Expander 6.4 Online Documentation

Introduction

EXPANDER (EXpression Analyzer and DisplayER) is a java-based tool for analysis of gene expression data. It is capable of (1) preprocessing (2) visualizing (3) clustering (4) biclustering and (5) performing downstream analysis of clusters and biclusters such as functional enrichment and promoter analysis (i.e. analysis of gene groups for enrichment of transcription factor binding sites in their promoters).

EXPANDER incorporates several conventional gene expression analysis algorithms and custom ones that have been developed in the computational genomics group in Tel-Aviv University, and provides them with an easy-to-operate user interface.

EXPANDER versions are available for Windows OS and for Linux/Unix OS and require the pre-installation of the Java Runtime Environment (JRE) 5.0 or later (Expander 6.05 is the first version that fully supports java 1.7). The Java Runtime Environment can be installed via: http://java.sun.com/javase/downloads/index.jsp.

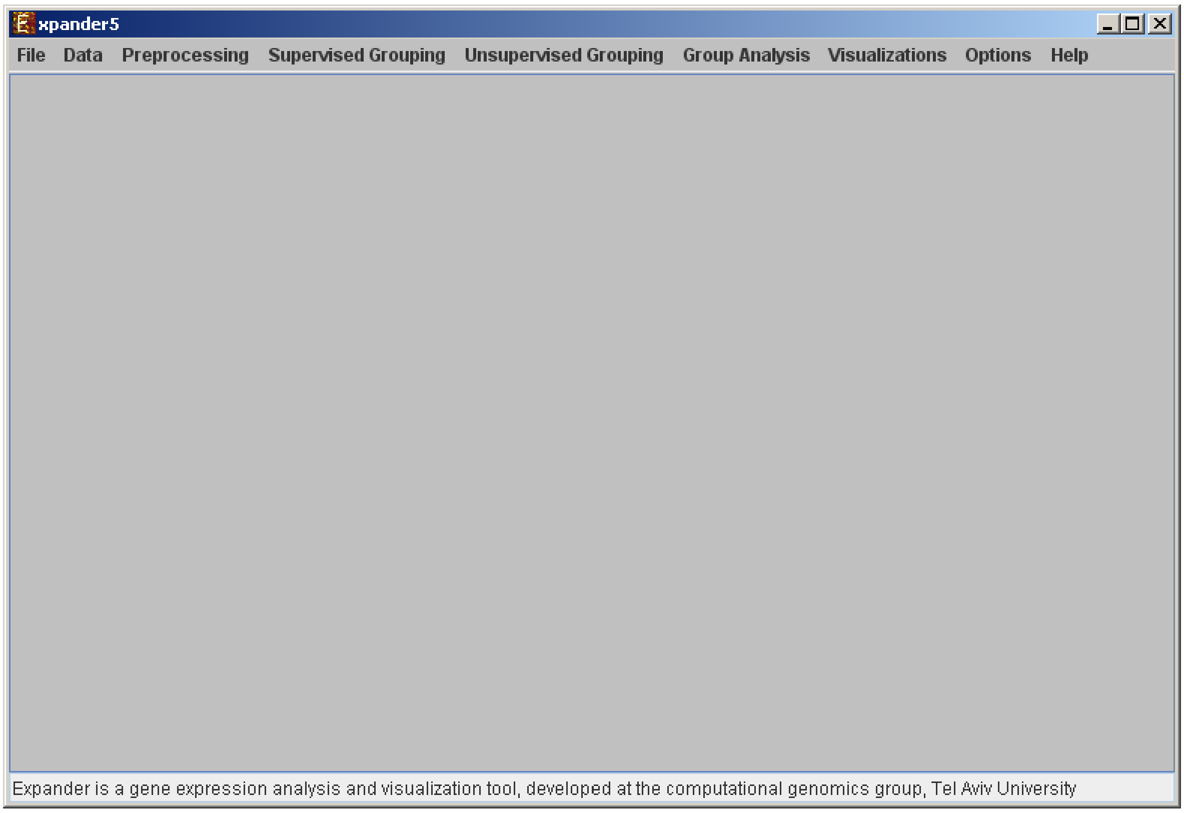
The CEL file preprocessing and the newly added SAM filter utilities require the pre-installation of one of the recent versions of R, a free software environment for statistical computing and graphics. For installation instructions, please refer to R External Application section.

Starting EXPANDER

Double click on the **Expander.bat** file, which is located under the Expander directory (alternatively, in Linux, open a Terminal window, cd into the Expander directory, and run the command: './Expander.bat').

When running on Linux/Unix OS, make sure that you have rwx permissions for the Expander directory and for the directory in which your data is located. Also make sure that you have rx permissions for all *.exe files that are under your Expander directory.

Upon running the program, the main menu bar appears:



Expander is a gene expression analysis and visualization tool, developed at the computational genomics group, Tel Aviv University

Input Data

Expander operates on the following types of data:

a) **Gene expression data** – For most of EXPANDER's steps for analysis of gene expression data, the technique used for obtaining the expression estimates doesn't make a difference. Whatever technique (e.g., **expression arrays, RNA-Seq**) was used, the input expression data should be summarized in a matrix (tab-delimited txt file; see File Formats section) in which rows correspond to probes/genes and columns – to samples.

Values can be either relative intensities data, expected as log 2 (R/G) values data (e.g. cDNA microarrays) OR absolute intensities data, expected as positive expression levels (E.g. High-density oligonucleotide data). Oligonucleotide data can be loaded with/without detection calls. Affymetrix data can also be loaded from CEL files (If R is installed).

When analyzing **RNA-Seq** data, one way to obtain gene expression matrix is to use TopHat (http://tophat.cbcb.umd.edu/tutorial.html) to align the sequenced reads to the relevant genome, and then use Cufflinks (http://cufflinks.cbcb.umd.edu/howitworks.html) or HTSeq (http://www-huber.embl.de/users/anders/HTSeq/doc/count.html#count) to obtain gene (or transcript) expression estimates from TopHat output.

If one wishes to perform functional analysis or promoter analysis, an **ID conversion file** should be loaded along with the data file. The conversion file maps each probe ID (first column) in the data file into a corresponding conventional gene ID that is used in the GO annotation and TF fingerprint files that are supplied with EXPANDER. The conversion file can be loaded in the middle of the session too, by Data >> Load Conversion File.

b) **Similarity data** – a pre-calculated similarity matrix

c) **Gene groups data** – contains predefined groups of genes. In this data type, the conventional gene IDs that are used by EXPANDER in the GO annotation and TF fingerprint files are expected.

For details regarding the Gene ID convention that is used for each organism, refer to the Supplied Files section.

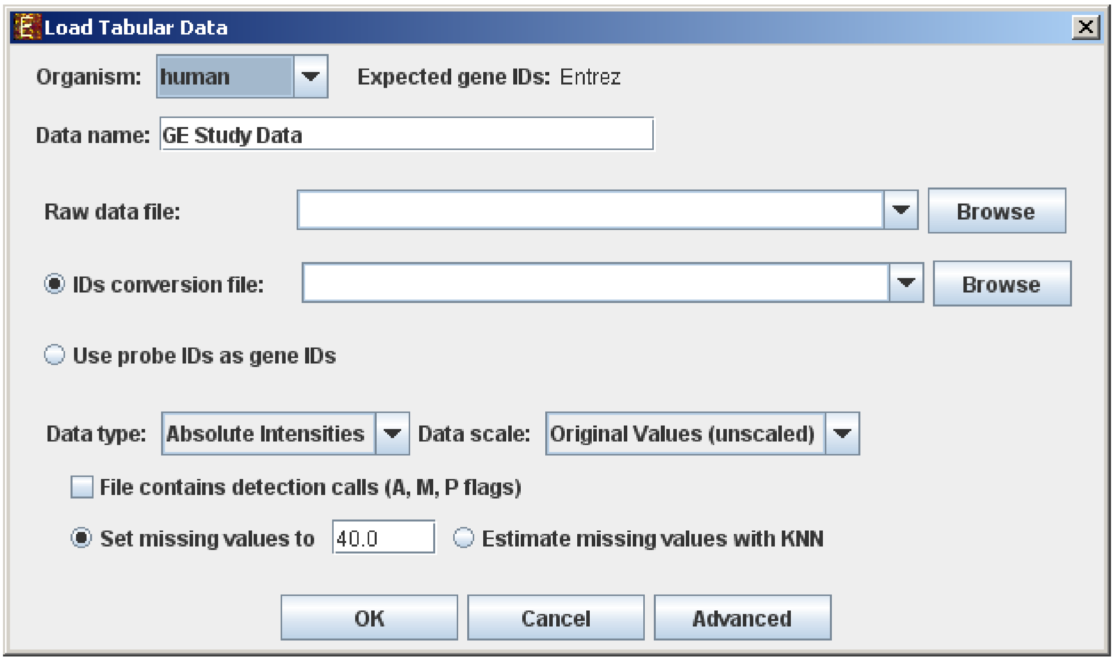
For details regarding the data files formats see the File Formats section.

Loading gene expression data:

Tabular Data File

To load tabular expression data, select: *File >> New Session*. From the submenu select *Expression Data >> Tabular Data File*.

When selecting *Tabular Data File,* the following dialog box will appear:



Organism: human
Expected gene IDs: Entrez

Data name: GE Study Data

Raw data file:

● IDs conversion file:

○ Use probe IDs as gene IDs

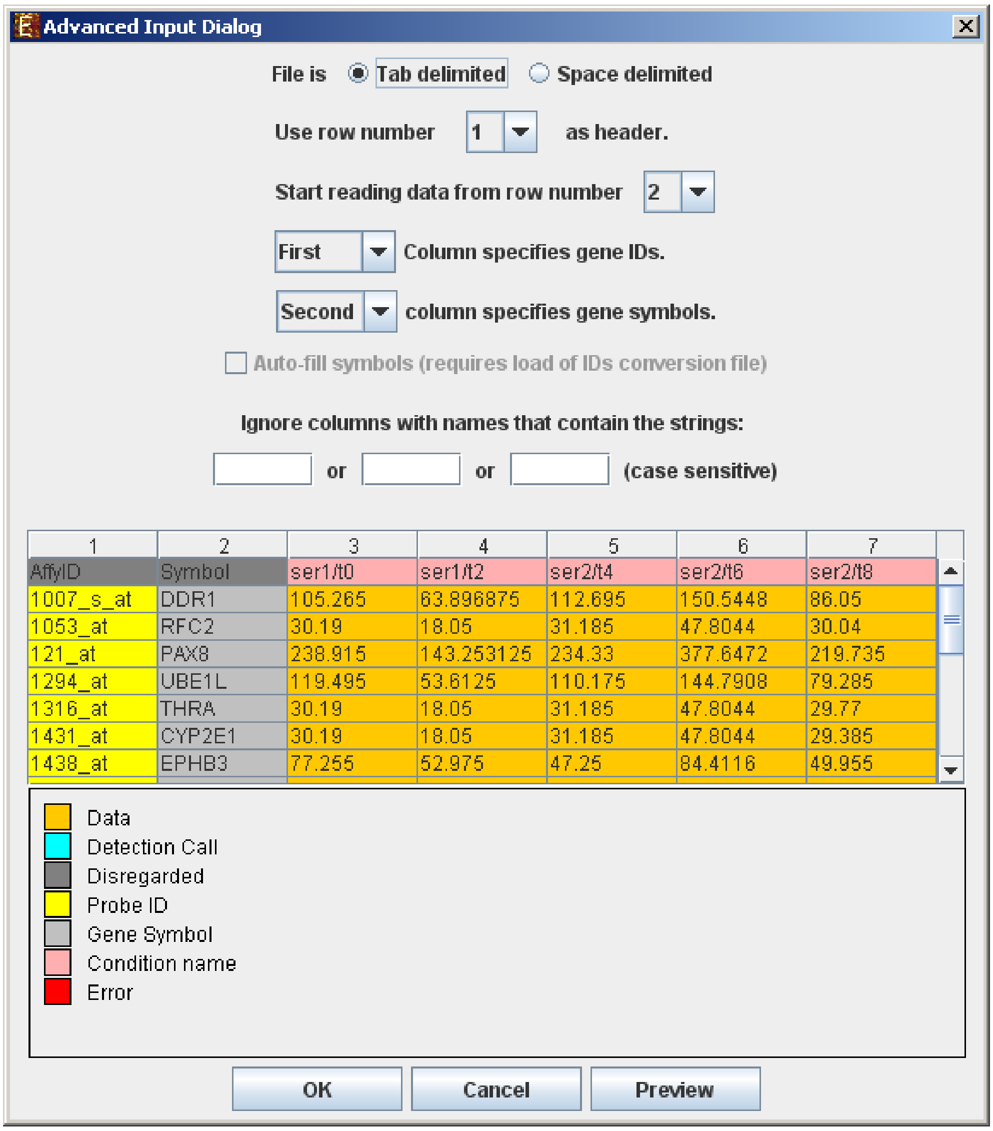
Data type: Absolute Intensities
Data scale: Original Values (unscaled)

□ File contains detection calls (A, M, P flags)

● Set missing values to 40.0
○ Estimate missing values with KNN

Data type and scale are to be determined according to the input file. If the file contains missing values, these values will be estimated upon loading the data either by setting them to and arbitrary value (if the 'Set missing value to ____' option is selected) or by utilizing the KNN (K-Nearest Neighbors) method (if the 'Estimate missing values with KNN' option is selected). If the file contains Affymetrix detection calls data, the relevant check box must be checked. You may change / erase the default floor value, to which all entries that are below that value will be set (this option is available only for absolute intensities data).

<u>Advanced Input Dialog</u>: Upon pressing the 'Advanced' button after filling the 'Raw Data File' field, an 'Advanced Input Dialog' appears. This dialog box can be used in order to facilitate the data load of files that are not in the required format. The first few rows and columns of the data are displayed in a table, demonstrating the way the data is read by the program according to the current input values.



File is ● Tab delimited ○ Space delimited

Use row number 1 as header.

Start reading data from row number 2

First Column specifies gene IDs.

Second column specifies gene symbols.

□ Auto-fill symbols (requires load of IDs conversion file)

Ignore columns with names that contain the strings:

or or (case sensitive)

1	2	3	4	5	6	7
AffyID	Symbol	ser1/t0	ser1/t2	ser2/t4	ser2/t6	ser2/t8
1007_s_at	DDR1	105.265	63.896875	112.695	150.5448	86.05
1053_at	RFC2	30.19	18.05	31.185	47.8044	30.04
121_at	PAX8	238.915	143.253125	234.33	377.6472	219.735
1294_at	UBE1L	119.495	53.6125	110.175	144.7908	79.285
1316_at	THRA	30.19	18.05	31.185	47.8044	29.77
1431_at	CYP2E1	30.19	18.05	31.185	47.8044	29.385
1438_at	EPHB3	77.255	52.975	47.25	84.4116	49.955

🟧 Data
🟦 Detection Call
⬛ Disregarded
🟨 Probe ID
⬜ Gene Symbol
🟫 Condition name
🟥 Error

CEL Files

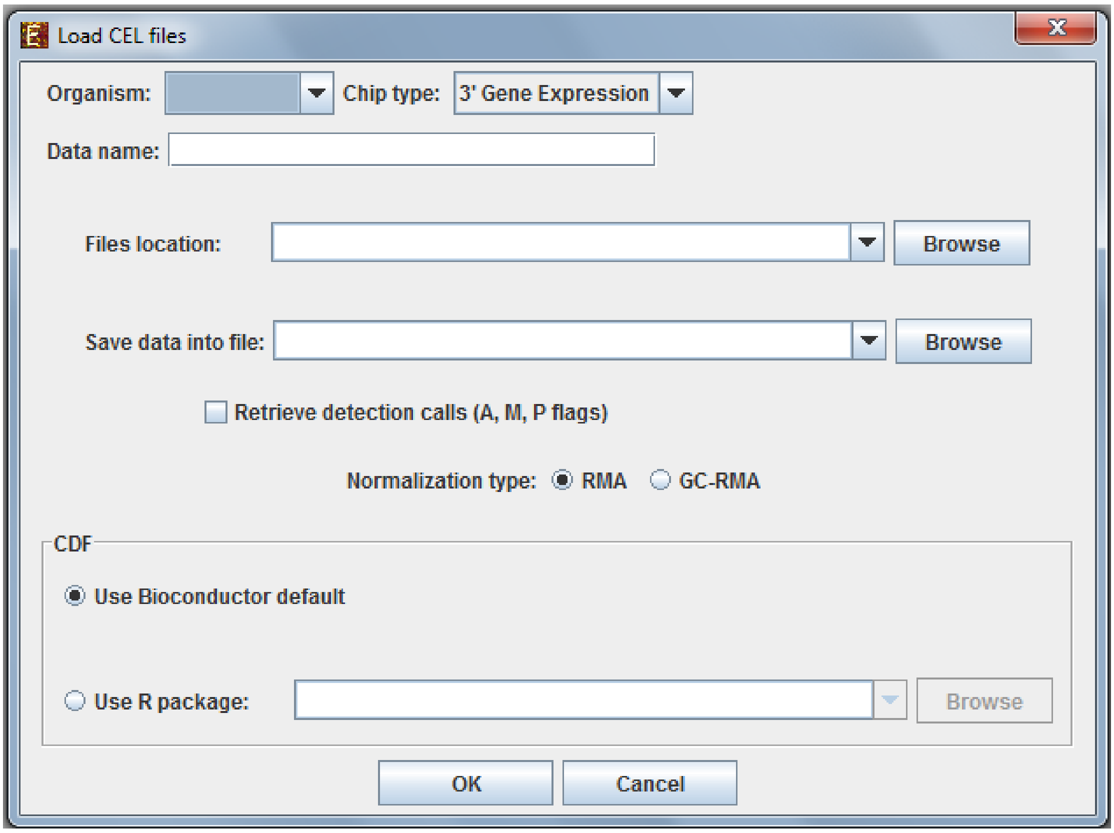
To load expression data from CEL files, select: *File >> New Session*. From the submenu select *Expression Data >> CEL Files*.

The load of CEL requires installation of R software (see R External Application section) along with specific packages, as detailed below. An open internet connection is also required for this operation.

Expander supports CEL files of three chip types:

1. **3' Gene Expression** - requires Bioconductor “affy” package
2. **Whole-Transcript Gene Expression** (Gene 1.0 chips) – requires the prior installation of a cdf package for the used chip (see links below).
3. **Alternative Splicing** (Exon 1.0 chips) requires the prior installation of a cdf package for the used chip (see links below). * Please note that we estimate the overall expression for the transcript, not exon-by-exon. Therefore, this becomes 'gene data' rather than 'alternative splicing data'.

When selecting *CEL Files,* the following dialog box will appear:



Organism:

Chip type: 3' Gene Expression

Data name:

Files location:

Save data into file:

□ Retrieve detection calls (A, M, P flags)

Normalization type: ● RMA ○ GC-RMA

CDF

● Use Bioconductor default

○ Use R package:

Please choose the relevant organism and chip type. Then browse to the folder where the CEL files are located (*Files location*), and choose where to save the expression file resulting from the CEL files preprocessing.

Preprocessing and normalization method: The default method in Expander is RMA. However, for 3' gene expression arrays, you may select GC-RMA instead (taking into account GC-content bias). Before using GC-RMA, please make sure you have the “gcrma” R package installed (see R External Application section).

CDF environment choice: You may use the default Bioconductor CDF environment for the chips or browse to an alternative CDF package which you have already installed in R. For whole transcript and alternative splicing chips (for which there is no default Bioconductor CDF environment), you will need to supply an alternative CDF package (see links below).

Note: GC-RMA requires the probe sequence information of the chip. If you decide not to use the default Bioconductor CDF environment, and have GC-RMA as the preprocessing method, you must have the suitable probe package installed in addition to the CDF alternative package.

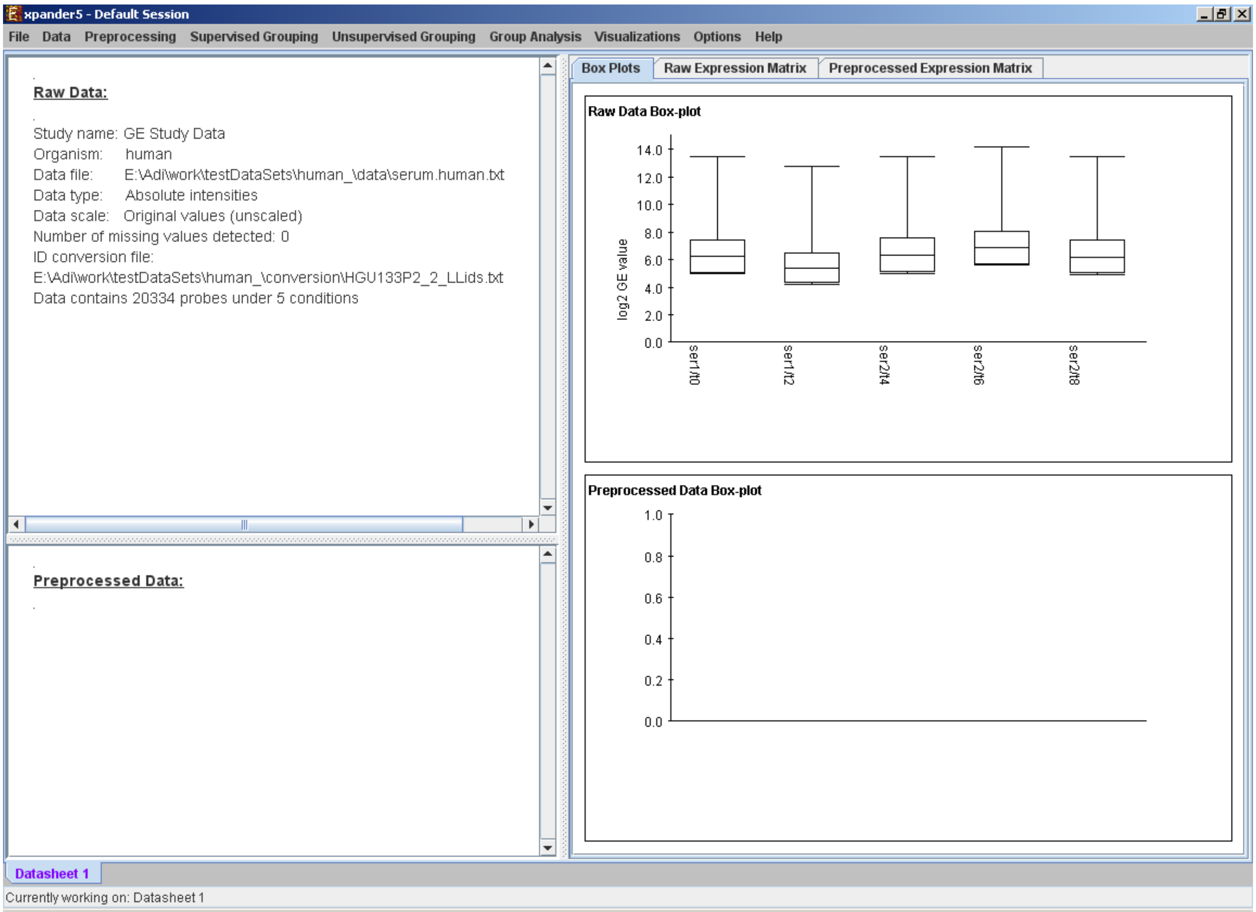
Link for downloading CDF environment packages (for 2nd option):

http://www.bioconductor.org/packages/release/data/annotation/

If Expander cannot find your R software, a window will appear, asking you to specify its location. Please browse to the location of your R software. In Windows, R.exe file is likely to be located in the 'bin' folder of R software. In Linux, you may type 'which R' in the command line to find R path. If you have a few versions of R installed, please make sure to point Expander to a version in which the Bioconductor “affy” package has been installed.

Once the CEL files preprocessing is done, a corresponding tabular data file is generated and a 'Load Study' dialog will appear, as in loading Tabular Data.

After loading a gene expression data set, a ‘Session Data’ display tab is added to the main window (see example below). It contains information regarding the raw data file, a box plot chart, and an expression matrix visualization of the raw data. If detection calls exist in the data file, their statistics for each probe appear in 3 columns in the heat maps (expression matrices), in a scale between 0 and 1, corresponding to the relative part of each of the detection calls (P, M and A). The detection calls statistics for each condition are displayed in a separate tab in two tables (one for the raw data and another for the preprocessed data) and are presented in percent.



Raw Data:

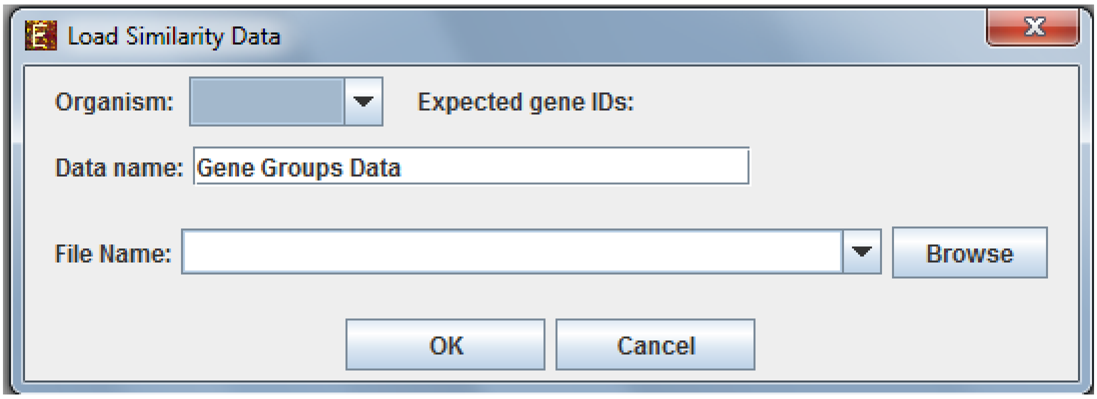
Study name: GE Study Data
Organism: human
Data file: E:\Adi\work\testDataSets\human_\data\serum.human.txt
Data type: Absolute intensities
Data scale: Original values (unscaled)
Number of missing values detected: 0
ID conversion file:
E:\Adi\work\testDataSets\human_\conversion\HGU133P2_2_LLids.txt
Data contains 20334 probes under 5 conditions

Preprocessed Data:

Currently working on: Datasheet 1

Working on similarity data no associated expression data

To start working on similarity data (no expression data associated) select File>>New Session>> Similarity Data...
The following dialog box will appear:



Organism:

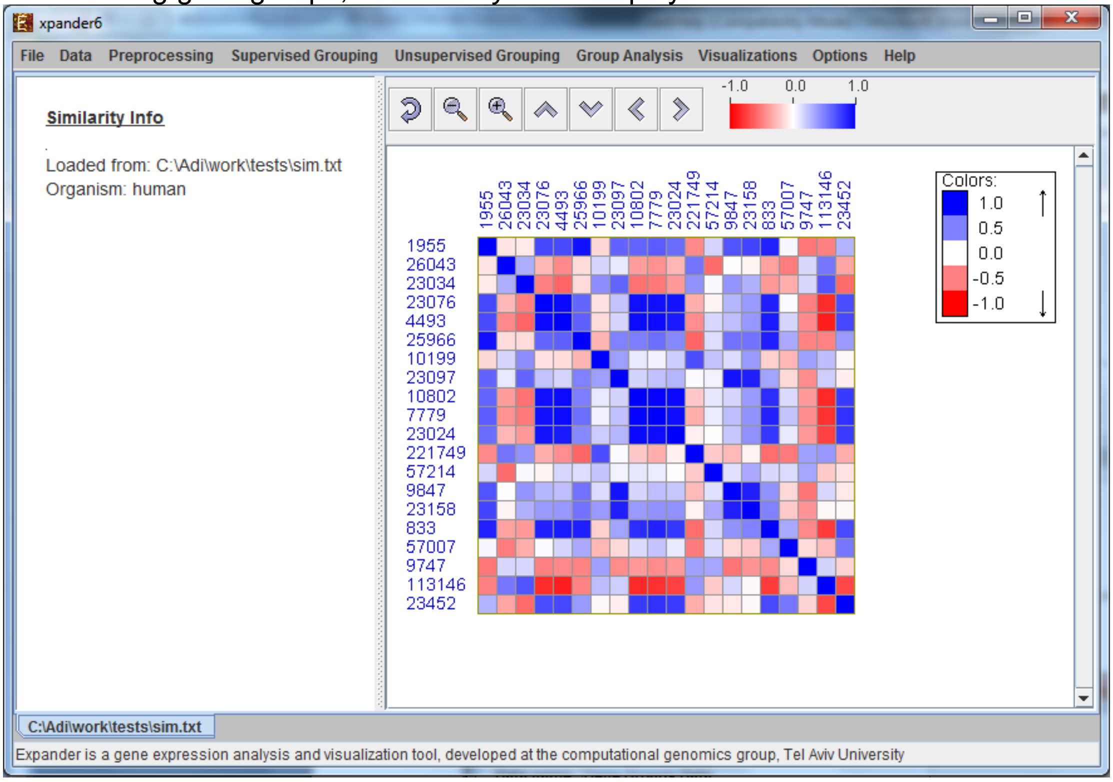
Expected gene IDs:

Data name: Gene Groups Data

File Name:

For details regarding the data files formats see the File Formats section.

After loading gene groups, a 'Similarity Data' display tab is added to the main window



Similarity Info

Loaded from: C:\Adi\work\tests\sim.txt
Organism: human

C:\Adi\work\tests\sim.txt

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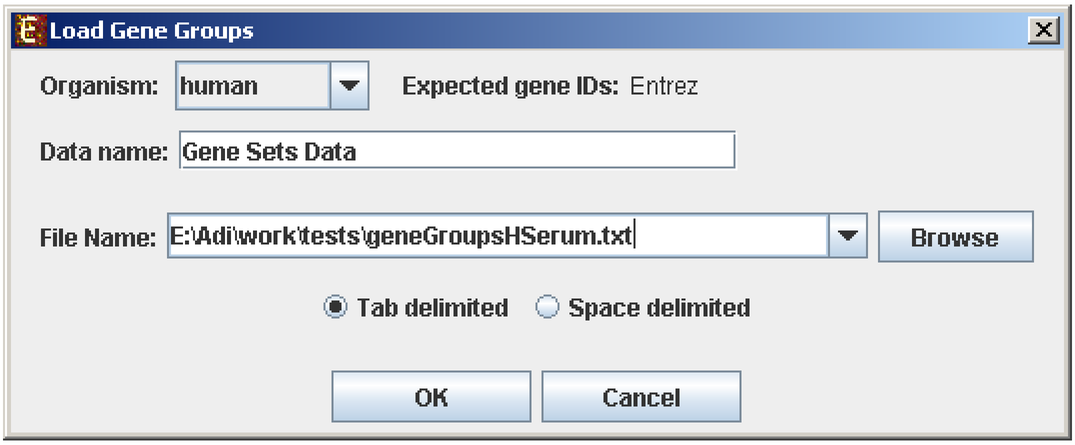
Currently similarity data can only be clustered using the Hierarchical clustering procedure by selecting *Unsupervised Grouping>>Hierarchical Clustering>>Cluster...* The resulting tree can be used to generate groups (for further details see Hierarchical Clustering).

Working on Gene Groups with no associated expression data

To start working on gene groups (no expression data associated) select *File>>New Session*. From the submenu select *Gene Groups*.

The following dialog box will appear:



Load Gene Groups

Organism: human

Expected gene IDs: Entrez

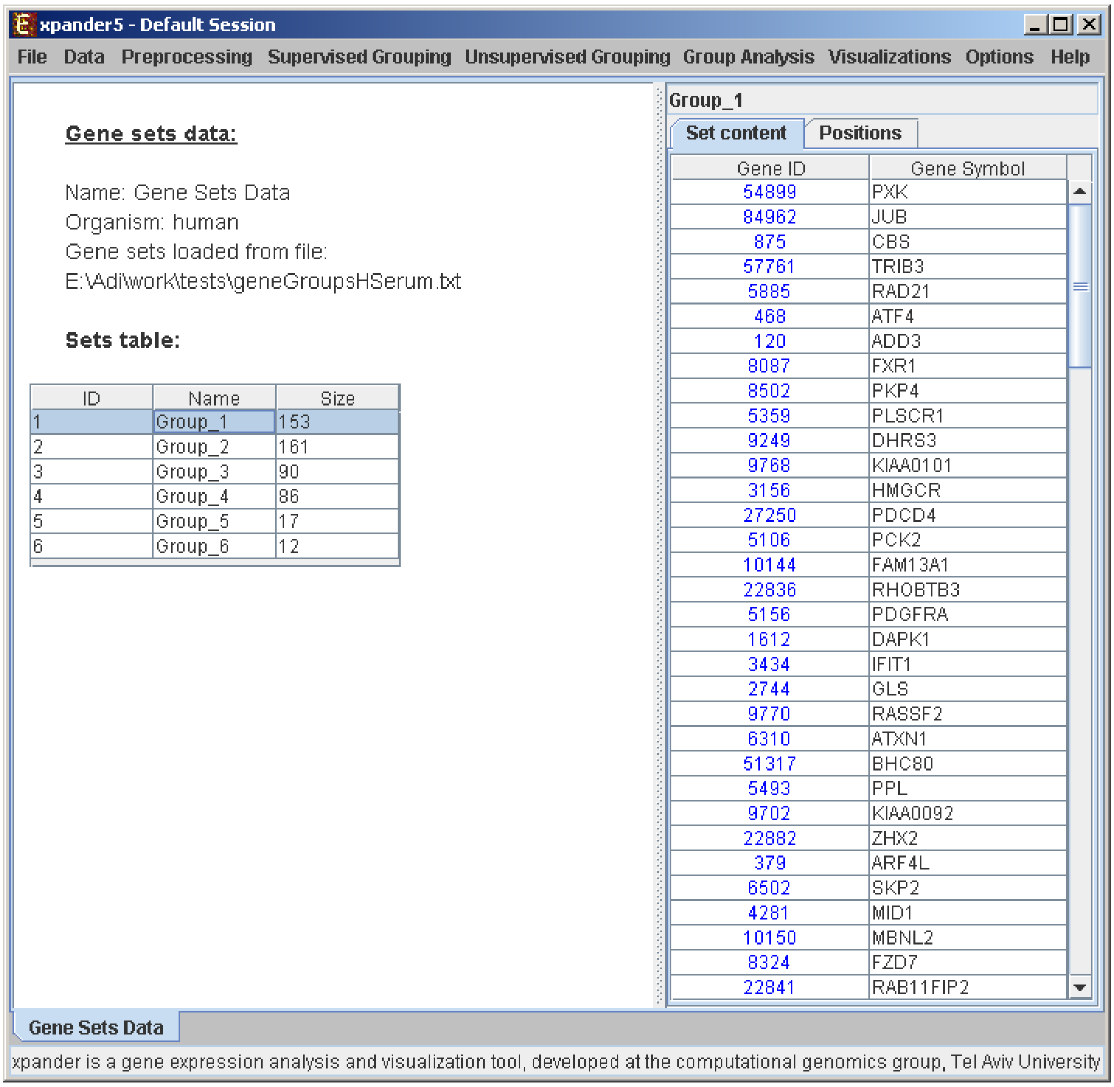
Data name: Gene Sets Data

File Name: E:\Adi\work\tests\geneGroupsHSerum.txt

● Tab delimited ○ Space delimited

For details regarding the data files formats see the File Formats section.

After loading gene groups, a ‘Session Data’ display tab is added to the main window (see example below). It contains information regarding the data file, and a table describing the different groups (serial number, name and size). Group names can be modified, by editing the corresponding cell in the table. Upon clicking on a row in the table, the corresponding group pane appears on the right. It contains a list of the genes in the group and a view of their chromosomal positions. If a network file has been loaded (via *Data>>Load Network*), the sub-graph, induced by the group is displayed as well.


xpander5 - Default Session
File Data Preprocessing Supervised Grouping Unsupervised Grouping Group Analysis Visualizations Options Help
Group_1
Set content
Positions
Gene Sets Data

Gene sets data:

Name: Gene Sets Data
Organism: human
Gene sets loaded from file:
E:\Adi\work\tests\geneGroupsHSerum.txt

Sets table:

ID	Name	Size
1	Group_1	153
2	Group_2	161
3	Group_3	90
4	Group_4	86
5	Group_5	17
6	Group_6	12

Gene ID	Gene Symbol
54899	PXK
84962	JUB
875	CBS
57761	TRIB3
5885	RAD21
468	ATF4
120	ADD3
8087	FXR1
8502	PKP4
5359	PLSCR1
9249	DHRS3
9768	KIAA0101
3156	HMGCR
27250	PDCD4
5106	PCK2
10144	FAM13A1
22836	RHOBTB3
5156	PDGFRA
1612	DAPK1
3434	IFIT1
2744	GLS
9770	RASSF2
6310	ATXN1
51317	BHC80
5493	PPL
9702	KIAA0092
22882	ZHX2
379	ARF4L
6502	SKP2
4281	MID1
10150	MBNL2
8324	FZD7
22841	RAB11FIP2

xpander is a gene expression analysis and visualization tool, developed at the computational genomics group, Tel Aviv University

Preprocessing GE Data

The following preprocessing operations can be performed using EXPANDER:

1) **Flooring** (*Preprocessing* >> *Floor Data*): setting all expression values that are bellow a certain threshold (set by the user) into that threshold. This can be done either by setting the floor value itself, or by setting the percentile that should be used as floor value.

2) **Merging conditions** (*Preprocessing* >> *Merge conditions*): merging a selected set of condition profiles (columns) in the dataset into one profile, in which each entry holds the average value of the merged entries.

3) **Merging probes according to gene ID** (*Preprocessing* >> *Merge Probes by Gene ID*): automatically shrinks the matrix so that all rows of probes from the same gene are merged into one average row, identified by the corresponding gene ID.

4) **Normalization**: required in order to remove systematic variation, i.e. variation arising from reasons other than biological differences between RNA samples. Expander performs normalization only for absolute intensities data, since it is assumed that the relative intensities data (e.g. cDNA microarrays) is already normalized, as it is input after performing log ratio (log2R/G).

Normalization can be performed using the following schemes:

a) **Quantile normalization** (*Preprocessing >> Normalization >> Quantile*), in which the whole data is used.

b) **Non-linear baseline normalization** (*Preprocessing >> Normalization >> Non Linear Baseline*), which uses a baseline array (can be selected by the user). In this scheme a normalization function is calculated using pseudo Loess regression of the M vs. A scatter plot. The subset of genes that are used to evaluate the normalization function can be set to 'all genes' (recommended when most genes in the dataset are expected to be constantly expressed) or a 'rank invariant set' of genes (recommended when there can be a large number of differentially expressed genes).

For more details regarding the normalization schemes see the References section.

5) **Condition filtration**: the conditions used in the analysis can be manually filtered by selecting: *Preprocessing* >> *Filter Conditions*. This will bring up a dialog box in which the user can select the required conditions from a list.

6) **Gene (probe) filtration**: can be performed in order to filter out some of the constantly expressed genes, and perform downstream analysis on a smaller informative subset of the genes.

