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Ultrafast, efficient separations of large-sized dsDNA in a blended polymer matrix by microfluidic chip electrophoresis: A Design of Experiments approach

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Abstract

Double-stranded (ds) DNA fragments over a wide size range were successfully separated in blended polymer matrices by microfluidic chip electrophoresis. Novel blended polymer matrices composed of two types of polymers with three different molar masses were developed to provide improved separations of large dsDNA without negatively impacting the separation of small dsDNA. Hydroxyethyl celluloses (HECs) with average molar masses of ~27 kDa and ~1 MDa were blended with a second class of polymer, high-molar mass (~7 MDa) linear polyacrylamide (LPA). Fast and highly efficient separations of commercially available DNA ladders were achieved on a borosilicate glass microchip. A distinct separation of a 1 Kb DNA extension ladder (200 bp to 40,000 bp) was completed in 2 minutes. An orthogonal Design of Experiments (DOE) was used to optimize experimental parameters for DNA separations over a wide size range. We find that the two dominant factors are the applied electric field strength and the inclusion of a high concentration of low-molar mass polymer in the matrix solution. These two factors exerted different effects on the separations of small dsDNA fragments below 1 kbp, medium dsDNA fragments between 1 kbp and 10 kbp, and large dsDNA fragments above 10 kbp.

Keywords

blended polymer; chip electrophoresis; Design of Experiments; large DNA separation

Introduction

Large-sized double-stranded (ds) DNA separation techniques evolved from gel electrophoresis [1–9] to capillary electrophoresis (CE) [10–13] to capillary array electrophoresis [14] and to microfluidic chip electrophoresis [15–18], progressing to more efficient, higher throughput, and more cost-effective platforms. A “lab-on-a-chip” is attractive for electrophoretic separations for both dsDNA and single-stranded (ss) DNA due to its miniature size, minimal sample consumption and the potential for higher resolution

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Conflict of interest statement

The authors declare no competing financial interests

separations, shorter analysis times, and lower cost. If plastic microfluidic chips could be implemented for large-scale DNA sequencing (*e.g.*, with 96-channel devices), specifically for the separation of ssDNA Sanger fragments < 1000 bases in length, the cost per lane could in principle be reduced by 96% relative to glass chips, and 99.6% relative to capillary array systems [19]. Similarly, if the separation of very large dsDNA fragments (> 10 kbp) can be achieved by microfluidic chip electrophoresis, the cost per lane could be substantially reduced and the required analysis time shortened. However, achieving good separation of large dsDNA molecules, quickly, still remains a challenge; there is no fast, reliable, and reproducible electrophoresis method that gives excellent resolution and high-efficiency peaks for both small and large molecules simultaneously.

In previous work, Barron *et al.* employed ultra-dilute solutions of hydroxyethyl cellulose (HEC) polymers and successfully separated large dsDNA molecules from 2.0 to 23.1 kbp by CE, with moderate resolution [10]. In such an ultra-dilute concentration regime, the separation mechanism was described as “transient entanglement coupling,” which was different from the Ogston [20] and reptation [21] models suitable for agarose gel electrophoresis. One of the advantages of large-sized DNA separation in dilute polymer solutions by CE was that the “transient entanglement coupling” mechanism did not appear to have an obvious upper size limitation for DNA separated in dilute polymer matrices [10].

Later, it was shown that “blended” polymer matrices (*i.e.*, solutions comprising mixtures of the same types of polymers, but with different average molar masses) are useful for separating mixtures of both large and small DNA fragments in a single CE analysis. Bünz and Barron worked with mixtures of low-and high-molar mass HECs at various concentrations, and optimized their blended HEC polymer matrix to achieve dsDNA separations over a wide range of sizes [22]. The blended polymer matrix was composed of 27 kDa/105 kDa HEC at 0.3/0.025 wt%, which showed a superior separation of dsDNA fragments sized from 72 bp to 23 kbp in a single run, with high-resolution separation seen over the entire range. In addition, the blended polymer matrix provided a five-fold viscosity reduction relative to a comparator matrix based on only one HEC molar mass, which can be extremely important for matrix loading in microchip electrophoresis. Blended polymer matrices seemed promising as a platform for dsDNA separation over a broad range of sizes, with flexibility for tailoring separation properties by substituting matrix components and varying their concentrations.

