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Chip PCR. II. Investigation of different PCR amplification systems in microfabricated silicon–glass chips

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ABSTRACT

We examined PCR in silicon dioxide-coated silicon–glass chips (12 μl in volume with a surface to volume ratio of ~17.5 mm²/μl) using two PCR reagent systems: (i) the conventional reagent system using *Taq* DNA polymerase; (ii) the hot-start reagent system based on a mixture of TaqStart antibody and *Taq* DNA polymerase. Quantitative results obtained from capillary electrophoresis for the expected amplification products showed that amplification in microchips was reproducible (between batch coefficient of variation 7.71%) and provided excellent yields. We also used the chip for PCR directly from isolated intact human lymphocytes. The amplification results were comparable with those obtained using extracted human genomic DNA. This investigation is fundamental to the integration of sample preparation, polynucleotide amplification and amplicate detection on a microchip.

INTRODUCTION

Microfabrication technology has been applied to a range of analytical problems. More recently silicon chip devices (1–7) have found several applications in DNA analyses. Microfabricated silicon chips have been used for the separation of DNA molecules by means of an array of 2 000 000 posts constructed on a 2.7 × 2.7 mm silicon chip (8), polymerase chain reaction (PCR) and ligase chain reaction (LCR) (9–11), DNA sequencing by hybridization (12–16) and chip capillary electrophoresis separation of antisense DNA oligonucleotides and restricted DNA fragments (17,18).

Previously we have described the fabrication and successful testing of silicon–glass microchips for both PCR and LCR (9,11). In this paper we present results from comparative studies of conventional PCR and hot-start PCR using *Taq* DNA polymerase antibody in both microfabricated silicon–glass chips and ordinary plastic tubes. Several reports previously published by different groups failed to show any quantitative comparison between the use of antibody and non-antibody systems (19–22). Through the use of entangled solution capillary electrophoresis (ESCE) we were able, for the first time, to quantitate the amplifications of the antibody and non-antibody systems in silicon–glass chips and tubes. We further apply this PCR microchip technology to perform a direct PCR amplification of DNA from isolated intact human white blood cells contained in a microchip. This fundamental study is essential to the integration of sample preparation (i.e. microfiltration), nucleic acid amplification (i.e. PCR, RT–PCR and LCR) and amplicate detection (with or without separation). The surface of the silicon chips used in this study was thermally treated to produce a layer (2000 Å) of silicon dioxide, in order to reduce non-specific adsorption of PCR reagents and therefore minimize inhibition of the amplification reaction (23,24).

MATERIALS AND METHODS

Buffers and chemicals

A 1× phosphate-buffered saline (PBS) buffer containing 150 mM sodium chloride, 150 mM sodium phosphate, pH 7.2 (Sigma, St Louis, MO) and a 1× TBE buffer containing 100 mM Tris, 90 mM boric acid, 1.0 mM EDTA, pH 8.3 (Life Technologies, Grand Island, NY) were prepared for cell isolation and electrophoresis. Tween 20® was purchased from Sigma. The dilution buffer for TaqStart antibody, containing 50 mM KCl, 10 mM Tris–HCl, pH 7.0, and the storage buffer, containing 50 mM KCl, 10 mM Tris–HCl, pH 7.0, 50% glycerol, were purchased from Clontech (Palo Alto, CA).

Microfabrication of silicon–glass chips

Silicon chips were fabricated by the Alberta Microelectronic Center (Edmonton, Alberta, Canada) using standard photolithographic procedures (25). Each 14 × 17 mm chip was etched to a depth of 115 μm. The chips were then thermally treated to produce a surface layer of silicon dioxide with a depth of 2000 ± 10 Å. The surface-polished Pyrex™ glass cover (Bullen Ultrasonics Inc., Eaton, OH) was essential to guarentee good anodic bonding and to minimize non-specific absorption. The procedures for washing and bonding of silicon chips and Pyrex glass covers were as described previously (23,24).

