CHAPTER 1

INTRODUCTION

India is an agro-based developing country where livestock act as a vital instrument for economic growth and development. This sector supports crop agriculture by providing manure to enrich soil fertility, preparing land for crop production, giving power for harvesting and threshing in addition to providing milk, meat and eggs to fulfill the national demand for animal protein. The development of ancient Indian civilization pivoted around cattle and agriculture. At that time, cows were the measure of wealth and symbol of prosperity. A cow is very important because everything that a living cow produces has some important use. An individual who had larger number of cow was considered to be a wealthy person in their community (Elekes 2014).

1.1 Indian livestock:

Our country is a great reservoir of diverse livestock genetic resources. This is manifested from the availability of almost all species of farm animals and with a great number of genetic variants in each species (Notter 1999). World's best breeds of draught cattle and dairy buffaloes are found in India (Borghese and Mazzi 2005). The buffaloes and cattle breeds of Indian origin are very popular due to their supremacy for resistance to most of the common tropical diseases, utilizing inferior quality of feed and superior quality of adaptation to withstand heat (Sejian 2011). But there have been serious deterioration in the quality and quantity of Indian livestock biodiversity due to lack of selection based on their performance (Dairy India Yearbook 2007).

India possesses major shares; both in the number of breeds and in the total numbers of livestock in the world (Laible 2009, Thornton 2010). India possesses the largest livestock population in the world. As per 19th Livestock census, 2012 (http://dahd.nic.in/sites/default/files/Livestock%20%205.pdf) the total livestock (buffalo, cattle, goat, sheep, horses, ponies, mules, pig, donkeys, camels, mithun, and yak) population of our country is 512.057 million (Melkamu and Bannor 2015). Out of total livestock population, 190.9 (37.28%) million cattle, 108.7 (21.23%) million buffaloes, 65.1 (12.71%) million sheep and 135.2 (26.40%) million goats were recorded in that census. This country possesses 57 percent of word buffalo and 13 percent of the world cattle

population (Fig-1.1 and Table-1.1). Out of 190.9 million cattle, total indigenous cattle population is 151.172 million.

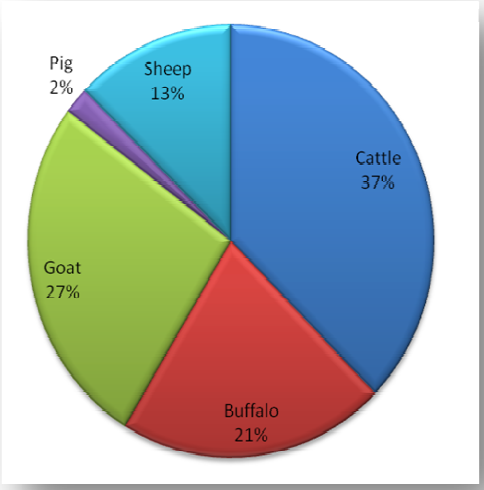

Fig-1.1: Distribution of Livestock Population in India (19th Livestock Census-2012).

Table-1.1: Indian animal genetic resources in respect of world perspective.

Name of Species	Total number of breeds in world	Total number of breeds in India	World population (million)	India Population (million)	Percentage in respect of world population
Cattle (*Bos taurus & Bos indicus)*	1019	40	1400	190.9	13
Buffalo (*Bubalusbubalis*)	123	13	168	108.7	57
Goat (*Capra hircus*)	576	26	924	135.2	15
Pig (*Sus scrofa*)	543	2	966	10	1
Sheep (*Ovis aries*)	1155	42	1000	65.1	6.5
Horses (*Equus caballus*)	694	6	58	0.625	1
Camel(*Camelus dromedarius*)	85	9	19	0.4	2.5
Donkey (*Equus asinus*)	157	1	53	0.319	0.26

Source: Statistics Division (Food and Agriculture Organization Corporate Statistical Database) & Ministry of Agriculture-India (2016).

