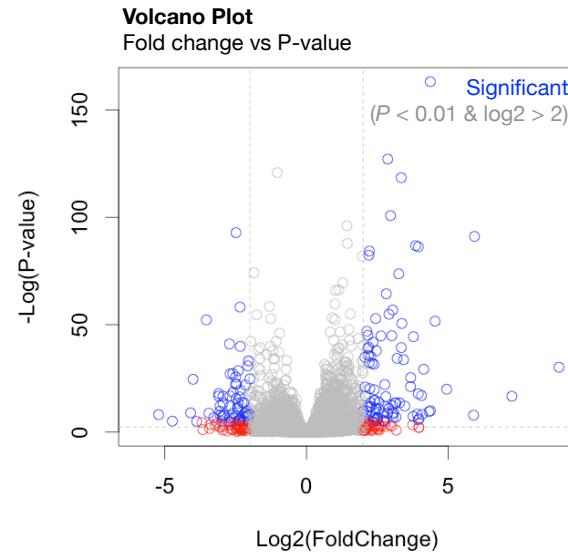


X	baseMean	log2FoldChange	IfSe	stat	pvalue	padj	symbol
ENSG00000152583	954.77093	4.3683590	0.23713648	18.421286	8.867079e-76	1.342919e-71	SPARC1
ENSG00000179094	743.25269	2.8638885	0.17555825	16.313039	7.972621e-60	6.037267e-56	PER1
ENSG00000116584	2277.91345	-1.0347000	0.06505273	-15.905557	5.798513e-57	2.927283e-53	ARHGEF2
ENSG00000189221	2383.75371	3.3415441	0.21241508	15.731200	9.244206e-56	3.500088e-52	MAOA
ENSG00000120129	3440.70375	2.9652108	0.20370277	14.556557	5.306416e-48	1.607313e-44	DUSP1
ENSG00000148175	13493.92037	1.4271683	0.10036663	14.219550	6.929711e-46	1.749175e-42	STOM
ENSG00000178695	2685.40974	-2.4890689	0.17806407	-13.978501	2.108817e-44	4.562576e-41	KCTD12
ENSG00000109906	439.54152	5.9275950	0.42819442	13.843233	1.397758e-43	2.646131e-40	ZBTB16
ENSG00000134686	2933.64246	1.4394898	0.10582729	13.602255	3.882769e-42	6.533838e-39	PHC2
ENSG00000101347	14134.99177	3.8504143	0.28490701	13.514635	1.281894e-41	1.941428e-38	SAMHD1
ENSG00000096060	2630.23049	3.9450524	0.29291821	13.468102	2.409807e-41	3.317866e-38	FKBP5
ENSG00000166741	7542.25287	2.2195906	0.16673544	13.312050	1.970000e-40	2.486304e-37	NNMT
ENSG00000125148	3695.87946	2.1985636	0.16700546	13.164621	1.402400e-39	1.633797e-36	MT2A
ENSG00000162614	5646.18314	1.9711402	0.15020631	13.122885	2.434854e-39	2.633990e-36	NEXN
ENSG00000106976	989.04683	-1.8501713	0.14778657	-12.519211	5.861471e-36	5.918132e-33	DNM1
ENSG00000187193	199.07694	3.2551424	0.26090711	12.476250	1.006146e-35	9.523804e-33	MT1X
ENSG00000256235	1123.47954	1.2801193	0.10547438	12.136779	6.742862e-34	6.007096e-31	SMIM3
ENSG00000177666	2639.57020	1.1399947	0.09606884	11.866436	1.768422e-32	1.487930e-29	PNPLA2
ENSG00000164125	7257.00808	1.0248523	0.08657600	11.837603	2.494830e-32	1.988642e-29	FAM198B
ENSG00000198624	2020.04495	2.8141014	0.24063429	11.694515	1.359615e-31	1.0295569e-28	CCDC69
ENSG00000123562	5008.55294	1.0045453	0.08901501	11.285123	1.554241e-29	1.120904e-26	MORF4L2
ENSG00000144369	1283.77980	-1.3090041	0.11714863	-11.173875	5.473974e-29	3.768333e-26	FAM171B
ENSG00000196517	241.91536	-2.3456877	0.21047366	-11.144804	7.591120e-29	4.998588e-26	SLC6A9
ENSG00000135821	19973.40000	3.0413943	0.27601796	11.018828	3.100706e-28	1.956675e-25	GLUL



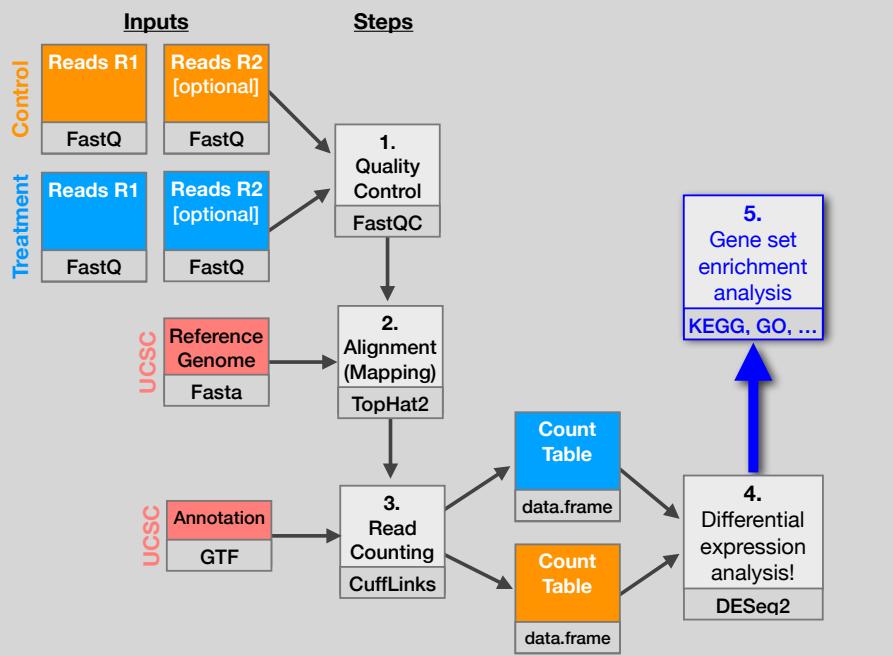
My high-throughput experiment generated a long list of genes/proteins...

