

A SIMPLE R-BANDING TECHNIQUE BY BrdU-HOECHST TREATMENT AND GIEMSA STAINING FOLLOWING HEATING AND ULTRAVIOLET EXPOSURE

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Summary A simple R-banding technique was presented. Peripheral leukocytes were incorporated with 5'-bromodeoxy uridine for 6 to 8 hr prior to harvesting. R-banded metaphases were obtained by Giemsa staining following 33258 Hoechst staining for 15 min and heating at 60°C for 80 min in 2×SSC with exposure to ultraviolet. This method has many advantages; 1) the possibility of obtaining a permanent R-banding preparation immediately after harvest, 2) feasible demonstration of the late-replicating X chromosome, and 3) no requirement of a fluorescence microscope.

INTRODUCTION

R-banding by the heat-denaturation method of Dutrillaux and Lejeune (1971) and several modifications of this method (Eiberg, 1975; Verma and Lubs, 1975) have been widely applied. The method of 5'-bromodeoxy uridine (BrdU) pretreatment in culture (RBA method) has been commonly used for the studying the replication of the X chromosome, and for demonstration of the R band with a high reproducibility (Dutrillaux, 1975; Pai and Thomas, 1980). The principal limitations of the RBA method are that a fluorescence microscope is required, and that slides should be processed for R-banding within 6-9 days after the harvest. Pai and Thomas (1980) pointed out the possibility of an R-banding technique with Hoechst-Giemsa staining. Camargo and Cervenka (1982) demonstrated an R-banding technique using cell synchronization combined with BrdU pulsing and fluorochrome-photolysis-Giemsa (FPG) differential staining. This method was essentially developed for the demonstration of sister chromatid exchange in the metaphase chromosome (Perry and Wolff, 1974; Korenberg and Freedlender, 1974). But this technique is too complicated for routine use.

We report here a simple R-banding technique using the BrdU-Hoechst 33258-Giemsa method. Permanent R-banded preparations are obtained immediately by our method.

Received May 16, 1983

MATERIALS AND METHODS

Whole blood samples from 60 consecutive patients referred to us for cytogenetic diagnosis were cultured for 72 hr at 37°C in Dulbecco's modified Eagle's medium with 0.1% glucose and 0.37% NaHCO₃ supplemented with 10% fetal bovine serum, 2.5% phytohemagglutinin (Gibco, M form) and anti-biotics. BrdU was added to a final concentration of 50 µg/ml during the last 6 to 8 hr of culture. Corcemid (Gibco, 0.03 µg/ml) and ethidium bromide (Sigma, 3 µg/ml) were added to the culture 90 min prior to fixation. Chromosome preparations were obtained by the ignition technique following hypotonic treatment with 3 : 1 0.075 M KCl : 1% sodium citrate for 15 min at 37°C, and fixation with a 3 : 1 methanol : acetic acid fixative. The slides were immediately stained in Hoechst 33258 (Wako Pure Chemicals; 50 µg/ml distilled water) for 15 min and then rinsed briefly in deionized water. The preparations were mounted in 2×SSC (0.3 M sodium chloride–0.03 M trisodium citrate) and the coverslips ringed with nail enamel to prevent evaporation. The slides were placed on a hot plate (surface temperature of approximately 60°C) and exposed to a ultraviolet (UV) light (distance of about 17 cm) for about 80 min. The coverslips were then removed and the slides rinsed in running water. The slides were then stained with phosphate-buffered Giemsa (3%, pH 6.8) for 20 min.

RESULTS AND DISCUSSION

Figure 1 shows an R-banded karyotype of a patient with 48,XXXXY after 33258 Hoechst and Giemsa staining. The Giemsa preparation is permanent, and can be easily studied with a compound microscope. The majority of cells on any given slide showed the typical R-banding pattern. This technique provided excellent-quality karyotypes of the R-band in all patients studied. Occasionally differences of the staining intensity between euchromatic regions of homologues were observed. But they were negligible when more than two metaphases were analyzed. When the R-banding pattern was not obtained at the first attempt, it was necessary to alter the duration of UV exposure and heating time to 60–90 min. Since a better-quality karyotype of the R-band was observed in less contracted chromosomes, ethidium bromide was added during the last 90 min according to the method of Ikeuchi and Sasaki (1979) with slight modifications. R-banding was feasible within three weeks of slide storage at room temperature. The period of BrdU pretreatment was changed from 3 to 12 hr. On 3 to 4 hr treatment, R-banding patterns were faintly visible and nearly all of the metaphases darkly stained. With the longest treatment (more than 10 hr), weakly stained metaphases were observed in many cells. In the 6–8 hr treatment group, R-banding of the best quality was observed. This phenomenon was also observed for the RBA method and discussed by Dutrillaux *et al.* (1976) and Camargo and Cervenka (1982).