As per approval of breed registration committee meeting held on 21st June, 2016 at New Delhi; altogether 160 indigenous livestock and poultry breeds (40 cattle, 13 buffalo, 26 goat, 42 sheep, six horse & pony, nine camel, six pig, one donkey and 17 chicken) have been registered in our country. Besides the registered breeds, many uncharacterized population of many species are also found in large numbers. This wide spread biodiversity of domesticated livestock and poultry breeds developed due to evolution within species as a result of adaptation and selection for meeting up the local needs (Henson1992).

1.2 Indian cattle biodiversity:

Our country is a wealthy reservoir of varied animal genetic resources and hardly any countries in the whole world have such wholesome number of breeds of farm animals with a great genetic diversity (Bhatia and Arora 2005). Most of the crop growing and livestock rearing areas of this country are dry with medium to low input production systems and such systems favour conservation of animal genetic resources. Our country have been played a significant role for enhancement of animal production in the world and also for improvement in the international gene pool of livestock (Lal et al. 2005). The India cattle breeds are broadly categories in to dual-purpose, milch and draught breeds. The 40 descriptive breeds of cattle in India comprise about 20% of the total cattle population. In addition, a large number of non-descript cattle and crossbred cattle comprise about 80% of the total cattle population. The western and northern regions of our country are the breeding track of different milch breeds of cattle like Sahiwal, Gir, Tharparkar and Red Sindhi. Kankrej, the heaviest cattle breed of India, originated in Gujarat. On the other hand the southern region of our country is also the breeding track of many other indigenous breeds of cattle like Kangayam, Hallikar, Khillari, Amrithmahal and dual purpose breeds of cattle like Deoni, Ongole and Krishanavalley. In the north-eastern part of India, the hilly cattle breed, Siri is found in Sikkim and West Bengal. Besides these, small sized cattle breeds like Punganur, Malnad, Gidda, Vechur and other breeds like Bargur, Umblacherry and Alambadi are also found (Singh 2016).

1.3 Indigenous cattle and its importance:

Both the descriptive breeds and non-descript cattle of indigenous origin are considered as indigenous cattle. Out of total indigenous cattle (151.172 million), the major part is non-descriptive cattle, contributing 113.25 million (74.92%) and rest 37.91 million (25.06%) are pure and graded indigenous cattle.

Indigenous cattle in our country act as savings and insurance for the cattle owners. Besides providing a means of livelihood diversification, livestock enables cattle owners to handle with fluctuations of income generation from labor wages or crop productions (Upton 2004). Besides these, the cattle also supply dung, which is used as fertilizer in the fields as well as fuel in the form of dung cakes in village areas. It was found that fermentation of 75 percent of the collected animal dung may produce an estimated about 195 million MW energy. Additionally, nearly 236 million tons of organic manure would provide around 35 million tons of nitrogen, more than the existing nitrogen chemical fertilizer manufacturing capacity in India (Smith 2011). The cow dung and urine are used to increase the soil fertility and maintain soil structure. Cattle urine is used as organic pesticide. In Ayurveda system of medicine, the medicinal properties of milk, urine and dung of indigenous cattle are well established in our country (Ghotge 2004).

The economic potential of cattle cannot be measured by simply looking at performance. Rare or endangered cattle breeds are highly adapted and their comparative performance should be evaluated within their own environmental conditions. The indigenous breeds of cattle should not be evaluated with the other foreign breeds of cattle kept under rigorous management system and modified superior conditions. Moreover, those indigenous breeds of cattle should be valued under which condition they developed and evaluated with the products for which they were selected (Henson 1992). The milk production of the dairy cattle can be partially fulfilled through two ways, by increasing the numbers of indigenous cattle and through crossbreeding with exotic cattle. These cross-breeding practices are harmful either to the environment or to the genetic resources of indigenous cattle (Hall and Ruane 1993). Replacing local

cattle with exotic cattle breeds is economically productive, labor saving and environmentally efficient for short term but it may not be viable for future (Mendelsohn 2003).

