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Evaluation and validation of the ABI 3700, ABI 3100, and the MegaBACE 1000 capillary array electrophoresis instruments for use with short tandem repeat microsatellite typing in a forensic environment

The demand for high-throughput DNA profiling has increased with the introduction of national DNA databases and has led to the development of automated methods of short tandem repeat (STR) profile production; however, a potential bottleneck still exists at the gel electrophoresis stage. Capillary electrophoresis sequencers capable of processing 96 samples with considerably reduced manual intervention are now available. In this paper, we compare the ABI Prism 377 slab-gel sequencer currently used by the Forensic Science Service with three leading capillary array electrophoresis instruments: the ABI Prism 3700, the Amersham MegaBACE 1000 and the 16-capillary ABI Prism 3100. We describe the experiments used to evaluate and validate these platforms for forensic use with the STR multiplex Ampf/STR SGMplus [1, 2], along with comparative data from the ABI Prism 377 sequencer.

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1 Introduction

Polyacrylamide electrophoresis in a slab-gel format is a well-established method of separating short tandem repeats (STRs) for forensic DNA profiling [3–5]. The ABI Prism 377 sequencer is widely used for this process and has been the platform of choice within the Forensic Science Service (FSS) for both casework and databasing since 1995 [1]. However, the increased demand for forensic profiling resulting from the rapid expansion of the National DNA Database (NDNADB) has led to the introduction of automated processes for DNA extraction, quantification and PCR, which has, in turn, resulted in a bottleneck at the gel running stage. This has highlighted a number of limitations of the ABI 377 instrument for current needs. With the introduction of automation into the process of generating STR profiles an instrument that is fully compatible with the higher throughput and which requires less manual intervention would allow samples to be processed faster and at a lower cost. These advances are offered by capillary electrophoresis (CE) instruments that have been evaluated for use by the FSS. The capillary array instruments separate DNA by size, through the application of a current across a non-cross-linked polymer matrix. This technology allows the simultaneous resolution of between 1 and 96 DNA samples within separate capillaries, depending on the instrumentation used. Capillary array electrophoresis (CAE) has been used to increase sequence throughput [7–10] and sequence generated by these means was successfully used in court as early as 1997 [14]. Recent developments have improved ease of use and robustness and, although principally designed for sequencing applications, CE instruments are being increasingly used for genotyping [11–13].

In this paper, we compare the ABI Prism 377 slab-gel sequencer currently used by the FSS with three leading CAE instruments: the ABI Prism 3700, the Amersham MegaBACE 1000 and the 16-capillary ABI Prism 3100. We describe the experiments used to evaluate and validate these platforms for forensic use with the STR multiplex Ampf/STR SGMplus [1, 2], along with comparative data from the ABI Prism 377 sequencer. A number of key acceptance criteria were examined, including correct designation of known sample profiles, precision, resolution, and sensitivity.

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Abbreviations: CAE, capillary array electrophoresis; **FSS**, Forensic Science Service; **PMT**, photomultiplier tube; **rfu**, relative fluorescence units

2 Materials and methods

2.1 Instrumentation

2.1.1 ABI Prism 3700

The ABI Prism 3700 instrument has a total of 110 capillaries, 96 of which are active at one time, 8 of which are spare, and 6 of which control the sheath flow system. The capillaries are usually made from a silica resin with an external surface coating of plastic making them flexible and also creating a protective sheath. Each capillary is 50 cm in length with an internal diameter of 50 μm. The inner surface is not coated. Sample DNA is transferred indirectly from a microtitre plate, *via* a robotic arm and load bar, for electrokinetic injection into the capillaries, prior to electrophoresis. The detection system is comparable to that found on the ABI Prism 377 sequencer, where the emissions of fluorophore-labeled DNA fragments are detected by a CCD camera following laser excitation. The ABI Prism 3700 instrument requires less time to process 96 samples than the ABI Prism 377 instrument; a batch of 96 samples has a processing time of approximately 2.75 h as opposed to approximately 3.5 h (not including gel preparation), and requires significantly less manual intervention. The ABI 3700 instrument can also run four plates from each setup allowing overnight runs without supervision. Electrophoretic separation of fluorescently labeled DNA fragments is monitored within the sheath flow system [6, 10]. The beam from the fixed argon laser emits at 488 nm and 514.5 nm and passes through each capillary's spatial position from 96 to 1, with excitation of fluorescently labelled DNA taking place externally at the capillary tips within the sheath flow. This arrangement may lead to a reduction of laser intensity toward the lower-numbered capillaries, resulting in a lower sensitivity at this end of the array. Emitted light from the excited fluorophores is detected *via* the CCD camera between 530 nm and 700 nm enabling five dye colors to be detected within each data collection time frame.

2.1.2 ABI 3100

The ABI 3100 instrument is a 16-capillary sequencer, which electrokinetically injects DNA directly from the sample preparation plate. The capillaries are not coated and each capillary is 36 cm in length and has an internal diameter of 50 μm. The instrument is able to electrophorese 16 samples per run with a complete run time of approximately 45 min. Therefore, the time taken to process one 96-well microtitre plate is approximately 4.5 h. The instrument uses the same signal source laser system as the ABI 3700 but, unlike the ABI 3700, array illumination is performed from both sides with the use of beam splitters and internal laser reflection. The detection cell is the same as the ABI 3700 instrument, although DNA detection takes place from within capillaries and not within a detection cuvette.

2.1.3 MegaBACE 1000

The MegaBACE 1000 is a 96-capillary array instrument with DNA injection into the capillaries carried out directly from the microtitre plate. The capillaries are 40 cm in length, are coated and have an internal diameter of 75 μm. The MegaBACE 1000 is also able to electrophorese 96 samples within one run, but requires greater manual intervention with the user responsible for exchanging buffer and sample troughs before each run. However, run times are greatly reduced with each plate taking approximately 1.75 h to process. Excitation of fluorescently labeled DNA is *via* two scanning lasers and detection is performed within capillaries. The forward scanning laser is a blue argon laser emitting at 488 nm while the other is a green solid-state laser emitting at 532 nm. Each laser scan leads to the excitation and detection of two dye colours, the blue laser detecting 6-carboxyfluoroscein (6-FAM) and 6-carboxyrhodamine X (ROX), and the green laser 6-carboxy-4',5'-dichloro-2',7'-dimethyluronium (JOE) and NED-2 amidite (NED) fluorescent dyes (blue, red, green, and yellow, respectively). The light emitted from each of these fluorescent dyes is collected and converted to a digital signal by photomultiplier tubes (PMTs).

2.2 Interpretation

2.2.1 Allele designation

Correct designation was defined as an absence of differences between the ABI Prism 377 and CE sequencer in the assignment of fragments to allele classes. This was checked in an approximately 1000-sample profile study, which also validated the various sizing regimes, and was used to produce the interpretation guidelines used for ABI Prism 3700 and ABI 3100 instrument data. The number of full sample profiles compared was 1129 for the ABI 3100 instrument and 1112 for the ABI 3700 instrument.

2.2.2 Instrument precision

A precision study, consisting of a number of allelic ladder runs, gave an indication of the frequency of misdesignation. Previous precision studies on a number of different CE platforms have demonstrated a decreased sizing accuracy with increased DNA fragment size [12, 13] and the importance of an internal size standard migrating at a linear rate in achieving precise sizing. Sizing precision is an indicator that the instrument is performing consistently rather than sizing accuracy, which is a measure of how

closely fragments are sized to their true length. Differences are observed in assigned allele sizes when using different instruments, separation matrices, and size standards [12, 13]. Sequence variation within STR allele length classes might affect migration and thus precision [20], consequently multiple injections and runs of the same allelic ladder were used to measure precision.

2.2.3 Resolution

Resolution studies involved samples or ladders with alleles differing by a single base pair, *e.g.*, HUMTH01 9.3/10. Visual comparison provided an indication of how easily peaks could be distinguished from one another by an interpreter, an important factor that could result in misgenotyping and the reprocessing of samples.

2.2.4 Sensitivity

Instrument sensitivity is extremely important for interpretation, as poor signal strength can result in poor morphology and the potential for errors in sizing. This impacts on the upstream processes if more DNA is required for profiling. Little or no change in sensitivity within an instrument, from run to run, and also between instruments of the same type is also critical when an automated process is being developed with little scope for day-to-day changes in sample preparation.

