Typing Human DNA Using Capillary Electrophoresis: Comparison of Slab Gel and Capillary Formats

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Capillary electrophoresis (CE) is a high resolution microanalytical technique which can be used to separate biological molecules such as DNA (1-3). Compared to conventional slab gel electrophoresis, CE provides rapid run times and similar run-to-run precision. Slab gel electrophoresis of PCR products generally requires 2-5 hours for separation and detection. Thin-walled fused silica capillaries allow for greater heat dissipation and consequently higher voltages (as high as 30,000 Volts). An increase in voltage results in run times of approximately 30-50 minutes for PCR amplified short tandem repeat (STR) alleles or even larger restriction fragments (4). Instruments available on the market to date, however, only provide single column capabilities. Therefore, with a single column instrument 29-48 injections can be made in a twenty-four hour period. With auto-sampling capabilities, the amount of hands-on time is minimized. Nevertheless, the current level of through-put cannot compete with manual or automated slab gel analysis. Development of multi-capillary instruments (24-96 capillary formats), will allow for high through-put analysis in the near future. Consequently, on a multi-capillary instrument greater than 3000 injections could be made in a single day.

The results presented in this short paper attempt to illustrate the precision and reproducibility of the Dionix CES1A CE. The CE results were then compared to data generated on the Applied Biosystems (AB) 373A Gene Sequencer. Comparisons were made using the short tandem repeat locus HUMTHO1 (5,6). This locus has received a favorable evaluation by the forensic community and was chosen as a model for the development of CE for forensic casework. PCR conditions for CE experiments were as follows; 50 μl PCR containing 10 mM Tris-HCl, pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$, 0.2 mM dATP, dCTP, dGTP, dTTP, 2.5 units of Taq DNA Polymerase (Cetus), 5-10 ng of target DNA, 0.01% gelatine, and 1 X HUMTHO1 Primer Pair (Promega; a HUMTHO1 allele specific ladder was also provided by Promega). Thermal cycler parameters (Perkin Elmer 9600) were as follows; 95°C hold for 2 minutes, 10 cycles of 94°C for 30 seconds, 64°C for 30 seconds, and 70°C for 45 seconds, and 20 cycles of 90°C for 30 seconds, 64°C for 30 seconds, and 70°C for 45 seconds. Separation of PCR product was performed on a non-denaturing μPage3^{TM} (3%C,3%T) polyacrylamide gel column with a 40 cm effective length, using μPage3^{TM} buffer (J&W Scientific). Ethidium bromide was added (10 μM) to the buffer prior to separation. PCR product or allelic ladder was subjected to dialysis to remove excess salts using VS 0.025 μm membranes (Millipore) according to Williams (1992). Nanoliter (nl) volumes of PCR product were loaded onto the column using electrokinetic injection. Separations were performed at 210 V/cm in the presence of a molecular weight marker (Boehringer Mannheim, DNA Marker XI, 50-1000 base pairs).

Initial questions were addressed concerning the accuracy and reproducibility of CE when sizing AmpFLP alleles. The data presented in Table 1 suggests that when using non-denaturing polyacrylamide gel capillaries (3%T,3%C), the accuracy of sizing HUMTHO1

alleles using the molecular weight marker is relatively poor. Nevertheless, the reproducibility of the sizing information from run-to-run is excellent. While the run-to-run data is reproducible, the day-to-day reproducibility is variable. Consequently, allele specific ladders are required as a daily standard in order to accurately type DNA samples. The results from Figure 1 illustrate that when using the allele specific ladder in conjunction with the PCR-based molecular weight marker, individual genotypes can be accurately and reproducibly determined. CE profiles of the allelic ladder and three individuals were overlaid, and based on retention times and size determinations, allele designations were made. The genotypes determined for the three individuals were consistent with the AB Gene Sequencer results.

