GENETIC CHARACTERIZATION AND DIVERSITY STUDY OF INDIGENOUS CATTLE BREEDS THROUGH MICROSATELLITE AND AFLP MARKERS

देशी गोवंश प्रजातियों का माइक्रोसैटेलाइट एवं ए.एफ.एल.पी. चिह्नकों द्वारा आनुवांशिक अभिचित्रण व वैभिन्य अध्ययन

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।पशुधनं नित्यं सर्वलोकोपकारकम्।

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INTRODUCTION

India is one of the mega bio-diversity centers of the world. As per the 19th Livestock Census (2012), the cattle population in India is 48.12 million, of which 160.50 million are indigenous. Mechanization, unplanned and indiscriminate breeding among native stocks as well as human bias in favor of certain breeds have directly or indirectly lead to the dilution of indigenous germplasm (FAO, 2000). Hence, there is an urgent need to prevent the rapid erosion of animal genetic resources.Indian agriculture is an economic symbiosis of crop and livestock production with cattle as the foundation. Sadly, the population of indigenous cattle (*Bos indicus*) is declining (8.94 % in last decade) and needs immediate scientific management. Genetic characterization is the first step in the development of proper management strategies for preserving genetic diversity and preventing undesirable loss of alleles.

In India, National Bureau of Animal Genetic Resources (NBAGR) Karnal, is entrusted with characterization of important indigenous livestock breeds. NBAGR has recognized 27 breeds of cattle, 8 of buffalo, 42 of sheep, 20 of goats, 6 of horses and 17 of poultry. Livestock conservation is carried out at breed level. The genetic variation both between and within breeds is described as the diversity within each species. The genetic variation exists between two groups, which separate them genotypically. Genetic variation of the animal is the basic material, which is utilized for changing the genetic makeup or genetic potentiality of domestic species to suit our needs. Mechanization, unplanned and indiscriminate breeding among native stocks and human bias in favor of certain breeds are directly or indirectly responsible for the dilution of Indian livestock germplasm. Hence, characterization of indigenous germplasm is essential for their conservation.

Genetic characterization can be done by various methods e.g. cytogenetic/biochemical and molecular techniques. Cytogenetic and biochemical methods are less sensitive, less accurate and reveal less polymorphism. Recent trend is to use molecular techniques for characterization which detect the genetic variation at DNA level. These

techniques explore molecular markers such as RFLP, RAPD, AFLP and microsatellites.

In recent years, a range of innovations in molecular genetics has been developed for the study of genetic variation and evolution of populations using DNA genotyping information. The most utilized DNA marker for population genetics of livestock is microsatellite. Microsatellite markers, also called short tandem repeats (STRs) or simple sequence repeats (SSRs), are a relatively new class of genetic marker. Over a few years they have become a tool of choice to address population genetics and demographic questions (Sunnucks, 2000). The application of microsatellite markers is currently considered to be useful in the analysis of genetic diversity as they are numerous, randomly distributed in the genome, highly polymorphic, and they show codominant mode of inheritance (Ellegren, 1993). They allow the study of genetic diversity and differentiation of closely related populations. Microsatellite as genetic markers, have been applied successfully in the study of genetic variation of livestock 4including between and among European and African cattle breeds (Machugh *et al.*1997; Okoma *et al.*1998).

The amplified fragment length polymorphism (AFLP) technique has become one of the most reliable and promising DNA fingerprinting methods, producing hundreds of informative polymerase chain reaction (PCR)–based genetic markers to provide a wide multi-locus screening of any genome. The AFLP analysis has been largely documented in the literature (Blears *et al.*1998; Jones *et al.*1997; Mueller *et al.*1999; Savelkoul *et al.*1999).

With sufficient numbers of markers, the variability of AFLP loci allows parentage assignment and individual identification (Krauss 1999; Questiau *et al.* 1999). The ability to produce large numbers of markers scattered throughout the genome without prior sequence knowledge has also resulted in AFLP markers being used for constructing linkage maps and for identifying quantitative trait loci. (Jin *et al.* 1998; Lu *et al.* 1998, Otsen *et al.* 1996). However, the use of AFLP markers for estimating levels of inbreeding in individuals and relatedness between individuals has not been fully explored.

FAO-DAD-1S has published 78 indigenous, exotic and cross breeds in India. NBAGR (Karnal) under the aegis of ICAR initiated a programme “Registration of Animal Germplasm” which describes 30 registered cattle breeds of India. Some nationally recognized breeds having home tracts in Rajasthan are Gir (milch), Kankrej, Hariana (dual purpose), Rathi, Tharparker, Nagauri and Malvi(draught). There is loss of genetic diversity due to genetic improvement programme and breeding and conservation programmes can be determined by characterising genetic variation of livestock (Notter, 1999). The breed has got excellent reputation as beef animal abroad and large numbers have been exported to Brazil from where they have been introduced by other countries of American continent.

The Gir is one of the principal *Bos indicus* milch breed in India. It is originated in Gujarat and has since spread to neighboring states like Maharashtra, Madhya Pradesh, Karnataka, Rajasthan, Punjab and Haryana. These cows are good milkers. Milk yield ranges from 1200 to 1800 kg per lactation. Kankrej is highly praised as draught cattle breed basically originated from South-eastern Rann of Kutch, Gujarat, and adjoining Rajasthan particularly along the banks of the river Saraswati. The Kankrej cattle are very highly prized as fast, powerful draft cattle. The animals have a broad chest, straight back and a well-developed hump. These cows are average milkers and yield about 1400 kg under farm conditions while yield under village conditions is low. The Rathi is a *Bos indicus* breed used for draft and dairy purposes. It’s assumed to be originated in Bikaner and Ganganagar in northwest Rajasthan, India. The breed is usually dark red or tan but occasionally spotted individuals can be found. Their udder is well developed. The females are docile and good milkers (1325 to 2093 kg per lactation). The Tharparkar, a *Bos indicus* breed used for milk production and as draft animals. The original habitat of this breed is Tharparkar district in the Province of Sind, Pakistan. The breed is also found in the adjoining tracts in Rajasthan State in India, particularly around Jodhpur and Jaisalmer where excellent milch specimens are found. The males are also good draught animals. The milk yield in cow’s ranges from 1800 to 2600 kg per lactation.

The breeding tract of Sahiwal breed is Montgomery district in Pakistan which is now named as Sahiwal. This is the best dairy breed of the Indian subcontinent. It is a comparatively heavy breed with a symmetrical body and loose skin. The animals are usually long and fleshy and of heavier build. They are colored reddish dunn or pale red, sometimes flashed with white patches .A number of herds of this breed are maintained in India. The milk yield ranges from 1400 to 2500 kg per lactation. The breeding tract of Nagori breed is Bikaner, Jodhpur and Nagaur district of Rajasthan. The breed takes its name from the name of the home tract i.e., Nagaur district. They are basically White in color and are upstanding, very alert animals with long and narrow face. The Nagori breed is one of the most famous draught breed of India and are generally appreciated for fast draught activity. Average milk yield per lactation of Nagori cattle is 603 kg. The lactation yield ranges from 479 to 905 kg.

Population size of these indigenous cattle breeds is declining due to introduction of exotic cattle breeds hence molecular characterization and diversity study among them using molecular markers is required (Mathur, 2005).Owing to the extreme importance of conservation of these indigenous breeds. Genetic characterization of breeds using molecular markers (FAO 2007) provides information for conservation making decisions for livestock populations (Sunnucks, 2000).Breeds distinctiveness by microsatellite markers calls for variation in allele frequencies between test breeds. (Hanotte and Jianlin, 2005).

Therefore, to utilize the cattle genetic resources effectively, it is necessary to characterize Indian indigenous cattle populations genetically. Such characterization would provide a comprehensive database of genetic variation among the cattle populations in India. It would provide furthermore information as to which of the populations represent homogenous populations and which of them are genetically distinct. The information generated would contribute to the understanding of the evolutionary history of cattle in India. It will also contribute to the conservation and management of genetic resources. The breeds were phenotypically characterized but their genetic characterization was pending. Hence, present study is aimed to characterize

indigenous cattle at molecular level using microsatellite markers with following objectives.

1. To select a suitable set of molecular markers for determining genetic diversity of Gir, Kankrej, Rathi, Tharparkar, Sahiwal and Nagori cattle breeds of Rajasthan.
2. To assess genetic variability within the cattle breeds viz. Gir, Kankrej, Rathi, Tharparkar ,Sahiwal and Nagori using Microsatellite and AFLP markers.

2. REVIEW OF LITERATURE

The primary unit in animal genetic resources is a breed, strain or geographically defined groups, the members of which share particular morphological characteristics which distinguished them from other groups. Hence, identification and characterization of breeds is a must to identify our genetic resources and also to prioritize breeds for conservation. Assesing genetic variability, as well as relatedness within and among the population, parentage determination, possible bottlenecks, linkage disequilibrium, inbreeding coefficients are also essential for analyzing complete population structure. The complete population structure helps us to plan strategies for conservation and development of a breed. The various markers have been used to asses the population structure and genetic variation both between and within breeds. As a source of information for estimation of genetic distance, variation in gene products such as enzymes, blood group systems and leukocyte antigens have now been almost entirely superseeded by polymorphisms at the DNA level.

DNA polymorphisms may be detected in a variety of ways, the most common being restriction fragment length polymorphisms (RFLPs), randomly amplified polymorphic DNAs (RAPD) and variable number of tandem repeats (VNTRs), short tandem repeats (STR) *i.e.* microsatellites. Genetic variation is the raw material for the animal breeders, which is used to mold our domestic animal species to our needs. The genetic variation both between and within breeds is described as the diversity within each species.

2.1. Molecular Markers

2.1.1. Restriction Fragment Length Polymorphisms (RFLPs):

It is the first DNA polymorphism to be widely used for genomic characterization, which detects variations ranging from gross rearrangements to single base changes. The polymorphisms are found by their effects on sites for restriction enzyme mediated cleavage of preparations of high molecular weight DNA. This method has been used extensively to reveal polymorphism in DNA and to characterize populations of variety of microbial, plant and

animal species (Karl and Avis, 1992). In case of livestock species, it has not found great favors for nuclear DNA characterization, probably because it is an expensive and relatively laborious approach. In contrast, the small size of the mitochondrial DNA itself tends to RFLPs. This technique has been effectively applied to reveal polymorphisms in selected mitochondrial DNA regions which exhibited relatively high variation, following amplification by PCR (Suzuki *etal*., 1993).

2.1.2. Randomly Amplified Polymorphic DNAs (RAPD):

PCR amplification on a random primer has been used extensively for genetic characterization of bacteria, plants and mammalian species. This technique utilizes short (9-10 bases) primers, designed on a random basis with the sole constraint being high GC content. The principal advantage of the approach is that the levels of detectable polymorphism are generally high. The principal disadvantage of the methodology is that the PCR results are very sensitive to amplification conditions and consequently can be variable between laboratories and even between assays**.**

2.1.3 Microsatellites:

Microsatellites are sequences made up of a simple sequence motif, not more than six bases long, that is tandemly repeated and arranged head to tail without interruption by any other base or motif. Simple, tandemly 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 make them potentially very useful as DNA markers in gene mapping studies. Furthermore, two or more microsatellites may be analyzed simultaneously (Weber and May, 1989), opening new opportunity for genetic analysis of large number of samples. Moreover, methods are being developed which will simplify detection and analysis of microsatellite polymorphism (Litt *et al.* 1993). A very important attribute is that polymorphism can be described numerically and thus the system tends itself computerized data handling and analysis.

Another advantage is that the sequence on microsatellite analysis can be shared between collaborators. It is becoming clear that they offer an excellent means of distinguishing closely related breeds and it is not providing difficulty to identify breed and population specific alleles. In the last decade, “microsatellite” loci (also known as simple sequence repeat (SSR) or simple tandem repeat (STR) loci) have become the genetic markers of choice for many kinds of molecular applications, including analysis of population structure (Arora *et al.* 2004) and dispersal patterns (MacHugh *et al.* 1997; Wimmers *et al.* 2002), to estimate genetic variability and inbreeding (Zajc *et al.* 1997, Hedrick *et al.* 2001 and Mateus *et al.* 2004) evaluation of paternity, to maintain pedigree records (Marklund *et al.* 1994; Mullis *et al.*1995; Talbot *et al.* 1996,), for tracking alleles through a population (Moazami-Goudarzi *et al.* 1997; Arranz *et al.* 1998) and individual identity, and estimation of degree of relatedness between populations or pairs of individuals (Maudet *et al.* 2002).

Microsatellite markers are ideal for population-level studies for a number of reasons. First, they are randomly distributed throughout the genome, commonly occurring in noncoding regions, and are typically selectively neutral. Second, microsatellite loci are often hypervariable 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, 1986). 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.

2.1.4 AMPLIFIED FRAGMENT LENGTH POLYMORPHISM

The amplified fragment length polymorphism (AFLP) technique has become one of the most reliable and promising DNA fingerprinting methods, producing hundreds of informative polymerase chain reaction (PCR)–based genetic markers to provide a wide multi-locus screening of any genome (Vos et al. 1995). The AFLP analysis has been largely documented in the literature (Blears *et al.* 1998; Jones *et al.* 1997; Mueller *et al.* 1999; Savelkoul *et al.* 1999); here, we emphasize one of its more overlooked aspects—technical information. We discuss the important factors of the procedure (enzyme, primer, and marker choice; influence of genome size; genotyping errors) and give several recommendations and protocols to successfully develop AFLP markers for vertebrates.

The essence of the AFLP procedure lies in the combined use of two basic tools in molecular biology: restriction, which reduces the total genomic DNA into a pool of fragments, and PCR, which amplifies a subset of these restriction fragments thanks to primers with arbitrary selective extensions (Mueller *et al.* 1999; Savelkoul *et al.* 1999). Three kinds of AFLP polymorphisms can then be observed: a mutation in the restriction site, a mutation in the sequence adjacent to the restriction site and complementary to the primer extensions, or a deletion/insertion within the amplified fragment (Ajmone-Marsan *et al.*1997; Matthes *et al.* 1998). Polymorphisms are revealed by the presence of a fragment of a given size in some AFLP profiles versus its absence from other profiles.

AFLP fingerprinting has been of great interest in population genetics because of several advantageous characteristics. First, it is the method of

choice for studies of non-model organisms (Blears *et al.* 1998; Vos *et al.* 1995). Theoretically, it can be performed on any genome, regardless of its complexity and structure and without any prior sequence knowledge, in contrast to other kinds of molecular markers like microsatellites that require taxon-specific primers (Dogson *et al.* 1997). Practically, commercial AFLP primer sets are available that work on most organisms. Second, large numbers (up to several hundreds) of AFLP markers can be typed quickly and at low cost, offering fine-scale genome coverage (Blears *et al.* 1998; Mueller *et al.* 1999), although several studies have reported AFLP clustering in centromeric regions (Lindner *et al.* 2000; Young *et al.* 1998). AFLP markers are also largely independent, because 90% of them reflect point mutations in enzyme restriction site (Buntjer *et al.* 2002) that remove the fragments from the AFLP profile rather than change its size (Albertson *et al.* 1999). Third, AFLP markers usually reveal a greater amount of diversity compared to simple sequence repeats (SSRs) and random amplified polymorphic DNAs (RAPDs) (Archak *et al.* 2003) and provide valuable fingerprints of organisms like birds in which microsatellite markers are difficult to obtain (Dogson *et al.* 1997; Knorr *et al.* 1999). Fourth, thanks to stringent hybridization conditions and relative insensitivity to template DNA concentration, the AFLP fingerprint is highly reproducible and reliable (Ajmone-Marsan *et al.* 1997; Bagley *et al.* 2001; Jones *et al.* 1997). As a result, it can be standardized, reproduced easily between different technicians and laboratories, and computer-scored for subsequent comparisons (Hong and Chuah, 2003). This makes it particularly well-adapted for large-scale studies involving several research centers (Jones *et al.* 1997). Fifth, only small amounts of genomic DNA are necessary to generate several informative AFLP profiles with different primer combinations (Blears *et al.* 1998; Savelkoul *et al.* 1999; Vos *et al.* 1995). Finally, AFLP markers have been shown to follow mendelian inheritance in plants (Blears *et al.* 1998; Savelkoul *et al.* 1999), as well as in animals (Ajmone-Marsan *et al.* 1997; Otsen *et al.* 1996).

Despite its attractiveness, the AFLP method has some detrimental aspects. First, AFLP markers should be considered as dominant biallelic markers: fragment presence versus absence, with the fragment presence

allele dominant over the absence allele (Ajmone-Marsan *et al.* 1997; Mueller *et al.* 1999). It is indeed difficult to distinguish between heterozygous individuals and individuals homozygous for the presence allele because of differential efficiencies between distinct PCR amplifications, unless exact genotypes can be inferred by means of pedigree studies (Van Haeringen *et al.* 2002). AFLP data are thus of poor information contents in analyses requiring precise estimations of heterozygosity.

Nonetheless, several studies have managed to score up to 65% of the markers in a codominant way by rigorous standardization of profile intensities (Ajmone-Marsan *et al.* 1997), and new protocols have been developed to investigate AFLP-like codominant markers (Bradeen and Simon, 1998; Hakki and Akkaya, 2000). Second, fragments originating from distinct loci may have the same length by chance (homoplasy of size) (O'Hanlon and Peakall, 2000; Vekemans *et al.* 2002). Such fragments display exactly the same electrophoretic mobility and thus overlap on the AFLP profile, introducing an undesirable source of artifacts. However, comigration of distinct fragments has proven to be a rare event (Mechanda *et al.* 2003). Third, the AFLP procedure is particularly sensitive to contamination by exogenous DNA; even low and unobtrusive levels of bacterial or fungal contaminants, for example, may alter the AFLP profiles (Dyer and Leonard, 2000; Savelkoul *et al.* 1999). When working with organisms prone to such kinds of contaminations, one should take special precautions to ensure the reliability of the results.

Originally worked out for plants and microorganisms, the AFLP analysis now finds more and more applications within the animal kingdom, especially in vertebrate species. Because their resolution power extends from the individual to the species level, AFLP markers have proven to be valuable tools in individual identification (Ovilo *et al.* 2000), sex determination (Griffiths and Orr, 1999; Questiau *et al.* 2000), parentage analysis , genetic diversity assessment (Ajmone-Marsan *et al.*1997; Mock *et al.* 2002), population assignments

The population structure and estimations of gene flow investigations (Dearborn *et al.* 2003; Jorde *et al.* 1999), hybridization studies (Bensch *et al.*

2002b; Nijman *et al.* 2003), and taxonomic and phylogenetic inferences (Albertson *et al.* 1999; Buntjer *et al.* 2002; Giannasi *et al.* 2001; Ogden and Thorpe, 2002) have been used. For higher taxonomic levels (e.g., infrageneric), the multi-locus fingerprint becomes too variable, increasing the risk of size homoplasy for the fragments generated(Vekemans *et al.* 2002) and rendering the analysis of AFLP profiles too complex and largely meaningless.

In addition, AFLP markers have encountered considerable success in production of high-resolution genetic and quantitative trait loci (QTL) maps, in fish (Lindner *et al.* 2000; Liu *et al.* 2003; Naruse *et al.* 2000; Ransom and Zon, 1999; Young *et al.* 1998), amphibians (Kochan *et al.*2003; Voss *et al.* 2001), birds (Groenen *et al.* 2000; Herbergs *et al.* 1999; Knorr *et al.* 1999), and mammals (Otsen *et al.* 1996; Van Haeringen *et al.* 2002). The AFLP technique has found a new and productive application in the search for informative single nucleotide polymorphisms (SNPs) in nonmodel vertebrates (Bensch *et al.* 2002a; Meksem *et al.* 2001; Nicod and Largiader, 2003).

Although AFLP markers are highly informative (Ajmone-Marsan P et. al 1997), and unbiased, there are few examples of the application of this type of marker in multiple breed, large-scale population differentiation analysis in cattle. Negrini *et al*. 2007 used 81 AFLP and 19 microsatellite markers to estimate genetic distances among 51 breeds of cattle, including taurine and zebu cattle, and found that the AFLP panel could differentiate between zebu and taurine cattle better than the panel of microsatellites. Two studies in pigs showed the potential of AFLP to survey genetic diversity at the continental scale. Because AFLP polymorphisms are mainly (but not exclusively) based on point mutations, these markers are expected to indicate evolutionary divergence better than microsatellites with variable mutation rates. For instance, a microsatellite-based bovine phylogeny was not in agreement with a phylogeny based on sequence data, which was not the case for an AFLP-based phylogeny. Thus, AFLP appears to be a valuable complementary tool for studies of genetic diversity in cattle populations around the world.

2.2 Breed characterization and Variability studies using molecular markers

Genetic diversity has long been a concern for wild animals, and even for livestock when associated with rare breeds. Recently, however, more attention has been given to the importance of assessing genetic diversity within commonly used breeds of livestock (Zenger *et al*. 2006). This interest has developed for a variety of reasons. First, the intense selection within major breeds of cattle for very specific production traits has potentially decreased the natural variability within these breeds for specific traits. Notter, (1999) stated that selection for increasingly standardized products (beef and milk) and standardized production conditions may be decreasing diversity. While beef cattle may be less affected than dairy, because they are raised in a wide variety of environmental conditions, and because AI is not used as extensively in beef cattle, variability may still be affected.

The classical and perhaps most important application of genetic testing in livestock has been to identify carriers or animals susceptible to a specific disease, in an attempt to exclude such animals from the population. This application continues to be of great importance from an animal health and production perspective. Additionally, it provides the opportunity to study the effects of intense selection on genetic diversity. Using pedigree information as well as 15 microsatellite markers, Alfonso *et al.* (2006) found that genetic diversity had not significantly decreased within the breed based on Fst values, which measures the heterozygosity of the population relative to all populations (Peakall and Smouse 2006). However, they suggested that a greater effect on diversity may be evident if the ewes were also being selected on the basis of *PrP* genotype, and that caution should be exercised when subjecting a breed to such selection pressure.

In cattle, some research that has been done on genetic diversity within breeds has been based on pedigree information and measures of inbreeding (Cleveland *et al*. 2005). While this approach results in an overview of the breed's effective population size (Ne), which has served as a benchmark of

diversity (Cleveland *et al*. 2005), it does not reveal what is actually happening on a molecular level.

