Published in final edited form as:
Nat Protoc. 2013 August ; 8(8): 1551–1566. doi:10.1038/nprot.2013.092.

Large-scale gene function analysis with PANTHER Classification System

Huaiyu Mi[*], **Anushya Muruganujan**, **John T. Casagrande**, and **Paul D. Thomas**
Division of Bioinformatics, Department of Preventive Medicine, Keck School of Medicine, University of Southern California, Los Angeles, California, 90089, USA.

Abstract

PANTHER Classification System (www.pantherdb.org) is a comprehensive system that combines gene function, ontology, pathways and statistical analysis tools to enable biologists to analyze large-scale genome-wide experimental data. The system is built with 82 complete genomes organized into gene families and subfamilies, and their evolutionary relationships are captured in phylogenetic trees, multiple sequence alignments and statistical models (Hidden-Markov Models, or HMMs). Genes are classified by their function in several different ways: families and subfamilies are annotated with ontology terms (Gene Ontology and PANTHER Protein Class), and sequences are assigned to PANTHER pathways. The PANTHER website includes a suite of tools to allow users to browse and query gene functions, and analyze large-scale experimental data with a number of statistical tests. It is widely used by bench scientists, bioinformaticians, computer scientists and systems biologists. In the 2013 release of PANTHER (v.8.0), besides the update of the data content, the website interface was redesigned to improve the user experience and analytical capability. This protocol will provide a detailed description of how to analyze genome-wide experimental data in the PANTHER Classification System.

INTRODUCTION

The PANTHER Classification System (www.pantherdb.org) is designed to be a comprehensive platform for the analysis of gene function on a genome-wide scale[1]. Although its initial development aimed to classify gene and protein functions[2,3], it has evolved through the years to also serve as an online resource for experimental data analysis[4]. The easy-to-use user interface and timely user support have made PANTHER one of the most widely used online resources for gene function classification and genome-wide data analysis.

PANTHER was initially released in 2003, and was the first database to combine both phylogenetic and functional data to define protein subfamilies of shared function and

[*] Corresponding author: Huaiyu Mi, huaiyumi@usc.edu, Tel: 1-323-442-7994, Fax: 1-323-865-0103.

Author contributions
AM developed the software code for the website. JTC maintained the database and web servers. HM generated the content of the system, supervised the project. PDT provided funding and supervised the project. HM wrote the manuscript with contributions from all authors.

Competing financial interests
The authors declare that they have no competing financial interests.

sequences[3]. It was also the first database to associate ontology terms describing function to statistical models (HMMs), which can be used to assign genes -- based on sequence information alone -- to subfamilies and functional classes. The novel concept was to annotate not single genes one at a time, but rather subfamilies of related genes that are likely to share function. We demonstrated the accuracy and comprehensiveness of the classifications on the *D. melanogaster* genome[5]. In 2005, we began providing annotations of biochemical pathways, viewable with the new PANTHER Pathway Applet[6,7]. These pathways were curated from the literature by expert biologists and diagrams were drawn with CellDesigner, a pathway-editing tool using controlled graphical notations to represent pathway knowledge[8]. In 2006, the first version of gene analysis tools was released[4]. These were primarily for the analysis of gene expression data. The tools were also designed to handle gene list data from any genome wide experiments. In the 2013 release of PANTHER 8.0, the web interface was redesigned to integrate multiple tools into one user-friendly and flexible interface, so that users can easily access all the tools and choose among them to perform gene list analysis at the genome-wide level[1].

Since the initial release in 2003, PANTHER has become one of the most popular online resources for genome wide data analysis. Currently, there are over 7000 registered users, 600–800 daily users (weekdays) and over 14,000 monthly users worldwide. These counts were based on unique Internet Protocol (IP) addresses accessing the site, so the actual number is even greater. PANTHER has been cited in over 3600 publications since 2003 based on Google Scholar, and is growing steadily. Users have successfully analyzed the data from gene expression [9,10], proteomics [11,12] and genome-wide association study (GWAS) experiments[13,14] in a diverse area of research, such as cancer research [10,11], neurological disorder studies [15,16], autoimmune diseases [9] and cardiac disease studies[14,17].

PANTHER is currently included in a number of large international consortia. PANTHER has been a member of the InterPro Consortium of protein annotation resources since 2005, so PANTHER annotations can be automatically generated from the highly-used InterProScan software[18]. More recently, PANTHER also became part of the Gene Ontology (GO) Consortium[19–21]. The phylogenetic-inferred curation paradigm has become part of the GO curation pipeline, and therefore, PANTHER annotation reflects a more up to date GO curation[22]. Finally, PANTHER Pathway is in the process of being integrated into Pathway Commons[23].

The PANTHER System is constructed with three functional modules (Figure 1). The core module is a large protein library that contains all protein-coding genes from 82 organisms organized first into families based on sequence homology, and then into subfamilies based on their shared functions (often a group of orthologous genes). Each family or subfamily is represented by a statistical model (HMM) annotated with ontology terms (GO terms and PANTHER Protein Class terms). The second module is the PANTHER pathway module, which contains 176 expert curated pathways. All pathways are connected, through manual curation, to individual proteins in the protein library through the pathway components, and therefore they are also linked to the phylogenetic information and statistical models. The last module is the website tool suite that contains a collection of bioinformatics tools and software, enabling users to not only query the data and classify genes and proteins, but also visualize, analyze and interpret genome-wide scale experimental data in the context of the enriched data content in the first two modules.

PANTHER protein library

The core of the PANTHER system is a collection of phylogenetically-defined protein families and subfamilies generated by computational algorithms, and curated by expert biologist using an extensive software system for associating ontology terms[1,3]. The current release contains over 640K proteins from 82 genomes, of which 79 are from the Reference Proteome Project (http://www.ebi.ac.uk/reference_proteomes/) (Figure 1). UniProt IDs are used as primary protein identifiers. These proteins are representatives of their respective genes. Therefore, each gene is represented by only one protein. In addition, UniProt IDmapping (http://www.uniprot.org/mapping/) is used to map the primary protein IDs to other IDs from different databases and resources, which expands the capability of PANTHER to support a wider range of ID types (see Supported IDs in Box 1). The proteins are divided into 7729 families, each of which is represented by a phylogenetic tree, an HMM, and a multiple sequence alignment (MSA) (Figure 1). Protein family trees are constructed computationally from sequence data using a phylogenetic tree inference algorithm called GIGA[24]. Nodes in the tree, corresponding to common ancestors of extant family members, are annotated by expert biologists with their inferred GO terms and PANTHER protein class terms, based on experiments performed on extant proteins. Subfamilies are determined based on the tree structure and often define the orthologous group, especially in organisms in the Deuterostomia superphylum. The functional annotations are propagated from the annotated ancestral nodes to the subfamily nodes, each of which is also represented by an HMM to allow classification of newly discovered protein sequences (Figure 2A). In addition, the HMM library and the scoring pipeline allow users to analyze genome-wide data from any organisms outside of the 82 within the PANTHER system (see below). Phylogenetic tree and functional annotation at the ancestral nodes have great advantage in annotation of previously unclassified genes. The PANTHER phylogenetic trees are currently used by the Gene Ontology Consortium in its curation pipeline [22].

PANTHER pathway

The PANTHER pathway dataset uses controlled vocabulary and graphical notation to describe pathway knowledge[7]. The graphical representation of pathways is generated by CellDesigner, a pathway-editing tool[8], and is compliant with the Systems Biology Graphical Notation Process Description standard (SBGN-PD) [25] (Figure 2B). Currently, there are 176 expert-curated pathways in PANTHER. The scope of the pathways is similar to those described in textbooks or review articles, e.g., glycolysis, the PDGF signaling pathway or the p53 pathway. Each pathway contains three key classes of data (Figure 1). First is the pathway component (or molecule class), which represents a specific class of molecules that play the same mechanistic role within a pathway. It can be a protein (e.g., PDGF, Jak), a DNA region, or a simple molecule (e.g., ATP or glucose). If a pathway component is a protein, gene or transcribed RNA, it is associated with the protein sequences in the PANTHER protein library through manual curation (Figure 1 and 2). The individual protein sequences are instances of the pathway component. In these cases, a pathway component is typically a group of homologous/orthologous proteins across various organisms that are involved in the same biochemical reaction within the pathway. The second class is reaction, which represents biochemical relationships among different pathway components. Since all PANTHER pathways are in compliance with SBGN-PD, a typical reaction is a transition of an input (or reactant) to an output (a product) controlled by a modifier (Figure 2B). The last class is the cell/tissue type and cellular component, which provides the location where the reaction occurs.

There are two key features of the PANTHER pathway data model. First, the association between the molecule classes and protein sequences links the pathway to the phylogenetic relationships and statistical models (HMMs) of the protein families. As a result, if a protein is associated with a pathway component via experimental evidence, its orthologs can be inferred to the same component based on the PANTHER phylogenetic tree. Second, PANTHER pathway supports community standards, and all pathways are available in SBML[26], BioPAX[27] and SBGN[25] formats.

PANTHER tools

PANTHER provides a number of useful research tools, including the PANTHER HMM Scoring Tool, cSNP Analysis Tool, and Gene List Analysis Tool. Both the PANTHER HMM Scoring Tool and cSNP Analysis Tools have online and downloadable versions. The online versions allow only one protein sequence to be analyzed at a time, while the downloadable versions allow large batch analyses. A brief description of the downloadable version of PANTHER HMM Scoring Tool can be found in Box 2. The details of how to use these tools can be found in the PANTHER help page (http://www.pantherdb.org/help/PANTHERhelp.jsp) and the user manual. In this protocol, we will focus on the Gene List Analysis Tool. Sample gene list files can be found in the Supplement materials for testing.

The Gene List Analysis Tool can be accessed directly from the PANTHER home page (www.pantherdb.org) (Figure 3). There are several options for users to input a list of genes (and optionally quantitative data) for analysis. The PANTHER database uses some database identifiers as the primary IDs for each gene, and they are the most common option for input file (e.g., a UniProt identifier, an Entrez Gene identifier, or even a gene symbol). The PANTHER database also maps identifiers from a number of different sources for 82 different organisms using the UniProt IDmapping mechanism, and the identifiers in the user’s list are automatically mapped to the primary IDs in the PANTHER database (Figure 1). A total list of the supported IDs can be found in Box 1. A report is generated that lists not only the mapping of each gene, but also the identifiers that could not be mapped, if any. Since each gene in the PANTHER database belongs to a phylogenetic tree that is annotated with GO and PANTHER ontology terms and pathways, the mapped IDs from the gene list will inherit these annotations. It needs to be pointed out that, in a very rare case, a single gene symbol or its synonym can be mapped to more than one gene from the IDmapping. We are currently working with UniProt to improve such mappings. However, this is so rare that we don’t think it affects significantly to the results from the statistical tests. If the gene list is not from the 82 organisms, a user can still use the PANTHER tools, but they need to map each of their own identifiers to PANTHER identifiers first, using the downloadable PANTHER HMM scoring tool (Figure 1 and Box 2), and creating the Generic PANTHER

Mapping File with two columns (the user's ID, and the ID of the best PANTHER HMM hit) (see Box 1 for more details of the file format). Each gene in the list will inherit the same annotations as those stored in the PANTHER database for the given HMM.

Once the gene list is classified with those functional terms, it can be analyzed in three different ways.

- Functional classification – This tool provides the functional classification results of the uploaded list, and displays them in either a gene list page or pie chart.
- Statistical overrepresentation test - This tool is based conceptually on the simple binomial test described previously[28]. It compares a test gene list uploaded by the user to a reference gene list, and determines whether a particular class (e.g., a GO biological process or PANTHER pathway) of genes is over- or under-represented. A more detailed description of the tool can be found in Box 3.
- Statistical enrichment test - This tool, based on the work by the PANTHER group[29] and Lander's group[30] for two different genomic data analyses at about the same time, uses the Mann-Whitney test[31] to determine if any ontology class or pathway has numeric values that are non-randomly distributed with respect to the entire list of values. The numerical data can be normalized raw readouts from the microarray experiments or, more commonly, the fold-change value for each gene in a differential expression experiment, or calculated p-values from GWAS experiment. A more detailed description can be found in Box 4.

