

iCycler iQ™ Quick Guide

Data Analysis

Allelic Discrimination

The **Allelic Discrimination** feature of the **Data Analysis** module is available post-run and offers flexibility for analyzing allelic discrimination data. You may display samples on a scatter plot based on threshold cycle or RFU (relative fluorescence units) values at any PCR cycle. You may choose to have the software automatically make allele calls, or you may manually make the allele calls.

1. Click on the **View Post-Run Data** tab in the **Library** module.
2. Select a data file and click on the **Analyze Data** button under **Data Analysis Operations**. The file will open in the **PCR Quantification** tab of the **Data Analysis** module.
3. Choose **PCR Base Line Subtracted** or **PCR Base Line Subtracted Curve Fit** in **Select analysis mode**.
4. If desired, make changes to the **Threshold Cycle Calculation** parameters to define the baseline cycles and the 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. (See “Data Analysis: PCR Quantification” Quick Guide for more details.)
5. You may select or deselect wells for analysis before proceeding to allelic discrimination options, by checking the **Select Wells** box.
6. In the **Select a Reporter** box, click on a fluorophore crayon that represents one of the alleles.
7. Click **Save for X-axis Allelic Analysis**.

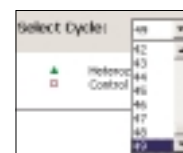


8. Click on another fluorophore pen that represents the other allele. Click **Save for Y-axis Allelic Analysis**.
9. Click on the **Allelic Discrimination** tab of the **Data Analysis** module, which is enabled once both axes have been assigned.
10. You can choose between two modes to display the allelic discrimination data:

- **Threshold Cycle** displays the distribution of samples on the scatter plot based on the threshold cycle. NOTE: Samples that do not cross threshold will be assigned the threshold cycle value of the last cycle run in the experiment



- **RFU** displays the distribution of samples on the scatter plot based on the RFU generated by each sample at the last PCR cycle number. Click on the drop-down menu by **Select Cycle** to generate the scatter plot based on RFU from a different cycle of the PCR experiment

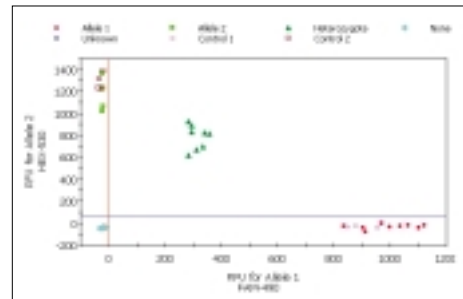


11. Click **Automatic Call** or **Manual Call**.

Automatic Call
 Manual Call

12. In **Automatic Call**, threshold bars are positioned automatically in one of two ways:

- Based on distribution of the control wells, when at least three wells have been assigned to **Control 1** and three to **Control 2**
- At 90% of the threshold cycle range or 10% of the RFU range on each axis if no controls are named



13. You may click and drag threshold bars directly on the plot to adjust **Automatic Call** assignment. Click **Recalculate** to reassign the wells to the new allele call in the scatter plot and in the data spreadsheet. Click **Undo All Changes** to return to default.

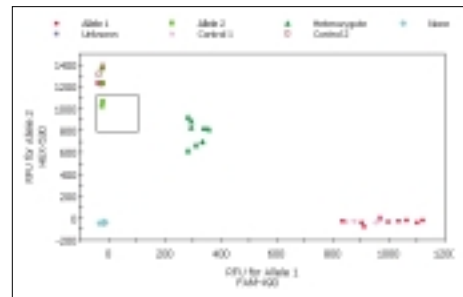
Undo All Changes
 Recalculate

14. **Manual Call** is the alternative analysis mode. Adjustments may be made either in the scatter plot or in the data spreadsheet

• **Scatter plot:**

Make a selection in the **Allele Call** box. Then click and drag the cursor over the corresponding sample(s) in the scatter plot. The samples are reassigned to the allele call selected by the radio button. The data spreadsheet is updated accordingly

Allele Call
 Allele 1
 Allele 2
 Heterozygote
 None
 Control 1
 Control 2
 Unknown



• **Data spreadsheet:**

Click in the **Call** cell of the spreadsheet. A pull-down menu will appear. Select an allele call for that sample, and the change is reflected also in the scatter plot

	RFU 1	RFU 2	Call
A1	963.46	13.70	Control 1
A2	877.94	-23.76	Allele 1
A3	954.77	-38.95	Heterozygote
A4	847.39	-21.73	None
A5	-34.75	1313.89	Unknown
A6	-37.47	1227.99	Control 1
A7	-29.24	1219.83	Control 2
A8	-19.99	1362.65	Control 2

15. Click **Reports** to obtain customized reports for the allelic discrimination data.