

An Introduction to Pathway Analysis

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Outline

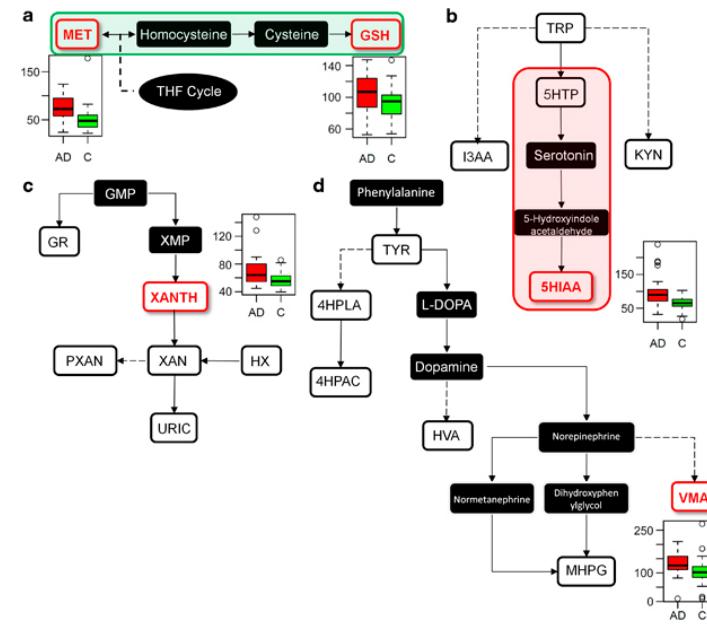
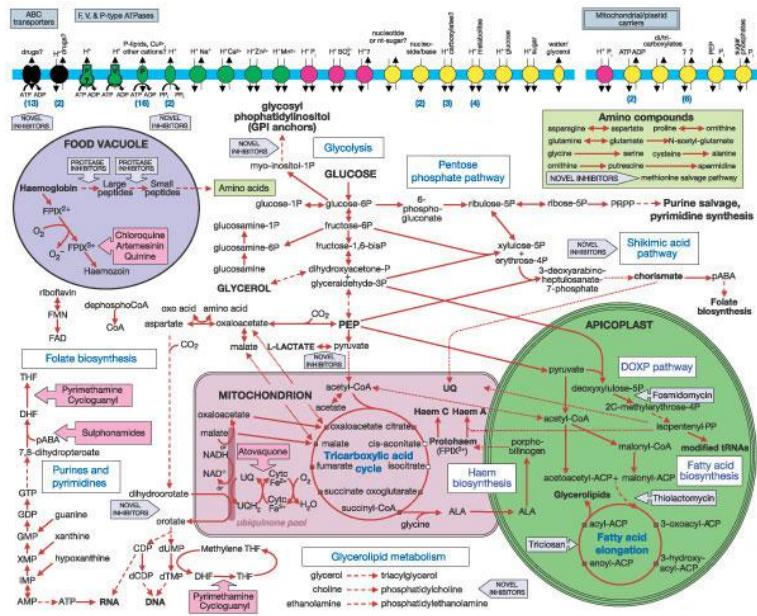
- Presentation
- Introduction and Background
- The problem: Interpreting gene lists
- Annotations and annotation databases
- The Gene Ontology Resource
- Gene list analysis using the GO and relatives
- Existing tools for pathway analysis

Introduction & Background

Health, disease and pathways

Metabolism is a complex network of chemical reactions within the confines of a cell that can be analyzed in self-contained parts called ***pathways***

One can generally assume that “normal” metabolism is what happens in healthy state or, reciprocally, that disease can *be associated with some type of alteration in metabolism*.



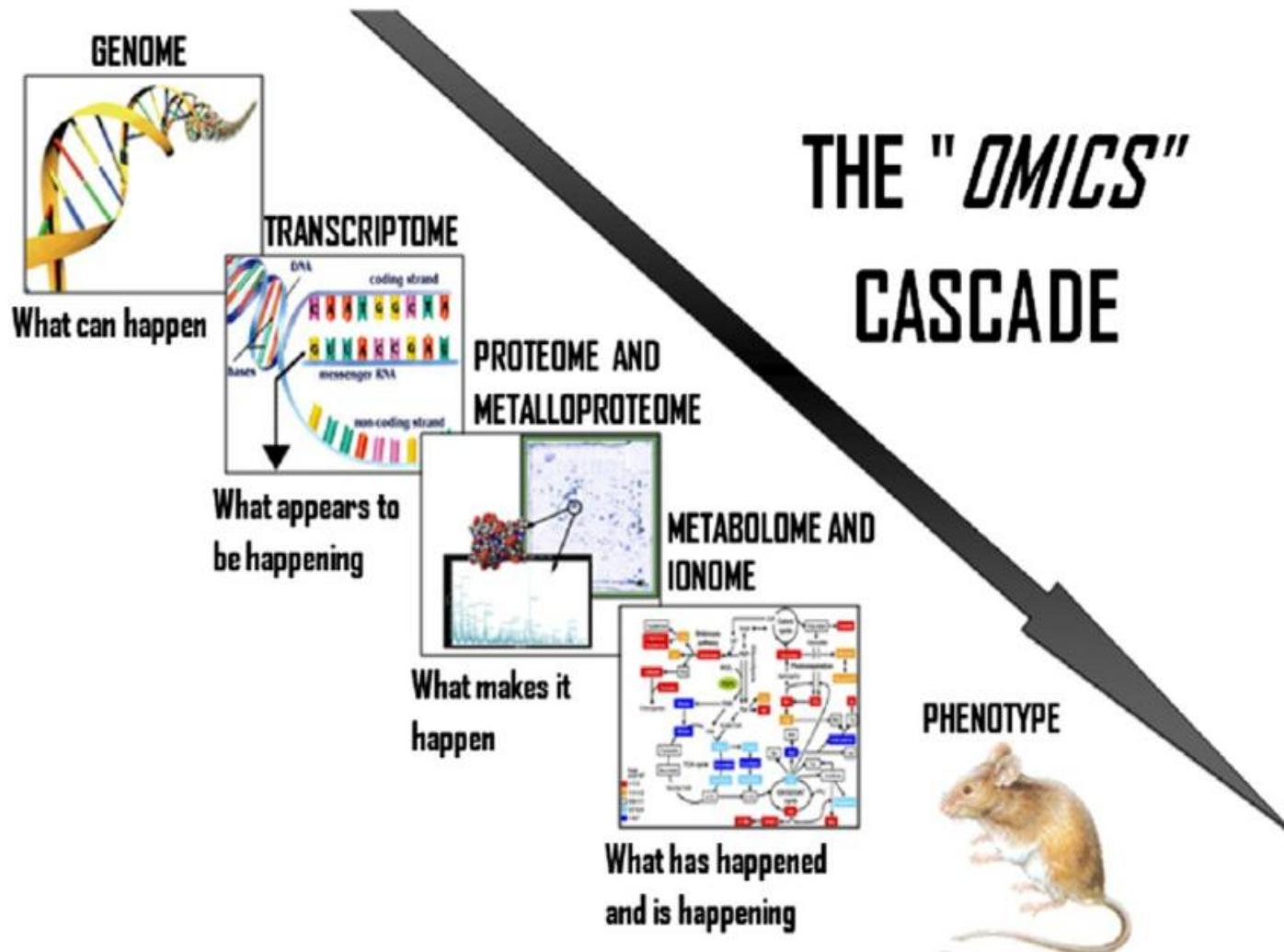
Pathways altered in ALZHEIMER disease

Characterization of disease can be attempted by studying how this affects or disrupts pathways
That's what Pathway Analysis is about (more or less)

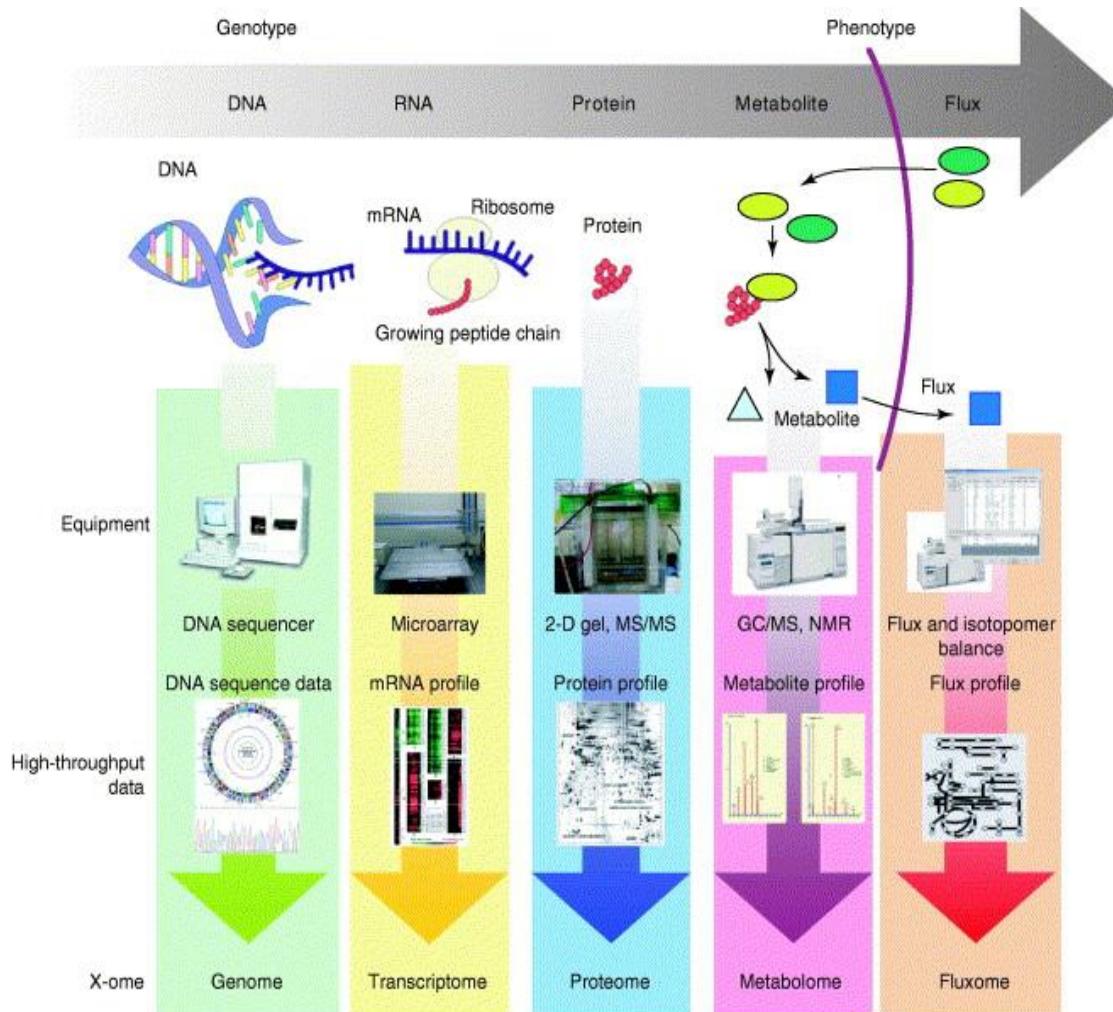
Pathway Analysis

- The term Pathway or Network Analysis denotes *any analytic technique that benefits from biological pathway or molecular network information to gain insight into a biological system.* (Creixell et alt, Nature Methods 2015 (12 (7))
- To be more specific, Pathway Analysis methods rely on high throughput information provided by omics technologies to:
 - Contextualize findings to help understand the mechanism of disease
 - Identify genes/proteins associated with the etiology of a disease
 - Predict drug targets
 - Understand how to therapeutically intervene in disease processes
 - Conduct target literature searches
 - Integrate diverse biological information

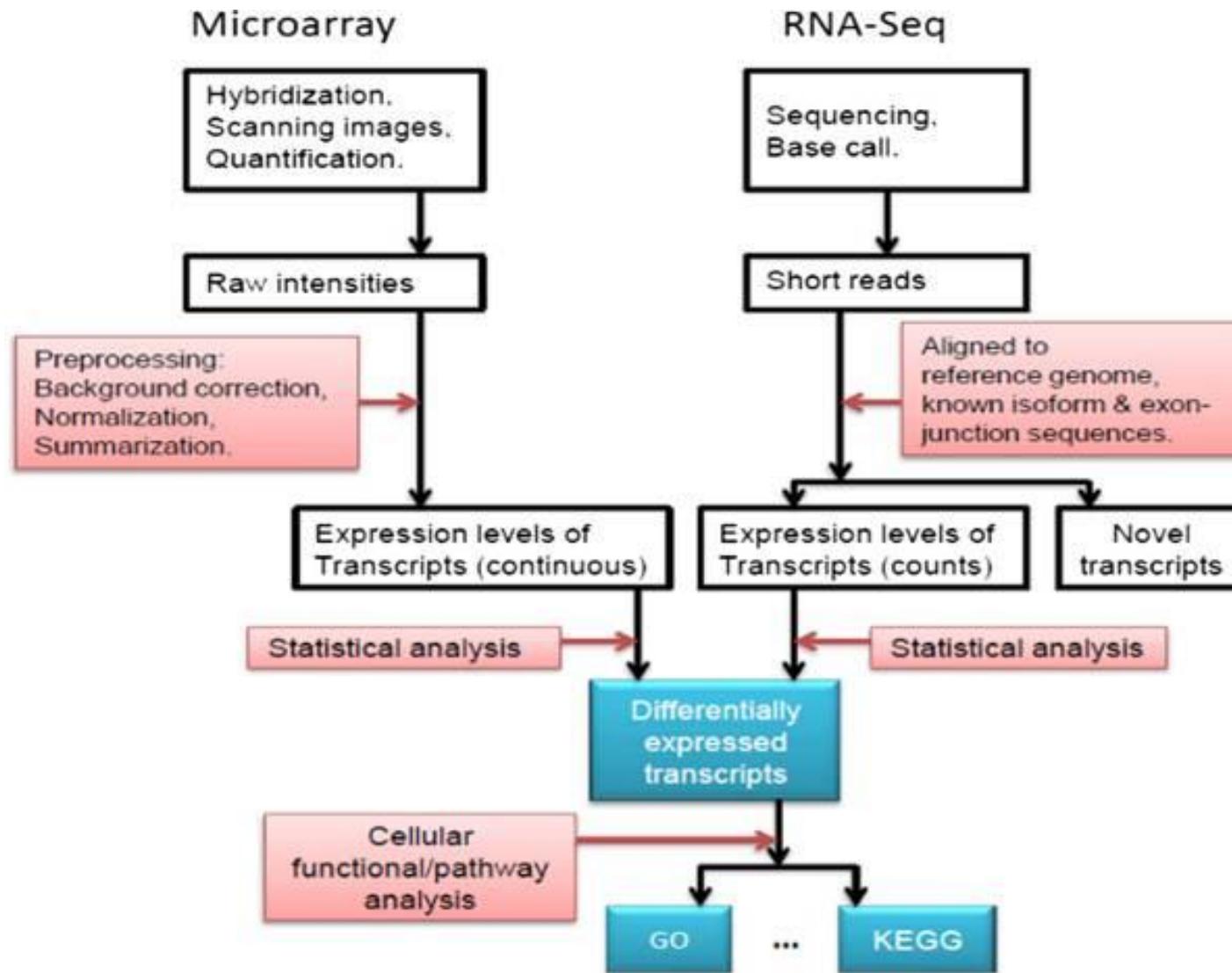
Biology: The “omes”



(Bio)technology: The “omics”

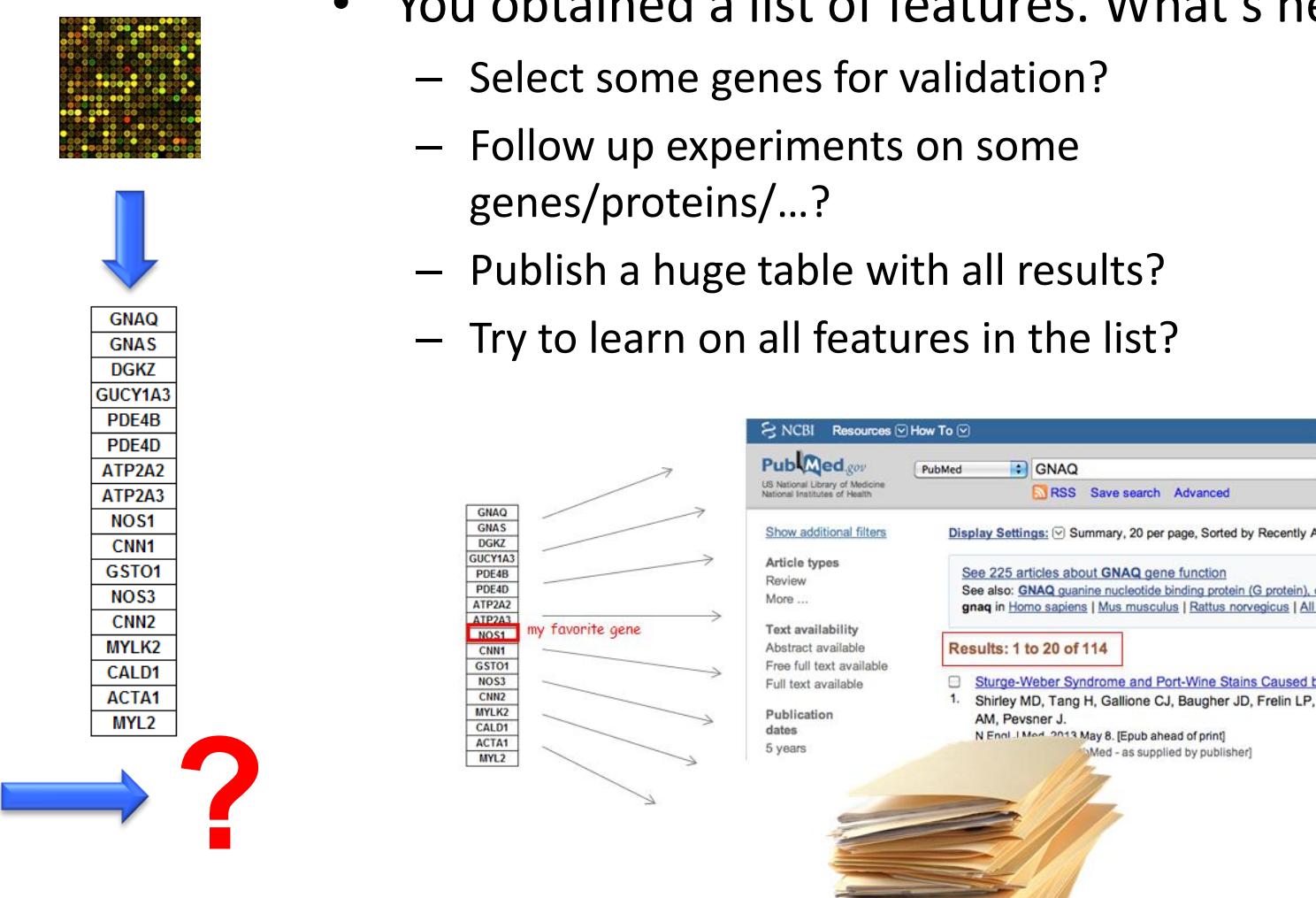


The life-cycle of an omics-based study



Managing Gene Lists

The (in)famous “*where to now?*” question



From gene lists to *Pathway Analysis*

- Gene lists contain useful information
 - This can be extracted from databases
 - Generically described as ***Gene Annotation***
- Besides, we may obtain information from the analysis of *gene sets*
 - Genes don't act individually, rather in groups → More ***realistic*** approach
 - There are less gene sets than individual genes → Relatively ***simpler*** to manage
 - Generically described as ***Pathway Analysis***

