A Comparison of the Validation Studies Conducted on the AmpF*l*STR™ Profiler Plus™ Kit

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ABSTRACT

The ABI-PE 310 Genetic Analyzer capillary electrophoresis (CE) unit was selected as the instrumentation to evaluate STR analysis for the Illinois State Police (ISP). The initial amplifications and subsequent analysis of samples was completed using the AmpF*l*STR™ Profiler and Profiler Plus™ PCR Amplification Kits (ABI-PE). In order to demonstrate reliability in house, analytical phases were designed to validate the use of the ABI-PE 310 in conjunction with the Profiler Plus™ kit. The areas of analysis included: polymer evaluation, reproducibility, sensitivity, precision, mixtures and non-probative casework analysis. Five forensic scientists worked on this project in the ISP R&D laboratory and five forensic scientists were assigned to the project at the ISP Forensic Science Center at Chicago (FSC-C). Information collected from these analytical phases was compared to data reported by ABI-PE in their AmpF*l*STR™ Profiler Plus™ User's Manual.

The AmpF*l*STR™ Profiler PCR Amplification Kit was used to evaluate different polymer concentrations available from ABI-PE. The polymer recommended for analysis is a 4% solution of a proprietary gel matrix (POP4). A 6% solution of the gel matrix is also available (POP6). The HUMTH01 locus amplified within the Profiler multiplex contains two alleles (9.3 and 10) that differ in length by a single base. A known HUMTH01 9.3, 10 sample was analyzed using both the POP4 and POP6 polymers. While neither polymer produced resolution such that the valley between peaks reached baseline, both solutions resolved the 9.3 and 10 alleles. Both ISP and ABI-PE data document resolution of the POP4 polymer at one base pair.

The remaining studies were carried out utilizing the AmpF*l*STR™ Profiler Plus™ PCR Amplification Kit. Reproducibility studies were designed to evaluate both intralaboratory and interlaboratory variations. Samples previously analyzed using the Hitachi FMBIO®-100 and STR Call™ software were evaluated in both laboratories participating in this study. The definition of a sample's genotype was not changed as a result of the detection method utilized or site of analysis in either study. Results were consistent with those reported by ABI-PE.

Sensitivity of the instrument was evaluated by analyzing a range of DNA utilized for amplification. Optimal input concentration of DNA identified by ISP and ABI-PE was found to be approximately 1 to 2.5 ng. This is consistent with ABI-PE's findings. Degradation affected peak height of larger loci in both studies.

Stutter is an inherent problem of the PCR process when amplifying STR's. Length of the locus, allele and/or repeat may affect the amount of stutter present. Samples were identified from previous analytical phases to define the amount of stutter that might be expected for alleles at the loci defined by the AmpF*l*STR™ Profiler Plus™ Kit. The results collected were compared with stutter results collected by ABI-PE. In both sets of data larger alleles produced a greater percentage of stutter.

The precision in determining fragment length using the ROX 500 Internal Sizing Standard (ABI-PE) was evaluated. Precision evaluations demonstrated that at three times the standard deviation, all size determinations were within one base pair of each other. In conjunction with the precision study, peak height ratios for heterozygotes were analyzed. This data demonstrated that peak height ratios for most heterozygous alleles were greater than or equal to 70%.

Using the stutter and precision data, the ability to resolve mixtures was evaluated in the ISP laboratories. Mixtures of samples with known genotypes were prepared at specific proportions to determine when the minor component of a mixture could be defined. ISP studies confirmed what was reported by ABI-PE; that beyond a ratio of 10:1, the minor component of a mixed sample is rarely detected.

In the final analytical phase, the data collected in earlier phases was used to evaluate DNA profiles obtained from non-probative casework samples. Blood and semen stains from forensic cases were collected and the STR profiles identified for each sample were compared to known reference standards. The frequency associated with single contributor profiles in the forensic samples ranged from approximately 1 in 1.4 billion to 1 in 310 trillion in the Caucasian population.

INTRODUCTION

Prior to April 1, 1998 the Illinois State Police Division of Forensic Services laboratories offered PCR analysis for the D1S80 locus [1] and the markers of the Amplitype PM kit [2]. Beginning in April of 1998 the Illinois State Police began analysis of the Short Tandem Repeats (STR's) defined in the AmpF*l*STR™ Profiler Plus™ PCR Amplification Kit [3]. The studies conducted to validate the use of this kit included an evaluation of reproducibility, sensitivity, stutter, precision, mixtures, and non-probative casework. The validation project was completed at the Research and Development Laboratory located in Springfield and the Forensic Science Center at Chicago (FSC-C).

METHODS

Sample extractions were carried out using a standard SDS/Proteinase K extraction buffer. Phenol/Chloroform/ Isoamyl alcohol was used to purify the extracted DNA. DNA samples were isolated using Microcon-100 tubes (Millipore). Samples were resolubilized in TE^{-4} buffer. The extracted DNA samples were quantified using yield gel analysis. Quantitation was also accomplished using the Quanti-Blot kit (Perkin-Elmer) with the alpha satellite D17Z1 to assess the concentration of total human DNA. If required further dilution of samples was made in distilled-deionized water. Amplifications were conducted as specified in the Profiler Plus™ kit.

