MOLECULAR GENETIC CHARACTERIZATION AND GENETIC DIVERSITY ANALYSIS OF SANGAMNERI BREED OF GOAT USING MICROSATELLITE MARKERS

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MASTER OF VETERINARY SCIENCE

IN

ANIMAL GENETICS AND BREEDING

BY

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I hereby declare that the experimental research work and interpretation of the thesis entitled **"MOLECULAR GENETIC CHARACTERIZATION AND GENETIC DIVERSITY ANALYSIS OF SANGAMNERI BREED OF GOAT USING MICROSATELLITE MARKERS"** or part thereof has not been submitted for any other degree or diploma of any University, nor the data have been derived from any thesis/publication of any University or scientific organization. The sources of materials used and all assistance received during the course of investigation have been duly acknowledged.

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Dedicated to

This work is dedicated
to my inspiration,
my friend, my guide, my
father Mr. Bhuban Chandra Nath
and my mother Arati Nath
and my elder sis Jaan & Pinky.

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(Sapna Nath)

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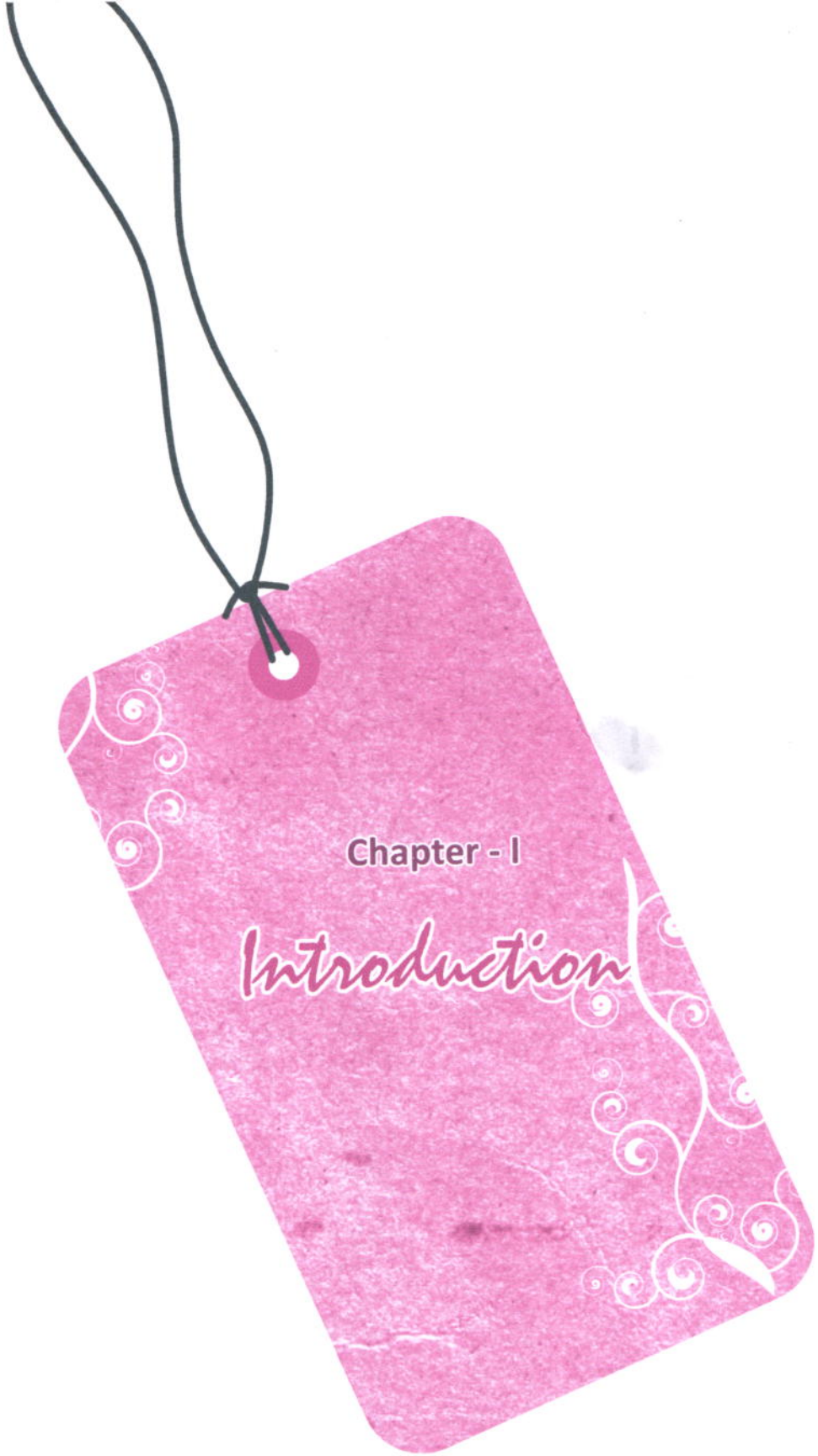
LIST OF ABBREVIATIONS

AFLP	Amplified Fragment Length Polymorphism
APS	Ammonium persulfate
bp	Base pair
dATP	Deoxyadenosine triphosphate
dCTP	Deoxycytosine triphosphate
dGTP	Deoxyguanosine triphosphate
dNTPs	Deoxynucleoside triphosphate
dTTP	Deoxytyrosine triphosphate
EDTA	Ethylene-diamine-tetraacetic acid
FAO	Food and Agricultural Organization
g	gram(s)
h	hour (s)
I	Shannon's Information index
IAM	Infinite Allele Model
ISAG	International Society of Animal Genetics
M	Molar
mA	mili Ampere
mg	milligram (s)
min	minute (s)
ml	milliliter (s)
mM	milimolar
ng	nanogram (s)
OD	Optical density
PAGE	Polyacrylamide gel electrophoresis
PCR	Polymerase Chain Reaction
PIC	Polymorphic Information content
pmole	Picomole(s)
RAPD	Randomly Amplified Polymorphic DNA
RBC	Red Blood Cell
RE	Restriction endonulease
RFLP	Restriction Fragment Length Polymorphism
rpm	Rotations per minute
SDS	Sodium dodecylsulphate
SNP	Single Nucleotide Polymorphism
SSR	Simple Sequence Repeat
STR	Simple Tandem Repeat
TBE	Tris Borate EDTA buffer
TE	Tris EDTA (buffer)
TEMED	N,N,N',N'-tetramethylethylenediamine
UV	Ultra violet
V	Volts
VNTR	Variable Number of Tandem Repeats
WBC	White Blood Cell
µl	Microliter


Chapter - I
Introduction

CHAPTER I

INTRODUCTION

Biological diversity is a global asset of paramount importance for the food security and socio-economic development of mankind. Biodiversity is the basis of human survival and economic well-being and provides to humankind enormous direct economic benefits, an array of indirect essential services through natural ecosystems and plays a prominent role in modulating ecosystem function and stability.

The life is greatly diversified into various forms to the extent that no two organism are alike how closely genetically related they are except clones and identical twins, this is called the biodiversity. The diversity can be realized in terms of species, breeds, strains, lines, herds, families, etc. Domestic animal biodiversity grabs our attention more than any other category as it is directly involved in the food security as well as food diversity. The genetic diversity has resulted due to the process of evolution over thousands of years during wild and domesticated stages for the efforts made by man to meet the market demand in present context. About 40 species of domestic animals and poultry contribute to meeting the needs of humankind, providing meat, fiber, milk, eggs, draught animal power, skins and manures and are an essential component of many mixed farming systems. Within these species, more than 7000 breeds and strains (FAO, 2007) constitute the animal genetic resources (AnGR) that are of crucial significance for food and agriculture.

India is bestowed with immense richness of agricultural biodiversity including livestock and poultry. The farm animal genetic resources in India are represented by a broad spectrum of native breeds of cattle, buffalo, goat, sheep, equines, camels and poultry. These breeds evolved over several generations of selection and domestication and have remarkable attributes of adaptability to tropical environment, genetic resistance too many diseases besides subsistence on poor quality of crop residue based feed and fodder. Intensive livestock development programmes are increasingly promoting the universal use of very few 'improved' breeds resulting in reduction in population numbers for many of the indigenous breed as well as the genetic variability within a species. According to the recently

published State of the World's Animal Genetic Resources for Food and Agriculture (FAO, 2007), in the past century, approximately 10% of farm animal breeds have become extinct and an additional 15% are considered rare or endangered. Moreover, the situation is presently unknown for 34% of the breeds, most of which are reared in developing countries.

India harbors proportionately more livestock diversity (8%) than its share of land in the world (2.4%). By virtue of varying climate and ecosystems prevalent in different regions of the country, there are well defined breeds and several strains in almost all the livestock species used for food, work, fiber and other valuable products. The enormous and diverse goat genetic resources of India are signified in the form of 20 documented breeds of goat (Acharya, 1982). These have been found by centuries of human and natural selection. The rich biological heritage of these animals are fast getting eroded and trends in the last few decades are alarming. While India ranks second in goat population with about 124.3 millions, i.e. 21 per cent of world's goat population, caprine diversity is shrinking rapidly and it appears that a very serious situation has arisen due to erosion of breeds of this species at quantitative as well as qualitative level. There is a marked decline in the population of unique animals conforming to true attributes of native breeds. Widespread use of crossbreeding, destruction of traditional production systems and reduction of pastures placed this precious germ pool under threat. It is only since last decade that concerted conservation efforts are really being made to preserve the genetic diversity of goat in India. Diverse attributes of a population are effective in its characterization, taking account of phenotypic traits, reproduction, geographic distribution, origin and habitat. Genetic characterization of populations or breeds allows the evaluation of genetic variability, a fundamental element in working out breeding strategies and genetic conservation plans.

Recent trend is to use molecular techniques for characterization which detects the genetic variation at DNA level. These techniques explore molecular markers such as RFLP, RAPD and Microsatellite. A marker is an identified genomic site and marker alleles represent polymorphism at this site. Among these markers, microsatellite markers are widely acceptable. Microsatellites are made up of Simple Tandem Repeat Sequence motif not more than six bases long. Tandemly

repeated di and tri nucleotide sequences (dC-dA)n is an example to be polymorphic in length in a number of eukaryotic genome. Microsatellites are highly polymorphic, dispersed throughout genome at a frequency of one at every 6kb sequence and amenable to PCR amplification makes them potentially very useful as DNA markers or in gene mapping studies. Microsatellite markers are very useful to study genetic variation, percentage control study, study of gene flows and genetic diversity.

Maharashtra State of India has two important descript breeds of goat i.e. Osmanabadi and Sangamneri along with other lesser known goat populations. The Sangamneri goat is a dual purpose breed of India. It has made its place in rural economy of Nasik, Ahmednagar and Pune district of Maharashtra. The breed has derived its name from Sangamner town of Ahmednagar district of Maharashtra. Sangamneri goats are medium sized animals. The coat color is complete white, however animals with admixture of black and brown color were also seen. The farmers of rural area of this region keep small flocks of Sangamneri goats to meet out family requirements of milk and finally sell the live animals for meat. This breed has proved its adaptability in semi-arid region of Maharashtra and performs well under poor quality range condition.

In view of the paramount importance of this breed in India, present research work was undertaken to characterize Sangamneri goat at molecular level using microsatellite markers with following objectives.

1) To evaluate genetic diversity in Sangamneri goat using microsatellite markers.
2) To estimate the allelic frequency of different microsatellite loci and to establish a microsatellite gene profile for Sangamneri goat using polymorphic markers.
3) To estimate percent heterozygosity and Polymorphic Information Content (PIC) values for these microsatellite markers in Sangamneri goat population.


Chapter - II
Review of Literature

CHAPTER II

REVIEW OF LITERATURE

The makers revealing variations at DNA level are referred to as the molecular marker. Almost unlimited number of genetic polymorphisms at the DNA sequence level had provided number of markers like Restriction fragment length polymorphism (RFLP) (Botstein *et al.,* 1980), Minisatellites or variable number of tandem repeats (VNTRs) (Jeffrey *et al.,* 1985), Random Amplified Polymorphic DNA (RAPD markers) (Williams *et al.* 1990) and Microsatellites (Litt and Luty, 1989). Molecular markers offer a unique opportunity to address questions on domestication and breeding at the genotype rather than phenotypic level besides helping characterize animal genetic resources at molecular level.

2.1 Microsatellites

Microsatellites are sequences made up of a simple sequence motif, not more than six bases long, that is tandem repeated and arranged head to tail without interruption by any other base or motif. Simple, tandem repeated di- and tri- nucleotide sequences have been demonstrated to be polymorphic in length in a number of eukaryotic genome (Litt and Luty, 1989). The frequency with which they occur (once every 50,000-60,000 bp), the high degree of polymorphism displayed, and their random distribution across the genome (Luty *et al.*, 1990) make them potentially very useful as DNA markers in gene mapping studies.

Microsatellite markers are ideal for population-level studies for a number of reasons. First, they are randomly distributed throughout the genome, commonly occurring in non-coding regions and are typically selectively neutral. Second, microsatellite loci are often hyper-variable within populations and show much higher mutation rates than other nuclear regions (Weber and Wong, 1993). Variation seen at microsatellite loci arises from differences among alleles in the number of times the basic motif is repeated with new alleles probably being generated through polymerase slippage and slipped-strand mispairing during DNA replication (Levinson and Gutman,

1987; Kruglyak *et al.* 1998; Toth *et al.* 2000), which results in the addition or loss of one or a small number of repeats. Third, microsatellite alleles show codominant inheritance making them relatively easy to score directly. Finally and most important for field applications microsatellite marker genotyping requires only miniscule amounts of template DNA, since it is based on PCR (Mullis and Faloona, 1987). Sufficient DNA for microsatellite analyses can be extracted from small pieces of tissue or minute quantities of blood as well as from single shed hairs or from the epithelial cells sloughed off in urine, faeces, or saliva. Once a microsatellite locus has been identified in the genome oligonucleotide primers can be designed from the DNA sequences upstream and downstream of the microsatellite to amplify that fragment of the genome by PCR. Then microsatellite marker variation can be assayed directly by electrophoresis and visualization of these PCR products in denaturing polyacrylamide gels because alleles vary in the number of repeats of the microsatellite motif, heterozygous individuals will show two PCR product bands while homozygotes will only display a single band.

2.2 Breed Characterization and Variability Studies Using Microsatellite Markers

Kemp *et al.* (1993) reported a set of six new bovine microsatellite polymorphisms based on (CA)n repeats. They were highly polymorphic and thus represented valuable markers for the genome mapping. Four of the six were polymorphic in sheep and two were in goat.

Amigues *et al.* (1994) carried out a comparison of blood typing and microsatellites for verifying the efficiency of microsatellites in parentage control in two French breeds (Alpine and Saanen) of goat. Four microsatellites (INRA005, INRA006, INRA023, β-cas) were used. One hundred seventy-eight out of 185 parentage controls were compatible with blood typing and microsatellite typing and five were incompatible with both the methods. The study showed that the practical efficiency of the microsatellite test was comparable to blood typing.

Arevalo *et al.* (1994) cloned the microsatellites SR-CRSP 1, 2, 3, 4 and 5 from caprine genomic library. Number of alleles, sample heterozygosity and PIC were calculated for the microsatellites and screened

in the unrelated parents of 15 distinct sire families of Anglo- Nubian, Toggenburg, Galla and small East African breeds. The number of alleles was 1-5 observed in these five microsatellites. Sample heterozygosity was 0.950-0.311 and PIC was 0.275- 0.886 for these five microsatellites.

Bhebhe *et al.* (1994) cloned microsatellites SR-CRSP 6, 7, 8, 9 and 10 from caprine genomic library. Number of alleles, sample heterozygosity and PIC were calculated for the microsatellites and screened in the unrelated parents of 15 distinct sire families of Anglo- Nubian, Toggenburg, Galla and small East African breeds. The number of alleles observed in SR-CRSP 6, 8, 9 and 10 were 3-7. Sample heterozygosity and PIC were 0.717-0.941and 0.626- 0.875 respectively for these five microsatellites.

Kemp *et al.* (1995) sequenced total of 197 clones. Out of these, 81 microsatellite markers yielded polymorphism in bovine, ovine and caprine. The mean polymorphic information content of the 97 markers determined in 20 cattle was 0.66. Thirty-nine of the markers were polymorphic in sheep and 32 were polymorphic in goat. This study identified a set of 18 robust markers that were polymorphic in all three species and that covered 14 bovine chromosomes. These formed a group of markers suited to genetic distance analysis and parentage control in cattle, sheep and goat.

Mattapallil and Ali (1999) studied the distribution and evolutionary pattern of the conserved microsatellite repeat sequences (CA)n, (TGG)6 and (GGAT) to determine the divergence time and phylogenic position. The result showed a high level of heterozygosity among the buffalo, cattle, sheep and goat. Result of these repeat loci suggested that the water buffalo genome shares a common ancestry with sheep and goat after the divergence of subfamily Bovinae from the Bovidae.

Saitbekova *et al.* (1999) studied genetic diversity in eight Swiss goat breeds using of 20 bovine microsatellites on 20-40 unrelated animals per breed. In addition the Creole, Ibex and Bezoar breeds of goat were included. Study on 352 animals revealed the average heterozygosity within population was higher in domestic goat (0.51-0.58) than in Ibex (0.17) and Bezoar goat (0.19). Twenty-seven per cent of the genetic diversity in the total population could be attributed to differences between the populations. However, with the

exclusion of Ibex from the total population, this proportion dropped to 17%. Principal component analysis showed that all Swiss goat breeds were closely related, whereas the Creole breed, Ibex and Bezoar goat were distinct from all eight Swiss goat breeds.

Yang *et al.* (1999) analyzed the microsatellite variation in five Chinese indigenous goat breeds Tibetan, Neimonggol, Liaoning, Taihang, Matou using six microsatellites. Allele frequencies, heterozygosity, PIC and effective allele number were calculated. The genetic relationship of five breeds corresponded to their history and geographic origins were very well assessed using the selected microsatellite.

Ganai and Yadav (2001) studied the parameters of genetic variation, genetic distances and time of divergence in three Indian goat breeds using 16 cattle microsatellite markers. The mean number of alleles and mean allele size (bp) per microsatellite marker in goats were 5.37 ± 0.78 and 143.9 ± 33.75 bp, respectively. The average values of heterozygosity and polymorphism information content were 0.54 ± 0.2 and 0.48 ± 0.20, respectively. Five of the eight genetic distance methods were highly correlated, revealing a closer relationship between Jamnapari and Barbari goats. A phylogenetic tree constructed from inter-individual distances revealed that the individuals clustered according to the breed to which they belonged, and the Jamnapari and Barbari goats formed a cluster. The divergence times between Sirohi and Jamnapari, and Sirohi and Barbari were approximately 2000 years, while its value between Barbari and Jamnapari goats was approximately 1370 years.

Thakkar *et al.* (2002) investigated five microsatellite markers ILSTS -005, ILSTS -001, ILSTS - 030, ILSTS -033 and ILSTS -034 in Zalawadi breed of goat that revealed heterozygocity values of 0.554, 0.524, 0.753, 0.693, 0.512 and PIC values of 0.490, 0.484, 0.705, 0.693 and 0.398 respectively. Numbers of alleles ranged from three to seven.

Behl *et al.* (2003) studied the genetic variability of 22 heterologus microsatellite markers in two Indian goat breeds (Black Bengal and Chegu). The evaluated microsatellite loci exhibited high mean heterozygosity (0.69 and 0.66), and PIC values (0.79 and 0.78) in Bengal and Chegu breeds, respectively.

Li *et al.* (2004) used eighteen microsatellites to investigate the genetic diversity and differentiation of eight Chinese indigenous goat breeds. The results indicated that there is a significant difference of genetic diversity between different loci. Chinese indigenous goat breeds have similar genetic diversity to other Asian goats, but with lower Fst. The genetic differentiation between populations is consistent with the results of archaeology, mt DNA and RAPD.

Maudet *et al.* (2004) tested 60 published microsatellite primer pairs from bovids (cattle, sheep and goat) on 49 individuals from 11 taxa including six wild goat-like species (Capra species), three divergent wild sheep (Ovis species) and two chamois (Rupicapra spp.) species. Approximately 30 microsatellites amplified and among them all the loci were polymorphic within most of the 11 species.

Patel (2004) studied fifteen bovine microsatellites selected from the available list of 25 microsatellites suggested by ISAG for estimation of genetic diversity in Surti breed of goat, amplified 2 (ETH-152 and ILSTS-065) to 11 (ILSTS-028) alleles. The heterozygosity values at these microsatellites ranged from 0.200 (ETH-152) to 0.720 (ILSTS-030). The polymorphic information content (PIC) values ranged from 0.164 (ETH-152) to 0.854 (ILSTS-087) on a set at 15 microsatellites revealed high degree of genetic variability in Surti goat indicating an important indigenous genetic resource on a set at 15 microsatellites.

Tantia *et al.* (2004) tested bottleneck in Black Bengal and Chegu breeds of Goats using 22 microsatellite loci. All the three models viz. IAM, TPM and SMM were subjected to Sign test, Standardized differences test and Wilcoxon rank test to know possible bottleneck. In Black Bengal the expected number of loci with heterozygosity excess was 13.21 out of 22 loci under IAM, 12.98 under TPM and 12.95 under SMM and loci observed with heterozygosity excess were 22, 22 and 15 loci, respectively. In Chegu breed the number of loci with heterozygosity excess were 22 and 21 with IAM and TPM model using sign test, which are significantly different from expected values of 13.12 and 13.06. Both the populations were found to have undergone bottleneck.

Kumar *et al.* (2005) studied genetic variation at 25 microsatellite loci in Marwari goat. The average polymorphism across the studied loci and the expected gene diversity in the population were 1.295 and 0.623 ± 0.041, respectively. The population was observed to be significantly differentiated into groups and showed a fairly high level of inbreeding (f = 0.264 ± 0.046) and global heterozygote deficit. The bottleneck analysis indicated the introduction of unique/rare alleles by the immigrants.

Mainguy *et al.* (2005) reported the results of a cross-species amplification test of 156 bovine, ovine and cervid microsatellite markers in a wild population of mountain goats, Oreamnos americanus, inhabiting Caw Ridge, Alberta, Canada. Twenty-nine markers were found to be low to moderately polymorphic with between two to nine alleles per locus. Observed heterozygosity ranged from 0.14 to 0.85 for a sample of 215 mountain goats.

Fatima (2006) studied microsatellite variation in three indigenous goat breeds- Gohilwadi, Surti and Zalawadi using 19 microsatellite markers selected from the list suggested by International Society for Animal observed that number of alleles ranged from four (in Oar JMP-29) to fifteen (in ILSTS-030 and -034) with total 178 alleles across three breeds. The overall heterozygosity, PIC and Shannon index values were 0.61, 0.60 and 1.50 indicating high gene diversity. The highest observed heterozygosity was found in Gohilwadi and minimum in Surti goat breed. Genetic distance was least (0.128) between Gohilwadi and Zalawadi and highest between Gohilwadi and Surti (0.1951). In all populations low inbreeding was indicated (mean FIS = 0.0192, FIT = 0.0914) within and among the breeds. Genetic differentiation between breeds was moderate with a mean FST value of 0.073 which showed that the average proportion of genetic variation explained by breed differences was 7.3 per cent. Deviations from Hardy-Weinberg equilibrium were noted for most of the locus.

Thilagam *et al.* (2006) studied 20 microsatellite markers using genomic DNA from 50 unrelated Kanniadu goats. The number of alleles ranged from 5 to 14 with allele sizes ranging from 90 to 222bp. The allele frequencies ranged from 0.0106 to 0.4480. Polymorphism information content ranged from 0.5710 to 0.8570. Except four loci, the population was not in Hardy-Weinberg equilibrium. The observed heterozygosity ranged from

0.7142 to 0.9778 while the expected heterozygosity ranged from 0. 6390 to 0.8702, indicating the heterogenous nature of the population distributed in the breeding tract.

Aggarwal *et al.* (2007) analysed the genetic variation at 25 microsatellite loci in 50 Mehsana goats. Estimation of effective number of alleles and gene diversity frequently observed in microsatellite markers revealed substantial genetic variation. The mean number of observed alleles per microsatellite marker was 12.28 and that of effective alleles was 6.23. The average observed and expected heterozygosity values were 0.652 and 0.765 respectively. The mean polymorphic information content value (0.724) further reflected high level of polymorphism across the loci.

Dixit *et al.* (2008) investigated genetic diversity within Kutchi goat using 25 microsatellite molecular markers. The average number of alleles observed across the studied microsatellite loci was 12.0 ± 1.02. The average expected gene diversity within the population was 0.79 ± 0.02, whereas observed heterozygosity was 0.59 ± 0.06. Thirteen out of the total 25 studied loci showed significant deviations from Hardy–Weinberg equilibrium. The Fis (inbreeding) value was 0.23 ± 0.07. The genetic differentiation among sub-populations of this breed was low (Fst = 0.05 ± 0.01). The Sign and Wilcoxon tests detected significant departure from mutation drift equilibrium in the population at most of the studied loci.

Sharma *et al.* (2008[a]) investigated genetic variation at 24 microsatellite loci and genetic bottleneck hypothesis for Barbari goat population. The estimates of genetic variability such as effective number of alleles and gene diversities revealed substantial genetic variation. Shannon's information index as indicator of polymorphism across studied loci, and Nei's expected heterozygosity were 1.183 and 0.58 ± 0.191, respectively. The population was observed to be significantly differentiated into different groups, and showed heterozygote deficiency (f=O.202±O.044).

Sharma *et al.* (2008[b]) investigated Beetal genetically utilizing microsatellite markers selected on the guidelines of ISAG and FAO's DADIS (Domestic Animal Diversity Information System) MoDAD programme. The observed heterozygosity in the population varied from 0.134 - 0.842 with the mean of 0.469 ± 0.191, indicating lower genetic variation in this population.

Bottleneck was examined assuming all three mutation models and was found to be absent. Population displayed heterozygote deficit to the tune of 22.3 per cent.

Cauveri *et al.* (2009) studied 5 microsatellite markers using genomic DNA from 30 unrelated animals of the three different goat breeds viz. Barbari, Kanniadu and Tellicherry. The observed and effective number of alleles in Barbari goats for the loci ETH 225, ILSTS 005, ILSTS 011, ILSTS 033 and INRA 035 were 6 (3.97), 6 (4.42), 8 (5.57), 14 (11.22) and 6 (5.56), respectively. The corresponding values in Kanniadu goats were 9 (6.94), 8 (5.62), 10 (8.1), 7 (5.78) and 9 (8.21) and Tellicherry goats were 8 (5.49), 10 (6.79), 11 (9.52), 6 (5.26) and 7 (4.53), respectively. the observed heterozygosity (H_o) ranged from 0.04 to 0.98 with overall H_o (Expected heterozygosity, H_E) being 0.69 (0.85). The polymorphic information content (PIC) ranged from 0.465 to 0.904 for all microsatellite loci indicating that these loci showed polymorphism in goats.

Kumar *et al.* (2009) conducted experiment on 50 genomic DNA samples of unrelated Gohilwari goat using 25 microsatellite markers selected from the list suggested by International Society for Animal Genetics (ISAG) and FAO's (DAD-IS). The observed number of alleles detected per locus ranged from 4-24 with an overall mean of 10.12 ± 5.46. Overall mean observed heterozygosity of 0.505 was lower than the overall mean expected heterozygosity of 0.684. Most of the loci showed the heterozygote deficit as also depicted by F_{is} value. Substantial genetic variation and polymorphism was observed across studied loci in the Gohilwari breed of goat. Also the population studied was not in Hardy-Weinberg equilibrium at most of the studied loci.

Ramamoorthi *et al.* (2009) studied the genetic diversity of Barbari goat using 21 microsatellite markers. The number of alleles ranged from 4 to 11, with allele sizes ranging from 88 to 220 bp. The distribution of allele frequencies was between 0.0104 and 0.5208. Polymorphism information content varied from 0.5563 to 0.8348. The population was not in Hardy-Weinberg equilibrium for all except two microsatellite loci (ILSTS044 and ILSTS060). The observed heterozygosity ranged from 0.8478 to 1.0000 while the expected heterozygosity ranged from 0.6208 to 0.8509.

Verma *et al.* (2009) analysed genetic diversity in Malabari goat. The microsatellite based genetic analysis indicated the number of alleles varying from 4 to 26 with an overall mean of 0.36. The average observed heterozygosity varied from 0.033 to 0.979 whereas expected heterozygosity ranged from 0.152 to 0.925 with mean of 0.689. The gene diversity varied from 0.153 to 0.927 and allelic richness varied from 3.61 to 20.96. The values of the FIS ranged from 0.028 (ILSTS002) to 0.0.924 (ETH 225).

Dixit *et al.* (2010) investigated the genetic diversity and relationship among goat breeds of southern India based on microsatellite markers. All five breeds of south India, namely Attappady, Osmanabadi, Sangamneri, Malabari and Kanniadu, along with Ganjam of eastern India were considered for the investigation. In total, 190 alleles were observed from 288 DNA samples analysed with 25 microsatellite loci across six breeds. The most diverse breed was Kanniadu to 0.61 in Osmanabadi. The genetic distance tended to be least (0.22) between Ganjam and Malbari and the widest (0.83) between Kanniadu and Malabari.

Garrine *et al.* (2010) described genetic diversity between and within the indigenous Landim and Pafuri goat breeds from Mozambique with reference to the unimproved South African Boer goat, the paternal ancestor of the Pafuri breed. Microsatellite polymorphism was used to quantify genetic diversity and drift. Heterozygosity (Hz) in three populations of the Landim breed were closely comparable (Hz = 0.588 - 0.623), with a higher Hz value of 0.672 in Pafuri, the latter most likely reflecting the influence of the Boer goat. Allelic richness values supported this trend. Drift among individual Landim populations was low (Fst = 0.046-0.059) compared to Fst = 0.077-0.091 between the Pafuri breed and individual Landim populations, again reflecting introgression of Boer goat genetic material.

Mishra *et al.* (2010) investigated the genetic diversity of Changthangi goat using a battery of 12 mirosatellite markers. The observed number of alleles varied from 5 (OarJMP 29) to 19 (Oar FCB304) with a mean of 10.4 ± 3.91. The effective number of alleles varied from 1.56 (OarJMP29) to 8.92 (OarFCB304) with a mean 4.59 ± 2.07. The value of Shannon information index (I) ranges from 0.774 (OarJMP29) to 2.487(OarFCB304)

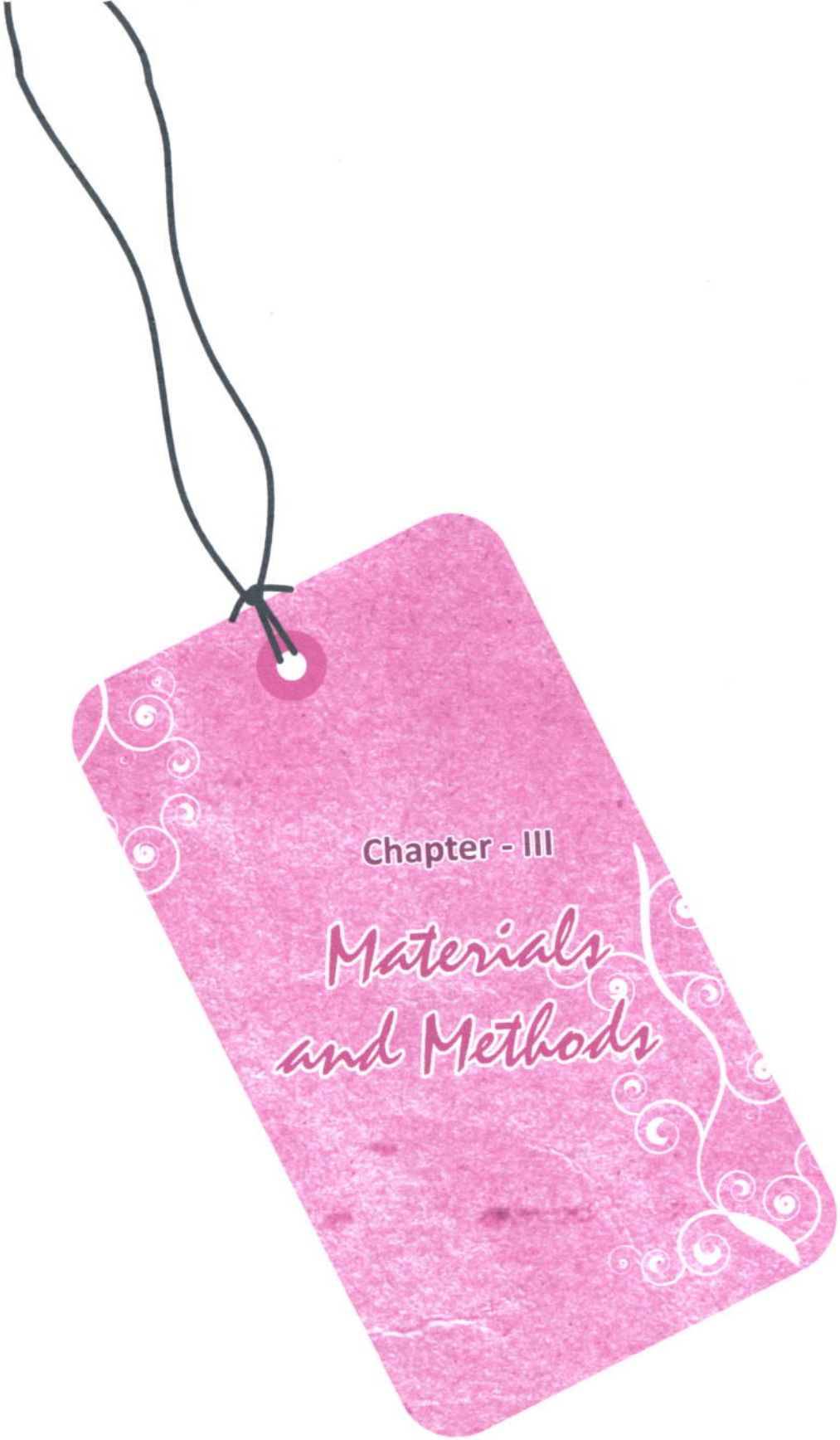
whereas poly information contents range from 0.343 (OarJMP29) to 0.846 (OarFCB304). The high value of I and PIC indicated the suitability of markers for studying the genetic variability in goat species. Observed heterozygosity ranged from 0.2368 (OarJMP29) to 0.9783 (OarFCB304). The mean observed heterozygosity was 0.3979. The expected heterozygosity was highest (0.8880) for locus Oarfeb 304 and lowest (0.3618) for locus OarJMP29. Nine of the 12 loci showed the positive inbreeding coefficient. Among the positive values the Fis varied from 0.0527 (ILSTS008) to 0.6491 (OMHC1) with a mean of 0.1773 however, ILST044, ILSTS002, OarFCB304 exhibited the negative values corresponding to 0.0471, 0.0855 and 0.1016 respectively. The L shaped curve obtained indicated that Changthangi population has not undergone any recent bottleneck.

Verma *et al.* (2010) studied the genetic variability in Sangamneri breed using 25 selected micro-satellite markers. The number of alleles observed varied from 3 (ILSTS 022) to 21 (OarFCB304) with an overall mean of 9.0. Most of the studied loci showed the polymorphic information content value greater than 0.5 except ILST008, OarJMP29, ILSTS005 and ILSTS029 locus. The PIC values varied from 0.271 (OarJMP29) to 0.878 (ILSTS 082) across loci with an average value of 0.711. The average expected gene diversity within the population ranged from 0.2769 (OarJMP 29) to 0.8880 (ILSTS 082) with an overall mean of 0.6970±0.033, whereas observed heterozygosity ranged from 0.0435 (ETH 225) to 0.8571 (ILSTS 002) with an average of 0.5399±0.0549. Sixteen out of total 25 studied loci showed significant heterozygote deficiency (positive Fis value). The significant positive values of the Fis ranged from 0.118 (OarFCB304) to 1.0 (ETH225) with an overall mean of 0.245.

