JOURNAL OF FORENSIC SCIENCES

J Forensic Sci, 2014
doi: 10.1111/1556-4029.12562
Available online at: onlinelibrary.wiley.com

TECHNICAL NOTE

CRIMINALISTICS

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Effectiveness of Single-Nucleotide Polymorphisms to Investigate Cattle Rustling*

ABSTRACT: Short tandem repeats (STR)s have been the eligible markers for forensic animal genetics, despite single-nucleotide polymorphisms (SNP)s became acceptable. The technology, the type, and amount of markers could limit the investigation in degraded forensic samples. The performance of a 32-SNP panel genotyped through OpenArrays™ (real-time PCR based) was evaluated to resolve cattle-specific forensic cases. DNA from different biological sources was used, including samples from an alleged instance of cattle rustling. SNPs and STRs performance and repeatability were compared. SNP call rate was variable among sample type (average = 80.18%), while forensic samples showed the lowest value (70.94%). The repeatability obtained (98.7%) supports the used technology. SNPs had better call rates than STRs in 12 of 20 casework samples, while forensic index values were similar for both panels. In conclusion, the 32-SNPs used are as informative as the standard bovine STR battery and hence are suitable to resolve cattle rustling investigations.

KEYWORDS: forensic science, forensic genetic identification, bovine, microsatellite, single-nucleotide polymorphism, cattle rustling, non-human forensic

Animal forensic genetics is defined as the application of relevant genetic techniques and theory to legal matters, or enforcement issues, concerning animal biological material (1). This area involves the DNA profiling of animal biological material to solve forensic investigations that involve animal victims (e.g., in animal cruelty, theft, poaching or trafficking cases), suspects (e.g., when an animal is suspected of attacking a person or another animal, causing an accident, or being responsible for property damage), or witness (e.g., when biological evidence from an animal can be used to link a human suspect to a crime scene or victim) (2–10).

During the last decades, animal DNA evidence has been successfully used in litigation (11–13). DNA technology provides reliability and accuracy in performing parentage testing and in individual identification (e.g., 6,12,14–18). Animal DNA profiles have been used as probative evidence to successfully resolve real legal cases, such as cattle rustling, horse doping, wildlife trafficking, and animal attack cases (2–4,8,19–24). Within criminal lawsuits that include animal forensic samples, rustling is the more frequent non-human forensic case in Argentina and in other undeveloped countries (2,3). More than 10,000 head of cattle are stolen each year only in Buenos Aires Province (25).

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*Supported by the Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Agencia Nacional de Promoción Científica y Tecnológica (ANPCyT) and Universidad Nacional de La Plata (UNLP).

Received 28 Feb. 2013; and in revised form 5 Oct. 2013; accepted 26 Oct. 2013.

Short tandem repeats (STRs) have been the DNA markers of choice for more than two decades because they are codominant, highly polymorphic, and found throughout the genome (26,27). However, recent advances in high-throughput DNA sequencing, computer software and bioinformatics, provide the tools for the single-nucleotide polymorphisms (SNPs) to become more popular (28,29). SNPs have some promising advantages over STRs, such as greater abundance and genetic stability (in mammals), simpler nomenclature and are more amenable to automated analysis and data interpretation (30–35).

Even though SNPs are becoming the markers of choice in parentage analysis (e.g., 36–38), the high-quality and high-quantity DNA required for the microarrays platforms generates some questions about their usefulness for certain types of forensic samples. Some studies have evaluated the use and performance of SNP arrays to genotype low-yield and/or low-quality DNA samples (39,40). Forensic DNA samples are usually purified from degraded biological samples, and as a consequence the quality and quantity of available DNA can limit the use of SNP-based technologies in this field.

Recently, the Parentage Recording Working Group of ICAR (International Committee for Animal Recording) developed a consensus SNP panel for identity and parentage testing in cattle, and certification of laboratories around the world was performed during the last years (41). Given the transition toward SNP-based testing and taking into account that there is greater experience in the use of STRs than SNPs (in terms of laboratory and statistical methods for analysis), the objective of the present study was to evaluate the performance of a panel of 32 SNPs to resolve forensic cases using the OpenArrays™ (Applied Biosystems, Foster

City, CA) microarrays technology to genotype DNA purified from different types of biological sources (including samples from animal forensic cases), and comparing the SNP-based results with those of the standard cattle forensic STR panel, with regard to their usefulness in investigating cattle rustling cases.