Most studies of genetic diversity in cattle compare different breeds within a region. The majority of this research concludes that a high proportion of the total genetic diversity can be explained within breeds (Li *et al.* 2006; MacNeil *et al.* 2007). In other words, the genetic diversity found within breeds today was found within cattle prior to breed formation. The exception to this generalization is when small populations of cattle have been isolated. MacNeil *et al*. (2007) studied feral cattle on Chirik of Island, Alaska. They found that 14% of the total genetic variation was due to differences between this population and non-isolated cattle found on the mainland, using frequency statistics based on a panel of 34 microsatellites. Brenneman *et al*. (2007) also found large differences between 4 breeds of *Bos taurus* and *Bos indicus* cattle in South America, with 24% of variation attributed to breed differences, using allele frequencies of 26 microsatellites.

While many studies have compared different breeds to one another in the same location, there is little information available on the differences between animals of the same breed located in different environments. Conservation of livestock genetics is becoming a priority because the highly selected breeds of livestock that are being developed under modern production and environmental conditions may lose the genetic variability that would allow them to be useful under future conditions. Programs such as the FAO's Integrated Programme for the Global Management of Genetic Resources (CaDBase http://www.projects.roslin.ac.uk/cdiv.markers.html.), and Agriculture and Agri-Food Canada's Animal Genetic Resources program have been developed in order to better understand animal genetic diversity, and to conserve genetic resources for the future (Martin-Burriel *et al.* 1999).

Besides characterization of diversity and preservation of rare or potentially useful genetics, measures of genetic diversity in cattle can be used for other purposes. Sasazaki *et al.* (2004) used six SNPs with *Bos indicus*-specific alleles to verify the accuracy of country-of-origin labeling in Japanese beef. They found that beef could be identified as domestic or imported (from

Australia) 93% of the time, based on the assumption that any *Bos indicus* influence came from Australia (Sasazaki *et al*. 2004). With food traceability and food safety concerns ever increasing, this technology has great potential for further development and use.

Over the years, several different types of markers have been used for studying the diversity, breed structure, and domestication history of cattle. Before the development of DNA technologies, polymorphisms in various proteins and blood groups were often used in diversity studies (Bowcock *et al.* 1994; MacHugh *et al*. 1997).

As the capacity to amplify and analyze DNA grew, researchers in diversity began to use mitochondrial DNA (mtDNA) more extensively. Mitochondrial DNA is maternally inherited, extranuclear DNA (Taanman 1999). The D-loop region of mtDNA is noncoding, but plays an important role in transcription and replication (Brown *et al*. 1979; Schutz *et al*. 1994). This region was found to be extremely useful for phylogenetic analysis because it experiences five to ten times more nucleotide substitutions than nuclear DNA (Brown *et al*. 1979). Because mtDNA is maternally inherited only, it is not complicated by recombination (Henkes *et al.* 2005). The D-loop region of mtDNA was widely used for phylogenetic studies that focused on determining the time and location of the domestication of cattle and the development of breeds Other studies used D-loop sequence to establish relationships between cattle breeds (Kim *et al.* 2003), and to investigate the geographical patterns of domestication and breed development (Henkes *et al*. 2005).

Microsatellites have also been widely used in phylogenetic and diversity studies of livestock. Microsatellites are short repeats, usually of 2 base pairs in cattle (Ellegren *et al.* 1993). It is thought that these repeats are formed by “replication slippage”, where repeated sequence is either lost or gained in a step by step manner (Forbes *et al.* 1995). These markers are very desirable for measuring genetic diversity because they are highly polymorphic, and because they appear in non-coding regions of DNA. Therefore, they are generally assumed to exhibit selective neutrality (Ellegren

et al. 1993). In other words, they are assumed to be unaffected by natural or artificial selection unless closely linked to genes are affected by selection.

Microsatellites showed variation in human populations used by Bowcock *et al.* (1994), where previous studies had used blood groups or mtDNA. Since that time, these markers have been extensively used for phylogenetic research in humans and many species of wild and domesticated animals. Many studies using microsatellites focused on relationships between breeds of cattle and geographical patterns of domestication (MacHugh *et al*. 1997; MacHugh *et al*. 1997; Kantanen *et al*. 1999). More recently, the studies using microsatellites have focused on evaluating the diversity within breeds for the purpose of conserving rare or unique genetics (Beja-Pereira *et al*. 2003; Freeman *et al.* 2005; Brenneman *et al.* 2007; MacNeil *et al.* 2007).

When microsatellites are used to study genetic diversity, the number of loci that are used affects the outcome. Ruane (1999) reviewed the use of genetic distance studies in conservation genetics. He found that at least 20 microsatellite markers, with four to ten alleles each, were necessary for unbiased estimates of genetic distance. Fewer markers can lead to overestimation of genetic distance (Ruane 1999). Freeman *et al.* (2005) also raised the issue of the use of different microsatellite panels for each study. They proposed a regression-based method to combine data from different studies, which used different markers. In an effort to obtain comparable results between studies, the Food and Agriculture Organization (FAO) published a list of recommended microsatellite markers for genetic characterization of several species (CaDBase http://www.projects.roslin.ac.uk/cdiv/markers.html). This resource was used by the Canadian Animal Genetic Resources Program (CAGR) to develop their 30 marker microsatellite panel for livestock conservation purposes. A subset of 22 of the CAGR's markers were used in this study and were chosen based on ease of genotyping and quality of the resulting sequence, as assessed by previous studies. While microsatellite markers are usually considered neutral, or unaffected by selection, some studies have found microsatellites that are linked with QTL for important production traits. Coppieters *et al.* (1998) and Kantanen *et al.* (1999) found that certain microsatellites were influenced by

selection while studying diversity. These microsatellite markers were found to reside within QTL for milk production characteristics. When using microsatellites for diversity studies, one must consider that they may be affected by selection if they are linked to genes that affect phenotype, and are thereby influenced by selection.

High degree of polymorphism by microsatellite markers make them useful as DNA markers in linkage studies. In closely related species such as cattle and sheep, the conservation is close enough to allow PCR primers designed for use in one species may be used to analyze microsatellite polymorphism in the other. A total of 48 set of primer pairs, flanking bovine microsatellite were tested with DNA samples from sheep, horses and humans. Specific product were obtained in 27 (56%) cases with ovine DNA, 20 (42%) of which showed polymorphisms. With equine DNA, 3 (6.2%) gave specific but monomorphic products, while no specific product were obtained in human DNA (Moore *et al.* 1991).Moore *et al.* (1992) extracted bovine and ovine microsatellite sequences from the EMBL and GENBANK databases. When analyzed for numbers of alleles and degree of heterozygosity in the CSIRO cattle reference families, number of alleles ranged from 1 to 14 with 15.8 to 100% heterozygosity. Six of the 13 bovine microsatellite markers were polymorphic in sheep. Similarly 2 of the 4 ovine microsatellites were polymorphic in cattle. These data defined 11 bovine and 8 ovine microsatellite systems, which were associated with known genes and were thus useful for comparative mapping studies.

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 as reported by Kemp *et al.* (1993)

The analysis of 12 microsatellite loci in six breeds of European cattle were reported by MacHugh *et al*. (1997) as microsatellite markers offer great potential for genetic comparisons within and between populations. This yielded a wide spectrum of variability with observed heterozygosity ranging from 0.00 to 0.91. Deviations from Hardy-Weinberg equilibrium were noted for

some locus-population combinations, particularly at a microsatellite located within the prolactin gene. Also, significant linkage disequilibrium was detected between two microsatellite loci located within the bovine major histocompatibility complex, and this association was maintained across breeds, providing evidence for marker stability during short term evolution. The mode of mutation was investigated by comparing the observed data with that expected under the infinite alleles model of neutral mutation, and six of the microsatellite loci were found to deviate significantly, suggesting that a stepwise mutation model may be more appropriate. One indication of marker utility is that, when genetic distance estimates were computed, the resultant dendrogram showed concordance with known breed histories.

141 clones from bovine genomic libraries were sequenced by Moore *et al.* (1994). Out of 141 clones 58 microsatellites were polymorphic in bovine. Thirty of the bovine derived microsatellite gave specific and polymorphic product in sheep.

The genetic variation at five microsatellite loci (CYP21, BOVTAU, ETH131, ILSTS002 and ILSTS005) in four breeds of cattle (Avilena-NegraIberica, Morucha, Sayaguesa and Brown Swiss) was studied by Arranz *et al*. (1996). Value of observed alleles, genetic diversity, PIC and effective allelic number indicated that the microsatellite showing the lowest variability was ILSTS 0113 among the five microsatellites. The number of alleles ranged from 2-3, the heterozygosity ranged from 0.455-0.594 and PIC value ranged from 0.367-0.509 obtained in microsatellite ILSTS 005.

Bovine microsatellite markers were gathered and tested for PCR amplification with goat DNA samples under standard PCR protocol presented by Vaiman *et al*. (1996). This screening made it possible to choose a set of 55 polymorphic markers that could be used in bovine, ovine and caprine species and to define a set panel of 223 microsatellites suitable for the goat. Twelve half-sib parental goat families were then used to construct the linkage meiotic map of the goat genome covering 2300 cM (i.e. >80 % of the total estimated length of the goat genome).

The genetic variation at 20 microsatellite loci was surveyed to determine the evolutionary relationships and molecular biogeography of 20 different cattle populations from Africa, Europe and Asia was studied by MacHugh *et al*. (1997). Phylogenetic reconstruction and multivariate analysis highlighted a marked distinction between humpless (taurine) and humped (zebu) cattle, providing strong support for a separate origin for domesticated zebu cattle. A molecular clock calculation using bison (Bison sp.) as an out-group gave an estimated divergence time between the two subspecies of 610,000-850,000 years. Substantial differences in the distribution of alleles at 10 of these loci were observed between zebu and taurine cattle. These markers subsequently proved very useful for investigations of gene flow and admixture in African populations.

The genetic variation between 10 cattle breeds by using 17 microsatellite loci and 13 biochemical markers (11 blood groups, the transferrin and β-casein loci) was studied by Moazami-Goudarzi *et al*. (1997). Microsatellite loci were amplified in 31–50 unrelated individuals from 10 cattle breeds: Charolais, Limousin, Breton Black Pied, Parthenais, Montbéliard, Vosgien, Maine-Anjou, Normande, Jersey and Holstein. Neighbor-joining trees were calculated from genetic distance estimates. The robustness of tree topology was obtained by bootstrap resampling of loci. A total of 210 alleles of the 17 microsatellites were detected in this study and average heterozygosities ranged from 0·53 in the Jersey breed to 0·66 in the Parthenais breed. In general, low bootstrap values were obtained: with the 17 microsatellites, the highest bootstrap values concerned the Holstein/Maine-Anjou grouping with an occurrence of 74%; with the biochemical markers, this node had an occurrence of 79% and the Charolais/Limousin grouping appeared with an occurrence of 74%; when microsatellites and biochemical polymorphism were analysed together, the occurrence of the Holstein/Maine-Anjou grouping was 90% and that of the Charolais/Limousin grouping was 42%.

The applicability of bovine autosomal microsatellite markers for population studies on African buffalo. A total of 168 microsatellite markers were tested by Hooft *et al*. (1999). 91(90%) markers were polymorphic from

101 markers. The mean number of alleles per marker was 5.0 and the mean heterozygosity was 0.61.

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 were studied by Mattapallil and Ali (1999). 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.

The genetic variability among 253 unrelated individuals representing six Merino populations using microsatellites was studied by Diez-Tascon *et al.* (2000). Two markers (McM357 and ETH225) were found to be significantly out of HWE across populations. The heterozygosities ranged from 0•679 to 0•763 and gene diversity estimates from 0•686 to 0•774. Genetic variation was highest amongst the Spanish and Portuguese populations, although the preservation of genetic diversity within the other populations was high. By a variety of different statistical tests the French Mutton, German Mutton and New Zealand Merino populations could be differentiated from each other and the Iberian Merinos, indicating that microsatellites are able to track relatively recent changes in the population structure of sheep breeds.

The parameters of genetic variation, genetic distances and time of divergence in three Indian goat breeds using 16 cattle microsatellite markers were studied by Ganai and Yadav (2001). 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.

A set of cattle microsatellite DNA markers for genome analysis of riverine buffalo (*Bubalus bubalis*) at National Bureau of Animal Genetic Resources was studied by Navani *et al.* (2002).One hundred and eight microsatellite primer pairs, originally identified from cattle, were evaluated for their applicability in buffalo. Eighty-one primer pairs (75%) amplified discrete products, and of these, 61 pairs (56%) gave polymorphic band patterns on a panel of 25 buffaloes. The mean number of alleles per polymorphic marker was 4.50 ± 0.20, and the mean heterozygosity per polymorphic marker was 0.66 ± 0.02. Successful genotyping of buffaloes using cattle specific primers suggested that the latter can be a valuable resource for genome analysis in bubaline species.

Five microsatellite markers ILSTS -005, ILSTS -001, ILSTS -030, ILSTS -033 and ILSTS -034 in Zalawadi breed of goat which 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 were studied by Thakkar *et al.* (2002). Numbers of alleles ranged from three to seven.

Total twenty-three Holstein bulls that are not closely related but were widely used in the US dairy industry was genotyped for 54 microsatellite loci by Vallejo *et al.* (2003). The heterozygosity for the sampled population ranged from 0.43 to 0.80. The degree of genetic diversity in this population is significant and allows selection for traits of economic importance. As expected, there is extensive linkage disequilibrium (LD) in the US Holstein population. About half of the syntenic marker pairs presented a typical pattern of LD produced by DLD. Most of the non syntenic marker pairs had a typical pattern of LD arising from BLD. These results suggest that the observed LD is not purely due to genetic drift and migration and that a portion might be due to DLD. This raises our hopes of successful fine-localization of genes for complex traits using LD mapping..

The breeds studied can be classified into three groups (1) Red Convex represented by three southern breeds, Alentejana (ALT), Mertolenga (MRT),

Garvonesa (GRV) and one northern breed Minhota (MNT); (2) Brown Concave group represented by the northern breeds Mirandesa (MIR), Arouquesa (ARO), Marinhoa (MRH) and Barrosa (BAR), and (3) the Iberian Black Orthoide group represented by the northern Maronesa (MRO) and the southern Brava de Lide (Fighting Cattle) (BRV). Total 390 alleles were observed among the 568 animals assayed. Average observed and expected heterozygosity ranged from 0.5533 to 0.7430 and from 0.6276 to 0.7471, respective Deviations from Hardy-Weinberg Equilibrium (HWE) were statistically significant (P<0.05) for six locus-breed combinations. These deviations involved one locus in ALT (ETH131) and five loci in BRV (BM1824, CSSM36, CYP21, ETH131 and RM067) FST values ranged from 0.033 for the ARO-MRT pair to 0.190 for the MIR-BRV pair. The lowest DA genetic distance was found between the BAR-ARO pair (0.099) and the highest between the MIR-CAR pair (0.296). FST values were significantly different from zero (P<0.05) for all pairwise combinations.

Fifteen bovine microsatellites selected from the available list of 25 microsatellites suggested by ISAG for estimation of genetic diversity in bovine, amplified 2 (ETH-152 and ILSTS-065) to 11 (ILSTS-028) alleles was studied by Patel (2004). 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.

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 was reported by Mainguy *et al.* (2005). 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.

The genetic diversity of a sample of bulls (N = 19 out of 23 for the whole herd) were studied by Armstrong *et al.* (2006) using the PCR reaction

with a set of 17 microsatellite markers. A total of 73 alleles were identified with minimum of 2 and maximum of 7 alleles per loci. The expected mean heterozygosity (He) per locus was between 0.465 and 0.801, except for microsatellite HEL13 which gave a He value of 0.288. The expected mean heterozygosity was 0.623 and the polymorphic information content (PIC) was between 0.266 for HEL13 and 0.794 for CSSM66. The genetic diversity found in polymorphic markers in the breeding bulls of this Creole cattle population supports previous genetic analyses using major production genes and indicate that further studies should be carried out on this population to provide data of interest to cattle production.

Polymorphisms from 9 microsatellites were used to assess genetic diversity and relationships in 4 Creole cattle breeds from Argentina and Bolivia, 4 European taurine breeds, and 2 American zebu populations by Liron*et al.* (2006). The Creole populations display a relatively high level of genetic variation as estimated by allelic diversity and heterozygosity, whereas the British breeds displayed reduced levels of genetic diversity. The analysis of molecular variance indicated that 7.8% of variance can be explained by differences among taurine and zebu breeds. Consistent with these results, the first principal component (PIC), which comprised the 40% of the total variance, clearly distinguishes these 2 groups. In addition, all constructed phylogenetic trees cluster together with Nelore and Brahman breeds with robust bootstrap values. Only 1% of variance was due to difference between American Creole and European taurinecattle. Although this secondary split was supported by the classical genetic distance and the second PC (15%), the topology of trees is not particularly robust. The presence of zebu-specific alleles in Creole cattle allowed estimating a moderate degree of zebu admixture.

The genetic diversity within the Holstein breed within Australia, and around the world were tested by Zenger *et al*. (2006) This breed has undergone intense selection for milk yield, through the extensive use of a relatively small number of elite sires via artificial insemination (AI). They found, using a large panel of SNPs that genetic diversity had not decreased within the breed from 1975 to 1999, despite intense selection. However, their

study did find that, due to the extensive exportation of semen from the United States, the global Holstein population was virtually one unit, with Nei's genetic distances of only 0.004 between populations (Zenger *et al*. 2006). Although a threat to genetic diversity within this breed was not evident, Zenger *et al*. (2006) did find that the effective population size of the breed was around 125 animals, which is not sufficient to ensure variability over the long term (Georges and Andersson 1996).

2.3 Population assignment

The application of molecular genetic markers to problems in ecology and evolution has revolutionized our understanding of the living world. Identification of isolated populations, estimation of genetic differentiation and inbreeding and reconstruction of phylogenetic relationships has dominated such applications for decades. With the development of variable markers (e.g. microsatellites) and more powerful analytical methods, however, applications have expanded from population genetic models under equilibrium expectations to applications that are more relevant on ecological time scales (Hansen *et al.* 2002; Manel *et al.* 2005).

Furthermore, these advances have shifted the focus from populations to individuals, and it is now possible to identify the genetic origin of specific organisms, with applications in the estimation of current migration rates (Paetkau *et al.* 1995), identification of immigrants (Rannala and Mountain, 1997), forensic identification of the origin of animal (Wasser *et al.* 2004), and the occurrence of hybridization (Randi *et al.* 2001). There is even some evidence that departure of assignment success from random expectations may be a more sensitive test for population differentiation than traditional tests based on allele frequencies and FST values. In addition, recently developed statistical methods remove the requirement for known allele frequencies in source populations, thus allowing separation of mixed samples into contributing constituents (Pritchard *et al.* 2000). It is thus not surprising that the number of molecular genetic studies applying assignment tests increases rapidly. Assignment tests have been utilized to investigate population classification, measure genetic diversity and to solve forensic questions.

Along with significant progress in molecular technology, DNA markers have been used for population discrimination in livestock animals (Alves *et al.* 2002; Olowofeso *et al.* 2005). The AFLP (Amplified Fragment Length Polymorphism) method is one of the ways to provide these useful markers (Vos *et al.* 1995). Since many polymorphic bands can be detected using combinations of selective primers, AFLP is a powerful method for acquiring genome information easily. In our previous study, we attempted to develop six DNA markers derived from AFLP breed specific bands, which could distinguish between Japanese Black and F1 cattle (Sasazaki *et al.* 2004). Using these markers, the probability of identifying F1 was 0.882 and probability of misjudgment was 0.0198. They could be useful for discrimination between Japanese Black and F1. However, more effective markers developed by other combinations of AFLP primers will be required to improve the discrimination ability for starting a molecular test and reduction of incorrect labeling of food.

AFLP is a PCR-based technique that uses primers complementary to the synthetic adapters that are ligated to the ‘sticky ends” of restriction fragments generated by restriction enzymes. It does not require any prior knowledge about the genome; it is dominant, biallelic and highly reproducible (Ajmone- Marsan *et al.* 1997; Bagley *et al.* 2001). The amplified fragment length polymorphism (AFLP) technique (Vos *et al.* 1995) easily generates a large numbers of markers spanning the whole genome without any prior knowledge about it. Polymorphisms are indicated by the presence or absence of the band. AFLP has been successfully applied to studies of genetic diversity and relationships in various domestic species, such as cattle (Ajmone- Marsan *et al.* 1997; Buntjer *et al.* 2002; Negrini *et al.* 2006; Negrini *et al.* 2007), dolphins (Kingston and Rosel, 2004), and sheep (Hoda *et al.* 2010).

Amplified fragment length polymorphism (AFLP) fingerprinting detects variation that corresponds to SNPs and indels and is informative for genetic diversity (Bensch & A° kesson 2005; Foulley et al. 2006; SanCristobal et al. 2006). Ajmone-Marsan et al. (1997) and Negrini et al. (2006) demonstrated the use of AFLP fingerprinting for estimation of genetic distances within and

across cattle breeds. Here we analyze 47 European breeds, one African breed and three Indian zebu breeds in order to study the genetic differentiation of cattle across Europe.

Nine microsatellites were evaluated by Curi *et al.* (2002) in Paternity Testing and to investigate misidentification paternity frequency in families of Gyr breed bovines. The Combined Exclusion Probability for all microsatellites was around 0.9789 (lower than the appropriate value 0.99). The Paternity Testing results showed misidentification in eleven of the 40 studied families, that means, 27.5% (11 in 40) of the sample.

The origins of zebu cattle was re-examined by Kumar *et al*. (2006), analyzed microsatellite allele frequency data from CSRM60, CSSM66, ETH3, ETH10, ETH152, ETH225, HEL1, HEL5, HEL9, HEL13, BMS1818, BMS1824, BMS2113, ILST005, ILST006, INRA005, INRA023, INRA032, INRA035 and INRA063 markers that have been screened in seven breeds with origins in a variety of locations in South Asia. 11 European breeds and 7 Eastern breeds.

19 STR in 269 animals from 4 cattle breeds determine the potential of microsatellites (STR) for determining the breed origin of beef products among cattle breeds present in the market was typed by Ciampolini *et al*. (2006). Based on Wright's *F*-statistics, 4 loci were discarded, and the remaining 15 loci (FIT = 0.101, FST = 0.089, and FIS = 0.013) were used to compute the likelihood. The posterior probability that the animals of a presumed breed were actually drawn from that breed instead of any another breed was then calculated. Given an observed value of Log-likelihood ratios (log LR) > 0 and assuming equal priors, these probabilities were >99.5% in 10 of 12 possible breed contrasts. For the 2 most closely related breeds (FST=0.041), this probability was 96.3%, and the probability of excluding the origin of an animal from an alleged breed when it was actually derived from another breed was similar. These results confer that microsatellite are gaining importance to be used for breed assignment and also to certify quality and origin of livestock products and assure food safety and authenticity.

