GE Healthcare

DYEnamic ET Terminator Matrix Standard for the ABI 3700

For use with ABI 3100, 3700 and 3730/3730XL

Product Booklet

Code: US84001

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Energy Transfer dyes and primers—US Patent numbers: 5,654,419, 5,688,648 and 5,707,804.

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2. Handling

2.1. Safety warnings and precautions

Warning: ***For research use only.*** Not recommended or intended for diagnosis of disease in humans or animals. Do not use internally or externally in humans or animals.

All chemicals should be considered as potentially hazardous. We therefore recommend that this product is handled only by those persons who have been trained in laboratory techniques and that it is used in accordance with the principles of good laboratory practice. Wear suitable protective clothing such as laboratory overalls, safety glasses and gloves. Care should be taken to avoid contact with skin or eyes. In the case of contact with skin or eyes wash immediately with water. See material safety data sheet(s) and/or safety statement(s) for specific advice.

2.2. Storage

Store at -15°C to -30°C.

3. Introduction

The DYEnamic ET Terminator Matrix Standard for the ABI 3700, ABI3730, ABI 3730XL (US84001) is formulated to create a spectral matrix with the ABI 3700 and ABI 3100 sequencing instruments. This spectral calibration must be performed prior to analysis of samples labelled with any dye set not previously used with the instrument. It is strongly recommended that the user read and thoroughly understand the section of the ABI 3100 user's manual (copyright, 2001) titled "Spectral Calibration" (pages 4-15 to 4-50). ABI 3730/3730x1 User's guide (copyright, 2002) titled "Spectral Calibration" (pages 4-11 to 4-28).ABI 3700 user's manual (copyright, 1999) titled "Performing a Spectral Calibration" (pages 6–21 to 6–56) before attempting to create a matrix for DYEnamic ET terminators.

4. Components of the kit

This reagent has been tested extensively and its concentration adjusted to meet rigorous standards. It is strongly recommended that the reagent be used exactly as described in this protocol.

The product consists of the following solution: **DYEnamic™ ET Terminator Matrix Standard for the ABI™ 3700, 3730, 3730XL (40 µl)**.

Each tube contains enough matrix standard to perform one spectral calibration on the ABI 3700 1 calibration on 3730, 1/2 calibration on 3730XL sequencing instrument, or eight calibrations on the ABI 3100. The product should be stored at -15°C to -30°C (not in a frost-free freezer). When not in a freezer, keep the reagent on ice prior to use.

5. Materials not supplied

Reagents

- **Water**—Only use deionized, distilled water with the DYEnamic ET Terminator Matrix Standard.

Equipment

- **Liquid-handling supplies**—Microcentrifuge tubes (200 µl), micropipettes, microcentrifuge, vortex mixer.
- **Instrument**—This product is designed for use with the ABI 3730, 3730XL, ABI 3700 and ABI 3100 sequencing instruments.

6. Protocols

6.1. Spectral calibration of the ABI 3700 for DYEnamic ET Terminators

6.1.1. Preparation of DYEnamic ET Terminator Matrix Standard

1. Briefly centrifuge the tube containing the DYEnamic ET Terminator Matrix Standard to bring the contents (40 µl) to the bottom of the tube.
2. Add 360 µl of distilled water to the tube that contains the matrix standard.
3. Mix the contents of the tube thoroughly by vortexing vigorously.
4. Briefly centrifuge the tube.
5. Transfer 200 µl of the matrix standard solution into each of two 200 µl microtubes as recommended by the instrument manufacturer.

6.1.2. Performing a spectral calibration

See pages 6–25 of the ABI 3700 user's manual for general guidelines for performing a default calibration. To create a spectral matrix for DYEnamic ET terminator chemistry, follow the protocol below.

1. Firmly place the two tubes containing the ET terminator matrix standard into the right bar in slot positions 9 and 10 as shown in Figure 1 and on pages 6–25 in the ABI 3700 user's manual.

Fig 1. Matrix standard loading position.

6.1.3. Performing a spectral calibration run for POP™-5

1. Open the ABI 3700 Data Collection software. Initiate a spectral calibration run by selecting the **Spectral Run** command under the **Run Setup** page. This command opens the **Calibration Module and Dye Set** dialog window from which the appropriate calibration module, dye set, and parameter files are selected.

