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TESE DE DOUTORADO

ANDRÉ ZORATTO GASTALDO

IDENTIFICAÇÃO GENÉTICA E ESTUDOS POPULACIONAIS UTILIZANDO MICROSSATÉLITES (STR) EM EQUINOS, BOVINOS E CANINOS DOMÉSTICOS PROVENIENTES DO URUGUAI, PARAGUAI E BRASIL

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Tese apresentada ao Programa de Pós-Graduação em Biologia Celular e Molecular da PUCRS como requisito para a obtenção do título de Doutor em Biologia Celular e Molecular.

Orientadora: Profa. Dra. Clarice Sampaio Alho

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RESUMO

O avanço da identificação molecular humana pelo estudo dos marcadores genéticos STR (*Short Tandem Repeats*) induziu e propiciou o crescimento também da identificação genética animal. Contudo, ao contrário da área humana, a identificação molecular animal ainda carece de validações dos sistemas de análise e de estudos populacionais para que seja considerada fidedigna no grau que se necessita. Apenas recentemente a ISAG (*International Society for Animal Genetics*) recomendou marcadores STR adequados para discriminação individual em espécies de animais domésticos. A identificação genética animal exata e precisa é buscada para fornecer, a entidades, associações, criadores e proprietários em geral, garantias de que seus animais são, de fato, oriundos de reprodutores confiáveis. Apenas registros genealógicos fidedignos e autênticos garantirão, por exemplo, eficiência nos processos de melhoramento genético. Mesmo sendo uma área promissora, neste momento há somente duas opções no mercado de *kits* comerciais para genotipagem animal. Mas seu uso tem sido preterido por laboratórios do ramo, os quais acabam preparando seus próprios painéis (*in house*) de identificação, utilizando os marcadores genéticos recomendados para atender suas demandas locais de forma customizada. Contudo, se não forem conhecidas as frequências dos alelos presentes nestes marcadores genéticos na população em estudo e não for estimado o real poder de discriminação que estes fornecem, as análises realizadas e os resultados obtidos poderão gerar interpretações equivocadas ou imprecisas. No presente trabalho, foram utilizados três painéis de marcadores genéticos STR com todos os *loci* recomendados pela ISAG, capazes de identificar indivíduos equinos, bovinos e caninos. Frequências alélicas e outros parâmetros como heterozigosidade, conteúdo de informação de polimorfismo, poder de exclusão e de discriminação foram obtidos a partir de amostras significativas em raças presentes no Rio Grande do Sul/Brasil, no Uruguai e no Paraguai, para que sejam usados na prática da genética animal com fins de identificação. Além disso, estudos populacionais comparativos dentro e entre raças também foram realizados, com o intuito de avaliar a diversidade genética das populações animais estudadas.

Palavras-chave: Identificação genética animal. ISAG. Marcadores STR. Estudos populacionais.

ABSTRACT

The evolution in human identification through the study of genetic markers STR (Short Tandem Repeats) induced and propitiated the growth also of animal genetic identification. However, contrary to what occurs in the human domain, animal molecular identification still lacks validation of analysis systems and population studies to be considered reliable to the degree required. Only recently the International Society for Animal Genetics (ISAG) has recommended STR markers suitable for individual discrimination in domestic animal species. Accurate animal genetic identification is sought to provide entities, associations, breeders and owners in general with assurances that their animals are in fact derived from reliable breeders. Only reliable and authentic genealogical records will ensure, for example, efficiency in genetic breeding processes. Even though it is a promising area, there are currently only two options in the market for commercial kits for animal genotyping. But its use has been deferred by specialized laboratories in the field, which end up preparing their own identification panels, using the recommended genetic markers to meet their local demands more specifically. However, if the frequencies of the alleles present in these genetic markers in the study population are not known and the real power of discrimination that they provide is not estimated, the analyzes performed and the results obtained may generate misleading or imprecise interpretations. In the present study, three panels of STR genetic markers were used with all loci recommended by ISAG, capable of identifying equine, bovine and canine individuals. Allelic frequencies and other parameters such as heterozygosity, polymorphism information content, power of exclusion and power of discrimination were obtained from significant samples in breeds present in Rio Grande do Sul (Southern Brazil), Uruguay and Paraguay, to be used in the practice of animal genetics for identification purposes. In addition, comparative population studies within and among breeds were also carried out, in order to evaluate the genetic diversity of the animal populations studied.

Keywords: Animal genetic identification. ISAG. STR markers. Population studies.

SUMÁRIO

1 INTRODUÇÃO GERAL

1.1 MARCADORES GENÉTICOS

Define-se como marcador genético um gene ou uma sequência de DNA com um local conhecido num cromossomo que pode identificar ou discriminar indivíduos ou espécies. Descreve-se como uma variação (a qual pode surgir devido a uma mutação ou alteração nos *loci* genômicos) passível de ser observada. Um marcador genético pode ser uma sequência de DNA curta, como uma sequência em torno de uma única mudança de pares de bases (por exemplo, os SNP – do inglês *Single Nucleotide Polimorphism*s), ou uma longa, como os minissatélites (VNTR - do inglês *Variable Number of Tandem Repeats*) e os microssatélites (STR - do inglês *Short Tandem Repeats*) (AL-SAMARAI; AL-KAZAZ, 2015).

Os marcadores genéticos podem ser divididos em duas classes: a) marcadores bioquímicos que detectam a variação no nível de um produto gênico, tais como alterações em proteínas e aminoácidos; e b) marcadores moleculares que detectam a variação no nível do DNA, tais como alterações dos nucleotídeos por deleção, duplicação, inversão e/ou inserção (BUTLER, 2005).

Estes últimos são amplamente utilizados na identificação de indivíduos, pois são altamente polimórficos, ou seja, apresentam muitas formas alternativas de sequências de nucleotídeos em uma dada região do DNA.

Vários tipos de polimorfimos são conhecidos e podem ser utilizados para a detecção ou para serem os próprios de marcadores genéticos moleculares, sendo que os mais comuns são: RFLP (do inglês *Restriction Fragment Length Polymorphism*) (SAMBROOK *et al.*, 1975), SSLP (do inglês *Simple Sequence Length Polymorphism*) (DIETRICH *et al.*, 1992), AFLP (do inglês *Amplified Fragment Length Polymorphism*) (VOS *et al.*, 1995), RAPD (do inglês *Random Amplified Polymorphic DNA*) (WILLIAMS, J. G. *et al.*, 1990), VNTR (JEFFREYS; WILSON; THEIN, 1985), STR (AKKAYA; BHAGWAT; CREGAN, 1992) e SNP (JORDAN, S. A. & HUMPHRIES, 1994).

Entre os marcadores genéticos moleculares mais utilizados na identificação de indivíduos estão os SNP, os VNTR e os STR.

1.1.1 *Single nucleotide polimorphisms* (SNP)

É uma variação da sequência de DNA que ocorre quando um nucleotídeo – A, T, C ou G –, no genoma ou em outra sequência partilhada, difere entre os membros de uma espécie biológica. Essas diferenças podem ocorrer através de transições, transversões, inserções e deleções de base única (JARNE; LAGODA, 1996). Para ser considerado um SNP, tal posição com sequências alternativas de DNA, deve ter o alelo menos frequente com frequência igual ou superior a 1%. Caracterizados por serem bi-alélicos, atualmente, os SNP estão entre as abordagens mais populares e mais utilizadas, ainda que sejam menos informativos quando comparados aos multi-alélicos mini e microssatélites. Esta popularidade dos SNP é devido a algumas características desejáveis: são abundantes em todo o genoma, são geneticamente estáveis e passíveis de análise automatizada de alto rendimento (VIGNAL *et al.*, 2008).

1.1.2 *Variable number of tandem repeats* (VNTR)

Os marcadores polimórficos conhecidos como VNTR (ou minissatélites) compreendem várias unidades de repetições sucessivas, cada uma com oito a oitenta pares de base (pb) de comprimento. O número exato de repetições, assim como o comprimento da região de VNTR, varia de um alelo para outro, e diferentes alelos podem ser identificados pelo seu comprimento (JEFFREYS; WILSON; THEIN, 1985). Altamente informativos e muito estudados e aplicados no início da identificação genética através da análise do DNA, a utilização destes marcadores moleculares está em desuso, devido ao tamanho dos fragmentos, pois para o isolamento destas regiões é necessário se obter uma grande quantidade de material biológico, o qual deve estar em bom estado de conservação para a extração de DNA. Em cenas de crime, em que há a necessidade de coletar vestígios biológicos para confronto com amostras de referência, estas condições geralmente não são encontradas (RAPLEY; WHITEHOUSE, 2007).

1.1.3 *Short tandem repeats* (STR)

STR (ou microssatélites) consistem em sequências de um a seis pb que estão repetidas várias vezes ao longo do genoma e são altamente polimórficas (TAUTZ, 1989). Os STR tornaram-se uma poderosa ferramenta para identificação genética, uma vez que, para sua detecção, pode-se utilizar a técnica da Reação em Cadeia da Polimerase (PCR), pela qual o DNA é amplificado a partir de oligonucleotídeos (*primers*) situados em regiões não repetitivas que flanqueiam os marcadores polimórficos (WEIR, 1992). Os STR são particularmente adequados para a análise de amostras forenses que contenham DNA degradado ou em concentração limitada (RAPLEY; WHITEHOUSE, 2007).

1.1.4 Utilização de marcadores genéticos STR para identificação genética

A fim de que um determinado STR possa ser considerado um marcador genético altamente informativo para a identificação de um indivíduo, é desejável que este possua características como: a) ser robusto (possuir estabilidade em diversos ambientes); b) não estar ligado a outros *loci*; c) ser altamente polimórfico; d) possuir unidades de repetição de quatro ou mais pares de bases; e e) apresentar baixa frequência de microvariâncias alélicas. A região de ligação do *primer* deve ser altamente conservada, seja gênero ou espécie-específico, além de produzir perfis genéticos limpos com poucos artefatos (WICTUM *et al*., 2013a). Além disso, dados populacionais devem ser reunidos para se ter o valor da frequência para cada possível alelo em cada marcador nas diferentes populações.

Desta forma, ao analisar um determinado grupo de STR que possua as características citadas acima e tendo estabelecido a frequência alélica destes, torna-se possível determinar a probabilidade de um genótipo particular ocorrer de forma aleatória em uma população (COYLE, 2007).

1.2 IDENTIFICAÇÃO GENÉTICA ANIMAL

A identificação genética animal exata e precisa sempre foi buscada na intenção de fornecer a associações, criadores e proprietários em geral garantias de

que seus animais realmente são oriundos daqueles reprodutores relatados como tendo sido utilizados no cruzamento. Em geral, com tais cruzamentos, objetiva-se o melhoramento genético de uma determinada espécie ou raça. Quanto maior a exatidão na identificação genética, maiores serão as informações referentes a um determinado animal, favorecendo, de forma significativa, a obtenção de registros genealógicos fidedignos e autênticos.

Aspectos como saúde pública, saúde animal, manejo animal, barreiras comerciais e demanda do consumidor também exigem uma identificação mais precisa e segura, de forma que seja possível rastrear os animais ao longo de suas vidas, desde o nascimento até a compra dos animais propriamente ditos ou de seus produtos (carne, leite, ovos etc.) (BOWLING *et al*., 2008).

Também no âmbito forense, a identificação genética animal tem destacada importância, pois, embora a maioria das genotipagens realizadas para investigações criminais envolva o DNA humano, este não é a única fonte de material genético que pode ser útil para demonstrar a culpa ou a inocência de um indivíduo suspeito de um crime. Animais domésticos como cães e gatos vivem em *habitats* humanos e, muitas vezes, depositam seus pelos em locais que podem ser usados para colocar um suspeito na cena do crime. A essa ocorrência, costuma-se dizer que o animal foi "testemunha" em uma cena de crime. Além dessa situação, os animais também podem ser vítimas de crimes. Casos como de abuso sexual, maus tratos ou roubo podem às vezes ser beneficiados pelo poder da análise do DNA. Os restos de um animal perdido podem ser identificados positivamente através da análise genética. Por fim, os animais também podem ser autores de crimes, quando, por exemplo, estão envolvidos num ataque a uma criança, a um outro cão, outro animal de estimação ou gado (COYLE, 2007). Nessa situação, a genotipagem do DNA pode ser utilizada para identificar o animal-autor (por exemplo, um pit bull). Se a vítima vem a óbito, então a análise de DNA pode ser a única prova que se tenha de que um animal sob suspeita cometeu o crime. As análises de DNA podem livrar animais inocentes do sacrifício/abate desnecessário (BUTLER, 2005).

Variadas formas de analisar e certificar a identificação genética foram elaboradas, especialmente nos últimos 50 anos, em decorrência dos avanços tecnológicos e científicos, os quais permitiram aos pesquisadores desenvolverem técnicas que se aproximam da exatidão máxima de identidade (próxima aos 100%)

(RAPLEY; WHITEHOUSE, 2007). Com a descoberta e a utilização de marcadores genéticos moleculares, como os minissatélites (VNTR) e os microssatélites (STR), com a finalidade de discriminar humanos ou animais, foi possível um relevante avanço na área de identificação genética nas duas últimas décadas. De fato, na maioria das vezes, nesta área da ciência, o conhecimento desenvolvido para os estudos de animais foi adquirido através das técnicas já utilizadas em seres humanos, fazendo-se assim uma adaptação para a espécie pesquisada.

Contudo os avanços verificados na identificação humana não são acompanhados com a mesma velocidade quando se refere à identificação genética animal. Enquanto na identificação humana há muito já se estabeleceram grupos de marcadores genéticos altamente polimórficos e informativos, que possibilitaram o surgimento de bancos de dados genéticos e de diversos *kits* comerciais (BUTLER, 2005), na identificação genética animal houve demora para um consenso sobre quais são os *loci* mais polimórficos e os alelos predominantes nas várias espécies de animais domésticos. Até pouco tempo, por exemplo, muitos tribunais, principalmente nos Estados Unidos, refutavam qualquer tipo de prova de DNA animal, justamente por não haver uma padronização no uso daqueles marcadores genéticos mais informativos, que estimulassem o surgimento de *kits* comerciais confiáveis, com boa reprodutibilidade e uniformidade de resultados e que possibilitassem estudos populacionais suficientes para a criação de bancos de dados genéticos. Por isso, sempre requisitavam testes mais apurados (KANTHASWAMY, 2009).

Devido a esta demanda e para iniciar um processo de padronização, a Sociedade Internacional de Genética Animal (ISAG, do inglês *International Society for Animal Genetics*) e seus membros chegaram a um consenso de quais seriam estas regiões para espécies de animais domésticos, entre eles os equinos, os bovinos e os caninos[1].

A ISAG, criada em 1988 – na 21ª Conferência Internacional de Genética Animal sobre Grupos Sanguíneos e Bioquímicos, realizada na cidade de Turim (Itália) –, é uma entidade sem fins lucrativos com foco em pesquisa básica e aplicada nos campos da imunogenética, genética bioquímica e genética molecular

[1] Disponível em: <http://www.isag.us/committees.asp>. Acesso em: 12 jan. 2017.

animal, apoiando o intercâmbio de ideias, resultados e aplicações de pesquisa. Além da organização de conferências e *workshops*, promovendo testes de comparação entre vários laboratórios do mundo, a ISAG proporciona a publicação de trabalhos pertinentes à área na revista *Animal Genetics*, o periódico oficial da Sociedade[2].

1.2.1 Marcadores genéticos recomendados pela ISAG

Conforme a conferência realizada no ano de 2014, em Xi'an (China), para a identificação genética de equinos, a ISAG recomenda que o painel de marcadores STR abranja os seguintes *loci*: AHT5, ASB2, ASB17, ASB23, CA425, HMS1, HMS2, HMS3, HMS6, HMS7, HTG4, HTG6, HTG7, HTG10, LEX3 e VHL20, totalizando 17 regiões (ISAG STANDING COMMITTEE, 2014). A Tabela 1 mostra todos os referidos marcadores genéticos, informando a localização, a estrutura e a sequência das repetições, a sequência dos *primers* e o comprimento dos fragmentos amplificados – *amplicons* – em pares de base (pb).

Para os bovinos, no encontro de comparação de resultados realizado no ano de 2016, em Salt Lake City (Estados Unidos), ainda que tenham sido incorporados marcadores genéticos STR adicionais, além de um painel de SNP, permanece sendo recomendado um painel de 12 *loci* STR, sendo estes: BM1818, BM1824, BM2113, ETH3, ETH10, ETH225, INRA023, SPS115, TGLA53, TGLA122, TGLA126 e TGLA227 (ISAG STANDING COMMITTEE, 2016a). Da mesma forma, a Tabela 2 apresenta todas as informações referentes a estes 12 marcadores genéticos.

No mesmo encontro, no *workshop* "Genética Aplicada para Animais de Companhia", a ISAG também recomendou que para a identificação genética de cães continue sendo utilizado o painel de marcadores genéticos STR que abranja os seguintes *loci*: AHT121, AHT137, INU055, REN54P11, AHTK253, AHTK211, INU005, REN64E19, AMEL, FH2848, REN247M23, CXX279, FH2054, REN162C04, AHTH171, AHTH260, INRA021, AHTH130, REN169O18, REN169D01 e REN105L03, totalizando 21 regiões localizadas em cromossomos autossômicos e uma região (AMEL – amelogenina) localizada nos cromossomos sexuais (X e Y). (ISAG STANDING COMMITTEE, 2016b). Na Tabela 3, são apresentados os dados referentes aos marcadores genéticos que compõem o painel, como o cromossomo

[2] Disponível em: <http://www.isag.us>. Acesso em: 12 jan. 2017.

de localização, o motivo da repetição (dinucleotídeos, trinucleotídeos, tetranucleotídeos etc.), a sequência das repetições, a sequência dos *primers* e o comprimento dos *amplicons*.

Tabela 1 – Painel de marcadores STR recomendados pela ISAG para a identificação genética de equinos

Locus	**Cromossomo**	**Estrutura de repetição**	**Sequência repetitiva**	**Sequências dos *primers* (*Forward e Reverse*)**	**Comprimento do amplicon (pb)**
AHT4	24	Composta	$(AC)_nAT(AC)_n$	F: AACCGCCTGAGCAAGGAAGT R: CCCAGAGAGTTTACCCT	144-164
AHT5	8	Simples	$(GT)_n$	F: ACGGACACATCCCTGCCTGC R: GCAGGCTAAGGAGGCTCAGC	126-144
ASB2	15	Simples	$(GT)_n$	F: CCACTAAGTGTCGTTTCAGAAGG R: CACAACTGAGTTCTCTGATAGG	216-250
ASB17	2	Simples	$(AC)_n$	F: ACCATTCAGGATCTCCACCG R: GAGGGCGGTACCTTTGTACC	87-129
ASB23	3	Simples e Composta	$(TG)_n$ e $(TG)_nTT(TG)_4$	F: GAGGGCAGCAGGTTGGGAAGG R: ACATCCTGGTCAAATCACAGTCC	175-211
CA425	28	Simples	$(GT)_n$	F: AGCTGCCTCGTTAATTCA R: CTCATGTCCGCTTGTCTC	226-246
HMS1	15	Simples	$(TG)_n$	F: CATCACTCTTCATGTCTGCTTGG R: TTGACATAAATGCTTATCCTATGGC	170-186
HMS2	10	Composta	$(CA)_n(TC)_2$	F: CTTGCAGTCGAATGTGTATTAAATG R: ACGGTGGCAACTGCCAAGGAAG	222-248
HMS3	9	Composta	$(TG)_2(CA)_2TC(CA)_n$ e $(TG)_2(CA)_2TC(CA)_nGA(CA)_5$	F: CCATCCTCACTTTTTCACTTTGTT R: CCAACTCTTTGTCACATAACAAGA	148-170
HMS6	4	Simples	$(GT)_n$	F: GAAGCTGCCAGTATTCAACCATTG R: CTCCATCTTGTGAAGTGTAACTCA	151-169
HMS7	1	Composta	$(AC)_2(CA)_n$	F: TGTTGTTGAAACATACCTTGACTGT R: CAGGAAACTCATGTTGATACCATC	165-185
HTG4	9	Complexa	$(TG)_nAT(AG)_5AAG(GA)_5ACAG(AGGG)_3$	F: CTATCTCAGTCTTGATTGCAGGAC R: CTCCCTCCCTCCCTCTGTTCTC	127-139
HTG6	15	Simples	$(TG)_n$	F: GTTCACTGAATGTCAAATTCTGCT R: CCTGCTTGGAGGCTGTGATAAGAT	84-102
HTG7	4	Simples	$(GT)_n$	F: CCTGAAGCAGAACATCCCTCCTTG R: ATAAAGTGTCTGGGCAGAGCTGCT	118-128
HTG10	21	Simples e Composta	$(TG)_n$ e $TATC(TG)_n$	F: TTTTTATTCTGATCTGTCACATTT R: CAATTCCCGCCCCACCCCCGGCA	95-115
LEX3	X	Simples	$(TG)_n$	F: ACATCTAACCAGTGCTGAGACT R: GAAGGAAAAAAAGGAGGAAGAC	142-164
VHL20	30	Simples	$(TG)_n$	F: CAAGTCCTCTTACTTGAAGACTAG R: AACTCAGGGAGAATCTTCCTCAG	87-105

Fonte: disponível em: <http://www.cstl.nist.gov/strbase/horseSTRs.htm>. Acesso em: 12 jan. 2017.

Tabela 2 – Painel de marcadores STR recomendados pela ISAG para a identificação genética de bovinos

Locus	**Cromossomo**	**Estrutura de repetição**	**Sequência repetitiva**	**Sequências dos *primers* (*Forward e Reverse*)**	**Comprimento do amplicon (pb)**
BM1818	23	Simples	$(TG)_n$	F: AGCTGGGAATATAACCAAAGG R: AGTGCTTTCAAGGTCCATGC	253-277
BM1824	1	Simples	$(GT)_n$	F: GAGCAAGGTGTTTTTCCAATC R: CATTCTCCAACTGCTTCCTTG	176-188
BM2113	2	Simples	$(CA)_n$	F: GCTGCCTTCTACCAAATACCC R: CTTCCTGAGAGAAGCAACACC	124-146
ETH3	19	Composta	$(GT)_nAC(GT)_6$	F: GAACCTGCCTCTCCTGCATTGG R: ACTCTGCCTGTGGCCAAGTAGG	100-128
ETH10	5	Simples	$(AC)_n$	F: GTTCAGGACTGGCCCTGCTAACA R: CCTCCAGCCCACTTTCTCTTCTC	206-222
ETH225	9	Composta	$(TG)_4CG(TG)(CA)_n$	F: GATCACCTTGCCACTATTTCCT R: ACATGACAGCCAGCTGCTACT	139-157
INRA023	3	Simples	$(AC)_n$	F: GAGTAGAGCTACAAGATAAACTTC R: TAACTACAGGGTGTTAGATGAACTC	201-225
SPS115	15	Composta	$(CA)_nTA(CA)_6$	F: AAAGTGACACAACAGCTTCACCAG R: AACCGAGTGTCCTAGTTTGGCTGTG	247-261
TGLA53	16	Composta	$(TG)_6CG(TG)_4(TA)_n$	F: GCTTTCAGAAATAGTTTGCATTCA R: ATCTTCACATGATATTACAGCAGA	151-187
TGLA122	21	Composta	$(AC)_n(AT)_n$	F: AATCACATGGCAAATAAGTACATAC R: CCCTCCTCCAGGTAAATCAGC	136-182
TGLA126	20	Simples	$(TG)_n$	F: CTAATTTAGAATGAGAGAGGCTTCT R: TTGGTCCTCTATTCTCTGAATATTCC	111-127
TGLA227	18	Simples	$(TG)_n$	F: GGAATTCCAAATCTGTTAATTTGCT R: ACAGACAGAAACTCAATGAAAGCA	76-104

Fonte: disponível em: <http:// http://www.cstl.nist.gov/strbase/cattleSTRs.htm>. Acesso em: 12 jan. 2017.

Tabela 3 – Painel de marcadores STR recomendados pela ISAG para a identificação genética de caninos

Locus	**Cromossomo**	**Motivo da repetição**	**Sequências dos *primers* (*Forward e Reverse*)**	**Comprimento do amplicon (pb)**
AHT121	13	di	F: TATTGCGAATGTCACTGCTT R: ATAGATACACTCTCTCTCCG	68-118
AHT137	11	di	F: TACAGAGCTCTTAACTGGGTCC R: CCTTGCAAAGTGTCATTGCT	126-156
AHTh130	36	di	F: GTTTCTCTCCCTTCGGGTTC R: GACGTGTGTTCACGCCAG	111-141
AHTh171	6	di	F: AGGTGCAGAGCACTCACTCA R: CCCATCCACAGTTCAGCTTT	215-239
AHTh260	16	di	F: CAGCAGCAGCCGCTATACCCACACCAGGAC R: CCACAGAGGAAGGGATGC	236-254
AHTk211	26	di	F: TTAGCAGCCGAGAAATACGC R: ATTCGCCCGACTTTGGCA	83-101
AHTk253	23	di	F: ACATTTGTGGCATTGGGGCTG R: TGCACATGGAGGACAAGCACGC	277-297
CXX0279	22	di	F: TGCTCAATGAAATAAGCCAGG R: GGCGACCTTCATTCTCTGAC	109-133
FH2054	12	tetra	F: GCCTTATTCATTGCAGTTAGGG R: ATGCTGAGTTTTGAACTTTCCC	135-179
FH2848	2	di	F: CAAAACCAACCCATTCACTC R: GTCACAAGGACTTTTCTCCTG	228-244
INRA021	21	di	F: ATGTAGTTGAGATTTCTCCTACGG R: TAATGGCTGATTTATTTGGTGG	87-11
INU005	33	di	F: CTTTCTACCAGCAAGGTTAC R: TTCCCATTTAATTGCCTCT	104-136
INU030	12	di	F: GGCTCCATGCTCAAGTCTGT R: CATTGAAAGGGAATGCTGGT	143-157
INU055	33	di	F: CCAGGCGTCCCTATCCATCT R: GCACCACTTTGGGCTCCTTC	204-220
REN105L03	11	di	F: GGAATCAAAAGCTGGCTCTCT R: GAGATTGCTGCCCTTTTTACC	231-249
REN162C04	7	di	F: TTCCCTTTGCTTTAGTAGGTTTTG R: TGGCTGTATTCTTTGGCACA	192-212
REN169D01	14	di	F: AGTGGGTTGCAAGTGGAAC R: AATAGCACATCTTCCCCACG	199-221
REN169O18	29	di	F: CACCCAACCTGTCTGTTCCT R: ACTGTGTGAGCCAATCCCTT	154-170
REN247M23	15	di	F: TGGTAACACCAAGGCTTTCC R: TGTCTTTTCCATGGTGGTGA	268-282

Locus	Cromossomo	Motivo da repetição	Sequências dos *primers* (***Forward e Reverse***)	Comprimento do amplicon (pb)
REN54P11	18	di	F: GGGGGAATTAACAAAGCCTGAG R: TGCAAATTCTGAGCCCCACTG	224-242
REN64E19	34	di	F: T TGTATTTTAATGTGGCAGTTT R: GACAAGGACAGGCAATACAGT	139-155
AMEL	X	-	F: GTGCCAGCTCAGCAGCCCGTGGT R: TCGGAGGCAGAGGTGGCTGTGGC	182-217

Fonte: (ISAG, 2005)

1.2.2 *Kits* comerciais para a genotipagem de equinos, bovinos e caninos

Devido ao fato de ainda ser de certa forma recente o consenso da ISAG quanto aos marcadores STR recomendáveis, não há muitas opções no mercado de *kits* comerciais para genotipagem de equinos, bovinos e caninos, existindo, no momento, apenas dois tipos de produtos para este fim: *StockMarks® Horse, Cattle, and Dog Genotyping Kit*s e *Equine, Bovine and Canine Genotypes™*. Originariamente, os *kits* StockMarks® foram desenvolvidos e eram comercializados pela empresa norte-americana *Applied Biosystems*, enquanto os produtos *Equine, Bovine and Canine Genotypes™* foram desenvolvidos e eram comercializados pela empresa finlandesa *Finnzymes Diagnostic*. Contudo, devido à ocorrência de fusões e aquisições, todos estes produtos são comercializados, desde 2014, pela empresa norte-americana *Thermo Fisher Scientific*.

1.2.2.1 StockMarks®

Desenvolvido no início dos anos 2000, os *kits* comerciais StockMarks® para equinos, bovinos e caninos foram os primeiros painéis contendo marcadores genéticos STR a serem lançados no mercado.

Utilizando a eletroforese capilar para a separação dos fragmentos (regiões do DNA de interesse), estes produtos contêm iniciadores (*primers*) marcados com compostos fluorescentes, amplificando marcadores STR recomendados pela ISAG (equinos e bovinos) e/ou pelo American Kennel Club (AKC).

Sintetizados para genotipar 100 animais utilizando a Reação em Cadeia da Polimerase (PCR) *multiplex* (várias regiões do DNA amplificadas na mesma reação), os componentes dos três *kits* são basicamente os mesmos, sendo estes:

- solução-tampão;
- $MgCl_2$ (apenas para o *kit* canino);
- *mix* de dNTP;
- *mix* de *primers* marcados e não marcados com fluorescência *(forward* e *reverse)* previamente misturados;
- DNA controle (concentração de 20 ng/µL para os *kits* equino e bovino e 10 ng/µL para o canino) para verificar a correta amplificação e detecção dos *loci* específicos de cada espécie animal;
- *AmpliTaq Gold DNA Polimerase* (versão modificada da enzima *AmpliTaq DNA Polimerase*) fornecida em estado inativo, sendo ativada através de um passo pré-PCR de 9-12 minutos a 95°C. Uma vez ativada, a enzima tem a mesma atividade, estabilidade térmica e meia-vida a 95°C que *AmpliTaq DNA Polymerase*.

As regiões do DNA que são amplificadas para cada espécie são as seguintes:

- *equinos*: VHL20, HTG4, AHT4, HMS7, HTG6, AHT5, HMS6, ASB23, ASB2, HTG10, HTG7, HMS3, HMS2, ASB17, LEX3, HMS1 e CA425, totalizando 17 regiões amplificadas na mesma PCR;
- *bovinos*: TGLA227, BM2113, TGLA53, ETH10, SPS115, TGLA126, TGLA122, INRA23, ETH3, ETH225 e BM1824, totalizando 11 regiões amplificadas na mesma PCR.
- *caninos*: PEZ1, FHC2054, FHC2010, PEZ5, PEZ20, PEZ12, PEZ3, PEZ6, PEZ8 e FHC2079, totalizando 11 regiões amplificadas na mesma PCR.

Os *kits* StockMarks® foram lançados no mercado anteriormente ao consenso da ISAG sobre os marcadores genéticos e nunca foram atualizados, tanto em relação à nomenclatura dos STR, como no caso do *kit* para cães, como a respeito do sistema de detecção de cinco fluorescências para os três produtos, comumente utilizado na comunidade forense. Além disso, os *kits* não incluem escadas alélicas, o que exige um nível de familiaridade e conhecimento científico incompatível com os laboratórios forenses (KANTHASWAMY, 2009).

1.2.2.2 Equine, Bovine and Canine Genotypes™

Os *kits Equine, Bovine and Canine Genotypes™* foram lançados entre 2007 e 2009, propiciando uma nova alternativa no mercado desse tipo de produto para a identificação genética dessas três espécies, além de possuir melhorias em relação aos *kits StockMarks®.*

As principais diferenças entre os *kits Genotypes™* e os *StockMarks®* referem-se aos componentes inclusos em cada produto (para as três espécies) e ao número de marcadores genéticos (bovinos e caninos).

Referente aos componentes, os *kits Genotypes™* para equinos, bovinos e caninos, percebe-se um avanço no desenvolvimento destes produtos, pois os reagentes para a reação de PCR foram agrupados em menos soluções, diminuindo consideravelmente os possíveis erros de manipulação e pipetagem. São componentes de cada *kit Genotypes™*:

- *Master Mix*: composto de solução-tampão, $MgCl_2$, dNTPs (dATP, dCTP, dGTP and dTTP) e *Phusion Hot Start DNA Polymerase*;
- *mix* de *primers* marcados e não marcados com fluorescência *(forward* e *reverse)* previamente misturados;
- DNA controle (concentração de 1,0 ng/µL para os *kits* equino e canino e 0,5 ng/µL para o bovino) para verificar a correta amplificação e detecção dos *loci* específicos de cada espécie animal.

As regiões do DNA que são amplificadas para cada espécie são as seguintes:

- *equinos*: VHL20, HTG4, AHT4, HMS7, HTG6, AHT5, HMS6, ASB23, ASB2, HTG10, HTG7, HMS3, HMS2, ASB17, LEX3, HMS1 e CA425, totalizando 17 regiões amplificadas na mesma PCR;
- *bovinos*: TGLA227, BM2113, TGLA53, ETH10, SPS115, TGLA126, TGLA122, INRA23, BM1818, ETH3, ETH225, BM1824, SPS113, RM067, CSRM60, MGTG4B, CSSM66 e ILSTS006, totalizando 18 regiões amplificadas na mesma PCR;
- *caninos*: AHTK211, CXX279, REN169O18, INU055, REN54P11, INRA21, AHT137, REN169D01, AHTH260, AHTK253, INU005, INU030,

Amelogenina, FH2848, AHT121, FH2054, REN162C04, AHTH171 e REN247M23, totalizando 19 regiões amplificadas na mesma PCR.

Da mesma forma que os *kits* StockMarks®, os *kits Genotypes™* não incluem escadas alélicas.

1.2.2.3 Painel de marcadores STR in house

Muitos laboratórios que realizam o serviço de genotipagem de animais preferem elaborar seus próprios painéis de marcadores STR *(in house*), sintetizando os *primers* dos *loci* recomendados pela ISAG. Esta prática é muitas vezes aplicada para contornar problemas que existem quando a oferta de produtos no mercado é limitada. Se por um lado os *kits* StockMarks® são obsoletos, sem nenhuma melhoria desde o seu lançamento, fornecendo resultados que despendem muito tempo de análise e interpretação, os *kits Genotypes™*, embora melhores se comparados aos primeiros, podem ter um custo elevado, prejudicando, muitas vezes, o orçamento do laboratório que desenvolve esta atividade. Além disso, nem sempre os produtos possuem todos os marcadores genéticos recomendados pela ISAG, como é caso do *kit* para a identificação canina.

