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ESCOLA DE CIÊNCIAS
PROGRAMA DE PÓS-GRADUAÇÃO EM BIOLOGIA CELULAR E MOLECULAR
DOUTORADO

EDUARDO FILIPE AVILA SILVA

APLICAÇÕES DA TECNOLOGIA DE SEQUENCIAMENTO MASSIVO PARALELO EM EXAMES GENÉTICOS DE INTERESSE FORENSE:
ALTERNATIVAS ANALÍTICAS PARA A CASUÍSTICA BRASILEIRA

Porto Alegre
2019

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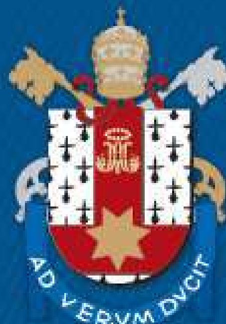

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Tese apresentada como requisito para a obtenção do grau de Doutor pelo Programa de Pós-Graduação em Biologia Celular e Molecular da Faculdade de Biociências da Pontifícia Universidade Católica do Rio Grande do Sul

Orientadora
Clarice Sampaio Alho

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RESUMO

A utilização de técnicas de biologia molecular na solução de problemas de interesse forense constitui importante parcela das ferramentas disponíveis na execução de perícias de interesse criminal ou cível, sendo particularmente relevante em casos como identificação de suspeitos de envolvimento em fatos delituosos, reconhecimento de vítimas em eventos de desastre de massa, determinação de paternidade ou outras relações de parentesco biológico, entre outros tipos de eventos de interesse judicial onde a genética forense é empregada. Nesse contexto, a perícia criminal muitas vezes se defronta com problemas relacionados ao tipo de amostra criminal cuja análise é alvo dos exames: vestígios biológicos com baixíssima quantidade de DNA ou com elevado grau de degradação do mesmo (devido à exposição a fatores ambientais), presença de misturas de mais de um doador, entre outros. Tais situações representam um desafio ao analista, onde a disponibilidade de novas tecnologias na solução de casos críticos pode ser a única alternativa disponível para a resolução do crime.

Técnicas de sequenciamento massivo paralelo (MPS) apresentam diversas vantagens para emprego em amostras criminais críticas, incluindo elevada sensibilidade, customização de painéis analíticos com inclusão de diferentes tipos de marcadores genéticos, capacidade de análise de amostras com elevado grau de degradação ou contaminação, quantidade significativa de informações geradas por uma única análise (possibilitando o processamento simultâneo de um grande número de amostras), entre outros. Dessa maneira, a inclusão das técnicas de MPS ao arsenal analítico disponível nas ciências forenses é indispensável, visando à solução de problemas forenses onde exames tradicionais são insuficientes ou não podem ser aplicados em razão das características da amostra.

O presente trabalho propôs investigar a adequação e conveniência da implementação de técnicas de MPS junto ao Laboratório Regional de Genética Forense da Polícia Federal, com o intuito de inclusão de tais técnicas no arsenal analítico disponível para execução de exames de rotina na casuística da Polícia Federal. Nesse contexto, foram realizados estudos de eficiência de genotipagem em um sistema de MPS com um painel comercial contendo 90 SNPs autossômicos informativos com elevada heterozigosidade, e 34 SNPs de clado superior do cromossomo Y. Os estudos incluíram a determinação das frequências alélicas associadas aos marcadores na população brasileira e a avaliação de sua eficiência nos processos de identificação humana. Amostras oriundas de casos forenses reais (*casework*) foram também examinadas, com objetivo de comparação da adequação da nova técnica proposta quando confrontada com resultados obtidos por métodos tradicionais, além da avaliação do impacto da natureza biológica da evidência criminal na qualidade e eficiência de genotipagem destas amostras. A pesquisa incluiu uma análise da variabilidade e estrutura genética dos haplótipos do cromossomo Y no Brasil, diferenciando as cinco regiões demográficas do país, e buscando caracterizar as diferentes linhagens patrilineares que contribuíram para a formação da população brasileira. Ao total, mais de 400 amostras criminais ou de referência das cinco regiões brasileiras foram avaliadas com sucesso por esta metodologia, sendo obtidas as frequências alélicas e outros parâmetros de interesse forense relevantes par auso em estudos de identificação humana.

Além do painel acima citado, o presente trabalho avaliou igualmente a eficiência de sequenciamento em linhagens uniparentais maternas de interesse forense. Assim, um painel comercial empregado no estudo do genoma mitocondrial completo foi avaliado quanto a sua capacidade de sequenciamento e adequabilidade para emprego em amostras da população brasileira. Este último estudo, um dos pioneiros na descrição de haplótipos de DNA mitocondrial com origem brasileira utilizando a sequência completa com um foco forense, incluiu um total de 96 amostras, no que pode ser considerado o embrião da organização de uma base de dados de genomas mitocondriais completos na população deste país, para uso em exames de interesse forense. Os resultados sugerem a adequabilidade dos métodos avaliados, os quais se mostraram apropriados para emprego na rotina analítica brasileira, com capacidade de geração de perfis genéticos ou haplótipos mitocondriais com adequada robustez, eficiência e confiabilidade.

PALAVRAS CHAVE: Genética forense; Sequenciamento massivo paralelo; População brasileira; Marcadores SNP; DNA mitocondrial; Cromossomo Y

ABSTRACT

Molecular biology techniques constitute a significant part of technical solutions available in forensic analysis for criminal or civil purposes. As such, these methods are particularly relevant in cases including crime suspects identification, mass disaster victim identification, paternity or other forms of kinship determination, among others. In this context, forensic analysis usually faces technical problems associated with the very nature of criminal biological evidence: samples with very low DNA amounts or in highly degraded conditions (usually due to environmental exposure), DNA mixtures including more than a single origin, background DNA contamination, inhibitors presence, and additional factors. This situation might represent a challenge to the analysts, where alternative technologies to traditional methods can be the only alternative for successful evaluation of some criminal evidences.

Massive Parallel Sequencing (MPS) techniques present advantages in challenging samples processing including high sensitivity, flexibility for panel design including different kind of forensic markers and polymorphisms, adequacy for use with degraded or contaminated samples, simultaneous processing of a high number of samples and/or polymorphisms (as a result of barcoding alternatives and humongous amount of data generated in a single run), along with other benefits. Therefore, inclusion of these techniques to routine analytical repertoire is key to efficient solution of current forensic problems caused by limits associated with traditional DNA genotyping, sequencing and analysis methods.

Adequacy and convenience of MPS techniques introduction and implementation in Brazilian Federal Police Forensic Genetics Regional Laboratory were evaluated in this project, in order to add such methods to available DNA analysis protocols in this facility. Genotyping efficiency studies were conducted in a commercial MPS panel including 90 highly heterozygous, informative SNP markers, as well as 34 high-clade Y-chromosome SNPs. Allelic frequencies for Brazilian populations and other relevant forensic descriptive indexes were estimated. In addition, efficiency of this method in real criminal casework samples was evaluated, and results compared to those obtained from traditional analysis methods. Impact of biological nature of criminal samples in genotyping efficiency was also analyzed. Finally, genetic variability and structure in Y-chromosome haplotypes distributions was assessed in Brazilian population, according to geopolitical regions, in order to describe paternal lineage contribution patterns to modern Brazilian populations. Over four hundred reference or criminal samples from all five Brazilian geopolitical regions were successfully evaluated, and resulting allele frequencies and forensic parameters obtained for human identification purposes.

A second commercial solution was also evaluated in its efficiency to generate complete DNA sequences of forensic relevant uniparental lineages. Thus, complete mitochondrial genomes were obtained through MPS techniques, whose efficiency and adequacy to Brazilian samples were explored. This study aims to lay the groundwork of a future database containing full-genome mitochondrial Brazilian sequences, for forensic purposes. Obtained results suggest adequacy of evaluated methods, which were found to be appropriate for routine use in Brazilian forensic casework processing, with ability to generate robust, efficient and trustworthy genetic profiles from Brazilian criminal samples.

KEYWORDS *Forensic genetics*; *Massive parallel sequencing*; *Brazilian population*; *SNP markers*; *mitochondrial DNA*; *Y-chromosome*

LISTA DE ABREVIATURAS

aDNA	Ácido desoxirribonucléico antigo
AIM	Marcador Informativo de Ancestralidade, do inglês, *Ancestry Informative Marker*
DNA	Ácido desoxirribonucléico
DVI	Identificação de Vítimas de Desastre, do inglês *Disasters Victims Identification*
dNTP	Deoxinucleotídeo Tri-Fosfato
emPCR	Reação em Cadeia da Polimerase de Emulsão, do inglês, *Emulsion Polymerase Chain Reaction*
LCN	do inglês *Low Copy Number*
MPS	Sequenciamento Massivo Paralelo, do inglês *Massive Parallel Sequencing*
NGS	Sequenciamento de Nova Geração, do inglês *Next Generation Sequencing*
PCR	Reação em Cadeia da Polimerase, do inglês, *Polymerase Chain Reaction*
PGM	Máquina Pessoal de Genoma, do inglês, *Personal Genome Machine*
RNA	Ácido ribonucléico
STR	Microssatélites, ou Repetições Curtas em Sequencia, do inglês *Short Tandem Repeat*
SWGDAM	Grupo Científico de Trabalho sobre Métodos de Análise de DNA, do inglês *Scientific Working Group on DNA Analysis Methods*

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CAPÍTULO 1 - APRESENTAÇÃO DO TEMA E OBJETIVOS

REFERENCIAL TEÓRICO

1. A Análise Molecular como Ferramenta Auxiliar à Justiça

O emprego de análises moleculares visando objetivos de identificação humana tem sido considerado uma ferramenta essencial e insubstituível no auxílio à Justiça, com aplicações abrangendo áreas distintas, incluindo a persecução penal e casos de interesse cível ou criminal (Murphy, 2018). Desde o trabalho pioneiro de *Sir* Alex Jeffreys e seus colaboradores, na década de 80 (Jeffreys et al., 1985), os avanços científicos nessa área do conhecimento tornaram possível demonstrar que polimorfismos de DNA podem ser empregados, com níveis significativos de precisão estatística, na individualização e na determinação da identidade de seres humanos (Butler, 2010). No contexto forense mais geral, a análise genética a partir de DNA coletado de evidências de natureza biológica integra uma variedade de áreas do conhecimento, incluindo técnicas de biologia molecular, genética de populações, genética clínica (em casos observados com ocorrência de padrões de herança distintos da mendeliana), genética animal ou vegetal, tecnologias avançadas de genômica e transcriptômica, ferramentas computacionais e estatísticas, entre outras. No aspecto jurídico-legal, esse conjunto de técnicas e conhecimentos podem auxiliar em uma ampla gama de casos de interesse cível ou criminal, incluindo a determinação de paternidade ou outros graus de parentesco biológico, investigação de crimes violentos ou outros de menor poder ofensivo, identificação de vítimas ou suspeitos de envolvimento nos mais diversos fatos delituosos, reconhecimento de vítimas de desastres em massa ou pessoas desaparecidas, assim como outros aspectos diversos, nos quais a identificação humana pelo DNA se faz necessária. Entre as análises envolvendo organismos não-humanos, tem-se como exemplo: i- a identificação de espécies animais ou vegetais com interesse legal, como aquelas envolvidas em crimes ambientais ou de biopirataria, ou na identificação de espécies proscritas ou com exploração comercial controlada, por exemplo); ii- individualização de organismos animais ou vegetais, como os utilizados na indústria e no comércio de alimentos (Dobrovolny et al., 2018); ou ainda iii- a determinação de origem nas investigações de rotas de tráfico ou comércio de espécies ilegais (Staats et al., 2016; Fett et al., 2019). Ferramentas moleculares podem ainda ser aplicadas como auxiliares em diversas etapas de exames periciais, como no emprego de análises de moléculas de RNA, para identificação e caracterização da natureza de vestígios biológicos em locais de crime (Døruma et al., 2018) ou em ensaios de metagenômica na caracterização de origem geográfica de determinados vestígios criminais (Clarke et al., 2017).

A relevância da análise de DNA na prática jurídica criminal se manifesta especialmente em três aspectos distintos: i- como elemento do conjunto probatório em casos criminais, reforçando a convicção, com elevado grau de probabilidade, nas provas de envolvimento de indivíduos; ii- na exoneração de

indivíduos erroneamente citados ou mesmo condenados, via relato testemunhal, eventos circunstanciais ou exames periciais de baixa fiabilidade ou interpretação improcedente – nestes casos de exoneração, a prova genética pode ser o único recurso para atestar-se a inocência de um indivíduo erroneamente envolvido ao delito (Hampikian et al., 2011); por fim, iii- na materialização de um suspeito via confronto positivo entre a amostra questionada e referências depositadas em bancos de perfis genéticos, os quais têm se mostrado eficazes na sugestão de identidade ou reconhecimento de potenciais autores, os quais de outra maneira teriam permanecido incógnitos (Struyf et al., 2019). Com relação a este tópico, o uso de bancos genéticos como apoio na investigação criminal tem crescido significativamente graças ao aumento no número de perfis agora disponíveis (BRASIL, 2019), ainda que questões jurídicas, legais e éticas ainda necessitem ampla e contínua reflexão (Hazel et al., 2018).

Mesmo considerando o atual estágio avançado de desenvolvimento das técnicas empregadas, a evolução e o aperfeiçoamento da análise molecular como ferramenta auxiliar à justiça estão focados em desenvolver, cada vez mais, protocolos analíticos mais seguros, confiáveis, de fácil uso e baixo custo. Tal avanço, contudo, necessita significativa qualificação e domínio científico e técnico por parte das equipes empenhadas em encontrar soluções alternativas, com intuito de superar atuais limitações exibidas pelos métodos correntes, além de simplificar e difundir de maneira mais abrangente o uso de ensaios de análise de DNA forense.

2. Elementos Técnicos do Exame de DNA no Contexto Criminal

O sucesso na utilização dos exames de investigação de DNA no interesse da justiça está diretamente relacionado ao aprimoramento contínuo dos protocolos empregados para a obtenção e análise de dados genéticos, os quais são resultantes da seleção criteriosa daqueles métodos e técnicas mais exitosos. Como consequência, tal prática acarreta a adoção, por parte das unidades de perícia e outras organizações técnico-científicas, das tecnologias de melhor desempenho para a rotina analítica, as quais são difundidas e adotadas como referência em um grande número de laboratórios forenses. (Rana, 2018). São objetos de seleção, por exemplo, características que incluem a redução do custo das análises, diminuição do tempo de processamento, aumento da eficiência e da robustez metodológica, elevada reprodutibilidade, possibilidade de emprego de automação na rotina, abrangência, bem como outras propriedades associadas às idiossincrasias e especificidades de determinados tipos de vestígios criminais de natureza biológica ou tipos de crimes avaliados (Shewale et al., 2014). Considerando este quadro, um conjunto bastante específico de alternativas analíticas foi selecionado como aquele mais próximo de reunir o maior número das vantagens acima referidas, o qual passou a constituir atualmente o referencial metodológico adotado em praticamente todos os laboratórios de investigação criminal no mundo (Linacre & Templeton, 2014). O cerne da genética forense aplicada mundialmente será brevemente explorado, por tópicos, como segue.

2.1 Polimorfismos Genéticos e Métodos Associados

A análise de marcadores genéticos do tipo microssatélite, também denominados STR (repetições curtas sequenciais, do inglês *short tandem repeat*), constitui a metodologia mais frequentemente usada na análise forense (Butler, 2010). Estes polimorfismos genéticos são caracterizados por repetições em sequência de um motivo de DNA, geralmente de quatro ou cinco nucleotídeos, onde as variantes se referem ao número de vezes em que o motivo é repetido (Marano & Fridman, 2019). Sua importância recai na alta heterozigosidade encontrada nas populações e elevada variabilidade entre indivíduos, o que permite que a análise de um reduzido número de *loci* seja suficiente para assegurar alta eficácia em procedimentos de individualização (Silva et al., 2012). A maior parte dos bancos de dados genéticos com fins forense em uso atualmente inclui sistemas que empregam quase que exclusivamente esse tipo de marcador genético, os quais são selecionados por critérios como neutralidade, variabilidade populacional e equilíbrio de ligação (Murphy, 2018). Os STR estão presentes em todos os cromossomos humanos, incluindo os sexuais, sendo seu uso igualmente difundido na caracterização de linhagens monoparentais paternas, na forma de avaliação de haplótipos do cromossomo Y (Alonso et al., 2018).

O protocolo analítico envolvendo os polimorfismos de STR segue, de maneira geral, as mesmas etapas mencionadas a seguir: i- extração do DNA de material biológico obtido a partir da evidência criminal; ii– etapa opcional de quantificação do DNA, com auxílio de técnicas específicas de PCR em tempo real; iii– amplificação das regiões polimórficas de interesse (STRs autossômicos e marcadores de cromossomo sexual) em PCR multiplex, usualmente através do uso de kits ou reagentes comerciais, que propiciam uma padronização dos procedimentos e marcadores utilizados; iv– análise de fragmentos, onde os produtos de PCR amplificados são submetidos à procedimentos de eletroforese capilar em analisadores genéticos, tendo como resultado a obtenção de um perfil genético; v– utilização do perfil obtido para os exames de confronto genético ou inclusão em bancos de dados, com execução das análises estatísticas consideradas apropriadas para cada caso (Linacre & Templeton, 2014).

Uma técnica alternativa de uso bastante frequente em laboratórios de análise forense é constituída pela análise de DNA de origem mitocondrial (mtDNA). A utilização dessa metodologia é especialmente comum em casos envolvendo determinados tipos de amostras criminais como pelos, amostras com baixa quantidade de DNA, ou amostras degradadas, onde o DNA nuclear muitas vezes propicia a obtenção de perfis de baixa qualidade, ou mesmo não é detectável (Pereira et al., 2018). Em situações como essas, o elevado número de cópias do genoma mitocondrial, comparativamente ao DNA facilita o sucesso na execução dos ensaios analíticos; entretanto, a herança materna exclusiva desses marcadores e a ausência de recombinação dificulta a identificação inequívoca de uma pessoa única, dado que todos indivíduos de mesma linhagem matrilinear em geral compartilham um haplótipo comum (Melton et al., 2012). Embora tenha sido proposta recentemente por Luo e colaboradores (2018), a possibilidade de herança paterna do DNA mitocondrial

ainda é considerada controversa (Lutz-Bonengel et al., 2018) e, como tal, este mecanismo ainda é visto com cautela comunidade forense.

No aspecto metodológico, a análise de mtDNA difere significativamente daquela usada na obtenção de perfis genéticos baseados em STR. Considerando que os polimorfismos do DNA mitocondrial são usualmente relativos a variações nos nucleotídeos presentes em posições determinadas do genoma desta organela, a aplicação de análise de fragmentos não é suficiente neste tipo de exame. Ao invés disso, são aplicadas técnicas alternativas como o uso do sequenciamento de Sanger, no qual a sequência de nucleotídeos do genoma mitocondrial é obtida e comparada a uma sequência de referência (Andrews et al., 1999), e apenas as variantes são reportadas (Parson et al., 2014).

2.2 Limitações Associadas às Técnicas Tradicionais de Análise Genética Forense

Os avanços tecnológicos e as melhorias integradas ao arsenal analítico disponível aos peritos forenses propiciaram o desenvolvimento de protocolos sensíveis, confiáveis e robustos na análise criminal de DNA, especialmente desenvolvidos e disponibilizados na forma de soluções comerciais amplamente utilizadas (Butler, 2014). Entretanto, as limitações associadas às técnicas tradicionais de análise genética, anteriormente mencionadas, são geralmente resultantes da natureza da evidência biológica cuja análise é o alvo. Dessa forma, a ocorrência de amostras criminais denominadas “críticas” continua sendo o grande desafio na obtenção de perfis genéticos a serem utilizados em exames de cotejo com interesse criminal (Bruijns et al., 2018).

Um dos exemplos mais relevantes de amostras críticas na rotina forense envolve amostra contendo reduzidas quantidades de DNA, também conhecidas como *low copy number* (abreviação LCN). A análise deste tipo de amostra apresenta dificuldades técnicas relevantes, as quais incluem a obtenção de perfis genéticos incompletos e/ou com sinais cuja intensidade dificulta a interpretação (Buckleton et al., 2016). A presença de fenômenos conhecidos como artefatos também é extremamente comum nestes casos, incluindo alelos *drop-in* ou *drop-out*, excesso de *stutter* nos produtos de PCR, desbalanço de heterozigoto ou sinais elevados de contaminação de background, (o que se assemelha e confunde-se com misturas de DNA), o que dificulta tanto a reprodutibilidade das análises quanto a capacidade de interpretação dos perfis genéticos resultantes (Marshall et al., 2015). Exemplos de amostras forenses usualmente associadas com características de LCN incluem principalmente as evidências biológicas de DNA epitelial, ou seja, aquele DNA depositado no suporte em decorrência do contato ou da manipulação de superfícies ou objetos. Este tipo de amostra, também conhecido como DNA de toque ou DNA de traço, representa um grande desafio na análise forense, em razão da variabilidade observada na ocorrência de fenômenos como transferência (incluindo eventos de transferência secundária, terciária ou de ordem superior), persistência, presença de inibidores ou contaminação com DNA ambiental humano ou de outras origens (Burril et al., 2019). O substrato ou suporte onde a amostra forense é depositada pode também afetar a qualidade da amostra de DNA, acelerando sua

degradação ou agregando a presença de contaminantes ou inibidores, afetando particularmente amostras de DNA de toque (Wood et al., 2017).

Além das baixas quantidades, o efeito da reduzida qualidade e/ou qualidade do DNA também pode impactar de maneira significativa o resultado de análises forenses. Os efeitos da degradação ambiental e química têm sido extensivamente investigados em estudos de validação de sistemas em DNA humano (Zavala et al., 2019). A possibilidade de obtenção de perfis genéticos completos é inversamente proporcional ao tempo em que a amostra fica exposta à atividade de nucleases ou a elementos, agentes e processos que degradam o DNA como fogo, salinidade, processos oxidativos e contaminantes (Wictum et al., 2013). A degradação do DNA em razão de mecanismos como a ação enzimática por nucleases de microrganismos (em condições de alta umidade), ou os efeitos ambientais como temperatura, umidade, radiação ultravioleta ou outros tipos de elementos nocivos à integridade molecular, constitui fator essencial na possibilidade de sucesso na obtenção de perfis genéticos de boa qualidade (Alaeddini et al., 2010). O tempo de duração da exposição do DNA ao agente degradante, bem como outros elementos adicionais também relevantes na eficiência de geração de resultados satisfatórios, agem prejudicando a amplificação ou em qualquer outra etapa analítica envolvida no exame (Hara et al., 2015). Os efeitos da degradação do DNA são particularmente limitantes na análise de marcadores do tipo STR, uma vez que a amplificação simultânea dos conjuntos gênicos por PCR *multiplex* envolve majoritariamente construções com *amplicons* longos, de tamanhos geralmente superiores a 200 pares de bases. Nessas condições, a degradação do DNA acarreta desbalanço de amplificação entre os diferentes marcadores afetando, particularmente, aqueles de maior tamanho, os quais em situações mais extremas podem não apresentar qualquer sinal ou resultado, impedindo a obtenção proficiente de perfis genéticos (Zavala et al., 2019).

Quando considerados os protocolos envolvendo análise de DNA mitocondrial, as maiores dificuldades técnicas estão associadas diretamente às peculiaridades apresentadas pelos métodos tradicionais de sequenciamento de Sanger. Tais metodologias são mais laboriosas, demoradas e com maior custo (Sultana & Sultan, 2018). Em razão disso, a maior parte dos laboratórios forenses adota como padrão o sequenciamento parcial do genoma mitocondrial, onde apenas os segmentos com maior variabilidade são sequenciados, como por exemplo a região controladora (localizada entre os nucleotídeos 16024 a 576 do genoma mitocondrial) ou ainda apenas as sequências hipervariáveis (Melton et al., 2012) localizadas dentro dessa, ignorando-se portanto a maior parte do genoma da organela. Considerando que as regiões ignoradas podem conter variantes gênicas ou polimorfismos importantes (não só para a investigação do haplótipo específico, mas também para diferenciar linhagens derivadas onde as variações estão localizadas unicamente na região codificante), a adoção de protocolos de análise incluindo o sequenciamento completo do genoma mitocondrial seria uma forma eficaz de trazer significativos benefícios à análise forense, aumentando a capacidade de discriminação entre linhagens maternas filogeneticamente próximas e beneficiando a investigação do haplótipo específico onde as mesmas são classificadas (van Oven, 2015).

2.3 Validação, Controle e Qualidade

A proximidade profissional entre as forças policiais, ou de segurança, e os órgãos oficiais de perícia pode ocasionar um fenômeno comumente observado entre peritos ou o pessoal com funções de natureza técnica: a identificação pessoal do analista com a missão de combate à criminalidade e a produção de provas de caráter acusatório ou condenatório. Estudos anteriores (Kassin et al., 2013) mostram que o problema do viés de confirmação pode se aplicar inclusive à análise genética, considerada uma referência em termos de rigor metodológico nas ciências forenses. A introdução de erros de julgamento ou de vieses contextuais é ainda mais significativa em situações envolvendo amostras de análise complexa, como casos de mistura, evidências com baixa quantidade de DNA ou na avaliação de perfis genéticos de baixa qualidade, cuja necessidade de tomada de decisão e a interpretação por parte do corpo técnico é mais pronunciada. A adoção de protocolos analíticos padronizados, os quais incluam a normatização de procedimentos em todas as etapas do processo laboratorial, especialmente as que envolvam tomada de decisão, é a única maneira de se contornar tais vieses (Cole, 2013).

Na busca de uniformidade e padronização de métodos na execução de exames de DNA de interesse forense, foi proposta a formação de um comitê científico ocupado com o estudo e publicação de orientações técnicas voltadas a diversos aspectos da análise forense envolvendo ensaios com DNA. Esse grupo, denominado SWGDAM (*Scientific Working Group on DNA Analysis Methods* ou Grupo Científico de Trabalho sobre Métodos de Análise de DNA), possui um significativo número de publicações contendo guias para validação de métodos, execução de ensaios analíticos e metodologias para interpretação de resultados, buscando uniformizar os procedimentos executados em laboratórios de genética forense. Dentre tais publicações, destaca-se o documento denominado "*SWGDAM Validation Guidelines for DNA Analysis Methods*", original de julho de 2003 e com última revisão publicada em dezembro de 2016 (SWGDAM, 2016), onde houve a inclusão de propostas específicas para a validação de métodos envolvendo técnicas de sequenciamento massivo paralelo. Conforme o SWGDAM, os processos de validação de métodos e reagentes deve incluir pelo menos duas etapas: validação de desenvolvimento e validação interna. A validação de desenvolvimento caracteriza-se pela proposição de testes para determinar as condições e as limitações de uma nova metodologia ou procedimento para a análise de DNA com fins forenses, sendo esta geralmente executada por fabricantes de reagentes com destinação comercial, ou por grupos de pesquisa ou entidades acadêmicas convidadas por estes. Métodos oriundos de pesquisas científicas podem ainda ter sua validação executada pelo próprio grupo ou equipe desenvolvedora. Já a validação interna é caracterizada por uma série de ensaios executados no próprio laboratório onde os métodos serão implementados, buscando demonstrar que os procedimentos previamente estabelecidos para análise de DNA com fins forenses reproduzem resultados conforme o esperado, nas condições específicas do laboratório, e incluem aspectos relacionados aos reagentes, equipamentos utilizados e protocolos analíticos específicos a serem empregados. Recomenda-se que, previamente à adoção de qualquer nova tecnologia ou método com objetivos de

aplicação forense, a equipe de analistas proceda a validação interna do ensaio em tela. Os laboratórios forenses são encorajados a sistematicamente rever os seus procedimentos operacionais padrão e protocolos de validação e de qualidade, à luz destas orientações, para atualização conforme necessário. O guia prevê ainda que as diretrizes nele contidas evoluam assim que tecnologias futuras venham a emergir. Nessas condições, é primordial que as etapas de validação sejam executadas quando da adoção de novos procedimentos ou da inclusão de métodos de investigação de DNA, adicionais ao repertório analítico já disponível aos peritos, buscando excelência e qualidade nos resultados obtidos e confiabilidade nas conclusões advindas destes. Destaca-se ainda a existência de diversas outras organizações internacionais cujo objetivo essencial é propor a padronização de exames periciais utilizando técnicas de DNA forense (Butler, 2015). No Brasil, a prerrogativa de regulamentação e normatização metodológica dos exames de genética forense no âmbito criminal é exercida pelo Comitê Gestor da Rede Integrada de Bancos de Perfis Genéticos, responsável pela padronização de técnicas nas etapas de coleta e análise de vestígios biológicos, e na administração dos perfis genéticos associados aos bancos de dados existentes nos laboratórios oficiais operados pelos diversos entes federativos (BRASIL, 2019b). Uma série de publicações é regularmente produzida e atualizada pelo referido corpo técnico, incluindo recomendações técnicas de operação dos laboratórios, tutoriais, manuais de procedimentos operacionais, bem como relatórios de auditoria externa, e da situação dos bancos de dados genéticos participantes da Rede Integrada (BRASIL, 2017).

3. Novas Tecnologias e Soluções na Investigação Genética Criminal

3.1 Marcadores e polimorfismos alternativos na investigação forense

As restrições técnicas apresentadas pela metodologia tradicional de análise de fragmentos acoplada à eletroforese capilar, com avaliação dos marcadores do tipo STR, acarretou o estudo de variações metodológicas visando especificamente a investigação de polimorfismos genéticos alternativos, cuja análise seja possível nos casos de amostras críticas, ou que apresentem outros fatores limitantes (Budowle & van Daal, 2018). Nesse contexto, os polimorfismos de nucleotídeo único (SNP) tornaram-se componentes importantes no campo da genética forense, despertando interesse no desenvolvimento de painéis para avaliação de DNA baseados nesses marcadores, especialmente em razão do custo, facilidade de estudo e baixas taxas de mutação (Schneider, 2012).

Os polimorfismos genéticos do tipo SNP correspondem a formas alternativas ou variantes presentes na sequência de nucleotídeos e, quando analisados no nível individual, são caracterizadas por diferenças em relação à qual base nitrogenada está presente em uma determinada posição do genoma. Os SNPs são, assim, o resultado de alteração em um único nucleotídeo em determinada posição do genoma humano, apresentando-se em geral na forma bialélica (Børsting et al., 2014). Mais de dois bilhões de SNPs foram

identificados no genoma humano, e sua avaliação pode ser feita pela ligação a segmentos flanqueadores à posição de um SNP específico, avaliando um fragmento curto do DNA (Kitts et al., 2013). Devido à natureza preponderantemente bialélica, este tipo de polimorsfimo possui um menor poder de discriminação individual se comparado com marcadores do tipo STR com uso forense, os quais são multialélicos. Assim, em aplicações de identificação humana, de forma geral são necessários de quatro a oito SNPs para se ter uma equivalência ao poder de discriminação de um único *locus* STR, com valores levemente superiores em exames de determinação de vínculo biológico (Mo et al., 2018). Estima-se que seja necessário entre 40 e 50 SNPs para se alcançar o mesmo poder de discriminação individual que é fornecido pelo conjunto padrão de 13 STRs inclusos no sistema CODIS original e designado para a individualização humana (Kidd et al., 2006).

O avanço das tecnologias de sequenciamento de DNA tem favorecido o aumento dos estudos focados em variantes polimórficas de uma única posição nucleotídica. Neste contexto, se observou que as análises de SNPs podem ser igualmente utéis, de acordo com o conjunto de marcadores investigado, e dependendo da distribuição das frequências populacionais dos mesmos em, pelo menos, quatro tipos de aplicações forenses distintas: i– em técnicas de identificação ou individualização humana, onde os SNPs empregados apresentam variabilidade intrapopulacional e alta heterozigosidade (Sanchez et al., 2006; Kidd et al., 2012; Huang et al., 2018); ii– na determinação da origem biogeográfica de indivíduos, e na avaliação dos índices de contribuição de diferentes populações ou povos ancestrais de origem continental distinta, com fins de avaliar os padrões de miscigenação e de ancestralidade no DNA de uma amostra, (com uso de SNPs classificados como AIMs, do inglês *Ancestry Informative Markers*) (Zhao et al., 2019); iii– na predição de caracteres fenotípicos e traços associados à aparência física, como aqueles verificados nos padrões de pigmentação da pele, olho e cabelos humanos (EVC, do inglês *Externally Visible Characteristics*, ou Características Externamente Visíveis), (Marano & Fridman, 2019); iv– na determinação de polimorfismos determinantes na classificação haplotípica de linhagens monoparentais (van Oven & Kayser, 2009; Willuweit & Roewer, 2015). Entre as aplicações não forenses, se destaca o estudo de SNPs com aplicações médicas, que incluem associação dos mesmos com manifestações clínicas ou patológicas, podendo ser também utilizadas no diagnóstico, prognóstico ou seleção de abordagem terapêutica para diversos tipos de enfermidades (Teama, 2018).

No âmbito da identificação humana, o emprego de marcadores SNPs vem ganhando espaço nos últimos anos em razão da disseminação e popularização das técnicas que permitem a genotipagem ou o sequenciamento simultâneo de um grande número de marcadores e/ou amostras (Shen et al., 2015). Em razão desse progresso tecnológico, as diversas vantagens na utilização dos marcadores SNP em identificação humana puderam ser explorados. Entre estas vantagens está a possibilidade de desenvolvimento de painéis incluindo *amplicons* de tamanho reduzido, o que permite sucesso na obtenção de perfis genéticos completos, mesmo a partir de amostras degradadas ou de baixa qualidade (Budowle & van Daal, 2018). Ainda que os marcadores do tipo STRs sejam mais frequentemente utilizados na rotina da prática forense devido ao seu

maior poder de discriminação, facilidade de interpretação de amostras de misturas e facilidade de combinação em reações de multiplex (onde o número inferior de marcadores para um mesmo poder de discriminação pode ser diferencial), a utilização de marcadores SNPs pode ter um papel significativo em casos forenses de difícil resolução, como aqueles que incluem amostras degradadas ou oriundas de material biológico antigo, ou em casos especializados, incluindo outros nichos forenses ainda a serem explorados (Gettings et al., 2015). Como exemplo, marcadores SNPs localizados em regiões genômicas muito próximas podem ser avaliados conjuntamente na forma de um microhaplótipo, e como tal oferece algumas vantagens em relação ao uso de SNPs independentes, avaliados separadamente (Oldoni et al., 2019). Ainda no campo da identificação humana, a utilidade dos SNPs foi demonstrada também em situações de difícil abordagem metodológica, como na identificação de laços de parentesco e relações biológicas em casos de reconstrução familiares complexos (Pontes et al., 2015; Morimoto et al., 2018), ou na individualização de gêmeos monozigóticos (Weber-Lehmann et al., 2014).

Como exemplo recente do uso de SNPs, a utilização de bases de dados de empresas especializadas em serviços de genealogia genética na busca de suspeitos em casos criminais gerou ampla discussão sobre os aspectos éticos e legais, incluindo direitos básicos como o da privacidade e de relações de consumo, diante desta abordagem investigativa conduzida por órgãos policiais dos Estados Unidos (Ram et al., 2018). Marcadores SNPs, que podem servir para a predição de características fenotípicas (EVC) e na busca por relações de parentesco ou na estimativa individual da ancestralidade biogeográfica, são por essas características buscados pelos consumidores. Tais marcadores, entretanto, também são informativos de discriminação individual tendo, portanto, potencial significativo para emprego em atividades de investigação criminal (Greitak et al., 2019). A abundância desse tipo de polimorfismo, e a possibilidade de caracterizar fenotipicamente um ser humano, são algumas das principais razões do grande interesse atual no campo forense pelos SNPs. Entre as EVCs, a variação de pigmentação em humanos tem sido um bom alvo. Variações do tipo SNPs, podem levar a substituições de aminoácidos na proteína, alterando as propriedades funcionais do produto traduzido, o qual pode se expressar em fenótipos distintos, ou mesmo estarem localizados em regiões não codificantes, mas em desequilíbrio de ligação com outras formas polimórficas, essas sim responsáveis pelo fenótipo (Bradbury et al., 2019). Os traços fenotípicos mais promissores para a identificação forense são aqueles relacionados à pigmentação da pele, olhos e cabelos, por serem características muito marcantes, de fácil visualização, e por constituírem um dos fenótipos amplamente variáveis na população humana (Liu et al., 2013). O desenvolvimento de técnicas quantitativas para mensurar a pigmentação proporcionou uma maior objetividade na classificação da variação de pigmentação existente, permitindo identificar de forma precisa os genes que, de alguma maneira, exercem influência sobre essa característica (Norton et al., 2016).

Apesar de promissora, a utilização de marcadores genéticos de traços fenotípicos na identificação humana para fins forenses, bem como a descrição de genes e do mecanismo pelo qual eles influenciam a

definição das características fenotípicas ou determinam sua associação com essas, é ainda de difícil elucidação, uma vez que essas características apresentam um padrão de herança complexo, por serem determinadas por múltiplos genes (poligenia) e por sofrerem forte influência do meio ambiente (Sturm & Larsson, 2009). Não obstante, estudos demonstram que a base genética da variação normal da pigmentação é passível de ser decifrada se forem avaliados os alvos adequados (Walsh et al., 2013). Estudos diversos buscam não apenas a verificação da característica pigmentação, mas também de outros elementos de predição fenotípica, incluindo relações biométricas de distância para predição de características de face (Kayser, 2015; Lippert et al., 2017). Alguns destes estudos possuem foco específico em populações latino-americanas, visando a caracterização de variantes próprias para esse grande grupo populacional, e suas peculiaridades (Claes et al., 2014; Adhikari et al., 2016).

Dentre as aplicações forenses de utilização de marcadores do tipo SNP, a avaliação da presença destas variantes polimórficas no cromossomo Y possui significativo interesse. Devido a sua natureza não recombinante, a análise do cromossomo Y permite a obtenção de informações não apenas referentes à identificação humana, mas igualmente no que tange aos dados filogeográficos das origens das linhagens de Y (Ochiai et al., 2016). A classificação dos haplótipos em haplogrupos é geralmente feita segundo um modelo hierárquico, onde a presença de alelos específicos em determinados *loci*, cuja mutação se acredita tenha ocorrido em um evento único, permite associar a linhagem patrilínea com sua origem geográfica, utilizando estudos de frequência populacional e análises de movimentos migratórios em nível local ou mundial (van Oven et al., 2014). Estudos diversos apontam para o amplo predomínio de linhagens do cromossomo Y de origem europeia na composição da população brasileira, devido basicamente a fatores históricos e sociais referentes à imigração e ocupação do território nacional, embora a presença de povos nativo-americanos, de africanos trazidos como escravos e de imigrantes recentes de origem principalmente européia e asiática tenham originado uma população altamente miscigenada, com elevado grau de diversidade genética (Grattapaglia et al., 2005; Pena et al, 2011). A maior parte dos estudos realizados no que se refere à variabilidade genética dos haplótipos de cromossomo Y já realizados no Brasil, se refere quase que exclusivamente a marcadores do tipo STR (Oliveira et al., 2014; Palha et al., 2012). Um pequeno número de estudos já foi realizado incluindo marcadores do tipo SNP, a maior parte limitados a pequenas populações e/ou a um reduzido número de marcadores pesquisados (Carvalho-Silva et al., 2001; Silva et al., 2006; Hünemeier et al., 2007; Gonçalves et al., 2008; Azevedo et al., 2009; Carvalho et al., 2010). Estudos mais abrangentes incluindo a população brasileira tem sido propostos apenas nos últimos anos (Resque et al. 2016). Oliveira e colaboradores (2014) demostraram que os marcadores Y-SNPs são capazes de detectar diferenças entre as frequências haplotípicas de populações de diferentes estados ou regiões brasileiras, não aparentes quando empregados marcadores do tipo Y-STR. Tais diferenças são importantes quando da utilização forense, sendo determinante na caracterização de frequências de haplótipos ou na composição de bancos de dados genéticos. Assim, a utilização de Y-SNPs não se limita à identificação de indivíduos do sexo

masculino em amostras criminais, mas pode ser utilizado como uma ferramenta de análise sobre a variabilidade genética da população brasileira, bem como sua origem histórica, visando à compreensão dos processos de miscigenação que deram origem ao moderno povo brasileiro.

3.2 MPS e outras tecnologias alternativas na análise de DNA

Sequenciamento Massivo Paralelo (do inglês *Massive Parallel Sequencing* ou *MPS*), também conhecido como Sequenciamento de Próxima Geração (do inglês *Next Generation Sequencing* ou *NGS*), é a terminologia usada para descrever diferentes tecnologias de análise de DNA recentes, entre as quais se destacam as diversas plataformas dos fabricantes Illumina (MiSeq, HiSeq, Solexa), Ion Torrent (Proton; *Personal Genome Machine* - PGM) e Pacific Biosciences (SMRT). Estas tecnologias, relativamente recentes e sua implementação e desenvolvimento, permitem o sequenciamento de DNA e RNA em uma grande quantidade de segmentos e/ou de amostras, de forma muito mais célere e econômica, quando comparado ao sequenciamento (ou análise de fragmentos) tradicional do tipo Sanger, e como tal têm revolucionado o estudo da genômica e da biologia molecular (Alonso et al., 2018). O sistema Ion Torrent, ao contrário das plataformas concorrentes Illumina (Solexa) e Pacific Biosciences, não faz uso de sinais ópticos em sua tecnologia de detecção (Bruijns et al., 2018). Ao invés disso, explora a peculiaridade bioquímica de que a adição de um dente a um polímero de DNA libera um íon hidrogênio (H^+). O processo de análise inicia adicionando adaptadores em segmentos de DNA alvo, os quais são amplificados individualmente em vesículas de uma emulsão (técnica de PCR de emulsão ou *emPCR*) depois de serem acoplados a partículas magnéticas (*beads*). Cada vesícula é isolada em poços de um microcondutor (*chip*), a qual é inundada por dNTPs, juntamente com tampões de polimerase, em ciclos que usam A, T, C e G individualmente e de maneira sequencial. O íon de hidrogênio liberado pela incorporação do dNPT altera o pH do poço onde está ocorrendo a síntese de DNA, o qual é detectado por ciclo em microreatores. As alterações do pH permitem determinar qual base se adicionou à sequência do DNA alvo a cada ciclo, de acordo com o a solução de nucleotídeos empregada no ciclo de extensão em tela. Tais variações são reconhecidas e registradas como sinais elétricos, revelando, por fim, a configuração de bases de cada sequência alvo (Goodwin et al., 2016).

As vantagens desta tecnologia para as finalidades forenses têm popularizado de maneira significativa o emprego da mesma nas investigações com fins criminais (de Knijff, 2019). Entre as vantagens encontradas é possível destacar a sensibilidade, uma vez que esta técnica permite a avaliação de quantidades bastante reduzidas de DNA, obtendo sucesso em ensaios com menos de 1ng de DNA inicial (Pereira et al., 2018). Amostras de DNA de baixa qualidade técnica, como aquelas apresentando elevado grau de degradação, podem ser também analisadas através da adoção de abordagens analíticas incluindo a redução do tamanho dos *amplicons* utilizados no design do painel de marcadores (Woerner et al., 2018). Além disto, a própria natureza da técnica, onde os resultados são a sequência de nucleotídeos que compõem o DNA alvo, possibilita um refinamento muito importante nos resultados de interesse forense (especialmente em

análises envolvendo marcadores STR) quando confrontados com aqueles obtidos a partir das técnicas tradicionais de análise de tamanho de fragmentos (obtidos pelos métodos de eletroforese capilar), uma vez que permite a detecção de variantes não apenas relacionadas ao tamanho de fragmento ou no número de nucleotídeos, mas sim na própria composição destes em um contexto individual (Butler, 2015b). Outras vantagens já descritas em estudos anteriores incluem: i- a simplicidade da rotina analítica, comportando o emprego de automatização dos processos (Kampmann et al., 2016); ii- a disponibilidade de plataformas computacionais para a construção de painéis de interesse (Ion AmpliSeq™ e Illumina AmpliSeq™, por exemplo); iii- a adaptação do rendimento de acordo com a necessidade (escalabilidade da metodologia, com uso de *chips* desenhados para níveis flexíveis de performance), o qual permite uma flexibilidade de adaptação para o número de amostras e de segmentos a serem avaliados; iv- a velocidade do processo e a capacidade de processamento simultâneo de um número significativo de amostras, através de técnicas de *barcoding*, permitindo a otimização no processamento da demanda analítica de um laboratório (Goodwin et al., 2016).

3.3 Potencial de uso da técnica de MPS em casos de interesse criminal

As aplicações forenses com potencial utilização da tecnologia de MPS são bastante amplas, apresentando algumas vantagens com relação os métodos de uso atual. Entre os exemplos, a identificação de vítimas de desastres em massa (DVI) pode se beneficiar de maneira particular desta técnica, em razão do volume de processamento e da enorme quantidade de dados gerados. Tendo como o exemplo a tragédia ocorrida em 2001 no World Trade Center, nos Estados Unidos, as abordagens periciais incluem não apenas a tipagem dos corpos de vítimas, mas também de pequenos fragmentos destes, além do enorme volume de amostras que inclui amostras de referência direta e de familiares (Biesecker et al., 2005). Outros exemplos como o tsunami no Oceano Índico em 2004, o incêndio ocorrido em 2004 no supermercado Ycuá-Bolaños em Assunção-Paraguai, o furacão Katrina em 2005, ou ainda os acidentes ocorridos em 2005 com o vôo 447 da Air France ou em 2007 com o vôo TAM JJ 3054, no Brasil, são um desafio para os peritos criminais, médicos e odonto-legistas, papiloscopistas e para os técnicos de laboratórios de biologia molecular encarregados da identificação das vítimas e dos restos mortais. Isto ocorre porque os elementos utilizados pelos médico-legistas, odonto-legistas e/ou papiloscopistas em suas técnicas de identificação, como impressões digitais, sexo, compleição física, grupo étnico, estatura e arcada dentária podem estar alterados a ponto de impossibilitar qualquer conclusão. Desta forma, resta como única possibilidade de identificação o uso das técnicas de DNA (Alonso et al., 2005; Tillmar et al., 2018).

Considerando o espectro das amostras antigas/ancestrais, esforços vêm sendo olvidados para que se obtenha DNA antigo (aDNA), a partir de ossos e/ou dentes, contornando a presença de muitas substâncias inibidoras, a degradação e a baixa quantidade de DNA. Uma vez que os métodos utilizados para extração de DNA a partir de restos de esqueletos antigos também têm um forte efeito sobre o sucesso de amplificação, é importante o uso de um procedimento de extração eficiente, capaz de remover todos os inibidores

possíveis, para se obter o máximo de DNA de boa qualidade disponível (Rohland et al., 2018). Uma experiência bem-sucedida foi identificação genética de Leopoldo III, na Áustria, e de cinco esqueletos datados com 300 anos encontrados no túmulo de Auersperg, Ljubljana, Eslovênia, foram também realizadas com sucesso através de marcadores autossômicos, além dos mitocondriais (Bauer et al., 2013). Estudos paleogenéticos mostram que a capacidade de obtenção de DNA antigo é cada vez mais ampla, sendo possível a avaliação de DNA de exemplares de restos humanos, ou de outras espécies de hominídeos, onde os genomas são proveitosos até mesmo na compreensão dos processos evolutivos da espécie (Marciniak & Perry, 2017). Além disso, o desenvolvimento de marcadores de ancestralidade específicos para uso com aDNA obtidos de restos humanos permitiu o estudo detalhado da estruturação genética existente em populações caucasianas ancestrais, fato não possível anteriormente com o uso de marcadores de ancestralidade baseados em populações modernas (Esposito et al., 2018)

Amostras com misturas de DNA de diferentes contribuintes são frequentes nos casos forenses, sendo que a maioria delas se concentra nas amostras coletadas a partir de crimes de agressão sexual. Estima-se no Brasil, que cerca de 30% das ocorrências deste tipo de crimes contra mulheres, o agressor é desconhecido à vítima (Cerqueira & Coelho, 2014), sendo esse um dos maiores desafios na identificação dos criminosos durante a investigação policial de estupro (Corovic et al., 2012). A capacidade de resolução de misturas com utilização de MPS é superior às técnicas tradicionais, baseado na capacidade de observação de alelos baseados não apenas no tamanho do amplicon, mas de sua sequência específica, o que permite um refinamento maior na diferenciação de componentes em uma mistura (Chan et al., 2018). Relatos recentes incluem a primeira condenação com utilização de DNA analisado por métodos de NGS, na Holanda. A utilização das técnicas em questão permitiu a deconvolução de uma mistura complexa, tarefa que não foi possível com o uso dos métodos tradicionais (Augestein, 2019).

Assim, amostras provenientes de material biológico em estado conservação ruim, em escassez, com misturas, ou antigos podem apresentar pouco DNA útil e/ou em estado elevado de degradação, de forma a não serem factíveis de obtenção de perfis genéticos a partir destes através das técnicas tradicionais de análise de STRs. Se a amplificação deste DNA com sistemas tradicionais tem eficácia insuficiente, portanto, o uso de sistemas de MPS passa a ser uma ferramenta alternativa e importante para esse fim. Os exemplos aqui discutidos não esgotam a variedade de aplicações forenses onde a técnica de MPS possui excelente potencial, incluindo as inúmeras alternativas incluindo DNA não-humano para investigação criminal (Arenas et al., 2017).

3.4 Painéis comerciais inclusos no presente estudo

O uso de painéis comerciais em laboratórios de investigação forense é geralmente preferível em relação ao emprego de técnicas ou marcadores desenvolvidos *in-house*, em razão das exaustivas etapas de validação pelas quais tais reagente são submetidos, os quais são executados tanto pelos desenvolvedores e

fabricantes quanto pela comunidade acadêmica (Eduardoff et al., 2014; Wai et al., 2018). Além disso, o uso de soluções comerciais facilita também a reprodutibilidade e uniformidade de condições sob as quais os exames genéticos são executados, os quais podem ser repetidos de maneira bastante similar em qualquer outro laboratório, o que facilita o acesso ao contraditório na produção da evidência genética (Murphy, 2018). Assim, as duas soluções comerciais escolhidas para serem avaliadas no presente estudo são a seguir brevemente descritas.

3.4.1 HID Ion Ampliseq Identity Panel

O kit comercial HID Ion Ampliseq Identity Panel, fabricado pela empresa Thermo Fisher, reúne 90 marcadores bialélicos do tipo SNP, especialmente selecionados para fornecer um elevado poder de discriminação em aplicações de identificação humana, comparável àqueles obtidos com os atuais reagentes disponíveis utilizando marcadores do tipo STR. Os marcadores SNP inclusos neste painel foram selecionados a partir de estudos prévios, sendo 43 deles propostos por Kidd e colaboradores (2006) e 47 inclusos no painel sugerido pelo consórcio SNPforID (Sanchez et al., 2006), e são caracterizados por apresentarem altos níveis de heterozigosidade intrapopulacional, conforme investigado em diversas populações mundiais. Além disso, foram inclusos 34 marcadores SNPs do cromossomo Y, os quais permitem a designação do clado superior no qual os haplótipos do cromossomo Y podem ser classificadas. Este kit foi originalmente projetado para possibilitar o processamento efetivo de amostras forenses críticas ou com características que dificultam o emprego de técnicas tradicionais, incluindo um design do painel limitado a *amplicons* de tamanho reduzido quando comparado aos marcadores tradicionais de PCR. Estudos explorando a validação de desenvolvimento ou aplicações forenses do reagente em tela foram publicados, os quais demonstraram que o mesmo possui características que favorecem seu emprego em exames envolvendo amostras de interesse forense (Børsting et al., 2014; Eduardoff et al., 2014; Buchard et al., 2016; Kampmann et al., 2016; Guo et al., 2016; Salata et al., 2016; Meiklejohn et al., 2017). Desde o desenvolvimento, a utilização desta solução comercial na identificação humana tem ensejado a verificação das frequências destes marcadores em algumas populações mundiais, incluindo dinamarqueses (Børsting et al., 2014), bascos (Garcia et al., 2017), chineses (Li et al., 2018; Liu et al., 2018) e somalis (van der Heijden et al., 2017).

3.4.2 Precision ID mtDNA Whole Genome Panel

O kit comercial Precision ID mtDNA Whole Genome Panel foi utilizado em uma versão pré-comercial, disponibilizado em condições de *early-access*. O reagente em tela realiza o sequenciamento do genoma mitocondrial completo, com o uso de 162 diferentes pares de primers, os quais são amplificados em duas distintas reações de multiplex. O resultado dessa amplificação é a geração de 162 *amplicons* curtos cujas extremidades são sobrepostas aos fragmentos contíguos, recobrindo a totalidade do genoma mitocondrial. Esta estratégia de sequenciamento foi recentemente utilizada com sucesso no processamento

de DNA mitocondrial antigo, altamente degradado, em mistura e incluindo diversos tipos de amostras críticas com interesse forense (Chaitanya et al., 2015; Churchill et al., 2017; Churchill et al., 2018; Strobl et al., 2018; Wai et al., 2018).

OBJETIVOS

Objetivo Geral

Este trabalho teve como objetivo avaliar as aplicações da tecnologia de sequenciamento massivo paralelo como alternativa analítica para casuística brasileira de exames criminais de interesse forense.

Objetivos Específicos

1- Avaliar o desempenho dos 124 marcadores SNP presentes no sistema comercial HID Ion Ampliseq Identity Panel (Thermo Fisher) para MPS nas cinco diferentes regiões brasileiras, a fim de identificar suas frequencias, bem como os parâmetros forenses e outros elementos de interesse.

2- Avaliar o desempenho dos 124 marcadores SNP presentes no sistema comercial HID Ion Ampliseq Identity Panel (Thermo Fisher) para MPS em amostras brasileiras criminais reais, em comparação com as técnicas tradicionais de análise.

3- Avaliar o sistema comercial Precision ID mtDNA Whole Genome Panel (Thermo Fisher) em amostras da população brasileira, com fins de avaliação da eficiência de genotipagem sob o ponto de vista forense, e também comparativamente aos métodos tradicionais de análise de DNA mitocondrial

CAPÍTULO 2 - Forensic characterization of Brazilian regional populations through massive parallel sequencing of 124 SNPs included in HID Ion Ampliseq Identity Panel

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Research paper

Forensic characterization of Brazilian regional populations through massive parallel sequencing of 124 SNPs included in HID ion Ampliseq Identity Panel

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ABSTRACT

Use of Massive Parallel Sequencing (MPS) techniques has been investigated by forensic community aiming introduction of such methods in routine forensic casework analyses. Interesting features presented by MPS include high-throughput, ability to simultaneous genotyping of significant number of samples and forensic markers, workflow automation, among others. Emergence of single nucleotide polymorphism (SNP) as forensic relevant markers was facilitated in this process, since concurrent typing of larger marker sets is necessary for obtaining same levels of individual discrimination provided by other marker categories. In this context, HID Ion Ampliseq Identity Panel is a commercial solution with forensic purposes comprising simultaneous analysis of 90 highly informative autosomal SNPs and 34 Y –chromosome superior clade SNPs for male lineage haplotyping. SNP typing can be obtained with smaller amplicons, and this panel was designed for efficient processing of critical or challenging forensic samples. In this work, a sample of 432 individuals from all five Brazilian geopolitical regions was evaluated with this panel, in order to access feasibility of this panel use in a national basis. Results obtained for all five regions, including forensic parameters, show that this marker set can be efficiently employed for Brazilian nationals in human identification or kinship determination applications, due to high levels of genetic discriminative information content displayed by Brazilians. Interpopulation comparison studies were executed among Brazilian regional populations and 26 worldwide populations, in order to access genetic stratification occurrence. Some levels of population structure were identified, and impact on database design was discussed. Y-chromosome haplotyping of Brazilian samples revealed high levels of European ancestry in Brazilian male lineages, and utility of haplotyping in real forensic casework is addressed. Finally, genotyping and sequencing efficiency with this panel were addressed, as an effort to appraise the adequacy of this panel use in Brazilian national forensic demands.

1. Introduction

Short Tandem Repeats (STR)-based length polymorphism analysis, associated with Capillary Electrophoresis (CE) techniques, has been the gold standard in forensic genetics for the past decades [1]. STR markers are highly polymorphic and informative, and as such were chosen as the core of most criminal genetic databases established worldwide [2]. Despite that, some limitations are still associated with these methods [3]. Relatively large amplicons are generated, posing a range of problems in successfully obtaining genetic profiles for a variety of biological and criminal samples. Low template DNA copy numbers, highly degraded DNA or environmental exposure can considerably impact genetic profile quality, with prominent effects on larger amplicons [4].

In order to circumvent such technical issues, the use of different genetic markers has been proposed [5]. Single Nucleotide Polymorphisms (SNPs) constitute genetic variations where a unique, specific nucleotide position in the genome can be found in multiple forms within a population. SNPs are widespread in human genome, and its use in forensic applications present some advantages over commonly used STRs, since SNPs have a lower mutation rate, can be typed with smaller amplicons and can also provide additional genetic information, as parental lineage determination, biogeographical ancestry or phenotypical traits assessment [6]. Since SNPs are usually found in biallelic forms in different populations, a larger number of different markers

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must be analyzed simultaneously to achieve similar power of discrimination or random match probability, when compared to their more polymorphic STRs counterparts [7]. Therefore, SNPs use in forensic DNA analysis for identification purposes have been limited due to the restricted capacity of simultaneous typing of different markers in a single run presented by CE method [8].

As an alternative, Massive Parallel Sequencing (MPS) techniques can be used for simultaneous typing of a large number of markers, with high throughput and consequent reduced analysis time [9]. Sample barcoding allows concurrent evaluation of significant number of samples, and process automation can be incorporated to minimize handling of criminal samples and improve workflow efficiency [10]. MPS achieves DNA typing on a single base resolution, and experimental design can be planned to assure high coverage. The HID-Ion AmpliSeq Identity Panel (Thermo Fischer Scientific, Waltham, MA, USA) commercial solution offers simultaneous amplification of 90 autosomal SNP markers and 34 Y upper-clade SNPs, designed to generate small size amplicons and successfully allow processing of highly degraded, low input and other forensic challenging samples [11]. Validation studies show that robust, efficient and reliable typing of forensic samples could be accomplished with different versions of this product, allowing its use in forensic individual identification or parentage testing [12–15]. Casework applications, however, demand proper knowledge regarding distribution and behavior of individual SNPs included in this panel in target populations, and several studies have been conducted to assess such features in specific populations [12,16–20].

Modern Brazilian population is a product of recurrent miscegenation processes occurred throughout national history, resulting in presently existent genetic structure where a high degree of genetic admixture is observed [21,22]. From the 16th century on, significant influxes of European (EUR) settlers (mainly from Iberian Peninsula) occurred, who interbred with Native American (NA) populations already present in Brazilian territory. Soon after, a large contingent of Africans (AFR), mostly from Western Africa Portuguese occupied territories, were forcedly brought to Brazil as slaves. Finally, late migratory movements took place in 19th and 20th centuries, with arrival of European (predominantly Germans, Italians, Portuguese and Spaniards) and Asian (essentially from Japan, Lebanon, Syria and other Mid-Western countries, then part of Ottoman Empire) migrants, giving rise to the highly admixed and multiethnic Brazilian population [23].

Brazilian territorial occupation and inland expansion were unevenly distributed over national history, following various patterns of multi-directional introgression according to social and historical conditions, and significantly vary for each distinct geographical region [23]. Heterogeneous processes of migratory flows led to marked differences in regional ethnical composition, and distinctive proportions of parental populations (NA, EUR and AFR) contribution in present-day geopolitical regions are noticeable [24]. Distribution of ethnic groups based on self-declared ancestry proportions differs considerably among regions, with relative higher Amerindian and African influence in Northern and Northeastern Brazil, respectively, while Southern Brazil displays prevalent European ancestry [25]. Asymmetrical contributions of ancestral populations to current genetic pool can also be observed in lineage markers, with predominant EUR Y-chromosome paternal contribution, and mitochondrial DNA (mtDNA) maternal lineages usually associated with AFR and NA origins [26,27]. Therefore, the possible occurrence of genetic structure concerning the specific SNP markers analyzed in this study in Brazilian populations must be investigated, with its inherent impact in forensic databases design, in order to avoid bias introduction in result interpretation. In the present study, Brazilian regional populations were compared to 26 different worldwide reference populations based on 88 ID-oriented SNPs included in HID-Ion AmpliSeq Identity Panel, aiming to scrutinize genetic diversity, similarity levels and population stratification for all investigated groups.

2. Materials and methods

2.1. Ethical statement

All samples analyzed in this study were voluntarily obtained following written informed consent from donors, and refer to a research-oriented human samples biobank available in PUC/RS. Anonymous samples were randomly selected from this bank, based on geographical origin only. This work follows the ethical principles stated in the Helsinki Declaration [28] of the World Medical Association, and was approved by Pontifical Catholic University of Rio Grande do Sul Institutional Review Board, under CAAE 52113715.9.0000.5336 number.

2.2. DNA samples, extraction and quantification

Oral swabs or peripheral blood were obtained from 432 unrelated volunteer donors from all five different Brazilian geopolitical regions. Male contributors were preferred, in order to assure a significant sample size for investigation of SNPs located in the Y chromosome included in this study. Number of samples obtained from each Brazilian region and its States are presented in Supplementary Figure S1. Genomic DNA was extracted using the automated platform Automate Express Forensic DNA Extraction System (Thermo Fischer Scientific Inc.) with the Prepfiler Automated Forensic DNA Extraction System (Thermo Fischer Scientific Inc.), or manually using DNA IQ System (Promega Corp., Madison, WI, USA). DNA was quantified with the Quantifiler Human DNA Quantification Kit (Thermo Fischer Scientific Inc.) on a 7500 Real-Time PCR System (Thermo Fischer Scientific Inc.) or using Qubit 2.0 Fluorometer with Qubit dsDNA HS Assay Kit (Thermo Fischer Scientific Inc.). All procedures followed manufacturer´s instructions.

2.3. Library preparation, emulsion PCR, and sequencing

DNA libraries were constructed using the Ion AmpliSeq Library Kit 2.0 (Thermo Fisher Scientific Inc.) combined with the HID-Ion AmpliSeq Identity Panel (Thermo Fisher Scientific Inc.), following manufacturer´s suggested protocol (HID-Ion Ampliseq Library Preparation, Revision C.0). Briefly, PCR amplification was performed in a final volume of 20 µL that contained 1 ng of template DNA, 4 µL of 5x Ion AmpliSeq™ HiFi Mix, and 10 µL of 2x Ion AmpliSeq™ primer pool (Identity Panel). Final volumes were adjusted with nuclease-free water. PCR was performed in a Veriti 96-well Thermal Cycler (Thermo Fischer Scientific Inc.) under the following conditions: enzyme activation for 2 min at 99 °C, 21 cycles of 15 s at 99 °C and 4 min at 60 °C, followed by a 10 °C hold. PCR amplicons were partially digested with 2 µL FuPa reagent, following incubation at 50 °C for 10 min, 55 °C for 10 min and 60 °C for 20 min, followed by a 1 h hold at 10 °C. Ligation of adaptors to the libraries were executed according to the manufacturer´s instructions, using a different barcodes for each sample in a same run (Ion Xpress Barcode Adaptors 1–96 Kit or IonCode Barcode Adapters 1-384 Kit) (Thermo Fisher Scientific Inc.), and resulting products were purified using Agencourt AMPure XP reagents (Beckman Coulter Inc., Brea, CA, USA), according to the manufacturer´s instructions. After purification, libraries were quantified using a 7500 Real-Time PCR System (Thermo Fischer Scientific Inc.) with the Ion Library TaqMan™ Quantitation Kit (Thermo Fischer Scientific Inc.). Libraries were pooled to a final concentration of 20 pM. Emulsion PCR (emPCR) was performed on the Ion OneTouch 2 instrument (Thermo Fisher Scientific Inc.) with the Ion PGM Hi-Q Template Kit (Thermo Fisher Scientific Inc.) following the manufacturer's protocol (Ion PGM Hi-Q Template Kit, Revision A.0). The emPCR products were enriched on the Ion OneTouch Enrichment System (Thermo Fisher Scientific Inc.). A final volume of 30 µL was loaded per chip. Sequencing was performed on the Ion PGM™ (Thermo Fischer Scientific Inc.) Sequencer with the Ion PGM Hi-Q Sequencing Kit (Thermo Fisher Scientific Inc.) following

manufacturer´s instructions (Ion PGM Hi-Q Sequencing Kit, Revision B.0) and using Ion 318 Chip Kit v2 (Thermo Fisher Scientific Inc.). A total of six chips were used in distinct runs for complete samples genotyping.

2.4. Data analysis and Y-chromosome haplogrouping

Sequencing data were analyzed using the Torrent Suite Software v5.0 (Thermo Fischer). Hg19 was used as reference genome data. The number of mapped reads was calculated by the Torrent Coverage Analysis v5.0 plugin (Thermo Fischer Scientific Inc.). SNP genotypes were called by the HID SNP Genotyper v4.3.1 plugin, with target regions file (iiSNPv3_FP.hotspot) and the hotspot regions file (IISNPv2.20140429.Designed) under default analysis settings. Minimum coverage was set for 6 reads per base position. All SNPs genotypes were independently reviewed by two different collaborators, and manual corrections applied. The Y-chromosome haplogroups were determined automatically by the plugin using Y-SNP combination, and confirmed according to the International Society of Genetic Genealogy (ISOGG) Haplogroup Tree 2018 version 13.227 (available online at https://isogg.org/tree/). Y markers nomenclature is presented as depicted by the plugin. Equivalency between reference SNP ID numbers (according to NCBI dbSNP) [29] and marker names as classified by HID SNP Genotyper plugin is listed in Supplementary Table S1. Only variations present in haplogroups identified in this study were listed. A comprehensive tree of all possible Y-chromosome superior-clade haplogroups generated by the 34 Y-SNP markers included in this panel can be found in [30]. Generated data was submitted to NCBI SRA database as raw sequence reads, under reference number SRP155858.

2.5. Statistical data analysis

Statistical data analysis and results report were performed according to previously reported [19]. HID-Ion AmpliSeq™ Identity Panel Sequencing performance was evaluated using four different statistical parameters [14]: Locus Balance (LB) assesses individual locus amplification efficiency, and is calculated as the coverage of a locus divided by the mean coverage of all locus, per sample; Locus Strand Balance (LSB) measures the balance between forward and reverse reads, and is shown as the number of forward reads divided by the total reads number. Heterozygote Balance (HB) estimates the ratio between two distinct alleles read counts in heterozygous samples, and it was plotted as the number of reads of the first base call in alphabetical order (A, C or G) divided by the number of reads of the alternate allele (C, G or T, respectively). Noise Level (NL) represents the amount of unspecific base call, calculated as the ratio of non-allele calls divided by total coverage.

Several forensic parameters were calculated for the sample set in question, using STRAF online software (available online at http://cmpg.unibe.ch/shiny/STRAF/), as well as allele frequencies [31]. Forensic parameters included observed heterozygosity (Ho), expected heterozygosity (He), probability matching (PM), probability of exclusion (PE), polymorphism information content (PIC), power of discrimination (PD) and typical paternity index (TPI). Markers linkage disequilibrium (LD), Hardy-Weinberg equilibrium (HWE), Analysis of Molecular Variance (AMOVA) and pairwise F_{ST} among five Brazilian regions alone or with 26 worldwide populations were performed with the software Arlequin 3.5.2.2 [32]. Same software was used to compute exact test of population differentiation among Brazilian regions, with number of steps for dememorization and Markov Chain equals to 10,000 and 100,000, respectively. Populations employed in the present study, obtained from 1000 Genomes Project (Phases 1 to 3, available online at https://www.ensembl.org/Homo_sapiens/Info/Index) along with abbreviations used here, are listed in Supplementary Table S2. Multidimensional Scaling Analysis (MDS) based on pairwise F_{ST} distances and Principal Components Analysis (PCA) of worldwide populations allele frequencies were executed using IBM SPSS Statistics Software v.17.0 (IBM Analytics Inc.) [33]. Individual genotypes PCA was performed using the above-cited STRAF online software.

Occurrence of genetic structure among 26 worldwide populations [34] and Brazilian dataset was assessed with Structure v.2.3.4 software [35], with six independent runs for each K value, ranging from 2 to 7. A 100,000 length Burn-In Period was applied, followed by a 100,000 steps Monte Carlo Markov Chain (MCMC) procedure, using the standard admixed model. Results were then processed through CLUMPAK SERVER online tool (available online at http://clumpak.tau.ac.il) [36], in order to generate representative graphics and estimate best K value fitting the data. Pairwise F_{ST} distances using complete Brazilian data or regional subsets were also used to build cladogram graphics using the Molecular Evolutionary Genetics Analysis v.7.0 (MEGA v.7.0) software [37], applying Neighbor-Joining (N-J) methods.

Y-Haplogroup frequencies were determined by direct counting. Y network tree graphics were designed by construction of median-joining [38] networks based on 14 Y-SNPs based haplotypes found in Brazilian population using NETWORK 5.0.0.1 software (available online at http://www.fluxus-engineering.com/sharenet.htm).

3. Results

The HID Ion Ampliseq Identity Panel was successfully used to generate genetic profiles for 432 Brazilian samples, from all five geopolitical regions. This commercial kit comprises 90 autosomal biallelic SNPs specially selected to provide enough discrimination power for individual identification, achieving match probabilities equivalent to current commercial STR-based genotyping solutions. SNP markers were selected from previous studies (43 SNPs proposed in Kidd´s panel [39] and 48 from SNPforID effort [40]) due to presenting high heterozygosity levels in different worldwide populations. In addition to autosomal markers, 34 Y-chromosome SNPs were included in the panel, which allow an upper-clade haplogroup designation for major Y-chromosome tree groups. The kit was designed to properly handle critical or difficult forensic samples, with smaller amplicons sizes than usual STR markers (average read size for this study was 106 bp). Brazilian population stratification, forensic parameters, allele frequencies, Y-chromosome haplotype distribution and other relevant descriptive indexes associated with included SNPs, along with sequencing performance, were investigated in the present study, in order to assess the adequacy of present panel introduction in Brazilian routine forensic casework.

3.1. Forensic parameters of 124 SNPs for Brazilian population

Complete SNP genotypes of all 432 Brazilian individuals, for 124 SNPs comprised in this panel, are presented in Supplementary Table S3 (Supplementary Material). Obtained genetic profiles were employed to further characterize regional populations, with individuals from South, Southeast, Northeast, North and Center-West geopolitical regions (sample sizes of 108, 83, 81, 80 and 80 individuals, respectively) included in this study. Resulting allele frequencies for 90 autosomal SNPs are listed in Supplementary Table S4, including national (all regions combined) and regional data. Pairwise F_{ST} and exact test of population differentiation based on genotype frequencies were performed for all five Brazilian regions, and results are presented in Supplementary Table S5. No evidence of significant differences among Brazilian regions was found after Bonferroni correction, based solely on allele frequencies of 90 autosomal SNPs evaluated in the present study. Forensic parameters estimations for Brazilian national and regional data, including Ho, He, PM, PE, PD, PIC, TPI are presented in Supplementary Table S6, as well as p-values for HWE tests for all loci. No locus presenting significant deviations of HWE were found in investigated regional or national populations, after Bonferroni correction. Despite that, four autosomal SNP marker pairs displayed significant linkage equilibrium, even after Bonferroni correction, for Brazilian combined dataset (pairs rs4288409-rs12997453; rs964681-rs159606; rs1736442-rs1454361 and rs221956-

Table 1
Forensic parameters for Brazilian national and region populations, based on 90 autosomal SNPs included in HID Ion Ampliseq Identity Panel.

Population	Combined PM	Combined PE
Br-S	2.97143×10^{-36}	0.999999951474895
Br-SE	2.21577×10^{-36}	0.999999928310930
Br-NE	1.62475×10^{-36}	0.999999815034317
Br-N	2.73451×10^{-36}	0.999999926102221
Br-CW	3.15661×10^{-35}	0.999999992066246
BRA (all regions included)	9.93154×10^{-37}	0.999999917134468

rs2016276). Pairwise LD test p-values for Brazilian national population are detailed in Supplementary Table S7. Average Ho for each Brazilian region is estimated as follows: 0.473 (South), 0.465 (Southeast and North), 0.45 (Northeast) and 0.49 (Center-West). Maximum and minimum Ho values for the complete dataset were found both in Northeastern Brazil (0.679 at rs6811238 and 0.235 at rs1357617, respectively). Marker rs938283 was found to have the lowest discrimination power for Southern, Southeastern and Northern samples (MP = 0.527, 0.566 and 0.563, respectively), while markers rs7704770 (MP = 0.351), rs729172 (MP = 0.342) and rs214955 (MP = 0.34) were the most discriminative for the aforementioned regions, in same order. At the same time, a single locus (rs2016276) possesses smallest power of discrimination values for both Northeastern (MP = 0.556) and Center-Western (MP = 0.573) populations. Although this specific marker is listed among SNPs presenting linkage disequilibrium in Brazilian population, its linked counterpart, rs221956, displays intermediate discrimination values for the same groups (MP = 0.432 and 0.401, respectively). Still regarding Northeast and Center-West regions, SNPs rs1335873 (MP = 0.342) and rs1498553 (MP = 0.354) are the most discriminatory for above-named Brazilian regions. Table 1 exhibits combined matching probability (CMP) and combined power of exclusion (CPE) for each Brazilian region and for the country as a whole. As a comparison parameter for such exquisite discrimination power presented by the present panel, CMP for a 15 STR markers panel (including all 13 CODIS core markers) in Brazilian population was estimated to be around 18 orders of magnitude smaller than CMP provided by Ion Ampliseq Identity Panel for Brazilian population [41]. Above results suggest that autosomal SNP set included in Ion HID Ampliseq Identity Panel shows enough polymorphism and informative value for Brazilian regional and national populations, and therefore can also be incorporated in forensic analytical repertoire, as a useful tool in kinship testing or individual identification exams.

3.2. *Y-haplotype frequencies distribution in Brazilian regional populations*

34 Y-chromosomes SNPs included in Ion HID Ampliseq Identity Panel were used to determine superior-clade haplotype frequencies for all five Brazilian regions, in a total of 388 male samples. Haplotype classification was automatically provided by HID SNP Genotyper v4.3.1 plugin, and outputs were confirmed according to International Society of Genetic Genealogy (ISOGG) Haplogroup Tree 2017 version 12.334. In fact, a single misclassification was observed, but it was due a Y-tree restructuration implemented after ISOGG Haplogroup Tree 2014. This older Y-tree version used C to T transition in Y-SNP P198 to categorize samples belonging to then-existing O3 subclade. Since 2015 Y-tree version, P198 has been used as a characteristic marker for O2 Y-haplogroup, and corrected, current classification is presented in this paper.

A total of 14 different Y-chromosome haplogroups were observed among Brazilians in this study. Regional populations presented different distributions of identified haplotypes, with numbers ranging from nine distinct Y-clades found in Southeastern and Center-Western Brazil to only six haplotypes detected in Northeastern Brazil. Both Southern and Northern regions featured eight variants each for Y-chromosome. European haplogroups showed higher prevalence for all regions, with a significant predominance of R1b haplotypes (relative frequencies ranging from 42.5% to 57.34% among all five Brazilian subpopulations) and representing around half Y-haplogroup observations in the whole country. In addition to R1b, only four other male clades were detected with an overall frequency over 5% in national population, here listed according to their prevalence: E, I, J and G. Unique, single occurrence haplotypes are present in Southern, Southeastern and Center-Western samples only. Countrywide Y-haplogroup frequencies ranged from 0.26% (B, DE, L, N and O2 groups) to 50.26% for the most prevalent R1b. Supplementary Figure S2 shows a Y-haplogroups tree for clades observed in this study only, with Y SNPs markers whose mutations are relevant in respective classification displayed. Branch ends feature a pie plot, with area proportional to haplogroup frequency, and relative contribution of each region to number of observed cases. Table 2 details complete Y-haplogroup diversity, including distribution among Brazilian regional and national populations. Relative contribution of E haplogroup is higher in Northeastern and Center-Western populations than other Brazilian regions. Besides that, the number of samples associated with typical NA haplogroups (Q) were found to have a low prevalence (1.55%), limited to Northern and Center-Western individuals. This results confirms previous observations regarding differential contributions of ancestral populations for different Brazilian regions, with AFR or NA ancestry ratios more substantial in specific Brazilian areas, as well as uneven male and female

Table 2
Y-chromosome haplotypes frequencies found in 388 male Brazilian samples, based on 34 superior-clade Y-SNPs included in Ion HID Ampliseq Identity Panel.

Y-haplogroup	Region count (%)					
	South	*Southeast*	*Northeast*	*North*	*Center-West*	*Brazil*
B	–	1 (1.23)	–	–	–	1 (0.26)
D	–	2 (2.47)	–	–	–	2 (0.52)
DE	–	–	–	–	1 (1.25)	1 (0.26)
E	8 (10.67)	13 (16.05)	21 (26.25)	9 (12.5)	18 (22.5)	69 (17.77)
G	6 (8.0)	5 (6.17)	3 (3.75)	5 (6.94)	4 (5.0)	23 (5.93)
I	10 (13.33)	9 (11.11)	11 (13.75)	8 (11.11)	5 (6.25)	43 (11.08)
J	5 (6.67)	4 (4.94)	8 (10.0)	6 (8.33)	10 (12.5)	33 (8.5)
L	1 (1.33)	–	–	–	–	1 (0.26)
T	1 (1.33)	1 (1.23)	3 (3.75)	1 (1.39)	2 (2.5)	8 (2.06)
N	–	–	–	–	1 (1.25)	1(0.26)
O2*	1 (1.33)	–	–	–	–	1 (0.26)
Q	–	–	–	2 (2.78)	4 (5.0)	6 (1.55)
R1a1	–	1 (1.23)	–	3 (4.17)	–	4 (1.03)
R1b	43 (57.34)	45 (55.57)	34 (42.5)	38 (52.78)	35 (43.75)	195 (50.26)
Total	75 (100)	81 (100)	80 (100)	72 (100)	80 (100)	388 (100)

O2 haplogroup marked with * was identified by plugin as O3, and reclassified according 2018 version of ISOGG Y-tree.

parental lineage proportions, with majoritarian EUR Y-chromosome contribution for the country as a whole, in contrast to relative higher AFR and NA-associated mtDNA lineages present in current Brazilian genetic pool [21–27].

3.3. Inter-population analysis and genetic stratification

A series of analyses was conducted to evaluate differences among Brazilian regions distributions in genotype and allele frequencies, in order to assess potential population genetic stratification for autosomal SNPs typed. Pairwise F_{ST} values for 90 overlapped autosomal SNPs for Brazilian regions only (presented in Supplementary Table S5) ranged from 0.00024 (Br-S and Br-N) to 0.00672 (Br-N and Br-NE pair). Overall, Northeastern Brazil was found to be the most genetically distinct Brazilian region, with higher pairwise F_{ST} average observed. Despite that, fixation indexes calculated are relatively low, demonstrating homogeneity in allele frequency distribution of 90 autosomal SNPs included in HID Ion Ampliseq Identity panel throughout Brazilian regions. Observed degree of genetic similarity between Southern and Northern Brazilian regions was not expected, due to very distinct historical colonization processes both regions went through. This unexpected founding can be explained by an overrepresentation of samples coming from Northern state of Acre in this regional subset. This particular Brazilian State, which comprises only 4.65% of Northern Brazilian geopolitical region population [25], represents almost three-quarters of total Northern samples evaluated in this study. Acre State has a unique colonization history in Brazil, with significant migratory waves of Southern region settlers in late 20th century [42]. In this case, expected elevated NA relative contribution in Northern region, which was consistently detected previously [21–27], might have been overlooked in this study due to sampling bias, also responsible for overstating EUR genetic heritage in Northern region habitants. Addition of samples from the two most populated Northern Brazilian States Amazonas and Pará is underway, aiming to improve present frequency database reliability.

Pairwise F_{ST} values were also calculated based on 88 overlapped autosomal SNPs among Brazilian national or regional populations and 26 worldwide populations included in 1000 Genomes Project, and results for regional datasets are displayed as a heat map in Fig. 1 (a heat map for national data can be found in Supplementary Figure S3). For Brazilian population as a whole, results ranged from 0.00283 (Puerto Ricans) to 0.072 (Mende from Sierra Leone). Brazilians showed higher similarity levels with EUR populations, followed by samples from AMR countries. Considering Brazilian populations, Br-NE presented markedly lower F_{ST} values with AFR samples (results between 0.02 and 0.052), followed by Br-CW (0.03 to 0.067), when compared to other regions results (ranging 0.037 to 0.095). An inverse approach shows same regions with higher genetic distance to EUR populations (minimum value for Br-NE and Br-CW is 0.013 and 0.006 with IBS and TSI, respectively), than its regional counterparts (with values as low as 0.0022 for Br-S and IBS pair), with FIN group classified as the most genetically distant of Brazilian populations among Europeans (0.015 for the whole country). When considered other American populations data, Brazilian regional values present markedly resemblance to PUR (from 0.002 to 0.005 for Br-S and Br-NE, in order) and CLM (Fst of 0.002 with Br-N and 0.007 with Br-NE), with PEL samples demonstrating higher genetic distances (from 0.034 for Br-S and Br-SE to 0.041 to Br-NE). Both Asian groups presented intermediate genetic resemblance with Brazilian populations, with SAS samples displaying an overall genetic similarity with investigated regions slightly higher than EAS (average pairwise F_{ST} values to Brazilian regions equivalent to 0.026 and 0.044, respectively).

In order to further examine above results and clarify eventual genetic relationship between 26 worldwide populations and Brazilian regions, a MDS plot based on pairwise F_{ST} values was drawn for obtained data, and is presented in Fig. 2. A clear pattern can be observed, with negative values for Dimension 1 as a characteristic feature for AFR populations. Both Asians groups (EAS and SAS) are grouped together in upper-right quadrant, with apparent distinct cluster separation between both geographical origins. As expected, AMR and EUR have close distribution, occupying the fourth quadrant. However, while EUR populations seem to be grouped relatively close to each other, AMR and BRA have broader dispersion, with a clear directional tendency of Br-NE and Br-CW towards negative values in Dimension 1 (trending to AFR cluster). PEL seems to be an outlier, with extreme negative values for Dimension 2. Wider dispersion displayed by AMR populations apparently reflect their admixed nature, phenomena also observed for other geographical origins (as AFR, for instance, where groups of samples with African ancestry collected outside Africa (ACB and ASW) show a tendency towards axis origins. Although discrete, Br-SE location represents the Brazilian population closer to Asian groups, maybe reflecting the significant historical presence of immigrant from these particular areas in Southeastern Brazil [23,25]. A cladogram was also drawn applying N-J methods, and is presented in Supplementary Figure S4 (equivalent figures were constructed considering Brazilian population as a whole, displaying complete combined dataset, which are presented in Supplementary Figures S5-S6). Resulting phylogenetic tree shows Br-N and Br-S populations positioned closer to EUR branches, followed by other AMR admixed populations. Br-SE and Br-CW occupy intermediate positions between AMR populations and a unique ramification gathering all Asian groups. Furthermore, Br-NE is the single population closer to the tree bottom, where AFR clades were positioned. PCA plots were also executed to help evaluate relevance of genetic distance between populations or individuals. Supplementary Figure S7 shows a PCA analysis for Brazilian and worldwide populations based on allele frequencies of 88 overlapped autosomal SNPs, and top two principal components account for a total of almost 80% of total variation (65.35% and 14.28%). Once again, AFR and EUR are plotted in extreme positions, and Asian populations occupy closed positioned clusters. AMR and BRA have a sparser distribution, with intermediate locations. Supplementary Figure S8 shows a PCA plot based on individual genotypes of all samples included in this study, grouped by its geographical origin. Once again, AMR and BRA individuals were plotted alongside EUR samples. However, first principal component (5.67%) seems to correctly discriminate AFR samples, while second (3.23%) and third (1.97%) principal components might be useful in Asian samples classification. Low values for variation accounted by each principal component in this analysis suggests that most part of genetic differences among samples is located in an individual level, and only a small fraction of total genetic variation is due to differences in samples distinct continental geographical origins. Results above are consistent among all employed statistical methods (PCA, MDS and phylogenetic analyses), and display genetic relationships among all populations that are supported by genetic, historical or ethnographic information, especially for Brazilian case [21–27].