It is worth pointing out that there are other similar tools to perform overrepresentation and enrichment tests, most notably, GSEA[32] and DAVID[33]. Although all three tools use very similar statistical algorithms in the back end, there are some differences among them. GSEA requires download and installation, so its targeted users are bioinformaticians who are more computer-savvy. Both DAVID and PANTHER are online tools and are more appealing to bench biologists. Compared to DAVID, PANTHER has a few advantages. First, PANTHER is currently part of the GO consortium, and integrates more updated GO curation data with the tools. In fact, DAVID downloads PANTHER data and integrate them in its analysis tools. Second, PANTHER allows users to analyze genome data from 82 organisms using the online tool, and any other organisms using the PANTHER scoring tool. Third, the phylogenetic trees in PANTHER protein library allow users to make more accurate ortholog prediction, and thus greatly enhance the capability of the tools. One weakness of PANTHER is that it cannot analyze data against resources outside of PANTHER, such as KEGG and Reactome.

Workspace

The Workspace is a unique feature in PANTHER that allows users to store their gene lists that they generate for future analysis. Although users do not have to register to use the PANTHER system, registration is required in order to user the workspace. Registration is free. In any PANTHER gene list display page, the user can easily send the list to the Workspace (see below).

MATERIALS

Equipment

Laptop or desktop computer with internet connection. High-speed internet connection is highly recommended.

System Requirements

- Operating System
  - Windows XP or Windows 7 for PC users
  - MacOS 10.5 or later for Mac users
  - Minimum of 2GB RAM recommended
- Browser
  - Microsoft Internet Explorer 8 or later (recommended for PC users)
  - Safari version 5 (recommended for Mac users)
  - Firefox version 19
  - Google Chrome version 26
- Java
  - Latest version of Java (can be downloaded from http://www.java.com/en/download/)
  - JavaScript, Java applets and cookies must be enabled in your browser
  - Java applet runtime parameters set to -ms128m -mx512m -Xss16m

PROCEDURE

Note: Sample gene list files can be found in the Supplement materials for you try the tools.

1. Access the PANTHER website by entering www.pantherdb.org in your web browser.

2. Prepare input file(s) according to option A if you are working with one of the 82 genomes in the PANTHER database, or option B if you are working with an organism other than the 82 genomes in the database.

   a. Prepare input file(s) in simple text file (.txt or .tab) with gene or protein identifiers as the first column, and numeric values as the second column if you want to use the statistical enrichment test. Detailed instruction of file format and supported IDs can be found in Box 1. (Timing: 15–30 minutes)

   b. Prepare input file(s) by mapping your sequence identifiers to the PANTHER HMM IDs using the procedure described in Box 2. (Timing: see Box 2 for details)

!! CRITICAL STEP The input file must be in simple text file format (.txt or .tab). It also must use IDs supported in the PANTHER system and the correct tab delimited format as described in Box 1.

3. Upload the gene list to the PANTHER tool system using option A if you have a small list for functional classification tool, option B if you have a large list and want to analyze using all the gene list analysis tools, or option C if you previously saved the list in the Workspace.

   a. Paste the ID list prepared in Step 2 into the Enter ID box. Alternatively you can also type IDs, one per line, into the box (blue arrow in Figure 3). Please note that this type of ID upload only supports *functional classification tools*.

   b. Upload the list file prepared in Step 2 by clicking the *Browse* button (red arrow in Figure 3), and follow the online instruction to locate the file.

   c. If you have previously saved your list into the Workspace, you can use it by clicking the *login* link (green arrow in Figure 3), and follow the online instruction to locate the file in the Workspace. Please note that numeric values cannot be saved in the Workspace, and therefore this type of upload does not support the *Statistical enrichment test*.

4. Select a corresponding list type in order for the tool to work properly. There are three list types that are supported by the tools: ID List, Previously exported text search results, and PANTHER Generic Mapping File. See Box 1 for details of the list types.

5. Select an organism from the drop-down menu, which lists the 12 model organisms first, followed by the rest 70 organisms ordered alphabetically.

6. Note: There are two purposes to select an organism at this point. First, some identifiers, such as gene symbols, are not organism specific. By selecting an organism here, it ensures that the IDs are mapped to those in the organism you are interested in. Second, if the statistical overrepresentation test is selected, the default reference gene list is based on the selected organism.

7. In the Select Analysis box, select one of the following 4 analyses by clicking the radial button, and then click the *submit* button.

   a. Select Functional classification viewed in gene list if you want to find out the functional classification of the genes in your list (Figure 4). See the next section for more details about how to interpret the results.

   b. Select *Functional classification viewed in pie chart* to get the functional classification of the genes in your list displayed as a pie chart (Figure 5).

   c. Select *Statistical overrepresentation test*.

i. On the next page (Figure 6), you can select additional gene lists for the analysis. A total of 4 test gene lists can be uploaded for this tool. To do so,

- **a.** Click *Browse* button
- **b.** Select the gene list from your computer
- **c.** Select the organism. The default is the organism selected when the first gene list is uploaded.
- **d.** Select the *List type*. The default is the one selected when the first gene list is uploaded.
- **e.** Click *Upload list*.

ii. After all lists are uploaded, click the *Finish selecting lists* button. The tool will take you to the next page, which allows you to make the following selections.

iii. Select a reference list. The default is always the entire proteome of the organism selected above. If you choose to change it, click the *Select reference list* button.

iv. You can then select a reference list from another organism, or upload your own using an interface similar to one to upload test gene list.

v. Select an ontology to analyze. There are five options.

- **a.** PANTHER Pathway
- **b.** GO molecular function
- **c.** GO biological process
- **d.** GO cellular location
- **e.** PANTHER protein class

vi. Multiple test correction (Bonferroni correction) is selected by default.

vii. Click the *Launch analysis* button.

viii. On the results page (Figure 7A), you can visualize the results with the following options:

- **a.** You can export the result table in a tab-delimited file by clicking the *Export results* button.
- **b.** You can also view the results in graphs by using the *View* drop-down menu.
- **c.** If your analysis is done in pathway as shown here, you can click the pathway name and display the

pathway diagram. The pathway components that have genes in your test list will be highlighted. The color of the highlighted component can be defined at the top of the page (Figure 7A, red circle). A total of 4 test lists can be analyzed and viewed at the same time.

d. Select *Statistical enrichment test*

i. After clicking the *submit button,* on the next page, select an ontology to analyze.

ii. Click the *Launch analysis* button.

iii. On the results page (Figure 8A), you can visualize the results with the following options:

a. You can export the result table in a tab-delimited file clicking the *Export results* button.

b. You can compare the distribution curve in graph view. To do so, you can check the box in front of the category or pathway of your interest, and then click the *Graph selected categories* button (Figure 8B).

c. If your analysis is done in pathway as shown here, you can click the pathway name and display the pathway diagram. The pathway components are colored in "heat-map" based on the input numeric values (Figure 8C). Click the *Specify color ranges of pathway diagrams* button on the result page to view or specify the color ranges.

Note: In order to use this tool, make sure that the uploaded gene list contains a second column with numerical values.

Timing

Step 1, launch website: instant.

Step 2A, prepare input file: 15–30 minutes

Step 2B, prepare input file by scoring the PANTHER HMM library: varies based on the number of sequences. On average, 180 sequences per hour, plus 1.5–3.5 hour tool installation.

Step 3–6, using online tools: 5 minutes.

TROUBLESHOOTING

Table 1 lists some of the common problems and the possible solutions.

ANTICIPATED RESULTS

A. Functional classification tool viewed in gene list page

This tool returns the results as a gene list webpage (Figure 5). The page displays all the IDs from the uploaded gene list and their mapped PANTHER sequence IDs as well as ontology and pathway terms. The page contains the following information (Figure 5).

- Gene ID – This is the identifier for genes in the PANTHER library. The format is as follows: organism|gene database source=gene id|protein database source=protein id. For example, HUMAN|ENSEMBL=ENSG00000111262|UniProtKB=Q09470 is a human se- quence, the gene sequence is from ENSEMBL with id ENSG00000111262, and the protein sequence is from UniProt with id Q09470.
- Mapped IDs – IDs from the uploaded gene list that are mapped to the gene ids in the first column.
- Gene Name/Gene Symbol – The Entrez gene definition and gene symbol.
- PANTHER Family/Subfamily – The name and identifier of the PANTHER family or subfamily where the gene in the first column is in.
- GO Molecular Function, Biological Process, Cellular Component: These are Gene Ontology terms from PANTHER GO Slim describing the function of the gene product.
- PANTHER Protein Class – This is a PANTHER Index terms describing protein classes.
- Pathway – Pathway and pathway component that are linked to the PANTHER subfamily in column 4. The subfamily is linked to the pathway component when at least one of its member genes is associated to the component directly by manual curation.
- Species – The organism of the gene in Column 1.

Once you reach the gene list page, you can make following changes to view the results.

- Sort the list – You can always sort the list by clicking on any of the underlined column names. A yellow triangle appears in front of the column name that you choose to sort. The orientation of the triangle indicates the sort is ascending or descending.
- Customize columns – You can click on the “x” button next to the column names to collapse the column.
- Converting a list to another list type – Select the genes you want to convert by clicking the checkboxes. The default is for all genes in the list.
- Saving the list. Select the genes you want to save by clicking the check- boxes. The default is for all genes in the list. You can select one of the followings from the pull-down menu as the destination:

  - Workspace – You need to register to save data to the workspace. The registration is free. When you make this selection, a pop-up window will ask you to name the list and add any comments. The name and comments can be edited at any time in the future from the Workspace page. Once the gene list is saved in your workspace, it can be returned to at any time. Only the IDs are stored, and they are mapped to the internal PANTHER gene ids, so when you access a list in the future, all information will be updated and current.

  - Exporting a list to a file – The list will be exported as a tab-delimited file. You can now import the file into Excel or perform any post-processing you wish.

  - View the list as text on the website.

- Use the pie chart view by clicking the colorful pie chart icon (see Glossary for the meaning of the abbreviations). See the next section below for details about how to interpret the pie chart.

B. Functional classification tool viewed in pie chart

This tool returns the results in a pie chart, which displays an overview of all ontology terms at the first (or most general) level within the same ontology (Figure 6). When a slice of the pie chart, which represents an ontology term, is clicked, a new pie chart will appear that contains its child ontology terms. Since one gene can be classified to more than one term, the pie chart is calculated based on the number of “hits” to the terms over the total number of class hits. Class hit means independent ontology terms. For example, if a gene is classified to 2 ontology terms that are not parent or child to each other, it counts as 2 class hits.

When you place the computer mouse pointer over a slice, the category name and a series of counts are displayed. In our example in Figure 6, the name is a GO term *apoptosis (GO: 0006915)* followed by

1. the number of genes (70) from the uploaded list that are classified to the term *apoptosis*;
2. the percent (14%) of genes classified to *apoptosis* (70) over the total number of genes (500);
3. the percent (4.7%) of genes classified to this *apoptosis* (70) over total number of class hits (1494).

C. Statistical overrepresentation test

The results of this analysis tool are displayed in a table (Figure 7A). If one test gene list is uploaded, the table contains six essential columns of data:

1. The first column contains the name of the PANTHER classification category. If you are doing this analysis in pathways, you can click on the pathway name to view the corresponding pathway diagram.

2. The second column contains the number of genes in the reference list that map to this particular PANTHER classification category.

3. The third column contains the number of genes in your uploaded list that map to this PANTHER classification category.

4. The fourth column contains the expected value (see Box 3), which is the number of genes you would expect in your list for this PANTHER category, based on the reference list.