Probe filtration can be performed using the following schemes:

a) **t-Test** (*Preprocessing* >> *Filter Probes* >> *t-Test*): When using this method, only probes that demonstrate differential expression between two condition subsets are selected.

b) **SAM -** Significance Analysis of Microarray (*Preprocessing* >> *Filter Probes* >> *SAM*): selects probes that demonstrate differential expression between conditions subsets. You may choose 2 or more subsets (multi-class tests are supported). This method uses permutations to get an 'empirical' estimate for the FDR of the reported differential genes (for details see the References section). Before using SAM, please make sure you have **R software along with the "samr" package** installed (see R External Application section).

c) **Fold Change** (*Preprocessing* >> *Filter Probes* >> *Fold Change*): when using this method only genes that are over/under expressed by at least n fold in at least k arrays are selected (n and k are determined by the user). The fold change can be calculated in relation to (a) a selected baseline array (b) the minimal expression value of the gene OR (c) the reference value when working on relative intensities (depending on the user's selection).

d) **Variation** (*Preprocessing* >> *Filter Probes* >> *Variation*): In this method, the k most variant genes are selected (k is determined by the user). Variance is used to measure variation for relative intensities data, and Coefficient of Variation is used to measure variation for absolute intensities data.

e) **Detection calls** (*Preprocessing* >> *Filter Probes* >> *Detection calls*): in this method probes/genes are filtered according to the number of expression signals for which the detection call is 'P' (Present). It can only be operated if the data file contains detection info.

f) **Load Probe Subset** (*Preprocessing* >> *Filter Probes* >> *Load Probe Subset*): the filtered set is loaded from an external txt file (for details regarding the format please see the File Formats section).

7) **Divide by Base** (*Preprocessing* >> *Divide by Base*) – Divides each entry in a profile (a column) by the corresponding entry in the profile of a selected base condition. This can be done for all conditions or for subsets of the conditions.

8) **Log data** (*Preprocessing* >> >> *Log Data*) – Performs log2 operation on each entry

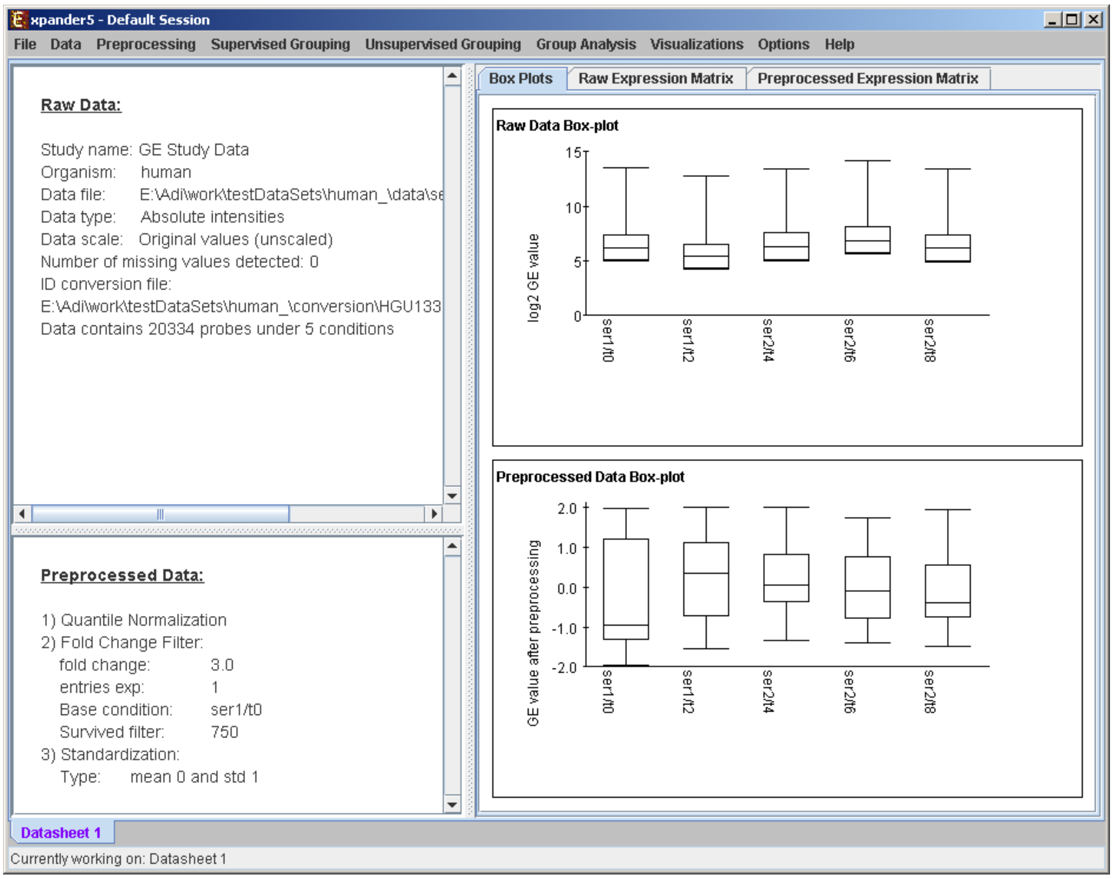
9) **Standardization**: When expression values between different genes are very different, but general expression patterns are similar (high Pearson Correlation values), we would expect to see this similarity when looking on a pattern display. Since the absolute values of expression are different, a manipulation is required, in order to view the patterns on the same scale. This manipulation is called standardization.

Standardization can be performed using the following schemes:

a) **Mean 0 and Variance 1** (*Preprocessing >> Standardization >> Mean 0 and Variance 1*) – normalizes each expression pattern to have a mean of 0 and a variance of 1. This method is appropriate in most cases when working on genes.

b) **Fixed norm** (*Preprocessing >> Standardization >> Fixed Norm*) - normalizes each expression pattern to have a fixed norm i.e. expression levels are divided by the norm of that expression vector (the root of sum of squares of that vector). This method is appropriate when different mean values or variances are expected for different patterns (e.g. when working on conditions and expecting larger variance in later phases of a response.

After performing a preprocessing operation, the information regarding the operation is added to the 'Preprocessed Data' section in the 'Session Data' tab. In addition, the 'Preprocessed Data box plot' and 'Preprocessed Expression Matrix' are automatically updated according to the new values in the data.



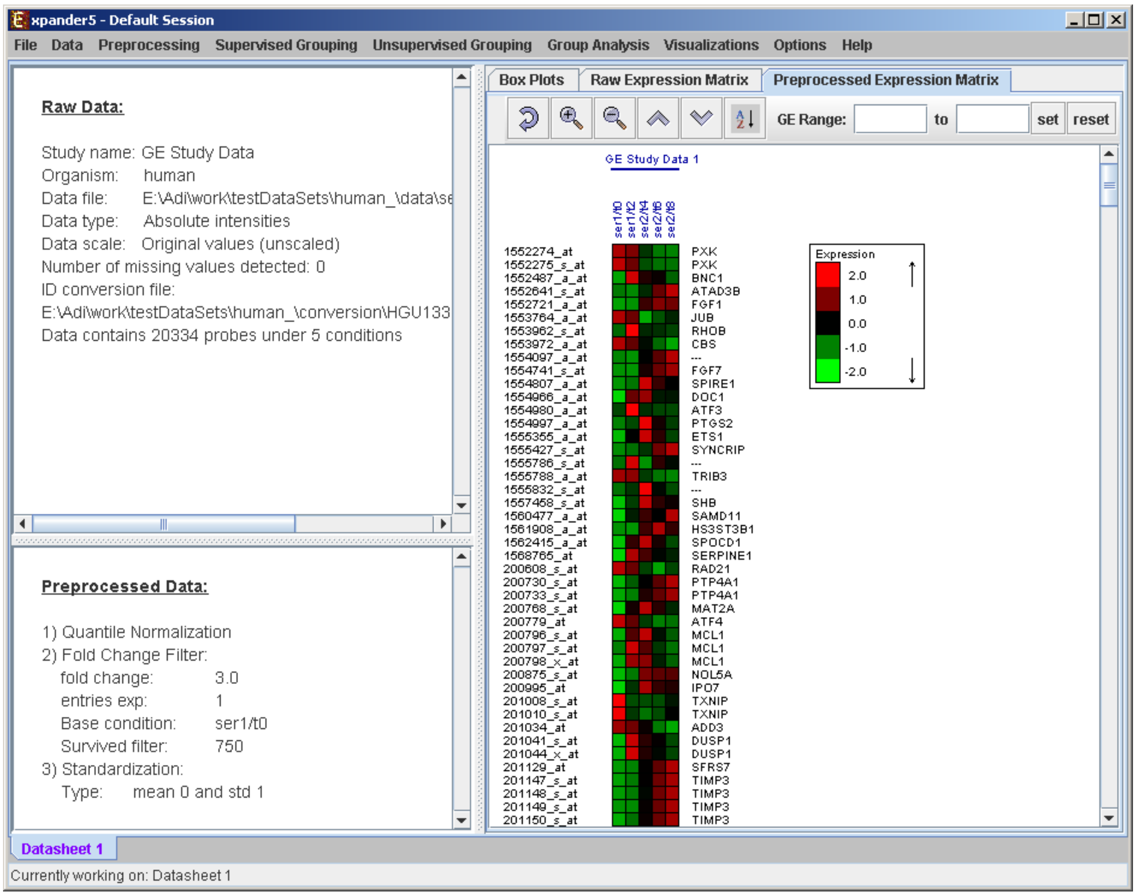
Raw Data:

Study name: GE Study Data
Organism: human
Data file: E:\Adi\work\testDataSets\human_\data\se
Data type: Absolute intensities
Data scale: Original values (unscaled)
Number of missing values detected: 0
ID conversion file:
E:\Adi\work\testDataSets\human_\conversion\HGU133
Data contains 20334 probes under 5 conditions

Preprocessed Data:

1) Quantile Normalization
2) Fold Change Filter:
fold change: 3.0
entries exp: 1
Base condition: ser1/t0
Survived filter: 750
3) Standardization:
Type: mean 0 and std 1

Currently working on: Datasheet 1



Raw Data:

Study name: GE Study Data
Organism: human
Data file: E:\Adi\work\testDataSets\human_\data\se
Data type: Absolute intensities
Data scale: Original values (unscaled)
Number of missing values detected: 0
ID conversion file:
E:\Adi\work\testDataSets\human_\conversion\HGU133
Data contains 20334 probes under 5 conditions

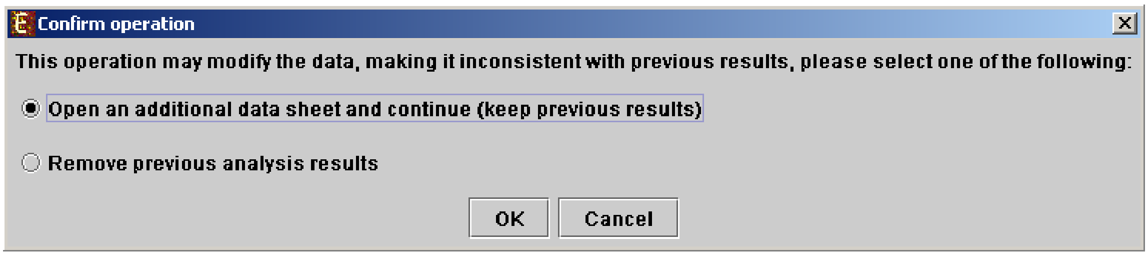
Preprocessed Data:

1) Quantile Normalization
2) Fold Change Filter:
fold change: 3.0
entries exp: 1
Base condition: ser1/t0
Survived filter: 750
3) Standardization:
Type: mean 0 and std 1

Currently working on: Datasheet 1

Upon selecting *Preprocessing >> Undo* the data is changed to be as it was before the most recent preprocessing operation was performed, and the corresponding information is removed from the 'Preprocessed Data' section. The 'Preprocessed Data box plot' and 'Preprocessed Expression Matrix' are automatically updated accordingly.

All the above operations can be performed before running further analysis on the data and generating displays. When attempting to perform further preprocessing operations after analysis results and visualizations have been generated, the following dialog box appears:



This operation may modify the data, making it inconsistent with previous results, please select one of the following:

● Open an additional data sheet and continue (keep previous results)

○ Remove previous analysis results

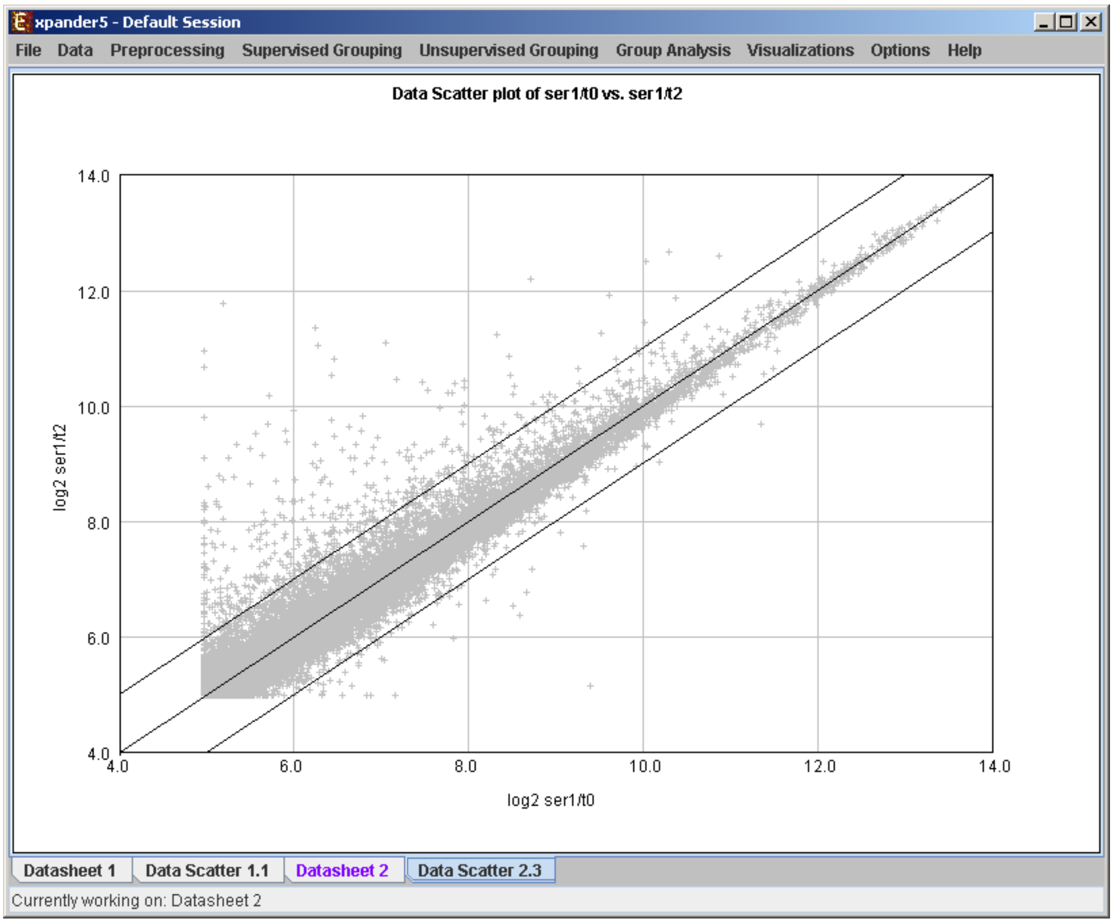
Upon choosing to open an additional data sheet, a new data set view tab called 'Data Sheet 2' is added to the main frame. The title of this tab is highlighted (colored in purple), indicating that it is now the active data sheet (i.e. all further operations refer to this data sheet). The active data sheet is automatically changed according to the selected (front) visualization tab.

Preprocessed gene expression data can be saved to a file at any time be selecting *Preprocessing >> Save Preprocessed Data*. The data is written in the same format defined for input GE data.

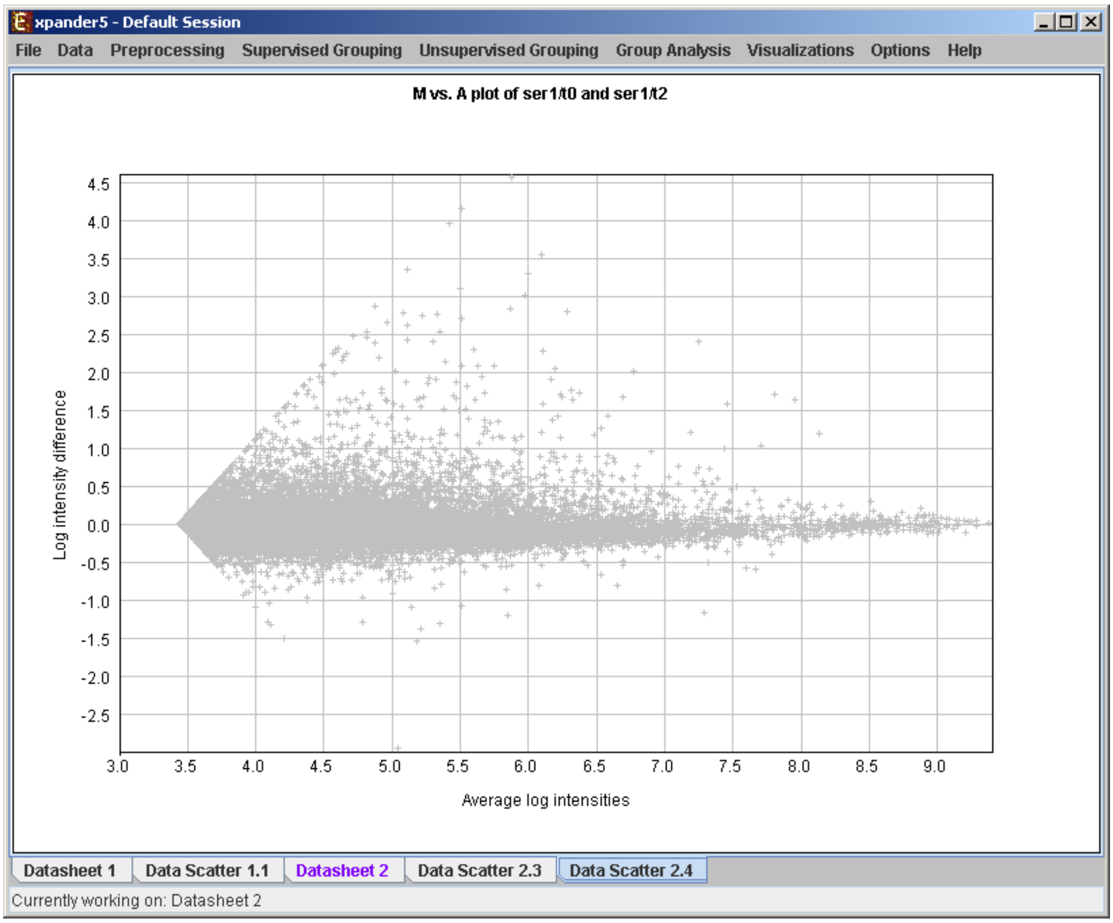
Viewing Data Plots

Expander provides two types of scatter plots visualizations that can be operated via *Visualization >> Scatter Plots...*

Simple plot - Displays a scatter plot of two arrays (selected by the user), in which the ith point (xi,yi) represents the expression value (log expression for un-logged data) of the ith gene in one array vs. the other. For normalized data, points should be located around the y=x line (marked on the scatter plot).



M vs. A plot (available only for absolute intensities data) - Displays a scatter plot in which each point (Ai,Mi) represents the log intensity difference of the i th probe in the two arrays (selected by the user) vs. the average log value of these intensities.



Differential Expression Analysis

The goal in this analysis is to detect groups of genes that demonstrate differential expression between two/more condition groups.

a) **t-Test** (*Supervised Grouping >> Differential Expression >> t-Test*): When using this method, genes can be assigned into one of two groups (up-regulated and down-regulated), depending on the definitions of t-test parameters.

b) **Wilcoxon rank sum test -** (*Supervised Grouping >> Differential Expression >> Ranksum test*): When using this method, genes can be assigned into one of two groups (up-regulated and down-regulated), depending on the definitions of Ranksum test parameters. This method is nearly as efficient as the t-test on normal distributions of expression values but has a greater efficiency than the t-test on non-normal distributions of expression values.

c) **Negative binomial (edgeR) –** Negative Binomial distribution test for RNA-seq count data (*Supervised Grouping >> Differential Expression >> NB (edgeR)*): this method is used to demonstrate differential expression between 2 condition subsets for RNA-seq count data where for each probe i and condition j in the expression matrix the value is a non-negative integer. As part of the test 3 different dispersion options are given: Tagwise – for a large amount of samples (>6), where a different dispersion is calculated for each probe, Common – for small amount of samples (<6), where the same dispersion is given for each probe, Poisson – a special case of NB where dispersion = 0 for all probes. The probes are then assigned into two groups (up-regulated and down-regulated). For further information regarding edgeR please refer to References. Before using edgeR, please make sure you have **R software along with the “edgeR” and "limma" packages** installed (see R External Application section).

d) **SAM -** Significance Analysis of Microarray (*Supervised Grouping >> Differential Expression >> SAM*): this method detects probes that demonstrate differential expression between conditions subsets. You may choose 2 or more subsets (multi-class tests are supported). The probes are then assigned into two groups (up-regulated and down-regulated) if 2 condition groups are tested or into one group of differentially expressed otherwise. SAM uses permutations to get an 'empirical' estimate for the FDR of the reported differential genes (for details see the References section). Before using SAM, please make sure you have **R software along with the "samr" package** installed (see R External Application section).

After performing differential expression grouping analysis, a solution visualization tab is added to the main window. It contains the following views:

Information regarding the algorithm, number of groups (can be either 1 or 2), number of un-grouped elements (non-differential), and numerical measures of the groups quality, including:

a) Overall average homogeneity - calculated as the average value of similarity between each element and the center of the group to which it has been assigned, weighted according to the size of the group.

b) Overall average separation – calculated as the average similarity between mean patterns of different groups, weighted according to their sizes.

c) Groups table - contains the number, name (label), size and homogeneity of each group.

Mean Patterns of the groups with error bars (±1 STD).

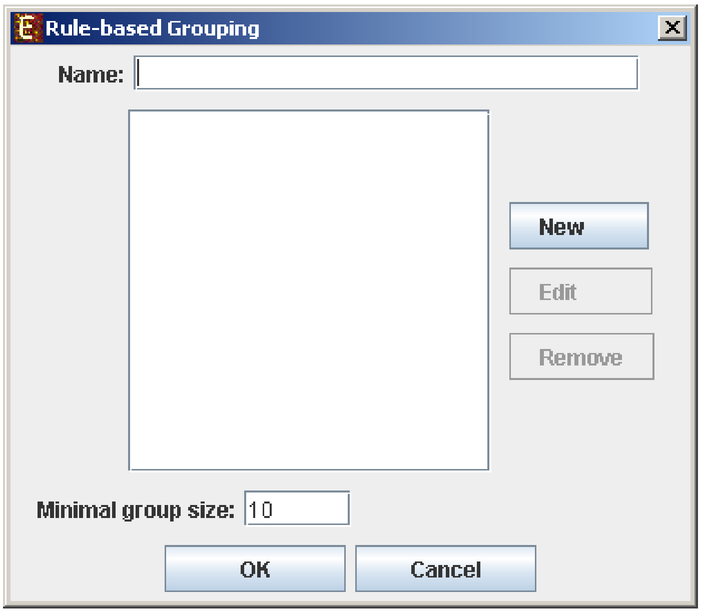
Upon selecting a group, the corresponding pane is displayed on the right. It contains a list of probes, p-values/q-values, fold-change, probe patterns, expression matrix (heat map) and the chromosomal locations of the genes. Similarity matrices for probes within the cluster as well as for conditions are also displayed in this tab, if the relevant options in the display settings are selected (see the Settings section). If a network file has been loaded (via Data>>Load Network), the sub-graph, induced by the cluster is also displayed in the group pane.

In order to allow comparison between groups and patterns, the displayed expression patterns are automatically standardized to have mean = 0 and STD = 1.

A differential expression solution can be saved using the File >> Export to text..., and reloaded using the Grouping Supervised Grouping >> Differential Expression >> Load Solution.

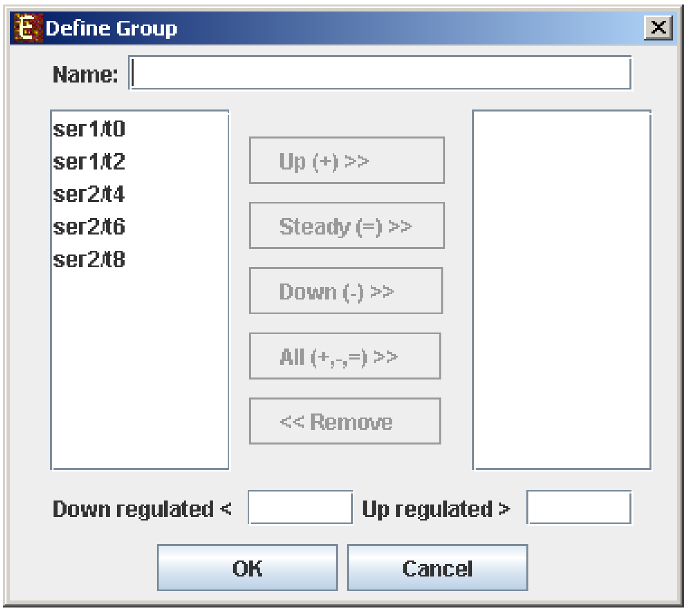
Defining a group according to a rule

This can be done by selecting *Supervised Grouping >> Rule-based Grouping.* The following input dialog box will appear:



Minimal group size: 10

Upon pressing the “New” button, the following dialog box will appear, to allow defining the group rule:



In the dialog box, name the new group and select the conditions of interest. For each condition define weather the expression level should be up-regulated, down-regulated or steady (between the up-regulation threshold and the down-regulation threshold). These thresholds should also be defined. A condition can also be added by pressing the “All” button. In this case a separate group will be defined for each of the options of that condition (i.e. a definition of a group using the “All” button can result in more than one group). The visualization for this operation is similar to the clustering results visualization (described below).

Defining a group according to similarity to a selected probe

This can be done by selecting *Supervised Grouping >> Group by Pattern Similarity.* An input dialog box allows setting the similarity measure (Pearson correlation, Spearman correlation or Euclidean distance) and reference probe ID as well as the expected group size. The visualization for this operation is similar to the clustering results visualization (described below).

Clustering GE Data

The goal of clustering is to partition the genes into distinct sets such that genes that are assigned to the same cluster should have similar expression patterns, while genes assigned to different clusters should have non-similar expression patterns. Usually there is no one solution that is the 'true' mathematical solution for this problem, but a good clustering solution should have two merits:

(1) High homogeneity (average similarity between genes from the same cluster).

(2) High separation (average distance/dissimilarity between genes from different clusters).

After operating one of the clustering algorithms a clustering results view appears. The view contains information about the solution and its quality including the method and parameters that were used to obtain it, number of clusters, number of singletons (probes that were not assigned to any cluster), overall homogeneity and separation, as well as the size and homogeneity of each cluster. This summary can be used to compare different solutions.

In order to apply a clustering algorithm to the data, select the required algorithm from the *Unsupervised Grouping >> Clustering* menu (options are: **KMeans**, **CLICK**, **SOM**). You can also use the agglomerative hierarchical clustering algorithm by extracting a partition from an existing hierarchical tree, by selecting *Unsupervised Grouping >> Hierarchical Clustering>> Generate Groups* (For details about building such a tree, please go to Hierarchical Clustering).

Currently similarity data can only be clustered using the Hierarchical clustering procedure by selecting *Unsupervised Grouping>>Hierarchical Clustering>>Cluster...* The resulting tree can be used to generate groups (for further details see Hierarchical Clustering).

An existing clustering solution can be loaded from a file by selecting *Unsupervised Grouping >> Clustering >>Load Solution* (For details regarding the clustering solution file format, refer to the File Formats section). The **CLICK** algorithm is not designed to find clusters under the size of 15 probes, so it might fail in clustering small datasets.

Fill the required input data in the algorithm input dialog box and press the 'Ok' button. The parameters required for each method are as follows:

Algorithm	Required parameters
KMeans	Expected number of clusters.
SOM	Grid width, grid length (width*length >= number of clusters) and number of iterations.
CLICK	Homogeneity value (0-1): allows the user control over the homogeneity of the resulting clustering, i.e. the average similarity between elements in the same cluster. This parameter serves as a threshold in various steps in the algorithm, including the definition of cluster kernels, singleton adoptions and kernel merging. The default value for this parameter is the estimated homogeneity of the true clustering. The higher the value assigned to this parameter the tighter the resulting clusters.
Hierarchical tree partition	Distance threshold (if extracting by distance): 0-1 the minimal tree distance that is reqiored for two nodes to be assigned to the same group • It is also possible to partition the tree according to manual node selection that is performed on the hierarchical view (see Hierarchical Clustering).

Details about the algorithms can be obtained through the relevant articles in the References section.

After clustering is performed, a clustering solution visualization tab is added to the main window. It contains the following views:

Information regarding the clustering algorithm, number of clusters, number of un-clustered elements (singletons), and numerical measures of the clustering quality, including:

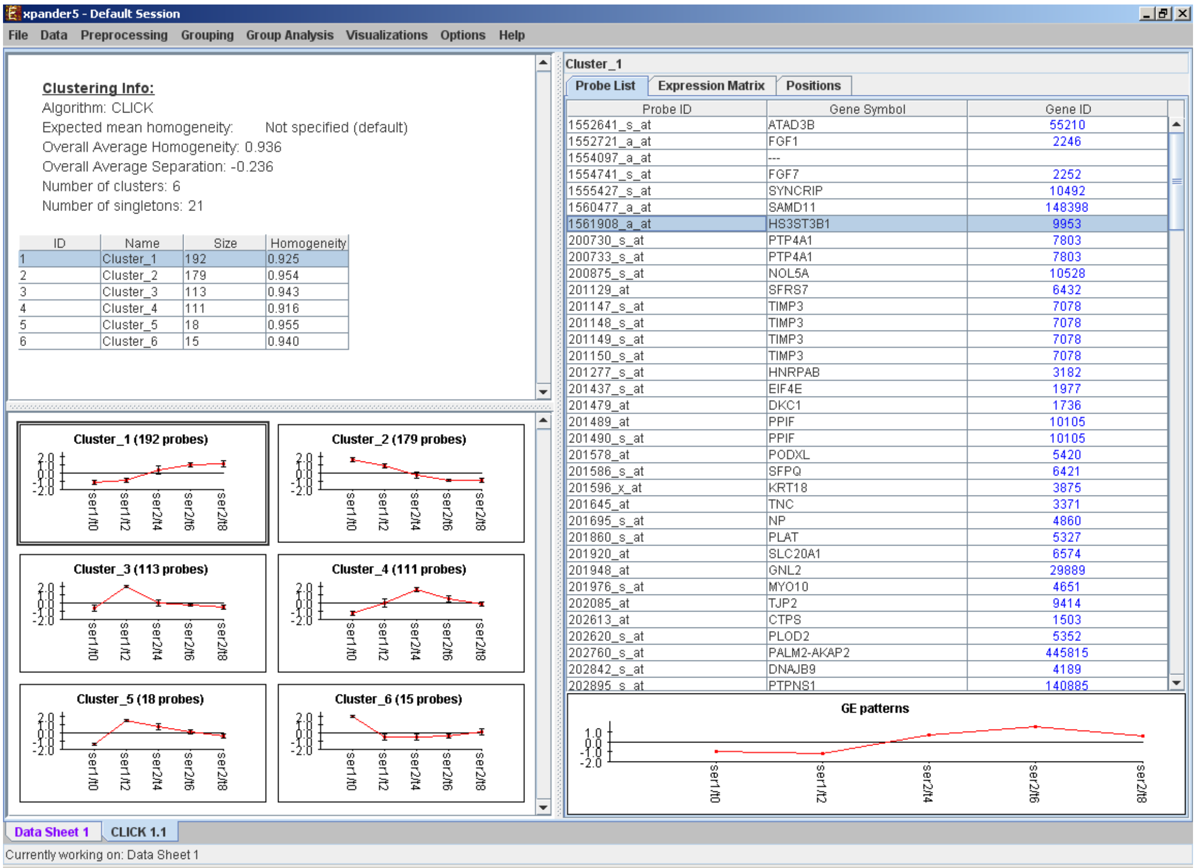
d) Overall average homogeneity - calculated as the average value of similarity between each element and the center of the cluster to which it has been assigned, weighted according to the size of the cluster.

e) Overall average separation – calculated as the average similarity between mean patterns of different clusters, weighted according to their sizes.

f) Clusters table - contains the number, name (label), size and homogeneity of each cluster. The name of a cluster can be changed by editing the corresponding cell in the table.

Mean Patterns of all clusters with error bars (±1 STD).

Upon selecting a cluster (from the clusters table or from the mean patterns view), the corresponding cluster pane is displayed on the right. It contains a list of probes, probe patterns, expression matrix (heat map) and the chromosomal locations of the genes. Similarity matrices for probes within the cluster as well as for conditions are also displayed in this tab, if the relevant options in the display settings are selected (see the Settings section). If a network file has been loaded (via Data>>Load Network), the sub-graph, induced by the cluster is also displayed in the cluster pane.

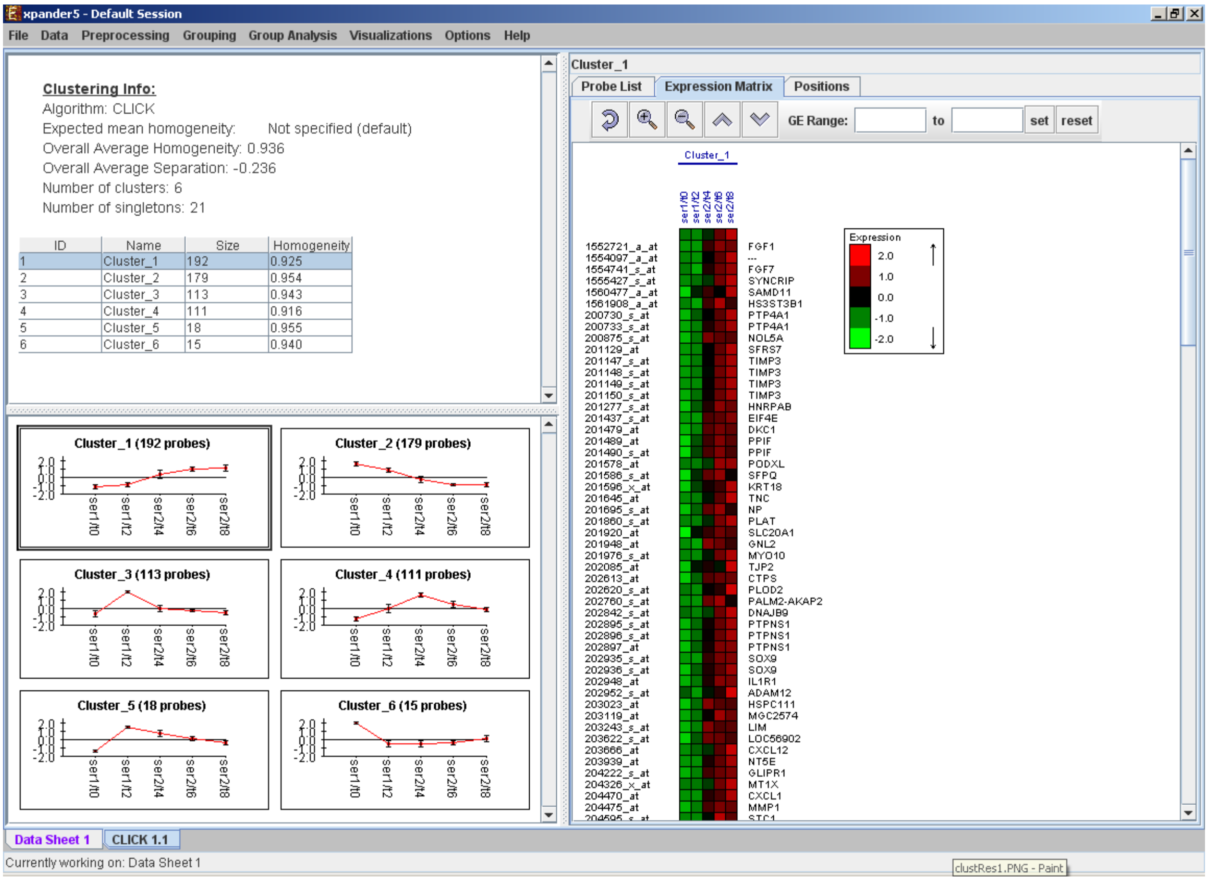

xpander5 - Default Session
File Data Preprocessing Grouping Group Analysis Visualizations Options Help
Cluster_1
Probe List Expression Matrix Positions

Clustering Info:
Algorithm: CLICK
Expected mean homogeneity: Not specified (default)
Overall Average Homogeneity: 0.936
Overall Average Separation: -0.236
Number of clusters: 6
Number of singletons: 21

ID	Name	Size	Homogeneity
1	Cluster_1	192	0.925
2	Cluster_2	179	0.954
3	Cluster_3	113	0.943
4	Cluster_4	111	0.916
5	Cluster_5	18	0.955
6	Cluster_6	15	0.940

Probe ID	Gene Symbol	Gene ID
1552641_s_at	ATAD3B	55210
1552721_a_at	FGF1	2246
1554097_a_at	---	
1554741_s_at	FGF7	2252
1555427_s_at	SYNCRIP	10492
1560477_a_at	SAMD11	148398
1561908_a_at	HS3ST3B1	9953
200730_s_at	PTP4A1	7803
200733_s_at	PTP4A1	7803
200875_s_at	NOL5A	10528
201129_at	SFRS7	6432
201147_s_at	TIMP3	7078
201148_s_at	TIMP3	7078
201149_s_at	TIMP3	7078
201150_s_at	TIMP3	7078
201277_s_at	HNRPAB	3182
201437_s_at	EIF4E	1977
201479_at	DKC1	1736
201489_at	PPIF	10105
201490_s_at	PPIF	10105
201578_at	PODXL	5420
201586_s_at	SFPQ	6421
201596_x_at	KRT18	3875
201645_at	TNC	3371
201695_s_at	NP	4860
201860_s_at	PLAT	5327
201920_at	SLC20A1	6574
201948_at	GNL2	29889
201976_s_at	MYO10	4651
202085_at	TJP2	9414
202613_at	CTPS	1503
202620_s_at	PLOD2	5352
202760_s_at	PALM2-AKAP2	445815
202842_s_at	DNAJB9	4189
202895_s_at	PTPNS1	140885

Currently working on: Data Sheet 1


xpander5 - Default Session
File Data Preprocessing Grouping Group Analysis Visualizations Options Help
Cluster_1
Probe List Expression Matrix Positions
GE Range: to set reset

Clustering Info:
Algorithm: CLICK
Expected mean homogeneity: Not specified (default)
Overall Average Homogeneity: 0.936
Overall Average Separation: -0.236
Number of clusters: 6
Number of singletons: 21

ID	Name	Size	Homogeneity
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2	Cluster_2	179	0.954
3	Cluster_3	113	0.943
4	Cluster_4	111	0.916
5	Cluster_5	18	0.955
6	Cluster_6	15	0.940

1552721_a_at	FGF1
1554097_a_at	---
1554741_s_at	FGF7
1555427_s_at	SYNCRIP
1560477_a_at	SAMD11
1561908_a_at	HS3ST3B1
200730_s_at	PTP4A1
200733_s_at	PTP4A1
200875_s_at	NOL5A
201129_at	SFRS7
201147_s_at	TIMP3
201148_s_at	TIMP3
201149_s_at	TIMP3
201150_s_at	TIMP3
201277_s_at	HNRPAB
201437_s_at	EIF4E
201479_at	DKC1
201489_at	PPIF
201490_s_at	PPIF
201578_at	PODXL
201586_s_at	SFPQ
201596_x_at	KRT18
201645_at	TNC
201695_s_at	NP
201860_s_at	PLAT
201920_at	SLC20A1
201948_at	GNL2
201976_s_at	MYO10
202085_at	TJP2
202613_at	CTPS
202620_s_at	PLOD2
202760_s_at	PALM2-AKAP2
202842_s_at	DNAJB9
202895_s_at	PTPNS1
202896_s_at	PTPNS1
202897_at	PTPNS1
202935_s_at	SOX9
202936_s_at	SOX9
202948_at	IL1R1
202952_s_at	ADAM12
203023_at	HSPC111
203119_at	MGC2574
203243_s_at	LIM
203622_s_at	LOC56902
203666_at	CXCL12
203939_at	NT5E
204222_s_at	GLIPR1
204326_x_at	MT1X
204470_at	CXCL1
204475_at	MMP1
204595_s_at	STC1

Currently working on: Data Sheet 1

After performing enrichment analysis (for details see the Enrichment Analysis Tools), if enrichment has been detected in the selected cluster, the corresponding histogram and analysis information are added to the single cluster view.