2.2.5 Contamination

Because of the likelihood of data produced on the sequencer being presented as evidence in court there must be no possibility of errors occurring in interpretation of the data. Therefore, any instrument used for a forensic application must be examined for the potential of contamination occurring, and if contamination does occur this must be fully identified and characterized. Contamination can be both physical, as in persistence of DNA within a capillary, and signal, as in capillary-to-capillary cross-talk of fluorescent light.

2.3 DNA extraction

DNA was extracted from buccal swabs (Whatman International, Maidstone, UK) using an automated Qiagen (Hilden, Germany) procedure [21, 22] on a Packard Automated Liquid Handling Station (Instrument, Downers Grove, IL, USA). In brief, 300 μL PBS, 0.01 M phosphate buffer, 0.0027 M KCl, 0.137 M NaCl, pH 7.4 and 300 μL AL lysis buffer (Qiagen, proprietary) was added to the swab and incubated at 70°C for 10 min. Following incubation, 300 μL of absolute ethanol was added and the solution applied to the QIAamp columns. The bound DNA was then washed twice with 500 μL of AW buffer (Qiagen), and eluted in 300 μL TE (1 mM Tris, 0.1 mM EDTA).

2.4 Amplification

The Amp*f*/STR SGM Plus kit (Applied Biosystems, Foster, City, CA, USA) was used for multiplex amplification of ten STR loci and the sex marker amelogenin [2]. Amplification reactions were prepared on a Hamilton Liquid Handling Station (Hamilton, Reno, NV, USA). Between 1.5 and 2.5 ng of DNA template was used in a 25 μL polymerase chain reaction volume (except where stated). Reactions were amplified on ABI 9600 Thermocyclers (Applied Biosystems) under the following parameters: 95°C for 11 min; 28 cycles of 94°C for 1 min, 59°C for 1 min and 72°C for 1 min; 60°C for 45 min final extension. Samples generated manually were also set up in 25 μL reaction volumes.

2.5 Post-amplification sample preparation and electrophoresis

2.5.1 ABI 377 instrument

PCR product (1.5 μL) was mixed with 1.5 μL GS-ROX XL 500 size standard with formamide/dextran blue (supplied by Applied Biosystems). Formamide/dextran blue and size standard were mixed at a 1:10 ratio. Allelic ladder control (1.5 μL) was added to the size standard formamide (BDH, Poole, Dorset, UK)/dextran blue mixture (Sigma, St.Louis, MO, USA). The samples were denatured at 90°C for 2 min and then snap-cooled on ice. An aliquot of 0.8 μL of the sample or allelic ladder mix was then loaded onto a 48-lane slab gel and electrophoresed at 3 kV and 50°C, for approximately 2.5 h through a 5% polyacrylamide gel (Long Ranger; BioWhittaker, Walkersville, MD, USA). This protocol has been optimized for use with STR multiplex systems [1, 2].

2.5.2 ABI 3700 instrument

PCR product (1.5 μL) was added to 13.5 μL HIDI formamide + GS-400HD ROX size standard mix (both components supplied by Applied Biosystems), prepared in a 1:40 ratio. Allelic ladder control (1.5 μL) was mixed with 10.5 μL size standard/HIDI formamide for electrophoresis. Samples were injected into the capillaries for 11 s at 10 kV, and electrophoresed at 7.5 kV and 50°C for a total of 2.75 h, using a cuvette temperature optimized for the specific instrument (either 46°C or 47°C). All other run parameters we left at default. DNA separation took place through POP-6, Performance Optimized Polymer (Applied Biosystems). Samples set up manually for the ABI Prism 3700 sequencer comprised 1 μL PCR product

added to 9 μL HIDI formamide/size standard mix, and 1 μL allelic ladder added to 7 μL HIDI formamide/size standard mix. Reagent preparation and run conditions were optimized beforehand (data not shown).

2.5.3 ABI 3100 instrument

PCR product (1.5 μL) was added to 13.5 μL HIDI formamide + GS-400HD ROX size standard mix, prepared in a 1:38 ratio. Allelic ladder control (1.5 μL) was mixed with 10.5 μL size standard/HIDI formamide for electrophoresis. Samples were injected into the capillaries in batches of 16 samples directly from the microtitre plate for 10 s at 3 kV. Electrophoresis was performed at 15 kV and 60°C for 45 min at default run conditions. Reagent preparation and run conditions were optimized beforehand (data not shown).

2.5.4 MegaBACE 1000 instrument

PCR product (3.5 μL) was added to 7.5 μL HIDI formamide + ET-ROX 400 size standard mix (ET-ROX supplied by Amersham Biosciences, Piscataway, NJ, USA). Samples were injected directly from the microtitre plate at 5 kV for 90 s and electrophoresed at 10 kV for 65 min at 60°C. Reagent preparation and run conditions were optimized beforehand (data not shown).

2.6 Data manipulation and analysis

Data obtained from the ABI Prism 377 sequencer were analysed using Genescan 2.1 software (Applied Biosystems) applying the local Southern sizing algorithm [23]. Data produced on the ABI Prism 3700 and ABI Prism 3100 instruments were autoextracted and analysed on the instrument, using ABI Genescan Collection and Analysis software Version 3.5 (Applied Biosystems), again using the local Southern sizing method. Analysed data from the ABI machines were then typed using Genotyper 1.1.1 or Genotyper 3.5nt software (Applied Biosystems) and relevant information on designation, molecular weight (base pairs), peak height (relative fluorescence units, rfu), and/or peak area (rfu) was exported to Microsoft Excel for manipulation. Data obtained on the MegaBACE 1000 were extracted and analysed on the instrument with Genetic Profiler Version 1.1 (Amersham Biosciences) using the Cubic Spline sizing algorithm. Information on molecular weight (bp), peak height (rfu) and peak width (rfu) were exported to Microsoft Excel for statistical manipulation and evaluation. Data from the MegaBACE 1000 instrument were also analysed using an automated analysis software, True Allele™. The software applies a series of sizing algorithms and rule sets to size and designate the detected DNA [24]. Because of differences in precision between the instruments, a novel method of sizing was introduced for the ABI 3700 CE platform [25, 28]. Averaging the positions of each allele from between five and eight ladders produced a virtual ladder; four ladders were used on some occasions to test the robustness of the method. These averages were then imported into Genotyper 1.1.1 to size sample alleles. Sizing within the ABI 3100 instrument was carried with one ladder within each run of 16 capillaries. Sizing for MegaBACE 1000 data was carried out using one ladder per 96 capillaries, but this was more a reflection of software restrictions rather than sizing precision.

3 Results

3.1 Concordance study

Concordance studies were carried out with the ABI Prism 3700, the ABI Prism 3100 and the ABI Prism 377 instruments. The MegaBACE 1000 instrument was excluded from this due to the unavailability of software capable of typing the collection of reference samples. Comparisons were made of 1112 sample profiles generated on the ABI Prism 3700 and 1129 from the ABI Prism 3100 instruments with corresponding profiles generated on the ABI Prism 377 sequencer. For the ABI 3700 and ABI 3100 instruments, an additional set of 36 and 61 samples, respectively, showing rare STR alleles, mutations and trisomy, were analysed and compared with the ABI 377 and again no differences were observed (Fig. 1).

The second part of the concordance study compared the allele peak height and peak area (in rfu) of full sample profiles between the instruments. Direct comparison was made between the ABI 3700 and ABI 377 instruments and the ABI 3100 and ABI 3700 instrument (Fig. 2) and it was demonstrated that peak balance was comparable between all three instruments. Peak characteristics were analysed initially by peak area, and then peak height. MiniTab™ was used to generate statistics for the data set, with peak balance measured as the ratio obtained from peaks from a heterozygote profile for each locus. Ideally peak balance should equal 1 indicating peaks of the same height and area. Again, the MegaBACE 1000 was excluded from this part of the study due to unavailability of suitable software.