Case study 1

- Lists *AvsB*, *AvsL* and *BvsL* contain the IDs of genes selected by being differentially expressed between three types of breast cancer tumors.
 - Farmer P, Bonnefoi H, Becette V, Tubiana-Hulin M et al. Identification of molecular apocrine breast tumours by microarray analysis. *Oncogene* 2005 Jul 7;24(29):4660-71. PMID: [15897907](#)
- See the analysis that generates the list in:
https://github.com/alexsanchezpla/scripts/tree/master/Exemple_Analisis_BioC

Gene Lists and Annotations

Gene and Protein Identifiers

- Identifiers (IDs) are ideally unique, stable names or numbers that help track database records
 - E.g. Social Insurance Number, Entrez Gene ID 41232
- But, information on features is stored in many databases...
 - Genes have many IDs
- Records for: Gene, DNA, RNA, Protein
 - Important to recognize the correct record type
 - E.g. Entrez Gene records don't store sequence. They link to DNA regions, RNA transcripts and proteins e.g. in RefSeq, which stores sequence.

GNAQ
GNAS
DGKZ
GUCY1A3
PDE4B
PDE4D
ATP2A2
ATP2A3
NOS1
CNN1
GSTO1
NOS3
CNN2
MYLK2
CALD1
ACTA1
MYL2

Common Identifiers

Gene

Ensembl ENSG00000139618

Entrez Gene 675

Unigene Hs.34012

RNA transcript

GenBank BC026160.1

RefSeq NM_000059

Ensembl ENST00000380152

Protein

Ensembl ENSP00000369497

RefSeq NP_000050.2

UniProt BRCA2_HUMAN or

A1YBP1_HUMAN

IPI IPI00412408.1

EMBL AF309413

PDB 1MIU

Species-specific

HUGO HGNC BRCA2

MGI MGI:109337

RGD 2219

ZFIN ZDB-GENE-060510-3

FlyBase CG9097

WormBase WBGene00002299 or ZK1067.1

SGD S000002187 or YDL029W

Annotations

InterPro IPR015252

OMIM 600185

Pfam PF09104

Gene Ontology GO:0000724

SNPs rs28897757

Experimental Platform

Affymetrix 208368_3p_s_at

Agilent A_23_P99452

CodeLink GE60169

Illumina GI_4502450-S

Red =

Recommended

Identifier Mapping

- There are many IDs!
 - Software tools recognize only a handful
 - May need to map from your gene list IDs to standard IDs
- Four main uses
 - Searching for a favorite gene name
 - Link to related resources
 - Identifier translation
 - E.g. Proteins to genes, Affy ID to Entrez Gene
 - Merging data from different sources
 - Find equivalent records

ID Challenges

- Avoid errors: map IDs correctly
 - Beware of 1-to-many mappings
- Gene name ambiguity – not a good ID
 - e.g. FLJ92943, LFS1, TRP53, p53
 - Better to use the standard gene symbol: TP53
- Excel error-introduction
 - OCT4 is changed to October-4 (paste as text)
- Problems reaching 100% coverage
 - E.g. due to version issues
 - Use multiple sources to increase coverage

Zeeberg BR et al. *Mistaken identifiers: gene name errors can be introduced inadvertently when using Excel in bioinformatics*
BMC Bioinformatics. 2004 Jun 23;5:80

Retraction: Hes1 is a target of microRNA-23 during retinoic-acid-induced neuronal differentiation of NT2 cells

Hiroaki Kawasaki & Kazunari Taira

Nature **423**, 838–842 (2003).

In this Article, the messenger RNA that is identified to be a target of microRNA-23 (miR-23) is from the gene termed human 'homolog of ES1' (HES1), accession number Y07572, and not from the gene encoding the transcriptional repressor 'Hairy enhancer of split' HES1 (accession number NM_00524) as stated in our paper. We incorrectly identified the gene because of the confusing nomenclature. The function of HES1 Y07572 is unknown but the encoded protein shares homology with a protein involved in isoprenoid biosynthesis. Our experiments in NT2 cells had revealed that the protein levels of the repressor Hes1 were diminished by miR-23. Although we have unpublished data that suggest the possibility that miR-23 might also interact with Hes1 repressor mRNA, the explanation for the finding that the level of repressor Hes1 protein decreases in response to miR-23 remains undefined with respect to mechanism and specificity. Given the interpretational difficulties resulting from our error, we respectfully retract the present paper. Further studies aimed at clarifying the physiological role of miR-23 will be submitted to a peer-reviewed journal subject to the outcome of our ongoing research.

Use ID converters to prepare list

DAVID Bioinformatics Resources 2007
National Institute of Allergy and Infectious Diseases (NIAID), NIH

Gene Accession Conversion Tool

Summary
The possible choices for ambiguous genes
The possible choices for each individual ambiguous gene

Conversion Summary
ID Count In DAVID DB Conversion
157 IDs Yes Successful
0 IDs Yes None
0 IDs No NA
1 IDs Ambiguous Pending
Total Unique User IDs: 166

Genes that have been converted. Right-click to Download the list Help Save the results
Submit the converted genes to DAVID for other analytical tools!!

From To Species David Gene Name

"1112_G_AT	5684	HOMO SAPIENS	NEURAL CELL ADHESION MOLECULE 1
"1331_S_AT	8718	HOMO SAPIENS	TUMOR NECROSIS FACTOR RECEPTOR SUPERFAMILY, MEMBER 25
"1355_G_AT	4915	HOMO SAPIENS	NEUROTROPHIC TYROSINE KINASE, RECEPTOR, TYPE 2
"1372_AT	7130	HOMO SAPIENS	TUMOR NECROSIS FACTOR, ALPHA-INDUCED PROTEIN 6
"1391_S_AT	1572	HOMO SAPIENS	CYTOCHROME P450, FAMILY 4, SUBFAMILY A, POLYPEPTIDE 11
"1403_S_AT	6332	HOMO SAPIENS	CHEMOKINE (C-C MOTIF) LIGAND 5
"1419_G_AT	4843	HOMO SAPIENS	NITRIC OXIDE SYNTHASE 2A (INDUCIBLE, HEPATOCYTES)
"1575_AT	5243	HOMO SAPIENS	ATP-BINDING CASSETTE, SUB-FAMILY B (MDR/TAP), MEMBER 1
"1645_AT	3814	HOMO SAPIENS	KISS-1 METASTASIS-SUPPRESSOR
"1786_AT	10461	HOMO SAPIENS	C-MER PROTO-ONCOGENE TYROSINE KINASE
3558	ENTREZ GENE ID	Convert	FIBROBLAST GROWTH FACTOR 3 (MURINE MAMMARY TUMOR VIRUS INTEGRATION SITE V-INT-2...)
3558	GI_ACCESSION	Convert	GROWTH DIFFERENTIATION FACTOR 15

Summary of Ambiguous Gene IDs
ID Count Possible Source Convert All
1 ENTREZ_GENE_ID
1 GI_ACCESSION

Possible Sources for Ambiguous IDs
Ambiguous ID Possibility Convert
3558 ENTREZ_GENE_ID
3558 GI_ACCESSION

Species of converted gene IDs
Converted gene IDs
Users' input gene IDs
*Users' decision for ambiguous IDs

g:Profiler

Welcome! Contact FAQ R / APIs Beta Archive

[?] Organism
Homo sapiens

[?] Target database
ENSG

[?] Output type
Table (HTML)

Convert IDs Clear

[?] Query (genes, proteins, probes, term)
Interpret query as chromosome
Numeric IDs treated as
AFFY_HUEX_1_0_ST_V2

J. Reimand, T. Arak, P. Adler, L. Kolberg, S. Reisberg, H. Peterson, J. Vilu: g:Profiler -- a web server for functional interpretation of gene lists (2016 update) Nucleic Acids Research

Recommendations

- For proteins and genes
 - (doesn't consider splice forms)
 - Map everything to Entrez Gene IDs or Official Gene Symbols using an appropriate tool, such as R/Bioc, or a spreadsheet if no other option.
- If 100% coverage desired, manually curate missing mappings using multiple resources
- Be careful of Excel auto conversions – especially when pasting large gene lists!
 - Remember to format cells as 'text' before pasting

The Gene Ontology (at last)

Where is pathway information?

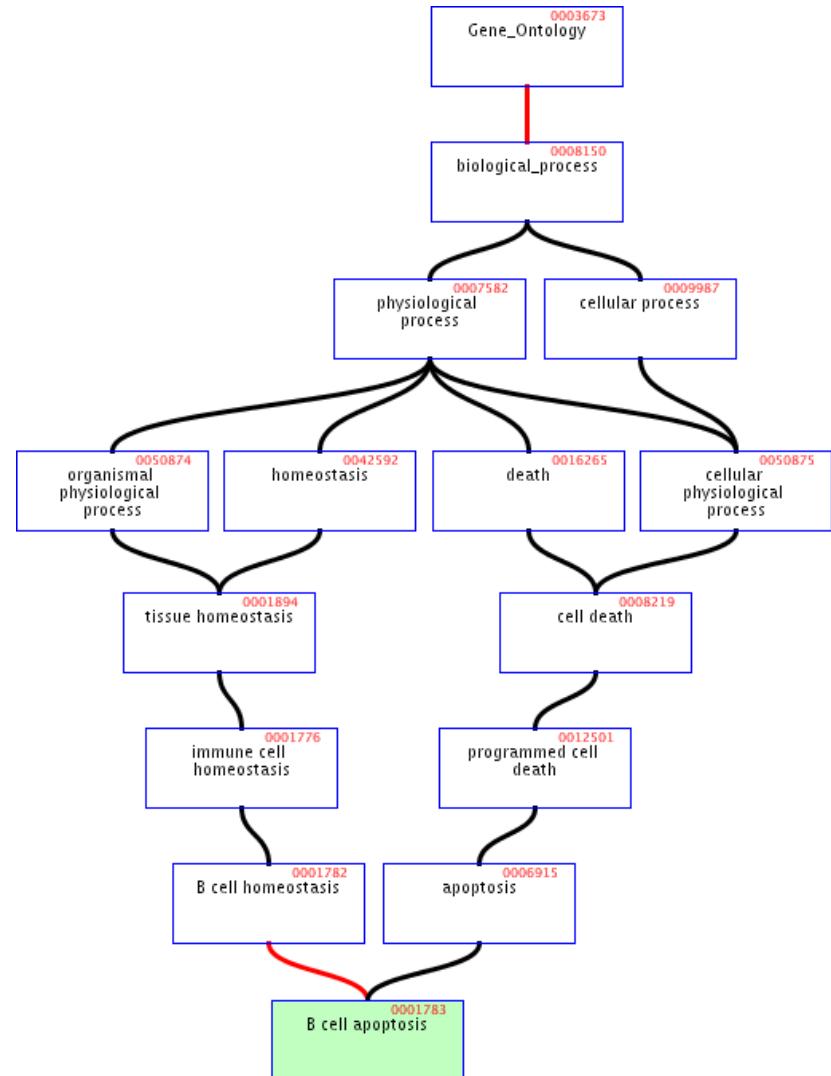
- Pathways
 - Gene Ontology biological process, pathway databases e.g. Reactome
- Other annotations
 - Gene Ontology molecular function, cell location
 - Chromosome position
 - Disease association
 - DNA properties (TF binding sites, gene structure (intron/exon), SNPs, ...)
 - Transcript properties (Splicing, 3' UTR, microRNA binding sites, ...)
 - Protein properties (Domains, 2ry and 3ry structure, PTM sites)
 - Interactions with other genes

What is the Gene Ontology (GO)?

- Set of biological phrases (terms) which are applied to genes:
 - protein kinase
 - apoptosis
 - membrane
- Dictionary: term definitions
- Ontology: A formal system for describing knowledge
- www.geneontology.org

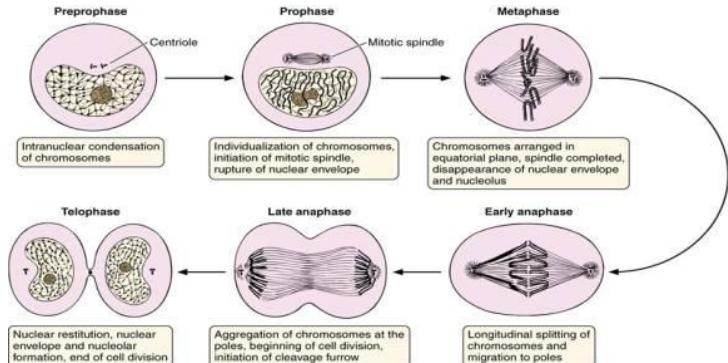
GO Structure

- Terms are related within a hierarchy
 - is-a
 - part-of
- Describes multiple levels of detail of gene function
- Terms can have more than one parent or child

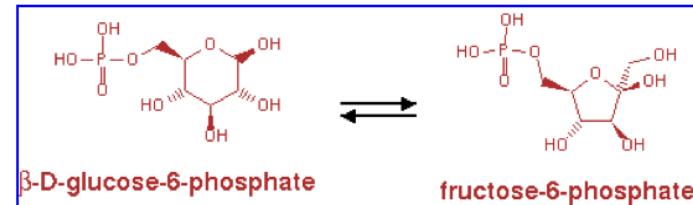
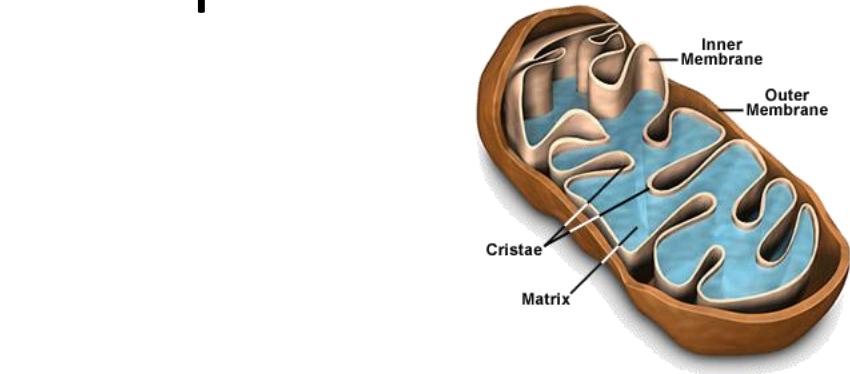


What is covered by the GO?