A desvantagem em elaborar e utilizar o próprio painel de marcadores genéticos na rotina laboratorial é que a maioria não é elaborada para amplificar todos os *loci* necessários em apenas uma reação de PCR, requisitando maior quantidade de DNA, além de aumentar o custo de cada genotipagem (WICTUM *et al.*, 2013a). Além disso, muitas vezes são observados resultados divergentes de laboratório para laboratório, por motivos diversos e também porque nem todos adotam programas de controle de qualidade internos e externos rígidos e consistentes o suficiente para certificação de que os resultados condizem com o que seria esperado. Assim, existe uma necessidade de que os painéis *in house* sejam validados por normas que possuam confiabilidade e aceitação científica.

1.3 ESTUDOS GENÉTICOS POPULACIONAIS

Para que a identificação genética animal e suas tantas aplicações (como identificação individual, testes de vínculo genético, rastreamento de produtos de

origem animal, programas de melhoramento genético etc.) possam ter sucesso e sejam realmente eficientes e informativas, é fundamental que se realizem estudos e análises com o maior número de indivíduos semelhantes da mesma população, para a criação de um banco de dados genéticos. Nos estudos populacionais, devem ser calculados e obtidos alguns índices e parâmetros que permitam determinar a probabilidade de uma amostra pertencer, de fato, ao animal testado ou a qualquer outro indivíduo da população, extrapolando ao máximo a possibilidade de coincidência e casualidade.

Os bancos de dados genéticos são repositórios de armazenamento a longo prazo de amostras ou perfis de DNA. Cada perfil constante em um banco é único, ou seja, pertence a um só indivíduo (à exceção ocorre quando há genótipos de gêmeos univitelinos) e podem ser utilizados para estimar a probalidade de uma determinada amostra pertencer ou não a certa pessoa ou a outro ser vivo (MCEWEN; REILLY, 1994; SCHECK; CARDOZO, 1994). O principal objetivo ao criar um banco de dados genéticos de uma população é encontrar os alelos mais comuns e testá-los várias vezes para estimar, de forma confiável, a frequência em que aparecem no grupo populacional em consideração. O ideal seria que tal banco contivesse os genótipos de todos os indivíduos de uma população específica, para permitir avaliações extremamente precisas sobre a frequência dos alelos. Contudo, por questões práticas, como o custo e o tempo necessários para o estabelecimento de um banco de dados genéticos com os dados de toda a população, é possível estimar com confiança as frequências dos alelos e dos genótipos em toda a população a partir de um subgrupo que represente o grupo maior de forma homogênea (BUTLER, 2005).

Assim, a primeira etapa a ser cumprida quando se quer realizar um estudo populacional e o estabelecimento de um banco de dados genéticos é a determinação do número de amostras a serem testadas, com grupos étnicos ou raciais bem definidos em que, preferencialmente, os indivíduos participantes não sejam relacionados, ou seja, não tenham nenhum tipo de vínculo genético (BUTLER, 2005).

Embora não se tenha uma regra ou um consenso sobre o número mínimo, em populações humanas, por exemplo, estima-se que a análise de 500 indivíduos pode fornecer uma amostragem adequada para um determinado marcador genético (CARRACEDO *et al.*, 2014). Todavia, sabe-se que quanto mais indivíduos da

mesma população forem usados no estudo, maior será a precisão na contagem dos alelos e na estimativa de suas frequências (FOREMAN; EVETT, 2001).

A recomendação de que os indivíduos participantes do estudo populacional não sejam relacionados tem por finalidade principal aumentar a precisão das estimativas de frequência de alelos, isto é, observar os que mais representam a população estudada (BUTLER, 2005). Contudo, quando se trata de animais domésticos e estes são de uma determinada raça, é muito mais difícil selecionar os participantes do estudo, pois, devido à pressão de seleção para a manutenção dos padrões genéticos raciais, há muita chance de que os indivíduos, de certa forma, sejam relacionados ou apresentem algum grau de consanguinidade. Posteriormente, será abordada a questão da consaguinidade e dos endocruzamentos.

Uma vez obtidos os genótipos dos indivíduos que representam uma significativa amostra da população estudada, é necessário assegurar que o banco de dados genético que está sendo gerado seja útil quando a identificação individual for requisitada. Através de testes estatísticos, são confirmadas a validade e a utilidade dos bancos de dados, sendo os mais importantes o Teste de Equilíbrio de Hardy-Weinberg e o Equilíbrio de Ligação (LE, do inglês *Linkage Equilibrium*). Outros parâmetros como a heterozigosidade (esperada e observada), o Conteúdo de Informação de Polimorfismo, o Poder de Exclusão, o Poder de Discriminação e a Probabilidade de Identidade também são índices que devem medidos quando são realizados estudos populacionais utilizando um grupo de marcadores genéticos STR (painel) para estimar o quão informativo pode ser cada *locus*, além de ser conhecido o real poder de discriminação individual quando o painel for utilizado para este fim.

1.3.1 Teste de Equilíbrio de Hardy-Weinberg

O equilíbrio de Hardy-Weinberg (HWE, do inglês *Hardy-Weinberg equilibrium*), ou o princípio do equilíbrio gênico, prevê a estabilidade das frequências dos alelos e genótipos de uma geração a outra. A principal finalidade para realizar o teste HWE, quando se está estimando frequências dos alelos, é determinar se, dentro de um *locus*, estes são independentes. Quando um *locus* é geneticamente estável, as frequências não devem mudar ao longo de muitas gerações.

As condições necessárias para que uma população se mantenha em equilíbrio gênico, segundo Hardy e Weinberg, são as seguintes:

- a população deve ser muito grande (teoricamente, quanto maior, melhor), de modo que possam ocorrer todos os tipos de cruzamento possíveis, de acordo com as leis de probabilidades;
- a população deve ser panmítica, isto é, os cruzamentos entre indivíduos de diferentes genótipos devem ocorrer ao acaso, sem qualquer preferência.

Assim, uma população que possua essas características, e na qual não ocorra nenhum fator evolutivo, tais como mutação, seleção ou migração, permanecerá em equilíbrio gênico, ou seja, as frequências dos alelos não sofrerão alteração ao longo das gerações. No entanto, é muito difícil que as populações atendam a todas as condições do princípio de HWE, pois o violam em algum grau, ocasionando a alteração da frequência dos alelos com o passar do tempo.

Sendo os alelos herdados de forma mendeliana, é possível prever a ocorrência destes seguindo um padrão previsível de probabilidade. As frequências de alelos são utilizadas para gerar frequências genotípicas esperadas que são então comparadas com as frequências genotípicas observadas. Se os valores observados e esperados forem semelhantes, então presume-se que os alelos dentro do *locus* são estáveis e estão em equilíbrio.

Outra finalidade de realizar o teste de HWE é procurar quaisquer indicações de excesso de homozigose, quando o número de homozigotos observados apresenta diferença significativa em relação número de homozigotos esperados (BUTLER, 2005).

Normalmente, quando são realizados testes para estimar o HWE em um determinado *locus*, utiliza-se o nível de significância (valor de *P*) igual a 0,05. Assim, após a realização do teste de HWE, se o *locus* apresentar valores maiores que 0,05, pode-se dizer que a população está em equilíbrio neste marcador genético. Entretanto, testes de HWE com valores abaixo de 0,05 indicam desequilíbrio, e o resultado é estatisticamente significativo. Contudo, quando são realizados testes independentes múltiplos, como é o caso do estabelecimento de frequências alélicas e genotípicas de marcadores genéticos STR de uma população, pode ser feita a correção de Bonferroni para ajustar o nível de significância (valor de *P*) entre todos

os testes realizados. Assim, aplica-se a fórmula *P* = 0,05/m, sendo “m” o número de testes realizados e o novo valor de *P* obtido é utilizado para avaliar se os resultados dos testes de HWE são estatisticamente significativos ou não (HARTL; CLARK, 2010).

1.3.2 Teste de Desequilíbrio de Ligação

Combinar as informações de múltiplos *loci* fortalece as análises estatísticas e torna o uso dos marcadores genéticos STR uma poderosa ferramenta de identificação individual. No entanto, para que isso ocorra, é preciso que os *loci* sejam independentes uns dos outros, ou seja, que ocorra a recombinação entre eles, durante o processo meiótico. Quando os *loci* STR ou qualquer outra sequência são transferidos independentemente de outro segmento de DNA durante a meiose, diz-se que essas duas estão em equilíbrio de ligação.

Este equilíbrio de ligação pode ser alcançado quando todas aquelas condições do equilíbrio de Hardy-Weinberg (cruzamento aleatório, população grande sem mutação, migração ou seleção) são verificadas. Porém, ao contrário do HWE, que precisa de apenas de poucas gerações para ocorrer, o equilíbrio de ligação ocorre de forma lenta e gradual. A velocidade para atingir o equilíbrio de ligação irá depender da taxa de recombinação para os genótipos heterozigotos para ambos os *loci* envolvidos.

A frequência de recombinação entre os *loci* irá depender se estiverem localizados no mesmo cromossomo e da distância física entre eles. Quanto mais próximos os *loci* estiverem um do outro, menor será a chance da quebra e de a união ocorrer na região entre os dois, e assim a migração destes no processo meiótico ocorre em bloco, sem o acontecimento da recombinação cromossômica. Quando esta situação ocorre, se diz que há um desequilíbrio de ligação entre os *loci.* Por consequência, quanto maior a distância, mais provável é a ocorrência da recombinação entre os dois locais, favorecendo o equilíbrio de ligação. É importante salientar, porém, que para ocorrer o desequilíbrio de ligação não é necessário que os *loci* estejam fisicamente ligados. Há também desequilíbrio de ligação entre *loci* localizados em cromossomos distintos (HARTL; CLARK, 2010). Por isso, quando são estimadas as frequências alélicas de um grupo de marcadores genéticos, é

recomendável que seja feito algum tipo de teste de desequilíbrio de ligação entre todos os *loci* utilizados para verificar se está ocorrendo. Se não há certeza de que todos os marcadores genéticos e seus alelos foram herdados de forma independente, os resultados obtidos, quando se utilizam estes dados, podem perder o devido valor e significado.

1.3.3 Heterozigosidade esperada (He) e heterozigosidade observada (Ho)

A heterozigosidade esperada (He), também conhecida como diversidade genética, é um dos parâmetros calculados para estudar a variação genética em uma população. Uma das interpretações aplicadas aos valores da He é a probabilidade que um indivíduo tem em ser heterozigoto em um dado *locus*. Ela fornece informações sobre a estrutura e a história de uma população. Valores altos de heterozigosidade significam que existe mais diversidade de alelos e, portanto, há menos chance de que a correspondência a uma determinada amostra tenha ocorrido de forma aleatória. Valores muito baixos de heterozigosidade, por outro lado, podem indicar que o tamanho de dada população é pequeno ou que a variabilidade genética é reduzida (BUTLER, 2005; NEI, 1978). O valor pode variar de 0 (sem heterozigose) a quase 1 (quanto mais alto, mais as frequências alélicas estarão próximas da igualdade). A heterozigosidade observada (Ho) é calculada dividindo-se o número de amostras contendo alelos heterozigóticos pelo número total de amostras. O valor de Ho geralmente é comparado com a He para ajustes ao teste de HWE.

1.3.4 Conteúdo de Informação de Polimorfismo

Podemos definir polimorfismo, do ponto de vista genético, como a variação genotípica existente dentro das populações (HARTL; CLARK, 2010). No nível molecular, o termo polimorfismo é mais frequentemente usado para descrever a variação genotípica, incluindo a variabilidade de alelos e alterações dentro de um gene ou de um genoma. O Conteúdo de Informação de Polimorfismo (PIC, do inglês, *Polymorphism Information Content*), termo que passou a ser utilizado nas análises de vínculo genético através do DNA, reflete a probabilidade de que uma

determinada prole ou um indivíduo, de um progenitor portador de um alelo raro num *locus*, permita a dedução do genótipo parental quando os genótipos destes forem analisados (BOTSTEIN *et al*., 1980). O valor de PIC será quase 0 se não houver variação alélica e pode atingir um máximo de 1 se um genótipo tiver apenas novos alelos, o que é um fenômeno raro. É usado, principalmente, para avaliar a diversidade de um gene ou segmento de DNA em uma população.

1.3.5 Poder de Exclusão, Poder de Discriminação e a Probabilidade de Identidade

Quando são realizados estudos populacionais para fins de identificação, seja na área forense ou em programas de melhoramento genético, é recomendável estimar parâmetros que indiquem quais são os *loci* com maior poder para diferenciar indivíduos de uma mesma população.

Assim, parâmetros como o Poder de Exclusão (PE), o Poder de Discriminação (PD) e a Probabilidade de Identidade (P_{ID}) são geralmente calculados e aparecem em publicações de estudos com as mais diversas populações e espécies, especialmente aquelas para o uso em análises de vínculo, de parentesco e em testes de paternidade.

O Poder de Exclusão de um marcador genético expressa a probabilidade que indivíduos de uma população possuam o genótipo diferente daquele selecionado randomicamente. Através da multiplicação (produto) de todos os valores de PE (PE combinado) de um grupo de marcadores STR, podemos estimar o quão poderoso é o painel em excluir um genótipo em particular selecionado ao acaso dentro de um banco de dados genético (HUSTON, 1998).

Já o Poder de Discriminação (PD) está relacionado com a correspondência aleatória, a probabilidade de identificar corretamente um indivíduo selecionado ao acaso (REIS *et al*., 2008). O produto de todos os PD (PD combinado) de um grupo de marcadores STR expressa o quão poderoso é o painel para a individualização de uma determinada amostra entre todas as testadas.

A Probabilidade de Identidade (P_{ID}) pode ser definida como a chance de que dois indivíduos selecionados aleatoriamente a partir de uma população tenham o mesmo genótipo em múltiplos *loci* (WAITS; LUIKART; TABERLET, 2001).

1.3.6 Estrutura genética de populações

As populações, em sua maioria, são agrupadas em subpopulações menores e são nestas que, geralmente, ocorrem os cruzamentos. Esse agrupamento é chamado de subdivisão populacional ou estrutura populacional, sendo observado na natureza na forma de rebanhos, bandos, cardumes, colônias etc. Quando há subdivisão populacional, é comum observar que as frequências dos alelos são diferentes em cada subpopulação, o que pode ser chamado de diferenciação genética. Esta pode ser resultado de seleção natural em favor de diferentes genótipos, mas também de processos aleatórios na transmissão dos alelos de uma geração para a próxima ou de diferenças casuais na frequência alélica entre os fundadores iniciais das subpopulações (HARTL; CLARK, 2010). Em algumas espécies de animais domésticos, a subdivisão populacional ocorre na forma das raças, em que indivíduos da mesma espécie foram e são selecionados artificialmente pelo homem, a fim de que determinadas características fenotípicas ou genotípicas sejam transmitidas às proles, conforme a necessidade ou a intenção. Essas características e habilidades podem ser em relação ao peso, ao tamanho, à conformação anatômica, à fertilidade, à produção de leite e à qualidade da carne (no caso dos bovinos, por exemplo), além de comportamento e temperamento etc. (LUSH, 1964).

Para que animais possam ser considerados de mesma raça, é fundamental que compartilhem dos mesmos traços, principalmente fenotípicos, que os caracterizem como pertencentes ao mesmo subgrupo populacional. Em programas de melhoramento genético animal, recomenda-se que sejam utilizados para a manutenção e a pureza da raça aqueles animais que melhor expressam os chamados padrões raciais, garantindo assim que as proles herdem essas características e estas possam ser transmitidas às próximas gerações (LUSH, 1964).

Assim, para garantir que as características desejáveis sejam transmitidas às gerações seguintes, é muito comum nas mais variadas raças de animais domésticos que ocorram os endocruzamentos, que é a ocorrência de cruzamentos entre indivíduos aparentados, pois a probabilidade de que a prole receba aqueles caracteres de seleção é maior (HARTL; CLARK, 2010; LUSH, 1964). Um dos principais efeitos do endocruzamento ou da endogamia é a redução de genótipos

heterozigotos, diminuindo consequentemente a variabilidade genética. Além disso, com a homozigosidade aumentada (maior proporção de indivíduos homozigotos na população), aumentam os alelos recessivos raros, que podiam estar sendo ocultados pelos dominantes, e os efeitos indesejáveis normalmente ligados a estes podem começar a aparecer, tornando os animais endogâmicos com menor mérito (menos qualidade em suas características) do que aqueles que são frutos de cruzamentos entre indivíduos não aparentados (LUSH, 1964). Portanto, em um sistema regular de cruzamentos, que adota um padrão sistemático e repetido de endocruzamentos como o retrocruzamento de uma linhagem-padrão (HARTL; CLARK, 2010) de uma determinada raça, é importante saber com que rapidez e com que intensidade a endogamia está ocorrendo em uma população, raça e até mesmo em rebanhos. Uma das formas de estimar os níveis de endogamia de uma população é a utilização das Estatísticas de F de Wright.

1.3.6.1 Estatísticas F de Wright

As estatísticas F (F_{IS}, F_{ST} e F_{IT}), também chamadas de índices de fixação, foram elaboradas por Sewall Green Wright para descrever as propriedades das populações subdivididas. Considerando a população como um todo (*T*), suas subpopulações (*S*) e seus indivíduos (*I*), foram definidos os seguintes parâmetros: F_{IT} como a correlação entre os gametas que se unem para formar os indivíduos em relação à população, F_{ST} como a correlação entre gametas tomados ao acaso nas subpopulações e mede o nível de diferenciação genética entre subpopulações e F_{IS} como a correlação entre gametas que se unem para produzir indivíduos com relação à subpopulação (WRIGHT, 1951). O coeficiente de endocruzamento (F_{IS}) pode ser definido também como a probabilidade que dois alelos de um *locus* em um indivíduo endocruzado sejam idênticos por descendência.

Os índices de fixação são úteis indicadores de diferenciação genética, permitindo uma comparação objetiva do efeito geral da estrutura populacional entre diferentes organismos ou indivíduos, sem entrar em detalhes de frequências alélicas, níveis observados de heterozigosidade etc. (HARTL; CLARK, 2010).

F_{ST} é a estatística informativa usada para examinar o nível de divergência genética entre subpopulações, e embora tenha um mínimo teórico de 0 (nenhuma

divergência genética) e um máximo teórico de 1 (fixação de alelos alternativos em diferentes populações), geralmente, na prática, os valores observados são bem menores que 1 (HARTL; CLARK, 2010). Para a interpretação dos valores de F_{ST}, Wright sugeriu as seguintes orientações (WRIGHT, 1978):

- a amplitude de 0 a 0,05 pode ser considerada indicativa de pequena diferenciação genética;
- a amplitude de 0,05 a 0,15 indica moderada diferenciação genética;
- a amplitude de 0,15 a 0,25 indica grande diferenciação genética;
- valores de F_{ST} acima de 0,25 indicam diferenciação genética muito grande.

1.3.7 Distância genética

A análise da distância genética revela a relação entre as populações e é útil para reconstruir também a relação entre indivíduos. Semelhanças genéticas e dissimilaridades podem ser observadas entre duas populações ou indivíduos, e ser usadas para a caracterização de diferentes raças e para a avaliação da variação das espécies através do tempo (NAQVI, 2007). Na análise da distância genética, a diferença genética entre populações é avaliada com base na diferença entre distribuições de frequências de alelos em vários *loci* ou com base na distribuição de tamanho de alelos (LAVAL; SANCRISTOBAL; CHEVALET, 2002).

Diversas medidas de distância genética foram desenvolvidas para a análise de dados de marcadores moleculares e podem ser classificadas em duas categorias principais: aquelas sem suposições biológicas, também chamadas distâncias geométricas como Cavalli-Sfroza (CAVALLI-SFORZA; EDWARDS, 1967); e as com suposições biológicas ou modelos como o de Nei (NEI, 1972, 1978), a distância de Reynold (REYNOLDS; WEIR; COCKERHAM, 1983) e a distância de Goldstein (GOLDSTEIN *et al.*, 1995). As medidas de distância com pressupostos biológicos baseiam-se nas forças evolutivas que poderiam resultar em variação alélica. Duas forças evolutivas, como mutação e a deriva genética, são usadas para fazer suposições ou modelos. A seleção natural, outra força evolutiva, não é geralmente usada para fazer suposições porque assumimos que estamos usando marcadores genéticos que não estão sujeitos à seleção natural.

2 OBJETIVOS

2.1 OBJETIVOS GERAIS

Os objetivos gerais desta tese são:

a) avaliar se os painéis de marcadores STR, recomendados pela Sociedade Internacional de Genética Animal (ISAG) para a identificação genética de equinos, bovinos e caninos são, de fato, altamente informativos e se estão em número suficiente para discriminar individualmente animais da mesma espécie e da mesma raça com um grau de certeza que se aproxime ao máximo dos 100%; e
b) estudar a estrutura genética de raças equinas, bovinas e caninas de animais criados no Uruguai, no Paraguai e no Brasil (Rio Grande do Sul), utilizando marcadores genéticos STR.

2.2 OBJETIVOS ESPECÍFICOS

Os objetivos específicos são:

a) obter a frequência alélica e outros índices de interesse forense em 17 marcadores STR em equinos de cinco raças presentes no Uruguai e estimar a distância genética existente entre as diferentes raças;
b) obter a frequência alélica e outros índices de interesse forense em 12 marcadores STR em bovinos de dez raças presentes no Uruguai e seis raças presentes no Paraguai e estimar a distância genética existente entre animais de raças distintas e mesma região geográfica, bem como a distância genética existente entre animais de mesma raça, mas de regiões geográficas diferentes; e
c) obter a frequência alélica e outros índices de interesse forense em 21 marcadores STR em cães de seis raças presentes no Rio Grande do Sul e estimar a distância genética existente entre as diferentes raças.

3 DESENVOLVIMENTO

3.1 ARTIGO CIENTÍFICO PUBLICADO[3]

Dear Editor,

It is known that horses (*Equus caballus*) had an important role in many activities that influenced the development of various civilizations around the world. For many reasons, men set the selection of animals according to ability, skills and disposition desired, giving rise of specific groups of horses which have distinctive characteristics transmitted to offspring consistently, the breeds. It is estimated that there are currently about 300 breeds of horses[1]. Due to this ancient and close interaction between horses and humans, relevant forensically cases such as theft horses, identity fraud and, more recently, the sale of adulterated or counterfeited semen doses, and doping control are the most observed crimes. Therefore, it is recommended that forensic analysis techniques become more efficient as possible with increasingly improved accuracy.

Uruguay, a small country in the southeastern region of South America bordered by Argentina and Brazil, is characterized by the strength of the agricultural sector and livestock production. As the most recent data collected by OIE (World Organization for Animal Health), it is estimated that Uruguay has about 400,000 horses in its territory[2]. The Rural Association of Uruguay, the oldest institution of this country in agricultural matters, and the Uruguayan Stud Book, responsible for the registration and identification of all Thoroughbreds born in the country or imported, are pioneering institutions in Latin America in establishing typification of horses through DNA analysis[3-4]. For this reason, both institutions have grouped into their records thousands of genotypes of horses of various breeds representing significantly the horse population of Uruguay.

The aim of this paper is to present a genetic study of forensic interest of a total group up to 32,053 purebred horse of five different breeds: Appaloosa (n=109),

[3] GASTALDO, A. *et al.* Population genetic study over 32,000 equines from Uruguay using seventeen forensically informative STR loci. **Forensic Science International: Genetics**, v. 26, p. e19-e22, Jan. 2-17. Disponível em: <http://www.fsigenetics.com/article/S1872-4973(16)30196-X/abstract>. As cópias das páginas da revista que traz este artigo publicado podem ser verificadas no Anexo A desta tese.

Arabian (n=1,661), Uruguayan Criollo (n=5,906), Thoroughbred (n=23,203) and Quarter Horse (n=1,174), using the seventeen STR loci recommended by International Society for Animal Genetics (ISAG) for genotyping horses (http://www.isag.us/Docs/EquineGenParentage2014.pdf). All samples were obtained from animals subjected to pedigree registration on Rural Association of Uruguay or Uruguayan Stud Book. DNA was isolated from hair root or semen straws samples using proteinase K in two types of extraction buffer: Buffer 1 to hair root samples ($MgCl_2$, PCR Buffer and Tween 20) and Buffer 2 to semen straws samples (EDTA, SDS, Tris, NaCl and DTT). Final concentration after DNA extraction: 50-100 ng/µL of equine genomic DNA. The seventeen STR loci were amplified in a single PCR multiplex performed on Veriti™ Thermal Cycler (Applied Biosystems, Foster City, CA, USA) or GeneAmp 9700 PCR System (Applied Biosystems) using fluorescent labeled primers which the sequences are available on http://www.cstl.nist.gov/strbase/horseSTRs.htm. The other reagents for PCR amplification (PCR Buffer, AmpliTaq Gold DNA Polymerase, dNTP mix) were carried out using the StockMarks® for Horses Genotyping Kit (Applied Biosystems). The amount of equine genomic DNA was 25-50 ng/µL per sample. Thermocycling conditions were: pre-incubation for 10min at 95ºC, followed by thirty cycles of 30s at 95ºC, 30s at 60ºC and 60s at 72ºC, with a final incubation for 60min at 72ºC. The electrophoresis and typing were performed on an ABI 3500 Genetic Analyzer (Applied Biosystems) using GeneScan™ 500 LIZ® Size Standard as internal lane standards according to the manufacturer's protocols. The data was collected by Data Collection v1.0 software (Applied Biosystems) and analyzed using GeneMapper ID-X v1.3 software (Applied Biosystems).

Calculations of allele frequencies, observed (Ho) and expected (He) heterozygosity, polymorphism information content (PIC) and p-values of the Hardy-Weinberg equilibrium (HWE) test for all seventeen loci, were assessed using CERVUS version 3.0.3[5]. Bonferroni's correction was used for HWE test, which assumes that a 0.05 significance level obtained for seventeen tests (one per locus) yields an actual significance threshold of 0.0029[6]. Power of discrimination (PD), power of exclusion (PE) and probability of identity (P_{ID}) were estimated with PowerStats (Promega Corporation)[7]. Estimated coefficients of inbreeding (F_{IS}) within breeds, fixation indices (F_{ST}) among breeds, and total inbreeding (F_{IT}) using an

analysis of molecular variance (AMOVA) were performed with ARLEQUIN version 3.1[8] and GENEPOP version 4.5.1[9]. UPGMA trees were built from Nei's genetic distance matrix using the PHYLIP software package version 3.69[10] and visualized with TreeView software version 1.6.6[11]. The allele frequencies and forensically informative parameters are available in Supplementary Data (Tables S1-S5).

The Hardy-Weinberg equilibrium test showed significant deviation ($P<0.05$) in various loci in all five horse breeds of this study. Even after applying Bonferroni's correction using the total number of loci analyzed ($P<0.0029$), the differences observed were also statistically significant in many loci on four of the five breeds, except Appaloosa, as seen in Table S1. Regarding heterozygosity, in general, the observed (Ho) were lower than the expected (He) in almost all loci of five breeds. The loci with lower polymorphism information content (PIC) was HTG7 (Appaloosa, Arabian and Uruguayan Criollo) and HTG4 (Thoroughbred and Quarter Horse). The loci with higher PIC was ASB17 (Appaloosa and Uruguayan Criollo), VHL20 (Arabian), ASB2 (Thoroughbred) and LEX3 (Quarter Horse). The probability of identity (P_{ID}), that indicates probability of two individuals within the population sharing the same genotype, showed the following overall values for each breed: 2.15×10^{-17} (Appaloosa), 2.41×10^{-15} (Arabian), 1.15×10^{-18} (Uruguayan Criollo), 2.28×10^{-15} (Thoroughbred) and 5.95×10^{-18} (Quarter Horse). The values obtained for combined Power of Discrimination (PD) and combined Power of Exclusion (PE) for seventeen markers were, respectively: 0.99999999999999999 and 0.99999 in Appaloosa breed; 0.999999999999999 and 0.9999 in Arabian breed; 0.999999999999999999 and 0.99999 in Uruguayan Criollo breed; 0.999999999999999 and 0.9999 in Thoroughbred breed; 0.99999999999999999 and 0.99999 in Quarter Horse breed.

In Table 1 the values of F_{IS}, F_{ST} and F_{IT} for each of the seventeen loci and the overall can be found. The positive values of F_{IS} (Table 2) indicate heterozygote deficiency and sub-structuration in all five horse breeds, being Quarter Horse and Thoroughbred the breeds with higher level of inbreeding. Based on genotypic frequencies of the seventeen STR of this study, pairwise genetic distances were calculated between the five breeds, using Nei's formulas implemented in PHYLIP software (Table 3). The analysis showed a clear separation between these breeds (Figure 1). The major genetic distance in these five breeds is between Arabian and Thoroughbred breeds, while the breeds which are closer from each other are

Appaloosa and Quarter Horse, corroborating the historical data that indicate the time and the way of each horse breed arose[12-21].

Based on the largest publication and one of the most important on allelic frequencies of the seventeen STR markers present in our study[1], we compared the most frequent alleles at each locus in three horse breeds that are common in both works (Appaloosa, Arabian and Thoroughbred). In many loci, the most frequent allele of the reference publication is not the same as our study. Regarding the comparison for Uruguayan Criollo and Quarter Horse breeds, since there were no publications with this set of seventeen STR loci, it was made with studies using common genetic markers with the present study (fewer markers)[17,22-24]. In all studies, in many loci, the most frequent allele is not the same as our study.

This study presents the allele frequencies and other forensically informative parameters of seventeen STR loci that have not been published yet with horses from Uruguay. The presentation of these data, with these set of genetic markers, for Uruguayan Criollo and Quarter Horse breeds, is one of the first publications in the scientific community. However, considering the large number of animals for both breeds used in this study, and the results obtained, it is feasible to assume that these data can be used for forensic and kinship analysis, genetic identification and phylogenetic reconstruction. Finally, we consider important to determine the allelic frequencies and other parameters of forensic interest and genetic identification with animals in the same breed, in significant sample size and a certain geographic region, similar to what is recommended for human populations[25-26]. Thus, common and rare alleles of the location will be known, allowing to identify characteristics of each population as the occurrence or not of sub-structuration, linkage to traits under selection, Wahlund effect, bottleneck effect, etc.[22].

This paper follows the guidelines for publication of population data requested by the journal[27], the recommendations of International Society for Forensic Genetics (ISFG) regarding the use of non-human (animal) DNA in forensic genetic investigations[28] and the proposed allele nomenclature for seventeen equine-specific STR loci[29] as recommended by the ISFG for the nomenclature of human STR[30].

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Appendix A. Supplementary data

Supplementary data associated with this paper can be found in the online version.

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Table 1

F_{IS}, F_{ST} and F_{IT} coefficients for five horse breeds to each of the seventeen loci and the overall.

Marker	**F_{IS}**	**F_{ST}**	**F_{IT}**
VHL20	0.0041	0.0448	0.0487
HTG4	0.0024	0.1416	0.1437
AHT4	0.0036	0.0251	0.0286
HMS7	0.0029	0.0404	0.0432
HTG6	0.0063	0.1665	0.1717
AHT5	0.0112	0.0938	0.1039
HMS6	-0.0011	0.0414	0.0403
ASB23	0.0085	0.0738	0.0817
ASB2	0.0198	0.0548	0.0735
HTG10	0.1141	0.0744	0.1800
HTG7	0.0106	0.1173	0.1267
HMS3	0.0226	0.1007	0.1210
HMS2	0.0103	0.2353	0.2431
ASB17	0.0021	0.0598	0.0618
LEX3	0.3653	0.0979	0.4274
HMS1	-0.0077	0.0314	0.0240
CA425	0.0217	0.0406	0.0614
All	**0.0368**	**0.0845**	**0.1182**

Table 2

Inbreeding coefficient (F_{IS}) estimated for five horse breeds.

Breed	**F_{IS}**
Appaloosa	0.0183
Arabian	0.0240
U. Criollo	0.0332
Thoroughbred	0.0374
Quarter Horse	0.0491

Table 3

Genetic distances (F_{ST} analysis) among five breeds of horses from Uruguay.

Breed	Appaloosa	Arabian	U. Criollo	Thoroughbred	Quarter Horse
Appaloosa	*	0.0677	0.0321	0.0607	0.0165
Arabian	0.0677	*	0.0765	0.1047	0.0720
U. Criollo	0.0321	0.0765	*	0.0876	0.0414
Thoroughbred	0.0607	0.1047	0.0876	*	0.0612
Quarter Horse	0.0165	0.0720	0.0414	0.0612	*

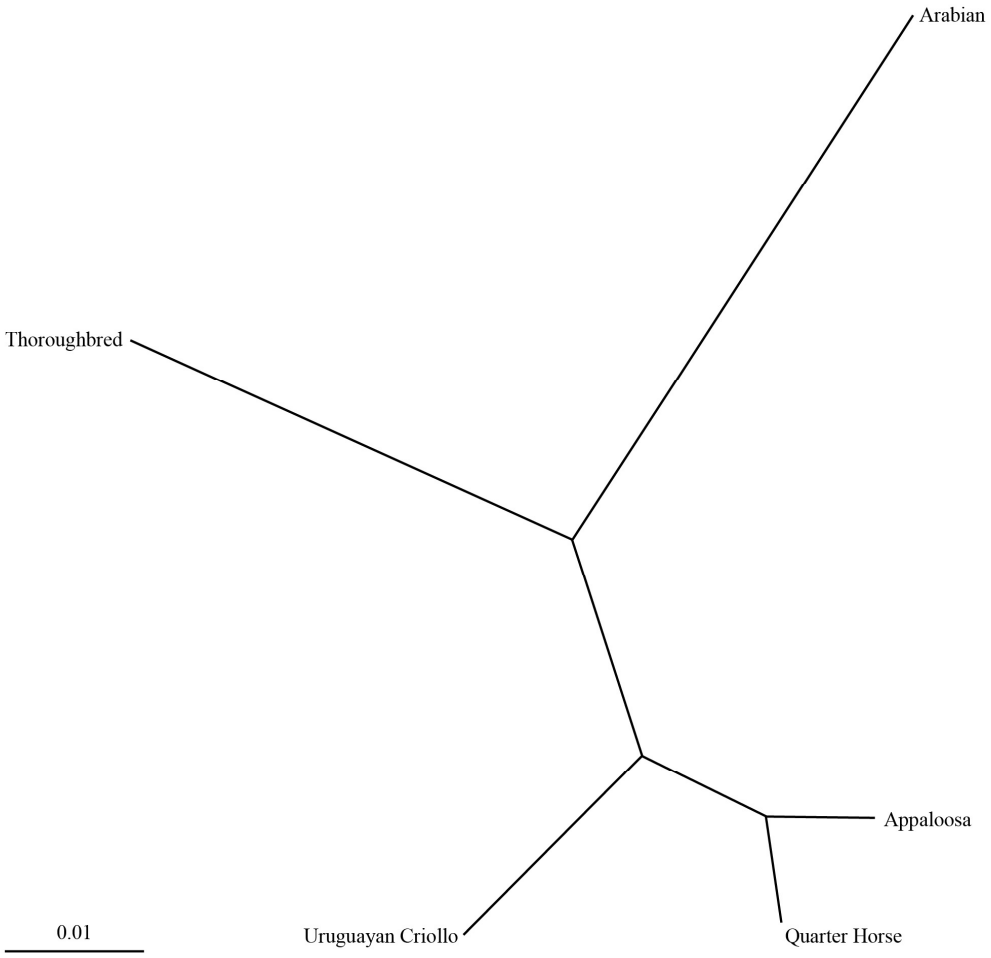

Figure 1

Neighbour-joining tree based on pairwise Nei's genetic distances calculated between five breeds of horses from Uruguay.

