User Bulletin #4

ABI Prism® 7700 Sequence Detection System

May 21, 1998 (updated 10/2000)

SUBJECT: Generating New Spectra Components

Overview

This user bulletin contains procedures for calibrating the ABI PRISM® 7700 Sequence Detector with the Sequence Detection Systems Spectral Calibration Kit (P/N 4305822). The kit features two new fluorescent dye standards (VIC and SYBR Green) which will require you to make three adjustments to your Sequence Detection Systems (SDS) software.

IMPORTANT Follow the three procedures in this bulletin in the order the document presents them. The SDS software will not permit you to create files out of the order described in this user bulletin.

The procedures are as follows:

This bulletin is a supplement to the *ABI PRISM 7700 Sequence Detection Systems User's Manual* (P/N 904989).

Applied Biosystems

Archiving Current Spectra Component Files

About Spectra Component Files

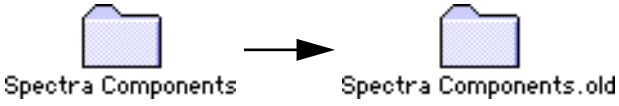
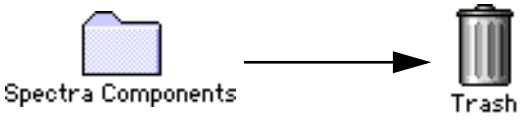
Spectra component files contain the background component and pure dye standards for your instrument. The ABI PRISM 7700 instrument uses these files during multicomponenting and data analysis as a basis to gauge the progress of the PCR reaction. Refer to page C-40 of the *ABI PRISM 7700 Sequence Detection Systems User's Manual* for more information on background component and pure dye files.

IMPORTANT You must archive or delete your existing Spectra Components folder before creating a new one.

Archiving or Deleting the Spectra Components Folder

To archive or delete the Spectra Components folder:

Step	Action
1	Double-click your hard disk icon.
2	Double-click the System Folder icon. 
3	Double-click the Preference folder icon. 
4	Double-click the SDS folder icon. 
5	Save or delete the existing Spectra Components file.

If...	Then...
you want to save the spectra components.	Click the Spectra Component icon text and enter a new name for the icon. 
you want to delete the spectra components.	Drag the Spectra Component folder icon into the trash. 

Generating a Background Component File

Overview

The SDS software refers to the background component file during multicomponenting to determine the contribution of background signal in each of the 96 wells. See page 3-7 of the *ABI PRISM 7700 Sequence Detection Systems User's Manual* for more information on the background component file.

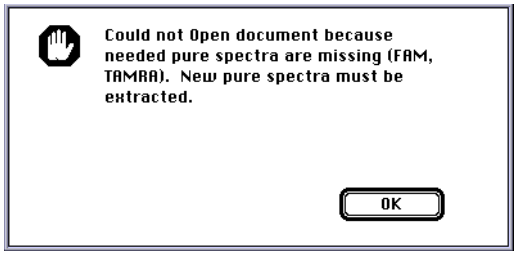
Hardware Setup

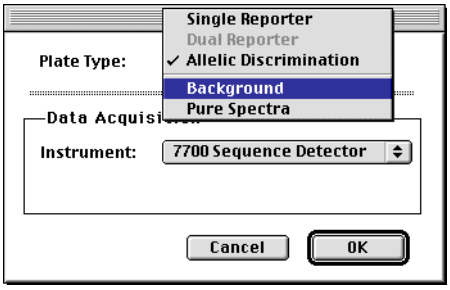
To prepare the instrument:

Step	Action
1	Pipet 50 µL of deionized water to each well in a MicroAmp® Optical 96-Well Reaction Plate (P/N 801-0560).
2	Cap the plate with MicroAmp® Optical Caps (P/N 801-0935).
3	Place the 96-well plate in the Sequence Detector sample block.
4	Slide the cover over the block and tighten the lid.

