

Use Transcriptome Analysis Console (TAC) to analyze QuantiGene™ Plex Data

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Applied Biosystems™ Transcriptome Analysis Console (TAC) 4.0.2 Software provides powerful and interactive visualizations to help simplify complex gene expression analysis. In combination with the QuantiGene™ Plex Data Analysis Software (<https://apps.thermofisher.com/apps/quantigene>), TAC software offers additional tools for in-depth analysis of multiplex data sets. QuantiGene™ Plex Data Analysis Software allows for the export of normalized expression data for each sample as individual CHP files. These CHP files can then be imported into the TAC software, which can be downloaded at <https://www.thermofisher.com/us/en/home/life-science/microarray-analysis/microarray-analysis-instruments-software-services/microarray-analysis-software/affymetrix-transcriptome-analysis-console-software.html>.

This quick reference provides instructions on how to:

- Export CHP files from the QuantiGene™ Data Analysis Software as a .zip file
- Extract the .zip file with 3rd-party software
- Install the library and annotation files into the TAC software
- Import the CHP files into TAC and set up basic comparisons
- Review analysis results and visualizations including volcano plots, heat maps, and Venn diagrams

For more detailed instructions on how to use TAC 4.0.2 software to analyze gene expression data, the user guide can be downloaded at https://assets.thermofisher.com/TFS-Assets/LSG/manuals/tac_user_manual.pdf.

Export CHP files

To create and export CHP files, first use the QGP Data Analysis Software (**QGP Data Analysis Software**) to import raw data, QC, and normalize. After running your normalization of the gene expression data, the **Review** screen (Fig. 1) shows the normalized values in the left pane. For detailed instructions, refer to the HELP section within the QGP Data Analysis Software by clicking **Help** in the top right corner. It is important to note that TAC analysis is dependent on accurate normalization of expression data

- To export, click  **Download CHP** to download a .zip file containing individual CHP files for each sample along with a TAC parameter folder that contains required library and annotation files.

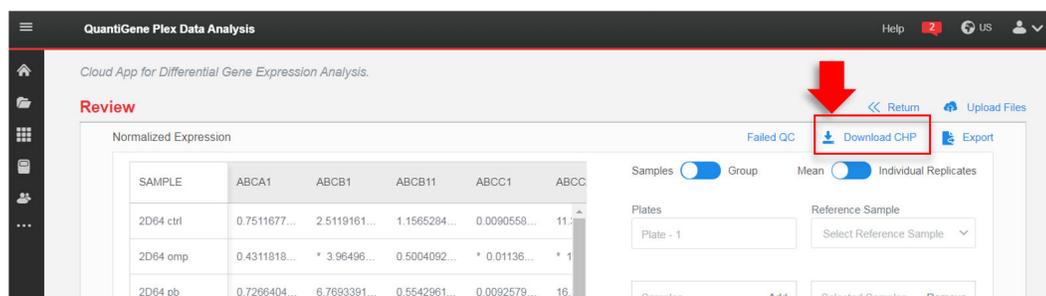


Figure 1 Download CHP files from QGP Data Analysis Software

Extract CHP files

It is important to note that the .zip file downloaded from the QuantiGene™ Data Analysis Software cannot be extracted with the default Microsoft™ Windows™ .zip file support. The file needs to be extracted by 3rd-party software that supports multiple archive types such as the open source application 7-zip (available at 7-zip.org) or a similar application.

The extracted contents of the .zip file include individual CHP files for each sample and a "TAC parameter files" folder containing the library and annotation files.

Install a TAC library

Before performing TAC analyses, a TAC Library File must be imported/installed from the **TAC parameter files** folder that is exported from the QuantiGene™ Data Analysis Software.

1. Click  on the **Preferences** tab.
2. Click  **Browse**.
3. Navigate to the **TAC parameter files** folder stored on your computer.
4. Click  **Select Folder**.

After assigning the **TAC parameter files** folder as the TAC library, the **Net Affx Username** and **Password** are automatically generated and the QuantiGene™ configuration file will appear as an **Installed Array Type**.