In order to allow comparison between groups and patterns, the displayed expression patterns are automatically standardized to have mean = 0 and STD = 1.

A clustering solution can be saved using the *File >> Export to text* option (with the corresponding clustering view as the selected tab) OR by using the *File>>Save All* option, which will export all solutions within a session to text and image files. A clustering solution can be reloaded using the *Unsupervised Grouping >> Clustering >> Load Solution*.

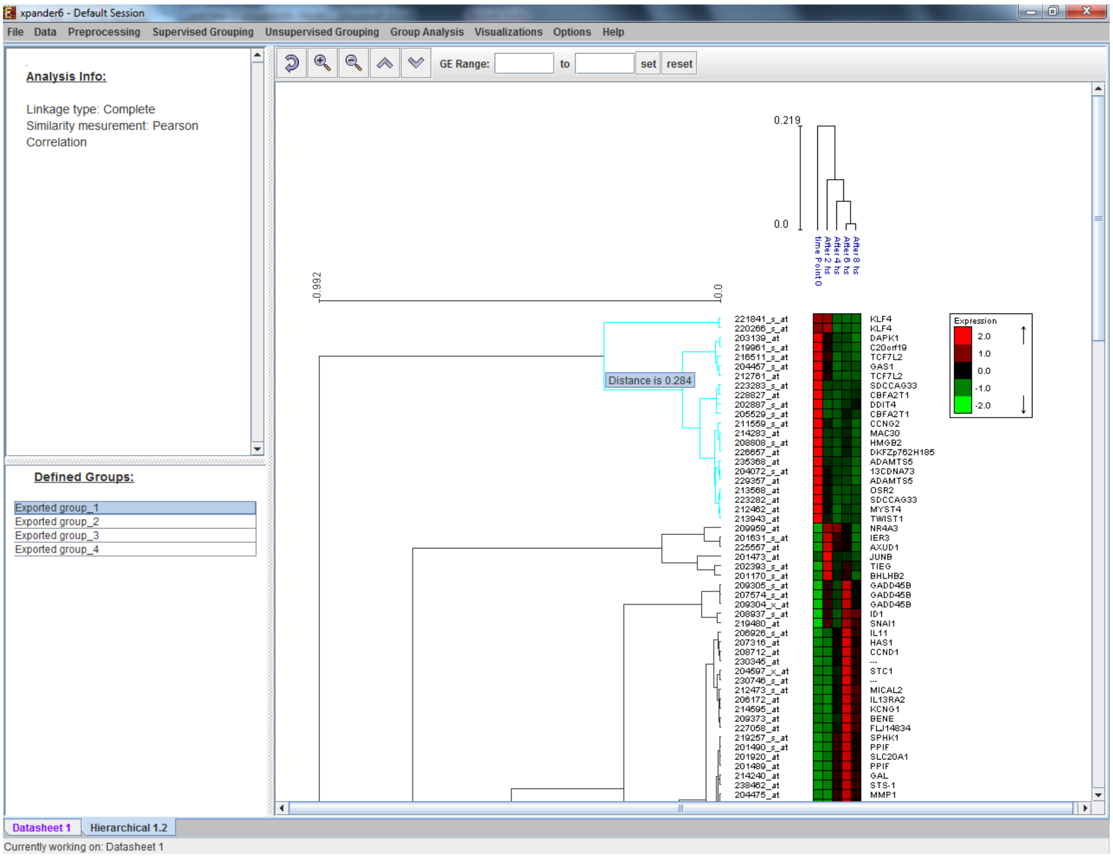
Hierarchical Clustering and Visualization

This tool uses the agglomerative algorithm to calculate a dendrogram tree for all expression patterns (probe patterns) and/or profiles (condition profiles). The type of linkage (manner in which the distance between a new node and the rest of the nodes is calculated) used in the algorithm can be set via an input dialog (for details regarding the algorithms refer to the References section). Note that it does not generate a partition of the probes to clusters. The distance measurement used in the algorithm is (1-Pearson Correlation)/2.

To perform hierarchical clustering, select *Unsupervised Grouping >> Hierarchical Clustering*. Upon selecting this option, a dialog box appears in which the 'linkage type' parameter, used in the algorithm can be set. After pressing 'OK', the algorithm will be operated both on the probe patterns and on the condition profiles.

The resulting trees are displayed next to an expression matrix so that the probe tree appears vertically on the left and the condition tree appears horizontally above the matrix. The scale next to each tree indicates the range of distance values between vectors corresponding to the leaves. The tool tip indicates the distance value corresponding to the cursor location on the tree.

If condition attributes file has been loaded for the analyzed dataset, a matrix representation of these attributes will be displayed below the expression matrix (heatmap).



Analysis Info:

Linkage type: Complete
Similarity mesurement: Pearson Correlation

Defined Groups:

Exported group_1
Exported group_2
Exported group_3
Exported group_4

Currently working on: Datasheet 1

Upon clicking on the vertical tree, a corresponding sub tree is highlighted (selected) and can be defined as a group by right clicking on the same location and selecting the “Export group” option from the right click menu. The sub tree is then added as a group of the bottom left panel of the display.

Upon selecting one of the groups that have been previously defined and added to the list on the bottom left panel, the corresponding sub tree is selected.

A previously selected sub tree can be removed from the list by right clicking on the corresponding group in the bottom left panel and selecting remove group.

Manually selected groups can then be defined as a grouping solution by selecting *Unsupervised Grouping>>Hierarchical Clustering>>Generated Groups>> From Selected subtrees.*

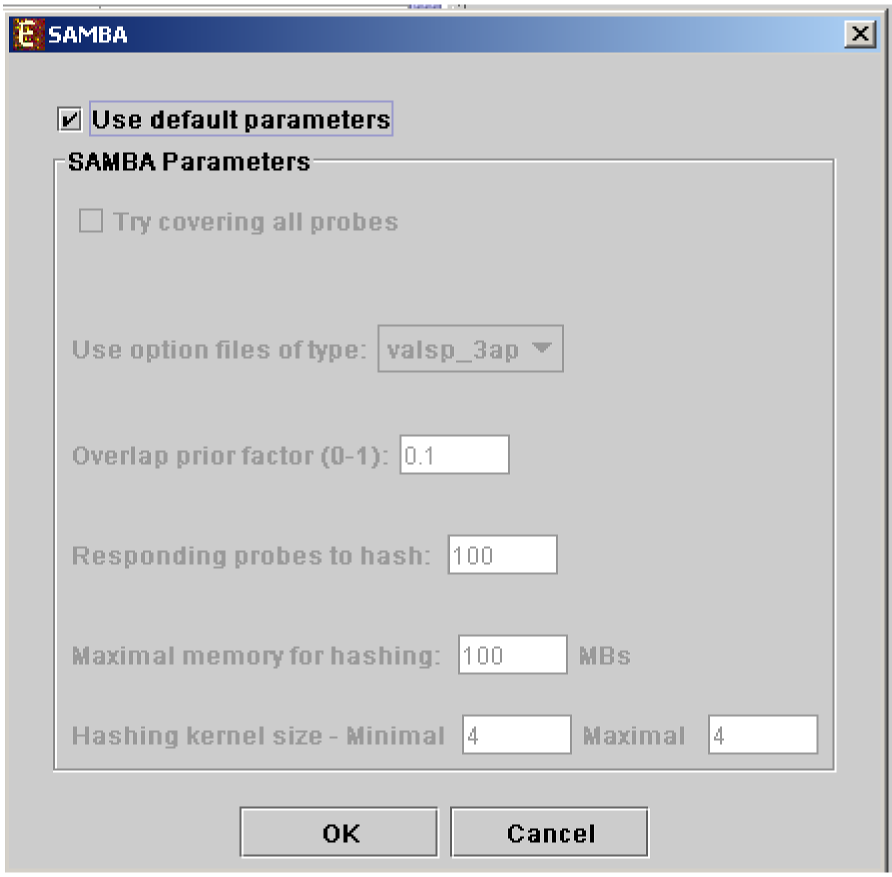
Biclustering GE Data

Biclustering is clustering of both genes and conditions of the data into subgroups that are not necessarily disjoint. It enables the user to detect genes that are co-regulated in only a subgroup of the conditions, and does not force genes to belong exclusively to one cluster. It is useful when working on datasets which contain a large number of conditions.

Expander incorporates two Biclustering algorithms: ISA (Iterative Signature Algorithm) and SAMBA algorithm (for details see the References section). Before using ISA, please make sure you have **R software along with the "eisa" package** installed (see R External Application section).

In order to apply the ISA algorithm to the data select *Unsupervised Grouping>>Bi-Clustering>>ISA*. This operation does not require parameter input.

In order to apply the SAMBA algorithm to the data select *Unsupervised Grouping>>Bi-Clustering>>SAMBA*. The following dialog box will appear:



SAMBA

☑ Use default parameters

SAMBA Parameters

□ Try covering all probes

Use option files of type: valsp_3ap

Overlap prior factor (0-1): 0.1

Responding probes to hash: 100

Maximal memory for hashing: 100 MBs

Hashing kernel size - Minimal 4 Maximal 4

It enables the configuration of some of the parameters for the algorithm. The following table specifies the different parameters that can be set via this dialog box:

Field	Description

<table>
<tr><td>Use default parameters</td><td>When checked, biclustering parameters (described below) are set automatically (this option is recommended unless the user is familiar with the parameters).</td></tr>
<tr><td>Option files type</td><td>The user can select one out of 6 options. The following table describes the advantages and disadvantages of each option:
<table>
<tr><th>Option name</th><th>fast performance</th><th>less memory required:</th><th>Flexible</th><th>Robust- can handle normalization problems and non gene-expression data</th></tr>
<tr><td>valsp_1</td><td>+</td><td>+</td><td>-</td><td>-</td></tr>
<tr><td>valsp_2</td><td>0</td><td>0</td><td>0</td><td>-</td></tr>
<tr><td>valsp_3</td><td>-</td><td>-</td><td>+</td><td>-</td></tr>
<tr><td>valsp_1ap</td><td>+</td><td>+</td><td>-</td><td>+</td></tr>
<tr><td>valsp_2ap</td><td>0</td><td>0</td><td>0</td><td>+</td></tr>
<tr><td>valsp_3ap</td><td>-</td><td>-</td><td>+</td><td>+</td></tr>
</table>
We recommend the valsp_3ap option (set as default), since it is very flexible, and produces good results also for data that was not normalized properly or for non gene-expression data.</td></tr>
<tr><td>Always cover all genes</td><td>When checked, the solution will cover each gene at least once (each gene will be included in one or more biclusters).</td></tr>
<tr><td>Always cover all conditions</td><td>When checked, the solution will cover each condition at least once (each condition will be included in one or more biclusters). Un checking this option will cause a reduction in the number of biclusters, and the algorithm will run faster.</td></tr>
<tr><td>Overlap prior factor</td><td>Can take values between 0 and 1, describes extent of overlap that is permitted between two different biclusters in the same solution. The higher this parameter is, the more strict the algorithm will be regarding adding a new bicluster (will require less overlap between the new bicluster and the existing ones).</td></tr>
<tr><td>Number of responding genes to hash</td><td>Can take values between 1 and the number of genes in the dataset. Default value is set to 100 (recommended unless data set size < 100). Has impact over the hashing stage in the algorithm.</td></tr>
<tr><td>Maximum hash size (in MB)</td><td>Described the maximum memory size that can be used for the hashing part of the algorithm (the whole algorithm will take up about twice this size of memory).</td></tr>
<tr><td>Maximum hash size</td><td>This parameter determines the number of condition kernel options that are tested and scored in the hashing stage. It can take values from 1 to 7. The default value is 4. In datasets with many conditions raising this number will significantly increase the algorithm run time (may also produce better results).</td></tr>
</table>

Minimum hash size	This parameter determines the minimal size of condition kernel in the hashing stage. It can take values from 1 to 7 and must be <= Maximum hash size. The default value is 4.

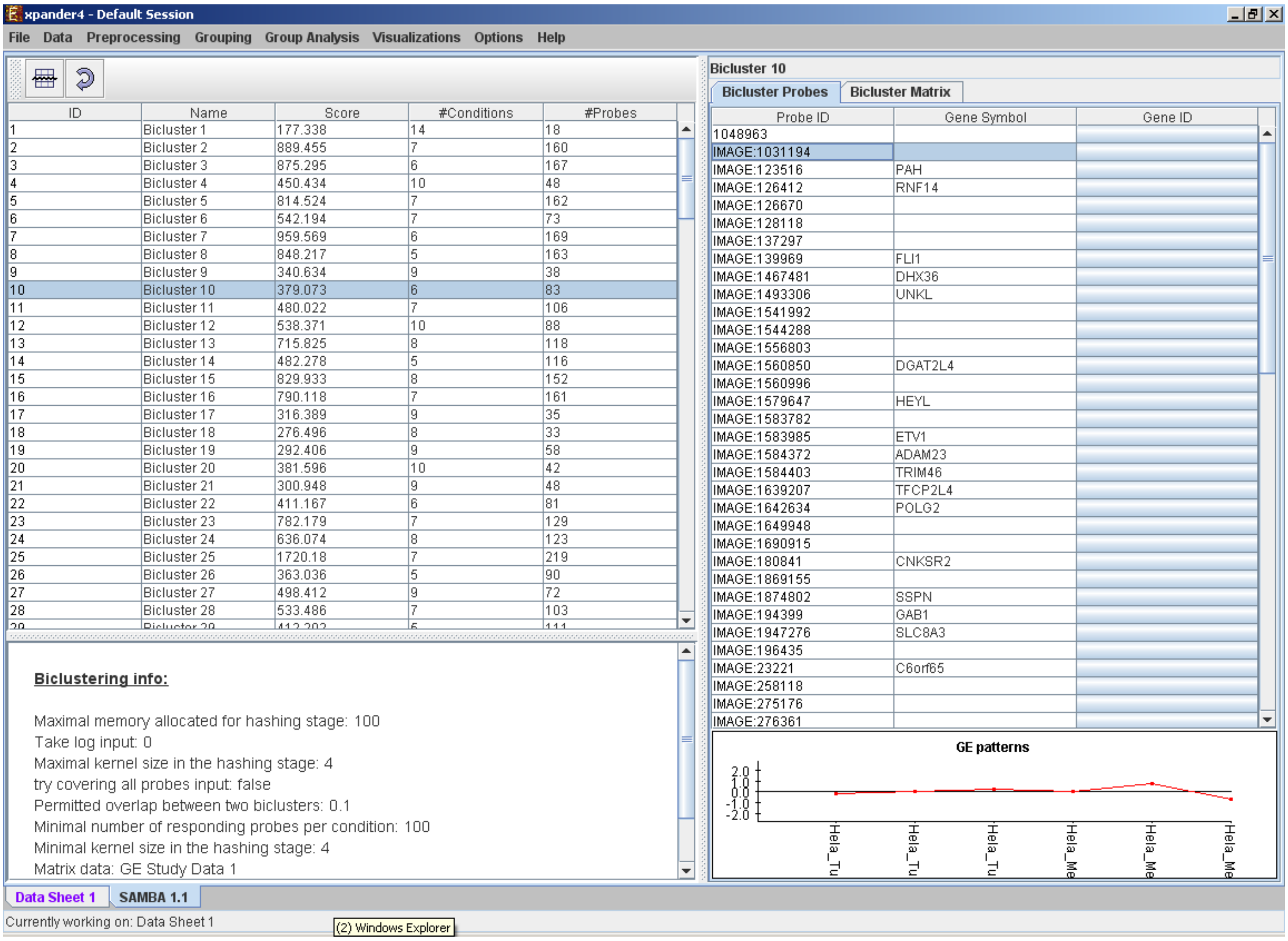
Upon clicking ‘OK’ in the dialog box, the SAMBA algorithm is operated on the dataset.

After biclustering is performed a biclustering solution visualization tab is added to the main window. It contains the following views:

a) Information regarding the biclustering algorithm, and number of resulting biclusters.

g) Biclusters table – contains the following information for each bicluster: serial number, name, score, number of probes genes and number of conditions. The name of a bicluster can be changed by editing the corresponding cell in the table. The score is given by the SAMBA algorithm and is size-dependent, thus, it is not recommended to use it to compare the quality of two biclusters of different sizes. The table can be filtered to display a subset of the biclusters by clicking on the ‘Filter’ (▦) button in the toolbar. Filtering can be performed according to: Score, number of probes and number of conditions.

Upon selecting a bicluster (from the biclusters table), the corresponding pane is displayed on the right. It contains a list of probes, probe patterns, expression matrix (heat map) and the chromosomal locations of the genes. Similarity matrices for probes within the cluster as well as for conditions are also displayed in this tab, if the relevant options in the display settings are selected (see the Settings section). If a network file has been loaded (via *Data>>Load Network*), the sub-graph, induced by the cluster is also displayed in the cluster pane.



ID	Name	Score	#Conditions	#Probes
1	Bicluster 1	177.338	14	18
2	Bicluster 2	889.455	7	160
3	Bicluster 3	875.295	6	167
4	Bicluster 4	450.434	10	48
5	Bicluster 5	814.524	7	162
6	Bicluster 6	542.194	7	73
7	Bicluster 7	959.569	6	169
8	Bicluster 8	848.217	5	163
9	Bicluster 9	340.634	9	38
10	Bicluster 10	379.073	6	83
11	Bicluster 11	480.022	7	106
12	Bicluster 12	538.371	10	88
13	Bicluster 13	715.825	8	118
14	Bicluster 14	482.278	5	116
15	Bicluster 15	829.933	8	152
16	Bicluster 16	790.118	7	161
17	Bicluster 17	316.389	9	35
18	Bicluster 18	276.496	8	33
19	Bicluster 19	292.406	9	58
20	Bicluster 20	381.596	10	42
21	Bicluster 21	300.948	9	48
22	Bicluster 22	411.167	6	81
23	Bicluster 23	782.179	7	129
24	Bicluster 24	636.074	8	123
25	Bicluster 25	1720.18	7	219
26	Bicluster 26	363.036	5	90
27	Bicluster 27	498.412	9	72
28	Bicluster 28	533.486	7	103
29	Bicluster 29	412.202	5	111

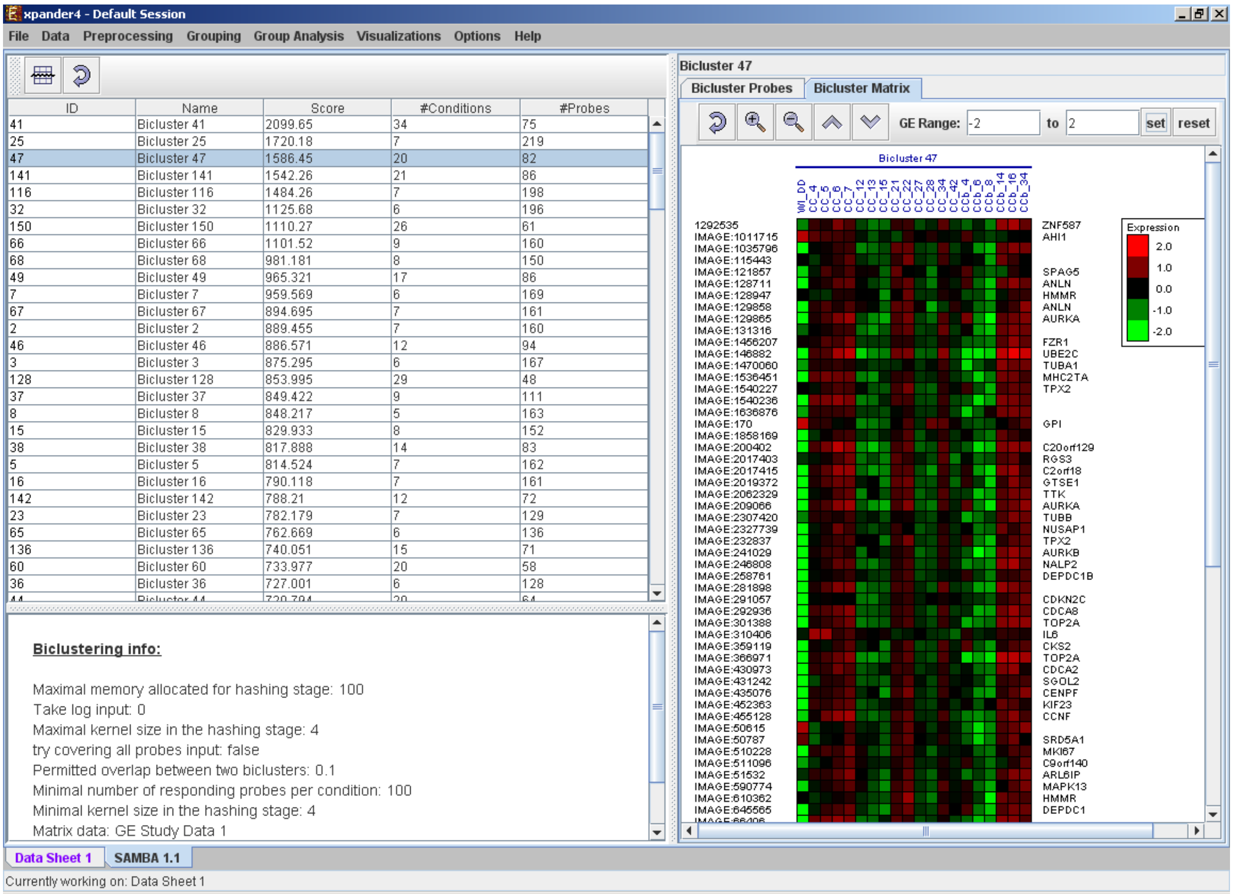
Biclustering info:

Maximal memory allocated for hashing stage: 100
Take log input: 0
Maximal kernel size in the hashing stage: 4
try covering all probes input: false
Permitted overlap between two biclusters: 0.1
Minimal number of responding probes per condition: 100
Minimal kernel size in the hashing stage: 4
Matrix data: GE Study Data 1

Bicluster 10

Probe ID	Gene Symbol	Gene ID
1048963		
IMAGE:1031194		
IMAGE:123516	PAH	
IMAGE:126412	RNF14	
IMAGE:126670		
IMAGE:128118		
IMAGE:137297		
IMAGE:139969	FLI1	
IMAGE:1467481	DHX36	
IMAGE:1493306	UNKL	
IMAGE:1541992		
IMAGE:1544288		
IMAGE:1556803		
IMAGE:1560850	DGAT2L4	
IMAGE:1560996		
IMAGE:1579647	HEYL	
IMAGE:1583782		
IMAGE:1583985	ETV1	
IMAGE:1584372	ADAM23	
IMAGE:1584403	TRIM46	
IMAGE:1639207	TFCP2L4	
IMAGE:1642634	POLG2	
IMAGE:1649948		
IMAGE:1690915		
IMAGE:180841	CNKSR2	
IMAGE:1869155		
IMAGE:1874802	SSPN	
IMAGE:194399	GAB1	
IMAGE:1947276	SLC8A3	
IMAGE:196435		
IMAGE:23221	C6orf65	
IMAGE:258118		
IMAGE:275176		
IMAGE:276361		

Currently working on: Data Sheet 1

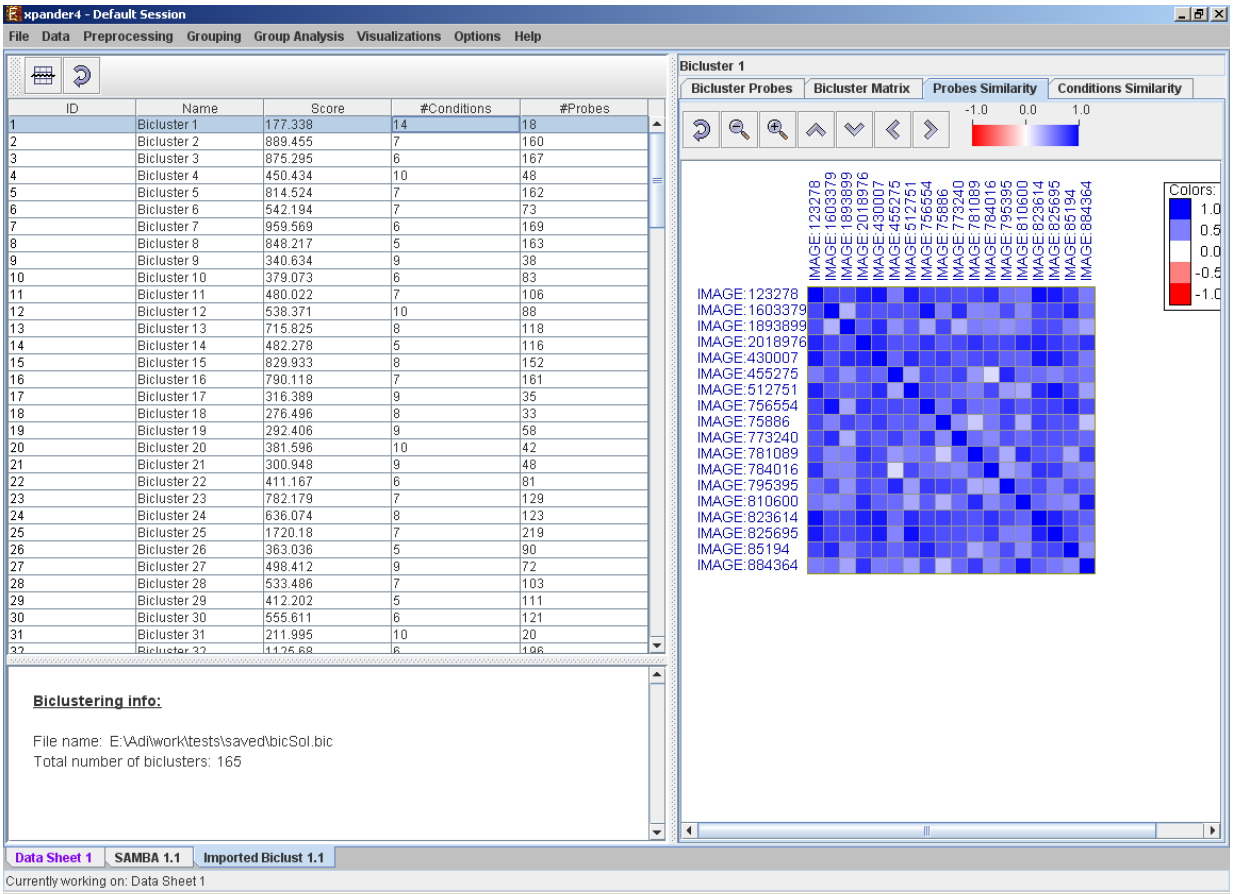


ID	Name	Score	#Conditions	#Probes
41	Bicluster 41	2099.65	34	75
25	Bicluster 25	1720.18	7	219
47	Bicluster 47	1586.45	20	82
141	Bicluster 141	1542.26	21	86
116	Bicluster 116	1484.26	7	198
32	Bicluster 32	1125.68	6	196
150	Bicluster 150	1110.27	26	61
66	Bicluster 66	1101.52	9	160
68	Bicluster 68	981.181	8	150
49	Bicluster 49	965.321	17	86
7	Bicluster 7	959.569	6	169
67	Bicluster 67	894.695	7	161
2	Bicluster 2	889.455	7	160
46	Bicluster 46	886.571	12	94
3	Bicluster 3	875.295	6	167
128	Bicluster 128	853.995	29	48
37	Bicluster 37	849.422	9	111
8	Bicluster 8	848.217	5	163
15	Bicluster 15	829.933	8	152
38	Bicluster 38	817.888	14	83
5	Bicluster 5	814.524	7	162
16	Bicluster 16	790.118	7	161
142	Bicluster 142	788.21	12	72
23	Bicluster 23	782.179	7	129
65	Bicluster 65	762.669	6	136
136	Bicluster 136	740.051	15	71
60	Bicluster 60	733.977	20	58
36	Bicluster 36	727.001	6	128
44	Bicluster 44	720.704	20	64

Biclustering info:

Maximal memory allocated for hashing stage: 100
Take log input: 0
Maximal kernel size in the hashing stage: 4
try covering all probes input: false
Permitted overlap between two biclusters: 0.1
Minimal number of responding probes per condition: 100
Minimal kernel size in the hashing stage: 4
Matrix data: GE Study Data 1

Currently working on: Data Sheet 1



ID	Name	Score	#Conditions	#Probes
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21	Bicluster 21	300.948	9	48
22	Bicluster 22	411.167	6	81
23	Bicluster 23	782.179	7	129
24	Bicluster 24	636.074	8	123
25	Bicluster 25	1720.18	7	219
26	Bicluster 26	363.036	5	90
27	Bicluster 27	498.412	9	72
28	Bicluster 28	533.486	7	103
29	Bicluster 29	412.202	5	111
30	Bicluster 30	555.611	6	121
31	Bicluster 31	211.995	10	20
32	Bicluster 32	1125.68	6	196

Biclustering info:

File name: E:\Adi\work\tests\saved\bicSol.bic
Total number of biclusters: 165

Currently working on: Data Sheet 1

After performing enrichment analysis (for details see the Enrichment Analysis Tools section), if enrichment has been detected in the selected bicluster, the corresponding histogram and analysis information are added to the single bicluster view, and a column is added to the expression matrix display for each enrichment class, stating for each probe, whether it belongs to that class.

A biclustering solution can be saved using the *File >> Export to text* option (with the corresponding biclustering view as the selected tab) OR by using the *File>>Save All* option, which will export all solutions within a session to text and image files. A biclustering solution can be reloaded by selecting *Unsupervised Grouping >> Bi-Clustering >> Load Solution*. For a format of the solution file, please refer to the File Formats section:

Network Based Grouping of GE Data

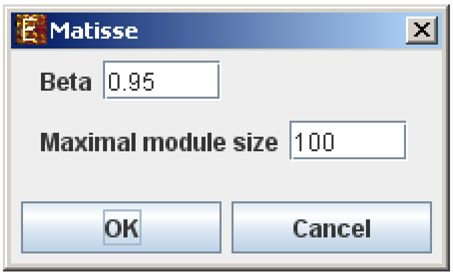
The goal here is to detect groups of genes that demonstrate similar expression patterns and are also highly connected in a given interactions network.

In order to operate these tools, an interactions network in .SIF format needs to be loaded. This can be done either by selecting *Data>>Load Network…* or via the dialog boxes of the tools.

In order to perform network based grouping Expander incorporates two algorithms: Matisse and Degas (for details see the References section). The DEGAS algorithm is relevant when the expression dataset compares two groups of heterogeneous samples (as in case-control studies). The groups detected by these tools are referred to as "modules" and may contain also genes that exist in the network, but are not present in the filtered GE data (referred to as "Back nodes").

To use the more advanced, stand-alone versions of MATISSE and DEGAS (with higher flexibility), please refer to the Matisse home page.

In order to apply the Matisse algorithm to the data select *Unsupervised Grouping>>Network >>Matisse*. The following dialog box will appear:


Matisse

Beta 0.95

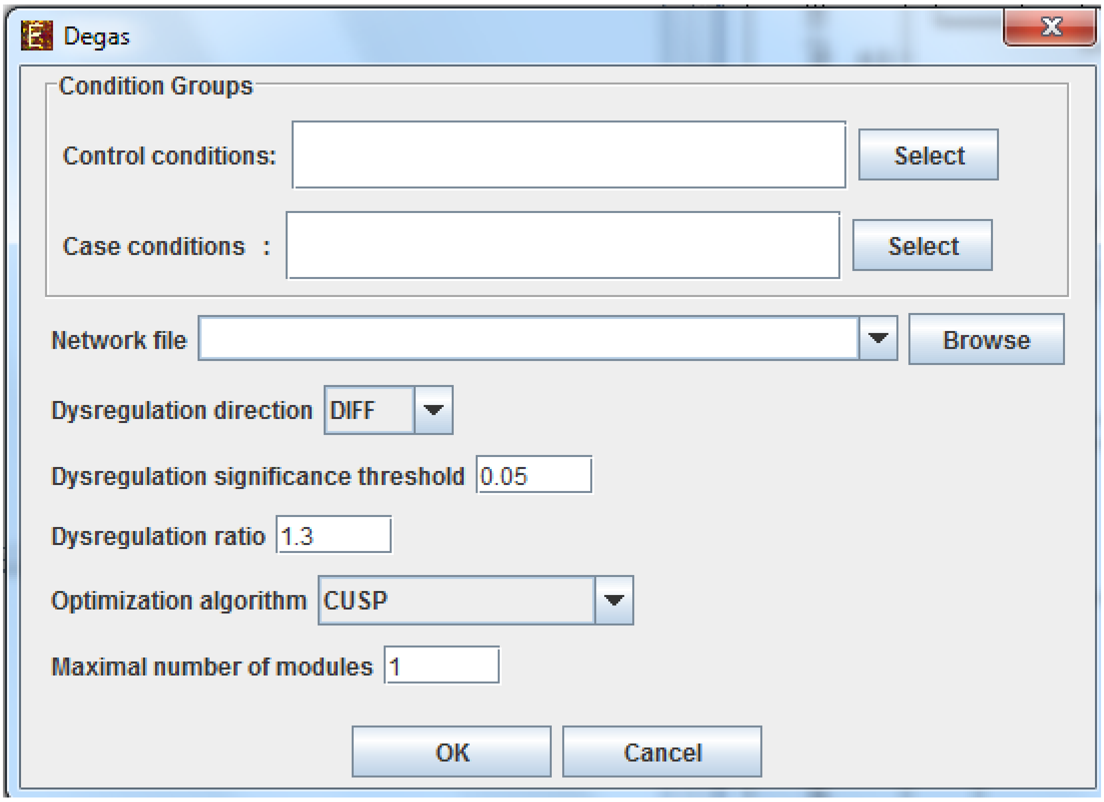
Maximal module size 100

It enables the configuration of some of the parameters for the algorithm:

Field	Description
Beta	The fraction of gene pairs that are expected to be strongly co-expressed in each module
Maximal module size	The maximum size for a detected module.

Upon clicking 'OK' in the dialog box, the Matisse algorithm is operated on the dataset.

In order to apply the Degas algorithm to the data select *Supervised Grouping>>Network >>Degas*. The following dialog box will appear:



Control conditions:

Case conditions :

Network file

Dysregulation direction DIFF

Dysregulation significance threshold 0.05

Dysregulation ratio 1.3

Optimization algorithm CUSP

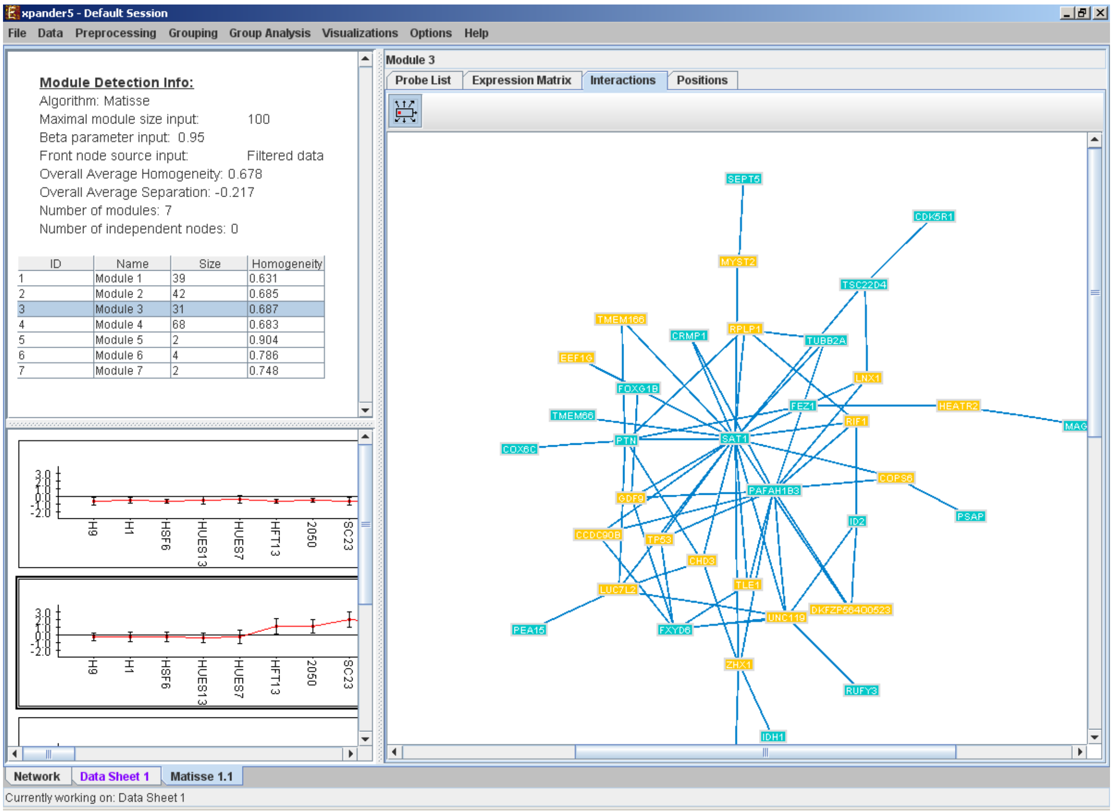
Maximal number of modules 1

It enables the configuration of some of the parameters for the algorithm:

Field	Description
Case conditions	The case conditions
Control conditions	The control conditions
Dysregulation direction	This parameter will determine which direction of dysregulation will be sought (up/down-regulation/both).
Dysregulation significance threshold	This threshold will be used to identify which genes are differentially expressed in each 'case' sample compared to the controls
Dysregulation ratio	The minimal threshold for the ratio between the gene expression in any of the case conditions and the average expression in the control conditions. Above this threshold a case condition is designated as dysregulated.
Optimization algorithm	The algorithm used to identify dysregulated pathways (DPs). See the DEGAS manuscript for details. CUSP is the recommended option
Maximal number of modules	After DEGAS identifies a significant DP, it removes it from the input data and attempts to identify additional DPs. This parameter specifies the total number of DPs that will be sought.

Upon clicking ‘OK’ in the dialog box, the Matisse algorithm is operated on the dataset.

After running network-based clustering, the solution is displayed in a new tab, which is added to the main window. The view is similar to the clustering results display. In the display, back nodes (genes that appear In the network, but not in the GE data) are marked in yellow.