Bayesian inference methods were also used to further characterize differences among populations investigated in this study. STRUCTURE software applies MCMC methods, where admixing levels in different populations can be inferred. Fig. 3 presents STRUCTURE plot of Brazilian samples alongside 26 worldwide populations, with inferred K clusters ranging from 2 to 5. As the number of selected K clusters increases, diverse strata comprising general genetic structure of all populations can be accessed. Although ideal number of clusters was designated as K = 3 using Evanno´s method [43], traditional estimations based on Probability of Data for K = k suggests optimum population numbers as 5. Since this last estimative seems to reflect actual differences in evaluated groups, according to similarities presented by populations sharing a same geographical origin as identified by clustering patterns, it was adopted in this paper. For K = 5, investigated Brazilian populations shows clustering patterns similar to others AMR admixed populations, while AFR, EUR, EAS and SAS populations display a clear predominance of distinct clusters in overall components associated with

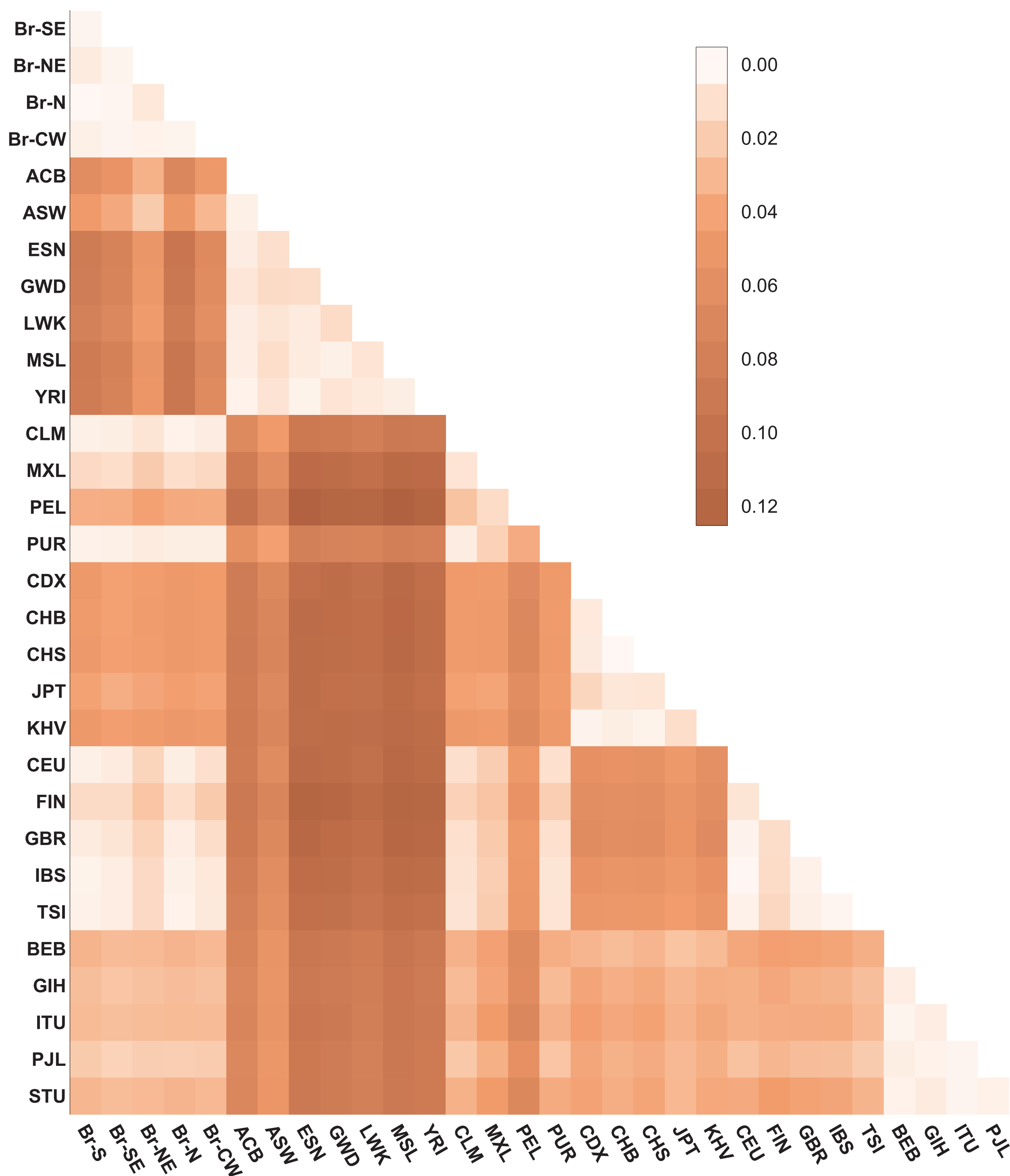


Fig. 1. Heat map of pairwise F_{ST} values for 88 overlapped autosomal SNPs included in HID Ion Ampliseq Identity Panel among 26 worldwide populations and Brazilian regional populations. Population full names described in Supplementary Table S2.

populations with the same geographical origin. Supplementary Table S8 summarizes relative contributions of each cluster for all Brazilians and worldwide populations evaluated, as well as show population geographical origin more strictly linked to each inferred cluster. Noteworthy, with K = 5, components associated with Cluster 2 (represented in red color in Fig. 3) seems to represent residual genetic structure not associated with clusters previously identified with smaller K numbers. Interesting enough, at least part of this residual genetic structure appears to be related to genetic contributions of NA ancestral populations, since admixed AMR populations present higher ratios of this particular cluster association. Brazilian populations show predominant affiliation with EUR and AMR clusters, with roughly 72% to 80% of genetic structure linked to both inferred groups. Above results for genetic Bayesian population inference are consistent with previously mentioned MDS, phylogeny and PCA analyses.

Finally, since occurrence of genetic stratification seems to be clearly

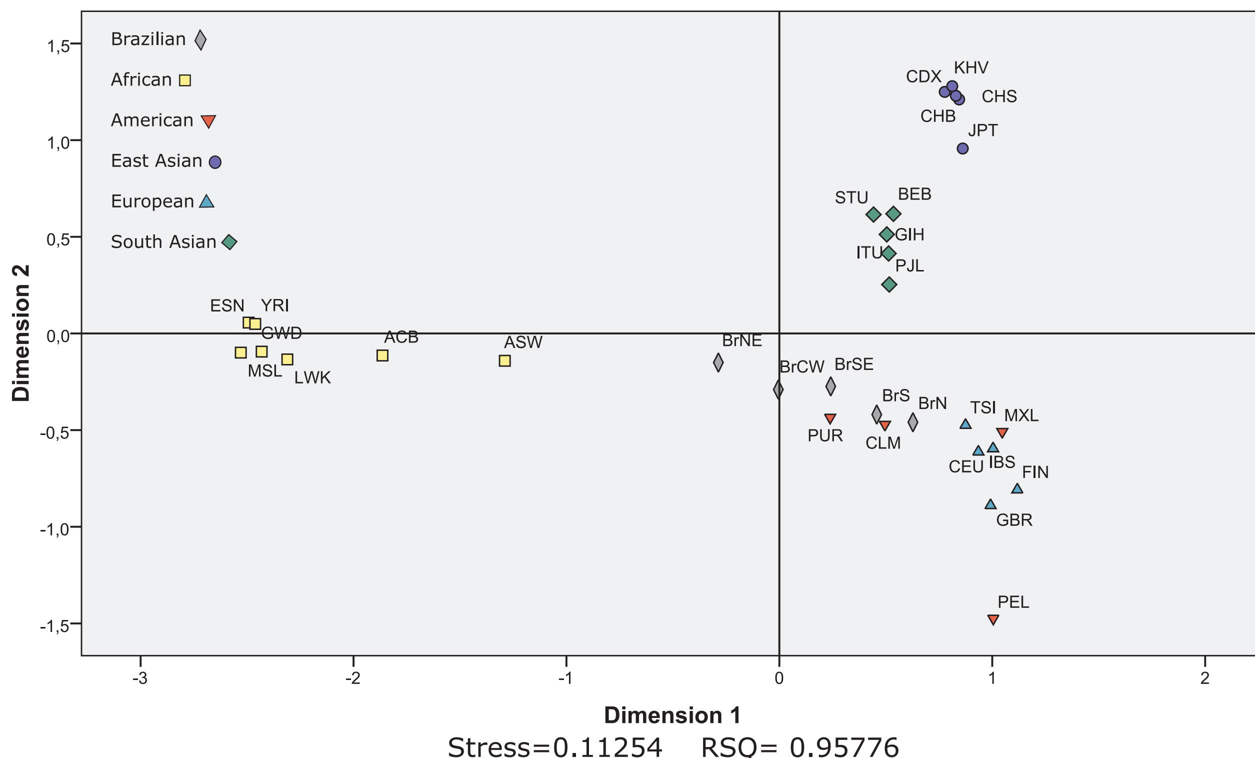


Fig. 2. Genetic distance evaluation for inter population analysis of 26 worldwide populations and Brazilian regions, presented as a MDS plot based on pairwise F_{ST} values for 88 overlapped autosomal SNPs included in HID Ion Ampliseq Identity Panel. Supplementary Figure S4 presents a phylogenetic tree build with Neighbor-joining methods, based on same pairwise F_{ST} values obtained.

defined for worldwide and, to a lesser extent, Brazilian regional populations, statistical tests were performed to evaluate the amount of genetic variance associated exclusively with verified genetic structure. Global AMOVA tests were executed with all five Brazilian regional and 26 worldwide populations, arranged according to geographical origin (Brazilian populations formed a separate group from remaining AMR populations) for 88 autosomal SNPs including in this panel, and results are detailed in Table 3. Within individuals variation accounts for an estimate of around 94% of total genetic variability, with a little over 5% of total genetic differences explained by among populations divergence. These results support convenience of employing HID Ion Ampliseq Identity Panel in forensic identification studies, since population genetic structure associated with autosomal SNP markers included in the kit seems to have a limited impact on affecting genetic diversity at

Table 3
Fixation indexes and global AMOVA results for Brazilian and 26 worldwide populations, based on individual genotypes of 88 autosomal SNPs included in HID Ion Ampliseq Identity Panel.

Source of Variation	Relative Variation (%)	Fixation Indexes
Among groups	5.11	F_{CT}: 0.05111
Among populations within groups	0.61	F_{SC}: 0.00641
Among individuals within populations	0.16	F_{IS}: 0.00170
Within individuals	94.12	F_{IT}: 0.05878
(Among populations)*	**94.28**	**F_{ST}: 0.05719**

* Among populations subsumes both categories accessing individual level variation.

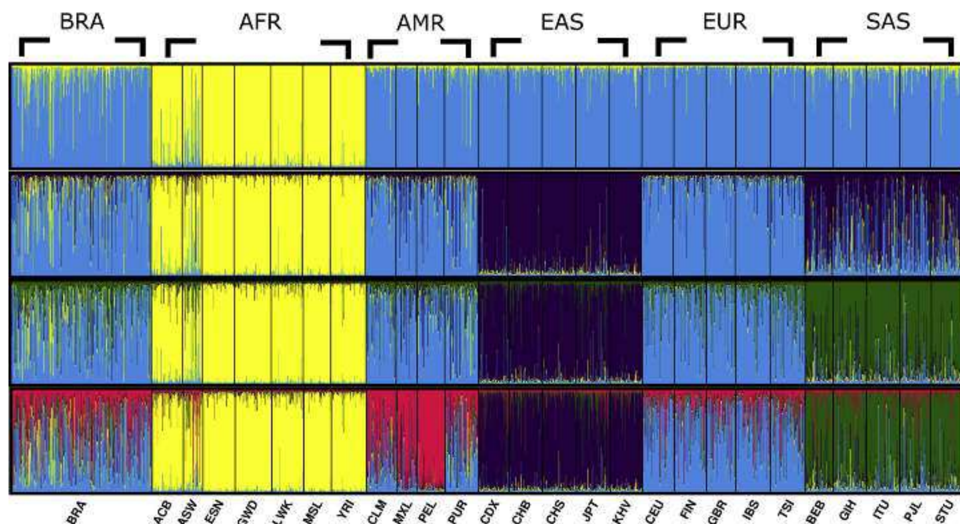


Fig. 3. Population structure of investigated Brazilian along with 26 worldwide populations based on 88 autosomal SNP markers included in HID Ion Ampliseq Identity Panel. Structure plots are presented from K cluster number ranging from 2 to 5 (top to bottom). Each vertical line stands for an individual, with each color representing relative proportion of association with each inferred cluster, assigned by distinct colors. Populations and geographical regions abbreviations are listed in Supplementary Table S2.

individual levels.

3.4. MPS sequencing performance

All samples in this study were genotyped through Ion PGM Sequencer, employing native data interpretation solutions provided by the manufacturer. HID SNP Genotyper plugin was the main pipeline for data calling. Additionally, Coverage Analysis plugin was used to access sequencing performance for all genotyping procedures. Since sample size largely exceed suggested maximum number of samples to be included in a single chip, all 432 samples were analyzed in six independent runs. Data regarding each individual experiment is presented in Supplementary Table S9. Maximum number of samples recommended by manufacturer to be included in a single run using 318v2 model chips (77 samples, calculated to ensure mean depth of 600x per locus) was slightly surpassed in three experiments, where 80 samples each per run were included. Despite that, mean depth per locus per sample range from 276x to 740x, with an average value of 476x, which can be considered satisfactory for reliable genotyping of single-source reference samples [13,14,44], where proposed coverage of as low as 20 reads might be considered sufficient for genotype determination [13]. Supplementary Figure S9 represents a heat map graphic representation of complete sample set (482 individuals) coverage for each evaluated locus. As expected, overall coverage for haploid Y-SNPs is consistently lower than diploid autosomal SNPs. Samples with no coverage for all Y-SNPs represents female individuals (44 total). Differences in coverage numbers can be noted for both individuals and markers, with low performance SNP markers identified by vertical lines presenting lighter color shades.

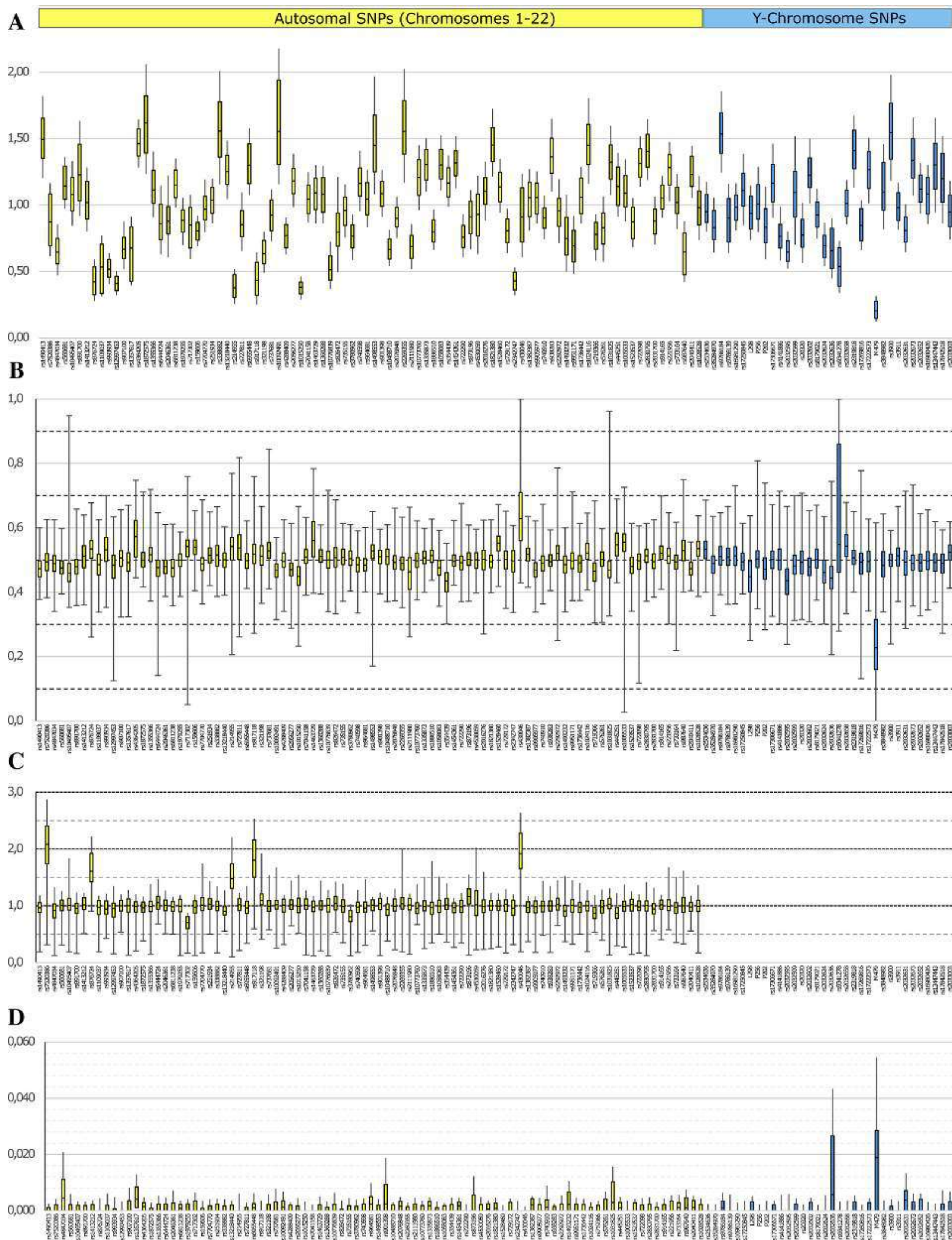
A series of metrics were applied to evaluate genotyping efficiency using HID Ion Ampliseq Panel, and results are summarized in Fig. 4. Average coverage across all 90 autosomal loci (527 ± 316 reads) is nearly twice as high as Y-SNPs (257 ± 172 reads). Therefore, LB calculations were performed independently for autosomal and Y-chromosome SNPs. As shown in Fig. 4A, LB median values for autosomal SNPs ranged from 0.38 at rs214955 and rs1015250 to 1.62 at rs1872575, while Y-SNP median LBs varies from 0.2 at M479 to 1.55 at rs3900. Overall average LB values for all 124 loci is 1.00 (Supplementary Table S10 presents details for individual markers). Six autosomal (rs876724, rs12997453, rs214955, rs917118, rs1015250 and rs2342747) and one Y (M479) markers provided poor LB performance, with values under 0.50 threshold, meaning coverage for this six loci are below half the average coverage. Overall inter locus coverage imbalance has been described for this panel, phenomena observed for different products versions [11–13,15–20,30]. This persistent issue might present obstacles with real forensic casework samples, especially concerning challenging samples, and efforts for achieving optimization of primers efficiency balance and global panel design must be addressed by manufacturer. LSB results ranged from 0.23 at M479 to 0.63 at rs430046, with an average value across all 124 loci equivalent to 0.50 ± 0.03, consistent with expected values (Fig. 4B and Supplementary Table S10). When manufacturer´s proposed thresholds are considered (0.50 ± 0.20), only outlier SNP among all loci is M479. As shown in Fig. 4C and Supplementary Table S10, average HB of all median values for 90 autosomal SNPs is 1.03 ± 0.19, with minimum and maximum values of 0.71 at rs717302 and 2.08 at rs7520386, respectively. Previous validation studies for this panel [13,14] propose HB acceptable thresholds in a 60:40 heterozygote ratio (HB values in 0.66 to 1.5 range), and obtained results show four SNPs (rs7520386, rs876724, rs917118 and rs430046) outside such conditions. Once again, impact of markers presenting low performance for HB metrics in forensic cases may be significant, especially in low-source DNA samples or mixture analysis, and data regarding this particular SNPs should be carefully scrutinized. Base calling efficiency, assessed by NL measures, where generally good, with most median values near zero (Fig. 4D and Supplementary Table S10) across all 124 loci. In a general manner, Y SNPs performed poorly than autosomal markers, including two SNPs (rs2032636 and M479) with 3rd quartile above 0.02, and overall larger median values, standard deviations and maximum observed values. Average median values were different than 0.00 for 22 markers (18 autosomal and 4 Y-SNPs), and only rs1357617 displays 1 st quartile value above zero. Highest NL median and relative value (25%) observed were both seen in M479 Y-SNP. Results found show that sequencing performance of HID Ion Ampliseq Identity Panel was generally good, with some problems observed for specific markers. Even though revised versions of this product have been released by manufacturer, all of them seem to present issues regarding locus and heterozygous balance [11,16–20]. Such questions must be addressed by manufacturer in order to deliver a more polished product, suitable for efficient use in routine forensic casework, especially considering critical sample analysis.

4. Discussion

Present study aims to evaluate utility of HID Ion Ampliseq Identity Panel in Brazilian regional populations, in order to develop a frequency database that allows use of this commercial solution in real forensic casework. 432 samples collected from all five Brazilian geopolitical regions were evaluated. Forensic statistical parameters obtained for all national populations show high polymorphism levels, suitable for human identification purposes.

Investigation of genetic stratification among Brazilian regions shows evidence of some degree of genetic structure nationwide, considering all 90 autosomal SNP markers included in the panel. However, the impact of observed stratification levels in database planning and construction is yet to be determined. A comprehensive analysis of available data for regional STR frequencies demonstrated that unified frequency databases can be used for Brazilian population as a whole, although some corrections might be necessary to adjust for observed levels of genetic structure existent for these markers [45]. Although no such statement can yet be made for polymorphisms investigated in this study, due to lack of sufficient information available for autosomal SNPs discussed here, present frequency database can be the foundation of a representative, accurate Brazilian SNP frequency database that allows reliable use of this set of markers in human identification applications and parentage testing.

Y-haplotypes frequency of major clades was also investigated for Brazilian population, through analysis of 34 Y-SNPs included in this panel. Findings are in accordance with previous studies for Brazilian populations, that demonstrate differential proportions in genetic contributions of immigrants from distinct geographical origins for all five Brazilian regions, according to historical colonization processes [21–27]. Furthermore, gender bias concerning male and female parental lineages distribution in Brazilian modern population, identified as predominance of Y-chromosome haplotypes with EUR origin or AFR and NA mtDNA-associated lineages relative overrepresentation, with diverse proportions in different Brazilian regions [26,27], was also verified within evaluated sample set. Obtained data for Y-haplotype frequencies supports above conclusions, with considerable preponderance of male lineages with EUR origin. In this context, it is important to question the relevance and utility of Y-chromosome haplotyping in forensic identification procedures focusing in Brazilian naturals. High levels of admixture are presented by Brazilians, and studies performed with ancestry informative markers (AIMs) suggest that most of identified Brazilian genetic variance is observed between individuals, rather than among population groups [46,47]. Same studies show geographical region, self-declared ethnicity or phenotypical features are bad ancestry predictors for Brazilian populations. Under such assumptions, the significant predominance of Y-chromosome EUR haplogroups (with recurrent R1b haplogroup representing over half national Y-haplogroup diversity) renders Y chromosome examination counterproductive, since no considerable amount of information on



(caption on next page)

Fig. 4. Sequencing performance of HID Ion Ampliseq Identity Panel. Data is presented as box-plots, with 1st and 3rd quartiles separated by median value in black. Open whiskers represent values for average plus standard deviation, while capped bars indicate maximum or minimum values. Autosomal SNPs presented in yellow, Y-chromosome in blue. (A) Locus balance for all SNPs, measured as depth of each locus divided by mean depth of all locus per sample. LB was calculated independently for autosomal and Y SNPs, and ideal value is equivalent to 1. (B) Locus strand balance for all SNPs, estimated as forward strand coverage divided by locus total coverage. 0.5 values are optimal, while 0.3 and 0.7 ratios (represented by dotted line) were considered lower and upper thresholds, respectively [13]. (C) Heterozygous balance for autosomal SNPs only. HB was calculated as coverage ratio of one allele to the other, in alphabetical order, for heterozygous genotypes only. Once again, optimal value equals 1. (D) Percentage of noise for all SNPs, measured as coverage of non-alleles divided by total locus coverage. Ideal value equals 0. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

genetic diversity is added to results. Information on Y-haplogrouping in a forensic context might be useful for highly structured populations as North Americans [48]. In such cases, specific ethnic groups inside the country display different allele frequencies for forensic relevant markers, leading to construction of independent databases for each group [49]. No evidence of such degree of genetic stratification was found in present work, nor described so far for populations from Brazil. Therefore, from a Brazilian perspective, substitution of Y-haplogroup SNPs included in HID Ion Ampliseq Identity Panel for other highly-informative, forensic relevant markers might be advantageous, in order to improve discrimination efficiency without reducing overall genotyping capacity (measured as expected depth for each locus in a multi-sample run) and subsequent sequencing performance. Evaluation of possible genetic structure occurrence among different Y-haplogroups was considered, but executed analyses (not shown) were not able to find proof of such divergences, if present. This situation is affected by low diversity in Y-haplogroups observed in Brazilian populations, and low number of non-AFR and non-EUR samples identified. Database expansion is underway, and new samples addition will be helpful in testing this hypothesis.

Inter population analysis of allele frequencies among Brazilian regions and 26 worldwide populations also shed light on interesting issues about investigated sample set. Despite being designed to be neutral and have no significant variations among geographical regions [39,40], autosomal SNPs included in this panel have some capacity to differentiate intercontinental populations through inter populations analysis. This phenomenon has been previously described [19], but application to Brazilian regional context revealed that different colonization processes distinct regions went through had implications on genetic structure presented by people in these areas, at least to some extent. As verified, sample selection bias might have masked such effect for Brazilian Northern region. However, performed tests were able to evaluate deeper levels of genetic structure not discussed by Liu et al. [19], especially concerning higher numbers of inferred populations. Results of interpopulation structure analysis seem to suggest that, at K = 5, some of the genetic structure observed might be derived from NA ancestral genetic contribution to modern AMR populations. Cluster presented in red color in Fig. 3 is distributed across all continental origins, and can be seen as the residual genetic diversity not detected with K = 4. Despite some relevance in European populations (around 20% of relative contribution for EUR group), this particular cluster comprises most of the genetic variation in AMR populations, with values of especially in 34.5%, 52.7%, 67.5% and 90.8% for PUR, CLM, MXL and PEL populations, respectively. For Brazilians, this cluster encompasses 30.3% of genetic structure identified. These results are in general agreement with a meta-analysis of comparative ancestry in American populations, based on AIM data obtained from several studies [50]. NA relative admixture proportions for above-mentioned populations are, in order: 14%, 44%, 62% and 92%, while overall estimate for Brazil is 17%. Despite discrepancies for BRA and PUR populations (where excess of this particular cluster contribution, when compared to AIM-based NA ancestry, might have origins in EUR ancestry component of both groups, due to fraction of this cluster contribution to EUR observed genetic structure), overall correlation between NA ancestry and this cluster contribution for AMR populations is acceptable.

In conclusion, results described in present paper suggest that HID Ion Ampliseq Identity Panel might be a useful tool to be incorporated to Brazilian forensic analytical repertoire. However, some issues still need to be addressed, including not only technical optimizations in panel design (especially aiming improvements regarding inter and intralocus balance). Adequacy of this reagent use in Brazilian real forensic casework must include attention to national genetic particularities, including but not limited to regional idiosyncrasies, which can significantly influence questions like database conception, implementation cost, and other relevant questions.

Conflict of interest

Authors declare they have no conflict of interest.

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CRediT authorship contribution statement

Eduardo Avila: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Resources, Data curation, Writing - original draft, Visualization, Project administration. **Aline Brugnera Felkl:** Formal analysis, Writing - review & editing. **Pietra Graebin:** Investigation, Resources, Data curation. **Cláudia Paiva Nunes:** Investigation, Project administration. **Clarice Sampaio Alho:** Conceptualization, Resources, Data curation, Writing - review & editing, Project administration, Supervision, Funding acquisition.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.fsigen.2019.02.012.

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CAPÍTULO 2 - Forensic characterization of Brazilian regional populations through massive parallel sequencing of 124 SNPs included in HID Ion Ampliseq Identity Panel

Artigo publicado no periódico Forensic Science International: Genetics

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Material Suplementar.

Disponível em https://www.sciencedirect.com/science/article/pii/S1872497318305234

Supplementary Figures

Supplementary Figure S1. Sample origins in Brazilian populations. Geopolitical regions divided by colors and named in boxes. States name, abbreviation and sample number indicated by lines.

Supplementary Figure S2. N-J phylogenetic tree of 14 Y-chromosome haplogroups found in Brazilian population. Y-SNP markers mutations relevant for haplogroup classification are presented in each branch. Pie chart area proportional to each haplogroup frequency, with colors indicating Brazilian region of occurrence. O2 group (marked with *) was identified by plugin as O3 group. SRY indicates SRY 1532.2 Y-SNP marker.

Supplementary Figure S3. Pairwise F_{ST} heat map values among Brazilian combined national population and 26 worldwide populations based on 88 overlapped autosomal SNPs.

Supplementary Figures S4-S6. Genetic distance evaluation for inter population analysis of 26 worldwide populations and Brazilian regional or national data based on pairwise Fst values of 88 overlapped autosomal SNPs.

Supplementary Figure S4. N-J phylogenetic tree for 5 Brazilian regional and 26 worldwide populations. Distances in Fst units.

Supplementary Figure S5. MDS plot for Brazilian national and 26 worldwide populations.

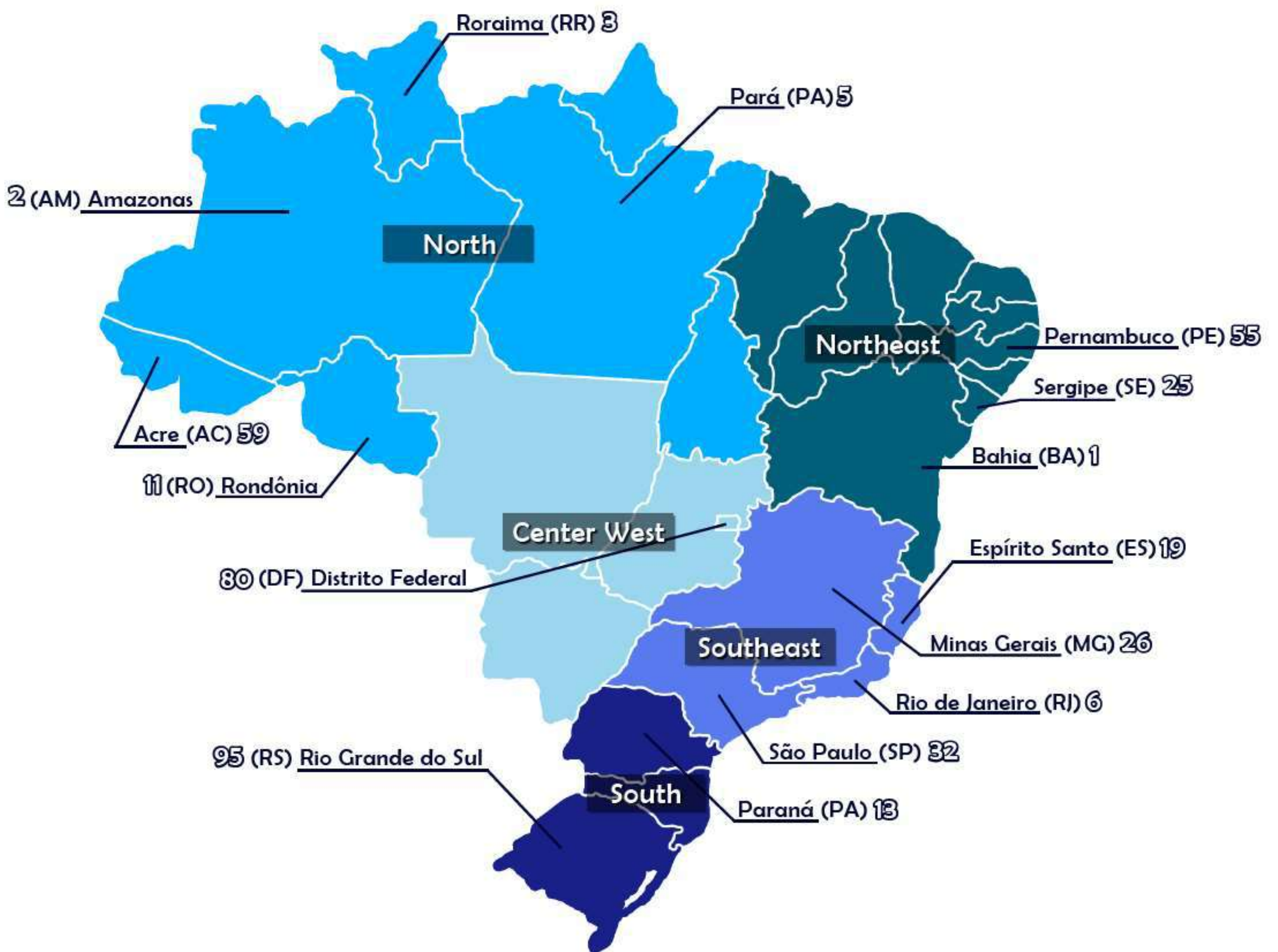
Supplementary Figure S6. N-J phylogenetic tree for Brazilian national and 26 worldwide populations. Distances in Fst units.

Supplementary Figures S7-S8. PCA plots of 26 worldwide populations and investigated Brazilian populations, based on 88 autosomal SNPs data.

Supplementary Figure S7. First and second components of PCA plot based on allele frequencies for all populations. Both axes were moved for graphic clarity.

Supplementary Figure S8. First and second (upper) and second and third components (lower) PCA plots of individual genotypes, with samples grouped by geographical origin.

Supplementary Figure S9. Coverage heat map of 124 SNP markers included in HID Ion Ampliseq Identity Panel for 432 Brazilian samples. Samples were arranged (top to bottom) according to mean coverage for all locus, in ascending order. SNPs are distributed (left to right) in same order as presented by plug in (as displayed in Supplementary Table S3).



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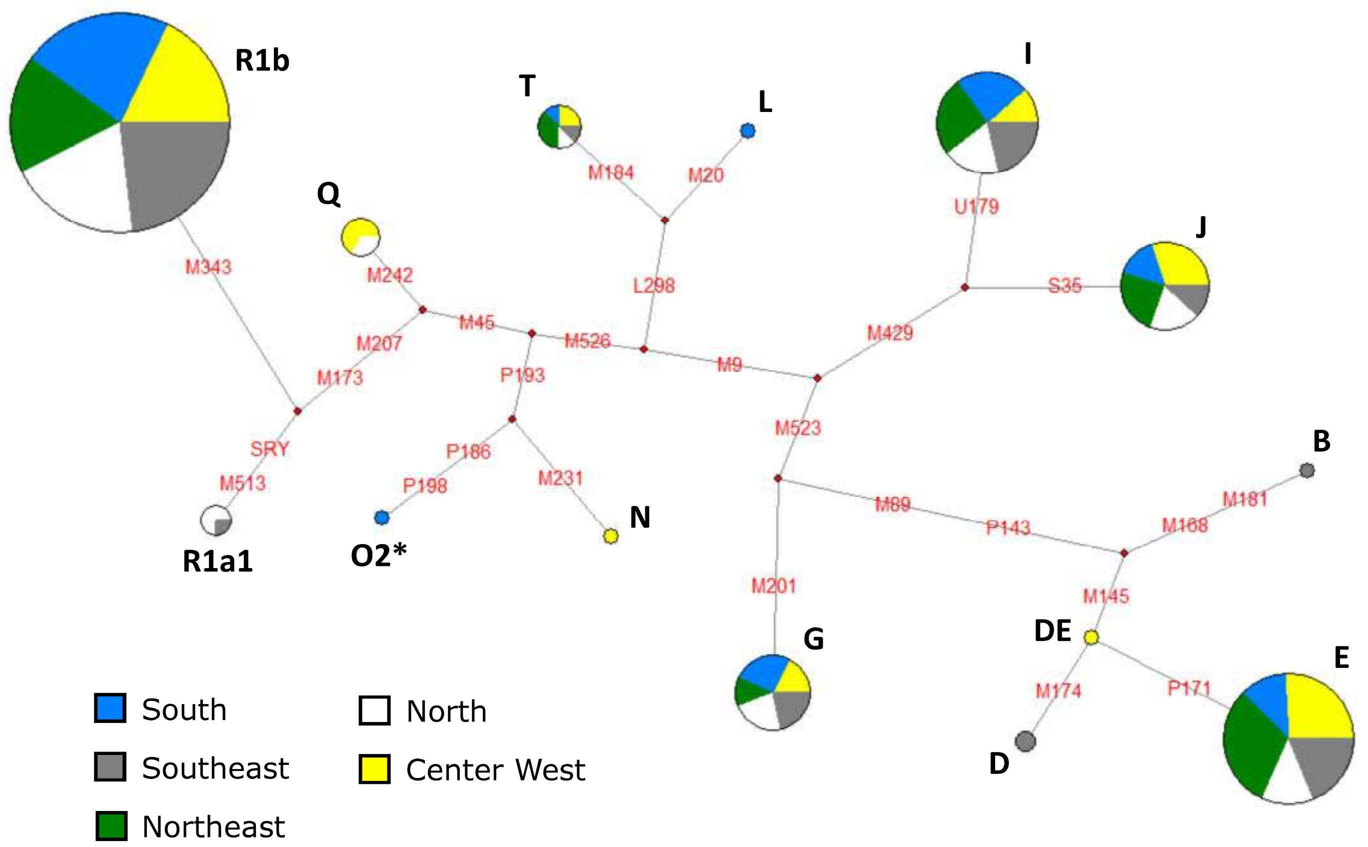
Bahia (BA) 1
11 (RO) Rondônia
Center West
Espírito Santo (ES) 19
80 (DF) Distrito Federal
Southeast
Minas Gerais (MG) 26
Rio de Janeiro (RJ) 6
95 (RS) Rio Grande do Sul
São Paulo (SP) 32
South
Paraná (PA) 13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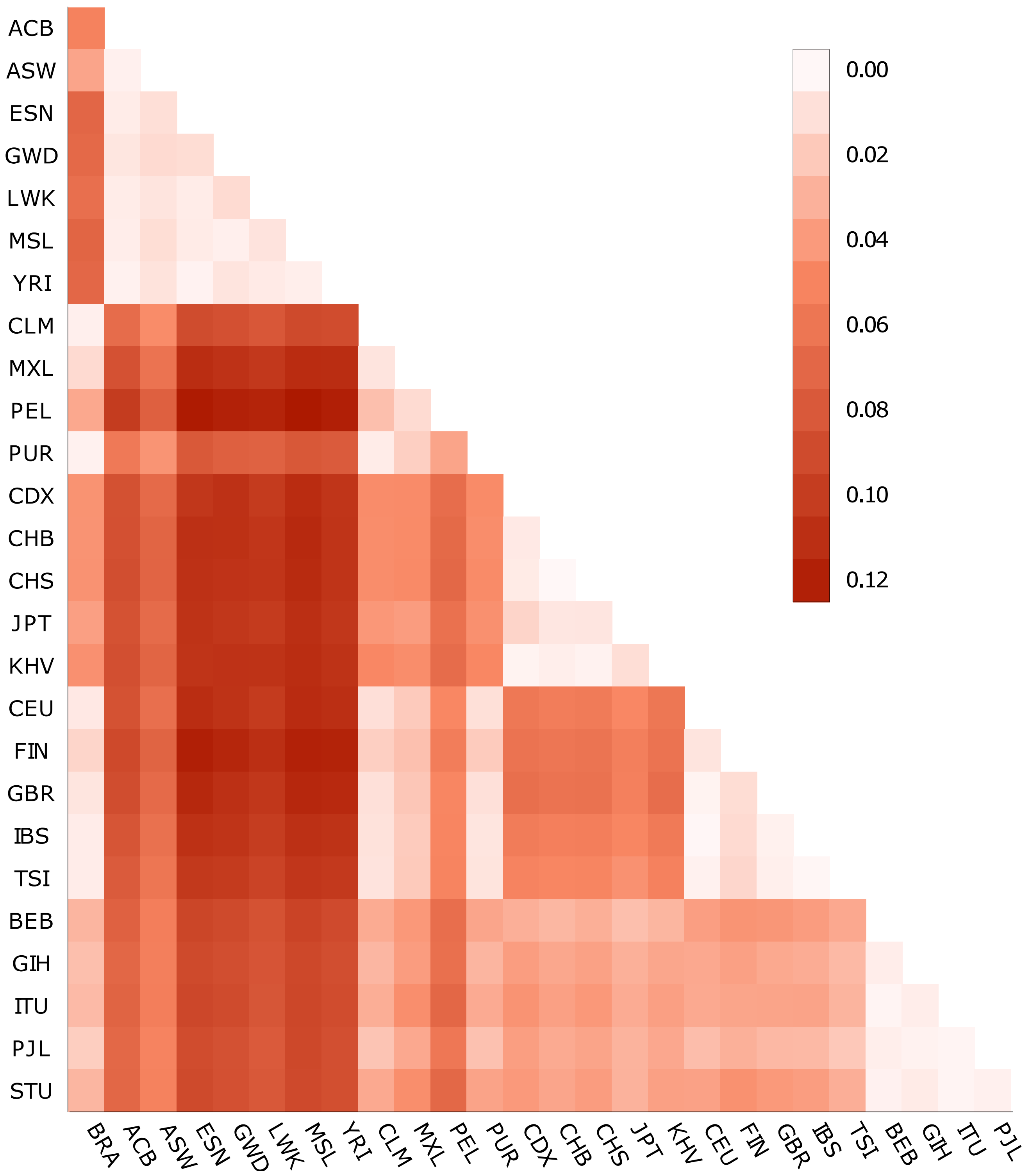

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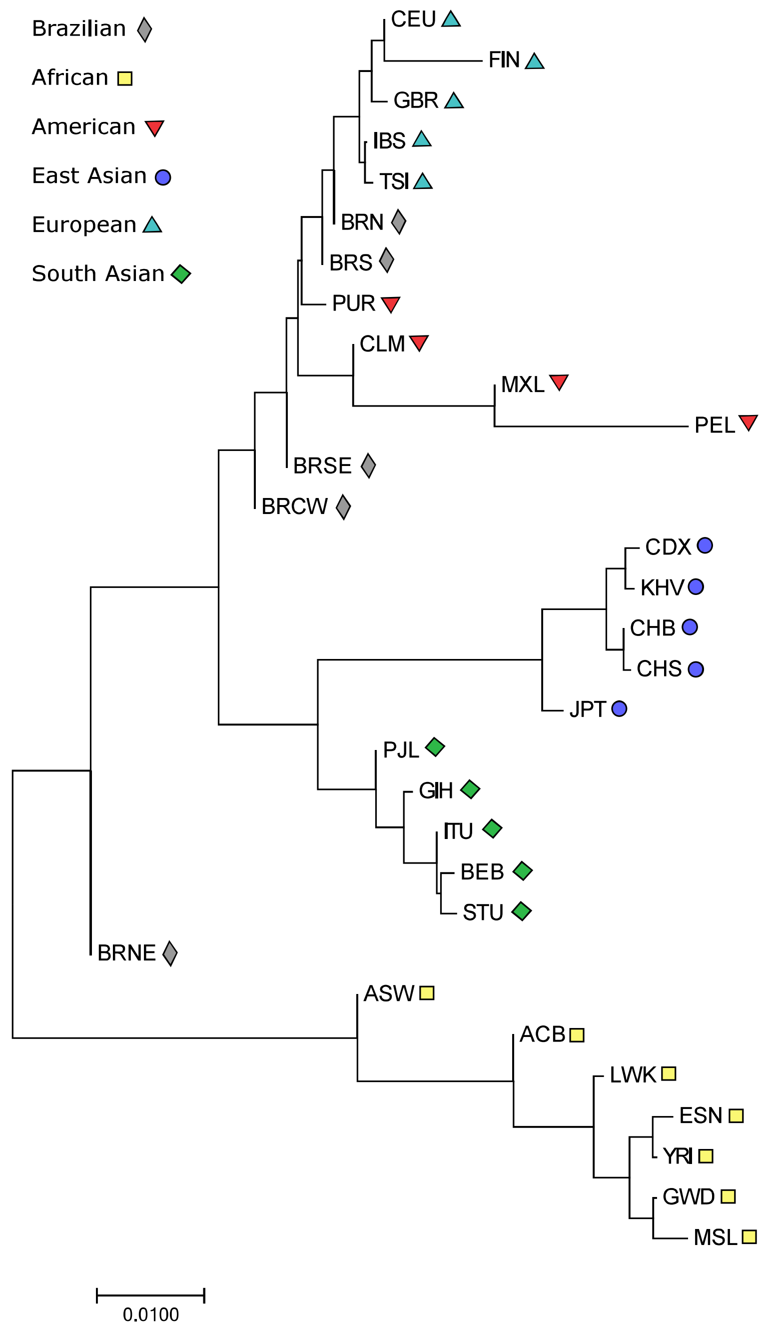

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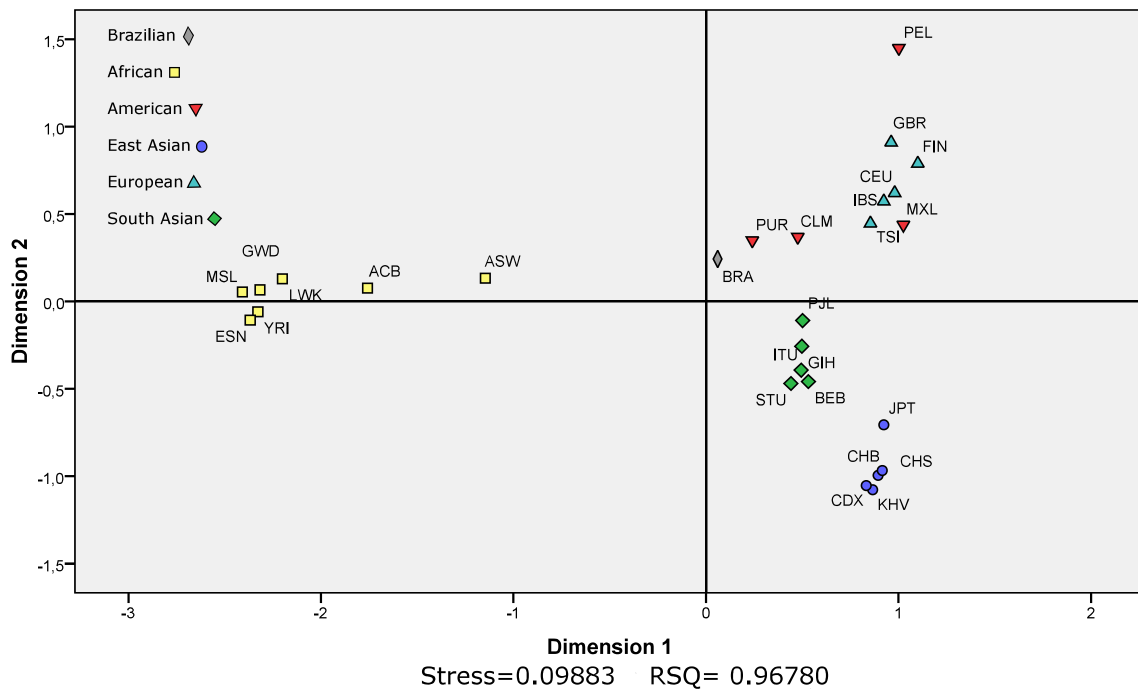
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South Asian
PEL
GBR
FIN
CEU
IBS
MXL
TSI
PUR
CLM
BRA
GWD
MSL
ACB
ASW
LWK
ESN
YRI
PJL
ITU
GIH
STU
BEB
JPT
CHB
CHS
CDX
KHV
Dimension 2
Dimension 1
1,5
1,0
0,5
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-1,0
-1,5
-3
-2
-1
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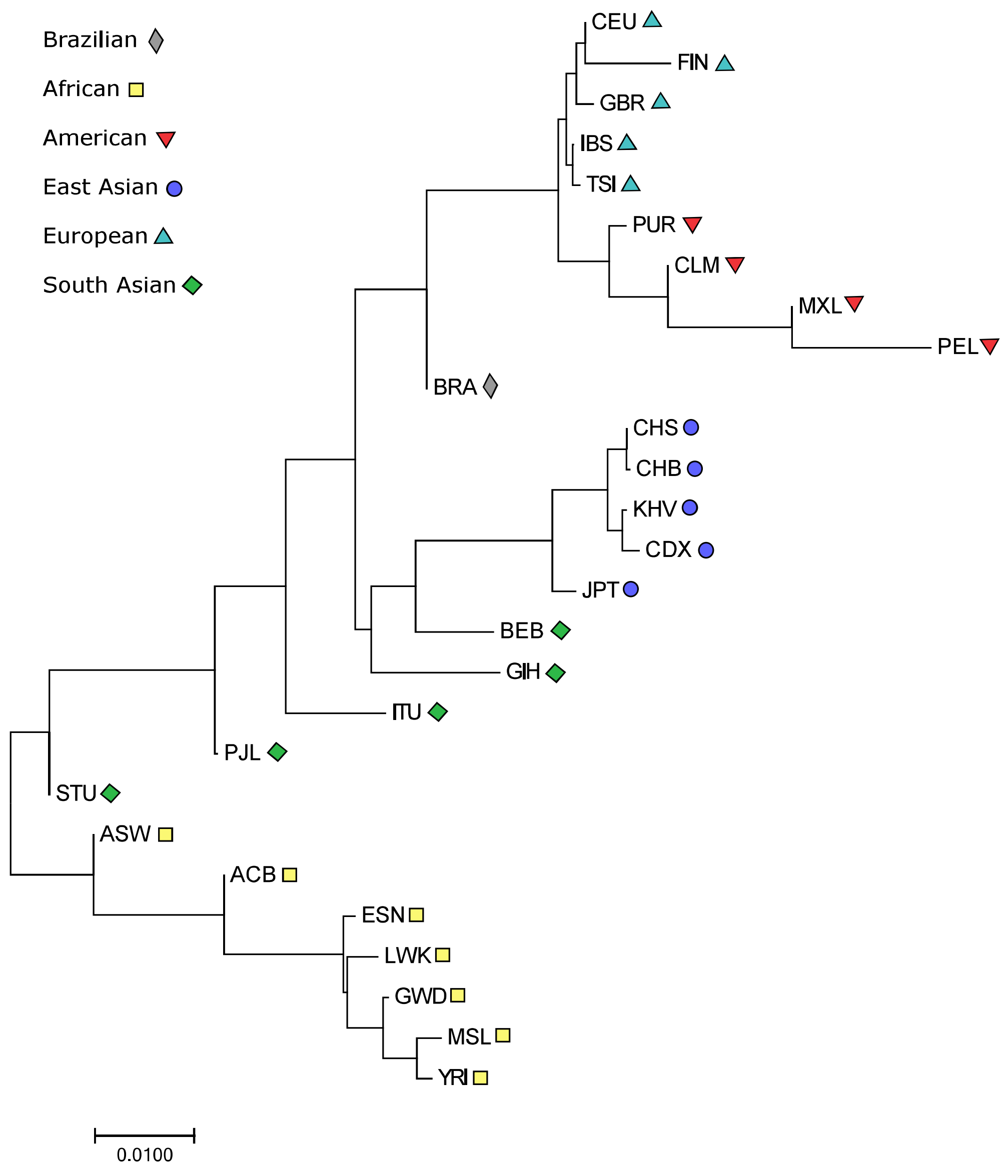

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CEU
FIN
GBR
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TSI
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CHB
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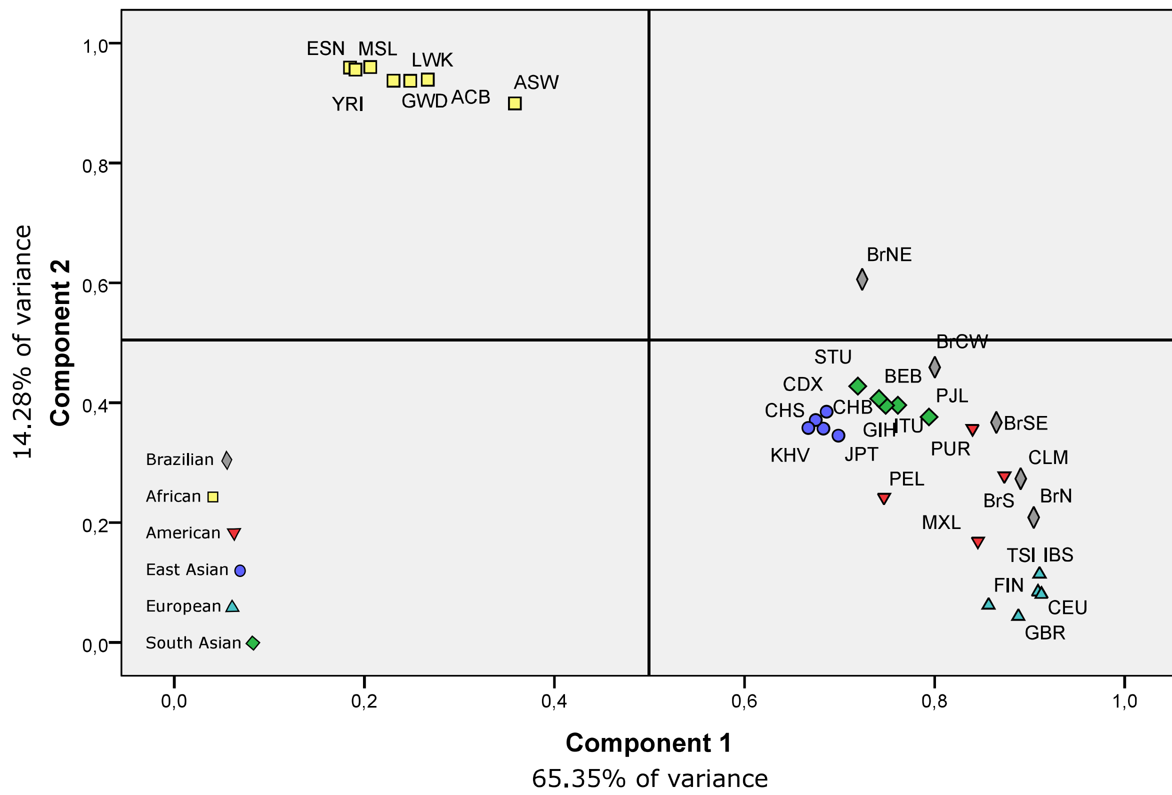
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KHV
JPT
PUR
CLM
PEL
BrS
BrN
MXL
TSI
IBS
FIN
CEU
GBR
Brazilian
African
American
East Asian
European
South Asian
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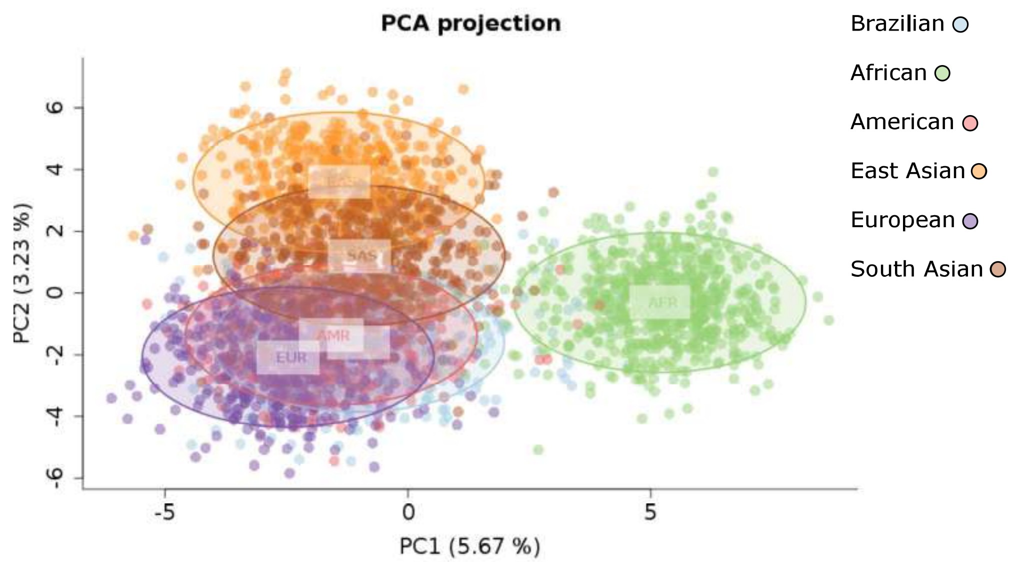
0,6
0,4
0,2
0,0
Component 2
14.28% of variance
Component 1
65.35% of variance


PCA projection
Brazilian
African
American
East Asian
European
South Asian
PC2 (3.23 %)
PC1 (5.67 %)
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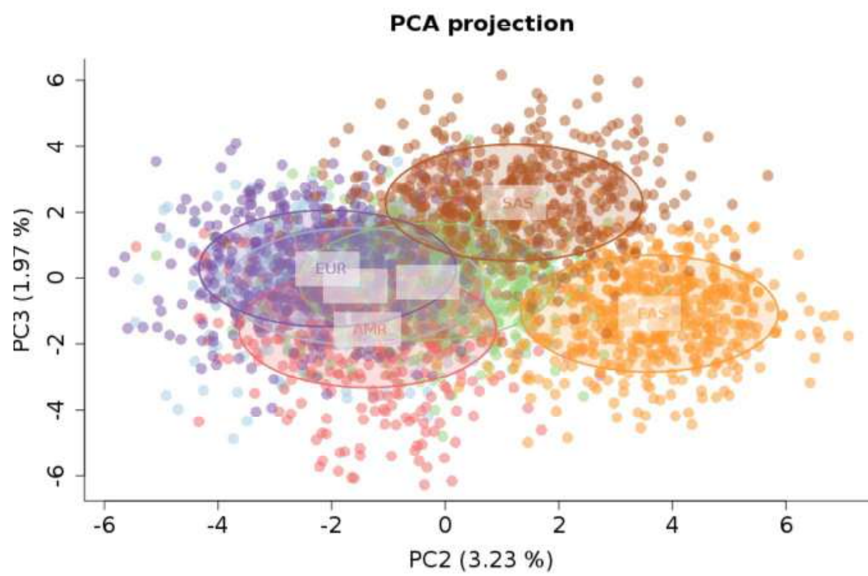
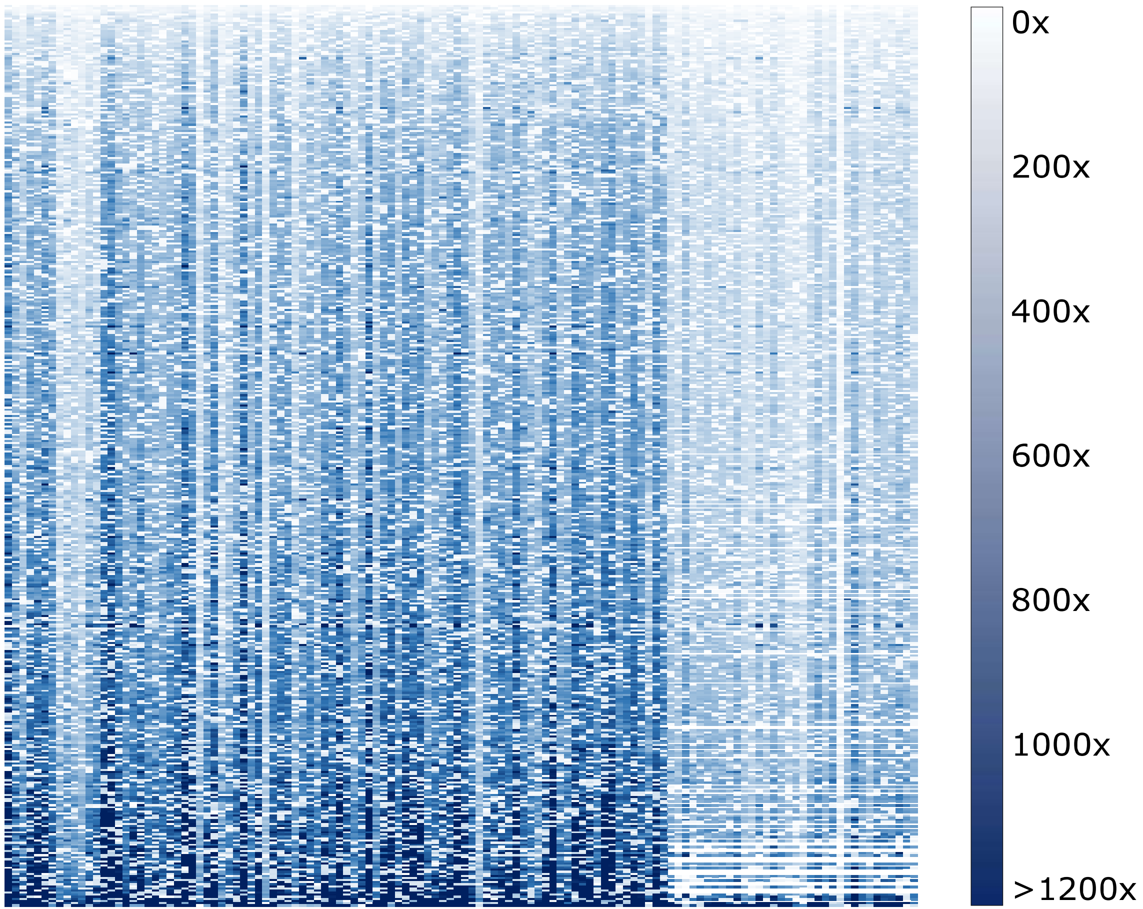
-4
-2
0
2
4
6
-5
0
5
SAS
AMR
EUR
AFR


PCA projection
PC3 (1.97 %)
PC2 (3.23 %)
-6
-4
-2
0
2
4
6
SAS
EUR
AMR
EAS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200x
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Supplementary Table S1.	Y-chromosome SNPs markers names correspondence between results presented by HID SNP Genotyper plugin v. 12.334 and NCBI dbSNP, for variations found in present study only. A comprehensive tree of all possible Y-chromosome superior-clade haplogroups generated by the 34 Y-SNP markers included in this panel can be found in [29].
Supplementary Table S2.	Metadata of populations used in the present study.
Supplementary Table S3.	Complete genotypes of 124 markers (90 autosomal and 34 Y-chromosome SNPs) included in HID Ion Ampliseq Identity Panel for 432 Brazilian samples. Y-genotypes marked as "-" . represents female samples. (**n**South=108; **n**Southeast=83; **n**Northeast=81; **n**North=80; **n**Center-west=80; **n**Total=432)
Supplementary Table S4.	Allele frequency of 90 autosomal ID-oriented SNP markers included in HID Ion Ampliseq Identity Panel for Brazilian and regional poplations. Reference allele presented in bold, alternative in plain font. (**n**South=108; **n**Southeast=83; **n**Northeast=81; **n**North=80; **n**Center-west=80; **n**Total=432)
Supplementary Table S5.	Pairwise FST and exact differentiation test for Brazilian regions based on allele frequencies of 90 autosomal SNPs included in HID Ion Ampliseq Identity Panel. Differentiation tests are presented in lower-left diagonal as p-values, while upper-right diagonal exhibits FST values.
Supplementary Table S6.	Forensic Parameters of 90 autosomal ID-oriented SNP markers included in HID Ion Ampliseq Identification Panel for Brazilian national and regional populations.
Supplementary Table S7.	Pairwise linkage desequilibrium p-values among 90 autosomal ID-oriented SNP markers included in HID Ion Ampliseq Identity Panel for Brazilian population. Marker pairs where LD was observed, for a significance level of 0.05 and after Bonferroni correction, are highlighted in bold.
Supplementary Table S8.	Relative contribution of each cluster in each population composition, based on Structure software analysis of 88 autosomal SNPs included in HID Ion Ampliseq Identity Panel, for K=5. Next to cluster number, associated geographical origin of higher relevance is informed. Population names and geographical origin abbreviations are listed in Supplementary Table S2.
Supplementary Table S9.	MPS performance data for each individual run using HID Ion Ampliseq Identity Panel with an ION PGM Sequencer.
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Supplementary Table S1. Y-chromosome SNPs markers names correspondence between results presented by HID SNP Genotyper plugin v. 12.334 and NCBI dbSNP, for variations found in present study only. A comprehensive tree of all possible Y-chromosome superior-clade haplogroups generated by the 34 Y-SNP markers included in this panel can be found in [29].

Y-chromosome marker name according to		Alleles		Major Y-Clade affiliation
HID SNP Genotyper plugin	*Reference SNP ID number*	*Ancestral*	*Variation*	
SRY1532.2	rs2534636	C	T	R1a1
M343	rs9786184	C	A	R1b
M523	rs9786184	A	G	IJK
P186	rs16981290	C	A	O
S35	rs17250845	G	C	J
L298	rs372687543	T	C	LT
M429	rs17306671	T	A	IJ
P143	rs4141886	G	A	CF
M168	rs2032595	C	T	CT
M181	rs2032599	T	C	B
M184	rs20320	G	A	T
M174	rs2032602	T	C	D
M242	rs8179021	C	T	Q
M173	rs2032624	A	C	R1
M201	rs2032636	G	T	G
M231	rs9341278	G	A	N
M207	rs2032658	A	G	R
U179	rs2319818	G	A	I
P198	rs17269816	C	T	O2*
M513	rs17222573	A	G	R1a
M145	rs3848982	C	T	DE
M9	rs3900	C	G	K
M20	rs3911	A	G	L
M45	rs2032631	G	A	P1
M89	rs2032652	C	T	F
P193	rs16980426	T	G	NO1
P171	rs17842518	G	T	E
M526	rs2033003	A	C	K2

O2 haplogroup (marked with *) was reported by plugin as O3.

Supplementary Table S2. Metadata of populations used in the present study.

Population number	Name and description	Sample size	Geographical Origin	Abbreviation
1	Brazilians from southern Brazil - Rio Grande do Sul and Parana states	108	Brazil (BRA)	Br-S
2	Brazilians from southeastern Brazil - Rio de Janeiro, Espirito Santo, Sao Paulo and Minas Gerais states	83	Brazil (BRA)	Br-SE
3	Brazilians from northeastern Brazil - Bahia, Pernambuco and Sergipe states	81	Brazil (BRA)	Br-NE
4	Brazilians from northern Brazil - Para, Amazonas, Roraima, Rondonia and Acre states	80	Brazil (BRA)	Br-N
5	Brazilians from Distrito Federal, Center-West region	80	Brazil (BRA)	Br-CW
6	African Carribbeans in Barbados	96	Africa (AFR)	ACB
7	Americans of African Ancestry in SW USA	61	Africa (AFR)	ASW
8	Esan in Nigeria	99	Africa (AFR)	ESN
9	Gambian in Western Divisions in the Gambia	113	Africa (AFR)	GWD
10	Luhya in Webuye, Kenya	99	Africa (AFR)	LWK
11	Mende in Sierra Leone	85	Africa (AFR)	MSL
12	Yoruba in Ibadan, Nigeria	108	Africa (AFR)	YRI
13	Colombians from Medellin, Colombia	94	America (AMR)	CLM
14	Mexican Ancestry from Los Angeles USA	64	America (AMR)	MXL
15	Peruvians from Lima, Peru	85	America (AMR)	PEL
16	Puerto Ricans from Puerto Rico	104	America (AMR)	PUR
17	Chinese Dai in Xishuangbanna, China	93	East Asia (EAS)	CDX
18	Han Chinese in Beijing, China	103	East Asia (EAS)	CHB
19	Southern Han Chinese, China	105	East Asia (EAS)	CHS
20	Japanese in Tokyo, Japan	104	East Asia (EAS)	JPT
21	Kinh in Ho Chi Minh City, Vietnam	99	East Asia (EAS)	KHV
22	Utah Residents (CEPH) with North and Western European Ancestry	99	Europe (EUR)	CEU
23	Finnish in Finland	99	Europe (EUR)	FIN
24	British in England and Scotland	91	Europe (EUR)	GBR
25	Iberian Population in Spain	107	Europe (EUR)	IBS
26	Toscani in Italia	107	Europe (EUR)	TSI
27	Bengali from Bangladesh	86	South Asia (SAS)	BEB
28	Gujarati Indian from Houston, Texas	103	South Asia (SAS)	GIH
29	Indian Telugu from the UK	102	South Asia (SAS)	ITU
30	Punjabi from Lahore, Pakistan	96	South Asia (SAS)	PJL
31	Sri Lankan Tamil from the UK	102	South Asia (SAS)	STU

Populations 6 to 31 were obtained from 1000 Genomes Project, Phases 1-3.

Supplementary Table S3. Complete genotypes of 124 markers (90 autosomal and 34 Y-chromosome SNPs) included in HID Ion Ampliseq Identity Panel for 432

Sample name	State	Region	Y-Haplogroup	Autosomal SNP marker name				
				rs1490413	*rs7520386*	*rs4847034*	*rs560681*	*rs10495407*
PR17	PR	Southern Brazil (Br-S)	-	AA	AG	GG	AA	AG
PR18	PR	Southern Brazil (Br-S)	-	AG	AG	GG	AA	AG
PR19	PR	Southern Brazil (Br-S)	I	AG	AG	AG	AA	GG
PR20	PR	Southern Brazil (Br-S)	R1b	AG	AA	GG	AA	GG
PR21	PR	Southern Brazil (Br-S)	-	GG	AG	AG	AG	AG
PR22	PR	Southern Brazil (Br-S)	-	GG	AG	AG	GG	AG
PR23	PR	Southern Brazil (Br-S)	-	GG	GG	AG	AA	AA
PR24	PR	Southern Brazil (Br-S)	-	AG	AA	AG	AG	GG
PR26	PR	Southern Brazil (Br-S)	-	AG	AG	AG	AA	AG
PR27	PR	Southern Brazil (Br-S)	R1b	AG	AA	AG	GG	AG
PR28	PR	Southern Brazil (Br-S)	G	AA	AA	GG	AA	AG
PR29	PR	Southern Brazil (Br-S)	R1b	AA	AG	AA	AG	GG
PR30	PR	Southern Brazil (Br-S)	G	AA	AG	AG	AG	AG
CR01	RS	Southern Brazil (Br-S)	R1b	GG	GG	GG	AA	AG
CR02	RS	Southern Brazil (Br-S)	R1b	AG	AA	GG	AG	AG
CR03	RS	Southern Brazil (Br-S)	R1b	AG	AG	GG	AG	GG
CR04	RS	Southern Brazil (Br-S)	R1b	GG	AG	GG	AG	GG
CR05	RS	Southern Brazil (Br-S)	R1b	GG	AA	AG	AG	AG
CR06	RS	Southern Brazil (Br-S)	O3	GG	AA	AG	AG	AG
CR07	RS	Southern Brazil (Br-S)	I	GG	AA	AA	GG	AA
CR08	RS	Southern Brazil (Br-S)	R1b	AA	AG	GG	AA	GG
CR09	RS	Southern Brazil (Br-S)	R1b	AG	AG	AA	AA	AG
CR10	RS	Southern Brazil (Br-S)	R1b	GG	AG	AG	AG	AG
CR11	RS	Southern Brazil (Br-S)	E	AG	AG	AG	AG	AA
CR12	RS	Southern Brazil (Br-S)	I	AG	AA	GG	AA	AG
CR13	RS	Southern Brazil (Br-S)	E	AG	AG	GG	AA	AG
CR14	RS	Southern Brazil (Br-S)	J	AG	AG	AG	AG	GG
CR15	RS	Southern Brazil (Br-S)	I	GG	GG	AA	AG	AG
CR16	RS	Southern Brazil (Br-S)	R1b	AG	GG	GG	AG	AG

Supplementary Table S4. Allele frequencies of 90 autosomal ID-oriented SNP markers included in HID Ion Ampliseq Identification Panel for Brazilian and regional populations. Reference allele presented in bold, alternative in plain font (**n**$_{South}$=108; **n**$_{Southeast}$=83; **n**$_{Northeast}$=81; **n**$_{North}$=80; **n**$_{Centerwest}$=80; **n**$_{Total}$=432).

Marker	Chr.	Alleles	Region											
			Brazil		*South*		*Southeast*		*Northeast*		*North*		*Center West*	
rs1490413	1	A/**G**	0.456	0.544	0.5046	0.4954	0.4639	0.5361	0.3951	0.6049	0.4875	0.5125	0.4125	0.5875
rs7520386	1	A/**G**	0.5405	0.4595	0.5926	0.4074	0.5	0.5	0.5741	0.4259	0.5125	0.4875	0.5063	0.4938
rs4847034	1	**A**/G	0.3796	0.6204	0.3611	0.6389	0.4157	0.5843	0.3889	0.6111	0.3938	0.6063	0.3438	0.6563
rs560681	1	**A**/G	0.6748	0.3252	0.713	0.287	0.6627	0.3373	0.6605	0.3395	0.7188	0.2813	0.6063	0.3938
rs10495407	1	A/**G**	0.316	0.684	0.375	0.625	0.2651	0.7349	0.2222	0.7778	0.375	0.625	0.325	0.675
rs891700	1	**A**/G	0.4907	0.5093	0.4537	0.5463	0.5	0.5	0.4815	0.5185	0.5063	0.4938	0.525	0.475
rs1413212	1	C/**T**	0.6829	0.3171	0.7037	0.2963	0.6687	0.3313	0.679	0.321	0.7125	0.2875	0.6438	0.3563
rs876724	2	**C**/T	0.7095	0.2905	0.7778	0.2222	0.6807	0.3193	0.6852	0.3148	0.6375	0.3625	0.7438	0.2563
rs1109037	2	A/**G**	0.4815	0.5185	0.4954	0.5046	0.4578	0.5422	0.5432	0.4568	0.4875	0.5125	0.4188	0.5813
rs993934	2	**A**/G	0.6308	0.3692	0.625	0.375	0.6386	0.3614	0.642	0.358	0.6063	0.3938	0.6438	0.3563
rs12997453	2	**A**/G	0.3692	0.6308	0.3472	0.6528	0.3735	0.6265	0.3457	0.6543	0.3813	0.6188	0.4063	0.5938
rs907100	2	C/**G**	0.5324	0.4676	0.5185	0.4815	0.5542	0.4458	0.6049	0.3951	0.4375	0.5625	0.55	0.45
rs1357617	3	**A**/T	0.2512	0.7488	0.2917	0.7083	0.241	0.759	0.2037	0.7963	0.2938	0.7063	0.2125	0.7875
rs4364205	3	G/**T**	0.5984	0.4016	0.5787	0.4213	0.5843	0.4157	0.5617	0.4383	0.6063	0.3938	0.6688	0.3313
rs1872575	3	A/**G**	0.5336	0.4664	0.5602	0.4398	0.5181	0.4819	0.4383	0.5617	0.6063	0.3938	0.5375	0.4625
rs1355366	3	C/**T**	0.3819	0.6181	0.3935	0.6065	0.4096	0.5904	0.3395	0.6605	0.3688	0.6313	0.3938	0.6063
rs6444724	3	C/**T**	0.4769	0.5231	0.4074	0.5926	0.4277	0.5723	0.4691	0.5309	0.5438	0.4563	0.5625	0.4375
rs2046361	4	A/**T**	0.3738	0.6262	0.3981	0.6019	0.3193	0.6807	0.4198	0.5802	0.3563	0.6438	0.3688	0.6313
rs6811238	4	G/**T**	0.4873	0.5127	0.5278	0.4722	0.4518	0.5482	0.5	0.5	0.4438	0.5563	0.5	0.5
rs1979255	4	**C**/G	0.36	0.64	0.3194	0.6806	0.3614	0.6386	0.3951	0.6049	0.3938	0.6063	0.3438	0.6563
rs717302	5	A/**G**	0.566	0.434	0.5741	0.4259	0.5301	0.4699	0.537	0.463	0.6125	0.3875	0.575	0.425
rs159606	5	**A**/G	0.3912	0.6088	0.3611	0.6389	0.4398	0.5602	0.3395	0.6605	0.3688	0.6313	0.4563	0.5438
rs7704770	5	A/**G**	0.544	0.456	0.5324	0.4676	0.5361	0.4639	0.5309	0.4691	0.5375	0.4625	0.5875	0.4125
rs251934	5	**A**/G	0.6308	0.3692	0.6296	0.3704	0.6506	0.3494	0.6605	0.3395	0.5938	0.4063	0.6188	0.3813
rs338882	5	A/**G**	0.5012	0.4988	0.5	0.5	0.5361	0.4639	0.537	0.463	0.4563	0.5438	0.475	0.525
rs13218440	6	A/**G**	0.4317	0.5683	0.412	0.588	0.4157	0.5843	0.4136	0.5864	0.45	0.55	0.475	0.525
rs214955	6	**C**/T	0.5081	0.4919	0.5648	0.4352	0.5181	0.4819	0.5123	0.4877	0.4875	0.5125	0.4375	0.5625
rs727811	6	**G**/T	0.4988	0.5012	0.4815	0.5185	0.4578	0.5422	0.5123	0.4877	0.4625	0.5375	0.5875	0.4125
rs6955448	7	**C**/T	0.6759	0.3241	0.7222	0.2778	0.6687	0.3313	0.6173	0.3827	0.675	0.325	0.6813	0.3188
rs917118	7	**C**/T	0.6123	0.3877	0.588	0.412	0.6988	0.3012	0.4815	0.5185	0.6625	0.3375	0.6375	0.3625
rs321198	7	C/**T**	0.5648	0.4352	0.6111	0.3889	0.5602	0.4398	0.5432	0.4568	0.5188	0.4813	0.575	0.425
rs737681	7	C/**T**	0.5961	0.4039	0.6389	0.3611	0.5723	0.4277	0.5247	0.4753	0.6125	0.3875	0.6188	0.3813
rs10092491	8	C/**T**	0.5683	0.4317	0.6343	0.3657	0.5964	0.4036	0.5679	0.4321	0.5313	0.4688	0.4875	0.5125
rs4288409	8	**A**/C	0.2708	0.7292	0.3009	0.6991	0.2831	0.7169	0.2284	0.7716	0.2563	0.7438	0.275	0.725
rs2056277	8	**C**/T	0.7639	0.2361	0.75	0.25	0.7651	0.2349	0.784	0.216	0.7625	0.2375	0.7625	0.2375
rs1015250	9	C/**G**	0.2847	0.7153	0.2546	0.7454	0.2831	0.7169	0.3457	0.6543	0.2125	0.7875	0.3375	0.6625
rs7041158	9	**C**/T	0.6539	0.3461	0.5972	0.4028	0.6566	0.3434	0.6543	0.3457	0.7	0.3	0.6813	0.3188
rs1463729	9	**C**/T	0.3958	0.6042	0.4537	0.5463	0.3614	0.6386	0.3642	0.6358	0.3688	0.6313	0.4125	0.5875
rs1360288	9	**C**/T	0.7106	0.2894	0.6991	0.3009	0.7229	0.2771	0.7407	0.2593	0.6875	0.3125	0.7063	0.2938
rs10776839	9	**G**/T	0.5301	0.4699	0.5093	0.4907	0.5	0.5	0.5988	0.4012	0.4938	0.5063	0.5563	0.4438
rs826472	10	C/**T**	0.6794	0.3206	0.6435	0.3565	0.747	0.253	0.716	0.284	0.6813	0.3188	0.6188	0.3813
rs735155	10	**C**/T	0.5567	0.4433	0.588	0.412	0.506	0.494	0.5494	0.4506	0.5688	0.4313	0.5625	0.4375
rs3780962	10	**A**/G	0.4838	0.5162	0.4722	0.5278	0.5301	0.4699	0.4877	0.5123	0.4	0.6	0.5313	0.4688
rs740598	10	A/**G**	0.6146	0.3854	0.6343	0.3657	0.5964	0.4036	0.5617	0.4383	0.6375	0.3625	0.6375	0.3625
rs964681	10	C/**T**	0.3646	0.6354	0.338	0.662	0.3614	0.6386	0.3025	0.6975	0.3875	0.6125	0.4438	0.5563
rs1498553	11	**C**/T	0.456	0.544	0.4213	0.5787	0.4578	0.5422	0.4506	0.5494	0.4625	0.5375	0.5	0.5
rs901398	11	**C**/T	0.3356	0.6644	0.3194	0.6806	0.3554	0.6446	0.3519	0.6481	0.35	0.65	0.3063	0.6938
rs10488710	11	**C**/G	0.39	0.61	0.3472	0.6528	0.4337	0.5663	0.358	0.642	0.3938	0.6063	0.4313	0.5688
rs2076848	11	**A**/T	0.6273	0.3727	0.5833	0.4167	0.6566	0.3434	0.679	0.321	0.575	0.425	0.6563	0.3438
rs2269355	12	**C**/G	0.5162	0.4838	0.5139	0.4861	0.4639	0.5361	0.4877	0.5123	0.575	0.425	0.5438	0.4563
rs2111980	12	C/**T**	0.4884	0.5116	0.4537	0.5463	0.4819	0.5181	0.4383	0.5617	0.575	0.425	0.5063	0.4938
rs10773760	12	**A**/G	0.5984	0.4016	0.5833	0.4167	0.5723	0.4277	0.6049	0.3951	0.5875	0.4125	0.65	0.35
rs1335873	13	A/**T**	0.5903	0.4097	0.6343	0.3657	0.5663	0.4337	0.4444	0.5556	0.675	0.325	0.6188	0.3813
rs1886510	13	A/**G**	0.419	0.581	0.4444	0.5556	0.4639	0.5361	0.358	0.642	0.425	0.575	0.3938	0.6063
rs1058083	13	**A**/G	0.3889	0.6111	0.412	0.588	0.3855	0.6145	0.358	0.642	0.3813	0.6188	0.4	0.6
rs354439	13	**A**/T	0.5521	0.4479	0.6296	0.3704	0.5723	0.4277	0.4753	0.5247	0.5313	0.4688	0.525	0.475
rs1454361	14	A/**T**	0.4201	0.5799	0.4213	0.5787	0.4518	0.5482	0.3765	0.6235	0.4188	0.5813	0.4313	0.5688
rs722290	14	C/**G**	0.4803	0.5197	0.5185	0.4815	0.488	0.512	0.4877	0.5123	0.4313	0.5688	0.4625	0.5375
rs873196	14	**C**/T	0.3484	0.6516	0.375	0.625	0.3012	0.6988	0.2963	0.7037	0.3938	0.6063	0.3688	0.6313
rs4530059	14	A/**G**	0.3889	0.6111	0.4074	0.5926	0.4096	0.5904	0.3519	0.6481	0.4	0.6	0.3688	0.6313
rs2016276	15	C/**T**	0.1898	0.8102	0.2269	0.7731	0.1928	0.8072	0.1667	0.8333	0.1938	0.8063	0.1563	0.8438
rs1821380	15	**C**/G	0.5417	0.4583	0.5278	0.4722	0.5542	0.4458	0.537	0.463	0.5438	0.4563	0.55	0.45
rs1528460	15	**C**/T	0.4433	0.5567	0.338	0.662	0.3916	0.6084	0.5864	0.4136	0.4375	0.5625	0.5	0.5
rs729172	16	**G**/T	0.5903	0.4097	0.6019	0.3981	0.506	0.494	0.6358	0.3642	0.5813	0.4188	0.625	0.375
rs2342747	16	**A**/G	0.3808	0.6192	0.375	0.625	0.3976	0.6024	0.3642	0.6358	0.3688	0.6313	0.4	0.6
rs430046	16	**C**/T	0.581	0.419	0.5602	0.4398	0.5904	0.4096	0.5802	0.4198	0.5688	0.4313	0.6125	0.3875
rs1382387	16	A/**C**	0.6516	0.3484	0.6991	0.3009	0.5904	0.4096	0.6235	0.3765	0.7125	0.2875	0.6188	0.3813
rs9905977	17	**A**/G	0.2859	0.7141	0.2917	0.7083	0.2711	0.7289	0.3086	0.6914	0.25	0.75	0.3063	0.6938
rs740910	17	**A**/G	0.7373	0.2627	0.7176	0.2824	0.7048	0.2952	0.8086	0.1914	0.7063	0.2938	0.7563	0.2438
rs938283	17	C/**T**	0.1725	0.8275	0.1898	0.8102	0.1687	0.8313	0.1728	0.8272	0.1625	0.8375	0.1625	0.8375
rs2292972	17	C/**T**	0.3866	0.6134	0.4306	0.5694	0.3193	0.6807	0.3642	0.6358	0.4438	0.5563	0.3625	0.6375
rs1493232	18	A/**C**	0.6157	0.3843	0.6296	0.3704	0.5904	0.4096	0.6358	0.3642	0.5625	0.4375	0.6563	0.3438
rs9951171	18	A/**G**	0.4167	0.5833	0.3889	0.6111	0.4699	0.5301	0.4012	0.5988	0.4438	0.5563	0.3875	0.6125
rs1736442	18	C/**T**	0.6192	0.3808	0.5787	0.4213	0.6325	0.3675	0.6235	0.3765	0.6375	0.3625	0.6375	0.3625
rs1024116	18	**C**/T	0.4757	0.5243	0.4213	0.5787	0.5723	0.4277	0.4877	0.5123	0.4063	0.5938	0.5063	0.4938
rs719366	19	A/**G**	0.7037	0.2963	0.6713	0.3287	0.7349	0.2651	0.6975	0.3025	0.675	0.325	0.75	0.25
rs576261	19	**A**/C	0.5729	0.4271	0.5602	0.4398	0.5783	0.4217	0.5741	0.4259	0.5688	0.4313	0.5875	0.4125
rs1031825	20	**A**/C	0.3333	0.6667	0.3194	0.6806	0.3193	0.6807	0.4074	0.5926	0.3	0.7	0.325	0.675
rs445251	20	C/**G**	0.5995	0.4005	0.5833	0.4167	0.6145	0.3855	0.6481	0.3519	0.5563	0.4438	0.6	0.4
rs1005533	20	A/**G**	0.4769	0.5231	0.4861	0.5139	0.4759	0.5241	0.5062	0.4938	0.475	0.525	0.4375	0.5625
rs1523537	20	C/**T**	0.397	0.603	0.3704	0.6296	0.4759	0.5241	0.4321	0.5679	0.3625	0.6375	0.35	0.65
rs722098	21	A/**G**	0.647	0.353	0.6528	0.3472	0.6747	0.3253	0.5617	0.4383	0.7375	0.2625	0.6063	0.3938
rs2830795	21	**A**/G	0.7373	0.2627	0.7176	0.2824	0.7289	0.2711	0.7346	0.2654	0.75	0.25	0.7625	0.2375
rs2831700	21	**A**/G	0.5775	0.4225	0.6204	0.3796	0.6265	0.3735	0.5802	0.4198	0.5688	0.4313	0.475	0.525
rs914165	21	A/**G**	0.4931	0.5069	0.4537	0.5463	0.5	0.5	0.5679	0.4321	0.4125	0.5875	0.5438	0.4563
rs221956	21	C/**T**	0.6331	0.3669	0.6389	0.3611	0.6145	0.3855	0.6111	0.3889	0.625	0.375	0.675	0.325
rs733164	22	A/**G**	0.3345	0.6655	0.3657	0.6343	0.3133	0.6867	0.3272	0.6728	0.35	0.65	0.3063	0.6938
rs987640	22	A/**T**	0.4259	0.5741	0.4352	0.5648	0.3976	0.6024	0.3951	0.6049	0.4563	0.5438	0.4438	0.5563
rs2040411	22	A/**G**	0.6725	0.3275	0.6806	0.3194	0.6145	0.3855	0.6605	0.3395	0.6625	0.3375	0.7438	0.2563
rs1028528	22	**A**/G	0.6389	0.3611	0.6528	0.3472	0.6566	0.3434	0.6049	0.3951	0.7125	0.2875	0.5625	0.4375

Supplementary Table S5. Pairwise F_{ST} and exact differentiation test for Brazilian regions based on allele frequencies of 90 autosomal SNPs included in HID Ion Ampliseq Identity Panel. Differentiation tests are presented in lower-left diagonal as p-values, while upper-right diagonal exhibits F_{ST} values.

