

Sequential QFQ-RBA-banding pattern in prometaphase chromosomes of cattle (*Bos taurus* L)

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Summary – A sequential QFQ-RBA banding procedure, for the first time performed on prometaphase chromosomes of cattle (*Bos taurus* L), is presented with the aim of establishing correlations between Q and R bands. The results of the present investigation allowed the standardization of the first QFQ-banded karyotype of this species, especially useful for identification of cattle chromosomes in gene mapping studies.

sequential banding / QFQ-banding / RBA-banding / standardization / cattle

Résumé – Application d'une technique séquentielle de bandes QFQ-RBA aux chromosomes prométaphasiques bovins (*Bos taurus* L). Une technique séquentielle de bandes QFQ-RBA est présentée pour la première fois sur les chromosomes prométaphasiques bovins dans le but d'établir des corrélations entre les bandes Q et R. Les résultats de la présente recherche ont permis l'établissement du premier caryotype standard QFQ bovin au niveau prométaphasique, utilisable pour la localisation des gènes dans l'espèce *Bos taurus* L.

technique de bandes séquentielles / bande Q / bande R / standardisation / bovin

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INTRODUCTION

Q-banding on chromosomes of cattle (*Bos taurus* L) has so far not received as much attention as the G- or the R-banding techniques. The lack of a standard Q-banded karyotype has been one of the major drawbacks which hampered the application of this technique for routine analysis of cattle chromosomes.

More recently, the utilization of Q-banding for chromosome identification in cattle gene mapping (Fries *et al*, 1989) has renewed the interest toward this technique and prompted the organizers of the 2nd International Conference for Standardization of Banded Karyotypes of Domestic Animals (Jouy-en-Josas, Paris) to standardize also a Q-banded karyotype (ISCNDA, 1989).

The present paper reports a sequential QFQ-RBA-banding technique for the first time performed on prometaphase chromosomes of cattle in order to establish correlations between Q- and R-bands; these correlations were used for the definition of the standard QFQ-banded karyotype of cattle at the Paris Conference.

MATERIALS AND METHODS

Peripheral blood (1 ml), drawn from the jugular vein of four young bulls of the Italian Friesian breed, was cultured at 37.5°C for 72 h in 9 ml of RPMI 1640 medium (Flow, Dutch modification), supplemented with 10% fetal calf serum (Gibco), 0.1% L-glutamine and 0.1 ml of pokeweed mitogen (Gibco). 6.5 h before the end of the culturing time, 5'-bromodeoxyuridine (BUdR, Sigma) was added at a final concentration of 20 µg/ml. The colcemid treatment (final concentration of 0.03 µg/ml) lasted 1 h. In order to facilitate the spreading of the prometaphase chromosomes, the cell suspension was treated with a more hypotonic solution (0.05 M, KCl) at 37.5°C for 15 min and fixed with methanol-acetic acid solution (3:1) for 1 h, centrifuged, fixed again and left overnight in the refrigerator. Air-dried slides were prepared.

Sequential QFQ-RBA banding procedure

Soon after the preparation, the air-dried slides were examined under phase contrast in order to preselect the best prometaphase plates. Subsequently, the slides were stained with a fresh solution of quinacrine dihydrochloride (Sigma) (0.1% in phosphate buffer, pH 7.0) for 15 min, washed in tap water for a few seconds mounted in the same buffer without sealing the coverslip, and examined promptly with a Leitz Dialux photomicroscope under epifluorescent optics (filter E3). The spreads were relocated and the best Q-banded prometaphase plates photographed with a Kodak microfilm 1454. After microphotography, the coverslip was removed under tap water, the slide destained gently in 30% ethanol for 10 min, washed in distilled water, air dried and stained again with acridine orange solution (0.2% in phosphate buffer) for 15 min, washed in tap water, mounted in the same buffer and sealed with paraffin. The prometaphase plates previously examined for QFQ-banding were relocated and photographed again under UV light for RBA-banding (filter I2/3) with the same Kodak microfilm 1454. Kodabrome papers n2 and n3 were used for printing.

RESULTS

Figure 1 shows in (A) a QFQ-banded prometaphase plate of cattle ($2n = 60,XY$) and in (B) the same plate sequentially stained for RBA-banding. The R-banded chromosomes from figure 1B were assembled into the karyotype shown in figure 2, according to the standard RBA-banded karyotype as defined at the Paris Conference (ISCNDA, 1989); subsequently, the Q-banded chromosomes from figure 1A were arranged into the Q-banded karyotype shown in figure 3.

These results were the basis upon which the final QFQ-banded karyotype, obtained from unlabelled prometaphase chromosomes, was constructed and established as standard at the Paris Conference.

