Comparison of PowerPlex™ 16, PowerPlex™1.1/2.1, and ABI Amp*fl*STR™ Profiler Plus™/COfiler™ for Forensic Use

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Aim. Several amplification and detection formats for the analysis of short tandem repeat loci are readily available to the forensic laboratory. Careful consideration must be given to the throughput, sensitivity, concordance, data interpretation, facility requirements, and costs of operation. The Pennsylvania State Police DNA Laboratory sought to establish that of any of the amplification or detection formats generally used in the United States generates concordant results and that the use of several formats within one laboratory provides a solution to the interpretation of difficult evidentiary samples.

Methods. Validation work consisting of sensitivity, precision, mixture, and substrate studies was performed by use of each of three detection formats (ABI Prism®310 Genetic Analyzer, ABI Prism®377 DNA Sequencer, and the Hitachi FMBIO®II Fluorescent Scanner) and three amplification systems (*GenePrint*® PowerPlex™ 16, *GenePrint*® PowerPlex™ 1.1/2.1, and Amp*fl*STR™ ProfilerPlus/COfiler). The results generated in each of the formats were compared, along with the problems incurred.

Results. All allele calls were concordant, with the exception of primer region variants, and all detection systems were sensitive and reliable. Even with the use of multiple formats, a general protocol can be written with only one set of interpretation guidelines.

Conclusion. National databases can be used with input data from any of these formats. The use of several detection formats allowed the forensic scientist to select a system, based on sample quality, quantity, and throughput requirements. Interpretation issues resulting from complex mixtures, degraded samples, rare microvariants, internal primer variants, unusual heterozygote ratios, above or below ladder alleles, and potential tri-alleles can be verified.

Key words: alleles; criminology; DNA; fluorescent dyes; forensic medicine; laboratories, forensic; polymerase chain reaction; polymorphism; tandem repeat sequences

The Pennsylvania State Police (PSP) DNA laboratory uses several detection formats and manufacturer's amplification kits in their analysis of forensic casework and convicted offender samples. The laboratory needs a system that will accommodate high throughput for the large number of convicted offender samples received yearly. We also need a system that can offer the sensitivity, reproducibility, and precision necessary for forensic casework analysis. Sequence capability for mitochondrial DNA analysis is also desirable. Sample preparation times, ease of instrument operation, and "user-friendly" software are considerations taken into account in instrument selection.

Each of the more commonly used laser detection instruments for short tandem repeat (STR) analysis offers a different approach (capillary electrophoresis vs gel-based electrophoresis) to the analysis of database and casework samples. The choice of instrument will dictate which manufacturer's STR amplification kit can be used. This is due to the compatibility of the primer dyes to the instrument laser and filter set. The ABI Prism® 310 Genetic Analyzer and the ABI Prism®377 DNA Sequencer (Applied Biosystems, Foster City, CA, USA) can be used with the ABI Amp*fl*STR™ nine-locus Profiler Plus™ and the ABI Amp*fl*STR™ seven-locus COfiler™ (Applied Biosystems), or the sixteen locus *GenePrint*® Power Plex™ 16 amplification kit (Promega Corporation, Madison, WI, USA). The Hitachi FMBIO®II Fluorescent Scanner (Hitachi, Alameda, CA, USA) is used with the eight-locus *GenePrint*®PowerPlex™ 1.1 and the nine-locus *GenePrint*® PowerPlex™ 2.1 (Promega Corporation). The choice between the STR amplification system and detection instrument can be difficult to make. In a high throughput laboratory more than one of any instrument may be needed. The choice could be made to purchase several of a particular instrument model or several different instruments. The

choice of only one model, however, does not offer the flexibility that may be desired.

The DNA laboratory made the decision to use the ABI Prism®310 Genetic Analyzer and the ABI Prism®377 DNA Sequencer for casework analysis and the Hitachi FMBIO®II Fluorescent Scanner as the workhorse for the analysis of the large quantity of database samples (1). We used newly hired scientists to validate the instruments and the amplification kits, whereas the casework qualified forensic scientists continued doing casework using restriction fragment length polymorphism (RFLP) technology. These new scientists accomplished their training and validation at the same time. Although we initially pondered the feasibility of a multiple instrument format, it quickly became evident that the decision allowed the laboratory much flexibility, less "down" time, and the ability to check difficult samples with a different set of primers and detection formats. Each of the instruments offered their own advantages and disadvantages.

