National Institute of Environmental Health Sciences
Your Environment. Your Health.

Pathway analysis

Biostat & Bioinfo short course series

02/08/2019

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An outline of the pathway analysis course

- Background of pathway analysis
- Random variable distribution and its usage in the pathway analysis
- A parametric approach
  - Hypergeometric distribution in details
  - An example: IPA
- Non-parametric approach
  - Experimental layout
  - An example: GSEA
- Pathway analysis summary
  - Making a right choice
  - Pros and cons
- Hands on practice
  - R and basic distribution
  - GSEA

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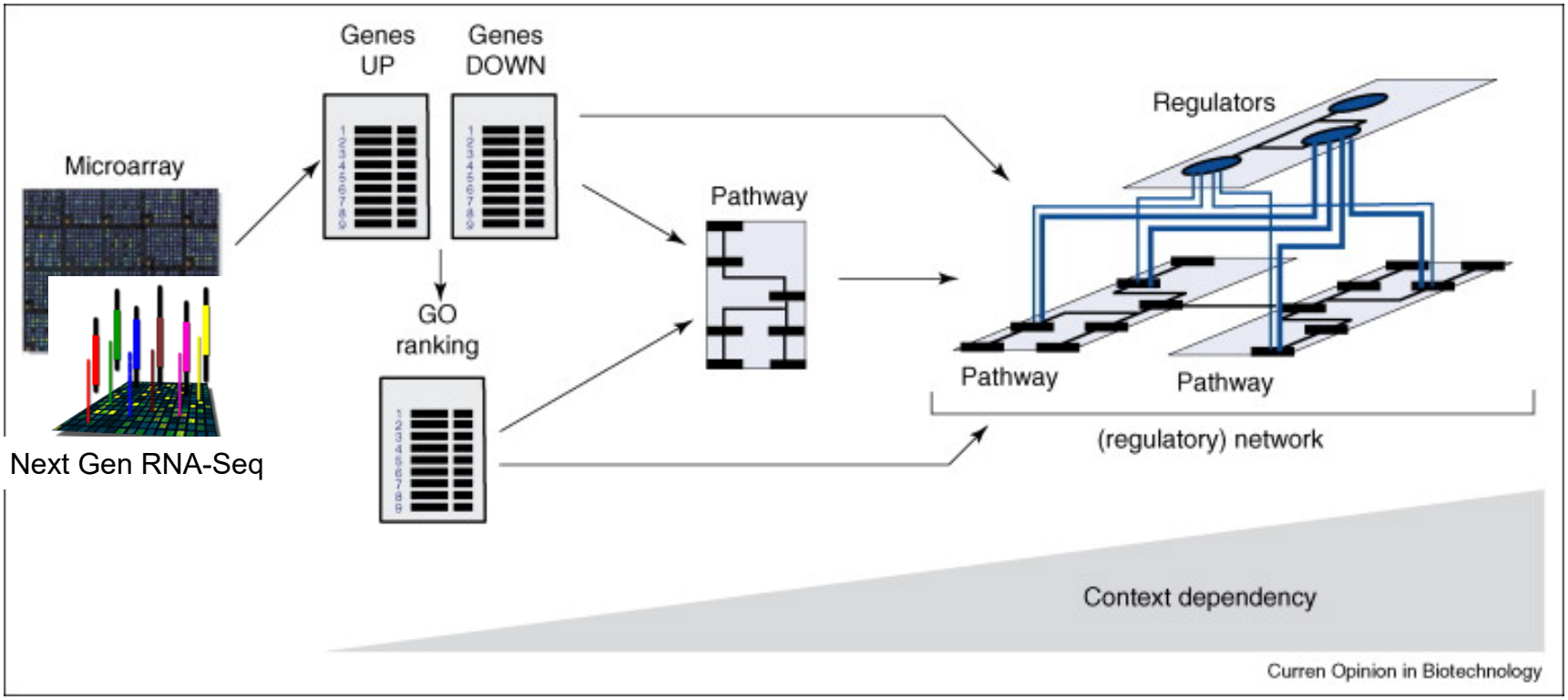
The Road to Pathway Analysis



Adapted from Werner (2008), Current Opinion in Biotech., 19:50-64

Why pathway analysis?

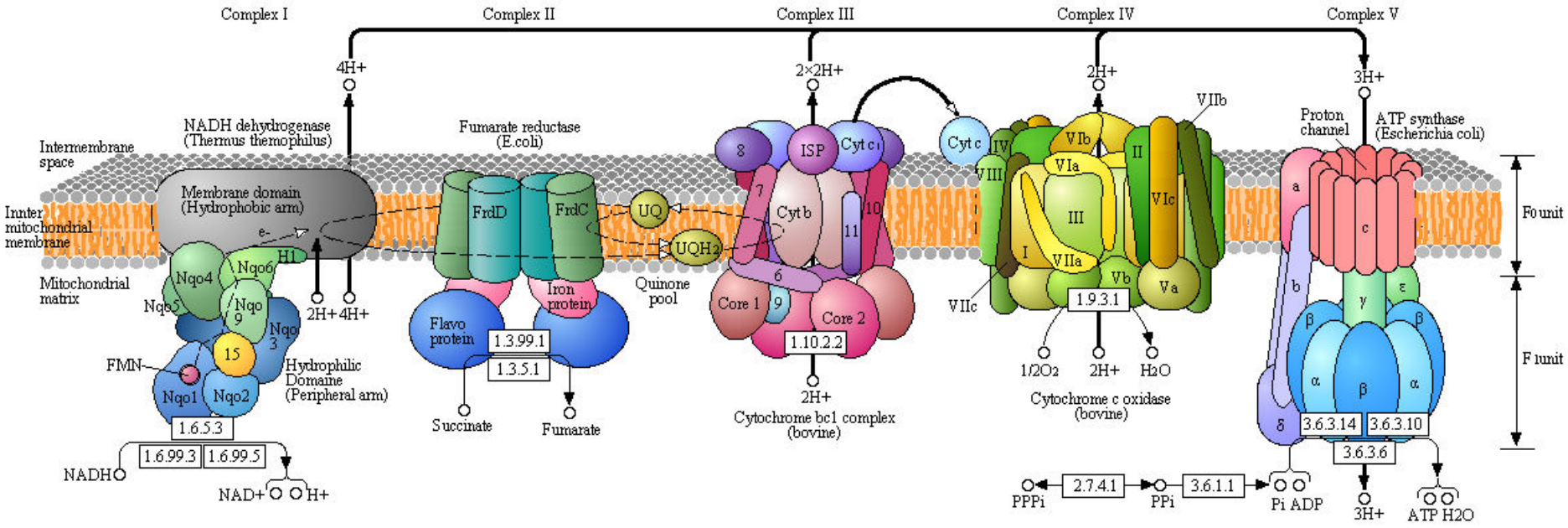
- Biological responses are systematic and collaborative
- Modern technologies provide high-throughput measurement
- Rich knowledge-based accumulation available
- Statistical frame works provide the analytical component

Common experimental rationale

- An established measurement of gene expression profile:
  - Microarray
  - RNAseq
  - Nano-string
  - Etc.
- Samples obtained from two conditions (e.g. treated vs. control)
- Several biological replicates at each condition
- Our focus is “how would a study object respond differently at two conditions?)
- Gene-centered analysis often
  - Asses statistical difference between conditions via some testing, t-test, limma etc.
  - Correct for multiple testing issues
  - Obtain a set of differentially expressed genes (DEGs)
- Where is the biology??

The oxidative phosphorylation

- A set of molecules in a cell that work together through a series of actions to achieve a particular outcome



http://employees.csbsju.edu/hjakubowski/classes/ch331/oxphos/olcouplingoxphos.html

Rich knowledge-based accumulation available

- GeneOntology (originated since 1998)
  - Molecular function describing activities, such as catalytic or binding activities, at the molecular level
  - Biological process referring to a biological objective to which the gene product contributes
  - Cellular component referring to the place in the cell (i.e. the location) where a gene product is found
- KEGG pathway (originated since 1996)
  - Metabolism: carbohydrates, energy, lipid, nucleotides, amino acid, xenobiotics
  - Human diseases
  - Genetic information processing
- Transfac/Transpath
  - Data on transcription factors, their experimentally-proven binding sites, and regulated genes
  - Protein-protein interactions and directed modification of proteins involved in signal transduction pathways,
- It is a knowledge based/driven approach
- Pathway --- gene sets are interexchange

Gene sets – generalized definition

Gene sets are sets of genes that have something in common, e. g., that they are

- part of the same pathway
- coding for proteins that are part of the same cellular component
- co-expressed under certain conditions
- putative targets of the same regulatory factor
- on the same cytogenetic band
- have come up as hits in some published assay
- Etc.

Molecular Signatures Database (MSigDB v5.2)

- Free database (http://www.broadinstitute.org/gsea/msigdb)

H **hallmark gene sets** are coherently expressed signatures derived by aggregating many MSigDB gene sets to represent well-defined biological states or processes.

C1 **positional gene sets** for each human chromosome and cytogenetic band.

C2 **curated gene sets** from online pathway databases, publications in PubMed, and knowledge of domain experts.

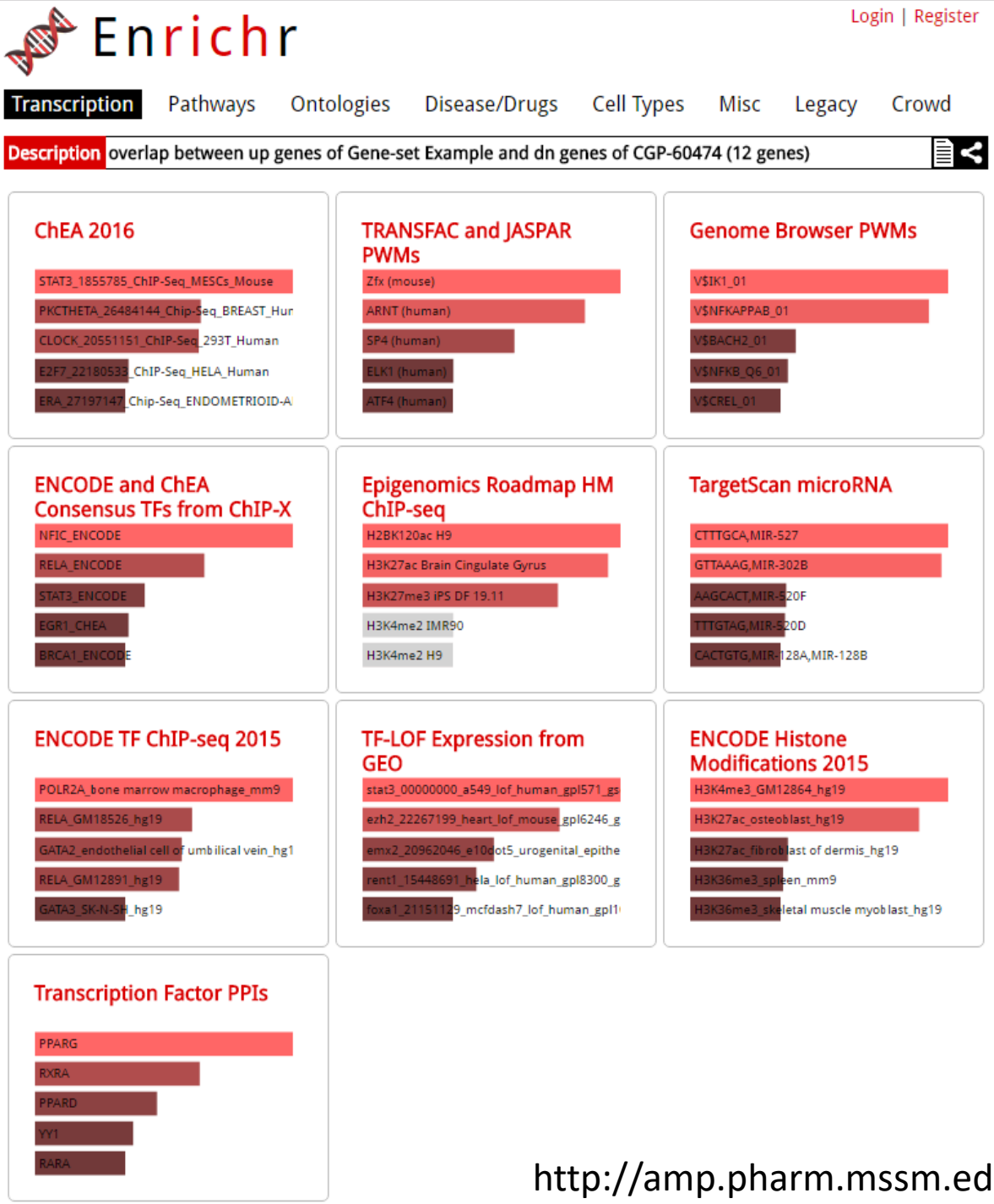
C3 **motif gene sets** based on conserved cis-regulatory motifs from a comparative analysis of the human, mouse, rat, and dog genomes.

C4 **computational gene sets** defined by mining large collections of cancer-oriented microarray data.

C5 **GO gene sets** consist of genes annotated by the same GO terms.

C6 **oncogenic signatures** defined directly from microarray gene expression data from cancer gene perturbations.

C7 **immunologic signatures** defined directly from microarray gene expression data from immunologic studies.



http://amp.pharm.mssm.edu/Enrichr/enrich

The statistical framework

- Well-established statistical and computation algorithms
- Parametric method
  - Hypergeometric test
  - Fisher's exact test
- Non-parametric approaches
  - GSEA
  - GSA
  - Etc.
- Other algorithms
  - **Auto expand :** Draws sub-networks around the selected objects
  - **Shortest paths:** Uses Dijkstra's shortest paths algorithm to find the shortest directed paths between the selected objects.
  - **Self regulation :**Finds the shortest directed paths containing transcription factors between the selected objects
  - Etc.

A good place to start

- A simple example:
  - 5 patients with the disease *D* and 5 healthy control subjects
  - Checked for elevated levels of the blood constituent *C*.
  - 4 of the patients, but only 2 of the healthy subjects show an elevated level of *C.*
- May we infer that the concentration of *C* is elevated inpatients with disease *D* more often than in healthy subjects?
- Or could our result have been mere coincidence?

