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IPA Upload and Analyze Your Data Tutorial

Overview:

Use IPA to upload and analyze your 'omics data.

Scenario:

You have RNA-seq, microarray, miRNA, proteomic, genomic, SNP, or metabolic data that you want to analyze with IPA. You need to know the rules, restrictions, and best practices for preparing the data for being uploaded into IPA and how to start an analysis

Tasks:

- Format the data in a way that IPA can upload.
- Set Upload options and assign the ID and Observation columns (measurement values).
- Set analyisis parameters: If the datasets are large, adjust expression cutoff values(s) to restrict their size.
- Run analysis

To understand more about interpreting the results of the analysis,see Analysis Results Tutorial

1) Prepare your data for uploading (as spreadsheet format).

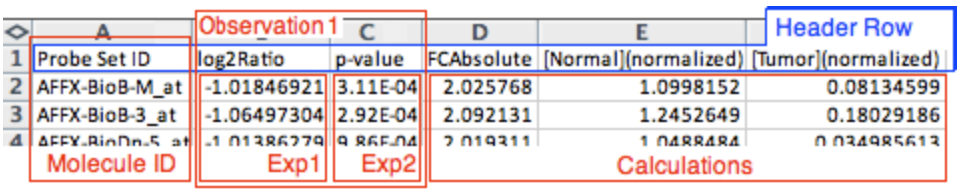
a) If your data is not in an Excel spreadsheet or tab delimited text file, transfer your data into one. IPA can also upload CuffDiff formatted files directly. See Cuffdiff file import.

b) If using Excel, perform basic calculations in the spreadsheet if necessary. Example: compute average ratio for experiment samples vs. control samples and p-values for replicates. Ideally, upload fold changes, log ratios, or log fold changes (which are the same thing as log ratios).

c) Make sure there is only one header row. (IPA can be set to ignore the first row when doing the import.)

d) Move the molecule IDs to the first column. If ID's are in the first column, IPA will scan down approximately 100 rows to guess the ID type(s) in the column. See Data Upload definitions for the types of IDs that are recognized.

e) IPA uses text in the header row to guess the column types. For example if it detects the text "fold change" it will assign it to Expr Fold Change.



f) IPA allows a maximum of 20 observations per imported file. An observation is one "comparison" between an experiment and control. For example if you have a dataset with three time points ratio'ed to a 0 time point, and each with a set of fold changes and a set of p-values for each time point, that would be three observations. See Data Upload definitions for more on this topic. If your data has more than 20 observations, then reduce the data to 20 observations in the spreadsheet or pick and choose up to 20 to import during the upload process.

g) IPA allows up to 8 "measurement values" per observations. For example: fold change, p-values, and the average intensity of expression would equate to three measurements. Any more than 8 will be excluded during the upload process.

2) Launch IPA

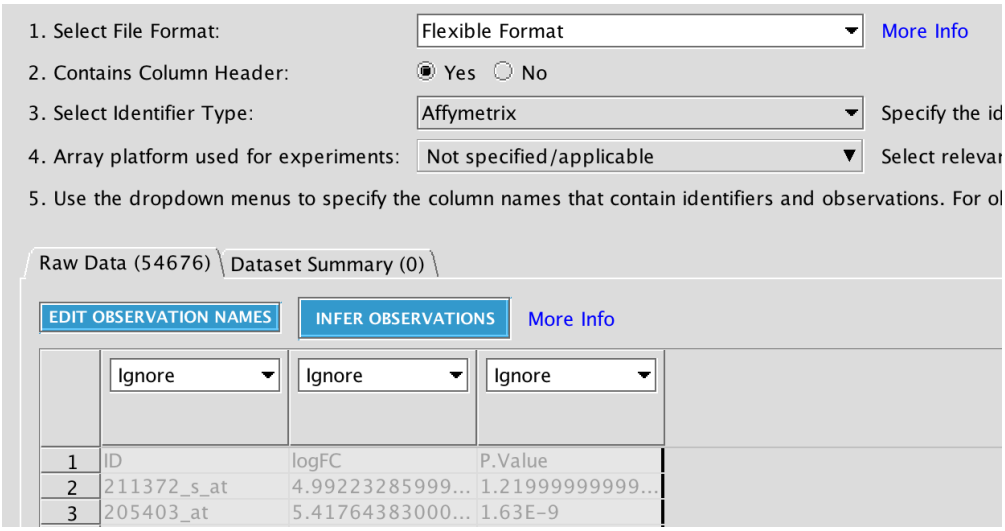
3) Upload your data into IPA.

a) Select **File>Upload Dataset...** from the menu.

b) Select the dataset that you modified in Step 1.

c) Click **Open**.

Your data appears in the "Dataset Upload - …" window.



d) Select "Flexible Format" if it's not already selected (The other options are for legacy support).

e) You can try clicking the Infer Observations button to see if IPA can guess the columns in your dataset. If that works well, skip to the Save step below. Otherwise, click the button again and continue with the next step.

f) If you have one header row in your dataset, make sure the Contains Column Header is set to **Yes**.

g) Select the appropriate Identifier Type(s). Note: IPA will guess the appropriate ID types from the IDs in the first column, but it is always a good idea to double check. Do **not** select all identifier types at once. This can lead to mis-mapping.

g) If the data came from a commonly known array platform, select it from the pull down menu, otherwise leave the value "Non Specified/applicable". Setting the array platform properly here will automatically select the same platform as “reference” in

