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Allelic diversity and forensic performance of a multiplex set of 11 microsatellite markers in Nelore and Gir cattle populations (*Bos indicus*)

DNA typing with microsatellite markers is rapidly substituting blood typing and has become the international standard for individual identification and determination of paternity and maternity relationships in bovine populations. Brazil, however, only now is starting to adopt this technology. A number of advantages derive from the use of DNA markers including (1) the much higher power of discrimination and exclusion and in paternity testing especially when testing does not involve the cow; (2) the possibility of declaring paternity even in situations of known common ancestry; (3) the utility of the DNA data for population and mating management and (4) the possibility of typing from various tissues other than blood. We have optimized the amplification and three-color fluorescent detection of 11 dinucleotide microsatellite markers in two PCR reactions for bovine high throughput genetic fingerprinting. The multiplex system includes all nine internationally recommended loci for bovine typing by the International Society of Animal Genetics: BM1824, BM2113, ETH10, ETH225, SPS115, TGLA122, TGLA227, TGLA126, INRA23, plus two additional loci ETH3, TGLA53 and the sex determining locus Amelogenin. We have typed these loci in proficiency ring trials run annually by the International Society of Animal Genetics. Based on ISAG confirmed test samples we constructed virtual allelic ladders using the ABI Genotyper software to allow precise allelic declaration in conformity to the ISAG standards. Allele frequency data have been collected from 96 genetically unrelated animals for each one of the two *Bos indicus* races, Nelore and Gir. Private low frequency (<5%) alleles were observed at all loci, most of them therefore subject to sampling effects. Interestingly however, a combination of two relatively higher frequency private alleles at loci SPS115 and INRA23 were observed in Nelore that could allow the estimation of a likelihood of an animal belonging to this race. No such alleles were observed in Gir. In Nelore, with the exception of locus INRA23, at all other markers the observed heterozygosity was lower than the expected one indicating a significant level of inbreeding. In Gir on the other hand, with the exception of loci TGLA227 and TGLA126, at all other loci the observed heterozygosity was slightly higher than the expected one. Accordingly, multilocus inbreeding coefficients were significantly different from zero in Nelore ($F_{is}=0.11$) (confidence interval at 95% by “bootstrap”) and not significantly different from zero in Gir ($F_{is}= -0.008$). In 37 out of 55 tests, significant linkage disequilibrium ($p<0.05$) was detected. In Nelore, power of paternity exclusion (PE) for the loci varied from a lowest 2.3% for locus TGLA227 in Gir (TGLA227 is essentially fixed for allele 77 in *Bos indicus*) to a highest 68,4% for TGLA122 in Nelore. Power of Discrimination (PD) varied from 0.31 for TGLA227 to 0.96 for TGLA122. For the large majority of markers loci PE was between 30 and 50%. The 11 loci combined yielded a PE above 99% in both races. This multiplex system is routinely used in our laboratory for efficient bovine typing and parentage confirmation required in elite animal registration. ■