Separation of Long DNA Molecules in a Microfabricated Entropic Trap Array

J. Han and H. G. Craighead*

A nanofluidic channel device, consisting of many entropic traps, was designed and fabricated for the separation of long DNA molecules. The channel comprises narrow constrictions and wider regions that cause size-dependent trapping of DNA at the onset of a constriction. This process creates electrophoretic mobility differences, thus enabling efficient separation without the use of a gel matrix or pulsed electric fields. Samples of long DNA molecules (5000 to ~160,000 base pairs) were efficiently separated into bands in 15-millimeter-long channels. Multiple-channel devices operating in parallel were demonstrated. The efficiency, compactness, and ease of fabrication of the device suggest the possibility of more practical integrated DNA analysis systems.

Gel electrophoresis is the standard method for separation of DNA by length. The efficiency of gel electrophoresis deteriorates seriously, however, for DNA molecules longer than about 40,000 base pairs (40 kbp). Slab gel pulsed-field gel electrophoresis (PFGE), using time-varying drive voltages, can be used to separate longer double-stranded DNA (dsDNA) fragments, but generally the process is slow, and recovery of separated DNA from gel is complex. Efficient separation has been reported with pulsed-field capillary gel electrophoresis (PFCGE) (*1–3*). However, only one sample could be run at a time in PFCGE, and so multiple capillary systems would be required for large-scale genome sequencing or DNA fingerprinting (*4*). Moreover, with respect to future integrated bioanalysis systems (*5, 6*)—the so-called micro total analysis systems (μ-TAS)—it could be cumbersome to introduce a foreign sieving matrix into the channel of a highly integrated device.

A variety of microfabricated systems (*7–12*) have been studied for separation of dsDNA. However, early artificial gel systems (*8, 10*) with arrays of pillars showed poor dc electrophoretic separation for long DNA molecules, and the use of pulsed electric fields was required (*9*). More recently, a single-molecule DNA sizing device (*11*) and a diffusion sorting array (*12*) were reported. Despite the advantages of these new systems, it is still unclear how these systems might be incorporated into established bioanalysis protocols.

Recently we introduced an entropic trap array system with lithographically defined constrictions comparable to molecular dimensions; this system can be used with static (dc) electric fields to rapidly separate large DNA fragments (*13*). A spatially varying but static electric field and an array of constrictions act as size-dependent traps for DNA motion. Separation matrices such as gels or polymer solutions are not used, and the methods used to fabricate this array are compatible with silicon-based processing. Therefore, this device could easily be integrated into a larger total analysis system. The basic theory of operation of the device has been described (*14*). Here, we demonstrate one application of the entropic trap array device by using it in a way similar to conventional slab gel PFGE methods.

The basic design of the entropic trap array (Fig. 1A) consists of alternating thin and thick regions in a microfabricated channel. The channel depth of the thin region is smaller than the radius of gyration (R_o) of DNA molecules being separated, and thus it serves as a molecular sieve. In the thick region, DNA molecules can form spherical equilibrium shapes because the thickness here is larger than R_o, whereas in the thin region DNA molecules are deformed. When driven by an electric field, DNA molecules travel through alternating thick and thin regions and repeatedly change their conformation. This conformation change costs entropic free energy, so DNA molecules are temporarily trapped at the entrance of the thin regions. The entropic trapping limits the overall mobility of DNA molecules in the channel, and the mobility of DNA becomes length-dependent. Interestingly, longer DNA molecules actually have higher mobility in this channel (*13*). In the escape of a DNA molecule from an entropic trap, only the part of the molecule that is in contact with the boundary of the thin region plays a crucial role. Whenever a sufficient number of DNA monomers are introduced into the high-field thin region (by Brownian motion), the escape of the whole molecule is initiated (*14*). Longer DNA molecules, with larger R_o, have a larger surface area in contact with the boundary and therefore have a higher probability to escape per unit time (due to a higher escape attempt frequency), which leads to a shorter trapping time and a higher overall mobility (Fig. 1B).

