Polyacrylamide Gel Electrophoresis

Applications for the Use of Minigel-Twin

SSCP analysis of the lipoprotein lipase gene (Single Strand Conformation Polymorphism)

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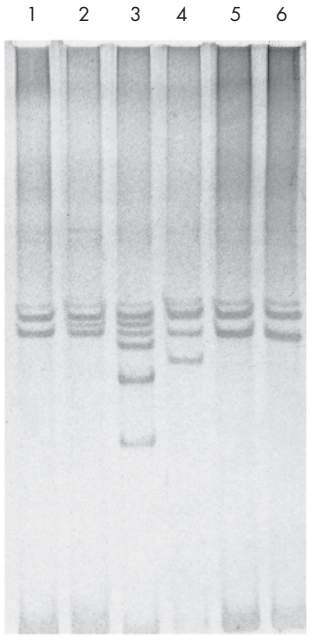
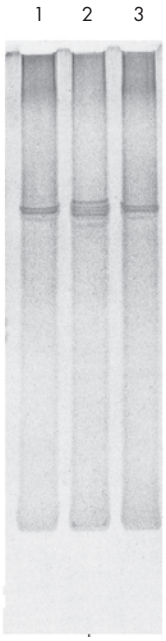
Lipoprotein lipase (LpL) plays a key role in the metabolism of triglyceride rich lipoproteins. Patients who are homozygous or compound heterozygotes for mutations in the LpL gene present with Type I Hyperlipoproteinemia (HPL) which is characterized by fasting chyclomicronemia with very high levels of triglycerides, pancreatitis and eruptive cutaneous xanthomatosis. Type I HLP is a rare desease with a frequency of 1 in a million, however heterozygotes occur with a frequency of up to 1 in 500. Heterozygosity for mutations in the LpL gene has been implicated in familial combined hyperlipidemia, Type III HLP and with elevated serum triglycerides. These conditions are associated with an increased risk of arthereosclerosis. Heterozygotes are also at increased risk of hypertriglyceridemia in the presence of environmental stress such as pregnancy, obesity and diabetes. The experiment shown here describe the use of SSCP electrophoresis to analyse the LpL gene of patients with the elevated serum triglycerides. 42 of a total of 88 patients (48 %) had mutations, and 27 patients (31 %) had mutations with amino acid changes.

SSCP analysis is performed non-radioactively. The Biometra Minigel-Twin has the advantage of high capacity, 40 samples can be processed simultaneously, with little sample needed and separation being very rapid. Silver staining also needs only one hour so that with a Minigel-Twin 80 samples can be screened in one day. Exon 1-9 and the exon-intron bonds were amplified by PCR. For the SSCP analysis the PCR products were diluted 1 in 20 in denaturing SSCP analysis buffer (1 x TBE, 3.5 M urea), incubated at 96 °C for 5 min. and cooled immediately on ice. Products were loaded onto 10 % polyacrylamide gels in 1 x TBE containing 10 % glycerol. Gels were poured the Minigel-Twin with 0.6 mm spacers. Electrophoresis was at 10 mA for 3 hours at room temperature. DNA was visualized by staining with silver.



Exon 3
Lanes 1, 2 and 6: controls;
Lane 3: Ser-88-mutation;
Lane 4: Del-560->564-mutation;
Lane 5: Val-69-Leu-mutation.



Exon 6
Lanes 1 and 3: controls;
Lane 2: Ser-266-Pro-mutation.

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Purification of DNA oligonucleotides with the Minigel-Twin electrophoresis instrument

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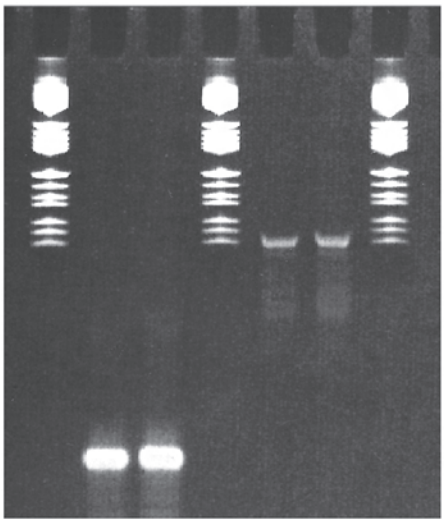
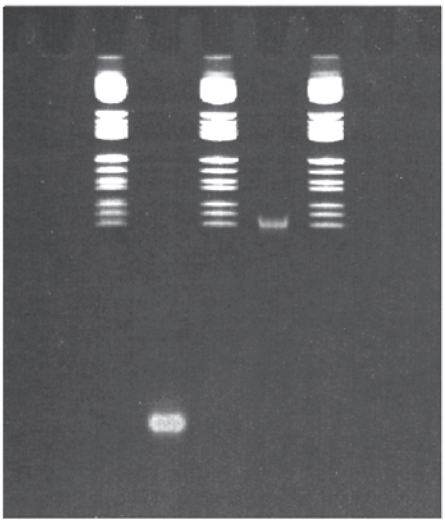
Crude oligonucleotides produced with automatic DNA synthesizers also contain fragments which are smaller than the full length products, down to decamer size or even smaller (left fig.). In order to separate these undesired products the raw products were separated using Biometra's Minigel-Twin electrophoresis instrument with a 12 % denaturing (8 M urea) polyacrylamide gel and with TBE as a running buffer for 2 h at 100 V (const.). After separation the full length products were visualized by UV shadowing using a TLC plate illuminated with short-wave UV light (254 nm).

The full length products were cut out and eluted from the gel strip using an elution buffer (0.1 % SDS, 0.5 M ammonium acetate, 10 mM magnesium acetate). The right figure shows the purified oligonucleotides after further electrophoretic separation.



Separation of crude DNA synthesis products. Lanes 1, 4, and 7: molecular weight markers; Lanes 2 and 3: 20mer DNA oligonucleotide (2 µg/lane); Lanes 5 and 6: 60mer DNA oligonucleotide (2 µg/lane).



Separation of DNA synthesis products purified by PAGE. Lanes 1, 3, and 5: molecular weight markers; Lane 2: 20mer DNA oligonucleotide (1 µg/lane); Lane 4: 60mer DNA oligonucleotide (1 µg/lane).