Isolation of human lymphocytes

Fresh human blood was drawn and mixed with EDTA. The anti-coagulated blood (5 ml) was spun at 150 *g* for 30 min. The

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plasma (supernatant) was carefully removed. The packed cells were then washed by resuspending in 1× PBS buffer. Centrifugation was repeated and the supernatant discarded. The cells were resuspended in 1× PBS buffer to the initial blood volume. The resuspended cells were centrifuged at 150 *g* for 30 min in a dumb-bell-shaped glass tube fabricated out of a glass pipette, specially designed to improve the recovery of lymphocytes. After centrifugation at 150 *g* for 30 min the purified lymphocytes were carefully removed by pipetting and mixed with 10 µl fresh 1× PBS buffer. A cell count was then performed using a Coulter counter STKS (Coulter, Hialeah, FL).

Extraction of DNA

The templates used in the present study were human genomic DNA and DNA from the bacterium *Campylobacter jejuni*. Extraction of human genomic DNA from human whole blood (200 µl) was performed using the ReadyAmp™ Genomic DNA purification system from Promega (Madison, WI). Extracted DNA was suspended in 200 µl of the supplied buffer used to store the ReadyAmp™ resin. The concentration of the extracted single-strand DNA was 25 ng/µl. *Campylobacter jejuni* DNA was donated by Dr I. Nachamkin and the extracted DNA was dissolved in water to a final concentration of 5 ng/µl.

PCR amplification reaction in the silicon–glass chips

PCR using Taq DNA polymerase and C.jejuni bacterial DNA as template. Each of the 10 silicon–glass chips was filled with 12 µl PCR reaction mixture containing 200 µM each dNTP, 0.6 U *Taq* DNA polymerase (Perkin-Elmer, Norwalk, CT), 0.6 µM each primer and 1.2 ng *C.jejuni* DNA. The primer sequences were: primer 1 (5′-CTTCAGGGATGGCGATAGCAGATAG-3′); primer 2 (5′-GCACTGAACCAATGTCGGCTCTGAT-3′). The reaction mixture was initially heated to 94°C for 1 min and cycled for 28 cycles: 15 s at 94°C, 1 min at 55°C and 1 min at 72°C. A final extension was performed at 72°C for 10 min.

PCR using Taq DNA polymerase and human genomic DNA as template. Each of the silicon–glass chips was filled with 12 µl PCR reaction mixture containing 200 µM each dNTP, 0.4 U *Taq* DNA polymerase and 1× reaction buffer (Perkin-Elmer), 0.6 µM each primer and 125 ng human genomic DNA. The primer sequences used were: primer 3 (5′-GTTTTCCTGGATTATGCC-TGGCACC-3′) and primer 4 (5′-GTTGGCATGCTTTGATGA-CGCTTC-3′). These amplify the *CFTR* gene of human genomic DNA containing the Δ508 mutation site. The reaction mixture was initially heated to 94°C for 6 min and then amplified for 35 cycles: 30 s at 94°C, 30 s at 53°C and 2 min at 65°C. A final extension was performed at 65°C for 5 min.

Hot-start PCR using TaqStart antibody. Each of the 10 silicon–glass chips was filled with 12 µl PCR reaction mixture containing 200 µM each dNTP, 0.6 U *Taq* DNA polymerase and 1× reaction buffer (Perkin-Elmer), 132 ng TaqStart antibody and 0.48 µl dilution buffer (Clontech), 0.6 µM each primer and 1.2 ng *C.jejuni* DNA. The primer sequence information and the thermal cycling conditions were as described above.

PCR amplification directly from human lymphocytes. Samples containing 1500 and 3000 lymphocytes were analyzed. Chips were filled with 12 µl PCR reaction mixture containing 200 µM each dNTP, 0.4 U *Taq* DNA polymerase and 1× reaction buffer (Perkin-Elmer), 0.2 µM each primer and human lymphocytes (1500 or 3000). The primer sequence information and the thermal cycling conditions were the same as described above for the amplification of human DNA.

PCR amplification reaction in GeneAmp™ reaction tubes

All of the microchip PCR amplifications were run in parallel in a GeneAmp™ PCR System 9600 in GeneAmp™ reaction tubes (Perkin-Elmer) under identical thermal cycling conditions using aliquots (12 µl) of the same reaction mixture.