In contrast to CE, the day-to-day, gel-to-gel reproducibility of the AB Gene Sequencer is excellent as reported in Table 2a. In addition, reproducibility is consistent across most of the STR loci tested (STR loci which exhibit micro-heterogeneity are more difficult to analyze). PCR conditions were similar to those described above, with the exception of the relative position of the primer pair and the thermal cycler parameters (7). Allele size determination is more precise when using the Gene Sequencer, although the observed values are still slightly different from the expected values. In general, the observed values differ from the expected by 1-2 base pairs in denaturing gels, and by 3.5-6 base pairs within native (non-denaturing) gels (8). This is consistent with the data presented here from native capillary gels. Nevertheless, the genotypes determined for the three individuals using a binning technique were identical to the genotypes determined by CE analysis (Table 2b). The Gene Sequencer and CE results were verified by conventional vertical polyacrylamide gel electrophoresis and silver staining. The PCR product was run next to a lane containing the identical allelic ladder used in the CE study.

The development of CE for forensic casework is continuing. Software specifically targeted for sizing STR alleles using retention times of allelic and molecular weight markers may enhance the day-to-day precision. Numerous generations of AB 672 Gene Sequencer software have dramatically improved sizing reproducibility. In addition, data management software (similar to the AB GenoTyper), which allows for unbiased typing and minimizes transcription errors, must be developed for CE. Through fluorescence detection and multi-column instrumentation, CE should be a powerful method for rapid, precise typing of DNA in the near future.

TABLE 1: Dionix CES1A CE Precision Data

Allele Number (Repeat Units)(Expected Size Value)	Day 1 (Mean & Standard Deviation)(7 Data Points)	Day 2 (Mean & Standard Deviation)(3 Data Points)	Day 3 (Mean & Standard Deviation)(3 DataPoints)
5 (179 bp Expected Size)	179.23 bp & 0.24 bp	178.36 bp & 0.03 bp	177.25 bp & 0.02 bp
6 (183 bp Expected Size)	183.34 bp & 0.15 bp	182.02 bp & 0.13 bp	179.84 bp & 0.15 bp
7 (187 bp Expected Size)	186.09 bp & 0.16 bp	184.75 bp & 0.21 bp	182.60 bp & 0.05 bp
8 (191 bp Expected Size)	189.01 bp & 0.18 bp	187.90 bp & 0.13 bp	186.36 bp & 0.04 bp
9 (195 bp Expected Size)	192.75 bp & 0.10 bp	191.61 bp & 0.05 bp	189.12 bp & 0.03 bp
10 (199 bp Expected Size)	195.82 bp & 0.27 bp	194.42 bp & 0.05 bp	191.84 bp & 0.02 bp
11 (203 bp Expected Size)	199.03 bp & 0.35 bp	197.44 bp & 0.04 bp	195.24 bp & 0.02 bp

TABLE 2a: Applied Biosystems 373A Gene Sequencer Precision Data

Allele Number (Repeat Units)(Expected Size Value)	Size Variance (Largest Difference)	Mean & Standard Deviation (309 Data Points, 6 Gels on 6 Different Days)
6 (159 bp Expected Size)	0.48 bp	157.97 bp & 0.089 bp
9 (171 bp Expected Size)	0.42 bp	170.41 bp & 0.074 bp

TABLE 2b: DNA Typing of Three Individuals (MMH, RKR, DLF) Using the Applied Biosystems 373A Gene Sequencer

DNA Identifier	Allele Size Value	Allele Number Designations Using the Binning Listed Below*
MMH	173.56 bp (173.56)	10,10
RKR	166.37 bp 170.56 bp	8,9
DLF	158.16 bp 170.42 bp	6,9

*Bin Boundaries: Allele 5 - 153.00-155.15; Allele 6 - 157.00-159.15; Allele 7 - 161.00-163.15; Allele 8 - 165.00-167.15; Allele 9 - 169.00-171.15; Allele 10 - 173.00-175.15; Allele 11 - 177.00-179.15.

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FIGURE 1: CE Typing Data - The CE traces for MMH, DLF, and RKR were overlaid onto the HUMTHO1 allele specific ladder run on the same day, on the same capillary. The asterisk in each overlay represent the alleles which line up with the ladder. Each set of overlays is labeled with one of the three individuals and the resulting genotype.