2x2 contingency table

	Patient with disease D	Healthy control subject	Total
Elevated level of compound C	4	2	6
Normal level of compound C	1	3	4
Total	5	5	10

$$E_{r,c} = \frac{(\text{Sum of row } r) \times (\text{Sum of column } c)}{\text{Sample size}}$$

	Patient with disease D	Healthy control subject	Total
Elevated level of compound C	4 (3)	2 (3)	6
Normal level of compound C	1 (2)	3 (2)	4
Total	5	5	10

Hypergeometric distribution

Probability to get this 2×2 table without an association between *D* and *C*:

$$\frac{\begin{array}{c}Number\ of\ ways\ to\\ choose\ 4\ out\ of\ 5\\ patiens\ to\\ have\ elevated\ C\end{array} \times \begin{array}{c}Number\ of\ ways\ to\\ choose\ 2\ out\ of\ 5\\ controls\ to\\ have\ elevated\ C\end{array}}{\begin{array}{c}nuber\ of\ ways\ to\\ choose\ 6\ our\ of\ 10\\ persons\ to\ have\\ elevated\ C\end{array}} = \frac{\binom{5}{4}\binom{5}{2}}{\binom{10}{6}}$$

in R:

```
> dhyper( 4, 5, 5, 6 )
[1] 0.2380952
```

Hypergeometric distribution

Under the null hypothesis, i.e., the assumption that there is no association between elevated levels of compound *C* and presence of disease *D, t*he probability that all 5 patients have elevated levels of C would be,

	Patient with disease D	Healthy control subject	Total
Elevated level of compound C	5	1	6
Normal level of compound C	0	4	4
Total	5	5	10

in R:

```
> dhyper( 5, 5, 5, 6 )
[1] 0.02380952
```

Hypergeometric distribution

Under the null hypothesis, i.e., the assumption that there is no association between elevated levels of compound *C* and presence of disease *D, t*he probability that 4 or even more of the patients have elevated levels of C,

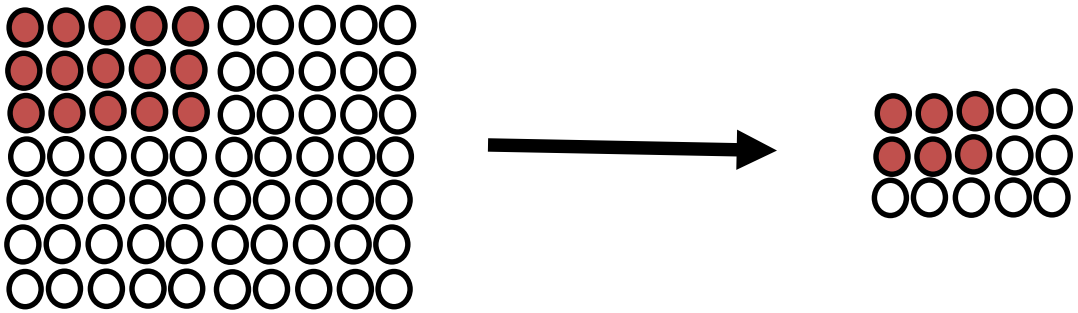
$$p = \frac{\binom{5}{4}\binom{5}{2}}{\binom{10}{6}} + \frac{\binom{5}{5}\binom{5}{2}}{\binom{10}{6}} = 0.26$$

This is insignificant

in R:

```
> 1 - phyper(3, 5, 5, 6 )
[1] 0.2619048
```

Hypergeometric Test (Right-tailed)



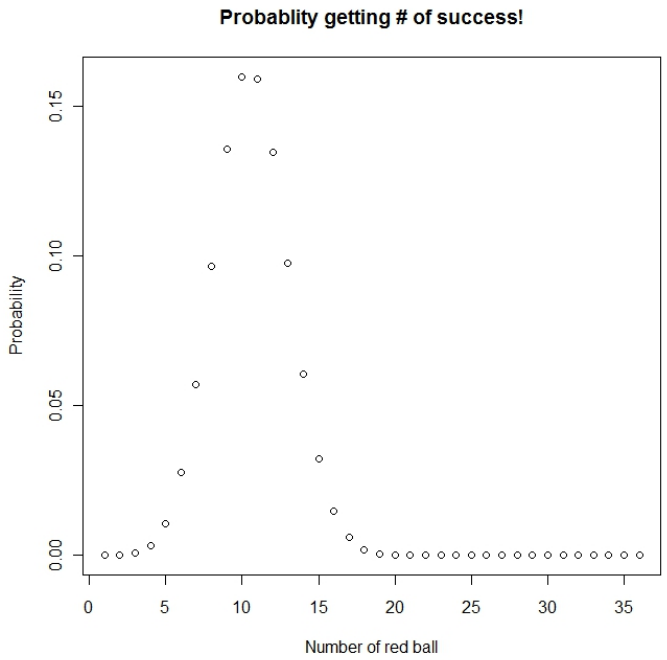
- An urn with two types of marbles:
  - **N total # of marbles**
  - **Of which, m # of red marbles**
  - **Drawing a red marble is a success!**
  - **Drawing a white marble is a failure!**
- n is the # of marbles randomly drawn
- k is the # of successes (red marbles) in the sample
- Hypergeometric distribution gives the probability

$$P(X = k) = \frac{\binom{m}{k}\binom{N-m}{n-k}}{\binom{N}{n}}.$$

for $k = 0,1,2,\ldots,n$
$k<=m,\ n-k<=N-m$

Hypergeometric distribution

- Number of red ball: 50 ← m
- Number of white ball: 120 } N
- Number of ball drawn (without replacement): 36 ← n
- Possible number of success ??
  - (0,1, 2, ….36),
- Probability to get 20 red balls is: 0.0001494571 $p(k=20, 36, 50, 170)$



http://www.aiaccess.net/English/Glossaries/GlosMod/e_gm_hypergeometric.htm

Pathway analysis (behind the scene)

Contingency table

	DEG	Not DEGs	totals
In a GO category	**x**	m-x	**m**
Not in GO category	k-x	n-k+x	n
totals	k	m+n -k	m + n (genes on array)

So, now you are probably given something like the following:

N
```
x  <- 5     #num_of_DEG in GO
m <- 20    #num_of_gene on chip in GO
n  <- 500  #num_of_gene on chip NOT in GO
k  <- 40    #num_of_DEG
```

Hypergeometric test vs. FET, we shall get same result

- ***phyper((x-1), m, n, k, lower.tail=FALSE)***
- ***(fisher.test(matrix(c(x,(k-x), (m-x), (n-k+x)),2,2), alternative='greater'))$p.value***


Quick Start
INGENUITY PATHWAY ANALYSIS
Start Here
Learning IPA
Shortcuts
Explore
Datasets
Annotate and filter datasets and use them directly for hypothesis generation when exploring pathways and gene lists.
› Annotate Datasets
› Filter datasets
Compare
Identify the union, unique, and common molecules across lists, pathways, biomarkers, and analyses.
› Compare data
Pathways
Create pathways from your datasets, targets, biomarkers, diseases and biological functions. Communicate pathways and network results through visually enhanced representations.
› Build pathways
› Design pathways
Analyze
Core
Interpret your data in the context of biological processes, pathways, and networks.
› Analyze dataset
› Compare analyses
IPA-Tox
Assess toxicity and safety of test compounds in the context of toxicological processes, pathways, and networks.
› Analyze dataset
› Compare analyses
IPA-Biomarker
Filter your datasets and identify and prioritize potential biomarker candidates.
› Analyze dataset
› Compare analyses
IPA-Metabolomics
Explore genotype-phenotype relationships and environmental influences via metabolite data.
› Analyze dataset
› Compare analyses
Do Not Show at Startup | Tutorials and Help | Training Schedule | Customer Support | How to Cite IPA
CLOSE

Ingenuity Pathway Analysis (IPA)

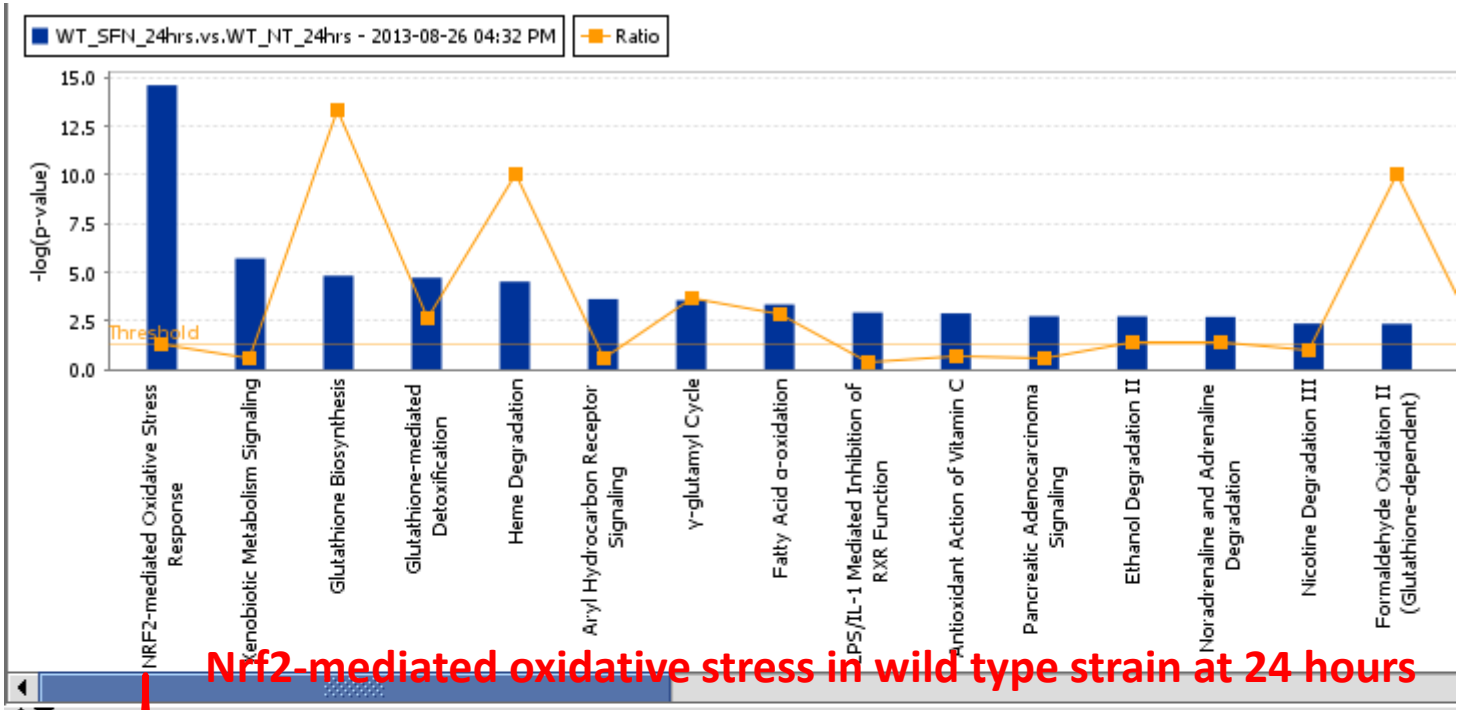
--Knowledgebase

- Desktop Java application utilizing a remote server for data, analysis and file management
- IPA Ontology: Curation of the scientific literature and content extraction of the IPA repository of molecular interactions, regulatory events, biological processes, gene-to-phenotype associations, and chemical knowledge

Ingenuity® Expert Findings	Experimentally demonstrated Findings that are manually curated for accuracy and contextual details from the full-text of articles in top journals.
Ingenuity® ExpertAssist Findings	Manually reviewed, automatically extracted Findings from the abstracts of a broad range of recently published journal articles.
Ingenuity® Expert Knowledge	Knowledge modeled by Ingenuity experts such as pathways, toxicity lists, and more.
Ingenuity® Supported Third Party Information	Manually reviewed content from selected sources and databases such as BIND, Argonaute 2, etc.

IPA Enrichment Analysis

- Uses the Fisher's exact test to determine the significance of a functional group or pathway
  - # molecules in a list that are associated with a function/pathway (**k**)
  - total # of molecules that are associated with a function/pathway (**m**)
  - # of molecules in all possible functions/pathways (**N**)
  - # of molecules in a list (**n**)



Nrf2-mediated oxidative stress in wild type strain at 24 hours

12 molecule(s) associated with **NRF2-mediated Oxidative Stress Response** at **WT_SFN_24hrs.vs.WT_NT_24hrs - 2013-08-26 04:32 PM**

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

Symbol	Entrez Gene Name	Identifier	Exp Val		
		Affymetrix	p-value	False Discovery Rate	Fold Change
ABCC1	ATP-binding cassette, sub-	1421378_s_at	8.68E-07	6.63E-05	↑2.054
ABCC4	ATP-binding cassette, sub-	1443870_at	3.60E-15	2.68E-12	↑2.962
AOX1	aldehyde oxidase 1	1419435_at	2.15E-11	6.91E-09	↑2.756
EPHX1	epoxide hydrolase 1,	1422438_at	7.19E-17	6.69E-14	↑2.347
GCLC*	glutamate-cysteine ligase,	1424296_at*	1.80E-17	2.24E-14	↑3.268
GCLM*	glutamate-cysteine ligase,	1418627_at*	1.24E-21	4.63E-18	↑3.343
GSR*	glutathione reductase	1421816_at*	8.32E-12	3.04E-09	↑2.604
GSTA5	glutathione S-transferase	1421040_a_at	1.58E-24	1.47E-20	↑17.533
GSTM5*	glutathione S-transferase mu	1416416_x_at*	4.93E-17	4.83E-14	↑2.879
HMOX1	heme oxygenase (decycling)	1448239_at	2.28E-08	3.05E-06	↑2.507
NQO1	NAD(P)H dehydrogenase,	1423627_at	1.49E-26	2.78E-22	↑6.419
TXNRD1	thioredoxin reductase 1	1421529_a_at	2.11E-19	3.57E-16	↑2.240

All the DEGs in the pathway are up-regulated

Hypergeometric Test (Right-tailed)

- Used in Ingenuity Pathway Analysis (IPA)
  - Commercial software (http://www.ingenuity.com/products/ipa)
  - Pros:
    - Great source of clean, expertly curated gene sets
  - Cons:
    - Not free
    - Throws away information by only using DEG list

Hypergeometric Test (Right-tailed)

- Used in Database for Annotation, Visualization and Integrated Discovery (DAVID)
  - Free software (https://david.ncifcrf.gov)
  - Pros:
    - Easy to use (web-based)
    - Large collection of gene sets
  - Cons:
    - Gene sets are not as clean
    - Throws away information by only using DEG list

Sampling over genes

- Hypergeometric testing for gene sets has been criticized on the ground of it sampling over genes (observation) instead of over microarrays (subjects)
- Hence, the meaning of the *p* values is quite unclear.
- Especially: Correlations between genes inflate the apparent sample size, causing potentially severe over-estimation of significance.
- Increasing the number of replicates influences significance only indirectly.

