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Scaling of Nucleic Acid Assays on Microelectrophoresis Array Devices: High-Dynamic Range Multi-gene Readout from less than 10 Transcripts

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Abstract

In this paper we describe progress in using the prodigious data-collecting ability of multilane microelectrophoresis instruments to bear on problems in scaled nucleic acid assays. We emphasize compound stacking and Solid-Support loading as means to concentrate <100 pg samples for direct injection. Reaction Mapping is applied to readout of quantitative polymerase chain reaction (qPCR) gene-expression and as a way to practically overcome difficulty in interpreting amplification curves of multiplexed qPCR at 20–50 gene/well complexity. We demonstrate multiplexed readout of gene expression over an abundance range of 9Log2 units starting with reverse-transcribed samples as small as 5 molecules in each sample.

1. Introduction

Commonly perceived opportunities in microfabricated bioassay devices include process integration, portability, point-of-care assay, and disposability. These are ideas that stem from the ability, at least in principle, for microfabrication to cost effectively produce high functional complexity in a small low-power package. In this paper we discuss a quite distinct set of specific motivations, namely scaling in sample size and channel multiplicity for more or less conventional column electrophoretic separations. While reduced sample size and increased channel multiplicity are clearly germane to the goals of integration, we argue that these relatively simple scaling opportunities have great power in their own right. We argue that when applied with current microelectrophoresis arrays, sample-scaling and parallelism-scaling can already supply needed improvement in nucleic acid assays for clinical and network biology applications. This paper will give some examples to make these points. However we hasten to add that the literature in microfluidic assay devices has truly exploded in the last ten years. Specifically, it is not possible in our current format to provide a complete review of the research in sample injection into microdevices. We also do not treat the important aspect of scaling (dramatically reducing) assay time on microfluidic devices.

This paper will summarize the much narrower topic of sample concentration/injection, and (re-thought) parallel assay design as applied in our own studies on highly parallel semi- or fully automated microelectrophoresis instruments.

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2. Materials and Methods

2.1. Chip fabrication and preparation

Micromachined glass microdevices at various complexity were fabricated as described previously [1–5]. Plates of 1.1-mm-thick alumina silicate glass (Corning 1730, Corning Co., Corning NY) were patterned by photolithography, HF etching and laser drilling. These were then fusion bonded to an un-patterned plate and diced into microfluidic devices of 1–384-lanes with separation lengths of 10–40 cm. A large variety of device geometries were made, typically including a four-ported double-T injector and separation channel, however sometimes operated non-conventionally as described below. In our standard configuration channels are 40–60 μm deep. Channel surfaces were coated with linear polyacrylamide (LPA) using a modified Hjerten procedure. More details can be found in the cited references [1–5]. Traditional injections used platinum electrodes of 0.5-mm diameter. For the solid-support loading, one end of a permalloy-80 wire (0.5-mm diameter, 5-cm length, ESPI International, Ashland, OR) was ground to hemispherical shape using lapping paper.

2.2. DNA sample preparation

For the solid-support-loading and sample-scaling experiments, samples were prepared with the Applied Biosystems (ABI) Big Dye Terminator 3.1 cycle sequencing kit (Applied Biosystems, Foster City, CA) using the GeneAmp PCR System 9700 with 20 μL reactions. One reaction contained 1 μL Big Dye Terminator reaction mix, 3.5 μL 5x ABI Sequencing Buffer, 3.2 pmol M13 forward primer (Gene Link, Inc. Hawthorne, NY), and 100 ng M13 mp18 template (New England BioLabs, Beverly, MA).