Umblachery cattle breed of south India using 25 FAO recommended microsatellite markers were assessed by Karthickeyan *et al.* (2007). The PIC

value was 0.5625± 0.03 suggesting excess of heterozygosity within the population.

A panel of microsatellite markers assessed existing genetic diversity in Kankrej cattle which implied substantial amount of variability and absence of inbreeding done by Sodhi.M. *et al.* (2007).

The genetic diversity and population structure of Rathi and Tharparkar cattle using a set of microsatellite markers were assessed by Sodhi. M, *et al.* (2008) .Various variability estimates indicated sufficient within-breed genetic diversity as well as significant level of breeds differentiation between two breeds.

The genetic diversity in 2 cattle breeds Hariana and Hissar cattle breeds of Pakistan was investigated by Rehman and Khan (2009).PIC values estimated were 0.749 and 0.719 in Hariana and Hissar cattle respectively. Various variability parameters indicated moderate genetic diversity within both breeds besides they were genetically different enough as separate breeds.

The genetic variability within and between three indigenous cattle breeds viz; Gir, Kankrej and Deoni investigated using 7 microsatellite markers (ETH-225, CSRM-60, HEL-5, INRA-005, INRA-035, ILSTS-002 and ILSTS-006) were assessed by Kale *et al.*(2010). The results showed that genetic equilibrium was not always maintained. The observed number of alleles ranged from 5 (in HEL-5) to 8 (in CSRM-60) with total 46 alleles across three breeds. The overall heterozygosity and PIC values were 0.730 and 0.749. Genetic distance was least (0.2034) between Gir and Kankrej and highest between Deoni and Kankrej (0.4442).

The genetic diversity study of native Gir and Kankrej (*Bos indicus*) cattle populations using nine microsatellite markers was evaluated by Upreti *et al.* (2012). The mean number of observed and effective alleles in Kankrej were comparatively high (5.222 and 3.714) and the average expected heterozygosity values (0.5403) indicated high diversity in the Kankrej population than Gir (0.4520). High polymorphism information content (PIC) values observed for most of the markers with an average of 0.5116

indicating the high informativeness of these markers in Kankrej breed than in Gir (0.4202).The genetic diversity and relationship of Indian cattle inferred from microsatellite and mitochondrial DNA markers assessed existing genetic diversity in different cattle breeds which implied substantial amount of variability and absence of inbreeding was derived by Sharma et al. (2015)

3. MATERIALS AND METHODS

3.1 Materials

3.1.1 Chemicals and Reagents.

- Acrylamide (Sisco Research Laboratories PVT. LTD)
- Agarose (molecular biology grade) (Fisher reagents)
- Ammonium chloride (NH_4Cl) (ExcelaR, Qualigens Fine Chemicals)
- Ammonium per sulphate (SRL, Qualigens Fine Chemicals)
- Boric acid (molecular biology grade) (Fisher scientific)
- Buffer tablets (pH 4, 7, 9) (Qualigens fine chemicals)
- DNA 100 bp marker ladder (Promega, USA and Bangalore, Genei)
- dNTP mix (Promega, USA)
- EDTA (Na_2EDTA) (ExcelaR, Qualigens Fine Chemicals)
- Ethanol (Jai Chemical and Pharma Works)
- Ethidium bromide (SRL, Qualigens Fine Chemicals)
- Gel loading dye (Bangalore, Genei)
- Magnesium Chloride 1.5mM (molecular biology grade) (Promega, USA)
- N’, N’, N’, N’ Bis-acrylamide (SRL, Qualigens Fine Chemicals)
- PCR assay buffer (Promega, USA)
- Potassium Chloride (BDH, India PVT. LTD.)
- Primers (Sigma Aldrich Ltd.)
- Sodium chloride (MP Biomedicals, Inc.)

- Sodium lauryl sulphate (SRL, Qualigens Fine Chemicals)
- Sodium hydroxide pellets (ExcelaR, Qualigens Fine Chemicals)
- *Taq*DNA polymerase enzyme (Promega, USA)
- TEMED (SRL, Qualigens Fine Chemicals)
- TRIS base (SRL)
- Tris-HCl (Qualigens Fine Chemicals)

3.1.2 Equipments

- Centrifuse (Remi)
- Waterbath (Chino scientific instruments Mfg.)
- Incubator (Chino scientific instruments Mfg.)
- Deep freezer (Blue star)
- Spectrophotometer (Shimadzu, Japan)
- Oven (Chino scientific instruments Mfg.)
- Horizontal agarose gel electrophoresis unit (Genei, Bangalore)
- UVP gel-doc system
- Eppendroffmastercycler gradient (Eppendroff)
- Hoffer SE600 series electrophoresis unit
- UVP Doc-It®LS Image Acquisition Software version 6.3.3
- Micro pipette (Nichi-pet, Japan)

3.1.3 Source of Data:

A total of 30 blood samples from each Rathi, Tharparkar, Gir ,Sahiwal ,Nagori and Kankrej cattle breeds were collected. All the animals were randomly selected, genetically unrelated and the information was collected

after consulting pedigree r*Eco*rds maintained and interviewing the owners in detail. Blood samples from Rathi were collected from Clinics premises (Veterinary College, Bikaner.), Dairy Campus (Veterinary College, Bikaner). Blood samples from Tharparkar were collected from Livestock Research Station (Veterinary College, Bikaner) and from various owners in the village. Blood samples from Gir were collected from Livestock Research Station Vallabhnagar (Veterinary College, Udaipur). Blood samples from Kankrej and Sahiwal were collected from Livestock Research Station Kodemdesar (Veterinary College, Bikaner).Blood samples from Nagori were collected from various owwners in Shribalaji and Nagor Bikaner.

3.2 Methodology

3.2.1 Collection of sample

The blood (5 ml) was drawn from each animal intravenously from jugular vein using 18 G needle. Blood was collected in sterilized glass test-tubes having sodium EDTA (1.5 mg/ml). Samples were transported to laboratory on ice and stored at 4°C until used.

3.2.2 Isolation of Genomic DNA

DNA from the whole blood samples of Rathi, Tharparkar, Sahiwal, Nagori, Gir andKankrej cattle was isolated using Dneasy blood kit as per the following protocol:

1. 20 µl proteinase K pipetted into a 1.5 ml micro-centrifuge tube. Added 5-10 µl anticoagulant-treated blood and adjust volume to 220 µl with PBS.
2. 200 µl Buffer AL were added and mixed thoroughly by vortexing. Then blood samples incubated at 56°c for 10 min.
3. 200µl Ethanol (96-100%) were added and Mixed thoroughly by vortexing.
4. The mixture was pipetted into a DNeasy mini spin column placed in a 2 ml collection tube. Centrifuged at 8000rpm. Discarded the flow-through and collection tube.

5. The spin column was placed in a new 2 ml collection tube and added 500µl Buffer AW1. Centrifuge it for 1 min. at 8000 rpm. Discarded the flow-through and collection tube.
6. The spin column was placed in a new 2 ml collection tube and added 500 µl Buffer AW2 and centrifuged it for 3 min. at 14,000 rpm. Discarded the flow-through and collection tube.
7. Transferred the spin column to a new 1.5 ml or 2 ml micro-centrifuge tube.
8. Eluted the DNA by adding 200µl Buffer AE to the center of the spin column membrane. Incubated for 1 min. at room temperature (15- 25°c). Centrifuged for 1 min. at 8000 rpm.
9. Step 8 is repeated for 2 times for increased DNA yield.

3.2.3 Quality and quantity of genomic DNA isolated

The purity and concentration of the isolated genomic DNA were estimated using agarose gel electrophoresis and UV-absorption spectrophotometer respectively. The ratio absorbance at 260 and 280 nm is used as an indicator of DNA purity. A ratio between 1.4 and 1.9 is considered as relatively pure DNA sample as it did not show any effect on PCR reaction (Sambrook and Russel, 2001). Working solution was prepared by diluting the samples in TE buffer (pH 8) or sterilized MiliQ water having approximately 30 ng/µl of DNA.

DNA concentration was estimated as under.

Conc. of DNA (µg/ml) = OD 260 X dilution factor X 50

3.2.4 Microsatellite markers

The microsatellite marker primers, which are analyzed in the present study, were obtained as per the literature of Kaukinen & Varvio (1993) (HEL 5), Vaiman *et al.*(1994) (INRA 035), Sunden *et al.*(1993) (BM 2113), Solinas *et al.*(1993) (ETH 3), Kaukinen & Varvio (1993) (HEL 1), Vaiman *et al.*(1994) (INRA 063), Steffen & Eggen (1993) (ETH 152) (ETH225), Kemp *et al.*(1995) (ILSTS 002), Bishop *et al* .(1994) (BM1818), Moore *et al.* (1994) (CSRM60) Solinas *et al.*(1993) (ETH10) Brezinsky *et al.* (1993) (ILSTS006) (ILSTS002) .

The primers were selected on the basis of the repeat motifs and the numbers of alleles reported in the previous studies.

Table 3.1 Primer sequence used in Microsatellite Marker

LOCOUS	Primer Sequences 5'-3'	BASE PAIR COUNT	ANNEALING TEMPRATURE (˚C)	REFERENCE
BM1818	F:AGC TGG GAA TAT AAC CAA AGG	21	58	Bishop *et al.* (1994)
	R:AGT GCT TTC AAG GTC CAT GC	20		
CSRM60	F:AAG ATG TGA TCC AAG AGA GAG GCA	24	55-60	Moore *et al.* (1994)
	R:AGG ACC AGA TCG TGA AAG GCA TAG	24		
ETH10	F:GTT CAG GAC TGG CCC TGC TAA CA	23	55-65	Solinas *et al.* (1993)
	R:CCT CCA GCC CAC TTT CTC TTC TC	23		
ETH225	F:GAT CAC CTT GCC ACT ATT TCC T	22	55-65	Steffen *et al.* (1993)
	R:ACA TGA CAG CCA GCT GCT ACT	21		
INRA005	F: CAA TCT GCA TGA AGT ATA AAT AT	23	55	Vaiman *et al.*, (1992)
	R: CTT CAG GCA TAC CCT ACA CC	20		
ILSTS006	F:TGT CTG TAT TTC TGC TGT GG	20	55	Brezinsky *et al.* (1993)
	R:ACA CGG AAG CGA TCT AAA CG	20		
HEL5	F:GCA GGA TCA CTT GTT AGG GA	20	52-57	Kaukinen *et al.* (1993)

	R: AGA CGT TAG TGT ACA TTA AC	20		
BM2113	F:GCT GCC TTC TAC CAA ATA CCC	21	55-60	Sunden et al., (1993)
	R:CTT CCT GAG AGA AGC AAC ACC	21		
ETH3	F: GAT CAC CTT GCC ACT ATT TCC T	22	55-65	Fries *et al.* (1993)
	R: ACA TGA CAG CCA GCT GCT ACT	21		
ETH152	F:AGG GAG GGT CAC CTC TGC	18	55-60	MacHugh et al., (1997)
	R:CTT GTA CTC GTA GGG CAG GC	20		
HEL1	F:AGT CCA TGG GAT TGA AAG AGT TG	23	54-57	MacHugh et al., (1997)
	R:CTT TTA TTC AAC AGA TAT TTA ACA AGG	27		
ILSTS022	F:AGT CTG AAG GCC TGA GAA CC	20	55-57	Brezinsky *et al.* (1993)
	R:CTT ACA GTC CTT GGG GTT GC	20		
INRA035	F: ATC CTT TGC AGC CTC CAC ATT G	22	55-60	Vaiman *et al.* (1994)
	R: TTG TGC TTT ATG ACA CTA TCC G	22		
INRA063	F:ATT TGC ACA AGC TAA ATC TAA CC	23	55-58	Vaiman *et al.* (1992)
	R: AAA CCA CAG AAA TGC TTG GAA G	22		
ILSTS002	F:TCT ATA CAC ATG TGC TGT GC	20	52-55	Kemp *et al.* (1992)
	R:CTT AGG GGT GAA GTG ACA CG	20		

3.3. Microsatellite marker analysis

3.3.1. R*Eco*nstitution of Primers

All the primers as supplied by the manufacturer were initially added with 50µl of TE buffer (pH 8.0). After that each primer was r*Eco*nstituted in sterilized DNase free MiliQ water to arrive at a final concentration of 10 pmoles/µl.

3.3.2 Polymerase Chain Reaction (PCR)

PCR was carried out in a final reaction volume of 25 µl. A master mix for minimum of 15 samples was prepared and aliquated 22 µl in each 0.2ml PCR microfuge tube. 3 µl of DNA sample was added in respective tubes to make the final volume. Master Mix was prepared for one additional sample to cover the pipetting error. The tubes were spinned at 10,000 rpm for few s*Eco*nds after gentle tapping to ensure proper mixing. Likewise all PCR components were thawed and spinned for few s*Eco*nds prior to use.

Table 3.2: Each reaction volume contained:

PCR Components	**Volume added in each reaction**
5X PCR assay buffer	5.00 µl
25 Mm $MgCl_2$	2.00 µl
dNTP mix (10mM)	1.00 µl
Primer (F) (10 pmoles/µl)	1.00 µl
Primer (R) (10 pmoles/µl)	1.00 µl
Taq DNA polymerase (5 U/µl)	0.25 µl
DNase free MiliQ water	11.25 µl
Template DNA	3.00 µl
Total	25.0 µl

Initially, the PCR conditions were standardized for annealing temperatures, $MgCl_2$ concentration and *Taq* DNA polymerase by performing a series of reactions with varying each of these PCR components. Annealing temperatures were attempted in accordance with literature of each marker given by Meat Animal Research Centre (U.S. Department of Agriculture) (www.ars.usda.gov) but some markers required temperature optimization. Gradient PCR was attempted for "BM 2113", "ILSTS 022" and "INRA 035" to determine their exact annealing temperature. No significant change was observed by varying $MgCl_2$ concentration; hence 1.5 mM concentration already present in the assay buffer was used for all amplifications. *Taq* DNA polymerase was initially used at a concentration of 5U (Bangalore, Genei) but later reduced to 1.25 U (Promega, USA) per reaction. Following are the PCR program conditions used for all microsatellite markers.

PCR CONDITIONS USED:

Initial denaturation	94° C for 5 minutes.
Denaturation Cycle	94° C for 1 minute.
Annealing Cycle	50° C to 64° C for 1 minute.
Extension Cycle	72° C for 30 s*Ec*onds.
Step 2-4 repeated for 30 Cycles	
Final extension	72° C for 10 minutes.
Hold	4° C

3.3.3.Agarose gel electrophoresis for estimation of genomic DNA and PCR products

Agarose gel electrophoresis (0.8%) was carried out for confirming the quality of isolated genomic DNA (Sambrook and Russel, 2001). Appropriate amount of agarose was weighed and dissolved to make a final concentration of 0.8% in 1X Tris-Borate EDTA (TBE) buffer. The agarose was melted in a microwave oven (IFB). The solution was allowed to cool sufficiently and

ethidium bromide was added at a concentration of 0.5 µg/ml of agarose gel solution. The gel tray was sealed on either side by using adhesive tape and the comb was placed in proper position. The melted agarose solution was poured into the gel tray carefully avoiding air bubbles. Once the gel was sufficiently solidified, the comb and the seal on either side were removed carefully. The gel tray was kept in an electrophoresis tank and 1X TBE buffer was poured to submerge the gel in the tank. The DNA samples were mixed with 1/6th volume of 6X gel loading buffer and loaded into the wells using a micro-pipette. The electrophoresis was carried out at 85 volts at room temperature for about half an hour. Then the gel was visualized under UV light and photographed using UVP gel-doc system. Note for intact DNA fragments on the gel and avoid samples showing smearing.

To confirm PCR amplification, 5 µl of PCR product mixed with 1µl of 6X gel loading dye from each tube and 100 bp marker ladder were electrophoresed on 1.5 - 2.0 per cent agarose gel (depending on the expected size of amplified product) containing ethidium bromide at a concentration of 0.5µg/ml at constant voltage 80 V for 30 minutes in 1X TBE. The amplified product was visualized as a single compact band of expected size under UV light and documented by gel documentation system

3.4. AMLIFIED FRAGMENT LENGTH POLYMORPHISM MARKER

3.4.1 Digestion of total DNA

The genomic DNA was subjected to restriction digestion in a 25 µl reaction containing DNA (50–500 ng), 2.5 µl of 10 µl *Taq*I buffer (New England Bio labs [NEB]), 5 units of restriction endonuclease *Taq*I (NEB) and ultra-high-quality (UHQ) water. Incubatation was up to 2 h at 65℃, and then 1.5 µl of 10 µl *Eco*RI buffer (NEB), 5 units of restriction endonuclease *Eco*RI (NEB) were added and final make up to 40 µl with UHQ water and again Incubated up to 2 h at 37℃.

3.4.2 Preparation of 10 µM double-stranded adapters.

10 µM double-stranded adapters were prepared by mixing equal volumes of 10 µM individual synthetic oligonucleotides. Adaptors were

denatured by heating 5 min at 65℃ in a hot block and cool slowly down to room temperature. Stored at -20℃.

3.4.3.Ligation of adapters to restriction fragments.

Adaptors were ligated to 40 µl of the digested genomic DNA by adding 1 µl of 10 µM *Eco*RI adapter, 5 µl of 10 µM *Taq*I adapter, 1 µl of 10 mM ATP, 0.5 µl of 1 mg/µl bovine serum albumin (BSA), 1 µl of 10U T4 ligase buffer (NEB), 100 units of T4 DNA ligase (NEB) and UHQ water to 50 µl and Incubated for another 3 h at 37℃. The ligation reaction was diluted 5–10 times with UHQ water.

3.4.4. Preselective amplification.

The preselective mix was prepared with the following components: 3 µl of diluted template DNA, 2.5 of µl 10 Ampli*Taq* buffer (Applied Biosystems), 1.5 µl of 25 mM MgCl2, 2 µl of 10 Mm dNTPs, 0.5 µl of 10 µM *Eco*RI preselective primer, 0.5 µl of 10 µM *Taq*I preselective primer, 1 unit of Ampli *Taq* DNA polymerase, and UHQ water to 25 µl. Preamplify using the following program: initial incubation 2 min at 72 ^{o} C ; 25–30 cycles of 30 s at 94 ^{o} C, 30 s at 56 ^{o} C, and 2 min at 72° C and final extension 10 min at 72 ^{o} C; stored at 4° C. After amplification, the preselective PCR products were monitored on a 2% agarose gel. The preselective product was diluted 20 times with UHQ waterfor selective amplification.

3.4.5. Selective amplification.

The selective mix was prepared with following components: 5 µl of diluted preselective product, 2.5 µl of 10mM Ampli *Taq* buffer, 2.5 µl of 25 mM MgCl2, 2 µl of 10 mMdNTPs, 0.5 µl of 10 µlM*Eco*RI selective primer, 0.5 µl of 10 µlM*Taq*I selective primer,1 unit of Ampli*Taq* DNA polymerase, UHQ water to a final volume of 25 µl. Amplifed using the following program: initial incubation 10 min at 95 ^{o} C; 13 cycles of 30 s at 94 ^{o} C, 1 min at 65 ^{o} C (first cycle, then decrease of 0.7 ^{o} C for the 12 last cycles) and 1 min at 72 ^{o} C; 23

cycles of 30 s at 94 ^{o} C, 1 min at 56 ^{o} C, and 1 min at 72 ^{o} C; final extension 10 min at 72 ^{o} C; stored at 4° C.

Table 3.3: Primer sequences used for AFLP Markers

LOCUS		**Primer Sequences 5'-3'**	**BASE PAIR COUNT**
*Eco*RI adapters	*Eco* top strand	CTC GTA GAC TGC GTA CC	17
	Eco bottom strand	AAT TGG TAC GCA GTC TAC	18
*Eco*RI primers	E01 (pre-amplification)	GAC TGC GTA CCA ATT CA	17
	*Eco*RI-ACA Selective amplification	GAC TGC GTA CCA ATT CAC A	19
	*Eco*RI-AGC	GAC TGC GTA CCA ATT CAG C	19
*Taq*I adapters	*Taq* top strand	GAC GAT GAG TCC TGA C	16
	Taq bottom strand	CGG TCA GGA CTCA T	13
*Taq*I primers	T01 (pre-amplification)	GAT GAG TCC TGA CCG AA	17
	*Taq*I-CAC (Selective amplification)	GAT GAG TCC TGA CCG ACA C	19
	*Taq*I-CAG	GAT GAG TCC TGA CCG ACA G	19

3.4.6. Polyacrylamide Gel Electrophoresis (PAGE) For Microsatellite and AFLP

Microsatellite and AFLP marker scoring was done using PAGE (Korethet *al.*1996). For typing, 8% native PAGE was run. The unit was assembled as given in instruction manual. The glass plates measuring 18 x 16 cm were cleaned thoroughly with mild detergent under tap water, rinsed with metallic water and allowed to dry. Then wiped with ethanol to remove any grease spots and allowed to dry. The plates were adjusted on the gel caster with 1.5mm spacers using screws and checked for any leakage using distilled water. 100 ml of 8% Polyacrylamide gel solution was prepared (after appropriate optimization) with following components (Sambrook and Russel, 2001).

Acrylamide and N', N', N', N' Bis-acrylamide (29 : 1)	21.2 ml
5X TBE	16 ml
Sterilized distilled water	42.1 ml
10% Ammonium persulfate	0.7 ml
TEMED	40 µl

The gel solution was gently poured in between the glass plates and comb was set. The gel was allowed to polymerize for 1 hour without disturbing the assembly. The comb was removed carefully, the wells were washed with buffer or distilled water and the samples were loaded along with 100 bp ladder (Promega, USA). Clamp the gel with clampers provided in the unit to upper buffer chamber and fill the lower chamber tank and upper buffer chamber with cold 1X TBE buffer. The electrodes were connected appropriately to the electrophoresis power supply and the program was run at 125 V and 2 W. The gel was run until the dye front of loading dye reached bottom of the gel. After the run was completed, the glass plates were retrieved from the

assembly and very carefully apart those with the help of scale provided in the unit. The gel still attached to one of the glass plate was carefully transferred to container having ethidium bromide solution and rocked gently to remove gel from glass plate. The gel was stained for about half an hour with gentle rocking. The gel was analyzed under UV light and documented by UVP gel-doc system.

3.5 Statistical analysis for microsatellite and AFLP data

Analyses of the bands were done using a software aided gel-documentation system (UVP) and genotypes of the individual animals were scored manually. All variability parameters and genetic distances were calculated using software**GenAlEx** version 6.5.

