



Promega

Technical Bulletin

PowerPlex[®] Matrix Standards, 310/377

INSTRUCTIONS FOR USE OF PRODUCT DG3640.



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PowerPlex[®] Matrix Standards, 310/377

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1. Description

Proper generation of a matrix file is critical to evaluate multicolor systems with the ABI PRISM[®] 310 Genetic Analyzer and ABI PRISM[®] 377 DNA Sequencer.

To prepare a matrix, four standards are run under the same capillary electrophoresis (CE) or gel conditions that are used for samples and allelic ladders. The PowerPlex[®] Matrix Standards, 310/377, consist of DNA fragments labeled with four different fluorescent dyes: one tube contains DNA fragments labeled with fluorescein, one tube contains DNA fragments labeled with carboxy-tetramethylrhodamine (TMR), two tubes contain DNA fragments labeled with 6-carboxy-4',5'-dichloro-2',7'-dimethoxyfluorescein (JOE), and one tube contains DNA fragments labeled with carboxy-X-rhodamine (CXR).

1. Description (continued)

Use the Fluorescein Matrix, JOE Matrix, TMR Matrix and CXR Matrix for the blue, green, yellow and red standards, respectively. The PowerPlex® Matrix Standards, 310/377, includes both JOE A- and JOE B-labeled fragments, but this protocol uses only JOE A. The PowerPlex® Matrix Standards, 310/377, is used with the PowerPlex® 1.2 System or any of the *GenePrint®* Fluorescent STR Systems (fluorescein-labeled). A matrix should be generated for each individual instrument and Promega chemistry. Protocols for operation of the fluorescence-detecting instrumentation should be obtained from the manufacturer.

For information on Promega's other fluorescent STR systems, refer to the *GenePrint® Fluorescent STR Systems Technical Manual #TMD006*, *PowerPlex® 1.1 System Technical Manual #TMD008*, *PowerPlex® 1.2 System Technical Manual #TMD009*, *PowerPlex® 2.1 System Technical Manual #TMD011*, *PowerPlex® 16 System Technical Manual #TMD012*, *PowerPlex® ES System Technical Manual #TMD017* and *PowerPlex® Y System Technical Manual #TMD018*. These Technical Manuals and additional product information are available upon request from Promega or at: www.promega.com

For the PowerPlex® 16 HS, PowerPlex® 16, PowerPlex® S5, Y and ES Systems, the PowerPlex® Matrix Standards, 310 (Cat.# DG4640), is required for matrix standardization for the ABI PRISM® 310 Genetic Analyzer and ABI PRISM® 377 DNA Sequencer.

2. Product Components and Storage Conditions

Product	Cat.#
PowerPlex® Matrix Standards, 310/377	DG3640

Not for Medical Diagnostic Use. Includes:

- 50µl Fluorescein Matrix
- 50µl JOE Matrix A
- 50µl JOE Matrix B
- 50µl TMR Matrix
- 50µl CXR Matrix
- 1ml Blue Dextran Loading Solution

Storage Conditions: Store all components at -20°C. The DNA fragments in the matrix standards are light-sensitive and must be stored in the dark. We strongly recommend that the matrix standards be stored with post-amplification reagents (away from pre-amplification materials) and used separately with different pipettes, tube racks, etc.

Additional product information and ordering information for accessory components and related products is available upon request from Promega or at www.promega.com


3. Detection of Amplified Fragments Using the ABI PRISM® 310 Genetic Analyzer

Materials to Be Supplied by the User

(Solution compositions are provided in Section 6.)

- dry heating block, water bath or thermal cycler
- crushed ice
- 310 capillaries, 47cm × 50µm
- Performance Optimized Polymer 4 (POP-4™)
- sample tubes and septa
- aerosol-resistant pipette tips
- 10X genetic analyzer buffer
- deionized formamide with conductivity <100µS/cm (Hi-Di™ Formamide, Applied Biosystems, Cat.# 4311320)

Note: The quality of the formamide is critical. Use deionized formamide with a conductivity <100µS/cm. Aliquots of formamide can be made and frozen at -20°C. Multiple freeze-thaw cycles or long-term storage at 4°C can cause a breakdown of the formamide. Formamide with a conductivity >100µS/cm can contain ions that compete with DNA during injection. This results in lower peak heights and reduced sensitivity. A longer injection time might not increase the signal.

