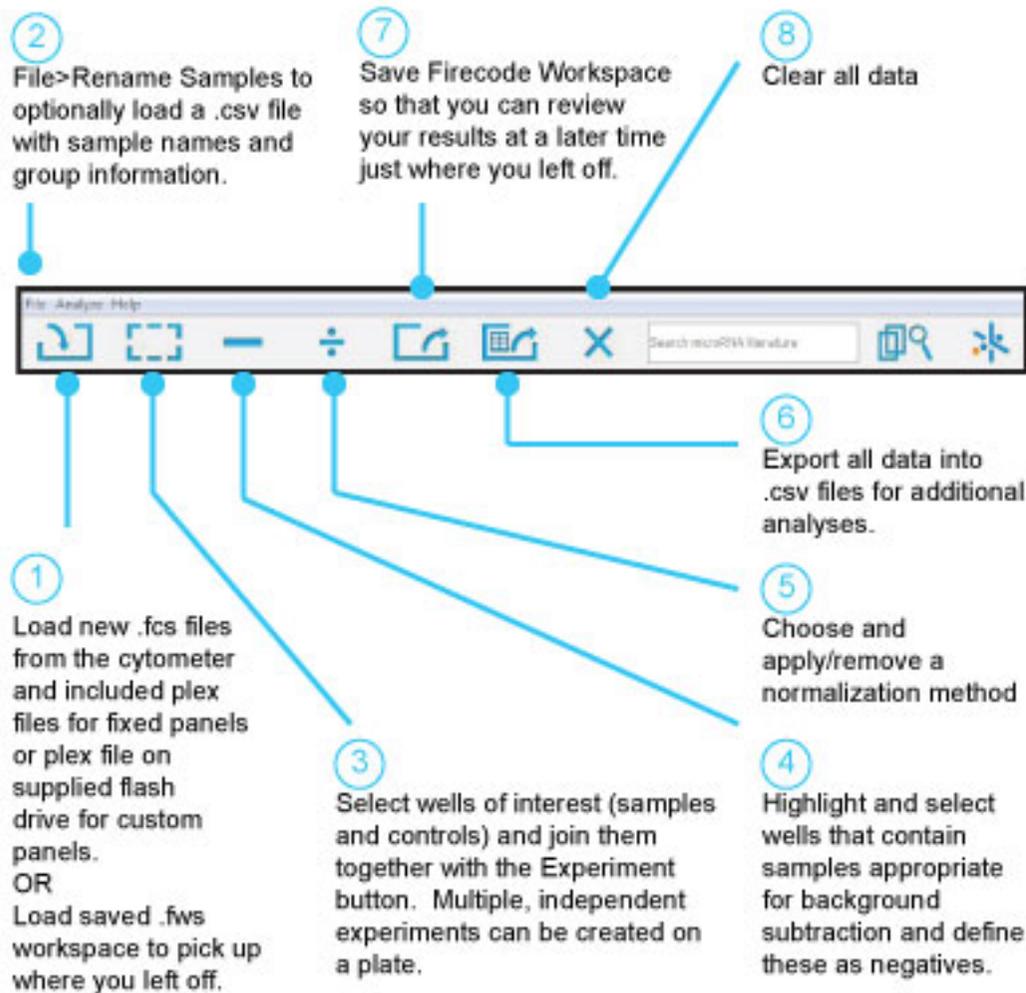


# Firefly™ Analysis Workbench

## Quick start guide



## Software Installation

### First Time Use

- At the Firefly site <http://www.fireflybio.com>, find the Software tab at the top. Choose 'Analysis' from pop up menu.
- Click the blue "Download the Firefly Analysis Workbench" to access additional instructions and a blue "Launch" button.
- Clicking the blue "Launch" button downloads a short Java web-start script and launches it.

### Subsequent Use

- Whenever the analysis workbench is updated, the application will download the new version, otherwise it will use the version it has already downloaded in order to save time.
- An internet connection is not needed for subsequent use, except for update purposes.

### Troubleshooting

- Depending on your browser and system configuration, the web-start script (suffix .jnlp) may start automatically or may need to be manually started. If it does not start automatically, go to the downloads folder of your browser and double-click the "firecode.jnlp" file to download and launch the software.
- On some machines, system security may prevent the application from running with a double-click; proceed by right-clicking the application and selecting Open with Java Web Start.
- Java security may ask if you want to run the program either after the web-start program has been downloaded or after the Workbench has been downloaded. Click OK at the prompts.
- You may receive a warning that an application is requesting access to your system. If you do, check the details of the certificate and click "Allow." This is needed so the Analysis Workbench can open your data files.
- You may also be asked to allow a shortcut to be installed on your desktop. Accept to run the Workbench without an internet connection.
- On some systems, Java Web Start may ask for permission to access the Internet to check for a new version of Java. Although not required for the Analysis Workbench unless your Java is older than 2006, it is recommended to stay up to date for security purposes.

## Frequently asked questions

### How do I tell the software what panel I am using?

If it's a fixed panel, it will be in the "PLX history" drop down. If it's a custom panel, enter the barcode for the panel.

