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Investigating DNA Migration in Pulsed Fields Using a Miniaturized Field Inversion Gel Electrophoresis System

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Abstract

Pulsed field gel electrophoresis (PFGE) is a well-established technique for fractionation of DNA fragments ranging from kilobases to megabases in length. But many of these separations require an undesirable combination of long experiment times (often approaching tens of hours) and application of high voltages (often approaching tens of kV). Here we present a simple miniaturized field inversion gel electrophoresis (FIGE) apparatus capable of separating DNA fragments up to 32.5 kb in length within 3 hours using a modest applied potential of 20 V. The device is small enough to be imaged under a fluorescence microscope, permitting the migrating DNA bands to be observed during the course of the separation run. We use this capability to investigate how separation performance is affected by parameters including the ratio of forward and backward voltage, pulse time, and temperature. We also characterize the dependence of DNA mobility on fragment size N , and observe a scaling in the vicinity of $N^{-0.5}$ over the size range investigated. The high speed, low power consumption, and simple design of this system may help enable future studies of DNA migration in PFGE to be performed quickly and inexpensively.

Keywords

pulsed field gel electrophoresis; field inversion gel electrophoresis; DNA

As DNA fragment lengths approach tens of kilobases, a point is reached where individual molecules become much larger than the average pore size of the gel matrix. In order to sustain electrophoretic migration in this regime, the DNA molecules must adopt extended conformations preferentially aligned in the electric field direction. But this mode of migration is accompanied by a drastic reduction (and ultimately saturation) in the size dependence of electrophoretic mobility, making separation of long DNA fragments under continuous electric fields extremely challenging if not impossible. One way to overcome these limitations involves introducing a dynamic reorientation process by periodically changing the direction and magnitude of the applied electric field. When the electric field direction is switched, smaller fragments are able to reorient more quickly than larger molecules and thus migrate faster. The size dependent nature of this reorientation process restores the ability to distinguish different sized fragments based on their electrophoretic mobility. These *pulsed field gel electrophoresis* (PFGE) techniques have greatly extended the range of DNA fragment sizes that can be separated, even up to tens of Mb [1,2].

PFGE methods are important in a variety of applications including analysis of microbial genomes [3–5], large-scale restriction mapping and analysis of chromosomes [6], detection

of chromosome breaks, and purification of genomic DNA by extracting fractionated bands after electrophoresis. PFGE can also be used to identify different DNA topological forms (e.g., linear, open circular, supercoiled) [7–11]. But despite the instrumental role of PFGE techniques in broadening the range of DNA fragment sizes that can be effectively separated, some important drawbacks remain. First, PFGE can be extremely time consuming, with run times generally ranging in the tens of hours. A major reason for this is that the extent to which the electric field can be increased to drive faster DNA migration is limited by the onset of Joule heating effects. Some progress has been reported toward performing separations at higher electric fields in both capillary [12,13] and slab gel-based instruments [14], but these methods rely on high voltage power supplies that have been custom modified to permit rapid field switching. A second drawback is that most PFGE instruments employ slab gel-based designs that rely on post-stain detection to visualize the separated bands. This not only adds to the total experiment time, but also means that the separation process cannot be observed while the run is in progress.

These limitations restrict the extent to which the details of DNA migration can be quantitatively studied in order to understand the fundamental phenomena and rationally optimize separation performance. Moreover, many commercial PFGE instruments are relatively expensive, a factor that can limit their accessibility both for routine analytical and preparative use as well as for performing fundamental studies. Thus, a need exists for a simplified experimental platform that would enable rapid PFGE separations to be performed while at the same time incorporating the ability to continuously observe the migrating DNA fragment zones during the course of the run.

We have attempted to address these needs by constructing a miniaturized instrument to perform *field inversion gel electrophoresis* (FIGE), a type of PFGE whereby the electric field direction periodically alternates by 180° between the forward and reverse direction of DNA migration. FIGE methods are attractive because the electric field remains uniform throughout the gel minimizing distortion of the shape and spacing of the separated bands, and they are also relatively straightforward to adapt for use in capillary-based formats. A caveat, however, is that electrophoretic mobility may exhibit a non-monotonic dependence on fragment length (or even mobility inversions) depending on details associated with the pulsed field profile and experiment conditions [15–18] (these anomalous effects can also be harnessed to provide enhanced separation performance under specific conditions within a particular range of fragment sizes [19–22]).