5. The fifth column has either a + or −. A plus sign indicates over-representation of this category in the test list: you observed more genes than expected based on the reference list (for this category, the number of genes in your list is greater than the expected value). Conversely, a negative sign indicates under-representation, i.e. fewer genes than expected.

6. The sixth column is the p-value as determined by the binomial statistic (see Box 3). This is the probability that the number of genes you observed in this category occurred by chance (randomly), as determined by your reference list. A small p-value indicates that the number you observed is significant and potentially interesting. A cutoff of 0.05 is recommended as a starting point.

If more than one test list is uploaded, columns 3 to 6 are repeated for each list.

From this result page, various statistics can be exported by using the drop-down menu next to the “Export results” button. The list of genes/proteins in any functional group can be viewed by clicking on the listed counts. When pathways are chosen as the functional categories, clicking on the pathway name brings up pathway diagrams colored according to preferences specified by the user (Figure 7B). The resulting pathway diagram can be exported as an image file (.png) by choosing “File -> Export image” from the Applet menu.

D. Statistical enrichment test

The returned results are displayed in a table with four essential columns of data (Figure 8A):

1. The first column contains the name of the PANTHER classification category. If you are doing this analysis in terms of pathways, you can click on the pathway name to view the pathway diagram. The genes in the pathway diagram are colored according to the numeric value provided in the uploaded gene list file, and the rules for this can be specified by clicking on the ‘Specify color ranges’ button.

2. The second column contains the number of genes that map to this particular PANTHER classification category.

3. The third column has either a + or −. A plus sign indicates that for this category, the distribution of values for your uploaded list is shifted towards greater values than the overall distribution of all genes that were uploaded. A negative sign indicates that the uploaded list is shifted towards smaller values than the overall list.

4. The fourth column contains the p-value as calculated from the Mann-Whitney U Test (Wilcoxon Rank-Sum test) (Box 4). A large p-value indicates that the genes for this category have a distribution that is similar to randomly choosing genes from the overall distribution. In other words, the values of the uploaded genes for this category have a similar distribution to the overall list of values that were input. A small, significant p-value indicates that the distribution for this category is non-random and different than the overall distribution. A cutoff of 0.05 is recommended as a starting point.

To have a visual representation of these distributions, select the checkboxes of the categories of interest, and click on the ‘Graph selected categories’ button. The graph will be displayed in a new window (Figure 8B). The x-axis is your uploaded value. The y-axis is the cumulative fraction. The blue curve is the overall distribution for all genes. The red curve is the selected functional category. In this case, it is PDGF signaling pathway. If you look at the data point x = −2.5, y is 0.3 for the red curve and 0.1 for the blue curve. This means that 30% of your uploaded genes have a value of −0.25 or smaller, but only 10% of the overall genes have a value of −0.25 or smaller. In other words, it shows that the distribution of the category tends to be smaller than the overall distribution. We find that this is critical for interpreting any deviation between the functional category distribution and the overall distribution.

The genes/proteins in each category can also be viewed from the output page by clicking on the listed counts. In addition, for pathways, clicking on the pathway name will bring up an interactive Java applet that colors the pathway using a “heat map” derived from the input values (Figure 8C). The resulting pathway diagram can be exported as an image file (.png) by choosing “File -> Export image” from the Applet menu.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

The authors would like to thank the Reference Proteome team, especially Craig McAnulla and Maria Martin, for their support to provide up-to-date Reference Proteome dataset, and Yukiko Matsuoka and Katoh Manami from the SBI Japan for their support on CellDesigner and pathway file update. This work is supported by NIH/NIGMS GM081084 to P.D.T.]. Funding for open access charge: University of Southern California.

Glossary

BioPAX	Biological Pathway Exchange format
BP	biological process or GO biological process
CC	cellular component or GO cellular component
cSNP	coding SNP
HMM	Hidden Markov Model

GO	gene ontology
GWAS	genome-wide association study
ID	identifier
IP	internet protocol
MF	molecular function or GO molecular function
MSA	multiple sequence alignment
NGS	next generation sequencing
PANTHER	Protein Annotation through Evolutionary Relationship
PC	protein class or PANTHER protein class
PDGF	platelet-derived growth factor
Png	portable network graphics
SBGN	Systems Biology Graphical Notation
SBGN-PD	Systems Biology Graphical Notation Process Description
SBML	Systems Biology Markup Language
SNP	single nucleoside polymorphism
VEGF	vascular endothelial growth factor

REFERENCES

1. Mi H, Muruganujan A & Thomas PD PANTHER in 2013: modeling the evolution of gene function, and other gene attributes, in the context of phylogenetic trees. Nucleic Acids Research 41, D377–D386 (2013). [PubMed: 23193289]
2. Venter JC, Adams MD, Myers EW, Li PW, et al. The sequence of the human genome. Science 291, 1304–1351 (2001). [PubMed: 11181995]
3. Thomas PD, Campbell MJ, Kejariwal A, Mi H, et al. PANTHER: a library of protein families and subfamilies indexed by function. Genome Res 13, 2129–2141 (2003). [PubMed: 12952881]
4. Thomas PD, Kejariwal A, Guo N, Mi H, et al. Applications for protein sequence-function evolution data: mRNA/protein expression analysis and coding SNP scoring tools. Nucleic Acids Res 34, W645–W650 (2006). [PubMed: 16912992]
5. Mi H, Vandergriff J, Campbell M, Narechania A, et al. Assessment of genome-wide protein function classification for Drosophila melanogaster. Genome Res 13, 2118–2128 (2003). [PubMed: 12952880]
6. Mi H, Lazareva-Ulitsky B, Loo R, Kejariwal A, et al. The PANTHER database of protein families, subfamilies, functions and pathways. Nucleic Acids Res 33, D284–D288 (2005). [PubMed: 15608197]
7. Mi H & Thomas P PANTHER pathway: an ontology-based pathway database coupled with data analysis tools. Methods Mol Biol 563, 123–140 (2009). [PubMed: 19597783]
8. Funahashi A, Matsuoka Y, Jouraku A, Morohashi M, et al. CellDesigner 3.5: A Versatile Modeling Tool for Biochemical Networks. Proceedings of the IEEE 96, 1254–1265 (2008).

9. van Baarsen LGM, Bos WH, Rustenburg F, van der Pouw Kraan TCTM, et al. Gene expression profiling in autoantibody-positive patients with arthralgia predicts development of arthritis. Arthritis & Rheumatism 62, 694–704 (2010). [PubMed: 20131234]
10. Verma G, Bhatia H & Datta M Gene expression profiling and pathway analysis identify the integrin signaling pathway to be altered by IL-1β in human pancreatic cancer cells: role of JNK. Cancer Lett 320, 86–95 (2012). [PubMed: 22313544]
11. Boyer AP, Collier TS, Vidavsky I & Bose R Quantitative proteomics with siRNA screening identifies novel mechanisms of trastuzumab resistance in HER2 amplified breast cancers. Mol Cell Proteomics 12, 180–193 (2013). [PubMed: 23105007]
12. Stützer I, Selevsek N, Esterhazy D, Schmidt A, et al. Systematic proteomic analysis identifies beta-site amyloid precursor protein cleaving enzyme 2 and 1 (BACE2 and BACE1) substrates in pancreatic beta-cells. Journal of Biological Chemistry (2013).
13. Shi Y, Zhao H, Shi Y, Cao Y, et al. Genome-wide association study identifies eight new risk loci for polycystic ovary syndrome. Nature Genetics 44, 1020–1025 (2012). [PubMed: 22885925]
14. den Hoed M, Eijgelsheim M, Esko T, Brundel BJJM, et al. Identification of heart rate–associated loci and their effects on cardiac conduction and rhythm disorders. Nature Genetics (2013).
15. Feng J, Zhou Y, Campbell SL, Le T, et al. Dnmt1 and Dnmt3a maintain DNA methylation and regulate synaptic function in adult forebrain neurons. Nat Neurosci 13, 423–430 (2010). [PubMed: 20228804]
16. Hek K, Demirkan A, Lahti J, Terracciano A, et al. A Genome-Wide Association Study of Depressive Symptoms. Biological Psychiatry 73, 667–678 (2013). [PubMed: 23290196]
17. Neely GG, Kuba K, Cammarato A, Isobe K, et al. A Global In Vivo Drosophila RNAi Screen Identifies NOT3 as a Conserved Regulator of Heart Function. Cell 141, 142–153 (2010). [PubMed: 20371351]
18. McDowall J & Hunter S InterPro protein classification. Methods Mol Biol 694, 37–47 (2011). [PubMed: 21082426]
19. Gene Ontology Consortium The Gene Ontology: enhancements for 2011. Nucleic Acids Res 40, D559–D564 (2012). [PubMed: 22102568]
20. Mi H, Dong Q, Muruganujan A, Gaudet P, et al. PANTHER version 7: improved phylogenetic trees, orthologs and collaboration with the Gene Ontology Consortium. Nucleic Acids Res 38, D204–D210 (2010). [PubMed: 20015972]
21. Reference Genome Group of the Gene Ontology Consortium The Gene Ontology’s Reference Genome Project: a unified framework for functional annotation across species. PLoS Comput Biol 5, e1000431 (2009). [PubMed: 19578431]
22. Gaudet P, Livstone MS, Lewis SE & Thomas PD Phylogenetic-based propagation of functional annotations within the Gene Ontology consortium. Brief Bioinform 12, 449–462 (2011). [PubMed: 21873635]
23. Cerami EG, Gross BE, Demir E, Rodchenkov I, et al. Pathway Commons, a web resource for biological pathway data. Nucleic Acids Research 39, D685–D690 (2010). [PubMed: 21071392]
24. Thomas PD GIGA: a simple, efficient algorithm for gene tree inference in the genomic age. BMC Bioinformatics 11, 312 (2010). [PubMed: 20534164]
25. Le Novere N BioModels Database - A Database of Annotated Published Models Available on the internet at: http://www.ebi.ac.uk/biomodels-main/static-pages.do?page=home (2011).
26. Hucka M, Finney A, Sauro HM, Bolouri H, et al. The systems biology markup language (SBML): a medium for representation and exchange of biochemical network models. Bioinformatics 19, 524–531 (2003). [PubMed: 12611808]
27. Demir E, Cary MP, Paley S, Fukuda K, et al. The BioPAX community standard for pathway data sharing. Nat Biotech 28, 935–942 (2010).
28. Cho RJ & Campbell MJ Transcription, genomes, function. Trends in Genetics 16, 409–415 (2000). [PubMed: 10973070]
29. Clark AG, Glanowski S, Nielsen R, Thomas PD, et al. Inferring nonneutral evolution from human-chimp-mouse orthologous gene trios. Science 302, 1960–1963 (2003). [PubMed: 14671302]

30. Mootha VK, Lepage P, Miller K, Bunkenborg J, et al. Identification of a gene causing human cytochrome c oxidase deficiency by integrative genomics. Proc Natl Acad Sci U S A 100, 605–610 (2003). [PubMed: 12529507]

31. Mann HB & Whitney DR On a Test of Whether one of Two Random Variables is Stochastically Larger than the Other. The Annals of Mathematical Statistics 18, 50–60 (1947).

32. Subramanian A, Tamayo P, Mootha VK, Mukherjee S, et al. Gene set enrichment analysis: a knowledge-based approach for interpreting genome-wide expression profiles. Proc Natl Acad Sci U S A 102, 15545–15550 (2005). [PubMed: 16199517]

33. Sherman B, Huang D, Tan Q, Guo Y, et al. DAVID Knowledgebase: a gene-centered database integrating heterogeneous gene annotation resources to facilitate high-throughput gene functional analysis. BMC Bioinformatics 8, 426 (2007). [PubMed: 17980028]

Box 1: Input file

File format

The input file is a tab-delimited text file (.txt or .tab). Only the data in the columns specified below will be used in the analyses. Data in additional columns are ignored. Microsoft Excel file is not accepted by the tool. Below are three file types that can be used.