Table S1: Allele frequencies and other forensic parameters for seventeen equine-specific STR loci of Appaloosa breed from Uruguayan equine population.

Alelle	VHL20	HTG4	AHT4	HMS7	HTG6	AHT5	HMS6	ASB23	ASB2	HTG10	HTG7	HMS3	HMS2	ASB17	LEX3	HMS1	CA425
9									0.0234								
10									0.0047								
11																	
12					0.1574												0.0093
13	0.3426						0.1651							0.0238	0.1373		
14					0.0046		0.0826							0.1524		0.0841	0.0140
15					0.1852	0.0047	0.2202	0.0872			0.1822		0.1869	0.0429	0.0441	0.2477	0.0327
16	0.1991			0.1193		0.2523			0.0280				0.0421	0.0286	0.0147	0.0561	
17	0.1852			0.0092		0.2009	0.0596	0.1743	0.0047	0.1456	0.0234		0.0374	0.0048			0.0093
18	0.1157			0.3349	0.0093	0.0093	0.4633	0.1422	0.3832		0.1168		0.2336	0.0238	0.0882	0.5841	0.2290
19	0.0509			0.2110	0.0093	0.2757	0.0092	0.2477	0.0047	0.0443	0.6776		0.3411		0.1275	0.0280	0.5000
20	0.0185			0.1422	0.5046	0.2290		0.1743	0.1729	0.1329			0.0888	0.1476	0.2941		0.2056
21	0.0093			0.1514	0.1065	0.0280			0.1075	0.1013		0.2523		0.2143	0.1029		
22	0.0787			0.0092					0.0280	0.0316				0.0381	0.0245		
23				0.0229	0.0231				0.0047	0.2405			0.0093	0.0095	0.1667		
24									0.1682	0.0190				0.0762			
25			0.2804						0.0701	0.0127		0.2523	0.0607	0.2190			
26			0.0140							0.2722		0.1284		0.0190			
27			0.3271					0.0688				0.0321					
28			0.0841									0.2661					
29			0.0140					0.1055				0.0367					
30		0.2804										0.0321					
31		0.1168	0.0047														
32		0.4065	0.2430														
33		0.0514	0.0140														
34		0.0794	0.0187														
35		0.0607															
36		0.0047															
Locus	**VHL20**	**HTG4**	**AHT4**	**HMS7**	**HTG6**	**AHT5**	**HMS6**	**ASB23**	**ASB2**	**HTG10**	**HTG7**	**HMS3**	**HMS2**	**ASB17**	**LEX3**	**HMS1**	**CA425**
N	108	107	107	109	108	107	109	109	107	79	107	109	107	105	102	107	107
HWE	1.74E-02	9.25E-03	2.05E-01	4.16E-01	5.32E-01	3.94E-01	3.58E-01	4.69E-01	8.67E-01	4.77E-01	1.86E-01	9.31E-03	3.94E-02	5.48E-01	4.08E-02	8.09E-02	2.46E-01
Ho	0.8889	0.7103	0.7009	0.7706	0.6667	0.7850	0.7339	0.7890	0.8037	0.7342	0.4206	0.7890	0.7850	0.8286	0.6471	0.6822	0.6542
He	0.7898	0.7332	0.7508	0.7889	0.6773	0.7702	0.7024	0.8380	0.7800	0.8207	0.4958	0.7856	0.7829	0.8536	0.8336	0.5892	0.6569
PD	0.9055	0.8692	0.8974	0.9168	0.8517	0.8993	0.8597	0.9481	0.9213	0.9354	0.6947	0.8972	0.9002	0.9529	0.9396	0.7571	0.8292
PE	0.7728	0.4443	0.4297	0.5457	0.3786	0.5716	0.4827	0.5788	0.6060	0.4831	0.1269	0.5788	0.5716	0.6531	0.3512	0.4013	0.3610
PIC	0.7571	0.6905	0.7046	0.7549	0.6348	0.7264	0.6579	0.8129	0.7497	0.7912	0.4490	0.7479	0.7484	0.8324	0.8095	0.5357	0.6011
P_{ID}	0.0945	0.1308	0.1026	0.0832	0.1483	0.1007	0.1403	0.0519	0.0787	0.0646	0.3053	0.1028	0.0998	0.0471	0.0604	0.2429	0.1708

HWE - P value for Hardy and Weinberg equilibrium, Ho - observed heterozygosity, He - expected heterozygosity,
PD - power of discrimination, PE - power of exclusion, PIC - polymorphism information content, P_{ID} - Probability of Identity (Matching Probability).
Statistically significative after Bonferroni's correction (<0.0029).

Table S2: Allele frequencies and other forensic parameters for seventeen equine-specific STR loci of Arabian breed from Uruguayan equine population.

Alelle	VHL20	HTG4	AHT4	HMS7	HTG6	AHT5	HMS6	ASB23	ASB2	HTG10	HTG7	HMS3	HMS2	ASB17	LEX3	HMS1	CA425
9									0.0023								
10									0.0013								
11																	
12					0.5122												
13	0.1649						0.1383							0.0352	0.2551		0.0013
14	0.0022				0.0013		0.3182				0.0010			0.0538	0.0006	0.0771	
15	0.0006				0.1141	0.0012	0.1141	0.0009			0.2238		0.1018	0.0016	0.3107	0.5337	
16	0.1761			0.1998		0.2516			0.0180				0.0181	0.0009	0.0251	0.0054	0.0901
17	0.0719			0.2324		0.0854	0.0726	0.3849	0.0010	0.0443	0.0068		0.0067		0.0003	0.0241	
18	0.2092			0.3922	0.0219	0.0028	0.3567	0.1912	0.0511	0.0004	0.0807		0.0812	0.0031	0.0241	0.3571	0.0234
19	0.0019			0.0370	0.0010	0.1387		0.1636	0.0007	0.0727	0.6873		0.3922	0.0214	0.0199	0.0013	0.0609
20	0.0313			0.0895	0.3483	0.4716		0.1381	0.0882	0.2882	0.0003	0.0003	0.1309	0.2692	0.1459	0.0003	0.7076
21	0.0257			0.0488	0.0010	0.0486		0.0003	0.0180	0.0246		0.0958	0.0006	0.1528	0.1848		0.1013
22	0.3162				0.0003				0.1584	0.0004				0.0585	0.0048	0.0010	0.0138
23				0.0003					0.0023	0.4127			0.1224	0.0016	0.0286		0.0013
24									0.6554	0.0221		0.0088	0.0003	0.0487			0.0003
25			0.0648						0.0030			0.2599	0.1458	0.3519			
26			0.0688							0.0894		0.2524		0.0013			
27			0.2631					0.0990		0.0451		0.0062					
28			0.1459									0.3580					
29			0.0003					0.0220				0.0179					
30		0.3253	0.1311									0.0007					
31		0.1257	0.0037														
32		0.5038	0.3211														
33		0.0363	0.0012														
34		0.0019															
35		0.0028															
36																	
37		0.0041															
Locus	**VHL20**	**HTG4**	**AHT4**	**HMS7**	**HTG6**	**AHT5**	**HMS6**	**ASB23**	**ASB2**	**HTG10**	**HTG7**	**HMS3**	**HMS2**	**ASB17**	**LEX3**	**HMS1**	**CA425**
N	1613	1583	1621	1609	1556	1604	1612	1611	1496	1197	1548	1535	1577	1590	1556	1575	1520
HWE	1.41E-01	1.21E-01	2.72E-01	5.03E-01	5.34E-01	2.31E-01	**4.31E-04**	2.60E-01	**4.25E-06**	1.55E-01	1.46E-02	**4.14E-27**	3.25E-01	2.87E-02	ND*	4.18E-01	2.76E-02
Ho	0.7960	0.6279	0.7773	0.7526	0.5951	0.6870	0.7469	0.7654	0.5274	0.7009	0.4935	0.6365	0.7819	0.7761	0.5501	0.5905	0.4757
He	0.7915	0.6234	0.7805	0.7407	0.6030	0.6856	0.7343	0.7595	0.5344	0.7285	0.4710	0.7312	0.7757	0.7702	0.7807	0.5813	0.4767
PD	0.9252	0.7903	0.9188	0.8917	0.7714	0.8564	0.8827	0.9076	0.7425	0.8878	0.6653	0.8816	0.9228	0.9122	0.9162	0.7446	0.7039
PE	0.5917	0.3257	0.5576	0.5143	0.2851	0.4085	0.5045	0.5364	0.2126	0.4297	0.1819	0.3370	0.5658	0.5555	0.2353	0.2796	0.1671
PIC	0.7613	0.5571	0.7482	0.7014	0.5288	0.6403	0.6903	0.7257	0.5026	0.6904	0.4167	0.6837	0.7498	0.7382	0.7475	0.5030	0.4533
P_{ID}	0.0748	0.2097	0.0812	0.1083	0.2286	0.1436	0.1173	0.0924	0.2575	0.1122	0.3347	0.1184	0.0772	0.0878	0.0838	0.2554	0.2961

HWE - P value for Hardy and Weinberg equilibrium, Ho - observed heterozygosity, He - expected heterozygosity,
PD - power of discrimination, PE - power of exclusion, PIC - polymorphism information content, P_{ID} - Probability of Identity (Matching Probability).
* Not Determined

Table S3: Allele frequencies and other forensic parameters for seventeen equine-specific STR loci of Uruguayan Criollo breed from Uruguayan equine population.

Alelle	VHL20	HTG4	AHT4	HMS7	HTG6	AHT5	HMS6	ASB23	ASB2	HTG10	HTG7	HMS3	HMS2	ASB17	LEX3	HMS1	CA425
6											0.0001						
7																	
8																	
9									0.0141								
10									0.0220								
11														0.0004			
12					0.1312												
13	0.3001						0.1107						0.0001	0.1223	0.1962		
14	0.0112				0.0991		0.0884				0.0005			0.0899	0.0003	0.0310	0.0571
15	0.0033				0.1949	0.0005	0.1965	0.0035			0.2389		0.3598	0.0718	0.0644	0.4858	0.0046
16	0.2545			0.0321	0.0003	0.1077	0.0299		0.0060				0.2042	0.0032	0.0283	0.0093	0.1845
17	0.1730			0.0305	0.0001	0.1993	0.1163	0.0992		0.0951	0.1287		0.0164	0.0011	0.0003	0.0018	0.0008
18	0.0813			0.3083	0.0066	0.0332	0.4385	0.0876	0.1771	0.0006	0.0649		0.0880	0.0119	0.1132	0.3560	0.1183
19	0.0183			0.2566	0.0003	0.1252	0.0197	0.5076	0.0120	0.0344	0.5663		0.1323	0.0095	0.2181	0.0973	0.1417
20	0.0643			0.1208	0.5108	0.2689		0.1118	0.2951	0.0535	0.0006	0.0003	0.0368	0.1400	0.2718	0.0003	0.4813
21	0.0401			0.2267	0.0517	0.2485		0.0001	0.2179	0.2240		0.2396		0.1801	0.0562		0.0110
22	0.0537			0.0035		0.0001			0.0611	0.0479			0.0001	0.0699	0.0021	0.0186	
23	0.0002			0.0214	0.0052	0.0167			0.0015	0.3695			0.0202	0.0139	0.0492		0.0007
24									0.1706	0.0238			0.0003	0.0718			
25			0.1636					0.0033	0.0220	0.0128		0.1732	0.1336	0.2013			
26			0.0401					0.0002		0.1044		0.0327	0.0084	0.0130		0.0001	
27			0.2247					0.0457		0.0336		0.1406					
28			0.1162					0.0043		0.0003		0.2786					
29			0.0370					0.1363	0.0005			0.0913					
30		0.0004	0.0284					0.0004				0.0429					
31		0.2269	0.1275									0.0008					
32		0.3148	0.2598														
33		0.3130	0.0027														
34		0.0844															
35		0.0116	0.0001														
36		0.0478															
37		0.0006															
38		0.0006															
Locus	**VHL20**	**HTG4**	**AHT4**	**HMS7**	**HTG6**	**AHT5**	**HMS6**	**ASB23**	**ASB2**	**HTG10**	**HTG7**	**HMS3**	**HMS2**	**ASB17**	**LEX3**	**HMS1**	**CA425**
N	5834	5844	5845	5835	5824	5852	5818	5815	5693	4637	5696	5814	5791	5818	5643	5783	5674
HWE	**2.22E-13**	1.18E-01	7.63E-03	2.88E-01	1.58E-02	**1.52E-04**	**1.00E-08**	**8.90E-07**	**4.00E-08**	**1.98E-25**	**2.53E-05**	**1.12E-12**	9.12E-02	**6.37E-08**	ND*	5.56E-01	**1.42E-10**
Ho	0.7732	0.7454	0.8027	0.7719	0.6662	0.7787	0.7107	0.6791	0.7790	0.7410	0.5836	0.7721	0.7755	0.8520	0.6614	0.6244	0.6621
He	0.7996	0.7420	0.8218	0.7707	0.6714	0.7977	0.7343	0.6917	0.7999	0.7852	0.6015	0.8040	0.7837	0.8687	0.8168	0.6265	0.6969
PD	0.9319	0.8882	0.9451	0.9106	0.8535	0.9294	0.8971	0.8771	0.9319	0.9303	0.7900	0.9349	0.9254	0.9685	0.9429	0.7925	0.8701
PE	0.5503	0.5019	0.6041	0.5480	0.3777	0.5601	0.4450	0.3968	0.5607	0.4942	0.2717	0.5483	0.5543	0.6988	0.3710	0.3210	0.3721
PIC	0.7726	0.6975	0.7982	0.7343	0.6332	0.7676	0.7025	0.6636	0.7715	0.7604	0.5490	0.7762	0.7561	0.8548	0.7925	0.5587	0.6603
P_{ID}	0.0681	0.1118	0.0549	0.0894	0.1465	0.0706	0.1029	0.1229	0.0681	0.0697	0.2100	0.0651	0.0746	0.0315	0.0571	0.2075	0.1299

HWE - P value for Hardy and Weinberg equilibrium, Ho - observed heterozygosity, He - expected heterozygosity,
PD - power of discrimination, PE - power of exclusion, PIC - polymorphism information content, P_{ID} - Probability of Identity (Matching Probability).
* Not Determined
Statistically significative after Bonferroni's correction (<0.0029).

Table S4: Allele frequencies and other forensic parameters for seventeen equine-specific STR loci of Thoroughbred breed from Uruguayan equine population.

Alelle	VHL20	HTG4	AHT4	HMS7	HTG6	AHT5	HMS6	ASB23	ASB2	HTG10	HTG7	HMS3	HMS2	ASB17	LEX3	HMS1	CA425
7											<0.0001						
8									<0.0001								
9					<0.0001		<0.0001		0.0231					<0.0001			
10								<0.0001	0.0003								
11							<0.0001		0.0005								
12	0.0001				0.3434		0.0001									<0.0001	0.0001
13	0.2641			<0.0001	<0.0001		0.1062	<0.0001			<0.0001			0.0002	0.0074	0.0001	<0.0001
14	0.0004				0.0002		0.0455	<0.0001			0.0002		0.0001	0.3060	0.0004	0.1552	<0.0001
15	0.0001			<0.0001	0.4995	0.0002	0.2957				0.1367		0.0641	0.0016	0.3105	0.4157	0.0367
16	0.2071			0.1112	0.0001	0.1857	<0.0001	<0.0001	0.0003	0.0001	0.0001		0.0005	0.0002	0.0001	0.0003	0.1966
17	0.3222			0.0015	<0.0001	0.4039	0.0076	0.1140	0.0002	0.3128	0.0043		0.0438	0.0005	0.0001	0.0001	0.0049
18	0.2009			0.1859	0.0055	0.0011	0.5446	0.2708	0.2011	0.0015	0.4321		0.1588	0.0002	0.0005	0.4279	0.0186
19	0.0045			0.2766	0.0002	0.2609	<0.0001	0.2331	0.0007	0.0793	0.4265	<0.0001	0.6853	0.0001	0.0308	0.0005	0.0253
20	0.0001			0.1751	0.1321	0.1130	<0.0001	0.1639	0.1724	0.1564			0.0466	0.0504	0.1935	0.0001	0.5627
21	0.0002			0.2495	0.0049	0.0347	<0.0001	<0.0001	0.1421	0.2215	<0.0001	0.5402	0.0001	0.2473	0.1067	<0.0001	0.1544
22	0.0003			<0.0001	0.0001	0.0003		<0.0001	0.0739	0.0006	<0.0001	<0.0001	0.0001	0.1883	0.0762		0.0001
23					0.0140	0.0002	<0.0001	0.0001	0.0194	0.1156		<0.0001	0.0001	0.0008	0.2736		0.0004
24			0.0001			<0.0001			0.2011	0.0005		0.0001	0.0001	0.0018	0.0001		0.0002
25			0.1755					<0.0001	0.1648	0.0010		0.1124	0.0004	0.2023	<0.0001		
26			0.0002					<0.0001	0.0001	0.1085		0.0328		0.0002	<0.0001		
27		<0.0001	0.2310					0.1977		0.0022		0.0942	<0.0001				
28		<0.0001	0.2229						<0.0001			0.2032					
29		0.0001	0.0050					0.0202				0.0007					
30		0.5851	0.0001									0.0162					
31		0.0014	0.0004												0.0001		
32		0.3616	0.3648														
33		0.0290	0.0001									<0.0001					
34		0.0001	<0.0001														
35		0.0224															
36		0.0004															
Locus	**VHL20**	**HTG4**	**AHT4**	**HMS7**	**HTG6**	**AHT5**	**HMS6**	**ASB23**	**ASB2**	**HTG10**	**HTG7**	**HMS3**	**HMS2**	**ASB17**	**LEX3**	**HMS1**	**CA425**
N	23170	23164	23150	23115	22443	22986	23097	22565	21694	14333	22489	23051	21641	22492	22039	21979	21375
HWE	5.03E-02	**6.90E-07**	2.31E-01	6.26E-03	1.39E-02	1.44E-02	9.70E-02	4.38E-02	**1.54E-06**	ND*	3.81E-01	**3.39E-11**	5.31E-03	2.42E-02	ND*	3.36E-01	**4.46E-06**
Ho	0.7461	0.5231	0.7361	0.7795	0.6118	0.7125	0.6091	0.7883	0.8212	0.6861	0.6079	0.6395	0.4922	0.7696	0.4482	0.6255	0.6097
He	0.7432	0.5256	0.7331	0.7836	0.6149	0.7204	0.6025	0.7930	0.8357	0.7972	0.6127	0.6440	0.4969	0.7663	0.7731	0.6200	0.6185
PD	0.8868	0.6830	0.8812	0.9187	0.7795	0.8765	0.7781	0.9253	0.9518	0.9322	0.7694	0.8333	0.7131	0.9053	0.9013	0.7729	0.8100
PE	0.5032	0.2083	0.4861	0.5615	0.3049	0.4477	0.3018	0.5773	0.6384	0.4051	0.3002	0.3409	0.1803	0.5435	0.1455	0.3222	0.3024
PIC	0.6963	0.4348	0.6858	0.7488	0.5430	0.6747	0.5403	0.7609	0.8140	0.7690	0.5309	0.6044	0.4651	0.7271	0.7381	0.5396	0.5752
P_{ID}	0.1132	0.3170	0.1188	0.0813	0.2205	0.1235	0.2219	0.0747	0.0482	0.0678	0.2306	0.1667	0.2869	0.0947	0.0987	0.2271	0.1900

HWE - P value for Hardy and Weinberg equilibrium, Ho - observed heterozygosity, He - expected heterozygosity,
PD - power of discrimination, PE - power of exclusion, PIC - polymorphism information content, P_{ID} - Probability of Identity (Matching Probability).
* Not Determined
Statistically significative after Bonferroni's correction (<0.0029).

Table S5: Allele frequencies and other forensic parameters for seventeen equine-specific STR loci of Quarter Horse breed from Uruguayan equine population.

Alelle	VHL20	HTG4	AHT4	HMS7	HTG6	AHT5	HMS6	ASB23	ASB2	HTG10	HTG7	HMS3	HMS2	ASB17	LEX3	HMS1	CA425
9									0.0048								
10									0.0139								
11																	
12					0.2660												
13	0.3415						0.0439							0.0275	0.1455		
14	0.0038				0.0261		0.0627				0.0009			0.1614	0.0021	0.0437	
15					0.2126		0.2086		0.0004		0.2494		0.1521	0.0052	0.1438	0.2581	0.0101
16	0.1077			0.1532	0.0004	0.3862	0.0282	0.0013	0.0265				0.0284	0.0056	0.0429	0.0946	0.1255
17	0.2577			0.0209	0.0004	0.1587	0.1237	0.0988	0.0009	0.2071	0.1473		0.0305	0.0060	0.0202	0.0021	
18	0.1397			0.3127	0.0060	0.0064	0.5320	0.1480	0.2685	0.0010	0.1628		0.4055	0.0004	0.0416	0.5638	0.0092
19	0.0457			0.1310	0.0060	0.1411	0.0009	0.2981	0.0321	0.0277	0.4371		0.2354	0.0082	0.1326	0.0372	0.2100
20	0.0231			0.1122	0.4153	0.2275		0.1882	0.1607	0.1192	0.0026		0.0193	0.1099	0.2000		0.4698
21	0.0286			0.2509	0.0560	0.0783			0.1677	0.1316		0.1985		0.3674	0.0906		0.1737
22	0.0521			0.0073		0.0004			0.0378	0.0123			0.0215	0.0747	0.0691	0.0004	
23				0.0119	0.0111	0.0013			0.0056	0.2708			0.0215	0.0064	0.1116		0.0017
24									0.1946	0.0211		0.0004	0.0004	0.0459			
25			0.1985					0.0009	0.0830	0.0092		0.4073	0.0855	0.1751			
26			0.0043					0.0047		0.1932		0.0897		0.0064			
27			0.2929					0.1861		0.0067		0.0679					
28			0.1238									0.1567					
29			0.0171					0.0697	0.0035			0.0376					
30		0.2568	0.0004					0.0043				0.0418					
31		0.0495	0.0034														
32		0.5776	0.3399														
33		0.0311	0.0196														
34		0.0205															
35		0.0550															
36		0.0085															
37		0.0009															
Locus	**VHL20**	**HTG4**	**AHT4**	**HMS7**	**HTG6**	**AHT5**	**HMS6**	**ASB23**	**ASB2**	**HTG10**	**HTG7**	**HMS3**	**HMS2**	**ASB17**	**LEX3**	**HMS1**	**CA425**
N	1170	1172	1171	1172	1169	1169	1172	1169	1151	973	1161	1171	1164	1165	1165	1168	1143
HWE	4.49E-03	6.70E-01	9.85E-02	2.12E-01	7.54E-01	2.57E-01	4.89E-01	2.30E-02	1.27E-02	**3.20E-10**	5.73E-01	**2.89E-06**	6.66E-02	7.83E-03	ND*	1.41E-01	4.28E-01
Ho	0.7521	0.5853	0.7139	0.7850	0.6989	0.7716	0.6382	0.7802	0.7889	0.7338	0.6848	0.7096	0.7139	0.7588	0.5820	0.6156	0.6702
He	0.7800	0.5937	0.7435	0.7858	0.7078	0.7481	0.6518	0.8049	0.8262	0.8138	0.6988	0.7546	0.7470	0.7879	0.8715	0.6035	0.6894
PD	0.9244	0.7874	0.8929	0.9213	0.8671	0.8977	0.8383	0.9337	0.9486	0.9396	0.8625	0.9071	0.9033	0.9287	0.9631	0.7897	0.8577
PE	0.5134	0.2736	0.4501	0.5715	0.4265	0.5474	0.3393	0.5627	0.5786	0.4825	0.4051	0.4433	0.4501	0.5249	0.2698	0.3100	0.3836
PIC	0.7497	0.5438	0.6998	0.7539	0.6588	0.7102	0.6123	0.7773	0.8036	0.7877	0.6492	0.7237	0.7132	0.7625	0.8576	0.5514	0.6442
P_{ID}	0.0756	0.2126	0.1071	0.0787	0.1329	0.1023	0.1617	0.0663	0.0514	0.0604	0.1375	0.0929	0.0967	0.0713	0.0369	0.2103	0.1423

HWE - P value for Hardy and Weinberg equilibrium, Ho - observed heterozygosity, He - expected heterozygosity,
PD - power of discrimination, PE - power of exclusion, PIC - polymorphism information content, P_{ID} - Probability of Identity (Matching Probability).
* Not Determined
Statistically significative after Bonferroni's correction (<0.0029).

3.2 ARTIGO CIENTÍFICO A SER SUBMETIDO 1[4]

Title of the manuscript: Genetic identification of cattle and comparative population study among taurines, zebuines and hybrid breeds in more than 28,000 animals raised in Uruguay and Paraguay using forensically informative STR loci

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[4] Artigo a ser submetido ao periódico científico *Forensic Science International: Genetics*.

Abstract: Allele frequencies and other forensic parameters for 12 STR genetic markers recommended by ISAG for genotyping cattle (TGLA227, BM2113, TGLA53, ETH10, SPS115, TGLA126, TGLA122, INRA23, ETH3, ETH225, BM1824 and BM1818) were determined from a pool of 28,683 bovines raised in Uruguay and Paraguay, consisting of ten breeds (Angus, Braford, Brahman, Brangus, Hereford, Holstein-Friesian, Jersey, Limousin, Nelore and Wagyu). The forensically informative parameters (Ho, He, HWE test, PIC, PD, PE and P_{ID}) as well as the coefficients of inbreeding (F_{IS}), fixation indices (F_{ST}), and total inbreeding (F_{IT}) were ascertained. Based on genotypic frequencies of the 12 STR of this study, pairwise genetic distances were calculated among the breeds showing a clear separation between cattle of different breeds in the same country, but also genetic diversity in animals of the same breed, but of distinct geographic location. The presentation of these data, with these set of genetic markers, for Angus, Brangus, Braford and Jersey breeds, is one of the first publications in the scientific community.

Keywords: Allele frequencies; Bovine-specific STR loci, Uruguayan cattle, Paraguayan cattle, genetic distances among breeds.

1 INTRODUCTION

Cattle (*Bos taurus*), since it was domesticated by man, thousands of years ago, have always been part of human conviviality, being very important for the growth and evolution of populations, used as transport and traction animals, as food through supply of meat and milk, and providing leather, so used for the manufacture of clothes, shoes, and other garments that wrapped and wore men and women through the ages. For all these utilities and so many others, cattle herds have become very valuable, desired by many civilizations, reason for quarrels and battles for their conquest. Over the last 300 years, cattle have been selected, according to need and exigency, being chosen those animals that best expressed the characteristics of interest (e.g., meat quality, weight gain, milk production, resistance to adverse environments) that were transmitted to the offspring, giving rise to specific groups, being visibly distinguished from the others, the breeds[1-2]. Cattle breeds of the subspecies *Bos taurus taurus* (taurines), which originate mainly in Europe, are characterized by the quality of their products, especially meat and milk. Among the

taurine breeds with great world expression are: Angus, Hereford, Holstein-Friesian, Jersey and Limousin. The zebu (subspecies *Bos taurus indicus*), of Asian and African origins, gained prominence due to rusticity and better environmental adaptation. When came to the American continent, especially Brazil and United States, breeds like Nelore and Brahman emerged and their populations rapidly expanded – currently, they are the two breeds of zebu beef cattle with the highest production in the world. However, while zebu breeds are highly adaptable, with very high production responses, wide tolerance to parasitic diseases and extreme temperatures, they present some problems that influence animal production, especially those related to the aspects of meat tenderness and sexual precocity. With taurine breeds, in general, the advantages and disadvantages are opposite to zebu breeds. Thus, in order to merge the advantages of these two subspecies of bovines, through the combination of different forms of crosses, the hybrid or composite breeds began to appear, with the high growth potential of the European continental breeds, the high quality of meat of British breeds and resistance to parasites and adaptability to the tropical climate of zebu breeds. Among these hybrid breeds are Braford and Brangus.

By the mid-twentieth century it was estimated that there were about 800 registered breeds of cattle. Although, after Second World War, the emphasis on productivity favored an increase in the specialization of breeds, which made it favorable for some of them to become more prevalent than the others. Some breeds have simply been extinguished due to the abandonment of breeders or because they have been replaced by more efficient ones. The process has been greatly accelerated by the introduction of new reproductive techniques, particularly artificial insemination, as well as increased exchange of breeding stocks from different regions, which caused the decline of genetic diversity in the most varied races. It was only in the 1980s that conservation efforts were actually undertaken to preserve the genetic diversity of livestock. Importantly, it is necessary to determine which breeds should be conserved (using objective criteria) because loss of variation will restrict the options available to meet future unknown requirements[2].

The meat of cattle is top-ranked as the highest producing and one of the most consumed types of meat in the world. Because of all the importance and value of this species, it is not uncommon to be targeted for crimes such as cattle rustling,

clandestine slaughter, theft and identity forgery[3-4]. Besides that, genetic identification of cattle, accurate and safe from failures, has always been sought with the intention of providing associations and breeders assurances that animals actually come from those parents who are reported as the true sire and dam. Aspects such as public health, animal health, animal management, trade barriers and consumer demand also require secure identification so that animals can be traced throughout their lives, from birth until purchase of themselves or of their products (meat, milk, etc.)[5]. Failure in identification or traceability processes in some point on the production chain can trigger crises of profound impact on worldwide livestock production[6].

Uruguay (UY) and Paraguay (PY), are countries located in South America, close to each other but not border, nearby of Brazil and Argentina. Both have similar economic policies, oriented to the primary sector very forceful in the agricultural production. The importance of this sector is so great for both countries that the bovine population of Uruguay and Paraguay is much larger than the human population. While in Uruguay there are more than 12 million cattle for a population of approximately 3 million people, in Paraguay there are around 14 million cattle for a population of just over 6 million[7]. These two countries have institutions to take care of the agricultural interests, the Rural Association of Uruguay and the Rural Association of Paraguay, nonprofit entities that gather cattle breeders of many breeds and have a mission to receive all the genetic information of the animals with pedigree, in order to carry out the genealogical record. Knowing the provenance and genealogy of the animals, through strict identification and traceability methods, guarantees quality both in the internal commercialization as in the export of products. Among the methods of identification adopted by these rural associations is the DNA typing of all animals with request for genealogical registration, which is only provided to the breeder applicant after verifying the previously provided information and the inclusion of the genotypes in the entity database[8-9]. For this reason, these institutions have grouped into their records thousands of genotypes of bovines of many breeds representing significantly the cattle population of Uruguay and Paraguay.

Thus, following the recommendation that large-scale population studies should be conducted as widely done in humans and has already been started in other non-human species relevant in forensic casework[3], this article aims to: (1) publish the

allelic frequencies and other forensic parameters of the 12 STR genetic markers recommended by the International Society for Animal Genetics (ISAG) in 28,683 bovines of ten breeds, being six of animals from Uruguay and Paraguay and the remaining four breeds of animals exclusively from Uruguay; (2) estimate the inbreeding coefficient (F_{IS}) and verify if any of the ten cattle breeds are substructure; (3) estimate the index of fixation (F_{ST}) of the ten cattle breeds to calculate the genetic distance among the animals of different breeds and of the same country, as well as to verify if there is significant genetic diversity between animals of the same breed and different geographic regions (Uruguay and Paraguay).

2 MATERIALS AND METHODS

2.1 Samples and DNA extraction

Samples were obtained from 28,683 bovines of ten different breeds of animals raised in Uruguay and Paraguay: Angus, Braford, Brahman, Brangus, Hereford, Holstein-Friesian, Jersey, Limousin, Nelore and Wagyu. Table 1 shows the abbreviation used to designate the ten breeds, the origin of cattle (Uruguay - UY; Paraguay - PY) and the number of animals of each breed used for the present study. All samples were obtained from animals subjected to pedigree registration on Rural Association of Uruguay or on Rural Association of Paraguay. DNA was isolated from hair root or semen straw samples using proteinase K in two types of extraction buffer: Buffer 1 to hair root samples ($MgCl_2$, PCR Buffer and Tween 20) and Buffer 2 to semen straw samples (EDTA, SDS, Tris, NaCl and DTT). Final concentration after DNA extraction: 50-100 ng/µL of bovine genomic DNA.

Table 1: Abbreviation, origin and number of animals of ten cattle breeds used in the study.