Dixit *et al.* (2011) examined genetic variation at 25 microsatellite loci in Kanniadu goats of Tamil Nadu. The observed number of alleles ranged from 5 (RM4) to 13 (RM088, OarE129) with an average value of 8.64±0.48. The effective number of alleles ranged from 1.45 (ILSTS34) to 7.89 (ILSTS033 and OMHC1) with the overall mean value of 4.22 ± 0.34. The average observed and expected heterozygosity values were 0.53±0.03 and 0.73 ± 0.02, respectively. The polymorphic information content value ranged from 0.30 to 0.86. These high values of PIC indicated higher polymorphism in

the breed. The high level of genetic variability suggested the scope for further genetic improvement of Kanniadu goats.

Musthafa (2011) studied genotypic characterization of Ardi goat using 14 microsatellite markers recommended by ISAG/FAO to measure the genetic diversity of Ardi goat breed found in Riyadh region. Mean number of alleles was observed as 6.643 ± 2.061 with the range of three (MAF209 and SRCRSP3) to nine (OarFCB20 and OarAE54) with 93 total alleles. The mean expected heterozygosity, observed heterozygosity and Nei's heterozygosity values were 0.675 ± 0.137, 0.553 ± 0.194 and 0.665 ± 0.135 respectively, indicating high genetic diversity among the studied loci. Estimated values of polymorphic information content and Shannon index showed that the most of the loci in this study were highly polymorphic and the values found to be 0.628 ± 0.138 and 1.390 ± 0.346 respectively. Deviation from Hardy-Weinberg equilibrium was observed for six of the studied loci.


Chapter - III
Materials and Methods

CHAPTER III

MATERIALS AND METHODS

3.1 Experimental Material

Experimental material for the present study comprised of 50 blood samples of Sangamneri goat (Plate 1). The Sangamneri goat is a dual purpose breed of India. Samples were collected at random from unrelated animals from different villages of Sangamner and Rahuri Taluka of Ahmednagar and owners were questioned in detail in order to avoid close relationships. The samples were also collected from All India Co-ordinated Research Project on Sangamneri Goat, Department of Animal and Husbandry, Mahatama Phule Krishi Vidyapeeth, Rahuri.

3.1.1 Sample collection, transportation and storage

Approximately 10 ml of venous blood samples from each animal were collected aseptically in sterile polypropylene vial containing 0.5 ml of 0.5 M EDTA as anticoagulant and then transported to laboratory in a thermocol box containing ice cool packs. The blood samples were kept at -20°C till the isolation of DNA.

3.1.2 DNA extraction from blood samples

The standard Phenol: Chloroform extraction method (Sambrook and Russell, 2001) was employed to isolate genomic DNA from frozen blood samples. The procedure is given follows:

Procedure of Isolation of Genomic DNA

1) Blood samples stored in the freezer were thawed at room temperature.
2) Thawed samples were transferred to sterile polypropylene centrifuge tubes and centrifuged @ 4000 rpm for 20 minutes at room temperature.
3) The reddish supernatant containing the plasma and lysed RBC was discarded by pipetting.
4) Chilled RBC lysis buffer twice the volume of pellet was added and mixed gently and kept in ice for 10 minutes.

Sangamneri Female goat

Sangamneri Male goat

Plate 1. Adult Sangamneri goat

5) Centrifugation was repeated @ 4000 rpm at room temperature and the black tarry coloured supernatant, containing lysed RBC was discarded by pipetting.

6) Steps 4 and 5 were repeated 3-4 times till the WBC pellet becomes free of reddish tinge.

7) DNA extraction buffer @ 3 ml per 10 ml of blood was added and vortexed to disperse the WBC pellet gently in the extraction buffer.

8) WBC pellet mixed with DNA extraction buffer was incubated at 370C for 30 minutes.

9) Subsequently, 10% SDS @ 200 µl per 10 ml blood was added and mixed gently by inverting the tubes once or twice.

10) Proteinase K (20 mg/ml solution) was added in two pulses. Half of the requirement (20 µl per 10 ml blood) was added in first pulse, mixed gently end to end, and incubated at 50°C overnight.

11) On the second day, contents of the tubes were transferred to clean sterile autoclaved 100 ml conical flask and then equal volume of tris-saturated phenol (pH 7.8) was added.

12) The flasks were kept on shaker and rotated for 15 minutes to mix the contents properly.

13) Subsequently, the contents of the flasks were transferred to 15 ml fresh, sterile centrifuge tubes and @ 4000 rpm for 10 minutes at room temperature.

14) The upper aqueous phase containing DNA was transferred to another conical flask with the help of 1 ml wide bore (3 mm diameter) microtip.

15) Similarly, extraction as in step 11 to 14 was done once with phenol: chloroform: isoamyl alcohol (25:24:1) and once with chloroform: isoamyl alcohol (24:1).

16) Finally the aqueous solution was transferred to 50 ml polypropylene centrifuge tube and 3 M sodium acetate @ 100 µl/ ml aqueous phase was added by gentle mixing.

17) More than two volumes of chilled isopropanol was added and mixed gently by swirling the tube once or twice. The tube was left at room temperature to allow precipitation of DNA.

18) The precipitated DNA was transferred to a sterile 1.5 ml eppendorf tube (using a wide bored microtip of 1 ml capacity) along with 500 µl and centrifuged @ 10000 rpm for 10 minutes at room temperature.

19) Supernatant was discarded without interfering with the DNA pellet.

20) The DNA pellet was then washed twice with 70% ethanol.

21) Finally, the DNA pellet was air dried for 1 hour to remove traces of ethanol and subsequently dissolved in 200 µl TE buffer.

22) The eppendorf tubes were kept in water bath at 60°C for 2 hours to inhibit DNase activity and to dissolve the pellet properly in TE buffer.

23) After 2 hours of water bath, DNA was cooled and stored at -20°C for further use.

The composition and preparation of buffers and solutions used in the procedure is given in the Appendix I.

3.1.3 Quality check and quantification of DNA

The quality and purity of DNA were checked and quantification was done by agarose gel electrophoresis.

The DNA samples were diluted at different dilutions 1:40 1:50 1:80 and 1:100 (20 µl of DNA in 980 µl nuclease free water) and subjected to agarose gel electrophoresis for quality check. Agarose 0.8 per cent in 1X TBE buffer (pH 8.0) was used for gel electrophoresis. Agarose 0.8 g was dissolved in 0.5X TBE buffer to a final volume of slightly more than 100 and was heated in microwave oven. Molten Agarose was taken out carefully. Ethidium bromide (1%) was added @ of 5 µl/100 ml of gel solution. Molten agarose was poured into sealed gel casting tray with the comb positioned appropriately. Once the gel got solidified, it was transferred to electrophoresis tank filled with 1X TBE buffer. The level of buffer was kept at least 1 cm above the gel. The wells were carefully charged with 3.0 µl DNA mixed with 0.5 µl 6X gel loading dye. Electrophoresis was carried out at 80V for 30-60 min. On completion of electrophoresis, the gel was visualized under UV transilluminator and quality and purity was judged. DNA appeared as single compact pink fluorescent band free from degradation (whole lane fluorescent) or RNA contamination (fluorescent spot migrated away from well). The dilution 1:50 yielded a hair thick band and was selected for use in PCR.

3.2 Microsatellite Primers

3.2.1 Chromosomal locations of microsatellite markers

Chromosomal location for different microsatellites used to estimate genetic variability among Sangamneri goat given in Table 3.1.

Table 3.1. Chromosomal location for different microsatellites used to estimate genetic variability among Sangamneri goat

Sr. No.	Microsatellite Markers	Chromosomal Location	Reference
1	ETH -225	Chromosome number 09	Steffen *et al.* (1993)
2	ILSTS-002	Chromosome number 14 (Sheep)	Kemp *et al.* (1992)
3	ILSTS-005	Chromosome number 10	Luikart *et al.* (1999)
4	ILSTS-008	Chromosome number 14	Saitbekova *et al.* (1999)
5	ILSTS -019	Chromosome number 25	Kemp *et al.* (1995)
6	ILSTS -022	Chromosome number 05	Kemp *et al* (1995)
7	ILSTS-029	Chromosome number 3	Luikart *et al.* (1999)
8	ILSTS -030	Chromosome number 02	Kemp *et al.* (1995)
9	ILSTS -033	Chromosome number 12	Kemp *et al.* (1995)
10	ILSTS -034	Chromosome number 05	Kemp *et al.* (1995)
11	ILSTS -044	Chromosome number 03	Kemp et al. (1995)
12	ILSTS-049	Chromosome number 11	Schibler *et al.* (1998)
13	ILSTS -058	Chromosome number 17	Kemp *et al.* (1995)
14	ILSTS -059	Chromosome number 13	Kemp *et al.* (1995)
15	ILSTS -065	Chromosome number 24	Kemp *et al.* (1995)
16	ILSTS082	Chromosome number 2	Schibler *et al.* (1998)
17	ILSTS -087	Chromosome number 06	Kemp *et al.* (1995)
18	Oar AE 129	Chromosome number07	Lumsden et al. (1995)
19	Oar HH 64	Chromosome number 4	Schibler *et al.* (1998)
20	Oar JMP 29	Chromosome number 24	Lumsden et al. (1995)
21	OMHC1	Chromosome number 14 (Sheep)	Groth and Wetherall, (1994)
22	RM4	Chromosome number 15	Crawford *et al.* (1995)

3.2.2 Primers sequence

Oligo primers specific to various microsatellite loci custom synthesized at Eurofins mwg/ operon, Genetix Biotech Asia Pvt. Ltd. and utilized in the study are listed below in the Table 3.2

Table 3.2. Primer sequences for different microsatellites used to estimate genetic variability among Sangamneri goat breed

Micro-satellite Markers	Primer Sequence (5'-3')	Repeats	Reference
ETH225	GATCACCTTGCCACTATTTCCT	(CA)18	Steffen *et al.* (1993)
	ACATGACAGCCAGCTGCTACT		
ILSTS002	F: TCTATACACATGTGCTGTGC	(CA)17	Kemp *et al.* (1992)
	R: CTTAGGGGTGAAGTGACACG		
ILSTS005	GGAAGCAATGAAATCTATAGCC	(nn)39	Luikart *et al.* (1999)
	TGTTCTGTGAGTTTGTAAGC		
ILST008	F: GAATCATGGATTTTCTGGGG	(CA)12	Saitbekova *et al.* (1999)
	R: TAGCAGTGAGTGAGGTTGGC		
ILSTS019	AAGGGACCTCATGTAGAAGC	(TG)10	Kemp *et al.* (1995)
	ACTTTTGGACCCTGTAGTGC		
ILSTS022	F: AGTCTGAAGGCCTGAGAACC	(GT)21	Kemp *et al.* (1995)
	R: CTTACAGTCCTTGGGGTTGC		
ILSTS029	F: TGTTTTGATGGAACACAGCC	(CA)19	Luikart *et al.* 1999
	R: TGGATTTAGACCAGGGTTGG		
ILSTS30	CTGCAGTTCTGCATATGTGG	(CA)13	Kemp *et al.* (1995)
	CTTAGACAACAGGGGTTTGG		
ILSTS033	TATTAGAGTGGCTCAGTGCC	(CA)12	Kemp *et al.* (1995)
	ATGCAGACAGTTTTAGAGGG		
ILSTS34	F: AAGGGTCTAAGTCCACTGGC	(GT)29	Kemp et al. (1995)
	R: GACCTGGTTTAGCAGAGAGC		
ILST044	F: AGTCACCCAAAAGTAACTGG	(GT)20	Kemp *et al.* (1995)
	R: ACATGTTGTATTCCAAGTGC		
ILSTS049	F: CAATTTTCTTGTCTCTCCCC	(CA)26	Schibler *et al.* (1998)
	R: GCTGAATCTTGTCAAACAGG		
ILSTS058	F: GCCTTACTACCATTTCCAGC	(GT)15	Kemp *et al.* (1995)
	R: CATCCTGACTTTGGCTGTGG		
ILSTS059	F:GCTGAACAATGTGATATGTTCAGG	(CA)4 (GT)21	Kemp *et al.* (1995)
	R:GGGACAATACTGTCTTAGATGCTGC		
ILSTS065	F: GCTGCAAAGAGTTGAACACC	(CA)22	Kemp *et al.* (1995)
	R: AACTATTACAGGAGGCTCCC		
ILSTS082	F: TTCGTTCCTCATAGTGCTGG	(GT)17	Schibler *et al.* (1998)
	R: AGAGGATTACACCAATCACC		
ILSTS087	F: AGCAGACATGATGACTCAGC	(CA)14	Kemp *et al.* (1995)
	R: CTGCCTCTTTTCTTGAGAGC		
OarAE129	F:AATCCAGTGTGTGAAAGACTAATCCAG	(CA)14	Lumsden *et al.* (1995)
	R:GTAGATCAAGATATAGAATATTTTTCAACACC		
Oar HH 64	F:CGTTCCCTCACTATGGAAAGTTATATATGC	(GT)17	Schibler *et al.* (1998)
	R: CACTCTATTGTAAGAATTTGAATGAGAGC		
Oar JMP29	F:GTATACACGTGGACACCGCTTTGTAC	(CA)21	Lumsden *et al.* (1995)
	R:GAAGTGGCAAGATTCAGAGGGGAAG		
OMHC1	F: ATCTGGTGGGCTACAGTCCATG	(CA)n	Groth and Wetherall, (1994)
	R: GCAATGCTTTCTAAATTCTGAGGAA		
RM4	F: CAGCAAAATATCAGCAAACCT	(CA)13	Crawford *et al.* (1995)
	R: CCACCTGGGAAGGCCTTTA		

3.2.3 Primer dilution

Oligos supplied in freeze dried powder form were reconstituted in nuclease free water to the volume (in µl) equivalent to the mass (µg) of primer and further diluted to give a final concentration 20 pmoles /µl.

3.3 PCR Amplification of Microsatellite Loci

3.3.1 PCR composition

PCR was carried out in a final reaction volume of 25 µl. Each reaction volume contained.

PCR components	Final Concentration	1 X	1/2X	50X
10X PCR buffer (with $MgCl_2$ 20 mM)	2.5 µl	2.5	1.25	62.5
dNTPs (2 mM each)	-	2.5	1.25	62.5
Forward Primer (20 pmole/µl)	10 pmol/µl	0.5	0.25	12.5
Reverse Primer (20 pmole/µl)	10 pmol/µl	0.5	0.25	12.5
Taq DNA polymerase (5 U/µl)	1.0 U/ µl	0.2	0.1	5.0
DNase free water	15.30 µl -	17.8	8.9	445
Total		**24**	**12**	**600**

Mastermix was prepared for one additional sample to cover pipetting error. All the reactions were carried out in 0.2 ml thin wall PCR tubes. PCR tubes containing mixture were tapped gently and quickly spinned @ 10,000 rpm for few seconds. The tubes were placed in a thermal cycler (Eppendorf) and subjected to PCR.

3.3.2 PCR Protocol-Berglund modified touchdown PCR

The 'touchdown' PCR protocol was used with initial denaturation of 95°C for 1 min, 3 cycles of 95°C for 45 s and 60°C for 1 min and 72°C for 1min, 3 cycles of 95°C for 45 s and 57°C for 1 min and 72°C for

1min, 3 cycles of 95°C for 45 s and 54°C for 1 min and 72°C for 1min, 3 cycles of 95°C for 45 s and 51°C for 1 min and 72°C for 1min, 20 cycles of 95°C for 45 s and 48°C for 1 min and 72°C for 5min,4°C for infinity.

3.3.3. Agarose gel electrophoresis

PCR amplification was confirmed by running, 5 µl of PCR product mixed with 1µl of 6X gel loading dye from each tube on 2.0 per cent agarose gel (depending on the expected size of amplified product) at a constant voltage 80 V for 30 min in 1 X TBE buffer. Ethidium bromide was incorporated @ 5µl of 1% solution /100 gel solution in the gel itself. The amplified product was visualized as a single compact fluorescent band of expected size under UV light and documented by gel documentation system (Biorad, Quantity One Software).

The chemicals were used for submarine gel electrophoresis are briefed in Appendix II.

3.4 Microsatellite Typing

3.4.1 PolyAcrylamide Gel Electrophoresis (PAGE) of PCR amplicons of microsatellite loci

Microsatellite alleles were resolved on 6-7 per cent denaturing polyacrylamide gel. For this, bonded and outer glass plates of the sequencing cell were cleaned thoroughly with mild detergent using soft sponge. Outer glass plates with heavy stains were cleaned thoroughly after soaking them in 1.0 M NaOH solution for several hours. Cleaned glass plates were washed thoroughly with tap water and rinsed with distilled water for several times. The glass plates were wiped with tissue paper, soaked in ethanol and allowed to dry. The surface of polycarbonate panel bonded glass plate was treated with 250µl repel silane (Methyldichlorosilane) and the opposite glass plate was treated with bind silane (3-methacryloyloxy propyl-trimethoxysilane) 10µl diluted to 1 ml in ethanol and allowed them to dry. The spacer of 0.4 mm thickness was positioned and the gel sandwich was assembled. The sandwich assembly inserted into cam-operated precision caster base, the gel sandwich was laid flat on lab bench and PAGE solution was injected between glass plates. Primarily comb was inserted between the glass plates with its

straight border oriented inward and clamps were immediately applied over it. The gel was allowed to polymerize for 1 hour. The comb was removed after polymerization.

The gel assembly was placed on DNA sequencing cell and electrophoresis chambers were filled with 1X TBE. The upper straight gel surface was flushed with tank buffer. Initially, a pre-run was made at 80W for 60 minutes loading only 6X buffer. The denatured PCR samples were loaded @ 2-6 µl per well on polyacrylamide gel and run at 100V. Runtime was decided based on length of PCR amplified fragment. The 100 bp ladder was used either in centre of PAGE or one side of PAGE when two plates were run at a time then one plate had ladder in centre and other plate had ladder in one side.

Solutions used in PAGE are given in Appendix III.

3.4.2 Silver staining of polyacrylamide gels and gel documentation

The gels were stained with Rapid Silver Staining Kit from GenAxy. The procedure follows.

Fixing step

1) Pour approximately 100 ml Fixing Solution in the Staining Tray.
2) Remove the gel from the electrophoresis apparatus and immerse into the Fixing Solution.
3) Cover the tray and gently mix on a shaker for 10 minutes.
4) Decant the fixing solution and add approximately 100 ml re-hydration Solution.
5) Leave on shaker for 5 minutes.

Reducing step

1) Rinse the gel thoroughly with water 2-3 times (2 to 3 minutes each wash).
2) Add 100 ml of water and two drops of Reagent A (Reducing Solution) directly in the Staining Tray.
3) Leave the gel on shaker for 10 minutes.
4) Acetic acid interferes with the silver staining therefore washing the gel thoroughly is important.

Staining step

1) Decant the Reducing Solution and quickly rinse the gel with water.
2) Add 100 ml water and 2 ml Reagent B (Silver Solution) to the Staining Tray.
3) Leave on the shaker for 10 minutes.

Developing step

1) Prepare 100 ml developing solution by mixing 20 ml of Reagent C (Developer Solution) with 80 ml water in a small beaker or flask and add two drops of Reagent D (developer enhancer).
2) Decant the Silver Solution and quickly rinse the gel with water.
3) Add approximately 50 ml of the developing solution, mix for 15 – 30 seconds.
4) Decant completely, add remaining 50 ml of developing solution.
5) Mix gently until bands are clearly visible (1 – 5 minute depending on the amount of the protein).
6) Immediately add 1-2 ml glacial acetic acid to the developer solution and mix to stop the reaction.
7) The staining procedure is now complete.

The stained gel can be stored in re-hydration solution.

Preparation of solutions

Fixing solution : 50% methanol + 10% acetic acid.

Mix 500ml methanol and 100ml glacial acetic acid and add water to make 1 litre.

Rehydration solution : 5% methanol + 7% acetic acid.

Mix 50ml methanol and 70ml glacial acetic acid and add water to make 1 litre.

The amplified product was visualized and documented by gel documentation system (Biorad, Quantity One Software).

3.4.3 Scoring of allele

The scoring of allele was carried out following get documentation on a BioRedTm gel-doc system, where the image capture and analysis was done by Quantity oneTm software. While the scoring of the bands was aided by the software, the final allele calling was manually accomplished by the joint observation of two independent observers. On the occasions, where stuffer bands were encountered along with the alleles in close migration ranges, a repeat PCR was performed and the allele – sizing and genotyping was determined in a consensus manner considering the pattern of both PCR reactions. Each sample exhibited one (in case of homozygote) or two (in case of heterozygote) pairs of bands (in heterozygote case) migrated as per their size. The alleles were numbered as 1, 2, 3, in order of their ascending size. Many a times these bands also appeared associated with 'stutter' (shadow) bands. Such samples were improved by optimization of PCR conditions and protocol. Each allele was assigned size by comparing with 100 bp DNA ladder co-migrated in the same gel.

3.4.4 Sizing of Allele and lane matching

The size of alleles was determined using the Quantity-one software for the PCR products resolved on gel, keeping 2 molecular weight sizing ladders in consideration. Using the lane-matching algorithm, a 5% level of tolerance was allowed for matching of alleles across the lanes. A judicious blend of manual as well as machine logic was applied by the independent observers, so as to score. The alleles in each lane, with respect to within and between population comparison of samples. After matching of the samples, the genotyping table was prepared in as M.S. Excel format which was processed further for feeding into the various population analysis software (GenAlex and MS-tools).

3.5 Statistical Analysis for Microsatellite Data

The population parameters drawn out of molecular analysis was derived using the software packages: GenAlex and MS-Tools.

3.5.1 Allele frequency

Allele frequency was calculated using formula

$$\text{Allele frequency} = \frac{\text{Number of a particular allele at a locus}}{\text{Total number of alleles in that locus}}$$

3.5.2 Effective number of alleles

It was calculated using the formula

$$Ne = 1 / \sum P_i^2$$

Where, P_i is the frequency of i^{th} allele.

3.5.3 Heterozygosity

The heterozygosity was calculated from allelic frequencies. The heterozygosity (H) for a marker locus in the Sangamneri breed of goat was estimated using the formula

$$H = 1 - \sum P_i^2$$

Where Σ stands for summation over all alleles (Nei, 1978) and P_1 is the frequency of the i^{th} allele at a locus in a population. The average heterozygosity per locus (H), defined as the mean of H over all structural loci in the genome was also calculated.

However, the unbiased estimate of the expected heterozygosity at a locus in the Sangamneri population studied was calculated by following formula.

$$\hat{H}_E = \frac{2N}{2N-1}\left[1 - \sum_{i=1}^{n} \hat{P}_i^2\right]$$

Where, P_j is the frequency for the j^{th} allele at i^{th} locus with I alleles in a population, and N is the number of individuals, assuming that the populations were in Hardy-Weinberg equilibrium (Nei, 1978).

The heterozygosity values were estimated using GenAlEx software version 6.4. (Peakall and Smouse, 2010).

3.5.4 Polymorphic information content (PIC)

The PIC was also calculated using marker allelic frequencies using following equation:

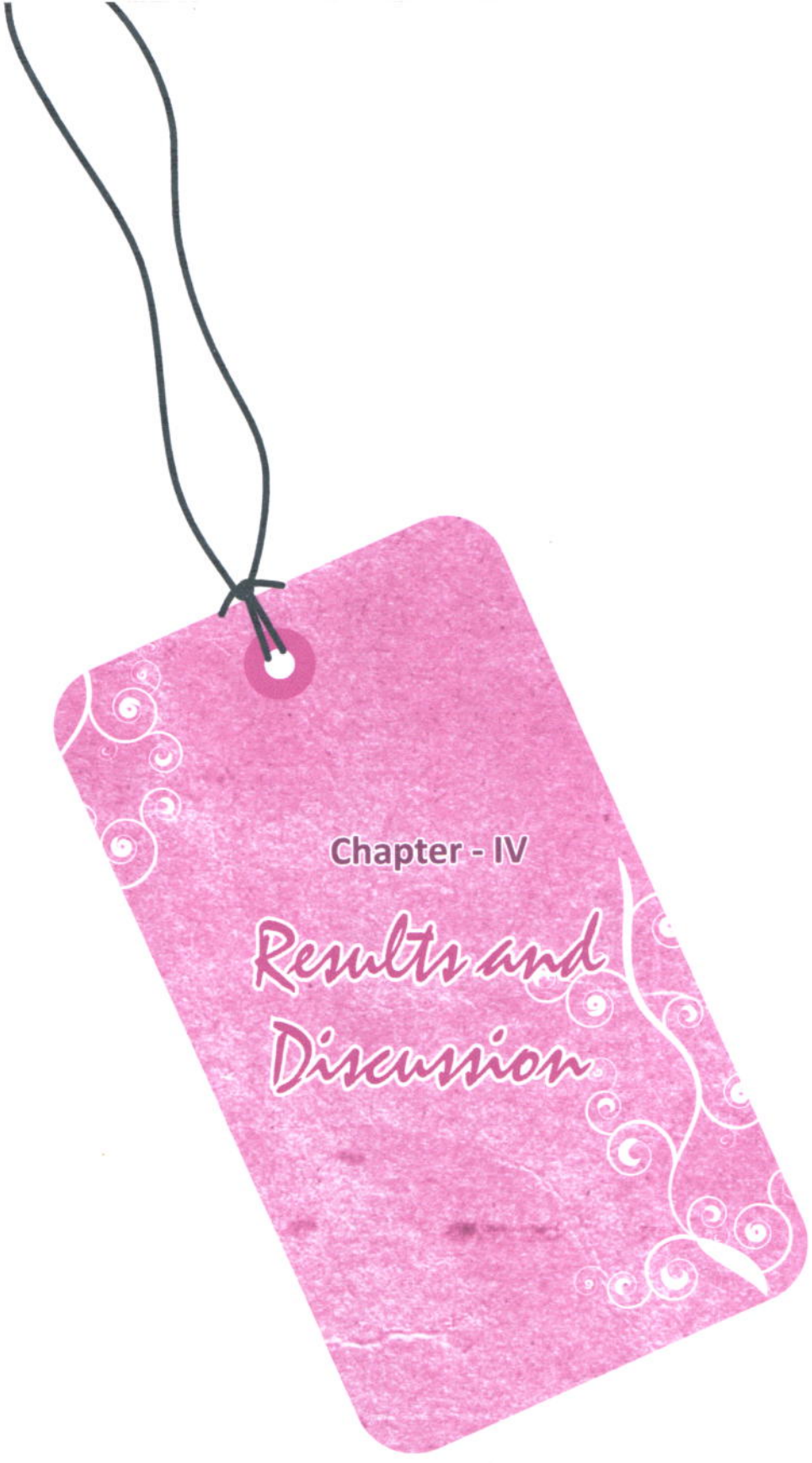
$$PIC = 1 - \left(\sum_{i=1}^{n} P_i^2\right) - \sum_{i=1}^{n-1} \square \sum_{j=i+1}^{n} 2P_i^2 P_j^2$$

Where, P_i and P_j are the frequencies of the ith and jth alleles at a locus with alleles in a population, respectively (Botstein et al., 1980).

The PIC values were estimated using MS-tools software (Stephen Park, 2001).

3.5.5 Shannon's Information Index

Shannon index was also estimated for the microsatellite markers studied. It was calculated after Shannon, C.E. and W. Weaver., (1949) by GenAlex software.


Chapter - IV
Results and
Discussion

CHAPTER IV

RESULTS AND DISCUSSION

Microsatellites are among the most useful markers and are being widely and successfully applied in conservation studies. Microsatellites are single locus, simple tandem repeats of less than six bases long. Tandemly repeated di- and tri- nucleotide sequences of which (dC-dA)n have been demonstrated to be polymorphic in length in a number of eukaryotic genome (Litt and Lutty, 1989). The principal objective of the present study was to study genetic variability within Sangamneri breed of goat using microsatellite markers. Twenty Two microsatellites suggested by International Society of Animal genetics (ISAG) were selected for the present study.

4.1 Microsatellite Analysis

4.1.1 Microsatellite ETH 225

Microsatellite ETH 225 contains (CA)18 repeats, and is located at nucleotide position in 43 -78 in the 189 bp sequence (Steffen *et al.*, 1993). Microsatellite ETH 225 is located at 11 cM from the beginning of INRA 136 on chromosome 9 in goat and cattle (Schibler *et al.*, 1998).

Sangamneri breed exhibited 17 alleles (Plate 2, Table 4.1) and the range of alleles size for the microsatellite locus ETH 225 was found in between 146 and 180 bp. Total number of alleles observed in the present study were at higher side than reported by Kumar *et al.* (2005) in Marwari goat (3), Gour *et al.* (2006) in Jamunapari goat (2), Sharma *et al.* (2008[a]) in Barbari goat (2), Dixit *et al.* (2010) in Southern Indian goat breed (8), Mishra *et al.* (2010) in Changthangi (10) and Dixit *et al.* (2011) in Kanniadu (7). Allele 9 (162 bp) was most predominant allele in the population for ETH 225.

The allelic size (146 to 180 bp) for the locus in Sangamneri goat was comparable to the allelic size for this locus reported in other breeds of goat as Kumar *et al.* (2005) reported the 151–155 bp in Marwari goat, Gour *et al.* (2006) reported the 154-156 bp in Jamunapari goat, Sharma *et al.* (2008[a]) reported the 151-153 bp in Barbari goat, while Dixit *et al.* (2010) reported 146-160 bp in Southern Indian goat breed which includes Ganjam, Attapady, Malbari, Kanniadu, Sangamneri and Osmnanabadi goat breed.

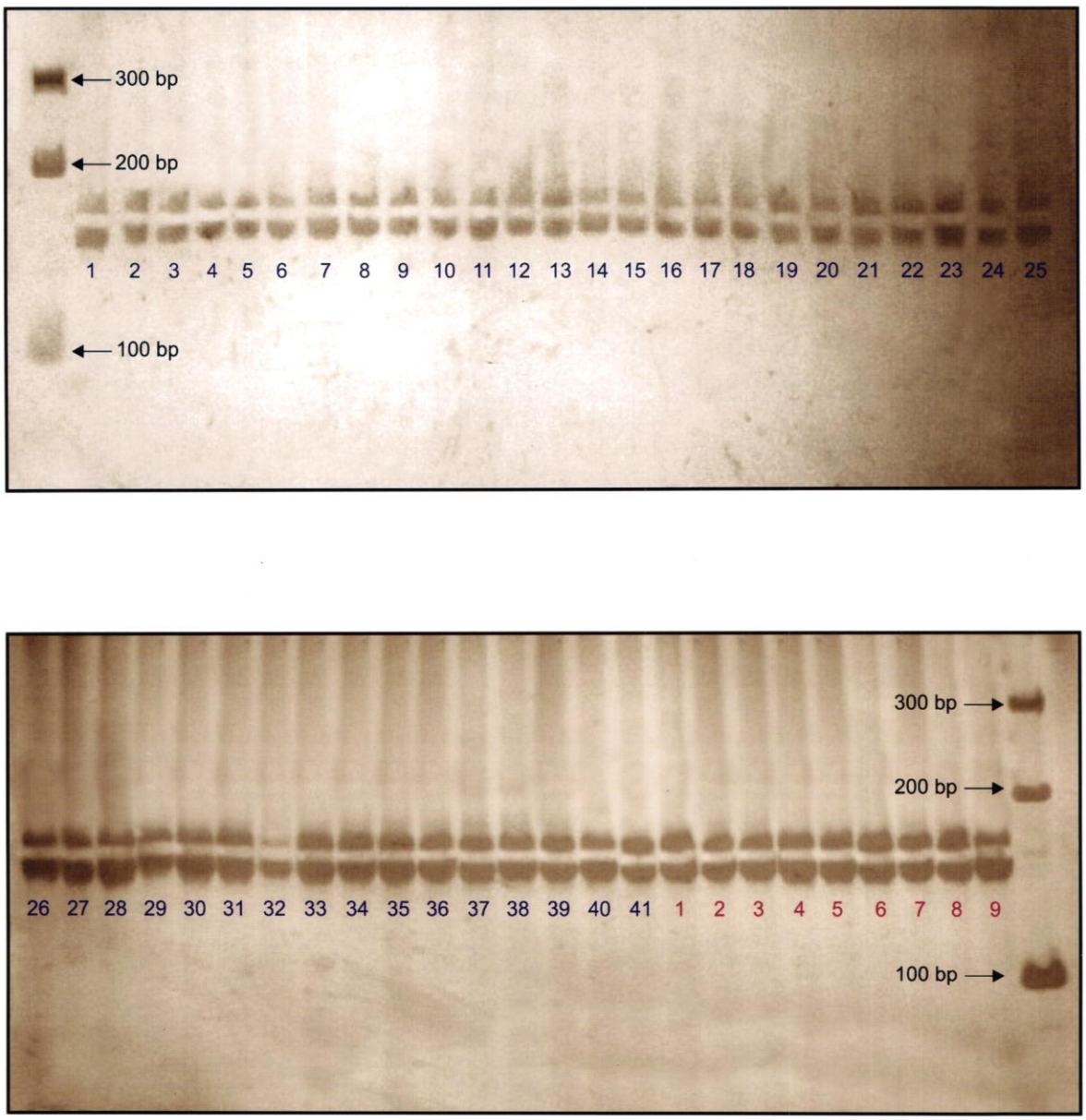


Plate 2. Resolution of PCR products of microsatellite locus ETH225 on 8% urea polyacrylamide gel

Blue colour numbers are Female (F1 to F41) = 1 to 41
Red colour numbers are Male (M1 to M9) = 1 to 9

Table 4.1. Frequency distribution and allele size of microsatellite ETH 255 in Sangamneri goat

Allele No.	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**
Allele size (bp)	146	148	150	152	154	156	158	160	162	164	166	168	170	174
Allelic frequency	0.020	0.100	0.060	0.060	0.020	0.050	0.010	0.110	0.120	0.090	0.060	0.030	0.070	0.030
Allele No.	**15**	**16**	**17**											
Allele size (bp)	176	178	180											
Allelic frequency	0.080	0.070	0.020											

Table 4.2. Genotype and genotypic frequency distribution of microsatellite ETH 225 in Sangamneri goat

Genotype	**5,5**	**6,6**	**2,8**	**3,8**	**2,9**	**4,9**	**9,9**	**2,10**	**4,10**	**10,10**	**2,11**	**3,11**	**4,11**	**2,12**
Observed count	1	1	1	1	3	1	1	1	3	1	1	4	1	1
Genotypic frequency	0.020	0.020	0.020	0.020	0.060	0.020	0.020	0.020	0.060	0.020	0.020	0.080	0.020	0.020
Genotype	**3,12**	**4,12**	**1,13**	**2,13**	**6,13**	**6,14**	**8,14**	**8,15**	**9,15**	**7,16**	**8,16**	**9,16**	**10,16**	**10,17**
Observed count	1	1	2	3	2	1	2	5	3	1	2	3	1	2
Genotypic frequency	0.020	0.020	0.040	0.060	0.040	0.020	0.040	0.100	0.060	0.020	0.040	0.060	0.020	0.040
Chi Square value	267.716**													

** Significant (P<0.01)

The genotypes and genotypic frequencies distribution of microsatellite ETH 225 in the Sangamneri goat is presented in Table 4.2. Total 28 different genotypic combinations were observed and genotype 8, 15 (160 bp and 176 bp) with a frequency of 0.100 represented the most predominant genotypes for the ETH 225 locus in the Sangamneri population.

