Development of PowerPlex® Matrix and Sample Protocols on the ABI PRISM® 3100

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We have developed a set of PowerPlex® matrix standards, which allows Promega's PowerPlex® Systems to be run on the ABI PRISM® 3100 Genetic Analyzer.

INTRODUCTION

Analysis of short tandem repeat (STR) polymorphisms is becoming the standard technique for DNA typing adopted by forensic laboratories around the world (1). Promega's PowerPlex® 16 System[b,c,d] provides increased efficiency and higher throughput by allowing for the amplification of the thirteen CODIS loci, plus Amelogenin and two additional highly polymorphic pentanucleotide STR loci, in a single reaction. Analysis of this system requires only a single capillary electrophoresis injection or gel lane. The advent of the 16-capillary detection platform in the ABI PRISM® 3100 Genetic Analyzer provides increased efficiency in sample detection. Use of the PowerPlex® 16 System on the ABI PRISM® 3100 reduces labor and time required for sample detection and analysis, providing higher throughput for both database and casework applications.

We have developed a set of matrix standards that allows the PowerPlex® Systems to be run on the ABI PRISM® 3100 Genetic Analyzer. The PowerPlex® Matrix Standards, 3100, contains matrix fragments labeled with the four fluorescent dyes used in the PowerPlex® Systems: 6-carboxy-4′,5′-dichloro-2′,7′-dimethoxyfluorescein (JOE), Fluorescein (FL), carboxy-tetramethylrhodamine (TMR), and carboxy-X-rhodamine (CXR). The PowerPlex® Matrix Standards, 3100, is intended for customers who are using the PowerPlex® 16 System, the PowerPlex® 1.2 System[b,c] or any of the *GenePrint*® Fluorescent STR Systems[b,c] on the ABI PRISM® 3100.

GENERATING A POWERPLEX® SPECTRAL CALIBRATION FILE

Proper generation of a spectral calibration file is critical to evaluate multicolor systems with the ABI PRISM® 3100 Genetic Analyzer. Once a spectral calibration file is generated using the PowerPlex® dye set, it is applied during the detection of the PowerPlex® data to compensate for the spectral overlap between different fluorescent dye colors.

For each set of dyes run on the instrument, the 3100 data collection software uses a different parameter file for calibration (2). To calibrate the PowerPlex® dye set, a parameter file must first be customized to specify a Q-value and a condition bounds range. These values are determined empirically and provide the software with information regarding the amount of spectral overlap and tolerance for pull-up and pull-down between the dyes within the set (2). We have determined the appropriate Q-value and condition bounds for the PowerPlex® Matrix Standards, 3100, and provide instructions on how to use these values to customize a PowerPlex® dye set parameter file. Once the parameter file has been customized, the fragments in the PowerPlex® Matrix Standards, 3100, can be used to create a spectral calibration file.

MATRIX SAMPLE PREPARATION

The PowerPlex® Matrix Standards, 3100, are provided as four separate dye fragments. Before the fragments are mixed together, an aliquot of each separate dye fragment is diluted 1:10 in nuclease-free water. The four dye fragments are then mixed together before loading onto the ABI PRISM® 3100 Genetic Analyzer.

The volume and intensity of each dye fragment have been optimized for injection with water instead of formamide. We observed dramatic differences (100-fold) in the intensity of matrix fragment detection when different lots of formamide were tested for injection. Differences in conductivity or pH of the formamide used can affect the efficiency with which DNA fragments are electrokinetically injected. This effect is more pronounced when fewer fragments are injected. With only four fragments in the matrix mix, we found that the most consistent results

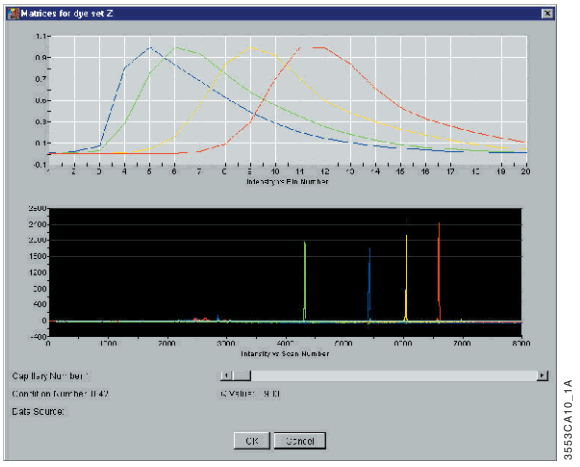

Figure 1. Spectral calibration of dye set Z using the PowerPlex® Matrix Standards, 3100.

were obtained by removing the formamide variable and injecting the matrix mix with water, which is provided with the product.

