GENETIC DIVERSITY OF Y CHROMOSOME IN PUNGANUR CATTLE

BY

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CERTIFICATE

SHAIK. REHMAN, GVM/2018-004 has satisfactorily prosecuted the course of research and that the thesis entitled **"GENETIC DIVERSITY OF Y CHROMOSOME IN PUNGANUR CATTLE"** submitted is the result of original research work and is of sufficiently high standard to warrant its presentation to the examination. I also certify that the thesis or part thereof has not been previously submitted by his for a degree of any university.

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CERTIFICATE

This is to certify that the thesis entitled **"GENETIC DIVERSITY OF Y CHROMOSOME IN PUNGANUR CATTLE"** submitted in partial fulfillment of the requirements for the degree of **Master of Veterinary Science** of **Sri Venkateswara Veterinary University, Tirupati** is a record of the bonafide research work carried out by **SHAIK. REHMAN, GVM/2018-004** under our guidance and supervision. The subject of the thesis has been approved by the Student's Advisory Committee.

No part of the thesis has been submitted by the student for any other degree or diploma. The published part has been fully acknowledged. All the assistance and help received during the course of investigation have been duly acknowledged by the author of the thesis.

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CONTENTS

LIST OF FIGURE

LIST OF TABLES

LIST OF SYMBOLS AND ABBREVIATIONS

%	Per cent
^{0} C	Degrees centigrade
A	Annealing
AMELY	Amelogenin, Y-linked
bp	Base Pair
cm	Centimeter(s)
CpG	Cytosine's followed by Guanine residues
D	Denaturation
DDX3Y	Dead box polypeptide 3, Y-linked
dh_20	Distilled Water
DNA	Deoxyribo Nucleic Acid
E	Extension
EDTA	Ethylene Diamine Tetra Acetic acid
EIF1AY	Eukaryotic translation initiation factor 1A, Y-linked
EIF2S3Y	Eukaryotic translation initiation factor 2, subunit 3, structural gene Y-linked
FAO	Food and Agricultural Organization
FE	Final Extension
FISH	Fluorescence In Situ Hybridization
GC	Guanine Cytosine content
GOI	Government of India
g	grams
hr	Hour
HSFY	Heat Shock transcription Factor, Y-linked; >100 copies
ID	Initial Denaturation
i.e.	That is
LRS	Livestock Research Station
M	Morality
µl	Microliter
ml	Milliliter
mm	Millimeter
min	Minute
mtDNA	Mitochondrial DNA

NCBI	National Center for Biotechnology Information
nm	Nanometer
No	Number
OFD1Y	Oral Facial Digital syndrome 1, Y-linked
PAB	Pseudo Autosomal Boundary
PAR	Pseudo Autosomal Region
PCR	Polymerase Chain Reaction
pmol	Picomole
RBC	Red Blood Corpuscle
RBMY	RNA Binding Motif protein, Y-linked
RFLP	Restriction Fragment Length Polymorphism
RH	Radiation Hybrid
rpm	Rotations Per Minute
SDS	Sodium Dodecyl Sulphate
SNP	Single Nucleotide Polymorphism
SRY	Sex Determine Region
STR	Short Tandem Repeats
SVVU	Sri Venkateswara Veterinary University
Ta	Annealing Temperature
TAE	Tris Acetate EDTA
TBE	Tris Borate EDTA
TSPY	Testis Specific Protein, Y-linked; multicopy
U	Unit
UBE1AY	Ubiquitin activating enzyme E1, Chr Y
UTY	Ubiquitously Transcribed tetratricopeptide repeat gene, Y-linked
USP9Y	Ubiquitin Specific Peptidase 9, Y-linked
V	Volts
ZFY	Zinc Finger Protein, Y-linked
ZNF280A	Zinc Finger Protein 280A, Y-linked; >100 copies, pseudo genes
ZNF280B	Zinc Finger Protein 280A, Y-linked; >100 copies

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Date :

Place : Gannavaram **(*Sk. Rehman)***

DECLARATION

I, **SHAIK. REHMAN** hereby declare that the thesis entitled **"GENETIC DIVERSITY OF Y CHROMOSOME IN PUNGANUR CATTLE"** submitted to **Sri Venkateswara Veterinary University, Tirupati** for the degree of **MASTER OF VETERINARY SCIENCE** is the result of original research work done by me. I also declare that the materials contained in this thesis have not been published earlier.

Date :

Place : Gannavaram

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ABSTRACT

Punganur is a miniature cattle originated from Chittoor district of Andhra Pradesh. Studies so far aimed at characterization of the breed at phenotypic level and molecular level using autosomal microsatellite markers and mitochondrial DNA. In addition to autosomal microsatellite markers and mitochondrial DNA, the Y-chromosomal variation also plays a key role in understanding population admixture to trace paternal lineage. Differentiation of paternal lineages via analysis of Y-chromosomal variation adds significantly to what can be inferred from mtDNA and autosomal variation in cattle. So far, the Y-chromosomal markers of Punganur cattle is not explored.

Hence, the present study is proposed with the following objectives.

Objectives of investigation:

1. To characterize Y- chromosome of Punganur cattle using Y chromosome specific markers
2. To determine Y chromosome haplogroups in Punganur cattle

A total of 60 Punganur bulls covering different geographical regions in and around Andhra Pradesh and Telangana were screened to identify four Y chromosome specific SNPs (ZFY9:120>C/T; USP9Y: 76426>C/T, SRY: 1748>G/T and UTY19:

423>G/T). To detect these SNPs, a PCR-Restriction Fragment Length Polymorphism (RFLP) strategy was designed for screening three SNPs (USP9Y: 76426>C/T, SRY: 1748>G/T and UTY19: 423>G/T) and Allele specific PCR method was adopted for screening SNPs in ZFY9 (120>C/T) in the present study. The resulting restriction profiles and Allele specific genotype were combined to yield haplotype (TCTT) specific for Punganur cattle representing Y3 haplogroup of cattle. These results indicated that all the animals in the present study belong to Y3 haplogroup which underpin the purity of paternal lineage of Punganur cattle. The Y chromosome specific haplotype combination (TCTT) deciphered in our study can be exploited as a marker in Punganur breed conservation and improvement studies for detecting the purity/indigenous origin of paternal lineages.

CHAPTER – I

INTRODUCTION

India is endowed with the largest livestock population in the world. Livestock plays an integral part of India's farming system. A wide variety of agro-ecological zones (20 No's. and 60 sub-zones) existing in India have helped to develop a large numbers of breeds of different livestock species, including poultry, for the benefit of local people under different farming systems. Indian zebu cattle (*Bos indicus*) evolved over time and bestowed with many desirable attributes like disease resistance, tolerance to heat stress, adaptability to environmental fluctuations and extremities.

According to 20th livestock census, India possessed 532.82 million number of livestock in 2018-2019 comprising 192.5 million cattle, 109.85 million buffaloes, 74.26 million sheep, 148.88 million goat, 9.06 million pig and the rest being constituted by other species such as yak, mithun, camel, donkey and mules (GOI, 2019). The Food and Agriculture Organization (FAO) data base indicates that India comprises 70 cattle, 20 buffalo, 62 sheep, 34 goat, 8 pig, 3 ass, 7 horse, 9 camel and 30 poultry breeds in addition to many more not characterized and accredited breeds. Out of which 50 cattle, 17 buffalo, 44 sheep, 34 goat, 9 camel, 7 horse, 10 pig, 3 ass and 19 poultry breeds were registered in National Bureau of Animal Genetics Resources, Karnal.

A plethora of attempts were made to improve the local genetic merit of animals by importing the exotic germplasm which results in dilution of Indian breeds. Punganur is one such breed of Indigenous cattle on the brink of extinction with only few of them left. Therefore, there is an ardent need to develop strategies for conservation of animal genetic resources in order to fully utilize the genetic merit of the animals.

The Punganur breed is named after the town of its origin, Punganur, in Chittoor district situated in south-eastern tip of Deccan plateau and was developed by Raja of

Punganur. Its average height is 70-90 cms. The breed is known for its short stature, high milk production efficiency and efficient reproductive characters. Study of historical reports and recent literature reveals it as a miniature or small sized milch cattle, rarely exceeding 12 to 14 hands in height and with much of appearance of Mysore breed (Shortt, 1876). It was recorded to be mainly raised in the Poonganoor zamindari estate in the vicinity of Vellore, Kunneambaddy, Gooriattum and Kungoonthy of the Madras Presidency.

Wallace (1888) expressed Punganur cattle as a small variety of Mysore with good milking ability. Slater (1918) reported that Punganur breed is almost extinct with few survivors got crossed with inferior cattle. Littlewood (1936) highlighted the breed's vulnerable status in his book "Livestock of South India" as in the state of practical disappearance because of farmer efforts to increase the size of their cattle by crossing with bulls of Mysore origin. This endangered breed is now being conserved at Livestock Research Station, Palamaner.

In the mid 1950's an attempt was made at Government Livestock farm, Palamanery, Chittoor district to put together a herd of cattle and bred them (Nath, 1993). In 1995 cross breeding of Punganur with Kerry was undertaken as a pilot study by the Government of Andhra Pradesh at Government livestock farm Palamaner, A.P and after some years they terminated it (Report, 1990). Later demographic survey in 1995 revealed that none of the Punganur cattle were available in its breeding tract and a total of 29 cows and 5 bulls were procured form Kakinada in order to conserve and develop the breed (Report, 1993-1994).

The efforts made by the scientific workers and with the advent of cultural and fancy preferences of progressive farmers, identification and breeding of the animals disbursed elsewhere in the state which eventually resulted in an increase in number from 733 (Report, 2007) to 2772 (pure Punganur) and 56 (Punganur graded) (Report, 2014).

Numerous studies have been conducted during the recent times, to incorporate information from nuclear, mitochondrial, and Y chromosome specific markers to delineate genetic diversity among cattle. Compared to mtDNA and autosomal DNA, bovine Y chromosome phylogeny is still poorly defined. In bovines, it is clear that novel Y-specific biallelic markers will be instrumental to the clarification of some issues related to cattle domestication from a male perspective. Further studies of male lineages contribute towards the better understanding of origin and relationships among different breeds. Though information on cattle Y chromosome sequence and polymorphism is restricted, microsatellites (STR) and Single nucleotide Polymorphism (SNPs) mapped to non-recombining region of Y chromosome have been employed to study the event of domestication and differentiation among bovid species. Some of these markers have also been used to detect introgression and to distinguish between *Bos taurus* and *Bos indicus* patrilines.

Genetic studies pertaining to cattle Y chromosome have mainly focused on the assessment of migration patterns, differences in diversity among different breeds and admixture of *Bos taurus* and *Bos indicus*. Recent discovery of five polymorphic sites on Y-chromosome paved the way for the identification of three haplotypes (Y1, Y2 and Y3) in contemporary cattle, with Y1 being more frequent in North-West Europe *B taurus*, while Y2 is predominant among southern European *B taurus* and Anatolian cattle, and Y3 being exclusively to *Bos indicus*. The Y3 haplogroup specific to *Bos indicus* can be distinguished from Y1 and Y2 haplogroups by identifying sequence variants in genes like *USP9Y, UTY, ZFY* and *SRY* (Gotherstrom *et al*., 2005, Li *et al*., 2007)..

In India genetic studies pertaining to Y chromosome are scanty and many studies were carried out till now aimed at characterization of the breed at phenotypic level and molecular level using autosomal microsatellite markers and mitochondrial DNA. In addition to autosomal microsatellite markers and mitochondrial DNA, the Y

chromosomal variation also plays a key role in understanding population admixture to trace paternal lineage. Differentiation of paternal lineages via analysis of Y-chromosomal variation adds significantly to what can be inferred from mtDNA and autosomal variation in cattle (Edwards *et al*., 2000, Lindgren *et al*., 2004, Gotherstrom *et al*., 2005, Anderung *et al*., 2005, Li *et al*., 2007). Though many microsatellites (STRs) and SNPs revealing Y chromosomal genetic diversity were reported in the literature, majority of the STRs were found to reveal very little information about the polymorphism, hence the present study was designed with major emphasis on four Y chromosome specific genes like *ZFY9*, *USP9Y*, *UTY* and *SRY* to delineate Y-haplotypic diversity in Punganur cattle breed.

Keeping in view of the above circumstances the present study is proposed with the following objectives.

Objectives:

1. To characterize Y chromosome of Punganur cattle using Y specific markers.
2. To determine Y chromosome haplogroups in Punganur cattle.

CHAPTER - II

REVIEW OF LITERATURE

2.1 ORIGIN AND DOMESTICATION OF CATTLE

Cattle were originally identified as three separate species: *Bos taurus*, European or "taurine" (including related species from Africa and Asia), *Bos indicus*, the Zebu; and the extinct *Bos primigenius*, the aurochs. These have been reclassified, with three subspecies: *Bos taurus primigenius*, *Bos taurus indicus*, and *Bos taurus taurus*, as one species, *Bos taurus*. (Wilson and Reeder, 2005). During the Holocene and Pleistocene period, the aurochs or the wild ox (*Bos primigenius*) was found to be widespread throughout Europe, Northern Africa and Southern Asia. Archeological findings revealed that Zebu cattle were domesticated 8,000–9,000 years ago and dispersed across northwestern South Asia (Patel, 2009).

Genetic studies pertaining to cattle domestication have shown that modern cattle (*Bos taurus* and *Bos indicus*) is resulted from at least two genetically distinct auroch populations (*Bos primigenius* and *Bos nomadicus*) (Chen *et al*., 2010; Bradley *et al*., 1996). According to the mtDNA analysis the time of divergence between *Bos taurus* and *Bos indicus* is estimated to be ranges between 117000 to 275000 years (Bradley *et al*., 1996) and according to microsatellite data analysis it is between 610000 to 850000 years (MacHugh *et al*., 1997).

2.2 ASSESSMENT OF GENETIC DIVERSITY IN CATTLE

Molecular characterization of animal genetic resources is an important step in order to assess genetic status and to make conservation plans. With the advent of technologies, molecular markers proved to be invaluable resources for characterization of livestock populations (Gamaniel and Gwaza, 2017). The genetic diversity of the population can be

assessed by using various types of molecular markers like microsatellites, mitochondrial markers and Y chromosomal markers (Hanotte and Jianlin, 2005; Joshi *et al*., 2012).

2.2.1 MOLECULAR MARKERS

Recently, molecular markers emerged as an instrument to understand the patterns and processes, resulting in global biological diversity (Rubinoff and Holland, 2005). Protein polymorphisms were the first molecular markers used in livestock. The level of polymorphism observed in proteins is often low thereby reduced the general applicability of protein typing in diversity studies. The DNA based markers took advantage over the protein based markers due to high polymorphism exhibited by these markers, co-dominance nature and ease of genotyping and made the polymorphic DNA based markers as the markers of choice for molecular-based surveys of genetic variation (Hanotte and Jianlin, 2005). DNA markers are advantageous as they show genetic differences at a detailed level with no hindrance from environmental factors (Saker *et al.*, 2005).

2.2.2 DNA MARKERS

Polymorphic DNA markers include both nuclear and mitochondrial DNA (mtDNA) markers. The mtDNA (D-loop, cytochrome-b), Y chromosome (SNP, microsatellite), and autosomal microsatellite markers, shows different patterns of Mendelian inheritance namely maternal, paternal and biparental inheritance respectively. The idea about centers of origins, migration routes and geographic areas of admixture amongst populations of different genetic origins are provided by these markers (Hanotte and Jianlin, 2005).

Comprehension of the haplotype structure is very useful for tracing back the evolutionary history of the animals in order to recognize various variants of important physical and economic characteristics. As far as an individual's evolutionary tracing back is concerned, mtDNA and Y chromosomal haplotypes are of special significance due to their respective descending matri- and patri-lineage.

In India, genetic studies pertaining to Y chromosome are scanty and many studies carried out till now aimed at characterization of the breed at phenotypic level and molecular level using autosomal microsatellite markers and mitochondrial DNA. In addition to autosomal microsatellite markers and mitochondrial DNA, the Y chromosomal variation also plays a key role in understanding population admixture to trace paternal lineage. Differentiation of paternal lineages via analysis of Y-chromosomal variation adds significantly to what can be inferred from mtDNA and autosomal variation in cattle (Edwards *et al*., 2000, Lindgren *et al*., 2004, Gotherstrom *et al*., 2005, Anderung *et al*., 2005, Li *et al*., 2007).

2.3 GENETIC DIVERSITY BASED ON Y CHROMOSOME IN CATTLE

2.3.1 CYTOGENETIC ANALYSIS

The bovine (*B. taurus*) chromosome complement includes 29 pairs of autosomes, all acrocentrics, and the submetacentric X and Y chromosomes, which can be readily distinguished from the autosomes in metaphase preparations.

Cytogenetic banding techniques (Evans *et al*. 1973) have been used to identify each of the autosomes and sex chromosomes. Banding techniques Giemsa (G-banding) and Reverse to Giemsa (R- banding) have also been used for band pattern comparisons among cattle, sheep, and goats, and results have supported the hypothesis of a common origin of all bovids as proposed by Wurster and Benirschke (1968).

Y-chromosome morphology and size differ among bovis; it is submetacentric in cattle (BTAY), sheep (OARY), and goats (CHIY) and, acrocentric in zebu (BINY) and in river buffalo (BBUY). Because of their small size and the fact that they are largely heterochromatic, Y-chromosome banding techniques do not offer enough resolution for chromosome rearrangement and evolutionary studies among bovids. This led to develop a bovine Y chromosome-specific DNA library and chromosome painting probe.

The availability of Y chromosome-specific molecular probes permitted Di Meo *et al*. (2005) to synergistically use chromosome banding and gene/marker localizations by FISH to infer Y-chromosome similarities and possible evolutionary patterns within and between Bovinae (BTAY, BINY, BBUY), and Caprinae (OARY, CHIY).

Di Meo *et al*. (2005) hypothesized that Y chromosomal rearrangements between these species are the result of a pericentric inversion or a centromeric transposition between BTAY and BINY, pericentric inversion between BTAY and BBUY, pericentric inversion with a major loss of heterochromatin between BBUY and OARY/CHIY, and a centromere transposition with loss of heterochromatin between BTAY and OARY/CHIY.