In practice, however, the largest resolved DNA size in uncrosslinked polymer solutions in steady-field capillary electrophoresis was not larger than 25 kbp [23]. For the separation of DNA fragments larger than 23.1 kbp, low electric field strength and pulsed electric fields were applied, to both gel electrophoresis and capillary electrophoresis. Pulsed field capillary electrophoresis (PFCE) could potentially be 10–200 times faster than pulsed field gel electrophoresis (PFGE), which could take up to 162 hours to complete [5], but PFCE tended to suffer from chronic irreproducibility of results [11–13].

Alternative, non-gel-based approaches to large DNA separations have also been investigated. These include self-assembling magnetic matrices that can separate 15 kbp to 48.5 kbp in several minutes [24] and 48.5 kbp to 168.9 kbp in 2.5 minutes [25].

Microfabricated anisotropic sieving structures have been successfully used to separate 2 kbp to 23 kbp of DNA in a minute [26] while dielectrophoresis using microfabricated posts [27] can separate 7 kbp from 14 kbp DNA and 48.5 kbp from 164 kbp DNA. A sparse, ordered array of posts was able to separate 2.7 kbp from 48.5 kbp in less than 60 seconds [28]. While these methods produce Gaussian peak shapes, they were not able to generate baseline separation for all the DNA separations shown. In addition, the production of such microfabricated structures was costly and complicated and after fabrication there was no flexibility for tailoring the device to different DNA separation range applications [29]. Note that separation matrices based on a polymer matrix of core-shell nanoparticles have been used for 1–15 kbp separations [18] on microfluidic chip electrophoresis. An interesting double pressurization procedure during the microfluidic chip electrophoresis was required to obtain adequate DNA separation with this nanosphere matrix, possibly increasing the concentration of packed nanospheres. Tseng et al., reported separation matrices composed gold nanoparticles and polymer composites, which separated DNA from 564 bp to 23,130 bp and from 8.3 kbp to 38.4 kbp within 5 minutes on capillary electrophoresis [30]. Here though we discuss a method of polymer matrix using commercially available reagents with no need for special microfabrication of pillars or posts. Good separation can be achieved for DNA ranging from 200 bp to 40 kbp in one single run, covering a different size range than many of the above mentioned strategies. While the commercially available Agilent Bioanalyzer can obtain DNA separations up to 12 kbp the polymer composition is proprietary.

A combination of dilute polymer solutions and microfluidic chip electrophoresis could combine the strengths of flexibility and fast analysis time. In the present study, we developed novel blended polymer matrices for separation of a wide size range of dsDNA fragments by microfluidic chip electrophoresis, with high speed and high resolution. We also performed a systematic investigation of the contributions of each polymer component in the matrices and the effect of electric field strength on the separation performance of DNA fragments for different size ranges using Design of Experiments, and offer some mechanistic rationale for our observations.

Experimental

Samples and reagents

ΦX174 RF DNA/*Hae* III Fragments, λ DNA/*Hind* III Fragments, a 1Kb DNA ladder and 1 Kb DNA Extension Ladder were obtained from Invitrogen, Inc. (San Diego, CA). See Supporting Information for specific DNA sizes for each ladder. The buffer used in all experiments was 0.5X TTE; TTE buffer consists of 50 mM tris(hydroxymethyl)aminomethane (Tris) and 50 mM *N*-tris(hydroxymethyl)methyl-3-aminopropanesulfonic acid (TAPS) with 2 mM ethylenediaminetetraacetic acid (EDTA). Tris was obtained from ISC BioExpress Inc. (Kaysville, UT), TAPS from Fisher Scientific (Pittsburgh, PA), and EDTA from Sigma (St. Louis, MO). Matrix polymers included two molar masses of hydroxyethyl cellulose (HEC) purchased from Polysciences, Inc. (Warrington, PA), and high molar mass linear polyacrylamide (LPA) that was synthesized in our own laboratory. The average molar masses of the two commercially obtained HEC

samples were nominally 24,000–27,000 g/mol and 1,000,000 g/mol respectively. The molar mass of the synthesized LPA was 7.01M g/mol as determined by a batch MALLS method [31]. The corresponding abbreviation for these three polymers are 27 kDa HEC, 1 MDa HEC and 7 MDa LPA, respectively. A dynamic, physically adsorbed coating of poly(*N*-hydroxyethylacrylamide) (PHEA)[32] coating polymers, also synthesized in our own laboratory, was used to suppress electroosmotic flow (EOF) in microfluidic chip electrophoresis. Ethidium bromide (EtBr) dye was obtained from Fisher Scientific (Pittsburgh, PA).