Breed	Abbreviation	Origin	Number
Angus	ANG	UY	6,030
Braford	BRF	UY; PY	238; 2,457
Brahman	BMN	UY; PY	46; 3,874
Brangus	BRG	UY; PY	237; 5,256
Hereford	HF	UY	7,008
Holstein-Friesian	HST	UY; PY	1,700; 464
Jersey	JE	UY; PY	264; 49
Limousin	LIM	UY; PY	193; 62
Nelore	NE	UY	670
Wagyu	WAG	UY	135
Total			**28,683**

2.2 PCR amplification

The 12 STR genetic markers recommended by ISAG for genotyping cattle (TGLA227, BM2113, TGLA53, ETH10, SPS115, TGLA126, TGLA122, INRA23, ETH3, ETH225, BM1824 and BM1818) were amplified in a single PCR multiplex performed on Veriti™ Thermal Cycler (Thermo Fisher Scientific, Waltham, MA, USA) or GeneAmp 9700 PCR System (Thermo Fisher Scientific) using fluorescent-labeled primers which the sequences are available on http://www.cstl.nist.gov/strbase/cattleSTRs.htm. The other reagents for PCR amplification (PCR Buffer, DNA Polymerase and dNTP mix) were carried out using the Platinum® Multiplex PCR Master Mix, 2x (Thermo Fisher Scientific). The amount of bovine genomic DNA was 25-50 ng/µL per sample. Thermocycling conditions were: pre-incubation for 15 minutes at 95°C, followed by thirty-one cycles of 45s at 95°C, 45s at 58°C and 60s at 72°C, with a final incubation for 30min at 72°C.

2.3 Detection, typing, and analysis of PCR products

Electrophoresis and typing were performed on an ABI 3500 Genetic Analyzer (Thermo Fisher Scientific) using GeneScan™ 500 ROX™ Size Standard as internal

lane standards according to the manufacturer's protocols. Data was collected by Data Collection v1.0 software (Thermo Fisher Scientific) and analyzed using GeneMapper ID-X v1.3 software (Thermo Fisher Scientific).

2.4 Quality control

Genia Laboratory is a member of International Society for Animal Genetics (ISAG), and since 2003 has participated of biannual Program of Quality Control from this institution. In addition, it provides services to Uruguayan Stud Book, Rural Association of Uruguay and Rural Association of Paraguay, and adopts a strict internal quality control throughout the laboratory management system.

2.5 Analysis of data

Calculations of allele frequencies, observed (Ho) and expected (He) heterozygosity, polymorphism information content (PIC) and *P*-values of the Hardy-Weinberg equilibrium (HWE) test for all 12 loci were assessed using CERVUS version 3.0.3[10]. Bonferroni's correction was used for HWE test, which assumes that a 0.05 significance level obtained for 12 tests (one per locus) yields an actual significance threshold of 0.0042[11]. Power of discrimination (PD), power of exclusion (PE) and probability of identity (P_{ID}) were estimated with PowerStats (Promega Corporation)[12]. Estimated coefficients of inbreeding (F_{IS}) within breeds, fixation indices (F_{ST}) among breeds, and total inbreeding (F_{IT}) using an analysis of molecular variance (AMOVA) were performed with ARLEQUIN version 3.1[13] and GENEPOP version 4.5.1[14]. Neighbour-joining trees were built from Nei's genetic distance matrix using the PHYLIP software package version 3.69[15] and visualized with TreeView software version 1.6.6[16]. The allele frequencies and forensically informative parameters are available in Supplementary Data (Tables S1-S16).

3 RESULTS AND DISCUSSION

Among the ten cattle breeds from Uruguay present in the study, eight of them showed deviation in, at least one of 12 STR loci, according to HWE test ($P<0.05$): Angus, Braford, Brangus, Hereford, Holstein-Friesian, Limousin, Nelore and Wagyu. After applying the Bonferroni's correction ($P<0.0042$), five breeds still showed at least

one locus in disequilibrium, with emphasis on Angus and Hereford breeds (see Tables S1 and S4), which presented, respectively, nine and six loci with *P*-value statistically significative. Regarding to the group of animals raised in Paraguay, represented by six breeds, with the exception of the Jersey breed, in the other five at least one locus showed deviation from HWE. When applied the Bonferroni's correction, Braford, Brahman and Brangus breeds still had many loci in disequilibrium, especially the last two of them (see Tables S12 and S13). Two breeds of Uruguayan cattle (Angus and Hereford) and two breeds of animals raised in Paraguay (Brahman and Brangus) that presented the highest number of loci in deviation from HWE are also the four in which the observed heterozygosity (Ho) was lower than expected heterozygosity (He) in most of the genetic markers studied. This fact corroborate that there is low genetic variability, intense selection for individuals that best represent the breeds and high rate of inbreeding which favors the increase of homozygotes in these breeds. The loci with highest polymorphism information content (PIC) in most breeds were TGLA227 and TGLA53, while the least polymorphic genetic markers were ETH3, ETH225 and SPS115. The combined power of discrimination (PD) and the combined power of exclusion (PE) among all ten cattle breeds from Uruguay were, respectively, 0.9999999999 and 0.9986. Among six breeds of animals raised in Paraguay combined PD and combined PE were, respectively, 0.999999999 and 0.9995. These values of combined PD and combined PE did not consider the hypothesis of one or both parents known, as was done in the publication by Van de Goor *et. al*, who obtained values similar to those found in the present study, assuming, however, the hypothesis that the two parents were known[3]. The combined Probability of Identity (P_{ID}) showed the following overall values between the breeds from Uruguay and Paraguay, respectively, 1.75×10^{-10} 1.29×10^{-10}.

Tables 2 and 3 present the values of F_{IS}, F_{ST} and F_{IT} for each of the 12 loci and the overall in cattle from Uruguay and Paraguay, respectively. The total F_{IS}, F_{ST} and F_{IT} values show that the cattle population from Uruguay (all the ten breeds) has a higher rate of inbreeding, more genetic differentiation and higher deficit of heterozygotes than in the Paraguayan cattle population. In Tables 4 and 5, positive values of F_{IS} (> 0) indicates heterozygote deficiency and sub-structuration in nine of ten cattle breeds from Uruguay (except Brahman), and in four of six breeds from

Paraguay (except Jersey and Limousin). The negative F_{IS} values, indicating that there is no excess of homozygotes in these three breeds, can be explained, perhaps, due to the small number of animals used (less than 100 animals per breed). It may be that when analyzing a larger group of animals, positive values of F_{IS} are obtained, indicating the occurrence of inbreeding as shown by the population of Brahman cattle (n = 3,874) from Paraguay and the populations of Jersey (n = 264) and Limousin (n = 193) from Uruguay.

On the other hand, Angus and Hereford were the breeds with higher level of inbreeding in Uruguayan group, while Brahman and Holstein-Friesian on Paraguayan cattle.

Table 2: F_{IS}, F_{ST} and F_{IT} coefficients for ten cattle breeds of bovines raised in Uruguay to each of the 12 loci and the overall.

Marker	**F_{IS}**	**F_{ST}**	**F_{IT}**
TGLA227	0.0336	0.1197	0.1493
BM2113	0.0195	0.0744	0.0924
TGLA53	0.0588	0.0923	0.1457
ETH10	0.0178	0.1671	0.1820
SPS115	0.0086	0.1004	0.1081
TGLA126	-0.0007	0.0575	0.0568
TGLA122	0.0185	0.1823	0.1974
INRA23	0.0141	0.1891	0.2005
ETH3	0.0183	0.1581	0.1735
ETH225	0.0226	0.0957	0.1161
BM1824	0.0126	0.1034	0.1147
BM1818	0.0269	0.0537	0.0791
All	**0.0213**	**0.1181**	**0.1368**

Table 3: F_{IS}, F_{ST} and F_{IT} coefficients for six cattle breeds of bovines raised in Paraguay to each of the 12 loci and the overall.

Marker	F_{IS}	F_{ST}	F_{IT}
TGLA227	0.0034	0.1113	0.1143
BM2113	-0.0061	0.0996	0.0941
TGLA53	0.0357	0.0965	0.1287
ETH10	-0.0006	0.0974	0.0968
SPS115	0.0205	0.0844	0.1032
TGLA126	0.0023	0.0900	0.0921
TGLA122	0.0041	0.0824	0.0862
INRA23	-0.0136	0.0922	0.0798
ETH3	0.0080	0.0772	0.0846
ETH225	0.0060	0.1289	0.1342
BM1824	0.0020	0.1845	0.1862
BM1818	0.0323	0.0871	0.1166
All	**0.0077**	**0.1024**	**0.1094**

Table 4: Inbreeding coefficient (F_{IS}) estimated for ten cattle breeds of bovines raised in Uruguay.

Breed	F_{IS}
Angus	0.0214
Braford	0.0064
Brahman	-0.0055
Brangus	0.0112
Hereford	0.0256
Holstein-Friesian	0.0117
Jersey	0.0070
Limousin	0.0198
Nelore	0.0206
Wagyu	0.0114

Table 5: Inbreeding coefficient (F_{IS}) estimated for six cattle breeds of bovines raised in Paraguay.

Breed	F_{IS}
Braford	0.0056
Brahman	0.0108
Brangus	0.0069
Holstein-Friesian	0.0150
Jersey	-0.0377
Limousin	-0.0287

Based on genotypic frequencies of the 12 STR of this study, pairwise genetic distances were calculated using Nei's formulas implemented in PHYLIP software. First, the genetic distance among cattle breeds of animals belonging to the same geographical region (Uruguay or Paraguay) was calculated, and the values obtained can be observed in Tables 6 and 7. In both geographic regions, the 12 STR loci used in the present study allow a clear separation of animal groups according to their breeds (Figures 1 and 2). It is also possible to perceive the separation of the breeds according to the subspecies (*Bos taurus taurus* – ANG, HF, HST, JE and LIM or *Bos taurus indicus* – BRM or NE), in addition to hybrid breeds (BRF and BNG). The breeds with the lowest genetic distance presented among ten breeds of cattle raised in Uruguay were Braford and Brangus, while the greatest occurred between Brahman and Jersey breeds. Among six breeds of cattle from Paraguay, the lowest observed genetic distance was also between Braford and Brangus, while the greatest was between Brahman and Limousin.

Subsequently, the genetic distance among six cattle breeds with animals from both countries was calculated, and values obtained can be observed in Table 8. Figure 3 clearly shows that animals belonging to the same breed but from different geographic region are clustered into six distinct groups. However, in some breeds, such as Brahman, Jersey and Limousin, although the animals are of the same breed, there is a small genetic distance between groups of different countries. In the other three breeds (Braford, Brangus and Holstein-Friesian), genetic distance between groups is almost non-existent.

Table 6: Genetic distances (F_{ST} analysis) among ten cattle breeds of bovines raised in Uruguay.

Breed	Angus	Braford	Brahman	Brangus	Hereford	H. Friesian	Jersey	Limousin	Nelore	Wagyu
Angus	*	0.0819	0.2061	0.0567	0.1093	0.0886	0.1172	0.0684	0.1983	0.1166
Braford	0.0819	*	0.1038	0.0410	0.0480	0.0851	0.1210	0.0670	0.1016	0.1163
Brahman	0.2061	0.1038	*	0.1046	0.2215	0.2062	0.2494	0.1898	0.0547	0.2201
Brangus	0.0567	0.0410	0.1046	*	0.1065	0.0945	0.1269	0.0783	0.1127	0.1280
Hereford	0.1093	0.0480	0.2215	0.1065	*	0.1117	0.1524	0.1112	0.2054	0.1567
H. Friesian	0.0886	0.0851	0.2062	0.0945	0.1117	*	0.1234	0.0934	0.1948	0.1226
Jersey	0.1172	0.1210	0.2494	0.1269	0.1524	0.1234	*	0.1476	0.2252	0.1707
Limousin	0.0684	0.0670	0.1898	0.0783	0.1112	0.0934	0.1476	*	0.1869	0.1134
Nelore	0.1983	0.1016	0.0547	0.1127	0.2054	0.1948	0.2252	0.1869	*	0.2242
Wagyu	0.1166	0.1163	0.2201	0.1280	0.1567	0.1226	0.1707	0.1134	0.2242	*

Table 7: Genetic distances (F_{ST} analysis) among six cattle breeds of bovines raised in Paraguay.

Breed	Braford	Brahman	Brangus	H. Friesian	Jersey	Limousin
Braford	*	0.1264	0.0448	0.0850	0.1161	0.0864
Brahman	0.1264	*	0.1182	0.2158	0.2202	0.2243
Brangus	0.0448	0.1182	*	0.0812	0.0871	0.0713
H. Friesian	0.0850	0.2158	0.0812	*	0.0892	0.0794
Jersey	0.1161	0.2202	0.0871	0.0892	*	0.1259
Limousin	0.0864	0.2243	0.0713	0.0794	0.1259	*

Figure 1: Neighbour-joining tree based on pairwise Nei's genetic distances calculated among ten cattle breeds of bovines raised in Uruguay.

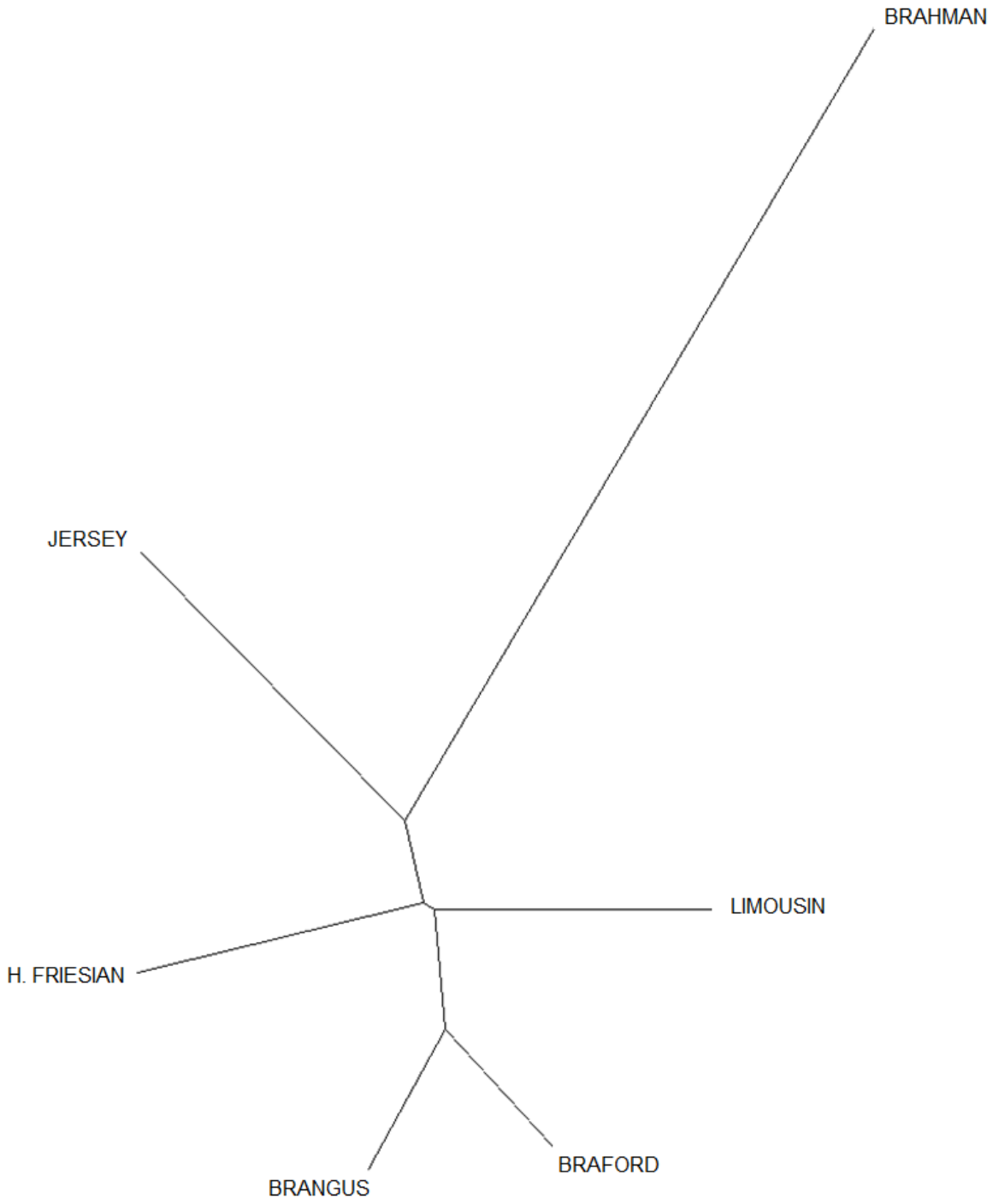

Figure 2: Neighbour-joining tree based on pairwise Nei's genetic distances calculated among six cattle breeds of bovines raised in Paraguay.

Table 8: Genetic distances (F_{ST} analysis) among six cattle breeds of bovines raised in Uruguay and Paraguay.

Breed	BRF_PY	BRF_UY	BMN_PY	BMN_UY	BNG_PY	BRG_UY	HST_PY	HST_UY	JER_PY	JER_UY	LIM_PY	LIM_UY
BRF_PY	*	0.0096	0.1264	0.0972	0.0448	0.0443	0.0850	0.0910	0.1161	0.1309	0.0864	0.0812
BRF_UY	0.0096	*	0.1316	0.1038	0.0405	0.0410	0.0782	0.0851	0.0965	0.1210	0.0755	0.0670
BMN_PY	0.1264	0.1316	*	0.0333	0.1182	0.1358	0.2158	0.2204	0.2202	0.2334	0.2243	0.2095
BMN_UY	0.0972	0.1038	0.0333	*	0.0974	0.1046	0.2004	0.2062	0.2346	0.2494	0.2182	0.1898
BNG_PY	0.0448	0.0405	0.1182	0.0974	*	0.0080	0.0812	0.0870	0.0871	0.1081	0.0713	0.0725
BRG_UY	0.0443	0.0410	0.1358	0.1046	0.0080	*	0.0888	0.0945	0.1050	0.1269	0.0730	0.0783
HST_PY	0.0850	0.0782	0.2158	0.2004	0.0812	0.0888	*	0.0029	0.0892	0.1183	0.0794	0.0892
HST_UY	0.0910	0.0851	0.2204	0.2062	0.0870	0.0945	0.0029	*	0.0988	0.1234	0.0855	0.0934
JER_PY	0.1161	0.0965	0.2202	0.2346	0.0871	0.1050	0.0892	0.0988	*	0.0190	0.1259	0.1200
JER_UY	0.1309	0.1210	0.2334	0.2494	0.1081	0.1269	0.1183	0.1234	0.0190	*	0.1582	0.1476
LIM_PY	0.0864	0.0755	0.2243	0.2182	0.0713	0.0730	0.0794	0.0855	0.1259	0.1582	*	0.0265
LIM_UY	0.0812	0.0670	0.2095	0.1898	0.0725	0.0783	0.0892	0.0934	0.1200	0.1476	0.0265	*

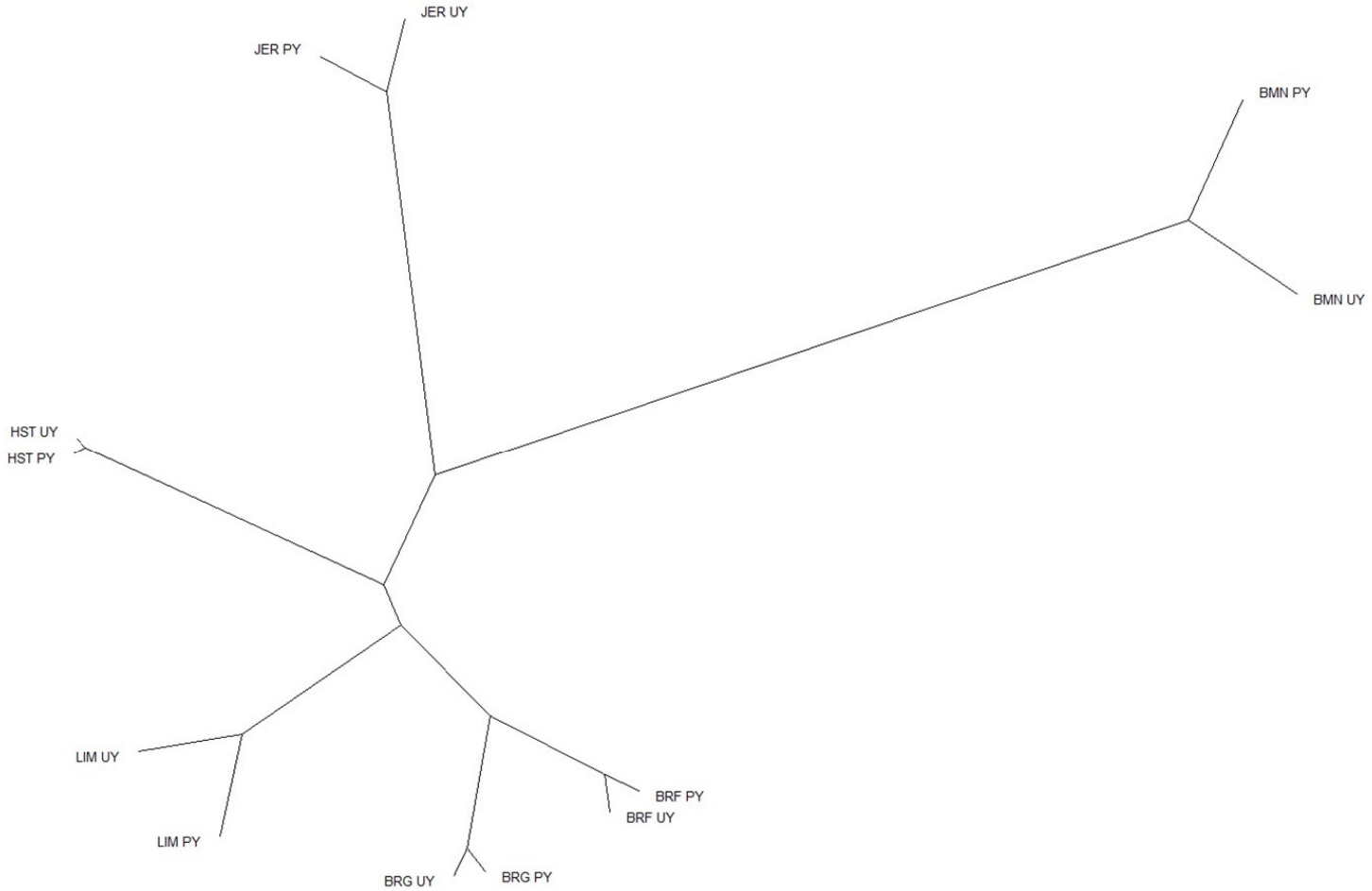

Figure 3: Neighbour-joining tree based on pairwise Nei's genetic distances calculated among animals belonging to the same breed but from different geographic region (Uruguay or Paraguay).

The 12 STR loci used in the present study for genetic identification of cattle, paternity testing and kinship verification were already recommended by ISAG in the 1990s. Since then, conferences, workshops, congresses and other meetings are organized by the entity to validate interlaboratory comparison tests for routine use of bovine kinship analysis[3]. Nevertheless, few studies have been published with the purpose of divulging and sharing the allelic frequencies and several parameters of forensic interest of these STR loci, besides the phylogenetic analyzes among varied breeds. Another limitation in the few publications available for comparison of results, is the fact that the number of animals used is relatively low, and some results can be misinterpreted. Most of these studies used a total population of cattle less than 1,000, and those who did comparative analysis among breeds involved about 50 to 100 animals per breed[1-3,17-21]. Thus, the results obtained in the present study have a limited comparison in some breeds due to the lack or use of other STR genetic markers than those recommended by ISAG. However, for most analysis and comparisons among populations and breeds, we used the study that involved the largest number of animals and several breeds, and which served as a guide for this publication[3]. For the breeds that the mentioned article did not publish data, we used sporadic references found in the most varied scientific journals of forensic interests, of genetics and animal breeding and of agricultural subjects.

Considering the allelic frequencies of Hereford breed, estimated only in animals raised in Uruguay and comparing them with the publication of van de Goor *et al*.[3], could be noted that the most frequent alleles of each marker are the same, with frequencies up to similar, except for marker TGLA53. In Wagyu breed, which is also present only in the Uruguayan cattle population, it can be seen that in half of the markers (six) that make up the STR panel recommended by ISAG the most frequent alleles are not the same (differences found in BM1818, BM1824, BM2113, SPS115, TGLA122, TGLA53).

In Holstein-Friesian and Limousin breeds, it was possible to compare the frequencies of alleles of animals raised in Uruguay and Paraguay with the article cited as reference. In Holstein-Friesian, described as the breed with the lowest genetic diversity among the bovine breeds of commercial interest[21-22], although in most genetic markers the most frequent alleles are the same among the three populations, there were some exceptions. Among cattle Holstein-Friesian from

Uruguay and Paraguay in only one locus, there was divergence among the most frequent alleles in both populations (BM1818). However, in comparison of allelic frequencies of the Holstein-Friesian breed of van de Goor *et al.* article[3] with those of the present study, there were disagreements in the allele more frequent in three markers for each geographic region, two in common (BM1824 and TGLA53) and locus INRA23 for Uruguayan cattle and locus BM1818 for the Paraguayan cattle of this breed. In Limousin breed, greater differences were observed when the most frequent alleles of the 12 loci were compared between the animals from Uruguay and Paraguay. The divergence occurred in four STR markers (BM2113, TGLA53, INRA23 and ETH225). In relation to the reference study[3], the divergence occurred in loci BM1824, BM2113, ETH10 and TGLA227 in cattle from Uruguay and at loci BM1824, BM2113, ETH10, ETH225, INRA23 and TGLA53 in Paraguayan cattle.

Nelore, a zebu breed, is responsible for containing the highest number of cattle in Brazil, a country bordering Uruguay and Paraguay and the largest producer of beef in the world. The breed is also responsible for assisting in the formation of new breeds such as Brahman and also for the emergence and maintenance of hybrid breeds (Braford and Brangus). Two studies were conducted using the Brazilian Nelore to estimate the frequencies of the alleles on 11 and 10 STR loci, respectively, in common with the present article[21,23]. In the first article, it can be noted that in all markers there are differences between the most frequent allele between the Brazilian cattle and the cattle of the same breed raised in Uruguay. In this Brazilian study, however, in some loci it seems that there was a mistake in determining the length of PCR amplicons fragments, which could perhaps determine that in three loci the most common allele between the two populations was the same[21]. In the second article published with Brazilian Nelore cattle with ten STR loci in common with the present study, the most frequent allele was the same in both populations in six genetic markers (TGLA53, ETH225, INRA23, BM1824, TGLA126, BM2113)[23].

In relation to the Brahman, another zebu breed, which in this study has representatives from Uruguay and Paraguay, we have based a published study with more than 3,000 zebus from Colombia to compare the most frequent alleles in 11 common STR in all three populations[24]. With Brahman cattle from Uruguay in all 11 loci, the most frequent alleles were the same as the animals raised in Colombia. However, for the Brahman zebus of Paraguay, in which number of genotyped

animals resembled the study carried out in Colombia, three loci did not present the same more frequent alleles (TGLA53, TGLA122 and TGLA126).

For the other four breeds (Angus, Brangus, Braford and Jersey) present in the study, no allelic frequency publications were found with the same STR loci group to allow comparison of the most frequent alleles in each genetic marker. Therefore, this article can be considered, especially for the first three breeds, as one of the first in the scientific community and can also be regarded as the largest ever for these breeds in relation to the expressive number of cattle used for the study.

It is necessary to mention, finally, that the use of this group of 12 STR loci recommended by ISAG is questioned, since many of genetic markers have been shown to be associated in some way with important economical traits such as milk protein yield, milk fat yield, somatic cell score, milk fat percentage, body weight at birth and body weight at weaning. This fact may contribute to the increase or decrease in allelic frequency at these loci, depending on the desired characteristic for the selection, resulting in a reduced overall informativeness and exclusion power of the marker panel[22]. Besides that, in some situations, only with the 12 STR loci recommended by the ISAG, it is not possible to perform reliable analysis for the resolution of forensic cases due to the limitation in the power of exclusion in some bovine breeds. Interrupting its use, however, does not seem to be the best way forward, since its selection was a long process, involving several rounds of interlaboratory tests, being extensively used, generating extensive genetic databases. Therefore, an alternative suggestion to overcome these limitations is that to the group of 12 STR loci already recommended four more loci should be added to the panel, making the set of genetic markers more powerful for resolving forensic parentage and kinship analysis. Van de Goor *et al.* has been proven that the inclusion of STR CSRM60, CSSM66, HAUT27 and ILSTS006 in the laboratory routine for genetic linkage analysis is easily applicable and in fact makes the tool for the genetic identification of cattle for forensic purposes more powerful[3].

4 CONCLUSIONS

The present article is the first study carried out with cattle raised in Uruguay and Paraguay that uses the analysis of the 12 STR loci recommended by ISAG,

estimating allelic frequencies and other parameters of forensic interest, as well as calculating the genetic distance between ten breeds. In addition, through the analysis of fixation index (F_{ST}) it was measured that there is genetic differentiation between animals of the same breed but that were born and were raised in different geographic regions. It should also be noted that, in at least six breeds, all these parameters were estimated from truly expressive populations (over 1,000 animals), which certainly provide more accurate results and provide better information on the genetic variability of a given breed.

In the same way as it has been motivated in other articles, we also encourage more population studies like this one to be carried out, first for better comparisons on the data published here. It would be interesting to be able to make a global or broader study on the genetic variability of the bovine breeds around the world, allowing a greater monitoring of the diversity, comparing the allelic frequencies of animals belonging to the same group, but of very different geographic regions. Secondly, for the creation of large databases of the most varied breeds from the most diverse locations, allowing the use of this information for the purpose of individual genetic identification, paternity tests, linkage analysis and for improvement in traceability systems, providing safe guarantees of provenance to the demanding markets of international trade, the food industry and consumers.

This paper follows the guidelines for publication of population data requested by the journal[25], the recommendations of International Society for Forensic Genetics (ISFG) regarding the use of non-human (animal) DNA in forensic genetic investigations[26] and the proposed allele nomenclature for standardization in forensic bovine DNA typing[27] as recommended by the ISGF for the nomenclature of human STR[28].

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Appendix A. Supplementary data

Supplementary data associated with this article can be found in the online version.