Material and Methods

Instrument Overview

ABI Prism® 310 Genetic Analyzer. The ABI Prism® 310 Genetic Analyzer is a single capillary electrophoresis system using a 96-well tray format (2,3). A full tray of samples may be loaded, the instrument parameters set, and electrophoresis of the samples occurs, while the analyst is free to do other functions. Sample preparation is minimal. Continual monitoring is generally unnecessary. However, in our laboratory, it was quickly recognized that the ABI Prism® 310 was not an instrument for rapid throughput for the thousands of convicted offender samples that needed to be analyzed. It took approximately two and one-half days for a full 96-well tray to be analyzed (30 min/sample). Due to the position of the capillary on the door of the instrument, minor changes in the temperature of the room affects the migration of the sample through the capillary, resulting in a change of the peak position. Maintaining a constant temperature is critical and may be difficult in areas were extreme temperature changes occur over the seasons of the year. In our laboratory, this is extremely difficult, especially over the cold winter months. Even though thermostats are set at a constant temperature, both day and night, the heating and cooling system has difficulty maintaining a constant temperature. Consequently, we see a change in the sizing precision (drift) of the instrument. After the completion of the entire 96 sample run, but before turning off the instrument, the analyst reviews the internal lane standard, monitoring the position of the 250 base pair peak and the allelic ladders on the instrument. If it is apparent that the peak positions have changed over the length of the run, the analyst reinjects the set of samples affected, taking care that there is sufficient polymer and buffer in the instrument to handle the additional injections. If the drift is not too bad, sub-projects may be created to assist in data interpretation. Although the review process and reinjection are easy steps, they add time to the analysis and decrease throughput. The instrument should be placed in an area where temperature changes are kept at a minimum.

Quality control procedures on the instrument generally require a new matrix file to be generated with each new lot of polymer and capillary to insure consistent precision and sensitivity. The matrix file is used by the instrument software to correct for spectral overlap of the dyes in the virtual filter set. Changes in laser alignment, pump force, or camera model can result in sensitivity changes and could require a sensitivity check after certain maintenance procedures. We found that different cameras resulted in dramatically different sensitivity. Additionally, the conductivity of the formamide added to the samples before injection is critical and the conductivity of each new lot should be checked. Spiking artifacts caused by electronic surges or precipitated polymer in the capillary, appear as very sharp lines in the electropherogram and can be present in a position of a true allele.

This changes the peak height values and could potentially cause interpretation difficulties in mixtures. Sometimes, these can take the shape of a true peak and the samples must be reinjected. However, the vast majority of spikes are easily recognized because they are normally present in the same position in all three dye-layers. Other anomalies can include noisy baseline from dirty capillaries, or defective capillary holder, old buffer or old polymer. Analysts found that the instrument's greatest advantage was that sample preparation was very minimal, and the collection software and the data analysis software very "user-friendly" and much simpler than the FMBIO®II Fluorescent Scanner.

ABI Prism® 377 DNA Sequencer. The ABI Pris® 377 DNA Sequencer was purchased for its sequencing abilities, but was initially validated to do casework analysis in STR loci (4). At the time of purchase, this instrument was capable of analyzing 32 samples, but can now be purchased in a 96-lane format. This instrument does necessitate the preparation of ultrathin polyacrylamide gels and gel loading. Gel preparation can become much easier with the use of the 5% Long Ranger™ denaturing polyacrylamide Singel™ Packs (BioWhittaker Molecular Applications, Rockland, ME, USA). These gels are packaged with pre-measured ingredients, so that one needs only to break the barrier between the packets, mix the gel, and pour it. There is no weighing of ingredients, exposure to the acrylamide is reduced, and gels are more consistent. The gel undergoes electrophoresis, laser excitation, and detection within the instrument. At the lower portion of the gel, a laser beam continuously scans across the gel as the fluorescent-labeled amplified products pass by during electrophoresis. The software is the same as with the ABI Prism® 310 Genetic Analyzer. Quality control measures include checking each new lot of gels and making conductivity checks of the formamide. In approximately three hours, all 32 samples are ready to analyze, which is especially important when a case takes on a priority status and time is of the essence. In our hands, this instrument was the most precise of the three instruments and the easiest to analyze.

