Development of Standard DNA Size Markers for Short Tandem Repeat Loci BM1818 and BM1824 of Cattle DNA Typing in Sri Lanka

N.W.N.P. Nugapola, P.B.A.I.K. Bulumulla, T.S.R. Fernando
Uva Wellassa University, Badulla, Sri Lanka

and

R.J. Illeperuma
Genetech, Molecular Diagnostics, No.54, Kitulwatte road, Colombo 08

Introduction

Cattle are the most common type of livestock animal raised for meat, dairy, as draft animals and various other purposes. Sri Lankan cattle population mainly consist of indigenous cattle "*Sinhala batu* cattle", European pure breeds (Jersey, Friesian, Ayrshire), Indian cattle breeds (Sahiwal, Sindhi, Tharparkar), and their crosses. Identification and traceability of cattle and their products is extremely important for proven of ownership, exploration of pedigree and animal disease control. Traditional identification methods are less effective and associated with many economic, cultural and ethical concerns and are not reliable methods of establishing maternity or paternity of cattle. Deoxyribo Nucleic Acid (DNA) based identification is based on the unique, unalterable, inherited DNA profile of an individual animal as an identifier. Testing the Short Tandem Repeats (STR) of DNA in establishing the identity and parentage is widely used for humans in Sri Lanka. However DNA based identity testing methods are needed to be developed for cattle in order to use it as an accurate method than conventional methods to address problems of cattle identity and traceability. Currently there is no proper procedure of DNA based of identification in Sri Lanka due to the unavailability of standard DNA size markers and there is an urgent need of developing standard DNA size markers for cattle STR (allelic ladders) for accurate genotyping of an individual. Therefore the present study was conducted to develop standard DNA size markers for STR loci BM1818 and BM1824.

Methodology

Selection of loci

Two bovine specific STR markers (BM1818, BM1824) recommended by the Food and Agriculture Organization (FAO) and the International Society for Animal Genetics (ISAG) (FAO, 2011) and previous studies done by Goor and Van de (2011) were selected for this study.

Sample collection and DNA extraction

Blood samples (n=38) and tissue samples (n=12) were randomly collected from unrelated animals from different areas of the country according to the recommendations provided by FAO (FAO, 2011). DNA extraction performed by Chelex® 100 extraction protocol described by Phillips (2009) with minor modifications. DNA from tissue samples was extracted by ChargeSwitch® Forensic DNA Purification Kit (Invitrogen™, Life Technologies Corporation) according to the kit's manual.

PCR amplification

PCR amplification of extracted DNA samples was carried out with 5 µL of genomic DNA in a 50 µL reaction volume, consisted of 5 µL of 10X STR buffer (DreamTaq Green Buffer - Thermo Scientific), 5 µL of each dNTP (Guangzhou Geneshun Biotech Ltd.), 5 µL of primer (FAO, 2011 and Goor and Van de 2011) (Integrated DNA Technology), 0.30 µL of Taq DNA polymerase (DreamTaq-Thermo scientific) and 30 µL of sterilized distilled water. The PCR reaction was carried out in a Veriti 96 well thermal cycler (Applied Biosystem, USA) under the following PCR cycling conditions (Sodhi *et al.*, 2006): 2 min at 94 °C, followed by 30 cycles of 1 min at 94 °C, 1 min at annealing temperature (56 to 62 °C) of each primer, 1 min at 72 °C, and final extension of 10 min at 72 °C. Subsequent to PCR, the products were subjected to 3 % w/v agarose gel electrophoresis to verify the success of the PCR reaction. Then the PCR products were subjected to 7 % denaturing polyacrylamide gel electrophoresis. Amplified products were detected using DNA Silver Staining procedure described by Bassam *et al*. (1991) and Promega technical manual.

Allelic ladder construction

Allelic ladders were constructed by two methods as follows (1) DNA samples that can contribute to maximum number of polymorphic alleles to each locus were selected, pooled and amplified by PCR reaction (100 µL reaction volume), (2) PCR products that can contribute to maximum number of polymorphic alleles to each locus were selected and pooled to create the allelic ladders.

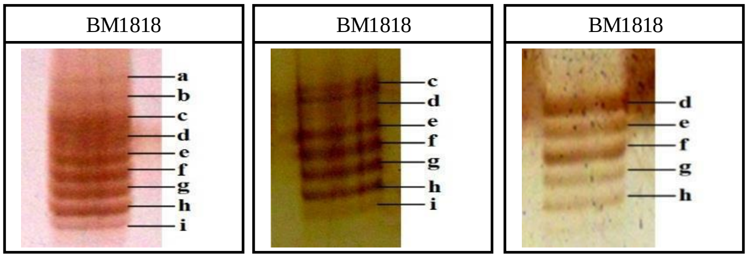
Results and Discussion

According to the Table 1, allelic range of the BM1818 locus was from allele 11 to 21. The highest frequency was shown by allele No.18 at a percentage of 35.0 percent. Allele 11 had the lowest frequency with 1.3 percent. When considering BM1824 locus, allelic range was from allele 11 to 19. The highest frequency was shown by allele No.13 at a percentage of 34.1 percent. Two alleles (11 and 18) showed the lowest allelic frequency of 1.2 percent.