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Alelle	TGLA227	BM2113	TGLA53	ETH10	SPS115	TGLA126	TGLA122	INRA23	ETH3	ETH225	BM1824	BM1818
10												0.0003
11								0.0001				
12	0.0015										0.3801	
12.1											0.0001	
13		0.0002						0.0009			0.1228	0.0001
14	0.1435	0.1659		0.0037		0.0002	0.0001	0.0782			0.2492	0.0323
14.1	0.0002											
15	0.0147	0.0287		0.0001				0.1482	0.0013		0.0003	0.0028
16	0.0004	0.0016		0.0020	0.0002	0.0745	0.0079	0.0001				0.2682
16.1								0.0003				
17	0.1449	0.0004		0.0794	0.0052	0.3898	0.1901	0.4584			0.2468	0.0907
17.1	0.0002											
18	0.2520	0.2312		0.5156	0.0001	0.3869		0.1997	0.0004		0.0006	0.4158
18.1				0.0002								
19	0.0732	0.2726	0.0152	0.1934	0.0001	0.0017	0.0025	0.0029		0.0513	0.0002	0.0357
19.1										0.0008		
20	0.1231	0.0574	0.0002	0.1890	0.0002	0.0139	0.0028	0.0034	0.0001			0.1536
20.1				0.0002					0.0001			
21	0.0010	0.1772	0.0132	0.0161	0.5225	0.1317	0.6066	0.1029	0.0055	0.2072		0.0004
22	0.2436	0.0643	0.4315	0.0004	0.0009	0.0009	0.0048	0.0049	0.4953	0.2054		0.0001
23	0.0003	0.0006	0.0365		0.0495	0.0003	0.0698		0.0650	0.2669		
24	0.0004		0.0394		0.2505		0.0219		0.2315	0.2663		
25	0.0008		0.0017		0.0144		0.0002		0.0039			0.0001
25.1							0.0001					
26			0.1346		0.0030		0.0854		0.1372	0.0015		
27			0.1286		0.1534		0.0002		0.0595			
28			0.0072						0.0003			
29			0.0059							0.0005		
30			0.1739				0.0002			0.0001		
31			0.0016				0.0002					
32							0.0001					
33			0.0101									
34			0.0002									
35			0.0003									
37							0.0071					
Locus	**TGLA227**	**BM2113**	**TGLA53**	**ETH10**	**SPS115**	**TGLA126**	**TGLA122**	**INRA23**	**ETH3**	**ETH225**	**BM1824**	**BM1818**
N	6001	6019	5872	6025	6015	6023	6026	6023	6018	6026	6024	5482
HWE	**4.00E-07**	**1.22E-16**	**7.96E-18**	**1.26E-05**	**1.94E-08**	1.16E-01	**1.12E-05**	**4.74E-06**	**1.35E-06**	**2.47E-06**	3.78E-02	4.08E-01
Ho	0.7914	0.7792	0.7168	0.6410	0.6342	0.6736	0.5665	0.6880	0.6637	0.7516	0.7118	0.7114
He	0.8149	0.8051	0.7455	0.6545	0.6380	0.6753	0.5831	0.7113	0.6746	0.7701	0.7175	0.7211
PD	0.9412	0.9352	0.9042	0.8345	0.8133	0.8325	0.7851	0.8789	0.8528	0.9092	0.8684	0.8801
PE	0.5829	0.5608	0.4546	0.3427	0.3339	0.3885	0.2520	0.4102	0.3738	0.5112	0.4466	0.4458
PIC	0.7892	0.7772	0.7169	0.6084	0.5859	0.6156	0.5461	0.6743	0.6320	0.7310	0.6663	0.6789
P_{ID}	0.0587	0.0648	0.0957	0.1654	0.1866	0.1675	0.2148	0.1210	0.1471	0.0908	0.1315	0.1198

Table S1: Allele frequencies and other forensic parameters for 12 bovine-specific STR loci of Angus breed from Uruguayan cattle population: HWE - *P* value for Hardy-Weinberg equilibrium, Ho - observed heterozygosity, He - expected heterozygosity, PD - power of discrimination, PE - power of exclusion, PIC - polymorphism information content, P_{ID} - Probability of Identity (Matching Probability). **Statistically significative after Bonferroni's correction (<0.0042).**

Alelle	TGLA227	BM2113	TGLA53	ETH10	SPS115	TGLA126	TGLA122	INRA23	ETH3	ETH225	BM1824	BM1818
11								0.0315				
12	0.2119							0.0252			0.2616	
13	0.0975							0.0126			0.2321	
14	0.0381	0.0084		0.1450			0.0340	0.0021			0.3439	0.0052
15	0.0805	0.0717		0.0798				0.0399	0.0316			0.0628
16		0.0865		0.2080		0.0063	0.0340					0.2435
17	0.0530	0.0105		0.0483		0.3650	0.3723	0.1912			0.1371	0.1152
17.1												
18	0.1610	0.1160		0.1239		0.1688		0.0945			0.0084	0.3927
18.1												
19	0.2161	0.2468	0.0907	0.2038		0.0401	0.1106	0.0777		0.1688	0.0169	0.0707
20	0.0911	0.0422		0.1912	0.0169	0.1814	0.0362	0.0042		0.0211		0.0969
21	0.0042	0.2447	0.0042		0.2797	0.1456	0.1851	0.4580	0.2384	0.1055		0.0079
22	0.0466	0.1709	0.3312		0.0466	0.0675	0.0383	0.0399	0.5042	0.0675		
23		0.0021	0.1688		0.1208	0.0253		0.0231	0.1688	0.2911		
24			0.0338		0.0339		0.0021		0.0105	0.1371		0.0026
25					0.0911					0.0063		0.0026
26					0.0466		0.1426		0.0232	0.0338		
27			0.1055		0.3623							
27.1												
28			0.0549						0.0232			
29			0.1435				0.0021			0.1646		
30			0.0063		0.0021		0.0064			0.0021		
31			0.0105									
32			0.0021									
33												
34												
34.1			0.0169									
35												
36			0.0021									
37			0.0295				0.0362					
38										0.0021		
Locus	**TGLA227**	**BM2113**	**TGLA53**	**ETH10**	**SPS115**	**TGLA126**	**TGLA122**	**INRA23**	**ETH3**	**ETH225**	**BM1824**	**BM1818**
N	236	237	237	238	236	237	235	238	237	237	237	191
HWE	3.40E-01	**3.71E-03**	2.08E-01	**1.71E-03**	6.00E-01	7.84E-01	4.37E-02	1.03E-01	7.30E-01	4.17E-01	2.78E-01	7.55E-01
Ho	0.8559	0.8397	0.7722	0.8319	0.7542	0.7932	0.7957	0.7773	0.6371	0.7890	0.7004	0.7801
He	0.8536	0.8237	0.8181	0.8353	0.7635	0.7790	0.7898	0.7347	0.6596	0.8253	0.7419	0.7568
PD	0.9582	0.9401	0.9437	0.9455	0.9109	0.9183	0.9340	0.8934	0.8364	0.9456	0.8895	0.9028
PE	0.7066	0.6733	0.5484	0.6596	0.5170	0.5866	0.5912	0.5576	0.3378	0.5788	0.4289	0.5627
PIC	0.8349	0.7986	0.7961	0.8115	0.7288	0.7479	0.7641	0.7075	0.6101	0.8016	0.6948	0.7223
P_{ID}	0.0418	0.0599	0.0563	0.0545	0.0891	0.0817	0.0660	0.1066	0.1636	0.0544	0.1105	0.0972

Table S2: Allele frequencies and other forensic parameters for 12 bovine-specific STR loci of Braford breed from Uruguayan cattle population: HWE - *P* value for Hardy-Weinberg equilibrium, Ho - observed heterozygosity, He - expected heterozygosity, PD - power of discrimination, PE - power of exclusion, PIC - polymorphism information content, P_{ID} - Probability of Identity (Matching Probability). **Statistically significative after Bonferroni's correction (<0.0042).**

Alelle	TGLA227	BM2113	TGLA53	ETH10	SPS115	TGLA126	TGLA122	INRA23	ETH3	ETH225	BM1824	BM1818
12	0.6087							0.0217			0.0543	
13	0.0435							0.0435			0.4783	
14	0.2283			0.3696			0.0761				0.3478	
15	0.1196	0.1087		0.1630				0.1196	0.0761			0.1250
16		0.2826		0.3804				0.0435				
17		0.0109				0.2174	0.2174				0.0326	0.0625
18		0.0109				0.0435	0.0435	0.0978	0.0109			0.3750
19		0.0652		0.0761		0.0652		0.0652		0.0109	0.0870	0.3125
20				0.0109	0.2444	0.0435	0.0761	0.0217		0.0109		0.1250
21		0.0978			0.4222	0.3913	0.3152	0.4348	0.4891	0.0217		
22		0.3913	0.4432		0.2111	0.2391	0.1630	0.1522	0.3587			
23		0.0326	0.1705									
24			0.0227		0.0556							
25					0.0333							
26			0.2386				0.0326		0.0652	0.0761		
27					0.0333							
28			0.0341									
29										0.8804		
30							0.0761					
33			0.0795									
36			0.0114									
Locus	**TGLA227**	**BM2113**	**TGLA53**	**ETH10**	**SPS115**	**TGLA126**	**TGLA122**	**INRA23**	**ETH3**	**ETH225**	**BM1824**	**BM1818**
N	46	46	44	46	45	46	46	46	46	46	46	8
HWE	8.38E-01	6.62E-01	4.97E-01	4.97E-01	7.44E-01	7.31E-01	N.D.	4.72E-01	8.67E-01	N.D.	1.87E-01	N.D.
Ho	0.5870	0.7609	0.7045	0.6304	0.7556	0.7826	0.8696	0.7826	0.5870	0.2391	0.6087	0.7500
He	0.5674	0.7482	0.7176	0.6937	0.7201	0.7425	0.8153	0.7633	0.6288	0.2207	0.6457	0.7750
PD	0.7429	0.8653	0.8533	0.8544	0.8652	0.8629	0.9159	0.9140	0.7987	0.3951	0.8157	0.6667
PE	0.2755	0.6252	0.4353	0.3290	0.5193	0.5672	0.7338	0.5672	0.2755	0.0414	0.3014	N.D.
PIC	0.5089	0.7026	0.6677	0.6281	0.6666	0.6928	0.7819	0.7315	0.5528	0.2083	0.5752	0.6816
P_{ID}	0.2571	0.1347	0.1467	0.1456	0.1348	0.1371	0.0841	0.0860	0.2013	0.6049	0.1843	0.3333

Table S3: Allele frequencies and other forensic parameters for 12 bovine-specific STR loci of Brahman breed from Uruguayan cattle population: HWE - *P* value for Hardy-Weinberg equilibrium, Ho - observed heterozygosity, He - expected heterozygosity, PD - power of discrimination, PE - power of exclusion, PIC - polymorphism information content, P_{ID} - Probability of Identity (Matching Probability).

Alelle	TGLA227	BM2113	TGLA53	ETH10	SPS115	TGLA126	TGLA122	INRA23	ETH3	ETH225	BM1824	BM1818
10												0.0023
11	0.0021							0.0128				
12	0.2564	0.0021						0.1043			0.4915	
13	0.0106	0.0021					0.0021	0.0106			0.1340	
14	0.2055	0.1519		0.1477			0.0591	0.0149			0.2957	0.0413
15	0.0169	0.0696		0.0527				0.0681	0.0511			0.0069
16	0.0021	0.0359		0.2743		0.0702	0.0063					0.1950
17	0.1017	0.0042		0.0717		0.1936	0.2236	0.2489			0.0766	0.1078
18	0.1525	0.0549		0.1814		0.2702	0.0063	0.1787			0.0021	0.4862
19	0.0487	0.3586	0.0066	0.1624		0.1043	0.0084	0.0191		0.1404		0.0505
20	0.0212	0.0570		0.1097	0.0299	0.0532	0.0148	0.0085		0.0021		0.0894
21		0.1013	0.0328		0.2885	0.1383	0.4177	0.3255	0.1213	0.1638		0.0023
22	0.1758	0.1371	0.2380		0.0085	0.1638	0.0717	0.0085	0.5128	0.0532		
23	0.0064	0.0253	0.0502		0.0427	0.0064	0.0021		0.0404	0.1851		0.0183
24			0.0721		0.3098		0.0042		0.1830	0.1021		
25			0.0349		0.1774							
26			0.1834		0.0107		0.1266		0.0787	0.0085		
27			0.0371		0.1325		0.0169		0.0128			
28			0.0197									
29			0.0022				0.0042			0.3447		
30			0.2336				0.0127					
31.1			0.0022									
32							0.0042					
33			0.0742									
35			0.0022									
36			0.0109									
37							0.0190					
Locus	**TGLA227**	**BM2113**	**TGLA53**	**ETH10**	**SPS115**	**TGLA126**	**TGLA122**	**INRA23**	**ETH3**	**ETH225**	**BM1824**	**BM1818**
N	236	237	229	237	234	235	237	235	235	235	235	218
HWE	3.96E-01	1.43E-01	1.09E-01	5.18E-02	7.04E-02	9.31E-02	1.04E-01	2.61E-02	8.18E-02	2.51E-01	4.87E-01	1.60E-01
Ho	0.8220	0.7764	0.7991	0.8101	0.7393	0.8681	0.7426	0.8340	0.6809	0.7489	0.6596	0.6651
He	0.8260	0.8079	0.8395	0.8254	0.7705	0.8266	0.7512	0.7853	0.6797	0.7887	0.6485	0.7029
PD	0.9428	0.9385	0.9487	0.9391	0.9086	0.9377	0.8976	0.9156	0.8555	0.9233	0.8103	0.8718
PE	0.6406	0.5559	0.5974	0.6180	0.4917	0.7308	0.4972	0.6637	0.3993	0.5079	0.3685	0.3764
PIC	0.8011	0.7862	0.8187	0.8007	0.7329	0.8022	0.7201	0.7530	0.6453	0.7582	0.5890	0.6696
P_{ID}	0.0572	0.0615	0.0513	0.0609	0.0914	0.0623	0.1024	0.0844	0.1445	0.0767	0.1897	0.1282

Table S4: Allele frequencies and other forensic parameters for 12 bovine-specific STR loci of Brangus breed from Uruguayan cattle population: HWE - *P* value for Hardy-Weinberg equilibrium, Ho - observed heterozygosity, He - expected heterozygosity, PD - power of discrimination, PE - power of exclusion, PIC - polymorphism information content, P_{ID} - Probability of Identity (Matching Probability).

Alelle	TGLA227	BM2113	TGLA53	ETH10	SPS115	TGLA126	TGLA122	INRA23	ETH3	ETH225	BM1824	BM1818
10												0.0001
11											0.0001	
12	0.0022							0.0001			0.0001	
13	0.0256			0.0002				0.0004			0.0288	
14	0.0380	0.0911		0.0014			0.0016	0.0008			0.5036	0.0022
15	0.0691	0.0381		0.0002				0.0004	0.0003			0.0413
15.1				0.0001								
16	0.0008	0.0010		0.0145		0.0029	0.1178	0.0002				0.3093
16.1						0.0001						
17	0.0391	0.0259		0.0736		0.3162	0.4886	0.2162			0.2176	0.0139
18	0.2255	0.1392		0.2337		0.4606	0.0001	0.1313			0.0341	0.5749
18.1							0.0001					
19	0.3949	0.1860	0.1067	0.1840		0.0157	0.0285	0.0026	0.0001	0.2361	0.0002	0.0217
20	0.1492	0.0268		0.4890	0.0003	0.1296	0.0118	0.0076		0.0014		0.0367
20.1									0.0001			
21	0.0013	0.3465	0.0011	0.0013	0.2752	0.0743	0.0785	0.6399	0.0023	0.0848		
22	0.0542	0.1448	0.3206	0.0019	0.0009	0.0006	0.0005	0.0004	0.5399	0.1173		
23	0.0001	0.0004	0.3080	0.0001	0.1742		0.0006	0.0001	0.4385	0.4080		
24		0.0001	0.0230		0.0373		0.0006		0.0086	0.1496		
25	0.0001		0.0007		0.1638		0.0004		0.0001	0.0019		
26			0.0053		0.0315		0.1889		0.0074	0.0004		
27			0.0233		0.3162		0.0006		0.0018			
28		0.0001	0.1918		0.0004				0.0007			
29			0.0009		0.0001				0.0001	0.0006		
30			0.0115		0.0001		0.0208			0.0001		
31			0.0001				0.0001					
32			0.0004				0.0001					
33			0.0001									
34			0.0064									
35							0.0013					
36							0.0003					
37							0.0589					
Locus	**TGLA227**	**BM2113**	**TGLA53**	**ETH10**	**SPS115**	**TGLA126**	**TGLA122**	**INRA23**	**ETH3**	**ETH225**	**BM1824**	**BM1818**
N	6784	6956	6831	6978	6914	6987	7000	6991	6995	7002	7002	4896
HWE	**0.00E+00**	1.43E-01	**6.87E-27**	3.83E-02	**2.15E-05**	1.19E-02	**2.79E-05**	4.32E-02	1.44E-02	2.14E-02	**1.52E-03**	**0.00E+00**
Ho	0.7297	0.7872	0.6968	0.6535	0.7524	0.6670	0.6834	0.5177	0.4969	0.7189	0.6398	0.5478
He	0.7596	0.7939	0.7529	0.6668	0.7648	0.6653	0.7007	0.5265	0.5161	0.7345	0.6506	0.5702
PD	0.9108	0.9319	0.9009	0.8388	0.9068	0.8281	0.8789	0.7196	0.6568	0.8916	0.8248	0.7433
PE	0.4757	0.5756	0.4232	0.3588	0.5137	0.3790	0.4025	0.2031	0.1843	0.4573	0.3412	0.2316
PIC	0.7292	0.7678	0.7129	0.6171	0.7267	0.6085	0.6696	0.4724	0.4038	0.6955	0.5972	0.5037
P_{ID}	0.0892	0.0681	0.0991	0.1612	0.0932	0.1719	0.1211	0.2804	0.3432	0.1084	0.1752	0.2567

Table S5: Allele frequencies and other forensic parameters for 12 bovine-specific STR loci of Hereford breed from Uruguayan cattle population: HWE - *P* value for Hardy-Weinberg equilibrium, Ho - observed heterozygosity, He - expected heterozygosity, PD - power of discrimination, PE - power of exclusion, PIC - polymorphism information content, P_{ID} - Probability of Identity (Matching Probability). **Statistically significative after Bonferroni's correction (<0.0042).**

Alelle	TGLA227	BM2113	TGLA53	ETH10	SPS115	TGLA126	TGLA122	INRA23	ETH3	ETH225	BM1824	BM1818
12	0.0003										0.2323	
13		0.0006						0.0038			0.2205	
14	0.0508	0.2275		0.0304				0.0097			0.1244	0.0181
14.1	0.0006											
15	0.1080	0.2177		0.0003			0.0041	0.2115			0.0003	0.0029
16	0.0012	0.0015		0.0547		0.0012	0.0027	0.0006				0.4148
17	0.0367			0.0047		0.3349	0.3451	0.2516			0.3738	0.0581
18	0.1703	0.0107		0.2134		0.5100		0.0372			0.0486	0.4429
19	0.1948	0.3527	0.0364	0.5124		0.0418	0.0024	0.2569		0.1264		0.0448
20	0.0361	0.1038		0.0284	0.0009	0.0736	0.2215	0.0009		0.0091		0.0186
20.1										0.0003		
21	0.0031	0.0841	0.1264	0.0381	0.6213	0.0383	0.0522	0.2274	0.0027	0.0324		
22	0.2890	0.0015	0.1610	0.1176	0.0024	0.0003	0.0168	0.0003	0.4131	0.0321		
23	0.0312		0.2929		0.1560		0.0009		0.0106	0.3430		
23.1										0.0003		
24			0.0054		0.0705				0.0041	0.3736		
25	0.0780		0.0265		0.1201		0.0035		0.0021	0.0822		
25.1			0.0003									
26			0.0912		0.0105		0.0923		0.1399			
27			0.0063		0.0182		0.1242		0.1340			
28			0.0102						0.2900			
29							0.0003		0.0035	0.0003		
30			0.1415				0.0003					
30.1										0.0003		
31			0.0015				0.0608					
32			0.0003				0.0165					
33			0.0003									
34			0.0202									
35			0.0795									
36							0.0006					
37							0.0558					
38												
Locus	**TGLA227**	**BM2113**	**TGLA53**	**ETH10**	**SPS115**	**TGLA126**	**TGLA122**	**INRA23**	**ETH3**	**ETH225**	**BM1824**	**BM1818**
N	1635	1677	1661	1692	1673	1699	1695	1693	1698	1697	1696	1050
HWE	1.22E-02	1.96E-02	**1.97E-09**	3.86E-01	8.68E-03	3.99E-01	1.13E-02	4.15E-01	4.25E-01	9.18E-02	8.33E-01	4.59E-01
Ho	0.7914	0.7454	0.7694	0.6785	0.5918	0.6086	0.8053	0.7891	0.7203	0.6971	0.7341	0.6057
He	0.8258	0.7587	0.8353	0.6721	0.5699	0.6193	0.7980	0.7730	0.7077	0.7181	0.7400	0.6261
PD	0.9494	0.9038	0.9549	0.8589	0.7797	0.7963	0.9351	0.9074	0.8616	0.8781	0.8905	0.7935
PE	0.5832	0.5019	0.5436	0.3970	0.2811	0.3013	0.6089	0.5790	0.4603	0.4238	0.4829	0.2978
PIC	0.8046	0.7208	0.8166	0.6354	0.5345	0.5543	0.7738	0.7341	0.6589	0.6719	0.6972	0.5538
P_{ID}	0.0506	0.0962	0.0451	0.1411	0.2203	0.2037	0.0649	0.0926	0.1384	0.1219	0.1095	0.2065

Table S6: Allele frequencies and other forensic parameters for 12 bovine-specific STR loci of Holstein-Friesian breed from Uruguayan cattle population: HWE - *P* value for Hardy-Weinberg equilibrium, Ho - observed heterozygosity, He - expected heterozygosity, PD - power of discrimination, PE - power of exclusion, PIC - polymorphism information content, P_{ID} - Probability of Identity (Matching Probability). **Statistically significative after Bonferroni's correction (<0.0042).**

Alelle	TGLA227	BM2113	TGLA53	ETH10	SPS115	TGLA126	TGLA122	INRA23	ETH3	ETH225	BM1824	BM1818
12	0.0596										0.0304	
13								0.3726			0.6692	0.0030
14	0.3423	0.0057						0.0309			0.1198	0.0268
15	0.0038	0.0209						0.0058				0.1696
16		0.0684					0.0019					0.1815
17		0.0019		0.1686		0.2452	0.4830	0.2394			0.0399	0.0506
18	0.0038	0.1312		0.4356		0.6844		0.1371			0.1407	0.5565
19	0.1827	0.7110	0.0058	0.2367		0.0019	0.0019	0.0058		0.0985		0.0119
20	0.3615	0.0608		0.1402		0.0399	0.0852					
21	0.0154		0.0039	0.0189		0.0285	0.1970	0.1042	0.0019	0.3239		
22	0.0173		0.2819		0.2538				0.7159	0.0019		
23			0.0811		0.0076			0.1004	0.0038	0.2140		
24	0.0135				0.4223		0.0038	0.0019	0.0644	0.3617		
25			0.0019		0.1288			0.0019	0.0019			
26			0.2876		0.0038		0.0947		0.2121			
27			0.2548		0.0038							
28			0.0154		0.1780							
29					0.0019							
30							0.1307					
33			0.0598									
34			0.0077				0.0019					
Locus	**TGLA227**	**BM2113**	**TGLA53**	**ETH10**	**SPS115**	**TGLA126**	**TGLA122**	**INRA23**	**ETH3**	**ETH225**	**BM1824**	**BM1818**
N	260	263	259	264	264	263	264	259	264	264	263	168
HWE	1.11E-01	4.59E-01	1.52E-01	6.79E-01	4.20E-01	4.71E-01	9.72E-01	5.49E-01	5.84E-01	1.38E-01	9.73E-01	1.89E-01
Ho	0.6846	0.4715	0.7104	0.7348	0.6894	0.4563	0.7008	0.7761	0.4508	0.7386	0.5095	0.6071
He	0.7158	0.4693	0.7639	0.7071	0.7102	0.4699	0.6959	0.7646	0.4391	0.7101	0.5165	0.6270
PD	0.8682	0.6964	0.9084	0.8632	0.8656	0.6612	0.8687	0.9049	0.6382	0.8484	0.7310	0.8206
PE	0.4049	0.1637	0.4446	0.4842	0.4120	0.1520	0.4294	0.5554	0.1479	0.4905	0.1960	0.2995
PIC	0.6648	0.4417	0.7233	0.6596	0.6613	0.4101	0.6577	0.7301	0.3875	0.6542	0.4819	0.5824
P_{ID}	0.1318	0.3036	0.0916	0.1368	0.1344	0.3388	0.1313	0.0951	0.3618	0.1516	0.2690	0.1794

Table S7: Allele frequencies and other forensic parameters for 12 bovine-specific STR loci of Jersey breed from Uruguayan cattle population: HWE - *P* value for Hardy-Weinberg equilibrium, Ho - observed heterozygosity, He - expected heterozygosity, PD - power of discrimination, PE - power of exclusion, PIC - polymorphism information content, P_{ID} - Probability of Identity (Matching Probability).

Alelle	TGLA227	BM2113	TGLA53	ETH10	SPS115	TGLA126	TGLA122	INRA23	ETH3	ETH225	BM1824	BM1818
12	0.0302										0.3316	
13											0.2306	
14	0.2555	0.0053						0.1927			0.0130	0.0404
15	0.1621	0.0559						0.0026				0.0184
16	0.0165			0.0285		0.0984						0.1801
17	0.0137	0.2899		0.0777		0.4948	0.1114	0.2578			0.4249	0.0882
18	0.2940	0.1755		0.2150		0.0674		0.3359	0.0208			0.5625
19	0.1566	0.2048	0.0829	0.2979			0.1062			0.4041		0.0993
20	0.0385	0.0665		0.2539		0.0751	0.1114	0.0208				0.0110
21		0.1330		0.0959	0.5311	0.2124	0.3731	0.1771	0.0026	0.1166		
22	0.0302	0.0027	0.2927	0.0311		0.0518	0.1632	0.0052	0.5078	0.0285		
23		0.0665	0.0207		0.0803			0.0078	0.1458	0.2124		
24	0.0027		0.0959		0.0233					0.2383		
25			0.1606		0.0648		0.0363		0.0078			
26			0.0492		0.0026		0.0622		0.0807			
27					0.2979				0.2109			
27.1			0.0466									
28			0.0415						0.0026			
29			0.0285						0.0208			
30			0.1218									
31			0.0596									
32							0.0130					
34							0.0078					
35							0.0155					
Locus	**TGLA227**	**BM2113**	**TGLA53**	**ETH10**	**SPS115**	**TGLA126**	**TGLA122**	**INRA23**	**ETH3**	**ETH225**	**BM1824**	**BM1818**
N	182	188	193	193	193	193	193	192	192	193	193	136
HWE	5.86E-01	4.40E-02	2.70E-01	9.68E-01	8.28E-02	9.57E-01	2.96E-01	8.49E-01	3.04E-01	2.61E-01	3.82E-01	8.00E-01
Ho	0.8022	0.7819	0.8187	0.7876	0.5492	0.6943	0.7668	0.7708	0.6719	0.7047	0.6425	0.6250
He	0.7959	0.8157	0.8487	0.7856	0.6196	0.6893	0.7945	0.7536	0.6707	0.7222	0.6579	0.6337
PD	0.9227	0.9354	0.9543	0.9167	0.7972	0.8689	0.9324	0.8939	0.8474	0.8678	0.8103	0.8376
PE	0.6031	0.5659	0.6341	0.5762	0.2343	0.4195	0.5390	0.5461	0.3861	0.4355	0.3450	N.D.
PIC	0.7642	0.7889	0.8313	0.7510	0.5596	0.6519	0.7699	0.7095	0.6283	0.6742	0.5855	0.5969
P_{ID}	0.0773	0.0646	0.0457	0.0833	0.2028	0.1311	0.0676	0.1061	0.1526	0.1322	0.1897	0.1624

Table S8: Allele frequencies and other forensic parameters for 12 bovine-specific STR loci of Limousin breed from Uruguayan cattle population: HWE - *P* value for Hardy-Weinberg equilibrium, Ho - observed heterozygosity, He - expected heterozygosity, PD - power of discrimination, PE - power of exclusion, PIC - polymorphism information content, P_{ID} - Probability of Identity (Matching Probability).

Alelle	TGLA227	BM2113	TGLA53	ETH10	SPS115	TGLA126	TGLA122	INRA23	ETH3	ETH225	BM1824	BM1818
10.1								0.0007				
11	0.0022							0.0679				0.0008
12	0.6662							0.0978			0.1799	0.0008
13	0.1744			0.0030			0.0022	0.0134			0.5134	
14	0.1040	0.0239		0.2619		0.0007	0.2710				0.2828	0.0339
14.1				0.0007				0.0007				
15	0.0082	0.0075		0.5127			0.0007	0.0724	0.0650			0.0566
15.1				0.0007								
16		0.3239		0.1784			0.0090	0.0261				0.0106
17	0.0022	0.0022			0.0007	0.1398	0.2350	0.0313			0.0022	0.3974
18	0.0007	0.0015		0.0022	0.0015	0.0374	0.0464	0.0463	0.0007	0.0007		0.3469
19	0.0015	0.2030	0.0008	0.0403	0.0030	0.0732	0.0966	0.0903	0.0007	0.0958	0.0172	0.0234
20	0.0007	0.0030	0.0038		0.1684	0.3393	0.0030	0.0007		0.0419	0.0045	0.0271
21		0.0231	0.0015		0.5052	0.1614	0.1040	0.5507	0.4410	0.0090		0.1018
22	0.0397	0.1970	0.5113		0.0412	0.2444	0.0906	0.0015	0.4178	0.0015		
23		0.2142	0.0559		0.0037	0.0030			0.0112	0.0127		
24		0.0007	0.0098		0.0449	0.0007	0.0030					
25			0.0023		0.1751		0.0180		0.0007	0.0539		
26			0.1216		0.0097		0.1198		0.0628	0.1916		
26.1										0.0015		
27			0.0506		0.0464					0.0052		
28			0.0264							0.0060		0.0008
29							0.0007			0.5636		
30			0.0272							0.0165		
31			0.0355									
32			0.0038									
33			0.0038									
34			0.0113									
35			0.0008									
36			0.1284									
37			0.0045									
38			0.0008									
Locus	**TGLA227**	**BM2113**	**TGLA53**	**ETH10**	**SPS115**	**TGLA126**	**TGLA122**	**INRA23**	**ETH3**	**ETH225**	**BM1824**	**BM1818**
N	668	670	662	670	668	669	668	670	669	668	670	663
HWE	8.67E-01	1.93E-01	7.66E-02	1.97E-02	4.05E-01	4.09E-01	1.21E-02	3.38E-02	4.45E-01	**6.69E-03**	5.79E-01	6.30E-02
Ho	0.5030	0.7522	0.6677	0.5851	0.6766	0.7907	0.8234	0.6597	0.6278	0.6048	0.6254	0.6637
He	0.5137	0.7686	0.6991	0.6356	0.6802	0.7734	0.8267	0.6656	0.6232	0.6317	0.6242	0.7061
PD	0.7229	0.9082	0.8806	0.8111	0.8621	0.9130	0.9451	0.8632	0.7787	0.8313	0.7910	0.8677
PE	0.1901	0.5136	0.3800	0.2734	0.3931	0.5820	0.6431	0.3687	0.3256	0.2967	0.3224	0.3743
PIC	0.4745	0.7305	0.6766	0.5767	0.6440	0.7391	0.8047	0.6452	0.5487	0.5975	0.5591	0.6585
P_{ID}	0.2771	0.0918	0.1194	0.1889	0.1379	0.0870	0.0549	0.1368	0.2213	0.1687	0.2090	0.1323

Table S9: Allele frequencies and other forensic parameters for 12 bovine-specific STR loci of Nelore breed from Uruguayan cattle population: HWE - *P* value for Hardy-Weinberg equilibrium, Ho - observed heterozygosity, He - expected heterozygosity, PD - power of discrimination, PE - power of exclusion, PIC - polymorphism information content, P_{ID} - Probability of Identity (Matching Probability). **Statistically significative after Bonferroni's correction (<0.0042).**

Allele	TGLA227	BM2113	TGLA53	ETH10	SPS115	TGLA126	TGLA122	INRA23	ETH3	ETH225	BM1824	BM1818
12											0.1037	
13								0.4444			0.3037	0.1555
14	0.4887	0.0556						0.0074			0.1593	0.0126
15		0.2333						0.0037				
16				0.0858								0.3109
17	0.1165	0.0259		0.0075		0.4259	0.2111	0.2370			0.4259	
18	0.0865			0.1978		0.2519	0.0074	0.0333			0.0074	0.5168
19	0.0526	0.1296	0.0444	0.0560		0.0148				0.0037		
20	0.0940	0.2630			0.0075		0.0889	0.1333				0.0042
21		0.2926		0.0149	0.3955	0.0704	0.4296	0.1296		0.1519		
22	0.1617		0.3815	0.6381	0.0784		0.2630	0.0111		0.0741		
23			0.1630		0.0261				0.3778	0.4074		
24					0.0336				0.1407	0.3630		
25			0.0037		0.1754							
26			0.1667						0.2778			
27			0.1111		0.2836				0.2037			
30			0.0037									
32			0.0704									
34			0.0519									
35			0.0037									
Locus	**TGLA227**	**BM2113**	**TGLA53**	**ETH10**	**SPS115**	**TGLA126**	**TGLA122**	**INRA23**	**ETH3**	**ETH225**	**BM1824**	**BM1818**
N	133	135	135	134	134	135	135	135	135	135	135	119
HWE	3.00E-01	1.01E-01	6.74E-01	4.41E-01	5.56E-01	6.92E-01	1.95E-02	2.96E-01	2.48E-01	7.26E-02	1.91E-02	6.82E-02
Ho	0.6391	0.7185	0.7926	0.5522	0.7537	0.6889	0.7407	0.7185	0.6889	0.7630	0.6222	0.5630
He	0.7050	0.7731	0.7810	0.5450	0.7271	0.6964	0.6963	0.7130	0.7215	0.6762	0.6927	0.6145
PD	0.8759	0.9032	0.9201	0.7552	0.8662	0.8487	0.8240	0.8708	0.8698	0.8014	0.8454	0.7775
PE	0.3404	0.4628	0.5854	0.2374	0.5162	0.4113	0.4941	0.4575	0.4113	0.5322	0.3184	0.2488
PIC	0.6718	0.7328	0.7518	0.5015	0.6803	0.6402	0.6409	0.6693	0.6682	0.6127	0.6364	0.5425
P_{ID}	0.1241	0.0968	0.0799	0.2448	0.1338	0.1513	0.1760	0.1292	0.1302	0.1986	0.1546	0.2225

Table S10: Allele frequencies and other forensic parameters for 12 bovine-specific STR loci of Nelore breed from Uruguayan cattle population: HWE - *P* value for Hardy-Weinberg equilibrium, Ho - observed heterozygosity, He - expected heterozygosity, PD - power of discrimination, PE - power of exclusion, PIC - polymorphism information content, P_{ID} - Probability of Identity (Matching Probability).