1. ID list – The first column must be the gene or protein identifiers. See below for the supported IDs. A second column of numerical values is required if a user wants to run the *Statistical enrichment test*.

2. Previously exported text search results – A text search result can be viewed as a gene list, which can be saved as a text file (see Section A under Anticipated Results). This file contains the gene or protein identifiers in the first column. This file type is not associated with numeric values, so it cannot be used for *Statistical enrichment test*.

3. PANTHER Generic Mapping File – For IDs from organisms other than the 82 organisms in the PANTHER database, a user-generated data containing mappings between those IDs and their corresponding PANTHER IDs can be used (see Box 2 for details about mapping). The file must be tab-delimited and contain the following columns:

   - The first column can contain a list of unique IDs from the user.
   - The second column should be corresponding PANTHER family or subfamily ID (ex: PTHR10078 or PTHR10078:SF1), and is used to look up the association to GO and PANTHER terms (molecular function, biological process, and pathway).
   - If you are uploading data for the “Statistical enrichment test” tool, a third column is required that contains the numeric value of the experiment.

Supported IDs

If the “ID list” file type is used, the IDs in the first column of the file must be from one of the following databases that are supported in the PANTHER system.

- **Ensembl**: Ensembl gene identifier. Example: “ENSG00000126243”
- **Ensembl_PRO**: Ensembl protein identifier. Example: “ENSP00000337383”
- **Ensembl_TRS**: Ensembl transcript identifier. Example: “ENST00000391828”
- **Gene ID**: EntrezGene IDs. Examples: “10203” (for Entrez gene GeneID: 10203)
- **Gene symbol**: for example, “CALCA”
- **GI**: NCBI GI numbers. Example: “16033597”

- **HGNC**: HUGO Gene Nomenclature ids. Example: "HGNC:16673"
- **IPI**: International Protein Index ids. Example: "IPI00740702"
- **UniGene**: NCBI UniGene ids. Examples: "Hs.654587", "At.36040"
- **UniProtKB**:UniProt accession. Example: "O80536"
- **UniProtKB-ID**: UniProt ID. Example: "AGAP3_HUMAN"

If you are not certain about the ID type in your uploaded gene list, or when you find that your IDs are not mapped to any PANTHER IDs in the result page, you can simply search your ID at NCBI (http://www.ncbi.nlm.nih.gov/) or a search engine website such as Google. You can find the ID type based on the database source on the result page.

Box 2 PANTHER HMM Scoring Tool

This is the downloadable version of the PANTHER Scoring Tool to allow users to submit a large number of protein sequences in fasta file format, and score against the PANTHER HMM library so that the sequence identifiers can be mapped to PANTHER HMM IDs and used in the gene list analysis tools.

UNIX and Perl are required on your computer in order to use the tool. The user needs to have the basic knowledge of using UNIX and Perl in order to complete the procedures described in this Box. If you don't feel you have adequate knowledge in these areas, you may want to get help from your colleague who has the technical expertise and knowledge, such as a bioinformatics support person. You can also send email to feedback@pantherdb.org for help.

Procedures:

1. Download the following scripts and data.

  1. pantherScore script (ftp://ftp.pantherdb.org//hmm_scoring/current_release/).

  2. (Timing: 2 minutes)

  3. PANTHER HMM library (ftp://ftp.pantherdb.org/panther_library/current_release/)

  4. (Timing: 15 minutes)

  5. HMMER2 (ftp://selab.janelia.org/pub/software/hmmer/2.3.2/hmmer-2.3.2.tar.gz).

  6. (Timing: 5 minutes)

  7. Note: Please note that this is an archived version of HMMER2. The current release is HGMMER3. The panther scoring script does not support HMMER3.Install PANTHER scoring tool

  8. BLAST (ftp://ftp.ncbi.nlm.nih.gov/blast/executables/blast+/2.2.24/ncbi-blast-2.2.24+-x64-linux.tar.gz)

(Timing: 5 minutes)

4. Decompress all four downloads. (Timing: 30 minutes)

5. Install HMMER2 and BLAST according to the installation instruction coming with the two algorithms. (Timing: 15 minutes)

6. Define the location of the HMMER and BLAST binaries in the $PATH variables on your computer. This is usually done quite differently depending on the UNIX shell environment on your computer. Basically, you need to update the $PATH on the UNIX shell files, such as .cshrc (for C shell) or .profile (for Bourne shell). If you are not familiar with $PATH, you need to consult someone with IT knowledge to help you. (Timing: 15 minutes for expert, 1–2 hours for others).

7. Run the script on UNIX command line. (Timing: each sequence takes 20 seconds to score, therefore, 180 sequences in an hour)

   1. cd pantherScore

   2. source panther.cshrc

   3. ./pantherScore.pl -l <panther_hmm_library> -D B -V -i <fasta file> -o <output file> -n

where

-l The path to the PANTHER HMM library downloaded above.

-D display type for results.

Options: B (best hit), A (all hits)

-i input fasta file to score. A sample fasta file is included in the downloaded called test.fasta

-o the output file

-n to display family and subfamily names in the output file.

Note: If you have a lot of sequences, you can split the fasta file and run the script on multiple computers.

The output file is a tab-delimited file in the following format:

col 1 - sequence ID

col 2 - PANTHER accession if (containts :SF, is a subfamily HMM)

col 3 - PANTHER family or subfamily name

col 4 - HMM evalue score, as reported by HMMER

col 5 - HMM score, as reported by HMMER (not used by PANTHER)

col 6 - alignment range of protein for this particular HMM

This file can be used as PANTHER Generic Mapping File for the gene list analysis tool. If the Statistic enrichment test is used, the numeric values need to be inserted in the 3rd column.

Box 3

Statistic Overrepresentation Test

The input (or test) list is usually a list of genes of your interest. It can be a list of genes that are up-regulated in the gene expression experiment, or have significant p-values from your GWAS experiment. The list is divided into groups based on GO or PANTHER classification (molecular function, biological process, cellular component, PANTHER Protein Class or PANTHER Pathway). As many as four test lists can be uploaded for each analysis. A reference list, which usually contains all the genes/proteins from which the list was drawn, is divided into groups in the same way. PANTHER provides reference proteome data set as default reference lists for all 82 genomes, so uploading a reference list is optional. Then, for each functional category, e.g., protein kinase for molecular function, cell proliferation for biological process, or apoptosis signaling pathway for pathway, the binomial test is applied to determine whether there is a statistical over- or under-representation of genes/proteins in the test list relative to the reference list.

P-value calculation in the overrepresentation test

The *expected value* is the number of genes you would expect in the test list for a particular PANTHER category, based on the reference list. For example, there are 20,000 genes in the reference list (ex: the human genome). 440 of these genes map to the GO term *induction of apoptosis*. Based on this, 2.2% (440 divided by 20,000) of the genes in the reference list are involved in *induction of apoptosis*. Now a test list that contains 500 genes is uploaded. Based on the reference list, it is **expected** that 11 genes (500 multiplied by 2.2%) in the test list would be involved in *induction of apoptosis*.

If for this biological process more genes are observed in the test list than expected, you have an over-representation (+) of genes involved in *induction of apoptosis*. If fewer genes are observed than expected, you have an under-representation (−). A p-value is calculated then to determine whether the over- or under- representation is significant or not. For example, let’s assume that 21 genes are observed in the test list are involved in *induction of apoptosis*. Although this is almost twice as the expected value, the p-value is large and not significant (the p-value would be 0.722). Alternatively, if 35 genes are observed, this is very different than the expected value, so you would expect a small, significant p-value (the p-value would be 6.21e-7). This small p-value indicates that the result is non-random and potentially interesting, and worth looking at in closer detail. A p-value cutoff of 0.05 is recommended as a start point.

The statistical method used in this test is the binomial test. In the binomial test we assume that under the NULL hypothesis, genes in the test list are sampled from the same general population as genes from the reference set, i.e. the probability p(C) of observing a gene from a particular category C in the test list is the same as in the reference list. We first estimate the probability p(C) from the reference set assuming that it is large and representative:

$p(C)=n(C)/N,$

where n(C) is the number of genes mapped to category C, and N is the total number of genes in the reference set.

We then use the above estimate to find the p-value: the probability of observing k(C) genes (or a more extreme number) in the uploaded list of size K. Under the NULL hypothesis, the number of genes of mapped to C is distributed binomially with probability parameter p(C) and thus the p-value would be

$$p - value = \sum \binom{K}{k} p(c)^{k} (1 - p(c))^{K - k}$$

where the sum runs from k(C) to K in the case of over-representation (i.e. when the number of observed genes k(C) is greater than expected p(C)*K under the NULL hypothesis), and 0 to k(C), in the case of under-representation (i.e. when k(C) is smaller than p(C)*K).

When developing this analysis tool, we tested other statistic methods also. We decided to use the Binomial, since other methods tends to be not as accurate when the population sizes or the expect number is small.

Box 4

Mann-Whitney Rank-Sum Test (U-Test)

The statistical test is general enough to handle any numerical data, continuous or discontinuous, generated by experiments such as gene expression, proteomics or GWAS. First, a reference distribution is generated using all values from the input data. Then the entire list is divided into groups based on GO or PANTHER classification (molecular function, biological process, cellular component, PANTHER Protein Class or PANTHER Pathway), and the distributions for each group are generated. The probability that the functional category distribution was drawn randomly from the reference distribution is estimated using the Mann-Whitney Rank-Sum Test (U-Test) [29].

To perform the rank sum test, first the values of the genes that map to a given category are combined with the overall list of values that were input. Then, all the values are ranked from smallest to largest, with the smallest value getting a rank of 1. If multiple values are identical, the average of the ranks for these values is used.

Then the rank sum is calculated for this category, by summing up the ranks for all of the genes that map to this category. The average rank, R1 is then calculated by dividing the rank sum by the number of genes, n1, that map to the category. Likewise, the rank sum is calculated for the list of all IDs uploaded, and the average rank, R2, is calculated by dividing the rank sum by the total number of genes uploaded, n2.

Next, the Mann Whitney U statistic is calculated for both populations:

$U1 = n1 * n2 + (n1 * (n1 + 1)) / 2 - R1$

$U2 = n2 * n2 + (n1 * (n2 + 1)) / 2 - R2$

The larger of these two values is the Mann Whitney U-statistic, U, whose distribution for small sample sizes can be found in most statistic books. In our case, our application is for large sample sizes, so we use the normal approximation:

$\text{Z-score} = (U - (n1 * n2)/2)/\text{sqrt}(n1 * n2 * (n1 + n2 + 1)/12)$.

It follows that the p-value is the integral under the standard normal density.