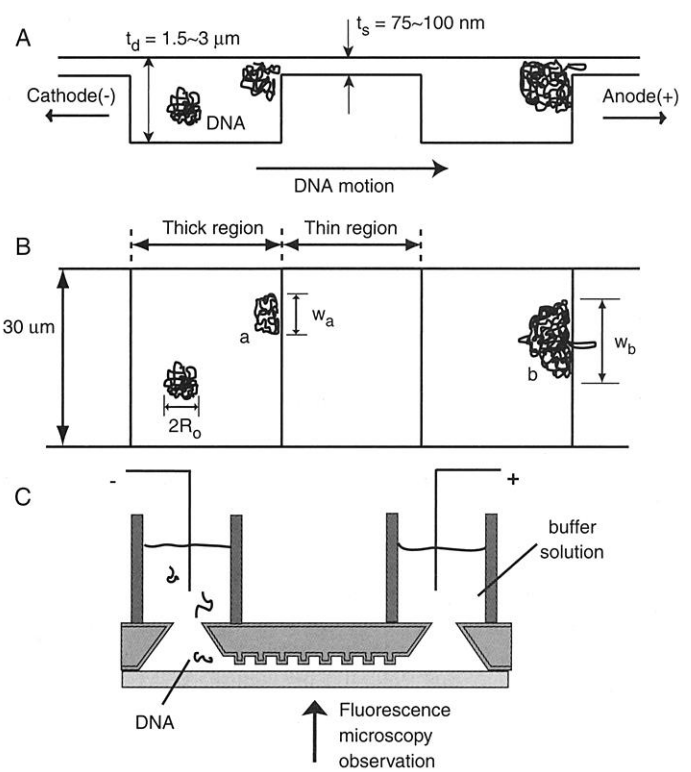

Fig. 1. Nanofluidic separation device with many entropic traps. **(A)** Cross-sectional schematic diagram of the device. Electrophoresed DNA molecules are trapped whenever they meet a thin region, because their radius of gyration (R_o) is much larger than the thin region depth (here, t_d and t_s are the thick and thin region depths, respectively). **(B)** Top view of the device in operation. Trapped DNA molecules eventually escape, with a probability of escape proportional to the length of the slit that the DNA molecule covers (w_a and w_b). Larger molecules have a higher escape probability because they cover wider regions of the slit ($w_b > w_a$). **(C)** Experimental setup. Reservoirs are made at both ends of the channel and filled with DNA solution.

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The channels were fabricated using photolithography and etching techniques on a Si substrate (*15*). Fluorescently labeled DNA solutions (*16*) were loaded into the reservoirs, then introduced into the channel by electrophoresis (Fig. 1C). The motion of individual DNA molecules as well as DNA bands were observed with optical microscopy (*17*). Several different dsDNA samples, ranging from 5 to 164 kbp, were separated and analyzed in this experiment.

A useful separation device must have an effective sample collection and launching system as well as a good separation mechanism. The entropic trap system could be used as an effective DNA focusing and launching system (Fig. 2). Band launching can be easily achieved in this device by controlling the electric field. At very low electric fields, DNA molecules are trapped indefinitely. In this condition DNA molecules from the solution enter the device and pile up at the first entropic barrier of the channel, forming a high-density band (Fig. 2A). The amount of DNA in a band was controlled by changing the collection time. The typical width of the initial DNA band was 10 to 50 μm. After collection, the band was launched by suddenly increasing the applied voltage to a higher value. DNA molecules escaped the first barrier and traveled through the channel as a narrow and well-defined band (Fig. 2B). As they migrated, DNA molecules with different lengths eluted as individual bands because of the mobility differences caused by the size-dependent entropic trapping. DNA molecules were allowed to travel the whole length of the channel, and the arrival of DNA molecules at the other end of the channel was recorded as video data (Fig. 2C). The band separation of different DNA molecules was analyzed by measuring the averaged fluorescence intensity of a region at the end of the channel (*18*). Individual DNA molecules, as well as their size and conformation, could be resolved in the video, which provided additional information for peak assignments.

