VACUUM TRANSFER OF DNA TO FILTERS FOR DETECTING INTERINDIVIDUAL POLYMORPHISM BY SOUTHERN'S BLOTTING HYBRIDIZATION METHOD

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In research in molecular genetics experiments on hybridization of DNA molecules immobilized on filters with labeled samples of nucleic acids are nowadays widely used. The method of transfer of restricted DNA from gel to nitrocellulose filters (blotting), suggested for this purpose by Southern [5], has had a great influence on the development of structural studies of individual genes and whole genomes of different organisms.

However, in the study of the genome organization of repeating sequences of DNA in higher eukaryotes, which are characterized by a structure of high complexity and by diversity of the different classes of DNA, standard genome blotting procedures [3] by no means always give clear results. This is most often because of considerable diffusion of DNA fractions during the prolonged transfer of DNA from gel to filter.

The aim of this investigation was to make Southern's blotting procedure more efficient by shortening the period of DNA transfer and increasing the degree of resolution of the hybridization patterns on nitrocellulose filters. This, in turn, would ensure a more effective solution to the problem of detecting population polymorphism in the structural organization of repeating sequences of DNA in higher eukaryotes, including man.

EXPERIMENTAL METHOD

Fragments of restricted DNA were separated by gel electrophoresis in 1-2% agarose gel and the gel was then treated in accordance with the standard formula: with 0.5 N NaOH and 1.5 M NaCl for 30-45 min, then with 1 M Tris-HCl (pH 7.5) and 1.5 M NaCl for 45-60 min with gentle mixing of the solution. The gel treated in this way was placed on a vacuum blotting apparatus, directly on a nitrocellulose filter (Fig. 1). The filter must be very slightly larger than the gel. Both the filter itself and the Whatman 3MM filter lining it were soaked beforehand in 15 × SSC (2.25 M NaCl and 225 mM CH_3COONa). When the gel had been placed on the filter (making sure that no air bubbles had appeared between them) it was covered with a sheet of Whatman 3MM paper soaked in 15 × SSC (equal in size to the disk of gel), and above it was placed a sheet of finely porous Porolon sponge 3-4 cm thick, completely soaked with 15 × SSC. At the end of these procedures the solution began to flow from the sponge through the gel and filter under a weak vacuum (not more than 0.1 atm). After 1.5-2 h transfer of DNA from gel to nitrocellulose filter was complete or nearly complete, with minimal diffusion of material of individual bands on the filter.

EXPERIMENTAL RESULTS

The results of experiments to study hybridization of human DNA, completely restricted by endonuclease Pst 1 and transferred to a Millipore HAWP 00010 nitrocellulose filter, from 1% agarose gel (LKB 2206-103 agarose) in accordance with the method described above, are given below. The radioactive probe was DNA of recombinant plasmids (pKN 001) containing Pst 1 fragment of the human genome, belonging to the class of primate alphoid DNA repeating sequences [2, 4]. It will be clear from Fig. 2a that many (more than 10) minor hybridization bands can be resolved in the region of high-molecular-weight fragments (more than 3000 nucleotide pairs in length), which usually are not revealed during genome blotting hybrid-

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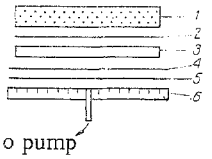

Fig. 1. Diagram of apparatus for vacuum transfer of DNA. 1) Finely porous Porolon, 2, 5) Whatman 3MM, 3) gel, 4) nitrocellulose filter, 6) carrying disk (a network of intersecting channels for collecting the solution is provided on the top side of disk facing the filter).

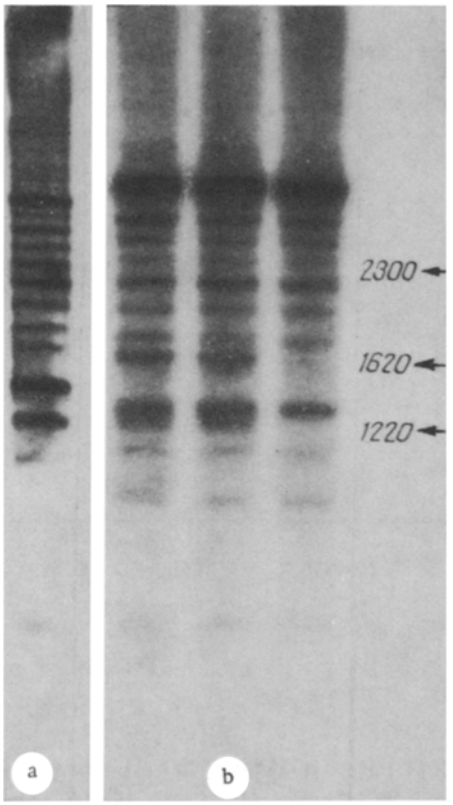

Fig. 2. Hybridization of [^{32}P]-DNA of plasmid pHS53 (a: DNA fragment of juxtacentromeric region of human 11th chromosome, cloned in plasmid pKN 001) and hybridization of [^{32}P]-DNA of recombinant plasmid pHS05 with DNA from three different individuals (b). Arrows — Pst 1 restriction fragments, exhibiting individual polymorphism of hybridization with the given fragment. Electrophoresis of human DNA, completely split by Pst 1 (12 μg per lane) in 1% agarose for 18 h with voltage 50 V (1.5 V/cm). DNA transferred from agarose to HAWP 00010 nitrocellulose filter (Millipore) under negative pressure of 0.1 atm for 90 min. Hybridization with labeled DNA ($5 \cdot 10^8$ cpm/μg) carried out for 12 h in 6 × SSC with 0.1% sodium dodecylsulfate at 68°C.

ization of DNA of higher eukaryotes in the standard version of Southern's method. The discovery of such bands makes it possible to reveal interindividual differences at the molecular level, as regards both the degree of copying and the structural organization of DNA repeating sequences in the genome.

An example of differences discovered between individual DNAs (from the placentas of three different individuals) as regards sequences complementary to the cloned fragment of the human genome (pH 0.5), belonging to the group of special chromosomal markers, is given

in Fig. 2b. The creation of a collection of such markers is nowadays regarded as an important task for research in human genetic mapping [1]. The modification of Southern's method given in this paper, in the writers' view, will facilitate the development of this research.

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