1.4 Cattle population trend:

There are 4.1% and 8.94% decrease in total and indigenous cattle population respectively noticed in Indian livestock census, 2012 with respect to 2007 livestock census. But the exotic/crossbreed cattle population have increased by 20.18% from 2007 to 2012 (Table-1.2). There is a noticeable increase in a limited number of specialized cattle breeds, while several indigenous cattle breeds have declined in quantity and in quality over the years (Dairy India Yearbook, 2007).

Table-1.2: Changes in cattle population from 2007 to 2012.

Category of cattle		Population (2007) (million)	Population (2012) (million)	Population Change (%)
Exotic and Crossbred	Male	6.844	5.971	-12.75
	Female	26.216	33.760	28.78
Total Exotic and Crossbred		33.060	39.732	20.18
Indigenous	Male	76.779	61.949	-19.32
	Female	89.236	89.224	-0.01
Total Indigenous		166.015	151.172	-8.94
Total Cattle		199.075	190.904	-4.10

Source: 19th Livestock census report-2012, Ministry of Agriculture, Government of India.

1.5 Eastern sub-Himalayan region:

The Eastern sub-Himalayan region, covering the areas lying between 82.70°E to 100.31°E longitude and 21.95°N to 29.45°N latitude comprising total areas of 524,190 sq. km. This region is situated between Kali Gandaki River in central Nepal in the west and Myanmar in the east. It includes North Bengal, Sikkim, Bhutan, Nepal, South-East Tibet and North-East India (Chettri et al. 2010). These vast areas have been well known to the scientific community as

a biodiversity hotspot. The extensive range of Eastern Himalayan biodiversity is an useful important area for studying the association between loss of ecosystem services and loss of biodiversity. The Eastern Himalayan region is subdivided into five distinguishing areas, a) Darjeeling Himalayan region, b) Sikkim Himalayan region, c) Bhutan Himalayan region, d) Nepal Himalayan region (Central, Eastern and Southern Nepal) and e) Arunachal Himalayan region (https://en.wikipedia.org/wiki/Eastern_Himalaya).

1.5.1 Climate:

The climate of Eastern Himalayan region is very chill in winter and cool in summer. The warm season generally commences in the middle part April and attains maximum temperature in the month of June. This season lasts up to the end of August. The average temperature and average rainfall in the Eastern Himalayan region are found to be 20°C and 500 mm respectively. In the winter season, snowfall is very common phenomenon. For this reason snow is accumulated in the valleys of Chumbi , Rangeet and Teesta (https://en.wikipedia.org/wiki/Climate_of_India).

1.5.2 Livestock:

North East hilly regions (NEH) of India are the home tract to diverse animal genetic resources including cattle, buffalo, sheep, goat, equines, pigs, mithuns, yaks, etc. As per 19th livestock census report-2012 (http://dahd.nic.in/sites/default/files/Livestock%20%205.pdf), the total livestock population in that NEH regions is around 21 million, out of which 11.49 million cattle, 0.84 million buffaloes, 4.37 million goats, 0.23 million sheep, 3.82 million pigs, 54 thousand mithuns and 16 thousand yaks were recorded. Most of the livestock populations of different species in that region are nondescript in nature. Several native breeds or populations like one cattle breed (Siri), three buffalo breeds (Assamese, Manipuri and Sikamese), one goat population (Assamese hill goat), two sheep breeds (Banpala and Tibetan), two chicken population (Miri and Chittagong), two duck populations (Nageshwari and Pati/Desi), two pig breeds (Ghungroo and Dome), one equine breed (Manipuri), two yak populations (Arunachali and Sikkim) and two mithun

populations (Arunachali and Nagami) are also seen in this NEH regions (http://www.kiran.nic.in/genetic_animal.html).

1.5.3 Agriculture:

The Eastern sub-Himalayan soil is acidic in nature and the hill slopes are embarked into successive steps or terraces on which the spring crops are grown plentifully. Shifting type of cultivation is generally noticed in these areas and agriculture is supplemented by fishing, animal husbandry, barter trade and hunting. Agricultural production is not sufficient for meeting the local demand for feed (Bhasin 2011). The majority of farmers of these areas operate mixed crop-livestock type of farming systems. The raising of livestock is incorporated with food crop production. The crops provide feed and fodder and on the other hand livestock provide milk, meat and milk products such as cheese and ghee. Livestock act as subsistence and also as a source of cash income. In this area, livestock also supplies draught power for ploughing the agricultural land and provide power for other agricultural operations such as transport and threshing (Tulachan and Neupane 1999).