For the reaction mapping experiments, total RNA extracted from human neutrophils [6] was prepared following the TRI reagent protocol. The cDNA was created using the Retroscript kit (Ambion, Austin, TX) on a Perkin Elmer GeneAmp PCR System 9600 following the manufacturer protocol. A 300-ng sample, total RNA, served as template for the reverse transcription, random decamers were used as primers. Three fluorophores carboxyfluorescein, 6-carboxy-4',5'-dichloro-2',7'-dimethoxyfluorescein (6-JOE), and tetramethylrhodamine (TMR) were used to provide labels. Twelve primer pairs were designed with the Primer3 software around gene exon boundaries with a PCR product range of 100 to 400 bp and were used to spread the multiplex products across the read-out electropherogram [6].

2.3. Separation matrix preparation

The LPA separation matrix was purchased from Dakota Biosciences, LLC (Sioux Falls, SD). 4% LPA solutions were prepared with 1x TTE (50 mM Tris/50 mM TAPS/2 mM EDTA) and 7 M urea. The solutions were ready for use after 3 days of slow stirring in a glass jar.

2.4. Optical detection system

A five-photomultiplier (PMT) cascade detector with a collection aperture of 0.5 NA (numerical aperture) was used. This was operated over greater than six decades of linear dynamic range using multiple channels on a single 16-bit digitizing board (one channel per PMT) [4,6]. Fluorescence was apportioned across the PMT channels using a combination of spectrally-neutral and dichroic beam splitters. To normalize for injection variations a carboxy-x-rhodamine (CXR)-dyed ladder was run as an internal lane standard on one optimized PMT channel (P1 channel, configured to optimize CXR-red signal).

2.5. Preelectrophoresis, injection and run conditions

The voltage sequences for preconditioning the channels and for separation are given below and, in more detail, in Refs. 1–8.

3. Results

3.1. Sample-Size Scaling

The first scaling opportunity that was investigated was straightforward reduction of sample size using microchip injectors. Smaller sample reduces front-end costs of the analysis and also reduces “noise” due to amplification of the original nucleic acid sample. Enzymatic amplification, particularly with low-copy-number starting material results in significant statistical noise. This is encountered, for example, in DNA forensics analysis of “touch” evidence [9] The new opportunities in sample scaling of microdevices over capillary array electrophoresis (CAE), almost entirely stem from, (i) the many novel sample loading configurations that can be created with lithography in two- and three-dimensional channel networks, and (ii) new sample transfer methods made-possible in miniaturized planar format. Sample scaling and sample concentration methods are intimately related, since the actual injection volume needed on a microdevice is typically only several nanoliters, much less than minimum volumes that can be handled externally by common fluid-handling automation.

3.1.1 Two-Stage Stacking—The most straightforward method of sample concentration onto packed-column microdevices is a variant of sample stacking, i.e., the same physics that is the mainstay of injection into sieving capillaries. For a microdevice, with cross injector, there are two interfaces which can be used sequentially to concentrate the sample and narrow the injection plug (Fig. 1). The first of these [1] is at the “gel” interface in the sample well (analogous to CAE). The second [2,7,8] is at the parting intersection of the loading arm and the separation channel, where a second conductivity discontinuity can be induced using ion depletion with pre-loading currents and low-conductivity buffer. Valezques [7,8] took real-time videos of the stacking phenomena at the second interface and showed that a very appreciable narrowing to plug lengths on the order of 10- μ m length can be achieved using short-term high voltages (~ 500 V/cm) timed with sample injection out of the loading channel. Voltage during the separation step is turned down to 100–200 V/cm to avoid biased reptation during separation. Even without high injection voltages Aborn [3] showed that careful conditioning of the first and second interfaces using pre-loading currents could be used to achieve routine injections into a 768-lane microdevice sequencer with a factor of eight reduction in required sample mass relative to contemporary CAE Sanger sequencing. Stacking and autogating effects at the first interface, which confound the experimental reproducibility, were reproduced accurately by a numerical model [1]. In practice, the uniform conditioning of both the first and second stacking interfaces is among the very most important aspects needed to get uniform results out of an cross-injected array microdevice [3].

