**ADDIS ABABA UNIVERSITY**

**COLLEGE OF NATURAL SCIENCES**

**BIOTECHNOLOGY PROGRAM**

# Genetic Diversity Study of Five Ethiopian Indigenous Bovine Breeds Using Microsatellite Markers

---

**A thesis submitted to the School of Graduate Studies of Addis Ababa University in partial fulfillment of the requirements for the degree of Masters of Science in Biotechnology**

**BY**

**Solomon Diriba**

**Supervisors:**

**Kassahun Tesfaye (PhD)**

**Tadelle Dessie (PhD)**

**May 2011**

## ACKNOWLEDGMENTS

I take the privilege in expressing my deepest gratitude and appreciation to my supervisors Dr Kassahun Tesfaye and Dr Tadelle Dessie for the most invaluable guidance and constructive criticism as well as active persuasion and encouragements during the pursuit of the project. It would not have been possible to come up with this thesis without their consistent help.

I would like to pass my special gratitude to Dr. Jean Hanson for allowing me to execute my lab work with comfort at the Molecular Biology Laboratory of ILRI, Addis Ababa. I also like to thank Mr. Temeselew Mamo for his patient presence in need of assistance during the course of my lab work. I would also like to take this opportunity to express my gratefulness to Mr. Eshetu Zerihun and Mr. Michael Temesgen for their assistance during blood sample collection. I am also thankful to the staff members of the Biotechnology Program and my classmates for creating friendly environment during my course work.

Special thanks go to the International Livestock Research Institute (ILRI) for accepting me as a graduate fellow and providing me the financial support to this project through project No. BTO3. I am also beholden to Haramaya Unviersity for the sponsorship to pursue my study. Indeed, I am so grateful to Addis Ababa University for admitting me to pursue my master degree in Biotechnology.

I have no words to express my deepest thanks to my revered family for their support, patience and love. I also want to express my heartfelt thanks to my wife Nolawit Dawit for the moral and unrelenting support. Finally, I thank the almighty God for putting me on this road, giving me the opportunities and fulfilling my dreams.

## LIST OF FIGURES

## LIST OF TABLES

## LIST OF ABBREVIATIONS

| | |
|---|---|
| AFLP | Amplified Fragment Length Polymorphism |
| Bp | Base pair |
| DAGRIS | Domestic Animal Genetic Resources Information System |
| Df | degree of freedom |
| EARI | Ethiopian Agricultural Research Institute |
| EDTA | Ethylene Diamine Tetraacetic Acid |
| FAO | Food and Agricultural Organization |
| HWE | Hardy-Weinberg Equilibrium |
| IBC | Institute of Biodiversity Conservation |
| ILRI | International Livestock Research Institute |
| mtDNA | Mitochondrial DNA |
| Na | Observed numbers of alleles |
| NCBI | National Center of Biotechnology Information |
| Ne | Effective numbers of alleles |
| PCR | Polymerase Chain Reaction |
| PIC | Polymorphic Information Content |
| QTL | Quantitative Trait Loci |
| RAPD | Random Amplified Polymorphic DNA |
| RFLP | Restriction Fragment Length Polymorphism |

| | |
|---|---|
| SNP | Single Nucleotide Polymorphism |
| SSLP | Simple Sequence Length Polymorphism |
| SSR's | Simple Sequence Repeats |
| SSTR | Simple Sequence Tandem Repeats |
| STR | Short Tandem Repeats |
| TEMED | Tetramethylethylenediamine |
| UPGMA | Unweighted Pair-Group Method with Arithmetic mean |
| UV | Ultraviolet |

## ABSTRACT

*The current study describes the genetic variability within and among five Ethiopian indigenous bovine breeds viz; Abigar, Boran, Horro, Guraghe and Sheko based on nine microsatellite markers (TGLA227, ETH3, ETH10, ETH152, ETH225, BM2113, BM1824, ILSTS049 and TGLA53). The Abigar breed represents the Sanga (cross between Bos taurus and Zebu breed) cattle breed, the Boran and Guraghe highland are grouped under Zebu breed, the Horro breed fall under Zenga (cross between Sanga and Zebu breed) cattle and the Sheko cattle belongs to humpless shorthorn (Bos taurus).*

*Blood samples were taken randomly from10 unrelated animals from each cattle breed and DNA was isolated for PCR amplification. The PCR products were resolved on eight or 10% non-denaturing polyacrylamide gels, based on the size of the products, and visualized by GelRed staining. Allelic frequencies, hetrozygosity values, polymorphic information content (PIC), genetic differentiation and genetic distance were estimated. The allelic frequencies were also tested for deviation from Hardy-Weinberg equilibrium (HWE).*

*The result of the current study indicated that the total numbers of alleles observed overall loci in Abigar, Boran, Guraghe, Horro and Sheko were 67, 65, 54, 58 and 59, respectively. The breeds showed deviation from HWE at least at one of the loci examined, except the Boran breed which was found to be at HWE for all loci studied. The overall observed and expected hetrozygosity values and PIC were 0.782±0.079, 0.847±0.050 and 0.828±0.057, respectively. The mean observed hetrozygosity value in Abigra, Boran, Guraghe, Horro and Sheko were 0.800±0.071, 0.803±0.157, 0.761±0.109, 0.781±0.133 and 0.763±0.084, respectivey, The Nei's expected hetrozygosity values in these breeds were found to be 0.820±0.036, 0.798±0.065, 0.788±0.070, 0.780±0.063 and 0.783±0.049, respectively. Generally, the genetic differentiations between the breeds were low. Moreover, non-significant (P>0.05) differentiation were noticed in the study (between Boran and Guraghe, between Guraghe and Horro and between Boran and Horro).The partitioning of genetic variability based on overall $F_{ST}$ values indicated 2.2 % of variation was due to between the breeds studied whereas the remaining (97.8%) was due to variation among individuals. The inbreeding coefficients within and among were small, 0.070±0.031and 0.090±0.27, respectively. Genetic distance based on Nei's corrected distance was found to be least (0.027) between Boran and Guraghe and highest (0.229) between Boran and Sheko. Generally, a dendrogram following UPGMA clustering generated based on Nei's genetic distance matrix agreed with the evolutionary history of the breeds studied.*

**TABLE OF CONTENTS**

# 1. ` INTRODUCTION

## 1.1 GENERAL BACKGROUND

Ethiopia stands first in the number of cattle population in Africa and third in the world. Ethiopian cattle population accounts 17% of the total cattle population in Africa (IBC, 2007). Ethiopia has diverse climatic and topographic conditions. These have contributed a lot for the evolution of different cattle breeds that can adapt to their respective ecological conditions in the country. Currently, in Ethiopia 25 indigenous cattle breeds are identified and recorded (IBC, 2007).

Domestic farm animals are crucial for food and agriculture, providing 30 to 40 percent of the agricultural sector's global economic value (FAO, 2000). According to IBC (2007), livestock in Ethiopia has contributed about 40% of the agricultural GDP, 18% of the total GDP and 19% from the export earnings.

Despite their contribution to the global economy, there is a rapid loss of genetic resource of farm animals. As reported by FAO (2000), the world loses two breeds of its valuable domestic diversity every week. The FAO global data bank for farm animal genetic resources, which contains information on 6,379 breeds from 30 mammalian and birds, 750 breeds were recorded as extinct and 32% are classified at high risk of loss and under threat of extinction. Since 1995, the number of mammalian breeds at risk of extinction has risen from 23 to 35%. There is a speculation that in 2020 more than 2000 domestic animal breeds could be lost (FAO, 2000). The report has also indicated the number of extinct cattle breeds in Africa, Europe and Near East as 23, 171, and 87, respectively.

A number of factors such as crossbreeding, breed substitution, famine, drought, war, human population growth, and disease outbreak are associated with the rapid reduction of genetic diversity of indigenous cattle populations in Africa. Among these factors, crossbreeding and breed substitution play the most significant role. For example, the rapid substitution of Ankole cattle breeds of Uganda by Holstein-Friesians breeds is believed to result in the extinction of Ankole breeds within twenty years (www.physorg.com/news.html). Okomo-Adhiambo (2002) mentioned the rapid human population growth in pastoral area is resulting in reduction of grazing

lands that in turn sped up admixtures and interbreeding among breeds. Disease epidemics potentially threaten farm animal genetic resource as a result of livestock death. For example, the 2001 epidemic of foot and mouth disease in United Kingdom has resulted in the loss of 6.5 million of sheep, cattle and pig (Anderson, 2002).

Hanotte and Jialin (2005), indicate an urgent need to document the diversity of livestock genetic resources and design appropriate strategies for conservation and sustainable use in developing countries. Conserving animal genetic diversity is important because it functions as a safe-guard against the future challenges mentioned above.

Characterization of genetic resource lays the foundation for making appropriate conservation and sustainable utilization strategies. Rege and Lipner (1992) indicated that characterization of African cattle breeds is largely based on phenotypic traits such as yield, type and morphology. However, these phenotypic traits have little power to detect genetic variation among populations and thus are not reliable to make decision on conservation and utilization. Genetic characterization based on DNA markers are the most invaluable way of getting information for making appropriate decision on conservation and sustainable utilization of farm animal genetic resources. DNA markers enable us to study variation or polymorphism at DNA level. Generally, molecular variation or polymorphism at DNA level is due to the substitution of purine by another purine or substitution of pyrimidine by another pyrimidine (transition) and the substitution of one pyrimidine by purine or vise versa (transversion). Polymorphism can also occur due to insertion or deletion of single or multiple nucleotides. It can also occur due to variation in the number of tandem repeats of repeated nucleotide sequences which results in polymorphism among individuals (Mburu and Hanotte, 2005).

Most commonly used DNA based markers include restriction fragment length polymorphism (RFLP), random amplified polymorphic DNA (RAPD), single nucleotide polymorphism (SNP), amplified fragment length polymorphism (AFLP) and microsatellite markers (SSR). Among these DNA based markers, microsatellites markers, which are sequence of varying numbers of short tandem repeated nucleotides of 1- 6 base pairs at a specific physical location in the genome, have recognized to be the most suitable markers to study variation within and between

cattle breeds. Some of the outstanding features that make microsatellites more suitable than other molecular markers in breed characterization include that they are: highly polymorphic, found abundantly in the genome, co-dominantly inherited and amenable to PCR amplification (Weber and May, 1989).

Genetic variability among five indigenous bovine breeds of Ethiopia viz., Horro, Sheko, Guraghe, Arsi and Abigar were studied using only three RAPD markers (Hassen *et al.*, 2007). However, RAPD markers are associated with a number of drawbacks such as limited ability for detection of polymorphism and inconsistent reproducibility. Moreover, their being dominant markers limit them for the differentiation of homozygotes from hetrozygotes and allele frequencies cannot be estimated. The small numbers of RAPD markers used in their study also limit detection of polymorphism among the cattle breeds. This entails further genetic diversity study of these breeds with a relatively outstanding DNA based marker to evaluate the present genetic variability among them.

### 1.2 OBJECTIVES OF THE STUDY

The current study aimed to evaluate the genetic diversity among five Ethiopian bovine breeds namely, Abigar, Boran, Guraghe highland, Horro and Sheko using microsatellite markers with the following specific objectives.

1. To measure the within genetic variability in the five indigenous bovine breeds viz., Horro, Sheko, Guraghe, Abigar and Boran using microsatellite markers.
2. To evaluate the genetic diversity and relationship among these indigenous bovine breeds using microsatellite markers.
3. To evaluate the informativeness of microsatellite markers and select those markers suitable for routine screening or analysis of indigenous cattle breeds in Ethiopia.

# 2. LITERATURE REVIEW

Breed identification and characterization are important steps towards creating a sustainable conservation and utilization strategies. Studying genetic variability within and between breeds, evaluating level of inbreeding, effective population size and possible bottlenecks are some of the practices carried out in breed characterization (Barker *et al*., 1997).

A number of genetic markers have been developed to study variation among livestock population over years that could be classified into three types as morphological, biochemical and molecular markers (Collard *et al.*, 2005). The major drawbacks of morphological (size, shape, color *etc*) and biochemical markers (blood proteins, isozymes and allozymes) are, the variability of limited age dependent markers and could also be influenced by environmental factors. In the case of biochemical markers, detection of polymorphism is based on electrophoretic separation of protein/enzymes based on their molecular weight and charge and then followed by specific staining for visualization (Barker *et al.*, 1966; Tanksley and Orton, 1983). Blood proteins such as transferrin, esterase, albumin and haemoglobin were often used to characterize farm animals (Nie *et al*., 1999; Ibeagha-Awemu *et al*., 2004).

These days, molecular markers are chosen over biochemical markers and morphological markers to study variation in farm animals as they are found relatively abundant, independent of growth and physiological state and provide a more powerful source of genetic polymorphism (Beckmann and Soller, 1986). Brief descriptions of these molecular markers used to study genetic variation in livestock are given below.

## 2.1 MOLECULAR MARKERS

### 2.1.1 RESTRICTION FRAGMENT LENGTH POLYMORPHISM (RFLP)

RFLP analysis was one of the first techniques to be widely used for detection of variation at DNA level. The technique consists of isolation of DNA, digestion of the DNA with restriction enzyme, separation of the DNA fragments with gel electrophoresis according to their size followed by hybridization using fluorescently or radioactively labeled probes (Southern, 1975).

Botstein *et al.* (1980) also stated that the difference among individuals in the length of restriction fragments is largely because of the loss of a cleavage site or formation of a new one. Insertion or deletion of certain DNA sequence can also alter the size of restriction fragments. RFLP can be used to study genetic distance, population variation, gene flow, effective population size, and analyses of parentage and relatedness. Some of the advantages of RFLP include: it gives reproducible results within and among laboratories and produces co-dominant markers. According to Collard *et al* (2005), polymorphic markers are described as co-dominant if they can distinguish between homozygotes and hetrozygotes. However, there are some drawbacks associated with RFLP analysis such as the requirement of relatively high quality and large quantity of DNA (2-10mg), labor intensive and the requirement of probes for detection. The development of PCR based RFLP analysis (PCR-RFLP) has made the technique to be less laborious with relatively small quantity of DNA and possibility of detection without use of probes. Several studies indicated the use of PCR-RFLP markers to evaluate cattle diversity (Braunschweig, 2008; Rachagani and Gupta, 2008; Curi *et al*., 2005; Amoli *et al*., 2000).

### 2.1.2 RANDOM AMPLIFIED POLYMORPHIC DNA (RAPD)

RAPD is molecular technique related to PCR and was first developed by Williams *et al* (1990). The technique utilizes the use of short arbitrary primers (10-12 bases) that bind randomly to multiple places in the genome and subsequently amplify these regions by PCR. The presence or absence of bands is used for detecting polymorphism among individuals. Even though RAPD lacks reproducibility within and among laboratories, it has been used for molecular characterization of cattle in different breeds as it is cost effective, simple and quick method as compared to other molecular techniques (Guneren *et a*., 2010; Zulu 2008; Hassen *et al*., 2007; Yu *et al*., 2004; Gwakisa *et al*., 1994).

Hassen *et al*. (2007) have reported genetic variability among five indigenous cattle breeds of Ethiopia (Horro, Arsi, Sheko, Guraghe and Abigar) using three RAPD primers. It has been reported that the RAPD markers used in the above study generated 33 loci and were useful to differentiate the breeds studied, but failed to differentiate between Guraghe Highland and Abigar. Sheko breed was found to be genetically distant from the rest of the breeds under study.

Their study has also indicated that the gene diversity within breed is much higher than between breed variation.

Guneren *et al*. (2010) also studied the genetic variability among four local cattle breeds of Turkey, namely, Anatolian Black (AB), East Anatolian Red (EAR), South Anatolian Red (SAR) and Turkish Grey Steppe (TG) using ten primers. UPGMA clustering have indicated that AB breed is more closely related to EAR breed than to SAR breed while TG breed was determined as a distinct group from other breeds. Yu *et al.* (2004) reported the genetic diversity and relationship of Yunana native cattle (DeHong cattle and DiQing) and introduced beef cattle breeds (Brahman, Simmental, MurryGrey and ShortHorn) using RAPD technique. Ten primers were used and found 83 loci of which 75% of the fragments observed to be polymorphic. The result depicted that Yunnnan DeHong cattle breed is closely related to the Brahman (*Bos indicus*), and the Yunnan DiQing cattle breed is closely related to the Simmental, ShortHorn, and MurryGrey (*Bos taurus*) breeds (Yu *et al*., 2004). Kantanen *et al*. (1995) studied the application of RAPD markers to detect genetic variation in five Finnish cattle. Out of 11 primers used, three of them showed polymorphism but failed to show significant differentiation among the Finnish breeds. Zulu (2008) characterized four indigenous cattle breeds of Zambia, namely, Angoni, Barotse, Tonga, and Baila using 10 arbitrary RAPD primers. The result indicated an average of 7.1 bands per primer with an average of 68% polymorphism. Breed differentiation was highest between the Tonga and the Barotse and lowest between the Tonga and the Baila breeds.

### 2.1.3 MITOCHONDRIAL DNA (mtDNA) MARKERS

It is a known fact that mitochondria are the only source of mammalian cytoplasmic genetic information. Wallace (1986) stated that mammalian mitochondrial DNA is a small circular DNA with size of 15-25 kb. Each mammalian mitochondrion DNA contains 13 protein-coding genes, 2 ribosomal RNA (rRNA) genes and 22 transfer RNA (tRNA) genes (Gray, 1989). Each mitochondrial DNA molecule contains a non-coding region called displacement loop (D loop) that regulates initiation of replication and transcription (Clayton, 1991). Cattle mtDNA codes for rRNA and tRNA required for the synthesis of intra-mitochondrial proteins, subunits of mitochondrial electron transport system and ATP synthesis complex (Brown *et al.*, 1988).

A number of features make mtDNA a useful genetic marker in population genetics studies and phylogenetics; these include maternal inheritance, lack of recombination and fast rate of evolution (Avise, 1991). The rate of evolution of the mtDNA is different for different parts of the molecule. The rRNA genes evolve approximately 100-fold quicker than their nuclear counterparts and the D-loop evolves 5-times faster than the rest of the mtDNA (Avise, 1991).

Dadi *et al*. (2008) studied variation in mitochondrial DNA and maternal genetic ancestry of 10 Ethiopian cattle populations. The result indicated that all mitochondrial DNA sequences from the populations converged into a maternal lineage corresponds to African *Bos taurus* cattle. The result has also shown none of the haplotypes were *Bos indicus* type. The rare zebu mtDNA might have lost due to destructive droughts and renderpest epidemics coupled with initial male led introgression of Zebu during its first arrival in African continent.

### 2.1.4 SINGLE NUCLEOTIDE POLYMORPHISMS (SNPs)

SNPs are variations in a DNA sequence that occur when a single nucleotide in the sequence is altered at least in one percent of the population (Syvanen, 2001). SNPs are the most frequent type of variation found in DNA (Brookes, 1999). SNPs are primarily detected by DNA sequencing, but RFLP and primer extension with allele specific probes are also employed for SNPs analysis (Syvanen, 2001). They are the most abundant molecular marker systems ever known in the genome with high genetic polymorphism (Gupta *et al.*, 2001). SNPs are highly reproducible and very informative. However, they are relatively expensive and require prior knowledge of sequence. A large number of SNPs have already been developed in human but the limited number of SNPs for many species creates a hurdle to their application in population genetic studies (Gupta *et al,* 2001). Currently, several SNPs are also characterized for *Bos taurus* and *B. indicus* and deposited in gene banks (http://www.ncbi.nlm.nih.gov/sites/entrez).

### 2.1.5 AMPLIFIED FRAGMENT LENGTH POLYMORPHISM (AFLP)

AFLP is a molecular technique that combines restriction based RFLP marker and PCR based RAPD marker. The technique first uses the restriction enzymes to digest or cut genomic DNA, followed by ligation of double-stranded adaptors to the sticky ends of the restriction to generate

template DAN (Vos *et al*., 1995). The nucleotide sequence of the adapters and the adjacent restriction sites serve as primer binding sites during selective PCR amplification of some of these fragments. The amplified fragments are separated by electrophoresis on polyacrylamide gels and visualized either through autoradiography or fluorescence methods. The major advantage of AFLP is its ability to detect large numbers of polymorphism in a population. In addition it has higher reproducibility than RAPD (Vos *et al*., 1995). However, the technique is expensive, labour intensive, technically complex and inherited as dominant markers. AFLP is used in DNA finger printing and in cattle population genetic studies (Ajmone-Marsan *et al.*, 2002).

### 2.1.6 MICROSATELLITE MARKERS

Microsatellite is a sequence of short tandem repeated segments of 1-6 base pairs at a unique physical location in the genome. Polymorphism among individuals occurs due to variation on the number of repeats. The primary mutational mechanism leading to changes in microsatellite length is DNA polymerase template slippage caused by mismatches between DNA strands while being replicated (Kruglyak *et al*., 1998). During replication of a repetitive region, DNA strands may dissociate and then reassociate incorrectly. Renewed replication in this misaligned state leads to insertion or deletion of repeat units, thus altering allele length. Microsatellite loci are also known as simple sequence repeats (SSR's), simple sequence tandem repeats (SSTR) and simple sequence length polymorphism (SSLP).

Several important characters make microsatellites as informative marker to study variation within and among populations. Microsatellites are variable and exhibit a high level of allelic variation, co-dominantly inherited and thus applied to study genetic variability, population structure and gene flow among populations. Microsatellites have different applications such as forensics, disease diagnosis and population genetic studies (Kenneth *et al*., Loudianos et al., 1994; Gras *et al*., 1999; Li *et al*., 2008). Currently, microsatellite markers are found to be suitable molecular markers to study cattle genetic resources (FAO, 2005). Various studies have also confirmed this fact (Hanotte *et al*., 2000; Hanotte *et al*., 2002; Guo *et al*., 2005; Li *et al*., 2008; Cervini *et al*., 2006).

Dadi *et al*. (2008) also recently evaluated genetic diversity, population structure and degree of admixture of 10 indigenous bovine breeds of Ethiopia based on 30 microsatellite markers. This finding indicated that Ethiopian indigenous cattle populations have high within genetic diversity but low genetic structure. Table1 summarizes the most commonly used markers.

Table 1: Comparison of most commonly used molecular (DNA) markers in population genetics

| **Feature** | **RFLPs** | **RAPDs** | **AFLPs** | **SSRs** | **SNPs** |
|---|---|---|---|---|---|
| DNA quality | high | high | moderate | moderate | high |
| PCR based | no | yes | yes | yes | yes |
| Frequency of occurrence in genome | low | limited | limited | high | very high |
| Polymorphisms | low | Low | high | high | moderate |
| Codominant/dominant | codminant | dominant | dominant | codominant | biallelic |
| Ease of use | not easy | easy | easy | easy | easy |
| Amenable to Automation | low | moderate | moderate | high | high |
| Reproducibility | high | unreliable | high | high | high |
| Development cost | low | low | moderate | high | high |
| Cost per analysis | high | low | moderate | low | low |

Source: Tang (2008)

### 2.2 MEASURING GENETIC VARIABILITY

As mentioned above, measuring the level of genetic variability within and among breeds is an essential step to plan strategies for conservation and sustainable development of breeds. There are various parameters developed so far to measure genetic variability within and among breeds. It is also possible to evaluate genetic variability of a given species through studying genetic differentiation and population structure. This measure of genetic variability often gives us a picture about the level of gene flow between subpopulations. Generally, gene flow increases genetic variability within population but decreases genetic variability between subpopulations,

i.e., it decreases the level of genetic differentiation between subpopulations (Nei, 1973; Slatikin and Barton, 1989).

