OBJECTIVES

Briefly objectives were divided into two parts:

Section-I: Chromosomal Localization, Copy Number Assessment and Transcriptional Status of BamHI Repeat Fractions in Water Buffalo Bubalus bubalis.

- To identify and isolate satellite DNA fragments from buffalo, Bubalus bubalis, using Restriction Fragment length polymorphism (RFLP) with BamH1 enzyme.

- To clone and characterized the recombinant plasmids representing different satellite fractions.

- Homology search within and across the species using default server (www.ncbi.nih.gov/BLAST). Multiple sequence alignment and Phylogenetic tree construction using ClustalW program (www.ebi.ac.uk/clustalw/).

- Zoo-blot analysis of repeat fragments to ascertain their conservation among different animals.

- Relative expression and copy number assessment of candidate satellite fractions using Real time PCR and their chromosomal organization employing Fluorescent in situ Hybridization (FISH) with buffalo metaphase chromosomes.

Section-II: Molecular mining of exonic sequences tagged with consensus of 33.6 repeat loci in Buffalo Bubalus bubalis.

- In silico analysis of minisatellite 33.6 related 5’ CCTCCAGCCCT 3’ repeat in several species at the National Center for Biotechnology Information (NCBI; http://www.ncbi.nlm.nih.gov) using default server www.ncbi.nih.gov/BLAST.

- To uncover repeat-tagged transcribing sequences in water buffalo, Bubalus bubalis, using consensus sequence of 33.6 repeat loci (CCTCCAGCCCTCCTCCAGCCCT) and cDNA from different somatic tissues.
Objectives

and spermatozoa employing Minisatellite Associated Sequence Amplification (MASA).

- Cloning, sequencing and database search (www.ncbi.nih.gov/BLAST) for different mRNA transcripts uncovered by MASA with 33.6 repeat loci. Multiple sequence alignment and Phylogenetic tree construction using ClustalW program (www.ebi.ac.uk/clustalw/).

- RNA slot-blot hybridization and RT PCR for MASA amplified sequences.

- Somatic tissue, gonad and spermatozoa specific expression study of few MASA generated candidate transcripts with Real time PCR.

- Cross-hybridization of MASA amplified orthologues in different species.

- Generation of full-length sequence of MASA generated Peroxisomal Membrane Protein-4 (PXMP-4) gene and their homology status across the species.