1.6 Siri cattle:

Siri cattle breed is the specialized cattle breed of the sub-Himalayan region with high adaptation to a wide range of temperature variations (8° to 24°C in summer and 0° to 14°C in winter) and high rainfall areas. Out of 40 indigenous cattle breeds in our country, the Siri is the only one indigenous descriptive cattle breed of West Bengal and Sikkim. This breed is the only Indian cattle breed with the cervico-thoracic type of hump. This breed has high adaptation to a wide range of hilly terrain (altitudes 150-2500 m) of the Himalayas. These cattle carry a thick hair coat all around the year to give protection from severe cold and heavy rains (Sharma et al. 2008). Siri cattle are a very important component of the survival farming system as a source of milk and milk products, draught power and manure. Siri breeders are communities in remote hilly areas of Sikkim and West Bengal where farmers live under extreme poverty and remain in food-insecure condition.

1.6.1 Origin and distribution of Siri cattle:

Siri cattle breed is the main cattle resource of Bhutan and said to be the real home tract of this breed. Presumably, Siri cattle have some lineage from the cattle in Tibet (Joshi and Phillips 1953). Similar type of black colour with white markings small cattle have been found in Sikong, province of China in the northeast of Bhutan (Nivsarkar et al. 2000). Crossing of Siri cattle with Nepali cattle look like Siri, but those crossed cattle can be identified by their colour and position of hump and horns. Those crossed cattle are known as Kachcha Siri or imitation Siri cattle (Joshi and Phillips 1953).

1.6.2 Location and topography:

The breeding tract of Siri cattle lies between 27° and 28° 1' north latitude, and 88° and 90° east longitude and between 1,200 m and 3,000 m above MSL (mean sea level) (Nivsarkar et al. 2000). Siri rearing places in Sikkim and in Darjeeling and Jalpaiguri districts of West Bengal are situated in the eastern Himalayas between 26°43' and 28°10' north latitude and between 89°4' and 88°58' east longitude.

1.6.3 Population:

As per 19th livestock census (2012), the total Indian population of Siri cattle is 17749. The Siri population in West Bengal and Sikkim are 5479 and 11254 respectively. But the population of Siri cattle is declining day by day due to the intensification of animal husbandry, widespread introduction of exotic breeds over the years and lack of proper initiative for the conservation of the genetic resource.

1.7 Breed characterization of cattle:

A large number of cattle in India are nondescript in nature and cannot be designated as recognized cattle breed. To overcome this situation, there is an urgent need of extensive evaluation and description of Indian genetic resources

through widespread field studies. Description and identification of the cattle breeds were initially performed in our country in the early thirties on the basis of a few undefined subjective parameters. There is an urgent necessity to evaluate cattle genetic resources in India on the basis of physical conformation, performance and genetic makeup of the cattle (Drucker et al. 2001). Attempts should also be taken to study and evaluate the morphological, biochemical and molecular markers in order to establish as independent cattle breeds.

1.7.1 Phenotypic characterization:

The term 'phenotypic characterization of Animal Genetic Resources (AnGR)' is the process of identifying breed populations and describing their characteristics of their production environments. Geographical distribution of breed populations is also an important part of phenotypic characterization. The Government of India in 1984, realizing the importance of animal genetic resources in our country, established the National Bureau of Animal Genetic Resources (NBAGR) at Karnal, Haryana, with the authorization of identification, characterization, evaluation, conservation and utilization of livestock and poultry genetic resources. Additionally, recognizing the requirement of authentic national documentation system of precious indigenous livestock genetic resources with identified characteristics, a system for "Registration of Animal Germplasm" has been initiated at Karnal through National Bureau of Animal Genetic Resources (NBAGR), by the Indian Council of Agricultural Research (Bhatia and Arora 2005). This novel initiation would protect to the precious animal genetic diversity and make possible for improvement of animal genetic resources.

