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ESTIMATION OF GENETIC VARIABILITY PARAMETERS IN INDIAN HF CATTLE USING MICROSATELLITE MARKERS

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Abstract: The study was undertaken with an objective to estimate polymorphism of 12 microsatellite markers in Indian HF cattle. Allele frequencies, polymorphism information content, heterozygosity and exclusion probability were calculated. A panel of 12 microsatellite markers (BM1824, BM2113, INRA023, SPS115, TGLA122, TGLA126, TGLA227, ETH10, ETH225, BM1818, ETH3, TGLA53) was amplified in a single multiplex reaction and analyzed by capillary electrophoresis on an automated DNA sequencer. The expected heterozygosity ranged from 0.599 to 0.892 (mean 0.7734). The total exclusion probability using 12 microsatellite loci with 1 known parent was 0.9988. Nine out of 12 microsatellite loci revealed relatively high polymorphic information content (>0.7). The results of this study showed a relatively low pedigree error rate of 4.34%.

Keywords: Cattle, Microsatellites, Multiplex Polymerase Chain Reaction, Fragment Analysis, Parentage Verification.

Introduction

The knowledge of correct parentage is a prerequisite in breeding programmes. Failure to record correct parentage can cause bias in sire evaluation, by introducing errors in estimates of heritabilities and breeding values. Misidentification reduces genetic gain with sire models (Gelderman et al., 1986) and may have an even greater effect with animal models that account for all assumed genetic relationships (Wiggans et al., 1988). Accurate pedigree information is essential to maintaining the quality of breed improvement programs and molecular markers have become an important genetic tool in animal genetics studies, allowing the analysis of genetic variability within and between herds. Microsatellites markers have been widely used as genetic markers in bovine population studies and pedigree verification (Visscher et al. 2002. Microsatellites have been effective in evaluating differences within cattle breeds and in determining population substructures (Ciampolini et al., 1995). More than 1400 microsatellites have been mapped in the cattle genome (Luikart et al., 1999) and some of them have been employed in population genetics studies and parentage verification. Many microsatellite loci have been used in cattle improvement programs

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worldwide. However, to date, there have been no reports of pedigree verification studies using microsatellite markers in Indian HF cattle population.

The use of DNA technology has opened a new possibility for developing more sophisticated and more accurate methods that are based on DNA analysis. Most informative and most commonly used are the microsatellite markers (Short Tandem Repeats) which are highly polymorphic and are located on the noncoding intron regions of the bovine genome. The advantage of microsatellite based tests is that theoretically any sample containing nuclear DNA can be used for analysis, and when genotyping recommended set of markers, the accuracy of the test is much higher as the probability of detecting mistaken parentage is a direct function of the polymorphism of the markers used. The aim of present study was to characterize Indian HF cattle through the analysis of the genetic variability of 12 microsatellite markers and to evaluate informativeness of these markers in parentage analysis.

Materials and Methods

Sample collection: Experimental material for the present study comprised of 96 samples of cattle (46 dams, 46 daughters, and 4 sires) of field progeny testing program conducted by National Dairy Development Board. Semen samples were collected from the bulls used in the program. The blood samples were collected in EDTA vaccutainer tubes. Minimum 5 ml. blood volume of each sample was collected and properly labelled to avoid misidentification of the sample. Calf and Dam samples were packed together for easy identification of the sample pairs. 5 Frozen semen doses of each individual sire were kept together to avoid misplacing or mixing with the doses of other sires. All the samples were carried in insulated box with cooled gel pack to maintain the low temperature during transport.

Extraction of DNA from Blood and Semen: The genomic DNA was isolated from blood samples using QIAamp Kits (QIAGEN, USA) while DNA from semen samples was extracted using QIAamp® DNA Investigator Kit (QIAGEN, USA) following manufacturer's instructions. The quantity and quality of DNA was checked by spectrophotometer (Biospec Nano) and agarose gel electrophoresis respectively.