Goeman and Buhlmann, 2007

Sampling over subjects

- Instead of using the hypergeometric distribution to get a *p* value from out statistic, we should better use subject permutation:
  - Let L_0 be the list of differential expressed genes and $m=|L_0|$ its size.
  - For ***N* permutations** σ_i (i=1,...,N) of the *subject* labels, calculate the DE statistic and let *Li* be the list of the *m* top ranking genes.
  - Let k_i be the number of differentially expressed genes in the gene set, i.e. the size of the intersection $L_i \cap S$.
  - The *p* value for gene set *S* is now the fraction of permutation that had a larger gene set than the correct sample assignment, i.e.,

$$p = \frac{|\{i|k_i > k_0\}|}{N}$$

Goeman and Buhlmann, 2007

The universe matters

- It is important to choose the universe correctly

Case 1: universe is all genes in the genome

	DEGs	Non-DEGs	Total
In GO	10	30	40
Not in GO	390	3570	3960
Total	400	3600	4000

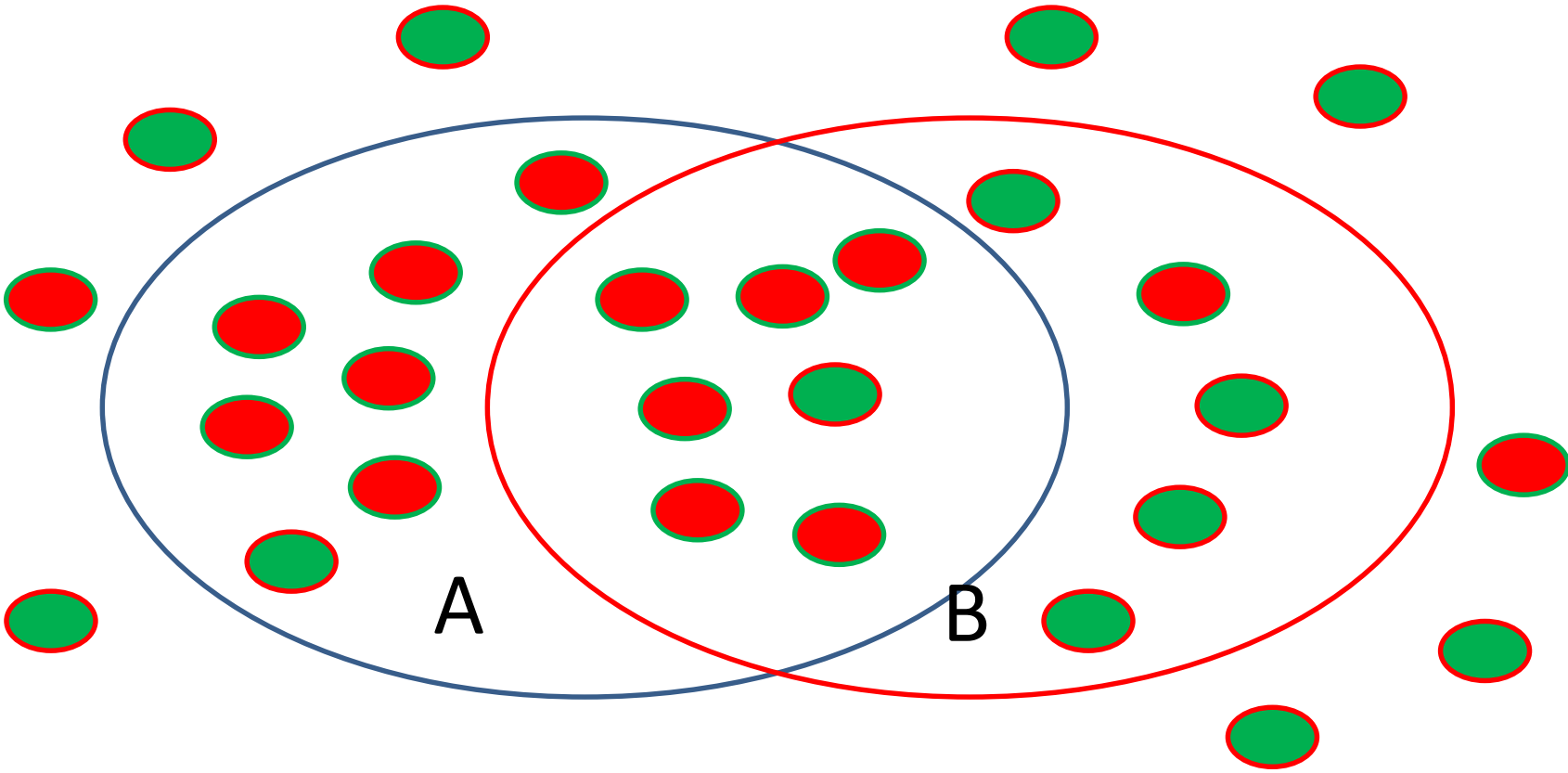
p=0.049

Case 2: universe is only expressed genes

	DEGs	Non-DEGs	Total
In GO	10	30	40
Not in GO	390	570	960
Total	400	600	1000

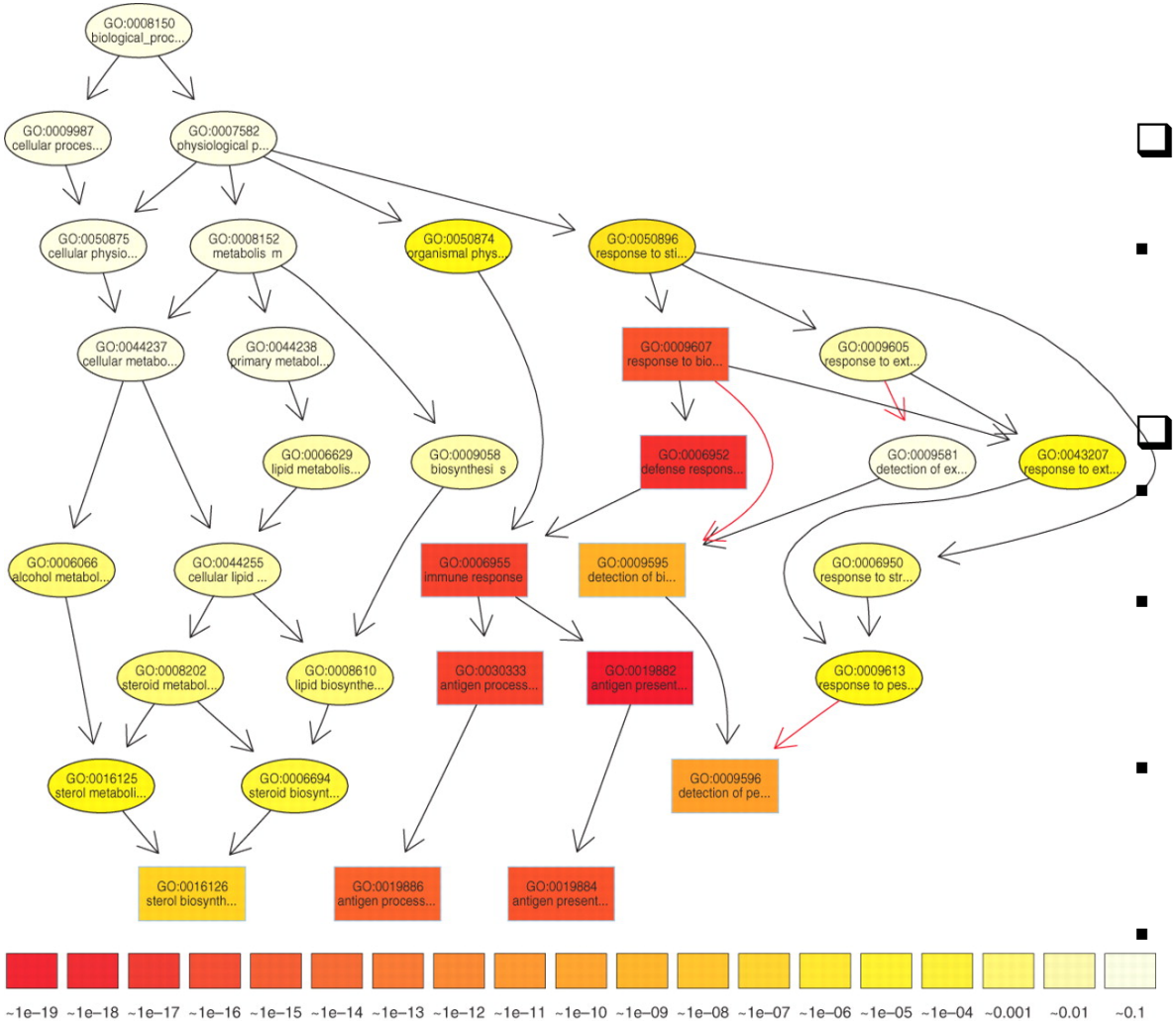
p=0.0048

Sets are overlapped



Set B is enriched only because of it overlap with set A

The subgraph induced by the 10 most significant GO terms identified by a current state-of-the-art method for scoring GO terms for enrichment.



❑ TopGO's elimination algorithm

- Test the leaf sets first. If significant, remove its "genes" before testing its ancestor sets

❑ TopGO's weight algorithm

- The genes are weighted by their relevance in the significant nodes.
- The enrichment score of a parent (gene node u) is compared with the scores of its children.
- Children with a better score than u represent the interesting genes better. Therefore, their significance is increased
- Children with a lower score than u have their significance reduced.

Alexa A et al. Bioinformatics 2006;22:1600-1607

Bioinformatics

A non-parametric approach

- Compare a sample empirical distribution function to the reference distribution
- Or, compare two sample empirical distribution functions
- Required data:
  - Ranked list of genes sorted by differential expression (includes all genes)
  - A gene set
- If the genes in the gene set tend to fall near either end of the ranked list, the gene set is considered significantly enriched
- The test significance is obtained from empirical distribution
  - Permutation based
  - Bootstrapping
  - Etc.

A non-parametric approach (GSEA)

- Mootha *et al.* [2003] suggest to use Kolmogorov-Smirnov (K-S) test
  - Sort all genes by LFC.
  - Go through the list, increasing a running sum for each gene in the gene set by (*N*-*n*), and decreasing it for each gene not in the gene set by *n*.

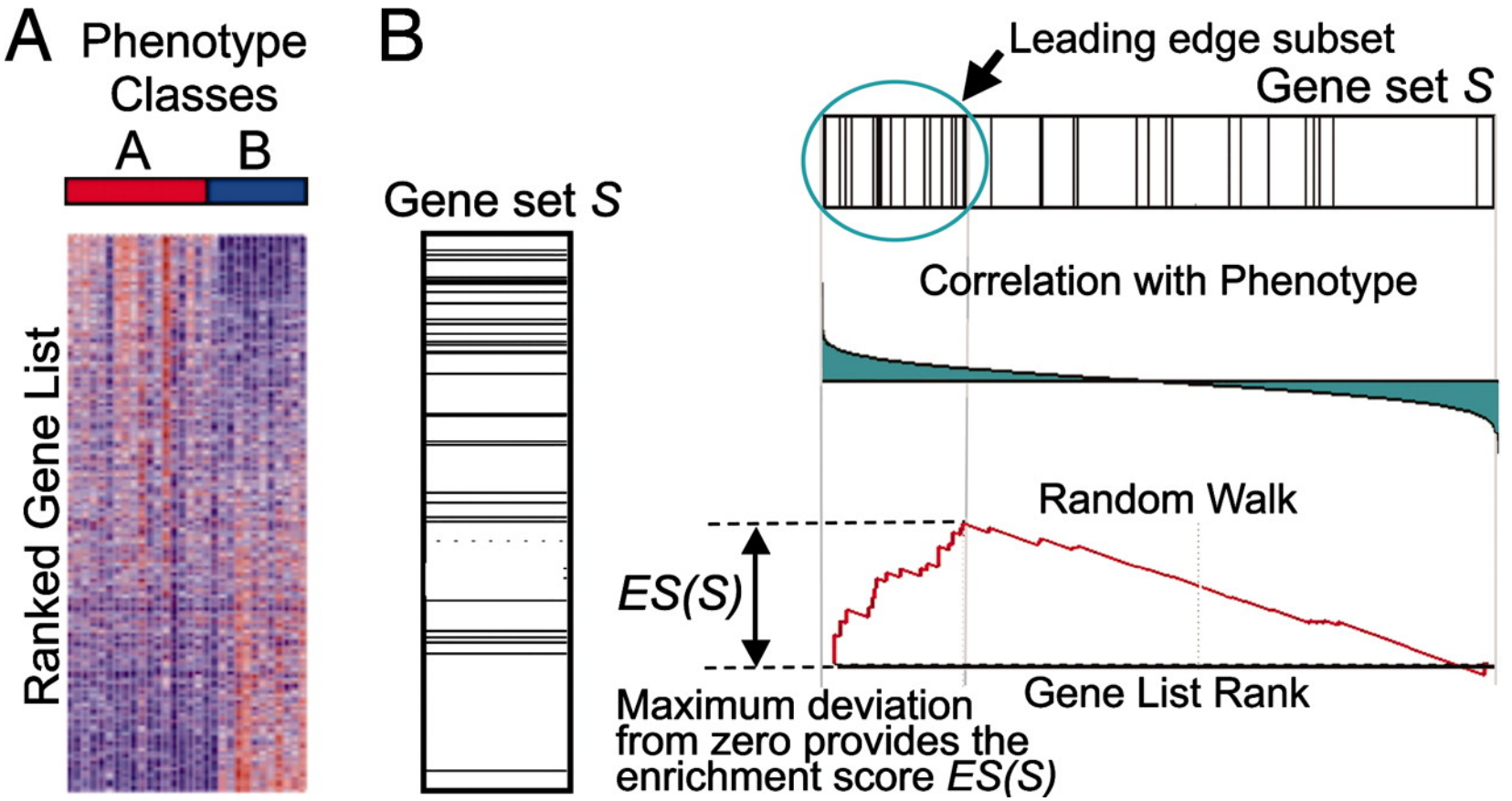
    [*N*: number of genes, *n*: size of gene set]
  - The maximum value of the running sum is the enrichment score (ES).

A non-parametric approach

- Assessing significance
  - To get *p* values, we do not use the KS distribution but rather estimate the null by subject permutation

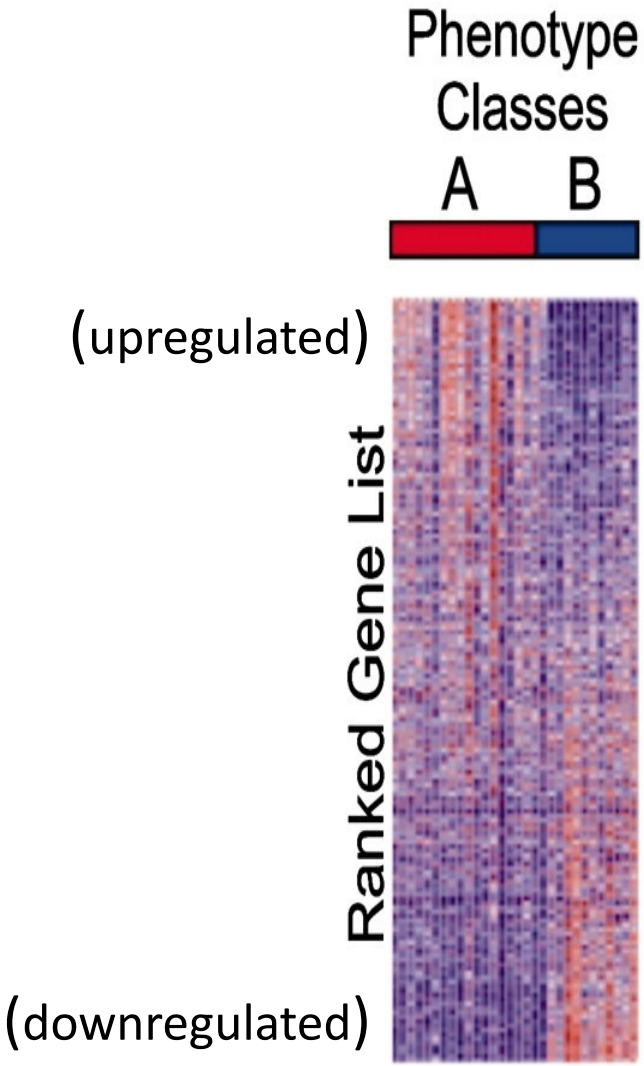
- Improved enrichment score
  - The KS statistic tests whether distributions are different, but this difference may not have a clear direction, making biological interpretation difficult
  - The updated GSEA algorithm [Subramanian *et al.*, PNAS **102** (2005) 15545] weights the in-/decrements of the running sum by the LFC.