Note: For a schematic of the procedure, see pages 6–26 in the ABI 3700 user's manual.

2. Using the pull-down menu under **Calibration Module**, choose the **SpecSQ1_1POP5DefaultModule** file for spectral calibration for POP-5.

Note: For a schematic of the procedure, see pages 6–26 in the ABI 3700 user's manual.

3. IMPORTANT! Under **Dye Set**, choose **F** from the pull-down menu.

3.4. IMPORTANT! Under **Parameter File**, choose **SeqStd (AnyDyeSet).par** from the pull-down menu.

5. Click **OK** to accept these chosen fields.

6. The spectral run will be displayed in the run queue. This action will simultaneously engage the **Start Run** button. Activate the run by clicking on **Start Run**.

7. After electrophoresis is completed (< 3 hours), a dialog window displays "Spectral Calibration Result" as shown on pages 6–29 in the ABI 3700 user's manual. This display indicates the number of capillaries (caps) that passed spectral calibration. For a schematic, see pages 6–29 in the user's manual. Accept the result by clicking **OK**. The software will automatically assign proper calibration values to failed caps from adjoining successful caps. The capillary status bar will indicate passed caps in black and questionable caps in yellow.

Note: For further information on the significance of color coding in the capillary status bar, see pages 6–29 and 5–68 in the ABI 3700 user's manual.

8. Upon completion of the spectral run and data processing, the quality of the spectral profile (the emission spectra for all four dyes) for each capillary must be examined. The *condition number* is a measure of the spectral overlap of the dyes. As the spectral overlap of a dye set decreases, so does the condition number. A condition number of 1.0 indicates no spectral overlap for a particular dye set. The expected condition number for ET dyes analyzed on the ABI 3700 is 7.3 ± 0.5. The *Q-value* is a measure of how well the spectral calibration fits the data it was created from. A Q-value of 1.0 represents a perfect fit. Any spectral calibration with a Q-value less than 0.92 will automatically fail the calibration and be replaced with spectral calibration data from an adjacent capillary. Those caps that have a questionable matrix should be replaced with data from a successful matrix.

Note: For further information and a schematic, see "Reviewing and Overriding the Spectral Calibration Profiles" on pages 6–34 of the ABI 3700 user's manual.

To review the calibration profile, open the Data Collection software. Go to the **Data Acquisition** menu and open the **Override Spectral Calibration** function. A dialog window titled **Select the dye set to display** will appear.

9. Select **F** from the pull-down menu **(IMPORTANT!)**. Click **OK**. This action opens the dialog window titled **Spectral Calibration Profile for F**. The fluorescence emission spectra for all four dyes in a particular capillary will be displayed as shown in Figure 2.

10. Examine the results for each cap and verify that the profile is similar to that shown in Figure 2.

Note: For additional schematic illustrations, see pages 6–35 of the ABI 3700 user's manual.

11. Although the software assigns values from successful caps to failed caps, in some instances the profile may not be similar to the one shown in the illustration. When this occurs, replace the values manually by using the values from a successful adjacent matrix. At the bottom left of the dialog box, under **Override matrix from another source**, click on the button **From capillary**, which allows the choice of values from any capillary. Choose any acceptable capillary.

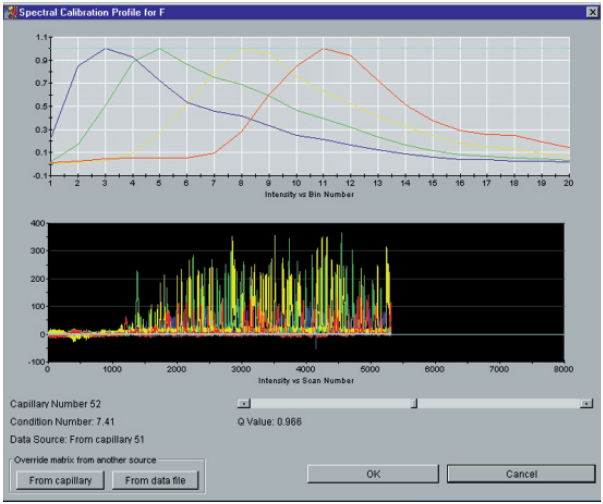

Fig 2. Spectral calibration profile for F using POP-5 with the ABI 3700.

