

ultimately help in physical mapping of genes in these less explored species. The investigation was carried out on 40 mithuns, 6 mithun cattle crossbreds, 8 cattles and 8 gaurs. The metaphase spreads prepared conventionally were hybridized with centromeric and telomeric probes. The chromosomal localization was highlighted by FISH signals in all species. In addition, the bovine chromosome painting probes were also used to delineate the chromosomal rearrangements. The centromeric DNA sequences were similar in all acrocentric mithun chromosomes; no signals in submetacentric chromosome of mithun crossbred and gaur were observed, while in cattle the signals were present in all chromosomes. The preliminary study supports that the Indian mithun and gaur are more closely related ancestral species than cattle.

## O14

### Multicolor FISH with 10 specific painting probes for the rapid identification of the sub-metacentric river buffalo autosomes (*Bubalus bubalis*, 2n=50)

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Multiplex-FISH (M-FISH), spectral karyotyping (SKY) and combined binary ratio labeling FISH (COBRA-FISH) are the current methods used for the simultaneous visualization of chromosomes in different colors. Their application in human clinical cytogenetics makes easier the identification of chromosomal abnormalities. In animal cytogenetics the use of these methods is still very limited, mainly for the lack of species specific-probes. In this work we propose a multi-color approach based on the simultaneous hybridization of specific river buffalo chromosome painting probes. Ten specific autosomal probes were prepared through conventional microdissection and DOP-PCR. Probes were labeled with Spectrum Green and Spectrum Orange in the second DOP-PCR step. Five sequential rounds of FISH were performed on the same slides. Each round was realized by using two probes simultaneously hybridized on the

mitosis. Slides were counterstained with DAPI in antifade. After each hybridization, the slide was washed twice in PBST for 10 min, air dried and reused again for the subsequent step. Digital images were captured in gray-scale and pseudo-colored by the software. All the five pairs of biarmed river buffalo autosomes (BBU 1p, 1q, 2p, 2q, 3p, 3q, 4p, 4q, 5p and 5q) were identified with a different color. The simultaneous hybridization of these probes allowed to develop the first multi-color FISH in the river buffalo species. Given the remarkable extent of chromosome banding homology within the Bovidae family, these probes might be utilized for cross-species hybridization experiments within the family, thus opening further opportunities for cytogenetic investigation also in other species.

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## O15

### Genomics and Evolution of TR loci in *Tursiops truncatus*

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The dolphin (*Tursiops truncatus*) is a mammal that has fully adapted to the aquatic environment during its evolution. Despite its popularity and even iconic status, our knowledge about dolphin genomics and evolution is very limited. We employed the GenBank and the EMBL-EBI draft genome assemblies (Ttru\_1.4 and turTru1, respectively) to identify T cell receptor (TR) loci in *Tursiops truncatus*. The dolphin gamma (TRG) locus is the smallest and simplest of all mammalian TRG loci yet studied because it only contains a single constant (TRGC), two variable (TRGV1, V2) and three joining (TRGJ1, J2, J3) genes only. Expression analysis to evaluate the gamma chain repertoire in the peripheral blood from animals in a marine monitored environment identified all the possible V-J rearrangements, some of which are preferentially expressed. Although there is not a definitive scenario because of gaps in the available assemblies, the dolphin beta (TRB) locus is also very simple in comparison to human and ovine. A significant bias for expression of the variable TRBV17S1 gene,