High-resolution liquid chromatography of DNA fragments on non-porous poly(styrene-divinylbenzene) particles

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ABSTRACT

DNA restriction fragments and PCR products were separated by means of ion-pair reversed-phase high-performance liquid chromatography on alkylated non-porous poly(styrene-divinylbenzene) particles with a mean diameter of 2.1 μm . Optimum resolution was obtained by using an acetonitrile gradient in 100 mM of triethylammonium acetate and a column temperature of 50 °C. This allowed the separation of DNA fragments differing in chain length by 1 – 5% up to a size of 500 base pairs. PCR products could be analyzed directly in less than two minutes with a concentration sensitivity of at least 300 ng/ml. Compared with anion-exchange chromatography or gel electrophoresis no desalting of the purified DNA molecules is required because the volatile buffer system can be readily evaporated. Subsequently, the method was used for the semiquantitative evaluation of the expression of multidrug resistance genes in mononuclear white blood cells.

INTRODUCTION

Restriction enzyme mapping and polymerase chain reaction have gained tremendous popularity in molecular biology and clinical diagnostics. The standard method for the separation of DNA fragments is electrophoresis in polyacrylamide and agarose gels (1). Despite impressive advances, however, modern electrophoresis is still, for the most part, a collection of manually intensive methodologies that cannot be run unattended and that are prone to irreproducibility and poor quantitative accuracy.

The recent introduction of fused silica capillaries as the migration channel in electrophoresis has been a big step forward in putting electrophoretic separations on the same instrumental footing as high-performance liquid chromatography (2, 3). With regard to the separation of double-stranded DNA fragments, three different approaches have been reported. The first one is based on the use of low- or zero-crosslinked polyacrylamide-filled capillaries, which allowed the realization of up to five million theoretical plates per meter and, hence, the resolution of DNA

fragments that differed by less than 10 base pairs, if their overall length was not larger than approximately 500 base pairs (4, 5). However, gel-filled capillaries are difficult to prepare and, due to the destruction of the gel matrix at high current densities, resolving power cannot be maintained for long periods of time. Moreover, it is not possible to deliver the same amount of each DNA species, because samples can only be injected electrokinetically. Therefore, several investigators have explored the feasibility of separating DNA fragments in free solution using micellar buffer systems containing either cetyltrimethylammonium bromide (6) or sodium dodecyl sulfate (7). However, this approach does not yield the same degree of resolution as obtained by means of gel-filled capillaries. Finally, the combined use of hydroxyethylcellulose and ethidium bromide was found to offer both the same resolution as seen with gel-filled capillaries as well as the advantage of hydrostatic injection (8).

However, all capillary electrophoretic approaches to the analysis of DNA restriction fragments and, especially, polymerase chain reaction (PCR) products require their desalting because otherwise the salts will cause considerable band broadening due to the conductivity difference between the sample zone and the surrounding buffer. In this respect, liquid chromatographic techniques are far less affected. The method most commonly used for the separation of DNA molecules is anion-exchange chromatography. The earliest attempts were carried out on columns filled with polychlorotrifluoroethylene powder coated with a trialkylmethylammonium chloride (9–11). However, insufficient coating of the matrix with trialkylmethylammonium chloride resulted in considerable loss of DNA. The subsequently introduced porous anion-exchange supports Mono Q and Mono P allowed the separation of DNA restriction fragments within a wide range of size, but resolution of fragments larger than 200 base pairs generally declined rapidly and separations of DNA molecules up to a length of 500 base pairs took almost four hours (12). A considerable reduction in time of analysis to less than 15 minutes as well as a significant enhancement in resolution was achieved by the introduction of non-porous particles to which diethylaminoethyl groups had been bonded chemically (13, 14). However, retention behaviour was

not found to be a direct function of chain length and, secondly, the isolated DNA fragments were eluted in a salt gradient which had to be removed before the DNA molecules could be employed in subsequent experiments such as DNA sequencing.

Ion-pair reversed-phase high-performance liquid chromatography (IP-RP-HPLC) is a well-established technique for separating mono- and oligonucleotides (15–18). The principal feasibility of separating DNA restriction fragments by means of IP-RP-HPLC on uncoated polychlorotrifluoroethylene powder has been demonstrated as early as 1979 (19), but attempts to transfer the results to commercial packing materials were not successful till 1986, when Eriksson et al. (20) published the separation of DNA molecules on a PepRPC (C_2/C_{18}) column, the packing of which is based on porous silica. However, complete separation of restriction fragments up to a size of 600 base pairs took more than 3 hours, which makes the method impractical for both analytical as well as preparative purposes. Nevertheless, both studies confirmed that separation of restriction fragments in IP-RP-HPLC depends mainly on the chain length.