Methods

Sample and DNA Extraction

The present study involved 570 bovine DNA samples, including 550 from a reference dataset and 20 from forensic cases as described below:

Reference Dataset

Of 550 DNA samples extracted from different biological sources including blood (n = 392), hair (n = 48), muscle (n = 94), semen (n = 6), bone (n = 2), and nasal swabs (n = 10) from 15 pure and mixed breeds (more details in supplementary materials, available upon request) were collected as reference cattle populations. These breeds were selected because they represent the major Argentinian breeds. There are two main breeding areas in Argentina: the temperate Pampa and the northeast subtropical regions. In the Pampa, Holstein and British breeds, such as Angus and Hereford, are predominant, while Zebuine and Creole cattle are more common in the subtropical northeast. Despite their origin, dataset also included 80 Chinese samples, given that they represent a different available type of DNA source (lyophilized muscle store at room temperature).

Bovine Rustling Case Studies

We analyzed twenty forensic samples (18 muscles and two bones). These samples belong to six cattle rustling cases where DNA analysis was performed on the reference and evidence samples. The reference samples, which were the remains of the stolen animals that were discarded by the rustlers at the owner's farm, were used for comparison with the evidence samples sequestered from the suspect of the rustling. All samples were submitted to our laboratory by the Buenos Aires Province Court (Argentina) for DNA comparative analysis. The seizure process tends to take months, making preservation of the biological samples challenging (details of analyzed forensic samples in supplementary materials, available upon request).

DNA was extracted from blood samples using the Wizard® Genomic DNA purification kit (Promega, Madison, WI) following the manufacturer's instructions. DNA was purified from meat and other tissue samples according to the methods previously reported (2). To extract DNA from hair, the bulbs were incubated at 95°C with NaOH (200 mM) for 10 min, followed by a subsequently neutralization step with Tris–HCl (100 mM, 200 mM, pH = 8.5). Two microliters of this nonpurified extraction were used for PCR. To recover DNA from bones, the materials were pulverized and incubated in 0.5 M EDTA—20% N-lauryl-sarcosine solution until complete demineralization. The obtained materials were purified using organic method. The assayed DNA was either freshly extracted or stored for up to 15 years at −20°C. DNA quality and quantity were measured using a spectrophotometer NanoView (GE Healthcare, Piscataway, NJ, USA).

Genotyping

DNA genotyping was performed with STRs and SNPs. For SNP genotyping, a set of 32 markers (details in supplementary material, available upon request) was used. These SNPs are included within the 116 markers that were selected for the consensus panel for cattle identification developed by the Parentage Recording Working Group of ICAR (42) because they exhibit the higher gene diversity in a previous study (43). SNP genotyping was performed using the OPENARRAYS PLATFORM (Life technologies, Foster City, CA). The results were analyzed using the TAQMANGENOTYPER_V1.2 software (Life technologies). Samples were genotyped in two independent runs (replicates) to evaluate the confidence of the technology.