REPRODUCIBILITY

To evaluate reproducibility 50 samples were identified for which STR types had been previously characterized using the FMBIO® in the R&D and FSC-C labs. No differences were observed in the genotypes defined using the two instruments. A second study conducted during the reproducibility phase was a comparison of the amount of input DNA. Ten previously characterized samples were amplified at 1 and 2 ng of input DNA. While comparable results were obtained when PCR product was analyzed on the 310 instrument the 2 ng amplifications resulted in relative fluorescence units (rfu) values which were closer to the target range of 1000 to 2000 rfu.

SENSITIVITY

Since the unknown samples associated with forensic cases are expected to have limitations, 2 ng amplifications of high molecular weight human DNA may not always be possible. A sensitivity study was designed to examine the effect of amplifying various concentrations of DNA with the AmpF*l*STR™ Profiler Plus™ kit and analyzing this data with the 310 Capillary Electrophoresis instrument. A dilution series was created for five samples ranging from 10 ng to 0.036 ng. The lower limit detected at FSC-C was 0.078 ng and the lower limit for detection of all loci in the R&D Laboratory was 0.30. The upper end of the concentration range was evaluated to determine the maximum concentration of input DNA which could be amplified and when analyzed would not result in artifacts due to incomplete matrix correction. The upper limit defined in the R&D Laboratory Study was 5.0 ng and the upper limit observed at FSC-C was 2.5 ng.

From the dilution series study comparisons were also conducted on the results of each locus and/or each dye as input DNA concentration was changed. With respect to dye sensitivity the results of 5-FAM and JOE were approximately equal. Loci labeled with the NED dye exhibited allele and locus dropout. This allele and locus dropout could be attributed to DNA fragment size and dye label. When allele or locus dropout was indicated increased injection time was evaluated to determine if results improved. Injection time studies of samples with rfu values within the target range indicated that an increase of injection time from 5 to 10 seconds would result in an rfu increase of approximately 50%. Doubling the injection time for samples which indicated allele or locus dropout was effective in providing conclusive results for loci which initially would be graded as inconclusive. When available 1-2.5 ng of human DNA is recommended for amplification using the Profiler Plus™ kit. When less human DNA is available increased injection time is recommended.

STUTTER

Stutter is a PCR artifact associated with STR's. An examination of stutter was completed for incorporation into interpretation guidelines. Five observations of high molecular weight alleles were selected at each locus. Heterozygous samples were separated by at least two repeats. The observations made in this study correlated well with the report issued by Perkin-Elmer in the AmpF*l*STR™ Profiler Plus™ user's manual. Areas where differences were noted were attributed to increase in allele size for example: at the FGA locus Perkin-Elmer noted a maximum of <10% stutter for the 28 allele; FSC-C noted a maximum of <11.39% stutter for the 29 allele; and the R&D Laboratory identified a >30 allele with a maximum of < 12.21% stutter.

PRECISION

Precision was evaluated in two phases. The first phase of the precision study examined expected peak height ratios for known heterozygous individuals. For amplifications of input DNA resulting in PCR product

which produced RFU's in the target range of approximately 1000-2000 rfu the maximum peak height variation noted was 70%. Evaluations of peak height ratios outside the linear range of the instrument were noted outside the value of 70%. In the second phase of the precision study the 310 was evaluated to test its sizing capabilities. Five samples were run 20 times and the allele sizes were recorded. All size determinations were within 1 bp.

MIXTURES

Given information from sensitivity, stutter, and peak height ratio studies an evaluation of known mixtures was completed. Two samples were selected such that most loci contained non-overlapping alleles. The samples were mixed at ratios of 1:200 to 1:1 and then the series was reversed. The minor contributor could be detected at 1:20 but could fit the stutter model and was therefore not accepted as a true allele. At 1:10 and greater the minor component could be detected and defined. At low fluorescence levels peak height ratios were noted which did not fit the 70% model. Given the results of the above described studies, non-probative cases were collected to evaluate the Profiler Plus™ kit and the 310 for application to casework analysis.

NON-PROBATIVE CASEWORK

Nineteen non-probative cases were analyzed which contained at a minimum the victim's standard and a blood or semen stain associated with the case. Nine of the cases had previously been analyzed using the Amplitype PM/DQA1 and D1S80 marker systems. The same match assessments were made when the samples were amplified using Profiler Plus™, Amplitype PM/DQA1 and D1S80. Of the 19 cases analyzed 16 matches and three exclusions were obtained. The reported power of discrimination of 1 in 310 trillion was obtained using the AmpF*l*STR™ Profiler Plus™ PCR Amplification Kit.

CONCLUSION

Some of the validations on the AmpF*l*STR™ Profiler Plus™ kit have been described. These studies included reproducibility sensitivity, precision, mixtures and non-probative casework analysis. Comparison of the genotype results obtained in the validation studies conducted in the Illinois State Police laboratories with those obtained by Perkin-Elmer serves to verify that the AmpF*l*STR™ Profiler Plus™ PCR Amplification kit in conjunction with the Applied Biosystems 310 capillary electrophoresis unit can be reliably utilized in forensic casework.

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