Hitachi® FMBIO®II Fluorescent Scanner. The FMBIO®II Fluorescent Scanner is used to scan gels that have previously undergone electrophoresis on a 43-cm long polyacrylamide gel (5) in a Model SA 43-cm vertical sequencing gel electrophoresis apparatus (Life Technologies, Rockville, MD, USA). We use the 5% Long Ranger™ Singel™ Packs for the gel composition. The gel takes approximately two hours to electrophorese and may be reused several times after scanning. This is accomplished by reversing the polarity on the gels after initial electrophoresis and running the gel backwards for a period of one half hour plus the time it took for the initial sample electrophoresis. The gel is then ready for the next set of samples. Validation work was completed on several sets of gels and no contamination from previous runs was observed. These gels may be used from three to five times. Our laboratory has five electrophoresis tanks, so that several gels can be running at the same time. The electrophoresis gel (sandwiched between glass plates) is placed on a platform in the instrument, the door closed, and the gel is scanned by a laser moving across the surface of the glass plates and recording the position of the amplified product. The scan time is approximately 20 to 30 minutes. The FMBIO®II Fluorescent Scanner is a very durable and reliable instrument, with very little routine maintenance. Temperature fluctuations do not affect the analysis and there are no specific facility requirements. Several gels can undergo electrophoresis at the same time while the instrument is scanning other gels. Gel loading is easy. However, the analysis software can be cumbersome and is, typically, more time consuming then the ABI software. Quality control procedures only involve the checking of each new lot of gels.

Instrument Precision

Precision testing. Each of the instruments was tested for precision. Twenty-two allelic ladders were compared over five separate injection runs on the ABI Prism® 310 Genetic Analyzer. Twenty-one ladders over eight gels were used for the ABI Prism® 377 DNA Sequencer and 21 ladders over 9 gels were used for the FMBIO®II Fluorescent Scanner. The amount of variability in the base pair size range of each allele was measured in three standard deviations. Our validations indicated that the ABI Prism® 377 DNA Sequencer exhibited the best precision, whereas the

ABI Prism® 310 Genetic Analyzer and the Hitachi FMBIO® II Fluorescent Scanner had similar precision (Table 1).

Testing the sensitivity of instruments. The sensitivity of the instruments was tested with dilutions of 1 ng, 0.5 ng, 0.25 ng, 0.125 ng, 0.0625 ng, and 0.03125 ng on three samples. Each sensitivity run was repeated three times. All alleles were detected on all instruments at the 0.5 ng of amplified target DNA.

Testing the amplification systems. Each of the amplification kits was also validated. The amplification kits used for the ABI Prism® 310 Genetic Analyzer and the ABI Prism® 377 DNA Sequencer included the ABI Amp*fl*STR™ nine-locus Profiler Plus™ and the ABI Amp*fl*STR™ seven-locus COfiler™ (6,7). The amplification kits used for the FMBIO®II Fluorescent Scanner included the eight-locus *GenePrint*® PowerPlex™ 1.1 and the nine-locus *GenePrint*® PowerPlex™ 2.1 (8,9). Each of the four kits was tested with the same extracted DNA and the same dilutions. Various single source samples (blood, saliva, vaginal, perspiration, teeth, bone, and semen), mixtures of physiological fluid samples in ratios of 20:1, 10:1, 5:1, 3:1, 2:1, and 1:1, head and pubic hairs, and animal bloods were studied. Various substrates, such as glass, stamps, envelopes, dirty tires, green leaves, leather shoes, wood, denim fabric, tennis shoes, and rusty metal were tested. Concordance studies (120 population samples and 19 external proficiency tests) were performed between all detection formats and amplification kits. All samples were concordant. The results of all of the validations performed showed an equal performance with these kits (10). After the validation process, the same target value of DNA for all amplification kits was chosen for casework analysis.