 **Caution:** Formamide is an irritant and teratogen; avoid inhalation and contact with skin. Read the warning label and take the necessary precautions when handling this substance. Always wear double gloves and safety glasses when working with formamide.

3.A. Instrument Preparation

1. Refer to the ABI PRISM® 310 Genetic Analyzer User's Manual for instructions on cleaning the pump block, installing the capillary, calibrating the autosampler and adding polymer to the syringe.
2. Open the ABI PRISM® 310 data collection software.
3. Prepare a GeneScan® sample sheet as described in the ABI PRISM® 310 Genetic Analyzer User's Manual. Enter the appropriate sample information in the "sample info" column. Create a new GeneScan® injection list. Select the appropriate sample sheet by using the pull-down menu.

3.A. Instrument Preparation (continued)

4. Select the "GS STR POP4 (1ml) A" Module using the pull-down menu. Change the run time to 30 minutes, and keep the settings for the remaining parameters as shown below:

Inj. Secs:	5
Inj. kV:	15.0
Run kV:	15.0
Run °C:	60
Run Time (minutes):	30

Note: The injection time may need to be optimized for individual instruments.

5. Select "none" for the matrix file.

3.B. Sample Preparation

1. For each matrix sample, combine 2µl of the matrix standard with 25µl deionized formamide or water.
2. Denature each sample for 3 minutes at 95°C, and immediately chill on crushed ice for 3 minutes.
3. Assemble the tubes in the appropriate autosampler tray (48-tube or 96-tube).
4. Place the autosampler tray in the instrument and close the instrument doors.

3.C. Capillary Electrophoresis and Detection

1. After loading the sample tray and closing the doors, click on the "Run" button to start the capillary electrophoresis system.
2. Monitor the electrophoresis by observing the raw data and status windows.
3. Each sample will take approximately 40 minutes for syringe pumping, sample injection and sample electrophoresis.

3.D. Matrix Generation for the ABI PRISM® 310 Genetic Analyzer

1. Open the GeneScan® project.
2. Review the raw data from the individual matrix samples. Highlight the Sample File name, then go to the “sample” menu and select “raw data.” Move the cursor beyond the primer peak, so the crosshair is on a flat portion of the baseline. Record the X value number shown at the bottom of the window. Select an area that includes at least 5 peaks; matrix generation requires a minimum of 5 peaks. With newer versions of GeneScan® Analysis Software, an acceptable matrix can be generated with fewer than 5 peaks. However, for optimal results, use as many peaks as possible. See Figure 1.

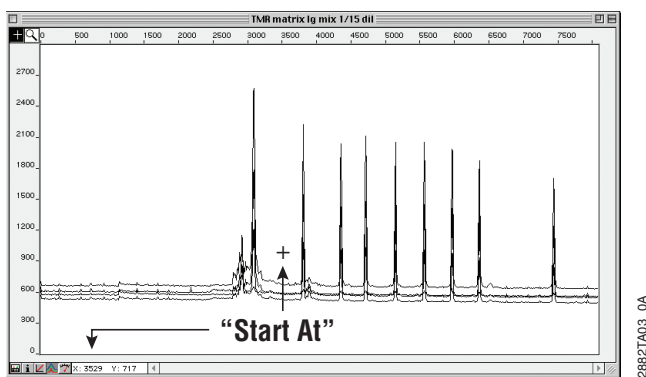


Figure 1. TMR Matrix raw data. The TMR Matrix standard was run on an ABI PRISM® 310 Genetic Analyzer. GeneScan® Analysis Software was used to view the “raw data” (option under “sample”). The cursor was placed on the baseline, and the “start at” value of 3529 was determined by using the readout in the lower left hand corner of the window

3. Under the File Menu, select “New”, then click the Matrix icon. The “points” field should have the default value of 100,000. Click on the dye color for each matrix, and indicate the sample file that corresponds to that dye. Enter the X value recorded from Step 2 in the “start at” field.

