

Joan M. Bienvenue,¹ M.S.; Kate L. Wilson,¹ B.S.; James P. Landers,^{1,2} Ph.D.;
and Jerome P. Ferrance,¹ Ph.D.

Evaluation of Sieving Polymers for Fast, Reproducible Electrophoretic Analysis of Short Tandem Repeats (STR) in Capillaries*

ABSTRACT: Efficient capillary electrophoretic STR analysis requires rapid, reproducible and robust separation of DNA fragments with reasonable capillary longevity—this is currently accomplished using proprietary commercial polymeric sieving matrices specifically developed for this separation. These matrices, while effective, are costly and do not provide adequate resolution of STR DNA fragments in capillaries with shorter effective separation lengths, increasing the time required to accomplish the separation and minimizing the potential extrapolation to other miniaturized platforms. As the forensic community looks toward next generation microchip technology as a means of processing casework more rapidly, new sieving polymers need to be evaluated for utilization in this platform. The research presented here describes the assessment of commercially-available polymeric sieving matrices for STR analysis, with consideration given to feasibility of incorporation into a microdevice. Polymer composition, molecular weight, and concentration were evaluated, along with an assessment of the effects of buffer composition, separation temperature, and capillary length. These variables were evaluated individually or collectively on the ability to resolve STR DNA fragments and the reproducibility of the separations and the results compared to a proprietary commercial product. A 600,000 Da MW poly(ethylene oxide) (PEO) solution at a 3% (w/v) concentration was determined to be the most suitable matrix for these separations. This polymer, in coated capillaries, provided highly robust and reproducible separations, with near baseline resolution of fragments having single base differences. Reductions in the temperature of the separation, from 60°C to 40°C, and the urea concentration of the buffer, from 7 M to 3.5 M, provided increased longevity of the PEO polymer for repeated separations. Comparison of this polymer with currently specified commercial products used for STR analysis showed that the optimized PEO matrix provided superior separations under all conditions tested. In addition, PEO could be utilized in shorter capillary systems, with a concurrent decrease in analysis time, highlighting its potential for use in shortened capillary or microdevice systems.

KEYWORDS: forensic science, DNA, DNA separations, polymers

Capillary electrophoresis is now a sanctioned method for separation and analysis of short tandem repeat (STR) DNA fragments for forensic applications and genetic mapping. Increased speed, separation efficiency, and automation have made the technique indispensable in the forensic laboratory. In order to accomplish the high-resolution separations required for STR analysis, a limited number of polymeric solutions have been evaluated systematically for suitability in this application (1,2,3–5). Linear polyacrylamides are commonly developed as commercial STR separation matrices because of their stability, reliability, and ability to yield high-resolution separations of DNA fragments differing in length by a single base. However, the high cost of these commercial products and their poor resolution in shorter separation lengths, which could provide faster separations, has created a need for alternative polymers.

Advances in analytical microchip technology have created an intense interest in microchips as the next generation platform for STR and SNP typing. A decade of literature has shown that

electrophoretic separations can be accomplished in microdevices rapidly and efficiently for both sequencing and size-based genetic analysis (1,6–15). In addition, there is a growing interest in integrating sample preparatory steps, such as DNA extraction and PCR amplification, into the same device used for the separation and detection. These advances represent the first steps towards realizing a micro-total analysis system (μ TAS), capable of comprehensive genetic analysis with sample in–answer out capabilities in one self-contained device. In order to translate forensic genetic analysis from bench-top instrumentation to the microchip, however, a variety of challenges exist. With post-amplification separation and detection of STR fragments, issues associated with separation length are crucial. Standard effective capillary lengths are 36 cm in order to resolve the fragments in linear polyacrylamide, however, if these separations are to be carried out effectively on a chip, comparable resolution must be obtained over shorter effective separation distances. This was achieved by Mathies and coworkers (10,11,16) who showed sequencing of both genomic and mitochondrial DNA, separations of STRs and genotyping on microchips using hydroxyl ethyl cellulose (HEC) and linear polyacrylamide (LPA) as sieving matrices. Erhlich and coworkers (1,6,8,9,13,20) have also demonstrated sequencing and forensic genotyping in microfabricated devices using LPA as the separation matrix. The devices reported, have often utilized extended separation channels to achieve the required resolution, but short distance separations (less than 20 cm) of DNA are preferred to minimize chip area and analysis time. In addition, LPA polymers produce viscous solutions that are difficult to load into microdevices.

¹ Department of Chemistry, McCormick Road, University of Virginia, Charlottesville, VA 22904.

² Department of Pathology University of Virginia Health Science Center, Charlottesville, VA 22901.

* Research funded by the U.S. Department of Justice, Federal Bureau of Investigation under contract J-FBI-03-084 and presented, in part, at HPCE, 2004. The views and conclusions contained in this document are those of the authors and should not be interpreted as necessarily representing the official policies, either expressed, or implied, of the U.S. Government.

Received 18 Sept. 2004; and in revised form 9 March 2005; accepted 12 March 2005; published 25 May 2005.

