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Research Article

BANDING PATTERN INDUCED BY RESTRICTION ENDONUCLEASES ON BUFFALO (BUBALUS BUBALIS) CHROMOSOMES

R. S. PRIYA[2], R. K. PATEL[1], K. R. S. SAMBASIVA RAO[2] and K. M. SINGH
Research and Development (Biotechnology Division)
National Dairy Development Board, Anand-388 001 (Gujarat)

ABSTRACT

The karyotype of reverine buffalo is composed of 24 pairs of autosomes and a pair of sex chromosomes (2n=50) The first five pairs of autosomes are submetacentric where as all other chromosomes are acrocentric including sex chromosomes, which are many times difficult to distinguish individually, even in well-banded chromosomes. The metaphase chromosomes preparations are susceptible to restriction enzymes. The present study was conducted to generate restriction enzyme banding profile on buffalo chromosomes to identify the homologous chromosome and structural abnormalities in addition to the conventional G-, R-, and C- banding. Chromosomal slides prepared by whole blood culture techniques were treated with various restriction enzymes; *MboI, HinfI, HaeIII, AvaI, AvaII* and *TaqI,* which revealed G- and C-like bands on buffalo chromosomes. Restriction enzymes treated cause the loss of DNA which is directly related to a decreased Giemsa stain that generates a characteristic staining pattern through out the length of the chromosomes. The protocols, quantity of enzyme used, banding pattern produced on chromosomes and preparation of karyotypes have been discussed in the present article.

Key words: Chromosomes, karyotype, restriction enzyme, DNA, banding

Chromosomal aberrations associated with reduced fertility have caused concern in various countries and many of them have been screening their breeding animals (Kovacs and Szepeshelyi, 1987, Patel, 2000). It is known that several domestic animals have chromosomes, which are very difficult to distinguish individually. In buffaloes most of autosomes and sex chromosomes are acrocentric in structure, therefore, identification problems are encountered while comparing with international standard (Gustavsson, 1999). As a result, many aberrations particularly structural that do not change the shape and size of chromosomes, have escaped our attention. A few autosomal reciprocal translocations (Kovacs *et al.*, 1992) and inversions (Roldan *et al.*, 1984) have so far been found in cattle, probably due to identification problems. Identification of reciprocal translocations not producing drastic changes in length/shape is difficult even in well-banded chromosomes preparations. The metaphase chromosome preparations are susceptible to restriction enzymes (Alfi *et al.*, 1973). The present study was conducted to generate restriction enzyme banding profile through out the length of chromosomes in buffalo using the enzymes: *Mbo* I, *Hinf* I, *Hae* III, *Ava* I, *Ava II* and *Taq* I so that these bands could help to identify the homologous chromosomes and structural abnormalities in addition to conventional G-, R- and C– bandings.

[1]Corresponding author, e-mail: rkpatel@ndbd.coop
[2]Centre for Biotechnology, Acharya Nagarjuna University, AP

MATERIALS AND METHODS

Lymphocyte culture: Chromosome preparation was made by using standard whole blood culture in RPMI-1640 medium supplemented with antibiotics, 15% fetal calf serum and pokeweed mitogen as described by Patel (1999).

Restriction endonuclease/ Giemsa banding: As metaphase chromosomes prepared by methanol-acetic acid fixation and air-drying technique are susceptible to DNases (Alfi *et al.*, 1973), 40 µl to 60 µl solution containing 0.8 unit/ il of restriction enzyme and 10X assay buffer provided with enzyme, was placed on a slide,