TECHNICAL MANUAL

VersaPlex™ 6C Matrix Standard

Instructions for Use of Product **DG4960**

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VersaPlex™ 6C Matrix Standard

All technical literature is available at: www.promega.com/protocols/
Visit the web site to verify that you are using the most current version of this Technical Manual.
E-mail Promega Technical Services if you have questions on use of this system: genetic@promega.com

1. Description

Proper generation of a spectral calibration file is critical to evaluate multicolor systems with the Applied Biosystems® 3500 and 3500xL Genetic Analyzers. The VersaPlex™ 6C Matrix Standard[a,b] consists of DNA fragments labeled with six different fluorescent dyes (FL, JOE, TMR, CXR, TOM and WEN) in one tube. The spectral calibration is performed using the J6 dye set. Once generated, the spectral calibration file is applied during sample detection to calculate the spectral overlap and separate the raw fluorescent signals into individual color signals.

The VersaPlex™ 6C Matrix Standard was developed for use with the VersaPlex™ 27PY System (Cat.# DC7020) and is compatible with the Applied Biosystems® 3500 and 3500xL Genetic Analyzers. A protocol to operate the Applied Biosystems® 3500 or 3500xL Genetic Analyzer should be obtained from the manufacturer.

A spectral calibration must be generated for each individual instrument. A new matrix should be run after major maintenance on the system, such as changing the laser, calibrating or replacing the CCD camera or changing the polymer type or capillary array. We also recommend that you generate a new matrix after the instrument is moved to a new location. In some instances, a software upgrade may necessitate generation of a new matrix. Individual labs should determine the frequency of matrix generation.

2. Product Components and Storage Conditions

PRODUCT	SIZE	CAT.#
VersaPlex™ 6C Matrix Standard	**5 preps**	**DG4960**

Not For Medical Diagnostic Use. Includes:

- 150µl 6C Matrix Mix
- 5 × 200µl Matrix Dilution Buffer

Storage Conditions: Upon receipt, store all components at –30°C to –10°C in a nonfrost-free freezer. After the first use, store the VersaPlex™ 6C Matrix Standard components at 2–10°C. We strongly recommend storing the VersaPlex™ 6C Matrix Standard with post-amplification reagents. The VersaPlex™ 6C Matrix Standard is light-sensitive; dilute the 6C Matrix Mix in the Matrix Dilution Buffer in the provided amber tube. Store the diluted 6C Matrix Mix at 2–10°C for up to 1 week.

3. Instrument Preparation and Spectral Calibration Using the Applied Biosystems® 3500 and 3500xL Genetic Analyzers

Materials to Be Supplied by the User

- centrifuge compatible with 96-well plates
- aerosol-resistant pipette tips
- 3500/3500xL capillary array, 36cm
- POP-4® polymer for the 3500 or 3500xL
- anode buffer container with 1X buffer
- cathode buffer container with 1X buffer
- MicroAmp® optical 96-well plate and septa
- Hi-Di™ formamide (Applied Biosystems® Cat.# 4311320)

For additional information on performing spectral calibration, refer to the *Applied Biosystems® 3500/3500xL Genetic Analyzer User Guide*.

3.A. Matrix Sample Preparation

1. At the first use, thaw the 6C Matrix Mix and Matrix Dilution Buffer completely. After the first use, store the reagents at 2–10°C.
2. Vortex the 6C Matrix Mix for 10–15 seconds prior to use. Add 10µl of the 6C Matrix Mix to one tube of Matrix Dilution Buffer. Vortex for 10–15 seconds. Label the tube with the date of dilution. The diluted 6C Matrix Mix can be stored for up to 1 week at 2–10°C.
3. Add 10µl of the diluted 6C Matrix Mix prepared in Step 2 to 500µl of Hi-Di™ formamide. Vortex for 10–15 seconds.

4. For the Applied Biosystems® 3500xL Genetic Analyzer, wells A1 through H3 of the 96-well plate are used for spectral calibration. Add 15µl of the 6C Matrix Mix with formamide prepared in Step 3 to each of the 24 wells. After placing the septa on the plate, briefly centrifuge the plate to remove bubbles. Do not heat denature.

   For the Applied Biosystems® 3500 Genetic Analyzer, wells A1 through H1 of the 96-well plate are used for spectral calibration. Add 15µl of the 6C Matrix Mix with formamide prepared in Step 3 to each of the eight wells. After placing the septa on the plate, briefly centrifuge the plate to remove bubbles. Do not heat denature.

