TECH TIPS

Spectral Calibration or Making a Matrix

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Q: What is spectral calibration, and is it different from making a matrix?

A: Spectral calibration is used to correct emission overlap of dyes on an ABI PRISM® 3100 Genetic Analyzer. The end result is similar to matrix generation using the ABI PRISM® 310 Genetic Analyzer or the ABI PRISM® 377 DNA Sequencer, but the process is different.

The ABI PRISM® 3100 Genetic Analyzer requires that all matrix fragments be analyzed in a single injection, while the ABI PRISM® 310 Genetic Analyzer and the ABI PRISM® 377 DNA Sequencer require a separate injection or lane for each color of the matrix. Spectral calibrations must be performed before samples can be detected with the ABI PRISM® 3100.

The ABI PRISM® 3100 collection software creates and evaluates the spectral calibration automatically, determining if each capillary meets the Q and C limits set by the user. C, or condition bounds, represents the amount of overlapping signal between the different dyes in the calibration dye set and is used to identify data that falls outside of expected parameters. The Q value determines the tolerance for pull up and pull down peaks and is typically set at 0.95.

For the ABI PRISM® 310 Genetic Analyzer and the ABI PRISM® 377 DNA Sequencer, the matrix is created in GeneScan® analysis software. The user selects starting points for matrix generation. At least five dye peaks within each color are required for matrix generation.

Q: Are matrix standards interchangeable?

A: No, each instrument requires specific matrix samples. The ABI PRISM® 310 Genetic Analyzer and the ABI PRISM® 377 DNA Sequencer use the Matrix FL-JOE-TMR-CXR (Cat.# DG2860), which consists of 4 series of 8 DNA fragments. One tube contains DNA fragments labeled with carboxy-tetramethylrhodamine (TMR), one tube contains DNA fragments labeled with 6-carboxy-4´,5´-dichloro-2´,7´-dimethoxy-fluorescein (JOE), one tube contains DNA fragments labeled with carboxy-X-rhodamine (CXR) and one tube contains DNA fragments labeled with fluorescein. Each dye is run in a separate injection or lane, and the matrix is generated manually by the user with GeneScan® software.

The ABI PRISM® 3100 Genetic Analyzer uses different algorithms to generate a spectral calibration. On this instrument four DNA fragments of distinct lengths, one containing each fluorophore label, are injected simultaneously during a spectral calibration run. The ABI PRISM® 3100 Genetic Analyzer collection software analyzes the spectral calibration raw data and generates a spectral calibration, which is essentially a matrix.

Q: How do I run matrix samples on my instrument?

A: Each instrument platform has different requirements for sample setup. Promega matrix samples include instrument-specific instructions for matrix generation. The *Matrix FL-JOE-TMR-CXR Technical Bulletin* #TBD015 and the *PowerPlex® Matrix Standards, 3100 Technical Bulletin #*TBD016 are available at: www.promega.com/tbs/tbs.htm

The PowerPlex® Matrix Standards, 3100 (Cat.# DG3380) are designed to be used in water, while the Matrix FL-JOE-TMR-CXR can be diluted in deionized formamide or in water. Matrix samples for the ABI PRISM® 377 DNA Sequencer should be mixed with an equal volume of Blue Dextran Loading Solution.

Important considerations for all matrix samples include:

- Matrix standards should be completely thawed and mixed with a vortex mixer for 10–15 seconds prior to removing an aliquot. Tubes should not be spun down after mixing. If necessary tubes can be tapped on the benchtop to collect the liquid at the bottom.
- Samples should be denatured by heating to 95°C for 2 minutes followed by cooling on crushed ice or an ice-water bath for 3 minutes.

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• There are differences in sensitivity between instruments. If a matrix is not generated or if peak heights are low or excessively high, different dilutions of the matrix standards may be required to obtain a good matrix.

Q: How do I know if I have generated a good matrix?

A: The best way to evaluate a matrix is to apply it either to matrix samples or to amplified samples. If sample peak heights are ≤2,000RFU, the background in each dye lane should be relatively flat. Some bleedthrough may be observed from the yellow channel (TMR) into the red channel (CXR). Samples with peak heights >2,000RFU may exhibit higher bleedthrough and raised baseline, since this amount of fluorescence can exceed the linear detection range of the instrument. For the ABI PRISM® 3100, the matrix is evaluated by the collection software and compared to Q and C values. For the PowerPlex® Matrix Standards, 3100, the condition number bounds should be set at [6.0, 9.0]. A spectral calibration that passes on a minimum of 12 capillaries will provide an effective matrix file.

Q: What would happen if I run a matrix designed for the ABI PRISM® 310 Genetic Analyzer on an ABI PRISM® 3100 Genetic Analyzer?

A: The spectral calibration will fail. The Matrix FL-JOE-TMR-CXR, which is the recommended matrix for the ABI PRISM® 310 Genetic Analyzer, is designed to run 8 dye fragments in each dye color, with each dye color as a separate injection. The PowerPlex® Matrix Standards, 3100 are designed to run one dye fragment of each dye color simultaneously in a single injection. An ABI PRISM® 3100 spectral calibration should only show 4 peaks in the run when the correct PowerPlex® Matrix Standards, 3100 are used.

Q: How often should I run a new matrix?

A: A new matrix should be run after major maintenance on the system, such as changing the laser, calibrating the CCD camera and replacing the CCD camera. It is advisable to make a new matrix after the instrument is moved to a new location. In some instances, a software upgrade may necessitate the generation of a new matrix. A new matrix does not need to be made after changing capillaries or polymer. Individual labs should determine the frequency of matrix generation.

Unexpected peaks that results from bleedthrough of one dye into another are a good indication that it may be time to create a new matrix. However, bleedthrough can also be caused by excessive signal. We recommend peak heights of ≤2,000RFU. Peak heights of ≥2,000RFU can saturate the instrument and cause elevated baseline and bleedthrough.

Q: My instrument is also used for sequencing. Do I need to run a new matrix each time I switch from sequencing to fragment analysis?

A: No, a new matrix needs to be created only when physical changes are made to the instrument or bleedthrough is observed. As long as the appropriate run modules are selected and the appropriate matrix is assigned to the samples, multiple chemistries can be used on the same instrument for different applications.

Q: I have a matrix from another manufacturer. Can I use it with the PowerPlex®(b–e) Systems?

A: No, matrix standards must contain the same fluorophores as the samples so that appropriate spectral overlap can be calculated. Using other dyes for matrix or spectral calibration will likely cause an elevated baseline or inverted peaks in sample runs.