2.3.2 CHROMOSOME GENETIC AND PHYSICAL MAPS

There are now over 5307 genetic markers mapped on the bovine genome. Of these, 1507 markers are type I (INRA, bovine genome databases, http://locus.jouy.inra.fr/cgi-bin/bovmap/intro.pl), and 3800 are type II (Ihara *et al*. 2004). However, none of these maps include the male-specific Y (MSY) region and are only limited to the RH map of the PAR.

This MSY region represents about 95% of the chromosome length and essentially comprises repetitive sequences making physical mapping and chromosome sequencing difficult. This nonrecombining region also makes genetic mapping impossible. Because of these limitations, a first-generation BTAY RH7000 map was generated (Liu *et al*. 2002). Thirteen markers were localized in the PAR region and 46 markers in the MSY. The *AMELY* gene was localized in the MSY close to the pseudoautosomal boundary (PAB) region and both the *SRY* and *TSPY* genes in the MSY region. Retention frequencies of Y-chromosome markers ranged from 18.5% to 76.5%. Retention frequencies higher than 55% were indicative of multiple marker copies making the map order of these markers difficult to achieve.

2.3.3 CHARACTERIZATION OF Y-CHROMOSOME

Sex chromosomes evolved from a pair of autosomes (Muller, 1914) and are believed to be the result of genetic sex determination that originated when a sex-determining gene was acquired by one member of the pair to become the sex specific determining chromosome. This gave origin to the male heterogamety, XX female: XY male, and female heterogamety, ZW female: ZZ male, systems. The former is observed in mammals, some species of turtles, insects, lizards, and even some plants and the latter in birds, amphibians, snakes, and some species of fish, turtles, insects, and lizards (Modi and Crews, 2005).

Y-chromosome is present in different shapes and sizes in different species. In humpless cattle (*Bos taurus*), it is a small submetacentric, whereas in humped zebu (*Bos indicus*), it is a small acrocentric with visible small p-arm ("p" for "petite," means small); in river buffalo (*Bubalus bubalis*), it is a small acrocentric chromosome, whereas in both sheep (*Ovis aries*) and goat (*Capra hircus*), it is very small and metacentric.

2.3.3.1 *The Pseudoautosomal Region*

In general, the mammalian Y-chromosome has two arms that harbor two pseudoautosomal regions (PAR1 and PAR2), which recombine with their homologous regions on X-chromosome; and a male-specific Y region (MSY) (or non-recombining region on Y [NRY]), which does not recombine with the X-chromosome.

These PAR1 and PAR2 consist of 5% of the entire chromosome. Being male-specific, the holandric genes are mostly involved in male sex determination, fertility, and development. The PAR is the region with highest homology between the X and Y chromosomes. Only one PAR region has been observed in Bovinae by FISH analysis (Ponce de Leo´n and Carpio, 1995; Robinson *et al*. 1998) and synaptonemal complex analysis (Switonski and Stranzinger, 1998).

Van Laere *et al*. (2008) have compared PAR, X-specific, and autosomal sequences of the bovine genome (Btau_4.0 build) to the available Y-chromosome sequences and comparable sequences obtained from the human genome NCBI 36 build. Their results indicate that there is a good correlation between GC and CpG island content with recombinational activity being higher in the PAR, next in the autosomes, followed by X-specific, and lower on the Y-specific sequences, in that order. There is also higher recombination rate in the human PAR than in the bovine. As in humans, the density of CpG islands in bovine is highest in autosomes, followed by the X chromosome, Y chromosome and PAR.

2.3.3.2 *Pseudoautosomal boundary*

Van Laere *et al*. (2008) identified the bovine PAB to be located between the *SHROOM2*and *GPR143* genes on the X chromosome. Sequence homology between X and Y PAR sites was found to be near perfect at 99.97%. Fine alignment of sequences and comparison between X- and Y-specific regions adjacent to the PAR allowed the identification of a 413 bp fragment with reduced homology at 86.20% separating the PAR from the nonhomologous gonosome-specific regions.

Van Laere *et al*. (2008) concluded that the bovine PAB occurred after ruminants diverged from other mammals and further proved that the bovine PAB is ruminant specific by comparing PAB sequences of other ruminants (bison, yak, banteng, zebu, and sheep) as well as by comparing the female to male gene copy ratio of the *SHROOM2* and *GPR143* genes in ruminants (cattle), nonruminants (horse, cats, dogs, mice, and humans) and cetacean (porpoises) assumed to be a close relative of ruminants

2.3.3.3 *The MSY region*

The male-specific region, comprising 95% of the DNA content of the Y chromosome, can be divided into two regions, (1) euchromatic and (2) heterochromatic. According to the human Y-chromosome sequence, the euchromatic region contains at

least four different types of sequences: (1) X-transposed (99% similarity to the Xq21), (2) X-degenerate (60%–96% to the X), (3) ampliconic, and (4) centromere repetitive sequences (Skaletsky *et al*., 2003). This euchromatic region also harbors all genes of the MSY, whereas the heterochromatic region contains Y-specific repetitive sequences.

The absence of recombination makes genetic mapping of the MSY virtually impossible, and the depth, breadth, and complexity of the repetitive sequences make sequencing extremely difficult making the mammalian Y chromosome a difficult target for linkage mapping and sequencing (Tilford *et al*., 2001; Liu and Ponce de Leo´n, 2007). The variation observed in MSY sequences and gene content is believed to be generated through two different mechanisms. One mechanism is the differential retention of genes from the proto-X/Y chromosomes during the process of Y-chromosome degeneration in different lineages.

Independent Y-chromosome decay during evolution (Graves, 2006; Wilkerson *et al*., 2008) led to different lineages retaining different subsets of Y genes and a diverse and lineage-specific Y-chromosome gene content. The second mechanism that led to diverse Y gene content is the autosome-to-Y transposition.

It is believed that the autosome-to-Y transposition of male fertility genes is a recurrent theme in mammalian Y-chromosome evolution (Hurst, 1994; Saxena *et al*., 1996; Graves, 2000). As a result, the content of male-beneficial genes in MSY has increased in spite of a 95% loss of the ancestral Y-chromosome genes due to absence of recombination.

Autosome-to-Y transposition events apparently occurred separately in different lineages with newly acquired Y-chromosome genes from diverse genomic locations (Murphy *et al*., 2006). This resulted in lineage-specific Y-chromosome genes (families) that account for a significant portion of the gene (and sequence) variation among mammalian Y chromosomes.

Recently reported the bovid lineage-specific Y-chromosome genes, *ZNF280BY*, *ZNF280AY*, and *PRAMEY*, which were derived from a transposition of a gene block (*ZNF280B-ZNF280A-PRAME*) on BTA17 (Liu *et al*., 2009b; Yang *et al*., 2011; Chang and Liu, 2010).

The gene content of the bovine MSY is, however, still unknown. Earlier investigations were focused on the development of Y chromosome-specific markers and the identification of bovine MSY genes by a comparative mapping approach. To accelerate this process, Ponce de Le´on and Carpio (1995) generated a BTAY-specific DNA phage library. This library has proven to be a very important resource not only for generating Y-specific markers and building the first-generation BTAY RH-map.

To date, a total of 15 orthologs ((1) *UBE1AY*, (2) *AMELY*, (3) *DDX3Y*, (4) *USP9Y*, (5) *UTY*, (6) *EIF1AY*, (7) *EIF2S3Y*, (8) *OFD1Y*, (9) *RBMY*, (10) *ZFY*, (11) *TSPY*, (12) *HSFY*, (13) *SRY*, (14) *DAZ*, and (15) *CDY*) of human or mouse Y chromosome-related genes have been identified in cattle.

These genes except for *DAZ* and *CDY* are physically mapped on BTAY either by a comparative approach (*RBM1A1*, *ZFY*, *DDX3Y*) (Liu *et al*. 2009a), or by restriction mapping (*AMELY*, *TSPY*), or by FISH and/or RH mapping (*SRY*, *DDX3Y*, and *UTY*) (Liu *et al*. 2002, 2009b), or by testis direct cDNA selection and male-specific polymerase chain reaction (PCR) (*UBE1AY*, *AMELY*, *DDX3Y*, *USP9Y*, *UTY*, *EIF1AY*, *EIF2S3Y*, *OFD1Y*, *RBMY*, *ZFY*, *TSPY*, *HSFY*) (Liu *et al*. unpublished data). The *DAZ* and *CDY* gene families are not present on BTAY.

2.4 BOVINE Y-CHROMOSOME PHYLOGENY

Bovine phylogenetic studies based on mitochondrial DNA sequences indicate that domestication of taurine (*Bos taurus*) in the Near East and zebuine (*Bos indicus*) in the Indus Valley required at least two genetically distinct auroch (*Bos primigenius*) species.

In *Bos indicus* breeds, Y-chromosome research information is scanty. Few studies have until now focused primarily on the use of Y-specific microsatellite loci as an alternatives to evaluate semen quality in Crossbred bulls and four Y chromosomal spermatogenesis copy number variations are associated candidate genes in crossbred and purebred indicine bulls.

Differentiation of paternal lineages via analysis of Y-chromosomal variation adds significantly to what can be inferred from mtDNA and autosomal variation (Underhill *et al*. 2000). The absence of interchromosomal recombination outside the pseudoautosomal region (PAR) preserves original arrangements of mutational events, and thus male lineages can be traced both within and among populations.

Genetic drift is relatively strong due to the effective population sizes of Y-chromosomes being, at most, 25% of the autosomal effective population size (Jobling and Tyler-Smith, 2003). Effective population size is often reduced further by the relatively high variability of male reproductive success. As a result, the Y-chromosome is a sensitive indicator of recent demographic events, such as population bottlenecks, founder effects and population expansions.

A comparison of European, Southwest-Asian and Indian cattle reveals a gradual autosomal indicine-taurine cline from India to Anatolia and a sharper cline of the mtDNA and Y-chromosomal markers (Loftus *et al*., 1999; Troy *et al*., 2001; Kumar *et al*., 2003; Edwards *et al*., 2007). A meta-analysis of different microsatellite datasets revealed patterns of diversity and taurine–zebu admixture over Europe, South-West Asia and Africa (Freeman *et al*. 2004).

Zhai *et al*. (1993) used the cloned fragment as a probe to hybridize to the Southern blot of HindIII-digested cattle genomic DNA, a 1.7-kb male-specific fragment was detected, which is considered to contain the 200-bp fragment sharing high homology with the known SRY gene.

Edwards *et al*. (2000) observed the assessment of a panel of four Y-specific microsatellites markers for polymorphism in a range of cattle and related species. They concluded the genetic dichotomy often observed between zebu and taurine cattle is useful for detecting gene flow and introgression between the two taxa. These microsatellites were found to provide additional evidence concerning the origins of African cattle.

Some of the characteristics that make the Y chromosome unique among all other nuclear chromosome are the absence of recombination with the X chromosome at meiosis in the MSY region, the abundance of repercussions of the Y specific sequence, the evolutionary trend in its genes and its genetic content coherence in male development, spermatogenesis and fertility (Lahn and Page, 1997).

The evolutionary history of a particular Y chromosome on which it is located is being recorded by a set of linked markers or haplotype which serves as an important tool for investigating population or evolutionary processes. Y-haplotypes frequency and geography provide powerful information concerning migration, sexual flow and population relations (Jorde *et al*., 2000; Su *et al*., 2000; Underhill *et al*., 2000; Hammer *et al*., 2001; Kayser *et al*., 2001; Jobling and Tyler- Smith, 2003).

Microsatellites (STRs) mapped to the nonrecombining region (Bishop *et al*., 1994; Vaiman *et al*., 1994; Kappes *et al*., 1997; Liu *et al*., 2003) have been used to study domestication and differentiation among bovid species and to detect introgression and to distinguish between *Bos taurus* and *Bos indicus* patrilines (Edwards *et al*., 2000; Giovambattista *et al*., 2000; Hanotte *et al*., 2000; Li *et al*., 2007).

Tanaka (2000) reported a polymorphism in the bovine SRY gene (causal of a replacement Cys to Phe at position 214) which distinguishes *B. taurus* and *B. indicus* haplotypes.

Hanotte *et al*. (2000) described distribution of taurine, indicine and mixed phenotypes in regards to correlation with the Y-chromosomal *INRA124* microsatellite

alleles. A polymorphism with 2 alleles was detected; Friesians and N´Dama had only the 132-bp allele, the Sahiwal had only the 130-bp allele and the Barotses had both.

Cheng *et al*. (2001) reported the cloning and sequences of the SRY genes of yak and Chinese native cattle showing SRY genes in Bovidae are less divergent, especially in the coding and 3' regions.

Microsatellite genotyping allowed a reconstruction of zebu migration routes (Hanotte *et al.* 2002). In West Africa, zebu introgression is counteracted by the tsetse resistance of the native taurine breeds (Freeman *et al.* 2004; Ibeagha-Awemu *et al.* 2004).

The inheritance of Y chromosome as a single block of haplotype suggests lack of recombination in the male specific area of the Y (MSY). For population studies and to analyze genetic diversities within and between populations the Y polymorphic markers have been recognized to be ideally suited (Jobling and Tyler-Smith 2003; Mukherjee *et al*., 2001).

Analysis of mtDNA, Y-chromosomal DNA and microsatellites indicated a purely banteng origin of Indonesian Bali cattle. However, mtDNA and nuclear DNA in a Bali cattle population kept in Malaysia was of mixed zebu-banteng origin (Nijman *et al.* 2003).

Verkaar *et al*. (2004) described assays based on mutations in *SRY* (sex-determining region Y-chromosome) and in the multicopy *TSPY* (testis-specific protein, Y encoded) (Jakubiczka *et al*., 1993; Vogel *et al*., 1997).

Gou *et al*. (2010) reported Diqing cattle being humpless, and adapting to the cold weather and high altitude as yaks have, their paternal lineages were almost *B. indicus* types. Wenshan cattle being humped and adapting to tropic weather, their maternal lineages contain appreciable *B. taurus* type in contrast, to Nujiang cattle which receive almost equal influences from *B. taurus* and *B. indicus*.

The *SRY* gene of yak was differentiated from that of the other species by the presence of an SNP at position 226 (Kikkava *et al*., 2003). Analysis of same SRY region

reported discordance existed between male and female markers for the animals suggesting that introgression occurred.

Mannen *et al* (2004) examined mtDNA loop sequence variation and frequencies of *Bos taurus* and *Bos indicus* Y chromosome haplotypes and revealed 20% of Mongolian cattle carried *B.indicus* mitochondrial haplotypes and but Japanese and Korean cattle carried only *Bos taurus* haplotypes attributing to importing of zebu cattle and subsequent crossing with native taurine cattle.

A demographic study of the native Portuguese cattle breed Alentejana shows that only 24 Y chromosomes with an effective number of 2.73 males are currently represented out of the original number of 671 founder sires (Carolino and Gama 2008).

Gotherstrom *et al*. (2005) found two haplogroups, Y1 and Y2, to be dominant in northern and southern Europe respectively in an initial survey of European breeds.

Despite facing limitations, studies on the origin and relations among domestic races contribute to a better understanding of male lines (Edwards *et al*., 2000; Lindgren *et al*., 2004; Anderung *et al*., 2005; Gotherstrom *et al*., 2005; Li *et al*., 2007).

Assessment of Y haplotype diversity among species and the taxonomic origins of the genes can be best facilitated by combined investigation of Y-chromosome SNPs and microsatellite alleles, which are highly conserved (i.e., ~10−8 per site per generation in humans) and highly mutable (i.e., ~10−3 per locus per generation in humans), respectively (Hurles and Jobling, 2001).

Y chromosome-specific single nucleotide polymorphisms (SNPs) and microsatellites markers were therefore combined and used to investigate the genetic diversity and origins in cattle (Bradley *et al*., 1994; Budowle *et al*., 2005; Cai *et al*., 2006; Yang *et al*., 2011), dogs (Bannasch *et al*., 2005; Erdogan *et al*., 2013) and sheep (Niemi *et al*., 2013).

The Y chromosome's genetic diversity was estimated to be lower than that of autosomal chromosomes. (Liu *et al.*, 2003; Hellborg and Ellegren, 2004; Ginja *et al.*, 2009). In several mammalian species, including cattle, relatively low levels of Y-chromosome genetic diversity have been reported (Hellborg and Ellegren 2004; Lindgren *et al.* 2004; Bannasch *et al.*, 2005; Meadows *et al.*, 2006; Li *et al.*, 2007).

The mitochondrial macrohaplogroup T (*B. taurus*) has six haplogroups (T, T1, T2, T3, T4 and T5) and the Q haplogroup of European auroch origin identified in the Near East cattle populations (Troy *et al*. 2001; Mannen *et al*. 2004; Achilli *et al*. 2008). Two separate haplogroups P and R of European auroch ancestry were identified in an animal from Korea and animals from the Italian peninsula, respectively. Haplogroups Q, P, and R are found at low frequencies while the T3 haplogroup predominates in European cattle, which originated from the expansion of a small cattle population domesticated in the Near East. T4 was found in Japanese cattle and is a derived clade within T3 suggesting its origin from either the same genetic source as the T3 founder sequence(s), or at most from a genetically (and geographically) closely related population of aurochs. The T1 haplogroup was found mainly in Northern Africa while the T2 haplogroup was found mainly in Continental Europe, Anatolia, and the Middle East.

Y-chromosome phylogenetic studies are rare (Verkaar *et al*., 2004), and most have been focusing on taurine and zebuine crosses (Hanotte *et al*., 2000; Anderung *et al*., 2005; Edwards *et al*., 2007).

Comparison of the Y3 sequence with an *SRY* sequence from an Indian Sahiwal zebu (GenBank accession number AY079145) (Verkaar *et al*., 2004) revealed three additional differences downstream of the open-reading frame, indicating zebu-specific Y-chromosomal SNPs.

Guiyun and Hongquan (2004) performed pcr based method for cattle SRY-Specific sequence to amplify a 163-bp region resulting all male samples had bands representing SRY-specific, whereas all female samples and control samples had no band.

In combination with a SNP in *UYT19* (Perez-Pardal *et al*., 2010a; Ginja *et al*., 2010; Gotherstrom *et al*., 2005; Kantanen *et al*., 2009) three cosegregating mutations differentiate the taurine Y1 and Y2 haplogroups. A composite microsatellite in *DBY* (Gotherstrom *et al*. 2005), with one major allele in both Y1 and Y2 and only present in the Italian Maremmana, was not used for differentiation of haplotypes.

