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User Bulletin

ABI PRISM 310, 377, 377 with XL Upgrade

August 15, 2000 (updated 01/2001)

SUBJECT: ABI PRISM dRhodamine Matrix Standards Kit

Introduction

Matrix standards are used to generate the multicomponent matrix required for four-color fluorescence detection on the Applied Biosystems ABI PRISM® 310 Genetic Analyzer, the ABI PRISM® 377 DNA Sequencer, and the ABI PRISM 377 DNA Sequencer with XL Upgrade ("ABI PRISM 377XL").

Sequencing Analysis software uses this multicomponent matrix to analyze samples that are labeled with four different fluorescent dyes but are run in a single capillary injection or gel lane. A set of four matrix standards only needs to be run once to generate a matrix file that is used with all samples run under similar conditions.

For more information on the use of matrix standards, refer to the user's manual for your instrument.

Note Matrix files are called instrument files in the ABI PRISM 377 Collection software versions 2.0 and 2.1 and in the Sequencing Analysis software.

New Dyes

Applied Biosystems has designed four new dichlororhodamine (dRhodamine) fluorescent dyes—dichloro[R110] (dR110), dichloro[R6G] (dR6G), dichloro[TAMRA] (dTAMRA), and dichloro[ROX] (dROX).

They are used with the following new cycle sequencing chemistries:

- dRhodamine Terminators
- BigDye Primers
- BigDye Terminators

Applied Biosystems

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The new primers and terminators are labeled as follows:

Table 1. Dye Labels

Base	dRhodamine Terminators	BigDye Primers	BigDye Terminators
A	dR6G	dR6G	dR6G
C	dTAMRA	dR110	dROX
G	dR110	dTAMRA	dR110
T	dROX	dROX	dTAMRA

dRhodamine Matrix Standards

Using the new dRhodamine sequencing chemistries requires making instrument (matrix) files from the new matrix standards found in the ABI PRISM® dRhodamine Matrix Standards Kit (P/N 403047). The new matrix standards are the following:

Table 2. dRhodamine Matrix Standards

Tube Label	Color of Raw Data on ABI PRISM 310 Electropherogram	Color of Raw Data on ABI PRISM 377 Gel Image
dR110 Matrix Standard	blue	blue
dR6G Matrix Standard	green	green
dTAMRA Matrix Standard	black	yellow
dROX Matrix Standard	red	red

The dRhodamine matrix standards are provided in a ready-to-use format and are premixed with a blue dye for convenience in gel loading. Matrix standards are stable for 6 months at 2–6 °C. Avoid freeze-thaw cycles.

IMPORTANT The ABI PRISM dRhodamine Matrix Standards Kit is for use with the ABI PRISM 310 Genetic Analyzer, the ABI PRISM 377 DNA Sequencer, and the ABI PRISM 377 DNA Sequencer with XL Upgrade.

IMPORTANT The dRhodamine sequencing chemistries are not designed for use with the ABI™ 373 DNA Sequencer or the ABI 373 DNA Sequencer with XL Upgrade.

Filter Set E

You must use run modules and dye set/primer (mobility) files for virtual Filter Set E when sequencing with the dRhodamine-based cycle sequencing chemistries.

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Installing Run Modules and Dye Set/Primer Files

Overview

Run modules and dye set/primer (mobility) files are found on the diskette supplied with the dRhodamine Matrix Standards Kit. They can also be obtained from the Applied Biosystems site on the World Wide Web www.appliedbiosystems.com/techsupport or from your local Field Applications Specialist (call Applied Biosystems Technical Support or your local sales office for more information).

Run Modules

Use the appropriate run module for your run parameters on your instrument as shown in Table 3.

Table 3. Run Modules

Instrument	Configuration	Run Module
ABI PRISM 310	DNA Sequencing Polymer,[a] 250-µL syringe	Seq Run (250 uL) E
	POP-6 polymer, 1-mL syringe	Seq POP6 (1 mL) E
	POP-6 polymer, 1-mL syringe, Rapid Sequencing	Seq POP6 (1 mL) Rapid E
ABI PRISM 377[b]	2X, 36-cm wtr, 36-well	Seq Run 36E-1200
	4X, 36-cm wtr, 36-well	Seq Run 36E-2400
	48-cm wtr, 36-well	Seq Run 48E-1200
ABI PRISM 377 with XL Upgrade[b]	2X, 36-cm wtr, 36-, 48-, or 64-well	Seq Run 36E-1200
	4X, 36-cm wtr, 36-, 48-, or 64-well	Seq Run 36E-2400
	48-cm wtr, 36-, 48-, or 64-well	Seq Run 48E-1200

a. The DNA Sequencing Polymer is not supported for use with the BigDye Primers and BigDye Terminators.

b. Use any plate check and prerun module on the ABI PRISM 377 DNA Sequencer and ABI PRISM 377 DNA Sequencer with XL Upgrade.