Minor peaks, which appear one repeat unit shorter than the major allele peak, will occur at certain genetic loci due to polymerase slippage during the elongation step of the amplification process. These are known as stutter peaks and are generally very low in peak intensity compared to the true allele. However, it is imperative that the laboratory determines the normal ratio of the stutter peak height to the true allele at each genetic locus for each system used. This is especially important to determine the presence of a true allele in single source and mixture samples. Although the stutter values were somewhat different on the amplification systems, depending on loci, they were all under 15% (Table 2). Peak height ratios of the two peaks present in heterozygote samples were also determined. These ratios are helpful in determining the major and minor components in mixtures. The average heterozygote ratios for the ABI Prism 310 Genetic Analyzer and the ABI Prism® 377 DNA Sequencer were 87%, but the range of values extended from 60% to 100%. Heterozygote ratios are not normally used on the FMBIO®II Fluorescent Scanner due to the variance in the optical density of the gel background. Visual inspection of the band intensities is used instead. The stutter ratios and the heterozygote ratios were determined from approximately 200 population samples.

Training across All Detection and Amplification Formats

Training on all of the instruments was not as difficult as one might expect. The extraction process and the quantitation methods are the same. Since the same amplification target values were used, the samples could be prepared with the same dilutions. Each analyst was already familiar with polymerase chain reaction (PCR) technology and had previous qualifications in RFLP and the DQA1/PM™ (Applied Biosystems) based systems. Consequently, the amount of training was not as extensive as their initial training. Initial training involves a minimum of 100 samples that include a wide range of physiological fluids, hairs, teeth, bone, stamps, envelopes and other substrates, and complex mixtures. Initial training was according to the Scientific Working Group for DNA Analysis Methods (SWGDAM) Training Guidelines (11). Cross training in the STR systems involved 30 single source stains and 10 mixture samples, a mock case, and an external proficiency test. The reason for the extensive single source stains was to familiarize the analyst with the detection instrument software nuances. Each of the manufactures' amplification kit directions was very easy to follow.

Protocol Development

Protocol manuals are necessary and can be somewhat cumbersome to write. However, the generation of a PCR Manual for STR analysis was not as difficult as initially expected. Various amplification and detection systems were used. The PCR protocols for the extraction and quantitation phases of forensic analysis are

Table 1. Instrument precision for ABI Prism® 310 Genetic Analyzer (ABI Prism® 310), ABI Prism® 377 DNA Sequencer (ABI Prism® 377), and Hitachi® FMBIO®II Fluorescent Scanner (Hitachi® FMBI®II) expressed in three standard deviations of the average base pair size of each allelic ladder set[a]

| Locus | 3 SD values of the measurements on instruments | | |
	ABI Prism® 310 (n = 22)	ABI Prism® 377 (n = 21)	Hitachi® FMBI®II (n = 21)
D3S1358	0.48	0.24	0.43
VWA	0.83	0.16	0.39
D7S820	1.07	0.15	0.38
D16S539	0.49	0.10	0.76
D8S1179	0.79	0.20	0.38
FGA	1.06	0.27	0.86
D18S51	1.60	0.26	0.97
D21S11	0.42	0.17	0.58
D13S317	0.78	0.18	0.37
D5S818	0.68	0.18	0.66
CSF1PO	1.22	0.14	1.44
THO1	0.58	0.17	0.75
TPOX	0.38	0.16	0.40
PENTA E			0.91

[a]The 3 standard deviation (SD) values were determined by first measuring the base pair size of each allele present in the allelic ladder used at each locus. Since the allelic ladder contains all of the alleles normaly found in each locus, this gave a good estimation across all of the loci for the entire electrophoresis run. The mean value of the measurements was determined and the SD (spread) around the mean was determined by the formula $\sqrt{1/n[(x_1-\bar{x})^2+\ldots+(x_n-\bar{x})^2]}$, where x = the allele size measured, $\bar{x}$ = the mean of the allele size measurements, and n = the number of measurements. Three SDs will account for 99% of the values. For example, at D3S1358, in the ABI Prism 310, the measurement of the same allele could vary by as much as plus or minus 0.48 base pairs. One percent of the time a sample may fall out of the range due to sampling error.