Total variation over the entire set of populations and within population variation are calculated using some parameters such as % polymorphic loci (P), allele per locus (Ap), effective number of alleles ($A_e$), expected hetrozygosity (He) and observed hetrozygosity (Ho) (Nei, 1973 and 1978; Hartl and Clark, 1989). Testing for deviation from HWE (per population) is also carried out as it tells about those primary forces viz., natural selection, mutation, genetic drift, nonrandom mating and genetic migration that derive evolutionary change. Among population variation is usually investigated using Writght's F-statistics (Wright 1951, 1965) genetic distance and phylogenetic or tree building approach. The following section briefly describes some important parameters to evaluate genetic variability in a population.

### 2.2.1 HARDY WEINBERG EQUILIBRIUM (HWE)

The Hardy Weinberg equilibrium states that for independently segregating alleles, the allelic and genotypic frequencies remain unchanged from generation to generation for a very large, randomly mating population in the absence of natural selection, mutation, gene flow and immigration. It is considered as a simple model that serves as a starting point for examining the genetic structure of populations. The deviation from HWE provides a lot of information about the population under study. It tells non random mating, mutation, natural selection and gene flow as these are the factors that cause deviation from HWE. Non random mating disrupts allelic frequencies by increasing the frequencies of certain alleles. Mutation can introduce new alleles or eliminates the existing alleles in a population and hence result in deviation from HWE. Natural selection also disrupts allelic frequencies, especially for those genes related to fitness. Genotyping errors, particularly in microsatellite markers, can also be the cause for deviation from HWE. Sometimes homozygotes can be considered as hetrozygotes due to stutter bands leading to excess hetrozygosity and thus deviation from Hardy-Weinberg proportion. Likewise hetrozygotes can also be genotyped as homozygotes due to allelic drop out (Chakarborty, 1992; Rehman and Khan, 2009).

*TESTING HARDY-WEINBERG EQUILIBRIUM*

When the observed and expected genotypic frequencies at a particular locus are not statistically different for a given population, then the population is said to be at HWE for that specific locus. There are different statistical tests used to test the allelic frequencies for fit to HWE. However, they can be grouped into three categories namely asymptotic, (Chi-square test and G-test, also known as log-likelihood test or a likelihood ratio test), exact (complete enumeration) and approximation (Markov Chain Monte Carlo). If the genotypic counts and/or the sample size are small, the asymptotic tests are not preferred as the distributions of the test statistics do not fit the chi-square distribution. In these situations, the exact test of HWE is chosen over the asymptotic tests (McDonald, 2009; Guo and Thompson, 1992).

### 2.2.2 OBSERVED NUMBER OF ALLELES ($N_a$)

The observed number of alleles (Na) per locus is total number of alleles that have been observed in the population under study. It is one of the basic parameter presented in population genetics studies. It depicts allelic richness of a locus for multi-allelic system such as microsatellites (Weir, 1996).

### 2.2.3 EFFECTIVE ($A_e$) NUMBER OF ALLELES

Effective number of alleles ($A_e$) is a measure of the number of equally frequent alleles it would take to achieve a given level of genetic diversity. The effective number of alleles at a given locus is calculated as the reciprocal of the expected homozygosity. The formula is:

$A_e = \frac{1}{r} \sum_{j=1}^{r} \frac{1}{1-Dj}$ where, $D_j$ is the expected hetrozygosity of the $j^{th}$ of the r loci.

The effective number of alleles will have the highest value (equal to the actual number of alleles) at the highest value of expected hetrozygosity *i.e.* when the frequencies of all alleles at that particular locus are equal. Thus, the effective number of alleles tells us whether the alleles at a given locus have the same or different allelic frequencies (Hartl and Clark, 1989, Nassiry *et al*., 2009).

### 2.2.4 EXPECTED HETROZYGOSITY (He)

Expected hetrozygosity (also known as gene diversity) is one of the parameters calculated to study genetic variation in natural population. It is the probability of non-identity of two randomly chosen genes in a population (Nei, 1973). It can also be viewed as the probability that an individual will be heterozygous at a given locus. It provides information on the structure and history of a population. For example, very low value of hetrozygosity might indicate the effect of small population sizes. Its value ranges from zero (no hetrozygosity) to nearly one (highest when allelic frequencies are equal) (Nei, 1973, 1978). For *k* number of alleles at a single locus, the expected hetrozygosity is calculated as:

$$He = 1 - \sum_{i=1}^{k} Pi^2$$

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

### 2.2.5 OBSERVED HETROZYGOSITIES (Ho)

This parameter is calculated first by determining the proportions of genes that are heterozygous and the number of hetrozygote individuals at a given locus. The formula is given by:

$$Ho = \frac{\text{number of hetrozygotes at a locus}}{\text{Total number of individual studied}}$$

This value is usually compared with the expected hetrozygosity to test for fit to HWE (Nassiry *et al*., 2009).

### 2.2.6 ALLELIC FREQUENCY

Allelic frequency is defined as a measure of the commonness of a specific allele in a population. Its value ranges from zero (no individual carries that allele in the population) to one (complete fixation of the allele *i.e.* all individuals in the population are homozygous for that allele). Allelic frequency is computed as:

$$P_i = \frac{ni}{2N}$$

Where $n_i$ is the number of times that the $i^{th}$ allele is observed and N is the total number of individuals in a given population (Weaver and Hedrick, 1997).

### 2.2.7 F-STATISTICS

F-statistics ($F_{IS}$, $F_{ST}$ and $F_{IT}$), also called fixation indexes, were coined by S Wright (Wright, 1951) to describe the properties of subdivided populations. $F_{IT}$ is correlation between two uniting gametes with respect to the whole population and $F_{IS}$ is the correlation between two uniting gametes within subpopulations. $F_{IS}$ can be viewed as a measure of inbreeding within subpopulations whereas $F_{IT}$ as a measure of total level of inbreeding. $F_{ST}$ is defined as the correlation between random gametes from different individuals within subpopulations with respect to the total population. It is referred as a measure of level of differentiation of subpopulations. Wright's F-statics are applicable to any population if there are only two alleles at a locus. However, Nei (1973) proposed a new way of measuring genetic differentiation between subpopulations irrespective of the number of alleles at a locus or to the patterns of evolutionary forces based on identities of two randomly chosen genes within and between populations. Weir and Cockerham (1984) proposed a variance approach to estimate F-statistics. They described a measure of $\theta_{ST}$ (an equivalent to $F_{ST}$) that estimate the correlation of pairs of alleles between individuals within subpopulations through the analysis of the partitioning of variance of allele frequencies. It is given by:

$$\theta_{ST} = \frac{\sum a_i}{\sum(a_i+b_i+c_i)}$$

Where, $a_i$ is variance of allele frequencies between population, bi is variance of allele frequencies within population and $c_i$ is variance of allele frequencies within individuals.

### 2.2.8 GENETIC DISTANCE

Analysis of genetic distance reveals relatedness among populations and is useful for reconstructing relationship among populations and individuals as well. It tells genetic similarities and dissimilarities between two populations or individuals, and can be used for characterization of different breeds and for evaluation of the change in variation in species overtime (Naqvi,

2007). In the analysis of genetic distance, the genetic difference between populations is assessed based on the difference between allele frequencies distributions at several loci or based on allele size distributions (Laval *et al*., 2002).

A number of genetic distance measures have been developed for the analysis of molecular marker data and could be classified into two main categories, i.e., the one with no biological assumptions, also called geometric distances like Cavalli-Sfroza chord distance (Cavalli-Sforza and Edwards, 1967) and the others are with biological assumptions or models like Nei's (1972, 1978), Reynold's distance (Reynold *et al*., 1983) and Goldstein's distance (Goldstein *et al*., 1995) also called delta mu squared ($\delta\mu^2$). Distance measures with biological assumptions are based on the evolutionary forces that could result in allelic variation. The two evolutionary forces, viz. mutation and genetic drift are the significant once that are used to make assumptions or models. Natural selection, one of the evolutionary forces, is not usually used to make assumptions because we assume that we are using markers that are not subject to natural selection.

Under the genetic distance measures with biological assumptions, there are two basic models namely the Infinite Allele model (IAM) and the Stepwise Mutation model (SMM). The assumption under the IAM model is that new mutant alleles are always different from the existing once in the population (Kimura and Crow, 1964). This model is believed to work with the classical genetic markers like allozymes. Nei's genetic distance measurements (1972, 1978 and 1983) and Reynold's distance are based on IAM. However, SMM assumes that the entire sequence of allelic states can be expressed by integers and that, if an allele changes a state by mutation, it moves either one step in the positive direction or one step in the negative direction in the allele space (Kimura and Ohta, 1978). The majority of microsatellite loci are believed to show a mutation in stepwise fashion. Goldestein's distance ($\delta\mu^2$) is one of the genetic distance measurements specifically designed for microsatellite markers. One of the information ignored by the IAM is the difference in repeat score between alleles which carries information about the amount of time that has passed since they share a common ancestral allele. However, Nei and Takezaki (1996) demonstrated that distances based on IAM perform well for phylogenetic tree

reconstruction as compared to those distances based on SMM for microsatellite data. Some distance measurements are briefly discussed below.

Nei's standard genetic distance (Nei, 1972) is one of the most widely used measures of genetic distance. It is based on allele frequencies and is given by:

$D_{ST} = -\ln[J_{XY}/\sqrt{JxJy}\,]$

Where Jx, Jy, and Jxy, are sample gene identities which are the averages of $\sum x_i^2$ (sample gene identity in population X), $\sum y_i^2$ (sample gene identity in population Y) and $\sum x_i y_i$ (sample gene identity in both population X and Y), respectively. Populations with equal gene frequencies, regardless of their respective genotype frequencies, will have genetic distance of zero, since ln(1) is equal to zero. This genetic distance is also called Nei's standard genetic distance without sample size corrected.

Nei modified the $D_{ST}$ with sample size correction and this distance is known as Nei's standard genetic distance with sample size correction or the Nei's unbiased genetic distance (Nei, 1978). The unbiased estimators for $J_X$ ($J'x$ ) and $J_Y$ ($J'y$) are given by:

$$J'x = \frac{1}{r}\left(\sum_{j}^{r}\left(n_{xj}\ \sum_{i}^{mj} x_{ij}^2\ - 1\right)/(n_{xj} - 1)\right)$$

$$J'y = \frac{1}{r}\left(\sum_{j}^{r}\left(n_{yj}\ \sum_{i}^{mj} y_{ij}^2\ - 1\right)/(n_{yj} - 1)\right)$$

Where, $n_{xj}$ and $n_{yj}$ are the number of chromosomes examined at the $j^{th}$ locus for populations X and Y. Thus the unbiased estimate of $D_{ST}$ is calculated by substituting $J_X$ and $J_Y$ by $J'_X$ and $J'_Y$, respectively.

Cavalli-Sforza chord distance, Dc, (Cavalli-Sforza and Edwards, 1967) is one of the early measures of genetic distance. They defined Dc as the chord distance between two populations on the surface of multidimensional hyperspare (a shere with greater than three dimensional) using

allele frequencies. For two populations X and Y examined at $j^{th}$ locus with alleles i = 1 to m, the formula is given by:

$$Dc = (2/\pi r)\sum_j^r \sqrt{2(1 - cos\theta)}$$

Where, $cos\theta = \sum_i^m \sqrt{x_{ij}y_{ij}}$ and $x_{ij}$ and $y_{ij}$ are allele frequencies for population X and Y at the jth locus, respectively and r is the number of loci studied.

Goldstein et al. (1995) suggested that mutation tends to change allelic sizes by a small amount in microsatellite loci. They argued that though the distributions of allele size do not fit the expectations of a strict stepwise mutation model, the infinite allele model is less applicable implying that distances based on IAM may not be suitable with microsatellite data. The Goldstein's distance ($\delta\mu^2$) is given by:

$$\delta\mu^2 = \sum_j^r (\mu x_j - \mu y_j)^2/r$$

where, $\mu x_j = \sum_i ix_{ij}$ and $\mu y_j = \sum_i iy_{ij}$ are average allelic states at the $j^{th}$ locus and xij and yij are the frequencies of the allele in state at the $j^{th}$ locus in populations X and Y, respectively.
However, due to large variance in allelic size difference, this measure of genetic distance has less efficiency in making tree topology for constructing phylogenetic trees (Takezaki and Nei, 1996).

## 2.3 MOLECULAR CHARACTERIZATION OF BOVINE BREEDS USING MICROSATELLITE MARKERS

With the invention of PCR technique (Mullis *et al*., 1986), microsatellites became a marker of choice for genetic linkage map construction (Ott, 1991). These DNA markers are extremely abundant, highly polymorphic and easy to assay through PCR and gel electrophoresis (Ott 1991, Weber and May 1989). A number of microsatellite markers were characterized from cattle genome that were used for molecular characterization and construction of genetic linkage maps (Bishop *et al*., 1994, Barendse *et al*., 1994).

Kemp *et al*. (1995) described a panel of 81 polymorphic bovine microsatellite markers along with 16 previously reported markers. The report has also indicated that 18 of the characterized markers were polymorphic in sheep and goat. Moore *et al*. (1992) reported 13 bovine microsatellite markers which were extracted from EMBL and GENEBANK data base. The number of alleles range from 1 to 14 with hetrozygosities ranging from 15.8% to 100%.

A total of 25 microsatellite markers were also used to characterize two Austrian cattle breeds (Carinthian Blond and Waldviertler Blond) and one Hungarian cattle breed (Hungarian Grey) (Manatrinon *et al*., 2008). A total of 213 alleles were detected across all loci and 54 of them were recorded as private alleles. The number of alleles per locus ranged from 4 (INRA005) to 14 (TGLA53) with means of 8.52 ± 2.52. The result for exact test of HWE showed that INRA32 locus was not at equilibrium ($P<0.01$) in Hungarian Grey and two loci (INRA23 and INRA32) were not at HWE ($P<0.05$) in Waldviertler Blond. The observed and expected hetrozygosities across the breeds ranged from 0.658 (in Hungarian Grey) to 0.675(in Carinthian Blond) and 0.631 (in Waldviertler Blond) to 0.679 (in Hungarian Grey), respectively. The genetic variation because of between breeds difference was 6% and the remaining 94% was due to differences among individuals. The report has also indicated that the exact test for population differentiation based on allele frequency variation were significantly different from each other ($P<0.001$).

Sun *et al*. (2007) evaluated the genetic polymorphism of Chinese local cattle (Quinchi cattle) using 12 microsatellite markers. The number of alleles observed range from 13 (INRA005) to 33 (HEL13) giving a mean number of 21 alleles per locus. Mean observed hetrozygosity and expected hetrozygosity were 0.9117 and 0.9047, respectively. Polymorphism information content values range from 0.7653 (*INRA005*) to 0.9420 (*HEL13*). The mean fixation index was -0.0076 depicting deviation from HWE were not significant.

Rehman and Khan (2009) studied the genetic diversity of Hariana and Hissar cattle from Pakistan using 27 bovine microsatellite markers. A total of 128 alleles were observed across the 27 loci studied with an average of 4.59 and 4.37 alleles per locus in Hiriana and Hissar cattle,

respectively. The markers showed PIC values of 0.749 in Hiriana and 0.719 in Hissar. The mean observed (Ho) and expected hetrozygosity (He) were 0.51 and 0.67 in Hariana and 0.47 and 0.63 in Hissar breeds, respectively. Significant hetrozygosity deficit ($F_{IS}$) was observed in both breeds ranging from 25.2% in Hariana to 25.9% in Hissar. The study has also indicated that the breeds are genetically different enough as separate breeds.

Karthickeyan et al (2009) used 25 bovine microsatellites to characterize Kangayama cattle breeds of Tamilnadu (India). The mean number of alleles in the breed was 4.04± 0.09 with a range of 2 to 6 alleles. The mean $F_{IS}$ value (-0.084) suggested excess hetrozygotes in the population.

Machado *et al*. (2003) studied the genetic diversity of four cattle breeds of Brazil (Gyr, Nellore, Guzerat and Holstein) using nine microsatellite markers. A total of 64 alleles scored in all breeds. The average number of alleles per locus was 7.11 ± 3.21. Hetrozygosity deficit was observed which might be due to a high level of endogamy among the animals sampled within each breed. UPGMA clustering using Nei's genetic distance has shown Nellore and Guzerat breeds are more closely related than the rest of the breeds studied.

Steigleder *et al*. (2004) evaluated the genetic diversity of Brazilian Creole cattle based on 14 microsatellite loci. The number of observed alleles range from 4 (BMS3004) to 13 (RM088). Most loci deviated from HWE except IDVGA51 though there is no indication of inbreeding in the population. The expected and observed Hetrozygosities range from 0.51 to 0.99 and 0.59 to 0.89, respectively. The PIC varies from 0.55 (BM3004) to 0.87 (TGLA227).

Metta et *al*. (2004) characterized two Indian cattle breeds namely Ongole and Deoni using 5 di- and 5 tri- nucleotide repeat loci. The di-nucleotide repeats were more polymorphic (100%) than the tri-nucleotide repeats which is 60%. The average hetrozygosity value was 0.46 ±0.1 and 0.50 ±0.1 in Ongole and Deoni breeds, respectively. Moderate level of genetic differentiation was observed with $F_{ST}$ value of 0.117.

Hansen *et al*. (2002) studied the genetic diversity among Canadienne, Brown Swiss, Holstein and Jersey cattle breeds of Canada using 15 microsatellite markers. The average number of alleles per locus for all of the microsatellites was 6.3 ± 0.64 for Canadienne, 6.3 ± 0.49 for Brown Swiss, 4.9 ± 0.47 for Jersey, and 6.1 ± 0.49 for Holstein cattle. Private alleles were also reported for each breed. Exact test of HWE across all loci showed that 13 loci were at HWE; the exceptions were BM2113 and BM6501. Nei's standard genetic distance ranged from 0.156 to 0.427. The lowest genetic distance was found between Canadienne and Holstein (0.156). The Brown Swiss and Jersey were found to be distantly related. Goldstein's genetic distance (Goldstein *et al*., 1995) measurement indicated that the Brown Swiss breed was more closely related to Holstein (1.301); Canadienne breed was more closely related to the Holstein breed (3.136) followed by Brown Swiss (3.385). The Jersey breed was found to be distantly related to Canadienne (3.717) and Brown Swiss (3.672). Pair-wise $F_{ST}$ values computed following the principles of drift only model (Raynolds *et al*., 1983) ranged from 0.079 (between Canadienne and Holstein) to 0.190 (between Jersey and Brow Swiss).

A total of 125 alleles were observed in characterization of five Romanian cattle breeds namely; Romanian Spotted, Romania Black Spotted, Romanian Brown, Montbeliarde and Romanian Grey Steppe based on 11 microsatellite loci (Georgescu *et al*., 2009). All loci were found to be polymorphic and the number of alleles range between 4 and 12. The observed hetrozygosity ranged from 0.593 (± 0.163) in Romanian Spotted breed to 0.690 (± 0.140) in Romanian Brown. The expected hetrozygosity ranged from 0.711 (± 0.127) in Romanian Spotted breed to 0.778 (± 0.083) in Romanian Black Spotted breeds. An exact test of HWE showed that 9 loci were at HWE when pooled across all breeds. However all breeds were at HWE across all loci. The overall $F_{ST}$ value was found to be 0.07 which indicate less differentiation among breeds. Thus 7% of the total genetic variation was due to between breed differences and the remaining 93% was because of differences among individuals. The overall $F_{IS}$ and $F_{IT}$ values across all loci were 0.151 and 0.21, respectively. The $F_{IS}$ values range between -0.04 at ETH225 locus to 0.593 at TGLA53 locus. The results of Reynolds's genetic distance (Reynolds *et al*., 1983) ranged from

0.056757 (between Montheliarde and Romanian Spotted) to 0.131480 (between Romanian Brown and Romanian Spotted).

One advantage of microsatellite markers is their ability to be used in paternity testing. Stevanovic *et al*. (2010) used 11 microsatellite loci (TGLA227, BM2113, TGLA53, ETH10, SPS115, TGLA126, TGLA122, INRA023, ETH3, ETH225, BM1824) to evaluate their use for paternity testing in Yugoslav Pied cattle population in Serbia. These microsatellite loci were found to be suitable for paternity testing in Yugoslav Pied cattle population in Serbia. The mean number of alleles per locus was found to be 8.723. The most informative loci were TGLA53 (14 alleles, PIC = 0.88), TGLA227 (11 alleles, PIC = 0.82), INRA023 (11 alleles, PIC = 0.86) and BM2113 (9 alleles, PIC = 0.80).

Okomo-Adhiambo (2002) reported genetic diversity among seven east African cattle breeds namely Abigar, Danakil, Sheko, Arado, Kenyan Boran, Kavirondo Zebu and Kilimanjaro Zebu using 18 autosomal microsatellite markers. They used Sahiwal, N'Dama and Holstein Friesian as reference breeds. Mean observed hetrozygosity ranged from 0.511 (N'Dama) to 0.660 (Holstein Friesian) and the mean number of alleles ranged from 4.3 (N'Dama) to 7.7 (Kenyan Boran). Eight loci showed deviation from HWE at Kenyan Boran whereas Arado breed was at HWE for all studied loci. Nei's standard genetic distance between breeds ranged from 0.023±0.009 (Danakil and Abigar) to 0.868±0.200 (N'Dama and Sahiwal).

The genetic diversity of eight British cattle breeds was quantified based on 30 microsatellite loci (Wiener *et al*., 2004). The mean observed and expected hetrozygosity values ranged from 0.59 (Jersey) to 0.67 (Ayrshire) and 0.56 (Highland breed), respectively. Private alleles for each breed were reported where Friesian breeds had the most private alleles (10) and Highland and Dexter had the least (3). The genetic variation because of between breeds difference was 13% and 87% was due to within breed variation. Their study also showed that the relationship between breeds was not in match with the geofigureical origin of the breeds.

Canon *et al*. (2001) investigated the genetic diversity of 18 European local breeds (from Spain, France, and Portugal) using 16 microsatellite primers. A total of 173 alleles were observed across all loci. The mean number of alleles per locus per breed was 6.5. Observed and expected heterozygosities per breed ranged from 0.54 (Pirenaica) to 0.72 (Barrosã), and from 0.61 (Aubrac) to 0.71 (Asturiana de Montaña, Barrosã, Morucha and Sayaguesa), respectively. Moreover, 7% of the total genetic variability was due to differences among breeds while the remaining 93% corresponded to differences within the breeds.

Czernekova *et al*. (2006) studied the genetic distance of central European cattle breeds namely; Czech Red cattle, German Red, Czech Pied or Czech Simmental, Polish Red, Czech Black and White, and German Black and White cattle breeds using thirteen microsatellite and five protein markers (Prolactin, Casein kappa, Lactoglobulin beta, Growth hormone 1, Pituitary growth factor). The protein loci were genotyped using PCR/RFLP method. The average hetrozygosities for microsatellite and protein loci ranged from 0.403 (Czech Red) to 0.506 (Czech Simmental) and 0.272 (German Red) to 0.434 (Czech Red), respectively. The lowest value of genetic distance (Nei 1983) based on microsatellite data was between Czech and German Black and White breeds (0.0210) and the highest was between German Red and Czech Black and White (0.1101). However, the genetic distance based on the coding loci the lowest genetic distance was observed between German Red and Polish Red (0.0084) and the highest was between Czech Red and German Red (0.0434).

Genetic polymorphism of 423 unrelated males from 25 indigenous cattle populations representing the three major groups of Chinese cattle (northern, central and southern groups) was analyzed based on two Y-chromosome microsatellite markers (UMN2404 and UMN0103). Taurine and indicine specific alleles were observed at each locus. The frequency of taurine alleles was predominant in northern cattle breeds while indicine alleles were predominant in the southern group. The geographicalcal distribution of these two types of alleles reveals the declining south to north and east to west gradient of male indicine introgression in China (Cai *et al*., 2006).

