

VIII. Data Import into the Plexor® Analysis Software


The Plexor® Analysis Software (Cat.# A4071) is available for download at: www.promega.com/plexorresources/. It is also available free-of-charge on CD-ROM by request. Software installation instructions are given in Section XI.B.

When exporting data for use with the Plexor® Analysis Software, be sure to assign descriptive names to the files, so that related amplification curve and melt curve files (e.g., files generated using the same data) can be easily identified during data import.

Note: Closing other programs before launching the Plexor® Analysis Software can increase operating speed.

1. To launch the Plexor® Analysis Software, go to the “Start” menu and select “Programs”, then “Plexor”; select “Analysis Desktop”.

Note: A shortcut can be placed on the desktop by right-clicking on “Analysis Desktop”, selecting “Copy”, then right-clicking on the Windows® desktop and selecting “Paste Shortcut”.

2. In the “File” menu, select “Import New Run” or select the icon: 
3. **Optional:** Enter an assay name in the “Assay Setup” screen, Step 1 (Figure 6). This screen is used to enter general information about the type of instrument and dyes used.

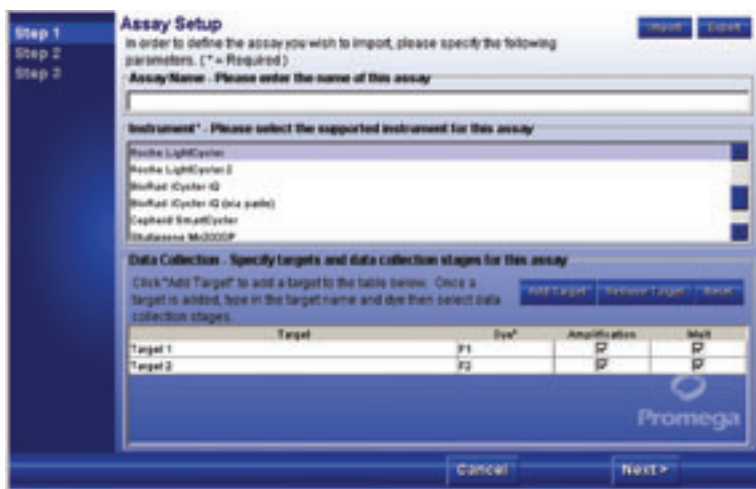
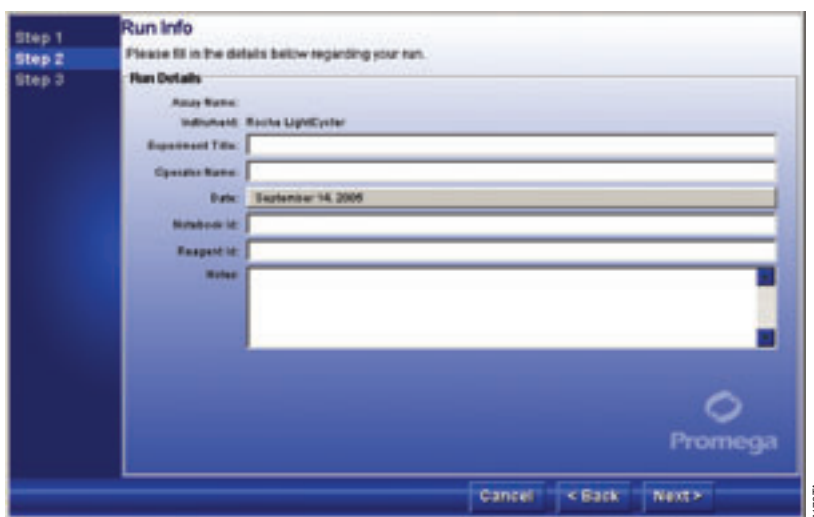


Figure 6. The Assay Setup screen.

4. Select “Roche LightCycler” as the instrument.
5. Select “Add Target” for each fluorescent dye used in your assay. For each dye, assign a target name, enter the dye name and indicate that there is amplification data and dissociation (melt) data to be analyzed for that dye. The name of the dye must be the same as that in the data file (i.e., “F1” or “F2”).
Note: For frequently run assays, a template with the target information and dyes can be saved (Section XI.C).
6. Select “Next”.
7. Enter information specific to your experiment in the “Run Info” screen, Step 2 (Figure 7). Details (date, notes, title, name of the person performing the experiment, etc.) can also be entered in the provided windows.



5172 TA

Figure 7. The Run Info screen.

8. Select “Next”.

VIII. Data Import into the Plexor® Analysis Software (continued)

9. Use the “File Import” screen, Step 3 (Figure 8), to specify the data files exported from the instrument in Section VII. Use “Browse” to locate the appropriate exported amplification and dissociation data files.

! When analyzing data with the Plexor® Analysis Software, be sure to choose the amplification curve file and melt curve file generated using the same data. The file names assigned when exporting data must be descriptive so that the appropriate files can be easily identified and imported into the Plexor® Analysis Software.