Slab gel electrophoresis

A 2.0% agarose gel (Perkin Elmer) was prepared using 1× TBE buffer. PCR products amplified from *C.jejuni* DNA (3 µl) were loaded onto the gel. DNA molecular weight marker VI (75 ng) (Boehringer-Mannheim, Indianapolis, IN) was used in each size marker lane of the electrophoresis gel. Electrophoresis was performed at 200 V for 50 min. The gel was post-stained with SYBR™ Green I fluorescent dye (Molecular Probes, Eugene, OR) at a final dilution of 40 000 times for 30 min on a reciprocal shaker (Lab Line Instruments, Melrose Park, IL). The electrophoresis results were recorded using Polaroid film 57 (Polaroid, Cambridge, MA).

Another 1.5% mini agarose gel (Sigma) was prepared using 1× TBE buffer. PCR products amplified from human genomic DNA (10 µl) were loaded onto the gel. DNA molecular weight marker VI (75 ng) (Boehringer-Mannheim) was used in each size marker lane of the electrophoresis gel. Electrophoresis was performed at 100 V for 60 min. The gel was pre-stained with ethidium bromide. The electrophoresis results were recorded using Polaroid film 57.

Capillary electrophoresis

Entangled solution capillary electrophoresis (ESCE) was performed on a Bio-Focus 3000™ with a UV detector (BioRad, Hercules, CA) in reversed polarity mode (negative potential at the injection end of the capillary column). Detection was achieved with the UV wavelength set at 260 nm. The external temperature of the capillary column was 25°C and the carousel temperature was 20°C. Before analysis 1 µl of each PCR sample was removed from the chip and GeneAmp™ reaction tube respectively and then diluted with 9 µl deionized distilled water. Sample injections were conducted at 5 kV for 20 s and the separation was performed at a field strength of 260 V/cm for 8 min. Post-run analysis of the data was performed using Bio-Focus 3000 Integrator version 3.01. The buffer system used for separation consisted of 1× TBE to which 0.5% (w/v) (hydroxypropyl)methyl-cellulose (HPMC) was added. The viscosity of a 2% aqueous solution for this cellulose derivative (H-7509; Sigma) was 4000 cP at 25°C. HPMC was dissolved in the buffer using the method recommended by Ulfelder *et al.* (26). Glycerol (5.0% v/v) was added to the buffer to further improve resolution. The buffer was filtered using a 2.0 µm filter and then degassed for 15 min by sonication before use. Samples and reagents (dNTPs, primers and DNA size marker) were analyzed on a surface-modified fused silica capillary column (DB-1; Perkin-Elmer). The capillary column (25 × 100 µm) was conditioned with 5 vol distilled water, followed by 5 vol separation buffer and then subjected to voltage equilibration

Figure 1. Microfabricated silicon–glass chips used for PCR (reaction volume 12 µl, surface area 210 mm^2). The central six-sided reaction chamber is connected by two small channels to the square entry and exit ports.

for 15 min until a stable baseline was achieved. After each run the column was washed with separation buffer for 25 s.

RESULTS AND DISCUSSION

Microfabricated silicon–glass PCR chips are shown in Figure 1. A feature of the chip is its high surface to volume ratio [210 mm^2:12 µl, surface to volume ratio (SVR) 17.5]. This is much higher than a glass capillary reaction tube (80 mm^2:10 µl, SVR 8) or a conventional plastic reaction tube (77 mm^2:50 µl, SVR 1.54). A high surface to volume ratio is advantageous, allowing for more efficient thermal conduction and dissipation. This high thermal transfer should translate into faster cycling times in the microchips compared with what is currently achievable in the GeneAmp™ reaction tubes or in glass capillary tubes. The shortest cycling time for PCR achieved by conventional PCR hardware using a positive displacement plastic tip as the reaction vessel is 30 min for a total of 30 cycles (27). The SVR of the reaction tip is 4.7 mm^2/µl (total reaction volume 10 µl) and is smaller than the SVR of the microchip. It is therefore anticipated that by using the PCR microchip, which has a larger SVR, an even shorter thermal cycling time may be obtained. However, a higher SVR increases the significance of surface chemistry, which may reduce the efficiency or inhibit PCR in the microchips. A silicon dioxide surface minimizes this effect (23,24). All chips used in this study were used only once to avoid contamination. After use the chips were discarded. It is anticipated that the chips will be inexpensive, especially under large scale industrial production.