Software Setup

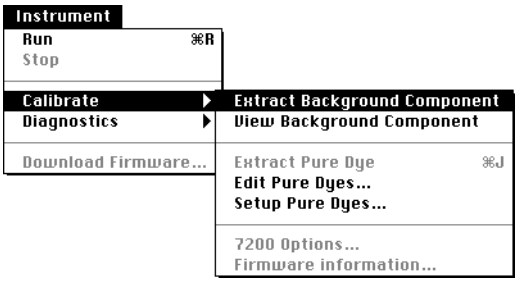
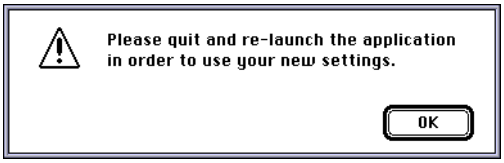
To prepare the software:

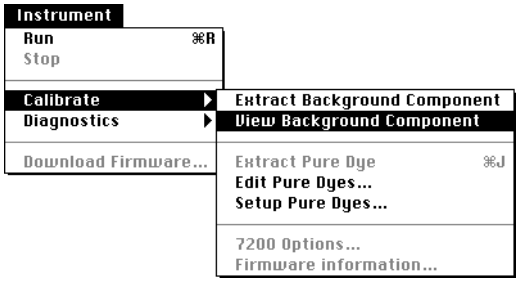
Step	Action
1	Launch the SDS software. A warning appears stating that the program is unable to access the pure spectra file. 
2	Click OK.
3	Select New Plate from the File menu.  The new plate dialog box appears.

4	Select Background from the Plate Type pop-up menu. 
5	Click OK. The background plate window appears.
6	Click Show Analysis. The software displays the plate document in the Analysis view.
7	Click Run. The ABI PRISM® Sequence Detector begins the run.

Creating a Background Component File

To save the background component file after the run is complete:

Step	Action
1	From the Calibrate submenu of the Instrument menu, select Extract Background Component.  The SDS software places the new background component file in a new Spectra Component folder. The SDS software displays an error message requesting you to quit and re-launch the application.
2	Click OK.  **Note** Do not quit and restart the SDS software at this time.

Step	Action
3	From the Calibrate submenu of the Instrument menu, select View Background Component to verify the uniformity of the background component for all 96 wells. 
4	You have successfully created a new background component file. If you wish to save the background plate document, do so now by selecting Save As.... from the File menu. See page C-17 of the *ABI PRISM 7700 Sequence Detection Systems User's Manual* for more information on saving the plate document. Otherwise, proceed on to the next step.
5	Select Quit from the File menu. The SDS software shuts down. The new background components will take effect when you restart the software.

Generating a Pure Spectra File

About the Pure Spectra File

The SDS software algorithm uses the dye standards contained within the pure spectra file during data analysis. The program uses the predetermined dye standards as a basis to evaluate relative signal strength from each well. Refer to the Pure Dye Spectra Calibration procedure on page 3-9 in the *ABI PRISM 7700 Sequence Detection Systems User's Manual* for more information on the pure spectra file.

IMPORTANT The following procedure requires you to create a Pure Spectra plate document. However, the SDS software will not allow you to open more than one plate document at a time. Therefore, close any open plate document before you proceed to the next section.

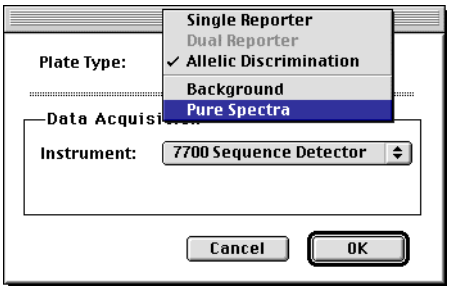
Setting up the Hardware

To prepare the instrument:

Step	Action
1	Remove a Sequence Detection Systems Spectral Calibration Kit (P/N 4305822) from the freezer and allow it to warm to room temperature. **IMPORTANT** Do not apply heat to the SDS Spectral Calibration Kit to thaw it.
2	Pipet 50 µL of each dye standard into four wells in a MicroAmp® Optical 96-Well Reaction Plate.
3	Cap the plate using MicroAmp® Optical Caps.
4	Place the 96-well reaction plate in the Sequence Detector sample block.
5	Slide the cover over the block and tighten the lid.