3.1.2 Gated Loading—As a specialized application, sample stacking at the first interface can be used with the intrinsic sample dispersion in the loading arm (the column length between first and second interface) to select a particular range of analyte peaks. Several authors have used this type of cascaded separation to sequentially purify bands [10,11]. The approach can lead to an improved signal-to-noise ratio by increasing the absolute concentration of the specific analyte of interest injected without overloading the separation channel. Goedecke [4] showed an appreciable increase in signal for a DNA forensics microdevice when used in this manner.

3.1.3. Solid-Support Sample Injection—Solid-Support injection [5] was designed completely independently of sample stacking for four reasons;

- i. to circumvent the minimum-practical-volume limitations of automated pipettors (currently $\sim 1 \mu\text{l}$)
- ii. to concentrate low-quantity samples out of dilute solution and
- iii. to purify samples, while efficiently injecting them into an electrophoresis column in a short plug.
- iv. to potentially simplify the congested injection channel network in big cross-injected array devices

The method relies on nonspecific adsorption of DNA onto micrometer-sized paramagnetic beads. DNA is adsorbed to carboxyl-modified microspheres in the presence of divalent cations. As the microspheres are paramagnetic they can be collected out of suspension by applying an external magnetic field. A wash step removes the unincorporated nucleotides, enzymes and excess salt but not the extension products. Release of the extension product can be by selective elution with pure water (Fig. 2).

The method was first demonstrated for Sanger sequencing samples, where it was shown that highly reliable sequencing results could be obtained with less than 300-pg of DNA sample. [5]. This is an order of magnitude reduction compared to sequencing with the same signal-to-noise ratio in the best cases using optimized stacking out of solution on our cross-injected microdevices. In principle the same paramagnetic beads can be used for several of the preliminary steps in the Sanger sample preparation. Therefore, through integration of many steps onto solid-support handling, there is not only an economy in the sample injection at readout, but also potential further economies in sample preparation prior to this last step. Others have investigated novel sample preparation steps that are highly relevant. [12–19].

The first steps in Solid-Support loading are the incubation of the beads with Sanger sequencing reaction and removal of unincorporated dideoxynucleotides (Fig. 2). The beads are then re-suspended in 70% ethanol and captured with a magnetized permalloy wire. Next the bead-coated wire (which is to serve as an electrode) is positioned over the sample port by means of a micromanipulator. Care must be taken to not immerse the electrode into water as this would elute the DNA from the beads and result in a dilution of the sample. For injection, high-voltage is applied to the electrode, *then* water is added with a pipette to complete the electrical circuit and to simultaneously elute the DNA from the captured beads. In some experiments a short high-voltage “release voltage” is applied immediately after the circuit is completed.

Figure 3 shows the injection peaks for 4 different release voltages for direct injection into a 12-cm-long channel filled with linear polyacrylamide (LPA) as separation matrix. 0.2 microliter of a 1/8 Sanger sequencing reaction (Big Dye Terminator 3.1 cycle sequencing kit, Applied Biosystems, Foster City, CA) served as DNA sample. The magnetic beads, with adsorbed ssDNA, were collected with a Permalloy80 electrode and subsequently injected from the tip into the separation channel. The electrode tip was polished to a hemisphere to maximize field uniformity during release. Fluorescence was detected 4 mm away from the injection port. Single peaks of high concentration are eluted in short uniform injection plugs (see Table 1).

With an electrostatic moded [5] it is clear that many release-pulse conditions inject sample plugs $\sim 1.6\text{s}$ FWHM or less. All non-idealities including DNA exfoliation into low-field regions, DNA desorption kinetics, sum to an injection pulse width of $\sim 0.7\text{--}1.1$ FWHM for typical DNA sequencing conditions. Therefore Solid-Support loading may be highly useful

as a general technique for DNA assays on both capillaries and microdevices. The method is geometrically ideal for the open wells of microdevices.

