The Use of PowerPlex® 16 and PowerPlex® ES Systems with the ABI PRISM® 3100-*Avant* Genetic Analyzer

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*We report the successful generation and application of a matrix to analyze PowerPlex® 16 and PowerPlex® ES System data on the ABI PRISM® 3100-*Avant *Genetic Analyzer.*

INTRODUCTION

Multiplex short tandem repeat (STR) typing is, at present, the main technology in forensic identification and paternity testing. Alleles of STR loci, which differ by the number of repeat units, can be distinguished by electrophoretic separation. Capillary electrophoresis (CE) instruments, such as the DNA sequencers from Applied Biosystems, allow laser-induced fluorescence detection of the labeled STR loci. Nearly all descriptions of making a matrix on the ABI PRISM® 3100 Genetic Analyzer apply to the STR amplification kits from Applied Biosystems, but CE instruments from Applied Biosystems can also be used to analyze PowerPlex® Systems. Here, we report the successful generation and application of a matrix to analyze PowerPlex® 16 System[c,e,f] and PowerPlex® ES System[c] data on the ABI PRISM® 3100-*Avant* Genetic Analyzer, a four- or sixteen-capillary CE instrument with low to medium throughput.

Although the dyes in a dye set fluoresce at different wavelengths, the emission spectra of the dyes overlap. This spectral overlap must be eliminated for proper data analysis. Performing a spectral calibration produces a mathematical description of the spectral overlap of a given set of dye labels. This mathematical description is also called a matrix. A matrix must be generated for each dye set and each individual instrument. Once generated, the matrix is applied during detection to correct spectral overlap for each capillary. To perform a spectral calibration on the ABI PRISM® 3100-*Avant* Genetic Analyzer, all dyes must be analyzed in a single injection. On the ABI PRISM® 3100-*Avant* Genetic Analyzer using the data collection software version 1.0, there are 6 dye sets (D, E, F, Z, E5

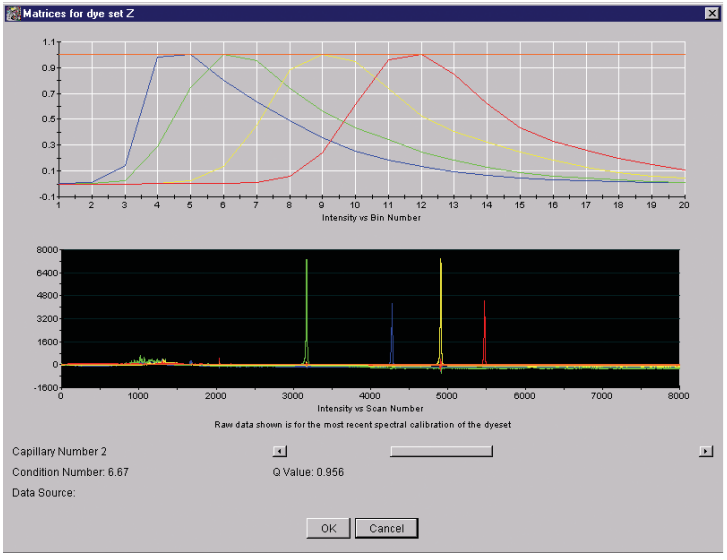

Figure 1. The spectral profile for dye set Z.

3100-AVANT

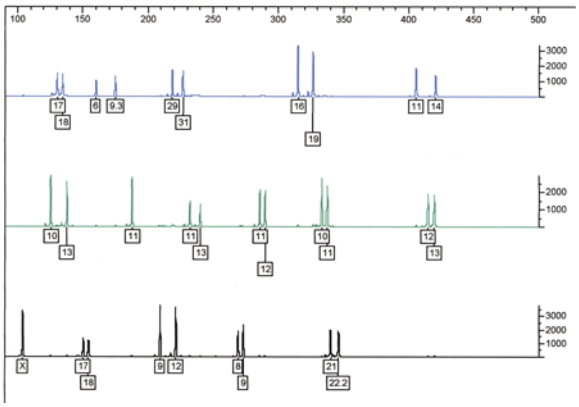

Figure 2. A DNA profile obtained with the PowerPlex® 16 System and analyzed using the ABI PRISM® 3100-*Avant* Genetic Analyzer.