STR genotyping was performed in three multiplex reactions: mix 1 included *ETH3*, *ETH10*, *ETH225*, *INRA023*, RM067, *TGLA126, and SPS115 markers*; mix 2 comprised *BM1818*, *BM1824*, *BM2113*, *BRR, HEL1,TGLA122,* and *TGLA227 STRs*; and mix 3 contained *CSRM60, CSSM66, HAUT27, and TGLA53* markers (41). Briefly, PCR was carried out in a total volume of 12.5 μL, containing 20 mM Tris–HCl (pH = 8.4), 50 mM KCl, 1.5 mM $MgCl_2$, 0.16 mM of each dNTP, 0.6 U Taq polymerase (Metabion, Martinsried, Germany), 0.12–0.48 μM of each primer, and 50 ng of DNA. The cycling conditions were as follows: a denaturation step of 1 min at 94°C, followed by 15 cycles of 20 sec at 94°C, 1.15 min at 60°C, and 30 sec at 72°C, and followed by 20 cycles of 20 sec at 94°C, 1.15 min at 58°C, and 30 sec at 72°C with a final elongation step of 5 min at 72°C. (More details in supplementary materials, available upon request.). Fragments were resolved in an automatic DNA sequencer MegaBACE 1000 (GE Healthcare). Allele sizes were determined using the ISAG nomenclature (2011/2012 Bovine ISAG Comparison Test, 41). The STRs used were selected because they belong to the standard FAO (Food and Agriculture Organization of the United Nations) panel of 30 STRs (44) or/and are those recommended by the International Society for Animal Genetics (ISAG, 40). Furthermore, they were previously used to successfully resolve forensic cases (1,2).

Performance of SNP Genotyping

The average performance of the 32 SNPs per sample for reference and casework samples, and for each analyzed sample types, was estimated using three values: (i) the number (total calls) and percentage (call rate) of successful genotyped data; (ii) the number and percentage of nonamplify (NON-AMP, failed RT-PCR reactions) data points (each SNP per sample reaction); and (iii) the number and percentage of undetermined genotypes (UND, positive amplifications with poor genotype cluster separation). Furthermore, the average performance of each SNP assay for reference and casework samples, and for each analyzed sample types, was also estimated using these parameters. Repeatability of SNP-based OpenArrays assay was calculated as the number and percentage of concordant calls between replicates.

Statistical Analysis

Allele frequencies were determined by direct counting. Hardy–Weinberg equilibrium (HWE) was estimated by F_{IS}, using the exact test implemented in GENEPOP 4 (45,46). Genetic differentiation, measured through the F_{ST} index, for each SNP and for the whole marker set among population was calculated using ARLEQUIN 3.5 (47).

Assignment Test

To select the reference gene frequencies for forensic index estimation, casework samples were allocated to putative source populations: the bovine breed group (European, Zebuine, Asian, and Mixed) or breed. Three assignment algorithms were used including the frequency-based method developed by Paetkau et al. (48), the Bayesian-based methods of Rannala & Mountain (49; hereafter referred to as R&M), and the Baudouin & Lebrun (50; hereafter referred to as B&L). All these algorithms are included in GENECLASS 2 package (51).

Forensic Index

This index was calculated for SNP and STR markers under three different expectations concerning the gene frequencies and structure of the reference populations (θ correction; Balding and Nichols' estimator; 52,53). The considered scenarios were the following: S1. The forensic index was estimated considering the whole population gene frequencies with θ correction as reference population; S2. Casework samples were assigned to their putative breed group of origin, and their gene frequencies with θ correction were used to estimate the forensic index; and S3. Casework samples were assigned to their putative breed of origin, and its gene frequencies without θ correction were used to calculate the forensic index. For S1 and S2, the conditional probability that evidence and reference sample share the same homozygote genotype for a single locus was calculated using the following equation (54):

$$R^i = \frac{(\theta + (1-\theta)pA)(3\theta + (1-\theta)pA)}{(1+\theta)(1+2\theta)}$$

when both samples share a heterozygote genotype for a single locus, the probability was estimated using this equation (54):

$$R^i = 2\frac{(\theta + (1-\theta)pA)(\theta + (1-\theta)pB)}{(1+\theta)(1+2\theta)}$$

where *pA* and *pB* were the gene frequencies of alleles *A* and *B*. Finally, the probability for the multiple analyzed loci was calculated using the product rule, as following:

$$LR = \frac{1}{\pi Pi}$$

In S3, forensic index was estimated using the assigned breed gene frequencies and the product rule method, as if the biological evidence could be assigned *a priori* to their putative breed of origin. The θ correction was applied as if the population was divided into an unknown number of subpopulations and the biological evidence corresponded to a general structured population (defined here as the whole bovine reference database or assigned group reference database) (52,53).