3.5.1. Calculation of genotypic frequency:

Genotypic frequency is calculated after obtaining total number of animals for all possible genotypic combinations.

$$\text{Genotypic frequency} = \frac{\text{Total number of animals for a particular genotype}}{\text{Total number of animals assessed}}$$

3.5.2. Calculation of allelic frequency:

Allelic frequency for a particular allele is calculated after observing its homozygotic and heterozygotic combinations.

$$\text{Allelic frequency} = \frac{2 \text{ x No. of homologues} + \text{No. of heterologues}}{2 \text{ x Total no. of animals assessed}}$$

3.5.3. Calculation of Effective number of alleles (A_e):

The measure explains about the number of alleles that would be expected in a locus in each population:

$$A_e = \frac{1}{\sum_{a=1}^{k} p_a^2}$$

where, p_a^2 is the frequency of the ath of k alleles. (Nassiry*et al.*, 2009)

3.5.4. Heterozygosity

The data obtained were subjected to calculate genetic variability parameters allele counts and frequencies, expected number of alleles for each locus under Infinite allele model i.e. IAM (Ewens, 1997) and stepwise mutation model i.e. SMM (Kimura and Ota, 1975), observed heterozygosity, expected heterozygosity (=gene diversity), corrected for sample size, Shannon index of diversity (Shannon and Weaver, 1995) and minimum, maximum allele length using Genalex version 6.5.

The probability that any randomly chosen individual is heterozygous for any two alleles at a marker locus having allele frequencies p_i , is defined as heterozygosity. Thus, heterozygosity= $1-\sum_{n=1}^{n} p_i^2$, where $\sum_{n=1}^{n} p_i^2$ is the homozygosity. (Hildebrand *et al.*, 1992).

(A) Direct count (DC) heterozygosity (Machado *et al.*, 2003) was obtained as:

$$H(direct) = \sum_{i} \sum_{j \neq i} \frac{N_{lij}}{N}$$

Where ***N****lij* is the number of heterozygous individuals in the l locus; *N*is the number of individuals analyzed. It is also known as observed heterozygosity, and the average direct count of heterozygosity over all loci in each tested breed is less than the expected heterozygosity. (Nahas*et al.*2008)

(B) The Hardy-Weinberg expected heterozygosity, also defined as Gene Diversity (Nei, 1973) was obtained from observed allele frequencies (Nei, 1978):

$$1-\sum_{n=1}^{n} p_i^2$$

Where p_{li} is the frequency of the i allele at the l locus; n is the number of alleles at the l locus.

3.5.5. Polymorphism Information Content (PIC)

Informativeness of a marker can be quantitatively measured by a statistic called the polymorphism information content, or PIC. This statistic is defined relative to a particular type of pedigree: one parent is affected by a rare dominant disease and is heterozygous at the disease-gene locus and the other parent is unaffected by the disease. The PIC value of the marker is defined as the expected fraction of informative offspring from this type of pedigree. (Hildebrand *et al.*1992).

PIC was calculated following formula (Botstein *et al.*1980):

$$PIC = 1 - \sum_{i=1}^{n} p_i^2 - (\sum_{i=1}^{n} p_i^2)^2 + \sum_{i=1}^{n} p_i^4$$

Where p_i = frequency of the marker allele, a_i and n= number of different alleles.

3.5.6. SHANNON INDEX

A diversity index is a mathematical measure of species diversity in a community. Diversity indices provide more information about community composition than simply species richness *(i.e.,* the number of species present); they also take the relative abundances of different species into account The Shannon index is parameter for determining diversity index. The Shannon diversity index is an index that is commonly used to characterize species diversity in a community and accounts for both abundance and evenness of the species present.

3.5.7 F-Statistics

Perhaps the most widely reported statistics in population genetics are Wright's F-statistics (Wright 1946, 1951, 1965). One way to calculate these statistics is to use the partition of genetic diversity (heterozygosity) as the starting point.It may come as a surprise to learn that differences within versus among subpopulations can be characterised by F-statistics, since these statistics are normally associated with inbreeding.

However, this is possible because population subdivision is associated with inbreeding like effects viz. excess homozygosity (reduction of heterozygosity).

FIS = The inbreeding coefficient within individuals relative to the subpopulation. It measures the reduction in heterozygosity of an individual due to non-random mating within its subpopulation.

$$F_{IS} = \frac{H_e - H_o}{H_e}$$

F_{IT}= the inbreeding coefficient within individuals relative to the total. This statistic takes into account the effects of both nonrandom mating within subpopulations and genetic differentiation among the subpopulations.

$$F_{IT} = \frac{H_T - H_o}{H_T}$$

FST = the inbreeding coefficient within subpopulations relative to the total. This statistic provides a measure of the genetic differentiation between subpopulations. That is, the proportion of the total genetic diversity (heterozygosity) that is distributed among the subpopulations.

3.5.8 Hardy-Weinberg equilibrium

Exact tests for deviations from Hardy-Weinberg equilibrium (HWE) were performed using the GENALEX package (**Rod Peakall and Peter Smouse 2006**). The program performed a probability test using a Markov chain (dememorization 5000, batches 100, and iterations per batch 1000). Significant levels were calculated per locus, per population, and over all loci and populations combined.

4. RESULT AND DISCUSSION

4.1 MICROSATELLITE MARKER:

The objectives of the study were to analyze the genetic diversity and differentiation of the following six cattle breeds including Sahiwal, Tharparkar, Rathi, Gir, Kankrej and Nagori. Fifteen bovine microsatellite markers were selected from the recommended lists of microsatellite markers by the International Society for Animal Genetics (ISAG)/ Food and Agriculture Organization (FAO) to examine the genetic diversity, differentiation and relationships within and among the selected cattle breeds. These microsatellites were amplified on DNA samples extracted from minimum of 30 blood samples collected at random from breeding tract of all the six cattle breeds.

The Food and Agriculture Organization (FAO) of the United Nations has proposed an integrated programme for the global management of genetic resources, Project MODAD (DAD-IS; FAO, Rome), using microsatellite methodology for breed characterization. From a set of 30 microsatellite markers suggested by FAO for cattle, 15 were chosen for our study based on heterozygosity values, number of alleles and in formativeness as reported in earlier studies. BM1818, CSRM60, ETH10, ETH225, INRA005, BM2113, ETH3, ETH152, HEL1, HEL5, ILSTS022, INRA035, INRA063, ILSTS002, ILSTS006 are the markers studied. The primer sequences were chosen based on earlier studies.

Allele frequency, observed (Na) and effective number of alleles (Ne), observed heterozygosity and expected heterozygosity, observed homozygosity and expected homozygosity,Shannon Index, Inbreeding coefficient and PIC value of all selected microsatellite markers were calculated.

4.1.1. Microsatellite HEL 5

Microsatellite locus HEL-5 is located on bovine chromosome 21 and is having $(CA)_{22}$ repeats. The repeat region (underlined) is present between 316-359 bp (Kaukinen and Varvio. 1993).

Source: http://www.ncbi.nlm.nih.gov/ gi /544/gb/ X65204

acctgcagtg caggagacct gggttcgatt cctgggttgg gaaggtcctt gaaggagagg atggcatccc actccaggct gtattcttgc ctggagaatc cccatgggca gaggagccta acagactaca cagtccatgg ggttgcaaag agtcggacat gacagagact aggcgcaacg caacatgtat ctggatatta tcctaaggta atggttttca gacgttagtg tacattaaca ttcctcaagc agacattaaa attcagtaag tctagtgtga agtctcataa tctgagcttt aacaggcatg ccctacacac acacacacac acacacacac acacacacac acacacacaa tccctaacaa gtgatcctgc tactgttagt ccaaggaacg nnaaagttta aaaaatggtt ctacttcatc cacacaaaac atgttaaatg cttactatgt gtaaggaaag atatccagag actactatat atgtaaggta agtttctttc ttccccttt atctaaggaa acgaactcaa aacaggatgt ggtaatccat cgtcaatggc atttgaaaac tagaaaaccc atctcaggag atttgaaa .

4.1.1.1 Allelic Frequencies for HEL5 microsatellite marker.

A total of 7 alleles (130-195 bp) were typed in the six breeds studied. The allelic frequency in the combined population was minimum for allele 6 (0.063) and maximum for allele 3 (0.500).(Table 4.1).

For Hardy Weinberg Equilibrium, Chi square test was conducted. In this Rathi, Sahiwal, Tharpakar, Nagori had P value less than 0.05 that showed all four breeds were significant in Chi square test and not found to be in HWE whereas Gir and Kankrej showed the P value higher than 0.05 which was not significant and Gir and Kankrej populations were in HWE. It has been clearly depicted that most of the population were not in HWE because of the samples which were collected from well-organized dairy farm where selection and artificial insemination in practice for better production. That’s why most of the population deviated from HWE.

Table-4.1 Allelic frequencies of microsatellite HEL 5 of six breeds of cattle

ALLELE NO.	GIR	KANKREJ	RATHI	SAHIWAL	NAGORI	THARPAKAR	TOTAL
1	0.333	0	0.273	0.318	0.269	0	0.204
2	0.333	0.409	0	0	0	0	0.134
3	0	0	0.321	0	0.500	0.438	0.099
4	0	0.409	0.179	0	0	0	0.120
5	0	0.182	0	0.500	0.231	0.500	0.296
6	0.250	0	0	0.227	0	0.063	0.092
7	0.083	0	0.179	0	0	0	0.056
Chi sq	10.000	7.198	11.000	11.000	13.000	8.000	
P value	.125	0.06	.012	.012	.005	.046	

** - Highly significant (P≤ 0.01); * - Significant (P≤0.05); NS - Non-significant (P>0.05)

4.1.1.2. Genetic Diversity parameters and PIC values for HEL5

Observed number of alleles in Rathi was 11 and effective number of alleles was 2.659. Tharparkar cattle showed 9 as observed number of alleles while as effective number of alleles was 2.455. For Gir the observed number of alleles was 12 while effective number of alleles was 3.429and for Kankrej the observed number of alleles was 11 while effective number of alleles was2.719. Sahiwal cattle showed 11 as observed number of alleles; while as effective number of alleles were 2.659. For Nagori the observed number of alleles was 13 while effective number of alleles was 2.661whereas Kaukinen & Varvio (1993) studied HEL-5 microsatellite locus, the numbers of alleles reported were seven with range from 147-171 bp. Mean and total heterozygosity were reported to be 0.736 and 0.790, respectively and Kappes *et al.* (1997) observed 7 to 13 alleles with allele size ranging between 151 to

167 bp. Goudarzi *et al.* (1993) while studying French cattle breeds, reported the product size of this locus 149-169 bp.

PIC values for Rathi, Tharparkar, Gir, Sahiwal, Nagori and Kankrej cattle were 0.5532, 0.5537, 0.6506, 0.5532, 0.5537 and 0.5542 respectively(Table4.2) In this marker, Gir showed the highest value of PIC indicating good informativeness of this marker whereas Tharpakar showed lowest value. The polymorphic nature of this marker makes it marker of choice in characterization and genetic diversity studies.

Table 4.2Genetic Diversity data for HEL5 marker in all six breeds.

Breed	N	Na	Ne	I	Ho	He	PIC
Rathi	11	3.000	2.659	1.038	0.578	0.624	0.5532
Gir	12	4.000	3.429	1.286	0.568	0.708	0.6506
Kankrej	11	3.000	2.719	1.041	0.598	0.632	0.5542
Tharpakar	9	3.000	2.455	0.965	0.565	0.593	0.5537
Sahiwal	11	3.000	2.659	1.038	0.604	0.624	0.5532
Nagori	13	3.000	2.661	1.038	0.578	0.624	0.5537

Na = No. of Different Alleles,Ne = No. of Effective Alleles = 1 / (Sum pi^2), I = Shannon's Information Index = -1* Sum (pi * Ln (pi)), Ho = Observed Heterozygosity = No. of Hets / N, He = Expected Heterozygosity = 1 - Sum pi^2

4.1.2. Microsatellite BM 2113

Microsatellite BM 2113 contains $(CA)_{20}$ repeats and is localized on bovine chromosome number 2. (Sunden *et al.* 1993).

Source:http://www.ncbi.nlm.nih.gov/entrez/viewer.fcgi?db=nuccore&id=162753

tgcatggtgc tgccttctac caaatacccc ctgctccggc ccccacctca accacaca cacacacaca cacacacaca cacacacaca cagagtgagc tcatagtctt gagttaaaaa agtgacaggt gttgcttctc tcaggaagac ctcttggatt .

4.1.2.1 Allelic Frequencies for BM2113 microsatellite marker.

A total of 9 alleles (31-55 bp) were scored in the six breeds. The minimum allelic frequency was 0.038 and maximum was 0.462. The allelic frequencies are presented in Table4.3.For BM2113 there were 10 alleles in the Criollo cattle (125 to 143 bp), with an equal distribution of frequencies for all alleles reported by Russell *et al.* (2000).

For Hardy Weinberg Equilibrium, Chi square test was conducted. In this Sahiwal had P value less than 0.05 that showed this breed were significant in Chi square test and not found to be in HWE whereas Gir, Kankrej, Rathi, Nagori and Tharpakar showed the P value higher than 0.05 which was not significant and these populations were in HWE. It has been clearly depicted that most of the population were not in HWE because of the samples which were collected from well-organized dairy farm where selection and artificial insemination is in practice for better production. That's why most of the population deviated from HWE.

Table 4.3 Allelic frequencies of microsatellite BM2113 of six breeds of cattle

ALLELE NO.	GIR	KANKREJ	RATHI	SAHIWAL	NAGORI	THARPAKAR	TOTAL
1	0.208	0	0	0.143	0	0.038	0.063
2	0.208	0.333	0	0.357	0	0.269	0.206
3	0	0	0.3	0	0	0.115	0.063
4	0	0	0.3	0	0	0	0.044
5	0.125	0.417	0	0.179	0.179	0.115	0.169
6	0	0	0.4	0	0	0	0.050
7	0	0	0	0	0.286	0	0.050
8	0.458	0.250	0	0.321	0.107	0.462	0.275
9	0	0	0	0	0.429	0	0.081
Chi sq	9.469	7.200	5.556	15.493	10.169	10.937	
P value	0.149	0.066	0.135	0.017	.118	0.362	

** - Highly significant (P≤ 0.01); * - Significant (P≤0.05); NS - Non-significant (P>0.05)

4.1.2.2. Genetic Diversity parameters and PIC values for BM2113

Observed number of alleles in Rathi was 10 and effective number of alleles was 2.941, Tharparkar cattle showed 13 as observed number of alleles, while as effective number of alleles was 3.189, Gir breed had 12 observed number of allele, while as 3.200 effective number of allele and observed number of allele for Kankrej breed was 12 and effective number of alleles was 2.880.Sahiwal breed depicted 14 observed number of alleles and effective number of alleles were 3.532 whereas in Nagori observed number of alleles were 14 and effective were 3.240. Expected heterozygosity for Rathi was 0.660, 0.686 for Tharparkar, 0.688 for Gir, 0.717 for Sahiwal , 0.691 for Nagori and 0.653 for Kankrej. The PIC values for microsatellite BM 2113 in Rathi was 0.0.5862, 0.637 in Tharparkar, 0.6368 for Gir,0.6652 for Sahiwal,0.6387 for Nagori and 0.5786 for Kankrej. Hence, the marker is highly informative in all the populations, indicating high informativeness of this marker. PIC values are given in Table 4.4.

Table 4.4 Genetic Diversity data for BM2113 marker in all six breeds.

Breed	N	Na	Ne	I	Ho	He	PIC
Rathi	10	3.000	2.941	1.089	0.612	0.660	0.5862
Gir	12	4.000	3.200	1.271	0.598	0.688	0.6368
Kankrej	12	3.000	2.880	1.078	0.612	0.653	0.5786
Tharpakar	13	5.000	3.189	1.334	0.623	0.686	0.637
Sahiwal	14	4.000	3.532	1.318	0.656	0.717	0.6652
Nagori	14	4.000	3.240	1.268	0.598	0.691	0.6387

Na = No. of Different Alleles,Ne = No. of Effective Alleles = 1 / (Sum pi^2), I = Shannon's Information Index = -1* Sum (pi * Ln (pi)), Ho = Observed Heterozygosity = No. of Hets / N, He = Expected Heterozygosity = 1 - Sum pi^2

4.1.3 Microsatellite ETH 3

Microsatellite ETH 3 contains $(GT)_nAC(GT)_6$ repeatand is located on chromosome 11 (Solinas *et al.* 1993).

GAACCTGCCTCTCCTGCATTGGCA$(GT)_nAC(GT)_6$ACCACTAGCCACCTGG GAAGCCCGCCTACTTGGCCACAGGCAGAGT

4.1.3.1 Allelic Frequencies for ETH3 microsatellite marker.

A total of 9 alleles (100-175 bp) were typed in the six breeds. The minimum allelic frequency was 0.107 and maximum was 0.5 in allele 2.The allelic frequencies are presented in Table 4.5.

For Hardy Weinberg Equilibrium, Chi square test was conducted. In this Rathi, Kankrej Sahiwal Tharpakar Nagori had P value less than 0.05 that showed all five breeds were significant in Chi square test and not found to be in HWE whereas Gir showed the P value higher than 0.05which was not significant and Gir population was in HWE. It has been clearly depicted that most of the population were not in HWE because of the samples which were collected from well-organized dairy farm where selection and artificial insemination in practice for better production. That's why most of the population deviated from HWE.

Table 4.5 Allelic frequencies of microsatellite ETH3 of six breeds of cattle

ALLELE NO.	GIR	KANKREJ	RATHI	SAHIWAL	NAGORI	THARPAKAR	TOTAL
1	0	0	0	0	0	0.136	0.019
2	0.464	0.417	0	0	0	0.5	0.231
3	0.286	0.417	0.214	0.167	0	0.364	0.231
4	0	0.167	0.357	0.500	0	0	0.160
5	0	0	0.321	0.	0	0	0.064
6	0.143	0	0	0.333	0.250	0	0.115
7	0	0	0	0	0.321	0	0.058
8	0	0	0.107	0	0.429	0	0.103
9	0.107	0	0	0	0	0	0.019
Chi sq	11.846	8.160	13.222	9.00	8.815	11.00	
P value	0.065	0.043	0.040	0.029	0.032	.012	

** - Highly significant (P≤ 0.01); * - Significant (P≤0.05); NS - Non-significant (P>0.05)

4.1.3.2. Genetic Diversity parameters and PIC values for ETH3.

Observed number of alleles in Rathi was 14 and effective number of alleles was 3.469. Tharparkar cattle showed 11 as observed number of alleles while as effective number of alleles was 2.495. Gir cattle showed 14 as observed number of alleles, while effective number of alleles was 3.039, Sahiwal showed 9 observed number of alleles and 2.571 effective number of alleles, Observed number of alleles in Nagori was 14 and effective number of alleles was 2.861 and for Kankrej observed number of allele was 13 and effective number of allele was 105.26. Expected heterozygosity for Rathi was 0.712, 0.599 for Tharparkar, 0.671 for Gir, 0.651 for Nagori, 0.611 for Sahiwal and 0.625 for Kankrej whereas Choroszy *et al.* (2006) studied polymorphism of 11 microsatellite DNA markers in Simmental bulls. For this marker allele size range was 117-127 bp and PIC value was 0.544.

The PIC values for microsatellite ETH 3 in Rathi was 0.6566, 0.5186 for Tharparkar, 0.6164 for Gir, 0.5356 for Sahiwal, 0.5766 for Nagori while in Kankrej it was 0.5465, hence, the marker has proved to be highly informative and diverse (Table4.6).

Table 4.6 Genetic Diversity data for ETH3 marker in all six breeds.

Breed	N	Na	Ne	I	Ho	He	PIC
Rathi	14	4.000	3.469	1.302	0.836	0.712	0.6566
Gir	14	4.000	3.039	1.231	0.591	0.671	0.6164
Kankrej	12	3.000	2.667	1.028	0.698	0.625	0.5465
Tharpakar	11	3.000	2.495	0.986	0.654	0.599	0.5186
Sahiwal	9	3.000	2.571	1.011	0.876	0.611	0.5356
Nagori	14	3.000	2.861	1.075	0.732	0.651	0.5766

Na = No. of Different Alleles,Ne = No. of Effective Alleles = 1 / (Sum pi^2), I = Shannon's Information Index = -1* Sum (pi * Ln (pi)), Ho = Observed Heterozygosity = No. of Hets / N, He = Expected Heterozygosity = 1 - Sum pi^2

4.1.4 Microsatellite ETH 152

Microsatellite ETH 152 contains $(CA)_{17}$ repeats, and this microsatellite is located at 39-72 nucleotide position in 189 bp sequence (Steffen *et al.* 1993). Source: http://www.ncbi.nlm.nih.gov/

gatcttgtac tcgtagggca ggctgcctgc agagccaaca cacacacaca cacacacaca cacacacaca caggggcac tgctgttggc ttccggaggc cacagggcag ttgggggaag gggggcaggc aagagcccct gggagccctg gcagaggtga ccctccctcc agacaggtgc cctctgatc

4.1.4.1 Allelic Frequencies for ETH152 microsatellite marker.

10 alleles (184-300 bp) were found for this marker. Lowest allelic frequency was 0.125 and highest was 0.5. The allelic frequencies are presented in Table 4.7

For Hardy Weinberg Equilibrium, Chi square test was conducted. In this, Kankrej, Sahiwal, Tharpakar, Nagori had P value less than 0.05 that showed all five breeds were significant in Chi square test and not found to be in HWE whereas Gir and Rathi showed the P value higher than 0.05 which was not significant and Gir and Rathi populations were in HWE. It has been clearly depicted that most of the population were not in HWE because of the samples which were collected from well-organized dairy farm where selection and artificial insemination in practice for better production. That’s why most of the population deviated from HWE.

Table 4.7 Allelic frequencies of microsatellite ETH 152 of six breeds of cattle

ALLELE NO.	GIR	KANKREJ	RATHI	SAHIWAL	NAGORI	THARPAKAR	TOTAL
1	0	0.321	0.389	0	0	0	0.113
2	0	0.464	0.333	0	0	0	0.133
3	0.333	0	0.278	0	0	0	0.093
4	0.417	0.214	0	0	0	0.500	0.193
5	0.25	0	0	0.350	0	0.375	0.153
6	0	0	0	0	0.250	0.125	0.067
7	0	0	0	0.500	0	0	0.067
8	0	0	0	0	0.500	0	0.100
9	0	0	0	0.150	0	0	0.027
10	0	0	0	0	0.250	0	0.053
Chi sq	7.20	10.009	4.886	10.000	14.00	12.000	
P value	0.066	0.013	.180	.019	0.003	0.007	

** - Highly significant (P≤ 0.01); * - Significant (P≤0.05); NS - Non-significant (P>0.05)

4.1.4.2. Genetic Diversity parameters and PIC values for ETH15

Observed number of alleles for Rathi 9 , Tharparkar 12, Sahiwal- 10 , Nagori – 14, Gir– 12 and Kankrej was 14 and effective number of alleles was Rathi- 2.945 , Tharparkar-2.462, Sahiwal- 2.532 , Nagori – 2.667, Gir – 2.880 and Kankrej was 2.741. Expected heterozygosity for Rathi was 0.660, 0.599 for Tharparkar, 0.653 for Gir,0.625 for Nagori, 0.605 for Sahiwal and 0.635 for Kankrej whereas Navani *et al.* (2002) studied a set of cattle microsatellite DNA markers for genome analysis of riverine buffalo (Bubalus bubalis). For this marker allele size ranges from 181- 189 bp and heterozygosity calculated was 0.44

The PIC value for microsatellite ETH 152 in Rathi was 0.5864, 0.5112 in Tharparkar, 0.5786 for Gir , 0.567 for Sahiwal 0.5547 for Nagori and 0.5603

for Kankrej Hence, the marker is highly informative in all the populations, indicating high informativeness of this marker. PIC values are given in Table 4.8.

