

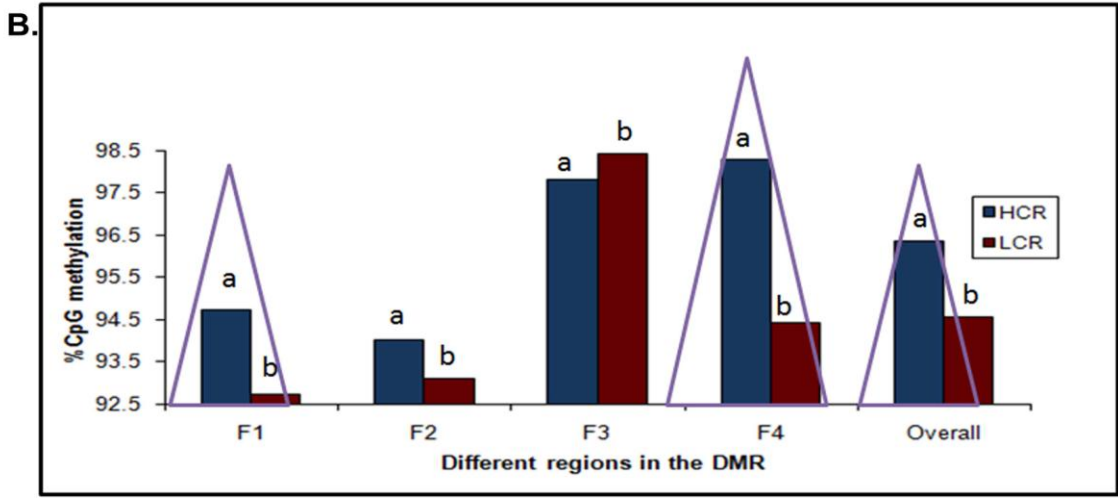
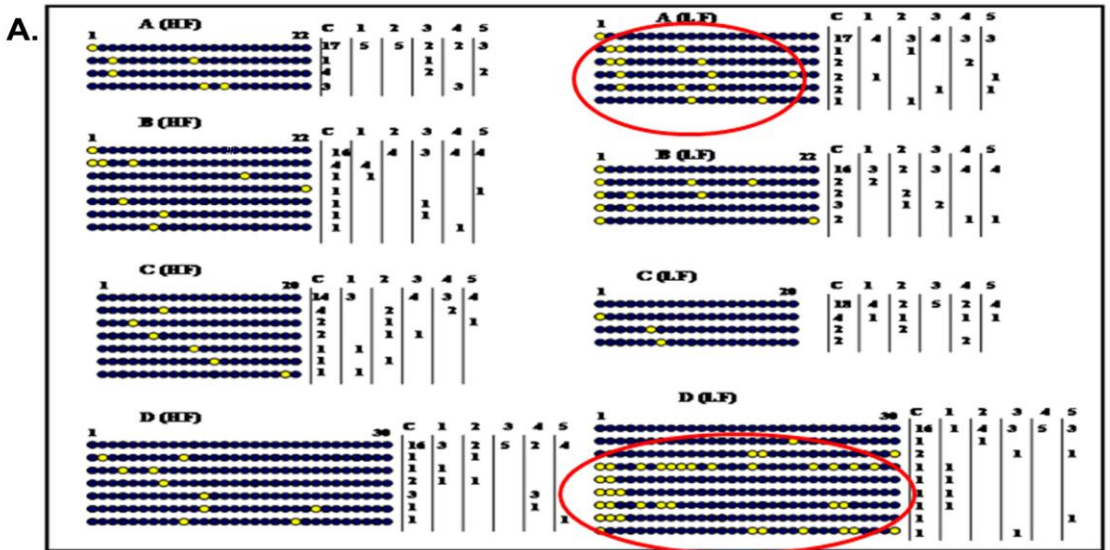
### Research Achievements/Innovations: From inception to March, 2011