Cattle Y chromosomal DNA studies are comparatively rare (Verkaar *et al*., 2004) and have primarily focused on evaluation of male mediated patterns of migration and admixture between *B. taurus* and *B. indicus* or the assessment of differences in diversity (Ginja *et al*., 2009; Kantanen *et al*., 2009).

The effective Y chromosome contribution tends to minimize in case of domesticated animals because of common use in breeding schemes of a few selected males that produce a large number of offspring (Hellborg and Ellegren, 2004).

Zebu (*B. indicus*) origin of domestication has been determined on the basis of the two major haplogroups I1 and I2, which are well represented in India suggesting either a single domestication event followed by introgression of wild auroch females into protodomesticated herds, or more probably that domestication included two different wild female populations (Chen *et al*., 2010).

Gotherstrom *et al*. (2005) identified five SNPs that permitted the identification of contemporary breeds into three Y-chromosome haplogroups (Y1, Y2 and Y3). The Y1 haplogroup was found to be prevalent among cattle in Northwestern Europe, Y2 was prevalent in Southern Europe, and Anatolian cattle and the Y3 haplogroup was identified only in zebu. These findings indicated that the Y2 haplotypes represent cattle domesticated in the Near East while the Y1 haplotype represents European aurochs

demonstrating the male lineage genetic influence of European aurochs in the formation of contemporary European cattle.

Gotherstrom *et al*. (2005) screened 3.5 kb of non-coding Y chromosome sequence in 20 animals and found two co-segregating sites an A/C SNP in UTY intron 19. Twenty showed the UTY 'C allele' of Y1 while only one showed the 'A allele' of Y3 indicating that Y1 was at high frequency among European aurochsen prior to the arrival of domestic cattle and that it was also frequent among aurochsen contemporary with the first domestic cattle.

The synergistic use of Y-specific microsatellites and the SNPs described by Gotherstrom *et al*. (2005) increased analytical resolution and allowed at least two different Y2-haplotypic subfamilies to be distinguished, one of them in Northern Italy and the other restricted to the African continent (Perez-Pardal *et al*. 2010b). Studies have further suggested that there has been introgression of wild sire European auroch genetics into domesticated herds, that cattle domestication in Africa most probably included local Y2 wild auroch sires, and that the high genetic similarity found in Asian zebu supports a single domestication event.

Sun and Zhang (2006) Compared swamp and river buffalo SRY gene sequences revealing a single nucleotide polymorphism (SNP, C/G) at the 202 bp site of the coding region differentiating swamp and river buffaloes.

Two Y chromosome specific microsatellites *UMN*2404 and *UMN*0103 were genotyped and assessed for polymorphisms. In the southern group, the indicative alleles dominated (92.4 percent), while the taurine alleles dominated in the northern group (95.5 percent). Cai *et al*. (2006) reported the origin of Chinese zebu may have been in Hainan Island, and Tibetan cattle were likely independently domesticated from another Bos primigenius breed. The geographical distribution of these frequencies shows a

pattern of male indicine introgression and a hybrid zone of indicine and taurine cattle in China.

Li *et al*. (2007) analyzed Y chromosome variation in 7 populations from north Ethiopia using five Y chromosome microsatellite markers. They reported north Ethiopian cattle populations with one exception (Abergelle) are characterized by a general low Y-chromosome haplotype diversity and reduced interpopulation variance stating limited, paternal origin of the north Ethiopian cattle populations and strong male-mediated gene flow among them.

Five diagnostic haplotypes of indicine and taurine origins on both the X and Y chromosomes were analyzed to be useful in identifying the variations among them and level of indicine admixture in African cattle breeds (Anderung *et al*., 2007).

The use of these markers (IMMs) increased the resolution of detection by identifying a subhaplogroup within the Y2 haplogroup in cattle Y chromosomes sampled from Northern Italy, Northern Atlantic Europe, Mongolia, and Japan, which might correspond to the mitochondrial haplogroup Q identified by Achilli *et al*. (2008).

Svensson and Gotherstrom (2008) observed a single nucleotide polymorphism (SNP) in the intron of the Y-chromosomal gene *UTY19* displays a north–south gradient in modern cattle and also did Comparison of cattle Y-chromosome variations over time and suggests that the frequency and distribution of these patrilines varied, which could be attributed to distinct breeding strategies.

Bollongino *et al*. (2008) analyzed the ancient DNA of 59 Neolithic skeletal samples. After initial molecular sexing, two segregating Y-SNPs were identified in 13 bulls indicating G allele at UTY19 is always connected with a deletion in the ZFY5 locus.

Yoon *et al*. (2008) identified the partial fragments of SRY gene after successful amplification by PCR with a product size of 801 bp and subjected to restriction enzyme

digestion with MseI in order to identify Bos indicus and Bos taurus alleles, which were used for breed differentiation between imported and Hanwoo beefs.

Y-chromosomal fragments comprising the *ZFY* (1,219 bp and 1,003 bp), *SRY* (2,644 bp) and *DBY* (also known as *DDX3Y*; 406 bp) genes were sequenced (Nijman *et al*., 2008). These sequences contained five mutational differences when compared to the zebu Y3 sequences (Ginja *et al*., 2009), and all European animals carried either the Y1 or Y2 taurine haplogroups.

More recently, in Portuguese native cattle breeds, 13 Y-chromosome specific haplotypes were identified by the combination of five SNPs, one indel, and seven STRs (Ginja *et al*., 2009). three previously described patrilines (Y1, Y2, and Y3) and 10 new haplotypes within *Bos taurus* were included in the 13 Y-haplotypes. Much of the diversity of seven haplotypes (H2Y1, H3Y1, H5Y1, H7Y2, H8Y2, H10Y2, and H12Y2) was identified in native cattle. H6Y2 and H11Y2 occurred at high frequency among races, including exotics, and therefore had a common genetic signature (Ginja *et al*., 2009).

In cattle, microsatellite analysis has identified several Y-haplotypes in Portuguese (Ginja *et al*. 2009), northern and eastern European (Kantanen *et al*., 2009), western-continental, British and Sub-Saharan Africa breeds (Perez-Pardal *et al*., 2010b), as well as in American Creole breeds (Ginja *et al*. 2010). Even though different sets of markers were used in these studies, and each only partially covered the diversity pattern of the paternal lineages, they have confirmed that Y-markers exhibit a strong phylogeographic structure in cattle.

Perez-Pardal *et al*. (2010a) analyzed polymorphism UTY19 on the African Y2 samples to confirm the haplogroup assignment and observed UTY G/T polymorphism (AY936543:g.423) differentiates Y1 and Y2 haplogroup.

Mohamad *et al*. (2009) suggested Y-chromosomal typing as a probe of the paternal lineage does not completely parallel the mtDNA results. PCR-RFLP of a Y-

chromosomal *SRY* gene segment, in which a *Bfa*I site indicates a banteng origin and absence of the *Bfa*I site in undigested samples and were confirmed by sequencing the same *SRY* fragment, indicating second banteng-specific mutation and differentiation between zebu and taurine origin.

Balaresque *et al*. (2010) explains how technological and cultural change is linked with the expansion of a Y-chromosomal lineage, and the contrast of this pattern with that shown by maternally inherited mitochondrial DNA suggesting a unique role for males in the transition.

To increase the marker coverage of the Y chromosome and the robustness of bovine Y-chromosome phylogenetic studies, Perez-Pardal *et al*. (2010a, 2010b) used a set of interspersed multilocus microsatellites (IMMs) described by Liu *et al*. (2003).

Based on Y-chromosome regions the reduced median network of *SRY* gene sequences was constructed. Two mutations were identified at sites 748 and 1100 bp of 1305-bp fragment from *SRY*, which are specific to *B. taurus* and *B. indicus*, respectively (Gou *et al*. 2010). By analyzing mtDNA and *SRY* sequences in native Yunnan cattle all paternal lineages were almost *Bos indicus* and all maternal lineages contained both *Bos taurus* and *Bos indicus*.

Winaya *et al*. (2011) analyzed the variance of partly sex related Y (SRY) gene sequence in Bali cattle bull to determine the genetic variation and phylogenetic relationship and found Bali cattle bull from Singosari has relatively closed genetic relationship with Baturiti which supported that in early data some Bali bulls of Singosari were came from Baturiti.

Mukhopadhyay *et al*. (2011) did exploration of Y-chromosome specific markers SRY, TSPY and confirmed single strand polymorphism followed by sequencing of different band patterns revealing only a single novel snp.

Edwards *et al*. (2011) collected haplogroup data and resolved further by genotyping microsatellites *INRA189* (10 alleles) and *BM861* (2 alleles). He proposed that the homogeneous Y1 and Y2 regions reflect founder effects associated with the development and expansion of two groups of dairy cattle, the pied or red breeds from the North Sea and Baltic coasts and the spotted, yellow or brown breeds from Switzerland, respectively.

Edwards *et al*. (2011) suggested Locus *INRA189* is the most informative marker with 10 alleles, differentiated among five Y1 and nine Y2 haplotypes. For 21 bulls belonging to the Indian and Southwest Asian breed groups, haplogroup Y3 was identified via the *INRA189*-88 bp allele. Microsatellite marker *BM861* defines one additional Y1 and three Y2 additional haplotypes. Haplotype Y1-98-158 is the most frequent within the Y1 haplogroup and is detected in 82% of the animals from this haplogroup across all geographic breed groups with the exception of the Indian and Podolian. Within the Y2 haplogroup, Y2-102-158 and Y2-104-158 haplotypes account for 62% and 29% of the animals respectively.

Cortes *et al*. (2011) reported Y chromosome haplotype pattern supported the hypothesis of two main paternal influences in the extant Lidia breed, corresponding to the two founding migrations that arrived in the Iberian Peninsula from domesticated origins represented by the clearly differentiated Y1 and Y2 haplogroups.

Ramesha *et al*. (2012) analyzed multiple sequence alignment of the DNA sequences of the SRY gene revealing that there exist no variation among the Indian yaks and the template. But, variation has been observed between the Indian yaks, yak hybrids, and hill cattle population at various positions viz., 61, 122 and 197.

Bonfiglio *et al*. (2012) identified a new sequence-tagged site (STS) within intron 26 of the bovine USP9Y gene, showing an 81-base pair insertion (g.76439_76440ins81 in sequence with Gen- Bank accession FJ195366) able to distinguish Y2 and Y3 Bos Y

haplogroups from Y1. Also reported four Y3-specific sequence variants which allow a distinction from haplogroup Y2 and typing of a *Bison bison* Y chromosome indicating that the ancestral allele for the USP9Y 81-bp insertion is the short Y1 version.

Syed-Shabthar *et al*. (2013) analyzed SRY of Y chromosome obtained from five species of the *Bos* genus (*B. javanicus, Bos gaurus, Bos indicus, Bos taurus,* and *Bos grunniens*) and reported the sequence variation is low (with parsimony informative characters: 2/660) resulting an unresolved Neighbor-Joining tree.

Zhang *et al*. (2013) investigated 4 Chinese native breeds using 5 single nucleotide polymorphisms (DDX3Y-1, DDX3Y-7, ZFY9, ZFY10, UTY19) specific to the bovine Y chromosome and detected low level of haplotype diversity when compared to some foreign breeds.

To determine the Y chromosome genetic diversity and paternal origin of Chinese cattle, Xin *et al*. (2013) investigated 369 bulls from 17 Chinese native cattle breeds and 30 bulls from Holstein and four bulls from Burma using USP9Y marker that could distinguish between taurine and indicine cattle more efficiently. In total, the taurine Y1, Y2 haplogroup and indicine Y3 haplogroup were detected in 7 (1.9 %), 193 (52.3 %) and 169 (45.8 %) individuals of 17 Chinese native breeds, respectively, among these, Y2 dominates in northern China (91.4 %), while Y3 dominates in southern China (81.2 %). Central China is an admixture zone with Y2 predominating overall (72.0 %). The results demonstrate that Chinese cattle have two paternal origins, one from *B. taurus* (Y2) and the other from *B. indicus* (Y3).

Mukherjee *et al*. (2013) suggested that the structural organization of Y chromosome varies between crossbred and Indicine bulls. Absolute copy number of *DDX3Y* and *USP9Y* do not vary between these two bovine subspecies probably because of their single-copy presence in the genome. *TSPY*, a multicopy gene in bovine genome, varies substantially between crossbred and Indicine bulls.

Niemi *et al*. (2013) analyzed UTY19 allele frequencies and observed significant increase of Y1 and decrease of Y2 in Finnish bulls from the Post-Medieval period to the present is in accordance with a similar temporal shift of paternal lines detected in Swedish cattle suggesting that this replacement of Y2 with Y1, resulting in an almost complete fixation of the Y1 type in the contemporary Fennoscandia.

African cattle contained unique paternal lineages, with 13 and four exclusive Y2 and Y3 haplotypes, respectively. Y-haplotype diversity in Creoles was high, with several Y1 (7), Y2 (9) and Y3 (7) haplotypes represented. The sharing of specific patrilines corroborates influence of Iberian (two Y1 and one Y2 haplotypes) and African (one Y2 haplotype) cattle in American Creoles, even though the major influence was from Indicine haplotypes (Ginja *et al*., 2016).

Svensson *et al*. (2014) performed analysis of the SNP in the Y-chromosomal intron UTY19 that divide modern taurine cattle in two major haplogroups (Y1 and Y2) showing that the mediaeval cattle belonged to the haplogroup Y2 with one single exception of a Y1.

Hartatik *et al*. (2014) investigated the specific markers of the paternally transmitted Y-chromosome for the direct detection of male-mediated introgression. A 211 bp PCR product were cut with *PstI* and *BfaI* restriction enzyme. The polymorphism of Y-chromosome was recognized by *Bfa*I restriction enzymes at the position 432 bp and 544 bp.

Yue *et al*. (2014) revealed all 25 male Mongolian cattle samples are identified with *Bos taurus* Y chromosome haplotype and no *Bos indicus* haplotypes were found and indicated zebu introgression to Mongolian cattle based on Historical and archeological records.

Anwar *et al*. (2014) determined the origin and genetic diversity of Pakistani cattle by analyzing *SRY* gene sequences of samples and gene bank and found no snps.

Margawati *et al*. (2015) identified 16 nucleotide differences in sequenced samples to UTY gene reference and found 100% similarity of UTY sequence for sequenced samples to UTY *Bos Taurus* and *Bos indicus*.

Prusak *et al*. (2015) investigated Y chromosome genetic diversity and relationships among six Polish cattle breeds using five cattle Y specific microsatellite markers. They observed three haplotypes in the paternal pool of analyzed population and reported that haplotype diversity is low, which reflected the low male effective population size.

Ginja *et al*. 2016 genotyped genetic polymorphisms located on the non-recombining region of the Y-chromosome, including five STRs (DDX3Y1, BM861, INRA189, UMN0103 and UMN0307), two indels (ZFY10, USP9Y) and one SNP (UTY19) and analyzed Y-haplogroups such as Y1 (587) and Y2 (824) which are known to be predominant in Northern and Southern European cattle, respectively, and the Y3-lineage (347) of Indicine cattle.

Kumar *et al*. (2017) analyzed the Y-chromosome variation in Indian native cattle breeds and crossbred population in 10 native cattle breeds and 50HF crossbred by screening of five SNPs of bull MSY and validated allele-specific PCR. They identified three haplotypes (Y1, Y2 and Y3). Indian native cattle breeds had pure indicine paternal origin (Y3). Whereas, HF crossbreds showed both the *Bos taurus* (Y1 and Y2) paternal lineages with predominance of Y1 (0.98) haplogroup.

Pelayo *et al*. (2017) identified a new haplogroup, distinct from those described by Gotherstrom *et al*. (2005), and named Y1.2 by genotyping the animals with five Y-specific microsatellites (*INRA189, UMN0103, UMN0307, BM861* and *BYM1*), two indels (*ZFY10* and *USP9Y*) and one SNP (*UTY19*)

Devi *et al*. (2017) investigated genetic diversity and bottle neck studies in 21 Punganur cattle breed using 17 microsatellite markers and revealed that there is no mode

shift in the frequency distribution of alleles and a normal L-distribution was obtained indicating that the population is non-bottlenecked.

Ma *et al*. (2017) analyzed polymorphisms of the Y chromosome USP9Y gene in 62 Qaidam cattle males with size of PCR products of Qaidam cattle USP9Y genes were 471 bp and 552 bp, respectively, and the 552-bp PCR products could not be digested by SspI revealing the above two belt types represented Y1 and Y2 cattle haplogroups, respectively, indicating that Qaidam cattle had two patrilineal branches with two paternal origins.

Hartatik *et al*. (2018) obtained five snps from the promoter region of SRY gene sequences alignment of 19 cattle. Three SNPs revealed on Bali cattle were at -966 C/G, -907 T/deletion, and - 402 C/T, located before CAAT box, TATA box, SRY-binding, and Sp1-binding region. Two SNPs revealed on Nellore cattle (*Bos indicus*; GenBank Acc. No. NC_032680.1) was at -140 G/A and -117 G/A (Table 2), located after CAAT box, TATA box, SRY-binding, and Sp1-binding region.

Prihatin *et al*. (2018) analyzed sequences showing the deletion and mutation were exist in Madura bull SRY gene, indicating the diversity of SRY gene within Madura bulls.

Chen *et al*. (2018) identified two distinct sub-haplogroups in each of the Y2 (Y2a/b) and Y3 (Y3a/b) haplogroups.

Thu *et al*. (2018) determined the Y-chromosomal haplotype of the Vietnamese Yellow cattle by sequencing the 1,062-bp segment of the SRY gene on Y chromosome.

Vinod *et al.* (2019) estimated the genetic variability parameters in 50 Punganur cattle breed using 20 microsatellite markers and concluded that the Punganur cattle population deviated from Hardy-Weinberg equilibrium with high inbreeding, shortfall of heterozygote's and little genetic variation.

Indriawati *et al*. (2020) suggested UTY and SRY primers are suitable for sex determination and the pooled-DNA could be used as an efficient PCR method both in consumables and PCR process for sex determination.

Ganguly *et al*. (2020) identified new alleles and haplotypes by analyzing Y chromosome markers in 301 bulls representing 19 native Indian cattle (*Bos indicus*) and described that native *B. indicus* cattle from India retain high levels of paternal genetic diversity which appears to have been lost in transboundary commercial indicine cattle and such diversity should be maintained through management and conservation plans.