Genetic diversity, introgression and relationship among nine African *Bos indicus* and three *Bos taurus* cattle breeds of Cameroon and Nigeria were studied at 16 microsatellite, 7 milk protein and 5 blood protein polymorphic loci (Ibeagha-Awemu *et al*., 2004). Three European and two Indian cattle breeds were used to evaluate the level of relationship with the African cattle breeds based on 13 microsatellite markers. Overall, 184 microsatellite, 21 blood protein and 20 milk protein alleles were detected in the study. The mean observed and expected hetrozygosities at the microsatellite loci across all *Bos indicus* breeds ranged from 0.652 (Wadara) to 0.697 (Red Bororo) and 0.703 (Ngaoundere) to 0.744 (White Fulani), respectively. In the *Bos taurus* breeds, these values were in the range of 0.506 (Muturu) to 0.605 (N'Dama) and 0.512 (Muturu) to 0.656 (Namchi), respectively. The result revealed that the level of Indian Zebu genetic admixiture proportion in the African zebu ranged from 58.1% (Ngauondere) to 74% (Nigerian Red Bororo). Namchi, African taurine breed, was found to be highly introgressed (30.2%) by the Indian Zebu genes. The European taurine influence was also recognized in the African Zebu (1.5 to 37.5%) and taurine breeds (4.5 to 50.1%).

A microsatellite genetic marker for the bovine prolactin gene was used to characterize seven breeds of North America namely; Simbrah, Simental, Santa Gertrudis, American Breed, Salers, Parthenais, and Hotlander (Yim et al., 2000). Three marker alleles; A (156bp), B (159bp) and C (162bp), were observed with five genotypes (no AC type). Genotype BB was predominant with genotypic frequency of 86.2%. Genotypes AB and BC were intermediate in frequencies at 9.0% and % and 3.0%, respectively. Allele B had the highest frequency across all breeds ranging from 0.75 (in American breeds) to 1.00 (in Salers). Allele C was not observed in Salers and Hotlander.

Maudet *et al*. (2002) studied the genetic diversity of six French and Holstein cattle breeds based on 23 microsatellite loci. A total of 215 alleles were detected in the study. The mean number of alleles per locus across the breeds ranged from 5.61 in Villard de Lans to 6.52 in Abodance. The result showed that no locus had a significant deviation ($P< 0.05$) from HWE in more than three breeds. The mean expected and observed hetrozygosities 0.65 to 0.699 and 0.643 to 0.693,

respectively. Pair-wise genetic differentiation indicated that Abodance and Holstein were the most differentiated pairs of breeds with $F_{ST}$ value of 0.112. The least genetic differentiation was observed between Montbeliarde and Villard deLans with $F_{ST}$ value of 0.043.

# 3. MATERIALS AND METHODS

## 3.1 EXPERIMENTAL MATERIALS

This study was conducted on Abigar, Boran, Guraghe, Horro and Sheko breeds of Ethiopian cattle. Blood samples of Abigar, Guraghe, Horro and Sheko were brought from Tollay cattle breeding station of the International Livestock Research Institute (ILRI) and Ethiopian Institute of Agricultural Research (EIAR) joint project. Samples for Boran were taken from Debrezeit research center of the Ethiopian Institute of Agricultural Research (EIAR). Phenotypic descriptions of the breeds are briefly presented in Appendix 1.

## 3.2 BLOOD COLLECTION AND EXTRACTION OF DNA

Blood was collected at random from ten unrelated animals from each cattle breed by veno-puncture with 10ml vaccutainer tube containing EDTA as anticoagulant. The blood was gently mixed with the EDTA and placed into an ice box containing ice and brought to the laboratory, where it was stored at -20$^0$C until DNA was extracted. Extraction of genomic DNA was carried out according to Bruford *et al.* (1992).

Briefly, blood samples were first thawed at room temperature and about 5ml blood was poured into 50ml centrifuge tube. Exactly 15ml of ice cold EL buffer (0.155M NH4Cl; 10mM KHCO3; 1mM EDTA, pH 7.4) was added into the centrifuging tube and blood samples were mixed gently by inversion. The solution was placed on ice for 15 min and mixed occasionally (3 to 4 times). Then it was centrifuged at 2550 rpm for exactly 10 min. The supernatant was removed carefully and the pellet was re-suspended with the EL buffer and centrifuged again at 2550 rpm. This step was repeated if any sign of haemoglobin was seen in the pellet. After discarding the supernatant, the pellet was re-suspended in 3ml KL buffer (10mM Tris; 2mM EDTA; 0.4M NaCl, pH 5.2). Exactly 100 ul of Proteinase K (20mg/ml) and 150 ul of 20% SDS were added and mixed very carefully until the solution becomes viscous. The mixture was then incubated overnight in an oven set at 37$^0$C. Approximately 1.5ml of 6M NaCl was added and mixed by shaking for about 15 sec and then centrifuged at 3700 rpm for exactly 15 min. The supernatant (0.6 ml) was transferred into 2ml eppendorf tube and 1.2 ml of absolute ethanol was added and the solution was mixed by inversion until the genomic DNA was precipitated. The precipitate was then rinsed

with 0.8 ml of 70% ethanol in 1.5 ml eppendorf tube by inverting the solution for 5 minutes and ethanol was carefully poured off using tissue paper. The DNA was allowed to air dry for 30 minutes. Finally, the extracted DNA was allowed to dissolve in 500 ul TE buffer (10mM Tris-HCl; 0.1mM EDTA, pH 7.4) overnight and stored at $4^0$C.

Table 2: List of cattle samples and source of blood samples

| No. | Breed type | Sources of Blood samples | Remark |
|---|---|---|---|
| 1 | Abigar | ILRI-EIAR Cattle Breeding station (Tollay) | Sanga |
| 2 | Boran | EIAR Cattle Breeding station (Debrezeit) | Large east African Zebu |
| 3 | Guraghe Highland | ILRI-EIAR Cattle Breeding station (Tollay) | Small east African Zebu |
| 4 | Horro | ILRI-EIAR Cattle Breeding station (Tollay) | Zenga |
| 5 | Sheko | ILRI-EIAR Cattle Breeding station (Tollay) | African taurine |

### 3.3 GENOMIC DNA QUANTITY AND QUALITY MEASURMENTS

The quantities of the extracted DNA were measured by UV spectrophotometry (GeneQuant) and quality of DNA were checked by UV spectrophotometry (GeneQuant) and agarose gel electrophoresis, respectively. Then 10 ul DNA samples were dissolved in 990 ul distilled deionized and autoclaved water. Optical density (OD) at 260nm and 280nm were measured and the ratio of 260: 280nm readings were computed. DNA samples with 1.6 to 2.00 were further analyzed by agarose gel electrophoresis to check their qualities.

Gel electrophoresis was carried out with 0.6 % agarose in 1X TAE buffer (pH 8.0). Agarose gel with 40 ml solution was prepared by dissolving 0.24g of agarose in 1X TAE buffer and melted in microwave-oven for 60 sec. Four micro litters of GelRed (Bioteam) was added into the melted

agarose and poured gently into casting gel tray and allowed to solidify for 40 min. After the gel is solidified, the combs were removed and transferred into the electrophoresis chamber. Then, 4.5 ul of DNA samples were mixed with 0.5 ul of 10X loading dye and load onto the gel and allowed to run for 40 minutes. After completion of electrophoresis, the gel was transferred and visualized under UV transilluminator (UV tech). Those samples showing thick single bands were considered as good quality DNA and were used for subsequent PCR.

### 3.4 MICROSATELLITE SELECTION

Nine microsatellite markers were chosen based on published articles of Bishop *et al*. (1994); Toldo *et al*. (1993); Barendse *et al*. (1994) and Kemp *et al*. (1995). These markers are also highly recommended by the International Society of Animal Genetics (ISAG) and Food and Agriculture Organization's Domestic Animal Diversity Information Service (FAO's DADIS) program for the study of bovine breeds characterization. Some features of the markers are shown in the table below (Table 3).

Primers supplied in lypholized powdered form (Bioteam) were dissolved using distilled, deionized, sterile water to a final concentration of 100 pmol/ul according to the manufacturere's instruction and 10 pmol/ul of primer stock solution was prepared by mixing 20 ul of 100pmol/ul in 180 ul sterile dd$H_2O$ and stored at -20$^0$C.

### 3.5 PCR REACTION

Polymerase chain reaction was carried out on about 85-120 ng of genomic DNA in a total of 25 µl reaction volume. Master mix was prepared for 30 samples and 24ul of the master mix was aliquated to each reaction tube and 1 ul of template DNA was added to make the final volume. Optimization was carried out for the concentration of $MgCl_2$ and primers. Initially 1.5mM $MgCl_2$ was used and was increased to 2.5mM by a factor of 0.5mM. Primers were also optimized from 4pmlo/ul to 7pmol/ul by a factor of 1pmol/ul. Colored PCR buffer (Promega) was used for all reactions thus avoided the use of loading dye for later applications. The table below (Table 4) elucidates the components of the reaction mixture with their respective final concentrations

Table 3: Microsatellite markers, their primer sequences, annealing temperature ($T_a$) and size range

| **Locus** | **Chr. no** | **Expected PCR product size (bp)** | $T_a$ ($^oC$) | **Primer sequences** | Gene bank acc. no. | Reference |
|---|---|---|---|---|---|---|
| BM1824 | 1 | 178-192 | 58 | *F: AGCAAGGTGTTTTTCCAATC*<br>*R: CATTCTCCAACTGCTTCCTTG* | G18394 | Bishop *et al*., 1994 |
| BM2113 | 2 | 123-143 | 58 | *F: CTGCCTTCTACCAAATACCC*<br>*R: TTCCTGAGAGAAGCAACACC* | M97162 | Bishop *et al*., 1994 |
| ETH10 | 5 | 212-224 | 58 | *F:GTTCAGGACTGGCCCTGCTAACA*<br>*R:CCTCCAGCCCACTTTCTCTTCTC* | Z22739 | Toldo *et al*., 1993 |
| ETH3 | 19 | 105-125 | 60 | *F: GAACCTGCCTCTCCTGCATTGG*<br>*R:ACTCTGCCTGTGGCCAAGTAGG* | Z22744 | Toldo *et al*., 1993 |
| ETH225 | 9 | 141-159 | 60 | *F: GATCACCTTGCCACTATTTCCT*<br>*R: ACATGACAGCCAGCTGCTACT* | Z14043 | Barendse *et al.*, 1994 |
| TGLA53 | 16 | 152-186 | | *F:GCTTTCAGAAATAGTTTGCATTCA*<br>*R:ATCTTCACATGATATTACAGCAGA* | L37208 | Barendse *et al*., 1994 |
| ILSTS049 | 11 | 159-169 | 58 | *F: CAATTTTCTTGTCTCTCCCC*<br>*R: GCTGAATCTTGTCAAACAGG* | L37261 | Kemp *et al*., 1995 |
| ETH152 | 5 | 157-175 | 56 | *F: TACTCGTAGGGCAGGCTGCCTG*<br>*R:GAGACCTCAGGGTTGGTGATCAG* | G18414 | Barendse *et al*., 1994 |
| TGLA227 | 18 | 76-104 | 56 | *F:CGAATTCCAAATCTGTTAATTTGC T*<br>*R:ACAGACAGAAACTCAATGAAAGC A* | - | Barendse *et al*., 1994 |

N.B = F: Forward primer; R: Reverse primer; Gene bank acc. no: Gene bank accession number

Table 4: PCR reaction components with their final concentration

| PCR Components | Final concentration | 1X (µl) | 30X (µl) |
|---|---|---|---|
| 5X PCR buffer (with 1.5mM $MgCl_2$) | 1X | 5 | 150 |
| $MgCl_2$ (25mM) | 1.5mM | 1.5 | 45 |
| Forward primer (10 pmol/µl) | 0.7uM | 1.75 | 52.5 |
| Reverse Primer (10 pmol/µl) | 0.7uM | 1.75 | 52.5 |
| dNTPs (200 µM) | 200uM | 0.5 | 15 |
| Taq pol (5U/µl) | 1U | 0.2 | 6 |
| Sterile dd$H_2O$ | _ | 13.3 | 399 |
| DNA template | _ | 1 | _ |
| Total | | 25 | 720 |

### 3.6 PCR PROTOCOL

The above PCR components were added sequentially as shown in the table on ice to prepare the master mix. After template DNA was added, the PCR reaction tubes were plucked to mix the components well and were tapped to settle the components at the bottom of the reaction tubes. The thermocycler (Elmener ) was set with an initial denaturation of 95°C for 1 min, and followed by 35 cycles of denaturation at 94$^oC$ for 30 sec, annealing at the temperature shown in table 3 for 30 sec and extension at 72$^oC$ for 30 sec, and a final elongation step at 72$^oC$ for 10 min. At the end of the reaction, the PCR products were stored at -20$^0C$ and were later subjected to agarose gel electrophoresis to ensure amplification.

### 3.7AGAROSE GEL ELECTROPHORESIS

To check amplification, 6 ul of the PCR product was loaded on 2% agarose gel prepared by dissolving 2g of agarose in 100ml 1XTBE buffer. Electrophoresis was carried out at 80 V for 45 minutes. After completion of electrophoresis, the gel was taken out of the electrophoresis

chamber and was submerged into EtBr solution (5ul/100ml) for staining. Staining was allowed for approximately 20 minutes followed by de-staining for another 20 minutes and then gel picture was taken under UV trans-illuminator by Biodoc Analyse 2.0 with digital cannon camera.

### 3.8 MICROSATELLITE GENOTYPING

The PCR products were resolved on 8 or 10% non denaturing polyacrylamide gel, depending on the size of the products, using vertical gel electrophoresis unit, 22 cm high, (SIGMA-ALDRICH). First, the glass plates were cleaned with mild detergents (LARGO) and were rinsed using tap water followed by repeated washings using distilled water. The glass plates then were further cleaned by 95% ethanol and were allowed to air dry. The polyacrylamide gel was cast in a glass plate sandwich separated by 1.0mm thick spacer on each vertical side. Gel wrap gasket was assembled accordingly to prevent any lick of the gel during casting. Moreover, clips were used to make the glass plate sandwich. Approximately 40 ml of gel solution was needed to cast each gel. The stock solution for acrylamide-bisacrylamide (19:1) was prepared by dissolving 38 g of acrylamide and 2 g of bisacrylamide in 100 ml of sterile distilled, deionized water thus to make 40% stock solution. The stock solution was stored at $4^0$C. Gel solution constituents are indicated in table 5.

Table 5: Polyacrylamide gel solution components

| **Gel components** | **8%** | **10%** |
|---|---|---|
| Acrylamide-bisacrylamide (19:1) | 8 ml | 10 ml |
| 10X TBE buffer (pH 8.2) | 2 ml | 2 ml |
| Ammonium persulfate (10%) | 200 ul | 200 ul |
| Steril dd$H_2O$ (to make the final volume) | _ | _ |
| TEMED | 40 ul | 40 ul |

After preparing the gel solution, it was poured between the glass plates and the comb was inserted and polymerization was allowed for an hour at room temperature. At the end of polymerization, the gel gaskets were removed and the glass plates were mounted on the

electrophoresis apparatus using clamps. TBE buffer with 0.5X was added in both the upper and lower chambers. The wells were flushed using TBE buffer after removing the comb. Four micro litters of the PCR products were diluted with 10 ul of autoclaved sterile water and 4 ul was taken to load onto the gel. DNA marker (phi X174 DNA/Hinf, promega) was loaded on the outer lanes. The gel was then allowed to run for about four to five hours at 300 volt depending on the size of the products.

At the end of electrophoresis, the gel was taken out of the glass plates and was submerged in 3X gelred staining solution for an hour. Finally, gel images were taken using UV tranilluminator and images were analyzed using Quantity One software package to determine the size of the PCR products.

### 3.9 STATISTICAL ANALYSIS

Hetrozygosity values (expected and observed) were calculated to determine the genetic variation. The observed hetrozygosity were carried out according to the algorithm of Levene (1949) using the POPGENE version 1.31 software package (Yeh *et al*., 1999). The estimation of expected or gene diversity, which shows the mean theoretical hetrozygosity if the population is in Hardy-Weinberg equilibrium, was computed based on Nei's (1973), and based on the following equation.

$H_{nb} = \frac{(n\sum(p_i)^2 - 1)}{n-1}$ Where, $H_{nb}$ is the expected hetrozygosity, n is the sample size and $p_i$ is allelic frquency.

This helps to analyze the genetic variation within the breeds. The effective number of alleles, which estimates the reciprocal of homozygosity, were computed according to Hartl and Clarck (1989) using the POPGENE software. The observed number of alleles was also determined using the above software package.

The Hardy-Weinberg equilibrium (HWE), which is the deviation of the observed hetrozygosity of an individual relative to the hetrozygosity expected under random mating, was analyzed. The

GENPOP package Version 3.4 (Rousset, 2007b) was also used to calculate an exact test for deviation from Hardy-Weinberg equilibrium.

The polymorphic information content (PIC) per locus was estimated as described by Botstein *et al*. (1980). A marker with PIC>0.5 can be considered as highly informative, whereas, 0.5>PIC>0.25 recognized as reasonably informative and below 0.25 is measured as slightly informative (Botstein *et al*., 1980). The PIC values were calculated using Cervus 3.0 software (Marshall *et al*., 1998) in order to assess how informative (polymorphic) the microsatellite markers are based on the following formula:

$$PIC = 1 - (\sum x_i^2) - (\sum 2x_i^2 x_j^2)$$

Where: $x_i$ and $x_j$ are the frequencies of the $i^{th}$ and $j^{th}$ alleles in a population.

F-statistics values were computed according to Weir and Cockerham (1984) using FSTAT version2.9.3.2 (Goudet, 2002). In their method, $F_{ST}$ ($\theta$) estimate the correlation of pairs of alleles between individuals within a subpopulation via the analysis of the partitioning of variance of allele frequency (between population, within population and within individuals). Generally, $F_{IS}$ is interpreted as a measure of inbreeding within the subpopulations (negative values indicate low level of inbreeding) and $F_{IT}$ as a measure of total inbreeding in the population whereas $F_{ST}$ is a measure of genetic differentiation between subpopulations.

Exact test of pair-wise $F_{ST}$ values was carried out to test for significant differentiation between the sampled populations using GENEPOP software package (Rousset, 2007b).

Genetic distances between populations were computed according to Nei's (1978) using Poptree2 software package. For the purpose of comparison with other studies, Goldstein *et al*. (1995) genetic distances was also computed using the same software.

A genetic relationship was estimated by constructing Unweighted Pair-wise Group method with arithmetic mean (UPGMA) tree based on Nei' (1978) and Goldstein *et al*. (1995) genetic distance using Poptree2 software package.

# 4. RESULTS

A t first a total of 10 microsatellite markers were tested to estimate the genetic variability among these breeds and to determine relationship based on these loci. However, one of the markers, BM1818, was found to be difficult for genotyping because of poor PCR amplification and non specific amplicons in some samples and hence excluded from the analysis.

## 4.1 ANALYSIS OF OBSERVED AND EFFECTIVE NUMBERS OF ALLELES AND ALLELE FREQUENCIES

### 4.1.1 MICROSATELLITE LOCUS TGLA227

Microsatellite analysis of TGLA 227 locus revealed a total of nine alleles whose size ranged from 73 to 93 bp across the entire population in the study (Appendix 2-A). The mean numbers of observed alleles and effective number of alleles per breed were 6.20 ±1.30 and 4.50 ± 1.37, respectively. The observed and effective numbers of alleles were 8 and 6.67, respectively in Abigar. Similarly, these values were 7 and 4.17 in Boran breeds, respectively. The least number of observed alleles were found in the Horro and Guraghe cattle breeds which were 5 and the effective numbers of alleles in these breeds were 3.92 and 3.00, respectively. In Sheko breeds these estimates were found to be 6 and 4.76, respectively. The effective and observed numbers of alleles in each cattle breed are summarized in figure 1.

The allelic frequencies at TGLA227 locus were found to range from 0.050 (for alleles 79 bp and 85 bp) to 0.200 (for alleles 73 and 75 bp) in Abigar. In Boran breeds this value ranged from 0.050 (for alleles 73, 77 and 81 bp) to 0.400 (for allele 75 bp) and similarly for Guraghe highland cattle breeds it ranged from 0.056 (for allele 73 and 77 bp) to 0.500 (for allele 75 bp). The least and the most frequent alleles in Horro breeds were 77 bp (with frequency of 0.050) and 75 and 83 bp (with frequency of 0.300), respectively and in Sheko allele 73 bp was the most frequent (0.300) and allele 83 bp was the least frequent (0.050). The overall allelic frequencies indicated that allele 75 bp was the most frequent one with frequency of 0.327 followed by allele 83 bp with frequency of 0.184. The least observed allele in these breeds was allele 93 bp with frequency of 0.020. Allelic frequencies for each breed are presented in table 6.

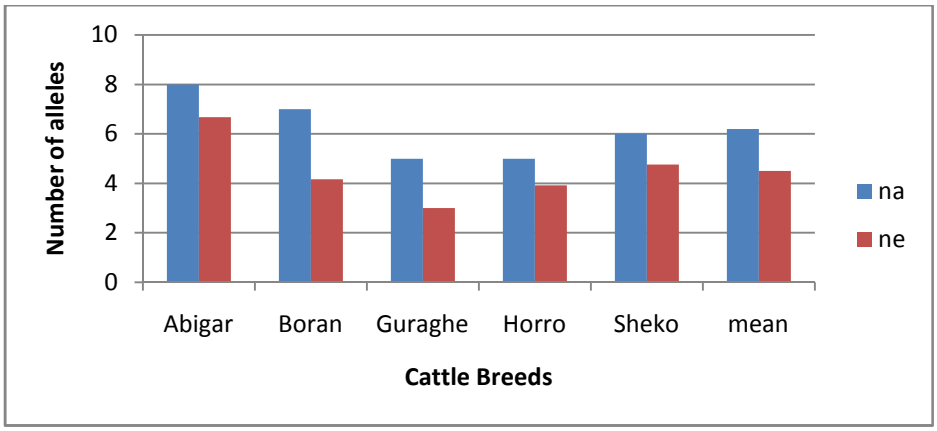



Figure 1: The effective (ne) and observed (na) numbers of alleles at TGLA227 locus in five indigenous bovine breeds of Ethiopia.

Table 6: Alleles and allelic frequencies at TGLA227 locus in the five cattle breeds of Ethiopia

| | **Allele Frequencies** | | | | | |
|---|---|---|---|---|---|---|
| **Alleles (bp)** | **Abigar** | **Boran** | **Guraghe** | **Horro** | **Sheko** | **Total** |
| 73 | 0.200 | 0.050 | 0.056 | 0.250 | 0.300 | 0.174 |
| 75 | 0.200 | 0.400 | 0.500 | 0.300 | 0.250 | 0.327 |
| 77 | | 0.050 | 0.056 | 0.050 | 0.100 | 0.051 |
| 79 | 0.050 | 0.100 | | | | 0.031 |
| 81 | 0.100 | 0.050 | | | 0.150 | 0.061 |
| 83 | 0.150 | 0.200 | 0.222 | 0.300 | 0.050 | 0.184 |
| 85 | 0.050 | 0.150 | 0.167 | 0.100 | 0.150 | 0.122 |
| 87 | 0.150 | | | | | 0.031 |
| 93 | 0.050 | | | | | 0.020 |

### 4.1.2 MICROSATELLITE LOCUS ETH3

A total of seven alleles were detected in combined analysis for microsatellite locus ETH 3. The size of the alleles ranged from 97 bp to 119 bp (Appendix 2-B). The observed and effective numbers of alleles were found to range from 5 to 7 and 3.45 to 5.13, respectively. The mean observed and effective numbers of alleles per breed were 5.80 ±0.84 and 4.26 ±0.94, respectively. The least numbers of observed alleles were scored in Boran and Horro breeds. In these breeds the observed numbers of alleles were 5 and effective numbers of alleles were 3.86 and 3.45, respectively. The maximum numbers of observed and effective numbers of alleles were scored in Guraghe cattle breeds with maximum values of 7 and 5.40, respectively. Six alleles were observed in Abigar and Sheko, where the effective numbers of alleles found to be 5.13 and 3.45, respectively. The effective and observed numbers of alleles in each cattle breed are summarized in figure 2.