The same data set was used to make comparisons of artefacts and again showed very little difference between the instruments, with regard to stutter peaks and *n*-peaks. Stutter peaks are allelic in origin, and are thought to arise

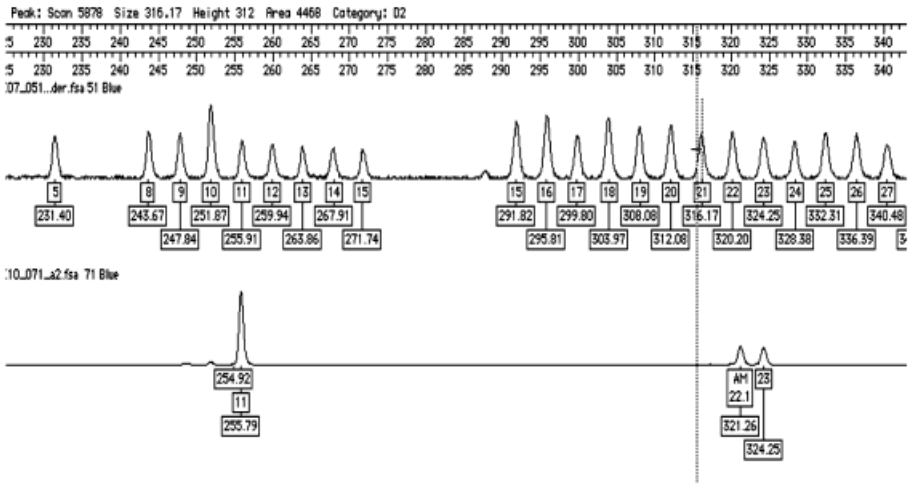

Figure 1. Electropherogram of a rare allele, D2S1338 22.1, generated using the Amp*f*/STR SGM Plus Kit at 28 cycles from Qiagen extracted DNA, detected on the ABI Prism 3700 platform sizing approximately 1.06 bp away from the control allele on the allelic ladder.

from slippage of the *Taq* polymerase enzyme, or referred to as slip strand displacement. Stutters are normally 4 bp lower in molecular weight than the allelic peak, however stutters 2 bp lower than the allelic peak have been observed occasionally and may be seen in conjunction with 4 bp stutters. Stutters normally occur in pairs when the locus is heterozygous. From experimental observation [26], 4 bp stutters tend to be $<$15% the size of the associated allelic peak. The more unusual 2 bp stutter peaks are generally smaller and those observed were always $<$6% of the associated allele peak. Stutter characteristics did show some variation on the ABI 3700 instrument, and this was attributed to raised baselines increasing the peak area recorded for the stutter (Fig. 3a). Peaks between 15–20% were examined carefully to ensure that they were stutters and not contaminants or genetic anomalies, such as somatic mutations (data not shown).

During amplification *Taq* polymerase may add an additional nucleotide, adenosine, to the end of the amplification product, an "*n*+1" peak. These results in the formation of two DNA fragments separated by a base pair, the "*n*"-peak and the "*n*+1" peak. The amplification parameters of the SGMplus system are designed to encourage the formation of "*n*+1" peaks by incorporating an additional extension at the end of the PCR; consequently if "*n*"-peaks are produced they tend to be at a much lower level then the "*n*+1" peak, usually at less than 15% of the "*n*+1" peak.

Fewer *n*- peaks were observed in the ABI 377 instrument data although the data obtained from the ABI 3100 instrument was relatively closer to this than the ABI 3700 instrument (Fig. 3b). As the signal intensity is greater on the ABI 3700 instrument due to the absence of scan averaging, the corresponding *n*-peaks on the 377 instrument may not be large enough to be detected. Typically, *n*-peaks were approximately less than 20% of the area of the major allele. In the majority of cases, the *n*-peak should not cause problems with interpretation especially if a heterozygote is present. At THO1 where the possibility of a 9.3/10 must be considered, the smaller allelic peak should be within 50% of its partner allele and therefore should not be confused with an *n*-peak (data not shown). The differences in the presence or absence of *n*-peaks for specific loci on the different instruments may be related to the differences in resolution exhibited by the instruments, as *n*-peaks may be lost when resolution is poor.

3.2 Precision

Precision of the CAE instruments was investigated by running of a number of allelic ladders, and examining their relative migration, as demonstrated by ranges and standard deviations. Previous studies using the FSS SGM STR system have established precision data for the ABI Prism 377 sequencer [1].

The ABI 3700 instrument in combination with our novel sizing regime was able to size to single base pair precision. However, precision deteriorated in the high-molecular-weight portion of the profile, affecting the D18S51 and HUMFIBRA (FGA) loci in particular (Table 1). Within a run, the rare 47.2 high-molecular-weight FGA allele varied by a maximum 0.92 bp (standard deviation = 0.21, Table 1). This allele demonstrated a range of 0.3 bp and 0.5 bp on the ABI 3100 and MegaBACE 1000 instruments. Other high-molecular-weight rare alleles within FGA, on the ABI 3700 instrument, were similarly affected as was a high-molecular-weight D18S51 allele, with a sizing range cov-

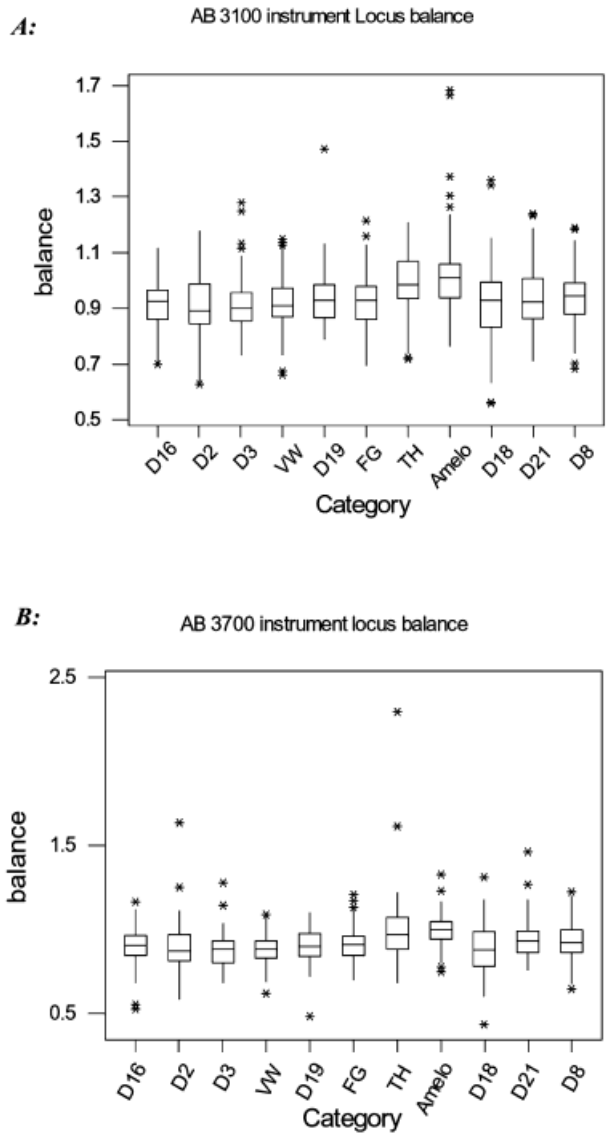

Figure 2. Peak area balance within locus**,** obtained from 84 sample profiles for each locus of the SGMplus STR system on the (A) ABI 3100 and (B) ABI 3700 instrument. Data obtained from samples extracted using Qiagen protocol and amplified using the Amp*f*/STR SGM Plus Kit at 28 cycles. Data manipulation was carried out using MiniTab software. Horizontal line within box represents median data point, box represents area between 1st and 3rd quartile (Q1 and Q3), vertical line (whisker) represents upper limit or Q3 + 1.5sd (Q3-Q1) and lower limit or Q1ä-1.5sd (Q3 − Q1) whilst asterisks represent data outliers.

