# INSTRUMENTATION

## Comparison of Multiple STR Platforms and Instrumentation

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In 1998, with the anticipated shift from RFLP and DQA1/PM typing systems to STR-based systems, our laboratory installed a Hitachi FMBIO® II Fluorescence Imaging System, an ABI PRISM® 310 Genetic Analyzer and an ABI PRISM® 377 DNA Sequencer. These instruments were subsequently validated and used for casework and convicted-offender samples. The original validated platforms included Profiler Plus™ for the ABI PRISM® 310 and ABI PRISM® 377 and PowerPlex® 1.1[c,d] for the Hitachi FMBIO® II. In order to satisfy CODIS requirements for sample upload to NDIS, COfiler® was added for the ABI instruments and PowerPlex® 2.1[c,d,e] was validated for the FMBIO® II instrument. These systems were used for both casework and convicted-offender samples until the 16-loci megaplex PowerPlex® 16 System[c,e,f] and PowerPlex® 16 BIO[c,e,f,g] Systems became available. These multiplexes are currently in use in our laboratory for casework and convicted offenders, while Profiler Plus™ and COfiler® are reserved to verify microvariants and off-ladder alleles whenever appropriate.

*We would like to share our experiences and compare the ABI PRISM® 310 Genetic Analyzer, ABI PRISM® 377 DNA Sequencer and the Hitachi FMBIO® II Fluorescence Imaging System with the two new ABI PRISM® 3100s installed in our laboratory.*

Two proficiency tests are analyzed each year by each analyst; one proficiency test is amplified using the PowerPlex® 16 System and run on either the ABI PRISM® 310 or 377, while the second proficiency test is amplified using the PowerPlex® 16 BIO System and analyzed using the FMBIO® II. Analysis using the individual ABI PRISM® 377 and 310 instruments is rotated each year. This allows the analyst to be proficiency tested on the ABI and FMBIO® software every year. Recently two ABI PRISM® 3100 Genetic Analyzers were purchased and validations begun.

For laboratories considering changing their current detection systems or supplementing those instruments with the ABI PRISM® 3100, we would like to share our experiences and compare our original instruments with the two new ABI PRISM® 3100s installed in our laboratory. The following comparisons are summaries and are not an all-encompassing review of our experiences in a multiplatform laboratory performing analysis on both casework and convicted-offender samples. We hope that our experiences will provide insight into instrument and kit choice.

### STOCHASTIC LEVEL

As part of our work to satisfy the FBI Standards requirements for validation, we performed sensitivity studies for each system and for each instrument. Our stochastic level is defined as the minimum dilution where a full profile at all loci is observed. This statistic gives an indication of instrument sensitivity and signal-to-background ratio. The stochastic level for the STR systems on the ABI PRISM® 3100 was equal to that on the FMBIO® II and twice as sensitive as that for PowerPlex® 16 on the ABI PRISM® 310 and 377 (Table 1).

### PRECISION

We completed precision studies for each instrument using ladders from various runs. The average precision was calculated as 3X the standard deviation (SD) for each allele at each locus. The ABI PRISM® 3100 had tighter precision than the ABI PRISM® 310 and 377 running the PowerPlex® 16 or the FMBIO® II running the PowerPlex® 16 BIO System (Table 1).

## MATRICES

Due to the overlap in the emission spectra of the fluorophores used in each dye set, a matrix needs to be generated for each instrument. A matrix is a mathematical algorithm defining the amount of dye-to-dye bleedthrough (spectral overlap). For the PowerPlex® 16 BIO System, there are a number of ways to produce a matrix. A band defining the dye color (i.e., an allele band) can be boxed and used as a basis for another dye's target. This is done for each color in the system, and the software then produces a matrix. The matrix dye sample cocktail included in the kit is loaded on the gel, and this sample lane is used to produce the matrix.

**Table 1. Stochastic Limit, Precision and Amplification Cycles for STR Amplification Kits on Instruments in Use at the Pennsylvania State Police DNA Laboratory.**

| System | Instrument | Stochastic Limit | Amplification Cycles | Average Precision (3X SD) |
|---|---|---|---|---|
| PowerPlex® 1.1/2.1[1] | FMBIO® II | 0.25ng | 10/20 | 0.66bp |
| Profiler Plus™/ COfiler® [1] | ABI PRISM® 310/377 | 0.25ng | 28 | 0.80bp (310)<br>0.18 bp (377) |
| PowerPlex® 16 | ABI PRISM® 310/377 | 0.25ng | 10/22 (310)<br>10/20 (377)[2] | 0.18bp (310)<br>0.21 bp (377) |
| PowerPlex® 16 BIO | FMBIO® II | 0.125ng | 10/22 | 0.45bp |
| PowerPlex® 16 | ABI PRISM® 3100 | 0.125ng | 10/20 | 0.15bp |