Based on recent advances in the synthesis of non-porous styrene-divinylbenzene copolymers and their application to the rapid analysis of proteins (21) and in combination with an ion-pairing reagent to the separation of oligonucleotides (17, 18), we now explore the applicability of non-porous alkylated poly(styrene-divinylbenzene) particles (PS-DVB) to the separation of DNA restriction fragments and PCR products.

MATERIALS AND METHODS

Chemicals

Styrene, divinylbenzene (DVB), polyvinylalcohol (PVA), and 1-chlorooctadecane were purchased from Riedel-de Haen (Seelze, FRG). HPLC gradient-grade acetonitrile and methanol as well as tetrahydrofuran and aluminium chloride were obtained from Merck (Darmstadt, FRG). Buffers were prepared using a stock solution of 2 M HPLC-grade triethylammoniumacetate (TEAA, Applied Biosystems, San Jose, CA, USA) and high-purity water (Epure, Barnstead Co., Newton, MA, USA). Acrylamide, Tris, boric acid, and EDTA were purchased from Bio-Rad Laboratories (Richmond, CA, USA).

DNA size standards

Size standards of double-stranded DNA were purchased from Boehringer Mannheim (pBR322 DNA-*Hae*III digest, Mannheim, FRG) and USB (Φ X-174 DNA-*Hinc*II digest, United States Biochemical, Cleveland, OH, USA).

Isolation of blood cells

Peripheral venous blood from controls and patients suffering from B-CLL was collected in 10-ml tubes containing heparin as anticoagulant. Mononuclear cells were isolated by density gradient centrifugation (Lymphoprep, Nycomed AS, Oslo, Norway). After removing these cells from the plasma-Ficoll interface, they were washed three times with MOPS (DMEM buffered with 25 mM 3-N-morpholinopropanesulfonic acid).

RNA isolation and reverse transcription

Following two washes with ice-cold phosphate buffered saline, total RNA was isolated from mononuclear cells by the guanidine thiocyanate-acid phenol extraction method (22). Subsequently, first-strand synthesis of cDNA was accomplished in a 0.6 mL Eppendorf tube by adding 500 ng of RNA to a total volume of

50 μ L of 50 mM Tris·HCl, pH 8.3, 50 mM KCl, 8 mM $MgCl_2$, 5 μ g BSA (Pharmacia, Uppsala, Sweden), 1 mM of each dNTP, 250 pmol random hexanucleotide primers, 20 mM β -mercaptoethanol, 1.25 U rRNasin® ribonuclease inhibitor (Cat. No. N2511, Promega, Madison, WI, USA), and 120 U M-MLV reverse transcriptase (M5301, Promega). The reaction was carried out at 37 °C for 1 hour and then stopped by boiling the sample for 2 min and cooling it on ice afterward.

Polymerase chain reaction

PCR was performed in a 0.6 mL Eppendorf tube by adding 2 μ L of cDNA or 20 ng of total RNA to a total volume of 50 μ L of 10 mM Tris·HCl, pH 9.0, 50 mM KCl, 1.5 mM $MgCl_2$, 0.2 mM of each dNTP, 0.1 mg/ml gelatin, 0.1 % Triton X-100, 0.5 μ M of each primer, and 3 U TaqDNA Polymerase (Cat. No. M1861, Promega). The sequences of the primers used for the amplification of β_2 -microglobulin (5'-ACCCCACTG-AAAAAGATGA and 5'-ATCTTCAAACCTCCATGATG) as well as the multidrug resistance (MDR) gene 1 (5'-CCCATCATTGCAATAGCAGG and 5'-GTTCAAACCT-CTGCTCCTGA) were exactly as described by Noonan et al. (23). Together with the primers used for the amplification of MDR-3, which were MDR-3/2061, 5'-TGTCAGAAGAGCC-TTGATGTGG, and MDR-3/2193, 5'-TGGCAATGGCACATA-CTGTTCC, respectively, they were synthesized on an automated DNA Synthesizer (Model 381 A, Applied Biosystems, San Jose, CA, USA) using phosphoramidite chemistry and purified by means of oligonucleotide purification cartridges (Cat. No. 400771, Applied Biosystems). Thirty cycles of amplification were carried out in a thermocycler (MJ Research Inc., MA, USA) with a 94°C denaturation step for 35 s, a 57°C annealing step for 30 s, and a 73°C extension step for 60 s. Beginning at cycle 16 each following DNA synthesis step was elongated for 5 s. Finally, samples were cooled to room temperature.

