CFX Manager™ Software Gene Expression Analysis
Quick Guide

With stringently qualified controls, you can use the Gene Expression Module of CFX Manager software to evaluate relative differences in a target's concentration between samples. The most common use of this application is for evaluating the target concentration in cDNA samples to infer steady-state mRNA levels. Typically, the message level(s) of one or more reference genes is used to normalize the values of a gene of interest. Reference genes correct for loading differences or other sampling variations present in each sample. The expression level of reference genes should not vary across the biological conditions being studied.

Gene Expression Analysis Setup

The example wells shown in Figure 1 contain a single fluorophore loaded in the wells with four replicate groups containing two different target names and two different sample names.

Assigning Reference Genes

1. Click **Experiment Settings** in the Plate Editor or the Bar Chart tab of the Gene Expression Module. The Experiment Settings window opens (Figure 2).

2. Select the **Targets** tab.

3. Click the appropriate checkbox(es) to indicate the target(s) that will be used as references.

4. Click the **Show Analysis Settings** checkbox in order to use a reaction efficiency other than 100% for any target.

5. The Auto Efficiency checkbox is checked by default. If a standard curve has been included in the run, then the efficiency calculated from the standard curve will be used in the calculations. Alternatively, type a reaction efficiency value into the cell corresponding to the appropriate target to use this efficiency in the calculations.

6. Check the sample type(s) that should be excluded from gene expression calculations.

Note: Changes in efficiency made in the bar chart settings window will be reflected in the values calculated in all the other plot types.

	1	2	3	4
A	Unk-1 Target 1 Sample 1	Unk-2 Target 1 Sample 2	Unk-3 Target 2 Sample 1	Unk-4 Target 2 Sample 2
B	Unk-1 Target 1 Sample 1	Unk-2 Target 1 Sample 2	Unk-3 Target 2 Sample 1	Unk-4 Target 2 Sample 2
C	Unk-1 Target 1 Sample 1	Unk-2 Target 1 Sample 2	Unk-3 Target 2 Sample 1	Unk-4 Target 2 Sample 2

Fig. 1. Representative wells for gene expression.

Fig. 2. Assigning reference target(s).

Assigning Control Samples

1. In the Experiment Settings window, select the **Samples** tab.

2. Click the appropriate checkbox to indicate the sample that will be used as the control in the calculations (Figure 3). The control sample is assigned a value of 1 for every gene, while all other samples are presented with values relative to the control.

Note: The control sample and references can also be selected using the Setup Wizard, which is available in the Editing Tools dropdown menu in the Plate Editor window.

Gene Expression Analysis

There are several ways to view gene expression data: bar chart, clustergram, scatter plot, volcano plot, and heat map. These options can be selected from the tabs on the left side of the analysis window (Figure 4). The Results tab combines gene expression calculations from the different plots into one window.

Bar Chart

Gene expression is represented by bars of varying height depending on the level of expression for each sample and target combination.

Select the analysis mode from the Mode dropdown menu (Figure 5):

- Normalized expression ΔΔCq — relative quantity of gene(s) of interest is normalized to relative quantity of the reference gene(s) across samples

- Relative quantity ΔCq — relative quantity of gene(s) of interest is not normalized; the results express the gene concentration relative to other samples in the experiment

Graphing options for bar charts:

- Select from the Graph Data dropdown menu (Figure 5) to display graph data relative to control (originating at 1) or relative to zero. In log scale, the Graph Data option defaults to relative to control

- Click the **Show Chart Settings** box and select from the X-Axis dropdown menu (Figure 5) to display Sample or Target on the x-axis

- Select from the Y-Axis dropdown menu (Figure 5) to use Linear, Log 2, or Log 10 as the y-axis scale

- When needed, select from the Scaling dropdown menu — Highest, Lowest, or Unscaled

- Select from the Error Type dropdown menu to display Standard Error of the Mean or Standard Deviation

- Choose the **Sort** option on the right-click graph menu (Figure 5) to customize the presentation order on the x-axis