Population	*South*	*Southeast*	*Northeast*	*North*	*Center-West*
South	*	0.00143	0.00525	0.00024	0.0035
Southeast	0.12654	*	0.00179	0.00096	0.0013
Northeast	0.04001	0.53635	*	0.00672	0.00237
North	0.11608	1.0000	0.58444	*	0.00187
Center West	0.11782	1.0000	0.47637	1.0000	*

Supplementary Table S6. Forensic Parameters of 90 autosomal ID-oriented SNP markers included in HID Ion Ampliseq Identification Panel for Brazilian national and regional populations.

Locus	Ho						He		
	Brazil	*South*	*Southeast*	*Northeast*	*North*	*Center West*	*Brazil*	*South*	*Southeast*
rs1005533	0.50462963	0.509259259	0.493975904	0.543209877	0.525000000	0.450000000	0.499506459	0.501937984	0.501861993
rs10092491	0.502314815	0.398148148	0.590361446	0.493827160	0.512500000	0.550000000	0.491242329	0.466106804	0.484337349
rs1015250	0.412037037	0.416666667	0.349397590	0.469135802	0.350000000	0.475000000	0.407782928	0.381352283	0.408397225
rs1024116	0.474537037	0.490740741	0.397590361	0.456790123	0.487500000	0.537500000	0.499396485	0.489879414	0.492515517
rs1028528	0.444444444	0.453703704	0.421686747	0.444444444	0.400000000	0.500000000	0.461954423	0.455426357	0.453669222
rs1031825	0.439814815	0.416666667	0.373493976	0.444444444	0.500000000	0.475000000	0.444959444	0.436821705	0.437312888
rs10488710	0.493055556	0.509259259	0.433734940	0.469135802	0.487500000	0.562500000	0.476371722	0.455426357	0.494194962
rs10495407	0.460648148	0.546296296	0.433734940	0.296296296	0.475000000	0.525000000	0.432768443	0.470930233	0.391967871
rs1058083	0.458333333	0.509259259	0.409638554	0.469135802	0.437500000	0.450000000	0.475859405	0.486778639	0.476670318
rs10773760	0.520833333	0.481481481	0.590361446	0.518518519	0.550000000	0.475000000	0.481199841	0.488372093	0.492515517
rs10776839	0.486111111	0.500000000	0.469879518	0.382716049	0.512500000	0.562500000	0.498766147	0.502153316	0.503030303
rs1109037	0.518518519	0.527777778	0.457831325	0.518518519	0.525000000	0.562500000	0.499892708	0.502282515	0.499452355
rs12997453	0.465277778	0.472222222	0.409638554	0.444444444	0.462500000	0.537500000	0.466329235	0.455426357	0.470828770
rs13218440	0.502314815	0.490740741	0.469879518	0.432098765	0.600000000	0.525000000	0.491242329	0.486778639	0.488718510
rs1335873	0.449074074	0.472222222	0.506024096	0.370370370	0.400000000	0.487500000	0.484260332	0.466106804	0.494194962
rs1355366	0.439814815	0.453703704	0.457831325	0.407407407	0.437500000	0.437500000	0.472672847	0.479543497	0.486600949
rs1357617	0.377314815	0.379629630	0.409638554	0.234567901	0.462500000	0.400000000	0.376590597	0.415116279	0.368017525
rs1360288	0.388888889	0.435185185	0.409638554	0.296296296	0.425000000	0.362500000	0.411731256	0.422695952	0.403066813
rs1382387	0.474537037	0.453703704	0.506024096	0.506172840	0.450000000	0.462500000	0.454548625	0.422695952	0.486600949
rs1413212	0.481481481	0.500000000	0.445783133	0.493827160	0.425000000	0.537500000	0.433618729	0.418949182	0.445783133
rs1454361	0.493055556	0.527777778	0.445783133	0.432098765	0.562500000	0.487500000	0.487809	0.489879414	0.498357065
rs1463729	0.5	0.537037037	0.506024096	0.506172840	0.462500000	0.475000000	0.478852839	0.498018949	0.464403067
rs1490413	0.490740741	0.564814815	0.518072289	0.493827160	0.400000000	0.450000000	0.49670615	0.502282515	0.500401606
rs1493232	0.490740741	0.444444444	0.506024096	0.530864198	0.500000000	0.487500000	0.473756491	0.468561585	0.486600949

Supplementary Table S7. Pairwise linkage desequilibrium p-values among 90 autosomal ID-oriented SNP markers included in HID Ion Ampliseq Identity Panel for I

Locus	rs1490413	rs7520386	rs4847034	rs560681	rs10495407	rs891700	rs1413212	rs876724	rs1109037	rs993934	rs12997453
rs1028528	0,0121	0,1671	0,0218	0,9953	0,0273	0,9068	0,6448	0,1272	0,8847	0,1698	0,7299
rs2040411	0,0783	0,4592	0,3064	0,4919	0,3046	0,7792	0,1711	0,8586	0,3643	0,6706	0,9118
rs987640	0,5885	0,1261	0,5331	0,7127	0,3369	0,9362	0,1180	0,2204	0,8668	0,4527	0,0098
rs733164	0,0353	0,4586	0,1800	0,5246	0,6931	0,0206	0,3554	0,2930	0,6685	0,8731	0,3847
rs221956	0,1930	0,0896	0,3401	0,9311	0,9406	0,7620	0,4922	0,6803	0,4323	0,6896	0,0495
rs914165	0,0259	0,4479	0,9515	0,5846	0,4270	0,9317	0,8927	0,3062	0,1239	0,6426	0,7143
rs2831700	0,8320	0,6369	0,9885	0,0548	0,3493	0,7120	0,3287	0,0988	0,8788	0,9609	0,7961
rs2830795	0,5903	0,6256	0,5210	0,3682	0,4359	0,5120	0,8023	0,0138	0,0414	0,0683	0,2918
rs722098	0,2109	0,1705	0,4781	0,2619	0,9777	0,3249	0,1314	0,1318	0,2860	0,2612	0,2565
rs1523537	0,2631	0,7676	0,5349	0,5835	0,6251	0,0644	0,3188	0,2964	0,2328	0,4126	0,8537
rs1005533	0,2769	0,9509	0,1702	0,3446	0,1498	0,6124	0,3584	0,9681	0,2883	0,3094	0,0473
rs445251	0,0565	0,6760	0,4460	0,3192	0,5404	0,2886	0,5223	0,0944	0,7675	0,5620	0,3116
rs1031825	0,8130	0,3677	0,5250	0,3596	0,0033	0,5753	0,0587	0,9414	0,1671	0,8622	0,2773
rs576261	0,8836	0,6727	0,6435	0,8294	0,3821	0,1974	0,7345	0,8610	0,8108	0,8788	0,2733
rs719366	0,8710	0,8486	0,1687	0,2241	0,3894	0,6569	0,4366	0,9759	0,4786	0,5857	0,1505
rs1024116	0,6284	0,4583	0,3466	0,7337	0,9392	0,1112	0,7737	0,4873	0,0879	0,8089	0,0099
rs1736442	0,3864	0,5744	0,8973	0,3442	0,0650	0,0463	0,5305	0,6854	0,3132	0,5544	0,9154
rs9951171	0,5159	0,4949	0,1138	0,0753	0,8706	0,8000	0,9409	0,1426	0,9735	0,7768	0,9061
rs1493232	0,9036	0,4637	0,1170	0,9960	0,4197	0,1969	0,9610	0,5042	0,3182	0,0167	0,7417
rs2292972	0,3879	0,7561	0,7675	0,3284	0,5713	0,3240	0,2021	0,5706	0,6258	0,3609	0,8004
rs938283	0,1630	0,7857	0,2494	0,0979	0,0604	0,3987	0,5111	0,5243	0,6452	0,2944	0,8970
rs740910	0,2713	0,3301	0,8689	0,4258	0,9462	0,1113	0,1020	0,9896	0,9633	0,2396	0,7641
rs9905977	0,3326	0,5684	0,7553	0,7484	0,6128	0,0204	0,9331	0,5057	0,4369	0,3994	0,2796
rs1382387	0,7162	0,8645	0,0462	0,6375	0,2402	0,3883	0,6301	0,1822	0,2524	0,8933	0,0647
rs430046	0,7541	0,8128	0,6725	0,9543	0,2579	0,3479	0,6184	0,4717	0,7927	0,6329	0,0092
rs2342747	0,3986	0,4042	0,5405	0,4893	0,1583	0,8283	0,6862	0,6309	0,7597	0,0090	0,5124
rs729172	0,1070	0,5860	0,3082	0,1841	0,7141	0,8035	0,0457	0,0961	0,0807	0,0123	0,0115
rs1528460	0,2306	0,7006	0,0721	0,6177	0,1700	0,0667	0,3836	0,6416	0,5745	0,8601	0,7552
rs1821380	0,3428	0,9936	0,2284	0,2411	0,0028	0,6061	0,5820	0,5482	0,2395	0,9554	0,4154
rs2016276	0,2389	0,0233	0,2390	0,5264	0,5437	0,3913	0,3935	0,9228	0,3115	0,5297	0,0504

Supplementary Table S8. Relative contribution of each cluster in each population composition, based on Structure software analysis of 88 autosomal SNPs included in HID Ion Ampliseq Identity Panel, for K=5. Next to cluster number, associated geographical origin of higher relevance is informed. Population names and geographical origin abbreviations are listed in Supplementary Table S2.

Population	Geographic origin	Population sample size	Cluster 1 (AFR)	Cluster 2 (AMR)	Cluster 3 (EUR)	Cluster 4 (EAS)	Cluster 5 (SAS)
BRA (combined)	BRA	432	0.096	0.303	0.445	0.052	0.104
Br-S	BRA	108	0.072	0.308	0.491	0.039	0.090
Br-SE	BRA	83	0.078	0.280	0.467	0.083	0.092
Br-NE	BRA	81	0.182	0.285	0.354	0.061	0.118
Br-N	BRA	80	0.047	0.313	0.496	0.042	0.102
Br-CW	BRA	80	0.103	0.327	0.399	0.045	0.126
ACB	AFR	96	0.873	0.035	0.034	0.029	0.029
ASW	AFR	61	0.704	0.110	0.057	0.044	0.085
ESN	AFR	99	0.950	0.012	0.010	0.015	0.012
GWD	AFR	113	0.940	0.014	0.012	0.016	0.016
LWK	AFR	99	0.918	0.018	0.023	0.019	0.022
MSL	AFR	85	0.950	0.013	0.012	0.011	0.014
YRI	AFR	108	0.944	0.013	0.012	0.013	0.018
CLM	AMR	94	0.038	0.527	0.300	0.040	0.095
MXL	AMR	64	0.014	0.675	0.195	0.066	0.050
PEL	AMR	85	0.019	0.908	0.027	0.027	0.019
PUR	AMR	104	0.076	0.345	0.432	0.064	0.083
CDX	EAS	93	0.019	0.052	0.031	0.842	0.056
CHB	EAS	103	0.020	0.032	0.031	0.839	0.077
CHS	EAS	105	0.022	0.031	0.023	0.872	0.052
JPT	EAS	104	0.028	0.054	0.040	0.760	0.118
KHV	EAS	99	0.019	0.036	0.020	0.876	0.049
CEU	EUR	99	0.015	0.194	0.685	0.030	0.076
FIN	EUR	99	0.017	0.180	0.678	0.038	0.088
GBR	EUR	91	0.019	0.209	0.671	0.030	0.072
IBS	EUR	107	0.016	0.204	0.698	0.027	0.055
TSI	EUR	107	0.022	0.175	0.650	0.037	0.116
BEB	SAS	86	0.030	0.069	0.036	0.131	0.734
GIH	SAS	103	0.031	0.076	0.086	0.086	0.722
ITU	SAS	102	0.028	0.046	0.094	0.055	0.778
PJL	SAS	96	0.024	0.083	0.129	0.089	0.675
STU	SAS	102	0.032	0.044	0.058	0.064	0.803

Supplementary Table S9. MPS performance data for each individual run using HID Ion Ampliseq Identity Panel with an ION PGM Sequencer.

Run designation	*Chip model*	*Samples per run*	*Loading (%)*	*Usable reads (%)*	*Aligned reads*	*Average mapped reads per sample (sd)*	*Mean depth per locus per sample (sd)*
Chip Z	318v2	40	65	54	3,825,722	95,643 (27,680)	740x (220x)
Chip A	318v2	80	87	69	6,503,967	81,300 (26,494)	582x (197x)
Chip B	318v2	78	73	64	5,058,526	64,819 (15,917)	488x (145x)
Chip C	318v2	74	67	44	2,954,944	40,056 (13,583)	276x (102x)
Chip D	318v2	80	78	61	5,118,798	66,477 (66,851)	415x (470x)
Chip E	318v2	80	81	48	4,190,246	52,378 (8,796)	357x (63x)
Average	**-**	**-**	**72.8**	**56.7**	**4,608,701**	**66,779 (26,553)**	**476x (200x)**

Table S10. HID Ion Ampliseq Identity Panel sequencing performance on four different evaluated MPS metrics, including Locus balance, Locus strand balance, Heterozygote balance and Noise level, for all six independent runs combined. Reference allele in bold, alternative in plain text.

locus	**Chr.**	**Alleles**	**Median (min; max)**			
			Locus balance	*Locus strand balance*	*Heterozygote balance*	*Noise*
rs1490413	1	A/**G**	1.49 (0.57; 2.85)	0.47 (0.38, 0.6)	0.97 (0.19, 2.25)	0 (0; 0,01861)
rs7520386	1	A/**G**	0.87 (0.2; 1.82)	0.49 (0.38, 0.63)	2.08 (0.32, 7.23)	0 (0; 0,02767)
rs4847034	1	**A**/G	0.65 (0.16; 2.04)	0.49 (0.34, 0.63)	0.92 (0.12, 5.89)	0,0045 (0; 0,08616)
rs560681	1	**A**/G	1.14 (0.39; 2.37)	0.48 (0.4, 0.6)	1.01 (0.31, 2.98)	0 (0; 0,04553)
rs10495407	1	A/**G**	1.08 (0.42; 2.24)	0.46 (0.35, 0.95)	1.01 (0.19, 7.36)	0 (0; 0,01493)
rs891700	1	**A**/G	1.23 (0.59; 3.47)	0.48 (0.36, 0.66)	0.96 (0.16, 3.12)	0 (0; 0,02239)
rs1413212	1	C/**T**	1.02 (0.26; 1.98)	0.51 (0.36, 0.64)	1.02 (0.51, 2.04)	0 (0; 0,02703)
rs876724	2	**C**/T	0.42 (0.08; 0.89)	0.53 (0.26, 0.68)	1.61 (0.91, 5.65)	0 (0; 0,02419)
rs1109037	2	A/**G**	0.53 (0.11; 1.51)	0.49 (0.34, 0.63)	0.97 (0.19, 1.7)	0 (0; 0,04234)
rs993934	2	**A**/G	0.52 (0.08; 0.99)	0.53 (0.35, 0.7)	0.94 (0.43, 2.45)	0 (0; 0,0365)
rs12997453	2	**A**/G	0.41 (0.11; 0.78)	0.48 (0.13, 0.63)	0.95 (0.16, 5.58)	0 (0; 0,02247)
rs907100	2	C/**G**	0.67 (0.25; 1.62)	0.51 (0.32, 0.66)	1 (0.54, 1.9)	0 (0; 0,04378)
rs1357617	3	**A**/T	0.68 (0.14; 1.64)	0.49 (0.32, 0.67)	1 (0.13, 3.01)	0,00406 (0; 0,04762)
rs4364205	3	G/**T**	1.46 (0.67; 1.9)	0.57 (0.44, 0.75)	0.99 (0.21, 2.88)	0,00147 (0; 0,02597)
rs1872575	3	A/**G**	1.62 (0.72; 4.53)	0.5 (0.42, 0.71)	0.97 (0.38, 2.04)	0 (0; 0,025)
rs1355366	3	C/**T**	1.11 (0.56; 2.54)	0.52 (0.37, 0.72)	0.99 (0.15, 4.22)	0 (0; 0,06102)
rs6444724	3	C/**T**	0.86 (0.24; 2.17)	0.48 (0.14, 0.65)	1.05 (0.68, 5.19)	0 (0; 0,03333)
rs2046361	4	A/**T**	0.88 (0.12; 3.52)	0.48 (0.39, 0.61)	1.01 (0.51, 2.93)	0 (0; 0,05618)
rs6811238	4	G/**T**	1.15 (0.65; 2.03)	0.48 (0.36, 0.61)	0.98 (0.5, 1.78)	0,00085 (0; 0,02154)
rs1979255	4	**C**/G	0.88 (0.21; 1.58)	0.5 (0.39, 0.59)	1 (0.13, 1.63)	0 (0; 0,03366)
rs717302	5	A/**G**	0.85 (0.18; 1.72)	0.54 (0.05, 0.76)	0.71 (0.17, 1.16)	0 (0; 0,02459)
rs159606	5	**A**/G	0.81 (0.33; 1.26)	0.54 (0.4, 0.65)	0.97 (0.12, 1.97)	0 (0; 0,02303)
rs7704770	5	A/**G**	0.97 (0.49; 2.18)	0.49 (0.36, 0.69)	1.01 (0.17, 8.17)	0 (0; 0,02983)
rs251934	5	**A**/G	1.04 (0.31; 1.61)	0.51 (0.42, 0.65)	1.03 (0.5, 4.85)	0 (0; 0,03307)
rs338882	5	A/**G**	1.56 (0.28; 4.56)	0.52 (0.39, 0.67)	0.99 (0.17, 5.36)	0 (0; 0,03807)
rs13218440	6	A/**G**	1.25 (0.47; 2.18)	0.5 (0.39, 0.6)	0.91 (0.55, 3.78)	0 (0; 0,01373)
rs214955	6	**C**/T	0.38 (0.05; 0.82)	0.54 (0.21, 0.77)	1.48 (0.11, 5.17)	0 (0; 0,05556)

CAPÍTULO 3 - Brazilian forensic casework analysis through MPS applications: Statistical weight-of-evidence and biological nature of criminal samples as an influence factor in quality metrics

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Brazilian forensic casework analysis through MPS applications: Statistical weight-of-evidence and biological nature of criminal samples as an influence factor in quality metrics

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ABSTRACT

Real forensic casework biological evidence can be found in a myriad of different conditions and presenting very distinct features, including key elements such as degradation levels, the nature of biological evidence, mixture presence, and surface or substrate deposition, among others. Technical protocols employed by forensic DNA analysts must consider such characteristics in order to improve the chances of successfully genotyping these materials. MPS has been used as a very useful tool for forensic sample processing and genetic profile generation. However, it is not completely clear how different features encountered with real forensic samples impact sequencing quality and, consequently, profile accuracy and reliability. In this context, the present study analyzes a set of 47 real forensic casework samples, obtained from semen, saliva, blood and epithelial evidence, as well as reference oral swabs, aiming to evaluate the impact of a sample's biological nature in profiling success. All DNA extracts from samples were standardized according to sample conditions, as assessed by traditional forensic profiling methods (real-time PCR quantitation and capillary electrophoresis-coupled STR fragment analysis). Samples were separated into groups according to their biological nature, and the resultant sequencing quality was evaluated through a series of well-established statistical tests, applied specifically to six different MPS quality metrics. The results showed that certain groups of samples, especially epithelial and (to a lesser extent) saliva samples, exhibited significantly lower quality in terms of some of the evaluated metrics. A number of reasons for such unexpected behavior are discussed. In addition, a series of calculations was performed to assess the weight of genetic evidence in Brazilian samples, and reflexes in data analysis and national allele frequency database construction are discussed. Overall, the results indicate that a unified national allele frequency database can be used nationwide. Besides this, MPS genetic profiles obtained from samples with particular biological origins may benefit from meticulous manual review, and visual inspection could be important as an additional step to avoid genotyping errors or misinterpretation, leading to more trustworthy and reliable results in real criminal forensic casework analysis.

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1. Introduction

The highly variable aspects associated with biological evidence collected from crime scenes or other samples with forensic interest pose a potential challenge to efficient processing of such materials [1]. In order to generate DNA profiles with appropriate quality and necessary attributes to be admitted in court as genetic evidence for human identification or be inserted in genetic databases, forensic DNA analysis must deal with a huge variety of different biological tissues, fluids, secretions and materials, as well as a plethora of objects or deposition surfaces where such evidence can be collected [2]. All these factors can affect the levels and quality of obtained DNA to be used in genetic human identification methods as features like DNA levels can greatly vary among different biological materials [3]. Furthermore, real forensic casework analysis usually involves handling samples where phenomena, like low template DNA (also called low-copy numbers, or LCN), poor quality DNA owing to degradation or

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environmental exposure to elements, presence of inhibitors or occurrence of distinct contributor mixtures in biological evidence, can considerably impact genetic profile quality [4,5]. Hence, a proper appraisal of how these inconsistencies can influence genetic profiles is crucial, aspiring to satisfy quality standards in genetic data generation for forensic purposes.

In order to overcome technical issues limiting the ability to properly process biological evidence, especially those presenting critical conditions or other characteristics that notoriously hamper genetic profile yield or interpretation, or possibly leading to statistical weight of evidence decrease, alternative methodological solutions have been proposed as possible solutions for challenging samples [6]. When compared to traditional forensic human identification DNA methods, which employ Short Tandem Repeat (STR) evaluation coupled with Capillary Electrophoresis (CE) fragment analysis, possible approaches include analysis of different genetic markers sets, evaluation of alternative DNA polymorphisms types, improvements in kit chemistry leading to higher sensitivity or tolerance to inhibitors, as well as introduction of technological alternatives to standard methods, specifically regarding equipment for DNA sequencing or genotyping and software used in data processing and analysis [4,7]. In this context, the HID-Ion AmpliSeq Identity Panel (Thermo Fisher Scientific, Waltham, USA) commercial solution is presented as an option for criminal forensic casework processing and profiling. This reagent comprises simultaneous amplification and evaluation of 90 autosomal biallelic single nucleotide polymorphisms (SNPs) specially selected to provide enough discrimination power for individual identification based on presentation of high heterozygosity levels in different worldwide populations [8,9]. In addition, a set of 34 Y upper-clade SNPs was included in the kit, aiming to identify male lineages associated with masculine samples. This kit was designed to be utilized in Massive Parallel Sequencing (MPS) platforms, where sequence variations can be assessed on an individual nucleotide basis.

MPS technologies offer advantages for forensic use, such as high-throughput capacity, quick and efficient simultaneous typing of a large number of samples and forensic markers through barcoding approaches, and possibility of workflow automation, among others [10]. The HID-Ion AmpliSeq Identity Panel, in particular, features a peculiar design especially conceived to aid in human identification forensic procedures, where amplified fragments possess relative small sizes when compared to typical STR markers. This unique trait allows successful analysis of highly degraded, low-input, critical condition DNA, which might be, otherwise, unable to generate a robust quality genetic profile [11]. Several validation studies were conducted to appraise key features of this panel, as sensitivity, specificity, reproducibility, accuracy, and ability to handle samples with conditions such as low DNA input, mixtures or degraded DNA [12–14]. In addition, population data regarding genetic markers included in the panel were determined for a relevant number of worldwide populations [11,13,15–20]. Reported conclusions for the studies described indicate robust, efficient and reliable typing of forensic samples could be accomplished with different versions of this product. Intra- and inter-population analysis also determined a high degree of genetic variability among tested individuals, emphasizing the adequacy of this chemistry's use in forensic individual identification or parentage testing. However, most published results are based on data generated from pristine condition DNA, like commercially available control DNA, NIST standards or reference, single-source samples. In addition, investigations reporting mixture or degraded sample evaluations were designed through controlled environments, employing mixtures with known contributor ratios or artificially degraded DNA, to infer effects in amplification performance [12–14,21]. A limited number of studies actually describe results from real forensic casework [21] obtained with the present kit, but some questions related to the forensic origin of samples remain to be more thoroughly investigated - those related to the number of unaligned sequences (probably related to bacterial or other organisms from environmental contamination of real criminal samples) or variations associated with the biological evidence's place of collection (as the substrate or surface of deposition where such samples were deposited) [21].

In this study, we applied a series of well-known statistical tests to evaluate the effect of the nature of biological evidence and biological origin of real forensic criminal casework DNA in obtained results' quality, indicated by different sequencing or base-calling quality metrics associated with each produced genetic profile. To achieve this, criminal samples collected from real crime scenes and identified as blood, saliva, semen or epithelial evidence were grouped together, and effects of evidence type on obtained sequences were estimated. In order to eliminate or minimize effects of other possible interfering elements associated with samples, such as degradation levels or mixture occurrence, samples were normalized according to characteristics presented by their Real-Time PCR (RT-PCR) quantitation and degradation level results, as well as correspondent STR genetic profile, generated by fragment size analysis through traditional CE applications. All samples employed were submitted to identical procedures across all workflow phases as there is evidence that adoption of different methods and protocols in criminal evidence-sample processing can directly affect MPS results as evidenced by impact analysis in MPS quality results in steps as diverse as DNA extraction [22], amplification [23,24], chip-based sequencing [25,26] or data analysis [27]. Seeing that all investigated samples were actual criminal cases investigated by the Brazilian Federal Police (BFP) regional DNA laboratory, a basic evaluation of obtained results was also performed concerning genetic weight of evidence, taking into consideration recently reported population data and allele frequency for Brazilian national and regional populations [20]. In this analysis, generated profiles were used to estimate Random Match Probabilities (RMP) or Likelihood Ratios (LRs) of criminal samples, not only for Brazilian national frequencies but also regional and even worldwide populations. A discussion of the obtained results and their association with possible genetic stratification of the Brazilian population, in light of previously described Brazilian population features, is also presented.

2. Materials and methods

2.1. Ethical statement

All reference, known-origin, single-source samples analyzed in this study were voluntarily obtained following written informed consent from donors, and refer to a research-oriented human samples biobank at PUC/RS. Anonymous samples were randomly selected from this bank. This work follows the ethical principles stated in the Helsinki Declaration [28] of the World Medical Association, and was approved by Pontifical Catholic University of Rio Grande do Sul Institutional Review Board under number CAAE 52113715.9.0000.5336. Criminal samples were selected among unidentified, unknown-source specimens available in the BFP regional laboratory, where no suspects had ever been identified or presented as suggested sample origins. No information regarding case or sample identification was disclosed. Individual profiles or Y-chromosome haplogroups were not reported for criminal samples as they might express, at least to some extent, biogeographical ancestry and other genetic characteristics associated with an individual who originated criminal evidence [20].

2.2. DNA samples

2.2.1. Reference samples

Ten single-source, known-origin reference samples were employed as experimental controls (CT) for the present work. All samples belonged to the same regional population as criminal samples, and were collected from internal oral mucosa swabs. This collection method was selected owing to being the choice method for reference DNA collection according to Brazilian criminal forensic doctrine [29]. Two sterile, DNA-free dry swabs were utilized for sampling of internal oral mucosa, one for each cheek. DNA extraction was performed with a single swab only, per individual.

2.2.2. Criminal samples

Thirty-seven criminal samples were chosen among forensic casework counter-samples available in the BFP regional laboratory. Samples were randomly selected among those that fulfill the following criteria: a) no suspect or individual was proposed as connected to the case in question; b) the crime scene was processed over the period between 2014 and 2016; c) samples were previously evaluated by STR analysis using CE methods in the laboratory, specifically with commercial kits, and a DNA report was produced as a result; d) biological evidence generated a single-source, complete DNA profile based on STR markers; e) quantity of extracted DNA from evidence was sufficient to assure at least 0.5 ng of DNA per μL, as assessed by Real-Time PCR (RT-PCR) quantitation; f) samples should have minimal to null levels of degradation and PCR inhibition, according to applied techniques.

Samples satisfying the selection criteria were randomly selected according to their biological nature as follows: 10 samples each for blood (BL), saliva (SA) and epithelial or touch DNA (EP) evidence cases, and 7 samples containing semen (SE) biological evidence. Based on the national legal and criminal system structure (where a restricted number of violent crimes are considered federal jurisdiction), the BFP has a very limited number of cases involving rape, sexual assault or other criminal activity involving semen evidence. Therefore, a lesser number of semen evidence samples was available at the laboratory, all of them obtained from ejaculates collected from used condom interiors. The biological origin of each sample was determined according to information stated in the original crime scene documentation referring to each case, exactly as reported by the crime scene expert attending the scene or by the serology laboratory. No additional presumptive or confirmatory tests were performed to ratify the evidence considered in this study.

Following sample selection, analysis of STR profile obtained by CE was executed. STR profiles presenting overall lower *rfu* signals (classified by at least half of observed allele signals presenting peak heights inferior to 4x analytical thresholds (AT) settings) were categorized as low-signal samples. Samples presenting at least three discernible peaks with sizes between half AT and AT values (and, as such, not treated as real allele signals) were classified as low-level mixtures, where the major contributor is easily identified. Such samples were presented as mixture samples even though minor contributor allelic peaks did not reach AT, and, as such, not classified as an actual mixture for STR profiles. A comprehensive description of all selected samples, including the nature of biological evidence, collection method employed, surface or substrate where evidence was deposited and additional characteristics presented by samples is located in Table 1.

2.3. DNA extraction and quantitation

Genomic DNA was extracted from all samples using the automated platform, Automate Express Forensic DNA Extraction System (Thermo Fisher Scientific Inc., Waltham, USA) with the Prepfiler Automated Forensic DNA Extraction System (Thermo Fisher Scientific Inc.). DNA extracts were quantified using the Quantifiler Trio Human DNA Quantification Kit (Thermo Fisher Scientific Inc.) on a 7500 Real-Time PCR System (Thermo Fisher Scientific Inc.). All procedures followed the manufacturer's instructions, with elution volumes of 50 μL. A sample was considered not degraded/inhibited when the degradation index (DI) <1.5 and no IPCCT flag was triggered in Quantifiler Trio assay.

2.4. STR fragment analysis

Criminal samples were submitted to STR fragment analysis including 22 autosomal STR markers, one Y-chromosome STR locus and amelogenin for gender determination. STR analysis was executed using commercial reagent PowerPlex Fusion System (Promega Corp. Madison, USA). Fragment sizes were determined by CE in a 3500 Genetic Analyzer (Thermo Fisher Scientific Inc.) and GeneMapper Id-X v.2 software was used for DNA profile evaluation. All analyses followed the manufacturers' protocols. Quantity of extracted DNA from criminal samples used in each PCR reaction was equivalent to 0.5 ng.

2.5. MPS library preparation, emulsion PCR and sequencing

DNA libraries were constructed using the Ion AmpliSeq Library Kit 2.0 (Thermo Fisher Scientific Inc.) combined with the HID-Ion AmpliSeq Identity Panel (Thermo Fisher Scientific Inc.), following the manufacturer's suggested protocol (HID-Ion Ampliseq Library Preparation, Revision C.0). Briefly, PCR amplification was performed in a final volume of 20 μL that contained 1 ng of template DNA, 4 μL of 5x Ion AmpliSeq™ HiFi Mix and 10 μL of 2x Ion AmpliSeq™ primer pool (Identity Panel). Final volumes were adjusted with nuclease-free water. PCR was performed in a Veriti 96-well Thermal Cycler (Thermo Fisher Scientific Inc.) under the following conditions: enzyme activation for 2 min at 99 °C, 21 cycles of 15 s at 99 °C and 4 min at 60 °C, followed by a 10 °C hold. PCR amplicons were partially digested with 2 μL FuPa reagent, with incubation thereafter at 50 °C for 10 min, 55 °C for 10 min and 60 °C for 20 min, followed by a 1 h hold at 10 °C. Ligation of adaptors to the libraries was conducted based on the manufacturer's instructions using different barcodes (Ion Xpress Barcode Adaptors 1–96 Kit or IonCode Barcode Adapters 1–384 Kit) for each sample in the same run (Thermo Fisher Scientific Inc.), and resulting products were purified using Agencourt AMPure XP reagents (Beckman Coulter Inc., Brea, USA) according to the manufacturer's protocol. After purification, libraries were quantified using a 7500 Real-Time PCR System (Thermo Fisher Scientific Inc.) with the Ion Library TaqMan™ Quantitation Kit (Thermo Fisher Scientific Inc.). All samples generated high-quality libraries, which were pooled to a final concentration of 20 pM. Emulsion PCR (emPCR) was performed on the Ion OneTouch 2 instrument (Thermo Fisher Scientific Inc.) with the Ion PGM Hi-Q Template Kit (Thermo Fisher Scientific Inc.) following the manufacturer's protocol (Ion PGM Hi-Q Template Kit, Revision A.0). The emPCR products were enriched on the Ion OneTouch Enrichment System (Thermo Fisher Scientific Inc.). A final volume of 30 μL was loaded per chip. Sequencing was carried out on the Ion PGM™ (Thermo Fisher Scientific Inc.) Sequencer with the Ion PGM Hi-Q Sequencing Kit (Thermo Fisher Scientific Inc.) following the manufacturer's instructions (Ion PGM Hi-Q Sequencing Kit, Revision B.0) and using the Ion 318 Chip Kit v2 (Thermo Fisher Scientific Inc.). All samples were evaluated in a single run, in a chip loaded to maximum capacity (77 distinct samples, not all of them included in present study), as suggested by the manufacturer.

Table 1
Criminal samples used in this study and their descriptive data.

Sample Id	Evidence Features					
	Type	State	Method	Surface/Substrate	Location	Classif.
BL.07	Blood	Patent	SC	Cotton t-shirt	Dry stain in sleeve	Normal
BL.09	Blood	Patent	WS	Shattered Glass	Dry stain	Normal
BL.11	Blood	Patent	DS	Laptop plastic shell	Wet stain	Normal
BL.20	Blood	Patent	DS	Metalic car door surface	Wet stain	Normal
BL.22	Blood	Patent	WS	Rendered brick wall	Dry stain	Normal
BL.24	Blood	Latent*	SC	Car Internal rooftop	Stain on headliner	LS
BL.25	Blood	Patent	WS	Glass window	Dry stain	Normal
BL.26	Blood	Patent	DS	Basalt pavement	Wet stain on sidewalk	Normal
BL.27	Blood	Patent	DS	Keramic tiles	Wet stain on wall	Normal
BL.30	Blood	Patent	DS	Keramic pavement	Wet stain on floor	Normal
EP.01	Epithelial	Latent	SC	Sports cap	Inner sweatband	Normal
EP.08	Epithelial	Patent	WS	Glass Surface	Smudged fingerprint	Normal
EP.10	Epithelial	Latent	SC	Cotton male underware	Inner waistband	Normal
EP.13	Epithelial	Latent	WS	Survaillance camera	Body	Mix
EP.14	Epithelial	Latent	WS	Laptop power cable	Connector jack	Mix
EP.15	Epithelial	Latent	WS	Car door handle	Inner side	Normal
EP.17	Epithelial	Latent	WS	Metal padlock	Shackle	Normal
EP.18	Epithelial	Latent	SC	Cotton bath towel	Lining stitches	Normal
EP.21	Epithelial	Latent	DS	Metalic nail	Shank	Normal
EP.28	Epithelial	Latent	WS	Plastic surface	Smudged fingerprint	LS
SA.02	Saliva	Latent	WS	PET bottle	Inner and outter finish	Normal
SA.03	Saliva	Latent	SC	Cigarrete butt	Tipping paper	Normal
SA.04	Saliva	Latent	WS	PET bottle	Inner and outter finish	Mix
SA.05	Saliva	Latent	WS	Stainless steel mug	Mug edge	Mix
SA.06	Saliva	Latent	SC	Cigarrete butt	Tipping paper	Normal
SA.12	Saliva	Latent	WS	PET bottle	Inner and outter finish	Normal
SA.16	Saliva	Latent	SC	Cigarrete butt	Tipping paper	Normal
SA.19	Saliva	Latent	SC	Tootbrush bristles	Free extremity	LS
SA.23	Saliva	Latent	SC	Cigarrete butt	Tipping paper	Normal
SA.29	Saliva	Latent	WS	Dry chewing gun	External surface	LS/Mix
SE.02	Semen	Patent	DS	Used condom	Internal ejaculate	Normal
SE.03	Semen	Patent	DS	Used condom	Internal ejaculate	Normal
SE.07	Semen	Patent	DS	Used condom	Internal ejaculate	Normal
SE.22	Semen	Patent	DS	Used condom	Internal ejaculate	Normal
SE.26	Semen	Patent	DS	Used condom	Internal ejaculate	Normal
SE.27	Semen	Patent	DS	Used condom	Internal ejaculate	Normal
SE.30	Semen	Patent	DS	Used condom	Internal ejaculate	Normal

* Luminol revealed stain; SC = substrate cutting; WS = wet swab; DS = dry swab; LS = Low signal; Mix = Mixture.

2.6. MPS data analysis

Sequencing data were analyzed using the Torrent Suite Software v5.0 (Thermo Fisher Scientific Inc.)). Hg19 was employed as reference genome data. The number of mapped reads was calculated by the Torrent Coverage Analysis v5.0 plugin (Thermo Fisher Scientific Inc.). SNP genotypes were called by the HID SNP Genotyper v4.3.1 plugin, with target regions file (iiSNPv3_FP. hotspot) and the hotspot regions file (IISNPv2.20140429.Designed) under default analysis settings. Minimum coverage was set to 6 reads per base position, and a minor allele standard threshold of 10% was utilized for heterozygous calls. All SNP genotypes were independently reviewed by two different collaborators with manual corrections applied.

2.7. Statistical data analysis

2.7.1. Weight of genetic evidence

All statistical analysis was performed according to BFP internal routine procedures. Weight of genetic evidence is reported for criminal samples as likelihood ratios (LRs) for a traditional match hypothesis following a logical Bayesian approach [30] or RMP in reference to a specific population. Population substructure correction was applied for data generated in our laboratory, with a 0.01 value for theta index. LR calculations were executed with validated, internal use computational tools and confirmed with Familias software v.3.2.2 [31] for STR markers only. Population data for STR [32–34] and SNP [20] markers were used in accordance with current BFP technical protocols.

2.7.2. MPS performance metrics assessment

Statistical data analysis and reports concerning quality metrics results consolidation were performed according to previously reported techniques [18,20]. HID-Ion AmpliSeq™ Identity Panel sequencing performance was evaluated using six different descriptive parameters [13,18,20]: Read Depth (RD), reported as the number of reads for each marker; Locus Balance (LB), which assesses individual locus amplification efficiency and is calculated as the read depth of a locus divided by the mean read depth of all loci, per sample. As Y-chromosome markers are expected to have around half the autosomal read numbers, LB values were assessed independently for autosomal and Y SNPs; Locus Strand Balance (LSB) measures the balance between forward and reverse reads and is expressed as the number of forward reads divided by the total reads number; Heterozygote Balance (HB) estimates the ratio between two distinct allelic read counts in heterozygous samples, and it was plotted as the number of reads of the first base call in alphabetical order (A, C or G) divided by the number of reads of the alternate allele (C, G or T, respectively). For LB, LSB and HB, calculations were performed not with actual measured estimates, but with the absolute residual value of each read, where employed predicted value was defined as the ideal value for each metric in a fully balanced system (equivalent to 1, 0.5 and 1, respectively). Noise Level (NL) represents the amount of unspecific base call,

calculated as the ratio of non-allele calls divided by total read depth. Finally, QS (Quality Score) is a phred-scale marginal (unconditional) probability of the called genotype, and can be found in plugin results as a GQ (General Quality) measure. These six particular metric indexes were chosen among other possible options because they are either directly indicated by analysis and data reporting plug-in (some of them are even flagged when values are below selected thresholds) or easily assessed by simple calculations based on results presented in the plugin main tab. In addition, same measures were employed to evaluate panel performance in validation studies [12–14]. Additional accessory metrics were directly obtained from plugin reports, nominally Reads on Target (RT), equivalent to the ratio of total reads successfully mapped to any targeted sequence of the human reference genome, and Mean Read Length (MRL), described as the average size (in base pairs) of mapped amplicons, per sample.

For each quality metric, per sample data was consolidated in a single value, comprised by the mean value of a particular metric for all markers. Such an approach was opted for in order to aggregate the performance information into one single value. Once markers are expected to perform similarly among samples (considering sample conditions are similar and inter-marker variation seems to be dependent on panel design [12–14]), and heterozygosis occurrence probability for samples and markers is random within the boundaries of population allele frequencies, the mean value is expected to merge all markers' information with no significant bias.

2.7.3. Statistical analysis

All calculations were executed using IBM® Statistical Package for the Social Sciences (SPSS®), version 22. Single-factor analysis of variance (ANOVA) tests were performed for genetic weight-of-evidence in the form of samples' RMP values to verify if resulting values differ for regional Brazilian populations [20] and across worldwide frequency databases. The same statistical test was applied to evaluate accessory descriptive statistics data (nominally RMP and MRL) in order to identify difference among groups.

Quality metrics were analyzed by the Kruskal–Wallis (KW) and Median test, both coupled with Dunn-Bonferroni *Post-Hoc* analysis (where pairwise differences are evaluated for all groups with the goal of verifying which sample type presents significant difference from each other). These methods were employed to compare the distribution among samples and median measurement among samples, respectively. Results with *p-values* < 0.05 were considered statistically significant, except in *post-hoc* cases, where Bonferroni correction was applied. Outlier samples, classified as far-out extreme values (differing from median at least 3x the IQR (inter-quartile range) value) or out values (different from median in the 1.5x to 3x IQR range), were identified for each evaluated metric. This samples were not included in the correspondent statistical hypothesis test.

KW testing checks the null hypothesis of equal distribution among classes (grouped according to sample nature), while the median test verifies if groups' median are significantly different from the gran median (GM), i.e., the median of all combined samples. Although KW and Median testing may seem redundant, they both reflect distinct features, with both central tendency and variance established. In addition, both tests were applied once KW was more sensitive to outliers, but also more powerful in the absence of such anomalies. Therefore, this simultaneous analysis was performed to avoid bias owing to spurious data. Additionally, KW/Median tests were chosen rather than ANOVA once the obtained data did not have enough evidence of normal distribution among all variables. Bonferroni correction after the Dunn test constitutes a conservative approach regarding the rejection of the null hypothesis. That means our analysis is more prone to Type II errors (when the test does not reject a null hypothesis that should be rejected). Such course of action is employed based on the exploratory nature of this study, where findings require stronger evidence to refute the current understanding of the forensic interpretation of MPS genotyping results regarding real casework samples, where evidence of differences in performance for distinct types of criminal samples has not been previously systematically proposed.

3. Results and discussion

The HID Ion Ampliseq Identity Panel successfully generated genetic profiles for all 37 criminal Brazilian samples, as well as for 10 control reference samples included in the present study. All samples were both collected and analyzed in Brazil's southernmost state (Rio Grande do Sul), and, as such, were considered as belonging to the Southern Brazilian geopolitical regional population [20]. A complete description of samples evaluated in this study, including features like nature of biologic evidence, collection method, surface or substrate where evidence was found, as well as associated weight of genetic evidence related to obtained SNP and STR profile features, is presented in Table 1. Even though this kit was specifically designed to properly handle critical or difficult forensic samples, through a strategy of small-size amplicon usage, this study focused on regular forensic DNA cases, where appropriate amounts of low-degradation DNA were extracted from criminal biological evidence of different origins. All criminal casework samples generated genetic profiles where most genotypes were considered reliable and presenting enough quality to be used in forensic match comparisons. However, distinct performance in quality metrics was observed for different types of biological evidence, and implications of these findings will be discussed in this paper.

3.1. Comparative evaluation of statistical weight of evidence for Brazilian populations

Ninety autosomal SNP markers genotypes of all 47 Brazilian criminal and reference samples were used for determination of the statistical weight of genetic evidence generated with present panel use on the Ion PGM MPS platform. Results were compared with equivalent descriptive statistics obtained from traditional STR fragment analysis for profiles assessed through CE applications. When considered Brazilian national allele frequencies, hypothetical simulations of match exams between sample genotypes and their identical, fully equivalent profiles were calculated. Average LR for all criminal samples was estimated at 1.0715×10^{39} (s.d. = $\pm 2.1345 \times 10^{39}$) when the 90-SNP marker set was considered. These results show that SNP markers largely outperform alternative traditional STR method counterparts, where the obtained average LR for 22 autosomal microsatellite markers is equivalent to 8.6135×10^{30} (s.d. = $\pm 2.1282 \times 10^{31}$). Differences in LR for both marker sets, for each sample, ranged from less than 6 to over 13 orders of magnitude, with SNP markers consistently presenting superior LR values. Supplementary Table S1 presents individual SNP- and STR-simulated LR values for each criminal sample evaluated. It has been previously proposed that inclusion of SNP polymorphism results in addition to STR marker genotypes significantly improves statistical robustness of biological relatedness tests for variant kinship degrees [35]. In the present paper, the obtained results suggest that autosomal SNP markers included in the present panel are able to provide far superior genetic weight of evidence in criminal sample-suspect match cases than standard applied forensic methods (STR markers coupled with CE fragment analyses) in Brazilian criminal casework. Such results can even be improved in terms of resulting likelihood ratios, with inclusion of Y-haplotype matching probabilities in overall

calculations [36,37] as the Ion HID Ampliseq Identity Panel also includes 34 high-clade Y-chromosome SNPs which can also enhance statistical conclusions.

Brazilian national and regional allele frequencies were described in a previous study for SNP markers included in the panel discussed in this paper [20]. No evidence of strong regional genetic stratification in these particular SNP marker set was reported, as reflected by low fixation indexes (represented by F_{st} values and other descriptive statistics). In order to verify if significant disparities would be observed in statistical results when biological evidence is assumed to come from different territorial regions (possessing, therefore, alternative geographical origins), we evaluated RMP values considering allele frequencies for the whole country (national database) or specific geopolitical regions (regional databases). In addition, worldwide populations based on the 1000-Genomes Project [38] were used to leverage RMP results for populations with diverse continental biogeographical origin, also based on their individual allele frequencies. RMP values for these continental populations are depicted as presented by the plugin, and therefore might feature some levels of skewing owing to not using theta for substructure correction, as oppose to what is employed in Brazilian population calculations.

The resulting RMP values show that no significant differences in RMP values are obtained when the individual originating evaluated criminal evidence is assumed to come from Brazilian national or regional populations when considering allele frequencies proposed for such groups. Fig. 1 and Supplementary Table S2 display complete RMP estimates for all 37 criminal and 10 reference samples based on Brazilian and worldwide population frequency databases, including 88 autosomal SNP markers included with the HID Ion Ampliseq Panel. Fig. 1A depicts RMP values for worldwide and Brazilian national populations, while Fig. 1B portrays a similar scenario, including Brazilian national and regional populations exclusively. The average value for the difference between the largest and smallest RMP considering Brazilian populations (national and regional) is 2.6399×10^{-34}, which is over five orders of magnitude smaller than the same estimation based on RMP values available for worldwide populations, equivalent to 1.0876×10^{-28}. In a similar analysis, ratios between per sample largest and smallest RMP values, when Brazilian national and regional frequencies are considered, are all situated within three orders of magnitude, with only less than 8.5% of samples presenting ratios above the 10^2 range. However, when the same calculations are performed based on allele frequencies of worldwide populations,

Fig. 1. Random Match Probabilities (RMP) for 47 Brazilian criminal and reference samples based on 88 autosomal SNPs included in HID Ion Ampliseq Panel. RMP values were obtained using distinct population frequencies databases (see Supplementary Table S2 for details on populations). Fig. 1A shows worldwide populations with distinct continental biogeographical origins, and Brazilian national database. Fig. 1B shows RMP values based on Brazilian national population (also included in Fig. 1A) and five distinct regional databases, equivalent to Brazilian five geopolitical regions.

differences between the smaller and largest RMP values per sample can reach over seven orders of magnitude, with 63.83% of samples showing results over 10^3. In addition, Fig. 1B depicts a very similar pattern for RMP obtained for all Brazilian populations, contrasting results presented in Fig. 1A for worldwide frequencies, where higher variation and a wider range of distribution is observed. These results show that variability of possible RMP values based on the Brazilian frequencies database is significantly smaller than observed in worldwide populations, suggesting an overall homogeneity in SNP polymorphism frequency distribution across national territory.

In order to further explore these results, variance analysis was carried out to compare RMP values for Brazilian, worldwide or totality of populations. Criminal samples included in the present study originate from cities belonging exclusively to the Southern Brazilian geopolitical region. Therefore, if a significant discrepancy in overall allele frequencies among regions can be distinguished, one would expect to observe differences in average RMP values for each region, or among RMP estimates calculated for a single sample using distinct frequency databases. In both cases, the largest RMP values are expected to be evidenced in the Southern Brazil database. Notably, smaller average RMP values for the current sample set were found to be reported for American populations. Such a fact is not surprising as modern American populations are similar to Brazilian ones, presenting high admixture levels and including a similar presence or contribution of ancestor populations [39]. One-way ANOVA for Brazilian populations resulted in no significant difference in RMP values among national and all five regional databases (*p-value* = 0.827313). The same conclusions were achieved for worldwide data (*p-value* = 0.479719) and all populations combined (*p-value* = 0.418685). Population-based evaluations of Brazilian and worldwide datasets for this particular kit have already reported appreciable differences in allele frequencies, which were robust enough to discern distinct continental origins [18,20]. Notwithstanding this, the bulk of observed genetic variation was still suggested to pertain to individual levels as expected in human identification forensic applications. Therefore, the results presented here are in agreement with such observations and support current BFP doctrine of a single national allele frequency database for use in forensic casework genetic analysis [32–34]. Even though, at the present time, statistical applications of a genetic national database have been limited to STR markers or (in rare cases) to uniparental lineages, it seems reasonable to propose that the same approach can be valid for SNP markers included in the HID Ion Ampliseq Panel. Applications of computational methods have been allocated to validate the use of a single national STR frequency database for the entire Brazilian territory, with evaluation of adjustment methods for population structure and sample size [40]. However, the proposed methods rely on extensive data availability, with specific information regarding allele frequency distribution regarding local, state, geopolitical regional or national levels. Present SNP marker frequencies should be further explored and spatially refined, including a necessary step of database expansion in both sample number and coverage area. This step is essential to appraise if proposed SNP markers for identification purposes can also be roughly considered as symmetrically distributed along Brazilian territory, and therefore suitable for unified database adoption. Further discussion on the adequacy and convenience of developing a Brazilian national SNP database has been presented in previous studies [20].

3.2. Evaluation of the nature of biological evidence's impact on quality metrics

A series of well-established statistical analyses were executed on six different quality metrics obtained through genotyping assays conducted with the Ion HID Ampliseq Identity Panel, and results for both Median and KW tests comprising all groups, as well as the *post-hoc* pairwise comparisons of all combined types of evidence are found in Table 2. Chosen metrics were selected among other possible criteria because they can be directly evaluated through analysis plugin results and provide an easily identifiable assessment of both sample and genotype quality for each genotyped polymorphism [11,12,14]. Plugin design includes quality thresholds, where sample or specific marker results not reaching previously defined values are flagged as low confidence data. In these cases, a thorough appraisal of obtained results and validation in a forensic context is necessary, including manual review of automatically generated findings for consistency verification [20,21].

Initial result evaluation of genotyping data of real criminal casework samples revealed differences in genotyping performance among samples based on their quality metrics as presented by the plugin. This result was somehow unexpected considering all samples selected for inclusion in the present research were specifically chosen to provide normalized DNA features presented by samples as revealed by their STR fragment analysis and quantitation results. Hence, all library preparation reactions included the same amount of non-degraded, non-inhibited, single-source human DNA, which was classified as such by previous examination of STR profiles generated with CE techniques, as well as quantitation procedures, which have also been

Table 2
Obtained p-values for statistical tests used in quality metrics comparison among groups of criminal evidences. Significant values in bold. Significance level was 0.05, with Bonferroni correction applied to post-hoc tests.

Metrics	LSB		HB		LB		NL		RD		QS	
Test	KW	Median	KW	Median	KW	Median	KW	Median	KW	Median	KW	Median
p-value	**0.030**	**0.032**	**0.001**	**0.004**	0.102	0.147	**0.009**	**0.016**	**0.000**	**0.000**	**0.003**	**0.005**
Pairwise Comparison	Post-Hoc p-value											
CT – SA	0.732	0.371	0.203	0.371	–	–	0.034	0.371	0.009	0.007	0.119	0.074
CT – SE	0.444	0.092	0.134	0.201	–	–	0.144	0.092	0.618	0.772	0.104	0.024
CT – EP	0.015	0.074	**0.004**	0.007	–	–	**0.000**	**0.000**	**0.000**	**0.000**	0.028	0.074
CT – BL	0.014	0.074	0.203	0.074	–	–	0.260	0.371	**0.001**	0.007	0.198	0.371
SA – SE	0.649	0.486	0.008	0.024	–	–	0.643	0.772	0.062	0.008	0.831	0.772
SA – EP	0.037	0.007	0.103	0.371	–	–	0.161	0.371	0.075	0.007	0.525	0.074
SA – BL	0.034	0.007	0.819	0.371	–	–	0.320	0.371	0.557	0.074	**0.004**	0.007
SE – EP	0.150	0.201	**0.000**	**0.001**	–	–	0.083	**0.001**	**0.000**	**0.000**	0.716	0.486
SE – BL	0.142	0.024	0.014	0.024	–	–	0.660	0.772	0.016	0.008	0.005	0.024
EP – BL	0.974	0.024	0.063	0.074	–	–	0.017	0.007	0.234	1.000	**0.000**	0.007

proposed as a reliable predictor of resultant STR profile quality [41]. As the main differences presented by samples refer just to the nature of biological evidence, as well as substrates or surfaces where such material was collected in crime scenes, obtained quality metric results were grouped according to the biological nature of evidence collected in crime scenes, and statistical tools were applied to evaluate these disparities. The results for all six quality metrics can be compared in Fig. 2, which features box plot graphics of each evidence group included in present study. Supplementary Tables S3-S8 contain data for all six appraised quality metrics, including all individual values for each SNP marker, obtained from all evaluated samples. Information regarding complete run performance for the chip was obtained from the results summary presented by Ion Torrent Suite and is reported in Supplementary Table S9.

3.2.1. Locus balance (LB) metrics

LB metrics results for each group are presented in Fig. 2A. Statistical analysis results show that no significant differences were observed among samples with a distinct biological nature for both median and distribution evaluations, indicating there is evidence that the present SNP panel performs similarly throughout a complete sample set in terms of this metric. Previous studies analyzing equivalent metrics in different biological tissues [11,12,14,20] have confirmed differences in amplicon efficiency for different SNP markers included with this reagent are related to panel design owing to primer construction features where sequence-related effects are important for amplification efficiency or pH-based nucleotide addition detection used for base calling and quality determination. Therefore, achievement of more balanced results would depend on changes in panel design itself as the observed imbalance among amplicons is an intrinsic feature of the present commercial solution. However, as this efficiency disequilibrium can be markedly significant to a specific number of SNP markers, it is important that manual inspection and results review are conducted by forensic analysts to verify data reliability and adequacy for inclusion in forensic sample analysis on a case-by-case basis as suggested in previous studies [12–14,20]. Alternatively, it has been suggested that low-quality markers should be excluded from results when analysis included criminal samples [12]. Removal of a few poorly performing SNPs should have limited impact in overall genetic weight of evidence as the

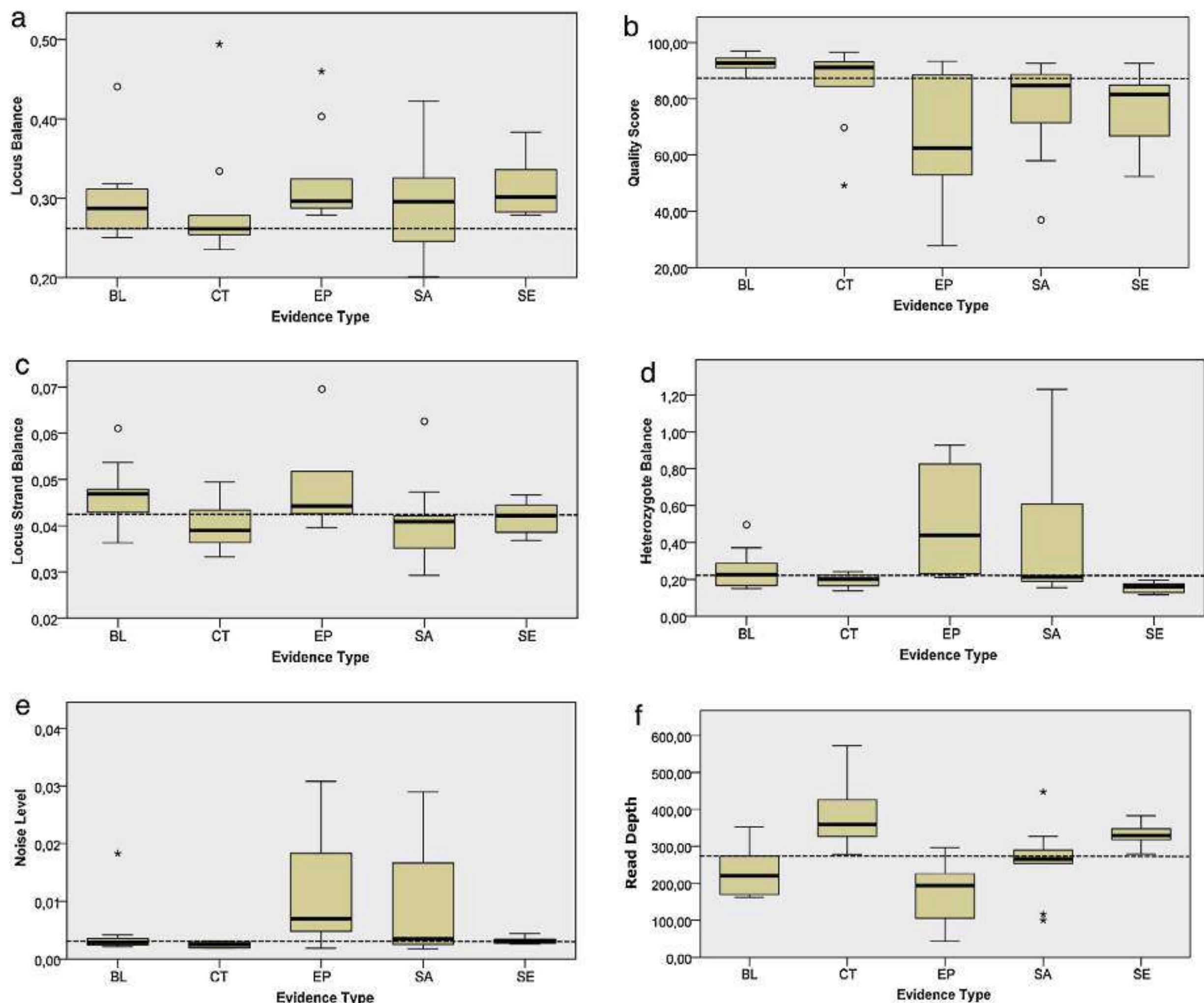

Fig. 2. Box plot distribution of six quality metrics for all tested evidence groups, with black line indicating median value and box representing 1st and 3rd quartiles. Upper and lower bars represent maximum and minimum values, respectively. Dotted line indicates gran median (GM), equivalent to the median value for complete sample set (all groups combined). Sample number is equal to 10 for all groups, except semen, where n = 7. Outlier results are indicated as stars (far out extreme values, differing from median at least 3x the IQR (inter-quartile range) value) or circles (out values, difference from median in the 1.5x to 3x IQR range). Outliers values are not included in any performed statistical hypothesis test. LB, LSB and HB values are plotted as the absolute residues for each value, as previously described, and as such indicates a target value of zero in Figs. 2A, 2C and 2D. BL: blood; CT: oral swabs (reference control); EP: epithelial; SA: saliva; SE: semen. 2A: locus balance, GM = 0.287; 2B: quality score, GM = 87.258; 2C: locus strand balance, GM = 0.043; 2D: heterozygous balance, GM = 0.217; 2E: noise Level, GM = 0.003; 2F: read depth, GM = 273.339.

elevated number of evaluated polymorphisms would still assure LR values above those obtained through traditional STR-CE techniques.

3.2.2. Quality score (QS) metrics

Concerning QS, analysis of the presented results (as in Fig. 2B) with respect to blood group determined it as the most consistent type of criminal evidence with a higher median and low variance. Owing to this exquisite behavior, the BL group yields significant distribution differences with both EP and SA samples, which displayed markedly lower performance in sequence quality. EP distribution shows several samples with overall lower QS, while SA and SE feature plainly bad sequencing performance for the obtained 50% lower values, located between the median and observed minimum. In contrast, the median among sample materials does not show a significant difference even though the obtained *p-values* approach significance levels in terms of pairwise comparisons for BL-EP and BL-SA evaluations (*p-value* for both comparisons was 0.07). Therefore, QS differs among sample groups mainly on with regard to weakly performing samples as low-quality events are markedly skewed in the first quartile and minimum values in SA and EP samples. No evidence of trends regarding individual sample features, as the surface where evidence was deposited, was found to be associated with QS metrics. Additionally, variations in library preparation or workbench manipulation do not seem to explain the findings as all libraries had high quality (as assessed by quantitation) and were diluted to the same concentration in the pool. As notable in Fig. 2B, poorly performing samples belonging to the SE group do not seem to significantly affect QS metrics for this tissue as visually identified differences were not supported by statistical hypothesis rejection.

QS metrics were found to have significantly different median values and distribution among all analyzed groups. Under this assumption, it is important to highlight that median values for all groups, except epithelial, were located above the 80 phred-score threshold. This has singular relevance as it suggests that, despite fluctuations in some of evaluated quality metrics presented by certain sample groups, overall confidence in base calling is still very significant with an estimated median accuracy equivalent to an error rate equivalent to a little less than one in a billion for called genotypes in all groups (gran median = 87.2 phred). Despite the fact particular specific markers consistently showed low QS, overall confidence in base calling for forensic samples is quite strong and seems to be appropriate for forensic applications. This suggestion is supported by the finding that roughly 68% of the total sample set presented median QS values equivalent to 99 when base calls for all markers are considered. The blood group displayed particularly robust results with a median value for the QS metric equivalent to the upper limit in all 10 analyzed samples. Thus, inter-sample evidenced variation in QS metrics was not shown to be associated with the nature or origin of biological evidence as all sample groups presented overall similar results for genotype reliability. Furthermore, no particular type of biological evidence was found to be more error-prone than others. However, outlier results were identified (with the EP.PF17 epithelial sample as the most extreme example with a median phred-score of 21, meaning a genotype base calling accuracy slightly above 99% for at least half of SNP marker set). Results suggest that base-calling efficiency seemed to vary among individual forensic samples. This finding emphasizes the need for execution of meticulous manual inspection of automatically generated genotypes by plugin. This step is recommended in order to assure low-quality results are not taken as adequate for genotype determination, and resulting genetic profiles are not effectively employed in real criminal casework.

3.2.3. Locus strand balance (LSB), heterozygote balance (HB) and noise level (NL) metrics

Besides LB index, all other metrics evaluated in the present study were found to have statistically significant differences among groups of biological evidence types. These variations were found in either median comparison or KW distribution tests, or in some cases, for both statistical tests, just as shown in Table 2. As for such metrics, LSB indicates that there is significant difference among groups of samples for both KW and median evaluations (see Fig. 2C). Therefore, at least one pairwise comparison was expected to display differences among tested groups. However, *post-hoc* analysis could not identify where proposed discrepancies lied. Without Bonferroni correction application, four significant differences would be highlighted. Owing to the conservative approach employed in this study, such differences were not considered significant, and therefore we conclude that, even though some level of association between LSB metrics and sample nature can be implied from the results, it is not clear how different groups contribute individually to this phenomenon.

Regarding the HB metrics, KW *post-hoc* analysis indicates that CT and SE yield significantly better results than EP. In the analysis featured in Fig. 2D, it can be seen that the epithelial group's first quartile approaches maximum value for the CT and SE groups. Median testing indicates that differences among CT and EP relies on the intragroup variability observed in EP samples, rather than on median values, while the discrepancy in the SE and EP results was strong enough to be detected by both statistical methods employed. Such results were considered sufficient to indicate EP samples presenting lower performance than all other kinds of evidence. Upon NL metrics analysis, again, we observed CT and SE performing better than EP, just as shown in Fig. 2E. Based on an employed conservative approach of Bonferroni correction, *post-hoc* analysis indicated a significant difference between SE and EP only on the median, mainly as a result of higher amplitude and maximum value of the SE distribution. On the other hand, CT-EP pairwise comparison indicates a significant difference for both median and distribution tests.

A simple examination of the results displayed in the box plot depicted in Figs. 2A-F shows that semen and control oral swab groups, followed by blood samples, usually had better quality median values and more balanced distributions for most of the measures included in analysis, with intragroup variability typically lesser than observed with other types of evidence (with the exception of QS distribution for semen samples, which displayed somewhat unexpected behavior). On the other hand, epithelial samples had lower performance for the same measures and, to a lesser extent, the same conclusion could be extended to the saliva group. Uniformity in the results presented by semen and control groups might be explained by low variation in overall conditions of such samples because of relatively low exposure to elements or environmental conditions, surfaces or substrates of deposit heterogeneity and low mixture probability. As semen sample availability in BFP laboratories is limited, all samples included belonged to cases where ejaculates were obtained from the interior of condoms, and therefore less subject to contamination or influence of idiosyncrasies with regard to substrate where these samples were collected. Result consistency obtained for both kinds of evidence indicate that such types of samples may be preferable for MPS-generated genetic profile analysis, when different tissues or biological fluids can be used as criminal DNA sources from a single individual. This fact may impact particularly known-source reference sample collection as results indicate that oral swabs provide more stable results. As such, there could be some advantages in choosing oral swabs as the choice method for reference material collection from suspects, victims or individuals involved in kinship tests. However, robust performance and low

variability displayed by the BL group in sequencing quality suggest that this biological material might also be appropriate for reference sampling. Previous studies employing this same SNP panel [42] reported that direct amplification of oral swabs preserved in FTA paper is possible, with results equivalent to extracted DNA from the same material. Authors also describe variations in RD, LB and HB between extracted or directly amplified DNA with a significant degree of intra-group variation. Such variations are linked with varying amounts of DNA in FTA punches, as well as a less precise fluorescence-based quantitation method for extracted DNA, and as such do not coincide with overall quality metric stability featured by control reference samples herein described. Relevant aspects regarding casework processing in forensic laboratories, such as per sample cost, throughput capacity and possibility of analytical process automation, are also affected by such identified features.

3.2.4. Read depth (RD) metrics

Read depth levels per samples, evaluated by RD metrics, is one of the evaluated indices that seems to be considerably influenced by the biological nature of evidence, as presented in Fig. 2F. Both median and distribution of samples vary among groups. Pairwise comparison among sample types demonstrates that, even though BL and SA also presented overall lower values than SE/CT, such differences are not statistically significant. However, RD analysis highlights again the difference between SE and CT with EP for both distribution and median tests - epithelial sample performance is markedly different than both high-quality groups. Without Bonferroni correction, several other post-hoc comparisons would indicate significant median difference, but this effect is not observed in distribution. This can be explained by high RD variance. Kruskal-Wallis testing shows that BL read depth distributions among samples also differed significantly from the CT group. Curiously enough, significant differences were also indicated between CT and BL results distribution. While presenting lower RD values, statistical evaluation elicited no difference in blood sample median from other groups, probably as a consequence of the conservative approach employed. Lower performance in depth metrics for blood samples have been previously reported for the present panel also in the form of reduced number of reads and allele imbalance [21] when compared to control 007 DNA. However, the same authors propose that such variation may be caused by inhibition during panel PCR steps or because of the presence of non-human DNA within the sample. Although hemoglobin and other *heme* compounds present in blood samples have been widely described as PCR inhibitors [43], such effects cannot fully explain these types of observations as analytical protocols include library quantitation and mixing in equimolar concentrations in a pool, where they are posteriorly co-amplified during the emulsion PCR phase. Inhibitions in primary PCR would be therefore attenuated by posterior PCR steps for library pool amplification, in which equal amounts of library for each sample is added. Moreover, if inhibitors present in blood samples are not removed during DNA extraction, we would expect them to impact not only blood samples during library preparation, but also affect library pool amplification in emulsion PCR steps. However, it is reasonable to expect that inhibition would affect all samples equally as they were all mixed together in the library pool. Non-human DNA presences in a sample, however, is a factor to be considered as a source of PCR bias and might be implicated in observed variations among groups. Nanopore membrane-based direct sequencing of unamplified total DNA extracted from forensic samples showed that latent forensic evidence can have as low as 10% of obtained sequences identified as possessing human origin, while almost half of the obtained sequences were classified as belonging to *Escherichia* genus [44]. In ancient DNA samples (which may present certain features resembling forensic evidence, including environmental or bacterial contamination), strong PCR bias favoring amplification of GC-rich bacterial sequences was reported in detriment to endogenous human DNA [23]. This event seems to be highly dependent on the employed polymerase, with a reduction of endogenous DNA fraction sequences to around half.

3.2.5. Sources of observed variation among groups

Several forms of error and bias introduction in MPS data is a widely known phenomenon, and its impact in forensic applications must be considered owing to the critical nature associated with these criminal evidence forms, which may include low-template copy numbers, high degradation levels or mixture occurrence in variable DNA donor contribution ratios [45]. Sequencing bias has been discussed and thoroughly studied primarily in clinical MPS applications as cancer and other pathology investigations (where only relatively small numbers of cells may be affected by mutational events leading to genetic polymorphisms) might be particularly interested in minor, less frequent genetic variants [46]. Forensic samples, however, still have been less explored as sources of genetic data for error evaluations.

MPS errors in general can be connected with experimental conditions, such as sample characteristics, PCR-introduced bias, analysis protocols or instrument-related technical limitations, but also to computational interpretation of generated data [46]. Variations in adopted post-sequencing pipelines include quality control of raw sequence reads, reference genome or sequence alignment, mutations or polymorphism detection (in variant-calling or genotyping steps), as well quality control procedures following each of the previous stages [27]. Sources of error in MPS applications as a result of PCR artifacts are well-known, and include phenomena like stochasticity, several forms of PCR bias (including amplicon length and CG content), polymerase errors and template switches [24]. Considering the employed sample set and its varying preservation or collection conditions, we considered the hypothesis that groups of samples might differ in amounts of non-human DNA present in extracts after evidence processing, which is expected to be highly variable among samples and not directly assessed by utilized RT-PCR quantitation methods. For such evaluation, the number of unaligned sequences were determined for each sample and differences among groups was evaluated. No statistical variation was observed for evidence groups in a single factor ANOVA test (*p-value* = 0.459218) with average values of on-target reads ranging from 91.84% for the semen group to 88.77% in epithelial samples (data not shown). Although a more detailed exploration of data regarding unaligned sequences associated with forensic samples is currently underway, inter- and intra-group overall homogeneity in the number of off-target reads with forensic samples suggests that bacterial contamination present in real casework forensic samples does not seem to be the main reason responsible for observed variation in quality metrics included in this study. It has been proposed in the literature that simultaneous analysis of reference and questioned criminal samples in a single run is not recommended in certain MPS applications as competition effects among specimens presenting varying DNA quality might favor sequencing of high-quality targets [47,48]. In forensic applications, this effect can have a significant impact on analysis owing to preferential amplification of reference samples in detriment to questioned criminal DNA, leading to overall lesser coverage (represented as average read counts) for the latter group. The current results seem to corroborate this hypothesis with higher quality biological evidence (represented by semen and reference oral swab groups) yielding better performance in sequencing quality metrics than other kinds of samples. Even though most of the previously reported impact is related to variations in read-depth values, other

metrics might also be concurrently affected by sample quality. Despite this, the executed analysis indicates genetic profiles can be simultaneously obtained for a large number of different samples (in this experiment, 77 distinct DNA extracts were included in a single run) through barcoding techniques with sufficient quality to support this method's use in real casework forensic applications. However, sequencing efficiency is still to be determined for critical real DNA evidence as highly degraded or low DNA template samples in order to fully assess potential and capability of the present panel in generating reliable, trustworthy genetic profiles to be employed in criminal justice procedures. If necessary, protocols can be adapted for low-quality criminal samples and smaller sample numbers, aiming for superior sequencing depth per genotyped marker.

Regarding LSB, HB and NL metrics, the performed analyses indicated there to be statistically significant differences among groups, for all three metrics, for both median and distribution comparisons. Curiously, LSB metrics' *p-values* indicated the null hypothesis was rejected for the test as a whole (including all groups), while pairwise evaluation of specific pairs of sample groups revealed no significant difference between all possible group combinations. For the remaining metrics, epithelial samples were found to have the most divergent results, with significance always associated with pairwise testing against semen and/or control groups. Even though no statistical significance was established, the saliva and, to a lesser extent, blood groups also displayed more erratic behavior in quality metrics measures than semen and control (oral swab) groups. This suggests an effect associated with environmental contamination as samples belonging to referred groups were collected in less stable or controlled conditions. It is not clear if this highly variable pattern presented by some quality metrics is caused by factors associated with conditions in which criminal biological evidence was collected (including but not limited to exposure to elements as UV radiation or high humidity, presence of PCR inhibitors, human or other species contaminating DNA presence, elements associated with surface or substrates where evidence was deposited, and others). Nonetheless, the observed results suggest these factors cannot be clearly identified or inferred through traditional forensic DNA analytical tools as all sample conditions were normalized according to RT-PCR quantitation and STR-CE fragment analysis results. Certain samples presented small-sized peaks in terms of STR genetic profiles, which could be associated with minor contributors in a mixture (described in Table 1). However, this pattern was found across almost all sample type groups, and manual inspection of the results suggested that the presence of minor mixture contributors in forensic samples does not present a marked impact in any of the investigated metrics, especially in those where low-level mixture impact is expected to have particular relevance (like NL and HB metrics). In addition, no association was found between the described results and the substrate or surface where biological evidence composing a sample set was collected, or even the collection method, although the evaluated sample number was not sufficient to provide an adequate number of observations for each evaluated deposition spot owing to the large variety of places or objects where evidence was located in respective crime scenes. Considering that these factors are highly diverse among real casework samples evaluated in forensic genetic laboratories, their features are associated with specific crime scenes and processing, and therefore cannot be controlled or managed by DNA laboratory staff. Notwithstanding, as the described elements might have significant repercussions in terms of quality aspects of DNA genotyping, their influence must be taken into consideration by forensic DNA analysts in results interpretation and profile generation.

A different approach to explain the differences observed among groups in quality metrics results might be related not only to variable environmental conditions or structural integrity of DNA obtained from such samples, but the very biological nature of human tissues and fluids from where this evidence originates and its intrinsic features. Thus, epithelial samples displayed a persistently inconsistent and erratic behavior with regards to quality metrics and were characterized as the only evidence type to differ with statistical significance from other sample groups (mainly to semen and/or reference oral swabs) for more than a single metric, both for median and distribution values. A recent review on trace DNA deposits [49] discusses factors influencing DNA recovery from "touch DNA" evidence and cellular composition of epithelial deposits. While circumstances surrounding the amounts of trace DNA (where shedders classification is discussed), transfer and persistence of genetic material, timing and place of deposition are considered, and such factors could have a limited impact on samples used in the present study as included cases comprise only DNA evidence where traditional methods (RT-PCR quantitation and STR-CE fragment analysis) were employed with success. As an example, considering touch DNA is widely reported as degraded [49], this effect was minimized in our sample set by selecting only evidence where degradation levels were undetectable. A possible explanation for the observed fluctuation might refer to the fraction of DNA present in touch deposits not related to cellular components deposited by contact, but to cell-free DNA (cfDNA) existent in epithelial evidence [50]. While the presence of cfDNA was reported for all different types of human tissues or fluids included in the present study (and therefore would be a component present in all evaluated samples) [51], its relevance may be more pronounced in epithelial deposits, whereas cfDNA can account for most recoverable DNA in manipulated objects, ranging from 84 to 100% of total DNA [52]. cfDNA presence in human fluids is believed to be mostly derived from hematopoietic apoptotic cells, with variable quantities according to tissue and deep sequencing of these DNA fractions featuring fragments subject to severe DNA damage and containing variant levels of sequence bias, introduced during programmed cell-death processes, leading to necessity of protocol modification for enhanced library preparations [53,54]. As most studies focus on degradation levels and fragment size of cfDNA components [49], it is not clear if this fraction of total epithelial DNA components would be more subject to the effects impacting PCR or sequencing quality. However, if such effects associated with DNA damage are potentially dependent on cfDNA fractions derived from criminal evidence, it is expected that large-sized amplicons would present lower amplification efficiency because of highly damaged patterns presented by this fraction. To test this hypothesis, a Kruskal-Wallis test was executed for all five sample groups with MRL additional metrics, or mean read length of sequenced amplicons (presented in Supplementary Table S10). It is important to notice that MRL metrics, as presented by the plugin, are provided as a unique number for the whole sample. Considering panel design prioritizes smaller amplicons, the effect of larger amplicon degradation can be harder to identify as smaller amplicons are not as affected by degradation as larger ones. Therefore, differences in samples for this metric are expected to be restricted to just a few base pairs. Yielded results confirm that distribution of amplicon size is not the same among all groups (*p-value* = 0.0051), and pairwise analysis showed that, after Bonferroni correction, significant differences are observed between epithelial-control (*p-value* = 0.0037) and saliva-control (*p-value* = 0.0005) groups. ANOVA testing also indicates differences in variance among groups (*p-value* = 0.0483). It is expected that control groups have higher overall values as collection processes (internal oral mucosa swabbing) promotes enrichment of cellular fractions in biological material used for DNA obtention through mechanical abrasion of oral mucosa. The described results are in accordance with the hypothesis of contribution of damaged cfDNA fraction of forensic evidence to the observed lowering of quality

metrics identified in specific groups tested. In addition to this, there is also a possibility that DNA quality in forensic evidence is being affected by external factors acting on DNA evidence only subsequent to its deposition. As such, additional environmental effects might increase DNA damage already present in cfDNA fractions or impacting overall conditions of genetic material present in evidence. It is possible that cfDNA fractions are more susceptible to diverse types of chemical alterations of nucleotide bases as a result of tautomeric isomerism (which are highly dependent on surrounding pH) [55], temperature, UV radiation or oxidative agent-mediated covalent bonds breaking/formation and other forms of structural changes to DNA, which seem plausible to a more exposed, cell-free fraction of total DNA found in criminal evidence. Despite being speculative, this hypothesis' verification would require additional experiments under controlled conditions in order to determine the molecular and/or chemical mechanisms involved in such DNA modifications, and how they would impact posterior steps in DNA genotyping of forensic samples.

Finally, we considered the possibility that variation observed in quality metrics for some sample types, which was markedly evident in epithelial criminal evidence, is a result of endogenous factors or particular features presented by this precise human tissue. As an example of such factors, accumulation of post-zygotic mutations in cells belonging to normal human tissues is a factor to be considered, especially in highly proliferative tissues, such as skin [56]. Somatic mosaicism manifests as single nucleotide variations (SNVs), epigenetic alterations, copy number variations (CNVs), different forms of *in-del* polymorphisms or even chromosomal variations that can be found widespread in human tissues and compatible with normal development and ageing [57]. Healthy tissue evaluation by deep-coverage sequencing revealed an average of 1035 benign SNVs found for each human fibroblast cell [56]. Extensive intra-individual variation has been described among different types of tissues, and experiments in skin cells suggest high levels of somatic mosaicism with at least 30% of cells presenting CNV polymorphisms not originally observed in early developmental phases [58,59]. Conceptually, it seems reasonable to assume somatic mosaicism would have at least some kind of impact on quality metrics, with measures such as NL and HB being particularly sensitive to these mutations' presence. However, considering the nature of groups included in the present study, one would expect the control group (constituted by oral mucosa swabs) also display the same susceptibility to such somatic variations taking into account the similar features presented by this kind of tissue and human skin. Nucleotide epigenetic differences between both groups of samples could explain this phenomenon as an alternative form of somatic natural variation as both tissues are subjected to discrepant environmental exposure to diverse external factors that can differentially affect distinct tissues in a single individual, with elements as aging or sun exposure already posited to alter methylation profiles of human skin cells [60,61]. Such an explanation is, however, very unlikely as it is not clear which molecular mechanisms might be involved in such an outcome considering no evidence of repercussions on PCR quality of efficiency based on methylated nucleotide presence in PCR templates is available. Therefore, lower performance in certain quality metrics presented by epithelial and, to a lesser extent, saliva and blood criminal casework are more likely to be associated with factors like environmental contaminants, as well as background DNA presence (as an element of relevance in DNA transfer studies) [62] than to variant features presented by distinct human tissues.

4. Conclusion

The results obtained in this study indicate there is enough evidence to propose the biological nature of criminal samples as a source of variation in certain MPS quality metrics considering the technical solutions employed in an analytical workflow. Such variations seem to be difficult to foresee as they are not directly apparently by analysis of criminal sample features using traditional profiling techniques. Epithelial samples were found to present relatively higher variation for certain parameters and overall lower sequencing performance than other types of criminal evidence. On the other hand, samples like oral swabs, semen and even blood presented overall robust sequencing quality and uniformity among samples. Some level of intragroup discrepancies was also detected, suggesting individual features presented by distinct samples might have a certain level of impact on sequencing quality metrics. A number of possible reasons for such variations were presented, albeit no conclusive explanations were proposed for intra- or inter-group result discrepancies. While different types of evidence were found to differ in their ability to generate high-quality genetic profiles, it seems plausible to propose group disparities in metrics do not prevent this technology's use in forensic cases, but rather draws attention to the need for human intervention in results interpretation and validation through a careful, meticulous expert review of reported results and considering variability. This particular follow-up step may be facilitated by technical modifications, improvement in analysis protocols or appropriate data processing, all seeking to minimize need for human intervention, yet such seems necessary under current technical conditions.

The present work also supports the previously proposed [20] nationwide use of a single allelic frequencies database for the whole Brazilian population through genetic weight-of-evidence evaluation in a forensic context. Once again, no evidence of a significant stratification for the Brazilian population was found taking into account the human identification-oriented SNP markers included with this reagent and its associated regional distribution frequencies. This observation is in agreement with current practices adopted by BFP and other Brazilian regional law enforcement agencies for data regarding STR frequency distributions [63]. Conservative approaches are employed for all statistical calculations, including corrections for substructures within populations [64], and it is highly recommended that such concepts are also adopted in SNP-based genotyping procedures.

It is possible that the distinct behavior of different types of criminal evidence for evaluated quality metrics may merely consist of mathematical artifacts, specifically resulting from nature or characteristics presented by the studied variables and employed calculation methods. However, the strict, conservative approach applied during statistical analysis supports the conclusion that at least some degree of asymmetry in quality metrics among groups is associated with the nature of human tissue where evidence originates. The present analysis was proposed as an exploratory, preliminary survey of this dataset, and additional investigation is necessary in order to ascertain proposed diversity in tested indices among groups. Thus, further scrutiny in real forensic casework is necessary, including evaluation of DNA transfer effects, impact of the substrate or deposition surface for each sample and other particular aspects of individual samples' nature. In order to achieve this objective, a significant increase in sample number is necessary with the objective of encompassing as much individual sample variability as possible. A proposed increased in sample number can reduce the influence of external factors and mathematical, spurious or stochastic effects, leading to a reliable, comprehensive appraisal of the nature of biological evidence's meaning in MPS genotyping of real criminal casework samples.