- GO terms divided into three aspects:
 - cellular component
 - molecular function
 - biological process



**Cell
division**



**glucose-6-phosphate
isomerase activity**

Part 1/2: Terms

- Where do GO terms come from?
 - GO terms are added by editors at EBI and gene annotation database groups
 - Terms added by request
 - Experts help with major development

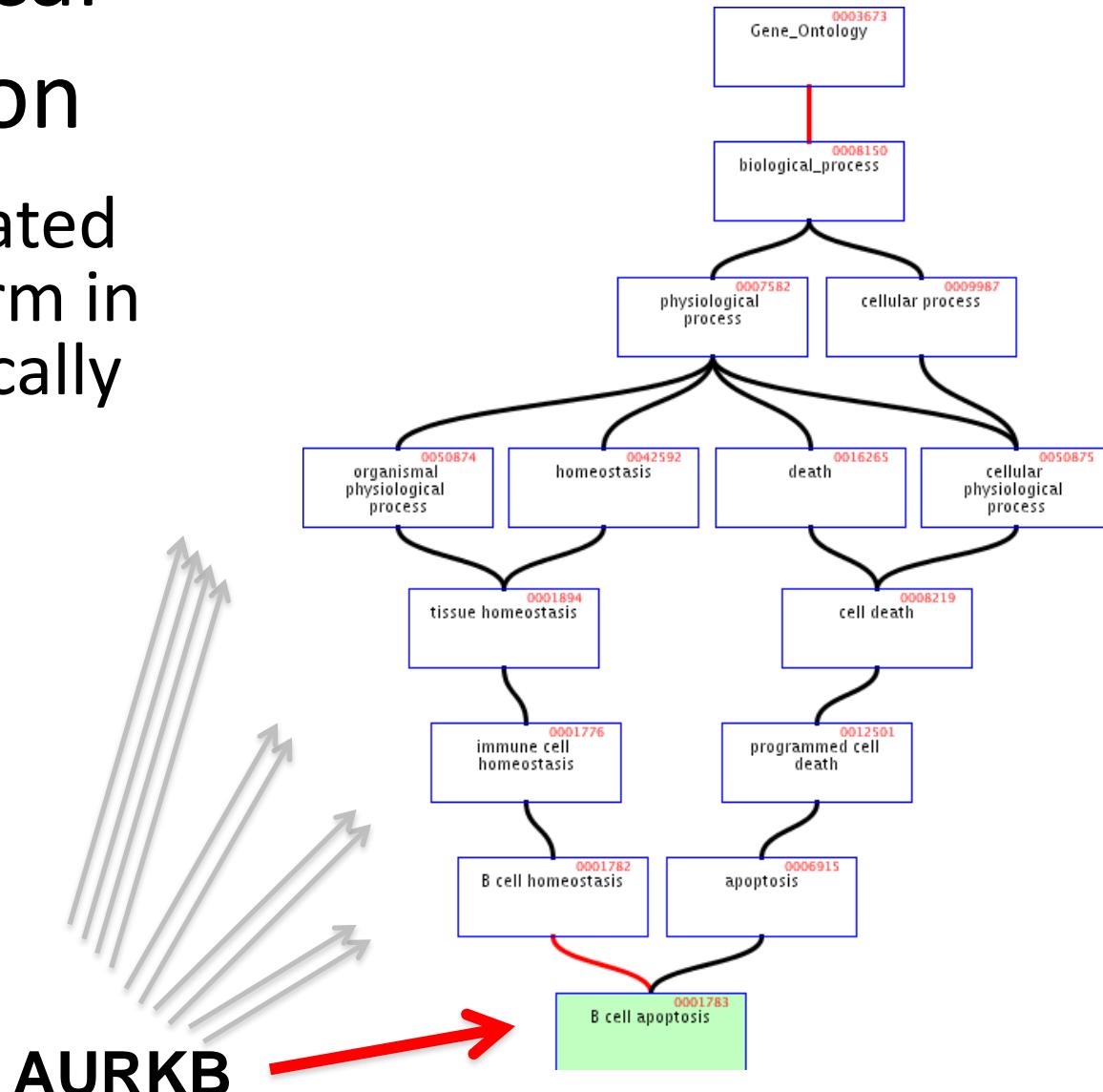
	<u>Jun 2012</u>	<u>Apr 2015</u>	<u>increase</u>
Biological process	23,074	28,158	22%
Molecular function	9,392	10,835	15%
Cellular component	2,994	3,903	30%
total	37,104	42,896	16%

Part 2/2: Annotations

- Genes are linked, or associated, with GO terms by trained curators at genome databases
 - Known as ‘gene associations’ or GO annotations
 - Multiple annotations per gene
- Some GO annotations created automatically (without human review)

Hierarchical annotation

- Genes annotated to specific term in GO automatically added to all parents of that term



Annotation Sources

- Manual annotation
 - Curated by scientists
 - High quality
 - Small number (time-consuming to create)
 - Reviewed computational analysis
- Electronic annotation
 - Annotation derived without human validation
 - Computational predictions (accuracy varies)
 - Lower ‘quality’ than manual codes
- Key point: be aware of annotation origin

Evidence Types

- **Experimental Evidence Codes**
 - EXP: Inferred from Experiment
 - IDA: Inferred from Direct Assay
 - IPI: Inferred from Physical Interaction
 - IMP: Inferred from Mutant Phenotype
 - IGI: Inferred from Genetic Interaction
 - IEP: Inferred from Expression Pattern
- **Computational Analysis Evidence Codes**
 - ISS: Inferred from Sequence or Structural Similarity
 - ISO: Inferred from Sequence Orthology
 - ISA: Inferred from Sequence Alignment
 - ISM: Inferred from Sequence Model
 - IGC: Inferred from Genomic Context
 - RCA: inferred from Reviewed Computational Analysis
- **Author Statement Evidence Codes**
 - TAS: Traceable Author Statement
 - NAS: Non-traceable Author Statement
- **Curator Statement Evidence Codes**
 - IC: Inferred by Curator
 - ND: No biological Data available
- **IEA: Inferred from electronic annotation**

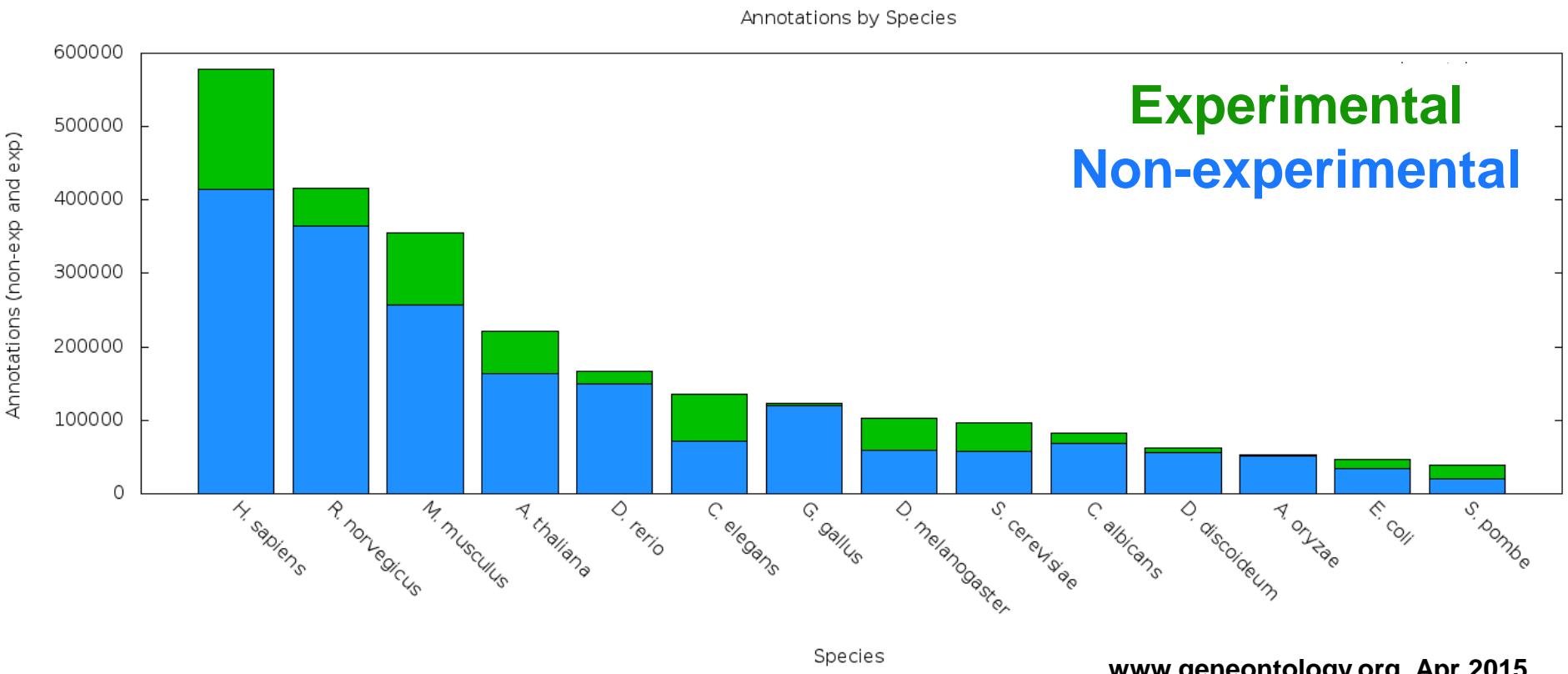


<http://www.geneontology.org/GO.evidence.shtml>

Species Coverage

- All major eukaryotic model organism species and human
- Several bacterial and parasite species through TIGR and GeneDB at Sanger
- New species annotations in development
- Current list:
 - <http://www.geneontology.org/GO.downloads.annotations.shtml>

Variable Coverage



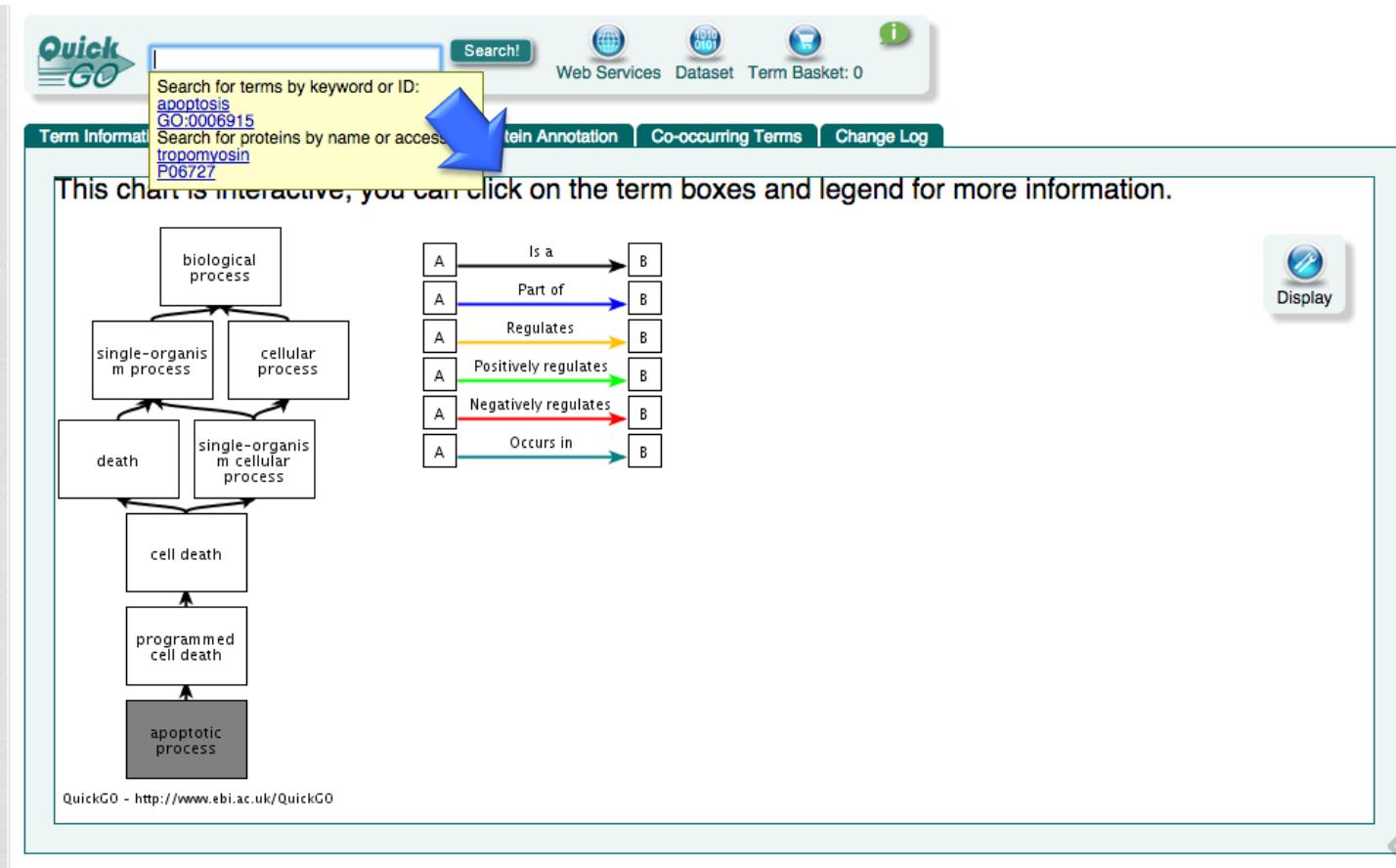
Contributing Databases

- [Berkeley *Drosophila* Genome Project \(BDGP\)](#)
- [dictyBase](#) (*Dictyostelium discoideum*)
- [FlyBase](#) (*Drosophila melanogaster*)
- [GeneDB](#) (*Schizosaccharomyces pombe*, *Plasmodium falciparum*, *Leishmania major* and *Trypanosoma brucei*)
- [UniProt Knowledgebase](#) (Swiss-Prot/TrEMBL/PIR-PSD) and [InterPro](#) databases
- [Gramene](#) (grains, including rice, *Oryza*)
- [Mouse Genome Database \(MGD\)](#) and [Gene Expression Database \(GXD\)](#) (*Mus musculus*)
- Rat Genome Database (RGD) (*Rattus norvegicus*)
- [Reactome](#)
- [Saccharomyces Genome Database \(SGD\)](#) (*Saccharomyces cerevisiae*)
- [The Arabidopsis Information Resource \(TAIR\)](#) (*Arabidopsis thaliana*)
- [The Institute for Genomic Research \(TIGR\)](#): databases on several bacterial species
- [WormBase](#) (*Caenorhabditis elegans*)
- [Zebrafish Information Network \(ZFIN\)](#): (*Danio rerio*)

GO Software Tools

- GO resources are freely available to anyone without restriction
 - ontologies, gene associations and tools developed by GO
- Other have used GO to create versatile tools
 - <https://omictools.com/gene-ontologies-category>
 - <https://omictools.com/gene-set-analysis-category>

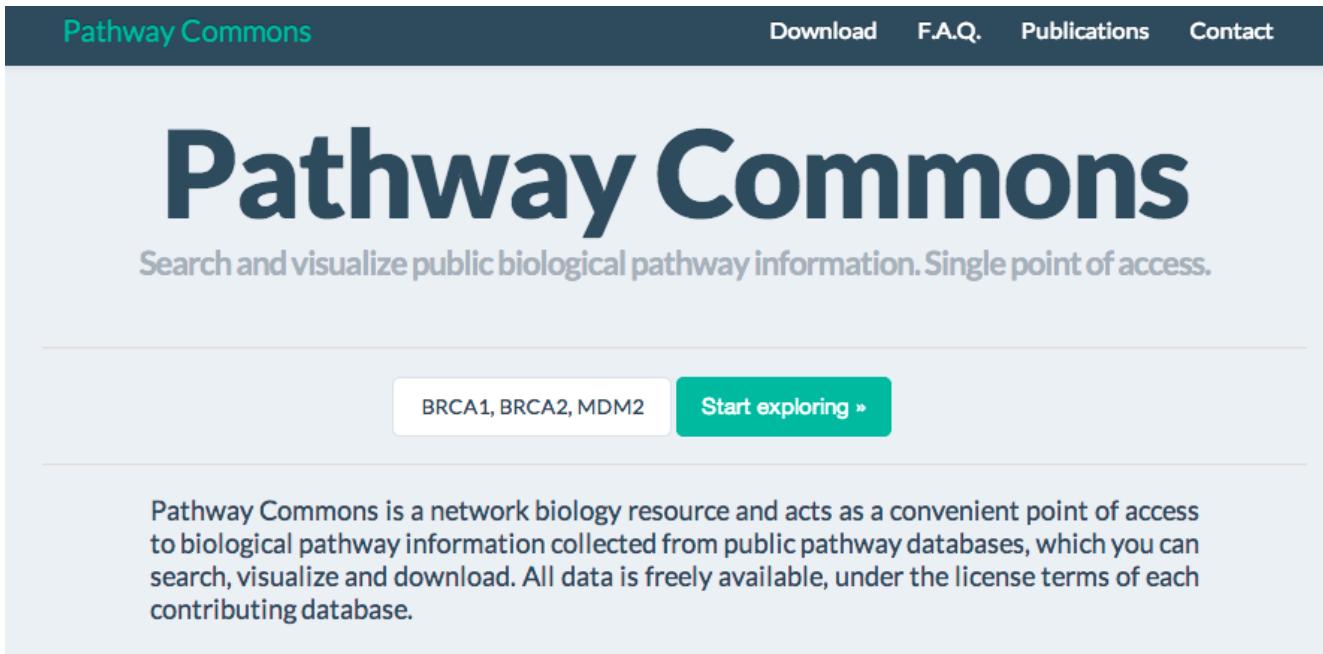
Accessing GO: QuickGO



<http://www.ebi.ac.uk/QuickGO/>

Pathway Databases

- <http://www.pathguide.org/> lists ~550 pathway related databases
- MSigDB: <http://www.broadinstitute.org/gsea/msigdb/>
- <http://www.pathwaycommons.org/> collects major ones



The screenshot shows the homepage of Pathway Commons. At the top, there is a dark header bar with the "Pathway Commons" logo in white. To the right of the logo are links for "Download", "F.A.Q.", "Publications", and "Contact". Below the header, the main title "Pathway Commons" is displayed in a large, bold, dark blue font. Underneath the title, a subtitle reads "Search and visualize public biological pathway information. Single point of access." A horizontal line separates this from the search interface below. The search interface includes a text input field containing "BRCA1, BRCA2, MDM2" and a green "Start exploring »" button. Another horizontal line is located below the search area. At the bottom of the page, a descriptive paragraph explains the purpose of Pathway Commons: "Pathway Commons is a network biology resource and acts as a convenient point of access to biological pathway information collected from public pathway databases, which you can search, visualize and download. All data is freely available, under the license terms of each contributing database."

Sources of Gene Attributes

- Ensembl BioMart (general)
 - <http://www.ensembl.org>
- Entrez Gene (general)
 - <http://www.ncbi.nlm.nih.gov/sites/entrez?db=gene>
- Model organism databases
 - E.g. SGD: <http://www.yeastgenome.org/>

Ensembl BioMart

- Convenient access to gene list annotation

The screenshot shows the Ensembl BioMart search interface. On the left, a sidebar lists datasets: 'Homo sapiens genes (GRCh37)' and 'Ensembl Gene ID' and 'Ensembl Transcript'. The main area has two dropdown menus: 'Ensembl Genes 58' and 'Homo sapiens genes (GRCh37)'. Below these are sections for 'REGION', 'GENE', 'TRANSCRIPT EVENT', 'GENE ONTOLOGY', 'EXPRESSION', 'MULTI SPECIES COMPARISONS', 'PROTEIN DOMAINS' (with options for 'Limit to genes ...', 'Limit to genes with these family or domain IDs', 'Transmembrane domains', 'Signal domains'), and 'VARIATIONS'. To the right, three large arrows point downwards from the top section to the bottom section, which contains a summary of selected attributes: 'Features' (radio button selected), 'Homologs', 'Structures', 'Variations', 'Transcript Event', 'Sequences', 'GENE', 'EXTERNAL', 'EXPRESSION', and 'PROTEIN DOMAINS'.