Since our miniaturized FIGE device is small enough to be placed under a fluorescence microscope, the separation process can be monitored during the course of the run. Mini-slab gels have been previously introduced for continuous field DNA separations [23–31], but have not been widely investigated for PFGE. Our apparatus (Figure 1a) consists of an outer buffer reservoir created by affixing four 8 mm-tall Plexiglas strips in a rectangular arrangement on top of a glass base plate using epoxy. Next, a set of four holes are drilled through each of two smaller 5 mm-tall strips in order to allow two 28 gauge platinum wires to be inserted into each strip and extended along its length (i.e., parallel to the bottom glass surface, approximately 2 cm long). These plastic strips are then mounted to the glass plate using epoxy and separated by a distance of 2.6 cm yielding two independently addressable pairs of horizontal electrodes oriented at 180° with respect to each other. Finally, two additional 5 mm tall strips are placed outside the electrode strips in order to create sidewalls that enclose an area for casting the agarose gel. These sidewalls are not permanently affixed to the lower glass plate so that the gel can be removed for further analysis if necessary.

We tested separation performance using a 2.5 kb dsDNA ladder containing fragment sizes ranging from 2.5 to 32.5 kb (2.5 kb Molecular Ruler; Bio-Rad Laboratories, Inc. Hercules,

CA). Samples were prepared by mixing 7 μL of the DNA ladder with 3 μL of 100x SYBR-Green fluorescent dye (Invitrogen/Molecular Probes, Carlsbad CA) and 1 μL of 6x Orange Loading Dye solution (Fermentas, Hanover MD). Electrophoresis gels were prepared by dissolving an appropriate amount of agarose powder (Certified Megabase Agarose; Bio-Rad Laboratories, Inc. Hercules, CA) in 1x Tris/Boric Acid/EDTA (TBE) buffer solution (Extended Range TBE; Bio-Rad Laboratories, Inc. Hercules, CA). Gels were then cast by pipetting a sufficient amount of molten agarose to cover the electrodes mounted on the plastic strips in the casting area (~ 2.5 mL). A homemade plastic comb with 1.6×0.5 mm teeth was then inserted to define several loading wells, and the gel was left to cure at room temperature for at least 15 minutes. The comb was then gently removed, an 0.8 μL aliquot of the DNA sample mixture was pipetted into each injection well, and 0.5x TBE running buffer solution was added to cover the gel.

Once the gel was cast and loaded, the entire device was placed on a thermally controlled stage whose temperature was regulated using a thermoelectric element. Runs were performed at 15 $^{\circ}\text{C}$. The two electrodes were connected to the two outputs of a DC power supply (Model E3646A, dual output, 0 – 20V, 1.5A; Agilent Technologies, Inc., Santa Clara CA) that was controlled using a custom program written in LabVIEW (National Instruments; Austin, TX) to enable the field direction to be periodically alternated. Detection was performed by observing the gel using an Olympus SZX-12 (Olympus America, Inc., Center Valley PA) fluorescence stereoscope (zoom with 1x objective, Hg arc lamp illumination, and GFP filter set (excitation: 460 – 490 nm band pass; emission: 510 nm long pass)) and a CCD-300 cooled CCD camera (Dage-MTI, Michigan City IN).

Since the magnitude, direction, and timescale of the applied voltage are periodically changed during the course of a separation run, it is useful to define an average electric field in terms of the difference between the forward and reverse components weighted by the time period over which each field component is applied. [32]:

$$E_{avg} = E_{fwd} \left(\frac{t_{fwd}}{t_{total}} \right) - E_{rev} \left(\frac{t_{rev}}{t_{total}} \right) = \frac{E_{fwd}t_{fwd} - E_{rev}t_{rev}}{t_{fwd} + t_{rev} + t_0} \quad (1)$$

Here, E_{fwd} and E_{rev} are the electric field magnitudes in the forward and reverse directions; t_{fwd} and t_{rev} represent the pulse time (i.e., the time during which the electric field is applied) in the forward and reverse directions; and t_0 expresses the time interval associated with switching the electric fields between the forward and reverse directions (the electric field strength is zero during this time). We used a value of $t_0 = 500$ ms to account for the 250 ms zero-field delay between each forward and reverse pulse in our setup. Thus, a positive value of E_{avg} indicates net migration in the forward direction. The relative magnitudes of forward and reverse potential are expressed in terms of a voltage ratio $\alpha = E_{fwd}/E_{rev}$.