Declaration of Competing Interest

Authors declare they have no conflict of interest.

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CRediT authorship contribution statement

E. Avila: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Resources, Data curation, Writing - original draft, Visualization, Project administration. **C.P. Cavalheiro:** Validation, Formal analysis, Investigation, Writing - review & editing. **A.B. Felkl:** Validation, Formal analysis, Investigation, Writing - review & editing. **P. Graebin:** Investigation, Resources, Data curation. **A. Kahmann:** Formal analysis, Resources. **C.S. Alho:** Conceptualization, Resources, Data curation, Writing - review & editing, Project administration, Supervision, Funding acquisition.

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Appendix A. Supplementary data

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CAPÍTULO 3 - Brazilian forensic casework analysis through MPS applications: Statistical weight-of-evidence and biological nature of criminal samples as an influence factor in quality metrics

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Supplementary Table S1. Obtained LR for a hypothetical genetic match analysis between identical genetic profiles belonging to a suspect and the evaluated criminal samples profiles, considering 90 autosomal SNP markers allele frequencies for Brazilian national database.

Samples		LR value	
Name	*Type*	*SNP*	*STR*
BL.PF07	Blood	1.23743810441685E+37	7.85701875144952E+28
BL.PF09	Blood	4.14820560759361E+36	3.71932157150648E+30
BL.PF11	Blood	1.4345311796194E+38	4.58748556894663E+28
BL.PF20	Blood	2.75719044017885E+37	1.56425810013325E+31
BL.PF22	Blood	1.09545310378634E+39	7.70397827735359E+30
BL.PF24	Blood	1.56819497508843E+37	2.37774791023571E+27
BL.PF25	Blood	4.07220768553542E+39	1.03503695508056E+28
BL.PF26	Blood	3.12456179848935E+36	3.56520098298984E+29
BL.PF27	Blood	3.39262406942654E+38	1.30802930227513E+29
BL.PF30	Blood	1.77516092793831E+39	2.99467201161844E+29
EP.PF01	Epithelial	3.26926401097262E+37	3.32255985898169E+28
EP.PF08	Epithelial	1.17484746563587E+39	1.54893836112809E+31
EP.PF10	Epithelial	1.99947382256186E+32	4.39297917186597E+30
EP.PF13	Epithelial	6.83952525636543E+32	7.782220761751E+26
EP.PF14	Epithelial	2.38776678800251E+38	1.88256399423795E+31
EP.PF15	Epithelial	1.08042144132441E+40	3.649804332947E+28
EP.PF17	Epithelial	1.9660231893874E+36	2.49658467288601E+30
EP.PF18	Epithelial	9.48516305883352E+37	1.29725631015582E+29
EP.PF21	Epithelial	8.50203109630223E+36	1.02893347504436E+29
EP.PF28	Epithelial	1.9815186701859E+37	1.5027467258249E+27
SA.PF02	Saliva	5.25598051249326E+36	1.86155297443082E+28
SA.PF03	Saliva	5.61424781594623E+37	6.0681165799896E+28
SA.PF04	Saliva	8.76132710427335E+37	3.1216468321729E+31
SA.PF05	Saliva	1.4612360455102E+37	3.74990994240452E+31
SA.PF06	Saliva	3.42790907451864E+37	6.16777774807285E+29
SA.PF12	Saliva	3.52151911170592E+39	6.17160124275116E+30
SA.PF16	Saliva	1.05973265766127E+39	6.50545394726864E+28
SA.PF19	Saliva	3.37924815478977E+35	9.42050477081E+18
SA.PF23	Saliva	2.62278931773178E+33	1.09768581524535E+32
SA.PF29	Saliva	3.78271744299509E+39	2.43295280496775E+26
SE.PF02	Semen	5.02939310860896E+39	4.89430558223412E+27
SE.PF03	Semen	1.27602943005643E+37	5.097051773143214E+30
SE.PF07	Semen	2.72985944705282E+39	1.21439091483628E+29
SE.PF22	Semen	9.10128366065498E+38	1.89456301211238E+29
SE.PF26	Semen	2.41322838856896E+39	3.663567399510522E+30
SE.PF27	Semen	5.86305163241493E+37	6.428173948307680E+29
SE.PF30	Semen	6.52225374219512E+37	8.632746992179203E+29
CT.PF02	Control (Oral Swab)	3.7426055644298E+35	-
CT.PF03	Control (Oral Swab)	8.44000797708389E+38	-
CT.PF04	Control (Oral Swab)	7.57062167597672E+38	-
CT.PF07	Control (Oral Swab)	6.52188563735941E+36	-
CT.PF08	Control (Oral Swab)	6.06851134083192E+37	-
CT.PF09	Control (Oral Swab)	9.682528301493E+38	-
CT.PF10	Control (Oral Swab)	1.07012529602384E+38	-
CT.PF11	Control (Oral Swab)	2.20165141320078E+37	-
CT.PF12	Control (Oral Swab)	2.34852303598364E+36	-
CT.PF13	Control (Oral Swab)	1.69872695879596E+38	-
Average	-	**9.060359E+38**	**7,17564E+30**

- : Does not apply

Supplementary Table S2. RMP values for all evaluated samples, based on worldwide and Brazilian national and regional populations. RMP values for worldwide populatic

Population	Region	Average RMP	RMP value per sample							
			BL.PF07	*BL.PF09*	*BL.PF11*	*BL.PF20*	*BL.PF22*	*BL.PF24*	*BL.PF25*	*BL.PF26*
Europe	Worldwide	7,19696E-29	1,244E-39	8,107E-39	8,7E-35	1,298E-38	1,32E-39	2,578E-37	1,286E-36	2,144E-39
America	Worldwide	1,05081E-28	1,314E-39	1,43E-37	5,31E-35	1,883E-38	4,08E-39	7,936E-37	1,075E-36	6,61E-40
South Asia	Worldwide	5,43469E-31	3,851E-42	2,024E-39	1,269E-41	5,9E-38	1,267E-39	3,33E-38	5,191E-38	1,449E-38
East Asia	Worldwide	8,52280E-33	2,366E-43	6,314E-41	1,432E-41	9,471E-42	5,615E-41	2,055E-38	2,727E-40	1,663E-39
Africa	Worldwide	1,42918E-32	2,24E-47	5,46E-40	5,229E-46	3,06E-38	2,885E-35	1,584E-43	1,001E-45	1,174E-40
National	Brazilian	1,45806E-34	2,456E-40	2,948E-39	3,059E-38	5,047E-38	1,781E-38	6,8435E-38	2,9172E-38	2,8397E-40
Southern	Brazilian	1,87924E-34	1,002E-39	2,116E-39	4,622E-38	1,046E-38	2,613E-39	8,7695E-38	6,3661E-38	6,1379E-40
Southeastern	Brazilian	6,33525E-35	6,801E-41	2,972E-39	4,637E-38	6,152E-39	1,664E-38	3,4757E-38	7,7743E-39	3,4071E-40
Northeastern	Brazilian	4,46565E-35	9,805E-41	7,969E-39	2,509E-39	1,013E-37	6,407E-37	2,2195E-38	2,3562E-39	3,0622E-40
Northern	Brazilian	6,34079E-35	1,218E-40	1,624E-40	2,057E-38	1,613E-38	8,566E-40	4,5862E-38	2,6852E-38	4,7196E-41
Center-Western	Brazilian	2,00021E-34	3,034E-41	1,392E-39	1,04E-38	2,959E-37	7,604E-39	2,3986E-38	2,7252E-38	2,3714E-41

ons as depicted by plugin. Brazilian data was calculated with a Θ=0.01.

BL.PF27	*BL.PF30*	*EP.PF01*	*EP.PF08*	*EP.PF10*	*EP.PF13*	*EP.PF14*	*EP.PF15*	*EP.PF17*	*EP.PF18*	*EP.PF21*	*EP.PF28*
2,412E-39	1,878E-38	2,214E-35	3,358E-33	9,667E-38	3,815E-28	3,916E-30	4,47E-36	9,91E-37	2,588E-37	2,015E-36	7,421E-32
7,406E-40	6,844E-41	5,536E-37	3,316E-35	5,57E-39	2,087E-28	1,698E-29	8,276E-36	7,86E-35	1,256E-36	5,056E-37	5,996E-32
2,167E-40	3,048E-41	7,959E-39	9,233E-36	7,791E-37	2,578E-30	2,746E-31	1,764E-38	1,24E-39	2,039E-37	5,983E-39	2,427E-35
2,899E-44	4,408E-44	3,029E-42	2,425E-36	1,317E-39	4E-32	4,454E-32	3,4E-39	3,883E-40	3,352E-39	8,532E-40	4,669E-37
9,981E-44	2,28E-49	3,214E-45	4,393E-38	3,236E-42	6,666E-31	2,492E-36	2,542E-40	4,175E-39	1,004E-43	1,452E-38	1,536E-43
9,4363E-40	2,6436E-40	8,0812E-38	3,2004E-37	5,6333E-40	5,0013E-33	1,4621E-33	4,188E-39	5,0864E-37	1,0543E-38	1,9026E-37	2,9592E-36
1,7415E-39	2,0089E-39	4,508E-37	2,387E-37	1,2489E-40	2,8476E-33	5,2485E-33	9,1812E-39	9,2614E-38	5,2846E-38	4,0733E-37	1,4373E-35
1,049E-39	8,1972E-41	1,7972E-38	2,9152E-37	4,3344E-40	2,4916E-33	3,4281E-34	1,819E-39	9,9909E-38	2,3021E-39	6,7229E-38	1,1406E-36
4,9571E-40	2,7298E-41	2,9519E-39	1,1834E-37	3,9372E-40	1,6366E-33	4,3047E-34	3,294E-39	9,1133E-37	3,2279E-39	2,5126E-37	3,4845E-37
9,9502E-40	2,7984E-40	2,981E-38	1,8467E-37	3,9497E-40	1,8518E-33	6,5656E-34	1,5616E-39	1,2313E-37	3,7049E-39	1,3053E-38	3,6539E-36
3,8482E-41	2,8049E-41	1,6748E-37	1,6132E-37	6,8806E-40	8,4173E-33	5,8391E-34	6,8712E-40	2,871E-36	3,0166E-39	1,0678E-37	3,2807E-37

SA.PF02	*SA.PF03*	*SA.PF04*	*SA.PF05*	*SA.PF06*	*SA.PF12*	*SA.PF16*	*SA.PF19*	*SA.PF23*	*SA.PF29*	*SE.PF02*	*SE.PF03*
1,492E-32	1,638E-36	5,527E-36	2,59E-36	2,564E-37	4,17E-38	2,761E-40	2,242E-36	1,862E-37	2,997E-27	4,861E-35	4,049E-35
4,14E-32	2,049E-37	1,813E-35	4,338E-38	1,159E-36	7,584E-38	1,404E-40	6,587E-38	8,024E-38	4,713E-27	2,374E-36	2,155E-35
2,756E-35	3,929E-38	1,591E-35	7,406E-38	5,618E-40	6,11E-40	2,537E-41	4,515E-38	5,282E-39	2,269E-29	5,674E-35	1,237E-34
1,483E-37	1,405E-41	7,992E-39	5,619E-42	3,712E-40	2,516E-42	9,994E-42	3,031E-39	4,395E-40	3,159E-31	9,161E-37	5,408E-35
2,124E-36	5,916E-48	3,921E-33	4,718E-47	5,71E-42	4,717E-46	4,29E-43	4,045E-43	6,923E-48	2,061E-37	9,181E-39	7,353E-37
2,4107E-37	6,9709E-39	3,6269E-38	9,1286E-40	6,3768E-38	8,5117E-40	9,2556E-41	1,1762E-37	1,1414E-38	3,8127E-34	1,9883E-40	7,8368E-38
1,1605E-37	3,8133E-38	2,6078E-38	9,9667E-40	5,3196E-38	6,4483E-39	4,8194E-41	2,4325E-37	3,8287E-38	7,1839E-34	5,0331E-41	1,2887E-38
3,7109E-37	1,3627E-39	2,6883E-38	6,4645E-40	2,9106E-38	1,1244E-40	1,2036E-41	5,828E-38	5,8233E-39	1,3964E-34	4,0958E-40	1,1507E-37
1,378E-37	4,3621E-39	2,2572E-37	3,6724E-40	1,1975E-37	1,0105E-40	7,8274E-41	7,6847E-39	9,1602E-40	2,2691E-35	4,6734E-40	4,3012E-38
7,7224E-38	1,1312E-39	8,8597E-40	3,9689E-40	7,2952E-39	1,2384E-39	6,5625E-41	7,0358E-38	4,3779E-38	4,6619E-34	5,6294E-41	5,4695E-38
1,0901E-37	2,0231E-39	2,5843E-38	3,1445E-40	4,2734E-38	1,1688E-40	1,2355E-40	1,3091E-37	8,7334E-40	3,9184E-34	4,4759E-41	6,9275E-38

SE.PF07	*SE.PF22*	*SE.PF26*	*SE.PF27*	*SE.PF30*	*CT.PE02*	*CT.PE03*	*CT.PE04*	*CT.PE07*	*CT.PE08*	*CT.PE09*	*CT.PE10*
6,165E-37	1,242E-35	6,706E-37	2,343E-35	3,16E-35	6,087E-32	3,374E-35	4,209E-35	1,19E-34	7,504E-35	7,093E-36	9,576E-37
7,544E-38	6,821E-37	2,328E-37	8,646E-36	3,133E-35	7,959E-33	7,78E-36	1,519E-35	7,482E-34	1,691E-35	2,945E-37	2,15E-34
2,11E-40	1,595E-39	6,632E-37	5,251E-38	3,199E-39	8,912E-35	3,925E-36	4,657E-36	4,748E-35	3,913E-36	1,472E-37	2,7E-36
8,492E-43	2,491E-40	2,072E-38	6,188E-37	1,689E-39	6,595E-38	8,144E-39	3,354E-39	1,16E-37	4,913E-38	6,503E-40	7,241E-35
1,63E-39	3,084E-40	1,156E-37	9,055E-40	4,51E-40	1,675E-37	1,152E-44	5,993E-40	1,078E-35	6,732E-38	3,879E-41	1,146E-33
3,6632E-40	1,0987E-39	4,1438E-40	1,7056E-38	1,5332E-38	2,6719E-36	1,1848E-39	1,3209E-39	1,5333E-37	1,6479E-38	1,0328E-39	9,3447E-39
1,682E-40	1,0211E-39	3,9573E-40	2,3266E-38	7,448E-39	1,1765E-36	1,5058E-39	1,4828E-40	5,2263E-38	8,8703E-40	2,6299E-39	2,8396E-39
4,7918E-41	4,3517E-40	3,1292E-40	6,2792E-39	1,5137E-39	8,9421E-37	4,6681E-40	6,599E-40	6,4337E-38	2,4146E-38	3,7703E-40	1,2834E-39
3,3776E-39	1,8995E-39	1,2702E-39	1,2049E-38	8,6254E-39	4,4475E-36	8,8988E-40	1,4881E-39	9,4426E-37	1,7611E-38	2,419E-40	1,9719E-37
5,3588E-41	1,1466E-40	4,9748E-41	2,8649E-39	6,6566E-38	7,6775E-37	3,9709E-40	4,8455E-40	2,9281E-38	3,8826E-38	1,0983E-39	7,2878E-40
2,575E-40	7,8203E-40	8,1972E-41	1,1476E-38	9,151E-39	3,0569E-36	5,2921E-40	6,9776E-39	8,1119E-38	1,0486E-38	1,9519E-40	1,2406E-38

CT.PE11	*CT.PE12*	*CT.PE13*
1,734E-35	3,221E-33	4,969E-35
1,205E-35	2,902E-33	3,092E-35
3,066E-37	5,169E-36	2,992E-36
9,597E-38	8,769E-38	1,882E-38
7,8E-38	5,985E-40	3,116E-41
4,542E-38	4,258E-37	5,8868E-39
7,4423E-39	2,7227E-37	2,0004E-38
8,4594E-38	9,041E-38	3,1972E-39
8,6444E-38	3,6607E-37	5,2485E-39
9,3977E-39	3,2074E-37	7,7571E-40
3,5326E-38	3,2087E-37	9,4476E-40

Supplementary Table S3. Read depth metrics values, stated as number of reads of each SNP marker for all 132 polymorphisms included in HID Ion Ampliseq Identity Panel, in Brazilian criminal samples.

Samples	**Locus**																	
Blood	*rs1005533*	*rs10092491*	*rs1015250*	*rs1024116*	*rs1028528*	*rs1031825*	*rs10488710*	*rs10495407*	*rs1058083*	*rs10773760*	*rs10776839*	*rs1109037*	*rs12997453*	*rs13218440*	*rs1335873*	*rs1355366*	*rs1357617*	*rs1360288*
BL.PF07	429	466	158	520	491	502	166	483	494	442	169	245	145	457	465	392	256	504
BL.PF09	351	215	74	279	260	231	118	240	333	209	157	209	73	245	100	237	168	267
BL.PF11	195	205	66	301	256	260	125	190	265	264	87	173	95	207	241	253	143	203
BL.PF20	336	496	123	470	328	447	216	278	430	407	196	200	119	421	416	395	223	285
BL.PF22	306	293	95	291	371	265	124	285	437	289	143	210	77	307	250	242	220	286
BL.PF24	127	168	68	174	351	142	94	93	277	284	92	139	29	325	251	119	44	227
BL.PF25	260	370	94	367	343	407	148	306	380	354	130	192	99	284	300	298	188	302
BL.PF26	236	349	98	274	376	413	135	262	401	372	94	314	66	487	346	214	218	416
BL.PF27	481	580	137	562	586	557	182	375	722	476	213	350	134	482	439	389	371	488
BL.PF30	179	268	84	256	275	235	132	181	278	209	70	149	61	278	231	150	134	219
Epithelial																		
EP.PF01	241	327	110	266	488	156	186	210	356	337	92	180	90	456	348	266	103	270
EP.PF08	118	72	60	173	182	116	80	112	163	162	69	81	33	158	117	191	97	128
EP.PF10	39	49	6	32	48	58	33	21	64	44	37	36	17	60	31	42	65	95
EP.PF13	176	120	52	165	185	135	108	130	158	136	69	131	42	125	101	94	70	175
EP.PF14	245	198	89	292	287	174	92	149	317	228	101	220	79	234	253	185	158	236
EP.PF15	89	64	34	58	82	48	51	54	126	65	20	62	27	89	54	70	19	84
EP.PF17	168	344	97	335	339	304	140	270	374	297	141	160	66	366	323	237	156	216
EP.PF18	376	530	132	365	485	407	153	323	460	451	220	282	91	575	444	351	233	372
EP.PF21	174	300	122	325	301	300	211	221	334	322	78	177	96	391	298	231	138	180
EP.PF28	129	185	104	200	510	204	159	148	441	352	69	348	39	465	263	146	134	229
Saliva																		
SA.PF02	369	324	131	471	384	409	178	244	379	430	104	219	113	378	342	242	246	363
SA.PF03	166	122	49	154	153	89	58	96	187	160	52	115	16	188	140	84	76	151
SA.PF04	437	613	227	679	638	626	362	528	644	648	209	366	187	802	609	520	429	591
SA.PF05	382	241	124	255	552	443	190	161	505	470	282	477	40	627	401	177	150	402
SA.PF06	290	273	141	296	510	344	199	266	410	436	132	223	88	442	350	299	177	467
SA.PF12	298	374	192	334	354	460	217	330	417	464	108	230	128	442	398	383	212	335
SA.PF16	275	330	163	341	409	288	216	221	307	512	100	218	92	484	437	307	230	338
SA.PF19	408	234	65	390	450	344	161	315	397	284	178	289	59	356	338	206	221	432
SA.PF23	252	433	228	460	535	445	257	294	537	544	130	229	98	544	390	286	209	410
SA.PF29	145	141	43	157	212	183	80	91	194	204	68	175	37	193	181	82	86	172
Semen																		
SE.PF02	262	511	176	620	357	660	340	279	447	436	118	241	200	522	456	596	349	295
SE.PF03	172	593	270	556	238	700	312	245	438	369	78	201	153	605	505	664	341	263
SE.PF07	392	703	191	484	375	680	274	454	553	403	182	296	172	610	482	585	406	354
SE.PF22	419	396	142	440	499	504	288	301	590	437	284	496	153	459	381	421	350	502
SE.PF26	425	589	246	613	468	793	275	415	545	434	162	307	251	601	486	709	488	307
SE.PF27	293	346	144	320	607	374	181	166	501	586	93	393	76	572	378	278	222	319
SE.PF30	268	518	234	524	345	679	300	260	506	379	155	266	191	547	454	524	306	345
Control (Oral Swab)																		
CT.PE02	408	450	147	537	469	413	240	366	549	396	103	241	133	495	536	397	283	423
CT.PE03	454	574	153	630	541	554	250	559	700	517	222	331	138	567	576	433	378	547
CT.PE04	870	914	196	887	760	761	335	727	929	704	425	493	199	799	853	662	472	879
CT.PE07	500	632	149	651	470	589	286	494	582	521	260	263	144	532	593	492	302	589
CT.PE08	519	660	169	603	510	614	298	588	671	613	266	285	173	725	593	499	394	608
CT.PE09	455	463	110	491	461	539	185	423	541	409	192	226	120	457	488	389	290	436
CT.PE10	472	405	125	449	374	198	185	372	539	283	153	195	140	391	351	305	258	461
CT.PE11	391	501	102	493	418	543	244	425	463	415	167	185	112	454	441	366	222	450
CT.PE12	457	590	196	320	731	619	289	462	856	1123	578	298	67	1303	1215	563	279	767
CT.PE13	508	416	95	397	527	396	161	312	579	417	383	307	86	426	439	318	281	714

rs1382387	*rs1413212*	*rs1454361*	*rs1463729*	*rs1490413*	*rs1493232*	*rs1498553*	*rs1523537*	*rs1528460*	*rs159606*	*rs1736442*	*rs1821380*	*rs1872575*	*rs1886510*	*rs1979255*	*rs2016276*	*rs2040411*	*rs2046361*	*rs2056277*	*rs2076848*
355	389	435	562	573	212	449	363	495	386	517	519	701	237	298	367	531	317	494	305
272	195	295	108	362	111	181	260	247	151	235	373	332	162	218	254	303	196	307	192
302	209	220	276	339	107	210	258	166	133	201	277	300	124	133	230	252	98	204	171
399	356	555	342	461	247	364	337	282	223	305	594	471	206	313	347	451	327	376	254
360	290	308	374	323	126	264	289	227	145	259	369	319	174	214	316	429	145	222	206
219	320	252	300	145	184	146	183	131	107	119	142	138	105	280	275	235	58	143	269
330	347	367	373	343	182	285	301	295	230	215	375	380	179	252	293	402	223	297	218
390	359	273	365	260	232	278	344	261	228	314	363	320	204	274	318	404	131	258	235
545	432	522	520	585	225	454	483	410	379	434	593	558	299	342	432	643	307	412	338
238	225	241	228	224	114	223	214	179	127	162	273	283	130	162	202	326	86	254	178
377	405	379	391	279	238	294	323	234	157	215	318	276	136	370	408	455	162	228	348
81	174	196	195	167	80	149	151	89	87	98	141	146	49	119	141	195	59	88	75
65	82	80	61	40	29	51	41	116	56	66	64	64	35	43	82	67	23	58	33
161	149	139	180	167	95	88	152	115	72	159	120	127	74	153	185	153	60	124	107
293	331	249	271	163	150	217	151	196	152	185	160	289	116	246	358	271	112	239	203
109	100	106	69	71	59	41	79	34	43	59	60	90	36	83	119	87	28	68	62
376	286	387	348	340	195	306	274	174	179	245	317	337	164	267	366	320	180	263	249
455	498	454	453	436	210	462	335	276	237	326	429	517	241	318	426	506	173	311	306
422	411	362	339	239	239	329	244	229	202	161	245	313	151	317	412	445	134	180	276
463	396	218	426	193	159	226	269	161	160	259	164	222	121	387	455	423	44	126	229
380	345	315	354	431	227	287	334	346	250	348	442	426	193	248	367	428	205	326	275
142	178	133	119	138	80	115	142	109	70	112	143	207	60	113	169	227	55	66	104
680	641	677	683	591	410	626	525	525	436	547	665	678	332	522	707	800	323	469	475
499	427	500	555	330	271	300	431	141	215	298	422	282	127	348	411	450	39	198	342
431	387	320	453	280	287	337	278	206	161	286	248	329	183	206	421	472	157	201	313
416	329	426	461	331	224	386	340	348	309	392	357	419	237	343	390	439	290	323	267
457	548	510	471	271	260	407	394	276	240	253	276	448	227	354	429	416	203	243	297
441	266	365	411	398	128	321	368	317	184	394	422	431	163	265	362	469	152	345	321
595	545	494	500	325	310	414	371	338	333	297	378	469	240	447	411	650	189	288	294
211	181	175	225	113	62	103	138	120	89	110	174	125	87	154	181	174	55	111	114
511	574	672	370	251	403	376	384	384	353	366	608	546	287	455	595	402	299	388	302
344	659	749	321	201	703	386	358	393	307	251	502	518	224	482	393	332	348	276	290
466	529	856	487	441	373	463	444	480	338	418	587	705	338	494	465	474	399	434	300
644	470	499	478	396	293	364	490	401	261	357	596	438	255	477	556	472	215	326	378
521	622	931	388	378	460	394	500	459	385	379	624	671	332	507	875	496	325	448	322
595	546	466	425	243	282	218	485	196	178	170	448	336	154	402	578	447	89	293	355
508	584	790	398	309	479	349	429	378	316	274	562	505	281	453	581	396	296	341	330
456	349	497	489	521	248	524	325	331	305	491	501	571	318	408	473	456	362	429	280
564	431	482	454	492	277	545	440	409	380	534	609	518	398	401	546	556	367	576	411
786	652	811	881	1020	315	894	552	651	438	769	938	960	593	566	744	865	546	842	614
529	386	557	588	645	284	669	396	446	348	543	647	693	385	416	568	547	387	542	420
583	510	657	614	681	294	624	458	575	374	561	668	680	357	468	530	579	399	567	490
473	403	554	450	574	193	498	430	351	281	503	550	704	298	360	471	567	265	465	348
415	296	420	389	495	168	436	313	351	317	430	514	440	251	280	312	402	280	407	269
437	376	499	473	526	229	495	324	451	395	421	549	570	334	363	376	459	315	418	345
699	1080	1002	1124	1025	561	835	436	346	320	484	635	559	179	762	871	658	123	479	885
392	334	382	519	686	204	390	410	254	247	491	505	522	222	271	447	489	195	406	286

rs2111980	rs214955	rs221956	rs2269355	rs2292972	rs2342747	rs251934	rs2830795	rs2831700	rs321198	rs338882	rs354439	rs3780962	rs4288409	rs430046	rs4364205	rs445251	rs4530059	rs4847034	rs560681
277	166	558	719	351	182	384	535	329	227	437	493	282	346	392	534	507	291	252	471
185	55	200	317	214	99	263	412	201	82	345	239	118	260	180	263	194	126	94	272
210	42	244	340	192	87	262	324	192	198	319	196	162	168	85	320	236	207	154	188
244	115	346	424	303	121	286	560	323	199	493	377	298	223	297	479	374	279	267	243
304	76	327	264	291	110	297	421	332	147	316	251	145	215	112	277	235	226	171	319
164	103	327	185	458	97	250	166	254	76	372	176	211	152	335	172	97	260	110	216
171	100	381	291	376	90	246	473	327	182	352	302	267	191	202	385	248	263	179	314
305	75	495	280	277	138	439	467	327	214	388	312	181	280	168	405	231	338	218	356
425	151	551	628	402	212	460	625	404	223	532	557	329	377	190	633	426	383	318	561
141	103	266	233	266	74	175	315	238	109	281	206	120	156	113	306	203	194	136	209
232	134	319	270	392	166	283	414	336	147	429	305	266	215	359	368	316	345	234	314
119	47	217	132	214	40	152	205	164	113	247	117	98	105	100	208	102	163	94	213
54	6	76	100	51	17	63	111	79	28	93	53	53	54	98	63	45	44	28	66
132	23	214	140	195	79	143	187	169	80	168	129	103	135	95	187	140	109	92	175
199	109	278	291	249	97	278	314	224	117	277	166	239	172	199	231	279	290	190	252
66	27	86	101	75	47	74	84	83	29	93	60	69	58	90	89	65	89	29	55
168	74	377	329	254	104	243	309	240	194	368	262	133	167	354	384	293	312	194	325
332	107	436	590	525	104	445	445	393	205	548	378	265	243	362	488	343	461	234	373
226	145	361	160	319	132	254	285	428	216	461	312	207	172	286	385	286	274	230	268
401	109	404	150	426	171	252	226	307	131	551	167	215	207	303	294	98	298	125	249
258	105	363	345	311	135	286	431	349	221	431	352	271	258	288	416	348	251	222	363
179	75	153	139	170	65	112	171	108	75	157	118	90	80	104	150	65	160	57	171
425	246	711	554	618	214	542	855	660	324	666	613	452	406	473	754	577	536	470	542
289	176	430	371	567	124	390	448	317	148	617	289	250	264	230	383	244	385	207	320
261	144	466	386	419	151	442	322	364	244	488	292	307	255	181	386	311	324	282	340
289	161	428	363	327	136	377	510	350	243	381	462	366	262	314	531	356	283	315	375
240	120	432	384	320	149	331	446	345	225	380	235	234	216	326	445	307	290	362	317
262	68	446	438	347	223	362	462	258	103	427	300	140	346	260	367	318	335	106	375
302	239	517	285	398	178	424	460	490	313	575	395	357	276	253	587	415	409	396	430
160	45	202	159	208	82	165	218	160	58	248	118	110	112	117	175	99	201	81	145
302	116	303	349	302	170	357	719	447	347	364	616	434	239	381	553	348	282	409	408
185	245	256	253	246	86	297	742	510	434	301	609	540	171	155	474	350	279	591	368
283	130	470	548	344	123	393	929	518	342	545	597	422	305	358	638	481	378	555	441
433	133	441	552	567	274	416	709	398	251	545	477	283	420	305	472	320	499	336	436
434	254	452	490	289	168	390	1044	517	434	496	705	477	328	322	691	444	287	540	409
434	194	569	228	441	163	356	505	426	277	475	286	422	271	331	411	156	361	333	436
273	190	388	312	259	126	325	820	536	424	437	509	448	219	485	560	357	319	473	414
299	141	522	323	380	187	427	488	416	200	594	415	312	307	441	576	440	463	245	488
414	157	597	733	439	145	488	674	407	322	612	555	316	353	244	672	535	487	391	642
559	154	883	1202	659	289	827	944	563	351	951	745	564	533	553	957	729	757	413	925
293	138	595	780	472	199	512	714	435	324	606	597	298	353	250	717	476	514	276	522
373	160	652	903	473	190	522	844	490	303	679	553	364	419	374	679	493	512	295	632
340	118	518	536	366	159	450	571	331	259	451	446	331	355	433	660	468	399	274	455
288	69	342	579	295	173	360	519	353	204	351	433	229	293	248	449	385	290	223	417
260	119	493	558	382	137	412	599	341	247	502	528	269	266	335	279	444	366	252	501
575	221	1186	658	818	163	569	610	510	371	1493	289	467	396	1007	898	571	1177	206	446
329	109	499	951	720	166	483	610	336	211	895	432	176	338	386	429	325	777	225	457

rs576261	rs6444724	rs6811238	rs6955448	rs7041158	rs717302	rs719366	rs722098	rs722290	rs727811	rs729172	rs733164	rs735155	rs737681	rs740598	rs740910	rs7520386	rs7704770	rs826472	rs873196
455	402	429	467	445	303	242	488	285	254	314	464	392	364	436	384	521	443	324	316
151	291	252	271	237	177	136	262	167	132	151	221	279	267	271	134	205	231	113	168
204	199	213	360	234	152	171	314	135	195	175	214	236	194	143	215	174	195	145	215
226	379	430	402	341	384	162	425	215	260	192	321	270	303	262	276	150	277	225	245
261	269	305	261	206	232	154	293	138	166	174	286	331	311	188	177	237	228	127	217
421	318	136	273	150	177	234	189	93	98	72	404	302	284	120	81	349	157	52	280
209	299	295	343	318	263	207	360	195	221	196	296	256	285	272	252	283	241	178	247
341	317	237	346	240	134	152	333	163	205	193	263	360	374	249	170	214	281	93	364
385	489	538	488	422	189	275	550	334	402	340	432	515	463	477	371	415	462	246	410
205	216	227	226	211	202	119	264	79	153	116	238	237	220	169	160	307	220	84	166
315	427	272	265	277	219	224	326	244	211	162	330	338	333	257	173	194	183	147	359
166	132	115	135	143	100	137	126	55	67	88	153	82	159	127	121	93	131	98	117
49	21	66	49	45	51	19	32	39	37	18	63	68	44	56	30	38	72	36	63
133	151	122	111	111	176	112	156	107	78	129	162	173	154	88	87	85	154	85	113
246	198	211	245	161	204	164	236	97	77	164	188	248	254	211	144	222	222	95	217
86	101	67	88	62	65	68	61	38	41	35	93	74	64	43	49	49	56	32	86
257	256	307	288	219	182	171	321	127	191	178	290	271	227	233	180	340	182	167	317
420	372	290	414	373	255	243	478	161	232	194	497	409	389	334	227	389	312	196	329
323	453	258	339	266	315	269	301	134	193	218	305	298	255	200	224	242	165	169	354
566	503	162	291	177	206	288	278	108	133	155	421	488	383	133	167	287	165	48	280
383	335	320	340	335	276	210	397	197	213	204	305	411	301	283	294	336	288	195	380
122	135	139	144	117	119	86	167	73	74	90	231	174	111	104	58	152	108	62	142
614	708	616	604	580	463	344	625	374	401	336	664	582	542	431	421	513	470	372	631
437	326	327	460	257	280	286	437	102	95	137	432	431	505	178	143	395	209	28	408
478	361	269	388	262	431	318	325	171	222	195	537	426	413	273	239	526	213	162	486
399	418	381	369	378	413	327	403	263	305	243	320	391	354	268	268	406	307	260	427
429	509	351	278	285	333	250	394	163	246	234	414	235	302	184	223	152	227	178	438
360	346	312	289	320	216	284	340	231	223	214	435	435	411	294	213	345	301	80	203
471	558	454	387	350	565	420	448	223	275	293	462	423	355	352	320	352	344	273	602
231	225	138	159	125	94	133	197	55	114	137	173	245	142	118	125	96	102	49	154
258	659	484	385	511	579	263	659	440	366	190	360	343	309	318	232	387	310	307	289
204	630	494	255	453	542	175	828	507	443	145	219	198	216	210	197	224	295	315	333
320	669	502	465	644	623	264	791	443	320	151	339	358	379	330	273	333	419	290	403
398	684	360	470	470	282	193	530	378	241	197	476	519	489	310	249	578	419	157	310
230	739	564	423	704	679	262	780	414	455	158	324	425	420	315	309	460	410	421	314
349	606	284	366	242	488	280	427	139	194	95	395	391	421	212	145	480	323	103	332
239	663	494	366	579	544	153	739	391	334	150	339	289	364	268	250	427	355	277	347
449	365	431	483	417	377	263	559	290	290	349	450	365	387	455	354	400	395	202	408
492	482	482	474	517	374	412	578	390	381	358	487	477	457	413	384	403	464	309	485
666	597	701	886	727	446	445	912	548	517	537	737	724	791	775	546	829	758	363	660
426	507	519	561	518	228	260	646	316	318	382	528	411	412	525	330	692	480	285	437
513	505	693	584	548	407	421	707	404	360	351	616	572	495	539	446	313	496	272	515
357	485	501	391	393	340	222	416	295	317	240	457	430	415	391	291	261	414	202	381
246	349	357	377	338	182	207	414	261	300	249	349	353	323	390	342	436	337	233	254
399	363	557	510	387	386	263	469	323	322	329	396	376	342	426	393	377	384	290	405
704	525	423	1101	397	709	468	703	132	176	158	1025	820	636	437	224	988	391	69	556
347	320	335	604	328	214	305	408	174	193	241	529	404	473	386	251	572	348	164	430

rs876724	*rs891700*	*rs901398*	*rs907100*	*rs914165*	*rs917118*	*rs938283*	*rs964681*	*rs987640*	*rs9905977*	*rs993934*	*rs9951171*	*L298*	*M479*	*P202*	*P256*	*rs13447443*	*rs16980426*	*rs16981290*	*rs17222573*
132	328	391	242	400	176	459	404	272	498	219	369	154	38	121	211	232	259	188	268
51	180	285	155	237	45	290	291	93	250	113	272	97	11	59	165	65	108	56	139
87	202	162	125	158	74	255	158	113	276	90	162	127	17	78	124	63	107	99	129
111	377	430	210	406	149	419	317	169	333	141	204	191	24	133	204	186	183	168	211
74	175	307	139	273	158	251	243	89	300	105	257	108	26	74	154	80	122	106	164
65	191	270	64	223	164	183	390	93	246	55	193	139	29	20	111	52	115	81	150
105	224	351	172	322	165	303	324	167	308	141	239	114	27	60	152	139	188	151	236
118	212	235	102	313	60	310	337	141	434	108	409	179	27	58	215	66	173	88	289
137	367	527	250	452	178	472	409	136	459	215	374	207	26	128	248	139	239	204	293
98	138	248	121	211	65	241	227	71	260	69	177	102	14	62	112	70	108	77	172
106	260	309	141	260	124	291	359	126	318	162	252	143	25	42	181	138	174	107	208
41	90	165	76	119	91	143	138	82	143	88	146	68	21	30	67	20	93	53	69
24	53	87	33	82	22	41	29	22	50	30	38	12	2	14	9	9	38	31	18
55	141	213	51	127	148	132	155	64	184	55	193	75	13	33	122	79	77	26	73
118	171	311	114	252	147	227	245	97	306	102	204	87	18	60	138	69	164	84	136
23	60	104	31	61	58	55	90	18	63	37	107	41	7	5	49	24	29	34	58
101	266	317	106	253	90	247	285	201	289	115	174	129	25	131	140	104	164	112	195
166	277	470	223	403	80	356	422	156	444	153	263	169	39	128	201	153	196	139	260
163	320	435	119	253	156	184	313	177	233	183	251	118	62	73	135	136	233	133	209
87	145	280	89	250	168	171	421	68	376	49	423	141	21	33	226	33	145	124	152
169	259	390	200	362	154	389	298	249	402	155	312	154	31	65	216	144	235	115	238
61	89	180	102	133	72	137	111	29	170	38	145	44	18	38	67	41	62	64	113
271	508	637	253	588	132	457	621	216	553	304	498	260	58	159	348	242	319	243	392
157	230	447	80	466	246	415	419	234	599	60	341	200	44	62	217	94	167	87	271
186	277	435	139	329	225	278	378	238	426	138	340	184	73	65	172	133	179	108	240
224	360	491	160	331	184	309	353	295	318	215	284	207	105	117	193	212	176	177	254
162	363	497	140	278	134	289	295	203	339	197	338	225	42	76	191	156	241	94	277
68	266	415	128	347	92	352	418	64	432	152	430	133	13	78	225	62	124	131	222
181	423	486	162	355	329	367	410	326	447	206	368	177	81	79	271	186	308	123	287
66	125	190	83	151	83	83	182	51	234	65	188	58	7	6	79	41	57	50	88
126	605	457	162	336	215	288	239	254	315	235	328	196	52	83	341	118	241	128	248
116	647	407	140	287	194	160	181	342	235	233	254	188	57	105	274	167	309	85	216
144	538	516	185	413	150	328	386	179	416	247	325	209	40	112	306	201	291	167	288
77	315	449	195	446	71	361	396	104	473	198	531	192	23	78	256	92	214	152	201
193	670	449	159	389	283	276	300	230	355	312	411	229	59	99	364	138	285	131	292
139	202	446	120	408	217	238	302	165	397	115	474	214	45	52	296	67	191	83	249
136	515	413	153	357	134	326	257	252	349	243	316	219	54	98	333	138	244	143	252
158	448	435	199	479	227	467	464	157	432	193	328	219	45	141	203	156	193	163	269
183	484	525	310	437	283	528	517	325	519	205	449	270	64	148	276	227	255	186	404
247	738	892	440	725	278	913	774	244	771	340	577	267	62	261	321	261	334	330	486
89	550	560	270	556	247	663	554	147	578	230	356	199	30	166	263	238	227	205	311
107	492	527	279	588	283	665	533	169	590	272	393	284	42	209	247	244	240	255	348
127	369	543	232	441	160	438	432	86	442	200	353	156	27	146	250	180	246	169	309
115	329	357	200	319	109	372	317	123	398	317	316	133	45	116	181	181	185	151	220
147	461	484	254	360	223	501	381	284	376	229	291	183	55	159	241	246	204	153	239
196	397	831	197	1201	138	1183	1116	277	846	46	319	454	78	205	342	112	229	214	428
135	297	445	234	460	104	528	630	115	656	110	401	210	28	136	228	109	168	150	290

rs17250845	*rs17269816*	*rs17306671*	*rs17842518*	*rs20320*	*rs2032595*	*rs2032599*	*rs2032602*	*rs2032624*	*rs2032631*	*rs2032636*	*rs2032652*	*rs2032658*	*rs2032673*	*rs2033003*	*rs2319818*	*rs2534636*	*rs35284970*	*rs3848982*	*rs3900*
217	136	208	259	153	114	215	249	140	188	149	259	223	246	214	250	171	160	213	179
117	91	133	104	90	45	102	146	49	128	36	117	84	147	158	133	124	70	99	116
188	95	104	78	108	71	60	145	74	91	26	101	81	62	182	140	71	84	95	126
186	153	175	244	152	79	137	233	124	135	109	203	168	265	157	230	144	121	163	222
175	132	111	183	145	61	89	172	63	128	63	153	90	176	178	157	101	101	130	130
181	106	51	173	121	39	17	70	53	57	60	112	44	76	79	184	68	109	188	46
149	92	110	179	147	83	114	167	91	130	105	177	127	205	153	194	126	119	120	180
216	170	106	161	179	88	74	116	65	147	54	159	157	189	172	184	137	127	223	168
226	219	215	248	138	109	153	253	113	186	79	291	222	272	208	258	194	192	214	279
108	113	94	77	90	43	58	88	39	87	44	138	104	121	77	159	88	82	86	110
183	185	130	199	159	65	97	180	95	73	77	137	157	171	178	188	125	119	189	132
63	45	32	66	76	40	48	69	49	22	32	68	80	110	46	81	65	56	68	43
27	9	34	31	40	18	23	52	33	32	22	23	36	54	41	6	27	17	2	48
68	55	105	91	58	38	54	74	23	45	27	71	40	85	79	86	55	33	49	50
94	126	92	141	121	43	47	80	37	88	61	114	88	130	107	149	85	78	84	125
41	20	26	59	34	9	22	26	18	50	19	27	29	30	33	74	53	22	24	26
152	147	182	188	115	73	59	135	53	97	71	187	148	150	123	227	138	140	133	159
291	122	230	249	157	71	108	194	120	168	112	275	167	212	170	244	149	139	231	189
173	150	116	167	221	67	95	141	86	77	87	205	136	220	177	242	118	114	160	117
244	149	93	200	207	38	25	87	35	71	19	172	99	186	125	189	76	164	201	76
204	196	143	158	178	101	89	209	107	141	140	171	172	212	160	194	144	106	124	214
86	49	49	113	57	21	21	50	22	44	27	81	44	55	53	77	12	49	53	48
360	263	304	418	263	167	203	311	170	210	138	322	257	287	310	382	228	261	290	313
260	95	112	273	178	36	16	75	40	68	86	213	62	78	189	184	83	186	173	70
195	169	134	252	160	77	47	154	53	79	79	186	137	154	153	246	91	172	168	178
218	185	178	200	177	61	157	167	127	124	156	236	169	172	137	230	159	166	177	246
204	199	158	288	166	71	102	161	59	125	88	249	159	206	187	263	142	151	158	186
234	132	177	156	117	76	103	194	59	169	26	190	117	185	207	201	110	138	155	146
295	254	189	332	229	105	120	171	103	118	117	226	205	205	253	349	160	184	220	277
125	58	40	115	74	24	20	59	28	77	20	84	43	46	75	108	70	59	85	90
245	234	138	255	244	128	172	239	132	146	156	209	219	264	195	218	206	207	224	242
213	252	139	257	212	113	178	215	183	70	161	212	253	345	156	216	195	145	178	156
271	163	177	373	222	128	157	277	128	156	121	267	180	279	222	260	156	189	199	218
304	204	187	277	243	110	124	214	74	177	41	240	184	273	169	182	193	211	236	174
296	237	130	332	291	113	194	291	121	175	141	251	244	389	190	156	241	214	225	286
287	182	104	291	241	77	49	145	55	106	56	268	138	178	160	143	135	175	220	108
263	230	131	333	249	93	143	253	146	120	163	226	211	281	131	190	157	201	219	245
199	140	231	265	127	120	208	250	87	176	57	208	189	232	220	268	236	207	205	337
253	206	257	202	242	126	179	308	147	194	115	313	185	311	255	286	219	153	227	347
398	324	410	495	234	188	257	435	171	292	138	454	288	422	368	434	294	284	358	499
295	244	293	217	198	146	199	239	139	205	102	276	249	306	282	325	182	209	275	317
379	274	289	336	214	144	257	286	135	221	117	363	243	293	324	372	215	206	298	374
263	180	229	216	221	117	153	248	117	184	63	228	192	302	278	249	199	163	238	182
173	165	200	173	102	89	172	252	90	173	86	244	139	197	160	195	137	144	190	244
189	155	228	207	147	102	207	266	157	175	152	251	192	261	243	263	215	165	206	363
600	216	254	687	277	121	106	295	38	120	49	448	176	264	274	533	205	459	921	229
274	148	173	195	141	87	116	189	87	125	68	243	137	193	194	201	149	124	165	157

rs3911	*rs4141886*	*rs8179021*	*rs9341278*	*rs9786139*	*rs9786184*
180	156	168	181	275	314
103	90	89	55	130	168
60	113	56	58	56	149
179	118	139	132	129	239
94	100	115	67	134	193
50	122	66	31	111	244
135	130	143	125	153	205
136	145	113	82	174	253
157	148	159	158	260	296
90	91	85	44	103	141
117	202	117	95	149	202
31	69	88	29	56	102
18	19	16	5	33	85
43	63	38	42	70	89
64	88	99	97	128	156
28	37	30	12	42	39
68	127	90	75	104	225
131	136	107	120	222	339
100	191	84	104	153	178
63	238	85	55	150	189
169	127	147	114	140	251
45	43	79	45	39	79
257	222	226	187	288	440
56	153	85	66	154	272
87	154	87	107	235	308
155	132	146	134	196	243
133	160	116	160	167	238
121	127	152	67	275	227
142	211	156	109	254	275
56	97	59	29	101	86
228	203	174	115	171	283
137	224	148	107	127	206
201	182	141	146	185	299
190	203	179	100	178	338
201	194	167	141	165	374
124	172	90	64	113	270
207	204	162	109	115	267
208	160	132	111	198	315
174	187	161	168	215	371
377	246	288	194	349	531
259	188	158	129	225	323
270	213	234	163	271	435
190	160	215	157	229	291
153	131	154	110	200	249
221	162	164	156	185	313
107	225	189	48	197	856
157	110	140	100	204	259

Supplementary Table S4. Locus balance metrics values for all 132 SNP markers included in HID Ion Ampliseq Identity Panel, in Brazilian criminal samples.

Samples	**Locus**																	
Blood	*rs1005533*	*rs10092491*	*rs1015250*	*rs1024116*	*rs1028528*	*rs1031825*	*rs10488710*	*rs10495407*	*rs1058083*	*rs10773760*	*rs10776839*	*rs1109037*	*rs12997453*	*rs13218440*	*rs1335873*	*rs1355366*	*rs1357617*	*rs1360288*
BL.PF07	1,11460739	1,21073903	0,41050808	1,35103926	1,27569284	1,30427252	0,4312933	1,25490762	1,2834873	1,14838337	0,43908776	0,63654734	0,3767321	1,18735566	1,20814088	1,01847575	0,66512702	1,30946882
BL.PF09	1,66114529	1,01751065	0,35021297	1,32039754	1,23047799	1,09323237	0,5584477	1,13582584	1,57595835	0,989115	0,7430194	0,989115	0,34548036	1,15948888	0,47326077	1,12162802	0,79507809	1,26360625
BL.PF11	0,98540146	1,03593487	0,33352049	1,52105559	1,29365525	1,31386861	0,6316676	0,96013476	1,33913532	1,33408198	0,43964065	0,87422796	0,48006738	1,04604155	1,21785514	1,27849523	0,72262774	1,02582819
BL.PF20	1,07013943	1,57972963	0,39174747	1,49692123	1,04465992	1,42366763	0,68794678	0,88541298	1,36952367	1,29627008	0,624248	0,63698776	0,37900771	1,34085923	1,32493453	1,25805082	0,71024135	0,90770755
BL.PF22	1,27393838	1,21981682	0,39550375	1,21149042	1,54454621	1,10324729	0,51623647	1,18651124	1,81931724	1,20316403	0,59533722	0,87427144	0,32056619	1,27810158	1,04079933	1,00749376	0,91590341	1,19067444
BL.PF24	0,66210971	0,87586167	0,35451544	0,90714244	1,82992527	0,74031165	0,49006546	0,484852	1,44412906	1,4806233	0,47963853	0,72467126	0,15119041	1,69437525	1,30857904	0,62040202	0,22939234	1,18345595
BL.PF25	0,97151872	1,38254588	0,35124139	1,37133605	1,28165739	1,52080047	0,55301835	1,14340281	1,41991198	1,32276011	0,48575936	0,71742921	0,36992444	1,06119738	1,12098314	1,11350992	0,70248277	1,12845636
BL.PF26	0,86397657	1,27766027	0,35876993	1,00309144	1,37650504	1,511959	0,49422389	0,95916043	1,46802799	1,36186137	0,34412626	1,14952815	0,24162057	1,78286691	1,26667751	0,78343638	0,79808005	1,52294175
BL.PF27	1,16990514	1,41069643	0,33321623	1,3669162	1,42528984	1,35475502	0,44266681	0,91208821	1,75607383	1,15774397	0,5180661	0,85128233	0,32591952	1,17233738	1,06775126	0,9461395	0,90235927	1,18693079
BL.PF30	0,94503432	1,41491171	0,44347979	1,35155746	1,45186836	1,24068751	0,69689681	0,95559336	1,46770693	1,10341996	0,36956649	0,78664868	0,3220508	1,46770693	1,21956943	0,7919282	0,70745586	1,15621517
Epithelial																		
EP.PF01	0,89321748	1,21195898	0,40769262	0,98587489	1,80867273	0,57818227	0,68937117	0,77832228	1,31944158	1,24902195	0,34097929	0,66713339	0,33356669	1,69007124	1,28979121	0,98587489	0,38174855	1,00070008
EP.PF08	0,95598164	0,58331083	0,48609236	1,4015663	1,47448015	0,93977856	0,64812314	0,9073724	1,3205509	1,31244937	0,55900621	0,65622468	0,2673508	1,28004321	0,9478801	1,547394	0,78584931	1,03699703
EP.PF10	0,77620522	0,9752322	0,11941619	0,63688633	0,9553295	1,15435648	0,65678903	0,41795666	1,27377267	0,87571871	0,73639982	0,71649713	0,33834586	1,19416188	0,61698364	0,83591331	1,29367536	1,8907563
EP.PF13	1,38316451	0,94306671	0,40866224	1,29671673	1,45389452	1,06095005	0,84876004	1,02165561	1,24170451	1,06880894	0,54226336	1,0295145	0,33007335	0,98236116	0,79374782	0,73873559	0,55012225	1,37530562
EP.PF14	1,19589977	0,96648226	0,4344289	1,42531728	1,40091116	0,8493329	0,44907257	0,72730231	1,54734787	1,11291897	0,49300358	1,07386918	0,38561666	1,14220631	1,23494956	0,90302636	0,77123332	1,15196876
EP.PF15	1,37322133	0,987485	0,52460141	0,89490828	1,26521516	0,74061375	0,78690211	0,83319047	1,94411109	1,00291445	0,30858906	0,95662609	0,41659523	1,37322133	0,83319047	1,08006172	0,29315961	1,29607406
EP.PF17	0,68298853	1,39850032	0,39434457	1,36191165	1,37817328	1,235884	0,56915711	1,09766013	1,52046255	1,20742615	0,57322251	0,65046526	0,26831692	1,48793929	1,31312675	0,96350167	0,63420363	0,87812811
EP.PF18	1,09788145	1,54754566	0,38542647	1,06576258	1,41615028	1,18839827	0,44674431	0,94312689	1,34315284	1,31687376	0,64237745	0,82341109	0,26571067	1,67894105	1,29643448	1,02488402	0,68033611	1,08620186
EP.PF21	0,67141142	1,1576059	0,47075973	1,25407306	1,16146459	1,1576059	0,81418282	0,85276968	1,28880123	1,242497	0,30097753	0,68298748	0,37043389	1,50874636	1,14988853	0,89135654	0,53249871	0,69456354
EP.PF28	0,52591049	0,75421272	0,42398985	0,8153651	2,07918101	0,8316724	0,64821526	0,60337018	1,79788005	1,43504258	0,28130096	1,41873528	0,15899619	1,89572386	1,07220511	0,59521652	0,54629462	0,93359304
Saliva																		
SA.PF02	1,22100077	1,07209824	0,43347182	1,55851318	1,27063495	1,35335858	0,58899224	0,80738262	1,25409022	1,42284643	0,3441303	0,72465899	0,37391081	1,25078128	1,13165925	0,80076473	0,81400051	1,2011471
SA.PF03	1,40784018	1,03467772	0,41556728	1,3060686	1,29758764	0,75480588	0,49189597	0,81417263	1,58594044	1,35695439	0,44101018	0,97531097	0,13569544	1,59442141	1,18733509	0,71240106	0,64455334	1,28062571
SA.PF04	0,84773893	1,18916239	0,44035867	1,31719619	1,23766004	1,21438117	0,70224598	1,02427038	1,24929948	1,2570591	0,40544036	0,7100056	0,36276243	1,55580463	1,18140277	1,00875113	0,83221968	1,14648446
SA.PF05	1,20712054	0,76156034	0,39184017	0,80580036	1,74432078	1,39988062	0,60040027	0,50876023	1,59580071	1,48520066	0,8911204	1,50732067	0,12640006	1,98132088	1,26716056	0,55932025	0,47400021	1,27032056
SA.PF06	0,94060833	0,88546922	0,45733026	0,96006919	1,65417327	1,11575609	0,64545192	0,86276488	1,32982557	1,41415598	0,42813896	0,72329537	0,28542598	1,43361684	1,13521695	0,96979963	0,57409543	1,51470376
SA.PF12	0,89531313	1,12364802	0,57684604	1,00347176	1,06355989	1,38202697	0,6519562	0,99145413	1,25283749	1,3940446	0,3244759	0,69101349	0,38456403	1,32794766	1,19575377	1,15068768	0,63693417	1,00647617
SA.PF16	0,90288925	1,08346709	0,53516708	1,11958266	1,34284255	0,94557128	0,70917846	0,72559463	1,00795272	1,68101561	0,32832336	0,71574493	0,30205749	1,58908507	1,43477309	1,00795272	0,75514373	1,10973296
SA.PF19	1,37998422	0,79146154	0,21985043	1,31910256	1,52204141	1,1635161	0,54455259	1,06542899	1,34277876	0,96057725	0,60205194	0,97748882	0,19955654	1,20410387	1,14322222	0,69675674	0,74749145	1,46115976
SA.PF23	0,66991582	1,15108551	0,60611431	1,22286221	1,42224191	1,18298626	0,6832078	0,78156845	1,42755871	1,44616748	0,34559149	0,60877271	0,26052282	1,44616748	1,03677448	0,76030128	0,55560479	1,0899424
SA.PF29	1,05908132	1,02986528	0,31407239	1,14672943	1,54844993	1,33663366	0,58432073	0,66466483	1,41697776	1,49001785	0,49667262	1,27820159	0,27024834	1,40967375	1,32202565	0,59892875	0,62814478	1,25628956
Semen																		
SE.PF02	0,69259238	1,35081948	0,46525289	1,63895906	0,9437232	1,74469835	0,898784	0,73753157	1,18163661	1,1525583	0,31193092	0,63707925	0,52869647	1,37989779	1,20542795	1,57551548	0,92257534	0,77982729
SE.PF03	0,48509918	1,67246404	0,7614929	1,56811131	0,67124189	1,97424086	0,87994735	0,6909843	1,23531071	1,04070697	0,21998684	0,56688916	0,43151264	1,70630817	1,42427376	1,87270847	0,96173733	0,74175049
SE.PF07	0,92768867	1,66368656	0,45201157	1,14541152	0,88745727	1,60925585	0,64843545	1,07441494	1,30870366	0,95372075	0,4307126	0,70049961	0,40704707	1,44359716	1,14067841	1,38443334	0,9608204	0,83775966
SE.PF22	1,0767825	1,01767511	0,3649239	1,13075012	1,28237343	1,29522287	0,74012735	0,77353588	1,51623312	1,12304046	0,72984781	1,27466377	0,39319266	1,17957797	0,97912681	1,08192228	0,89946032	1,29008309
SE.PF26	0,95303351	1,32079233	0,55163822	1,37461069	1,04945808	1,77824841	0,61666874	0,93060919	1,22212533	0,9732154	0,36327395	0,68842656	0,56285038	1,34770151	1,08982185	1,58988414	1,09430671	0,68842656
SE.PF27	0,89923274	1,06189258	0,44194373	0,98209719	1,8629156	1,14782609	0,55549872	0,50946292	1,53759591	1,79846547	0,28542199	1,20613811	0,23324808	1,75549872	1,1601023	0,85319693	0,68132992	0,97902813
SE.PF30	0,7041719	1,36104867	0,61483666	1,37681371	0,90648994	1,78407731	0,78825212	0,68315184	1,32951858	0,99582518	0,4072636	0,69891688	0,50185385	1,43724637	1,19288821	1,37681371	0,80401717	0,90648994
Control (Oral Swab)																		
CT.PE02	1,36865149	1,05078809	0,64360771	1,28196147	0,9614711	1,17688266	0,91681261	0,4150613	0,63309982	0,50700525	0,34938704	0,52276708	0,74343257	1,51313485	1,5	1,04290718	0,95884413	0,95096322
CT.PE03	1,09927758	0,90042452	0,87361287	1,43442318	1,24897594	1,08140314	0,96298503	0,40887763	0,73955463	0,45803232	0,30833395	0,69263424	0,84456692	1,5014523	1,15736948	0,96745364	1,07693453	0,81998957
CT.PE04	1,53091855	1,24424655	0,61987192	1,388333	1,09115469	1,1076646	0,97858715	0,37072243	0,73994397	0,51030618	0,29867921	0,66039624	0,70842506	1,43636182	1,44086452	0,99359616	0,89603762	0,8194917
CT.PE07	1,42639507	1,53033393	0,6103644	1,15438484	1,09246382	1,21630587	0,85362557	0,1968204	0,58161535	0,508637	0,31845099	0,59709561	0,6678625	1,58562056	1,5325454	1,08804089	1,12121287	0,85583704
CT.PE08	1,39225842	0,63990732	0,60310754	1,29208123	1,20212621	1,00586071	1,04266049	0,21875426	0,58266321	0,55608559	0,35368679	0,57039662	0,80550634	1,38816955	1,39021398	1,02017173	1,03243833	0,8157285
CT.PE09	1,50371125	0,68374327	0,71779945	1,19196623	1,10813564	0,96667152	1,05574152	0,33270266	0,59205356	0,5239412	0,31436472	0,60777179	0,75971474	1,72900597	1,84427303	1,01906564	1,27055742	0,69422209
CT.PE10	1,54098928	1,35731581	0,69422345	1,29816672	1,15807679	1,02421308	0,92148046	0,35800761	0,60705638	0,98685576	0,43583535	0,62262193	0,80318229	1,39778623	1,36976825	0,94949844	1,08647527	0,8716707
CT.PE11	1,40370645	1,00607858	0,67249815	1,33699036	1,13417346	1,23024463	1,00340993	0,39229059	0,49369904	0,61111935	0,29888807	0,67783543	0,59243884	0,74455152	1,52112676	0,9767235	0,96871757	0,84062268
CT.PE12	1,71481151	1,652911	0,34463529	0,74615213	0,77291992	0,66417578	1,80682579	0,32790542	0,49855008	0,07695739	0,11209012	0,32957841	0,46676333	1,50234218	0,93519964	0,94189159	0,87831809	0,20577738
CT.PE13	1,794298	1,4961202	0,58850882	1,1953268	0,81606556	0,77683164	0,87360865	0,35310529	0,80298759	0,28771542	0,22494115	0,61204917	0,73498213	1,12209015	1,36534046	0,83175913	0,83699032	0,51004098

rs1382387	*rs1413212*	*rs1454361*	*rs1463729*	*rs1490413*	*rs1493232*	*rs1498553*	*rs1523537*	*rs1528460*	*rs159606*	*rs1736442*	*rs1821380*	*rs1872575*	*rs1886510*	*rs1979255*	*rs2016276*	*rs2040411*	*rs2046361*	*rs2056277*	*rs2076848*
0,92234411	1,01068129	1,1301963	1,46016166	1,48874134	0,55080831	1,16657044	0,94312933	1,28608545	1,00288684	1,3432448	1,34844111	1,82130485	0,61576212	0,77424942	0,95352194	1,37961894	0,82361432	1,2834873	0,79243649
1,28726929	0,9228585	1,39611926	0,51112163	1,71320398	0,52531945	0,85660199	1,23047799	1,16895409	0,71462376	1,1121628	1,76526266	1,57122575	0,76668244	1,03170847	1,20208235	1,43398012	0,9275911	1,45291055	0,90866067
1,52610893	1,05614823	1,11173498	1,39472207	1,71308254	0,54070747	1,06120157	1,30376193	0,83885458	0,67209433	1,0157215	1,39977541	1,51600225	0,62661426	0,67209433	1,16226839	1,27344189	0,4952274	1,03088153	0,86412128
1,27079057	1,13383821	1,76764102	1,08924906	1,46825678	0,78667988	1,15931772	1,07332437	0,89815274	0,71024135	0,97140633	1,89185363	1,50010616	0,65609739	0,99688584	1,10517376	1,43640739	1,04147498	1,19753698	0,80897445
1,49875104	1,20732723	1,28226478	1,5570358	1,34471274	0,52456286	1,0990841	1,20316403	0,9450458	0,60366361	1,07826811	1,53621982	1,32805995	0,72439634	0,89092423	1,31557036	1,78601166	0,60366361	0,92422981	0,85761865
1,14174825	1,66830794	1,3137925	1,5640387	0,75595204	0,95927707	0,7611655	0,9540636	0,68296356	0,55784047	0,62040202	0,74031165	0,7194578	0,54741354	1,45976945	1,43370214	1,22516364	0,30238081	0,74552511	1,40242136
1,23308146	1,29660384	1,37133605	1,39375571	1,28165739	0,68006311	1,06493399	1,12471975	1,10230009	0,85942041	0,80337125	1,40122893	1,41991198	0,66885328	0,94162584	1,09482687	1,50211741	0,83326414	1,10977331	0,81458108
1,42775789	1,31426944	0,99943052	1,33623495	0,95183859	0,8493329	1,01773511	1,25935568	0,95549951	0,83468923	1,14952815	1,32891311	1,17149365	0,7468272	1,00309144	1,16417182	1,47901074	0,47958021	0,94451676	0,86031565
1,3255682	1,05072562	1,26962679	1,26476232	1,42285761	0,54725293	1,10423479	1,17476961	0,99721644	0,92181715	1,05559009	1,44231549	1,35718726	0,72723833	0,83182445	1,05072562	1,56392725	0,74669621	1,00208091	0,82209551
1,25652607	1,1878923	1,27236464	1,20373086	1,18261278	0,60186543	1,17733326	1,12981756	0,94503432	0,67049921	0,85528245	1,44130932	1,49410453	0,68633777	0,85528245	1,06646331	1,72112395	0,45403883	1,34099842	0,9397548
1,39727381	1,50105012	1,40468641	1,44916197	1,03405675	0,88209859	1,0896512	1,1971338	0,8672734	0,58188856	0,79685377	1,17860231	1,02293786	0,50405634	1,37132974	1,51216901	1,68636495	0,60042005	0,84503562	1,28979121
0,65622468	1,40966784	1,5879017	1,57980016	1,35295706	0,64812314	1,20712935	1,22333243	0,721037	0,70483392	0,79395085	1,14231704	1,18282474	0,39697543	0,96408318	1,14231704	1,57980016	0,47799082	0,71293546	0,60761545
1,29367536	1,63202123	1,59221583	1,21406457	0,79610792	0,57717824	1,01503759	0,81601061	2,30871296	1,11455108	1,31357806	1,27377267	1,27377267	0,69659443	0,85581601	1,63202123	1,33348076	0,45776205	1,15435648	0,65678903
1,26528117	1,1709745	1,09238561	1,41460007	1,31243451	0,74659448	0,69158226	1,19455117	0,90377227	0,56584003	1,2495634	0,94306671	0,99807894	0,58155781	1,20241006	1,45389452	1,20241006	0,47153336	0,97450227	0,84090115
1,4301985	1,615685	1,21542467	1,32281158	0,79563944	0,73218353	1,05922551	0,73706476	0,95671982	0,74194598	0,90302636	0,78099577	1,41067361	0,56622193	1,200781	1,74747803	1,32281158	0,54669704	1,16661243	0,99088838
1,68181039	1,54294531	1,63552203	1,06463226	1,09549117	0,91033773	0,63260758	1,2189268	0,52460141	0,66346648	0,91033773	0,92576719	1,38865078	0,55546031	1,28064461	1,83610492	1,34236242	0,43202469	1,04920281	0,95662609
1,52859337	1,16270666	1,57331286	1,41476195	1,38223868	0,79275454	1,24401482	1,11392176	0,70738097	0,72770801	0,99602493	1,2887343	1,37004246	0,66672689	1,08546391	1,48793929	1,30093053	0,73177342	1,06920228	1,01228657
1,32855335	1,45410894	1,32563346	1,32271356	1,2730753	0,61317847	1,34899264	0,97816566	0,8058917	0,6920157	0,95188658	1,25263602	1,509587	0,70369529	0,9285274	1,24387633	1,47746812	0,50514226	0,90808812	0,89348863
1,62836563	1,58592008	1,39684445	1,30809467	0,92222603	0,92222603	1,2695078	0,94151946	0,88363917	0,77945464	0,6212485	0,94537815	1,20776882	0,58266164	1,22320357	1,58977877	1,71711542	0,51706397	0,69456354	1,06499743
1,88757021	1,6144229	0,88874796	1,73672767	0,78682732	0,64821526	0,92136257	1,09666606	0,65636891	0,65229208	1,05589781	0,66859938	0,90505526	0,49329589	1,57773147	1,85495561	1,72449719	0,17938032	0,51368001	0,93359304
1,25739917	1,14158609	1,04231773	1,17136659	1,42615537	0,75113056	0,94966727	1,10518769	1,14489503	0,8272363	1,15151292	1,46255377	1,40961065	0,63862642	0,82061841	1,21438288	1,41622854	0,67833376	1,07871613	0,90995992
1,20429702	1,50961176	1,12796834	1,00923483	1,17037316	0,6784772	0,97531097	1,20429702	0,92442518	0,59366755	0,94986807	1,21277799	1,75555974	0,5088579	0,95834904	1,43328308	1,92517904	0,46645307	0,55974369	0,88202035
1,3191361	1,24347976	1,31331638	1,32495581	1,14648446	0,79536147	1,21438117	1,01845066	1,01845066	0,84579903	1,06112859	1,2900375	1,31525628	0,6440488	1,01263094	1,37151356	1,55192482	0,62658965	0,90981592	0,92145536
1,5768407	1,3493206	1,5800007	1,75380078	1,04280046	0,85636038	0,94800042	1,36196061	0,4455602	0,6794003	0,94168042	1,33352059	0,8911204	0,40132018	1,09968049	1,29876058	1,42200063	0,12324005	0,62568028	1,08072048
1,39793859	1,2552256	1,03791264	1,46929508	0,90817356	0,9308779	1,09305175	0,90168661	0,66815626	0,5221998	0,92763442	0,8043823	1,06710394	0,59355629	0,66815626	1,36550382	1,53092115	0,50922589	0,65193888	1,0152083
1,24983309	0,98844973	1,27987715	1,38503138	0,99445854	0,67298705	1,15970089	1,0214982	1,04553345	0,9283616	1,17772733	1,07257311	1,25884631	0,71204433	1,03051142	1,17171852	1,31893444	0,87127787	0,97042329	0,80217653
1,50043776	1,79921202	1,67444915	1,54640304	0,88975631	0,85364074	1,33627608	1,29359405	0,90617248	0,78797607	0,83065811	0,90617248	1,47088866	0,74529403	1,1622647	1,40850722	1,36582519	0,66649642	0,79782577	0,97512039
1,49160059	0,89969559	1,2345447	1,39013116	1,34616107	0,43293622	1,08572288	1,24469165	1,07219362	0,62234582	1,33263182	1,42733662	1,45777744	0,55131722	0,89631328	1,22439776	1,58630539	0,51411177	1,16689842	1,08572288
1,58174568	1,44882588	1,31324767	1,32919805	0,86397873	0,82410279	1,10057599	0,98626495	0,89853788	0,8852459	0,78954364	1,00487373	1,24678777	0,63801506	1,18830306	1,0926008	1,72795747	0,50243686	0,76561808	0,78156845
1,54114592	1,32202565	1,27820159	1,64340205	0,82535303	0,45284856	0,75231294	1,00795325	0,87648109	0,65005681	0,803441	1,27089758	0,91300114	0,63544879	1,1248174	1,32202565	1,27089758	0,4017205	0,81074501	0,83265704
1,35081948	1,51735887	1,77642014	0,97808847	0,66351407	1,06532339	0,99394936	1,01509722	1,01509722	0,93314927	0,96751454	1,60723727	1,44334136	0,75867943	1,20278447	1,572872	1,0626799	0,79040122	1,02567115	0,79833167
0,97019836	1,85860675	2,11243772	0,90533045	0,56688916	1,98270189	1,08865282	1,00968318	1,10839522	0,86584563	0,70790636	1,41581273	1,46093823	0,63175707	1,35940585	1,10839522	0,93635424	0,98147974	0,77841497	0,81789978
1,10281357	1,25190639	2,02576913	1,15251118	1,04364975	0,88272417	1,09571391	1,05074941	1,13594531	0,79989482	0,98921904	1,38916645	1,66841967	0,79989482	1,16907704	1,10044702	1,12174599	0,94425454	1,02708388	0,70996582
1,655007	1,20784672	1,28237343	1,22840581	1,01767511	0,75297679	0,93543874	1,25924445	1,03052454	0,67074041	0,91744953	1,53165244	1,12561035	0,65532109	1,22583593	1,42885697	1,21298649	0,55252563	0,83778304	0,97141715
1,16830696	1,39479258	2,087704	0,87006354	0,84763922	1,03151862	0,88351813	1,1212159	1,02927619	0,86333624	0,84988165	1,39927744	1,50467173	0,74448736	1,13691292	1,96212782	1,11224617	0,72879033	1,00460944	0,72206304
1,82608696	1,67570332	1,43017903	1,30434783	0,74578005	0,86547315	0,66905371	1,48849105	0,60153453	0,54629156	0,52173913	1,37493606	1,03120205	0,47263427	1,23375959	1,77391304	1,37186701	0,27314578	0,89923274	1,08951407
1,3347736	1,53446413	2,07573059	1,04574782	0,81189969	1,25857589	0,91699997	1,12720054	0,99319768	0,83029224	0,71993694	1,47665898	1,32689108	0,73832949	1,19026071	1,52658161	1,0404928	0,7777421	0,89597991	0,86707734
1,13222417	1,07180385	0,99036778	0,80122592	1,03765324	1,12171629	1,56042032	1,30035026	0,3704028	0,76182137	1,26882662	0,59632224	0,52539405	1,01663748	1,1821366	0,80647986	1,12697023	0,38616462	1,09544658	1,28458844
1,07693453	0,89595591	0,8356297	0,84903553	1,03671706	1,09034036	1,36739406	1,26685038	0,35078573	0,85126983	1,0590601	0,63230804	0,71944589	1,02107693	1,2824905	0,78870932	1,28695911	0,34184851	1,15513518	1,01437402
1,05213128	0,84950971	0,66940164	0,65739444	1,13768261	1,24124475	1,42735641	1,19921953	0,23113868	0,77596558	1,32979788	0,41725035	0,52681609	1,18721233	1,37182309	0,79997999	1,26375825	0,29417651	1,09115469	1,32229338
1,14775045	0,91996953	0,50421407	0,7695899	1,0615033	1,13227019	1,34014792	1,1764995	0,3051822	0,70324594	1,24063199	0,54623191	0,71651473	0,91112367	1,39764602	0,78064722	1,19861415	0,32950832	1,14553898	1,30034155
1,4167916	0,95679433	0,83208396	0,76461769	1,01403844	1,06719368	1,38816955	1,48221344	0,32710917	0,73599564	1,19394848	0,57857435	0,619463	1,011994	1,34932534	0,85661715	1,15919313	0,34550906	1,12034892	1,25528145
1,31247271	0,94309416	0,89070004	0,73613739	1,08455829	1,1788677	1,18148741	1,19720565	0,30912531	0,83044681	1,02430505	0,41915296	0,67850386	1,08717799	1,21292388	0,92999563	1,2181633	0,28816766	1,02954446	1,1788677
1,11138015	0,8716707	0,56658596	0,98685576	1,04911795	1,12071947	1,09270149	1,21722587	0,21480457	0,9339329	1,17364234	0,33932895	0,63507437	1,00553442	1,26080941	0,91214113	1,26703563	0,38913871	1,05223106	1,21099965
1,4864344	0,96871757	1,03009637	1,05411416	1,02475908	1,0994811	1,33965901	1,21156412	0,31756857	0,85930319	1,36100815	0,59510749	0,65915493	0,91267606	1,33699036	0,70985915	1,11549296	0,27220163	1,03276501	1,26226835
0,70767343	1,27481597	1,18614767	0,53535579	0,65413785	0,95192951	2,49776935	2,17990185	0,36973009	0,29444568	1,84195851	0,23087218	0,62067812	1,06401963	0,98706224	0,66250279	0,8013607	0,32790542	0,66417578	1,88043721
0,87622424	0,70882618	0,55973728	0,64605191	0,91022698	1,26333227	2,34095731	1,11424337	0,28509983	0,50480979	1,57981923	0,27202185	0,55189049	1,23717632	1,08808742	0,88407103	1,06193147	0,2484815	0,85791508	1,35749368

rs2111980	*rs214955*	*rs221956*	*rs2269355*	*rs2292972*	*rs2342747*	*rs251934*	*rs2830795*	*rs2831700*	*rs321198*	*rs338882*	*rs354439*	*rs3780962*	*rs4288409*	*rs430046*	*rs4364205*	*rs445251*	*rs4530059*	*rs4847034*	*rs560681*
0,71968822	0,4312933	1,44976905	1,86807159	0,9119515	0,47286374	0,99769053	1,39001155	0,85479215	0,5897806	1,13539261	1,28088915	0,73267898	0,89896074	1,01847575	1,38741339	1,31726328	0,75606236	0,65473441	1,22372979
0,87553242	0,26029342	0,94652153	1,50023663	1,01277804	0,46852816	1,24467582	1,94983436	0,95125414	0,38807383	1,63274965	1,13109323	0,5584477	1,23047799	0,85186938	1,24467582	0,91812589	0,59630857	0,44486512	1,28726929
1,06120157	0,21224031	1,23301516	1,71813588	0,97024144	0,43964065	1,32397529	1,63728243	0,97024144	1,00056148	1,61201572	0,9904548	0,81864121	0,84896126	0,42953397	1,61706906	1,19258843	1,04604155	0,77821449	0,95002807
0,77712506	0,36626796	1,10198882	1,35041404	0,96503645	0,38537759	0,91089249	1,78356572	1,02873523	0,63380282	1,57017482	1,20072192	0,94911176	0,71024135	0,94592682	1,52558567	1,1911671	0,88859792	0,85037865	0,77394012
1,26561199	0,316403	1,36136553	1,0990841	1,21149042	0,45795171	1,23646961	1,75270608	1,38218152	0,61199001	1,31557036	1,04496253	0,60366361	0,89508743	0,4662781	1,15320566	0,97835137	0,9408826	0,71190674	1,32805995
0,85500782	0,53698662	1,70480218	0,96449053	2,38776574	0,50570584	1,30336558	0,86543474	1,32421943	0,39622314	1,93940798	0,91756937	1,10004055	0,79244627	1,74650988	0,89671552	0,50570584	1,3555002	0,57348086	1,12610786
0,63896039	0,37366105	1,42364859	1,08735365	1,40496554	0,33629494	0,91920618	1,76741676	1,22187163	0,68006311	1,31528689	1,12845636	0,997675	0,7136926	0,75479532	1,43859503	0,9266794	0,98272856	0,66885328	1,17329569
1,11657989	0,27456883	1,81215425	1,02505695	1,01407419	0,50520664	1,60714286	1,70964855	1,19712008	0,78343638	1,42043606	1,14220631	0,6626261	1,02505695	0,61503417	1,48267166	0,84567198	1,23739017	0,79808005	1,30328669
1,03369997	0,36726752	1,34016161	1,52744372	0,97775856	0,51563387	1,1188282	1,52014702	0,98262303	0,54238845	1,29394914	1,35475502	0,80020539	0,91695268	0,46212469	1,5396049	1,03613221	0,93154609	0,7734508	1,36448396
0,74441251	0,5437907	1,40435267	1,23012847	1,40435267	0,39068458	0,92391623	1,66304922	1,25652607	0,57546782	1,48354549	1,08758139	0,63354256	0,82360533	0,59658591	1,61553352	1,07174283	1,02422714	0,7180149	1,10341996
0,85986081	0,49664374	1,18230861	1,00070008	1,45286826	0,61524523	1,04888193	1,53440679	1,24531565	0,5448256	1,59000124	1,13042046	0,98587489	0,79685377	1,33056047	1,36391714	1,17118972	1,27867232	0,8672734	1,16377713
0,96408318	0,38077235	1,75803403	1,06940319	1,73372941	0,32406157	1,23143397	1,66081555	1,32865244	0,91547394	2,00108021	0,9478801	0,79395085	0,85066163	0,81015393	1,68512017	0,82635701	1,3205509	0,76154469	1,72562787
1,07474569	0,11941619	1,51260504	1,99026979	1,01503759	0,33834586	1,25386997	2,20919947	1,57231314	0,55727554	1,85095091	1,05484299	1,05484299	1,07474569	1,9504644	1,25386997	0,89562141	0,87571871	0,55727554	1,31357806
1,03737338	0,18075445	1,68180231	1,1002445	1,53248341	0,62085225	1,12382117	1,46961229	1,32815229	0,62871114	1,3202934	1,01379672	0,8094656	1,06095005	0,74659448	1,46961229	1,1002445	0,85661893	0,72301781	1,37530562
0,97136349	0,53205337	1,35698015	1,42043606	1,21542467	0,47347869	1,35698015	1,5327042	1,09339408	0,57110316	1,35209893	0,81028311	1,16661243	0,83957045	0,97136349	1,12756264	1,36186137	1,41555483	0,92743248	1,23006834
1,01834391	0,41659523	1,32693297	1,55837476	1,15720898	0,7251843	1,14177953	1,29607406	1,28064461	0,44745414	1,43493914	0,92576719	1,06463226	0,89490828	1,38865078	1,37322133	1,00291445	1,37322133	0,44745414	0,84861992
0,68298853	0,30084018	1,53265878	1,3375192	1,03261361	0,42280242	0,98789412	1,25621104	0,9756979	0,78868913	1,49607011	1,06513687	0,54069925	0,67892312	1,4391544	1,56111663	1,19116451	1,26840726	0,78868913	1,32125757
0,96940596	0,31242903	1,2730753	1,72273951	1,53294618	0,30366934	1,29935438	1,29935438	1,14751971	0,59857898	1,60010382	1,10372125	0,77377283	0,70953509	1,05700289	1,42490997	1,00152484	1,34607274	0,68325601	1,08912176
0,87206311	0,55950952	1,39298577	0,61738981	1,23092094	0,5093466	0,98010633	1,0997256	1,65151775	0,83347625	1,7788544	1,20391014	0,79874807	0,66369405	1,10358429	1,48559424	1,10358429	1,05728005	0,88749786	1,03412794
1,63480703	0,44437398	1,64703751	0,61152383	1,73672767	0,69713716	1,02736003	0,92136257	1,25158543	0,53406414	2,24633086	0,68082986	0,87651749	0,84390288	1,23527813	1,1985867	0,3995289	1,214894	0,50960319	1,01512955
0,85370786	0,34743924	1,2011471	1,14158609	1,02908195	0,4467076	0,94635832	1,42615537	1,15482187	0,73127689	1,42615537	1,1647487	0,89672414	0,85370786	0,95297621	1,3765212	1,15151292	0,83054524	0,73458583	1,2011471
1,51809273	0,63607237	1,29758764	1,17885413	1,44176404	0,55126272	0,94986807	1,45024501	0,91594421	0,63607237	1,3315115	1,00075386	0,76328685	0,6784772	0,88202035	1,27214474	0,55126272	1,35695439	0,483415	1,45024501
0,82446006	0,47721688	1,37927318	1,07470794	1,19886192	0,41513989	1,05142906	1,65861965	1,28033797	0,62852955	1,29197741	1,18916239	0,87683752	0,78760185	0,91757555	1,46268914	1,11932577	1,03978963	0,91175583	1,05142906
0,91324041	0,55616025	1,3588006	1,17236052	1,7917208	0,39184017	1,23240055	1,41568063	1,00172045	0,46768021	1,94972087	0,91324041	0,79000035	0,83424037	0,72680032	1,21028054	0,77104034	1,21660054	0,65412029	1,01120045
0,8465475	0,46706069	1,51146029	1,25198212	1,35901687	0,48976503	1,43361684	1,0443996	1,18062563	0,79140839	1,58281678	0,94709529	0,99574744	0,82708664	0,58706934	1,25198212	1,00872135	1,05088655	0,91466052	1,10278218
0,86827347	0,48370944	1,28588597	1,09059955	0,98244091	0,40859928	1,13266124	1,5322473	1,05154226	0,73007077	1,14467886	1,38803579	1,09961277	0,78715449	0,94338363	1,59533983	1,0695687	0,85024703	0,94638804	1,12665242
0,78797607	0,39398803	1,41835692	1,26076171	1,05063476	0,48920181	1,08675033	1,46432219	1,1327156	0,73872756	1,24762878	0,7715599	0,76827667	0,70917846	1,07033416	1,46103896	1,00795272	0,95213775	1,18853057	1,04078506
0,88616633	0,22999737	1,50851216	1,48145364	1,17366305	0,75425608	1,22439776	1,56262919	0,87263708	0,34837837	1,44424819	1,01469428	0,473524	1,17028073	0,87940171	1,24130933	1,07557593	1,13307528	0,35852531	1,26836785
0,80283562	0,63535667	1,37439078	0,75764289	1,05804165	0,47319451	1,12715995	1,22286221	1,30261409	0,83207798	1,52857776	1,05006646	0,94904741	0,73371732	0,67257421	1,56047851	1,10323438	1,08728401	1,05272486	1,14311032
1,16864145	0,32868041	1,47540984	1,16133745	1,51923389	0,59892875	1,2051615	1,59227398	1,16864145	0,42363253	1,81139425	0,86187307	0,803441	0,81804902	0,85456906	1,27820159	0,7230969	1,46810583	0,59162474	1,05908132
0,79833167	0,30664395	0,80097515	0,92257534	0,79833167	0,449392	0,9437232	1,90066381	1,18163661	0,91728837	0,96222757	1,62838513	1,14727134	0,63179228	1,00716677	1,46184574	0,91993186	0,74546202	1,08118428	1,0785408
0,52176366	0,6909843	0,72200808	0,71354705	0,69380464	0,24254959	0,83764219	2,09269531	1,43837548	1,22402933	0,84892357	1,71758955	1,5229858	0,48227884	0,43715333	1,33684309	0,98712043	0,786876	1,66682335	1,03788662
0,66973442	0,30765185	1,11227978	1,29687089	0,81409414	0,29108598	0,93005522	2,19852748	1,22587431	0,80936103	1,28977123	1,41283197	0,99868525	0,72179858	0,84722587	1,50986064	1,13831186	0,89455693	1,31343676	1,04364975
1,11276091	0,34179492	1,13332001	1,41857742	1,45712572	0,70414894	1,06907284	1,82204963	1,02281488	0,64504155	1,40058822	1,22583593	0,72727792	1,07935239	0,78381543	1,21298649	0,82236372	1,28237343	0,86348191	1,12047057
0,9732154	0,56957768	1,01357917	1,09879158	0,64806279	0,37672854	0,8745484	2,34109879	1,15933724	0,9732154	1,11224617	1,58091441	1,06963997	0,73551763	0,72206304	1,54952037	0,99563972	0,64357792	1,21091317	0,9171546
1,33196931	0,59539642	1,74629156	0,69974425	1,35345269	0,50025575	1,09258312	1,54987212	1,30741688	0,85012788	1,45780051	0,87774936	1,29514066	0,83171355	1,01585678	1,26138107	0,47877238	1,10792839	1,02199488	1,33810742
0,71730943	0,49922635	1,01947275	0,81978221	0,68052433	0,33106589	0,8539398	2,15455581	1,40834379	1,114063	1,14822059	1,3374011	1,17712317	0,57542405	1,27434093	1,47140396	0,93802003	0,83817476	1,24281085	1,08778793
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1,2221643	0,49601549	0,69039994	1,06576301	0,70604007	0,92276756	1,15513518	1,21769569	1,17300961	0,558576	0,91829895	1,63774484	0,92500186	1,15513518	1,28695911	0,889253	1,56401281	1,24003873	1,07693453	0,87137857
1,31929157	0,63788273	0,5448269	1,08665199	0,8465079	1,16319792	1,16169702	1,34180508	1,33880328	0,50280168	0,92155293	1,80408245	0,8390034	1,05663398	1,28026816	0,89003402	1,3943366	1,1181709	1,21723034	0,8224935
1,30255301	0,57498096	0,63026759	0,9089122	0,65901664	1,16101924	1,22515173	1,47947023	1,23842052	0,63247905	0,92881539	1,72494287	0,64795931	1,15217338	1,31139887	0,85141411	1,28707276	1,32024474	1,23178613	0,69882301
1,24301486	0,543819	0,55608559	1,16941529	0,74417337	1,10194903	1,08968243	1,27572577	1,07741584	0,60924083	1,00177184	1,84612239	0,76257326	1,25323702	1,21234837	0,72986234	1,37181409	1,13057108	1,34319204	0,82595066
1,14219182	0,50298355	0,52918061	1,12647358	0,86712269	1,02430505	1,131713	1,30461359	1,42250036	0,48464561	0,91165769	1,40416242	0,89070004	1,07145976	1,27841653	0,78067239	1,41726095	1,16838888	1,45131713	0,77281327
1,43514355	0,47630578	0,72535455	1,09892771	0,71290211	1,21411276	0,98685576	1,35731581	1,11138015	0,57592529	0,8374265	1,80249049	0,89657558	0,88101003	1,09270149	0,78139052	1,6779661	1,34797648	1,30750605	0,81252162
1,20088955	0,44566345	0,7739066	1,00340993	0,71786509	1,13684211	1,01675315	1,3209785	1,29162342	0,651149	0,92068199	1,48910304	0,69384729	1,10748703	1,17687176	0,89132691	1,23558191	1,40904374	1,33165308	0,86197183
1,28318091	0,96698639	0,11543609	1,37184921	0,78128485	0,73109525	1,86705331	1,39694401	1,39025206	0,4834932	1,48059335	1,10082534	0,96196743	1,87876422	2,03267901	0,29946464	1,43207673	0,4834932	1,67633281	0,22083426
1,86753466	1,00177279	0,42895754	1,05670028	0,46034468	1,00961958	1,6478247	1,02008196	1,16393967	0,42111076	0,7480601	2,48743061	0,86053068	1,09070301	1,1482461	0,58066204	1,51442936	1,12993694	0,9991572	0,45511349