6.1.4. Performing a spectral calibration run for POP-37

1. Open the ABI 3700 Data Collection software. Initiate a spectral calibration run by selecting the **Spectral Run** command under the **Run Setup** page. This command opens the **Calibration Module and Dye Set** dialog window from which the appropriate calibration module, dye set, and parameter files are selected.

Note: For a schematic of the procedure, see pages 6–26 in the ABI 3700 user's manual.

2. Use the pull-down menu under **Calibration Module** and choose the **SpecSQ1_2POP37DefaultModule** file for spectral calibration for POP-37.

Note: For a schematic of the procedure, see pages 6–26 in the ABI 3700 user's manual.

3. IMPORTANT! Under **Dye Set**, choose **F** from the pull-down menu.

4. IMPORTANT! Under **Parameter File**, choose **SeqStd(AnyDyeSet).par** from the pull-down menu.

5. Click **OK** to accept these chosen fields.

6. The spectral run will be displayed in the run queue. This action will simultaneously engage the **Start Run** button. Activate the run by clicking on **Start Run**.

7. After electrophoresis is complete (< 3 hours), a dialog window displays "Spectral Calibration Result" as shown on pages 6–29 in the ABI 3700 user's manual. This display indicates the number of capillaries (caps) that passed spectral calibration. For a schematic, see pages 6–29 in the user's manual. Accept the result by clicking **OK**. The software will automatically assign proper calibration values to failed caps from adjoining successful caps. The capillary status bar will indicate passed caps in black and questionable caps in yellow.

Note: For further information on the significance of color coding in the capillary status bar, see pages 6–29 and 5–68 in the ABI 3700 user's manual.

8. Upon completion of the spectral run and data processing, the quality of the spectral profile (the emission spectra for all four dyes) for each capillary must be examined. The *condition number* is a measure of the spectral overlap of the dyes. As the spectral overlap of a dye set decreases, so does the condition number. A condition number of 1.0 indicates no spectral overlap for a particular dye set. The expected condition number for ET dyes analyzed on the ABI 3700 is 7.3 ± 0.5. The *Q-value* is a measure of how well the spectral calibration fits the data it was created from. A Q-value of 1.0 represents a perfect fit. Any spectral calibration with a Q-value less than 0.92 will automatically fail the calibration and be replaced with spectral calibration data from an adjacent capillary. Those caps that have a questionable matrix should be replaced with data from a successful matrix.

Note: For further information and a schematic, see "Reviewing and Overriding the Spectral Calibration Profiles" on pages 6–34 of the ABI 3700 user's manual.

9. To review the calibration profile, open the Data Collection software. Go to the **Data Acquisition** menu and open the **Override Spectral Calibration** function. A dialog window titled **Select the dye set to display** will appear.

10. Select **F** from the pull-down menu **(IMPORTANT!)**. Click **OK**. This action opens the dialog window titled **Spectral Calibration Profile for F**. The fluorescence emission spectra for all four dyes in a particular capillary will be displayed as shown in Figure 2.

11. Examine the results for each cap and verify that the profile is similar to that shown in Figure 2.

Note: For additional schematic illustrations, see pages 6–35 of the ABI 3700 user's manual.

12. Although the software assigns values from successful caps to failed caps, in some instances the profile may not be similar to the one shown in the illustration. When this occurs, replace the values manually by using the values from a successful adjacent matrix. At the bottom left of the dialog box, under **Override matrix from another source**, click on the button **From capillary**, which allows the choice of values from any capillary. Choose any acceptable capillary.

6.1.5. Performing a spectral calibration run for POP-6

1. Open the ABI 3700 Data Collection software, and then open the **Module Editor** listed under the **Instrument Utilities** menu. Select the **Others** tab under the module menu within the **Module Editor**. Load the **SpectSQ1_1POP6DefaultModule** parameters into the **Module Editor** by clicking on its listing within the **Others** tab.
2. Scroll down to the **Run Time** parameter (number 15) listed in the **Module Parameters** window. Edit the **Run Time** by clicking on the default value of 2 700 seconds, and replace that with 4 000 seconds.
3. Click the **Save As** button and enter **SpecSQ1_1POP6Extended** as the new module file name. Click **OK** to save the new parameters. Click **Done** to exit the **Module Editor**.
4. In the Data Collection software, initiate a spectral calibration run by selecting the **Spectral Run** command within the **Run Setup** page. This opens the **Calibration Module and Dye Set** dialog window. From here, the appropriate type of calibration module, dye set, and parameter files can be selected for performing a spectral calibration.

