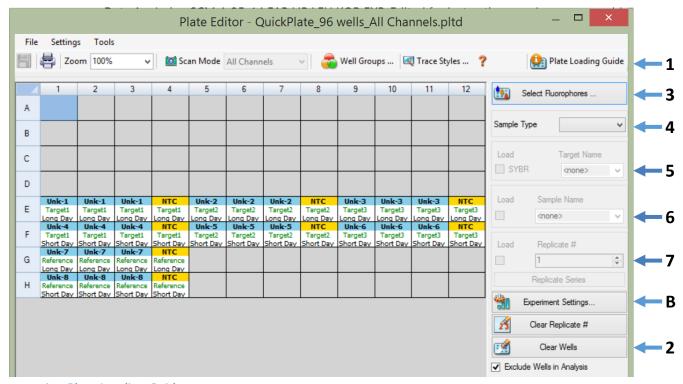## A. Editing a Plate

Click View/Edit Plate... Use the screen shot below to help guide you through the next steps.

1. Plate Loading Guide

There is a quick tool located at the top right of the window, called the Plate Loading Guide. This button brings up a mini-checklist to work you through the steps and can be helpful if you have not used the machine in a while and need a refresher!

2. Clear Wells

This is optional if you want to clear wells that do not contain any data. **NOTE:** Be careful with this button! If you clear a well that means the system will not read it, so make sure it is indeed empty.

3. Select Fluorophores

Select the appropriate wells and indicate which fluorophore you are using in your reactions. For SYBR Green-based assays, the only fluorophore you need checked is SYBR. Click OK.

4. Sample Type

You need to indicate what kind of sample type your template is. Select the wells you wish to label and choose the Sample Type dropdown menu. Select the appropriate sample type (Unknown, Standard, NTC, Positive Control, Negative Control, NRT).

5. Target Name

The target name is usually the name of the primer or the gene of interest. Select the wells you wish to label, then type the target name into the Target Name box and hit Enter on your keyboard.

6. Sample Name

The sample name indicates where the nucleic acid came from. Select the wells you wish to label, type the sample name into the Sample Name box, and hit Enter your keyboard.

7. Replicate Series

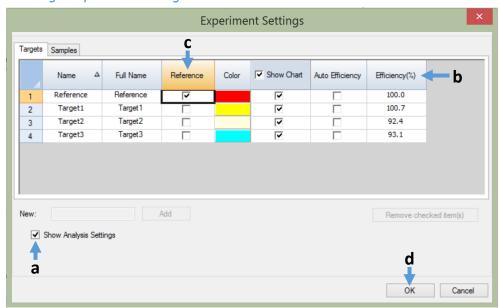You need to indicate how many technical replicates of each sample-target combination you prepared.

a. Select all the wells of the same Sample Type, and then click Replicate Series.

b. Input the Replicate Size (i.e. 3 if you set up three technical replicates).
c. If necessary, indicate the Starting Replicate number.
d. Select whether your technical replicates are set up horizontally or vertically.
e. Click Apply.

B. Annotating a Plate
In order for CFX Manager to calculate an accurate change in gene expression you will need to annotate your plate with your experiment settings

1. *Target Experiment Settings*

a. Check the box next to "Show Analysis Settings".
b. Input the correct efficiency values from your standard curves for each target.
c. Check the box in the "Reference" column next to the target that is your reference gene.
d. Click OK.