The overall allelic frequencies were found to range from 0.064 (for allele 97 bp) to 0.287 (for allele109 bp). The second most frequent alleles were 115 bp and 111 bp with a frequency of 0.192 and 0.149, respectively. The 109 bp allele was the most frequently observed allele in Abigar and Boran with frequencies of 0.250 and 0.389, respectively. Likewise, the frequency of this allele in Sheko was 0.444. However, in the Guraghe highland cattle breed the 111 bp allele was found to be the most frequent one with frequency of 0.278 followed by the 109 bp allele with a frequency of 0.222. In Horro breeds allele 115 bp was the most frequently recorded one with a frequency of 0.450. Allelic frequencies for each cattle breed are shown in table 7.

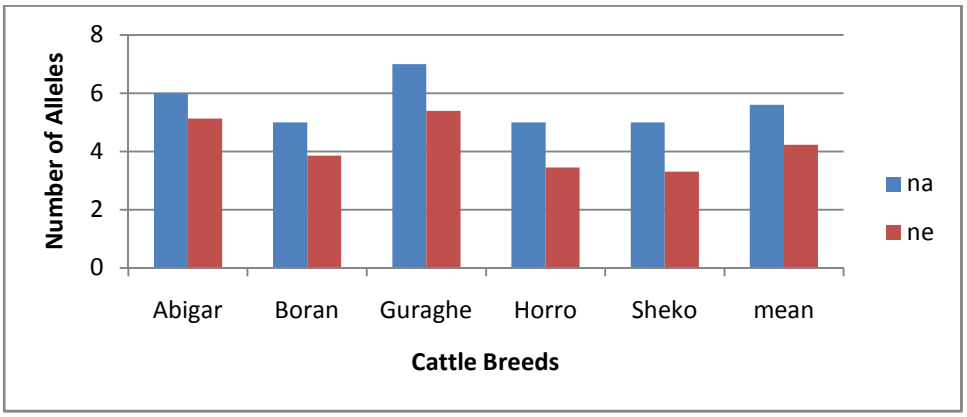



Figure 2: The effective (ne) and observed (na) numbers of alleles at ETH3 locus in five indigenous bovine breeds of Ethiopia

Table 7: Alleles and allelic frequencies at ETH 3 locus in the five cattle breeds of Ethiopia

| | **Allele Frequencies** | | | | | |
|---|---|---|---|---|---|---|
| **Alleles (in bp)** | **Abigar** | **Boran** | **Guraghe** | **Horro** | **Sheko** | **Total** |
| 97 | 0.200 | | 0.111 | | | 0.063 |
| 105 | 0.200 | | 0.167 | | | 0.075 |
| 107 | 0.200 | 0.056 | 0.111 | 0.050 | 0.056 | 0.096 |
| 119 | 0.250 | 0.389 | 0.222 | 0.150 | 0.444 | 0.287 |
| 111 | 0.100 | 0.167 | 0.278 | 0.150 | 0.056 | 0.149 |
| 115 | 0.050 | 0.167 | 0.056 | 0.450 | 0.222 | 0.192 |
| 119 | | 0.222 | 0.056 | 0.200 | 0.222 | 0.138 |

### 4.1.3 MICROSATELLITE LOCUS ETH10

In this study a total of 10 alleles whose size ranged from 209 to 227 bp were observed on microsatellite analysis at ETH 10 locus in overall populations analysis (Appendix 2-C). The overall effective numbers of alleles at this locus was found to be 5.95. The mean observed numbers of alleles and effective numbers of alleles per breed were 7.6 ± 1.67 and 6.03 ±1.73,

respectively. The least number of observed alleles were recorded in the Horro and Sheko breeds which was six. The highest number of observed alleles were recorded in Boran (10) followed by Abigar (8) and Guraghe(8) breeds. It was also the Boran breed which showed the highest effective numbers of alleles (8) followed by Guraghe cattle breed (7.04) whereas Sheko and Horro showed lower numbers of effective numbers of alleles which were 4.44 and 4.00, respectively. The effective and observed numbers of alleles in each cattle breed are summarized in figure 3.

The overall allelic frequencies at ETH 10 locus were ranged from 0.031 (for alleles 217 and 223 bp) to 0.255 (for allele 209 bp). In Abigar breeds the allelic frequencies ranged from 0.050 (for alleles 219 and 225 bp) to 0.200 (for alleles 209 and 215 bp). The least frequently observed alleles in Sheko breed was allele 227 bp with frequency of 0.050. Allele 209 bp was the most frequently observed allele in Boran and 221 bp in Guraghe Highland breeds with frequencies of 0.200 and 0.222, respectively. Similarly, in Horro breed it was allele 209 bp which was found to be the most frequently observed allele with frequency of 0.400. Table 8 presented allelic frequencies of ETH10 locus for each cattle breed in the study.

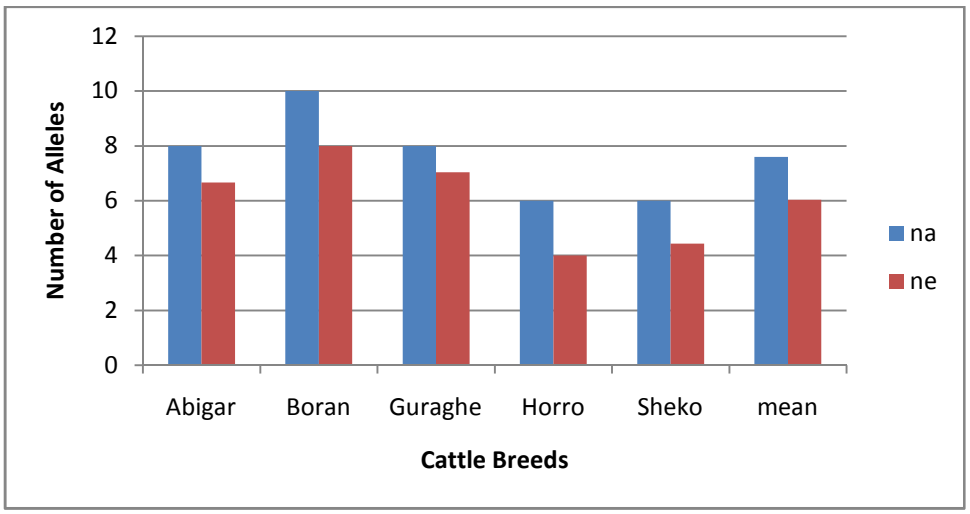



Figure 3: The effective (ne) and observed (na) numbers of alleles at ETH10 locus in five indigenous bovine breeds of Ethiopia

Table 8: Alleles and allelic frequencies at ETH10 locus in the five cattle breeds of Ethiopia

| | Allele Frequencies | | | | | |
|---|---|---|---|---|---|---|
| **Alleles** | **Abigar** | **Boran** | **Guraghe** | **Horro** | **Sheko** | **Total** |
| 209 | 0.200 | 0.200 | 0.111 | 0.400 | 0.350 | 0.255 |
| 211 | 0.100 | 0.100 | 0.111 | 0.150 | 0.200 | 0.133 |
| 213 | 0.100 | 0.100 | 0.167 | | | 0.071 |
| 215 | 0.200 | 0.050 | 0.111 | 0.050 | 0.200 | 0.122 |
| 217 | | 0.150 | | | | 0.031 |
| 219 | 0.050 | 0.050 | 0.111 | 0.150 | 0.100 | 0.092 |
| 221 | 0.150 | 0.100 | 0.222 | 0.200 | 0.100 | 0.153 |
| 223 | | 0.050 | 0.111 | | 0.100 | 0.031 |
| 225 | 0.050 | 0.150 | 0.056 | 0.050 | | 0.163 |
| 227 | 0.150 | 0.050 | | | 0.050 | 0.051 |

### 4.1.4 MICROSATELLITE LOCUS ILSTS049

A total of nine alleles were typed on microsatellite analysis of ILSTS049 locus in the five bovine breeds included in this study. The size of the alleles ranged from 149 to 169 bp (Appendix 2-D). The average numbers of alleles per breed were 7.00 ±1.22 and the effective numbers of alleles were 5.29 ±0.84. The observed and effective numbers of alleles in Abigar were 8 and 5.71, respectively, while these values were found to be 8 and 6.23, respectively in Guraghe highland breeds. Five alleles were typed in Boran cattle breed with 4.05 effective numbers of alleles. The observed numbers of alleles in Sheko and Horro breeds were 7 with 5.56 and 4.88 effective numbers of alleles, respectively. The observed and effective numbers of alleles for each cattle breed at microsatellite locus ILSTS049 are presented in figure 4.

The allelic frequencies at ILSTS049 locus were found to range from 0.050 (for alleles 157 and 161 bp ) to 0.300 (for allele 155 bp) in Abigar. In Boran breeds, this value ranged from 0.056 (for allele 161 bp) to 0.333 (for allele 169 bp) and for Guraghe Highland cattle breeds, it ranged

from 0.056 (for alleles 149, 159 and 163 bp) to 0.222 (for alleles 161 and 165bp). The most frequent allele in Horro breeds was 163 bp (with frequency of 0.300) and in Sheko allele 165 bp was the most frequent (0.300), while allele 167 bp was the least frequent (0.050) one. The overall allelic frequencies indicated that allele 163 bp was the most frequent one with frequency of 0.177 followed by allele 165 bp with frequency of 0.167. The least observed alleles in the overall population were alleles 157 and 159 bp with frequency of 0.042. Allelic frequencies at locus ILSTS049 for each breed are presented in table 9.

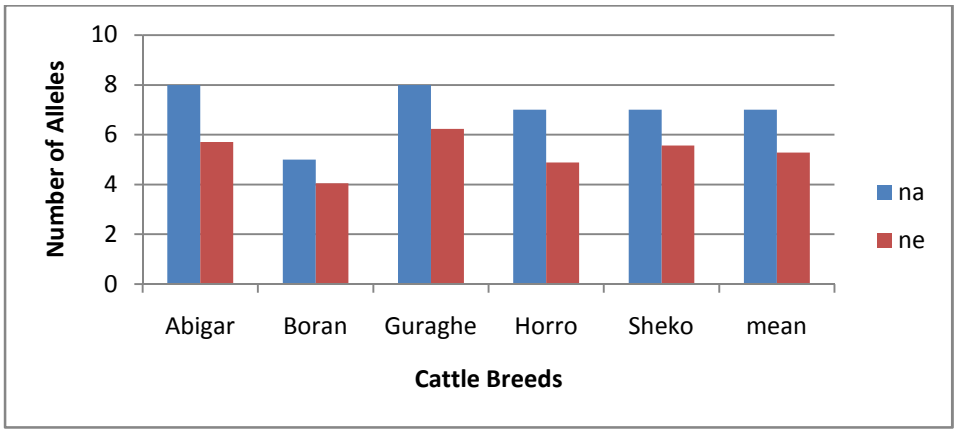



Figure 4: The effective (ne) and observed (na) numbers of alleles at ILSTS049 locus in five indigenous bovine breeds of Ethiopia

Table 9: Alleles and allelic frequencies at ILSTS049 locus in the five cattle breeds of Ethiopia

| | **Allele Frequencies** | | | | | |
|---|---|---|---|---|---|---|
| **Alleles** | **Abigar** | **Boran** | **Guraghe** | **Horro** | **Sheko** | **Total** |
| 149 | 0.100 | | 0.056 | | 0.150 | 0.063 |
| 155 | 0.300 | | 0.167 | 0.150 | 0.150 | 0.156 |
| 157 | 0.050 | | | 0.050 | 0.100 | 0.042 |
| 159 | 0.100 | | 0.056 | 0.050 | | 0.042 |
| 161 | 0.050 | 0.056 | 0.222 | 0.150 | 0.100 | 0.115 |
| 163 | 0.200 | 0.167 | 0.056 | 0.300 | 0.150 | 0.177 |

| | | | | | | |
|---|---|---|---|---|---|---|
| 165 | 0.100 | 0.167 | 0.222 | 0.050 | 0.300 | 0.167 |
| 167 | 0.100 | 0.278 | 0.111 | 0.250 | 0.050 | 0.156 |
| 169 | | 0.333 | 0.111 | | | 0.083 |

### 4.1.5 MICROSATELLITE LOCUS ETH152

Microsatellite analysis of ETH 152 locus revealed a total of six alleles whose size ranged from 189 to 205 bp across the entire population in the study (Appendix 2-E). The mean numbers of observed alleles and effective number of alleles per breed were 4.80 ±0.84 and 3.53 ±0.63, respectively. The observed and effective numbers of alleles were 5 and 3.92, respectively in Abigar. Similarly, these values were 5 and 3.18, respectively in Boran breeds. The observed numbers of alleles were found to be least in Guraghe and Horro cattle breeds which were 4 with 3.18 effective numbers of alleles. In the Boran and Sheko breeds the observed and effective numbers of alleles were 5 and 3.18, and 6 and 4.17, respectively. The effective and observed numbers of alleles at this locus for each cattle breed are summarized in figure 5.

The overall allelic frequencies at ETH 152 locus were ranged from 0.021 (for allele 193 bp) to 0.340 (for allele 191 bp). All alleles were genotyped in all breeds except alleles 193 and 205 bp. In Abigar breeds the allelic frequencies ranged from 0.100 (for allele 205 bp) to 0.400 (for allele 195 bp). In Sheko, the least frequently observed alleles were alleles 199 bp and 205 bp with frequency of 0.050 and the most frequent one was allele 189 with frequency of 0.350. Alleles 191 bp was the most frequently observed alleles in the three cattle breeds viz., Boran, Guraghe and Horro with frequencies of 0.500, 0.350 and 0.350, respectively. Table 10 presented allelic frequencies at this locus for each cattle breed in the study.

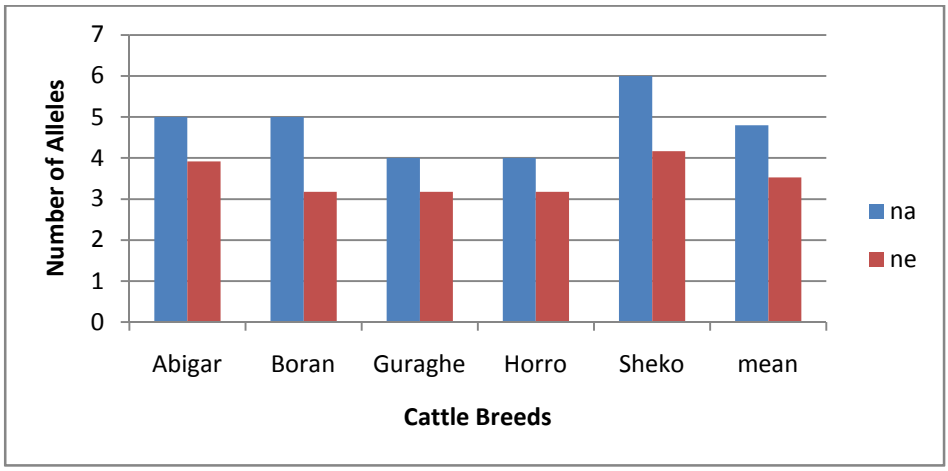



Figure 5: The effective (ne) and observed (na) numbers of alleles at ETH 152 locus in five indigenous bovine breeds of Ethiopia

Table 10: Alleles and allelic frequencies at ETH152 locus in the five cattle breeds of Ethiopia

| | Allele Frequencies | | | | | |
|---|---|---|---|---|---|---|
| **Alleles** | **Abigar** | **Boran** | **Guraghe** | **Horro** | **Sheko** | **Total** |
| 189 | 0.150 | 0.111 | 0.333 | 0.278 | 0.350 | 0.245 |
| 191 | 0.200 | 0.500 | 0.389 | 0.444 | 0.200 | 0.340 |
| 193 | | | | | 0.100 | 0.021 |
| 195 | 0.400 | 0.167 | 0.222 | 0.167 | 0.250 | 0.245 |
| 199 | 0.150 | 0.111 | 0.056 | 0.111 | 0.050 | 0.096 |
| 205 | 0.100 | 0.111 | | 0.050 | 0.050 | 0.053 |

### 4.1.6 MICROSATELLITE LOCUS BM2113

On microsatellite analysis of BM2113 locus, 10 alleles were genotyped with size range of 121 to 145 bp in the five cattle population (Appendix 2-F). The average effective numbers of alleles per breed was 5.76 ±1.26. The least effective numbers of alleles were observed in Sheko (3.77) and the largest effective numbers of alleles were recorded in Abigar breed (6.90). The mean observed

alleles per breed was 7.4 ±0.89. A total of 8 alleles were observed in Abigar, Boran and Horro breeds whereas 7 and 6 alleles were observed in Guraghe and Sheko cattle breeds, respectively. The observed and effective numbers of alleles for each cattle breed are presented in figure 6.

The allelic frequencies at BM2113 locus were found to range from 0.050 (for alleles 125, 127 and 129 bp) to 0.200 (for allele 145 bp) in Abigar. In Boran breeds this value ranged from 0.050 to 0.200 (for alleles 121 and 127 bp) and for Guraghe highland cattle it ranged from 0.056 (for alleles 121 and 133 bp) to 0.278 (for allele 131 bp). Allele 125 bp was the most frequent alleles in Horro breed (with frequency of 0.250) followed by allele 131 bp (with frequency of 0.200). In Sheko allele 135 bp was the most frequently observed alleles with frequency of 0.389 followed by allele 139 bp (0.278). The overall allelic frequencies indicated that alleles 125 and 131 bp were the most frequent one with frequency of 0.135 and the least observed allele in these breeds was allele 145 bp with frequency of 0.052. Allelic frequencies at this locus for each breed are presented in table 11.

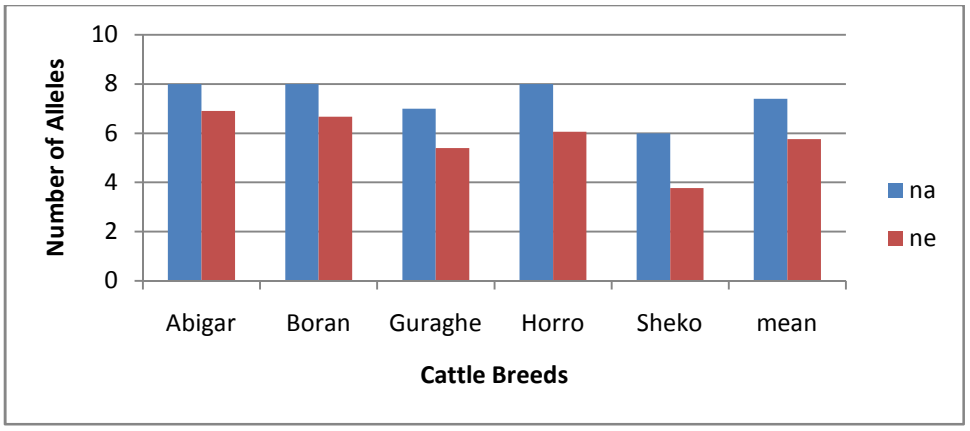



Figure 6: The effective (ne) and observed (na) numbers of alleles at BM2113 locus in five indigenous bovine breeds of Ethiopia

Table 11: Alleles and allelic frequencies at BM2113 locus in the five cattle breeds of Ethiopia

| | Allele Frequencies | | | | | |
|---|---|---|---|---|---|---|
| **Alleles** | **Abigar** | **Boran** | **Guraghe** | **Horro** | **Sheko** | **Total** |
| 121 | 0.150 | 0.200 | 0.056 | 0.050 | | 0.094 |
| 123 | 0.150 | 0.050 | 0.111 | 0.050 | | 0.073 |
| 125 | 0.050 | 0.150 | 0.111 | 0.250 | | 0.115 |
| 127 | 0.050 | 0.200 | 0.222 | 0.150 | 0.056 | 0.135 |
| 129 | 0.050 | 0.050 | | 0.150 | | 0.063 |
| 131 | | 0.150 | 0.278 | 0.200 | 0.056 | 0.135 |
| 133 | 0.100 | 0.100 | 0.056 | 0.100 | 0.167 | 0.115 |
| 135 | | 0.100 | 0.167 | 0.050 | 0.278 | 0.115 |
| 139 | 0.150 | | | | 0.389 | 0.104 |
| 145 | 0.200 | | | | 0.056 | 0.052 |

### 4.1.7 MICROSATELLITE LOCUS BM1824

Microsatellite analysis of BM1824 locus revealed a total of 8 alleles whose size ranged from 169 to 189 bp in the five cattle breeds (Appendix 2-G). The mean numbers of observed and effective numbers of alleles per breed were 6.80 ±0.45 and 4.76 ±1.02, respectively. Six alleles were genotyped in Guraghe breed with 4.05 effective numbers of alleles. A total of 7 alleles were observed in the rest of the breeds studied. The effective numbers of alleles ranged from 3.77 Sheko to 6.25 in Horro. These values are summarized in figure 7.

The overall allelic frequencies at BM1824 locus were ranged from 0.010 (for alleles 175 and 177) to 0.0.367 (for allele 179 bp). In Abigar breed the allelic frequencies ranged from 0.050 (for alleles 175, 193 and 197 bp) to 0.300 (for allele 179 bp). The least frequently observed alleles in Sheko breed were alleles 175 and 177 bp with frequency of 0.050. Allele 179 bp was the most frequently observed alleles in Boran with frequencies of 0.450. However, 175 bp allele was the

most frequent allele in Guraghe Highland cattle with frequencies of 0.389. Table 12 presented allelic frequencies at BM1824 locus for each cattle breed in the study.