- ✦ Cloning and sequencing of five bovine Y chromosomal genes have been completed.
- ✦ Ten non-coding gene markers were analyzed for their arrangement in the Y chromosome and found to be arranged in five clusters in the available BAC sequences of *Bos taurus* Y chromosomes.
- ✦ Bovine Y chromosomal haplotyping method was developed based on Y chromosome based SNPs and indel markers.
- ✦ A simplified bi-sulfite sequencing protocol was optimized to determine methylation status of CpG islands. The protocol allows accurate estimation of CpG methylation status.
- ✦ Differential methylation pattern was analyzed in the H19 DMR region of high (conception rate  $\geq 52\%$ ) and low (conception rate  $\leq 35\%$ ) fertile crossbred bulls. Overall methylation % revealed that the low fertile bulls are hypomethylated at H19 DMR region.
- ✦ Optimized RNA isolation from semen of crossbred bulls and optimized amplification of endogenous control gene for real time expression profiling of sperm transcript. Real time PCR based copy number estimation of Y chromosomal genes, SSH library preparations and microarray profiling are in process.

## Salient achievements/innovations

A simplified bi-sulfite sequencing protocol optimized to determine methylation status of CpG islands:

Genomic DNA methylation is an epigenetic event that affects cell function by altering gene expression and refers to the covalent addition of a methyl group, catalyzed by DNA methyltransferase (DNMT), to the 5-carbon of cytosine in a CpG dinucleotide. It is involved in various aspects of development and other biological phenomena of eukaryotic organisms. Several experimental approaches have been carried out to analyze DNA methylation. The main methodologies may broadly be classified into two categories, Polymerase Chain Reaction (PCR) based and non-PCR based methods. PCR based techniques are more powerful and reliable in analysis of genomic DNA methylation profiles. Three main processes are required in the PCR based analysis: detection of methylated cytosine and this is done by methylation-sensitive enzymes or sodium bisulphite treatment, then followed by PCR amplification and sequencing. All these methods are more complex and have their limitations. A simplified bi-sulfite sequencing protocol has been optimized to determine methylation status of CpG islands. The protocol allows accurate estimation of CpG methylation status. The present method is very simple and inexpensive, can convert more than 95% of unmethylated cytosine into uracil residue. These uracil residues could easily be detected subsequently.



- A. Methylation pattern of different overlapping fragments of 2 kb H19 DMR in high (conception rate  $\geq 52\%$ ) and low (conception rate  $\leq 35\%$ ) fertile crossbred bulls.
- B. Methylation distribution across four fragments of H19 DMR in HCR and LCR bulls. Mean Methylation at fragment 1 & 4 was significantly hypomethylated in low fertile bulls. Overall methylation % revealed that low fertile crossbred bulls are hypomethylated at H19 DMR.

Bovine Y chromosomal haplotyping method was developed based on Y chromosome based SNPs and indel markers

Y chromosome microsatellites (STRs) were used to distinguish between *B. taurus* and *B. indicus* ancestry (Bradley et al. 1994). Gotherstrom et al. (2005) used SNPs to define three cattle Y haplogroups (Y1 and Y2 in *B. taurus* and Y3 in *B. indicus*) and to investigate aurochs contributions to the genetic composition of modern breeds. Recently, Ginja et al. (2009) used a combination of SNPs and STRs specific to the non-recombining region of the Y chromosome to describe 13 haplotypes in European cattle. These markers are useful to detect introgression and to distinguish between *Bos taurus* and *Bos indicus* patriline. Characterization and conservation of domestic animal genetic resources is a priority, and efforts are being made to take into account information from nuclear, mitochondrial, and Y chromosome markers to define conservation priorities. The analysis of Y chromosome haplotypes can thus provide additional information for inferring the origins and genetic relationships of our cattle in India and help in classifying Indian cattle into different haplogroups and also establishment of a reference database on the lines of those available for humans or *Bos taurus* cattle.