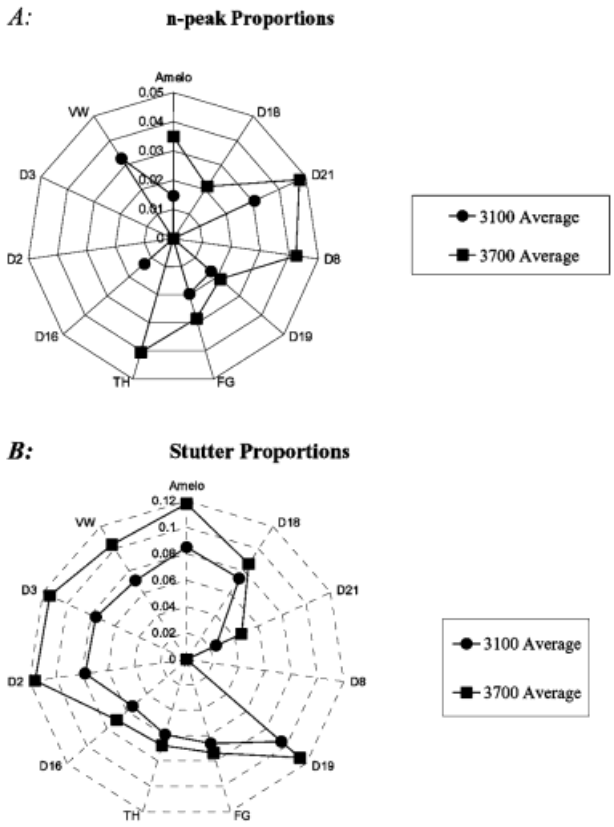

Figure 3. Allelic artefact proportions obtained from 84 samples amplified with Amp*f*/STR SGMplus system for each locus in the STR system. (A) Stutter proportions; (B) *n*-peak proportions, for the ABI3100 and ABI3700 instruments.

ering 0.66 bp, and standard deviation of 0.15. This relatively poor precision pattern for the high-molecular-weight markers was repeated for the ABI 3100 instrument. However, intrarun precision (within 16 capillaries) was better than the ABI 3700 instrument and MegaBACE 1000 instruments, although wider than the ABI 377 instrument. The highest range demonstrated on the ABI 3100 instrument was also for the FGA marker region, which had a sizing range of 0.66 bp or a standard deviation of 0.17 (Table 1). The largest range demonstrated on the MegaBACE 1000 instrument was for the FGA marker region at 0.9 bp or a standard deviation of 0.17.

Sizing across the array within a run was investigated by plotting the migration of specific alleles within the allelic ladder *versus* capillary number (Fig. 4). A general trend in migration was seen within the ABI 3700 instrument data that was not repeated within the ABI 3100 instrument data. This trend in the ABI 3700 instrument, can be seen from capillary number 1–96 as a decrease in base pair sizing for a specific allele, from approximately 338.86 bp within capillary 1 to 337.97 bp within capillary 94. This is a base pair range of 0.89 bp and a standard deviation of 0.16. The pattern observed within the ABI 3100 instrument did not follow a linear trend across the array but a U-shaped pattern could be seen with the lowest migration of the FGA 47.2 allele in capillaries 8 and 10 (Fig. 4).

Table 1. Data collected from 18 runs of 48 capillaries of Amp*f*/STR SGMplus allelic ladder on the ABI 3700; 60 runs of a maximum of 16 for the ABI 3100 instrument; 6 runs of a maximum of 96 for the MegaBACE 1000 instrument compared to allelic ladder data collected from the 377 sequencer

	Min range (bp)				Max range (bp)				Min std dev				Max std dev			
Locus	377	3100	3700	Mega-BACE	377	3100	3700	Mega-BACE	377	3100	3700	Mega-BACE	377	3100	3700	Mega-BACE
Amelogenin	0.24	0.15	0.15	0.25	0.34	0.21	0.31	0.47	0.07	0.05	0.03	0.05	0.07	0.06	0.06	0.09
D18S51	0.19	0.24	0.20	0.31	0.45	0.55	0.66	0.83	0.05	0.06	0.05	0.06	0.10	0.15	0.14	0.13
D21S11	0.14	0.13	0.06	0.16	0.30	0.39	0.40	0.45	0.04	0.04	0.03	0.04	0.07	0.11	0.07	0.07
D8S1179	0.10	0.19	0.12	0.27	0.31	0.50	0.53	0.50	0.04	0.06	0.04	0.05	0.08	0.14	0.12	0.09
D19S433	0.16	0.12	0.10	0.17	0.33	0.30	0.30	0.44	0.05	0.04	0.02	0.05	0.08	0.07	0.06	0.09
HUMFIBRA	0.20	0.40	0.26	0.51	0.46	0.66	0.92	0.90	0.06	0.11	0.06	0.10	0.10	0.17	0.21	0.17
HUMTH01	0.18	0.16	0.11	0.15	0.32	0.46	0.37	0.39	0.05	0.05	0.03	0.04	0.06	0.12	0.07	0.07
D16S539	0.17	0.25	0.13	0.16	0.35	0.42	0.39	0.34	0.05	0.08	0.03	0.04	0.07	0.11	0.08	0.06
D2S1338	0.19	0.25	0.16	0.22	0.31	0.50	0.52	0.62	0.04	0.07	0.04	0.05	0.07	0.14	0.12	0.09
D3S1358	0.17	0.11	0.13	0.18	0.36	0.31	0.41	0.53	0.05	0.03	0.03	0.05	0.09	0.07	0.09	0.09
HUMVWFA31	0.14	0.16	0.12	0.17	0.29	0.57	0.42	0.36	0.03	0.05	0.03	0.04	0.07	0.16	0.08	0.07

ABI Prism 377 instrument data derives from one run of 36 lanes of Amp*f*/STR SGMplus allelic ladder on a 36-lane slab gel and was used as a reference point for all comparisons. Ranges and standard deviations were calculated from the largest migrating fragment and smallest migrating fragment for each allele of every locus on a within run basis. Differences in sample size reflect the differing capacities of each platform.

A base pair range of 0.31 bp and standard deviation of 0.14 was observed here. The MegaBACE 1000 instrument showed a wide distribution of migration across the array but a general increase in allele size was seen from capillary number 1–96. The range here was less marked than the ABI 3700 at 0.47 bp and a standard deviation of 0.1.

3.3 Resolution

The ability to distinguish between peaks that are separated by a base pair is a prerequisite of any detection platform used where the STR system has the likelihood of displaying rare alleles differing by a single base pair. Although resolution is a product of capillary length and voltage, other factors may also effect the signal observed including EOF, the detection window and the detection integration time. Because of this, a standard method of measuring resolution within the forensic community is to calculate the peak width of specific alleles of the Amp*f*/STR SGMplus allelic ladder, within the low- and high-molecular-weight range. The peak heights (rfu) are then divided by peak width (*t*) to establish an indication of resolution (Table 2). Here, the smaller the peak width the better the resolution of the instrument.

The MegaBACE demonstrated the smallest mean peak widths at the low (D19)- and high (FGA)-molecular-weight loci. The peak height/peak width for the MegaBACE also demonstrated the greatest variation from D19 at 1169 to FGA at 139. Resolution was poorest on the ABI 3100 instrument with both D19 and FGA alleles demonstrating relatively similar resolution. A good visual indicator of resolution is the Amp*f*/STR SGMplus allelic ladder, specifically at HUMTH01 9.3 and HUMTH01 10 alleles. A series of electropherograms (Fig. 5) indicates the ABI 3700, the ABI 3100 (Fig. 4a) and the MegaBACE 1000 (Fig. 5b) sequencers are able to distinguish between these DNA fragments as effectively as the ABI Prism 377 sequencer (Fig. 5a).

3.4 Sensitivity

Sensitivity was examined using dilutions of PCR products. Comparison between the platforms was difficult, as different quantities of PCR product and size standards are required. In addition, the detection and averaging carried out by the instruments varies, such that the data output may not be a true representation of the sensitivity of the instrument. That aside, the instruments demonstrated a degree of sensitivity equal to each other, as measured by the ability to generate a full profile (Fig. 6). A drop in "full profile" across all three instruments was observed at 1.5 ng template level and is probably due to variations introduced during the PCR set-up procedure rather than instrument effects. A direct comparison of signal-to-noise ratio between the three instruments was carried out using data obtained from the template concentration titration