rs576261	*rs6444724*	*rs6811238*	*rs6955448*	*rs7041158*	*rs717302*	*rs719366*	*rs722098*	*rs722290*	*rs727811*	*rs729172*	*rs733164*	*rs735155*	*rs737681*	*rs740598*	*rs740910*	*rs7520386*	*rs7704770*	*rs826472*	*rs873196*
1,18215935	1,04445727	1,11460739	1,21333718	1,15617783	0,78724018	0,62875289	1,26789838	0,74047344	0,65993072	0,81581986	1,20554273	1,01847575	0,94572748	1,13279446	0,99769053	1,35363741	1,15098152	0,84180139	0,82101617
0,71462376	1,37718883	1,19261713	1,28253668	1,12162802	0,83767156	0,64363464	1,23994321	0,79034548	0,62470421	0,71462376	1,04590629	1,32039754	1,26360625	1,28253668	0,63416943	0,97018457	1,09323237	0,53478467	0,79507809
1,03088153	1,00561482	1,07636159	1,8192027	1,18248175	0,7681078	0,86412128	1,58674902	0,68220101	0,98540146	0,88433464	1,08141494	1,19258843	0,98034812	0,72262774	1,08646828	0,8792813	0,98540146	0,73273442	1,08646828
0,71979616	1,2070918	1,36952367	1,28034539	1,08606412	1,22301649	0,51596008	1,35359898	0,68476184	0,82808408	0,61150825	1,02236535	0,85993347	0,96503645	0,83445396	0,8790431	0,47774082	0,88222804	0,71661123	0,78031
1,0865945	1,11990008	1,26977519	1,0865945	0,85761865	0,96586178	0,64113239	1,21981682	0,57452123	0,69109076	0,72439634	1,19067444	1,37801832	1,29475437	0,7826811	0,73688593	0,98667777	0,94920899	0,52872606	0,90341382
2,19486764	1,65788102	0,70903088	1,42327521	0,78201935	0,92278283	1,21995018	0,98534438	0,484852	0,51091931	0,37536929	2,10623878	1,57446562	1,4806233	0,62561548	0,42229045	1,81949835	0,81851358	0,27110004	1,45976945
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1,24837293	1,1605109	0,86763749	1,26667751	0,87862024	0,49056297	0,55645949	1,21908558	0,59672958	0,75048812	0,70655711	0,96282135	1,31793036	1,36918321	0,9115685	0,622356	0,78343638	1,02871787	0,34046534	1,33257403
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1,04523227	1,18669228	0,95878449	0,87233671	0,87233671	1,38316451	0,8801956	1,22598673	0,84090115	0,61299336	1,01379672	1,27314006	1,35958784	1,21026895	0,69158226	0,68372337	0,66800559	1,21026895	0,66800559	0,88805449
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1,02281488	1,75780246	0,92515919	1,20784672	1,20784672	0,72470803	0,49598812	1,36203992	0,97141715	0,61934268	0,50626767	1,22326604	1,33377117	1,25667457	0,79666486	0,63990177	1,48539448	1,0767825	0,4034722	0,79666486
0,51575931	1,65715709	1,26473153	0,94854865	1,57867198	1,52261119	0,58751713	1,7490968	0,92836676	1,02030647	0,35430422	0,7265479	0,95303351	0,94182135	0,70636601	0,69291142	1,03151862	0,91939704	0,94406378	0,70412358
1,07109974	1,85984655	0,87161125	1,12327366	0,742711	1,49769821	0,85933504	1,31048593	0,42659847	0,59539642	0,2915601	1,21227621	1,2	1,29207161	0,65063939	0,44501279	1,47314578	0,99130435	0,31611253	1,01892583
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0,79072985	0,9028018	0,97129021	1,60013836	1,09270149	0,7751643	0,53856797	0,77205119	1,29194051	1,23901764	1,0646835	1,15807679	0,91836735	0,52300242	0,98374265	1,33863715	1,39778623	0,6444137	0,76582497	0,61639571
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0,57598438	0,83468923	1,51806053	0,55645949	1,23006834	0,71753986	1,10803775	1,19589977	0,47347869	1,49365441	0,4978848	0,99576961	0,9023795	0,18669921	0,62233069	1,43136059	0,71568029	1,70103722	0,87126297	1,41061623
0,35487742	0,92576719	1,60466312	0,47831305	0,94119664	0,89490828	0,84861992	1,38865078	0,27773016	0,97205555	0,57088977	1,65095148	1,29433612	0,22098422	0,15784587	1,54688951	0,75766017	0,91550604	1,0733519	1,83101207
0,4106062	1,0813985	1,2887343	0,43093324	1,0285482	0,36588671	1,00415575	1,15864125	0,81714699	1,17490288	0,46752191	0,70738097	1,00458085	0,19468621	1,02015575	1,09024279	0,80989464	1,27714155	0,87219423	1,51855245
0,48470298	0,8088116	1,37235182	0,65113714	1,17671868	0,2335918	1,0394835	1,23219674	0,45550401	1,29643448	0,44674431	0,76793304	0,96766588	0,22330751	0,7329067	1,15089256	0,87605254	1,12226339	0,79589087	1,48871674
0,62896587	1,23477963	1,67852855	0,45918367	0,97624764	0,60195507	0,70999829	1,20776882	0,68298748	0,89907392	0,7061396	0,96853027	0,83968188	0,44118878	0,51946421	0,96065299	0,96776894	1,65801591	0,9464211	1,48723315
0,35468382	0,5911397	1,14151114	0,36283747	1,01920638	0,68490669	0,69713716	1,71634354	0,27722413	1,53288639	0,19976445	1,72449719	1,16613963	0,17368037	0,2729263	1,8691316	0,2729263	1,1992216	1,02554123	1,25711506
0,55921174	0,8570168	1,29048862	0,66178904	1,19783816	0,50957756	1,28717968	0,98606566	0,82392735	1,33019596	0,5128865	1,0323909	0,98624976	0,1985308	0,41627425	1,38331136	0,92220757	1,50499152	0,73648521	1,52420418
0,51733886	0,75480588	1,52657369	0,86505842	1,12796834	0,61062948	1,1618922	0,94138711	0,24594798	1,44176404	0,32227667	1,22973992	0,83668904	0,34228188	0,72259508	1,27404922	0,77964206	1,17897092	1,21700224	2,14876957
0,52571453	0,98547226	1,23572014	0,49079622	1,14066474	0,25606759	0,88653705	1,20468164	0,4190197	1,07276803	0,58973143	0,9660732	0,97164212	0,21675093	0,59419653	1,30050561	0,90437459	1,19213014	0,90811167	1,46493735
0,49612022	0,72680032	1,41252063	0,25280011	1,47256065	0,77736035	1,31140058	1,32404059	0,73944033	1,89284084	0,18960008	1,07756048	1,54370034	0,33961407	0,47854711	1,67491487	0,72553916	1,28898978	0,67150965	2,09171396
0,60328672	0,89844313	1,4109125	0,4508433	1,06710394	0,72978233	0,90168661	1,22603431	0,77194753	1,38172121	0,44759983	1,10278218	1,24720893	0,49481659	0,44059011	1,16586922	0,90151515	1,21331738	0,73205742	1,62679426
0,67298705	1,08158633	1,47516357	0,48070503	0,99445854	0,55281079	0,9283616	1,06055548	0,88629991	0,95540126	0,64594739	0,85325144	1,19510953	0,60621498	0,67549669	1,11428086	1,22397691	1,01613177	1,02190525	1,4664629
0,53188385	1,1918138	1,63176711	0,45965271	0,91273895	0,43995331	0,89885452	0,96855392	0,66649642	1,1130162	0,64679702	1,10973296	1,36655949	0,2550911	0,46159343	1,16005716	0,94748124	1,46373705	0,57091819	1,68238657
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0,33307878	1,59930682	1,20807143	0,42824414	0,88821007	0,5683487	0,76132292	0,63179228	0,67144452	0,83269694	0,62121835	0,86706221	1,00150286	0,26570484	0,4241058	1,74241058	0,6029456	1,23143974	0,65404268	1,26720769
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0,43278934	1,5024293	1,00685187	0,35654666	0,87230597	0,6346082	0,61891117	0,67272954	0,51575931	0,79606329	0,69963872	0,92163947	1,05845568	0,27270256	0,45758564	1,68243611	0,63784666	1,31729201	0,60549212	1,34964655
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0,35734096	1,35316615	1,08516042	0,40200858	0,93802003	0,35208595	0,85656731	0,67526932	0,66213178	0,91699997	0,63848422	0,83029224	1,14027565	0,28116386	0,51026034	1,7338438	0,71852986	1,2704441	0,74456355	1,31209801
1,1558669	1,07180385	0,85376532	1,46847636	1,28196147	1,09281961	1,25831874	1,37127846	1,1821366	0,41243433	1,19789842	1,23204904	1,2392278	1,08694981	1,65405405	1,03969112	0,85590734	1,04494208	1,14996139	1,06594595
1,19535265	1,01437402	0,98309377	1,29142772	1,50592091	0,90936173	0,97639085	1,3338795	1,08810605	0,7261488	1,24227303	1,20875847	0,97575678	0,68169309	1,65299437	0,95793474	0,82872494	1,12724414	1,20298781	1,22972088
1,09415649	1,30578347	0,8284971	1,36882129	1,41685011	0,845007	1,08815289	1,32529518	1,1061637	0,36621973	1,29827897	1,14068441	0,90461538	0,87384615	1,63384615	1,07384615	1,01538462	1,22461538	0,82153846	0,98769231
1,05265744	1,10573261	0,87574023	1,42860653	1,57898617	0,96198737	1,22957466	1,31582181	1,16765364	0,32508539	1,20967147	1,03938865	0,81218008	0,93266833	1,44139651	1,00406878	0,91481822	1,31644573	0,88804305	1,17364484
1,00790514	1,06106038	0,93635001	1,44541366	1,72550089	1,00177184	1,20212621	1,33296988	1,25937031	0,34550906	1,18372632	1,04266049	0,83115406	0,79636157	1,68163729	1,0476407	0,98578738	1,46515065	1,09789653	0,95486072
1,22602241	1,19196623	1,12647358	1,0897977	1,49585213	0,86712269	1,15529035	1,35700771	1,19720565	0,22529472	1,48537331	1,20768447	1,00281607	0,8214021	1,46642952	1,15399437	0,85163776	1,32532978	0,78612717	1,25981918
1,19854722	1,46938776	0,97440332	1,2888274	1,61570391	1,09892771	0,99308198	1,0646835	1,08647527	0,38291249	1,25147008	1,16430301	0,84170582	0,88471269	1,52981568	1,22876762	0,92771955	1,06288399	0,81713047	1,11203469
1,18487769	1,04343958	0,86464047	1,25159377	1,59851742	0,91000741	0,96071164	1,31564122	1,0567828	0,75789474	1,22490734	1,11549296	1,07106227	0,82197802	1,5592674	0,92161172	0,7621978	0,94153846	0,91164835	1,20058608
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0,8500683	1,32872214	1,07239385	1,06716266	1,5955128	0,87883984	1,20317359	1,30518178	1,38364963	0,3007934	1,27902584	1,37841844	0,92868928	0,77286893	1,61429881	1,27149404	0,93492209	1,70779102	1,30889093	1,42108158

rs17250845	*rs17269816*	*rs17306671*	*rs17842518*	*rs20320*	*rs2032595*	*rs2032599*	*rs2032602*	*rs2032624*	*rs2032631*	*rs2032636*	*rs2032652*	*rs2032658*	*rs2032673*	*rs2033003*	*rs2319818*	*rs2534636*	*rs35284970*	*rs3848982*	*rs3900*
1,10316986	0,69138756	1,05741627	1,3166866	0,777811	0,57954545	1,09300239	1,26584928	0,71172249	0,95574163	0,75747608	1,3166866	1,13367225	1,25059809	1,08791866	1,27093301	0,86931818	0,81339713	1,08283493	0,90998804
1,16179907	0,9036215	1,32067757	1,03271028	0,89369159	0,44684579	1,01285047	1,44976636	0,48656542	1,27102804	0,35747664	1,16179907	0,83411215	1,45969626	1,56892523	1,32067757	1,23130841	0,69509346	0,98306075	1,15186916
1,98632691	1,00372902	1,09881914	0,82411436	1,14108142	0,75015538	0,63393412	1,53200746	0,78185208	0,96146675	0,27470479	1,06712244	0,85581106	0,65506526	1,9229335	1,47917961	0,75015538	0,88750777	1,00372902	1,33126165
1,12207239	0,92299503	1,05571327	1,47196593	0,91696238	0,47657913	0,82647268	1,40560681	0,74804826	0,81440738	0,65755855	1,2246274	1,01348474	1,59865153	0,94712562	1,38750887	0,86870121	0,72995032	0,9833215	1,33924769
1,4601227	1,10134969	0,92613497	1,52687117	1,20981595	0,50895706	0,74257669	1,43509202	0,52564417	1,06797546	0,52564417	1,27656442	0,75092025	1,46846626	1,48515337	1,30993865	0,84269939	0,84269939	1,08466258	1,08466258
1,95055468	1,14231379	0,5496038	1,86434231	1,30396197	0,42028526	0,18320127	0,75435816	0,57115689	0,61426307	0,64659271	1,20697306	0,47416799	0,81901743	0,85134707	1,98288431	0,73280507	1,17464342	2,02599049	0,49572108
1,07194245	0,6618705	0,79136691	1,28776978	1,05755396	0,5971223	0,82014388	1,20143885	0,65467626	0,9352518	0,75539568	1,27338129	0,91366906	1,47482014	1,10071942	1,39568345	0,90647482	0,85611511	0,86330935	1,29496403
1,50184049	1,18200409	0,73701431	1,1194274	1,24458078	0,61186094	0,51451943	0,80654397	0,45194274	1,02208589	0,37546012	1,10552147	1,09161554	1,31411043	1,19591002	1,2793456	0,95255624	0,88302658	1,55051125	1,16809816
1,14158372	1,10622493	1,08601991	1,25271134	0,69707324	0,55058684	0,77284207	1,27796761	0,57079186	0,9395335	0,39904918	1,46991532	1,1213787	1,37394146	1,05066112	1,30322389	0,97994354	0,96984103	1,08096865	1,40930025
1,19337017	1,24861878	1,03867403	0,85082873	0,99447514	0,47513812	0,64088398	0,97237569	0,43093923	0,96132597	0,48618785	1,52486188	1,14917127	1,33701657	0,85082873	1,75690608	0,97237569	0,90607735	0,95027624	1,21546961
1,31293522	1,32728424	0,93268622	1,42772737	1,14074699	0,46634311	0,69592741	1,29141169	0,68157839	0,52373919	0,55243722	0,98290779	1,12639797	1,22684111	1,27706267	1,34880777	0,89681367	0,85376662	1,35598227	0,94703524
1,09565217	0,7826087	0,55652174	1,14782609	1,32173913	0,69565217	0,83478261	1,2	0,85217391	0,3826087	0,55652174	1,1826087	1,39130435	1,91304348	0,8	1,40869565	1,13043478	0,97391304	1,1826087	0,74782609
1,03846154	0,34615385	1,30769231	1,19230769	1,53846154	0,69230769	0,88461538	2	1,26923077	1,23076923	0,84615385	0,88461538	1,38461538	2,07692308	1,57692308	0,23076923	1,03846154	0,65384615	0,07692308	1,84615385
1,13947758	0,92163627	1,75948743	1,52488911	0,97190734	0,63676688	0,90487925	1,24001971	0,38541153	0,75406604	0,45243963	1,18974864	0,67028093	1,42434697	1,32380483	1,44110399	0,92163627	0,55298176	0,82109414	0,83785116
0,97498475	1,30689445	0,95424039	1,46247712	1,25503356	0,44600366	0,48749237	0,82977425	0,38377059	0,91275168	0,63270287	1,18242831	0,91275168	1,34838316	1,10982306	1,54545455	0,88163514	0,8090299	0,87126297	1,29652227
1,29433612	0,63138347	0,82079851	1,86258124	1,0733519	0,28412256	0,69452182	0,82079851	0,56824513	1,57845868	0,5998143	0,85236769	0,91550604	0,94707521	1,04178273	2,33611885	1,6731662	0,69452182	0,75766017	0,82079851
1,18369217	1,14475492	1,41731562	1,46404031	0,89555657	0,56848374	0,45945946	1,05130554	0,41273477	0,7553825	0,55290884	1,45625286	1,15254237	1,16811727	0,95785616	1,7677508	1,07466789	1,09024279	1,03573065	1,23820431
1,66621758	0,6985517	1,31694173	1,42573257	0,89895588	0,40653419	0,61839003	1,11081172	0,68710003	0,96194005	0,64129336	1,57460424	0,95621421	1,21387673	0,97339171	1,3971034	0,85314921	0,79589087	1,32266756	1,08218255
1,23105902	1,06739221	0,82544998	1,18836333	1,57262453	0,47676852	0,67601507	1,00334868	0,61197154	0,547928	0,61908748	1,45876936	0,96776894	1,56550858	1,25952281	1,72205944	0,83968188	0,81121808	1,1385517	0,83256593
2,01800049	1,23230358	0,76915592	1,65409876	1,71199222	0,31427876	0,20676234	0,71953296	0,28946728	0,58720506	0,15713938	1,42252493	0,81877889	1,53831185	1,03381172	1,56312333	0,62855753	1,35636098	1,66236925	0,62855753
1,30646073	1,25522697	0,91580335	1,01186664	1,13995103	0,64682614	0,56997551	1,33848182	0,68525146	0,90299491	0,8965907	1,09512149	1,10152571	1,35769448	1,02467508	1,24241853	0,92220757	0,67884724	0,79412319	1,37050292
1,63534676	0,93176734	0,93176734	2,14876957	1,08389262	0,39932886	0,39932886	0,950783	0,41834452	0,83668904	0,51342282	1,54026846	0,83668904	1,0458613	1,00782998	1,46420582	0,22818792	0,93176734	1,00782998	0,91275168
1,34535063	0,98285337	1,13607386	1,56210156	0,98285337	0,62409321	0,75862827	1,16223346	0,63530446	0,78478787	0,51571774	1,20334139	0,96043086	1,07254342	1,15849637	1,4275665	0,8520554	0,9753792	1,08375467	1,16970763
2,00681044	0,73325766	0,86447219	2,10715096	1,3738933	0,27786606	0,12349603	0,57888763	0,30874007	0,52485812	0,66379115	1,64404086	0,47854711	0,60204313	1,45879682	1,42020431	0,64063564	1,43564132	1,33530079	0,54029512
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1,64075067	0,92555166	1,24108064	1,09383378	0,82037534	0,53289338	0,72221077	1,36028047	0,41369355	1,1849866	0,18230563	1,33223345	0,82037534	1,29717468	1,45143329	1,40936276	0,77129305	0,96762219	1,08682203	1,02371623
1,48131738	1,27543937	0,94904741	1,66710973	1,149904	0,52724856	0,60256978	0,85866194	0,51720573	0,59252695	0,58750554	1,13483976	1,02939005	1,02939005	1,27041796	1,75247379	0,80342638	0,92394033	1,10471127	1,39093192
2,01040681	0,93282876	0,64333018	1,84957427	1,19016083	0,38599811	0,32166509	0,94891202	0,45033113	1,2384106	0,32166509	1,35099338	0,69157994	0,73982971	1,20624409	1,73699149	1,12582781	0,94891202	1,36707663	1,4474929
1,25187857	1,19567178	0,70513977	1,30297565	1,24676886	0,65404268	0,87886985	1,22122032	0,67448151	0,74601743	0,79711452	1,06792907	1,11902615	1,34896303	0,99639315	1,11391644	1,05259994	1,05770965	1,14457469	1,23654944
1,16825294	1,38215841	0,76238103	1,40958219	1,16276819	0,61977738	0,9762865	1,17922246	1,00371028	0,38393289	0,88304565	1,16276819	1,38764317	1,89224068	0,85562187	1,18470721	1,06952734	0,79528956	0,9762865	0,85562187
1,33323687	0,80191	0,8707857	1,83504558	1,0921719	0,62972074	0,77239184	1,36275503	0,62972074	0,76747215	0,59528288	1,3135581	0,88554478	1,37259441	1,0921719	1,27912024	0,76747215	0,92982202	0,97901896	1,07249313
1,66360856	1,1163689	1,02333816	1,51585385	1,32979237	0,60196362	0,67857718	1,17109287	0,40495735	0,9686142	0,22436826	1,31337518	1,00692097	1,49396427	0,92483502	0,99597618	1,05617254	1,15467568	1,29148559	0,95219701
1,36813486	1,0954323	0,60087004	1,53452964	1,34502447	0,52229473	0,89668298	1,34502447	0,55927134	0,80886351	0,65171289	1,16014138	1,12778684	1,79798804	0,87819467	0,72104405	1,11392061	0,98912452	1,03996737	1,32191408
1,8954934	1,2020202	0,68686869	1,92191142	1,59168609	0,50854701	0,32362082	0,95765346	0,36324786	0,7000777	0,36985237	1,77000777	0,91142191	1,17560218	1,05672106	0,94444444	0,89160839	1,15578866	1,45299145	0,71328671
1,36937213	1,19754977	0,6820827	1,7338438	1,29647779	0,48422665	0,74456355	1,31730475	0,76018377	0,62480858	0,84869832	1,17672282	1,09862175	1,46309342	0,6820827	0,98928025	0,81745789	1,04655436	1,14027565	1,27565084
0,7403861	1,21297297	0,84015444	0,63011583	1,09220077	0,66687259	1,31274131	0,69312741	0,45683398	0,29930502	0,58285714	0,99243243	1,40725869	0,73513514	1,41250965	0,23629344	1,07644788	1,76957529	1,09220077	0,92416988
0,65941554	1,14506618	0,83318045	0,56139431	0,79753636	1,07823352	1,37229721	0,71733718	0,65496003	0,5123837	0,74852575	0,82426943	1,27427598	0,91783515	1,80002621	0,28515267	1,01140086	1,54606212	0,77525881	0,86436902
0,80307692	1,26153846	0,75692308	0,57846154	0,79076923	0,72	1,33846154	0,88615385	0,52615385	0,42461538	0,59692308	0,88615385	1,33538462	0,99692308	1,49538462	0,19076923	1,10153846	1,53538462	1,16	0,89846154
0,74077963	1,30752067	0,83895524	0,65152907	0,88804305	0,88358052	1,06654417	0,70507941	0,62029138	0,45517784	0,5756661	1,11116944	1,45032156	1,08885681	1,38784617	0,13387584	1,22719517	1,41462134	1,15579472	0,91481822
0,80795907	1,1172257	0,8234224	0,55667993	0,99351904	0,82728823	1,1056282	0,90460489	0,52188744	0,45230244	0,63013076	0,93939738	1,43808982	1,0592382	1,34530984	0,16236498	1,15201819	1,44582149	1,04377487	0,85434906
0,7357344	1,15399437	0,80628427	0,58959538	0,77100934	1,11368015	1,24974063	1,08344449	0,58959538	0,31747443	0,79116644	0,96754113	1,2547799	0,90706981	1,55713651	0,13606047	1,19934786	0,91714836	0,95746258	0,92722692
0,71268522	1,22876762	0,80484279	0,54680159	1,05674015	0,62667149	1,5482472	0,94615107	0,55294543	0,52837008	0,67582219	0,85399349	1,19804843	1,01373329	1,35164438	0,27647271	1,16732924	1,49909649	0,94000723	1,06288399
0,79208791	1,13582418	0,80703297	0,50813187	1,03120879	0,73230769	1,32512821	0,81699634	0,78212454	0,75721612	0,77714286	0,95648352	1,31018315	0,77216117	1,19062271	0,27399267	1,02622711	1,80835165	1,10095238	0,87179487
0,70008035	0,86741663	0,76838088	0,41321816	0,36199277	0,94596223	1,0074327	0,64543994	0,12977099	0,16733628	0,16392125	0,6010446	1,82020892	0,73764564	1,46163118	0,26637204	3,14523905	0,78204098	0,36540779	0,40980313
0,84766269	1,07827681	0,68560953	0,54225481	0,72300642	0,87882676	1,17800183	0,87259395	0,54225481	0,42383135	0,62328139	0,85389551	1,2527956	0,92245646	1,80751604	0,17451879	1,0284143	0,97855179	0,97855179	0,77910174

rs3911	*rs4141886*	*rs8179021*	*rs9341278*	*rs9786139*	*rs9786184*
0,91507177	0,7930622	0,85406699	0,9201555	1,39802632	1,59629187
1,02278037	0,89369159	0,88376168	0,54614486	1,29088785	1,6682243
0,63393412	1,19390926	0,59167185	0,61280298	0,59167185	1,57426973
1,07984386	0,71185238	0,83853797	0,79630944	0,7782115	1,4418027
0,78429448	0,83435583	0,9595092	0,5590184	1,11803681	1,61030675
0,53882726	1,31473851	0,71125198	0,3340729	1,19619651	2,62947702
0,97122302	0,9352518	1,02877698	0,89928058	1,10071942	1,47482014
0,94560327	1,00817996	0,78568507	0,57014315	1,20981595	1,7591002
0,7930471	0,7475858	0,80314961	0,79809835	1,3133264	1,49517159
0,99447514	1,00552486	0,93922652	0,48618785	1,13812155	1,55801105
0,8394176	1,4492509	0,8394176	0,68157839	1,0690019	1,4492509
0,53913043	1,2	1,53043478	0,50434783	0,97391304	1,77391304
0,69230769	0,73076923	0,61538462	0,19230769	1,26923077	3,26923077
0,720552	1,05569246	0,63676688	0,70379497	1,17299162	1,49137506
0,6638194	0,91275168	1,02684564	1,00610128	1,3276388	1,61805979
0,88393686	1,16805942	0,94707521	0,37883008	1,32590529	1,23119777
0,5295465	0,98900596	0,70087036	0,58405863	0,80989464	1,7521759
0,7500842	0,77871337	0,6126642	0,68710003	1,27113506	1,9410576
0,71159481	1,35914609	0,59773964	0,7400586	1,08874006	1,26663876
0,52104111	1,96837752	0,70299197	0,45487716	1,24057407	1,56312333
1,08231305	0,81333584	0,94142023	0,73008099	0,8965907	1,60745903
0,8557047	0,81767338	1,50223714	0,8557047	0,74161074	1,50223714
0,96043086	0,82963289	0,84458123	0,69883491	1,0762805	1,64431743
0,4322361	1,18093076	0,65607264	0,50942111	1,18864926	2,09943246
0,58971292	1,04385965	0,58971292	0,72527911	1,59290271	2,0877193
0,89488878	0,76209883	0,84292749	0,77364578	1,13160129	1,40295466
0,8077885	0,97177563	0,70453733	0,97177563	1,01429082	1,44551626
0,84842236	0,89049289	1,06578676	0,46978759	1,92823263	1,59166839
0,71304091	1,05951853	0,78334072	0,54733422	1,27543937	1,38088909
0,90066225	1,56007569	0,94891202	0,46641438	1,6244087	1,38315989
1,16501353	1,03727081	0,88908927	0,58761647	0,87376014	1,44604749
0,75141152	1,22858526	0,81174383	0,58686885	0,69656396	1,12985965
0,98885834	0,89538417	0,69367675	0,71827521	0,91014325	1,47098828
1,03975535	1,11089651	0,97955899	0,54723966	0,97408659	1,84967005
0,92903752	0,89668298	0,7718869	0,65171289	0,76264274	1,72865688
0,81895882	1,13597514	0,59440559	0,42268842	0,74630925	1,78321678
1,07779479	1,06217458	0,84349158	0,56753446	0,59877489	1,39019908
1,21822394	1,09220077	1,01343629	0,81915058	1,39150579	1,15521236
1,38566374	1,39457476	1,13615516	1,01140086	0,9000131	1,13615516
1,29846154	1,39692308	1,02769231	0,80307692	1,52307692	1,13230769
1,36553353	1,2316577	1,01299383	1,06208164	0,96836855	1,25843287
1,13268903	1,40329733	0,92779989	0,94326322	1,29891984	1,25252985
1,52186157	1,14895509	1,23966207	0,90706981	1,08848377	1,40091893
1,2103361	1,49909649	1,13661005	1,11203469	1,06288399	0,98301409
1,30021978	1,25040293	1,01626374	1,22549451	1,03120879	1,21054945
0,90156689	1,5299317	0,78204098	0,38248292	2,34612294	0,93571716
1,20293309	1,51457379	1,04711274	0,67937672	1,21539872	1,2091659

Supplementary Table S5. Strand balance metrics values for all 132 SNP markers included in HID Ion Ampliseq Identity Panel, in Brazilian criminal samples.

Samples	Locus																	
Blood	*rs1005533*	*rs10092491*	*rs1015250*	*rs1024116*	*rs1028528*	*rs1031825*	*rs10488710*	*rs10495407*	*rs1058083*	*rs10773760*	*rs10776839*	*rs1109037*	*rs12997453*	*rs13218440*	*rs1335873*	*rs1355366*	*rs1357617*	*rs1360288*
BL.PF07	0,54778555	0,44849785	0,52531646	0,50192308	0,51120163	0,44820717	0,39156627	0,5010352	0,51821862	0,5520362	0,52662722	0,53469388	0,37931034	0,54923414	0,54408602	0,60459184	0,45703125	0,47222222
BL.PF09	0,51566952	0,38604651	0,52702703	0,45519713	0,51538462	0,45454545	0,5	0,4875	0,42642643	0,53110048	0,59235669	0,53110048	0,43835616	0,52244898	0,5	0,61181435	0,45238095	0,46441948
BL.PF11	0,50769231	0,50243902	0,43939394	0,59136213	0,58984375	0,54230769	0,496	0,57368421	0,43396226	0,53787879	0,45977011	0,47976879	0,42105263	0,52173913	0,53941909	0,53754941	0,46153846	0,54187192
BL.PF20	0,57142857	0,4516129	0,41463415	0,54255319	0,53658537	0,47203579	0,46296296	0,51798561	0,45348837	0,5012285	0,54591837	0,495	0,43697479	0,52019002	0,49759615	0,51139241	0,42152466	0,49122807
BL.PF22	0,49346405	0,4334471	0,42105263	0,49140893	0,47978437	0,45283019	0,46774194	0,49473684	0,4416476	0,53287197	0,58741259	0,50952381	0,45454545	0,56677524	0,596	0,61157025	0,44090909	0,43706294
BL.PF24	0,59055118	0,31547619	0,54411765	0,55172414	0,50997151	0,56338028	0,5106383	0,5483871	0,47292419	0,54577465	0,60869565	0,61870504	0,4137931	0,49538462	0,53386454	0,60504202	0,38636364	0,49339207
BL.PF25	0,51923077	0,47837838	0,38297872	0,53405995	0,60349854	0,47174447	0,5472973	0,51633987	0,48947368	0,51694915	0,45384615	0,50520833	0,32323232	0,52464789	0,48333333	0,58053691	0,45212766	0,39403974
BL.PF26	0,60169492	0,48710602	0,45918367	0,50364964	0,54787234	0,50121065	0,51111111	0,49236641	0,5286783	0,44086022	0,4893617	0,47133758	0,45454545	0,52566735	0,54046243	0,60280374	0,43119266	0,47355769
BL.PF27	0,54054054	0,47241379	0,51094891	0,51423488	0,53071672	0,46858169	0,56043956	0,47733333	0,47368421	0,54621849	0,5399061	0,56	0,46268657	0,48962656	0,50797267	0,52956298	0,46091644	0,5102459
BL.PF30	0,53072626	0,44776119	0,32142857	0,515625	0,57090909	0,45531915	0,45454545	0,47513812	0,5	0,54066986	0,45714286	0,52348993	0,37704918	0,51079137	0,5974026	0,61333333	0,39552239	0,53424658
Epithelial																		
EP.PF01	0,60995851	0,51376147	0,37272727	0,52631579	0,50204918	0,49358974	0,5483871	0,48095238	0,4494382	0,49851632	0,5326087	0,49444444	0,5	0,54824561	0,58908046	0,57518797	0,45631068	0,48148148
EP.PF08	0,44067797	0,5	0,51666667	0,46820809	0,53296703	0,50862069	0,5125	0,47321429	0,49079755	0,45679012	0,53623188	0,50617284	0,54545455	0,55063291	0,52991453	0,48167539	0,53608247	0,53125
EP.PF10	0,41025641	0,51020408	0,5	0,40625	0,52083333	0,63793103	0,54545455	0,61904762	0,5625	0,47727273	0,56756757	0,61111111	0,58823529	0,58333333	0,58064516	0,54761905	0,43076923	0,51578947
EP.PF13	0,46590909	0,44166667	0,46153846	0,53333333	0,54594595	0,52592593	0,5462963	0,54615385	0,52531646	0,57352941	0,43478261	0,45801527	0,21428571	0,52	0,55445545	0,37234043	0,45714286	0,48
EP.PF14	0,57142857	0,43939394	0,4494382	0,53767123	0,56794425	0,47701149	0,52173913	0,44295302	0,47318612	0,49561404	0,51485149	0,51818182	0,39240506	0,56410256	0,55731225	0,52972973	0,5	0,49152542
EP.PF15	0,53932584	0,34375	0,5	0,5	0,42682927	0,47916667	0,49019608	0,55555556	0,3968254	0,50769231	0,55	0,58064516	0,40740741	0,42696629	0,55555556	0,55714286	0,47368421	0,5
EP.PF17	0,53571429	0,36046512	0,42268041	0,55223881	0,54867257	0,49342105	0,5	0,53333333	0,47593583	0,54882155	0,62411348	0,56875	0,42424242	0,56557377	0,55108359	0,4978903	0,44230769	0,56944444
EP.PF18	0,55319149	0,41698113	0,43181818	0,55890411	0,52371134	0,48648649	0,56862745	0,47368421	0,50217391	0,50554324	0,51818182	0,5248227	0,51648352	0,53043478	0,56531532	0,54700855	0,49356223	0,47043011
EP.PF21	0,48850575	0,36333333	0,43442623	0,54769231	0,46511628	0,54666667	0,48815166	0,47511312	0,52694611	0,49068323	0,46153846	0,45762712	0,42708333	0,56777494	0,52348993	0,52813853	0,53623188	0,46666667
EP.PF28	0,5503876	0,44864865	0,50961538	0,555	0,58039216	0,44117647	0,44654088	0,5472973	0,47165533	0,46306818	0,47826087	0,55172414	0,48717949	0,48172043	0,46768061	0,56164384	0,38059701	0,52401747
Saliva																		
SA.PF02	0,56368564	0,42283951	0,4351145	0,53503185	0,50260417	0,50366748	0,50561798	0,50409836	0,49868074	0,49534884	0,53846154	0,57534247	0,3539823	0,54232804	0,52046784	0,54958678	0,43089431	0,43250689
SA.PF03	0,48192771	0,5	0,46938776	0,56493506	0,56862745	0,3258427	0,51724138	0,57291667	0,43850267	0,53125	0,55769231	0,53913043	0,125	0,44148936	0,50714286	0,54761905	0,53947368	0,54966887
SA.PF04	0,49656751	0,42903752	0,4845815	0,51693667	0,54545455	0,45527157	0,45027624	0,50378788	0,47826087	0,47993827	0,51674641	0,51092896	0,40641711	0,52493766	0,50738916	0,59807692	0,45221445	0,46362098
SA.PF05	0,47905759	0,53526971	0,51612903	0,50588235	0,48369565	0,44243792	0,47368421	0,42236025	0,52871287	0,5106383	0,53191489	0,53459119	0,55	0,54545455	0,47880299	0,5480226	0,44	0,46517413
SA.PF06	0,54137931	0,42857143	0,43262411	0,56418919	0,49803922	0,41860465	0,50251256	0,40601504	0,47073171	0,5206422	0,43939394	0,53811659	0,5	0,52036199	0,54571429	0,5451505	0,53672316	0,48394004
SA.PF12	0,54026846	0,48395722	0,48958333	0,54491018	0,49435028	0,52608696	0,47004608	0,47878788	0,49880096	0,53448276	0,44444444	0,48695652	0,4765625	0,52262443	0,57537688	0,47519582	0,48113208	0,48059701
SA.PF16	0,52	0,47272727	0,50920245	0,54545455	0,53056235	0,48263889	0,56018519	0,47058824	0,44625407	0,52539063	0,55	0,51834862	0,45652174	0,50206612	0,54004577	0,54071661	0,53043478	0,51183432
SA.PF19	0,49509804	0,47863248	0,49230769	0,53846154	0,54222222	0,48255814	0,55900621	0,46984127	0,43576826	0,54929577	0,57303371	0,52249135	0,3559322	0,4747191	0,56804734	0,58737864	0,4841629	0,47916667
SA.PF23	0,51984127	0,45265589	0,43859649	0,51956522	0,5046729	0,51011236	0,49027237	0,51360544	0,46182495	0,48897059	0,5	0,55458515	0,47959184	0,51654412	0,46410256	0,53846154	0,48803828	0,49268293
SA.PF29	0,53793103	0,4822695	0,46511628	0,53503185	0,49056604	0,49726776	0,45	0,51648352	0,53092784	0,48039216	0,52941176	0,53142857	0,43243243	0,59067358	0,5801105	0,56097561	0,54651163	0,50581395
Semen																		
SE.PF02	0,5610687	0,38551859	0,52272727	0,51612903	0,54621849	0,49393939	0,47941176	0,5483871	0,41834452	0,51376147	0,39830508	0,47302905	0,415	0,50957854	0,56798246	0,48825503	0,47277937	0,48813559
SE.PF03	0,50581395	0,47048904	0,51111111	0,55935252	0,4789916	0,52142857	0,55128205	0,47346939	0,50228311	0,54200542	0,57692308	0,51243781	0,50980392	0,5107438	0,54653465	0,55120482	0,50146628	0,55513308
SE.PF07	0,54336735	0,3826458	0,48167539	0,50619835	0,544	0,475	0,48175182	0,52643172	0,47739602	0,52605459	0,53296703	0,55405405	0,44186047	0,53442623	0,5746888	0,59316239	0,44581281	0,46892655
SE.PF22	0,52505967	0,44949495	0,40140845	0,55227273	0,51302605	0,44444444	0,49305556	0,45847176	0,46610169	0,48741419	0,51760563	0,53024194	0,39215686	0,54684096	0,4855643	0,55106888	0,44	0,44621514
SE.PF26	0,55294118	0,4991511	0,43495935	0,57096248	0,52777778	0,48928121	0,51636364	0,49156627	0,4733945	0,50691244	0,52469136	0,52117264	0,37450199	0,47587354	0,52057613	0,56699577	0,46721311	0,44299674
SE.PF27	0,5665529	0,46531792	0,46527778	0,540625	0,55518946	0,47058824	0,48618785	0,4939759	0,50698603	0,49829352	0,6344086	0,52671756	0,35526316	0,49300699	0,52380952	0,71942446	0,46396396	0,47021944
SE.PF30	0,5261194	0,42084942	0,47863248	0,54007634	0,48695652	0,49779087	0,49333333	0,47692308	0,46047431	0,50395778	0,54193548	0,53007519	0,35602094	0,50639854	0,54405286	0,53435115	0,50653595	0,51304348
Control (Oral Swab)																		
CT.PE02	0,4877451	0,47111111	0,47619048	0,51024209	0,47334755	0,52542373	0,47916667	0,55464481	0,51183971	0,55050505	0,50485437	0,55186722	0,51879699	0,54949495	0,59514925	0,50125945	0,48409894	0,51536643
CT.PE03	0,52202643	0,43902439	0,49673203	0,57460317	0,49537893	0,53971119	0,568	0,49910555	0,46	0,48742747	0,44144144	0,57099698	0,49275362	0,48853616	0,54861111	0,49884527	0,46825397	0,49177331
CT.PE04	0,52643678	0,4321663	0,37755102	0,5535513	0,51315789	0,50722733	0,51343284	0,44291609	0,48439182	0,49431818	0,45882353	0,5010142	0,46733668	0,53817272	0,52754982	0,48640483	0,44915254	0,46530148
CT.PE07	0,548	0,41297468	0,46308725	0,56989247	0,50638298	0,51612903	0,53146853	0,49595142	0,50343643	0,49520154	0,48846154	0,52091255	0,38194444	0,53947368	0,52107926	0,5304878	0,48344371	0,48896435
CT.PE08	0,52601156	0,38181818	0,44970414	0,52902156	0,49411765	0,55374593	0,46308725	0,48979592	0,45603577	0,49102773	0,53383459	0,51929825	0,46820809	0,50896552	0,54637437	0,58717435	0,47461929	0,49506579
CT.PE09	0,51428571	0,41036717	0,43636364	0,50916497	0,55748373	0,432282	0,44864865	0,48463357	0,46950092	0,49877751	0,4375	0,54424779	0,34166667	0,50547046	0,52663934	0,58868895	0,45517241	0,52293578
CT.PE10	0,49788136	0,44691358	0,504	0,51893096	0,44385027	0,44949495	0,54594595	0,43817204	0,49907236	0,50883392	0,52287582	0,52820513	0,43571429	0,52685422	0,58119658	0,51147541	0,45348837	0,48156182
CT.PE11	0,46803069	0,38722555	0,45098039	0,53955375	0,49043062	0,53406998	0,5	0,49411765	0,47516199	0,50120482	0,50898204	0,54594595	0,49107143	0,52643172	0,53741497	0,53825137	0,54504505	0,47777778
CT.PE12	0,57768053	0,54745763	0,42346939	0,584375	0,5376197	0,58319871	0,60553633	0,48051948	0,43107477	0,46749777	0,47750865	0,47315436	0,49253731	0,54105909	0,54897119	0,53108348	0,45878136	0,47457627
CT.PE13	0,52559055	0,38221154	0,34736842	0,45843829	0,56166983	0,52020202	0,46583851	0,48397436	0,42659758	0,51079137	0,57441253	0,53094463	0,37209302	0,53286385	0,54441913	0,60062893	0,43060498	0,50980392

rs1382387	rs1413212	rs1454361	rs1463729	rs1490413	rs1493232	rs1498553	rs1523537	rs1528460	rs159606	rs1736442	rs1821380	rs1872575	rs1886510	rs1979255	rs2016276	rs2040411	rs2046361	rs2056277	rs2076848
0,51267606	0,44215938	0,51494253	0,45907473	0,47469459	0,48584906	0,43429844	0,39393939	0,58585859	0,59585492	0,52611219	0,52793834	0,54778887	0,51898734	0,5	0,52043597	0,48022599	0,49211356	0,48582996	0,47213115
0,48529412	0,48717949	0,54576271	0,42592593	0,48342541	0,5045045	0,43646409	0,45	0,51012146	0,51655629	0,50638298	0,42091153	0,56325301	0,51234568	0,50458716	0,55905512	0,43234323	0,54591837	0,4723127	0,47916667
0,48013245	0,44976077	0,53181818	0,52898551	0,49852507	0,57943925	0,45714286	0,46124031	0,55421687	0,52631579	0,45771144	0,49819495	0,54	0,56451613	0,4887218	0,52608696	0,48809524	0,45918367	0,42647059	0,45614035
0,50626566	0,46067416	0,52432432	0,48538012	0,54880694	0,44129555	0,48351648	0,47774481	0,53191489	0,59641256	0,48196721	0,48653199	0,57324841	0,47572816	0,48881789	0,52161383	0,50332594	0,48318043	0,47340426	0,50787402
0,48611111	0,47241379	0,49025974	0,45989305	0,49535604	0,50793651	0,40151515	0,4083045	0,57709251	0,54482759	0,48648649	0,50948509	0,51724138	0,58045977	0,46261682	0,51265823	0,47319347	0,54482759	0,50900901	0,49514563
0,57990868	0,403125	0,56746032	0,47666667	0,45517241	0,52717391	0,47945205	0,46448087	0,51908397	0,42990654	0,57142857	0,54225352	0,45652174	0,57142857	0,48214286	0,50545455	0,50638298	0,46551724	0,42657343	0,5464684
0,54848485	0,45821326	0,52316076	0,48257373	0,50728863	0,52197802	0,44210526	0,40531561	0,6	0,58695652	0,57209302	0,45066667	0,56578947	0,45251397	0,47619048	0,61774744	0,50497512	0,51569507	0,46464646	0,49541284
0,51794872	0,50417827	0,47619048	0,54520548	0,55384615	0,52586207	0,42805755	0,38081395	0,57854406	0,55701754	0,52229299	0,50964187	0,55625	0,55882353	0,47810219	0,55660377	0,5	0,46564885	0,43410853	0,4893617
0,52477064	0,4537037	0,52490421	0,44230769	0,43589744	0,52	0,45814978	0,50931677	0,56341463	0,56200528	0,48156682	0,50084317	0,53405018	0,55518395	0,51461988	0,48611111	0,4681182	0,49511401	0,48300971	0,51183432
0,51680672	0,44888889	0,43153527	0,50877193	0,53571429	0,46491228	0,46188341	0,39719626	0,53631285	0,5984252	0,45679012	0,48351648	0,55477032	0,46923077	0,48765432	0,53960396	0,50306748	0,59302326	0,46850394	0,52247191
0,54907162	0,49135802	0,53562005	0,47058824	0,57706093	0,5210084	0,43197279	0,41486068	0,66666667	0,5477707	0,4744186	0,54716981	0,55072464	0,52205882	0,50540541	0,45833333	0,44835165	0,5308642	0,44736842	0,56609195
0,50617284	0,51149425	0,56632653	0,52307692	0,46107784	0,4	0,45637584	0,52317881	0,52808989	0,57471264	0,45918367	0,4964539	0,54109589	0,6122449	0,49579832	0,5248227	0,50769231	0,49152542	0,43181818	0,53333333
0,41538462	0,45121951	0,5125	0,49180328	0,6	0,55172414	0,43137255	0,36585366	0,57758621	0,55357143	0,48484848	0,40625	0,5625	0,51428571	0,48837209	0,42682927	0,46268657	0,56521739	0,43103448	0,48484848
0,51552795	0,4966443	0,5323741	0,43333333	0,41916168	0,54736842	0,53409091	0,47368421	0,52173913	0,54166667	0,44654088	0,54166667	0,59055118	0,51351351	0,50980392	0,55675676	0,49673203	0,6	0,61290323	0,53271028
0,5221843	0,46223565	0,45381526	0,50922509	0,45398773	0,51333333	0,46082949	0,45695364	0,60714286	0,59210526	0,52432432	0,4625	0,55017301	0,45689655	0,46747967	0,55027933	0,4095941	0,39285714	0,50627615	0,4729064
0,55963303	0,41	0,62264151	0,46376812	0,47887324	0,52542373	0,17073171	0,41772152	0,52941176	0,53488372	0,44067797	0,45	0,71111111	0,44444444	0,45783133	0,47058824	0,54022989	0,57142857	0,47058824	0,66129032
0,49734043	0,47902098	0,51162791	0,39655172	0,46764706	0,51794872	0,4869281	0,45985401	0,57471264	0,55865922	0,51428571	0,52681388	0,54302671	0,51829268	0,4906367	0,53825137	0,53125	0,48888889	0,52091255	0,48192771
0,46813187	0,42168675	0,55947137	0,49889625	0,48623853	0,53809524	0,45670996	0,42686567	0,6557971	0,50632911	0,46932515	0,55011655	0,57253385	0,48547718	0,48742138	0,52112676	0,51383399	0,53757225	0,45659164	0,4379085
0,51895735	0,45498783	0,50276243	0,48377581	0,48117155	0,42677824	0,50455927	0,45081967	0,56768559	0,58910891	0,46583851	0,49795918	0,55591054	0,49006623	0,49526814	0,58009709	0,46741573	0,52238806	0,47222222	0,47463768
0,50539957	0,4520202	0,52293578	0,47887324	0,51295337	0,47798742	0,52654867	0,43866171	0,57763975	0,6	0,49420849	0,48780488	0,48198198	0,47933884	0,46770026	0,56703297	0,47281324	0,5	0,46031746	0,58515284
0,51052632	0,4173913	0,4952381	0,51412429	0,45939675	0,55506608	0,51219512	0,4491018	0,56358382	0,564	0,47701149	0,50452489	0,49295775	0,48186528	0,47983871	0,52588556	0,48831776	0,50731707	0,46319018	0,47636364
0,6056338	0,4494382	0,46616541	0,60504202	0,44927536	0,5875	0,4	0,45774648	0,62385321	0,5	0,5625	0,3986014	0,61352657	0,5	0,51327434	0,55621302	0,44493392	0,4	0,28787879	0,53846154
0,53529412	0,3900156	0,49335303	0,47144949	0,50084602	0,47560976	0,42811502	0,4952381	0,52761905	0,5412844	0,51736746	0,49774436	0,56637168	0,43975904	0,46743295	0,56718529	0,51875	0,54489164	0,46481876	0,51368421
0,46893788	0,47540984	0,55	0,49009009	0,52121212	0,46863469	0,54333333	0,48027842	0,5177305	0,50232558	0,46308725	0,52132701	0,56382979	0,48031496	0,50574713	0,50608273	0,46666667	0,43589744	0,42424242	0,52339181
0,45707657	0,5245478	0,55625	0,54746137	0,51071429	0,4738676	0,51038576	0,44604317	0,52912621	0,50310559	0,44755245	0,625	0,51671733	0,57377049	0,49029126	0,51306413	0,47457627	0,43949045	0,43781095	0,48242812
0,50721154	0,50759878	0,51643192	0,47722343	0,51057402	0,45982143	0,53626943	0,49117647	0,53448276	0,52427184	0,49489796	0,50980392	0,54653938	0,55696203	0,51603499	0,48205128	0,51025057	0,43103448	0,46130031	0,45692884
0,53610503	0,45437956	0,5254902	0,50318471	0,53505535	0,50384615	0,46437346	0,40609137	0,53985507	0,5125	0,47035573	0,50724638	0,52678571	0,55066079	0,52259887	0,56643357	0,49759615	0,5320197	0,45679012	0,52861953
0,56235828	0,45488722	0,50958904	0,46958637	0,48492462	0,4296875	0,47040498	0,47826087	0,50788644	0,53804348	0,48477157	0,49052133	0,52668213	0,46625767	0,45283019	0,5718232	0,50319829	0,48026316	0,53333333	0,4953271
0,50420168	0,49908257	0,51619433	0,454	0,47384615	0,47741935	0,48309179	0,50134771	0,5887574	0,48948949	0,50505051	0,47619048	0,56076759	0,50416667	0,43400447	0,51824818	0,51230769	0,40740741	0,48611111	0,53061224
0,507109	0,44198895	0,56571429	0,44444444	0,56637168	0,5	0,52427184	0,4057971	0,625	0,51685393	0,5	0,53448276	0,512	0,43678161	0,45454545	0,51933702	0,5	0,50909091	0,53153153	0,57894737
0,53033268	0,50348432	0,50446429	0,48918919	0,4940239	0,48883375	0,47606383	0,44791667	0,55729167	0,54390935	0,46174863	0,52960526	0,52197802	0,56097561	0,46593407	0,51596639	0,50497512	0,48160535	0,44329897	0,5
0,52616279	0,48710167	0,48865154	0,44859813	0,49751244	0,49502134	0,43523316	0,46648045	0,57760814	0,47882736	0,51394422	0,47211155	0,50579151	0,61607143	0,44190871	0,51399491	0,51807229	0,48850575	0,49637681	0,4862069
0,54935622	0,44234405	0,54439252	0,50924025	0,53968254	0,50134048	0,42116631	0,44369369	0,59166667	0,52071006	0,53349282	0,49744463	0,52907801	0,5147929	0,45748988	0,55053763	0,48734177	0,53383459	0,51612903	0,43666667
0,55745342	0,44893617	0,49498998	0,46443515	0,50757576	0,45392491	0,43131868	0,46326531	0,49625935	0,54022989	0,53221289	0,47986577	0,50456621	0,5372549	0,51572327	0,50719424	0,47881356	0,50232558	0,49386503	0,50793651
0,48560461	0,48231511	0,52953813	0,54896907	0,47883598	0,49130435	0,40101523	0,446	0,56427015	0,54025974	0,50131926	0,55929487	0,5290611	0,51204819	0,47140039	0,51771429	0,48387097	0,47076923	0,47767857	0,52795031
0,49243697	0,42307692	0,57725322	0,52941176	0,47325103	0,5035461	0,38990826	0,43917526	0,55612245	0,47752809	0,55882353	0,50669643	0,5327381	0,49350649	0,50248756	0,56055363	0,51230425	0,57303371	0,49488055	0,52112676
0,47244094	0,44691781	0,49240506	0,54020101	0,51456311	0,49060543	0,44985673	0,44755245	0,4973545	0,51898734	0,48905109	0,53380783	0,52277228	0,53736655	0,53421634	0,52151463	0,46212121	0,51689189	0,41935484	0,52121212
0,46710526	0,51289398	0,48088531	0,44785276	0,49712092	0,45564516	0,52671756	0,47384615	0,56495468	0,59016393	0,4114053	0,58483034	0,53590193	0,52515723	0,50980392	0,50105708	0,5	0,48066298	0,46620047	0,47857143
0,5212766	0,49419954	0,49585062	0,47136564	0,55284553	0,50902527	0,53944954	0,44772727	0,53300733	0,57368421	0,42509363	0,53858785	0,56756757	0,49246231	0,47381546	0,53663004	0,47841727	0,52316076	0,42708333	0,486618
0,49872774	0,52760736	0,53020962	0,46992054	0,49705882	0,50793651	0,47651007	0,44746377	0,57757296	0,52968037	0,4473342	0,53624733	0,52604167	0,49915683	0,51236749	0,50672043	0,47861272	0,53479853	0,45486936	0,51140065
0,45746692	0,46373057	0,52603232	0,51530612	0,51937984	0,44014085	0,49775785	0,43434343	0,57847534	0,52873563	0,45856354	0,47604328	0,51226551	0,50649351	0,53846154	0,54929577	0,47531993	0,5503876	0,42619926	0,53333333
0,53001715	0,49411765	0,51598174	0,49511401	0,51101322	0,48979592	0,42628205	0,44104803	0,56173913	0,49197861	0,42245989	0,53143713	0,54558824	0,4929972	0,48290598	0,56226415	0,49050086	0,44862155	0,44973545	0,57142857
0,53065539	0,47146402	0,55595668	0,52222222	0,51393728	0,47668394	0,4437751	0,46046512	0,50997151	0,58007117	0,54870775	0,46727273	0,56960227	0,51677852	0,48333333	0,53503185	0,49559083	0,5245283	0,4688172	0,43390805
0,52048193	0,4527027	0,50238095	0,50642674	0,4989899	0,48214286	0,50229358	0,42172524	0,51282051	0,5488959	0,44651163	0,49027237	0,56363636	0,53386454	0,48214286	0,53525641	0,44029851	0,46428571	0,45454545	0,49814126
0,50114416	0,47074468	0,51102204	0,49471459	0,53992395	0,44978166	0,47878788	0,45987654	0,57427938	0,51898734	0,44418052	0,50455373	0,52982456	0,51796407	0,4600551	0,50531915	0,47276688	0,48571429	0,45454545	0,51884058
0,50214592	0,43611111	0,5259481	0,49644128	0,51121951	0,50623886	0,48982036	0,44495413	0,60115607	0,609375	0,46900826	0,57480315	0,54203936	0,51396648	0,48950131	0,49712974	0,49240122	0,5203252	0,43006263	0,55028249
0,54591837	0,41017964	0,52879581	0,48554913	0,48542274	0,54411765	0,37692308	0,45853659	0,64566929	0,55465587	0,50712831	0,52277228	0,5440613	0,46846847	0,48339483	0,57270694	0,4805726	0,54871795	0,4729064	0,53846154

rs2111980	rs214955	rs221956	rs2269355	rs2292972	rs2342747	rs251934	rs2830795	rs2831700	rs321198	rs338882	rs354439	rs3780962	rs4288409	rs430046	rs4364205	rs445251	rs4530059	rs4847034	rs560681
0,53790614	0,46987952	0,53942652	0,50904033	0,5014245	0,41208791	0,52864583	0,47663551	0,55015198	0,51982379	0,48741419	0,44624746	0,5070922	0,54046243	0,5	0,50374532	0,48323471	0,48453608	0,53571429	0,55414013
0,47567568	0,43636364	0,475	0,49526814	0,54205607	0,43434343	0,49429658	0,47572816	0,56218905	0,63414634	0,4173913	0,46025105	0,52542373	0,53461538	0,47777778	0,51711027	0,47938144	0,55555556	0,34042553	0,55882353
0,44285714	0,4047619	0,56557377	0,55882353	0,47916667	0,51724138	0,50763359	0,4845679	0,515625	0,53535354	0,45141066	0,51530612	0,53703704	0,49404762	0,96470588	0,521875	0,49576271	0,47826087	0,43506494	0,54787234
0,50409836	0,47826087	0,47398844	0,53537736	0,48184818	0,53719008	0,44755245	0,5	0,49535604	0,49246231	0,44421907	0,48806366	0,43959732	0,50672646	0,64309764	0,55114823	0,49465241	0,54480287	0,46067416	0,51440329
0,47697368	0,57894737	0,52599388	0,52272727	0,50171821	0,40909091	0,52525253	0,51543943	0,47590361	0,48979592	0,43987342	0,49003984	0,53793103	0,43255814	0,94642857	0,5198556	0,44680851	0,57964602	0,47368421	0,48275862
0,53658537	0,52427184	0,49235474	0,54054054	0,50655022	0,48453608	0,52	0,5	0,50393701	0,55263158	0,42741935	0,42045455	0,52132701	0,53947368	0,65970149	0,47093023	0,44329897	0,48846154	0,54545455	0,53240741
0,49707602	0,43	0,51181102	0,53608247	0,46276596	0,56666667	0,53658537	0,46300211	0,49847095	0,48351648	0,44034091	0,43046358	0,56179775	0,59685864	0,56435644	0,5038961	0,48387097	0,47528517	0,54189944	0,48726115
0,53442623	0,6	0,53333333	0,55357143	0,55595668	0,43478261	0,49886105	0,46895075	0,48012232	0,51401869	0,43298969	0,36538462	0,52486188	0,48928571	0,92857143	0,50617284	0,55844156	0,45857988	0,49541284	0,51404494
0,51764706	0,51655629	0,53357532	0,50796178	0,52736318	0,53301887	0,47826087	0,4624	0,4529703	0,47982063	0,4981203	0,4524237	0,52583587	0,4801061	0,95789474	0,492891	0,49765258	0,49608355	0,47798742	0,54010695
0,42553191	0,46601942	0,43984962	0,53648069	0,46992481	0,54054054	0,53142857	0,42222222	0,61344538	0,49541284	0,4911032	0,44174757	0,55833333	0,52564103	0,90265487	0,50980392	0,51724138	0,51546392	0,44117647	0,49760766
0,47413793	0,44029851	0,56426332	0,45555556	0,50765306	0,47590361	0,4664311	0,52173913	0,4702381	0,49659864	0,47319347	0,47213115	0,41353383	0,49767442	0,52924791	0,50543478	0,48734177	0,52173913	0,44871795	0,55095541
0,49579832	0,5106383	0,52073733	0,49242424	0,55140187	0,55	0,47368421	0,49268293	0,52439024	0,52212389	0,46963563	0,52991453	0,53061224	0,57142857	0,49	0,47596154	0,44117647	0,48466258	0,4787234	0,4600939
0,35185185	0,5	0,60526316	0,49	0,43137255	0,52941176	0,55555556	0,48648649	0,51898734	0,42857143	0,56989247	0,45283019	0,56603774	0,53703704	0,42857143	0,49206349	0,46666667	0,43181818	0,53571429	0,45454545
0,50757576	0,56521739	0,42990654	0,52142857	0,46153846	0,46835443	0,46153846	0,50802139	0,47337278	0,45	0,49404762	0,48062016	0,46601942	0,52592593	0,86315789	0,51336898	0,52142857	0,47706422	0,5	0,50285714
0,51256281	0,56880734	0,55395683	0,45017182	0,46586345	0,48453608	0,48201439	0,50318471	0,54910714	0,46153846	0,49097473	0,43975904	0,48953975	0,47674419	0,5879397	0,46320346	0,53046595	0,5137931	0,47368421	0,50793651
0,37878788	0,59259259	0,60465116	0,52475248	0,46666667	0,53191489	0,5	0,5	0,57831325	0,44827586	0,39784946	0,53333333	0,55072464	0,46551724	0,6	0,56179775	0,49230769	0,48314607	0,55172414	0,47272727
0,51190476	0,47297297	0,52519894	0,5106383	0,48425197	0,47115385	0,48148148	0,43042071	0,50833333	0,46391753	0,48641304	0,46564885	0,48120301	0,54491018	0,44915254	0,5390625	0,49829352	0,50641026	0,50515464	0,49538462
0,51506024	0,53271028	0,57110092	0,47118644	0,47238095	0,50961538	0,47865169	0,46741573	0,4605598	0,5804878	0,38686131	0,48148148	0,43773585	0,52263374	0,64917127	0,49180328	0,51895044	0,46203905	0,47863248	0,52010724
0,53539823	0,44827586	0,51246537	0,50625	0,56739812	0,45454545	0,47244094	0,47719298	0,55373832	0,46296296	0,45119306	0,44551282	0,55555556	0,47093023	0,47902098	0,47012987	0,43006993	0,45620438	0,45217391	0,49626866
0,52618454	0,55963303	0,54950495	0,63333333	0,5258216	0,48538012	0,48412698	0,37168142	0,45276873	0,48854962	0,4845735	0,41916168	0,51627907	0,50241546	0,55775578	0,50680272	0,55102041	0,47315436	0,52	0,44578313
0,52325581	0,58095238	0,53168044	0,47246377	0,47588424	0,4	0,43356643	0,45475638	0,52148997	0,54751131	0,47331787	0,46306818	0,49815498	0,48449612	0,61458333	0,51442308	0,5	0,47410359	0,53153153	0,52066116
0,48603352	0,53333333	0,5620915	0,39568345	0,5	0,58461538	0,57142857	0,51461988	0,47222222	0,57333333	0,5477707	0,44915254	0,53333333	0,625	0,64423077	0,50666667	0,43076923	0,41875	0,38596491	0,49707602
0,52470588	0,46341463	0,51898734	0,52888087	0,51779935	0,49065421	0,46678967	0,4877193	0,48181818	0,5	0,43693694	0,4812398	0,51548673	0,51477833	0,61099366	0,53050398	0,48006932	0,4738806	0,46170213	0,50922509
0,56747405	0,47727273	0,50930233	0,54447439	0,50793651	0,52419355	0,48461538	0,46428571	0,47949527	0,45945946	0,49756888	0,48442907	0,472	0,48106061	0,82173913	0,48302872	0,53688525	0,44415584	0,46376812	0,48125
0,59770115	0,49305556	0,52575107	0,48963731	0,51789976	0,43046358	0,52036199	0,47204969	0,51098901	0,54098361	0,42827869	0,46575342	0,51465798	0,48235294	0,79005525	0,46891192	0,49517685	0,4691358	0,42907801	0,5
0,48096886	0,48447205	0,50700935	0,53168044	0,54740061	0,49264706	0,48275862	0,43333333	0,46571429	0,50617284	0,42519685	0,45670996	0,51912568	0,57251908	0,62101911	0,47834275	0,50561798	0,44522968	0,52380952	0,55466667
0,5375	0,525	0,51388889	0,5	0,5	0,59731544	0,52265861	0,48206278	0,48115942	0,54666667	0,46315789	0,46808511	0,50854701	0,55092593	0,60736196	0,5258427	0,49185668	0,51724138	0,46132597	0,46687697
0,52671756	0,57352941	0,51793722	0,50456621	0,45533141	0,51121076	0,50276243	0,47619048	0,46899225	0,47572816	0,42388759	0,45666667	0,51428571	0,54624277	0,65769231	0,45776567	0,49685535	0,45970149	0,5	0,49333333
0,42715232	0,40585774	0,53965184	0,47017544	0,48241206	0,45505618	0,46698113	0,43043478	0,4755102	0,4600639	0,49043478	0,50379747	0,51820728	0,51811594	0,88537549	0,51788756	0,54216867	0,50855746	0,48989899	0,54186047
0,51875	0,44444444	0,54455446	0,48427673	0,51442308	0,51219512	0,53939394	0,5	0,55625	0,4137931	0,51209677	0,43220339	0,53636364	0,58928571	0,58119658	0,45714286	0,51515152	0,46766169	0,58024691	0,50344828
0,55960265	0,5	0,47854785	0,51002865	0,47350993	0,52941176	0,45938375	0,47705146	0,53914989	0,44668588	0,48076923	0,47077922	0,49769585	0,57322176	0,50918635	0,48462929	0,50862069	0,45035461	0,48410758	0,51470588
0,52432432	0,49387755	0,51953125	0,50592885	0,52439024	0,43023256	0,48148148	0,49730458	0,52941176	0,44930876	0,43853821	0,4729064	0,48148148	0,46783626	0,92903226	0,52953586	0,51714286	0,55197133	0,49238579	0,50271739
0,50530035	0,46923077	0,52340426	0,48175182	0,44767442	0,4796748	0,51653944	0,48546825	0,49227799	0,47368421	0,48807339	0,45393635	0,48104265	0,47868852	0,62849162	0,51567398	0,5031185	0,45238095	0,48108108	0,59863946
0,55658199	0,56390977	0,49206349	0,51992754	0,4638448	0,5729927	0,44471154	0,52468265	0,55276382	0,54581673	0,45321101	0,45283019	0,48056537	0,46904762	0,63934426	0,49788136	0,475	0,44088176	0,49404762	0,55733945
0,50230415	0,47637795	0,50442478	0,49183673	0,55363322	0,54761905	0,49487179	0,4894636	0,49516441	0,51152074	0,44758065	0,45106383	0,51991614	0,49390244	0,6552795	0,54558611	0,46171171	0,45993031	0,52222222	0,52567237
0,52304147	0,51546392	0,46924429	0,55701754	0,45351474	0,5398773	0,49157303	0,45544554	0,45305164	0,50180505	0,45894737	0,41608392	0,58293839	0,48708487	0,68882175	0,47445255	0,42948718	0,50138504	0,4954955	0,5206422
0,60805861	0,54210526	0,51030928	0,51923077	0,4980695	0,48412698	0,43076923	0,52317073	0,50186567	0,52122642	0,45995423	0,4459725	0,47544643	0,53424658	0,44536082	0,50535714	0,4929972	0,4169279	0,51374207	0,50724638
0,46153846	0,44680851	0,54789272	0,49845201	0,50263158	0,50802139	0,51990632	0,46516393	0,45913462	0,535	0,45622896	0,45060241	0,43910256	0,49185668	0,44444444	0,49131944	0,475	0,47084233	0,50204082	0,47336066
0,49033816	0,47133758	0,52931323	0,47203274	0,51936219	0,46896552	0,46311475	0,47922849	0,5012285	0,50621118	0,49346405	0,47207207	0,5	0,52691218	0,88934426	0,4985119	0,53084112	0,47843943	0,47826087	0,45794393
0,51520572	0,43506494	0,51642129	0,48252912	0,50075873	0,49134948	0,53204353	0,4904661	0,53108348	0,50712251	0,42586751	0,44026846	0,48758865	0,50844278	0,64014467	0,49738767	0,49245542	0,46895641	0,46489104	0,47459459
0,50170648	0,48550725	0,55630252	0,47820513	0,5190678	0,48743719	0,48242188	0,5	0,48735632	0,52469136	0,44719472	0,49916248	0,48657718	0,52407932	0,884	0,50488145	0,49579832	0,47859922	0,51086957	0,49616858
0,45040214	0,45625	0,59509202	0,50276855	0,52008457	0,52631579	0,45402299	0,52369668	0,46938776	0,52805281	0,44477172	0,44846293	0,49725275	0,49164678	0,69251337	0,55375552	0,43407708	0,51171875	0,50508475	0,49683544
0,53235294	0,51694915	0,51737452	0,46828358	0,50273224	0,50314465	0,48888889	0,46409807	0,50151057	0,52509653	0,45454545	0,41704036	0,55891239	0,48450704	0,47344111	0,51515152	0,51923077	0,52130326	0,48175182	0,49010989
0,53819444	0,42028986	0,47076023	0,4835924	0,50508475	0,42196532	0,46388889	0,51830443	0,46458924	0,43137255	0,52421652	0,4965358	0,55895197	0,50511945	0,66129032	0,54788419	0,51168831	0,50344828	0,47085202	0,46522782
0,43461538	0,46218487	0,51926978	0,49820789	0,4947644	0,54014599	0,50485437	0,46577629	0,50439883	0,50607287	0,46015936	0,44886364	0,52416357	0,42857143	0,64477612	0,50537634	0,4954955	0,52185792	0,49206349	0,500998
0,4173913	0,40723982	0,6222597	0,46808511	0,52200489	0,4601227	0,49209139	0,55245902	0,45294118	0,56603774	0,42397857	0,4567474	0,40899358	0,43686869	0,58788481	0,45768374	0,49211909	0,49617672	0,46116505	0,52242152
0,48328267	0,51376147	0,5490982	0,50998948	0,47361111	0,52409639	0,54658385	0,47868852	0,48809524	0,52132701	0,45363128	0,40509259	0,58522727	0,4704142	0,61917098	0,51981352	0,46461538	0,4967825	0,47555556	0,54485777

rs576261	rs6444724	rs6811238	rs6955448	rs7041158	rs717302	rs719366	rs722098	rs722290	rs727811	rs729172	rs733164	rs735155	rs737681	rs740598	rs740910	rs7520386	rs7704770	rs826472	rs873196
0,54725275	0,45771144	0,45687646	0,52462527	0,50337079	0,45874587	0,42975207	0,48565574	0,44210526	0,5511811	0,4044586	0,39224138	0,52295918	0,57142857	0,47706422	0,41666667	0,53550864	0,50112867	0,52469136	0,51265823
0,50993377	0,45017182	0,49206349	0,53874539	0,51898734	0,44067797	0,33823529	0,49618321	0,43712575	0,59848485	0,43708609	0,40271493	0,51612903	0,54307116	0,49815498	0,50746269	0,5902439	0,4978355	0,54867257	0,51190476
0,42647059	0,4321608	0,48826291	0,53055556	0,51709402	0,56578947	0,43859649	0,51273885	0,52592593	0,61025641	0,50857143	0,47663551	0,51694915	0,5257732	0,4965035	0,46511628	0,51724138	0,5025641	0,50344828	0,48837209
0,42035398	0,50395778	0,51627907	0,47014925	0,51319648	0,48958333	0,35802469	0,50823529	0,48837209	0,62692308	0,47395833	0,47040498	0,44814815	0,55775578	0,54580153	0,49637681	0,51333333	0,4765343	0,49777778	0,46530612
0,5440613	0,41263941	0,46229508	0,52873563	0,48543689	0,49568966	0,38311688	0,51194539	0,52173913	0,57228916	0,43678161	0,48601399	0,50151057	0,55305466	0,43085106	0,47457627	0,58227848	0,55701754	0,50393701	0,52073733
0,49643705	0,49371069	0,41176471	0,50549451	0,62	0,55367232	0,47863248	0,51851852	0,48387097	0,62244898	0,43055556	0,43069307	0,4602649	0,55633803	0,575	0,54320988	0,5530086	0,36305732	0,65384615	0,57142857
0,45454545	0,51505017	0,47457627	0,56559767	0,52201258	0,51711027	0,44444444	0,5	0,46666667	0,59728507	0,50510204	0,43581081	0,49609375	0,54736842	0,48897059	0,44444444	0,51590106	0,4813278	0,56741573	0,46963563
0,46041056	0,47318612	0,43037975	0,49132948	0,49583333	0,51492537	0,32894737	0,4984985	0,46625767	0,6	0,52849741	0,4904943	0,51388889	0,56417112	0,53815261	0,51176471	0,56542056	0,49466192	0,55913978	0,48901099
0,44675325	0,53374233	0,45724907	0,55122951	0,5450237	0,48148148	0,51272727	0,47636364	0,47904192	0,60447761	0,5	0,45601852	0,45825243	0,57235421	0,53668763	0,49326146	0,50120482	0,54761905	0,48373984	0,47560976
0,48780488	0,41203704	0,51101322	0,54424779	0,58293839	0,45049505	0,49579832	0,42045455	0,55696203	0,77777778	0,4137931	0,47478992	0,47257384	0,54545455	0,44378698	0,4375	0,53420195	0,49545455	0,58333333	0,53012048
0,57460317	0,48711944	0,45955882	0,51698113	0,49097473	0,52054795	0,48660714	0,4601227	0,49180328	0,35545024	0,49382716	0,53333333	0,54142012	0,55855856	0,49027237	0,51445087	0,54639175	0,57377049	0,40816327	0,49582173
0,52409639	0,41666667	0,46086957	0,43703704	0,53146853	0,51	0,40875912	0,48412698	0,52727273	0,65671642	0,42045455	0,49673203	0,56097561	0,56603774	0,59055118	0,36363636	0,47311828	0,42748092	0,65306122	0,51282051
0,30612245	0,57142857	0,53030303	0,51020408	0,6	0,58823529	0,68421053	0,40625	0,46153846	0,64864865	0,5	0,53968254	0,55882353	0,70454545	0,57142857	0,5	0,55263158	0,38888889	0,41666667	0,53968254
0,4887218	0,50993377	0,54098361	0,62162162	0,54054054	0,49431818	0,375	0,53205128	0,51401869	0,74358974	0,36434109	0,5	0,50289017	0,56493506	0,46590909	0,50574713	0,54117647	0,45454545	0,64705882	0,37168142
0,5	0,48989899	0,54976303	0,50204082	0,50310559	0,53431373	0,30487805	0,54661017	0,49484536	0,81818182	0,5	0,47340426	0,51209677	0,51968504	0,48815166	0,45138889	0,54054054	0,51351351	0,56842105	0,48847926
0,47674419	0,46534653	0,49253731	0,53409091	0,53225806	0,46153846	0,51470588	0,44262295	0,5	0,68292683	0,51428571	0,40860215	0,5	0,640625	0,46511628	0,51020408	0,51020408	0,51785714	0,6875	0,39534884
0,51361868	0,5078125	0,46905537	0,52083333	0,57077626	0,51098901	0,43274854	0,46728972	0,53543307	0,64397906	0,3988764	0,51034483	0,5498155	0,56387665	0,57081545	0,48888889	0,51764706	0,48351648	0,52694611	0,50788644
0,5047619	0,51075269	0,48275862	0,55797101	0,46648794	0,48627451	0,41152263	0,48953975	0,55900621	0,54741379	0,5	0,48490946	0,48166259	0,57069409	0,52095808	0,48898678	0,53213368	0,44230769	0,47959184	0,54103343
0,48297214	0,49889625	0,51162791	0,56932153	0,52631579	0,46349206	0,50929368	0,4717608	0,50746269	0,59067358	0,46788991	0,51803279	0,47651007	0,56078431	0,54	0,48660714	0,52479339	0,49090909	0,56804734	0,41525424
0,54416961	0,50894632	0,57407407	0,50859107	0,50847458	0,54368932	0,43055556	0,53956835	0,5	0,4962406	0,55483871	0,50356295	0,47745902	0,57441253	0,58646617	0,49101796	0,54703833	0,4	0,5625	0,475
0,49347258	0,48059701	0,496875	0,50294118	0,54626866	0,54347826	0,4	0,48866499	0,54314721	0,69014085	0,42647059	0,49508197	0,55474453	0,49501661	0,53710247	0,42176871	0,5	0,43402778	0,55384615	0,55789474
0,52459016	0,48888889	0,47482014	0,45138889	0,63247863	0,61344538	0,44186047	0,46107784	0,52054795	0,58108108	0,46666667	0,44588745	0,48275862	0,54054054	0,5	0,46551724	0,55263158	0,55555556	0,53225806	0,54225352
0,47882736	0,47175141	0,4724026	0,54966887	0,55862069	0,52699784	0,4244186	0,4704	0,44919786	0,58104738	0,47916667	0,45481928	0,50343643	0,56642066	0,51972158	0,48931116	0,52241715	0,49574468	0,58870968	0,54199683
0,45995423	0,47546012	0,52905199	0,55652174	0,53307393	0,56071429	0,53146853	0,55148741	0,6372549	0,49473684	0,51094891	0,43287037	0,48491879	0,6039604	0,51123596	0,48251748	0,48860759	0,59330144	0,57142857	0,47794118
0,5376569	0,5734072	0,44981413	0,53865979	0,48473282	0,54756381	0,47484277	0,46769231	0,4502924	0,63513514	0,46153846	0,48789572	0,48122066	0,54237288	0,57509158	0,48117155	0,53422053	0,51173709	0,5	0,47736626
0,48621554	0,49521531	0,46456693	0,48780488	0,51587302	0,49636804	0,49541284	0,55831266	0,50570342	0,53442623	0,52263374	0,4625	0,46547315	0,5480226	0,4738806	0,48507463	0,48275862	0,50814332	0,53461538	0,5175644
0,51748252	0,46954813	0,48148148	0,47841727	0,49473684	0,50750751	0,444	0,47969543	0,47239264	0,64227642	0,47863248	0,48792271	0,56170213	0,60596026	0,50543478	0,47982063	0,51315789	0,48017621	0,49438202	0,48630137
0,425	0,4132948	0,50641026	0,50865052	0,565625	0,46296296	0,47887324	0,50882353	0,41991342	0,67264574	0,50934579	0,46436782	0,49655172	0,5863747	0,5	0,4741784	0,49275362	0,4551495	0,55	0,46305419
0,54989384	0,49462366	0,45154185	0,51937984	0,47714286	0,51504425	0,5	0,484375	0,47982063	0,56	0,48122867	0,47186147	0,52955083	0,54366197	0,53409091	0,528125	0,55397727	0,47965116	0,52747253	0,49501661
0,54545455	0,45333333	0,53623188	0,55974843	0,456	0,54255319	0,46616541	0,53299492	0,52727273	0,53508772	0,53284672	0,46242775	0,42857143	0,53521127	0,55084746	0,432	0,45833333	0,53921569	0,48979592	0,51948052
0,46511628	0,47040971	0,46487603	0,49350649	0,51663405	0,55785838	0,4904943	0,50379363	0,46136364	0,68579235	0,41578947	0,46944444	0,45772595	0,59223301	0,50943396	0,48706897	0,52971576	0,52580645	0,57003257	0,49480969
0,55882353	0,54126984	0,52834008	0,52156863	0,48565121	0,49630996	0,48571429	0,50483092	0,52071006	0,60045147	0,39310345	0,456621	0,55050505	0,53240741	0,4952381	0,44162437	0,48660714	0,56949153	0,54603175	0,5045045
0,496875	0,50523169	0,50796813	0,51397849	0,53416149	0,52808989	0,45454545	0,51201011	0,52370203	0,653125	0,45033113	0,42182891	0,53351955	0,60686016	0,51515152	0,40659341	0,56456456	0,48210024	0,63793103	0,48883375
0,42964824	0,49415205	0,54722222	0,57234043	0,52978723	0,4751773	0,39896373	0,46037736	0,47883598	0,58091286	0,46192893	0,45798319	0,477842	0,58691207	0,58387097	0,40963855	0,56055363	0,42243437	0,55414013	0,5483871
0,4826087	0,44925575	0,35815603	0,4751773	0,52272727	0,49631811	0,4351145	0,5025641	0,45169082	0,53626374	0,47468354	0,52777778	0,48705882	0,55	0,5015873	0,42394822	0,55	0,40731707	0,53206651	0,5
0,43839542	0,45544554	0,45774648	0,53278689	0,55371901	0,5102459	0,44285714	0,47540984	0,50359712	0,6443299	0,48421053	0,48607595	0,49616368	0,63895487	0,49528302	0,44137931	0,55	0,46439628	0,62135922	0,49096386
0,50627615	0,46907994	0,47975709	0,60655738	0,50259067	0,55147059	0,36601307	0,52097429	0,47314578	0,68263473	0,4	0,4660767	0,48096886	0,57417582	0,58208955	0,436	0,52693208	0,48732394	0,59927798	0,4610951
0,46993318	0,49589041	0,50116009	0,53416149	0,51798561	0,55702918	0,46768061	0,53667263	0,53103448	0,65517241	0,48997135	0,47555556	0,48767123	0,58139535	0,53626374	0,47457627	0,5075	0,48860759	0,68316832	0,5245098
0,57926829	0,50414938	0,4813278	0,52531646	0,50096712	0,50534759	0,46359223	0,50346021	0,5025641	0,61154856	0,47765363	0,4476386	0,47798742	0,58643326	0,47215496	0,45833333	0,51116625	0,46982759	0,61165049	0,51752577
0,53903904	0,50251256	0,50071327	0,53950339	0,49105915	0,50672646	0,46516854	0,50328947	0,53832117	0,59381044	0,46554935	0,47354138	0,55801105	0,52465234	0,54709677	0,47435897	0,52834741	0,52110818	0,57575758	0,48939394
0,58685446	0,52268245	0,47013487	0,49554367	0,48069498	0,46491228	0,42692308	0,51393189	0,51582278	0,70440252	0,42670157	0,50757576	0,52554745	0,52427184	0,51047619	0,46363636	0,52312139	0,48125	0,61052632	0,49885584
0,50682261	0,4990099	0,48340548	0,47431507	0,50364964	0,55773956	0,45368171	0,4893918	0,51237624	0,71111111	0,45584046	0,50162338	0,48426573	0,6020202	0,47124304	0,50224215	0,53354633	0,4858871	0,54411765	0,46019417
0,51260504	0,43917526	0,48702595	0,57289003	0,52417303	0,5	0,4954955	0,50721154	0,4	0,72239748	0,4	0,43763676	0,49767442	0,55421687	0,51662404	0,4467354	0,5210728	0,42270531	0,61881188	0,50918635
0,48373984	0,48710602	0,48739496	0,55437666	0,51183432	0,54945055	0,45410628	0,50966184	0,49042146	0,69333333	0,41365462	0,42979943	0,50141643	0,57275542	0,50769231	0,46783626	0,5733945	0,56676558	0,6223176	0,5511811
0,49122807	0,5399449	0,51346499	0,52745098	0,49870801	0,56994819	0,49809886	0,45202559	0,46749226	0,63043478	0,46808511	0,50252525	0,50531915	0,53216374	0,53051643	0,46819338	0,50928382	0,4453125	0,60689655	0,54320988
0,49147727	0,47809524	0,46335697	0,47956403	0,47103275	0,55148096	0,3974359	0,43669986	0,65909091	0,26136364	0,62658228	0,51707317	0,55121951	0,57075472	0,50114416	0,42410714	0,50506073	0,48849105	0,33333333	0,51079137
0,48126801	0,44375	0,4119403	0,5115894	0,5304878	0,51869159	0,39344262	0,44852941	0,4137931	0,61658031	0,45228216	0,48582231	0,50990099	0,60887949	0,50777202	0,41832669	0,52797203	0,49425287	0,59146341	0,51860465