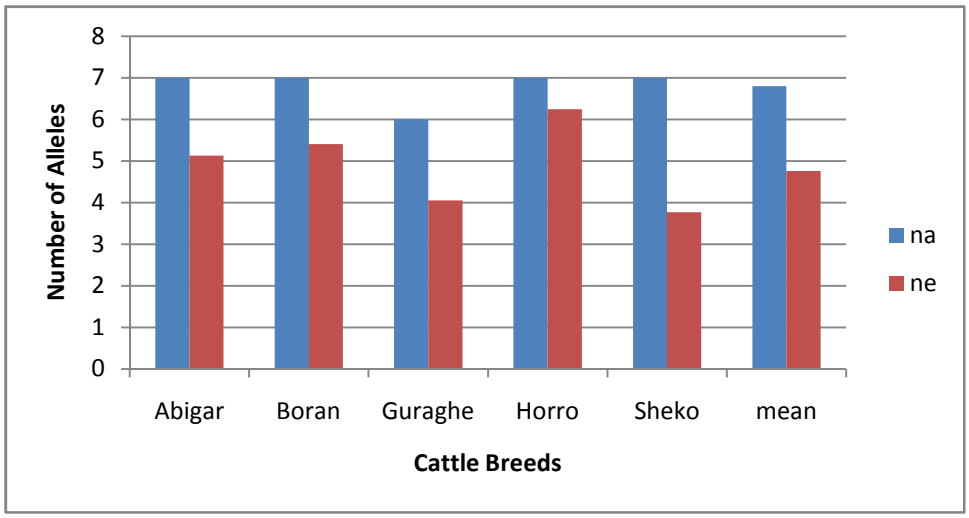



Figure 7: The effective (ne) and observed (na) numbers of alleles at BM1824 locus in five indigenous bovine breeds of Ethiopia

Table 12: Alleles and allelic frequencies at BM1824 locus in the five cattle breeds of Ethiopia

| | **Allele Frequencies** | | | | | |
|---|---|---|---|---|---|---|
| **Alleles** | **Abigar** | **Boran** | **Guraghe** | **Horro** | **Sheko** | **Total** |
| 169 | 0.250 | 0.050 | 0.056 | | | 0.071 |
| 173 | 0.200 | 0.250 | 0.222 | 0.150 | 0.150 | 0.194 |
| 175 | 0.250 | 0.250 | 0.389 | 0.200 | 0.050 | 0.225 |
| 177 | 0.050 | | 0.056 | 0.200 | 0.050 | 0.071 |
| 179 | 0.050 | 0.100 | | 0.100 | 0.100 | 0.071 |
| 185 | | 0.150 | 0.167 | 0.150 | 0.350 | 0.163 |
| 187 | 0.050 | 0.150 | 0.111 | 0.050 | 0.100 | 0.092 |
| 189 | 0.150 | 0.050 | | 0.150 | 0.200 | 0.112 |

### 4.1.8 MICROSATELLITE LOCUS ETH225

Total of10 alleles whose size ranged from 137 to 157 bp were typed at microsatellite ETH225 locus in the five cattle populations (Appendix 2-H). The average numbers of observed and effective numbers of alleles per breed were found to be 6.60 ±0.89 and 4.61 ±0.31, respectively. The maximum numbers of alleles (eight) were recorded in Boran and Abigar breeds. The effective numbers of alleles in these breeds were 6.06 and 5.00, respectively. Seven alleles were typed in Guraghe and Sheko, whereas a total of six alleles were observed in Horro cattle breeds. The effective numbers of alleles in these breeds were found to be 5.59, 5.41 and 4.63, respectively. The summery of these values are shown in figure 8.

The allelic frequencies at ETH 225 locus were found to range from 0.050 (for alleles 141, 143 bp, and 153 bp) to 0.350 (for allele 153 bp) in Abigar. In Boran breeds this value ranged from 0.050 (for alleles 143, 149 and 157 bp) to 0.250 (for allele 155 bp). The most frequent allele in Horro breeds was 157 bp with frequency of 0.333, and in Sheko breed allele 143 bp was the most frequent one (frequency of 0.300). The overall allelic frequencies indicated that allele 155 bp was the most frequent one with frequency of 0.188 followed by allele 157 bp with frequency of 0.177. The least observed allele in these breeds was allele 137 bp with total frequency of 0.031. Allelic frequencies at Eth225 locus for each cattle breed are presented in table 13.

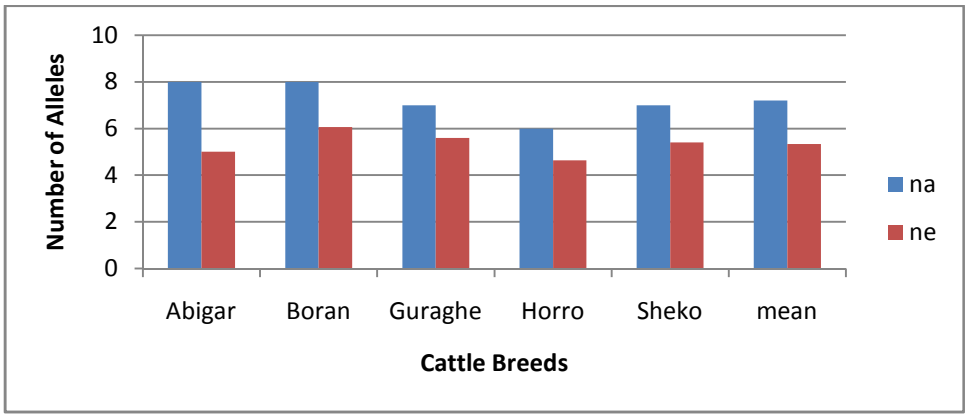



Figure 8: The effective (ne) and observed (na) numbers of alleles at ETH225 locus in five indigenous bovine breeds of Ethiopia

Table 13: Alleles and allelic frequencies at ETH 225 locus in the five cattle breeds of Ethiopia

| | **Allele Frequencies** | | | | | |
|---|---|---|---|---|---|---|
| **Alleles** | **Abigar** | **Boran** | **Guraghe** | **Horro** | **Sheko** | **Total** |
| 137 | | | 0.056 | | 0.100 | 0.031 |
| 141 | 0.050 | 0.100 | 0.222 | | | 0.073 |
| 143 | 0.050 | 0.150 | 0.222 | 0.111 | 0.300 | 0.167 |
| 145 | | 0.200 | | | | 0.042 |
| 147 | 0.100 | | 0.111 | | 0.100 | 0.063 |
| 149 | 0.100 | 0.050 | 0.222 | 0.222 | 0.050 | 0.125 |
| 151 | 0.100 | 0.150 | | 0.111 | | 0.073 |
| 153 | 0.050 | 0.050 | | 0.056 | 0.150 | 0.063 |
| 155 | 0.200 | 0.250 | 0.111 | 0.167 | 0.200 | 0.187 |
| 157 | 0.350 | 0.050 | 0.056 | 0.333 | 0.100 | 0.177 |

### 4.1.9 MICROSATELLITE LOCUS TGLA53

A total of 14 alleles were genotyped on microsatellite analysis of TGLA53 locus in overall population evaluation. The size of the alleles ranged from 155 bp to 183 bp (Appendix 2-I). The observed and effective numbers of alleles were found to range from 8 to 10 and 6.10 to 8.10, respectively. The mean observed and effective numbers of alleles per breed were 9.20 ±0.84 and 7.10 ± 0.81, respectively. The same numbers of observed alleles (9) were scored in Abigar and Sheko breeds; whereas 10 alleles were genotyped in Boran and Horro cattle breeds and 8 alleles were typed in Guraghe cattle. The effective numbers of alleles in Abigar, Boran and Guraghe were 6.45, 7.41 and 6.10, respectively. Similarly, these values were 8.10 and 7.41 in Horro and Sheko breeds, respectively. The effective and observed numbers of alleles in each cattle breed are summarized in figure 9.

The overall allelic frequencies were found to range from 0.032 (for allele 183 bp) to 0.149 (for allele167 bp). The second most frequent allele was 155 bp with a frequency of 0.138. The 155 bp

allele was the most frequently observed allele in Abigar and Boran with frequencies of 0.250. Allele 169 bp was the most frequent allele in Guraghe and Horro cattle breeds with frequency of 0.250 and 0.222, respectively. In the Sheko cattle allele 171 bp was found to be the most frequent one with frequency of 0.200. Allelic frequencies at this locus for each cattle breed are shown in table 14.

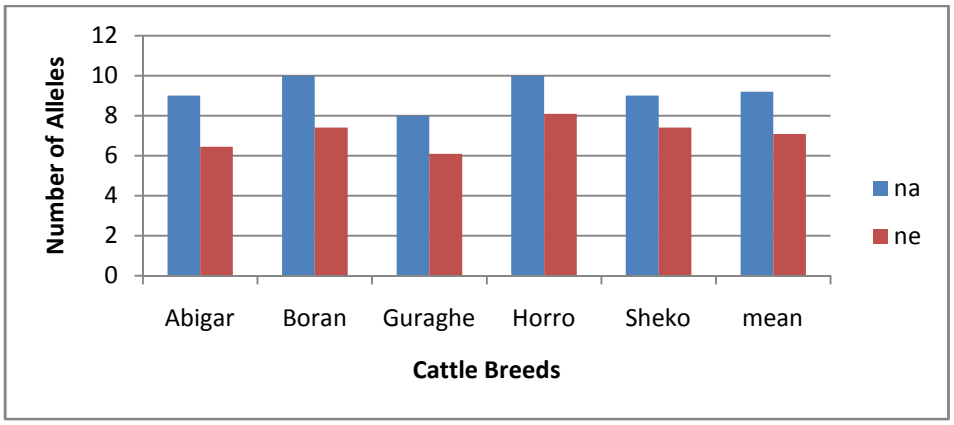



Figure 9: The effective (ne) and observed (na) numbers of alleles at TGLA53 locus in five indigenous bovine breeds of Ethiopia

Table 14: Alleles and allelic frequencies at TGLA53 locus in the five cattle breeds of Ethiopia

| | **Allele Frequencies** | | | | | |
|---|---|---|---|---|---|---|
| **Alleles** | **Abigar** | **Boran** | **Guraghe** | **Horro** | **Sheko** | **Total** |
| 155 | 0.250 | 0.250 | 0.063 | 0.111 | | 0.138 |
| 157 | 0.050 | | 0.188 | 0.111 | | 0.064 |
| 161 | 0.050 | 0.050 | 0.063 | 0.111 | | 0.053 |
| 163 | 0.100 | 0.150 | | 0.056 | 0.050 | 0.075 |
| 165 | | | 0.188 | 0.056 | 0.100 | 0.064 |
| 167 | 0.200 | 0.200 | 0.063 | 0.111 | 0.150 | 0.149 |
| 169 | | 0.100 | 0.250 | 0.222 | 0.050 | 0.117 |
| 171 | | | | | 0.200 | 0.043 |

| | | | | | | |
|---|---|---|---|---|---|---|
| 173 | 0.100 | 0.0.50 | 0.063 | 0.056 | 0.100 | 0.075 |
| 175 | 0.150 | 0.100 | | 0.111 | | 0.075 |
| 177 | | | | | 0.050 | 0.011 |
| 179 | 0.050 | 0.050 | | | 0.150 | 0.053 |
| 181 | | | 0.125 | | 0.150 | 0.053 |
| 183 | 0.050 | 0.050 | | 0.056 | | 0.032 |

### 4.2 HARDY WEINBERG EQUILIBRIUM (HWE) TEST

The estimation of exact P-Values by the Markov chain method with dememorization of 10000, 100 batches and 1000 iterations per batch was used to test for HWE using GENEPOP software package.

The exact test demonstrated that loci TGLA227, BM1824, BM2113, ETH152 and ETH10 are in Hardy-Weinberg equilibrium in all the populations analyzed. However, significant deviation from HWE was observed in Sheko and Guraghe at TGLA53 locus. The Horro breed was not at HWE when ILSTS049 and ETH225 loci were examined and Abigar breed showed deviation from HWE at ETH3 locus. Moreover, the Abigar breed also showed deviation from HWE at ETH225 locus. Overall, the Boran breed was the only one consistently at HWE at all loci analysis in the study. The summery of HWE test of the breeds at each locus is given in table 15.

Table 15: P-values with their SE for exact test of HWE at each locus

| Locus | Abigar | Boran | Guraghe | Horro | Sheko |
|---|---|---|---|---|---|
| TGLA227 | 0.0930 (0.0106) | 0.3405 (0.0165) | 0.5394 (0.0111) | 0.5486 (0.0087) | 0.0832 (0.0064) |
| ETH3 | 0.0272* (0.0032) | 0.5332 (0.0092) | 0.4023 (0.0162) | 0.7513 (0.0072) | 0.9587 (0.0034) |
| ETH152 | 0.0726 (0.0067) | 0.5011 (0.0090) | 0.5258 (0.0064) | 0.2405 (0.0053) | 0.1919 (0.0106) |
| ETH10 | 0.0746 (0.0084) | 1.0000 (0.0000) | 0.4112 (0.0174) | 0.2584 (0.0112) | 0.0726 (0.0067) |
| TGLA53 | 0.2692 | 0.0655 | 0.0093** | 0.2258 | 0.0338* |

| | | | | | |
|---|---|---|---|---|---|
| | (0.0217) | (0.0144) | (0.0031) | (0.0210) | (0.0074) |
| ILSTS049 | 0.1691<br>(0.0144) | 0.3100<br>(0.0099) | 0.0604<br>(0.0107) | 0.0156*<br>(0.0039) | 0.2642<br>(0.0161) |
| BM1824 | 0.7467<br>(0.0113) | 0.2951<br>(0.0139) | 0.3393<br>(0.0130) | 0.4154<br>(0.0139) | 0.1847<br>(0.0103 ) |
| BM2113 | 0.1029<br>(0.0120) | 0.0820<br>(0.0103) | 0.1294<br>(0.0108) | 0.1295<br>(0.0121) | 0.7124<br>(0.0129) |
| ETH225 | 0.0347*<br>(0.0075 ) | 0.0530<br>(0.0080) | 0.3580<br>(0.0135) | 0.0150*<br>(0.0035) | 0.0959<br>(0.0091) |

*P < 0.05, **P <0.01

### 4.3 HETROZYGOSITY AND PIC

The observed hetrozygosity and Nei's expected hetrozygosity values for each breed at specific locus and the entire population overall loci were calculated using POPGENE software. These results are presented in tables 16 and 17. The PIC values for all loci across all individuals are also presented in table 17.

The observed hetrozygosity values for Abigar breed ranged from 0.700 (ETH225 and TGLA53) to 0.900 (BM2113 and BM1824) and Nei's expected hetrozygosity ranged from 0.745 (ETH152) to 0.850 (TGLA227). In Boran breed, these values ranged from 0.556 (ILSTS049) to 1.000 (ETH3 and ETH10) and 0.741 (ETH3) to 0.875 (ETH10), respectively. The minimum and the maximum observed hetrozygosity values in the Guraghe breed were 0.625 (TGLA53) and 0.889 (ETH3, ETH10 and ETH225). In this breed Nei's expected hetrozygoaity ranged from 0.667 (TGL227) to 0.850 (ETH10). Similarly, these values were found to range from 0.600 (ILSTS049) to 0.900 (BM2113 and ETH3) and 0.685 (ETH152) to 0.877 (TGLA53) in Horro breed, respectively. In Sheko breed, the observed and Nei's expected hetrozygosities ranged from 0.700 (TGLA227 and ILSTS049) to 0.889 (ETH225 and ETH3) and 0.735 (BM2113) to 0.865 (TGLA53), respectively. The analysis of the whole population overall loci indicated that the observed and Nei's expected hetrozygosities ranging from 0.690 (ILSTS049) to 0.894 (ETH3) and 0.752 (ETH152) to 0.910 (TGLA53), respectively.

Table 16: Summary of hetrozygosity statistics for all loci of five indigenous cattle breeds of Ethiopia

| Locus | Abigar | | Boran | | Guraghe | | Horro | | Sheko | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Het obs | Nei's exp | Het obs | Nei's exp | Het obs | Nei's exp | Het obs | Nei's exp | Het obs | Nei's exp |
| BM1824 | 0.900 | 0.805 | 0.700 | 0.815 | 0.778 | 0.753 | 0.800 | 0.840 | 0.800 | 0.790 |
| BM2113 | 0.900 | 0.855 | 0.900 | 0.850 | 0.667 | 0.815 | 0.900 | 0.835 | 0.778 | 0.735 |
| ETH3 | 0.800 | 0.805 | 1.000 | 0.741 | 0.889 | 0.815 | 0.900 | 0.710 | 0.889 | 0.698 |
| ETH10 | 0.800 | 0.850 | 1.000 | 0.875 | 0.889 | 0.858 | 0.700 | 0.750 | 0.800 | 0.775 |
| ETH152 | 0.800 | 0.745 | 0.667 | 0.658 | 0.667 | 0.685 | 0.556 | 0.685 | 0.600 | 0.760 |
| TGLA53 | 0.800 | 0.845 | 0.800 | 0.865 | 0.625 | 0.836 | 0.889 | 0.877 | 0.800 | 0.865 |
| TGLA227 | 0.700 | 0.850 | 0.700 | 0.760 | 0.667 | 0.667 | 0.800 | 0.745 | 0.700 | 0.790 |
| ILST049 | 0.800 | 0.825 | 0.556 | 0.753 | 0.778 | 0.840 | 0.600 | 0.795 | 0.700 | 0.820 |
| ETH225 | 0.700 | 0.800 | 0.900 | 0.835 | 0.889 | 0.821 | 0.889 | 0.784 | 0.800 | 0.815 |
| Mean | 0.800 | 0.820 | 0.803 | 0.798 | 0.761 | 0.788 | 0.781 | 0.780 | 0.763 | 0.783 |
| St. Dev | 0.071 | 0.036 | 0.157 | 0.065 | 0.109 | 0.070 | 0.133 | 0.063 | 0.084 | 0.049 |

Table 17: Summary of hetrozygosity statistics and PIC for the pooled population overall loci.

| Locus | Observed Hetrozygosity | Nei's expected Hetrozygosity | PIC |
|---|---|---|---|
| BM1824 | 0.796 | 0.849 | 0.831 |
| BM2113 | 0.833 | 0.892 | 0.882 |
| ETH3 | 0.894 | 0.821 | 0.798 |
| ETH10 | 0.837 | 0.857 | 0.842 |
| ETH152 | 0.656 | 0.752 | 0.711 |
| TGLA53 | 0.787 | 0.910 | 0.902 |
| TGLA227 | 0.714 | 0.806 | 0.782 |

| | | | |
|---|---|---|---|
| ILST049 | 0.690 | 0.865 | 0.849 |
| ETH225 | 0.833 | 0.869 | 0.855 |
| Mean | 0.782 | 0.847 | 0.828 |
| St. Dev | 0.079 | 0.050 | 0.057 |

### 4.4 F-STATISTICS

The method of Weir and Cockerham (1984) was used to calculate the F-statistics ($F_{IS}$, $F_{ST}$ and $F_{IT}$) using FSTAT software package. The F-statistics values across each locus and over all loci was computed and summarized in table 19. The $F_{IS}$ value across each locus in the sampled population is indicated in table 18. The $F_{IS}$ value overall loci indicated that the Guraghe breed was relatively inbreed (0.094) followed by Sheko and Abigar breeds with inbreeding value 0.079 and 0.077, respectively. On the other hand, the Boran breed showed relatively low level of inbreeding (0.046) followed by the Horro breed (0.053).

Table 18: $F_{IS}$ value per breed across each locus and overall loci

| **Locus** | **Abigar** | **Boran** | **Guraghe** | **Horro** | **Sheko** |
|---|---|---|---|---|---|
| BM1824 | -0.066 | 0.192 | 0.026 | 0.100 | 0.040 |
| BM2113 | 0.000 | -0.006 | 0.238 | -0.025 | 0.000 |
| ETH3 | 0.059 | 0.059 | -0.032 | -0.218 | -0.219 |
| ETH10 | 0.111 | -0.091 | 0.023 | 0.119 | 0.020 |
| ETH152 | -0.021 | 0.086 | 0.086 | 0.245 | 0.260 |
| TGLA53 | 0.106 | 0.106 | 0.314 | 0.045 | 0.127 |
| TGLA227 | 0.227 | 0.131 | 0.059 | 0.021 | 0.166 |
| ILSTS049 | 0.083 | 0.316 | 0.132 | 0.294 | 0.197 |
| ETH225 | 0.176 | -0.025 | -0.024 | -0.076 | 0.071 |
| All | 0.077 | 0.046 | 0.094 | 0.053 | 0.079 |

Table 19: F statistics ($F_{IS}$, $F_{ST}$ and $F_{IT}$) for each locus across the whole population

| **Locus** | **$F_{IT}$** | **$F_{ST}$** | **$F_{IS}$** |
|---|---|---|---|
| BM1824 | 0.075 | 0.016 | 0.061 |
| BM2113 | 0.085 | 0.048 | 0.038 |
| ETH3 | -0.067 | 0.056 | -0.131 |
| ETH10 | 0.034 | 0.000 | 0.034 |
| ETH152 | 0.134 | 0.002 | 0.132 |
| TGLA53 | 0.147 | 0.015 | 0.134 |
| TGLA227 | 0.125 | 0.007 | 0.119 |
| ILSTS049 | 0.218 | 0.021 | 0.201 |
| ETH225 | 0.057 | 0.030 | 0.028 |
| Jackknifing over all loci | 0.090 (0.027) | 0.022 (0.007) | 0.070 (0.031) |

Table 19 showed the F-statistics values of the whole population at each locus. It can be inferred from this table that ETH3 locus showed relatively low level of inbreeding (-0.131) followed by locus ETH225 (0.028). A higher level of inbreeding was observed at locus ILSTS049 (0.201). The genetic differentiation at locus ETH3 was higher (0.058) followed by locus BM2113 (0.046). However, locus ETH10 showed lower level of genetic differentiation (0.000). The $F_{IT}$ value ranged from -0.067 (ETH3) to 0.218 (ILSTS049). The mean $F_{IS}$, $F_{ST}$ and $F_{IT}$ values obtained by jackknifing overall loci were 0.070 ±0.031, 0.022 ±0.007 and 0.090±0.27, respectively.

Pair-wise genetic differentiation ($F_{ST}$) between population was computed based on allele identity and was tested for the level of significance (Fisher's method of exact test) using GENEPOP software package. The result has revealed that there was significant genetic differentiation ($P < 0.05$) between Sheko and the other four breeds. There was also significant genetic differentiation ($P < 0.05$) between Abigar and the other three cattle breeds. However, there was no significant

genetic differentiation between the remaining breeds in the sampled populations. Table 20 showed pair-wise genetic differentiation between pairs of population.

Table 20: Pair-wise genetic differentiation ($F_{ST}$) between pairs of cattle populations

| | Abigar | Boran | Guraghe | Horro | Sheko |
|---|---|---|---|---|---|
| Abigar | | | | | |
| Boran | 0.022* | | | | |
| Guraghe | 0.024* | $0.001^{NS}$ | | | |
| Horro | 0.021* | $0.006^{NS}$ | $0.010^{NS}$ | | |
| Sheko | 0.031* | 0.036* | 0.033* | 0.030* | |

*P < 0.05, $^{NS}$: Not significant

### 4.5 GENETIC DISTANCE AND DENDROGRAM

The results of the genetic distances between the five cattle breeds in this study based on Nei's unbiased standard genetic distance and Goldstein's distance (delta mu) are presented in table 21.

Based on Nei's unbiased standard genetic distance, the Sheko breed was consistently distant from the other populations in the study followed by the Abigar. The smallest distance was observed between Boran and Guraghe (0.027) followed by Boran and Horro (0.046). The largest distance was observed between Boran and Sheko (0.214). However, based on delta mu, the smallest distance was noted between Boran and Horro (1.246) followed by Boran and Guraghe (1.531). The Sheko breed was also found to be consistently distant from the other four breeds. The results are indicated in table 21.

Dendrogram was constructed for both distance types using UPGMA clustering method after 1000 replications and recovered similar grouping. The Boran and Guraghe cattle breeds were clustered together based on Nei's corrected standard genetic distance (fig. 10) and then with the Horro breed. The Sheko breed was clustered away from the rest of the breeds. However, the Horro and the Boran breeds were clustered together based on delta mu genetic distance (fig. 11).