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Dye Set/Primer Files

Use the correct dye set/primer (mobility) file for your instrument as shown in Table 4.

Table 4. Dye Set/Primer Files

Sequencing Chemistry	Instrument	Dye Set/Primer File
dRhodamine Terminators	ABI PRISM 310, DNA Sequencing Polymer[a]	DT DSP{dR Set-AnyPrimer}
	ABI PRISM 310, POP-6 polymer	DT POP6{dR Set-Any Primer}
	ABI PRISM 310, POP-6 polymer, Rapid Sequencing	DT POP6{dR Set-Any Primer}
	ABI PRISM 377[b]	DT {dR Set Any-Primer}
	ABI PRISM 377 with XL Upgrade[b]	DT {dR Set Any-Primer}
BigDye Primers	ABI PRISM 310, POP-6 polymer, –21 M13 primers	DP POP6 {BD Set-21M13}
	ABI PRISM 310, POP-6 polymer, M13 Reverse primers	DP POP6 {BD Set-M13 Reverse}
	ABI PRISM 377[b]	DP5%LR{BD M13 FWD & REV}
	ABI PRISM 377 with XL Upgrade[b]	DP5%LR{BD M13 FWD & REV}
BigDye Terminators	ABI PRISM 310, POP-6 polymer	DT POP6{BD Set-Any Primer}
	ABI PRISM 310, POP-6 polymer, Rapid Sequencing	DT POP6{BD Set-Any Primer}
	ABI PRISM 377[b]	DT {BD Set Any-Primer}
	ABI PRISM 377 with XL Upgrade[b]	DT {BD Set Any-Primer}

a. DSP = DNA Sequencing Polymer. The DNA Sequencing Polymer is not supported for use with the BigDye Primers and BigDye Terminators.

b. The dye set/primer file can be used with 5 and 5.5% Long Ranger gels and 4 and 4.25% polyacrylamide gels (19:1, acrylamide:bis).

IMPORTANT Mobility shifts and dye set/primer file names for the dRhodamine Terminators are similar to those for the BigDye Terminators. Their respective mobility files can be mistaken for each other easily without noticeably affecting the base spacing in the data.

If a mobility file for the wrong sequencing chemistry is used, some bases will be miscalled because of differences in which terminators are labeled with which dyes (see Table 1 on page 2) and because of the mobility shifts.

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Installing Run Modules and Dye Set/Primer Files

To install the run modules and dye set/primer (mobility) files:

Step	Action
1	Copy the run modules for your instrument into the Module folder within the Collection software folder. The modules are on the diskette supplied with the dRhodamine Matrix Standards Kit.
2	Copy the mobility files for your instrument into the ABI folder within the System folder. The mobility files are on the diskette supplied with the dRhodamine Matrix Standards Kit.
3	Relaunch the Collection and/or Sequencing Analysis software if either was open while the files were installed. **Note** Sometimes it is necessary to restart the Macintosh to use the new run modules and dye set/primer files.

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Making Instrument (Matrix) Files

Running Standards on the ABI PRISM 310

To run standards:

Step	Action	
1	Prepare a separate loading cocktail for each of the four matrix standards as shown below. **IMPORTANT** The matrix standards can precipitate in the tube, leading to very low signal strength. Mix each matrix standard thoroughly before using by vortexing or pipetting up and down.	
	Component	**Volume (µL)**
	Matrix standard	1
	Deionized formamide	12
	Total volume	13
	! WARNING ! CHEMICAL HAZARD Formamide is a teratogen and is harmful by inhalation, skin contact, and ingestion. Use in a well-ventilated area. Use chemical-resistant gloves and safety glasses when handling.	
2	Heat each sample at 95 °C for 2 minutes. Place on ice until ready to load.	
3	Run each matrix standard sample on the ABI PRISM 310 in a separate injection. Refer to the ABI PRISM 310 *Genetic Analyzer User's Manual* or *User Bulletin #1* (P/N 904261) for instructions on setting up the instrument.	
4	Examine the electropherogram of the raw data. The matrix standards should display the following colors:	
	Matrix Standard	**Color in Electropherogram**
	dR110	blue
	dR6G	green
	dTAMRA	black
	dROX	red