Reduction of separation length transfers much of the burden onto the separation polymer to provide the requisite resolution over a shorter distance, while having a reasonably low viscosity for ease of use and efficiency of microdevice filling. Due to these requirements, higher viscosity, uncrosslinked polyacrylamides, such as POP-4 (Applied Biosystems, Foster City, CA), currently used in commercial instruments, may not translate effectively to the microdevice. POP-4 requires long, thermostated capillaries to produce the high-resolution separations necessary for forensic casework analysis. As a result, it is unlikely that this polymer, although demonstrated as suitable for conventional STR analysis, is capable of effectively resolving single-base pair differences in short channels and amenable to facile translation into the microdevice. For these reasons, other commercially available polymeric separation matrices are being systematically evaluated for this use in microdevices.

The work presented in this report describes the evaluation of poly(ethylene oxide) (PEO) for separation of STR fragments in capillaries with a view towards use in the microchip platform and as a potential alternative to commercially available sieving matrices. Accurate, reproducible separation of single base pair differences is reported using a low-viscosity, denaturing solution of low-molecular weight PEO in short capillaries. In addition, the effects of capillary temperature, buffer composition, polymer weight and concentration, and capillary length are detailed. Resolution was better than results obtained using conventional commercial polymers, with markedly shorter run times. Finally, feasibility and demonstration of inclusion of this separation matrix in a typical microdevice are also discussed.

Methods

Preparation of Polymeric Sieving Matrices

A concentrated solution of 10X TBE buffer (890 mM tris, 890 mM boric acid, 20 mM EDTA) was prepared by dissolving 5.39 g of Trizma (Sigma, MO), 2.75 g of boric acid (Sigma, St. Louis, MO), and 0.38 g of EDTA (Amresco, Solon, OH) in 50 mL of deionized water. For polymeric solutions the appropriate percentage w/v of poly(ethylene oxide) (Acros, New Jersey), poly(vinylpyrrolidone) (Polysciences, Inc., Warrington, PA), or hydroxyethyl cellulose (Aldrich, Milwaukee, WI) with the desired molecular weight was weighed out. To the polymer, 5 mL of 10X TBE and either 21.0 g (7 M) or 10.51 g (3.5 M) of urea (Fisher, Fair Lawn, NJ) were added, then diluted to 50 mL with deionized water and stirred at room temperature until the solution was homogenous. Polymeric solutions were filtered through a 5 μ m filter before use.

Sample Preparation

DNA allelic ladder samples from Applied Biosystems AmpF ℓ STR[®] COfiler[®] (Applied Biosystems, Foster City, CA) were used in all experiments. Samples were prepared by combining 25 μ L of Hi-Di formamide containing 1 μ L of the GeneScan-500 ROX size standard, with 1.5 μ L of AmpF ℓ STR allelic ladder and mixed by pipetting up and down. Samples were then heat denatured at 95°C for 3 min in a water bath, followed by snap cooling on ice for 3 minutes.

Capillary Electrophoresis

Electrophoresis was accomplished using an ABI Prism[®] 310 Genetic Analyzer (Applied Biosystems, Foster City, CA). Fused-

silica capillaries, (Polymicro Technologies, Phoenix, AZ), 50 μ m ID, 47 cm, unless otherwise stated, were coated using a 0.2% w/v solution of epoxy poly(dimethacrylamide) (EPDMA), prepared as previously described (21,22). Coating was performed before installation in the 310 using pressure flushing (550 kPa) with an in-house constructed device. The capillary was flushed for 10 min with 1 M NaOH, 20 min with deionized water and 20 min with 0.2% EPDMA. After flushing, the capillary was allowed to sit at room temperature with EPDMA for 30 min before installation in the 310 instrument. Samples separated using the POP-4 polymer were analyzed in uncoated capillaries according to the manufacturer's instructions. Sample ladders were analyzed using the GS STR POP-4F module—2 min capillary flush with polymer, 5s injection at 15.0 kV, separation at 15 kV, 60°C, 24 min—unless otherwise stated. The capillary temperature was varied from 30–60°C for temperature optimization studies and the sample analysis time was shortened when applicable. After the ABI color matrix was applied to the data, allele sizes were estimated using the local Southern method from GeneScan[®] 3.7 analysis software (Applied Biosystems, Foster City, CA). No manipulation of the raw data was employed other than the default light smoothing incorporated in the GeneScan[®] software.

Results and Discussion

The separation of DNA is essential to a wide variety of biomedical and forensic applications, including DNA sequencing and STR typing. In order to achieve the high resolution, high throughput separations required for these particular applications, a wide variety of replaceable and non-replaceable sieving matrices have been explored and described in the literature for use in capillaries. Replaceable matrices that can easily be loaded into the capillary and removed following separation have become the sieving polymers of choice for DNA separations. An array of polymer properties are considered when choosing the most appropriate matrix for a particular application—including polymer molecular weight and concentration, cost, capillary coating properties, viscosity, and ease of preparation and use. Polymer molecular weight and concentration is of particular importance, since it is well accepted that large DNA fragments are best separated with less concentrated polymer solutions of higher molecular weight, while smaller DNA fragments are more easily resolved with more concentrated polymer solutions of low molar mass (23,24). For sequencing applications, mixtures of high and low molecular weight polymers have been employed for separating DNA fragments over a large molecular weight range. STR applications, however, have a smaller, more defined molecular weight range and, as a result, single polymer solutions can often be utilized effectively for separation.