5. Place the plate in the 3500 series 96-well standard plate base and cover with the plate retainer. Do not start the spectral calibration run until the oven is preheated to 60°C.

3.B. Instrument Preparation and Spectral Calibration

We have found that the use of fresh polymer and a new capillary array results in an optimal spectral calibration. Representative data are shown in Figure 1.

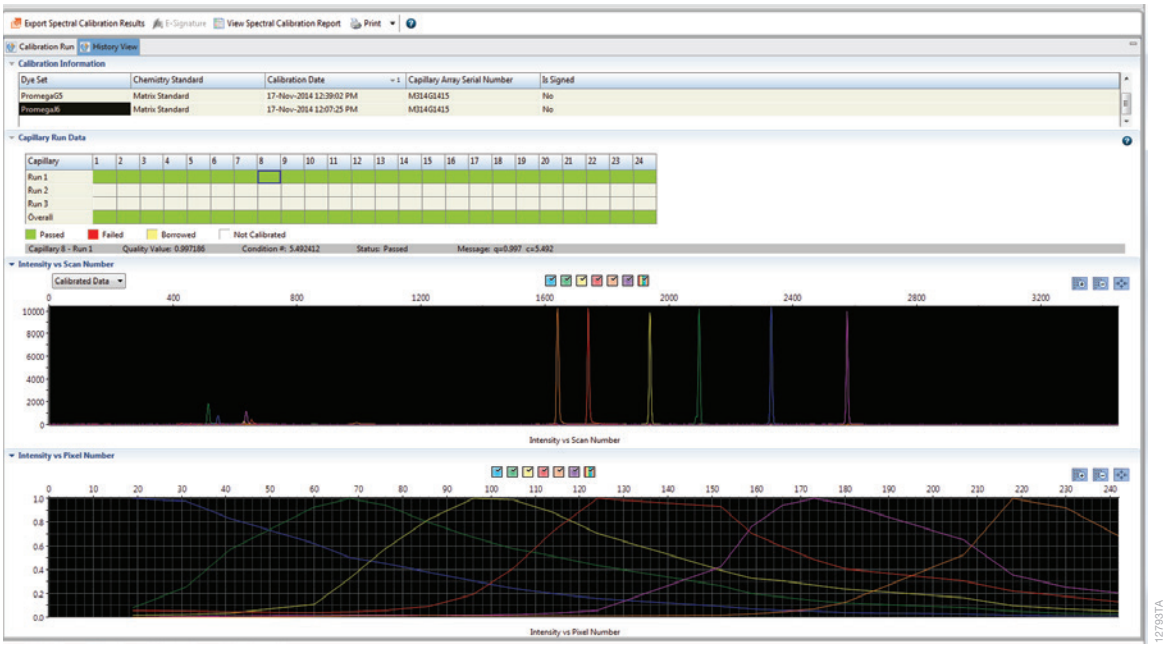

Figure 1. Representative data for the VersaPlex™ 6C Matrix Standard on the Applied Biosystems® 3500xL Genetic Analyzer using POP-4® polymer and Data Collection Software, Version 2.0.

3.B. Instrument Preparation and Spectral Calibration (continued)

1. Set the oven temperature to 60°C, and then select the Start Pre-Heat icon at least 30 minutes prior to the first injection to preheat the oven.

2. To perform a spectral calibration for the VersaPlex™ 27PY System (Cat.#DC7020), a new dye set should be created. If a new dye set was created previously, proceed to Step 2c.

   a. To create the new dye set, navigate to the Library, highlight "Dye Sets" and select "Create".

   b. The Create a New Dye Set window will appear (Figure 2). Name the Dye Set (e.g., Promega J6), select "Matrix Standard" for the Chemistry and select "J6 Template" for the Dye Set Template.

      Under Parameters, change the After Scan number to 800 from the default number of 500. Select "Save".

   c. To perform the spectral calibration, go to the Maintenance tab, select "Spectral", and under the Calibration Run tab, choose the appropriate fields: Choose "Matrix Standard" from the Chemistry Standard drop-down menu and the new Promega 6-color dye set (i.e., Promega J6) created in Step 2b from the Dye Set drop-down menu.

   d. Select "Start Run".