We separated a mixture of T2 (164 kbp) and T7 (37.9 kbp) DNA, which formed separate bands (Fig. 3). Video data verified that the first band is composed of larger T2 DNA and the second band is composed of smaller T7 DNA. In this run, the separation took about 15 min, and thus was about two orders of magnitude faster than conventional slab gel PFGE (typically 12 to 24 hours). As the electric field was increased, the entropic trapping effect became negligible (*13, 14*) and separation was not achieved (28 V/cm, Fig. 3).

The efficiency of a molecular separation system is usually characterized by the theoretical plate number N (*19*). Typical N values for this channel are 10^3 to 10^4 (10^6 to 10^7 plates/m), depending on the electric field. The N values at 24.5 V/cm are larger than at 21.0 V/cm, which means that peak dispersion is increased at lower voltages (*20*). However, the separation resolution between two peaks at 21.0 V/cm is better than at 24.5 V/cm (1.95 versus 0.89). The resolution of separation is given as $R_s = (\Delta V/V)\sqrt{N/16}$, where $\Delta V/V$ is the fractional band velocity difference between two peaks. In our case, $\Delta V/V$ is greatly increased with a lower electric field because entropic trapping effects dominate the DNA migration at lower electric fields and enhance mobility differences between DNA lengths. The better separation resolution at lower electric fields is the result of increased selectivity (represented by $\Delta V/V$), which wins out over the additional band broadening (represented by N) caused by increased trapping. There is also a trade-off between the resolution and the speed of the separation; with a lower electric field, more highly resolved separation can be obtained but the speed of separation is drastically decreased.

In slab gel electrophoresis, the presence of too many or too few DNA molecules can cause problems. We varied the amount of DNA in a launched band by changing the collection time of the DNA at the same collection voltage. Higher concentrations of DNA in a band increased the overall band mobility, but this shift was reasonably small (about 5 to 7% for an approximate fivefold concentration change). In addition, the concentration change did not seem to affect the separation capability (*21*), although higher concentration resulted in slightly wider bands in the final electrophoregram.

In many DNA analyses, multiple samples are electrophoresed simultaneously so that bands from an unknown sample can be compared to the bands from a well-known sample (DNA ladder). This strategy is effective in calibrating individual electrophoresis runs. To realize parallel sample analysis, we designed and fabricated a device that has two separate lanes with the same sieving structure (Fig. 4A), in which two samples could be loaded independently and then collected and launched at the same time. The central collection chamber is

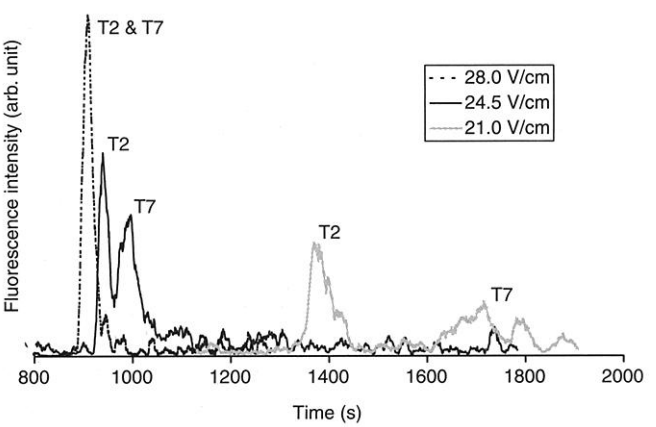

Fig. 3. Electric field dependence of the separation. T2-T7 DNA mixture is separated through a channel with 90-nm thin regions, 650-nm thick regions, and 4-μm channel period. At 21.0 V/cm (gray line), the theoretical plate number (N) was 4900 for the T2 peak and 970 for the T7 peak, and the resolution was 1.95. At 24.5 V/cm (black line), N = 8500 for the T2 peak and 3400 for the T7 peak, and the resolution was 0.89. At 28.0 V/cm, no separation was achieved (broken line).