Purification of PCR products

In order to obtain a standard for the construction of a calibration curve several PCR samples containing a 404-bp product were pooled and purified by means of size-exclusion chromatography, for which prepacked Bio-Spin 30 (Bio-Rad Laboratories, Hercules, CA, USA) columns were used. The columns were washed three times with TE buffer, before 60 μ L of PCR sample were applied to the top of the gel bed. The column was centrifuged in a swinging bucket at 1100×g for 4 min. This procedure was repeated two times with 80 μ L of TE buffer. The eluted DNA was then precipitated with 0.1 volume of 3 M sodium acetate, pH 5.0, and 1 volume of isopropanol, stored at –20 °C overnight, and centrifuged at 15000×g for 30 min. The supernatant was discarded and the pellet was rinsed with 75 % ethanol, dried under vacuum and usually resuspended in 22 μ L of TE buffer prior to analysis both by means of spectrophotometry (Lambda 2 UV/VIS Spectrophotometer, Perkin Elmer, Überlingen, FRG), in order to determine the content of purified PCR product, as well as by high-performance liquid chromatography.

High-performance liquid chromatography

The HPLC system consisted of a high-precision pump (Model 480 GT, GynkoteK, Germering, FRG), a degasser (Liliput, GynkoteK), a column oven (Model STH 585, GynkoteK), a variable wavelength UV-monitor (Model UVD-160, GynkoteK), a biocompatible sample injection valve (Model 9125, Rheodyne Inc., Cotati, CA, USA) with a 10- μ L sample loop, and a PC-based data system (GynkoSoft, Version 3.12, GynkoteK).

PS-DVB beads were prepared by a two-step microsuspension method (17, 24). Subsequently, part of the material was alkylated under Friedel–Crafts conditions (Austrian patent filed, No. 2285/92). Depending on the dimensions of the stainless steel column used for packing, either 0.6 g (30×4.6 mm I.D.) or 1.1 g (50×4.6 mm I.D.) of the modified beads were suspended in tetrahydrofurane. Then the slurry was sonicated and packed into the column at a pressure of 70 MPa with the use of an air-driven pump (Model Maximator MSF 111, Ammann Technik, Klliken, CH) and methanol as the driving solvent. Thereafter, methanol was replaced with water at the same inlet pressure.

Gradient profiles are given on each chromatogram. The aqueous buffer was 0.1 M TEAA, pH 7.0, throughout the study. In order to keep the concentration of TEAA constant and not to be affected by volume contraction during mixing of organic solvents with water, the mobile phase was prepared as follows: for a 25% solution of acetonitrile in 0.1 M TEAA, 50 ml of the 2 M TEAA stock solution were added to 250 ml of acetonitrile in a 1000 ml volumetric flask and the final volume was made up to 1000 ml by the addition of water.

Polyacrylamide gel electrophoresis

Ten μL of each PCR sample or chromatographically isolated DNA restriction fragment were separated in 10% polyacrylamide gels in TBE buffer (90 mM Tris-borate, 1 mM EDTA, pH 8.3). Separations were carried out in a small vertical Mini-Protein II electrophoresis cell (Bio-Rad Laboratories) at a constant voltage of 80 V for 90 min. Subsequently, the gels were stained in a solution of 0.5 $\mu\text{g}/\text{mL}$ ethidium bromide in TBE buffer for 25 min and photographed.