Table 21: Pair-wise genetic distance; delta mu above the diagonal and Nei's corrected standard genetic distance below the diagonal

| | Abigar | Boran | Guraghe | Horro | Sheko |
|---|---|---|---|---|---|
| Abigar | **** | 3.912 | 2.245 | 3.389 | 5.271 |
| Boran | 0.166 | **** | 1.531 | 1.246 | 5.427 |
| Guraghe | 0.180 | 0.027 | **** | 2.360 | 4.484 |
| Horro | 0.137 | 0.046 | 0.070 | **** | 3.362 |
| Sheko | 0.214 | 0.229 | 0.218 | 0.181 | **** |

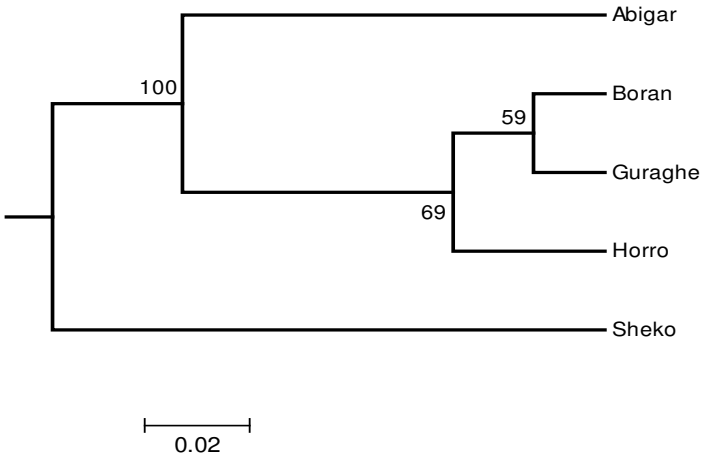



Figure 10: Dendrogram showing genetic relationship between the sampled populations using UPGMA based on Nei's corrected standard genetic distance.

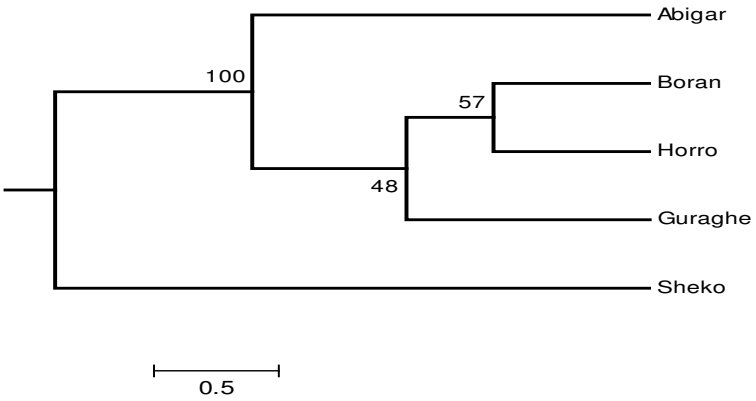



Figure 211: Dendrogram showing genetic relationship between the sampled populations using UPGMA method based on delta mu genetic distance.

# 5. DISCUSSION

## 5.1 ALLELIC RICHNESS AND DIVERSITY OF MICROSATELLITE LOCI

Generally, the observed numbers of alleles at a particular locus is calculated to depict the allelic richness of that locus in the analyzed population. This parameter is one way of measuring the genetic diversity of the population under study, i.e. the larger the observed numbers of alleles, the more genetic diversity of the population. The effective numbers of alleles is also another important measure of the number of alleles at a locus. More importantly, this tells us the allelic frequency distribution in the population. i.e., if the effective numbers of allele is closer to the number of observed alleles, then the alleles have similar allelic frequency in the population studied. These population genetic parameters were used in many studies to measure genetic diversity in cattle populations (Rehman and Khan, 2009; Radko *et al*., 2005; Karthickeyan *et al*., 2009; Stevanovic *et al*., 2010; Caruthers, 2009; Sun *et al*., 2008).

### 5.1.1 MICROSATELLITE TGLA227 LOCUCS

Microsatellite TGLA227 locus contains (TG)n repeats that are located on chromosome 18 and specifically at 84.7cM (http://www.ncbi.nlm.nih.gov/genome/sts/sts.cgi?uid=250914).

The result of the current study has indicated that a total of nine alleles whose size ranged from 73 to 93 bp were genotyped in the five sampled cattle populations. This observed alleles size range also agreed with Dadi *et al*. (2008) report where a total of 12 alleles whose size ranged from 71 to 93 bp in 10 cattle population of Ethiopia were observed. The number of individuals sampled per population and the number of populations studied might be the main cause for the higher numbers of observed alleles in Dadi's *et al*. (2008) study. The observed numbers of alleles at this locus were almost similar across the breeds. Likewise, the effective numbers of alleles across the breeds were similar. Barendse *et al*. (1994) characterized this locus in *Bos taurus* and reported the size to range from 64 to 114 bp. Stevanovich *et al*. (2010) genotyped 11 alleles whose size ranged from 78 to 114 bp in the Yugoslav Pied cattle breed in Serbia. A total of 31 alleles, whose size ranged from 65 to 125 bp were genotyped in eight Chinese cattle breeds (Sun et al., 2008). Cervini *et al*. (2006) reported 6 alleles at TGLA227 locus whose size ranged from 75 to 97 bp in

the characterization of Brazilian Nellore cattle (*Bos indicus*). Eleven and eight alleles were observed in the comparison of Canadidan and international Angus breeds at TGLA227 locus, respectively. The effective numbers of alleles were reported to be 6.341 in Canadian and 2.998 in the international Aungus breeds (Carruthers, 2009).

### 5.1.2 MICROSATELLITE LOCUS ETH 3

Microsatellite locus ETH3 (D19S2) contains a compound repeating structure, $(GT)_nAC(GT)_6$, (http://www.cstl.nist.gov/strbase/cattleSTRs.htm) at chromosome 19 in bovine genome (Toldo *et al*., 1993).

The result depicted a total of seven alleles ranging from 97-119 bp in the five cattle population. The observed numbers of alleles in these breeds ranged from 5 to 7. This showed the allelic richness of this locus in these breeds. The effective numbers of alleles indicated the presence of some alleles at a relatively higher frequency than the others. This could be notably observed in the Sheko breed where the observed and effective numbers of alleles were 5 and 3.45, respectively. Similarly, Dadi *et al*. (2008) reported a total of eight alleles ranging from 95 to 121 bp in 10 cattle breeds of Ethiopia. Toldo et al. (1993) characterized ETH3 locus and reported 8 alleles ranging from 90-135 bp. In Moroccon Oulmès-Zaer and Tidili cows, 11 alleles were observed with size ranging from 97-127 bp (Boujenane and Ouragh, 2010). On molecular characterization of Gaolao and Kenketha (*Bos indicus*) a total of eight and six observed numbers of alleles with 2.05 and 1.17 effective numbers of alleles were reported with size ranged from 103-121 bp (Chaudari *et al*., 2009).

### 5.1.3 MICROSATELLITE LOCUS ETH10

Microsatellite locus ETH10 (D5S3) contained a simple repeating structure of (AC)n (http://www.cstl.nist.gov/strbase/cattleSTRs.htm) at chromosome 5 of the bovine genome (Toldo *et al*., 1993).

The microsatellite analysis in the current study has showen a total of 10 alleles ranging from 209-227 bp in the five cattle population. However, Dadi *et al*. (2008) reported less numbers of (8) alleles with the size ranging from 211-227 bp in 10 bovine breeds of Ethiopia. Toldo *et al*.

(1993) reported the allelic size range at this locus to be 212-224 bp in *Bos taurus*. The International Society of Animal Genetic Resource (ISAG) also confirmed a wide size range of 198-234 bp for this microsatellite locus. The breeds showed a higher level of diversity in terms of the observed and effective numbers of alleles. The effective numbers of alleles in the breeds indicated the presence of few alleles which were not at relatively similar allelic frequencies.

### 5.1.4 MICROSATELLITE LOCUS ILSTS049

The ILSTS049 locus (accession number: L37261) contained $(CA)_n$ repeats stretched from 61-114 bp at the 546 bp complete nucleotide sequence of the locus at chromosome 11 in the bovine genome (Kemp *et al*., 1995).

The result of the present study indicated a total of nine alleles ranging from 149-169 bp in the five cattle population. The allelic size range reported by Kemp *et al*. (1995) was 159-169 bp in *Bos tuarus*. The observed numbers of alleles in these breeds ranged from 5 (Boran) to 8 (Abigar and Guraghe) which showed the allelic richness of this locus in these cattle population. In addition, the effective numbers of alleles depicted the higher level of genetic diversity of the analyzed populations at this locus. Even though the observed numbers of alleles were the same for Abigar and Guraghe cattle breeds, their respective effective numbers of alleles were not the same (5.71 for Abigar and 6.23 for Guraghe). This variation was observed due to the fact that the alleles in Guraghe cattle showed a relatively similar allelic frequency than the Abigar breeds. This was also observed in Sheko and Horro breeds where the observed numbers of alleles were the same but different effective numbers of alleles.

### 5.1.5 MICROSATELLITE LOCUS ETH152

ETH152 locus is a $(CA)_n$ repeat located at 39-72 bp nucleotide position in the 189 bp sequence (accession number: Z14040) at chromosome five of the bovine genome (Steffen and Eggen, 1993).

In this study a total of six alleles ranging from 189-205 bp were observed in the five cattle breeds studied. The observed numbers of alleles in Abigar and Boran were equal (5) but different numbers of effective alleles (3.92 and 3.18, respectively). This showed that the Abigar breed has

a much more balanced distribution of allelic frequencies than the Boran breed. The distribution of allelic frequencies in Guraghe and Horro were equally balanced as their effective numbers of alleles were equal (3.18).

The size of alleles reported by Dadi *et al*. (2008) ranged from 191-203 bp in10 local cattle population of Ethiopia. In Sistanie and Taleshie cattle breeds of Iran 6 and 5 numbers of observed alleles with 3.3 and 3.4 effective numbers of alleles were reported (Mirhoseinie *et al*., 2005). On the assessment of genetic variability in Tharpakar breed of Indian Zebu a total of 5 alleles with 2.87 effective numbers of alleles ranging from 190-204 bp were typed (Sodhi *et al*., 2006). However, Sun *et al*. (2007) found a total of 22 alleles ranging from 185 to 235 bp with 15.18 effective numbers of alleles in Qinchuan cattle breeds of China.

### 5.1.6 MICROSATELLITE LOCUS BM2113

BM2113 locus (D2S26) is a $(CA)_n$ repeat located at 52-92 bp nucleotide position in the 160 bp sequence (accession number: M97162) at chromosome 2 of the bovine genome (Sunden *et al*., 1993).

The numbers of observed alleles in this study were 10 with 121-145 bp range. The Abigar, Boran and Horro showed equal numbers of observed alleles. However, their effective numbers of alleles were not the same due to inequity of the distribution of the allelic frequencies within these breeds. More balanced allelic frequencies were observed in Abigar breed. In Sheko breed the effective numbers of alleles were smaller which showed the presence of few alleles at higher frequency.

Dadi *et al*. (2008) reported the same numbers of alleles (10) ranging from 122-146 bp in 10 bovine breeds of Ethiopia. Bishop *et al*. (1994) recorded the size of the alleles at this locus in the range of 123-145 bp. A total of 11 alleles ranging from 123-143 bp were reported in the genetic diversity study among Canadienne, Brown Swiss, Holstein and Jersy cattle breeds of Canada (Hansen *et al*., 2002). Georgescu *et al*. (2009) typed 12 alleles at BM2113 locus in five Romanian cattle breeds whose size ranged from 116-140 bp.

### 5.1.7 MICROSATELLITE LOCUS BM1824

The repeating nucleotide unit of BM1824 locus (D1S34) is a $(TG)_n$ unit stretching from 91-121 bp on the 269 bp nucleotide sequence (accession number: G18394) at chromosome 1 of the bovine genome (Barendse *et al*., 1994).

In this study eight alleles ranging from 169-189 bp were genotyped in the five cattle population. This agrees with Dadi *et al*. (2008) report. A total of seven alleles were typed in the four cattle population, except the Guraghe breed (6 alleles). This indicated the allelic richness of this locus in these cattle population. Though the numbers of alleles were greater in Sheko compared to Guraghe cattle, the effective numbers of alleles in Sheko were smaller than the Guraghe breed. This showed a relatively balanced distribution of allelic frequency in Guraghe than Sheko. More importantly, the effective numbers of alleles measure genetic diversity since it is computed as reciprocal of homozygosity. Thus, the Guraghe breed showed lower level of homozygosity than the Sheko breed.

Riojas-Valdes *et al*. (2009) reported 10 alleles in the range of 150-188 bp in Brangus cattle breed of Mexico. A total of five alleles ranging from 176-197 bp were genotyped in the genetic diversity analysis of Hariana and Hisar cattle breeds of Pakistan with 3.51 and 4.48 effective numbers of alleles, respectively (Rehman and Khan, 2009).

### 5.1.8 MICROSATELLITE LOCUS ETH225

ETH225 locus (D9S2) contained a compound repeating structure, $(TG)_4CG(TG)(CA)_n$, (http://www.cstl.nist.gov/strbase/cattleSTRs.htm) at chromosome 9 of the bovine genome (Steffen and Eggen, 1993).

The current study has shown that there were 10 alleles ranging from 137-157 bp in the five cattle population analyzed and 6-8 alleles were observed in these populations. These observed numbers of alleles indicated the allelic richness of ETH225 locus in the population studied. Dadi *et al*. (2008) also reported the same allelic size range in10 bovine breeds of Ethiopia. However, the total numbers of alleles genotyped in Dadi *et al*. (2008) were nine alleles. The effective numbers of alleles indicated the presence of a relatively similar level of allelic distribution among these

cattle breeds at this locus. The allelic size range reported by Fries *et al*. (1993) was 131-155 bp. The total numbers of alleles observed at this locus in six north Ethiopian cattle breeds were eight (Zerabruk *et al*., 2007). Higher numbers of alleles (21) were observed in Brangus breed of Mexico at this locus which ranged from 108-161 bp (Riojas-Valdes *et al*., 2009).

### 5.1.9 MICROSATELLITE LOCUS TGLA53

This locus is characterized by having a $(TG)_6CG(TG)_4(TA)_n$ repeating unit at chromosome 16 of the bovine genome (http://www.cstl.nist.gov/strbase/cattleSTRs.htm).

The mean observed and effective numbers of alleles per breed at this locus were higher than the rest of the loci analyzed in the study. This showed the highest allelic richness of this locus. In addition, this locus was found to show the lowest level of expected homozygosity (as explained by higher effective numbers of alleles) than the other markers in the study. The study also depicted that there were 14 alleles ranging from 155-183 bp in the five cattle population. This allelic size range agreed with Dadi *et al*. (2008) where a total of 19 alleles ranging from 145-183 bp were observed in 10 local bovine breeds of Ethiopia. The allele size reported by Georgesm and Massey (1992) ranged from 151-187 bp. A total of 17 alleles ranging from 154-188 bp were observed in the genetic diversity analysis of seven European cattle breeds at TGLA53 locus (Stevanovich *et al*., 2010). In the genetic variability study among Polish-Red, Herford and Holstein-Fiesian cattle breeds, 9, 6 and 11 alleles ranging from 154-186 bp were genotyped (Radok *et al*., 2005).

## 5.2 HARDY WEINBERG EQUILIBRIUM (HWE) TEST

Hardy Weinberg equilibrium test is considered as a simple but basic model that serves as a starting point to examine the genetic structure of a population. In the test, the observed and expected genotypic frequencies are compared to check whether there exists a statistically significant difference between them.

In the study, five loci (TGLA227, BM1824, BM2113, ETH152 and ETH10) were found to be at HWE in the five cattle populations. The rest of the loci analyzed showed deviation from HWE at least in one of the breeds, except the Boran breed, where all loci were found to be at HWE.

Generally, deviation from HWE could be due to the violation of one or more of the assumptions (large population size, random mating, absence of natural selection, absence of mutation and gene flow). However, the most probable reason for the observed deviation in this study could be due to small sample size which might have introduced bias into the parameter estimate. Moreover, genotyping error (the presence of null alleles i.e. some hetrozygote individuals might be falsely genotyped as homozygotes because of the allelic drop out during PCR amplification) might have played a role in the observed deviation from HWE (Chakraborty *et al*., 1992).

The genetic analysis of Ongole cattle breeds of India using 25 microsatellite markers indicated that 17 loci were at HWE and the rest showed deviation from HWE (Karthicheyan *et al*., 2007). In the study of genetic diversity among Romanian cattle breeds using 11 microsatellite markers, none of the breeds were found to deviate from HWE (Georgescu et al., 2009). Ciampolini *et al*. (1995) indicated that the HWE was not always maintained in the genetic variability study of four Italian beef cattle breeds using 17 microsatellite markers. Out of the 18 loci analyzed in East African bovine breeds eight of them showed significant deviation from HWE in the Kenyan Boran breeds (Okomo, 1997). An examination of 15 microsatellite markers for deviation from HWE across four breeds of Canada cattle breeds revealed 13 makers were at HWE; the exceptions were BM2113 and BM6501 (Hansen *et al*., 2002).

## 5.3 HETROZYGOSITY AND GENETIC DIVERSITY OF ETHIOPIAN CATTLE

Hetrozygosity values (expected and observed hetrozygosities) are used to determine the genetic variation within breeds under study. The expected hetrozygosity can be explained as the probability of an individual in a population to be heterozygous at a given locus or it is a probability of nonidentity of two randomly chosen genes in a population (Nei, 1973). Generally, the hyper-variability nature of microsatellite markers make them to show higher values of expected hetrozygosity than the biallelic markers such as biochemical markers and RFLP (Bruford and Wayne, 1993). The observed hetrozygosity tells the proportion of individuals found to be heterozygous at a given locus.

In this study it has been observed that the Guraghe cattle showed a relatively lower value of observed hetrozygosity than the rest of the breeds. A relatively lower observed hetrozygosity was

also noticed in Sheko and Horro breeds. A higher value of observed hetrozygosity was seen in Boran and Abigar. Generally, the observed hetrozygosity values examined in the sampled populations were higher showing a higher genetic diversity within the breeds. The result has also indicated that the expected hetrozygosity values were higher in Abigar and Boran breeds. The higher expected hetrozygosity noticed in Abigar might be explained by its evolutionary history that it is a cross between a taurine and a zebu cattle. However, Dadi *et al*. (2008) indicated the Sheko breed was the one which showed the highest expected hetrozygosity values based on 30 microsatellite loci. This difference could be explained by sample size and the number of loci used in the present study. More importantly, the overall mean observed hetrozygosity values for each breed were lower than the corresponding expected hetrozygosities, which might indicate the presence of overall loss in hetrozygosity within the examined cattle populations. Dadi *et al*. (2008) also noticed that the observed hetrozygosity values were lower than the expected hetrozygosities in 10 indigenous bovine breeds of Ethiopia. Moreover, the larger values of expected hetrozygosity than the observed hetrozygosity might be due to the large numbers of observed alleles at each locus (Kalinwski, 2002).

The mean observed and expected hetrozygosity values (0.78 and 0.85, respectively) in current study were almost similar with Teneva *et al*. (2005) study in Bulgarian Grey cattle based on 11 microsatellite markers which were 0.78 and 0.86, respectively. Stevanovich *et al*. (2010) reported the mean observed and expected hetrozygosity values to be 0.651 and 0.750, respectively in Yugoslav Pied cattle of Serbia.

### 5.4 PIC AND UTILITY OF MICROSATELLITE MARKERS/LOCI

PIC values are usually calculated to evaluate the level of polymorphism of a marker. As described by Botstein *et al*., (1980) a marker with PIC > 0.5 is said to be highly informative and between 0.5 and 0.25 considered as reasonably informative where below 0.25 is said to be slightly informative.

The present work depicted all the nine microsatellite loci used in the study were having PIC values greater than 0.5. This indicated their usefulness for routine analysis of cattle genetic diversity of the populations under study. The high value of PIC could also indicate the genetic

heterogeneity of the sampled populations (Rehman and Khan, 2009). The markers used in this study were also found to show higher PIC values in many cattle breeds (Stevanovic *et al*., 2010; Czernekova *et al*., 2006; Rehman and Khan, 2009; Radko *et al*., 2005; Karthickeyan *et al*., 2009; Egito *et al*., 2007).

## 5.5 F-STATISTICS AND INTER-POPULATION GENETIC DIVERSITY

F-statistics ($F_{IT}$, $F_{IS}$ and $F_{ST}$), also known as fixation indexes, were coined by Wright (1951) to describe the properties of a subdivided population. $F_{IS}$ and $F_{IT}$ are defined as the correlations between two uniting gametes relative to the subpopulation and relative to the total population, respectively and $F_{ST}$ is the correlation between two gametes drawn at a random from each subpopulation. The variance based method of estimating F-statistics that have been developed by Weir and Cockerham (1984) was used in the present study.

According to the result of this study, the Guraghe breed showed a relatively higher level of inbreeding (0.094) than the other breeds in the study though the other breeds showed similar level of inbreeding. The overall $F_{IS}$ and $F_{IT}$ were found to be very low, which were very close to zero indicating the low level of inbreeding within and among populations. This low level of inbreeding can also be an indication of low level of genetic differentiation between the populations under study.

The overall (multilocus) $F_{ST}$ value (0.022) indicated that around 2.2% of the total genetic variation could be explained by population difference and the remaining 97.8% was due to differences among individuals within breeds. According to Wright (1951), $F_{ST}$ value below 0.05 is generally considered as a very low level of genetic differentiation. Thus, the breeds under study showed a very low level of genetic differentiation. A higher level of inter-populational gene flow due to long distance migrations across localities, deliberate exchange of breeding stocks and utilization of communal grazing lands might have played the major role for the low level of genetic differentiation observed in the studied populations. In addition, lack of breed improvement and uncontrolled mating practices could result in gene flow among populations. This result was found to agree with Dadi's *et al*. (2008) where they reported a very low level of genetic differentiation (0.013) among 10 indigenous bovine breeds of Ethiopia.

The pair-wise $F_{ST}$ analysis indicated that there was non-significant genetic differentiation between Guraghe and Boran and between Boran and Horro. This might imply that there could be genetic admixture and interbreeding among the breeds in the studied population (Okomo, 2002). Dadi's *et al* (2008) report has also indicated insignificant level of genetic differentiation between 13 pairs of cattle populations in their study. The non-significant genetic differentiation between Guraghe and Boran might be explained by the fact that both breeds are grouped under the east African zebu cattle (DAGRIS, 2007). However, the small sample size might have contributed too. It was less likely to observe non-significant genetic differentiation between Horro and Boran breeds as the Horro breed is classified into Zenga (Sanga and Zebu cross breed). Moreover, the observed insignificant differentiation between these breeds do not undergo with their geographic localities as the Horro breed mainly inhabit the north-western Ethiopia whereas the Boran breed inhabits the southern rangelands of Ethiopia. Thus, there might have been gene flow from one population to the other due to livestock movement as a result of human migration in search of favorable environments for themselves and their cattle. Interestingly, the Sheko breed significantly differentiated from the other breeds as it is classified as the only east African *Bos taurus* where the others share common zebu genetic background (DAGRIS, 2007). The significant genetic differentiation of Abigar with the rest of the breeds was expected as it is classified into Sanga cattle breed (DAGRIS, 2007). Moreover, the geographic locality of the breed with the other breeds, particularly with Boran, Horro and Guraghe could also contribute to the observed significant differentiation (DAGRIS, 2007).

Hassen *et al*. (2007) reported insignificant genetic differentiation between Guraghe and Arsi breeds and also between Guraghe and Abigar based on three RAPD primers. However, in this study significant genetic differentiation was observed between Sheko and Guraghe. It is not surprising to observe significant genetic differentiation between these breeds as they belong to Sanga and zebu cattle, respectively. On the study of genetic diversity, introgression, and relationship among West and central African cattle breeds, a slightly higher level of genetic differentiation ($F_{ST}$ = 0.06) were observed (Ibeagha-Awemu *et el*., 2004). A relatively higher level of genetic differentiation (0.13) was reported in eight cattle breeds of British. Such higher

level of differentiation might be due to selection and/or higher level of inbreeding (Wiener et al., 2004).