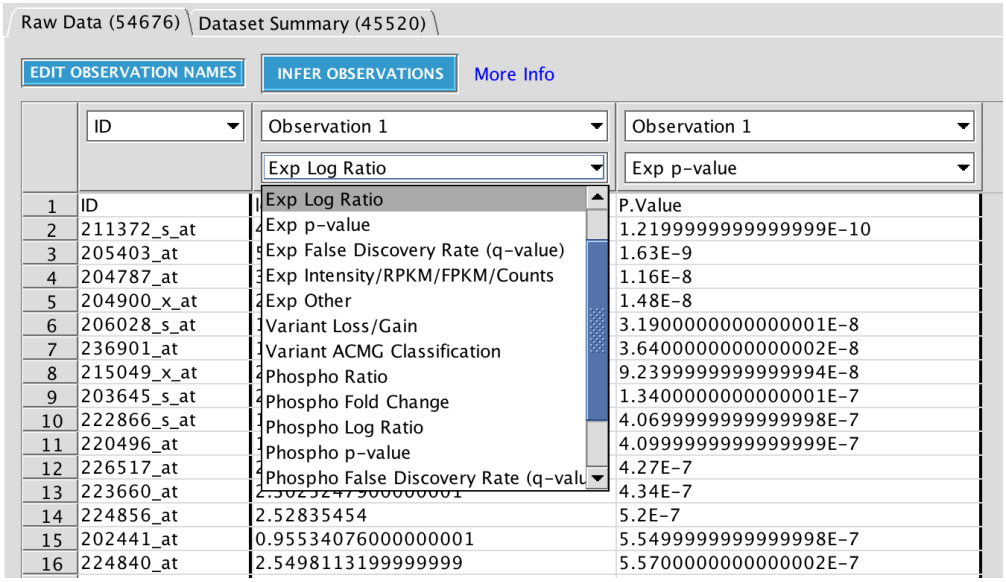
the create core analysis page and will improve the accuracy of the p-value calculations used in analysis.

h) Select the appropriate ID and Observation labels from the dropdown menus for each of the columns.

i. Set the column with your molecule identities to **ID**. Check the Dataset Summary tab to view the breakdown of mapped vs. unmapped molecules from your uploaded data.

ii. Set the column with the first observation value to Observation 1

A second pull down menu appears.



iii. Set the new dropdown menu to the measurement type (i.e. Expr Fold Change, Expr p-value, etc.).

iv. Repeat the last two steps for each of the observations if there is more than one.

⚠ Note: If there is more than one measurement type per observation, be sure to assign each batch of measurement columns to the **same** observation. For example, if the data set has log ratio and p-value columns like the example shown above, this is NOT two observations-- do NOT assign one column to Observation 1 and the other to Observation 2. This is one observation (Observation 1) with two measurements as shown above.

If there are multiple observations, there are shortcuts available to make assigning the batches easier (as described below).

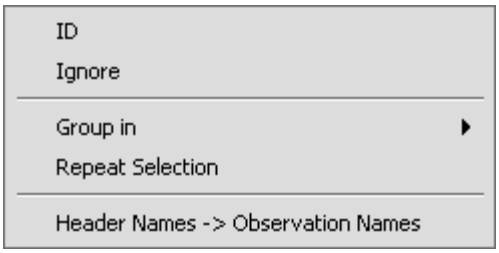
Shortcuts:

- Ignore: Right-click a column and select Ignore.
- Repeat Selection: Select a column(s), right-click the selected columns and select Repeat Selection. The

assignments made for the selected columns will be repeated for the columns to the right of the selected ones.

- Header Names -> Observation Name: This selection will take the name found in the column header and use it to label the observation.

- Group In: Select more than one column, right-click the selected columns and select Group In. The selected columns will be grouped as an observation.



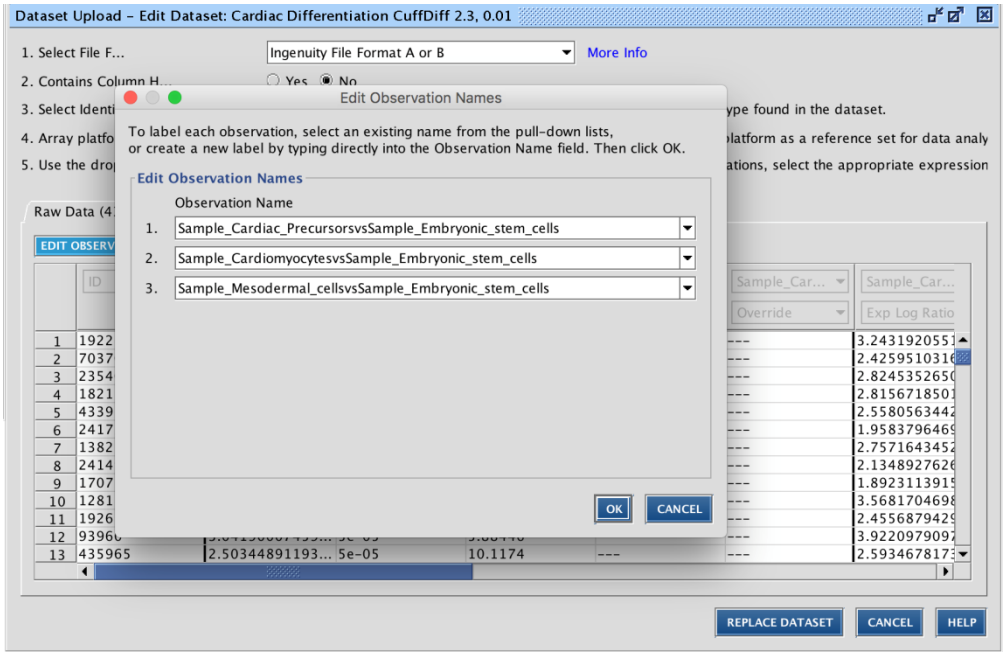
v. Leave any extraneous columns set to **Ignore**.

i) You can customize the names of the column headers.

i. Click the **EDIT OBSERVATION NAMES** button. This is especially important to do if uploading a multi-observation dataset like the example below.

ii. Select the name from the drop down menu or type in a new one.

iii. Click **OK**.



j) Click **Save** at the bottom of the window.