Alelle	TGLA227	BM2113	TGLA53	ETH10	SPS115	TGLA126	TGLA122	INRA23	ETH3	ETH225	BM1824	BM1818
10				0.0002								
11	0.0004							0.0298				
12	0.1690	0.0002						0.0660			0.2177	
13	0.0184			0.0002				0.0102			0.1491	
14	0.0906	0.0379		0.2074		0.0004	0.0457	0.0014			0.4631	0.0055
15	0.0597	0.0674		0.0926			0.0002	0.0408	0.0898	0.0002	0.0006	0.0316
15.1				0.0002								
16	0.0002	0.0623	0.0002	0.2686		0.0022	0.0349		0.0002		0.0002	0.2052
16.1				0.0004				0.0031				
17	0.1090	0.0081	0.0002	0.0214		0.3635	0.2996	0.1292		0.0002	0.1456	0.0900
17.1	0.0002					0.0002						
18	0.1663	0.0790	0.0002	0.0508		0.1613	0.0090	0.0611	0.0031	0.0002	0.0114	0.5111
18.1	0.0002											0.0765
19	0.1794	0.2656	0.0550	0.1507	0.0004	0.0279	0.1305	0.0475	0.0006	0.1554	0.0116	
20	0.1581	0.0448		0.2033	0.0564	0.1674	0.0514	0.0114	0.0002	0.0065	0.0006	0.0763
20.1				0.0002		0.0002						
21	0.0059	0.2218	0.0066	0.0029	0.3398	0.1935	0.2366	0.5872	0.3217	0.0697		0.0037
22	0.0421	0.2053	0.2843	0.0008	0.0280	0.0815	0.0279	0.0029	0.4088	0.0864		
22.1						0.0002						
23	0.0002	0.0075	0.2925		0.1146	0.0014	0.0002	0.0094	0.1314	0.2534		
23.1			0.0002							0.0004		
24			0.0225	0.0004	0.0793	0.0002	0.0010		0.0140	0.2375		
25			0.0050		0.1069		0.0012		0.0047	0.0024		
26			0.1340		0.0225		0.1351		0.0202	0.0350		0.0002
27			0.0506		0.2517		0.0004		0.0031	0.0002		
28			0.0783						0.0021	0.0002		
28.1									0.0002			
29							0.0014			0.1495		
30			0.0098		0.0002		0.0041			0.0029		
31			0.0014				0.0004					
32			0.0002									
33			0.0116									
34			0.0064									
35			0.0025				0.0006					
36			0.0325									
37			0.0025		0.0002		0.0198					
38			0.0005									
39			0.0011									
40			0.0009									
41			0.0009									
42			0.0002									
Locus	**TGLA227**	**BM2113**	**TGLA53**	**ETH10**	**SPS115**	**TGLA126**	**TGLA122**	**INRA23**	**ETH3**	**ETH225**	**BM1824**	**BM1818**
N	2444	2455	2202	2452	2447	2455	2453	2453	2428	2455	2455	2439
HWE	9.22E-01	7.44E-02	**3.05E-05**	6.15E-01	6.10E-02	2.33E-02	**7.69E-04**	3.53E-02	1.02E-02	2.80E-01	6.39E-01	3.59E-02
Ho	0.8727	0.8073	0.7643	0.8120	0.7850	0.7784	0.8182	0.6294	0.7055	0.8098	0.6929	0.6478
He	0.8609	0.8201	0.8022	0.8094	0.7860	0.7691	0.8119	0.6253	0.7036	0.8195	0.6945	0.6760
PD	0.9636	0.9448	0.9357	0.9360	0.9224	0.9122	0.9388	0.8401	0.8634	0.9427	0.8621	0.8597
PE	0.7402	0.6127	0.5346	0.6215	0.5716	0.5596	0.6332	0.3277	0.4368	0.6172	0.4173	0.3522
PIC	0.8448	0.7967	0.7771	0.7822	0.7577	0.7359	0.7881	0.6040	0.6545	0.7951	0.6502	0.6410
P_{ID}	0.0364	0.0552	0.0643	0.0640	0.0776	0.0878	0.0612	0.1599	0.1366	0.0573	0.1379	0.1403

Table S11: Allele frequencies and other forensic parameters for 12 bovine-specific STR loci of Braford breed from Paraguayan cattle population: HWE - *P* value for Hardy-Weinberg equilibrium, Ho - observed heterozygosity, He - expected heterozygosity, PD - power of discrimination, PE - power of exclusion, PIC - polymorphism information content, P_{ID} - Probability of Identity (Matching Probability). **Statistically significative after Bonferroni's correction (<0.0042).**

Alelle	TGLA227	BM2113	TGLA53	ETH10	SPS115	TGLA126	TGLA122	INRA23	ETH3	ETH225	BM1824	BM1818
9	0.0001											0.0001
10								0.0001				0.0001
10.1								0.0001				
11	0.0012							0.0408				
12	0.5802			0.0006		0.0001		0.1293			0.0101	
12.1	0.0001			0.0003								
13	0.0106			0.0006				0.0628	0.0001		0.6987	0.0003
14	0.2521	0.0021		0.2227		0.0167		0.0004			0.2482	0.0071
14.1						0.0001						
15	0.1371	0.0397		0.3319				0.0561	0.0465		0.0014	0.0897
15.1	0.0001			0.0004								
16	0.0010	0.1254		0.3801		0.0005		0.0089			0.0014	0.1359
16.1	0.0001							0.0003				
17	0.0019	0.0006		0.0019		0.2097		0.0048	0.0001		0.0229	0.2953
17.1				0.0003								
18	0.0047	0.0021	0.0001	0.0144	0.0005	0.0466		0.0426	0.0014	0.0001	0.0008	0.1428
18.1	0.0004							0.0001				0.0001
19	0.0018	0.0965	0.0024	0.0410	0.0016	0.0875		0.1345	0.0018	0.0048	0.0164	0.2907
19.1	0.0003			0.0001				0.0003				0.0003
20	0.0006	0.0096	0.0004	0.0057	0.3028	0.0576		0.0014	0.0010	0.0026	0.0001	0.0135
21	0.0006	0.0845	0.0018		0.3625	0.2556		0.3343	0.5035	0.0150		0.0240
22	0.0070	0.5650	0.3010		0.2134	0.3223	0.0001	0.1819	0.4262	0.0014		0.0001
23		0.0741	0.0321		0.0045	0.0013	0.0004	0.0004	0.0112	0.2147		
23.1								0.0001		0.0001		
24		0.0005	0.0110		0.0253	0.0012	0.0003		0.0009	0.0040		
24.1						0.0003				0.0001		
25			0.0201		0.0298				0.0001	0.0008		
25.1						0.0001			0.0003			
26			0.5170		0.0183				0.0059	0.0787		
26.1						0.0001						
27			0.0074		0.0410				0.0003	0.0023		
27.1						0.0003						
28			0.0029		0.0003				0.0007	0.0009		
28.1								0.0004				
29			0.0003				0.0003			0.6686		
29.1								0.0003				
30			0.0016		0.0001		0.0001			0.0050		
31			0.0004							0.0009		
32			0.0001				0.2402					
33			0.0008				0.2402					
33.1			0.0003									
34			0.0424				0.0004					
35			0.0025				0.0302					
36			0.0441				0.0755					
37			0.0034				0.0442					
38			0.0013				0.1509					
39							0.2298					
40			0.0001				0.0659					
41			0.0025				0.0008					
41.1			0.0003									
42			0.0021				0.0022					
43			0.0007									
44			0.0001				0.0256					
45							0.0045					
46			0.0008									
47			0.0001				0.0536					
48							0.0672					
49							0.0005					
51							0.0049					
52							0.0003					
53							0.0003					
Locus	**TGLA227**	**BM2113**	**TGLA53**	**ETH10**	**SPS115**	**TGLA126**	**TGLA122**	**INRA23**	**ETH3**	**ETH225**	**BM1824**	**BM1818**
N	3867	3869	3801	3866	3857	3863	3869	3859	3841	3871	3872	3859
HWE	**2.03E-03**	6.16E-03	**1.21E-05**	**3.51E-10**	**1.42E-03**	1.10E-02	**2.00E-08**	**2.49E-03**	**1.03E-05**	**1.26E-25**	**4.20E-06**	**1.56E-04**
Ho	0.5710	0.6575	0.6127	0.6981	0.7120	0.7771	0.8403	0.8093	0.5475	0.4859	0.4411	0.7642
He	0.5809	0.6415	0.6368	0.6939	0.7279	0.7735	0.8458	0.8098	0.5626	0.5005	0.4493	0.7807
PD	0.7627	0.8480	0.8175	0.8442	0.8815	0.9132	0.9582	0.9401	0.7151	0.7002	0.6314	0.9184
PE	0.2575	0.3657	0.3058	0.4254	0.4470	0.5572	0.6758	0.6164	0.2326	0.1755	0.1410	0.5344
PIC	0.5229	0.6152	0.5845	0.6356	0.6815	0.7391	0.8286	0.7877	0.4684	0.4528	0.3881	0.7480
P_{ID}	0.2373	0.1520	0.1825	0.1558	0.1185	0.0868	0.0418	0.0599	0.2849	0.2998	0.3686	0.0816

Table S12: Allele frequencies and other forensic parameters for 12 bovine-specific STR loci of Brahman breed from Paraguayan cattle population: HWE - *P* value for Hardy-Weinberg equilibrium, Ho - observed heterozygosity, He - expected heterozygosity, PD - power of discrimination, PE - power of exclusion, PIC - polymorphism information content, P_{ID} - Probability of Identity (Matching Probability). **Statistically significative after Bonferroni's correction (<0.0042).**

Alelle	TGLA227	BM2113	TGLA53	ETH10	SPS115	TGLA126	TGLA122	INRA23	ETH3	ETH225	BM1824	BM1818
11	0.0002							0.0188				
12	0.2663					0.0002		0.0462			0.3508	0.0003
13	0.0174							0.0141			0.1900	0.0001
14	0.1914	0.1110		0.1456		0.0001	0.0882	0.0073			0.3115	0.0205
15	0.0332	0.0332		0.0516		0.0003		0.0764	0.0379		0.0002	0.0042
16	0.0004	0.0595		0.2250		0.0913	0.0054	0.0048			0.0004	0.2262
16.1				0.0001								
17	0.1245	0.0103		0.0925	0.0001	0.1668	0.1446	0.3191			0.1341	0.1488
18	0.1180	0.1280	0.0002	0.2636		0.3734	0.0148	0.1901	0.0065		0.0010	0.4015
18.1		0.0001										
19	0.0170	0.2687	0.0183	0.1362	0.0002	0.0851	0.0067	0.0217	0.0002	0.0880	0.0106	0.0574
19.1					0.0001							
20	0.0281	0.0906	0.0005	0.0807	0.0307	0.0568	0.0245	0.0010	0.0003	0.0024	0.0016	0.1201
20.1										0.0001		
21	0.0008	0.0901	0.0316	0.0044	0.3872	0.1321	0.4840	0.2921	0.1738	0.2006		0.0025
22	0.2025	0.1797	0.3374	0.0002	0.0279	0.0921	0.0503	0.0084	0.4698	0.0512		0.0001
23	0.0003	0.0288	0.0452		0.0448	0.0016	0.0283	0.0001	0.0770	0.1698		0.0110
24			0.0451		0.2532	0.0002	0.0152		0.1396	0.1479		0.0002
25	0.0001		0.0495		0.1002		0.0015		0.0066	0.0003		0.0004
26			0.1516		0.0076		0.1084		0.0847	0.0373		0.0004
27			0.0760		0.1479		0.0082		0.0025	0.0004		
28			0.0062		0.0001				0.0011	0.0006		0.0064
29			0.0019				0.0067			0.2974		
30			0.1625				0.0010			0.0038		
31			0.0032				0.0001			0.0003		
32			0.0055				0.0026					
33			0.0421				0.0001					
34			0.0073									
35			0.0011				0.0005					
36			0.0124									
37			0.0013				0.0087					
38							0.0001					
39			0.0005									
40			0.0006									
41			0.0001									
43.1							0.0003					
Locus	**TGLA227**	**BM2113**	**TGLA53**	**ETH10**	**SPS115**	**TGLA126**	**TGLA122**	**INRA23**	**ETH3**	**ETH225**	**BM1824**	**BM1818**
N	5235	5252	5147	5246	5230	5253	5255	5252	5210	5248	5254	5231
HWE	**2.83E-03**	2.48E-01	**2.13E-07**	4.76E-01	**2.70E-05**	**3.00E-08**	**2.82E-03**	**1.82E-06**	2.09E-02	**1.80E-06**	1.88E-01	**1.73E-08**
Ho	0.8189	0.8509	0.7972	0.8220	0.7262	0.7752	0.7146	0.7934	0.7098	0.8133	0.7278	0.7178
He	0.8196	0.8450	0.8212	0.8224	0.7504	0.7881	0.7207	0.7677	0.7150	0.8089	0.7258	0.7472
PD	0.9433	0.9584	0.9489	0.9449	0.9031	0.9302	0.8984	0.9070	0.8886	0.9366	0.8745	0.9011
PE	0.6343	0.6966	0.5929	0.6404	0.4699	0.5536	0.4505	0.5869	0.4436	0.6239	0.4726	0.4564
PIC	0.7955	0.8277	0.8033	0.7991	0.7151	0.7638	0.6981	0.7326	0.6838	0.7831	0.6767	0.7125
P_{ID}	0.0567	0.0416	0.0511	0.0551	0.0969	0.0698	0.1016	0.0930	0.1114	0.0634	0.1255	0.0989

Table S13: Allele frequencies and other forensic parameters for 12 bovine-specific STR loci of Brangus breed from Paraguayan cattle population: HWE - *P* value for Hardy-Weinberg equilibrium, Ho - observed heterozygosity, He - expected heterozygosity, PD - power of discrimination, PE - power of exclusion, PIC - polymorphism information content, P_{ID} - Probability of Identity (Matching Probability). **Statistically significative after Bonferroni's correction (<0.0042).**

Alelle	TGLA227	BM2113	TGLA53	ETH10	SPS115	TGLA126	TGLA122	INRA23	ETH3	ETH225	BM1824	BM1818
11								0.0011				
12	0.0097										0.2306	
13	0.0043	0.0011						0.0130			0.2241	0.0022
14	0.0691	0.2328		0.0357			0.0011	0.0054			0.1196	0.0044
15	0.1080	0.1907		0.0032		0.0022	0.0011	0.1641	0.0011			0.0044
16	0.0011	0.0011		0.0530		0.0011	0.0054	0.0011			0.0022	0.4858
17	0.0821	0.0011		0.0152		0.3276	0.3125	0.2711			0.3966	0.0722
18	0.1706	0.0119		0.1742		0.4978	0.0011	0.0378	0.0011		0.0259	0.3818
19	0.1210	0.3879	0.0370	0.5227		0.0183	0.0022	0.2419		0.1263	0.0011	0.0208
19.1								0.0032				
20	0.0475	0.1121		0.0335	0.0032	0.0970	0.2198			0.0162		0.0274
21		0.0550	0.0882	0.0714	0.5324	0.0528	0.0690	0.2592	0.0022	0.0529		
22	0.2765	0.0022	0.1558	0.0909	0.0011	0.0032	0.0226	0.0011	0.4212	0.0119		
23	0.0259	0.0043	0.2843		0.2451				0.0227	0.3499		
24			0.0011		0.0421			0.0011	0.0097	0.3467		
25	0.0842		0.0327		0.1145				0.0054	0.0918		0.0011
26			0.1253		0.0313		0.0485		0.1415	0.0022		
27			0.0120		0.0302		0.1293		0.1523			
28			0.0109				0.0011		0.2397			
29			0.0011				0.0043		0.0032	0.0022		
30			0.1035									
31			0.0033				0.0668					
32							0.0237					
33			0.0022									
34			0.0349									
35			0.1068									
36			0.0011				0.0011					
37							0.0905					
Locus	**TGLA227**	**BM2113**	**TGLA53**	**ETH10**	**SPS115**	**TGLA126**	**TGLA122**	**INRA23**	**ETH3**	**ETH225**	**BM1824**	**BM1818**
N	463	464	459	462	463	464	464	463	463	463	464	457
HWE	2.42E-01	1.25E-01	2.99E-01	1.83E-01	1.62E-02	9.73E-01	5.55E-01	5.51E-01	2.27E-01	9.31E-02	6.59E-01	8.17E-01
Ho	0.8143	0.7349	0.7996	0.6450	0.6328	0.6379	0.8060	0.7408	0.7451	0.7365	0.7500	0.5952
He	0.8474	0.7440	0.8463	0.6783	0.6404	0.6330	0.8173	0.7731	0.7221	0.7306	0.7251	0.6125
PD	0.9594	0.8917	0.9592	0.8606	0.8171	0.8006	0.9452	0.9122	0.8763	0.8765	0.8740	0.7793
PE	0.6258	0.4843	0.5982	0.3484	0.3321	0.3389	0.6103	0.4942	0.5015	0.4870	0.5098	0.2851
PIC	0.8301	0.7043	0.8292	0.6493	0.5940	0.5701	0.7950	0.7344	0.6793	0.6866	0.6787	0.5380
P_{ID}	0.0406	0.1083	0.0408	0.1394	0.1829	0.1994	0.0548	0.0878	0.1237	0.1235	0.1260	0.2207

Table S14: Allele frequencies and other forensic parameters for 12 bovine-specific STR loci of Holstein-Friesian breed from Paraguayan cattle population: HWE - *P* value for Hardy-Weinberg equilibrium, Ho - observed heterozygosity, He - expected heterozygosity, PD - power of discrimination, PE - power of exclusion, PIC - polymorphism information content, P_{ID} - Probability of Identity (Matching Probability).

Alelle	TGLA227	BM2113	TGLA53	ETH10	SPS115	TGLA126	TGLA122	INRA23	ETH3	ETH225	BM1824	BM1818
12	0.0306										0.0510	
13								0.3265			0.5612	
14	0.2653	0.0204						0.0102			0.0918	0.0510
15		0.0102										0.1327
16	0.0102	0.0510		0.0102								0.4286
17				0.3673		0.2347	0.3469	0.3571			0.1735	0.0918
18	0.0102	0.2143		0.3367		0.6531		0.0714			0.1224	0.2857
19	0.1939	0.6122		0.1735		0.0102		0.0408		0.0918		
20	0.3469	0.0918		0.0816		0.0918	0.2041					0.0102
21	0.0102			0.0306	0.2959	0.0102	0.2755	0.0918		0.2449		
22	0.1224		0.2813						0.7245			
23			0.0938		0.2857			0.0306		0.2959		
24	0.0102				0.1429				0.0714	0.3673		
25			0.0104					0.0714				
26			0.2188				0.0306		0.1735			
27			0.3333		0.2755							
28			0.0104						0.0306			
30							0.1429					
33			0.0521									
Locus	**TGLA227**	**BM2113**	**TGLA53**	**ETH10**	**SPS115**	**TGLA126**	**TGLA122**	**INRA23**	**ETH3**	**ETH225**	**BM1824**	**BM1818**
N	49	49	48	49	49	49	49	49	49	49	49	49
HWE	2.47E-01	9.12E-01	7.91E-01	9.15E-01	N.D.	3.76E-01	3.76E-01	8.61E-01	N.D.	5.54E-02	8.45E-01	1.45E-01
Ho	0.6939	0.5918	0.7500	0.6939	0.7347	0.5918	0.8367	0.7347	0.5510	0.7755	0.6122	0.8163
He	0.7631	0.5735	0.7581	0.7212	0.7421	0.5150	0.7484	0.7522	0.4435	0.7164	0.6354	0.7132
PD	0.8946	0.7613	0.8898	0.8247	0.8738	0.6914	0.8588	0.8846	0.6539	0.8272	0.8247	0.8172
PE	0.4189	0.2812	0.5098	0.4189	0.4839	0.2812	0.6689	0.4839	0.2362	0.5544	0.3058	0.6297
PIC	0.7165	0.5235	0.7088	0.6640	0.6845	0.4545	0.6959	0.7070	0.4007	0.6540	0.5916	0.6603
P_{ID}	0.1054	0.2387	0.1102	0.1753	0.1262	0.3086	0.1412	0.1154	0.3461	0.1728	0.1753	0.1828

Table S15: Allele frequencies and other forensic parameters for 12 bovine-specific STR loci of Jersey breed from Paraguayan cattle population: HWE - *P* value for Hardy-Weinberg equilibrium, Ho - observed heterozygosity, He - expected heterozygosity, PD - power of discrimination, PE - power of exclusion, PIC - polymorphism information content, P_{ID} - Probability of Identity (Matching Probability).

Alelle	TGLA227	BM2113	TGLA53	ETH10	SPS115	TGLA126	TGLA122	INRA23	ETH3	ETH225	BM1824	BM1818
12											0.4194	
13											0.0887	
14	0.2903							0.0403			0.0081	0.0323
15	0.0161	0.1210										
16	0.0242	0.0161		0.0161		0.0323						0.2742
17	0.1048	0.2097		0.0323		0.4355	0.0887	0.4355			0.4839	0.1048
18	0.2984	0.0161		0.2258		0.0323		0.1935				0.4597
19	0.2419	0.5000	0.1210	0.4516			0.0403			0.2339		0.1290
20		0.0242		0.1048		0.1694	0.0968					
21		0.0403		0.1532	0.5887	0.2419	0.4758	0.3306		0.1613		
22	0.0242		0.2258	0.0161		0.0887	0.1048		0.6290	0.0323		
23		0.0726			0.0645				0.2177	0.2903		
24			0.1129		0.1290					0.2823		
25			0.1210		0.0161		0.0161					
26			0.0403				0.0242		0.0403			
27			0.0161		0.2016				0.1129			
28			0.0484									
29			0.0887									
30			0.2258									
34							0.0081					
37							0.1452					
Locus	**TGLA227**	**BM2113**	**TGLA53**	**ETH10**	**SPS115**	**TGLA126**	**TGLA122**	**INRA23**	**ETH3**	**ETH225**	**BM1824**	**BM1818**
N	62	62	62	62	62	62	62	62	62	62	62	62
HWE	9.20E-01	8.87E-01	N.D.	2.71E-01	**8.88E-03**	9.22E-02	7.12E-01	3.93E-02	5.11E-01	7.60E-01	2.55E-01	8.48E-01
Ho	0.7742	0.6613	0.9032	0.7581	0.7419	0.7742	0.7258	0.6129	0.5645	0.7581	0.5806	0.6935
He	0.7619	0.6890	0.8508	0.7148	0.5965	0.7190	0.7276	0.6673	0.5469	0.7604	0.5868	0.6904
PD	0.8913	0.8548	0.9365	0.8730	0.7716	0.8538	0.8887	0.8163	0.7268	0.8892	0.7274	0.8439
PE	0.5521	0.3710	0.8020	0.5237	0.4961	0.5521	0.4693	0.3067	0.2504	0.5237	0.2683	0.4184
PIC	0.7146	0.6478	0.8254	0.6692	0.5471	0.6712	0.6963	0.5970	0.4922	0.7110	0.4932	0.6362
P_{ID}	0.1087	0.1452	0.0635	0.1270	0.2284	0.1462	0.1113	0.1837	0.2732	0.1108	0.2726	0.1561

Table S16: Allele frequencies and other forensic parameters for 12 bovine-specific STR loci of Limousin breed from Paraguayan cattle population: HWE - *P* value for Hardy-Weinberg equilibrium, Ho - observed heterozygosity, He - expected heterozygosity, PD - power of discrimination, PE - power of exclusion, PIC - polymorphism information content, P_{ID} - Probability of Identity (Matching Probability).

3.3 ARTIGO CIENTÍFICO A SER SUBMETIDO 2[5]

Title of the manuscript: Genetic database and individual identification of dogs from Rio Grande do Sul, Southern Brazil, using twenty-one forensically informative STR loci

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Abstract: Allele frequencies and other forensic parameters for twenty-one canine-specific STR loci were determined from a pool of 648 dogs, consisting of six very popular breeds in Rio Grande do Sul, Southern Brazil (Dachshund, German Shepherd, Labrador Retriever, Poodle Standard, Shih Tzu and Yorkshire Terrier). Forensically informative values (Ho, He, HWE test, PIC, PD, PE and P_{ID}) were ascertained from this population sample. Each breed population could be distinguished from the others by comparing the allele frequency values using F-statistics and calculating F_{ST} indices with pairwise comparisons of inter-population molecular variance (AMOVA). The accuracy of assigning individuals to the breed of origin based on genotype data was studied using individual assignment tests. Results showed that these 21 STR loci can be adequate for individual identification in forensic cases even in relatively inbred dog populations.

Keywords: Allele frequencies; Canine-specific STR loci, Genetic database, individual identification.

1 INTRODUCTION

Dogs (*Canis lupus familiaris*), more than any other animal species, have been a part of human history and is the closest companion of the man and most popular pet. Since their domestication, in East Asia about 15,000 years ago, in several independent processes from wild gray wolves (*Canis lupus*), dogs were selected by man according to the desired morphological characteristics, behavioral traits and the ability to learn and perform different tasks. Thus, breeds emerged for the most varied purposes: guarding, hunting, herding, guinding, pulling, etc. Over 1,000 breeds have been reported, but nowadays, approximately 500 internationally recognized breeds exist and most of them are relatively young. These ones have been developed in the last 200-400 years through intense selection for morphological and behavioral traits, from a small number of animals, from recent genetic founders, and therefore, purebred dogs have a limited genetic pool, being a species characterized by having high degree of isolation and narrower bottleneck[1-3].

No other animal species can vary much as dogs in morphological aspects such as weight, height, body mass, shape, behavior. Even so, dogs of almost all breeds can mate with other breeds and produce fertile offspring[1]. According to the

American Pet Products Association, it is estimated that in the United States there are more than 83 million dogs and that in Europe, according to the International Federation for Animal Health-Europe (IFAH-Europe), this number is around 60 million. The estimated worldwide canine population is around 223 million animals[4].

As a consequence of close integration and abundant interaction with the social life of humans, it is common to involve dogs in forensically relevant cases, when they are the offenders (such as accidents, dog attacks to children, other pets and livestock)[1]. They can also be "witnesses" to crimes, when traces of blood, urine, feces, hair and saliva of dogs are found in a crime scene or in the victim, and these biological materials may aid in linking to a possibly involved person[4-5]. In other situations, they may be victims in cases of cruelty to animals, having been beaten, tortured or mutilated, as in dog fighting and other crimes that contribute to the death of thousands of dogs every year[4]. Besides that, dogs get lost, are stolen, abandoned, poisoned, and this occurs oftentimes in large urban centers. Because the dog is of a breed that is in evidence or fashionable, or the person is no longer able to raise it, or it started to bother or it barks too much, in short, there are many justifications that people arrange to mistreat or neglect the one who has always been considered man's best friend. Many owners and guardians who really care about dogs get distress and apprehension as they often cannot find their pets again.

Individual genetic identification of dogs can assist in solving many of these misfortunes. When faced with one of the situations described above, investigators, police officers, judges and even dog owners may request the confrontation of any evidence or vestige with the registered genetic profile of a particular animal suspected of being involved in any crime or inconvenient event. To that matter, it is necessary to register dog’s profile in a genetic database accessible to those seeking identification.

Instead of what occurs in humans, in which millions of genetic profiles are in national forensic databases around the world, animal genetic databases exist, but are smaller and with limited range. They are typically owned by the genetic testing laboratories that generated them, and may be accessible to other laboratories only if databases are uploaded onto public domains such as STRBase[4].

In Brazil, the largest country in South America, according to the most recent data collected by World Organization for Animal Health, it is estimated that around 24

million dogs live in Brazilian households and 58.6% of which are in the South Region. In Rio Grande do Sul, Southern Brazil, there are almost 2 million pet canines inhabiting residences and only in the metropolitan region of Porto Alegre there are more than 500,000 abandoned dogs in the streets, municipal kennels or voluntary shelters[6].

In order to initiate a genetic database and individual identification system of dogs raised in Rio Grande Sul, the present study aims to estimate the allelic frequencies and other forensic parameters of 21 STR loci recommended by International Society for Animal Genetics (ISAG) from six canine breeds very popular in Brazil. In addition, index of inbreeding (F_{IS}) and fixation (F_{ST}) were obtained, allowing to verify substructure levels of each breed, as well as the genetic distance among them.

2 MATERIAL AND METHODS

2.1 Population

A total group of 648 dogs of six different breeds: Dachshund (n=106), German Shepherd (n=127), Labrador Retriever (n=102), Poodle Standard (n=102), Shih Tzu (n=105) and Yorkshire Terrier (n=106). All samples were obtained from animals subjected to routine tests in veterinary clinics or clinical pathology laboratory.

2.2 DNA extraction

Canine genomic DNA was isolated and purified from dried blood samples preserved in FTA cards (Whatman Bioscience, Cambridge, UK) following the manufacturer's instructions.

2.3 PCR amplification

Two sets of STR genetic markers were prepared by the Genia Laboratory (Montevideo, Uruguay) for the amplification of twenty-one loci recommended by ISAG in two PCR multiplex. First set: AMEL, AHT137, CXX0279, FH2848, REN169D01, REN105L03, REN169018, REN247M23, REN162C04, INRA21, INU030, AHTh171, REN54P11, AHT121, AHTh260, AHTk253 and AHTH130. Second set: FH2054, AHTk211, REN64E19, AHTk253 and INU005. Both sets were

performed on Veriti™ Thermal Cycler (Thermo Fisher Scientific, Waltham, MA, USA) or GeneAmp 9700 PCR System (Thermo Fisher Scientific) using fluorescent labeled primers, which sequences are available on www.isag.us/Docs/AppGenCompAnim2014_cor.pdf. The other reagents for PCR amplification (PCR Buffer, DNA Polymerase and dNTP mix) were carried out using the Platinum® Multiplex PCR Master Mix, 2x (Thermo Fisher Scientific). The amount of canine genomic DNA was 25-50 ng/µL per sample. Thermocycling conditions to amplify both set of STR loci were: pre-incubation for 15min at 95°C, followed by thirty cycles of 30s at 95°C, 30s at 59°C and 60s at 72°C, with a final incubation for 60min at 72°C.

2.4 Detection, typing, and analysis of PCR products

Electrophoresis and typing were performed on an ABI 3500 Genetic Analyzer (Thermo Fisher Scientific). Since there is no proposed allele nomenclature for the 21 STR loci, as recommended by the International Society for Forensic Genetics (ISFG)(OLAISEN *et al.*, 1998) the designation of the amplified DNA fragments were performed according to the length of PCR products using GeneScan™ 500 LIZ® Size Standard, as internal lane standards according to the manufacturer's protocols and a reference sample. Data was collected by Data Collection v1.0 software (Thermo Fisher Scientific) and analyzed using GeneMapper ID-X v1.3 software (Thermo Fisher Scientific).

2.5 Quality control

Genia Laboratory is a member of International Society for Animal Genetics (ISAG) and since 2003 has participated of biannual Program of Quality Control of this institution. In addition, it provides services to Uruguayan Stud Book, Rural Association of Uruguay and Rural Association of Paraguay, and adopts a strict internal quality control throughout the laboratory management system.

2.6 Analysis of data

Calculations of allele frequencies, observed (Ho) and expected (He) heterozygosity, polymorphism information content (PIC) and *P*-values of the Hardy-

Weinberg equilibrium (HWE) test for all twenty-one loci were assessed using CERVUS version 3.0.3[8]. Bonferroni's correction was used for HWE test, which assumes that a 0.05 significance level obtained for twenty-one tests (one per locus) yields an actual significance threshold of 0.0024[9]. Power of discrimination (PD), power of exclusion (PE) and probability of identity (P_{ID}) were estimated with PowerStats (Promega Corporation)[10]. Estimated coefficients of inbreeding (F_{IS}) within breeds, fixation indices (F_{ST}) among breeds, and total inbreeding (F_{IT}) using an analysis of molecular variance (AMOVA) were performed with ARLEQUIN version 3.1[11] and GENEPOP version 4.5.1[12]. Neighbour-joining trees were built from Nei's genetic distance matrix using the PHYLIP software package version 3.69[13] and visualized with TreeView software version 1.6.6[14]. The accuracy of assigning individuals to the breed of origin based on genotype data was studied using individual assignment tests, implemented in the program GeneClass v.2.0h[15]. Assignment success was measured using groups of at least 15 individuals per breed, all having a complete 21-locus genotype. The allele frequencies and forensically informative parameters are available in Supplementary Data (Table S1-S6).

3 RESULTS AND DISCUSSION

Among the six canine breeds present in the study, five of them showed deviation in at least one of twenty-one STR loci, according to HWE test ($P<0.05$), with exception Dachshund breed. Even after applying the Bonferroni's correction ($P<0.0024$), four breeds still showed at least one locus in disequilibrium (German Shepherd, Labrador Retriever, Shih Tzu and Yorkshire Terrier). *P*-values were not determined on loci AHT121 and FH2054 in Dachshund breed, on locus AHTK253 in German Shepherd and on loci AHT121 and AHTH171 in Poodle Standard and thus the HWE test could not be performed in these loci.

In all six breeds the observed heterozygosity was lower than the expected heterozygosity in almost all markers, corroborating there is low genetic variability and high rate of inbreeding which favors the increase of homozygotes in those breeds. The overall heterozygosity ranged from 0.5933 (German Shepherd) to 0.7360 (Dachshund). Similar results to these, in relation to heterozygosity, were found in a study using 15 STR loci in dogs from the United States[2].The locus with the highest

PIC was different for each of the six breeds and thus the most polymorphic loci for Dachshund, German Shepherd, Labrador Retriever, Standard Poodle, Shih Tzu and Yorkshire Terrier, respectively, were: FH2054, REN169O18, AHTH130, AHTH171, AHT137, AHT121. Meanwhile, the least polymorphic genetic markers were REN64E19 (Dachshund), AHTK253 (German Shepherd and ShihTzu), REN247M23 (Labrador Retriever and Poodle Standard) and REN54P11 (Yorkshire Terrier). The overall PIC ranged from 0.5393 (German Shepherd) to 0.6954 (Dachshund).

The probability of incorrect parentage assignment (Probability of Exclusion - PE) is a direct indicator of the usefulness of STR markers for parentage verification and genetic identification. Using the highest possible number of markers increases the probability of correct identity. A study with Borzoi dogs using 18 STR loci showed that PE combined (PEc) values was 0,99998 and PEc based on estimation of 20 STR with two dog breeds increased the probability of parentage assignment to 0.999994[16]. With the twenty-one STR loci recommended by ISAG presented in this study, the probability of exclusion combined (PEc) and the probability of discrimination combined (PDc) among all six canine breeds were, respectively, 0.99991 and 0.999999999999999. Table 1 shows the PEc and PDc for each breed. Probability of Identity (P_{ID}), that indicates probability of two individuals within the population sharing the same genotype, showed the following overall values for each breed: $6.48x10^{-22}$ (Dachshund), $3.10x10^{-15}$ (German Shepherd), $3.33x10^{-18}$ (Labrador Retriever), $8.25x10^{-22}$ (Poodle Standard), $2.37x10^{-19}$ (Shih Tzu) and $6.03x10^{-20}$ (Yorkshire Terrier).

Table 1: Probability of exclusion combined (PEc) and Probability of Discrimination combined (PDc) among six canine breeds.

Breed	PEc	PDc
Dachshund	0.999999281976191	0.999999999999999
German Shepherd	0.999589309856314	0.999999999999997
Labrador	0.999984055610843	0.999999999999999
Poodle	0.999995570630392	0.999999999999999
Shih Tzu	0.999900290025969	0.999999999999999
Yorkshire	0.999999999999999	0.999981564382281

Table 2 shows the values of F_{IS}, F_{ST} and F_{IT} for each of the twenty-one loci and the overall. In Table 3, the positive values of F_{IS} (> 0) indicate heterozygote deficiency and sub-structuration in all six breeds, being Shih Tzu and Yorkshire the breeds with higher level of inbreeding.

Based on genotypic frequencies of the twenty-one STR of this study, pairwise genetic distances were calculated among the six breeds, using Nei's formulas implemented in PHYLIP software (Table 4). Analysis showed a clear separation between these breeds (Figure 1). The major genetic distance in these six breeds is between German Shepherd and Shih Tzu, while the breeds which are closer from each other are Dachshund and Poodle Standard, corroborating the historical data that indicate the way of each dog breed arose[17-28]. In a comparison with a study that calculated the genetic distance between several canine breeds in the United States using 15 STR loci different from those presented in this article (with the exception of the locus FH2054), the results obtained with that population sample showed that breeds which are closest to each other are Dachshund and Shih Tzu, while the largest distance observed was between the German Shepherd and Poodle Standard breeds[2].

Table 2: F_{IS}, F_{ST} and F_{IT} coefficients for six canine breeds to each of the twenty one loci and the overall.

MARKER	F_{IS}	F_{ST}	F_{IT}
AHT121	0.1950	0.1422	0.3095
AHT137	0.0166	0.1569	0.1709
INU030	0.0715	0.1462	0.2073
INU055	0.0277	0.0730	0.0987
REN54P11	0.0914	0.1776	0.2528
AHTK253	0.0224	0.1986	0.2166
AHTK211	0.0240	0.1456	0.1661
INU005	0.1425	0.1110	0.2377
REN64E19	0.1658	0.2199	0.3492
FH2848	0.0247	0.1122	0.1341
REN247M23	0.0666	0.1937	0.2474
CXX279	0.0387	0.1139	0.1482
FH2054	0.0195	0.0702	0.0883
REN162C04	0.0316	0.1207	0.1485
AHTH171	0.0561	0.1784	0.2245
AHTH260	0.0161	0.1162	0.1304
INRA21	0.0418	0.0681	0.1071
AHTH130	0.0843	0.1040	0.1796
REN169018	0.0718	0.1213	0.1844
REN169D01	0.0514	0.1009	0.1471
REN105L03	0.0661	0.1941	0.2473
All	**0.0624**	**0.1367**	**0.1906**

Table 3: Inbreeding coefficient (F_{IS}) estimated for six canine breeds.