Evaluation of Candidate Polymers as Separation Matrices for STR Analysis

In considering alternative polymers for separation of STR products, a literature search was performed to identify potential candidates. The self-coating polymer poly(vinylpyrrolidone) (PVP) was first explored—specifically, the 1,000,000 MW polymer described by Gao et al. (25,26). Since it had been determined that a 6.2% w/v solution of PVP was a suitable separation matrix for STR markers, a range of 5–7% PVP was explored in this work. Although reasonably high resolution could be obtained with this polymer, it was noted that extraneous peaks not specific to alleles appeared in a spurious, unpredictable manner (data not shown). This would be highly problematic for casework samples, where extraneous peaks may

result in incorrect typing. In addition, use of PVP with the pump block system employed in the ABI 310 resulted in contamination of the syringe, rendering it ineffective for future separations. For these reasons, PVP was quickly eliminated as a candidate for separations.

In addition to PVP, hydroxyethyl cellulose (HEC) and hydroxypropyl cellulose (HPC) were also explored as candidate polymers. While these polymers have been shown to have excellent self-coating properties and provide reasonable resolution of DNA fragments (24–28), both polymers gave marginal resolution of the allelic ladder, and baseline-resolved, single base resolution could not be achieved with either system (data not shown). In addition, because HPC is thermo-responsive (23) and would be affected by microchip or capillary temperature, it was decided to forego exploring these two polymers further. PEO was also evaluated as a sieving polymer in these separations. Preliminary results in EPDMA-coated capillaries, to minimize electroosmotic flow (EOF), utilizing low percentage (1–2%), low molecular weight (600,000 Da) solutions of PEO were encouraging, with partial resolution of single base pair differences in the allelic ladder at the TH01 loci (data not shown). As such, PEO became the leading candidate for further evaluation in these separations.

PEO as an STR Separation Matrix

It was not surprising that PEO was found to be one of the better candidates since its utility as a matrix for high resolution separations of DNA was well-established (29–34). For both sequencing (29,34) and for the separation of STR fragments (31) a polymer solution of 1.5–1.6% 8,000,000 MW PEO and 1.4–1.5% 600,000 MW PEO was utilized in the presence of 3.5 M urea. However, a critical evaluation of these conditions was warranted for two reasons. First, the DNA fragment size range associated with STR analysis is much narrower than in standard sequencing analysis—consequently, the need for the higher (8,000,000 Da) MW polymer, which increases the viscosity and complexity of the sieving matrix, was questioned. Second, the extensive capillary preparation steps involved (flushing before and in-between runs) in order to exploit the self-coating properties of PEO for elimination of electroosmotic flow (EOF) raised concerns for use in microchips. This was also found to be problematic with the capillary electrophoresis instrument most widely used for STR analysis (ABI 310), because the instrument does not have a pumping mechanism that allows for facile flushing of the capillary. While these flushing steps would be necessary to employ the self-coating properties of any PEO matrix, it could be avoided through the use of a coated capillary. Consequently, this study employed EPDMA as an adsorbed capillary coating to suppress EOF with all of the PEO matrices tested. EPDMA-coated capillaries were prepared off-instrument using a pressure-flush device as described, then the capillaries installed, eliminating the between run flushings required for self-coating. EPDMA has been demonstrated to be a stable, easily utilized, coating for these purposes; however, capillaries coated to minimize

EOF using other coating procedures should also be appropriate for this application.

A separation using the approximate matrix concentration described by Zhang et al. (3)—1.5% 8,000,000 MW, 1.4% 600,000 MW—was performed in an ABI 310 using an EPDMA-coated capillary, but with 3.5 M urea and 40°C, conditions more consistent with those expected for microchip separations. The results shown in Fig. 1 were utilized as a standard for comparison with results obtained using other PEO formulations. Although partial resolution of the 9.3/10 alleles of TH01 was achieved, the peaks were not baseline resolved. In addition, later runs on the same coated capillary showed the presence of extra peaks which could not be identified. Additional separations were performed in which the ratio of the 8,000,000 and 600,000 MW PEO polymers was varied; however, improvements in the separation were not observed (data not shown).

In an effort to reduce the complexity of the separation matrix and decrease the viscosity, single low molecular weight PEO polymer matrices were evaluated to determine their efficacy for STR separations, based on the assumption that the narrow size range of the fragments would allow use of a homogenous polymer solution. Separation matrices in 7 M urea containing a PEO polymer in the molecular weight range from 100,000 – 600,000 Da and at various concentrations were used to separate the fragments in the DNA allelic ladder, in capillaries thermostated at 60°C (Fig. 2A and B). The 100,000 MW PEO was evaluated from 2–7% w/v, the 200,000 MW, from 2–6% w/v and the 600,000 MW from 1–4% w/v with viscosity and separation efficiency trends determining the range of percentages explored with each molecular weight. Figure 2A depicts optimization of polymer percentage using 600,000 MW PEO, from 1 to 4% w/v. From these studies, it was clear that 600,000 molecular weight PEO at 3% gave the best resolution, as this polymer weight and percentage achieved near baseline resolution of the 9.3/10 alleles of TH01 while maintaining resolution of the other alleles in the ladder. This is in good agreement with the results of Fung et al. (29), who noted that approximately 3% total polymer weight mixtures had resulted in optimal resolution. Figure 2B provides exemplary electropherograms, at the PEO concentration that provided the best resolution, for each of the polymer molecular weights tested. It is clear that the 3% (w/v) 600,000 MW PEO provides near baseline single base resolution, while even at their optimal concentration, the other molecular weights did not provide resolution of that quality. Mixtures of low molecular weight polymers were also explored, with the rational that lower molecular weight mixtures might provide equal resolution in shorter analysis times. Keeping the total polymer percentage at 3% (w/v), mixtures of 600,000 and 100,000 MW PEO were made at 75:25, 50:50, and 25:75 ratios. The 50:50 blend gave the best resolution of the mixtures evaluated, however, it did not produce results that surpassed the optimum 600,000 molecular weight results detailed in Fig. 2 (data not shown).