Select genome

Select filters

Select attributes to download

Dataset
Homo sapiens genes (GRCh37)
Filters
[None selected]
Attributes
Ensembl Gene ID
Ensembl Transcript

Ensembl Genes 58

Homo sapiens genes (GRCh37)

REGION:
GENE:
TRANSCRIPT EVENT:
GENE ONTOLOGY:
EXPRESSION:
MULTI SPECIES COMPARISONS:
PROTEIN DOMAINS:
Limit to genes ...
with Protein feature scanprosite ID(s) Only
Excluded
Limit to genes with these family or domain IDs:
Ensembl Protein Family ID(s) [e.g. ENSFM00250000000002]
Transmembrane domains
Signal domains
VARIATIONS:

Features Homologs
Structures Variations
Transcript Event Sequences

GENE:
EXTERNAL:
EXPRESSION:
PROTEIN DOMAINS:

www.ensembl.org

Pathway Analysis

Overrepresentation Analysis

Gene Set Enrichment Analysis

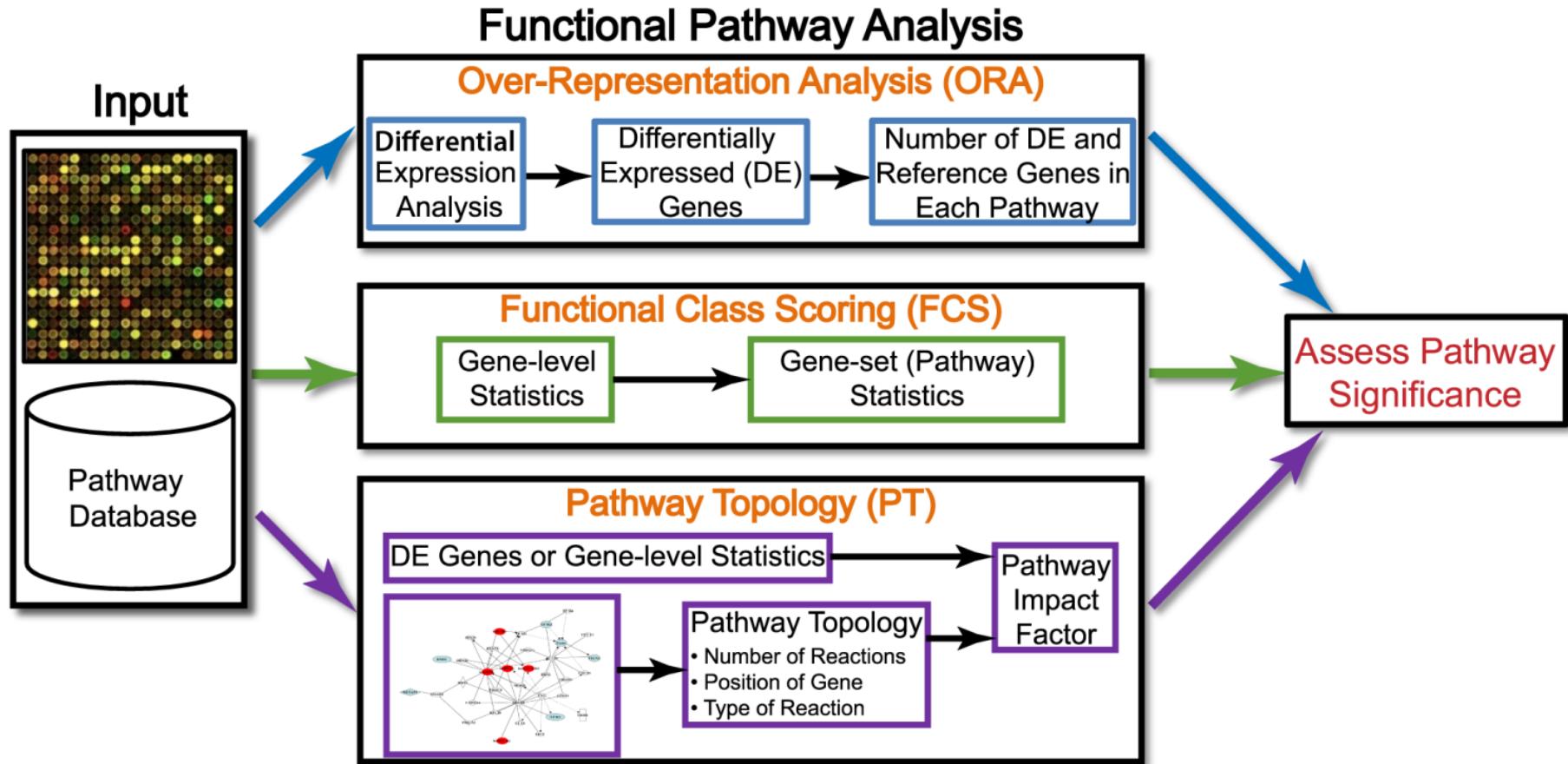
Pathway and Network Analysis

- Any type of analysis that involves pathway or network information
- Helps gain mechanistic insight into ‘omics’ data
 - Identifying a master regulator, drug targets, characterizing pathways active in a sample
- Most commonly applied to help interpret lists of genes
- Most popular type is pathway ***enrichment analysis***, but many others are useful

Benefits of Pathway Analysis

- Easier to interpret
 - Familiar concepts e.g. cell cycle
- Identifies possible causal mechanisms
- Predicts new roles for genes
- Improves statistical power
 - Fewer tests, aggregates data from multiple genes into one pathway
- More reproducible
 - E.g. gene expression signatures
- Facilitates integration of multiple data types

Types of Pathway Analysis



Khatri et alt. 10 years of Pathway Analysis

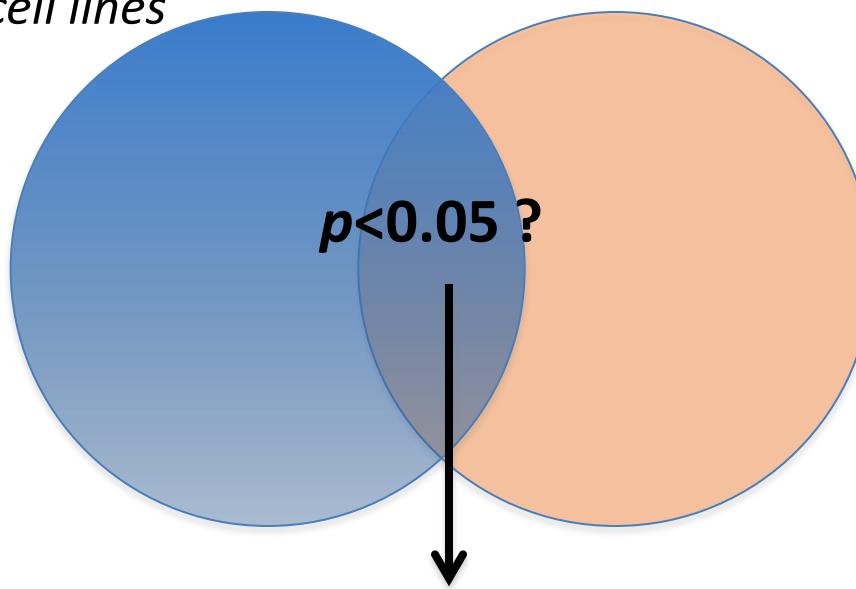
Types of input for Pathway Analysis

- **Gene list** (e.g. expression change > 2-fold)
 - Answers the question: **Are any gene sets surprisingly enriched (or depleted) in my gene list?**
 - Statistical test: Fisher's Exact Test (aka Hypergeometric test)
- **Ranked list** (e.g. by differential expression)
 - Answers the question: **Are any gene set ranked surprisingly high or low in my ranked list of genes?**
 - Statistical test: minimum hypergeometric test (+ others we won't discuss)

Over-representation analysis

Gene list from experiment:

Genes down-regulated in drug-sensitive brain cancer cell lines



Pathway information:
All genes known to be involved in Neurotransmitter signaling



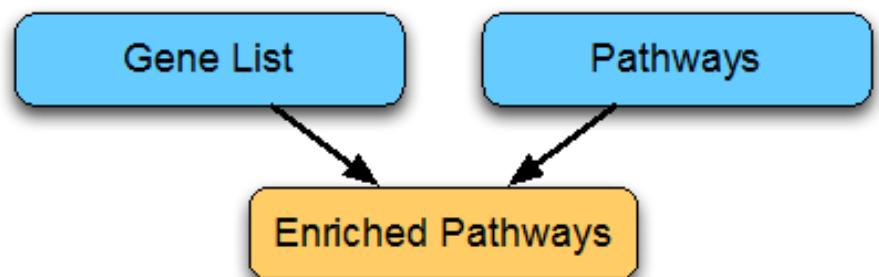
Test many pathways

Statistical test: are there more annotations in gene list than expected?

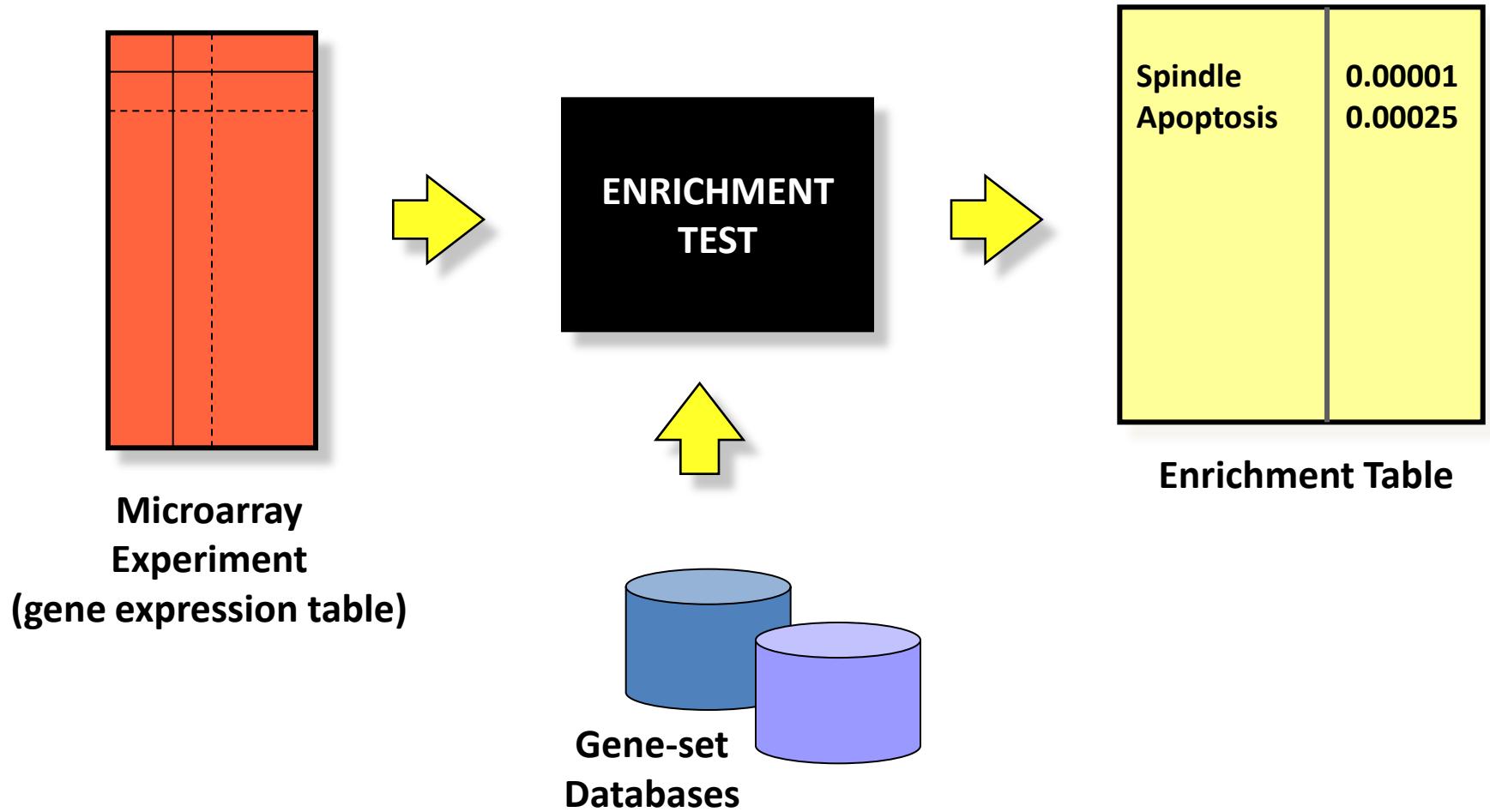
Hypothesis: drug sensitivity in brain cancer is related to reduced neurotransmitter signaling

Over-representation analysis

- Combines
 - Gene(feature) lists ← (Gen)omic experiment
 - Pathways and other gene annotations
 - Gene Ontology
 - Ontology Structure
 - Annotation
 - BioMart
 - Other resources