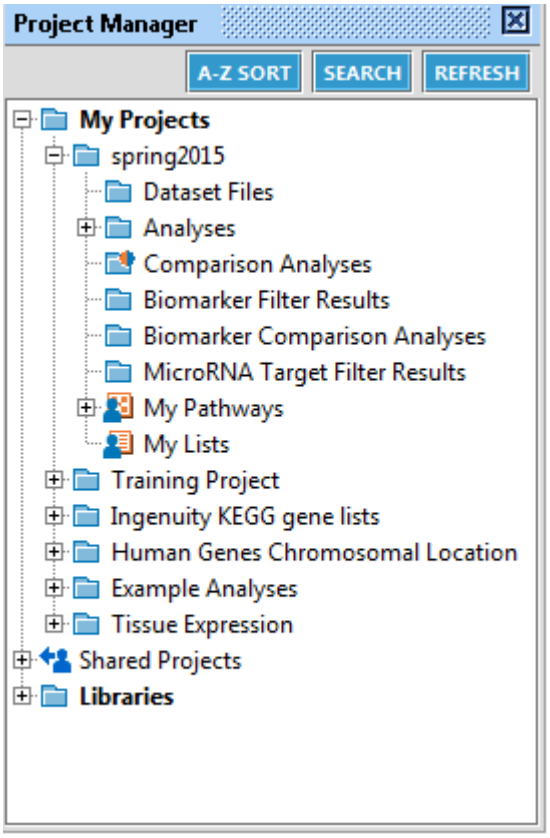
A **Save Dataset** dialog opens.

k) Click **New Project** to create a new one.

l) Enter a name for your dataset.

m) Click **Save**.

IPA saves the data in the Project Manager under the project folder that you selected.



4) Start a Core Analysis on your dataset.

a) Select **File>New>Core Analysis**… from the application menu.

A **Create Core Analysis** dialog appears.

b) Select the dataset that you saved in step 3 and click **Next**.

5) Set **Filters and General Settings for Analysis** parameters.

a) Set the **Reference Set** parameter to the molecule set (reference set) that should be viewed as the complete universe when calculating the statistical significance. You may have already set this when uploading the data.

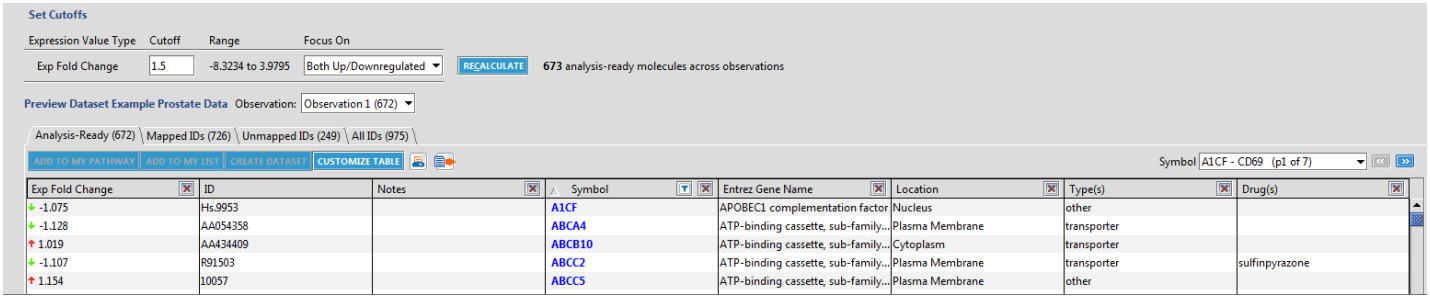
b) You may either leave the Network Analysis parameters, Optional Analysis, Data Source, Confidence, Species, and Tissue & Cell Lines optionson their default settings or provide appropriate filters of your choice.

6) Set **Cutoffs**

It is important to analyze the most significant molecules in your dataset. For example, if you uploaded the data for an entire microarray, you need to set a cutoff so that IPA analyzes only the significantly differentially expressed genes.

a) Enter values for the **Cutoffs**.
b) Click the **Recalculate** button to recalculate the "analysis ready" molecules (i.e. that passed your cutoffs)

Note: We recommend that the number of "analysis ready" molecules should ideally be less than ~3000 in order to focus on the most relevant molecules in your dataset. This will minimize noise in the results. Adjust the Cutoff values more stringently to restrict how many are analyzed. Also, the maximum number of molecules that can be analyzed in one analysis is 8000. You may use the expression value cutoff and also the analysis filter options to keep your “analysis ready” molecule size within the allowable limits.



7) Click the **Run Analysis** button at the bottom right of the window.

The **Start Analysis** dialog appears

a) Select the same project that contains your dataset.
b) Set the analysis name. It is often helpful to include the cutoff values used in the analysis in the name.
c) Click OK.