Note: For a schematic of the procedure, see pages 6–26 in the ABI 3700 user's manual.

5. Using the pull-down menu under **Calibration Module** choose the **SpecSQ1_1POP6Extended** file for spectral calibration for POP-6.

Note: For a schematic of the procedure, see pages 6–26 in the ABI 3700 user's manual.

6. **IMPORTANT!** Under **Dye Set**, choose **F** from the pull-down menu.
7. **IMPORTANT!** Under **Parameter File**, choose **SeqStd (AnyDyeSet).par** from the pull-down menu.
8. Click **OK** to accept these chosen fields.
9. The spectral run will be displayed in the run queue. This action will simultaneously engage the **Start Run** button. Activate the run by clicking on **Start Run**.
10. After the run is completed (< 3 hours), a dialog window will display "Spectral Calibration Result" as shown on pages 6–29 in the ABI 3700 user's manual. This display indicates the number of caps that passed spectral calibration. For a schematic, see pages 6–29 in the user's manual. Accept the result by clicking **OK**. The software automatically assigns proper calibration values to failed caps from the adjoining successful caps. The capillary status bar indicates passed caps in black and questionable caps in yellow.

Note: For further information on the significance of color coding in the capillary status bar, see pages 6–29 and 5–68 in the ABI 3700 user's manual.

11. Upon completion of the spectral run and data processing, the quality of the spectral profile (the emission spectra for all four dyes) for each capillary must be examined. The *condition number* is a measure of the spectral overlap of the dyes. As the spectral overlap of the dyes decrease, so does the condition number. A condition number of 1.0 indicates no spectral overlap for a

particular dye set. The expected condition number for ET dyes on the ABI 3700 is 7.3 ± 0.5. The *Q-value* is a measure of how well the spectral calibration fits the data it was created from. A Q-value of 1.0 represents a perfect fit. Any spectral calibration with a Q-value less than 0.92 will automatically fail the calibration and be replaced with spectral calibration data from an adjacent capillary. Those caps that have a questionable matrix should be replaced with data from a successful matrix. For further information and a schematic, see "Reviewing and Overriding the Spectral Calibration Profiles" on pages 6-34 of the ABI 3700 user's manual.

To review the calibration profile, open the Data Collection software. Go to the **Data Acquisition** menu and open the **Override Spectral Calibration** function. A dialog window titled **Select the dye set to display** will appear.

12. Select **F** from the pull-down menu **(IMPORTANT!)**. Click **OK**. This action opens a dialog window titled **Spectral Calibration Profile for F**. The fluorescence emission spectra for all four dyes in a particular capillary is displayed as shown in Figure 2.

13. Examine the results for each cap and verify that the profile is similar to that shown in Figure 4.

Note: For additional schematic illustrations, see pages 6–35 of the ABI 3700 user's manual.

14. Although the software assigns values from successful caps to failed caps, in some instances the profile may not be similar to the one shown in the illustration. When this occurs, replace the values manually by using the values from a successful adjacent matrix. At the bottom left of the dialog box, under **Override matrix from another source**, click on the button **From capillary** which allows the selection of values from any capillary. Choose any acceptable capillary.

6.2. Spectral calibration of the ABI 3100 for DYEnamic ET Terminators

The DYEnamic ET Terminator Matrix Standard is formulated for creating a spectral matrix with the ABI 3100 sequencing instrument. This spectral calibration must be performed prior to analysis of samples labelled with any dye set not previously used with the instrument. It is strongly recommended that the user read and thoroughly understand the section of the ABI 3100 user's manual (copyright, 2001) titled "Performing a Spectral Calibration" (pages 4–15 to 4–49) before attempting to create a matrix for DYEnamic ET terminators.