- Right click the graph to copy and paste it into other applications or to save a copy of the graph as an image file. An alternative copying option is to click the icon in the top right corner of the chart and drag it into a Microsoft Word or PowerPoint document
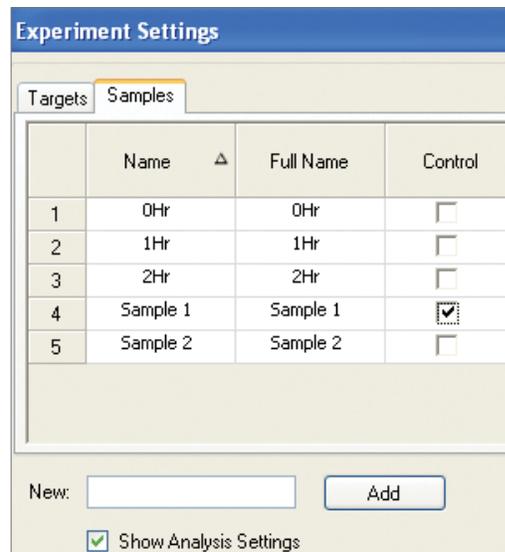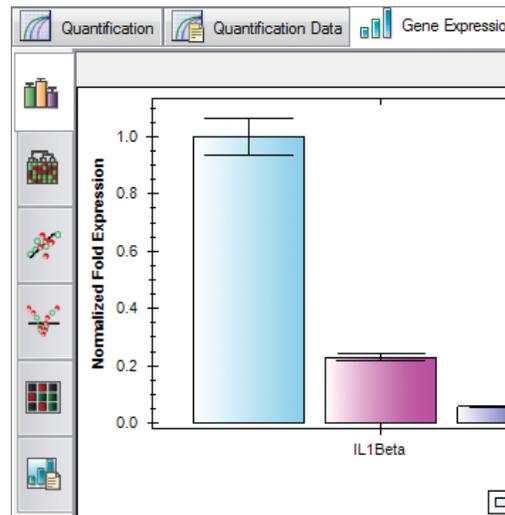
Fig. 3. Assigning the control sample.

Fig. 4. Gene expression data analysis selection tabs.

Clustergram

Samples and targets are clustered in a hierarchy based on the similarity of relative gene expression. The data point color indicates greater expression (red), lower expression (green), or no change in expression (black). The lighter the color, the greater the expression level.

Graphing options for clustergrams:

- Select to cluster or label data by target, sample, both target and sample, or neither target nor sample by choosing from these options in the appropriate dropdown menu
- Adjust the size of the plot image using the Size slider
- By default, average values are plotted; to display values for replicates, select **Split Out Replicates**

Scatter Plot

The relative difference in expression of a target for a control versus an experimental sample is displayed. The data point color indicates upregulation (red), downregulation (green), or no change in regulation (black) based on the threshold setting.

Graphing options for scatter plots:

- Select the control and experimental samples from the respective dropdown menus
- To make a change in the Regulation threshold field, enter a numeric value, use the up and down arrows, or click and drag the lines within the plot
- The targets displayed can be changed using the checkboxes in the first column

Volcano Plot

The regulation of a target and its significance is displayed for an experimental sample compared to a control sample based on where regulation and *P* value cutoffs have been set. The data point color indicates upregulation (red), downregulation (green), or regulation that is within the specified threshold setting (black).

Graphing options for volcano plots:

- Select the control and experimental samples from the respective dropdown menus
- To change the position of the threshold(s), enter a numeric value in the Regulation threshold or P-Value threshold field, use the up and down arrows, or click and drag the lines within the plot
- The targets displayed can be changed using the checkboxes in the first column of the spreadsheet

