Oncogene detection by enzymatic amplification on flow sorted chromosomes

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Direct spot blot hybridization on flow sorted chromosomes has allowed gene mapping and characterization of chromosomal rearrangements (1,2). This technique still requires a relatively high number of sorted chromosomes and several concentrations in order to get an unambiguous positive signal especially when probing single copy gene. Here the "Polymerase Chain Reaction" (PCR, ref. 3) was used to amplify directly on a few flow sorted chromosomes a specific sequence which can be then reliably identified.

Metaphasic chromosomes were prepared from a human lymphoblastoid cell line ICB 101 and sorted with an ATC 3000 flow cytometer (2). The sorted chromosome suspension was directly submitted to the PCR reaction without any further purification. The first exon of *c-Ki-ras* proto-oncogene, located on human chromosome 12p12.1, was amplified from either 1 µg genomic DNA (Lane 1), 8,000 (Lane 2) or 4,000 (Lane 3) chromosomes from the 9-12 peak (2), using 100 pM of each previously described primers (4) in 100 µl of 50 mM KCl, 10 mM Tris-HCl (pH 8.4), 25 mM MgCl₂, 200 µg/ml gelatin, 200 µM of each dNTP and 2 units of *Taq* polymerase (Biolabs, New England). Control (Lane 4) was performed with 5,000 chromosomes from the 1 to 8 region (2). The solution was denatured (95°C, 7 min), the primers were annealed (40°C, 1 min) and the reaction proceeded at 65°C for 3 min. In subsequent cycles, denaturation lasted 30 s. After 15 cycles, 2 other units of *Taq* polymerase were added and 15 more cycles were performed. A fifth of the reaction volume of each sample was resolved on a 4% Nusieve agarose gel (FMC, New Jersey) and stained with ethidium bromide (Fig. A). The 108 bases amplified DNA was only visible in Lane A1.

Southern transfer was performed as described (5) onto Hybond N membrane (Amersham). A probe specific for the amplified DNA (4) was ³²P 5' end labelled and hybridized onto the filter. After stringent washing, the membrane was autoradiographed for 4 h with intensification screens (Fig. B). Although the specific hybridization signal was much stronger in Lane B1, where the starting material corresponded to 600,000 *Ki-ras* gene copies, it was still perfectly noticeable in Lanes B2 and B3 where the copie numbers were 4,000 and 2,000 respectively. No amplification was detected in the control (Lane B4) under the same conditions. The 2 bands corresponding to the single and double stranded amplified DNA segments were observed when one strand was more efficiently amplified than the other (data not shown). The 50 bp band on the gel (Fig.A) corresponded to an aberrant amplified primer sequence which did not cross-hybridize with the *Ki-ras* probe used.

These results show that PCR reduces dramatically the requirement of flow sorted material for the detection of unique DNA sequence on specific chromosomes. With the use of several types of primers and an automated microwell sorting device, the combination of PCR and flow cytometry should enable the rapid and precise characterization of chromosomal rearrangements on short term blood sample culture and opens potential diagnostic applications.

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