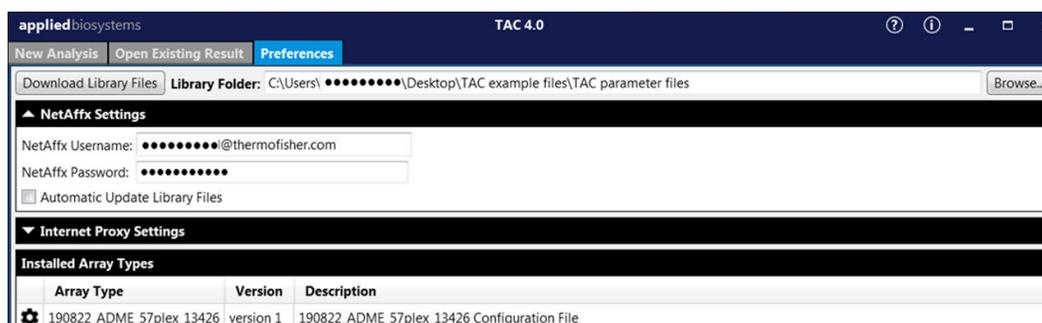


Figure 2 Install TAC library under the Preferences tab

Set up a new analysis

Each sample from your QuantiGene™ analysis will be exported as an individual CHP file. These files can be directly drag-dropped into the window under the **New Analysis** tab. You can also select **Import CHP file** under the **Import Files** drop-down.

Different experimental conditions or sample attributes can then be compared across samples during the differential expression analysis.

1. Click **Comparison** (Fig. 3) from the drop-down menu in the header of the column you would like to compare (the title of column can be changed by right clicking on the header).
2. Type to assign condition values for each sample. Multiple samples can be selected and assigned values simultaneously.
3. Additional conditions can be added to the analysis by right-clicking in the header next to the previous condition/column and selecting **Add new column** and **Comparison** in the drop-down menu.

All conditions will be automatically compared in the analysis, but the **Comparison Setup Wizard** can be used to customize the comparisons prior to running the analysis.

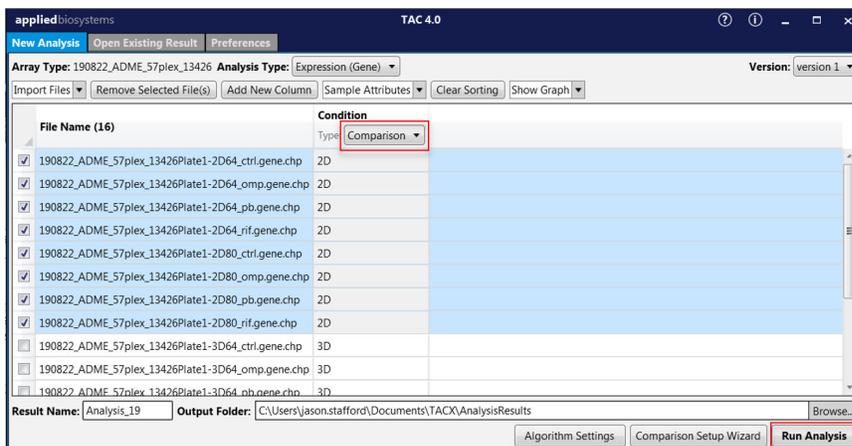


Figure 3 Set up comparisons under New Analysis tab.

Review analysis results

Sample QC View

After the analysis is complete, the results automatically open in the TAC Viewer. Principal Component Analysis (PCA) of your QuantiGene™ data is shown under the **Sample QC View** tab (Fig. 4). PCA identifies 3 variables (PCA1, PCA2, and PCA3) that account for the majority of variance in the data set.

Signal Box plots are also shown under **Sample QC View** and allow you to quickly assess overall signals for each sample and identify any outliers.