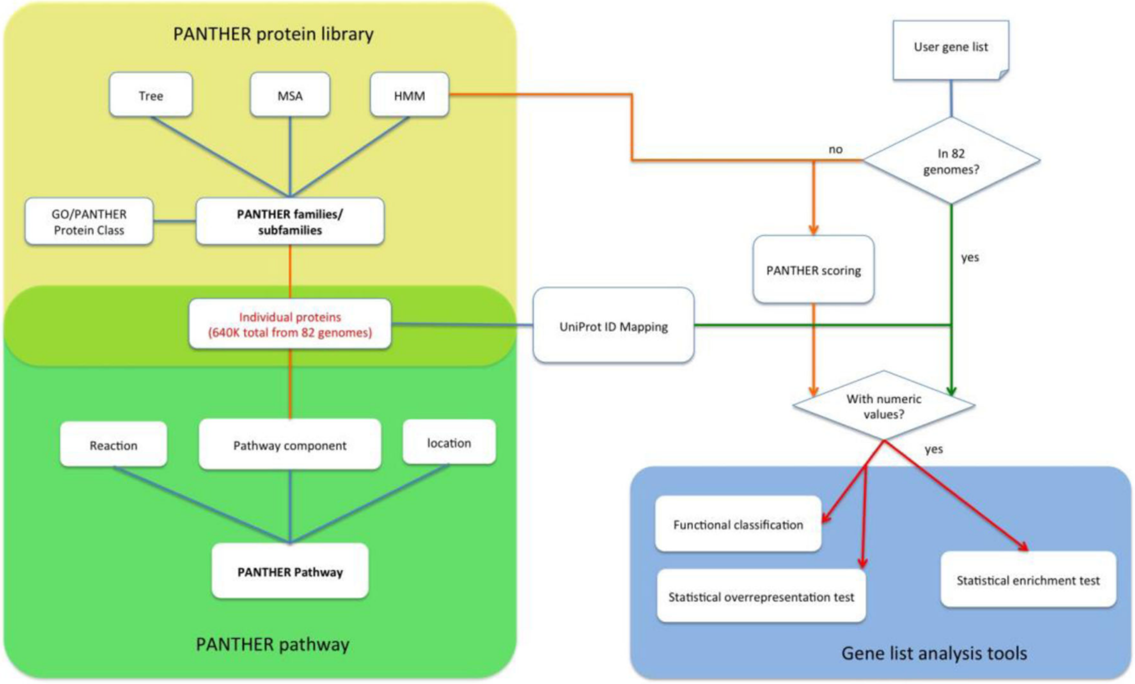
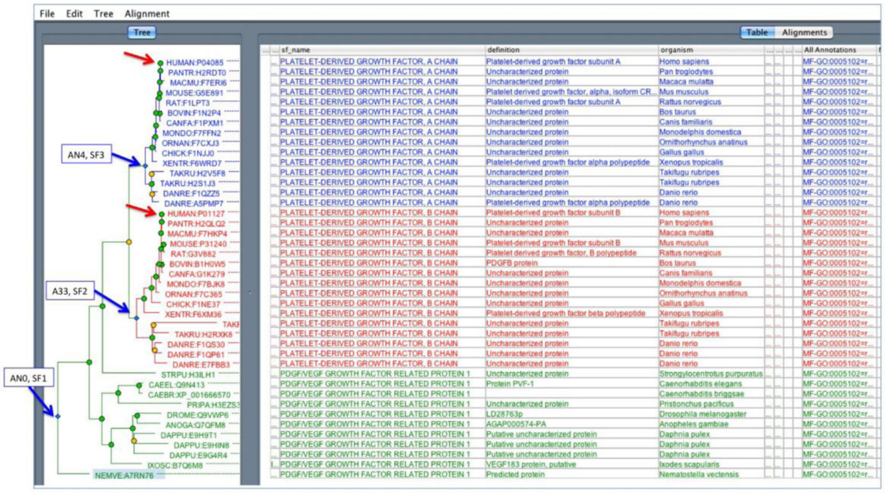


Figure 1. Overview of PANTHER infrastructure.

PANTHER is consisted of three modules. The core module is the PANTHER protein library (yellow shade) that contains a collection of PANTHER families and subfamilies, each of which is represented by a phylogenetic tree, an MSA and an HMM. The second module is the pathway that contains 176 expert-curated pathways (green shade). The pathway components are associated to the protein sequences that are used to build the protein library (the light green shade), and therefore, the pathways are also linked to the subfamilies and HMMs. The third module is the tool suite. In this diagram, the gene list analysis tool is used as an example (blue shade). When the user uploads a gene list to the tool, and if the IDs in the list are from one of 82 organisms in PANTHER, the tool will map the IDs to the IDs in the PANTHER protein library (green arrows). If the uploaded IDs are not from one of the 82 organisms, the user can score the sequences against the PANTHER HMM library and generate the PANTHER Generic Mapping file (see Box 2) (orange arrows). There are three tests in the tool: functional classification, statistical overrepresentation test and statistical enrichment test. Numeric values must be provided in order to use the statistical enrichment test.



	sf_name	definition	organism	All Annotations
HUMAN:P04085	PLATELET-DERIVED GROWTH FACTOR, A CHAIN	Platelet-derived growth factor subunit A	Homo sapiens	MF-GO:0005102=r...
PANTR:H2RDT0	PLATELET-DERIVED GROWTH FACTOR, A CHAIN	Uncharacterized protein	Pan troglodytes	MF-GO:0005102=r...
MACMU:F7ERI6	PLATELET-DERIVED GROWTH FACTOR, A CHAIN	Uncharacterized protein	Macaca mulatta	MF-GO:0005102=r...
MOUSE:G5E891	PLATELET-DERIVED GROWTH FACTOR, A CHAIN	Platelet derived growth factor, alpha, isoform CR...	Mus musculus	MF-GO:0005102=r...
RAT:F1LPT3	PLATELET-DERIVED GROWTH FACTOR, A CHAIN	Platelet-derived growth factor subunit A	Rattus norvegicus	MF-GO:0005102=r...
BOVIN:F1N2P4	PLATELET-DERIVED GROWTH FACTOR, A CHAIN	Uncharacterized protein	Bos taurus	MF-GO:0005102=r...
CANFA:F1PXM1	PLATELET-DERIVED GROWTH FACTOR, A CHAIN	Uncharacterized protein	Canis familiaris	MF-GO:0005102=r...
MONDO:F7FFN2	PLATELET-DERIVED GROWTH FACTOR, A CHAIN	Uncharacterized protein	Monodelphis domestica	MF-GO:0005102=r...
ORNAN:F7CXJ3	PLATELET-DERIVED GROWTH FACTOR, A CHAIN	Uncharacterized protein	Ornithorhynchus anatinus	MF-GO:0005102=r...
CHICK:F1NJJ0	PLATELET-DERIVED GROWTH FACTOR, A CHAIN	Uncharacterized protein	Gallus gallus	MF-GO:0005102=r...
XENTR:F6WRD7	PLATELET-DERIVED GROWTH FACTOR, A CHAIN	Platelet-derived growth factor alpha polypeptide	Xenopus tropicalis	MF-GO:0005102=r...
TAKRU:H2V5F8	PLATELET-DERIVED GROWTH FACTOR, A CHAIN	Uncharacterized protein	Takifugu rubripes	MF-GO:0005102=r...
TAKRU:H2S1J3	PLATELET-DERIVED GROWTH FACTOR, A CHAIN	Uncharacterized protein	Takifugu rubripes	MF-GO:0005102=r...
DANRE:F1QZZ5	PLATELET-DERIVED GROWTH FACTOR, A CHAIN	Uncharacterized protein	Danio rerio	MF-GO:0005102=r...
DANRE:A5PMP7	PLATELET-DERIVED GROWTH FACTOR, A CHAIN	Platelet-derived growth factor alpha polypeptide	Danio rerio	MF-GO:0005102=r...
HUMAN:P01127	PLATELET-DERIVED GROWTH FACTOR, B CHAIN	Platelet-derived growth factor subunit B	Homo sapiens	MF-GO:0005102=r...
PANTR:H2QLQ2	PLATELET-DERIVED GROWTH FACTOR, B CHAIN	Uncharacterized protein	Pan troglodytes	MF-GO:0005102=r...
MACMU:F7HKP4	PLATELET-DERIVED GROWTH FACTOR, B CHAIN	Uncharacterized protein	Macaca mulatta	MF-GO:0005102=r...
MOUSE:P31240	PLATELET-DERIVED GROWTH FACTOR, B CHAIN	Platelet-derived growth factor subunit B	Mus musculus	MF-GO:0005102=r...
RAT:G3V882	PLATELET-DERIVED GROWTH FACTOR, B CHAIN	Platelet derived growth factor, B polypeptide	Rattus norvegicus	MF-GO:0005102=r...
BOVIN:B1H0W5	PLATELET-DERIVED GROWTH FACTOR, B CHAIN	PDGFB protein	Bos taurus	MF-GO:0005102=r...
CANFA:G1K279	PLATELET-DERIVED GROWTH FACTOR, B CHAIN	Uncharacterized protein	Canis familiaris	MF-GO:0005102=r...
MONDO:F7BJK8	PLATELET-DERIVED GROWTH FACTOR, B CHAIN	Uncharacterized protein	Monodelphis domestica	MF-GO:0005102=r...
ORNAN:F7C365	PLATELET-DERIVED GROWTH FACTOR, B CHAIN	Uncharacterized protein	Ornithorhynchus anatinus	MF-GO:0005102=r...
CHICK:F1NE37	PLATELET-DERIVED GROWTH FACTOR, B CHAIN	Uncharacterized protein	Gallus gallus	MF-GO:0005102=r...
XENTR:F6XM36	PLATELET-DERIVED GROWTH FACTOR, B CHAIN	Platelet-derived growth factor beta polypeptide	Xenopus tropicalis	MF-GO:0005102=r...
TAKI	PLATELET-DERIVED GROWTH FACTOR, B CHAIN	Uncharacterized protein	Takifugu rubripes	MF-GO:0005102=r...
TAKRU:H2RXK8	PLATELET-DERIVED GROWTH FACTOR, B CHAIN	Uncharacterized protein	Takifugu rubripes	MF-GO:0005102=r...
DANRE:F1QS30	PLATELET-DERIVED GROWTH FACTOR, B CHAIN	Uncharacterized protein	Danio rerio	MF-GO:0005102=r...
DANRE:F1QP61	PLATELET-DERIVED GROWTH FACTOR, B CHAIN	Uncharacterized protein	Danio rerio	MF-GO:0005102=r...
DANRE:E7FBB3	PLATELET-DERIVED GROWTH FACTOR, B CHAIN	Uncharacterized protein	Danio rerio	MF-GO:0005102=r...
STRPU:H3ILH1	PDGF/VEGF GROWTH FACTOR RELATED PROTEIN 1	Uncharacterized protein	Strongylocentrotus purpuratus	MF-GO:0005102=r...
CAEEL:Q9N413	PDGF/VEGF GROWTH FACTOR RELATED PROTEIN 1	Protein PVF-1	Caenorhabditis elegans	MF-GO:0005102=r...
CAEBR:XP_001666570	PDGF/VEGF GROWTH FACTOR RELATED PROTEIN 1		Caenorhabditis briggsae	MF-GO:0005102=r...
PRIPA:H3EZS3	PDGF/VEGF GROWTH FACTOR RELATED PROTEIN 1	Uncharacterized protein	Pristionchus pacificus	MF-GO:0005102=r...
DROME:Q9VWP6	PDGF/VEGF GROWTH FACTOR RELATED PROTEIN 1	LD28763p	Drosophila melanogaster	MF-GO:0005102=r...
ANOGA:Q7QFM8	PDGF/VEGF GROWTH FACTOR RELATED PROTEIN 1	AGAP000574-PA	Anopheles gambiae	MF-GO:0005102=r...
DAPPU:E9H9T1	PDGF/VEGF GROWTH FACTOR RELATED PROTEIN 1	Putative uncharacterized protein	Daphnia pulex	MF-GO:0005102=r...
DAPPU:E9HIN8	PDGF/VEGF GROWTH FACTOR RELATED PROTEIN 1	Putative uncharacterized protein	Daphnia pulex	MF-GO:0005102=r...
DAPPU:E9G4R4	PDGF/VEGF GROWTH FACTOR RELATED PROTEIN 1	Putative uncharacterized protein	Daphnia pulex	MF-GO:0005102=r...
IXOSC:B7Q6M8	PDGF/VEGF GROWTH FACTOR RELATED PROTEIN 1	VEGF183 protein, putative	Ixodes scapularis	MF-GO:0005102=r...
NEMVE:A7RN76	PDGF/VEGF GROWTH FACTOR RELATED PROTEIN 1	Predicted protein	Nematostella vectensis	MF-GO:0005102=r...