3.2. Multiplicity and Internal Cross-Correlation

Microfabrication allows massive parallelism at a relatively low additional cost. However there are many different ways to use channel multiplicity. Not all ways are useful since “input/output” fluid interfaces and detector must support the additional channel count.

3.2.1 Simple Parallelism—The most obvious way to effectively use channel multiplicity is for applications that require automation of highly repetitive assays. An example is long-read DNA Sanger sequencing for read-lengths beyond the (quality-related) practical limits currently achieved in sequencing-by-synthesis (SBS) [20,21]. Since sequence “assembly” costs explode with low quality factors (Q-factor) or for short read length, *de novo* sequencing of mammalian genomes is still well beyond SBS. An automated 768-lane microelectrophoresis system [22–24] can produce >5 Mbases of long-read DNA sequencing in a day, 6–8 times the practical productivity of a 96-lane Sanger CAE machine for comparable data. This is what would be expected based on simple channel count. Furthermore mostly because of their monolithic structure (mechanical rigidity, and better heat sinking) and sample-injection, we believe multi-channel microfabricated devices outperform CAE machines in data quality factors [4,22,23]. Reduction in the sample preparation cost (see sections above) and disposable cost enter into the calculation that would motivate conversion from existing 96-lane CAE machines.

3.2.2 Reaction Mapping—Beyond simple scale up, some of the more interesting ways to use massive parallelism in electrophoresis involve mixed-mode encoding of analyte identity, high-dynamic range quantization, and internal calibration. The raw information content that is available from a 384-lane long-read sequencing device (384 lanes, each reading 1100 peaks, $\times$ four colors) is on the order of 1.6M peaks per plate read. A 16-lane forensics device (16 lanes $\times$ 600 peaks, $\times$ 4 colors) is making 38,000 readings. Clearly there should be novel ways to use this information bandwidth outside sequencing. We chose to address a perceived gap in the technology available for routine expression analysis, i.e. technology available for either routine clinical analysis of expression (where existing solutions are too costly), or for scale-up research applications of disease (where accuracy and cost both come to bear).

Although *qualitative* gene expression analysis has been very highly multiplexed on hybridization arrays, these arrays are only weakly quantitative with a dynamic range of about $5\log_2$ units. [25]. Furthermore the major commercial hybridization arrays are contradictory in assigning gene ratios even at this low level of quantitation [25]. The gold standard for *quantitative* analysis of nucleic acids is currently real-time quantitative PCR (qPCR), which is implemented usually as monoplex reactions using multi-well (96- to 384-well) fluorometer readout. A principle limitation of qPCR can be that it becomes excessive in reagent cost and sample requirement when scaled to multi-gene analysis.

In the Reaction Mapping method [6] we have explored an approach that would extend a much higher degree of multiplexing to qPCR, ultimately 20–50 genes per reaction. Our approach (Fig. 4) is to use array electrophoresis with a complex precalibration, mapping, step. By modifying a four-color laser-induced fluorescence (LIF) detector to report up to 6 decades in range, we retain the high dynamic range of the qPCR and make good use of the array column separation to multiplex many gene products. Through the pre-calibration, we extend the multiplex and compensate for non-exponential effects in PCR.

This approach modifies the notion of a qPCR “standard curve” to encompass the broader concept of mapping the entire interacting multiplex. PCR in multiplexes, notoriously, can have numerous non-idealities due to interferences and saturations of the amplification chemistry. In Ref. 22 we suggest how Reaction Mapping can increase the productivity of a single 96-well PCR amplification from current practice (typically 32 genes) by a factor of 60X (to analysis of 1920 genes). Therefore there is a dramatic reduction in the enzyme and sample preparation costs that dominate routine qPCR analysis.