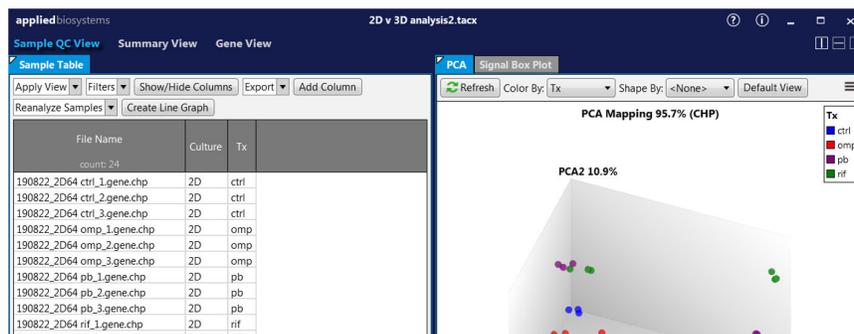


Figure 4 Principal Component Analysis (PCA) shown under Sample QC View.

Summary view

Selecting the **Summary View** tab opens the tabs for **Analysis Summary** and **Gene Lists and Venn Diagram**.

Analysis Summary provides data viewing and comparison. It includes:

- Summary of parameters used in the current analysis
- Comparison table showing sample and gene count across comparisons
- Graph of differently expressed genes that provides the gene count for each comparison
- Graph of sources of variation indicating the fraction of total variance (0-1) that can be explained by the attribute

Gene Lists and Venn Diagrams (Fig. 5) provide a convenient way to visualize the intersection of genes from the lists generated by your comparisons.

- From the **Gene List**, click  on 2-3 gene list checkboxes to compare.

- Click  on the checkbox of any region of the Venn diagram, then click  on **Create Gene List from Selection(s)** to export the results of your analysis. The newly created Gene List will appear at the bottom of the **Gene List** in the left pane.

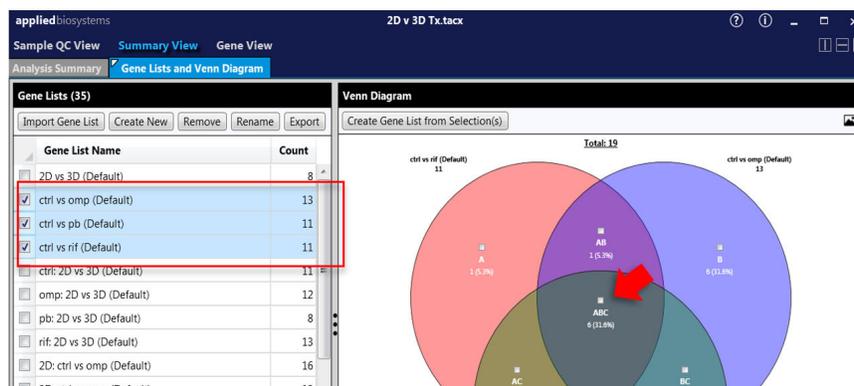


Figure 5 Gene List and Venn Diagrams.

Gene View

The **Gene Table** and **Scatter Plot** (Fig. 6) are shown by default under the **Gene View** tab. Condition pairings made under the **Comparison** drop-down menu (upper left) generate 5 types of differential expression analysis graphs for QuantiGene™ Plex data:

- **Scatter Plot**
- **Volcano Plot**
- **Hierarchical Clustering**
- **WikiPathways**
- **Sample Signals**

An All Conditions comparison only generates Hierarchical Clustering and Sample Signals graphs.

Note: Graphs can be exported as a PNG (96-600 dpi) by clicking on  **Options**.