Table 2. Stutter values calculated for the ABI Amp*fl*STR® nine-locus Profiler Plus and the seven-locus COfiler on the ABI Prism® 310 (ABI Prism® 310) and the ABI Prism® 377 (ABI Prism® 377), and for the Promega *GenePrint*® eight-locus PowerPlex 1.1 and the nine-locus PowerPlex 2.1 (Hitachi® FMBI®II)[a]

| Locus | Stutter (%) | | |
	ABI Prism® 310	ABI Prism® 377	Hitachi® FMBI®II
D3S1358	12	15	14
VWA	10	14	15
FGA	12	13	11
D8S1179	9	12	13
D21S11	9	16	11
D18S51	14	15	11
D5S818	10	13	13
D13S317	8	11	14
D7S820	9	12	14
D16S539	11	15	14
TH01	6	8	7
TPOX	7	13	8
CSF1PO	11	11	9
PENTA E			0

[a]Stutter values are calculated by dividing the peak height of a minor peak (n-4 peak) by the peak height of the true allele (n). The maximum ratio at each genetic locus is the value given in the table. Approximately 200 single source samples exhibiting a minor peak (less than 15%) occurred one repeat unit shorter than the major allele peak. They are used to make these estimates of stutter. This illustrates that any peak appearing at one repeat unit from another peak that is less than the stutter ratio is most likely due to slippage occurring during the elongation step of the amplification process, and not a true allele.

the same for all formats. The protocol is sectionalized for each of the two amplification formats and the three detection instruments. However, only one interpretation, statistics, report writing, and review sections exists. Whether peaks or bands, the interpretations are identical. Major and minor components are determined in the same manner as is the determination of a match. The usage of peaks and bands is interchangeable. Threshold values can be set for either instrument; however, band visualization is normally the threshold for the Hitachi FMBIO®II. The SWGDAM in the United State recognized that the interpretations were basically the same and established generic guidelines applicable to all systems (12).

Results

Although there is a variety of differences with detection and amplification formats, we have found that all gave correct allele calls after repeatedly typing the same training samples and checking difficult single source and mixture samples. Cases analyzed in one year on one instrument or amplification format were linked to case results generated in the following year in another format. Database "hits" have also been made across formats. We have also found the flexibility of the variety of formats useful when alleles between, above, or below the ladder are present. In these instances, the samples are reamplified with another set of primers. In addition, we have the ability to check for true homozygotes when it is suspected that a variant has occurred in the primer region, resulting in one of the alleles not being detected. The use of multiple formats also assists the laboratory. When kits are difficult to obtain from one manufacturer due to insufficient stock, quality control problems, or when instrument maintenance problems occur, the laboratory can still proceed on the other format. Tracing problems with amplifications is also easier with the different detection formats. Since both the ABI Prism® 310 and 377 use the ABI Amp*fl*STR™ Profiler Plus and COfiler kits, the same amplified product can be checked on both instruments to determine if the problem is instrument, sample, or amplification related.

The laboratory is now using the sixteen-locus *GenePrint*® PowerPlex™ 16 amplification kit. This amplification kit is compatible with the ABI Prism® 310 Genetic Analyzer and the ABI Prism® 377 DNA Sequencer. The internal lane standard is excellent and the sensitivity superb (unpublished data). The single amplification kit reduces analysis time. The amplification preparation time is cut in half, there is only one set of tubes to label, one set to amplify, less injections or gels per case, less sample used, and quality control on only one kit. It is not only time-efficient but also very cost-effective. We will, however, still maintain other amplification kits to confirm microvariants or resolve problems that may occur with degraded DNA or very dilute samples.

Conclusion

In conclusion, we can assure that any of the formats used will provide reliable results (13-19). Each has its own nuances but all are accurate and reliable. The allele calls are the same unless primer variants are present. This could occur with both amplification platforms. Data can be confirmed on any of the instruments. No matter which format is used, data input into Combined DNA Index System (CODIS) and the search engine employed by CODIS will make an accurate and reliable match. All three of the instruments in our laboratory are in constant use. Detection format selection is based on sample source and condition and instrument availability. After one-year use of all systems, we have found no reason to change the general interpretation guidelines used for all instruments and amplification formats. We feel we have gained a considerable amount of knowledge on the analysis of STR-based systems through the use of several formats and have gained a confidence in the reproducibility of results. We are satisfied with the pathways chosen and need not wonder "if the grass is greener" in another format.

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