Technical note

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A simple technique for obtaining prometaphase chromosomes from lymphocytes

The human chromosomes of 'prophases' and 'prometaphases' reveal more bands that can be seen even in the best banded 'metaphase' chromosome. The earlier techniques in obtaining prometaphase chromosomes are rather complex¹ and require several steps. This report describes a simple method for synchronising peripheral blood culture and obtaining sufficient cells at the appropriate stage of division for high resolution banding.

Lymphocytes from peripheral blood from normal donors (10 females and 10 males) were cultured for 68 to 72 hours at 37°C in 5 ml of 1A medium (Cat No 120–1670 Grand Island Biological) supplemented with fetal calf serum and phytohaemagglutinin. After 68 to 72 hours' incubation, the cultures were treated with 5-bromo-2-deoxyuridine (200 µg/ml, Sigma) and 0.3 µg/ml of thymidine (Sigma) for exactly 4½ hours. Before harvest, cultures were treated for an additional half hour with colcemid (0.03 µg/ml, Gibco Diagnostic). It is important to mention that BrdU and thymidine are added at the

same time and no washing is required. Colcemid was also added without removing the other two chemicals. The cells were then harvested by the standard techniques. GTG banding² was performed immediately after harvest and good quality bands were obtained. For RFA banding³ slides were allowed to age for 7 to 10 days. In the figure, chromosome 1 is shown as an example of GTG and RFA banding. The diagrammatic pattern is reproduced from the recent nomenclature on high resolution banding.⁴

Several concentrations of BrdU and thymidine with variable durations of treatment were tried. A brief exposure of 4½ hours to BrdU (200 µg/ml) and thymidine (0.03 µg/ml) produced the best results. By this method it is possible to obtain prophase or prometaphase spreads suitable for G banding within 75 hours. Reduction in exposure time to BrdU produces more mitoses. A lower concentration of colcemid (0.03 µg/ml) is also an important factor in obtaining good preparations. This improved technique is a valuable tool in the analysis of prometaphase chromosomes.

J V SHAH, R S VERMA, J RODRIGUEZ, AND H DOSHI
*Departments of Laboratories and
Medicine, The Jewish Hospital and
Medical Center of Brooklyn and SUNY
Downstate Medical Center,
Brooklyn, NY 11238, USA.*

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FIGURE High resolution
GTG and RFA bands in
human chromosome 1.

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Correspondence and requests for reprints to Dr Ram S Verma, Division of Cytogenetics, The Jewish Hospital and Medical Center, 555 Prospect Place, Brooklyn, NY 11238, USA.