In Reaction Mapping, a multiplex that represents the biological network of interest is designed with PCR amplicons that are dispersed at different base-pair product lengths across the “gel”. This full multiplex is amplified in a single well at several different cycle numbers, peak areas are acquired for each of these cycle numbers, and then the whole data set is reduced together to produce a standard curve for the full multiplex. Once the reaction map is generated, it is generally possible to run single-time-point, single-well reactions to generate gene ratios for the full multiplex. There is an “overhead” expense in the primer-design and mapping steps. However this overhead can be generously compensated by much lower costs in routine quantitative analysis of gene networks in scale-up studies or clinical applications.

There are several corollary benefits that come with this approach, namely (i) extensive internal calibration and (ii) important increased sensitivity/linearity compared to fluorometric qPCR read out. First, there is room in the multiplex for inclusion one or more housekeeping gene to control for PCR amplification irreproducibility (a large consideration). Second there is room for a doped (known absolute concentration) of labeled DNA ladder for each electrophoresis lane (post PCR). The latter provides an absolute reference in molecular density and in peak assignment. These internal standards come almost for free given the large unused detection bandwidth in the electrophoresis array. After that, because all genes are amplified in the same PCR well, the approach intrinsically maintains an internal cross-referencing between all genes. Given the relatively high randomness of PCR amplifications, particularly for low-copy-number alleles,¹ this internal calibration should provide a reproducibility in assigning gene ratios that monoplex fluorometric qPCR simply cannot match.

Our initial investigation of Reaction Mapping was done with 7-plex and 12-plex qPCRs on a 16-lane microdevice, 20-cm effective column length, with LPA matrix, and a four-color LIF detector modified to cover 6 decades of dynamic range. Figure 5 illustrates the process involved in mapping a 7plex. In this example the multiplex, itself, is somewhat artificial (composed of genomic DNA rather than transcripts), but the example is instructive since absolute copy number is known for each allele in this artificial construction. Hence the assertion that gene ratios are accurately assigned through internal cross-correlation can be tested.

Templates were generated by 7 singleplex PCRs with human genomic DNA as template and primers which amplify 2 kbp regions surrounding short tandem repeats (STRs) that were available in a commercial forensics multiplex (Promega Powerplex 1.2). Primers were chosen so that only homozygous regions were amplified. The amplicons were spin column purified, diluted appropriately and then combined to yield concentrations from 5 to 5000000 copies per reaction. For the reaction map, the Promega Powerplex 1.2 kit was used to generate 7 amplicons of 139 bp to 307 bp in length carrying two different fluorescent labels. In Fig. 5, microchip electropherograms after 10, 20, 30, 40 and 60 cycles are presented. The electrophoretic readout also allows for relatively high cycle numbers because amplicons are not only discriminated by fluorescent tags but also by size in contrast to real-time PCR where only fluorescence intensity is recorded. As a consequence detection of as low as 5

template copies is readily possible using our approach (Fig. 5). An example of the follow-through extension to allele-ratio measurement is shown in Fig. 6.

The second important corollary benefit of the Reaction Mapping method is a very substantial increase in detection sensitivity that works to extend the dynamic range of absolute detection and to reduce PCR “noise”. PCR irreproducibility increases monotonically with increased cycle number during enzymatic amplification. The advantage of the new method stems from the more perfect detection of LIF and column detection compared to fluorimetry. This reduces the total number of cycles that are needed to get a detectable signal.

A direct comparison was made between our (more or less standard) LIF detector [6] and Taqman detection in a commercial qPCR machine. A single gene (HSPH1) was chosen out of larger study of expression in human neutrophils stimulated by the fungal pathogen *C. albicans*. We made a direct comparison of the threshold cycle for the detection of a fixed starting quantity of DNA. Real-time PCR with the ABI Prism readout was used as a standard with one microliter of a reverse transcription reaction as template. This was compared to a 12plex PCR readout on our microdevice using 12 microliters of the same reverse transcription reaction, i.e. the amount of template/gene is the same for both the qPCR and our multiplex approach. The results are shown in Fig. 7. The sensitivity of our detector allows readings after 10–11 cycles, compared to 16 cycles for the qPCR measurement. This advantage appears to stem from the high numerical aperture and stray-light rejection of our detector, combined with the sample-concentrating effects of microfluidic injection when compared to open-well detection, even in a highly optimized fluorometer.