Note: “Advanced Options” can be used to create templates for routine capillary setups and analysis conditions. See Section XI.C for details concerning these advanced options and an explanation of the default analysis settings.

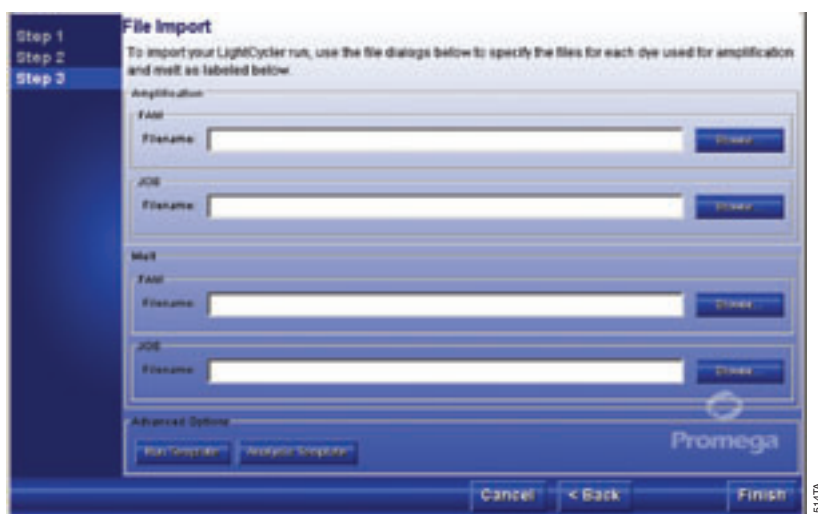


Figure 8. The File Import screen.

10. Select “Finish” to complete the data import and open “Analysis Desktop”.

Note: If the names of the dyes you have entered do not match those listed in the data files, an error message will appear (Figure 9). If this message appears, return to the “Assay Setup” screen, Step 1 (Figure 6). Click on the “Dye” field under the “Data Collection” heading and enter the dye names exactly as shown in the error message after “Found:”. Any dye name entered must exist in the data file. You do not need to enter all dyes. Only those entered will be analyzed. Repeat Steps 5–10 of this protocol.

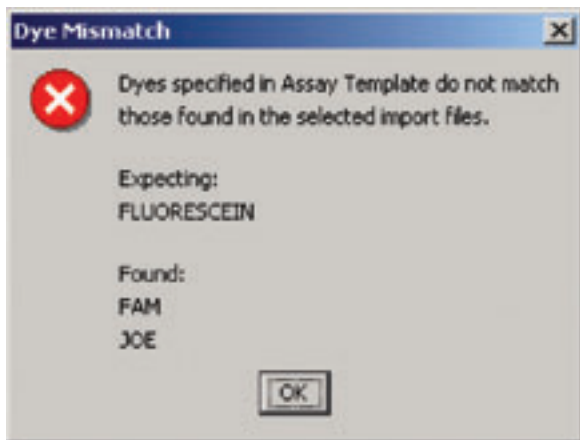


Figure 9. The Dye Mismatch error message.

IX. Data Analysis with the Plexor® Analysis Software

After data import is complete (Section VIII), the “PCR Curves” tab of the “Analysis Desktop” is displayed (Figure 10).

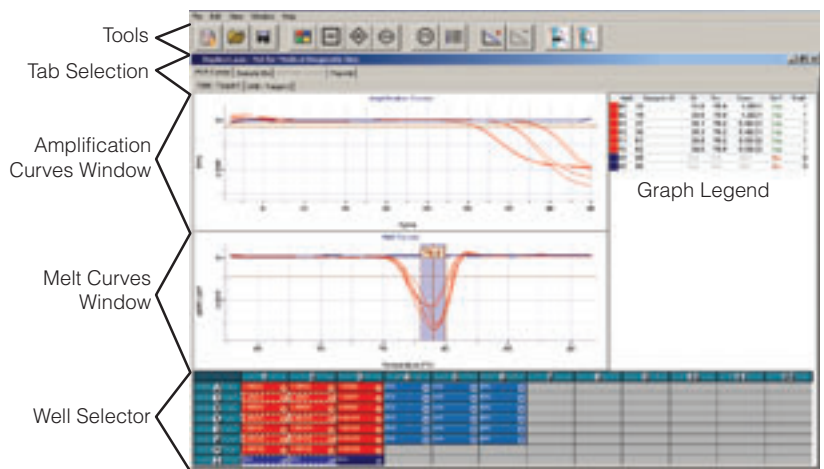


Figure 10. The PCR Curves tab of the analysis desktop. The amplification curves window, melt curves window and well selector are indicated.

IX.A. Sample Definition

1. Use the Well Selector, which is shown in Figure 10, to select and define each capillary or group of capillaries. Choose one of the icons shown in Figure 11 to define the samples. See Notes 1-5.