CIBTech Journal of Biotechnology ISSN: 2319-3859 (Online)
An Online International Journal Available at http://www.cibtech.org/cjb.htm
2012 Vol. 2 (1) January-March, pp.34-37/Thiagarajan
Research Article

ALLELES AND THEIR FREQUENCIES FOR EIGHT MICROSATELLITES IN ONGOLE BREED OF CATTLE

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ABSTRACT
Eight microsatellite primers developed from genomic library of N'Dama bull were used in 50 DNA samples of Ongole cattle. The microsatellite primers were ILSTS 005, ILSTS 006, ILSTS008, ILSTS 010, ILSTS 011, ILSTS 012, ILSTS 013 and ILSTS 014. The microsatellite loci were found to be highly polymorphic and the number of alleles were found to be 15, 17, 13, 11, 11, 9,7 and 7 respectively and are comparable with alleles found in n N'Dama , Friesian, Boran and Zebu cattle. The level of polymorphism displayed by the eight microsatellites could be useful in fixing breed specific alleles for genetic characterization of Indian zebu breeds. The accuracy in genetic analysis and measurement of variability could be further improved by employing more number of microsatellite loci for genetic analysis in this breed.

Key Words: *Microsatellite Primers, Alleles, Polymorphism, Characterization and Ongole*

INTRODUCTION
It was estimated that about 800 types of breeds of cattle existed which differ in their capacities as draught animals or producers of meat or milk. At initial stages, strong emphasis was placed on productivity that favored prevalence of few breeds. Only a limited number of breeds were considered for economical rearing which in turn favored increased exchange of breeding stocks among regions. Reduction of diversity has been felt recently, primarily in terms of loss of breeds and strains. It is only since 1980s that concerted conservation efforts have really been made to preserve the genetic diversity of cattle (FAO, 1981). As variation is the main tool for geneticists for future genetic experiments, breeds should be chosen in order to cover the widest range of variability. In this study, we are concerned with Ongole breed because of its ability for heavy draft work. There are many different sources of data relevant to genetic differences between breeds. However, concurrently with the development of RFLP and RAPD, another class of DNA polymorphism was characterized based on tandem arrays of repeated sequences. Tandem sequence repetition is widespread in eukaryotic genomes and many types of repeat motifs have been described (Payne, 1997). Sub classes of repetitive loci were described with repeat units of two to six base pairs and were entitled as microsatellites or Short Tandem Repeats (STRs). Microsatellites often have multiple alleles and may have heterozygosity frequencies of 70 per cent or more. This makes them highly informative for genetic analysis. Keeping the back ground information in mind, this study was undertaken to identify the DNA markers in Ongole cattle and to characterize the Ongole cattle by developed DNA markers.

MATERIALS AND METHODS
Primers
A total of eight microsatellite primers were used for microsatellite analysis (Table 1). These microsatellites were originally developed by Brezinsky *et al.,* (1993a,b,c) and Kemp *et al.,* (1993) from the genomic library of N' Dama (*Bos taurus*) bull. The primers were reconstituted in TE with a

concentration of 40 n moles/ml as stock solution and 40 p moles/ml as working solution. All primers were tested with similar conditions on tested DNA samples.

Genomic DNA

High molecular weight template was prepared from peripheral blood mononuclear cells collected from 50 Ongole cattle maintained at University Research Farm, Madhavaram, Tamil Nadu Veterinary and Animal Sciences University and from farmers herd in and around Thirupati. The DNA samples were dissolved in TE buffer (pH8.0) to make uniform concentration of 50 ng/ml.

PCR Amplification

The amplification reactions were carried out in 0.2 ml microfuge tubes using a programmable thermal cycler (MJ Research). Each 20µl reaction mix comprised of primers each at 20mMoles, DNTPs each at 50mMoles, 0.5 units of Taq DNA polymerase, 200ng template genomic DNA, 10mM Tris (pH8.3), 50mM KCl, 0.001 Nonidet P40 and 1.5mM $MgCl_2$.