An **Analysis Running** dialog appears to tell you that the analysis is in progress. When the analysis is complete an email is sent to

you and the name of the analysis appears in the **Project Manager** window in bold text.

8) Open the analysis and explore the results.

a) When the analysis is finished, open it by double clicking on it in the **Project Manager**. If you analyzed a multi-observation dataset, the analysis will appear in a folder.

Core Analysis Results Tutorial

Note: This article pertains to a Core Analysis based on **expression data**. For details on the other types, see Core Analysis Types.

Overview

A **Core Analysis**has multiple ways of helping you find biological insights by automatically using the curated information from the QIAGEN Knowledge Base to put molecules in your 'omics dataset into context. For this tutorial, we will walk through the interpretation of a transcriptomics dataset.

The tabs in the analysis are as follows:

- The **Summary** tab displays the top results for all the analyses. See Analysis Summary Page.
- **Canonical Pathways** tab displays your molecules of interest within well-established signaling or metabolic pathways. See Canonical Pathways Overview.
- **Upstream Analysis tab** predicts which upstream regulators (any molecule that can influence the transcription or expression of another molecule) might be activated or inhibited to explain the expression changes in your dataset. See Upstream Regulator Analysis. It also connects upstream regulators into signaling cascades called Mechanistic Networks. If you have Advanced Analytics in IPA, this tab can also display Causal Networks, or hierarchical networks or regulators controlled by a master regulator.
- **Diseases & Functions**relates molecules in your dataset to known disease states and biological functions. See Downstream Effects Analysis Tutorial.
- **Regulator Effects** displays hypotheses for how a phenotype, function or disease is regulated in your dataset by activated or inhibited upstream regulators.See Regulator Effects.
- **Networks** displays non-directional interaction networks of molecules based on known relationships in the QIAGEN Knowledge Base to your molecules of interest. See What are Networks?
- **Molecules** displays all of the molecules from the analyzed dataset in a table format. "Analysis-ready" molecules will have their identifier displayed in bold font, and are all those that passed the filters and cutoffs you may have applied and were evaluated for overlap with

Canonical Pathways, as targets of Upstream Regulators etc in the Core Analysis.

- **Analysis Match** (requires additional licensing). Displays matches against your own analysis and >50,000 other analyses. See Analysis Match.

Use these tools to find insights that are most relevant to your experimental model or question. Note that this tutorial will draw on only a *subset* of these features, due to length considerations.

Scenario

You have RNA microarray data from the white blood cells drawn from children with childhood exacerbated asthma compared to the convalescent state. For this example, we will use expression data from PBMCs from PMID 19620293. You would like to know if (and how) the data supports involvement of immune/inflammatory responses in acute asthma attack. You can also find novel gene-to-disease associations that can be followed up and confirmed with future wet bench experiments.

Tasks

- Open the **Core Analysis**for your microarray data.
- Use the **Summary Page**to quickly identify promising directions for exploration.
- Explore the results for areas of importance to your research:
  - View **Canonical Pathways** that contain significant numbers of genes from your dataset.
  - Use **MAP** (Molecule Activity Predictor) to predict effects on functional endpoints in a canonical pathway.
  - Overlay **Biomarkers** that identify genes in the TREM1 Signaling Pathway that are used as efficacy indicators for asthma treatments.
  - Explore the impact on downstream **diseases and functions**.
  - View **Upstream Regulators** to find which and immune regulators may have been activated to drive the observed gene expression changes.
  - Use **Regulator Effects** to see a hypotheses of how activation of certain upstream regulators may lead to outcomes like asthma.
  - Evaluate and explore similarity and differences with other analyses with **Analysis Match** (if available on your IPA license).

Steps

1) Start IPA

2) In the **Project Manager**, double click My Projects > Example Analyses > Analyses > Childhood exacerbated asthma GSE16032 with FDR GEO2R. The analysis opens in a new window displaying the **Summary**tab:

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Top Canonical Pathways

Name	p-value	Overlap
Phagosome Formation	3.74E-07	14.9 % 18/121
Iron homeostasis signaling pathway	5.38E-06	13.0 % 17/131
IL-12 Signaling and Production in Macrophages	2.30E-05	11.6 % 17/146
Th1 and Th2 Activation Pathway	5.23E-05	10.5 % 18/171
Eicosanoid Signaling	8.41E-05	15.9 % 10/63

Top Upstream Regulators

Upstream Regulators

Name	p-value	Predicted Activation
dexamethasone	9.81E-25	Activated
filgrastim	6.62E-24	Activated
Immunoglobulin	5.30E-17	Inhibited
GATA2	4.21E-16	
lipopolysaccharide	8.66E-16	Activated

Causal Network

Name	p-value	Predicted Activation
fluticasone propionate	3.63E-25	Activated
filgrastim	6.62E-24	Activated
BTB 09588	4.53E-23	Activated
RDR 03785	4.53E-23	Activated
DFP 00129	4.53E-23	Activated

Top Diseases and Bio Functions

Diseases and Disorders

Name	p-value range	# Molecules
Inflammatory Response	2.41E-05 - 8.50E-19	278
Organismal Injury and Abnormalities	3.00E-05 - 1.59E-16	486
Connective Tissue Disorders	2.93E-05 - 1.72E-16	154
Inflammatory Disease	2.93E-05 - 1.72E-16	213
Skeletal and Muscular Disorders	2.93E-05 - 1.72E-16	149