The 250 ms delay time between pulses was an inherent attribute of our power supply's switching speed and was not readily adjustable. The possibility exists that this feature of the pulse sequence could play a role in the separation if it becomes comparable to the DNA relaxation time. Experimental studies of electrophoretically stretched DNA relaxation in agarose gels suggests that the timescale associated with the most rapid relaxation mode scales approximately as $N^{1.5}$ (where N is the DNA fragment length in base pairs), consistent with Zimm-like behavior [33,34]. But the absolute value of the relaxation time is more challenging to predict quantitatively because of sensitivity to experimental details including the concentration and composition of the gel and buffer. Kantor et. al. obtained a relationship for the relaxation time τ (s) = $7.6 \times 10^{-7} N^{1.45}$ based on fluorescence imaging of individual molecules in the 245 – 980 kb range [33], while Sturm and Weill obtained τ (s) =

$4 \times 10^{-8} N^{1.5}$ based on birefringence measurements of DNA in the 10 – 50 kb range [34]. Taking a 30 kb fragment length as representative of the upper limit of sizes in our DNA ladder, these scalings predict relaxation times of about 2.4 and 0.21 s respectively. Although these predictions differ by an order of magnitude, they serve to establish a characteristic range of values and suggest that our 250 ms delay time is comparable to the DNA relaxation time for the longest fragments in the DNA ladder.

Electrophoretic migration was characterized by analyzing digitized images recorded at regular time intervals during the course of the run in order to determine the incremental distance traveled by each migrating band. Displacements were measured in units of pixels, converted to units of distance using a calibration standard, and then used to calculate mobility $\mu = v/E_{avg}$, where v is the migration velocity (i.e., displacement $\div$ time) and E_{avg} is the average electric field strength during the pulse sequence (equation 1). Mobilities computed over several time intervals were then averaged to obtain a final value. Image analysis and calculations were performed using in-house code written in MATLAB (The Mathworks, Inc., Novi MI). Measurements were taken after 3 h of run time unless otherwise noted, and in most cases data from at least three separation runs were averaged.

The digitized images were also used to characterize the ability to distinguish adjacent migrating bands in terms of a separation resolution parameter R defined as the distance between two peaks relative to the sum of their half widths ($R = (x_2 - x_1)/(w_1 + w_2)$, where x_i is the position of band i and w_i is the half width of band i). Resolution calculations were performed using an in-house MATLAB code. Briefly, a spatially averaged intensity profile is plotted across a user-defined region spanning multiple bands and the location of the maximum intensity value of each peak is manually selected along with the minima on either side. The selected peaks are then fitted to a Gaussian distribution function using an optimization procedure, after which the standard deviation σ is calculated. The total width of each peak is defined as 2σ , yielding an expression for separation resolution of the form $R = (x_2 - x_1)/(\sigma_1 + \sigma_2)$. When plotting data, the calculated resolution between two peaks is assigned to an intermediate average fragment length (e.g., the resolution value between the 10 and 12.5 kb bands is plotted as corresponding to an intermediate fragment size of 11.25 kb at the midpoint between the two bands). We note that this peak analysis is performed on a snapshot of bands in the gel at a single point in time, as opposed to finish-line detection where data is acquired at a fixed downstream location.

Fluorescence images representative of typical separation conditions are shown in Figure 1b for runs performed under both continuous field and FIGE modes. In the continuous field separation, bands corresponding to fragments longer than about 12.5 kb experience compression and are difficult to resolve. These compression effects are an indication that the DNA fragments have entered a migration regime where they become oriented in the electric field direction, thereby losing their size dependent mobility and making it unlikely that longer running times will improve resolution. In contrast, the same bands are clearly distinguishable when the separation is performed under FIGE conditions. In both cases, bands corresponding to the shortest fragment sizes (2.5 and 5 kb) appear fainter than the others. We attribute this to a combination of the increased rate of band broadening by diffusion experienced by the smallest DNA fragments and non-uniform illumination effects as these faster moving fragments reach the edge of the region where the excitation light is brightest. The reduced fluorescence intensity of these fragments also makes it difficult to reproducibly locate and fit their peak shapes, so we have not included the 2.5 and 5 kb bands in the quantitative analysis shown in Figures 2 and 3.