Module Detection Info:

Algorithm: Matisse

Maximal module size input: 100

Beta parameter input: 0.95

Front node source input: Filtered data

Overall Average Homogeneity: 0.678

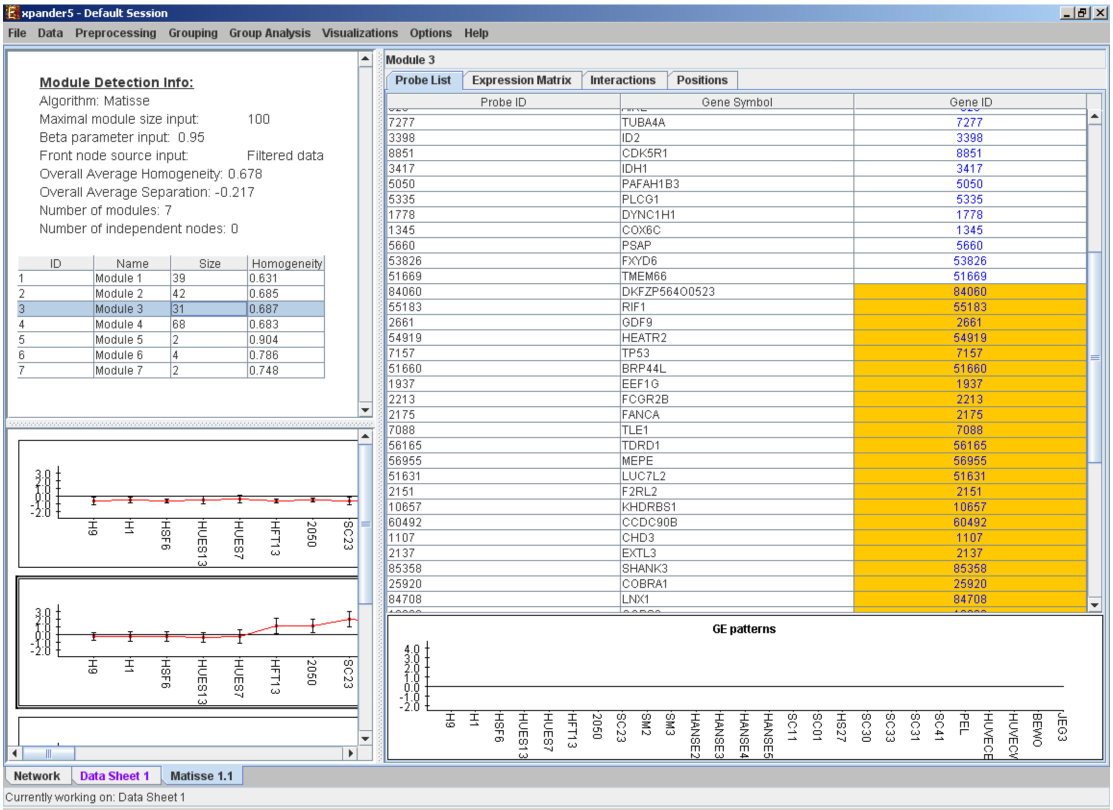
Overall Average Separation: -0.217

Number of modules: 7

Number of independent nodes: 0

ID	Name	Size	Homogeneity
1	Module 1	39	0.631
2	Module 2	42	0.685
3	Module 3	31	0.687
4	Module 4	68	0.683
5	Module 5	2	0.904
6	Module 6	4	0.786
7	Module 7	2	0.748

Currently working on: Data Sheet 1



Module Detection Info:

Algorithm: Matisse

Maximal module size input: 100

Beta parameter input: 0.95

Front node source input: Filtered data

Overall Average Homogeneity: 0.678

Overall Average Separation: -0.217

Number of modules: 7

Number of independent nodes: 0

ID	Name	Size	Homogeneity
1	Module 1	39	0.631
2	Module 2	42	0.685
3	Module 3	31	0.687
4	Module 4	68	0.683
5	Module 5	2	0.904
6	Module 6	4	0.786
7	Module 7	2	0.748

Probe ID	Gene Symbol	Gene ID
7277	TUBA4A	7277
3398	ID2	3398
8851	CDK5R1	8851
3417	IDH1	3417
5050	PAFAH1B3	5050
5335	PLCG1	5335
1778	DYNC1H1	1778
1345	COX6C	1345
5660	PSAP	5660
53826	FXYD6	53826
51669	TMEM66	51669
84060	DKFZP564O0523	84060
55183	RIF1	55183
2661	GDF9	2661
54919	HEATR2	54919
7157	TP53	7157
51660	BRP44L	51660
1937	EEF1G	1937
2213	FCGR2B	2213
2175	FANCA	2175
7088	TLE1	7088
56165	TDRD1	56165
56955	MEPE	56955
51631	LUC7L2	51631
2151	F2RL2	2151
10657	KHDRBS1	10657
60492	CCDC90B	60492
1107	CHD3	1107
2137	EXTL3	2137
85358	SHANK3	85358
25920	COBRA1	25920
84708	LNX1	84708

Currently working on: Data Sheet 1

After performing enrichment analysis (for details see the Enrichment Analysis Tools section), if enrichment has been detected in the selected module, the corresponding histogram and analysis information are added to the single module view, and a column is added to the expression matrix display for each enrichment class, stating for each probe, whether it belongs to that class.

A network-based grouping solution can be saved using the *File >> Export to text* option (with the corresponding grouping view as the selected tab) OR by using the *File>>Save All* option, which will export all solutions within a session to text and image files. A network-based grouping solution can be reloaded using the *Unsupervised Grouping >> Network >> Load Solution* option or via *Supervised Grouping >> Network >> Load Solution*. For a format of the solution file, please refer to the File Formats section:

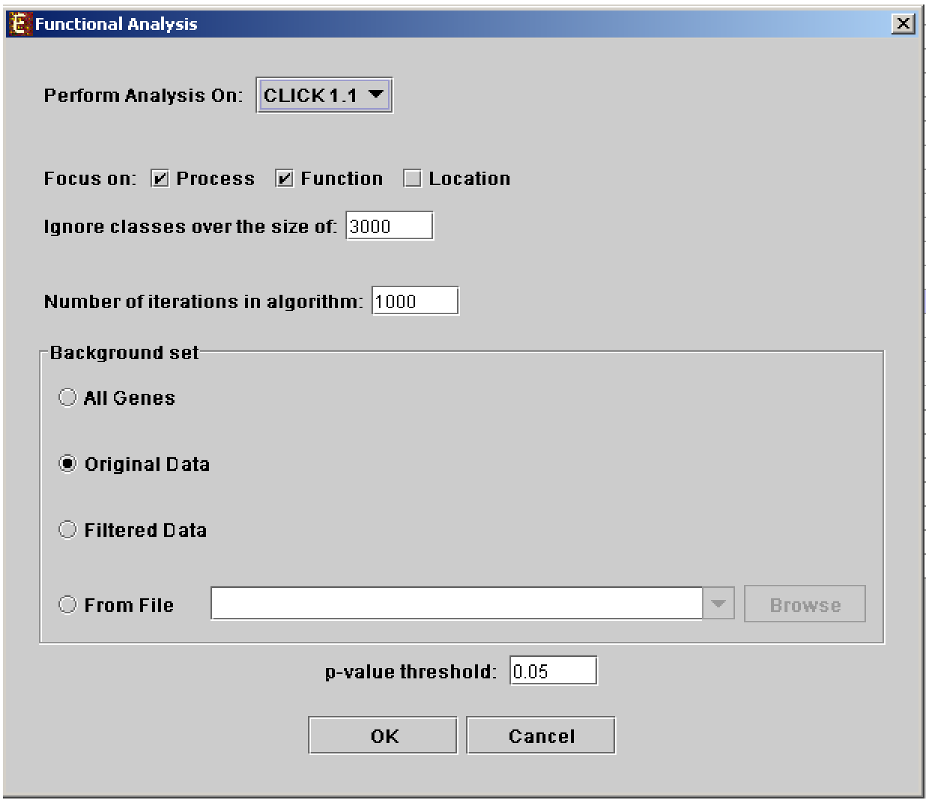
Group Enrichments Analysis Tools

The following analysis can be performed on gene sets, clusters, biclusters, network based modules, similarity based groups, or the filtered dataset (the analyzed set of probes as one set). Before operating any of the enrichment analysis operation (not including the “General enrichment analysis”), the data files for the relevant organism should be downloaded. Download can be done by selecting *Help >> Download Data for Organism*. Upon starting a new session, automatic data download will be suggested if Expander did not detect data for relevant organism.

Functional Analysis

This tool performs basic statistical analysis on the distribution of functions of genes within each cluster. The functions of the genes are determined according to annotation files (GO), which can be downloaded from the EXPANDER download page (see the Supplied Files section). To perform this analysis, Expander utilizes the TANGO software, which performs hyper-geometric enrichment tests and corrects for multiple testing by bootstrapping and estimating the empirical p-value distribution for the evaluated sets.

Before operating functional analysis the annotation files for the relevant organism should be downloaded from the download page (more details at introduction of Group Analysis Tools). To perform the analysis, select *Enrichment Analysis* >> *Functional Analysis* >> *TANGO*. The following dialog box will appear:



Functional Analysis

Perform Analysis On: CLICK 1.1

Focus on: ☑ Process ☑ Function □ Location

Ignore classes over the size of: 3000

Number of iterations in algorithm: 1000

Background set

○ All Genes

● Original Data

○ Filtered Data

○ From File

p-value threshold: 0.05

The following table specifies the different parameters that can be set via this dialog box:

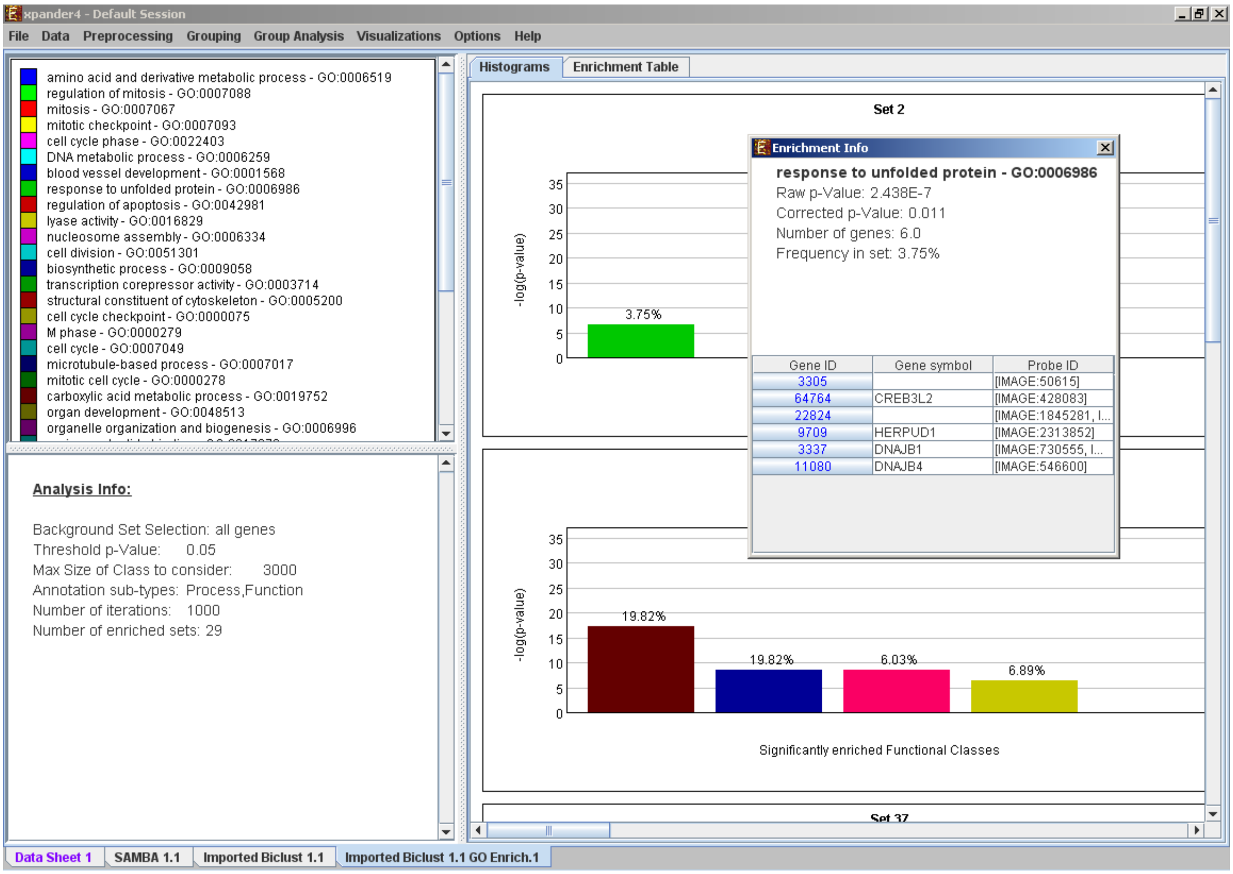
Field	Description
Perform analysis on	The grouping solution on which the analysis will be performed.
Include back nodes	Include genes that are part of the module' but not included in the GE data (Relevant only if the analysis is performed on modules, detected by network based algorithm)
Focus on	Can be used to select annotation subtypes that are of interest (Process, Function and Location). And the analysis will focus on these types only.
Ignore classes over the size of	This parameter states the level in the GO tree at which annotations are too general (class size

	indicates how general it is) and are thus no longer interesting.
Number of iterations in algorithm	The number of random sampling performed by the algorithm. Increasing this parameter, will increase runtime and will provide higher resolution on corrected p-Values. I.e., corrected p-Values will range between 1/<#iterations> and 1.
Background set	Determines the set of genes that will be used as background in the analysis. Options are: all genes (of the relevant organism), original input data, filtered data or background set from file (see the Files Format section for details regarding the format of an external background set).
Corrected p-value threshold	A functional class will be considered significantly enriched in a cluster/bicluster if its corrected p-value is lower than this threshold. The value in this field should be at least 1/1000, since the TANGO algorithm performs 1000 bootstraps in order to estimate the corrected p-value.

Upon clicking ‘OK’ in the dialog box, the TANGO algorithm is operated.

After functional analysis is performed a functional analysis solution visualization tab is added to the main window. It contains general information regarding the analysis, a sort-able table holding all detected enrichments (set ID, functional class, p-value, etc.) and a multi-histogram panel along with a color index (mapping each color to a corresponding functional class). The multi-histogram panel contains one histogram for each probe/gene set/group in which enrichment has been detected. Each histogram contains a column for each significant (more frequent than would be expected by random) functional class. The definition of significant depends on the user’s selection of threshold p-value i.e., a functional class is considered significantly enriched in a cluster/bicluster if its corrected p-value is lower than the preset threshold p-value.

The height of the column is proportional to the significance of this enrichment (i.e. height = -log(raw p-value)). The frequency in set (frequency of genes of a functional class within the examined set, in %) is written on top of the column. Upon clicking on a column, a dialog box is displayed containing the class name, raw p-value, corrected p-value, and a list of the genes in the cluster/bi-cluster that belong to the class. Upon clicking on one of the gene Ids in the table, a relevant web page with information regarding this gene is displayed. The display tool tip shows the cluster number, size and homogeneity.



- 🟦 amino acid and derivative metabolic process - GO:0006519
- 🟩 regulation of mitosis - GO:0007088
- 🟥 mitosis - GO:0007067
- 🟨 mitotic checkpoint - GO:0007093
- 🟪 cell cycle phase - GO:0022403
- 🟦 DNA metabolic process - GO:0006259
- 🟦 blood vessel development - GO:0001568
- 🟩 response to unfolded protein - GO:0006986
- 🟥 regulation of apoptosis - GO:0042981
- 🟨 lyase activity - GO:0016829
- 🟪 nucleosome assembly - GO:0006334
- 🟦 cell division - GO:0051301
- 🟦 biosynthetic process - GO:0009058
- 🟩 transcription corepressor activity - GO:0003714
- 🟥 structural constituent of cytoskeleton - GO:0005200
- 🟨 cell cycle checkpoint - GO:0000075
- 🟪 M phase - GO:0000279
- 🟦 cell cycle - GO:0007049
- 🟦 microtubule-based process - GO:0007017
- 🟩 mitotic cell cycle - GO:0000278
- 🟥 carboxylic acid metabolic process - GO:0019752
- 🟨 organ development - GO:0048513
- 🟪 organelle organization and biogenesis - GO:0006996

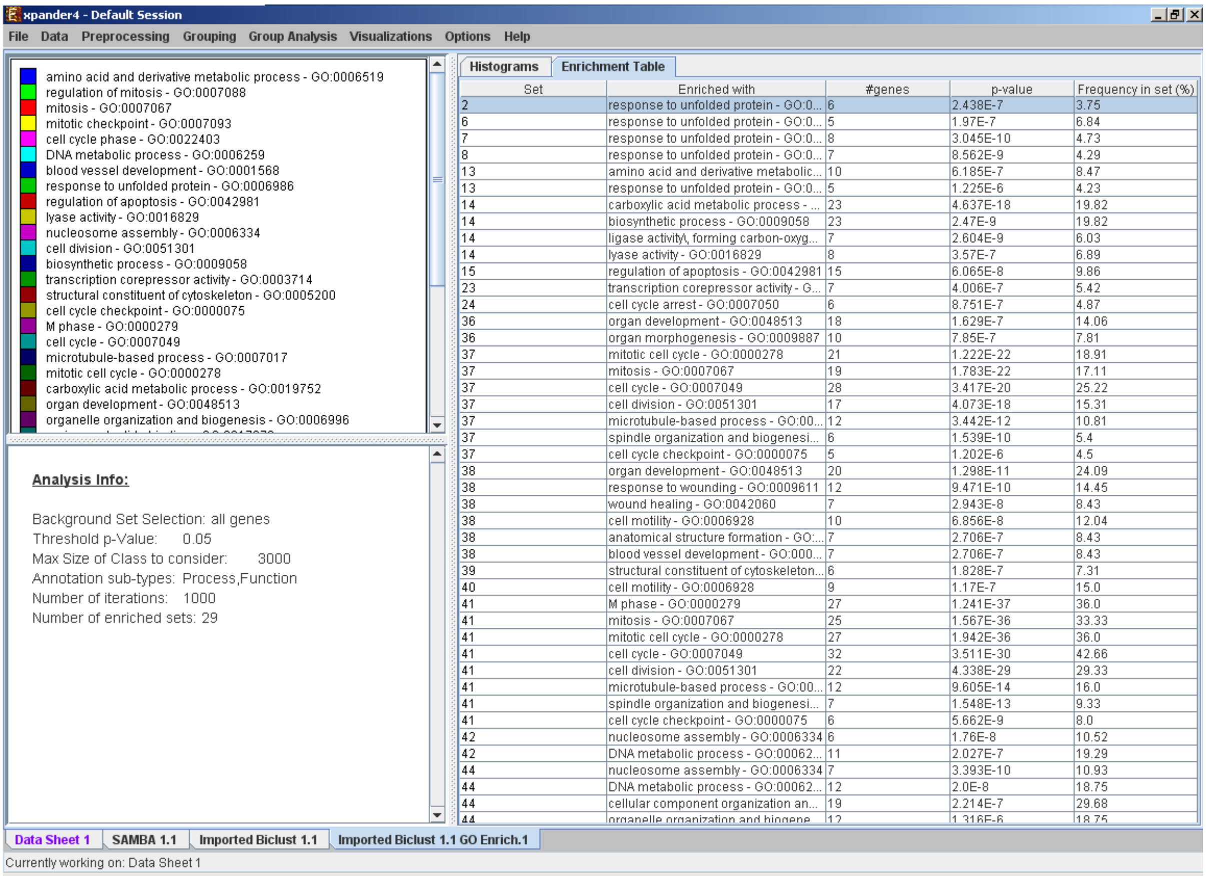
Analysis Info:

Background Set Selection: all genes
Threshold p-Value: 0.05
Max Size of Class to consider: 3000
Annotation sub-types: Process,Function
Number of iterations: 1000
Number of enriched sets: 29

Enrichment Info

response to unfolded protein - GO:0006986
Raw p-Value: 2.438E-7
Corrected p-Value: 0.011
Number of genes: 6.0
Frequency in set: 3.75%

Gene ID	Gene symbol	Probe ID
3305		[IMAGE:50615]
64764	CREB3L2	[IMAGE:428083]
22824		[IMAGE:1845281, I...
9709	HERPUD1	[IMAGE:2313852]
3337	DNAJB1	[IMAGE:730555, I...
11080	DNAJB4	[IMAGE:546600]



- 🟦 amino acid and derivative metabolic process - GO:0006519
- 🟩 regulation of mitosis - GO:0007088
- 🟥 mitosis - GO:0007067
- 🟨 mitotic checkpoint - GO:0007093
- 🟪 cell cycle phase - GO:0022403
- 🟦 DNA metabolic process - GO:0006259
- 🟦 blood vessel development - GO:0001568
- 🟩 response to unfolded protein - GO:0006986
- 🟥 regulation of apoptosis - GO:0042981
- 🟨 lyase activity - GO:0016829
- 🟪 nucleosome assembly - GO:0006334
- 🟦 cell division - GO:0051301
- 🟦 biosynthetic process - GO:0009058
- 🟩 transcription corepressor activity - GO:0003714
- 🟥 structural constituent of cytoskeleton - GO:0005200
- 🟨 cell cycle checkpoint - GO:0000075
- 🟪 M phase - GO:0000279
- 🟦 cell cycle - GO:0007049
- 🟦 microtubule-based process - GO:0007017
- 🟩 mitotic cell cycle - GO:0000278
- 🟥 carboxylic acid metabolic process - GO:0019752
- 🟨 organ development - GO:0048513
- 🟪 organelle organization and biogenesis - GO:0006996

Analysis Info:

Background Set Selection: all genes
Threshold p-Value: 0.05
Max Size of Class to consider: 3000
Annotation sub-types: Process,Function
Number of iterations: 1000
Number of enriched sets: 29

Set	Enriched with	#genes	p-value	Frequency in set (%)
2	response to unfolded protein - GO:0...	6	2.438E-7	3.75
6	response to unfolded protein - GO:0...	5	1.97E-7	6.84
7	response to unfolded protein - GO:0...	8	3.045E-10	4.73
8	response to unfolded protein - GO:0...	7	8.562E-9	4.29
13	amino acid and derivative metabolic...	10	6.185E-7	8.47
13	response to unfolded protein - GO:0...	5	1.225E-6	4.23
14	carboxylic acid metabolic process - ...	23	4.637E-18	19.82
14	biosynthetic process - GO:0009058	23	2.47E-9	19.82
14	ligase activity\, forming carbon-oxyg...	7	2.604E-9	6.03
14	lyase activity - GO:0016829	8	3.57E-7	6.89
15	regulation of apoptosis - GO:0042981	15	6.065E-8	9.86
23	transcription corepressor activity - G...	7	4.006E-7	5.42
24	cell cycle arrest - GO:0007050	6	8.751E-7	4.87
36	organ development - GO:0048513	18	1.629E-7	14.06
36	organ morphogenesis - GO:0009887	10	7.85E-7	7.81
37	mitotic cell cycle - GO:0000278	21	1.222E-22	18.91
37	mitosis - GO:0007067	19	1.783E-22	17.11
37	cell cycle - GO:0007049	28	3.417E-20	25.22
37	cell division - GO:0051301	17	4.073E-18	15.31
37	microtubule-based process - GO:00...	12	3.442E-12	10.81
37	spindle organization and biogenesi...	6	1.539E-10	5.4
37	cell cycle checkpoint - GO:0000075	5	1.202E-6	4.5
38	organ development - GO:0048513	20	1.298E-11	24.09
38	response to wounding - GO:0009611	12	9.471E-10	14.45
38	wound healing - GO:0042060	7	2.943E-8	8.43
38	cell motility - GO:0006928	10	6.856E-8	12.04
38	anatomical structure formation - GO:...	7	2.706E-7	8.43
38	blood vessel development - GO:000...	7	2.706E-7	8.43
39	structural constituent of cytoskeleton...	6	1.828E-7	7.31
40	cell motility - GO:0006928	9	1.17E-7	15.0
41	M phase - GO:0000279	27	1.241E-37	36.0
41	mitosis - GO:0007067	25	1.567E-36	33.33
41	mitotic cell cycle - GO:0000278	27	1.942E-36	36.0
41	cell cycle - GO:0007049	32	3.511E-30	42.66
41	cell division - GO:0051301	22	4.338E-29	29.33
41	microtubule-based process - GO:00...	12	9.605E-14	16.0
41	spindle organization and biogenesi...	7	1.548E-13	9.33
41	cell cycle checkpoint - GO:0000075	6	5.662E-9	8.0
42	nucleosome assembly - GO:0006334	6	1.76E-8	10.52
42	DNA metabolic process - GO:00062...	11	2.027E-7	19.29
44	nucleosome assembly - GO:0006334	7	3.393E-10	10.93
44	DNA metabolic process - GO:00062...	12	2.0E-8	18.75
44	cellular component organization an...	19	2.214E-7	29.68
44	organelle organization and biogene...	12	1.316E-6	18.75

Currently working on: Data Sheet 1

Annotation files are currently supplied with EXPANDER for yeast, human, mouse, rat, fly, zebrafish, c-elegans, Arabidopsis, chicken and E. coli, and are updated on a regular basis (for more information, refer to the Supplied Files section).

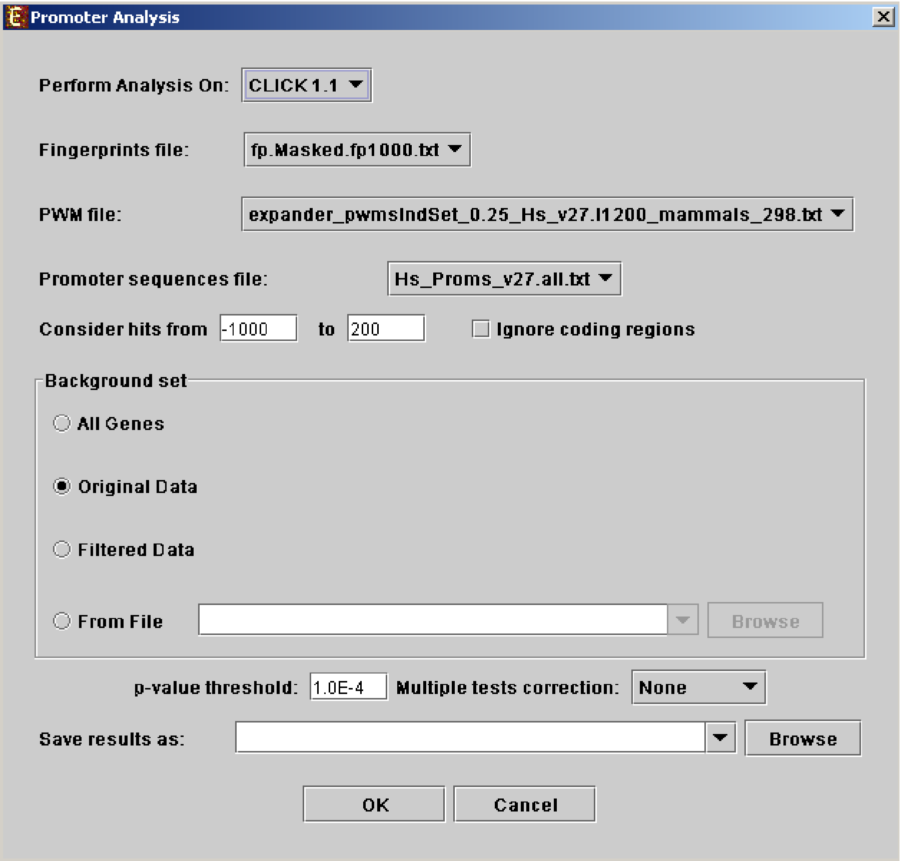
The results of this analysis can be exported to a text file by selecting *File>>Export to text* when the corresponding view is the selected tab OR by using the *File>>Save All* option, which will export all solutions within a session to text and image files.

Promoter Analysis

PRIMA

This tool identifies TFs whose binding sites are significantly over-represented in a given set of promoters (i.e. cluster or bicluster). To perform this analysis Expander utilizes the PRIMA (PRomoter Integration in Microarray Analysis) software which performs a statistical analysis on the distribution of transcription factor motifs in the promoters of genes within each cluster or bicluster. To achieve this, PRIMA uses preprocessed TF fingerprint files, which can be downloaded from the EXPANDER download-page (see the Supplied Files section), and are updated on a regular basis. For details regarding the PRIMA software see the References section.

Before operating promoter analysis, the TF fingerprint file for the relevant organism should be downloaded from the download page (more details at introduction of Enrichment Analysis Tools). To perform the analysis, select *Enrichment Analysis >> Promoter Analysis >> PRIMA*. The following dialog box will appear:



Promoter Analysis

Perform Analysis On: CLICK 1.1

Fingerprints file: fp.Masked.fp1000.txt

PWM file: expander_pwmsIndSet_0.25_Hs_v27.l1200_mammals_298.txt

Promoter sequences file: Hs_Proms_v27.all.txt

Consider hits from -1000 to 200 □ Ignore coding regions

Background set

○ All Genes

● Original Data

○ Filtered Data

○ From File

p-value threshold: 1.0E-4 Multiple tests correction: None

Save results as:

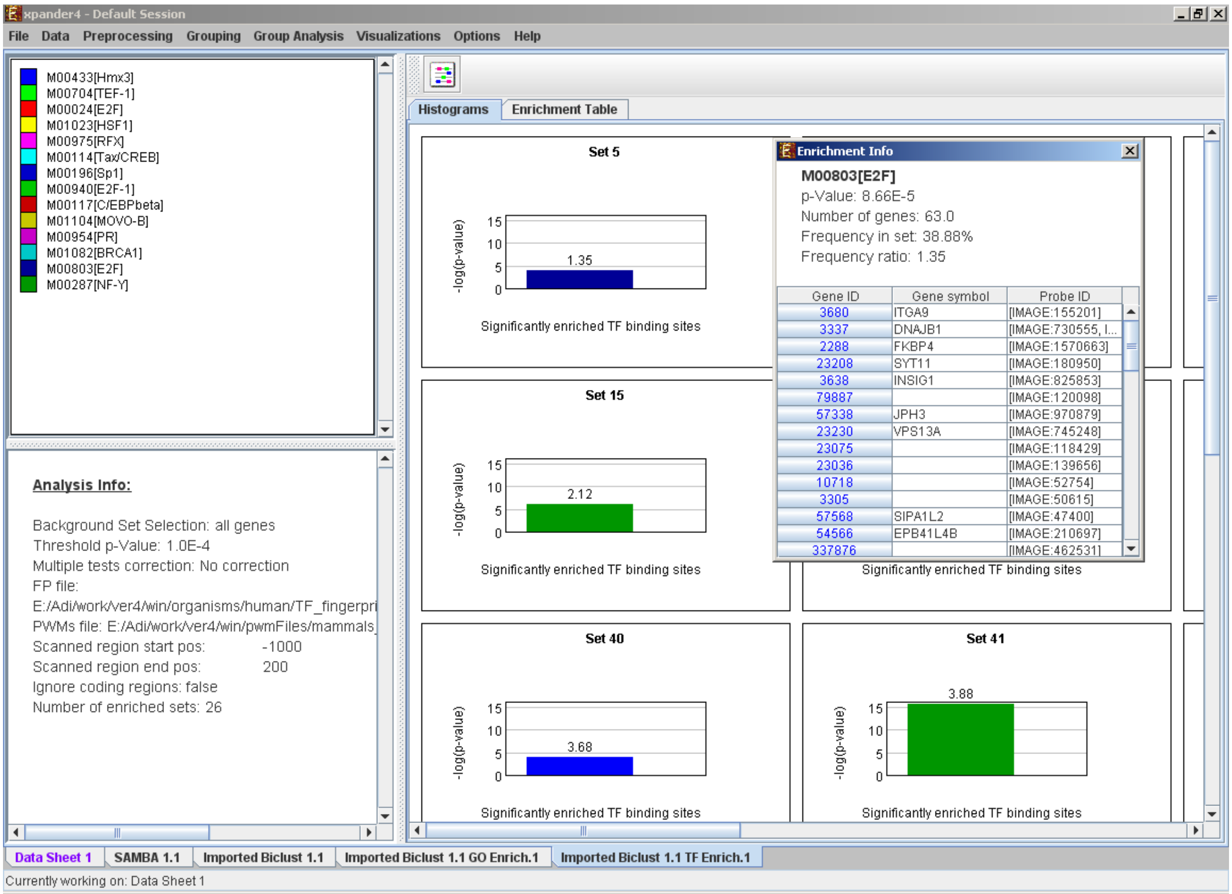
The following table specifies the different parameters that can be set via this dialog box:

Field	Description
Perform analysis on	The grouping solution on which the analysis will be performed.
Include back nodes	Include genes that are part of the module' but not included in the GE data (Relevant only if the analysis is performed on modules, detected by network based algorithm)
Fingerprints file	Automatically set according to the selection of the organism.
PWM file	Automatically set according to the selection of the organism.
Promoter sequences file	Contains the gene sequences that are used for the TF binding sites display. Automatically set according to the selection of the organism.
Hits range	Determines which regions of the gene are to be analyzed. The possible range depends on the investigated organism (i.e. on the information provided in the TF fingerprint files), and is specified in the Supplied Files section.
Background set	Determines the set of genes that will be used as background in the analysis. Options are: all genes (of the relevant organism), original input data, filtered data or background set from file (see the Files Format section for details regarding the format of an external background set).
Threshold p-value	A TF's binding site will be considered significantly enriched in a cluster if its corrected p-value is lower than this threshold.
Multiple tests correction	Can be set to Bonferroni or None (when set to Bonferroni the corrected p-values are the ones that are compared to the threshold p-value).
Save results as	When filled, the program results are saved in stated txt file.

After promoter analysis is performed, a promoter analysis solution visualization tab is added to the main window. It contains general information regarding the analysis, a sort-able table holding all detected enrichments (set ID, TF binding site, p-value, etc.) and a multi-histogram panel along with a color index (mapping each color to a corresponding TF binding site). The multi-histogram panel contains one histogram for each probe/gene set/group in which enrichment has been detected. Each histogram contains a column for each significant (more frequent than would be expected by random) TF binding site. The definition of significant depends on the user's selection of threshold p-value. i.e., a TF binding site is considered significantly enriched in a cluster/bicluster if its corrected p-value is lower than the preset threshold p-value.

The height of a column is proportional to the significance of this enrichment (i.e. height = -log(p-value)), and the frequency ratio (frequency in set divided by frequency in background) is written on top of the column. Upon clicking on a column, a dialog box is displayed containing:

TF accession number in TRANSFAC DB [TF name], p-value, % of covered promoters in cluster, relative frequency (frequency in cluster divided by frequency in background set) and a list of the genes in the cluster which contain the motif in their promoters. Upon clicking on one of the gene Ids in the table, a relevant web page with information regarding this gene is displayed. The display tool tip shows the cluster number, size and homogeneity.


xpander4 - Default Session
File
Data
Preprocessing
Grouping
Group Analysis
Visualizations
Options
Help
Histograms
Enrichment Table
Set 5
15
10
5
0
1.35
-log(p-value)
Significantly enriched TF binding sites
Set 15
15
10
5
0
2.12
-log(p-value)
Significantly enriched TF binding sites
Significantly enriched TF binding sites
Set 40
15
10
5
0
3.68
-log(p-value)
Significantly enriched TF binding sites
Set 41
3.88
15
10
5
0
-log(p-value)
Significantly enriched TF binding sites
Data Sheet 1
SAMBA 1.1
Imported Biclust 1.1
Imported Biclust 1.1 GO Enrich.1
Imported Biclust 1.1 TF Enrich.1
Currently working on: Data Sheet 1

🟦 M00433[Hmx3]
🟩 M00704[TEF-1]
🟥 M00024[E2F]
🟨 M01023[HSF1]
🟪 M00975[RFX]
🟦 M00114[Tax/CREB]
🟦 M00196[Sp1]
🟩 M00940[E2F-1]
🟥 M00117[C/EBPbeta]
🟨 M01104[MOVO-B]
🟪 M00954[PR]
🟦 M01082[BRCA1]
🟦 M00803[E2F]
🟩 M00287[NF-Y]

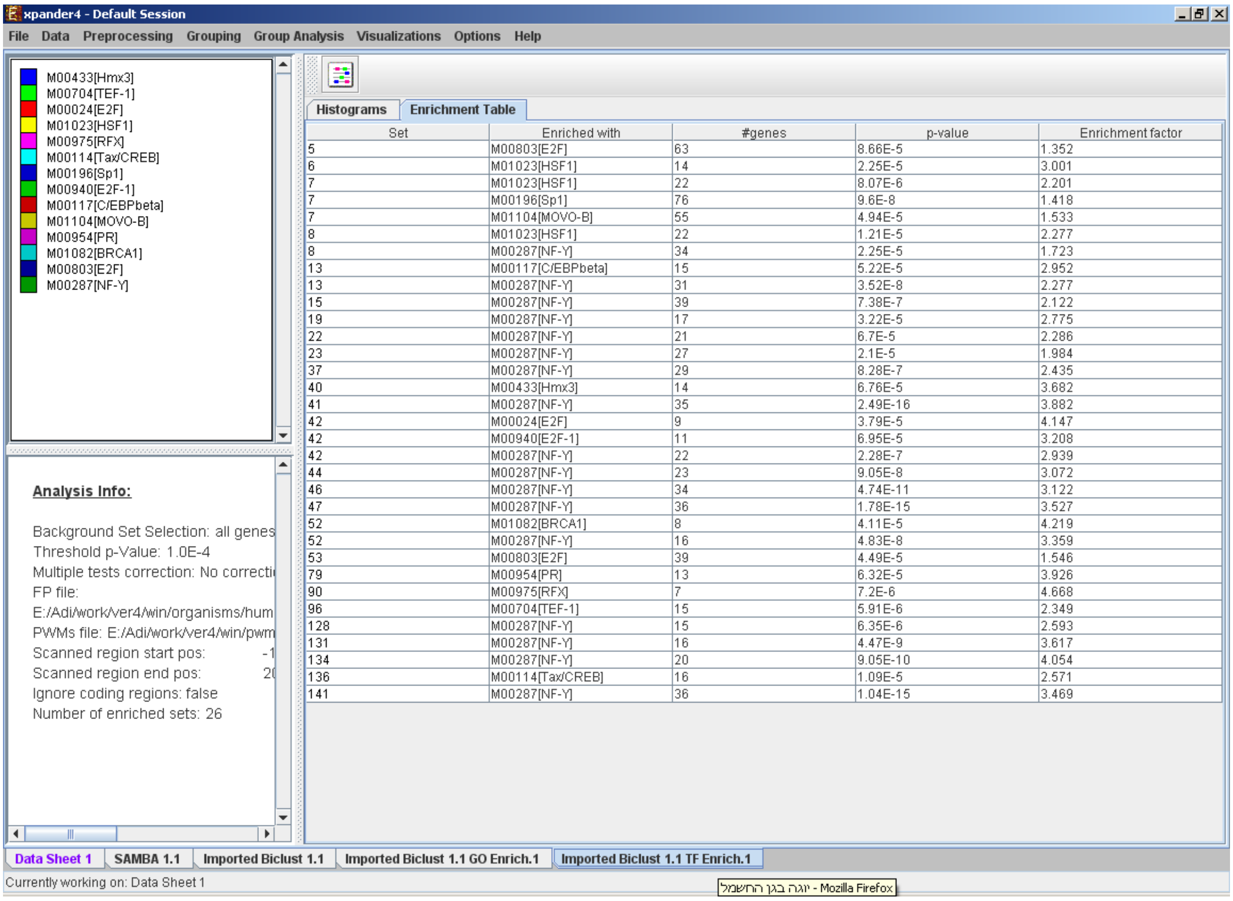
Analysis Info:

Background Set Selection: all genes
Threshold p-Value: 1.0E-4
Multiple tests correction: No correction
FP file:
E:/Adi/work/ver4/win/organisms/human/TF_fingerpri
PWMs file: E:/Adi/work/ver4/win/pwmFiles/mammals_
Scanned region start pos: -1000
Scanned region end pos: 200
Ignore coding regions: false
Number of enriched sets: 26

Enrichment Info

M00803[E2F]
p-Value: 8.66E-5
Number of genes: 63.0
Frequency in set: 38.88%
Frequency ratio: 1.35

Gene ID	Gene symbol	Probe ID
3680	ITGA9	[IMAGE:155201]
3337	DNAJB1	[IMAGE:730555, I...
2288	FKBP4	[IMAGE:1570663]
23208	SYT11	[IMAGE:180950]
3638	INSIG1	[IMAGE:825853]
79887		[IMAGE:120098]
57338	JPH3	[IMAGE:970879]
23230	VPS13A	[IMAGE:745248]
23075		[IMAGE:118429]
23036		[IMAGE:139656]
10718		[IMAGE:52754]
3305		[IMAGE:50615]
57568	SIPA1L2	[IMAGE:47400]
54566	EPB41L4B	[IMAGE:210697]
337876		[IMAGE:462531]


xpander4 - Default Session
File
Data
Preprocessing
Grouping
Group Analysis
Visualizations
Options
Help
Histograms
Enrichment Table
Data Sheet 1
SAMBA 1.1
Imported Biclust 1.1
Imported Biclust 1.1 GO Enrich.1
Imported Biclust 1.1 TF Enrich.1
Currently working on: Data Sheet 1
יוגה בגן החשמל - Mozilla Firefox