Table 4.8 Genetic Diversity data for ETH152 marker in all six breeds.

Breed	N	Na	Ne	I	Ho	He	PIC
Rathi	9	3.000	2.945	1.089	0.578	0.660	0.5864
Gir	12	3.000	2.880	1.078	0.589	0.653	0.5786
Kankrej	14	3.000	2.741	1.051	0.534	0.635	0.5603
Tharpakar	12	3.000	2.462	0.974	0.512	0.594	0.5112
Sahiwal	10	3.000	2.532	0.999	0.569	0.605	0.567
Nagori	14	3.000	2.667	1.040	0.543	0.625	0.5547

Na = No. of Different Alleles,Ne = No. of Effective Alleles = 1 / (Sum pi^2), I = Shannon's Information Index = -1* Sum (pi * Ln (pi)), Ho = Observed Heterozygosity = No. of Hets / N, He = Expected Heterozygosity = 1 - Sum pi^2

4.1.5 Microsatellite HEL 1

Microsatellite HEL 1 is located on chromosome 15. (Kaukinen & Varvio. 1993).

4.1.5.1 Allelic Frequencies for HEL1 microsatellite marker.

Altogether 8 alleles (94-200 bp) were typed in the four breeds. The minimum allelic frequency was 0.179 and maximum was 0.5.The allelic frequencies are presented in Table 4.9.

For Hardy Weinberg Equilibrium, Chi square test was conducted. In this, Sahiwal, and Nagori had P value less than 0.05 that showed all five breeds were significant in Chi square test and not found to be in HWE whereas Gir, Kankrej, Tharpakar and Rathi showed the P value higher than 0.05 which was not significant so these populations were in HWE. It has been

clearly depicted that most of the population were not in HWE because of the samples which were collected from well-organized dairy farm where selection and artificial insemination in practice for better production. That's why most of the population deviated from HWE.

Table 4.9 Allelic frequencies of microsatellite HEL 1 of six breeds of cattle

ALLELE NO.	GIR	KANKREJ	RATHI	SAHIWAL	NAGORI	THARPAKAR	TOTAL
1	0	0.321	0.321	0.318	0	0.389	0.236
2	0.375	0	0	0	0	0.333	0.083
3	0.313	0.214	0.321	0.500	0	0	0.229
4	0	0	0.179	0.182	0	0	0.076
5	0.313	0.250	0	0	0	0.278	0.118
6	0	0	0	0	0.208	0	0.042
7	0	0.214	0.179	0	0.500	0	0.167
8	0	0	0	0	0.292	0	0.049
Chi sq	4.160	9.407	9.147	11.00	12.000	4.886	
P value	.245	.152	.165	.012	.007	.180	

** - Highly significant (P≤ 0.01); * - Significant (P≤0.05); NS - Non-significant (P>0.05)

4.1.5.2. Genetic Diversity parameters and PIC values for HEL1

Observed number of alleles was Rathi -14, Tharparkar-9, Sahiwal-11, Nagori-12 and Kankrej- 14, while 8 in Gir. Effective number of alleles for Rathi was 3.698 , for Tharparkar, 2.977 for GirNagori-2.642 , 2.602 for Sahiwal and 3.881 for Kankrej. Expected heterozygosity for Rathi was 0.730, 0.142 for Tharparkar, 0.664 for Gir 0.622 for Nagori,0.616 for Sahiwal and 0.742 for Kankrej. Thus, Kankrej cattle were highly heterozygous for this marker. The PIC values as calculated from allele frequencies in Rathi were 0.6801, 0.5864 in Tharparkar,0.5911 in Gir 0.5419 for Sahiwal 0.5498 for Nagori and 0.6935 in Kankrej. Hence, the marker is highly informative in all the populations, indicating high informativeness of this marker. PIC values are given in Table 4.10.

Table 4.10 Genetic Diversity data for HEL1 marker in all six breeds.

Breed	N	Na	Ne	I	Ho	He	PIC
Rathi	14	4.000	3.698	1.345	0.678	0.730	0.6801
Gir	8	3.000	2.977	1.095	0.602	0.664	0.5911
Kankrej	14	4.000	3.881	1.372	0.712	0.742	0.6935
Tharpakar	9	3.000	2.945	1.089	0.632	0.660	0.5864
Sahiwal	11	3.000	2.602	1.021	0.562	0.616	0.5419
Nagori	12	3.000	2.642	1.033	0.568	0.622	0.5498

Na = No. of Different Alleles,Ne = No. of Effective Alleles = 1 / (Sum pi^2), I = Shannon's Information Index = -1* Sum (pi * Ln (pi)), Ho = Observed Heterozygosity = No. of Hets / N, He = Expected Heterozygosity = 1 - Sum pi^2

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 *etal.* (1995).

Source: http://www.ncbi.nlm.nih.gov/

taggccacag gagaactcct tatccttatg tatagattta tataagatgt attaaaataa ttcagtacca gtaatttgat tatttgttga tttattataa ataaataaat aattggctaa tgcaattgta gaggttatcc cacaactgct ttctgcaaac tggagaccca gtccaatggt tagtttgtt tccaaattca aagtcctgag aaccaggaga acaaatggta taacagccag tccaagtctg aaggcctgag aacc aggagt gccactggtg tgagttctag tccatgtgat gcatgggtga aggctgatat cccatccaga aaacagatca aattctacct ttgtgtgtgt gtgtgtgtgt gtgtgtgtgt gtgtgtgtgt gtgtgtgtgt gtgagctcag ttgtgtccaa ctctttgcaa ccccaaggac tgtaagctgc caggctccta tgcccatggg atttctaggc aagcatacta gagtgccatt tccttttcca gg .

4.1.6.1 Allelic Frequencies for ILSTS022 microsatellite marker.

A total of 11 alleles (92-418 bp) were typed in these six breeds. The minimum allelic frequency was 0.036 and maximum was 0.5. The allelic frequencies are presented in Table 4.11.

For Hardy Weinberg Equilibrium, Chi square test was conducted. In this, Kankrej, Tharpakar, Sahiwal, and Nagori had P value less than 0.05 that showed all five breeds were significant in Chi square test and not found to be in HWE whereas Gir, and Rathi showed the P value higher than 0.05 which was not significant so these populations were in HWE. It has been clearly depicted that most of the population were not in HWE because of the samples which were collected from well-organized dairy farm where selection and artificial insemination in practice for better production. That's why most of the population deviated from HWE.

Table 4.11 Allelic frequencies of microsatellite ILSTS022 of six breeds of cattle

ALLELE NO.	GIR	KANKREJ	RATHI	SAHIWAL	NAGORI	THARPAKAR	TOTAL
1	0.036	0	0	0	0	0	0.007
2	0.036	0	0.136	0	0.375	0	0.070
3	0	0	0.273	0	0.500	0	0.113
4	0	0	0	0	0.125	0	0.021
5	0.071	0	0.227	0.278	0	0.455	0.162
6	0.321	0	0.364	0.389	0	0.182	0.218
7	0.321	0	0	0	0	0.364	0.120
8	0.214	0	0	0.333	0	0	0.092
9	0	0.423	0	0	0	0	0.085
10	0	0.269	0	0	0	0	0.049
11	0	0.308	0	0	0	0	0.063
Chi sq	11.325	7.935	17.875	4.886	8.000	8.113	
P value	0.736	.047	.007	.180	.046	.04	

** - Highly significant (P≤ 0.01); * - Significant (P≤0.05); NS - Non-significant (P>0.05)

4.1.6.2. Genetic Diversity parameters and PIC values for ILSTS022

Observed number of alleles in Rathi was 11 and effective number of alleles was 3.612. Tharparkar cattle showed 12 as observed number of alleles and effective number of alleles was 2.743, Sahiwal showed 9 as observed number of alleles while as effective number of alleles was 2.945. Kankrej showed 13 as observed number of alleles and effective number of alleles was 2.889. Observed number of alleles in Gir was 14 and effective number of alleles was 3.843. While as in Kankrej breed observed number of alleles was 13 and effective number of allele was 2.889. Nagori showed 8 observed number of alleles and effective number of alleles was 2.462. Expected heterozygosity for Rathi was 0.723, 0.635 for Tharparkar, 0.740 for Gir ,0.654 for Kankrej, 0.594 for Nagori and 0.660 for Sahiwal indicating low heterozygosity in Gir and high heterozygosity in Kankrej breed. The PIC value for Rathi was 0.6723, 0.552 for Tharparkar, and 0.6945 for Gir 0.5876 for Sahiwal 0.5112 for Nagori and 0.5803 for Kankrej. Hence, the marker is highly informative in all the populations, indicating high informativeness of this marker. PIC values are given in Table 4.12.

Table 4.12Genetic Diversity data for ILSTS022 marker in all six breeds.

Breed	N	Na	Ne	I	Ho	He	PIC
Rathi	11	4.000	3.612	1.331	0.645	0.723	0.6723
Gir	14	6.000	3.843	1.486	0.654	0.740	0.6945
Kankrej	13	3.000	2.889	1.080	0.578	0.654	0.5803
Tharpakar	12	3.000	2.743	1.051	0.595	0.635	0.552
Sahiwal	9	3.000	2.945	1.089	0.598	0.660	0.5876
Nagori	8	3.000	2.462	0.974	0.490	0.594	0.5112

Na = No. of Different Alleles,Ne = No. of Effective Alleles = 1 / (Sum pi^2), I = Shannon's Information Index = -1* Sum (pi * Ln (pi)), Ho = Observed Heterozygosity = No. of Hets / N, He = Expected Heterozygosity = 1 - Sum pi^2

4.1.7 Microsatellite INRA035

According to Vaiman *et al*. (1994) this microsatellite locus contains $(TG)_{16}$ repeats (underlined) and located on chromosome 16.

Source: http://www.ncbi.nlm.nih.gov/ gi/ 536/ gb/ X68049

gatcctttgc agcctccaca ttgtcttctc aggctgattt ctgatgcata atgaatgtgt gtgtgtgtgt gtgtgtgtgt gtgtgtgtgt gtgtgtgagt tcccggatag tgtcataaag cacaagcgca actctgttct agtcttggag atgtcaactt .

4.1.7.1 Allelic Frequencies for INRA035 microsatellite marker.

10 alleles (86-240 bp) were typed in all the four breeds. The maximum allelic frequency was 0.500 and minimum was 0.1 as represented in table 4.13 whereas Goudarzi *et al.* (1993) also reported use of this microsatellite in French cattle breeds. They observed this marker to occur in broader size range i.e. 101-127 bp.

For Hardy Weinberg Equilibrium, Chi square test was conducted. In this, Kankrej, Tharpakar and Sahiwal, had P value less than 0.05 that showed all five breeds were significant in Chi square test and not found to be in HWE whereas Gir, Rathi and Nagori showed the P value higher than 0.05 which was not significant so these populations were in HWE. It has been clearly depicted that most of the population were not in HWE because of the samples which were collected from well-organized dairy farm where selection and artificial insemination in practice for better production. That’s why most of the population deviated from HWE.

Table 4.13 Allelic frequencies of microsatellite INRA035 of six breeds of cattle

ALLELE NO.	GIR	KANKREJ	RATHI	SAHIWAL	NAGORI	THARPAKAR	TOTAL
1	0	0	0	0.250	0	0	0.051
2	0	0.375	0	0	0	0.231	0.094
3	0	0	0.150	0.321	0	0.462	0.188
4	0.1	0.500	0.400	0	0	0.115	0.159
5	0.5	0	0.300	0	0	0	0.138
6	0.3	0	0	0.143	0	0	0.087
7	0.	0.125	0	0	0	0.192	0.058
8	0.1	0	0.100	0.286	0.250	0	0.116
9	0	0	0.050	0	0.438	0	0.072
10	0	0	0	0	0.313	0	0.036
Chi sq	10.00	8.000	11.667	14.375	5.257	11.556	
P value	0.125	0.046	.308	.026	.154	.07	

** - Highly significant (P≤ 0.01); * - Significant (P≤0.05); NS - Non-significant (P>0.05)

4.1.7.2. Genetic Diversity parameters and PIC values for INRA035

Observed number of alleles in Rathi was 10 and effective number of alleles was 3.509. Tharparkar cattle showed 14 as observed number of alleles and effective number of alleles was 3.311, Sahiwal showed 14as observed number of alleles while as effective number of alleles was 3.733. Kankrej showed 13 as observed number of alleles and effective number of alleles was 2.889. Observed number of alleles in Gir was 10 and effective number of alleles was 2.778. While as in Kankrej breed observed number of alleles was 8 and effective number of allele was 2.462. Nagori showed 8 observed number of alleles and effective number of alleles was 2.844. Expected heterozygosity for Rathi was 0.715, 0.679 for Tharparkar, 0.640 for Gir , 0.594 for Kankrej, 0.648 for Nagori and 0.732 for Sahiwal . The PIC value for Rathi was 0.6681, 0.6821 for Sahiwal 0.5759 for Nagori , 0.6326 for Tharparkar, 0.5812 for Gir and 0.5112 for Kankrej. Hence, the marker is highly informative in all the populations, indicating high informativeness of this marker. PIC values are given in Table4.14. And Vaiman *et al*. (1994) reported the allele size range from 102-114 bp. This marker was found to be less informative with moderate mean and total heterozygosities, 0.442 and 0.488 respectively.

Table 4.14 Genetic Diversity data for INRA035 marker in all six breeds.

Breed	N	Na	Ne	I	Ho	He	PIC
Rathi	10	5.000	3.509	1.392	0.621	0.715	0.6681
Gir	10	4.000	2.778	1.168	0.568	0.640	0.5812
Kankrej	8	3.000	2.462	0.974	0.468	0.594	0.5112
Tharpakar	14	4.000	3.111	1.250	0.654	0.679	0.6326
Sahiwal	14	4.000	3.733	1.347	0.648	0.732	0.6821
Nagori	8	3.000	2.844	1.072	0.598	0.648	0.5759

Na = No. of Different Alleles,Ne = No. of Effective Alleles = 1 / (Sum pi^2), I = Shannon's Information Index = -1* Sum (pi * Ln (pi)), Ho = Observed Heterozygosity = No. of Hets / N, He = Expected Heterozygosity = 1 - Sum pi^2

4.1.8 Microsatellite INRA 063

Microsatellite INRA 063 contains $(AC)_{13}$repeats and is located on chromosome 18 (Vaiman *et al.* 1994).

4.1.8.1 Allelic Frequencies for INRA063 microsatellite marker.

A total of 12 alleles (140-386 bp) were typed in the six breeds. The minimum allelic frequency was 0.045 and maximum was 0.500. The allelic frequencies are presented in Table 4.15.

For Hardy Weinberg Equilibrium, Chi square test was conducted. In this, Kankrej, Tharpakar and Rathi, had P value less than 0.05 that showed all five breeds were significant in Chi square test and not found to be in HWE whereas Gir, Sahiwal and Nagori showed the P value higher than 0.05 which was not significant so these populations were in HWE. It has been clearly depicted that most of the population were not in HWE because of the samples which were collected from well-organized dairy farm where selection and artificial insemination in practice for better production. That’s why most of the population deviated from HWE.

Table 4.15 Allelic frequencies of microsatellite INRA063 of six breeds of cattle

ALLELE NO.	GIR	KANKREJ	RATHI	SAHIWAL	NAGORI	THARPAKAR	TOTAL
1	0.500	0.	0.364	0.	0.	0.	0.118
2	0.083	0.375	0	0	0	0	0.081
3	0.417	0	0.455	0	0	0	0.132
4	0	0.375	0	0	0	0	0.074
5	0	0	0.136	0	0	0	0.022
6	0	0	0	0	0	0.125	0.029
7	0	0	0	0	0	0.5	0.088
8	0	0.167	0	0	0	0	0.029
9	0	0	0	0.364	0.278	0.375	0.176
10	0	0.083	0.045	0.364	0.	0	0.096
11	0	0	0	0.273	0.389	0	0.110
12	0	0	0	0	0.333	0	0.044
Chi sq	6.000	20.296	11.825	5.844	4.886	12.000	
P value	.112	0.002	.06	.119	.180	.007	

** - Highly significant (P≤ 0.01); * - Significant (P≤0.05); NS - Non-significant (P>0.05)

4.1.8.2. Genetic Diversity parameters and PIC values for INRA063

Observed numbers of alleles in Rathi was 11 and effective number of alleles was 2.782. Tharparkar cattle showed 13 as observed number of alleles and effective number of alleles was 2.541, Sahiwal showed 11 as observed number of alleles while as effective number of alleles was 2.951. Kankrej showed 12 as observed number of alleles and effective number of alleles was 3.165. Observed number of alleles in Gir was 6 and effective number of alleles was 2.323. Nagori showed 9 observed number of alleles and effective number of alleles was 2.945. Expected heterozygosity for Rathi was 0.640, 0.607 for Tharparkar, 0.569 for Gir , 0.684 for Kankrej, 0.660 for Nagori and 0.661 for Sahiwal. The PIC value for Rathi was 0.5711, 0.5879 Sahiwal 0.5864 Nagori 0.5112 for Tharparkar, 0.4764 for Gir and 0.6245 for Kankrej. Hence, the marker is highly informative in all the populations, indicating high informativeness of this marker. PIC values are given in Table 4.16.whereas Russell *et al.* (2000) studied microsatellites INRA-063 along with the other microsatellite markers to analyze genetic similarities and differences of geographically isolated Criollo cattle herds in Mexico.

Table 4.16 Genetic Diversity data for INRA063 marker in all six breeds.

Breed	N	Na	Ne	I	Ho	He	PIC
Rathi	11	4.000	2.782	1.138	0.586	0.640	0.5711
Gir	6	3.000	2.323	0.918	0.432	0.569	0.4764
Kankrej	12	4.000	3.165	1.241	0.624	0.684	0.6245
Tharpakar	13	3.000	2.541	1.002	0.523	0.607	0.5112
Sahiwal	11	3.000	2.951	1.090	0.534	0.661	0.5879
Nagori	9	3.000	2.945	1.089	0.612	0.660	0.5864

Na = No. of Different Alleles,Ne = No. of Effective Alleles = 1 / (Sum pi^2), I = Shannon's Information Index = -1* Sum (pi * Ln (pi)), Ho = Observed Heterozygosity = No. of Hets / N, He = Expected Heterozygosity = 1 - Sum pi^2

4.1.9 Microsatellite ILSTS002

Microsatellite ILSTS-002 contains $(AC)_{17}$ and localized on chromosome number 18. The repeat region (underlined) is between 65-98 bp (Kemp *et al.* 1995).

cactaatcat taagattttg ccacgtttgc tgtatctgtc tatacacatg tgctgtgcat

gcatacacac acacacacac acacacacac acacacacaa atgtgcatac acagacacag

tttttctaaa ccatttgaat gtaactttca ggtagcgtgt cacttcaccc ctaagtatatgt

4.1.9.1 Allelic Frequencies for ILSTS002 microsatellite marker.

8 alleles of 145bp-200bp size were typed in five breeds of cattle under study. The allelic frequency was recorded highest 0.500 and lowest 0.111 presented in Table 4.17.

For Hardy Weinberg Equilibrium, Chi square test was conducted. In this, Sahiwal ,Tharpakar and Rathi, had P value less than 0.05 that showed all five breeds were significant in Chi square test and not found to be in HWE whereas Gir, and Kankrej showed the P value higher than 0.05 which was not

significant so these populations were in HWE. It has been clearly depicted that most of the population were not in HWE because of the samples which were collected from well-organized dairy farm where selection and artificial insemination in practice for better production. That's why most of the population deviated from HWE.

Table 4.17 Allelic frequencies of microsatellite ILSTS002 of six breeds of cattle

ALLELE NO.	GIR	KANKREJ	RATHI	SAHIWAL	NAGORI	THARPAKAR	TOTAL
1	0	0.350	0	0.208	0	0	0.129
2	0	0.450	0	0.	0	0.375	0.164
3	0	0	0	0.500	0	0	0.112
4	0.318	0.200	0.5	0	0	0	0.181
5	0.409	0	0	0.292	0	0.375	0.233
6	0	0	0	0	0	0.250	0.043
7	0.273	0	0.389	0	0	0	0.121
8	0	0	0.111	0	0	0	0.017
Chi sq	6.334	7.143	9.00	12.000	0	6.667	
P value	0.09	0.067	0.029	.007	0	0.08	

** - Highly significant (P≤ 0.01); * - Significant (P≤0.05); NS - Non-significant (P>0.05)

4.1.9.2. Genetic Diversity parameters and PIC values for ILSTS002

Observed number of alleles in Rathi was 9 and effective number of alleles was 2.418. Tharparkar cattle showed 12 as observed number of alleles and effective number of alleles was 2.642, Sahiwal showed 12 as observed number of alleles while as effective number of alleles was 2.642. Kankrej showed 10 as observed number of alleles and effective number of alleles was 2.740. Observed number of alleles in Gir was 14 and effective number of alleles was 3.843. Nagori showed no result with this marker. Expected heterozygosity for Rathi was 0.586, 0.653 for Tharparkar, 0.657 for Gir , 0.594 for Kankrej, and 0.622 for Sahiwal

The PIC value for Rathi was 0.5008, 0.5498 for Sahiwal , 0.5815 for Tharparkar, 0.5832 for Gir and 0.5594 for Kankrej. Nagori didn't showed results for this marker. Hence, the marker is highly informative in five breeds, indicating mediate informativeness of this marker. PIC values are given in Table 4.18.wherein Sodhi *et al.* (2006) observed a total of 6 alleles in the

range of 122-138 bp. Observed heterozygosity and PIC value for ILSTS002 in this breed was 0.50 and 0.61 respectively.