We investigated the effect of changing the relative magnitudes of applied potential in the forward and reverse directions. These effects were isolated by holding the pulse time in both

the forward and reverse directions constant at 800 ms, while the forward potential was maintained at 20 V ($E_{fwd} = 7.7$ V/cm). It is evident from the data in Figure 2a that after 3 h of run time, the separation resolution remains relatively unchanged over the range of α investigated, with the exception of the lowest and highest values (1.5 and 6.0 respectively) where resolution is noticeably lower for fragments shorter than 20 kb. This deviation can be explained in terms of the two limiting cases these conditions represent. When α becomes small ($E_{rev} \gg E_{fwd}$), the DNA's forward migration speed also becomes small resulting in much longer run times and degraded resolution. In the opposite limit ($E_{fwd} \gg E_{rev}$), the DNA migration occurs faster but begins to approach continuous field behavior also resulting in degraded resolution (see Figure 1b). This illustrates the goal of optimizing the voltage ratio between these limits.

A second parameter related to the electric field is the pulse time, or the time over which the potential is held constant in each direction. When separation resolution is plotted as a function of pulse time, a transition (local maximum) is evident in the vicinity of 800 ms (Figure 2b). Finally, the data in Figure 2c suggest that resolution does not change appreciably as the run temperature is increased from 15 to 20 °C, but a decrease in resolution is evident as the temperature is increased further to 35 °C. In most pulsed field methods, separations are performed at temperatures in the vicinity of 15 °C in order to minimize the effects of band broadening by diffusion during the long run times required. In the case of our miniaturized system, these results suggest the possibility of running at higher electric fields to achieve even faster separations because the thin slab gel format promotes more efficient heat transfer to dissipate the effects of Joule heating.

In continuous field electrophoresis, DNA migration is expressed in terms of a mobility $\mu = v/E$, where v is the migration velocity of a given fragment. Since the direction and magnitude of the electric field are periodically changing in a FIGE experiment, we use the average electric field defined in equation 1 to calculate mobility. Figure 3 shows the size dependence of mobility, where it is evident that the mobility of a given fragment length does not appear to change significantly over the range of agarose gel concentrations studied. Furthermore, we observe a mobility scaling close to $\mu \propto N^{-0.5}$ as opposed to the N^{-1} normally observed in continuous field gel electrophoresis (N can be taken as the DNA fragment length) [35].

In order to compare these scalings with those reported in literature, it is often necessary to convert data reported in terms of displacement and/or velocity into mobility values using the electric field and pulse parameters employed in each study (Table 1). Data are sometimes expressed in terms of displacements or velocities of the migrating bands in order to avoid the need to specify an average electric field (see equation 1). Birren et. al. investigated electrophoresis of linear dsDNA fragments in terms of relative migration distance using three different sets of pulsing parameters with values of E_{avg} in the vicinity of 6 – 8 V/cm [36]. A scaling of $\mu \propto N^{-0.58}$ was observed under conditions where $E_{fwd} = E_{rev}$ ($\alpha = 1$) with $t_{fwd} > t_{rev}$, while a stronger size dependence of electrophoretic mobility was observed at longer pulse times when $E_{fwd} > E_{rev}$ ($\alpha = 1.5$) and $t_{fwd} = t_{rev}$. Sorbal et. al. investigated FIGE migration of both linear and supercoiled DNA ladders at agarose concentrations of 0.8, 1.0 and 1.2% with pulse times varying over a wide range from 0.12 to 120 s [15]. Considering the case of linear dsDNA with fragment sizes in the range of 1 – 12 kb and run conditions closest to those employed in our experiments (i.e., $E_{fwd} = E_{rev} = 5.7$ V/cm, $t_{fwd} = 1.2$ s, $t_{rev} = 0.4$ s) yields mobility scalings very close to $\mu \propto N^{-0.5}$ at all three agarose concentrations, consistent with our observations.