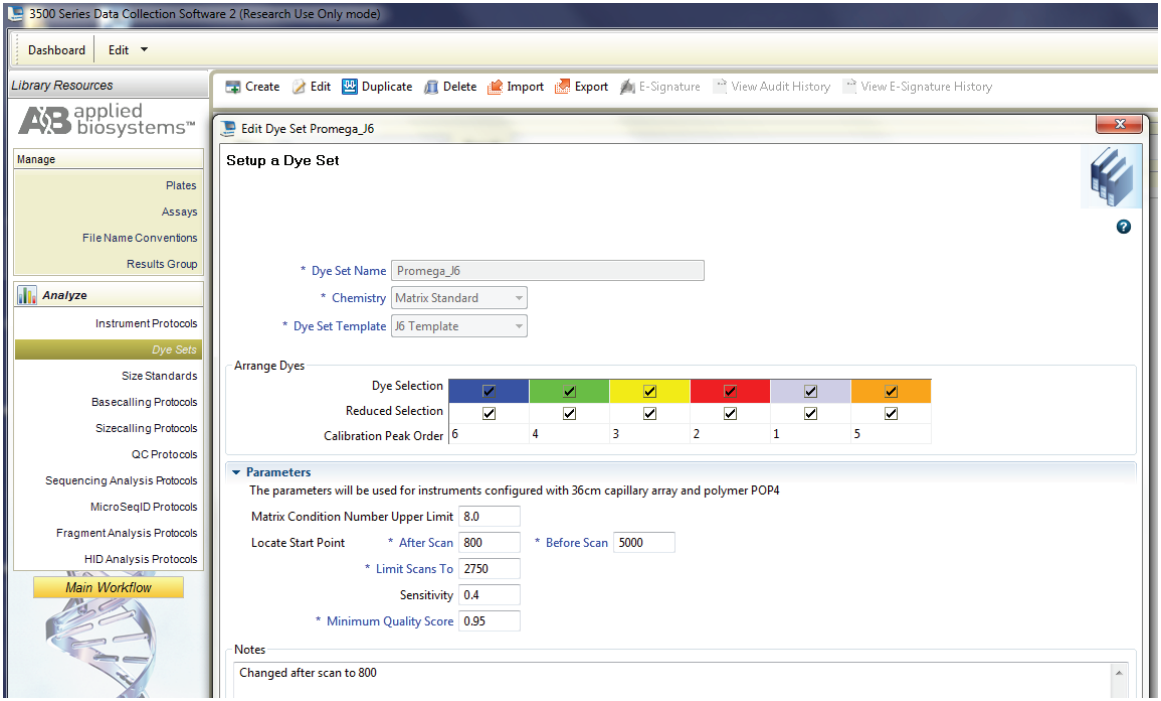

Figure 2. The Create New Dye Set window.

3. Upon completion of the spectral calibration, check the quality of the spectral in the Capillary Run Data display and choose either "Accept" or "Reject".

   Note: Refer to the *3500 Series Data Collection Software User Manual* for the criteria recommended when accepting or rejecting a spectral calibration.

4. Troubleshooting

For questions not addressed here, please contact your local Promega Branch Office or Distributor. Contact information available at: **www.promega.com**. E-mail: **genetic@promega.com**

Symptoms	Causes and Comments
Fewer than the recommended number of capillaries passed the spectral calibration	Poor-quality formamide was used. The quality of formamide is critical. Use Hi-Di™ formamide. Freeze formamide in aliquots at –20°C. Multiple freeze-thaw cycles or storage at 4°C may cause breakdown of formamide. Poor-quality formamide may contain ions that compete with DNA during injection, which results in lower peak heights and reduced sensitivity.
	Matrix standard was too dilute. Matrix standard that is too dilute will result in low spectral calibration peak heights, which may result in spectral calibration failure. Increase the volume of diluted 6C Matrix Mix added to the Hi-Di™ formamide during sample preparation.
	Matrix standard was too concentrated. Matrix standard that is too concentrated may result in spectral calibration failure due to saturated peaks, bleedthrough or oversubtraction in other dye colors. Decrease the volume of diluted 6C Matrix Mix added to the Hi-Di™ formamide during matrix sample preparation.
	Reboot the CE instrument and the instrument's computer. Repeat the spectral calibration.
	Ensure that the oven is preheated to 60°C prior to spectral calibration.
All capillaries failed spectral calibration	For best spectral calibration results, use fresh polymer, fresh buffer and water, and a capillary array with fewer than 100 injections.

[a]TMR, CXR, TOM and WEN dyes are proprietary.

[b]U.S. Pat. No. 9,139,868, European Pat. No. 2972229 and other patents pending.

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All prices and specifications are subject to change without prior notice.

Product claims are subject to change. Please contact Promega Technical Services or access the Promega online catalog for the most up-to-date information on Promega products.

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