[1]amplified at half reaction volumes
[2]low-level samples may be amplified at 10/22 cycles

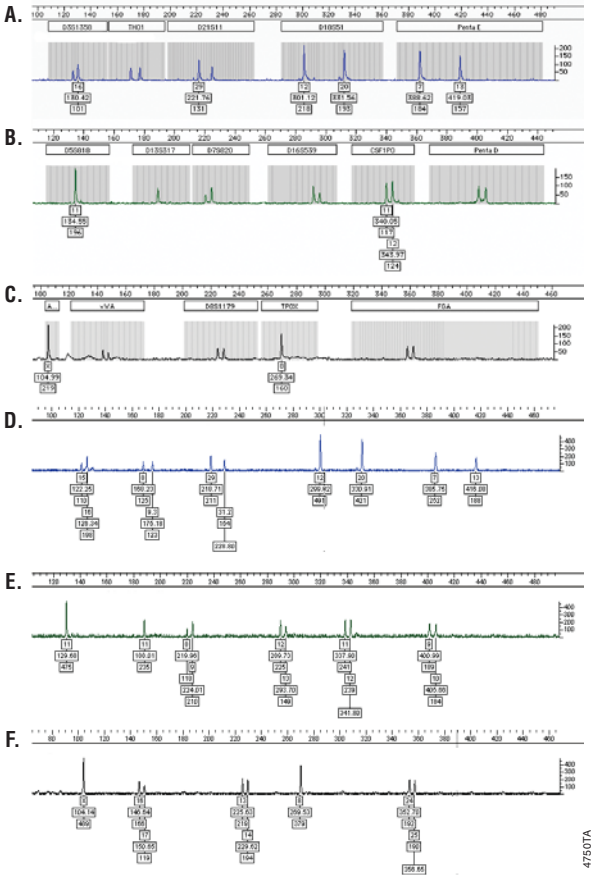


**Figure 1. A nonprobative case run on the ABI PRISM® 377 and 3100.** An aliquot of the same PowerPlex® 16 amplified product was analyzed on both the ABI PRISM® 377 and 3100. **Panels A–C.** Electropherograms showing the fluorescein-, JOE- and TMR-labeled loci of the PowerPlex® 16 System run on the ABI PRISM® 377. **Panels D–F.** Electropherograms showing the fluorescein-, JOE-, and TMR-labeled loci of the PowerPlex® 16 System run on the ABI PRISM® 3100.

We produce a new matrix for each gel run, and the resulting matrix usually requires some manual changes. For the ABI PRISM® 377, we make a matrix for each new lot of gel and at least once every three months. The appropriate set of dye-labeled fragments is purchased, mixed with buffer and separated by electrophoresis. A matrix is produced using the GeneScan® software and rarely needs to be altered. For the ABI PRISM® 310, we create a matrix for each new lot of polymer and each new lot of capillary. As with the PowerPlex® 16 BIO System, the matrix often needs some manual changes. We tested the new PowerPlex® Matrix Standards, 310/377, (Cat.# DG3640) on the ABI PRISM® 310, and no correction was needed after the matrix had been generated. For the ABI PRISM® 3100, we generate a new spectral (matrix) at least annually, whenever decreased color separation is observed or whenever service is performed on the laser or CCD camera. Low-level bleedthrough (approximately 4.5%) from the green layer is frequently observed in the blue channel, especially at D18S51 and Penta E.

## CONCORDANCE

We have run several hundred samples, including, but not limited to, training validation, proficiency, database, convicted-offender, NIST and nonprobative case samples on each instrument and have shown concordance.

## COMPARISON OF PLATFORMS

As part of the PowerPlex® 16 BIO System validation for the FMBIO® II, we re-analyzed 14 nonprobative cases previously analyzed using Profiler Plus™/COfiler® on the ABI PRISM® 310, PowerPlex® 16 on the ABI PRISM® 310 or 377, or PowerPlex® 1.1/2.1 on the FMBIO® II (Table 2). The PowerPlex® 16 BIO System was similar in sensitivity to PowerPlex® 16 on the ABI PRISM® 377, PowerPlex® 1.1/2.1 on the FMBIO® II, and Profiler Plus™/COfiler® on the ABI PRISM®

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310. PowerPlex® 16 on the ABI PRISM® 310 may be slightly more sensitive; however, the overall sensitivity is similar for all kits and all instruments.