Table 1. Allelic frequencies of BM1818 and BM1824 allelic ladders

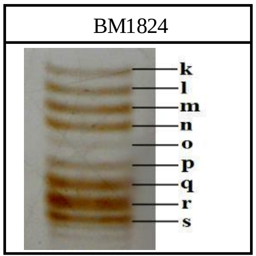
	Frequency	
Allele	BM1818	BM1824
11	1.3%	1.2%
12	0.0%	12.2%
13	2.5%	34.1%
14	6.3%	25.6%
15	3.8%	0.0%
16	12.5%	0.0%
17	23.8%	22.0%
18	35.0%	1.2%
19	6.3%	3.7%
20	3.8%	0.0%
21	3.8%	0.0%
22	0.0%	0.0%
23	0.0%	0.0%

Nine common alleles form the BM1818 locus and eight common alleles form the BM1824 locus were pooled to construct allelic ladders (shown in Figure 1 and Figure 2 respectively).



A=PCR products pooled **B**= PCR products pooled **C=**DNA pooled

Figure 1. BM1818 Allelic ladders



D=DNA pooled

Figure 2. BM1824 Allelic ladder

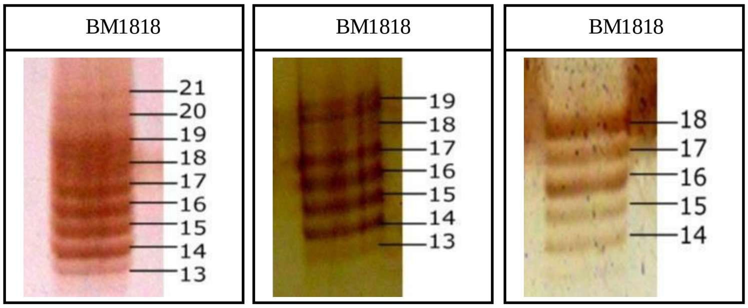
Allele labeled as "d" in BM1818 locus (Figure 1 – C) and allele labeled as "p" in BM1824 locus (Figure 2) were isolated and sequenced to determine the number of repeating units at each locus. According to the sequence results, allele "d" have 18 "TG" tandemly repeating units (Figure 3), therefore it was identified as allele 18 of the BM1818 ladder. And allele "p" has the 14 "GT" tandemly repeating units (Figure 4); therefore it was identified as allele 14 of the BM1824 ladder.

AAAGATACCTGCACCCCTATGTTCATAGCAGCATTATTTATACTAGCCAAGCAAGCCATGGAA
ACCGCACCTAAGTTATCTCCATTCATCAAGGGATGAATGGAGAAATTGTGTGTGTGTGTGTGT
GTGTGTGTGTGTGTGTGTGTAAGGGGGCAGCTTTGAACATAGGGGTGCAGGTATTTTTTTGAG
TGTTTGTTTTAGTTTCCTGTATTTATATTGATATAA

Figure 3. Sequence of the allele “d” of the BM1818 locus (18 TG repeating units are highlighted)

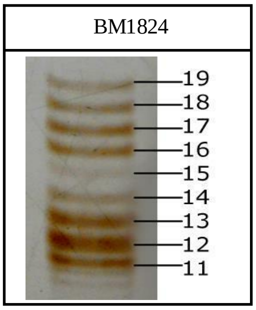
TTGAGCAAGGTGTTTTTCCAATCTAACTTTCTGTGTGTGTGTGTGTGTGTGTGTGTGTGTTAGT
TGCTTAGTCATGTCACTTAGCCAAATTTCCAAAAAAGTGTGAGTAGGAATGAAAACTTATTTT
AATATTCATGTCGGCGGCGTACCAGTCGATGTCCTC

Figure 4. Sequence of the allele “p” of the BM1824 locus (14 GT repeating units are highlighted)



A=PCR products pooled **B**= PCR products pooled **C**=DNA pooled

Figure 5: BM1818 Allelic ladders



D=DNA pooled

Figure 6: BM1824 Allelic ladder

After the determination of the accurate number of repeating units of alleles, BM1818 ladder (Figure 5) and BM1824 ladders (Figure 6) were labeled.

Conclusions

Two DNA size markers (BM1818 & BM1824) for cattle STRs were created and allelic frequencies of the two STR loci were also determined. Newly constructed DNA size markers (allelic ladders) were successfully applied in cattle maternity tests and the results of these STR analyses have been accepted by courts.

References

Bassam, B.J., Caetano-Anolles, G., Gresshoff, P.M. 1991. Fast and sensitive silver staining of DNA in polyacrylamide gels. Analytical Biochemistry 196, 80-83.

FAO. 2011. Molecular genetic characterization of animal genetic resources. FAO Animal Production and Health Guidelines. No. 9. Rome.

Goor, L.H.P. van de, 2011. Bovine and Equine forensic DNA Analysis. Wageningen: Utrecht University.

Phillips, S.M., 2009. A comparative study of DNA extraction methodologies: Variation in DNA yield and effects on downstream PCR analysis. Master of Science. Boston University School of Medicine.

Sodhi, M., Mukesh, M., Prakash, B., Ahlawat, S.P.S., Sobti, R.C., 2006. Microsatellite DNA typing for assessment of genetic variability in Tharparkar breed of Indian zebu (*Bos indicus*) cattle, a major breed of Rajasthan. Journal of Genetics, 85(3), 165–170.