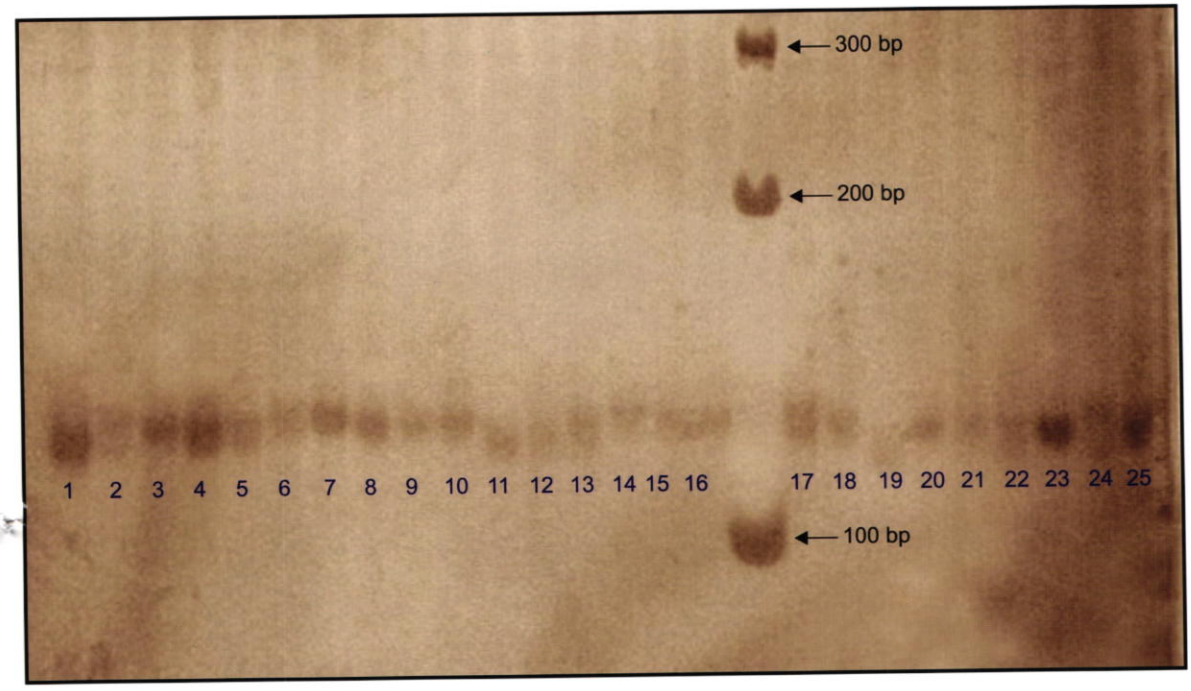
4.1.2 Microsatellite ILSTS 002

Microsatellite ILSTS 002 contained (CA)17 repeats, and is located at 66-99 bp nucleotide position in 182 bp sequence. It is located on chromosome five in goat (Kemp *et al.*, 1992) and on chromosome no 14 in sheep (Maddox *et al.*, 2001).

ILSTS-002 was found to exhibit 8 alleles (Plate 3, Table 4.3) in Sangamneri goat breed. Similar number of allele were also reported by Kumar *et al.* (2005) in Marwari goat (7), Gour *et al.* (2006) in Jamunapari goat (6), Sharma *et al.* (2008[a]) in Barbari goat (7) and Dixit *et al.* (2011) in Kanniadu goat (8), while Dixit *et al.* (2010) reported more number (16) of allele in southern Indian goat breed and Mishra *et al.* (2010) in Changthangi goat (10). Allele 4 (126 bp) was the most predominant allele, while the alleles 7 (132 bp) were present in the population with the least frequency of 0.023 for this microsatellite locus (Table 4.3).

The range of alleles size for the microsatellite locus was found in between 118 and 134 bp. The allelic size observed for Sangamneri goat was comparable to the allelic size for this locus reported by Kumar *et al.* (2005) in Marwari (118-130), Gour *et al.* (2006) in Jamunapari (119-129), Sharma *et al.* (2008[a]) for Barbari (114-128) and Dixit *et al.* (2010) for Southern Indian goat breed (113-135).

The genotypes and genotypic frequency distribution of microsatellite locus ILSTS 002 in the Sangamneri goat is depicted in Table 4.4. Total 8 different genotypic combinations were observed for this locus and the genotype 4, 4 (126 bp and 126 bp) with a frequency of 0.363 represented the most predominant genotypes for the locus in the Sangamneri goat population.



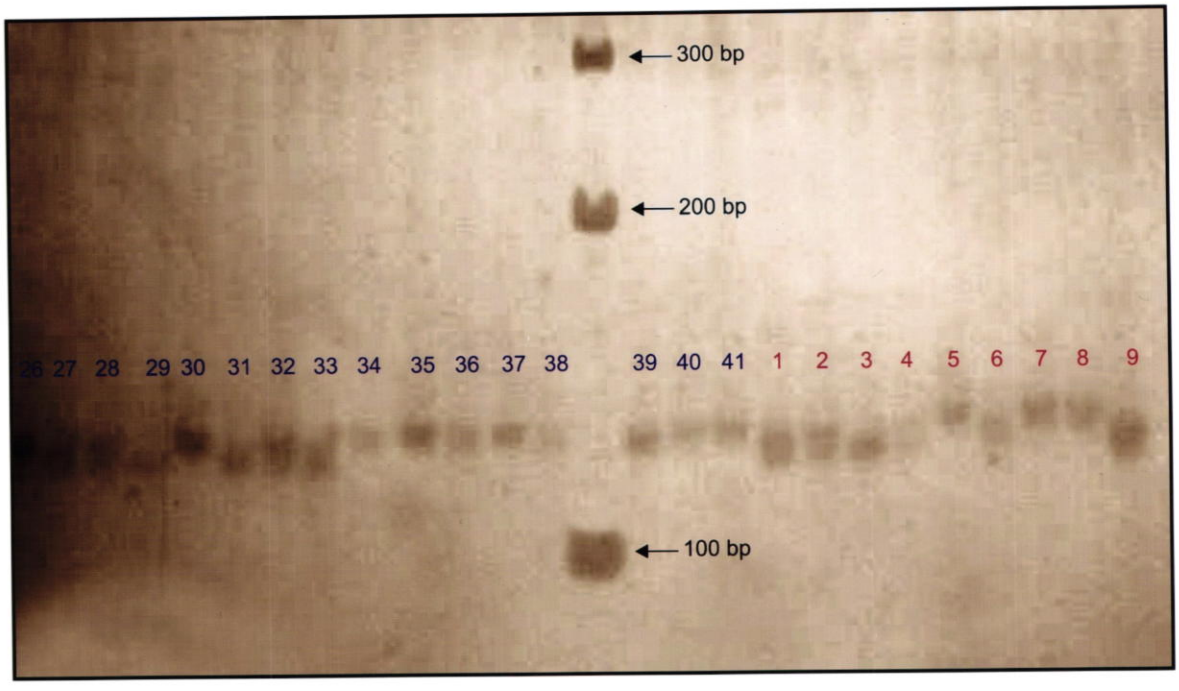


Plate 3. Resolution of PCR products of microsatellite locus ILSTS 002 on 8% urea polyacrylamide gel

Blue colour numbers are Female (F1 to F41) = 1 to 41
Red colour numbers are Male (M1 to M9) = 1 to 9

Table 4.3. Frequency distribution and allele size of microsatellite ILSTS002 in Samgamneri goat

Allelle number	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Allele size (bp)	118	122	124	126	128	130	132	134
Allelic frequency	0.045	0.114	0.159	0.364	0.136	0.114	0.023	0.045

Table 4.4. Genotypes and genotypic frequency distribution of microsatellite ILSTS002 in Sangamneri goat

Genotype	**1,1**	**2,2**	**3,3**	**4,4**	**5,5**	**6,6**	**7,7**	**8,8**
Observed count	2	5	7	16	6	5	1	2
Genotypic frequency	0.045	0.113	0.159	0.363	0.136	0.113	0.022	0.045
Chi Square value	308.00**							

** Significant (P<0.01)

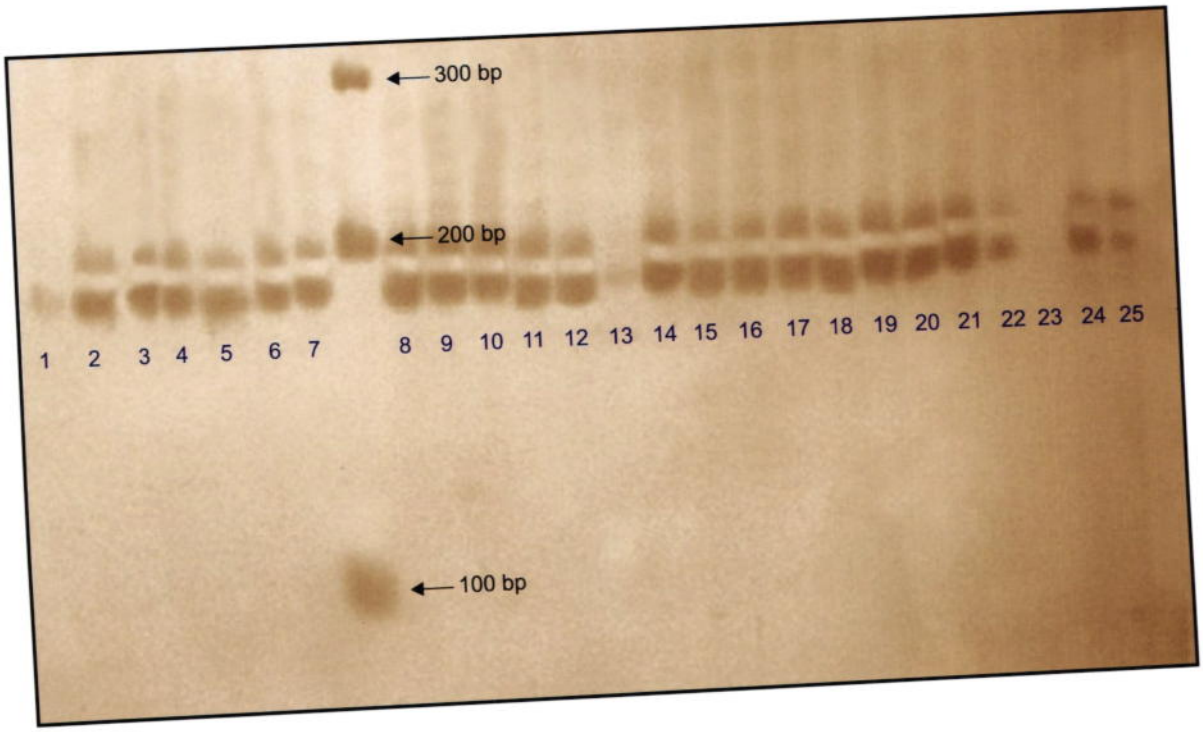
4.1.3 Microsatellite ILSTS 005

Microsatellite ILSTS 005 contains (nn)39 repeats. The microsatellite is located at nucleotide position 61-128 in the 280 bp sequence (Kemp *et al.*, 1993). Microsatellite ILSTS 005 is located at 31 cM from the beginning of BP31 and at 12 cM from the beginning of MCM 185 on chromosome 10 in goat (Schibler *et al.*, 1998), while in sheep this microsatellite is located on chromosome 7 (Kemp *et al.*, 1995).

For ILSTS 005 microsatellite locus total 18 alleles were reported for Sangamneri goat population (Plate 4 and Table 4.5). Total number of allele observed in the present study were at higher side than reported by Kumar *et al.* (2005) in Marwari goat (3), Gour *et al.* (2006) in Jamunapari goat (3), Sharma *et al.* (2008[a]) in Barbari goat (4) and Dixit *et al.* (2010) in Southern Indian goat breed (9). Mishra *et al.* (2010) in Changthangi goat (9) and Dixit *et al.* (2011) in Kanniadu (6).

The size of alleles locus ranged between 177 and 211 bp. The narrow range of allelic size were observed for other goat breed as compare to Sangamneri goat and reported between 179-185 bp in Marwari goat (Kumar *et al.*, 2005), 177-183 bp in Jamunapari goat (Gour *et al.*, 2006) and 172-190 bp in Barbari goat (Sharma *et al.* 2008[a]) and Dixit *et al.* (2010) for Southern Indian goat breed (113-135).

The genotypes and genotypic frequencies observed in the Sangamneri goat population in the present study for the microsatellite locus ILSTS 005 are presented in Table 4.6 and total 21 different genotypic combinations were found at this microsatellite locus. The genotype 4,11 with a frequency of 0.125 represented the most predominant genotypes for the locus in the Sangamneri population.



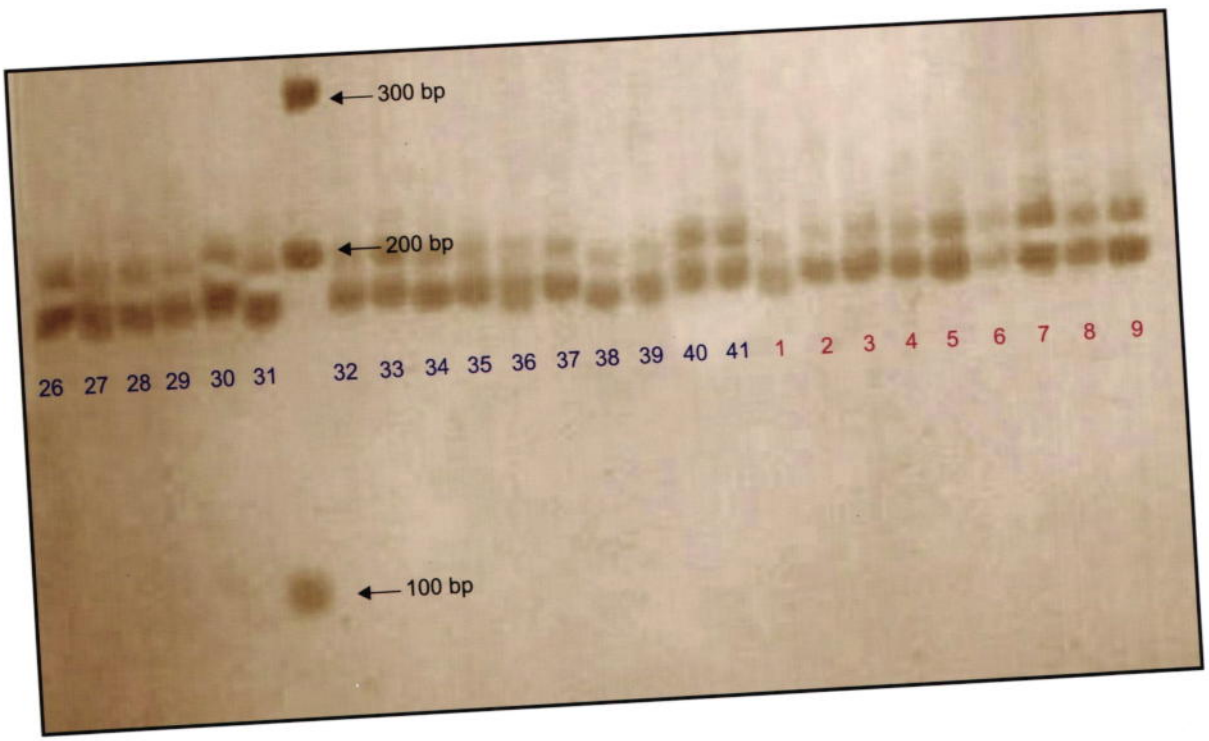


Plate 4. Resolution of PCR products of microsatellite locus ILSTS 005 on 8% urea polyacrylamide gel

Blue colour numbers are Female (F1 to F41) = 1 to 41
Red colour numbers are Male (M1 to M9) = 1 to 9

Table 4.5. Frequency distribution and allele size of microsatellite ILSTS005 in Sangamneri goat

Allelle number	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
Allele size (bp)	177	179	181	183	185	187	189	191	193
Allelic frequency	0.010	0.021	0.073	0.135	0.083	0.042	0.052	0.052	0.063
Allelle number	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**
Allele size (bp)	195	197	199	201	203	205	207	209	211
Allelic frequency	0.052	0.094	0.083	0.073	0.021	0.073	0.021	0.021	0.031

Table 4.6. Genotypes and genotypic frequency distribution of microsatellite ILSTS 005 in Sangamneri goat

Genotype	**4,4**	**3,8**	**1,9**	**2,9**	**2,10**	**3,10**	**3,11**	**4,11**	**4,12**
Observed count	1	1	1	1	1	3	3	6	4
Genotypic frequency	0.021	0.021	0.021	0.021	0.021	0.062	0.062	0.125	0.083
Genotype	**5,12**	**5,13**	**6,13**	**6,14**	**7,14**	**4,15**	**7,15**	**8,15**	**8,16**
Observed count	4	4	3	1	1	1	4	2	2
Genotypic frequency	0.083	0.083	0.062	0.021	0.021	0.021	0.083	0.042	0.042
Genotype	**9,17**	**9,18**	**10,18**						
Observed count	2	2	1						
Genotypic frequency	0.042	0.042	0.021						
Chi Square value	330.118**								

** Significant (P<0.01)

4.1.4 Microsatellite ILSTS 008

Microsatellite ILSTS 008 contains (CA)12 repeats, and is located at 83-104 nucleotide position in 179 bp sequence (Pepin *et al.*, 1995). It is located on chromosome 9 in sheep (Kemp *et al.*, 1995) and on chromosome 14 in goat (Saitbekova *et al.*, 1999).

The studied Sangamneri population exhibited 32 alleles for this locus (Plate 5 and Table 4.7). Total number of allele observed for Microsatellite ILSTS 008 locus were at higher side than reported by Kumar *et al.* (2005) in Marwari goat (8), Gour *et al.* (2006) in Jamunapari goat (4), Sharma *et al.* (2008[a]) in Barbari goat (5), Dixit *et al.* (2010) in Southern Indian goat breed (11), Mishra *et al.* (2010) in Changthangi (6) and Dixit *et al.* (2011) in Kanniadu (10). Allele 11 (182 bp) was the most predominant allele with a frequency of 0.112.

The allelic size for the ILSTS 008 microsatellite locus ranged between 162 and 238 bp Narrow range of allelic size were observed by Kumar *et al.* (2005) for Marwari goat (172-190), Gour *et al.* (2006) for Jamunapari goat (174-189), Sharma *et al.* (2008[a]) for Barbari goat (171-181) and Dixit *et al.* (2010) for Southern Indian goat breed (167-195).

Genotypes and genotypic frequency distribution of microsatellite locus ILSTS008 in Sangamneri goat are presented in Table 4.8. Total 36 different genotypic combinations were observed at this microsatellite locus. The most predominant genotypes for the locus in the Sangamneri population was the genotype 11,11 with a frequency of 0.082.

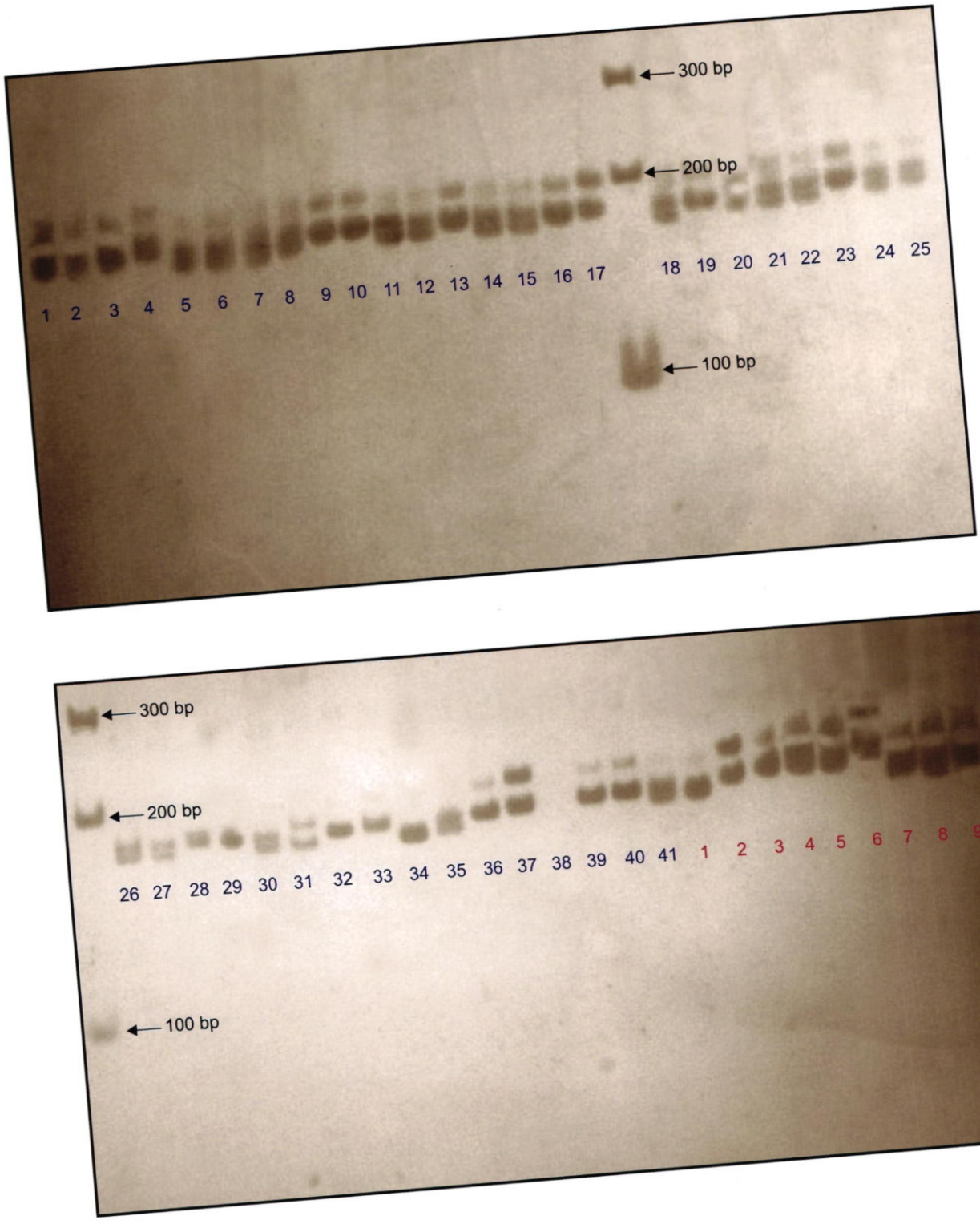


Plate 5. Resolution of PCR products of microsatellite locus ILSTS 008 on 8% urea polyacrylamide gel

Blue colour numbers are Female (F1 to F41) = 1 to 41
Red colour numbers are Male (M1 to M9) = 1 to 9

Table 4.7. Frequency distribution and allele size of microsatellite ILSTS008 in Sangamneri goat

Allelle number	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
Allele size (bp)	162	164	166	168	170	172	174	176	178	180	182	184
Allelic frequency	0.020	0.040	0.020	0.041	0.031	0.010	0.031	0.102	0.041	0.041	0.112	0.041
Allelle number	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**22**	**23**	**24**
Allele size (bp)	186	188	190	192	194	196	198	200	202	204	206	210
Allelic frequency	0.031	0.020	0.020	0.092	0.010	0.051	0.020	0.010	0.031	0.020	0.031	0.020
Allelle number	**25**	**26**	**27**	**28**	**29**	**30**	**31**	**32**				
Allele size (bp)	212	214	218	220	222	226	228	238				
Allelic frequency	0.010	0.010	0.020	0.020	0.010	0.010	0.020	0.010				

Table 4.8. Genotypes and genotypic frequency distribution of microsatellite ILSTS 008 in Sangamneri goat

Genotype	**1,1**	**3,3**	**4,4**	**7,7**	**2,8**	**8,8**	**10,10**	**2,11**	**11,11**	**12,12**	**4,13**	**8,14**
Observed count	1	1	1	1	1	2	2	3	4	2	1	2
Genotypic frequency	0.020	0.020	0.020	0.020	0.020	0.041	0.041	0.061	0.082	0.041	0.020	0.041
Genotype	**4,15**	**5,16**	**6,16**	**7,16**	**16,16**	**5,17**	**5,18**	**8,18**	**9,18**	**9,19**	**8,20**	**9,21**
Observed count	1	1	1	1	2	1	1	2	2	1	1	1
Genotypic frequency	0.020	0.020	0.020	0.020	0.041	0.020	0.020	0.041	0.041	0.020	0.020	0.020
Genotype	**13,23**	**15,24**	**16,25**	**16,26**	**21,27**	**19,28**	**22,28**	**21,29**	**22,30**	**23,31**	**24,31**	**27,32**
Observed count	2	1	1	1	1	1	1	1	1	1	1	1
Genotypic frequency	0.041	0.020	0.020	0.020	0.020	0.020	0.020	0.020	0.020	0.020	0.020	0.020
Chi Square value	728.648**											

** Significant ($p<0.01$)

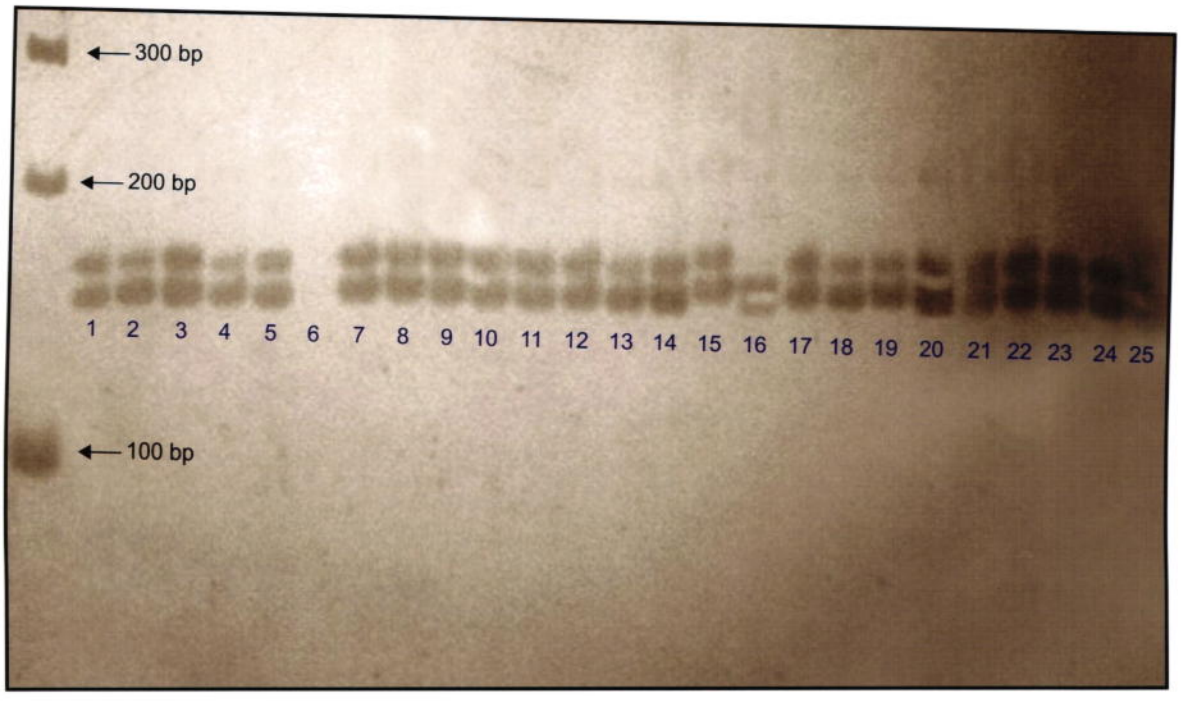
4.1.5 Microsatellite ILSTS 019

Microsatellite ILSTS 019 contains (TG)10 repeats and is located at 103 – 122 nucleotide position in 227 bp sequence (Kemp *et al.*, 1995). Microsatellite ILSTS 019 is located on chromosome 25 in goat (Kemp *et al.*, 1995).

Frequency distribution and allele size of microsatellite ILSTS 019 in Sangamneri goat are presented in Table 4.9. In the Sangamneri goat population 19 alleles were observed for this locus(Plate 6). Higher number of allele were observed in the present study than reported by Kumar *et al.* (2005) in Marwari goat (6), Gour *et al.* (2006) in Jamunapari goat (6), Sharma *et al.* (2008[a]) in Barbari goat (5), Verma *et al.* (2009) in Malabari (10) and Dixit *et al.* (2010) in Southern Indian goat breed (9). Allele 9 (161 bp) and 12 (167 bp) was the most predominant allele with a frequency of 0.120.

The size of alleles for the microsatellite locus was found in the range between 145 and 183 bp. Narrow range of allelic size were observed by Kumar *et al.* (2005) in Marwari goat (147-157), Gour *et al.* (2006) in Jamunapari goat (145-155), Sharma *et al.* (2008[a]) for Barbari goat (148-156) and Dixit *et al.* (2010) for Southern Indian goat breed (142-162).

The genotypes and genotypic frequencies observed for the microsatellite locus ILSTS 019 in the Sangamneri goat population in the present study are given in Table 4.10. Total 30 different genotypic combinations were found at this microsatellite locus and the genotype 6,12 (155 bp, 167 bp) with a frequency of 0.080 represented the most predominant genotypes in the Sangamneri population for the locus ILSTS 019.



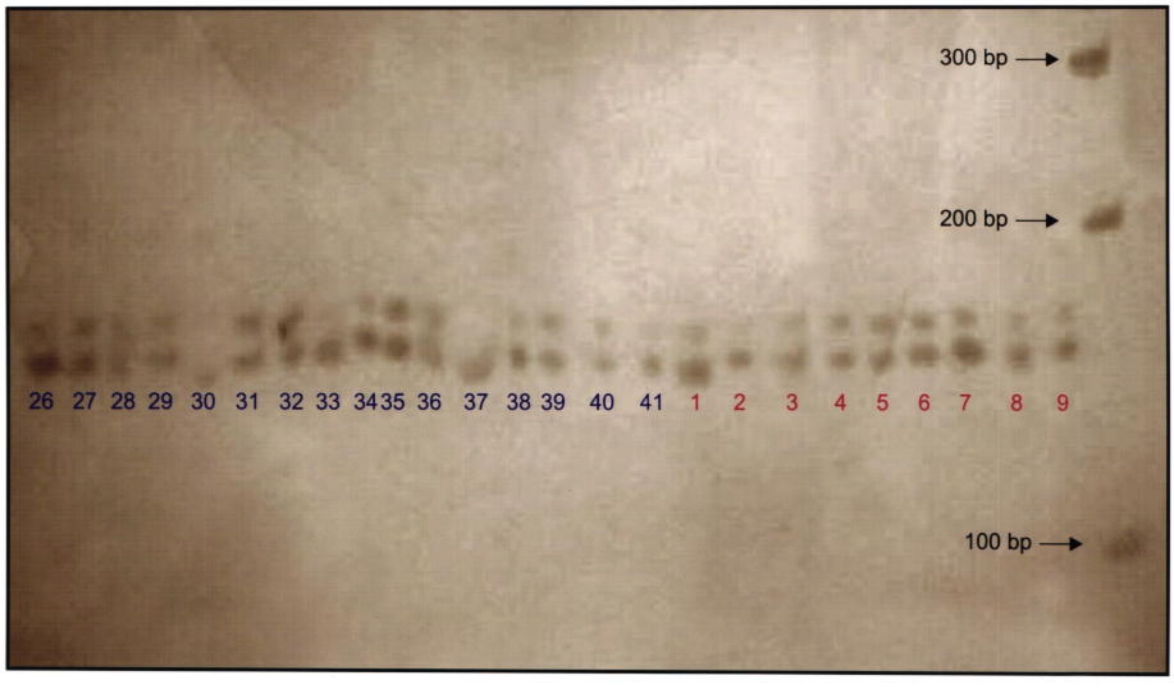


Plate 6. Resolution of PCR products of microsatellite locus ILSTS 019 on 8% urea polyacrylamide gel

Blue colour numbers are Female (F1 to F41) = 1 to 41
Red colour numbers are Male (M1 to M9) = 1 to 9

Table 4.9. Frequency distribution and allele size of microsatellite ILSTS019 in Sangamneri goat

Allelle number	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
Allele size (bp)	145	147	149	151	153	155	157	159	161	163
Allelic frequency	0.020	0.010	0.080	0.090	0.070	0.070	0.030	0.080	0.120	0.050
Allelle number	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	
Allele size (bp)	165	167	169	171	173	175	179	181	183	
Allelic frequency	0.030	0.120	0.030	0.040	0.060	0.010	0.040	0.030	0.020	

Table 4.10. Genotypes and genotypic frequency distribution of microsatellite ILSTS019 in Sangamneri goat

Genotype	**1,1**	**3,3**	**4,4**	**5,5**	**6,6**	**7,7**	**2,8**	**3,8**	**3,9**	**4,9**
Observed count	1	2	3	1	1	1	1	1	3	1
Genotypic frequency	0.020	0.040	0.060	0.020	0.020	0.020	0.020	0.020	0.060	0.020
Genotype	**9,9**	**4,10**	**5,10**	**5,11**	**7,11**	**5,12**	**6,12**	**9,12**	**6,13**	**9,13**
Observed count	1	2	2	1	1	2	4	1	1	1
Genotypic frequency	0.020	0.040	0.040	0.020	0.020	0.040	0.080	0.020	0.020	0.020
Genotype	**8,14**	**10,14**	**8,15**	**9,15**	**9,16**	**11,17**	**12,17**	**13,17**	**12,18**	**14,19**
Observed count	1	1	3	3	1	1	2	1	3	2
Genotypic frequency	0.020	0.020	0.060	0.060	0.020	0.020	0.040	0.020	0.060	0.040
Chi Square value	309.927**									

** Significant (P<0.01)

4.1.6 Microsatellite ILSTS 022

Microsatellite ILSTS 022 contains (TG)21 repeats, and is located at 352 – 393 nucleotide position in 502 bp sequence published by Kemp *et al.* (1995). Microsatellite ILSTS 022 is located on chromosome 5 in goat (Kemp *et al.,* 1995), While in sheep, this microsatellite is located on chromosome 3 (Kemp *et al.,* 1995).

Frequency distribution and allele size of microsatellite ILSTS 022 in Sagamneri goat are presented in Table 4.11. In the Sangamneri population studied, at this microsatellite locus 21 alleles observed (Plate 7) and allele 4 (197 bp) with a frequency of 0.204 was the most predominant allele followed by alleles 5 (199 bp) with a frequency of 0.112 for ILSTS 022 microsatellite locus. Higher number of allele were observed in the present study than reported by Kumar *et al.* (2005) in Marwari goat (3), Gour *et al.* (2006) in Jamunapari goat (4), Sharma *et al.* (2008[a]) in Barbari goat (6), Dixit *et al.* (2010) in Southern Indian goat breed (7) and Dixit *et al.* (2011) in Kanniadu (7).

The allelic size for the microsatellite locus ranged between 191 and 225 bp (Plate 7). The allelic size observed for Sangamneri goat was comparable to the allelic size for this locus reported between 191-197 bp in Marwari goat (Kumar *et al.,* 2005), 191-198 bp in Jamunapari goat (Gour *et al.*, 2006) and 190-204 bp in Barbari goat (Sharma *et al.* 2008[a]) and 186-202bp for Southern Indian goat breed (Dixit *et al.,* 2010).

