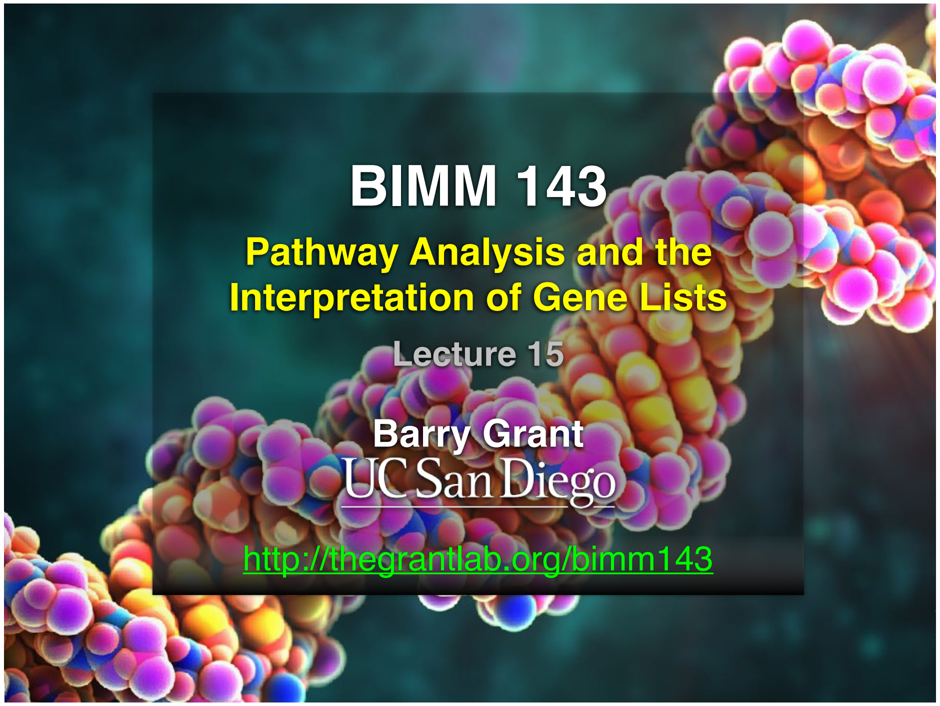


BIMM 143

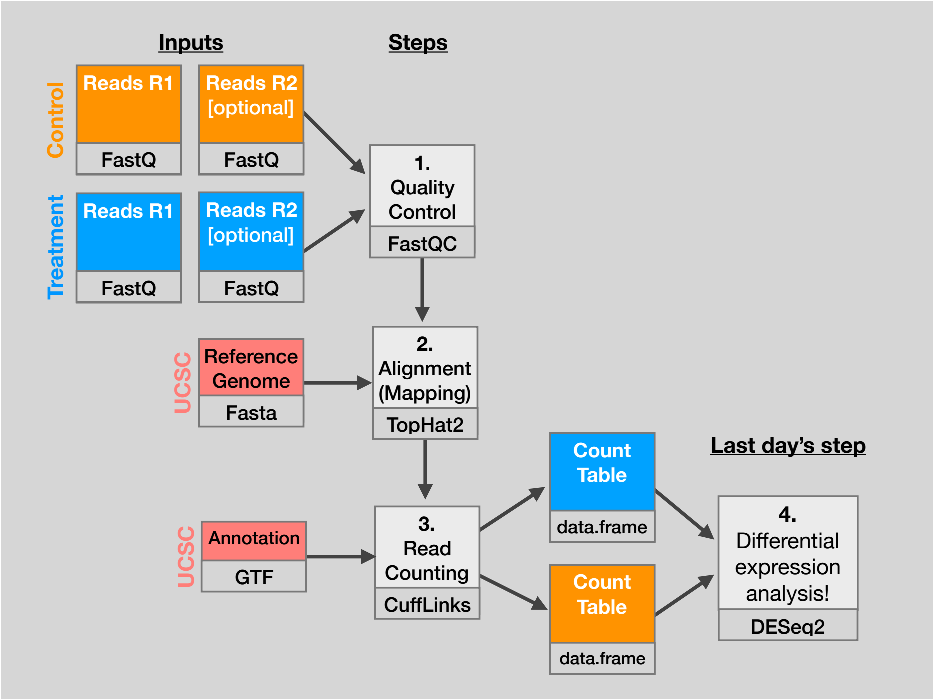
Pathway Analysis and the Interpretation of Gene Lists