Dye Color	Corresponding Matrix
Blue	Fluorescein Matrix
Green	JOE Matrix A
Yellow	TMR Matrix
Red	CXR Matrix

4. Select “OK” and the matrix file will be generated.

3.D. Matrix Generation for the ABI PRISM® 310 Genetic Analyzer (continued)

5. Save the matrix file in the Matrix Standards Folder located in the GeneScan® folder. Be sure to use a meaningful name that indicates with which STR system the matrix file will be used. For the Macintosh® version of the software, a copy of the matrix file is automatically saved in the GS Matrix folder. For the Windows NT® version of the software, store a copy of the matrix file in the matrix folder at:
C:\appliedbio\shared\analysis\sizecaller\matrix.

Note: To generate a matrix in GeneMapper™ *ID*, open the “GeneMapper Manager” and click on the “Matrices” tab. Click on the “New” button. Select the appropriate sample files to be used for each dye color, and enter the X value in the “start at” field as described in Steps 2 and 3. Click on the “Create” button, then on the “OK” button to generate and save the matrix.

6. A new matrix can be applied to previously run samples by highlighting the sample in the GeneScan® project. Under the “sample” menu, select “install new matrix,” highlight the new matrix and click on “open.” The new matrix will be applied to the sample file, and the samples can be analyzed using the new matrix.
7. The quality of a matrix can be verified. Apply the new matrix file to the samples used to generate the matrix. Analyze the matrix samples using all four dye colors. The matrix samples should have peaks between 1,000–4,000RFU in the dye colors listed in Step 3. The baselines for the other three dye colors should be relatively flat. A small amount of bleedthrough may be seen with the TMR (yellow) into the CXR (red) channel.

4. Detection of Amplified Fragments Using the ABI PRISM® 377 DNA Sequencer

Materials to Be Supplied by the User

(Solution compositions are provided in Section 6.)

- dry heating block, water bath or thermal cycler
- crushed ice
- Long Ranger® gel solution (Lonza Cat.# 50611) or Long Ranger Singel® pack for ABI sequencers 377-36cm (Lonza Cat.# 50691)
- 10% ammonium persulfate (Cat.# V3131)
- TEMED
- Urea (Cat.# V3171)
- TBE 10X buffer
- Nalgene® tissue culture filter (0.2 micron)
- aerosol-resistant pipette tips
- gel-loading pipette tips
- 36cm front and rear glass plates
- 36cm gel spacers (0.2mm thick)
- 36-well sharkstooth comb or 34-well squaretooth comb (0.2mm thick)
- clamps (e.g., large office binder clamps)
- Liqui-Nox® or other detergent

4.A. Polyacrylamide Gel Preparation

Hazardous reagents are used in the preparation and use of gels for the ABI PRISM® 377 DNA Sequencer. The reagents and their hazards are listed in Table 1.

Table 1. Hazardous Reagents.

Reagents for ABI PRISM® 377 DNA Sequencer	Hazard
acrylamide (Long Ranger® gel solution)	suspected carcinogen, toxic
ammonium persulfate	oxidizer, corrosive
formamide (contained in the Blue Dextran Loading Solution)	irritant, teratogen
TEMED	corrosive, flammable
urea	irritant

The following protocol is for preparation of a 36cm denaturing polyacrylamide gel for use with the ABI PRISM® 377 DNA Sequencer. Low-fluorescence glass plates are recommended and can be obtained from the instrument manufacturer.

1. Thoroughly clean the glass plates with hot water and a 1% Liqui-Nox® solution or another dilute laboratory detergent solution. Rinse extremely well using deionized water. Allow the glass plates to air-dry in a dust-free environment.