A GSEA overview illustrating the method.



Subramanian A et al. PNAS 2005;102:15545-15550

Kolmogorov–Smirnov (KS) Test



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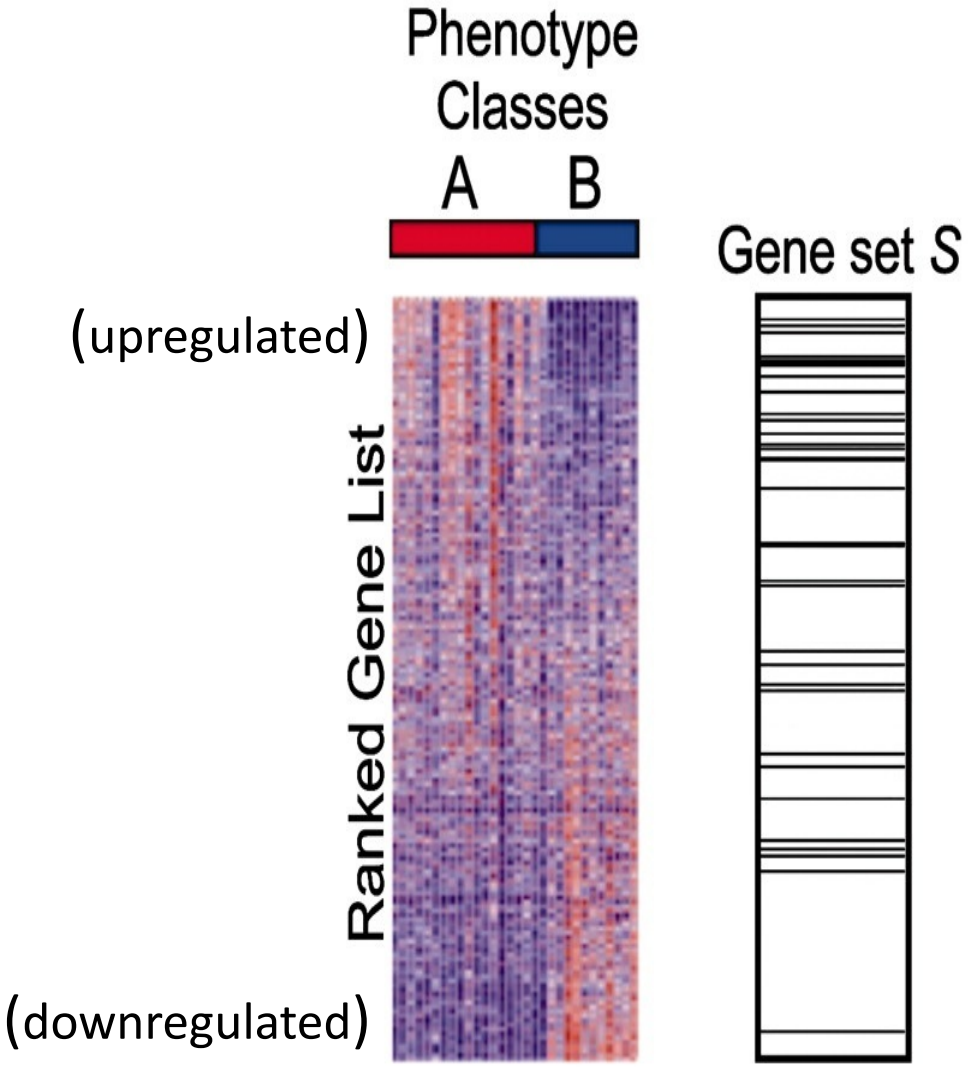
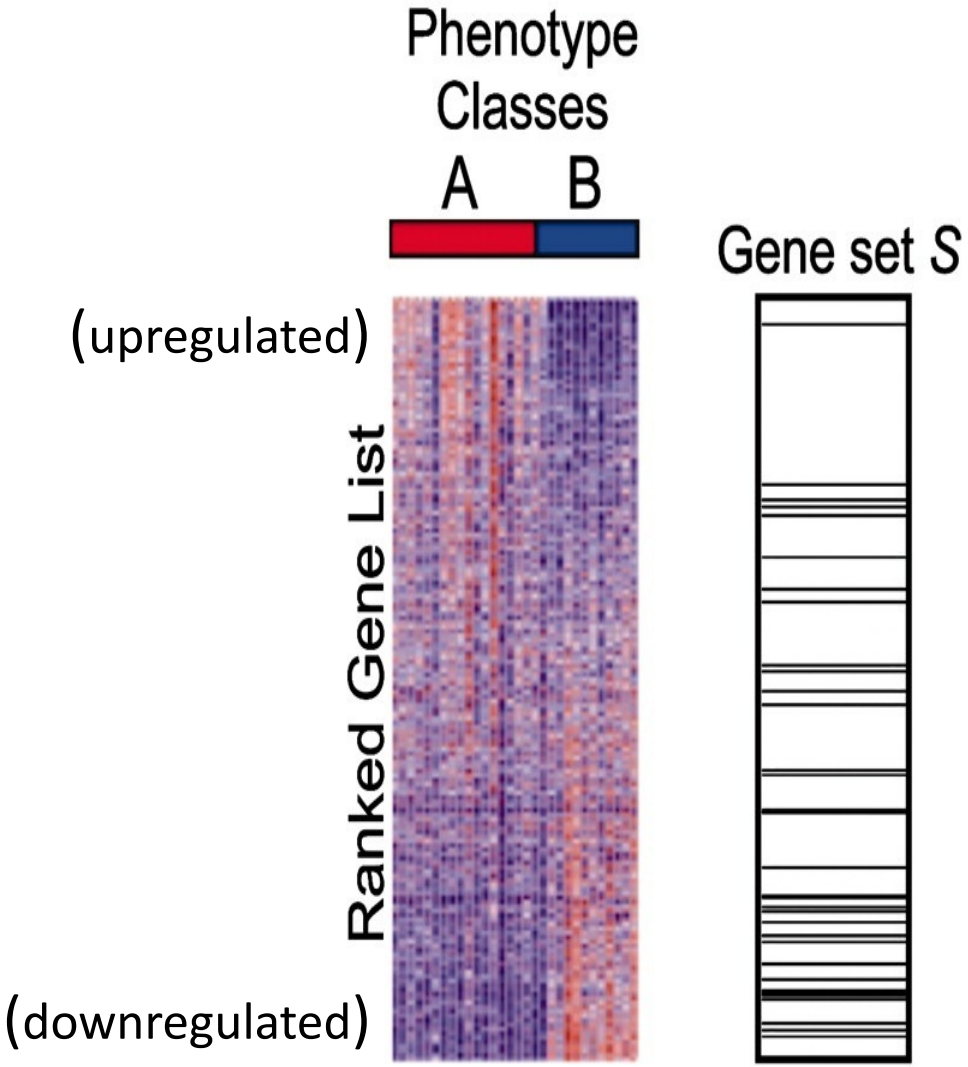


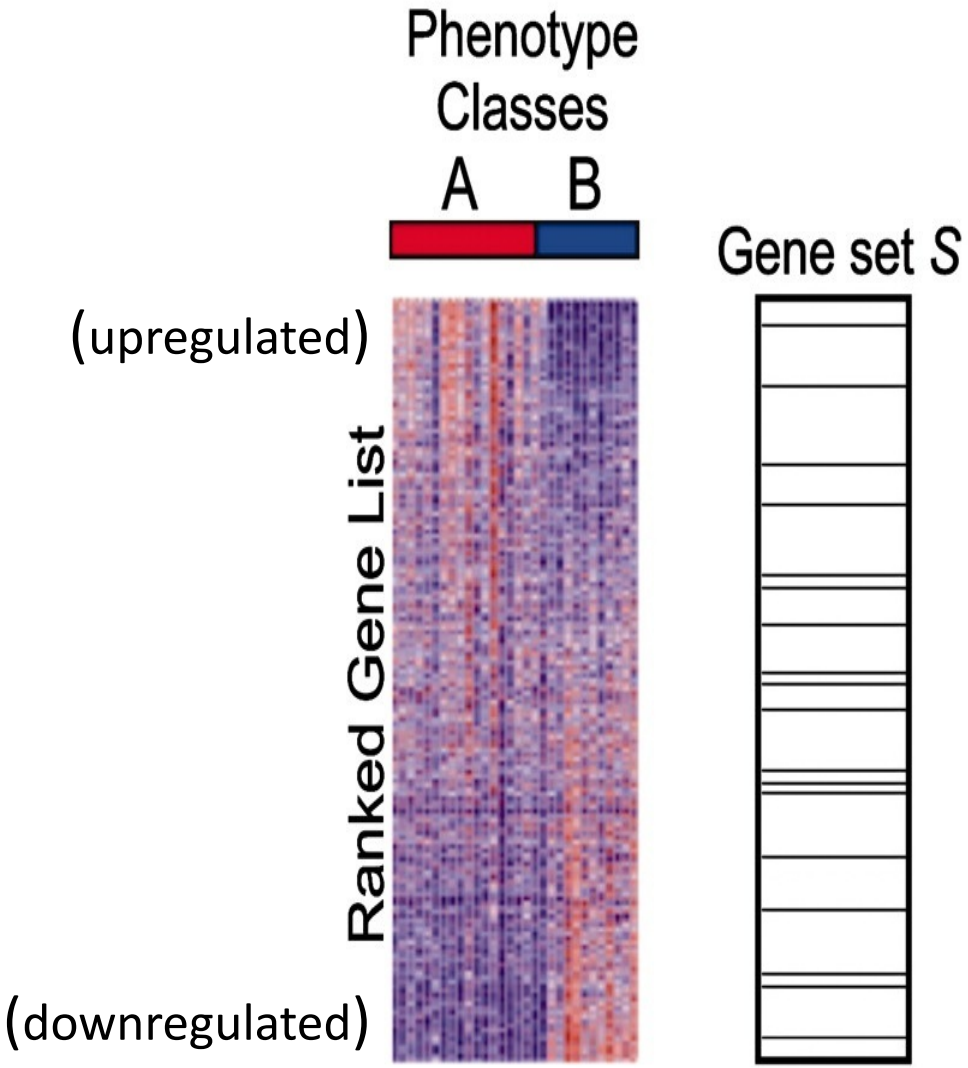
Large, positive enrichment score

Kolmogorov–Smirnov (KS) Test



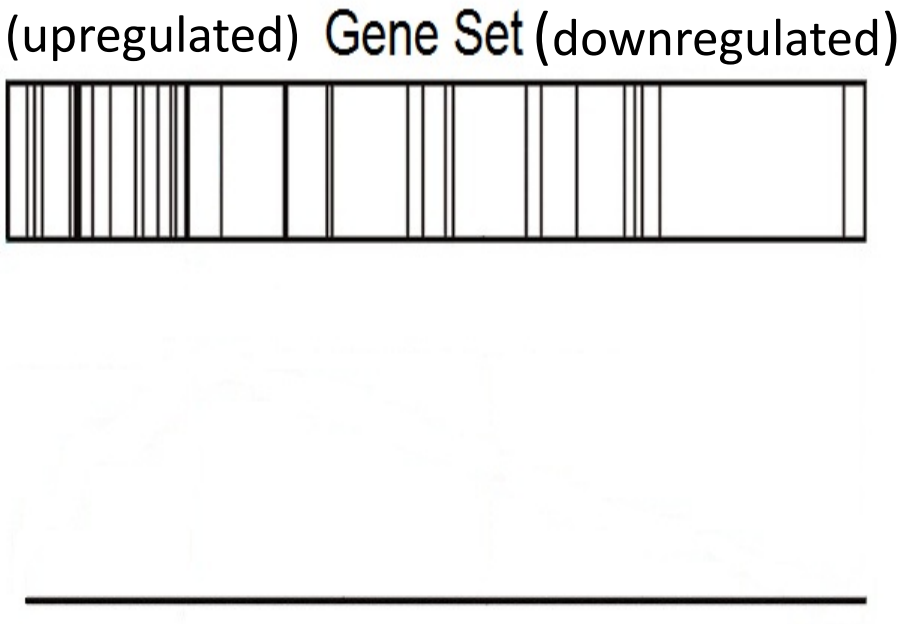
Large, negative enrichment score

Kolmogorov–Smirnov (KS) Test

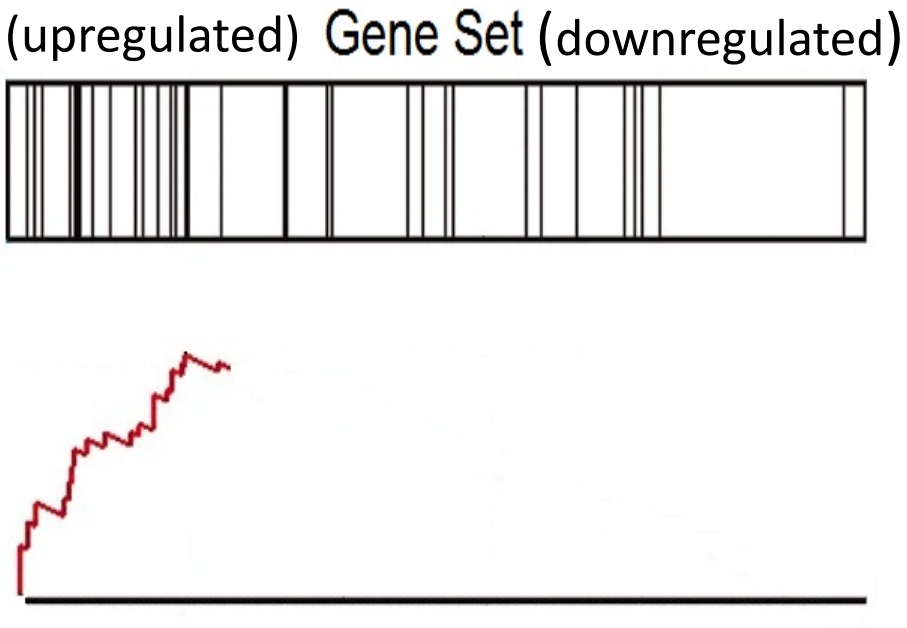


Near zero
enrichment score

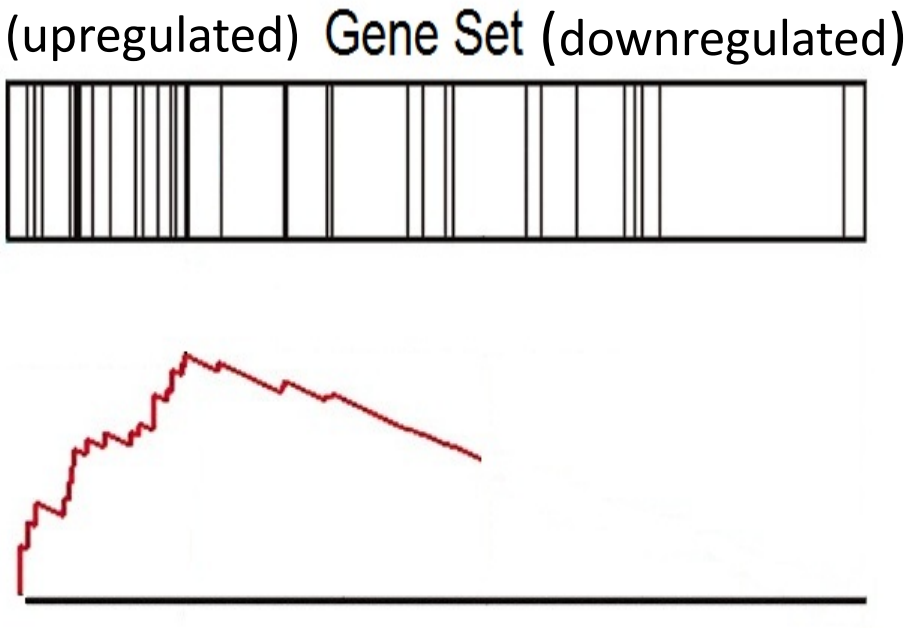
Kolmogorov–Smirnov (KS) Test



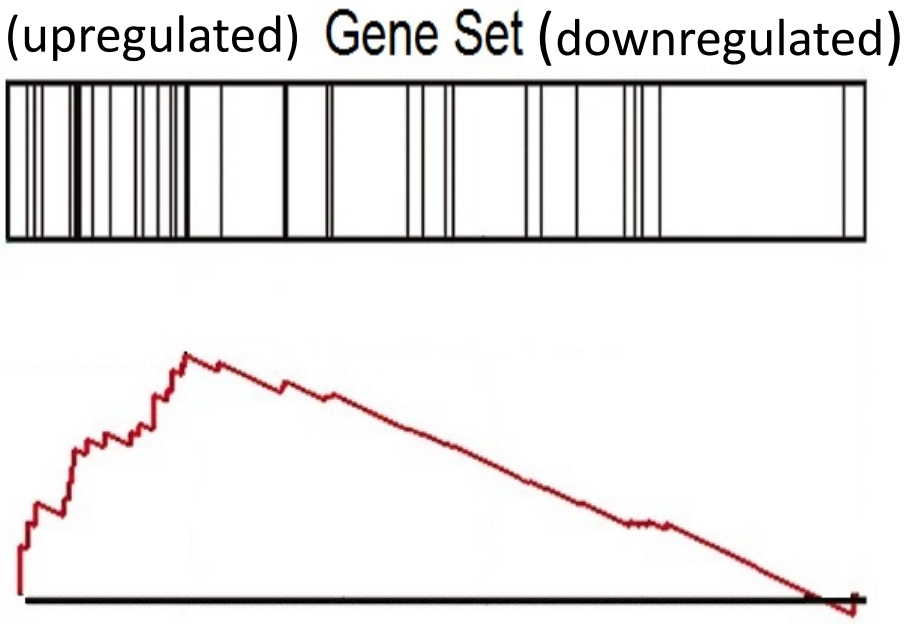
Kolmogorov–Smirnov (KS) Test



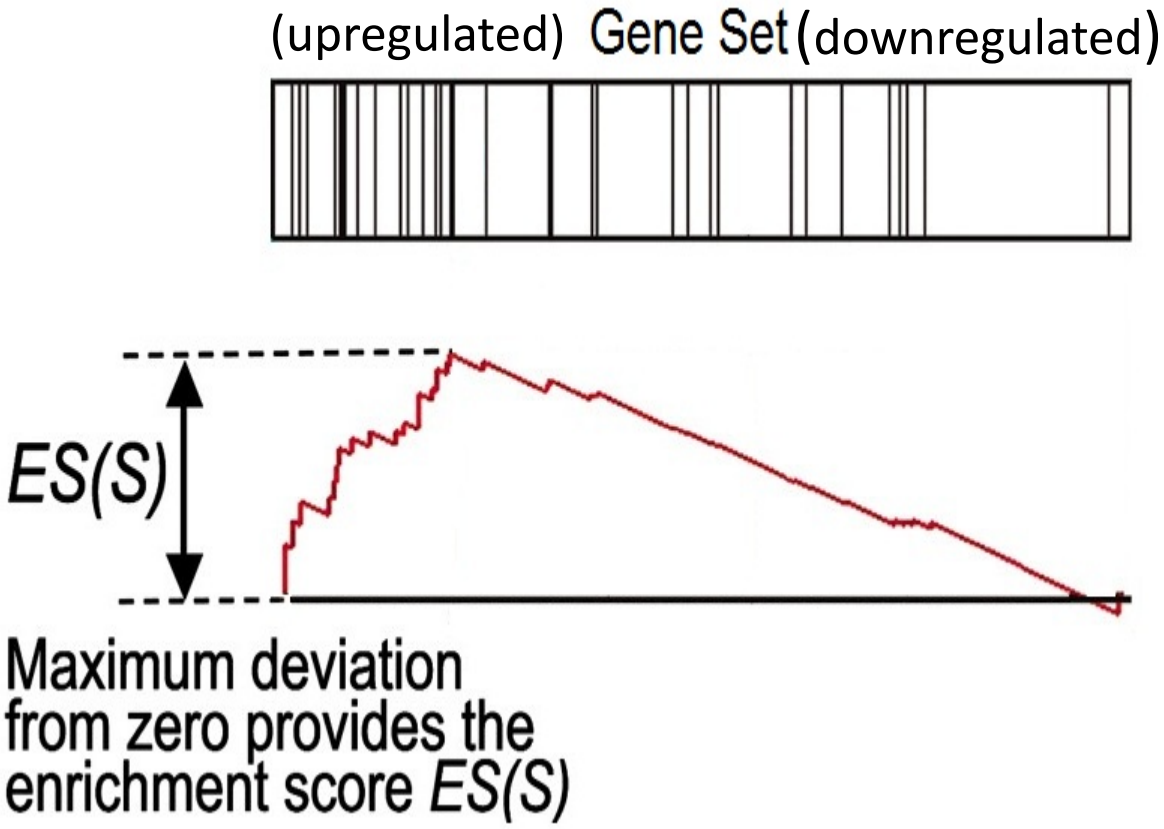
Kolmogorov–Smirnov (KS) Test



Kolmogorov–Smirnov (KS) Test

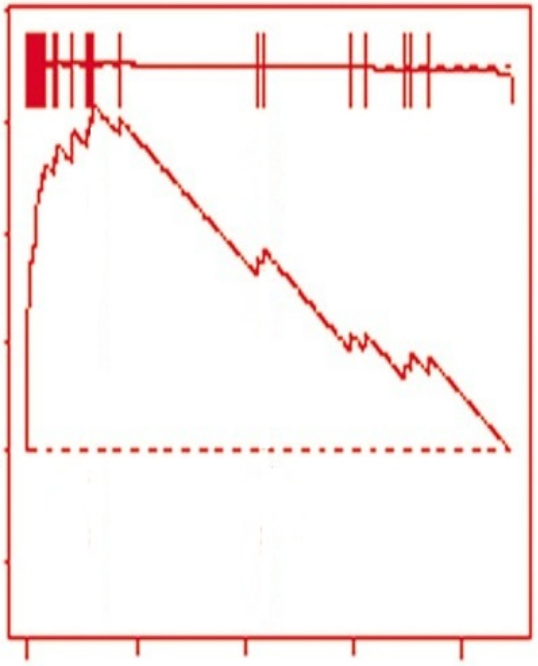


Kolmogorov–Smirnov (KS) Test



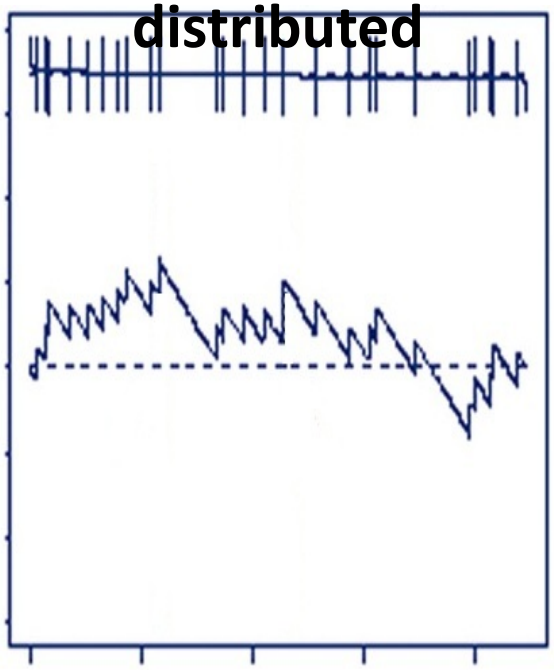
Kolmogorov–Smirnov (KS) Test

Many genes upregulated



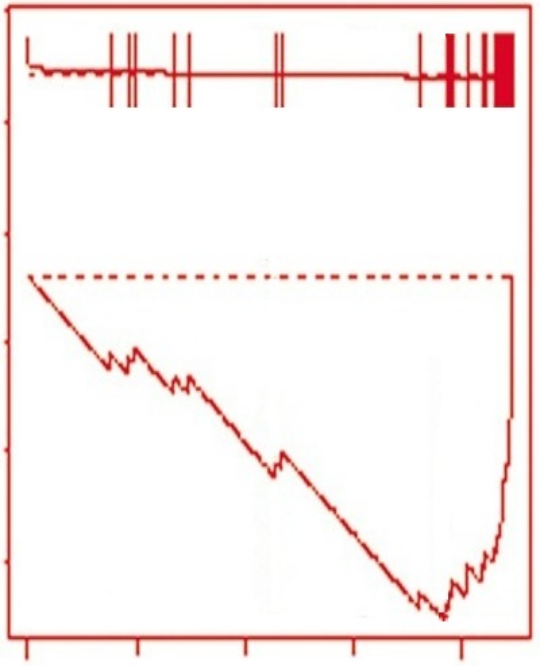
Large positive enrichment score

Genes randomly distributed

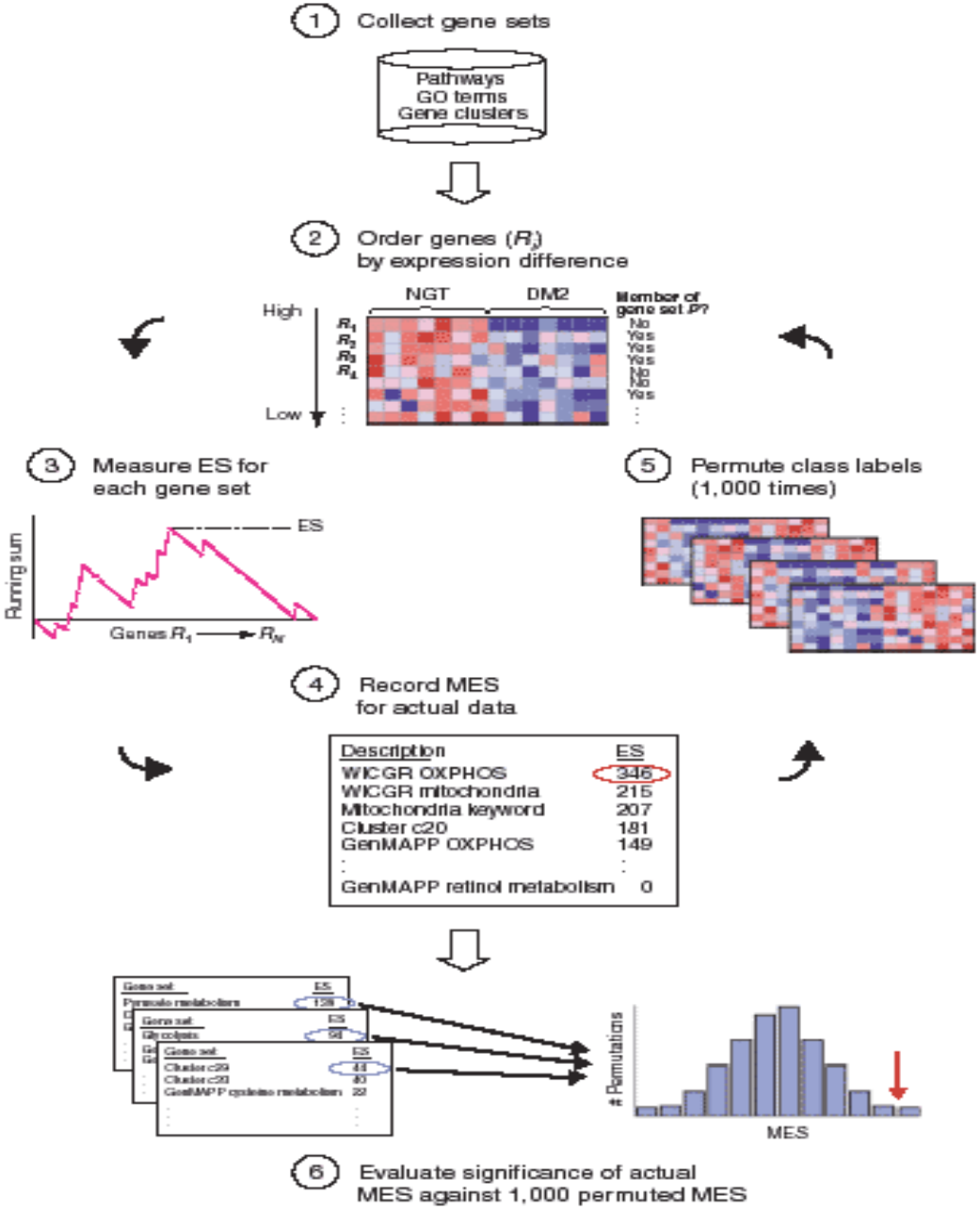


Near zero enrichment score

Many genes downregulated



Large negative enrichment score



Mootha et al., Nature Genetics, 2003, 34(3):267-273

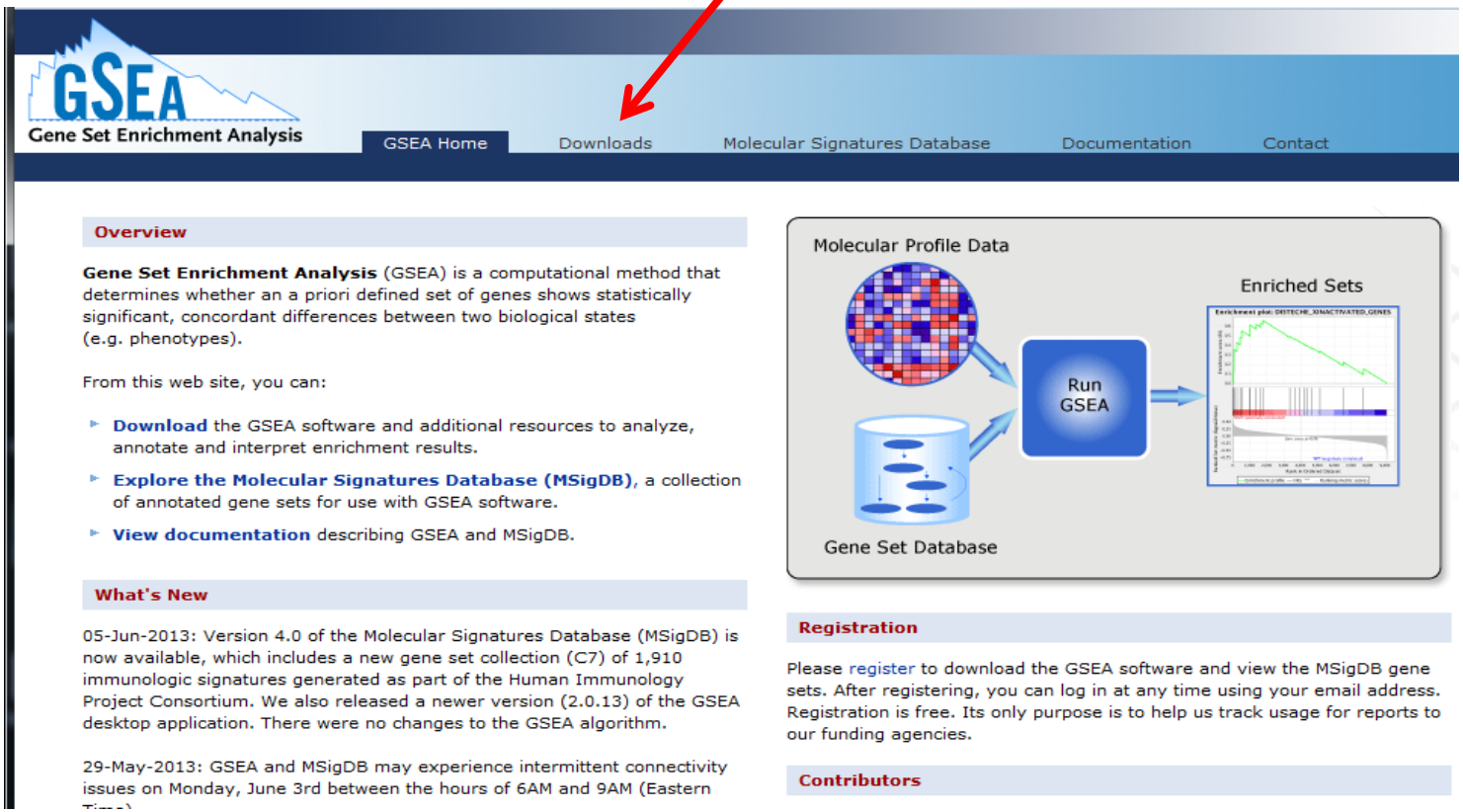
Kolmogorov–Smirnov (KS) Test

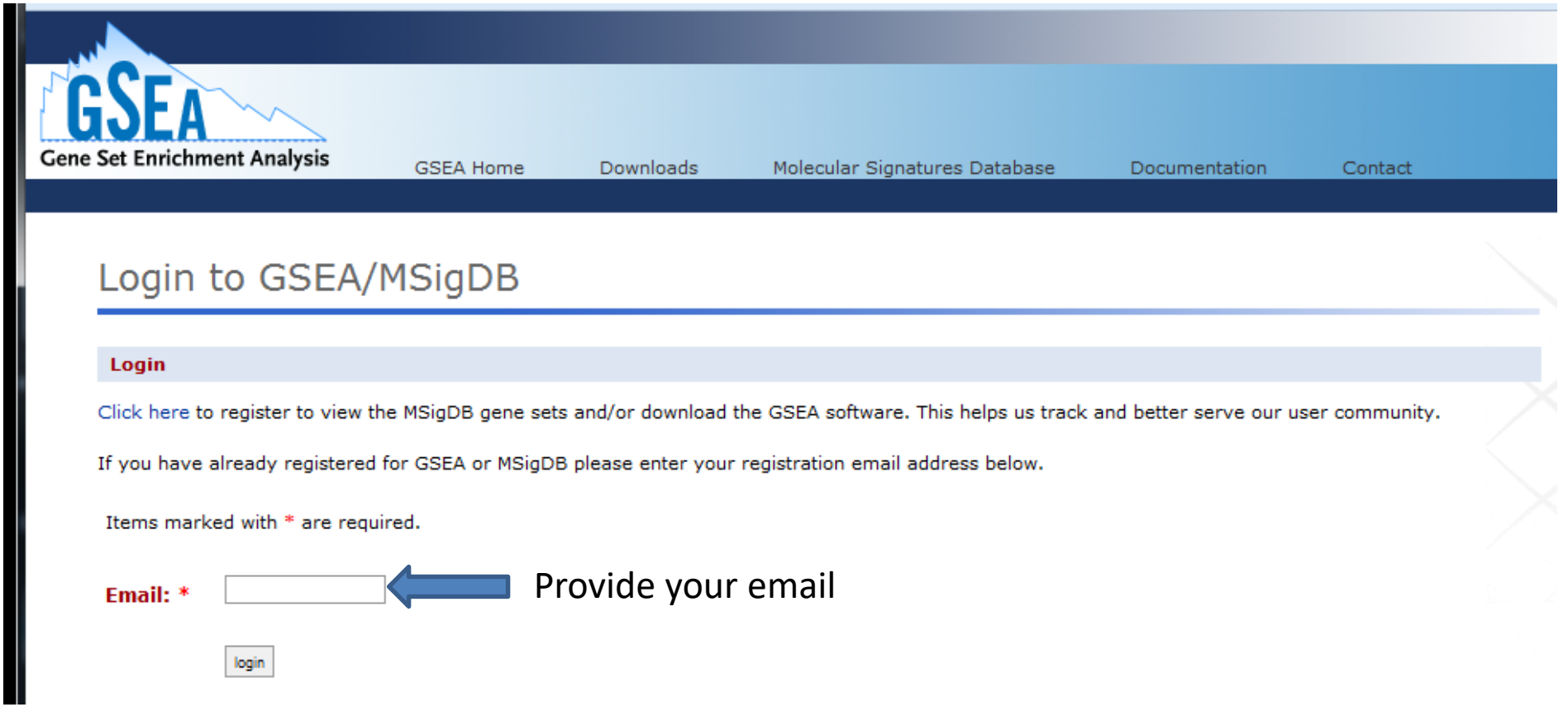
- Used in Gene Set Enrichment Analysis (GSEA)
  - Free software (http://www.broadinstitute.org/gsea)
  - Pros:
    - Large collection of gene sets
    - Uses more information than methods that only use DEG list
    - Enrichment plot improves interpretability
  - Cons:
    - Permutation-based p-values

Hands on practice on both parametric and non-parametric

NOW, IT IS YOUR TURN

Click to download




GSEA
Gene Set Enrichment Analysis
GSEA Home
Downloads
Molecular Signatures Database
Documentation
Contact
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Login
Click here to register to view the MSigDB gene sets and/or download the GSEA software. This helps us track and better serve our user community.
If you have already registered for GSEA or MSigDB please enter your registration email address below.
Items marked with * are required.
Email: *
Provide your email
login

GSEA Example

Downloads

The GSEA software and source code and the Molecular Signatures Database (MSigDB) are freely available to individuals in both academia and industry for internal research purposes. Please see the GSEA/MSigDB license for more details.