1.7.2 Genetic characterization:

Since 1990, molecular data have become more important and appropriate for the characterization of animal genetic diversity (Groeneveld et al. 2010). In 1993, a FAO (Food and Agriculture Organization) working group projected a program for characterization of animal genetic resources globally (Rege and Gibson 2003). Scientists in many countries in the world worked independently to characterize locally available breeds of livestock. The FAO has attempted to

construct worldwide comprehensive molecular datasets through breed characterization for most livestock species. The study of the molecular diversity of livestock at the genetic level has become a most effective and important area of research. The molecular works were based on the use of neutral genetic marker data (Boettcher et al. 2010). This molecular data worked as an estimate of the likelihood genetic variation within breeds or in between breed. The tasks are as follows: a) Searching and identification of the wild ancestral species of most livestock, b) recording the site of domestication of livestock, c) determination of the genetic constitution of breeds through quantitative measures of the diversity, subdivision or admixture, assortative mating, introgression and inbreeding, d) reconstruction of the phylogenetic relationships of livestock populations, e) enlightening evolutionary history of species and population and f) investigation of algorithms that obtain a prioritization of breeds for conservation from molecular data (Groeneveld et al. 2010).

Most studies have concentrated on the well-recognized breeds in developing countries with relatively little attention to many local breeds which have stayed largely free of systematic selection and contain much of the original genetic diversity. Identification of genomic regions for determining the functional diversity, quality of adaptation, disease resistance capacity and productive traits are the most important areas for those studies.

1.8 Markers:

1.8.1 Morphological markers:

Morphological markers are used for identification, classification, and characterization of different species or populations. Morphological markers refer to external characteristics of the animal and it includes body shape, anatomical characteristics, coat colour and skin structure through measurement and direct visual observation (Van Wezel and Rodgers 1996, Gizaw et al. 2007). An animal's phenotype is mainly determined by its genetic makeup and its existing environment. This marker is efficiently used to evaluate qualitative traits and to

characterize phenotypic differences between individuals through direct observations and measurements.

1.8.2 Cytogenetic markers:

Cytogenetic markers are very useful method to assess farm animal genetic resources on the basis of chromosomes numbers, morphology and abnormalities of farm animals. This marker includes chromosome numbers, structures, karyotypes, banding patterns, repeats, deletions, inversions and translocations (Saccone et al. 2002). Chromosomes carries of genetic material and any changes or mutations in it may causes genetic variation in individual animal. This type of marker is widely used to determine the specific location of a gene on the specific chromosome and its relative position with respect to other genes (Dobzhansky and Dobzhansky 1937, Ferguson-Smith 2014).

1.8.3 Biochemical markers:

Blood type and isozymes are mainly used as biochemical markers and represent biochemical traits. Biochemical markers are analyzed through protein electrophoresis. Protein electrophoresis is an effective, rapid, straight forward and economic technique for polymorphism study. This marker provides more detailed representation of polymorphisms than other markers like morphological and cytogenetic markers. For this reason, it is still extensively used for the origin and classification of species (Jonker et al. 1982).

1.8.4 Molecular markers (DNA-based markers):

Molecular markers are mainly used for assessing DNA level genetic variations between individuals and different populations. With the advancement of molecular biotechnology, molecular markers became very useful and rapid tools. Mutations within individual genome are the main basis of molecular markers and also the most reliable and effective markers till date. Now a day different types of molecular markers have been effectively used to determine DNA polymorphisms (Bruford et al. 2003). Polymerase chain reaction (PCR) (Mullis et al. 1986) can amplify a fragment of DNA *in vitro*. Since the invention

of PCR, a series of techniques have emerged in combination with it, e.g. PCR—RFLP, AFLP, RAPD, simple sequences repeats (SSRs), single nucleotide polymorphisms (SNPs), DNA barcoding and D-loop polymorphisms (Dubey 1993).