The genotypes and genotypic frequencies observed in the Sangamneri goat population for the microsatellite locus ILSTS 022 are presented in Table 4.12. Total 32 different genotypic combinations were observed and the most predominant genotypes for the locus in the Sangamneri population was the genotype 4, 4 that presented a frequency of 0.10.

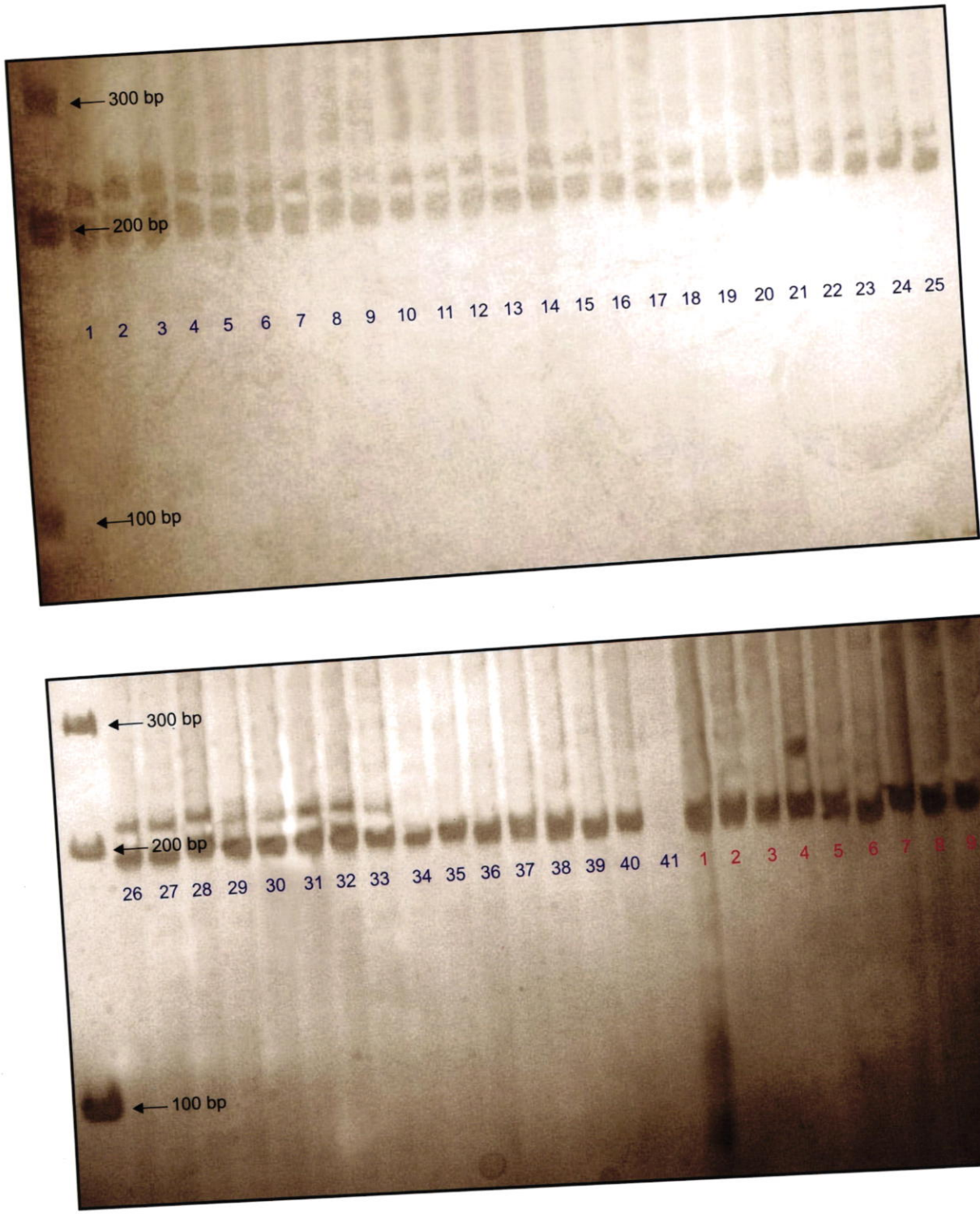


Plate 7. Resolution of PCR products of microsatellite locus ILSTS 022 on 8% urea polyacrylamide gel

Blue colour numbers are Female (F1 to F41) = 1 to 41
Red colour numbers are Male (M1 to M9) = 1 to 9

Table 4.11. Frequency distribution and allele size of microsatellite ILSTS022 in Sangamneri goat

Allelle number	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
Allele size (bp)	191	193	195	197	199	201	203	205	207	209	211	213
Allelic frequency	0.010	0.031	0.031	0.204	0.112	0.092	0.102	0.031	0.051	0.041	0.031	0.041
Allelle number	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**			
Allele size (bp)	215	217	219	221	223	225	227	237	239			
Allelic frequency	0.041	0.061	0.051	0.010	0.020	0.010	0.010	0.010	0.010			

Table 4.12. Genotypes and genotypic frequency distribution of microsatellite ILSTS 022 in Sangamneri goat

Genotype	**4,4**	**5,5**	**6,6**	**7,7**	**2,9**	**5,9**	**1,10**	**2,10**	**4,10**	**3,11**	**4,11**	**5,11**
Observed count	5	3	3	4	1	1	1	1	1	1	1	1
Genotypic frequency	0.102											
Genotype	**4,12**	**5,12**	**6,12**	**3,13**	**4,13**	**4,14**	**5,14**	**6,14**	**7,14**	**2,15**	**4,15**	**5,15**
Observed count	2	1	1	2	2	2	1	1	2	1	2	1
Genotypic frequency												
Genotype	**6,15**	**8,16**	**8,17**	**9,17**	**10,18**	**9,19**	**9,20**	**8,21**				
Observed count	1	1	1	1	1	1	1	1				
Genotypic frequency												
Chi Square value	318.76**											

** Significant (P<0.01)

4.1.7 Microsatellite ILSTS 029

Microsatellite ILSTS 029 contains (AC)19 repeats, and this microsatellite is located at 227-256 nucleotide position in 511 bp sequence in bovine (Kemp *et al.*, 1995). It is located on goat chromosome 3 (Luikart *et al.*, 1999).

At this microsatellite locus 19 alleles were found and the allele size for the microsatellite locus varied in the range between 142 and 184 bp (Plate 8, Table 4.13). Similar number of alleles were observed by Dixit *et al.* (2010) in Southern Indian goat breed (17), while, Kumar *et al.* (2005), Gour *et al.* (2006), Sharma *et al.* (2008[a]), Verma *et al.* (2009) and Dixit *et al.* (2011) reported less number of allele for Marwari (8), Jamunapari (3), Barbari (4), Malabari (14) and Kanniadu (10) goat, respectively.

The allelic size observed for Sangamneri goat was comparable to the allelic size for this locus reported by Kumar *et al.* (2005) in Marwari (154-186 bp), Gour *et al.* (2006) in Jamunapari (155-171 bp), Sharma *et al.* (2008[a]) for Barbari (153-167 bp) and Dixit *et al.* (2010) for Southern Indian goat breed (148-191).

Genotypes and genotypic frequency distribution of microsatellite locus ILSTS 008 in Sangamneri goat are presented in Table 4.14. Total 36 different genotypic combinations were found in the Sangamneri goat population studied and genotype 3,8 (146 bp and 156 bp) with a frequency of 0.125 represented the most predominant genotypes for the locus in the Sangamneri population.

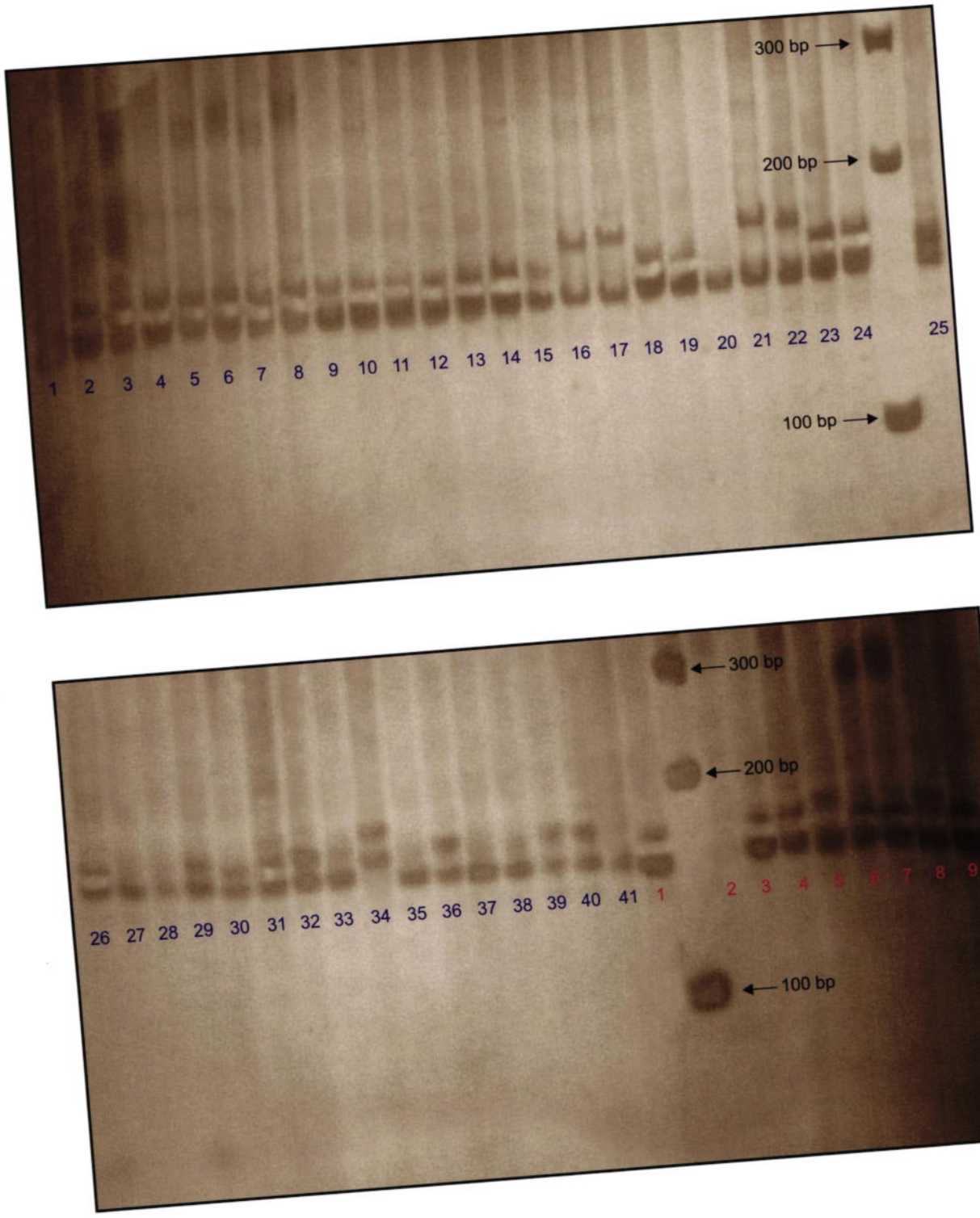


Plate 8. Resolution of PCR products of microsatellite locus ILSTS 029 on 8% urea polyacrylamide gel

Blue colour numbers are Female (F1 to F41) = 1 to 41
Red colour numbers are Male (M1 to M9) = 1 to 9

Table 4.13. Frequency distribution and allele size of microsatellite ILSTS029 in Sangamneri goat

Allelle number	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**
Allele size (bp)	142	144	146	148	150	152	154	156	158	160	162	164	166
Allelic frequency	0.021	0.031	0.094	0.073	0.021	0.104	0.052	0.198	0.052	0.031	0.042	0.031	0.042
Allelle number	**14**	**15**	**16**	**17**	**18**	**19**							
Allele size (bp)	168	170	172	174	178	184							
Allelic frequency	0.042	0.031	0.083	0.031	0.010	0.010							

Table 4.14. Genotypes and genotypic frequency distribution of microsatellite ILSTS029 in Sangamneri goat

Genotype	**4,4**	**1,5**	**1,6**	**2,6**	**6,6**	**2,7**	**3,7**	**7,7**	**2,8**	**3,8**	**4,8**	**8,8**	**4,9**
Observed count	1	1	1	1	1	1	1	1	1	6	1	2	2
Genotypic frequency	0.021	0.021	0.021	0.021	0.021	0.021	0.021	0.021	0.021	0.125	0.021	0.042	0.042
Genotype	**4,10**	**6,10**	**6,11**	**4,12**	**6,12**	**7,13**	**8,13**	**3,14**	**6,14**	**9,14**	**3,15**	**8,15**	**9,15**
Observed count	1	1	1	1	1	1	2	1	2	1	1	1	1
Genotypic frequency	0.021	0.021	0.021	0.021	0.021	0.021	0.042	0.021	0.042	0.021	0.021	0.021	0.021
Genotype	**5,16**	**6,16**	**8,16**	**9,16**	**10,16**	**11,16**	**11,17**	**12,17**	**8,18**	**13,19**			
Observed count	1	1	3	1	1	1	2	1	1	1			
Genotypic frequency	0.021	0.021	0.063	0.021	0.021	0.021	0.042	0.021	0.021	0.021			
Chi Square value	192.273**												

** Significant (P<0.01)

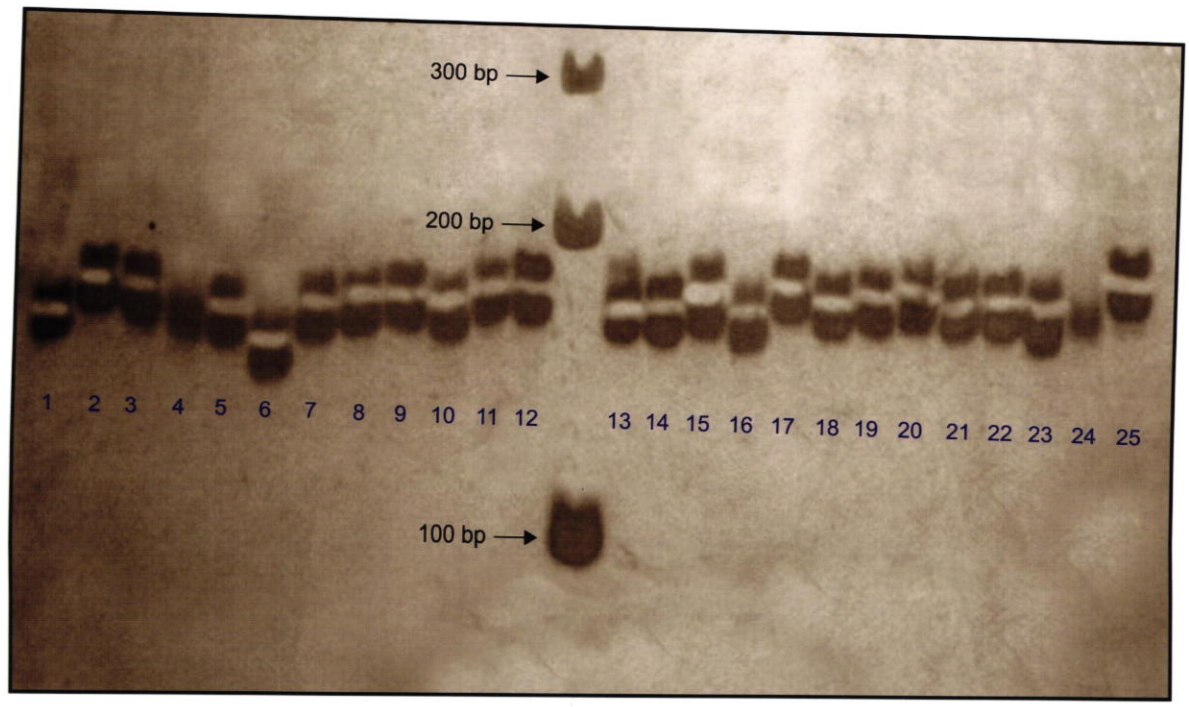
4.1.8 Microsatellite ILSTS 030

Contains (GT)13 repeat and is located at 445 – 471 nucleotide position in 556 bp sequence (Kemp *et al.*, 1995).Microsatellite ILSTS 030 is located at 15 cM from CSSM 42 and at 32 cM from the beginning of INRA 040 (Schibler *et al.*, 1998) on chromosome 2 in goat.

Sangamneri breed exhibited 25 alleles for Microsatellite ILSTS 030 (Plate 9 and Table 4.15). Kumar *et al.* (2005) in Marwari goat (6), Gour *et al.* (2006) in Jamunapari goat (7), Sharma *et al.* (2008[a]) in Barbari goat (6), Verma *et al.* (2009) in Malabari goat (9) and Dixit *et al.* (2010) in Southern Indian goat breed (11) reported lower number of alleles than the present study.

The size of alleles for the microsatellite locus ILSTS 030 ranged between 140 bp and 204 bp. Kumar *et al.* (2005), Gour *et al.* (2006), Sharma *et al.* (2008[a]) and Dixit *et al.* (2010) reported narrow range of allele size for Marwari (164-174 bp), for Jamunapari (151-173 bp), for Barbari (153-171 bp) and for Southern Indian goat breed (159-179 bp), respectively.

The genotypes and genotypic frequencies observed for the microsatellite locus ILSTS 030 in the Sangamneri goat population in the present study are given in Table 4.16. Total 41 different genotypic combinations were observed at this locus.



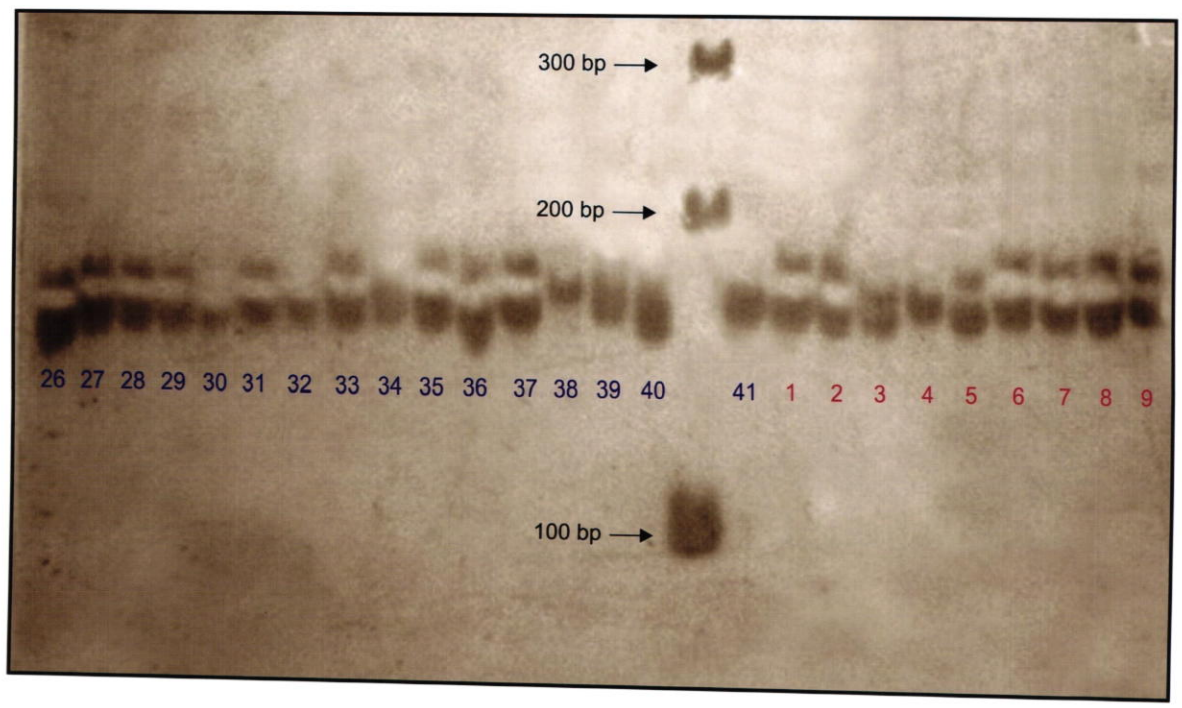


Plate 9. Resolution of PCR products of microsatellite locus ILSTS 030 on 8% urea polyacrylamide gel

Blue colour numbers are Female (F1 to F41) = 1 to 41
Red colour numbers are Male (M1 to M9) = 1 to 9

Table 4.15. Frequency distribution and allele size of microsatellite ILSTS030 in Sangamneri goat

Allelle number	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**
Allele size (bp)	140	144	148	150	152	154	156	158	160	162	164	166	168
Allelic frequency	0.010	0.010	0.010	0.020	0.010	0.090	0.060	0.070	0.070	0.050	0.050	0.080	0.080
Allelle number	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**22**	**23**	**24**	**25**	
Allele size (bp)	170	172	174	176	178	180	182	184	186	188	190	204	
Allelic frequency	0.080	0.030	0.030	0.060	0.020	0.030	0.040	0.010	0.020	0.050	0.010	0.010	

Table 4.16. Genotypes and genotypic frequency distribution of microsatellite ILSTS030 in Sangamneri goat

Genotype	**1,4**	**6,6**	**7,7**	**8,8**	**9,9**	**3,10**	**10,10**	**4,11**	**6,12**	**12,12**	**6,13**	**7,13**	**13,13**
Observed count	1	2	1	2	1	1	2	1	1	1	1	1	1
Genotypic frequency	0.020	0.040	0.020	0.040	0.020	0.020	0.040	0.020	0.020	0.020	0.020	0.020	0.020
Genotype	**6,14**	**7,14**	**9,14**	**5,15**	**6,15**	**9,15**	**8,16**	**9,16**	**8,17**	**9,17**	**11,17**	**17,17**	**9,18**
Observed count	2	2	1	1	1	1	1	1	2	1	1	1	1
Genotypic frequency	0.040	0.040	0.020	0.020	0.020	0.020	0.020	0.020	0.040	0.020	0.020	0.020	0.020
Genotype	**11,18**	**11,19**	**12,19**	**12,20**	**13,20**	**14,20**	**13,21**	**14,22**	**11,23**	**12,23**	**13,23**	**16,23**	**14,24**
Observed count	1	1	1	1	2	1	1	1	1	2	1	1	1
Genotypic frequency	0.020	0.020	0.020	0.020	0.040	0.020	0.020	0.020	0.020	0.040	0.020	0.020	0.020
Genotype	**19,25**												
Observed count	1												
Genotypic frequency	0.020												
Chi Square value	354.135**												

** Significant (P<0.01)

4.1.9 Microsatellite ILSTS 033

Microsatellite ILSTS 033 contains (CA)12 repeats and is located at 133 – 156 nucleotide position in 288 bp sequence (Kemp *et al.*, 1995). Microsatellite ILSTS 033 is located at 108 cM from the beginning of BMS 2252 on chromosome 12 of the goat map (Schibler *et al.*, 1998).

In the studied Sangamneri population total 13 alleles reported and the allelic size for the microsatellite ILSTS 033 locus ranged between 161 and 191 bp (Plate 10 and Table 4.17). Higher number of alleles were observed by Verma *et al.* (2009) in Malabari goat (15) and Dixit *et al.* (2010) in Southern Indian goat breed (21), while, Kumar *et al.* (2005), Gour *et al.* (2006) and Sharma *et al.* (2008[a]) reported less number of allele for Marwari (9), Jamunapari (5) and Barbari (6) goat, respectively. Allele 4 (171 bp) was the most predominant allele with a frequency of 0.130 for this microsatellite locus in the Sangamneri population studied.

The allelic size for the locus in Sangamneri goat was comparable to the allelic size for this locus reported in some other breeds of goat Kumar *et al.* (2005) reported the 159-182 bp allele size in Marwari goat, Gour *et al.* (2006) reported the 156-182 bp in Jamunapari goat, Sharma *et al.* (2008[a]) reported the 170-182 bp in Barbari goat and Dixit *et al.* (2010) reported 151-187 bp in Southern Indian goat breed which includes Ganjam, Attapady, Malbari, Kanniadu, Sangamneri and Osmnanabadi goat breed.

The genotypes and genotypic frequencies observed in the Sangamneri goat population in the present study for the microsatellite locus ILSTS 033 are presented in Table 4.18. In all 15 different genotypic combinations were observed at this microsatellite locus. The genotype 4,11 with a frequency of 0.140 represented the most predominant genotypes for the locus in the Sangamneri population.

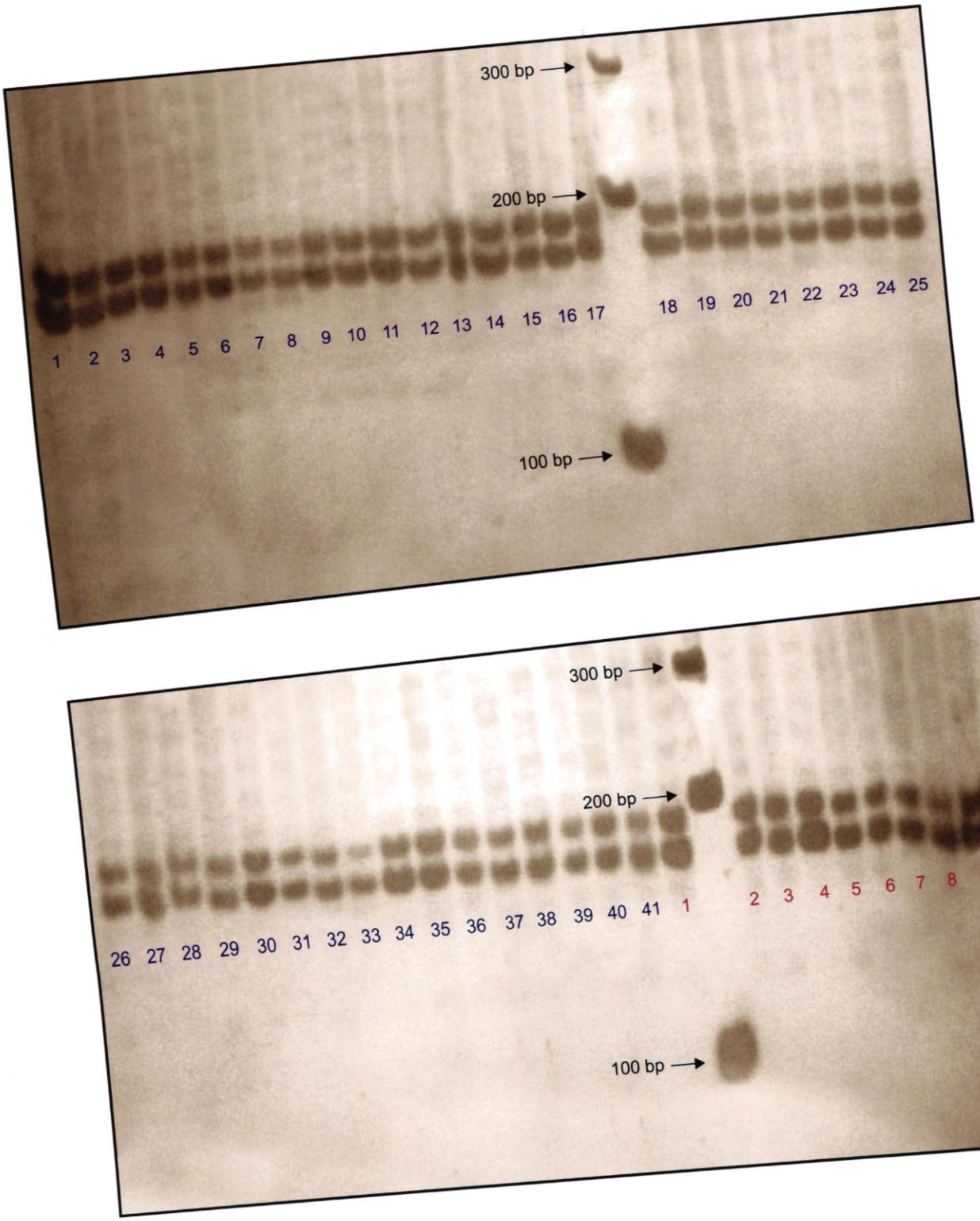


Plate 10. Resolution of PCR products of microsatellite locus ILSTS 033 on 8% urea polyacrylamide gel

Blue colour numbers are Female (F1 to F41) = 1 to 41
Red colour numbers are Male (M1 to M9) = 1 to 9

Table 4.17. Frequency distribution and allele size of microsatellite ILSTS033 in Sangamneri goat

Allele No.	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Allele size (bp)	161	167	169	171	173	175	177	181
Allelic frequency	0.020	0.020	0.100	0.130	0.080	0.110	0.060	0.010
Allele No.	**9**	**10**	**11**	**12**	**13**			
Allele size (bp)	183	185	187	189	191			
Allelic frequency	0.070	0.110	0.110	0.080	0.100			

Table 4.18. Genotypes and genotypic frequency distribution of microsatellite ILSTS033 in Sangamneri goat

Genotype	**1,6**	**1,7**	**2,8**	**2,9**	**3,9**	**3,10**	**4,10**	**5,10**
Observed count	1	1	1	1	6	4	6	1
Genotypic frequency	0.020	0.020	0.020	0.020	0.120	0.080	0.120	0.020
Genotype	**4,11**	**5,11**	**5,12**	**6,12**	**7,12**	**6,13**	**7,13**	
Observed count	7	4	3	4	1	6	4	
Genotypic frequency	0.140	0.080	0.060	0.080	0.020	0.120	0.080	
Chi Square value	258.476**							

** Significant (P<0.001)

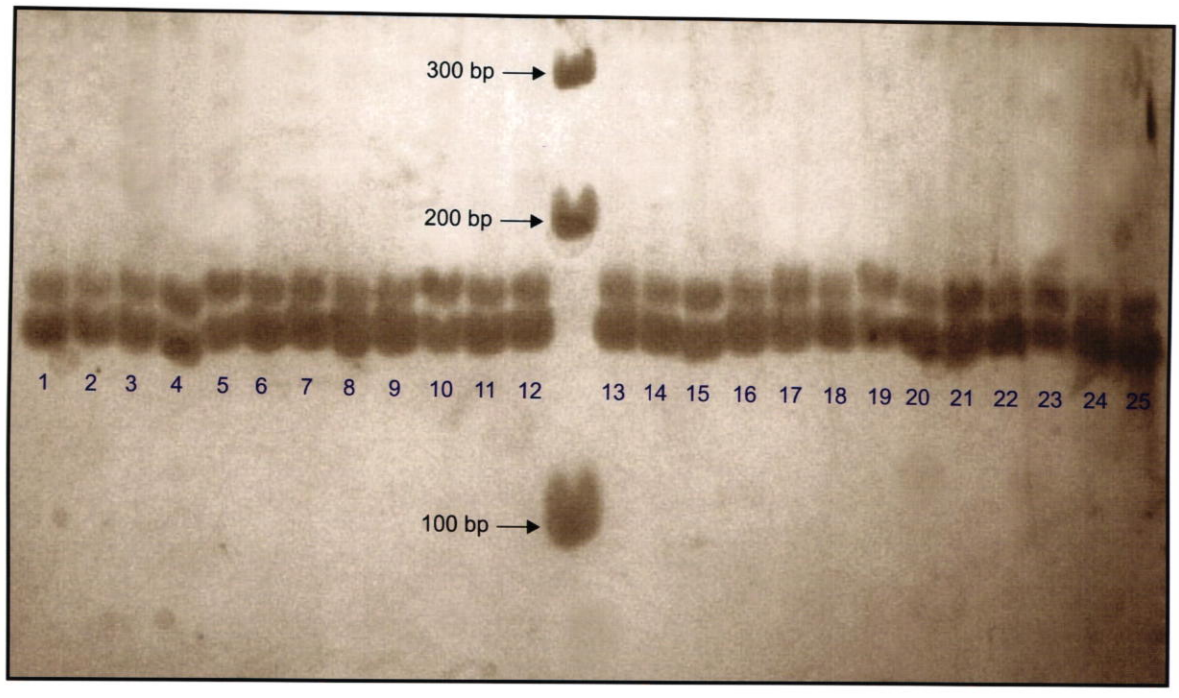
4.1.10 Microsatellite ILSTS 034

Microsatellite ILSTS 034 contains (GT)19 repeats, and this microsatellite is located at 257 – 394 nucleotide position in 557 bp sequence (Kemp *et al.*, 1995). It is located at 58 cM from the beginning of BM 0321 and 21 cM from BM 2830 on chromosome 5 in goat map (Schibler *et al.*, 1998).

Frequency distribution and allele size of microsatellite ILSTS 034 in Sangamneri goat are presented in Table 4.19. Sangamneri goat was found to be exhibited 13 alleles (Plate 11). Similar number of alleles were also observed by Dixit *et al.* (2010) in Southern Indian goat breed while, Kumar *et al.* (2005), Gour *et al.* (2006), Sharma *et al.* (2008[a]), Verma *et al.* (2010) and Dixit *et al.* (2011) reported less number of allele for Marwari (6), Jamunapari (3), Barbari (5), Malabari (8) and in Kanniadu (6) goat breed, respectively. Allele 5 (156 bp) with a frequency of 0.170 was the most predominant allele for this microsatellite locus in the Sangamneri goat population studied (Table 4.19).

The size of alleles for the microsatellite locus ILSTS 034 ranged between 148 bp and 184 bp. The allelic size for the locus in Sangamneri goat was comparable to the allelic size for this locus reported in some other breeds of goat as Kumar *et al.* (2005) reported the 158-180 bp in Marwari goat, Gour *et al.* (2006) reported the 156-178 bp in Jamunapari goat, Sharma *et al.* (2008[a]) reported the 151-171 bp in Barbari goat and Dixit *et al.* (2010) reported 153-185 bp in Southern Indian goat breed which includes Ganjam, Attapady, Malbari, Kanniadu, Sangamneri and Osmnanabadi goat breed.

The genotypes and genotypic frequencies observed in the Sangamneri goat population in the present study for the microsatellite locus ILSTS 034 are given in Table 4.20. Total 17 different genotypic combinations at this locus and the genotype 4,10 (154 bp, 170 bp) and 5,10 (156 bp, 170 bp) with a frequency of 0.160 represented the most predominant genotypes for the locus in the Sangamneri population.