Results

DNA was extracted from different biological sources and genotyped by two replicates, resulting in a total of 18,240 data points. First, we evaluated the performance of the used SNP set through different values: number and percentage (call rate) of successful calls, NON-AMP, UND, and repeatability. The results for the reference samples exhibited average successful calls of 25.66 (call rate = 80.18%; 14,625 of 17,600 successful data points) SNPs per sample, ranging from 0 to 100% of genotypes call rate among individual samples (Table 1, Fig. 1). The failed reactions were explained by a 4.28 (13.37%) and 2.10 (6.56%) of average NON-AMP and UND data points per sample, respectively. No correlation between UND and NO-AMP among samples was observed ($R = 0.33$; Fig. 2*a*). Forensic casework results showed an average call rate value of 70.94% (454 of 640 successful data points), ranging from 0% to 100% of genotype calls among individual samples (Table 1). In concordance with reference sample results, the failed data points were explained more by NON-AMP reactions (19.84%) than by UND ones (3.59%). Regarding repeatability, the SNPs exhibited a high percentage of concordant calls between replicates (98.7%), in both reference and casework samples.

Second, we analyzed two variables that can account for the successful results: assay design and DNA biological sources. Figure 3*a–c* shows the average performance (call rate, UND, and NON-AMP) obtained for each individual SNP assay among samples. As expected, the percentage of unsuccessful results was unevenly distributed. Four markers (rs290102811, rs29012691, rs29011266, and rs17871190) exhibited the highest percentage of average UND due to poor genotype cluster separation, while more than ten markers had average NON-AMP values between 15 and 22% (Fig. 3*a–c*, details on the performance of each individual SNP assay in supplementary materials, available upon request). No correlation between UND and NON-AMP among SNP assays was observed ($R = 0.11$; Fig. 2*b*). When performance of SNP typing using different DNA sources was compared, significant differences were observed ($p < 0.05$). While four of five sample types exhibited an average successful calls higher than 25 (average call rate >75%), unpurified DNA

TABLE 1—*Performance of the single-nucleotide polymorphism set obtained for reference and casework samples and for each analyzed sample types was estimated through the following values: number (call rate between brackets) of successful genotyped data; number of nonamplify reactions (NON-AMP); and number of undetermined genotypes (UND, positive amplifications with poor genotype cluster separation) among samples.* n = *sample size.*

Group	*n*	Average Call Rate	Average Nonamplified	Average Indeterminate
Reference	550	25.66 (80.18%)	4.28 (13.37%)	2.1 (6.56%)
Casework	20	22.70 (70.94%)	6.35 (19.84%)	1.15 (3.59)
Sample Type	***n***	**Average Call Rate**	**Average Nonamplified**	**Average Indeterminate**
Blood	392	26.89 (84.02%)	3.58 (11.20%)	1.52 (4.76%)
Hair	48	16.40 (51.24%)	8.60 (26.89%)	6.98 (21.81%)
Beef	112	24.57 (76.79%)	4.70 (14.69%)	2.06 (6.45%)
Swab	10	31.30 (97.81%)	0.20 (0.65%)	0.5 (1.56%)
Semen	6	30.50 (95.31)	1.00 (3.12%)	0.5 (1.56%)
Bone	2	30.00 (93.75)	1.00 (3.12%)	1.00 (3.12%)

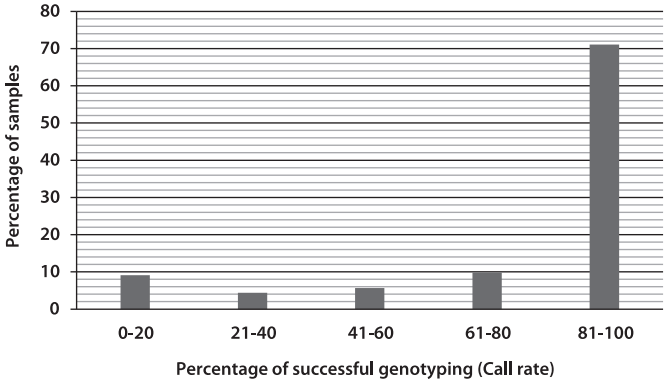

FIG. 1—*Average performance (in percentage) of the 32 single-nucleotide polymorphism set among samples. Samples were classified in five groups according their percentage (call rate) of successful genotyped data.*