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Running Standards on the ABI PRISM 377 or ABI PRISM 377 with XL Upgrade

To run standards:

Step	Action	
1	Prepare a separate loading cocktail for each of the four matrix standards as shown below. **IMPORTANT** The matrix standards can precipitate in the tube, leading to very low signal strength. Mix each matrix standard thoroughly before using by vortexing or pipetting up and down.	
	Component	**Volume (µL)**
	Matrix standard	2
	Deionized formamide	2
	Total volume	4
	! WARNING ! CHEMICAL HAZARD Formamide is a teratogen and is harmful by inhalation, skin contact, and ingestion. Use in a well-ventilated area. Use chemical-resistant gloves and safety glasses when handling.	
2	Heat the cocktails at 95 °C for two minutes. Place on ice until ready to load. **IMPORTANT** DNA samples should not be stored in formamide for more than a few hours.	
3	Load each of the four matrix standard cocktails into a separate lane of the gel as shown below.	
	Instrument Platform	**Loading Volume (µL)**
	ABI PRISM 377	1
	ABI PRISM 377XL-48	1–1.5
	ABI PRISM 377XL-64	1
4	Perform electrophoresis according to your instrument user's manual.	
5	After electrophoresis, examine the raw data. The matrix standards should display the following colors in the gel image:	
	Matrix Standard	**Color on Gel Image**
	dR110	blue
	dR6G	green
	dTAMRA	yellow
	dROX	red
6	Check the lane tracking for the matrix standards before making the matrix.	

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Making the Matrix

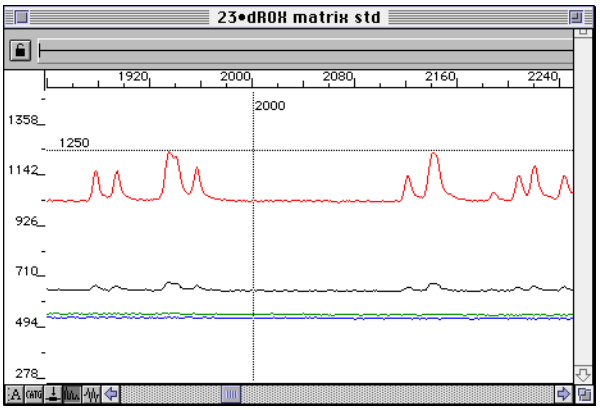
You must put the correct data file for each matrix standard into the correct "box" in the Data Utility application (Table 5).

Table 5. Placement of Standards in the Data Utility Application

Box	Dye Primer Matrix	Taq Terminator Matrix	T7 Terminator Matrix
C…	dR110	dROX	dR6G
A…	dR6G	dR6G	dTAMRA
G…	dTAMRA	dR110	dROX
T…	dROX	dTAMRA	dR110

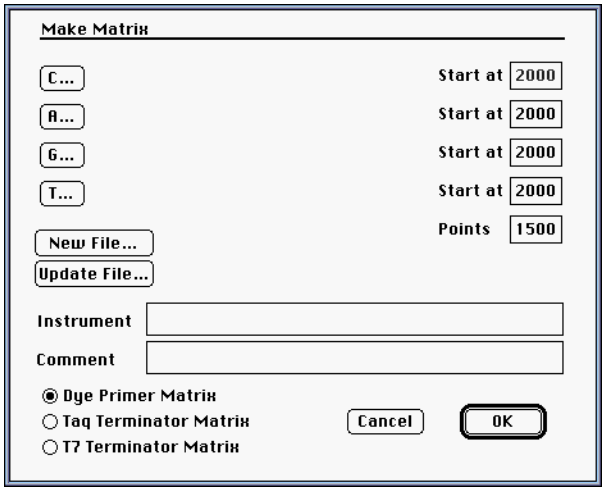
IMPORTANT You need to make all three matrix files, even if you are only using one dRhodamine-based chemistry. The Collection software will not run with only a terminator matrix in the file. An error message will appear saying, "Tag not found. Cannot start the run."

To make the Dye Primer Matrix:

Step	Action
1	Set the analysis start point and the number of data points to analyze. a. In the Sequencing Analysis software, examine the raw data for one of the matrix standard samples as shown below. b. Select a starting point where there are no peaks and the baseline is flat. c. Select a number of data points to analyze such that no peaks in the range are off-scale, *i.e.*, above 4000 relative fluorescence units (RFU), and that the baseline at the end of the range is flat. A typical number of data points is 1500. 

