

CHROMOSOME DIGESTION WITH THE RESTRICTION ENZYME ALU I: A SIMPLE METHOD OF C-BANDING

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ABSTRACT

Treatment of freshly prepared human chromosomes with low concentrations (10 U/ml) of the restriction enzyme Alu I, followed by Giemsa staining, consistently produces good-quality C-banding. This CAG (C-bands by Alu I using Giemsa) banding is simple, quick and inexpensive, thus being well suited for the routine cytogenetics laboratory.

INTRODUCTION

A general feature of primate genomes is the presence of a class of highly repetitive DNA, called alpha satellite, localized in the centromeric region of the chromosomes. In 1971 Arrighi and Hsu described a cytological technique for specific staining of this chromatin in human chromosomes. The technique was based on denaturation of the chromosomes with sodium hydroxide followed by a short term incubation in saline and staining with Giemsa. The preferential staining of the centromeric chromosomal regions was thought to be due to preferential reannealing of the short and highly repetitive DNA sequences located there (Pardue and Gall, 1970), but alternative explanations have also been proposed (Comings, 1978). In 1972 Sumner refined this technique by substituting the less destructive barium hydroxyde for the sodium hydroxyde and established the basic procedure which

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became subsequently known as CBG (constitutive heterochromatin bands by barium hydroxyde using Giemsa) or more simply, C-banding.

C-banding stains the secondary constriction (h) regions of chromosomes 1, 9 and 16, the centromeres of all chromosomes and the satellites of the acrocentric chromosomes. One feature of C-bands is their considerable heteromorphism of size and position which makes them invaluable inherited markers for human genetics studies (reviewed by Verma and Dosik, 1980; Erdtmann, 1982).

In 1983 Miller *et al.* showed that a C-banding pattern could be obtained by Giemsa staining of human chromosomes digested *in situ* with some restriction enzymes of 4 or 5 base pair recognition sequences, particularly Alu I, Dde I, Hae III and Eco RII. This effect was presumably due to removal of DNA from the chromosomes by the endonucleases, which was shown to be true for Alu I (Mezzanote *et al.*, 1985).

In the present article we show that chromosomal digestion with Alu I followed by Giemsa staining can be used as a simple, quick and inexpensive method for the production of C-bands in the routine cytogenetics laboratory.

MATERIALS AND METHODS

Peripheral blood lymphocytes were cultured for 72 h in McCoy's 5A modified medium containing 10% fetal calf serum and 20 $\mu\text{g/ml}$ phytohemagglutinin. The cells were then arrested in metaphase with colcemid, swollen in KCl, fixed in methanol:acetic acid (3:1) and dropped onto ice cold glass slides to obtain the metaphase spreads. The preparations were then air dried and treated immediately with the restriction enzyme.

Alu I, the restriction enzyme from *Arthrobacter luteus*, was purchased from Sigma (St Louis, MO, USA) or Bethesda Research Laboratory (BRL - Gaithersburg, MD, USA) at a concentration of 5 U/ μl . Aliquots of 1 μl were put in 0,5 ml polypropylene tubes, covered with Parafilm and stored at -20°C . The reaction buffer was 50 mM Tris-Hcl pH 8.0 containing 10 mM MgCl_2 and 4 mM spermidine trihydrochloride. For the banding procedure, 500 μl of buffer was added to a tube containing the enzyme, so as to make a final concentration of 10 U/ml. 50 μl of the enzyme was dispensed on top of the glass slide, which was then covered with a coverslip and incubated for four hours in a humid chamber at 37°C . After this incubation period, the coverslip was carefully floated off in a dish containing saline and the chromosomes were stained with Giemsa.

RESULTS

Two representative karyotypes obtained with Alu I digestion of chromosomes are shown in Figure 1. The banding pattern was typical of C-banding, with staining