Dadi *et al*. (2008) reported that the overall $F_{IT}$ and $F_{IS}$ values in 10 bovine breeds to be 0.083 ± 0.017 and 0.071 ± 0.017, respectively. These values are more or less similar with the one in this study (0.090 ± 0.027 and 0.070 ± 0.031, respectively).

## 5.6 GENETIC DISTANCE AND INTER-BREED GENETIC RELATIONSHIP

The genetic relationship among populations can be evaluated using genetic distance measurements. There are so many methods developed so far to examine genetic distance between populations (Takezaki and Nei, 1996; Goldstein *et al*., 1995; Nei 1978, 1972 and Cavalli-Sforza and Edwards, 1967). However, Nei and Takezaki (1996) demonstrated the superiority of Nei's standard genetic distance with sample size corrected and Goldstein's distance ($\delta\mu^2$) to show evolutionary time.

Generally, the genetic distance observed between the breeds in the current study was small. This was also observed in East African cattle breeds that ranged from 0.023 ± 0.009 between Danakil and Abigar to 0.868 ± 0.200 N'Dama and Sahiwa (Okomo, 1997). The present study also revealed the fact that the Sheko breed is consistently distant from the other populations as it is classified as *Bos tuarus* where the others share a zebu genetic background (Rege, 1999; DAGRIS, 2007). It has been also shown that the genetic distance between Guraghe and Boran was found to be the smallest one (0.027) followed by Horro and Boran (0.046). The smallest distance observed between Guraghe and Boran could be explained by the fact that both of them belong to the east African zebu breeds (DAGRIS, 2007). From the evolutionary history of the breeds, the Horro breed is expected to be closer to the zebu than the taurine breeds as it belongs to the Zenga (cross between Zebu and Sanga) cattle (DAGRIS, 2007). This result also revealed the fact that the Horro breed is closer to the Zebu (Guraghe and Boran) than the Sheko breed. The Abigar breed was expected (both evolutionary and geographically) to show the smallest genetic distance with Sheko. However, the result indicated that the Horro breed was much closer than the Abigar though the Abigar breed was the second closest breed. The closer genetic distance between Abigar and Sheko could be explained by the fact that the Abigar breed is

believed to be the cross between taurine and zebu cattle (Rege *et al*., 1999) and hence they share a common ancestor. Moreover, the geographical proximity of their natural habitats might have created the possibility of interbreeding between them and thus reducing the genetic distance. The smallest distance observed between Sheko and Horro might support the fact that the Horro breed has a taurine blood as it belongs to Zenga cattle (Rege, 1999). The largest genetic distances were observed between Sheko and Boran and between Guraghe and Sheko. This difference is most probably due to the fact that the Boran and Guraghe breeds belong to the Zebu cattle (*Bos indicus*) whereas the Sheko belongs to the African taurine breed (DAGRIS, 2007). Moreover, the distance between Sheko and Boran could be explained with geographic distance as Sheko and Boran breeds found in west and East of Great Rift valley, respectively. Similarly, Hassen *et al*. (2007) also reported the highest distance between Guraghe Highland and Sheko (0.0354) followed by Sheko and Arsi (0.0233). In contrast, Dadi *et al*. (2008) reported that the Sheko breed exhibited the smallest distance with Boran than the rest of the breeds studied.

Results from Goldstein's genetic distance do not fit with both the evolutionary history and geographical localities of the breeds under study. This might be due to the size of sampled populations. Wiener *et al*. (2004) used Nei's standard genetic distance to construct phylogenetic trees in eight British cattle breeds and the result indicated the smallest distance between Ayshire and Friesian (0.1944) and the maximum distance was observed between Highland and Gurnsey (0.4446). Genetic distances among Gir, Kankrej and Deoni cattle breeds of India were determined from seven microsatellite markers based on Nei's standard genetic distance and the distance matrix was used to construct dendogram. The dendogram indicated that the clustering was in accordance with the evolutionary and geographical locations of the breeds (Deepak, 2004).

# 6. CONCLUSION AND RECOMMENDATION

All the microsatellite loci used gave PCR amplification products in the five cattle breeds included viz., Abigar, Boran, Horro, Sheko and Guraghe. More importantly, the markers depicted high level of polymorphism indicating their application and utilization for routine analysis of genetic diversity of the cattle population studied.

The genetic variability across all loci in the studied cattle breeds were estimated to be in range of 4-10 alleles with observed and expected hetrozygosity values ranging from 0.556 to 1.000 and 0.658 to 0.877, respectively. These indicated the presence of diversity within the breeds studied, which is very important for future breed improvement schemes.

The low mean $F_{IT}$ (0.090) and $F_{IS}$ (0.070) values clearly indicated the presence of diversity within and among Ethiopian breeds with low inbreeding depression septum. These low inbreeding values are important features for future breed development programs.

The analysis has also implicated the presence of low level of genetic differentiation ($F_{ST}$ = 0.022) between the breeds studied. This depicted the loss of genetic resources of the indigenous cattle population that might be due to drought (lack of grazing land), disease and introduction of exotic breed. Thus, this study urges conservation strategy should be put in place before Ethiopia losses its cattle diversity irreversibly.

The validation of the observed genetic relationship among the breeds might require the use of large sample size and many microsatellite loci.

This study can be extended by comparing the genetic diversity of the breeds at economically important traits such as growth rate, feed utilization efficiency, milk yield, disease resistance and carcass quality to further analyze their genetic relationships. Moreover, study on phylogenetic relationship of Ethiopian breeds should be carried out using mtDNA and Y chromosome markers so as to get a clear maternal and paternal lineage of all breed.

# 7. REFERENCES


Ajmone-Marsan, P., Negrini, R., Milanesi, E., Bozzi, R., Nijman, I.J., Buntjer, J.B.,Valentini, and Lenstra, J.A. (2002). Genetic distances within and across cattle breeds as indicated by biallelic AFLP markers. *Animal Genetics*, 33: 280-286.

Amoli, M., Ollier, W. and Hajeer, A.H. (2000). A novel PCR-RFLP assay for the detection of a polymorphism in the 39 of STAT6 gene. *Genes and Immunity*, 1: 349-350.

Anderson, I. (2002). Foot and mouth disease 2001: lessons to be learned inquiry report: Presented to the Prime Minister and the Secretary of State for Environment, Food and Rural Affairs and the devolved administrations in Scotland and Wales.

Avise, J.C. (1991). Ten unorthodox perspectives on evolution prompted by comparative population findings on mitochondrial DNA. *Annual Review of Genetics*, 25: 45-69.

Baker, C.M., Manwell, C., Labrisky, R.F. and Harper, J.A. (1966). Molecular genetics of avian proteins: Egg, blood and tissue proteins of the Ringnecked pheasant, Phasianus colchicus L. *Comparative Biochemistry and Physiology*, 17: 467-499.

Barendse, W., Armitage, S.M., Kossarek, L.M., Shalom, A., Kirpatrick, B.W., Ryan, A.M., Clayton, D., Li, L., Nebergs, H.L., Zhang, N., Grosse, W.M., Weiss, J., Creighton, P., McCarthy, F., Ron, M., Teale, A.J., Fries, R., McGrow, R.A., Moore, S.S., Georges, M., Soller, M., Womack, J.E. and Hetzel D.J.S. (1994). A genetic linkage map of the bovine genome. *Nature genetics.* 6: 227-237.

Barker JSF, Moore SS, Hetzel DJS, Evans D, Tan SG, Byrne K (1997). Genetic diversity of Asian water buffalo (Bubalus bubalis): microsatellite variation and a comparison with protein-coding loci. *Animal Genetics*, 28: 103–115.

Bishop, M.D., Kappes, S.M. and Keele, J.W. (1994). A genetic linkage map for cattle. *Genetics*, 136: 619-39.

Botstein, D., White, R.L., Skolnick, M., Davis, R.W. (1980). Construction of a genetic linkage

map in man using restriction fragment length polymorphisms. *Human Genetics*, 32: 314-331.

Braunschweig. M.H. (2008). Association between two paternal casein haplotypes and milk yield traits of Swiss Fleckvieh cattle. *Journal of Applied Genetics.* 49: 69-74.

Bruford, M.W. and Wayne, R.K. (1993). Microsatellites and their application to population genetic studies. *Current Opinion in Genetics and Development*, 3: 939-943.

Bruford, M.W., Hanotte, O., Brookfield, J.Y., Burke, T. (1992). Single-locus and multilocus DNA fingerprinting. In: Hoelzel AR (ed), Molecular Genetic Analysis of Populations: A practical Approach. pp. 225-269.

Cai, X., Chen, H., Wang, S., Xue, K. and Lei, C. (2006). Polymorphisms of two Y chromosome microsatellites in Chinese cattle. *Genetics Selection Evolution*. 38: 525- 534.

Canon, J., Alexandrino, P., Bessa, I., Carleos, C., Carretero, Y., Dunner, S., Feran, N., Garcia, D., Jordana, J., Laloe, D., Pereira, A., Sanchez, A., Goudarzi, K.M. (2001). Genetic diversity measures of local European beef cattle breeds for conservation purposes. *Genetics Selection Evolution*, 33: 311-332.

Cavalli-Sfrozal, L. and. Edwards, A. W. F. (1967). Phylogenetic analysis: models and estimation procedures. *American Journal of Human Genetics*, 19:233-257.

Cervini, M., Henrique-Silva, F., Mortari, N., Matheucci, J.R. (2006). Genetic variability of 10 microsatellite markers in the characterization of Brazilian Nellore cattle. *Genetics and Molecular Biology*, 29(3): 486-490.

Chakraborty, R., De Andrade, M., Daiger, S. P. and Budovle, B. (1992). Apparent heterozygote deficiencies observed in DNA typing data and their implications in forensic applications. *Annals of Human Genetics*, 56: 45.

Chaudhari, M.V., Parmer, S.N., Joshi, C.G., Bhong, C. D., Fatima, M. S. Thakur and Thakur, S. S. (2009). Molecular characterization of Kenkatha and Gaolao (*Bos indicus*) cattle breeds using microsatellite markers. *Animal Biodiversity and Conservation*, 32(2): 50-54.

Clayton, D.A (1991). Replication and transcription of vertebrate mitochondrial DNA. *Annual Review of Cell Biology*, 7:453-478.

Collard, B.C.Y., Jahufer, M.Z.Z., Brouwer, J.B., Pang,E.C.K. (2005). An introduction to markers, quantitative trait loci (QTL) mappintg and marker-assisted selection for crop improvement: The basic concepts. *Euphytica*, 142:169-196.

Curi,R.A., Henrique, N. De Oliveira., M.A., Gimenes, S., Antonio, C.S. and Catalina, R.L. (2005). Effects of CSN3 and LGB gene polymorphisms on production traits in beef cattle. *Genetics and Molecular Biology*, 28: 262-266.

Czernekova, V., Kott, T., Dudkova, G., Sztankoova, Z. and Soldat, J. (2006). Genetic diversity between seven central European cattle breeds as revealed by microsatellite analysis. *Czech Journal of Animal Science* 51: 1-7.

Dadi, H., Tibbo, M., Takahash, Y., Nomura, K., Handa, H. and Amano, T. (2008). Microsatellite analysis reveals high genetic diversity but low genetic structure in Ethiopian indigenous cattl populations. *Animal Genetics*, 39: 425-431.

DAGRIS (2007). Domestic Animal Genetic Resources Information System (DAGRIS). (*eds. S. Kemp, Y. Mamo, B. Asrat and T. Dessie*). International Livestock Research Institute, Addis Ababa, Ethiopia. http://dagris.ilri.cgiar.org

Egito, A.A., Paiva, S.R., Albuquerque, M.S., Mariante, A.S., Almeida, L.D., Castro, S.R. and Grattapaglia, D. (2007). Microsatellite based genetic diversity and relationships among ten Creole and commercial cattle breeds raised in Brazil. *BMC Genetics*, 8: 83-89.

FAO (2000). World watch list for domestic animal diversity. 3rd edition.

Georgescu, S.E., Manea, M.A., Zaulet, M. and Costache, M. (2009). Genetic diversity among Romanian cattle breeds with a special focus on the Romanian Grey Steppe Breed. *Romanian Biotechnological Letters*, 14 (1): 4194- 4200.

Georgesm, M. and J. Massey, J. (1992). Polymorphic DNA markers in Bovidae (World Intellectual Property Org., Geneva) WO Publishing. No. 92/13102.

Goldstein, D.B., Linares, A.R. and Feldman, M.W (1995a). An evaluation of genetic distances for use with microsatellite loci. *Genetics*, 139 463-471.

Goudet, J., (2002). FSTAT, a program to estimate and test gene diversities and fixation indices (version 2.9.3.2). Available from http://www.unil.ch/izea/softwares/fstat.html.

Gras E, Matias-Guiu, X, Catasus L, Arguelles R and Cardona D (2000) Application of microsatellite PCR techniques in the identification of mixed up tissue specimens in surgical pathology. *Journal of Clinical Pathology*, 53:238–240.

Gray, M.W. (1989). Origin and evolution of mitochondrial DNA. *Annual Review of Cell Biology*, 5:25–50.

Guneren, G., Akyuz, B. and Ertugrul, O. (2010). Use of RAPD-PCR for genetic analyses on the native cattle breeds in Turkey. *Ankara Üniversitesi Veteriner Fakultesi Dergisi,* 57: 167-172.

Guo, L.Z., Hai, G.L., Qi, Z., Shan, L.G. and Yu, H.W. (2005). Genetic diversity analysis of five cattle breeds native to China using microsatellites. *Journal of Genetics*, 84:1, 77-80.

Guo, S.W. and Thompson, E. A (1992). Performing the exact test of Hardy-Weinberg proportion for multiple alleles. *Biometrics*, 48: 361-372.

Gupta, P.K., Roy, J.K. and Prasad, M. (2001). Single nucleotide polymorphisms: A new paradigm for molecular marker technology and DNA polymorphism detection with emphasis on their use in plants. *Current Science*, 80: 4-11.

Gwakisa, P. S., Kemp, S .J. and Teale, A.J. (1994). Characterization of Zebu cattle breeds in Tanzania using Random Amplified Polymorphic DNA markers. *Animal Genetics*, 25: 89-94.

Hannotte, O. and Jianlin, H. (2005). Genetic characterization of livestock populations and its use in conservation decision making. In: Ruane J. and A. Sannino, eds. pp. 89-96.The role of biotechnology in exploring and protecting genetic resources, Rome, Italy.

Hanotte,O., Bradley, D.G., Ochieng, J.W., Verjee, Y. and Rege, J.E.O. (2002). African Pastoralism: Genetic Imprints of Origins and Migrations. *Science*, 296: 336-339.

Hanotte, O., Tawah, C.L., Bradley, D.G., Okomo, M., Verjee, Y., Ochieng, J.W. and Rege J.E.O. (2000). Geographic distribution and frequency of a taurine *Bos Taurus* and an indicine *Bos indicus* Y specific allele amongst sub-Saharan African cattle breeds. *Molecular Ecology*, 9: 387-396.

Hansen, C., Shrestha, J.N, Parker, R.J., Crow, J.H., McAlpine, P.J. and Derr, J. (2002). Genetic diversity among Canadienne, Brown Swiss, Holstein, and Jersey cattle of Canada based on 15 bovine microsatellite markers. *Genome*, 45: 897–904.

Hartl, D.L. and Clark, A.G. (1989). Principles of population genetics. 2nd ed. Sinauer Associates. Sunderland, MA.

Hassen, F., Bekele, E., Ayalew, W. and Dessie, T, (2007). Genetic variability of five indigenous Ethiopian cattle breeds using RAPD markers. *African Journal of Biotechnology*, 6: 2274-2279.

Ibeagha-Awemu, E.M., Jager, S., Erhardt, G. (2004). Polymorphism in blood proteins of *Bos indicus* and *Bos taurus* cattle breeds of Cameroon and Nigeria, and description of new albumin variant. *Biochemical Genetics*, 42: 181-97.

Institute of Biodiversity Conservation (2007). Domestic Animal Genetic Resources of Ethiopia. http://www.ibc-et.org/.

Kalinwski, S.T. (2002). How many alleles per locus should be used to estimated genetic distances. *Heredity*, 88: 62-65.

Kantenen, J., Vikki, J., Elo, K. and Tanila ,M. (1995). Random amplified polymorphic DNA in cattle and sheep: application for detecting genetic variation. *Animal Genetics*, 26: 315-320.

Karthickeyan, S.M., Sivaselvam ,S.N., Selvam, R. and Thangaraju, P. (2009). Microsatellite analysis of Kangayam cattle (Bos indicus) of Tamilandu. *Indian journal of Science and Technology*, 2: 38-40.

Kemp, S.J., Hishida, O., Wambogu, J., Rink, A., Longeri, M.L., Ma, R.Z., Da, Y., Lewin, H.A,

Berandse, W. and Teale, A.J. (1995). A panel of polymorphic bovine, ovine and caprine microsatellite markers. *Animal Genetics*, 26: 299-306.

Kenneth C. J, Kenneth F. L and James D (2002) Characterization of 11 polymorphic tetranucleotide microsatellites for forensic applications in California elk (Cervus elaphus canadensis). *Molecular Ecology*, 2: 425-427.

Kimura, M., and J. F. Crow (1964). The number of alleles that can be maintained in a finite population. *Genetics*, 49: 725-738.

Kimura, M. and Ohta ,T. (1978). Stepwise mutation model and distribution of allelic frequencies in a finite population. *Proceedings of National Academy of Sciences of USA*, 75:2868-2872.

Kruglyak, S., Durrett, R., Schug, M.D., Aquadro, C.F. (1998). Equilibrium distribution of microsatellite repeats length resulting from a balance between slippage events and point mutations**.** *Proceedings of National Academy of Sciences of USA*, 95: 10074-10078.

Laval G., SanCristobal M., Chevalet C. (2002). Measuring genetic distances between breeds: use of some distances in various shortterm evolution models. *Genetics Selection Evolution*, 34: 481–507.

Lemecha, H., Mulatu, W., Hussein, I., Rege, E., Tekle, T., Abdicho, S., Ayalew, W. (2006). Response of four indigenous cattle breeds to natural tsetse and trypanosomosis challenge in Ghibe valley of Ethiopia. *Veterinary Parasitology*, 141: 165-176.

Levene, H. (1949). On a matching problem in genetics. *Annals of Mathematics and Statistics.* 20: 91- 94.

Li, Y., Chen, H., Lan, X.Y., Kong, X.J. and Min, L.J. (2008). Genetic diveristy of five cinese goat breeds assessed by microsatellite markers. *Czech Jornal of Animal Sciences.* 53: 315-319.

Loudianos G., Figus A.L., Loi A., Angius A., Dessì V., Deiana M., De Virgiliis S, Monni G., Cao A. and Pirastu M. (1994). Improvement of prenatal diagnosis of Wilson disease using microsatellite markers. *Prenatal Diagnosis*, 10: 999-1002.

Machado, M.A., Schuster, I., Martinez, M.L. and Campos, A.L. (2003). Genetic diversity of four cattle breeds using microsatellite markers. *Brasil Zootec*, 32(1): 93-98.

Manatrinon, S. Fischereleitner, F. and Baumung, R. (2008). Genetic characterization among some Austrian and Hungarian cattle breeds. *Arch. Tierz., Dummerstorf*, 51(5): 426-437.

Margaret, O. (2002). Characterization of the genetic diversity of East African cattle using microsatellite DNA markers. International Livestock Research Institute (ILRI), Nairobi, Kenya.

Maudet, C., Luikart, G. and Taberlet P. (2002). Genetic diversity and assignment tests among seven French cattle breeds based on microsatellite DNA analysis. *Journal of Animal Sciences*, 80: 942-950.

Mburu, D. and Hanotte, O. (2005). A practical approach to microsatellite genotyping with special refence to livestock population genetics. ILRI, Nairobi, Kenya.

McDonald, J.H (2009). Handbook of Biological Statistics. Sparky House Publishing, Baltimore, Maryland. 39-45.

Metta, M., Kanginakudru, S., Gudiseva, N and Nagaraju (2004). Genetic characterization of the Indian cattle breeds, Ongole and Deoni (*Bos indicus*), using microsatellite markers – a Preliminary study. *BMC Genetics*, 5:16 (http://www.biomedcentral.com/1471-2156/5/16).

Mirhoseinie, S. Y., Vahidie, S.F. and Gharehyazie, B. (2005). Survey of efficiency of six microsatellite loci in Iranian indigenous cattle and buffalo populations. *Iranian Journal of Biotechnology*, 3(1): 5-10.

Moore, S.S., Barendse, W., Berger, K.T., Armitage, S.M. and Hetzel, D.J.S. (1992). Bovine and ovine DNA microsatellites from the EMBL and GENEBANK databases. *Animal Genetics*,

23: 463-467.

Mullis, K., Faloona, F., Scharf, S., Saiki, R., Horn, G. and Erlich, H. (1986). Specific enzymatic amplification of DNA in vitro: the polymerase chain reaction. Cold Spring Harbor Symposia. *Quantitative Biology*, 51**:** 263-273.

Naqvi, A. N. (2007). Application of Molecular Genetic Technologies in Livestock Production: Potentials for Developing Countries. *Advances in Biological Research*, 1 (3-4): 72-84.

Nassiry, M.R., Javanmard, A. and Tohidi, R. (2009). Application of Statistical Procedures for Analysis of Genetic Diversity in Domestic Animal Populations. *American Journal of Animal and Veterinary Sciences*, 4(4): 136-141.

Nei M (1973). Analysis of Gene Diversity in Subdivided Populations. *Proceedings of National Academy of Sciences of* USA, 7: 3321-3323.

Nei,M. (1978). Estimation of average heterozygosity and genetic distance from a small number of individuals. *Genetics*, 89: 583-590.

Nei, M. (1972). Genetic distance between populations. *American Naturalist*, 106: 283-292.

Nei, M., Tajima, F. and Tateno, Y. (1983). Accuracy of estimated phylogenetic trees from molecular data. *Journal of Molecular Evolution*, 19: 153-170.

Nie, L., Yu,Y., Zhang, X.Q., Yang, G.F., Wen, J.K., Zhang, Y.P. (1999). Genetic diversity of cattle in south China as revealed by blood protein electrophoresis. *Biochemical Genetics* 37: 257-654.

Ott, J.(1991). Analysis of human genetic linkage, rev ed. The Johns Hopkins University Press, Baltimore.

Rachagani, S and Gupta I. D. (2008). Bovine kappa-casein gene polymorphism and its association with milk production traits. *Genetics and Molecular Biology* Online Ahead of Print ISSN 1678-4502.

Radko, A., Agata, Y., Tomas, Z. and Ewa, S. (2005). Genetic variability among Polish Red,

Hereford and Holstein-Friesian cattle raised in Poland based on analysis of microsatellite DNA sequences. *Journal of Applied Genetics*, 46(1): 89-91.

Radok, A. (2010). Application of a complementary set of 10 microsatellite DNA markers for parentage verification in Polish Red cattle. *Annals of Animal* Sciences, 10: 9-15.

Rege, J.E.O. and Lipner, M.E. (1992). African animal genetic resources: Their characterization, conservation and utilization. Proceedings of the Research Planning Workshop, 19–21 February 1992, Addis Ababa, Ethiopia, pp 172. International Livestock Centre for Africa, Addis Ababa, Ethiopia.

Rehman, M.S. and Khan M.S. (2009). Genetic diversity of Hiriana and hissar cattle from Pakistan using microsatellite analysis. *Pakistan vet. Journal*, 29 (2): 67-71.

Reynolds, J., Weir, B.S., and Cockerham, C.C (1983). Estimation for the coancestry coefficient: basis for a short-term genetic distance. *Genetics*, 105:767-779.

Rousset, F. (2008). GENEPOP (version 4.0). Available on http://www.cecill.info/index.en.html.