Conventional PCR

PCR amplification results using *Taq* DNA polymerase from both microchips (lanes 14–18) and GeneAmp™ reaction tubes (lanes 20–24) are shown in Figure 2. The PCR product yields from five individual, randomly chosen microchips and their corresponding

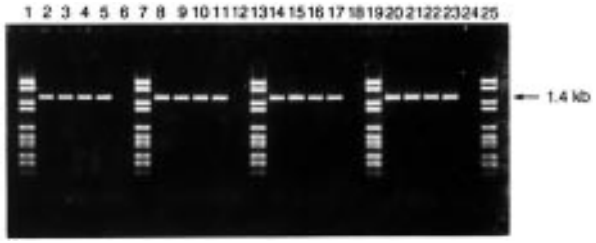

Figure 2. Agarose gel (2% in 1× TBE) electrophoresis of the PCR products amplified from *C.jejuni* DNA. Lanes 2–5, amplification products using the hot-start procedure and chosen randomly from a series of 10 chips. Lane 6, negative control for hot-start chip PCR. Lanes 8–11, positive controls from the GeneAmp™ reaction tubes using the hot-start procedure. Lane 12, negative control for hot-start PCR in a GeneAmp™ reaction tube. Lanes 14–17, amplification products using the conventional procedure and chosen randomly from a series of 10 chips. Lane 18, negative control for the conventional chip PCR. Lanes 20–23, positive controls from the GeneAmp™ reaction tubes using the conventional PCR procedure. Lane 24, negative control for conventional PCR in a GeneAmp™ reaction tube. Lanes 1, 7, 13, 19 and 25, molecular weight marker VI. The gel was run at 200 V for 50 min.

GeneAmp™ reaction tube controls were examined. Visual inspection of the gel electrophoresis results indicated that the yield of chip PCR amplification products was equivalent to that obtained using GeneAmp™ reaction tubes. The amplification products from the microchips have the same size of ~1.4 kb. Lanes 18 and 24 were the negative controls (containing no DNA template) for the chips and the GeneAmp™ reaction tubes respectively. In order to assess the reliability of thermal modification of the silicon dioxide surface of the microchips ESCE was employed for quantitative analysis of the amplified PCR product and the data are shown in Table 1. The quantitative ESCE data indicate that the yield of the product generated from the microchip is ~50% that from the GeneAmp™ reaction tube. The higher coefficient of variation (CV) for PCR performed in the chips may imply that the chip surface has non-specific binding sites that may be adsorbing molecules involved in the PCR reaction.

Hot-start PCR

Hot-start PCR is generally recognized as the most reliable way to perform PCR (27). A hot-start PCR procedure prevents primer extension before the PCR reagents attain a high temperature. This ensures adequate stringency in the first thermal cycle. The simplest way of conducting hot-start PCR is to use anti-*Taq* DNA polymerase antibodies capable of inhibiting *Taq* DNA polymerase activity until they become denatured at high temperature (21). The efficiency of the desired amplification can be enhanced through the use of the hot-start method by minimizing non-specific primer extension. Hot-start PCR results using TaqStart antibody from both microchips (lanes 2–6) and GeneAmp™ reaction tubes (lanes 8–12) are also shown in Figure 2. The four amplification products shown in lanes 2–5 were chosen randomly from the 10 chips used in this study. Samples in lanes 8–11 were the corresponding GeneAmp™ reaction tube controls. Lanes 6 and 12 were the negative controls (without DNA template) amplified in a microchip and a GeneAmp™ reaction tube respectively. All four samples from the microchips (lanes 2–5) gave similar yields. These were similar to that obtained in the GeneAmp™ reaction tube as determined by visual evaluation. Examination of the ESCE results shown in Table 1 indicates that all 10 microchips

produced PCR amplification products with a consistant and higher yield (nearly double) than that from the GeneAmp™ reaction tubes. The reproducibility of the amplification results from the microchip using the hot-start procedure was excellent (CV 7.71%). The reason for the increased yield of PCR product and improved reproducibility may be due to the hot-start antibody maintaining the integrity of the *Taq* DNA polymerase active site and protecting it from deactivation by non-specific binding inside the microchip. Non-specific binding may occur at either a non-active site of the *Taq* DNA polymerase or at the TaqStart antibody. Although an immobilized *Taq* DNA polymerase would have limited access to the target DNA, either situation would leave the active site of the *Taq* DNA polymerase intact and operational after becoming dissociated from the antibody during the initial heat denaturation process. Without the TaqStart antibody the active site of the *Taq* DNA polymerase may be non-specifically bound to the glass surface of the microchip, thereby completely losing activity.