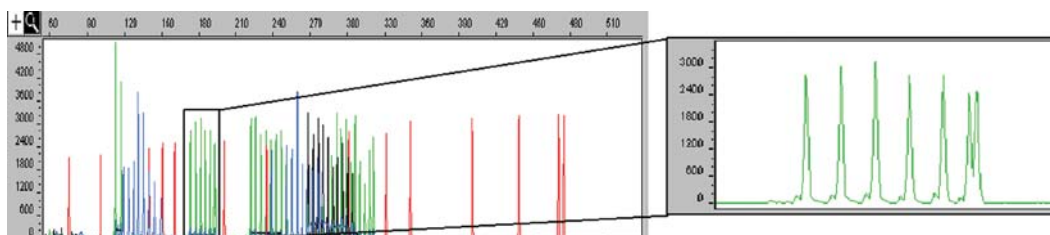


FIG. 1—The TH01 alleles from the Cofiler[®] allelic ladder as separated using a mixture of 1.5% 8,000,000 MW PEO and 1.4% 600,000 MW PEO. Inset shows resolution of the single base difference between the 9.3/10 alleles. Conditions: 47 cm capillary, 36 cm effective length, 40°C, 3.5 M urea.

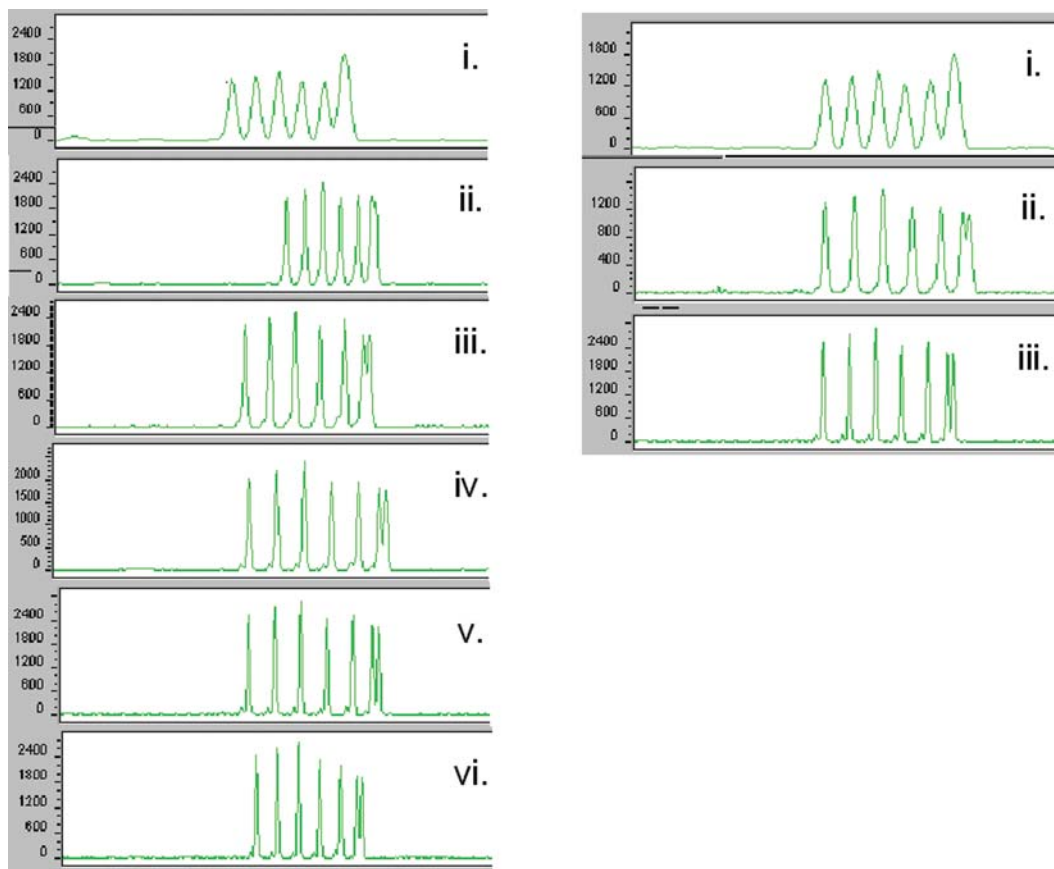


FIG. 2—A) Increasing 600,000 MW PEO polymer concentration (w/v) in buffer: i) 1%; ii) 1.5%; iii) 2%; iv) 2.5%; v) 3%; vi) 4%. B) Resolution obtained with each PEO molecular weight at its optimal concentration (w/v). i) 100,000 MW PEO, 3%; ii) 200,000 MW PEO at 5%; iii) 600,000 MW, 3%. Conditions: 47 cm capillary, 36 cm effective length, 60°C, 7.0 M urea.