RESULTS AND DISCUSSION

Characterization of the stationary phase

Because of their uniform size ($2.1 \pm 0.12 \mu\text{m}$, $N=257$), no sieving of the unmodified non-porous PS-DVB particles was required following their synthesis. Both the narrow size distribution as well as the non-porosity of the particles allow a minimization of the length of diffusion paths. This yields both an optimum of resolution as well as a significant reduction in time of analysis compared to a porous stationary phase (20). Another major advantage of the non-porous PS-DVB adsorbent is that the totally organic polymer is operable over a wide pH-range, typically 1–13, without any damage occurring to the packing. This results in high column life-time and allows the easy regeneration of a deteriorated column with aqueous sodium hydroxide or organic solvents. As seen in Figure 1a, dsDNA restriction fragments larger than approximately 100 base pairs in length could not be resolved sufficiently on unmodified PS-DVB particles. As in the case of oligonucleotides (18), an improvement in resolving power was obtained upon incorporation of polyvinyl alcohol during polymerisation (Fig. 1b). An even higher degree of resolution, which even allowed the efficient separation of dsDNA fragments larger than 200 base pairs in size, was achieved by alkylating the PS-DVB particles with octadecyl groups (Fig. 1c). This is a consequence of the effective shielding of the aromatic rings of the PS-DVB matrix which otherwise unwantedly interact with the nucleobases that represent the hydrophobic moieties of the amphiphilic polynucleotides.

Optimization of the chromatographic conditions

Figure 2 shows, that the resolution of DNA restriction fragments increases with increasing concentration of TEAA in the mobile

Figure 1. Comparison of uncoated (a) and chemically modified (b,c) PS-DVB particles in the analysis of DNA restriction fragments. Columns: (a) PS-DVB, (b) PS-DVB-PVA and (c) PS-DVB- C_{18} , $2.1 \mu\text{m}$, $30 \times 4.6 \text{ mm}$ I.D.; mobile phase: 0.1 M TEAA, pH 7.0; linear gradient from 7.5–13.75% acetonitrile in 4 min, followed by 13.75–16.25% acetonitrile in 6 min; flow-rate: 1 ml/min; temperature: 50°C ; detection: UV, 254 nm; sample: pBR322 DNA-*Hae*III digest, 0.5 μg .

Figure 2. Effect of the concentration of TEAA in the eluant on the chromatographic separation of DNA restriction fragments. Column: PS-DVB- C_{18} , $2.1 \mu\text{m}$, $30 \times 4.6 \text{ mm}$ I.D.; mobile phase: (a) 0.025 M, (b) 0.05 M, (c) 0.075 M, (d) 0.1 M, and (e) 0.125 M TEAA, respectively, pH 7.0; linear gradient from 7.5–13.75% acetonitrile in 4 min, followed by 13.75–16.25% acetonitrile in 6 min; flow-rate: 1 ml/min; temperature: 50°C ; detection: UV, 254 nm; sample: pBR322 DNA-*Hae*III digest, 0.5 μg .

phase. This is especially true for fragments larger than 300 base pairs in length. In this connexion, it is important to understand the principal mechanisms involved in IP-RP-HPLC (15). Firstly, the retention sequence is controlled by the charge of the polynucleotides, thus determining the number of ion pairs formed. This enables IP-RP-HPLC to separate polydeoxynucleotides reliably according to their chain length, largely irrespective of their base composition (19, 25). Secondly, the degree of retention is directly proportional to the chain length of the *n*-alkyl group of the ion pairing reagent. However, triethylammonium acetate

Figure 3. Percentage of acetonitrile needed for elution of restriction fragments from pBR322 DNA cleaved with *HaeIII*, depending on the TEAA concentration. Chromatographic conditions as in Fig. 2.

Figure 4. Effect of column temperature on the chromatographic separation of DNA restriction fragments. Column: PS-DVB- C_{18} , 2.1 μ m, 30 $\times$ 4.6 mm I.D.; mobile phase: 0.1 M TEAA, pH 7.0; linear gradient from 7.5–13.75% acetonitrile in 4 min, followed by 13.75–16.25% acetonitrile in 6 min; flow-rate: 1 ml/min; temperature: 20–70 $^{\circ}$ C; detection: UV, 254 nm; sample: pBR322 DNA-*HaeIII* digest, 0.5 μ g.

was not only chosen because it allows the faster elution of polynucleotides compared to the more commonly used tetrabutylammonium salts but also because it can be removed

Figure 5. Effect of eluant flow-rate on the resolution of selected pairs of DNA restriction fragments from the pBR322 DNA-*HaeIII* digest. Column: PS-DVB- C_{18} , 2.1 μ m, 30 $\times$ 4.6 mm I.D.; mobile phase: 0.1 M TEAA, pH 7.0; linear gradient from 7.5–13.75% acetonitrile in 4 min, followed by 13.75–18.75% acetonitrile in 12 min; flow-rate: 0.2–1 ml/min; temperature: 50 $^{\circ}$ C; detection: UV, 254 nm; sample: Φ X-174 DNA-*HincII* digest, 0.65 μ g.

