RT^2 Profiler PCR Array Data Analysis v3.5

http://pcrdataanalysis.sabiosciences.com/pcr/arrayanalysis.php

revision date 9 November 2012

Overview

The RT^2 Profiler PCR Array Data Analysis Quick Reference Card contains instructions for analyzing the data returned from using qRT-PCR Assays or RT^2 Profiler PCR Arrays. For more information on the bench procedure for these products, please refer to the appropriate user manual or handbook. If you require additional assistance, please contact your local QIAGEN technical representative.

Important Notes:

- Please note that you must complete all of your work with the Data Analysis Web Portal in the same session. The data is not stored on a server, so all work is lost once the session (or the web browser) is closed. **Be sure to export all processed data and results** to an Excel file saved on your local computer.
- Please set the screen resolution to 1024 × 768 or greater, if possible.
- Turn off any window pop-up blockers. The software will launch separate windows for viewing the plots and charts.

Setting baseline and threshold setting on the real-time thermocycler:

The RT^2 Profiler PCR Array Data Analysis Webportal analyzes C_T values to calculate changes in gene expression. The C_T values must be exported from your qPCR instrument and formatted into an Excel spreadsheet. All of the plates/wells must be copied into a single worksheet.

1. Calculate the threshold cycle (C_T) for each well using the real-time cycler software, as described in the following steps.
    Note: If using the Roche LightCycler 480, there are 2 options for data analysis: using the second derivate max setting (in this case there is no need to calculate the C_T) or using "Fit Points" (in this case the C_T should be defined manually as described in step 3).

2. Define the baseline by choosing the automated baseline option if the cycler has the adaptive baseline function. If the cycler does not have the adaptive baseline function, set the baseline manually. To set the baseline manually, use the linear view of the amplification plots to determine the earliest visible amplification. Set the cycler to use the readings from cycle number 2 through 2 cycles before the earliest visible amplification, but no more than cycle 15. The earliest amplification will usually be visible between cycles 14 and 18.
3. Manually define the threshold by using the log view of the amplification plots. Choose a threshold value above the background signal but within the lower one-third to lower one-half of the linear phase of the amplification plot.
    Note: Ensure that the threshold values are the same across all RT^2 Profiler PCR Array runs in the same analysis. The absolute position of the threshold is less important than its consistent position across arrays. If the RNA sample is of sufficient quality, the cycling program has been carried out correctly, and threshold values have been defined correctly, the value of C_T PPC should be 20±2 for all arrays or samples.
4. Export or copy the C_T values from each experimental run into an Excel Spreadsheet. C_T values for each sample should be in a single column.

Formatting data for the Data Analysis Webportal:

The RT^2 Profiler PCR Array Data Analysis Webportal analyzes C$_T$ values to calculate changes in gene expression. For the RT^2 Profiler PCR Array Data Analysis Webportal to correctly read/import your data, the C$_T$ values must be exported from your qPCR instrument and then formatted into a new Excel spreadsheet. The RT^2 Profiler PCR Array Data Analysis Webportal <u>only</u> accepts Excel spreadsheets for analysis. All of the plates/wells must be copied into a single worksheet. <u>Please use the templates below as guides.</u>

1. If you are using a catalogued PCR Array where 1 sample was tested on a 96 well plate, 100 well ring or 384 well plate (the 1x384 layout), the Raw C$_T$ values should be in a single column that corresponds to the correct well location on the PCR Array. A blank spreadsheet is available at: http://www.sabiosciences.com/pcr/template.xls

2. If you are starting with a 384 well plate which is in the 4x96 layout, then you will need to separate each sample's data into a separate column, by using the 384-Well Format E/G Data Analysis Patch tool located at http://www.sabiosciences.com/pcrarraydataanalysis.php

3. If you are using a custom PCR Array, the Raw C$_T$ values for each sample should be in a single column that corresponds to the correct well location (column A) on the PCR Array. The gene tested in each well must be supplied by the user in column B. A sample spreadsheet is available at: http://pcrdataanalysis.sabiosciences.com/pcr/templates/customarray_template.xls

4. If you are analyzing data from qPCR primer, primer/probe or Taqman gene expression experiments, the Raw C$_T$ values for each sample should be in a single column that corresponds to the correct well location (column A) on the PCR Array. The gene tested in each well must be supplied by the user in column B. A sample spreadsheet is available at: http://pcrdataanalysis.sabiosciences.com/pcr/templates/assay_template.xls

Upload and annotate the data:

1. Choose the experiment that was performed: Standard/Cataloged RT^2 Profiler PCR Array, Custom RT^2 PCR Array or individual assays

   a. If you selected **Standard RT² PCR Array** then Enter the PCR Array Pathway Number from the drop-down list.
   Note: The RT^2 Profiler PCR Array System was upgraded to Version 4.0 on May 25, 2012. The Version 4.0 arrays added a "Z" to their pathway numbers. Therefore, a Version 3.0 PCR Array has the following number PAXX-###, while a Version 4.0 PCR Array has the following number PAXX-###**Z**. The XX denotes the species (HS = Human, MM = Mouse, RN = Rat, …) the ### denotes the pathway/gene list. The letters (A, C, D, E, F, H, G, R) that follow the ### or the "Z" can be ignored, they represent the plate format.

   b. If you selected **Custom RT² PCR Array** then Enter the Custom Array ID (ex. CAPX####) in the text field.
   c. If your experiment used individual primer assays then select **Single or Multi-Gene qPCR Assays**.
2. Browse and select the MS Excel file that you prepared in the above section (*Formatting data for the Data Analysis Webportal*) that contains all of your PCR data with a maximum number of 100 samples. Click **Upload**.