Results are shown in Fig. 8 for a 12-gene multiplex taken as part of the same study. To control for the possibility of template loading artifacts, Reaction Maps were assembled from data collected over five cycle numbers, using stimulated and unstimulated neutrophil samples. The results, collected from full 12plex PCR reactions, are displayed as separated panels in Fig. 8. Note that all the amplifications remain highly exponential, nonetheless the loading of DNA template from the stimulating organism appears to cause a shift in the slopes for several genes. This artifact which might easily be missed using conventional standard curves and monoplexes is easily managed using the strictly empirical data reduction of our method.

It is immediately obvious from Fig. 8, that genes like HSPH1, CCL20 and CXCL2 are up-regulated whereas others (e.g. IL1B, TNF and LGALS3) are virtually unchanged. This is confirmed by a more thorough data analysis.

4. Discussion

In this short review we have given examples as to how the new opportunities of array microelectrophoresis devices for reduced sample-size and increased column multiplicity can be highly useful in scaled analysis of nucleic acids. Some of the opportunities (e.g., Reaction Mapping) are equally feasible by novel usage of CAE instruments. We have emphasized compound injection stacking and Solid-Support loading as means to effectively bring concentrated ~100 pg sample plugs into the assay device. We have discussed current simple column-number scaling as applied successfully to increasing the capacity of automated instruments. Sanger sequencing is only an initial application. Using the example of Reaction Mapped highly multiplexed qPCR readout, we have suggested that the raw information bandwidth in current array electrophoresis machines is not yet well exploited for more general assays (outside sequencing). We argue that the combination of sample scaling with

mixed-mode analysis, e.g., combining Solid-Support loading with Reaction Mapping of low-cycle-number qPCR, could multiply the strengths of array microelectrophoresis into areas that are urgently needed for multi-parameter measurement of low-copy-number nucleic acids at high dynamic range and high throughput. These are important unmet needs for study of biological networks.

In our first iteration we have been able to show $>9\log_2$ dynamic range in readout of internally cross-correlated expression analysis starting with as few as 5 initial molecular copies [6]. We have demonstrated a 5- to 6-cycle reduction in required amplification relative to fluorometric qPCR, which, particularly where it is combined with the new loading methods, would increase the integrity of the assay. We have demonstrated this in 12plexes but the data capacity is several times larger than this, and is certainly great enough to represent a small signaling pathway or similar small gene network (20–50 genes) in a single well reaction and single electrophoretic read. A 96- or 384-lane electrophoresis array would permit the expression analysis for the full gene network across 96 (or 384) samples (20–50 genes) in several hours (including PCR) in a sample size that would be limited by the smallest cell number that could be handled in the transcription step.