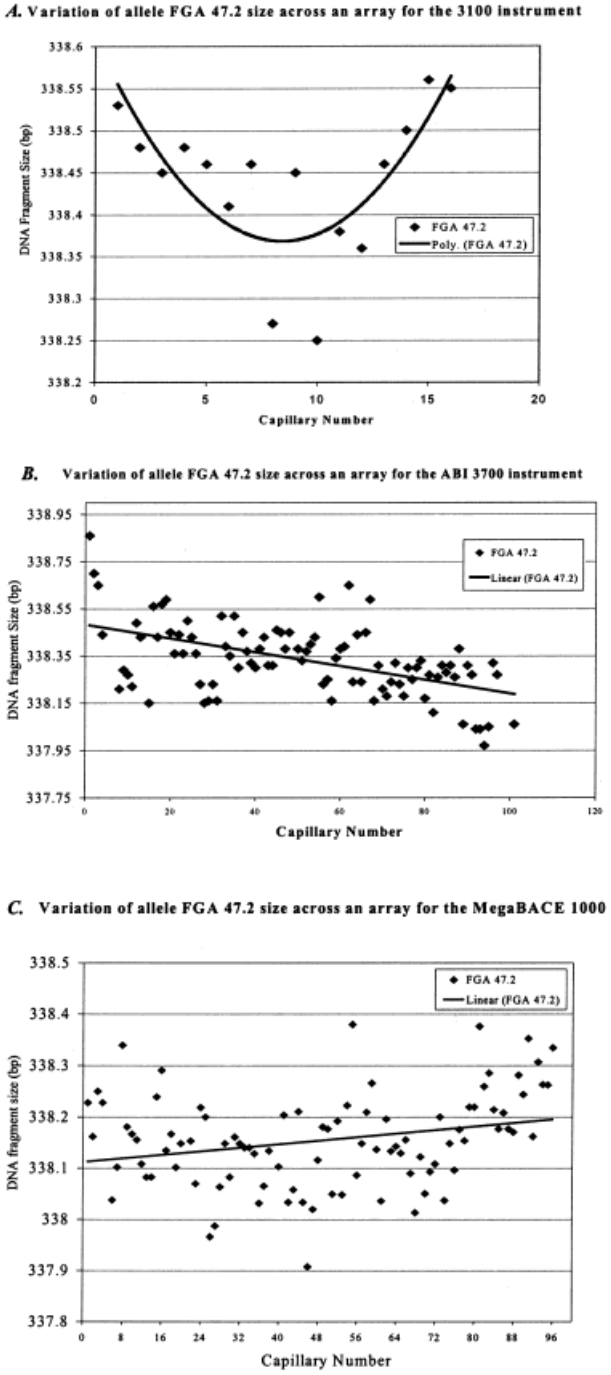

Figure 4. Variation in migration of "worst performing alleles" across the capillary arrays for (A) ABI 3100 (FGA 47.2); (B) ABI 3700 (FGA 47.2); (C) MegaBACE 1000 instrument (FGA 47.2). Data collated using Amp*f*/STR SGMplus allelic ladder in each capillary for a single run. Sizes in bp; solid line represents trend of data series.

(Table 3). The mean signal-to-noise ratio obtained from the three instruments were comparable, with the highest mean observed within the MegaBACE 1000 instrument in the yellow dye set. Although the ABI 3700 instrument demonstrated mean values from each dye set that were comparable with the other two instruments, the lowest signal-to-noise ratio was obtained here. Signal-to-noise ratios of 2.2, 1.7 and 1.6, for yellow, blue and green dye sets were calculated and were identified as being specific to the high-molecular-weight alleles. This is probably due to the greater degree of signal "tail off", from low-to-high molecular weight, demonstrated within the ABI 3700 instrument. This again must be treated with a degree of caution, as we have previously shown that there is a variability of sensitivity between instruments of the same model, and across the capillary array within the same instrument due to differences in spatial calibration and cuvette temperature (Fig. 7).

Table 2. Resolution calculated from 12 Amp*f*/STR SGMplus allelic ladder samples for ABI 3700, MegaBACE 1000, and ABI 3100 instruments

	Mean peak width D19	Mean height/ width D19	Mean peak width FGA	Mean height/ width FGA
3100	15.68	85.25	16.93	61.91
MegaBACE	3.88	1169.45	12.10	139.92
3700	17.23	434.27	21.08	138.50

Peak widths for ABI 3100 and 3700 instruments were calculated using the formula (peak area/peak height) $\times$ 2. Peak widths for MegaBACE 1000 instrument are calculated within instrument analysis software.

The temperature within the cuvette system of the ABI Prism 3700 instrument had a significant impact on the quality of data produced. The data were analysed for the high- and low-molecular-weight markers in the yellow fluorescent dye set. This was repeated for the ABI 3100 and MegaBACE 1000 instruments. Changes in temperature significantly altered the sensitivity of the ABI 3700 instrument and the relative sensitivity of each capillary (Fig. 7). Spatial calibrations also had an effect on sensitivity for each capillary for the ABI Prism 3700 instrument. The spatial calibration is carried out to map a capillary to a spatial position on the CCD camera and therefore define the position where data is collected for each capillary. Altering both the spatial calibration and the cuvette temperature increased peak heights of the 400 bp size standard peak, and also affected the relative sensitivity of the capillaries. At the lower cuvette temperature, 40°C, a distinct "left-to-right" variation across the capillary array was observed, with the lower-numbered capillaries demonstrating lower sensitivity. This differential across the array was reduced with a higher cuvette temperature of 46°C–50°C (Fig. 7). Linear regressions were carried out for the 100 bp and 400 bp size standard peaks at a cuvette tem-

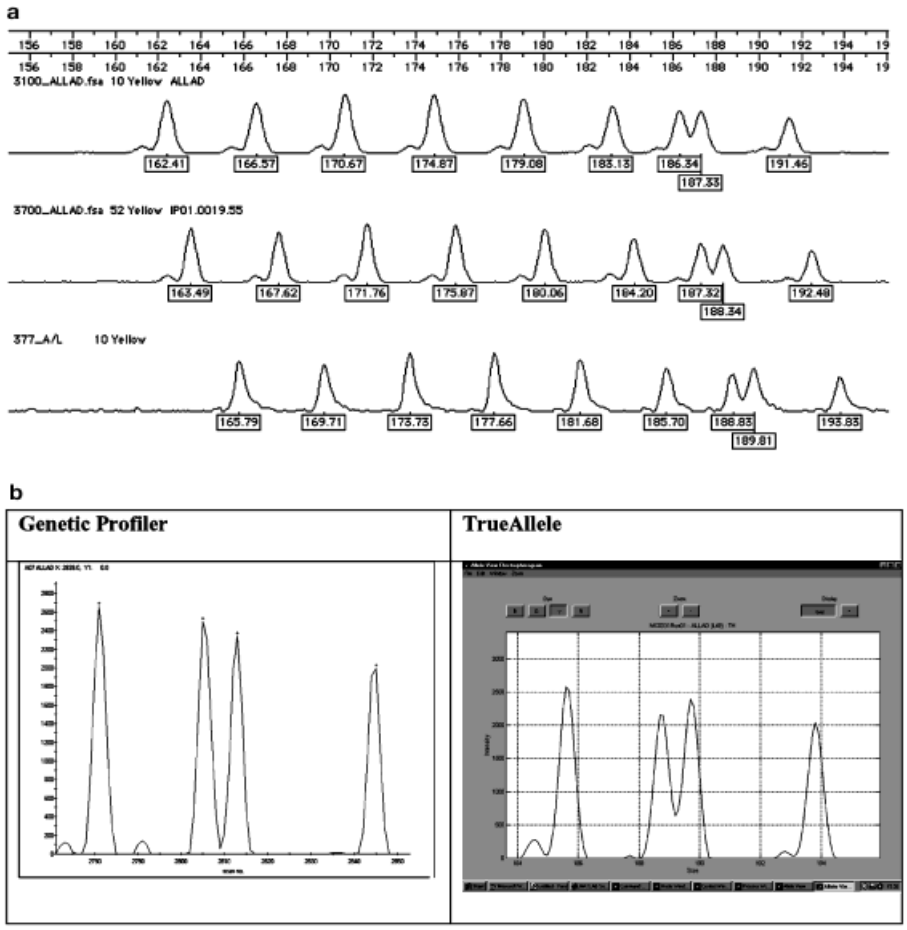

Figure 5. (a) Electropherogram of HUMTH01 locus obtained by electrophoresis of the Amp*f*/STR SGMplus allelic ladder on the ABI Prism 3700, ABI Prism 3100 and ABI Prism 377 instruments, demonstrating 1 bp resolution between HUMTH01 9.3 and 10. Data analysed within Genescan 3.5 software prior to display within Genotyper 1.1.1. (b) Same as (a), except electrophoresis on MegaBACE 1000 instrument and data analysed within Genetic Profiler and True Allele.