Microfluidic chip electrophoresis

Microfluidic chip electrophoresis was conducted using a custom-built system in our laboratory with multi-color laser-induced fluorescence (LIF) detection [33]. Glass microchips (T8050) were obtained from Micronit (Enschede, The Netherlands), and had a standard 100 μm “offset T” injector, a 8 cm separation distance, and 50 μm wide $\times$ 20 μm deep channels. Microchannels were dynamically coated with 0.1 wt% PHEA aqueous solution, after a pretreatment rinse with 1M HCl. DNA samples were diluted in DI water to a final concentration 5 $\mu\text{g/ml}$. The amount of matrix polymer materials that was added into 0.5X TTE buffer was varied to obtain good separations of both small and large dsDNA fragments. Both DNA samples and polymer matrix solutions were mixed with EtBr, to a final concentration 1 μM of EtBr. DNA samples were electrokinetically injected with applied voltage 200 V for 20 s. The separation electric field strength was varied for obtaining good DNA separation performance.

Orthogonal analysis

To screen individual contributions of each polymer in our blended polymer matrices, we employed a Design of Experiment approach for orthogonal analysis to the study of four factors. Orthogonal design is a good approach to minimize the number of experimental runs while improving the screening for an optimal formulation in a complicated system with multiple variables [34]. In the current study, we have four factors: **(A)** the concentration of 27 kDa HEC, **(B)** the concentration of 1 MDa HEC, **(C)** the concentration of 7 MDa LPA, and **(D)** the applied electric field for the electrophoretic separation (abbreviated as E). A minimal 16-run combination of $L_{16}(4^2)$ was employed instead of a conventional $4^4 = 256$ running combination, according to orthogonal design of experiments. Table 1 lists the four levels and factors used for polymer matrix and separation field for DNA separation. All 16-runs were analyzed by peak fitting (see below). Many blended matrix separations were tested and repeated three times. The specific 64 runs discussed herein were run once each in a short timeframe to minimize systematic errors. Since these results match earlier electropherograms, we are confident of their reproducibility and reliability.

Peak fitting

We used Peak Fit Version 4.0 to fit our electropherograms with the use of the chromatographic analysis function. A normal distribution fit of the Gaussian function was employed to analyze symmetric peaks, and an exponentially modified Gaussian (EMG) function [35] was employed for asymmetric or tailed/fronted peaks. We calculated the

resolution between pairs of peaks using the standard approach, and the full width at the peak base, rather than the peak width at the half-maximum; taking a more conservative approach to estimating resolution.

Results and Discussion

Preliminary results separating the 1 kbp DNA ladder with a blended matrix of 27 kDa and 1 MDa HEC showed excellent separation below 9,162 bp. Separation of the same ladder with 7 MDa LPA alone showed good separation from 75 bp to 12,216 bp but with reduced resolution for small dsDNA fragments (see Supporting Information). These results are fundamentally similar to what was seen in a previous report by Bünz *et al.*[22], where it was found that a high concentration of low molar mass HEC polymers is most appropriate for separating small dsDNA fragments, while a low concentration of high-molar mass HEC polymers is appropriate for separating large dsDNA fragments. A mixture of the low and high molar mass polymers we used—in this case, very different chemical classes of polymers, which is different from the Bünz *et al.* study that used only HEC—was predicted to show a synergistic effect on the matrix's ability to separate both small and large dsDNA fragments, in a single electrophoretic analysis. A substantially improved separation of the 1 Kb DNA ladder in microfluidic chip electrophoresis was obtained using a blended matrix of 27 kDa HEC, 1 MDa HEC and 7 MDa LPA, at concentrations of 0.45 w/v %, 0.15 w/v % and 0.15 w/v %, respectively (see Supporting Information).