Breed	F_{IS}
Dachshund	0.0459
German Shepherd	0.0452
Labrador Retriever	0.0430
Poodle Standard	0.0612
Shih Tzu	0.0950
Yorkshire Terrier	0.0825

Table 4: Nei's genetic distances (F_{ST} analysis) among six canine breeds.

Breed	Dachshund	G. Shepherd	Labrador	Poodle	Shih Tzu	Yorkshire
Dachshund	*	0.1625	0.1186	0.0577	0.0997	0.0763
G. Shepherd	0.1625	*	0.2089	0.1791	0.2211	0.1809
Labrador	0.1186	0.2089	*	0.1136	0.1556	0.1451
Poodle	0.0577	0.1791	0.1136	*	0.0781	0.0766
Shih Tzu	0.0997	0.2211	0.1556	0.0781	*	0.1228
Yorkshire	0.0763	0.1809	0.1451	0.0766	0.1228	*

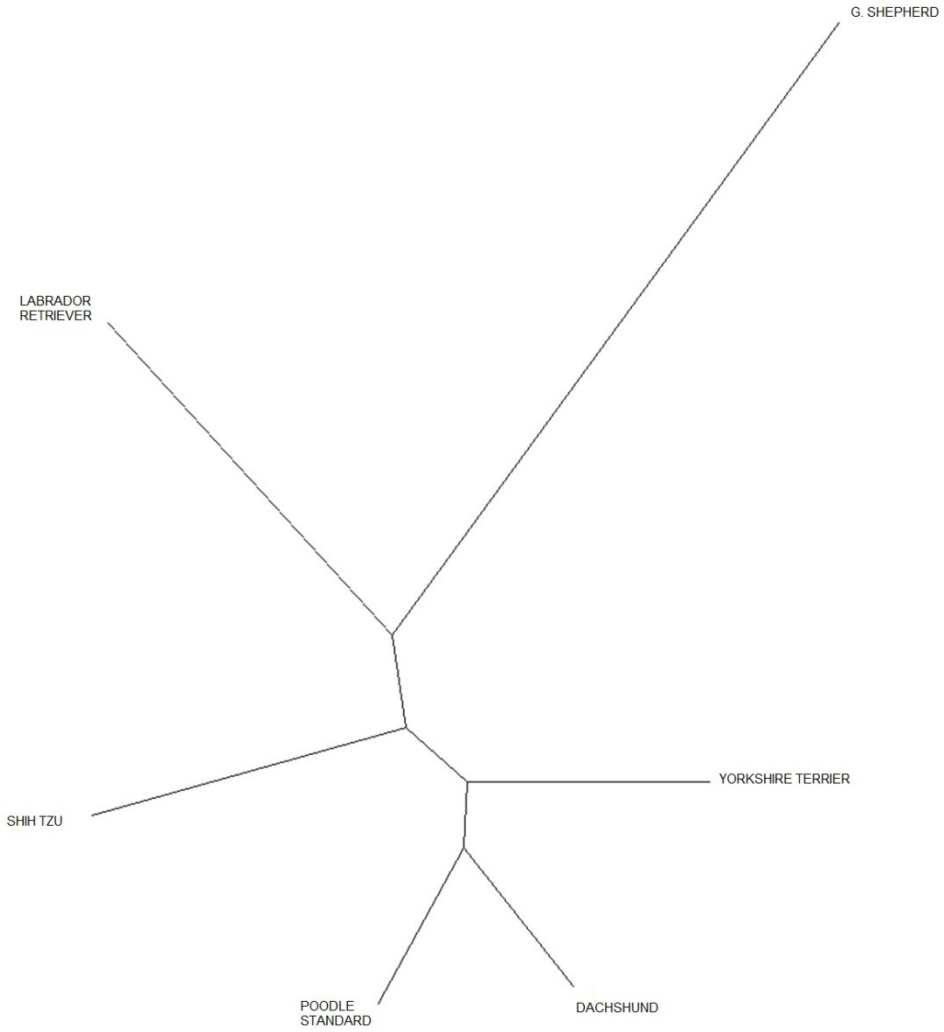

Figure 1: Neighbour-joining tree based on pairwise Nei's genetic distances calculated between six breeds of dogs raised in Rio Grande do Sul, Southern Brazil.

To perform individual assignment, a genotype bank was created from the genetic profile of the animals used for the present study. The genotype of fifteen dogs from each breed was randomly excluded from the genetic database created and a blind test was performed to estimate the accuracy in individual breed assignment. The test has provided excellent results of success in assignment, in which the lowest rate obtained was of 93.30% on Poodle Standard breed. Table 5 shows the range within-breed assignment success. Three other tests were performed to estimate the accuracy of breed assignment when dealing with groups of animals. Groups of five, ten and fifteen dogs of each breed were created, and the genotypes of these were excluded from the database created. In all three tests, there was 100% accuracy in the attribution to which breed each group belonged.

Table 5: Range within-breed assignment success for six dog breeds.

Breed	**Range of breed assignment probabilities (%)**
Dachshund	97.13-100
German Shepherd	97.92-100
Labrador Retriever	98.21-100
Poodle Standard	93.30-100
Shih Tzu	99.23-100
Yorkshire Terrier	99.73-100

4 CONCLUSION

This is one of the first publications in the scientific community to present allelic frequencies and other forensic parameters using the twenty-one canine-specific STR loci recommended by ISAG for the genetic identification of dogs. Certainly, it is the first publication with animals raised in Rio Grande do Sul and most probably in Brazil, because, until the elaboration of the present article, no similar studies were found. Thus, the comparison with other canine populations, from other regions of the world, was somewhat impaired, because it would be interesting to discuss and evaluate if the data found in our study are consistent with similar research. However, the richness of the results obtained cannot be underestimated and it is hoped that future similar works can be carried out, allowing a wide debate on the usefulness and informativeness of these twenty-one STR selected by ISAG.

Considering the diverse approaches of the present study and the results shown, it is feasible to assume that this group of STR genetic markers can be used by police authorities, public security agencies, kennel clubs, breeders, etc. for several proposes: forensic and kinship analysis, genetic identification and phylogenetic reconstruction. The creation of a genetic database and the verification that through this genetic markers it is possible to predict with a high degree of confidence the breed of a certain dog – although it has been carried out in few dog breeds, among so many existing – generates positive expectations that reliable identification systems can be developed and used successfully, perhaps helping to identify an animal involved in a crime, such as an attack on a child, or allowing owners to find their dogs. Finally, as we have already suggested in other studies, it is important to determine the allelic frequencies and other parameters of forensic interest and genetic identification with animals in the same breed, in significant sample size and a certain geographic region, similar to what is recommended for human populations[29-30]. Thus, common and rare alleles of the location will be known, allowing to identify characteristics of each population as the occurrence or not of sub-structuration, linkage to traits under selection, Wahlund effect, bottleneck effect, etc.

This paper follows the guidelines for publication of population data requested by the journal[31] and the recommendations of International Society for Forensic Genetics (ISFG) regarding the use of non-human (animal) DNA in forensic genetic investigations[32].

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Appendix A. Supplementary data

Supplementary data associated with this article can be found in the online version.

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Alelle	AHT121	AHT137	INU030	INU055	REN54P11	AHTK253	AHTK211	INU005	REN64E19	FH2848	REN247M23
87							0.1476				
89							0.3905				
91							0.3667				
92	0.0971										
93							0.0238				
94	0.0243										
95							0.0524				
96	0.0291										
97							0.0190				
98	0.1602										
99											
100	0.1359										
101	0.0049										
102	0.1845										
103											
104	0.0485							0.0048			
105											
106	0.1408										
108	0.0437										
110	0.0049							0.1857			
112	0.0631										
114	0.0388										
116											
117											
118	0.0049										
119											
120	0.0194										
121											
122								0.0810			
123											
124								0.5048			
125											
126								0.1381			
127											
128								0.0810			
129											
130								0.0048			
131		0.2452									
132											
133		0.0288									
135											
137		0.0529									
139		0.0048							0.0095		
140											
141		0.0721									
142											
143		0.0144							0.0381		
144			0.3786								
145		0.2115							0.4333		
146			0.0971								
147		0.0817							0.1381		
148			0.0194								
149		0.0240							0.0238		
150			0.2427								
151		0.2308									
152		0.0048	0.2379								
153		0.0288							0.3476		
154			0.0049								
155									0.0095		
156			0.0194								
160											
162											
164											
166											
168											
170											
172											
174											
176											
180											
196											

Allele	AHT121	AHT137	INU030	INU055	REN54P11	AHTK253	AHTK211	INU005	REN64E19	FH2848	REN247M23
200											
202											
204											
206											
208				0.0196							
210				0.4559							
212				0.1225							
214				0.1127							
216				0.2451							
217											
218				0.0245							
219											
220				0.0196							
221											
223					0.0049						
224											
225											
226					0.2745						
227											
228					0.0049						
229											
230										0.0051	
231											
232					0.3137					0.0202	
233											
234					0.3284					0.1010	
235											
236					0.0637					0.2828	
237											
238					0.0049					0.1818	
239											
240					0.0049					0.2626	
241											
242										0.0707	
244										0.0707	
246										0.0051	
248											
250											
252											
268											0.4350
270											0.2650
272											0.2250
274											0.0200
276											0.0200
278											0.0350
280						0.0150					
286						0.2350					
288						0.1900					
290						0.3650					
292						0.1950					
Locus	**AHT121**	**AHT137**	**INU030**	**INU055**	**REN54P11**	**AHTK253**	**AHTK211**	**INU005**	**REN64E19**	**FH2848**	**REN247M23**
N	103	104	103	102	102	100	105	105	105	99	100
HWE	ND*	1.13E-01	2.79E-01	5.82E-01	8.64E-01	8.49E-01	3.70E-01	4.35E-01	2.47E-01	5.13E-01	7.27E-01
Ho	0.6602	0.7212	0.7476	0.6275	0.6961	0.7100	0.7143	0.6286	0.6095	0.7980	0.6600
He	0.8852	0.8287	0.7345	0.7065	0.7177	0.7409	0.6909	0.6817	0.6733	0.8014	0.6914
PD	0.9650	0.9442	0.8621	0.8733	0.8597	0.8864	0.8245	0.8557	0.8432	0.9173	0.8454
PE	0.3694	0.4617	0.5056	0.3251	0.4222	0.4439	0.4507	0.3266	0.3025	0.5953	0.3691
PIC	0.8694	0.8025	0.6859	0.6620	0.6595	0.6917	0.6318	0.6417	0.6115	0.7686	0.6338
P_{ID}	0.0350	0.0558	0.1379	0.1267	0.1403	0.1136	0.1755	0.1443	0.1568	0.0827	0.1546

Alelle	CXX0279	FH2054	REN162C04	AHTH171	AHTH260	INRA021	AHTH130	REN169O18	REN169D01	REN105L03
87										
89										
91						0.1218				
92										
93										
94										
95						0.2179				
96										
97						0.0833				
98										
99						0.1474				

100								
101					0.4167			
102								
103					0.0064			
104								
105					0.0064			
106								
108								
110								
112								
114								
116	0.3317							
117						0.0096		
118	0.1442							
119						0.0192		
120	0.0096							
121						0.1683		
122								
123						0.1058		
124	0.2212							
125						0.0288		
126	0.2308							
127						0.1250		
128	0.0048							
129						0.2981		
130	0.0577							
131						0.0962		
132						0.0048		
133						0.1154		
135						0.0240		
137						0.0048		
139								
140		0.0048						
141								
142		0.0048						
143								
144		0.0337						
145								
146								
147								
148		0.0721						
149								
150								
151								
152		0.1587						
153		0.0096						
154								
155								
156		0.1202						
160		0.0192					0.0097	
162							0.1699	
164		0.1106					0.2039	
166							0.0777	
168		0.1394					0.1942	
170		0.0481					0.2913	
172		0.1635					0.0534	
174		0.0048						
176		0.0962						
180		0.0144						
196			0.0049					
200			0.0631					
202			0.0728					0.0728
204			0.1990					
206			0.4320					
208			0.1942					0.0049
210			0.0340					0.1262
212								0.2524
214								0.0194
216								0.2233
217				0.0097				
218								0.1893
219				0.3544				
220								0.1068

221				0.0097						
223				0.0049						
224									0.0049	
225				0.3786						
226										
227				0.0049						0.1127
228										
229				0.0680						0.0294
230										
231				0.0485						0.1716
232										
233				0.0437						0.3627
234										
235				0.0534						0.0196
236										
237				0.0243						0.0147
238					0.0340					
239										0.0343
240										
241										0.2500
242					0.3301					
244					0.0340					0.0049
246					0.3981					
248					0.0388					
250					0.1553					
252					0.0097					
268										
270										
272										
274										
276										
278										
280										
286										
288										
290										
292										

Locus	**CXX0279**	**FH2054**	**REN162C04**	**AHTH171**	**AHTH260**	**INRA021**	**AHTH130**	**REN169O18**	**REN169D01**	**REN105L03**
N	104	104	103	103	103	78	104	103	103	102
HWE	6.69E-01	ND*	8.30E-01	4.78E-01	6.80E-01	9.51E-01	1.98E-01	9.52E-01	5.88E-01	3.72E-01
Ho	0.7404	0.8654	0.7282	0.6408	0.7184	0.7179	0.8462	0.7767	0.8447	0.7549
He	0.7672	0.8876	0.7291	0.7220	0.7080	0.7400	0.8356	0.8019	0.8215	0.7649
PD	0.9007	0.9684	0.8760	0.8815	0.8527	0.8826	0.9440	0.9279	0.9294	0.8970
PE	0.4935	0.7254	0.4731	0.3427	0.4574	0.4566	0.6873	0.5565	0.6844	0.5182
PIC	0.7254	0.8720	0.6881	0.6756	0.6548	0.6984	0.8125	0.7685	0.7925	0.7263
P_{ID}	0.0993	0.0316	0.1240	0.1185	0.1473	0.1174	0.0560	0.0721	0.0706	0.1030

Table S1: Allele frequencies and other forensic parameters for 21 canine-specific STR loci of Dachshund breed raised in Rio Grande do Sul, Southern Brazil: HWE - *P* value for Hardy-Weinberg equilibrium, Ho - observed heterozygosity, He - expected heterozygosity, PD - probability of discrimination, PE - probability of exclusion, PIC - polymorphism information content, P_{ID} - Probability of Identity (Matching Probability). ND*: Not Determined.

Alelle	AHT121	AHT137	INU030	INU055	REN54P11	AHTK253	AHTK211	INU005	REN64E19	FH2848	REN247M23
87							0.3257				
89							0.3807				
91							0.0550				
92	0.0040										
94	0.0920										
95							0.2294				
97							0.0092				
98	0.0200										
99											
100	0.0320										
101	0.0040										
102	0.6880										
103											
104	0.1080										
106	0.0280										
108	0.0080										
110								0.0865			
112	0.0040										
114	0.0120										
115											
116											
117											
118											
119											
121											
122								0.0096			
123											
124								0.3702			
125											
126								0.5192			
127											
128								0.0048			
129											
130								0.0096			
131		0.5119									
133		0.0238									
135		0.0079									
137		0.4048									
139		0.0079									
141		0.0040									
143									0.0046		
144			0.0675								
145									0.0231		
146			0.4405								
147		0.0238							0.0602		
148			0.0040								
149		0.0040									
150			0.4841								
151		0.0079									
152			0.0040								
153		0.0040							0.1250		
155									0.7824		
156											
157									0.0046		
158											
160											
162											
164											
166											
168											
170											
172											
174											
178											
198											
200											
202											
204											
206											

208				0.0040							
210				0.4520							
212											
214				0.0760							
216											
218				0.3560							
220				0.1120							
221											
222											
223											
224											
225											
226					0.3120						
227											
228					0.0040						
229											
231											
232					0.0560					0.1098	
233											
234					0.6160						
235											
236										0.0081	
237											
238					0.0120					0.0366	
239											
240										0.4390	
241											
242										0.3862	
244										0.0203	
246											
248											
250											
252											
266											0.0041
268											0.1545
270											0.6829
272											0.0122
276											0.0041
278											0.1423
282						0.0040					
284						0.0080					
286						0.0200					
288						0.8040					
290						0.0200					
292						0.0200					
294						0.1240					

Locus	**AHT121**	**AHT137**	**INU030**	**INU055**	**REN54P11**	**AHTK253**	**AHTK211**	**INU005**	**REN64E19**	**FH2848**	**REN247M23**
N	125	126	126	125	125	125	109	104	108	123	123
HWE	2.06E-02	2.10E-01	4.28E-01	9.62E-01	1.03E-01	ND*	3.84E-01	2.86E-01	**1.61E-03**	2.40E-01	5.72E-01
Ho	0.4480	0.6429	0.5159	0.6640	0.4480	0.3280	0.6789	0.5385	0.2870	0.6748	0.4553
He	0.5061	0.5750	0.5693	0.6532	0.5220	0.3383	0.6964	0.5885	0.3697	0.6469	0.4913
PD	0.6990	0.6804	0.7341	0.8250	0.6956	0.5295	0.8424	0.7581	0.5273	0.7923	0.6944
PE	0.1459	0.3455	0.2018	0.3748	0.1459	0.0757	0.3964	0.2234	0.0583	0.3904	0.1513
PIC	0.4824	0.4857	0.4721	0.5866	0.4429	0.3154	0.6342	0.5055	0.3436	0.5772	0.4470
P_{ID}	0.3010	0.3196	0.2659	0.1750	0.3044	0.4705	0.1576	0.2419	0.4727	0.2077	0.3056

Alelle	**CXX0279**	**FH2054**	**REN162C04**	**AHTH171**	**AHTH260**	**INRA021**	**AHTH130**	**REN169O18**	**REN169D01**	**REN105L03**
87										
89										
91						0.0640				
92										
94										
95						0.4560				
97						0.0480				
98										
99						0.1800				
100										
101						0.2520				
102										
103										
104										
106										

108								
110	0.0119							
112								
114								
115	0.0040							
116	0.4484							
117	0.0040							
118	0.1548							
119					0.0079			
121					0.0238			
122								
123					0.1627			
124	0.0119							
125					0.1032			
126	0.3532							
127					0.1905			
128								
129					0.0913			
130	0.0119							
131					0.4048			
133					0.0040			
135					0.0119			
137								
139								
141								
143								
144								
145								
146								
147								
148		0.0083						
149								
150								
151								
152		0.3625						
153								
155								
156		0.0250						
157								
158						0.1825		
160		0.0500				0.0119		
162						0.1071		
164		0.2708				0.0992		
166						0.1548		
168		0.2708				0.3294		
170						0.0595		
172		0.0125				0.0437		
174						0.0079		
178						0.0040		
198			0.0041					
200			0.2276				0.0080	
202			0.0122				0.0040	
204			0.0813					
206			0.4553				0.0040	
208			0.0285					
210							0.0080	
212			0.1911				0.2600	
214							0.0040	
216							0.6880	
218							0.0080	
220							0.0080	
221				0.0120				
222							0.0040	
223				0.5000				
224							0.0040	
225				0.0800				
226								
227								0.5280
228								
229				0.0120				0.0080
231								0.1280
232								
233				0.2920				0.0800

234					0.0040					
235				0.0040						0.0720
236										
237				0.0040						0.0040
238					0.4600					
239										0.0040
240					0.0040					0.0040
241										0.1680
242					0.1920					
244										0.0040
246					0.1520					
248					0.0320					
250					0.0040					
252					0.1520					
266										
268										
270										
272										
276										
278										
282										
284										
286										
288										
290										
292										
294										

Locus	**CXX0279**	**FH2054**	**REN162C04**	**AHTH171**	**AHTH260**	**INRA021**	**AHTH130**	**REN169O18**	**REN169D01**	**REN105L03**
N	126	120	123	125	125	125	126	126	125	125
HWE	8.77E-01	7.09E-01	2.07E-01	8.88E-01	6.39E-01	8.25E-01	4.37E-01	6.69E-01	5.78E-02	8.95E-01
Ho	0.5952	0.6917	0.7236	0.6240	0.6640	0.7120	0.7143	0.7540	0.3920	0.6400
He	0.6524	0.7215	0.6997	0.6514	0.7071	0.6925	0.7567	0.8105	0.4606	0.6676
PD	0.8157	0.8681	0.8462	0.8244	0.8667	0.8461	0.9038	0.9392	0.6318	0.8544
PE	0.2852	0.4155	0.4657	0.3207	0.3748	0.4471	0.4507	0.5166	0.1091	0.3417
PIC	0.5837	0.6674	0.6519	0.5954	0.6640	0.6419	0.7220	0.7838	0.3944	0.6315
P_{ID}	0.1843	0.1319	0.1538	0.1756	0.1333	0.1539	0.0962	0.0608	0.3682	0.1456

Table S2: Allele frequencies and other forensic parameters for 21 canine-specific STR loci of German Shepherd breed raised in Rio Grande do Sul, Southern Brazil: HWE - *P* value for Hardy-Weinberg equilibrium, Ho - observed heterozygosity, He - expected heterozygosity, PD - probability of discrimination, PE - probability of exclusion, PIC - polymorphism information content, P_{ID} - Probability of Identity (Matching Probability). ND*: Not Determined. **Statistically significative after Bonferroni's correction (<0.0024).**

Alelle	AHT121	AHT137	INU030	INU055	REN54P11	AHTK253	AHTK211	INU005	REN64E19	FH2848	REN247M23
87							0.1238				
89							0.0297				
91							0.1782				
93							0.0198				
94	0.0102										
95							0.5248				
96	0.0408										
97							0.1238				
98	0.0306										
99											
100	0.0612										
101											
102	0.4184										
103											
104	0.0204										
106	0.3878										
108	0.0255										
110								0.0297			
112	0.0051										
114											
116											
118											
119											
121											
123											
124								0.5297			
125											
126								0.4158			
127											
128								0.0050			
129											
130											
131		0.0357									
132								0.0099			
133		0.0969									
135		0.0255									
137		0.0663									
139		0.0051							0.0050		
141		0.0153							0.0050		
143									0.0050		
144			0.6134								
145									0.1980		
146			0.0206								
147		0.1327							0.2030		
148			0.0052								
149		0.4286							0.0693		
150			0.2784								
151		0.0204							0.0050		
152			0.0567								
153		0.1735							0.5000		
154			0.0052								
155									0.0099		
156			0.0206								
160											
162											
164											
166											
168											
170											
172											
200											
202											
204											
206											
208				0.1211							
210				0.3263							
212				0.0263							
214				0.0316							
216				0.0632							

218				0.3263							
220				0.0947							
221											
222				0.0053	0.1330						
223											
224				0.0053							
225											
226					0.3245						
227											
228					0.0106						
229											
231											
232					0.3723					0.1429	
233											
234					0.1170					0.0165	
235											
236					0.0319						
237											
238					0.0106					0.2198	
239											
240										0.1978	
241											
242										0.1099	
244										0.3132	
245											
246											
248											
250											
252											
254											
256											
266											0.0056
268											0.7389
270											0.0167
272											0.1556
274											0.0056
276											0.0722
278											0.0056
284						0.1848					
286						0.0489					
288						0.5489					
290						0.1141					
292						0.1033					

Locus	**AHT121**	**AHT137**	**INU030**	**INU055**	**REN54P11**	**AHTK253**	**AHTK211**	**INU005**	**REN64E19**	**FH2848**	**REN247M23**
N	98	98	97	95	94	92	101	101	101	91	90
HWE	5.08E-01	1.49E-01	5.94E-01	2.04E-01	6.83E-01	6.06E-01	4.39E-01	7.80E-02	3.19E-01	4.85E-01	1.61E-01
Ho	0.6020	0.7857	0.4948	0.8000	0.7021	0.6848	0.5941	0.4752	0.5941	0.8022	0.3556
He	0.6705	0.7561	0.5449	0.7617	0.7273	0.6420	0.6643	0.5481	0.6679	0.7860	0.4266
PD	0.7926	0.9080	0.7319	0.9013	0.8762	0.8240	0.8485	0.7174	0.8313	0.9083	0.6148
PE	0.2933	0.5728	0.1830	0.5990	0.4316	0.4051	0.2838	0.1667	0.2838	0.6031	0.0891
PIC	0.6095	0.7259	0.4801	0.7219	0.6778	0.6000	0.6233	0.4473	0.6179	0.7481	0.3915
P_{ID}	0.2074	0.0920	0.2681	0.0987	0.1238	0.1760	0.1515	0.2826	0.1687	0.0917	0.3852

Alelle	**CXX0279**	**FH2054**	**REN162C04**	**AHTH171**	**AHTH260**	**INRA021**	**AHTH130**	**REN169O18**	**REN169D01**	**REN105L03**
87										
89										
91						0.0085				
93										
94										
95						0.3559				
96										
97						0.0339				
98										
99						0.0424				
100										
101						0.5085				
102										
103						0.0508				
104										
106										

108									
110									
112									
114	0.0101								
116	0.1465								
118	0.1162								
119						0.2550			
121						0.1150			
123						0.2000			
124	0.4394								
125						0.0450			
126	0.0960								
127						0.1400			
128									
129						0.2050			
130	0.1919								
131						0.0400			
132									
133									
135									
137									
139									
141									
143									
144									
145									
146									
147									
148		0.1040							
149									
150									
151									
152		0.2871							
153									
154									
155									
156		0.3267							
160		0.0099					0.0051		
162							0.0612		
164		0.1040					0.2347		
166							0.0153		
168		0.1485					0.5306		
170		0.0099					0.1327		
172		0.0050					0.0102		
200			0.1563						
202			0.6094					0.0990	
204			0.0573					0.0104	
206			0.0781					0.0052	
208			0.0208						
210			0.0677					0.0208	
212			0.0104					0.2240	
214								0.2969	
216								0.1563	
218								0.0052	
220								0.0729	
221				0.0105					
222								0.0052	
223				0.2737					
224								0.0938	
225				0.1684					
226								0.0104	
227				0.1789					0.0108
228									
229				0.0158					
231									0.1075
232									
233				0.0263					0.0484
234									
235				0.2368					0.6989
236									
237				0.0211					0.0484
238					0.0372				
239									0.0269

240					0.1702					
241										0.0430
242					0.0319					
244					0.0319					0.0054
245										0.0108
246					0.4415					
248					0.1702					
250					0.0213					
252					0.0426					
254					0.0266					
256					0.0266					
266										
268										
270										
272										
274										
276										
278										
284										
286										
288										
290										
292										

Locus	**CXX0279**	**FH2054**	**REN162C04**	**AHTH171**	**AHTH260**	**INRA021**	**AHTH130**	**REN169O18**	**REN169D01**	**REN105L03**
N	99	101	96	95	94	59	100	98	96	93
HWE	1.38E-01	8.06E-01	3.69E-01	2.16E-01	2.38E-01	8.85E-01	2.70E-02	**1.25E-03**	8.92E-01	8.80E-01
Ho	0.6768	0.8119	0.5208	0.8000	0.7553	0.6441	0.6900	0.5918	0.8125	0.4624
He	0.7295	0.7707	0.5928	0.8067	0.7440	0.6144	0.8206	0.6448	0.8169	0.4951
PD	0.8862	0.9030	0.7884	0.9194	0.8911	0.7527	0.9362	0.8007	0.9342	0.7196
PE	0.3932	0.6213	0.2064	0.5990	0.5189	0.3471	0.4130	0.2812	0.6224	0.1566
PIC	0.6900	0.7315	0.5600	0.7740	0.7121	0.5393	0.7910	0.5957	0.7886	0.4736
P_{ID}	0.1138	0.0970	0.2116	0.0806	0.1089	0.2473	0.0638	0.1993	0.0658	0.2804

Table S3: Allele frequencies and other forensic parameters for 21 canine-specific STR loci of Labrador Retriever breed raised in Rio Grande do Sul, Southern Brazil: HWE - *P* value for Hardy-Weinberg equilibrium, Ho - observed heterozygosity, He - expected heterozygosity, PD - probability of discrimination, PE - probability of exclusion, PIC - polymorphism information content, P_{ID} - Probability of Identity (Matching Probability). **Statistically significative after Bonferroni's correction (<0.0024).**

Alelle	AHT121	AHT137	INU030	INU055	REN54P11	AHTK253	AHTK211	INU005	REN64E19	FH2848	REN247M23
80	0.0098										
87							0.1186				
89							0.3144				
91							0.5052				
92	0.0490										
93							0.0103				
94	0.0049										
95							0.0412				
96	0.0441										
97							0.0103				
98	0.1078										
99											
100	0.1225										
101											
102	0.1961										
104	0.1765										
105											
106	0.1324										
107											
108	0.1176										
109											
110	0.0196							0.2907			
112	0.0147										
114	0.0049										
116											
117											
118											
119											
120											
121											
122								0.0407			
123											
124								0.2616			
125											
126								0.2733			
127											
128								0.0930			
129											
130								0.0233			
131		0.1471									
132								0.0174			
133		0.0931									
135		0.0196									
137		0.0539									
139									0.0449		
141		0.0294							0.0056		
143		0.1029							0.0169		
144			0.2598								
145		0.0147							0.5337		
146			0.0539								
147		0.5147							0.2079		
148			0.2549								
149		0.0049							0.1011		
150			0.3676								
151		0.0196							0.0112		
152			0.0392								
153									0.0674		
154			0.0147								
155									0.0112		
156			0.0098								
158											
160											
162											
164											
166											
168											
170											
172											
174											

176											
180											
200											
202											
204				0.0049							
206											
208				0.1078							
210				0.4118							
212				0.1275							
214				0.1225							
215				0.0049							
216				0.0343							
217											
218				0.1667							
219											
220											
221											
222				0.0196	0.0049						
223											
225											
226					0.2941						
227											
228					0.0147					0.0049	
229											
230					0.0049					0.0931	
231											
232					0.4461					0.2059	
233											
234					0.1275					0.0343	
235											
236					0.0147					0.0735	
237											
238					0.0931					0.2549	
239											
240										0.2255	
241											
242										0.0980	
244										0.0098	
246											
248						0.0049					
250											
252											
256											
266											0.0245
268						0.0049					0.5735
270											0.0588
272											0.2794
276											0.0049
278											0.0588
280						0.1029					
284						0.0735					
286						0.0686					
288						0.4608					
290						0.1275					
292						0.1569					

Locus	**AHT121**	**AHT137**	**INU030**	**INU055**	**REN54P11**	**AHTK253**	**AHTK211**	**INU005**	**REN64E19**	**FH2848**	**REN247M23**
N	102	102	102	102	102	102	97	86	89	102	102
HWE	**ND***	7.70E-01	7.08E-01	8.49E-01	7.06E-01	9.37E-01	9.60E-01	1.04E-02	8.59E-01	3.76E-01	9.26E-01
Ho	0.7353	0.6961	0.6471	0.7157	0.6471	0.7157	0.6186	0.6279	0.6292	0.7745	0.5392
He	0.8716	0.6928	0.7312	0.7619	0.6925	0.7296	0.6332	0.7657	0.6583	0.8208	0.5883
PD	0.9629	0.8722	0.8868	0.9068	0.8601	0.8937	0.7975	0.8981	0.8398	0.9393	0.7826
PE	0.4849	0.4222	0.3512	0.4529	0.3512	0.4529	0.3137	0.3257	0.3274	0.5526	0.2242
PIC	0.8530	0.6638	0.6805	0.7298	0.6399	0.6971	0.5682	0.7217	0.6184	0.7920	0.5279
P_{ID}	0.0371	0.1278	0.1132	0.0932	0.1399	0.1063	0.2025	0.1019	0.1602	0.0607	0.2174

Alelle	**CXX0279**	**FH2054**	**REN162C04**	**AHTH171**	**AHTH260**	**INRA021**	**AHTH130**	**REN169O18**	**REN169D01**	**REN105L03**
80										
87										
89										
91						0.0299				

92									
93									
94									
95					0.2612				
96									
97					0.3060				
98									
99					0.2463				
100									
101					0.1269				
102									
104									
105					0.0149				
106									
107					0.0075				
108									
109					0.0075				
110									
112									
114									
116	0.2843								
117						0.0294			
118	0.3480								
119						0.1471			
120	0.0098								
121						0.3284			
122									
123						0.1422			
124	0.1324								
125						0.0196			
126	0.0441								
127						0.1422			
128	0.0196								
129						0.1225			
130	0.1618								
131						0.0441			
132									
133						0.0049			
135						0.0196			
137									
139									
141									
143									
144		0.0052							
145									
146									
147									
148		0.0619							
149									
150									
151									
152		0.1753							
153									
154									
155									
156		0.2732					0.0049		
158		0.0052					0.0098		
160		0.1082					0.0392		
162							0.4265		
164		0.0515					0.0735		
166							0.1127		
168		0.1546					0.1422		
170							0.1618		
172		0.1392					0.0147		
174							0.0147		
176		0.0103							
180		0.0155							
200			0.0147					0.0245	
202			0.2794					0.0980	
204			0.2304						
206			0.2892						
208			0.1667						
210			0.0049					0.1373	

212			0.0147						0.1078	
214										
215										
216									0.3284	
217				0.0245						
218									0.0931	
219				0.1569						
220									0.1667	
221				0.0343						
222									0.0441	
223				0.0441						
225				0.2108						
226										
227				0.0147						0.0147
228										
229				0.0441						
230										
231				0.0637						0.1716
232										
233				0.1176						0.2255
234										
235				0.0686						0.0931
236										
237				0.1765						0.0245
238					0.1127					
239				0.0441						0.0049
240					0.0539					
241										0.4657
242					0.0196					
244					0.1520					
246					0.5637					
248					0.0245					
250					0.0294					
252					0.0392					
256					0.0049					
266										
268										
270										
272										
276										
278										
280										
284										
286										
288										
290										
292										

Locus	**CXX0279**	**FH2054**	**REN162C04**	**AHTH171**	**AHTH260**	**INRA021**	**AHTH130**	**REN169O18**	**REN169D01**	**REN105L03**
N	102	97	102	102	102	67	102	102	102	102
HWE	9.52E-01	8.08E-01	1.37E-01	**ND***	7.11E-01	1.59E-01	4.39E-01	6.69E-01	2.21E-01	3.95E-01
Ho	0.7451	0.7629	0.6667	0.7941	0.6863	0.7015	0.7353	0.7941	0.7941	0.6667
He	0.7556	0.8371	0.7607	0.8737	0.6432	0.7659	0.8155	0.7552	0.8171	0.6968
PD	0.8931	0.9459	0.8883	0.9592	0.8439	0.8920	0.9389	0.9014	0.9325	0.8595
PE	0.5014	0.5321	0.3786	0.5882	0.4073	0.4306	0.4849	0.5882	0.5882	0.3786
PIC	0.7126	0.8124	0.7146	0.8561	0.6123	0.7200	0.7891	0.7242	0.7912	0.6499
P_{ID}	0.1069	0.0541	0.1117	0.0408	0.1561	0.1080	0.0611	0.0986	0.0675	0.1405