Multiplex PCR amplification: ISAG has recommended use of minimum 12 markers for routine verification of cattle pedigrees. We used nine microsatellites recognized as "international marker set" which need to be included in parentage panels for verification of cattle pedigrees along with 3 additional set of markers (Table-1).

Table 1: Microsatellite Markers

Locus		Primer Sequence (5'-3')	Dye	Primer range
BM1824	F	GAG CAA GGT GTT TTT CCA ATC	NED	170-218
	R	CAT TCT CCA ACT GCT TCC TTG		
BM2113	F	GCT GCC TTC TAC CAA ATA CCC	6 FAM	116-46
	R	CTT CCT GAG AGA AGC AAC ACC		
INRA023	F	GAG TAG AGC TAC AAG ATA AAC TTC	6 FAM	194-236
	R	TAA CTA CAG GGT GTT AGA TGA ACT C		
SPS115	F	AAA GTG ACA CAA CAG CTT CTC CAG	6 FAM	240-270
	R	AAC GAG TGT CCT AGT TTG GCT GTG		
TGLA122	F	CCC TCC TCC AGG TAA ATC AGC	VIC	133-193
	R	AAT CAC ATG GCA AAT AAG TAC ATA		
TGLA126	F	CTA ATT TAG AAT GAG AGA GGC TTC T	VIC	104-132
	R	TTG GTC TCT ATT CTC TGA ATA TTC C		
TGLA227	F	CGA ATT CCA AAT CTG TTA ATT TGC T	6 FAM	63-115
	R	ACA GAC AGA AAC TCA ATG AAA GCA		
ETH10	F	GTT CAG GAC TGG CCC TGC TAA CA	6 FAM	198-234
	R	CCT CCA GCC CAC TTT CTC TTC TC		
ETH225	F	GAT CAC CTT GCC ACT ATT TCC T	6 FAM	132-166
	R	ACA TGA CAG CCA GCT GCT ACT		
BM1818	F	AGCTGGGAATATAACCAAAGG	VIC	248-276
	R	AGTGCTTTCAAGGTCCATGC		
ETH3	F	GAACCTGCCTCTCCTGCATTGG	NED	89-131
	R	ACTCTGCCTGTGGCCAAGTAGG		
TGLA53	F	GCTTTCAGAAATAGTTTGCATTCA	6 FAM	147-197
	R	ATCTTCACATGATATTACAGCAGA		

Custom tailed oligos synthesized at Invitrogen (USA) were utilized in the present study. The 5′-end of the forward primer was labeled with one of the 3 fluorescent dyes: Carboxyfluorescein (FAM), Carboxyhexachlorofluorescein (HEX) andVIC. Oligos supplied in freeze-dried powder form were reconstituted in milliQ water to the volume (µl) equivalent to the mass (µg) of primer and further diluted in MiliQ water to give a final concentration 10

pmoles/µl. Various combinations of primers and DNA were tested in a final volume of 15 µl containing 2x Multiplex PCR master mix (Qiagen, USA) 10 pmole of each primer and 60-90 ng of DNA template. Amplification was performed in Master Cycler gradient thermocycler (Applied Biosystems Veriti, USA) with the following cycling conditions: after an initial denaturation at 95°C for 15 minutes, 38 cycles were programmed as follows:95°C for 30 seconds, 57°C for 90 seconds, 72°C for 60 seconds and final extension at 72°C for 30 minutes.

Fragment Analysis: The PCR product was diluted 8 times to obtain optimum peak height. Each 1µl of PCR product was mixed with 0.3 µl of size standard fluorescent dye GS Liz 500 (PE- Applied Biosystems) and finally made the volume up to 10 µl with Hi-Di formamide. Samples were denatured for 5 min at 95 °C and snap chilled on ice for 5 minutes before being run on ABI-3500 XL genetic analyzer. The reaction mix of PCR product was prepared