The late-replicating X chromosomes were shown as an unbanded or variably

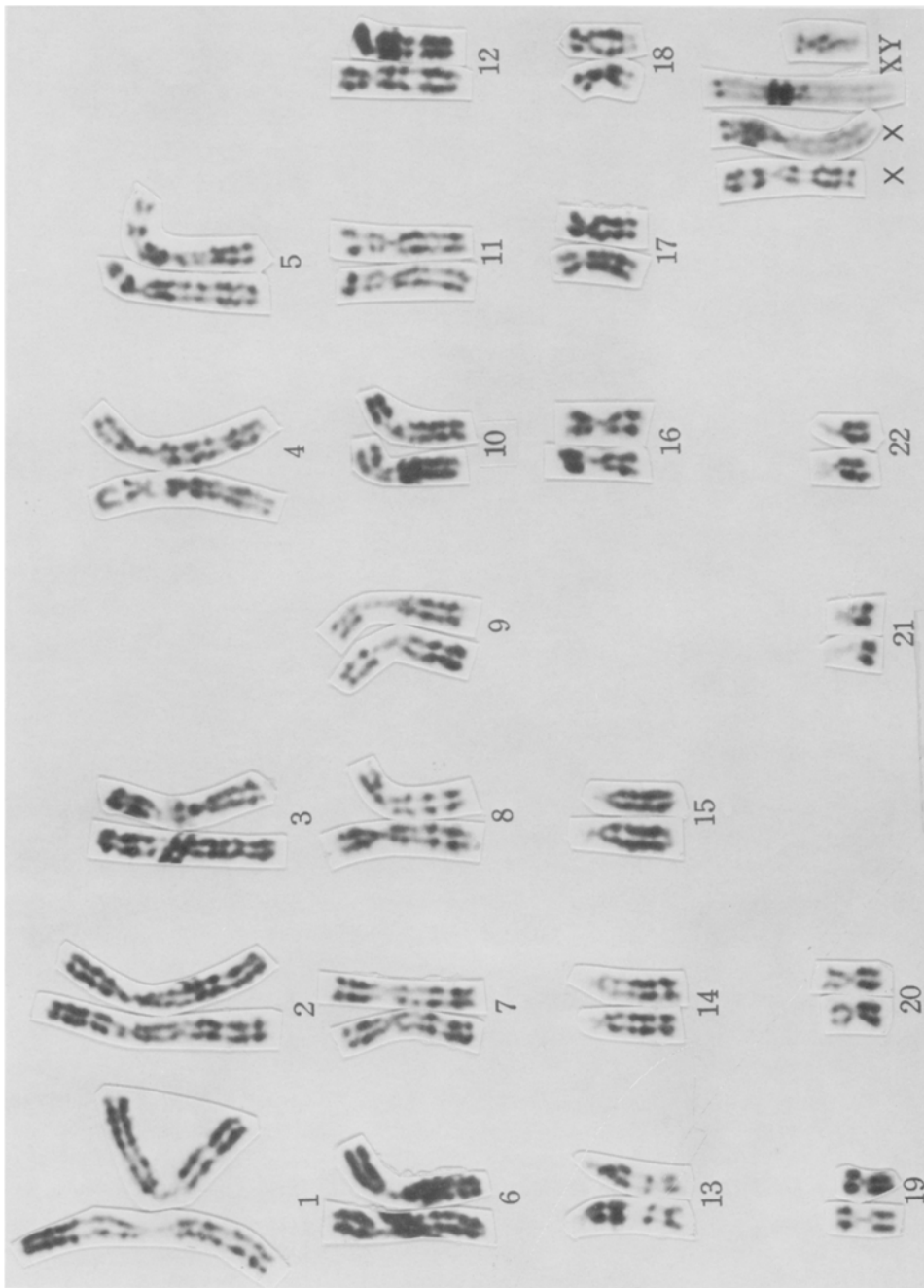


Fig. 1. An R-banded karyotype of 48,XXXYY. The late-replicating X chromosomes are lightly stained and elongated. The regions of constitutive heterochromatin are also lightly stained and elongated.