4.A. Polyacrylamide Gel Preparation (continued)

2. Assemble the glass plates by placing 0.2mm side gel spacers between the front and rear glass plates. Hold the plates together using binder clamps (4 clamps on each side). Place the assembly horizontally on a test tube rack or similar support.
3. Prepare a 5% Long Ranger® acrylamide gel (total of 50ml) by combining the ingredients listed in Table 2. Stir the solution until the urea has dissolved.

Table 2. Preparation of a 5% Long Ranger® Polyacrylamide Gel.

Component	5% Gel	Final Concentration
urea	18g	6M
deionized water	26ml	-
10X TBE	5ml	1X
50% Long Ranger® gel solution	5ml	5%
total volume	50ml	

Note: Long Ranger Singel® Packs can be used.

- !** **Caution:** Acrylamide (Long Ranger® gel solution) is a neurotoxin and suspected carcinogen; avoid inhalation and contact with skin. Read the warning label and take the necessary precautions when handling this substance. Always wear double gloves and safety glasses when working with acrylamide solutions.
4. Filter the acrylamide solution through a 0.2 micron filter (e.g., Nalgene® tissue culture filter) and degas for an additional 5 minutes.
 5. Add 35µl of TEMED and 250µl of fresh 10% ammonium persulfate to the 50ml of acrylamide solution and mix gently.
 6. Using a disposable 30cc syringe, pour the gel by starting at the well end of the plates and carefully injecting the acrylamide between the horizontal glass plates. Allow the solution to fill the top width of the plates. While maintaining a constant flow of solution, gently tap the glass plates to assist the movement of solution to the bottom of the plates.
 7. Insert a 36-well sharktooth comb or 34-well squaretooth comb between the glass plates. Sharktooth combs with 64 or 96 wells can also be used.
 8. Secure the comb with 3 evenly spaced clamps.
 9. Keep the remaining acrylamide solution as a polymerization control.
 10. Allow polymerization to proceed for >2 hours. Check the polymerization control to be sure that polymerization has occurred.

Note: The gel can be stored overnight if a paper towel saturated with deionized water and plastic wrap are placed around the top and bottom to prevent the gel from drying out (crystallization of the urea will destroy the gel).

4.B. Instrument Preparation

1. Open the ABI PRISM® 377 data collection software.
2. Prepare a sample sheet as described in the GeneScan® Analysis Software User's Manual. Enter the appropriate sample information in the "sample info" column.
3. Create a new GeneScan® run and use the following settings:

Plate Check Module:	Plate Check A
PreRun Module:	PR GS 36A-2400
Run Module:	GS 36A-2400
Collect Time:	3 hours
Well-to-Read Distance:	36cm
4. Select the appropriate sample sheet and comb selection by using the pull-down menus.
5. Select "none" for the gel matrix file.

4.C. Gel Prerun

1. Remove the clamps from the polymerized acrylamide gel. If necessary, clean any excess acrylamide from the glass plates with paper towels saturated with deionized water.
2. Shave any excess polyacrylamide away from the comb and remove the comb. If using a sharktooth comb, carefully insert the sharktooth comb teeth into the gel approximately 1-2mm.
3. Position the gel/glass plate unit in the 377 cassette.
4. Secure the cassette in the instrument and perform a plate check as recommended in the ABI PRISM® 377 DNA Sequencer User's Manual. If the horizontal line graph is not flat, remove the cassette, clean the plate surface, and repeat plate check.
5. Add TBE 1X buffer to the top and bottom buffer chambers of the instrument.
6. Using a 30cc syringe filled with buffer, remove any air bubbles and unpolymerized acrylamide from the well area of the gel and place the lid on the upper buffer chamber. Using a syringe with a bent 19-gauge needle, remove the air bubbles from the bottom of the gel.
7. Attach the heating plate, connect the water tubing, attach all electrodes, close the instrument door and click on the "PreRun" button. Allow the gel to prerun for 15-20 minutes or until the gel temperature is at least 40°C. Open the Status Window to monitor the temperature of the gel.
8. Prepare the matrix samples during the gel prerun.

