



Technical Bulletin

PowerPlex® Matrix Standards, 3100/3130

INSTRUCTIONS FOR USE OF PRODUCT DG4650.

IMPORTANT

We recommend using this product once, then discarding it.



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PowerPlex® Matrix Standards, 3100/3130

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Technical Bulletin. Please contact Promega Technical Services if you have questions on use
of this system. E-mail: techserv@promega.com.

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1. Description

Proper generation of a spectral calibration file is critical to evaluate multicolor systems with the ABI PRISM® 3100 and 3100-*Avant* and Applied Biosystems 3130 and 3130*xl* Genetic Analyzers. The PowerPlex® Matrix Standards, 3100/3130, includes individual fragments labeled with four different fluorescent dyes (Figure 1). Each matrix fragment is provided in a separate tube: one tube contains a fragment labeled with fluorescein (FL), one tube contains a fragment labeled with 6-carboxy-4',5'-dichloro-2',7'-dimethoxyfluorescein (JOE), one tube contains a fragment labeled with carboxy-tetramethylrhodamine (TMR), and one tube contains a fragment labeled with carboxy-X-rhodamine (CXR).

1. Description (continued)

These matrix fragments are mixed and used on the ABI PRISM® 3100 or 3100-*Avant* or Applied Biosystems 3130 or 3130*xl* Genetic Analyzer to perform a spectral calibration for a specified dye set. For data collection software, versions 1.0.1 and 1.1, the spectral calibration should be performed on dye set Z using dye set F parameters. For data collection software, version 2.0 and 3.0, the spectral calibration should be performed on dye set F. A spectral calibration file is generated for a specific set of dyes, array type (4 or 16 capillaries) and array length (36cm). Once generated, this file is applied during sample detection to calculate the spectral overlap between the four different dye colors and separate the raw fluorescent signals into individual dye signals. A matrix should be generated for each individual instrument. The PowerPlex® Matrix Standards, 3100/3130, was developed for use with the PowerPlex® 16, 16 HS, Y, ES and S5 Systems and can be used with any of the *GenePrint*® Fluorescent STR Systems. A matrix should be generated for each individual instrument. Protocols to operate the ABI PRISM® 3100 or 3100-*Avant* or Applied Biosystems 3130 or 3130*xl* Genetic Analyzer should be obtained from the manufacturer. Technical Manuals and additional product information are available on the Web site at: www.promega.com

2. Product Components and Storage Conditions

Product	Cat.#
PowerPlex® Matrix Standards, 3100/3130	DG4650

Not for Medical Diagnostic Use. Includes:

- 25µl Fluorescein Matrix
- 25µl JOE Matrix
- 25µl TMR Matrix
- 25µl CXR Matrix
- 1.25ml Nuclease-Free Water

Storage Conditions: Store all components at -20°C in a nonfrost-free freezer. Do not store reagents in the freezer door, where the temperature can fluctuate. The fragments in the matrix standards are light-sensitive and must be stored in the dark. We strongly recommend that these be stored with post-amplification reagents and used separately with different pipettes, tube racks, etc. We recommend using this product once, then discarding it.

3. Instrument Preparation and Spectral Calibration Using Data Collection Software, Version 2.0 or Version 3.0 (ABI PRISM® 3100 and 3100-*Avant* and Applied Biosystems 3130 and 3130*xl* Genetic Analyzers)

Materials to Be Supplied by the User

- Hi-Di™ formamide (Applied Biosystems Cat.# 4311320)
- dry heating block, water bath or thermal cycler
- crushed ice or ice-water bath
- 3100 or 3130 capillary array, 36cm
- Performance Optimized Polymer 4 (POP-4™) for the 3100 or 3130
- 10X genetic analyzer buffer with EDTA
- MicroAmp® optical 96-well plate
- aerosol-resistant pipette tips

3.A. Matrix Sample Preparation

There will be instrument-to-instrument variation in the sensitivity of detection. Matrix sample dilutions described here may need to be optimized in individual laboratories depending on the sensitivity of each ABI PRISM® 3100, 3100-*Avant*, 3130 or 3130*xl* Genetic Analyzer. The optimal dilution may differ for each dye fragment. You also may need to adjust injection time or voltage in Section 3.B to achieve a passing spectral calibration. Peak heights in the range of 1,000–4,000RFU are ideal. Peak heights above 750RFU are required.

