

MATRIX

The New PowerPlex® Matrix Standards, 3100—Custom

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INTRODUCTION

A spectral calibration on the ABI PRISM® 3100 Genetic Analyzer, also known as a matrix, is a mathematical description of the spectral overlap of a given set of fluorescent dye labels. It is used to correct emission overlap of dyes. A poor matrix can result in raised baseline and bleedthrough of fluorescent signal from one channel into another. Thus proper generation of a spectral calibration file is critical to evaluate multicolor systems, such as the PowerPlex® Systems.

THE NEW POWERPLEX® MATRIX STANDARDS, 3100—CUSTOM

Promega has developed the PowerPlex® Matrix Standards, 3100—Custom (Cat.# X3121), which includes four individual fragments labeled with four different fluorescent dyes, for the ABI PRISM® 3100 Genetic Analyzer. Each matrix fragment is provided in a separate tube: one tube contains a 375bp fragment labeled with fluorescein (FL), two tubes each contain a 350bp fragment labeled with 6-carboxy-4',5'-dichloro-2',7'-dimethoxyfluorescein (JOE A and JOE B), one tube contains a 300bp fragment labeled with carboxy-tetramethylrhodamine (TMR), and one tube contains a 275bp fragment labeled with carboxy-X-rhodamine (CXR).

The new PowerPlex® Matrix Standards, 3100—Custom, will be particularly useful for those applications that require higher peak heights, such as the analysis of DNA mixtures, and in forensic casework. In addition, these improved matrix standards accommodate more sensitive instruments with better resolution. An electropherogram image showing these new matrix standards is provided in Figure 1.

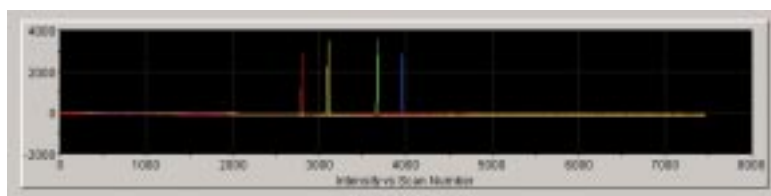


Figure 1. The new PowerPlex® Matrix Standards, 3100—Custom. Five microliters of each matrix standard was mixed with 480µl of HiDi™ formamide, and the mixture was injected onto an ABI PRISM® 3100-Avant Genetic Analyzer using a 5-second, 3kV injection.

WHAT'S NEW?

The new PowerPlex® Matrix Standards, 3100—Custom, includes two JOE matrix standards: JOE A Matrix, Custom, and JOE B Matrix, Custom. To increase the spectral calibration quality, use JOE A Matrix, Custom, to generate a matrix for the PowerPlex®Y or PowerPlex®ES System, and use JOE B Matrix, Custom, for the PowerPlex®16 System.

The high-quality matrix generated with the PowerPlex® Matrix Standards, 3100—Custom, means significantly less bleedthrough and raised baseline and better spectral performance for casework on the ABI PRISM® 3100 Genetic Analyzer.

We have changed the concentration of the DNA fragments in the PowerPlex® Matrix Standards, 3100—Custom, eliminating the need to perform an initial dilution.

The recommended dye set and parameters used when generating the spectral calibration have changed from previous PowerPlex® Matrix Standards, 3100, protocols, depending upon your version of the ABI PRISM® 3100 data collection software. These recommended changes have been made to take advantage of software updates that are present in data collection software versions 1.1 and newer. These software updates are not present in the data collection software version 1.0.1; therefore, users of this software may not see significant improvement in spectral performance.

SUMMARY OF PROTOCOL CHANGES

Preparing the Matrix Standards: To generate a spectral calibration with the new custom matrix standards, add 5µl of each dye fragment to 480µl of Hi-Di™ formamide. This new dilution scheme eliminates the initial 1:10 dilution of each matrix standard required for the PowerPlex® Matrix Standards, 3100 (Cat.# DG3650). The solvent used to prepare the matrix standards has also changed. We find that using HiDi™ formamide rather than water with these new matrix standards results in a higher quality matrix.

Data Collection Software Parameters: For data collection software version 1.1, we still recommend using dye set Z, but the recommended dye set parameters have changed from dye set D parameters to dye set F parameters. For data collection software version 2.0, the recommended dye set has changed from “any 4 dyes” to dye set F. For data collection software version 1.0.1,