The spectral calibration run calibrates dye set Z (which allows for user-defined parameters) with the PowerPlex® Matrix Standards, 3100, using a modified run module and a PowerPlex® dye set parameter file. The spectral calibration data should be reviewed when the run is completed. If a capillary fails calibration, it will automatically be assigned the spectral data from the nearest passing capillary to the left. To achieve an effective spectral calibration file, a minimum of 12 out of the 16 capillaries should pass calibration, as recommended by the instrument manufacturer.

DETECTION OF AMPLIFIED SAMPLES

Once the dye set has been calibrated using the PowerPlex® Matrix Standards, 3100, samples amplified using the Power-Plex® Systems can be run on the 3100 instrument.

The Internal Lane Standard 600 (ILS600) is included in the PowerPlex® 16 System as the internal lane standard for four-color detection and analysis of amplified samples. The Fluorescent Ladder (CXR), 60–400 Bases, is included in the PowerPlex® 1.2 System as the internal lane standard and can also be used with the *GenePrint*® Fluorescent STR Systems.

A loading mixture is prepared by combining 1µl of the appropriate internal lane standard with 9µl of deionized formamide for each sample to be run. Amplified product or allelic ladder is mixed with this loading mixture as described in Figures 2, 3 and 4.

Amplified sample peaks under 2,000rfu are ideal. There may be instrument-to-instrument variation in peak intensity detected using the same sample; therefore, injection time or the amount of product mixed with loading cocktail may need to be increased or decreased according to instrument sensitivity. The volume of internal lane standard used in the loading cocktail can also be varied to adjust the relative intensity of the internal lane standard peak heights to amplified sample peaks.

DATA ANALYSIS

Sample data collected on the ABI PRISM® 3100 is analyzed using the GeneScan® analysis software, as described in the technical manual that accompanies each amplification system.

BLEEDTHROUGH

Bleedthrough, or pull-up, is sometimes observed from the yellow (TMR) channel into the red (CXR) channel. If yellow-to-red bleedthrough is detected, the peak amplitude threshold can be increased to 100–200rfu for the red channel in the GeneScan® analysis parameters. This should not interfere with interpretation, as the internal lane standard fragments should be greater than 300rfu.

Overloading of amplified sample is often a cause of artifact peaks. Sample peak heights greater than 4,000rfu may generate bleed-through or oversubtraction (pull-down)

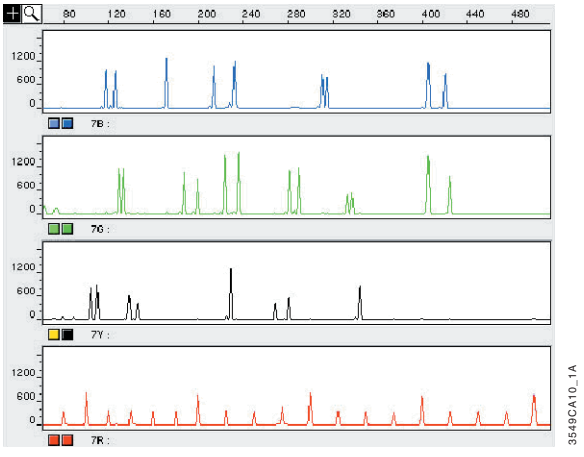

Figure 2. A 1ng genomic DNA sample amplified using the PowerPlex® 16 System and detected on the ABI PRISM® 3100 Genetic Analyzer. One microliter of amplified sample was mixed with 10µl of loading cocktail. The default 10-second injection time was used.