rs876724	rs891700	rs901398	rs907100	rs914165	rs917118	rs938283	rs964681	rs987640	rs9905977	rs993934	rs9951171	L298	M479	P202	P256	rs13447443	rs16980426	rs16981290	rs17222573
0,56060606	0,57926829	0,45012788	0,46280992	0,56	0,58522727	0,50980392	0,43069307	0,61029412	0,45381526	0,55707763	0,55826558	0,5	0,34210526	0,51239669	0,492891	0,48706897	0,47490347	0,5106383	0,44776119
0,58823529	0,59444444	0,40701754	0,48387097	0,57383966	0,51111111	0,50344828	0,48109966	0,56989247	0,44	0,38053097	0,50735294	0,36082474	0,45454545	0,52542373	0,52121212	0,52307692	0,48148148	0,55357143	0,38848921
0,55172414	0,51485149	0,5	0,496	0,55063291	0,60810811	0,51764706	0,60126582	0,53982301	0,4384058	0,55555556	0,45679012	0,51181102	0,29411765	0,5	0,35483871	0,55555556	0,42056075	0,50505051	0,48062016
0,52252252	0,52785146	0,49069767	0,5	0,56650246	0,51006711	0,51789976	0,45425868	0,5147929	0,42042042	0,5177305	0,54901961	0,48167539	0,375	0,48120301	0,59803922	0,40322581	0,55191257	0,52380952	0,43127962
0,67567568	0,58285714	0,52117264	0,50359712	0,49450549	0,50632911	0,5936255	0,43621399	0,64044944	0,51	0,51428571	0,52918288	0,52777778	0,61538462	0,44594595	0,59090909	0,5125	0,44262295	0,39622642	0,5304878
0,44615385	0,53403141	0,41851852	0,65625	0,5426009	0,57926829	0,56284153	0,46410256	0,53763441	0,51219512	0,56363636	0,50259067	0,53956835	0,37931034	0,5	0,52252252	0,44230769	0,55652174	0,54320988	0,46666667
0,44761905	0,51785714	0,43589744	0,45930233	0,54347826	0,55151515	0,54455446	0,52160494	0,67065868	0,4512987	0,5106383	0,54811715	0,49122807	0,33333333	0,45	0,52631579	0,58273381	0,43085106	0,61589404	0,44067797
0,66101695	0,55660377	0,49361702	0,46078431	0,54313099	0,41666667	0,53548387	0,48961424	0,55319149	0,48156682	0,51851852	0,53300733	0,44692737	0,51851852	0,43103448	0,53023256	0,56060606	0,38150289	0,43181818	0,5017301
0,54744526	0,55313351	0,48956357	0,5	0,53982301	0,43258427	0,47881356	0,5403423	0,47058824	0,54901961	0,50232558	0,50802139	0,46859903	0,23076923	0,3828125	0,50806452	0,56115108	0,56903766	0,53921569	0,51194539
0,58163265	0,54347826	0,49596774	0,52066116	0,5971564	0,46153846	0,593361	0,41409692	0,70422535	0,47307692	0,57971014	0,50847458	0,45098039	0,21428571	0,53225806	0,34821429	0,51428571	0,53703704	0,36363636	0,45930233
0,5754717	0,49230769	0,48220065	0,42553191	0,47307692	0,51612903	0,56013746	0,46796657	0,52380952	0,51257862	0,54320988	0,50396825	0,53146853	0,56	0,5952381	0,61325967	0,50724638	0,46551724	0,44859813	0,50961538
0,48780488	0,52222222	0,43030303	0,52631579	0,4789916	0,3956044	0,4965035	0,47101449	0,57317073	0,48251748	0,59090909	0,52054795	0,44117647	0,57142857	0,36666667	0,50746269	0,45	0,4516129	0,39622642	0,43478261
0,58333333	0,35849057	0,47126437	0,48484848	0,5	0,27272727	0,56097561	0,44827586	0,54545455	0,62	0,7	0,44736842	0,25	0,5	0,35714286	0,55555556	0,55555556	0,44736842	0,48387097	0,55555556
0,58181818	0,60283688	0,4600939	0,47058824	0,5511811	0,56081081	0,52272727	0,50322581	0,515625	0,49456522	0,58181818	0,48186528	0,45333333	0,46153846	0,36363636	0,55737705	0,46835443	0,44155844	0,73076923	0,50684932
0,49152542	0,54385965	0,46945338	0,43859649	0,56746032	0,4829932	0,5154185	0,46122449	0,55670103	0,4869281	0,54901961	0,49019608	0,55172414	0,27777778	0,65	0,48550725	0,44927536	0,48780488	0,5952381	0,43382353
0,26086957	0,51666667	0,49038462	0,32258065	0,55737705	0,55172414	0,58181818	0,52222222	0,5	0,42857143	0,48648649	0,57009346	0,53658537	0,28571429	N	0,48979592	0,375	0,34482759	0,55882353	0,44827586
0,63366337	0,5	0,47949527	0,49056604	0,5256917	0,48888889	0,48987854	0,49824561	0,56716418	0,4567474	0,53043478	0,54597701	0,49612403	0,24	0,45801527	0,55714286	0,60576923	0,4695122	0,60714286	0,47179487
0,55421687	0,42960289	0,47021277	0,49775785	0,51116625	0,6625	0,46067416	0,45260664	0,57051282	0,48198198	0,4379085	0,56273764	0,49704142	0,41025641	0,4375	0,54228856	0,46405229	0,43367347	0,47482014	0,46153846
0,48466258	0,515625	0,49885057	0,5210084	0,50988142	0,43589744	0,45652174	0,41533546	0,54237288	0,49785408	0,48087432	0,54183267	0,45762712	0,35483871	0,46575342	0,4962963	0,55882353	0,527897	0,52631579	0,48325359
0,57471264	0,53793103	0,44642857	0,50561798	0,56	0,50595238	0,53216374	0,4608076	0,55882353	0,53457447	0,55102041	0,52009456	0,53191489	0,42857143	0,51515152	0,47345133	0,45454545	0,42068966	0,45967742	0,52631579
0,55029586	0,57142857	0,50512821	0,51	0,51657459	0,51948052	0,53727506	0,47315436	0,60240964	0,46268657	0,49677419	0,53525641	0,44805195	0,38709677	0,50769231	0,47222222	0,47916667	0,5106383	0,46956522	0,52941176
0,54098361	0,51685393	0,43888889	0,56862745	0,60150376	0,44444444	0,46715328	0,5045045	0,65517241	0,48235294	0,60526316	0,53103448	0,5	0,44444444	0,42105263	0,52238806	0,46341463	0,5	0,625	0,40707965
0,55350554	0,57677165	0,43799058	0,50592885	0,54931973	0,58333333	0,51203501	0,50402576	0,61111111	0,46473779	0,51973684	0,51405622	0,49615385	0,27586207	0,5408805	0,57183908	0,49173554	0,47021944	0,58436214	0,42346939
0,49681529	0,54782609	0,50782998	0,55	0,54077253	0,51626016	0,53493976	0,55608592	0,51282051	0,46577629	0,65	0,56304985	0,49	0,45454545	0,48387097	0,5437788	0,59574468	0,53293413	0,5862069	0,48708487
0,53225806	0,54151625	0,50344828	0,41726619	0,58054711	0,49333333	0,54316547	0,48412698	0,5	0,4600939	0,48550725	0,52647059	0,48369565	0,38356164	0,50769231	0,53488372	0,52631579	0,48603352	0,55555556	0,48333333
0,52232143	0,44166667	0,47657841	0,50625	0,46827795	0,51630435	0,56634304	0,50424929	0,45084746	0,44654088	0,4744186	0,51408451	0,51690821	0,33333333	0,47863248	0,50777202	0,53301887	0,48295455	0,52542373	0,49212598
0,53703704	0,4600551	0,47082495	0,53571429	0,51438849	0,42537313	0,51903114	0,51525424	0,50738916	0,50147493	0,46192893	0,47337278	0,47111111	0,38095238	0,59210526	0,46073298	0,47435897	0,39419087	0,56382979	0,45848375
0,45588235	0,54887218	0,45060241	0,5390625	0,53314121	0,55434783	0,55681818	0,51913876	0,640625	0,49074074	0,48684211	0,45813953	0,48120301	0,30769231	0,5	0,52888889	0,46774194	0,46774194	0,5648855	0,40540541
0,59116022	0,52245863	0,52880658	0,49382716	0,46760563	0,54407295	0,51226158	0,45365854	0,54907975	0,47651007	0,47087379	0,48913043	0,47457627	0,43209877	0,50632911	0,49446494	0,51075269	0,48376623	0,44715447	0,48780488
0,5	0,56	0,45789474	0,39759036	0,53642384	0,5060241	0,5060241	0,47802198	0,52941176	0,51709402	0,46153846	0,47340426	0,63793103	0,57142857	0,83333333	0,50632911	0,43902439	0,43859649	0,5	0,48863636
0,5	0,54545455	0,49015317	0,51851852	0,55357143	0,52093023	0,54861111	0,49790795	0,55511811	0,45714286	0,4893617	0,55487805	0,50510204	0,42307692	0,54216867	0,44868035	0,40677966	0,48962656	0,4921875	0,45564516
0,57758621	0,49149923	0,47665848	0,47857143	0,48432056	0,58247423	0,4375	0,44751381	0,56140351	0,59148936	0,47639485	0,54724409	0,50531915	0,26315789	0,51428571	0,51459854	0,51497006	0,5210356	0,48235294	0,49074074
0,64583333	0,60223048	0,47868217	0,49189189	0,52542373	0,64	0,56402439	0,48186528	0,65363128	0,44711538	0,52226721	0,50769231	0,42105263	0,25	0,52678571	0,52614379	0,47263682	0,45360825	0,55688623	0,44097222
0,4025974	0,53333333	0,4454343	0,46153846	0,55829596	0,5915493	0,50969529	0,53030303	0,67307692	0,46300211	0,52020202	0,49717514	0,51041667	0,30434783	0,43589744	0,4609375	0,4673913	0,49065421	0,55263158	0,46766169
0,57512953	0,56119403	0,47438753	0,6163522	0,54498715	0,56183746	0,49637681	0,41333333	0,6	0,50422535	0,51923077	0,49635036	0,4279476	0,3559322	0,47474747	0,52747253	0,5	0,43157895	0,5648855	0,46917808
0,56834532	0,65841584	0,41704036	0,5	0,5122549	0,5483871	0,52521008	0,52649007	0,55757576	0,4861461	0,46086957	0,52320675	0,42056075	0,35555556	0,36538462	0,53378378	0,47761194	0,45026178	0,48192771	0,41767068
0,48529412	0,57087379	0,47941889	0,46405229	0,53221289	0,47014925	0,55521472	0,52140078	0,60714286	0,43266476	0,58024691	0,49683544	0,43378995	0,18518519	0,45918367	0,4954955	0,48550725	0,47540984	0,42657343	0,50396825
0,52531646	0,484375	0,49425287	0,48241206	0,51148225	0,55947137	0,46895075	0,52155172	0,50318471	0,50925926	0,4507772	0,55487805	0,51598174	0,35555556	0,44680851	0,62068966	0,54487179	0,48704663	0,52147239	0,42750929
0,58469945	0,51859504	0,48190476	0,44193548	0,56292906	0,50530035	0,4905303	0,48162476	0,54153846	0,47013487	0,48780488	0,49665924	0,51481481	0,265625	0,43243243	0,48550725	0,51101322	0,49411765	0,53763441	0,45544554
0,53846154	0,52574526	0,50224215	0,47272727	0,56413793	0,54676259	0,46330778	0,50258398	0,56967213	0,56679637	0,49117647	0,52512998	0,53932584	0,30645161	0,46360153	0,47040498	0,51340996	0,44610778	0,51212121	0,52263374
0,53932584	0,49272727	0,51607143	0,44814815	0,52877698	0,56680162	0,47209653	0,45487365	0,58503401	0,52768166	0,49565217	0,48033708	0,46231156	0,16666667	0,47590361	0,51330798	0,52941176	0,44493392	0,55121951	0,51768489
0,51401869	0,55894309	0,43833017	0,51971326	0,49489796	0,50176678	0,51578947	0,49530957	0,56213018	0,47288136	0,46691176	0,50381679	0,44014085	0,35714286	0,44976077	0,51821862	0,46311475	0,425	0,54901961	0,50287356
0,50393701	0,62059621	0,45303867	0,53017241	0,52380952	0,51875	0,52968037	0,45138889	0,6744186	0,56334842	0,51	0,50991501	0,39102564	0,18518519	0,56164384	0,48	0,43888889	0,5203252	0,55621302	0,4368932
0,52173913	0,56534954	0,4929972	0,52	0,49529781	0,4587156	0,48924731	0,43533123	0,56910569	0,48492462	0,49211356	0,49050633	0,44360902	0,26666667	0,47413793	0,52486188	0,46961326	0,45945946	0,49006623	0,48181818
0,57823129	0,48590022	0,5661157	0,47637795	0,53333333	0,46636771	0,47904192	0,55380577	0,51408451	0,4787234	0,50655022	0,47766323	0,52459016	0,25454545	0,42767296	0,46058091	0,41869919	0,45588235	0,54248366	0,41004184
0,52040816	0,46599496	0,43080626	0,48730964	0,5253955	0,5942029	0,48943364	0,46594982	0,49097473	0,50472813	0,58695652	0,52664577	0,54845815	0,57692308	0,43902439	0,55555556	0,52678571	0,43668122	0,52336449	0,50934579
0,58518519	0,58922559	0,42696629	0,46581197	0,49130435	0,55769231	0,55492424	0,5047619	0,65217391	0,48018293	0,54545455	0,50374065	0,44285714	0,35714286	0,47058824	0,50438596	0,41284404	0,45238095	0,48666667	0,52068966

rs17250845	rs17269816	rs17306671	rs17842518	rs20320	rs2032595	rs2032599	rs2032602	rs2032624	rs2032631	rs2032636	rs2032652	rs2032658	rs2032673	rs2033003	rs2319818	rs2534636	rs35284970	rs3848982	rs3900
0,47926267	0,41176471	0,39903846	0,48648649	0,46405229	0,38596491	0,59534884	0,48594378	0,36428571	0,46808511	0,38926174	0,51737452	0,53811659	0,53658537	0,5046729	0,456	0,52631579	0,50625	0,55399061	0,45810056
0,52136752	0,45054945	0,46616541	0,46153846	0,52222222	0,55555556	0,6372549	0,52739726	0,42857143	0,4375	0,27777778	0,54700855	0,51190476	0,57823129	0,52531646	0,42105263	0,54032258	0,54285714	0,51515152	0,29310345
0,40957447	0,45263158	0,47115385	0,42307692	0,42592593	0,42253521	0,38333333	0,42758621	0,39189189	0,56043956	0,65384615	0,54455446	0,4691358	0,53225806	0,51648352	0,52857143	0,4084507	0,5	0,52631579	0,53174603
0,53225806	0,50326797	0,50857143	0,45901639	0,57894737	0,53164557	0,52554745	0,472103	0,41129032	0,42962963	0,51376147	0,45320197	0,49404762	0,45283019	0,41401274	0,54347826	0,50694444	0,46280992	0,50306748	0,56756757
0,59428571	0,5	0,41441441	0,44808743	0,47586207	0,52459016	0,50561798	0,4244186	0,44444444	0,4453125	0,53968254	0,50326797	0,38888889	0,56818182	0,56179775	0,50955414	0,56435644	0,51485149	0,46153846	0,53846154
0,59116022	0,52830189	0,49019608	0,49132948	0,38016529	0,35897436	0,47058824	0,47142857	0,45283019	0,47368421	0,43333333	0,5625	0,43181818	0,48684211	0,49367089	0,52717391	0,48529412	0,55045872	0,58510638	0,23913043
0,47651007	0,39130435	0,4	0,43575419	0,55782313	0,43373494	0,49122807	0,46107784	0,37362637	0,50769231	0,41904762	0,44632768	0,58267717	0,51707317	0,51633987	0,45876289	0,54761905	0,50420168	0,53333333	0,45555556
0,52314815	0,5	0,44339623	0,45962733	0,46368715	0,43181818	0,40540541	0,46551724	0,38461538	0,47619048	0,57407407	0,49685535	0,59235669	0,60846561	0,44767442	0,42934783	0,45255474	0,46456693	0,5426009	0,52380952
0,52212389	0,48401826	0,37674419	0,58064516	0,50724638	0,37614679	0,49673203	0,47826087	0,49557522	0,53763441	0,50632911	0,48109966	0,52252252	0,5	0,54326923	0,53875969	0,49484536	0,53645833	0,52803738	0,49103943
0,53703704	0,45132743	0,40425532	0,51948052	0,57777778	0,55813953	0,68965517	0,375	0,58974359	0,54022989	0,27272727	0,52898551	0,50961538	0,50413223	0,55844156	0,44654088	0,45454545	0,46341463	0,44186047	0,47272727
0,53551913	0,41081081	0,52307692	0,4321608	0,51572327	0,49230769	0,59793814	0,47222222	0,54736842	0,38356164	0,46753247	0,45255474	0,50955414	0,54385965	0,47752809	0,5106383	0,544	0,4789916	0,54497354	0,5
0,50793651	0,62222222	0,53125	0,39393939	0,53947368	0,5	0,625	0,52173913	0,51020408	0,54545455	0,3125	0,63235294	0,4875	0,51818182	0,45652174	0,5308642	0,56923077	0,58928571	0,51470588	0,48837209
0,55555556	0,77777778	0,47058824	0,5483871	0,475	0,33333333	0,43478261	0,65384615	0,54545455	0,4375	0,45454545	0,52173913	0,33333333	0,46296296	0,58536585	0,5	0,66666667	0,41176471	0,5	0,4375
0,5	0,41818182	0,5047619	0,46153846	0,53448276	0,47368421	0,46296296	0,48648649	0,52173913	0,55555556	0,44444444	0,52112676	0,525	0,50588235	0,50632911	0,53488372	0,58181818	0,39393939	0,48979592	0,5
0,55319149	0,45238095	0,51086957	0,46808511	0,46280992	0,48837209	0,44680851	0,4875	0,54054054	0,56818182	0,44262295	0,46491228	0,59090909	0,44615385	0,55140187	0,48322148	0,55294118	0,56410256	0,45238095	0,52
0,46341463	0,35	0,46153846	0,59322034	0,58823529	0,66666667	0,63636364	0,30769231	0,38888889	0,34	0,36842105	0,51851852	0,4137931	0,73333333	0,45454545	0,41891892	0,66037736	0,59090909	0,45833333	0,5
0,51315789	0,42857143	0,50549451	0,55319149	0,43478261	0,64383562	0,54237288	0,45925926	0,47169811	0,43298969	0,47887324	0,56684492	0,5472973	0,61333333	0,50406504	0,46255507	0,57971014	0,53571429	0,56390977	0,46540881
0,56357388	0,13114754	0,46956522	0,4939759	0,52229299	0,4084507	0,58333333	0,44329897	0,45833333	0,49404762	0,41071429	0,52363636	0,4011976	0,38679245	0,57647059	0,48770492	0,56375839	0,46043165	0,6017316	0,5026455
0,52601156	0,54666667	0,45689655	0,55688623	0,52488688	0,52238806	0,52631579	0,5106383	0,43023256	0,35064935	0,44827586	0,51707317	0,58823529	0,49545455	0,53107345	0,52892562	0,49152542	0,43859649	0,5375	0,46153846
0,54098361	0,52348993	0,38709677	0,44	0,49758454	0,55263158	0,48	0,35632184	0,54285714	0,6056338	0,57894737	0,53488372	0,57575758	0,44623656	0,488	0,57671958	0,5	0,5304878	0,53233831	0,51315789
0,50980392	0,58163265	0,48251748	0,55696203	0,48876404	0,36633663	0,49438202	0,50239234	0,41121495	0,53191489	0,45	0,5380117	0,47093023	0,48113208	0,475	0,53092784	0,44444444	0,47169811	0,63709677	0,5
0,56976744	0,40816327	0,55102041	0,50442478	0,45614035	0,23809524	0,57142857	0,52	0,40909091	0,40909091	0,44444444	0,50617284	0,43181818	0,4	0,45283019	0,57142857	0,41666667	0,44897959	0,56603774	0,41666667
0,55555556	0,48669202	0,46381579	0,53827751	0,45627376	0,35329341	0,45812808	0,48553055	0,46470588	0,5047619	0,36956522	0,4689441	0,49805447	0,47735192	0,53225806	0,46335079	0,53947368	0,53639847	0,55517241	0,50798722
0,49615385	0,45263158	0,44642857	0,55677656	0,47191011	0,5	0,3125	0,42666667	0,575	0,33823529	0,45348837	0,57746479	0,51612903	0,51282051	0,44973545	0,51630435	0,53012048	0,52688172	0,52601156	0,52857143
0,50769231	0,56213018	0,52985075	0,49206349	0,49375	0,37662338	0,4893617	0,46103896	0,30188679	0,56962025	0,46835443	0,56989247	0,54744526	0,5	0,50326797	0,48373984	0,43956044	0,50581395	0,49404762	0,45505618
0,52752294	0,49189189	0,43820225	0,515	0,53107345	0,49180328	0,50955414	0,47904192	0,51968504	0,4516129	0,50641026	0,49576271	0,44970414	0,46511628	0,56934307	0,51304348	0,50314465	0,51807229	0,48587571	0,5
0,43627451	0,49246231	0,48101266	0,53472222	0,54216867	0,46478873	0,51960784	0,52173913	0,38983051	0,464	0,42045455	0,47791165	0,52830189	0,53398058	0,57754011	0,49429658	0,5915493	0,52980132	0,48101266	0,47849462
0,50854701	0,43939394	0,51977401	0,51282051	0,58974359	0,53947368	0,6407767	0,48969072	0,42372881	0,46153846	0,23076923	0,48947368	0,53846154	0,48108108	0,51207729	0,45771144	0,53636364	0,53623188	0,51612903	0,48630137
0,53220339	0,55511811	0,47089947	0,53012048	0,49344978	0,33333333	0,41666667	0,45614035	0,42718447	0,45762712	0,45299145	0,49115044	0,45365854	0,46829268	0,59288538	0,49570201	0,49375	0,53804348	0,53636364	0,4801444
0,48	0,51724138	0,525	0,56521739	0,45945946	0,375	0,7	0,40677966	0,53571429	0,5974026	0,4	0,47619048	0,55813953	0,45652174	0,46666667	0,5462963	0,68571429	0,37288136	0,45882353	0,48888889
0,5755102	0,44017094	0,5	0,46666667	0,47540984	0,4375	0,43023256	0,42677824	0,43181818	0,56849315	0,37820513	0,49282297	0,59817352	0,50757576	0,53333333	0,47706422	0,5	0,51207729	0,52232143	0,46694215
0,56807512	0,48412698	0,49640288	0,58365759	0,55188679	0,46017699	0,54494382	0,48372093	0,46994536	0,55714286	0,42236025	0,47169811	0,50592885	0,50724638	0,47435897	0,46759259	0,55384615	0,44137931	0,48314607	0,48717949
0,55350554	0,42944785	0,44067797	0,48793566	0,4954955	0,421875	0,54140127	0,51263538	0,40625	0,43589744	0,33884298	0,5505618	0,51111111	0,52688172	0,4954955	0,45769231	0,5	0,41798942	0,55778894	0,52293578
0,52302632	0,45588235	0,43315508	0,54512635	0,48971193	0,48181818	0,52419355	0,46728972	0,47297297	0,44067797	0,36585366	0,46666667	0,51086957	0,53479853	0,47928994	0,49450549	0,48186528	0,53554502	0,52966102	0,49425287
0,48648649	0,53164557	0,45384615	0,54819277	0,47766323	0,46017699	0,5	0,43986254	0,46280992	0,48	0,31914894	0,48605578	0,54098361	0,56041131	0,45789474	0,44871795	0,51037344	0,43457944	0,49333333	0,44055944
0,54703833	0,42857143	0,47115385	0,5532646	0,51452282	0,44155844	0,57142857	0,51724138	0,38181818	0,52830189	0,39285714	0,49253731	0,55072464	0,48876404	0,50625	0,5034965	0,44444444	0,48	0,50909091	0,51851852
0,49809886	0,52608696	0,45801527	0,55255255	0,57028112	0,49462366	0,48951049	0,50988142	0,4109589	0,6	0,42944785	0,42477876	0,49763033	0,5480427	0,49618321	0,49473684	0,43312102	0,44776119	0,57077626	0,44081633
0,55778894	0,57857143	0,51082251	0,49433962	0,49606299	0,38333333	0,51442308	0,508	0,52873563	0,47727273	0,43859649	0,50480769	0,4973545	0,42241379	0,46363636	0,51119403	0,54237288	0,52657005	0,52195122	0,52818991
0,52964427	0,47087379	0,49027237	0,47029703	0,53305785	0,53968254	0,48603352	0,52597403	0,51020408	0,51030928	0,4	0,55591054	0,52432432	0,52411576	0,54509804	0,51398601	0,47488584	0,47712418	0,48898678	0,45533141
0,52261307	0,53703704	0,49756098	0,48484848	0,52136752	0,48404255	0,51361868	0,45747126	0,47953216	0,55479452	0,42753623	0,48237885	0,51388889	0,53080569	0,53532609	0,47004608	0,46938776	0,48591549	0,58659218	0,50901804
0,51864407	0,50819672	0,50853242	0,48847926	0,46969697	0,44520548	0,55276382	0,45606695	0,43884892	0,50731707	0,39215686	0,51811594	0,53815261	0,46405229	0,45744681	0,48307692	0,51098901	0,48803828	0,51636364	0,52996845
0,51978892	0,44160584	0,4982699	0,55654762	0,54205607	0,38194444	0,45136187	0,51748252	0,43703704	0,49321267	0,30769231	0,46831956	0,5473251	0,45733788	0,54320988	0,48924731	0,53023256	0,45145631	0,48657718	0,5
0,52851711	0,5	0,42358079	0,52314815	0,49773756	0,42735043	0,54248366	0,44354839	0,47008547	0,52173913	0,33333333	0,51754386	0,53645833	0,58278146	0,48561151	0,48594378	0,50251256	0,49693252	0,5210084	0,53846154
0,55491329	0,4969697	0,44	0,43930636	0,53921569	0,4494382	0,51162791	0,48015873	0,46666667	0,53757225	0,44186047	0,52868852	0,66906475	0,50761421	0,49375	0,46153846	0,4379562	0,5	0,44210526	0,50409836
0,55026455	0,52903226	0,53947368	0,50724638	0,49659864	0,40196078	0,51207729	0,52631579	0,49681529	0,37142857	0,42763158	0,54183267	0,50520833	0,47509579	0,54320988	0,57794677	0,51627907	0,43636364	0,57281553	0,49035813
0,51	0,49537037	0,52755906	0,4643377	0,51263538	0,47933884	0,53773585	0,54576271	0,60526316	0,50833333	0,40816327	0,47991071	0,55681818	0,43560606	0,51094891	0,4727955	0,50243902	0,49673203	0,54723127	0,48034934
0,4379562	0,60135135	0,48554913	0,46153846	0,46808511	0,34482759	0,49137931	0,56084656	0,42528736	0,448	0,33823529	0,43621399	0,48175182	0,52331606	0,5257732	0,49751244	0,59060403	0,52419355	0,53333333	0,57961783

rs3911	*rs4141886*	*rs8179021*	*rs9341278*	*rs9786139*	*rs9786184*
0,48888889	0,52564103	0,57142857	0,37569061	0,55636364	0,50318471
0,52427184	0,43333333	0,52808989	0,52727273	0,51538462	0,55357143
0,45	0,53982301	0,48214286	0,51724138	0,53571429	0,40268456
0,52513966	0,45762712	0,50359712	0,41666667	0,44186047	0,55230126
0,43617021	0,52	0,56521739	0,52238806	0,52238806	0,49222798
0,46	0,5	0,57575758	0,29032258	0,55855856	0,4795082
0,4	0,56923077	0,66433566	0,472	0,50980392	0,51219512
0,52941176	0,57931034	0,55752212	0,45121951	0,54022989	0,44268775
0,42038217	0,5	0,47169811	0,43670886	0,50384615	0,46621622
0,37777778	0,40659341	0,63529412	0,43181818	0,44660194	0,5177305
0,45299145	0,41584158	0,37606838	0,55789474	0,46308725	0,51980198
0,61290323	0,52173913	0,54545455	0,31034483	0,64285714	0,55882353
0,66666667	0,47368421	0,5625	0,5	0,66666667	0,44705882
0,51162791	0,61904762	0,60526316	0,33333333	0,45714286	0,60674157
0,515625	0,5	0,54545455	0,40206186	0,4765625	0,48076923
0,46428571	0,51351351	0,63333333	0,33333333	0,4047619	0,46153846
0,51470588	0,53543307	0,38888889	0,44	0,38461538	0,50222222
0,51908397	0,58823529	0,54205607	0,44166667	0,47297297	0,51622419
0,57	0,54450262	0,57142857	0,27884615	0,47058824	0,53932584
0,52380952	0,56302521	0,67058824	0,45454545	0,52666667	0,51851852
0,47337278	0,46456693	0,57142857	0,3245614	0,39285714	0,48207171
0,46666667	0,30232558	0,46835443	0,4	0,51282051	0,39240506
0,52529183	0,53603604	0,57522124	0,44385027	0,51041667	0,54318182
0,42857143	0,58169935	0,55294118	0,54545455	0,45454545	0,52205882
0,47126437	0,55194805	0,45977011	0,31775701	0,54893617	0,60714286
0,47096774	0,53030303	0,47945205	0,3358209	0,48979592	0,54320988
0,48120301	0,5125	0,55172414	0,45	0,5748503	0,52521008
0,44628099	0,4488189	0,57894737	0,44776119	0,52727273	0,4845815
0,53521127	0,47867299	0,50641026	0,32110092	0,47637795	0,53818182
0,41071429	0,59793814	0,55932203	0,44827586	0,4950495	0,48837209
0,41666667	0,46305419	0,5	0,39130435	0,47953216	0,55123675
0,46715328	0,56696429	0,44594595	0,45794393	0,49606299	0,46116505
0,48258706	0,57142857	0,53900709	0,39041096	0,44864865	0,52173913
0,48947368	0,5270936	0,51955307	0,36	0,49438202	0,50591716
0,4278607	0,53092784	0,47305389	0,40425532	0,52727273	0,54278075
0,40322581	0,43604651	0,55555556	0,328125	0,46902655	0,50740741
0,53623188	0,54901961	0,39506173	0,29357798	0,46956522	0,51685393
0,43269231	0,4875	0,4469697	0,32432432	0,49494949	0,55873016
0,51724138	0,55614973	0,53416149	0,30952381	0,51162791	0,53908356
0,49867374	0,5	0,54861111	0,28350515	0,47277937	0,54237288
0,47490347	0,53723404	0,51265823	0,27906977	0,45333333	0,49845201
0,55185185	0,52112676	0,55982906	0,35582822	0,54612546	0,52413793
0,44210526	0,46875	0,46046512	0,40127389	0,49781659	0,51890034
0,59477124	0,52671756	0,47402597	0,32727273	0,5	0,50200803
0,50226244	0,47530864	0,49390244	0,3525641	0,48648649	0,5399361
0,47663551	0,50222222	0,51322751	0,625	0,5177665	0,55724299
0,50955414	0,52727273	0,5	0,31	0,5	0,52509653

Supplementary Table S6. Heterozigous balance metrics values for 90 autosomal SNP markers included in HID Ion Ampliseq Identity Panel, in Brazilian criminal samples. N indicates an homozygote genotype for SNP in question.

Samples	**Locus**																	
Blood	*rs1005533*	*rs10092491*	*rs1015250*	*rs1024116*	*rs1028528*	*rs1031825*	*rs10488710*	*rs10495407*	*rs1058083*	*rs10773760*	*rs10776839*	*rs1109037*	*rs12997453*	*rs13218440*	*rs1335873*	*rs1355366*	*rs1357617*	*rs1360288*
BL.PF07	1,03317536	N	0,79545455	N	1,00819672	N	N	N	N	N	0,92045455	0,77536232	N	0,85772358	0,85258964	1	1,00787402	N
BL.PF09	N	0,85344828	1,38709677	0,83552632	N	N	N	1,05128205	N	N	1,10810811	0,8173913	N	N	N	0,99159664	1,0875	0,99253731
BL.PF11	0,83962264	N	N	N	1,53465347	N	1,35849057	N	0,66666667	1,5631068	N	1,33783784	1,26190476	1,02941176	0,86821705	N	N	N
BL.PF20	1,08695652	N	0,80882353	1,03463203	0,89595376	1,02262443	N	0,95774648	0,96788991	N	N	0,8018018	1,42857143	0,69758065	1,11794872	N	0,92241379	N
BL.PF22	1,15492958	N	N	N	1,20833333	1,31578947	1	1,51327434	N	0,79503106	1,42372881	1,23404255	1,02631579	0,78947368	N	N	N	0,84516129
BL.PF24	1,64583333	N	N	N	0,86702128	1,67924528	0,44615385	2,1	1,14728682	1,13533835	N	N	0,52631579	N	N	0,45121951	N	1,00884956
BL.PF25	N	1,29813665	0,62068966	N	N	N	1,05555556	1,31060606	1,65034965	N	1,16666667	1,28571429	N	1,21875	N	N	N	N
BL.PF26	N	0,96067416	0,96	N	N	1,13989637	0,73076923	N	N	N	N	N	1,12903226	N	1,28476821	N	1,2371134	1,13917526
BL.PF27	0,95528455	0,7791411	N	1,02888087	N	1,1259542	1,04494382	N	N	0,7761194	0,82051282	N	N	1,04680851	N	N	N	N
BL.PF30	N	N	N	N	1,192	N	1,0625	N	N	N	N	N	N	0,94405594	N	N	N	N
Epithelial																		
EP.PF01	0,86821705	N	N	N	1,33653846	N	0,77142857	0,68	N	N	0,93617021	0,74757282	N	N	N	0,73856209	N	0,88811189
EP.PF08	0,53246753	1,4	N	N	N	N	0,37931034	N	N	N	0,91666667	N	0,375	N	0,98305085	N	N	N
EP.PF10	0,95	0,25641026	N	1,13333333	N	N	N	6	N	0,69230769	N	N	0,54545455	N	0,72222222	2,23076923	N	1,0212766
EP.PF13	N	1,66666667	0,96153846	1,29166667	0,36296296	0,17391304	0,8	6,22222222	N	0,97101449	0,91666667	0,84507042	0,75	N	0,24691358	4,22222222	1,12121212	0,68269231
EP.PF14	1,05882353	N	N	1,31746032	N	2,625	2,68	N	5,19607843	N	1,10416667	N	5,58333333	3,7755102	N	N	0,63157895	N
EP.PF15	1,02272727	N	0,78947368	N	1,15789474	N	N	0,20454545	N	0,75675676	N	N	0,8	1,40540541	N	0,89189189	0,58333333	N
EP.PF17	N	N	0,97959184	1,53787879	N	N	0,6091954	0,81208054	N	N	1,16923077	1,05128205	N	N	1,09803922	N	N	0,54285714
EP.PF18	0,74883721	1,08661417	1,31578947	N	1,24537037	N	1,35384615	N	N	N	1,12621359	N	N	N	N	N	0,94957983	0,94736842
EP.PF21	0,75757576	N	N	0,91176471	N	1,01351351	1,05882353	N	1,04907975	N	0,81395349	0,73529412	1,52631579	0,72246696	N	N	1,04477612	1
EP.PF28	5,14285714	N	N	N	N	0,13333333	N	3,45454545	0,79268293	1,21383648	0,17241379	N	N	N	0,99242424	N	0,2	N
Saliva																		
SA.PF02	N	1,4	N	0,90688259	0,88235294	N	1,02272727	1,05932203	N	0,76954733	N	0,99090909	N	0,88059701	N	1,26168224	N	0,84693878
SA.PF03	N	0,50617284	N	0,54	1,2173913	0,61818182	N	0,92	N	1,07792208	0,92592593	N	N	1,02150538	N	N	0,76744186	4,59259259
SA.PF04	0,90829694	0,96474359	N	N	0,93920973	1,02588997	N	N	N	1	N	N	N	N	1,0367893	0,91176471	N	N
SA.PF05	0,8817734	1,38613861	N	0,80851064	1,26229508	1,24873096	N	N	0,91287879	0,75373134	0,11904762	0,88142292	1,5	N	1,31791908	N	N	N
SA.PF06	N	N	7,17647059	N	1,15189873	1,18471338	0,97029703	0,98507463	N	N	1,12903226	0,99107143	0,79591837	N	N	N	N	N
SA.PF12	N	N	1,28571429	1,06832298	N	0,8699187	0,97272727	1,00613497	0,86098655	N	1,11764706	1,09174312	1,18965517	N	N	0,88669951	N	N
SA.PF16	0,93661972	0,96428571	0,98780488	1,17197452	N	N	1,13861386	1,02752294	N	N	N	0,84745763	0,95744681	N	N	1,14685315	N	N
SA.PF19	1,08163265	1,14678899	N	1,07446809	0,84016393	N	N	0,94444444	0,84651163	N	1,19753086	1,29365079	N	1,10650888	N	N	N	N
SA.PF23	N	N	N	N	N	0,95154185	0,76027397	N	0,90425532	N	0,68831169	1,29	N	0,91549296	1,15555556	0,68639053	0,78448276	1,20430108
SA.PF29	N	2,13333333	N	0,9625	1,03846154	N	0,9047619	0,75	N	1,10309278	0,94285714	N	0,15625	1,05319149	N	1,34285714	N	N
Semen																		
SE.PF02	N	N	N	N	N	N	N	N	N	N	N	0,85384615	0,81818182	N	1,02666667	0,90705128	N	N
SE.PF03	1,38888889	1,03780069	0,95652174	1,09811321	1,05172414	N	0,84615385	1,00819672	1,12621359	0,921875	0,90243902	0,77876106	N	0,96428571	0,9348659	0,93586006	N	N
SE.PF07	1,21468927	0,96368715	0,91	N	1,19298246	N	N	0,97816594	1,2388664	0,90995261	N	N	0,75510204	0,96141479	N	N	N	N
SE.PF22	0,92201835	1,24431818	0,89333333	0,92139738	N	N	N	1,42741935	N	0,69379845	N	N	1,25	N	N	N	0,93333333	1,1637931
SE.PF26	1,125	N	N	N	0,94190871	1,01269036	0,8707483	N	N	0,97272727	N	N	1,05737705	0,9704918	N	N	1,08119658	0,86060606
SE.PF27	N	0,88461538	N	1,03821656	1,03010033	N	1,02247191	N	0,91954023	N	N	N	1,23529412	N	N	N	1,02777778	1,06493506
SE.PF30	N	N	1,12727273	N	N	N	0,93548387	1,03937008	N	N	N	0,80952381	1,07608696	N	N	N	0,95512821	N
Control (Oral Swab)																		
CT.PE02	0,74358974	0,89873418	0,72941176	0,83276451	0,91020408	N	0,88976378	N	1,08745247	N	N	N	0,70512821	N	1,09375	1,12299465	N	0,92694064
CT.PE03	0,95689655	N	N	0,89189189	N	1,00724638	N	1,08646617	1,01149425	N	N	0,9702381	0,84	1,19767442	N	N	N	N
CT.PE04	1,06413302	N	N	1,09198113	N	N	N	N	0,858	N	0,83982684	0,95238095	N	N	N	1,26712329	N	N
CT.PE07	0,79856115	N	N	1,02803738	N	1,13454545	1,13432836	N	1,10108303	1,0511811	N	0,97744361	N	1,11155378	N	0,99190283	N	N
CT.PE08	1,01945525	N	1,22368421	N	N	0,96474359	1	N	N	N	0,87323944	1,09558824	0,96590909	1,00276243	1,16423358	N	1,17222222	0,92088608
CT.PE09	0,68518519	N	N	N	0,836	N	N	N	N	1,06565657	N	1,13207547	N	0,89211618	N	0,760181	N	N
CT.PE10	1	1,02	1,23214286	1,15865385	0,9893617	N	N	N	N	N	1,08219178	N	N	N	N	0,82634731	N	N
CT.PE11	1,31360947	0,79569892	N	0,94094488	0,97169811	1,27731092	N	N	N	N	0,94186047	1,22891566	1	1,02678571	1,1	1,04469274	1,03703704	N
CT.PE12	1,16587678	1,05574913	N	N	N	N	N	0,95762712	0,98372093	0,79807692	N	N	0,55813953	N	N	N	1	0,90773067
CT.PE13	0,86764706	N	N	N	0,94464945	N	1,0125	0,91975309	1,14444444	0,84513274	N	N	N	1,21875	N	0,84883721	N	N

rs1382387	rs1413212	rs1454361	rs1463729	rs1490413	rs1493232	rs1498553	rs1523537	rs1528460	rs159606	rs1736442	rs1821380	rs1872575	rs1886510	rs1979255	rs2016276	rs2040411	rs2046361	rs2056277	rs2076848
N	N	0,74	N	N	N	N	N	1,09745763	0,90147783	N	N	0,92582418	N	N	1,00546448	N	N	N	0,85365854
N	1,19101124	1,07746479	N	1,19393939	N	N	N	0,9296875	N	0,94214876	0,97340426	1,12820513	0,95180723	0,89565217	N	1,10416667	N	N	0,93939394
N	N	1,11538462	N	1,15923567	N	1,07920792	0,92537313	0,86516854	N	0,5037594	N	N	N	1,41818182	0,67883212	N	1,45	N	N
1,05670103	1	N	0,77202073	N	0,9296875	N	0,82162162	0,83116883	0,95614035	N	N	1,00425532	N	N	N	N	0,89534884	N	N
0,76470588	1,21374046	N	N	N	N	0,91304348	0,95918367	N	1,21538462	0,94736842	N	1,32116788	N	0,72580645	N	1,01886792	0,93333333	N	0,7826087
N	1,26950355	N	N	N	0,78640777	N	N	N	0,6984127	1,28846154	N	1,26229508	N	0,91780822	N	N	N	1,67924528	1,20491803
1,08860759	0,81675393	0,92146597	N	1,18471338	N	N	N	0,84375	0,72180451	1,5	1,04918033	1,13483146	0,79	N	1,09285714	N	0,90598291	N	1,18
N	N	N	N	0,92592593	N	N	1,30872483	0,86428571	N	1,61666667	0,92553191	0,77777778	N	0,97122302	1,17808219	1	1,51923077	N	N
0,7752443	1,13366337	N	0,88043478	1,19101124	0,74418605	N	0,9795082	N	N	N	N	N	N	N	N	0,94545455	N	0,93867925	N
N	0,85950413	1,17117117	1,23529412	0,96491228	1,19230769	N	N	N	N	1	1,37391304	N	0,68831169	N	1,19565217	1,01863354	2,26923077	0,67105263	1,61764706
1,05464481	1,20108696	0,8047619	N	N	N	N	1,16778523	1,14678899	N	1,17171717	N	0,62352941	1	N	N	N	N	N	N
N	1,38356164	1,48101266	0,65254237	N	N	N	1,04054054	0,30882353	N	1,04166667	N	N	0,75	0,38372093	N	0,83962264	N	1,2	0,2295082
0,80555556	N	N	0,17647059	N	3,14285714	N	N	N	N	N	N	0,82857143	1,05882353	N	N	N	1,09090909	2,625	1,0625
0,78888889	1,15942029	0,6547619	0,11801242	0,98809524	0,72727273	N	0,67032967	1,05357143	1,05714286	0,67368421	0,69014085	0,62820513	0,34545455	0,125	N	N	0,81818182	1,43137255	0,75409836
N	0,6974359	0,70547945	0,75974026	0,31451613	0,72413793	0,97272727	N	N	0,68888889	N	N	N	N	0,92913386	0,97790055	N	N	N	0,91509434
N	N	N	N	N	0,68571429	N	N	N	N	1,10714286	N	N	0,44	0,97619048	N	0,67307692	1,33333333	N	N
1,21301775	0,78125	0,69736842	1,12195122	N	N	N	N	N	0,98888889	N	1,10810811	1	1,18666667	N	N	N	0,8556701	N	N
N	0,74125874	1,01777778	N	0,85531915	N	N	0,94767442	N	1,12612613	N	1,26455026	0,77054795	N	N	0,81623932	0,81362007	0,67961165	N	1,09589041
0,91818182	0,85135135	N	N	N	1,0877193	1,13636364	N	0,56849315	1,37647059	2,26530612	N	1,28467153	0,85185185	0,8372093	N	N	1,06153846	N	N
N	1,58823529	N	N	2,015625	N	0,808	0,55491329	N	N	1,35454545	0,84269663	1,24242424	N	N	N	1,17525773	N	1,57142857	N
N	0,82539683	N	N	0,5962963	0,90756303	0,96575342	1	0,93296089	N	N	0,90517241	1,11940299	N	0,81021898	0,86734694	N	1,20430108	N	N
0,61363636	N	N	N	0,18965517	1,19444444	0,96551724	0,79746835	0,98181818	0,89189189	N	0,7654321	N	N	0,96491228	N	N	2,92857143	0,83333333	N
N	1,14046823	1,05151515	1,00882353	0,92508143	0,85520362	N	0,93014706	N	N	1,05660377	0,84444444	0,72081218	0,9702381	N	N	0,92067308	N	0,97457627	1,04741379
N	N	N	0,13496933	N	8,03333333	0,77514793	N	0,85526316	N	0,64640884	N	N	8,07142857	N	0,69135802	N	0,95	N	N
N	0,99484536	N	N	N	N	1,53383459	N	N	N	0,89403974	N	0,92397661	8,15	0,30573248	N	0,89558233	N	N	1,42635659
N	1,25342466	N	1,02643172	N	0,89830508	1,05882353	1,04819277	N	0,70165746	0,99489796	0,67942584	N	N	N	1,01030928	0,7804878	N	N	1,136
1,10138249	N	0,97674419	1,03017241	1,03759398	0,98473282	1,01485149	N	1,10687023	0,76470588	N	N	N	N	1,17177914	N	N	N	N	N
N	N	0,79802956	N	N	N	1,19863014	N	1,01910828	N	N	N	N	0,69791667	N	N	N	N	N	N
N	N	0,84328358	N	0,98170732	N	N	N	N	N	1,13669065	0,79146919	1,03030303	N	N	1,12953368	N	1,77941176	0,64942529	0,93421053
N	0,94623656	N	N	0,66176471	N	0,56060606	0,79220779	N	0,81632653	1,2244898	0,76530612	N	1,02325581	N	N	N	N	N	1,07272727
0,95038168	0,90066225	1,03636364	1,02185792	N	0,82352941	1,06593407	1,48701299	1,09836066	0,81958763	1,11560694	N	N	N	N	0,88888889	1,08290155	0,9044586	N	N
N	0,9969697	N	0,81355932	1,09375	N	N	0,98888889	1,00510204	N	0,83211679	0,89772727	0,79861111	N	N	0,90776699	N	0,94413408	N	1,04964539
1,1040724	1,06640625	0,99067599	1,09913793	0,67045455	N	N	1,00904977	1,06896552	N	N	N	0,92622951	0,98823529	N	N	N	N	1,1928934	0,91082803
N	1,10762332	N	0,80681818	N	0,67816092	1,01104972	1,05882353	0,92788462	N	N	1,05172414	1,02777778	N	1,07391304	N	N	N	0,96385542	N
0,9010989	N	N	N	N	0,81818182	0,90338164	N	N	0,97435897	1,04324324	N	N	N	1,03614458	N	N	N	N	1,04458599
N	1,1042471	1,10859729	N	0,91338583	0,91836735	N	N	0,66101695	1	0,97674419	N	N	0,87804878	N	0,9009901	0,97345133	N	0,8427673	1,0887574
N	0,92739274	N	0,96059113	N	N	0,81770833	N	N	N	N	0,95789474	N	1,00714286	N	1,00692042	N	N	N	N
1,06334842	N	N	N	0,92250923	N	0,87142857	N	0,9939759	N	N	0,97244094	1,15471698	N	N	N	0,80555556	0,97814208	N	N
N	N	N	0,94849785	N	N	N	N	N	N	0,89361702	N	N	N	0,93236715	1,19277108	0,8877551	1,18452381	0,83121019	N
0,86223278	0,88985507	0,96350365	1,17283951	N	N	1,10849057	1,28099174	1,17391304	N	N	0,99574468	0,93939394	N	N	N	N	N	N	N
N	N	N	N	1,04761905	0,67058824	N	1,19444444	N	N	N	N	N	N	N	N	1,07224335	N	1,07662835	N
N	N	1,29370629	1,00653595	N	1,15441176	1,11525424	1,0913242	N	N	0,86333333	N	0,72588832	0,97237569	N	N	0,98287671	0,82568807	N	1,20720721
0,92276423	N	N	1,12264151	N	0,83809524	0,81090909	N	N	0,84868421	N	0,93639576	0,8238342	N	N	N	N	1,08661417	N	N
1,01463415	1,34920635	1,12121212	N	1,19026549	0,95348837	1,15841584	N	N	N	0,95454545	0,99224806	N	1,12711864	N	0,96226415	N	N	N	N
N	1,02150538	0,88301887	0,92276423	0,99619772	N	1,12446352	N	N	1,03608247	N	N	0,84466019	1,03658537	N	N	N	0,95031056	N	N
0,98579545	1,0552381	N	N	0,99805068	0,93771626	N	N	0,98850575	0,77777778	1,0209205	1,17123288	N	N	N	1,15632754	N	0,80882353	N	1,10238095
0,8708134	N	1,14606742	0,96590909	N	N	N	1,02970297	0,92424242	0,65771812	N	N	0,97727273	N	N	0,84297521	0,86259542	N	1,09278351	N

rs2111980	rs214955	rs221956	rs2269355	rs2292972	rs2342747	rs251934	rs2830795	rs2831700	rs321198	rs338882	rs354439	rs3780962	rs4288409	rs430046	rs4364205	rs445251	rs4530059	rs4847034	rs560681
N	N	N	N	N	1,09195402	1,1396648	1,08171206	N	N	1,10096154	N	N	N	N	0,99253731	N	N	N	N
N	N	1	N	N	1,15217391	1,03875969	N	0,97058824	N	N	0,92741935	0,53246753	1,14049587	N	N	N	1,57142857	N	N
N	0,10526316	N	1,2972973	0,65517241	N	N	N	N	1,10638298	1,08496732	N	0,76086957	N	N	1,00628931	N	N	0,94871795	0,64912281
1,06779661	1,25490196	N	N	1,29545455	N	1,11111111	N	0,84571429	1,50632911	N	N	N	0,90598291	1,65178571	0,87058824	N	N	1,23076923	N
N	1,71428571	N	N	1,07857143	N	0,99328859	0,82251082	0,90804598	N	N	0,76760563	0,66666667	N	N	1,2892562	N	N	N	N
N	1,28888889	N	1,11627907	N	N	N	0,72916667	0,75172414	N	0,94240838	N	0,63565891	N	2,31683168	0,74489796	1,46153846	0,96969697	1,23913043	N
1,19230769	2,125	N	N	N	N	N	N	1,3028169	N	0,92349727	1,09722222	N	0,69026549	1,97058824	N	0,87878788	0,97744361	1,13095238	N
N	N	1,07983193	N	0,89726027	0,84	N	0,90612245	N	0,91071429	0,86538462	1,22857143	0,50833333	1,01438849	N	0,81981982	0,8852459	1,01190476	N	N
1,06829268	N	N	1,22340426	1,11578947	N	0,96995708	N	N	1,08411215	N	0,85666667	N	N	N	0,89221557	1,01895735	N	N	1,03649635
N	N	1,01515152	N	N	N	1,30263158	N	1,35643564	1,01851852	0,7345679	N	1	N	N	N	N	N	N	1,51807229
1,37113402	1,52830189	N	N	1	0,93023256	1,39830508	1,23783784	N	0,93421053	1,02358491	1,11805556	0,63190184	0,74796748	N	0,96256684	0,84795322	N	N	N
0,7	1,23809524	0,99082569	2	2,10144928	1,5	N	N	N	N	N	0,36046512	0,50769231	N	N	N	N	N	N	N
1	N	N	N	2	N	N	N	N	1,8	N	N	N	N	N	N	3,09090909	N	0,52941176	0,65
0,16814159	0,15	1,32608696	N	0,96969697	0,31666667	1,58181818	N	2,52083333	5,66666667	0,17482517	N	0,63492063	N	0,23376623	N	N	0,3625	N	N
1,13978495	N	1,3559322	0,19262295	5,88888889	N	1,18897638	N	1,11320755	2	0,95070423	1,26027397	0,53205128	0,84946237	1,88405797	1,40625	4,34615385	0,8125	0,72477064	0,79136691
N	0,92857143	N	0,85185185	1,02702703	N	N	N	N	N	0,78846154	N	0,27777778	N	1,8125	1,225	N	1,02272727	N	1,03703704
0,97647059	5,16666667	N	1,5625	N	1,36363636	N	N	N	0,97959184	N	0,75838926	N	N	N	1,14606742	N	1,01290323	0,76699029	N
0,96449704	N	0,83966245	0,98986486	N	1	1,32460733	N	N	1,22826087	0,90592334	N	0,51428571	N	2,04201681	1,07659574	N	0,94514768	1,03539823	N
N	1,37704918	N	N	N	0,92647059	1	1,05035971	1,02369668	1,22916667	N	0,92592593	N	N	N	N	0,51322751	1,71287129	0,54285714	N
0,94660194	1,31914894	0,96116505	N	0,84415584	N	1,31192661	0,12437811	N	0,95522388	1,00363636	N	0,38709677	N	2,88461538	0,80368098	0,38028169	N	5,88888889	0,76595745
N	1,69230769	0,96216216	N	1,17482517	N	0,66666667	N	1,04093567	1,14563107	0,90265487	0,71707317	0,94964029	N	2,09677419	0,58174905	1	N	N	N
N	N	N	N	1,2972973	0,54761905	N	1,16455696	0,86206897	0,78571429	0,58585859	N	N	N	1,41860465	N	N	1,10526316	N	1,01176471
N	N	1,01704545	1,12741313	N	N	0,8277027	N	N	N	0,90544413	1,08163265	0,63768116	N	1,90184049	0,85432099	N	1,06949807	N	1,08914729
0,15139442	N	5,93548387	N	N	1,25454545	N	N	N	N	N	N	N	N	0,2849162	1,04278075	N	N	N	1,06451613
1,55882353	1,48275862	6,28125	1,09782609	0,79059829	N	1,33333333	1,07741935	N	N	N	N	0,77456647	N	0,19078947	N	N	N	0,70731707	1,62790698
1,06428571	1,80701754	N	0,99450549	N	1,47272727	0,99468085	N	1	N	1,64583333	1,08108108	N	N	2,27083333	N	0,85416667	N	1,2919708	1,13714286
1,60869565	N	N	N	N	1,15942029	1,31468531	1,11374408	0,8852459	N	N	N	0,52941176	N	2,36082474	1,02739726	0,84337349	1,14925373	N	0,98125
1	N	0,92241379	0,87553648	1,23870968	0,784	N	0,85542169	N	1,45238095	0,98604651	1,11267606	0,47368421	N	2,46666667	1,00546448	1,06493506	1,09375	0,6	N
N	N	N	N	0,8952381	0,78	N	N	0,87022901	N	N	N	N	N	N	0,85396825	N	0,69709544	N	0,96330275
N	2	0,578125	N	1,47619048	N	0,5	1,03738318	0,88235294	8,5	5,35897436	N	0,96428571	1	N	0,20689655	1,13043478	5,93103448	1	1,04225352
1,01333333	N	N	N	N	1,26666667	N	1,01966292	1,01351351	0,97142857	N	1,09183673	0,75	N	N	N	N	0,90540541	0,96135266	N
1,20238095	1,72222222	N	N	1,25688073	1	N	N	0,9391635	N	N	1,00330033	N	1,03571429	N	1,03448276	0,85561497	1,46902655	N	0,90625
N	N	N	1,02214022	N	1,12068966	1,05235602	1,02396514	1,0234375	N	N	N	N	N	1,95867769	0,9186747	N	0,95854922	1,109375	0,99545455
0,99539171	N	N	N	N	1,04477612	N	1,01994302	0,73043478	1,42718447	0,82274247	N	0,59887006	N	2,01980198	1,05217391	0,93333333	0,95686275	N	1,10679612
0,99082569	N	N	N	0,9527027	0,80645161	0,89320388	N	N	N	0,94509804	N	0,66202091	N	2,31958763	0,85983827	0,8649789	N	0,89399293	N
1,23195876	1,30952381	1	N	1,03225806	N	N	N	N	1,13076923	0,91532258	N	N	1,07692308	2,03669725	N	N	N	N	1,10628019
N	1,08791209	0,89268293	0,71428571	1,03937008	N	N	N	N	1,28648649	N	1,24778761	0,96491228	N	N	0,80582524	0,86315789	0,94512195	N	0,91666667
0,94771242	1,71153846	N	4,046875	1,0212766	N	1,11386139	N	N	0,85185185	0,81097561	N	0,75280899	N	N	N	N	N	0,73381295	N
N	1,70689655	0,84829721	N	N	1,10144928	N	1,03012048	N	1,11842105	1,10309278	N	N	N	N	N	1,01509434	1,20361991	N	N
N	N	1,117506	N	N	N	N	N	0,74303406	N	1,14932127	N	N	1,09090909	1,71078431	1,12222222	0,87371134	N	0,84090909	N
0,9025974	1,19354839	1,17948718	0,95238095	N	1,01010101	N	N	0,84680851	N	0,99669967	1,27862595	0,54404145	N	N	1,03693182	N	N	1,03787879	1,0515873
1,03278689	1,42424242	N	1,01565996	0,95454545	N	N	0,91818182	0,6838488	N	0,92897727	N	0,70093458	N	2,11666667	N	1,0982906	N	0,66477273	1,10367893
0,92090395	N	0,8700361	0,98148148	N	N	1,02252252	N	N	N	0,90677966	0,81300813	N	1,13855422	N	N	1,00429185	N	N	N
N	1,875	N	1,25291829	N	0,78350515	N	N	1,04046243	1,10309278	1,20754717	N	0,86178862	N	1,88372093	N	N	N	0,9380531	1,00970874
N	2,60606061	N	N	1,02116402	N	N	1,26893939	N	N	1,13617021	N	1,06923077	N	2,35	N	0,83057851	N	N	1,30875576
N	1,83333333	0,90675241	N	N	1,17333333	0,95862069	N	0,67763158	1,01630435	N	0,9527027	0,76893939	N	2,52097902	0,90042373	N	1,09625668	0,51879699	N
1,08917197	N	N	0,75925926	1,12389381	1,10126582	1,08189655	N	1	N	N	1	0,67619048	N	2,67619048	N	0,78571429	N	0,8974359	N

rs576261	rs6444724	rs6811238	rs6955448	rs7041158	rs717302	rs719366	rs722098	rs722290	rs727811	rs729172	rs733164	rs735155	rs737681	rs740598	rs740910	rs7520386	rs7704770	rs826472	rs873196
1,09677419	1,12169312	0,84482759	1,04824561	0,98660714	0,74137931	0,79259259	1,02489627	1,24409449	N	N	1,00869565	1,06315789	N	N	N	N	0,87711864	N	N
N	1,18796992	0,96875	N	0,78195489	N	N	N	N	1,2	1,12857143	N	N	N	0,75974026	N	3,43478261	1,08108108	N	1,66666667
N	0,86792453	N	1,76153846	N	N	0,72727273	N	0,82432432	N	N	N	1,29126214	N	0,18181818	1,07843137	2,28301887	N	1,41666667	1,94520548
0,91525424	N	N	N	N	N	N	0,92307692	N	1,13114754	N	1,01898734	1,109375	0,96753247	N	N	N	0,76433121	N	N
N	N	N	N	N	N	0,79069767	N	0,68292683	0,86516854	N	0,83766234	N	0,84023669	0,60683761	N	3,38888889	1,10185185	N	N
0,78389831	1,27142857	1	0,96376812	N	1,05813953	0,73333333	N	N	0,58064516	2,13043478	N	N	N	0,62162162	N	N	0,68817204	0,85714286	N
1,22340426	1	1,02054795	N	N	0,47752809	0,656	N	0,96969697	N	N	0,87898089	1,07317073	0,93877551	N	0,86567164	2,32941176	N	1,28205128	N
N	N	N	N	N	N	N	1,01818182	N	0,97115385	1,08695652	0,68831169	N	1	1,33962264	N	N	N	N	N
0,925	N	0,91785714	N	N	N	N	N	1,56153846	N	1,17307692	N	1,08943089	N	N	0,89175258	2,33870968	N	N	N
1,44047619	N	0,84552846	0,90677966	N	N	N	N	N	N	N	N	N	0,92982456	1,22368421	0,63541667	N	N	0,86666667	N
N	N	N	N	0,99280576	0,68461538	N	1,34532374	N	N	1,61290323	0,80327869	1,26845638	N	N	1,01162791	N	0,81188119	N	N
N	0,75	0,65217391	1,97777778	0,98611111	N	0,73417722	2,40540541	2,05555556	N	N	N	0,36666667	1,69491525	0,47674419	N	1,81818182	N	N	N
3,08333333	N	N	N	N	0,41666667	N	0,77777778	N	N	N	N	N	4,5	1,24	N	6,6	N	0,8	1,73913043
0,64197531	1,12676056	1,06779661	0,734375	0,13265306	N	0,77777778	N	1,54761905	0,625	0,17272727	N	1,27631579	1,10958904	2,14285714	N	0,49122807	N	1,07317073	0,15306122
1,61702128	5,1875	1,77631579	N	N	0,31612903	0,97590361	1,65168539	0,46969697	N	N	0,74766355	0,85074627	N	1,39772727	N	3,03636364	0,17460317	N	0,24712644
N	1,97058824	1,16129032	0,95555556	0,87878788	0,71052632	N	N	N	1,15789474	N	N	N	N	N	N	N	N	N	1,52941176
1,27433628	1	N	N	N	N	0,81914894	1,34306569	1,01587302	N	2,23636364	0,66666667	0,72611465	1,12149533	1,13761468	1,29487179	4,63333333	N	0,83516484	N
0,71428571	1,08988764	N	1,21390374	N	N	N	1,08733624	N	N	N	0,91796875	0,84234234	1,04736842	N	N	2,40350877	0,74301676	N	N
0,99382716	0,84897959	N	N	N	0,76966292	0,74675325	0,82424242	N	1,19318182	0,71653543	0,64864865	1,27480916	0,90298507	N	N	N	1,26027397	0,79787234	N
N	1,04471545	N	N	1,05813953	0,50364964	1,01398601	0,17372881	1,03773585	1,33333333	1,21428571	7,36	0,63210702	N	1,46296296	1,03703704	2,4047619	8,16666667	N	0,12903226
0,81042654	N	1,22222222	N	1,09375	N	N	0,83796296	0,72807018	0,80508475	0,83783784	1	N	1,18115942	1,2109375	N	N	1,04255319	1,32142857	N
N	1,59615385	N	1,48275862	2,25	N	1,15	0,43965517	N	0,8974359	1,04545455	0,49350649	1,28947368	0,68253968	1,14583333	2,22222222	N	N	N	0,97222222
N	1,10746269	0,84684685	0,96732026	0,99312715	0,48874598	N	N	N	N	N	N	0,93355482	N	N	N	2,53103448	1,03478261	N	1,41762452
0,9	0,68393782	N	N	1,1779661	N	N	N	1,55	1,5	1,53703704	N	N	0,94980695	N	N	7,22916667	5,96666667	N	1,06060606
1,01687764	1,12352941	0,70253165	1	1,16528926	0,74493927	0,70053476	N	N	1,55172414	1,5	N	1,13	N	1,15873016	0,92682927	N	N	1,28169014	1,29245283
N	N	1,09340659	0,97311828	0,89949749	N	0,71578947	N	1,25862069	N	N	1,4351145	N	0,96666667	N	N	N	0,99350649	N	N
N	N	1,01724138	0,59770115	N	N	0,83823529	0,88516746	N	1,01639344	0,8	1,04455446	N	N	N	N	N	0,97391304	0,85416667	N
0,88481675	N	N	0,88888889	1	N	0,63218391	1,01183432	0,925	N	N	N	1,1969697	N	N	N	2,21495327	N	1,35294118	1,4047619
N	0,82352941	N	N	1,08333333	0,77358491	0,95348837	N	0,93859649	1,00729927	N	N	1,05339806	N	0,755	1,02531646	1,95798319	N	N	N
1,11926606	N	0,5	1,21428571	0,71232877	N	1,21666667	0,17261905	N	0,21276596	N	0,51754386	N	N	0,93442623	0,8115942	0,31506849	N	N	0,79069767
1,22413793	N	0,84030418	N	N	N	N	1,17161716	0,95555556	0,95721925	0,96875	N	N	N	N	1,01754386	N	N	N	1,2578125
N	N	N	N	0,94420601	0,74516129	1,13414634	0,844098	N	N	N	0,71653543	0,86792453	N	N	N	2,24637681	N	N	1,35460993
N	N	0,85925926	0,97854077	0,88856305	N	N	N	0,97767857	1,07792208	1,05479452	N	N	N	N	N	2,20192308	N	1,28346457	1,51875
N	N	N	N	N	0,43877551	N	0,96654275	N	N	N	0,83268482	N	0,94047619	N	0,91472868	N	N	1,30882353	N
N	N	1,00716846	1,08457711	1,17956656	0,71898734	N	0,89294404	N	0,93617021	N	0,88304094	N	N	N	N	N	N	N	N
1,05952381	1,02	1,0070922	0,91578947	0,90551181	N	N	1,03333333	N	1,39506173	N	0,9408867	1,04712042	0,99526066	0,88392857	N	N	1,01875	0,98076923	N
0,75735294	1,1704918	0,9	N	N	0,75728155	N	N	0,76470588	1,19736842	1,09859155	N	N	0,93617021	N	N	N	N	N	1,05325444
0,90254237	N	0,69291339	1,05106383	N	N	N	N	1,02097902	N	N	0,98666667	0,87179487	0,98461538	N	0,85263158	1,50943396	N	N	1,42857143
N	N	0,87548638	1,32178218	N	0,48605578	N	N	0,82242991	N	N	0,9755102	N	0,76447876	1,16842105	N	2,25	N	N	1,15555556
1,0846395	N	N	N	0,92572944	0,77689243	0,75889328	N	0,85762712	N	0,99256506	1,0109589	1,03370787	0,94348894	N	N	1,97841727	0,98167539	1,17365269	1,32394366
1,08333333	0,94252874	0,90774908	N	1,11428571	N	N	N	N	N	N	N	0,91162791	N	0,90217391	N	N	N	1,31707317	1,46892655
N	0,99209486	N	N	1,03717472	0,52434457	0,70445344	0,98314607	N	N	0,95530726	N	N	0,96428571	1,00371747	N	N	1,31775701	N	1,04365079
N	1,13656388	N	N	N	N	N	1,13333333	N	1,29710145	N	N	0,86147186	0,87782805	N	N	N	1	N	N
1,27777778	1,05294118	N	N	N	N	N	1,28729282	N	N	1,15652174	N	0,90810811	N	N	N	N	N	N	N
0,89099526	N	1,02545455	1,16170213	N	0,78703704	N	N	1,01875	1,28368794	0,78804348	N	N	N	1,31693989	1,14285714	1,71223022	1,23255814	N	N
1,13333333	N	N	0,79901153	1,1576087	N	0,67383513	0,8643617	N	1	N	N	0,85714286	0,9331307	0,93362832	N	2,26821192	N	0,53333333	N
0,9494382	N	0,73056995	0,84662577	1,24657534	0,36305732	N	0,95215311	N	N	0,92	N	N	N	1,19428571	0,90839695	2,48170732	1,10909091	N	N

rs876724	rs891700	rs901398	rs907100	rs914165	rs917118	rs938283	rs964681	rs987640	rs9905977	rs993934	rs9951171
2	N	1,27325581	1,00840336	1,05128205	2,0877193	N	1,13978495	1,54205607	N	N	0,845
N	N	1,14285714	N	0,94262295	N	N	N	1,96774194	N	N	0,8951049
2,95454545	N	N	N	1,22535211	2,2173913	N	5,7826087	N	N	N	1,16
N	0,82125604	0,91071429	0,77118644	N	N	N	0,86390533	N	N	N	N
1,64285714	N	N	N	N	N	N	N	1,2	0,80120482	0,875	N
1,95454545	0,66086957	N	N	1,16504854	2,4893617	N	0,69298246	N	N	N	0,82075472
2,88888889	N	1,47183099	1,20512821	0,93975904	N	0,92356688	N	N	0,75428571	N	N
N	0,98130841	1,21698113	N	0,80924855	N	1,10884354	N	1,56363636	N	0,89473684	1,045
1,79591837	N	1,04263566	N	N	2,86956522	0,88047809	N	N	0,84337349	N	1,04395604
N	1,12307692	1,23423423	N	1,11	1,5	N	N	0,97222222	0,83098592	0,64285714	1,02298851
N	N	1,13103448	N	N	2,17948718	0,72189349	0,91935484	1,42307692	0,80681818	0,7311828	N
N	0,69811321	N	N	N	1,67647059	0,55434783	N	N	N	N	1,21212121
N	0,51428571	N	N	1,48484848	N	N	1,6	N	N	0,5	0,9
N	1,47368421	0,8362069	1,125	1,18965517	N	N	1,23188406	N	N	N	0,32191781
N	N	0,77714286	0,96551724	1,04878049	2,58536585	N	N	1,15555556	0,61052632	0,85454545	N
N	0,57894737	1,31111111	1,58333333	1,9047619	2,625	0,77419355	N	1,25	N	N	N
2,25806452	0,61212121	0,86982249	N	1,45631068	N	N	0,95804196	N	1,03521127	1,3	1,23076923
N	N	1,09821429	1,29896907	0,88317757	N	1,17073171	N	0,81395349	N	N	N
N	1	N	N	1,5049505	N	N	N	0,93406593	0,94166667	N	1,39047619
3,14285714	N	0,57303371	0,81632653	0,59235669	3,54054054	3,17073171	1,42690058	1,06060606	0,74883721	1,33333333	N
N	N	1,03125	N	N	1,33333333	N	N	N	1,64473684	1,12328767	N
N	1,34210526	0,125	N	1,41818182	N	N	N	1,9	N	2,45454545	6,25
N	N	1,00946372	0,91666667	1	N	1,07727273	N	1,13861386	N	0,78823529	1,14224138
N	0,16161616	N	0,73913043	N	1,82758621	N	N	8	N	0,42857143	N
N	N	1,07142857	1,89583333	1,31690141	N	0,91724138	N	0,95081967	1,14070352	0,68292683	N
1,63529412	N	N	N	N	N	N	1,36734694	N	N	N	1,16793893
N	N	1,21428571	N	N	N	N	N	N	N	0,91262136	1,02409639
N	0,82191781	N	0,92424242	0,89617486	N	N	0,92056075	0,52380952	N	0,85365854	1,09756098
N	1,14213198	1,05063291	N	0,82051282	1,53076923	N	N	N	N	0,98076923	N
N	0,1682243	0,57024793	0,60784314	0,86419753	1,59375	N	N	N	0,54966887	1,16666667	0,8989899
1,73913043	1,05084746	N	1,3	0,94219653	N	0,92	0,888	0,67105263	1,00636943	0,8359375	N
N	N	N	N	0,81012658	N	N	1,05747126	N	N	N	0,78873239
2,13043478	N	1,14107884	0,96808511	N	1,94117647	N	0,85436893	N	1,15544041	1,1025641	N
2,08	N	1,245	1,11956522	N	N	N	0,94059406	N	N	N	N
N	N	N	N	N	N	0,97142857	0,97986577	1,14953271	0,90860215	1,05263158	0,85972851
2,08888889	N	N	N	N	1,64634146	N	0,93548387	N	0,93658537	1,875	N
N	1,14583333	0,91627907	N	0,99441341	N	N	N	1,12711864	1,08982036	0,8984375	N
N	N	1,10194175	1,23595506	0,98755187	2,15277778	N	N	0,89156627	0,82278481	N	N
2,26785714	1,06837607	N	N	0,87124464	1,50442478	N	0,82206406	1,40298507	N	N	N
N	0,91927083	N	N	0,76399027	2,08888889	N	N	N	1,18413598	N	1,01045296
N	N	1,03636364	1,31896552	0,81107492	N	N	0,94680851	0,75	0,71005917	N	N
N	N	1,19583333	0,9787234	N	N	N	N	N	N	0,83783784	1,17777778
1,59183673	N	N	N	N	3,44444444	0,90829694	N	N	0,88085106	N	0,98870056
N	1,05625	0,76732673	N	N	N	N	0,90909091	N	0,84651163	N	N
1,49152542	N	N	N	N	1,82278481	N	N	N	N	N	0,69005848
N	1,04639175	1,05693069	N	1,0321489	1,26229508	N	N	0,80392157	0,99528302	N	1,03184713
N	0,95394737	1,06976744	N	N	N	N	N	N	N	N	N

Supplementary Table S7. Noise level values for all 132 SNP markers included in HID Ion Ampliseq Identity Panel, in Brazilian criminal samples.