Table 1: Nucleotide sequence of microsatellite primers

Sl.No	Code	Nucleotide sequence
1.	ILSTS 005	5’ GGAAGCAATGAAATCTATACC 3’ 5’ TGTTCTGTGAGTTTGTAAGC3’
2.	ILSTS 006	5’ TGTCTGTATTTCTGCTGTGG 3’ 5’ ACACGGAAGCGATCTAAACG 3’
3.	ILSTS 008	5’ AGCACCTGCTGCATACTACC 3’ 5’ GAATCAGTGTCAGTGTTTCCC 3’
4.	ILSTS 010	5’ ATGGAGAGCAAATGGTCAGC 3’ 5’ ACTACAATGGACATGAGTCCG 3’
5.	ILSTS 011	5’ GCTTGCTACATGGAAAGTGC 3’ 5’ CTAAAATGCAGAGCCCTACC 3’
6.	ILSTS 012	5’ TCTACCACCGATACAGATGG 3’ 5’ GAAGTAGGTAGTGCTGGAGG 3’
7.	ILSTS 013	5’ CTTGATCCTTATAGAACCTGG 3’ 5’ ACACAAAATCAGATCAGT 3’
8.	ILSTS 014	5’ CTGACTATGGTGATAATCCC 3’ 5’ TCTTTTCCCTTTCCTTCCCC 3’

Thermal Cycling

ILSTS00 and ILSTS 006:

Amplification included an initial denaturing step of 45 seconds at 94ºC. This was followed by 19 cycles of 45 seconds at 89ºC, 1 min. at the annealing temperature of 55ºC and 20 seconds at 70ºC. Final extension was for 3 min. at 70ºC.

ILSTS 008:

Amplification reactions were carried out as same as that of ILSTS 005 and ILSTS 006 but the annealing temperature was 58ºC.

ILSTS 010, ILSTS 011, ILSTS012, ILSTS 013 and ILSTS 014:
Amplification reactions were carried out as same as that of ILSTS 008 but the temperature cycling after initial denaturation was 24 times instead of 19 times.

Analysis of PCR Products
The PCR products were made to run on a 6 per cent Poly Acrylamide gel electrophoresis together with100 bp DNA ladder at 100 volts for 90 min. The gel was stained by ethidium bromide for 10 min. and viewed by UV illumination. The alleles were photographed and were analysed by software aided gel documentation system (comprised of Ultra-lum scanner, Scion image capturing system and Sigma gel package).

RESULTS AND DISCUSSION

DNA samples of Ongole cattle were amplified with eight microsatellite primers and the products were analysed for alleles. The alleles and their frequencies scored in Ongole cattle for 8 different primers are depicted in Table 2.

Table 2: Alleles and their frequencies for different microsatellite primers

ILSTS005		ILSTS006		ILSTS008		ILSTS010		ILSTS011		ILSTS012		ILSTS013		ILSTS014	
*	**	*	**	*	**	*	**	*	**	*	**	*	**	*	**
185	0.03	183	0.03	110	0.05	305	0.05	312	0.15	90	0.03	142	0.33	151	0.15
188	0.05	195	0.03	112	0.05	333	0.10	324	0.08	92	0.15	143	0.03	154	0.15
193	0.05	207	0.03	115	0.08	344	0.08	326	0.10	96	0.08	145	0.15	158	0.20
201	0.03	213	0.05	120	0.05	355	0.10	328	0.08	98	0.18	160	0.15	160	0.05
203	0.05	224	0.08	123	0.10	366	0.05	329	0.13	101	0.05	162	0.13	162	0.13
207	0.05	237	0.05	128	0.05	377	0.03	346	0.08	104	0.05	163	0.15	163	0.18
212	0.085	248	0.05	131	0.10	383	0.15	348	0.05	106	0.20	164	0.08	166	0.15
215	0.13	255	0.08	136	0.13	389	0.08	349	0.05	110	0.15				
218	0.10	257	0.08	139	0.08	405	0.08	350	0.08	112	0.13				
220	0.05	284	0.08	142	0.13	427	0.13	352	0.13						
221	0.05	285	0.08	145	0.05	439	0.18	354	0.10						
227	0.05	299	0.05	153	0.05										
226	0.10	309	0.05	158	0.05										
240	0.10	315	0.05												
246	0.13	349	0.05												
354	0.05														
363	0.05														