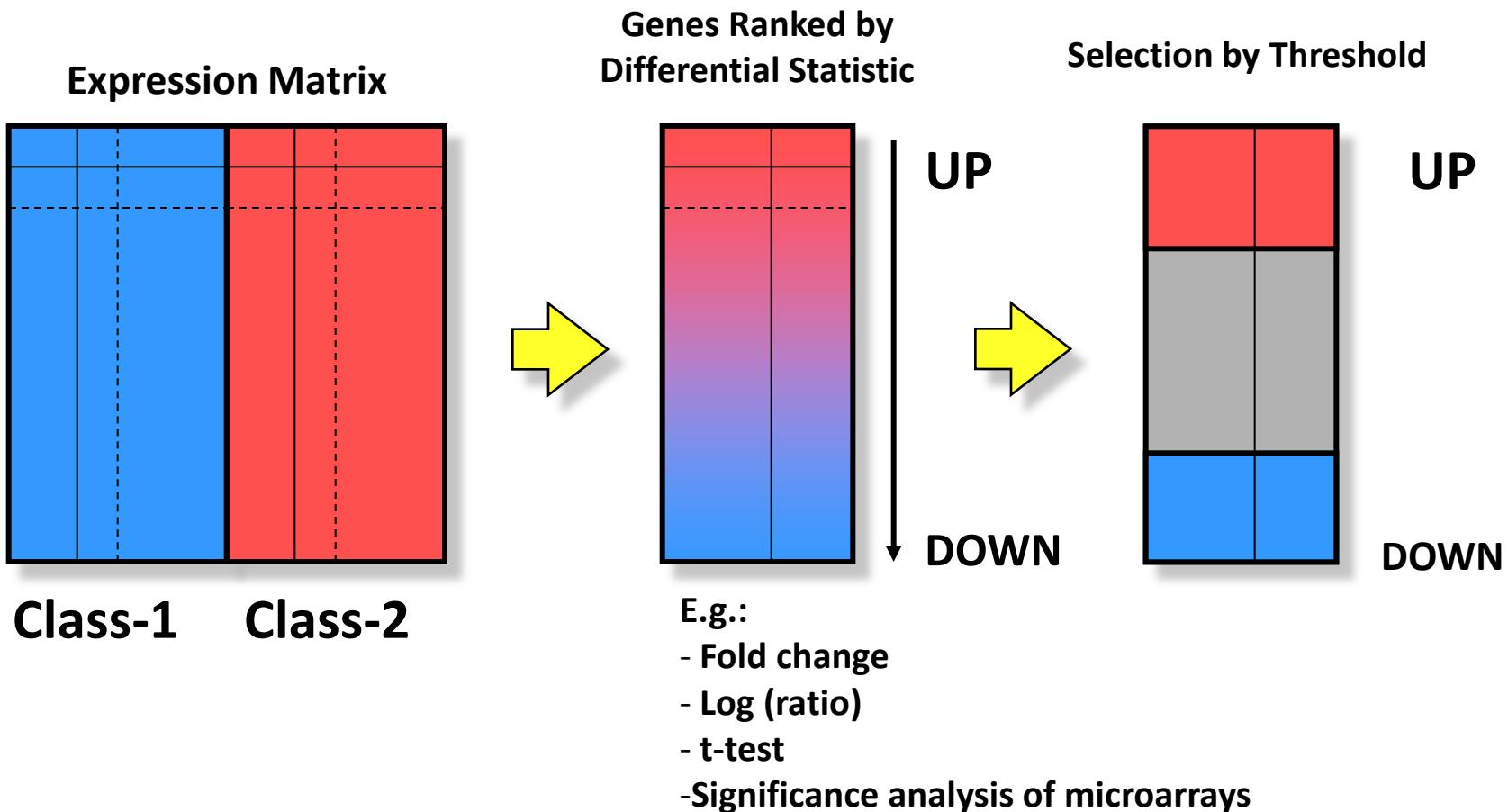
Enrichment Test



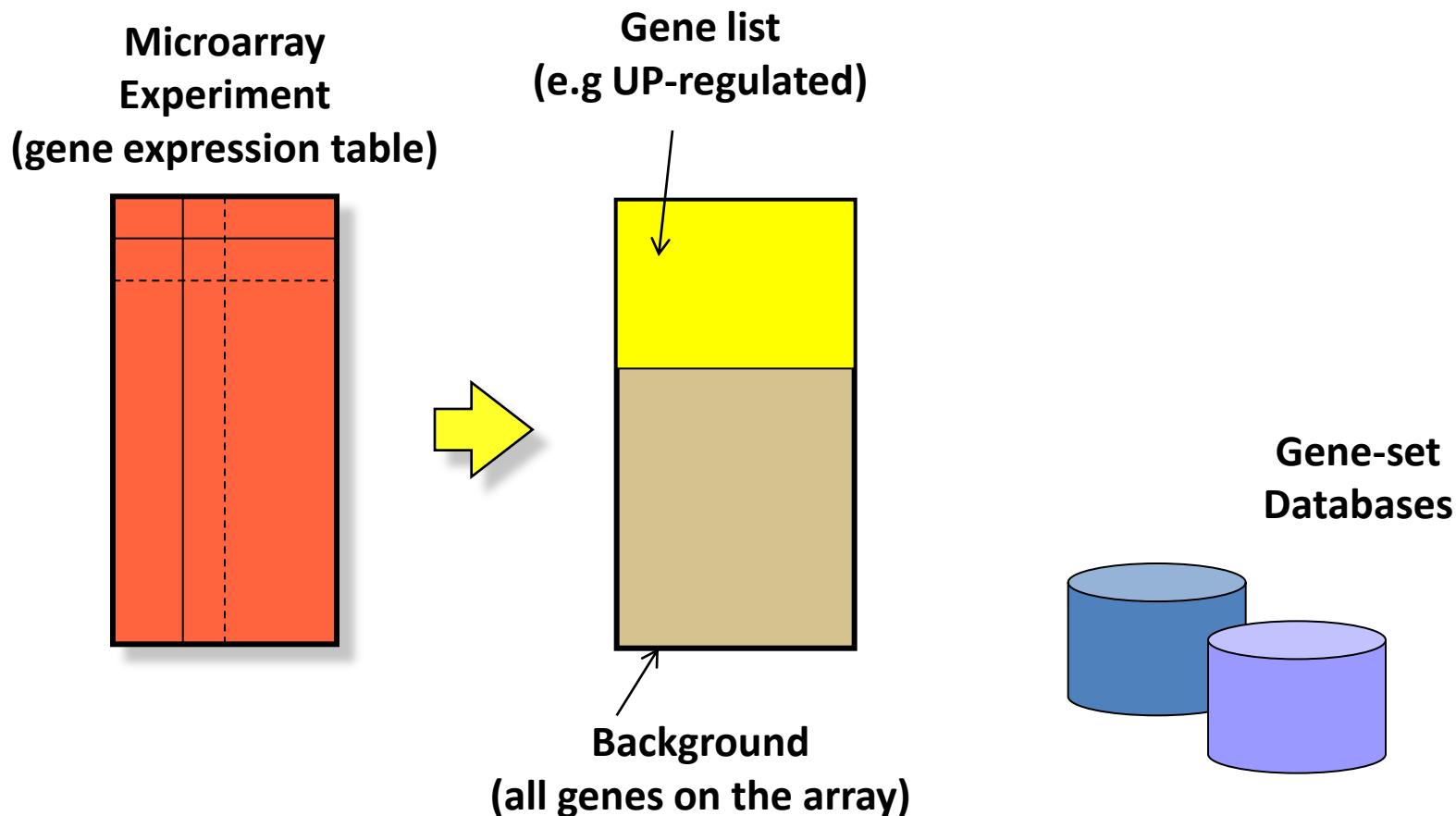
Gene list enrichment analysis

- Given:
 1. Gene list: e.g. RRP6, MRD1, RRP7, RRP43, RRP42 (yeast)
 2. Gene sets or annotations: e.g. Gene ontology, transcription factor binding sites in promoter
- Question: *Are any of the gene annotations surprisingly enriched in the gene list?*
- Details:
 - Where do the gene lists come from?
 - How to assess “surprisingly” (statistics)
 - How to correct for repeating the tests

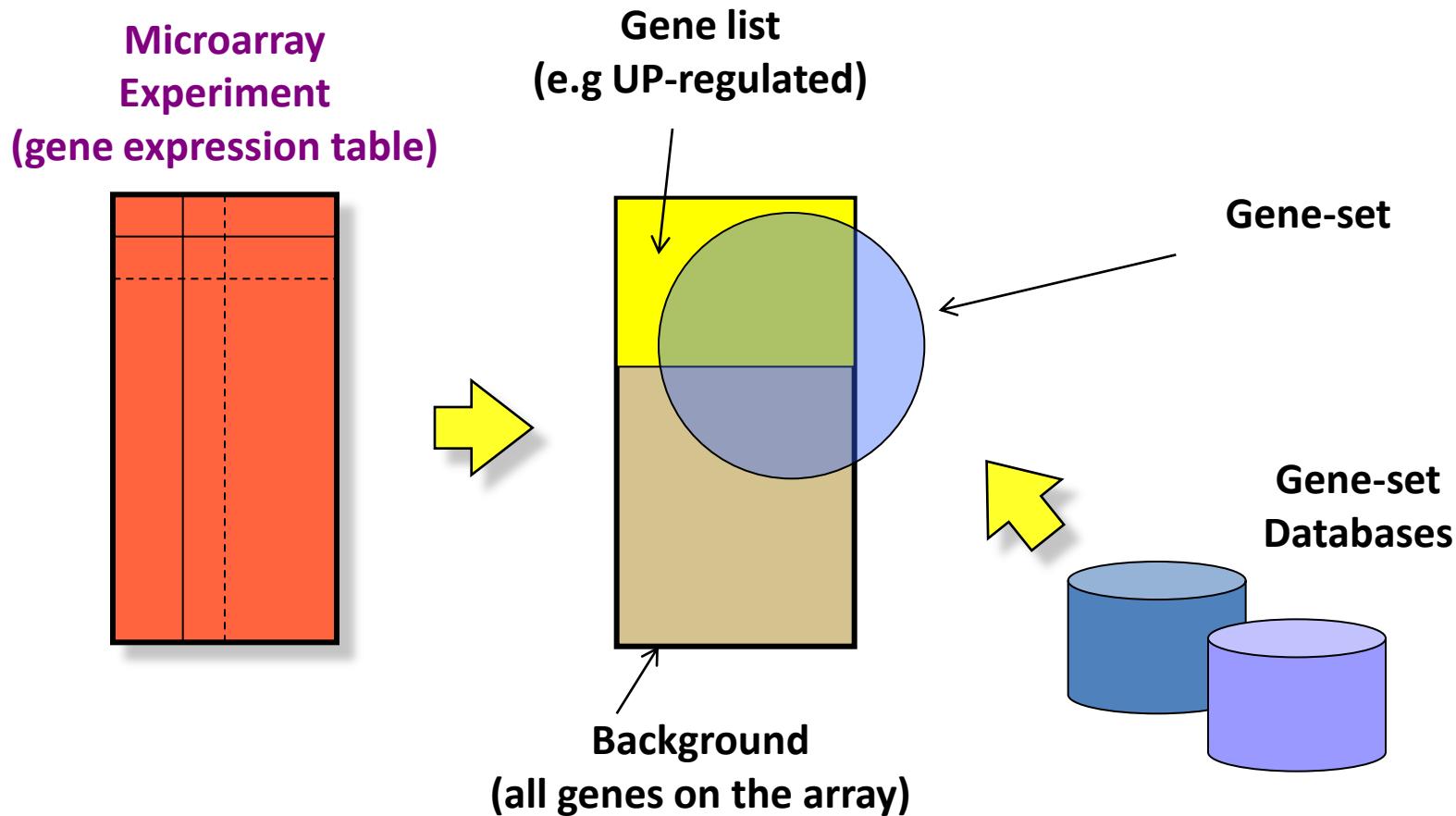
Two-class design for gene lists



Example gene list enrichment test



Example gene list enrichment test



Example gene list enrichment test

- Given a list of differentially expressed genes and a collection of gene sets apply the following strategy
 - For each gene set fill a 2x2 contingency table
 - Calculate p-value using Fisher test
 - Compute FDR to adjust p-values for doing many tests

	Differentially expressed	Not differentially expressed	TOTAL
In Gene Set	10	30	40
Not In Gene Set	390	3570	3960
TOTAL	400	3600	4000

Warning: Background must be carefully chosen!

Naive Analysis: A Fisher test using R

```
> GOnnnnCounts<- matrix(c(10, 30, 390, 3570),
+   nrow = 2, byrow=TRUE,
+   dimnames = list(GeneSet = c("In Gene Set", "Not in Gene Set"),
+                   Test =c("Differentially expressed", "Not Dif. Expr.")))
> GOnnnnCounts
      Test
GeneSet      Differentially expressed Not Dif. Expr.
  In Gene Set                      10            30
  Not in Gene Set                  390          3570
> fisher.test(GOnnnnCounts, alternative = "greater")
      Fisher's Exact Test for Count Data

data:  GOnnnnCounts
p-value = 0.004836
alternative hypothesis: true odds ratio is greater than 1
95 percent confidence interval:
 1.508343      Inf
sample estimates:
odds ratio
 3.049831
```

Recipe for gene list enrichment test

- **Step 1:** Define **gene list** (e.g. thresholding analyzed list) and **background list**,
- **Step 2:** Select gene sets to test for enrichment,
- **Step 3:** Run enrichment tests and correct for multiple testing, if necessary,
- **Step 4:** Interpret your enrichments
- **Step 5:** Publish! ;)

Possible problems with gene list test

- No “natural” value for the threshold
- Possible loss of statistical power due to thresholding
 - No resolution between significant signals with different strengths
 - Weak signals neglected
- Different results at different threshold settings
- Based on the wrong assumption of independent gene (or gene group) sampling, which increases false positive predictions

Alternative: Gene Set Testing

- A gene set
 - a group of genes with related functions.
 - sets of genes or pathways, for their association with a phenotype.
 - Examples: metabolic pathway, protein complex, or GO (gene ontology) category.
- Identified from a prior biological knowledge.
- May better reflect the true underlying biology.
- May be more appropriate units for analysis.

Gene Sets

Each row represents one gene set →

	A	B	C	D	E	F	G
1	chr10q24	Cytogenetic band	PITX3	SPFH1	NEURL	C10orf12	NDUFB8
2	chr5q23	Cytogenetic band	ALDH7A1	IL13	8-Sep	INF1	ACSL6
3	chr8q24	Cytogenetic band	HAS2	LRRC14	TSTA3	DGAT1	RECQL4
4	chr16q24	Cytogenetic band	RPL13	GALNS	FANCA	CPNE7	COTL1
5	chr13q14	Cytogenetic band	AKAP11	ARL11	ATP7B	C13orf1	C13orf9
6	chr7p21	Cytogenetic band	ARL4A	SCIN	GLCCI1	SP8	SOSTDC1
7	chr10q23	Cytogenetic band	SNCG	FER1L3	C10orf116	HHEX	TNKS2
8	chr14q12	Cytogenetic band	C14orf125	FOXG1C	HECTD1	SCFD1	AP4S1
9	chr13q13	Cytogenetic band	ALG5	RFXAP	DCAMKL1	MAB2L1	STOML3
10	chr1p34	Cytogenetic band	JMJD2A	MRPS15	HIVEP3	GJB3	CDCA8
11	chr10q21	Cytogenetic band	MBL2	C10orf70	DNAJC12	BICC1	CXXC6
12	chr10q21	Cytogenetic band	QUB1	QHAT2	LPIC2	QLC4CA1	QELQD2

If editing in excel, watch out for its tendency to auto-format gene sets (SEP8 becomes 8-Sep)

First column are gene set names. Duplicates are not allowed

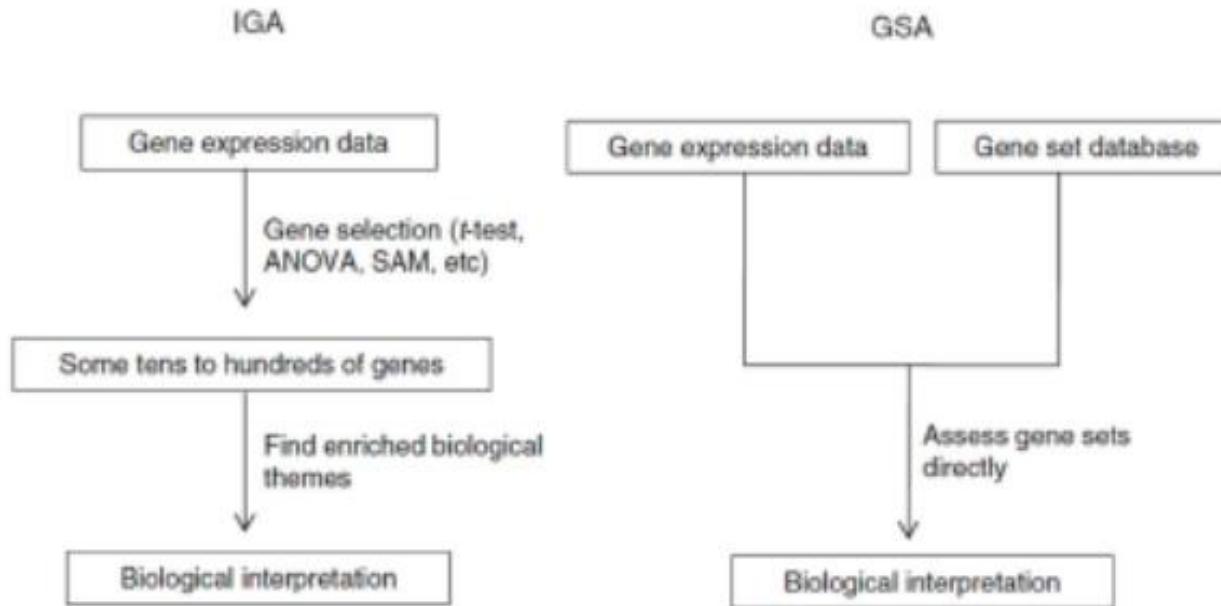
Second column contains a brief description. Its optional – you can fill in a dummy field (e.g. "na")

Unequal lengths (i.e # of genes) is allowed

MSigDB Collection	Subcollection	No. Gene Sets
C1: positional gene sets		326
C2: curated gene sets	CGP: chemical and genetic perturbations CP: Canonical pathways KEGG/Biocarta/REACTOME	3402
C3: motif gene sets	MIR: microRNA targets TFT: transcription factor targets	1320
C4: computational gene sets	CGN: cancer gene neighborhoods CM: cancer modules	221
C5: GO gene sets	BP: GO biological process CC: GO cellular component MF: GO molecular function	615
C6: oncogenic signatures		427
C7: immunologic signatures		431
		825
		233
		396
		189
		1910
	Total	10295

Gene Set (Enrichment) Analysis

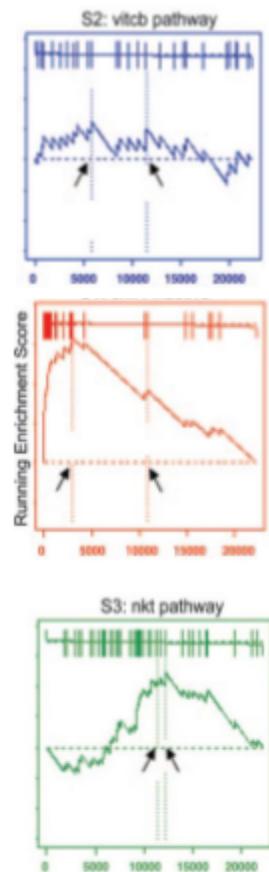
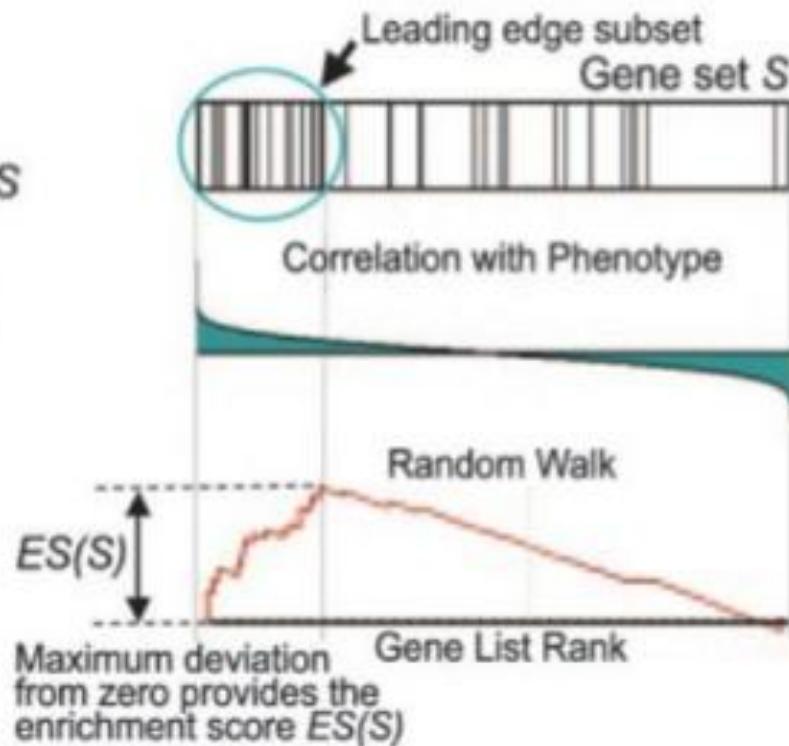
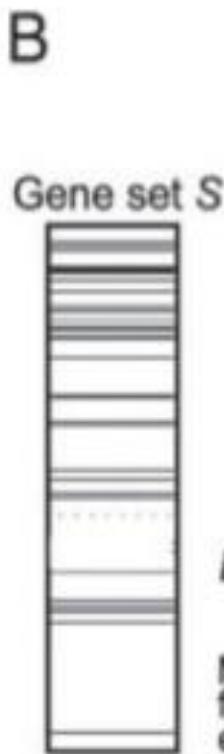
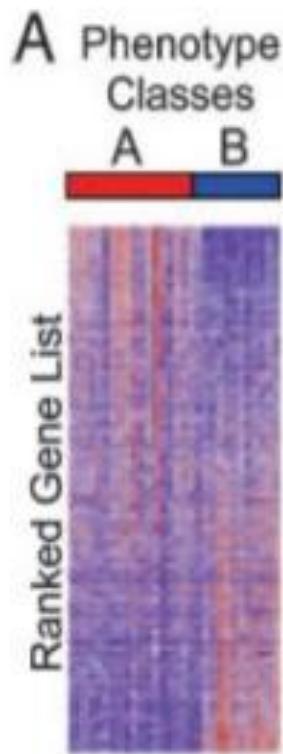
- Introduced by Mootha (2003) as an alternative to ORA.
- It aims to identify gene sets with *subtle but coordinated expression changes* that cannot be detected by ORA methods.
 - Weak changes in individual genes gathered to large gene sets can show a significant pattern.
- Results of GSA are not affected by arbitrarily chosen cutoffs.
- It does not provide information as detailed as ORA



The GSEA method

- Original GSEA method is based on comparing, for each gene group, the distribution of the test statistic within the group with the overall distribution of those statistics, i.e. the calculated for all genes.
- To do this, test statistics are ranked (from biggest to smallest) and a running sum is computed.
 - Let $N = \#$ genes in the array, $G = \#$ genes in the gene set.
 - If a gene belongs to the gene set a quantity $\sqrt{\frac{N-G}{G}}$ is added
 - If a gene does not belong to gene set a quantity $\sqrt{\frac{G}{N-G}}$ is subtracted
 - If there is no concentration of genes belonging to the gene set (this appear at random) the random sum behaves as a random walk
 - If, instead, genes in the gene set tend to be more abundant in the top part of the list the running sum will tend to increase deviating from the random walk distribution.
- The distribution of the running sum is compared with that of the random walk using a Kolmogorov-Smirnov test (K-S test) statistic
- P-values are computed based on a randomization.