Orthogonal analysis

An orthogonal analysis was employed to investigate correlations between experimental conditions and the DNA separation performance for a blended matrix. The experimental conditions include three polymers in the matrix solution—the two types of polymers of HEC and LPA, at three different molar masses and at various concentrations. In addition, the applied separation electric field strength was included as another experimental parameter. Values were chosen based on previous literature and preliminary experiments done in our laboratory. A good or less good separation is noted by the resolving ability of several sets of two adjacent dsDNA peaks, and an observation of the appearance/disappearance and peak shapes of the largest separated dsDNA fragment(s). Also, whether or not a given dsDNA fragment of large size is resolved is another indicator for good or less good separation performance. By correlating the experimental conditions and separation performance of the studied four commercially available DNA ladders, we determined how these factors affect the resolving abilities of various polymer networks for dsDNA fragments within different size ranges, *i.e.* small fragments below 1 kbp, medium fragments between 1 kbp and 10 kbp, and large fragments above 10 kbp. The results, which we found to be quite interesting and revealing, will be helpful in the development of novel polymer matrices and for optimizing the applied electric field for DNA separations over a broad size range.

Separation of 271/281 and 506/517 bp fragments—First, we studied how electrophoretic parameters impact the resolution of small DNA fragments below 1 kbp, with only a 10-bp difference in size, by determining the peak resolution between 271/281 bp in Φ X174 RF DNA/*Hae* III Fragments and 506/517 bp in 1 Kb DNA ladder and 1 Kb DNA

Extension Ladder. An average resolution was calculated for each level of the four factors. For each factor, the extreme difference of the average resolution values (R) is defined as the difference between the largest and the smallest average resolution at various levels. A large R value indicates that the given factor has a strong effect on the separation performance when varied. The calculated peak resolution and R for 271/281 and 506/517 bp are listed in Table 2. Higher concentration HEC and LPA lead to higher average peak resolutions, *i.e.* a better separation of small fragments with minor difference in size. For example, when the concentration of 27 kDa HEC decreased from 0.84 w/v % (level 1) to 0.21 w/v % (level 4), the resolution of 271/281 bp in Φ X174 RF DNA/*Hae* III fragments decreased from 1.66 to 0.53. Similar results were observed for 506/517 bp in 1 Kb DNA ladder and 1 Kb DNA extension ladder. At the concentration of 0.21 w/v % 27 kDa HEC, only one peak was present, indicating an inability to resolve a 10-bp difference in the 500 bp range. In addition, the extreme difference of resolution of factor A was highest, which meant 27 kDa HEC was the dominant factor in resolving such small length difference between DNA fragments. The optimal formulation is determined by selecting the level of each factor with highest average resolution. Therefore, the optimized formulation for separating 271/281 bp in Φ X174 RF DNA/*Hae* III Fragments is A1B1C2E3. The matrix contains 0.82 w/v % 27 kDa HEC, 0.28 w/v % 1 MDa HEC and 0.09 w/v % 7 MDa LPA, with applied electric field of 200 V/cm. Interestingly, the same optimal formulation was determined to obtain a good DNA separation of 506/517 bp in 1 Kb DNA ladder and in 1 Kb DNA extension ladder. Also, the order of R in a descending sequence for all these three separations between 271/281 and 506/517 bp are the same, $A > B > C > E$. That suggests the order of contribution from each factor. Therefore, the low molar mass polymer concentration (27 kDa HEC) is the most important factor to obtain a good separation on small DNA fragments around 200 bp and 500 bp range, all below 1 kbp. Separation electric field strength showed minimal effects on these separations, so a high electric field strength was preferred, for fast separation, as long as it does not cause too much Joule heating (which can lead to broader peaks).

Separations of 2027/2322 and 5000/5090 bp—Second, we studied peak resolution between 2027/2322 bp in λ DNA/Hind III Fragments. The calculated average peak resolution and R are listed in Table 3. The determined optimal formula for separating 2027/2322 bp in λ DNA/Hind III fragments is A1B1C2E3. The matrix contains 0.82 w/v % 27 kDa HEC, 0.28 w/v % 1 MDa HEC and 0.09 w/v % 7 MDa LPA, with applied electric field of 200 V/cm. Although the optimal formulation for separating 2027/2322 bp is coincidentally the same as that for separating 271/281 and 506/517 bp, the extreme difference of the average resolution values R shows a different trend ($A > E > C > B$). The high concentration of low molar mass HEC still plays a critical role in resolving this 295-bp difference in 2000 bp range. Also, the separation electric field strength shows a strong effect on the separation of 2027/2322 bp, stronger than that of both factor B and C. In all 16 runs of the current orthogonal analysis, only one peak was observed for 5000/5090 bp in 1 Kb DNA extension ladder.