Figure 6. High-resolution liquid chromatographic separation of 31 DNA restriction fragments. Column: PS-DVB- C_{18} , 2.1 μ m, 50 $\times$ 4.6 mm I.D.; mobile phase: 0.1 M TEAA, pH 7.0; linear gradient from 8.75–11.25% acetonitrile in 2 min, 11.25–14.5% acetonitrile in 10 min, 14.5–15.25% acetonitrile in 4 min, and 15.25–16.25% acetonitrile in 4 min; flow-rate: 1 ml/min; temperature: 50 $^{\circ}$ C; detection: UV, 254 nm; sample: pBR322 DNA-*HaeIII* digest, 0.75 μ g, and Φ X-174 DNA-*HincII* digest, 0.65 μ g, respectively.

by evaporation (20). Thirdly, polynucleotides are retained the less the higher the solvent strength of the mobile phase.

Figure 3 shows the effect of TEAA in detail. Above a concentration of 75 mM of TEAA, ion-pair formation has reached nearly its maximum with regard to DNA restriction fragments shorter than 200 base pairs, because their retention behaviour remains almost the same. This is reflected in the percentage of acetonitrile needed for their elution. Only for longer fragments, a further increase in retention is noted and therefore, more acetonitrile is required to elute them. As far as resolution is concerned, the increase in the concentration of TEAA from 25

Figure 7. High-resolution liquid chromatographic separation of three PCR products spiked with restriction fragments from a pBR322 DNA-*Hae*III digest. Column: PS-DVB- C_{18} , 2.1 μ m, 50 $\times$ 4.6 mm I.D.; mobile phase: 0.1 M TEAA, pH 7.0; linear gradient from 7.5–13.75% acetonitrile in 4 min, followed by 13.75–16.25% acetonitrile in 6 min; flow-rate: 1 ml/min; temperature: 50 $^{\circ}$ C; detection: UV, 254 nm; sample: 120, 132 and 167-bp PCR products, respectively, and 0.5 μ g of a pBR322 DNA-*Hae*III digest; U=unspecific PCR product. The isolated restriction fragments of the pBR322 DNA-*Hae*III digest were subsequently separated on a 10% polyacrylamide gel in TBE buffer for 90 min at 80 V. Gels were then stained in TBE buffer containing 0.5 μ g/mL ethidium bromide for 25 min and photographed.

Figure 8. Calibration curve for a 404-bp PCR product.

to 125 mM in the eluant resulted in a linear enhancement in separation efficiency.

Figure 4 illustrates the impact of column temperature on the resolution of DNA restriction fragments. It is evident that retention times increase with increasing column temperature. Concomitantly, resolution also improves and reaches its optimum at a temperature of 50 $^{\circ}$ C. The increase in retention is most probably the consequence of a defolding and a derotating of the double helix, which exposes more phosphate groups for ion-pair formation and, consequently, enables stronger interaction of the DNA molecules with the stationary phase. At even higher temperatures, however, a rapid decrease in separation efficiency is noted. This phenomenon is not related to the matrix, because similar results have been obtained with pure PS-DVB. The

Figure 9. Dependence of the peak half-width on sample loading. Column: PS-DVB- C_{18} , 2.1 μ m, 50 $\times$ 4.6 mm I.D.; mobile phase: 0.1 M TEAA, pH 7.0; linear gradient from 12.5–17.5% aceto-nitrile in 5 min; flow-rate: 0.75 ml/min; temperature: 50 $^{\circ}$ C; detection: UV, 254 nm; sample: 404-bp PCR product, purified by size exclusion chromatography.

Figure 10. High-speed chromatographic analyses of PCR-products. Column: PS-DVB- C_{18} , 2.1 μ m, 30 $\times$ 4.6 mm I.D.; mobile phase: 0.1 M TEAA, pH 7.0; linear gradient from 10–15% acetonitrile in 2 min; flow-rate: 1.5 ml/min; temperature: 50 $^{\circ}$ C; detection: UV, 254 nm; sample: (a) normal mononuclear white blood cells, (b) chronic lymphatic leukemia; 1 = unspecific PCR product, 2 = β^2 -microglobulin (120 bp), 3 = multidrug resistance gene III (132 bp), 4 = multidrug resistance gene I (167 bp).

decrease in resolution and the final failure to resolve the restriction fragments at all is rather due to the denaturation of the DNA molecules, which occurs in non-aqueous solutions earlier than in aqueous solutions (26).