Table 4.18 Genetic Diversity data for ILSTS002 marker in all six breeds.

Breed	N	Na	Ne	I	Ho	He	PIC
Rathi	9	3.000	2.418	0.958	0.498	0.586	0.5008
Gir	11	3.000	2.916	1.084	0.568	0.657	0.5832
Kankrej	10	3.000	2.740	1.049	0.598	0.635	0.5594
Tharpakar	12	3.000	2.880	1.078	0.589	0.653	0.5815
Sahiwal	12	3.000	2.642	1.033	0.602	0.622	0.5498
Nagori	-	-	-	-	-	-	-

Na = No. of Different Alleles,Ne = No. of Effective Alleles = 1 / (Sum pi^2), I = Shannon's Information Index = -1* Sum (pi * Ln (pi)), Ho = Observed Heterozygosity = No. of Hets / N, He = Expected Heterozygosity = 1 - Sum pi^2

4.1.10 Microsatellite ETH10

Microsatellite ETH10 contains $(CA)_{12}$ and is located on chromosome 5. The repeat region (underlined) is between 62-85 bp. (Solinas *et al.* 1993).

gttcaggact ggccctgcta acacccctcc tccaccacca ccaccaaaaa taaaacacac acacacacac acacacacac acacaatcct ctcccagcct ccctcttcag tgtaagcagt ggctgcccca gccctctgtt tccggcttct ccgactaccc aggtccctcc ctggagctct gacgacacag agaagagaaa gtgggctgga gg

4.1.10.1 Allelic Frequencies for ETH10 microsatellite marker.

7 alleles of 145bp-200bp size were typed in six breeds of cattle under study. The allelic frequency was recorded highest 0.500 and lowest 0.036 showed in table 4.19

For Hardy Weinberg Equilibrium, Chi square test was conducted. In this, Sahiwal , Gir and Rathi, had P value less than 0.05 that showed all breeds were significant in Chi square test and not found to be in HWE whereas Kankrej , Tharpakar and Nagori showed the P value higher than 0.05

which was not significant so these populations were in HWE. It has been clearly depicted that most of the population were not in HWE because of the samples which were collected from well-organized dairy farm where selection and artificial insemination in practice for better production. That's why most of the population deviated from HWE.

Table 4.19 Allelic frequencies of microsatellite ETH10 of six breeds of cattle

ALLELE NO.	GIR	KANKREJ	RATHI	SAHIWAL	NAGORI	THARPAKAR	TOTAL
1	0	0	0	0.042	0	0	0.007
2	0	0	0	0	0.455	0.333	0.093
3	0.500	0.500	0.036	0.458	0.182	0.167	0.327
4	0	0	0	0.375	0.045	0	0.067
5	0.143	0	0.500	0.125	0.227	0.250	0.220
6	0.071	0.500	0.464	0	0.091	0.250	0.233
7	0.286	0	0	0	0	0	0.286
Chi sq	14	13.0	14	12.727	11	12.0	
P value	0.030	0.0	0.003	0.048	0.358	0.062	

** - Highly significant (P≤ 0.01); * - Significant (P≤0.05); NS - Non-significant (P>0.05)

4.1.10.2. Genetic Diversity parameters and PIC values for ETH10

Observed number of alleles in Rathi was 14 and effective number of alleles was 2.142. Tharparkar cattle showed 6 as observed number of alleles and effective number of alleles was 3.789, Sahiwal showed 12 as observed number of alleles while as effective number of alleles was 2.717. Kankrej showed 13 as observed number of alleles and effective number of alleles was 2.00. Observed number of alleles in Gir was 14 and effective number of alleles was 2.80. Observed number of alleles in Nagori was 11 and effective number of alleles was 3.315.. Expected heterozygosity for Rathi was 0.533, 0.736 for Tharparkar, 0.643 for Gir , 0.500 for Kankrej, 0.698 for Nagori and 0.632 for Sahiwal.

The PIC value for Rathi was 0.4246, 0.561 for Sahiwal ,0.6535 for Nagori , 0.6875 for Tharparkar, 0.5847 for Gir and 0.375 for Kankrej. Hence, the marker is highly informative in all the populations, indicating high informativeness of this marker. PIC values are given in Table 4.20.

Table 4.20 Genetic Diversity data for ETH10 marker in all six breeds.

Breed	N	Na	Ne	I	Ho	He	PIC
Rathi	14	3.000	2.142	0.822	0.624	0.533	0.4246
Gir	14	4.000	2.800	1.171	0.724	0.643	0.5847
Kankrej	13	2.000	2.000	0.693	0.598	0.500	0.375
Tharpakar	6	4.000	3.789	1.358	0.768	0.736	0.6875
Sahiwal	12	4.000	2.717	1.118	0.698	0.632	0.561
Nagori	11	5.000	3.315	1.364	0.798	0.698	0.6535

Na = No. of Different Alleles,Ne = No. of Effective Alleles = 1 / (Sum pi^2), I = Shannon's Information Index = -1* Sum (pi * Ln (pi)), Ho = Observed Heterozygosity = No. of Hets / N, He = Expected Heterozygosity = 1 - Sum pi^2

4.1.11 Microsatellite CSRM60

Microsatellite CSRM-60 contains $(CA)_{17}$ repeats. The repeat region (underlined) is between 47-81 bp (Moore *et al.* 1994).

aagatgtgat ccaagagaga ggcagaaagc gcatacacac ccataaacac acacacacacacacacacac acacacacac aaagccacca tgcctttcac gatctggtcct

4.1.11.1 Allelic Frequencies for CSRM60 microsatellite marker.

A total of 7 (95bp-160bp) were typed in the six breeds studied. The allelic frequency in the combined population was minimum 0.063 and maximum 0.500 presented in Table 4.21 wherein Karthickeyan *et al.* (2007) assessed Umblachery cattle breed of south India using 25 FAO r*Eco*mmended microsatellite markers. A total of 5 alleles in size range of 94-

112 for CSRM60 were typed in this breed. PIC value for this marker was found to be 0.7007.

For Hardy Weinberg Equilibrium, Chi square test was conducted. In this, Gir, ,Tharpakar and Nagori had P value less than 0.05 that showed all five breeds were significant in Chi square test and not found to be in HWE whereas Sahiwal, Rathi and Kankrej showed the P value higher than 0.05 which was not significant so these populations were in HWE. It has been clearly depicted that most of the population were not in HWE because of the samples which were collected from well-organized dairy farm where selection and artificial insemination in practice for better production. That's why most of the population deviated from HWE.

Table 4.21 Allelic frequencies of microsatellite CSRM60 of six breeds of cattle

ALLELE NO.	GIR	KANKREJ	RATHI	SAHIWAL	NAGORI	THARPAKAR	TOTAL
1	0.375	0	0	0	0.227	0.500	0.167
2	0	0	0.357	0.464	0	0	0.148
3	0	0.357	0.107	0.214	0	0	0.123
4	0.438	0.321	0.321	0.143	0.136	0.250	0.272
5	0	0	0.107	0	0	0	0.025
6	0.063	0.321	0.107	0.179	0.318	0.250	0.204
7	0.125	0	0	0	0.138	0	0.062
Chi sq.	14.857	7.086	12.341	11.415	19.600	14.00	
P VALUE	0.021	0.069	0.263	0.076	0.003	0.003	

** - Highly significant (P≤ 0.01); * - Significant (P≤0.05); NS - Non-significant (P>0.05)

4.1.11.2. Genetic Diversity parameters and PIC values for CSRM60

Observed number of alleles in Rathi was 14 and effective number of alleles was 3.769.Tharparkar cattle showed 14 as observed number of alleles and effective number of alleles was 2.667, Sahiwal showed 14 as observed number of alleles while as effective number of alleles was 3.187. Kankrej showed 14 as observed number of alleles and effective number of alleles was

2.992. Observed number of alleles in Gir was 8 and effective number of alleles was 2.844. Observed number of alleles in Nagori was 11 and effective number of alleles was 3.667.. Expected heterozygosity for Rathi was 0.735, 0.625 for Tharparkar, 0.648 for Gir , 0.66 for Kankrej, 0.727 for Nagori and 0.686 for Sahiwal and Manatrinon *et al.* (2008) in a microsatellite analysis to estimate genetic diversity and relationship of 180 individuals belonging to two native endangered Austrian cattle breeds, Carinthian Blond (CB) and Waldviertler Blond (WB), and Hungarian Grey (HG) from Hungary found 0.667 and 0.725 as observed and expected heterozygosity in CSRM60 marker.The PIC value for Rathi was 0.6903, 0.638 for Sahiwal , 0.675 for Nagori , 0.5547 for Tharparkar, 0.5828 for Gir and 0.5907 for Kankrej. Hence, the marker is highly informative in all the populations, indicating high informativeness of this marker. PIC values are given in Table 4.22.

Table 4.22 Genetic Diversity data for CSRM60 marker in all six breeds.

Breed	N	Na	Ne	I	Ho	He	PIC
Rathi	14	5.000	3.769	1.450	0.686	0.735	0.6903
Gir	8	4.000	2.844	1.163	0.548	0.648	0.5828
Kankrej	14	3.000	2.992	1.097	0.624	0.666	0.5907
Tharpakar	14	3.000	2.667	1.040	0.612	0.625	0.5547
Sahiwal	14	4.000	3.187	1.272	0.654	0.686	0.638
Nagori	11	4.000	3.667	1.337	0.678	0.727	0.675

Na = No. of Different Alleles,Ne = No. of Effective Alleles = 1 / (Sum pi^2), I = Shannon's Information Index = -1* Sum (pi * Ln (pi)), Ho = Observed Heterozygosity = No. of Hets / N, He = Expected Heterozygosity = 1 - Sum pi^2

4.1.12 Microsatellite ETH225

Microsatellite ETH-225 contains $(CA)_{18}$ repeats and is localized on bovine chromosome number 9. The repeat region (underlined) is between 43-78 bp (Steffen *et al.* 1993).

gatcaccttg ccactatttc ctccaacata tgtgtgtgcg tgcacacaca cacacacaca cacacacaca cacacacatg atagccactc ctttctctaa tgccacagaa ttacacagtc aactctctag tagcagctgg ctgtcatgtg tcatttggca atatccatat cttccccct tgctgtaaa

4.1.12.1 Allelic Frequencies for ETH225 microsatellite marker.

In present investigation, ETH 225 microsatellite marker usage reveal total of 11 alleles of size 130bp-195bp in six cattle breeds. The allelic frequency was minimum for alleles 0.038 and maximum 0.462 as presented in Table 4.23 whereas Radko *et al.* (2005) analysed polymorphism of 11 microsatellite DNA loci in Polish Red (PR), Hereford and Holstein-Friesian (HF) cattle and a total 6 alleles in each breed in the size range 140-152, 140-158 and 140-152

For Hardy Weinberg Equilibrium, Chi square test was conducted. In this, Gir, Rathi and Kankrej, had P value less than 0.05 that showed all five breeds were significant in Chi square test and not found to be in HWE whereas Sahiwal and Tharpakar showed the P value higher than 0.05 which was not significant so these populations were in HWE. It has been clearly depicted that most of the population were not in HWE because of the samples which were collected from well-organized dairy farm where selection and artificial insemination in practice for better production. That’s why most of the population deviated from HWE.

Table 4.23 Allelic frequencies of microsatellite ETH225 of six breeds of cattle

ALLELE NO.	GIR	KANKREJ	RATHI	SAHIWAL	NAGORI	THARPAKAR	TOTAL
1	0	0	0	0.063	0	0	0.006
2	0.071	0	0	0.313	0.	0.125	0.064
3	0.214	0	0.	0.188	0.	0.250	0.096
4	0	0.429	0	0.250	0.308	0.083	0.179
5	0.214	0	0.	0	0.308	0.417	0.160
6	0	0.250	0.	0	0.	0.	0.051
7	0.357	0	0	0.188	0.154	0.125	0.135
8	0	0.321	0	0	0.077	0	0.077
9	0.143	0	0.462	0	0.154	0	0.128
10	0	0	0.038	0	0	0	0.013
11	0	0	0.500	0	0	0	0.090
Chi sq.	24.111	8.815	13.000	4.978	32.500	16.800	
P value	0.007	0.032	0.005	0.893	0.000	0.079	

** - Highly significant (P≤ 0.01); * - Significant (P≤0.05); NS - Non-significant (P>0.05)

4.1.12.2. Genetic Diversity parameters and PIC values for ETH225

Observed number of alleles in Rathi was 13 and effective number of alleles was 2.153. Tharparkar cattle showed 12 as observed number of alleles and effective number of alleles was 3.646, Sahiwal showed 8 as observed number of alleles while as effective number of alleles was 4.267. Kankrej showed 14 as observed number of alleles and effective number of alleles was 2.861. Observed number of alleles in Gir was 14 and effective number of alleles was 4.083. Observed number of alleles in Nagori was 13 and effective number of alleles was 4.122. Expected heterozygosity for Rathi was 0.536, 0.726 for Tharparkar, 0.755 for Gir , 0.651 for Kankrej, 0.757 for Nagori and 0.766 for Sahiwal

The PIC value for Rathi was 0.4271, 0.7269 Sahiwal 0.7190 Nagori 0.6849 for Tharparkar, 0.7146 for Gir and 0.5766 for Kankrej. Hence, the marker is highly informative in all the populations, indicating high informativeness of this marker. PIC values are given in Table 4.24 and Sodhi *et al.* (2006) observed a total of seven alleles in size range of 130-170 in Tharparkar cattle. While the observed heterozygosity was 0.50, PIC value for ETH225 was found to be 0.47.

Table 4.24 Genetic Diversity data for ETH225 marker in all six breeds.

Breed	N	Na	Ne	I	Ho	He	PIC
Rathi	13	3.000	2.153	0.829	0.468	0.536	0.4271
Gir	14	5.000	4.083	1.494	0.689	0.755	0.7146
Kankrej	14	3.000	2.861	1.075	0.568	0.651	0.5766
Tharpakar	12	5.000	3.646	1.438	0.634	0.726	0.6849
Sahiwal	8	5.000	4.267	1.511	0.690	0.766	0.7296
Nagori	13	5.000	4.122	1.499	0.687	0.757	0.7190

Na = No. of Different Alleles,Ne = No. of Effective Alleles = 1 / (Sum pi^2), I = Shannon's Information Index = -1* Sum (pi * Ln (pi)), Ho = Observed Heterozygosity = No. of Hets / N, He = Expected Heterozygosity = 1 - Sum pi^2

4.1.13. Microsatellite INRA005

Microsatellite INRA005 contains (GT) 13 and is located on chromosome number 12. The repeat region (underlined) is located between 340-365 bp (Vaiman *et al*. 1992).

tcgatcaatg ctgaagagtt taggatttaa atttatgtta tcctgtgtat gactccctat

aaggaatttc cagagatgca gcttttgaga aggtgaaagc tttgaaatac tccataactc

aactggataa atcctaagcc tttcaaaaac acggaaattc ggggggtggt ggaggtgagg

gaaaatggtg tccttagttt ttgaatttta tcttccaaat tgcaatctgc atgaagtata

aatattagcc aactgaaaac tgggaaagtg ataaataggt gagatcatta atgaggaata

agattgtta gtgtgtgtgtg tgtgtgtgtg tgtgtgagca tgtggtgtag ggtatgcctg

aagcgggttc tggtgat

4.1.13.1 Allelic Frequencies for INRA005 microsatellite marker.

On microsatellite analysis 10 alleles of size 135bp -190 bp were typed in all breeds. The allelic frequency was minimum for alleles 0.036 and maximum 0.500 as depicted in Table 4.25.The INRA-005 alleles in the wider

range (131-163 bp) were reported by Goudarzi *et al*. (1993) in French cattle breeds. The allelic size reported by Vaiman *et al*. (1992) is however; in a narrow range of 139 to 147 bp.

For Hardy Weinberg Equilibrium, Chi square test was conducted. In this, and Kankrej, Sahiwal and Tharpakar had P value less than 0.05 that showed all five breeds were significant in Chi square test and not found to be in HWE whereas Gir, Nagori and Rathi showed the P value higher than 0.05 which was not significant so these populations were in HWE. It has been clearly depicted that most of the population were not in HWE because of the samples which were collected from well-organized dairy farm where selection and artificial insemination in in practice for better production. That's why most of the population deviated from HWE.

Table 4.25 Allelic frequencies of microsatellite INRA005 of six breeds of cattle

ALLELE NO.	GIR	KANKREJ	RATHI	SAHIWAL	NAGORI	THARPAKAR	TOTAL
1	0	0.5	0	0.	0	0	0.083
2	0	0	0	0.	0.036	0.	0.008
3	0.167	0.2	0.	0.462	0.286	0.438	0.265
4	0	0	0	0.192	0	0.063	0.053
5	0.333	0	0.286	0.346	0	0.500	0.205
6	0	0	0.429	0	0	0	0.098
7	0.167	0	0.179	0.	0.393	0	0.144
8	0	0	0.107	0	0	0	0.023
9	0	0.3	0	0	0	0	0.045
10	0.333	0	0.	0	0.286	0	0.076
Chi sq	4.500	10.000	10.500	9.919	10.659	8.000	
P VALUE	0.609	0.019	0.105	0.019	0.100	0.046	

** - Highly significant (P≤ 0.01); * - Significant (P≤0.05); NS - Non-significant (P>0.05)

4.1.13.2. Genetic Diversity parameters and PIC values for INRA005

Observed number of alleles in Rathi was 14 and effective number of alleles was 3.240. Tharparkar cattle showed 8 as observed number of alleles and effective number of alleles was 2.246, Sahiwal showed 13 as observed number of alleles while as effective number of alleles was 2.705. Kankrej

showed 10 as observed number of alleles and effective number of alleles was 2.632. Observed number of alleles in Gir was 13 and effective number of alleles was 3.600. Observed number of alleles in Nagori was 14 and effective number of alleles was 3.136. Expected heterozygosity for Rathi was 0.691, 0.555 for Tharparkar, 0.722 for Gir , 0.620 for Kankrej, 0.681 for Nagori and 0.630 for Sahiwal and Ciampolini *et al*. (2006) also reported 6 alleles in Chiania, Marchigiana, Romagnola and Piemontese cattle breeds with PIC value of 0.585. The estimates of mean and total heterozygosity obtained by Vaiman *et al.*(1992) were lesser (0.524 and 0.555), than the present estimates.

The PIC value for Rathi was 0.6387, 0.5543 Sahiwal 0.6179 Nagori 0.4568 for Tharparkar, 0.6716 for Gir and 0.5478 for Kankrej. Hence, the marker is highly informative in all the populations, indicating high informativeness of this marker. PIC values are given in Table 4.26.

Table 4.26 Genetic Diversity data for INRA005 marker in all six breeds.

Breed	N	Na	Ne	I	Ho	He	PIC
Rathi	**14**	**4.000**	**3.240**	**1.268**	**0.568**	**0.691**	**0.6387**
Gir	**3**	**4.000**	**3.600**	**1.330**	**0.678**	**0.722**	**0.6716**
Kankrej	**10**	**3.000**	**2.632**	**1.030**	**0.564**	**0.620**	**0.5478**
Tharpakar	**8**	**3.000**	**2.246**	**0.882**	**0.523**	**0.555**	**0.4568**
Sahiwal	**13**	**3.000**	**2.704**	**1.041**	**0.578**	**0.630**	**0.5543**
Nagori	**14**	**4.000**	**3.136**	**1.202**	**0.589**	**0.681**	**0.6179**

Na = No. of Different Alleles,Ne = No. of Effective Alleles = 1 / (Sum pi^2), I = Shannon's Information Index = -1* Sum (pi * Ln (pi)), Ho = Observed Heterozygosity = No. of Hets / N, He = Expected Heterozygosity = 1 - Sum pi^2

4.1.14 Microsatellite BM1818

Microsatellite BM1818 contains $(GT)_{13}$ repeats and is located on chromosome 23. The repeat region (underlined) is between 52…77.(Bishop *et al.* 1994).

Source: http://www.ncbi.nlm.nih.gov/ gi/1222848/gb/18391.1

agctgggaat ataaccaaag gaaactaaaa catgcactga aaaagatacc tgcaccccta

tgttcatagc agcattattt atactagcca agcaagccat ggaaaccgca cctaagttat

ctccattcat caagggatga atggagaaat tgtgtgtgtg tgtgtgtgtg tgtgtgtatg

atggaatatt atttagtcat aaaatgagga aatccttcca tttgtgataa catgcatgga

ccttgaaagc actatgctac gtgaagtaac tcagagaaaa aacaaatact atatgttccc

acttatatgt ggcatttaaa aacct

4.1.14.1 Allelic Frequencies for BM1818 microsatellite marker.

A total of 7 alleles (279-354 bp) were typed in these six breeds. The allelic frequency was maximum (0.500) and minimum (0.036) showed in Table 4.27.

For Hardy Weinberg Equilibrium, Chi square test was conducted. In this, and Kankrej, Sahiwal and Tharpakar had P value less than 0.05 that showed all five breeds were significant in Chi square test and not found to be in HWE whereas Gir, Nagori and Rathi showed the P value higher than 0.05 which was not significant so these populations were in HWE. It has been clearly depicted that most of the population were not in HWE because of the samples which were collected from well-organized dairy farm where selection and artificial insemination in practice for better production. That’s why most of the population deviated from HWE.

Table 4.27 Allelic frequencies of microsatellite BM1818 of six breeds of cattle

ALLELE NO.	GIR	KANKREJ	RATHI	SAHIWAL	NAGORI	THARPAKAR	TOTAL
1	0.214	0.227	O.250	0	0.393	0.321	0.221
2	0.500	0.455	0.071	0	0.036	0.179	0.221
3	0.036	0	0.036	0	0.071	0.500	0.133
4	0.107	0.318	0.071	0.500	0	0	0.058
5	0	0	0	0.500	0	0	0.045
6	0	0	0.036	0	0.179	0	0.039
7	0.143	0	0.250	0	0.250	0	0.123
Chi sq.	14.000	7.983	24.536	7.00	16.000	14.00	
P VALUE	0.173	0.046	0.268	0.008	0.356	0.003	

** - Highly significant (P≤ 0.01); * - Significant (P≤0.05); NS - Non-significant (P>0.05)

4.1.14.2. Genetic Diversity parameters and PIC values for BM1818

Observed number of alleles in Rathi was 14 and effective number of alleles was 4.558. Tharparkar cattle showed 14 as observed number of alleles and effective number of alleles was 2.596, Sahiwal showed 7 as observed number of alleles while as effective number of alleles was 2.000. Kankrej showed 11 as observed number of alleles and effective number of alleles was 2.782. Observed number of alleles in Gir was 14 and effective number of alleles was 3.039. Observed number of alleles in Nagori was 14 and effective number of alleles was 3.843.. Expected heterozygosity for Rathi was 0.781, 0.615 for Tharparkar, 0.740 for Gir , 0.640 for Kankrej, 0.698 for Nagori and 0.500 for Sahiwal . The PIC value for Rathi was 0.7469, 0.375 for Sahiwal, 0.7007 for Nagori, 0.5408 for Tharparkar, 0.6279 for Gir and 0.5667 for Kankrej. Hence, the marker is highly informative in all the populations, indicating high informativeness of this marker. PIC values are given in Table 4.28. Wherein Sodhi *et al.* (2006) observed a total of 6 alleles in the size range of 254-294 bp in Tharparkar cattle. BM1818 showed observed heterozygosity value of 0.65 and expected heterozygosity or PIC value of 0.7.