Molecular and Cellular Functions

Name	p-value range	# Molecules
Cell-To-Cell Signaling and Interaction	2.04E-05 - 6.92E-19	194
Cellular Compromise	1.72E-05 - 8.50E-19	98
Cellular Function and Maintenance	2.26E-05 - 3.68E-17	210
Cellular Movement	2.16E-05 - 1.10E-14	197
Cellular Development	2.81E-05 - 1.78E-14	242

Physiological System Development and Function

Name	p-value range	# Molecules
Hematological System Development and Function	2.77E-05 - 6.92E-19	228
Immune Cell Trafficking	2.08E-05 - 1.32E-15	135
Tissue Morphology	2.34E-05 - 3.06E-15	167
Hematopoiesis	2.77E-05 - 1.78E-14	115
Lymphoid Tissue Structure and Development	2.77E-05 - 1.78E-14	141

Top Tox Functions

Assays: Clinical Chemistry and Hematology

Name	p-value range	# Molecules
Increased Levels of Creatinine	4.03E-01 - 9.02E-03	6
Increased Levels of AST	2.20E-02 - 2.20E-02	3
Increased Levels of Albumin	3.62E-02 - 3.62E-02	1
Increased Levels of ALT	6.01E-02 - 6.01E-02	3
Increased Levels of Red Blood Cells	6.20E-02 - 6.20E-02	7

Cardiotoxicity

Name	p-value range	# Molecules
Cardiac Infarction	3.81E-01 - 1.70E-08	30
Cardiac Inflammation	2.41E-01 - 3.46E-05	13
Cardiac Arteriopathy	4.25E-01 - 8.30E-05	29
Cardiac Dilation	6.17E-01 - 5.55E-04	24
Cardiac Enlargement	1.00E00 - 5.55E-04	36

Hepatotoxicity

Name	p-value range	# Molecules
Liver Inflammation/Hepatitis	3.58E-01 - 6.40E-09	41
Liver Damage	3.58E-01 - 1.21E-07	35
Hepatocellular Carcinoma	5.87E-01 - 1.26E-04	65
Liver Hyperplasia/Hyperproliferation	5.87E-01 - 1.26E-04	267
Liver Hyperbilirubinemia	7.11E-02 - 6.03E-04	5

Nephrotoxicity

Name	p-value range	# Molecules
Renal Necrosis/Cell Death	3.08E-01 - 6.77E-06	31
Kidney Failure	3.62E-01 - 2.45E-05	27
Renal Inflammation	5.87E-01 - 9.59E-05	27

Summary Tab

The tab (shown above) lists the top results for the different types of analyses that are included in Core Analysis. For this dataset:

- Several immune-related Canonical Pathways are involved, such as the Phagosome Pathway and the IL-12 Signaling and Production in Macrophages Pathway, and the TREM1 Signaling pathway is predicted to be activated.
- Key cytokines are activated as upstream regulators such as TNF and IL1B.
- Biological processes in the categories of Infectious Disease, Respiratory Disease, and Inflammatory Response are involved.
- Regulator Effects networks indicate that several upstream regulators appear to drive homing of leukocytes and adhesion of blood cells.
- Interaction networks are discovered that are involved in processes such as cell cycle and organismal injury.

The p-values associated with the results are indicated on a scale in the Summary with red dots. Many of the results represent categories that contain many sub-categories, so a cluster of red dots is shown with each red dot indicating the p-value for each sub-category.

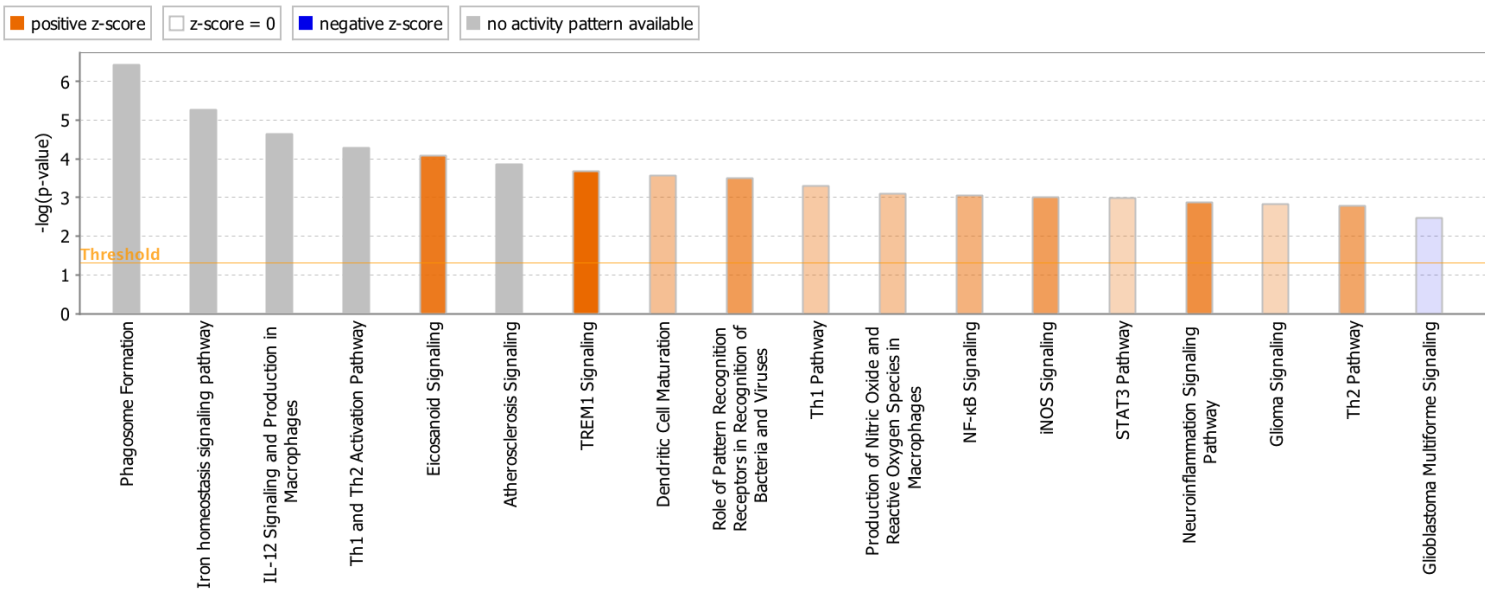
Note: The QIAGEN Knowledge Base is updated on a weekly basis and new pathways and other new assets are released quarterly. Details and information in these screen shots may not appear exactly the same as in the current version of this particular analysis.