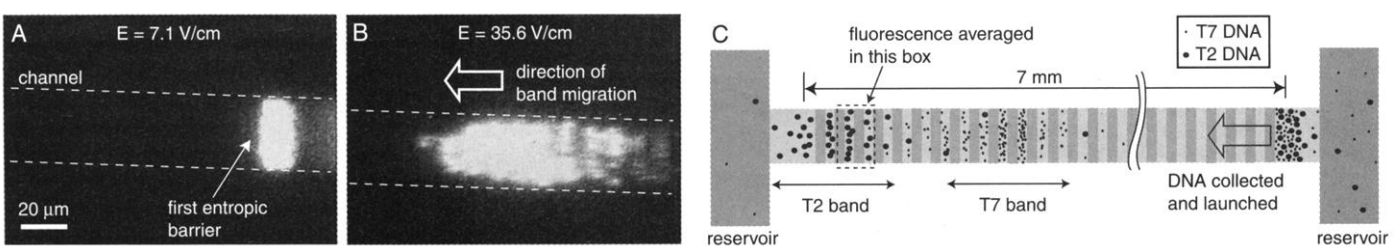

Fig. 2. Band launching and analysis of bands. The channel has 90-nm thin regions and 2.7-μm thick regions. The period of the channel structure is 10 μm. The device was filled with buffer solution. The connected reservoir had a DNA concentration of 0.3 μg/ml. (**A**) Collection of T2-T7 DNA mixture at the first entropic barrier. DNA molecules were collected by applying 7.1 V/cm from the DNA solution reservoir. (**B**) Migrating DNA band immediately after being launched by 35.6 V/cm. (**C**) Schematic diagram for launching and data analysis. The arrival of DNA molecules was monitored at the other end of the channel. A rectangular region of interest was defined, and the fluorescence signal from this area was summed.