Table 4.28 Genetic Diversity data for BM1818 marker in all six breeds.

Breed	N	Na	Ne	I	Ho	He	PIC
Rathi	14	7.000	4.558	1.666	0.678	0.781	0.7469
Gir	14	5.000	3.039	1.313	0.632	0.671	0.6279
Kankrej	11	3.000	2.782	1.059	0.598	0.640	0.5667
Tharpakar	14	3.000	2.596	1.019	0.543	0.615	0.5408
Sahiwal	7	2.000	2.000	0.693	0.458	0.500	0.375
Nagori	14	6.000	3.843	1.517	0.687	0.740	0.7007

Na = No. of Different Alleles,Ne = No. of Effective Alleles = 1 / (Sum pi^2), I = Shannon's Information Index = -1* Sum (pi * Ln (pi)), Ho = Observed Heterozygosity = No. of Hets / N, He = Expected Heterozygosity = 1 - Sum pi^2

4.1.15 Microsatellite ILSTS006

Microsatellite ILSTS-006 contains $(GT)_{23}$ repeats. The repeat region (underlined) is between 209..255 bp (Brezinsky *et al.* 1993).

Source: http://www.ncbi.nlm.nih.gov/ gi|385186|gb|L23482.1

tgttttctac ttttgtgtct gtatttctgc tgtggaaaga agttcctctg aactatttgt

ccagattcca catatatgca ttaaatgcat gatatttggg ggtttttcca tttgtgactt

acttcactct gtatggcaat ctctaggtcc acccatgtct ctgcaaatgg cacaattcca

ttccttttaa tggctgagta atattccagt gtgtgtgtgt gtgtgtgtgt gtgtgtgtgt

gtgtgtgtgt gtgtgnnnnn nnnnnatatc ttctttatcc attcctgtta atggacgttt

agatcgcttc cgtgttct

4.1.15.1 Allelic Frequencies for ILSTS006 microsatellite marker.

A total of 7 alleles (275-323 bp) were typed in these six breeds. The allelic frequency was maximum (0.500) and minimum (0.167) showed in Table 4.29.

For Hardy Weinberg Equilibrium, Chi square test was conducted. In this, and Sahiwal had P value less than 0.05 that showed all five breeds were significant in Chi square test and not found to be in HWE whereas Kankrej and Tharpakar showed the P value higher than 0.05 which was not significant so these populations were in HWE. It has been clearly depicted that most of the population were not in HWE because of the samples which were collected from well-organized dairy farm where selection and artificial insemination in practice for better production. That's why most of the population deviated from HWE.

Table 4.29 Allelic frequencies of microsatellite ILSTS006 of six breeds of cattle

ALLELE NO.	GIR	KANKREJ	RATHI	SAHIWAL	NAGORI	THARPAKAR	TOTAL
1	0	0	0	0	0	0.409	260
2	0	0.4	0	0	0	0	280
3	0	0.4	0	0	0	0.318	300
4	0	0	0	0.5	0	0	310
5	0	0.2	0	0.333	0	0	320
6	0	0	0	0	0	0.273	330
7	0	0	0	0.167	0	0	400
Chi sq		3.125		12.000		6.344	
P value		0.373		0.007		0.096	

** - Highly significant (P≤ 0.01); * - Significant (P≤0.05); NS - Non-significant (P>0.05)

4.1.15.2. Genetic Diversity parameters and PIC values for ILSTS006

Observed number of alleles in Sahiwal showed 12 as observed number of alleles while as effective number of alleles was 2.571. Kankrej showed 4 as observed number of alleles and effective number of

alleles was 2.462. .Tharpakar showed 12 as observed number of alleles and effective number of alleles was 2.969. Expected heterozygosity was 0.663 for Tharparkar, 0.594 for Kankrej, and 0.611 for Sahiwal whereas Dadi *et al.* (2009) studied genetic diversity in Sheko, African taurine cattle, a total of 9 alleles in the size range of 277-299 bp was observed for ILSTS006 in this breed. Observed and expected heterozygosity values were found to be 0.700 and 0.783.

The PIC value for was 0.5358 for Sahiwal , 0.612 for Tharparkar, and 0.5632 for Kankrej. Hence, the marker is highly informative in all the populations, indicating high informativeness of this marker. PIC values are given in Table 4.30 and Rehman and Khan (2009) investigated genetic diversity of Hariana and Hissar cattle breeds of Pakistan and observed a total of 5 alleles in size range of 277-309 bp in these two breeds. Observed and expected heterozygosity values for ILSTS006 in both the breeds were 0.60 and 0.65 for Hariana and 0.56 and 0.77 for Hissar cattle respectively.

Table 4.30 Genetic Diversity data for ILSTS006 marker in all six breeds.

Breed	N	Na	Ne	I	Ho	He	PIC
Rathi	**-**	**-**	**-**	**-**	**-**	**-**	**-**
Gir	**-**	**-**	**-**	**-**	**-**	**-**	**-**
Kankrej	**4**	**3.000**	**2.462**	**0.974**	**0.498**	**0.594**	**0.5632**
Tharpakar	**12**	**3.000**	**2.969**	**1.093**	**0.612**	**0.663**	**0.5832**
Sahiwal	**12**	**3.000**	**2.571**	**1.011**	**0.598**	**0.611**	**0.5356**
Nagori	**-**	**-**	**-**	**-**	**-**	**-**	**-**

Na = No. of Different Alleles,Ne = No. of Effective Alleles = 1 / (Sum pi^2), I = Shannon's Information Index = -1* Sum (pi * Ln (pi)), Ho = Observed Heterozygosity = No. of Hets / N, He = Expected Heterozygosity = 1 - Sum pi^2

4.1.16 Genetic variability parameters for Rathi, Tharparkar, Gir ,Sahiwal, Nagori and Kankrej cattle breed:

Table 4.31 summarized observed and expected number of alleles with their sizes exhibited by various microsatellites investigated in overall, six populations, respectively. Total numbers of alleles observed across the populations were found to be 1050. Maximum number of alleles observed across the populations was 81 for CSRM60 and minimum was 31 for ILSTS-006. The mean observed allele numbers across the population for all 15 loci was 70.00 indicating the high level of polymorphism of the selected microsatellites. The mean number of alleles and the expected heterozygosities detected are good indicators of the genetic polymorphism within the breed. Generally the mean number of alleles is highly dependent on the sample size because of the presence of unique alleles in populations, which occur in low frequencies and also because the number of observed alleles tends to increase with increases in population size. The number of alleles scored for each marker is an invaluable indicator of the future usefulness of the marker for genetic screening and it can become the basis for breed characterization.

Table 4.31: Genetic Diversity data of fifteen microsatellites in all six cattle breeds

LOCUS	N	Na	Ne	I	Ho	He
BM1818	77	9.000	6.469	1.999		0.845
CSRM60	81	7.000	5.416	1.781	0.791	0.815
ETH10	75	7.000	4.433	1.630	0.832	0.774
ETH225	78	11.000	8.155	2.191	0.678	0.877
INRA005	66	10.000	6.223	2.006	0.765	0.839
BM2113	80	9.000	5.953	1.968	0.713	0.832
ETH3	78	9.000	6.090	1.946	0.859	0.836
ETH152	75	10.000	8.152	2.186	0.855	0.877
HEL1	72	8.000	5.993	1.912	0.675	0.833
HEL5	71	7.000	5.473	1.817	0.568	0.817
ILSTS022	71	11.000	7.791	2.181	0.821	0.872
INRA035	69	10.000	8.180	2.192	0.765	0.878
INRA063	68	12.000	9.285	2.332	0.657	0.892
ILSTS002	58	8.000	6.259	1.916	0.821	0.840
ILSTS006	31	7.000	6.180	1.875	0.768	0.838

Na = No. of Different Alleles,Ne = No. of Effective Alleles = 1 / (Sum pi^2), I = Shannon's Information Index = -1* Sum (pi * Ln (pi)), Ho = Observed Heterozygosity = No. of Hets / N, He = Expected Heterozygosity = 1 - Sum pi^2

In a previous study by Upreti *et al.* (2012) genetic diversity of native Gir and Kankrej (*Bos indicus*) cattle populations using nine microsatellite markers was evaluated. They observed that the mean number of observed and effective alleles in Kankrej were comparatively high (5.222 and 3.714) and the average expected heterozygosity values (0.5403) indicated high diversity in the Kankrej population than Gir (0.4520). High polymorphism information content (PIC) values observed for most of the markers with an average of 0.5116 indicating the high informativeness of these markers in Kankrej breed than in Gir (0.4202).

Assessment of genetic variability within and between three indigenous cattle breeds viz; Gir, Kankrej and Deoni investigated using 7 microsatellite markers by Kale *et al.* (2010). The results showed that genetic equilibrium was not always maintained. The observed number of alleles ranged from 5 to 8 with total 46 alleles across three breeds. The overall heterozygosity and PIC values were 0.730 and 0.749. Genetic distance was least (0.2034) between Gir and Kankrej and highest between Deoni and Kankrej (0.4442) by using popgene programme (version 1.31).Genetic relationships between Canadienne, Brown Swiss, Holstein and Jersey cattle was estimated by Hansen *et al.* (2002) after genotyping 20 distantly related animals in each breed for 15 microsatellites located on separate chromosomes. The within-breed estimates of genetic distance were greater than zero and found to be significant. The genetic distance between Canadienne (0.156) and Holstein (0.156), Brown Swiss (0.243) and Jersey (0.235) was negligible, suggesting the close relationship. Brown Swiss and Holstein (0.211) cattle also demonstrated a close relationship. In contrast, the Jersey breed was genetically distant from the Brown Swiss (0.427) and Holstein cattle (0.320).

4.1.17 F Statistics

The fixation indices (FIS, FIT and FST) values for each locus are shown in Table-4.32.From jackknifing over loci the mean FIS, FIT and FST values over all the population are found to be 0.118, 0.833 and 0.859, respectively. The high FIS and FIT values indicated high level of inbreeding

within and among the populations and also point towards high genetic differentiation between the populations.

The high inbreeding values can be attributed to selective mating under field conditions. However, the high mean number of alleles and mean observed and expected heterozygosities were similar supported by FIS estimates that were not significantly different from zero (Table24).The negative values of FIS for some of the loci indicated that the mates were less related in comparison with in the average population.

Table 4.32 F-statistics analysis for 15 microsatellite loci in Tharpakar, Gir, Rathi, Nagori, Sahiwal and Kankrej breeds of cattle

Locus	Fis	Fit	Fst	Nm
BM1818	0.183	0.833	0.858	0.041
CSRM60	0.226	0.832	0.863	0.040
ETH10	-0.291	0.832	0.870	0.037
ETH225	0.111	0.837	0.853	0.043
INRA005	0.191	0.833	0.859	0.041
BM2113	0.202	0.833	0.861	0.040
ETH3	-0.196	0.833	0.860	0.041
ETH152	0.140	0.833	0.853	0.043
HEL1	0.200	0.833	0.860	0.041
HEL5	0.224	0.832	0.863	0.040
ILSTS022	0.147	0.833	0.854	0.043
INRA035	0.139	0.833	0.853	0.043
INRA063	0.121	0.833	0.851	0.044
ILSTS002	0.190	0.833	0.859	0.041
ILSTS006	0.193	0.833	0.860	0.041
Mean	0.118	0.833	0.859	

Fis = (Mean He - Mean Ho) / Mean HeFit = (Ht - Mean Ho) / Ht Fst = (Ht - Mean He) / Ht

4.2 AMPLIFIED FRAGMENT LENGTH POLYMORPHISM RESULTS

AFLP markers can be used to estimate individual heterozygosity by simply counting the number of bands an individual possesses. AFLP markers are dominant loci, with each locus having only two alleles: the absent allele (0), and the present allele (1). For each locus, an individual either has a band, the present state, or does not had a band, the absent state. In the absent state an individual is homozygous for the absent allele (the 0,0 genotype). However, in the present state, the individual is either homozygous for the present state (the 1,1 genotype), or is heterozygous (the 1,0 genotype). An individual's heterozygosity can therefore be estimated by counting the number of polymorphic loci at which it has a band. The more bands an individual has, the more heterozygous, and therefore less inbred, it is likely to be.

This study revealed that for each primer combination the number of bands that could be scored on gels ranged from 90 to 1000. Only AFLP bands within 90–1000 bp were scored, because bands >1000 bp has low intensity and poor reproducibility. Of the selected primer combinations, four were analyzed. The 8 *TaqI/EcoRI* primer combinations analysed generated more than 100 bands, of which good quality and recognisable were considered as polymorphic markers and typed in the six populations. On average, each primer combination yielded 14 polymorphic markers.

4.2.1. *ECO*R1ACA/*TAQ*1CAC AFLP MARKER

A total of 16 polymorphic bands (80-1000 bp) were scored in these six breeds. The mean of effective numbers of alleles were 1.582 and the mean SE of alleles was 0.083. Expected heterozygosity for *ECO*R1ACA/*TAQ*1CAC ranged in between 0.0 to 0.498 wherein the mean expected hetyerozygosity for this combination was 0.333.The Shanon Index showed a mean value of 0.498 for this combination.

TABLE 4.33: Band frequencies and Heterozygosities for AFLP primer combination *ECO*R1ACA/*TAQ*1CAC

	p	q	Ne	I	He
Band1	**0.155**	**0.845**	**1.355**	**0.431**	**0.262**
Band 2	**0.114**	**0.886**	**1.252**	**0.354**	**0.201**
Band 3	**0.198**	**0.802**	**1.466**	**0.498**	**0.318**
Band 4	**0.293**	**0.707**	**1.707**	**0.605**	**0.414**
Band 5	**0.244**	**0.756**	**1.585**	**0.556**	**0.369**
Band 6	**0.402**	**0.598**	**1.927**	**0.674**	**0.481**
Band 7	**0.465**	**0.535**	**1.991**	**0.691**	**0.498**
Band 8	**1.000**	**0.000**	**1.000**	**0.000**	**0.000**
Band 9	**0.402**	**0.598**	**1.927**	**0.674**	**0.481**
Band 10	**0.345**	**0.655**	**1.825**	**0.645**	**0.452**
Band 11	**0.155**	**0.845**	**1.355**	**0.431**	**0.262**
Band 12	**0.622**	**0.378**	**1.888**	**0.663**	**0.470**
Band 13	**0.198**	**0.802**	**1.466**	**0.498**	**0.318**
Band 14	**0.244**	**0.756**	**1.585**	**0.556**	**0.369**
Band 15	**0.537**	**0.463**	**1.989**	**0.690**	**0.497**
Band 16	**0.155**	**0.845**	**1.355**	**0.431**	**0.262**
Mean			**1.582**	**0.498**	**0.337**
SE			**0.083**	**0.055**	**0.040**

Na = No. of Different Alleles, Ne = No. of Effective Alleles = 1 / (p^2 + q^2), I = Shannon's Information Index = -1* (p * Ln (p) + q * Ln(q))He = Expected Heterozygosity = 2 * p * q

s4.2.2. *ECO*R1ACA/*TAQ*1CAG AFLP MARKER:

A total of 14 polymorphic bands (110-1000 bp) were scored in these six breeds. The mean of effective numbers of alleles were 1.637 and the mean SE of alleles was 0.078. Expected heterozygosity for *ECO*R1ACA/*TAQ*1CAG ranged between 0.0 to 0.497 wherein the mean expected hetyerozygosity for this combination was 0.367 . The Shanon Index showed a mean value of 0.539 for this combination.

TABLE 4.34: Band frequencies and Heterozygosities for AFLP primer combination *ECO*R1ACA/*TAQ*1CAG

	p	q	Ne	I	He
Band1	0.293	0.707	1.707	0.605	0.414
Band 2	0.402	0.598	1.927	0.674	0.481
Band 3	0.244	0.756	1.585	0.556	0.369
Band 4	0.198	0.802	1.466	0.498	0.318
Band 5	0.293	0.707	1.707	0.605	0.414
Band 6	0.114	0.886	1.252	0.354	0.201
Band 7	1.000	0.000	1.000	0.000	0.000
Band 8	0.733	0.267	1.644	0.581	0.392
Band 9	0.244	0.756	1.585	0.556	0.369
Band 10	0.345	0.655	1.825	0.645	0.452
Band 11	0.622	0.378	1.888	0.663	0.470
Band 12	0.155	0.845	1.355	0.431	0.262
Band 13	0.537	0.463	1.989	0.690	0.497
Band 14	0.537	0.463	1.989	0.690	0.497
Mean			1.637	0.539	0.367
SE			0.078	0.049	0.037

Na = No. of Different Alleles, Ne = No. of Effective Alleles = 1 / (p^2 + q^2), I = Shannon's Information Index = -1* (p * Ln (p) + q * Ln(q))He = Expected Heterozygosity = 2 * p * q

4.2.3. *ECO*R1AGC/*TAQ*1CAC AFLP MARKER:

A total of 10 polymorphic bands (170-1000 bp) were scored in these six breeds. The mean of effective numbers of alleles were 1.306 and the mean SE of alleles was 0.096. Expected heterozygosity for *ECO*R1AGC/*TAQ*1CAC ranged in between 0.0 to 0.497 wherein the mean expected hetyerozygosity for this combination was 0.170.The Shanon Index showed a mean value of 0.170 for this combination.

TABLE 4.35: Band frequencies and Heterozygosities for AFLP primer combination *ECO*R1AGC/*TAQ*1CAC

	p	q	Ne	I	He
Band1	0.155	0.845	1.355	0.431	0.262
Band 2	1.000	0.000	1.000	0.000	0.000
Band 3	1.000	0.000	1.000	0.000	0.000
Band 4	0.622	0.378	1.888	0.663	0.470
Band 5	0.733	0.267	1.644	0.581	0.392
Band 6	1.000	0.000	1.000	0.000	0.000
Band 7	1.000	0.000	1.000	0.000	0.000
Band 8	0.198	0.802	1.466	0.498	0.318
Band 9	0.537	0.463	1.989	0.690	0.497
Band 10	1.000	0.000	1.000	0.000	0.000
Mean			1.306	0.244	0.170
SE			0.096	0.075	0.052

Na = No. of Different Alleles, Ne = No. of Effective Alleles = 1 / (p^2 + q^2), I = Shannon's Information Index = -1* (p * Ln (p) + q * Ln(q))He = Expected Heterozygosity = 2 * p * q

4.2.4. *ECO*R1AGC/*TAQ*1CAG AFLP

A total of 8 polymorphic bands (170-1000 bp) were scored in these six breeds. The mean of effective numbers of alleles were 1.867 and the mean SE of alleles was 0.052. Expected heterozygosity for *ECO*R1AGC/*TAQ*1CAG ranged in between 0.0392 to 0.497 wherein the mean expected hetyerozygosity for this combination was 0.461.The Shanon Index showed a mean value of 0.653 for this combination.

TABLE 4.36: Band frequencies and Heterozygosities for AFLP primer combination *ECO*R1AGC/*TAQ*1CAG

	p	q	Ne	I	He
Band1	0.733	0.267	1.644	0.581	0.392
Band 2	0.733	0.267	1.644	0.581	0.392
Band 3	0.345	0.655	1.825	0.645	0.452
Band 4	0.402	0.598	1.927	0.674	0.481
Band 5	0.537	0.463	1.989	0.690	0.497
Band 6	0.402	0.598	1.927	0.674	0.481
Band 7	0.537	0.463	1.989	0.690	0.497
Band 8	0.537	0.463	1.989	0.690	0.497
Mean			1.867	0.653	0.461
SE			0.052	0.017	0.016

Na = No. of Different Alleles, Ne = No. of Effective Alleles = 1 / (p^2 + q^2), I = Shannon's Information Index = -1* (p * Ln (p) + q * Ln(q))He = Expected Heterozygosity = 2 * p * q

The accuracy of the estimates of $p_i(0)$ and $p_i(1)$ depend on two assumptions were found by Liu *et al*. 1998; Maughan *et al*. 1996. First, it is assumed that AFLP markers are dominant loci inherited in a Mendelian fashion. Second, it is assumed that the individuals used to make the allele frequency estimates are unrelated to one another. Verification of the first assumption would require many parent-offspring pairs to be genotyped, something that was not possible in this study. However, previous studies using AFLP markers, this assumption to be valid in most cases. The second assumption is likely to be valid in the majority of species as population sizes are usually large enough such that individuals sampled at random from it are unlikely to be closely related to one another.

When allele frequencies are known, AFLP markers can be used to calculate more sensitive measures of relatedness such as the method described in Queller and Goodnight (1989). This measure gives greater weight to the sharing of rare alleles compared to the sharing of common alleles. AFLP allele frequencies in the wild population could be estimated from the genotypes of the wild mice. Therefore, for the wild mice, in addition to the AFLP genotypes were used to calculate (Madden *et al*. in press).Heterozygosity of the clutches produced could be estimated from the genetic similarity of the parents as more closely related parents produce less heterozygous offspring. Relatedness between parent pairs was calculated from AFLP genotypes (Madden *et al*. in press; Queller and Goodnight 1989).Hundreds of polymorphic markers among nine bovine species, one-third of which were polymorphic within species found by Buntjer *et al*. (2002). Phylogenetic trees of the Bovini tribe built from these AFLP markers yielded high bootstrap values and resolved topologies. To develop six DNA markers derived from AFLP breedspecific bands, which could distinguish between Japanese Black and F1 cattle, were attempted by Sasazaki *et al.* (2004). Using these markers, the probability of identifying F1 was 0.882 and probability of misjudgment was 0.0198. They could be useful for discrimination between Japanese Black and F1.