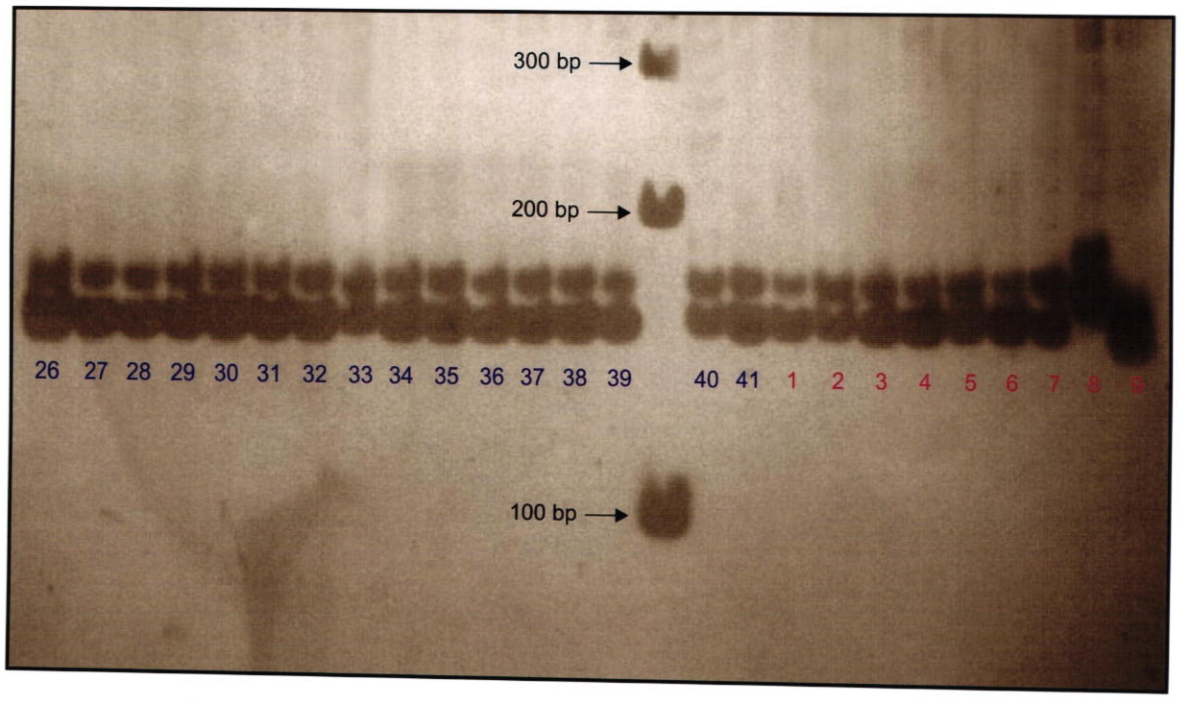


Plate 11. Resolution of PCR products of microsatellite locus ILSTS 034 on 8% urea polyacrylamide gel

Blue colour numbers are Female (F1 to F41) = 1 to 41
Red colour numbers are Male (M1 to M9) = 1 to 9

Table 4.19. Frequency distribution and allele size of microsatellite ILSTS034 in Sangamneri goat

Allele No.	**1**	**2**	**3**	**4**	**5**	**6**	**7**
Allele size (bp)	148	150	152	154	156	158	164
Allelic frequency	0.010	0.030	0.060	0.150	0.170	0.060	0.010
Allele No.	**8**	**9**	**10**	**11**	**12**	**13**	
Allele size (bp)	166	168	170	172	174	184	
Allelic frequency	0.020	0.150	0.230	0.080	0.020	0.010	

Table 4.20. Genotypes and genotypic frequency distribution of microsatellite ILSTS034 in Sangamneri goat

Genotype	**1,7**	**2,8**	**3,9**	**4,9**	**5,9**	**9,9**	**2,10**
Observed count	1	1	3	7	3	1	2
Genotypic frequency	0.020	0.020	0.060	0.140	0.060	0.020	0.040
Genotype	**3,10**	**4,10**	**5,10**	**6,10**	**3,11**	**5,11**	**6,11**
Observed count	2	8	8	3	1	5	2
Genotypic frequency	0.040	0.160	0.160	0.060	0.020	0.100	0.040
Genotype	**5,12**	**6,12**	**8,13**				
Observed count	1	1	1				
Genotypic frequency	0.020	0.020	0.020				
Chi Square value	243.073**						

** Significant (P<0.001)

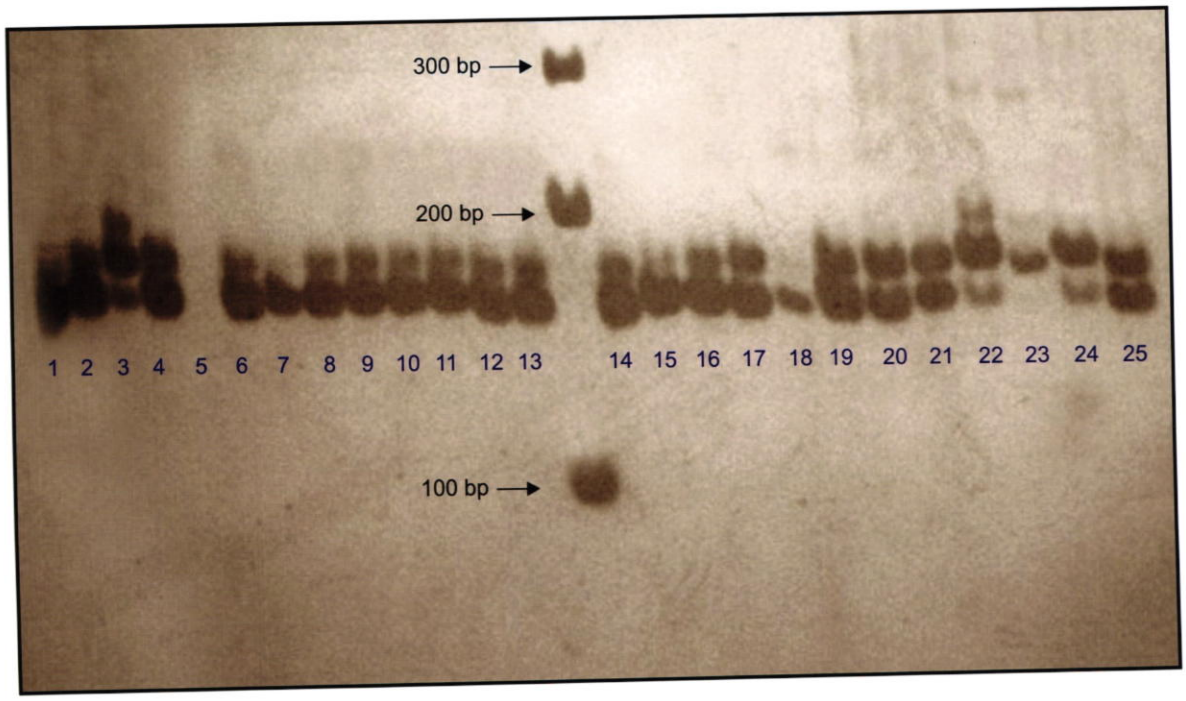
4.1.11 Microsatellite ILSTS 044

Microsatellite ILSTS 044 contains (GT)20 repeats, and this microsatellite is lsocated at 263 - 302 nucleotide position in 497 bp sequence (Kemp *et al.*, 1995). Microsatellite ILSTS 044 is located on chromosome 3 in goat (Kemp *et al.*, 1995).

In Sangamneri goat total 25 alleles were observed for microsatellite ILSTS 044 (Plate 12 and Table 4.22). More number of allele were observed in the present study than reported by Kumar *et al.* (2005) in Marwari goat (6), Sharma *et al.* (2008[a]) in Barbari goat (2), Sharma *et al* (2008[b]) in Beetal (6) and Dixit *et al.* (2010) in Southern Indian goat breed (16).

The allele size for the microsatellite ILSTS 044 locus varied in the range between 141 and 197 bp. The allelic size observed for the locus in Sangamneri goat had wide range as comparable to the findings of Kumar *et al.* (2005) reported 150-172 bp in Marwari goat, Sharma *et al.* (2008[a]) reported 155-157 in Barbari goat and Dixit *et al.* (2010) reported 145-177 in Southern Indian goat breed. Allele 15 (171 bp) was the most predominant allele for this microsatellite locus in the population studied with a frequency of 0.130.

The genotypes and genotypic frequencies observed in the present study for the microsatellite locus ILSTS 044 in the Sangamneri goat population are depicted in Table 4.22. Total 32 different genotypic combinations at this microsatellite locus and the genotype 6,5 and 8,15 with a frequency of 0.65 represented the most predominant genotypes for the locus in the Sangamneri population (Table 4.21).



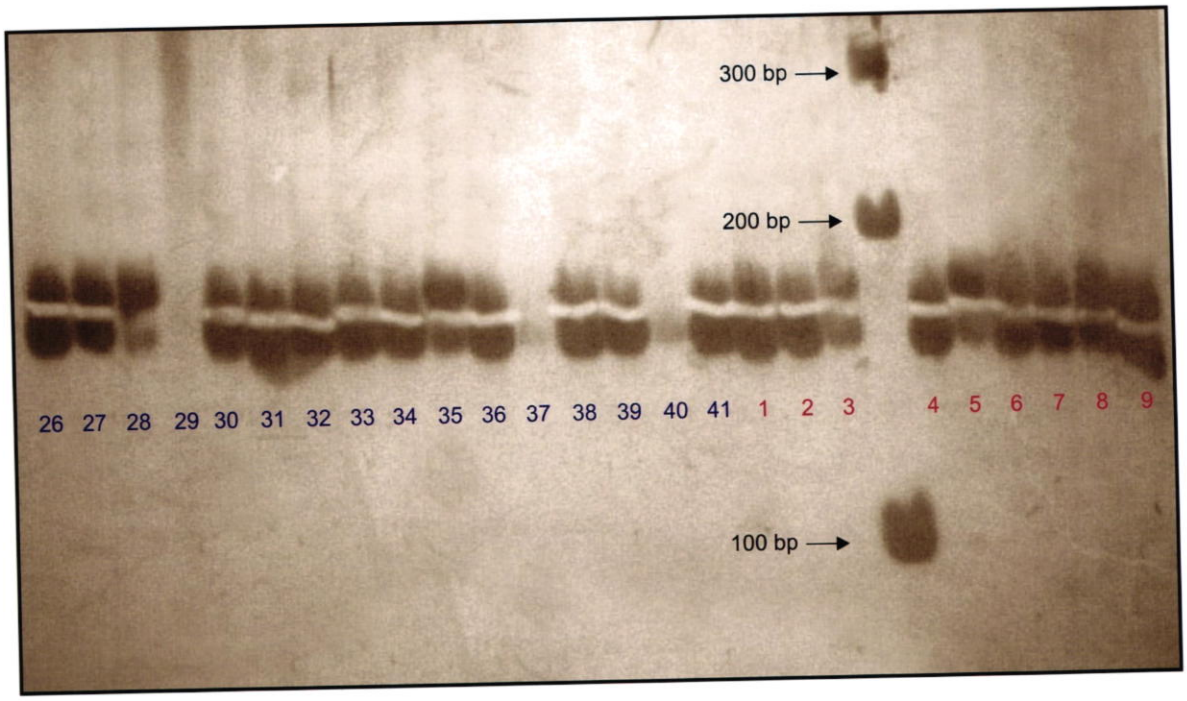


Plate 12. Resolution of PCR products of microsatellite locus ILSTS 044 on 8% urea polyacrylamide gel

Blue colour numbers are Female (F1 to F41) = 1 to 41
Red colour numbers are Male (M1 to M9) = 1 to 9

Table 4.21. Frequency distribution and allele size of microsatellite ILSTS044 in Sangamneri goat

Allelle number	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**
Allele size (bp)	141	143	145	149	151	153	155	157	159	161	163
Allelic frequency	0.011	0.011	0.043	0.022	0.033	0.054	0.043	0.098	0.087	0.043	0.043
Allelle number	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**22**
Allele size (bp)	165	167	169	171	173	175	177	179	181	183	185
Allelic frequency	0.022	0.065	0.054	0.130	0.065	0.043	0.022	0.011	0.022	0.011	0.022
Allelle number	**23**	**24**	**25**								
Allele size (bp)	189	195	197								
Allelic frequency	0.011	0.011	0.022								

Table 4.22. Genotypes and genotypic frequency distribution of microsatellite ILSTS 044 in Sangamneri goat

Genotype	**4,4**	**1,5**	**7,7**	**2,9**	**3,9**	**3,10**	**3,11**	**5,13**	**8,13**	**6,14**	**7,14**
Observed count	1	1	1	1	1	2	1	2	2	1	2
Genotypic frequency	0.021	0.021	0.021	0.021	0.021	0..043	0.021	0..043	0..043	0.021	0..043
Genotype	**8,14**	**6,15**	**8,15**	**9,15**	**15,15**	**6,16**	**8,16**	**9,16**	**10,16**	**8,17**	**9,17**
Observed count	2	3	3	2	2	1	1	2	1	1	2
Genotypic frequency	0..043	0.065	0.065	0..043	0..043	0.021	0.021	0..043	0.021	0.021	0..043
Genotype	**10,17**	**11,18**	**11,20**	**12,21**	**12,22**	**13,22**	**13,23**	**16,24**	**19,25**	**20,25**	
Observed count	1	2	1	1	1	1	1	1	1	1	
Genotypic frequency	0.021	0..043	0.021	0.021	0.021	0.021	0.021	0.021	0.021	0.021	
Chi Square value	443.129**										

** Significant (P<0.01)

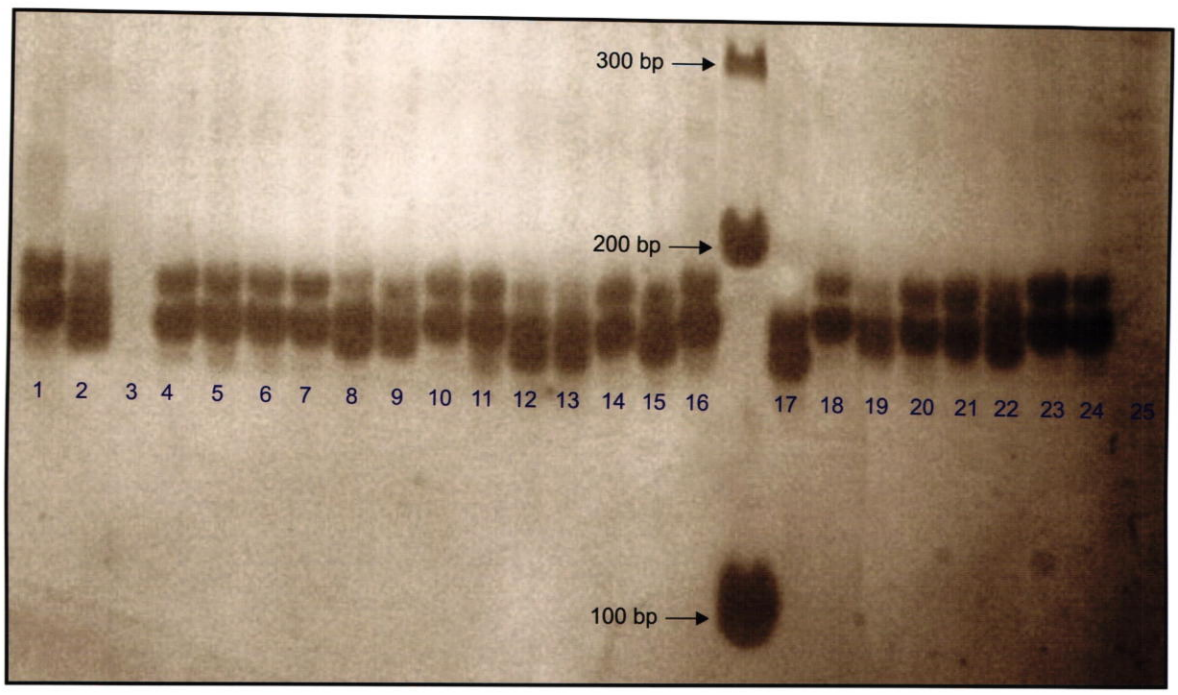
4.1.12 Microsatellite ILSTS 049

Microsatellite ILSTS 049 contained (CA)24 repeats located at 92-197 nucleotide position in the 546 sequence in bovine (Kemp *et al.*, 1995). Microsatellite ILSTS 049 is located on chromosome 11 in goat (Kemp *et al.*, 1995).

At this microsatellite ILSTS 049 locus 18 alleles were observed for microsatellite (Plate 13 and Table 4.23) Lower number of alleles were reported by Kumar *et al.* (2005) in Marwari goat (5), Gour *et al.* (2006) in Jamunapari goat (6), Sharma *et al.* (2008[a]) in Barbari goat (5), Verma *et al.* (2009) in Malabari goat (8), Dixit *et al.* (2010) in Southern Indian goat breed (9) and Dixit *et al.* (2011) in Kanniadu (6) than the present study. Allele 8 (172 bp), with a frequency of 0.219 was the most predominant allele for this microsatellite locus in the Sangamneri goat population studied

The size of alleles for the microsatellite locus ranged between 158 and 194 bp (Table 4.23). The allelic size for the locus in Sangamneri goat was at wide range as compare to the earlier worker in other breeds of goat. Kumar *et al.* (2005) reported 165-179 bp in Marwari goat, Gour *et al.* (2006) reported the 167-179 bp in Jamunapari goat, Sharma *et al.* (2008[a]) reported the 170-178 bp in Barbari goat and Dixit *et al.* (2010) reported 160-184 bp in Southern Indian goat breed which includes Ganjam, Attapady, Malbari, Kanniadu, Sangamneri and Osmnanabadi goat breed.

Genotypes and genotypic frequency distribution of microsatellite locus ILSTS049 in Sangamneri goat are presented in Table 4.24. Total 23 different genotypic combinations were found in the Sangamneri population studied and the genotype 6,13 (168 bp, 182 bp) with a frequency of 0.104 represented the most predominant genotypes for the locus in the Sangamneri population (Table 4.24).



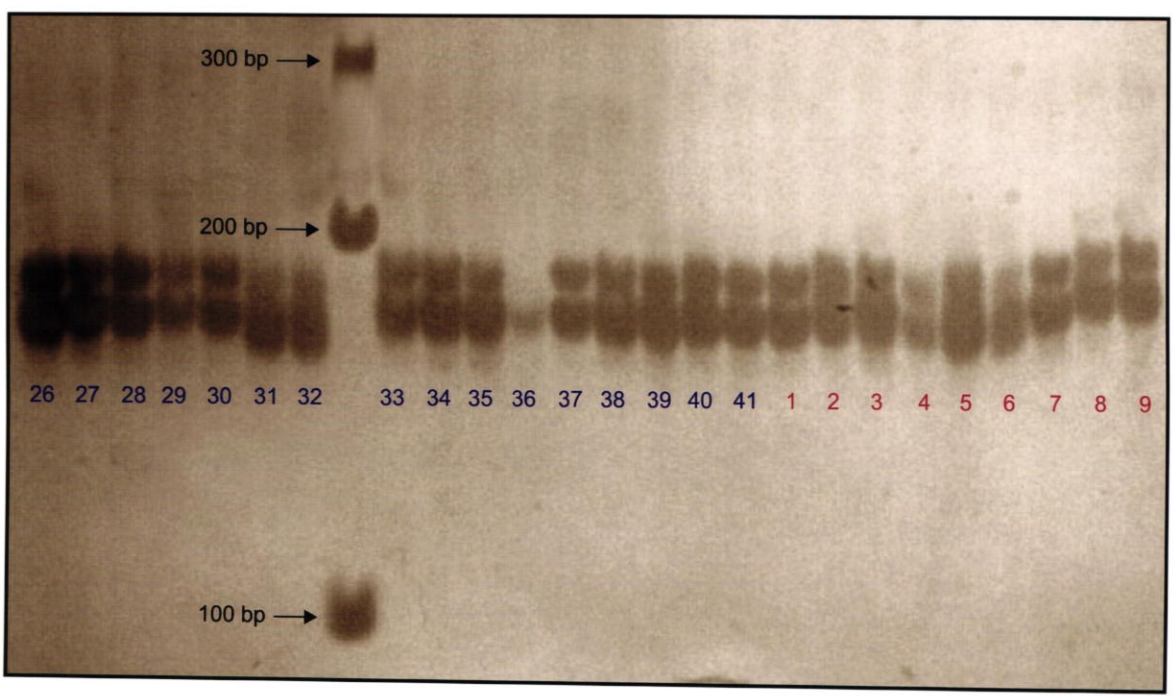


Plate 13. Resolution of PCR products of microsatellite locus ILSTS 049 on 8% urea polyacrylamide gel

Blue colour numbers are Female (F1 to F41) = 1 to 41
Red colour numbers are Male (M1 to M9) = 1 to 9

Table 4.23. Frequency distribution and allele size of microsatellite ILSTS049 in Sangamneri goat

Allelle number	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
Allele size (bp)	158	160	162	164	166	168	170	172	174
Allelic frequency	0.010	0.010	0.031	0.021	0.021	0.094	0.104	0.219	0.042
Allelle number	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**
Allele size (bp)	176	178	180	182	184	186	188	192	194
Allelic frequency	0.021	0.021	0.052	0.104	0.146	0.052	0.031	0.010	0.010

Table 4.24. Genotypes and genotypic frequency distribution of microsatellite ILSTS049 in Sangamneri goat

Genotype	**2,6**	**1,7**	**7,7**	**3,8**	**8,8**	**5,9**	**9,9**	**3,11**	**4,12**
Observed count	1	1	1	1	2	1	1	1	1
Genotypic frequency	0.021	0.021	0.021	0.021	0.042	0.021	0.021	0.021	0.021
Genotype	**6,12**	**4,13**	**6,13**	**7,13**	**8,13**	**5,14**	**7,14**	**8,14**	**7,15**
Observed count	3	1	5	3	1	1	2	11	1
Genotypic frequency	0.063	0.021	0.104	0.063	0.021	0.021	0.042	0.229	0.021
Genotype	**8,15**	**9,16**	**10,16**	**11,17**	**12,18**				
Observed count	4	1	2	1	1				
Genotypic frequency	0.083	0.021	0.042	0.021	0.021				
Chi Square value	291.446**								

** Significant (P<0.01)

4.1.13 Microsatellite ILSTS 058

Microsatellite ILSTS 058 contains (GT)15 repeats, and this microsatellite is located at 306 - 334 nucleotide position in 527 bp sequence (Kemp *et al.*, 1995). Microsatellite ILSTS 058 is located at 56 cM from the beginning of OarFCB048 and 4 cM from BL50 on chromosome 17 in goat (Schibler *et al.*, 1998).

For ILSTS 058 microsatellite locus total 18 alleles were reported for Sangamneri goat population (Plate 14, Table 4.25). More number of alleles were observed by Verma *et al.* (2009) in Malabari goat (26) and Dixit *et al.* (2010) in Southern Indian goat breed (25), while, less number of allele observed by Kumar *et al.* (2005) in Marwari goat (8), Gour *et al.* (2006) in Jamunapari goat (4), Sharma *et al.* (2008[a]) in Barbari goat (6), Sharma *et al.* (2008[b]) for Beetal goat (5) and Dixit *et al.* (2011) for Kanniadu (6) than the present study.

The allelic size for the microsatellite locus ranged between 158 and 210 bp. Kumar *et al.* (2005), Gour *et al.* (2006), Sharma *et al.* (2008[a]) reported and Dixit *et al.* (2010) reported narrow range of allelic size in Marwari, Jamunapari, Barbari and Southern Indian goat breed than the present findings. Allele 10 (178 bp) represented the most predominant allele for this microsatellite locus in the Sangamneri goat population studied.

Genotypes and genotypic frequency distribution of microsatellite locus ILSTS 058 in Sangamneri goat are presented in Table 4.26. The total 30 different genotypic combinations were observed at this microsatellite locus and The genotype 10,16 (178 bp, 190 bp) with a frequency of 0.104 represented the most predominant genotype for the locus in the Sangamneri population.

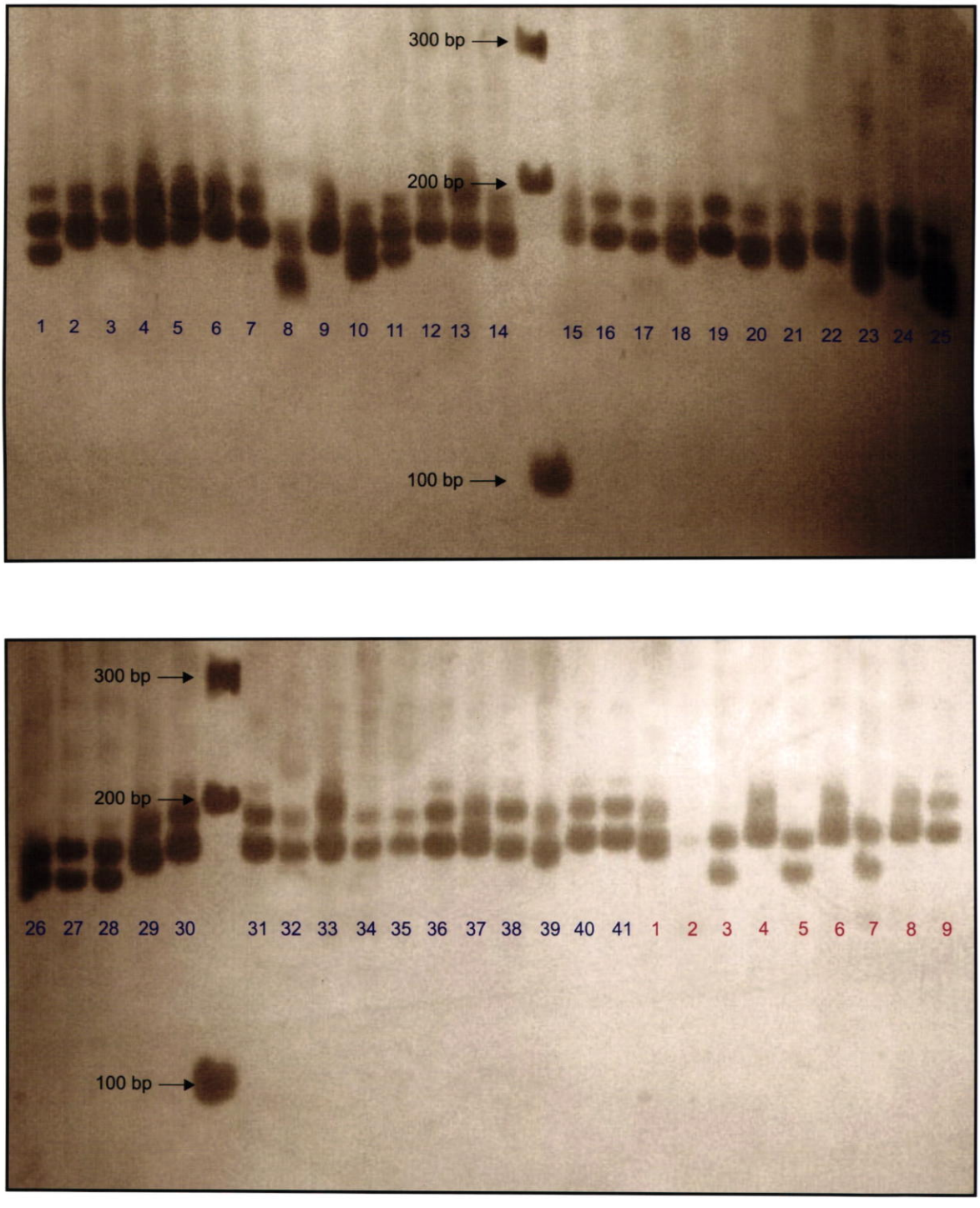


Plate 14. Resolution of PCR products of microsatellite locus ILSTS 058 on 8% urea polyacrylamide gel

Blue colour numbers are Female (F1 to F41) = 1 to 41
Red colour numbers are Male (M1 to M9) = 1 to 9

Table 4.25. Frequency distribution and allele size of microsatellite ILSTS058 in Sangamneri goat

Allele number	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
Allele size (bp)	158	162	164	166	168	170	172	174	176
Allelic frequency	0.021	0.021	0.010	0.021	0.021	0.010	0.073	0.083	0.042
Allele number	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**
Allele size (bp)	178	180	182	184	186	188	190	192	194
Allelic frequency	0.115	0.063	0.031	0.031	0.042	0.073	0.094	0.063	0.073
Allele number	**19**	**20**	**21**	**22**	**23**	**24**			
Allele size (bp)	196	198	200	206	208	210			
Allelic frequency	0.010	0.042	0.021	0.010	0.010	0.021			

Table 4.26. Genotypes and genotypic frequency distribution of microsatellite ILSTS 058 in Sangamneri goat

Genotype	**1,7**	**2,7**	**3,8**	**4,8**	**1,9**	**2,9**	**4,10**	**5,10**	**6,12**
Observed count	1	1	1	1	1	1	1	1	1
Genotypic frequency	0.021	0.021	0.021	0.021	0.021	0.021	0.021	0.021	0.021
Genotype	**5,13**	**7,14**	**7,15**	**8,15**	**8,16**	**9,16**	**10,16**	**9,17**	**10,17**
Observed count	1	2	3	4	2	1	5	1	2
Genotypic frequency	0.021	0.042	0.062	0.083	0.042	0.021	0.104	0.021	0.042
Genotype	**11,17**	**10,18**	**11,18**	**12,19**	**11,20**	**12,20**	**13,20**	**14,21**	**16,22**
Observed count	1	2	4	1	1	1	2	2	1
Genotypic frequency	0.021	0.042	0.083	0.021	0.021	0.021	0.042	0.042	0.021
Genotype	**17,23**	**17,24**	**18,24**						
Observed count	1	1	1						
Genotypic frequency	0.021	0.021	0.021						
Chi Square value	375.027**								

** Significant (P<0.01)

4.1.14 Microsatellite ILSTS 059

Microsatellite ILSTS 059 contains (CA)4 and (GT)21 repeats, and is located at 219 - 259 nucleotide position in 468 bp sequence (Kemp *et al.*, 1995). Microsatellite ILSTS 059 is located on chromosome 13 in goat Kemp *et al.* (1995).

Sangamneri breed exhibited 14 alleles for Microsatellite ILSTS 059 (Plate 15 and Table 4.27). Verma *et al.* (2009) in Malabari goat (11) and Dixit *et al.* (2010) in Southern Indian goat breed (12) reported slightly less number of alleles than the present study. Allele 11 (132 bp) was the most predominant allele with a frequency of 0.120 for this microsatellite locus in the Sangamneri goat population studied while the alleles 1 (108 bp) and 14 (138 bp) was present in the population with the least frequency of 0.011 for this microsatellite locus.

The allelic size for the microsatellite locus ranged between 108 and 138 bp. Similar range of allelic size were also reported by Dixit *et al.* (2010) for southern Indian goat breed (105-135).

The genotypes and genotypic frequencies observed in the Sangamneri goat population in the present study for the microsatellite locus ILSTS 059 are given in Table 4.28. Total 28 different genotypic combinations were observed for this locus and the genotype 5,11(120 bp, 132 bp) and 6,11 (122 bp, 132 bp) and 7,12 (124 bp, 134 bp) with a frequency of 0.086 represented the most predominant genotypes for the locus in the Sangamneri population.

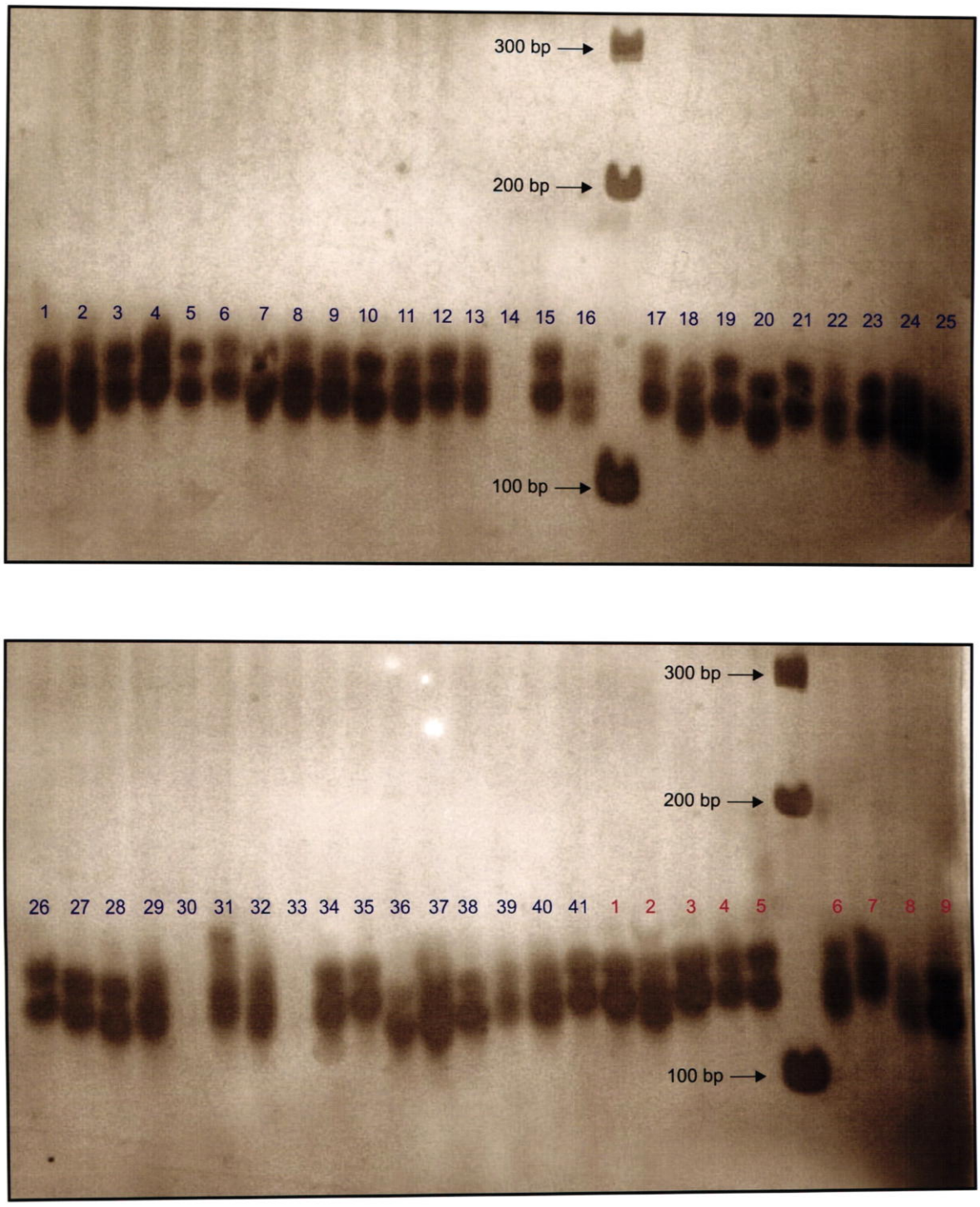


Plate 15. Resolution of PCR products of microsatellite locus ILSTS 059 on 8% urea polyacrylamide gel

Blue colour numbers are Female (F1 to F41) = 1 to 41
Red colour numbers are Male (M1 to M9) = 1 to 9

Table 4.27. Frequency distribution and allele size of microsatellite ILSTS059 in Sangamneri goat

Allelle number	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
Allele size (bp)	108	114	116	118	120	122	124	126	128	130
Allelic frequency	0.011	0.043	0.065	0.065	0.109	0.109	0.109	0.098	0.076	0.054
Allelle number	**11**	**12**	**13**	**14**						
Allele size (bp)	132	134	136	138						
Allelic frequency	0.120	0.065	0.065	0.011						

Table 4.28. Genotypes and genotypic frequency distribution microsatellite ILSTS059 in Sangamneri goat

Genotype	**2,2**	**3,3**	**1,4**	**4,4**	**5,5**	**2,6**	**6,6**	**3,7**	**3,8**	**4,8**
Observed count	1	1	1	1	1	1	1	1	2	1
Genotypic frequency	0.022	0.022	0.022	0.022	0.022	0.022	0.022	0.022	0.043	0.022
Genotype	**2,9**	**3,9**	**4,9**	**5,9**	**6,9**	**5,10**	**6,10**	**7,10**	**5,11**	**6,11**
Observed count	1	1	2	1	1	3	1	1	4	4
Genotypic frequency	0.022	0.022	0.043	0.022	0.022	0.065	0.022	0.022	0.086	0.086
Genotype	**7,11**	**8,11**	**7,12**	**8,12**	**6,13**	**7,13**	**8,13**	**9,14**		
Observed count	2	1	4	2	1	2	3	1		
Genotypic frequency	0.043	0.022	0.086	0.043	0.022	0.043	0.065	0.022		
Chi Square value	142.723**									

** Significant (P<0.001)

4.1.15 Microsatellite ILSTS 065

Microsatellite ILSTS 065 contains (CA)22 repeats, and this microsatellite is located at 130 – 173 nucleotide position in 615 bp sequence (Kemp *et al.*, 1995). Microsatellite ILSTS 065 is located at 24 cM from the beginning of BMS2526 and at 13 cM from ILSTS 031 on chromosome 24 in goat (Schibler *et al.*, 1998).