Next, the PEO polymer was compared to the commercially supplied polymer, POP-4 (Applied Biosystems, Foster City, CA), a standard separation matrix employed for STR analysis. Consecutive separations of ladder were completed with POP-4 using the manufacturer's recommended conditions (60°C) and compared to separations using 600,000 MW PEO at a concentration of 3.0%. Figure 3 shows typical electropherograms using both POP-4 (A) and PEO (B) with the same separation length and temperature conditions. Note that while POP-4 gave partial resolution of the 9.3/10 alleles, PEO provided a separation that is almost baseline. In addition, the time necessary to complete the full ladder separation is decreased by almost 3 min with the PEO, surpassing the commercial STR analysis polymer both in resolution and run time, issues of critical importance when considering a sieving matrix for inclusion in a microdevice. Thus, with 600,000 MW PEO being decidedly superior to both the commercial POP-4 polymer and other molecular weights of the PEO polymer, it was selected for additional optimization studies.

Having established the PEO molecular weight and concentration that provided optimal resolution, reproducibility of repeat separations was the next focus of this investigation. With the conditions originally used in this system (denaturing 7M urea at 60°C, POP-4 standard conditions) only 8–10 runs could be completed before a significant decrease in resolution was observed (data not shown). To increase the number of runs that could be accomplished before a loss in resolution, two factors—capillary temperature and urea concentration—were investigated. This was based on the observations of Fung et al. (29), who noted that interaction of urea decom-

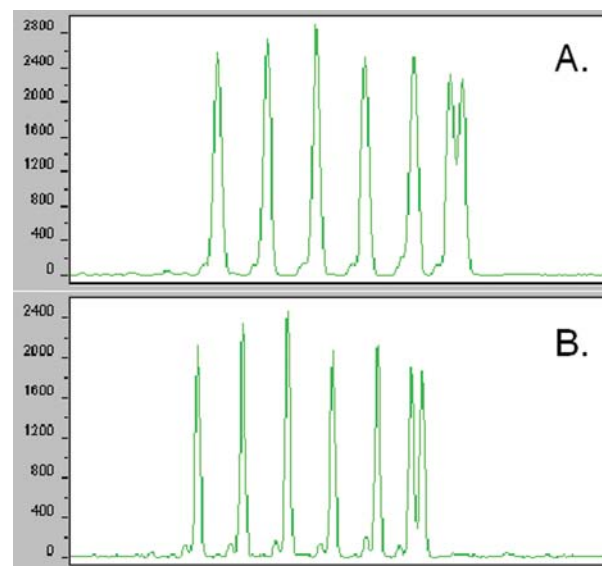


FIG. 3—Comparison of the commercial separation matrix, POP-4 (A), with PEO, 3% (w/v), 600,000 MW, 7.0 M urea (B). Conditions: 47 cm capillary, 36 cm effective length, 60°C.

position products resulted in compromised separation efficiency at higher urea concentrations using the PEO polymer system. High concentrations of urea in polymer solutions have also been reported to degrade separations over time in heated capillary systems (35)

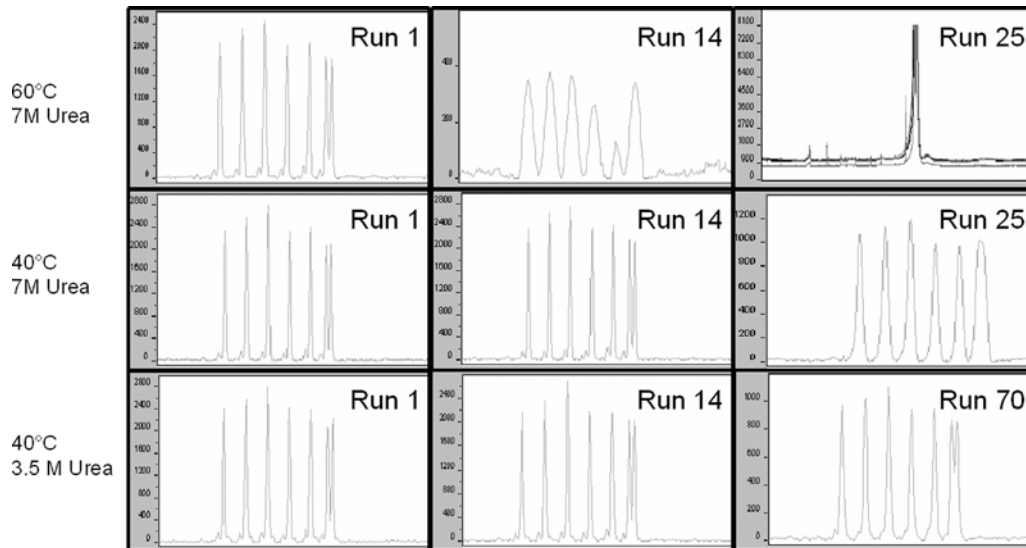


FIG. 4—The effect of temperature and urea concentration on run to run reproducibility. Decreased capillary temperature and urea concentration extends the lifetime of the separation matrix. Conditions: PEO 3%, 600,000 MW, 47 cm capillary, 36 cm effective length, capillary temperature and urea concentration as specified.