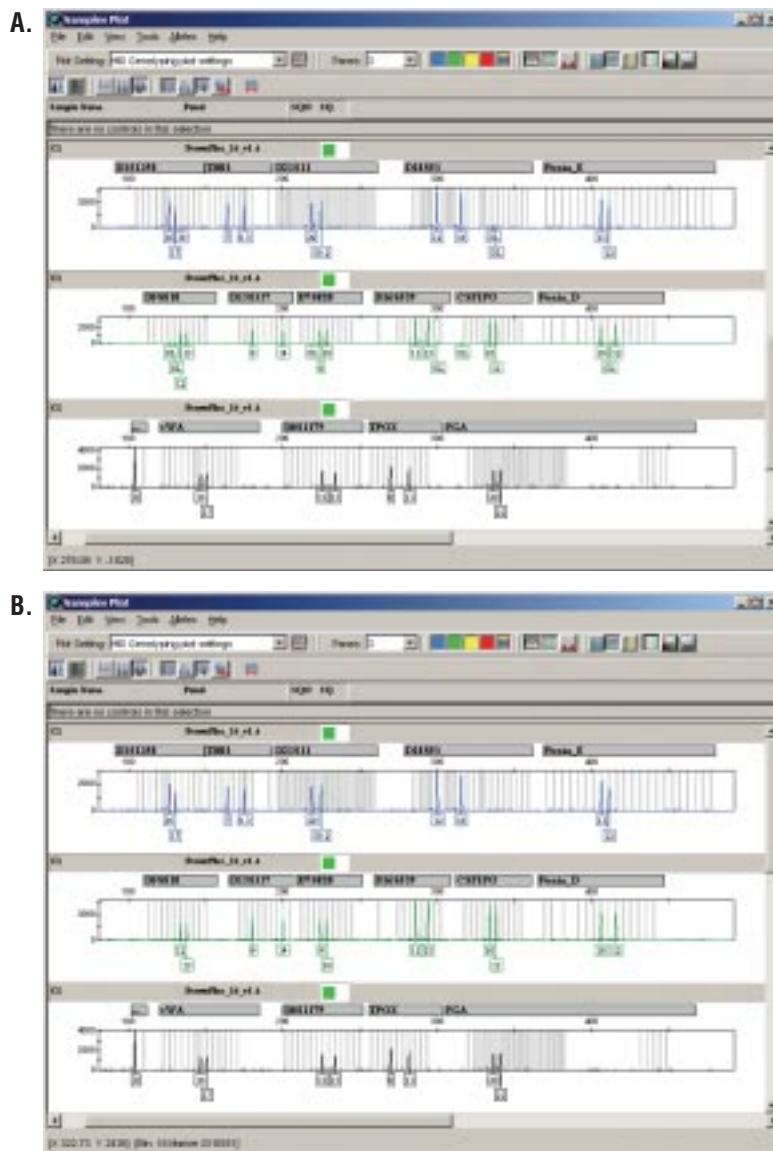


Figure 2. Comparison of spectral performance between the current PowerPlex® Matrix Standards, 3100 (Cat.# DG3650), and the new PowerPlex® Matrix Standards, 3100—Custom (Cat.# X3121). **Panel A.** Sample “C1” was analyzed on an ABI PRISM® 3100 Genetic Analyzer using a spectral created with the current PowerPlex® Matrix Standards, 3100 (Cat.# DG3650). **Panel B.** From the same well on a 96-well plate, sample “C1” was reanalyzed on the same instrument using a spectral created with the new PowerPlex® Matrix Standards, 3100—Custom (Cat.# X3121). This sample was analyzed on GeneMapper™ ID version 3.1 with a cutoff of 50RFU. Note the reduction of off-ladder peaks, which can be directly attributed to improved spectral resolution.

there is no advantage to changing these parameters for the PowerPlex® Matrix Standards, 3100—Custom.

Spectral Parameters: For data collection software versions 1.0.1

and 1.2, change the condition bounds range in the parameter file at: D:\appliedbio\Support Files\Data Collection Support Files\Calibration Data\Spectral Calibration\ParamFiles to [4.0, 11.0]. The condition number

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generated can vary between individual instruments. Therefore, you may need to optimize this range for your particular instrument.

Run Module: Change the run module to alter the data delay time (400 seconds) and lengthen the recommended run time (see the PowerPlex® Matrix Standards, 3100—Custom, manual for recommendations).

HOW THESE CHANGES BENEFIT YOU

The high-quality matrix generated with the PowerPlex® Matrix Standards, 3100—Custom, means less bleedthrough between dye channels (3% or less), allowing higher sample peak heights in the range of 4,000–6,000RFU (Figure 2). In addition, as shown in Figure 3, raised baseline is significantly reduced. This translates to better performance over the entire linear range of fluorescence detection. Analysts spend less time manually editing data, which saves time and increases laboratory throughput. Finally, and perhaps most importantly, these improvements result in better spectral performance for the analysis of casework samples with the ABI PRISM® 3100 Genetic Analyzer.

For more information about these improved matrix standards or for a protocol, contact Promega Technical Services at: genetic@promega.com. For those who do not want to switch from the existing set of matrix standards, the PowerPlex® Matrix Standards, 3100 (Cat.# DG3650), is still available.



Figure 3. Close-up view of the sample “C1” from Figure 2 analyzed with a cutoff of 50RFU. **Panel A.** Current PowerPlex® Matrix Standards, 3100 (Cat.# DG3650). **Panel B.** The new PowerPlex® Matrix Standards, 3100—Custom (Cat.# X3121). Note the reduction of bleedthrough from the fluorescein channel into the JOE channel and the reduction of the raised baseline in the fluorescein channel caused by the JOE channel.