Sangamneri breed exhibited a total of 8 alleles (Plate 16, Table 4.29), More number of allele were observed by Dixit *et al.* (2010) in Southern Indian goat breed (10), however less number of allele were observed by Kumar *et al.* (2005) in Marwari goat (2), Gour *et al.* (2006) in Jamunapari goat (2), Verma *et al.* (2009) in Malabari goat (4) and Dixit *et al.* (2011) in Kanniadu (7). Allele 4 (121 bp) was the most predominant allele and was present in the population with a frequency of 0.362 for this microsatellite locus.

The range of alleles size for the microsatellite locus was found in between 115 and 133 bp (Table 4.29). The allelic size observed for the locus in Sangamneri goat was comparable to the allelic size for this locus reported in some other breeds of goat. Kumar *et al.* (2005) reported the 110-130 bp in Marwari goat, Gour *et al.* (2006) reported the 119-121 bp in Jamunapari goat and Dixit *et al.* (2010) reported 105-135 bp in Southern Indian goat.

The genotypes and genotypic frequencies observed in the Sangamneri goat population in the present study for the microsatellite locus ILSTS-065 are given in Table 4.30. Total 8 different genotypic combinations were observed for this locus and the genotype 4,7 with a frequency of 0.212 represented the most predominant genotypes for the locus in the Sangamneri population (Table 4.29).

Table 4.29. Frequency distribution and allele size of microsatellite ILSTS065 in Sangamneri goat

Allelle number	1	2	3	4	5	6	7	8
Allele size (bp)	115	117	119	121	123	129	131	133
Allelic frequency	0.043	0.064	0.298	0.362	0.021	0.085	0.106	0.021

Table 4.30. Genotypes and genotypic frequency distribution of microsatellite ILSTS065 in Sangamneri goat

Genotype	1,1	2,2	3,3	4,4	5,5	3,6	4,7	4,8
Observed count	2	3	10	11	1	8	10	2
Genotypic frequency	0.042	0.0638	0.212	0.234	0.021	0.170	0.212	0.042
Chi Square value	197.691**							

** Significant (P<0.001)

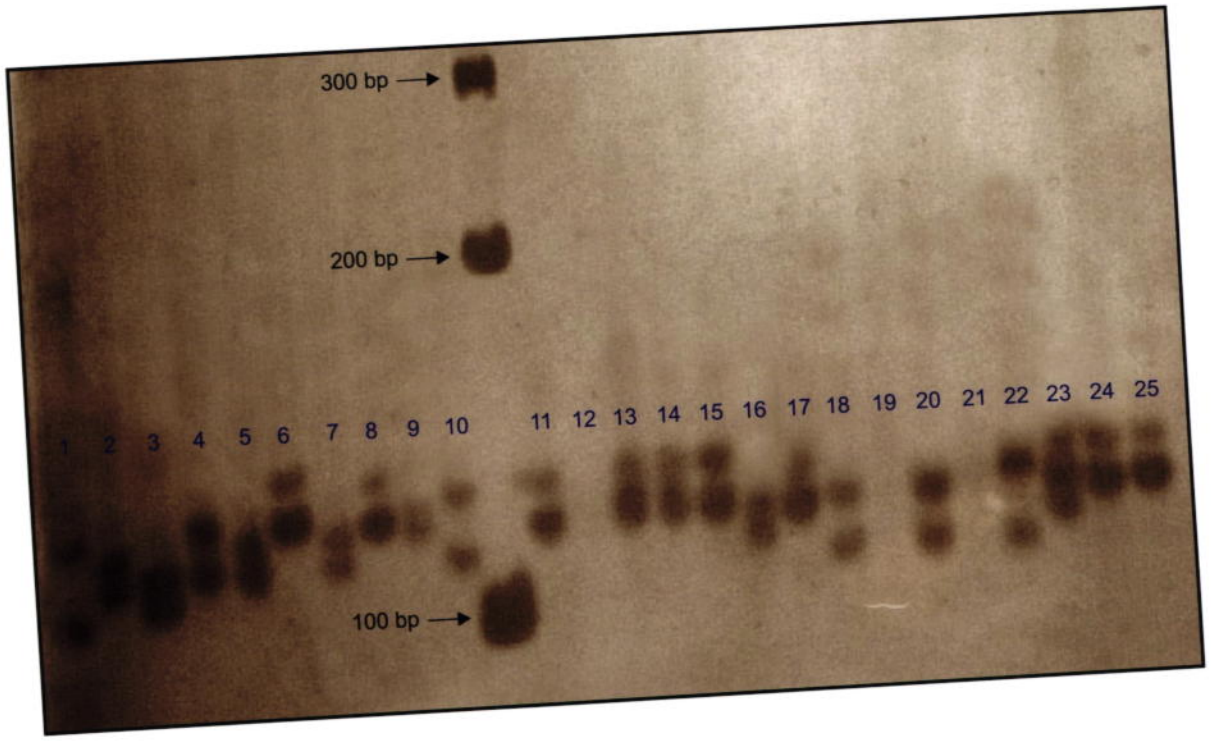
4.1.16 Microsatellite ILSTS 082

Microsatellite ILSTS 082 contained (GT)17 repeats, and is located at 164–197 nucleotide position in 560 bp sequence in bovine (Kemp *et al.*, 1995). It is located on chromosome 2 in goat (Schibler *et al.*, 1998).

For ILSTS 082 microsatellite locus total 15 alleles were reported for Sangamneri goat population (Plate 17, Table 4.31). More number of alleles were observed by Dixit *et al.* (2010) in southern Indian goat breed (18), while similar number of allele were observed by Verma *et al.* (2009) in Malabari goat (15). Less number of allele observed by Kumar *et al.* (2005) in Marwari goat (5), Gour *et al.* (2006) in Jamunapari goat (7), and Dixit *et al.* (2011) reported less number of allele for Kanniadu (12).

The size of alleles for the microsatellite locus ranged between 102 and 134 bp (Table 4.31). Similar range of allele locus were observed by Kumar *et al.* (2005) in Marwari goat (108-124 bp), Gour *et al.* (2006) in Jamunapari goat (108-136 bp) and Dixit *et al.* (2010) in Southern Indian goat breed (100-136 bp). Allele 8(118) and 9 (120 bp) was the most predominant allele with a frequency of 0.122, while the alleles 1 (102bp) and 2 (104 bp) were present in the population with the least frequency of 0.148 for this microsatellite locus (Table 4.31).

The genotypes and genotypic frequencies observed in the Sangamneri goat population in the present study for the microsatellite locus ILSTS 082 are presented in Table 4.32. Total 28 different genotypic combinations were observed at this microsatellite locus and the genotype 9,14 (120 bp, 130 bp) with a frequency of 0.111 represented the most predominant genotypes for the locus in the Sangamneri population.



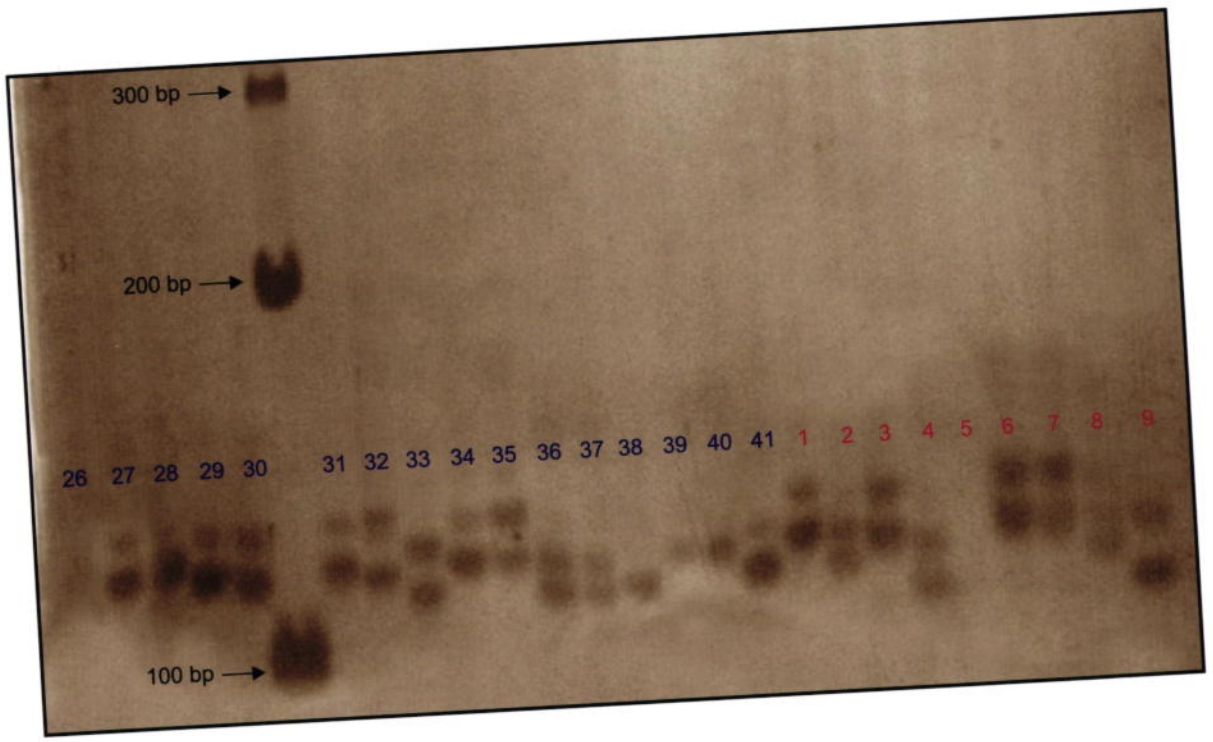


Plate 17. Resolution of PCR products of microsatellite locus ILSTS 082 on 8% urea polyacrylamide gel

Blue colour numbers are Female (F1 to F41) = 1 to 41
Red colour numbers are Male (M1 to M9) = 1 to 9

Table 4.31. Frequency distribution and allele size of microsatellite ILSTS082 in Sangamneri goat

Allelle number	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
Allele size (bp)	102	104	108	110	112	114	116	118	120	122
Allelic frequency	0.011	0.011	0.033	0.100	0.056	0.067	0.100	0.122	0.122	0.089
Allelle number	**11**	**12**	**13**	**14**	**15**					
Allele size (bp)	124	126	128	130	134					
Allelic frequency	0.056	0.056	0.056	0.100	0.022					

Table 4.32. Genotypes and genotypic frequency distribution of microsatellite ILSTS082 in Sangamneri goat

Genotype	**2,3**	**5,5**	**3,6**	**4,7**	**5,7**	**7,7**	**1,8**	**4,8**	**8,8**	**4,9**
Observed count	1	1	1	3	1	1	1	1	3	2
Genotypic frequency	0.022	0.022	0.022	0.066	0.022	0.022	0.022	0.022	0.066	0.044
Genotype	**5,9**	**6,9**	**4,10**	**6,10**	**7,10**	**3,11**	**6,11**	**4,12**	**5,12**	**7,12**
Observed count	1	1	2	1	1	1	2	1	1	1
Genotypic frequency	0.022	0.022	0.044	0.022	0.022	0.022	0.044	0.022	0.022	0.022
Genotype	**8,12**	**6,13**	**7,13**	**8,13**	**9,13**	**9,14**	**10,14**	**11,15**		
Observed count	2	1	1	1	2	5	4	2		
Genotypic frequency	0.044	0.022	0.022	0.022	0.044	0.111	0.088	0.044		
Chi Square value	172.717**									

** Significant ($p<0.001$)

4.1.17 Microsatellite ILSTS 087

Microsatellite ILSTS 087 contains (CA)14 repeats, and is located at 61 – 88 nucleotide position in 203 bp sequence Kemp *et al.* (1995). Microsatellite ILSTS-087 is located on chromosome 6 in goat (Kemp *et al.*, 1995).

For ILSTS 087 microsatellite locus total 17 alleles were reported for Sangamneri goat population (Plate 18 and Table 4.33). Total number of allele observed in the present study were at higher side than reported by Kumar *et al.* (2005) in Marwari goat (6), Gour *et al.* (2006) in Jamunapari goat (6), Sharma *et al.* (2008[a]) in Barbari goat (6), Verma *et al.* (2009) in Malabari goat (10), Dixit *et al.* (2010) in Southern Indian goat breed (9) and Dixit *et al.* (2011) in Kanniadu goat (7). Allele 7 (153 bp) was the most predominant allele at this microsatellite locus present in the Sangamneri goat population a frequency of 0.170 (Table 4.33).

The range of alleles size for the microsatellite locus was found in between 141 and 173 bp (Table 4.33). The allelic size observed for the locus in Sangamneri goat was comparable with the finding of Dixit *et al.* (2010) who reported 142-164 bp in Southern Indian goat breed which includes Ganjam, Attapady, Malbari, Kanniadu, Sangamneri and Osmnanabadi goat breed. While, Kumar *et al.* (2005), Gour *et al.* (2006) and Sharma *et al.* (2008[a]) reported the narrow range alleles size in Marwari goat (147-159 bp), Jamunapari goat (145-159 bp) and in Barbari goat (145-159 bp), respectively.

The genotypes and genotypic frequencies distribution of microsatellite ILSTS 087 in Sangamneri goat are given in Table 4.34. Total 24 different genotypic combinations were observed at this locus and the genotype 6,10 (151 bp, 159 bp) with a frequency of 0.120 represented the most predominant genotypes for the locus in the Sangamneri population (Table 4.33).

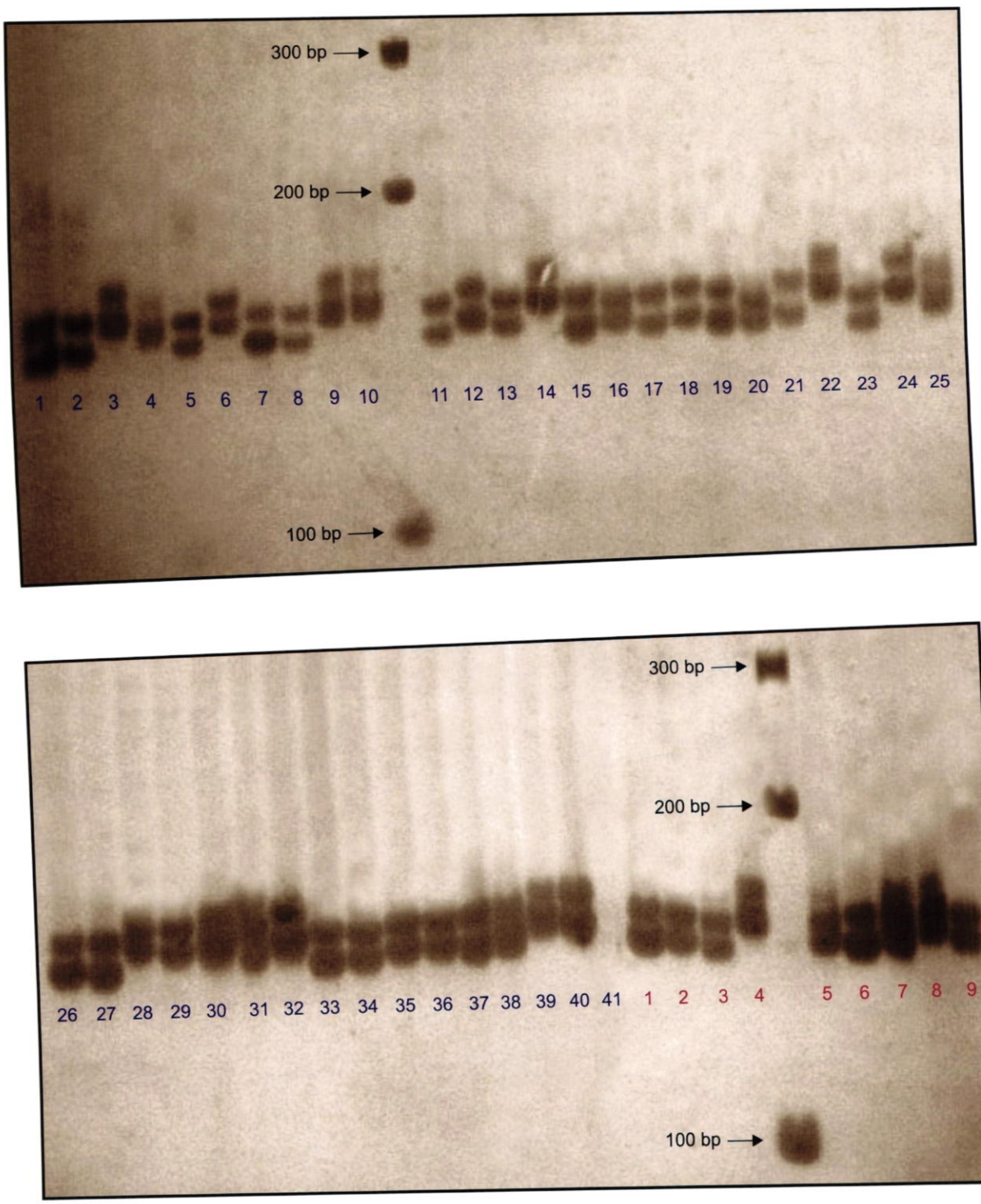


Plate 18. Resolution of PCR products of microsatellite locus ILSTS 087 on 8% urea polyacrylamide gel

Blue colour numbers are Female (F1 to F41) = 1 to 41
Red colour numbers are Male (M1 to M9) = 1 to 9

Table 4.33. Frequency distribution and allele size of microsatellite ILSTS087 in Sangamneri goat

Allelle number	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
Allele size (bp)	141	143	145	147	149	151	153	155	157
Allelic frequency	0.010	0.010	0.020	0.030	0.070	0.140	0.170	0.060	0.070
Allelle number	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	
Allele size (bp)	159	161	163	165	167	169	171	173	
Allelic frequency	0.150	0.080	0.080	0.020	0.030	0.030	0.020	0.010	

Table 4.34. Genotypes and genotypic frequency distribution of microsatellite ILSTS 087 in Sangamneri goat

Genotype	**5,5**	**1,7**	**2,7**	**3,7**	**3,8**	**4,8**	**5,9**	**6,9**	**5,10**
Observed count	1	1	1	1	1	3	3	3	2
Genotypic frequency	0.020	0.020	0.020	0.020	0.020	0.060	0.060	0.060	0.040
Genotype	**6,10**	**7,10**	**6,11**	**7,11**	**7,12**	**8,12**	**7,13**	**9,13**	**8,14**
Observed count	6	3	5	3	5	1	1	1	1
Genotypic frequency	0.120	0.060	0.100	0.060	0.100	0.020	0.020	0.020	0.020
Genotype	**10,14**	**7,15**	**10,15**	**10,16**	**12,16**	**12,17**			
Observed count	2	2	1	1	1	1			
Genotypic frequency	0.040	0.040	0.020	0.020	0.020	0.020			
Chi Square value	185.236**								

** Significant (P<0.01)

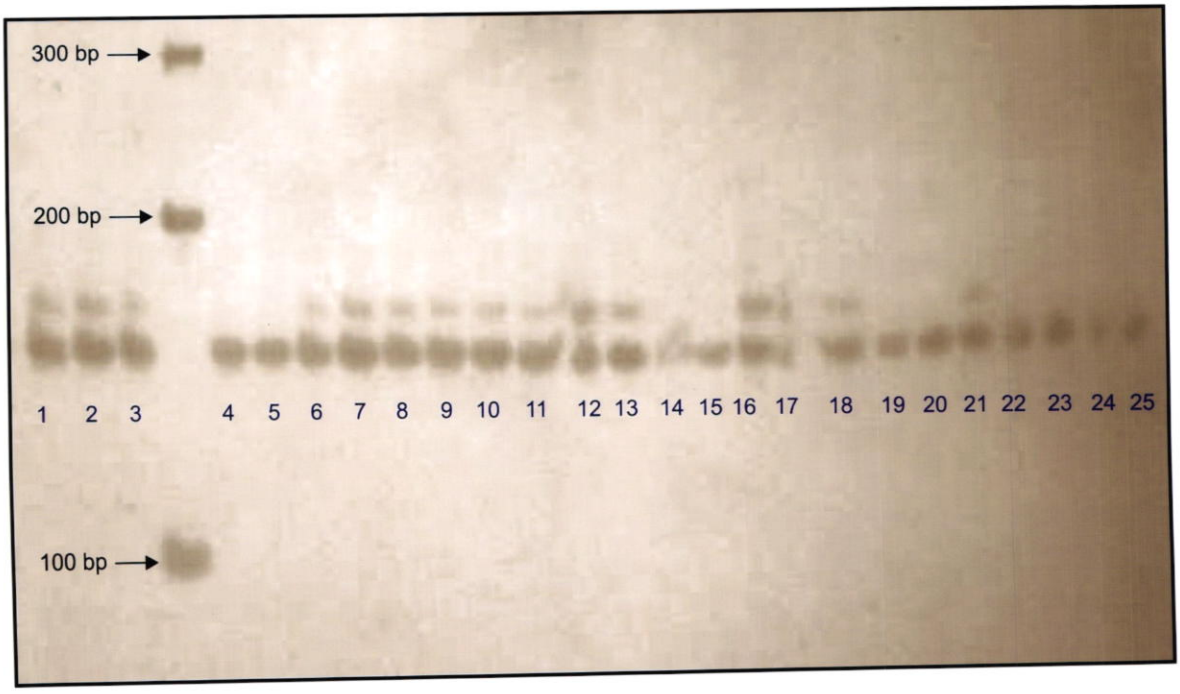
4.1.18 Microsatellite Oar AE 129

Microsatellite ILSTS 087 contains (CA)14 repeats, and is located at 61 – 88 nucleotide position in 203 bp sequence Kemp *et al.* (1995). Microsatellite ILSTS 087 is located on chromosome 6 in goat (Kemp *et al.*, 1995).

Frequency distribution and allele size of microsatellite Oar AE 129 in Sangamneri goat are presented in Table 4.35. Sangamneri goat exhibited total 19 alleles (Plate 19). Similar number of alleles were also observed by Dixit *et al.* (2010) in Southern Indian goat breed while, Kumar *et al.* (2005), Verma *et al.* (2009), and Dixit *et al.* (2011) reported less number of allele in Marwari (6), Malabari goat (15) and Kanniadu (13) goat breed, respectively. Allele 3 (141 bp) and 10 (155 bp) was the most predominant allele at this microsatellite locus present in the Sangamneri goat population with a frequency of 0.114, while the alleles 19 (173 bp) was present in the population with the least frequency of 0.011 for this microsatellite locus (Table 4.35).

The size of alleles for the microsatellite locus ranged between 133 and 173 bp. Similar range of allelic size were reported by earlier workers, Kumar *et al.* (2005) in Marwari goat (140-170 bp) and Dixit *et al.* (2010) in Southern Indian goat breed (130-175 bp).

Genotypes and genotypic frequency distribution of microsatellite locus Oar Ae 129 in Sangamneri goat are presented in Table 4.35. Total number of alleles in 26 different genotypic combinations were observed at this microsatellite locus and the genotype 3,3 (141 bp, 141 bp) with a frequency of 0.114 represented the most predominant genotypes for the locus in the Sangamneri population.



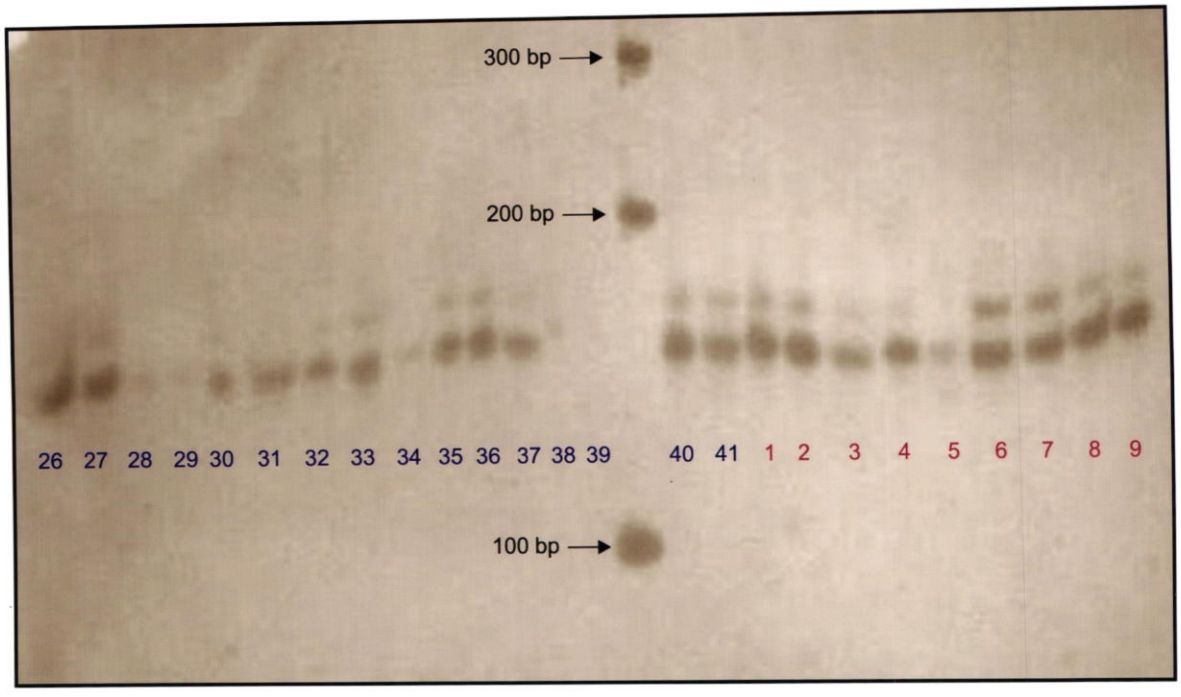


Plate 19. Resolution of PCR products of microsatellite locus OAR AE 129 on 8% urea polyacrylamide gel

Blue colour numbers are Female (F1 to F41) = 1 to 41
Red colour numbers are Male (M1 to M9) = 1 to 9

Table 4.35. Frequency distribution and allele size of microsatellite Oar AE 129 in Sangamneri goat

Allelle number	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
Allele size (bp)	133	139	141	143	145	147	149	151	153	155
Allelic frequency	0.023	0.023	0.114	0.057	0.034	0.023	0.091	0.045	0.068	0.114
Allelle number	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	
Allele size (bp)	157	159	161	163	165	167	169	171	173	
Allelic frequency	0.034	0.057	0.045	0.034	0.034	0.080	0.045	0.068	0.011	

Table 4.36. Genotypes and genotypic frequency distribution of microsatellite Oar AE 129 in Sangamneri goat

Genotype	**1,1**	**2,2**	**3,3**	**4,4**	**7,7**	**8,8**	**9,9**	**10,10**	**4,11**	**4,12**
Observed count	1	1	5	1	3	1	1	2	1	2
Genotypic frequency	0.023	0.023	0.114	0.023	0.068	0.023	0.023	0.045	0.023	0.045
Genotype	**5,12**	**5,13**	**6,13**	**13,13**	**6,14**	**7,14**	**8,15**	**15,15**	**8,16**	**9,16**
Observed count	2	1	1	1	1	2	1	1	1	4
Genotypic frequency	0.045	0.023	0.023	0.023	0.023	0.045	0.023	0.023	0.023	0.091
Genotype	**16,16**	**10,17**	**10,18**	**11,18**	**18,18**	**12,19**				
Observed count	1	4	2	2	1	1				
Genotypic frequency	0.023	0.091	0.045	0.045	0.023	0.023				
Chi Square value	395.057**									

** Significant (P<0.01)

4.1.19 Microsatellite Oar HH64

Microsatellite Oar HH64 contains (GT)17 repeats, and is located at 45-78 bp nucleotide position in 128 bp sequence It is located on chromosome number 4 in sheep (Henry *et al.*, 1993) and goat (Vaiman *et al.*, 1998).

The studied Sangamneri population exhibited 10 alleles for this locus (Plate 20 and Table 4.37). Similar number of alleles was observed by Dixit *et al.* (2010) in Southern Indian goat breed (10), while Verma *et al.* (2009) reported more number of allele in Malabari goat (13) than the present findings. However, less number of alleles were also observed for Microsatellite Oar HH64 locus by Kumar *et al.* (2005) in Marwari goat (5), Gour *et al.* (2006) in Jamunapari goat (6), Sharma *et al.* (2008[a]) in Barbari goat (4), Sharma *et al.* (2008 [b]) for Beetal goat (6), Mishra *et al.* (2010) in Changthangi (9) and Dixit *et al.* (2011) in Kanniadu (7). Allele 8 (135 bp) was the most predominant allele that presented a frequency of 0.263.

The allelic size for the Oar HH64 microsatellite locus ranged between 105 and 139 bp. Narrow range of allelic size were observed by Kumar *et al.* (2005) for Marwari goat (125.133), Gour *et al.* (2006) for Jamunapari goat (124-136), Sharma *et al.* (2008[a]) for Barbari goat (124-130) and Dixit *et al.* (2010) for Southern Indian goat breed (120-138).

Genotypes and genotypic frequency distribution of microsatellite locus Oar HH64 in Sangamneri goat are presented in Table 4.38. Total 10 different genotypic combinations were observed at this microsatellite locus and the genotype 8,8 with a frequency of 0.263 represented the most predominant genotypes for the locus in the Sangamneri population (Table 4.38).

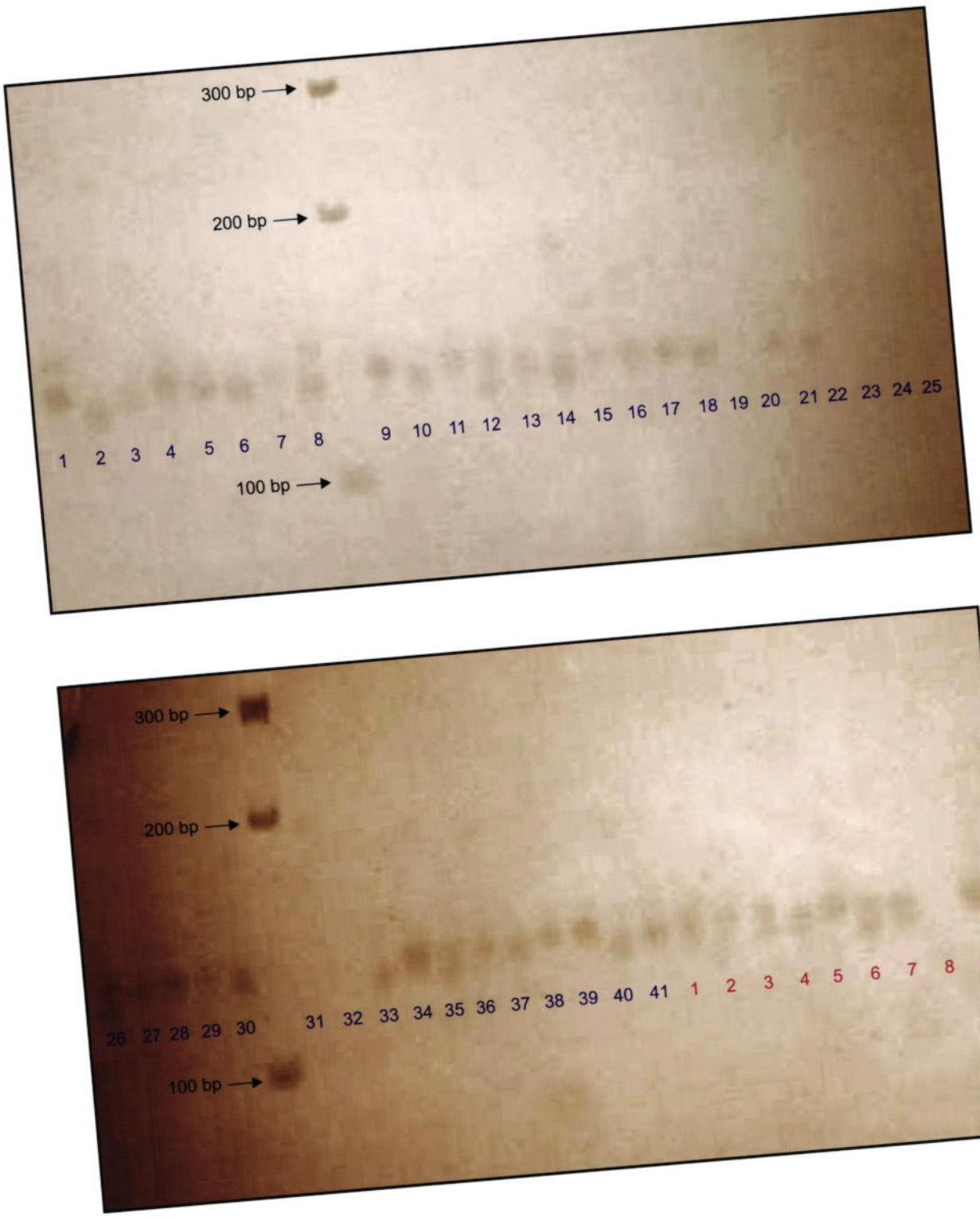


Plate 20. Resolution of PCR products of microsatellite locus OAR HH 64 on 8% urea polyacrylamide gel

Blue colour numbers are Female (F1 to F41) = 1 to 41
Red colour numbers are Male (M1 to M9) = 1 to 9

Table 4.37. Frequency distribution and allele size of microsatellite Oar HH 64 in Sangamneri goat

Allelle number	1	2	3	4	5	6	7	8	9	10
Allele size (bp)	105	123	125	127	129	131	133	135	137	139
Allelic frequency	0.013	0.026	0.026	0.053	0.158	0.092	0.132	0.263	0.184	0.053

Table 4.38. Genotypes and genotypic frequency distribution of microsatellite Oar HH 64 in Sangamneri goat

Genotype	2,2	3,3	4,4	5,5	1,6	6,6	7,7	8,8	9,9	10,10
Observed count	1	1	2	6	1	3	5	10	7	2
Genotypic frequency	0.026	0.026	0.052	0.157	0.026	0.078	0.131	0.263	0.184	0.052
Chi Square value	304.776**									

** Significant (P<0.01)

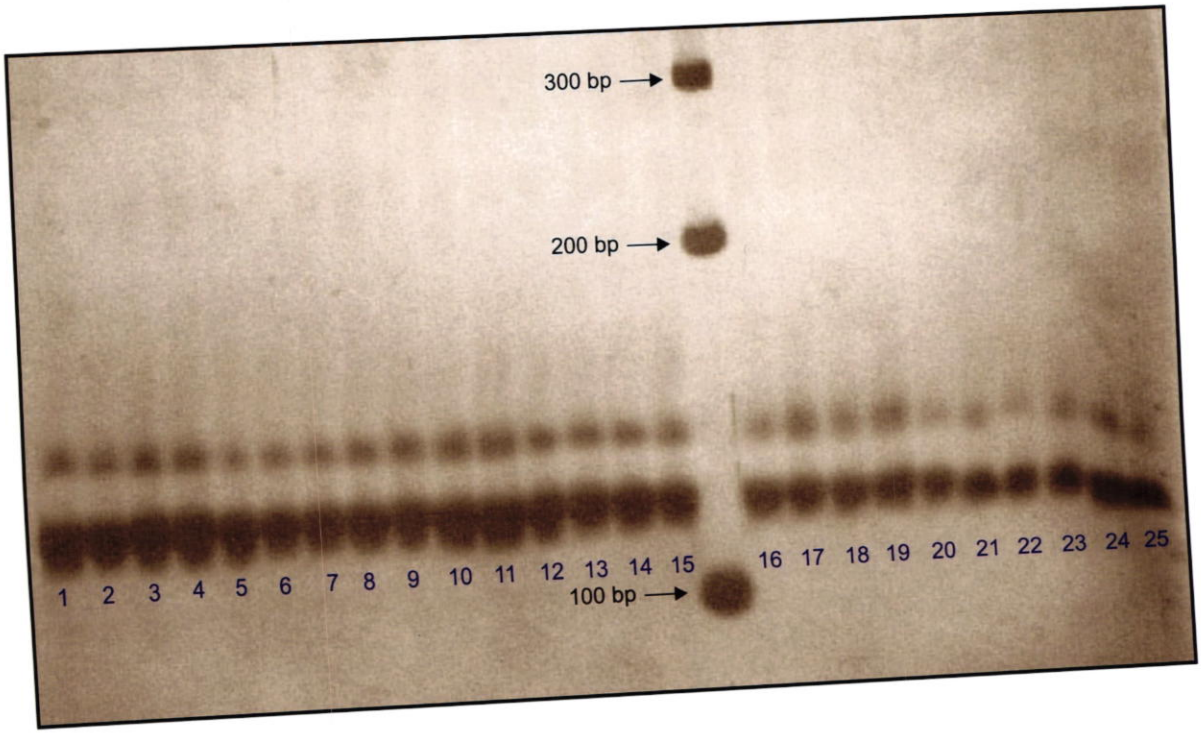
4.1.20 Microsatellite Oar JMP29

Microsatellite ILSTS 082 contained (GT)17 repeats, and is located at 164–197 nucleotide position in 560 bp sequence in bovine (Kemp *et al.*, 1995). It is located on chromosome 2 in goat (Schibler *et al.*, 1998).