What do I do now?



## Pathway analysis! (a.k.a. geneset enrichment)

Use bioinformatics methods to help extract biological meaning from such lists...

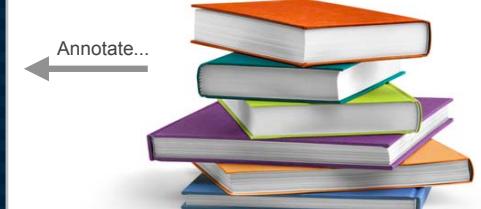


## Basic idea

### Differentially Expressed Genes (DEGs)

ENSG00000152881	9147.2093	4.3481590	0.21715644	18.421286	8.697079e-76	L142028-71	SPARC1
ENSG00000179094	741.25248	2.4938590	0.17555853	13.61313	7.972621e-60	L072707-56	PMS1
ENSG00000116584	227.716345	-1.0149144	0.05005729	13.505357	5.798118e-57	S202788-53	ARHGAP2
ENSG00000186221	238.737673	3.1950344	0.21454507	13.512100	9.246000e-56	S300515-52	MAGA
ENSG00000177357	1.3951254	0.00000000	0.00000000	13.512100	9.246000e-56	S300515-52	MAGA
ENSG00000141175	1349.62037	1.4271683	0.05006663	14.121950	6.039311e-46	L740174-42	STOM
ENSG00000178695	2618.40074	-2.4906649	0.17060460	13.972651	7.008181e-44	L562726-41	KCTD12
ENSG00000105969	419.54152	5.9737950	0.41618442	13.642123	3.397558e-41	L264310-40	ZEBR1
ENSG00000110174	1.4134.9617	3.4550143	0.24490701	13.514651	1.384084e-41	L441628-38	SAMHD1
ENSG000001101747	14134.9617	3.4550143	0.24490701	13.514651	1.384084e-41	L441628-38	SAMHD1
ENSG00000694674	2610.23049	3.9450524	0.29591831	13.641020	2.608007e-41	S317864-38	FBXRS
ENSG00000106741	7542.23287	2.2159510	0.16467314	13.121050	1.970000e-40	L448304-37	NMNT
ENSG00000125148	360.57946	2.1895336	0.16105046	13.161400	1.602400e-39	L131797-36	MTZA
ENSG00000106076	1.3951254	0.00000000	0.00000000	13.161400	1.602400e-39	L131797-36	MTZA
ENSG00000106076	988.66843	-1.8501713	0.14798537	12.531811	8.814784e-36	S301310-33	DNAI1
ENSG00000187131	139.07694	3.2551424	0.26099711	12.478250	1.008146e-31	S323804-33	MTLX
ENSG00000262635	1123.47954	1.2801201	0.15047433	12.118979	6.742826e-34	L037096-31	SMN3
ENSG00000377668	2619.57020	1.1599497	0.09068884	13.886458	1.764042e-32	L437936-32	PPML2
ENSG00000106741	7542.23287	2.2159510	0.16467314	13.121050	1.970000e-40	L448304-37	NMNT
ENSG00000188624	2020.04495	2.8161014	0.24681629	16.094515	1.559815e-31	L029598-28	CCDC89
ENSG00000123582	5098.555294	1.0045453	0.08001565	11.285123	1.5594241e-29	L123904-26	MORF4L2
ENSG00000144569	1203.77980	-1.3050041	0.11714681	11.171875	5.473974e-29	S376338-26	FAM171B
ENSG00000196157	201.81536	-2.3456577	0.21067936	11.144001	7.951220e-29	S495858-26	SCEAS
ENSG00000137093	1059.46020	3.941343	0.27603196	11.030023	4.107070e-25	L105072-25	GLB1

Gene-sets (Pathways, annotations, etc...)



## Basic idea

### Differentially Expressed Genes (DEGs)

	baseMean	logFoldChange	negLog10PValue	padj	symbol
ENSG0000011583	954.77093	3.463556	0.171134	0.000238	NP_0011583
ENSG00000117094	743.81209	2.893059	0.175142	0.000238	NP_00117094
ENSG0000011654	2277.81345	-1.034700	0.000527	0.053557	NP_0011654
ENSG00000182023	2383.73373	1.341554	0.212415	0.000238	NP_00182023
ENSG00000182128	1446.70373	2.961216	0.207027	0.000238	NP_00182128
ENSG0000014375	13493.8037	1.427748	0.100463	0.000238	NP_0014375
ENSG0000014376	13493.8037	1.427748	0.100463	0.000238	NP_0014376
ENSG00000139968	418.44142	5.927950	0.428184	0.000238	NP_00139968
ENSG00000142462	2933.64246	1.439488	0.158272	0.000238	NP_00142462
ENSG00000133487	1414.99177	3.850541	0.248970	0.000238	NP_00133487
ENSG00000133488	1414.99177	3.850541	0.248970	0.000238	NP_00133488
ENSG00000142679	2030.85709	3.893059	0.212415	0.000238	NP_00142679
ENSG00000142681	7642.52347	2.710898	0.146736	0.000238	NP_00142681
ENSG0000011148	3095.87946	2.189838	0.170504	0.000238	NP_001148
ENSG00000126126	1646.88318	1.971140	0.150283	0.000238	NP_00126126
ENSG00000143879	773.72862	1.895173	0.150283	0.000238	NP_00143879
ENSG00000182127	1121.47954	1.295119	0.156743	0.000238	NP_00182127
ENSG00000177668	2639.37020	1.110994	0.090084	0.000238	NP_00177668
ENSG00000141213	7257.20088	1.024852	0.086576	0.000238	NP_00141213
ENSG00000140674	2026.04495	2.814104	0.248970	0.000238	NP_00140674
ENSG00000140675	2026.04495	2.814104	0.248970	0.000238	NP_00140675
ENSG00000144985	1281.77980	-1.359004	0.137146	0.117787	NP_00144985
ENSG00000185157	241.85156	-2.345887	0.210473	0.117787	NP_00185157
ENSG00000115821	19075.40029	1.041394	0.270279	0.117787	NP_00115821