Ganguly *et al*. 2020 found all the *B. indicus* bulls restricted to the Y3 haplogroup through amplification by PCR specific to USP9Y resulting a 362 bp fragment for Y1 and a 443 bp fragment for Y2 and Y3 and done PCR-RFLP with enzyme Ssp I cleaving the Y3 into two distinct fragments of 337 bp and 107 bp, allowing to differentiate Y3 from Y2 with Y2 having no cutting site for *Ssp I.*

The summary of SNPs identified for Y chromosomal genes like ZFY_9, UTY_19 and USP9Y in various indigenous breeds of cattle is detailed below:

Gene	SNP	Breeds	Reference
ZFY_9	T	Gir, Kankrej, Khillar, Mewati, Nagori, Nimari, Rathi, Sahiwal, Tharparkar, Malnad Gidda	Kumar *et al*. 2017
ZFY_9	T	Dangi, Gir, Hariaya, Kangayam, Kankrej, Khillar, Krishna Valley, Malnad Gidda, Mewati, Nagori, Nimari, Ongole, Rathi, Red Sindhi, Sahiwal, Tharparkar, Vechur, Kherigarh	Ganguly *et al*. 2020
UTY_19	T	Gir, Kankrej, Khillar, Mewati, Nagori, Nimari, Rathi, Sahiwal, Tharparkar, Malnad Gidda	Kumar *et al*. 2017
UTY_19	T	Dangi, Gir, Hariaya, Kangayam, Kankrej, Khillar, Krishna Valley, Malnad Gidda, Mewati, Nagori, Nimari, Ongole, Rathi, Red Sindhi, Sahiwal, Tharparkar, Vechur, Kherigarh	Ganguly *et al*. 2020
USP9Y	C	Dangi, Gir, Hariaya, Kangayam, Kankrej, Khillar, Krishna Valley, Malnad Gidda, Mewati, Nagori, Nimari, Ongole, Rathi, Red Sindhi, Sahiwal, Tharparkar, Vechur, Kherigarh	Ganguly *et al*. 2020

CHAPTER - III

MATERIALS AND METHODS

The present study was conducted on Punganur cattle breed in order to understand the genetic diversity with respect to paternal lineage.

3.1 MATERIALS

3.1.1 Collection of samples

Data was recorded on 60 animals which were maintained in a conservation programme at Livestock Research Station (LRS), Palamaner, Chittoor district and different parts of Andhra Pradesh and Telangana. The details of animal's samples and their location were summarized in Table 1 and represented in Figure 1.

3.1.2 Experimental Animals

The present study does not warrant permission from Institutional Animal Ethics Committee as no experimentation was conducted on the animals. The blood samples were collected aseptically from the animals maintained at Livestock Research Station (SVVU), Palamaner summarized in Table 1 and represented in Figure 1.

3.1.3 Chemicals and Oligonucleotide Primers

The chemicals used in the present study were obtained from Himedia, Mumbai. The oligonucleotide primers were custom synthesized from Barcode Biosciences, Bangalore.

3.1.4 Consumables

All the consumables such as glassware and plasticware used for the present study were procured from Borosil, Mumbai and Tarson, Kolkata respectively.

Fig. 1: Location of Samples Collected in and around Andhra Pradesh

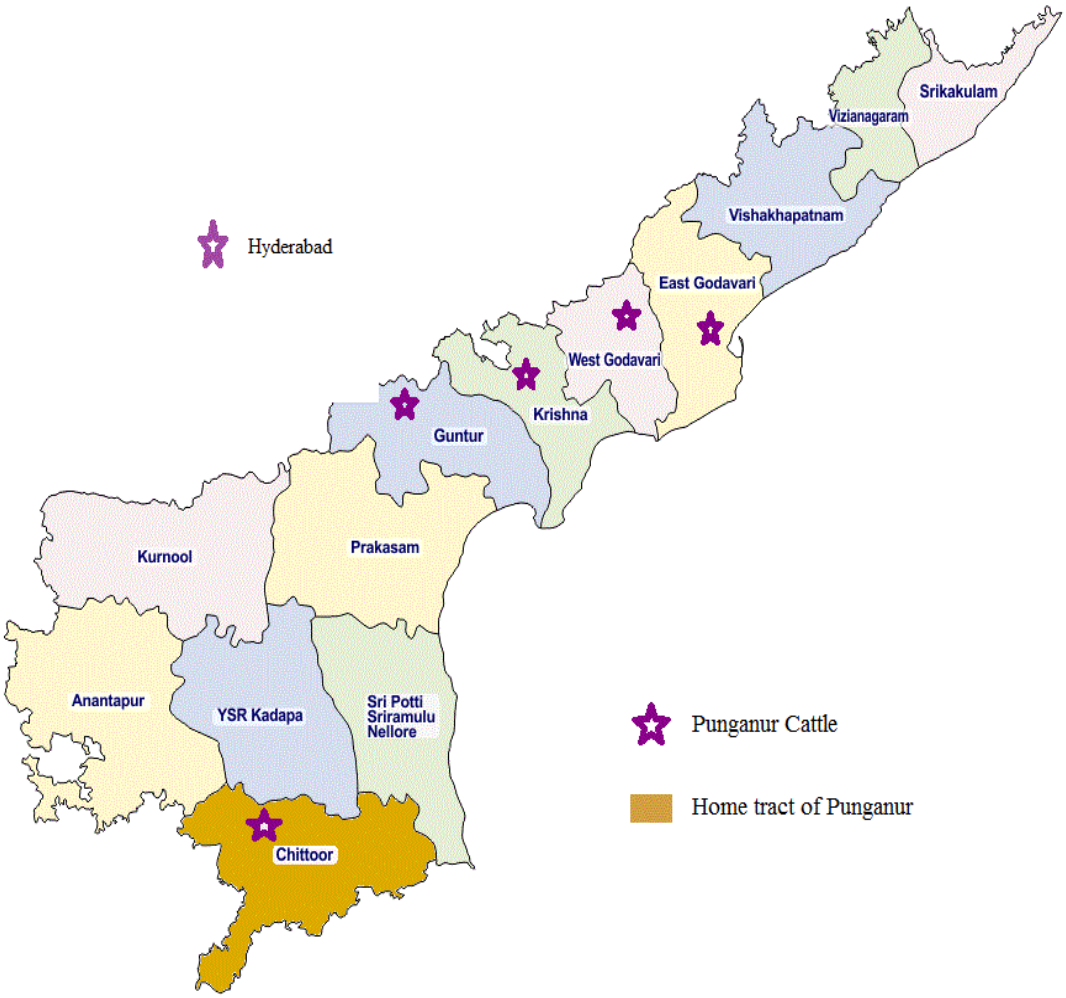


Fig.2. Photographs of Punganur Bulls

Table 1 Number of samples collected from each district

District	Location	No. of samples
Guntur	Guntur	1
	Bapatla	1
Krishna	Manikonda	1
	Elamaru	1
	Allapuram	1
West Godavari	Bhimavaram	4
East Godavari	Mandapeta	3
Chittoor	LRS, Palamaner	44
Hyderabad	Hyderabad	4
Grand Total		60

3.1.5 Preparation of Solutions / Reagents for DNA Isolation

The compositions of different solutions/reagents used in DNA isolation were given below.

Compositions of different solutions/reagents used in DNA isolation:

RBC LYSIS BUFFER

Ammonium chloride : 8.0235 g

Potassium chloride (10 mM) : 0.7455 g

EDTA : 0.0372 g

Triple distilled water to make up to 1000 ml, autoclaved and stored at 4°C

PROTEINASE-K

Proteinase-K : 20 mg

Triple distilled water to make up to 1 ml and stored at -20 ^{0}C

Tris-HCL (50 mM)

Tris-HCL	:	1.576 g
Triple distilled water to make up the volume	:	10 ml

pH adjusted to 8.0, autoclaved and stored at 4 ^{0}C

EDTA (20 mM)

EDTA (disodium, dihydrate)	:	0.74 g
Triple glass distilled water to make up the volume	:	100 ml

SODIUM DODECYL SULPHATE (SDS) (10%)

SDS	:	10 g
Triple glass distilled water	:	100 ml

LYSIS BUFFER

Tris-HCL (50 mM)	:	0.5 ml
EDTA (20 mM)	:	0.4 ml
SDS (10 %)	:	2 ml
Triple distilled water to make up the volume	:	10 ml

pH adjusted to 8.0, autoclaved and stored at 4 ^{0}C

SODIUM ACETATE (3 M)

Sodium acetate	:	12.3045 g
Triple distilled water to make up the volume	:	50 ml

SODIUM CHLORIDE (2 M)

Sodium chloride	:	1.1688 g
Triple distilled water to make up the volume	:	10 ml

CHLOROFORM : ISOAMYLALCOHOL (24:1)

Chloroform	:	24 parts
Isoamyl alcohol	:	1 part

ETHANOL (95 %)

Ethanol (99.9 %)	:	95 ml
Triple distilled water	:	5 ml

ETHANOL (70 %)

Ethanol (99.9 %)	:	70 ml
Triple distilled water	:	30 ml

3.2 METHODS

3.2.1 Blood sample collection, transport and storage

Four ml of whole blood was collected from jugular vein of each animal using K3 (EDTA) BD Vacutainers. Immediately after collection, the samples were labeled and transported to the laboratory in an ice packed container and stored at -20°C.

3.2.2 DNA Extraction

3.2.2.1 *Isolation of genomic DNA from whole blood*

DNA from blood samples was isolated following high salt method (Montgomery and Sise, 1990) with modifications to suit the lab conditions. The steps followed for isolation of DNA from blood samples are given below.

i. Half ml of blood and 1.5 ml of RBC lysis buffer were taken in a 2 ml micro centrifuge tube. This tube was shaken vigorously by using rotary sample mixer (Model: MS-NRK-30) for 10 minutes for complete lyses of red blood cells. Nuclear material was pelleted by centrifugation at 4000 rpm at room temperature for 15 minutes and the supernatant was discarded.

ii. Nuclear pellet was washed with 2 ml of RBC lysis buffer and centrifuged once again. This step was repeated for three to four times till the clear nuclear pellet was obtained.

iii. To the pellet 500 µl lysis buffer (pH 8) and 10 µl proteinase K were added and mixed. The tube was incubated at 56 ^{o}C overnight for digestion of protein.

iv. To this tube 50 µl of 2M NaCl was added and shaken vigorously for 10 minutes. To this mixture equal volume of Chloroform: Isoamyl alcohol (24:1) was added, mixed and centrifuged at 12,000 rpm for 10 minutes at 24 °C.

v. Aqueous upper phase was transferred to a fresh tube without disturbing the interphase.

vi. Finally, DNA was precipitated by adding double the volume of ice cold 95% ethanol and one-tenth volume of 3M sodium acetate to the supernatant collected and inverted several times for 10 minutes and centrifuged at 4,000 rpm for 10 minutes at 24 °C.

vii. The supernatant was discarded and 200 µl of 70 per cent ethanol was added to the pellet and centrifuged at 2,000 rpm for 5 minutes at 24 °C.

viii. The supernatant was discarded and the pellet was air dried until ethanol smell goes off.

ix. The dried DNA pellet was resuspended in 50µl of nuclease free water (pre warmed) and kept at 65 °C for 30 minutes for dissolution.

x. The resuspended pellet was stored at -20 °C until further use.

3.2.2.2 *Quality and quantity of genomic DNA*

The genomic DNA was verified using 1% agarose gel electrophoresis. A 1% agarose gel was prepared by boiling agarose in 0.5X TAE buffer. To the agarose, ethidium bromide was added at the rate of 0.5 µg/ml. The prepared gel was poured into an electrophoresis plate and left at room temperature for about 30 minutes for polymerization. One µl of 6x loading buffer containing bromophenol blue dye and six µl of distilled H_2O were mixed and to this 2µl of DNA sample was added and then loaded into the wells of the gel. The DNA samples were run on agarose gel at 100 V for about 60 minutes in 0.5X TAE buffer and gel was visualized under Gel Doc.

DNA was quantified by using NanoDrop™ 2000/2000c (Thermo Fisher Scientific) using the convention that one optical absorbance unit at 260 nm equals 40 µg per ml. Purity of DNA was judged on the basis of OD ratio at 260:280 and samples having the acceptable purity *i.e.* 1.7 to 2.0 were used for further analysis.

3.2.3 Polymerase chain reaction (PCR) for amplification of targeted region of bovine Y specific genes

Three target regions of different Y specific genes (SRY, USP9Y and UTY19), encompassing the putative SNP sites, were amplified by standardizing PCR program using genomic DNA and gene specific forward and reverse primers in a fixed reaction mixture with the help of Thermal cycler (Applied Bio systems, Germany) (Table 2). The gene-specific forward and reverse oligonucleotide primers were taken from the published literature (Verkaar *et al*., 2004; Kumar *et al*., 2017; Ganguly *et al*., 2020).

Table 2: Amplification of three target regions of different Y specific genes

Locus	Region	Size (bp)	Ta (°C)	Primer sequence (5'-3')	SNPs	GenBank Accession No:
SRY	MSY	520	58	F-ACGTCAAGCGACCCATGAAC R-TAAGCACAAGAAAGTCCAGGC	1748>G/T	DQ336527
USP9Y	Intron 26	471	53.5	F- GGGGCTTAGAGTGCTCCAGT R- ACAGCTCCTCAAAACCAGAAT	76426>C/T	FJ195366
UTY-19	Intron 19	278	52	F-GATGCCTATATTAGCCATTGACA R-AAATTCTTTATGATGTTCCATCC	423>G/T	AY936543

3.2.3.1 *Preparation of stock and working primer solution*

The oligonucleotide primers were custom synthesized by Barcode Biosciences, Bangalore. Oligos supplied in freeze dried powder form were reconstituted in nuclease and protease free water to a concentration of 100 pmol/µl of stock primer. The stock primer solution is further diluted in the ratio of 1:20 using nuclease free water to give a final concentration of 5 pmol/µl working solution.

3.2.3.2 *Optimization of PCR conditions*

The PCR was set up for two reactions, one each for both primers and the total volume of each reaction was 10µl. A reaction of 9µl comprising 2X master mix, nuclease free water, forward and reverse primers was aliquoted in each PCR tube and 1µl of DNA was

added to each tube to make the final volume. Various PCR components and their volume were summarized below in Table 3.

Table 3: PCR components and their volume

PCR Components	Volume
DNA Template	1.0µl
Primer Forward (5pmol/µl)	0.5µl
Primer Reverse (5pmol/µl)	0.5µl
2X PCR Master Mix	5.0µl
Nuclease free water	3.0µl
Total	10.0µl

PCR mix was prepared for two additional samples to cover pipetting error and negative control. The PCR conditions were optimized by setting different time-temperature combinations for annealing and extension process. The PCR was carried out in total volume for 10 µl in a 200 µl PCR tube. The combination that gave best result, in terms of yield and specificity of the product was further used for amplification. The PCR was carried out in a Proflex PCR system (Applied Biosystems, Germany).

3.2.4 Detection of PCR products

3.2.4.1 *Preparation of Reagents for Agarose Gel Electrophoresis*

(2 %) Agarose gel

Agarose - 0.8 g

1x TAE working solution - 40 ml

Preparation of 0.5X TAE buffer (working)

10 ml of 50X TAE stock solution was mixed with 990 ml of triple distilled water to prepare 1000 ml of 0.5X TAE working solution.

Three µl of each PCR amplicons were analyzed on 1.5 per cent (w/v) agarose gel containing 0.5 µg/ml ethidium bromide in O.5X TAE buffer and visualized on UV transilluminator (Gel Doc ™ XR+, Biorad, U.S.A). The horizontal gel electrophoresis was carried out at 80 volts for 45 minutes. The sizes and quantities of PCR products were verified by comparison with 100 bp DNA ladder (Genei Merck, Bangalore, India). The photograph of the gel was obtained by a gel image system (Omega Fluor™ Plus Documentation Systems, BioExpress, USA).

3.3 RESTRICTION FRAGMENT LENGTH POLYMORPHISM OF UTY-19, USP9Y AND SRY

The PCR products were subjected to RFLP to observe the polymorphic patterns and thus genotype of the genes.

3.3.1 Restriction enzyme digestion

Digestion of PCR products was done using specific restriction enzymes summarized in Table 4. The restriction enzymes used in present study were obtained from Fermentas and invitrogen, Thermoscientific.

3.3.2 Genotyping by RFLP analysis

The PCR products were digested with respective combinations of restriction enzyme and corresponding reaction buffer in sufficient quantity of nuclease and protease free water (Himedia, Mumbai) summarized in Table 5.

Table 4: Details of the restriction enzyme and their recognition sites for identified SNPs

S.No.	Gene	Enzyme	Recognition sequence
1	UTY-19	M1uCI	5' --- AATT --- 3' 3' --- TTAA --- 5'
2	*USP9Y*	*SspI*	5' ---AAT ATT--- 3' 3' ---TTA TAA--- 5'
3	*SRY*	*MseI*	5' ---T TA A--- 3' 3' ---A AT T--- 5'

Table 5: Protocol for restriction digestion of PCR products

Y-specific locus	***UTY-19***	***USP9Y***	***SRY***
Restriction enzyme	M1uCI	SspI	MseI
Concentration of Restriction Enzyme (10 units/µl)	10U (1ul)	2.5U (0.5ul)	10U (1ul)
10X Buffer	2ul	1.5ul	2ul
PCR Product	7ul	5ul	7ul
Nuclease free water	10ul	8ul	10ul
Incubation time / temperature	30min@37°C	4hr@37°C	30min@37°C

3.3.2.1 *Agarose gel electrophoresis of PCR-RFLP products*

The total volume of the digested samples was separated on 3 percent agarose gel for genotyping in 1X TBE buffer at 300V for 600 minutes. Gels were visualized with Omega Fluor™ Plus Documentation Systems (BioExpress, USA).

The sizes of PCR-RFLP products were verified by comparison with 100bp and 50bp DNA ladder (O'GeneRuler DNA Ladder, Thermoscientific). The photograph of the gel was obtained by a gel image system. The agarose gels were scored based on the pattern observed.

