ISOLATION OF DNA FROM EPITHELIAL CELLS OF THE INNER SURFACE OF THE CHEEK

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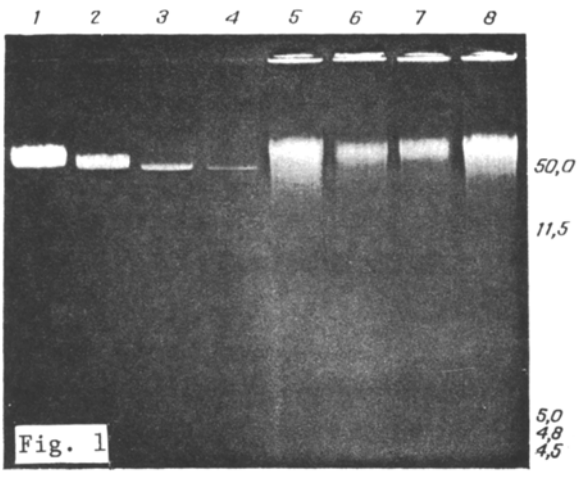
Mapping of the human genome by means of molecular genetic markers is based, as we know, on analysis of representative families or of population samples of individual DNA preparations.

It has hitherto been considered that the most readily accessible and convenient material for isolation of individual human genome DNA is peripheral blood lymphocytes. Yet the tak-



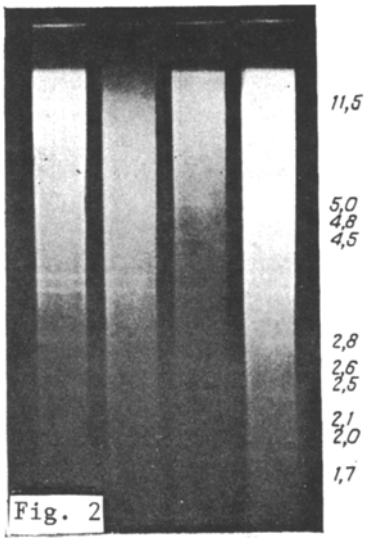


Fig. 1. Electrophoresis of samples of DNA of phage λ and genomic DNA from scrapings of the buccal epithelium. Samples of DNA of phage λ in double serial dilutions applied to lanes 1-4: 2, 1, 0.5, and 0.25 μg respectively. Samples of genomic DNA of epithelial cells applied to lanes 5-8: half the total quantity of DNA isolated from independent scrapings of epithelium was applied to each lane. Electrophoresis conducted in 0.4% agarose gel at 1 V/cm in Tris-borate-EDTA buffer for 16 h. Markers correspond to mobility of native DNA of phage λ and its restriction PstI-fragments. Here and in Fig. 2, numbers indicate kilobase-pairs.

Fig. 2. Electrophoresis of samples of restriction DNA from scrapings of buccal epithelial cells. Half of the DNA isolated from one scraping, hydrolyzed by restriction endonuclease EcoRI was applied to each lane. Restriction carried out in high-salt buffer with 0.02% Triton X-100 and 4 mM spermidine in the presence of 10 units of EcoRI for 2 h at 37°C. Electrophoresis conducted in 1% agarose gel at 1 V/cm in Tris-borate-EDTA buffer for 1 h. Markers correspond to mobility of restriction PstI-fragments of DNA of phage λ.

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ing of blood for isolation of lymphocytes is an invasive procedure. It is this state of affairs which frequently limits the sample of donors necessary for analysis, especially in cases when the intention is to study DNA from young children or from aged individuals.

As a result of improvements in methods of nucleic acid hybridization, it has now become possible to reduce to a minimum the quantity of material required to analyze restriction polymorphism of DNA. For instance, some investigators [3], in order to obtain a "fingerprint" of DNA, are content with only two or three drops of blood which can be taken from the finger, or even with a few hair bulbs. However, material obtained in this way as a rule is sufficient for only one experiment.

In this paper we suggest another, readily accessible source of individual human genomic DNA – namely the epithelium of the inner surface of the human cheek, which is traditional cytogenetic material for the detection of Barr's bodies in cell nuclei.

The epithelium can be collected by scraping with any suitable instrument: a blunt scalpel, a small spatula, and so on. However, it is better to use a special instrument for this purpose, which has a round hollow at its end. By moving such a "spoon" over the inside of the cheek with gentle pressure, it is possible to obtain from 20 to 100 mg or more of fresh biomass of epithelial cells.

DNA can be isolated from cells or cell nuclei thus collected by any known method [1-8], but the most effective procedure is that described by Gill and co-workers [3]. The biomass is transferred to a centrifuge tube with a capacity of 1.5 ml together with 250 µl of 0.01 M Tris-HCl, 0.01 M EDTA, 0.1 M NaCl (pH 8.0), 2% sodium dodecylsulfate, 50 µg/ml of proteinase K, and 0.04 M dithiothreitol (this solution can be made up beforehand and kept in aliquots at −20°C), gently mixed, and incubated at 37°C for 2-3 h. The DNA is then purified by extraction with phenol (once) and chloroform (once), and precipitated with 2-2.5 volumes of 96° ethanol. It is recommended that the residue be washed with an equal volume of 96° ethanol, dried, and dissolved in the necessary buffer, such as 10 mM Tris-HCl and 1 mM EDTA (pH 8.0). Phenol-chloroform extraction and dissolving of the DNA after precipitation with ethanol should be carried out without any vigorous shaking of the liquid.

The quality and quantity of DNA thus isolated are demonstrated by electrophoresis (Fig. 1).

The isolated DNA can be used directly for analysis by the blot-hybridization method, for in this case there are no difficulties with hydrolysis by restriction endonucleases (Fig. 2). The material obtained is quite sufficient for several experiments.

The accessibility of the epithelial cells, the exceptional simplicity and the absolute painlessness of the method of obtaining the material make DNA analysis possible in virtually all informative families and large population samples without any obvious limitations.

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