Figure 2A

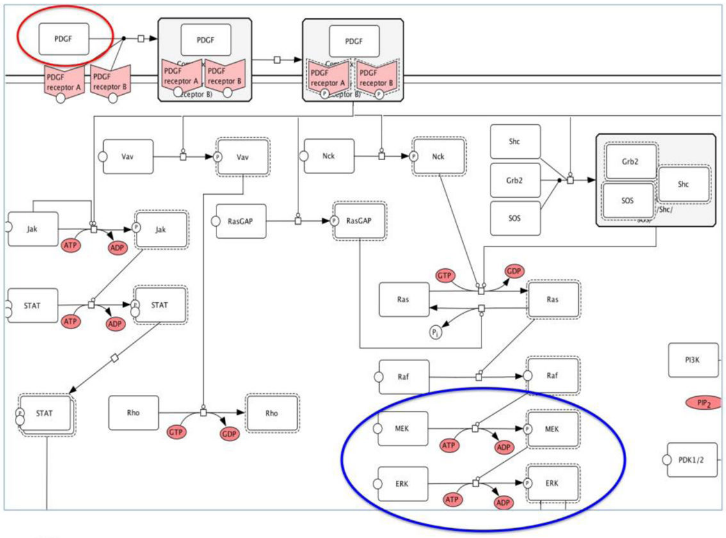
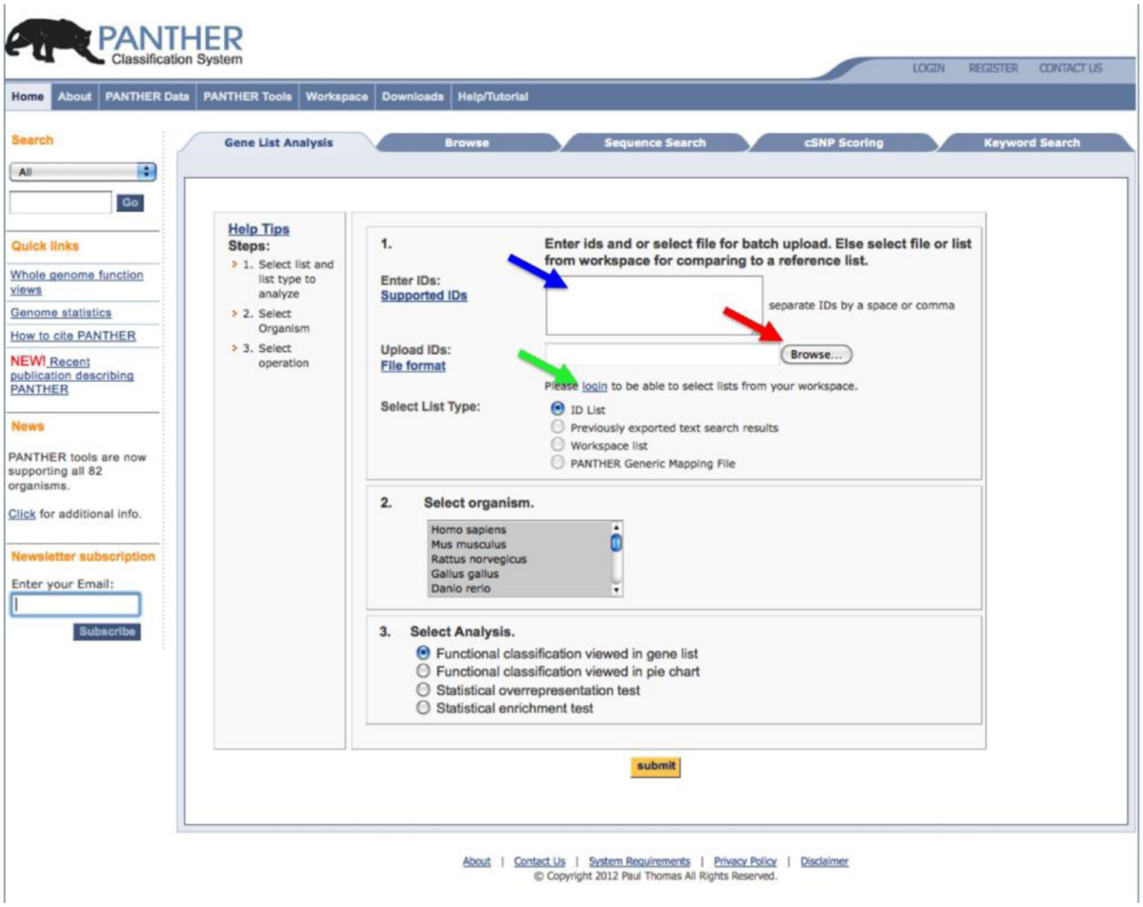


Figure 2B

Figure 2. Examples of PANTHER phylogenetic tree and pathway diagram

(A) A sample phylogenetic tree from PANTHER (PTHR11633, PLATELET-DERIVED GROWTH FACTOR). The family contains three subfamilies (blue arrows). SF1 is annotated as “PDGF/VEGF growth factor related protein 1” based on the annotation in the drosophila and c. elegans sequences (Q9VWP6 and Q9N143, respectively). There is a recent duplication that generates the PDGF A chain (SF3) and the PDGF B chain (SF2). Ontology terms are annotated to the node that represents the common ancestor if the extant family, in this case, AN0 (SF1). The classifications are propagated to all the descent nodes, including the AN4 (SF3) and AN33 (SF2).

(B) An example of PANTHER pathway diagram (P00047, PDGF signaling pathway). The diagram is shown in CellDesigner process diagram, which is similar to the SBGN-PD format, for example (blue circle), a transition of an input (e.g., ERK) to an output (eg., phosphorylated ERK) catalyzed by a modifier (eg., phosphorylated MEK). A pathway component (e.g, PDGF in red circle) is associated with the protein sequences in the protein library (red arrows in 2A) through expert curation. This association is supported by literature evidence. As a result, the pathway component of PDGF can be inferred to other orthologous protein sequences in the subfamilies in the library (SF2 and SF3 in 2A).


PANTHER
Classification System
LOGIN REGISTER CONTACT US
Home About PANTHER Data PANTHER Tools Workspace Downloads Help/Tutorial
Search
All
Go
Quick links
Whole genome function views
Genome statistics
How to cite PANTHER
NEW! Recent publication describing PANTHER
News
Newsletter subscription
Subscribe
Gene List Analysis
Browse
Sequence Search
cSNP Scoring
Keyword Search
Browse...
submit
About | Contact Us | System Requirements | Privacy Policy | Disclaimer

PANTHER tools are now supporting all 82 organisms.

Click for additional info.

Enter your Email:

Help Tips

Steps:

1. Select list and list type to analyze
2. Select Organism
3. Select operation

1. Enter ids and or select file for batch upload. Else select file or list from workspace for comparing to a reference list.

Enter IDs: Supported IDs

separate IDs by a space or comma

Upload IDs: File format

Please login to be able to select lists from your workspace.

Select List Type:

- ● ID List
- ○ Previously exported text search results
- ○ Workspace list
- ○ PANTHER Generic Mapping File

2. Select organism.

Homo sapiens
Mus musculus
Rattus norvegicus
Gallus gallus
Danio rerio

3. Select Analysis.

- ● Functional classification viewed in gene list
- ○ Functional classification viewed in pie chart
- ○ Statistical overrepresentation test
- ○ Statistical enrichment test

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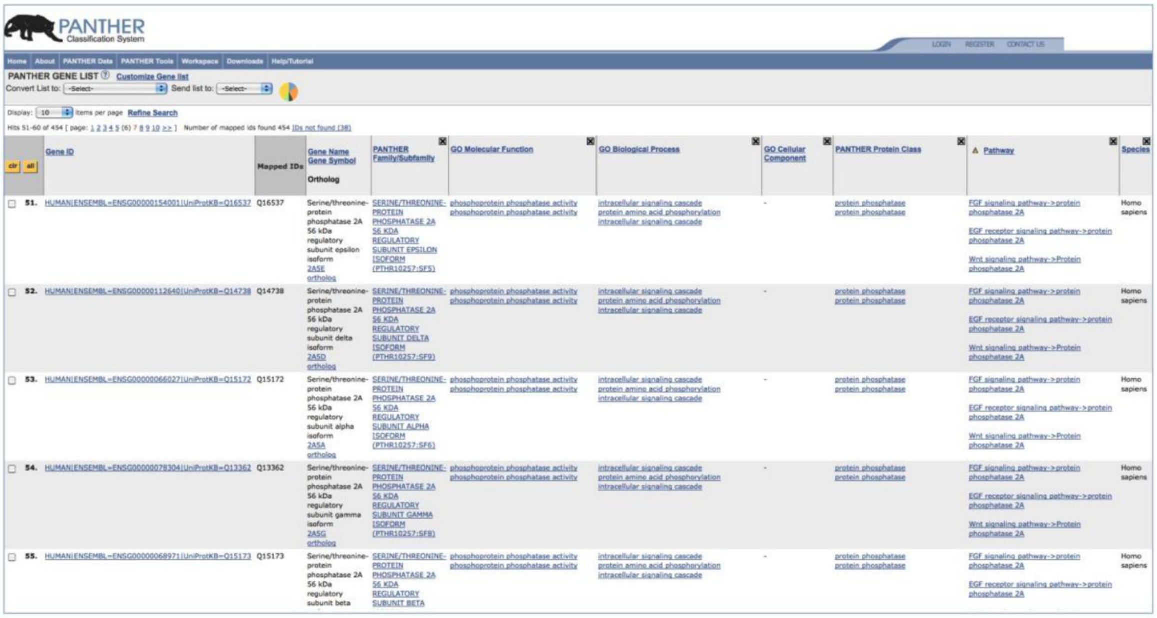
Figure 3.

The PANTHER home page with the Gene List Analysis Tools.



PANTHER GENE LIST ⓘ Customize Gene list

Convert List to: -Select- Send list to: -Select-

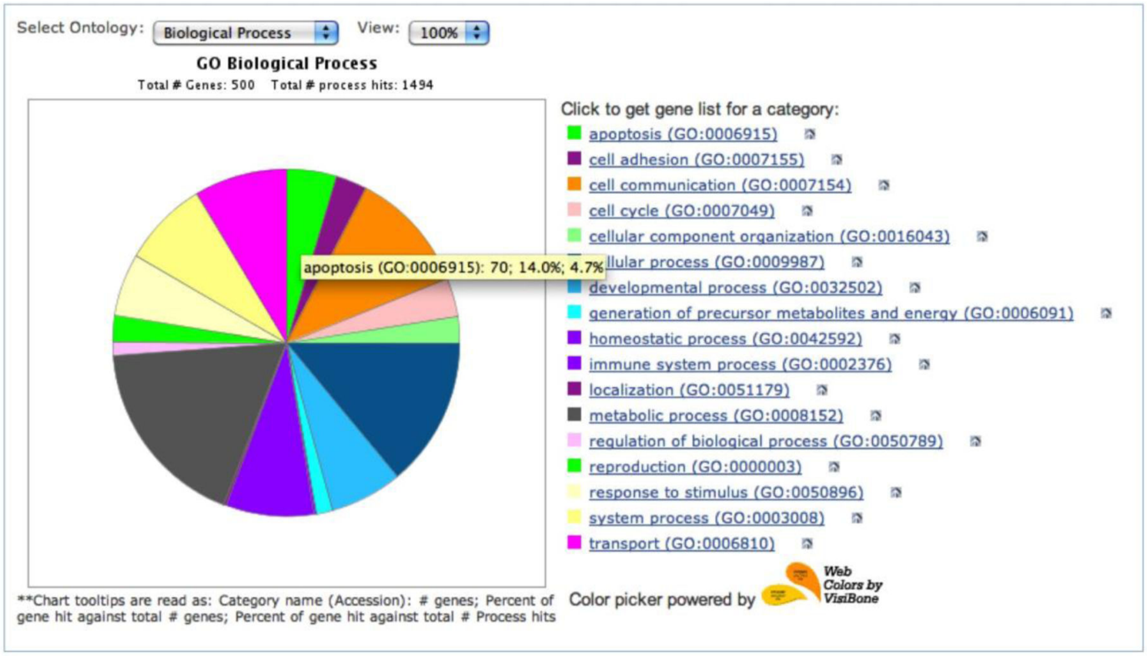
Display: 10 items per page Refine Search