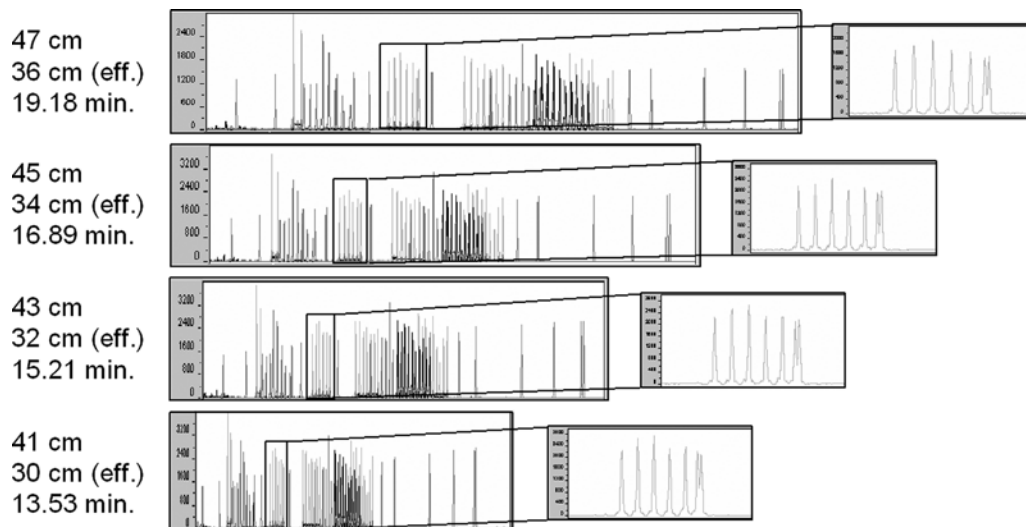


FIG. 5—The effect of decreasing the capillary length on resolution and separation time. Conditions: PEO 3%, 600,000 MW, Capillary length as specified, 40°C, 3.5 M urea.

and, as such, both capillary temperature and urea concentration were evaluated to determine optimal running conditions for repeat separations. As with normal processing using the GS STR POP-4F module on the ABI 310, new polymer in the syringe was flushed through the capillary prior to each separation (Fig. 4). It is undetermined at this point whether the decrease in resolution was the result of matrix degradation (polymer or urea components) during separations, EPDMA coating degradation, or a combination of both. The decreased resolution does not appear to be due to degradation of the sieving matrix in the syringe, but rather due to problems with contamination of the capillary over time. Stripping and recoating of these capillaries restored resolution in subsequent separations.

It is clear that the urea concentration and capillary temperature had dramatic effects on the separation reproducibility. At higher temperature (60°C) and urea concentration (7 M), resolution of the 9.3/10 alleles on TH01 began decreasing significantly by run 8 (Fig. 4, top panel). By lowering the capillary temperature to 40°C and decreasing the concentration of urea to 3.5 M, upwards of 70

consecutive runs could be achieved without appreciable deterioration of resolution or renaturation of single stranded DNA, as depicted in the bottom panel of Fig. 4. While 70 consecutive separations may not be sufficient for some crime labs to switch from the commercial polymer to PEO, it is believed that further evaluation of coatings and separation conditions will be able to extend the longevity of these capillaries.

In order to compare resolution with the commercially supplied STR analysis polymer, POP-4, separations with PEO were initially performed and optimized using a 47 cm capillary, the standard capillary length for STR analysis on the ABI 310. Once running conditions had been sufficiently optimized with the PEO, the capillary was systematically shortened to monitor resolution over the range of STR fragment sizes and the resolution of the 9.3/10 alleles of TH01. Decreasing the capillary length effected shorter run times and explored the feasibility for use of this polymer on a microdevice. Figure 5 details resolution of the 9.3/10 alleles as the capillary is shortened from 36 cm effective length (47 cm

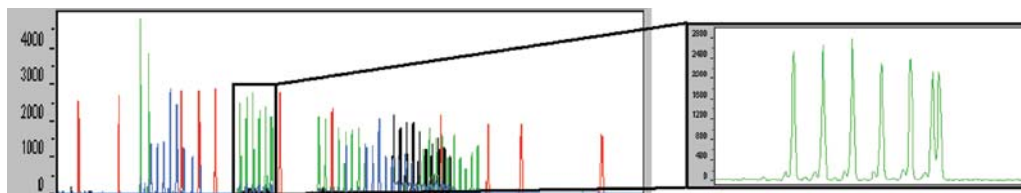


FIG. 6—Resolution of the 9.3/10 TH01 alleles from the COfiler[®] allelic ladder as separated using 3.0% 90,000 MW HEC. Conditions: 47 cm capillary, 36 cm effective length, 40°C, 3.5 M urea.

total), the length used in the previous experiments, to 30 cm effective (41 cm total length). In the microdevice, channels on the order of 17–20 cm would be acceptable for both chip design and speed of separation. Note that, as the capillary was shortened, there was a significant decrease in analysis time, from approximately 19 min to approximately 13.5 min (as determined using the 400 bp fragment of the ROX ladder), and that this decrease is accompanied by minimal degradation of resolution. Due to the constraints of the instrumentation, the capillary could not be shortened to less than 41 cm; however, it is believed that this represents the first step towards optimization of this polymer for short channel separations in microdevices.