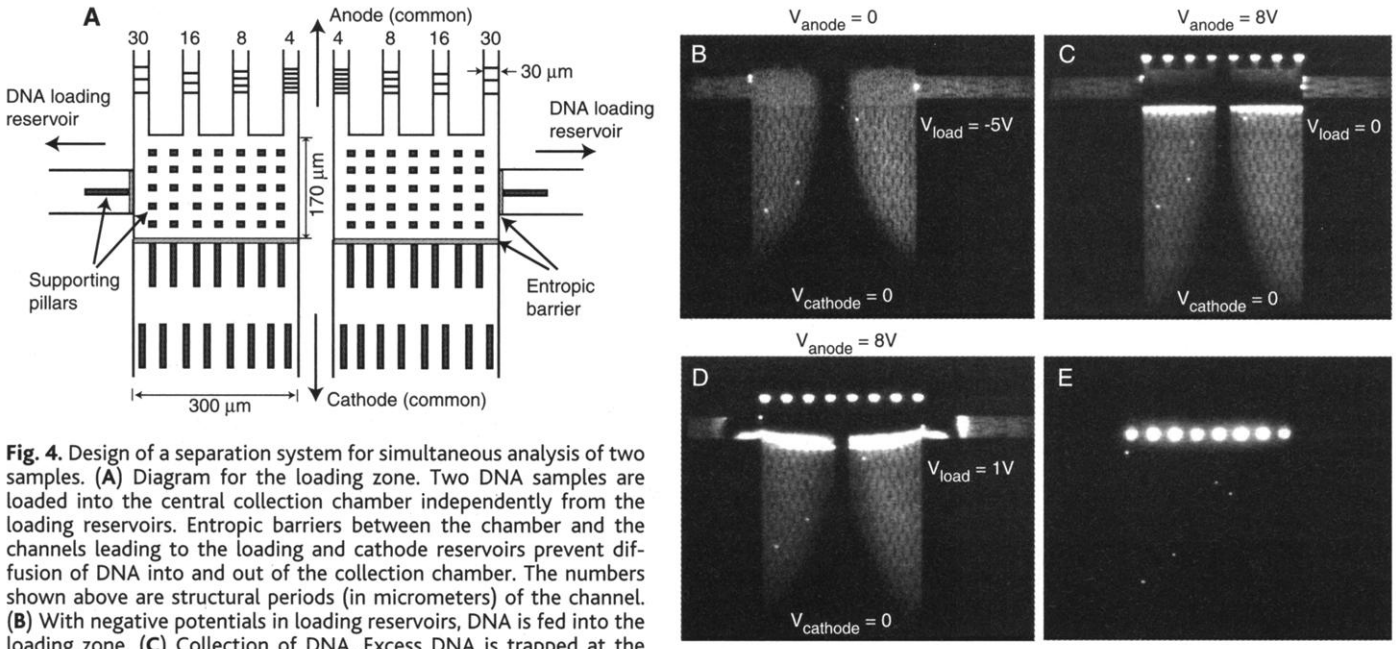

Fig. 4. Design of a separation system for simultaneous analysis of two samples. (**A**) Diagram for the loading zone. Two DNA samples are loaded into the central collection chamber independently from the loading reservoirs. Entropic barriers between the chamber and the channels leading to the loading and cathode reservoirs prevent diffusion of DNA into and out of the collection chamber. The numbers shown above are structural periods (in micrometers) of the channel. (**B**) With negative potentials in loading reservoirs, DNA is fed into the loading zone. (**C**) Collection of DNA. Excess DNA is trapped at the entropic barrier, and only DNA in the collection chamber is driven to the launching line. (**D**) With positive potential in the loading reservoir, excess DNA that is trapped is flushed back into the loading reservoir. (**E**) DNA collected at the first entropic trap of each channel, ready for launching.

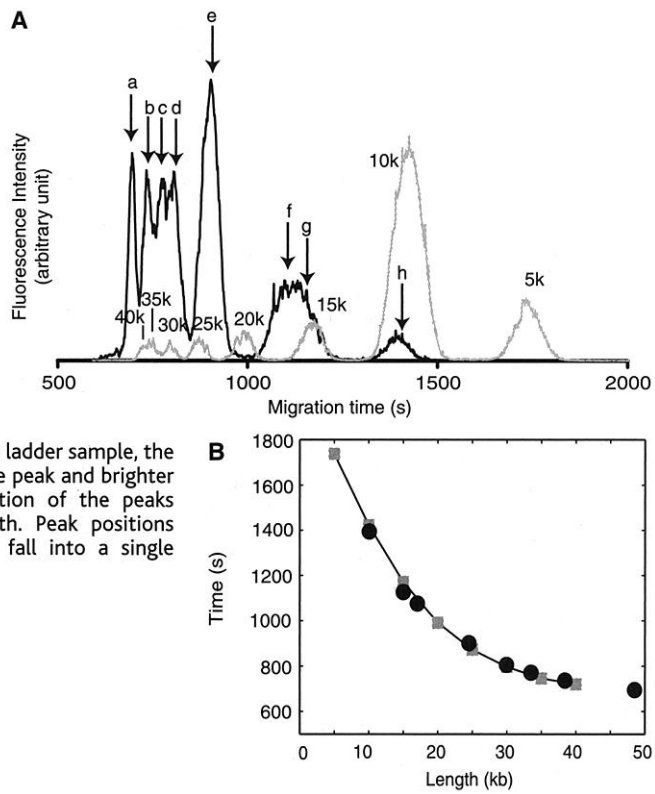

Fig. 5. (**A**) Simultaneous separation of the Mono Cut Mix sample (black line) and 5-kbp ladder sample (gray line) by the entropic trap array, run at 80 V/cm. The channel has 75-nm thin regions and 1.8-μm thick regions; the channel period is 4 μm and the length is 15 mm. Peak assignment for the Mono Cut Mix sample: (a) 48,502 bp, (b) 38,416 bp, (c) 33,498 bp, (d) 29,946 bp, (e) 24,508/23,994 bp (reference band), (f) 17,053 bp, (g) 15,004 bp, (h) 10,086 bp. For the 5-kbp ladder sample, the 10-kbp peak is the reference peak and brighter than the others. (**B**) Location of the peaks plotted against DNA length. Peak positions from both samples nicely fall into a single curve.