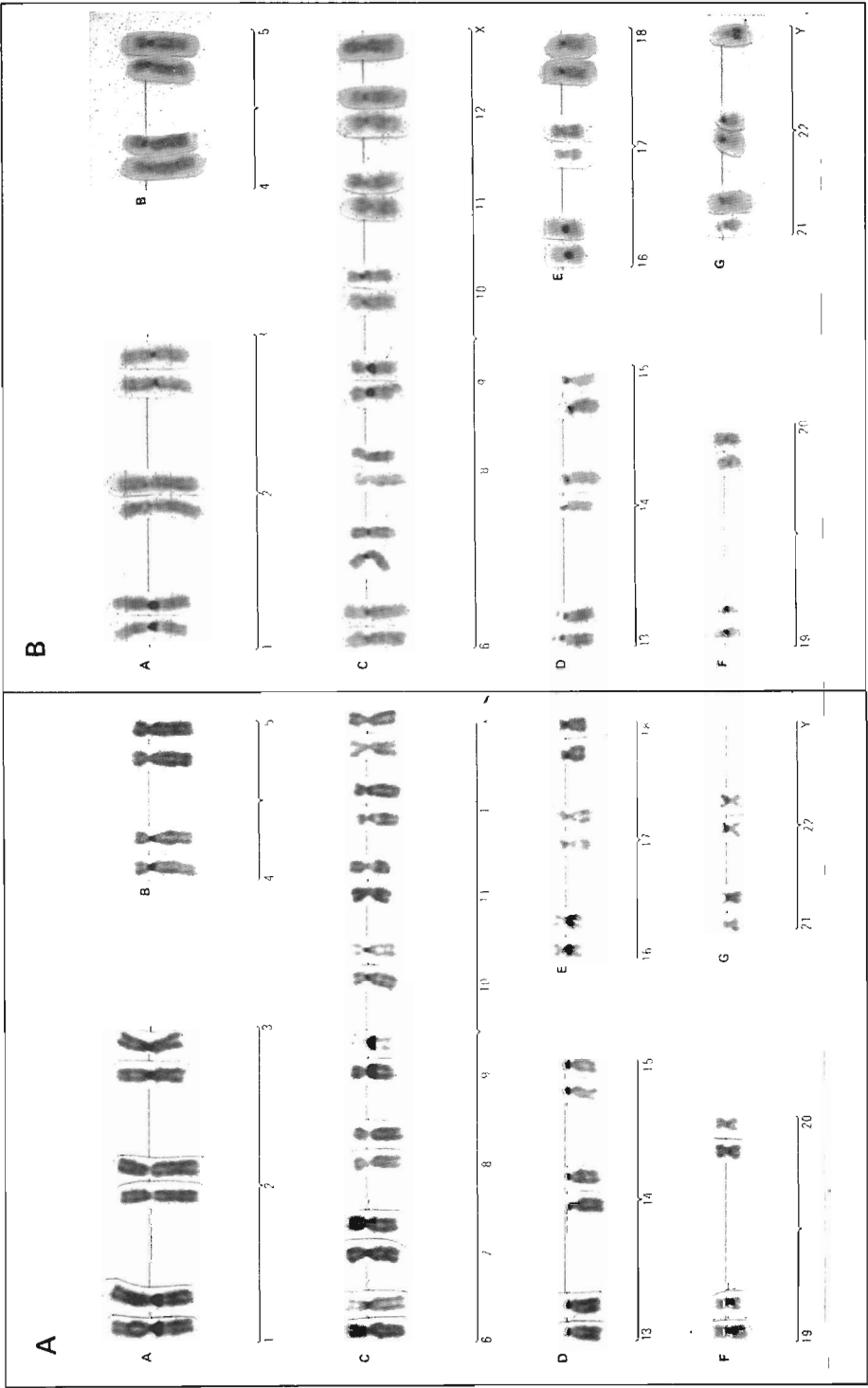


Figure 1 - Karyotypes of two normal individuals, a female (1A) and a male (1B), stained with Giemsa after chromosome digestion with Alu I.

of the secondary constriction (h) region of chromosomes 1, 9 and 16, of the centromeres of most chromosomes, of the satellites of the acrocentric chromosomes and of the long arm of chromosome Y. We have observed little or no staining of the centromeres of chromosomes 2, 8, 11 and 17, but the number of patients studied is small and there may be heteromorphism in these regions. In all preparations there is also faint residual G-banding, which is useful in the identification of individual chromosomes.

Best results are obtained when fresh slides are used, in contrast to CBG-banding in which the preparations need to be aged. However, old slides will also band well if the time of digestion is increased to overnight. We have found that the inclusion of spermidine hydrochloride in the enzyme buffer, although not essential, gives better and more reproducible results.

C-banding by Alu I reveals considerable heteromorphism, some of it not otherwise so well observed. For instance, there is variation at chromosome 4 that is not so clearly seen with the barium method (see Figure 1B). Also, heteromorphism at chromosome 19 becomes more frequent (see Figures 1A and 1B). One patient was heterozygous at chromosomes 19, 21 and 22 (Figure 1A) while the other was heterozygous at chromosomes 4, 15, 19 and 22 (Figure 1B).

DISCUSSION

The enzyme Alu I has the tetranucleotide recognition sequence AGCT. Its most common target in the human genome is within a prevalent family of short (approximately 300 base pairs) intercalated repetitive sequences which has been called the *Alu* family. There are $3-5 \times 10^5$ copies of the *Alu* sequence in the human genome, constituting 3-6% of the total (Kariya *et al.*, 1987). On the other hand, there appear to be few or no restriction sites for Alu I in the centromeric chromatin, which is mostly made up of alpha satellite sequences (Waye and Willard, 1987). Because of this, Alu I digestion removes a significant proportion of chromosomal DNA, leaving behind the centromeric blocks of heterochromatin that will be stained by Giemsa or any other DNA dye (Mezzanote *et al.*, 1985).

The C-banding pattern produced by Alu I digestion is essentially the same as that of the barium procedure described by Sumner (1972) with a few interesting differences, including a better definition of the heteromorphisms at chromosomes 4 and 19. The Alu I method is simple, quick and inexpensive. The slides are processed immediately after being prepared, without the need for "aging", and results are obtained in four hours. Good quality results are consistently obtained. The cost per slide is only US\$ 0.25, corresponding to 0.5 U of the restriction enzyme. Thus, C-banding by digestion with Alu I (CAG banding) is very well suited for routine use in clinical cytogenetic laboratories.

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RESUMO

O tratamento de preparações frescas de cromossomas humanos com baixas concentrações (10 U/ml) da enzima de restrição Alu I, seguido de coloração por Giemsa, produz reprodutivelmente bandas C de boa qualidade. Este bandamento CAG (bandas centroméricas com Alu I e Giemsa) é simples, rápido e barato, sendo assim, bem adaptado para o laboratório de rotina de citogenética.

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