Table 3. Signal-to-noise ratios obtained from PCR template concentration titration for MegaBACE 1000, ABI 3100 and ABI 3700 instruments using template concentrations from 0.5 to 4 ng

	Min range (S/N)			Max range (S/N)			Mean (S/N)		
Dye	MegaBACE	3100	3700	MegaBACE	3100	3700	MegaBACE	3100	3700
Yellow	19.930	7.163	2.234	92.395	29.980	82.359	42.814	14.880	26.605
Blue	8.598	11.980	1.713	52.978	60.122	64.574	21.648	27.027	19.480
Green	6.044	11.776	1.645	78.231	59.878	84.652	28.884	25.671	30.052

Noise level identified as highest peak, within analysis window, not attributed to PCR amplification, within each dye set. Signal to noise calculated for each dye set across the whole PCR fragment range from low to high molecular weight.

perature of 40°C and 47°C. At 40°C the linear correlation coefficients were calculated to be 0.66 for the 100 bp peaks and 0.83 for the 400 bp peaks, therefore demonstrating a weak positive linear correlation across the array from capillaries 1–104. At 47°C the 100 bp and 400 bp peaks demonstrated linear correlation coefficients of −0.26 and −0.29, demonstrating no linear correlation across the array. The difference between the 100 bp size standard peak and the 400 bp size standard peak was reduced at the higher temperature (Fig. 7) decreasing the "tailing off" in signal intensity seen within each capillary. As a result, all peaks in a sample lane fell within the acceptable level with peak heights of between 150 rfu and 9000 rfu, without further operator intervention. This

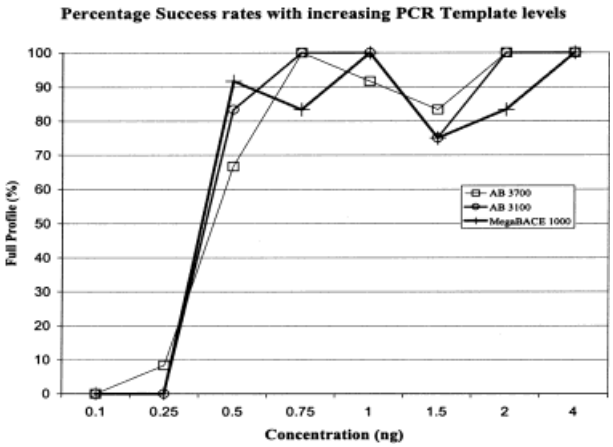

Figure 6. Quality of profile produced from Amp*f*/STR SGMplus PCR products produced with varying concentrations of template DNA. Electrophoresis carried out on the ABI Prism 3700, ABI 3100 and the MegaBACE 1000 sequencer using their relevant product setup protocols. ABI instrument data was analysed within Genescan 3.5 or Genescan 2.1 software then viewed and typed within Genotyper 1.1.1 software. Data produced on the MegaBACE 1000 instrument was analysed with Genetic Profiler. Data expressed as percentage full profile for each PCR template concentration.

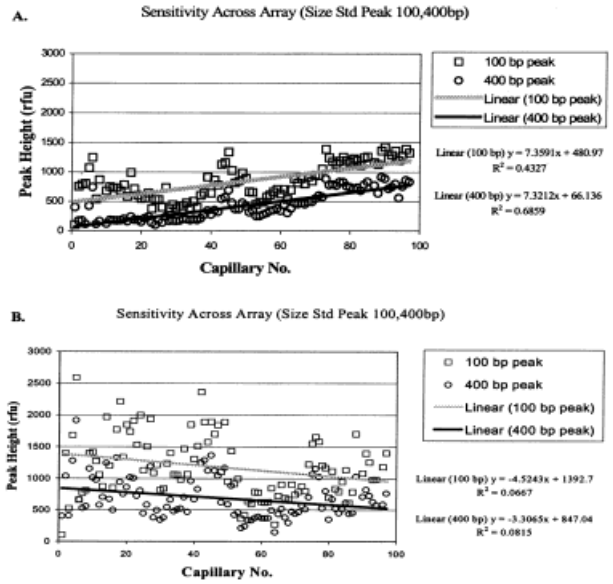

Figure 7. Heights (rfu) of 100 bp size standard peak and 400 bp standard peak, in relation to capillary number. Data obtained by electrophoresis of 96 capillaries of Amp*f*/STR SGMplus allelic ladder and GS 400 HD size standard. Data analysed by Genescan 3.5 and manipulated in Genotyper 1.1.1. Relevant peaks, for each capillary, were labeled in peak heights and compared to their spatial position within the array. The graph shows the variation in sensitivity within the same instrument and capillary array with varying spatial calibration and cuvette temperature parameters set at (A) 40°C; (B) 47°C.

difference in peak heights within a sample lane is sometimes assumed to be a product of preferential injection effect, but could also be associated with cuvette temperature because of the improvement that can be seen at the higher cuvette temperature. Changes in temperature within the cuvette could be effecting the laminar flow of the polymer at the capillary tip detection zone and the optical density of the polymer and result in the differences observed in the data at varying temperature. However, this is speculation and the precise mechanism is unknown. The marked decrease in the R^2 value from a cuvette temperature of 40°C–47°C is a result of a greater scatter of data points and therefore more variability in sensitivity (peak heights) between capillaries of the array.

Alterations to the cuvette temperature did not affect the ABI 3100 and MegaBACE 1000 instruments because of the instruments' different detection systems. This was reflected in the changes in sensitivity across the capillary array and within each capillary. These, unlike the ABI 3700 instrument, did not follow a distinct pattern from one side of the array to the other, and in addition did not demonstrate "tail off" of signal from the low to high molecular weight range of the profile. Although there was no distinct pattern across the array, for the MegaBACE 1000 instrument, there was a wide variation in sensitivity or amount of DNA detected for each capillary within an electrophoretic run. The ABI 3700 instrument exhibits an approximately 3-fold difference in the FGA marker, with the highest peak in capillary 79 at 4286 rfu and the lowest at 1444 rfu in capillary 26. This difference was also seen with D19S433 with the highest peak in capillary 79 at 5640 rfu and the lowest in capillary 27 at 1936 rfu. The MegaBACE 1000 instrument exhibited the lowest mean peak height for D19S433 was 1112 rfu and the highest was 4301, an approximately 4-fold difference (Fig. 8). FGA exhibited a larger variation, from 1187 rfu to 6236 rfu, an approximately 6-fold difference. In contrast, the ABI 3100 instrument showed a more even distribution of peak heights across the array, with an approximately 2-fold difference in FGA and a 3-fold difference with D19S433. The lowest performing capillary was number 2, with a peak height for FGA of 433 rfu and approximately 205 rfu for D19S433. The highest peak height was observed in capillary 1 with FGA at approximately 923 rfu and D19S433 at 685 rfu. The differences in sensitivity obtained for each capillary on the instruments is closely related to the array and calibrations performed on it. Therefore, a different array will demonstrate different sensitivities for each capillary. However, the general trend in the data obtained on the ABI3700 instrument, from low to high capillary numbers, with a cuvette temperature of 40°C does not alter with changes to array or calibrations.

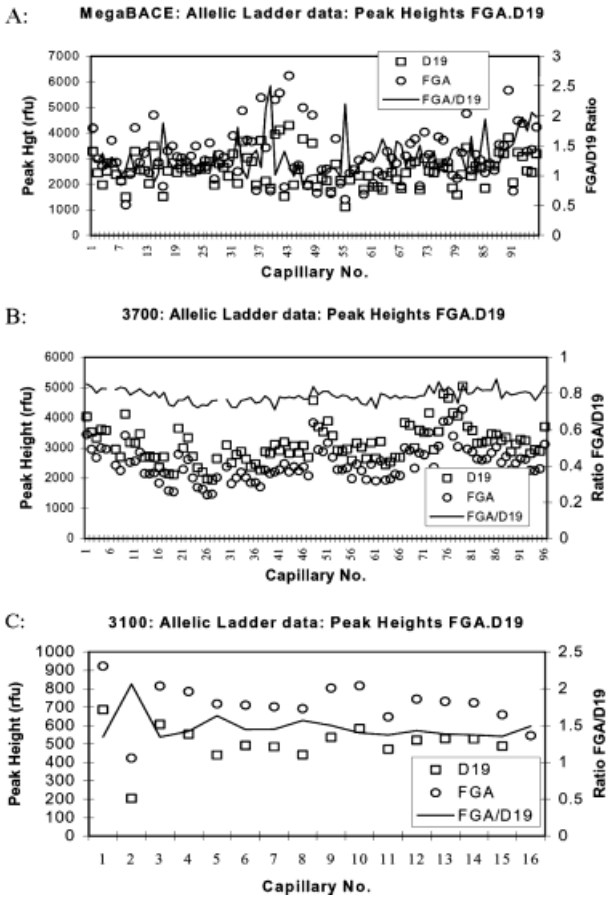

Figure 8. Sensitivity of (A) MegaBACE 1000; (B) ABI 3100 and (C) ABI 3700 instruments across the array and within capillary. Data generated using Amp*f*/STR SGMplus allelic ladder and GS 400 HD size standard for the ABI 3700 or ABI 3100 instrument. Amp*f*/STR SGMplus allelic ladder and ET Rox 400 size standard used on the MegaBACE 1000 instrument. Sensitivity across array measured using peak heights of SGMplus markers FGA and D19S433. Sensitivity calculated from ratio of peak heights of FGA/D19S433.