In Sangamneri goat total 14 alleles were observed for microsatellite Oar JMP 29 (Plate 21 and Table 4.39). More number of allele were observed in the present study than reported by Kumar *et al.* (2005) in Marwari goat (6), Gour *et al.* (2006) in Jamunapari goat (4), Sharma *et al.* (2008 [b]) for Beetal goat (3), Verma *et al.* (2009) in Malabari goat (4), Dixit *et al.* (2010) in Southern Indian goat breed (10) and Dixit *et al.* (2011) in Kanniadu goat (9). Allele 6 (120 bp) was the most predominant allele with a frequency of 0.310, while the alleles 1 (106 bp) and 2 (112 bp) was present in the population with the least frequency of 0.010 for this microsatellite locus (Table 4.39).

The allele size for the microsatellite Oar JMP 29 locus varied in the range between 106 and 138 bp. The allelic size observed for the locus in Sangamneri goat had wide range as comparable to the allelic size for this locus reported in some other breeds of goat as Kumar *et al.* (2005) reported 125-135 bp in Marwari goat, Gour *et al.* (2006) for Jamunapari goat (95-121), and Dixit *et al.* (2010) in Southern Indian goat breed (120-140).

The genotypes and genotypic frequencies observed in the present study for the microsatellite locus Oar JMP 29 in the Sangamneri goat population are depicted in Table 4.40. Total 22 different genotypic combinations at this microsatellite locus and genotype 6,6 (120 bp, 120 bp) with a frequency of 0.240 represented the most predominant genotypes for the locus in the Sangamneri population.



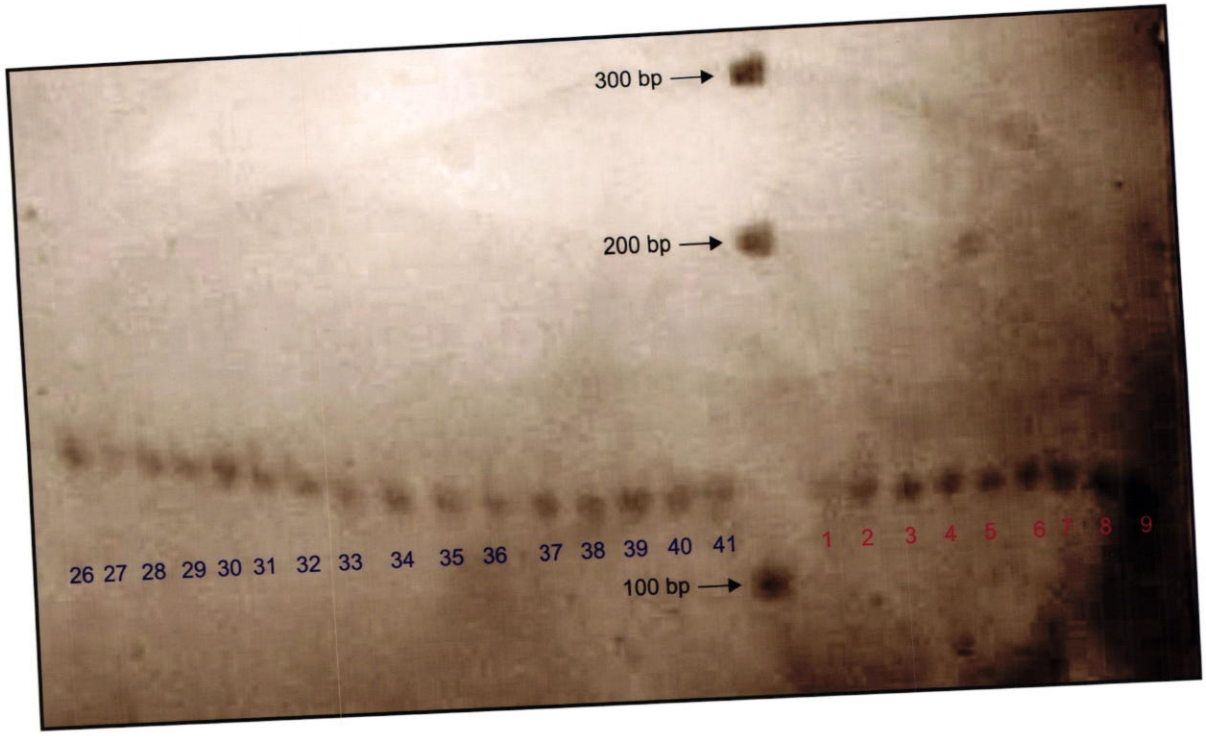


Plate 21. Resolution of PCR products of microsatellite locus OAR JMP 29 on 8% urea polyacrylamide gel

Blue colour numbers are Female (F1 to F41) = 1 to 41
Red colour numbers are Male (M1 to M9) = 1 to 9

Table 4.39. Frequency distribution and allele size of microsatellite Oar JMP 29 in Sangamneri goat

Allelle number	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Allele size (bp)	106	112	114	116	118	120	122	124
Allelic frequency	0.010	0.010	0.060	0.030	0.120	0.310	0.060	0.040
Allelle number	**9**	**10**	**11**	**12**	**13**	**14**		
Allele size (bp)	128	130	132	134	136	138		
Allelic frequency	0.040	0.050	0.040	0.120	0.070	0.040		

Table 4.40. Genotypes and genotypic frequency distribution of microsatellite Oar JMP 29 in Sangamneri goat

Genotype	**3,3**	**5,5**	**6,6**	**7,7**	**1,8**	**3,8**	**8,8**	**9,9**
Observed count	1	2	12	3	1	1	1	2
Genotypic frequency	0.020	0.040	0.240	0.060	0.020	0.020	0.020	0.040
Genotype	**3,10**	**5,10**	**10,10**	**4,11**	**5,11**	**11,11**	**2,12**	**3,12**
Observed count	2	1	1	1	1	1	1	1
Genotypic frequency	0.040	0.020	0.020	0.020	0.020	0.020	0.020	0.020
Genotype	**4,12**	**5,12**	**6,12**	**12,12**	**4,13**	**5,13**	**6,13**	**14,14**
Observed count	1	4	3	1	1	2	4	2
Genotypic frequency	0.020	0.080	0.060	0.020	0.020	0.040	0.080	0.040
Chi Square value	272.980**							

** Significant (P<0.01)

4.1.21 Microsatellite OMHC 1

It is a dinucleotide repeat marker with (CA)n repeats and located on chromosome number 20 in sheep within the MHC Class I region (Groth and Wetherall, 1994). Gruszczynska *et al.* (2002) identified 13 alleles of the gene in Polish Heath Sheep and nine in Polish Lowland Sheep. The allele size ranges from 180-208 bp in Polish Heath Sheep where as allele range found to be 186-202 bp in Polish Lowland Sheep. Similar results were obtained by Groth and Wetherall (1994), who proved the presence of eight OMHC1 alleles within a group of 20 Australian Merino Sheep – the amplified fragment was approximately 200 bp. In their further studies they confirmed the presence of this microsatellite sequence between 39 bp and 86 bp of the amplified fragment (Groth and Wetherall, 1995).

Microsatellite locus OMHC 1 was found to exhibit 18 alleles (Plate 22, Table 4.41) in Sangamneri goat breed. More number of allele were reported by Dixit *et al.* (2010) in southern Indian goat breed (22), while, Kumar *et al.* (2005) in Marwari goat (6), Gour *et al.* (2006) in Jamunapari goat (4), Sharma *et al.* (2008[a]) in Barbari goat (7), Sharma *et al.* (2008[b]) in Beetal goat (9), Verma *et al.* (2009) in Malabari goat (16), Mishra *et al.* (2010) in Changthangi goat (11) and Dixit *et al.* (2011) in Kanniadu (12) reported less number of alleles than present study. Allele 6 (190 bp) was the most predominant allele, present in the population with a frequency of 0.156 (Table 4.42).

The range of alleles size for the microsatellite locus was found in between 178 and 224 bp. The allelic size observed for Sangamneri goat was comparable to the allelic size for this locus reported by Kumar *et al.* (2005) in Marwari (184-204), Gour *et al.* (2006) in Jamunapari (187-199), Sharma *et al.* (2008[a]) for Barbari (189-201) and Dixit *et al.* (2010) for Southern Indian goat breed (179-209).

The genotypes and genotypic frequency distribution of microsatellite locus OMHC 1 in the Sangamneri goat is depicted in Table 4.42. Total 25 different genotypic combinations were observed for this locus and the genotype 6, 6 (190, 190 bp) with a frequency of 0.155 represented the most predominant genotypes for the locus in the Sangamneri population.

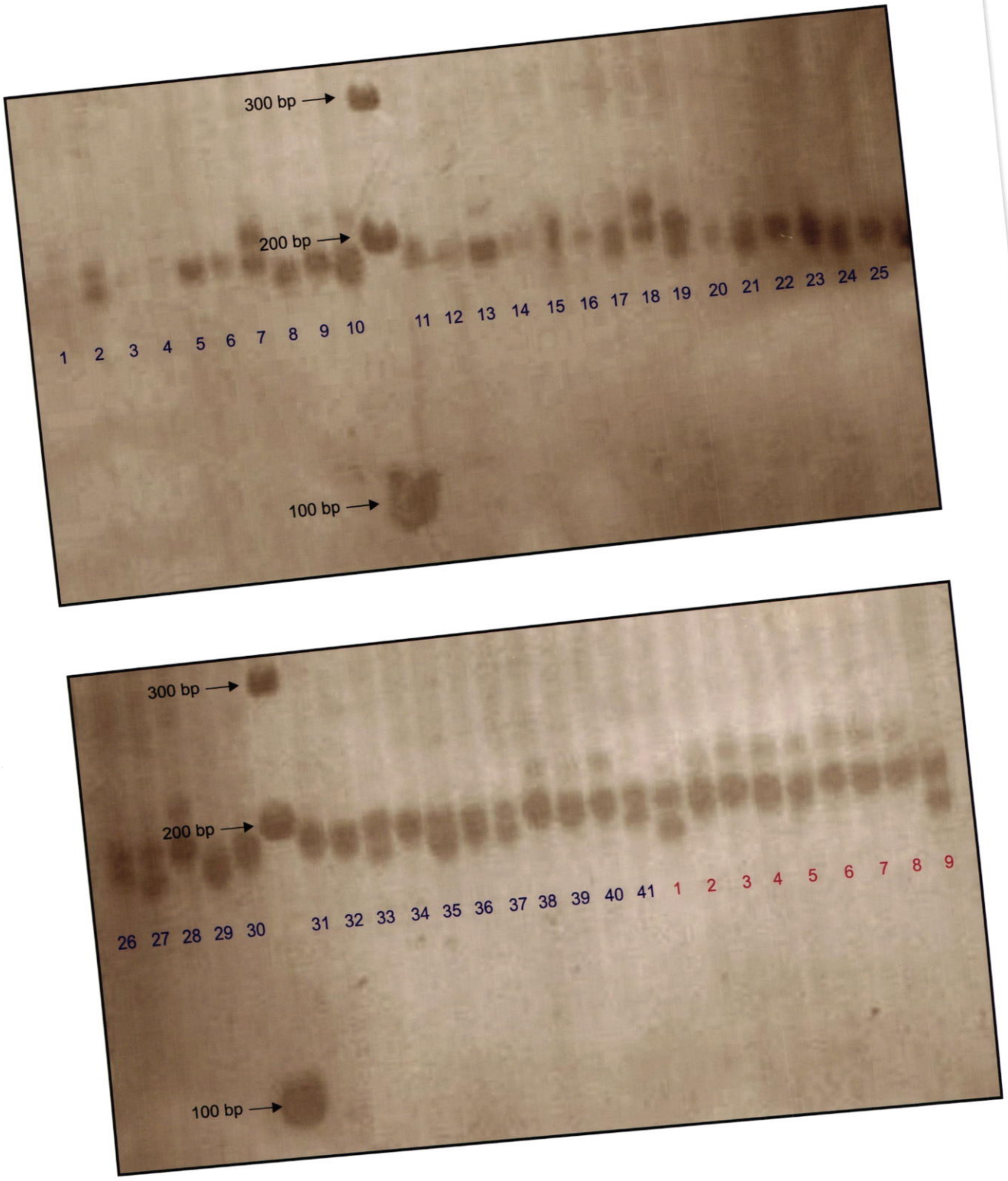


Plate 22. Resolution of PCR products of microsatellite locus OMH C1 on 8% urea polyacrylamide gel

Blue colour numbers are Female (F1 to F41) = 1 to 41
Red colour numbers are Male (M1 to M9) = 1 to 9

Table 4.41. Frequency distribution and allele size of microsatellite OMHC 1 in Sangamneri goat

Allelle number	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
Allele size (bp)	178	182	184	186	188	190	192	194	196
Allelic frequency	0.011	0.044	0.089	0.089	0.033	0.156	0.133	0.078	0.133
Allelle number	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**
Allele size (bp)	198	200	202	204	210	214	218	220	224
Allelic frequency	0.044	0.033	0.011	0.011	0.011	0.011	0.022	0.056	0.033

Table 4.42. Genotypes and genotypic frequency distribution of microsatellite OMHC 1in Sangamneri goat

Genotype	**2,2**	**3,3**	**4,4**	**6,6**	**1,7**	**3,7**	**7,7**	**4,8**	**8,8**
Observed count	2	2	3	7	1	2	3	1	2
Genotypic frequency	0.044	0.044	0.066	0.155	0.022	0.044	0.066	0.022	0.044
Genotype	**3,9**	**5,9**	**9,9**	**5,10**	**7,11**	**4,12**	**7,13**	**9,14**	**7,15**
Observed count	2	1	2	2	1	1	1	1	1
Genotypic frequency	0.044	0.022	0.044	0.044	0.022	0.022	0.022	0.022	0.022
Genotype	**8,16**	**9,16**	**8,17**	**9,17**	**10,17**	**10,18**	**11,18**		
Observed count	1	1	1	3	1	1	2		
Genotypic frequency	0.022	0.022	0.022	0.066	0.022	0.022	0.044		
Chi Square value	276.114**								

** Significant (P<0.01)

4.1.22 Microsatellite RM 004

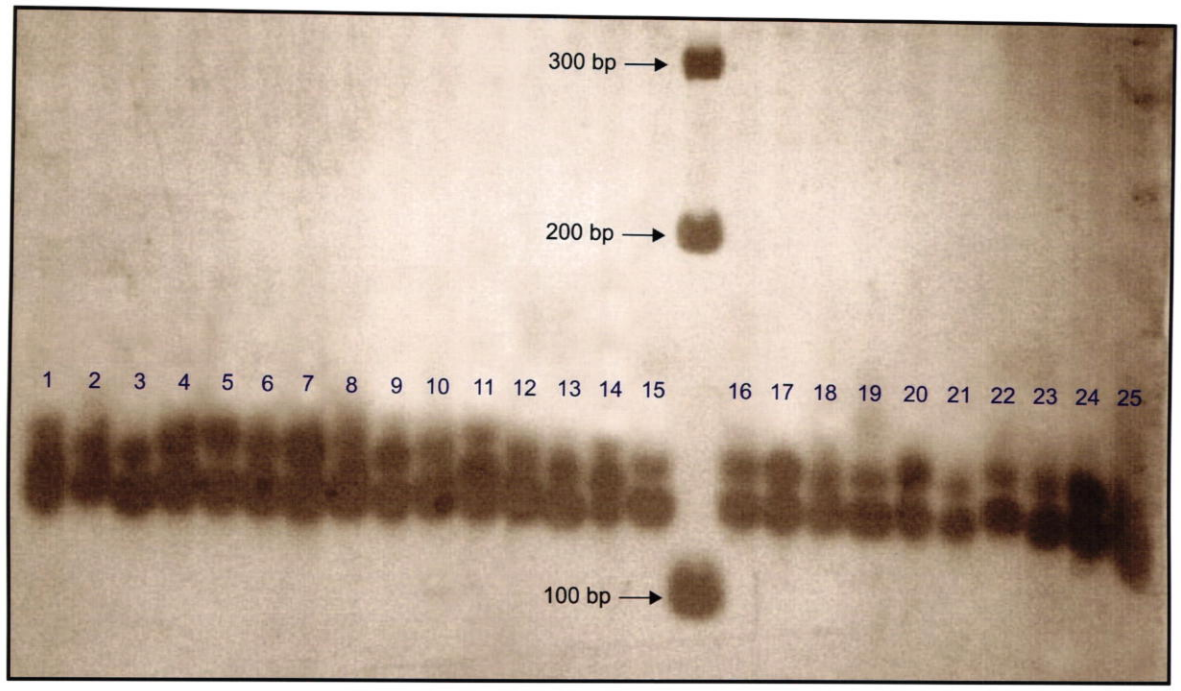
Microsatellite RM 004 contained (CA)13 repeats and is located at 70-95 and 101–106 nucleotide position in 192 bp sequence on chromosome number 15 in sheep (Kossarek *et al.*, 1993).

The studied Sangamneri population exhibited 12 alleles for this locus (Plate 23 and Table 4.43). Total number of allele observed for Microsatellite RM 004 locus were at higher side than reported by Kumar *et al.* (2005) in Marwari goat (4), Gour *et al.* (2006) in Jamunapari goat (3), Sharma *et al.* (2008[a]) in Barbari goat (5), Sharma *et al.* (2008[b]) in Beetal goat (4), Verma *et al.* (2009) in Malabari goat (8), Dixit *et al.* (2010) in Southern Indian goat breed (9) and Dixit *et al.* (2011) in Kanniadu (5). Allele 5 (118 bp) was the most predominant allele that presented a frequency of 0.230.

The allelic size for the RM 004 microsatellite locus ranged between 110 and 132 bp. Similar range were reported by Dixit *et al.* (2010) for Southern Indian goat breed (104-127), however, narrow range of allelic size were observed by Kumar *et al.* (2005) for Marwari goat (116-122), Gour *et al.* (2006) for Jamunapari goat (116-120), Sharma *et al.* (2008[a]) for Barbari goat (115-123).

Genotypes and genotypic frequency distribution of microsatellite locus RM 004 in Sangamneri goat are presented in Table 44. Total 22 different genotypic combinations were observed at this microsatellite locus and the genotype 5,11 (118 bp, 130 bp) with a frequency of 0.120 represented the most predominant genotypes for the locus in the Sangamneri population studied

The Sangamneri goat population studied showed a departure from Hardy Weinberg equilibrium at 21 out of 22 studied loci indicating that the population was under direct or indirect selection for these loci.



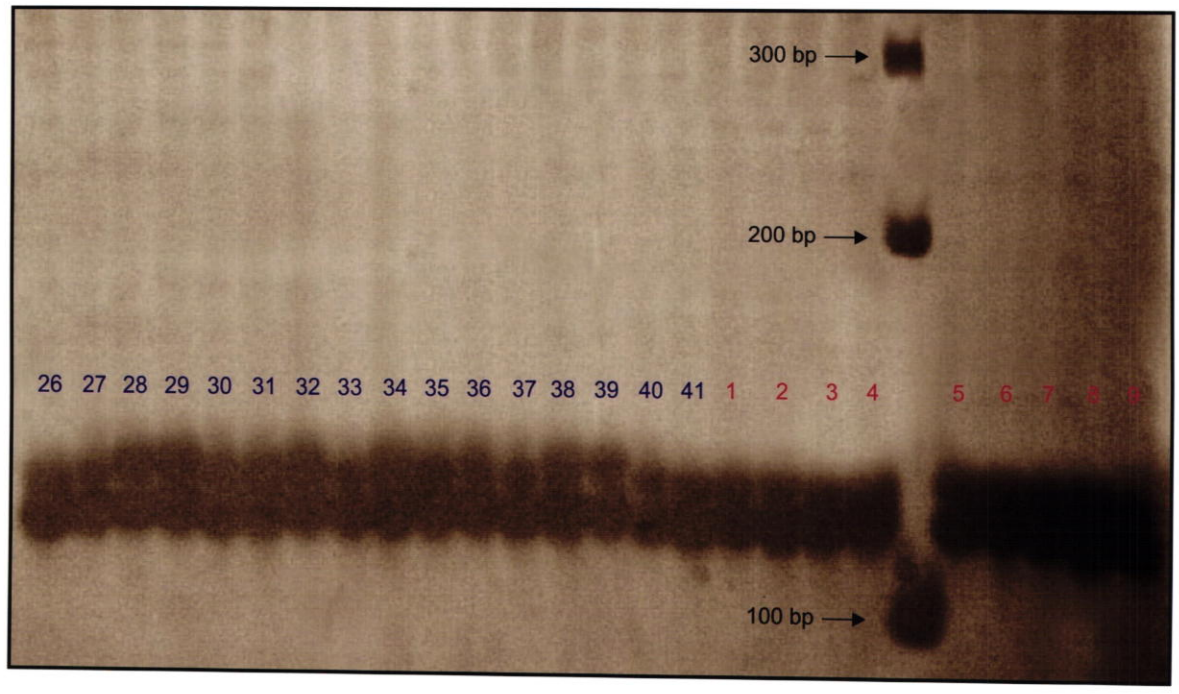


Plate 23. Resolution of PCR products of microsatellite locus RM004 on 8% urea polyacrylamide gel

Blue colour numbers are Female (F1 to F41) = 1 to 41
Red colour numbers are Male (M1 to M9) = 1 to 9

Table 4.43. Frequency distribution and allele size of microsatellite RM 004 in Sangamneri goat

Allelle number	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Allele size (bp)	110	112	114	116	118	120	122	124
Allelic frequency	0.020	0.010	0.030	0.120	0.230	0.110	0.090	0.100
Allelle number	**9**	**10**	**11**	**12**				
Allele size (bp)	126	128	130	132				
Allelic frequency	0.130	0.060	0.090	0.010				

Table 4.44. Genotypes and genotypic frequency distribution of microsatellite RM 004 in Sangamneri goat

Genotype	**4,4**	**1,5**	**5,5**	**1,6**	**6,6**	**2,7**	**3,7**	**7,7**
Observed count	1	1	3	1	2	1	1	2
Genotypic frequency	0.020	0.020	0.060	0.020	0.040	0.020	0.020	0.040
Genotype	**4,8**	**5,8**	**8,8**	**3,9**	**4,9**	**5,9**	**6,9**	**4,10**
Observed count	5	3	1	2	4	5	2	1
Genotypic frequency	0.100	0.060	0.020	0.040	0080	0.100	0.040	0.020
Genotype	**5,10**	**6,10**	**5,11**	**6,11**	**7,11**	**7,12**		
Observed count	2	3	6	1	2	1		
Genotypic frequency	0.040	0.060	0.120	0.020	0.040	0.020		
Chi Square value	103.606**							

** Significant (P<0.01)

4.2 Genetic Variation

Various measures of genetic variation across microsatellite loci in Sangamneri goat is summarised in Table 4.45.

4.2.1 Microsatellite allele variability

The observed and effective number of alleles for each locus is summarized in Table 4.45. The number of alleles observed in the samples for a given marker is supposed to represent the number of alleles in the whole population for that particular locus, since the DNA sample was drawn at random. Mean number of alleles observed over a range of loci in different population is considered to be a reasonable indicator of genetic variation within the population (MacHugh *et al.*, 1997).

The observed number of alleles varied from 8 (ILSTS002, ILSTS 065) to 32 (ILSTS 008) with a mean of 17.227 ± 1.221. The effective number of alleles varied from 4.083 (ILSTS 065) to 19.208 (ILSTS 008) with a mean 11.149 ± 0.842.

The effective number of alleles was found to be lesser than the observed number in all the loci studied. It may be mentioned that if all the alleles in a population have same frequency, the effective number of alleles would be the same as observed number of alleles. Otherwise, effective number of alleles would be the actual number of allele, the situation generally existing in natural population. Since most of the allele are normally represented once or twice in a population and contribute very little to the average heterozygosity or genetic variance in the population, the effective number of allele is more useful component in population genetic studies than the observed number.

The mean number of alleles observed in the present study for Sangamneri goat was found to be closer to the findings of Aggarwal *et al.* (2007) and Dixit *et al.* (2008) in Mehsana (12.28 ± 5.35) and Kutchi goat breed (12.00 ± 1.02) respectively, however, the effective number of allele for these goat breed was less than half of the observed number of alleles. In the present study the effective number of allele is above half of the observed number of each locus, this may be due to the fact that large number of alleles in respect of each locus is present with a very low frequency.

Table 4.45. Various measures of genetic variation across microsatellite loci in Sangamneri goat

Locus	Size (range) of alleles bp	Observed Number of Alleles	Effective Number of Alleles	Observed heterozygosity	Expected heterozygosity	PIC Value	Shannon's information index (I)	FIS value
ETH225	146-180	17	12.953	0.920	0.923	0.917	2.666	0.003
ILSTS002	118-134	8	4.840	0.000	0.793	0.770	1.793	1.00
ILSTS005	177-211	18	13.593	0.979	0.926	0.922	2.725	-0.057
ILSTS008	162-238	32	19.208	0.673	0.948	0.946	3.204	0.290
ILSTS019	145-183	19	13.624	0.780	0.927	0.922	2.747	0.158
ILSTS022	191-239	21	10.889	0.694	0.908	0.902	2.675	0.236
ILSTS029	142-184	19	11.406	0.896	0.912	0.906	2.679	0.018
ILSTS030	140-204	25	17.241	0.780	0.942	0.939	2.984	0.172
ILSTS033	161-191	13	10.482	1.000	0.905	0.897	2.416	-0.105
ILSTS034	148-184	13	7.022	0.980	0.858	0.842	2.148	-0.143
ILSTS044	141-197	25	15.970	0.913	0.937	0.931	2.966	0.026
ILSTS049	158-194	18	9.018	0.917	0.889	0.880	2.476	-0.031
ILSTS058	158-210	24	15.890	1.000	0.937	0.934	2.932	-0.067

contd...

Locus	Size (range) of alleles bp	Observed Number of Alleles	Effective Number of Alleles	Observed heterozygosity	Expected heterozygosity	PIC Value	Shannon's information index (I)	FIS value
ILSTS059	108-138	14	11.469	0.891	0.913	0.906	2.506	0.024
ILSTS065	115-133	8	4.083	0.426	0.755	0.720	1.650	0.436
ILSTS082	102-134	15	11.571	0.889	0.914	0.907	2.541	0.027
ILSTS087	141-173	17	9.862	0.980	0.899	0.890	2.495	-0.091
Oar AE 129	133-173	19	14.556	0.568	0.931	0.928	2.797	0.390
Oar HH 64	105-139	10	6.211	0.026	0.839	0.820	1.999	0.969
Oar JMP29	106-138	14	6.803	0.480	0.853	0.841	2.258	0.437
OMHC1	178-224	18	10.743	0.533	0.907	0.900	2.568	0.412
RM004	110-132	12	7.837	0.820	0.872	0.860	2.209	0.060
Mean		17.227	11.149	0.734	0.895	0.885	2.520	0.191
SE		1.221	0.842	0.061	0.010	0.012	0.081	0.066

The mean observed and effective number of alleles were found to be higher in the present study than that reported by Kumar *et al.* (2005) in Marwari, Gour *et al.* (2006) in Jamunapari, Sharma *et al.* (2008[a]) in Barbari, Sharma *et al.* (2008[b]) in Beetal, Verma *et al.* (2009) in Malabari, Kumar *et al.* (2009) in Gohilwari, Verma *et al.* (2010) in Sangamneri, Vijh *et al.* (2010) in Black Bengal and Chegu, Mishra *et al.* (2010) in Changthangi and Dixit *et al.* (2011) in Kanniaddu goat breed.

The total number of alleles observed and the minimum number of alleles at a locus demonstrated that all microsatellite loci were sufficiently polymorphic and markers used were appropriate since the number of allele resolved for each marker was either equal or more than the required number of allele (at least 4 alleles) recommended for microsatellite (Barker, 1994).

4.2.2 Heterozygosity measure

Heterozygosity is another measure for assessing the genetic variability within population. Observed and expected heterozygosity for the loci studied is presented in the Table 4.45. A more appropriate measure of genetic variation within a population is average expected heterozygosity gene diversity (Nei, 1978). The values of observed heterozygosity ranged from 0.000 (ILSTS 002) to 1.000 (ILSTS 033, ILSTS 058) with an average of 0.734 ± 0.061, whereas the expected heterozygosity varied from 0.755 (ILSTS 065) to 0.948 (ILSTS 008) with an overall mean of 0.895 ± 0.010. Vijh *et al.* (2010) reported similar estimate of mean observed and expected heterozygosity in Black Bengal and Chegu goat.

All the loci had greater expected heterozygosity than observed hertrozygosity except six loci (ILSTS 005, ILSTS 033, ILSTS 034, ILSTS 049, ILSTS 058 and ILSTS 087). This indicated that 73% loci showed positive deviation from Hardy-Weinberg equilibrium (HWE). The higher values of expected heterozygosity were also reported for ILSTS 034 and ILSTS 058 loci in Kanniadu goat breed by Dixit *et al.* (2011).

Sangamneri possesses more genetic variability when compared to Marwari (Kumar *et al.,* 2005), Jamunapari (Gour *et al.,* 2006), Mehsana (Aggarwal *et al.,* 2007), Barbari (Sharma *et al.,* 2008[a]), Beetal (Sharma *et al.,* 2008[b]), Malabari (Verma *et al.,* 2009), Gohilwari (Kumar *et al.*,

2009), Changthangi (Mishra *et al.*, 2010) and Kanniaddu (Dixit *et al.*, 2011) goat breeds.

4.2.3 Polymorphic Information Content (PIC)

The PIC values for microsatellite markers were calculated and presented in Table 4.45. The PIC value indicates the informative value of different microsatellite markers studied. According to Botstein *et al.* (1980) polymorphic markers are classified as highly informative that have a PIC value greater than 0.5, reasonably informative with the PIC value ranging between 0.25 to 0.5 and slightly informative if the value is below 0.25. The polymorphic nature of microsatellite makes them marker of choice in characterization and genetic diversity studies. The PIC values ranged from 0.72 (ILSTS 065) to 0.946 (ILSTS 008) with an average of 0.885 ± 0.011.Thus all the markers under study were found to be highly informative.

Aggarwal *et al.* (2007) in Mehsana (0.72) and Vijh *et al.* (2010) in Black Bengal (0.80) and Chegu goat (0.80) reported similar estimate of PIC, however Kumar *et al.* (2005) in Marwari, Gour *et al.* (2006) in Jamunapari, Sharma *et al.* (2008[a]) in Barbari, Sharma *et al.* (2008[b]) in Beetal, Verma *et al.* (2009) in Malabari, Kumar *et al.* (2009) in Gohilwari, Verma *et al.* (2010) in Sangamneri, and Dixit *et al.* (2011) in Kanniaddu goat breed reported lower estimate of PIC than the present study.

4.2.4 Shannon's Information Index (I)

The Shannon's information index represents the relative abundance of genetic information of a specific locus to the total information available of the loci (Shannon and Weaver, 1949). The values were found be in the range of 1.650 (ILSTS 065) to 3.204 (ILSTS 008) with an average of 2.520 ± 0.081. The high value of Shannon's information index indicated the suitability of markers for studying the genetic variability in goat species.

Kumar *et al.* (2005) in Marwari, Gour *et al.* (2006) in Jamunapari, Aggarwal *et al.* (2007) in Mehsana, Sharma *et al.* (2008[a]) in Barbari, Sharma *et al.* (2008[b]) in Beetal, Kumar *et al.* (2009) in Gohilwari, Mishra *et al.* (2010) in Changthangi and Dixit *et al.* (2011) in Kanniaddu goat breed reported lower estimate of Shannon's Information Index than the present study.

4.2.5 Heterozygote deficiency (F_{is})

The F_{is} values provide the non-random union of gametes in the population, i.e. the mating among the individuals in the population which are related more than the average relationship. More the values of F_{is}, more the inbreeding in the population. The negative values of F_{is} point towards out-breeding i.e. mating of individuals who are less related than the average relationship of the population.

Heterozygosity deficiency (positive F_{is} value) was observed in sixteen out of twenty two studied loci indicating departures from random mating and suggested that some of the studied loci were homozygous in the population. The positive value of F_{is} ranged from 0.003 to 1.00 with an average of 0.19 ± 0.07. This value is as per expectation as low genetic variations observed in this breed compared to other Indian breeds may be due to rate of inbreeding in this goat population. Similar high estimate were also reported in Indian goat populations *viz.,* Mehsana (Aggarwal *et al.*, 2007), Barbari (Sharma *et al.,* 2008[a]), Beetal goat (Sharma *et al.,* 2008[b]), Kutchi (Dixit *et al.,* 2008) and Malabari goat (Verma *et al.,* 2009).

The heterozygosity deficiency observed at these loci may be due to one or more of the reasons like population subdivision owing to the genetic drift, null alleles, selection against heterozygote's or inbreeding. However, pin-pointing the reason is generally difficult (Christiansen, 1974) but a reason observed here is that inbreeding which presumeably resulted from unplanned and indiscriminate mating prevailing in the breeding tract, led to the small effective population size. Due to the non availability of sufficient number of breeding buck same few bucks are used for whole village and also in the nearby villages.