Hits 51-60 of 454 [page: 1 2 3 4 5 (6) 7 8 9 10 >>] Number of mapped ids found 454 IDs not found (38)

clr all	Gene ID	Mapped IDs	Gene Name Gene Symbol Ortholog	PANTHER Family/Subfamily ☒	GO Molecular Function ☒	GO Biological Process ☒	GO Cellular Component ☒	PANTHER Protein Class ☒	⚠ Pathway ☒	Species ☒
□ 51.	HUMAN|ENSEMBL=ENSG00000154001|UniProtKB=Q16537	Q16537	Serine/threonine-protein phosphatase 2A 56 kDa regulatory subunit epsilon isoform 2A5E ortholog	SERINE/THREONINE-PROTEIN PHOSPHATASE 2A 56 KDA REGULATORY SUBUNIT EPSILON ISOFORM (PTHR10257:SF5)	phosphoprotein phosphatase activity phosphoprotein phosphatase activity	intracellular signaling cascade protein amino acid phosphorylation intracellular signaling cascade	-	protein phosphatase protein phosphatase	FGF signaling pathway->protein phosphatase 2A EGF receptor signaling pathway->protein phosphatase 2A Wnt signaling pathway->Protein phosphatase 2A	Homo sapiens
□ 52.	HUMAN|ENSEMBL=ENSG00000112640|UniProtKB=Q14738	Q14738	Serine/threonine-protein phosphatase 2A 56 kDa regulatory subunit delta isoform 2A5D ortholog	SERINE/THREONINE-PROTEIN PHOSPHATASE 2A 56 KDA REGULATORY SUBUNIT DELTA ISOFORM (PTHR10257:SF9)	phosphoprotein phosphatase activity phosphoprotein phosphatase activity	intracellular signaling cascade protein amino acid phosphorylation intracellular signaling cascade	-	protein phosphatase protein phosphatase	FGF signaling pathway->protein phosphatase 2A EGF receptor signaling pathway->protein phosphatase 2A Wnt signaling pathway->Protein phosphatase 2A	Homo sapiens
□ 53.	HUMAN|ENSEMBL=ENSG00000066027|UniProtKB=Q15172	Q15172	Serine/threonine-protein phosphatase 2A 56 kDa regulatory subunit alpha isoform 2A5A ortholog	SERINE/THREONINE-PROTEIN PHOSPHATASE 2A 56 KDA REGULATORY SUBUNIT ALPHA ISOFORM (PTHR10257:SF6)	phosphoprotein phosphatase activity phosphoprotein phosphatase activity	intracellular signaling cascade protein amino acid phosphorylation intracellular signaling cascade	-	protein phosphatase protein phosphatase	FGF signaling pathway->protein phosphatase 2A EGF receptor signaling pathway->protein phosphatase 2A Wnt signaling pathway->Protein phosphatase 2A	Homo sapiens
□ 54.	HUMAN|ENSEMBL=ENSG00000078304|UniProtKB=Q13362	Q13362	Serine/threonine-protein phosphatase 2A 56 kDa regulatory subunit gamma isoform 2A5G ortholog	SERINE/THREONINE-PROTEIN PHOSPHATASE 2A 56 KDA REGULATORY SUBUNIT GAMMA ISOFORM (PTHR10257:SF8)	phosphoprotein phosphatase activity phosphoprotein phosphatase activity	intracellular signaling cascade protein amino acid phosphorylation intracellular signaling cascade	-	protein phosphatase protein phosphatase	FGF signaling pathway->protein phosphatase 2A EGF receptor signaling pathway->protein phosphatase 2A Wnt signaling pathway->Protein phosphatase 2A	Homo sapiens
□ 55.	HUMAN|ENSEMBL=ENSG00000068971|UniProtKB=Q15173	Q15173	Serine/threonine-protein phosphatase 2A 56 kDa regulatory subunit beta	SERINE/THREONINE-PROTEIN PHOSPHATASE 2A 56 KDA REGULATORY SUBUNIT BETA	phosphoprotein phosphatase activity phosphoprotein phosphatase activity	intracellular signaling cascade protein amino acid phosphorylation intracellular signaling cascade	-	protein phosphatase protein phosphatase	FGF signaling pathway->protein phosphatase 2A EGF receptor signaling pathway->protein phosphatase 2A	Homo sapiens

Figure 4.

User interface of the *statistical overrepresentation test* to allow user to select additional test gene lists.



Select Ontology: Biological Process View: 100%

GO Biological Process

Total # Genes: 500 Total # process hits: 1494

apoptosis (GO:0006915): 70; 14.0%; 4.7%

Click to get gene list for a category:

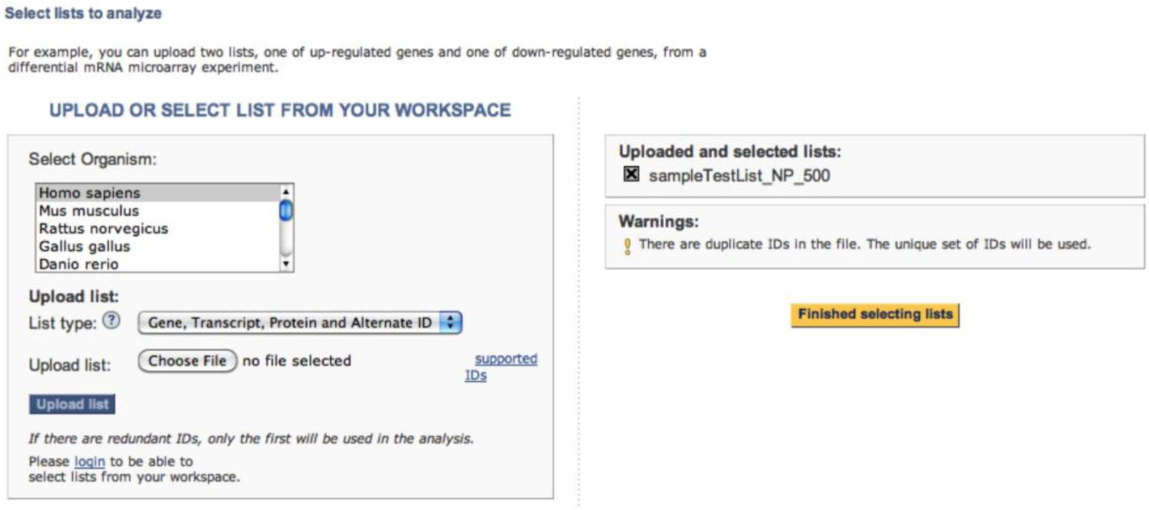
- ■ apoptosis (GO:0006915)
- ■ cell adhesion (GO:0007155)
- ■ cell communication (GO:0007154)
- ■ cell cycle (GO:0007049)
- ■ cellular component organization (GO:0016043)
- llular process (GO:0009987)
- ■ developmental process (GO:0032502)
- ■ generation of precursor metabolites and energy (GO:0006091)
- ■ homeostatic process (GO:0042592)
- ■ immune system process (GO:0002376)
- ■ localization (GO:0051179)
- ■ metabolic process (GO:0008152)
- ■ regulation of biological process (GO:0050789)
- ■ reproduction (GO:0000003)
- ■ response to stimulus (GO:0050896)
- ■ system process (GO:0003008)
- ■ transport (GO:0006810)

**Chart tooltips are read as: Category name (Accession): # genes; Percent of gene hit against total # genes; Percent of gene hit against total # Process hits

Color picker powered by Web Colors by VisiBone

Figure 5.

Results of functional classification displayed as a gene list page. The results are based on the sampleTestList_NP_500 file in the Supplemental Materials.



Select lists to analyze

For example, you can upload two lists, one of up-regulated genes and one of down-regulated genes, from a differential mRNA microarray experiment.

UPLOAD OR SELECT LIST FROM YOUR WORKSPACE

Select Organism:

Homo sapiens
Mus musculus
Rattus norvegicus
Gallus gallus
Danio rerio

Upload list:

List type: ? Gene, Transcript, Protein and Alternate ID

Upload list: Choose File no file selected

If there are redundant IDs, only the first will be used in the analysis.

Please login to be able to select lists from your workspace.

Uploaded and selected lists:

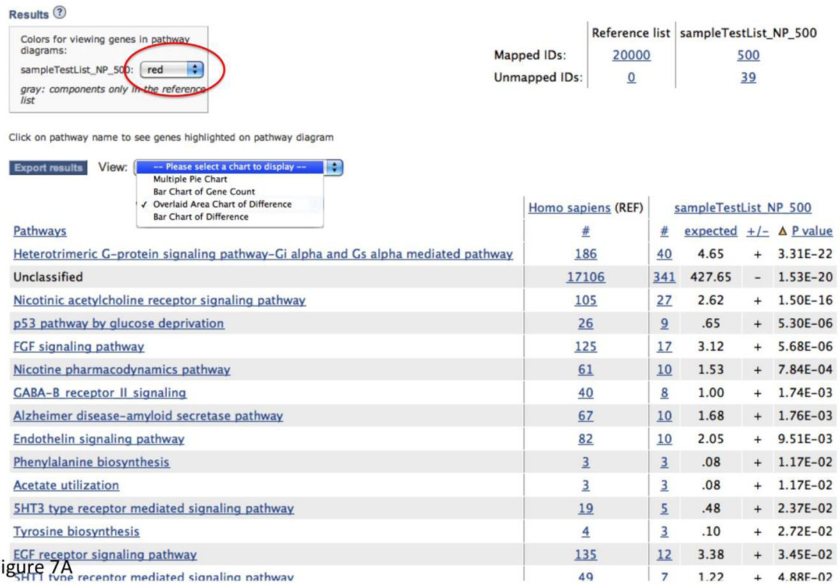
☒ sampleTestList_NP_500

Warnings:

There are duplicate IDs in the file. The unique set of IDs will be used.

Figure 6.

PANTHER pie chart results from the sampleTestList_NP_500 file in the Supplemental Materials. You can use the *Select ontology* drop-down menu to switch to the pie chart of different ontologies. Click on the pie chart section to display the child categories. Click on the legends on the right side to retrieve the list of the genes for that category.



Results ?