Separation of large-sized DNA fragments, above 10 kbp—Good separations were obtained for both the 1 Kb DNA ladder and the 1 Kb DNA extension ladder over a wide

range of DNA fragments, ranging from 75 bp up to 12,216 bp and 40,000 bp respectively. Figure 1A shows an overall separation of the 1 Kb DNA ladder from 75 bp to 12,216 bp, in the matrix of A1B2C2 which consists of 0.84 w/v % 27 kDa HEC, 0.21 w/v % 1 MDa HEC, and 0.09 w/v % 7 MDa LPA, with applied separation electric field of 250 V/cm. The separation is good at both ends of DNA length. Among all other electropherograms of this series of 16-run experiments, either the resolution of small fragments below 1 kbp was found to deteriorate, or the signal strength or peak shapes for the large-sized fragments deteriorated under different experimental conditions. (Data not shown) A similar result was obtained for the 1 Kb DNA extension ladder. Although we did not get a good overall separation covering all fragment peaks, Figure 1B shows the best separation obtained in this series of 16 different runs. The blended matrix was A4B4C1 consisting of 0.21 w/v % 27 kDa HEC, 0.07 w/v % 1 MDa HEC and 0.12 w/v % 7 MDa LPA with applied separation electric field strength at 150 V/cm. A baseline-resolved separation was obtained, up to the 40 kbp peak; however, peak shapes corresponding to fragments above 10 kbp were asymmetrical (fronted) with long tails off to the right-hand side of the peak. This phenomenon is frequently observed for large-sized dsDNA fragments, and either DNA stretching or DNA trapping in the sieving matrix may be the cause of this effect. Although several fragments below 200 bp cannot be separated and the difference between 506/517 bp cannot be distinguished in the experimental conditions, the separation is still a superior separation from 200 bp to 40,000 bp.

Since large DNA seemed to stretch progressively with increasing gel concentration and field strength, electrophoresis for separating large DNA fragments should be carried out at a low polymer concentration and low field strength [36]. In the series of 16-run experiments for separating λ DNA/Hind III Fragments, we found that the largest fragment peak (23 kbp) may be absent or present from the electropherograms depending on the experimental conditions. Figure 2 shows two typical electropherograms with both results showed good separation with baseline resolved abilities. However, there was no evidence of another peak after 10 seconds of the second largest 9,416 bp fragment in Figure 2A, while the peak for the 23-kbp fragment was clearly distinguishable immediately following the peak for the 9,416 bp fragment (Figure 2B). Taking a binary approach to tracking the results, the presence of the 23-kbp peak is defined as **1** and the absence of this peak is **0**. Then the averaged “presence” for each level of any factor was calculated to demonstrate the separation performance of the large-sized DNA fragment of 23 kbp. The extreme difference of the “presence” of the 23-kbp peak for each factor was defined as the difference between the largest and smallest average “presence” values at different levels, which suggested the extent of effect on the separating ability of the matrix for the 23-kbp peak by varying the level of a given factor (see Supporting Information). It was found that factor A (27 kDa HEC) and E (separation electric field strength) are two key parameters whereas factors B (1 MDa HEC) and C (7 MDa LPA) have much smaller effects on the presence of the 23-kbp fragment peak. The order of the extreme difference of the averaged “presence” is $E = A > B = C$. Furthermore, the averaged “presence” values of these two critical parameters, factor A and factor E, suggests that a low concentration of 27 kDa HEC and low separation electric field strength are favorable of obtaining the largest 23 kbp fragment peak.

That fact that low electric field strength was favorable for large-sized DNA separation is not surprising; however, this is the first proof that a low concentration of low molar mass polymer is critical to the resolution of large DNA fragments. An increase of the average “presence” value with decreasing concentration of 27 kDa HEC indicated that minimizing the concentration of low molar mass polymer is helpful to the appearance and separation of large-sized DNA fragments. How low the concentration must be, and if there is a maximum limiting concentration of 27 kDa HEC for separating a given large-sized DNA fragment, remains an interesting open question, although it is beyond the scope of this study. It would be simple enough to decrease the concentration of low mass polymer or remove it entirely from the matrix when separating large-sized DNA fragments, but a compromise is required to separate DNA fragments that cover a broad size range, since it is necessary for decent separations of small DNA fragments.