3.4 PCR PRODUCT PURIFICATION AND SEQUENCING

The amplified PCR products from representative samples (2 samples for each gene) were purified and sequenced. The obtained electropherograms of all the 6 sequences were quality checked, assembled and analyzed by using Codon Code Aligner with reference sequences. The reference sequences details comprises: UTY gene (intron 19, GenBank accession numbers AY936539), SRY gene (GenBank accession numbers DQ336526, DQ336527) and USP9Y gene (GenBank accession numbers FJ195364).

3.5. ALLELE SPECIFIC PCR

Allele Specific Primers were adopted from the article by Kumar *et al*. (2016). The genomic specificity of the primers was tested using the Primer-BLAST program (http://www.ncbi.nlm.nih.gov/tools/primer-blast/). One set of AS-PCR Primer forward and reverse gene-specific oligonucleotide primers were selected. The primers were checked for oligonucleotide melting temperature, oligonucleotide length, GC content, primer-dimer possibilities and PCR product size. Primers were procured from Barcode Biosciences Pvt. Ltd (Bangalore). The sequence of primers, their respective nucleotide numbers, target region and amplicon sizes were listed in Table 6.

In allele specific PCR, for each sample, two PCR reactions were run in parallel, say one PCR reaction with primer having C at 3’ end and second reaction with primer having T at 3’ end. The reverse primer was common for both the reaction (Fig. 2). To increase specificity, an extra mismatch is also intentionally introduced at the third position from the 3' end of each of the two allele specific primers. A reaction of 9µl comprising 2X master mix, nuclease free water, forward and reverse primers was aliquoted in each PCR tube and 1µl of DNA were added to each tube to make the final volume. Amplification was performed on a thermal cycler (Applied Biosystems, Germany) with specific cycling conditions as mentioned in Table 7. The PCR products were analyzed by electrophoresis on 1.5% Tris-acetate-EDTA/ethidium bromide agarose gels, visualized under gel image system and the presence or absence of specific band was indicative of presence or absence of a particular allele. A sample was considered negative for a particular allele when the amplicon was absent. Furthermore, template DNA is same for each allele specific genotyping reaction serves as a control for the false positive or negative reaction secondary to extraction failure or the presence of an inhibitor.

3.5.1 *Validation of the assay*

To rule out any false results arising from non-specific amplification of Allele specific PCR is very essential. To validate our present assays, in all the assays negative (no template) and a female DNA (*Bos indicus*) were used as control to rule out possible PCR contamination and non-specific amplification.

Fig. 3: Basic principle of allele specific PCR (Kumar *et al*. 2017)

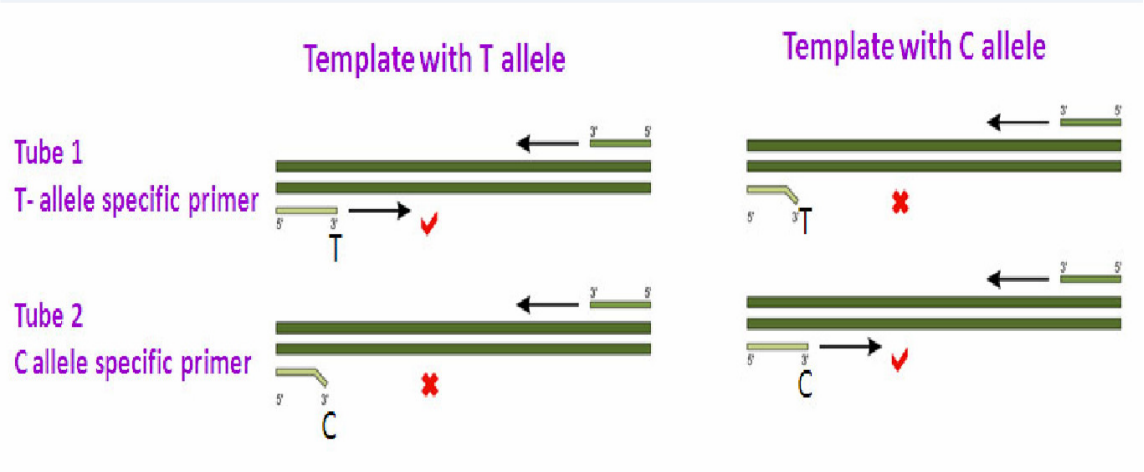


Table 6: Allele specific PCR primers detail for screening one SNP on bull Y-chromosomes

SNP	Position	Primer ID	Primer Sequence (5'-3')	Conc. (uM)	Ta (°C)	Product size (bp)
ZFY9	Intron 9 120>C/T AY928828	AS-ZFY-9 (T)	TACTAATGAACTGATTTAA GTAAAAAACA	1.0	52	86
		AS-ZFY-9 (C)	TACTAATGAACTGATTTAA GTAAAAAACG	1.0		
		ZFY9-CR	CATAAAGAAAGTTCCTATT AAAGTTAAA	1.0		

Table 7: PCR cycle profile for various Y-specific locus

Locus	**AS-ZFY9**	**SRY**	**USP9Y**	**UTY19**
Cycle Profile	95°C 5 min (ID) 30 cycles 95°C 25 sec (D) 52°C 15 sec (A) 72°C 25 sec (E) Final Extension 72°C 10 min 4°C Hold	95°C 2min (ID) 30 cycles 92°C 15 sec (D) 58°C 30 sec (A) 72°C 45 sec (E) Final extension 72°C 5 min 4°C hold	95°C 5mi (ID) 35 cycles 94°C 30 sec (D) 53.5°C 30 sec (A) 72°C 30 sec (E) Final extension 72°C 10 min 4°C hold	95°C 5mi (ID) 35 cycles 95°C 30 sec (D) 52°C 20 sec (A) 72°C 30 sec (E) Final extension 72°C 10 min 4°C hold

3.6 HAPLOTYPE ANALYSIS

The sequence data of all genes and allele specific pattern under study were analyzed to identify SNPs, and the identified SNPs were combined to construct haplotype specific to Y chromosome of Punganur population.

CHAPTER-IV
RESULTS

A total of sixty samples from Punganur cattle (bull) were included in the present study for genotyping Y chromosome variations (*ZFY9*, *USP9Y*, *UTY* and *SRY*) and to delineate Y-haplotypic diversity in Punganur cattle breed.

4.1 ISOLATION OF GENOMIC DNA

In the present study, the genomic DNA was isolated from blood samples of Punganur bulls successfully, according to the standard protocol of Proteinase-K: Phenol:Chloroform:Isoamylalcohol method (Sambrook and Russell, 2001) with slight modifications.

4.2 QUALITY AND QUANTITY ESTIMATION OF DNA

Quality and quantity of genomic DNA from extracted samples was evaluated by submarine horizontal agarose (0.8 %) gel electrophoresis. All the samples isolated showed distinct bands of genomic DNA. Approximately 2 µl DNA solution (dissolved in Molecular grade water) was used in NanoDrop spectrophotometer. The absorbance of the sample was measured at 260nm and 280nm wavelength. The ratio of optical density at the two wavelengths (OD260/OD280) indicates purity of sample. OD between 1.7 and 1.9 is expected for a good quality DNA. All the DNA samples in the present study were found within this range. The concentration of DNA in the samples ranged from 50.1 to 858.6 ng/µl.

4.3 OPTIMIZATION OF POLYMERASE CHAIN REACTION (PCR)

4.3.1 Template DNA concentration

In the present study, after a series of trial and errors, approximately 50ng of template DNA was optimized per 10µl of PCR reaction volume.

4.3.2 Primer concentration

The concentration of C-Allele and T-Allele of *ZFY9* gene with common reverse primer was optimized by setting up PCR trials. Finally, the optimum level of primer used was 5 pmol/µl.

4.3.3 Determining annealing temperature (Ta)

The annealing temperature (Ta) which affects both specificity and yield of PCR product was considered as one of the crucial factors. The annealing temperature was standardized by setting up PCR trials.

4.4 POLYMERASE CHAIN REACTION (PCR) FOR AMPLIFICATION OF TARGETED REGIONOF BOVINE Y SPECIFIC GENES

Four target regions of Y specific genes (Allele Specific *ZFY9*, *USP9Y*, *UTY19* and *SRY*) pertaining to the *MSY* of bovine Y chromosome, encompassing the putative SNP sites, were amplified by standardizing PCR program (Table 8).

4.5 AGAROSE GELELECTROPHORESIS OF PCR PRODUCTS

In order to confirm the amplification of the targeted sequences, the PCR product of Allele Specific *ZFY9*, *USP9Y*, *UTY19* and *SRY* genes were subjected to 2 percent agarose gel electrophoreses at 80V, 80A for about 45 minutes and later gel was visualized and captured under gel documentation system. The amplified product was visualized as a single compact band for all the genes. Amplicons with 87bp for *ZFY9*, 443bp for *USP9Y*, 278bp for *UTY19* gene and 520bp for *SRY* gene were recorded after comparison with 100bp and 50 bp DNA ladder (Genei Merck, Bangalore, India) and were shown in Figure 4, 5a, 6a and 7a.

4.6 SINGLE NUCLEOTIDE POLYMORPHISM (SNP) HAPLOTYPING

Out of four genes included in the present investigation, Allele specific PCR amplification was done for *ZFY9*, and PCR RFLP method was utilized for the remaining three genes (*SRY*, *USP9Y* and *UTY19*).

4.6.1 Detection of single nucleotide polymorphism by Allele specific PCR

One SNP for *ZFY_9* was studied through allele specific PCR amplification. The location of single nucleotide points and different primer positions were explained in Fig. 4. All SNP points were typed based on the presence or absence of allele specific amplification products.

4.6.1.1 *ZFY_9*

ZFY_9 SNP specific nucleotide is either 'C' or 'T'. All samples manifested amplification (87bp) in combination of T allele specific forward primer and common reverse primer (Figure 4), while no amplification was observed in combination of 'C' allele specific forward primer and common reverse primer. The allele specific gel pattern indicated that only 'T' allele was observed at the particular nucleotide position in all the Punganur bull samples.

4.6.2 IDENTIFICATINO OF POLYMORPHISM BY PCR RFLP

For the remaining three genes (*USP9Y*, *UTY_19* AND *SRY*) PCR-Restriction Fragment Length Polymorphism (RFLP) method was done. PCR-RFLP consists of several separate steps like design of primers, identification of an appropriate restriction enzyme, amplification, restriction enzyme treatment of amplified products and electrophoresis to resolve the restriction fragments.

4.6.2.1 PCR-RFLP of *USP9Y, UTY_19* AND *SRY* genes

The PCR product of *USP9Y, UTY_19* and *SRY* genes were digested with suitable restriction enzymes namely *SspI, M1ucI* and *MseI, respectively* which recognizes the SNP 76426C>T in the *USP9Y*, 423G>T in *UTY_19* and 1748G>T in *SRY* gene. During the execution of PCR-RFLP assays, 7µl of PCR product was digested with 2.5 units of *SspI* for *USP9Y*, likewise 10 units of *M1ucI* for *UTY_19* and 10 units of *MseI* restriction endonuclease enzyme for *SRY*. The digested products were incubated for 30mins at 37°C

in case of *SRY and UTY_19,* whereas *USP9Y* products were incubated for four hours at 37°C (Table 5 and 6).

4.6.2.2 Agarose gel electrophoresis of PCR –RFLP product of *USP9Y, UTY_19* and *SRY* genes

The allele identification for all the products was done by electrophoretic resolvement of the fragments. The electrophoresis of the enzyme digested PCR products were subjected to 2% gel, 70V for 45 minutes in case of *USP9Y* and *SRY* genes, while 3.5% gel, 70V for 60 minutes for *UTY19* gene. PCR-RFLP samples were loaded by using gel loading dye (6 X bromophenol blue). The restriction fragment sizes of 337bp and 107bp (C variant) was observed for USP9Y/SspI and for UTY_19/M1ucI restriction fragment sizes of 66bp and 157bp (T variant) was observed while 124bp and 360bp (T variant) were observed for SRY/MseI (Fig. 5b, 6b and 7b).

4.7 SEQUENCING

In the present study, representative PCR products of three SNPs (*USP9Y*, *UTY19* and *SRY)* were selected and sequenced using Sanger method of sequencing (Barcode Biosciences, Bangalore). The obtained electropherograms of all the 6 sequences were quality checked, assembled and analyzed by using Codon Code Aligner with reference sequences and electropherograms of three SNPs were shown in Fig.8 while results were mentioned according to the SNPs obtained from sequencing data in Table 8.

4.8 ANALYSIS OF HAPLOTYPES

Genetic structuring of native cattle breeds like estimation of PIC, allelic frequencies, was not carried out in the present investigation as all the Punganur were observed to have only one haplotype (Y3). Further, the results (Fixed Y3 haplotypes in native breed) also reflect that the native animals (bulls) included in the present study were of *Bos indicus* type (shown in Table 9).

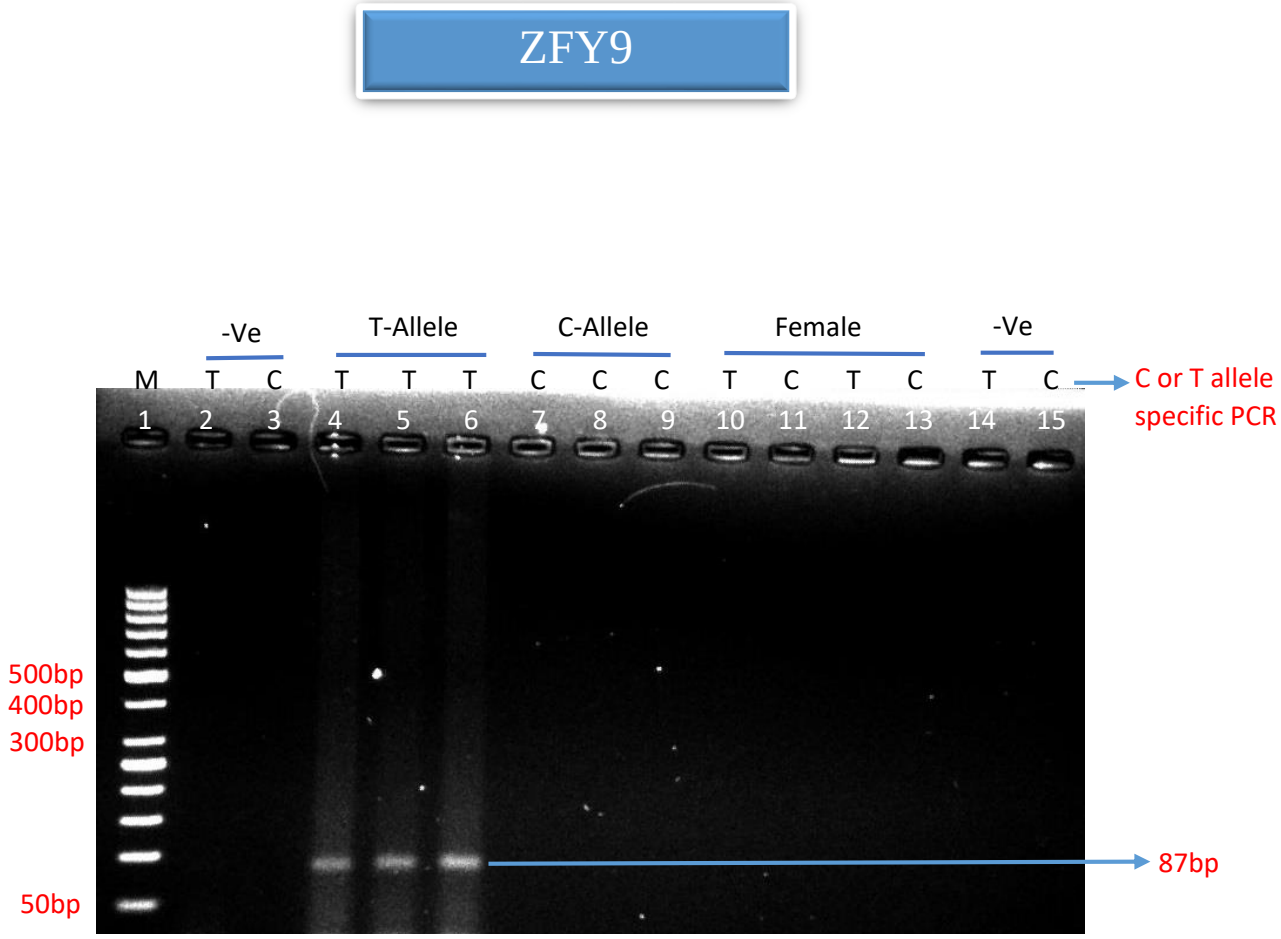


Fig. 4: Screening of SNP of ZFY9 (120> C/T) through allele specific PCR

Lane 1 (M): 50 bp DNA Ladder
Lane 2-3 and 14-15: Negative samples
Lane 4-6: T-Allele specific PCR product from Punganur bull sample
Lane 7-9: C-Allele specific PCR product from Punganur bull sample
Lane 10-13: Female samples from Punganur cattle

USP9Y

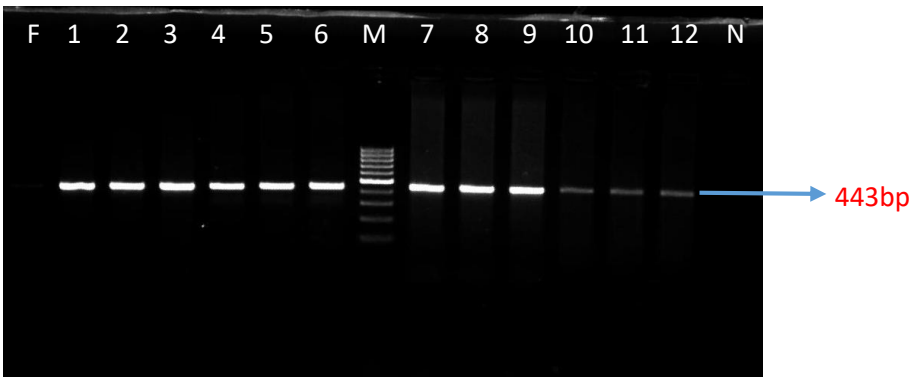


Fig. 5a: Amplification of SNP of USP9Y (76426C>T) through PCR

F: Female sample from Punganur Cattle
Lane 1-6: PCR product from Punganur bulls samples
M: 100bp DNA ladder
Lane 7-12: PCR product from Punganur bulls samples
N: -Ve sample