Canonical Pathways tab

3) Click on the **Canonical Pathways** tab. Canonical Pathways provide information about what is known from the literature to occur on the cellular level in signaling and metabolic cascades.
The TREM1 Signaling pathway is predicted to be activated (it has an orange colored bar in the bar chart) based on the expression pattern of the genes in this dataset. TREM1 is an important cell surface signaling molecule involved in the immune response. Click on the orange bar to display results from the TREM1 Signaling pathway.

The **upper pane** (see the graphic below) shows a bar chart where the height of the bars indicates the significance of the overlap of the molecules in your dataset to the pathways in the QIAGEN Knowledge

Base. Significance values calculated based on the Fisher's right-tailed exact test and the -log(p-value) are displayed on the y-axis of the bar chart. The taller the bar, the more significant the overlap of your dataset with the pathway. See Canonical Pathways for a Dataset to learn more about the calculations that are used. The color of the bars indicates whether the pathway is predicted to be activated (orange bars) or inhibited (blue bars), or if the pathway is *ineligible* for such an assessment (gray bars). See Pathway Activity Overlay for more details on the z-score calculation used to color the bars.



The lower pane:

Displays the molecules from your dataset that are members of the TREM1 pathway (because you clicked on that bar in the bar chart in the previous step). Note that some genes from the dataset may be members of groups or complexes that are on the pathway.

10 molecule(s) associated with **TREM1 Signaling** at **Childhood exacerbated asthma GSE16032 with FDR GEO2R** [Ratio: 10/70 (0.143)] [z-score: 2.53] [p-value: 2.08E-04]

ADD TO MY PATHWAY | ADD TO MY LIST | CREATE DATASET | CUSTOMIZE TABLE

Symbol	Entrez Gene N...	Identifier	Measurement		Add/Remove column(s)	Expected	Location	Type(s)	Bi
		Affymetrix	Expr False Disc...	Expr p-value	Expr Log Ratio				
AKT2*	AKT serine/threonine	236664_at*	3.93E-02	4.91E-04	-0.466	Up	Cytoplasm	kinase	
FCGR2B	Fc fragment of IgG re	210889_s_at	3.67E-02	4.19E-04	1.052	Up	Plasma Membrane	transmembrane re...	
IL10	interleukin 10	207433_at	2.44E-02	1.81E-04	0.858	Up	Extracellular Space	cytokine	di
NLRC4*	NLR family CARD do	1552553_a_at*	1.14E-02	4.16E-05	1.810	Up	Cytoplasm	other	
PLCG2*	phospholipase C gar	204613_at*	2.02E-02	1.23E-04	0.614	Up	Cytoplasm	enzyme	
TLR2	toll like receptor 2	204924_at	1.18E-02	4.45E-05	1.522	Up	Plasma Membrane	transmembrane re...	di
TLR4*	toll like receptor 4	232068_s_at*	1.03E-02	3.44E-05	1.828	Up	Plasma Membrane	transmembrane re...	ef
TLR6*	toll like receptor 6	239021_at*	4.97E-02	8.24E-04	0.614	Up	Plasma Membrane	transmembrane re...	
TLR8*	toll like receptor 8	229560_at*	6.41E-03	8.66E-06	1.903	Up	Plasma Membrane	transmembrane re...	
TREM1	triggering receptor e	219434_at	1.60E-02	7.74E-05	1.012	Up	Plasma Membrane	transmembrane re...	ef

Selected/Total molecules: 0 / 10

Note that the overall z-score for the TREM1 pathway is 2.53, indicating that it is likely activated (because positive values >2 are considered activating).