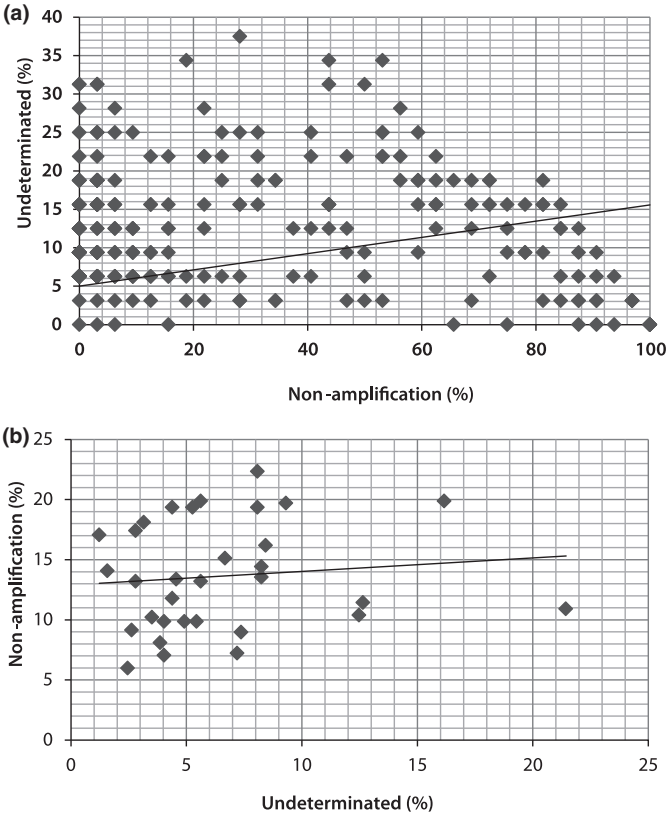

FIG. 2—*Comparison between the percentage of undetermined (UND) and nonamplified (NON-AMP) results. (a) Correlation between percentage of UND and NON-AMP obtained for the single-nucleotide polymorphism (SNP) set for each samples, (b) Correlation between percentage of UND and NON-AMP obtained for each SNP assay among samples.*