4.D. Sample Preparation and Loading

1. Combine 1.5µl of each matrix sample with 1.5µl of Blue Dextran Loading Solution.
2. Denature each sample for 2 minutes at 95°C and immediately chill on crushed ice for 3 minutes.

Note: Instrument detection limits vary; therefore, the amount of product mixed with loading cocktail may need to be increased or decreased.

3. After the 15- to 20-minute prerun, pause the instrument by clicking on the Pause button. When the prerun is paused, the water will continue to circulate to keep the gel warm during the sample loading.
4. Use a 30cc syringe filled with buffer to flush the urea from the well area.
5. Load 1.5µl of each denatured sample into the respective wells.
6. Place the lid on the upper buffer chamber, and close the instrument door.

4.E. Gel Electrophoresis and Detection

1. After loading, click on "Cancel" to stop the prerun. Make sure that the run time is set at 3 hours, then click on "Run" to begin electrophoresis.
2. Monitor the electrophoresis by observing the gel image and status windows.
3. Allow electrophoresis to proceed for 3 hours. The largest fragment will have migrated past the laser.
4. Track and extract the gel lanes.

4.F. Matrix Generation for the ABI PRISM® 377 DNA Sequencer

1. Open the GeneScan® project.
2. Review the raw data from the individual matrix samples. Highlight the sample file name, then go under the sample menu and select "raw data." Move the cursor beyond the primer peak so the crosshair is on a flat portion of the baseline. Record the X value number shown at the bottom of the window.

Matrix generation requires a minimum of 5 peaks. Select an area that includes 5 peaks in each color. See Figure 1. With newer versions of GeneScan® Analysis Software, an acceptable matrix can be generated with fewer than 5 peaks. However, for optimal results, use as many peaks as possible.

3. Under the File Menu, select “New” then click the matrix icon. The “points” field should have the default value of 100,000. Click on the dye color for each matrix and indicate the sample file that corresponds to that dye. Enter the recorded X value from Step 2 in the “start at” field.

<u>Dye Color</u>	<u>Corresponding Matrix</u>
Blue	Fluorescein Matrix
Green	JOE Matrix A
Yellow	TMR Matrix
Red	CXR Matrix

4. Click “OK” and the matrix file will be generated.
5. Save the matrix file in the Matrix Standards Folder located in the GeneScan® folder. A copy of the matrix file should be stored in the ABI folder located in the system folder.
6. A new matrix can be applied to previously run samples by highlighting the sample in the GeneScan® project. Under “sample” select “install new matrix,” highlight the new matrix and click on “open.” The new matrix will be applied to the sample file and the samples can be analyzed using the new matrix.
7. The quality of the matrix can be verified. Apply the new matrix file to the samples used to generate the matrix. Analyze the matrix samples using all 4 dye colors. The matrix samples should have peaks of 800-2,000RFU in the dye colors listed in Step 3. As you evaluate each sample, the baselines for the other three dye colors should be relatively flat.

4.G. Reuse of Glass Plates

Separate the glass plates and discard the gel. Clean the glass plates with hot water and a detergent such as 1% Liqui-Nox® detergent. Rinse extremely well with deionized water and allow the plates to air-dry. Do not scrape the plates with abrasive materials during this process.

Gel extrusion (gel expands into the comb during a run) can occur due to a build-up of residue. If this occurs, soak the plates in 2N HCl for 15 minutes, then rinse thoroughly.