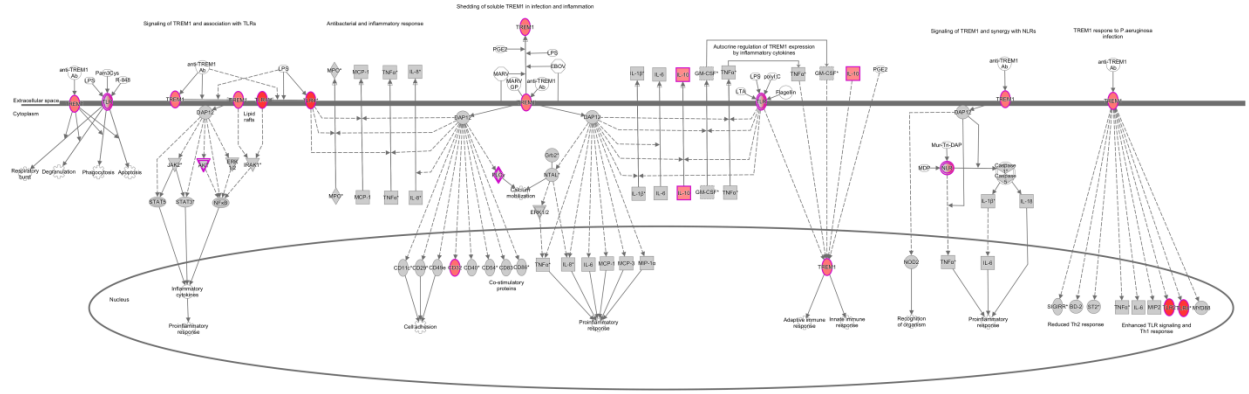
The table conveys the following information that can be useful in interpreting the results of your experiment:

- The Gene Symbol and Entrez Gene name, which identifies the molecules in your dataset that overlap (e.g. participate in) that particular pathway.
- The observed expression changes in the dataset, in this example, Expr False Discovery Rate, Expr p-value, Expr Log Ratio.
- The identifier uploaded from the dataset (Affymetrix IDs in this example).
- The expected "direction" (up or down regulated) for the molecule in the pathway (from the point of view of the activated state of the pathway). This column is available only for pathways that are eligible for Pathway Activity Analysis (see the Eligible Pathways List).
- The predominant cellular location of the molecule, and the molecule type.
- If the molecule has been identified as a biomarker it will indicate the type of biomarker (diagnosis, efficacy, etc.), which is helpful in determining if the molecule has been studied in a particular disease state that is relevant to the one your are studying.
- If there are any drugs that target a given molecule, information on how and in what biological context the molecule has been targeted.

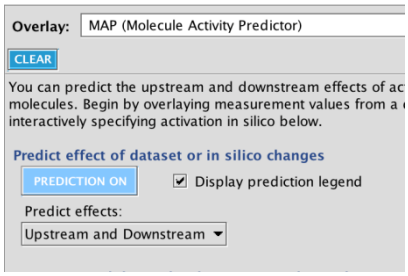
If the pane is too small, you can drag the vertical partition bar up to adjust it.

4) Click the **Open Pathway**button at the top right of the lower pane. A new window displays the canonical pathway diagram.

- Canonical pathways are usually directional, following the biological information flow in the cell. Arrows and top to bottom flow indicate upstream and downstream location, respectively.
- Dataset molecules that meet the filters and data value cutoff criteria for up- and down-regulation are shaded red and green, respectively.
- Dataset molecules that do not meet the cutoff or filter criteria are shaded grey in the pathway.
- Pathway molecules that are not in your dataset are white.
- The shapes and positions of the molecules in the pathway define gene type and cellular location, respectively. See Pathway Legend.
- Double outlined shapes represent groups of molecules (protein families, or protein complexes). Groups can be multicolored to indicate they include both up- and down-regulated molecules as their members. Right click and choose "Show Members/Membership" to expand groups to see their individual members.
- The pathway shows the biological picture of the activation of the TREM1 pathway, indicating that not only are several of the key receptors up-regulated in asthma, so are a number of downstream effectors.



- 5) To get a causal picture of the biology represented in the pathway, go to **Overlay > MAP (Molecule Activity Predictor**) and click the Start Prediction button.