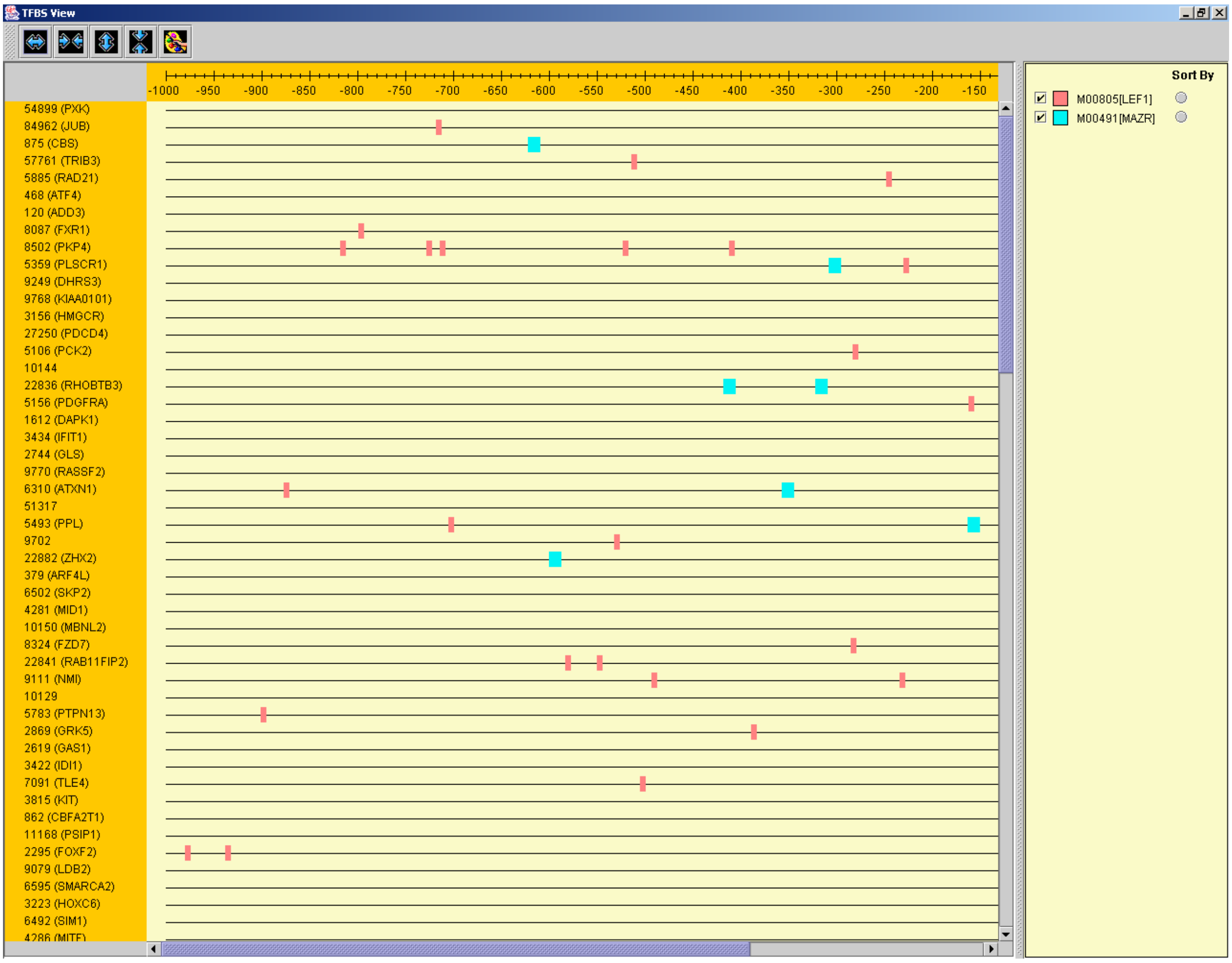
🟦 M00433[Hmx3]
🟩 M00704[TEF-1]
🟥 M00024[E2F]
🟨 M01023[HSF1]
🟪 M00975[RFX]
🟦 M00114[Tax/CREB]
🟦 M00196[Sp1]
🟩 M00940[E2F-1]
🟥 M00117[C/EBPbeta]
🟨 M01104[MOVO-B]
🟪 M00954[PR]
🟦 M01082[BRCA1]
🟦 M00803[E2F]
🟩 M00287[NF-Y]

Analysis Info:

Background Set Selection: all genes
Threshold p-Value: 1.0E-4
Multiple tests correction: No correcti
FP file:
E:/Adi/work/ver4/win/organisms/hum
PWMs file: E:/Adi/work/ver4/win/pwm
Scanned region start pos: -1
Scanned region end pos: 2(
Ignore coding regions: false
Number of enriched sets: 26

Set	Enriched with	#genes	p-value	Enrichment factor
5	M00803[E2F]	63	8.66E-5	1.352
6	M01023[HSF1]	14	2.25E-5	3.001
7	M01023[HSF1]	22	8.07E-6	2.201
7	M00196[Sp1]	76	9.6E-8	1.418
7	M01104[MOVO-B]	55	4.94E-5	1.533
8	M01023[HSF1]	22	1.21E-5	2.277
8	M00287[NF-Y]	34	2.25E-5	1.723
13	M00117[C/EBPbeta]	15	5.22E-5	2.952
13	M00287[NF-Y]	31	3.52E-8	2.277
15	M00287[NF-Y]	39	7.38E-7	2.122
19	M00287[NF-Y]	17	3.22E-5	2.775
22	M00287[NF-Y]	21	6.7E-5	2.286
23	M00287[NF-Y]	27	2.1E-5	1.984
37	M00287[NF-Y]	29	8.28E-7	2.435
40	M00433[Hmx3]	14	6.76E-5	3.682
41	M00287[NF-Y]	35	2.49E-16	3.882
42	M00024[E2F]	9	3.79E-5	4.147
42	M00940[E2F-1]	11	6.95E-5	3.208
42	M00287[NF-Y]	22	2.28E-7	2.939
44	M00287[NF-Y]	23	9.05E-8	3.072
46	M00287[NF-Y]	34	4.74E-11	3.122
47	M00287[NF-Y]	36	1.78E-15	3.527
52	M01082[BRCA1]	8	4.11E-5	4.219
52	M00287[NF-Y]	16	4.83E-8	3.359
53	M00803[E2F]	39	4.49E-5	1.546
79	M00954[PR]	13	6.32E-5	3.926
90	M00975[RFX]	7	7.2E-6	4.668
96	M00704[TEF-1]	15	5.91E-6	2.349
128	M00287[NF-Y]	15	6.35E-6	2.593
131	M00287[NF-Y]	16	4.47E-9	3.617
134	M00287[NF-Y]	20	9.05E-10	4.054
136	M00114[Tax/CREB]	16	1.09E-5	2.571
141	M00287[NF-Y]	36	1.04E-15	3.469

After performing promoter analysis, TF binding sites can be viewed by selecting *Enrichment Analysis >> Promoter Analysis >> View Binding Sites* OR by pressing the toolbar button (☰). After selecting the gene group (cluster/bi-cluster etc.) to be viewed, a separate frame is displayed, containing a line to represent each of the genes in the group, and a colored rectangle, to represent each binding site. A color index appears on the right, mapping each color to the corresponding TF (PWM). A check box next to each of the entries in the color index allows hiding any of the PWMs, and a radio button next to each of the entries in the color index allows sorting the genes in the display according to the number of hits of the corresponding TF. The toolbar contains tools for vertical and horizontal zooming. If a sequence file had been selected via the promoter analysis input dialog, the actual sequence will be displayed when the zoom factor (scale) allows it.

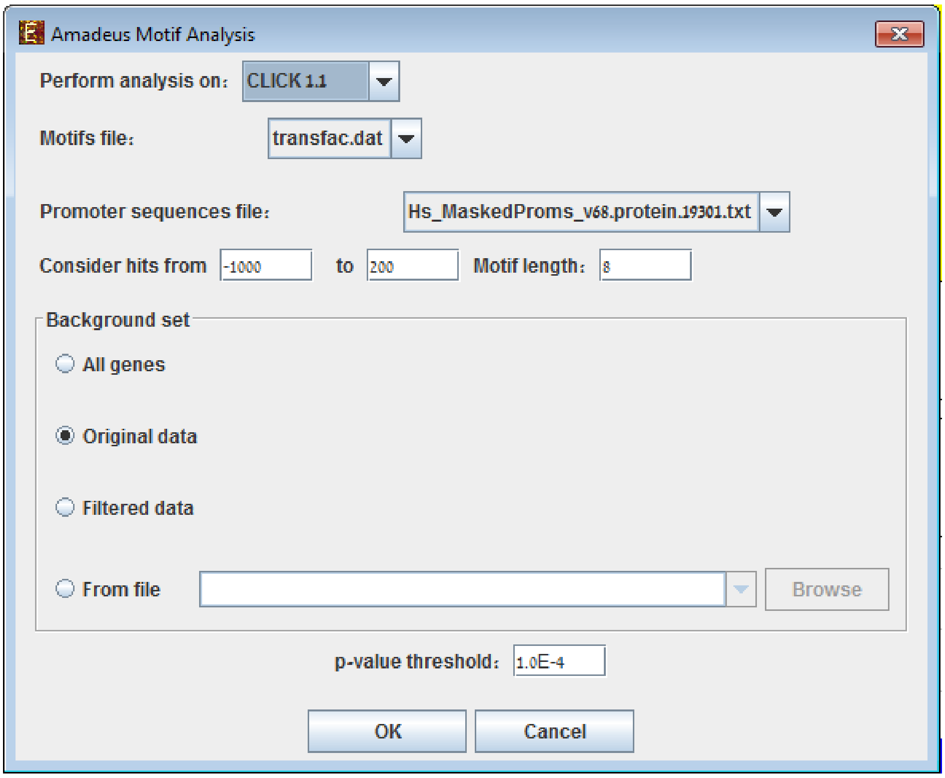


AMADEUS

Another option for performing promoter analysis, is finding enriched *de novo* motifs using AMADEUS integrated software in Expander. *Amadeus* is a software for *de novo* motif discovery. It seeks for motifs which are enriched in a target set of genes compared to the background set. These sets can be extended to any kind of sequences (e.g., ChIP-seq peaks).

In order to perform motifs enrichments analysis, select *Enrichment Analysis >> Promoter Analysis >> AMADEUS*.

The following dialog box will appear:



Amadeus Motif Analysis

Perform analysis on: CLICK 1.1

Motifs file: transfac.dat

Promoter sequences file: Hs_MaskedProms_v68.protein.19301.txt

Consider hits from -1000 to 200 Motif length: 8

Background set

○ All genes

● Original data

○ Filtered data

○ From file

p-value threshold: 1.0E-4

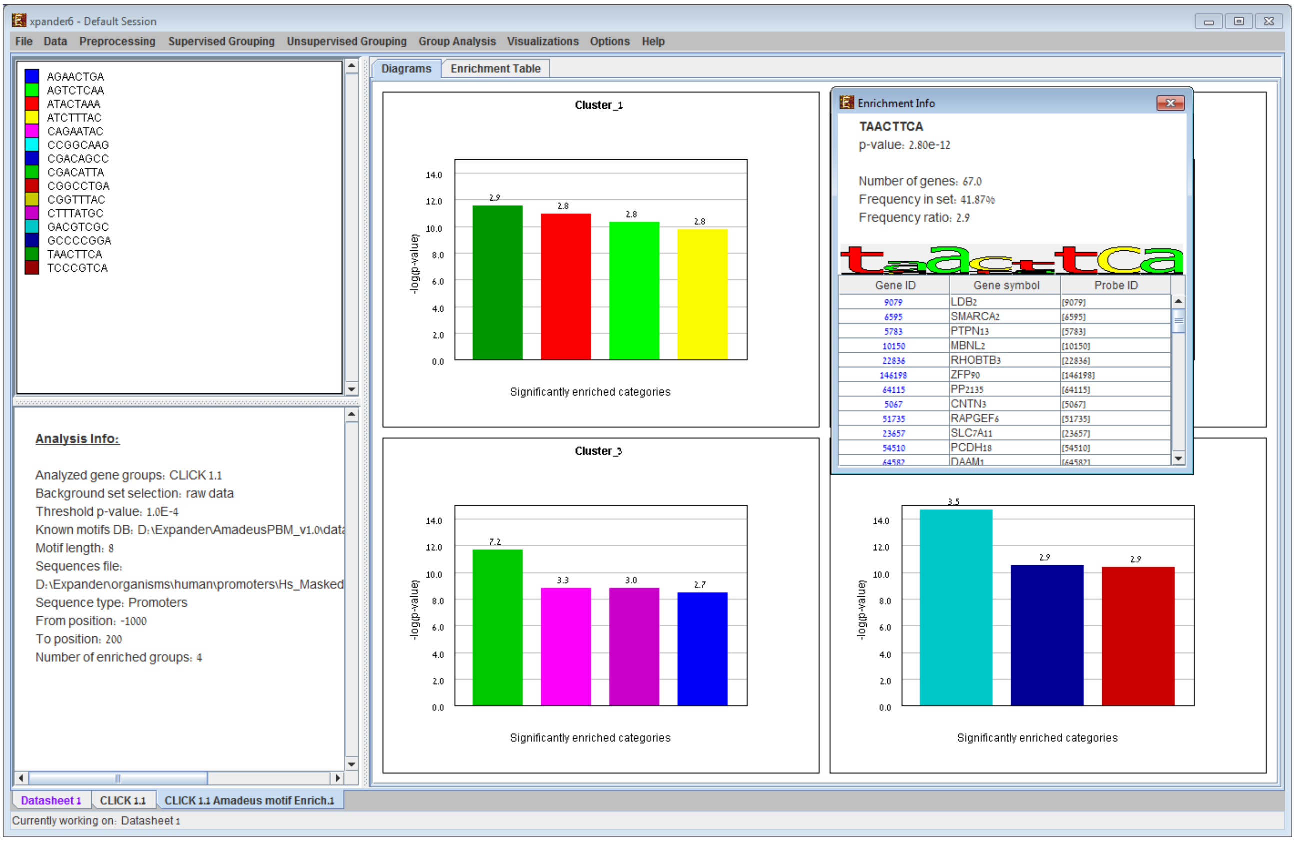
The following table specifies the different parameters that can be set via this dialog box:

Field	Description
Perform analysis on	The grouping solution on which the analysis will be performed.
Motifs file	A matrix table of known motifs in TRANSFAC format. The automatic default is transfac.dat, a public release of TRANSFAC.
Promoter sequences file	Contains the promoter sequences in fasta format. Automatically set according to the selection of the organism. Can be set to any set of sequences (e.g., ChIP-seq peaks).
Motif Length	The length of the motif to be searched for.
Hits range	Determines which sections of the sequences are analyzed. The range depends on the organism (i.e. the average length of a promoter sequence, on the information provided in the TF fingerprint files), and is specified in the Supplied Files section. It can be set manually.
Background set	Determines the set of genes, whose promoter sequences will be used as background in the analysis. Options are: all genes (of the relevant organism), original input data, filtered data or background set from file (see the Files Format section for details regarding the format of an external background set).

P-value threshold	A motif will be considered significantly enriched in a cluster if its corrected p-value is lower than this threshold.

After AMDEUS analysis is performed, an Amadeus motif solution visualization tab is added to the main window. It contains general information regarding the analysis, a sort-able table holding all detected enrichments (set ID, Motif binding site, p-value, etc.) and a multi-histogram panel along with a color index (mapping each color to a corresponding Motif binding site). The multi-histogram panel contains one histogram for each probe/gene set/group in which enrichment has been detected. Each histogram contains a column for each significant (more frequent than would be expected in the background set) motif binding site. The significance depends on the user's selection of p-value threshold. i.e., a motif binding site is considered significantly enriched in a cluster/bicluster if its corrected p-value is lower than the preset p-value threshold.

The height of a column is proportional to the significance of this enrichment (i.e. height = -log(p-value)), and the frequency ratio (frequency in the target set divided by frequency in the background set) is written on top of the column. Upon clicking on a column, a dialog box is displayed containing:



🟦 AGAACTGA
🟩 AGTCTCAA
🟥 ATACTAAA
🟨 ATCTTTAC
🟪 CAGAATAC
🟦 CCGGCAAG
🟦 CGACAGCC
🟩 CGACATTA
🟥 CGGCCTGA
🟨 CGGTTTAC
🟪 CTTTATGC
🟦 GACGTCGC
🟦 GCCCCGGA
🟩 TAACTTCA
🟥 TCCCGTCA

Analysis Info:

Analyzed gene groups: CLICK 1.1
Background set selection: raw data
Threshold p-value: 1.0E-4
Known motifs DB: D:\Expander\AmadeusPBM_v1.0\data
Motif length: 8
Sequences file:
D:\Expander\organisms\human\promoters\Hs_Masked
Sequence type: Promoters
From position: -1000
To position: 200
Number of enriched groups: 4

Enrichment Info

TAACTTCA
p-value: 2.80e-12

Number of genes: 67.0
Frequency in set: 41.87%
Frequency ratio: 2.9

Gene ID	Gene symbol	Probe ID
9079	LDB2	[9079]
6595	SMARCA2	[6595]
5783	PTPN13	[5783]
10150	MBNL2	[10150]
22836	RHOBTB3	[22836]
146198	ZFP90	[146198]
64115	PP2135	[64115]
5067	CNTN3	[5067]
51735	RAPGEF6	[51735]
23657	SLC7A11	[23657]
54510	PCDH18	[54510]
64582	DAAM1	[64582]

Currently working on: Datasheet 1

motif binding site, p-value, % of promoters containing the motif in the cluster, relative frequency (frequency in the cluster divided by frequency in background set), motif logo created from position weight matrix and a list of the genes in the cluster which contain the motif in their promoters. Upon clicking on one of the gene ids in the table, a relevant web page with information regarding this gene is displayed. The display tool tip shows the cluster number, size and homogeneity.

TF motif fingerprint files and promoter sequence files are currently supplied with EXPANDER for yeast, human, mouse, rat, fly, zebrafish, c-elegans, arabidopsis and chicken, and are updated on a regular basis (for more information, refer to the Supplied Files section).

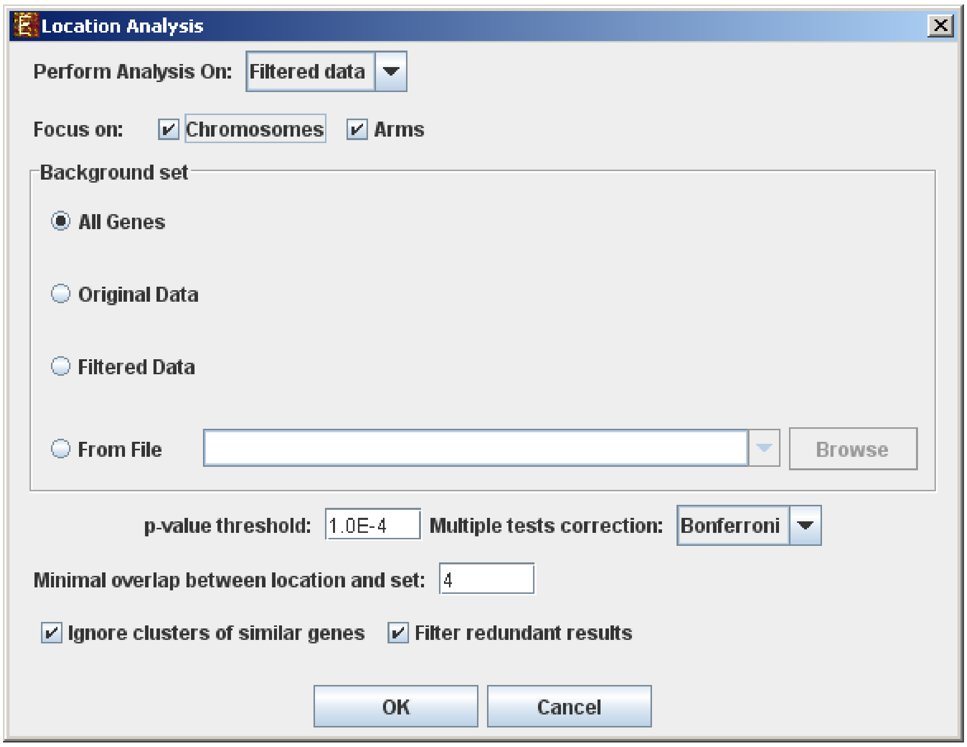
The results of this analysis can be exported to a text by selecting *File>>Export to text* when the corresponding view is the selected tab.

Location Enrichment Analysis

This tool performs basic statistical analysis on the distribution of chromosomal locations of genes within each group. The locations of the genes are specified in organism-specific data files, which can be downloaded from the EXPANDER download-page (see the Supplied Files section).

Before operating location analysis, the location data for the relevant organism should be downloaded from the download page (more details at introduction of Enrichment Analysis Tools). In this analysis, hyper-geometric enrichment tests are performed, and the results can be (if requested) corrected for multiple testing using the Bonferroni correction.

To perform the analysis, select *Enrichment Analysis >> Location Analysis >> Detect Enrichment*. The following dialog box will appear:



Location Analysis

Perform Analysis On: Filtered data

Focus on: ☑ Chromosomes ☑ Arms

Background set

● All Genes

○ Original Data

○ Filtered Data

○ From File

p-value threshold: 1.0E-4 Multiple tests correction: Bonferroni

Minimal overlap between location and set: 4

☑ Ignore clusters of similar genes ☑ Filter redundant results

The following table specifies the different parameters that can be set via this dialog box:

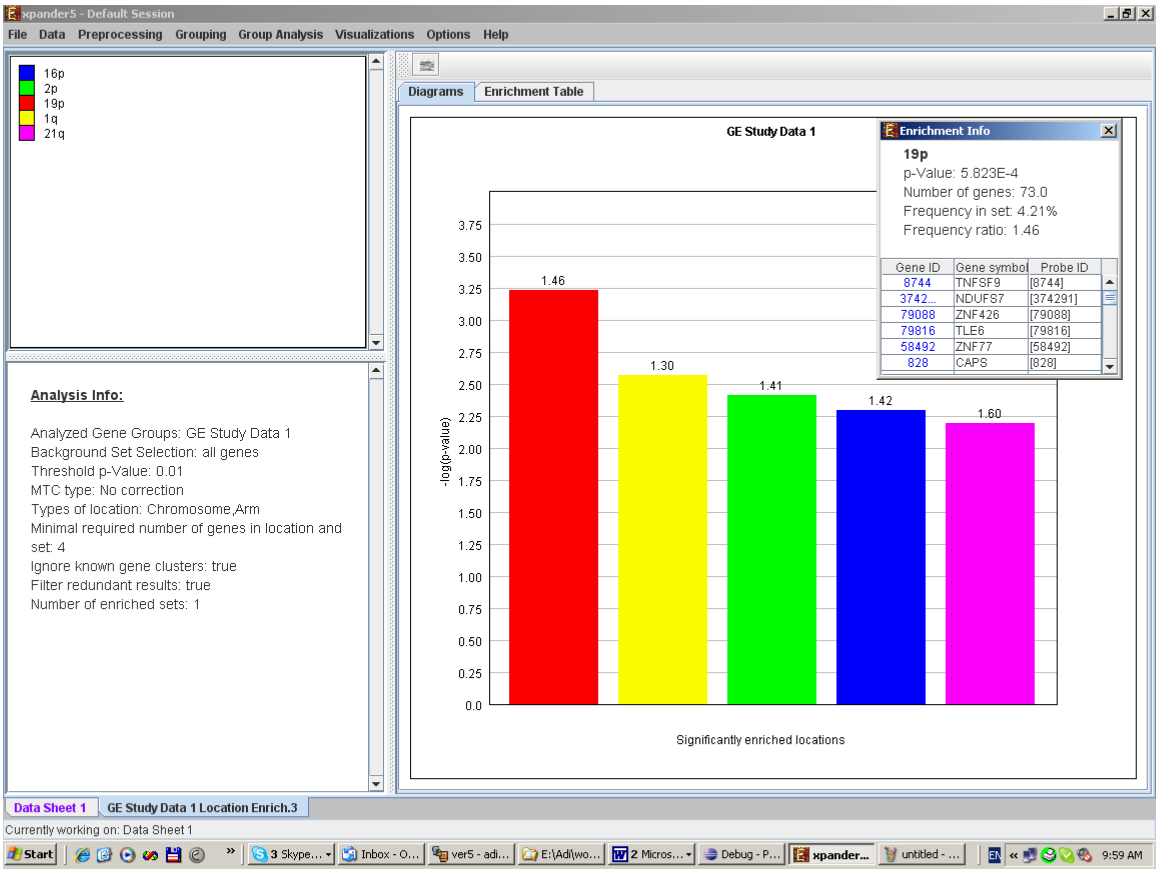
Field	Description
Perform analysis on	The grouping solution on which the analysis will be performed.
Include back nodes	Include genes that are part of the module' but not included in the GE data (Relevant only if the analysis is performed on modules, detected by

	network based algorithm)
Focus on (Chromosomes, Arms*, Bands*)	Location types to perform analysis on.
Background set	Determines the set of genes that will be used as background in the analysis. Options are: all genes (of the relevant organism), original input data, filtered data or background set from file (see the Files Format section for details regarding the format of an external background set).
p-value threshold	A category/attribute will be considered significantly enriched in a cluster/bicluster if its corrected p-value is lower than this threshold.
Multiple tests correction	Can be set to Bonferroni or None (when set to Bonferroni the corrected p-values are the ones that are compared to the threshold p-value).
Minimal overlap between category and set	The minimal number of genes from a group (cluster/bi-cluster/module etc.) expected to be categorized/attributed by an attribute in order for its enrichment to be accepted.
Ignore clusters of similar genes*	If selected, genes from known homology clusters are not included in the analysis.
Filter redundant results	If selected, the results are filtered, so that out of two enrichments of overlapping areas in the same group, only one is selected (the most significant one).

* If relevant data exists

After the analysis is performed an enrichment analysis solution visualization tab is added to the main window. It contains general information regarding the analysis, a sort-able table holding all detected enrichments (set ID, enrichment category, p-value, etc.) and a multi-histogram panel along with a color index (mapping each color to a corresponding location). The multi-histogram panel contains one histogram for each probe/gene group in which enrichment has been detected. Each histogram contains a column for each significant (more frequent than would be expected by random) location. The definition of significant depends on the user’s selection of threshold p-value i.e., a category is considered significantly enriched in a cluster/bicluster if its corrected p-value is lower than the preset threshold p-value.

The height of the column is proportional to the significance of this enrichment (i.e. height = -log(raw p-value)), and the frequency ratio (frequency in set divided by frequency in background) is written on top of the column. Upon clicking on a column, a dialog box is displayed containing the location, corrected p-value, and a list of the genes in the group that are mapped to this location. Upon clicking on one of the gene Ids in the table, a relevant web page with information regarding this gene is displayed.



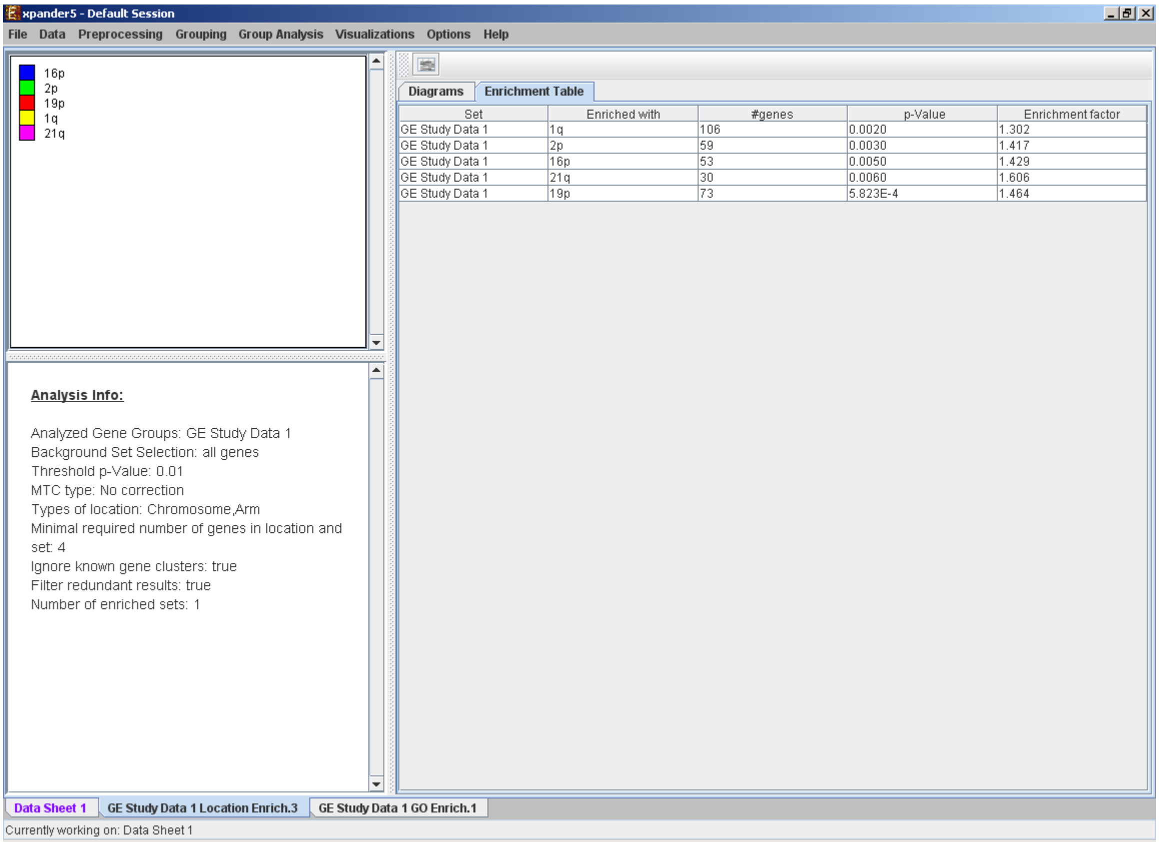
Analysis Info:

Analyzed Gene Groups: GE Study Data 1
Background Set Selection: all genes
Threshold p-Value: 0.01
MTC type: No correction
Types of location: Chromosome,Arm
Minimal required number of genes in location and set: 4
Ignore known gene clusters: true
Filter redundant results: true
Number of enriched sets: 1

19p
p-Value: 5.823E-4
Number of genes: 73.0
Frequency in set: 4.21%
Frequency ratio: 1.46

Gene ID	Gene symbol	Probe ID
8744	TNFSF9	[8744]
3742...	NDUFS7	[374291]
79088	ZNF426	[79088]
79816	TLE6	[79816]
58492	ZNF77	[58492]
828	CAPS	[828]

Currently working on: Data Sheet 1


xpander5 - Default Session
File Data Preprocessing Grouping Group Analysis Visualizations Options Help
🟦 16p
🟩 2p
🟥 19p
🟨 1q
🟪 21q
Diagrams
Enrichment Table
Data Sheet 1
GE Study Data 1 Location Enrich.3
GE Study Data 1 GO Enrich.1

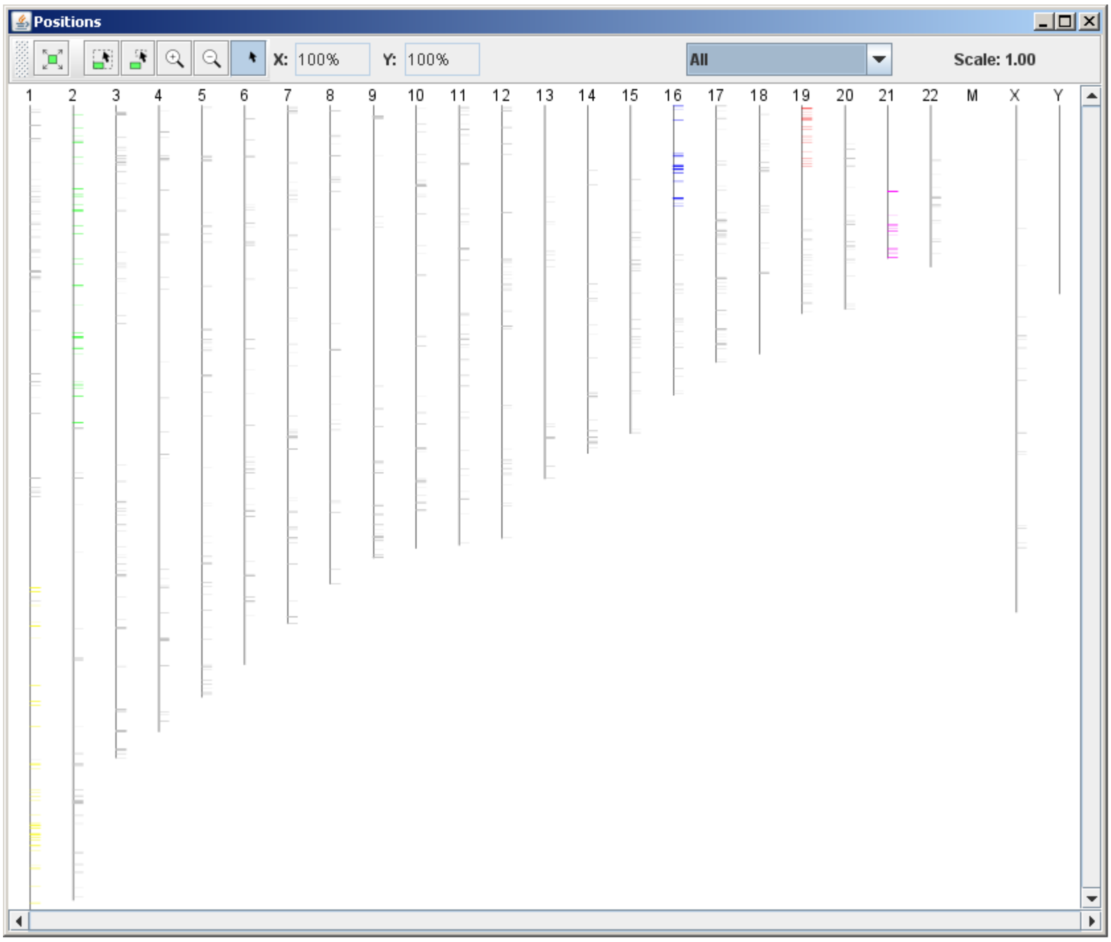
Set	Enriched with	#genes	p-Value	Enrichment factor
GE Study Data 1	1q	106	0.0020	1.302
GE Study Data 1	2p	59	0.0030	1.417
GE Study Data 1	16p	53	0.0050	1.429
GE Study Data 1	21q	30	0.0060	1.606
GE Study Data 1	19p	73	5.823E-4	1.464

Analysis Info:

Analyzed Gene Groups: GE Study Data 1
Background Set Selection: all genes
Threshold p-Value: 0.01
MTC type: No correction
Types of location: Chromosome,Arm
Minimal required number of genes in location and set: 4
Ignore known gene clusters: true
Filter redundant results: true
Number of enriched sets: 1

Currently working on: Data Sheet 1

After performing location enrichment analysis, the locations can be viewed by selecting *Enrichment Analysis* >> *Location Analysis* >> *View Locations* OR by pressing the toolbar button (▤). After selecting the gene group (cluster/bi-cluster etc.) to be viewed, a separate frame is displayed, containing an image of all chromosomes on which the positions of the genes in the group are marked. If the gene is located on an area that was identified to be enriched in that group, its position is marked in the same color to this area the enrichment results histogram.



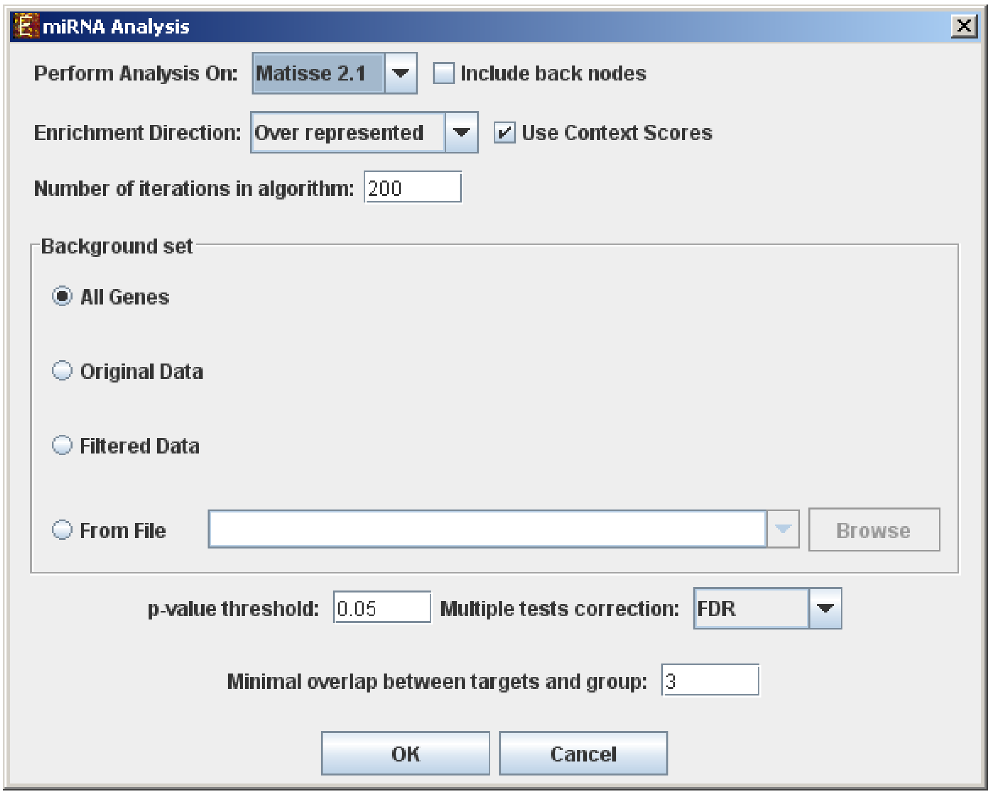
The results of this analysis can be exported to a text file by selecting *File>>Export to text* when the corresponding view is the selected tab OR by using the *File>>Save All* option, which will export all solutions within a session to text and image files.

miRNA Targets Enrichment Analysis

This tool performs a statistical analysis on the distribution of miRNA target gene within each group. The miRNA targets information is supplied in organism-specific data files, which can be downloaded from the EXPANDER download-page (see the Supplied Files section). For this analysis, Expander utilizes the FAME algorithm, which performs empirical tests using a sampling technique (random permutations) to estimate the empirical p-value distribution for the evaluated groups. This is done while accounting for biases in the 3' UTR sequences

Before operating miRNA enrichment analysis, the location data for the relevant organism should be downloaded from the download page (more details at introduction of Enrichment Analysis Tools). In this analysis, hyper-geometric enrichment tests are performed, and the results can be (if requested) corrected for multiple testing using the Bonferroni correction.

To perform the analysis, select *Enrichment Analysis* >> *miRNA Analysis* >> *FAME*. The following dialog box will appear:



Perform Analysis On: Matisse 2.1 □ Include back nodes

Enrichment Direction: Over represented ☑ Use Context Scores

Number of iterations in algorithm: 200

Background set

● All Genes

○ Original Data

○ Filtered Data

○ From File

p-value threshold: 0.05 Multiple tests correction: FDR

Minimal overlap between targets and group: 3

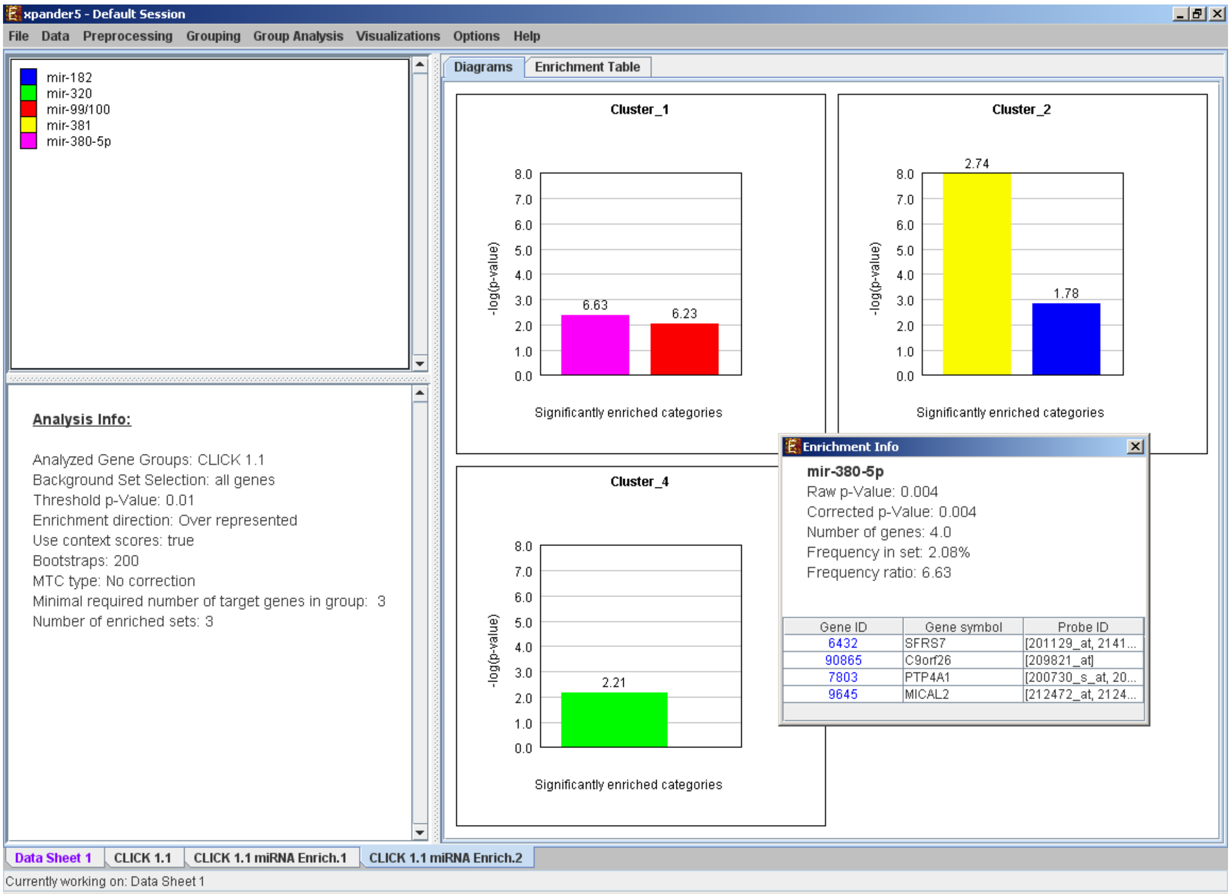
The following table specifies the different parameters that can be set via this dialog box:

Field	Description
Perform analysis on	The grouping solution on which the analysis will be performed.
Include back nodes	Include genes that are part of the module' but not included in the GE data (Relevant only if the analysis is performed on modules, detected by network based algorithm)
Enrichment Direction	Allows to choose between searching for over-represented targets and searching for under-represented targets.
Use context scores	If context scores are used, FAME will assign a higher weight to miRNA-gene pairs for which at least one target site has a high maximal context score (see References section for further details).
Number of Iterations	The number of random permutations used for the empirical tests.
Background set	Determines the set of genes that will be used as background in the analysis. Options are: all genes (of the relevant organism), original input

	data, filtered data or background set from file (see the Files Format section for details regarding the format of an external background set).
p-value threshold	A category/attribute will be considered significantly enriched in a cluster/bicluster if its corrected p-value is lower than this threshold.
Multiple tests correction	Can be set to FDR, Bonferroni or None (when set to Bonferroni/FDR the corrected p-values are the ones that are compared to the threshold p-value).
Minimal overlap between targets and group	The minimal number of genes from a group (cluster/bi-cluster/module etc.) expected to be categorized/attributed by an attribute in order for its enrichment to be accepted.

After the analysis is performed an enrichment analysis solution visualization tab is added to the main window. It contains general information regarding the analysis, a sort-able table holding all detected enrichments (group name, enriched miRNA target, p-value, etc.) and a multi-histogram panel along with a color index (mapping each color to a corresponding miRNA). The multi-histogram panel contains one histogram for each probe/gene group in which enrichment has been detected. Each histogram contains a column for each significant (more frequent than would be expected by random) miRNA target. The definition of significant depends on the user's selection of threshold p-value i.e., an mRNA target is considered significantly enriched in a group of genes if its corrected p-value is lower than the selected threshold p-value.

The height of the column is proportional to the significance of this enrichment (i.e. height = -log(raw p-value)), and the frequency ratio (frequency in set divided by frequency in background) is written on top of the column. Upon clicking on a column, a dialog box is displayed containing the miRNA name, corrected p-value, and a list of the genes in the group that are mapped to this location. Upon clicking on one of the gene Ids in the table, a relevant web page with information regarding this gene is displayed.



🟦	mir-182
🟩	mir-320
🟥	mir-99/100
🟨	mir-381
🟪	mir-380-5p

Analysis Info:

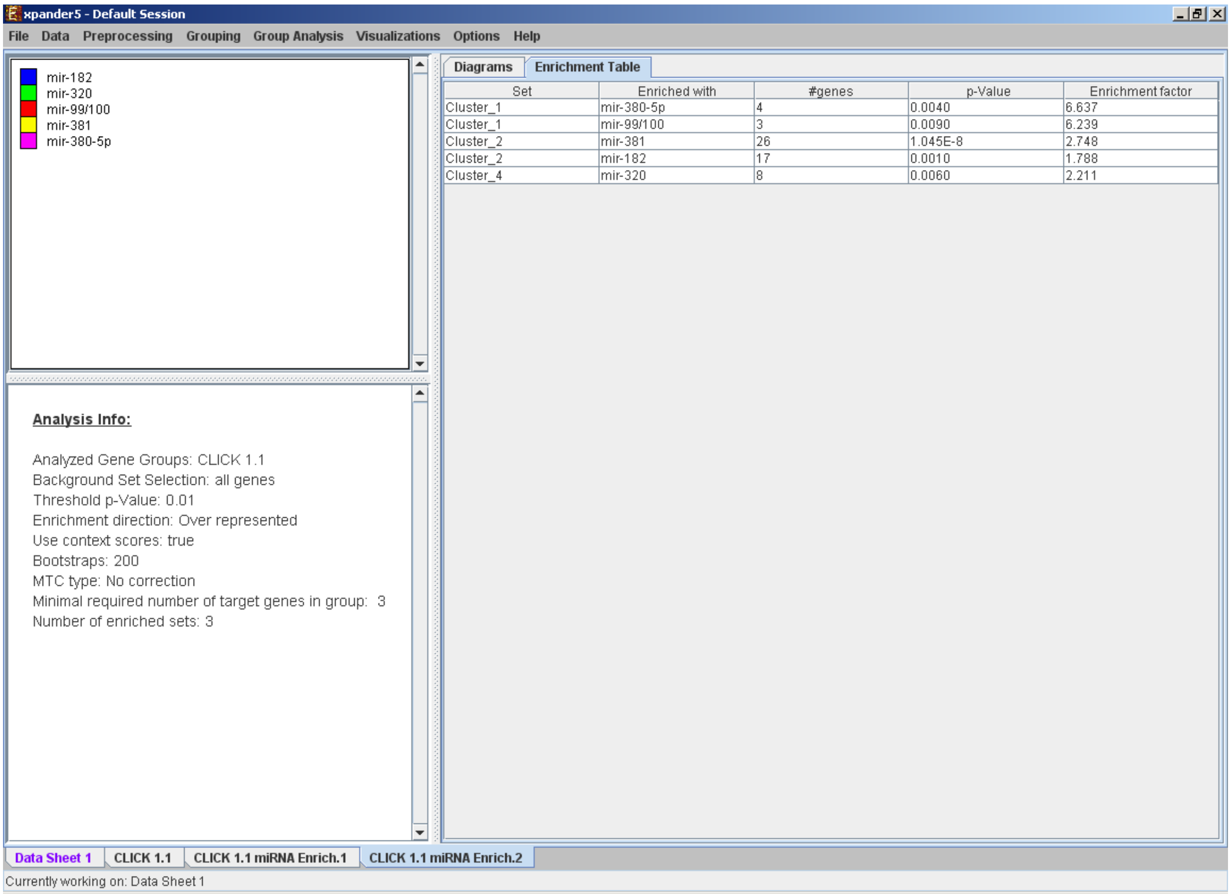
Analyzed Gene Groups: CLICK 1.1
Background Set Selection: all genes
Threshold p-Value: 0.01
Enrichment direction: Over represented
Use context scores: true
Bootstraps: 200
MTC type: No correction
Minimal required number of target genes in group: 3
Number of enriched sets: 3

Enrichment Info

mir-380-5p
Raw p-Value: 0.004
Corrected p-Value: 0.004
Number of genes: 4.0
Frequency in set: 2.08%
Frequency ratio: 6.63

Gene ID	Gene symbol	Probe ID
6432	SFRS7	[201129_at, 2141...
90865	C9orf26	[209821_at]
7803	PTP4A1	[200730_s_at, 20...
9645	MICAL2	[212472_at, 2124...

Currently working on: Data Sheet 1



🟦	mir-182
🟩	mir-320
🟥	mir-99/100
🟨	mir-381
🟪	mir-380-5p

Analysis Info:

Analyzed Gene Groups: CLICK 1.1
Background Set Selection: all genes
Threshold p-Value: 0.01
Enrichment direction: Over represented
Use context scores: true
Bootstraps: 200
MTC type: No correction
Minimal required number of target genes in group: 3
Number of enriched sets: 3

Set	Enriched with	#genes	p-Value	Enrichment factor
Cluster_1	mir-380-5p	4	0.0040	6.637
Cluster_1	mir-99/100	3	0.0090	6.239
Cluster_2	mir-381	26	1.045E-8	2.748
Cluster_2	mir-182	17	0.0010	1.788
Cluster_4	mir-320	8	0.0060	2.211

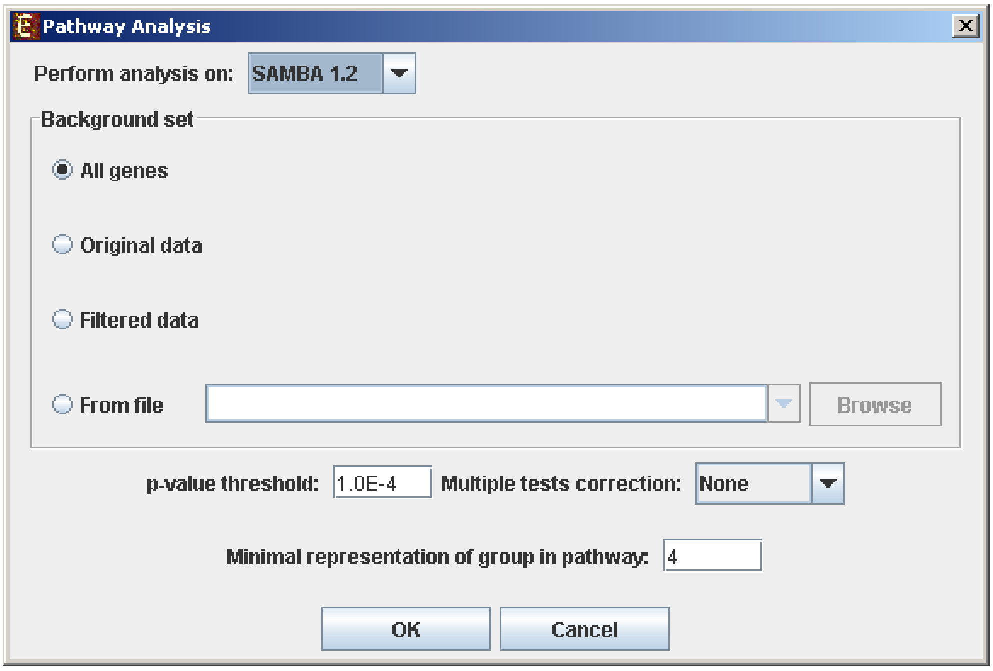
Currently working on: Data Sheet 1

The results of this analysis can be exported to a text file by selecting *File>>Export to text* when the corresponding view is the selected tab. OR by using the *File>>Save All* option, which will export all solutions within a session to text and image files.

Pathway Enrichment Analysis

This tool performs a statistical analysis on the representation of KEGG and WikiPathways pathway maps within each group. The KEGG and WikiPathways information is supplied in organism-specific data files, which can be downloaded from the EXPANDER download-page (see the Supplied Files section In this analysis, hyper-geometric enrichment tests are performed, and the results can be (if requested) corrected for multiple testing using the Bonferroni correction.

To perform the analysis, select *Enrichment Analysis >> Pathway Analysis >> KEGG or Enrichment Analysis >> Pathway Analysis >> WikiPathways* . The following dialog box will appear:



Pathway Analysis
Perform analysis on: SAMBA 1.2
Background set
● All genes
○ Original data
○ Filtered data
○ From file
Browse
p-value threshold: 1.0E-4
Multiple tests correction: None
Minimal representation of group in pathway: 4
OK
Cancel

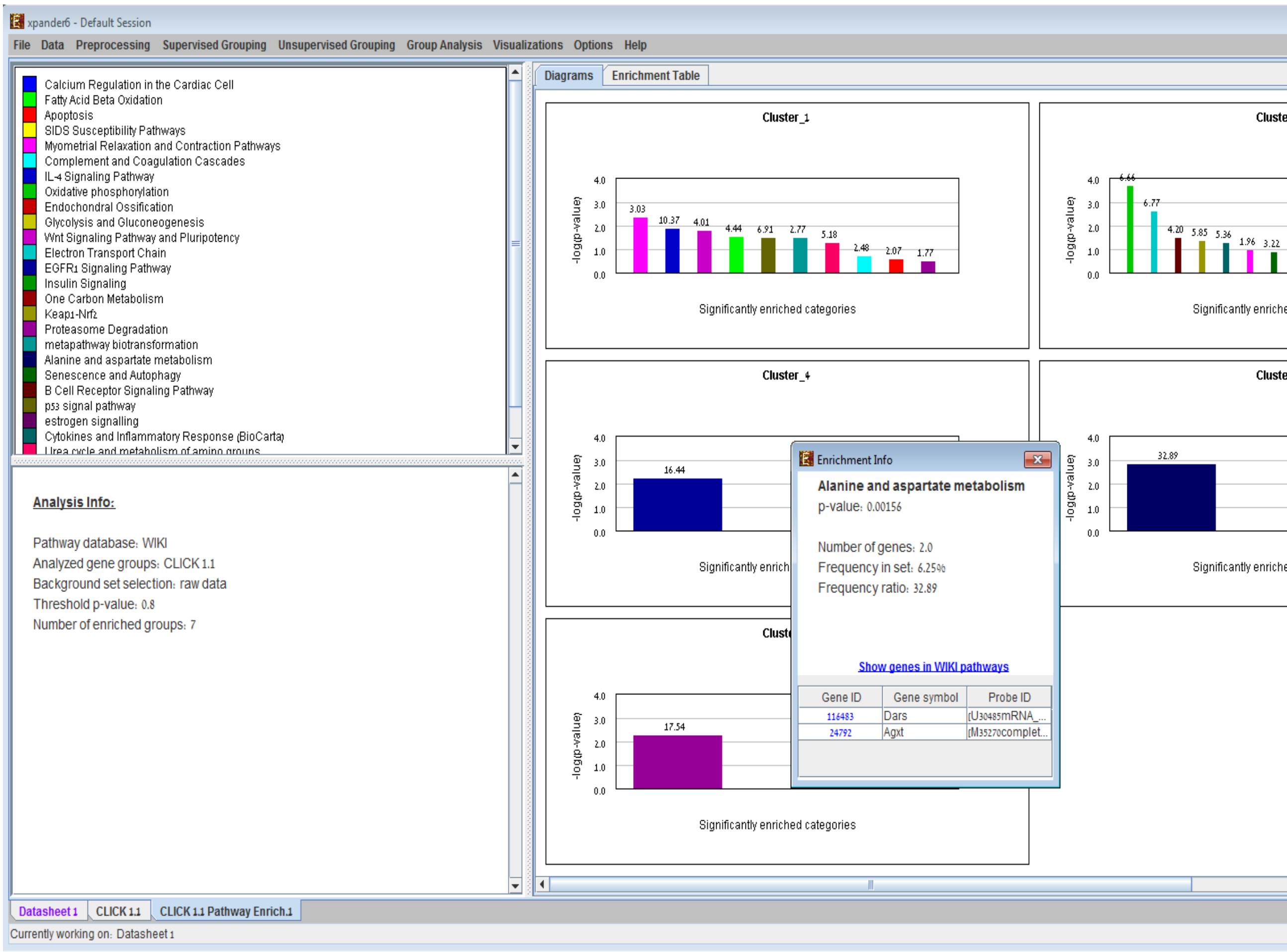
The following table specifies the different parameters that can be set via this dialog box:

Field	Description
Perform analysis on	The grouping solution on which the analysis will be performed.
Background set	Determines the set of genes that will be used as background in the analysis. Options are: all genes (of the relevant organism), original input data, filtered data or background set from file (see the Files Format section for details regarding the format of an external background set).
p-value threshold	A category/attribute will be considered significantly enriched in the pathway in a

	cluster/bicluster if its corrected p-value is lower than this threshold.
Multiple tests correction	Can be set to Bonferroni or None (when set to Bonferroni the corrected p-values are the ones that are compared to the threshold p-value).
Minimal overlap between category and set	The minimal number of genes from a cluster/bi-cluster expected to be categorized/attributed by an attribute in order for its pathway analysis to be accepted.

After the analysis was performed a Pathway analysis solution visualization tab is added to the main window. It contains general information about the analysis, a sorted table holding all detected pathways (group name, enriched pathway target, p-value, etc.) and multi-histogram panel along with a color index (mapping each color to a corresponding pathway). The multi-histogram panel contains one histogram for each probe/gene group in which enrichment has been detected. Each histogram contains a column for each significant (more frequent than would be expected by random) pathway target. The definition of significant depends on the user's selection of threshold p-value i.e., a pathway target is considered significantly enriched in a group of genes if its corrected p-value is lower than the selected threshold p-value.

The height of the column is proportional to the significance of this enrichment (i.e. height = -log(raw p-value)), and the frequency ratio (frequency in set divided by frequency in background) is written on top of the column. Upon clicking on a column, a dialog box is displayed containing the pathway name, corrected p-value, link to the relevant pathway map web page, and a list of the genes in the group that are included in the corresponding pathway. Upon clicking on one of the gene Ids in the table, a relevant web page with information regarding this gene is displayed.



- ■ Calcium Regulation in the Cardiac Cell
- ■ Fatty Acid Beta Oxidation
- ■ Apoptosis
- ■ SIDS Susceptibility Pathways
- ■ Myometrial Relaxation and Contraction Pathways
- ■ Complement and Coagulation Cascades
- ■ IL-4 Signaling Pathway
- ■ Oxidative phosphorylation
- ■ Endochondral Ossification
- ■ Glycolysis and Gluconeogenesis
- ■ Wnt Signaling Pathway and Pluripotency
- ■ Electron Transport Chain
- ■ EGFR1 Signaling Pathway
- ■ Insulin Signaling
- ■ One Carbon Metabolism
- ■ Keap1-Nrf2
- ■ Proteasome Degradation
- ■ metapathway biotransformation
- ■ Alanine and aspartate metabolism
- ■ Senescence and Autophagy
- ■ B Cell Receptor Signaling Pathway
- ■ p53 signal pathway
- ■ estrogen signalling
- ■ Cytokines and Inflammatory Response (BioCarta)
- ■ Urea cycle and metabolism of amino groups

Analysis Info:

Pathway database: WIKI
Analyzed gene groups: CLICK 1.1
Background set selection: raw data
Threshold p-value: 0.8
Number of enriched groups: 7

Enrichment Info

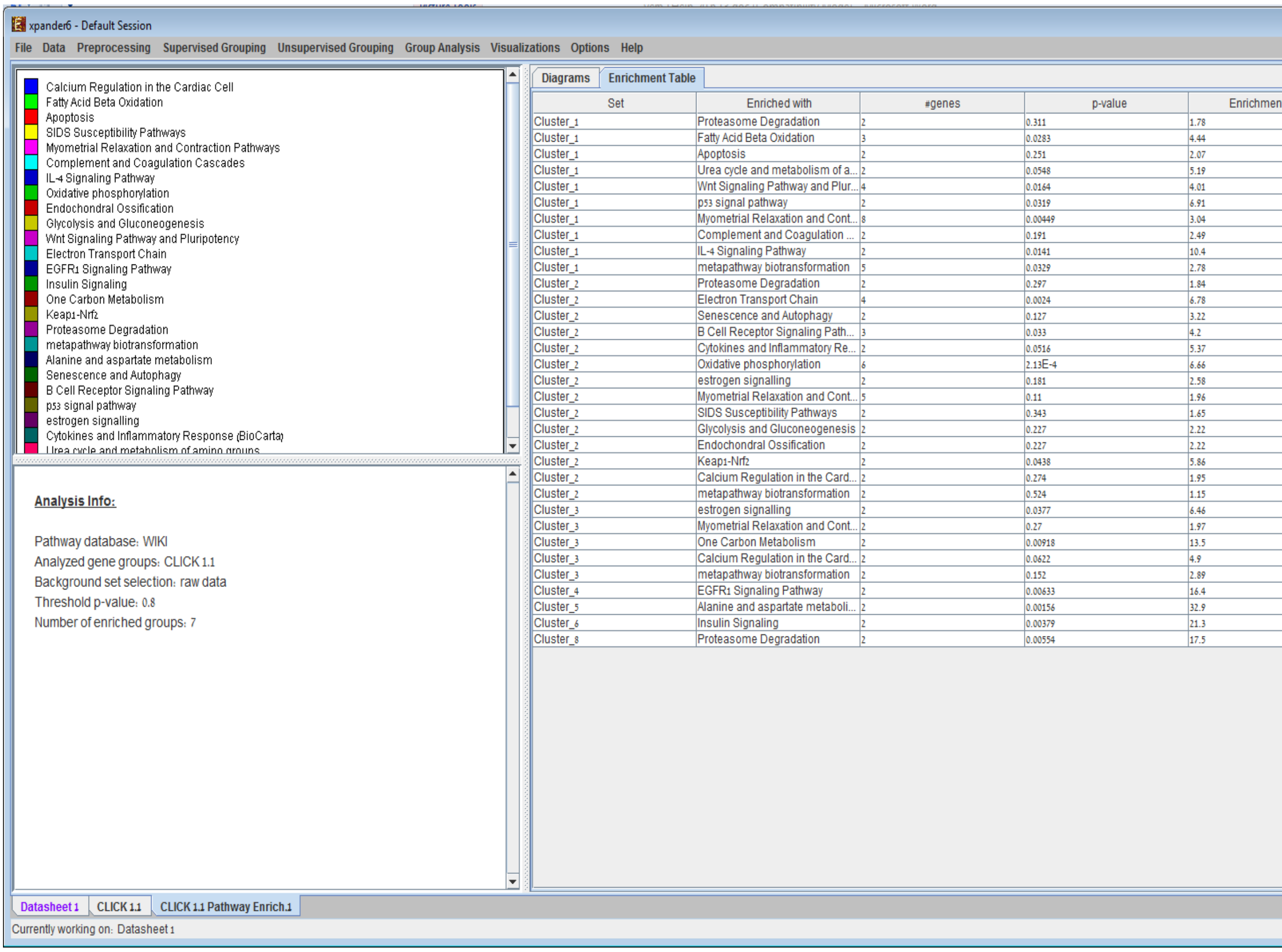
Alanine and aspartate metabolism
p-value: 0.00156

Number of genes: 2.0
Frequency in set: 6.25%
Frequency ratio: 32.89

Show genes in WIKI pathways

Gene ID	Gene symbol	Probe ID
116483	Dars	(U30485mRNA_...
24792	Agxt	(M35270complet...

Currently working on: Datasheet 1



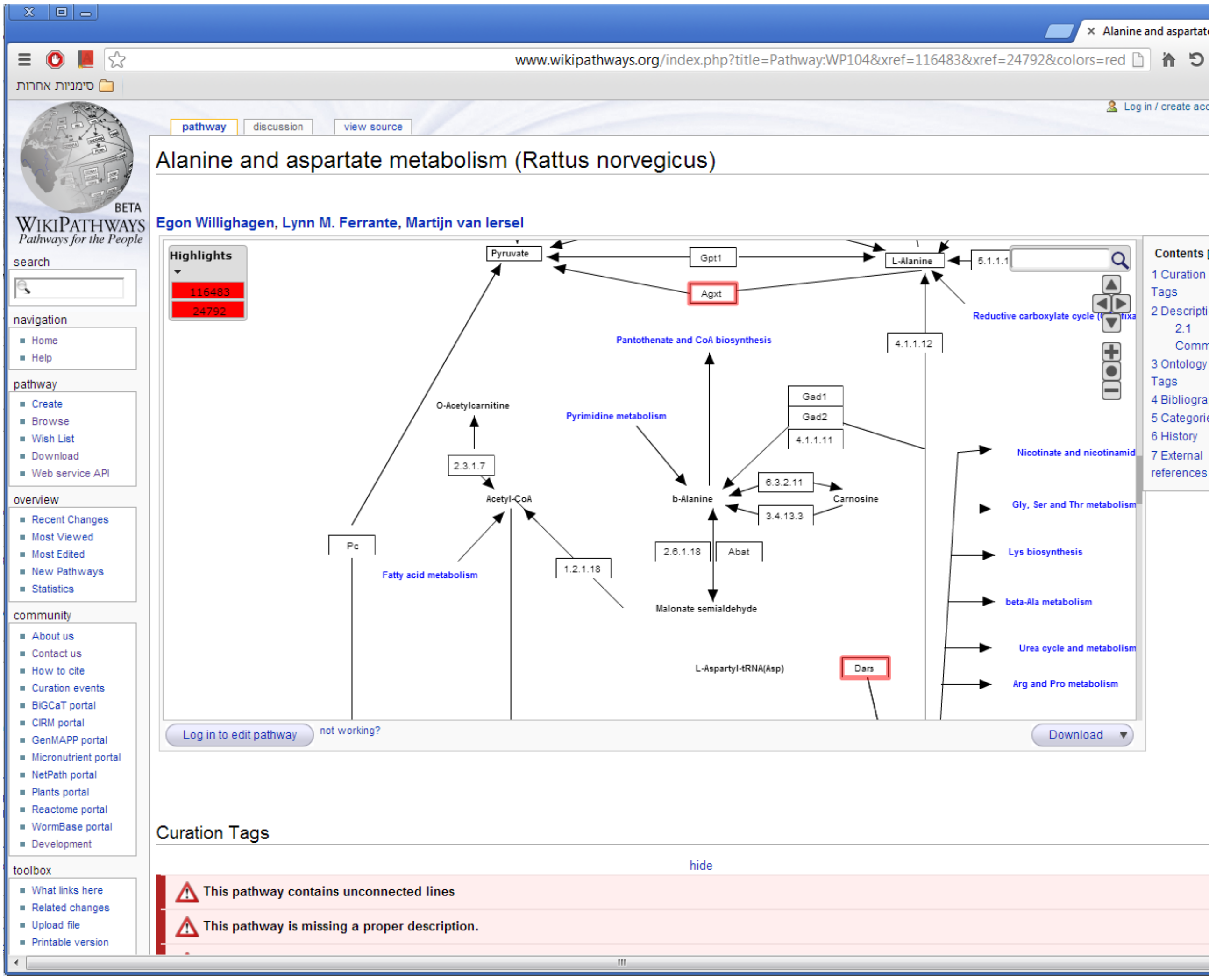
- ■ Calcium Regulation in the Cardiac Cell
- ■ Fatty Acid Beta Oxidation
- ■ Apoptosis
- ■ SIDS Susceptibility Pathways
- ■ Myometrial Relaxation and Contraction Pathways
- ■ Complement and Coagulation Cascades
- ■ IL-4 Signaling Pathway
- ■ Oxidative phosphorylation
- ■ Endochondral Ossification
- ■ Glycolysis and Gluconeogenesis
- ■ Wnt Signaling Pathway and Pluripotency
- ■ Electron Transport Chain
- ■ EGFR1 Signaling Pathway
- ■ Insulin Signaling
- ■ One Carbon Metabolism
- ■ Keap1-Nrf2
- ■ Proteasome Degradation
- ■ metapathway biotransformation
- ■ Alanine and aspartate metabolism
- ■ Senescence and Autophagy
- ■ B Cell Receptor Signaling Pathway
- ■ p53 signal pathway
- ■ estrogen signalling
- ■ Cytokines and Inflammatory Response (BioCarta)
- ■ Urea cycle and metabolism of amino groups

Analysis Info:

Pathway database: WIKI
Analyzed gene groups: CLICK 1.1
Background set selection: raw data
Threshold p-value: 0.8
Number of enriched groups: 7

Set	Enriched with	#genes	p-value	Enrichmen
Cluster_1	Proteasome Degradation	2	0.311	1.78
Cluster_1	Fatty Acid Beta Oxidation	3	0.0283	4.44
Cluster_1	Apoptosis	2	0.251	2.07
Cluster_1	Urea cycle and metabolism of a...	2	0.0548	5.19
Cluster_1	Wnt Signaling Pathway and Plur...	4	0.0164	4.01
Cluster_1	p53 signal pathway	2	0.0319	6.91
Cluster_1	Myometrial Relaxation and Cont...	8	0.00449	3.04
Cluster_1	Complement and Coagulation ...	2	0.191	2.49
Cluster_1	IL-4 Signaling Pathway	2	0.0141	10.4
Cluster_1	metapathway biotransformation	5	0.0329	2.78
Cluster_2	Proteasome Degradation	2	0.297	1.84
Cluster_2	Electron Transport Chain	4	0.0024	6.78
Cluster_2	Senescence and Autophagy	2	0.127	3.22
Cluster_2	B Cell Receptor Signaling Path...	3	0.033	4.2
Cluster_2	Cytokines and Inflammatory Re...	2	0.0516	5.37
Cluster_2	Oxidative phosphorylation	6	2.13E-4	6.66
Cluster_2	estrogen signalling	2	0.181	2.58
Cluster_2	Myometrial Relaxation and Cont...	5	0.11	1.96
Cluster_2	SIDS Susceptibility Pathways	2	0.343	1.65
Cluster_2	Glycolysis and Gluconeogenesis	2	0.227	2.22
Cluster_2	Endochondral Ossification	2	0.227	2.22
Cluster_2	Keap1-Nrf2	2	0.0438	5.86
Cluster_2	Calcium Regulation in the Card...	2	0.274	1.95
Cluster_2	metapathway biotransformation	2	0.524	1.15
Cluster_3	estrogen signalling	2	0.0377	6.46
Cluster_3	Myometrial Relaxation and Cont...	2	0.27	1.97
Cluster_3	One Carbon Metabolism	2	0.00918	13.5
Cluster_3	Calcium Regulation in the Card...	2	0.0622	4.9
Cluster_3	metapathway biotransformation	2	0.152	2.89
Cluster_4	EGFR1 Signaling Pathway	2	0.00633	16.4
Cluster_5	Alanine and aspartate metaboli...	2	0.00156	32.9
Cluster_6	Insulin Signaling	2	0.00379	21.3
Cluster_8	Proteasome Degradation	2	0.00554	17.5

Upon clicking on the link to the pathway map web page, the web browser displays the page with the relevant genes highlighted in it.



Alanine and aspartate metabolism (Rattus norvegicus)

Egon Willighagen, Lynn M. Ferrante, Martijn van Iersel

Curation Tags

This pathway contains unconnected lines

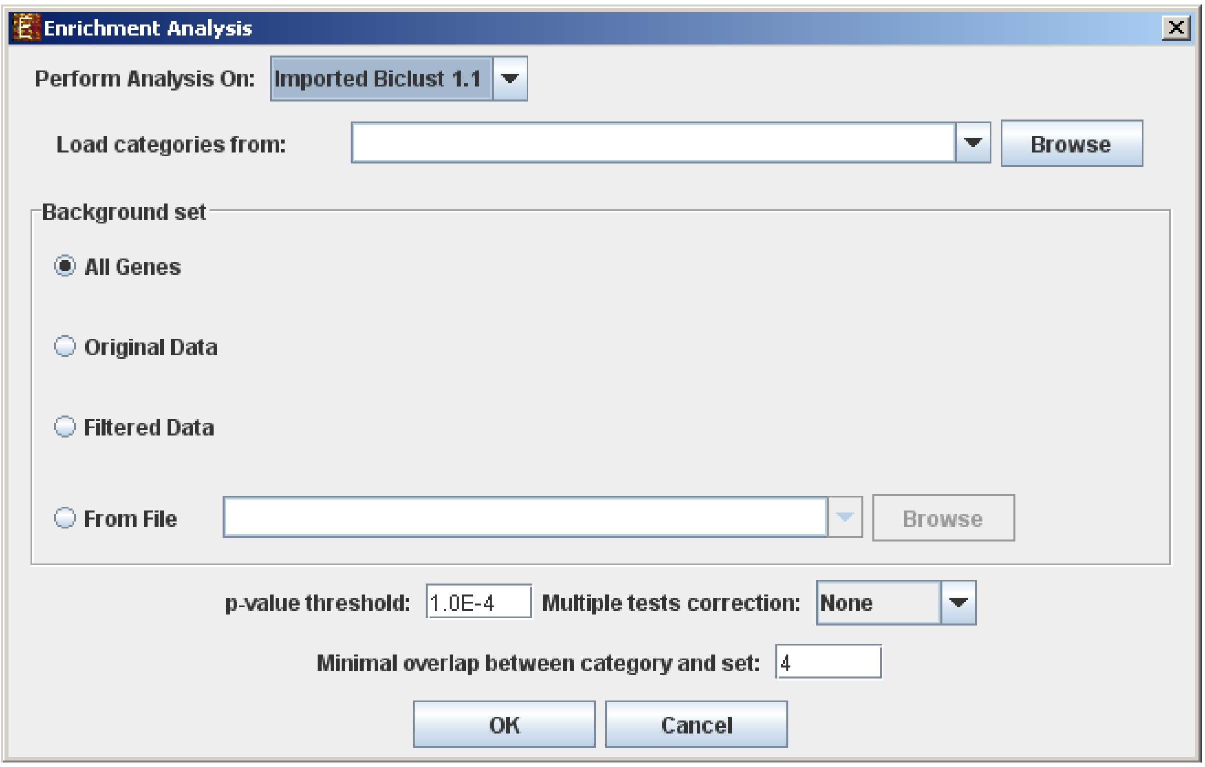
This pathway is missing a proper description.

The results of this analysis can be exported to a text file by selecting *File>>Export to text* when the corresponding view is the selected tab. OR by using the *File>>Save All* option, which will export all solutions within a session to text and image files.

General Enrichment Analysis

This tool performs basic statistical analysis on the distribution of categories/attributes of genes within each group. The categories/attributes of the genes are to be determined by the user and imported as a text (for details regarding the required format, see the File Formats section). In this analysis, hyper-geometric enrichment tests are performed, and the results can be (if requested) corrected for multiple testing using the Bonferroni correction.

To perform the analysis, select *Enrichment Analysis >> General Enrichment Analysis >> Detect Enrichment*. The following dialog box will appear:



Enrichment Analysis

Perform Analysis On: Imported Biclust 1.1

Load categories from:

Background set

● All Genes

○ Original Data

○ Filtered Data

○ From File

p-value threshold: 1.0E-4 Multiple tests correction: None

Minimal overlap between category and set: 4

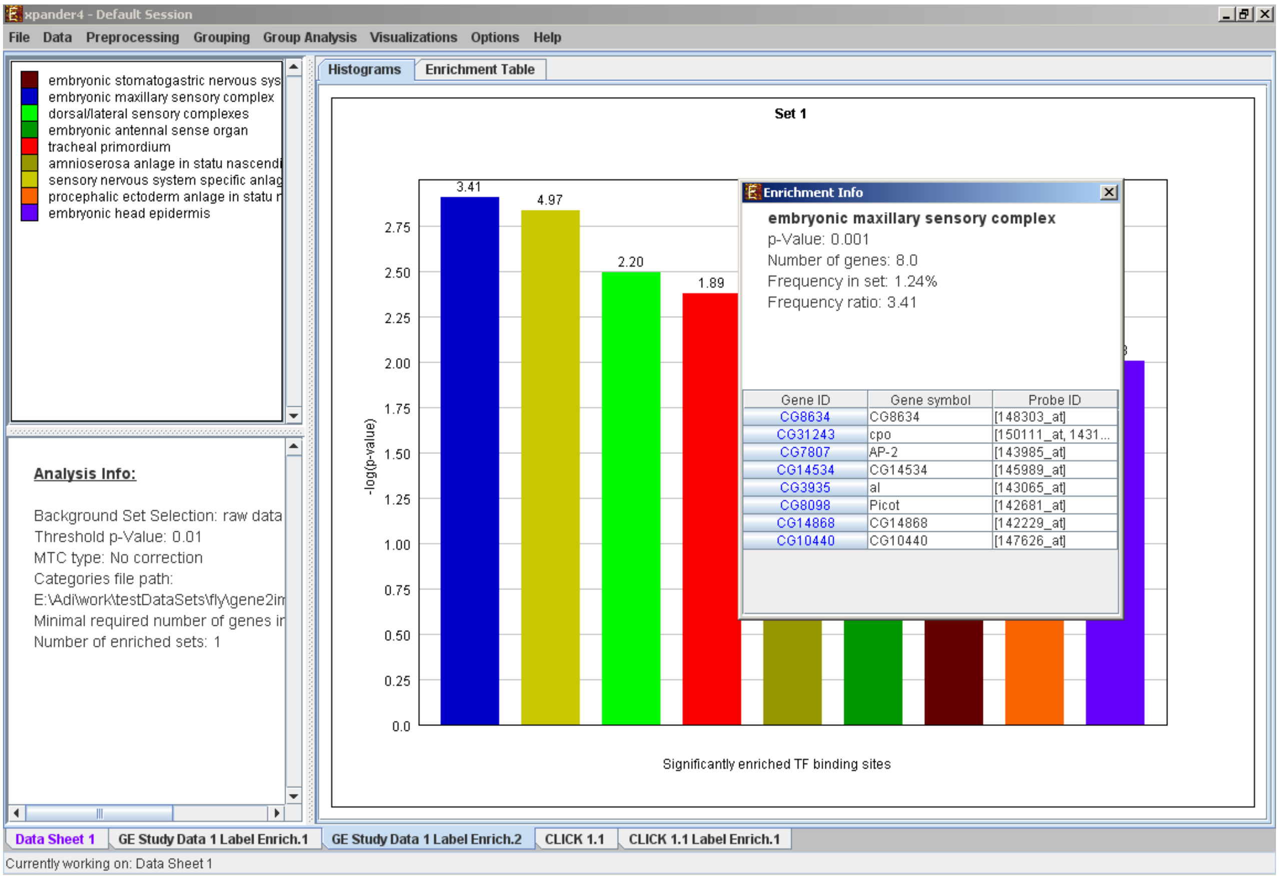
The following table specifies the different parameters that can be set via this dialog box:

Field	Description
Perform analysis on	The grouping solution on which the analysis will be performed.
Include back nodes	Include genes that are part of the module' but not included in the GE data (Relevant only if the analysis is performed on modules, detected by network based algorithm)
Load categories from	Input field for the file path, holding the gene categories/attributes.
Background set	Determines the set of genes that will be used as background in the analysis. Options are: all genes (of the relevant organism), original input data, filtered data or background set from file (see the Files Format section for details regarding the format of an external background set).
p-value threshold	A category/attribute will be considered significantly enriched in a cluster/bicluster if its corrected p-value is lower than this threshold.
Multiple tests correction	Can be set to Bonferroni or None (when set to Bonferroni the corrected p-values are the ones that are compared to the threshold p-value).
Minimal overlap between category and	The minimal number of genes from a cluster/bi-cluster expected to be categorized/attributed by

set	an attribute in order for its enrichment to be accepted.