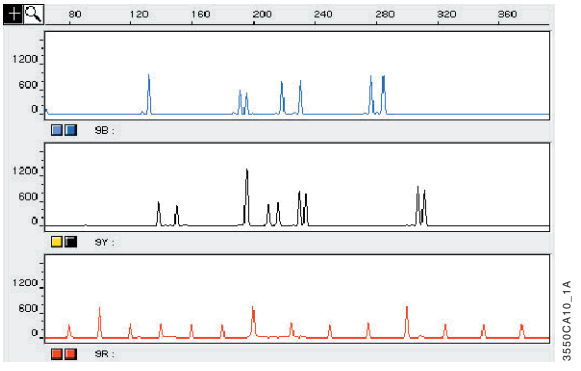

Figure 3. A 1ng genomic DNA sample amplified using the PowerPlex® 1.2 System and detected on the ABI PRISM® 3100 Genetic Analyzer. One microliter of amplified sample was mixed with 10µl of loading cocktail. The default 10-second injection time was used.

from one color to another. If the peak heights are too intense, samples can be diluted in Gold ST★R 1X Buffer before mixing with loading cocktail. However, best results are achieved by using less DNA template in the amplification reactions or by reducing the amplification program 2–4 cycles.

POWERTYPER™ MACRO

To facilitate analysis of the data generated with the PowerPlex® 16 and PowerPlex® 1.2 Systems, we have created files for each system to allow automatic assignment of genotypes using the Genotyper® software. After samples have been amplified using the PowerPlex® Systems, detected using the ABI PRISM® 3100, and analyzed using the GeneScan® analysis software, the sample files can be imported into the Genotyper® program and analyzed using the PowerTyper™ 16 Macro or the PowerTyper™ 1.2 Macro. No macro modifications are required for use with the ABI PRISM® 3100.

FOR MORE INFORMATION

The *PowerPlex® Matrix Standards, 3100, Technical Bulletin* #TBD016 provides additional information regarding the PowerPlex® Matrix Standards, 3100, and running PowerPlex® samples on the ABI PRISM® 3100 Genetic Analyzer. This Technical Bulletin and other product information is available on the Internet at www.promega.com/applications/hmnid/ or upon request from Promega.

ACKNOWLEDGMENTS

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1. Micka, K.A. *et al.* (1999) TWGDAM validation of a nine-locus and a four-locus fluorescent STR multiplex system. *J. Forensic Sci.* **44**, 6.
2. *ABI PRISM® 3100 Genetic Analyzer User's Manual*, Applied Biosystems.

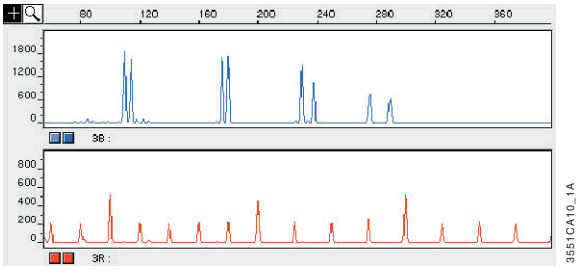

Figure 4. A 1ng genomic DNA sample amplified using the FFFL Fluorescent STR System and detected on the ABI PRISM® 3100 Genetic Analyzer. An aliquot of amplified sample was diluted 1:4 in 1X Gold ST★R Buffer, then 1µl of the diluted sample was mixed with 10µl of loading cocktail. A 5-second injection time was used.

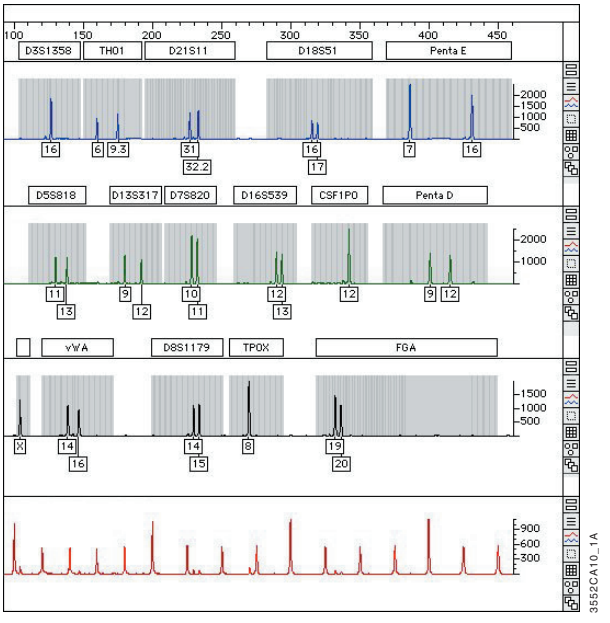

Figure 5. Analysis of a 1ng genomic DNA sample amplified using the PowerPlex® 16 System and detected on the ABI PRISM® 3100 Genetic Analyzer. The analyzed GeneScan® data was imported into the Genotyper® software, and genotyping was accomplished using the PowerTyper™ 16 Macro.