ESCE analysis of PCR products

Quantitative analysis using ESCE revealed substantial differences in the yields of PCR products and the reproducibility of the PCR reactions in tubes and chips (Table 1). The yields (based on peak area) of the products amplified in tubes using *Taq* DNA polymerase alone (Table1) were almost 60% greater than those from reactions run with the hot-start antibody in tubes (Table 1). This might be due to the altered buffer system used with the TaqStart antibody. However, the antibody system did improve the yields within the chips. Without the antibody system the product yields in the chips were ~30% lower than those run using reaction mixtures containing the antibody (Table 1). Additionally, reactions performed in the chips with the hot-start antibody were the most reproducible, with an interbatch CV of 7.71%. The results from the reactions performed with and without the hot-start antibody clearly indicate the importance of the antibody in improving yields and reproducibility in the chips by both maintaining activity of the *Taq* DNA polymerase and blocking non-specific binding sites. The highest yields were obtained in GeneAmp™ reaction tubes using *Taq* DNA polymerase alone (Table 1). This was approximately twice as great as yields obtained from the same reaction performed in the chips and 30% more than yields obtained from chips using the antibody system (Table 1). However, the reproducibility from batch to batch in both tubes and chips using *Taq* DNA polymerase alone was poor with CV valuess >10%.

An unexpected peak was observed in all electropherograms with a migration time of ~5.5 min (Fig. 3). Analyses of the individual PCR reagents identified the peak as Tween 20®, found in the reaction buffer (data not shown). The absence of any detectable primer dimers in all cases (Figs 2 and 3) may be due to the care taken in designing primers for this system. Matches between the two primers and between the individual primers themselves were minimized to avoid any primer dimer formation.

ESCE has the advantage of quantitation, automation (30 samples each batch), fast analysis (8 min), minimal sample band diffusion and low consumption of both sample (a few nanoliters) and chemical reagents (a few mililiters) compared with conventional gel electrophoresis methods. It eliminates the use of fluorescent dyes, which are usually carcinogenic and involve labor intensive manual operations. It is ideally suited to the analysis of PCR products amplified in microchips. A UV detector was used in the ESCE system to detect the amplification products. This detection system is not sensitive enough to detect non-specfically amplified products, which were present at very low concentrations. However, the use of a high sensitivity laser-induced fluorescence detector may detect these products.

Table 1. Quantitative results obtained from PCR using ESCE

Sample number	PCR chips		GeneAmp tubes	
	w/o Antibody product (area)	w/ Antibody product (area)	w/o Antibody product (area)	w/ Antibody product (area)
1	18922	23089	31424	14091
2	21669	21695	41980	11258
3	18098	22006	39031	13821
4	13193	23065	36110	13654
5	15893	19826	31106	15922
6	11533	24844	28438	15591
7	12630	24478	26017	11345
8	27705	22027	34117	11856
9	12614	23783	31952	16043
10	14057	26474	30762	15862
Avg.	16631	23129	33094	13944
Std. Dev.	4822	1784	4578	1821
C.V.	28.99%	7.71%	13.83%	13.06%

Lymphocyte PCR

PCR products amplified directly from isolated human lymphocytes and from extracted human genomic DNA using *Taq* DNA polymerase are shown in Figure 4. The size of the amplified product is ~100 bp. Lanes 2 and 3 were the products from reactions performed in GeneAmp™ reaction tubes. Lanes 4–6 were from reactions performed in the microchips. Lanes 2 and 4 show products amplified from the purified DNA and lanes 3, 5 and 6 show products from isolated lymphocytes (1500, 3000 and 1500 respectively). The results shown in Figure 4 illustrate that in the presence of either extracted DNA or intact lymphocytes PCR amplification in both microchips and GeneAmp™ reaction tubes generated positive signals with the expected product size. However, it was observed that a much smaller sized, non-specific product was present in all samples amplified from the lymphocytes. The existence of the interference band does not affect interpretation of the experimental results.