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References

1. El-Difrawy SA, Srivastava A, Gismondi EA, McKenna BK, Ehrlich DJ. Electrophoresis. 2006; 27(19):3779–3787. [PubMed: 16960830]
2. Srivastava A, Metaxas AC, So P, Matsudaira P, Ehrlich D, Georgiou G. Electrophoresis. 2005; 26(15–16):1130–1143. [PubMed: 15704245]
3. Aborn JH, El-Difrawy SA, Novotny M, Gismondi EA, Lam R, Matsudaira P, McKenna BK, O'Neil T, Streechon P, Ehrlich DJ. Lab on a Chip. 2005; 6:669–674. [PubMed: 15915260]
4. Goedecke N, McKenna B, El-Difrawy S, Gismondi E, Swenson A, Carey L, Matsudaira P, Ehrlich DJ. J Chroma A. 2006; 1111:206–213.
5. Ueberfeld J, El-Difrawy SA, Ramandie K, Ehrlich DJ. Anal Chem. 2006; 78:3632–3627. [PubMed: 16737217]
6. Ueberfeld J, McKenna B, Rubin-Berjerano I, Verstrepen K, Ehrlich DJ. Analytical Chemistry. 2008; 80:740–7436.
7. Vazquez M, McKinley G, Mitnik L, Desmarais S, Matsudaira P, Ehrlich DJ. J Chromatogr B. 2002; 779(2):163–171.
8. Vazquez M, McKinley G, Mitnik L, Desmarais S, Matsudaira P, Ehrlich D. Analytical Chem. 2002; 74(9):1952–1961.
9. Gill P, Whitaker J, Flaxman C, Brown N, Buckleton J. Forensic Sci Int. 2000; 112:17–40. [PubMed: 10882828]
10. Ermakov SV, Jacobson SC, Ramsey JM. Anal Chem. 2000; 72:3512. [PubMed: 10952536]
11. Zhang G, Du W, Liu B-F, Hisamoto H, Terabe S. Anal Chim Acta. 2007; 584:129–135. [PubMed: 17386595]
12. Wen J, Legendre LA, Bienvenue JM, Landers JP. Anal Chem. 2008; 80(17):6472–6479. [PubMed: 18754652]
13. Easley CJ, Karlinsey JM, Bienvenue JM, Legendre LA, Roper MG, Feldman SH, Hughes MA, Hewlett EL, Merkel TJ, Ferrance JP, Landers JP. Proc Nat Acad Sci. 2006; 103:19272–19277. [PubMed: 17159153]
14. Breadmore MC, Wolfe KA, Arcibal IG, Leung WK, Dickson D, Giordano BC, Power ME, Ferrance JP, Feldman SH, Norris PM, Landers JP. Anal Chem. 2003; 75:1880–1886. [PubMed: 12713046]

15. Paegel BM, Blazej RG, Mathies RA. *Curr Opin Biotechnol.* 2003; 14:42–50. [PubMed: 12566001]
16. Lagally ET, Mathies RA. *J Phys D: Appl Phys.* 2004; 37:R245–R261.
17. Paegel BM, Yeung SHI, Mathies RA. *Anal Chem.* 2002; 74:5092–5098. [PubMed: 12380835]
18. Gingeras TR, Higuchi R, Kricka LJ, Lo YMD, Wittwer CT. *Clin Chem.* 2005; 51:661–671. [PubMed: 15650028]
19. Kan C-W, Fredlake CP, Doherty EAS, Barron AE. *Electrophoresis.* 2004; 25:3564–3588. [PubMed: 15565709]
20. <http://www.illumina.com/pages.ilmn?ID=203>
21. <http://www.454.com/products-solutions/how-it-works/sequencing-chemistry.asp>
22. El-Difrawy SA, Lam R, Aborn JH, Novotny M, Gismondi EA, Matsudaira P, McKenna BK, O'Neil T, Streechon P, Ehrlich DJ. *Rev Sci Instrum.* 2005; 76(7):074301.
23. <http://www.shimadzu-biotech.jp/products/denova/index.html>
24. Kumagai H, Utsunomiya S, Nakamura S, Yamamoto R, Harada A, Kaji T, Hazama M, Ohashi T, Inami A, Ikegami T, Miyamoto K, Endo N, Yoshimi K, Toyoda A, Hattori M, Sakaki Y. *Electrophoresis.* 2008; 29:4723–4732. [PubMed: 19016243]
25. Kuo WP, et al. *Nat Biotechnol.* 2006; 24:832–836. [PubMed: 16823376]

Figure 1.

The geometry for typical double-T injection of DNA into a matrix-filled microdevice channel. The vertical separation channel (cathode to anode) and horizontal loading channel (sample-well to waste-well) are typically pre-conditioned with a separate currents in low-conductivity buffer to reduce matrix conductivity. Hence the two channels can be manipulated to have conductivity interfaces useful for sample stacking. The two most important dielectric interfaces are labeled.