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
Unable to generate a matrix (due to faint or no peaks)	<p>Poor capillary electrophoresis (CE) injection. Reinject the sample. Check the syringe for leakage. Check the laser power.</p> <hr/> <p>Poor-quality formamide used. Use only high-quality formamide when running samples on the ABI PRISM® 310 Genetic Analyzer. The conductivity of the deionized formamide should be less than 100µS/cm.</p> <hr/> <p>Samples degraded due to improper storage. Store matrices in the dark at -20°C.</p> <hr/> <p>Peak heights too low. Peak heights should be 1,000-4,000RFU for the ABI PRISM® 310 Genetic Analyzer and 800-2,000RFU for the ABI PRISM® 377 DNA Sequencer. To increase peak heights, increase the injection time or loading volume.</p> <hr/> <p>Samples not denatured. Heat denature the samples and immediately chill on crushed ice before loading the gel or capillary.</p>
Unable to generate a matrix (miscellaneous)	<p>Insufficient number of peaks. Choose an area that includes a minimum of 5 peaks for matrix standardization. With newer versions of GeneScan® Analysis Software, an acceptable matrix can be created with fewer than 5 peaks. However, for optimal results, use as many peaks as possible.</p>
Poor-quality matrix (extra peaks visible in one or all of the color channels)	<p>CE-related artifacts (“spikes”). Minor voltage changes or urea crystals passing by the laser can cause “spikes” or unexpected peaks. Spikes sometimes appear in one color but often are easily identified by their presence in more than one color. Reinject the samples to confirm.</p>

5. Troubleshooting (continued)

Symptoms	Causes and Comments								
Poor-quality matrix (extra peaks visible in one or all of the color channels; continued)	CE-related artifacts (contaminants). Contaminants in the water used with the ABI PRISM® 310 Genetic Analyzer and for diluting the 10X genetic analyzer buffer can generate peaks in the blue and green dye colors. Use autoclaved water to clean the pump block and to prepare sample dilutions. Change vials and wash the buffer reservoir.								
Poor-quality matrix (elevated baseline and/or inverted peaks in analyzed samples; see Figure 2)	<p>Matrix used was generated on another instrument. A matrix must be generated for each instrument.</p> <p>Wrong dye used. Generate the matrix using the same dyes as those contained in the samples.</p> <p>Oversubtraction of signal because signal is saturated. When generating a matrix, avoid choosing samples with peak heights that are higher than the recommended RFU values, as this can result in a matrix that causes inverted peaks or elevated baseline. An improvement in analyzed samples can be seen by diluting the matrix samples in water before preparation for use in this protocol (prior to Section 3.B, Step 1, or Section 4.D, Step 1).</p>								
Matrix baseline has inverted peaks. See Figure 3.	<p>Incorrect or no “start at” value entered. “Start at” value should have a flat baseline.</p> <p>Wrong colors assigned to the dyes. Confirm the dye and color selection:</p> <table data-bbox="542 1034 798 1134"> <tbody> <tr> <td>Fluorescein:</td> <td>Blue</td> </tr> <tr> <td>JOE A:</td> <td>Green</td> </tr> <tr> <td>TMR:</td> <td>Yellow</td> </tr> <tr> <td>CXR:</td> <td>Red</td> </tr> </tbody> </table>	Fluorescein:	Blue	JOE A:	Green	TMR:	Yellow	CXR:	Red
Fluorescein:	Blue								
JOE A:	Green								
TMR:	Yellow								
CXR:	Red								

5. Troubleshooting (continued)

Symptoms	Causes and Comments
Previously generated matrix no longer performs optimally	Changes to or aging of instrument components. The sensitivity of the instrument can change if the instrument has been moved or recently serviced (replacement or realignment of the laser, CCD camera, power supply or mirrors). The sensitivity also can change over time due to aging of the instrument. These changes can result in poor matrix performance. Generate a new matrix.