### Gene-sets (Pathways, annotations, etc...)

Annotate...



## Pathway analysis (a.k.a. geneset enrichment) Principle



- DEGs come from your experiment

> Critical, needs to be as clean as possible

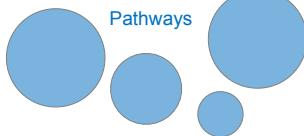
- Pathway genes ("geneset") come from annotations

> Important, but typically not a competitive advantage

- Variations of the math: overlap, ranking, networks... > Not critical, different algorithms show similar performances

### Differentially Expressed Genes (DEGs)

Overlap...



## Pathway analysis (a.k.a. geneset enrichment) Limitations

Side-note:

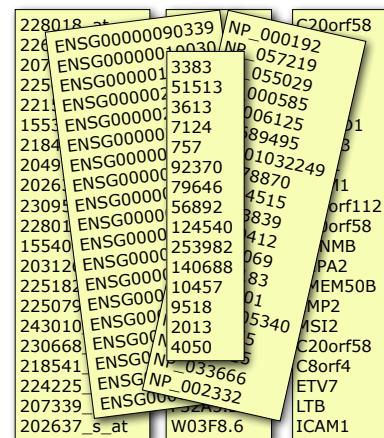
- **Geneset annotation bias:** can only discover what is already known
- **Non-model organisms:** no high-quality genesets available
- **Post-transcriptional regulation** is neglected
- **Tissue-specific** variations of pathways are not annotated
  - e.g. NF-κB regulates metabolism, not inflammation, in adipocytes
- **Size bias:** stats are influenced by the size of the pathway
  - Many pathways/receptors **converge** to few regulators  
e.g. Tens of innate immune receptors activate four TFs: NF-κB, AP-1, IRF3/7, NFAT

## Starting point for pathway analysis: Your gene list

- You have a list of genes/proteins of interest

- You have quantitative data for each gene/protein

- Fold change
- p-value
- Spectral counts
- Presence/absence



## Translating between identifiers

- Many different identifiers exist for genes and proteins, e.g. UniProt, Entrez, etc.
- Often you will have to translate one set of ids into another
  - A program might only accept certain types of ids
  - You might have a list of genes with one type of id and info for genes with another type of id

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- **Various web sites translate ids -> best for small lists**
  - UniProt <[www.uniprot.org](http://www.uniprot.org)>; IDConverter <[idconverter.bioinfo.cnio.es](http://idconverter.bioinfo.cnio.es)>

## Translating between identifiers: UniProt <[www.uniprot.org](http://www.uniprot.org)>

The screenshot shows the UniProt homepage. At the top, there is a search bar with dropdown menus for 'Search in' (Protein Knowledgebase (UniProtKB)) and 'Query'. Below the search bar are buttons for 'Search', 'Clear', 'Fields', 'Blast', 'Align', 'Retrieve', and 'ID Mapping'. The 'ID Mapping' button is highlighted with a red box. The main area is titled 'WELCOME' and 'NEWS'. Below this, there is a form for 'Identifiers' mapping. It has two dropdown menus: 'From' (set to 'EMBL/GenBank/DDBJ') and 'To' (set to 'UniProtKB AC'). There is also a file upload field labeled 'Choose File' with the placeholder 'no file selected'. To the right of the dropdowns are three buttons: 'Map', 'Swap', and 'Clear'.

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- **VLOOKUP in Excel - good if you are an excel whizz - I am not!**
  - Download flat file from Entrez, Uniprot, etc; Open in Excel; Find columns that correspond to the 2 IDs you want to convert between; Sort by ID; Use vlookup to translate your list

## Translating between identifiers: Excel VLOOKUP

VLOOKUP(lookup\_value, table\_array, col\_index\_num)