After the analysis is performed an enrichment analysis solution visualization tab is added to the main window. It contains general information regarding the analysis, a sort-able table holding all detected enrichments (set ID, enrichment category, p-value, etc.) and a multi-histogram panel along with a color index (mapping each color to a corresponding category). The multi-histogram panel contains one histogram for each probe/gene set/group in which enrichment has been detected. Each histogram contains a column for each significant (more frequent than would be expected by random) category. The definition of significant depends on the user's selection of threshold p-value i.e., a category is considered significantly enriched in a cluster/bicluster if its corrected p-value is lower than the preset threshold p-value.

The height of the column is proportional to the significance of this enrichment (i.e. height = -log(raw p-value)), and the frequency ratio (frequency in set divided by frequency in background) is written on top of the column. Upon clicking on a column, a dialog box is displayed containing the class name, corrected p-value, and a list of the genes in the cluster/bi-cluster that belong to the category. Upon clicking on one of the gene Ids in the table, a relevant web page with information regarding this gene is displayed. The display tool tip shows the cluster number, size and homogeneity.



🟥	embryonic stomatogastric nervous sys
🟦	embryonic maxillary sensory complex
🟩	dorsal/lateral sensory complexes
🟩	embryonic antennal sense organ
🟥	tracheal primordium
🟨	amnioserosa anlage in statu nascendi
🟨	sensory nervous system specific anlag
🟧	procephalic ectoderm anlage in statu r
🟪	embryonic head epidermis

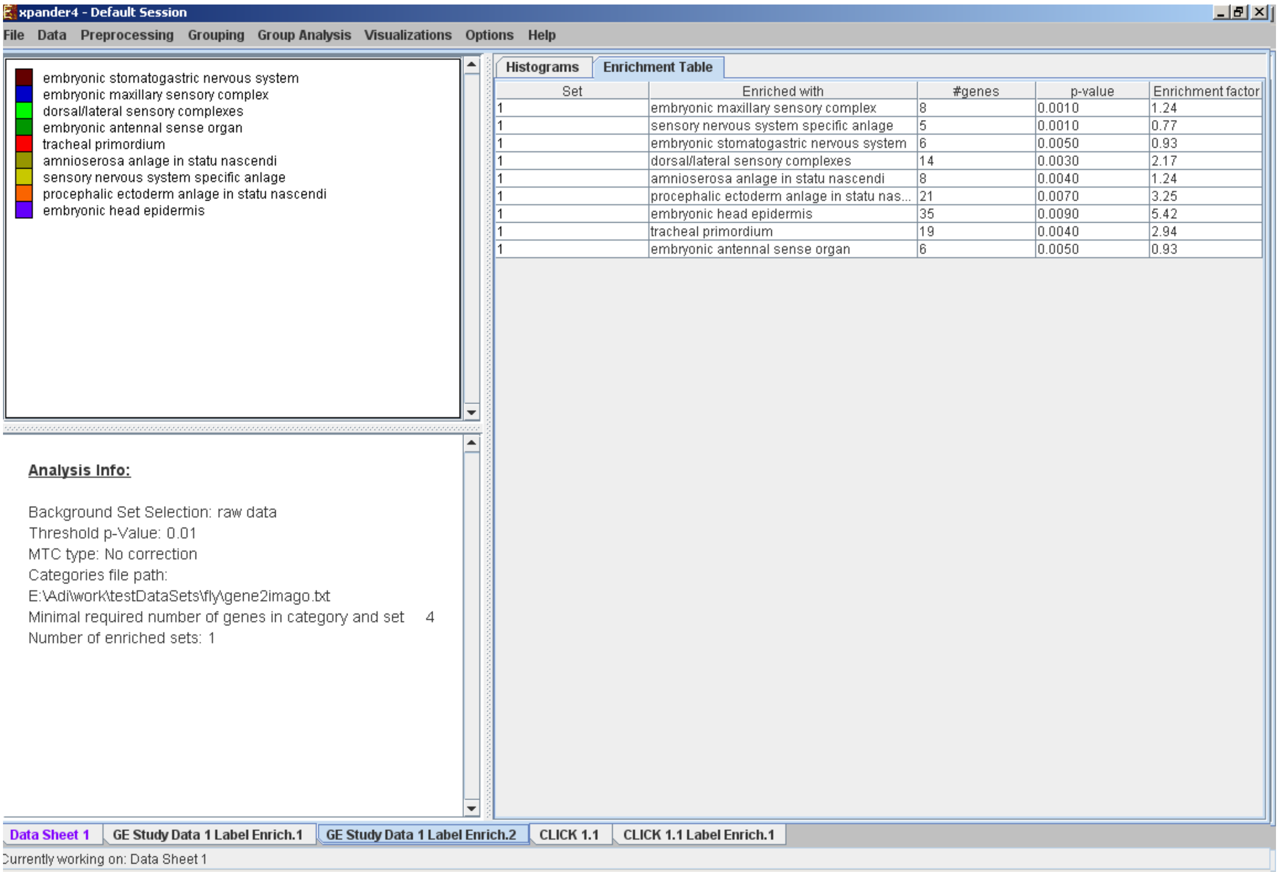
Analysis Info:

Background Set Selection: raw data
Threshold p-Value: 0.01
MTC type: No correction
Categories file path:
E:\Adi\work\testDataSets\fly\gene2im
Minimal required number of genes ir
Number of enriched sets: 1

embryonic maxillary sensory complex
p-Value: 0.001
Number of genes: 8.0
Frequency in set: 1.24%
Frequency ratio: 3.41

Gene ID	Gene symbol	Probe ID
CG8634	CG8634	[148303_at]
CG31243	cpo	[150111_at, 1431...
CG7807	AP-2	[143985_at]
CG14534	CG14534	[145989_at]
CG3935	al	[143065_at]
CG8098	Picot	[142681_at]
CG14868	CG14868	[142229_at]
CG10440	CG10440	[147626_at]

Currently working on: Data Sheet 1



🟫 embryonic stomatogastric nervous system
🟦 embryonic maxillary sensory complex
🟩 dorsal/lateral sensory complexes
🟩 embryonic antennal sense organ
🟥 tracheal primordium
🟫 amnioserosa anlage in statu nascendi
🟨 sensory nervous system specific anlage
🟧 procephalic ectoderm anlage in statu nascendi
🟪 embryonic head epidermis

Set	Enriched with	#genes	p-value	Enrichment factor
1	embryonic maxillary sensory complex	8	0.0010	1.24
1	sensory nervous system specific anlage	5	0.0010	0.77
1	embryonic stomatogastric nervous system	6	0.0050	0.93
1	dorsal/lateral sensory complexes	14	0.0030	2.17
1	amnioserosa anlage in statu nascendi	8	0.0040	1.24
1	procephalic ectoderm anlage in statu nas...	21	0.0070	3.25
1	embryonic head epidermis	35	0.0090	5.42
1	tracheal primordium	19	0.0040	2.94
1	embryonic antennal sense organ	6	0.0050	0.93

Analysis Info:

Background Set Selection: raw data
Threshold p-Value: 0.01
MTC type: No correction
Categories file path:
E:\Adi\work\testDataSets\fly\gene2imago.txt
Minimal required number of genes in category and set 4
Number of enriched sets: 1

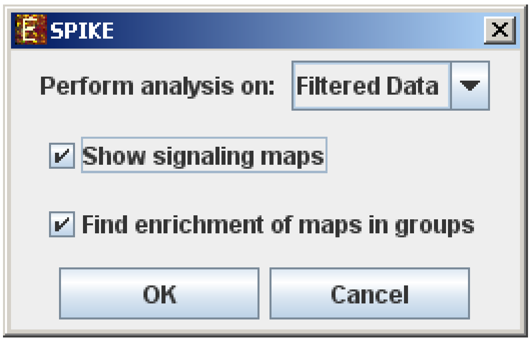
Currently working on: Data Sheet 1

The results of this analysis can be exported to a text file by selecting *File>>Export to text* when the corresponding view is the selected tab. OR by using the *File>>Save All* option, which will export all solutions within a session to text and image files.

Network Based Enrichment Analysis

This tool allows browsing through signaling data to view the sub-graphs that are induced by the analyzed gene groups. It also enables the user to search for statistical enrichment of these groups in highly curated signaling maps. To perform this task, Expander interfaces with the SPIKE software and database. For further information regarding the SPIKE software see the References section.

To perform the analysis on one/more of the gene groups defined in Expander (i.e. clusters, bi-clusters, modules, loaded gene sets or filtered data), select *Enrichment Analysis >> Network >> SPIKE>>Gene Groups*. The following dialog box will appear:



SPIKE

Perform analysis on: Filtered Data

☑ Show signaling maps

☑ Find enrichment of maps in groups

The following table specifies the different parameters that can be set via this dialog box:

Field	Description
Perform analysis on	The grouping solution on which the analysis will be performed.
Show signaling Maps	For each group display regulatory data induced by the genes included in the group.
Find enrichment of maps in groups	For each group, search for signaling maps that are enriched with genes included in the group.

Pressing OK in the dialog box will launch the SPIKE application. When operated for the first time, the launch takes a few minutes, since it has to build a local database. From this point on, please refer to page 12 in the SPIKE user manual.

SPIKE can also be operated on a sub-group of genes that is derived from an existing enrichment solution in Expander. I.e. a group of genes that has a common annotation that was found to be enriched by one of the enrichment analysis operations. In order to operate SPIKE on such a *group, select: Enrichment Analysis>>Network>> SPIKE>>Enrichment Derived Sets*.

Gene Set Enrichment Analysis (GSEA)

GSEA (Subramanian et al 2005) considers experiments with genomewide expression profiles from samples belonging to two classes. Genes are ranked based on the correlation between their expression and the differential expression between classes distinction or pre-ranked by the user.

Given an *a priori* defined set of genes *S*, the goal of GSEA is to determine whether the members of *S* are randomly distributed throughout the ranked list of genes (L) or primarily found at the top or bottom. It is expected that sets related to the phenotypic distinction will tend to show the latter distribution.

There are two key elements of the GSEA method in Expander:

Step 1: Calculation of an Enrichment Score. Enrichment score (*ES*) reflects the degree to which a set *S* is overrepresented at the extremes (top or bottom) of the entire ranked list *L*. The score is calculated by walking down the list *L*, increasing a running-sum statistic when we encounter a gene in *S* and decreasing it when we encounter genes not in *S.* The magnitude of the increment depends on the correlation of the gene with the phenotype. The enrichment score is the maximum deviation from zero encountered in the random walk. It corresponds to a weighted Kolmogorov–Smirnov-like statistic.

Step 2: Estimation of Significance Level of ES. An estimation of the statistical significance (nominal *P*-value) of the *ES* is done by using an empirical phenotype-based permutation test procedure that preserves the complex correlation structure of the gene expression data. Specifically, the phenotype labels are permuted again and the *ES* of the gene set for the permuted data is re-computed, which generates a null distribution for the *ES.* If the user provided a pre-ranked list of genes then a random shuffling of the ranked list is done instead. The empirical, nominal *P* value of the observed *ES* is then calculated relative to this null distribution. Importantly, the permutation of class labels preserves gene-gene correlations and, thus, provides a more biologically reasonable assessment of significance than would be obtained by permuting genes.

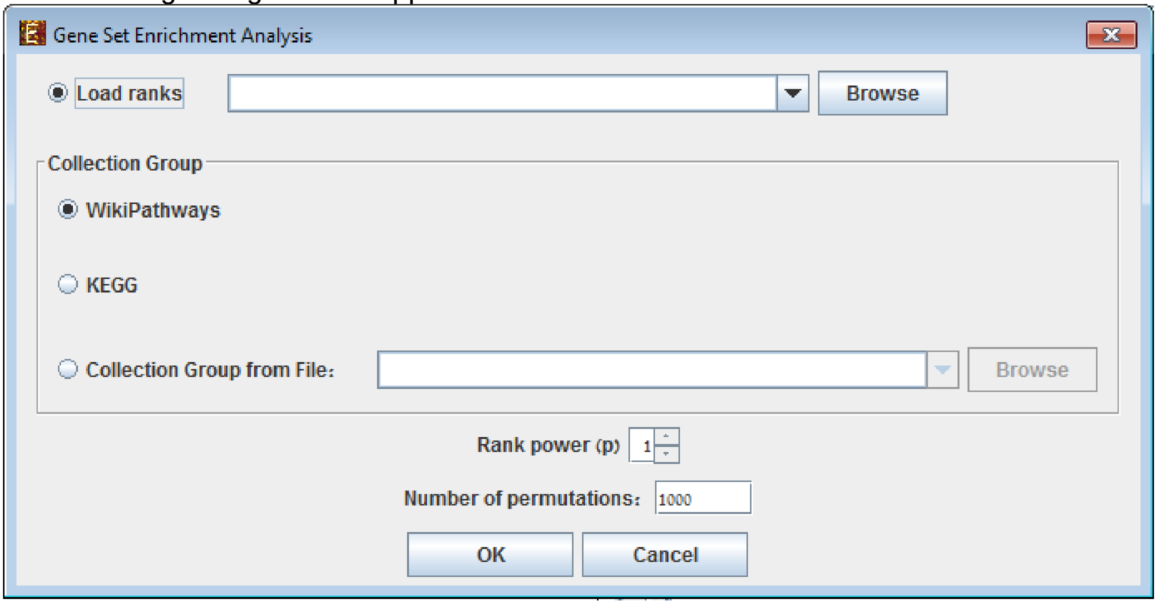
There are 2 ways to perform GSEA:

1. GSEA on a pre-Ranked list of Genes without loading gene expression data
2. GSEA on a gene expression data

<u>GSEA on a pre-Ranked list of Genes without loading gene expression data</u>

To perform analysis on a pre-ranked list of genes, select *File->New Session->Gene Ranking Analysis (GSEA).*

The following dialog box will appear:



Gene Set Enrichment Analysis

● Load ranks

Collection Group

● WikiPathways

○ KEGG

○ Collection Group from File:

Rank power (p) 1

Number of permutations: 1000

The following table specifies the different parameters that can be set via this dialog box:

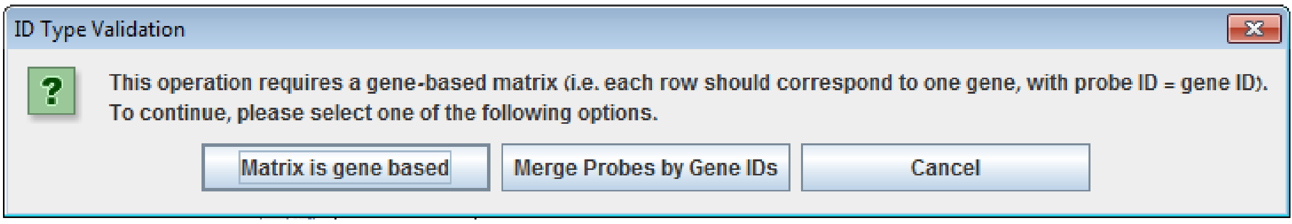
Field	Description
Load ranks	User pre-ranked list of genes file composed of two columns – first with genes and second with values
Collection Group	Can be chosen between: WikiPathways, KEGG or User pre-defined file with gene sets*
Rank Power (p)	If p=0 then ES is reduced to standard Kolmogorov–Smirnov statistic. If p=1then ES is a weighted Kolmogorov–Smirnov-like statistic.

Number of permutations	For estimation of the Significance Level of ES

Please refer to Results section in "GSEA on a gene expression data" to interpret the results.

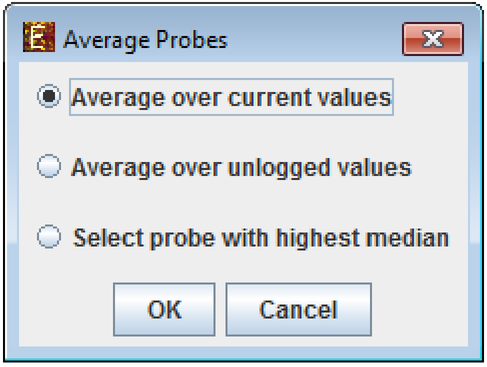
GSEA on a gene expression data

To perform the analysis on the gene expression, select *Enrichment Analysis* >> run GSEA… The following dialog box will appear:



This operation requires a gene-based matrix (i.e. each row should correspond to one gene, with probe ID = gene ID).
To continue, please select one of the following options.

The user can choose between "Matrix is gene based" (i.e each row should correspond to one gene, with probe ID = gene ID) or "Merge Probes by Gene IDs".
In case the user chose "Merge Probes by Gene IDs", a dialog box titled "Average Probes" will appear:

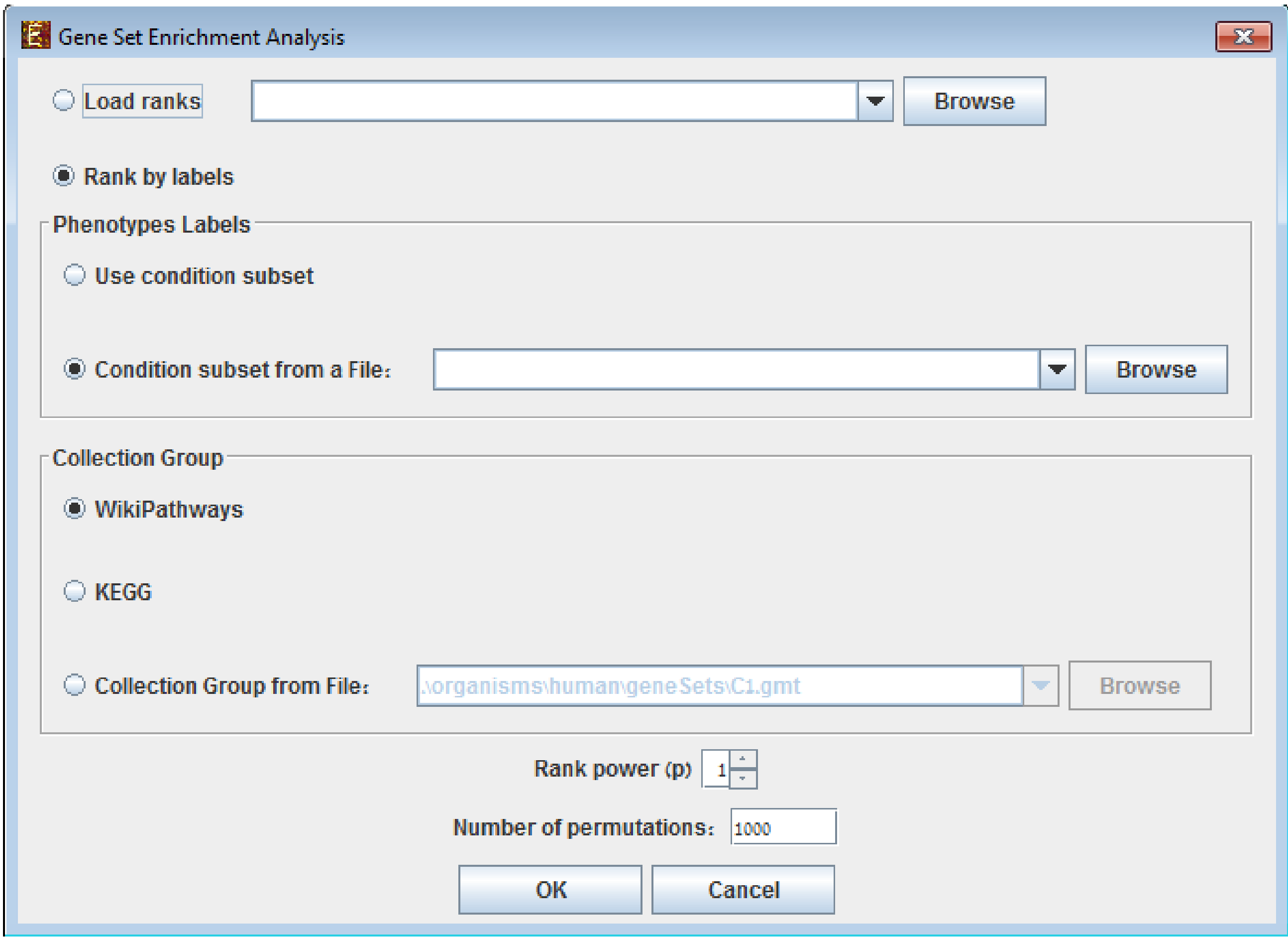


● Average over current values

○ Average over unlogged values

○ Select probe with highest median

After choosing the preferred merging option or "Matrix gene is based" in "ID Type Validation" dialog box, "Gene Set Enrichment Analysis" dialog box will appear:



Gene Set Enrichment Analysis

○ Load ranks

● Rank by labels

Phenotypes Labels

○ Use condition subset

● Condition subset from a File:

Collection Group

● WikiPathways

○ KEGG

○ Collection Group from File: .\organisms\human\geneSets\C1.gmt

Rank power (p) 1

Number of permutations: 1000

The following table specifies the different parameters that can be set via this dialog box:

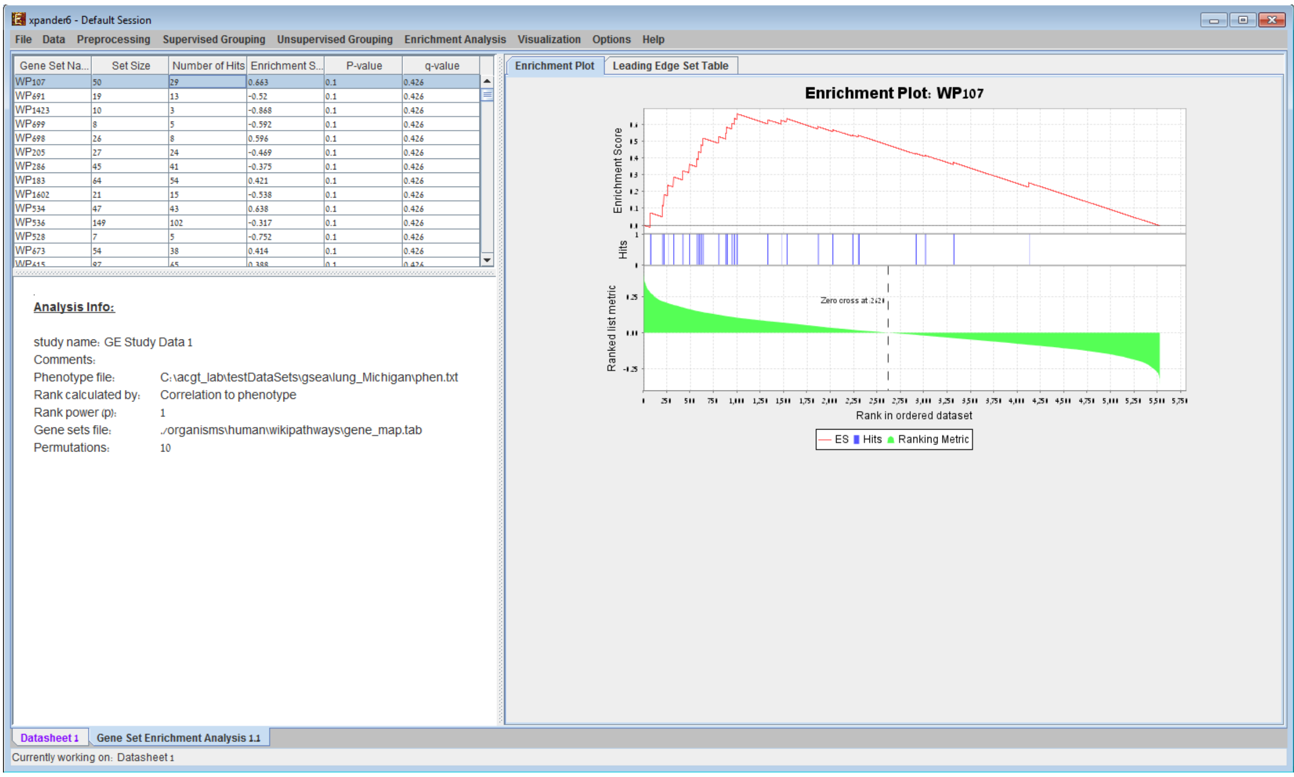
Field	Description
Load ranks	User pre-ranked list of genes file composed of two columns – first with genes and second with values
Use condition subset	Can be used when the matrix is composed of two condition subsets
Condition subset from a File	A file with a single tab delimited row which contains the phenotype for each condition
Collection Group	Can be chosen between: WikiPathways, KEGG or User pre-defined file with gene sets*
Rank Power (p)	If p=0 then ES is reduced to standard Kolmogorov–Smirnov statistic. If p=1then ES is a weighted Kolmogorov–Smirnov-like statistic.
Number of permutations	For estimation of the Significance Level of ES

*Collection Group File

More gene sets are available to download via MSigDB and files can be loaded via "Collection Group File" field in Collection Group.
The MSigDB gene sets are divided into 7 major collections:
C1.gmt – Positional gene sets
C2.gmt – Curated gene sets
C3.gmt – Motif gene sets
C4.gmt – Computational gene sets
C5.gmt – GO gene sets
C6.gmt – Oncogenic signatures
C7.gmt – Immunologic signatures
For further information, please refer to: http://www.broadinstitute.org/gsea/msigdb/index.jsp

Results

After the analysis is performed a gene set enrichment analysis solution visualization tab is added to the main window. It contains general information regarding the analysis, a sort-able table holding all gene sets (Gene set name, set size, Number of hits, Enrichment score, P-value and q-value(FDR)), an enrichment plot for each gene set selected in the table. The enrichment plot panel contains a graph of the enrichment score for each gene in the ranked list, a bar of hits of the genes in the gene set with the genes in the ranked list and a ranked list metric of the genes, and a tab - leading edge set table that contains Gene ID, Gene symbol, Ranke metric and Hit (if the gene was hit by a gene in the gene set). The leading edge set table contains only genes that appear before the maximum enrichment score.

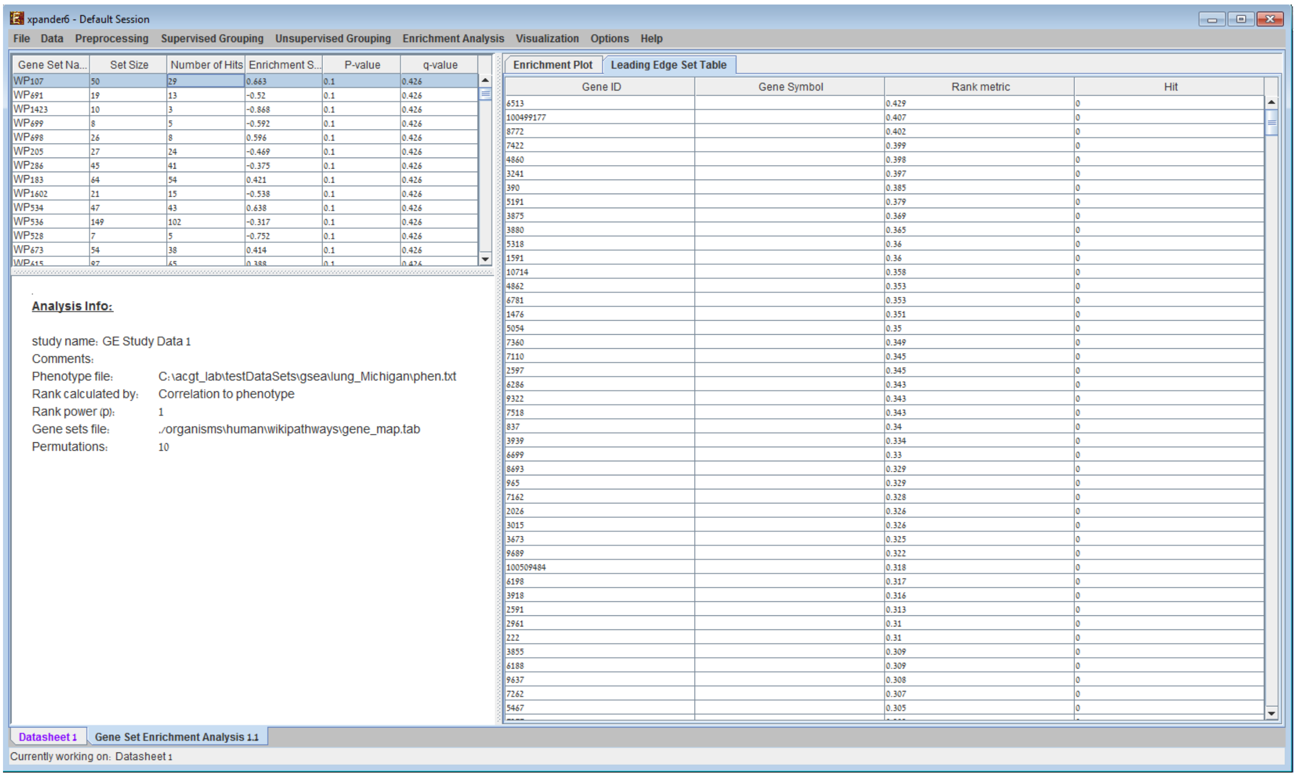


Gene Set Na...	Set Size	Number of Hits	Enrichment S...	P-value	q-value
WP107	50	29	0.663	0.1	0.426
WP691	19	13	-0.52	0.1	0.426
WP1423	10	3	-0.868	0.1	0.426
WP699	8	5	-0.592	0.1	0.426
WP698	26	8	0.596	0.1	0.426
WP205	27	24	-0.469	0.1	0.426
WP286	45	41	-0.375	0.1	0.426
WP183	64	54	0.421	0.1	0.426
WP1602	21	15	-0.538	0.1	0.426
WP534	47	43	0.638	0.1	0.426
WP536	149	102	-0.317	0.1	0.426
WP528	7	5	-0.752	0.1	0.426
WP673	54	38	0.414	0.1	0.426
WP615	97	65	0.388	0.1	0.426

Analysis Info:

study name: GE Study Data 1	
Comments:	
Phenotype file:	C:\acgt_lab\testDataSets\gsea\lung_Michigan\phen.txt
Rank calculated by:	Correlation to phenotype
Rank power (p):	1
Gene sets file:	./organisms\human\wikipathways\gene_map.tab
Permutations:	10

Currently working on: Datasheet 1



Gene Set Na...	Set Size	Number of Hits	Enrichment S...	P-value	q-value
WP107	50	29	0.663	0.1	0.426
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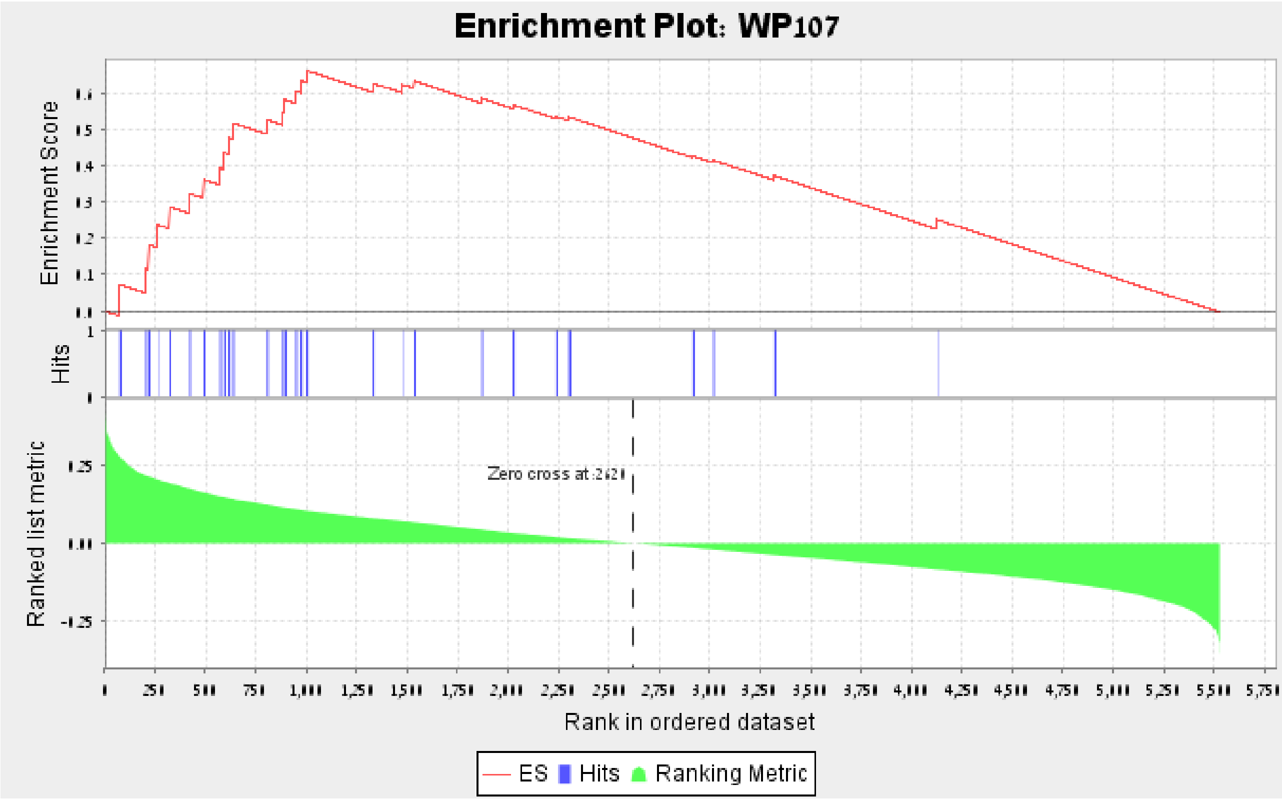
WP699	8	5	-0.592	0.1	0.426
WP698	26	8	0.596	0.1	0.426
WP205	27	24	-0.469	0.1	0.426
WP286	45	41	-0.375	0.1	0.426
WP183	64	54	0.421	0.1	0.426
WP1602	21	15	-0.538	0.1	0.426
WP534	47	43	0.638	0.1	0.426
WP536	149	102	-0.317	0.1	0.426
WP528	7	5	-0.752	0.1	0.426
WP673	54	38	0.414	0.1	0.426
WP615	97	65	0.388	0.1	0.426

Analysis Info:

study name: GE Study Data 1
Comments:
Phenotype file: C:\acgt_lab\testDataSets\gsea\lung_Michigan\phen.txt
Rank calculated by: Correlation to phenotype
Rank power (p): 1
Gene sets file: ./organisms\human\wikipathways\gene_map.tab
Permutations: 10

Gene ID	Gene Symbol	Rank metric	Hit
6513		0.429	0
100499177		0.407	0
8772		0.402	0
7422		0.399	0
4860		0.398	0
3241		0.397	0
390		0.385	0
5191		0.379	0
3875		0.369	0
3880		0.365	0
5318		0.36	0
1591		0.36	0
10714		0.358	0
4862		0.353	0
6781		0.353	0
1476		0.351	0
5054		0.35	0
7360		0.349	0
7110		0.345	0
2597		0.345	0
6286		0.343	0
9322		0.343	0
7518		0.343	0
837		0.34	0
3939		0.334	0
6699		0.33	0
8693		0.329	0
965		0.329	0
7162		0.328	0
2026		0.326	0
3015		0.326	0
3673		0.325	0
9689		0.322	0
100509484		0.318	0
6198		0.317	0
3918		0.316	0
2591		0.313	0
2961		0.31	0
222		0.31	0
3855		0.309	0
6188		0.309	0
9637		0.308	0
7262		0.307	0
5467		0.305	0

Currently working on: Datasheet 1



The enrichment plot can be saved as image file by right clicking on the graph->Save as…

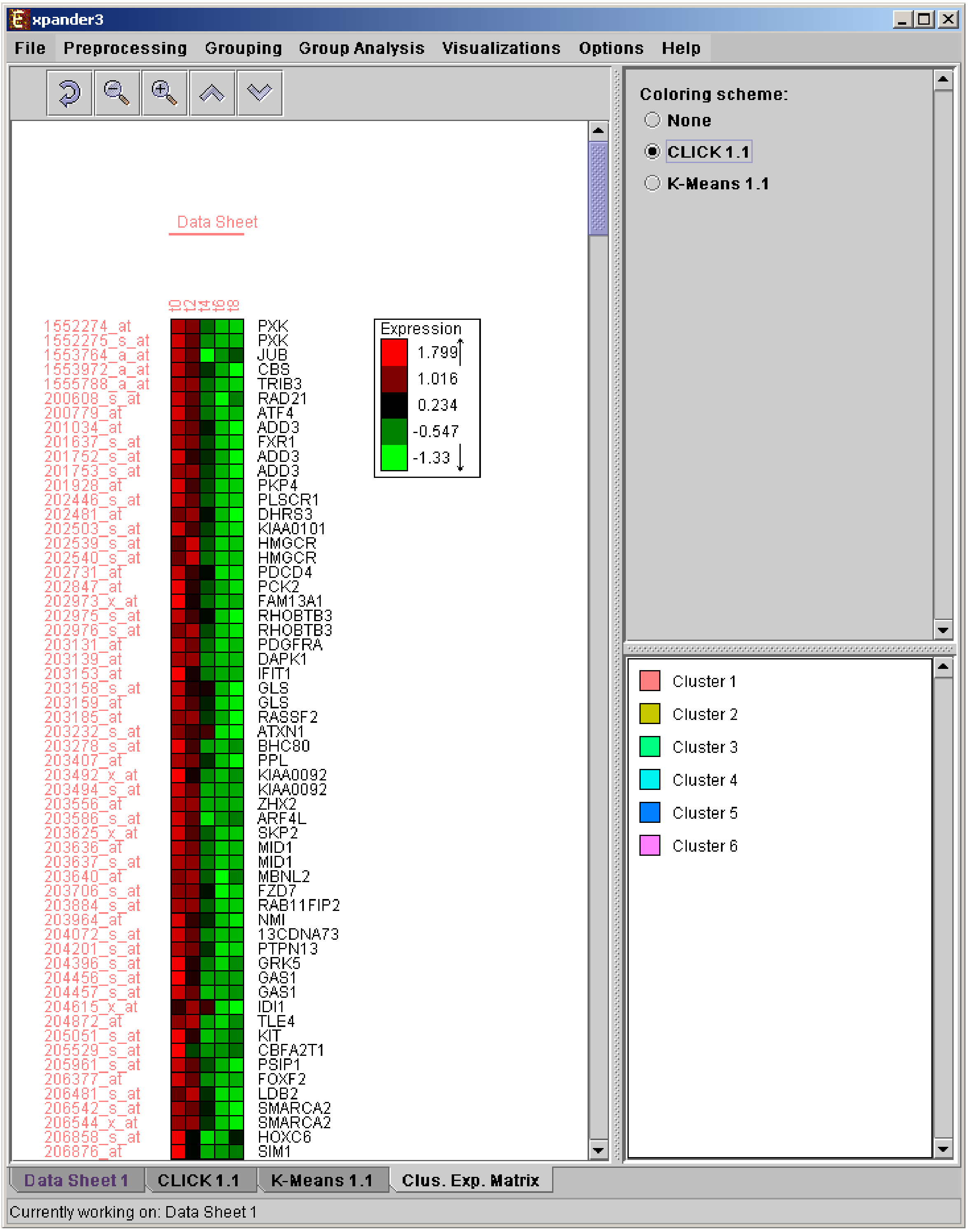
The results in the tables can be saved as image file by selecting *File>>Save As Image* when the corresponding view is the selected tab OR by using the *File>>Save All* option, which will export all solutions within a session to text and image files.