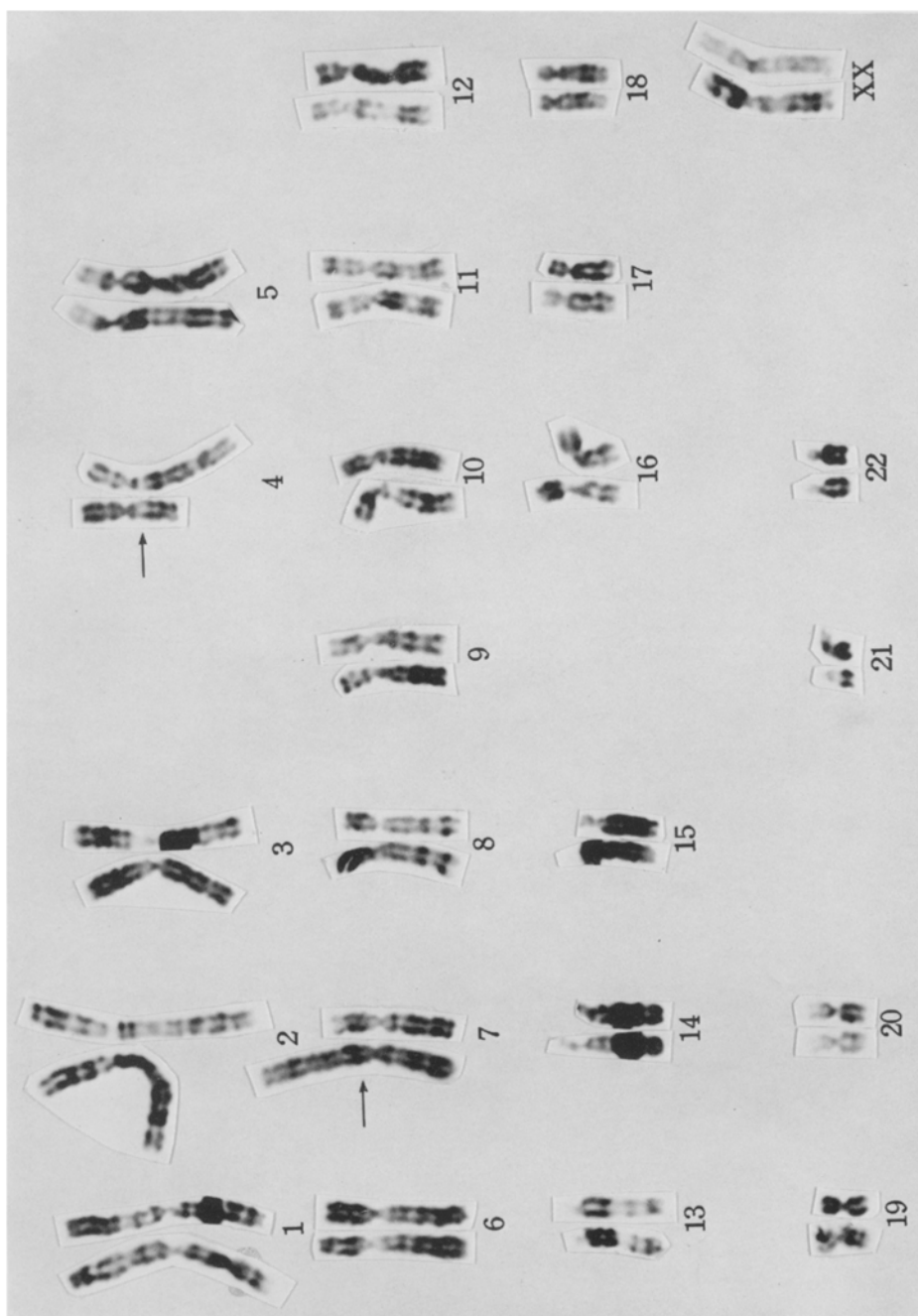


Fig. 2. An R-banded karyotype of 46,XX,t(4;7)(q21;p12). She showed mental retardation without any morphological abnormalities.

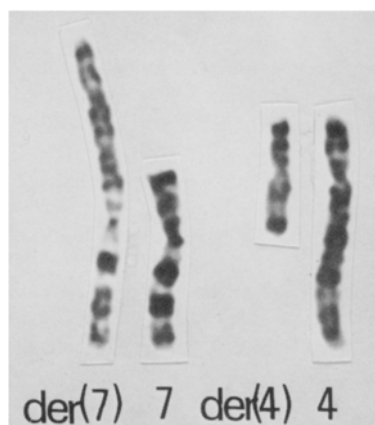


Fig. 3. A partial karyotype of 46,XX,t(4;7)(q21;p12) by G banding.

banded structure. Although it is necessary to count and analyze a large number of cells in a patient with mosaic Turner syndrome, mosaicism can be easily analyzed by this method. A late-replicating study of the X chromosome can also be performed easily. The pitfall of this method is that the structural abnormalities of the X chromosome may be misjudged.

Figure 2 shows an R-banded karyotype of 46,XX,t(4;7)(q21;p12). Figure 3 shows a partial karyotype. The combination with R and G band is believed to be the most informative. Since more than ten slides can be processed for R-banding at the same time, this method can save much time, effort and money, and can be adapted for routine use.

The advantages of this method are 1) the feasibility of getting permanent R-banding preparations 0–3 weeks after harvesting, 2) no necessity of a fluorescence microscope, and 3) the ability to demonstrate the late-replicating X chromosome. Our technique is simple and highly reproducible and may be of value for practical application.

Acknowledgement We are grateful to Dr. Y. Kuroki, Director of Div. of Medical Genetics, Kanagawa Children's Medical Center, for his kind advice and encouragement.

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