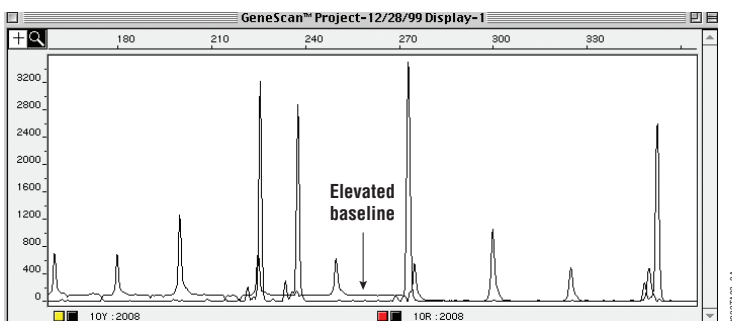


Figure 2. Elevated baseline. A sample was run on an ABI PRISM® 310 Genetic Analyzer and analyzed using GeneScan® Analysis Software. The resulting electropherogram shows an elevated baseline below 270 bases. An elevated baseline can be the result of using a matrix from another instrument, using a matrix made on the same instrument before service or using a matrix made with different dyes.

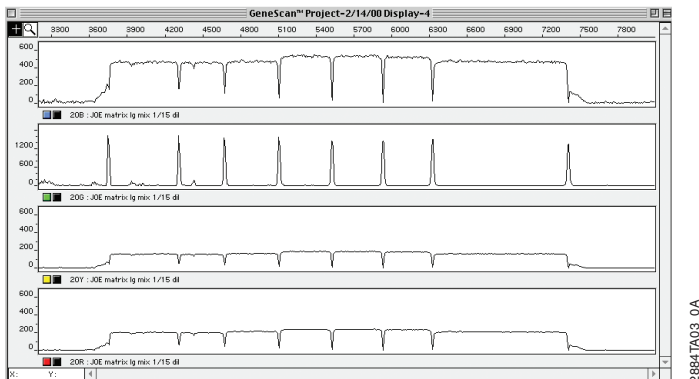


Figure 3. Inverted baseline. The four matrix samples from the PowerPlex® Matrix Standards, 310/377, were run on an ABI PRISM® 310 Genetic Analyzer. A matrix was made using the GeneScan® Analysis Software, but no “start at” point was entered for the matrix samples. The resulting matrix was applied to the JOE Matrix sample file, and analysis was done using all four colors. The result shows inverted peaks in the blue, yellow and red channels.

6. Composition of Buffers and Solutions

10% ammonium persulfate

Add 0.05g of ammonium persulfate to 500µl of deionized water. Use 250µl of 10% ammonium persulfate for each 50ml of acrylamide gel solution.

deionized formamide

Use ultra-pure grade formamide (Applied Biosystems Hi-Di™ Formamide Cat.# 431132). Use a conductivity meter to check the conductivity. The deionized formamide must have a conductivity <100µS/cm. Aliquots of deionized formamide can be made and frozen. Avoid multiple freeze-thaw cycles.

Blue Dextran Loading Solution

88.25% formamide
 15mg/ml blue dextran
 4.1mM EDTA (pH 8.0)

TBE 10X buffer

107.8g Tris base
 7.44g EDTA
 (Na₂EDTA • 2H₂O)
 ~55.0g boric acid

Dissolve the Tris base and EDTA in 800ml deionized water. Slowly add the boric acid and monitor the pH until the desired pH of 8.3 is obtained. Bring the volume to 1 liter with deionized water.

7. Related Products

Product	Size	Cat.#
PowerPlex® 1.2 System	100 reactions	DC6101
GenePrint® Fluorescent STR Systems	100 reactions	DC5171

Not for Medical Diagnostic Use.

Accessory Components

Product	Size	Cat.#
Internal Lane Standard 600*	150µl	DG1071
Fluorescent Ladder (CXR), 60–400 Bases*	65µl	DG6221
Gold ST★R 10X Buffer*	1.2ml	DM2411
Mineral Oil	12ml	DY1151
Nuclease-Free Water*	50ml	P1193

*For Laboratory Use.

Sample Preparation Systems

Product	Size	Cat.#
DNA IQ™ System*	100 reactions	DC6701
	400 reactions	DC6700
Plexor® HY System**	800 reactions	DC1000
	200 reactions	DC1001

*For Laboratory Use.

**Not for Medical Diagnostic Use.

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