2. *Sample Experiment Settings*

a. Make sure there is a check mark next to the "Show Analysis Settings" box.
b. Check the box in the "Control" column for the appropriate sample, if you want the software to set the expression level of a particular sample to 1 (i.e. time point 0, wild-type, untreated).
c. Click OK.

d.   Click OK in the Plate Editor Window.

e.   Click Yes to Apply changes and get back to the Data Analysis window.
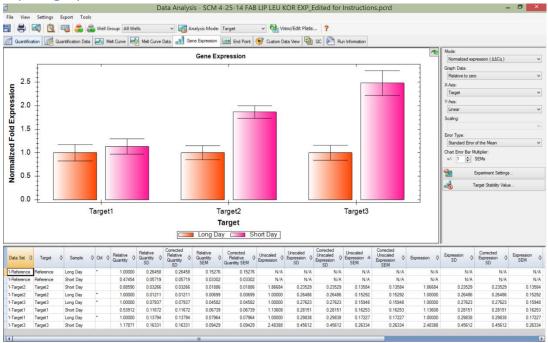
C.   Exporting Expression Data

Data Set	Target	Sample	Ctrl	Relative Quantity	Relative Quantity SD	Corrected Relative Quantity SD	Relative Quantity SEM	Corrected Relative Quantity SEM	Unscaled Expression	Unscaled Expression SD	Corrected Unscaled Expression SD	Unscaled Expression SEM	Corrected Unscaled Expression SEM	Expression	Expression SD	Corrected Expression SD	Expression SEM
1-Reference	Reference	Long Day	*	1.00000	0.26458	0.26458	0.15276	0.15276	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
1-Reference	Reference	Short Day		0.47454	0.05719	0.05719	0.03302	0.03302	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
1-Target2	Target2	Short Day		0.88590	0.03266	0.03266	0.01886	0.01886	1.86684	0.23529	0.23529	0.13584	0.13584	1.86684	0.23529	0.23529	0.13584
1-Target2	Target2	Long Day	*	1.00000	0.01211	0.01211	0.00699	0.00699	1.00000	0.26486	0.26486	0.15292	0.15292	1.00000	0.26486	0.26486	0.15292
1-Target3	Target3	Long Day	*	1.00000	0.07937	0.07937	0.04582	0.04582	1.00000	0.27623	0.27623	0.15948	0.15948	1.00000	0.27623	0.27623	0.15948
1-Target1	Target1	Short Day		0.53912	0.11672	0.11672	0.06739	0.06739	1.13608	0.28151	0.28151	0.16253	0.16253	1.13608	0.28151	0.28151	0.16253
1-Target1	Target1	Long Day	*	1.00000	0.13794	0.13794	0.07964	0.07964	1.00000	0.29838	0.29838	0.17227	0.17227	1.00000	0.29838	0.29838	0.17227
1-Target3	Target3	Short Day		1.17871	0.16331	0.16331	0.09429	0.09429	2.48388	0.45612	0.45612	0.26334	0.26334	2.48388	0.45612	0.45612	0.26334

a.   Select the "Gene Expression" tab.

b.   Make sure the "Analysis Mode:" is set to "Target".

c.   Set the "Mode:" to "Normalized expression (ΔΔCq)".

d.   Decide how you want to scale your Expression data. **NOTE:** The conclusion or interpretation of the data will remain the same for all scaling options. Scaling just displays the data slightly differently on the graph. Select one of these scaling options to calculate and present your gene expression data in a manner that best suits your experimental design:

   i.   If you selected a control sample in the "Experiment Settings" window, the software has already scaled your data so that the expression level of the control sample is set to 1. You will not be able to choose any other scaling option.

   ii.   Average: Scale the normalized gene expression to the average for each target by dividing the expression level of each sample (within that target) by the average expression level in all the samples of that target.

   iii. Highest: Scale the normalized gene expression to the highest for each target by dividing the expression level of each sample by the highest level of expression in all the samples within a target.

   iv.   Lowest: Scale the normalized gene expression to the lowest for each target by dividing the expression level of each sample by the lowest level of expression in all the samples within a target.

   v.   Unscaled: This option presents the unscaled normalized gene expression.

e.   Highlight the "Target", "Sample", "Expression", and "Expression SEM" columns by holding down the Ctrl key on your keyboard and clicking on the appropriate column headings. To copy these into an Excel Spreadsheet press "Ctrl" and "C" at the same time, then paste them into an open workbook.