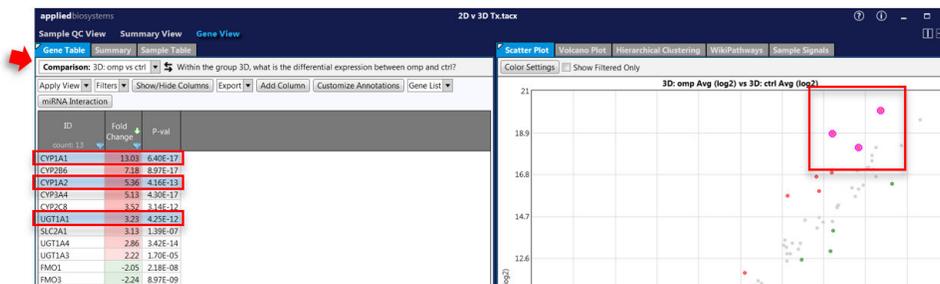


Figure 6 Gene Table and Scatter Plot. Scatter plots show differential gene expression between two conditions from the Comparison drop-down. The 1st condition is shown on the Y-axis and the 2nd condition is shown on the X-axis (log2 scale). Green points are down-regulated >2-fold in the 1st condition and red points are upregulated >2-fold in the 1st condition. You can lasso a gene or a group of genes by holding down the left mouse button. After closing the loop around your genes of interest they will be highlighted in magenta and also highlighted in blue inside the table view on the left.

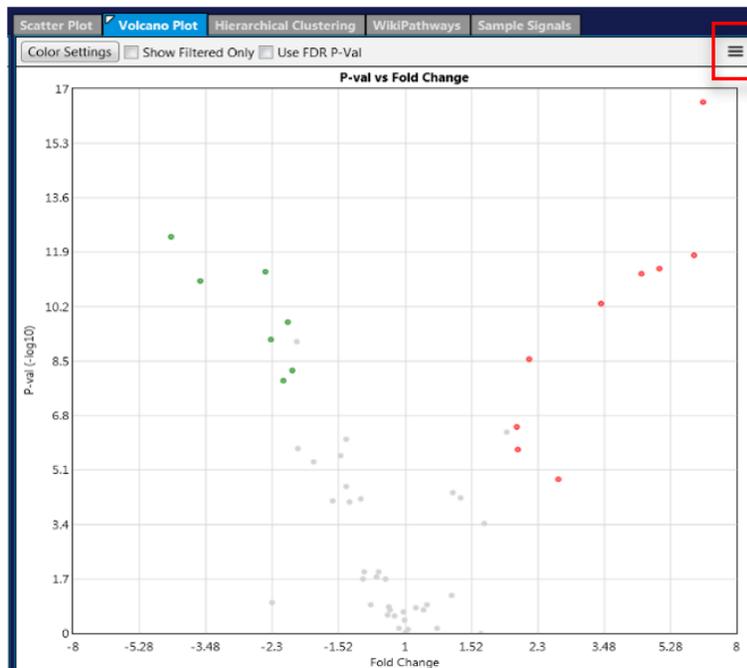


Figure 7 Volcano Plots are a type of scatter plot used to quickly identify statistically significant changes in large data sets by plotting significance versus fold-change. The X-axis is the linear fold change from the current condition pair and the Y-axis is the $-\log_{10}$ p-value of the ANOVA p-values. Genes of interest can be lassoed as described for Scatter Plots. Export a PNG (96-600 dpi) by clicking on \equiv Options.