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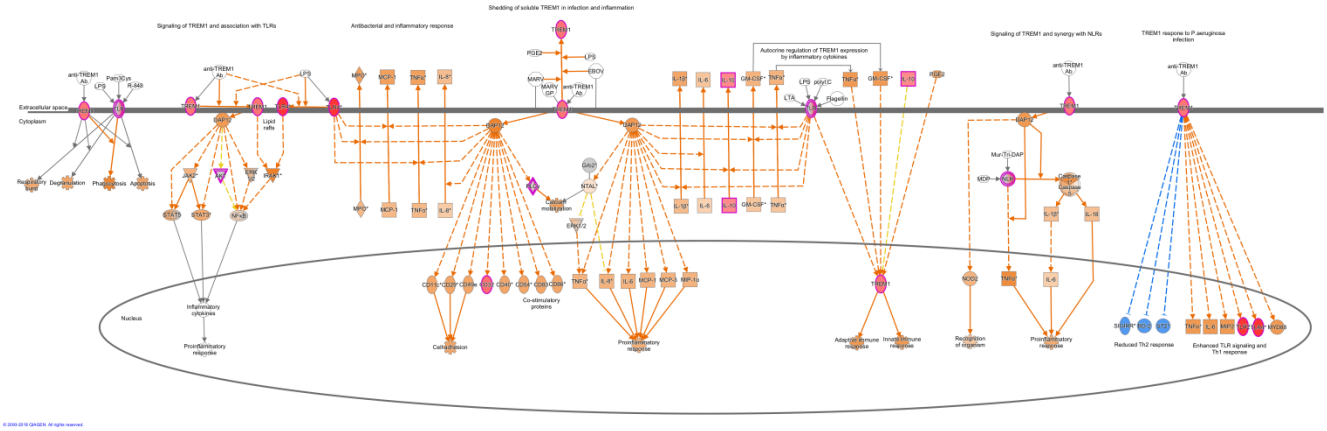
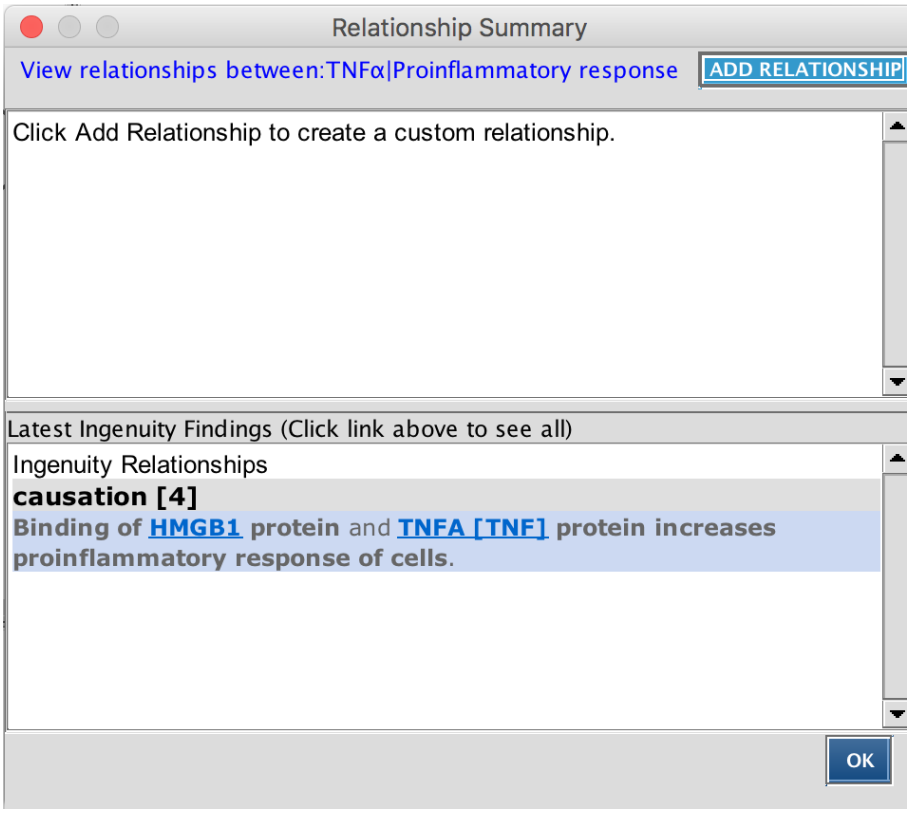
This predicts the activity of nodes on the pathway that are not part of your dataset-- i.e. it colors as many gray or white nodes in the pathway orange (activated) or blue (inhibited) as possible based on the expected influence of the molecules in the dataset that are up or down regulated (i.e. the red and green molecules on the pathway). For example, if a gene is upregulated in your dataset, and the literature states that it activates a downstream gene that it is connected to in the pathway diagram, then that downstream gene is predicted to be activated. Orange nodes indicate predictions of activation, and blue nodes indicate prediction of inhibition.



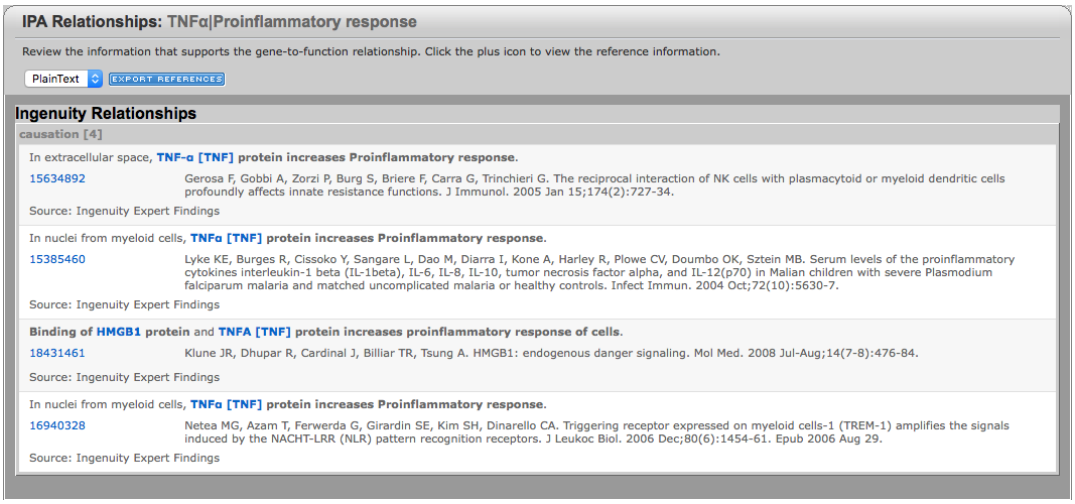
Zooming in on the bottom of the pathway, you can see that several biological functions related to immunity are predicted to be increased in the pathway. For example, both adaptive and immune responses as well as pro-inflammatory response are predicted to be increased.

6) Double click on any relationship on the pathway to view the curated findings and the literature support for it. Clicking on the line between TNFa and the biological function "Proinflammatory response" brings up a relationship summary, which shows the most recently added findings.



Click on the blue hyperlink at the top of the relationship summary to bring up a full listing of

the relationships:



- If desired, click the blue hyperlinks at the left side of each finding to go the NCBI record for the paper.

7) Click back to the main Core Analysis window then click VIEW REPORT bottom below the Canonical Pathway bar chart. This will open a web page with a detailed description of the TREM1 pathway.

The pathway report discusses the role of the TREM1 pathway in immune function, and shows that several of the proteins on the pathway are targets in drug trials for asthma: