

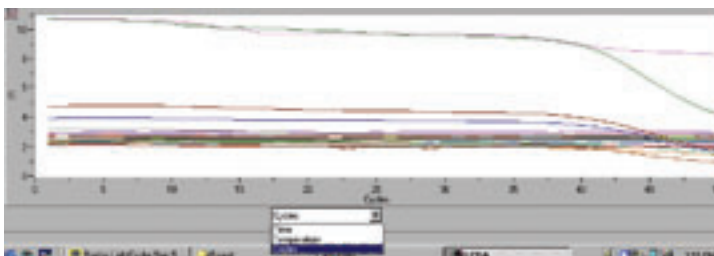
## VII. Data Export from the LightCycler® Data Analysis Software

Before the data can be analyzed using the Plexor® Analysis Software, the data must be exported from the LightCycler® software. Two \*.txt files must be exported for each color channel used: one with the amplification data and one with the melt/dissociation data. Be sure to use a descriptive name when naming these files so that it is clear which file contains the amplification data and which file contains the dissociation data, and that the files are related.

1. The LightCycler® software version 3.5 will automatically analyze after a run and open a data file in the LightCycler® software Main Window. (The Main Window can also be opened from Front by clicking on the Data Analysis button. Go to **File** → **Open** and **Select Data File to Analyze**. Click “Open” to analyze file.)
2. To export the amplification data from the Main Window, select “Select a Program” and select the “PCR” segment from the programs listed.



3. If this is a multiplex experiment, and a color compensation file was not chosen during the setup, a color compensation file must be applied before exporting. To apply a Color Compensation file (see Section III) to a multiplex experiment during post-run analysis:
  - a. Select "Color Compensation" from the top toolbar.
  - b. Select "Load Calibration Data" from the color compensation menu.
  - c. Choose the appropriate file from the list that appears.
4. After selecting the PCR program, make sure that the bottom graph in the Main Window displays “Fluorescence vs. Cycles” (chosen from the drop-down box).



5. Select “F1” from the “Fluorescence” drop-down box. For multiplex data (more than one dye), select “Color Compensation” if it is not already active (button will be highlighted when active).
6. Select “Quantification”. This will open the LightCycler® software Quantification screen.

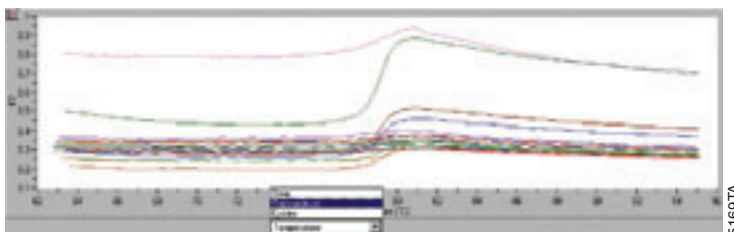
- Go to the **Quantification** menu → **Export** → **Baseline Adjustment**. Save the data to a \*.txt file.

For duplex reactions you must export both the F1 and the F2 channels separately by changing “Fluorescence” in LightCycler® software Main Window to F2 and selecting “Quantification” again to open a new LightCycler® software Quantification screen with data for the F2 file you want to export.

- Select the “Window” menu to return to the front analysis window.
- To export the melt data, from the Main Window, select “Select a Program” and select “MELT”. From the pull-down menu, select the “MELT” segment from the programs listed.



- After selecting the MELT program, make sure that the bottom graph in the Main Window displays “Fluorescence vs. Temperature”.



- Select “F1” from the “Fluorescence” drop-down box. Select “Melting Curve”. You will see the LightCycler® software “Melting Curve” screen.
- Go to the **Melting Curves** menu → **Export** → **Melting Curve Data**. Save data to a \*.txt file.

For duplex reactions you must export both the F1 and the F2 channels separately by changing “Fluorescence” in LightCycler® software Main Window to F2 and then selecting “Melting Curve” again to open a new LightCycler® software Melting Curve screen with data for the F2 file you want to export. To return to the main analysis window after each export, select the “Window” menu.

- If analysis will be done on a separate computer, save the files onto a removable media or an accessible network location. These exported files are now ready for use with the Plexor® Analysis Software.

**Note:** The Plexor® Analysis Software will use the greatest change in signal throughout the imported data to determine a signal threshold. This may affect sensitivity of assays with lower signal if multiple types of assays (in the same color) are being run simultaneously. This can be avoided by exporting the samples for each assay separately.