Results and Discussion

Allele Frequency Analysis using Cervus 3.0: The number of alleles per locus (NA) varied from 8 (TGLA126) to 21 (TGLA122). The mean number of alleles across 12 loci was 12.667. The expected heterozygosity (He) ranged from 0.599 (SPS115) to 0.892 (TGLA122). Among the tested 12 loci, BM1824, BM2113, INRA023, TGLA122, TGLA227, ETH10, ETH225, BM1818, TGLA53 showed higher polymorphism with PIC values higher than 0.7. These estimations were generally similar to those reported by Herráez et al. (2005), Rahimi et al. (2006), Řehout et al. (2006) and Ozkan et al. (2009). Exclusion probability value was greatest for marker SPS115 (0.790) and least for marker TGLA122 (0.363). Combined EP for the selected 12 markers was 0.9988 indicating parentage assignments with 99% of confidence. The cumulative exclusion probability is a measure of the ability of a certain panel of marker to identify genetic paternity, excluding all other candidates. The high genetic variability of markers implied their high effectiveness for parentage testing.

Table: 2 Allele Frequency Analysis of 12 microsatellite markers

Locus	(k)	(He)	(Ho)	(PIC)	(EP)
BM1818	11	0.729	0.744	0.702	0.652
BM1824	19	0.646	0.776	0.751	0.585
BM2113	13	0.844	0.801	0.769	0.57
ETH10	9	0.853	0.802	0.773	0.559
ETH225	10	0.723	0.785	0.748	0.6

ETH3	9	0.663	0.696	0.666	0.693
INRA23	10	0.726	0.785	0.749	0.598
SPS115	9	0.674	0.599	0.567	0.79
TGLA122	18	0.874	0.892	0.878	0.363
TGLA126	8	0.621	0.671	0.61	0.748
TGLA227	21	0.915	0.886	0.87	0.381
TGLA53	15	0.906	0.844	0.825	0.469

Where, (k): Number of alleles at the locus, (Ho): Observed heterozygosity, (He): Expected heterozygosity, PIC: Polymorphic information content, (EP): Exclsuion Probability.

Manual Parentage Verification: After allele scoring and genotyping of each individual, the results obtained were confirmed manually by matching samples of each daughter with her respective dam and sire. A mismatch at more than 2 locus was interpreted as wrong parentage. A confirmed match at all loci was considered as true parentage. Parentage was verified for each daughter likewise by comparing daughter dam–sire trio and marked as correct or wrong. We found 2 daughters having wrong parentage out of 46 daughters using the above procedure.

Accurate cattle pedigree information is essential for the optimal development of breed and selection programs, improving productivity in the animal industry. Misidentification of parentage can lead to breeding inaccuracy, causing great financial losses in herd management and in the beef industry. Microsatellites are the most widely used molecular markers in pedigree control. The use of microsatellites with high polymorphism information content would help to correctly identify individual cattle, allowing for the better operation of cattle breeding programs.

It is important to note that no study has been undertaken to evaluate efficacy of microsatellite markers for parentage verification in Indian HF cattle. Our objective of this study was to study polymorphism of 12 microsatellite markers and their efficacy for using in parentage analysis of HF cattle. Microsatellites suitable for this purpose are those showing multiple alleles as well as high heterozygosity. We used nine microsatellites recognized as “international marker set” in parentage panel for verification of cattle pedigrees along with 3 additional set of markers. The results of this study showed a relatively low pedigree error rate of 4.34%. Christensen et al. (1982) reported misidentification rates between 5 and 15% in Danish dairy cattle, Geldermann et al. (1986) estimated misidentification rates of 13% using blood group factors and biochemical polymorphisms in cattle. Ron et al. (1996) found a 5%

misidentification rate using microsatellite analysis in Israeli dairy cattle. Rosa (1997) reported a misidentification rate of 15% in Brazilian livestock, based on restriction fragment length polymorphism (RFLP) and microsatellite analysis.

Conclusion

The study shows that the microsatellite markers are highly polymorphic which makes them genetic markers of choice for paternity evaluation. A multiplex microsatellite panel consisting of 12 loci has been successfully evaluated. The use of this multiplex analysis proved efficient in characterization of HF cattle and can be used in pedigree verification. It is a fast, robust, reliable, and economic tool to verify the parentage as well as to assign the putative sire to daughters under progeny testing program with very high accuracy.

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