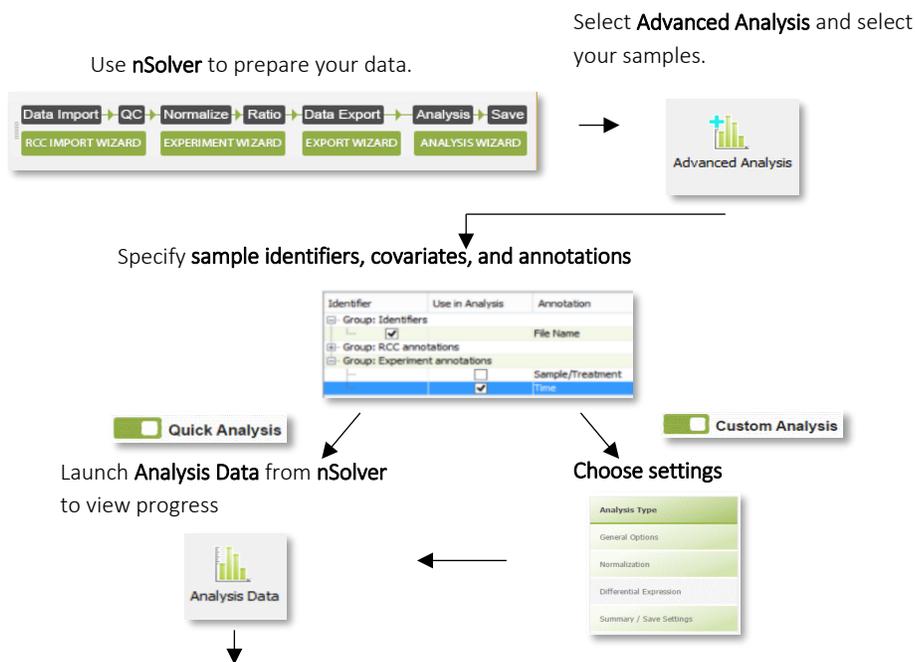


Advanced Analysis 2.0 Quick Start Guide

The Advanced Analysis software plugin (version 2.0 and later) provides a number of R-based statistical tools with minimal input from the user. Before beginning, ensure you have a reliable internet connection and security settings that allow pop-ups. First-time Advanced Analysis users should ensure they allow adequate time to download required R libraries (this ~750 MB file may take up to 1 hour to download). See the *Advanced Analysis 2.0 User Manual* installation instructions.

1. Experimental Design & nSolver™ Software 4.0 Data Preparation: **Import** your **RCC** and **RLF** files to nSolver version 4.0 or later and create an **Experiment**. For more on this topic, see the *nSolver 4.0 User Manual* or the *nSolver 4.0 Quick Start Guide*. Annotate samples, bearing in mind that the annotations will be used as variables in Advanced Analysis. On the **Experiments** tab, highlight the raw or normalized data and select **Advanced Analysis**.
2. Creating an Advanced Analysis: Highlight the desired **Advanced Analysis** version (if more than one installed), choose a **Name** for the analysis, and **Browse** for the location in which you would like it saved. Select an **Identifier** that is unique to each sample (including SNV references) and one or more **Covariate** by checking appropriate boxes. Use the drop-down menu in the **Categorical Reference** column to set a reference group as your baseline. Selecting **Quick Analysis** will result in Overview, Normalization, and Differential Expression analyses for expression data and variant call detection analyses for SNV and Fusion data. **Custom** may be selected when wanting to customize analysis settings; these settings are addressed on pages 3-4 of this guide.
3. Viewing Analysis: Return to the nSolver 4.0 dashboard, select your experiment, and expand the navigation tree. Highlight the Analysis data level and find your most recent analysis on the list. Highlight it and select **Analysis Data**. This will open a window in your browser; you may need to **Allow Blocked Content**, depending on your internet security settings. Libraries will load, status messages will dynamically appear in the browser, and ultimately, an analysis screen will appear. See next page for descriptions of plots and options available.

Workflow



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View **QC** results and **data analysis** in pathways of interest (this step continued from previous page).



Overview

Overview heatmaps display raw data, allowing you to identify gene sets with low counts and normalized data clusters, which gives you a high-level view of possible associations within the data. Choose to view only genes in particular gene sets along the left side of the window and choose to view Principal Component analysis, study design, and QC data along the top of the window.