Software

There are several options for GSEA software. All options implement exactly the same algorithm. Usage recommendations and installation instructions are listed below. Current Java implementations of GSEA require Java 6 or 7.

javaGSEA Desktop Application	▸ Easy-to-use graphical user interface ▸ Runs on any desktop computer (Windows, Mac OS X, Linux etc.) that supports Java 6 or 7 ▸ Produces richly annotated reports of enrichment results ▸ Integrated gene sets browser to view gene set annotations, search for gene sets and ma… gene sets between platforms	Launch with 1GB (for 32 or 64-bit Java) …memory: Launch
javaGSEA Java Jar file	▸ Command line usage ▸ Runs on any platform that supports Java 6 or 7 ▸ We recommend using the 'Launch' buttons above instead of this mode for most users	download gsea2-2.2.0.jar
R-GSEA	▸ Usage from within the R programming environment	download

Step 1

Step 2

Step 3

GSEA v2.0.12 (Gene set enrichment analysis -- Broad Institute)

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Leading edge analysis

Gene set tools

Chip2Chip mapping

Browse MSigDB

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Processes: click 'status' field for results

Name	Status

Show results folder

Home | Load data

Steps in GSEA

1. What you need for GSEA

- Expression data set
- Phenotype annotation
- Gene sets – use MSigDB or your own gene sets

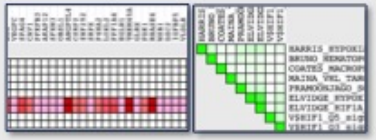
2. Run GSEA

- Start with default parameters
- If you want to collapse probes to genes, specify chip platform

3. View results

4. Leading edge analysis

- Leading edge finds genes driving enrichment results



Gene Set Tools

Chip2Chip mapping

- Convert gene sets between platforms

Chip2Chip mapping

Explore MSigDB gene sets

- Search the database of thousands of gene sets
- Browse the gene sets by name
- Find overlapping gene sets
- Export gene sets

Browse MSigDB

See also

- MSigDB online tools at: www.broadinstitute.org/msigdb

Getting Help

GSEA web site:
www.broadinstitute.org/gsea

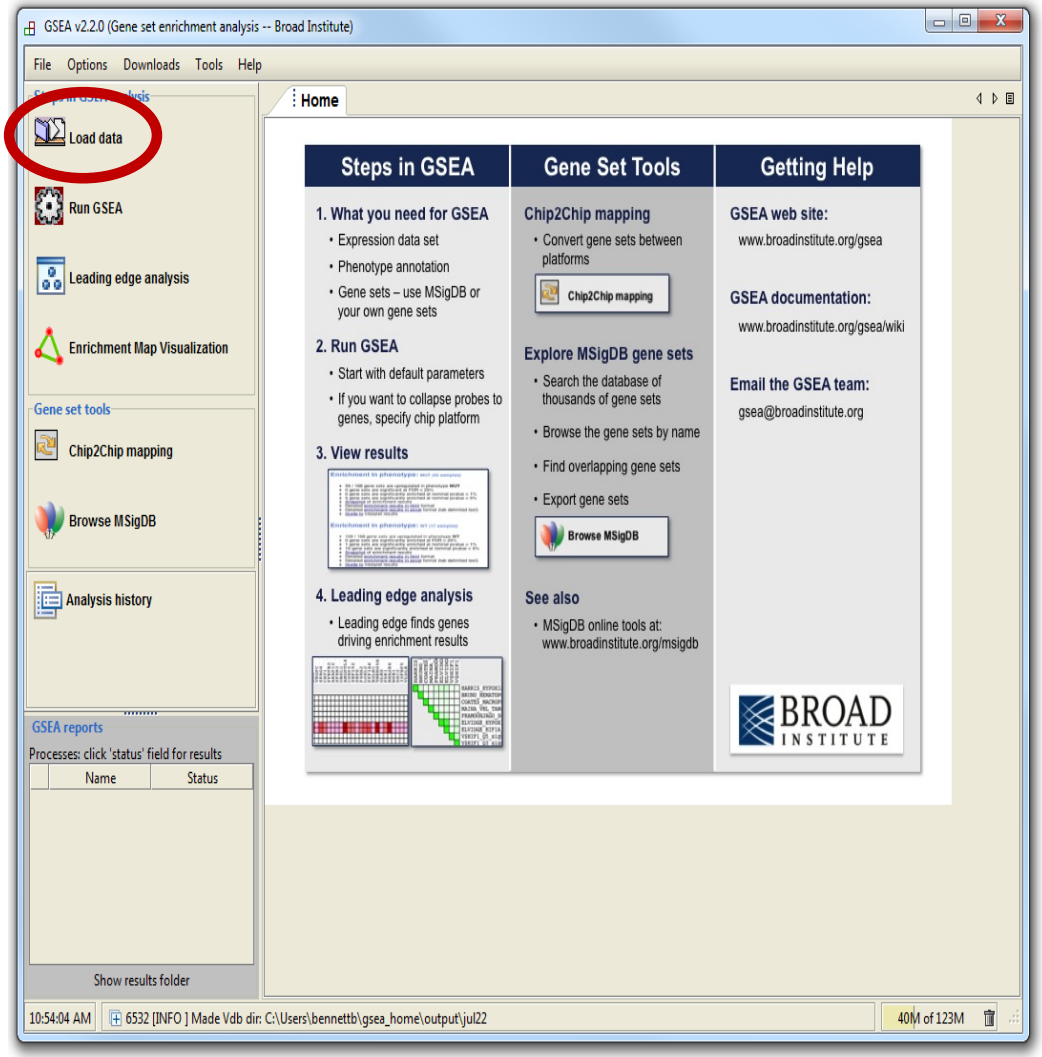
GSEA documentation:
www.broadinstitute.org/gsea/wiki