A	B	C	D	E	F	G	H	I	J	K
RefSeq	Symbol	Exp1	Exp2	Exp3		Annotation Table	RefSeq	Entrez ID	Unigene	RefSeq
NM_153103	Kif1c	2.31975457	1.24558927	2.78816871		NM_001001	Zfp85-rs1	22746	Mm.288396	NM_001
NM_146017	Gabbrp	4.15029735	3.08055836	1.18919962		NM_001001	Scap	235623	Mm.288741	NM_001
NM_018883	Camkk1	3.83282512	0.0522951	0.64684259		NM_001001	Scap	235623	Mm.288741	NM_001
NM_145936	Tspyl2	0.45449369	1.62761318	7.59770627		NM_001001	Fbxo41	330369	Mm.38777	NM_001
NM_026599	Cgnl1	4.84541871	2.84751796	1.61595768		NM_001001	Taf9b	407786	Mm.19440	NM_001
NM_013926	Cbx8	1.22903318	0.2863077	0.02952665		NM_001001	Taf9b	407786	Mm.19440	NM_001
NR_015566	A330023F24	1.44695053	0.98809479	1.59330144		NM_001001	BC051142	407788	Mm.73205	NM_001
NM_008623	Mpz	0.50749263	0.94350028	6.10581569		NM_001001	BC051142	407788	Mm.73205	NM_001
NM_183127	Fate1	2.45672795	4.87960794	3.60759511		NM_001001	BC048546	232408	Mm.259234	NM_001
NM_008943		4.78701069	4.15302647	0.85432314		NM_001001	Zfp941	407812	Mm.359154	NM_001
NM_025382		0.66397344	1.40664187	3.09539802		NM_001001	BC031181	407819	Mm.29866	NM_001
NM_182841		1.25528938	0.20505996	2.76879488		NM_001001	Baz2b	407823	Mm.486364	NM_001
NM_030061		0.17670108	2.75415469	2.98900691		NM_001001	Tmem204	407831	Mm.34379	NM_001
NM_133216		6.572343	0.59671282	3.84650536		NM_001001	Ccdc111	408022	Mm.217385	NM_001
NM_030063		7.05132762	0.65043627	1.68111836		NM_001001	BC048507	408058	Mm.177840	NM_001

## Translating between identifiers

- Many different identifiers exist for genes and proteins, e.g. UniProt, Entrez, etc.
  - Often you will have to translate one set of ids into another
    - A program might only accept certain types of ids
    - You might have a list of genes with one type of id and info for genes with another type of id
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    - UniProt < [www.uniprot.org](http://www.uniprot.org); IDConverter < [idconverter.bioinfo.cnio.es](http://idconverter.bioinfo.cnio.es) >
  - VLOOKUP in Excel -> good if you are an excel whizz - I am not!
    - Download flat file from Entrez, Uniprot, etc; Open in Excel; Find columns that correspond to the two ids you want to convert between; Use vlookup to translate your list
- Use the **merge()** or **mapIDs()** functions in R - fast, versatile & reproducible!
  - Also **clusterProfiler::bitr()** function and many others... [Link to clusterProfiler vignette]

Reminder

2. class-material (bash)

```
# Using the merge() function
> anno <- read.csv("data/annotables_grch38.csv") This is an annotation file

> merge(mygenes, anno, by.x="row.names", by.y= "ensgene")
This is our differential expressed genes
```

Reminder

2. class-material (bash)

```
# Using the merge() function
> anno <- read.csv("data/annotables_grch38.csv")

> merge(mygenes, anno, by.x="row.names", by.y= "ensgene")

# Using mapIDs() function from bioconductor
> library("AnnotationDbi")
> library("org.Hs.eg.db") Load the required Bioconductor packages

> mygenes$symbol <- mapIds( org.Hs.eg.db,
  column="SYMBOL",
  keys=row.names(mygenes),
  keytype="ENSEMBL" ) Annotation we want to add
                                         Our vector of gene names & their format
```

## bitr: Biological Id Translator

clusterProfiler provides `bitr` and `bitr_kegg` for converting ID types. Both `bitr` and `bitr_kegg` support many species including model and many non-model organisms.

```
x <- c("GPX3", "GLRX", "LBP", "CRYAB", "DEFB1", "HCLS1", "SOD2", "HSPA2",  
      "ORM1", "IGFBP1", "PTHLH", "GPC3", "IGFBP3", "T0B1", "MITF", "NDRG1",  
      "NR1H4", "FGFR3", "PVR", "IL6", "PTPRM", "ERBB2", "NID2", "LAMB1",  
      "COMP", "PLS3", "MCAM", "SPP1", "LAMC1", "COL4A2", "COL4A1", "MYOC",  
      "ANXA4", "TFPI2", "CST6", "SLPI", "TIMP2", "CPM", "GGT1", "NNMT",  
      "MAL", "EEF1A2", "HGD", "TCN2", "CDA", "PCCA", "CRYM", "PDHX",  
      "STC1", "WARS", "HMOX1", "FXYD2", "RBP4", "SLC6A12", "KDELR3", "ITM2B")  
eg = bitr(x, fromType="SYMBOL", toType="ENTREZID", OrgDb="org.Hs.eg.db")  
head(eg)
```

```
## SYMBOL ENTREZID  
## 1 GPX3 2878  
## 2 GLRX 2745  
## 3 LBP 3929  
## 4 CRYAB 1410  
## 5 DEFB1 1672  
## 6 HCLS1 3059
```

See package vignette:

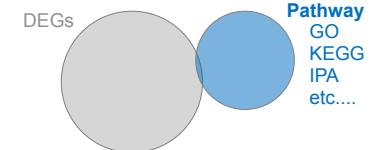
<https://bioconductor.org/packages/release/bioc/html/clusterProfiler.html>

Alternative...

## What functional set databases do you want?

- Most commonly used:

- **Gene Ontology (GO)**
- **KEGG Pathways** (mostly metabolic)
- **GeneGO MetaBase** 
- **Ingenuity Pathway Analysis (IPA)** 



- Many others...