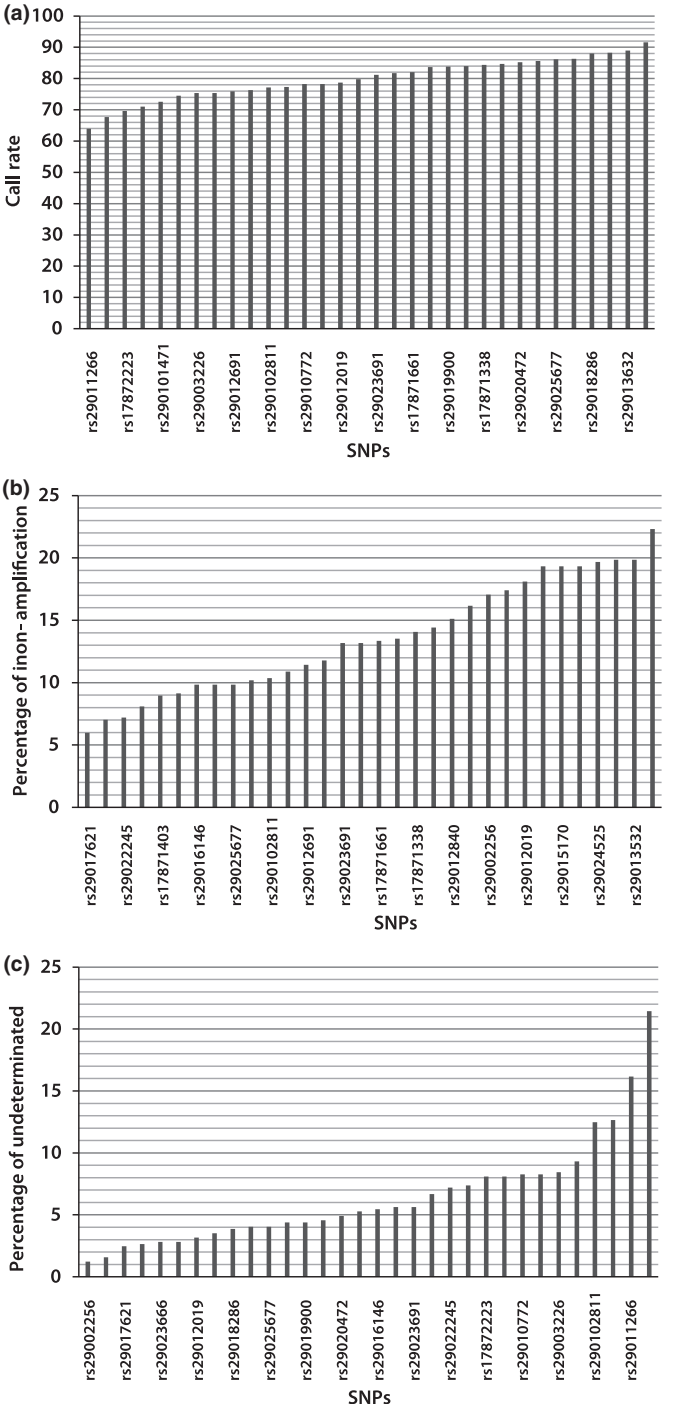

FIG. 3—*Average performance of each single-nucleotide polymorphism (SNP) assay among samples. (a) Percentage of successful genotyping per SNP (call rate) among samples, (b) percentage of nonamplification (NO-AMP) genotyping per SNP among samples, and (c) percentage of undetermined genotyping per SNP (UND) among samples. The SNPs were ordered by increasing call rate, NON-AMP and UND values.*

extracted from hair had a poor performance (average call rate = 51.24%) probably due to the presence of inhibitors of DNA amplification (e.g., hair pigments; Table 1). Excluding hair samples, our average call rate reached 83.11%, while when poorly performing samples were removed (call rate <10%), considering that in these cases, the problem was the DNA instead of the genotyping method, the mean call rate increased to 87.37%.

Finally, we evaluated SNPs and STRs genotyping performance and identification power in forensic samples from cattle rustling. Table 2 compares the call rates for SNP and STR sets obtained for each individual casework sample, showing that in 12 of 20 casework samples, SNP set presents a higher performance than STR one. To compare the identification power of both type of markers for resolving cattle rustling cases, we assigned each casework to a breed and breed group of origin, and then, the forensic index was estimated by the algorithms described in materials and methods section. Likely, the R&M,

TABLE 2—*Comparison of the number (call rate in brackets) of successful genotyping for microsatellite (Short tandem repeat) and single-nucleotide polymorphism (SNP) sets obtained for each casework sample (CW).*

Casework	Sample	STR	SNP
CW_1	1	11 (61%)	15 (47%)
	2	10 (56%)	15 (47%)
CW_2	3	17 (94%)	32 (100%)
	4	15 (83%)	31 (97%)
	5	16 (89%)	31 (97%)
CW_3	6	12 (67%)	31 (97%)
	7	12 (67%)	31 (97%)
CW_4	8	11 (61%)	6 (19%)
	9	10 (56%)	14 (44%)
	10	9 (50%)	0 (0%)
	11	12 (67%)	31 (91%)
	12	9 (50%)	0 (0%)
	13	8 (44%)	0 (0%)
	14	18 (100%)	28 (87%)
	15	16 (89%)	32 (100%)
CW_5	16	12 (67%)	31 (97%)
	17	15 (83%)	31 (97%)
CW_6	18	12 (67%)	32 (100%)
	19	14 (78%)	32 (100%)
	20	15 (83%)	31 (97%)