In summary, we have confirmed that a silicon dioxide surface in a silicon chip is capable of reducing non-specific adsorption of PCR reagents compared with other silicon surfaces. The TaqStart antibody reagent system is more suitable for chip-based PCR reactions than the *Taq* DNA polymerase reagent system, as judged by both the yield and consistancy of amplification. The PCR amplification results obtained directly from isolated human lymphocytes are comparable with those from human genomic DNA extracted from human lymphocytes. This result indicates that cell filtration combined with direct PCR of the filtered cells could be effectively combined on a PCR chip. Construction of a micro-DNA laboratory on a silicon chip requires three analytical

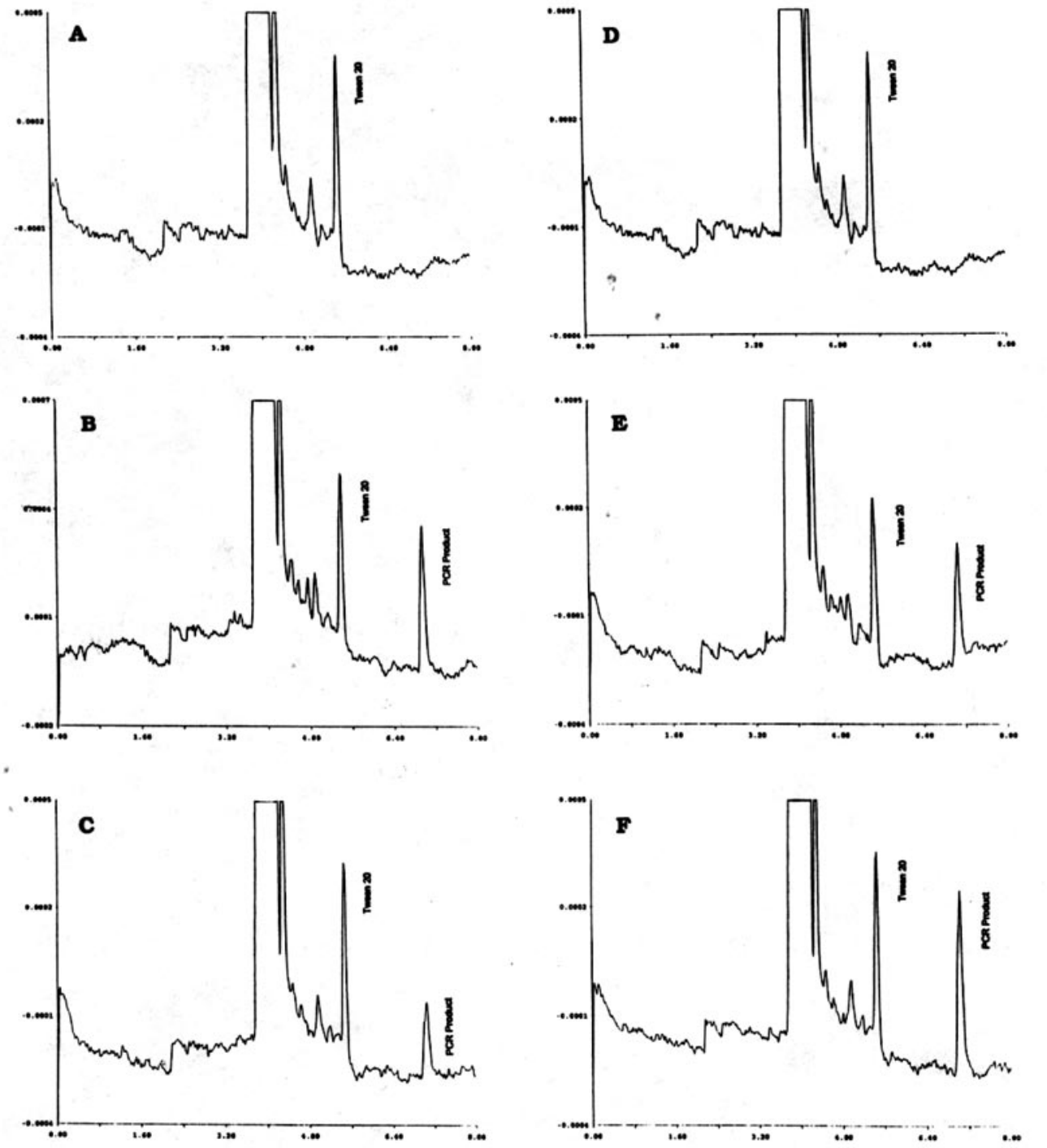

Figure 3. Electropherograms of samples of the amplification products from PCR reactions in both chips and GeneAmp™ reaction tubes. The PCR products were separated by ESCE in inverted mode and detected at 260 nm. (**A**) Negative amplification product from a silicon–glass chip using the hot-start procedure. (**B**) Positive amplification sample from a silicon–glass chip using the hot-start procedure. (**C**) Positive amplification sample from a GeneAmp™ reaction tube using the hot-start procedure. (**D**) Negative amplification product from a silicon–glass chip using the conventional procedure. (**E**) Positive amplification sample from a silicon–glass chip using the conventional procedure. (**F**) Positive amplification