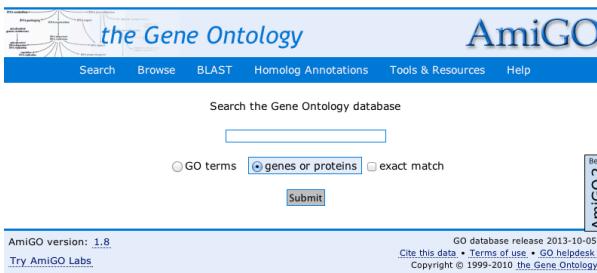
- **Enzyme Classification, PFAM, Reactome,**
- Disease Ontology, MSigDB, Chemical Entities of Biological Interest, Network of Cancer Genes etc...
- See: Open Biomedical Ontologies ([www.obofoundry.org](http://www.obofoundry.org))

## GO < [www.geneontology.org](http://www.geneontology.org) >

- What function does HSF1 perform?
  - response to heat; sequence-specific DNA binding; transcription; etc
- **Ontology** => a structured and controlled vocabulary that allows us to annotate gene products consistently, interpret the relationships among annotations, and can easily be *handled by a computer*
- GO database consists of 3 ontologies that describe gene products in terms of their associated **biological processes**, **cellular components** and **molecular functions**

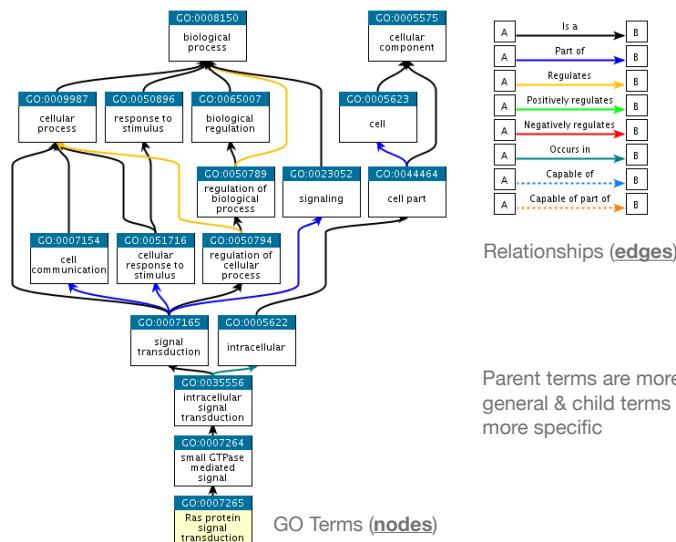
## GO Annotations

- GO is not a stand-alone database of genes/proteins or sequences
- Rather gene products get annotated with **GO terms** by UniProt and other organism specific databases, such as Flybase, Wormbase, MGI, ZFIN, etc.
- Annotations are available through AmiGO < [amigo.geneontology.org](http://amigo.geneontology.org) >



The screenshot shows the AmiGO web interface. At the top, there's a navigation bar with links for 'Search', 'Browse', 'BLAST', 'Homolog Annotations', 'Tools & Resources', and 'Help'. Below the navigation bar is a search bar labeled 'Search the Gene Ontology database'. Underneath the search bar are three radio buttons: 'GO terms' (selected), 'genes or proteins', and 'exact match'. A 'Submit' button is located below the search bar. At the bottom of the page, there's footer information including 'AmiGO version: 1.8', 'GO database release 2013-10-05', 'Try AmiGO Labs', 'Cite this data', 'Terms of use', 'GO Helpdesk', 'Copyright © 1999-2010 the Gene Ontology', and a 'Beta' badge.

**GO is structured as a “directed graph”**

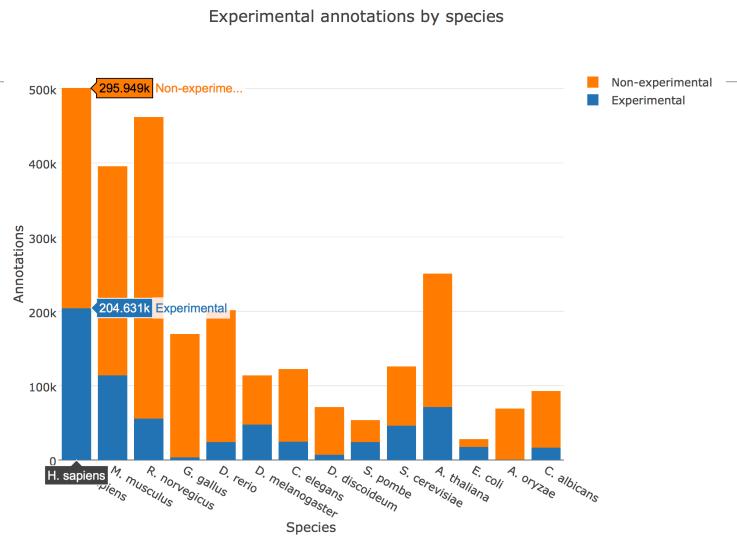


Parent terms are more general & child terms more specific

Evidence code	Evidence code description	Source of evidence	Manually checked	Current number of annotations*
IDA	Inferred from direct assay	Experimental	Yes	71,050
IEP	Inferred from expression pattern	Experimental	Yes	4,598
IGI	Inferred from genetic interaction	Experimental	Yes	8,311
IMP	Inferred from mutant phenotype	Experimental	Yes	61,549
IPI	Inferred from physical interaction	Experimental	Yes	17,043
ISS	Inferred from sequence or structural similarity	Computational	Yes	196,643
RCA	Inferred from reviewed computational analysis	Computational	Yes	103,792
IGC	Inferred from genomic context	Computational	Yes	4
IEA	Inferred from electronic annotation	Computational	No	15,687,382
IC	Inferred by curator	Indirectly derived from experimental or computational evidence made by a curator	Yes	5,167
TAS	Traceable author statement	Indirectly derived from experimental or computational evidence made by the author of the published article	Yes	44,564
NAS	Non-traceable author statement	No 'source of evidence' statement given	Yes	25,656
ND	No biological data available	No information available	Yes	132,192
NR	Not recorded	Unknown	Yes	1,185

\*October 2007 release

Can now do gene list analysis with GeneGO online!