Heat Map

Differences in the regulation of an experimental versus a control sample for each target are displayed in a grid that corresponds to the plate layout. The grid segment color indicates upregulation (red), downregulation (green), or no change in regulation (black). The lighter the color, the greater the degree of regulation.
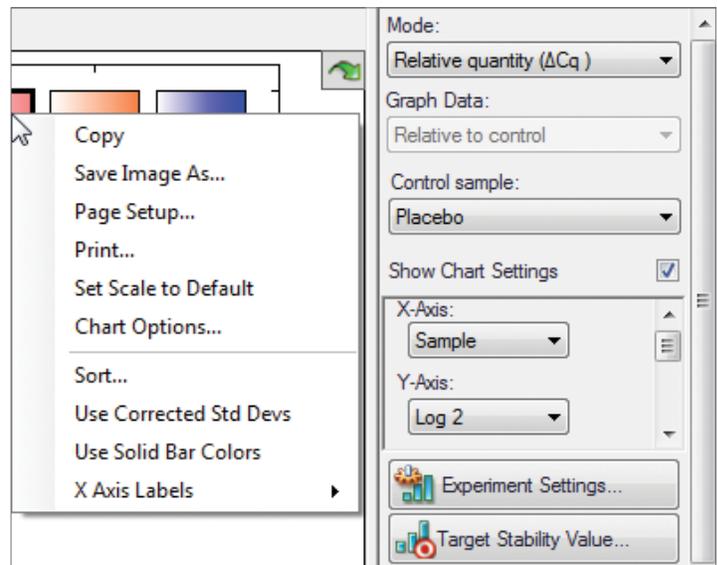

Fig. 5. Graphing options and right-click graph menu for bar charts.

Graphing options for heat maps:

- Select the control and experimental samples from the respective dropdown menus
- Adjust the size of the plot image using the Size slider
- If the data are spread over multiple plates, select a specific plate using the dropdown menu at the top of the window

Results

Gene expression data are also displayed in a spreadsheet format as shown in Figure 6. The Results tab combines data from all the analyses into a single spreadsheet to facilitate viewing and export.

Options for results spreadsheet (applicable to all data spreadsheets):

- Click any column header to display the data sorted by the column values
- Click and drag any header to reposition a column
- Right click the spreadsheet to select the columns to display, to choose a sorting order, or to export the results to a Microsoft Excel spreadsheet or an alternative format

Gene Study

Gene studies combine data from multiple runs and are well-suited to using the data analysis tools to quickly determine which targets are worth pursuing when many are being screened. Inter-run calibrators (wells with the same sample and target name) are used to normalize data from different plates for each target.

Prior to importing data into a gene study:

1. Adjust the baseline or threshold to optimize data.

2. Select the appropriate well group.

3. Make any changes to the plate layout.

To set up a gene study:

1. Select **File > New > Gene Study** from the main window.

2. In the Study Setup tab (Figure 7), click **Add Data Files** and browse to select files for inclusion.

3. Select the **Study Analysis** tab to analyze data. The same gene expression analysis choices that are available in the Data Analysis window are available here.

For more information, visit us at
www.bio-rad.com/web/ampSW3846E.

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Target	Sample	Ctrl	Expression	Expression SD	Corrected Expression SD
NGK	0Hr	*	1.00000	0.12920	0.12920
NGK	1Hr		2.57905	0.50071	0.50071
NGK	2Hr		18.46978	3.94070	3.94070
NGK	3Hr		163.08526	27.34259	27.34259
NGK	4Hr		1840.02543	250.03713	250.03713
NGK	5Hr		18083.88405	2972.66882	2972.66882

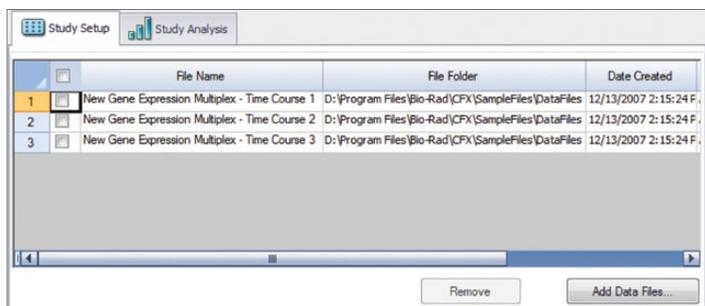Fig. 6. Gene expression results in spreadsheet format.

Fig. 7. Gene study containing three data files.