6.2.1. Preparation of DYEnamic ET Terminator Matrix Standard

1. Briefly centrifuge the tube containing the DYEnamic ET Terminator Matrix Standard to bring the contents (40 µl) to the bottom of the tube.
2. Transfer 5 µl of the DYEnamic ET Terminator Matrix Standard into a tube containing 195 µl of distilled water.
3. Mix the contents of the tube thoroughly by vigorous vortexing.
4. Briefly centrifuge the tube and dispense 10 µl of the DYEnamic ET Terminator Matrix Standard into a 96-well plate as shown below (Figure 3). It is crucial to centrifuge the plate to position the samples at the bottom of each well.
5. Assemble the plate for loading onto the ABI 3100 (see pages 3–9 of the ABI 3100 user's manual).

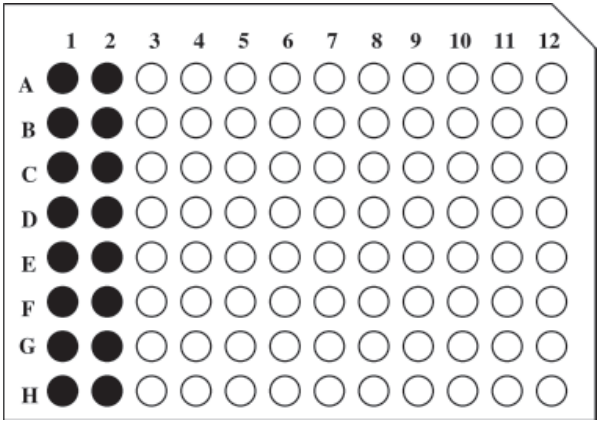

Fig 3. Matrix standard loading positions.

6.2.2. Performing a spectral calibration

For general guidelines on performing a default calibration, see pages 4–17 of the ABI 3100 user's manual. To create a spectral matrix for DYEnamic ET terminator chemistry, follow the protocol below.

1. Place the plate on the autosampler

Note: For a schematic of the procedure, see pages 4–24 in the ABI 3100 user's manual.

2. Open the 3100 Data Collection software and initiate a spectral calibration run by selecting **New** within the **Plate View** page. This opens up the **Plate Editor** dialog box. Name the plate, select **Spectral Calibration**, and select **96-Well plate** type. Click **Finish**. This opens the **Plate Editor** spreadsheet.

3. Within the **Plate Editor** spreadsheet, complete the following for the 16 samples in the sample plate:

3.1. Name the samples.

3.2. IMPORTANT! Select **Dye Set F**.

3.3. Select the run module appropriate for your capillary array size:
36 cm: **Spect36_POP6DefaultModule**
50 cm: **Spect50_POP6DefaultModule**
80 cm: **Spect80_POP4DefaultModule**

3.4. IMPORTANT! Select the spectral calibration parameters, **SeqStd(AnyDyeSet).par**.

3.5. Click **OK**.

4. The newly created plate record then appears in the **Pending Plate Records** table of the **Plate Setup** page. In the **Plate Setup** page, select the newly created plate from the **Pending Plate Records** table.

5. Click on the graphic that corresponds to the plate on the autosampler. The plate then moves from the **Pending Plate Records** table to the **Linked Plate Records** table. For a pictorial representation see pages 3–39 in the ABI 3100 user's manual.

6. Click the **Run Instrument** button on the toolbar to begin the run.

7. After completing the run (40–65 minutes), a dialog window displays "Spectral Calibration Result" as shown on pages 4–23 in ABI 3100 user's manual, indicating the number of caps that passed spectral calibration. Accept the result by clicking **OK**. The software then automatically assigns proper calibration values to failed caps from the adjoining successful caps.

8. Upon completion of the spectral run and data processing, the quality of the spectral profile (the emission spectra for all four dyes) for each capillary must be examined. Any capillary that generated a questionable matrix should be carefully examined and replaced with data from a successful matrix. Instructions for

matrix replacement are provided in the ABI 3100 user's manual on pages 4–27, "Overriding a Spectral Calibration Profile".

9. The *condition number* is a measure of the spectral overlap of the dyes. As the spectral overlap of a dye decreases, so does the condition number. A condition number of 1.0 indicates no spectral overlap for a particular dye set. The expected condition number for ET dyes on the ABI 3100 is 7.3 ± 0.5. The *Q-value* is a measure of how well the spectral calibration fits the data it was created from. A *Q-value* of 1.0 represents a perfect fit. Any spectral calibration with a *Q-value* less than 0.92 will automatically fail the calibration and be replaced with spectral calibration data from an adjacent capillary.