The GSEA method



Recipe for **ranked** list enrichment test

- **Step 1:** Rank **ALL** your genes,
- **Step 2:** Select gene sets to test for enrichment,
- **Step 3:** Run enrichment tests and correct for multiple testing, if necessary,
- **Step 4:** Interpret your enrichments
- **Step 5:** Publish! ;)

GSEA variants

- GSEA is not free from criticisms
 - Use of KS test
 - Null hypothesis is not clear
- Many alternative available
 - Efron's GSA
 - Limma's ROAST
 - Irizarry's simple GSA based on Wilcoxon...

Tools for Pathway Analysis

R/Bioconductor



Bioconductor
OPEN SOURCE SOFTWARE FOR BIOINFORMATICS

Home Install Help Developers About

Search:

[Home](#) » [BiocViews](#)

All Packages

Bioconductor version 3.4 (Release)

Autocomplete biocViews search:

Packages found under GeneSetEnrichment:

Show	All	entries	Search table:	<input type="text"/>
Package	Maintainer	Title		
DriverMutation (1)		Gene expression enrichment in human brain regions		
FunctionalPrediction (6)		An integrated analysis package of miRNA and mRNA expression data		
GenePrediction (3)		Gene Expression Atlas query and gene set enrichment package.		
GeneRegulation (45)		Methods to Find the Gene Expression Modules that Represent the Drivers of Kauffman's Attractor Landscape		
GeneSetEnrichment (74)	Steffi Grote			
GeneSignaling (2)	Ti-Tai Wang			
GeneTarget (10)	Simon Jupp			
GenomeAnnotation (27)				
GenomeAssembly (1)				
GenomeWideAssociation (14)				
GenomicVariation (19)				
HistoneModification (1)				
LinkageDisequilibrium (4)				
MotifAnnotation (12)				
Category	Bioconductor Package Maintainer	Chromosomal Aberrations Finder in Expression data		
		Category Analysis		

As of March 2017 there are 74 packages under the view “Gene Set Enrichment”

Other (non-R) pathway analysis tools

- DAVID
- Pathway Painter
- Babelomics
- GenMAPP (www.genmapp.com)
- WikiPathways (www.wikipathways.org)
- cPath (cbio.mskcc.org/cpath)
- BioCyc (www.biocyc.org)
- Pubgene (www.pubgene.org)
- PANTHER (www.pantherdb.org)
- WebGestalt (bioinfo.vanderbilt.edu/webgestalt/)
- ToppGeneSuite ([/toppgene.cchmc.org/](http://toppgene.cchmc.org/))
- GeneGo/MetaCore (www.genego.com)
- Ingenuity Pathway Analysis (www.ingenuity.com)
- Pathway Studio (www.riadnegenomics.com)

BABELOMICS (FATIGO et alt.)

WYSIWYG enrichment analysis. GSEAsuite: on the one hand, the role of the list within the interactions pool; on the other hand, the comparative behaviour of the list as a subnetwork module.

- Set enrichment analysis**
 - Gene set analysis
 - Proteins gene sets with common alterations coordinately associated to high or low values in a control gene list by protein analysis
 - Mann-Whitney
 - NetworkGeneCo
 - NetworkGeneCo analysis in the context of alterations obtained by interacting
 - GSEA
 - Gene set analysis (also known as Pathway Based Enrichment) is the context of genome-wide association analysis (GWAS) with SNPs or CNVs
 - NetworkGSEA
 - PPS Subnetwork enrichment analysis, finds significant subnetworks or protein-protein interactions within a list of ranked gene/proteins
- Module enrichment analysis**
 - Genomic
 - Proteins annotations that frequently co-occur in a list of genes and rank them by statistical significance
- Tissue phenotype-based profiling**
 - Affymetrix
 - Single enrichment analysis using expression values from public disease experiments (Affymetrix dataset)
 - SAGE
 - Single enrichment analysis using expression values from public disease experiments of SAGE
- Functional annotation**
 - ReactGO
 - A tool for functional annotation of gene lists and the analysis of association rates

Annotation enrichment analysis. GeneGo suite: analysis of biological processes, pathways, diseases, and gene networks. GeneGo suite provides a unique approach to gene network analysis and visualization. It uses the integrated knowledge of biological pathways, gene expression, and gene interaction data to predict the function of genes and their interactions. GeneGo suite provides a unique approach to gene network analysis and visualization. It uses the integrated knowledge of biological pathways, gene expression, and gene interaction data to predict the function of genes and their interactions.

Annotation enrichment analysis. DAVID: DAVID is a web-based gene expression analysis tool that integrates many different sources of public gene expression data. DAVID provides users with a way to analyze their own gene expression data in the context of large-scale gene expression studies. DAVID also provides users with a way to analyze their own gene expression data in the context of large-scale gene expression studies.

BIOINFORMATICS APPLICATIONS NOTE

FatiGO: a web tool for finding significant associations of Gene Ontology terms with groups of genes

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ABSTRACT

Summary: We present a system for parallel analysis to detect Gene Ontology (GO) terms that are significantly overrepresented in two lists of genes. The analysis of expression data experiments (DNA microarray, RNA sequencing, etc.) has provided a better understanding of gene regulation. However, the analysis of these data is often limited by the lack of knowledge about the regulatory mechanisms. One of the main challenges in this field is to identify the genes that are most likely to be involved in a specific biological process. This is particularly important in the context of disease diagnosis, where the performance of medical tests can be improved by identifying genes that are most likely to be involved in a specific disease. Thus, the performance of FatiGO in the GO enrichment web page (http://www.geneontology.org/fatiGO.html) is evaluated. Results show that FatiGO can correctly identify the most likely GO terms in a group of genes. Thus, FatiGO is able to identify the most likely GO terms in a group of genes. Thus, FatiGO is able to identify the most likely GO terms in a group of genes.

Gene list

Are targets for a specific regulator over-represented in my gene list with respect to the normal regulation in the genome?

Genome

• GATA1 = 40%

• SP1 = 20%

• GATA1 = 10%

• SP1 = 20%

The gene list is over-enriched for GATA1 targets.

	Gene list	Genome
Regulated by GATA1	4	2
Not regulated by GATA1	6	18

- Takes two lists of genes (ideally a group of interest and the rest of the genes in the experiment, although any two groups, formed in any way, can be tested against each other)
- Convert them into two lists of gene sets using the corresponding gene-gen set association table. The gene sets are functional termed grouped by Gene Ontology, KEGG pathways, InterPro motifs, Swissprot keywords, microRNA, TFBSS, BioCarta pathways and cisRED motifs.
- Then a Fisher's exact test for 2x2 contingency tables is used to check for significant overrepresentation of gene sets in one of the sets with respect to the other one.
- Multiple test correction to account for the multiple hypothesis tested (one for each term) is applied as previously described.

Upload gene list, set parameters

Define your comparison

Id list vs Id list
 Id List vs Rest of genome
 Id List vs Rest of ids contained in your annotations (complementary list)

Select your data

List 1: [browse server](#) Entrez from CinRn_vs_Cinlni

List 2: Rest of genome

Options

Filter exact test: [etailed](#)

Remove duplicates? [Remove on each list separately](#)

Databases

Organism: Human (homo sapiens)

GO biological process [\[options\]](#)
 GO molecular function [\[options\]](#)
 GO cellular component [\[options\]](#)
 GOSlim GOA [\[options\]](#)
 Interpro [\[options\]](#)
 KEGG pathways [\[options\]](#)
 Reactome [\[options\]](#)
 Biocarta [\[options\]](#)
 miRNA targets [\[options\]](#)
 Jaspar TFBS [\[options\]](#)

Your annotations [browse server](#) no data selected. This data format is:
Or go to Upload Data form: [Upload \[annotation\]](#)

Job

job name: [CinRn_vs_Cinlni from Entrez](#)
job description: [Full topTable entrezIds vs human genome](#)

[Run](#)

Obtain “significantly enriched” sets

Define your comparison

- Id list vs Id list
- Id List vs Rest of genome
- Id List vs Rest of ids contained in your annotations (complementary list)

Select your data

List 1: [browse server](#) Entrez from CinRn_vs_Cinlni

List 2: Rest of genome

Options

Fisher exact test: [Two tailed](#)

Remove duplicates? [Remove on each list separately](#)

Databases

Organism: Human (homo sapiens)

GO biological process [\[options\]](#)

GO molecular function [\[options\]](#)

GO cellular component [\[options\]](#)

GO Slim GOA [\[options\]](#)

Interpro [\[options\]](#)

KEGG pathways [\[options\]](#)

Reactome [\[options\]](#)

Biocarta [\[options\]](#)

miRNA targets [\[options\]](#)

Jaspar TFBS [\[options\]](#)

[browse server](#) no data selected. This data format is:
Or go to Upload Data form: [Upload \[annotation\]](#)

Job

job name: [CinRn_vs_Cinlni from Entrez](#)

job description: [Full topTable entrezIds vs human genome](#)

Input data

- Species : **hsa**
- Duplicates management : [Remove list 1 ids from genome](#)
- Fisher exact test : [Two tailed](#)
- List 1 (after duplicates managing) : [clean_list1.txt](#)
- Genome (after duplicates managing) : [clean_list2.txt](#)

Summary

Id annotations per DB :

DB	Count	Detail
GO biological process (levels from 3 to 9)	3581 of 6813 (52.56%) 9.88 annotations/id	8902 of 16805 (52.97%) 6.31 annotations/id
GO cellular component (levels from 3 to 9)	2399 of 6813 (35.21%) 1.38 annotations/id	5237 of 16805 (31.16%) 0.94 annotations/id
GO molecular function (levels from 3 to 9)	3446 of 6813 (50.58%) 3.28 annotations/id	8690 of 16805 (51.71%) 2.46 annotations/id
KEGG	1761 of 6813 (25.85%) 0.79 annotations/id	3405 of 16805 (20.26%) 0.51 annotations/id
miRNA target	4805 of 6813 (70.53%) 23.3 annotations/id	12339 of 16805 (73.42%) 19.17 annotations/id

Duplicates management :

Detail	List 1	Genome
Number of duplicates	870 of 7683 (0.11%)	6816 of 23621 (0.29%)
Number of finally used ids	6813	16805

Significant Results

Number of significant terms per DB :

DB	Number of significant terms
GO biological process (levels from 3 to 9)	641
GO cellular component (levels from 3 to 9)	56
GO molecular function (levels from 3 to 9)	126
KEGG	76
miRNA target	495

Visualize results

KEGG

KEGG significant terms (pvalue<0.05) : [significant_kegg_0.05.txt](#) ([download as EXCEL](#))

Term	Term size	Term size (in genome)	Term annotation % per list	Annotated ids	Odds ratio (log _e)	pvalue	Adjusted pvalue
Oxidative phosphorylation (hsa00190)	139	134	list 1: 1% list 2: 0.42%	list 1: 245973,1349,106... list 2: ENS000000006695,ENSG...	0.8655	4.414e-7	0.000009516
Epithelial cell signaling in Helicobacter pylori infection (hsa05120)	77	74	list 1: 0.53% list 2: 0.24%	list 1: 245973,4602,323... list 2: ENSG00000070831,ENSG...	0.7756	0.0006437	0.003122
MAPK signaling pathway (hsa04010)	300	289	list 1: 1.95% list 2: 0.99%	list 1: 5578,11184,5159... list 2: ENSG00000000283,ENSG...	0.6849	7.031e-9	2.273e-7
ErbB signaling pathway (hsa04012)	91	88	list 1: 0.53% list 2: 0.33%	list 1: 5578,53358,3296... list 2: ENSG00000051382,ENSG...	0.481	0.018	0.04655
Calcium signaling pathway (hsa04020)	190	176	list 1: 1.28% list 2: 0.61%	list 1: 5578,5159,89832... list 2: ENSG00000004468,ENSG...	0.7407	4.971e-7	0.000009643
Wnt signaling pathway (hsa04310)	163	156	list 1: 0.92% list 2: 0.6%	list 1: 5578,1144,7473... list 2: ENSG00000002745,ENSG...	0.4441	0.00435	0.01507
VEGF signaling pathway (hsa04370)	78	74	list 1: 0.54% list 2: 0.24%	list 1: 5578,5534,9317... list 2: ENSG00000051382,ENSG...	0.8032	0.0003834	0.002188
Focal adhesion (hsa04510)	110	104	list 1: 1.54% list 2: 0.68%	list 1: 5578,3371,55742... list 2: ENSG00000017427,ENSG...	0.8205	2.188e-9	1.415e-7
Gap junction (hsa04540)	62	58	list 1: 0.69% list 2: 0.31%	list 1: 5578,5159,114,5... list 2: ENSG00000061918,ENSG...	0.8056	0.0006547	0.0005522
Long-term potentiation (hsa04360)	100	95	list 1: 0.54% list 2: 0.23%	list 1: 5578,114,5534,2... list 2: ENSG000000005339,ENSG...	0.8533	0.0001996	0.001249

Search the term hsa04510 Focal adhesion

General databases
[Ensembl](#) [novoseek](#)

Functional databases
[KEGG](#)

Other info
hsa04510 pathway description

hsa04510 pathway description

GO molecular function (levels from 3 to 9) significant terms (pvalue<0.05) : [significant_go_molecular_function_3_9_0.05.txt](#) ([download as EXCEL](#))

Visualize results

KEGG

KEGG significant terms (pvalue<0.05) : [significant_kegg_0.05.txt](#) ([download as EXCEL](#))

Term	Term size
Oxidative phosphorylation (hsa00190)	139
Epithelial cell signaling in Helicobacter pylori infection (hsa05120)	77
MAPK signaling pathway (hsa04010)	300
ErbB signaling pathway (hsa04012)	91
Calcium signaling pathway (hsa04020)	190
Wnt signaling pathway (hsa04310)	163
VEGF signaling pathway (hsa04370)	78
Focal adhesion (hsa04510)	Search the term hsa04510
Gap junction (hsa04540)	General databases Ensembl novoseek
Long-term potentiation (hsa04420)	Functional databases KEGG
	Other info hsa04510 pathway diagram
	next 1 2 3 4 5 6

KEGG Focal adhesion - Homo sapiens (human)

[Pathway menu | Organism menu | Pathway entry | [Download GML](#) | Show description | User data mapping]

Homo sapiens (human) [Go](#) [100%](#)

FOCAL ADHESION

The diagram illustrates the 'Focal adhesion' pathway in Homo sapiens. It shows the interaction between the extracellular matrix (ECM) and intracellular proteins. Key components include ITGB, RhoA, PI3K, FAK, Rac, and various kinases like Src, PI3K, Akt, and MEK1/2. The diagram highlights several key pathways and their regulation:

- ECM-receptor interaction:** ECM interacts with ITGA and ITGB, which activate signaling cascades involving Src, PI3K, and FAK.
- RhoA signaling:** RhoA is activated by RhoGEF and inhibited by RhoGAP. It activates ROCK, which in turn regulates MLC and mDia1.
- PI3K-Akt signaling:** PI3K is activated by FAK and PTEN. Akt is activated by PI3K and inhibited by PTEN. Akt activates GSK-3β and Cdk4/2.
- MAPK signaling:** FAK activates Rac, which activates PAK. PAK activates JNK and c-Jun. JNK activates Bad and Bel-2. MEK1/2 activates ERK1/2, which activates Elk1.
- Cytokine-cytokine receptor interaction:** RTK (e.g., EGFR) activates Shc, Grb2, Sos, Ha-Ras, and Raf-1.
- Regulation of actin cytoskeleton:** Actin is regulated by MLCP, MLCK, and mDia1.
- Cell motility:** Filopodia, lamellipodia formation, and FA turnover are regulated by actin polymerization.
- Cell proliferation:** PI3K-Akt signaling promotes cell cycle progression through Cdk4/2 and DNA synthesis.
- Cell survival:** MAPK signaling and PI3K-Akt signaling both contribute to cell survival.

“Official” GSEA. BROAD Institute

The image displays three screenshots of biological databases. The left screenshot shows the GSEA (Gene Set Enrichment Analysis) homepage, featuring a central diagram illustrating the process from molecular profile data to enriched gene sets. The middle screenshot is from the Nature Genetics journal website, showing a search results page for gene sets. The right screenshot is the Molecular Signatures Database (MSigDB) v4.1 homepage, which includes a detailed description of its collection of gene sets across various categories.

