

Free Solution Hydrodynamic Separation of DNA Fragments from 75 to 106 000 Base Pairs in A Single Run

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We report here a new technique to separate a wide size range of DNA fragments in a single run without using polymer gels.

Analyzing the relative length of DNA fragments is fundamental in the field of molecular biology. For the majority of research laboratories, gel electrophoresis [including pulsed field gel electrophoresis (PFGE)¹] is utilized to separate DNA molecules based on relative size. However, gels can be difficult to work with, especially when a narrow capillary or microchannel electrophoresis is utilized to increase the throughput. Noolandi first proposed a free solution DNA separation approach² by tagging DNA molecules covalently with a charge-neutral monosized entity or a “drag-tag” to render the free solution electrophoretic mobilities of the tagged fragments dependent on the fragment size, and Heller et al. were the first to have experimentally demonstrated this concept.³ While promising results were obtained,⁴ intrinsically the resolution must decrease as the fragment size increases. Micro- or nanofabricated size-sorting structures have also been proposed for nongel DNA separations,⁵ but resolving power is limited. Chromatographic methods for DNA separations⁶ generally cannot compete with the resolving power of gel electrophoresis.

Recently we have observed that unusually efficient separation of proteins and DNA molecules is possible in very narrow bore open capillaries.⁷ In this communication, we separate a wide size range of DNA fragments in an uncoated open tube, 1–5 μm in inner radius (r_i). We first intercalate a fluorescent dye (YOYO, Molecular Probes, Eugene, OR) with double-stranded DNA. We then fill the capillary with a buffer solution (10 mM Tris-HCl, 1 mM- Na_2EDTA , pH 8.0), hydrodynamically inject the sample into the capillary, use pneumatic pressure between 20 to 500 psi for elution, and detect the separated DNA using an on-column laser-induced fluorescence detector. Experimental conditions and apparatus are detailed in the Supporting Information (SI).

Figure 1A presents three typical chromatograms of such separations. The DNA sample is synthesized by mixing a GeneRuler 1 kb DNA ladder plus (Fermentas Life Sciences Inc., Glen Burnie, MD), a lambda DNA mono cut mix (N3019L, New England Biolabs Inc., Beverly, MA), and a 105 968 kbp linearized bacterial artificial chromosome (BAC) DNA prepared from the *Arabidopsis* BAC clone T6H20. As can be seen from chromatogram (II), all fragments between 75 and 105 968 bp are well resolved in a capillary with $r_i = 2.5 \mu\text{m}$. It is also noticeable that the 5 μm r_i capillary is more effective than the 1 μm r_i capillary for separations of DNA fragments >20 kbp, while the reverse is true for separations of DNA fragments <5 kbp. For a capillary with the same diameter, increasing its length will improve the resolutions of DNA of all

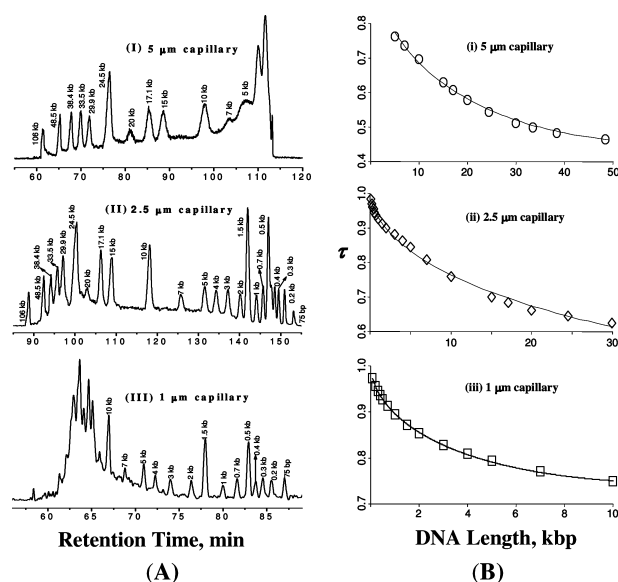


Figure 1. Typical DNA fragment separations in microcapillaries. (A) Chromatograms. (B) Fits to eq 3. Sample: mixture of 25 ng/ μL linearized T6H20 BAC DNA, 25 ng/ μL lambda DNA mono cut mix, and 25 ng/ μL GeneRuler 1 kb plus DNA ladder. (I) 5 μm r_i and 15 m long (14.95 m effective length, L_{eff}) at 350 psi, (II) 2.5 μm r_i and 445 cm long (440 cm L_{eff}) at 360 psi, and (III) 1 μm r_i and 75 cm long (70 cm L_{eff}) at 75 psi. Injection 10 s @ 100 psi, eluent 10 mM Tris-1 mM EDTA (pH 8.0).

sizes. This provides a guide for matching the capillary bore to the range of interest.

