AMPLIFICATION

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 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 on 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.

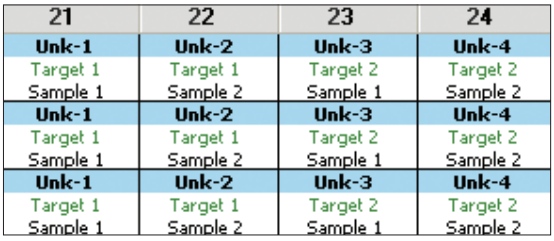

Fig. 1. Representative wells for gene expression.

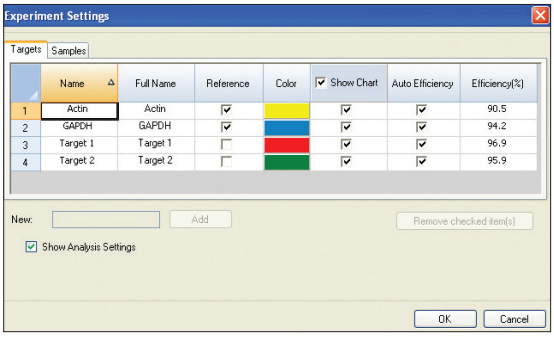

Fig. 2. Assigning reference target(s).

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.

Gene Expression Analysis

Selecting the Analysis Mode

1. Select the analysis mode from the Mode drop-down menu (Figure 4):
   a. Normalized expression $\Delta\Delta C_q$ — relative quantity of gene(s) of interest is normalized to relative quantity of the reference gene(s) across samples.
   b. Relative quantity ΔC_q — 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

1. Select from the Graph Data drop-down menu 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.
2. Select from the X-Axis drop-down menu to display Sample or Target on the x-axis.
3. Select from the Y-Axis drop-down menu to use Linear, Log 2, or Log 10 as the y-axis scale.
4. When needed, select from the Scaling drop-down menu — Highest, Lowest, or Unscaled.
5. Select from the Error Type drop-down menu to display Standard Error of the Mean or Standard Deviation.
6. Choose the Sort option on the right-click graph menu to customize the presentation order on the x-axis.
7. Right click on 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.

Spreadsheet Options

1. Gene expression analysis results are displayed in the spreadsheet (Figure 5).
2. Click on any column header to display the data sorted by the column values.
3. Right click on the spreadsheet to export the results to a Microsoft Excel spreadsheet or an alternative format.

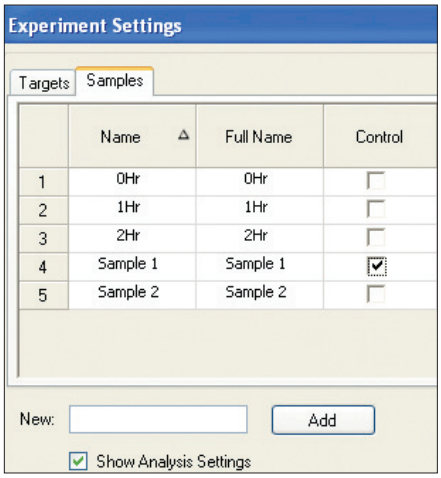

Fig. 3. Assigning the control sample.

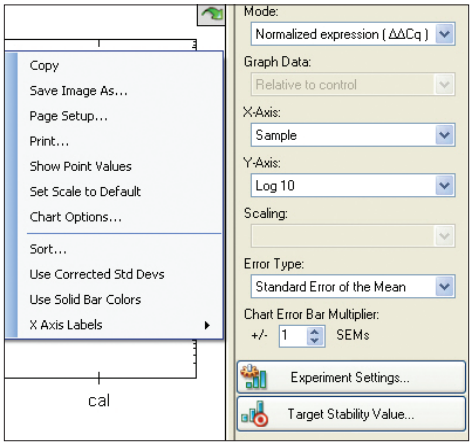

Fig. 4. Graphing analysis options and right-click graph menu.

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. 5. Gene expression results in spreadsheet format.

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