Samples	**Locus**																	
Blood	*rs1005533*	*rs10092491*	*rs1015250*	*rs1024116*	*rs1028528*	*rs1031825*	*rs10488710*	*rs10495407*	*rs1058083*	*rs10773760*	*rs10776839*	*rs1109037*	*rs12997453*	*rs13218440*	*rs1335873*	*rs1355366*	*rs1357617*	*rs1360288*
BL.PF07	0	0,00858369	0	0,00192308	0,00203666	0,02191235	0	0,00207039	0	0,00226244	0	0	0	0	0	0	0,00390625	0
BL.PF09	0	0	0	0	0,00384615	0,01298701	0	0	0,003003	0	0,00636943	0	0	0	0	0	0,00595238	0
BL.PF11	0	0,0097561	0	0,02325581	0	0,02692308	0	0,00526316	0	0	0,02298851	0	0	0	0	0,01976285	0,02797203	0,08374384
BL.PF20	0	0,01209677	0	0	0	0	0	0	0,00232558	0	0	0	0	0	0,00721154	0	0	0
BL.PF22	0	0,01365188	0	0	0	0,00377358	0	0,00350877	0	0	0	0	0	0,00325733	0	0	0,02272727	0
BL.PF24	0	0,00595238	0	0	0	0	0	0	0	0	0	0,00719424	0	0	0,00796813	0	0	0
BL.PF25	0	0	0	0,00544959	0,00291545	0,02211302	0	0,00326797	0,00263158	0	0	0	0	0	0,00666667	0	0,0212766	0
BL.PF26	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0,00289017	0	0,00458716	0,00240385
BL.PF27	0	0	0	0	0	0	0	0	0	0	0	0,00571429	0	0,00207469	0	0	0	0
BL.PF30	0	0,00746269	0	0	0,00363636	0	0	0,00552486	0,00359712	0	0	0	0	0	0	0	0,00746269	0
Epithelial																		
EP.PF01	0	0,03363914	0,02727273	0,03383459	0,00409836	0,08974359	0	0	0,04494382	0,06231454	0,01086957	0	0,02222222	0,06798246	0,01149425	0	0,04854369	0
EP.PF08	0	0	0	0	0	0,01724138	0	0,00892857	0	0	0	0	0	0	0	0	0,03092784	0
EP.PF10	0	0	0	0	0,04166667	0,01724138	0	0	0	0	0	0	0	0	0	0	0,03076923	0
EP.PF13	0,01136364	0	0,01923077	0	0,00540541	0	0	0	0,03797468	0	0	0	0	0,04	0	0	0	0
EP.PF14	0	0,02525253	0,01123596	0	0,05574913	0	0	0,0738255	0,00315457	0,07894737	0	0,12272727	0	0	0,0513834	0	0,01898734	0,04661017
EP.PF15	0	0	0	0	0	0,02083333	0,01960784	0,01851852	0	0	0	0	0	0	0,01851852	0	0	0,01190476
EP.PF17	0,00595238	0,01744186	0	0	0,00294985	0,01644737	0	0	0	0,01010101	0	0	0	0	0,00619195	0	0,00641026	0
EP.PF18	0	0	0	0	0	0,02702703	0	0,00309598	0	0	0,00454545	0,0070922	0	0,00695652	0	0,002849	0,00429185	0,00537634
EP.PF21	0	0,01333333	0	0	0,00332226	0,00666667	0,00473934	0	0	0	0	0	0	0	0	0	0,00724638	0,01111111
EP.PF28	0	0,0972973	0,01923077	0,06	0,00392157	0	0,09433962	0,00675676	0	0	0,01449275	0,03448276	0,12820513	0,00645161	0	0,10273973	0,01492537	0,00436681
Saliva																		
SA.PF02	0,09638554	0	0	0	0	0	0,05172414	0	0,0855615	0	0	0,06086957	0,0625	0	0,03571429	0,10714286	0	0
SA.PF03	0	0	0,00440529	0	0	0	0	0	0,00776398	0	0,00478469	0,00273224	0	0	0	0	0,01864802	0,00169205
SA.PF04	0	0	0,02419355	0	0	0	0,02105263	0,05590062	0	0	0	0,00209644	0	0,10685805	0	0,03954802	0,02666667	0,04726368
SA.PF05	0,03793103	0,1025641	0,0141844	0,03716216	0	0,00290698	0	0	0,04146341	0,02293578	0	0	0	0,06561086	0,01142857	0,00334448	0,03389831	0,04496788
SA.PF06	0,0033557	0,0026738	0	0,00299401	0	0	0	0,00909091	0,00479616	0,00215517	0	0,00869565	0,0078125	0	0,00753769	0	0,00943396	0
SA.PF12	0	0	0	0	0	0,02444988	0	0,00409836	0,00527704	0	0,00961538	0	0	0	0	0	0,00813008	0,00275482
SA.PF16	0	0	0	0	0,01711491	0,02430556	0	0	0	0,00390625	0,02	0	0	0,00413223	0,00228833	0	0	0
SA.PF19	0	0	0	0	0,00222222	0,01744186	0	0	0	0,00352113	0	0	0,01694915	0	0,00591716	0	0,02262443	0,00231481
SA.PF23	0	0,00461894	0	0	0,00373832	0,00449438	0	0	0	0,00919118	0	0	0,02040816	0	0,00512821	0,0034965	0,00956938	0
SA.PF29	0,0137931	0	0	0	0	0,03278689	0	0	0,1185567	0	0	0,12571429	0	0	0,02762431	0	0,08139535	0,02325581
Semen																		
SE.PF02	0	0,00587084	0,00568182	0	0,00280112	0,00454545	0	0	0	0,00229358	0	0	0	0,00383142	0	0,00167785	0	0
SE.PF03	0	0	0	0	0	0,04	0	0	0	0	0	0	0	0	0	0	0,02639296	0
SE.PF07	0	0	0	0	0	0,00735294	0	0,00220264	0	0	0	0,00337838	0	0	0,00207469	0,00512821	0,02216749	0
SE.PF22	0	0,00252525	0	0	0,00400802	0,02579365	0,00347222	0	0,00677966	0	0,00352113	0,01209677	0	0	0	0	0,00571429	0
SE.PF26	0	0	0	0,00163132	0	0	0	0,00240964	0,00183486	0	0,00617284	0,01302932	0	0	0	0,00141044	0,00204918	0
SE.PF27	0	0,00867052	0,00694444	0	0	0,03475936	0,00552486	0,0060241	0	0,00511945	0	0,00254453	0	0,01048951	0,00793651	0,01079137	0,01351351	0,0031348
SE.PF30	0	0,00772201	0	0,0019084	0,00289855	0,02798233	0	0,00384615	0,00197628	0	0	0	0	0,00731261	0	0	0,00326797	0,00289855
Control (Oral Swab)																		
CT.PE02	0	0	0	0	0,0021322	0,02905569	0	0,00273224	0	0,00757576	0	0	0	0	0	0	0,00706714	0,00236407
CT.PE03	0	0,00174216	0	0	0,00739372	0	0	0,00715564	0	0,00193424	0	0	0	0	0,00347222	0	0,00529101	0
CT.PE04	0,00114943	0,00328228	0	0	0,00131579	0,00262812	0	0,00137552	0	0,00142045	0	0,0020284	0	0,00750939	0,00234467	0	0,01271186	0
CT.PE07	0	0,00949367	0,00671141	0	0,00212766	0,00339559	0	0,00202429	0	0	0	0	0	0,0037594	0,00168634	0	0,01324503	0
CT.PE08	0	0,00606061	0	0,00165837	0	0,00162866	0	0	0,00149031	0,00163132	0	0	0	0	0	0,00400802	0,00761421	0,00164474
CT.PE09	0	0,01295896	0	0,00203666	0,00433839	0,02411874	0	0,00945626	0	0	0,015625	0	0	0,00218818	0,00204918	0	0,01724138	0,00458716
CT.PE10	0	0,00246914	0	0	0	0	0	0	0,00556586	0,00353357	0,00653595	0,01025641	0	0	0,002849	0	0,01550388	0
CT.PE11	0	0	0	0	0	0,00184162	0,00819672	0,00470588	0,00215983	0	0	0	0	0	0	0	0,00900901	0
CT.PE12	0	0	0	0	0,00547196	0,04200323	0,00346021	0	0,00350467	0,00089047	0,0017301	0	0	0,00613968	0,00658436	0	0,01075269	0,00260756
CT.PE13	0	0,00480769	0	0	0	0,00505051	0	0,00320513	0	0	0	0,00977199	0	0	0,00455581	0	0,01067616	0

rs1382387	*rs1413212*	*rs1454361*	*rs1463729*	*rs1490413*	*rs1493232*	*rs1498553*	*rs1523537*	*rs1528460*	*rs159606*	*rs1736442*	*rs1821380*	*rs1872575*	*rs1886510*	*rs1979255*	*rs2016276*	*rs2040411*	*rs2046361*	*rs2056277*	*rs2076848*
0,0056338	0,00257069	0	0	0,0017452	0	0,00668151	0,00550964	0	0	0,00386847	0,00192678	0	0	0	0	0,00188324	0,00630915	0,00202429	0,00327869
0	0	0	0,00925926	0	0,02702703	0,00552486	0,00384615	0	0,00662252	0	0,00536193	0	0	0	0,00393701	0	0	0	0
0,00331126	0,03827751	0	0,09057971	0	0,00934579	0	0	0	0	0,00497512	0,01083032	0,09	0	0	0	0,01190476	0	0	0,04678363
0	0	0,0036036	0	0	0	0,00549451	0	0	0	0	0,00841751	0	0	0	0,00288184	0,00443459	0,0030581	0	0
0	0	0	0,0026738	0,00309598	0,02380952	0	0,00346021	0	0,00689655	0	0,00271003	0,0031348	0	0	0	0,002331	0	0	0,00485437
0	0	0,00396825	0,00333333	0,00689655	0	0	0,01092896	0	0	0	0	0	0	0	0	0,00851064	0	0,00699301	0
0	0	0	0,01340483	0	0,00549451	0	0,0166113	0	0,00434783	0	0	0	0	0,00396825	0	0,00248756	0	0	0
0,00512821	0,00278552	0,003663	0	0	0	0	0	0	0,00438596	0	0,00275482	0	0	0	0	0,00990099	0	0,00387597	0
0	0,00231481	0	0,00192308	0	0	0,00440529	0	0	0	0,00460829	0	0	0	0	0,00462963	0,00155521	0,00325733	0,00242718	0
0,01260504	0	0	0	0	0	0	0	0	0,01574803	0	0	0	0	0,00617284	0	0,00306748	0,01162791	0	0
0,00265252	0	0	0,03324808	0,05376344	0,05462185	0,05102041	0	0	0,07006369	0	0,01572327	0	0	0,01351351	0,0245098	0,03296703	0,02469136	0,00877193	0,06609195
0,02469136	0	0	0	0,00598802	0	0	0	0	0	0	0	0	0	0	0	0	0,01694915	0	0
0	0	0	0,01639344	0	0	0	0	0	0	0	0	0	0	0	0,01219512	0	0	0	0
0	0	0	0	0	0	0,11363636	0	0	0	0	0	0	0	0	0,02702703	0,02614379	0	0	0
0,08191126	0	0	0	0	0	0	0,08609272	0	0	0,02162162	0	0,01384083	0,02586207	0,00406504	0	0,04059041	0,08928571	0,0209205	0
0,00917431	0,07	0	0,01449275	0	0	0	0	0	0	0	0	0	0	0	0,01680672	0	0	0,05882353	0,03225806
0,00531915	0,0034965	0	0	0	0,04102564	0,00326797	0,00364964	0	0	0,00408163	0,01577287	0,00890208	0	0	0	0,003125	0	0	0
0,0043956	0	0	0	0	0,0047619	0	0	0	0,00421941	0	0,002331	0	0	0,00314465	0,00234742	0	0	0	0
0	0	0	0,01474926	0	0,0041841	0	0	0	0	0,00621118	0,00408163	0	0,00662252	0,00315457	0,00485437	0,00898876	0	0	0,02173913
0,00215983	0	0	0,07042254	0	0,06289308	0	0	0	0,075	0	0	0	0,00826446	0,05943152	0,00879121	0,00236407	0	0	0,03930131
0	0,00561798	0,03007519	0,07563025	0	0,0125	0,00869565	0	0	0	0	0	0,11594203	0	0,00884956	0	0,00440529	0	0	0,04807692
0,00588235	0,00156006	0	0	0	0	0,00319489	0	0	0	0,00365631	0,00150376	0	0,00301205	0,00191571	0	0,00125	0,00928793	0,00639659	0
0,07214429	0,03512881	0,082	0	0,0030303	0	0	0,05800464	0	0,12093023	0	0,0521327	0,0177305	0	0,04022989	0	0,07777778	0	0,05050505	0,00584795
0,09280742	0	0,01875	0,09492274	0,01071429	0,07665505	0	0,04676259	0,00970874	0,01242236	0	0,03225806	0	0	0,00485437	0,0023753	0	0,11464968	0,04477612	0
0,00240385	0	0	0,0021692	0	0	0,00259067	0	0,00287356	0,00323625	0,00255102	0,01680672	0	0	0,0058309	0	0,0022779	0	0	0
0,00526316	0	0,0031746	0	0	0	0	0	0	0	0,00287356	0	0	0	0	0,0027248	0	0	0,00306748	0,00363636
0,00218818	0	0	0	0	0	0	0	0	0	0,00395257	0,01449275	0	0,01762115	0	0,002331	0	0,00985222	0,00823045	0
0	0,0037594	0	0,00243309	0,00251256	0,015625	0	0	0	0,00543478	0	0,00236967	0	0	0,00377358	0,00276243	0,00426439	0	0	0,00311526
0,00336134	0,00183486	0	0	0	0,00967742	0,00483092	0	0,00295858	0	0	0	0	0,00416667	0,00447427	0	0,00461538	0	0,00347222	0
0,00473934	0	0,03428571	0,00888889	0	0,01612903	0	0	0,04166667	0	0,00909091	0,00574713	0,088	0	0,00649351	0,00552486	0,0862069	0,01818182	0	0
0	0	0	0	0,00398406	0	0	0,00260417	0	0	0	0	0	0	0	0	0	0	0,00257732	0,00331126
0	0	0,00133511	0	0	0,00142248	0	0	0	0	0	0,00199203	0	0,00446429	0	0	0,0060241	0	0	0,00344828
0,00214592	0	0,00233645	0	0	0,01072386	0	0	0	0	0,00239234	0	0	0	0,00202429	0,00215054	0	0,01754386	0,00460829	0
0,00310559	0	0	0,00209205	0,00252525	0,00341297	0	0	0	0,00383142	0	0,00167785	0	0,00392157	0	0,00179856	0,00635593	0	0	0,0026455
0,00383877	0	0,00322234	0	0	0	0	0,002	0	0	0,00263852	0,00160256	0	0,0060241	0	0,00114286	0	0	0	0,00310559
0,00504202	0,0018315	0	0	0	0	0	0,00412371	0	0	0	0,02232143	0	0	0,00248756	0,00346021	0,00223714	0,05617978	0	0,0056338
0	0	0,00126582	0	0	0,02296451	0	0,002331	0	0,00316456	0,00729927	0,00711744	0,00594059	0	0,00441501	0,00172117	0,00757576	0	0	0,0030303
0	0	0,00804829	0	0	0,02016129	0	0	0	0	0,00610998	0	0	0	0	0,00422833	0,00219298	0	0	0
0,0035461	0	0,00207469	0	0	0,01444043	0,00183486	0	0	0	0	0,00656814	0	0	0,00249377	0	0,00179856	0	0,00173611	0
0,00254453	0	0,00493218	0,00113507	0	0,01269841	0	0	0,0015361	0,00228311	0,00260078	0	0	0	0,00530035	0,00268817	0,01040462	0,02197802	0,00950119	0,00651466
0,00378072	0,00518135	0,00359066	0,00170068	0	0	0,00149477	0,00252525	0	0,00287356	0,00184162	0,00309119	0	0,0025974	0	0,00704225	0,00365631	0,01550388	0	0,00238095
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0	0,00092593	0,000998	0,00533808	0	0,00178253	0,00479042	0,00458716	0	0	0,00206612	0,0015748	0,00178891	0	0,00524934	0,00229621	0,01519757	0	0,00208768	0,00225989
0,00255102	0	0	0	0,00437318	0,00490196	0,00512821	0	0	0	0	0,00990099	0	0	0,00369004	0,00223714	0,00204499	0	0	0

rs2111980	rs214955	rs221956	rs2269355	rs2292972	rs2342747	rs251934	rs2830795	rs2831700	rs321198	rs338882	rs354439	rs3780962	rs4288409	rs430046	rs4364205	rs445251	rs4530059	rs4847034	rs560681
0,00361011	0	0,00896057	0,00139082	0	0	0,00260417	0	0	0	0	0	0	0,00289017	0	0	0,00197239	0	0,00396825	0,01061571
0	0	0	0,00315457	0	0	0	0,00242718	0	0	0	0	0	0,00384615	0	0	0	0	0,0212766	0,00735294
0,03333333	0	0,08196721	0	0	0,01149425	0,1221374	0,09567901	0	0	0	0,04081633	0	0,04761905	0	0,003125	0,00423729	0,09661836	0,01298701	0
0	0	0,00289017	0	0	0	0,0034965	0,00178571	0	0,00502513	0	0,0132626	0,0033557	0	0	0,00417537	0,01069519	0,00716846	0,02247191	0,00823045
0	0	0,00917431	0	0	0	0	0	0	0,02721088	0,00632911	0	0	0	0	0	0,00425532	0,00442478	0,01754386	0,0031348
0	0	0	0,01621622	0,00218341	0,02061856	0,004	0	0	0,01315789	0,00268817	0	0	0	0	0,00581395	0,01030928	0	0,06363636	0,00462963
0	0	0,00787402	0	0,00531915	0	0	0,00211416	0	0	0	0	0,00749064	0	0	0	0	0	0	0
0,00327869	0	0	0	0	0	0,00455581	0	0	0	0	0	0	0	0	0,00246914	0,004329	0	0,05504587	0
0,00235294	0	0,01270417	0,00159236	0	0	0,00217391	0,0016	0,00247525	0	0,0018797	0	0	0	0	0,00157978	0	0	0,03459119	0,00534759
0	0	0	0,00429185	0,0075188	0,01351351	0	0	0	0	0	0	0	0	0	0,00326797	0	0,0257732	0,03676471	0
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0	0	0	0	0	0	0,09868421	0	0	0	0	0	0	0	0	0,00480769	0	0,04294479	0,07446809	0
0	0	0,01315789	0	0	0	0	0	0	0	0,02150538	0	0	0	0	0	0	0	0,07142857	0
0	0	0	0,07857143	0	0	0,00699301	0,06951872	0	0	0	0,03100775	0	0,03703704	0	0,09625668	0,11428571	0	0,04347826	0
0	0,00917431	0	0	0,00401606	0,01030928	0	0,05414013	0	0	0	0,0060241	0	0	0	0	0,00358423	0	0,01052632	0,01190476
0,03030303	0	0,01162791	0,00990099	0	0,0212766	0,02702703	0	0	0	0	0,01666667	0	0	0	0	0,04615385	0	0,03448276	0
0	0	0,00265252	0,00303951	0,01574803	0	0,00411523	0,00323625	0,00416667	0	0	0	0	0,00598802	0	0,00520833	0,00341297	0	0,06185567	0
0	0,00934579	0	0,00169492	0,00190476	0	0,00224719	0,00449438	0,00254453	0	0,00182482	0	0	0	0	0	0,00291545	0	0,01709402	0
0	0	0,00831025	0	0,00626959	0,00757576	0	0	0,00233645	0,00925926	0,0021692	0	0	0,01162791	0	0	0	0	0,06086957	0
0	0	0	0,04	0	0,02339181	0	0	0,06188925	0	0	0	0	0	0	0	0	0,08724832	0,008	0
0,02793296	0	0,00653595	0,04316547	0	0	0,08035714	0	0	0	0	0,00847458	0,05555556	0	0	0,03333333	0,01538462	0	0,01754386	0
0	0	0,00140647	0,00541516	0,00161812	0,0046729	0,00184502	0	0,0030303	0,00308642	0,0015015	0,00163132	0	0,00246305	0	0,00397878	0	0	0,04468085	0,00553506
0	0,04545455	0	0,06199461	0,00529101	0	0,0025641	0,04910714	0,11041009	0,08108108	0,06807131	0,06228374	0,072	0,06818182	0	0,00261097	0	0,0025974	0,04830918	0
0	0	0	0	0	0,03311258	0,00226244	0	0,06593407	0,10245902	0,05532787	0,05479452	0	0,05098039	0	0	0	0,00308642	0,0070922	0,00294118
0	0,00621118	0,00700935	0	0,0030581	0	0,00530504	0	0	0	0	0	0,00546448	0	0	0,0094162	0	0	0,0031746	0,00266667
0	0	0	0	0	0	0,0034965	0	0	0	0,00232019	0	0	0,00387597	0	0	0	0,00398406	0,03153153	0
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0,00331126	0	0,00967118	0	0	0	0	0,00217391	0	0,00319489	0,00173913	0	0,00280112	0,00362319	0,00395257	0,00511073	0,01204819	0	0,01262626	0,00465116
0,00625	0	0	0	0	0	0	0	0	0,01724138	0	0,10169492	0	0	0,05128205	0	0,01010101	0	0,03703704	0
0	0	0,00990099	0	0,00331126	0	0	0	0	0,00576369	0	0,00162338	0	0	0	0	0	0	0,00733496	0
0	0	0,00390625	0	0	0	0,003367	0,00269542	0	0,00230415	0	0,00328407	0,0037037	0	0,00645161	0,00421941	0,00857143	0	0,02538071	0,00543478
0	0,00769231	0,00425532	0	0	0	0,00254453	0	0	0	0,00183486	0,01675042	0,00473934	0,00327869	0	0,0015674	0,00623701	0	0,02702703	0,00453515
0	0	0,00226757	0,00362319	0	0	0,00240385	0	0	0,00398406	0	0,00628931	0	0	0	0	0,003125	0	0,04166667	0,00458716
0	0	0,00442478	0	0	0	0	0,00383142	0	0	0	0	0	0	0	0,00144718	0,0045045	0,00348432	0,00740741	0
0,00230415	0	0,00175747	0,00438596	0	0,00613497	0,00842697	0,00792079	0,00234742	0	0	0	0,00236967	0,00369004	0	0,00486618	0,01282051	0	0,03303303	0
0,00732601	0	0	0	0	0,00793651	0,00307692	0,00487805	0,00559701	0,00235849	0	0,00196464	0	0,00456621	0,00206186	0,00357143	0,00840336	0	0,00634249	0
0,00334448	0	0	0	0	0	0	0,00204918	0,00240385	0	0	0	0	0	0	0	0	0,00647948	0,01632653	0,00204918
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0,00178891	0,00649351	0	0,00499168	0,00151745	0,00346021	0,00241838	0	0	0,002849	0,00105152	0,00536913	0	0,00750469	0	0,00208986	0,00274348	0	0,01937046	0
0	0,01449275	0	0,00128205	0	0	0	0,00140056	0,00229885	0,00308642	0,00165017	0	0	0,00283286	0	0	0	0	0,02536232	0,00957854
0,00268097	0	0	0,00221484	0	0	0,00383142	0	0	0	0	0,00904159	0	0	0	0,00147275	0,0040568	0,00195313	0,00677966	0,00474684
0	0	0	0,00186567	0,00273224	0	0,00222222	0,00350263	0	0	0,00221729	0	0	0	0	0,00454545	0,00213675	0	0,04014599	0
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0	0	0,0040568	0,00179211	0	0	0,00242718	0	0	0,00809717	0	0,0094697	0	0,0037594	0	0,02150538	0,00225225	0	0	0
0,00347826	0	0	CONT	0,00611247	0	0,00175747	0,00163934	0	0	0,00133958	0	0	0	0	0,00111359	0,00875657	0,00084962	0,01941748	0,00224215
0,00303951	0	0,01002004	0,00105152	0	0	0	0,00327869	0	0	0,00446927	0	0	0	0	0	0	0,01158301	0,01333333	0

rs576261	*rs6444724*	*rs6811238*	*rs6955448*	*rs7041158*	*rs717302*	*rs719366*	*rs722098*	*rs722290*	*rs727811*	*rs729172*	*rs733164*	*rs735155*	*rs737681*	*rs740598*	*rs740910*	*rs7520386*	*rs7704770*	*rs826472*	*rs873196*
0	0,00248756	0,002331	0	0	0	0	0	0	0	0	0,00431034	0	0,00274725	0,00458716	0	0,00383877	0	0,00308642	0,03481013
0,02649007	0	0	0,01107011	0	0	0	0	0,00598802	0	0,01324503	0,00452489	0	0,00374532	0	0	0,00487805	0	0	0
0,02941176	0,00502513	0,01877934	0,00277778	0	0,01315789	0	0	0	0,01538462	0	0,0046729	0	0,05154639	0	0,01395349	0	0,00512821	0	0
0	0	0	0	0,00293255	0,00260417	0,00617284	0	0	0	0	0,00623053	0	0	0,01908397	0,00724638	0	0	0,00444444	0,02040816
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0	0,00334448	0	0	0	0	0	0	0	0	0,00510204	0,00337838	0,00390625	0	0	0,00793651	0	0	0	0,03643725
0,00293255	0,00315457	0,00843882	0,00578035	0	0,00746269	0,00657895	0	0	0	0,00518135	0,01140684	0	0	0,00401606	0	0	0	0	0
0	0,00204499	0,00185874	0	0,00236967	0	0	0	0,00299401	0,00497512	0,00294118	0,01388889	0,00194175	0,00647948	0,00209644	0,01078167	0,00240964	0,0021645	0	0,00243902
0	0	0	0,00442478	0	0,0049505	0	0,00757576	0,01265823	0	0	0,00420168	0	0	0	0,01875	0,00651466	0	0	0,03614458
0,02222222	0,04918033	0,01838235	0,01886792	0	0	0,02678571	0	0,02868852	0,02369668	0	0	0	0,03303303	0,07003891	0	0,09278351	0	0,00680272	0,02228412
0	0,04545455	0,00869565	0,00740741	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0,05128205
0	0	0	0,02040816	0	0	0	0	0	0	0	0,11111111	0	0	0	0	0	0	0	0
0	0	0	0	0	0,10227273	0	0,1025641	0	0	0	0,13580247	0	0	0	0,04597701	0	0,1038961	0	0
0	0	0	0,04489796	0,08695652	0	0	0	0	0,14285714	0,0304878	0,00531915	0	0,05511811	0	0	0	0	0	0
0	0	0	0	0	0	0	0,06557377	0	0	0	0	0	0	0,02325581	0	0,02040816	0,05357143	0,03125	0
0	0	0	0,00347222	0	0,01648352	0	0	0	0	0	0	0	0	0	0,00555556	0,00588235	0,00549451	0	0
0	0	0	0	0,00268097	0	0	0	0	0	0,00515464	0,01207243	0	0	0,00299401	0,01321586	0,00257069	0	0	0,0212766
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0,13934426	0	0	0	0	0,07563025	0	0	0,02739726	0	0	0,004329	0	0,04504505	0,00961538	0	0,00657895	0,12037037	0,08064516	0
0,00651466	0,00282486	0,00162338	0,00331126	0	0	0	0,0016	0,00534759	0	0	0,00301205	0	0,00922509	0,00232019	0,02375297	0,00194932	0,00425532	0	0
0	0,00306748	0,05504587	0,07173913	0	0,07857143	0,06643357	0,06636156	0	0	0	0,10185185	0,00232019	0	0,04494382	0	0	0	0,03571429	0
0	0	0	0,00515464	0	0	0	0,06153846	0,02923977	0	0	0,02979516	0	0,07990315	0,003663	0,0083682	0,00760456	0,02347418	0	0
0,01754386	0,00239234	0	0,00542005	0	0,00484262	0,0030581	0,00496278	0,00380228	0,00655738	0	0,003125	0	0	0	0	0,00492611	0	0	0,02576112
0,00261097	0	0	0	0	0	0	0	0	0	0	0,00327869	0,00486618	0	0	0	0,00297619	0	0	0,00263158
0,002331	0	0	0	0	0,00900901	0	0	0	0	0	0,00241546	0,00425532	0,00993377	0	0	0	0	0	0,02054795
0	0,00578035	0	0	0	0	0	0	0	0	0,00934579	0	0	0,00729927	0,01020408	0	0,00289855	0	0	0,00492611
0,00212314	0	0	0	0	0,00176991	0	0	0,00896861	0	0,00682594	0	0	0,01126761	0,00284091	0	0	0,00290698	0	0
0	0,05333333	0	0,02515723	0	0,08510638	0	0	0,03636364	0	0,04379562	0	0,11836735	0,01408451	0	0	0	0,06862745	0,02040816	0
0	0,00758725	0	0,02337662	0	0	0	0,00151745	0	0	0,00526316	0	0,0058309	0,00323625	0	0,00862069	0,00775194	0,00967742	0	0
0,00980392	0,00793651	0	0	0	0,00184502	0	0	0	0,00677201	0	0,00456621	0	0,00925926	0	0	0	0	0,00952381	0,003003
0,021875	0,0044843	0	0,00860215	0	0	0	0	0	0	0,00662252	0,00589971	0,0027933	0	0,00606061	0	0	0,00238663	0	0
0,00502513	0,00292398	0	0,00425532	0	0	0	0,00188679	0,0026455	0	0,00507614	0,0105042	0,00192678	0	0	0,00803213	0	0,00238663	0	0
0,00434783	0,00270636	0,0070922	0,00945626	0	0	0	0,0025641	0	0	0	0,00617284	0,00235294	0	0,0031746	0	0,00652174	0	0,00475059	0,03503185
0,00859599	0	0,00352113	0,00546448	0	0	0,00714286	0	0,00719424	0	0	0,00253165	0	0	0,00471698	0,02068966	0,00833333	0	0	0,01204819
0	0,0015083	0	0	0	0,00183824	0	0	0,00255754	0	0,00666667	0,00589971	0,00346021	0	0,01492537	0	0,00702576	0	0,00361011	0
0	0	0,00232019	0,00207039	0	0,00265252	0,00760456	0,00178891	0,00344828	0	0	0,00666667	0	0	0	0,00564972	0,0025	0,00759494	0	0
0,00813008	0	0	0,01054852	0	0,0026738	0	0,00519031	0	0,00262467	0	0,00616016	0,00209644	0	0,00242131	0,00260417	0	0	0	0
0,0015015	0,00167504	0,00142653	0	0,00137552	0	0	0,00109649	0	0	0,0018622	0,00407056	0	0	0,00258065	0	0,00120627	0,00131926	0	0
0,00234742	0	0,00385356	0	0	0,00438596	0	0	0	0	0,0052356	0	0	0,00242718	0	0,0030303	0,00433526	0,00208333	0	0
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0	0	0,00199601	0,00255754	0	0	0	0	0	0	0,00416667	0,00656455	0	0	0,01534527	0	0	0	0	0,04461942
0	0	0,00280112	0,01591512	0	0,01098901	0,00483092	0	0	0	0,00401606	0	0	0	0	0	0,00688073	0	0	0,02362205
0	0,00275482	0	0,00392157	0,00258398	0	0	0	0	0	0	0	0	0,00877193	0,00469484	0,00763359	0	0	0	0,01481481
0	0,00380952	0	0,00817439	0	0	0,00213675	0,00284495	0	0	0	0,00195122	0,00121951	0	0	0	0,00101215	0,00255754	0	0,02877698
0	0	0,00298507	0,00331126	0	0	0	0	0	0	0,00414938	0	0	0,01057082	0,00518135	0,00398406	0,00174825	0	0	0

rs876724	rs891700	rs901398	rs907100	rs914165	rs917118	rs938283	rs964681	rs987640	rs9905977	rs993934	rs9951171	L298	M479	P202	P256	s13447443	s16980426	s16981290	s17222573	s17250845	s17269816
0	0,00304878	0	0,01239669	0	0	0,00217865	0,01485149	0	0,00200803	0	0	0	0,052632	0	0	0	0	0	0,007463	0	0
0	0	0	0	0	0	0	0,02061856	0,01075269	0,008	0	0,00367647	0,010309	0	0	0	0	0	0	0	0	0
0	0	0,0308642	0	0	0	0,0627451	0,01265823	0	0,01449275	0	0	0,007874	0	0	0	0	0	0,010101	0	0	0
0	0	0,00465116	0,0047619	0,01231527	0	0	0,00630915	0	0,003003	0,0070922	0	0	0	0	0	0	0	0	0	0,037634	0
0	0	0,00977199	0,00719424	0,01098901	0,00632911	0,00398406	0,00411523	0,01123596	0,00333333	0	0	0	0	0	0	0	0,008197	0	0	0	0,007576
0	0	0	0,015625	0	0	0	0,01025641	0,01075269	0	0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0,00330033	0	0	0,00324675	0	0,0125523	0,008772	0	0	0	0	0	0	0	0	0,021739
0	0	0	0	0	0	0	0	0	0,00230415	0	0	0	0	0	0	0	0	0	0,00692	0	0
0	0,0027248	0	0,008	0	0	0	0,00244499	0,00735294	0	0	0,00534759	0	0	0	0	0	0	0	0	0	0
0	0	0	0,01652893	0	0	0	0,01321586	0	0	0	0,00564972	0	0,071429	0	0	0	0	0	0	0	0
0,03773585	0,04230769	0	0,0141844	0,06923077	0	0	0,00557103	0	0	0,00617284	0,03571429	0	0,08	0	0	0	0,045977	0,046729	0	0	0,048649
0	0	0,01212121	0	0,07563025	0	0	0	0	0	0,01136364	0	0	0	0	0	0	0	0	0	0	0
0	0	0,04597701	0	0	0	0	0,10344828	0,04545455	0	0	0	0	0	0	0	0	0	0	0	0	0
0,05454545	0	0	0	0	0,03378378	0,03030303	0,00645161	0,140625	0,07608696	0	0	0	0	0	0	0	0	0	0	0,014706	0
0,02542373	0,0994152	0	0	0	0	0,01321586	0,09387755	0	0	0	0,07352941	0,091954	0	0	0	0	0	0	0	0,202128	0
0	0	0	0	0	0	0	0,08888889	0	0,03174603	0	0,06542056	0	0	0	0	0	0	0	0	0,02439	0
0	0	0,00315457	0	0	0	0	0,01754386	0,00497512	0	0	0	0	0	0,007634	0,007143	0	0	0	0,005128	0	0
0	0,00361011	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0,006873	0
0	0	0,05057471	0,03361345	0	0,00641026	0	0,01277955	0,00564972	0	0	0	0	0,016129	0	0	0	0	0	0	0	0,006667
0	0,06206897	0	0	0	0	0	0,01425178	0	0	0	0,01891253	0	0	0	0	0	0	0	0	0	0
0	0	0	0,01960784	0	0,04166667	0,02919708	0,0990991	0	0	0	0	0,022727	0	0,026316	0	0	0	0	0,00885	0	0
0	0,00393701	0	0	0	0	0	0	0	0	0	0,00200803	0,003846	0,034483	0	0,005747	0	0	0,004115	0	0,008333	0
0	0	0,04026846	0	0,00643777	0	0,07710843	0,10501193	0	0,04507513	0	0,04692082	0	0	0	0	0	0	0	0	0,111538	0,031579
0,01075269	0,06859206	0	0	0	0,08888889	0	0,08201058	0	0	0	0,07941176	0	0,013699	0	0	0	0	0	0	0,133333	0
0	0	0,02647658	0,00625	0,00302115	0	0	0,01416431	0,00338983	0	0	0	0	0	0	0	0	0	0,011299	0,007874	0	0
0,00591716	0	0	0	0,00552486	0	0	0	0,00803213	0	0	0	0	0	0	0	0	0	0	0,004202	0	0
0	0	0,00201207	0,02857143	0,00719424	0,00746269	0	0,01355932	0	0,00294985	0	0,00591716	0	0,02381	0	0	0	0	0,010638	0	0,009804	0
0	0	0,03614458	0,0078125	0	0	0	0,01674641	0	0,00925926	0	0	0,007519	0	0	0,004444	0	0	0	0	0	0
0	0,00236407	0	0	0	0	0,00544959	0	0,00306748	0,00223714	0	0	0	0	0,012658	0	0	0	0	0,003484	0	0
0	0	0	0,01204819	0	0	0,02409639	0,05494505	0,07843137	0	0	0	0	0	0	0	0	0	0	0	0,064	0
0	0	0,04157549	0,00617284	0	0	0	0,0125523	0	0	0	0	0,005102	0,057692	0	0	0	0	0	0,004032	0	0
0	0,0015456	0,04422604	0	0,00348432	0	0	0,01104972	0,00584795	0,00851064	0	0	0,010638	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0,00304878	0,01036269	0,00558659	0	0,00404858	0,00307692	0,004785	0	0	0	0	0,003436	0	0	0	0
0	0,00634921	0	0	0,00896861	0,01408451	0	0,01010101	0,00961538	0,00845666	0	0,00376648	0	0	0	0	0	0	0	0	0,009868	0,004902
0	0	0,02895323	0	0	0	0	0,01666667	0	0	0	0	0,004367	0,016949	0,010101	0,002747	0	0	0	0	0,003378	0
0	0,0049505	0,02466368	0,01666667	0,01715686	0	0	0,00662252	0	0	0	0	0	0	0	0,003378	0	0	0	0	0	0
0	0	0,00242131	0,00653595	0	0	0,00306748	0,03501946	0,00396825	0	0	0	0,009132	0,018519	0	0,003003	0	0	0,006993	0,003968	0,007605	0
0	0	0,0045977	0	0	0	0	0,00215517	0	0	0	0,00304878	0	0	0,007092	0	0	0	0	0	0	0
0	0	0,03809524	0,00645161	0,00228833	0	0	0,00967118	0,00923077	0,00192678	0	0	0	0,015625	0	0	0,004405	0	0	0	0	0,004854
0	0,00135501	0,02914798	0	0	0	0,00109529	0,02325581	0	0	0,00588235	0	0,003745	0,016129	0	0	0,003831	0,005988	0	0	0,005025	0
0,01123596	0,00181818	0	0,0037037	0	0	0	0,00902527	0	0	0	0,00280899	0	0	0	0	0	0	0	0,003215	0	0
0,00934579	0,00203252	0	0	0,00510204	0	0	0,01313321	0,00591716	0,00338983	0	0,00254453	0	0	0	0	0	0	0	0	0	0
0	0,00271003	0,00368324	0,01724138	0	0	0,00228311	0	0	0	0	0,00283286	0	0	0	0	0,005556	0	0	0	0,003802	0
0	0	0	0,01	0	0	0,00268817	0,00630915	0,00813008	0,00251256	0,00630915	0	0	0	0	0,005525	0	0	0	0	0	0
0	0,0021692	0,02892562	0,01181102	0	0	0,00399202	0,00262467	0	0,00531915	0	0,00687285	0,005464	0,018182	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0,00089606	0,00361011	0	0	0	0	0,012821	0	0	0	0	0	0,002336	0	0
0,00740741	0	0	0,01282051	0,00217391	0	0,00378788	0,0015873	0	0,00152439	0	0	0	0	0	0	0,009174	0	0	0,003448	0,00365	0

rs17306671	rs17842518	rs20320	rs2032595	rs2032599	rs2032602	rs2032624	rs2032631	rs2032636	rs2032652	rs2032658	rs2032673	rs2033003	rs2319818	rs2534636	rs3528497	rs3848982	rs3900	rs3911	rs4141886	rs8179021	rs9341278	rs9786139	rs9786184
0	0	0	0	0	0,008032	0	0	0,040268	0,003861	0	0,004065	0,004673	0,012	0	0	0	0	0	0	0	0	0,003636	0,012739
0	0	0,011111	0	0	0	0,020408	0	0	0	0	0,013605	0	0	0	0	0	0	0	0	0	0	0	0
0	0,064103	0,055556	0	0	0,006897	0,054054	0,010989	0	0,108911	0,024691	0	0,104396	0	0	0	0,063158	0,039683	0	0,053097	0	0	0,142857	0,161074
0	0	0	0	0	0,004292	0	0	0,045872	0	0	0,011321	0	0	0	0	0	0	0	0,008475	0	0	0	0,008368
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0	0	0	0	0	0	0,010989	0	0,028571	0	0	0,004878	0	0,005155	0	0	0	0	0	0,007692	0	0	0,006536	0,014634
0	0	0,005587	0	0	0	0	0	0,055556	0	0	0,010582	0	0,005435	0	0	0	0	0,007353	0	0	0	0	0
0	0	0	0	0,006536	0	0,00885	0,005376	0,063291	0	0	0,011029	0	0,003876	0,005155	0	0	0	0	0	0	0	0	0
0	0,025974	0	0	0	0	0	0	0,068182	0,007246	0	0	0	0	0	0	0	0	0	0	0	0,022727	0,009709	0,014184
0	0,025126	0	0	0	0,011111	0,073684	0	0,012987	0,007299	0,11465	0	0	0	0	0	0	0,007576	0	0	0,008547	0	0	0,064356
0	0	0	0	0	0,014493	0	0	0,03125	0,014706	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0,029412	0	0	0	0	0	0	0	0,045455	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0	0,263736	0	0,026316	0	0	0,26087	0,044444	0,037037	0,112676	0	0	0,189873	0	0	0	0,346939	0,28	0	0,206349	0	0	0,257143	0,191011
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0	0	0	0	0	0	0	0	0,052632	0	0	0	0,060606	0,027027	0	0	0	0,076923	0	0	0	0	0	0
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0,004348	0	0	0	0	0	0	0,005952	0,017857	0	0	0,004717	0	0	0	0	0	0	0	0	0	0	0	0,00295
0	0,011976	0	0,014925	0	0	0	0,012987	0,068966	0,009756	0	0,018182	0,011299	0,004132	0	0	0	0	0	0,036649	0	0	0	0,005618
0,010753	0	0	0	0	0	0,057143	0,028169	0,052632	0	0	0,005376	0,024	0,005291	0	0	0	0	0	0,004202	0	0	0	0,031746
0	0	0	0	0	0	0	0	0,037037	0	0	0	0	0	0	0	0	0	0	0,093023	0	0	0	0
0	0,002392	0	0	0	0,003215	0	0	0,014493	0	0	0,006969	0	0	0	0	0	0	0	0	0	0	0	0
0,1875	0	0	0	0	0	0,1	0,117647	0,034884	0	0,129032	0,025641	0,148148	0,005435	0	0	0	0,057143	0	0,019608	0	0	0	0,158088
0,059701	0	0	0,025974	0	0,006494	0,037736	0,151899	0,037975	0,005376	0,124088	0,006494	0,150327	0,004065	0	0	0	0,140449	0,011494	0	0	0	0,012766	0,107143
0	0	0	0	0	0,005988	0	0,024194	0,038462	0	0,005917	0	0	0,004348	0	0	0	0	0	0	0	0	0	0,004115
0	0	0,005618	0	0	0	0	0	0,035714	0	0	0	0	0,015464	0	0	0	0	0	0	0	0	0	0
0	0	0,006024	0,014085	0	0	0	0	0,045455	0	0	0,009709	0,026738	0	0,007042	0	0	0	0	0	0	0	0	0
0,00565	0	0	0	0	0	0	0,017751	0	0	0	0,010811	0	0	0	0	0	0	0,016529	0	0	0	0	0
0,005291	0	0	0,009524	0	0	0	0	0,025641	0	0	0	0,003953	0,002865	0	0	0	0	0	0	0,00641	0	0	0,003636
0,125	0	0	0	0	0	0	0,168831	0,05	0	0	0	0,053333	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0,006849	0,025641	0	0,004566	0,003788	0	0,009174	0,004854	0	0	0,004132	0	0	0	0	0	0
0	0	0	0,00885	0	0	0	0	0,043478	0	0	0,002899	0	0,00463	0,005128	0	0	0	0	0	0	0	0	0
0	0	0	0,007813	0	0	0	0	0,066116	0	0	0,021505	0	0	0	0	0	0	0	0	0	0,006849	0,005405	0,003344
0,005348	0,00361	0	0	0	0,004673	0,013514	0,00565	0,097561	0	0	0,010989	0,011834	0,005495	0	0	0,004237	0	0,005263	0	0	0	0	0,002959
0	0	0	0	0	0	0	0,005714	0,049645	0	0,004098	0,002571	0	0,00641	0	0	0	0,003497	0	0	0	0	0	0,005348
0	0	0	0	0,020408	0	0	0	0,017857	0,003731	0	0	0	0,006993	0	0	0	0,009259	0,024194	0	0	0	0	0
0	0	0,004016	0	0	0,003953	0	0	0,030675	0,004425	0	0	0,007634	0,005263	0	0	0	0	0	0	0	0	0,008696	0
0,004329	0	0	0	0	0,004	0	0,011364	0,017544	0	0	0,008621	0	0,003731	0	0	0	0	0,004808	0	0	0	0	0
0	0,034653	0	0	0	0	0	0,005155	0	0,015974	0,005405	0,012862	0	0	0	0	0	0	0	0	0	0	0,013953	0,005391
0	0	0	0	0	0,004598	0	0,003425	0,057971	0	0	0,007109	0,002717	0,002304	0	0	0	0	0	0	0	0,005155	0	0,00565
0	0,009217	0	0	0	0	0	0	0,019608	0,01087	0	0,009804	0,003546	0,003077	0	0	0,003636	0	0	0,010638	0	0	0	0,021672
0	0	0	0,006944	0	0	0	0	0,042735	0	0	0,010239	0,003086	0	0	0	0	0,002674	0	0	0	0	0	0
0	0	0	0	0	0,004032	0	0	0,063492	0	0	0,013245	0	0,004016	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0,023256	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0,012048
0	0,009662	0	0	0	0,003759	0	0,005714	0,065789	0,015936	0	0,003831	0	0,003802	0,004651	0	0	0	0,004525	0	0	0	0	0,00639
0	0	0	0	0	0,00339	0	0	0,020408	0,002232	0	0	0,00365	0,022514	0	0	0,001086	0	0	0	0	0	0	0,001168
0	0	0	0,011494	0	0	0	0	0	0	0	0,020725	0	0,004975	0	0	0	0	0	0	0	0	0	0

Supplementary Table S8. Quality control scores for all genotyping reads of 132 SNP markers included in HID Ion Ampliseq Identity Panel, in Brazilian criminal samples.

Samples	**Locus**																	
Blood	*rs1005533*	*rs10092491*	*rs1015250*	*rs1024116*	*rs1028528*	*rs1031825*	*rs10488710*	*rs10495407*	*rs1058083*	*rs10773760*	*rs10776839*	*rs1109037*	*rs12997453*	*rs13218440*	*rs1335873*	*rs1355366*	*rs1357617*	*rs1360288*
BL.PF07	99	99	99	99	99	97	99	99	99	99	46	99	99	99	99	99	99	99
BL.PF09	99	99	69	99	99	99	99	99	99	99	99	99	99	99	99	99	99	99
BL.PF11	99	99	89	99	99	99	99	99	99	99	99	99	90	99	99	99	99	99
BL.PF20	99	99	58	99	99	99	99	99	99	99	99	99	65	99	99	99	99	99
BL.PF22	99	99	99	99	99	99	99	99	99	99	99	99	99	99	99	99	99	99
BL.PF24	99	99	50	99	99	99	84	99	99	99	60	99	55	99	99	99	99	99
BL.PF25	99	99	99	99	99	90	84	99	99	99	99	78	63	99	99	99	99	99
BL.PF26	99	99	46	99	99	99	99	99	99	99	99	99	99	99	99	99	99	99
BL.PF27	99	99	89	99	99	97	99	99	99	99	99	99	76	99	99	99	99	99
BL.PF30	99	99	43	99	99	99	99	99	99	99	99	99	39	99	99	99	99	99
Epithelial																		
EP.PF01	99	99	33	99	99	99	59	17	99	99	3	99	61	0	99	31	57	49
EP.PF08	49	0	5	51	99	99	99	99	60	99	99	99	99	28	99	99	64	60
EP.PF10	99	99	99	99	99	99	99	99	99	99	99	99	99	99	99	99	94	99
EP.PF13	99	99	99	99	99	99	75	99	99	99	99	99	66	99	99	99	99	99
EP.PF14	99	99	27	78	82	36	48	50	73	73	99	37	25	71	99	86	42	57
EP.PF15	99	99	99	99	99	77	53	99	99	95	99	99	33	99	45	99	99	99
EP.PF17	86	17	0	68	10	18	15	6	29	68	17	16	24	27	54	38	28	99
EP.PF18	99	74	30	76	99	60	99	79	99	99	24	99	99	99	99	71	47	0
EP.PF21	62	99	99	99	90	12	99	8	29	99	99	99	72	22	25	22	99	99
EP.PF28	99	99	59	99	99	99	99	99	99	99	38	99	51	99	99	99	99	99
Saliva																		
SA.PF02	99	49	32	99	28	94	53	9	27	10	99	0	10	52	29	84	99	32
SA.PF03	99	29	63	26	99	15	16	13	57	99	0	27	51	99	17	99	28	30
SA.PF04	99	99	99	99	99	79	99	99	99	99	36	99	99	99	99	99	99	99
SA.PF05	76	99	99	99	99	99	99	99	99	99	99	99	30	99	99	99	70	99
SA.PF06	99	99	99	99	99	97	99	99	99	99	99	99	41	99	99	99	99	99
SA.PF12	99	99	29	99	99	99	73	99	99	99	99	99	19	99	99	93	98	99
SA.PF16	99	99	99	99	99	99	98	99	99	99	89	99	99	99	99	99	99	99
SA.PF19	99	99	55	99	99	99	99	99	99	99	99	99	99	99	99	99	99	99
SA.PF23	99	99	99	99	99	99	99	99	99	99	99	99	30	99	99	99	99	99
SA.PF29	99	67	31	79	99	99	72	76	99	99	41	54	35	99	99	91	20	99
Semen																		
SE.PF02	99	99	99	99	99	99	99	99	99	99	99	99	45	99	99	99	83	99
SE.PF03	99	99	99	99	99	99	99	99	99	99	42	99	99	99	99	97	99	99
SE.PF07	99	99	62	99	99	99	99	99	99	99	99	99	61	99	99	99	99	99
SE.PF22	16	4	31	18	99	4	5	45	99	99	9	64	0	99	99	0	17	99
SE.PF26	48	99	19	99	99	42	99	99	0	99	99	0	6	99	48	99	5	45
SE.PF27	81	99	38	99	99	99	99	83	99	95	32	67	27	99	99	68	54	99
SE.PF30	99	99	43	99	99	99	99	99	99	99	99	99	99	99	99	99	97	99
Control (Oral Swab)																		
CT.PE02	99	99	70	99	99	99	99	99	99	99	53	99	99	99	99	99	99	99
CT.PE03	99	99	99	99	99	99	99	99	99	99	99	99	69	99	99	99	99	99
CT.PE04	99	99	99	99	99	99	99	99	99	99	82	99	99	99	99	99	99	99
CT.PE07	99	99	99	99	99	99	99	99	99	99	99	99	99	99	99	99	99	99
CT.PE08	99	99	99	99	99	99	99	99	99	99	64	99	99	99	99	99	99	99
CT.PE09	99	99	65	99	99	69	99	75	99	99	42	99	99	99	99	99	99	99
CT.PE10	99	99	99	99	99	99	99	99	99	99	70	99	99	99	99	99	99	99
CT.PE11	99	61	33	51	99	6	99	99	52	25	99	99	25	28	99	99	19	99
CT.PE12	0	99	22	99	99	99	0	99	7	99	99	12	3	99	27	6	99	24
CT.PE13	99	99	93	99	99	99	99	99	99	99	94	99	85	99	99	99	99	99

rs1382387	rs1413212	rs1454361	rs1463729	rs1490413	rs1493232	rs1498553	rs1523537	rs1528460	rs159606	rs1736442	rs1821380	rs1872575	rs1886510	rs1979255	rs2016276	rs2040411	rs2046361	rs2056277	rs2076848
99	99	99	99	99	69	99	99	99	99	74	90	99	99	99	99	99	99	99	99
99	99	99	99	99	89	99	99	99	99	84	99	99	99	99	99	99	99	99	99
99	99	99	99	99	99	99	99	99	99	99	99	99	99	99	99	99	99	99	99
99	99	99	99	99	99	99	99	99	99	93	99	99	99	99	99	99	99	99	99
99	99	99	99	99	99	99	99	99	99	99	99	99	99	99	99	99	99	99	99
99	99	99	99	99	99	99	99	99	99	76	99	99	99	99	99	99	99	99	99
99	99	99	99	99	99	99	99	99	99	68	78	99	99	99	99	99	99	99	99
99	99	99	99	99	94	99	99	99	99	69	85	99	99	99	99	99	99	99	99
99	99	99	99	99	99	99	99	99	99	95	99	99	99	99	80	99	99	99	99
99	99	99	99	99	83	99	99	99	99	68	99	99	99	99	99	99	99	99	88
25	75	14	8	99	3	99	42	99	0	39	0	99	47	82	3	58	99	21	86
8	99	91	7	98	15	99	36	75	53	49	0	99	50	99	3	69	99	99	0
99	99	99	99	99	99	99	99	99	99	62	98	99	99	99	99	99	99	99	99
99	99	99	99	99	96	99	99	99	99	85	98	99	99	99	99	99	99	99	99
28	99	99	99	66	36	67	99	36	39	24	28	99	64	66	88	69	64	99	19
99	99	99	47	99	28	72	99	99	59	25	53	99	99	99	99	99	99	99	88
99	37	36	9	18	18	23	18	52	25	14	12	30	29	99	76	19	29	30	52
99	36	99	6	99	40	99	99	99	60	36	85	99	99	7	56	99	99	99	99
99	99	99	3	99	99	0	99	99	99	12	23	99	99	99	39	3	44	38	99
99	99	99	99	99	99	99	99	99	99	52	92	99	99	99	87	99	99	99	99
12	99	99	99	61	99	99	0	89	99	38	25	50	72	93	30	99	99	43	0
49	8	48	23	32	88	18	36	16	19	15	12	99	27	41	32	99	37	99	50
99	99	99	99	99	99	99	99	99	99	34	74	99	96	99	68	99	99	99	78
99	99	99	99	99	41	99	99	79	99	51	69	99	99	99	99	99	99	99	99
99	99	99	99	99	95	99	99	99	99	63	99	99	99	99	99	99	99	99	99
99	99	99	99	99	41	99	99	99	83	59	99	99	99	99	99	99	99	99	69
99	99	99	99	99	99	99	99	99	99	76	33	99	99	99	93	99	99	99	99
99	99	99	99	99	99	99	99	99	99	60	78	99	87	99	99	99	99	99	99
99	99	99	99	99	99	99	99	99	99	55	99	99	99	99	99	99	99	99	99
99	99	99	99	56	99	66	82	59	99	37	82	99	65	99	47	99	99	99	26
99	99	99	99	99	73	99	99	99	99	68	67	99	99	99	99	99	99	99	99
99	99	99	99	99	99	99	99	99	99	40	98	99	99	99	92	99	99	99	99
99	99	99	99	99	99	99	99	99	99	92	99	99	99	99	99	99	99	99	99
99	99	99	22	99	14	99	99	73	9	56	99	99	99	99	54	41	99	99	20
96	99	40	83	99	20	99	99	21	99	23	11	99	99	5	99	61	81	10	17
99	99	99	99	99	99	99	97	81	41	35	49	99	99	99	99	73	99	99	60
99	99	99	99	99	33	99	99	99	99	48	79	99	99	99	79	99	99	99	99
99	99	99	99	99	99	99	99	99	99	57	99	99	99	99	99	99	99	99	99
99	99	99	99	99	99	99	99	99	99	38	96	99	99	99	92	99	99	99	99
99	99	99	99	99	99	99	99	99	99	75	99	99	99	99	99	99	99	99	99
99	99	99	99	99	99	99	99	99	99	69	99	99	99	99	99	99	99	99	97
99	99	99	99	99	99	99	99	99	99	59	99	99	99	99	99	99	99	99	99
99	99	99	99	99	99	98	99	99	99	37	99	99	99	99	99	99	99	99	17
99	99	99	99	99	99	99	99	99	99	64	99	99	99	99	99	99	99	99	99
99	99	99	74	29	25	38	99	99	11	16	83	99	98	99	99	99	98	99	47
99	71	30	8	15	99	99	99	99	99	29	39	51	99	0	27	99	76	99	30
99	99	99	99	99	99	99	99	99	99	99	99	99	99	99	99	99	99	99	99

rs2111980	rs214955	rs221956	rs2269355	rs2292972	rs2342747	rs251934	rs2830795	rs2831700	rs321198	rs338882	rs354439	rs3780962	rs4288409	rs430046	rs4364205	rs445251	rs4530059	rs4847034	rs560681
99	99	99	99	99	61	99	85	99	99	99	99	99	99	94	99	99	99	99	99
99	99	99	99	99	99	99	99	99	99	99	99	99	99	69	99	99	99	99	99
99	99	99	69	99	99	99	99	99	99	99	99	99	99	99	99	99	99	99	99
99	99	99	99	99	99	99	99	99	82	99	99	99	99	95	99	99	99	99	99
99	99	99	99	99	99	99	86	99	97	99	99	99	99	93	99	99	99	99	99
99	99	99	53	99	99	99	72	99	90	99	99	99	99	74	99	99	99	99	99
99	99	99	72	99	99	99	99	99	62	99	99	99	99	65	99	99	99	99	99
99	99	99	69	99	99	99	61	99	97	99	99	99	99	75	99	99	99	99	74
99	99	99	99	99	0	99	99	99	93	99	99	99	99	99	99	99	99	99	99
99	99	99	49	99	99	99	99	99	67	99	99	95	99	56	99	99	99	99	99
24	99	10	26	20	27	99	99	99	37	51	0	0	33	84	22	14	17	65	99
29	99	99	99	16	99	99	31	99	41	99	24	3	44	78	32	99	37	16	99
99	99	99	99	99	99	99	99	99	72	99	99	99	99	75	99	99	99	99	99
99	99	99	75	99	99	99	99	99	77	99	99	99	99	72	99	99	99	99	99
99	17	99	86	99	99	99	61	4	29	93	75	51	99	25	60	92	48	45	85
99	99	84	25	99	99	97	99	99	56	99	99	37	99	32	99	99	99	81	99
38	73	99	0	26	45	52	8	28	12	50	36	36	27	0	24	24	24	44	28
92	24	42	3	0	99	99	31	0	32	0	87	99	99	38	32	99	23	39	99
99	99	10	5	99	0	99	35	99	24	13	99	10	13	15	29	99	26	21	4
99	99	99	99	99	99	99	61	99	65	99	99	99	99	48	99	99	99	99	99
99	99	99	49	99	26	15	35	99	38	31	99	99	99	35	99	99	99	99	99
0	15	16	60	31	99	99	14	19	24	38	37	13	99	10	20	25	26	96	99
99	99	99	54	99	99	99	99	99	55	99	99	99	99	68	97	99	97	99	99
99	99	99	12	99	99	79	99	99	62	99	99	99	99	63	99	60	75	99	99
99	99	99	48	99	99	99	99	99	67	99	99	99	99	63	99	99	99	99	99
99	99	99	23	99	99	99	99	99	50	99	99	99	99	62	99	99	99	99	99
99	99	99	99	99	99	99	55	99	65	99	99	99	99	55	99	99	99	99	99
81	80	99	99	99	72	99	99	99	53	99	99	99	99	51	99	94	77	99	99
99	99	99	99	99	99	99	99	99	72	99	99	99	99	83	99	99	99	99	99
99	99	74	99	99	99	99	28	99	31	99	99	26	99	49	80	99	69	99	99
99	99	99	83	99	99	99	41	99	57	99	99	82	99	54	99	99	99	99	99
99	99	99	34	99	99	99	99	99	62	99	99	99	99	57	99	99	99	77	99
99	99	99	68	99	99	99	96	99	87	99	99	99	99	87	99	99	99	88	99
99	37	99	99	99	30	99	45	99	34	3	24	99	99	74	75	99	94	99	99
50	99	63	46	99	72	99	37	99	32	99	99	3	21	27	3	99	99	16	23
99	99	64	46	99	96	99	25	99	40	99	99	99	99	37	93	99	70	51	99
99	99	99	89	99	99	99	49	99	46	99	99	35	99	46	99	99	97	52	99
99	99	99	52	99	99	99	99	99	93	99	99	99	99	93	99	99	99	99	99
99	99	99	99	99	99	99	99	99	86	99	99	99	99	65	99	99	99	63	99
99	99	99	50	99	99	99	99	99	70	99	99	99	99	85	99	99	99	99	99
99	99	99	60	99	99	99	99	99	87	99	99	99	99	95	99	99	99	99	99
99	99	99	99	99	99	99	99	99	99	99	99	99	99	96	99	99	99	99	99
99	99	99	99	99	93	99	64	99	61	99	99	99	99	79	99	99	99	99	99
99	99	99	99	99	99	99	48	99	71	99	99	99	99	91	99	99	99	99	99
99	30	99	99	91	99	99	99	99	56	99	83	99	99	54	99	99	99	99	99
99	15	42	34	60	22	99	71	0	0	99	99	99	99	22	53	11	36	99	31
99	99	99	99	99	99	99	96	99	99	99	99	99	99	99	99	99	99	99	99

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99	99	99	99	99	99	99	85	99	99	77	99	34	99	99	99	99	99	99	99	99	99	99	78
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96	80	99	84	80	90	80	27	71	75	57	33	31	99	68	78	62	99	80	99	70	60	66	60
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0	17	0	0	9	14	18	0	10	23	15	14	8	10	16	24	18	0	0	22	0	0	0	0
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36	35	33	25	47	49	26	11	24	33	18	9	7	4	18	38	19	39	54	35	19	20	17	19
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28	56	99	60	80	70	53	34	47	88	27	51	12	86	53	83	94	90	70	66	54	57	69	30
84	83	95	69	79	99	69	36	62	99	56	61	29	92	76	99	71	99	74	99	81	53	63	60
61	99	95	59	52	58	99	23	43	64	39	27	19	92	61	97	79	99	72	52	45	35	38	47
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23	52	68	48	23	78	55	18	0	32	24	26	7	50	20	34	35	83	84	9	23	55	30	14
63	85	99	26	50	81	66	37	51	75	33	59	26	80	58	83	69	79	54	81	61	58	65	56
30	78	99	77	48	73	80	40	33	52	29	65	8	72	71	85	78	83	99	76	61	65	51	37
63	99	99	99	97	99	62	49	70	99	51	83	10	99	99	99	94	99	97	99	70	67	72	71
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18	26	40	26	5	52	33	11	0	27	13	13	4	38	20	21	11	49	38	41	25	44	27	13
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36	55	74	59	50	83	65	27	40	77	21	58	30	69	41	79	80	71	59	59	42	45	52	30
53	99	99	99	62	99	99	58	77	99	60	57	40	95	90	99	88	81	99	99	92	92	79	52
75	99	98	99	63	99	96	51	81	97	83	32	37	96	99	99	70	97	80	70	62	99	67	48
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41	97	91	92	76	99	99	50	56	96	25	70	5	99	84	99	75	82	99	79	86	92	81	45
62	99	99	99	59	99	99	51	87	99	55	78	28	99	99	99	86	70	99	99	91	88	75	64
30	86	99	82	47	99	99	35	15	65	25	48	26	99	62	80	72	56	99	40	39	78	41	29
62	99	99	99	59	99	99	42	65	99	66	54	46	93	95	99	59	85	99	99	94	92	73	49
62	29	94	24	59	49	72	29	43	80	7	33	27	61	0	77	80	85	85	59	53	91	52	43
18	28	50	22	22	51	26	0	0	23	10	20	6	37	20	25	24	35	24	22	20	0	36	20
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Median
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Supplementary Table S9. Summary of run performance obtained for the evaluated chip.

Run designation	*Chip model*	*Samples per run*	*Loading (%)*	*Usable reads (%)*	*Aligned reads*	*Average mapped reads per sample (sd)*	*Mean depth per locus per sample (sd)*
Anc	318v2	80	78	61	5,118,798	66,477 (66,851)	415x (470x)

Supplementary Table S10. Total number of reads and mean read lengths (in base pairs) for all samples.

Samples		Read Values	
Name	*Type*	*Reads*	*Mean read lenght (bp)*
BL.PF07	Blood	47495	102
BL.PF09	Blood	25468	100
BL.PF11	Blood	25616	100
BL.PF20	Blood	36572	98
BL.PF22	Blood	28873	96
BL.PF24	Blood	27131	86
BL.PF25	Blood	34379	99
BL.PF26	Blood	33395	95
BL.PF27	Blood	48048	98
BL.PF30	Blood	22810	96
EP.PF01	Epithelial	36960	95
EP.PF08	Epithelial	14599	94
EP.PF10	Epithelial	6441	98
EP.PF13	Epithelial	17437	98
EP.PF14	Epithelial	24529	94
EP.PF15	Epithelial	32446	117
EP.PF17	Epithelial	29138	95
EP.PF18	Epithelial	40514	95
EP.PF21	Epithelial	33628	93
EP.PF28	Epithelial	32508	86
SA.PF02	Saliva	14420	92
SA.PF03	Saliva	60948	96
SA.PF04	Saliva	35671	86
SA.PF05	Saliva	37785	91
SA.PF06	Saliva	41981	97
SA.PF12	Saliva	38715	99
SA.PF16	Saliva	36267	92
SA.PF19	Saliva	39690	100
SA.PF23	Saliva	46600	92
SA.PF29	Saliva	17445	92
SE.PF02	Semen	54660	102
SE.PF03	Semen	41399	97
SE.PF07	Semen	48663	98
SE.PF22	Semen	44496	93
SE.PF26	Semen	51511	97
SE.PF27	Semen	38259	88
SE.PF30	Semen	44013	95
CT.PF02	Control (Oral Swab)	46185	99
CT.PF03	Control (Oral Swab)	53531	99
CT.PF04	Control (Oral Swab)	79494	100
CT.PF07	Control (Oral Swab)	55736	101
CT.PF08	Control (Oral Swab)	62417	101
CT.PF09	Control (Oral Swab)	57739	102
CT.PF10	Control (Oral Swab)	48060	108
CT.PF11	Control (Oral Swab)	47300	102
CT.PF12	Control (Oral Swab)	69000	98
CT.PF13	Control (Oral Swab)	44502	96
Average	-	**39456.89**	**96.77**

- : Does not apply

CAPÍTULO 4 - Full mtDNA genome sequencing of Brazilian admixed populations: a forensic-focused evaluation of a MPS application as an alternative to Sanger sequencing methods

Artigo publicado no periódico Forensic Science International: Genetics

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Research paper

Full mtDNA genome sequencing of Brazilian admixed populations: A forensic-focused evaluation of a MPS application as an alternative to Sanger sequencing methods

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ABSTRACT

The use of Massive Parallel Sequencing (MPS) techniques have been proposed by the forensic community as an alternative to Sanger sequencing methods in routine forensic casework analysis regarding mitochondrial DNA (mtDNA). Interesting features of MPS include high throughput, ability to simultaneously genotype a significant number of samples by barcoding techniques, processing automation, reduced time and costs, among others. Advantages include the capability of generating full mtDNA genome sequences versus usual techniques, usually limited to hypervariable or control regions exclusively. In this work, 96 reference single-source samples from three different Brazilian cities were subjected to full mtDNA genome sequencing by MPS techniques using an early-access version of Precision ID mtDNA Whole Genome Panel on an Ion Torrent PGM platform (Thermo Fisher Scientific, Waltham, MA, USA). Complete, high-quality sequences were obtained and sequencing performance was evaluated via four different metrics. As a subset of evaluated samples have been previously submitted for Sanger sequencing of the control region, a comparative analysis of both methods' results was conducted in order to compare technique adequacy within a forensic context. Even though this study is one of the first to report full mtDNA genome sequences for Brazilian admixed populations, the observed haplotypes exhibit a predominance of Native American and African maternal lineages in the studied sample set, reproducing results described in the literature for control regions only. Interpopulation analysis among Brazilian and 26 worldwide populations was also carried out. The results indicate that MPS-generated full mtDNA genome sequences may have great utility in forensic real casework applications, with a pronounced gain of genetic information and discrimination power provided by coding region evaluation and the enhanced capacity of heteroplasmies determination. Database construction and other relevant factors concerning implementation of such techniques in Brazilian forensic laboratories are also discussed.

1. Introduction

The investigative use of mitochondrial DNA (mtDNA) genome sequencing has been widely employed worldwide in forensic laboratories, especially in cases involving biological evidence presenting critical conditions, including extremely low quantities, high degradation levels or ancient DNA, where short tandem repeat (STR) analysis might have limited efficiency [1]. Owing to the fact that human cells have multiple mtDNA genome copies, generally surpassing nuclear DNA quantities by a factor of thousands, evaluation of maternal lineage markers is sometimes the only possible technical approach to obtain genetic information from biological evidence [2]. However, limitations to this method include a lower individualization power compared to autosomal markers, taking into consideration individuals from a single maternal ancestry line may share a unique DNA haplotype. The usual analytical approach to forensic mtDNA analysis consists of Sanger sequencing (SS) capillary electrophoresis (CE) applications, aimed at identifying nucleotide polymorphism events in any of the three highly

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variable regions comprising most nucleotide polymorphisms, located inside a non-coding portion of the mitochondrial genome close to the replication origin, called the control region (CR) [3]. Hyper-variable regions I and II (HV-I and HV-II, respectively) encompass most of the individual variation among samples, even those belonging to the same haplotype, and as such are considered the most informative region of the mtDNA genome [4]. However, its complete sequence comprises over 16k base pairs, and ˜75% of total reported polymorphic variants are observed in nucleotide positions located in the coding region, usually not included in forensic mtDNA examination based on limitations inherent to SS applications [5,6].

Massive Parallel Sequencing (MPS) applications have been proposed in order to circumvent SS and CE technical restrictions in mtDNA full-genome analysis [7]. As a consequence of its high-throughput capacity and workflow automation solutions, this technique is able to provide simultaneous sequencing of a large number of samples through barcoding use with consequent reduced analysis time and significant gains in efficiency and the amount of information obtained from a criminal sample [7]. Efficient MPS of full mtDNA genomes has been achieved with various experimental designs [8–14]. However, it has been put forth that panel designs for forensic applications should account for the possibility of high degradation levels presented by certain forensic samples [15]. Therefore, among proposed solutions is the use of a large number of smaller amplicons, an analytical strategy which should provide efficient amplification of degraded DNA. The Precision ID mtDNA Whole Genome Panel (Thermo Fisher Scientific, Waltham, MA, USA) commercial solution offers simultaneous amplification of 162 small, overlapping amplicons comprising a full mtDNA genome. It was designed to generate small-sized amplicons and successfully allow processing of highly degraded, low-input and other challenging forensic samples. Validation studies show that robust, sensitive, efficient and reliable typing of forensic samples can be accomplished with different versions of this product, allowing its use in forensic individual identification or parentage testing [5,15–17].

The modern Brazilian population features a high degree of genetic admixture, products of recurrent miscegenation processes occurring throughout national history [18,19]. European (EUR) settler (from the Iberian Peninsula mainly) influx has occurred since the XVI century, with a notable gender bias toward masculine contributions. Such migrant men have interbred with local Native American (NA) populations, especially during the initial colonization phases. Later in Brazilian history, the slavery work force (then widely accepted in Portuguese society) adoption motivated forced transport of large contingents of African (AFR) individuals, with special relevance of Western African Portuguese colonies, settlements or commercial outposts (mostly located in Western Africa, with some minor Eastern Africa participation). Late migratory processes took place in the 19th and 20th centuries, as governmental policies for territory occupation and national economic growth promoted arrival of significant amounts of Asian (essentially from Japan and Mid-Western countries, as Lebanon and Syria, part of the then-existing Ottoman Empire) and Central and Southern European (predominantly Germans, Italians, Portuguese and Spaniards) individuals. Such diverse biogeographical groups shaped the current Brazilian population, forging a genetic and culturally diverse, highly admixed and multiethnic country [20].

The significant Brazilian territorial extension was consolidated through several distinct inland migratory flows, with expansion from coastal areas to continental interior direction following distinct patterns in spatial or temporal territorial occupancy. Multidirectional introgression was guided by social and historical conditions, which significantly varied for each distinct geographical region [20]. As a result of heterogeneous migratory flow dynamics, ethnic composition of regional or local groups may display distinct proportions of parental populations contribution (NA, EUR and AFR) among geopolitical regions. [21]. This phenomenon can be noticed from a genetic standpoint, but is also evidenced through contrasting individual self-declared ethnic or ancestry ratios among regional groups, with a relatively higher NA and AFR influence in Northern and Northeastern Brazil, respectively, while Southern Brazil exhibits a prevalent EUR ancestry [22]. Parental lineage markers clearly display a more markedly asymmetrical distribution of ancestral populations contribution to the current genetic pool [23–25]. Historical and cultural factors motivating admixture mechanisms in Brazil yielded current overall preponderance of the EUR Y-chromosome paternal contribution in contrast to predominance of AFR and NA mitochondrial DNA (mtDNA) maternal lineages [23–25], even when regional idiosyncrasies are considered.

In this work, an evaluation of Precision ID mtDNA Whole Genome Panel sequencing efficiency in 96 Brazilian samples was put forth. This commercially available product was designed for forensic (among other) applications, and the present analysis discusses not only technical considerations (including sequence performance) related to the product, but also some aspects involving convenience in this commercial solution adoption by Brazilian forensic laboratories. A comparative analysis with the current widely used mtDNA technique in Brazil (SS followed by CE of mitochondrial HVRs or CR) is also presented. The current work has a strict focus on single-source reference samples for the Brazilian population. Adoption of proposed analytical alternatives in current forensic casework also demands protocol validation with real evidence obtained from crime scenes. Several studies [5,15–17,26] have already evaluated present commercial solution (as well as posterior versions of the same product) efficiency in mock or real forensic cases with remarkable success. Some particular issues intrinsically linked to the very nature of biological evidence in criminal DNA analysis have also been addressed by several research groups regarding mtDNA evaluation, as mixture occurrence or samples presenting highly degraded DNA or DNA template low-copy numbers [5,15–17,26]. The reported results suggest this panel is a viable alternative for forensic mtDNA full-genome sequencing, with reliability and efficiency in accordance with forensic quality standards.

2. Materials and methods

2.1. Ethical statement

DNA samples included in the present study were obtained from voluntary adults following written informed consent. All selected subjects are part of a research-purposed human samples biobank available in PUC/RS comprised of Brazilian samples. This work was approved by the Pontifical Catholic University of Rio Grande do Sul Institutional Review Board under CAAE 52113715.9.0000.5336 number, and follows the ethical principles stated in the Helsinki Declaration [27] of the World Medical Association.

2.2. Samples description

Oral swabs or peripheral blood were obtained from 96 unrelated volunteer donors from three different Brazilian cities located in distinct geopolitical regions. Subjects from Porto Alegre (Southern Brazil, n = 9), Rio de Janeiro (Southeastern Brazil, n = 3) and Distrito Federal (Center-Western Brazil, n = 84) were evaluated. Sample size for individuals from Distrito Federal was chosen to be larger than other regions based on specific features presented by this population [22]. Besides, this particular sample set from Distrito Federal was thoroughly investigated for a significant number of genetic markers [23,28–30], including a series of forensically relevant polymorphisms, and therefore can be used for a comprehensive characterization of Brazilian genetic complexion. Aiming to contrast sequencing performance and forensic-focused data analysis between gold standard techniques (Sanger method) and MPS results, our sample set includes 65 individuals from Distrito Federal (of a total of 84) who had their mtDNA genome CR sequences described in a precedent report [28].

2.3. DNA extraction and quantitation

Total DNA was extracted using the automated platform Automate Express Forensic DNA Extraction System (Thermo Fisher Scientific Inc.) with the Prepfiler Automated Forensic DNA Extraction System (Thermo Fisher Scientific Inc.), or manually via the DNA IQ System (Promega Corp., Madison, WI, USA). Autosomal DNA was quantified with the Quantifiler Human DNA Quantification Kit (Thermo Fisher Scientific Inc.) on a 7500 Real-Time PCR System (Thermo Fisher Scientific Inc.). MtDNA quantities were estimated using a 1:100 genomic to mitochondrial DNA copies ratio according to the product manual. All procedures followed the manufacturer´s instructions.

2.4. Library preparation, emulsion PCR, and sequencing

DNA libraries were constructed using the Ion AmpliSeq Library Kit 2.0 (Thermo Fisher Scientific Inc.) combined with an early-access version of Precision ID mtDNA Whole Genome Panel (Thermo Fisher Scientific Inc.) v.2.2 following the manufacturer´s suggested protocol (Early Access Ampliseq Mitochondrial Sequencing User Manual, May 2015 Revision), applying the suggested Conservative Library Preparation Method. Briefly, PCR amplification was performed in two different reactions per sample, each one using 5 µL of Ion Precision primer pool A or B (Mitochondrial Sequencing Panel) at a final volume of 10 µL containing 1 ng of template DNA and 2 µL of 5x Ion AmpliSeq HiFi Mix. Total reaction volumes were adjusted with nuclease-free water. PCR was performed in a Veriti 96-well Thermal Cycler (Thermo Fisher Scientific Inc.) under the following conditions: enzyme activation for 2 min at 99 °C, 21 cycles of 15 s at 99 °C and 4 min at 60 °C followed by a 10 °C hold. At this point, both amplified mitochondrial PCR reactions for each sample were combined in a single 20 µL pool, where PCR amplicons were partially digested with 2 µL FuPa reagent following incubation at 50 °C for 10 min, 55 °C for 10 min and 60 °C for 20 min, all followed by a 1 h hold at 10 °C. Adaptor ligation to the libraries was executed according to the manufacturer´s instructions using different barcodes for each sample in a single run (Ion Xpress Barcode Adaptors 1–96 Kit or IonCode Barcode Adapters 1–384 Kit) (Thermo Fisher Scientific Inc.), and the resultant products were purified manually with Agencourt AMPure XP reagents (Beckman Coulter Inc., Brea, CA, USA) along with a DynaMag 96-Side Magnetic Rack according to the manufacturer's protocol. After purification, libraries were quantified employing a 7500 Real-Time PCR System (Thermo Fisher Scientific Inc.) with the Ion Library TaqMan™ Quantitation Kit (Thermo Fisher Scientific Inc.) as described in product manual. Libraries were pooled to a final concentration of 20 pM. Emulsion PCR (emPCR) was carried out on the Ion OneTouch 2 instrument (Thermo Fisher Scientific Inc.) with the Ion PGM Hi-Q Template Kit (Thermo Fisher Scientific Inc.) following the manufacturer's protocol (Ion PGM Hi-Q Template Kit, Revision A.0). The emPCR products were enriched on the Ion OneTouch Enrichment System (Thermo Fisher Scientific Inc.). A final volume of 30 µl was loaded per chip. Sequencing was performed on the Ion Torrent PGM™ (Thermo Fisher Scientific Inc.) Sequencer with the Ion PGM Hi-Q Sequencing Kit (Thermo Fisher Scientific Inc.) following the manufacturer´s instructions (Ion PGM Hi-Q Sequencing Kit, Revision B.0) and using Ion 318 Chip Kit v2 (Thermo Fisher Scientific Inc.). Positive and negative controls were evaluated in this study with the results also utilized for internal validation of reagents. A total of three chips were used in distinct runs for complete sample-set genotyping.

2.5. Data analysis and mtDNA haplogrouping

Sequencing data were analyzed using the Torrent Suite Software v5.0 (Thermo Fisher). A modified version of the Revised Cambridge reference sequence (rCRS + 80, based on Genbank NC_012920.1) [31], including repetition of the first 80 nucleotides at the mtDNA sequence end, was employed as reference genome data for alignment. The number of mapped reads was calculated by the Torrent Coverage Analysis v5.0 plugin (Thermo Fisher Scientific Inc.). Individual differences from the reference sequences were defined as called by Ion Torrent Variant Caller v5.0.0.7 plugin with the target regions file (rCRS_plus_80nts_mt_targets) and no designated hotspot regions file under default analysis settings. Minimum read number was set for six reads per base position. As the plugins used did not follow current nomenclature adopted by the forensic community [32–34], corrections were applied to variant reports, including revisions of the proposed alignment in identified polymorphisms (where differences from the reference sequence were aligned as close to the 3´ end of DNA as possible, rather than the usually reported 5´-end placement obtained with the employed plugins). MtDNA sequences were independently reviewed by two different collaborators through BAM file visualization with IGV (Integrative Genomes Viewer v2.3.80) software [35] and manual corrections were applied. mtDNA haplogroups were determined using Haplogrep 2 v2.1.13 [36] according to mtDNA phylotree Build 17 (available online at http://www.phylotree.org/) [37] and confirmed through EMPOP database v4/R11 [38]. The identified length heteroplasmies were not reported. Point heteroplasmy threshold was set to 15%, and variants above this number were reported in the final results. Sequence quality control was performed by EMPOP tools [38], and the generated data was submitted to the EMPOP database as rCRS variations, deposited under reference number EMP00750.

2.6. Sequencing performance and statistical data analysis

Precision ID mtDNA Whole Genome Panel sequencing performance was evaluated with four different statistical parameters: Read Depth (RD), equivalent to obtained number of reads per amplicon; Amplicon Balance (AB), which assesses individual amplification efficiency and is calculated as the number of reads for each amplicon, divided by the mean RD of all amplicons, per sample; Strand Balance (SB) that measures the balance between forward and reverse reads and was calculated as follows: most prevalent strand read number (forward or reverse) was divided by total read number for allele reads only. Expected values indicating no strand bias was equal to 0.5. The results are presented as deviations from this ideal value. Noise Level (NL) represents the amount of unspecific base call, calculated as the ratio of non-allele calls at a nucleotide position divided by total number of reads at that same nucleotide position. SB and NL were evaluated for reported variants only. Statistical evaluation of read-depth differences among distinct amplicons or primer pools was executed using Microsoft Excel 2016 (Microsoft Corporation, Redmond, USA) or R statistical packages. Additional information on the employed formulae is provided in Supplementary Material M1.

2.7. Interpopulation analysis

Haplotype frequencies, molecular diversity indices and other relevant population parameters were calculated for the sample set in question using Arlequin v3.5.2.2 [39] software. The same program was made use of to perform Analysis of Molecular Variance (AMOVA) and pairwise F_{ST} testing among Brazilian population and 26 worldwide populations. Populations employed in the present study were obtained from the 1000 Genomes Project (1kGP - Phase 3; available online at http://www.internationalgenome.org/data/) as VCF variant files [40]. Samples with previously described parental or fraternal relations sharing a unique haplotype had only one individual from each familial group included in this study. Variants were converted to full mitosequence FASTA files using Haplogrep 2 online software, and realigned against the Brazilian sample set using MAFFT v7 multiple sequence online alignment tool (available online at https://mafft.cbrc.jp/alignment/server/) under standard parameters [41]. Multidimensional Scaling Analysis (MDS) based on pairwise F_{ST} distances was executed using IBM SPSS Statistics Software v.17.0 (IBM Analytics

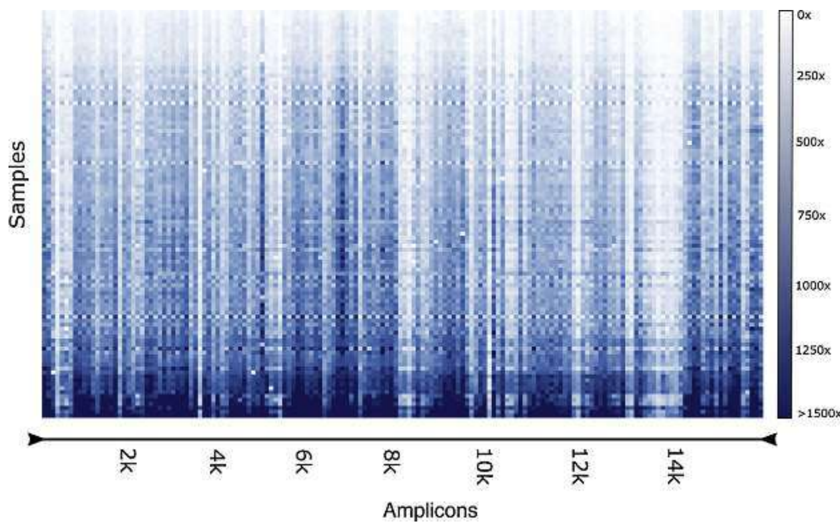

Fig. 1. Read Depth heat map of 162 amplicons included in Precision ID mtDNA Whole Genome Panel for 96 Brazilian samples. Samples were arranged (top to bottom) according to mean number of reads for all amplicons, in ascending order. Amplicons are distributed (left to right) in same order as mtDNA genome covered region (as displayed in Supplementary Table S2).

Inc., Chicago, USA). Populations used in this study, their metadata and employed abbreviations are detailed in Supplementary Table S1. Pairwise F_{ST} distances using Brazilian and worldwide data were also utilized to build cladogram graphics using the Molecular Evolutionary Genetics Analysis v7.0 (MEGA v.7.0) software [42], applying Neighbor-Joining (N-J) methods.

3. Results

The Precision ID mtDNA Whole Genome Panel was successfully used to generate full genome sequences for 96 Brazilian samples from three different cities located in distinct geopolitical regions. This commercial kit comprises 162 primer pairs amplified in two distinct multiplex reactions (A and B pools), generating a series of short, overlapping mtDNA amplicons covering the full mitochondrial genome. Supplementary Table S2 details mtDNA regions associated with each amplicon. Such a sequencing strategy has been demonstrated to be useful for successful amplification of ancient, highly degraded, mixed and other challenging forensic samples [5,15–17,26]. Therefore, the present kit was designed to properly handle critical or difficult forensic samples, although such an approach may be fraught with setbacks particularly related to nonspecific amplification and detection of nuclear copies of mtDNA sequences (also known as numts) [6,15,43,44]. The obtained results show an average amplicon size for all amplicons equivalent to 105 bp. Brazilian population haplotype distribution and frequencies, as well as other relevant descriptive indices connected with forensic applications of mtDNA analysis and sequencing performance, were investigated in the present study in order to assess distribution of mtDNA haplotype frequencies for the Brazilian population and evaluate the adequacy of the present panel introduction in Brazilian routine forensic casework.

3.1. MPS mtDNA sequencing performance

In order to properly assess sequencing efficiency using Precision ID mtDNA Whole Genome Panel, a series of analytical metrics were employed as described in the Material and Methods section. All samples in the present study were genotyped through Ion PGM Sequencer, employing native data interpretation solutions provided by the manufacturer. The Variant Caller plugin was the main pipeline for data calling. Additionally, the Coverage Analysis plugin was utilized to access or calculate sequencing performance metrics for all genotyping procedures. As the maximum number of samples suggested by the manufacturer in a single run is smaller than total investigated sample size, all 96 samples were analyzed in three independent runs along with other samples not included in this study, totaling no more than 48 samples per chip. Data regarding each individual experiment is presented in Supplementary Table S3. The best results were observed for chip mt3, where the slightly smaller number of samples included in the run and superior number of usable reads resulted in improved overall mean depth per sample. The number of usable reads obtained in first two experiments was severely affected by an unusually high polyclonal ISPs ratio, which was later corrected during the third run's execution. Performance improvements were achieved by using freshly opened reagents in library purification and ISP enrichment steps. In order to assess technique reproducibility, two specific samples were included in all three runs, and the resulting sequences showed no differences in sequence obtained for these samples across all three distinct experiments. In a similar manner, the employed positive controls also displayed complete concordance for all three distinct runs with reference sequences. Considering overall sequencing performance, features presented by control samples were found to be similar to other Brazilian samples, including read depth and other relevant elements.

3.1.1. Amplicon-based metrics evaluation

For the adopted protocol, inclusion of 48 samples in a single run using 318v2 model chips is proposed to ensure a mean depth of at least 400x per locus. Despite that, mean depth per sample ranged from 40x to 2,587x, with an average value of 430x (s.d. 392x), which can be considered satisfactory for reliable genotyping of single-source reference samples, where the proposed depth of as low as 10 reads might be considered sufficient for genotype determination [17]. Similar mean depth results were obtained with the same commercial kit for heat degraded samples [5], suggesting reliable use of real forensic casework evidence might be possible. Still, regarding RD metrics, when mean read depth is evaluated per amplicon instead of on a per sample basis, the observed values vary from 91x to 1032x, with average values of 430x (s.d. 185x). Supplementary Figure S1 depicts the mean read depth results obtained for each individual amplicon. This significant variance in amplification performance for different primer pairs has been previously reported, and was demonstrated to be sequence-dependent, where high CG content or homopolymeric regions performed comparatively worse than other regions [5]. Fig. 1 illustrates a heat map graphical representation of the complete sample set's (96 individuals) number of reads for each evaluated amplicon. Differences in RD values can be easily observed among distinct samples (depicted with horizontal lines), and relative amplification performance of each amplicon (vertically represented) can also be evaluated. Low-performance regions and amplicons, identified with vertical lines featuring lighter color shades, display smaller RD numbers, and detected variation

roughly reproduces results obtained with this reagent by other research groups [5,6,15–17,45]. To further explore these findings, less efficient amplicons were characterized using a previously proposed value of 100x for average read depth as a threshold for low-performing amplicons [5]. In our analysis, only three amplicons were found to have amplification levels under this value: amplicons mt_4, mt_101 and mt_139. All three amplicons were reported as challenging, with the former associated with inconsistencies during base calling across two different MPS systems [6] and the latter two identified as endowed with low amplification efficiency in previous studies [5,46]. It is important to note that some samples present a peculiar checkerboard pattern, with intercalated high and low depth values across the entire mtDNA genome. This feature is explained by discrepancies in PCR efficiency attained by the two different primer pools, A and B, employed, where uneven amplification performance leads to higher RD values for amplicons included in a specific pool when compared to its counterpart. This variation in efficacy between both pools comprising this kit have been previously described [6], and it was even considered to be instrument-dependent (where different equipment models or manufacturers consistently display preferential amplification of pool A or B in detriment of the other. However, even though variations between both primer pool´s quality were observed for certain samples in our results, no evidence of directional favoring of any of such pools was noted (0.46 *p-value* on a two-tailed binomial test). Thus, observed fluctuations in depth numbers may be explained by stochastic factors or inconsistencies in library preparation, as this latter step was carried out manually, considering no automation alternative was available in our laboratory.

Concerning AB values, a simple visual exam of Fig. 1 and Supplementary Fig. 1 allows for the conclusion that a significant variation in PCR efficiency among amplicons is observed. Supplementary Fig. 2 (A and B) presents AB values for all 162 amplicons, divided by primer pair pools included in the kit (A and B, respectively). As shown in Supplementary Fig. 2, AB median values for amplicons included in pool A ranged from 0.18 at mt_7 and 2.6 at mt_135 amplicons. For pool B, this statistic varies from 0.16 at mt_116 amplicon to 1.8 at mt_132. The overall average of AB values for each sample, considering all 162 amplicons, is 1.00. Thirty-one amplicons in total (mt_7, 11, 13, 35, 71, 103, 107, 143 and 161 for pool A and mt_2, 4, 10, 32, 40, 48, 50, 52, 56, 78, 80, 102, 104, 112, 114, 116, 118, 120, 122, 124, 126 and 154) exhibited poor AB performance with values under the 0.50 threshold, meaning the number of reads for these 31 regions are below half the average RD per sample. This low performance issue is minimized by overlapping design of primers included in both pools, where sequences located in both extremities for underachieving amplicons can be partially compensated by flanking amplicons in an alternative primer pool. However, a specific region comprising amplicons mt_102 to mt_104 (bases 10481 to 10775 in rCRS) was found to be particularly challenging as low RD values were obtained for tandem located amplicons. Homopolymeric regions have been proposed as the main cause for lower efficiency in Ion PGM and other platforms [14,45], possibly because of inaccurate flow-call and pH fluctuation detection in proton-based systems. As no significant homopolymeric stretches are found in this particular sequence, other causes might be implicated. Additional factors, such as sample quality and G/C content, have been demonstrated to have effects on base-calling accuracy [46], and some of them might be directly impacting amplification efficiency in this particular region. Therefore, a careful evaluation of suggested base calls and meticulous interpretation of detected variants is particularly important for regions described as problematic or demonstrating lower quality results under this analytic protocol, especially in forensic cases where critical biological evidence is involved, including challenging samples presenting degraded or a low quantity of DNA. Efforts to achieve improvement in primer balance and efficiency might also be considered by manufacturers in updated kit versions in order to obtain products suitable for reliable and trustworthy sequencing of mtDNA in real forensic casework.

3.1.2. Polymorphism-based metrics evaluation

Regarding SB results, overall performance of this panel was robust, with 58% of amplicons (94 total) displaying SB median values under the 0.1 threshold. However, roughly 14.2% (23 total) of primer pairs resulted in amplified fragments where at least one identified variation within such regions exhibits high imbalance, with observed SB median values above 0.4. Supplementary Figure S3A depicts a boxplot representation of results obtained for SB values for all detected variants identified in the evaluated samples organized by amplicon. Not only median values exhibit high heterogeneity among amplicons, but also variance presents a very broad distribution among amplicons (with standard deviation values ranging from 0.011 to mt_133 to 0.242 to mt_79 for amplicons with at least five variations detected for all samples). The impact of amplicons presenting low performance for SB metrics in forensic cases may be significant, especially in distinguishing real occurring variations in overlapped regions amplified by more than a single primer pair. Base call errors have been reported as a consequence of extreme strand imbalance, even though strand bias is suggested to be inherent to DNA sequence properties [5]. Therefore, visual analysis of BAM files for rCRS differences presenting significant strand imbalance is important for evaluating if such observations are real or an artifact originating from panel design. Finally, Supplementary Figure S3B exhibits NL measures distribution for all amplicons containing variants in our sample set. Overall, NL can be considered low with all median results under 5% noise-to-signal ratio. This result is consistent with previous studies [5,6,15–17,45] and consistent with high-quality demands involved in forensic DNA genotyping or sequencing. Only two amplicons (mt_15 and mt_86) presented median NL levels greater than 4%. Noise reads can be resulting from sequencing errors, polymerase errors in PCR, alignment mistakes and contamination by alternative DNA or numts sequences [17]. Analysis of NL not only in these two particular regions, but for most of the observed individual variants with increased base miscalling ratios, indicates that most of this problem is caused by the presence of nearby small or medium-sized homopolymeric regions (presenting three- to six-nucleotide stretches of a unique base) [17]. Even though a proportion of this observation might be caused by real-length heteroplasmic events, it is more likely that inaccurate flow-call or pH fluctuation detection, a feature inherent to Ion PGM system technology, might be responsible, at least partially, for reported NL. Visual inspection of BAM files allowed for identification of a significant number of small sequences (usually less than 40 nucleotides long) inside mtDNA, thereby presenting high noise levels (not depicted in Supplementary Figure S3B as such polymorphisms were not identified as rCRS variants) and usually distributed in several different concentrated areas. Such regions, which were found to be present in the same overall mtDNA locations throughout all evaluated samples, are widely distributed all over the mtDNA genome. Despite no specific assessments being performed to fully characterize these regions, such polymorphisms are probably associated with autosomal numt sequences, which are also amplified along with actual mtDNA as a consequence of a multiple, short, overlapping amplicon-sequencing strategy adopted in this panel. However, our data analysis suggests that these deviant polymorphisms were amplified (when compared to actual mitochondrial sequences) at a rate insufficient to significantly affect automatic base-calling performed by algorithms included in employed plugins. Previous works [6,15,43,44] have posited alternative approaches to verify the presence of numts impact in the present panel's sequencing efficiency as employing alignment of MPS sequences against the full human genome to confirm numts amplification by this kit in nuclear DNA. However, the same reports also indicate that numts co-amplification has a limited impact in rCRS variant identification. Yet, special attention to such regions is necessary when forensic samples are being evaluated, and this considers that common occurrence of DNA mixtures, environmental

contamination, low DNA quantity and/or quality or DNA degradation levels can be mistakenly identified as numts or have its genetic features marked or influenced by this very same numts presence in evaluated samples. The results indicated that sequencing performance of the Precision ID mtDNA Whole Genome Panel was usually favorable, with accuracy, reproducibility and efficiency adequate for forensic applications. Certain problems observed in specific amplicons have, in a general manner, no major influence in proper mtDNA full sequencing and rCRS variant determination. However, questions that must be addressed by the manufacturer include continuous development and revision of plugins along with base-calling algorithms in order to provide reliable, easy-to-use solutions that can be incorporated into analytical resources available in forensic genetics laboratories. Analytical tools and computational resources considering the nature of forensic analysis, including but not limited to uncertain sample quality or conditions, mixture occurrence, automation or need for high throughput and idiosyncrasies related to forensic data reports need to be provided, in order to maximize result reliability and facilitate data interpretation by analysts.