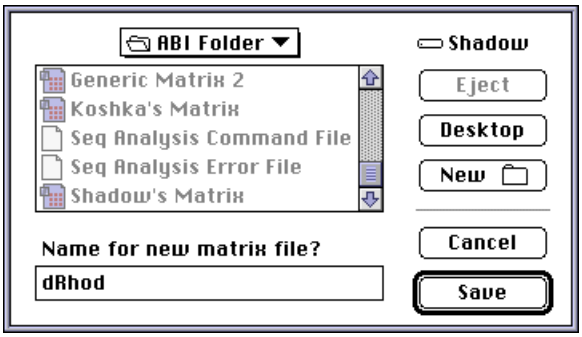
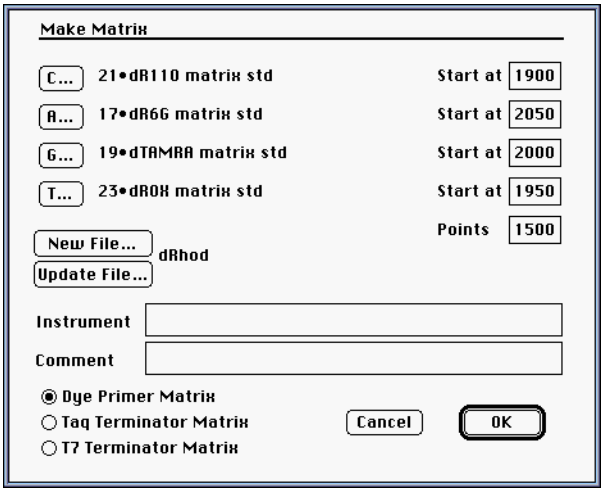
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To make the Dye Primer Matrix: *(continued)*

Step	Action
2	Repeat step 1 on page 8 for each matrix standard sample. Record the results for later use. **IMPORTANT** The number of data points analyzed is the same for each matrix standard. Choose starting points for each sample such that all peaks are less than 4000 RFU and that both the starting and ending points have flat baselines and no peaks.
3	Launch the Data Utility software.
4	From the Utilities menu, choose Make Matrix… The Make Matrix dialog box appears as shown below. Verify that the Dye Primer Matrix button at the lower left is selected. 
5	Click on the box for each nucleotide base and enter the data file that corresponds to the correct matrix standard as shown in Table 5 on page 8.
6	Enter the analysis start point for each matrix standard sample as determined in step 1 on page 8.

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To make the Dye Primer Matrix: *(continued)*

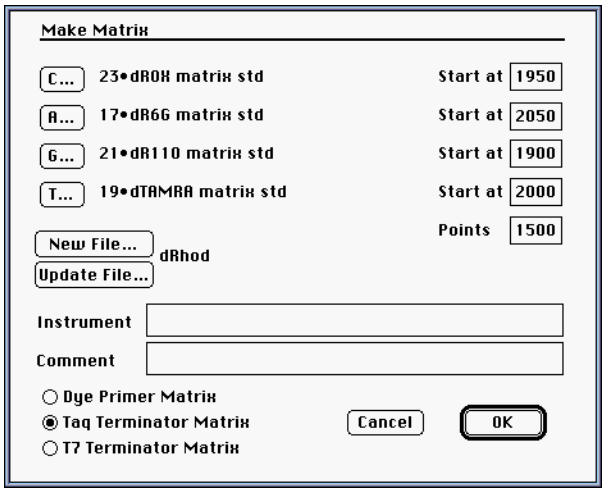
Step	Action
7	Click New File… A dialog window appears as shown below. Name the file dRhod and save it in the ABI folder within the System folder. 
8	The Make Matrix dialog box should look like that shown below. **Note** The numbers in the Start at and Points boxes below are typical values. Your numbers may vary. 
9	a. Click OK. The computer makes the matrix. When finished, a dialog window appears with the message “Make matrix successfully completed.” b. Click OK.

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To make the Dye Primer Matrix: *(continued)*

Step	Action
10	If the computer is unable to make a matrix, examine the raw data again in the Sequencing Analysis software. If many peaks are off-scale, dilute the matrix standards and rerun them.