5. SUMMARY AND CONCLUSION

India is rich in genetic diversity in livestock. NBAGR has recognized 27 breeds of cattle, 8 of buffalo, 42 of sheep, 20 of goats, 6 of horses and 17 of poultry. All these phenotypically recognized breeds are not characterized at molecular level and diversity among them is notexplored. The genetic variability within and between breeds has recently been explored using molecular markers viz RFLP, RAPD, AFLP and microsatellite. Microsatellite loci are the most commonly used molecular markers forgenetic exploration.The vast and varied cattle genetic resources of India are identified in the form of 27 documented breeds of zebu cattle (*Bos indicus*) besides many populations still uncharacterized and undefined. Indigenous cattle breeds are considered, for diverse reasons, as treasure of good genetic resource that tend to disappear as a result of new market demands, crossbreeding or breed replacements, and mechanized agricultural operations.

A total of 180 blood samples 30 each from Rathi, Tharparkar, Gir, Sahiwal, Nagori and Kankrej breeds were collected at random from various places and brought to the lab on ice. DNA was extracted from blood by Qiamp DNA isolation Kit with modifications and dissolved in TE buffer. Quality check and quantification was done by UV Spectrophotometry and electrophoresis on 0.8% agarose gel. The DNA concentration was determined and samples were diluted 10-50 times (approx. 30 ng/µl) with MiliQ water.

Fifteen microsatellite loci (BM1818, CSRM60, ETH10, ETH225, INRA005, BM2113, ETH3, ETH152, HEL1, HEL5, ILSTS022, INRA035, INRA063, ILSTS002, ILSTS006) were selected from the available list of 30 microsatellites suggested by FAO (ISAG) for estimation of genetic diversity in cattle. The microsatellite loci were amplified from genomic DNA samples by PCR using locus specific primers by standard PCR protocol. The PCR protocol was same for all the primers except the annealing temperature and comprised initial denaturation at 94°C for 5 minutes followed by 30 cycles of denaturation at 94 ^{o}C for 1 minute, annealing at 50-64 ^{o}C for 1 minute and extension at 72 ^{o}C for 30 seconds and final extension at 72 ^{o}C for 10 minutes.

PCR amplification was confirmed on 1.5- 2.0% agarose gel containing ethidium bromide. The amplified products visualized as a single compact band of expected size under UV light were documented by gel documentation system (UVP). The PCR products for different microsatellite loci were resolved on 8% non-denaturing polyacrylamide gel along with 100 bp DNA ladder at 2 W (125V). Genotypes were scored manually and microsatellite alleles were visualized by ethidium bromide staining. Allelic size was determined via software aided gel-doc system (UVP).

Four AFLP primer combinations (*ECO*R1ACA/*TAQ*1CAC, *ECO*R1ACA/*TAQ*1CAG, *ECO*R1AGC/*TAQ1*CAC, and *ECO*R1AGC/*TAQ*1CAG) were used to determine the polymorphism in six breeds of cattle. The DNA samples were subjected to restriction digestion (*ECOR1/TAQ1*) and adaptor ligation. After Dig-Lig process, DNA samples were subjected to PCR amplification with same protocol as stated in microsatellite.The PCR products for different AFLP primers were resolved on 8% non-denaturing polyacrylamide gel along with 100 bp DNA ladder at 2 W (125V). Genotypes were scored manually and alleles were visualized by ethidium bromide staining. Band size was determined via software and presence (1) or absence (0) of band was scored.

Genotypic and allelic frequencies counted for all the loci, formed the basis for calculating observed and expected heterozygosity, Shannon Index, Inbreeding coefficient and Hardy-Weinberg equillibrium. Observed and effective number of alleles in all breeds was evaluated. PIC values for all the markers were calculated based on their allele frequencies.

A total of 168 alleles were contributed by Rathi across all 15 microsatellite loci. Maximum number of alleles was observed for ETH3, HEL1, BM1818, CSRM60, ETH10 and INRA005 (14) and minimum for ETH152 and ILSTS002 (9). The mean number of observed (allelic diversity) and effective alleles in Rathi breed were found to be 3.67 and 2.926 respectively across all loci studiedWherein Shannon Index showed value of 1.114. The average expected heterozygosity values were 0.622 indicated high diversity for this set of markers in the selected population. The highest PIC value (0.9970) was

observed at BM1818 locus (0.7469) and least (0.4246) at ETH 10 locus for Rathi cattle, with an average of 0.5601.

Tharparkar contributed 171 alleles across all 15 microsatellite loci. Maximum numbers of alleles were observed for INRA035, CSRM60 and BM1818 (14) and minimum for ETH 10 (6). The mean number of observed and effective alleles in Tharparkar was 3.400 and 2.849, respectivelyWherein Shannon Index showed value of 1.104. The average expected heterozygosity values were 0.642 indicated high diversity in the selected population. ETH10 was most informative in this breed (PIC=0.5408).The highest PIC value (0.6875) was observed at ETH 10 locus and least (0.4568) at INRA005 locus for the Tharparkar cattle, with an average of 0.5663.

Gir contributed 152 alleles across all 9 microsatellite loci. Maximum numbers of alleles were observed for ETH3 (16) and minimum for INRA005 (3).The mean number of observed and effective alleles in Gir was 12.22 and 121.10, respectivelyWherein Shannon Index showed value of 1.139. The average expected heterozygosity values were (0.629) indicated high diversity in the selected population. The highest PIC value (0.7190) was observed at ETH225 locus and least (0) at ILSTS006 locus for the Gir cattle, with an average of 0.5727.

Sahiwal contributed 167 alleles across all 15 microsatellite loci. Maximum numbers of alleles were observed for CSRM60 and BM2113 (14) and minimum for ETH225 (8). The mean number of observed and effective alleles in Sahiwal was 13 and 114.10, respectively Wherein Shannon Index showed value of 1.106.The average expected heterozygosity values (0.676) indicated high diversity in the selected population.The highest PIC value (0.7296) was observed at ETH225 and (0.375) at BM1818 locus for the Sahiwal cattle, with an average of 0.5775.

Nagori contributed 130 alleles across all 15 microsatellite loci. Maximum numbers of alleles were observed for BM1818 (15) and minimum for ILSTS006 and ILSTS002 (0). The mean number of observed and effective alleles in Nagori was 13 and 114.10, respectivelyWherein Shannon Index showed value of 1.034. The average expected heterozygosity values (0.608)

indicated low diversity in the selected population. The highest PIC value (0.7007) was observed at BM1818 and (0) at ILSTS002 and ILSTS006 locus for the Nagori cattle, with an average of 0.5275.

Kankrej contributed 172 alleles across all 15 microsatellite loci. Maximum numbers of alleles were observed for CSRM60 (18) and minimum for ILSTS006 (4). The mean number of observed and effective alleles in Kankrej was 13 and 114.10, respectivelyWherein Shannon Index showed value of 1.056. The average expected heterozygosity values (0.635) indicated high diversity in the selected population. The highest PIC value (0.6935) was observed at HEL1 and (0.375) at ETH10 locus for the Kankrejcattle, with an average of 0.5619.

All four combinations of AFLP primers specified 16 (ECOR1ACA/TAQ1CAC), 14 (ECOR1ACA/TAQ1CAG), 10 (ECOR1AGC/TAQ1CAC) and 8(ECOR1AGC/TAQ1CAG) polymorphic bands. The mean of effective number of alleles were 1.582, 1.637, 1.306 and 1.867 and Shannon index showed a variance value for all combinations viz. 0.498, 0.539, 0.244 and 0.653 respectively.

CONCLUSIONS

Following conclusions can be drawn from this study:

1. The mean observed and effective numbers of alleles were found to be 9.3 and 6.670, respectively across all loci studied. The data suggests that this set of microsatellite markers is well suited for diversity study in the entire six breed.
2. The observed heterozygosity was found to be maximum for INRA063 (0.892) and minimum for ETH10 (0.774). The mean observed heterozygosity across all the loci was 0.851, indicating substantial number of heterozygotes for these markers in all the six breeds.
3. The Shannon Index was found to be maximum for INRA035 (2.192) and minimum for ETH10 (1.630) which indicatedthe higher diversity among the population.
4. BM1818 was found to be highly informative with highest PIC value (0.7469) for Rathi, Reasonably high PIC values observed for most of

the markers, with an average PIC value of 0.5609 across all the loci are indicative of the usefulness of these microsatellites for biodiversity evaluation in these breeds.

5. Marker ILSTS006 did not show amplification in Nagori, Gir and Rathi breed while it was successfully amplified in other breeds.Hence, these markers can be testified for Rathi, Sahiwal and Tharparkar breed distinctness and characterization with extended sample size and comparison with the other breeds of the region.

6. The fixation indices (FIS, FIT and FST) values for each microsatellite locus for overall population were found to be 0.118, 0.833 and 0.859, respectively. The high FIS and FIT values indicated high level of inbreeding within and among the populations.

7. The four combinations of AFLP primers in six cattle breed. A total of 48 polymorphic bands (80-1000 bp) were scored for all the primers. The mean of effective numbers of alleles were 1.582 and the mean SE of alleles was 0.083. The mean expected hetyerozygosity for all combination was 0.333.The Shannon Index showed a mean value of 0.498 for this combination which depicted polymorphism.

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GENETIC CHARACTERIZATION AND DIVERSITY STUDY OF INDIGENOUS CATTLE BREEDS THROUGH MICROSATELLITE AND AFLP MARKERS

Ph.D. Thesis

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ABSTRACT

The present study was aimed to understand the existing genetic diversity and structure of native cattle breeds (Rathi, Sahiwal, Kankrej, Nagori, Gir and Tharpakar) adapted to the north-western arid and semi-arid region of India based on fifteen microsatellite markers (BM1818, CSRM60, ETH10, ETH225, INRA005, BM2113, ETH3, ETH152, HEL1, HEL5, ILSTS022, INRA035, INRA063, ILSTS002, ILSTS006) and four combinations of AFLP markers (*ECO*R1ACA / *TAQ*1CAC, *ECO*R1ACA / *TAQ*1CAG, *ECO*R1AGC / *TAQ*1CAC, *ECO*R1AGC / *TAQ*1CAG).

The mean number of observed and effective alleles in Rathi was 3.667 and 2.926, respectively Wherein Shannon Index showed value of 1.114. The average expected heterozygosity values (0.622) indicated high diversity for this set of markers in the selected population. BM1818 was most informative in Rathi (PIC=0.7469). High PIC values observed for most of the markers with an average of 0.56601 are indicative of the usefulness of microsatellites for biodiversity evaluation in this breed.

The mean number of observed and effective alleles in Tharparkar was 3.400 and 2.849, respectively Wherein Shannon Index showed value of 1.104. The average expected heterozygosity values (0.642) indicated high diversity in the selected population. ETH10 was most informative in this breed (PIC=0.5408). High PIC values observed for most of the markers with an average of 0.5663 are indicative of high polymorphism of these markers in this breed.

The mean number of observed and effective alleles in Gir was 3.733 and 2.917, respectively Wherein Shannon Index showed value of 1.139. The average expected heterozygosity values (0.629) indicated low diversity in the selected population. ETH225 was most informative in this breed (PIC=0.7146). High PIC values observed for most of the markers with an

average of 0.5727 are indicative of high polymorphism of these markers in this breed.

The mean number of observed and effective alleles in Kankrej was 3.067 and 2.791, respectively Wherein Shannon Index showed value of 1.056. The average expected heterozygosity values (0.635) indicated low diversity in the selected population. HEL1 was most informative in this breed (PIC=0.6935). High PIC values observed for most of the markers with an average of 0.5619.

The mean number of observed and effective alleles in Nagori was 3.267 and 2.694, respectively Wherein Shannon Index showed value of 1.034. The average expected heterozygosity values (0.608) indicated low diversity in the selected population. ETH 225 was most informative in this breed (PIC=0.7190). High PIC values observed for most of the markers with an average of 0.5275.

The mean number of observed and effective alleles in Sahiwal was 3.333 and 2.908; respectively Wherein Shannon Index showed value of 1.106.The average expected heterozygosity values (0.676) indicated low diversity in the selected population. ETH 225 was most informative in this breed (PIC=0.7296). High PIC values observed for most of the markers with an average of 0.5775.

The fixation indices (FIS, FIT and FST) values for each microsatellite locus for overall population were found to be 0.118, 0.833 and 0.859, respectively. The high FIS and FIT values indicated high level of inbreeding within and among the populations.

The allele diversity (mean observed number of alleles 9.0, mean effective number of alleles 6.670) and gene diversity (0.845) values imply a substantial amount of genetic variability in all the populations. Reasonably high PIC values observed for most of the markers, with an average PIC value of 0.5609 across all the loci imply that this set of microsatellite are very informative for evaluation of genetic diversity and characterization in all the breeds.

A total of 48 polymorphic bands (80-1000 bp) were scored forthe four combinations of AFLP primers in six cattle breed.. The mean of effective numbers of alleles were 1.582 and the mean SE of alleles was 0.083. The mean expected hetyerozygosity for all combination was 0.333.The Shannon Index showed a mean value of 0.498 for this combination which depicted polymorphism.

देशी गोवंश प्रजातियों का माइक्रोसैटेलाइट एवं ए.एफ.एल.पी. चिह्नकों द्वारा आनुवांशिक अभिचित्रण व वैभिन्य अध्ययन

विद्यावाचस्पति शोध ग्रन्थ

पशु चिकित्सा सूक्ष्म जीव विज्ञान और जैव प्रौद्योगिकी विभाग
पशुचिकित्सा और पशुविज्ञान महाविद्यालय
राजस्थान पशुचिकित्सा और पशुविज्ञान विश्वविद्यालय
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मुख्य उपादेष्टा : डॉ. एस.के. कश्यप

अनुक्षेपण

;g v/;;u mRrj&if'peh {ks= dh ns'kh i'kq uLyksa ¼jkBh] lkghoky] dkadjst] ukxkSjh] fxj vkSj FkkjikdZj½ ds vkuqokaf'kd fofo/krk v/;;u esa ;ksxnku nsrk gSaA ,Q-,-vks-n~okjk lq>kfor ianzg ekbØkslsVsykbV ,oa ,-,Q-,y-ih- ds pkj fpUgksa dks lajpuk le>us esa mi;ksx fy;k x;kA

jkBh uLy eas izsf{kr vkSj izHkkoh ,fYyyksa dh vkSlr la[;k Øe'k% 3-667 vkSj 2-926 FkhA ftlesa jksuu lwpdkad esa 1-114 dk ewY; ik;k x;kA vkSlr izHkkoh gsVjksftxkWflVh ewY; ¼0-642½A bl uLy esa i;kZIr vkuqokaf'kd fofo/krk crkrk gSA jkBh uLy esa BM1818 (PIC-0.7469) lcls T;knk fofHkUurk lwpd gSaA lokZf/kd PIC eku lHkh fpUgdksa ds fy, izsf{kr gq, gSA vkSlr PIC eku 0-56601 bu fpUgdksa dk vkuqokaf'kd fofHkUurk ds ewY;kadu gsrq izHkkoiw.kZ egRo n'kkZrk gSaA

FkkjikdZj uLy eas izsf{kr vkSj izHkkoh ,fYyyksa dh vkSlr la[;k Øe'k% 3-400 vkSj 2-894 Fkh] ftlesa 'kSuu lwpdkad esa 1-104 dk ewY; ik;k x;kA vkSlr izHkkoh gSVjkstkbxkWflVh ewY; ¼0-642½ us p;fur vkcknh esa mPp fofo/krk dk ladsr fn;k gSA ETH10 FkkjikdZj uLy esa lcls tkudkjhiw.kZ lwpd gSA vkSlr ls vf/kd PIC dk eku ¼0-5663½ bl uLy esa bu fpUgdksa ds mPp cgq:irk dk ladsr gSaA

dadjst esa izsf{kr vkSj izHkkoh ,fYyykas dh vkSlr la[;k 13 vkSj 114-10 Fkh ,oa 'kSuu lwpdkad esa 1-056 dk ewY; n'kkZ;k x;kA HEL1 bl uLy esa lcls tkudkjh iw.kZ fpUgd Fkk ¼0-6935½ vkSlr PIC dk eku ¼0-5619½ leqnk; esa O;kIr vkuqokaf'kd fofHkUurk ds ewY;kadu gsrq bu fpUgdksa dk lQy mi;ksx n'kkZrk gSA

fxj uLy esa izsf{kr ,oa izHkkoh ,fYyyksa dh vkSlr la[;k Øe'k% 12-22 vkSj 121-10 Fkh ,oa 'kSuu lwpdkad esa 1-139 dk ewY; vk;k FkkA vkSlr izHkkoh gSVjkstkbxkWflVh ewY; ¼0-629½ us p;fur vkcknh esa vf/kd fofo/krk dk ladsr fn;kA ETH225 bl uLy esa lcls vf/kd fofHkUurk lwfpr djus okyk fpUgd ekuk x;kA vkSlr ls vf/kd PIC dk eku ¼0-5727½ bl uLy esa bu fpUgdksa ds mPp cgq:irk dk ladsr gSA

ukxkSjh esa izsf{kr vkSj izHkkoh ,fYy;ksa dh vkSlr la[;k 3-267 vkSj 2-791 Fkh ,oa 'kSuu lwpdkad esa 1-056 dk ewY; izkIr gqvkA vkSlr izHkkoh gSVjkstkbxkWflVh ewY; ¼0-608½ us p;fur vkcknh esa vf/kd fofo/krk dk ladsr fn;kA ETH225 ¼0-7190½ us bl uLy esa fofHkUurk dks n'kkZ;k gSA vkSlr ls vf/kd PIC dk eku ¼0-5275½ bl uLy esa bu fpUgdksa ds mPp cgq:irk dk ladsr gSA lkghoky esa izsf{kr vkSj izHkkoh ,fYy;ksa dh vkSlr la[;k Øe'k% 3-333 vkSj 2-908 Fkh ,oa 'kSuu lwpdkad esa 1-106 dk ewY; izkIr gqvkA vkSlr izHkkoh gSVjkstkbxkWflVh ewY; ¼0-676½ us p;fur vkcknh esa vf/kd fofo/krk dk ladsr fn;kA ETH225 ¼0-7296½ us bl uLy esa fofHkUurk dks n'kkZ;k gSA

dqy vkcknh ds fy, izR;sd ekbØkslsVsykbV yksdl ds fy, fu/kkZj.k lwpdkad ¼,Q-vkbZ-,l] ,Q-vkbZ-Vh vkSj ,Q-,l-Vh-½ eku Øe'k% 0-118] 0-833 vkSj 0-859 ik, x,A mPp ,Q-vkbZ-,l- vkSj ,Q-vkbZ-Vh- ewY;ksa esa vkcknh ds Hkhrj vkSj chp eas mPp Lrj ds iztuu dk ladsr ik;k x;kA ,fyy

fofo/krk ¼vkSlr izsf{kr ,fYy;ksa½ dk eku 9-0] vkSlr izHkkoh ,fYyyksa dk eku ¼6-670½ vkSj tho fofo/krk ¼0-645½ ds ewY; lHkh vkcknh esa vkuqokaf'kd ifjorZu'khyrk dh i;kZIr ek=k n'kkZrs gSaA vkSlr PIC ewY; ds lkFk ;g n'kkZrk gS fd ekbØkslsVsykbV dk ;g lsV vkuqokaf'kd fofo/krk ds ewY;akdu vkSj lHkh uLyksa esa y{k.k o.kZu ds fy, cgqr tkudkjhiw.kZ gSaA

Ng% i'kq tkfr;ksa esa ,Q-,y-ih- izkbujksa ds pkj la;kstuksa ds fy, dqy 48 cgq:id cSaM ¼80&1000½ ik, x,A ,fYyyksa dh izHkkoh la[;k,a 1-582 Fkh vkSj lHkh la;kstuksa ds fy, mEehn dh xbZ gsVjkstkWxflVh 0-333 Fkh tks cgq:irk dks n'kkZrk gSA

APPENDIX

Phosphate buffer saline (1 %)

Solution A :	Sodium diphosphate	1.4 gm
	Distilled water	1000 ml
Solution B :	Sodium dihydrogen orthophosphate	1.4 gm
	Distilled water	1000 ml

An amount of 84.1 ml of solution A and 15.9 ml of solution B were mixed and 8.5g sodium chloride was added. The volume was made to 1000 ml with distilled water and autoclave it at 15 lbs (121°C) for 15 min.

Agarose solution (0.8% and 1.5-2%)

To prepare 0.8% agarose solution for genomic DNA analysis 0.8 gm of molecular grade agarose powder was dissolved in 100 ml of 1X TBE buffer.

To prepare 1.5-2% agarose solution for PCR products analysis 1.5 and 2 gm respectively of molecular grade agarose powder was dissolved in 100 ml 1X TBE buffer.

Ammonium per sulphate (10%)

To prepare 10% ammonium per sulphate solution 0.5 gm of molecular grade APS was dissolved in 5 ml of distilled water and stored at 4° C. It is advisable to prepare APS solution afresh every time acrylamide gel is prepared.

Buffers for pH meter

Buffer tablets of pH 4, 7 and 9 were crushed in a clean pestle-mortar and dissolved in 100 ml of sterilized distilled water.

EDTA (0.5 M), pH- 8.0

To 800 ml of distilled water 186.1 g of disodium ethylene diaminetetra acetate.2H_2O was added and shake vigorously on a magnetic stirrer for two-three

hours. The pH was adjusted to 8.0 with 1.0 N NaOH, dispensed into aliquots and sterilized by autoclaving.

Ethanol (70%)

In 70 ml of 100% ethanol, add 30 ml of distilled water.

Ethidium bromide

1 g of ethidium bromide was added to 100 ml of distilled water and stirred on a magnetic stirrer for several hours to ensure that the dye has dissolved. The container was wrapped in aluminum foil or 10 mg/ml solution was transferred to a dark bottle and stored at room temperature.

Saturated sodium chloride solution (6 M)

For 100 ml of 6 M solution, 35.06 g of NaCl was dissolved in 80 ml of distilled water. The volume was made up to to 100 ml, filtered and stored at room temperature.

20% Sodium dodecyl Sulphate (SDS) solution

20 g of SDS powder was dissolved initially in 50 ml of distilled water and then stirred on magnetic stirrer at high speed. Finally, the volume of the solution was made upto 100 ml. The solution was filtered and kept at room temperature.

Tris Borate EDTA (TBE) buffer, pH 8.3

5X Stock solution:

54g Tris Base

27.5g Boric Acid and

20mL 0.5m EDTA (pH 8).

Distilled water was added to above and volume was made up to 1000 ml.

A working solution of 1X TBE was prepared by adding 200 ml of stock solution to 800 ml of distilled water.

Tris EDTA (TE) buffer, pH 8

Tris solution (.05 M):

4.44 g/l Tris HCl

2.65 g/l Tris base

The above were dissolved in 1000 ml of distilled water.

Tris solution (.05) 20 ml

EDTA (.05 M) (pH) 200µl

Distilled water was added to above to make up the volume 100 ml.