B&L, and Paetkau algorithms exhibited a complete agreement in their results, and the casework sample number 20, the unique with known breed origin, was correctly allocated (details about breed assignment of forensic samples in supplementary materials, upon request). Furthermore, with the exception of casework sample number 19, which was assigned to Brangus breed, all samples were allocated to *Bos taurus* breeds instead of *B. indicus*. Reference and evidence profiles from casework 2 and 6 did not match, so further analyses were not carried out. The visual inspection of Table 3 showed that both type of marker sets presented similar values of forensic index. As expected, the forensic index values obtained using the Balding and Nichols' correction were more conservative (by a factor of 1–7) than values calculated using the rule product in a non-sub-structured population using the breed-assigned gene frequencies (Table 3). These differences were more significant in STR than SNP, maybe due to the presence of several STR rare alleles with low gene frequencies.

Discussion

The present study focused on the analyses of two main issues related with the routine work in genetic forensic laboratories: (i) the level of performance (sensitivity and repeatability) of SNP analysis in DNA purified from different biological sources, as well as from 20 forensic evidentiary samples from closed cases; and (ii) comparison of the forensic index and performance (call rate) provided by SNPs and STRs to resolve cases of cattle rustling.

Previous studies showed that for match comparison purposes (e.g., traceability and forensic analysis), between two and three SNPs per STR were needed to obtain an equivalent statistical power (exclusion power, 15,16). Match probability values showed that *c*. 25 SNPs are equivalent to the 12 dinucleotide STRs of the minimal set recommended by ISAG (MP *c*. 10^{-11}; e.g., 15,16,43). Our average call rate was slightly higher than that of 25-SNP limit value which was proposed by different authors to get enough discrimination power for bovine genetic identification (15,16) which is the case of cattle rustling. Therefore, more than 70% of the analyzed samples achieved this call rate value. The failed genotyping reactions were explained by both NON-AMP and UND data points. These failed matters could have resulted from different features of DNA samples (presence of inhibitors of amplification, DNA degradation, etc.).

Comparison between forensic casework and reference results showed that the obtained average call rate value for casework was lower than that obtained for reference samples, being under the proposed limit of 25 SNPs for genetic identification mentioned above. Interestingly, reference samples duplicated casework ones in the average percentage of UND (6.56% vs. 3.59%). These results could be a consequence of an excess of DNA rather than poor DNA quality in some reference samples. Regarding repeatability, the high degree of concordance between replicates obtained in the present work was similar to those reported by other authors (38,39,55), showing the reliability of the positive results obtained by the used technology.

The analysis of variables (assay design, DNA biological sources) that could affect the results showed a significant effect of the SNP assay design on the genotyping call rate. This could be consequence of probes (poor genotype cluster separation) or primers (lack of amplification) design errors. Furthermore, the lack of correlation between UND and NO-AMP among SNP assays is not unexpected due to UND and NO-AMP can be consequence of different matters: DNA quality, primers and probe design, polymorphism in primer sequence, unspecific binding of the TaqMan probe, etc. Considering that we used a custom microarray, call rates could be greatly increased by improving the design of primers and probes of the used SNP assays or replacing some of them. When performance of SNP typing using different DNA sources was compared, significant differences were observed. These differences between call rates obtained

TABLE 3—*Forensic index values obtained for each casework using microsatellite (Short tandem repeat) and single-nucleotide polymorphism (SNP) sets were detailed. This index was calculated considering three scenarios: S1. The forensic index was estimated considering the whole population gene frequencies with θ correction as reference population; S2. Casework samples were assigned to their putative breed group of origin, and its gene frequencies with θ correction were used to estimate the forensic index; and S3. Casework samples were assigned to their putative breed of origin, and its gene frequencies without θ corrections were used to calculate the forensic index.*