Profile balance within each capillary was measured using the ratio of peak heights from the high (FGA)- and low (D19)-molecular-weight ranges of the Amp*f*/STR SGMplus allelic ladder. Peak heights obtained from Amp*f*/STR SGMplus allelic ladder on the MegaBACE 1000 instrument exhibited a distribution of ratios between 0.8 and 2.5 as compared to the ABI 3100 instrument with FGA/D19S433 ratios of 1.34–2.5. The ABI 3700 instrument demonstrated a ratio ranging from approximately 0.71 to 0.87 (Fig. 8).

Sensitivity was demonstrated to vary with the different instrumentation. The ABI 3700 and ABI 3100 instruments showed differences within the array and between different arrays and spatial calibrations. Spatial calibrations were

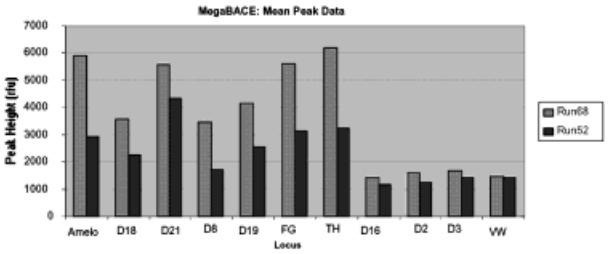

Figure 9. Differences in sensitivity on the MegaBACE 1000 instrument measured as peak heights, over lifetime of a capillary array. Data collated from Amp*f*/STR SGMplus allelic ladder and expressed as average peak height for all alleles of each locus.

not required for the MegaBACE 1000 instrument, but changes were exhibited with different capillary arrays on the same instrument and could be closely associated with adjustments made to the sensitivity of each PMT. The MegaBACE 1000 instrument showed differences in sensitivity for each of the runs exhibited by mean peak heights for each locus (Fig. 9). The markers labeled with the blue fluorescent dye showed no differences between runs but the other markers showed a drop in sensitivity.

3.5 Contamination

Contamination studies were carried out on the ABI 3100 and ABI 3700 instruments. Extensive contamination studies could not be carried out on the MegaBACE 1000 instrument due the unavailability of software capable of sizing and designating alleles and then displaying the results in EPG format of a number of capillaries adjacent to each other. Contamination within a run was apparent at default wash settings of the ABI Prism 3700 instrument and was a result of sample-to-sample contamination *via* the robotic loading needles. This contamination was eliminated through increasing these wash parameters between 4- and 6-fold. With increased use of the instrument a rare event termed "load bar" contamination was identified. This exhibited itself as sample mixtures with sample profiles originating from directly adjacent wells within the sample injection bar on the ABI 3700 sequencer (Fig. 10). It is thought to be a result of "leakage" on the sample loading bar from well to well *via* capillary action due to inefficient load bar drying steps, resulting in sample mixing between specific wells on the loading bar. This contamination is an ongoing problem with the ABI 3700 instrument and contamination assays should be performed on the instrument on a regular basis to monitor this.

Sample loading tip contamination was not observed on the ABI 3100 or the MegaBACE 1000 instrument because sample injection is carried out directly from the sample

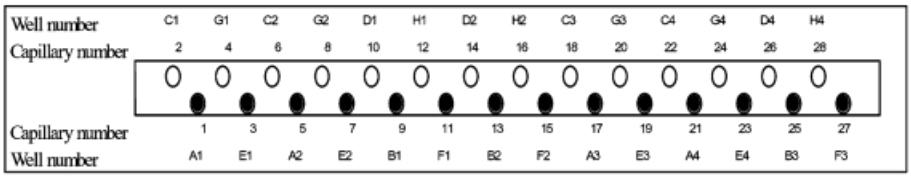

Figure 10. Diagrammatic representation of the load bar of the ABI 3700 instrument showing a sample of the capillary numbers and well positions on a 96-well microtitre plate. Contamination occurs from adjacent capillaries on the loading bar, *e.g.*, the loading well for capillary 3 could potentially contaminate the loading wells for capillaries 1 or 5, or the loading well for capillary 10 could contaminate the loading wells for capillaries 8 or 12. Because of the nature of this contamination it is relatively easy to detect within viewing software.

microtitre plate. However, primer flare, which includes unincorporated primer and primer dimer, was detected on the ABI 3100 instrument on a blank electrophoretic run which followed a run containing PCR product. This is defined as "carry-over" and is thought to be a result of contaminant remaining on the capillary tips after default tip wash protocols. Investigations were carried out in order to eliminate this "carry-over" using different wash parameters and solutions. This involved increasing the number of tip washes from 1 to 2 (this is the maximum) and increasing the time the tips remain within the wash solution (Table 4). In addition to this, water was replaced as the wash solution with a nonionic detergent, Tween 20, at a number of different concentrations (Fig. 11). A decrease in average height of primer flare carryover from

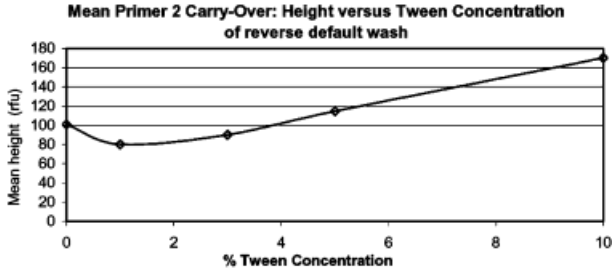

Figure 11. Mean carry-over peak height (rfu) of primer flare observed in blank runs, containing size standard in capillaries proceeding positive runs containing Amp*f*/STR SGMplus PCR products on the ABI Prism 3100 instrument. Average carry-over heights were calculated for each wash solution concentration.

Table 4. Reductions in peak height of primer flare on the ABI Prism 3100 instrument using different tip washing regimes

Experiment	Standard run	Expected run		
	Mean height	Mean height	SD	*t*-value
a. Old water wash	494.69	590.0625	1059.31	−0.36
b. New water wash	494.69	494.6875	972.25	1.28
c. Extra wash (2 steps)	494.69	152.125	889.20	1.54
d. Extra wash time (62 s)	494.69	264.25	955.94	0.96
e. Extra wash step (2 steps)+ time (62 s)	590.06	311.69	436.71	2.55

Data collated from runs of blank lanes containing HD 400 size standard preceding runs with capillaries containing sample product. Comparisons were carried out using default wash parameters as control and altered wash regimes of (a) old water wash, tip wash reservoir containing used wash solution, (b) new water wash, tip wash reservoir containing fresh wash solution, (c) extra wash (2 steps), addition of new reservoir wash step; (d) extra wash time (62 s), lengthening of time tips remaining in wash solution (from 9 s to 62 s); (e) extra wash step and time. A significant ($P < 0.05$) decrease in primer flare is demonstrated for any result with a positive *t*-value greater than 1.75 compared to standard run.

100 rfu in water, was observed when using 1% (80 rfu) and 3% Tween 20 (90 rfu). Statistical analysis was carried out on primer flare height using mean paired test and demonstrated this decrease not to be significant but a result of sampling variation. The addition of an extra wash step combined with an increase in wash time or each of these steps separately demonstrates a real reduction in primer height. A *t*-value of 2.55 was calculated and was shown to be significant, with a confidence level of 0.05.