### Where do I find my barcode?

It is on the side of the Firefly™ particles tube.

### What data are presented in the heatmap?

By default, the fold-change of a probe relative to the average of all samples is presented. Linear and logarithmic signal can also be presented.

### How do I assign names and groups to samples?

You can either edit the sample names in the table and add group names to them, or you can read a prepared plate layout CSV file.

### How can I reduce clutter from unwanted probes or samples?

Probes can be removed by selecting in the probes panel. Then they can be either deleted entirely, or temporarily hidden.

### What is p-value?

A multiplex assay requires more stringent screening for significance than when testing a single probe. Multiple methods are provided.

### How can I do further analysis, for instance, in R?

A CSV format file can be exported containing all the experiments defined.

### Can I restart analysis where I last left off?

Sure. Save a workspace and reload it.

### 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. Perhaps the cytometer had low throughput; rescanning may help.

### What are the columns of the export file?

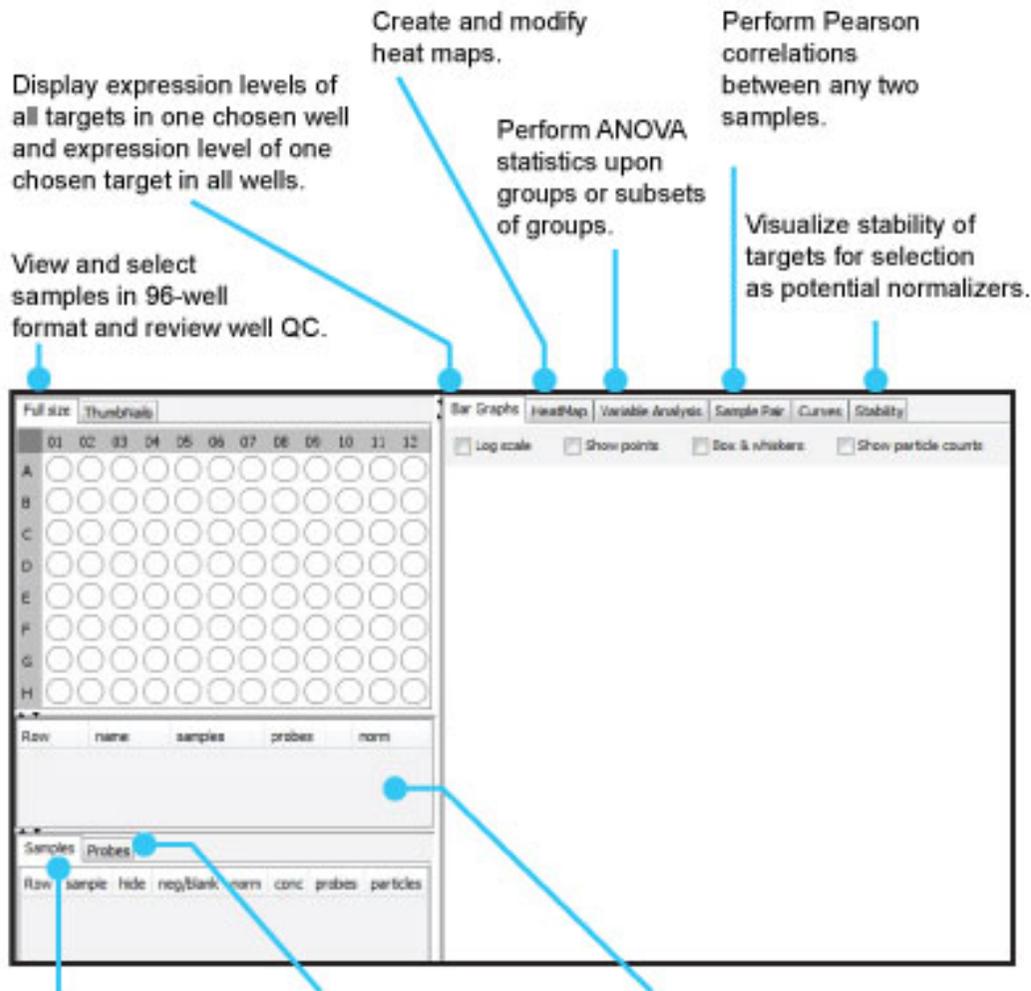
Mean			Standard error			Standard deviation			Coefficient of variation			Number of particles		
P1	P2	P3	P1	P2	P3	P1	P2	P3	P1	P2	P3	P1	P2	P3

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 GUI element such as a chart?

Hovering the mouse over an element provides a short message. Right-clicking brings up a more extensive menu with a help link.

Please contact [technical@abcam.com](mailto:technical@abcam.com) for further questions.



Display expression levels of all targets in one chosen well and expression level of one chosen target in all wells.

View and select samples in 96-well format and review well QC.

Create and modify heat maps.

Perform ANOVA statistics upon groups or subsets of groups.

Perform Pearson correlations between any two samples.

Visualize stability of targets for selection as potential normalizers.

View and edit sample traits such as name, status as a negative well, group name and if the sample is hidden.

View and edit probe traits such as name, status as normalizers, and if the probe is hidden.

View details of each selected experiment.