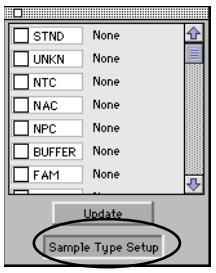
Setting up the Software

To prepare the software to receive new dyes:

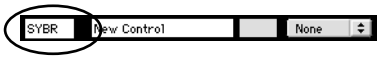
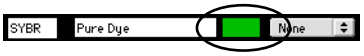
Step	Action
1	Double click the SDS software icon. A warning appears stating the program is unable to access the pure spectra file.
2	Click OK.
3	Select New Plate from the File menu. The new plate dialog box appears.
4	Select Pure Spectra from the Plate Type pop-up menu. 
5	Click OK. A new plate appears.

Adding New Dyes to the Dye Palette

To add new dyes to the dye palette:

Step	Action
1	From the Setup menu, select Sample Type Palette.  The Sample Type Palette dialog box appears.
2	Click Sample Type Setup.  The Sample Type Setup dialog box appears.

Step	Action
3	To add the new dye to the dye list in the Sample Type Setup dialog box:

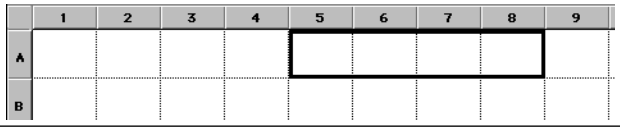
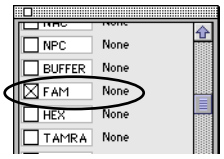
a.	Click Add.  A new row appears at the bottom of the dye list.
b.	Click the Acronym text field and enter a name for the new dye no more than five characters long (i.e. VIC or SYBR). 
c.	Click the Name text field and enter “Pure Dye.” 
d.	Click the Color field.  The Color pallet Dialog Box appears.
e.	Select a color for the new dye and click OK. See page C-34, “Editing sample attributes,” of the *ABI PRISM 7700 Sequence Detection Systems User’s Manual* for information on selecting a new dye color. The color field for the new dye fills with the new color.

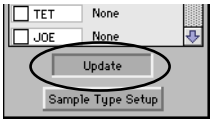
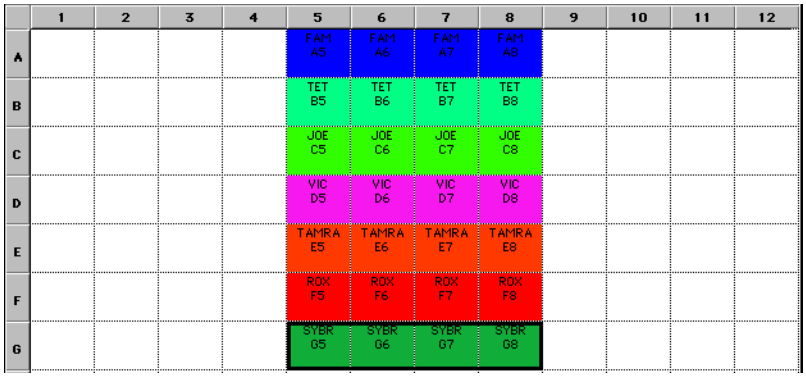
Repeat steps a-e to add other dyes to the list.