1. Thaw the concentrated matrix reagents. Mix the reagents by vortexing for 5–10 seconds before each use. Do not centrifuge the matrix reagents as this may cause the DNA to be concentrated at the bottom of the tube.
2. **Initial dilution of concentrated fragments:** Before mixing the dye fragments, dilute an aliquot of each dye fragment 1:10 in Nuclease-Free Water. Vortex for 5–10 seconds to mix.

	FL	JOE	TMR	CXR
Concentrated Dye	5µl	5µl	5µl	5µl
Nuclease-Free Water	45µl	45µl	45µl	45µl

3. **Fragment mix (using 1:10 dilutions of dye fragments):** After the initial dilution in Step 2, mix the dye fragments as listed below. Vortex each dye fragment before adding it to the HiDi™ formamide.

Component	Volume
Hi-Di™ formamide	480µl
FL from initial dilution	5.0µl
JOE from initial dilution	5.0µl
TMR from initial dilution	5.0µl
CXR from initial dilution	5.0µl

3.A. Matrix Sample Preparation (continued)

4. On the ABI PRISM® 3100 and Applied Biosystems 3130xl Genetic Analyzer, 16 wells are used for matrix detection on 16 capillaries (wells A1 through H2 of a 96-well tray). Vortex and load 25µl of the fragment mix prepared in Step 3 into each of the 16 wells. On the ABI PRISM® 3100-*Avant* or Applied Biosystems 3130 Genetic Analyzer, four wells are used for matrix detection on the four capillaries (wells A1 through D1 of a 96-well plate). Load 25µl of the fragment mix in each of the four wells.
5. Denature samples at 95°C for 3 minutes, then immediately chill on crushed ice or in an ice-water bath for 3 minutes. Denature the samples just prior to loading the instrument.

3.B. Instrument Preparation

1. Prepare the matrix samples as previously described in Section 3.A.

Note: Differences in instrument sensitivity may result in peak imbalance or reduced peak height of the matrix dyes. You may need to adjust injection time or voltage to achieve a passing spectral calibration. The same plate of matrix standards can be re-injected up to four times. To re-inject the same matrix standards plate, add an injection by selecting “Plate Manager”, then “Edit”. Select “Edit” again in the top left corner of the window, then select “Add single run”.

Alternatively, you may need to prepare a new plate and adjust the dilution of individual matrix dyes. Peak heights in the range of 1,000–4,000RFU are ideal. Peak heights above 750RFU and below the saturation point of the instrument are required.

2. Run the spectral calibration procedure as described in the ABI PRISM® 3100 or 3100-*Avant*, 3130 or Applied Biosystems 3130xl Genetic Analyzer user’s manuals with the following exceptions:
 - a. In the Module Manager, select “New.” Select “Spectral” in the Type drop-down list, and select “Spect36_POP4” in the Template drop-down list. Confirm or change the following settings:

Inj. kV:	3
Inj. Secs:	5
Data Delay Time:	100
Run Time:	800 seconds
 - b. In the Protocol Manager, under Instrument Protocols select “New.” Type a name for your protocol. Select “Spectral” in the Type drop-down list, select “F” in the DyeSet drop-down list and select “36” in the Array Length drop-down list. Select the spectral module you created in the previous step in the Run Module drop-down list. Finally, select “Edit Parameters.” Change the lower condition bound to 4.0, and change the upper condition bound to 12.0. Select “OK” in the Edit Parameters window, and select “OK” in the Protocol Manager.

Note: The condition number (“C value”) obtained when generating a spectral calibration will vary with the instrument. After obtaining a spectral calibration that performs acceptably, the condition bounds range in the previous step may be narrowed to more critically evaluate C values for subsequent calibrations.

- c. In the Plate Manager, create a new plate record as described in the instrument user’s manual. In the dialog box that appears, select “Spectral Calibration” in the Application drop-down list, and select “96-well” as the plate type. Add entries in the owner and operator windows, name the plate and select “OK.”
- d. In the spectral calibration plate editor dialog box, place sample names in the appropriate cells. In the Instrument Protocol column, select the protocol you created in Step 2.b. Ensure that this information is present for each row that contains a sample name. Select “OK.”
- e. Run your plate as described in the instrument user’s manual.
- f. Upon completion of the run, check the status of the spectral run in the Event Log window. For the ABI PRISM® 3100 and 3130*xl* Genetic Analyzers, we recommend that a minimum of 12 of the 16 capillaries pass calibration. For the ABI PRISM® 3100-*Avant* and 3130 Genetic Analyzers, we recommend that a minimum of three of the four capillaries pass calibration. If fewer than the recommended numbers of capillaries pass, the spectral calibration should be repeated.
- g. When repeating the spectral calibration run, samples in the same plate may be re-injected up to four times. There will be instrument-to-instrument variation in the sensitivity of detection; it may necessary to adjust the module injection voltage and/or injection time.