Differential Expression

This module isolates the effect of each variable on the data. It displays a linear regression of the differential gene expression for each variable as a volcano plot.

Share

This allows you to access to the Advanced Analysis report as a sharable zip file. Once it is saved to your computer, extract AdvAnalysisReport.zip and view the HTML report outside of nSolver. This folder also contains all the analysis output images and data files.

Gene Set Analysis (GSA)

GSA overlays differential expression data for sets of genes grouped by biological function, considering the covariates and relative to the baseline.

Normalization

This module allows you to normalize mRNA and protein data separately. It uses the geNorm algorithm for mRNA, choosing only the most stable housekeeping genes. Scatter plots display the effect of the chosen normalization settings on the data. Protein expression data is also displayed.

Analysis Parameters

Under this tab, you may view all analysis settings and details. You may also review the reasons behind any aborted analyses.



SNV- & Fusion-specific plots

SNV and Fusion variant detection call summaries can be found on these tabs. QC metrics specific to these assays can also be found in this section.

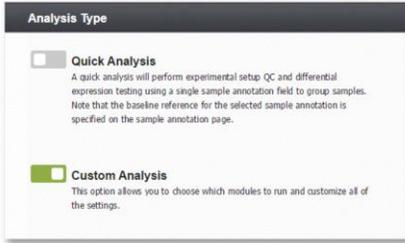
PathView

This module displays different KEGG pathways and highlights pathway members most differentially expressed in your data.

Custom Advanced Analysis Settings & Plots

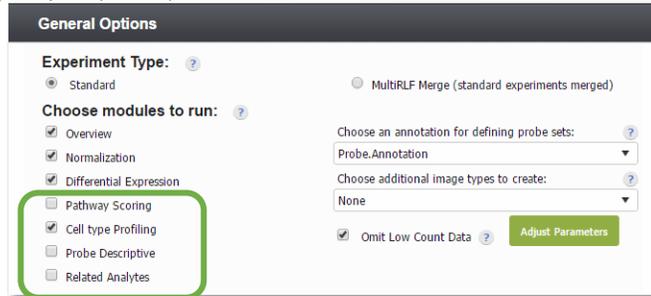
Analysis Type

Here, you choose between Quick and Custom Analysis.

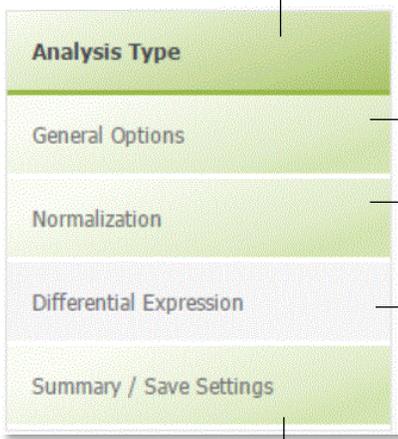


General Options

On this menu, choose the modules to run, confirm the experiment type, choose a probe annotation file, and determine any additional image types (.pdf, .jpg, etc.) to create. Use the check box to omit low count data and then Adjust Parameters to alter the thresholds (analyte-specific) that define low count.

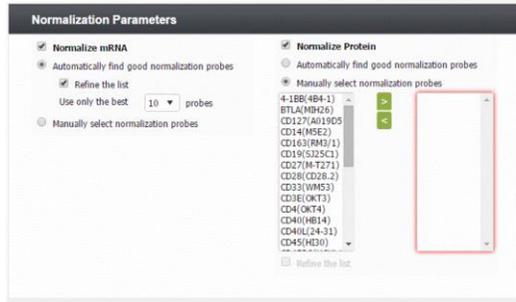


Selecting these modules adds them to the menu. See following page.



Normalization

Advanced Analysis allows you to normalize each analyte type with its own custom settings. Manually select probes or allow the software to automatically select the best performing probes. It can also refine the list of probes to the top 10 (or other number of your choice). See previous page for resulting plot.



Summary/Save Settings

This displays a summary of your settings for the current analysis and allows you to save them for a future application.