- No cut-off, uses "all" genes ranked
- For each functional annotation
 - Are genes randomly distributed in ranked list?
or
 - Are genes distributed towards the top/bottom?
- Calculate enrichment score (ES)
- Calculate significance of ES
- Correct for multiple testing



Upload data matrix (not gene list!)

#1.2	6811	7									
NAME	Description	Cf9	Cf10	Cf11	Cf4	Cf5	Cf7	Cf8			
18 ABAT		6.7100903124	6.2010968359	6.597489765	6.5013934729	6.8451356349	7.5118595998	6.4110123122			
19 ABCA1		5.4530779893	5.4355752984	5.4610085993	5.5476548331	5.4520121732	6.0489388288	5.0407680213			
22 ABCB7		7.3694752885	6.8977680549	7.3073544278	7.3465162227	7.5633266456	7.7642116686	7.1601794575			
30 ACAA1//OTTHUMG000001559		4.7248754828	5.0149574378	4.5595306969	4.706261494	4.8956576824	5.2365107743	4.257629554			
34 ACADM		8.9297666695	8.7971612382	8.5892949897	9.0426688459	9.6077263301	9.1942861042	8.6120161117			
36 ACADSB		5.7822772441	5.2725699164	6.0307174265	5.8207351756	6.0645458856	6.4805674854	5.7841453389			
39 ACAT2//LOC100129518//SOD2		6.9231386649	6.5549055906	6.4733739317	7.0506799232	7.4855891211	6.8006708513	5.9320368941			
41 ASIC1		4.7737457913	4.6841654133	5.1766921232	4.7347939833	4.4541148569	5.1399967599	4.7122823994			
43 ACHE		4.1624435064	3.6882333994	3.9935732941	3.7993435183	2.8043924676	3.6560631404	3.7848718218			
51 ACOX1		6.7826908205	7.0496507119	7.0241582248	6.4457072968	6.5778707815	6.8922885681	6.3313127929			
52 ACP1		7.4211781076	7.097706037	6.9938476336	7.5431622044	7.746785656	7.6525873676	7.0653335002			
53 ACP2		5.059193215	4.8278872873	5.1630183499	4.7642311305	4.3365637313	4.8455273799	4.4079333028			
54 ACP5		3.37202491	3.7503157578	3.7381985644	4.6264847891	3.4352961728	3.843873691	3.5304751138			
58 ACTA1		3.5406402898	3.9470695083	2.5025194518	2.7164043575	3.3941808636	3.7235832021	3.0603353631			
60 ACTB		6.2064344458	5.6953236783	5.7415926085	6.8800198082	5.7209218115	5.5140222137	6.9150639376			
71 ACTG1		10.9730301369	11.0722992954	10.7531966455	10.7612095627	10.6072998343	11.01349861	10.3146970978			
86 ACTL6A		7.306880239	7.3581695226	7.0414218102	7.3981514689	7.9485237615	7.4044889147	6.869460735			
88 ACTN2		4.8202948791	4.9049509308	4.7554192235	4.9223164025	5.2463745747	5.11490026	4.630485298			
90 ACVR1		5.0631062492	5.2052068044	4.6386208381	4.7515376273	4.9948847171	4.7537831842	4.3205454954			
94 ACVRL1		4.0012691816	3.5307672326	3.5967210171	4.4299320674	3.4079464211	4.335039134	3.5205318489			
97 ACYP1		5.7893977955	6.0223275581	6.3080750412	6.0767124941	6.1296880315	5.3047725532	5.8596962219			
100 ADA		3.283936647	3.5490351114	3.5513605652	3.5467692743	3.2251494559	3.5644893261	3.2357167769			
107 ADCY1		7.9872080552	7.5776132191	7.2672454373	7.3952269045	7.1706457374	7.7049262511	7.3400486978			
111 ADCY5		5.1798093059	5.0993243605	5.6976263009	5.3114479241	5.3256353917	5.6350976706	4.9818460015			
112 ADCY6//MIR4701		5.3929438612	5.4234156153	5.7267878509	5.8827262514	5.2677475971	5.4827914174	5.0904796544			

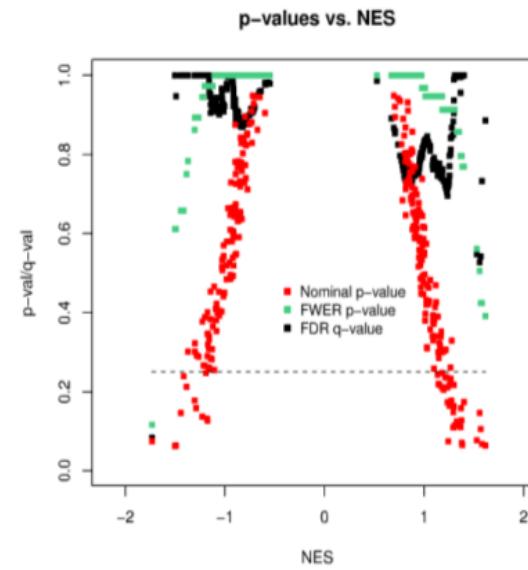
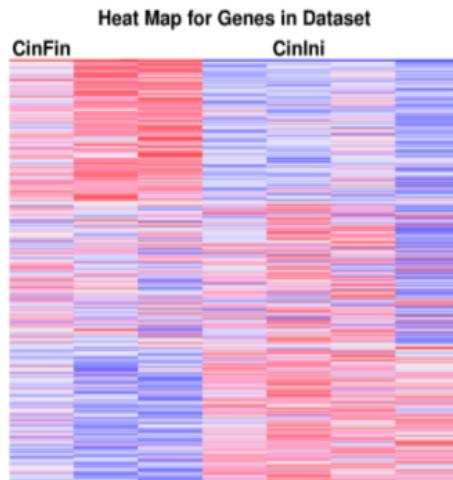
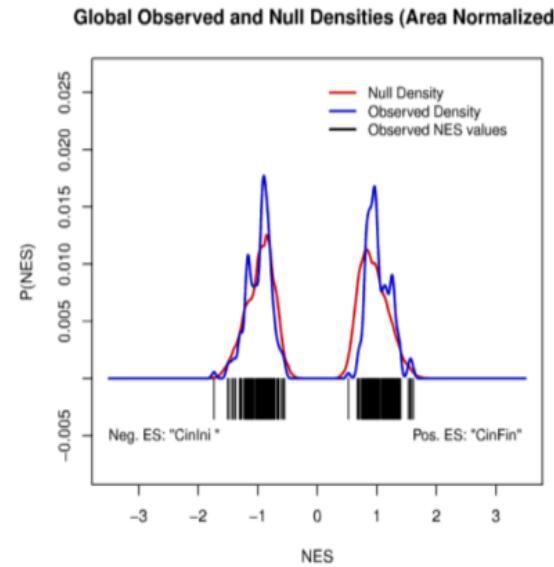
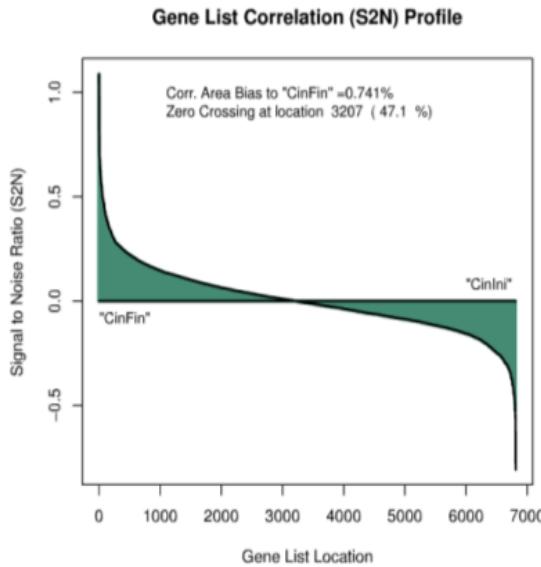
Set analysis parameters and gene sets

#1.2									
	NAME	Description	Cf9	Cf10	Cf11	Cf4	Cf5	Cf7	Cf8
18	ABAT		6.7100903124	6.2010968359	6.597489765	6.5013934729			
19	ABC1		5.4530779893	5.4355752984	5.4610085993	5.5476548331			
22	ABC87		7.3694752885	6.8977680549	7.3073544278	7.3465162227			
30	ACAA1//OTTHUMG000001559		4.7248754828	5.0149574378	4.5595306969	4.706261494			
34	ACADM		8.9297666695	8.7971612382	8.5892949897	9.0426688459			
36	ACADSB		5.7822772441	5.2725699164	6.0307174265	5.8207351756			
39	ACAT2//LOC100129518//SOD2		6.9231386649	6.5549055906	6.4733739317	7.0506799232			
41	ASIC1		4.7737457913	4.6841654133	5.1766921232	4.7347939833			
43	ACHE		4.1624435064	3.6882333994	3.9935732941	3.7993435183			
51	ACOX1		6.7826908205	7.0496507119	7.0241582248	6.4457072968			
52	ACP1		7.4211781076	7.097706037	6.9938476336	7.5431622044			
53	ACP2		5.059193215	4.8278872873	5.1630183499	4.7642311305			
54	ACP5		3.37202491	3.7503157578	3.7381985644	4.6264847891			
58	ACTA1		3.5406402898	3.9470695083	2.5025194518	2.7164043575			
60	ACTB		6.2064344458	5.6953236783	5.7415926085	6.8800198082			
71	ACTG1		10.9730301369	11.0722992954	10.7531966455	10.7612095627			
86	ACTL6A		7.306880239	7.3581695226	7.0414218102	7.3981514689			
88	ACTN2		4.8202948791	4.9049509308	4.7554192235	4.9223164025			
90	ACVR1		5.0631062492	5.2052068044	4.6386208381	4.7515376273			
94	ACVRL1		4.0012691816	3.5307672326	3.5967210171	4.4299320674			
97	ACYP1		5.7893977955	6.0223275581	6.3080750412	6.0767124941			
100	ADA		3.283936647	3.5490351114	3.5513605652	3.5467692743			
107	ADCY1		7.9872080552	7.5776132191	7.2672454373	7.3952269045			
111	ADCY5		5.1798093059	5.0993243605	5.6976263009	5.3114479241			
112	ADCY6//MIR4701		5.3929438612	5.4234156153	5.7267878509	5.8827262514			

```
Run.A276bis.R ✎ | Source on Save | Run | Source | 1:1 | Top Level | R Script |
```

```
1 # GSEA 1.0 -- Gene Set Enrichment Analysis / Broad Institute
2 #
3 # R script to run custom GSEA analysis of the UEB study ID #A276,
4 # based on the R script to run GSEA Analysis of the Leukemia ALL/AML vs C1 example
5
6 GSEA.program.location <- "/home/ferran/gsea_home/GSEA-P-R/GSEA.1.0.R" # R source program
7 source(GSEA.program.location, verbose=T, max.deparse.length=9999)
8
9 GSEA( # Input/Output Files :
10 # input.ds = "/home/ferran/estudis/microarrays/2015-01-MartaGarcia-StJdDeu-A161-A276/results/GSEA/Human"
11 input.ds = "/home/ferran/estudis/microarrays/2015-01-MartaGarcia-StJdDeu-A161-A276/results/GSEA/Human"
12 input.cls = "/home/ferran/estudis/microarrays/2015-01-MartaGarcia-StJdDeu-A161-A276/results/GSEA/VehFi"
13 # gs.db = "/home/ferran/estudis/microarrays/2015-01-MartaGarcia-StJdDeu-A161-A276/results/GSEA/geneSets"
14 gs.db = "/home/ferran/gsea_home/GSEA-P-R/GeneSetDatabases/c2.all.v4.0.entrez.gmt", # Gene set
15 # output.directory = "/home/ferran/estudis/microarrays/2015-01-MartaGarcia-StJdDeu-A161-A276/results/GS"
16 output.directory = "/home/ferran/estudis/microarrays/2015-01-MartaGarcia-StJdDeu-A161-A276/results/GSE"
17 # Program parameters :-
18 doc.string = "A276.VehFin.vs.VehInt", # Documentation string used as a prefix to name res
19 non.interactive.run = F, # Run in interactive (i.e. R GUI) or batch (R command line) m
20 reshuffling.type = "sample.labels", # Type of permutation reshuffling: "sample.labels" or "gene.l
21 nperm = 1000, # Number of random permutations (default: 1000)
22 weighted.score.type = 1, # Enrichment correlation-based weighting: 0=no weight (KS), 1
23 nom.p.val.threshold = -1, # Significance threshold for nominal p-vals for gene sets (de
24 fwer.p.val.threshold = -1, # Significance threshold for FWER p-vals for gene sets (defau
25 fdr.q.val.threshold = 0.25, # Significance threshold for FDR q-vals for gene sets (defau
26 topgs = 10, # Besides those passing test, number of top scoring gene sets
27 adjust.FDR.q.val = F, # Adjust the FDR q-vals (default: F)
28 gs.size.threshold.min = 25, # Minimum size (in genes) for database gene sets to be consid
29 gs.size.threshold.max = 1500, # Maximum size (in genes) for database gene sets to be consid
30 reverse.sign = F, # Reverse direction of gene list (pos. enrichment becomes neg
31 preproc.type = 0, # Preproc.normalization: 0=none, 1=col(z-score), 2=col(rank)
32 random.seed = 123456, # Random number generator seed. (default: 123456)
33 perm.type = 0, # For experts only. Permutation type: 0 = unbalanced, 1 = bal
34 fraction = 1.0, # For experts only. Subsampling fraction. Set to 1.0 (no resa
35 replace = F, # For experts only. Resampling mode (replacement or not repla
36 save.intermediate.results = F, # For experts only, save intermediate results (e.g. matrix of
37 OLD.GSEA = F, # Use original (old) version of GSEA (default: F)
38 use.fast.enrichment.routine = T # Use faster routine to compute enrichment for random permuta
39 )
40 #
41
42
```

Get results. Interpret output



DAVID

Gene-annotation enrichment:
typical batch annotation and gene-GO term
enrichment analysis to highlight the most relevant
GO terms associated with a given gene list.

- Huang DW, Sherman BT, Lempicki RA. Systematic and integrative analysis of large gene lists using DAVID Bioinformatics Resources. *Nature Protoc.* 2009;4(1):44-57. [PubMed]
- Huang DW, Sherman BT, Lempicki RA. Bioinformatics enrichment tools: paths toward the comprehensive functional analysis of large gene lists. *Nucleic Acids Res.* 2009;37(1):1-13. [PubMed]

Pathway mapping / Pathway Viewer:
can display genes from a user's list on KEGG
and BioCarta pathway maps to facilitate
biological interpretation in a network context.

Functional Annotation Clustering:
measures relationships among the annotation
terms based on the degrees of their co-association
genes to group the similar, redundant, and
heterogeneous annotation contents from the same
or different resources into annotation groups.

DAVID Bioinformatics Resources 6.7
National Institute of Allergy and Infectious Diseases (NIAID), NIH

Home Start Analysis Shortcut to DAVID Tools Technical Center Downloads & APIs Term of Service Why DAVID? About

Shortcut to DAVID Tools

- Functional Annotation**
Gene-annotation enrichment analysis, functional annotation clustering , BioCarta & KEGG pathway mapping, gene-disease association, homologue match, ID translation, literature match and more
- Gene Functional Classification**
Provide a rapid means to reduce large lists of genes into functionally related groups of genes to help unravel the biological content captured by high throughput technologies. More
- Gene ID Conversion**
Convert list of gene ID/acccessions to others of your choice with the most comprehensive gene ID mapping repository. The ambiguous accessions in the list can also be determined semi-automatically. More
- Gene Name Batch Viewer**
Display gene names for a given gene list; Search functionally related genes within your list or not in your list; Deep links to enriched detailed information. More

Welcome to DAVID 6.7

2003 - 2014

The Database for Annotation, Visualization and Integrated Discovery (DAVID) v6.7 is an update to the sixth version of our original web-accessible programs. DAVID now provides a comprehensive set of functional annotation tools for investigators to understand biological meaning behind large list of genes. For any given gene list, DAVID tools are able to:

- Identify enriched biological themes, particularly GO terms
- Discover enriched functional-related gene groups
- Cluster redundant annotation terms
- Visualize genes on BioCarta & KEGG pathway maps
- Display related many-genes-to-many-terms on 2-D view.
- Search for other functionally related genes not in the list
- List interacting proteins
- Explore gene names in batch
- Link gene-disease associations
- Highlight protein functional domains and motifs

What's Important in DAVID

- Current (v 6.7) release now available
- New requirement to cite DAVID
- IDs of Affy Exon and GenBank supported
- Novel Classification Algorithms
- Pre-built Affymetrix and GeneChip backgrounds
- User's customized gene lists
- Enhanced calculating speed

Statistics of DAVID

DAVID Bioinformatic Resource

Year	Users
2004	~10
2005	~150
2006	~300
2007	~500
2008	~700
2009	~1000
2010	~1800

> 10,000 Citations

Upload gene lists. Define background

 DAVID Bioinformatics Database

Functional Annotation Tool
DAVID Bioinformatics Resources 6.7, NIAID/NIH

Home Start Analysis Shortcut to DAVID Tools Technical Center Downloads & APIs Term of Service Why DAVID? About Us

Upload List Background

Gene List Manager

Select to limit annotations by one or more species [Help](#)

- Use All Species -
Homo sapiens(159)
Unknown(5)

Select Species

List Manager [Help](#)

demolist1

Select List to:

[View Unmapped Ids](#)

Annotation Summary Results

Current Gene List: demolist1 155 DAVID IDs [Help and Tool Manual](#)

Current Background: Homo sapiens Check Defaults

Disease (1 selected)
 Functional_Categories (3 selected)
 Gene_Ontology (3 selected)
 General Annotations (0 selected)
 Literature (0 selected)
 Main_Accessions (0 selected)
 Pathways (3 selected)
 Protein_Domains (3 selected)
 Protein_Interactions (0 selected)
 Tissue_Expression (0 selected)