1.8.4.1 RFLP marker:

RFLP (restriction fragment length polymorphism) was the first DNA-based marker and widely used markers in AnGR for constructing genetic linkage maps and breeding program development. This marker was established by Grodzicker et al. (1974). This method is commonly used to recognize DNA polymorphisms among different individuals. In this particular method, insertions, duplications, nucleotide base substitutions, deletions and inversions within the whole genome on an individual can be identified through electrophoresis method (Kristensen et al. 2001).

1.8.4.2 RAPD marker:

In RAPD (randomly amplified polymorphic DNA), the target genomic DNA amplifies with short arbitrary primers (commonly 10 bp) in a PCR reaction and used to produce DNA profiles for detecting amplified fragment length polymorphisms. RAPD was first developed in 1990 (Bardakci 2001, Tingey and del Tufo 1993). RAPD-PCR fingerprints are generally used successfully for detecting genetic diversity among different species (Yang et al. 2013).

1.8.4.3 AFLP marker:

AFLP (amplified fragment length polymorphism) markers is the combination of the PCR and RFLP methods and was first developed by Zabeau and Vos (1993). This is well established method for its effective, rapid and economical technique to distinguish a large number of polymorphic genetic resources. For genome typing, this AFLP method is an ideal molecular method. This method is extensively useful to distinguish genetic polymorphisms and to characterize and evaluate animal genetic resources (Ajmone et al. 2002, Negrini et al. 2007).

1.8 4.4 Microsatellite DNA marker:

Microsatellite DNA is also known as simple sequences repeats (SSRs) or short tandem repeats (STRs). This marker generally consist of motifs made up of 1–6 base pairs tandem repeated sequence several times (e.g. CACACACACACACACA) (Litt and Luty 1989, Tautz 1989). The tandem repeats at microsatellite loci are generally conservative and the repetition sequence is highly variable between different species and even also different individuals within same species. By designing specific primers for specific conserved sequences and amplifying the repeat sequences by way of PCR, genetic polymorphisms can be detected via electrophoresis (Gupta et al. 1994).

1.8.4.5 SNP markers and whole-genome sequencing:

SNP marker is coming after SSRs and RFLPs markers. In scientific community, it is considered as a third generation molecular marker technology (Gill 2001). This marker has been successfully applied to study the genetic variation among different species and breeds (Amaral et al. 2008, Brooks et al. 2010). SNP is a unique molecular marker technology first proposed by Lander (1996). Sequence polymorphism occurred due to a single nucleotide mutation at specific locations in the DNA sequence. This type of polymorphism includes single base transitions, insertions, deletions and transfusions (Lander 1996).

1.8.4.6 Mitochondrial DNA-based markers:

Mitochondrial DNA (mtDNA) sequences are the novel markers of choice for domestication studies. The separation of mtDNA lineage within a particular livestock population only took place through the series of process of domestication of wild female or due to the incorporation of female into the domestic stock. Those mtDNA based markers are commonly used in the scientific field to recognize the geographical origins, the number of maternal lineages and their probable wild progenitors. It may also provide important

information on the geographical distribution of livestock species (Guo et al. 2006).

1.9 Molecular characterization based on mitochondrial DNA:

Mitochondrial DNA was first discovered by Nass and Nass (1963). About twenty years later the human mtDNA was completely sequenced (Anderson et al. 1982a). The mitochondrial genomes in eukaryotes contain total 37 genes. Out of 37 genes, two genes encode ribosomal RNA (rRNA) molecules, 13 genes encode proteins and another 22 genes encode transfer RNA (tRNA) molecules. The protein coding genes in the mtDNA are concerned with primarily in the series of process of oxidative respiration. In all vertebrates, the total number of genes in the mtDNA is equal but may change the order of those genes (Linacre and Tobe 2011). On the other hand in mammalian species, the order of the loci of genes on the mtDNA is identical but may vary between taxonomic classes. The order is dissimilar between avian and mammalian mitochondrial genomes. There are two strands in the mtDNA of vertebrates, one is light stand (L-strand) and another is heavy stand (H-strand) (Fig-1.2). The H-strand holds eight tRNA genes and one protein-coding gene (ND6) and considers as the sense strand. On the other hand the L-strand holds 14 tRNA genes, two rRNA genes and 12 protein-coding genes (Xia et al. 2007). In comparison to the nuclear DNA, the mtDNA is inherited maternally in a haploid manner, has no introns and recombination rarely occure (Burton et al. 2013, Hebert et al. 2003a, Rubinoff and Le Roux 2008). Phylogenetics often utilizes mitochondrial DNA for its usefulness in studying species-level relationships and recently diverged taxa.