To make the Taq Terminator Matrix:

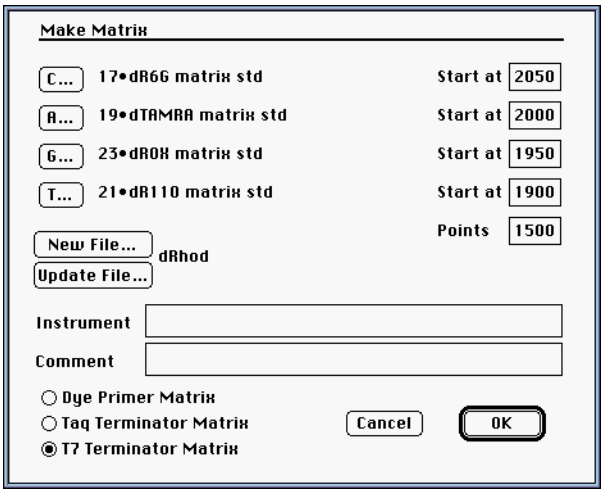
Step	Action
1	In the Data Utility application, choose Make Matrix… from the Utilities menu. The Make Matrix dialog box appears.
2	In the Make Matrix dialog box, click the Taq Terminator Matrix button at the lower left.
3	Click on the box for each nucleotide base and enter the data file that corresponds to the correct matrix standard as shown in Table 5 on page 8. **IMPORTANT** The order of matrix standard data files is different from that in the Dye Primer Matrix (see Table 5 on page 8).
4	Enter the same numbers for each matrix standard sample in the Start at and Points boxes as were used for the Dye Primer Matrix.
5	Click Update File… A dialog window appears.
6	Choose dRhod from the ABI folder within the System folder and click Save. The Make Matrix dialog box should look like that shown below. 

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To make the Taq Terminator Matrix: *(continued)*

Step	Action
7	a. Click OK. The computer makes the matrix. When finished, a dialog window appears with the message “Make matrix successfully completed.” b. Click OK.

To make the T7 Terminator Matrix:

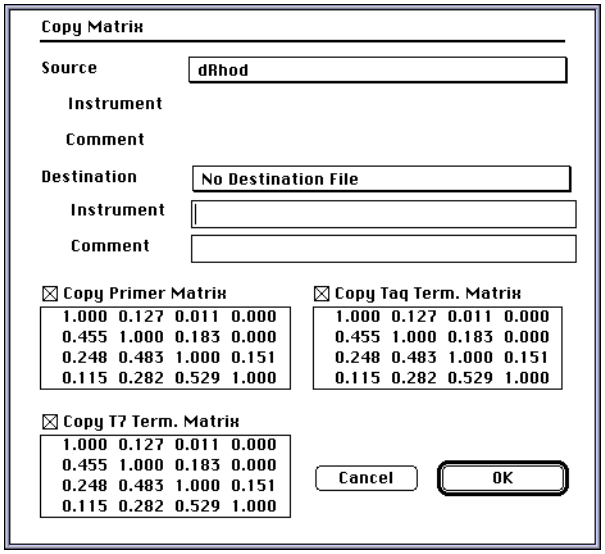
Step	Action
1	In the Data Utility application, choose Make Matrix… from the Utilities menu. The Make Matrix dialog box appears.
2	In the Make Matrix dialog box, click the T7 Terminator Matrix button at the lower left.
3	Click on the box for each nucleotide base and enter the data file that corresponds to the correct matrix standard as shown in Table 5 on page 8 (note the order of the matrix standard files).
4	Enter the same numbers for each matrix standard sample in the Start at and Points boxes as were used in the Dye Primer Matrix and Taq Terminator Matrix.
5	Click Update File… A dialog window appears.
6	Choose dRhod from the ABI folder within the System folder and click Save. The Make Matrix dialog box should look like that shown below. 

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To make the T7 Terminator Matrix: *(continued)*

Step	Action
7	a. Click OK. The computer makes the matrix. When finished, a dialog window appears with the message “Make matrix successfully completed.” b. Click OK.

To check the instrument file:

Step	Action
1	From the Utilities menu, choose Copy Matrix…
2	Under Source, select Instrument file and choose dRhod from the ABI folder within the System folder. The three matrix files within the dRhod instrument file appear as shown below. 
3	Make sure that all three matrix files have numbers that range from 0–1. The numbers on the diagonals from top left to bottom right should be 1. If not, then repeat the matrix-making procedure starting with “To make the Dye Primer Matrix:” on page 8. **Note** The corresponding numbers for all three matrix files will be the same.
4	Click Cancel.

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To check the instrument file: *(continued)*

Step	Action
5	Restart the Sequencing Analysis software and use dRhod as the instrument file to analyze your sequencing data.

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