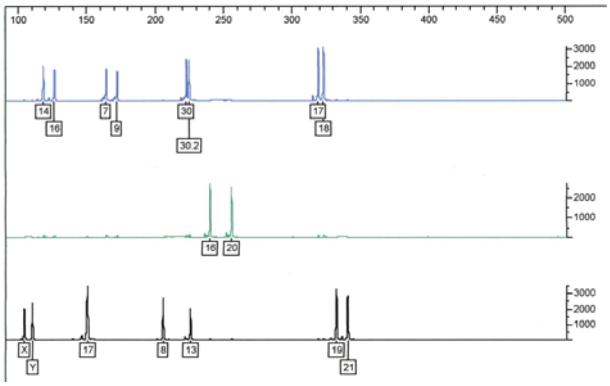

Figure 3. A DNA profile obtained with the PowerPlex® ES System and analyzed using the ABI PRISM® 3100-*Avant* Genetic Analyzer.

and G5). For the PowerPlex® Systems, the dye set "Z" should be used. Because the PowerPlex® 16, PowerPlex® ES and PowerPlex® Y(c) Systems use the same dyes, only one matrix is necessary.

SAMPLE PREPARATION

To generate a PowerPlex® spectral calibration file on the ABI PRISM® 3100-*Avant* Genetic Analyzer, the PowerPlex® Matrix Standards, 3100, (Cat.# DG3380) are required. We diluted each dye (JOE, FL, TMR and CXR) 1:10 in water and added 2.5µl of each dilution to 240µl of nuclease-free water. We loaded 15µl of this fragment mix into the wells, one for every capillary. For more information, refer to the *PowerPlex® Matrix Standards, 3100, Technical Bulletin* #TBD016.

INSTRUMENT PREPARATION

The run module and a special parameter file must be created for PowerPlex® applications. The run module is similar to the existing file "Spect36_POP4DefaultModule", but we changed the run time from 800 seconds to 2200 seconds. This module was saved with a new name: "Spect36_POP4PowerPlex".

Second, a new parameter file (MtxStd{Genescan_SetZPowerPlex}) with changed condition bounds range was created (see the *PowerPlex® Matrix Standards, 3100, Technical Bulletin* #TBD016). The following instrument settings are important:

Dye Set: Z

Spectral Run Module:
Spect36_POP4PowerPlex

Spectral Parameters:
MtxStd{Genescan_SetZPowerPlex}

After spectral calibration, the "spectral calibration result" window indicates which capillaries passed calibration. A "•" indicates the capillary passed; an "X" indicates the capillary failed. If a capillary fails spectral calibration, it is possible to override the failed run with data from a passed capillary (Figure 1), although Applied Biosystems does not recommend overwriting the capillary profiles on the ABI PRISM® 3100-*Avant* Genetic Analyzer. If fewer than 3 out of 4 capillaries on the ABI PRISM® 3100-*Avant* Genetic Analyzer pass, the spectral calibration should be repeated.

Genotyping of STR alleles was performed with an allelic ladder and internal lane standard. Fragment sizes of ladder alleles and sample alleles were calculated and subsequently compared. The Genotyper® Software was used for allele assignment.

We have successfully generated a spectral calibration on our ABI PRISM® 3100-*Avant* Genetic Analyzer, and we used this matrix for the PowerPlex® 16 System in paternity testing (Figure 2) and PowerPlex® ES System in forensic stain analysis (Figure 3) with good results.

Editor's Note: Promega has developed a new PowerPlex® Matrix Standards, 3100 (Cat.# DG3650), to replace the PowerPlex® Matrix Standards, 3100 (Cat.# DG3380). This new 3100 matrix standard is compatible with Data Collection Software 2.0. For more information about this new matrix, see the PowerPlex® Matrix Standards, 3100, Technical Bulletin #TBD019.