4	Click OK. The name of the new dye appears at the bottom of the dye list.

Assigning Dye Standards to the Plate Document

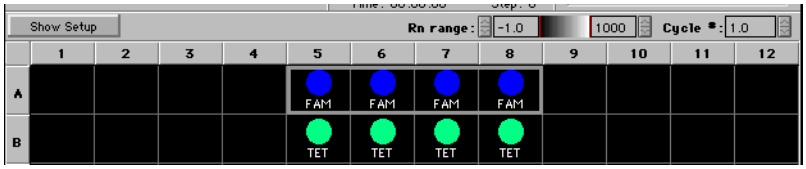
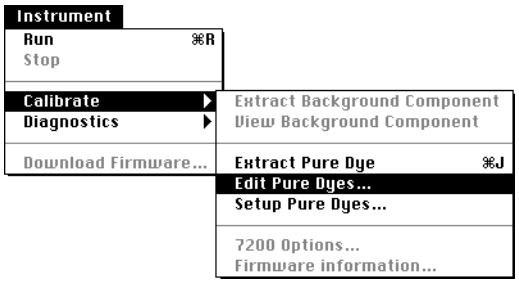
To assign dye standards to the plate document:

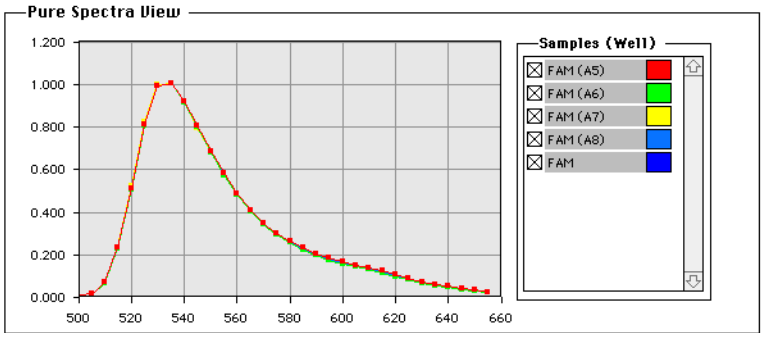
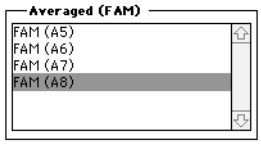
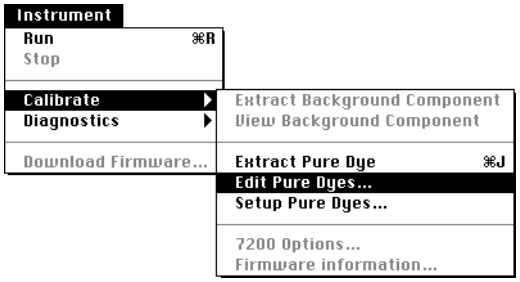
Step	Action
1	Select the four wells from the plate document that correspond to the wells on the MicroAmp® Optical 96-Well Reaction Plate containing the FAM standard. 
2	Click the FAM checkbox in the palette dialog box to label the wells. 

Step	Action
3	Click Update from the palette box.  SDS designates the selected row with the FAM dye.
4	Repeat steps 1–3 for each dye standard (TET, JOE, VIC, TAMRA, ROX, and SYBR Green) until your plate document resembles the figure below. 
5	Click Show Analysis from the Setup view.
6	Click Run.

Saving Data to a Pure Spectra File

When the instrument completes its run:

Step	Action
1	Select the four FAM wells from step 1 of the previous procedure. 
2	From the Calibrate submenu of the Instrument menu, select Extract Pure Dye. 

Step	Action
3	Inspect the pure spectra for uniformity. 
4	Contaminated wells may produce spectra that deviate from consensus peaks. Follow the procedure below to remove any outlying spectrum from the Averaged (sample) box. To remove an outlying spectrum: a. Click the outlying spectrum in the Averaged Box.  b. Click => to move the spectrum to the delete box.
5	Click OK.
6	Continue selecting wells and extracting dye spectra for each dye standard (TET, JOE, VIC, TAMRA, ROX, and SYBR Green). To view the extracted pure spectra components: a. Select the Calibrate submenu from the Instrument menu and select Edit Pure Dyes.  b. Click OK when you are finished.
7	Save your plate document with an identifying name, such as “Pure Dye.” See step 4 from “Creating a Background Component File” on page 5 for information on saving files to the hard disk.

Step	Action
8	Select Quit from the File menu. The SDS software closes. The new spectra components will take effect the next time you activate the software.

P/N 4306234B; Stock Number 777804-002