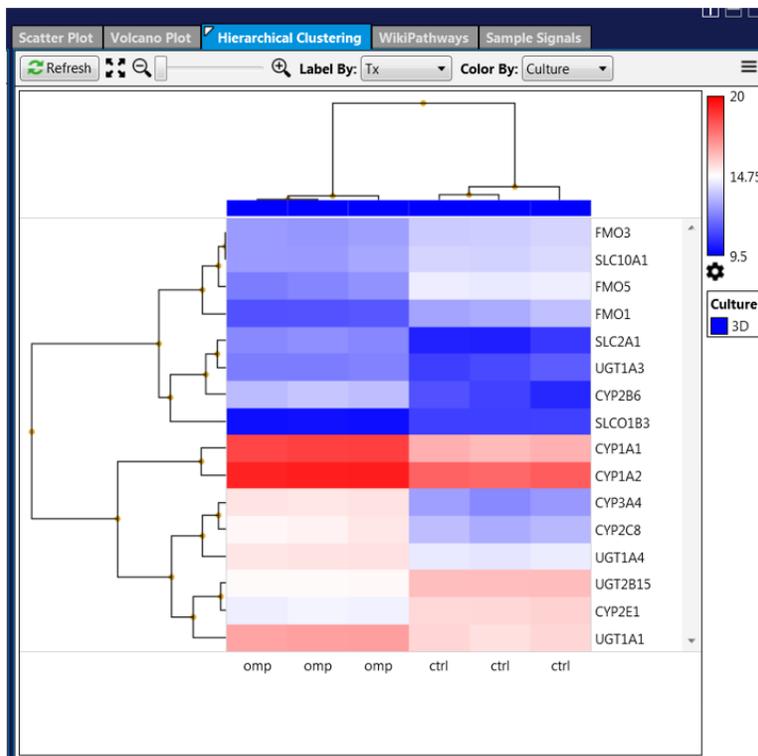


Figure 8 Hierarchical Clustering is a method of analysis that seeks to build a hierarchy of clusters to group different samples. TAC uses an agglomerative or “bottom-up” approach where each observation starts in its own cluster and pairs of clusters are merged as one moves up the hierarchy. Click the Label By drop-down to label the bottom of the graph. Click the Color By to select the attribute to color the graph. Mousing the cursor over the graph will display details about the sample ID and signal for each tab.

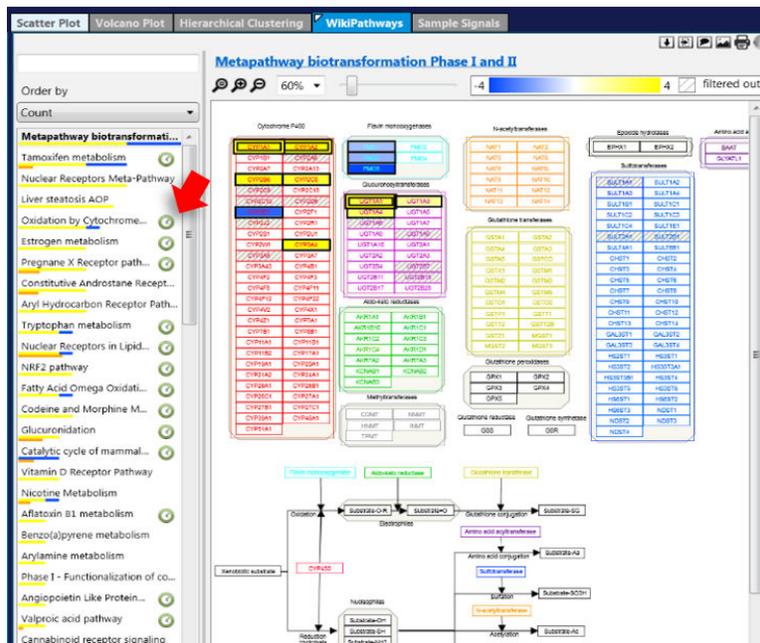


Figure 9 WikiPathways (wikipathways.org) is a biology community-maintained website displaying biological pathways. Each pathway has its own web page containing diagrams, descriptions, references, version histories, component genes, protein lists, etc. You must have an active internet connection to use WikiPathways. Upregulated genes found within the pathway are highlighted in yellow, whereas downregulated genes are highlighted in blue. The remaining gene targets from your QuantiGene™ panel that are not differentially expressed (based on filter criteria) between conditions are identified within the pathway as greyed-out boxes. A clock icon to the right of a pathway indicates an outdated pathway. Click on the clock to update the pathway. After the pathway is updated, the clock will be replaced with a New icon.



Figure 10 The Sample Signals bar graph is a graphical representation of the gene target signal intensity (following normalization) and standard deviations among conditions. The height of the bar represents the average normalized signal for each comparison and each point on the top of the bar represents the signal from a specific sample. Mouse over the sample to display the sample ID and signal. Clicking on a point to select a sample will also highlight the gene identity in the Sample Table tab.

Also available under the **Gene View** tab are **Summary** and **Sample Table** tabs. The **Summary** tab provides a summary of the comparison, including how many samples for each condition and filter criteria. It also provides a summary of differential gene expression in graphical and tabular display, which includes the total number of genes and the number of genes upregulated or downregulated by group.

The **Sample Table** tab allows you to quickly identify any outliers based on signal. Highlighting a sample in the table highlights the same sample in the **Sample Signals** graph.

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