Review the calibration profile using the following steps. Open the Data Collection software; select **Override Spectral Calibration** from the **File Menu**. A dialog window titled **Select the dye set to display** appears. Select **F** from the pull down menu **(IMPORTANT!)**. Click **OK**. This action opens the dialog window **Spectral Calibration Profile for F**. The fluorescence emission spectra for all four dyes in a particular capillary is displayed as shown in Figure 4. Examine the results for each cap and verify that it is similar to the one shown in this example and as illustrated on page 4-29 of the ABI 3100 user's manual. Although, the software assigns values from successful caps to failed caps in some instances, the profile may not be similar to the one shown in the illustration. When this happens, manually replace the values with the data from a successful adjacent matrix. At the bottom left of the dialog box, under **Override matrix from another source** click on the button **From capillary**. These commands allow you to select a value from any acceptable capillary.

10. Examine the results for each cap and verify that the profile is similar to that shown in Figure 4.

Note: For additional schematic illustrations, see pages 4–29 of the ABI 3100 user's manual.

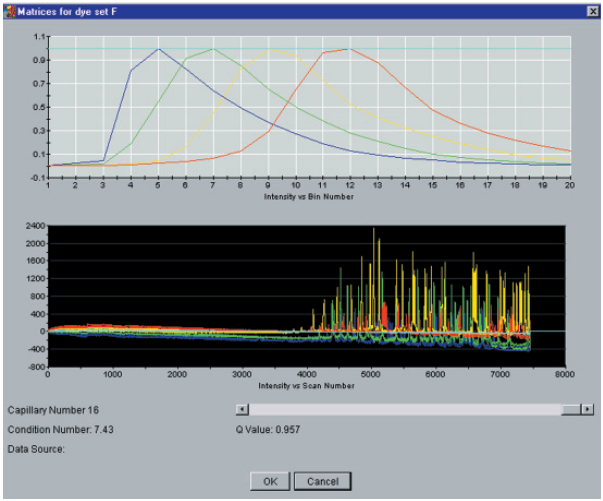

Fig 4. Spectral calibration profile for F using POP-6 with the ABI 3100.

6.3. Spectral Calibration of the ABI 3730/3730XL for DYEnamic ET Terminators

DYEnamic ET Terminator Matrix Standard for the ABI 3700 (US84001) is formulated for performing a spectral calibration and creating a spectral matrix for the ABI 3730 sequencing instrument.

6.3.1. Preparation of DYEnamic ET Terminator Matrix Standard

1. Briefly centrifuge the tube containing the DYEnamic ET Terminator Matrix Standard to bring the contents (40 µl) to the bottom of the tube (**2 tubes for 3730XL**).
2. Add 460 µl of distilled water to the tube containing the matrix standard. Mix the contents of the tube thoroughly by vigorous vortexing. Briefly centrifuge the tube.

Note: Combine 2 tubes for 3730XL.

3. Dispense 10 µl of the DYEnamic ET Terminator matrix standard into every other column of a 96 well plate (**all wells for 3730XL**): It is essential to centrifuge the plate to position the samples at the bottom of each well.

6.3.2. Creation of Spectral Protocol

1. Expand the view in the tree pane.

1.1 Click the **+ box** next to the GA intruments icon.

1.2 Click the **+ box** next to the ga3730 icon.

2. Click the **Protocol Manager** icon.

3. In the Instrument Protocols section, click **New**. The Protocol Editor dialog box opens.

4. Create a spectral protocol.

4.1. Type **ET_Spectral** or a similar name in the Name Field.

4.2. Select **SPECTRAL** from the Type drop-down list.

4.3. Select the appropriate run module from the drop-down list.

(i) **Spect36_SeqStd_POP7_ET** for 36-cm array with POP-7

Note: Prior to this step the Spect36_SeqStd_POP7 module must be edited to include a run time of 2000 seconds. The module can be edited in the Protocol Manager by creating a new module based on the Spect36_SeqStd_POP7 module.

(ii) **Spect50_SeqStd_POP7** for 50-cm array with POP-7

5. Select **E-BigDyeV1** or **Z-BigDyeV3** from the **Dye Set** drop-down list.

Note: Select a Dye Set not in use. An ET spectral calibration will overwrite any previous spectral calibrations per formed on the Dye Set selected.