Slatkin, M. and Barton, N.H. (1989). A comparison of three indirect methods for estimating average levels of gene flow. *Evolution*, 43: 1349-1368.

Sodhi, M., Mukesh, M., Ahlawat, S.P.S. and Sobti, R.C. (2006). Microsatellite DNA typing for assessment of genetic variability in Tharparkar breed of Indian zebu (*Bos indicus*) cattle, a major breed of Rajasthan. *Journal of Genetics*, 3: 14-19.

Southern, E.M. (1975). Detection of specific sequences among DNA fragments separated by gel electrophoresis. *Journal of Molecular Biology* 98: 503-517.

Steffen, P. and Eggen, A. (1993). Isolation and mapping of polymorphic microsatellites in cattle. *Animal Genetics*, 24: 121-124.

Steigleder, C.S., Almeida, E.A. and Weimer, T.A. (2004). Genetic diversity of a Brazilian Creole cattle based on fourteen microsatellite loci. *Arch. Zootec.* 53: 3-11.

Stevanovic, J., Stanimirovic, Z., Dimitrijevich, V. and Maletic, M. (2010). Evaluation of 11 microsatellite loci for their use in paternity testing in Yugoslav Pied cattle (YU

Simmental cattle). *Czech Jouranl of Animal Sciences* 55: 221-226.

Sun, W., Chen, H., Lei, C., Lei, X. and Zhang, Y. (2007). Study on population genetic characteristics of Qinchuan cows using microsatellite markers. *Journal of Genetics and Genomics*, 34 (1): 17-25.

Sunden, S.L.F., Stone, R.T., Bishop, M.D., Kappes, S.M., Keele, J.W. and Beattie, C.W. (1993). A highly polymorphic bovine microsatellite locus: BM2113. *Journal Animal Genetics*, 24: 69.

Syvanen, A.C. (2001). Assessing genetic variation: Genotyping single nucleotide polymorphisms. *Nature Genetics* 2: 930-942.

Takezaki, N. and Nei, M. (1996). Genetic distances and reconstruction of phylogenetic trees from microsatellite DNA. *Genetics*, 144: 389-399.

Tang, J. (2008). A bioinformatics approach to marker development. PhD thesis, Wageningen University. The Netherlands.

Tanksley, S.D. and Orton, T.J. (1983). Isozymes in Plant Genetics and Breeding. Amsterdam: Elsevier Science.

Vos, P., Hogers, R., Sleeker, M., Reijans, M., Lee, T., Homes, M., Freiters, A., Pot, J., Peleman J, Kuiper, M., Zabeau, M. (1995). AFLP: a new concept for DNA fingerprinting. *Nucleic Acids Research*, 23: 4407-4414.

Wallace, D.C. (1986). Mitochondrial genes and disease. *Hospital Practise*, 21: 77-92.

Weaver R. and Hedrick P (1997) Genetics. W.m. C. Brown publisher. USA, ISBN-0-697-16000-9. Pp 532-557.

Weber, J.L., May, P.E. (1989). Abundant class of human DNA polymorphisms which can be typed using the polymerase chain reaction. *American Journal of Human Genetics*, 44: 388–396.

Weir, B.S. (1996). Genetic Data Analysis II. Sunderland, MA: Sinauer Associates, Inc.

Weir, B.S. and Cockerham C.C. (1984). Estimating F-statistics for the analysis of population

structure. *Evolution*, 38: 1358-1370.

Wiener, P., Burton, D. and Williams, J.L. (2004). Breed relationships and definition in British cattle: a genetic analysis. *Heredity*, 93: 597-602.

Williams, J.G.K., Kubelik, A.R., Livak, J., Rafalski, J.A., Tingey, S.V. (1990). DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. *Nucleic Acids Research*, 18: 6531-6535.

Wright S (1951) The genetical structure of populations. *Annals of Eugenics*, 15: 323-354.

Wright S (1965) The interpretation of population structure by Fstatistics with special regard to systems of mating. *Evolution*, 19: 395-420.

Yeh, F.C. and Yang, R. (1999). Microsoft Window-based Freeware for Population Genetic Analysis. Available on ftp://ftp.microsoft.com/Softlib/MSLFILES/HPGL.EXE.

Yim, Y.S., Herring, A.D. and Miller, M.F. (2000). Characterization of diverse cattle breeds for a microsatellite genetic marker of the bovine prolactine gene. *Texas journal of Agriculature and Natural Resources*, 13.

Yu, Y., Lian, L., Kunwen, J., Shi, X., Zhu, F., Nie, L. and Zhang, Y. (2004). Genetic Diversity and Relationship of Yunnan Native Cattle Breeds and Introduced Beef Cattle Breeds. *Biochemical Genetics,* 42.

Zulu,D.N., Smith, J., Cassell, G., David, R.W. (2008). Genetic Characterization of Zambian Native cattle Breeds. M.Sc. thesis, Virginia Polytechnic Institute and State University. Blacksburg, Virginia.

## APPENDIX 1- PHENOTYPIC DESCRIPTION OF ABIGA, BORAN, HORRO, SHEKO AND GURAGHE HIGHLAND CATTLE BREEDS OF ETHIOPIA

**1. Abigar Breed:** this breed is also known as Anuak breed. Abigar breed is grouped under sanga type breed that has evolved in Ethiopia as a result of interbreeding of the Longhorn-shorthorn and zebu type cattle (DAGRIS, 2007). Abigar cattle breeds are bred by the Nuer and Anuak tribes of Southwest Ethiopia and Dinka, Nuer and Skilluk tribes of southeastern Sudan along the White Nile and the adjacent lowlands (Alberro and Haile-mariam, 1982). Generally Abigar breed has large body size, long horns, and small humps. The typical coat colors of this breed include; light in shade, white with red or black patches (DAGRIS, 2007).

Figure 12: Abigar cattle breed

**2. Horro Breed:** DAGRIS (2007) has placed this cattle breed under zenga group. Generally the zenga breeds are emerged from the crosses of Sanga and zebu cattle breeds. Horro was developed through the interbreeding of various Abyssinian Highland zebu and Nilotic sanga, particularly the Abigar. It inhabits the north-western Ethiopia, mainly in the Horro Gudru area of eastern Wollega as well as the adjoining part Shewa and Illubabor (DAGRIS, 2007). The coat color of the breed is mainly brown or reddish brown but slightly lighter around the muzzle and

on the abdominal floor. Males are usually darker in color, being almost black on the humps and withers. Their dewlap is moderate in size and thin of skin. Their horns are also moderate in size, but larger than the zebu (DAGRIS, 2007).

Figure 13: Horro cattle

**3. Guraghe Highland Breed:** It is placed under the small east African zebu cattle(under the sub-group Abyssinian shorthorn zebu) that are believed to be descended from the recent introduction of zebu into Africa from Asia (DAGRIS,2007). The small Abyssinian short-horned zebu inhabits the higher altitude wetter agricultural areas and hence they are referred to as Ethiopian highland zebu. The Guraghe highland zebu, which is one of them, is found on the wet central and south western highlands of Ethiopia mainly with the Guraghe and Hadiya tribes (DAGRIS, 2007). Their coat color is mainly red, chestnut and roan.

Figure 14. Guraghe highland cattle

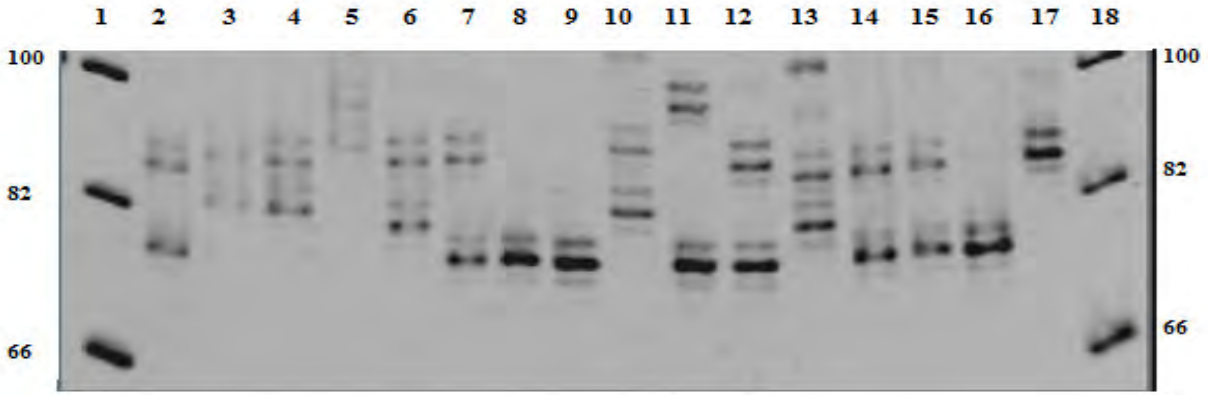
**4. Sheko Breed:** DAGRIS (2007) has placed the Sheko Breeds into humpless shorthorns *Bos taurus*. They are believed to be the last remnants of the original humpless (*Bos taurus*) cattle in eastern Africa. At present some of the Sheko manifest small humps that they inherited from zebu introgression. The breed is now considered endangered by gradual interbreeding with the zebu and Sanga, mainly with Abigar. Generally, they are small in size and humpless. Some of them have short horns and many are polled. Their coat color is brown or black and white. Sheko breeds inhabit the humid parts of south-western Ethiopia with the Sheko tribe around Bench zone. They are believed to have some level of trypano-tolerance (Lemecha *et al*., 2006).

Figure 15: Sheko cattle breed

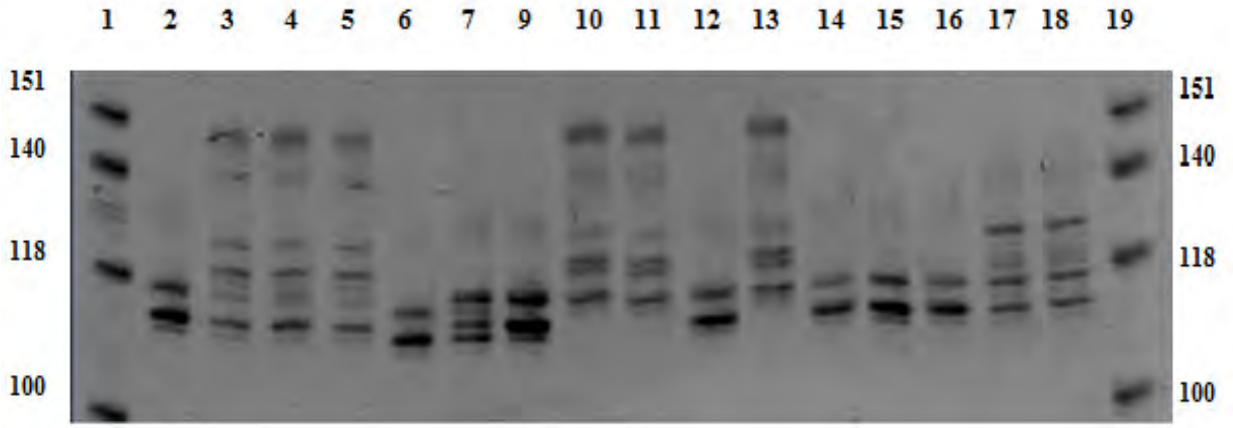
**5. Ethiopian Boran Breed**: are set under the large east African Zebu that are known to have descended from the secondary cattle domestication in the arid areas of the Fertile Crecent about 5000 BC. These breeds are characterized with their characteristic hump and pendulous dewlap. The main location of Ethiopian Boran is the southern rangelands of Ethiopia, around Liben, Mega and Arero plains with the Borana pastoralists and bordering area of northern Kenya (DAGRIS, 2007).

Figure 16: Boran cattle breed (Courtsy of DAGRIS, 2006)

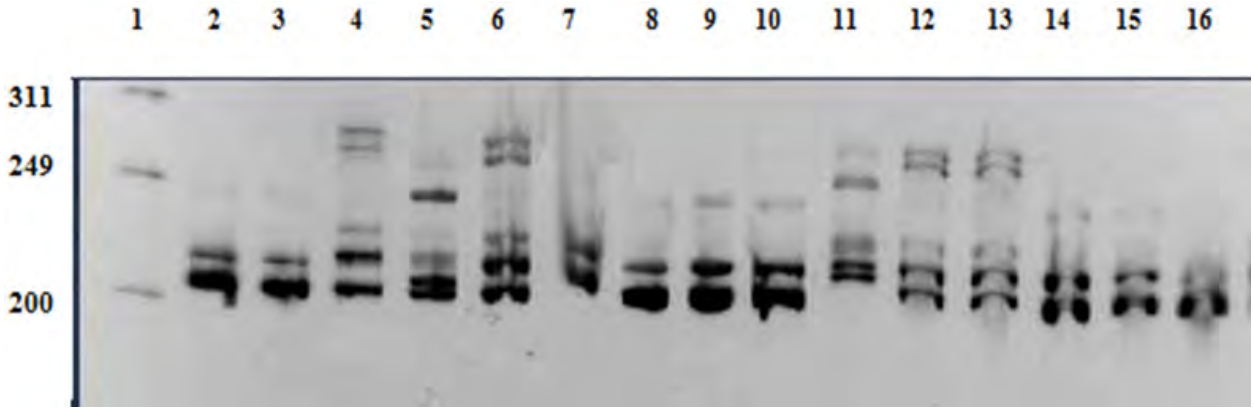
**APPENDIX 2-** SOME GEL IMAGES FROM PAGE ANALYSIS



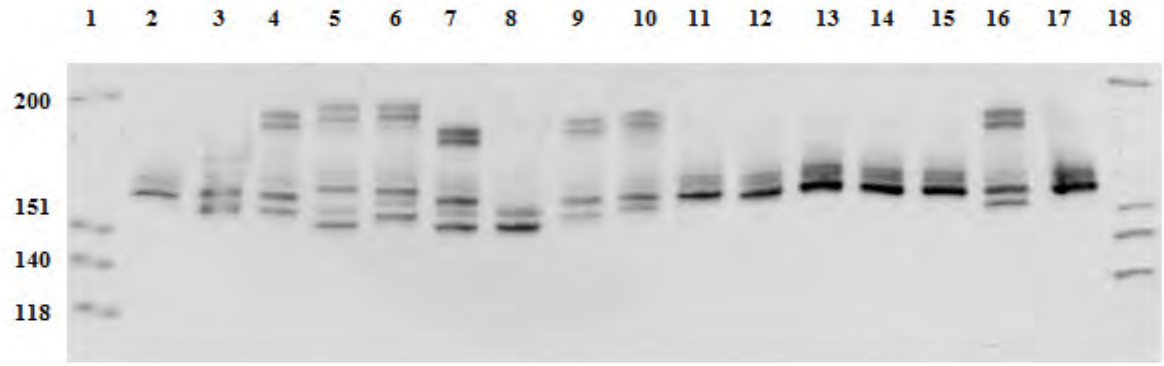

A: TGLA227 Microsatellite alleles resolved on 10% non-denaturing polyacrylamide gel after 5 hrs run at 300 volt. Markers (in bp) are loaded onto the outer lanes.



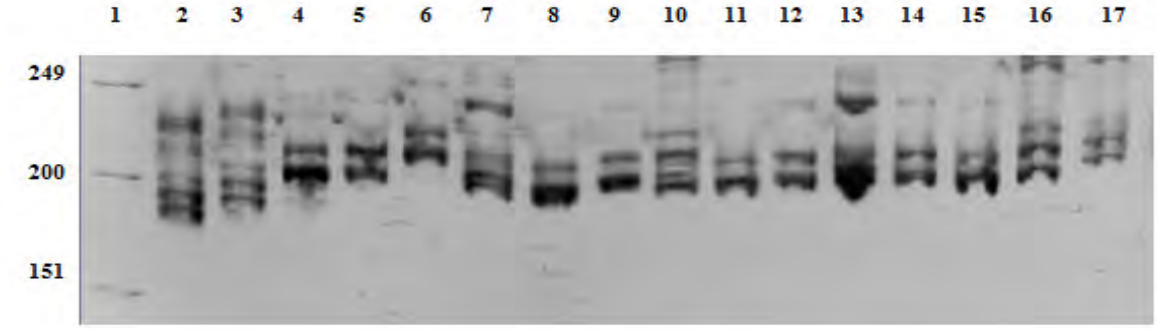

B: ETH3 Microsatellite alleles resolved on 10% non-denaturing polyacrylamide gel after 4.5 hrs run at 300 volt. Markers (in bp) are loaded onto the outer lanes.



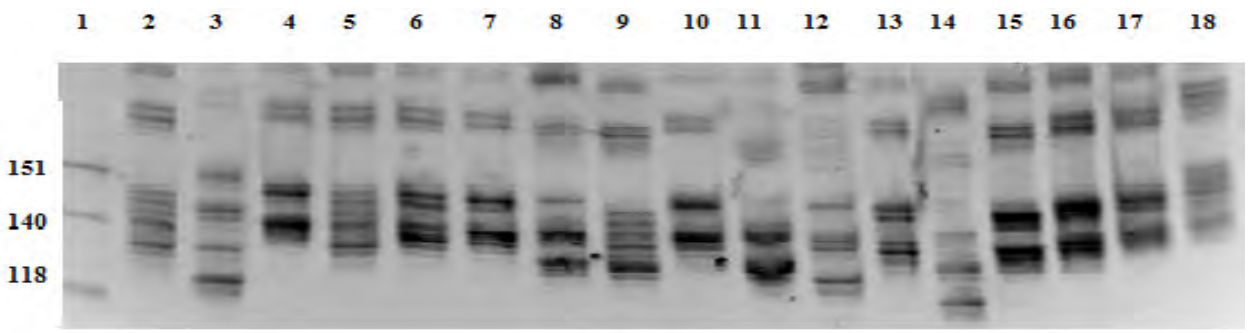

C: ETH10 Microsatellite alleles resolved on 8% non-denaturing polyacrylamide gel after 4.5 hrs run at 300 volt. Marker (in bp) is loaded onto the outer lane.



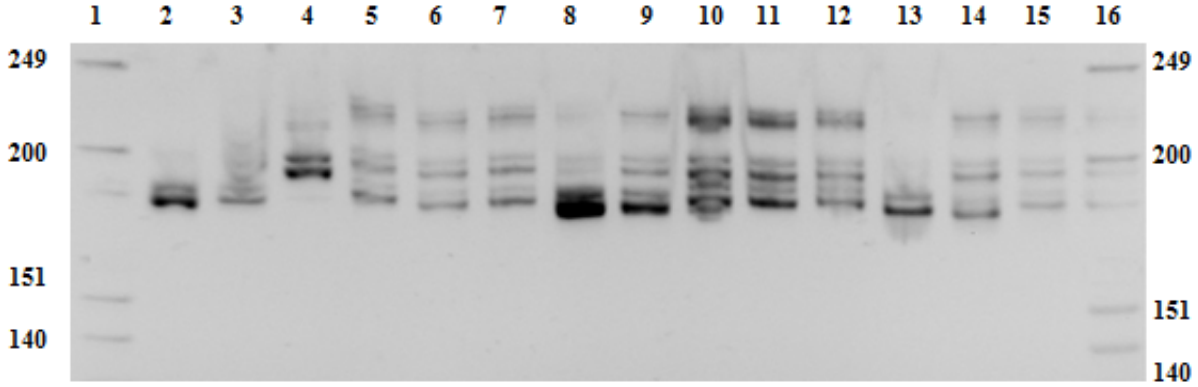

D: ILSTS049 Microsatellite alleles resolved on 8% non-denaturing polyacrylamide gel after 4 hrs run at 300 volt. Markers (in bp) are loaded onto the outer lanes.



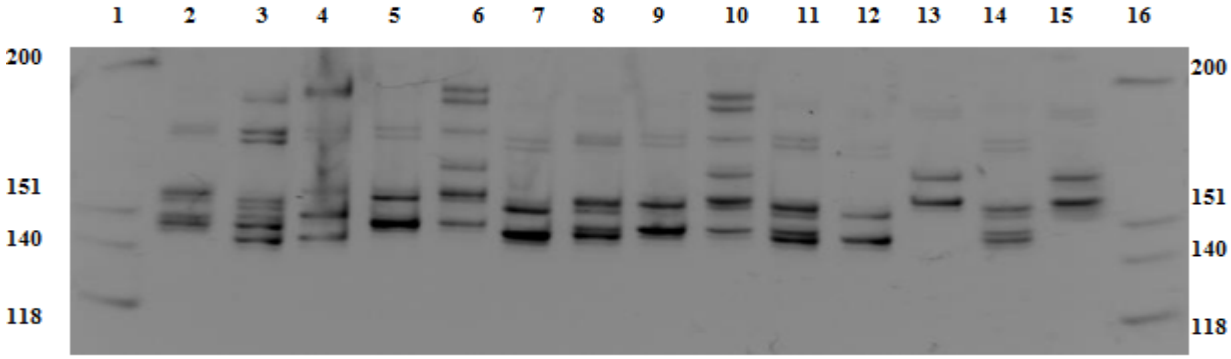

E: ETH152 Microsatellite alleles resolved on 8% non-denaturing polyacrylamide gel after 4.5 hrs run at 300 volt. Marker (in bp) is loaded onto the outer left lane



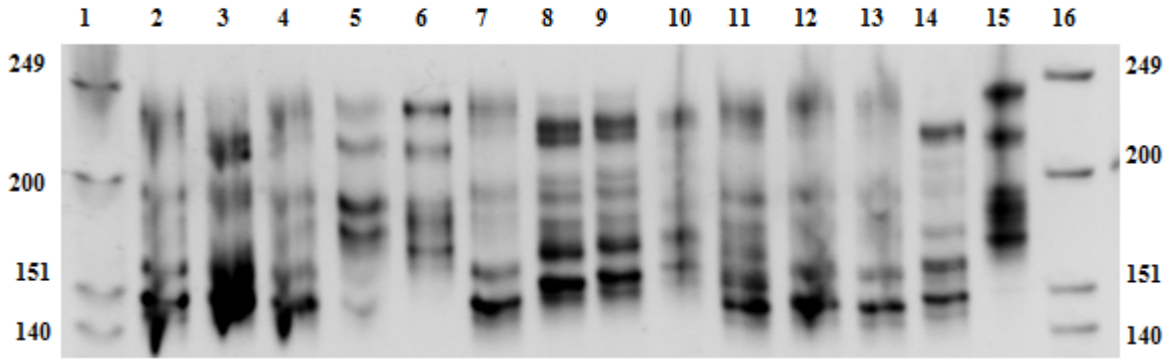

F: BM2113: Microsatellite alleles resolved on 8% non-denaturing polyacrylamide gel after 4.5 hrs run at 300 volt. Markers (in bp) are loaded onto the outer lanes.




G: BM1824 Microsatellite alleles resolved on 8% non-denaturing polyacrylamide gel after 4.5 hrs run at 300 volt.




H: ETH225 Microsatellite alleles resolved on 8% non-denaturing polyacrylamide gel after 4.5 hrs run at 300 volt. Markers (in bp) are loaded onto the outer lanes.




I: TGLA53 Microsatellite alleles resolved on 8% non-denaturing polyacrylamide gel after 4 hrs run at 300 volt. Markers (in bp) are loaded onto the outer lanes.

**Declaration**

I, undersigned, declare that this thesis is my original work. It has never been submitted in any institution and that all sources of materials used for the thesis have been duely acknowledged.

Name: **Solomon Diriba**

Signature:_____

Date:_____

This thesis has been submitted for examination with our approvals as:

Major Supervisor: **Kassahun Tesfaye (PhD)**

Signature:_____

Date:_____

Co-Supervisor: **Tadelle Dessie (PhD)**

Signature:_____

Date:_____