4. PowerPlex® Spectral Calibration File Generation Using Data Collection Software, Version 1.0.1 or Version 1.1 (ABI PRISM® 3100 and 3100-*Avant* Genetic Analyzers)

Note: To create a successful spectral calibration, two file modifications must be made; the first involves the run module, and the second involves the parameter file. These instructions only apply to data collection software, versions 1.0.1 and 1.1. For instructions to generate a spectral calibration using data collection software, version 2.0 or 3.0, see Section 3.

Materials to Be Supplied by the User

- Hi-Di™ formamide (Applied Biosystems Cat.# 4311320)
- dry heating block, water bath or thermal cycler
- crushed ice or ice-water bath
- 3100 capillary array, 36cm
- Performance Optimized Polymer 4 (POP-4™) for the 3100 instruments
- 10X genetic analyzer buffer with EDTA
- MicroAmp® optical 96-well plate
- aerosol-resistant pipette tips

4.A. Spectral Run Module

1. Open the ABI PRISM® 3100 data collection software.
2. Under Tools, open the module editor. Select the calibration tab, then select the "Spect36_POP4DefaultModule" module.
3. Confirm or change the settings for the following:

Inj. kV:	3
Inj. Secs:	5
Data Delay Time:	100
Run Time:	800 seconds

4. Select "Save As", and save the module as a new name (for example, Spect36_POP4PowerPlex).

Use this as the initial run module for all spectral calibration runs using the PowerPlex® Matrix Standards, 3100/3130.

Note: There will be instrument-to-instrument variation in the sensitivity of detection. You may have to modify the injection time or voltage, depending on the sensitivity of each ABI PRISM® 3100 or 3100-*Avant* Genetic Analyzer.

4.B. Spectral Parameters

1. To customize the spectral parameters, locate the parameter file at: My Computer/3100files (D:)\AppliedBio\Support Files\Data Collection Support Files\Calibration Data\Spectral Calibration\ParamFiles
2. Select "MtxStd{Genescan_SetF}". This will open the .par file in Microsoft® Notepad.
3. Change the condition bounds range to [4.0, 12.0]
4. Select "File Save As" to save the parameter file as a new name (for example, MtxStd{Genescan_SetZPowerPlex}.par). Use this as the parameter file for all spectral calibration runs using the PowerPlex® Matrix Standards, 3100/3130.

Note: The condition number ("C value") obtained when generating a spectral calibration will vary with the instrument. After obtaining a spectral calibration that performs acceptably, the condition bounds range in the previous step may be narrowed to more critically evaluate C values for subsequent calibrations.

4.C. Matrix Sample Preparation

There will be instrument-to-instrument variation in the sensitivity of detection. Matrix sample dilutions described here may need to be optimized in individual laboratories depending on the sensitivity of each ABI PRISM® 3100 or 3100-*Avant* Genetic Analyzer. The optimal dilution may differ for each dye fragment. You also may need to adjust injection time or voltage n Section 3.B to achieve a passing spectral calibration. Peak heights in the range of 1,000–4,000RFU are ideal. Peak heights above 750RFU are required.

1. Thaw the concentrated matrix reagents. Mix the reagents by vortexing for 5–10 seconds before each use. Do not centrifuge the matrix reagents as this may cause the DNA to be concentrated at the bottom of the tube.
2. **Initial dilution of concentrated fragments:** Before mixing the dye fragments, dilute an aliquot of each dye fragment 1:10 in Nuclease-Free Water. Vortex for 5–10 seconds to mix.

	FL	JOE	TMR	CXR
Concentrated Dye	5µl	5µl	5µl	5µl
Nuclease-Free Water	45µl	45µl	45µl	45µl

4.C. Matrix Sample Preparation (continued)

3. **Fragment mix (using 1:10 dilutions of dye fragments):** After the initial dilution in Step 2, mix the dye fragments as listed below. Vortex each dye fragment before adding it to the HiDi™ formamide.

