Purification of specific restriction fragments of human deoxyribonucleic acid by using liquid countercurrent chromatography

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Recent developments in cloning fragments of the eukaryotic genome larger than complimentary DNA copies of mRNA have led to interest in fractionation methods for restriction-enzyme digests of genomic DNA. Electrophoretic methods using agarose gels (Tonegawa et al., 1977) and column chromatography using RPC-5 (Hardies & Wells, 1976) are well known. The procedure described here exploits the differences in partition coefficients between different DNA restriction fragments dissolved in the immiscible phases formed by aqueous solutions of the polymers dextran and poly(ethylene glycol) (Albertson, 1971; Ohlsson et al., 1977). It has only been feasible to use this method since the development of the continuous-flow toroidal-coil centrifuge (Sutherland & Ito, 1978). This consists of a coil of plastic tube around the periphery of a flat centrifuge rotor. The apparatus is designed to allow fluid into and out of the coil while it is being rotated. During rotation the two immiscible phases separate out. The dextran-rich solution forms the lower phase, which is held stationary in the outermost part of the coil. The upper poly(ethylene glycol)-rich solution forms the mobile phase and is pumped through the stationary phase along with the sample.

Poly(ethylene glycol) 6000 (BDH) and dextran T-500 (Pharmacia) were used. The concentration of the stock solutions of the polymers was determined by freeze-drying known volumes. These stocks could then be diluted accurately to give a phase system of exactly 5% (w/v) poly(ethylene glycol) and 6% (w/v) dextran. The distribution of endonuclease-EcoRI-cleaved human DNA in this phase system, as measured by $A_{260}$, was assessed against variation in the pH of the solution buffered by 0.01 M-sodium phosphate at 4°C. For the fractionation procedure the pH of the system was chosen as that giving an equal partition of DNA between the upper and lower phases, i.e. log $K_D = 0$. This point was 7.4. For fractionation, 90 ml of this system was made up and the phases separated. Endonuclease-EcoRI-cleaved human DNA (5 mg) was dissolved in 0.5 ml of the poly(ethylene glycol)-rich phase. The stationary coil was filled with the lower, dextran-rich, phase. After starting the centrifuge and running at 1000 rev./min (200 g), 2 ml of mobile phase was pumped into the coil, followed by the DNA solution. The remainder of the upper phase was then used to elute the sample at 10 ml/h. Fractions of 1 ml were collected. The $A_{260}$ of fractions was determined and 1 µg of DNA from each fraction was precipitated with ethanol. After washing with 70% (v/v) ethanol the material was redisolved in 10 µl of electrophoresis buffer and fractionated on a 0.8% (w/v) agarose gel. After denaturation the DNA was transferred from the gel to a nitrocellulose filter by using the Southern (1975) technique. A recombinant plasmid, X11101, radiolabelled with $^{32}$P and containing sequences specific for 18 and 28S ribosomal DNA (Wellauer et al., 1976; Arnheim & Southern, 1977) was used in a hybridization assay to detect fractionation (Jeffreys & Flavell, 1977). Those phases showing an enrichment of ribosomal DNA fragments were identified by radioautography of the hybridized filters.

DNA was present in 37 out of 46 fractions collected. Fractionation of restriction fragments was not obvious from observation of the DNA made visible under U.V. light after staining with ethidium bromide. However, the hybridization assay revealed that ribosomal-specific DNA was concentrated in 9 out of the 37 fractions containing DNA. These fractions represented 29.7% of the total DNA used or a purification of 3.4-fold.

This model system has demonstrated that counter-current chromatography in the toroidal-coil centrifuge using aqueous polymer solutions can be used to purify restriction fragments of human DNA. Fractionation is not obviously based on the size of DNA fragments and so the procedure may be useful in purifying single-copy gene sequences in conjunction with a size-fractionation technique.

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Physical mapping of part of the tumorigenic deoxyribonucleic acid (T-DNA) region of the tumour-inducing (Ti) plasmid from Agrobacterium tumefaciens strain C58

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The transfer and integration of Ti-plasmid sequences into plant genomes is a complex process. To study the influence of the DNA sequence of both the Ti plasmid and the plant genome on the extent and site of insertion, the right-hand end of the T-DNA of plasmid pTiC58 (a Ti plasmid carrying nopaline anabolic and catabolic functions) cloned as a 9.55 x 106-dalton endonuclease-

EcoRI-produced fragment in vector plasmid pMB9 (recombinant plasmid pS595) has been extensively mapped by using restriction endonucleases, and subfractions have been cloned in plasmid pBR322 (Fig. 1).

A comparison by hybridization using the Southern (1975) technique with nick-translated (Maniatis et al., 1975) plasmid pTiB6S3 as a probe against nitrocellulose-bound multiple restriction-endonuclease digests of fragment pS595 show that the common region (Hepburn & Hindley, 1979) extends into the endonuclease-HindIII-produced fragment, which contains the end of the T-DNA (Schell et al., 1979). This confirms the evi

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