separated by entropic barriers to prevent diffusion of DNA from the loading channel to the collection chamber. Also, these entropic barriers provide a means to control exactly the amount of DNA being launched. The loading and collection operation is shown in Fig. 4. First, a DNA solution with known concentration is driven into the central collection chamber and toward the cathode reservoir (Fig. 4B). Then, DNA molecules in the collection chamber are driven into the separation channels by a low electric field across the channel (Fig. 4C). The existence of the entropic barrier, isolating the collection chamber from other regions, ensures that only DNA in the central chamber is collected. After all the DNA molecules in the collection chamber are collected, the DNA behind the collection chamber is drained to the loading channel (Fig. 4D). Finally, all the DNA molecules behind the launching bands are cleared out, leaving isolated, highly concentrated bands with a known amount of DNA. This process can be repeated as desired to yield even higher concentrations of DNA.

Using this device, we analyzed two different DNA samples simultaneously and compared the electrophoregrams from each lane. The separation result in our device of two standard DNA ladder systems for PFGE (*22*) shows distinct bands of DNA in both samples (Fig. 5A), and the two electrophoregrams compare well with each other (Fig. 5B). One peak from each sample is brighter than others for referencing purposes, which facilitated the peak assignment in our data. In these data the shortest 1.5-kbp DNA band is missing, probably because of the low gain of the intensified charge-coupled device (ICCD) camera (*23*). In this run, all of the peaks were eluted from the separation channel within about 30 min. The resolution of the 5- to 20-kbp range is better than that of the 20- to 48-kbp range (*24*).

A conventional slab gel PFGE separation of the same DNA, obtained after about an 11-hour run, showed resolution similar to our data. Thus, the separation speed of our system is an order of magnitude faster than that of slab gel PFGE. The resolution and speed of our device are comparable to those in recent dc capillary electrophoresis experiments (*25*). However, our device is only 1.5 cm long, and it is possible to achieve better separation through longer channels. Electrophoregrams

taken at three different points along the channel show that the separation resolution is improved as DNA bands migrate along the channel (*21*).

The separation capability of the device was retained even at higher average electric fields. Separation of peaks could still be observed even at electric fields as high as 128 V/cm (*26*). This value is much higher than typical running electric fields in PFGE (1 to 10 V/cm). The use of such a high electric field, as well as the use of a dc field (no pulsing as in PFGE), greatly enhances the speed of the separation in our device because the channel structure ensures that DNA molecules relax to equilibrium size before they meet another constriction. In PFGE, the relaxation of DNA is achieved by pulsing the electric field.

The above results demonstrate the potential of this device as an efficient separation system for long DNA molecules. In addition to more efficient separation, this nanofluidic channel device can separate and analyze small amounts of DNA. The recovery of DNA molecules after separation is straightforward. The device can be modified to separate smaller or larger DNA molecules as well as various proteins and other polymers. The separation mechanism we have used will be valid for molecules with a radius of gyration larger than the gap width. Several separation channels, optimized for different length ranges of DNA, can be integrated in parallel for single-run sorting and analysis of various DNA samples. The fabrication method does not require high-resolution lithography techniques. There should be no serious technical barrier in making much thicker or thinner constrictions required for application of the device to a wider range of DNA sizes (*27*). The simplicity of the device will make theoretical modeling easier for further improvement, ultimately enabling its mass production and integration into a future μ-TAS.