sample from a GeneAmp™ reaction tube using the conventional procedure. Samples were electrokinetically injected at 5 kV for 20 s. The separation was performed at 6.5 kV for 8 min inside a DB-1 coated capillary column with a total length of 25 cm.

steps: (i) sample preparation; (ii) chemical reaction; (iii) detection (with or without separation). We have studied sample preparation using silicon microfilters and demonstrated effective isolation of red blood cells and, more recently, white blood cells (28). Successful PCR linked to cell filtration on a chip are two key components in an integrated microchip PCR analyzer and we are now investigating other components of the PCR assay scheme, including on-chip detection. Integration of all of the steps in a PCR assay onto a single microchip will lead to low cost and convenient DNA amplification and detection.

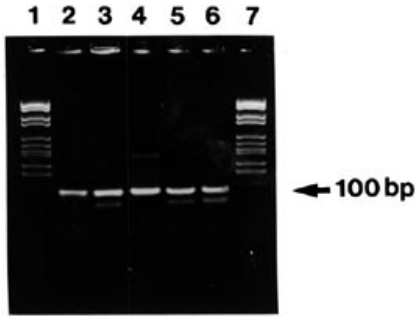

Figure 4. Agarose gel (1.5% in 1× TBE) electrophoresis of the *CFTR* gene containing the Δ508 mutation site amplified directly from isolated human lymphocytes and from extracted human genomic DNA using *Taq* DNA polymerase. The size of the amplified product was ~100 bp. Lanes 2 and 3, products from reactions performed in GeneAmp™ reaction tubes; lanes 4–6, from the microchips. Lanes 2 and 4, products amplified from the purified DNA; lanes 3, 5 and 6, from the isolated lymphocytes (1500, 3000 and 1500 lymphocytes respectively). Lanes 1 and 7, molecular weight marker VI. The gel was run at 100 V for 50 min.