Component	Volume
Hi-Di™ formamide	480µl
FL from initial dilution	5.0µl
JOE from initial dilution	5.0µl
TMR from initial dilution	5.0µl
CXR from initial dilution	5.0µl

4. On the ABI PRISM® 3100 Genetic Analyzer, 16 wells are used for matrix detection on 16 capillaries (wells A1 through H2 of a 96-well tray). Vortex and load 25µl of the fragment mix prepared in Step 3 into each of the 16 wells. On the ABI PRISM® 3100-*Avant* Genetic Analyzer, four wells are used for matrix detection on the four capillaries (wells A1 through D1 of a 96-well plate). Load 25µl of the fragment mix in each of the four wells.
5. Denature samples at 95°C for 3 minutes, then chill immediately on crushed ice or in an ice-water bath for 3 minutes. Denature the samples just prior to loading the instrument.

4.D. Instrument Preparation

These instructions apply only when using the data collection software, version 1.0.1 or 1.1.

Refer to the instrument user's manual for instructions on cleaning the pump blocks, installing the capillary array, performing a spectral calibration and adding polymer to the reserve syringe.

Differences in instrument sensitivity may result in peak imbalance or reduced peak height of the matrix dyes. You may need to adjust injection time or voltage to achieve a passing spectral calibration. The same plate of matrix standards can be re-injected up to four times. To re-inject the same matrix standards plate, add an injection by selecting "Plate Manager", then "Edit". Select "Edit" again in the top left corner of the window, then select "Add single run".

Alternatively, you may need to prepare a new plate and adjust the dilution of individual matrix dyes. Peak heights in the range of 1,000–4,000RFU are ideal. Peak heights above 750RFU and below the saturation point of the instrument are required.

1. Open the ABI PRISM® 3100 data collection software.
2. Open a new plate record. Name the plate, and select "Spectral Calibration". Select "96-well" as the plate type. Select "Finish".

3. Complete the plate record spreadsheet for the wells you have loaded. Enter appropriate information into the Sample Name column.
4. In the dye set column, select "Z" from the pull-down menu.
5. In the spectral run module column, select "Spect36_POP4PowerPlex" from the pull-down menu. This is the module that was created in Section 4.A.
6. In the spectral parameters column, select "mtxStd{Genescan_SetZ PowerPlex}" from the pull-down menu. This is the file that was created in Section 4.B.

The resulting spectral plate record should have the following settings:

Dye Set:	Z
Spectral Run Module:	Spect36_POP4PowerPlex
Spectral Parameters:	MtxStd{Genescan_SetZPowerPlex}

7. Select "OK". This new plate record will appear in the pending plate records table on the plate setup page of the data collection software.

4.E. Spectral Calibration Run

1. In the pending plate records table, click once on the name of the plate record you just created.
2. Once the plate record is highlighted, select the plate graphic that corresponds to the plate on the autosampler that contains your spectral calibration samples.
3. When the plate record is linked to the plate, the plate graphic will change from yellow to green, the plate record moves from the pending plate records table to the linked plate records table, and the Run Instrument button becomes enabled.
4. Select the Run Instrument button on the toolbar to start the spectral calibration run.

4.F. Spectral Calibration Results

1. After the spectral calibration run is completed, the Spectral Calibration Result window opens, indicating which capillaries passed calibration. A "." indicates the capillary passed. An "X" indicates the capillary failed.
2. Select "OK".
3. Review the spectral calibration data. In the Tools menu of the data collection software, select "Display Spectral Calibration".
4. Select the dye set button, select "Dye Set Z" from the pull-down menu and select "OK".

4.F. Spectral Calibration Results (continued)

5. The Matrices for Dye Set Z window displays the spectral calibration data for each capillary. Use the scroll bar to review the data for each of the 16 capillaries. For the PowerPlex® Matrix Standards, 3100/3130, the following conditions must be met for the capillary to pass calibration:
 - a. The Q-value must be greater than 0.95.
 - b. The condition number (or C-value) must fall within the range previously determined in Section 4.B, Step 3.
 - c. The peak height values must be above 750RFU and below saturation.
6. Select “OK” to close the window.
7. 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, we recommend that a minimum of 12 out of the 16 capillaries pass calibration for the ABI PRISM® 3100 Genetic Analyzer. For the ABI PRISM® 3100-*Avant* Genetic Analyzer, we recommend that a minimum of three of the four capillaries pass calibration. If fewer than the recommended number of capillaries pass, the spectral calibration should be repeated.