******* *Indicates allele size in bp and* ******** *indicates allele frequency*

Discussion
DNA samples of Ongole breed of cattle were amplified with ILSTS 005 and the product gave polymorphism with allele sizes varying from185 to 246. The allele frequencies ranged from 0.03 to 0.13. Out of 15 alleles observed, alleles 215 and 246 were more frequently detected in 50 Ongole animals. Karthickeyan *et al.*, (2008) used ILSTS 005 and observed 4 alleles viz, 182, 186, 190 and 194 in the same breed of cattle. Brezinsky *et al.*, (1993b) used ILSTS 005 and observed 4 alleles viz. 181, 183, 185 and 193 in N'Dama (5 animals) and Friesian(5 animals) cattle. They considered only full sib animals and hence the limited number of alleles. In the present study numbers of alleles were more because the samples collected were from unrelated animals in a large population distributed in a wider area.
The products after amplification with ILSTS 006 revealed polymorphism with allele sizes varying from 183 to 360. The observed allele frequencies ranged from 0.03 to 0.13. In this locus, frequency of allele

207 was more than any other allele in the population. Karthickeyan *et al.,* (2008) observed 4 alleles viz, 290, 292, 296 and 300 in with the same primer. Brezinsky *et al.,* (1993c) observed 7 alleles viz. 290, 292,294,296,298, 300 and 304 in N'Dama, Friesian, Boran and Zebu cattle.

DNA samples amplified with ILSTS 008 and the resultant product gave allele sizes ranged from 110 to 158 with allelic frequencies 0.05 to 0.13. Alleles 123, 131, 136 and 142 appeared frequently. These results were comparable with similar work carried out by Kemp *et al.,* (1993) with observed alleles such as 173, 175, 179 and 181 in 5 animals each of N'Dama, Friesian, Boran and Zebu breeds.

ILSTS 010 primer product showed polymorphism with allele size varying from 305 to 439 with allele frequencies ranging from 0.03 to 0.18. These results were comparable with similar work carried out by Brezinsky *et al.,* (1993a) in 5 animals each of N'Dama, Friesian, Boran and Zebu breeds and the observed allele sizes 281, 287 and 291.

Products of ILSTS 011 gave alleles from 312 to 354 with allele frequencies 0.05 to 0.15. Alleles 312, 329 and 352 were frequently detected in this breed. Similar reports were reported by Karthickeyan *et al.,* (2008) for ongole breed in different population. They observed allele sizes of 262, 268 and 274.

ILSTS 012 primed product of Ongole cattle revealed alleles such as 90, 92, 96, 98, 101, 104, 106, 110 and 112 with corresponding allele frequencies of 0.03, 0.15, 0.08, 0.18, 0.05, 0.05, 0.20, 0.15 and 0.13.Alleles 106 and 98 were more frequently detected in this breed group Brezinsky *et al.,* (1993a) found that alleles 85, 95, 97 and 99 were detected.

Products of ILSTS 013 showed polymorphism with allele sizes ranging from 142 to 164 with allele frequencies from 0.03 to 0.33. Alleles 142, 145, 160 and 163 were abundant in this breed. Brezinsky *et al.,* (1993a) observed alleles of microsatellite locus ILSTS 013 in N'Dama, Friesian, Boran and Zebu breed of South Africa as 121, 123, 125, 127 and 129.

DNA samples of cattle were amplified with ILSTS 014 and the products showed polymorphism. The allele sizes scored were 151, 154, 158, 160, 162, 163 and 166. Alleles 158 and 163 were predominant in this breed. Brezinsky *et al.,* (1993a) observed alleles of microsatellite locus ILSTS 014 in N'Dama, Friesian, Boran and Zebu breed of South Africa as 129, 131 and 133. They also observed Mendelian inheritance of the allele's 131 and 133 in 8 full siblings in N'Dama and Boran calves.

The level of polymorphism displayed by eight microsatellites in the Ongole breed could be useful in fixing breed specific alleles for genetic characterization of Indian Zebu breeds. The accuracy in genetic analysis and measurement of variability could be further improved by employing more number of microsatellite loci for genetic analysis in this breed.

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