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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 family Bovidae, these probes might be utilized for cross-species hybridization experiments within the family, thus opening further opportunities for cytogenetic investigation also in other species.

This study was supported by CISIA-VARIGEAV project, National Research Council (CNR) of Italy