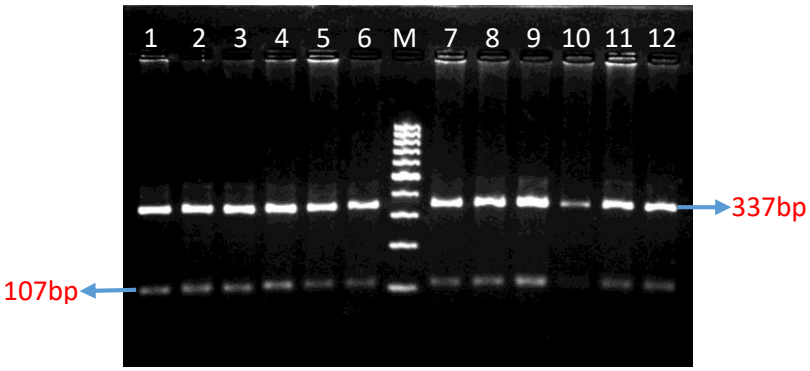


Fig. 5b: Screening of SNP of USP9Y (76426C>T) through PCR-RFLP method

Lane 1-6: PCR-RFLP product from Punganur bulls samples
M: 100bp DNA ladder
Lane 7-12: PCR-RFLP product from Punganur bulls samples

UTY19

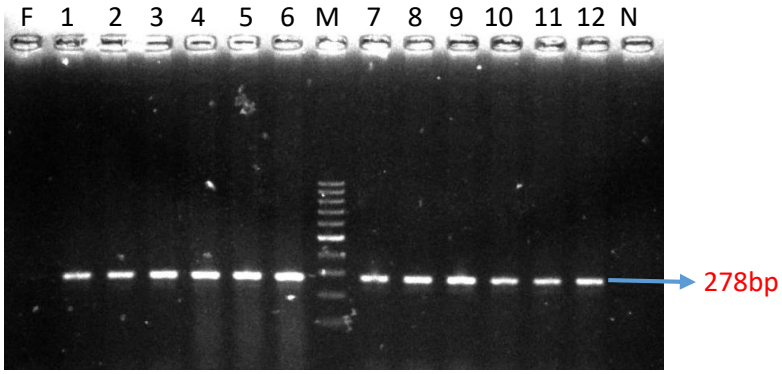


Fig. 6a: Amplification of SNP of UTY19 (423G>T) through PCR

F: Female sample from Punganur Cattle
Lane 1-6: PCR product from Punganur bulls samples
M: 100bp DNA ladder
Lane 7-12: PCR product from Punganur bulls samples
N: -Ve sample

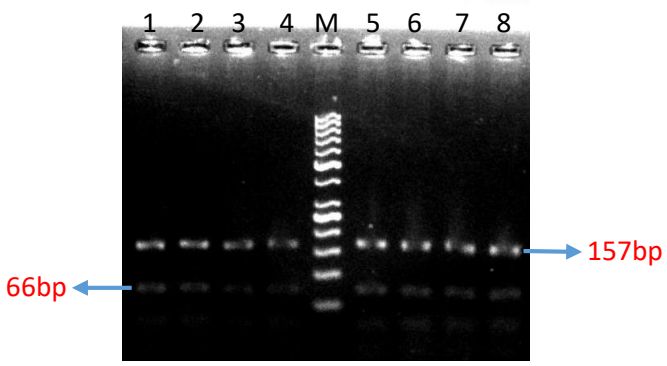


Fig. 6b: Screening of SNP of UTY19 (423G>T) through PCR-RFLP method

Lane 1-6: PCR-RFLP product from Punganur bulls samples
M: 50bp DNA ladder
Lane 7-12: PCR-RFLP product from Punganur bulls samples

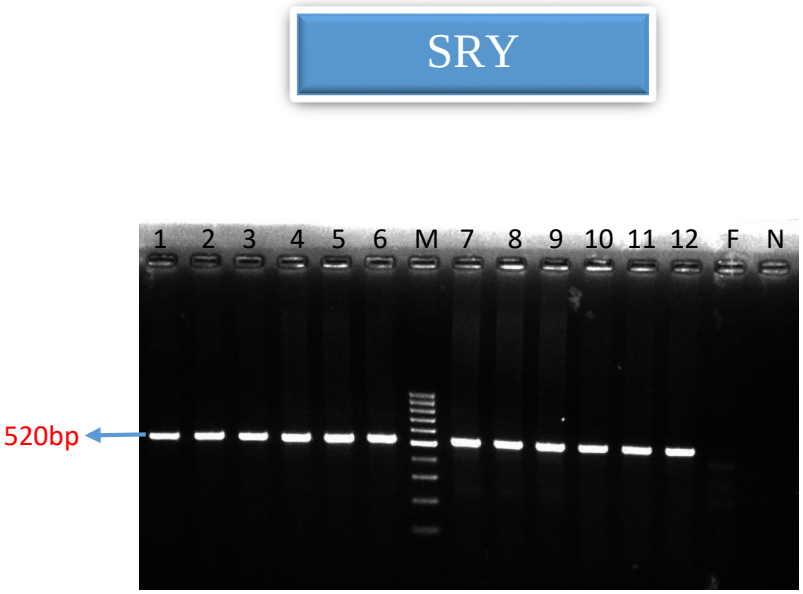


Fig. 7a: Amplification of SNP of SRY (1748G>T) through PCR

Lane 1-6: PCR product from Punganur bulls samples
M: 100bp DNA ladder
Lane 7-12: PCR product from Punganur bulls samples
F: Female sample from Punganur cattle
N: -Ve sample

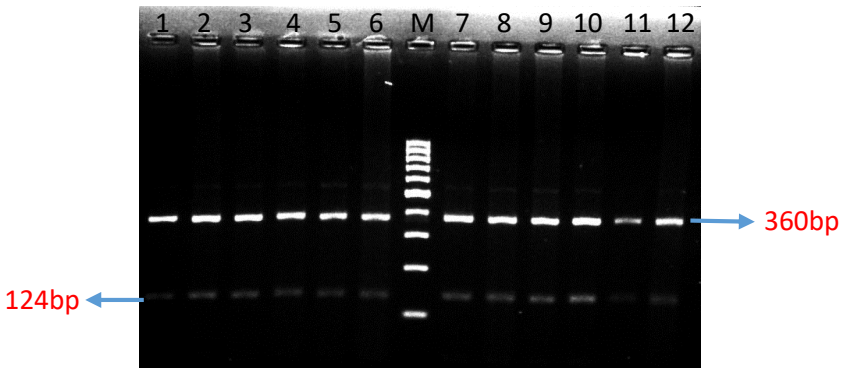
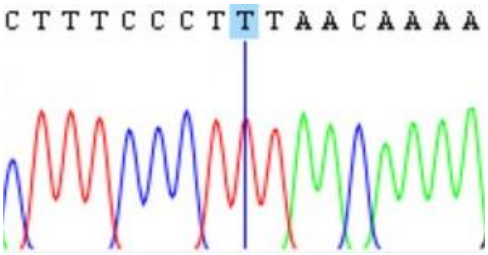
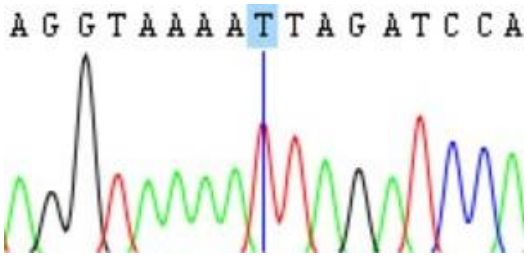


Fig. 7b: Screening of SNP of SRY (1748G>T) through PCR-RFLP method

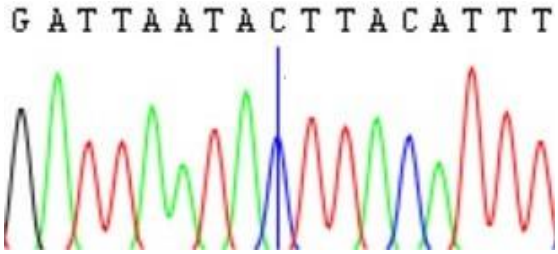
Lane 1-6: PCR-RFLP product from Punganur bulls samples
M: 100bp DNA ladder
Lane 7-12: PCR-RFLP product from Punganur bulls samples



A) Electropherogram of SRY



B) Electropherogram of UTY19



C) Electropherogram of USP9Y

Fig. 8: Validation of allele types through direct sequencing of representative samples pertaining to A) SRY, B) UTY19 and C) USP9Y

Table 8: Details of SNPs identified for all genes in 60 Punganur bulls

Sl No.	Sample	ZFY9	USP9Y	UTY19	SRY
1	P480	T	C	T	T
2	P351	T	C	T	T
3	P137	T	C	T	T
4	P114	T	C	T	T
5	P535	T	C	T	T
6	P426	T	C	T	T
7	P114	T	C	T	T
8	P120	T	C	T	T
9	P141	T	C	T	T
10	NP9	T	C	T	T
11	P461	T	C	T	T
12	P417	T	C	T	T
13	N22	T	C	T	T
14	P411	T	C	T	T
15	P531	T	C	T	T
16	N24	T	C	T	T
17	P127	T	C	T	T
18	P532	T	C	T	T
19	P100	T	C	T	T
20	P112	T	C	T	T
21	N26	T	C	T	T
22	P516	T	C	T	T
23	P121	T	C	T	T
24	P161	T	C	T	T
25	P139	T	C	T	T
26	N27	T	C	T	T
27	P158	T	C	T	T
28	P177	T	C	T	T
29	P208	T	C	T	T
30	P140	T	C	T	T
31	P125	T	C	T	T

Sl No.	Sample	ZFY9	USP9Y	UTY19	SRY
32	P136	T	C	T	T
33	P160	T	C	T	T
34	P154	T	C	T	T
35	P166	T	C	T	T
36	P265	T	C	T	T
37	P342	T	C	T	T
38	P640	T	C	T	T
39	P243	T	C	T	T
40	P964	T	C	T	T
41	P318	T	C	T	T
42	P353	T	C	T	T
43	P158	T	C	T	T
44	GNT3	T	C	T	T
45	BPT1	T	C	T	T
46	MK1	T	C	T	T
47	ELM5	T	C	T	T
48	ALP3	T	C	T	T
49	BHM1	T	C	T	T
50	BHM2	T	C	T	T
51	BHM3	T	C	T	T
52	BHM5	T	C	T	T
53	MP1	T	C	T	T
54	MP4	T	C	T	T
55	MP5	T	C	T	T
56	MP12	T	C	T	T
57	HYD1	T	C	T	T
58	HYD3	T	C	T	T
59	HYD4	T	C	T	T
60	HYD9	T	C	T	T

Based on the results obtained, the haplotype profile of Punganur bulls obtained by combining SNPS of all genes included in the present investigation was of single type, Y3 (TCTT) and was shown in Table 9.

Table 9: Haplotype profile of Male Punganur Cattle

Haplotype	**ZFY9**	**USP9Y**	**UTY19**	**SRY**	**Frequency**
Y3 (TCTT)	T	C	T	T	1.00

CHAPTER V
DISCUSSION

India was endowed with a wide reserve of rich biodiversity indigenous bovine communities. These breeds have evolved over generations, enduring as a result of their adaptability to various geographical conditions, consistency in performance even with low quality feed and fodder, disease tolerance etc. There have been ample attempts in the past breeding programmes to augment the genetic value of local animals through the import of exotic germplasms, finally resulting in the dilution of local Indian races. One such breed of indigenous cattle on the verge of extinction is the Punganur, with only few of them left. The Punganur cattle were the shortest indigenous breed. This breed of cattle has its origin in the Chittoor district of Andhra Pradesh in south-eastern India (Nath, 1993).

A Report (2014) estimated that 2772 pure Punganur cattle were distributed across the state, arising a question about the purity of the present Punganur animal. The measurement of genetic relationships between and within breeds is the task of molecular characterization (Report, 2011). The advancements in molecular tools like microsatellites (Mukesh *et al.,* 2004), mitochondrial DNA (Sharma *et al*., 2015) and Y chromosome specific markers (Gotherstrom *et al*., 2005) and their application in animal genetics is useful in apprehending the genetic variation in the present population under study. Conventional genetic improvement programmes embrace only a few males in their breeding plan, though sire is half of the herd. Therefore Y chromosome studies are of particular interest in livestock breeding schemes. Furthermore, molecular variation in the Y chromosome provides plenty of information about the pattern of distribution of paternal lineages. Therefore, the present study was undertaken for screening Y chromosome specific variation, and to explore the existing genetic diversity in Punganur based on Y chromosome specific SNPs/haplotypes.

5.1 Screening of Y chromosome specific variation/haplotypes

Four targeted regions, encompassing putative Single Nucleotide Polymorphism (SNP) sites (Allele specific ZFY9: 120>C/T, USP9Y: 76426>C/T, SRY: 1748>G/T and UTY19: 423>G/T) of cattle MSY were screened in 60 Punganur bulls covering different geographical regions in and around Andhra Pradesh. Allele specific PCR method and PCR-Restriction Fragment Length Polymorphism (RFLP) strategy was utilized in our study to detect these SNPs.

The resulting restriction profiles and Allele specific PCR genotypes indicated that all the animals in the present study belong to Y3 haplogroup, which coincides with results of various researchers (Kumar *et al.* 2017; Ganguly *et al.* 2020).

5.1.1 ZFY-9

One single nucleotide polymorphism (ZFY9-C/T) was analyzed by allele-specific PCR (AS-PCR) protocol and genotyped to differentiate zebu (Y3) and taurine specific (Y1 and Y2) Y haplogroups. All bull samples showed Y3 haplotype (T allele) indicating pure indicine paternal lineage. Results were validated with sequencing and found out to be in agreement with previous reports (Kumar *et al.* 2017; Ganguly *et al.* 2020).

Similar to our results, Ganguly *et al.* 2020 asserted that all the indigenous breeds included in their study shown T-allele and concluded that all the bulls belong to Y3 haplogroup, indicating pure indicine paternal lineage.

Gotherstrom *et al.* (2005) and Ginja *et al.* (2009) noticed a broader distribution of haplotype Y1 in northern European breeds and the presence of haplotype Y2 predominantly in southern Europe breeds and none of the breeds exhibited Y3 haplotype. Edwards *et al*., (2011) observed that European cattle contained only taurine haplotypes, while southwestern Asian cattle have substantial frequency of indicine haplotypes cattle.

5.1.2 USP9Y

Bonfiglio *et al*. (2012) identified a new sequence-tagged site (STS) within intron 26 of the bovine USP9Y gene, showing an 81-base pair insertion (g.76439_76440ins81: GenBank accession FJ195366), which is able to distinguish Y2 and Y3 haplogroups from Y1, which is further differentiated between Y2 and Y3 by enzyme *Ssp I* cleaving 443 bp fragment into two distinct fragments of 337 bp and 107 bp with Y2 having no cutting site for *Ssp I*. Accordingly, all the samples from Punganur bulls demonstrated two distinct fragments of 337 bp and 107 bp after PCR RFLP digestion with *Ssp I* enzyme, indicating that all the bulls were found to be restricted to the Y3 haplogroup, which corroborates with findings of earlier reports (Ganguly *et al*. 2020; Ma *et al*. 2017). The current study deciphered that, the enzyme SspI with PCR-RFLP can be used to differentiate the *Bos indicus* and *Bos taurus*.

Ganguly *et al*. 2020 also reported that all the indigenous breeds selected for screening SNPs by PCR RFLP, were restricted to the Y3 haplogroup.

5.1.3 UTY19

UTY19 (G/T) polymorphism was analyzed by PCR-RFLP with *M1ucI* enzyme cleaving the 278bp amplified fragment into 66bp and 157bp fragment in the polymorphic samples and *vice versa* in non-polymorphic samples. The results obtained were validated with sequencing and also found out to be in agreement with reports of researchers (Ganguly *et al*. 2020; Kumar *et al*. 2017)

Ganguly *et al*. (2020) and Kumar *et al*. (2017) also analyzed and identified T-allele through amplification by Allele specific PCR, and inferred that all the bulls in their study can be grouped under Y3 haplotype revealing indicine lineage.

Gotherstrom *et al*. (2005) screened 3.5 kb of non-coding Y chromosome sequence in 20 animals and found two co-segregating sites an A/C SNP in UTY intron 19. Twenty

showed the UTY ‘C allele’ of Y1 while only one showed the ‘A allele’ Y3 indicating that Y1 was at high frequency.

5.1.4 SRY

The SNP in SRY gene of Y chromosome was ascertained by PCR RFLP with *MseI* restriction enzyme and it was observed that all the samples displayed ‘T’ nucleotide (g.1748G>T) at position 1748, which is specific to Y3 haplogroup. The presence of T SNP proclaimed that, all the Punganur bulls comprises *Bos indicus* genetic constitution (Y3 haplotype). The results were validated with sequencing and were in accordance with previous reports (Hartatik *et al*. 2014; Mukhopadhyay *et al*. 2011; Guiyun and Hongquan, 2004).

Similarly, Tanaka (2000) reported a polymorphism in the bovine *SRY* gene (causal of a replacement Cys to Phe at position 214) which distinguishes *B. taurus* and *B. indicus* haplotypes.

Yoon *et al*. (2008) also conducted restriction enzyme digestion of SRY gene with *MseI* in order to identify *Bos indicus* and *Bos taurus* alleles, whose result was used to discriminate imported and Hanwoo beefs. Likewise, two mutations were identified at sites 748bp and 1100bp of 1305-bp fragment from *SRY*, which are specific to *B. taurus* and *B. indicus*, respectively (Gou *et al*. 2010).

The current study provided insightful knowledge on the present scenario in the times of ambiguity in confirming the purity of the *Bos indicus*. The present findings inferred that all the Punganur bulls can be grouped under Y3 haplogroup from which, it can be speculated that bulls in our study encompassed *Bos indicus* paternal genetic constitution (can be supported by further comparative studies with other indigenous breeds). The Y chromosome specific haplotype identified can be harnessed as a marker in genetic selection of bulls in Punganur breed conservation and improvement programme.