3.2. Genetic diversity and mtDNA haplogroup distribution in a Brazilian sample set

Successful full mtDNA genome sequencing was attained for 96 Brazilian individuals, and our experiment design showed that simultaneous amplification of a significant number of samples can be achieved with barcoding strategies. The capability of concurrent analysis of multiple sample can be a determining factor for technique adoption by forensic laboratories as reports of casework backlog and high-throughput needs are common [47]. Obtained haplotypes and haplogroups for all 96 samples are presented in Supplementary Table S4. When considering all 96 obtained Brazilian full mtDNA genome sequences, 94 different sequences were found, though we observed two distinct sample pairs presenting a shared, unique haplotype (belonging to B2 and L2a1a2c groups). The resulting haplotype diversity for all 96 samples is 0.9996 ± 0.0016, which is shown to be slightly superior than previously reported haplotype diversity for 306 individuals from Distrito Federal [28] for CR sequence only, but significantly less variable (0.9988 ± 0.7471). The number of polymorphic sites for the sample set is 737, with 668 transitions and 50 transversions observed. Mean number of pairwise differences among samples is 57.65 ± 25.12. Table 1 presents coarse haplotype frequency distribution for all Brazilian samples, including haplotype biogeographical origin. Complete refined, low-clade haplotype classification for each sample is reported in Supplementary Table S4.

Table 1
mtDNA coarse haplotype frequencies based on 96 full mtDNA genomes from Brazilian population.

Coarse haplogroup classification	Haplotype Biogeographic Origin	Observed samples	Frequency (%)
A2	Native American	13	13.54
B2	Native American	5	5.21
C1	Native American	14	14.58
D	Native American	5	5.21
H	Indo-European	11	11.47
J1	Indo-European	1	1.04
K	Indo-European	1	1.04
L0	African	2	2.08
L1	African	12	12.5
L2	African	12	12.5
L3	African	12	12.5
L4	African	1	1.04
M5	Asian	1	1.04
U	Indo-European	4	4.17
V9	Indo-European	1	1.04
X2	Indo-European	1	1.04
Total	–	**96**	**100**

Table 1 presents coarse haplogroup frequencies found in 96 unrelated individuals from the Brazilian population. When considering haplogroup frequencies in our sample set, the results obtained in this study reflects the general distribution of maternal lineages in the Brazilian population with a significant preponderance of AFR and NA mitochondrial DNA [25,28,48–55]. A significant gender bias and contribution imbalance has been reported for parental linages in the Brazilian population caused by a peculiar and uneven presence of EUR paternal lineages, along with elevated AFR and NA relative maternal contribution [21,23–25]. The two more frequent coarse haplogroups found in our experiment possess NA origin, specifically C1 (14.58%) followed by A2 (13.54%). In total, samples with NA precedence comprise 38.54% of all Brazilian samples evaluated. Africa was found to be the most frequent biogeographical origin of Brazilian maternal lineages, with 40.62% of the complete sample set. The most common coarse haplogroups of African ancestry were L1, L2 and L3, each with 12 identified samples. This finding agrees with historical events of the African slave trade and forced introduction of individuals from this continent in Brazil during the XVI and XIX centuries [54,55]. The remaining samples belong to groups with presumed biogeographical origin from other continents and worldwide populations. For instance, haplogroup H was the most common haplotype in the Brazilian population (11.47%) not belonging to Native or African lines. This clade is also the most frequent in Europe, especially in the Iberian Peninsula [56], also in agreement with Brazilian territorial occupation dynamics. Other unusual haplogroups with a single observation, as M5 or X2b4a, might reflect recent immigration (from XIX century on) of Asian (mainly Japanese) or Arabic (from then Ottoman Empire regions, like Lebanon and Syria) to Brazil. In this respect, full mtDNA genome sequencing provides an interesting tool to thoroughly analyze samples. As an example, we describe findings for sample RS09 classified in haplogroup J1b2. This lineage has a proposed semite origin with higher frequencies in the Middle East, Indo-Persian and other Arabian populations [57,58]. However, this specific sample presents mutations 6719C and 14927 G, which despite not being yet recognized as diagnostic for a distinct clade, have been closely associated to individuals with a Spanish or Iberian ancestry background [57,59], rather believed to have a European origin. Fernandes and collaborators [60] propose that these mutations can be characteristic of a new J1b2 subclade, called J1b2b. Therefore, despite a proposed Near-East origin for J1b2, this particular sample seems to belong to a new, undefined subclade with European origin that may be associated with populational groups in the Iberian Peninsula and their worldwide dispersal during the Great Navigations Age (from XV-XVI centuries on). This example is presented to illustrate the relevance of phylogenetic knowledge about worldwide mtDNA genome variations, and how biogeographical origin of already characterized or yet unknown subclades might be related to specific mutations, some of which are located in the mitochondrial coding region exclusively. Therefore, full mtDNA sequencing as a forensic tool can supply additional information with special consideration of highly ethnically diversified and admixed populations as Brazilian.

Most studies concentrating on Brazilian mitochondrial lines are limited to HV-I or HV-II sequence analysis, and therefore a more exhaustive analysis of results obtained in this study cannot be performed, especially concerning comparative evaluation of low-level mtDNA clades. However, the approximate frequencies of coarse haplogroup distribution mirrors previously reported results, with major AFR and NA contributions [25,28,48–55]. Supplementary Figure S4 features a complete phylogenetic tree including all samples and variants observed in the present study. Several elements supporting forensic casework analysis are based on consistently accepted phylogenetic data of worldwide mtDNA genome samples, including database construction and quality control procedures [61]. With that in mind, construction of a database of full mtDNA sequences based on local Brazilian populations is very relevant owing to the existence of unknown (or not yet

acknowledged as a unique clade) lineages. A large sample size for database composition is necessary in order to fully represent Brazilian mtDNA genetic diversity and assess any possible population substructure occurrence [62,63].

3.3. Comparative performance of MPS and Sanger sequencing in mtDNA genome haplotype determination and forensic resolution between CR x full mitogenome

A subset of total samples analyzed in the present study comprised of 65 individuals from Distrito Federal has been previously included in a Brazilian mitochondrial CR sequence database [28]. In order to evaluate overall sequencing performance and information gain provided by full sequence analysis, we compared the employed MPS technique results with current methods widely available in Brazilian forensic laboratories (SS). As described earlier, the additional information provided by inclusion of mitochondrial genome coding sequences in contrast to CR probing only was able to provide a higher haplotype diversity even with a significantly smaller sample size utilized in the present study (less than a third of the original total sample number). Even more significant than that, inclusion of extra genetic information was sufficient to discriminate two pairs of samples (DF05/DF84 and DF44/DF53) presenting distinct haplotypes, which were previously classified as sharing the same haplotype (inside each pair) considering CR genetic data exclusively. As the number of randomly selected samples included in this comparative analysis is relatively small (65), addition of mtDNA coding sequences to haplotype comparison was shown to increase relative discrimination capability significantly, therefore justifying its adoption in real forensic casework routine.

Haplotype classification differences observed between Sanger sequencing of CR and full mtDNA MPS were observed for 36 of 65 samples (55.4%). Such differences are expected and can be easily explained by additional information provided by coding region sequencing. As previously reported [64], changes in haplogroup assignment based on addition of polymorphisms located in coding regions, which are essential for haplogroup designation, have been observed. Such changes included not only reassignment to more refined, low-clade haplogroups, but also revisions of clade denomination based on the absence or presence of typical, diagnostic mutations for expected haplogroups.

Regarding sequencing consistency between SS and MPS results, we have identified five inconsistencies between two employed techniques for this sample set, just as presented in Supplementary Table S5. Three of the observed differences were shown to consist of heteroplasmic variations, unidentified by SS and evidenced with MPS techniques. This kind of observation is actually expected as MPS methods have a markedly superior ability to detect heteroplasmies versus traditional SS [43]. The remaining discrepancies refer to two mutations (belonging to a single sample, DF45, at positions 297 and 416) identified by Sanger sequencing and undetected in full mtDNA sequencing. The reasons for such discrepancies are unclear: they were at first thought as possibly related to algorithms used in base-calling as both mutations are located in primer-binding regions of the evaluated panel; thus, results would have been affected during data-processing steps, like primer trimming or other automated procedures. Manual review of data with IGV visualization software, however, did not reveal the presence of both polymorphisms in the DF45 sample as the overlapping strategy used with this panel design allowed for sequence evaluation in these regions by amplification with another primer pair. Therefore, reasons for the two mentioned inconsistencies remain uncertain, since raw data for SS was not available for additional review. However, the careful performed analysis in MPS data for this particular sample suggests that discrepancies are likely a result of misinterpreted results in SS evaluation. As a consequence, total rCRS variations detected inside the CR is equivalent to 1024, and the total disagreement ratio (including unidentified heteroplasmies) was less than 0.5% between both methods. In addition, no inconsistencies were detected for samples evaluated by MPS only and sequenced in duplicate across two distinct runs. This result suggests that MPS-obtained sequences might be as reliable as currently employed techniques in forensic laboratories, providing a highly informative and trustworthy source of genetic data with possible forensic applications, as stated by other research groups [26].

It has been proposed that the actual frequency of heteroplasmic polymorphisms in mtDNA samples is higher than previously estimated as MPS techniques have an increased ability to recognize and identify such occurrences besides Sanger sequencing, especially at lower levels (between 10–20% heteroplasmy thresholds) [8,65]. In this study, all three Sanger-identified point heteroplasmies were also reported by MPS processing. In addition to that, two heteroplasmic polymorphisms were identified with MPS inside CR and not previously reported as such by Sanger analysis. Considering the adopted threshold of 15% and minimal coverage of 40x for HP determination (as proposed by [44]), our analyses identified a total number of 20 HP observations with 15 of them located inside mtDNA CR. It is important to note that certain low-level heteroplasmies might have been undetected in our analysis, since they present low-quality scores in our analysis (as a result of marked strand imbalance, for instance) or were identified as noise (caused by a low number of reads within these particular nucleotides, insufficient for variant identification). Supplementary Table S6 details all identified PHP and relative contributions for each variant.

3.4. Inter-population analysis based on mtDNA haplotype frequencies

A series of analyses were conducted to evaluate the differences and genetic structure among Brazilian mtDNA haplotype frequencies and 26 worldwide populations included in 1kGP. Pairwise F_{ST} values were calculated based on haplotype frequency results that are displayed as a heat map in Fig. 2. For the Brazilian population as a whole, the results ranged from 0.06518 (for ASW group, comprised of Americans with AFR ancestry) to 0.1693 (EUR from Finland). Brazilians showed higher similarity levels with admixed populations where the AFR and/or NA contribution to the pool of maternal lineages is higher (in addition to ASW, Puerto Ricans (PUR), Mexicans (MXL) and African Caribbean (ACB) populations), followed by samples from African countries and remaining admixed Latin Americans. Further, EUR samples were found to have a higher pairwise distance from the BRA population. Both Asian groups presented intermediate genetic resemblance with the present sample set, with South Asians (SAS) displaying an overall genetic similarity with investigated regions slightly higher than East Asians (EAS) samples (average pairwise F_{ST} values to Brazilian population equivalent to 0.0926 and 0.1018, respectively). Complete pairwise F_{ST} values are presented in Supplementary Table S7 (with values obtained based on populations) and S8 (displaying differences based on biogeographic continental origin groups).

In order to further examine the aforementioned results and clarify the eventual genetic relationship between 26 worldwide populations and Brazilian regions, a MDS plot based on pairwise F_{ST} values was drawn for the obtained data and is presented in Fig. 3A. A clear pattern can be observed with negative values for Dimension 1 as a characteristic feature for non-AFR populations. Both Asians groups (EAS and SAS) are grouped approximately together around the axis center with an apparently distinct cluster separation between both geographical origins. As expected, AMR and Asians groups have a close distribution occupying the first quadrant. However, while EUR populations seem to be grouped relatively close to each other, AMR and Asians have a broader dispersion. The BRA population seem to be located in a very particular location, with a clear directional tendency towards positive values in Dimension 1 (trending to AFR cluster). PUR seems to be an outlier in the AMR group, exhibiting a similar tendency to the BRA population and close to the AFR cluster. A wider dispersion displayed by AMR populations seems to reflect their admixed nature, and how distinct relative contributions of parental populations affect modern population frequency distribution. A cladogram was also drawn

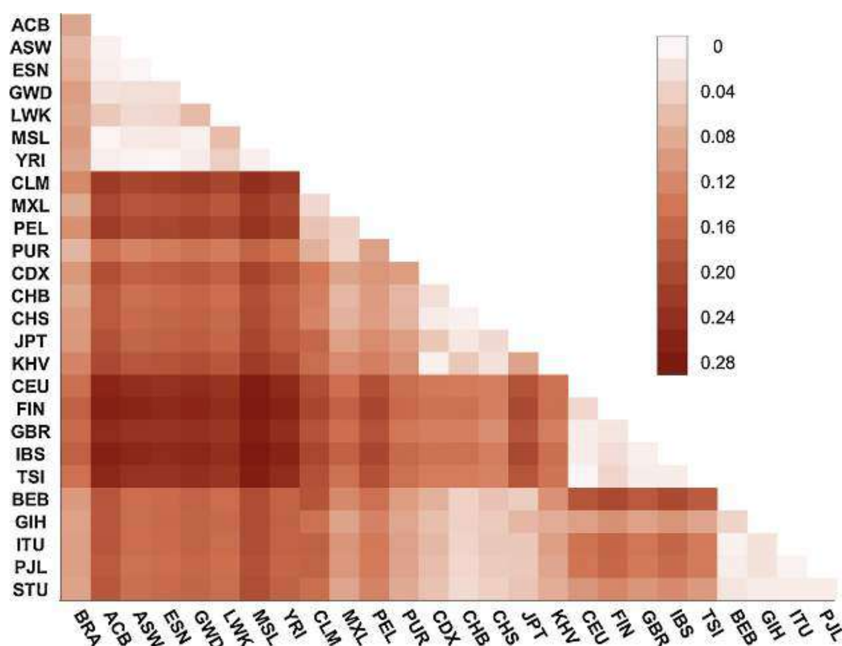

Fig. 2. Heat map of pairwise F_{ST} values based on mtDNA haplotype frequencies for 26 worldwide populations and Brazilian data set. Population full names described in Supplementary Table S1.

applying N-J methods, and is presented in Fig. 3B. The resulting phylogenetic tree shows the BRA population positioned between the AFR and AMR branches, reflecting higher parental contributions of individuals with such biogeographical origins for maternal lines. EUR populations are located at the treetop, featuring the largest distance to the Brazilian sample set. It is important to highlight that these findings are supported by previously reported genetic, historical or ethnographic information, especially for the Brazilian case [18–25,48–55].

Finally, as the occurrence of genetic stratification regarding mtDNA haplotype frequencies seems to be clearly defined for Brazilian and all other worldwide populations, statistical tests were performed to evaluate the amount of genetic variance associated exclusively with the verified genetic structure. Global AMOVA tests were executed with BRA and 26 worldwide populations, arranged according to geographical origin (BRA samples were categorized in a separate group from remaining AMR populations), and the results are detailed in Table 2. Among-group variation accounts for an estimate of roughly 85% of total genetic variability with less than 1% of total genetic differences explained by divergence found among populations within same groups. These results indicate that population genetic structure associated with mtDNA haplotypes are highly dependent on sample biogeographical origin, and that populations within the same continent present very similar haplotype distributions. Therefore, maternal lineage ancestral origins can be traced back to their estimated parental population with a certain degree of confidence. In this regard, even though high admixture levels are evidenced in BRA populations, uneven contribution levels of distinct parental populations in maternal and paternal lineages (assessed by mtDNA and Y-chromosome haplotypes) limit global diversity presented by mtDNA alone. Owing to historical interbreeding dynamics in BRA population formation, AFR and AMR possess relatively higher contributions to BRA mtDNA genetic pool, which does not necessarily reflect the overall contribution of parental populations from distinct biogeographical origins to the modern Brazilian population [18,19,21,54]. This fact introduces a certain degree of genetic structure and bias that should be considered when forensic applications or database constructions are designed. Till the present moment, genetic structure in Brazilian populations regarding mtDNA haplotype distribution is admitted, although very limited in magnitude, considering both spatial distribution in regional populations (estimated on a state or geopolitical region basis) or in distinct ethnic groups in the Brazilian population (based on self-declared assessment) [28,66,67]. As a final note, it is important to account for the fact that small, isolated groups within the national territory were observed to display higher levels of mtDNA genetic structure when compared to the national population [55,68–70]. This fact must be taken into consideration during forensic analysis, especially when criminal evidence is believed to originate from individuals belonging to these local communities.

4. Conclusion

The present study sought to evaluate the utility of the Precision ID mtDNA Whole Genome Panel in Brazilian populations in order to start the development of a frequency database that allows for use of this commercial solution in real forensic casework. A total of 96 samples collected from three Brazilian cities located in distinct geopolitical regions were evaluated. This is one of the first studies reporting complete mitochondrial genome sequences for Brazilian samples, and additional genetic information provided by coding region inclusion during analysis can be a determinant in correct haplotype classification and identification of new or undescribed subclades. Diversity indices and other relevant forensic statistical parameters obtained for this sample set exhibit high polymorphism levels that are suitable for human identification purposes. Full mtDNA haplotypes were shown to add valuable amounts of genetic information available to forensic investigators, and in a limited sample size (n = 65) were observed to properly discriminate two distinct pairs of samples, previously undistinguishable via traditional Sanger sequencing of CR techniques. Therefore, from a forensic perspective, inclusion of MPS analytical tools for mtDNA analysis can be demonstrated to be a very useful resource for law enforcement agencies not only because of full mtDNA sequencing capability, but also to high-throughput performance. Workflow automation should also be considered when casework volumes or existent case backlog is significant. However, some issues still need to be addressed, including technical optimizations in panel design and improvements in raw data processing and results presentation. Design and construction of a representative forensic national database is also essential, with a significant sample number addition in order to appraise evidence of possible genetic regional or ethnical stratification in the

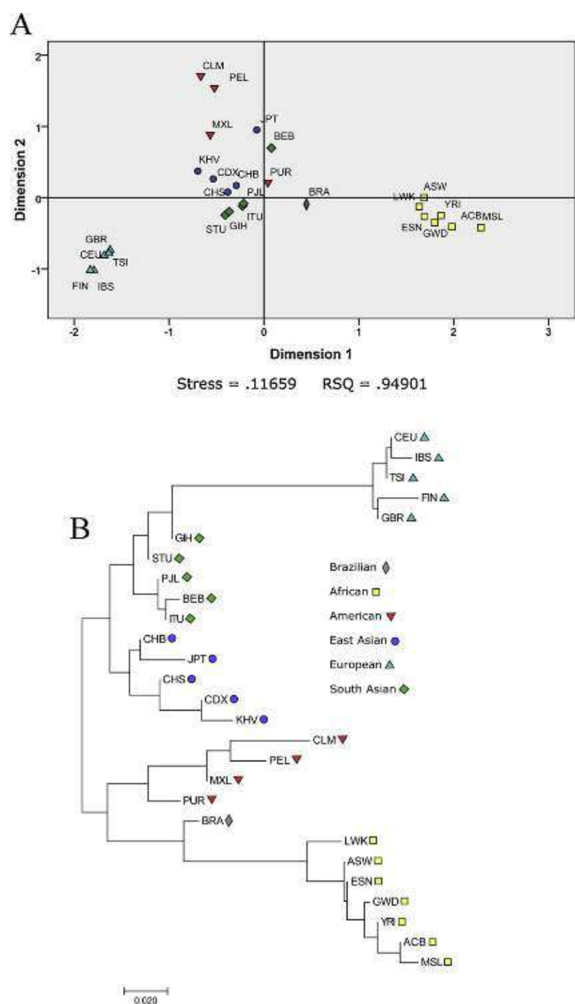

Fig. 3. Genetic distance evaluation for inter population analysis of 26 worldwide populations and Brazilian data ser. (A) MDS plot constructed based on pairwise F_{ST} values for full mtdna haplotype frequencies. (B) Phylogenetic tree build with Neighbor-joining methods, based on pairwise F_{ST} values obtained. Scale in F_{ST} units.

Table 2
Fixation indexes and global AMOVA results for Brazilian and 26 worldwide populations, based on full mtDNA haplotype frequencies.

Source of Variation	Relative Variation (%)	Fixation Indexes
Among groups	84.65	F_{CT}: 0.84645
Among populations within groups	0.32	F_{SC}: 0.02111
Within populations	15.03	F_{ST}: 0.84969

Brazilian population [63].

Proposed MPS metrics evaluated in this work can be used in addition to other computational resources to assess the overall quality of produced mtDNA results [71]. For this particular product version and equipment used, a great deal of effort is still necessary in data review, manual data quality verification and results evaluation as automatically generated reports are not in compliance with suggested forensic standards. In particular, handling and manual revision of primer-derived bias (caused by use of degenerated primers in reagent design) at the time of results interpretation was shown to be a meticulous task where subjective analyst intervention was crucial [72]. As such, a considerable degree of expertise in mtDNA data analysis is still necessary, and such proficiency levels for technical personnel might not be available in every national forensic laboratory. For instance, the recently proposed possibility of paternal heritage for mtDNA owing to defective elimination of spermatozoid carried organelles [73] in zygotes might be indistinguishable from a mtDNA mixture sample. Although there is insufficient scientific evidence to fully support this hypothesis [74], such a possibility might present a challenge to an inexperienced or inadequately trained lab workforce. Techniques for mixture deconvolution based on mtDNA data have been proposed [17,26,44], but these methods involve manual or visual data inspection and critical results evaluation, where analyst knowledge may be critical in phasing contributing samples and mixture parsing [17,26]. Even though software packages for automatic mtDNA mixture analysis have been released [75], such tools still require some level of operator judgment in data evaluation. Therefore, inclusion of MPS tools in Brazilian forensic routine casework processing would require intensive training and official governmental investment in order to assure uniform, consistent results production across different national forensic laboratories throughout the country. As an alternative, the constitution of a dedicated, specialized central laboratory (or even a small number of regional facilities, aiming to minimize logistical restraints resulting from Brazilian large territorial distribution) responsible for processing mtDNA-related biological evidence could be considered, where sufficient personnel training and expertise would be ensured and financial or human resource allocation could be optimized.

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Declaration of Competing Interest

Authors declare they have no conflict of interest.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.fsigen.2019.07.004.

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CAPÍTULO 4 - Full mtDNA genome sequencing of Brazilian admixed populations: a forensic-focused evaluation of a MPS application as an alternative to Sanger sequencing methods

Artigo publicado no periódico Forensic Science International: Genetics

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Material Suplementar.

Disponível em https://www.sciencedirect.com/science/article/pii/S1872497319301437?via%3Dihub

Supplementary Figures

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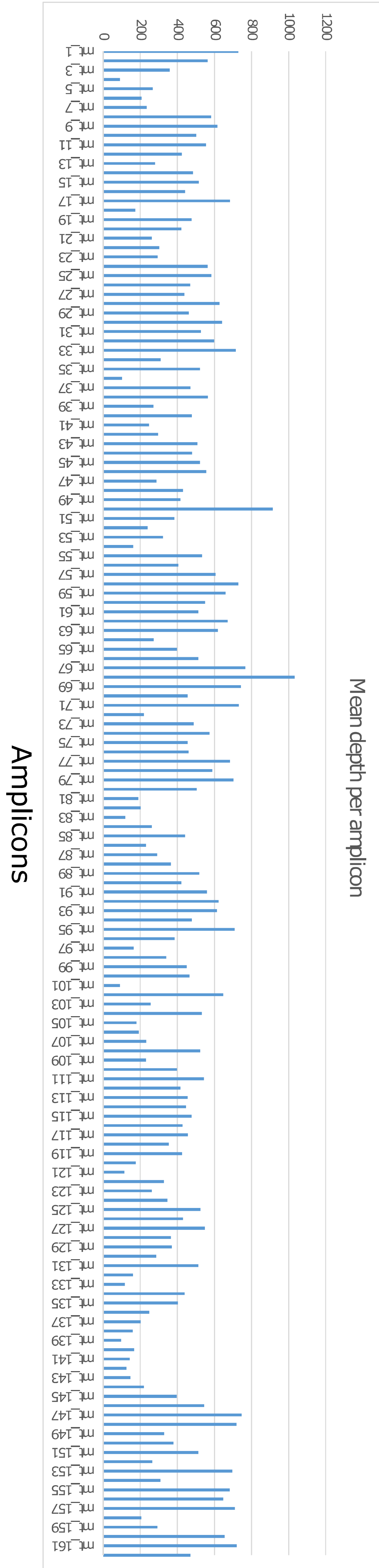
Supplementary Figure S3. Strand balance (SB) and Noise level (NL) metrics presented by 96 Brazilian full mtDNA sequences. Presented values were calculated for nucleotide positions identified as variants to rCRS [31] only. Number of variants occurrences identified in each amplicon is presented next to amplicon name, between parenthesis. Data is presented as box-plots, with 1st and 3rd quartiles separated by median value in black. Open whiskers represent minimal and maximum values. (A) Strand Balance (SB). Amplicons are distributed according to number of variants observations for all 96 samples, in ascending order (left to right). (B) Noise levels (NL) for variant positions identified in all 96 samples evaluated.

Supplementary Figure S4. Complete phylogenetic tree for mtDNA haplotype definition including all 96 analyzed samples. Tree was automatic generated by Haplogrep 2 v2.1.1.13 [36] according to mtDNA phylotree build 17 [37]. Boxes presents haplotypes and number indicate variants nucleotide positions relative to rCRS [31].



Depth
Mean depth per amplicon
Amplicons
0
200
400
600
800
1000
1200
mt_1
mt_3
mt_5
mt_7
mt_9
mt_11
mt_13
mt_15
mt_17
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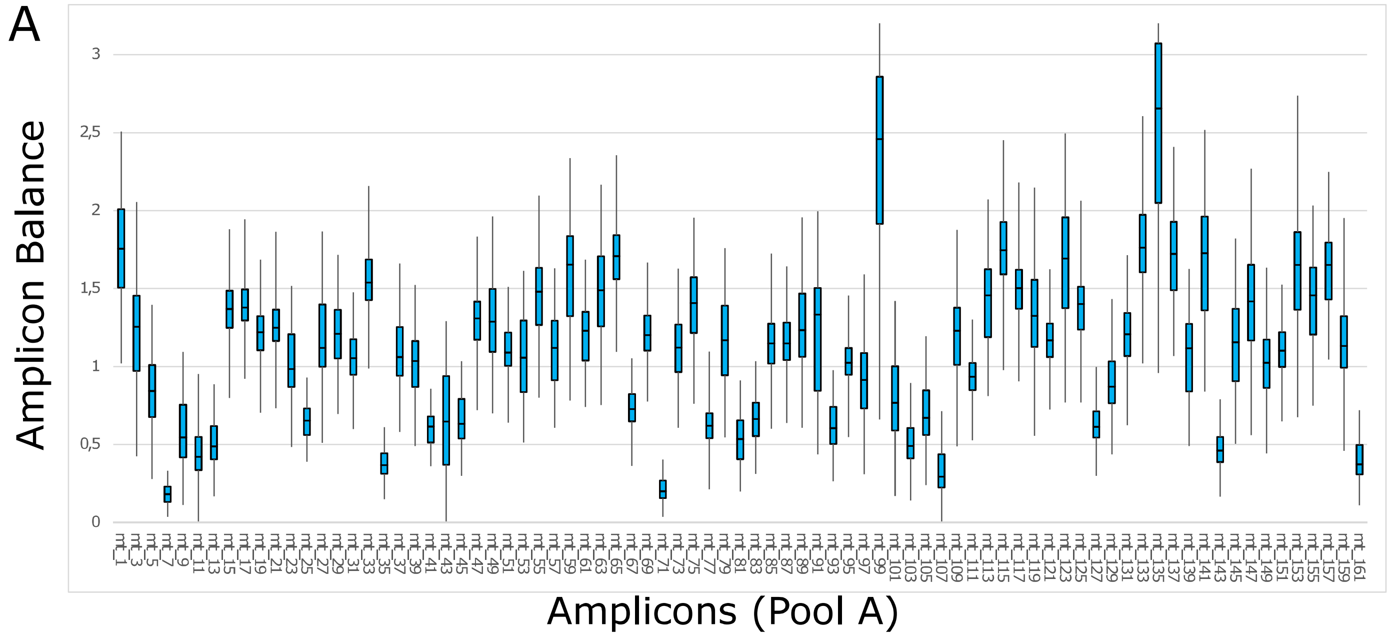
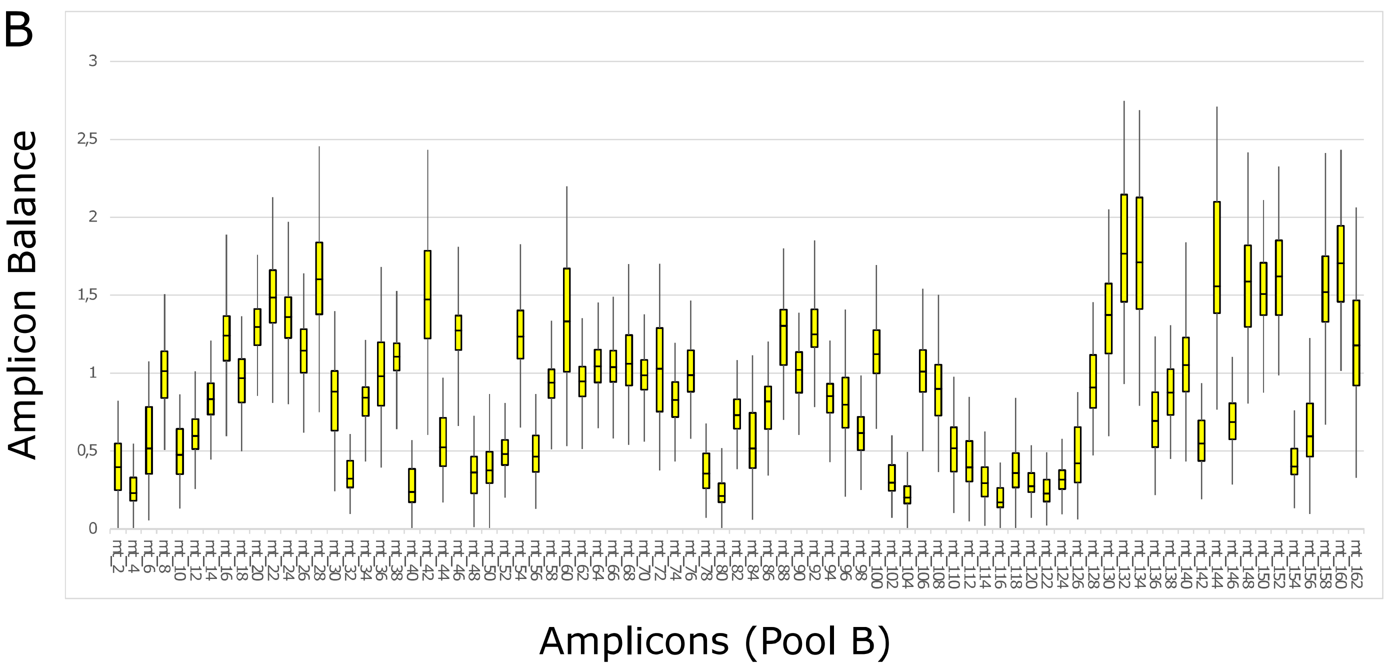
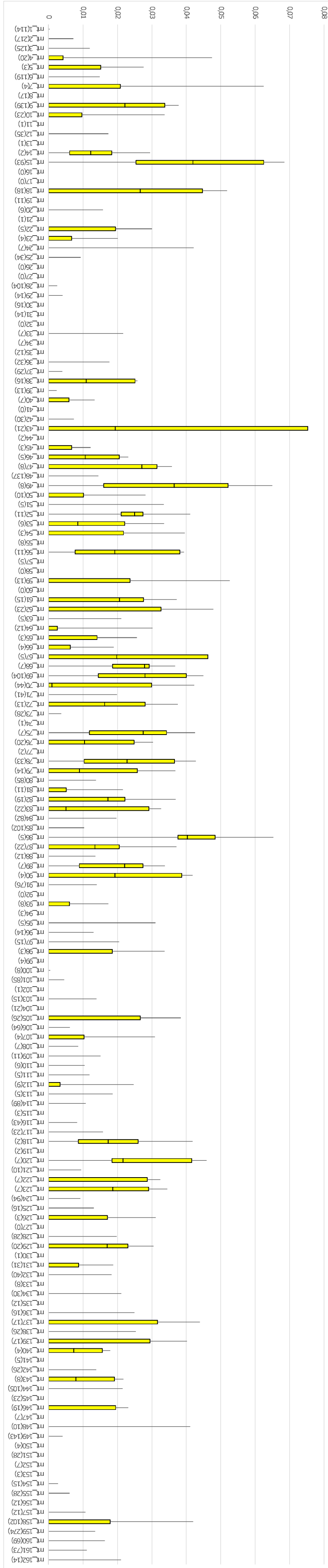
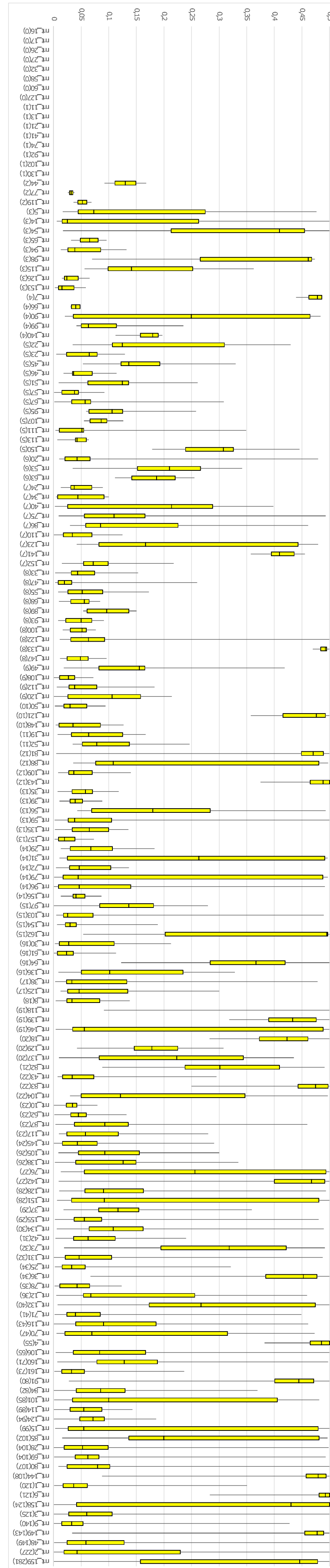
mt_161

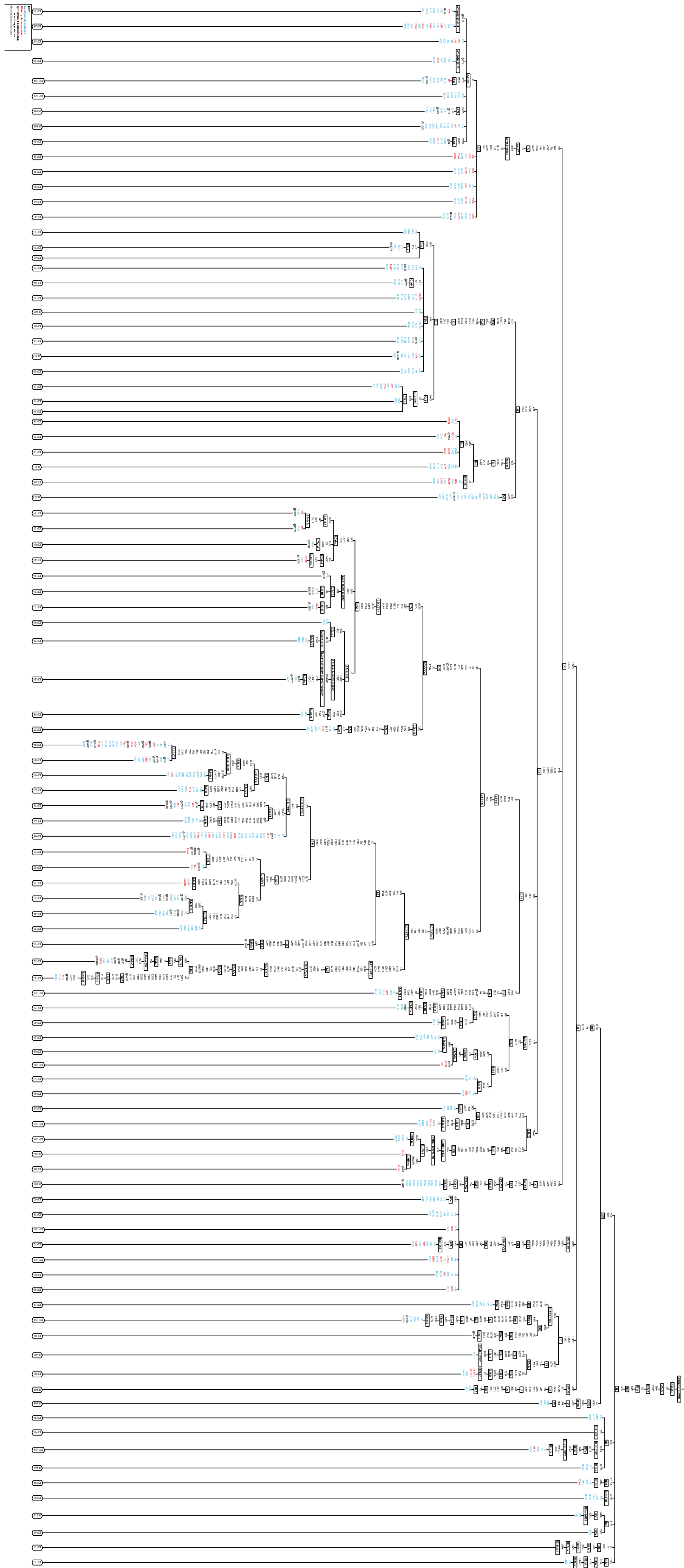




Amplicons (Pool B)


Noise Level
B
Amplicons


Strand Balance
A



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Supplementary Table S1. Metadata of populations used in the present study.

Population number	Name and description	Sample size	Geographical Origin	Abbreviation
1	Brazilians from Brasília (DF), Porto Alegre (RS) and Rio de Janeiro (RJ) cities	96	Brazil (BRA)	BRA
2	African Carribbeans in Barbados	96	Africa (AFR)	ACB
3	Americans of African Ancestry in SW USA	60	Africa (AFR)	ASW
4	Esan in Nigeria	99	Africa (AFR)	ESN
5	Gambian in Western Divisions in the Gambia	113	Africa (AFR)	GWD
6	Luhya in Webuye, Kenya	101	Africa (AFR)	LWK
7	Mende in Sierra Leone	85	Africa (AFR)	MSL
8	Yoruba in Ibadan, Nigeria	107	Africa (AFR)	YRI
9	Colombians from Medellin, Colombia	94	America (AMR)	CLM
10	Mexican Ancestry from Los Angeles USA	65	America (AMR)	MXL
11	Peruvians from Lima, Peru	85	America (AMR)	PEL
12	Puerto Ricans from Puerto Rico	104	America (AMR)	PUR
13	Chinese Dai in Xishuangbanna, China	98	East Asia (EAS)	CDX
14	Han Chinese in Beijing, China	103	East Asia (EAS)	CHB
15	Southern Han Chinese, China	105	East Asia (EAS)	CHS
16	Japanese in Tokyo, Japan	104	East Asia (EAS)	JPT
17	Kinh in Ho Chi Minh City, Vietnam	77	East Asia (EAS)	KHV
18	Utah Residents (CEPH) with North and Western European Ancestry	99	Europe (EUR)	CEU
19	Finnish in Finland	99	Europe (EUR)	FIN
20	British in England and Scotland	92	Europe (EUR)	GBR
21	Iberian Population in Spain	107	Europe (EUR)	IBS
22	Toscani in Italia	107	Europe (EUR)	TSI
23	Bengali from Bangladesh	86	South Asia (SAS)	BEB
24	Gujarati Indian from Houston, Texas	104	South Asia (SAS)	GIH
25	Indian Telugu from the UK	103	South Asia (SAS)	ITU
26	Punjabi from Lahore, Pakistan	97	South Asia (SAS)	PJL
27	Sri Lankan Tamil from the UK	102	South Asia (SAS)	STU

Populations 6 to 27 were obtained from 1000 Genomes Project, Phases 1-3.

Supplementary Table S2. Details of each individual amplicon included in Precision ID mtDNA Full Genome Panel. Nucleotide positions are reported according to rCRS [31]. Amplicon size in basepairs.

Amplicon Details				
Amplicon Name	*Starting Position*	*Ending position*	*Size*	*Pool*
mt_1	15	119	104	A
mt_2	118	248	130	B
mt_3	247	329	82	A
mt_4	298	411	113	B
mt_5	384	480	96	A
mt_6	459	543	84	B
mt_7	518	610	92	A
mt_8	596	709	113	B
mt_9	695	808	113	A
mt_10	793	903	110	B
mt_11	889	1001	112	A
mt_12	991	1118	127	B
mt_13	1117	1245	128	A
mt_14	1238	1351	113	B
mt_15	1340	1474	134	A
mt_16	1463	1574	111	B
mt_17	1563	1684	121	A
mt_18	1673	1787	114	B
mt_19	1774	1870	96	A
mt_20	1859	1988	129	B
mt_21	1977	2079	102	A
mt_22	2068	2199	131	B
mt_23	2188	2272	84	A
mt_24	2261	2352	91	B
mt_25	2341	2434	93	A
mt_26	2430	2547	117	B
mt_27	2535	2653	118	A
mt_28	2642	2772	130	B
mt_29	2772	2888	116	A
mt_30	2877	2995	118	B
mt_31	2984	3107	123	A
mt_32	3096	3197	101	B
mt_33	3182	3275	93	A
mt_34	3266	3394	128	B
mt_35	3383	3485	102	A
mt_36	3472	3596	124	B
mt_37	3585	3668	83	A
mt_38	3657	3785	128	B
mt_39	3776	3895	119	A
mt_40	3884	4004	120	B
mt_41	3993	4112	119	A
mt_42	4101	4216	115	B
mt_43	4205	4331	126	A
mt_44	4320	4412	92	B
mt_45	4401	4498	97	A
mt_46	4487	4617	130	B
mt_47	4606	4721	115	A
mt_48	4710	4835	125	B
mt_49	4824	4951	127	A
mt_50	4940	5040	100	B
mt_51	5029	5138	109	A
mt_52	5127	5253	126	B

mt_53	5242	5348	106	A
mt_54	5337	5465	128	B
mt_55	5454	5574	120	A
mt_56	5563	5688	125	B
mt_57	5677	5799	122	A
mt_58	5788	5869	81	B
mt_59	5858	5982	124	A
mt_60	5971	6081	110	B
mt_61	6070	6188	118	A
mt_62	6177	6308	131	B
mt_63	6297	6424	127	A
mt_64	6413	6521	108	B
mt_65	6510	6637	127	A
mt_66	6626	6752	126	B
mt_67	6741	6869	128	A
mt_68	6858	6991	133	B
mt_69	6980	7113	133	A
mt_70	7107	7233	126	B
mt_71	7222	7348	126	A
mt_72	7337	7468	131	B
mt_73	7451	7563	112	A
mt_74	7552	7657	105	B
mt_75	7646	7773	127	A
mt_76	7762	7888	126	B
mt_77	7877	8005	128	A
mt_78	7994	8117	123	B
mt_79	8106	8228	122	A
mt_80	8217	8340	123	B
mt_81	8329	8440	111	A
mt_82	8430	8560	130	B
mt_83	8548	8658	110	A
mt_84	8647	8779	132	B
mt_85	8769	8892	123	A
mt_86	8881	9011	130	B
mt_87	9000	9122	122	A
mt_88	9111	9239	128	B
mt_89	9228	9363	135	A
mt_90	9354	9479	125	B
mt_91	9468	9589	121	A
mt_92	9578	9651	73	B
mt_93	9640	9767	127	A
mt_94	9756	9852	96	B
mt_95	9839	9958	119	A
mt_96	9947	10062	115	B
mt_97	10050	10162	112	A
mt_98	10151	10248	97	B
mt_99	10235	10308	73	A
mt_100	10297	10404	107	B
mt_101	10393	10492	99	A
mt_102	10481	10577	96	B
mt_103	10566	10665	99	A
mt_104	10654	10775	121	B
mt_105	10764	10880	116	A
mt_106	10869	10996	127	B
mt_107	10985	11109	124	A
mt_108	11098	11185	87	B
mt_109	11174	11301	127	A
mt_110	11291	11384	93	B
mt_111	11369	11470	101	A
mt_112	11460	11587	127	B
mt_113	11576	11699	123	A

mt_114	11688	11782	94	B
mt_115	11771	11885	114	A
mt_116	11869	11993	124	B
mt_117	11982	12086	104	A
mt_118	12075	12201	126	B
mt_119	12190	12282	92	A
mt_120	12279	12387	108	B
mt_121	12351	12459	108	A
mt_122	12454	12559	105	B
mt_123	12548	12662	114	A
mt_124	12651	12772	121	B
mt_125	12757	12882	125	A
mt_126	12868	12973	105	B
mt_127	12962	13084	122	A
mt_128	13074	13202	128	B
mt_129	13191	13326	135	A
mt_130	13321	13453	132	B
mt_131	13442	13570	128	A
mt_132	13559	13696	137	B
mt_133	13685	13789	104	A
mt_134	13778	13901	123	B
mt_135	13891	14001	110	A
mt_136	13990	14074	84	B
mt_137	14063	14182	119	A
mt_138	14166	14286	120	B
mt_139	14275	14367	92	A
mt_140	14356	14435	79	B
mt_141	14424	14540	116	A
mt_142	14537	14629	92	B
mt_143	14612	14716	104	A
mt_144	14705	14839	134	B
mt_145	14836	14967	131	A
mt_146	14956	15065	109	B
mt_147	15054	15146	92	A
mt_148	15135	15257	122	B
mt_149	15242	15368	126	A
mt_150	15355	15466	111	B
mt_151	15451	15585	134	A
mt_152	15581	15693	112	B
mt_153	15682	15769	87	A
mt_154	15758	15859	101	B
mt_155	15848	15964	116	A
mt_156	15953	16069	116	B
mt_157	16055	16131	76	A
mt_158	16109	16225	116	B
mt_159	16221	16341	120	A
mt_160	16338	16458	120	B
mt_161	16447	16552	105	A
mt_162	16541	80	108	B

Supplementary Table S3. MPS performance data for each individual run using Precision ID mtDNA Whole Genome Panel in an ION PGM Sequencer.

Run designation	*Chip model*	*Samples per run**	*Loading (%)*	*Usable reads (%)*	*Aligned reads*	*Average mapped reads per sample (sd) ***	*Mean depth per amplicon per sample (sd) ***
Chip mt1	318v2	48	77	36	2,904,939	60,503 (79,553)	506x (1178x)
Chip mt2	318v2	48	77	28	2,198,441	55,805 (19,353)	376x (131x)
Chip mt3	318v2	44	76	52	3,055,039	119,356 (53,552)	836x (217x)
Average	**-**	**-**	**76.6**	**38.6**	**4,608,701**	**68,972 (53,056)**	**516x (629x)**

***** Total samples per run includes samples not pertaining to present study

****** Values presented for samples including in this study only, average adjusted by sample number per chip

Supplementary Table S4. Full mtDNA sequences of 96 Brazilian samples, presented as variations to rCRS [31]. Haplogroups classified by EMPOP [38]. Heteroplasn

Sample name	Origin	Range	Haplogroup	rCRS polymorphisms					
DF01	Distrito Federal	1-16569	*U8a1a*	73G	263G	282C	309.1C	315.1C	750G
DF03	Distrito Federal	1-16569	*U5b1f1*	73G	150T	263G	315.1C	533G	750G
DF04	Distrito Federal	1-16569	*L2a1a1*	73G	146C	152C	195C	263G	309.1C
DF05	Distrito Federal	1-16569	*C1b*	73G	249del	263G	290del	291del	309.1C
DF06	Distrito Federal	1-16569	*L3f1b4a*	73G	150T	189G	263G	309.1C	315.1C
DF07	Distrito Federal	1-16569	*B2*	73G	103A	152C	263G	309.1C	309.2C
DF08	Distrito Federal	1-16569	*L3e2a1b1*	73G	150T	195C	198T	263G	315.1C
DF09	Distrito Federal	1-16569	*L1c1a2*	73G	93G	95C	151T	152C	182T
DF10	Distrito Federal	1-16569	*D1*	73G	263G	309.1C	309.2C	315.1C	489C
DF11	Distrito Federal	1-16569	*C1d1*	73G	182T	194T	249del	263G	290del
DF12	Distrito Federal	1-16569	*H13a1a*	263G	315.1C	709A	750G	1438G	2259T
DF13	Distrito Federal	1-16569	*L2a1a2c*	73G	146C	152C	195C	263G	315.1C
DF14	Distrito Federal	1-16569	*H15a1a1*	55C	57C	263G	315.1C	750G	1438G
DF17	Distrito Federal	1-16569	*L2a1f3*	73G	146C	152C	195C	198T	263G
DF18	Distrito Federal	1-16569	*U3b1*	73G	150T	152C	263G	309.1C	315.1C
DF19	Distrito Federal	1-16569	*C1b*	73G	249del	263G	290del	291del	309.1C
DF20	Distrito Federal	1-16569	*L3e2a1b1*	73G	146C	150T	195C	198T	263G
DF21	Distrito Federal	1-16569	*L3e1f1a*	73G	150T	200G	263G	309.1C	315.1C
DF22	Distrito Federal	1-16569	*C1b*	73G	152C	249del	263G	290del	291del
DF23	Distrito Federal	1-16569	*L1c3c*	73G	93G	151T	152C	153G	182T
DF24	Distrito Federal	1-16569	*L2a1f1*	73G	146C	152C	195C	263G	309.1C
DF25	Distrito Federal	1-16569	*A2+(64)+@16111*	64T	73G	146C	152C	153G	186T
DF26	Distrito Federal	1-16569	*D1*	73G	195C	263G	315.1C	489C	573.1C
DF27	Distrito Federal	1-16569	*L2c*	73G	93G	146C	150T	152C	182T
DF28	Distrito Federal	1-16569	*L2a1c5*	73G	143A	146C	152C	195C	263G
DF29	Distrito Federal	1-16569	*L2a1q*	73G	143A	146C	152C	263G	315.1C
DF30	Distrito Federal	1-16569	*A2+64+@153*	59C	64T	73G	146C	204C	235G
DF31	Distrito Federal	1-16569	*A2*	73G	146C	153G	235G	263G	315.1C
DF32	Distrito Federal	1-16569	*L0a1a2*	93G	185A	189G	236C	247A	263G

DF33	Distrito Federal	1-16569	*A2+(64)*	64T	73G	146C	152C	153G	235G
DF34	Distrito Federal	1-16569	*L2a1f*	73G	146C	152C	195C	263G	315.1C
DF35	Distrito Federal	1-16569	*L2a1a2c*	73G	146C	152C	195C	263G	315.1C
DF36	Distrito Federal	1-16569	*D1*	73G	263G	315.1C	489C	750G	1438G
DF37	Distrito Federal	1-16569	*C1c*	73G	249del	263G	290del	291del	309.1C
DF38	Distrito Federal	1-16569	*A2*	73G	146C	153G	235G	263G	309.1C
DF39	Distrito Federal	1-16569	*A2+64+@16111*	59C	62T	64T	73G	153G	235G
DF40	Distrito Federal	1-16569	*B2*	73G	263G	315.1C	499A	750G	827G
DF41	Distrito Federal	1-16569	*L2a1a3a*	73G	143A	146C	152C	195C	263G
DF42	Distrito Federal	1-16569	*L3e1d1*	73G	150T	152C	189G	200G	263G
DF43	Distrito Federal	1-16569	*A2+(64)+@16111*	73G	146C	153G	235G	263G	309.1C
DF44	Distrito Federal	1-16569	*L1c3b1b*	73G	151T	152C	182T	186A	189C
DF45	Distrito Federal	1-16569	*L1c3c*	73G	93G	151T	152C	182T	186A
DF46	Distrito Federal	1-16569	*L3e2b*	73G	150T	195C	263G	309.1C	315.1C
DF47	Distrito Federal	1-16569	*L0a2a1b*	64T	73G	93G	152C	189G	236C
DF49	Distrito Federal	1-16569	*C1b*	73G	249del	263G	290del	291del	315.1C
DF50	Distrito Federal	1-16569	*A2ah*	73G	146C	153G	235G	263G	309.1C
DF52	Distrito Federal	1-16569	*L3e2b*	73G	150T	195C	263G	315.1C	709A
DF53	Distrito Federal	1-16569	*L1c3b1*	73G	151T	152C	182T	186A	189C
DF54	Distrito Federal	1-16569	*L1b1a*	73G	152C	182T	185T	195C	247A
DF57	Distrito Federal	1-16569	*L1c3b1b*	73G	151T	152C	182T	186A	189C
DF58	Distrito Federal	1-16569	*C1b*	73G	249del	263G	290del	291del	309.1C
DF59	Distrito Federal	1-16569	*C1d1*	73G	194T	249del	263G	290del	291del
DF61	Distrito Federal	1-16569	*A2*	73G	146C	153G	235G	263G	315.1C
DF63	Distrito Federal	1-16569	*C1c*	73G	249del	263G	290del	291del	309.1C
DF64	Distrito Federal	1-16569	*L1c4b*	73G	146C	152C	182T	186A	189C
DF65	Distrito Federal	1-16569	*H3u*	263G	750G	315.1C	1438G	4769G	6776C
DF69	Distrito Federal	1-16569	*L1c1b*	73G	151T	152C	182T	186A	189C
DF70	Distrito Federal	1-16569	*L1c3b2*	73G	151T	152C	182T	186A	189C
DF72	Distrito Federal	1-16569	*B2b+152*	73G	146C	152C	263G	315.1C	499A
DF73	Distrito Federal	1-16569	*C1d1*	73G	194T	249del	263G	290del	291del
DF74	Distrito Federal	1-16569	*C1c4*	73G	151T	214G	249del	263G	290del
DF75	Distrito Federal	1-16569	*L1c2b*	73G	151T	152C	182T	186A	189C

DF78	Distrito Federal	1-16569	*B2*	73G	103A	152C	263G	309del	315.1C
DF82	Distrito Federal	1-16569	*A2*	73G	146C	153G	207A	235G	263G
DF83	Distrito Federal	1-16569	*L3b2*	73G	146C	263G	315.1C	523del	524del
DF84	Distrito Federal	1-16569	*C1b9*	73G	249del	263G	290del	291del	309.1C
DF86	Distrito Federal	1-16569	*D4+195*	73G	195C	214G	263G	309.1C	315.1C
DF87	Distrito Federal	1-16569	*H+1378*	200G	263G	315.1C	750G	1438G	4769G
DF88	Distrito Federal	1-16569	*L2a1e1*	73G	146C	152C	195C	263G	309.1C
DF89	Distrito Federal	1-16569	*H10a*	146C	263G	315.1C	750G	1438G	4216C
DF90	Distrito Federal	1-16569	*L2a1c*	73G	143A	146C	152C	195C	263G
DF91	Distrito Federal	1-16569	*H1+152*	152C	263G	309.1C	315.1C	750G	1438G
DF92	Distrito Federal	1-16569	*B2o*	73G	152C	263G	309.1C	315.1C	460C
DF93	Distrito Federal	1-16569	*L1c1a1*	73G	151T	152C	182T	186A	189C
DF94	Distrito Federal	1-16569	*H1*	263G	309.1C	315.1C	750G	1438G	3010A
DF100	Distrito Federal	1-16569	*L3e2a1b*	73G	150T	195C	263G	315.1C	750G
DF101	Distrito Federal	1-16569	*A2+(64)*	64T	73G	146C	153G	235G	263G
DF103	Distrito Federal	1-16569	*K1a4a1*	73G	195C	263G	315.1C	497T	524.1A
DF104	Distrito Federal	1-16569	*A2x*	64T	73G	146C	153G	235G	263G
DF105	Distrito Federal	1-16569	*L3b1a11*	73G	257G	263G	315.1C	523del	524del
DF106	Distrito Federal	1-16569	*L3f1b4c*	73G	150T	189G	200G	263G	309.1C
DF107	Distrito Federal	1-16569	*L4b2b1*	73G	146C	152C	195C	244G	263G
DF108	Distrito Federal	1-16569	*H1b1a*	152C	200G	263G	315.1C	523del	524del
DF109	Distrito Federal	1-16569	*B2*	73G	263G	315.1C	499A	750G	827G
RJ01	Rio de Janeiro	1-16569	*D1*	73G	210G	228A	263G	309.1C	315.1C
RJ02	Rio de Janeiro	1-16569	*C1b*	73G	228A	249del	263G	290del	291del
RJ03	Rio de Janeiro	1-16569	*M5*	73G	200G	263G	315.1C	489C	523del
RS01	Porto Alegre	1-16569	*U5a1b+16362*	73G	263G	309.1C	315.1C	750G	1438G
RS02	Porto Alegre	1-16569	*L3f1b4a*	73G	150T	189G	200G	263G	309.1C
RS03	Porto Alegre	1-16569	*X2b4a*	73G	153G	195C	225A	226C	263G
RS04	Porto Alegre	1-16569	*H1ba*	263G	309.1C	315.1C	750G	1438G	3010A
RS06	Porto Alegre	1-16569	*A2h*	64T	73G	146C	**153R**	235G	263G
RS07	Porto Alegre	1-16569	*C1b*	73G	263G	249del	290del	291del	315.1C
RS08	Porto Alegre	1-16569	*V9*	72C	204C	207A	263G	315.1C	750G
RS09	Porto Alegre	1-16569	*J1b2*	73G	263G	295T	309.1C	315.1C	462T

RS10	Porto Alegre	1-16569	*H3v+16093*	199C	263G	315.1C	408A	750G	1438G

Supplementary Table S5. Sample equivalence for Distrito Federal subset between CR only profiles [28] and this study. Full mtDNA results presented in italic. Inconsistent haplotype classification highlighted in bold.

Full mtDNA (present study)		**CR only**		**Inconsistencies**	
Sample Name	*Haplotype*	*Sample Name*	*Haplotype*	*Haplotype*	*Polymorphisms*
DF01	*U8a1a*	1	U8a1a	No	n/a
DF03	*U5b1f1*	6	U5b1f1	No	n/a
DF04	*L2a1a1*	7	L2a1a1	No	n/a
DF05	***C1b***	8	**C1b8**	Yes	n/a
DF06	*L3f1b4a*	9	L3f1b4a	No	n/a
DF08	*L3e2a1b1*	11	L3e2a1b1	No	n/a
DF09	*L1c1a2*	12	L1c1a2	No	n/a
DF10	*D1*	13	D1	No	n/a
DF11	***C1d1***	14	**C1d+194**	Yes	n/a
DF12	***H13a1a***	15	**R0**	Yes	n/a
DF13	***L2a1a2c***	16	**L2a1a2**	Yes	n/a
DF14	*H15a1a1*	17	H15a1a1	No	n/a
DF17	*L2a1f3*	20	L2a1f3	No	n/a
DF18	***U3b1***	21	**U3**	Yes	n/a
DF20	*L3e2a1b1*	25	L3e2a1b1	No	n/a
DF22	*C1b*	29	C1b	No	n/a
DF23	*L1c3c*	30	L1c3c	No	n/a
DF24	***L2a1f1***	31	**L2a1+16189+(16192)**	Yes	n/a
DF25	***A2+(64)+@16111***	34	**A2+(64)**	Yes	n/a
DF27	***L2c***	37	**L2c3**	Yes	*513R* (513A)
DF28	***L2a1c5***	40	**L2a1c+16129**	Yes	n/a
DF29	*L2a1q*	41	L2a1q	No	n/a
DF30	*A2+64+@153*	43	A2+(64)+@153	Yes	n/a
DF31	***A2***	44	**A2al**	Yes	n/a
DF32	***L0a1a2***	47	**L0a1'4**	Yes	n/a
DF33	*A2+(64)*	48	A2+(64)	No	n/a
DF34	***L2a1f***	50	**L2a1+16189+(16192)**	Yes	n/a
DF35	***L2a1a2c***	51	**L2a1a2**	Yes	n/a
DF36	*D1*	52	D1	No	n/a
DF38	*A2*	54	A2	No	n/a
DF39	*A2+64+@16111*	55	A2+(64)+@16111	Yes	n/a
DF40	***B2***	56	**B4b**	Yes	n/a
DF41	*L2a1a3a*	59B	L2a1	Yes	n/a
DF42	*L3e1d1*	61	L3e1d	Yes	n/a
DF43	***A2+(64)+@16111***	62	**A2+(64)**	Yes	n/a
DF44	***L1c3b1b***	63	**L1c3b1a**	Yes	n/a
DF45	*L1c3c*	64	L1c3c	No	- (297G/416A)
DF46	*L3e2b*	65	L3e2b	No	n/a
DF47	***L0a2a1b***	66	**L0a2**	Yes	*16148Y* (-)
DF50	*A2ah*	69	A2ah	No	n/a
DF54	***L1b1a***	73	**L1b**	Yes	n/a
DF57	***L1c3b1b***	76B	**L1c3b**	Yes	n/a
DF59	***C1d1***	78	**C1d+194**	Yes	n/a
DF65	***H3u***	84	**H1**	Yes	n/a
DF70	*L1c3b2*	89	L1c3b2	No	*16119R* (-)
DF72	***B2b+152***	91	**B2**	Yes	n/a
DF73	***C1d1***	92	**C1d+194**	Yes	n/a
DF74	*C1c4*	93	C1c4	No	n/a
DF78	***B2***	97	**B2b+152**	Yes	n/a
DF82	*A2*	101	A2	No	n/a
DF84	***C1b9***	103B	**C1b8**	Yes	n/a
DF86	***D4+195***	105	**D1**	Yes	n/a
DF87	***H+1378***	106B	**HV13a**	Yes	n/a
DF88	***L2a1e1***	107	**L2a1**	Yes	n/a
DF89	***H10a***	108	**H**	Yes	n/a
DF90	***L2a1c***	109	**L2a1**	Yes	n/a

DF91	***H1+152***	110	**H**	Yes	n/a
DF100	***L3e2a1b***	119	**L3e2**	Yes	n/a
DF101	*A2+(64)*	121	A2+(64)	No	n/a
DF103	***K1a4a1***	125	**K1a**	Yes	n/a
DF104	***A2x***	126	**A2+(64)**	Yes	n/a
DF105	***L3b1a11***	127	**L3b1a**	Yes	n/a
DF106	*L3f1b4c*	128	L3f1b4c	No	n/a
DF107	*L4b2b1*	129	L4b2b1	No	n/a
DF108	***H1b1a***	131	**H1b1+16362**	Yes	n/a

n/a: no differences reported - : no variation from rCRS

Supplementary Table S6. Point Heteroplamies identified in 96 Brazilian full mtDNA genomes by MPS. rCRS reference nucleotide in bold. Relative contibution presents ratio of variant polymorphism in reference to rCRS sequence.

Sample		Point Heteroplasmy		Variant Relative Contribution (%)	
Sample Name	*Haplotype*	*Type*	*Location (nt)*	*Ratio*	*Read Number*
DF24	L2a1f1	R (**A**/G)	16309	64.0	322x
DF25	A2+(64)+@16111	M (A/**C**)	15386	26.5	211x
DF27	L2c	R (A/**G**)	513	22.0	89x
DF32	L0a1a2	Y (**C**/T)	4456	18.3	60x
DF36	D1	Y (C/**T**)	4117	20.5	210x
DF39	A2+64+@16111	R (**A**/G)	11002	25.6	222x
DF44	L1c3b1b	R (**A**/G)	1686	81.8	41x
DF47	L0a2a1b	Y (**C**/T)	5603	15.0	78x
DF47	L0a2a1b	Y (**C**/T)	16148	66.0	189x
DF57	L1c3b1b	R (**A**/G)	1686	83.3	95x
DF57	L1c3b1b	Y (**C**/T)	8468	81.4	42x
DF57	L1c3b1b	R (**A**/G)	12076	22.5	632x
DF58	C1b	R (**A**/G)	8923	74.9	203x
DF70	L1c3b2	R (**A**/G)	16119	37.3	719x
DF78	B2	Y (C/**T**)	8047	16.0	432x
DF93	L1c1a1	Y (**C**/T)	4056	36.6	202x
DF101	A2+(64)	Y (C/**T)**	16325	40.2	479x
DF103	K1a4a1	R (A/**G**)	16390	41.4	720x
DF105	L3b1a11	R (**A**/G)	4767	16.2	913x
RJ03	M5	R (**A**/G)	9254	52.4	232x
RS06	A2h	R (**A**/G)	153	17.9	864x

Supplementary Table S7. Pairwise F_{ST} and exact non-differentiation test for Brazilian and 26 worldwide populations based on full mtDNA haplotype frequencies. Applied significance level is 0.05, prior to Bonferroni correction. Exact non-differentiation tests are presented in lower-left diagonal as p-values, while upper-right diagonal exhibits F_{ST} values. Significant values are presented in bold (p-values for F_{ST} comparison and non-differentiation test variance not shown).

Population	BRA	ACB	ASW	ESN	GWD	LWK	MSL	YRI	CLM	MXL	PEL	PUR	CDX	CHB	CHS	JPT	KHV	CEU	FIN	GBR	IBS	TSI	BEB	GIH	ITU	PJL	STU
BRA	*	0.08548	0.06518	0.07519	0.0944	0.08884	0.09729	0.08699	0.11866	0.08094	0.11095	0.06857	0.1026	0.08479	0.09755	0.1011	0.12313	0.14979	0.16937	0.15431	0.16717	0.14713	0.09873	0.0894	0.09399	0.09299	0.08795
ACB	0.14031	*	0.00505	0.00793	0.02012	0.04793	0.00198	0.00808	0.22242	0.20451	0.22006	0.14816	0.19296	0.17772	0.17849	0.19015	0.20151	0.25705	0.26634	0.24783	0.26466	0.25263	0.18127	0.17992	0.18117	0.1759	0.18015
ASW	0.04291	0.08321	*	-0.00493	0.02268	0.02904	0.01297	0.00318	0.20485	0.18128	0.19959	0.12337	0.16743	0.14964	0.15464	0.16213	0.18066	0.24373	0.25877	0.23666	0.25509	0.23755	0.1527	0.1531	0.15177	0.14857	0.15108
ESN	0.12601	0.24461	0.08121	*	0.0231	0.03332	0.0137	0.00064	0.21067	0.18771	0.20531	0.13481	0.17262	0.15634	0.16111	0.16732	0.18461	0.23856	0.25155	0.23311	0.24885	0.23315	0.15579	0.15757	0.15564	0.15261	0.1562
GWD	0.05701	0.11961	0.04761	0.12191	*	0.06113	0.00582	0.01096	0.22022	0.19615	0.21328	0.14519	0.17953	0.16202	0.16647	0.17332	0.19337	0.24544	0.25695	0.23949	0.25504	0.24076	0.16573	0.16427	0.16321	0.1605	0.16264
LWK	0.00001	0.00001	0.00001	0.00001	0.00001	*	0.05988	0.04053	0.20431	0.17955	0.20157	0.13442	0.16869	0.15273	0.15688	0.16148	0.18005	0.23339	0.24566	0.22798	0.24199	0.22977	0.15402	0.15798	0.154	0.15096	0.15332
MSL	0.14541	0.23141	0.07351	0.30811	0.13581	0.00001	*	0.00717	0.24067	0.22117	0.23604	0.16558	0.20896	0.19293	0.19724	0.2043	0.22098	0.27768	0.29105	0.27172	0.28929	0.27271	0.19618	0.19525	0.19523	0.19089	0.19519
YRI	0.11631	0.21821	0.08071	0.24661	0.11341	0.00001	0.24201	*	0.22144	0.19891	0.215	0.14325	0.18349	0.16555	0.17072	0.1752	0.19716	0.25086	0.26404	0.24512	0.26105	0.24559	0.1652	0.16759	0.16511	0.16182	0.16645
CLM	0.00001	0.00001	0.00001	0.00001	0.00001	0.00001	0.00001	0.00001	*	0.03143	0.05274	0.07344	0.13899	0.12947	0.12417	0.1603	0.15312	0.19162	0.20469	0.18608	0.20286	0.19055	0.18463	0.14769	0.16932	0.16526	0.1502
MXL	0.12471	0.23991	0.06951	0.26321	0.13701	0.00001	0.24221	0.27851	0.00001	*	0.036	0.03483	0.08899	0.0666	0.07403	0.09124	0.11597	0.14911	0.16948	0.15098	0.16602	0.14763	0.11795	0.08889	0.10338	0.10147	0.08669
PEL	0.27171	0.47601	0.12291	0.44851	0.25401	0.00001	0.41771	0.51851	0.00001	0.47931	*	0.08984	0.10212	0.09648	0.09572	0.11525	0.12919	0.19277	0.20616	0.18829	0.20537	0.19028	0.14829	0.12516	0.137	0.13563	0.12484
PUR	0.00001	0.00001	0.00001	0.00001	0.00001	0.00001	0.00001	0.00001	0.00001	0.00001	0.00001	*	0.09528	0.06696	0.06996	0.09511	0.10832	0.15132	0.15742	0.13964	0.15713	0.14852	0.09494	0.08406	0.08955	0.08736	0.08099
CDX	0.00511	0.01141	0.00251	0.00361	0.00431	0.00001	0.00501	0.00731	0.00001	0.01491	0.01941	0.00001	*	0.02324	0.01217	0.04911	0.00615	0.13673	0.14584	0.12951	0.14681	0.13288	0.07407	0.05852	0.06417	0.05968	0.05394
CHB	0.23821	0.48821	0.14591	0.53311	0.25891	0.00001	0.48071	0.45591	0.00001	0.53261	1.00000	0.00001	0.01821	*	0.00537	0.01605	0.0472	0.13588	0.14729	0.13054	0.14828	0.13284	0.03793	0.03827	0.03482	0.03243	0.02878
CHS	0.07051	0.13681	0.04681	0.13181	0.05481	0.00001	0.14831	0.13211	0.00001	0.15661	0.24071	0.00001	0.00481	0.25981	*	0.02986	0.02079	0.12958	0.12941	0.11384	0.13196	0.12627	0.0544	0.04483	0.04885	0.0437	0.03952
JPT	0.15081	0.26841	0.08101	0.21151	0.12701	0.00001	0.22961	0.18791	0.00001	0.27071	0.52531	0.00001	0.01051	0.50261	0.12421	*	0.08834	0.1876	0.20104	0.18383	0.20151	0.18319	0.0431	0.06587	0.04846	0.04723	0.05173
KHV	0.11551	0.23941	0.05861	0.28881	0.13551	0.00001	0.26141	0.25031	0.00001	0.28961	0.50361	0.00001	0.01221	0.49871	0.13451	0.24471	*	0.147	0.14743	0.1302	0.15089	0.14335	0.10789	0.07872	0.09328	0.08605	0.07851
CEU	0.13661	0.26181	0.05851	0.24761	0.12301	0.00061	0.28421	0.26391	0.00001	0.22351	0.51141	0.00001	0.00521	0.52731	0.12981	0.22601	0.27241	*	0.03203	0.0096	0.00895	-0.00173	0.18328	0.09143	0.1444	0.14145	0.10375
FIN	0.00001	0.00001	0.00001	0.00001	0.00001	0.00001	0.00001	0.00001	0.00001	0.00001	0.00001	0.00001	0.00001	0.00001	0.00001	0.00001	0.00001	0.00001	*	0.0182	0.02517	0.03391	0.19912	0.10903	0.16172	0.15988	0.12123
GBR	0.01491	0.03201	0.01471	0.03811	0.01831	0.00001	0.02721	0.03701	0.00001	0.04111	0.06241	0.00001	0.00261	0.07171	0.02661	0.02401	0.03011	0.03601	0.00001	*	0.00704	0.00906	0.17748	0.09003	0.14038	0.13647	0.1034
IBS	0.09371	0.23191	0.08731	0.19791	0.10331	0.00001	0.25231	0.17961	0.00001	0.29051	0.56491	0.00001	0.00881	0.50861	0.09411	0.25281	0.31181	0.30171	0.00001	0.02551	*	0.00933	0.19866	0.10621	0.16079	0.15471	0.11695
TSI	0.06061	0.10901	0.04711	0.12941	0.06041	0.00001	0.11411	0.15251	0.00001	0.14831	0.26041	0.00001	0.00461	0.26981	0.06171	0.14021	0.14761	0.11741	0.00001	0.01111	0.11271	*	0.17521	0.08614	0.13597	0.13396	0.09892
BEB	0.20981	0.47511	0.14051	0.47341	0.26381	0.00001	0.50731	0.54971	0.00001	0.51351	1.00000	0.00001	0.02031	1.00000	0.27911	0.55121	0.52511	0.54431	0.00001	0.05001	0.48311	0.25111	*	0.03508	0.00473	0.00884	0.01814
GIH	0.00191	0.01101	0.00141	0.01121	0.00061	0.00001	0.00331	0.00641	0.00001	0.00771	0.01651	0.00001	0.00021	0.01061	0.00181	0.00271	0.01201	0.00701	0.00001	0.00001	0.00751	0.00241	0.00581	*	0.02104	0.02075	0.01002
ITU	0.01271	0.03941	0.02181	0.05201	0.01551	0.00001	0.02161	0.03201	0.00001	0.04131	0.06711	0.00001	0.00011	0.05941	0.01961	0.02211	0.03691	0.02431	0.00001	0.00141	0.02441	0.01381	0.07971	0.00001	*	0.00285	0.00873
PJL	0.04901	0.07841	0.01681	0.07681	0.02861	0.00001	0.04331	0.06481	0.00001	0.08281	0.13131	0.00001	0.00081	0.09711	0.02911	0.06521	0.05711	0.08651	0.00001	0.00651	0.06631	0.01721	0.12031	0.00381	0.00361	*	0.00968
STU	0.00061	0.00241	0.00161	0.00081	0.00201	0.00001	0.00561	0.00261	0.00001	0.00591	0.00291	0.00001	0.00111	0.00501	0.00011	0.00271	0.00291	0.00361	0.00001	0.00171	0.00251	0.00071	0.00651	0.00001	0.00061	0.00021	*

Supplementary Table S8. Pairwise F_{ST} and exact non-differentiation test for Brazilian and 26 worldwide populations based on full mtDNA haplotype frequencies, grouped by geographical origin. Applied significance level is 0.05, prior to Bonferroni correction. Exact non-differentiation tests are presented in lower-left diagonal as p-values, while upper-right diagonal exhibits F_{ST} values. Significant values are presented in bold (p-values for F_{ST} comparison and non-differentiation test variance not shown).

Population	*BRA*	*AFR*	*AMR*	*EAS*	*EUR*	*SAS*
BRA	*	**0.07839**	**0.08729**	**0.10748**	**0.19871**	**0.10746**
AFR	**0.00000**	*	**0.16130**	**0.15530**	**0.22807**	**0.14788**
AMR	**0.00000**	**0.00000**	*	**0.07499**	**0.15168**	**0.09611**
EAS	0.01443	**0.00000**	**0.00000**	*	**0.13222**	**0.03795**
EUR	**0.00126**	**0.00000**	**0.00000**	**0.00000**	*	**0.12561**
SAS	**0.00000**	**0.00000**	**0.00000**	**0.00000**	**0.00000**	*

Supplementary Material M1:

In this section authors provide breakdown and detailing of formulae used in to evaluate sequencing performance obtained in present work, as specified in Section 2.6 of present paper.

Read Depth (RD) metrics:

- Directly assessed by plugin results. Constitutes a simple count of the number of times any of the 162 amplicons was detected.

(1).

$$RD_{amp(X)} = C_{amp(X)}$$

where $RD_{amp(X)}$ = RD metrics value for any particular amplicon and $C_{amp(X)}$ = number of reads for any particular amplicon.

Amplicon Balance (AB) metrics:

- Calculated as the number of reads of each amplicon, divided by average number of reads for all amplicons in a sample. It is important to notice that, for each sample,

(2).

$$AB_{amp(X)} = \frac{RD_{amp(X)}}{\sum_{i=1}^{162} RD_{amp(i)}/162}$$

where $AB_{amp(X)}$ = AB metrics value for any particular amplicon and $\sum_{i=1}^{162} RD_{amp(i)}/162$ = average number of reads for any particular sample.

It is important to notice that in an ideal, fully balanced system (where all primer pairs have the same amplification efficiency) all different amplicons would be detected exactly the same number of times, and therefore $AB_{amp(X)}$ value would be equivalent to 1 across all primer pairs. At the same time, each bias or preferential amplification of any particular amplicon will be compensated, in this evaluation, by an equivalent decrease in performance of at least one other PCR segment, leading to an average $AB_{amp(X)}$ value for each sample equivalent to 1.

Strand Balance (SB) metrics:

- Calculated for each polymorphism as the number of reads of the most prevalent strand (forward or reverse) divided by total number of reads for this particular base position. Obtained value is expressed as the absolute residual value of each read, where employed predicted value was defined as the ideal value in a fully balanced system (0.5).

(3).

$$SB_{var(X)} = \frac{C_{prev(X)}}{C_{var(X)}} - 0.5$$

where $SB_{var(X)}$ = SB metrics value for any nucleotide position, $C_{prev(X)}$ = number of reads for the most prevalent strand (forward or reverse) in this same base and $C_{var(X)}$ = total number of reads for this particular nucleotide position.

Once again, an ideal balanced system would present same number of reads for forward or reverse reads, and therefore optimal value for this parameter is 0.5 (meaning each forward and reverse strands were detected the same amount of times). For plotting purposes, we present the result as the absolute residual. In this case, metric will range from 0 (a balanced system) to a maximum plotted value of 0.5 (where all observed reads belong to a single category, and therefore the ratio among prevalent strand/total reads is equal to 1).

Noise Level (NL) metrics:

- Estimated for each polymorphism as the ratio of non-specific allele calls.

(4).

$$NL_{var(X)} = \frac{C_{un(X)}}{C_{var(X)}}$$

where $NL_{var(X)}$ = NL metrics value for any nucleotide position and $C_{un(X)}$ = number of reads where detected nucleotide was different than the base called by plugin for this particular nucleotide position. A value of 0 for this metric is expected when no unspecific reads are present, with results increasing when noise or reading errors are identified.

In cases where more than a single base was called (meaning a point heteroplasmy was detected), non-specific base calls do not include any of the variants identified in the heteroplasmic position.

CAPÍTULO 5 – CONSIDERAÇÕES FINAIS

A busca por exatidão, alto grau de confiabilidade e reprodutibilidade e robustez exigida na execução de exames envolvendo amostras de interesse forense é uma tarefa continuamente executada por profissionais que atuam na área, envolvendo grupos de pesquisas vinculados tanto à academia quanto aos órgãos responsáveis pela repressão ao crime e persecução penal. Dessa forma, é responsabilidade conjunta dessas organizações a investigação criteriosa e permanente de todos os métodos e protocolos analíticos aplicados na identificação de suspeitos de envolvimento em eventos criminosos, onde o interesse da verdade e da justiça deve sempre ser o preponderante.

No contexto descrito acima, os resultados obtidos durante a realização dos estudos relatados neste trabalho trazem à luz peculiaridades associadas à possibilidade efetiva da incorporação, por parte da Polícia Federal e de outras unidades de perícia, da rotina analítica via técnicas de sequenciamento massivo paralelo. Como tal, o conhecimento da distribuição na população brasileira dos marcadores do tipo SNP com uso em aplicações de identificação humana é essencial para qualquer cálculo estatístico que permitem a valoração da prova examinada. A partir disto, os resultados apresentados aqui sugerem que a introdução das novas técnicas de análise de SNPs, de sequenciamento completo mtDNA e do uso de MSP na realidade dos laboratórios forenses brasileiros é uma possibilidade concreta, uma vez que se mostram adequadas para a resolução dos problemas típicos das amostras oriundas de cenários criminais.

Em uma abordagem crítica adicional, discussões complementares relevantes ainda devem ser propostas levando em consideração a realidade brasileira, incluindo questões éticas e legais. Benefícios e vantagens apresentados pela nova metodologia devem ser sopesados de acordo com os custos do uso da técnica e todos os aspectos que envolvem a sua implementação, bem como sua adequação ao contexto dos bancos de dados genéticos, já em utilização no Brasil, sempre tendo como foco principal o combate ao crime e administração da justiça em âmbito nacional.

CAPÍTULO 6 – PERSPECTIVAS

A quantidade de dados e de informações gerados pela técnica de Sequenciamento Massivo Paralelo é tão ampla que existe ainda uma quantidade significativa de resultados gerados durante o presente estudo que não puderam ser ainda processados e/ou avaliados. A estimativa é que, pelo menos, três outros artigos científicos sejam ainda elaborados e publicados utilizando tais dados. São eles:

1- Genetic analysis of human remains as a tool for studying Brazilian territorial expansion and urban occupation: the founding population of Brazilian southernmost capital (nome provisório)

A técnica de MPS foi utilizada na caracterização de marcadores SNPs relacionados à identificação e à ancestralidade biogeográfica em restos humanos recuperados em escavações arqueológias executadas no primeiro cemitério da cidade de Porto Alegre. O DNA mitocondrial destas amostras foi igualmente sequenciado. A análise foi efetuada com uma abordagem da genética de populações e na descrição histórica da formação populacional da população porto-alegrense. Resultados mostram que a população original e fundadora da cidade de Porto Alegre possuiu uma estruturação genética similar àquela observada na Porto Alegre moderna, com exceção dos marcadores de linhagem uniparental, onde a contribuição mitocondrial européia foi identificada em níveis inferiores aos encontrados atualmente.

Estágio atual: Manuscrito em preparação.

2- Comparative analysis of Brazilian male lineages through two different types of forensic markers - (nome provisório)

Resultados obtidos através da técnica de MPS para os haplótipos do cromossomo Y, utilizando marcadores do tipo SNP, foi comparado com aqueles obtidos através de perfis de polimorfismo do tipo STR no mesmo cromossomo. Uma análise descritiva da população brasileira é oferecida, assim como a comparação entre os resultadoso obtidos a partir dos dois marcadores. Comparações com populações mundiais e publicações anteriores objetivam avaliar a contribuição relativa de populações ancestrais na dinâmica de formação da população brasileira moderna, no tocante à e distribuição das linhagens masculinas.

Estágio atual: Depósito dos haplótipos em bases de dados internacionais para validação. Análises concluídas. Aguarda preparação do manuscrito.

3- The role of unaligned sequences in MPS forensic examination in biological evidence nature prediction - (nome provisório)

Sequencias não alinhadas com o genoma humano são geradas durante o processamento com as técnicas de MPS, em razão da presença de contaminantes e de outros organismos não-humanos tanto nos

vestígios criminais encontrados quanto nos suportes onde estes foram coletados. Neste estudo, estas sequencias não alinhadas foram utilizadas no desenvolvimento de um método preditivo que tem como objetivo a associação de determinados padrões de amplificação inespecífica de sequencias de organismos não-humanos com a natureza biológica da evidência criminal. Análises preliminares, baseadas em abordagens estatísticas ou em técnicas de aprendizado de máquina, permitiram verificar que o efeito do DNA de fundo existente no suporte onde esses vestígios foram depositados pode ser identificado, e seu impacto deduzido do restante das análises. Essa abordagem, ainda em desenvolvimento, tem potencial para complementar outras técnicas na determinação da natureza biológica de amostras criminais.

Estágio atual: Análises e processamento dos dados em execução.

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