The separation mechanism appears to follow the simple linear model⁸ of hydrodynamic chromatography. In this model, the relative retention (τ) of a particle, defined as the retention time of a particle (t) divided by the retention time of an unretained solute (t_0), is estimated from

$$\tau = t/t_0 = [(a - r)/a]^2 \quad (1)$$

where a and r are the radii of the capillary and the particle. For a polymer molecule, its radius (or effective radius) can be evaluated according to its molecular weight,⁹

$$r = c(M_w)^{0.567} \quad (2)$$

where c is a constant. For a polynucleotide, the molecular weight can be further replaced by the number of bases in the molecule (L). The relative retention of a DNA fragment can thus be approximated by,

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$$\tau = t/t_0 = 1 - k \cdot L^{0.567} + k' \cdot L^{1.134} \quad (3)$$

where k and k' are constants; t_0 can also be fit, rather than experimentally determined. Figure 1B shows excellent matches between the experimental data and the fitted lines using eq 3. The excellent correlation coefficients (r^2 values of 1.000, 0.996, and 0.996, respectively, for separations in $r_i=1$, 2.5, and 5 μm capillaries) suggest that the separation mechanism is indeed akin to hydrodynamic chromatography.

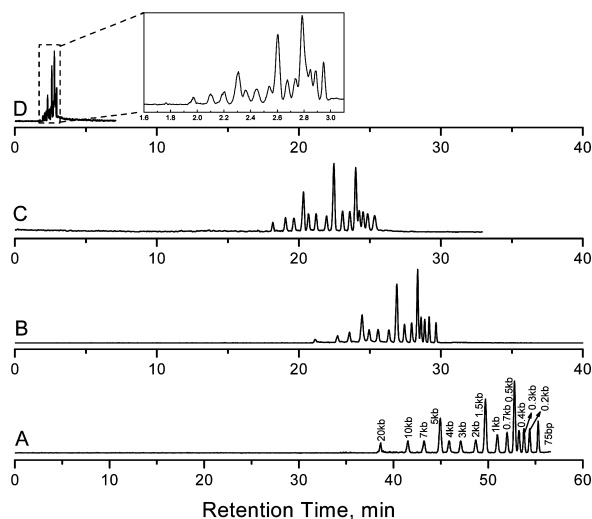


Figure 2. Effect of experimental parameters on separation speed. Sample: 50 ng/ μL GeneRuler 1 kb plus DNA ladder. (A) $r_i=1$ μm and 70 cm long capillary, 100 psi elution pressure (ΔP), 23 $^{\circ}\text{C}$; (B) same as A, 80 $^{\circ}\text{C}$; (C) same as A, 15 cm long capillary, $\Delta P = 20$ psi; (D) same as C, $\Delta P = 100$ psi.

The initial results in Figure 2 show that the separation speed can be increased by (a) reducing the capillary length, (b) increasing the elution pressure, and (c) elevating the separation temperature. As can be expected, the separation speed is directly proportional to the elution pressure (Figure 2C vs 2D) and inversely proportional to the length of the capillary (Figure 2A vs 2C). In Figure 2D, the separation of 75 to 20 000 bp fragments is completed in less than 3 min with the entire elution window being ~ 1 min. Interestingly, while an increase in ΔP or a reduction of column length can increase speed at the expense of resolution, faster separations are achievable at higher temperatures with little loss of resolution (Figure 2A vs 2B).

The ultimate test of a DNA separation system is the ability to collect fractions that can then be further analyzed or amplified. Figure 3 demonstrates that specific DNA fragments can be collected and amplified by polymerase chain reaction (PCR). The chromatogram in Figure 3C shows a mixture of the GeneRuler 1 kb DNA ladder plus and the two DNA fragments of 1100 and 625 bp. The two DNA fragments were originally PCR amplified from *Arabidopsis* genomic DNA. Chromatograms in Figure 3A and 3B present the DNA fragments that were collected and reamplified after separation.

In conclusion, we have demonstrated an uncoated microcapillary for separations of a wide range of DNA fragments in a single run in free solution by hydrodynamic rather than electrophoretic means. The capability of resolving a wide range of DNA fragments makes this technique suitable for important DNA assay and manipulation techniques, e.g., fingerprinting for the assembly of BAC contigs for genomic DNA sequencing. Since no gels or conduit wall

coatings are needed, it is readily implemented in the chip scale for high-speed and high-throughput DNA analysis without requiring a high voltage source.

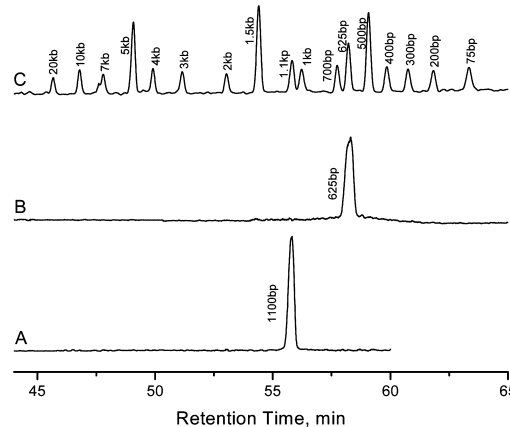


Figure 3. DNA collection and PCR amplification after nanocapillary separation. Chromatograms were obtained with an $r_i=0.5$ μm capillary with an effective length of 45 cm, $\Delta P = 100$ psi. Traces A and B respectively show the 1100 and 625 bp fragments after collection and PCR amplification. Trace C displays the result of the mixture of these fragments and 25 ng/ μL GeneRuler 1-kb plus DNA ladder. Injection 3 s @ 90 psi (A and B) and 20 s @ 90 psi (C).

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Supporting Information Available: Sample preparation and experimental methods. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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