- See AmiGO for details: [http://amigo.geneontology.org/amigo/base\\_statistics](http://amigo.geneontology.org/amigo/base_statistics)

pantherdb.org/webservices/go/overrep.jsp

# PANTHER Classification System

GENEONTOLOGY Unifying Biology

Home About PANTHER Data PANTHER Tools Workspace Downloads Help/Tutorial

New! PANTHER13.1 released.

Search

All

Quick links

Whole genome function views  
Genome statistics  
Data Version  
How to cite PANTHER  
**NEW!** Recent publication describing PANTHER

News  
PANTHER13.1 Released  
Click for additional info.

Newsletter subscription  
Enter your Email:

PostgreSQL Admin

Gene List Analysis      Browse      Sequence Search      cSNP Scoring      Keyword Search

Please refer to our article in [Nature Protocols](#) for detailed instructions on how to use this page.

Error parsing request, no input specified

**Help Tips**

**Steps:**

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

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

Enter IDs:   no file selected

separate IDs by a space or comma

Upload IDs:

2. Select organism.

ID List  
 Previously exported text search results  
 Workspace list  
 PANTHER Generic Mapping File  
 VCF File Flanking region 20 Kb

3. Select analysis.

Functional classification viewed in gene list

## Another popular online tool: **DAVID** at NIAID < david.abcc.ncifcrf.gov >

The screenshot shows the DAVID Analysis Wizard interface. At the top, it says "Analysis Wizard" and "DAVID Bioinformatics Resources 2008, NIAID/NIH". Below that is a navigation bar with links like Home, Start Analysis, Shortcut to DAVID Tools, Technical Center, Downloads & APIs, Term of Service, Why DAVID?, and About Us. The main area is titled "Analysis Wizard" and has a sub-section "Step 1: Submit your gene list through left panel". It includes a text input field for "Gene List" with a "Clear" button, a "Choose From a File" section, and a dropdown for "Step 2: Select Identifier" set to "AFFY\_ID". A note says "Note: Affy Exon IDs and Affy Gene Array IDs are now supported in DAVID, as \*affy\_id\* type." Below this is a list of gene identifiers: 1007\_s\_at, 1008\_s\_at, 117\_at, 121\_at, 1255\_g\_at, 1316\_at, 1320\_at, 1431\_at, 1438\_at, 1487\_at, 1494\_f\_at, 1598\_g\_at. At the bottom, there's a "Submit List" button.

## DAVID

- Functional Annotation Chart



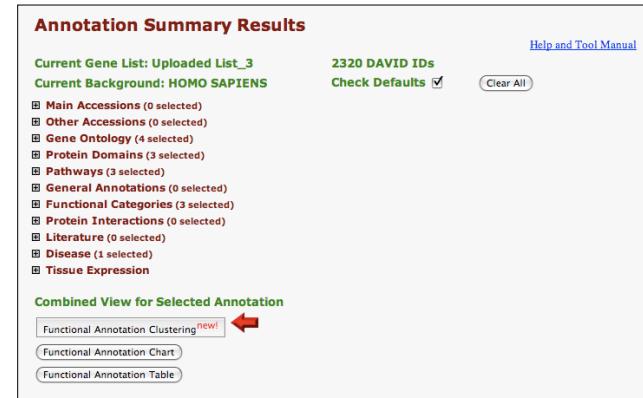
Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources  
Da Wei Huang, Brad T Sherman & Richard A Lempicki  
Nature Protocols 4, 44 - 57 (2009)

## Overlapping functional sets

- Many functional sets overlap
  - In particular those from databases that are hierarchical in nature (e.g. GO)
- Hierarchy enables:
  - Annotation flexibility (e.g. allow different degrees of annotation completeness based on what is known)
  - Computational methods to “understand” function relationships (e.g. ATPase function is a subset of enzyme function)
- Unfortunately, this also makes functional profiling trickier
  - Clustering of functional sets can be helpful in these cases

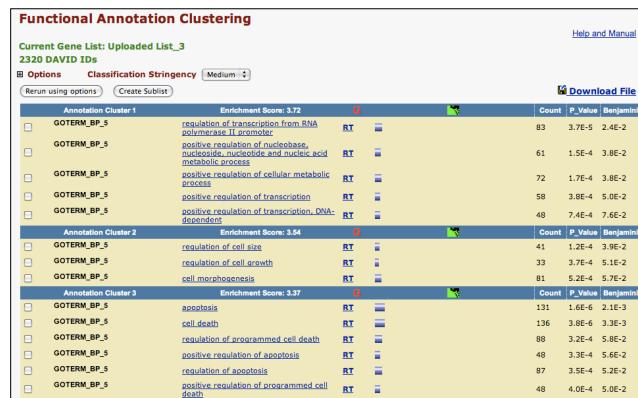
## DAVID

- DAVID now offers functional annotation clustering:



## DAVID Functional Annotation Clustering

- Based on shared genes between functional sets



Hands-on time!

[https://bioboot.github.io/bimm143\\_F18/lectures/#16](https://bioboot.github.io/bimm143_F18/lectures/#16)

Also: R Quiz Online

Want more?