	STR			SNP		
Casework #	S1	S2	S3	S1	S2	S3
CW_1	2.19E-08	3.99E-08	8.23E-11	6.20E-03	2.36E-03	7.07E-04
CW_2		No match			No match	
CW_3	2.33E-11	1.14E-11	4.91E-17	1.04E-11	7.56E-12	1.37E-12
CW_4	1.67E-10	8.05E-10	4.14E-15	5.24E-11	1.64E-11	1.79E-15
CW_5	4.04E-10	5.38E-10	7.38E-15	1.00E-11	8.79E-13	4.05E-15
CW_6		No match			No match	

from the analyzed biological type of samples are in agreement with previous reports that also showed different performance among different type of samples (39,40,56). Yokoyama et al. (39) found that DNA extracted from canine saliva and blood samples performed equally well on microarrays SNP genotyping platform, with average call rates >99%. Some works reported that the performance of buccal swab DNA on SNP array genotyping was modest (39,56). This is probably due to the high level of microbial DNA contamination in DNA extraction (39,56). By contrast, Rincón et al. (40) obtained a call rate higher than 99% using buccal DNA. In conclusion, these articles showed that when homogeneous DNAs with high concentration and high-quality are available, it is possible to reach call rates closed to 100% using SNP microarrays platforms.

The demand for high concentration (at least 50 ng/μL) of high-quality DNA for using on microarray SNP platforms analysis creates challenges for traditional sample ascertainment. The required amount of DNA is far from the current sensitivity of the standard STR genotyping methods. This condition is possible to obtain in the cases of parentage and association analysis, where the quantity and quality of the sample can be managed. Unfortunately, in routine forensic work, laboratories can only work with the biological samples submitted from a crime scene. Forensic samples are usually purified from degraded biological samples and usually exhibit low quality and quantity. In the case of hair DNA, as was used in the present work, it is necessary to include a step of DNA purification to increase performance.

Finally, we compared the analyzed SNP set with a standard STR set in their ability to resolve cases involving cattle rustling. To do this, we evaluated their genotyping performance and identification power, finding that in 12 of 20 casework samples, SNP set presented higher performance than STRs. In cases of cattle rustling, the source of the population (breed origin) of biological evidence and reference is usually unknown. For this reason, we considered the hypothesis that the estimation of the forensic index between biological evidence and reference under livestock robbery context could be significantly dissimilar if different reference populations are considered (3). Following this line of reasoning, we first assigned each casework to a breed and breed group of origin, and then, the forensic index was estimated by R&M, B&L, and Paetkau assignment algorithms described in materials and methods section. Likely, these algorithms exhibited a complete agreement in their results. With the exception of casework sample number 19, which was assigned to Brangus breed, all samples were allocated to *Bos taurus* breeds instead of *B. indicus*. This is not unexpected because in the temperate Buenos Aires Province (Argentina), Holstein and British breeds, such as Angus and Hereford, are the predominant raised breeds.

Obtained results showed that both type of marker sets presented similar values of forensic index. As expected, the forensic index values obtained using the Balding and Nichols' correction were more conservative than those calculated through the rule product in a non-sub-structured population using the assigned breed gene frequencies. These results are in agreement with the fact that bovine, as well as other domestic animals, exhibits significant levels of population subdivision. In this sense, the analysis of molecular variance (AMOVA) with genetic markers showed that variance among bovine breeds accounts for more than 10% of the total genetic variation (3). Thus, subpopulation error is larger for strongly subdivided populations than for more homogeneous ones. This type of uncertainty has a more considerable effect in domestic animals than it does in humans. In other words, in livestock, Balding and Nichols' correction, when applied to the whole population, has a strong bias in favor of the defendant. By the other hand, the forensic index calculated using the simpler product estimator introduces an important bias in favor of the prosecution.

Conclusion

In the present work, we obtained an average call rate higher than 80% even that heterogeneous reference and casework samples were genotyped using a TaqMan real-time-based microarrays technology. Furthermore, our results made evidence that the used 32 SNP panel is as informative as the standard bovine dinucleotide STR set to resolve cattle rustling cases. We believe that the obtained performance could be increased improving the design of some SNP TaqMan assays, including DNA purification step in the case of hair samples, as well as a real-time algorithm instead of final point one during the analysis of the results to reduce the number of UND data.

Acknowledgment

The authors acknowledge Dr. Sree Kanthaswamy for suggestions and helpful comments about the manuscript.

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