FIGE-mode mobility studies have also been performed in capillary electrophoresis systems. These systems are of interest due to their considerably shorter run times (generally less than

one hour) resulting from the ability to apply higher electric fields than are possible in benchtop-scale slab gel instruments. Different techniques are also used to detect the migrating bands, with slab gel instruments employing post-stain detection with intercalating dyes while capillary methods operate in a finish-line detection mode whereby dye is present in the running buffer and binds to the DNA fragments during the course of the separation. Heller et. al. investigated migration of linear dsDNA fragments in the 0.1 – 12 kb size range in a 1% HPC sieving matrix and observed a scaling of $\mu \propto N^{-0.18}$ using pulse conditions corresponding to $E_{fwd} = 243$ V/cm and $E_{rev} = 81$ V/cm ($\alpha = 3$) with $t_{fwd} = t_{rev}$ [12]. Kim and Morris also investigated FIGE-based separations of linear dsDNA fragments in the 8.3 – 48.5 kb size range using a capillary system containing ultradilute HEC and PEO sieving gels [13]. Remarkably, a level of resolution sufficient to distinguish all fragments in the DNA sample could be achieved in run times of under 4 min, although mobilities were found to be very weakly size dependent under these conditions with $\mu \propto N^{-0.07}$. A strong dependence on pulse time was also observed whereby mobilities exhibited a weak but uniform size dependence under some pulsing conditions, but developed a much stronger size dependence at fragment sizes between about 20 – 30 kb under other pulsing conditions.

Two observations can be made from the comparison between our data and the results of previous studies. First, the scalings of mobility with fragment size that we observe (e.g., $\mu \propto N^{-0.5}$) appear to be generally consistent with those previously observed in FIGE-based separations performed in slab gel instruments under similar conditions within a comparable DNA size range. Interestingly, the experiments of Birren et. al. with $t_{fwd} = t_{rev}$ incorporate pulse parameters most closely matching ours (in a 1% agarose gel) but yield a mobility scaling of $\mu \propto N^{-0.39}$ which deviates somewhat from our observations. This may be attributable to the different values of α employed or the switching delay t_0 included in our pulse sequence. Secondly, there is a significant difference in mobility scaling between experiments performed in capillary instruments as compared with slab gels, with the capillary data exhibiting a much weaker size dependence. This may be a consequence of the higher electric fields employed in the capillary experiments (about 100 times those used in slab gels) that would promote a more fully extended DNA conformation during a greater fraction of the total pulse time (this is at least partially counteracted by the use of considerably faster pulse times). Capillary methods also rely on specialized high voltage power supplies that have been custom modified to permit rapid field switching, and the finish-line detection mode may also limit the ability to resolve larger slower moving fragments.

In summary, we have used a simple miniature slab gel device to perform rapid FIGE-mode separations of dsDNA fragments in the 2 – 32.5 kb size range. In addition to faster run times, the compact design offers advantages of reduced reagent consumption and improved heat transfer, potentially making it possible to run at higher electric fields than are accessible in benchtop systems. This device allowed us to investigate the relationship between electrophoretic mobility and DNA fragment size, where there is a lack of systematic experimental data spanning a broad range of operating conditions. We hope that this kind of device design can offer a simple and convenient platform to help enable future studies of DNA migration in PFGE to be performed quickly and inexpensively.

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Abbreviations

FIGE	field inversion gel electrophoresis
PFGE	pulsed field gel electrophoresis
HEC	hydroxyethylcellulose
HPC	hydroxypropylcellulose
PEO	polyethylene oxide