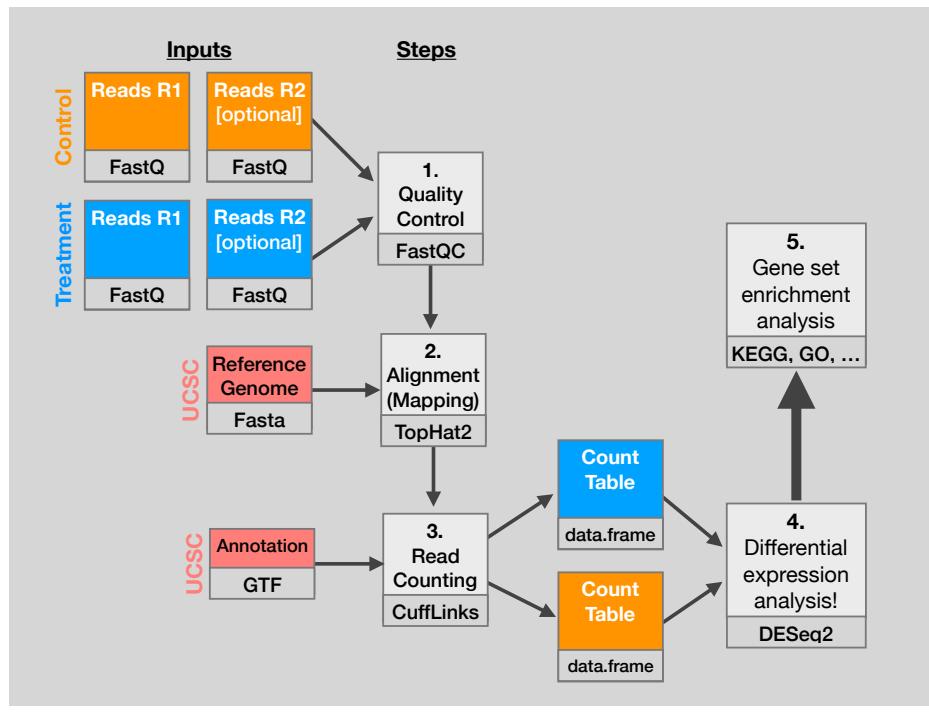
- GeneGO** < [portal.genego.com](http://portal.genego.com) >
  - MD/PhD curated annotations, great for certain domains (eg, Cystic Fibrosis)
  - Nice network analysis tools
  - Email us for access
- Oncomine** < [www.oncomine.org](http://www.oncomine.org) >
  - Extensive cancer related expression datasets
  - Nice concept analysis tools
  - Research edition is free for academics, Premium edition \$\$\$
- Lots and lots other R/Bioconductor packages in this area!!!**

Do it Yourself!

# Hands-on time!

[https://bioboot.github.io/bimm143\\_F18/lectures/#16](https://bioboot.github.io/bimm143_F18/lectures/#16)

Also: R Quiz Online



# Data structure: counts + metadata

## countData

gene	ctrl_1	ctrl_2	exp_1	exp_1
geneA	10	11	56	45
geneB	0	0	128	54
geneC	42	41	59	41
geneD	103	122	1	23
geneE	10	23	14	56
geneF	0	1	2	0
...	...	...	...	...

**countData** is the count matrix (number of reads coming from each gene for each sample)

First column of **colData** must match column names of **countData** (-1st)

## colData

id	treatment	sex	...
ctrl_1	control	male	...
ctrl_2	control	female	...
exp_1	treatment	male	...
exp_2	treatment	female	...

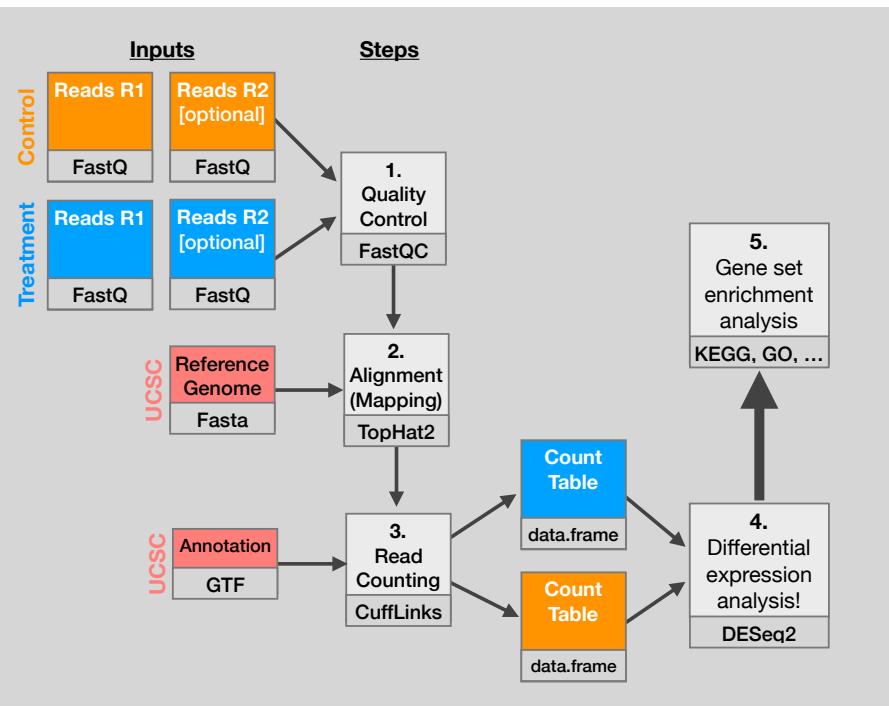
Sample names:  
**ctrl\_1, ctrl\_2, exp\_1, exp\_2**

**colData** describes metadata about the *columns* of countData

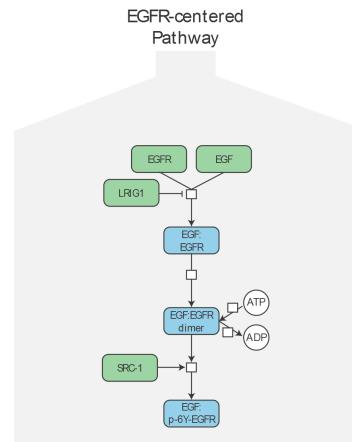
## Advice:

