1. Click the Load button and load an .fcs file

2. Select your custom panel in the pull-down menu

3. Draw rectangles around the wells to define your experiments and click the Experiment button

4. Select your negative controls and click the Negatives button

5. Rename your experiments as desired

6. If desired, rename your probes and/or samples

Click between samples and probes to view your results
Click the Export button to export your data in .csv format

Frequently Asked Questions

What if many of the wells are shown as red or yellow?
The most likely cause is failure to follow experimental protocol. However it can also occur if the wrong barcode was used.

Where do I find my barcode?
It is on the side of the Firefly particles tube

What if I have lost my barcode?
If the microRNA set is the same as a previous run, the barcode can be selected from the dropdown list

What if I select the wrong barcode by accident?
You can re-select using Analyze → Settings → Code → Preset

Why do some wells not appear in the graphs?
If their quality score was too low (well appears red) they are not used

What if the heatmap has low contrast?
If there is one microRNA probe is in much higher concentration than any other, it can dominate the heatmap. Switching to log scale can bring out the smaller elements. Alternatively, it can be removed from the probe list.

What if the tree branches are too close together?
Try reducing the tree branch depth display exponent

How I can avoid unwanted probes from cluttering the graph?
Probes can be removed by selecting and deleting in the probes panel

If I remove a probe, can I restore it?
Not in the same experiment, but re-selecting the wells and defining a new experiment will restore all the probes

Why does the chart go blank after designating a blank probe?
Immediately after designating a blank probe, that probe is selected. By definition, its values are all zero. Selecting a different probe will show its blank-subtracted profile

What are the columns of the export file?

Mean			Standard error			Standard Deviation			Coefficient of Variation			Number of Particles		
P1	P2	P3	P1	P2	P3	P1	P2	P3	P1	P2	P3	P1	P2	P3

In a nutshell, the columns values run across probes P1..Pn and the rows run across all the samples of the first experiment, with subsequent experiments following

How do I find out more about a menu item or button?
Hovering the mouse over controls provides more information

What is the x-control probe? Can I use it for normalization?
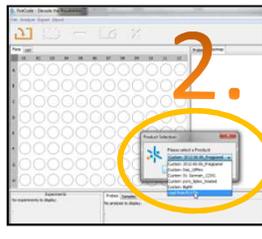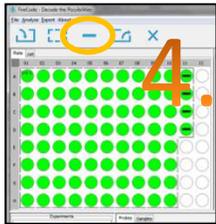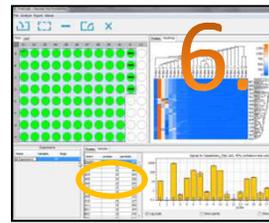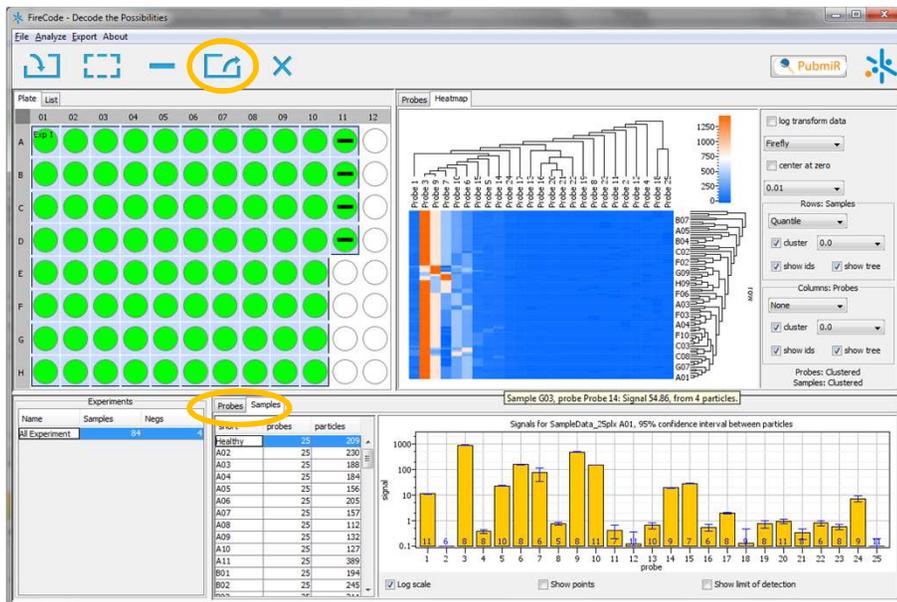X-control is for Firefly internal use. It should not be used for any purpose.

With other questions and concerns, contact the Firefly Support Department at
1-888-240-7789 or support@fireflybio.com

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