CHAPTER VI

SUMMARY

The present study is conducted to explore the genetic diversity by identifying the variation in Y chromosome of Punganur cattle. In Andhra Pradesh, the Punganur cattle breed is at the verge of extinction and its purity is at arguable based on the available literature. In the present study single nucleotide polymorphism in *ZFY9* was detected by Allele specific PCR and SNPs in *USP9Y*, *UTY19* and *SRY* genes were identified by PCR-RFLP method to genotype the animals within the Punganur breed.

Genomic DNA was isolated from the blood samples of 60 Punganur bulls present nearer to breeding tract by using modified high salt method. All the genes were amplified by utilizing the primers available in the published literature. Single nucleotide polymorphism analysis in *ZFY9* was carried out by Allele specific PCR and all the animals were found to have 'T' allele representing Y3 haplogroup of cattle.

Genotyping based on *SRY* by using *MseI* enzyme in Punganur animals revealed that all the animals were of TT genotype. Restriction enzyme digestion of *UTY19* PCR product with *M1ucI* restriction enzyme resulted in the 66bp and 157bp fragments among all the Punganur animals, indicating genotype TT. Analysis of USP9Y gene by *Ssp I* enzyme revealed two distinct fragments of 337 bp and 107 bp after RE digestion of 443bp product indicating the presence of Y3 haplogroup in Punganur cattle.

The amplicons were sequenced using Sanger method of DNA sequencing. The resultant electropherograms were quality checked, assembled and analyzed by using Codon Code Aligner software. The results of present study concluded that all the Punganur animals reflected pure indicine paternal lineage.

Genetic analyses may be conducted in large scale populations to ascertain the admixture proportions among Punganur cattle and their possible relationship with Y chromosome specific variation in the breed. Moreover, studies may be directed to explore

more SNPs in entire male specific region of cattle Y-chromosome in order to construct new haplotypes, which can be utilized as markers in selection of sires in Punganur breed conservation and improvement programmes.

LITERATURE CITED

Achilli, A., Olivieri, A., Pellecchia, M., Uboldi, C., Colli, L., Al-Zahery, N. and Torroni, A. (2008). Mitochondrial genomes of extinct aurochs survive in domestic cattle. Current Biology, **18**: R157-R158.

Anderung, C., Bouwman, A., Persson, P., Carretero, J. M., Ortega, A. I., Elburg, R. and Götherström, A. (2005). Prehistoric contacts over the Straits of Gibraltar indicated by genetic analysis of Iberian Bronze Age cattle. Proceedings of the National Academy of Sciences, **102**: 8431-8435.

Anwar, S., Babar, M. E., Muhammad, I., Asif, N., Tanveer, H., Shah, S. A. and Tauseef, A. (2014). Paternal lineage analysis in Sahiwal, Cholistani and Dajal cattle breeds of Pakistan through SRY gene. Global Veterinaria, **12**: 508-511.

Balaresque, P., Bowden, G. R., Adams, S. M., Leung, H. Y., King, T. E., Rosser, Z. H. and Jobling, M. A. (2010). A predominantly neolithic origin for European paternal lineages. PLoS Biol, **8**: e1000285.

Bannasch D.L., Bannasch, M, J., Ryun, J.R., Famula, T.R. and Pedersen, N. C. (2005). Y chromosome haplotype analysis in purebred dogs. Mamm Genome. **16**: 273–280.

Bishop, M. D., Kappes, S. M., Keele, J. W., Stone, R. T., Sunden, G. A., Hawkins, S. S., Toldo, R., Fries, M. D., Grosz, J., Yoo and Beattie, C. W. (1994). A genetic linkage map for cattle. Genetics. **136**: 619–639.

Bollongino, R., Elsner, J., Vigne, J. D. and Burger, J. (2008). Y-SNPs do not indicate hybridisation between European aurochs and domestic cattle. PloS one, **3**: e3418.

Bonfiglio, S., De Gaetano, A., Tesfaye, K., Grugni, V., Semino, O. and Ferretti, L. (2012). A novel USP9Y polymorphism allowing a rapid and unambiguous classification of Bos taurus Y chromosomes into haplogroups. Animal genetics, **43**: 611-613.

Bradley, D, G., MacHugh, D.E., Loftus, R.T., Sow, R.S., Hoste, C.H. and Cunningham, E.P. (1994). Zebu-taurine variation in Y chromosomal DNA: a sensitive assay for genetic introgression in West African trypanotolerant cattle populations. Animal Genetics, **25**: 7–12.

Bradley, D. G., MacHugh, D. E., Cunningham, P. and Loftus, R. T. (1996). Mitochondrial diversity and the origins of African and European cattle. Proceedings of the National Academy of Sciences, **93**: 5131-5135.

Budowle, B., Adamowicz, M., Aranda, X.G., Barna, C., Chakraborty, R., Cheswick, D., Dafoe, B., Eisenberg, A., Frappier, R. and Gross, A.M. (2005). Twelve short

tandem repeat loci Y chromosome haplotypes: genetic analysis on populations residing in North America. Forensic Science International, **150**: 1–15.

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.

Carolino, N. and Gama, L. T. (2008). Indicators of genetic erosion in an endangered population: the Alentejana cattle breed in Portugal. Journal of Animal Science, **86**: 47–56.

Chang, T.C. and Liu, W. S. (2010). The molecular evolution of PL10 homologs. BMC Evolutionary Biology, **10**: 1-12.

Chen, N., Cai, Y. and Chen, Q. (2018). Whole-genome resequencing reveals world-wide ancestry and adaptive introgression events of domesticated cattle in East Asia. Nature Communications, **9**: 1-13.

Chen, S., Lin, B. Z., Baig, M., Mitra, B., Lopes, R. J., Santos, A. M. and Beja-Pereira, A. (2010). Zebu cattle are an exclusive legacy of the South Asia Neolithic. Molecular biology and evolution, **27**: 1-6.

Cheng, H., Shi, H., Zhou, R., Guo, Y., Liu, L., Liu, J. and Sutou, S. (2001). Characterization of Bovidae sex-determining gene SRY. Genetics Selection Evolution, **33**: 1-8.

Cortes, O., Tupac-Yupanqui, I., Dunner, S., Fernández, J. and Cañón, J. (2011). Y chromosome genetic diversity in the Lidia bovine breed: a highly fragmented population. Journal of Animal Breeding and Genetics, **128**: 491-496.

Devi, K. S., Gupta, B. R. and Vani, S. (2017). Genetic diversity and bottleneck studies in Punganur cattle through microsatellite markers. International Journal of Science, **6**: 303-307.

Di Meo, G. P., Perucatti, A., Floriot, S., Incarnato, D., Rullo, R., Jambrenghi, A. C. and Iannuzzi, L. (2005). Chromosome evolution and improved cytogenetic maps of the Y chromosome in cattle, zebu, river buffalo, sheep and goat. Chromosome Research, **13**: 349-355.

Edwards, C. J., Baird, J. F. and MacHugh, D. E. (2007). Taurine and zebu admixture in Near Eastern cattle: a comparison of mitochondrial, autosomal and Y-chromosomal data. Animal Genetics, **38**: 520-524.

Edwards, C. J., Gaillard, C., Bradley, D. G. and MacHugh, D. E. (2000). Y-specific microsatellite polymorphisms in a range of bovid species. Animal Genetics, **31**: 127-130.

Edwards, C. J., Ginja, C., Kantanen, J., Pérez-Pardal, L., Tresset, A., Stock, F. and European Cattle Genetic Diversity Consortium. (2011). Dual origins of dairy cattle farming evidence from a comprehensive survey of European Y-chromosomal variation. PloS one, **6**: e15922.

Erdoğan, M., Tepeli, C., Brenig, B., Akbulut, M.D., Uğuz,. C, Savolainen, P. and Özbeyaz, C. (2013). Genetic variability among native dog breeds in Turkey. Turkish Journal of Biology. **37**: 176–183.

Evans, H. J., Buckland, R. A. and Sumner, A. T. (1973). Chromosome homology and heterochromatin in goat, sheep and ox studied by banding techniques. Chromosoma, **42**: 383-402.

Freeman, A. R., Meghen, C. M., Machugh, D. E., Loftus, R. T., Achukwi, M. D., Bado, A. and Bradley, D. G. (2004). Admixture and diversity in West African cattle populations. Molecular Ecology, **13**: 3477-3487.

Gamaniel, I. B. and Gwaza, D. S. (2017). Molecular characterization of animal genetics resources, it's potential for use in developing countries. Journal of Genetics and Genetic Engineering, **1**: 43-57.

Ganguly, I., Jeevan, C., Singh, S., Dixit, S. P., Sodhi, M., Ranjan, A. and Sharma, A. (2020). Y-chromosome genetic diversity of Bos indicus cattle in close proximity to the centre of domestication. Scientific reports, **10**: 1-9.

Ginja, C., Telo da Gama, L. and Penedo, M.C.T. (2009). Y Chromosome haplotype analysis in Portuguese cattle breeds using SNPs and STRs. Journal Heredity, **100**: 148-157.

Ginja, C., Penedo, C., Cortés, O., Martín-Burriel, I., Egito, A., Gama, L. T. D. and B Consortium. (2016). Origins and genetic structure of Creole cattle inferred from Y-chromosomal variation. Journal of Animal Science, **94**: 108-108.

Ginja, C., Penedo, M. C. T., Melucci, L., Quiroz, J., Martinez Lopez, O. R., Revidatti, M. A. and Gama, L. T. (2010). Origins and genetic diversity of New World Creole cattle: inferences from mitochondrial and Y chromosome polymorphisms. Animal genetics, **41**: 128-141.

Ginja, C., Telo da Gama, L. and Penedo, M. C. T. (2009). Y chromosome haplotype analysis in Portuguese cattle breeds using SNPs and STRs. Journal of Heredity, **100**: 148-157.

Giovambattista, G., Ripoli, M. V., De Luca, J.C., Mirol, P.M., Liron, J.P. and Dulout, F.N. (2000). Male-mediated introgression of Bos indicus genes into Argentine and Bolivian Creole cattle breeds. Animal Genetics, **31**: 302–305.

Götherström, A., Anderung, C., Hellborg, L., Elburg, R., Smith, C., Bradley, D. G. and Ellegren, H. (2005). Cattle domestication in the Near East was followed by hybridization with aurochs bulls in Europe. Proceedings of the Royal Society B: Biological Sciences, **272**: 2345-2351.

GOI (2019). 20th Livestock census, All India Report, Government of India. Ministry of Agriculture, Department of Animal Husbandry, Dairying and Fisheries, KrishiBhavan, New Delhi.

Gou, X., Wang, Y., Yang, S., Deng, W. and Mao, H. (2010). Genetic diversity and origin of Gayal and cattle in Yunnan revealed by mtDNA control region and SRY gene sequence variation. Journal of Animal breeding and Genetics, **127**: 154-160.

Graves, J.A.M. (2000). Human Y chromosome, sex determination, and spermatogenesis - a feminist view. Biology of Reproduction, **63**: 667–676.

Graves, J.A.M. (2006) Sex chromosome specialization and degeneration in mammals. Cell, **124**: 901–914.

Guiyun, H. and Hongquan, C. (2004). Cattle SRY-specific sequence amplification. Journal of Anhui Agricultural University, **31**: 421-423.

Hammer, M.F., Karafet, T.M., Redd, A.J., Jarjanazi, H., Santachiara-Benerecetti, S., Soodyall, H. and Zegura, S.L. (2001). Hierarchical patterns of global human Y chromosome diversity. Mol .Biol. Evol. **18**: 1189–1203.

Hanotte, O., Bradley, D. G., Ochieng, J. W., Verjee, Y., Hill, E. W. and Rege, J. E. O. (2002). African pastoralism: genetic imprints of origins and migrations. Science, **296**: 336-339.

Hanotte, O. and Jianlin, H. (2005). Genetic characterization of livestock populations and its use in conservation decision-making. The Role of Biotechnology in Exploring and Protecting Agricultural Genetic Resources. Food and Agriculture Organization of the United Nations, Rome: 89-96.

Hanotte, O., Tawah, C. L., Bradley, D. G., Okomo, M., Verjee, Y., Ochieng, J. and Rege, J.E. (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.

Hartatik, T., Priyadi, D. A., Agus, A., Bintara, S., Budisatria, I. G. S., Panjono, P. and Adinata, Y. (2018). SRY gene marker differences in native and crossbreed cattle. Buletin Peternakan, **42**

Hartatik, T., Widi, T. S. M., Volkandari, S. D. and Maharani, D. (2014). Analysis of DNA polymorphism in SRY gene of Madura cattle populations. Procedia Environmental Sciences, **20**: 365-369.

Hellborg, L. and Ellegren, H. (2004). Low levels of nucleotide diversity in mammalian Y chromosomes. Molecular Biology and Evolution, **21**:158–163.

Hurles, M. E. and Joblin, M. A. (2001). Haploid chromosomes in molecular ecology: lessons from the human Y. Molecular Ecology, **10**: 1599–1613.

Hurst, L. D. (1994) Embryonic growth and the evolution of the mammalian Y chromosome. I. The Y as an attractor for selfish growth factors. Heredity, **73**: 223–232.

Ibeagha-Awemu, E. M., Jann, O. C., Weimann, C. and Erhardt, G. (2004). Genetic diversity, introgression and relationships among West/Central African cattle breeds. Genetics Selection Evolution, **36**: 1-18.

Ihara, N., Takasuga, A., Mizoshita, K., Takeda, H., Sugimoto, M., Mizoguchi, Y. and Sugimoto, Y. (2004). A comprehensive genetic map of the cattle genome based on 3802 microsatellites. Genome Research, **14**: 1987-1998.

Indriawati, I., Volkandari, S. D. and Margawati, E. T. (2020). The Application of UTY and SRY Molecular Markers for Determination of Unknown Sex Samples in Bali Cattle. Journal of ILMU DASAR, **21**: 55-60.

Jakubiczka S, Schnieders F, Schmidtke J (1993). A bovine homologue of the human TSPY gene. Genomics **17**: 732–735

Jobling, M. A. and Tyler-Smith, C. (2003). The human Y chromosome: an evolutionary marker comes of age. Nature Reviews Genetics, **4**: 598-612.

Jorde, L. B., Watkins, W.S., Bamshad, M. J., Dixon, M. E., Ricker, C. E., Seielstad, M. T. and Batzer, M. A. (2000). The distribution of human genetic diversity: a comparison of mitochondrial, autosomal, and Y- chromosome data. The American Journal of Human Genetics, **66**: 979–988.

Joshi, B. K., Sodhi, M., Mukesh, M. and Mishra, B. P. (2012). Genetic characterization of farm animal genetic resources of India: A review. Indian Journal of Animal Sciences, **82**: 1259.

Kantanen, J., Edwards, C. J., Bradley, D. G., Viinalass, H., Thessler, S., Ivanova, Z. and Vilkki, J. (2009). Maternal and paternal genealogy of Eurasian taurine cattle (Bos taurus). Heredity, **103**: 404-415.

Kappes, S. M., Keele, J. W., Stone, R. T., McGraw, R. A., Sonstegard, T. S., Smith, T. P., Lopez-Corrales, N. L. and Beattie, C. W. (1997). A second- generation linkage map of the bovine genome. Genome Research, **7**: 235–249.

Kayser, M., Krawczak, M., Excoffier, L., Dieltjes, P., Corach, D., Pascali, V., Gehrig, C., Bernini, L.F., Jespersen, J. and Bakker, E. (2001). An extensive analysis of Y chromosomal microsatellite haplotypes in globally dispersed human populations. The American Journal of Human Genetics, **68**: 990–1018.

Kikkawa, Y., Takada, T., Sutopo, Nomura, K., Namikawa, T., Yonekawa, H. and Amano, T. (2003). Phylogenies using mtDNA and SRY provide evidence for male-mediated introgression in Asian domestic cattle. Animal Genetics, **34**: 96-101.

Kumar, P., Freeman, A. R., Loftus, R. T., Gaillard, C., Fuller, D. Q. and Bradley, D. G. (2003). Admixture analysis of South Asian cattle. Heredity, **91**: 43-50.

Kumar, S., Ganguly, I., Singh, S., Sodhi, M., and Bhakat, M. (2017). Y-chromosome variation in Indian native cattle breeds and crossbred population. Indian Journal of Animal Research, **51**: 1-7.

Lahn, B.T. and Page, D. C. (1997). Functional coherence of the human Y-chromosome. Science, **278**: 675–680.

Le, T. N., Vu, H. V., Okuda, Y., Duong, H. T., Nguyen, T. B., Nguyen, V. H. and Kunieda, T. (2018). Genetic characterization of Vietnamese Yellow cattle using mitochondrial DNA and Y-chromosomal haplotypes and genes associated with economical traits. Animal Science Journal, **89**: 1641-1647.

Li, M. H., Zerabruk, M., Vangen, O., Olsaker, I. and Kantanen, J. (2007). Reduced genetic structure of north Ethiopian cattle revealed by Y-chromosome analysis. Heredity, **98**: 214-221.

Lindgren, G., Backstrom, N., SwiLindgren, G., Backstrom, N., Swinburne, J., Hellborg, L., Einarsson,. A, Sandberg, K., Cothran, G., Vila, C., Binns, M. and Ellegren, H. (2004). Limited number of patrilines in horse domestication. Nature genetics, **36**: 335–336.

Littlewood, R. W. (1936). Livestock of southern India.

Liu, W.S., Beattie, C.W. and Ponce de Leon, F. A. (2003). Bovine Y chromosome microsatellite polymorphisms. Cytogenetic Genome Research, **102**: 53–58.

Liu, W. S., Mariani, P., Beattie, C. W., Alexander, L. J. and De León, F. A. P. (2002). A radiation hybrid map for the bovine Y Chromosome. Mammalian Genome, **13**: 320-326.

Liu, W. S. and Ponce de Leo´n, F. A. (2007). Mapping of the Bovine Y chromosome. Electronic Journal of Biology, **3**: 5-12.

Liu, W.S., Wang, A.H; Yang, Y., Chang, T.C; Landrito, E. and Yasue, H. (2009a) Molecular characterization of the DDX3Y gene and its homologs in cattle. Cytogenetic and Genome Research, **126**: 318–328.

Liu, W., Yang, Y. and Chang, T. C. (2009b). Isolation and Characterization of Male Fertility-Related Genes on the Bovine Y chromosome.

Loftus, R. T., Ertugrul, O., Harba, A. H., El-Barody, M. A. A., MacHugh, D. E., Park, S. D. E. and Bradley, D. G. (1999). A microsatellite survey of cattle from a centre of origin: the Near East. Molecular Ecology, **8**: 2015-2022.