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15. First, a thin channel was etched into the substrate, then an additional lithography process defined the thick regions in the channel. Fabrication does not require high-resolution lithography techniques, as the fine dimension is controlled by an etch depth. Loading holes were made by potassium hydroxide (KOH) wet etching, and the device was sealed by anodic bonding to a thin glass plate. The same device could also be made using more sophisticated methods (*10*). Small reservoirs were made at both ends of the channel and the device was filled with buffer solution.
16. DNA used in this work was purchased from Sigma, New England Biolabs, and Gibco BRL. DNA was labeled with YOYO-1 dye (Molecular Probes) at a dye/base pair ratio of 1:10. As a buffer solution, tris-borate-EDTA (TBE) buffer at 5× concentration was used. This high concentration of the buffer effectively quenched the electro-osmosis of the channel without using any other surface modification agents.
17. An inverted microscope (Olympus IX-70) with fluorescence filter set (XF-100, Omega Optical Inc.) was used to detect the fluorescence signal from dyed DNA. Microscope images were recorded by an ICCD camera (ICCD-350F, Videoscope Intl.) into video format.
18. To get an electrophoregram, we electronically defined a region of interest (typically 50 to 150 μm wide) at the end of the channel and summed the fluorescence signal from that area every 0.5 to 1 s. This analysis was done with a video image processor (DVP-32, Instrutech Co.).
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20. The main source of dispersion is not the diffusion of the molecules, but the statistical variation of the trapping time. The escape of DNA from a trap follows the same statistics as a radioactive decay. The standard deviation of trapping lifetime also increases with the average trapping lifetime. This increases the band dispersion at low fields. Diffusion of DNA in the channel is virtually blocked by the existence of the thin regions. In previous single-molecule experiments (*13*), we could not observe any diffusion across the thin region barrier in the absence of an applied field.
21. Additional data are available at www.sciencemag.org/feature/data/1046112.shl.
22. The DNA ladder samples used in the experiment were the Mono Cut Mix (New England Biolabs) and 5-kb ladder (Gibco BRL). The Mono Cut Mix sample contains DNA molecules ranging from 1.5 to 48 kbp; the 5-kb ladder contains 5-kbp DNA molecules and multiples up to 40 kbp. The concentration (6.38 μg/ml) and the dye/base pair ratio (1:10) of both DNA solutions were the same.
23. In other data with higher ICCD gain settings, the 1.5-kbp peak was identified as a tiny peak in the electrophoregram. In such a case, however, the ICCD was saturated by brighter peaks, yielding a poor electrophoregram in the 20- to 48-kbp region. This is due to the limited dynamic range of the ICCD camera. Other detectors would be used in a real separation device as opposed to a research system.
24. An increase in the thin region depth may bring about better separation with even longer DNA molecules. The thin region depth corresponds to the average pore size of a gel, which in a conventional gel can be controlled only by changing the concentration of the gel. However, in our system, one can reliably define the thin region depth to a high accuracy in the fabrication process.
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26. Smaller DNA can be separated at a higher electric field because of its shorter relaxation time.
27. The fabrication method used here is expected to work for channels less than 50 nm thick.

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Structure of the Hydrated α-Al_2O_3 (0001) Surface

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The physical and chemical properties of the hydrated α-Al_2O_3 (0001) surface are important for understanding the reactivity of natural and synthetic aluminum-containing oxides. The structure of this surface was determined in the presence of water vapor at 300 kelvin by crystal truncation rod diffraction at a third-generation synchrotron x-ray source. The fully hydrated surface is oxygen terminated, with a 53% contracted double Al layer directly below. The structure is an intermediate between α-Al_2O_3 and γ-$Al(OH)_3$, a fully hydroxylated form of alumina. A semiordered oxygen layer about 2.3 angstroms above the terminal oxygen layer is interpreted as adsorbed water. The clean α-Al_2O_3 (0001) surface, in contrast, is Al terminated and significantly relaxed relative to the bulk structure. These differences explain the different reactivities of the clean and hydroxylated surfaces.

The interaction of water with solid surfaces plays an important role in many natural and technological processes, from mineral dissolution and adsorption/desorption reactions and the reaction of water with sulfate aerosol particles in the troposphere to corrosion of metals and the cleaning of semiconductor surfaces (*1*). Many previous studies of solid-water interfacial reactions have assumed that the solid surface is a perfect termination of the bulk structure, unmodified by hydration, but this assumption is almost certainly not true, even after short exposures to a humid atmosphere (*1–4*). This is important because the reactivity of metal oxide surfaces is strongly influenced by the degree of surface hydroxylation.