Red annotation categories denote DAVID defined defaults

Combined View for Selected Annotation

Results

Functional Annotation Clustering

[Help and Manual](#)

Current Gene List: demolist1

Current Background: Homo sapiens

155 DAVID IDs

Options Classification Stringency Medium

[Rerun using options](#) [Create Sublist](#)

72 Cluster(s)

[!\[\]\(15611869979b36bd4de6926b60e559e0_img.jpg\) Download File](#)

Annotation Cluster 1		Enrichment Score: 4.81	G		Count	P Value	Benjamini
<input type="checkbox"/>	UP_SEQ_FEATURE	signal peptide	RT		50	6.5E-7	4.2E-4
<input type="checkbox"/>	SP_PIR_KEYWORDS	signal	RT		50	8.6E-7	2.8E-4
<input type="checkbox"/>	UP_SEQ_FEATURE	disulfide bond	RT		45	1.2E-6	4.0E-4
<input type="checkbox"/>	SP_PIR_KEYWORDS	disulfide bond	RT		46	1.7E-6	2.7E-4
<input type="checkbox"/>	GOTERM_CC_FAT	extracellular region	RT		40	6.9E-6	1.5E-3
<input type="checkbox"/>	GOTERM_CC_FAT	extracellular region part	RT		24	3.8E-5	4.0E-3
<input type="checkbox"/>	SP_PIR_KEYWORDS	Secreted	RT		29	7.2E-5	4.6E-3
<input type="checkbox"/>	GOTERM_CC_FAT	extracellular space	RT		19	9.4E-5	6.5E-3
<input type="checkbox"/>	SP_PIR_KEYWORDS	glycoprotein	RT		53	2.3E-4	7.5E-3
<input type="checkbox"/>	UP_SEQ_FEATURE	glycosylation site:N-linked (GlcNAc..)	RT		48	1.6E-3	1.6E-1
Annotation Cluster 2		Enrichment Score: 2.64	G		Count	P Value	Benjamini
<input type="checkbox"/>	GOTERM_BP_FAT	response to bacterium	RT		10	1.4E-4	9.1E-2
<input type="checkbox"/>	SP_PIR_KEYWORDS	antibiotic	RT		6	1.7E-4	7.9E-3
<input type="checkbox"/>	SP_PIR_KEYWORDS	Antimicrobial	RT		6	2.1E-4	8.5E-3
<input type="checkbox"/>	INTERPRO	Defensin_propeptide	RT		3	7.2E-4	2.5E-1
<input type="checkbox"/>	INTERPRO	Alpha_defensin	RT		3	7.2E-4	2.5E-1
<input type="checkbox"/>	INTERPRO	Alpha-defensin	RT		3	7.2E-4	2.5E-1
<input type="checkbox"/>	GOTERM_BP_FAT	defense response to bacterium	RT		7	8.9E-4	3.4E-1
<input type="checkbox"/>	PIR_SUPERFAMILY	PIRSF001875:alpha-defensin	RT		3	1.2E-3	1.2E-1
<input type="checkbox"/>	INTERPRO	Mammalian defensin	RT		3	2.0E-3	2.3E-1
<input type="checkbox"/>	SMART	DEFSN	RT		3	2.8E-3	2.5E-1
<input type="checkbox"/>	SP_PIR_KEYWORDS	fungicide	RT		3	3.0E-3	6.0E-2

Results

Functional Annotation Clustering

[Help and Manual](#)

Current Gene List

Current Background

155 DAVID IDs

Options

Clustering

Rerun using options

72 Cluster(s)

Annotation

UP_SEQ_FEAT

SP_PIR_KEYWORDS

UP_SEQ_FEAT

SP_PIR_KEYWORDS

GOTERM_CC_FAT

GOTERM_CC_FAT

SP_PIR_KEYWORDS

GOTERM_CC_FAT

SP_PIR_KEYWORDS

UP_SEQ_FEAT

Annotation

GOTERM_BP_FAT

SP_PIR_KEYWORDS

SP_PIR_KEYWORDS

INTERPRO

INTERPRO

INTERPRO

GOTERM_BP_FAT

PIR_SUPERFAMILY

INTERPRO

SMART

SP_PIR_KEYWORDS

Functional Annotation Chart

[Help and Manual](#)

Current Gene List: demolist1

Current Background: Homo sapiens

155 DAVID IDs

Options

Rerun Using Options

Create Sublist

211 chart records

[Download File](#)

Sublist	Category	Term	RT	Genes	Count	%	P-Value	Benjamini
	UP_SEQ_FEATURE	signal peptide	RT	██████████	50	32,3	6,5E-7	4,2E-4
	SP_PIR_KEYWORDS	signal	RT	██████████	50	32,3	8,6E-7	2,8E-4
	UP_SEQ_FEATURE	disulfide bond	RT	██████████	45	29,0	1,2E-6	4,0E-4
	SP_PIR_KEYWORDS	disulfide bond	RT	██████████	46	29,7	1,7E-6	2,7E-4
	GOTERM_CC_FAT	extracellular region	RT	██████████	40	25,8	6,9E-6	1,5E-3
	GOTERM_CC_FAT	extracellular region part	RT	██████████	24	15,5	3,8E-5	4,0E-3
	GOTERM_MF_FAT	oxygen binding	RT	██████████	6	3,9	3,8E-5	1,4E-2
	SP_PIR_KEYWORDS	heme	RT	██████████	8	5,2	4,0E-5	4,3E-3
	GOTERM_BP_FAT	iron	RT	██████████	11	7,1	6,9E-5	5,6E-3
	SP_PIR_KEYWORDS	Secreted	RT	██████████	29	18,7	7,2E-5	4,6E-3
	SP_PIR_KEYWORDS	extracellular space	RT	██████████	19	12,3	9,4E-5	6,5E-3
	GOTERM_MF_FAT	heme binding	RT	██████████	8	5,2	1,0E-4	1,9E-2
	SP_PIR_KEYWORDS	chromoprotein	RT	██████████	6	3,9	1,1E-4	5,9E-3
	GOTERM_BP_FAT	defense response	RT	██████████	18	11,6	1,3E-4	1,7E-1
	GOTERM_BP_FAT	response to bacterium	RT	██████████	10	6,5	1,4E-4	9,1E-2
	GOTERM_MF_FAT	tetrapyrrole binding	RT	██████████	8	5,2	1,5E-4	1,9E-2
	SP_PIR_KEYWORDS	antibiotic	RT	██████████	6	3,9	1,7E-4	7,9E-3
	SP_PIR_KEYWORDS	Antimicrobial	RT	██████████	6	3,9	2,1E-4	8,5E-3
	SP_PIR_KEYWORDS	chemotaxis	RT	██████████	6	3,9	2,3E-4	8,0E-3
	SP_PIR_KEYWORDS	glycoprotein	RT	██████████	53	34,2	2,3E-4	7,5E-3

Results

Functional Annotation Clustering

Current Gene List

Current Background

155 DAVID IDs

Options

Class

Rerun using option

72 Cluster(s)

Annotation

UP_SEQ_FEAT

SP_PIR_KEYW

UP_SEQ_FEAT

SP_PIR_KEYW

GOTERM_CC

GOTERM_CC

SP_PIR_KEYW

GOTERM_CC

SP_PIR_KEYW

UP_SEQ_FEAT

Annotation

GOTERM_BP

SP_PIR_KEYW

SP_PIR_KEYW

INTERPRO

INTERPRO

INTERPRO

GOTERM_BP

PIR_SUPERFA

INTERPRO

SMART

SP_PIR_KEYW

Functional Annotation Chart

[Help and Manual](#)

Current Gene List

Current Background

155 DAVID IDs

Options

Class

Rerun Using Option

211 chart records

Sublist

Category

Functional Annotation Table

[Help and Manual](#)

Current Gene List: demolist1

Current Background: Homo sapiens

155 DAVID IDs

150 record(s)

[Help and Manual](#)

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	37166_at	3-hydroxyanthranilate 3,4-dioxygenase	Related Genes	Homo sapiens
GOTERM_BP_FAT	coenzyme metabolic process, oxidoreduction coenzyme metabolic process, vitamin metabolic process, water-soluble vitamin metabolic process, nicotinamide metabolic process, coenzyme biosynthetic process, vitamin biosynthetic process, nucleotide biosynthetic process, alkaloid metabolic process, response to inorganic substance, response to metal ion, response to zinc ion, organic acid biosynthetic process, pyridine nucleotide biosynthetic process, NAD metabolic process, cellular homeostasis, secondary metabolic process, quinolinate biosynthetic process, nucleobase, nucleoside and nucleotide biosynthetic process, nucleobase, nucleoside, nucleotide and nucleic acid biosynthetic process, water-soluble vitamin biosynthetic process, homeostatic process, cellular amide metabolic process, dicarboxylic acid metabolic process, nitrogen compound biosynthetic process, carboxylic acid biosynthetic process, nicotinamide nucleotide metabolic process, response to cadmium ion, quinolinate metabolic process, cofactor metabolic process, cofactor biosynthetic process, oxidation reduction, anatomical structure homeostasis, neuron maintenance,			
GOTERM_CC_FAT	cell fraction, soluble fraction, mitochondrion, mitochondrial envelope, cytosol, organelle membrane, mitochondrial membrane, organelle envelope, envelope, mitochondrial part,			
GOTERM_MF_FAT	3-hydroxyanthranilate 3,4-dioxygenase activity, iron ion binding, ferrous iron binding, electron carrier activity, oxidoreductase activity, acting on single donors with incorporation of molecular oxygen, oxidoreductase activity, acting on single donors with incorporation of molecular oxygen, incorporation of two atoms of oxygen, oxygen binding, ion binding, cation binding, metal ion binding, transition metal ion binding,			
INTERPRO	3-hydroxyanthranilic acid dioxygenase, 3-hydroxyanthranilate 3,4-dioxygenase, metazoan,			
KEGG_PATHWAY	Tryptophan metabolism,			
PIR_SUPERFAMILY	PIRSF017681:3-hydroxyanthranilate 3,4-dioxygenase, animal type, PIRSF017681:3hydroanth_dOase_animal,			
SP_PIR_KEYWORDS	3d-structure, alternative splicing, complete proteome, cytoplasm, dioxygenase, iron, metal-binding, oxidoreductase, polymorphism, pyridine nucleotide biosynthesis,			
UP_SEQ_FEATURE	binding site:Dioxygen, binding site:Substrate, chain:3-hydroxyanthranilate 3,4-dioxygenase, helix, metal ion-binding site:iron; catalytic, sequence variant, splice variant, strand, turn,			
	34467_g_at	5-hydroxytryptamine (serotonin) receptor 4	Related Genes	Homo sapiens
GOTERM_BP_FAT	cell surface receptor linked signal transduction, G-protein coupled receptor protein signaling pathway, G-protein signaling, coupled to cyclic nucleotide second messenger, intracellular signaling cascade, second-messenger-mediated signaling, cyclic-nucleotide-mediated signaling,			
GOTERM_CC_FAT	cell fraction, membrane fraction, insoluble fraction, endosome, plasma membrane, integral to plasma membrane, integral to membrane, intrinsic to membrane, intrinsic to plasma membrane, plasma membrane part,			
GOTERM_MF_FAT	adrenoceptor activity, serotonin receptor activity, amine receptor activity,			
INTERPRO	7TM GPCR, rhodopsin-like, 5-Hydroxytryptamine 4 receptor, GPCR, rhodopsin-like superfamily,			
KEGG_PATHWAY	Calcium signaling pathway, Neuroactive ligand-receptor interaction,			
PIR_SUPERFAMILY	PIRSF038635:5-hydroxytryptamine receptor 4, PIRSF800006:rhodopsin-like G protein-coupled receptors,			
SP_PIR_KEYWORDS	alternative splicing, cell membrane, complete proteome, disulfide bond, endosome, G protein-coupled receptor, g-protein coupled receptor, glycoprotein, lipoprotein, membrane, neurotransmitter receptor, palmitate, polymorphism, receptor, transducer, transmembrane, transmembrane protein,			

Ingenuity Pathways

The image shows two views of the Ingenuity Pathway Analysis (IPA) platform. The top view is the website homepage, featuring a Newton's cradle graphic, a 'SIGN UP FOR IPA' button, and sections for 'OVERVIEW', 'FEATURES', 'ADVANCED', 'APPLICATIONS', 'WEBINARS', 'TRAINING', and 'RESOURCES'. A central text block states: 'Model, analyze, and understand the complex biological and chemical systems at the core of life science research with IPA'. Below this are three service cards: 'Market Leading Pathway Analysis' (with a network graph icon), 'Predictive Causal Analytics' (with a causal graph icon), and 'NGS/RNA-Seq Data Analysis' (with a scatter plot icon). The bottom view is a screenshot of the IPA software interface titled 'Start Here', showing tabs for 'Explore' and 'Analyze', and sections for 'Datasets', 'Compare', and 'IPA-Biomarker'.

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INGENUITY PATHWAY ANALYSIS

SIGN UP FOR IPA

SIGN UP TODAY

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OVERVIEW FEATURES ADVANCED APPLICATIONS WEBINARS TRAINING RESOURCES

Model, analyze, and understand the complex biological and chemical systems at the core of life science research with IPA

Market Leading Pathway Analysis

Predictive Causal Analytics

NGS/RNA-Seq Data Analysis

Start Here

Explore Analyze

Datasets

Annotate and filter datasets and use them directly for hypothesis generation when exploring pathways and gene lists.

Analyze dataset Compare analyses

Compare

Identify the union, unique, and common molecules across lists, pathways, biomarkers, and analyses.

Analyze dataset Compare analyses

IPA-Biomarker

Filter your datasets and identify and prioritize potential biomarker candidates.

Exercise

- Obtain a **gene list** and a **background list** from a differential expression analysis (background may be the list of **all** genes analyzed) (see next slide)
- Convert identifiers into “Entrez” ids if they are not already converted.
- Select two pathway analysis tools e.g. DAVID and Babelomics
- Do a Gene Enrichment Analysis with each tool.
- Compare the 5-10 top enriched categories and comment about the differences.
- Alternatively do it with R/Bioconductor with the code from the following slides.

R code to prepare the data

```
topTab <-  
  read.table(https://raw.githubusercontent.com/alexsanchezpla/scripts/master/Exemple\_Analisis\_BioC/results/ExpressAndTop\_AvsB.csv, head=TRUE, sep=";", dec=",")  
colnames(topTab)  
head(topTab)  
geneListUp <- unique(  
  topTab$EntrezsA [topTab$adj.P.Val<0.05 & topTab$logFC > 0] )  
length(geneListUp)  
geneListDown <- unique(  
  topTab$EntrezsA [topTab$adj.P.Val<0.05 & topTab$logFC < 0] )  
length(geneListDown)  
geneUniverse <- unique(topTab$EntrezsA)  
length(geneUniverse)  
write.csv(geneListUp, file="selectedAvsB.up.csv")  
write.csv(geneListDown, file="selectedAvsB.down.csv")  
write.csv(geneUniverse, file="geneUniverse.csv")
```

A quick ORA analysis with R

```
# GOAnalysis
require(GOstats)
## Creamos los "hiperparametros" en que se basa el analisis
GOparams = new("GOHyperGParams",
                geneIds=geneListUp, universeGeneIds=geneUniverse, annotation="org.Hs.eg.db", ontology="BP",
                pvalueCutoff=0.001, conditional=FALSE, testDirection="over")
KEGGparams = new("KEGGHyperGParams",
                geneIds=geneListUp, universeGeneIds=geneUniverse,
                annotation="org.Hs.eg.db",      pvalueCutoff=0.01, testDirection="over")
## Ejecutamos los analisis
GOhyper = hyperGTest(GOparams)
KEGGhyper = hyperGTest(KEGGparams)
cat("GO\n"); print(head(summary(GOhyper)))
cat("KEGG\n"); print(head(summary(KEGGhyper)))
# Creamos un informe html con los resultados
GOfilename =file.path(paste("GOResults.",".html", sep=""))
KEGGfilename =file.path(paste("KEGGResults.",".html", sep=""))
htmlReport(GOhyper, file = GOfilename, summary.args=list("htmlLinks"=TRUE))
htmlReport(KEGGhyper, file = KEGGfilename, summary.args=list("htmlLinks"=TRUE))
```

Expected output

Gene to GO BP test for over-representation

GOBPID	Pvalue	OddsRatio	ExpCount	Count	Size	Term
GO:0043436	0.000	1.947	42	73	889	oxoacid metabolic process
GO:0019752	0.000	2.003	37	67	792	carboxylic acid metabolic process
GO:0006082	0.000	1.909	42	73	904	organic acid metabolic process
GO:0044710	0.000	1.503	196	245	4164	single-organism metabolic process
GO:0006629	0.000	1.842	47	78	1000	lipid metabolic process
GO:1900101	0.000	10.270	1	7	21	regulation of endoplasmic reticulum unfolded protein response
GO:0044255	0.000	1.843	36	60	757	cellular lipid metabolic process
GO:0006631	0.000	2.443	13	29	278	fatty acid metabolic process

Gene to KEGG test for over-representation

KEGGID	Pvalue	OddsRatio	ExpCount	Count	Size	Term
04914	0.000	3.963	4	13	74	Progesterone-mediated oocyte maturation
04146	0.001	3.439	4	11	70	Peroxisome
05221	0.002	3.589	3	9	55	Acute myeloid leukemia
04910	0.003	2.460	7	15	128	Insulin signaling pathway
01100	0.003	1.538	48	66	912	Metabolic pathways
04114	0.009	2.492	5	11	92	Oocyte meiosis

Summary

- Pathway Analysis is a useful approach to help gain biological understanding from omics-based studies.
- There are many ways, many methods, many tools →
- Choice of the method should be guided by
 - a combination of availability, ease of use and usefulness ,
 - Usually obtained from a good understanding of how it
- Different methods may yield different results
 - Worth checking!

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