Email the GSEA team:
gsea@broadinstitute.org

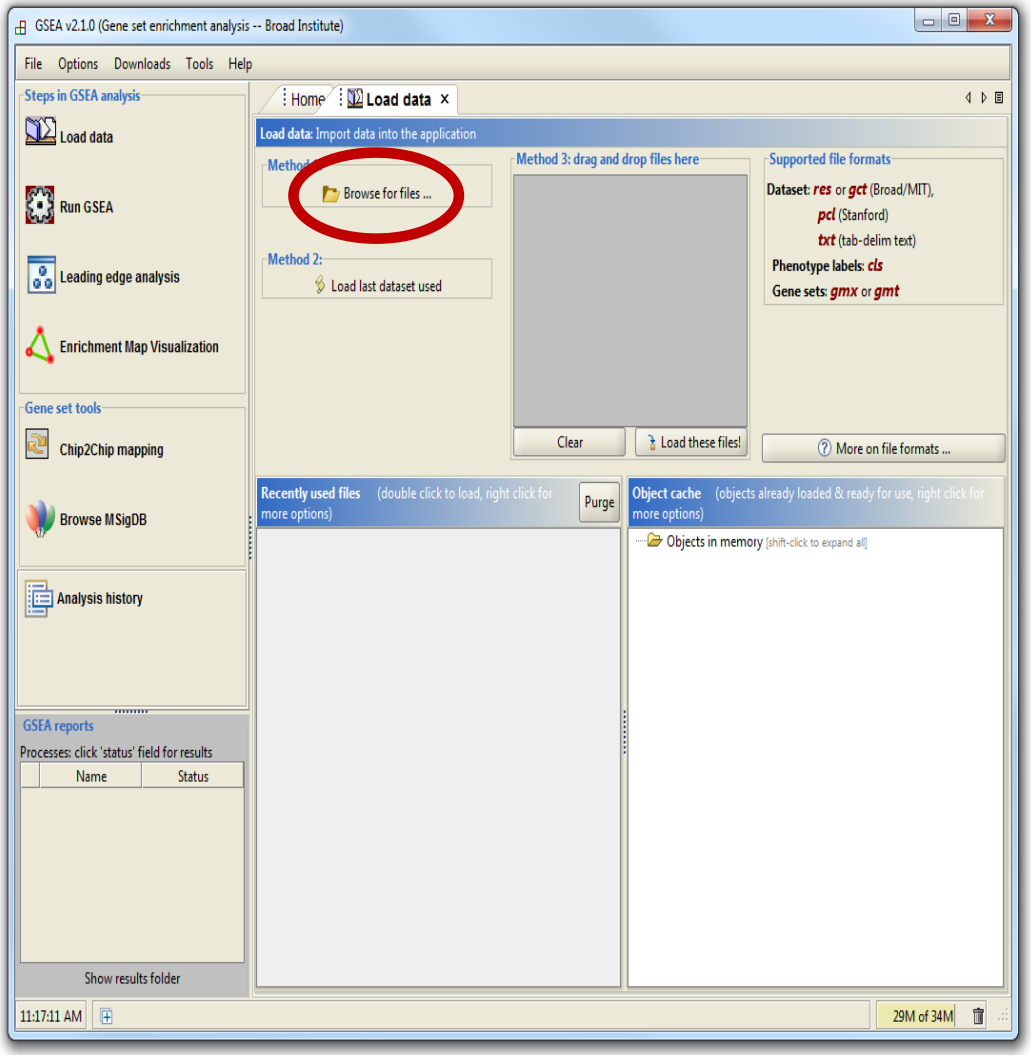
BROAD INSTITUTE

4:03:18 PM | 6510 [INFO] Made Vdb dir: C:\Users\li11\gsea_home\output\jul11 | 14M of 61M

GSEA Example



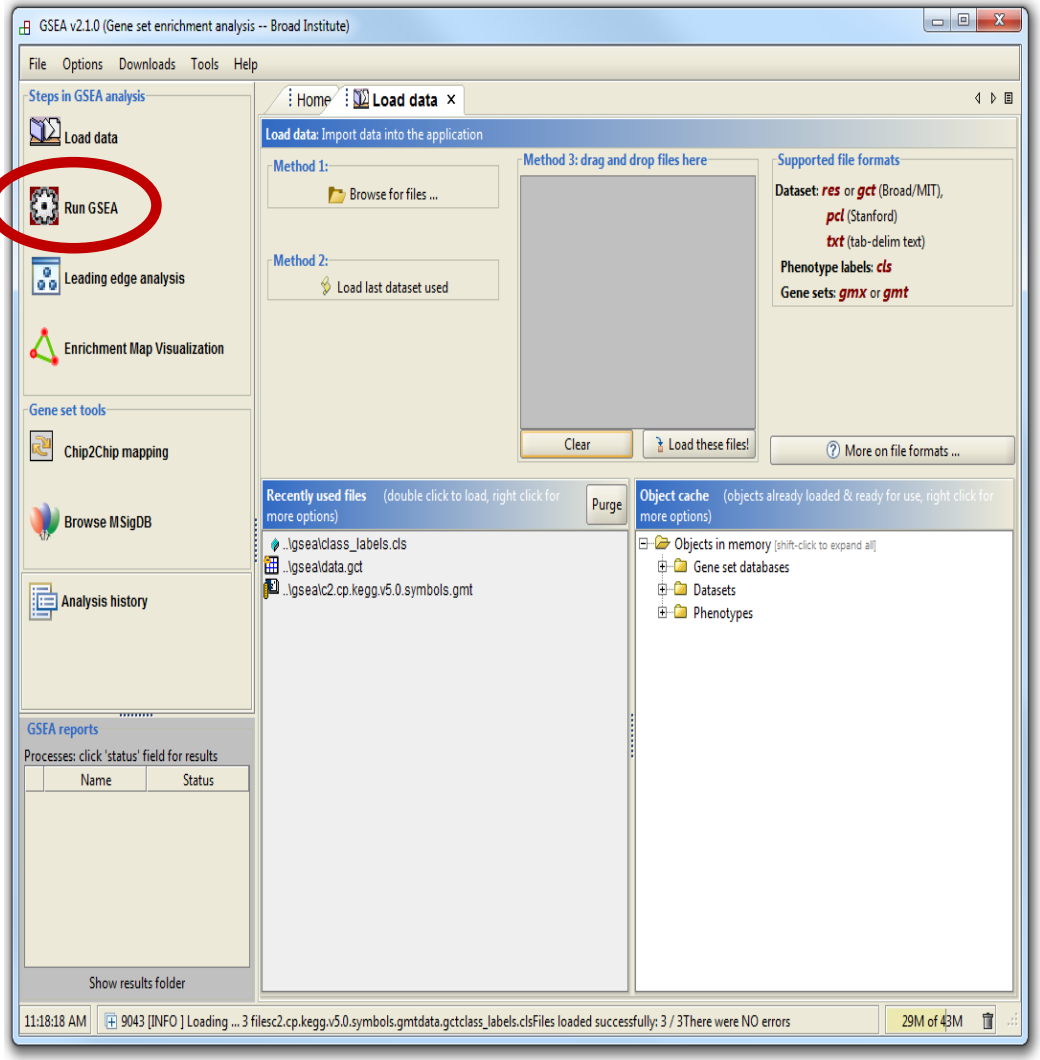
GSEA Example



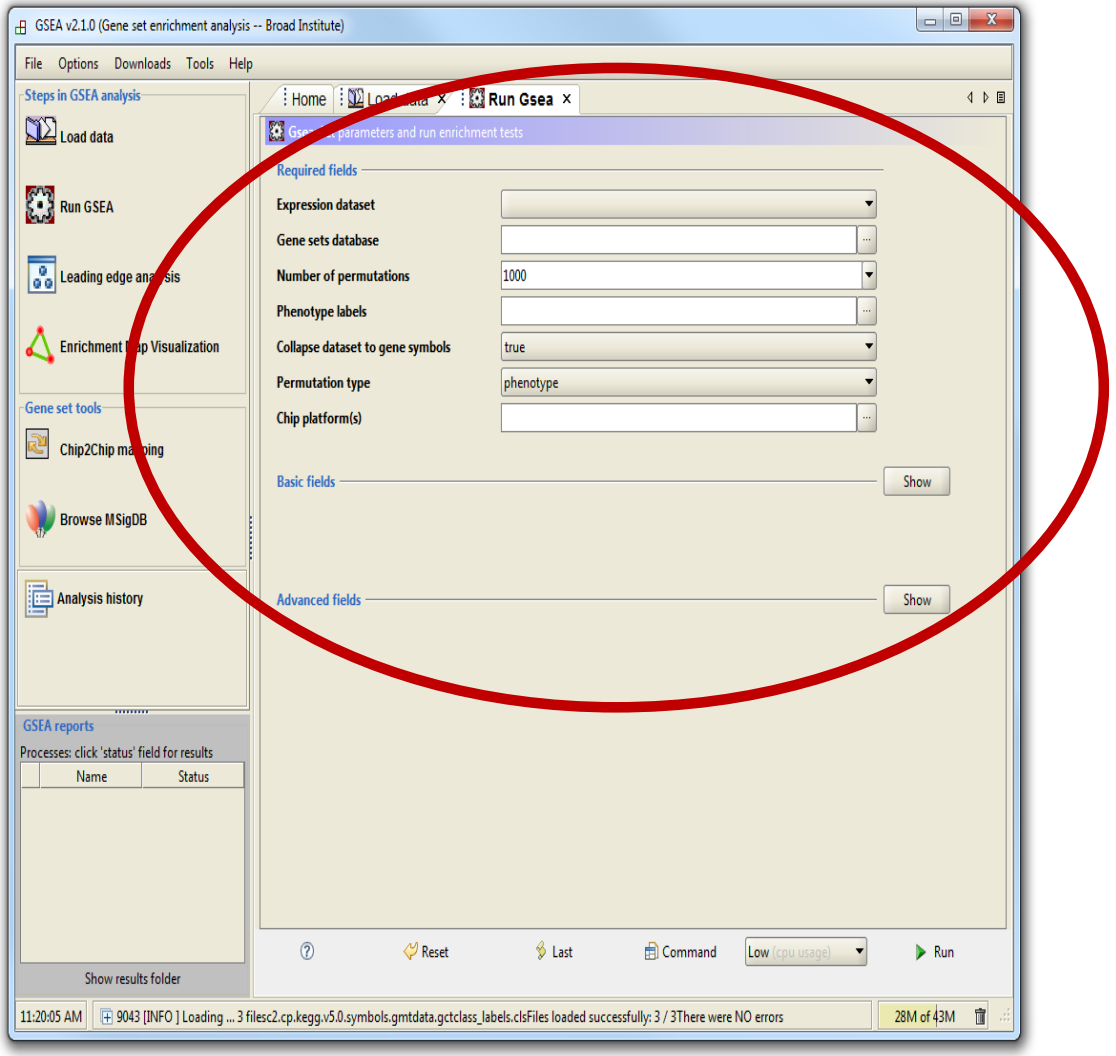
GSEA Example

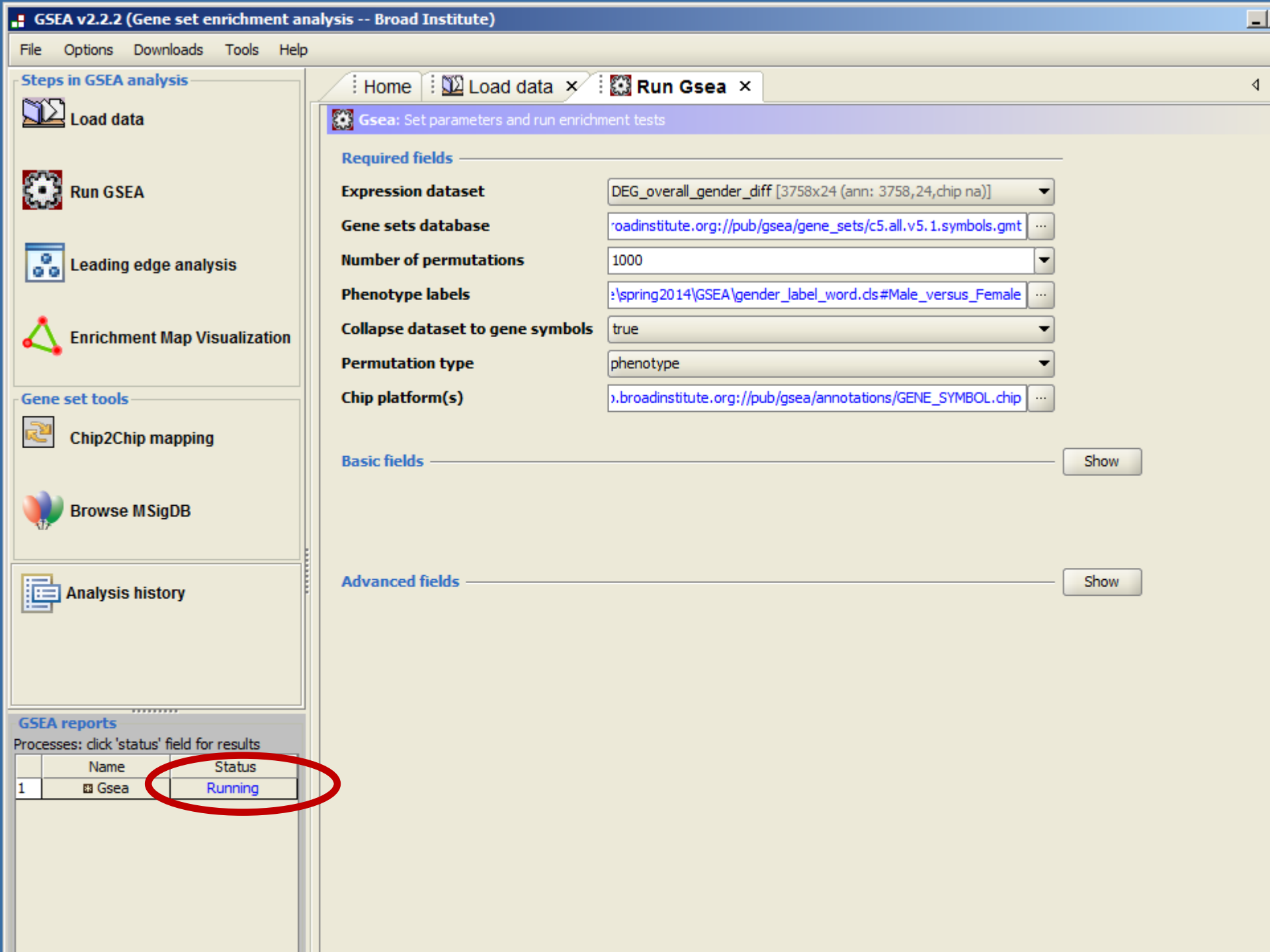
- Supported file types:
  - http://www.broadinstitute.org/cancer/software/gsea/wiki/index.php/Data_formats
- Required:
  - Expression data file
  - Class label file
- Optional:
  - Gene set file