Table S4: Allele frequencies and other forensic parameters for 21 canine-specific STR loci of Poodle Standard breed raised in Rio Grande do Sul, Southern Brazil: HWE - *P* value for Hardy-Weinberg equilibrium, Ho - observed heterozygosity, He - expected heterozygosity, PD - probability of discrimination, PE - probability of exclusion, PIC - polymorphism information content, P_{ID} - Probability of Identity (Matching Probability). ND*: Not Determined. **Statistically significative after Bonferroni's correction (<0.0024).**

Alelle	AHT121	AHT137	INU030	INU055	REN54P11	AHTK253	AHTK211	INU005	REN64E19	FH2848	REN247M23
87							0.0714				
89							0.3714				
91							0.4381				
92	0.0048										
93							0.0905				
94	0.0048										
95							0.0238				
97							0.0048				
98	0.0238										
99											
100	0.0143							0.0048			
101											
102	0.1000										
103											
104	0.1476										
105											
106	0.5762										
108	0.0714										
110	0.0190							0.3702			
111											
112	0.0190										
113											
114	0.0143										
116											
117											
118											
119											
120	0.0048							0.0048			
121											
122								0.0048			
123											
124								0.0433			
125											
126								0.3317			
127											
128								0.1731			
129											
130								0.0337			
131		0.3095									
132								0.0337			
133		0.0571									
135		0.0048									
137		0.0571									
139		0.0048							0.0048		
140		0.0048									
141		0.0429									
144			0.3571								
145		0.1524							0.3413		
146			0.0048								
147		0.1667							0.3365		
148			0.3476								
149		0.1476							0.0913		
150			0.2333								
151		0.0476							0.0048		
152			0.0333								
153		0.0048							0.2115		
154			0.0238								
155									0.0048		
156											
157									0.0048		
160											
162											
164											
166											
168											
170											
172											
176											
179											
200											
202											
204											

206											
208				0.1117							
210				0.3301							
212				0.0437							
214				0.3155							
216				0.0097							
218				0.0485							
219											
220											
221											
222				0.1408							
223											
224					0.0048						
225											
226					0.1000						
227											
228					0.0952					0.0048	
229											
230										0.0095	
231											
232					0.6238					0.2619	
233											
234					0.1048					0.0143	
235											
236					0.0619					0.1524	
237											
238					0.0095					0.3810	
239											
240										0.0476	
241											
242										0.0714	
244										0.0524	
246										0.0048	
248											
250											
252											
266											0.0048
268											0.6476
270											0.2381
272											0.0381
274											0.0095
276											0.0524
278											0.0095
284						0.0619					
286						0.0714					
288						0.1524					
290						0.0238					
292						0.6857					
294						0.0048					

Locus	**AHT121**	**AHT137**	**INU030**	**INU055**	**REN54P11**	**AHTK253**	**AHTK211**	**INU005**	**REN64E19**	**FH2848**	**REN247M23**
N	105	105	105	103	105	105	105	104	104	105	105
HWE	9.46E-01	2.24E-01	9.96E-01	1.57E-02	4.34E-01	5.41E-01	7.93E-01	**7.69E-05**	6.80E-02	3.85E-02	2.49E-01
Ho	0.6476	0.7810	0.6762	0.6990	0.5048	0.4857	0.6952	0.5288	0.6058	0.6762	0.4762
He	0.6324	0.8246	0.6988	0.7585	0.5796	0.4994	0.6594	0.7222	0.7205	0.7562	0.5220
PD	0.8339	0.9437	0.8521	0.8826	0.7815	0.7095	0.7958	0.8695	0.8781	0.9001	0.7189
PE	0.3520	0.5642	0.3924	0.4268	0.1917	0.1753	0.4209	0.2140	0.2979	0.3924	0.1675
PIC	0.6028	0.7995	0.6366	0.7171	0.5496	0.4658	0.5940	0.6714	0.6654	0.7177	0.4678
P_{ID}	0.1661	0.0563	0.1479	0.1174	0.2185	0.2905	0.2042	0.1305	0.1219	0.0999	0.2811

Alelle	**CXX0279**	**FH2054**	**REN162C04**	**AHTH171**	**AHTH260**	**INRA021**	**AHTH130**	**REN169O18**	**REN169D01**	**REN105L03**
87										
89										
91										
92										
93						0.0099				
94										
95						0.2723				
97						0.2228				
98										
99						0.1386				

100								
101					0.0990			
102								
103					0.2228			
104								
105					0.0347			
106								
108								
110								
111						0.0286		
112								
113						0.0143		
114	0.0429							
116	0.3048							
117						0.0095		
118	0.4619							
119						0.0238		
120	0.0190							
121						0.0524		
122	0.0429							
123						0.0429		
124	0.0429							
125						0.0143		
126	0.0571							
127						0.2095		
128								
129						0.5476		
130	0.0286							
131						0.0381		
132								
133						0.0190		
135								
137								
139								
140								
141								
144								
145								
146								
147								
148		0.0048						
149								
150								
151								
152		0.2981						
153								
154								
155								
156		0.2308						
157								
160		0.0337					0.1286	
162							0.3810	
164		0.0962					0.0333	
166							0.2048	
168		0.0288					0.0286	
170							0.2143	
172		0.3029					0.0048	
176		0.0048						
179							0.0048	
200			0.0096					0.0476
202			0.2163					0.0714
204			0.1923					
206			0.3990					
208			0.0529					
210			0.1154					0.3238
212			0.0144					0.1238
214								0.1333
216								0.2476
218								0.0333
219				0.0619				
220								0.0190
221				0.0190				
222								

223										0.0048
224										
225				0.0476						
226										
227				0.1429						0.2619
228										
229				0.0048						0.2571
230										
231										0.3143
232										
233				0.5333						0.0429
234										
235				0.1810						0.0286
236										
237				0.0095						0.0143
238					0.0286					
239										0.0048
240										
241										0.0714
242										
244					0.2571					
246					0.3905					
248					0.1810					
250					0.1381					
252					0.0048					
266										
268										
270										
272										
274										
276										
278										
284										
286										
288										
290										
292										
294										

Locus	**CXX0279**	**FH2054**	**REN162C04**	**AHTH171**	**AHTH260**	**INRA021**	**AHTH130**	**REN169O18**	**REN169D01**	**REN105L03**
N	105	104	104	105	105	101	105	105	105	105
HWE	5.12E-01	2.38E-01	9.55E-01	9.46E-01	1.12E-01	3.72E-01	3.82E-02	3.00E-01	4.16E-01	1.09E-01
Ho	0.6381	0.6731	0.7596	0.6000	0.6857	0.6931	0.5524	0.6095	0.7143	0.6190
He	0.6871	0.7585	0.7441	0.6590	0.7322	0.8003	0.6510	0.7521	0.7957	0.7621
PD	0.8492	0.9000	0.8916	0.8461	0.8815	0.9260	0.8292	0.9030	0.9281	0.9043
PE	0.3391	0.3879	0.5264	0.2909	0.4065	0.4176	0.2376	0.3025	0.4507	0.3144
PIC	0.6380	0.7141	0.7024	0.6198	0.6842	0.7657	0.6159	0.7105	0.7639	0.7190
P_{ID}	0.1508	0.1000	0.1084	0.1539	0.1185	0.0740	0.1708	0.0970	0.0719	0.0957

Table S5: Allele frequencies and other forensic parameters for 21 canine-specific STR loci of Shih Tzu breed raised in Rio Grande do Sul, Southern Brazil: HWE - *P* value for Hardy-Weinberg equilibrium, Ho - observed heterozygosity, He - expected heterozygosity, PD - probability of discrimination, PE - probability of exclusion, PIC - polymorphism information content, P_{ID} - Probability of Identity (Matching Probability). **Statistically significative after Bonferroni's correction (<0.0024).**

Alelle	AHT121	AHT137	INU030	INU055	REN54P11	AHTK253	AHTK211	INU005	REN64E19	FH2848	REN247M23
87							0.1762				
89							0.1095				
91							0.5810				
92	0.0048										
93							0.0333				
94	0.0476										
95							0.0286				
96	0.0048										
97							0.0714				
98	0.2048										
99											
100	0.1714										
101											
102	0.0952										
104	0.2524										
105											
106	0.1429										
108	0.0619										
110								0.1000			
112	0.0143										
116											
117											
118											
119											
120											
121											
122								0.0286			
123											
124								0.4952			
125											
126								0.1238			
127											
128								0.0381			
129											
130								0.1476			
131		0.2877									
132								0.0524			
133		0.0142									
134		0.0047									
135		0.0047									
136								0.0143	0.0048		
137		0.3632									
139		0.0094							0.1524		
140											
141									0.0095		
143		0.0047							0.0190		
144			0.2115								
145									0.2286		
146			0.0096								
147		0.1038							0.3667		
148			0.0673								
149									0.0571		
150			0.5529								
151		0.1462							0.0095		
152			0.0529								
153		0.0613	0.0144						0.0286		
154			0.0865								
155			0.0048						0.1238		
156											
160											
162											
164											
166											
168											
170											
172											
174											
176											
198											

Alelle	AHT121	AHT137	INU030	INU055	REN54P11	AHTK253	AHTK211	INU005	REN64E19	FH2848	REN247M23
200											
202											
204											
206											
208				0.0240							
210				0.5817							
212				0.0913							
214				0.1779							
216											
218				0.1202							
219											
220				0.0048							
221											
222					0.0534						
223											
225											
226					0.0388						
227											
230					0.0049						
231											
232					0.4175						
233											
234					0.0146						
235											
236					0.4466					0.3000	
237											
238					0.0146					0.1100	
239											
240					0.0049					0.3950	
241											
242					0.0049					0.0300	
244										0.1600	
246										0.0050	
248											
252											
254											
268											0.2723
270											0.1634
272											0.3267
276											0.0050
278											0.2327
280						0.0049					
284						0.0098					
286						0.0637					
288						0.3627					
290						0.2157					
292						0.2598					
294						0.0833					
Locus	**AHT121**	**AHT137**	**INU030**	**INU055**	**REN54P11**	**AHTK253**	**AHTK211**	**INU005**	**REN64E19**	**FH2848**	**REN247M23**
N	105	106	104	104	103	102	105	105	105	100	101
HWE	**3.06E-06**	9.28E-01	2.40E-01	8.21E-01	9.36E-02	1.53E-01	3.55E-01	9.61E-01	**6.01E-05**	8.55E-01	7.91E-01
Ho	0.4476	0.7170	0.5577	0.6250	0.5243	0.6961	0.5619	0.6381	0.5143	0.6900	0.7426
He	0.8331	0.7526	0.6375	0.6095	0.6244	0.7469	0.6154	0.7058	0.7738	0.7189	0.7419
PD	0.9261	0.8918	0.8175	0.7986	0.7863	0.8856	0.8116	0.8820	0.9020	0.8728	0.8832
PE	0.1456	0.4550	0.2432	0.3220	0.2096	0.4222	0.2476	0.3391	0.2003	0.4130	0.4971
PIC	0.8072	0.7109	0.5963	0.5676	0.5482	0.7015	0.5773	0.6748	0.7389	0.6676	0.6903
P_{ID}	0.0739	0.1082	0.1825	0.2014	0.2137	0.1144	0.1884	0.1180	0.0980	0.1272	0.1168

Alelle	CXX0279	FH2054	REN162C04	AHTH171	AHTH260	INRA021	AHTH130	REN169O18	REN169D01	REN105L03
87										
89										
91										
92										
93										
94										
95						0.4080				
96										
97						0.1724				
98										
99						0.1609				

100										
101					0.2414					
102										
104										
105					0.0172					
106										
108										
110										
112										
116	0.0613									
117						0.0094				
118	0.2358									
119						0.1651				
120	0.0142									
121						0.0660				
122	0.0566									
123						0.0236				
124	0.0613									
125						0.0189				
126	0.4009									
127						0.3962				
128	0.1226									
129						0.2406				
130	0.0472									
131						0.0094				
132										
133						0.0566				
134										
135						0.0094				
136										
137										
139						0.0047				
140		0.0048								
141										
143										
144										
145										
146										
147										
148		0.2571								
149										
150										
151										
152		0.3381								
153										
154										
155										
156		0.2286								
160		0.0952					0.0625			
162							0.4808			
164		0.0333					0.3510			
166							0.0337			
168		0.0238					0.0337			
170		0.0048					0.0337			
172		0.0095								
174							0.0048			
176		0.0048								
198			0.0721							
200			0.0962						0.1346	
202			0.1346						0.1250	
204			0.0673							
206			0.2644							
208			0.0625							
210			0.3029						0.0577	
212									0.1731	
214									0.0048	
216									0.3654	
218										
219				0.5721						
220									0.1394	
221				0.0096						
222										
223				0.0240						

225				0.1779						
226										
227										0.0049
230										
231				0.0721						0.0971
232										
233				0.1250						0.1456
234										
235				0.0144						0.2476
236										
237				0.0048						0.1893
238					0.1875					
239										0.0049
240					0.0096					
241										0.3107
242					0.1058					
244					0.0577					
246					0.3942					
248					0.0288					
252					0.2067					
254					0.0096					
268										
270										
272										
276										
278										
280										
284										
286										
288										
290										
292										
294										

Locus	**CXX0279**	**FH2054**	**REN162C04**	**AHTH171**	**AHTH260**	**INRA021**	**AHTH130**	**REN169O18**	**REN169D01**	**REN105L03**
N	106	105	104	104	104	87	106	104	104	103
HWE	9.57E-01	1.18E-01	2.86E-01	8.98E-01	8.82E-01	4.37E-01	1.74E-01	1.45E-01	8.65E-01	5.39E-01
Ho	0.7925	0.8381	0.7788	0.6346	0.7212	0.6897	0.6981	0.5673	0.7212	0.7476
He	0.7590	0.7600	0.8012	0.6223	0.7548	0.7235	0.7527	0.6414	0.7838	0.7794
PD	0.9057	0.8784	0.9279	0.8138	0.9000	0.8704	0.8998	0.8173	0.9199	0.9115
PE	0.5851	0.6715	0.5604	0.3345	0.4617	0.4124	0.4253	0.2535	0.4617	0.5056
PIC	0.7248	0.7173	0.7700	0.5828	0.7166	0.6732	0.7149	0.5762	0.7513	0.7402
P_{ID}	0.0943	0.1216	0.0721	0.1862	0.1000	0.1296	0.1002	0.1827	0.0801	0.0885

Table S6: Allele frequencies and other forensic parameters for 21 canine-specific STR loci of Yorkshire Terrier breed raised in Rio Grande do Sul, Southern Brazil: HWE - *P* value for Hardy-Weinberg equilibrium, Ho - observed heterozygosity, He - expected heterozygosity, PD - probability of discrimination, PE - probability of exclusion, PIC - polymorphism information content, P_{ID} - Probability of Identity (Matching Probability). **Statistically significative after Bonferroni's correction (<0.0024).**

4 CONCLUSÕES E CONSIDERAÇÕES FINAIS

No presente trabalho, reunimos números significativos de genótipos de animais, especialmente de equinos e bovinos, para poder estimar com maior precisão diversos índices que são fundamentais para a identificação genética utilizando marcadores STR. De fato, ainda que estes *loci* sejam, em sua maioria, repetições dinucleotídicas, apresentando maior quantidade de artefatos quando da amplificação na PCR, o que dificulta um pouco mais as análises, são as regiões de estudo recomendadas pela Sociedade Internacional de Genética Animal (ISAG). Muitas dessas regiões foram selecionadas ainda na década de 1990, quando a utilização de microssatélites para a identificação genética ainda dava os seus primeiros passos. Como não houve mudanças nas recomendações, apenas adições de mais marcadores, as bases ou os bancos de dados genéticos foram e são constituídos pelas informações geradas por esses *loci* em todo o mundo e em diversas espécies de animais domésticos.

Ainda assim, tivemos muitas dificuldades em encontrar publicações de frequências alélicas e demais índices de interesse forense, além dos estudos de estruturação populacional entre as raças, que tivessem utilizado esses grupos de marcadores genéticos, que compõem o painel recomendado para as espécies equina, bovina e canina. Tanto que a determinação desses parâmetros com esses STR nas raças equinas Quarto de Milha e Crioulo Uruguaio, nas raças bovinas Angus, Brangus, Braford e Jersey e nas seis raças de cães, pode ser considerada com uma das primeiras a serem publicadas na comunidade científica.

Dentre as conclusões mais significativas que podemos citar, estão:

1) Os três painéis de marcadores STR utilizados no estudo conseguem discriminar os indivíduos de acordo com a raça.
2) Tendo em vista que os animais de todas as raças e das três espécies do estudo apresentaram algum grau de subestruturação, é recomendável utilizar números significativos de animais de cada raça para que a variabilidade genética seja corretamente estimada, pois grupos populacionais pequenos podem mascarar ou descaracterizar a real distribuição dos alelos. A avaliação correta da variabilidade genética de uma raça possibilita o estabelecimento de bons programas de

melhoramento animal, uma vez que a ocorrência em excesso de cruzamentos endogâmicos ou de indivíduos muito parecidos geneticamente pode resultar no aparecimento de características indesejáveis ou prejudiciais para a manutenção do padrão racial e na qualidade genética do rebanho ou da prole.

3) Foram observadas diferenças genéticas entre bovinos de mesma raça e de regiões geográficas distintas, mesmo sendo Uruguai e Paraguai países muito próximos. Seria interessante avaliar outros grupos populacionais com mesmas raças só que de países mais longínquos, para verificar se os achados no presente estudo tiveram algum viés (como o tamanho amostral de alguma população) ou se de fato as frequências dos alelos podem variar conforme a localização.
4) O grupo de marcadores genéticos recomendados pela ISAG para a identificação genética de bovinos é composto por um painel de 12 STR. De fato, os índices que determinam o quão poderoso é o painel para a discriminação individual (PE, PD e P_{ID}) foram bem menores que aqueles das outras espécies, embora tal comparação não seja a mais adequada a se fazer. Contudo, no trabalho de Van de Goor *et al.*, que serviu como referência para várias comparações no artigo de raças bovinas, além dos 12 STR recomendados pela ISAG, foram utilizados mais quatro STR e as melhoras em PE, PD e P_{ID} foram significativas – e assim os autores sugerem que tais regiões sejam incluídas no referido painel, sugestão a qual corroboramos.
5) No trabalho realizado com cães provenientes do Rio Grande do Sul, iniciamos um projeto que pretendemos dar continuidade: a criação de um banco de dados genético para as mais variadas raças caninas. Sua utilização estaria ligada, principalmente, aos serviços de testes de paternidade e de análises de vínculo e de parentesco solicitados pelos *Kennels Clubs* e criadores, mas também para a identificação individual, podendo ser útil no caso de animais perdidos ou envolvidos em ataques a seres humanos, ou até mesmo presentes em algum local de crime. O painel de 21 marcadores mostrou-se eficiente e proporcionou dados que

permitiram predizer a raça de um determinado animal, escolhido aleatoriamente, com uma precisão próxima aos 100%.

6) O conhecimento e a divulgação dos alelos mais frequentes de cada marcador STR dos três painéis recomendados pela ISAG utilizados neste estudo, pode propiciar o surgimento de *kits* comerciais para fins de identificação genética que apresentem, entre seus componentes, uma escada alélica, algo que ainda é ausente nos produtos disponíveis no mercado. Isto facilitaria e, certamente, ajudaria a popularizar a utilização destes marcadores genéticos, tornando a interpretação dos resultados mais segura e confiável, permitindo a sua implementação na rotina de laboratórios especializados em identificação genética animal. Com certeza, o surgimento de um produto mais prático, mais fácil de aplicar à rotina laboratorial e que propicie resultados seguros e confiáveis será um dos principais propulsores para que a identificação genética animal atinja os mesmos patamares da identificação humana, respeitando, obviamente, os propósitos aos quais cada área se destina.

Por fim, consideramos que o trabalho desenvolvido proporcionou resultados importantes e significativos na área da identificação genética animal, sendo que seus dados podem ser utilizados tanto na área forense, em testes de paternidade, análise de vínculo e de parentesco, em programas de rastreabilidade bovina e de produtos cárneos, como também para estudos filogenéticos. Conforme já mencionado, estão disponíveis poucas publicações na comunidade científica de trabalhos semelhantes a este, e por isso, talvez, o presente estudo possa servir de inspiração para a realização de pesquisas semelhantes, em outras raças e espécies, propiciando maior conhecimento sobre a variabilidade genética das raças e sobre a estruturação das populações, contribuindo de forma consistente para o estabelecimento e gerenciamento de bons programas de melhoramento genético animal, tão necessários para a manutenção dos padrões raciais exigidos pelas associações de criadores, mas também para o aperfeiçoamento daquelas características de maior interesse, desejadas para a propagação entre os rebanhos ou proles.

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ANEXO A – Páginas do artigo publicado na revista Forensic Science International: Genetics

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Correspondence

Population genetic study over 32,000 equines from Uruguay using seventeen forensically informative STR loci

Dear Editor,

It is known that horses (*Equus caballus*) had an important role in many activities that influenced the development of various civilizations around the world. For many reasons, men set the selection of animals according to ability, skills and disposition desired, giving rise of specific groups of horses which have distinctive characteristics transmitted to offspring consistently, the breeds. It is estimated that there are currently about 300 breeds of horses [1]. Due to this ancient and close interaction between horses and humans, relevant forensically cases such as theft horses, identity fraud and, more recently, the sale of adulterated or counterfeited semen doses, and doping control are the most observed crimes. Therefore, it is recommended that forensic analysis techniques become more efficient as possible with increasingly improved accuracy.

Uruguay, a small country in the southeastern region of South America bordered by Argentina and Brazil, is characterized by the strength of the agricultural sector and livestock production. As the most recent data collected by OIE (World Organization for Animal Health), it is estimated that Uruguay has about 400,000 horses in its territory [2]. The Rural Association of Uruguay, the oldest institution of this country in agricultural matters, and the Uruguayan Stud Book, responsible for the registration and identification of all Thoroughbreds born in the country or imported, are pioneering institutions in Latin America in establishing typification of horses through DNA analysis [3,4]. For this reason, both institutions have grouped into their records thousands of genotypes of horses of various breeds representing significantly the horse population of Uruguay.

The aim of this paper is to present a genetic study of forensic interest of a total group up to 32,053 purebred horse of five different breeds: Appaloosa (n = 109), Arabian (n = 1661), Uruguayan Criollo (n = 5906), Thoroughbred (n = 23,203) and Quarter Horse (n = 1174), using the seventeen STR loci recommended by International Society for Animal Genetics (ISAG) for genotyping horses (http://www.isag.us/Docs/EquineGenParentage2014.pdf). All samples were obtained from animals subjected to pedigree registration on Rural Association of Uruguay or Uruguayan Stud Book. DNA was isolated from hair root or semen straws samples using proteinase K in two types of extraction buffer: Buffer 1 to hair root samples ($MgCl_2$, PCR Buffer and Tween 20) and Buffer 2 to semen straws samples (EDTA, SDS, Tris, NaCl and DTT). Final concentration after DNA extraction: 50–100 ng/mL of equine genomic DNA. The seventeen STR loci were amplified in a single PCR multiplex performed on Veriti™ Thermal Cycler (Applied Biosystems, Foster City, CA, USA) or GeneAmp 9700 PCR System (Applied Biosystems) using fluorescent labeled primers which the sequences are available on http://www.cstl.nist.gov/strbase/horseSTRs.htm. The other reagents for PCR amplification (PCR Buffer, AmpliTaq Gold DNA Polymerase, dNTP mix) were carried out using the StockMarks™ for Horses Genotyping Kit (Applied Biosystems). The amount of equine genomic DNA was 25–50 ng/mL per sample. Thermocycling conditions were: pre-incubation for 10 min at 95 °C, followed by thirty cycles of 30 s at 95 °C, 30 s at 60 °C and 60 s at 72 °C, with a final incubation for 60 min at 72 °C. The electrophoresis and typing were performed on an ABI 3500 Genetic Analyzer (Applied Biosystems) using GeneScan™ 500 LIZ™ Size Standard as internal lane standards according to the manufacturer's protocols. The data was collected by Data Collection v1.0 software (Applied Biosystems) and analyzed using GeneMapper ID-X v1.3 software (Applied Biosystems).

Calculations of allele frequencies, observed (Ho) and expected (He) heterozygosity, polymorphism information content (PIC) and p-values of the Hardy-Weinberg equilibrium (HWE) test for all seventeen loci, were assessed using CERVUS version 3.0.3 [5]. Bonferroni's correction was used for HWE test, which assumes that a 0.05 significance level obtained for seventeen tests (one per locus) yields an actual significance threshold of 0.0029 [6]. Power of discrimination (PD), power of exclusion (PE) and probability of identity (P_{ID}) were estimated with PowerStats (Promega Corporation) [7]. Estimated coefficients of inbreeding (F_{IS}) within breeds, fixation indices (F_{ST}) among breeds, and total inbreeding (F_{IT}) using an analysis of molecular variance (AMOVA) were performed with ARLEQUIN version 3.1 [8] and GENEPOP version 4.5.1 [9]. UPGMA trees were built from Nei's genetic distance matrix using the PHYLIP software package version 3.69 [10] and visualized with TreeView software version 1.6.6 [11]. The allele frequencies and forensically informative parameters are available in Supplementary data in the online version DOI: 10.1016/j.fsigen.2016.10.011 (Tables S1–S5).

The Hardy-Weinberg equilibrium test showed significant deviation ($P < 0.05$) in various loci in all five horse breeds of this study. Even after applying Bonferroni's correction using the total number of loci analyzed ($P < 0.0029$), the differences observed were also statistically significant in many loci on four of the five breeds, except Appaloosa, as seen in Table S1 in Supplementary data in the online version DOI: 10.1016/j.fsigen.2016.10.011. Regarding heterozygosity, in general, the observed (Ho) were lower than the expected (He) in almost all loci of five breeds. The loci with lower polymorphism information content (PIC) was HTG7 (Appaloosa, Arabian and Uruguayan Criollo) and HTG4 (Thoroughbred and Quarter Horse). The loci with higher PIC was ASB17 (Appaloosa and Uruguayan Criollo), VHL20 (Arabian), ASB2 (Thoroughbred) and LEX3 (Quarter Horse). The probability of

http://dx.doi.org/10.1016/j.fsigen.2016.10.011

Table 1
F_{IS}, F_{ST} and F_{IT} coefficients for five horse breeds to each of the seventeen loci and the overall.

Marker	F_{IS}	F_{ST}	F_{IT}
VHL20	0.0041	0.0448	0.0487
HTG4	0.0024	0.1416	0.1437
AHT4	0.0036	0.0251	0.0286
HMS7	0.0029	0.0404	0.0432
HTG6	0.0063	0.1665	0.1717
AHT5	0.0112	0.0938	0.1039
HMS6	−0.0011	0.0414	0.0403
ASB23	0.0085	0.0738	0.0817
ASB2	0.0198	0.0548	0.0735
HTG10	0.1141	0.0744	0.1800
HTG7	0.0106	0.1173	0.1267
HMS3	0.0226	0.1007	0.1210
HMS2	0.0103	0.2353	0.2431
ASB17	0.0021	0.0598	0.0618
LEX3	0.3653	0.0979	0.4274
HMS1	−0.0077	0.0314	0.0240
CA425	0.0217	0.0406	0.0614
All	0.0368	0.0845	0.1182

Table 2
Inbreeding coefficient (F_{IS}) estimated for five horse breeds.

Breed	F_{IS}
Appaloosa	0.0183
Arabian	0.0240
U. Criollo	0.0332
Thoroughbred	0.0374
Quarter Horse	0.0491

Table 3
Genetic distances (F_{ST} analysis) among five breeds of horses from Uruguay.

Breed	Appaloosa	Arabian	U. Criollo	Thoroughbred	Quarter Horse
Appaloosa	*	0.0677	0.0321	0.0607	0.0165
Arabian	0.0677	*	0.0765	0.1047	0.0720
U. Criollo	0.0321	0.0765	*	0.0876	0.0414
Thoroughbred	0.0607	0.1047	0.0876	*	0.0612
Quarter Horse	0.0165	0.0720	0.0414	0.0612	*

identity (P_{ID}), that indicates probability of two individuals within the population sharing the same genotype, showed the following overall values for each breed: 2.15×10^{-17} (Appaloosa), 2.41×10^{-15} (Arabian), 1.15×10^{-18} (Uruguayan Criollo), 2.28×10^{-15} (Thoroughbred) and 5.95×10^{-18} (Quarter Horse). The values obtained for combined Power of Discrimination (PD) and combined Power of Exclusion (PE) for seventeen markers were, respectively: 0.99999999999999999 and 0.99999 in Appaloosa breed; 0.999999999999999 and 0.9999 in Arabian breed; 0.999999999999999999 and 0.99999 in Uruguayan Criollo breed; 0.999999999999999 and 0.9999 in Thoroughbred breed; 0.99999999999999999 and 0.99999 in Quarter Horse breed.

In Table 1 the values of F_{IS}, F_{ST} and F_{IT} for each of the seventeen loci and the overall can be found. The positive values of F_{IS} (Table 2) indicate heterozygote deficiency and sub-structuration in all five

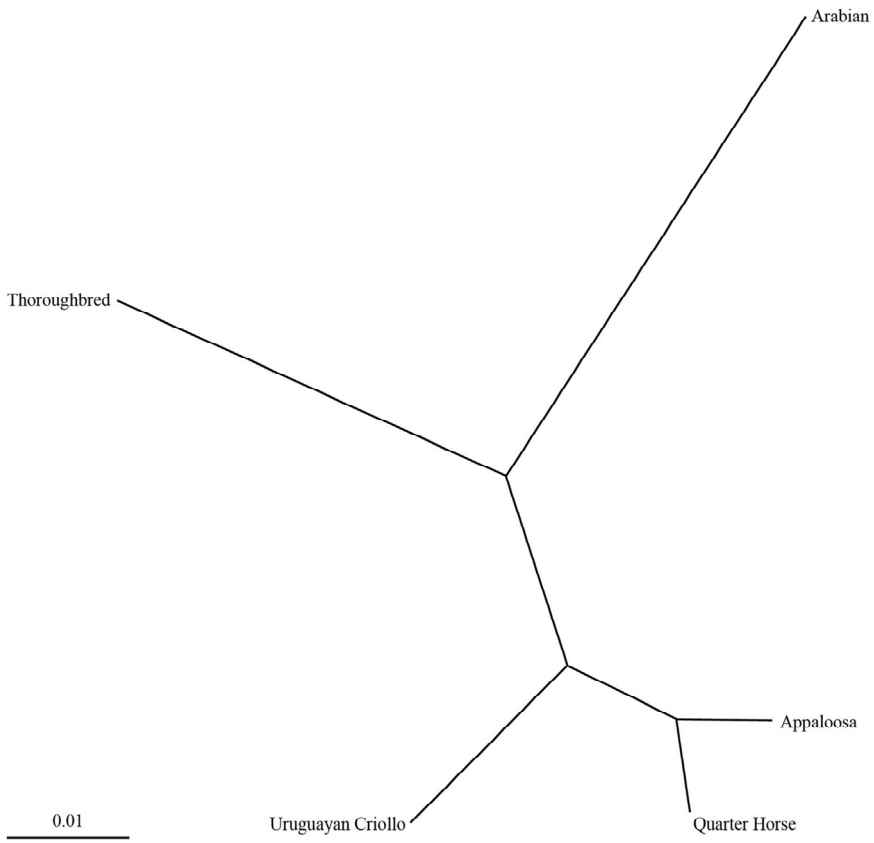

Fig. 1. Neighbour-joining tree based on pairwise Nei's genetic distances calculated between five breeds of horses from Uruguay.

horse breeds, being Quarter Horse and Thoroughbred the breeds with higher level of inbreeding. Based on genotypic frequencies of the seventeen STRs of this study, pairwise genetic distances were calculated between the five breeds, using Nei's formulas implemented in PHYLIP software (Table 3). The analysis showed a clear separation between these breeds (Fig. 1). The major genetic distance in these five breeds is between Arabian and Thoroughbred breeds, while the breeds which are closer from each other are Appaloosa and Quarter Horse, corroborating the historical data that indicate the time and the way of each horse breed arose [12–21].

Based on the largest publication and one of the most important on allelic frequencies of the seventeen STR markers present in our study [1], we compared the most frequent alleles at each locus in three horse breeds that are common in both works (Appaloosa, Arabian and Thoroughbred). In many loci, the most frequent allele of the reference publication is not the same as our study. Regarding the comparison for Uruguayan Criollo and Quarter Horse breeds, since there were no publications with this set of seventeen STR loci, it was made with studies using common genetic markers with the present study (fewer markers) [17,22–24]. In all studies, in many loci, the most frequent allele is not the same as our study.

This study presents the allele frequencies and other forensically informative parameters of seventeen STR loci that have not been published yet with horses from Uruguay. The presentation of these data, with these set of genetic markers, for Uruguayan Criollo and Quarter Horse breeds, is one of the first publications in the scientific community. However, considering the large number of animals for both breeds used in this study, and the results obtained, it is feasible to assume that these data can be used for forensic and kinship analysis, genetic identification and phyloge- netic reconstruction. Finally, we consider important to determine the allelic frequencies and other parameters of forensic interest and genetic identification with animals in the same breed, in significant sample size and a certain geographic region, similar to what is recommended for human populations [25,26]. Thus, common and rare alleles of the location will be known, allowing to identify characteristics of each population as the occurrence or not of sub-structuration, linkage to traits under selection, Wahlund effect, bottleneck effect, etc. [22].

This paper follows the guidelines for publication of population data requested by the journal [27], the recommendations of International Society for Forensic Genetics (ISFG) regarding the use of non-human (animal) DNA in forensic genetic investigations [28] and the proposed allele nomenclature for seventeen equine-specific STR loci [29] as recommended by the ISFG for the nomenclature of human STRs [30].

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