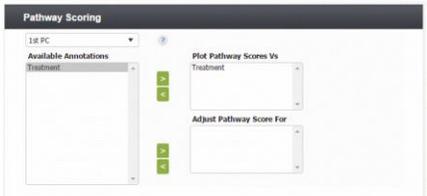
Differential Expression

On this menu, choose one or more variables to include in your differential expression model. Predictors and confounders are treated equally in this model, but results will only be shown for predictors. Choose to run DE using the **Optimal** or **Fast/Approximate** method. The Optimal method is robust for estimating differential expression when probe counts are low or near background but computationally demanding. The Fast/Approximate method works well for probe counts observed significantly above noise. The PathView plots can be colored by either t-statistic or fold-change. See previous page for resulting plot.



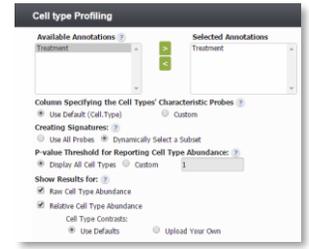
Pathway Scoring

Use the green arrows to select variables to plot against pathway scores and variables to adjust for before calculating pathway scores. See below for plot.



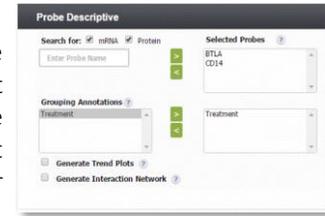
Cell Type Profiling

Use the green arrows to move at least one covariate from **Available Annotations** to **Selected** to analyze cell population abundance. See below for resulting plot.



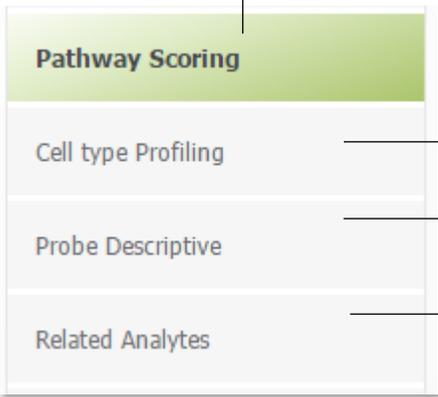
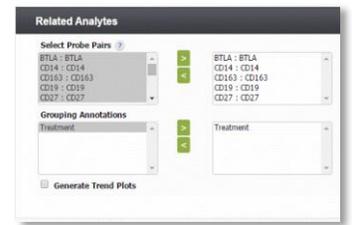
Probe Descriptive

Search for probe names to calculate detailed metrics on a smaller subset of genes. At least 5 genes need to be entered for Principal Component Analysis biplots. See below for resulting plot.



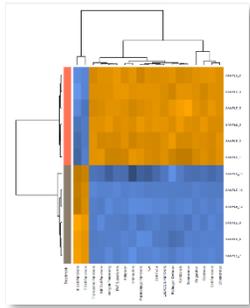
Related Analytes

Related probes for different analytes will be listed. Use the green arrows to move the probe pairs of interest. See below for resulting plot.



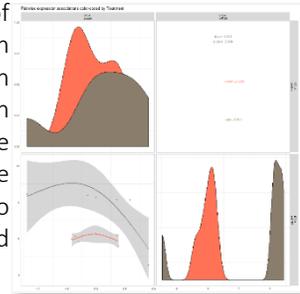
Pathway Score

The Pathway Score summarizes the data from a pathway's genes with a single score. The heatmap of Pathway scores shows a high-level overview of how the pathway scores change across samples.



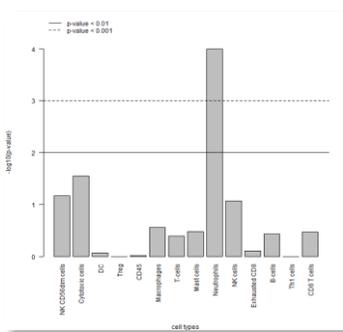
Related Analytes

This module compares the expression levels of multiple analytes when they have been linked in the probe annotation file. It applies all the tools of the Probe Descriptive Module to each pair of related analytes.



Cell Type Profiling

This module quantifies various cell types using cell type-specific marker genes.



Probe Descriptive

This module provides detailed descriptive analysis of 1–15 genes selected using univariate and correlation plots. When at least 5 probes are selected, PCA biplots and parallel coordinate plots will also be generated.