Figure out “**What do I want to do with my list?**”

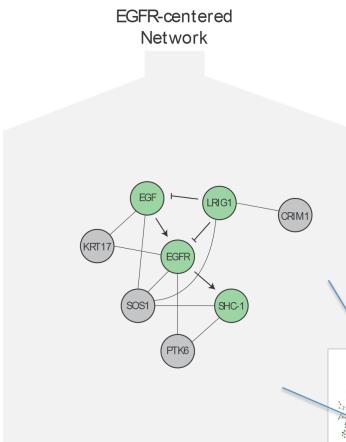
- Organize/summarize data for presentation or manuscript
  - DAVID: GO\_FAT -> Functional Annotation Clustering -> Pick threshold
- Infer biological processes from the list
  - DAVID: Functional Annotation Chart -> explore functional databases and see which make sense
  - GSEA: Select MSigDB sets of interest -> e.g., immunologic signatures
  - Use domain specific database it at all possible!
- Find “missing” genes/proteins not detected by experiment
  - ConceptGen: Gene-gene enrichment



## Pathways vs Networks



- Detailed, high-confidence consensus
- Biochemical reactions
- Small-scale, fewer genes
- Concentrated from decades of literature



- Simplified cellular logic, noisy
- Abstractions: directed, undirected
- Large-scale, genome-wide
- Constructed from omics data integration

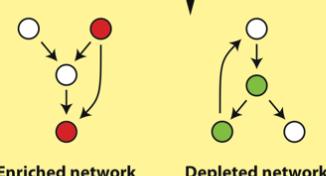
Next Class

## Goal

### 1 Enrichment of fixed gene sets

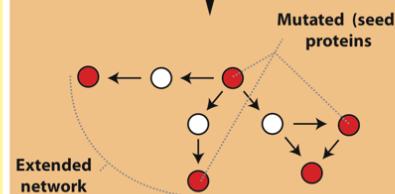
Identification of pre-built pathways or networks that are enriched in a set of mutated or differentially expressed genes

## Output



### 2 De novo sub-network construction and clustering

Construction of specific sub-networks from the set of mutated or differentially expressed genes to identify an extended list of putative cancer genes



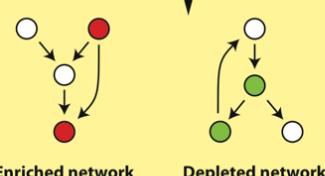
Next Class

## Goal

### 1 Enrichment of fixed gene sets

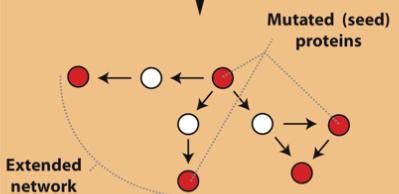
Identification of pre-built pathways or networks that are enriched in a set of mutated or differentially expressed genes

## Output



### 2 De novo sub-network construction and clustering

Construction of specific sub-networks from the set of mutated or differentially expressed genes to identify an extended list of putative cancer genes



Next Class

What biological process is altered in this cancer?

Are NEW pathways altered in this cancer? Are there clinically relevant tumor subtypes?

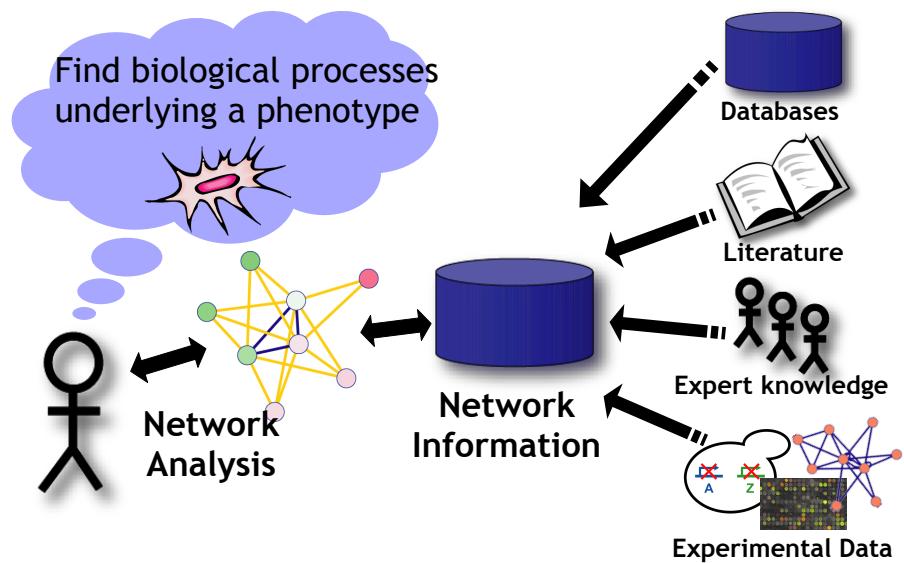
## Pathway analysis (a.k.a. geneset enrichment)

### Limitations

Side-note:

- **Geneset annotation bias:** can only discover what is already known
- **Non-model organisms:** no high-quality genesets available
- **Post-transcriptional regulation** is neglected
- **Tissue-specific** variations of pathways are not annotated
  - e.g. NF-κB regulates metabolism, not inflammation, in adipocytes
- **Size bias:** stats are influenced by the size of the pathway
  - Many pathways/receptors **converge** to few regulators  
e.g. Tens of innate immune receptors activate four TFs: NF-κB, AP-1, IRF3/7, NFAT

## Pathway & Network Analysis Overview



Do it Yourself!

# R Knowledge Check

## Quiz

This will be marked but not graded  
(i.e. will not factor into your course grade)