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REFERENCES

1 Service,R.F. (1995) *Science*, **268**, 26–27.
2 Manz,A, Harrison,D.J., Verpoorte,E. and Widmer,H.M. (1993) In Brown,P.R. and Grushka,E. (eds), *Progress in Chromatography.* Marcel Dekker, New York, NY, pp. 1–66.
3 Kricka,L.J., Nozaki,O. and Wilding,P. (1994) *J. Int. Fedn Clin. Chem.*, **6**, 54–59.
4 Kricka,L.J. and Wilding,P. (1995) in Price,C.P. and Newman,D.J. (eds), *Principles and Practice of Immunoassay,* 2nd Edn. Macmillan Reference Books, London, UK, in press.
5 Kricka,L.J., Ji,X., Nozaki,O. and Wilding,P. (1994*) J. Biolumin. Chemilumin.*, **9**, 135–138.
6 Kricka,L.J., Faro,I., Heyner,S., Garside,W.T., Fitzpatrick,G. and Wilding,P. (1995) *Clin. Chem.*, **41**, 1358–1359.
7 Manz,A., Miyahara,Y. Miura,J., Watanabe,Y., Miyagi,H. and Sato,K. (1990) *Sensors Actuators*, **B1**, 249–255.
8 Volkmuth,W.D. and Austin,R.H. (1992) *Science*, **358**, 600–602.
9 Wilding,P., Shoffner,M.A. and Kricka,L.J. (1994) *Clin. Chem.*, **40**, 1815–1818.
10 Northrup,M.A., Gonzalez,C., Lehew,S. and Hills,R. (1994) In van den Berg,A. and Bergveld,P. (eds.), *Micro Total Analysis Systems*, Kluwer, Dordrecht, The Netherlands, p. 139.
11 Cheng,J., Shoffner,M.A., Mitchelson,K.R., Kricka,L.J. and Wilding,P. (1995) *J. Chromatogr. A*, in press.
12 Fodor,S.P.A., Rava,R.P., Huang,X.C., Pease,A.C., Holems,C.P. and Adams,C.L. (1993) *Nature*, **364**, 555–556.
13 Maskos,U and Southern,E.M. (1993) *Nucleic Acids Res.*, **21**, 4663–4669.
14 Drmanac,R., Drmanac,S., Strezoska,Z., Paunesku,T., Labat,I., Zeremski,M., Snoddy,J., Funkhouser,W.K., Koop,B., Hood,L. and Crkvenjakov,R. (1993) *Science*, **260**, 1649–1652.
15 Eggers,M., Hogan,M., Reich,R.K., Lamture,J., Ehrlich,D., Hollis,M., Kosicki,B., Powdrill,T., Beattie,K., Smith,S., Varma,R., Gangadharan,R., Mallik,A., Burke,B. and Wallace,D. (1994) *BioTechniques*, **17**, 516–524.
16 Beattie,K.L., Beattie,W.G., Meng,L., Turner,S.L., Coral-Vazquez,R., Smith,D.D., McIntyre,P.M. and Dao,D.D. (1995) *Clin. Chem.*, **41**, 700–706.
17 Jacobson,S.C., Hergenröder,R, Moore,A.W.,Jr and Ramsey,J.M. (1994) *Anal. Chem.*, **66**, 4127–4132.
18 Woolley,A.T. and Mathies,R.A. (1994) *Proc. Natl. Acad. Sci. USA*, **91**, 11348–11352.
19 Scalice,E.R., Sharkey,D.J. and Daiss,J.L. (1994) *J. Immunol. Methods*, **172**, 147–163.
20 Findlay,J.B., Atwood,S.M., Bergmeyer,L., Chemelli,J., Christy,K., Cummins,T., Donish,W., Ekeze,T., Falvo,J., Patterson,D., Puskas,J., Quenin,J., Shah,J., Sharky,D., Sutherland,J.W.H., Sutton,R., Warren,H. and Wellman,J. (1993) *Clin. Chem.*, **39**, 1927–1933.
21 Sharkey,D.J., Scalice,E.R., Christy,K.G., Altwood,S.M. and Daiss,J.L. (1994) *BioTechnology*, **12**, 506–509.
22 Kellogg,D.E., Rybalkin,I., Chen,S., Mukhamedova,N., Vlasik,T., Siebert,P.D. and Chenchik,A. (1994) *BioTechniques*, **16**, 1134–1137.
23 Wilding,P., Shoffner,M.A., Cheng,J., Hvichia,G. and Kricka,L.J. (1995) *Clin. Chem.*, **41**, 1367–1368.
24 Shoffner,M.A, Cheng,J., Hvichia,G.E., Kricka,L.J. and Wilding,P. (1996) *Nucleic Acids Res.*, **24**, 375–379.
25 Wallis,G. (1970) *J. Am. Ceram. Soc.*, **53**, 563–567.
26 Ulfelder,K.J., Schwartz,H.E., Hall,J.M. and Sunzeri,F.J. (1992) *Anal. Biochem.*, **200**, 260–267.
27 Taylor,G.R. and Logan,W.P. (1995) *Curr. Opin. Biotechnol.*, **6**,24–29
28 Wilding,P., Pfahler,J, Bau,H.H., Zemel,J.N. and Kricka,L.J. (1994) *Clin. Chem.*, **40**, 43–47.