In a final set of experiments, once the effective conditions for single stranded DNA separations with PEO had been determined, HEC, originally investigated only using the high-temperature (60°C), high-urea concentration (7 M) of traditional separations, was re-evaluated. As depicted in Fig. 6, low molecular weight HEC (90,000 Da) at 3% provided reasonably high resolution of the 9.3/10 alleles of TH01, without compromising separation of the larger alleles, using the lower temperature and urea concentration. In addition, the time required to achieve this separation was essentially the same as with PEO, approximately 19 min in the 36 cm effective length capillary. When compared with PEO, HEC provided almost equivalent resolution with optimized PEO separations, however, the solution was more viscous. As highlighted before, viscosity is of great concern when considering a matrix for inclusion in a microdevice, thus PEO is the more favorable separation matrix at this time. However, the separation provided by HEC, like PEO, was superior in both run time and resolution to the commercial polymer POP-4. This suggests that there may be a number of other polymeric matrices that could be optimized and tailored for effective separation of STR fragments in both capillaries and microdevices, and the ones explored here do not represent an exhaustive list.

Conclusions

With a view to STR analysis on microdevices employing relatively short separation lengths, a variety of polymeric separation matrices have been evaluated to determine polymers capable of effecting reproducible, high-resolution separations of STR DNA fragments. Although HEC and PVP, commonly used polymers for DNA separations, provided resolution of STR fragments, 600,000 MW poly(ethylene oxide) (PEO) at a 3% (w/v) concentration was determined to be the most suitable matrix for these separations. This polymer, in coated capillaries, provided highly robust and reproducible separations, with near baseline resolution of fragments having single base differences. Reductions in the temperature of the separation, from 60°C to 40°C, and the urea concentration of the buffer, from 7 M to 3.5 M, provided impressive longevity of the PEO polymer for repeated separations. In fact, comparison of this polymer with currently specified commercial products used for STR analysis showed that the optimized PEO matrix provided superior separations under all conditions tested, indicating that this polymer

might be useful in current conventional CE analyses. In addition, PEO could be utilized in shorter capillary systems, at lower temperatures with limited loss of resolution, making it a viable candidate for extrapolation to microdevices.

References

- Schmalzing D, Koutny L, Adourian A, Belgrader P, Matsudaira P, Ehrlich D. [DNA typing in thirty seconds with a microfabricated device](#). *Proc Natl Acad Sci* 1997;94:10273–8. [[PubMed](#)]
- Mansfield ES, Robertson JM, Vainer M, et al. Analysis of multiplexed short tandem repeat (STR) systems using capillary array electrophoresis. *Electrophoresis* 1998;19:101–7. [[PubMed](#)]
- Zhang N, Yeung ES. [Simultaneous separation and genetic typing of four short tandem repeat loci by capillary electrophoresis](#). *J Chromatogr A* 1997;768:135–41.
- Butler JM, McCord BR, Jung JM, Allen RO. Rapid analysis of the short tandem repeat HUMTH01 by capillary electrophoresis. *Biotechniques* 1994;17:1062–4, 1066, 1068 passim. [[PubMed](#)]
- Buel E, LaFontain M, Schwartz M, Walkinshaw M. Evaluation of capillary electrophoresis performance through resolution measurements. *J Forensic Sci* 2001;46:341–5. [[PubMed](#)]
- Schmalzing D, Adourian A, Koutny L, Ziaugra L, Matsudaira P, Ehrlich D. [DNA sequencing on microfabricated electrophoretic devices](#). *Anal Chem* 1998;70:2303–10. [[PubMed](#)]
- Schmalzing D, Koutny L, Salas-Solano O, Adourian A, Matsudaira P, Ehrlich D. [Recent developments in DNA sequencing by capillary and microdevice electrophoresis](#). *Electrophoresis* 1999;20:3066–77. [[PubMed](#)]
- Schmalzing D, Belenky A, Novotny MA, et al. [Microchip electrophoresis: a method for high-speed SNP detection](#). *Nucleic Acids Res* 2000;28:E43. [[PubMed](#)]
- Schmalzing D, Koutny L, Adourian A, Chisholm D, Matsudaira P, Ehrlich D. Genotyping by microdevice electrophoresis. *Methods in Mol Bio* 2001;163:163–73.
- Paegel BM, Emrich CA, Wedemayer GJ, Scherer JR, Mathies RA. [High throughput DNA sequencing with a microfabricated 96-lane capillary array electrophoresis bioprocessor](#). *Proc Natl Acad Sci* 2002;99:574–9. [[PubMed](#)]
- Paegel BM, Blazej RG, Mathies RA. [Microfluidic devices for DNA sequencing: sample preparation and electrophoretic analysis](#). *Curr Opin Biotechnol* 2003;14:42–50. [[PubMed](#)]
- Medintz IL, Paegel BM, Mathies RA. [Microfabricated capillary array electrophoresis DNA analysis systems](#). *J Chromatogr. A.* 2001;924:265–70. [[PubMed](#)]
- Koutny L, Schmalzing D, Salas-Solano O, et al. [Eight hundred-base sequencing in a microfabricated electrophoretic device](#). *Anal Chem* 2000;72:3388–91. [[PubMed](#)]
- Ehrlich DJ, Matsudaira P. [Microfluidic devices for DNA analysis](#). *Trends in Biotechnology* 1999;17:315–9. [[PubMed](#)]
- Woolley AT, Mathies RA. [Ultra-high-speed DNA sequencing using capillary electrophoresis chips](#). *Anal Chem* 1995;67:3676–80. [[PubMed](#)]
- Kheterpal I, Mathies RA. Capillary array electrophoresis DNA sequencing. *Analytical Chemistry* 1999;71:31A–37A. [[PubMed](#)]
- Blazej RG, Paegel BM, Mathies RA. [Polymorphism ratio sequencing: a new approach for single nucleotide polymorphism discovery and genotyping](#). *Genome Res* 2003;13:287–93. [[PubMed](#)]
- Wang Y, Wallin JM, Ju J, Sensabaugh GF, Mathies RA. High-resolution capillary array electrophoretic sizing of multiplexed short tandem repeat loci using energy-transfer fluorescent primers. *Electrophoresis* 1996; 17:1485–90. [[PubMed](#)]

