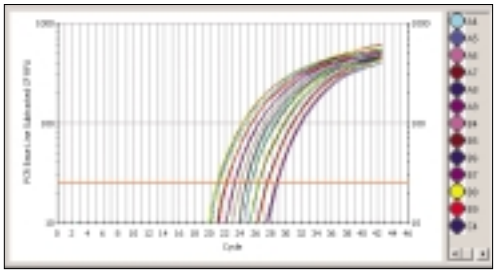
iCycler iQ™ Quick Guide
Data Analysis
PCR Quantification

The **PCR Quantification** screen is displayed during the PCR run and presents data as they are being collected in real time. This screen is also enabled post-run when you open your data file.

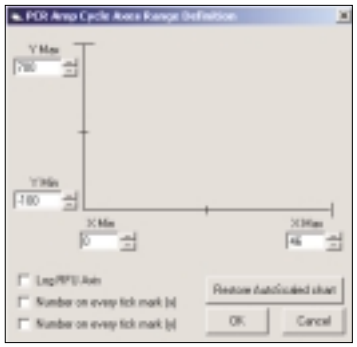
1. Select the **Library** module and click on the **View Post-Run Data** tab.
2. Select a data file and click on the **Analyze Data** button under **Data Analysis Operations**.
3. The data file will open in the **PCR Quantification** tab of the **Data Analysis** module.
4. **PCR Base Line Subtracted** or **PCR Base Line Subtracted Curve Fit** must be selected in order to calculate threshold value.
5. Threshold cycle calculation is automatically calculated or can be user defined
   - **Auto Calculated** — The software automatically defines baseline cycles and a threshold position
   - **User Defined** — When selected you may define the baseline cycles and threshold position. You may also click and drag the threshold bar directly on the amplification plot. Click **Recalculate Threshold Cycles** to update threshold cycle values



6. Threshold cycle (C_T) values and sample name identifiers are displayed in the spreadsheet to the right of the amplification plot.
7. Check the **Select Wells** box to select or deselect wells for analysis.
8. In the **Select a Fluorophore** box, click on fluorophore crayons to view other dye layers.

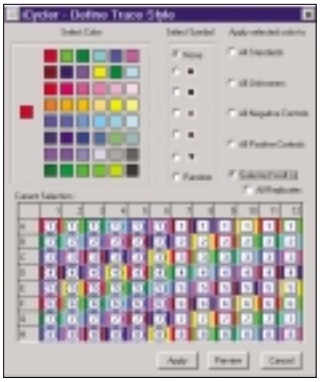
Graph Options

1. Right-click on the amplification plot to view the context menu.
2. Select **Adjust Graph** to make changes to the graph, e.g., changing to **Log RFU Axis** view or changing the min and max values for the x and y axes.



BIO-RAD

3. Select **Define Trace Style** to customize the colors and symbols of the sample well traces
   - Choose the type of trace to be modified (e.g., all standards) or click selected wells to modify on a well-by-well basis
   - If you choose to apply the selected color to selected well(s), the plate layout with colors is displayed so that you can click on the wells to change the well color
   - Click **Preview** to see changes to the traces in the graph
   - Click **Apply** to apply changes to the traces in the graph



4. You may choose options to copy and print data and graphs from this menu.

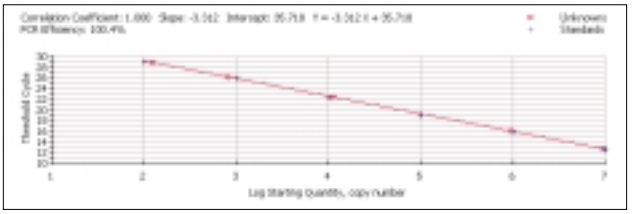
Reports in PCR Quantification

1. Click **Reports** to obtain customized reports of the PCR quantification data.
2. You may choose to sort the data by threshold cycle, well, sample identifier, or gradient setpoint.
3. Save or print reports containing information about the run.
4. Close **Report Viewer** to continue data analysis.

Standard Curve

When there are standards present in the amplification run, the **PCR Standard Curve** tab is available.

1. Click on the **PCR Standard Curve** tab of the **Data Analysis** module to view the standard curve plot containing the correlation coefficient, the slope of the line, and the PCR efficiency.
2. A data spreadsheet displays all information for all samples, including starting quantities, threshold cycles, and calculated concentrations for unknowns.



3. Click **Reports** on this page to obtain customized reports of the standard curve data or the PCR quantification plot.

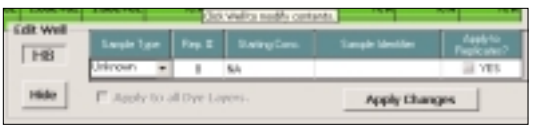
Save Data

1. Click on the **View/Save Data** tab of the **Data Analysis** module for a whole plate view of results based on **Threshold Cycle**, **Calculated Concentration**, or **Standard Quantities**.
2. Click **Reports** to obtain summarized results in this plate view.
3. Click on **Save OPD File** to view **Data Analysis Settings**, **Modified Well Data**, and **Melt Peak Data**. Notes may also be written about the data OPD file. Check the **Autosave to OPD** box to save the settings automatically.

Post-Run Editing

You may modify some attributes of a well post-run in the **View/Save Data** tab of the **Data Analysis** module.

1. Click on a well to to bring up information on that well and modify its contents. You may change the sample type, replicate number, starting concentration, and sample identifier.



2. Click **Apply Changes** to complete changes. Click **Restore Original Definitions** to undo changes. Information on the modified wells is recorded in the reports.