Figure 2.

Solid-Support Loading. Left to right: (i) Functionalized paramagnetic beads are added to scavenge analyte out of solution. (ii) Beads can be immobilized to the vessel wall for wash steps. (iii) The beads are collected by magnetizing an electrode (permanent or electromagnet) then, (iv) transported on the magnetized electrode to the microdevice where the analyte is released, typically using a voltage pulse in water. The method permits purification and concentration of the analyte and circumvents the small-volume limits of liquid-handling pipettors.

Figure 3. Injection peaks for ssDNA into LPA channels, 4 different injection voltages using the Solid-Support loading method. Detection by laser-induced fluorescence 4 mm down stream from Interface 1 (Fig. 1).

Figure 4.

Reaction Mapping is used for scale up of highly multiplexed qPCR. The initial step is to design primers that spread their amplicons over the four-color “gel” readout, then, 1(a)–1(c) to characterize the interacting PCR multiplex using a high-dynamic-range detector. The synthesized data from several cycle numbers of amplification constructs a “Reaction Map” which is a form of standard curve. The Reaction Map is an empirical model and does not make any assumptions on the multiplex reactions. The scale-up assay, step 2(a)/2(b), becomes very efficient and can read more than 20 genes per well over a high dynamic range in gene abundance.

Figure 5.

Reaction map data from a 7plex PCR with templates ranging from 5 to 5×10^6 copies. Primers were taken from the forensics kit Powerplex 1.2. Samples of the 7plex PCR were taken at 10, 20, 30, 40, and 60 cycles, respectively, purified and analyzed electrophoretically. Signals of the 540-nm and the 580-nm channel for the same cycle number are superposed. Loci names and amplicon lengths (in bp) are given in the corresponding color at the bottom of the picture. The relatively prominent peaks 4 bp shorter than the desired amplicons are stutter peaks caused by enzyme errors. They do not hamper data analysis. (See Ref. 22 for details on sample preparation and electrophoresis.)

Figure 6.

Detection of a mix of 10.0 ng/μl K562 genomic DNA and 0.03 ng/μl 12244 genomic DNA using the Reaction Mapping method. Shown are signal intensities for the D13S317 locus (blue channel) corresponding to 173 bp (K562) and 188 bp (12244) peaks. PCRs were run with the Promega Powerplex 1.2 kit. Signals were normalized to the 180 bp ladder peak.

Figure 7. Comparison of microchip detection (green channel) to a real-time qPCR (dots: microdevice. fluorescence intensity for HSPH1 in a 12plex PCR at different cycle numbers); (squares: ABI prism) Template amount per reaction was identical. Note the lower threshold cycle using the microfluidic device.

Fig. 8.

Electrophoresis data collected for a study of expression of 12 genes monitored in human neutrophil cells, with and without stimulation by the yeast pathogen, *C. albicans*. Primer pairs were designed for every gene, followed by single-well amplification, carried out as 12plex PCRs. One map is constructed using total RNA isolated from neutrophils without *C. albicans* (“neutrophils”), and a second map with total RNA taken from neutrophils cultured in the presence of *C. albicans* (“neutrophils + yeast”). Gene annotations, amplicon lengths and color of the fluorescent labels are given in the 12 amplification curve titles.

Table 1

Full width at half maximum (FWHM) and integrated peak area for different injection voltages into an electrophoretic microchip using the Solid-Support DNA loading method. Measured peak areas range from 4.2 to 6.8 RFU·s. The cause for the low injected amplitude for the 15.0 kV pulse is unknown. Optimum injection (smallest FWHM and the highest peak area) is at 2.3 kV.

	Injection voltage			
	0.1 kV	2.3 kV	15.0 kV	30.0 kV
FWHM (s)	1.8	1.6	1.9	1.6
Peak area (RFU·s)	4.2	6.8	1.6	5.8