

Technical methods

A simple method for chromosome banding

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Various techniques have been described which produce characteristic bands along the length of chromosomes. There are basically three ways of achieving this. The first relies on the preferential staining of the chromosomes by Quinacrine derivatives (Caspersson, Zech, and Johansson, 1970), the second involves denaturation followed by renaturation of the chromosomal nucleoprotein, and the third method uses enzymatic hydrolysis.

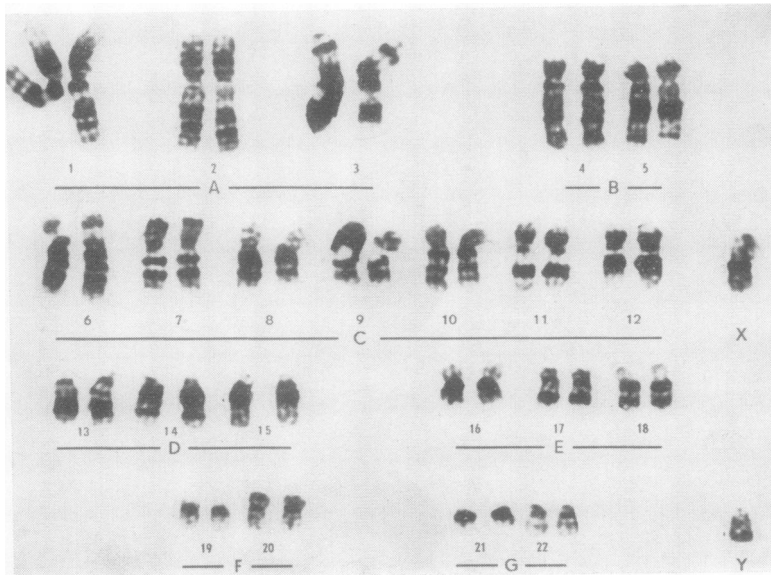
In our experience, satisfactory banding has not been obtained consistently using either a trypsin method (Seabright, 1971), the acetic-saline-Giemsa method (Sumner, Evans, and Buckland, 1971), or the Giemsa pH 9.0 method (Patil, Merrick, and Lubs, 1971). The following is basically a modification of the acetic-saline-Giemsa technique but the reagents used are easier to make, the incubation is at a lower temperature, and the total time involved is less. This method has proved to be very reliable and the results reproducible.

Received for publication 27 April 1972.

Chromosome preparations from blood lymphocyte cultures are obtained using conventional methods incorporating hypotonic treatment—four minutes in 0.075 M KCl at 37°C, using as fixative 3 parts methanol to 1 part glacial acetic acid, and spread by dropping the cell suspension onto grease-free dry slides, and air dried.

Three buffer tablets pH 7.0 100 ml size (G. T. Gurr) are dissolved in 100 ml of deionized water and the temperature raised to 37°C. The slides are incubated at 37°C in the concentrated buffer initially for 10 minutes, stained in Leishman (G. T. Gurr), diluted 1 in 4 with dilute buffer pH 7.0 for five minutes, differentiated in the same buffer, air dried, and mounted in DPX. If the bands are indistinct, the time of buffer incubation is increased up to one and a half hours. The chromosomes illustrated in Figs. 1 and 2 were incubated for 10 minutes. Slides which had been stored for some months required longer buffer-incubation times. The maximum number of bands is distinguishable on elongated chromosomes but overlapping of the chromosomes poses a major problem, eg, more bands are distinguishable in Fig. 1 than in Fig. 2 but there is overlapping of a number 3 and a number 9 chromosome.

The proportions of analysable metaphases in which the chromosomes are banded sufficiently to allow identification, varies from culture to culture,



Figs. 1 and 2 *Karyotypes of two male human metaphases from the same preparation. More bands are distinguishable in Fig. 1 than in Fig. 2 because the chromosomes are less contracted.*

Fig. 1.

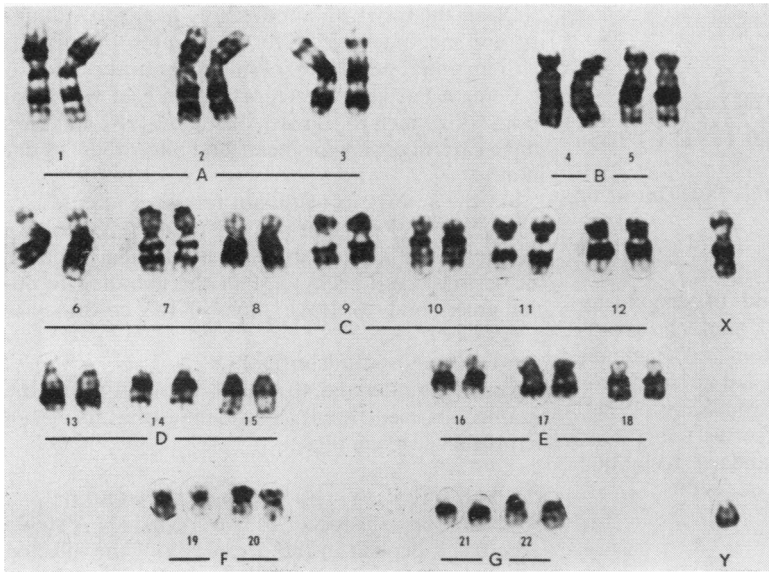


Fig. 2.

the lowest being approximately 20% and the highest 70%. Using this method, we have been able to identify a familial $t(3q+; 7q-)$ in the balanced and unbalanced forms and the extra chromosome in a $46,XY/47,XY,11\pm$ mosaic.

References

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