19. Huang XC, Quesada MA, Mathies RA. [DNA sequencing using capillary array electrophoresis](#). *Anal Chem* 1992;64:2149–54. [\[PubMed\]](#)
20. Salas-Solano O, Schmalzing D, Koutny L, et al. [Optimization of high-performance DNA sequencing on short microfabricated electrophoretic devices](#). *Anal Chem* 2000;72:3129–37. [\[PubMed\]](#)
21. Chiari M, Cretich M, Damin F, Ceriotti L, Consonni R. [New adsorbed coatings for capillary electrophoresis](#). *Electrophoresis* 2000;21:909–916. [\[PubMed\]](#)
22. Chiari M, Cretich M, Stastna M, Radko SP, Chrambach A. [Rapid capillary coating by epoxy-poly\(dimethylacrylamide\): Performance in capillary zone electrophoresis of protein and polystyrene carboxylate](#). *Electrophoresis* 2001;22:656–9. [\[PubMed\]](#)
23. Kan CW, Barron AE. [A DNA sieving matrix with thermally tunable mesh size](#). *Electrophoresis* 2003;24:55–62. [\[PubMed\]](#)
24. Bunz AP, Barron AE, Prausnitz JM, Blanch HW. [Capillary electrophoretic separation of DNA restriction fragments in mixtures of low- and high-molecular-weight hydroxyethylcellulose](#). *Ind & Eng Chem Res* 1996;35:2900–8. [\[PubMed\]](#)
25. Gao Q, Pang HM, Yeung ES. [Simultaneous genetic typing from multiple short tandem repeat loci using a 96-capillary array electrophoresis system](#). *Electrophoresis* 1999;20:1518–26. [\[PubMed\]](#)
26. Gao Q, Yeung ES. [A matrix for DNA separation: genotyping and sequencing using poly\(vinylpyrrolidone\) solution in uncoated capillaries](#). *Anal Chem* 1998;70:1382–8. [\[PubMed\]](#)
27. Barron AE, Soane DS, Blanch HW. [Capillary electrophoresis of DNA in uncross-linked polymer-solutions](#). *J Chromatogr A* 1993;652:3–16. [\[PubMed\]](#)
28. Barron AE, Sunada WM, Blanch HW. [Capillary electrophoresis of DNA in uncrosslinked polymer solutions: Evidence for a new mechanism of DNA separation](#). *Biotechnol and Bioeng* 1996;52:259–70. [\[PubMed\]](#)
29. Fung EN, Pang HM, Yeung ES. [Fast DNA separations using poly\(ethylene oxide\) in non-denaturing medium with temperature programming](#). *J Chromatogr A* 1998;806:157–64. [\[PubMed\]](#)
30. Preisler J, Yeung ES. [Characterization of nonbonded poly\(ethylene oxide\) coating for capillary electrophoresis via continuous monitoring of electroosmotic flow](#). *Anal Chem* 1996;68:2885–9. [\[PubMed\]](#)
31. Zhang NY, Yeung ES. [Simultaneous separation and genetic typing of four short tandem repeat loci by capillary electrophoresis](#). *J Chromatogr A* 1997;768:135–41. [\[PubMed\]](#)
32. Chang HT, Yeung ES. [Poly\(ethyleneoxide\) for high-resolution and high-speed separation of DNA by capillary electrophoresis](#). *J Chromatogr B* 1995;669:113–23. [\[PubMed\]](#)
33. Fung EN, Yeung ES. [High-speed DNA-sequencing by using mixed poly\(ethylene oxide\) solutions in uncoated capillary columns](#). *Anal Chem* 1995;67:1913–9. [\[PubMed\]](#)
34. Wei W, Yeung ES. [Improvements in DNA sequencing by capillary electrophoresis at elevated temperature using poly\(ethylene oxide\) as a sieving matrix](#). *J Chromatogr B* 2000;745:221–30. [\[PubMed\]](#)
35. Todorov TI, Yamaguchi Y, Morris MD. [Effect of urea on the polymer buffer solutions used for the electrophoretic separations of nucleic acids](#). *Anal Chem* 2003;75:1837–43. [\[PubMed\]](#)

Additional information and reprint requests:

Jerome P. Ferrance, PhD.
Department of Chemistry
P.O. Box 400319
McCormick Road
University of Virginia
Charlottesville, VA 22904