MtDNA possess several amazing characteristics that make it a very useful molecular marker for study of evolution. The evolutionary rate of the mtDNA is not homogenous, but it shows variation in different regions that are subject to strong functional constraints. Generally, the slowest evolving mitochondrial genes are found in the areas those encoding the two ribosomal RNAs (rRNAs) and the 22 transfer RNAs (tRNAs). It has been establish that all the genes responsible for three subunits of cytochrome c oxidase, 16S rRNA, cytochrome b and some tRNA are highly conserve (Tobe and Linacre 2010). Mitochondrial genes are highly conserved at the amino acid level. Usually, the

mutations are narrowed at third codon position, with a predominance of transitions than transversions (Hebert et al. 2003a). It was reported that some nucleotides of mtDNA are more susceptible to mutations than others. The frequency of mutation for all four nucleotides is not random (Baer et al. 2007).

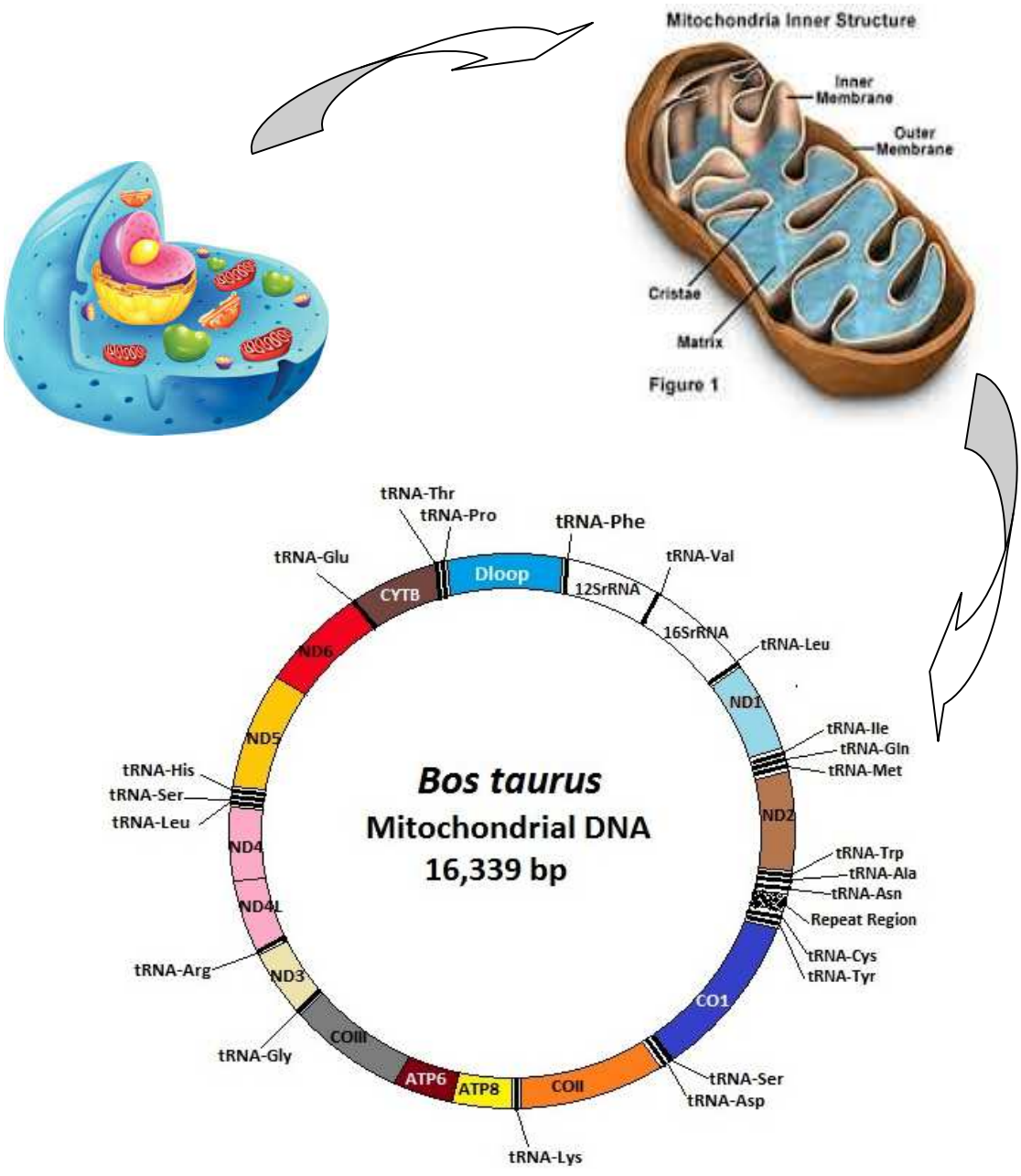

Fig-1.2: Schematic diagram of Mitochondrial Genome.

1.9.1 DNA barcoding:

DNA barcoding involves a 650 base pair fragment sequence of the mitochondrial gene, *COI* (cytochrome c oxidase subunit I). Various authors have proposed different purposes for DNA barcoding, but the most prevalent concept of DNA barcoding is the making of a library of sequences that can be implemented to identify previously described taxa (Francis et al. 2010, Galimberti et al. 2013, Rubinoff and Le Roux 2008). The barcoding region is a segment of a gene within a protein-coding region of the mitochondrial genome. The changes in nucleotide will have an effect on the amino acids and ultimately the protein production. But the third positions of codons are not under strong selection to remain constant because of the redundancy of the amino acid coding system. Another advantage of using a protein-coding regions instead of any RNA-encoding genes is the relative rarity of indels (Hebert et al. 2003a).

1.9.2 Displacement loop (D-loop):

D-loop is a non-coding control region of mtDNA expression about 1,100 bp long. It is well established that the variations study of sequences in D-loop region is very useful tool for explaining the diversification and origin of modern cattle populations (Matisoo-Smith and Horsburgh 2012).