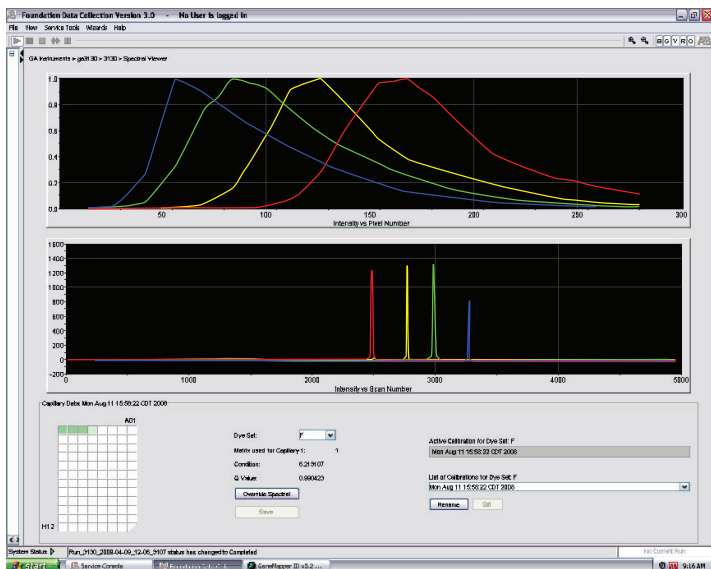


Figure 1. PowerPlex® Matrix Standards, 3100/3130, on the Applied Biosystems 3130 Genetic Analyzer using data collection software, version 3.0. Figure shows the CXR (red), TMR (yellow), JOE (green) and FL (blue) peaks in the spectral viewer from one of the four capillaries.

8. When repeating the spectral calibration run, samples in the same plate may be re-injected up to four times. There will be instrument-to-instrument variation in the sensitivity of detection; it may be necessary to adjust the module injection voltage and/or injection time.

5. Troubleshooting

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

Symptoms	Causes and Comments
Fewer than the recommended number of capillaries passed the spectral calibration	<p>Differences in instrument sensitivity may result in peak imbalance or reduced peak height of the matrix dyes. You may need to adjust injection time or voltage to achieve a passing spectral calibration. The same plate of matrix standards can be re-injected up to four times. Alternatively, you may need to prepare a new plate and adjust the dilution of individual matrix dyes. Peak heights in the range of 1,000–4,000RFU are ideal. Peak heights above 750RFU and below the saturation point of the instrument are required.</p>
	<p>Matrix standards were too dilute. Matrix samples that are too dilute will result in low peak heights. If matrix peak heights are below 1,000RFU, decrease the dilution of each fragment in Step 2 of Section 3.A or 4.C.</p>
	<p>Matrix standards were too concentrated. Matrix sample peak heights that are too high may result in bleedthrough or oversubtraction in other dye colors, which may result in spectral calibration failure. If matrix sample peak heights are too intense (>6,000RFU), increase the dilution of each fragment in Step 2 of Section 3.A or 4.A and repeat the spectral calibration run. Another option is to decrease the injection time, so less sample is injected.</p>
	<p>Incomplete denaturation can cause extra peaks in one or all color channels. Heat denature samples for the recommended time, and cool on crushed ice or in an ice-water bath immediately prior to loading the capillary. Do not cool samples in a thermal cycler or -20°C freezer.</p>
	<p>For best spectral calibration results, use a fresh bottle of polymer, fresh buffer and water, and a capillary array with fewer than 100 injections.</p>

5. Troubleshooting (continued)

Symptoms	Causes and Comments
All capillaries failed spectral calibration	Monitoring fragment migration in the Capillaries Viewer during the spectral calibration run can provide information that might be useful for troubleshooting purposes. Reinject the spectral calibration plate, and monitor the Capillaries Viewer during the run. Note any unusual peak formations, extremely high or low peak heights.

6. Related Products

Product	Size	Cat.#
PowerPlex® 16 HS System	100 reactions	DC2101
	400 reactions	DC2100
PowerPlex® 16 System	100 reactions	DC6531
	400 reactions	DC6530
PowerPlex® S5 System	100 reactions	DC6951
	400 reactions	DC6950
PowerPlex® Y System	50 reactions	DC6761
	200 reactions	DC6760
PowerPlex® 1.2 System	100 reactions	DC6101
PowerPlex® ES System	100 reactions	DC6731
	400 reactions	DC6730

Not for Medical Diagnostic Use.

Accessory Components

Product	Size	Cat.#
Nuclease-Free Water	50ml	P1193

For Laboratory Use.

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