Setup and quality check the uploaded data:

1. The uploaded raw C$_T$ data is displayed in the window underneath the **Analysis Setup** tab.
2. In the **Basic Setup** section, assign samples to different groups. At least two groups are needed, where one of those groups must be the control group. Click **Update** when finished. You may exclude samples from the analysis by selecting **Exclude** on the drop down menu.
   a. Review the **Data QC** section to assess each groups' PCR reproducibility, reverse transcription efficiency, and the presence of genomic DNA contamination.
   b. The **Select Housekeeping Genes** section allows you to remove or add preferred housekeeping genes for data normalization by clicking the appropriate checkboxes. Click **Update** when finished.

   Note: The user must click on **Perform Normalization** before proceeding with the analysis.

   c. Review the **Data Overview** section to see each group's distribution of threshold cycle values and the average of the raw data in each group.

Analysis page:

1. See the **Average C$_T$, 2^(- C$_T$)**, **Fold Change**, **p-value**, and **Fold Regulation** sections for the results processed by the software from your data. The **Fold Change** and **p-value** results are used by the software in subsequent graphical analyses.

Note: The fold change (fold difference) is calculated by the equation $2(-\Delta\Delta C_T)$. For the fold regulation, the software transforms fold change values less than 1 (meaning that the gene is down regulated) by returning the negative inverse. The values of the numbers are the identical; the software has only changed the representation of the value. For example if ABL1 has a fold change value of 0.31, this is equivalent ABL1 having a fold regulation of -3.2 fold. (ABL1 expression is decreased 3.2 fold).

Plots and Charts:

1. Heat Map
   a. Define Groups to be compared and whether to report the fold-change results as a log transformation or not (default recommended). Click **Update** once changes have been made. Click **Export Data** to download the results as an Excel file.
   b. To save the figure:
      1. Using Windows: Right-click on the figure and save the image/picture
      2. Using OS X: Hold down the Control key & Click and save the image/picture
2. Scatter Plot
   a. Define Groups to be compared. Choose the fold-change boundary (and p-value for Volcano Plot) of interest. Click **Update**.
   b. Click on symbols to identify gene and fold-change (and p-value for Volcano Plot); mouse over table entries to point to symbol on plot.
   c. Click check boxes to remove genes from or add genes to plot. Click **Update**.
   d. Click **Export Data** to download the results as an Excel file.
   e. To save the figure:
      1. Using Windows: Right-click on the figure and save the image/picture
      2. Using OS X: Hold down the Control key & Click and save the image/picture
3. Volcano Plot
   a. Define Groups to be compared. Choose the fold-change boundary (and p-value for Volcano Plot) of interest. Click **Update**.
   b. Click on symbols to identify gene and fold-change (and p-value for Volcano Plot); mouse over table entries to point to symbol on plot.
   c. Click check boxes to remove genes from or add genes to plot. Click **Update**.

       d.   Click **Export Data** to download the results as an Excel file.

       e.   To save the figure:
1. Using Windows: Right-click on the figure and save the image/picture
2. Using OS X: Hold down the Control key & Click and save the image/picture

4. Clustergram
   a. Sort samples by "Array" or by "Group".
   b. Select "Join Type". The default of "Average" is recommended.
   c. Cluster in "1-D" by genes only or in "2-D" by genes and samples.
   d. Color code the graph by "Genes", "Samples" or "Entire Dataset". The default of "Genes" is recommended.
   e. Choose whether or not to display the gene symbols and array or group names.
   f. Click check boxes to remove genes from or add genes to plot.
   g. Click **Update**.
   h. To save the figure:
      1. Using Windows: Right-click on the figure and save the image/picture
      2. Using OS X: Hold down the Control key & Click and save the image/picture
5. Multigroup Plot
   a. Click check boxes to add genes to or remove genes from plots.
   b. Choose results to plot on the y-axis, either "AVG Delta C_T", "2 ^ Delta C_T", or "Fold Change".
   c. Click **Update**.
   d. Review either the line plot (above) or the column chart (below).
   e. To save the figure:
      1. Using Windows: Right-click on the figure and save the image/picture
      2. Using OS X: Hold down the Control key & Click and save the image/picture

Export the Results:

1. Click **Export Data** to download a MS Excel file containing all raw and processed data from the "Readout" and "Analysis Result" sections.

Note: The RT^2 Profiler PCR Array Data Analysis Webportal does not save any results. Please ensure that you have exported or copied any figures or tables from each session.

What's Next

The Data Analysis software delivers a list of expression changes in the samples from the supplied data. In order to assist in further analysis, the software utilizes the latest bioinformatics tools to analyze the data. Please use each sub-menu to examine the results.

1. Gene Expression
   a. This tool will help define a panel of genes based of experiment's results. The tool is designed to deliver a list of gene expression assays that would allow the user to follow-up the results of the analyzed experiment.
2. miRNA Regulation
   a. This tool will identify candidate miRNA regulators in your experimental system. The tool delivers a list of miRNAs that could be targeting the genes that had observed changes in expression in your selected samples.

RT^2 Profiler PCR Arrays, RT^2 PCR Assays, and RT^2 SYBR Mastermixes are intended for molecular biology applications. These products are not intended for the diagnosis, prevention, or treatment of a disease.

For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit handbook or user manual. QIAGEN kit handbooks and user manuals are available at www.qiagen.com or can be requested from QIAGEN Technical Services or your local distributor.