Investigations were also carried out on the ABI 3100 and ABI 3700 instruments into signal contamination from adjacent capillaries at the detector. This is defined as "cross-talk" and results in signal from a capillary containing labeled DNA being detected in an adjacent capillary. It was observed in both instruments and characterized as percentage "cross-talk" (Table 5). The highest level of cross-talk that was observed on the ABI 3700 instrument was approximately 0.54% at a height of 70 rfu, and was from a contributory peak of 13 049 rfu in height. The highest level of "cross-talk" on the ABI 3100 instrument was 0.53% with the highest peak height of 39 rfu and a contributory peak of 7405 rfu in height. This highlighted the need to check adjacent capillaries for signal cross-talk. On the ABI 3700 instrument peaks with heights of between 9000 and 12 000 rfu should be investigated as they

Table 5. Data generated by electrophoresis of Amp*f*/STR SGMplus PCR products on the ABI Prism 3700 and ABI Prism 3100 instruments adjacent to wells containing GS 400HD size standard and formamide only, to form a chequerboard pattern of sample, size standard, sample, *etc.*

Peak No.	Contributory peak height (rfu)	Cross-talk peak height	%Cross-talk
3700			
1	13 149	54	0.41
2	12 900	58	0.45
3	13 049	70	0.54
4	13 564	66	0.49
5	12 811	68	0.53
6	12 750	58	0.46
7	13 037	62	0.48
3100			
1	6 116	27	0.44
2	7 442	30	0.40
3	7 474	33	0.44
4	5 574	17	0.30
5	7 603	31	0.41
6	7 405	39	0.53
7	7 399	34	0.46
8	4 066	21	0.52
9	3 181	17	0.53
10	6 251	28	0.53

Data analysed in Genescan 3.5 and manipulated in Genotyper 1.1.1. Amelogenin was used for data analysis and defining level of cross-talk as this locus reproducibly gives higher peaks than the STR loci. Cross-talk was calculated as the percentage of signal from the "contaminant peak" compared to the "contributory peak"

could result in contaminant "peaks" at heights of approximately 45–60 rfu. Sample profiles exhibiting heights between 6000 and 7000 rfu will result in contaminant peaks with heights of approximately 30–35 rfu.

4 Discussion

This study aimed to determine the suitability of present CE systems for forensic use with the STR multiplex Amp*f*/STR SGMplus, in comparison with the ABI Prism 377 sequencer. This involved examining correct designation of known sample profiles, precision, resolution, and sensitivity and contamination. Our data highlighted a decrease in sizing precision with increasing molecular weight on all the CAE instruments. The ranges were greatest on the ABI 3700 and MegaBACE 1000 instruments in particular at the D18S51 and FGA loci. Using the sizing system for the ABI Prism 377 instrument could potentially lead to alleles falling outside of their sizing bins and moving into the next, leading to misdesignation of a profile. However, within run precision was at a level to allow sizing of sample profiles from a virtual or average ladder created from the same run. Our results have shown that forming a composite ladder from between five and eight allelic ladder data points is the most accurate method for sizing alleles on the ABI Prism 3700 instrument. This sizing regime was not required for the ABI 3100 instrument as intrarun precision was at a level that allows sample alleles to be sized with one allelic ladder from each run. This results in a minimum of six allelic ladders being electrophoresed for every 96-well microtitre sample plate. The sizing regime used for the MegaBACE 1000 instrument was not fully validated because of software and data quality issues raised during its evaluation. A fully functional software package was not available for sizing and typing MegaBACE 1000 instrument data making an extensive concordance study extremely difficult. In addition, data quality issues were observed in the data itself, and were principally exhibited as poor peak morphology, or split peaks. This was a recurrent problem with the MegaBACE 1000 instrument and the extent of the problem varied from 1% to 25% of capillaries for each run. Amersham have recently produced a new loading solution to replace formamide and resolve this problem, but this was not tested at the time of this study.

The absence of errors over a broad range of samples demonstrates the sizing robustness of the instruments with each sizing regime. Although on the ABI 3700 and ABI 3100 instrument, migration patterns have been shown to differ between arrays and even within the same array over time, there is no evidence to show this will result in a typing error. Allelic ladders have been positioned within each instrument array to maximize precision and no errors in typing were observed using this sizing regime. Interestingly, an occurrence of a rare allele falling outside our size-calling ranges on the ABI Prism 3700 instrument was similarly affected on the ABI Prism 377 instrument (data not shown). This example was a rare D8 5-allele, as profiled on the ABI 377 sequencer, which was preliminarily called a 4.3-allele on the ABI 3700 sequencer. However, when the band shift correlation was carried out it was demonstrated to be a 5-allele. The band shift test is calculated from the distance a sample allele differs from the same allele of an allelic ladder. Alleles electrophoretically separated within the same sample lane should shift in the same direction and at a similar rate [27]. When the sample profile was studied on the ABI Prism 377 instrument, it was also found to be out of the allelic sizing range, and could potentially be designated a 4.3-allele. Again, the correlation rule established it to be a 5-allele. In this instance, the ABI Prism 3700 instrument

was found to vary by minus 0.44 bp from the center of the size-calling range and the same fragment on the ABI Prism 377 instrument varied by minus 0.36 bp from the center of the same sizing range.

Different sensitivities were measured for each instrument both between arrays and within the same array (Figs. 7–9). The upper and lower levels of sensitivity were such to allow detection and analysis of the broad range of samples encountered in forensic science. Sensitivity within each capillary and across the profile was demonstrated to be more even on the ABI 3100 and MegaBACE 1000 instrument. This is a measure of how comparable peak height/area are across sample profile and is important for interpretation. A drop off in signal from low to high molecular weight, seen on the ABI 3700 instrument, may cause allelic peak heights to fall below safe-calling thresholds. This was also observed when calculating signal-to-noise ratios. With a greater range or ratios obtained on the ABI 3700 instrument due to a drop in signal at the high-molecular-weight region, signal-to-noise ratios calculated for all three instruments indicate that single-sample source data interpretation will not be difficult, although the "tail off" in signal will create difficulties in interpretation of sample mixtures, with mixture ratio calculations impossible because of the unpredictable nature of this signal loss.

Any instrumentation used for forensic applications must be accurate and allow correct allelic designation of samples processed. The presence of contaminating peaks increases the likelihood of getting this wrong. The initial presence of "tip carry-over" on the ABI 3700 instrument was of major concern but this was eliminated with increased stringency of the wash protocols. Run-to-run "carry-over" was identified on the ABI 3100 instrument. This was characterised using the "primer flare" and the level of this observed in negative runs. Work in increasing the stringency of washing parameters between each run has gone some way into reducing this primer "carry-over" but its elimination could not be achieved. Because of the relatively high concentration of primer flare compared to sample, and the very low level of primer "carry-over", no sample "carry-over" will occur under normal operational use of the ABI3100 instrument.

Signal contamination was observed between adjacent capillaries at an approximate average of 0.5% of the contributory peak. This could result in an allelic peak occurring above our calling thresholds in allelic positions when the contributory peak is between 9000 and 12 000 rfu in height on the ABI 3700 instrument and between 4000 and 6000 rfu peak height on the ABI 3100 instrument. Therefore, capillaries adjacent to capillaries exhibiting allelic peaks at these heights are examined for "cross-talk" peaks.

All three instruments were found to be capable of processing sample prepared using our current STR multiplex system. The absence of errors in the extensive concordance studies carried out on the ABI 3100 and ABI 3700 instruments has allowed us to integrate these instruments into our current Criminal Justice sample DNA profiling systems with a high degree of confidence. Extensive contamination studies and changes to default wash parameters have also eliminated the possibility of a sample being associated with the wrong STR designation by either of these instruments. The sizing regimes used for the capillary array instruments tested here were found to be precise, and eliminated misdesignations. This level of confidence in our fragment-sizing methods is required for forensic applications where accuracy of result is of prime importance. The instruments evaluated here demonstrated a broad range of sensitivities that would allow us to detect DNA at the levels regularly encountered in forensic casework. Sensitivity at the lower end of the concentration range would allow detection and genotyping of DNA from the wide variety of forensic substrate types where DNA is present in minute quantities.

In conclusion, we found that although all three capillary array instruments showed good precision, resolution and gave advantages in time and lower manual intervention. The three instruments evaluated here differ in many ways. These include the mechanism by which sample DNA is introduced into the capillaries, the capillaries themselves, *i.e.*, capillary diameter, length, and internal coating, the mechanism of excitation (although both use laser-induced fluorescence), where the DNA is detected and the method of detection itself. These will have effect of on all the criteria evaluated within this study including precision, accuracy, sensitivity, and resolution and makes comparisons between them very difficult. However, in our hands, ABI 3100 was found to be the more suitable instrument for use for forensic applications and in a casework environment. This instrument demonstrated the greatest precision and therefore sizing accuracy; data quality, including peak morphology and baseline noise was also superior on this instrument. In addition, the contamination issues present within the ABI 3700 instrument were not present within this instrument.

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