A similar situation exists for the applied electric field strength, for fast separation of large-sized DNA. Large-sized DNA easily hooks up with entangled “knots” in the polymer matrix [37], and may not be able to be released in an electric field with high strength. When a relatively low electric field is applied, there is enough time for large DNA fragment to relax according to its viscoelastic properties, and thus it can be released from the entangled “knots” and can migrate through and separate in such a sieving matrix. Appropriate electric field strength is critical for large DNA fragments to obtain an adequate electrophoretic mobility and enough relaxation time. Therefore, both dilute low molar mass polymers and low electric field strength are important factors in separating the 23-kbp fragment. Since our evaluation only considered the two possibilities of presence and absence of the peak and did not take into account the signal strength and peak shape in any quantitative way, we did not expect a finer category of those studied factors. Among the studied four factors, we can determine that a low concentration of 27 kDa HEC at 0.21 w/v % and low electric field strength of 150 V/cm are optimal conditions, while the concentration ranges of 1 MDa HEC and 7 MDa LPA show minimal effects for separating large DNA fragment like 23 kbp. The same method was employed to study the presence of 12 kbp in 1 Kb DNA ladder and the presence of 20 kbp in 1 Kb DNA extension ladder (see Supporting Information). Similar results were obtained, in that the most significant factors are factors A and E. Furthermore a stronger dependence on electric field strength was observed compared to the 27 kDa HEC. Hence, the critical parameter for separating large DNA fragments above 10 kbp is the applied electric field strength. To reduce the capture of large DNA fragment and facilitate its release further, a pulsed field may offer an alternative or additional method (and may very well improve the peak shape). A further investigation on pulsed-field (PF) microfluidic chip electrophoresis will be carried out in our lab for the separation of extremely large dsDNA molecules.

Concluding remarks

We systematically investigated a blended polymer matrix and optimized the experimental conditions for resolving DNA fragments up to 40,000 bp. A good separation of DNA fragments from 200 bp to 40,000 bp was achieved in the blended matrix A4B4C1 consisting of 0.21 w/v % 27 kDa HEC, 0.07 w/v % 1 MDa HEC and 0.12 w/v % 7 MDa LPA with applied electric field strength of 150 V/cm. Individual contributions of each factor, including

the concentration of blended polymer matrix and the separation electric field strength, were screened by the use of an orthogonal analysis. Concentrated, low molar mass HEC was the dominant factor for resolving small DNA fragments with a size below 1 kbp. A 10-bp difference can be resolved in this size range. Both low molar mass HEC concentration and applied electric field were dominant factors in resolving medium-sized DNA fragments from 1 kbp to 10 kbp, with the resolving ability of a 295-bp difference. For DNA fragments above 10 kbp, the applied separation electric field became the dominant factor needing optimization. We were pleased to find that an orthogonal design of experiments provided us with an effective, highly quantitative way to analyze several key factors in a complex system for DNA separation, and could help us to identify a reasonably well optimized experimental condition that can achieve highly reproducible separations of very large dsDNA molecules by microfluidic chip electrophoresis. With DOE, more variables can be introduced such as additional polymer sizes, to study the separation ability and factor dependence in even more complex blended matrices, potentially bringing us closer to a universal blended polymer matrix suitable for all sizes.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1.

Microfluidic electrophoregram of (A) 1 Kb DNA ladder with peak identification. The polymer matrix was A1B2C2 containing 0.82 w/v % 27 kDa HEC, 0.28 w/v % 1 MDa HEC and 0.09 w/v % 7 MDa LPA and the applied electric field was 250 V/cm and (B) 1 Kb DNA Extension Ladder with peak identification. The polymer matrix was A4B4C1 containing 0.21 w/v % 27 kDa HEC, 0.07 w/v % 1 MDa HEC and 0.12 w/v % 7 MDa LPA and the applied electric field was 150 V/cm.

Figure 2.