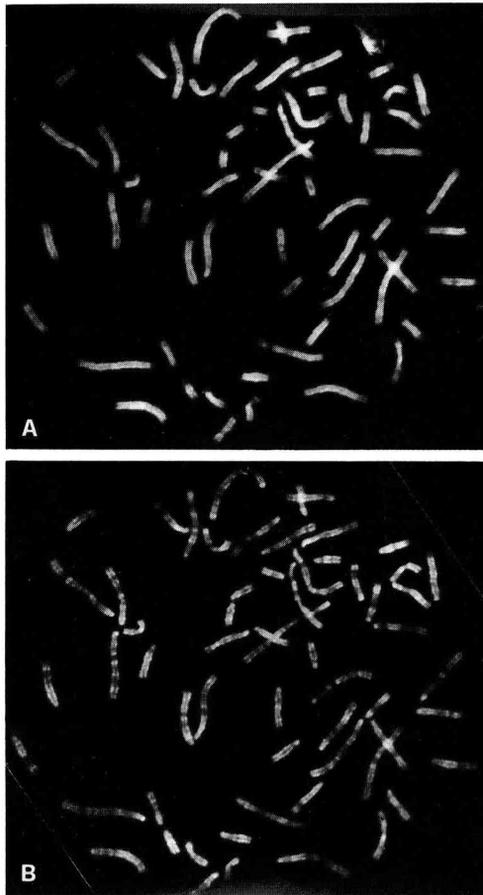


Fig 1. A. QFQ-banded prometaphase plate of cattle ($2n = 60,XY$); B. the same plate sequentially stained for RBA-banding.

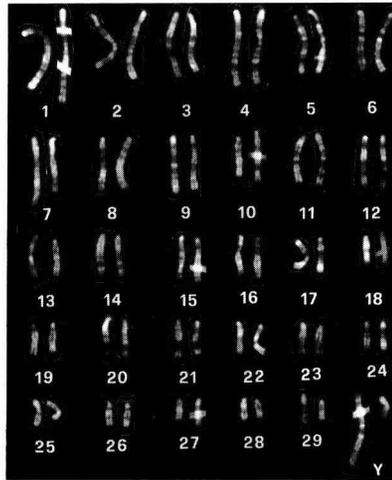


Fig 2. RBA-banded prometaphase karyotype of cattle, arranged according to the RBA-banded karyotype defined as standard at the Paris Conference, from the plate shown in figure 1B.

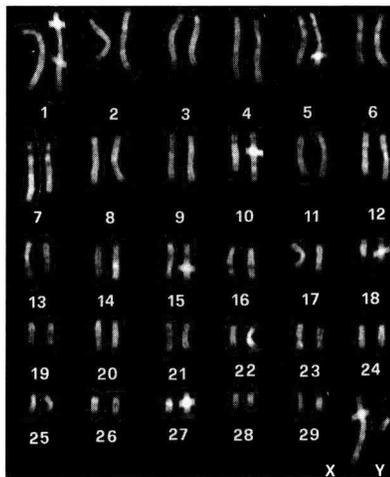


Fig 3. QFQ-banded prometaphase karyotype of cattle, arranged according to figure 2, from the plate shown in figure 1A.

DISCUSSION

Q-bands were first visualized in *Vicia faba* and subsequently in human chromosomes (Caspersson *et al*, 1969, 1971). In cattle, the first reports on Q-banding were provided by Hansen (1971, 1972, 1973), Popescu (1975) and Gustavsson (1976).

So far, the use of Q-banding for identification of cattle chromosomes in routine analysis has been limited by the fact that better chromosome identification can be achieved with other methods. The Reading Conference (Ford *et al*, 1980) provided a G-banded standard karyotype with a verbal description of the main G-bands; this description, together with the G-banded idiogram provided by Lin *et al*, (1977), has served for more recent studies (Di Berardino *et al*, 1979, 1980, 1981) and for some of the gene assignments by *in situ* hybridization following Q-band staining (Fries *et al*, 1986, 1988; Hediger, 1988).

The use of Q-banding for identification of cattle chromosomes in somatic cell hybrids and in *in situ* hybridization experiments has renewed the interest toward this technique. This has prompted the organizers of the 2nd International Conference for Standardization of Banded Karyotypes of Domestic Animals (ISCNDA, 1989) to improve – on the prometaphase level – the Reading G-banded standard karyotype and to provide for the first time the RBA and QFQ standard karyotypes. For these purposes, it was decided to examine the correlations between G- and R-bands (Di Berardino *et al*, in press) and those between Q- and R-bands.

This investigation, therefore, was carried out in order to trace correlations between Q- and R-bands, thus providing the necessary information for the definition of the new standard QFQ-banded karyotype for the species.

In the sequential procedure reported in the present paper, the late BUdR incorporation, necessary to achieve the RBA banding, did not seem to affect the Q-banding pattern; by comparing different spreads with and without BUdR treatment merely shows that the same patterns are present but it does not prove that they are the same chromosomes. Similarly, the quinacrine dihydrochloride staining used for QFQ-banding did not significantly affect the quality of the RBA-banding pattern. This technique, therefore, can also be used for tracing correlations between Q- and R-bands in other domestic species such as goat and sheep, for which such studies are still lacking.

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