Ma, Z., Hu, S., Li, R., Chen, S., Lei, C. and Chao, S. (2017). Y chromosome haplogroups and paternal origin of Qaidam cattle based on polymorphism analysis of USP9Y gene. Chinese Journal of Animal Science, **53**: 36-39.

MacHugh, D. E., Shriver, M. D., Loftus, R. T., Cunningham, P. and Bradley, D. G. (1997). Microsatellite DNA variation and the evolution, domestication and phylogeography of taurine and zebu cattle (Bos taurus and Bos indicus). Genetics, **146**: 1071-1086.

Mannen, H., Kohno, M., Nagata, Y., Tsuji, S., Bradley, D. G., Yeo, J. S. and Amano, T. (2004). Independent mitochondrial origin and historical genetic differentiation in North Eastern Asian cattle. Molecular phylogenetics and evolution, **32**: 539-544.

Margawati, E. T., Indriawati, I., Volkandari, S. D. and Ridwan, M. (2015). Identification of pure breed Bali cattle by using molecular approach. In International Seminar on Tropical Animal Production (ISTAP), 426-431.

Meadows, J. R. S., Hanotte, O., Drogemuller, C., Calvo, J., Godfrey, R., Coltman, D., Maddox, J.F., Marzanov, N., Kantanen, J. and Kijas, J. W. (2006). Globally dispersed Y chromosomal haplotypes in wild and domestic sheep. Animal Genetics, **37**: 444–53.

Modi, W. S. and Crews, D. (2005). Sex chromosomes and sex determination in reptiles: Commentary. Current Opinion in Genetics and Development, **15**: 660-665.

Mohamad, K., Olsson, M., van Tol, H. T., Mikko, S., Vlamings, B. H., Andersson, G. and Lenstra, J. A. (2009). On the origin of Indonesian cattle. PLoS One, **4**: e5490.

Montgomery, G. W. and Sise, J. (1990). Extraction of DNA from sheep white blood cells. New Zealand Journal of Agricultural Research, **33**: 437-441.

Mukesh, M., Sodhi, M., Bhatia, S. and Mishra, B. P. (2004). Genetic diversity of Indian native cattle breeds as analysed with 20 microsatellite loci. Journal of Animal Breeding and Genetics, **121**: 416-424.

Mukherjee, A., Dass, G., Gohain, M., Brahma, B., Datta, T. K. and De, S. (2013). Absolute copy number differences of Y chromosomal genes between crossbred (Bos taurus× Bos indicus) and Indicine bulls. Journal of animal science and biotechnology, **4**: 1-7.

Mukherjee, N., Nebel, A., Oppenheim, A. and Majumder, P.P. (2001). High-resolution analysis of Y-chromosomal polymorphisms reveals signatures of population movements from Central Asia and West Asia into India. Journal of Genetics, **80**: 125-135.

Mukhopadhyay, C. S., Gupta, A. K., Yadav, B. R. and Mohanty, T. K. (2011). Exploration of Y-chromosome specific markers to discover SNP associated with sub fertility traits in dairy bulls.

Muller, H. J. (1914). A gene for the fourth chromosome of. The Journal of Experimental Zoology, **17**: 325.

Murphy, W. J., Pearks Wilkerson, A. J., Raudsepp, T., Agarwala, R., Schaffer, A. A., Stanyon, R. and Chowdhary, B. P. (2006). Novel gene acquisition on carnivore Y chromosomes. PLoS Genetics, **2**: e43.

Nath, M. N. (1993). "Punganur"-The Miniature *Bos indicus* Cattle. Animal Genetic Resources, **11**: 57-59.

Niemi, M., Bläuer, A., Iso-Touru, T., Nyström, V., Harjula, J., Taavitsainen, J. P., Storå J., Lidén, K. and Kantanen, J. (2013). Mitochondrial DNA and Y-chromosomal diversity in ancient populations of domestic sheep (Ovisaries) in Finland: comparison with contemporary sheep breeds. Genetics Selection Evolution, **45**: 1-14.

Nijman, I. J., Otsen, M., Verkaar, E. L. C., De Ruijter, C., Hanekamp, E., Ochieng, J. W. and Lenstra, J. A. (2003). Hybridization of banteng (Bos javanicus) and zebu (Bos indicus) revealed by mitochondrial DNA, satellite DNA, AFLP and microsatellites. Heredity, **90**: 10-16.

Nijman, I. J., Van Boxtel, D. C., Van Cann, L. M., Marnoch, Y., Cuppen, E. and Lenstra, J. A. (2008). Phylogeny of Y chromosomes from bovine species. Cladistics, **24**: 723-726.

Patel, A. K. (2009). Occupational histories, settlements, and subsistence in western India: what bones and genes can tell us about the origins and spread of pastoralism. Anthropozoologica, **44**: 173-188.

Pelayo, R., Penedo, M. C. T., Valera, M., Molina, A., Millon, L., Ginja, C. and Royo, L. J. (2017). Identification of a new Y chromosome haplogroup in Spanish native cattle. Animal genetics, **48**: 450-454.

Pérez-Pardal, L., Royo, L. J., Beja-Pereira, A., Chen, S., Cantet, R. J., Traoré, A. and Goyache, F. (2010a). Multiple paternal origins of domestic cattle revealed by Y-specific interspersed multilocus microsatellites. Heredity, **105**: 511-519.

Pérez-Pardal, L., Royo, L. J., Beja-Pereira, A., C˘urik, I., Traoré, A., Fernández, I., and Goyache, F. (2010b). Y-specific microsatellites reveal an African subfamily in taurine (Bos taurus) cattle. Animal Genetics, **41**: 232-241.

Ponce de Leo´n, F. A, Ambady, S., Hawkins, G.A., Kappes, S.M. and Bishop, M. D. (1996). Development of a bovine X chromosome linkage group and painting probes to assess cattle, sheep, and goat X chromosome segment homologies. Proc. Natl. Acad. Sci. USA, **93**: 3450–3454.

Ponce de León, F.A. and Carpio, C. (1995). Identification of the bovine X chromosome pseudoautosomal region. 9th North American Colloquium on Domestic Animal Cytogenetics and Gene Mapping, Texas AandM University (Abstract) pp. 8.

Prihatin, K. W., Maylinda, S. and Hakim, L. (2018). The Sry gene variations amongst selected madura cattle populations. Jurnal Kedokteran Hewan, December, **12**: 101-103.

Prusak, B., Sawicka-Zugaj, W., Korwin-Kossakowska, A. and Grzybowski, T. (2015). Y chromosome genetic diversity and breed relationships in native Polish cattle assessed by microsatellite markers. Turkish Journal of Biology, **39**: 611-617.

Ramesha, K. P., Jayakumar, S., DAS, S., Biswas, T. K., Krishnan, G., Chouhan, V. S. and Kataktalware, M. A. (2012). Genetic variation of SRY gene in Yak and related bovines. International Journal of Pharmacology and Biosciences, **3**: 81-86.

Report. (1990). Commissioned paper in the Thematic Working Group on Domesticated Bio – Diversity, National Biodiversity Strategy and Action Plan, Ministry of Environment and Forestry, Government of India.

Report. (1993-1994). Annual Progress Report. Andhra Pradesh Agricultural University, Hyderabad.

Report, (2007). Livestock census, Bulletin of Bureau of Economic and Statistics, Government of Andhra Pradesh, Hyderabad, India.

Report. (2011). Food and Agricultural Organization. Draft Guidelines on Molecular Characterization of Animal Genetic Resources. Commission on Genetic Resources for Food and Agriculture, Rome.

Report. (2014). 19th Livestock Census, All India Report, Ministry of Agriculture Department of Animal Husbandry, Dairying and Fisheries, Krishi Bhawan, New Delhi.

Robinson, T.J., Harrison, W.R., Ponce de Leon, F.A., Davis, S.K. and Elder, F.F. (1998). A molecular cytogenetic analysis of X chromosome repatterning in the Bovidae: transpositions, inversions, and phylogenetic inference. Cytogenetics and Cell Genetics, **80**: 179–184.

Rubinoff, D. and Holland, B. S. (2005). Between two extremes: mitochondrial DNA is neither the panacea nor the nemesis of phylogenetic and taxonomic inference. Systematic Biology, **54**: 952-961.

Saker, M. M., Youssef, S. S., Abdallah, N. A., Bashandy, H. S. and El Sharkawy, A. M. (2005). Genetic analysis of some Egyptian rice genotypes using RAPD, SSR and AFLP. African Journal of Biotechnology, **4**: 882-890.

Saxena, R., Brown, L. G., Hawkins, T., Alagappan, R. K., Skaletsky, H., Reeve, M. P. and Page, D. C. (1996). The DAZ gene cluster on the human Y chromosome arose from an autosomal gene that was transposed, repeatedly amplified and pruned. Nature genetics, **14**: 292-299.

Sharma, R., Kishore, A., Mukesh, M., Ahlawat, S., Maitra, A., Pandey, A. K. and Tantia, M. S. (2015). Genetic diversity and relationship of Indian cattle inferred from microsatellite and mitochondrial DNA markers. BioMed Central Genetics, **16**: 73.

Shortt, J. (1876). A manual of Indian cattle and sheep: their breeds, management and diseases. Higginbotham.

Skaletsky, H., Kuroda-Kawaguchi, T., Minx, P. J., Cordum, H. S., Hillier, L., Brown, L. G. and Page, D. C. (2003). The male-specific region of the human Y chromosome is a mosaic of discrete sequence classes. Nature, **423**: 825-837.

Slater, G. (1918). Economic studies. Vol. I: some south Indian villages.

Su, B., Xiao, C., Deka, R., Seielstad, M. T., Kangwanpong, D., Xiao, J., Lu, D., Underhill, P., Cavalli-Sforza, L., Chakraborty, R. and Jin L. (2000). Y-Chromosome haplotypes reveal prehistorical migrations to the Himalayas. Human Genetics, **107**: 582–590.

Sun, D., Yu, Y. and Zhang, Y. (2006). A Y-linked SNP in SRY gene differentiates Chinese indigenous swamp buffalo and introduced river buffalo. Asian-australasian journal of animal sciences, **19**: 1240-1244.

Svensson, E. M., Häsler, S., Nussbaumer, M., Rehazek, A., Omrak, A. and Götherström, A. (2014). Medieval cattle from Bern (Switzerland): An archaeozoological, genetic and historical approach. Schweiz Arch Tierheilkd, **156**: 17-26.

Svensson, E. and Götherström, A. (2008). Temporal fluctuations of Y-chromosomal variation in Bos taurus. Biology Letters, **4**: 752-754.

Switonski, M. and Stranzinger, G. (1998). Studies of synaptonemal complexes in farm animals: A review. Journal of Heredity **89**: 473–480.

Syed-Shabthar, S. M. F., Rosli, M. K. A., Mohd-Zin, N. A. A., Romaino, S. M. N., Fazly-Ann, Z. A., Mahani, M. C. and Md-Zain, B. M. (2013). The molecular phylogenetic signature of Bali cattle revealed by maternal and paternal markers. Molecular biology reports, **40**: 5165-5176.

Tanaka, K. (2000). An assay for paternal gene flow between the taurus-type and indicus-type cattle in Laos and Vietnam using variation in SRY gene. Report of the Society for Researches on Native Livestock, **18**: 59-64.

Tilford, C. A., Kuroda-Kawaguchi, T., Skaletsky, H., Rozen, S., Brown, L. G., Rosenberg, M. and Page, D. C. (2001). A physical map of the human Y chromosome. Nature, **409**: 943-945.

Troy, C. S., MacHugh, D. E., Bailey, J. F., Magee, D. A., Loftus, R. T., Cunningham, P. and Bradley, D. G. (2001). Genetic evidence for Near-Eastern origins of European cattle. Nature, **410**: 1088-1091.

Underhill, P. A., Shen, P., Lin, A. A., Jin, L., Passarino, G., Yang, W. H., Kauffman, E., Bonne-Tamir, B., Bertranpetit, J., Francalacci, P., Ibrahim, M., Jenkins, T., Kidd, J. R., Mehdi, S. Q., Seielstad, M. T., Wells, R. S., Piazza, A., Davis, R. W., Feldman, M. W., Cavalli-Sforza, L. L. and Oefner, P. J. (2000). Y-Chromosome sequence variation and the history of human populations. Nature Genetics, **26**: 358– 361.

Vaiman, D., Mercier D., Moazami-Goudarzi, K., Eggen, A., Ciampolini, R., Lepingle, A., Velmala, R., Kaukinen, J., Varvio, S. L., Martin, P., Leveziel, H. and Guerin, G. (1994). A set of 99 cattle microsatellites: characterization, synteny mapping, and polymorphism. Mammalian Genome. **5**: 288–297.

Van Laere, A.S., Coppieters, W. and Georges, M. (2008). Characterization of the bovine pseudoautosomal boundary: Documenting the evolutionary history of mammalian sex chromosomes. Genome Research, **18**: 1884–1895.

Verkaar, E.L.C., Isaac, J., Nijman, Maurice Beeke, Eline Hanekamp and Johannes A. Lenstra. (2004). Maternal and paternal lineages in cross-breeding bovine species has wisent a hybrid origin. Molecular Biology Evolution, **21**: 1165-1170

Vinod, U., Kumari, P., Vinoo, R., Gangaraju, G. and Bharathi, G. (2019). Estimation of genetic variability parameters in Punganur cattle by microsatellite markers. The Pharma Innovation Journal, **8**: 63-67.

Vogel T, Dechend F, Manz E, Jung C, Jakubiczka S, Fehr S. (1997). Organization and expression of bovine TSPY. Mammal Genome, **8**: 491–496.

Wallace, R. (1888). India in 1887 as seen by Robert Wallace. Oliver and Boyd.

Wilkerson, A. J. P., Raudsepp, T., Graves, T., Albracht, D., Warren, W., Chowdhary, B. P., Skow, L. C. and Murphy, W. J. (2008). Gene discovery and comparative analysis of X-degenerate genes from the domestic cat Y chromosome. Genomics **92**: 329–338.Wilson, D. E. and Reeder, D. M. (2005). Mammal species of the world: a taxonomic and geographic reference. JHU Press.

Winaya, A., Rahayu, I. D., Amin, M. and Herliantin, H. (2011). The Genetic Variation of Bali Cattle (Bos javanicus) Based on Sex Related Y Chromosome Gene. Animal Production, **13**.

Wurster, D. H. and Benirschke, K. (1968). Chromosome studies in the superfamily Bovoidea. Chromosoma, **25**: 152-171.

Xin, Y., Zan, L., Liu, Y., Tian, W., Wang, H., Cheng, G. and Yang, W. (2013). Y-STR Haplotypes and Their Genetic Relationship of Nine Chinese Native Cattle Breeds, 114-121.

Yang, Y., Chang, T. C., Yasue, H, Bharti, A. K., Retzel, E.F. and Liu, W. S. (2011). ZNF280BY and ZNF280AY: autosome derived Y-chromosome gene families in Bovidae. BMC Genomics, **12**: 13.

Yoon, D., Kwon, Y. S., Lee, K. Y., Jung, W. Y., Sasazaki, S., Mannen, H. and Lee, J. H. (2008). Discrimination of Korean cattle (Hanwoo) using DNA markers derived from SNPs in bovine mitochondrial and SRY genes. Asian-Australasian Journal of Animal Sciences, **21**: 25-28.

Yue, X., Li, R., Liu, L., Zhang, Y., Huang, J., Chang, Z. and Lei, C. (2014). When and how did Bos indicus introgress into Mongolian cattle?. Gene, **537**: 214-219.

Zhai, W., Chen, X., Wu, D., Xu, J., Gao, Y. and Zhu, L. (1993). Amplification and analysis of a male-specific SRY homologous sequence of cattle. Chinese journal of biotechnology, **9**: 79-84.

Zhang, R., Cheng, M., Li, X., Chen, F., Zheng, J., Wang, X. and Meng, Q. (2013). Y-SNPs haplotype diversity in four Chinese cattle breeds. Animal biotechnology, **24**: 288-292.

ANNEXURE

QUANTITY AND QUALITY OF DNA SAMPLES

S.No	Sample	Concentration of the DNA (ng/µl)	λ 260/λ280
1	P480	234.477	1.76
2	P351	206.904	1.76
3	P137	117.546	1.59
4	P114	263.194	1.75
5	P535	242.55	1.76
6	P426	125.229	1.74
7	P114	61.196	1.72
8	P120	162.388	1.76
9	P141	165.733	1.7
10	NP9	210.6	1.71
11	P461	352.286	1.76
12	P417	165.608	1.74
13	N22	523.615	1.75
14	P411	366.4	1.78
15	P531	233.775	1.75
16	N24	249.173	1.73
17	P127	209.257	1.75
18	P532	433.975	1.77
19	P100	338.734	1.77
20	P112	180.416	1.73
21	N26	70.681	1.7
22	P516	66.762	1.72
23	P121	222.561	1.77
24	P161	102.585	1.74
25	P139	119.728	1.72
26	N27	243.615	1.75
27	P158	355.91	1.72
28	P117	282.91	1.71
29	P208	165.217	1.7

S.No	Sample	Concentration of the DNA (ng/µl)	λ 260/λ280
30	P140	236.564	1.74
31	P125	251.12	1.74
32	P136	128.58	1.7
33	P160	248.197	1.75
34	P154	50.145	1.72
35	P166	134.422	1.73
36	P265	114.827	1.7
37	P342	132.985	1.73
38	P640	106.099	1.72
39	P243	210.715	1.74
40	P964	145.076	1.75
41	P318	858.69	1.71
42	P353	71.08	1.72
43	P158	355.91	1.72
44	GNT3	90.03	1.74
45	BPT1	648.778	1.76
46	MK1	131.608	1.81
47	ELM5	136.543	1.77
48	ALP3	85.369	1.95
49	BHM1	562.002	1.84
50	BHM2	159.997	1.85
51	BHM3	90.991	1.71
52	BHM5	65.283	1.81
53	MP1	131.608	1.84
54	MP4	129.934	1.73
55	MP5	168.73	1.83
56	MP12	69.906	1.71
57	HYD1	160.049	1.73
58	HYD3	205.654	1.74
59	HYD4	183.357	1.74
60	HYD9	85.136	1.77