References

1. Schwartz DC, Cantor CR. Cell. 1984; 37:67–75. [PubMed: 6373014]
2. Burmeister, M.; Ulanovsky, L., editors. Pulsed-Field Gel Electrophoresis. Humana Press; Totowa, NJ: 1991.
3. Orbach MJ, Vollrath D, Davis RW, Yanofsky C. Molecular and Cellular Biology. 1988; 8:1469–1473. [PubMed: 2967910]
4. Ribot E, Fitzgerald C, Kubota K, Swaminathan B, Barrett TJ. Journal of Clinical Microbiology. 2001; 39:1889–1894. [PubMed: 11326009]
5. Terajima J, Izumiya H, Iyoda S, Mitobe J, Miura M, Watanabe H. Foodborne Pathogens and Disease. 2006; 3:68–73. [PubMed: 16602981]
6. Barros TSL, Zhao Y, Lin SP, Roe BA, Dally EL, Davis RE. Canadian Journal of Microbiology. 2006; 52:857–867. [PubMed: 17110978]
7. Wang M, Lai E. Electrophoresis. 1995; 16:1–7. [PubMed: 7737080]
8. Chu G. Electrophoresis. 1989; 10:290–295. [PubMed: 2670544]
9. Hightower RC, Metge DW, Santi DV. Nucleic Acids Research. 1987; 15:8387–8398. [PubMed: 2823228]
10. Garvey EP, Santi DV. Science. 1986; 233:535–540. [PubMed: 3726545]
11. Hightower RC, Santi DV. Electrophoresis. 1989; 10:283–290. [PubMed: 2670543]
12. Heller C, Pakleza C, Viovy JL. Electrophoresis. 1995; 16:1423–1428. [PubMed: 8529608]
13. Kim Y, Morris MD. Electrophoresis. 1996; 17:152–160. [PubMed: 8907533]
14. Wagner L, Lai E. Electrophoresis. 1994; 15:1078–1083. [PubMed: 7859711]
15. Sobral BWS, Atherly AG. Nucleic Acids Research. 1989; 17:7359–7369. [PubMed: 2798097]
16. Heller C, Pohl FM. Nucleic Acids Research. 1989; 17:5989–6003. [PubMed: 2528121]
17. Lalonde M, Noolandi J, Turmel C, Rousseau J, Slater GW. Proc Natl Acad Sci USA. 1987; 84:8011–8015. [PubMed: 3317398]
18. Ito T, Hohjoh H, Sakaki Y. Electrophoresis. 1993; 14:278–282. [PubMed: 8500458]
19. Griess GA, Rogers E, Serwer P. Electrophoresis. 2000; 21:859–864. [PubMed: 10768770]
20. Griess GA, Serwer P. Electrophoresis. 2001; 22:4320–4327. [PubMed: 11824597]
21. Griess GA, Rogers E, Serwer P. Electrophoresis. 2001; 22:981–989. [PubMed: 11358152]
22. Griess GA, Choi H, Basu A, Valvano JW, Serwer P. Electrophoresis. 2002; 23:2610–2617. [PubMed: 12210164]
23. Szoke M, Sasvari-Szekely M, Barta C, Guttman A. Electrophoresis. 1999; 20:497–501. [PubMed: 10217162]
24. Guttman A, Ronai Z. Electrophoresis. 2000; 21:3952–3964. [PubMed: 11192118]
25. Maurer HR, Dati FA. Analytical Biochemistry. 1972; 46:19–32. [PubMed: 5017657]
26. Guttman A, Ronai Z, Khandurina J, Lengyel T, Sasvari-Szekely M. Chromatographia. 2004; 60:S295–S298.
27. Sasvari-Szekely M, Gerstner A, Ronai Z, Staub M, Guttman A. Electrophoresis. 2000; 21:816–821. [PubMed: 10733227]
28. Lengyel T, Guttman A. Journal of Chromatography A. 1999; 853:511–518. [PubMed: 10486760]

29. Guttman A, Barta C, Szoke M, Sasvari-Szekely M, Kalasz H. *Journal of Chromatography A*. 1998; 828:481–487. [PubMed: 9916326]
30. Shandrick S, Ronai Z, Guttman A. *Electrophoresis*. 2002; 23:591–595. [PubMed: 11870770]
31. Berdichevsky M, Khandurina J, Guttman A. *American Biotechnology Laboratory*. 2003; 21:22–23.
32. Mouradian S, Brumley RL Jr, Smith LM. *Electrophoresis*. 1994; 15:1084–1090. [PubMed: 7859712]
33. Kantor RM, Guo X-H, Huff EJ, Schwartz DC. *Biochemical and Biophysical Research Communications*. 1999; 258:102–108. [PubMed: 10222242]
34. Sturm J, Weill G. *Physical Review Letters*. 1989; 62:1484–1487. [PubMed: 10039686]
35. Lumpkin OJ, Zimm BH. *Biopolymers*. 1982; 21:2315–2316. [PubMed: 7171739]
36. Birren BW, Hood L, Lai E. *Electrophoresis*. 1989; 10:302–309. [PubMed: 2527739]

Figure 1.