GSEA Example



GSEA Example

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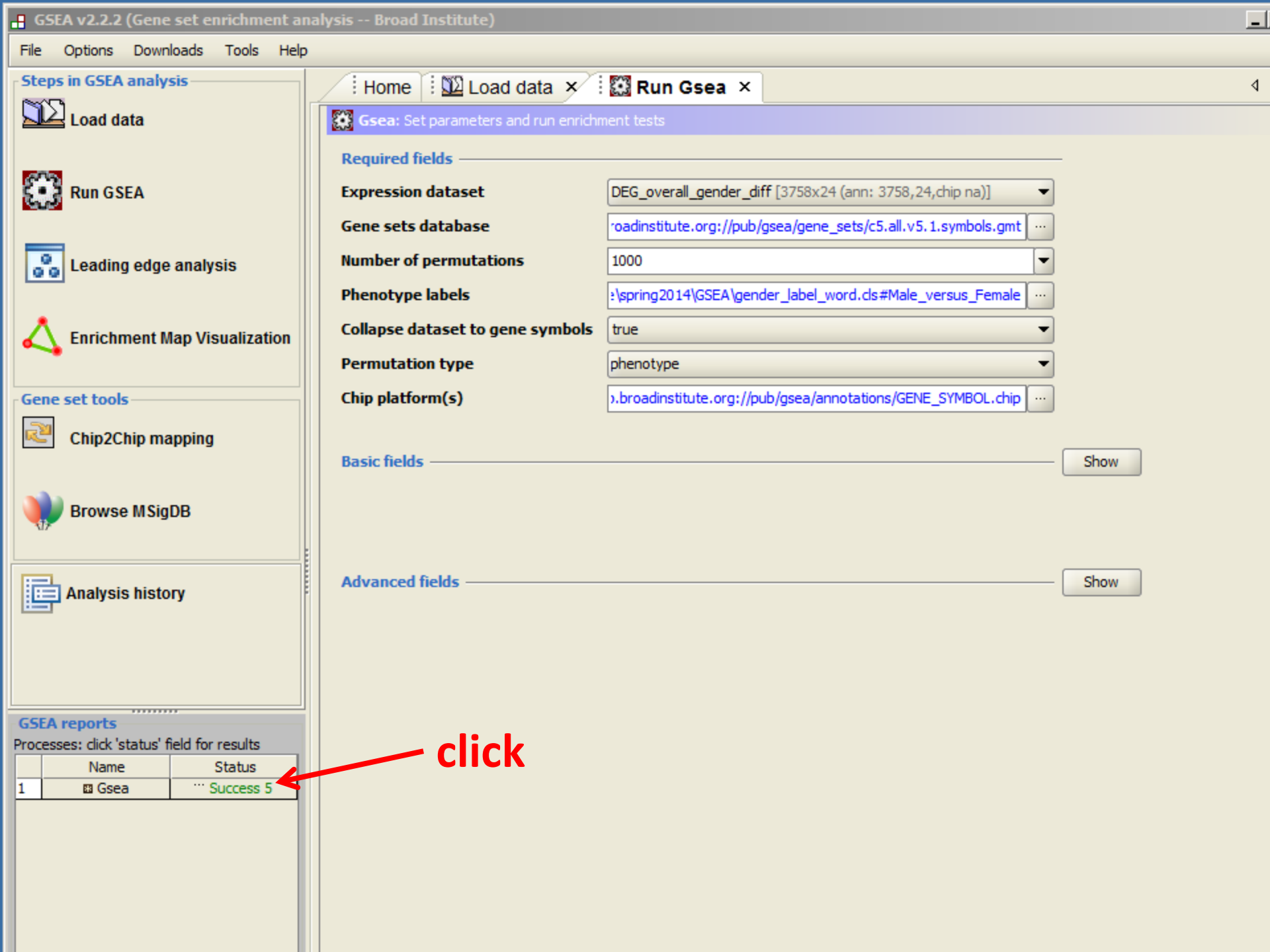
Gsea: Set parameters and run enrichment tests
Required fields
Expression dataset
DEG_overall_gender_diff [3758x24 (ann: 3758,24,chip na)]
Gene sets database
oadinstitute.org://pub/gsea/gene_sets/c5.all.v5.1.symbols.gmt
Number of permutations
1000
Phenotype labels
:\spring2014\GSEA\gender_label_word.cls#Male_versus_Female
Collapse dataset to gene symbols
true
Permutation type
phenotype
Chip platform(s)
).broadinstitute.org://pub/gsea/annotations/GENE_SYMBOL.chip
Basic fields
Show
Advanced fields
Show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Number of permutations
1000
Phenotype labels
:\spring2014\GSEA\gender_label_word.cls#Male_versus_Female
Collapse dataset to gene symbols
true
Permutation type
phenotype
Chip platform(s)
).broadinstitute.org://pub/gsea/annotations/GENE_SYMBOL.chip
Basic fields
Show
Advanced fields
Show

GSEA Report for Dataset DEG_overall_gender_diff

Enrichment in phenotype: Male (12 samples)

- 31 / 378 gene sets are upregulated in phenotype **Male**
- 0 gene sets are significant at FDR < 25%
- 0 gene sets are significantly enriched at nominal pvalue < 1%
- 1 gene sets are significantly enriched at nominal pvalue < 5%
- Snapshot of enrichment results
- Detailed enrichment results in html format
- Detailed enrichment results in excel format (tab delimited text)
- Guide to interpret results

Enrichment in phenotype: Female (12 samples)

- 347 / 378 gene sets are upregulated in phenotype **Female**
- 4 gene sets are significantly enriched at FDR < 25%
- 4 gene sets are significantly enriched at nominal pvalue < 1%
- 5 gene sets are significantly enriched at nominal pvalue < 5%
- Snapshot of enrichment results
- Detailed enrichment results in html format
- Detailed enrichment results in excel format (tab delimited text)
- Guide to interpret results

Dataset details

- The dataset has 3750 native features
- After collapsing features into gene symbols, there are: 2736 genes

Gene set details

- Gene set size filters (min=15, max=500) resulted in filtering out 1076 / 1454 gene sets
- The remaining 378 gene sets were used in the analysis
- List of gene sets used and their sizes (restricted to features in the specified dataset)

Gene markers for the Male *versus* Female comparison

- The dataset has 2736 features (genes)
- # of markers for phenotype **Male**: 310 (11.3%) with correlation area 10.8%
- # of markers for phenotype **Female**: 2426 (88.7%) with correlation area 89.2%
- Detailed rank ordered gene list for all features in the dataset
- Heat map and gene list correlation profile for all features in the dataset
- Buttefly plot of significant genes

	GS follow link to MSigDB	GS DETAILS	SIZE	ES	NES	NOM p-val	FDR q-val	FWER p-val	RANK AT MAX	LEADING EDGE
1	STEROID_METABOLIC_PROCESS	Details ...	1	-0.59	-1.86	0.000	0.247	0.093	326	tags=35%, list=12%, signal=40%
2	MONOCARBOXYLIC_ACID_METABOLIC_PROCESS	Details ...	27	-0.47	-1.84	0.004	0.146	0.107	644	tags=37%, list=24%, signal=48%
3	CELLULAR_LIPID_METABOLIC_PROCESS	Details ...	64	-0.45	-1.81	0.000	0.136	0.138	575	tags=31%, list=21%, signal=39%
4	FATTY_ACID_METABOLIC_PROCESS	Details ...	19	-0.59	-1.76	0.008	0.184	0.217	644	tags=47%, list=24%, signal=62%
5	LIPID_METABOLIC_PROCESS	Details ...	78	-0.39	-1.65	0.014	0.410	0.415	675	tags=29%, list=25%, signal=38%
6	CARBOXYLIC_ACID_METABOLIC_PROCESS	Details ...	51	-0.32	-1.45	0.090	1.000	0.696	696	tags=24%, list=25%, signal=31%
7	ORGANIC_ACID_METABOLIC_PROCESS	Details ...	51	-0.32	-1.45	0.090	1.000	0.696	696	tags=24%, list=25%, signal=31%
8	LIPID_BIOSYNTHETIC_PROCESS	Details ...	22	-0.46	-1.40	0.150	1.000	0.747	575	tags=36%, list=21%, signal=46%
9	GOLGI_APPARATUS	Details ...	30	-0.43	-1.39	0.076	1.000	0.753	379	tags=37%, list=14%, signal=42%
10	GTPASE_ACTIVITY	Details ...	15	-0.50	-1.38	0.092	1.000	0.761	195	tags=33%, list=7%, signal=36%
11	CELL_MIGRATION	Details ...	22	-0.48	-1.35	0.121	1.000	0.781	969	tags=64%, list=35%, signal=98%
12	OXIDOREDUCTASE_ACTIVITY	Details ...	66	-0.27	-1.34	0.119	1.000	0.783	284	tags=12%, list=10%, signal=13%
13	TRANSMEMBRANE_RECEPTOR_PROTEIN_KINASE_ACTIVITY	Details ...	15	-0.53	-1.34	0.105	1.000	0.794	795	tags=67%, list=29%, signal=93%

GSEA Example

Table: GSEA Results Summary

Dataset	DEG_overall_gender_diff_overall_gender_diff_collapsed_to_symbols.gender_label_word.cls #Male_versus_Female.gender_label_word.cls #Male_versus_Female_repos
Phenotype	gender_label_word.cls#Male_versus_Female_repos
Upregulated in class	Female
GeneSet	STEROID_METABOLIC_PROCESS
Enrichment Score (ES)	-0.5875557
Normalized Enrichment Score (NES)	-1.8563647
Nominal p-value	0.0
FDR q-value	0.24662763
FWER p-Value	0.093

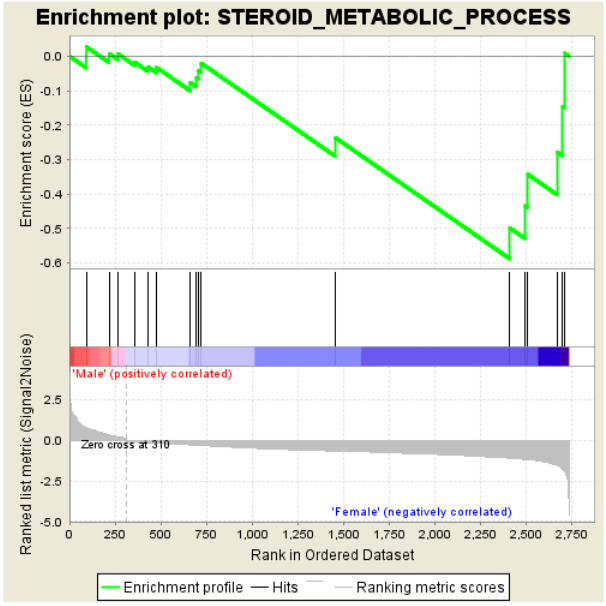
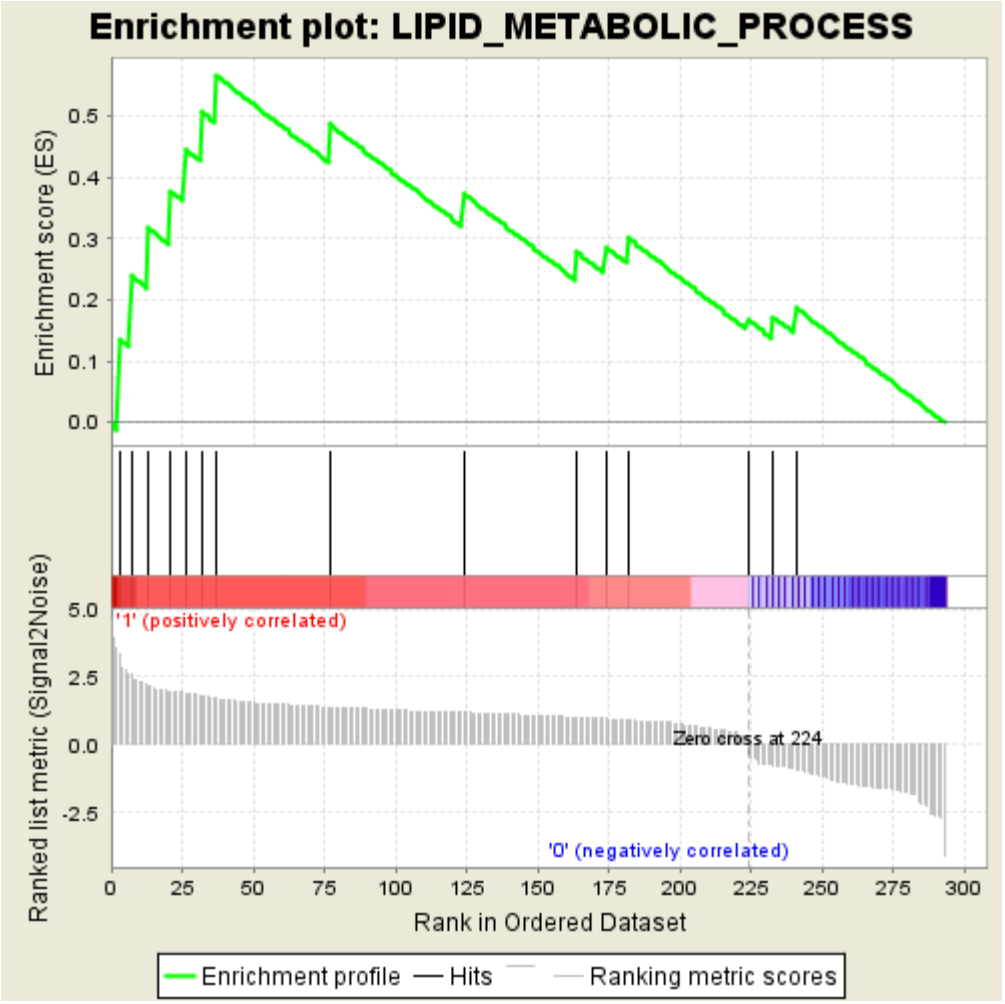
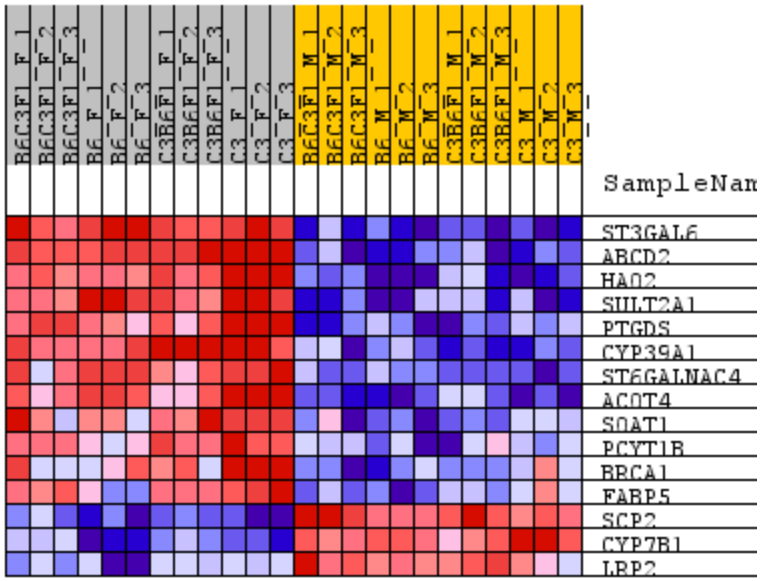


Fig 1: Enrichment plot: STEROID_METABOLIC_PROCESS
Profile of the Running ES Score & Positions of GeneSet Members on the Rank Ordered List

Lipid metabolism enriched -- GSEA





- http://www-stat.stanford.edu/~tibs/GSA/
- http://www.netsci.org/Resources/Software/Bioinform/pathwayanalysis.html
- http://www.broadinstitute.org/gsea/index.jsp
- http://david.abcc.ncifcrf.gov/
- http://www.biocarta.com/
- http://web.expasy.org/pathways/
- http://www.genmapp.org/
- http://www.genome.jp/kegg/
- http://www.ingenuity.com/
- http://www.genego.com/metacore.php
- http://www.geneontology.org/
- *http://omicslab.genetics.ac.cn/**GOEAST**/tutorial.php*
- *http://expressome.kobic.re.kr/GAzer/document.jsp*
- *http://www.biobase-international.com/products*
- *http://jaspar.genereg.net/*

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