As part of our ABI PRISM® 3100 training, we re-analyzed amplified product from nonprobative cases previously analyzed using the ABI PRISM® 377. Coupled with the above comparisons for the FMBIO® and PowerPlex® 16 BIO System, we could indirectly compare sensitivity, precision and concordance of each platform. The ABI PRISM® 3100 exhibits increased sensitivity and precision when compared to the ABI PRISM® 377. Product analyzed on the ABI PRISM® 3100 demonstrated higher peak heights, and in many cases, additional alleles were above threshold. One case originally had only 11 alleles (over 8 loci) above threshold when run on the ABI PRISM® 377; however when we re-analyzed the same amplified product using the ABI PRISM® 3100, we obtained a full, 16-loci profile (Figure 1). This increase in sensitivity was seen, to a lesser extent, with additional cases and a proficiency sample (additional alleles from the male were in a female fraction).

Our overall experience indicates that the ABI PRISM® 3100s appear to be more sensitive instruments regardless of the instrument, laser or camera age.

## ROBOTIC WORKSTATION

We have outsourced the majority of our convicted-offender samples to help reduce our backlog; however, these liquid blood samples still need to be inventoried, plated and prepared for shipment and the data reviewed before uploading to NDIS. The use of a Packard BIOScience MultiPROBE® II Workstation has greatly reduced the hazards involved with plating large numbers of convicted-offender blood samples, as this population has a higher risk for certain blood-borne diseases than the population at large. In addition to plating blood samples, DNA purification and contamination validations were done with this instrument (data not shown). DNA extractions using the DNA IQ™ System[(b)] were optimized to provide average yields of 0.7ng/µl. Cross-contamination studies (done in triplicate) were performed in 96-well, U-bottom plates with convicted-offender blood samples and blanks arranged in a checkerboard pattern. The blanks showed no visible bands in post-amplification gels, and blanks chosen at random and analyzed on the ABI PRISM® 310 contained no detectable DNA. With the conclusion of the robot validations and the ABI PRISM® 3100s high throughput, we plan to analyze these convicted-offender samples in-house.

## DISCUSSION

Several laboratories are contemplating changing or supplementing their detection platforms. Our years of casework and convicted-offender experience has allowed us to compare six amplification kits across four available platforms. The ABI PRISM® 310 and 377 platforms running Profiler Plus™/COfiler® and PowerPlex® 16 have comparable sensitivity to the FMBIO® II running PowerPlex® 1.1/2.1 and PowerPlex® 16 BIO. The ABI PRISM® 3100s running PowerPlex® 16 have tighter precision and increased sensitivity but have matrix issues that need to be resolved. With the use of better dyes and enhanced spectral (matrix), the high throughput and eventual integration of our robotic workstation would allow the ABI PRISM® 3100 to be a versatile and efficient instrument.

## FUTURE PLANS.

We should complete ABI PRISM® 3100 training for each DNA analyst, including single-source samples, nonprobative cases and proficiency samples soon. Future plans include the use of the ABI PRISM® 3100 for casework analysis and continued automation with the Packard BIOScience MultiPROBE® II Workstation to allow direct amplification of convicted-offenders' DNA extracted from whole blood. This will allow analysis of convicted offenders in-house. A second robot (to be purchased) would be ideal for casework samples, initially validated for known standards, and eventually used for appropriate evidence samples.

**Table 2. Comparison of Cases Originally Analyzed Using Various STR Amplification Kits Run on the ABI PRISM® 310, ABI PRISM® 377 and FMBIO® II.**

| Gel # | Amount of DNA Amplified (ng) | Amount Loaded on FMBIO® II (µl) | Result | Initial Analysis |
|---|---|---|---|---|
| 1 | 0.5 | 2.5 | Less Sensitive | PowerPlex® 16/ABI 310 |
| 2 | 0.5 | 2.5 | Same | PowerPlex® 16/ABI 377 |
| 3 | 0.5 | 3.0 | Same | PowerPlex® 1.1/2.1/FMBIO® II |
| 4 | 0.5 | 2.0 | Same | PowerPlex® 1.1/2.1/FMBIO® II |
| 5 | 0.5 | 2.0 | Same | PowerPlex® 1.1/2.1/FMBIO® II |
| 6 | 0.5 | 2.0 | Less Sensitive | PowerPlex® 16/ABI 310 |
| 7 | 0.5 | 2.0 | Same | PowerPlex® 16/ABI 310 |
| 8 | 0.5 | 2.0 | More Sensitive | PowerPlex® 1.1/2.1/FMBIO® II |
| 9 | 0.5 | 2.5 | More Sensitive | Profiler Plus™/COfiler® /ABI 310 |
| 10 | 0.5 | 2.5 | Same | PowerPlex® 1.1/2.1/FMBIO® II |
| 11 | 0.5 | 2.5 | Same | PowerPlex® 16/ABI 310 |
| 12 | 0.5 | 3.0 | Same | PowerPlex® 16/ABI 310 |
| 13 | 0.5 | 2.0 | More Sensitive | PowerPlex® 16/ABI 310 |