4.2.6 Suitability of microsatellite markers

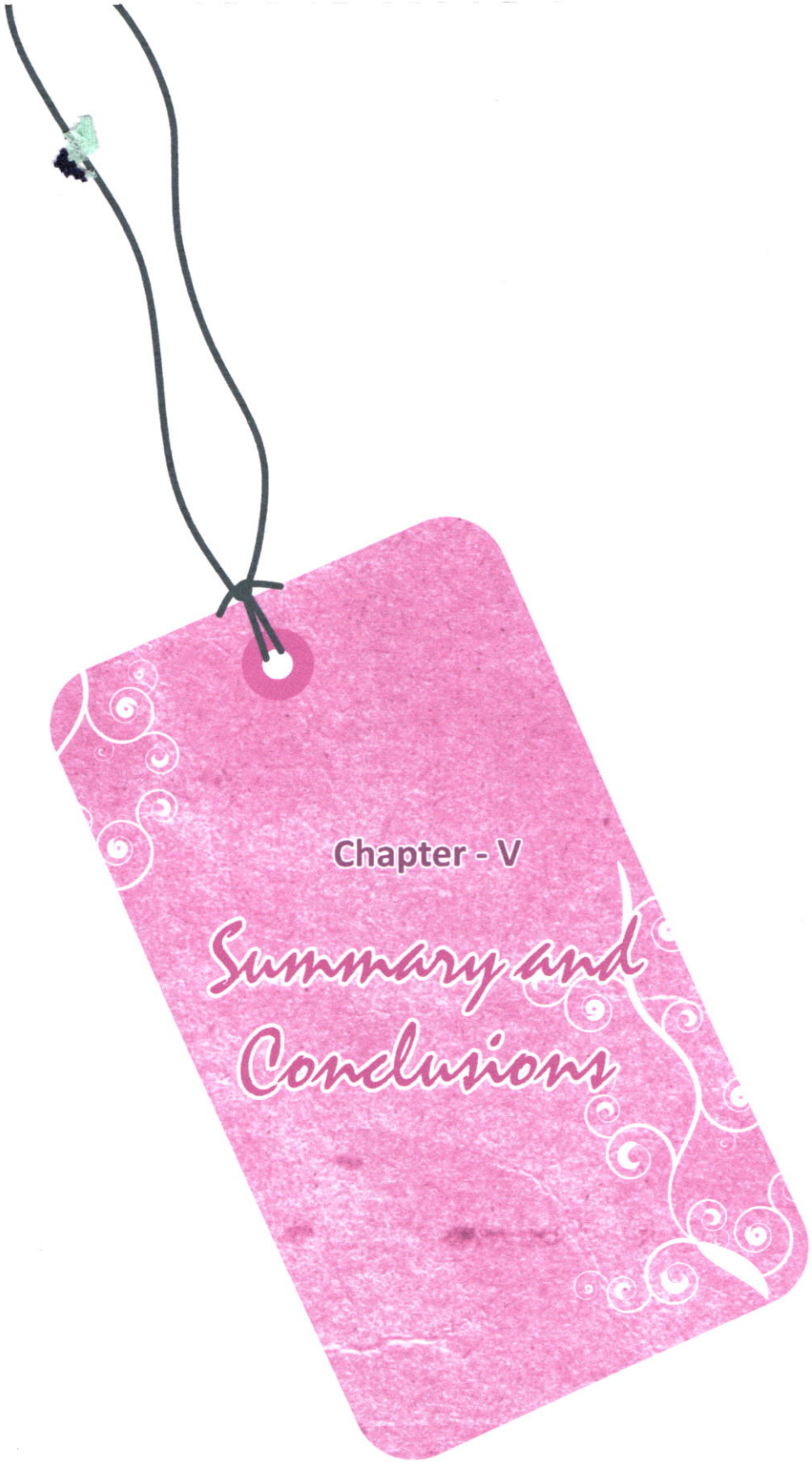
All the loci could be amplified successfully. The observed number of alleles varied from 8 (ILSTS 002, ILSTS 065) to 32 (ILSTS 008) with a mean of 17.227 ± 1.221. The effective number of alleles varied from 4.083 (ILSTS 065) to 19.208 (ILSTS 008) with a mean 11.149 ± 0.842. At all the studied in Sangamneri breed of goat the observed number of alleles was higher than the effective number of alleles. The total number of alleles observed and the minimum number of alleles at a locus demonstrated that all

microsatellite loci were sufficiently polymorphic and markers used were appropriate since the number of alleles resolved for each marker was more than the required number of alleles (at least 4 alleles) recommended for microsatellite markers to be used in the estimation of genetic distance.

All the loci showed the polymorphic information content greater than 0.5. The high value of PIC indicated the suitability of markers for studying the genetic variability in goat species. Shannon's information index, a measure of biodiversity, was sufficiently high in Sangamneri goat population with the overall mean of (Table 4.45).

Takezaki and Nei (1996) determined that for the markers to be useful for measuring genetic variation, they should have expected average heterozygosity between 0.3 and 0.8 in the population. The values of observed heterozygosity ranged from 0.000 (ILSTS 002) to 1.000 (ILSTS 033, ILSTS 058) with an average of 0.734 ± 0.061. The higher values of the average expected gene diversity suggests the suitability of the selected markers.

All the parameters (observed number of alleles, effective number of alleles, Shannon's information index and PIC value and gene diversity) estimated to know the genetic variation showed that all the microsatellite markers used were highly informative, indicating the suitability for genetic diversity studies in goats.


Chapter - V
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CHAPTER V

SUMMARY AND CONCLUSIONS

Fifty blood samples of unrelated Sangamneri goat were collected at random from different villages of Sangamner and Rahuri Taluka of Ahmednagar. The samples were also collected from All India Co-ordinated Research Project on Sangamneri Goat, Department of Animal Husbandry, Mahatama Phule Krishi Vidyapeeth, Rahuri to study genetic variability within Sangamneri breed of goat using microsatellite markers.

Blood samples were brought to the lab on ice. DNA was extracted from blood by standard Phenol: Chloroform extraction method with modifications and dissolved in Tris-EDTA buffer. Quality check and quantification was done by electrophoresis on 0.8% agarose gel containing ethidium bromide. A set of 22 microsatellite markers (ETH 225, ILSTS 002, ILSTS 005, ILSTS 008, ILSTS 019, ILSTS 022, ILSTS 029, ILSTS 030, ILSTS 033, ILSTS 034, ILSTS 044, ILSTS 049, ILSTS 058, ILSTS 059, ILSTS 065, ILSTS 082, ILSTS 087,Oar AE 129, Oar HH 64, Oar JMP 29, OMHC1 and RM 004,) were selected for the study. The microsatellite loci were amplified from genomic DNA samples by PCR using locus specific primers by Touchdown PCR protocol.

Microsatellite profiling was carried out on 8% urea polyacrylamide gel electrophoresis. After completion of PCR and electrophoresis the DNA profiles were scored from the gel by two independent observers, that were further analysed using standard population analysis software (GenAlex and MS-tools) used internationally for such purpose.

The allele size (bp) of the 22 microsatellite loci *viz.,* ETH 225, ILSTS 002, ILSTS 005, ILSTS 008, ILSTS 019, ILSTS 022, ILSTS 029, ILSTS 030, ILSTS 033, ILSTS 034, ILSTS 044, ILSTS 049, ILSTS 058, ILSTS 059, ILSTS 065, ILSTS 082, ILSTS 087,Oar AE 129, Oar HH 64, Oar JMP 29, OMHC1 and RM 004 were 145-180, 118-134, 177-211, 162-238, 145-183, 191-239, 142-184, 140-204, 161-191, 148-184, 141-197, 158-194, 158-210, 108-138, 115-133, 102-134, 141-173, 133-173, 105-139, 106-138, 178-224 and 110-132, respectively.

The observed number of alleles for the loci ETH 225, ILSTS 002, ILSTS 005, ILSTS 008, ILSTS 019, ILSTS 022, ILSTS 029, ILSTS 030, ILSTS 033, ILSTS 034, ILSTS 044, ILSTS 049, ILSTS 058, ILSTS 059, ILSTS 065, ILSTS 082, ILSTS 087, Oar AE 129, Oar HH 64, Oar JMP 29, OMHC1 and RM 004 were17, 8, 18, 32, 19, 21, 19, 25, 13, 13, 25, 18, 24, 14, 8, 15, 17, 19, 10, 14, 18, and 12, respectively with a mean of 17.227± 1.221.

The effective number of alleles for the loci ETH 225, ILSTS 002, ILSTS 005, ILSTS 008, ILSTS 019, ILSTS 022, ILSTS 029, ILSTS 030, ILSTS 033, ILSTS 034, ILSTS 044, ILSTS 049, ILSTS 058, ILSTS 059, ILSTS 065, ILSTS 082, ILSTS 087,Oar AE 129, Oar HH 64, Oar JMP 29, OMHC1 and RM 004 were 12.953, 4.840, 13.593, 19.208, 13.624, 10.889, 11.406, 17.241, 10.482, 7.022, 15.970, 9.018, 15.890, 11.469, 4.083, 11.571, 9.862, 14.556, 6.211, 6.803, 10.743 and 7.837 with a mean 11.148 ± 0.842.

The values of observed heterozygosity ranged from 0.000 (ILSTS002) to 1.000 (ILSTS033, ILSTS058) with an average of 0.734 ± 0.061, whereas the expected heterozygosity varied from 0.755 (ILSTS065) to 0.948 (ILSTS008) with an overall mean of 0.895 ± 0.010.

The PIC values ranged from 0.72 (ILSTS065) to 0.946 (ILSTS 008) with an average of 0.885 ± 0.011 and thus all the markers under study were found to be highly informative. The Shannon's information index values were found be in the range from 1.650 (ILSTS 065) to 3.204 (ILSTS008) with an average of 2.520 ± 0.08, the high value of indicated the suitability of markers for studying the genetic variability in goat species.

Heterozygosity deficiency (positive F_{IS} value) was observed in sixteen out of twenty two studied loci indicating departures from random mating and suggested that some of the studied loci were homozygous in the population. The positive value of F_{is} ranged from 0.003 to 1.00 with an average of 0.19 ± 0.07. This value is as per expectation as low genetic variations observed in Sangamneri goat breed which may be due to rate of inbreeding in this goat population.

All the parameters (observed number of alleles, effective number of alleles, Shannon's information index and PIC value and gene diversity) estimated to know the genetic variation showed that all the

microsatellite markers used were highly informative, indicating the suitability for genetic diversity studies in goats.

Considering the primary aim to evaluate genetic diversity in Sangamneri goat, the results provided a good scientific validation of genetic and genomic estimates.

1) All the microsatellites could be successfully amplified in Sangamneri goat using the selected primers.
2) All the microsatellite loci used in the present study were polymorphic.
3) Sangamneri goat exhibited substantial amount of genetic variation at the studied loci.
4) The microsatellite markers studied were highly informative for studying genetic variations in goats as indicated by high values of Polymorphic Information Content and Shannon's Information Index values obtained.


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Appendix

APPENDIX - I

Buffers and solutions used in isolation of DNA from Blood by Phenol : Chloroform extraction method

A) 2.7 % EDTA solution (pH 8.0)

EDTA disodium salt	:	2.7 g
Double distilled water	:	100 ml

Adjust pH 8.0 using NaOH pellets. Sterilize by autoclaving and store at room temperature.

B) 0.5 M EDTA solution (pH 8.0)

EDTA disodium salt	:	186.1 g
Double distilled water	:	100 ml

Adjust pH 8.0 using NaOH pellets. Sterilize by autoclaving and store at room temperature.

C) RBC lysis buffer (1X)

Ammonium chloride	:	8.3 g
Potassium bicarbonate	:	1.0 g
0.5 M EDTA (pH 8.0)	:	299 µl
Double distilled water (upto)	:	100 ml

Adjust pH 8.0 using NaOH pellets. Sterilize by autoclaving and store at room temperature.

D) 5 M NaCl solution

Sodium chloride	:	186.1 g
Double distilled water (upto)	:	100 ml

Sterilize by autoclaving and store at room temperature.

E) DNA Extraction buffer

1 M tris buffer (pH 8.0)	:	5 ml
5 M NaCl	:	40 ml
0.5 M EDTA	:	2 ml
Double distilled water (upto)	:	500 ml

Autoclave in batches of 100 ml and store at room temperature.

F) 10 % SDS

SDS (Sodium dodecyl sulphate) : 100 g

Double distilled water (upto) : 1000 ml

Adjust pH 7.2 using concentrated HCl. Heat in water bath at 60^0C to dissolve and then store at room temperature.

G) 3 M sodium acetate

Sodium acetate (anhydrous) : 24.6 g

Double distilled water (upto) : 1000 ml

Adjust pH 5.5 using glacial acetic acid. Autoclave in batches of 20 ml.

H) TE Buffer

1M Tris-HCl (PH-8.0) 1.0 ml (10 mM)

0.5 M EDTA 0.2 ml (1 mM)

Make up to 100 ml with autoclaved double distilled water.

APPENDIX II

Chemicals used in the Agarose Gel Electrophoresis

A) 10X TBE, pH 8.3

0.9M Tris HCl

0.9M Boric acid

20 mM EDTA

B) Gel loading dye (6X)

0.25% bromophenol blue

0.25% Xylene cyanol FF

15% Ficoll.

Stored at room temperature

C) Ethidium bromide (1%)

10 mg Ethidium bromide

1.0 ml distilled water

APPENDIX III

Solutions used for 8 per cent Denaturing Page

A) Gel solution

Acrylamide and N', N', N', N' Bis-acrylamide (19:1)	:	6-7%
Urea	:	42%
10X TBE	:	10%
Autoclaved distilled water to make volume 100 ml		
Ammonium persulphate (10 %)	:	700 µl
TEMED	:	35 µl

B) 2X sequencing gel loading dye

Formamide	:	98%
EDTA, PH 8.0	:	10 mM
Xylene cyanol FF	:	0.025%
Bromophenol blue	:	0.025%

APPENDIX - IV

Genotypic Distribution Of Sangamneri Goat Population At Different Microsatellite Loci

Females (F1 to F41) were coded serially as 1 to 41
Males (M1 to M9) were coded serially as 42 to 50

LOCUS ETH 225

Genotype	Sample number
1,13	42,45
2,8	39
2,9	28,34,40
2,10	37
2,11	35
2,12	48
2,13	36,47,50
3, 8	41
3,11	26,27,32,33
3,12	44
4,9	43
4,10	38,46,49
4,11	31
4,12	30

Genotype	Sample number
5,5	1
6,6	2
6,13	3,29
6,14	4
7,16	13
8,14	11,21
8,15	5,6,7,20,22
8,16	18,19
9,9	23
9,15	8,10,24
9,16	16,17,25
10,10	14
10,16	9
10,17	12,15

LOCUS ILSTS 002

Genotype	Sample number
1,1	1,19
2,2	3,4,5,11,12
3,3	2,8,9,31,33,42,44
4,4	6,7,10,13,15,17,18,20,21,25, 26,28,32,39,47,50
5,5	14,16,30,36,37,43
6,6	24,34,35,40,41
7,7	49
8,8	46,48

LOCUS ILSTS 005

Genotype	Sample number
1,9	27
2,9	28
2,10	26
3,8	29
3,10	2,5,12
3,11	11,31,38
4,4	1
4,11	4,6,7,8,9,10
4,12	18,32,34,39
4,15	3
5,12	13,16,30,35

Genotype	Sample number
5,13	15,33,36,37
6,13	17,19,42
6,14	20
7,14	22
7,15	21,40,41,43
8,15	25,45
8,16	44,46
9,17	23,47
9,18	48,49
10,18	50

LOCUS ILSTS 008

Genotype	Sample number
1,1	5
2,8	1
2,11	2,3,6
3,3	7
4,4	8
4,13	11
4,15	4
5,16	12
5,17	14
5,18	15
6,16	9
7,7	27
7,16	10
8,8	30,34
8,14	20,31
8,18	13,16
8,20	21
9,18	18,19

Genotype	Sample number
9,19	17
9,21	22
10,10	26,29
11,11	25,28,32,35
12,12	24,33
13,23	23,36
15,24	37
16,16	41,42
16,25	39
16,26	40
19,28	43
21,27	48
21,29	49
22,28	50
22,30	44
23,31	46
24,31	45
27,32	47

LOCUS ILSTS 019

Genotype	Sample number
1,1	45
2,8	42
3,3	30,41
3,8	47
3,9	40,44,46
4,4	37,43,50
4,9	48
4,10	1,49
5,5	7
5,10	26,28
5,11	27
5,12	2,39
6,6	38
6,12	3,4,29,31
6,13	5

Genotype	Sample number
7,7	36
7,11	32
8,14	35
8,15	6,10,11
9,9	34
9,12	16
9,13	9
9,15	8,12,13
9,16	14
10,14	25
11,17	18
12,17	15,17
12,18	19,20
13,17	21
14,19	22,23

LOCUS ILSTS 022

Genotype	Sample number
1,10	1
2,9	4
2,10	9
2,15	3
3,11	12
3,13	26,27
4,4	34,35,37,39,47
4,10	6
4,11	18
4,12	7,10
4,13	11
4,14	29
4,15	31,32
5,5	36,38,40
5,9	16
5,11	17

Genotype	Sample number
5,12	19
5,14	14
5,15	28
6,6	43
6,12	13
6,14	8
6,15	2
7,7	42,44,48,50
7,14	5,20
8,16	15
8,17	25
8,21	45
9,17	23
9,19	24
9,20	21
10,18	22

LOCUS ILSTS 029

Genotype	Sample number
1,5	3
1,6	2
2,6	7
2,7	4
2,8	9
3,7	5
3,8	6,8,10,11,12,15
3,14	16
3,15	17
4,4	20
4,8	13
4,9	14,19
4,10	18
4,12	42
5,16	21
6,6	37
6,10	25
6,11	50

Genotype	Sample number
6,12	23
6,14	44,49
6,16	22
7,7	41
7,13	24
8,8	35,38
8,13	47,48
8,15	45
8,16	36,39,40
8,18	46
9,14	30
9,15	31
9,16	32
10,16	33
11,16	29
11,17	27,28
12,17	26
13,19	34

LOCUS ILSTS 030

Genotype	Sample number
1,4	6
3,10	26
4,11	5
5,15	36
6,6	4,30
6,12	7
6,13	28
6,14	3,29
6,15	10
7,7	32
7,13	27
7,14	8,31
8,8	40,41
8,16	37
8,17	14,33
9,9	34
9,14	13
9,15	2
9,16	9
9,17	16

Genotype	Sample number
9,18	35
10,10	39,44
11,17	43
11,18	46
11,19	42
11,23	15
12,12	38
12,19	11
12,20	12
12,23	23,49
13,13	45
13,20	47,48
13,21	22
13,23	21
14,20	18
14,22	50
14,24	20
16,23	17
19,25	25

LOCUS ILSTS O33

Genotype	Sample number
1,6	1
1,7	2
2,8	3
2,9	29
3,9	4,26,27,30,32,33
3,10	5,28,31,34
4,10	6,14,35,36,37,49
4,11	7,8,9,10,12,38,42

Genotype	Sample number
5,10	50
5,11	11,13,39,40
5,12	15,16,41
6,12	18,43,46,48
6,13	20,21,22,24,25,44
7,12	45
7,13	17,19,23,47

LOCUS ILSTS 034

Genotype	Sample number
1,7	4
2,8	25
2,10	20,26
3,9	1,3,31
3,10	5,27
3,11	50
4,9	2,6,8,9,15,24,32
4,10	7,10,14,21,30,36,45,46
5,9	28,29,33

Genotype	Sample number
5,10	12,16,23,34,37,39,42,43
5,11	13,17,22,35,41
5,12	19
6,10	38,40,44
6,11	18,47
6,12	48
8,13	49
9,9	11

LOCUS ILSTS 044

Genotype	Sample number
1,5	25
2,9	24
3,9	21
3,10	20,22
3,11	19
4,4	18
5,13	14,50
6,14	17
6,15	16,31,48
6,16	15
7,7	23
7,14	47,49
8,13	33,34
8,14	41,45
8,15	38,39,42
8,16	30

Genotype	Sample number
8,17	13
9,15	36,43
9,16	35,44
9,17	28,46
10,16	32
10,17	27
11,18	12,26
11,20	11
12,21	10
12,22	9
13,22	8
13,23	6
15,15	1,7
16,24	4
19,25	3
20,25	2

LOCUS ILSTS 049

Genotype	Sample number
1,7	17
2,6	12
3,8	13
3,11	2
4,12	9
4,13	26
5,9	15
5,14	27
6,12	4,5,31
6,13	6,7,11,14,46
7,7	19
7,13	10,32,45

Genotype	Sample number
7,14	16,20
7,15	43
8,8	36
8,13	35
8,14	1,21,28,29,30,33,34,38, 41,42
8,15	18,37,40,44
9,9	47
9,16	24
10,16	23,48
11,17	49
12,18	50

LOCUS ILSTS 058

Genotype	Sample number
1,7	8
1,9	25
2,7	26
2,9	27
3,8	28
4,8	10
4,10	1
5,10	11
5,13	23
6,12	24
7,14	20,22
7,15	14,21,44
8,15	17,18,29,46
8,16	9,19
9,16	15

Genotype	Sample number
9,17	48
10,16	2,3,4,7,12
10,17	31,32
10,18	5,13
11,17	30
11,18	6,34,35,39
11,20	33
12,19	36
12,20	38
13,20	37,42
14,21	40,41
16,22	45
17,23	50
17,24	47
18,24	49

LOCUS ILSTS 059

Genotype	Sample number
1,4	25
2,2	36
2,6	37
2,9	50
3,3	22
3,7	24
3,8	20,23
3,9	29
4,4	49
4,8	18
4,9	28,38
5,5	35
5,9	8
5,10	21,27,32

Genotype	Sample number
5,11	10,19,42,44
6,6	39
6,9	31
6,10	2
6,11	1,26,34,40
6,13	43
7,10	47
7,11	9,11
7,12	12,13,15,17
7,13	7,41
8,11	4
8,12	3,48
8,13	5,45,46
9,14	6

LOCUS ILSTS 065

Genotype	Sample number
1,1	1,3
2,2	4,5,6
3,3	7,8,11,17,18,22,23,25,31,46
3,6	14,26,28,29,30,42,43,47

Genotype	Sample number
4,4	10,12,16,19,20,21,32, 33,39,45,50
4,7	15,34,35,36,37,38,41, 44,48,49
4,8	13,40
5,5	24

LOCUS ILSTS 082

Genotype	Sample number
1,8	1
2,3	3
3,6	2
3,11	27
4,7	5,36,37
4,8	45
4,9	18,33
4,10	20,50
4,12	22
5,5	38
5,7	7
5,9	4
5,12	10
6,9	16

Genotype	Sample number
6,10	41
6,11	29,30
6,13	32
7,7	49
7,10	43
7,12	28
7,13	31
8,8	9,39,40
8,12	11,34
8,13	35
9,13	8,17
9,14	6,15,23,24,44
10,14	13,14,25,42
11,15	47,48

LOCUS ILSTS 087

Genotype	Sample number
1,7	1
2,7	2
3,7	5
3,8	27
4,8	7,8,26
5,5	4
5,9	33,34,44
5,10	11,47
6,9	20,29,46
6,10	13,15,35,36,43,50
6,11	17,23,31,37,48
7,10	6,16,28

Genotype	Sample number
7,11	3,19,42
7,12	18,30,32,38,49
7,13	21
7,15	40,41
8,12	12
8,14	45
9,13	9
10,14	10,25
10,15	39
10,16	14
12,16	24
12,17	22

LOCUS Oar AE 129

Genotype	Sample number
1,1	26
2,2	30
3,3	15,31,32,33,37
4,4	17
4,11	27
4,12	35,36
5,12	13,16
5,13	47
6,13	48
6,14	12
7,7	44,45,46
7,14	11,18
8,8	19

Genotype	Sample number
8,15	49
8,16	43
9,9	4
9,16	10,40,41,42
10,10	5,20
10,17	3,6,9,50
10,18	2,7
11,18	1,8
12,19	21
13,13	22
15,15	23
16,16	24
18,18	25

LOCUS Oar HH64

Genotype	Sample number
1,6	10
2,2	2
3,3	26
4,4	1,3
5,5	5,6,8,14,31,35
6,6	4,27,30

Genotype	Sample number
7,7	28,34,37,40,44
8,8	9,15,17,18,19,22,36, 41, 42,47
9,9	12,20,38,39,43,45,48
10,10	46,50

LOCUS Oar JMP 29

Genotype	Sample number
1,8	25
2,12	11
3,3	50
3,8	1
3,10	3,24
3,12	10
4,11	6
4,12	5
4,13	12
5,5	38,39
5,10	15
5,11	2
5,12	7,8,21,22

Genotype	Sample number
5,13	13,14
6,6	16,35, 36,37,40,41,42, 43,44,45,46,49
6,12	4,17,23
6,13	9,18,19,20
7,7	34,47,48
8,8	33
9,9	31,32
10,10	30
11,11	29
12,12	28
14,14	26,27

LOCUS OMHC 1

Genotype	Sample number
1,7	27
2,2	24,29
3,3	20,23
3,7	19,26
3,9	33,42
4,4	17,22,25
4,8	35
4,12	50
5,9	36
5,10	2,37
6,6	8,10,11,12,13,30,32
7,7	9,16,31
7,11	41

Genotype	Sample number
7,13	18
7,15	28
8,8	5,34
8,16	43
8,17	39
9,9	6,14
9,14	7
9,16	45
9,17	38,40,46
10,17	44
10,18	48
11,18	49

LOCUS RM 004

Genotype	Sample number
1,5	25
1,6	50
2,7	24
3,7	49
3,9	17,48
4,4	23
4,8	18,19,21,40,41
4,9	13,15,16,20
4,10	12
5,5	30,33,35
5,8	14,44,45

Genotype	Sample number
5,9	22,26,27,42,43
5,10	1,28
5,11	6,7,10,29,38,39
6,6	31,32
6,9	8,11
6,10	3,9,37
6,11	36
7,7	46,47
7,11	2,4
7,12	5
8,8	34


Vita

VITA

The author **Dr. Sapna Nath** was born on 6th November, 1985 in Haleswar Borphukhuripar (Tezpur), Distt. Sonitpur of Assam State. She completed her Secondary School Certificate examination in 2001 from K.V. No. 2 (C.B.S.E.), Air Force Station, Tezpur in first class and Higher Secondary School Certificate examination in 2003 from K.V. No. 2 (C.B.S.E.), AFS, Tezpur in first class. He was selected for National games in Badminton and represented her college at national level in 2005 at Kerala.

Later she joined Veterinary profession and completed B.V.Sc. & A.H. from College of Veterinary Sciences, Khanapara, Guwahati in 2009. She joined postgraduate (M.V.Sc.) studies in the discipline of Animal Genetics and Breeding in the Post Graduate Institute of Veterinary and Animal Sciences, Akola in 2010.

She is a Life member of Indian Society of Animal Genetics and Breeding and presented papers in national conferences.


Thesis Abstract

THESIS ABSTRACT

a)	**Title of the thesis (in Capital letters)**	: **"MOLECULAR GENETIC CHARACTERIZATION AND GENETIC DIVERSITY ANALYSIS OF SANGAMNERI BREED OF GOAT USING MICROSATELLITE MARKERS"**
b)	**Full name of student**	: **Sapna Nath**
c)	**Name and address of Major Advisor**	: **Dr. S. V. Kuralkar** Head, Department of Animal Genetics and Breeding, Post Graduate Institute of Veterinary and Animal Sciences, Akola.
d)	**Degree to be awarded**	: M.V.Sc.
e)	**Year of award of degree**	: 2012
f)	**Major subject**	: Animal Genetics and Breeding
g)	**Total number of pages in the thesis**	: 79
h)	**Number of words in the abstract**	: 441
i)	**Signature of Student**	:
j)	**Signature, Name and address of forwarding authority**	:

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ABSTRACT

Fifty blood samples from unrelated Sangamneri goat were collected at randomly and DNA was extracted from blood. A set of 22 microsatellite markers were selected for the study. The microsatellite loci were amplified from genomic DNA samples by PCR using locus specific primers by Touchdown PCR protocol. Microsatellite profiling was carried out on 8% urea polyacrylamide gel electrophoresis. After completion of PCR and electrophoresis the DNA profiles were scored from the gel, that were further analysed using standard population analysis softwares (GenAlex and MS-tools) used internationally for such purpose.

The allele size (bp) of the 22 microsatellite loci *viz.,* ETH 225, ILSTS 002, ILSTS 005, ILSTS 008, ILSTS 019, ILSTS 022, ILSTS 029, ILSTS 030, ILSTS 033, ILSTS 034, ILSTS 044, ILSTS 049, ILSTS 058, ILSTS 059, ILSTS 065, ILSTS 082, ILSTS 087,Oar AE 129, Oar HH 64, Oar JMP 29, OMHC1 and RM 004 were 145-180, 118-134, 177-211, 162-238, 145-183, 191-239, 142-184, 140-204, 161-191, 148-184, 141-197, 158-194, 158-210, 108-138, 115-133, 102-134, 141-173, 133-173, 105-139, 106-138, 178-224 and 110-132, respectively.

The observed number of alleles varied from 8 (ILSTS002, ILSTS065) to 32 (ILSTS008) with a mean of 17.227 ± 1.221. The effective number of alleles varied from 4.083 (ILSTS065) to 19.208 (ILSTS008) with a mean 11.149 ± 0.842.

The values of observed heterozygosity ranged from 0.000 (ILSTS002) to 1.000 (ILSTS033, ILSTS058) with an average of 0.734 ± 0.061, whereas the expected heterozygosity varied from 0.755 (ILSTS065) to 0.948 (ILSTS008) with an overall mean of 0.895 ± 0.010.

Twenty one out of 20 loci studied showed significant deviations from the Hardy Weinberg equilibrium and 73 per cent loci showed heterozygosity deficiency.

The PIC values ranged from 0.72 (ILSTS065) to 0.946 (ILSTS 008) with an average of 0.885 ± 0.011 and the Shannon's information index values were found be in the range from 1.650 (ILSTS 065) to 3.204 (ILSTS008) with an average of 2.520 ± 0.08, the high value of indicated the suitability of markers for studying the genetic variability in goat species.

Heterozygosity deficiency (positive F_{IS} value) was observed in sixteen out of twenty two studied loci indicating departures from random mating and suggested that some of the studied loci were homozygous in the population. The positive value of F_{is} ranged from 0.003 to 1.00 with an average of 0.19 ± 0.07.

All the parameters (observed number of alleles, effective number of alleles, Shannon's information index and PIC value and gene diversity) estimated to know the genetic variation showed that all the microsatellite markers used were highly informative, indicating the suitability for genetic diversity studies in goats. All the studied loci were polymorphic and reflected substantial genetic diversity in Osmanabadi goat population.


प्रबंध सारांश

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१.	प्रबंधाचे शिर्षक	: ''मायक्रोसॅटेलाईट मार्करचा उपयोग करुन संगमनेरी शेळीमध्ये अनुवांशिकी गुणांचा व अनुवांशिकी तफावतीचा अभ्यास''
२.	विद्यार्थ्याचे पूर्ण नांव	: सपना नाथ
३.	मुख्य मार्गदर्शकाचे नांव व पत्ता	: डॉ. एस. व्ही. कुरळकर विभाग प्रमुख, पशु अनुवांशिकी व प्रजननशास्त्र विभाग, स्नातकोत्तर पशुवैद्यक व पशुविज्ञान संस्था, अकोला.
४.	प्रदान केली जाणारी पदवी	: एम.व्ही.एस्सी.
५.	पदवी प्रदान करण्याचे वर्ष	: २०१२
६.	मुख्य विषय	: पशु अनुवांशिकी व प्रजननशास्त्र विभाग
७.	पबंधामधील एकुण पाणे	: ७९
८.	प्रबंध सारांशामधील एकुण शब्द	: २४८
९.	विद्यार्थ्याची सही	:
१०.	प्रबंधक कार्यवाहीस्तव पाठविणाऱ्या अधिकाऱ्याची सही, नाव व पत्ता	:

(एस. व्ही. कुरळकर)
विभाग प्रमुख
पशु अनुवांशिकी व प्रजनन शास्त्र विभाग,
स्नातकोत्तर पशुवैद्यकीय व पशुविज्ञान संस्था,
अकोला.

सारांश

संगमनेरी शेळीमध्ये अनुवांशिकी गुणांचा व अनुवांशिकी तफावतीचा अभ्यास करण्यासाठी २२ मायक्रोसॅटेलाईट मार्करर्सची (निशान चिन्ह) निवड करुन सदर शोध कार्यात उपयोग करण्यात आला. ज्या उस्मानाबादी शेळ्यामध्ये कुठलेही नातेसंबंध नाही अश्या एकुण ५० उस्मानाबादी शेळीचे रक्ताने नमुने घेण्यात आलेत. प्रत्येक रक्ताच्या नमुन्यातील जनुके मिळविण्यात आले व तद्नंतर प्रमाणित

पीसीआर द्वारे रुपरेषा ठरविण्यात आली आहे. त्यानंतर ८% युरीया पॉलीएक्रीएमाईड जेल चा वापर करुन मायक्रोसॅटेलाईटच्या आराखड्याचे वैशिष्टकरण करण्यात आले.

अलीलची दृष्यरुपी संख्या ही ८ (आयएलएसटीएस ००२ व ०६५) ते ३२ (आयएलएसटीएस ००८) पर्यंत असुन सरासरी १७.२२७ ± १.२२१ एवढी आहे, परंतू अलील ची उपयोगी असणारी संख्या ७.०८३ (आयएलएसटीएस ०६५) ते १९.२०८ (आयएलएसटीएस ००८) एवढी असून सरासरी ११.१४९ ± ०.८४२ एवढी आहे.

उस्मानाबादी शेळ्यांमध्ये जनुकाची अपेक्षित सरासरी तफावत ही ०.७५५ (आयएलएसटीएस ०६५) ते ०.९४८ (आयएलएसटीएस ००८) एवढी काढण्यात आली व त्याची सरासरी ही ०.८९५ ± ०.०१० एवढी होती. २२ पैकी २१ मायक्रोसॅटेलाईट हे हार्डीवेनबर्ग एक्वीलीब्रीयम पासुन दुर आहे. सदर संशोधनात पीआसी ची संख्या काढण्यात आली ही संख्या ०.७२ (आयएलएसटीएस ०६५) ते ०.९४६ (आयएलएसटीएस ००८) पर्यंत असून त्याची सरासरी ०.८८५ ± ०.०११ एवढी आहे. शॉनान माहितीदर्शक तक्त्यानुसार संख्या १.६५० (आयएलएसटीएस ६५) ते ३.२०४ (आयएलएसटीएस ००८) एवढी असुन सरासरी २.५२० ± ०.०८ एवढी आहे.

सगळ्या घटकांचा विचार केला असता उदा. अलील ची दृष्यरुपी संख्या, अलील ची उपयोगी असणारी संख्या, जनुकाची अपेक्षित सरासरी ची तफावत, पीक संख्या व शॉनान माहितीदर्शक तक्त्यानुसार असे निदर्शनास येते की, या संशोधनात उपयोगात असलेले मायक्रोसॅटेलाईट चिन्ह हे अनुवांशिक तफावतीचा अभ्यास करण्याकरीता अतिशय उपयुक्त व माहिती दर्शक आहे. यावरुन असे दिसते की, सदर मॉयक्रोसॅटेलाईट चिन्ह शेळ्यांमध्ये अनुवांशिकी तफावतीचा अभ्यास करण्यास सोयीस्कर आहे.