6. Select **SeqStd {Any4DyeSet). par** from the **Params** drop-down list.

Note: If the SeqStd{Any4DyeSet}. par is not available an existing parameter file must be edited to be compatible with the DYEnamic ET terminators. See the Editing Spectral Calibration Section at the end of this protocol.

7. Click **OK**.

The module is saved and displayed in the Instrument Protocols section of the Protocol Manager view.

6.3.3. Creating a Plate Record

1. Expand the view in the tree pane.

1.1. Click the **+ box** next to the GA Instruments icon.

1.2. Click the **+ box** next to the ga3730 icon.

2. Click the **Plate Manager** icon.

The Plate Manager view opens.

3. Click **New**.

The New Plate Dialog opens.

4. Complete the plate information

4.1. Type a name for the plate ID in the ID (barcode) field.

4.2. Type a name for the plate in the Name field.

4.3. Select **Spectral Calibration** from the Application drop-down list.

4.4. Select **96-Well** from the Plate Type drop-down list.

4.5. Select Heat Sealing or Septa from the Plate Sealing drop-down list.

4.6. Type a name for the owner and operator in the appropriate fields.

5. Click **OK**.

A blank plate record opens.

6. Complete the plate record.

6.1. In the Sample Name column, type a name

6.2. In the Instrument Protocol 1 column, select the protocol created in the "Creation of Spectral Protocol" section.

6.3. Select the Sample Name and Instrument Protocol 1 columns, and fill down.

7. Click **OK**.

6.3.4. Adding a Plate to the Run Scheduler

1. Click the **Run Scheduler** icon.

The Run Scheduler view opens.

2. In the Input Stack section, click Search.

A search dialog box opens.

3. Search for the spectral calibration record.

4. Add the plate record.

4.1. Select the plate you want to use in the Name column.

4.2. Click **Add**.

4.3. Click **Done**.

The plate is added to the Run Scheduler view.

5. Click on the **Run Instrument** button.

6.3.5. Evaluating Spectral Calibration Results

To view the pass/fail status of each capillary:

1. Locate the log file at the following location:
E:\AppliedBiosystems\UDC\DataCollection\Data\ga 3730\instrument name\SpectralCalMcIFiles\E-BigDyeV1

2. Open the file in Notepad.

3. View the results.

The condition number (c) is a measure of the spectral overlap of the dyes. As the spectral overlap on the dyes decreases, so does the condition number. A condition number of 1.0 would indicate no spectral overlap for a particular dye set. The expected condition number for ET dyes on the ABI 3730 is 6.7 ± 0.5. The Q-value is a measure of how well the spectral calibration fits that data it was created from. A Q-value (q) of 1.0 represents a perfect fit. Any spectral calibration with a Q-value less than 0.92 will automatically fail the calibration and be replaced with spectral calibration data from an adjacent capillary.

6.3.6. Editing Spectral calibration Files

If the **SeqStd{Any4DyeSet}.par** is not available when setting up the ET terminator spectral calibration protocol on the instrument, an existing parameter file must be edited to be compatible with the DYEnamic ET terminators.

1. Locate the Spectral Calibration ParamFiles folder in the following Path:

E:/Appliedbiosystems/UDC/ga3730/CalibrationData/

SupportFiles/SpectralCalibration/ParamFiles

Note: Search for files with a ".par" extension if the ParamFiles folder cannot be located.

2. Edit the **SeqStd{E}.par** if you are performing the ET spectral calibration on Dye Set E, and edit the **SeqStd{Z}.par** if the calibration to be performed on Dye Set Z.

3. Rename the existing SeqStd{E or Z}.par file OrgSeqStd{E or Z}.par.

4. Make a copy of the file to be edited by copying and pasting the file into the ParamFiles folder.

5. Edit the copied parameter file to include the following:
minQ = 0.92

ConditionBounds = [1.0, 10.0]
maxScansAnalyzed = 5000

6. Save the file.
7. Rename the edited file, **SeqStd{E}.par** or **SeqStd{Z}.par** as appropriate.

Note: The instrument will only recognize the files if named exactly as described above.

8. Proceed with the ET spectral calibration protocol.
9. If at a later date a calibration must be performed with a sequencing chemistry other than the DYEnamic ET terminators, the edited parameter file must be renamed and the **OrgSeqStd{E or Z}.par** file must be renamed to its original file name.

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