Matrix Visualizations

An expression matrix (Heat-map) visualization is integrated in many of EXPANDER's displays. This visualization is similar to the red-green matrix representation of Eisen et al (1998). All it does is to render the gene-expression data on the screen in color, where green indicates under expression, and red indicates over expression. Color rendering can be configured by the user in one of the following manners: (a) by setting the range (top and bottom values) of rendered values (default values are set according to the data scale, e.g. 40-1000 for non-standardized absolute intensities data) or (b) by setting the percent of values, which are to be disregarded as extreme values from each edge (by default set to 5%). The manner of color scale configuration (i.e. (a) vs. (b)) can be set via the 'Data Matrix View' tab in the 'Display Settings' dialog box, available from *Options* >> *Settings*. The red/green coloring scheme can be changed to blue/yellow (using *Options* >> *Settings* >> *Display* >> 'Data Matrix View' tab).

A color scale appears next to the matrix (upper right side). The displayed tool tip shows the probe ID and condition title corresponding to the row and column on which the cursor is placed, and the expression value in that position. The matrix toolbar contains zoom in, zoom out, reset scale (to reset zoom factor), shorten condition title and Elongate condition title tools.

Upon selecting *Visualization >> Clustered Expression Matrix*, a clustered expression matrix visualization tab is added to the main window. The probes are ordered in their original order. If a clustering solution has been previously created, its' name appears next to a radio button in the top right panel. Upon pressing this button, the order of the probes in the display changes and probe IDs are colored according to the clusters. The color index at the bottom right panel, maps each color to the index of the corresponding cluster.



Coloring scheme:

○ None

● CLICK 1.1

○ K-Means 1.1

🟥 Cluster 1

🟨 Cluster 2

🟩 Cluster 3

🟦 Cluster 4

🟦 Cluster 5

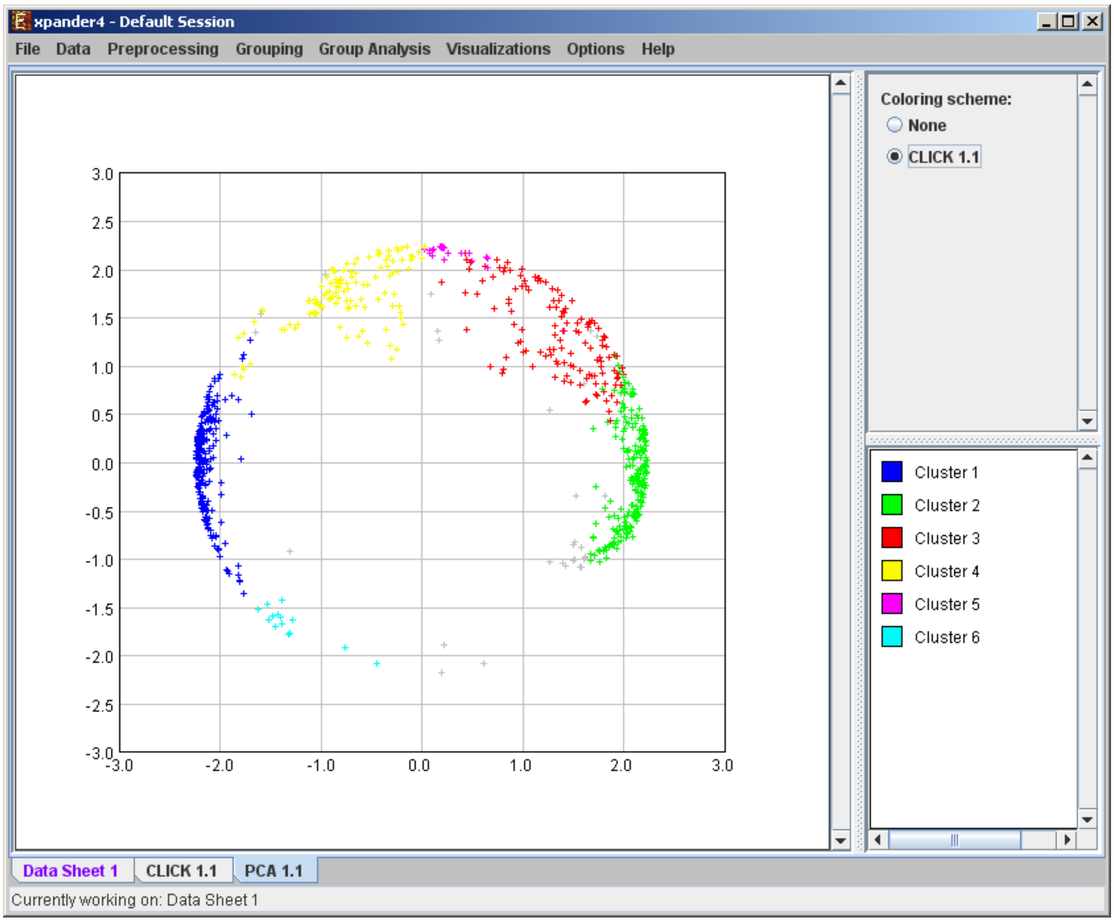
🟪 Cluster 6

Currently working on: Data Sheet 1

PCA Transformation

This tool transforms the original data from a k (original pattern length) to a 2 dimensional space, so that each expression vector is represented by a dot on an XY scatter chart. The transformation is based on the PCA (Principal Component Analysis) algorithm. To operate the tool, select *Visualization* >> *PCA*.

If a clustering solution has been previously created, its' name appears next to a radio button in the top right panel. Upon pressing this button, the color of each dot in the display changes according to the cluster assignment of the corresponding probe. The color index at the bottom right panel, maps each color to the index of the corresponding cluster.



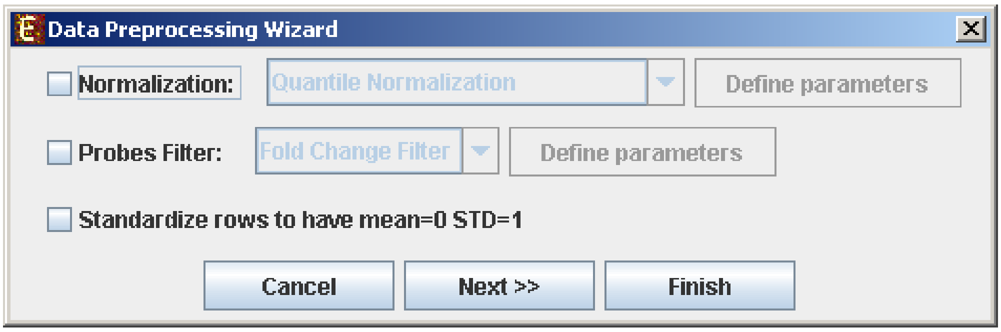
Coloring scheme:
○ None
● CLICK 1.1

🟦 Cluster 1
🟩 Cluster 2
🟥 Cluster 3
🟨 Cluster 4
🟪 Cluster 5
🟦 Cluster 6

Currently working on: Data Sheet 1

Analysis Wizard

Expander allows performing an automatic analysis on a loaded dataset by using the analysis wizard to predefine the analysis stages and parameters.
To use this tool, go to *Data>> Analysis Wizard*.
Upon selecting this option, the following dialog box will appear, allowing to define the required preprocessing operations:

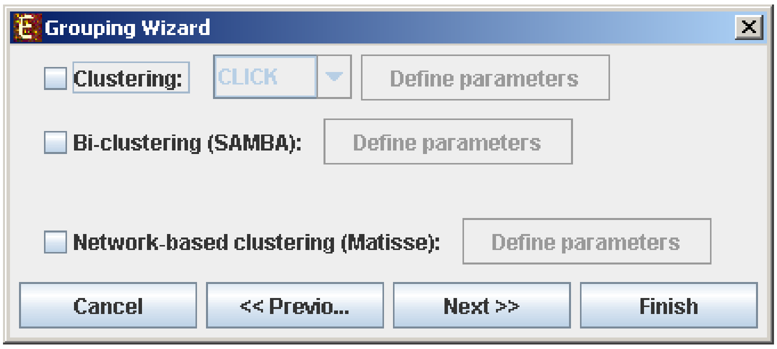


□ Normalization: Quantile Normalization

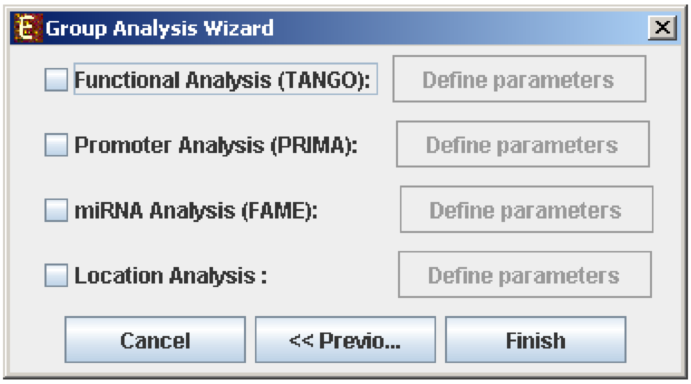
□ Probes Filter: Fold Change Filter

□ Standardize rows to have mean=0 STD=1

For some of the stages, parameters can be defined by pressing the corresponding “Define parameters” button. Upon pressing the “Next>>” button, the following dialog box will appear, allowing to define the required grouping operations:



Upon pressing the "Next>>" button, the following dialog box will appear, allowing to define the required enrichment analysis operations:

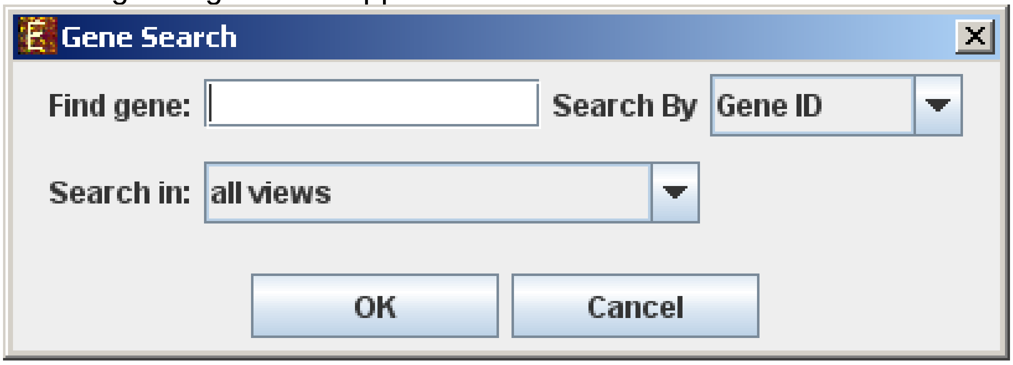


Upon pressing the "Finish" button (in any one of the dialog boxes) the entire set of operations defined by the user is performed by Expander, and the corresponding visualizations are generated.

Additional Options

<u>Searching for a gene/probe in the display</u>

A gene can be detected in a display by selecting *Options* >> *Search Gene.* The following dialog box will appear:



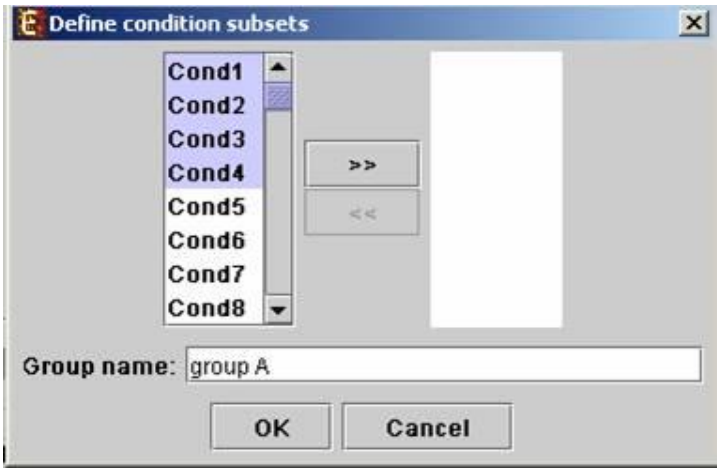
Search By Gene ID

Search in: all views

Please type the ID of the gene (can also be symbol/probe ID depending on the selection in the "Search By" combo-box) in the corresponding text box. Note that you must type the entire name or ID, not part of it. After pressing the “OK” button, a window will appear containing text describing the number of items detected in each of the searched views (number of “hits” in each view). In addition, the corresponding elements will be highlighted in all searched displays.

Defining condition subsets

You may group several conditions under a common subset name, by selecting Data >> Define Condition Subsets. This partition is used for visualization purposes. In the dialog box, select the relevant conditions, type a group name and click on the arrows.



Group name: group A

I addition to subset definitions, multiple condition annotations can be loaded using the option Data>>Load Condition Attributes. The file should be in a tabular (tab delimited), in which rows correspond to attributes (first column will contain attribute names) and columns correspond to conditions (first row will contain condition labels in the same order as in your expression data). Values can be numeric and/or textual.

Saving and loading sessions

A set of analysis operations performed on one data set can be saved by selecting *File >> Save Session*. It can later be reloaded by selecting *File >> Load Session*. Loading a previously saved session will bring up all analysis output and visualizations that had been generated in that session, and the user will be able to continue working where he had previously stopped.

Closing views

The user can close all open views by selecting *File >> Close All*.

Closing a single view can be performed either by selecting *File>>Close* when the relevant view is selected OR by right clicking on the tab title of the relevant view and selecting *Close* from the popup menu.

Docking a view into a separate frame

Can be performed either by selecting *Options >> Dock into external frame* when the relevant view is selected OR by right clicking on the tab title of the relevant view and selecting *Dock into external frame* from the popup menu.

Upon creating the separate frame, the view will be removed from the main window. Upon closing the separate frame generated in this manner, the view will be retrieved into the main window.

Accessing the EXPANDER download page

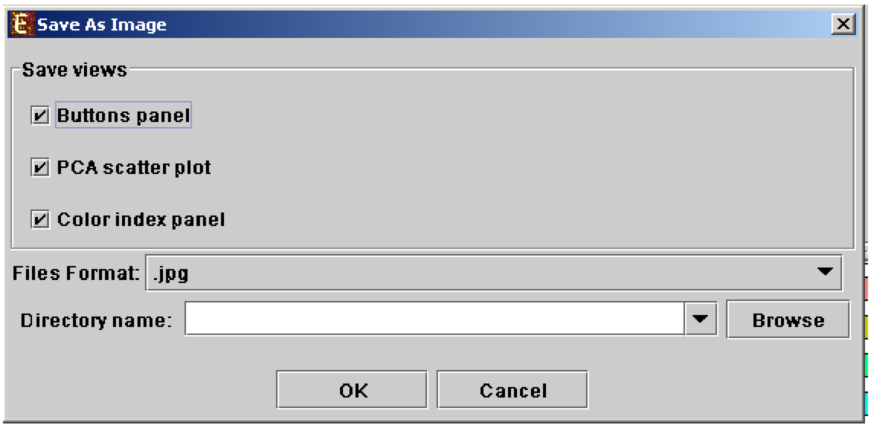
The Expander download page can be accessed directly by selecting *Help >> Open Download Page*, while the machine is connected to the Internet.

Printing the display

Each display can be printed by selecting *File >> Print* while its tab is selected.

Exporting display into image files

Each display can be exported into image files of type .jpg, .png or .eps (post-script). This can be done by selecting *File >> Save As Image*. Upon selecting this option, a dialog box, similar to the following is displayed. In the dialog box the saved images (sections of the view), image files format, and destination directory name are input.



Save views

☑ Buttons panel

☑ PCA scatter plot

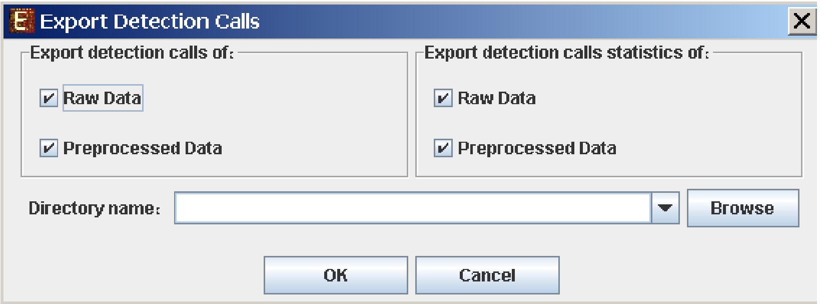
☑ Color index panel

Files Format: .jpg

Directory name:

Exporting detection calls information

The detection calls info of the raw and preprocessed data can be exported into text files, by selecting *Data >> Export Detection Calls*. Upon selecting this option, the following dialog box is displayed. You may export the detection calls and also the statistics of detection calls (percent of P, M and A calls per condition), for raw data and for preprocessed data.



Export detection calls of:

☑ Raw Data

☑ Preprocessed Data

Export detection calls statistics of:

☑ Raw Data

☑ Preprocessed Data

Directory name:

File Formats

Expression data file format:

1) Suffix: no limitations.
2) Separating token: tab delimiter.
3) Format:

1st line: contains a string like ‘probeId’ and a tab delimiter, followed by a string like ‘geneSymbol’ and a tab delimiter, followed by the names of all conditions separated by tab delimiters. The symbol column is optional – if the file does not contain a symbol column, please specify it in the Advanced Input Dialog box (see Input Data section).

2nd line (**optional**): contains the string ‘>SERIES’, a tab delimiter followed by the string ‘SYMBOL ‘ (if there is a symbol column), a tab delimiter and then all series names corresponding to the condition (one series assigned for each condition) separated by tab delimiters.

Next lines: Each subsequent line consists of the probe ID (an identifier string that is unique to each probe in the chip), followed by a string, which represents the gene full name (if missing can be left empty by adding an additional tab delimiter), followed by its expression values (all tab delimited). If the expression file contains missing values, Expander either replaces them with a preset value (0 by default), or estimates them using the KNN (K-Nearest Neighbors) method, depending on the user selection in the data load dialog box.

*For example see files ‘expressionData1.txt’ and ‘expressionData2.txt’ in the Expander/sample_input_files/ directory.

If the data is not in the above format, it may be possible to load it using the ‘Advanced’ dialog box, which appears upon pressing the ‘Advanced’ button in the Expression Data load dialog box (see Advanced Input Dialog box in Input Data section).

Expression data with detection calls file format:

1) Suffix: no limitations.
2) Separating token: tab delimiter.
3) Format:

1st line: contains a string like ‘probeId’ and a tab delimiter, followed by a string like ‘geneSymbol’ and a tab delimiter, followed by the names of all conditions and detection signals columns alternately, separated by tab delimiters. Each title of condition is followed by a title of its detection column.

The symbol column is optional – if the file does not contain a symbol column, please specify it in the Advanced Input Dialog box (see Input Data section).

Next lines: Each subsequent line consists of the probe ID (an identifier string that is unique to each probe in the chip), followed by a string, which represents the gene full name (if missing can be left empty by adding an additional tab delimiter), followed by its expression values and detection calls values, alternately (all tab delimited). Each expression value is followed by its detection value (P, M or A). If the expression file contains missing values, Expander either replaces them with a preset value (0 by default), or estimates them using the KNN (K-Nearest Neighbors) method, depending on the user selection in the data load dialog box.

*For example see files ‘expressionWithDetection.txt’ in the Expander/sample_input_files/ directory.

If the data is not in the above format, it may be possible to load it using the ‘Advanced’ dialog box, which appears upon pressing the ‘Advanced’ button in the Expression Data load dialog box (see Advanced Input Dialog box in Input Data section).

Gene Sets file format:

1) Suffix: no limitations

2) Format: Each line contains a gene ID, a gene symbol (optional) and the name/number of its set (separated by tabs/spaces). The gene IDs are expected to be of the same convention used

in the GO annotation and TF fingerprint files. For details regarding the Gene ID convention that is used for each organism, refer to the Supplied files section.

*For example see file ‘geneSetsData1.txt’ under the Expander/sample_input_files/ directory (see Sample input files for more details).

Probes Filter file format:

Each line contains a single identifier. Identifiers can be probe Ids, gene Ids OR gene symbols (but not a mixture of these identifier types).

ID conversion file format:

1) Suffix: Currently, there are no limitations regarding the file name suffix.

2) Format: Each line contains the probe id as it appears in the data file, a tab separator and the corresponding gene ID (e.g. Entrez/Locus-Link ids for mouse and human genes and ORF codes for yeast). The second field can be left blank, indicating no conversion for that probe ID.

* It is possible that several probe IDs in the data file will be mapped to the same gene ID (e.g.: several ESTs from the same gene).

Clustering files format:

1) Suffix: no limitations.

2) Format: Each line contains the probeID, a tab separator and name/number of its cluster. The number 0 is reserved for probes that are left unclustered. The file does not have to contain all probes in the data. If a probe does not appear in the file, it is automatically set as unclustered.

*For example see file ‘expressionData1Clustering.sol’ (a clustering solution for the data file’ expressionData1.txt’) under the Expander/sample_input_files/ directory (see Sample Input Files section for more details).

Biclustering files format:

1) Suffix: `.bic`.
2) Format: the file is composed of two parts, presented here.

Part 1 presents a summary of the biclusters found.

- It begins with the string: `[Bick]` in the first line.
- Following lines contain the bicluster's id followed by its' score, separated by a tab delimiter (a line for each bicluster).

Part 2 presents the probesets and the conditions contained in each bicluster.

- It begins with the string: `[Bicd]` in the first line.
- Following lines contain the bicluster id, type of element ('0' for condition, '1' for probe) and element id (name of condition or probe ID), separated by tab delimiters.

Background set files format:

1) Suffix: no limitation.

2) Format: each line should contain one gene ID. The gene IDs are expected to be of the same convention used in the annotation and TF fingerprint files for the organism you are working on (please refer to the Supplied Files section).

Gene annotations/categories files format (for the general enrichment analysis):

1) Suffix: no limitation.

2) Format: each line should contain one gene ID and an annotation/category name separated by a tab delimiter. The gene Ids are expected to be of the same convention used in the annotation and TF fingerprint files for the organism you are working on (please refer to the Supplied Files section).

Sample Input Files

Several sample files are provided under Expander/sample_input_files/. These files include:

expressionData1.txt – A gene expression data file that was generated using the cDNA microarray technology. This is a partial dataset extracted from a yeast cell cycle dataset generated by Spellman et al 1998 (see the References section). Gene identifiers in this set are yeast ORFs, which are the same identifiers used in the annotation and TF fingerprint files that are supplied with Expander. Thus, no conversion file is required.

ExpressionData2.txt – A gene expression data file that was generated in the Affymetrix technology. This dataset was generated in an experiment that was conducted in out laboratory on human cells, and has not yet been published. Affymetrix chips of type HG-Focus were used for this experiment and thus, the HG-Focus conversion file is required for the analysis (can be downloaded from the download page).

ExpressionData3.txt – taken from Murray JI, Whitfield ML, Trinklein ND, Myers RM, Brown PO, Botstein D: Diverse and specific gene expression responses to stresses in cultured human cells. Mol Biol Cell 2004, 15:2361-2374. A corresponding conversion file (from clone-Ids to LL-Ids) is available at the same directory under the name Data3Conversion.txt.

expressionWithDetection.txt – A gene expression data file with detection calls, that was generated in the Affymetrix technology. This dataset was generated in an experiment that was conducted in our laboratory on human cells. Affymetrix GeneChip HGU133 Plus 2.0 arrays were used for this experiment.

expressionData1Clustering.sol – A clustering solution that was generated by Expander for the dataset in ‘expressionData1.txt’.

geneSetsData1.txt – Contains sets of human genes (in Entrez/Locus-Link Ids).

Data3Conversion.txt - A conversion file for expressionData3.txt.

Supplied Files

The following files include gene info files: Gene ID conversion files, GO annotation files, TF fingerprint files, promoter sequences, miRNA target scan files, chromosomal position files, and biological pathway files, taken from the KEGG database*. These files should be extracted into "Expander/organisms" directory.

Organism	**Size after extraction**	**Origin of GO annotations**	**Origin of sequences used for generating TF-fingerprint files**
Human	431MB	NCBI - Oct 2009,GOA@EBI - Nov 2008	Ensembl release 53
Baker's yeast	30.6MB	NCBI - October 2009	SGD database 1.01
S. pombe	15.7MB	NCBI - November 2008	Sanger GeneDB - October 2008
Listeria monocytosenes EGD-e	1.74MB	Blast2GO - February 2009	Not available
Mouse	338MB	NCBI - Oct 2009	Ensembl release 53

Rat	280MB	NCBI - Oct 2009	Ensembl release 56	
Fly	218MB	NCBI - Oct 2009	Ensembl release 53	
C-elegans	278Mb	NCBI - Oct 2009	Ensembl release 56	
Arabidopsis	267MB	NCBI - Oct 2009	TAIR - December 2006	
Zebra Fish	254Mb	NCBI - Oct 2009	Ensembl release 53	

Chicken	205Mb	NCBI - Oct 2009	Ensembl release 56
Tomato	1.7Mb	EBI - March 2009	-
A.Fumigatus	72.5Mb	EBI - Jan 2010	Kevin Verstrepen's lab (via private communication with Nir Osherov)
E. coli	1.25MB	EBI - April 2008	Not available yet
Rice	170MB	GO DB Dec 2010	Bioinformatics Core database Washington State University
Leishmania	2MB	Zilberstein D. lab Technion - Israel Nov 2011	-
V.vinifera (grape)	11MB	GO DB July 2012	-

* Users of this product may not download large quantities of KEGG Data.

Gene ID conversion files:

Gene ID conversion files for many of the Affymetrix chips can be downloaded from the Expander download page. The files map each Affymetrix Id into the corresponding gene Id. Conversion files are generated and added to the download page according to user requests. If you can't find the file you need here, please look it up in the download page, and contact us if it's not there.

Organism	**Chip name**
Human	HG-Focus
Human	HGU1332
Human	HG-U95E
Human	HG-U133A
Human	HT_HG-U133A
Human	HG-U133Plus2
Human	Hu-35KsubB
Human	HuGene-1_0-ST
Mouse	MGU74Av2
Mouse	MGU430_2
Mouse	MG430A2
Mouse	MoGene-1_0-ST
Rat	RGU34A
Rat	Rat230_2
Rat	Agilent
C-elegans	C. elegans Genome Chip
Arabidopsis	ATH1
Zebra-Fish	GeneChip Zebrafish Genome Array
Chicken	Affymetrix Chicken Genome Chip
E. coli	Affymetrix E. coli Antisense Genome Array
E. coli	Affymetrix E. coli Genome 2.0 Array

Network files :

Organism	**File name**	**Network origin**

Human	Expander.hsa.RualNature05.sif	Towards a proteome-scale map of the human protein-protein interaction network by Rual JF et al. *Nature*. 437(7062):1173-8 (2005)
Human	Expander.hsa.IntAct.sif	IntAct database (http://www.ebi.ac.uk/intact/)
Mouse	Expander.mmu.IntAct.sif	IntAct database (http://www.ebi.ac.uk/intact/)
Rat	Expander.rno.IntAct.sif	IntAct database (http://www.ebi.ac.uk/intact/)
Worm	Expander.cel.SimonisNatMethods08.sif	Empirically controlled mapping of the *Caenorhabditis elegans* protein-protein interactome network by Simonis N. et al. *Nature Methods* 6, 47 - 54 (2009)
Fly	Expander.dme.DroID.sif	DroID database (http://www.droidb.org/)
Yeast	Expander.sce.United.sif	1. High-Quality Binary Protein Interaction Map of the Yeast Interactome Network by Yu et al. *Science* 322(5898):104 – 110 (2008) 2. Comprehensive curation and analysis of global interaction networks in Saccharomyces cerevisiae by Reguly et al. *Journal of Biology* 5(4):11 (2006) 3. Toward a comprehensive atlas of the physical interactome of Saccharomyces cerevisiae by Collins SR et al. *Molecular Cell Proteomics* 6(3):439-50 (2007)
Arabidopsis	Expander.ath.TAIR.sif	TAIR database (http://www.arabidopsis.org/)
E. coli	Expander.eco.Arifuzzaman06.txt	Large-scale identification of protein–protein interaction of Escherichia coli K-12 by Arifuzzaman M et al. *Genome Research* 16(5):686-91. (2006)

Settings

The Settings are accessible from the *Options* menu, and contain *Display* settings *External applications* settings.

The **Display** dialog box contains the following tabs:

Clustering Results View – Contains check boxes that configure the following parameters:

- A common Y-axis scale for all cluster patterns (vs. cluster specific)
- Visible x axis
- Connect all points in a pattern
- Display similarity matrix for probes (using Pearson correlation)
- Display similarity matrix for conditions (using Pearson correlation)

Enrichment Analysis Results View – Contains a check box that configures whether the Y-axis scale of all histograms is common OR cluster specific.

Data Matrix View – Allows selection between:

- Range control and extreme values control when rendering expression matrix values.
- The red/green coloring scheme can be changed to blue/yellow.

The ***External applications*** dialog box allows specification of the location of the R executable (required for CEL files loading). In Windows, R.exe file is likely to be located in the 'bin' folder of R software. In Linux, you may type 'which R' in the command line to find R path.

R External Application

The CEL file preprocessing and the newly added SAM filter utilities require the pre-installation of one of the recent versions of R, a free software environment for statistical computing and graphics. R can be installed from: http://cran.r-project.org/.

Upon the first time that Expander uses R external application, a window will pop, asking you to specify your R software location. Please browse to the location of your R software. In Windows, R.exe file is likely to be located in the 'bin' folder of R software. In Linux, you may type 'which R' in the command line to find R path. If you have a few versions of R installed, please make sure to point Expander to a version in which the necessary packages have been installed.

You may also specify R location from the menu: *Options >> Settings >> External applications*.

To use R utilities, please make sure there are no white spaces in the path of Expander directory (or the CEL files directory, if loading CEL files). For example, if the name of the Expander folder is 'Expander 5', change it to 'Expander_5'. If Expander is under "Program Files" it should be moved to another location, because of the space between "Program" and "Files". The R software does not cope well with spaces in the path.
Also, please make sure to have 'write' permission to the Expander\Rscripts directory. If you are loading CEL files, check also that you have 'write' permission to the *Files location* which you specified in the 'Load CEL Files' dialog box.

After specifying R software location, a window will pop, asking you to approve or disapprove automatically installation of R packages when needed.

If you approve automatically installation of R packages then when R utility is used in Expander, Expander will automatically install the needed R packages for the used R utility.

If you disapprove automatically installation of R packages then please refer to "Manually installation of R packages" section.

Manually installation of R packages

After installing R, please do the following to install the Bioconductor “affy” package , "gcrma" package and the "samr" package:

1. Run R.
2. In the R frame\window type the text: **source("http://bioconductor.org/biocLite.R")**
3. Press ‘Enter’.
4. In the R frame\window type the text: **biocLite("affy")**
5. Press ‘Enter’.

To install the 'samr' package:

6. In the R frame\window type the text: **install.packages("samr")**
7. R frame\window type the text: **install.packages("impute")**
8. Press ‘Enter’.

To install the ‘eisa’ package:

9. In the R frame\window type the text: **biocLite("eisa")**
10. Press ‘Enter’.

To install the 'gcrma' package:

11. In the R frame\window type the text: **source("http://bioconductor.org/biocLite.R")**
12. Press ‘Enter’. In the R frame\window type the text:
13. **biocLite("gcrma")**
14. Press ‘Enter’.

To install the 'limma' package:

15. In the R frame\window type the text: **source("http://bioconductor.org/biocLite.R")**
16. Press ‘Enter’. In the R frame\window type the text:
17. **biocLite("limma")**
18. Press ‘Enter’.

To install the 'limma' package:

19. In the R frame\window type the text: **source("http://bioconductor.org/biocLite.R")**
20. Press ‘Enter’. In the R frame\window type the text:
21. **biocLite("edgeR")**
22. Press ‘Enter’.

You may install only one of the packages, depending on what you wish to use (to install only "samr", follow instructions number 1, 6 and 7).

FAQ

Linux/Unix problems

Click clustering, Samba bi-clustering, Tango and Prima Promoter analysis algorithms fail when running on Linux/Unix.

CEL Files Loading Problems

How do I install R and the Bioconductor "affy" package?

Loading of CEL files or performing SAM filter continue for ever

Loading of CEL files fail.

Clustering

When I try to run Biclustering on my data I get a failure notice.

How can I save the clustering expression patterns charts?

Grouping Analysis (functional and promoter analysis)

When I run Functional Analysis, Expander gets stuck.

When I load a session with that contains Functional Analysis results, Expander gets stuck,

When I try to run Promoter Analysis no values appear in the Fingerprints file field of the input dialog box.

When I try to run the promoter\functional analysis, I get a failure message box.

Promoter\Functional analysis produces no results (the resulting view is empty).

How can I save the bar charts produced by Expander, displaying the enrichments?

Why do certain Transcription Factors have a few accession numbers or\and a few gene IDs?

Saving sessions

When I try to save a session Expander fails and returns an XStream error message.

Others

Can I run Expander on Mac OS?

Click clustering, Samba bi-clustering, Tango and Prima Promoter analysis algorithms fail when running on Linux/Unix.

Answer: Make sure that you have write permission in the Expander directory, and execution permissions on the files: click.exe, samba.exe, annot_sets.exe and analyzeFingerprints.exe, which are under the Expander directory. If the problem still occurs, open the file expanderLog.txt and search for the text: “libstdc++.so.5”. If this text appears (along with a message indicating it has not been found), please contact your system administrator and report this problem (this is a system problem). If you do not have a system administrator, and fail to install this library, please contact us (expander@cs.tau.ac.il) and we will try to assist.

How do I install R and the Bioconductor "affy" package?

Answer: please refer to R External Application section.

Loading of CEL files or performing SAM filter continue forever.

Answer: If the operation continues forever (the 'processing, please wait' window is displayed), please check if there is a folder with a space in its name somewhere in the path of Expander (or the CEL files) directory. For example, if the name of the Expander folder is 'Expander 4', change it to 'Expander_4'. The R software used for preprocessing CEL files has a problem dealing with spaces in the path. If this is the problem, then in the expanderLog.txt file (in your Expander directory) there should be a message about arguments being ignored.

Loading of CEL files fail.

Answer: Make sure you have R along with the Bioconductor “affy” package installed in the R version which is specified in the settings “External Applications” tab (from the menu select *Options* >> *Settings* >> *External applications*). If R location is not defined in the settings, please define it (In Windows, R.exe file is likely to be located in the 'bin' folder of R software. In Linux, you may type 'which R' in the command line to find R path). If you are using an R package as cdf source, please make sure that the package is a folder located under your R library directory and that it is the correct package for your chip. If loading of CEL files still fails, please make sure that the *Files location* which you specified in the 'Load CEL Files' dialog box, is a folder which contains CEL files and that you have write permission to that folder.

When I try to run Biclustering on my data I get a failure notice.

Answer: Make sure that the ‘Use option files of type’ field in the SAMBA input dialog box is not empty (if it is, please re-download Expander). Also make sure that the following files exist in your Expander directory: ibic.opt, samba.exe.

How can I save the clustering expression patterns charts?

When the clustering results tab is open, please go to *File* >> *Save As Image*.

When I run Functional Analysis, Expander gets stuck.

Answer: If you are working with Expander version 4.0 or 4.0.1, please update to a higher version (4.0.2 and on).

When I load a session with that contains Functional Analysis results, Expander gets stuck.

Answer: If you are working with Expander version 4.0 or 4.0.1 (and the session was created with a version < 4.0), please update to a higher version (4.0.2 and on).

When I try to run Promoter Analysis no values appear in the Fingerprints file field of the input dialog box.

Answer: Fingerprint files are not placed in the right directory. Fingerprint files should be placed under the ‘TF_fingerprints’ directory that is under the Expander/organism/<org name> directory. For example, the human FP file should be placed under: …/Expander/organisms/human/TF_fingerprints/. When downloading the organism specific data zip, it should be extracted into the Expander/organisms/ directory. This will automatically put them in the right place.

Enrichment analysis leads to a failure message box.

Answer: Errors while running group (enrichment) analysis can be caused by the following problems:

a) Organism specific data (Fingerprint\annotation files) is not in the right directory. The organism specific data zip should be extracted into the Expander/organisms/ directory. You may download the relevant data by selecting from the menu: *Help >> Download Data for Organism*.

b) Data contains elements that do not appear in the background set (this is only relevant when the background set is loaded from an external file).

Enrichment analysis produces no results (the resulting view is empty).

Answer*:* This can be caused by one of the following:

a) You are using the wrong conversion file or a conversion file that is not in the right format or does not map the probes to the expected type of gene Ids. The conversion file maps each probe ID in your data file to a gene ID that is used for enrichment analysis. A conversion file is required when the probe Ids in your data file do not match the ones in the enrichment files (for example annotation and TF_fingerprint files that we supply).

b) You did not set the organism field in the input dialog to the organism type of your data.

c) You are trying to analyze only one set (e.g. the filtered data set) which you are using also as background (in this case the analysis has no meaning since it is trying to detect enrichments in the cluster/bicluster in comparison to the background set).

d) You set the threshold p-value to be too strict (low).

e) Biological reason i.e., there is nothing to report regarding this specific clustering/biclustering solution or this gene sets data.

How can I save the bar charts produced by Expander, displaying the enrichments?

When the results tab is open, please go to *File >> Save As Image*.

Why do certain Transcription Factors have a few accession numbers or\and a few gene IDs?

Answer: The transcription factors (TFs) found enriched by Prima are presented in the following way:

Accession Num. in TRANSFAC DB [TF name]. For example, M00287[NF-Y]

It is possible that a TF will have a few accession numbers in TRANSFAC, which represent different PWMs (position weight matrices specify the probability for observing each nucleotide at each position of the binding site, based on a set of empirically validated binding sites of the respective TF).

It is also possible that a TF will have a few Entrez gene IDs, since a TF may be composed of a few proteins. For example, NF-Y is a trimer, composed of 3 subunits.

Can I run Expander on Mac OS?

Expander is not designed for Mac OS. You can probably use it partially – without running its features that require the execution of exe files (CLICK, SAMBA, TANGO and PRIMA). The exe files are only suitable for Windows and Linux / Unix.

When I try to save a session, Expander fails and returns an XStream error message

If you are using java version 1.7, please switch to version 6. We currently have no solution for this problem, that occurs with java1.7 and XStream, which is an external package that we are using. We will do our best to resolve it in the coming future. In order to configure the Expander.bat files to use a java 6 version do the following:

1) Make sure that jave 6 (or 5) is installed on your PC by exploring the “Program Files (x86)/Java” (or “Program Files/Java”) directory. In it there should be a subdirectory by the name jre6 or jre5 (otherwise please install java6 from http://java.sun.com/javase/downloads/index.jsp.)
2) In the Expander directory right click on one of the Expander.bat files (the one you are using) and select “Edit”.
3) In the file type the path of the java6 exe file instead of the word java. E.g. if your path is C:\Program Files (x86)\Java\jre6 then put the text: “C:\Program Files (x86)\Java\jre6\bin\java.exe” (including the quotes (“”)) instead of the word java.
4) Remove the text “–client” from the file
5) Save and close the file

This section will be updated as we get user feedbacks and problems.

Please refer all questions/comments to Expander@cs.tau.ac.il.

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