1.10 Aim and objective of current research:

Only few studies were conducted regarding the physical characteristics, particularly the colour patterns and their relevance to reproductive as well as productive parameters of Siri cattle. The methodical study of the pattern and extent of genetic variability among different livestock breeds of diverse species is a prerequisite for scientific conservation of animal genetic resources and also may support in the development of scientific breeding programs (Kim et al. 2002, Vasconcellos et al. 2003). In the present study, analysis of mtDNA sequence variations particularly *COI* and D-loop of Siri cattle has been done to elucidate the genetic diversity as well as the ancestry of this breed. The

cytogenetic and phenotypic character studies will be helpful for categorization Siri cattle whether this breed is *B. indicus, B. taurus* or cross of these. This would also help to establish adequate planning for conservation, selection for better performance, breeding strategy and breed improvement. As such, interventions to improve the productivity of this cattle breed shall directly contribute to increasing rural livelihood and poverty alleviation. Considering all the above issues and immense literature review on the present scenario of the importance of Siri cattle at a national and international level, the present study was designed focusing on the following objectives:

A. Phenotypic characterization of Siri cattle for breed registration.
B. Identification of species generating the bar-code sequence for Siri cattle and breed specific mitochondrial DNA sequence marker in Siri cattle.
C. To generate mitochondrial DNA D-loop sequences of Siri cattle from different places of Eastern sub-Himalayan region.
D. Phylogenetic study of Siri cattle with other indigenous and exotic cattle breed.
E. Study the molecular evolution of Siri cattle based on mtDNA sequences.
F. Measurement of genetic distances and divergence times of Siri cattle with other cattle breed and other close related species.