(a) Photograph of the miniaturized FIGE apparatus. After casting the gel and loading a DNA sample, the entire device is placed on a temperature controlled stage and observed under a fluorescence microscope. (b) Comparison of separation results for gel electrophoresis performed in continuous field and field inversion modes (2.5 kb molecular ruler dsDNA sample, 1% agarose gel, $T = 15^{\circ}\text{C}$). (a) Continuous field mode, $E = 5 \text{ V/cm}$, 45 min run time. Bands corresponding to fragment sizes larger than 12.5 kb experience compression and are difficult to resolve. (b) Field inversion mode with $E_{fwd} = 7.7 \text{ V/cm}$, $E_{rev} = 3.0 \text{ V/cm}$ ($\alpha = 2.5$), $t_{fwd} = t_{rev} = 800 \text{ ms}$, and a 3 h run time. Nearly all bands are resolvable.

Figure 2.

(a) Effect of changing the ratio of forward and reverse electric field strength ($\alpha = E_{fwd}/E_{rev}$) on separation resolution. The value of E_{fwd} was held constant at 7.7 V/cm with $t_{fwd} = t_{rev} = 800$ ms. Experiments were performed in a 0.8% agarose gel, at $T = 15$ °C for a run time of 3 h. (b) Resolution as a function of pulse time ($t_{fwd} = t_{rev}$). Experiments were performed in a 0.8% agarose gel, at 15 °C for 3 hours with $E_{fwd} = 7.7$ V/cm, $E_{rev} = 2.2$ V/cm ($\alpha = 3.5$). (c) Effect of temperature on separation resolution. Experiments were performed in a 0.8% agarose gel, at 15 °C for 3 hours with $E_{fwd} = 7.7$ V/cm, $E_{rev} = 2.2$ V/cm ($\alpha = 3.5$), and $t_{fwd} = t_{rev} = 800$ ms.

Figure 3.

Double logarithmic plot of mobility versus DNA fragment size at different agarose gel concentrations. Experiments were performed at 15 °C for 3 hours with $E_{fwd} = 7.7$ V/cm, $E_{rev} = 2.2$ V/cm ($\alpha = 3.5$), and $t_{fwd} = t_{rev} = 800$ ms.

Table 1

Comparison between size dependence of DNA mobility observed in this work and the results of comparable experiments reported in literature (i.e., FIGE-based separations of double-stranded DNA in a comparable size range). The figure numbers in the original manuscripts from which mobility scalings have been computed are indicated below the citation of each paper in the first column.

Reference	Measurement	Format	DNA Size Range (kb)	Gel	Buffer	T (°C)	Run Time	E_{fwd} (V/cm)	a	t_{fwd} (ms)	t_{rev} (ms)	E_{avg} (V/cm)	Mobility Scaling*
This work (Figure 3)	Mobility ($\mu = v/E_{avg}$)	Miniature slab gel	2.5 – 32.5	0.6% agarose 0.8% agarose 1.0% agarose 1.3% agarose	0.5x TBE	15	3 h	7.7	5.7	800	800	4.0	-0.53 -0.47 -0.50 -0.47
Birren et. al. [36] (Figure 2b)	Displacement	Slab gel	1 – 50	1.0% agarose	0.5x TBE	13	10 h	9 9 6	1.5 1.5 1.0	800 200 500	800 200 200	7.5 7.5 6.0	-0.39 -0.61 -0.58
Sorbal et. al. [15] (Figure 3d–f)	Mobility ($\mu = v/E_{avg}$)	Slab gel	1 – 12	0.8% agarose 1.0% agarose 1.2% agarose	0.5x TBE	13	7 – 10 h	5.7	1.0	1200	400	5.7	-0.50 -0.47 -0.52
Heller et. al. [12] (Figure 1c)	Velocity	Capillary	0.1 – 12	1% HPC	1x TBE	25	1 h	243	3.0	10	10	162	-0.18
Kim & Morris [13] (Figure 6)	Mobility ($\mu = v/E_{avg}$)	Capillary	8.3 – 48.5	0.0033% HEC	0.5x TBE	Not stated	4 min	200	2.4	14	14	142	-0.07

*The mobility scaling values in the last column indicate the exponent z associated with the mobility size dependence $\mu \propto N^z$.