The flow-rate of the eluant also exerts a significant impact on resolution (Fig. 5). In the case of relatively small double-stranded polynucleotides up to a chain length of 200 base pairs an increase in the flow-rate of the eluant allows a concomitant enhancement in resolution due to the minimization of dispersion. But the longer the DNA molecules grow, the more prominent becomes their mass transfer resistance to move between the stationary and the mobile phase. This causes an increase in peak width with increasing flow-rate. Therefore, a flow-rate as low as 0.4 ml/min

is preferable for the separation of double-stranded polynucleotides larger than approximately 600 base pairs.

Figure 6 shows the separation of a mixture of 31 DNA restriction fragments obtained by the cleavage of pBR322 DNA with *Hae*III and Φ X-174 DNA with *Hinc*II, respectively. It can be clearly seen that under optimized chromatographic conditions one base-pair resolution is obtained for fragments shorter than approximately 150 base pairs. Thereafter, separation efficiency decreases gradually.

Quantitation of PCR products

Figure 7 shows the separation of three PCR products spiked with a pBR322 DNA-*Hae*III digest. Subsequently, the individual restriction fragments were isolated and subjected to polyacrylamide gel electrophoresis in order to confirm their elution order. It is evident that IP-RP-HPLC allows the separation of DNA molecules according to their size. This is in contrast to anion-exchange chromatography (12, 13) which failed to separate the same restriction fragments according to their length, because AT-pairs have been shown to interact more strongly with the stationary phase than GC-pairs (12).

The chromatogram also demonstrates the high sensitivity of on-column UV detection, which permitted the determination of a PCR amplified 404-bp gene fragment with a lower mass detection limit of 0.006 μ g. This is equal to a minimum detectable concentration of 300 ng/ml using a 20- μ l sample loop. This value compares favourably to the concentration sensitivity reported for on-column UV absorbance detection in capillary zone electrophoresis (8). Moreover, the calibration curve (Fig. 8), which had been obtained for the 404-bp PCR product, showed excellent linearity ($r=0.957$) over a range of more than 2 magnitudes (0.006–1.8 μ g), as assessed by the evaluation of a log (signal) versus log (concentration) plot (27). The relative standard deviation in peak area measurements was less than 5% as determined with ten repeated injections of the 404-bp PCR product.

The loading capacity was evaluated by injecting increasing amounts of a 404-bp PCR product purified chromatographically by means of a polyacrylamide size exclusion gel. Subsequently, the peak half-width was plotted against the sample load (Fig. 9). It remained constant at sample loads up to approximately 0.5 μ g, and then increased with further increase in the sample load. However, for semipreparative purposes at least 5 μ g of DNA can be applied with little decrease in resolution.

The recovery of the 404-bp PCR product from the PS-DVB- C_{18} column was determined by measuring its peak area both with the column in place as well as after its replacement with an internal union. The recovery rate was 97.5%. Furthermore, no cross-contamination was detected. Therefore, IP-RP-HPLC on non-porous PS-DVB- C_{18} beads can be considered an excellent tool for the rapid analysis and micropurification of double-stranded polynucleotides without any prior sample pretreatment, as confirmed by the evaluation of the expression of β -microglobulin and the multidrug resistance genes I and III in mononuclear white blood cells obtained from a control (Fig. 10a) as well as from a patient suffering from chronic lymphatic leukemia (Fig. 10b). Furthermore, analyses can be carried out with a high degree of reproducibility, the mean relative standard deviation of the retention times has been 3.4% for 42 measurements.

CONCLUSIONS

It is concluded that IP-RP-HPLC on alkylated non-porous styrene-divinylbenzene copolymers represents the first real alternative to gel electrophoresis. Allowing an equal sample throughput, no time-consuming and laborious pouring of gels as well as no staining with the mutagenic ethidium bromide are required. Moreover, quantitation can be achieved directly by means of UV absorbance at 254 nm without the need for an expensive two-dimensional gel scanner. And in comparison with capillary zone electrophoresis, not only faster separations are achieved, but PCR products may be also analyzed without prior purification.

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