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SHORT COMMUNICATION

A MODIFIED R-BANDING TECHNIQUE

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ABSTRACT

The present report describes a new modification of an R-banding technique using 5-BrdU incorporation and Hoechst 33258 and Giemsa staining (RBG), which has produced good banding quality and has proved to be highly reproducible.

INTRODUCTION

The R-banding technique permits chromosome identification and is especially used for the study of regions showing negative staining by Q- and G-banding. Originally described by Dutrillaux and Lejeune (1971), this technique results in positive staining of Q- and G-negative bands and of the telomeric regions of all chromosomes. These bands were originally obtained by heat denaturation in solutions at acid pH and by Giemsa or acridine orange staining, but showed little reproducibility. New methods were later proposed using 5-bromodeoxyuridine (BrdU) incorporation during the final hours of culture and acridine orange staining (Dutrillaux *et al.*, 1973) or Giemsa staining after Hoechst 33258 staining and treatment with 2SSC (Perry and Wolff, 1974).

BrdU incorporation during the final stage of DNA synthesis occurs in late-replicating regions (G-positive bands), making them undercondensed. The late-replicating X chromosome incorporates BrdU later than the early-replicating X, thus becoming more undercondensed.

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In our laboratory we developed a modification of the techniques of Perry and Wolff (1974) and Korenberg and Freedlender (1974) for visualization of R bands (RBG) which has produced good banding quality and has proved to be highly reproducible (Figure 1) (Ribeiro and Melaragno, 1986).

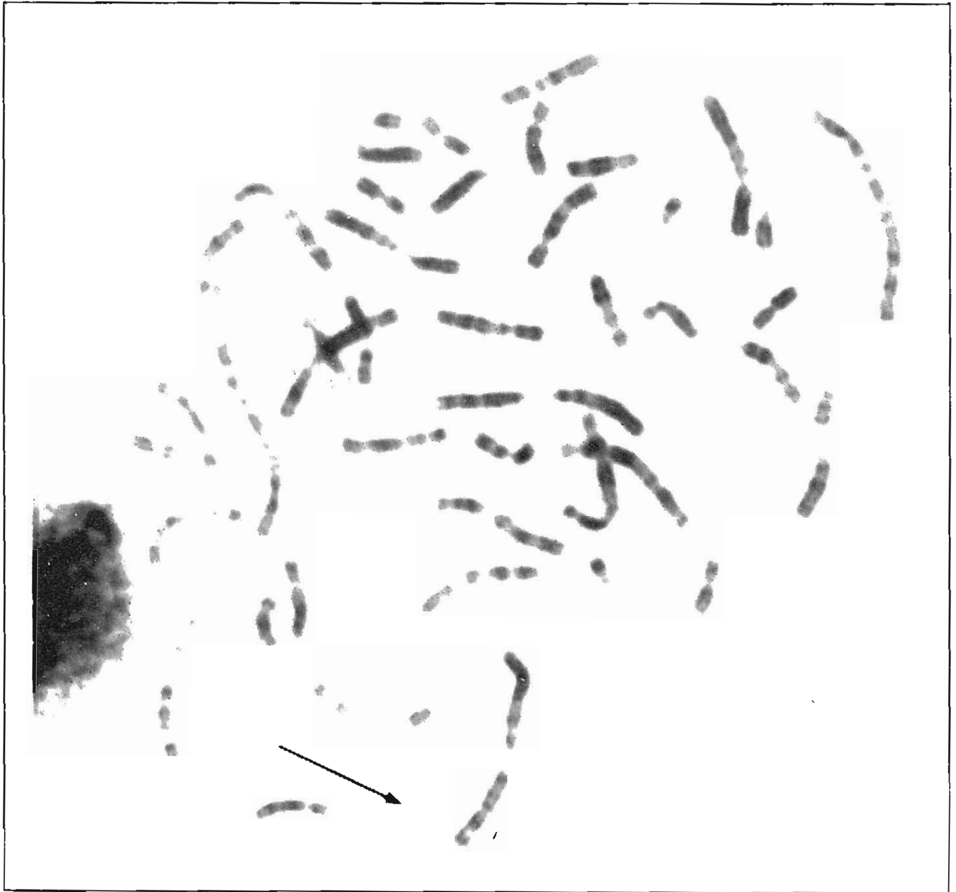

Figure 1 - RBG-Banded chromosomes. The arrow indicates the nonstaining late-replicating X chromosome.

PROCEDURE

a) Cultures - Cultures are prepared as usual in dark glass bottles and 5-BrdU (Sigma) is added during the last 7 hours of culture at a final concentration of 50 μg/ml. Cytological preparation and slide mounting are performed by routine methods.

b) Slide treatment - Slides aged 2 or 3 days are used preferentially.

1. Slides are placed on a flat surface covered with aluminum paper 10 cm below a germicidal ultraviolet lamp (254 nm). A thin layer of Hoechst 33258 solution at a concentration of 5 μg/ml is placed on each slide (± 3 ml per slide) and the preparation is exposed to UV light for 5 to 15 minutes. The slides are then washed with deionized water and dried.
2. Slides are incubated in 1.0 M phosphate buffer (NaH_2PO_4), pH 8.0-8.3, on a water bath at 89°C for 3 to 5 minutes, and washed with deionized water.

c) Staining - Slides are stained with 5% buffered Giemsa for 7 minutes.

Alternatively, slides may be stained with acridine orange diluted in phosphate buffer, pH 6.7, to a 50 μg/ml concentration, for 20 minutes. In this case, analysis is carried out using a fluorescence microscope. Prolonged exposure of metaphase preparations results in destaining.

NOTES

- Slides stored in a freezer in plastic boxes conserve the characteristics of newly-mounted slides and can be used later.
- Slides showing no differentiation should be treated with the buffer for a longer period of time.
- When the slide is faded, this means that buffer treatment was too long.
- In very old slides, the chromosomes have a worn appearance.
- In general, metaphases showing good differentiation of pairs 21 and 22, 19 and 20 are the best for analysis.
- When the 1 M phosphate buffer bottle is left in an incubator at 37°C, salt sedimentation will be avoided.
- The NaH_2PO_4 solution is brought to pH 8.0-8.3 with the aid of a 10 N NaOH solution.
- The 1 M phosphate buffer solution may be used for more than one series of slides.

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RESUMO

No presente trabalho descrevemos uma modificação da técnica de bandamento R, que

utiliza incorporação de 5-BrdU, tratamento pelo Hoechst 33258 e coloração pelo Giemsa (RBG). Esta técnica produz boa qualidade de bandamento além de alta reprodutibilidade.

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