Microfluidic electrophoregram of λ DNA/Hind III Fragments with peak identification. The polymer matrices were (A) A1B3C3 containing 0.84 w/v % 27 kDa HEC, 0.14 w/v % 1 MDa HEC and 0.06 w/v % 7 MDa LPA with applied E of 200 V/cm and (B) A4B4C1 containing 0.21 w/v % 27 kDa HEC, 0.07 w/v % 1 MDa HEC and 0.12 w/v % 7 MDa LPA with applied E of 200 V/cm.

Table 1

Orthogonal design of experiment: 16-run combination of four factors (27 kDa HEC (A), 1 MDa HEC (B), 7 MDa LPA (C) and Microfluidic chip electrophoresis separation electrical field(E)) and four levels (various concentrations of each polymer in matrix and applied electric field strength).

Run	Code	Factor A	Factor B	Factor C	Factor E
		27 kDa HEC (w/v)%	1 MDa HEC (w/v)%	7 MDa LPA (w/v) %	E (V/cm)
1	A1B1C1E1	0.84	0.28	0.12	300
2	A1B2C2E2	0.84	0.21	0.09	250
3	A1B3C3E3	0.84	0.14	0.06	200
4	A1B4C4E4	0.84	0.07	0.03	150
5	A2B1C2E3	0.63	0.28	0.09	200
6	A2B2C1E4	0.63	0.21	0.12	150
7	A2B3C4E1	0.63	0.14	0.03	300
8	A2B4C3E2	0.63	0.07	0.06	250
9	A3B1C3E4	0.42	0.28	0.06	150
10	A3B2C4E3	0.42	0.21	0.03	200
11	A3B3C1E2	0.42	0.14	0.12	250
12	A3B4C2E1	0.42	0.07	0.09	300
13	A4B1C4E2	0.21	0.28	0.03	250
14	A4B2C3E1	0.21	0.21	0.06	300
15	A4B3C2E4	0.21	0.14	0.09	150
16	A4B4C1E3	0.21	0.07	0.12	200

Average resolution of each level for the studied four factors and their extreme difference for separation of 271/281 bp fragments in Φ X174 RF DNA/*Hae* III Fragments and 506/517 bp fragments in 1 Kb DNA ladder and 1Kb DNA Extension Ladder.

Table 2

	Levels	Factor A	Factor B	Factor C	Factor E
		27 kDa HEC (w/v)%	1 MDa HEC (w/v)%	7 MDa LPA (w/v) %	E (V/cm)
271/281 bp in Φ X174 RF DNA/ <i>Hae</i> III	1	1.66	1.34	0.94	0.97
	2	1.18	1.10	1.03	0.93
	3	0.41	1.02	1.02	1.00
	4	0.53	0.80	0.80	0.90
	R	1.25	0.55	0.23	0.11
	Levels	Factor A	Factor B	Factor C	Factor E
		27 kDa HEC (w/v)%	1 MDa HEC (w/v)%	7 MDa LPA (w/v) %	E (V/cm)
506/517 bp in 1 Kb DNA ladder	1	0.53	0.28	0.16	0.16
	2	0.12	0.16	0.28	0.16
	3	0	0.14	0.14	0.26
	4	0	0.07	0.07	0.07
	R	0.53	0.21	0.21	0.19
	Levels	Factor A	Factor B	Factor C	Factor E
		27 kDa HEC (w/v)%	1 MDa HEC (w/v)%	7 MDa LPA (w/v) %	E (V/cm)
506/517 bp in 1 Kb DNA Extension Ladder	1	0.40	0.35	0.24	0.11
	2	0.28	0.30	0.32	0.17
	3	0.09	0.21	0.21	0.27
	4	0	0	0	0.22
	R	0.40	0.35	0.32	0.17

Table 3

Average resolution of each level for the studied four factors and their extreme difference for separation of 2027/2322 bp fragments in λ DNA/Hind III Fragments.

Levels	Factor A	Factor B	Factor C	Factor E
	27 kDa HEC (w/v)%	1 MDa HEC (w/v)%	7 MDa LPA (w/v) %	E (V/cm)
1	1.05	0.71	0.69	0.42
2	0.78	0.53	0.98	0.93
3	0.31	0.53	0.53	0.96
4	0.59	0.54	0.54	0.42
R	0.74	0.18	0.45	0.55