Colors for viewing genes in pathway diagrams:

sampleTestList_NP_500: red

gray: components only in the reference list

	Reference list	sampleTestList_NP_500
Mapped IDs:	20000	500
Unmapped IDs:	0	39

Click on pathway name to see genes highlighted on pathway diagram

	Homo sapiens (REF)	sampleTestList_NP_500			
Pathways	#	#	expected	+/-	▲ P value
Heterotrimeric G-protein signaling pathway-Gi alpha and Gs alpha mediated pathway	186	40	4.65	+	3.31E-22
Unclassified	17106	341	427.65	-	1.53E-20
Nicotinic acetylcholine receptor signaling pathway	105	27	2.62	+	1.50E-16
p53 pathway by glucose deprivation	26	9	.65	+	5.30E-06
FGF signaling pathway	125	17	3.12	+	5.68E-06
Nicotine pharmacodynamics pathway	61	10	1.53	+	7.84E-04
GABA-B receptor II signaling	40	8	1.00	+	1.74E-03
Alzheimer disease-amyloid secretase pathway	67	10	1.68	+	1.76E-03
Endothelin signaling pathway	82	10	2.05	+	9.51E-03
Phenylalanine biosynthesis	3	3	.08	+	1.17E-02
Acetate utilization	3	3	.08	+	1.17E-02
5HT3 type receptor mediated signaling pathway	19	5	.48	+	2.37E-02
Tyrosine biosynthesis	4	3	.10	+	2.72E-02
EGF receptor signaling pathway	135	12	3.38	+	3.45E-02
5HT1 type receptor mediated signaling pathway	49	7	1.22	+	4.88E-02

Figure 7A

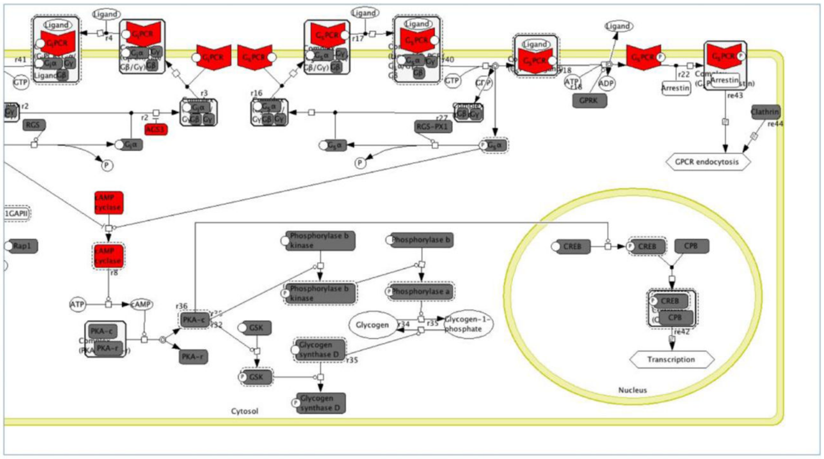
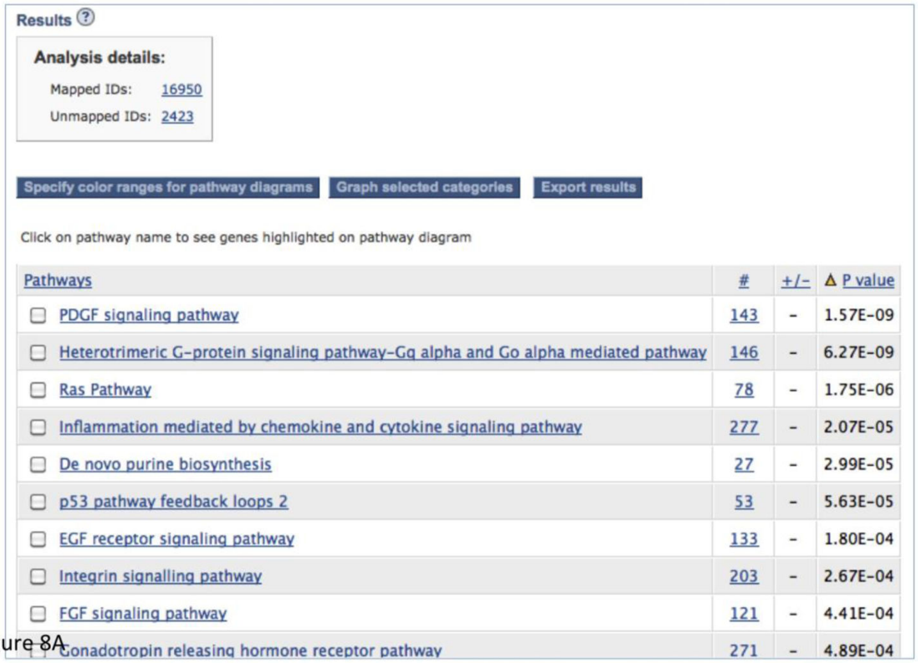


Figure 7B

Figure 7. Result from the *statistical overrepresentation test*. The results are based on the sampleTestList_NP_500 file in the Supplemental Materials.

(A) The summary of the results is displayed in a table. You can export the table in a tab-delimited file by clicking the *Export results* button. You can also view the results in other views by using the *View* drop-down menu. If your analysis is done in pathway as shown here, you can click the pathway name and display the pathway diagram. The pathway components that have genes in your test list will be highlighted. The color of the highlighted component can be defined at the top of the page (red circle). A total of 4 test lists can be analyzed and viewed at the same time.

(B) The results viewed in the PANTHER pathway *Heterotrimeric G-protein signaling pathway – Gi alpha and Gs alpha mediated pathway* (P00026). The components that contain the genes in the test gene list are highlighted in red.



Results ?

Analysis details:

Mapped IDs: 16950

Unmapped IDs: 2423

Specify color ranges for pathway diagrams
Graph selected categories
Export results

Click on pathway name to see genes highlighted on pathway diagram

Pathways	#	+/-	▲ P value
□ PDGF signaling pathway	143	-	1.57E-09
□ Heterotrimeric G-protein signaling pathway-Gq alpha and Go alpha mediated pathway	146	-	6.27E-09
□ Ras Pathway	78	-	1.75E-06
□ Inflammation mediated by chemokine and cytokine signaling pathway	277	-	2.07E-05
□ De novo purine biosynthesis	27	-	2.99E-05
□ p53 pathway feedback loops 2	53	-	5.63E-05
□ EGF receptor signaling pathway	133	-	1.80E-04
□ Integrin signalling pathway	203	-	2.67E-04
□ FGF signaling pathway	121	-	4.41E-04
□ Gonadotropin releasing hormone receptor pathway	271	-	4.89E-04

Figure 8A

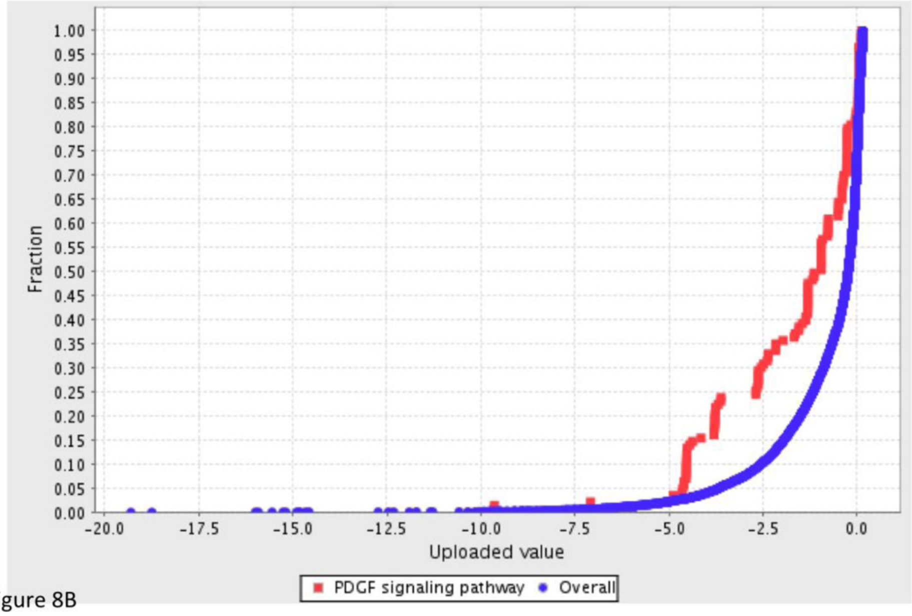


Figure 8B

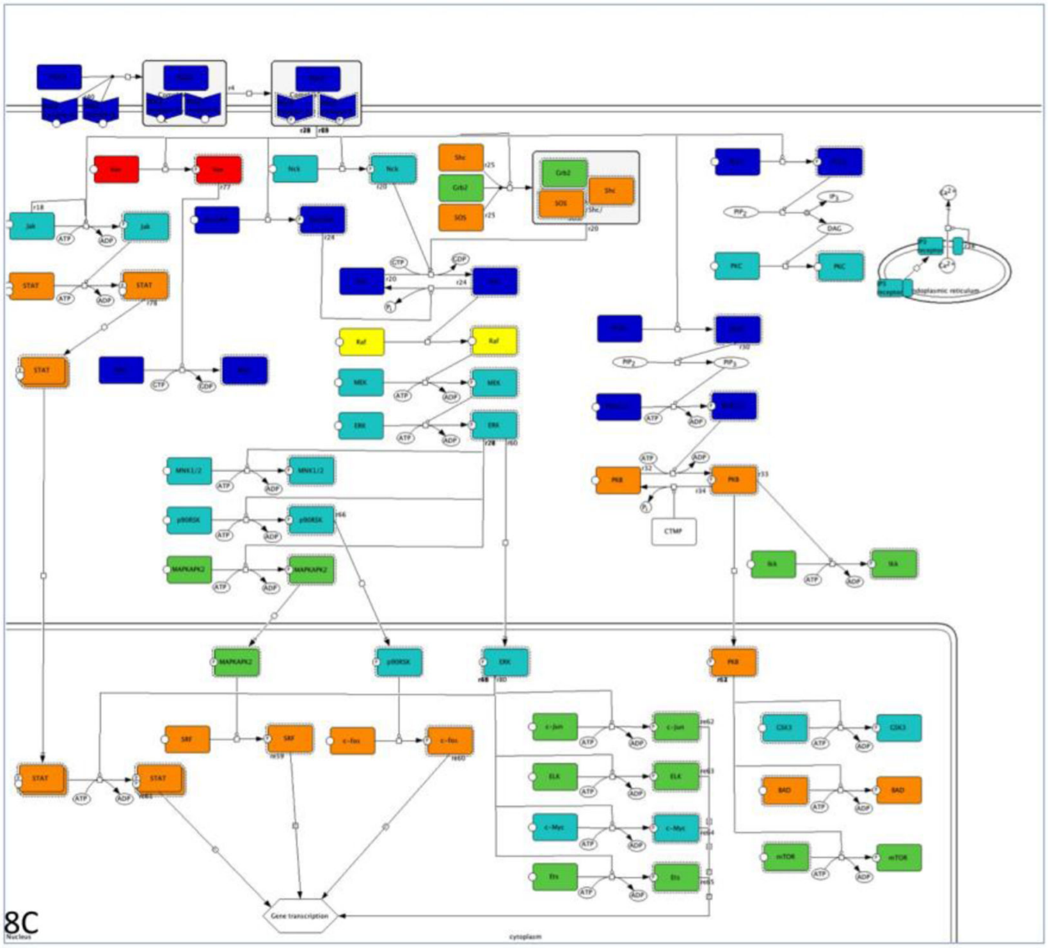


Figure 8C

Figure 8.

The results from the *statistical enrichment test.* The results are based on the sampleTestList_NP file in the Supplemental Materials. (A) The output of the tool with a list of P-values for each comparison between a functional category distribution and the reference distribution. (B) Comparison of the distributions from PDGF signaling pathway (red) and reference (blue) in graph view. (C) A pathway diagram of PDGF signaling pathway that is visualized using an interactive pathway Java applet that colors the pathway using a "heat map" derived from the input values.

Table 1

Troubleshooting

Step	Problem	Possible reasons and solutions
3	Fail to upload the file	This is usually because the input file is in the wrong file format. Possible solutions: 1. Make sure that your file is in simple text format (.txt or .tab). 2 If you are uploading a file with numeric values for the enrichment test, make sure that the second column contains only numeric numbers. Any rows with no values should be removed instead of leaving it blank or mark it as “n/a”, etc. 3. Make sure that there is no blank rows in the first column.
6A, 6Cvii, 6Dii.	IDs in the uploaded file don’t have a mapped ID in PANTHER	The current PANTHER data is based on the April 2012 release of Reference Proteome Project and its ID mapping. It is possible that a small fraction of the IDs may not map due to the outdated data either in the PANTHER database or your uploaded file. There is no solution to this. If you believe that you are using the current IDs, please do the followings: 1. Make sure that the IDs in the uploaded file are supported by PANTHER. Refer to Box 1 for Supported IDs. 2. IDs from certain database may contain a version number at the end of them, e.g., NP_000242.1. Do not include the version number “.1” in the ID, and just use NP_000242. 3. Send a feedback to feedback@pantherdb.org.
6Cviiic, 6Diiic	Pathway Applet can not be launched to view the pathway diagrams	This is most likely that your Java plug-in is outdated. Read the system requirement and make sure that your computer has the most updated Java version.