Lecture 15

Barry Grant

UC San Diego

http://thegrantlab.org/bimm143



Inputs

Steps

Control

Reads R1
FastQ

Reads R2 [optional]
FastQ

Treatment

Reads R1
FastQ

Reads R2 [optional]
FastQ

1. Quality Control
FastQC

UCSC

Reference Genome
Fasta

2. Alignment (Mapping)
TopHat2

UCSC

Annotation
GTF

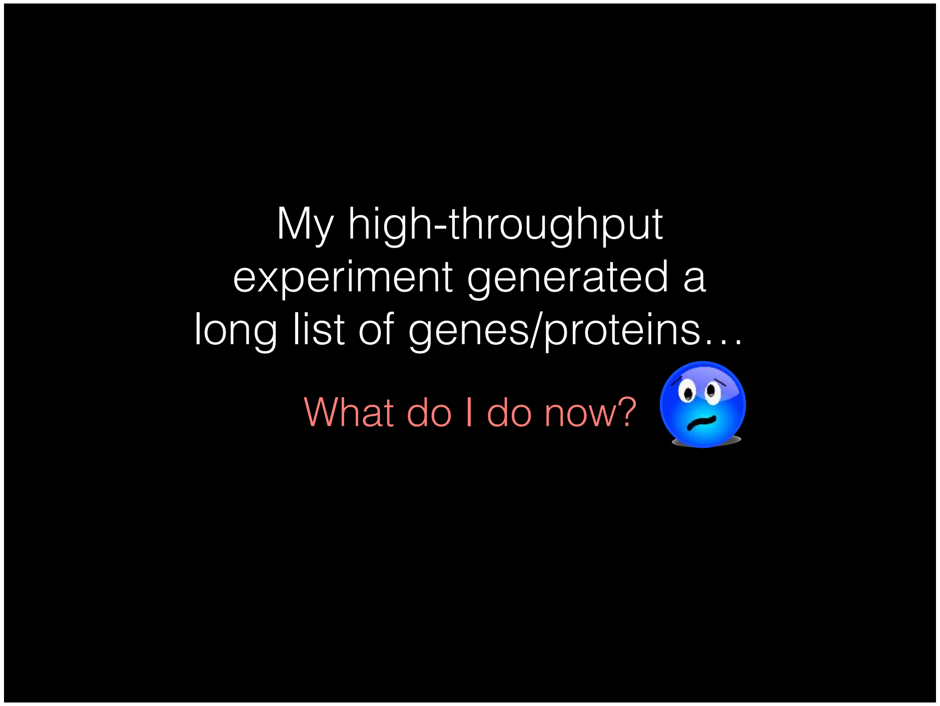
3. Read Counting
CuffLinks

Count Table
data.frame

Count Table
data.frame

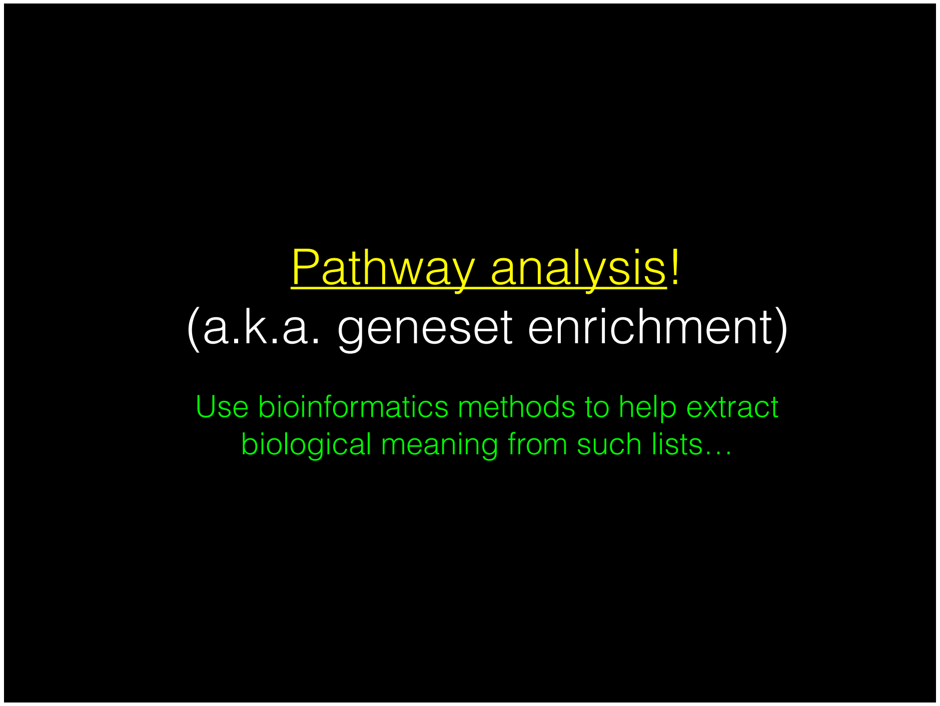
Last day's step

4. Differential expression analysis!
DESeq2



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

What do I do now?

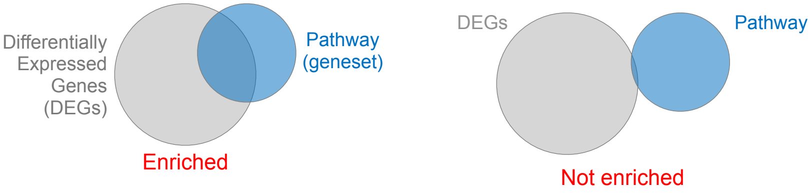


Pathway analysis!

(a.k.a. geneset enrichment)

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

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



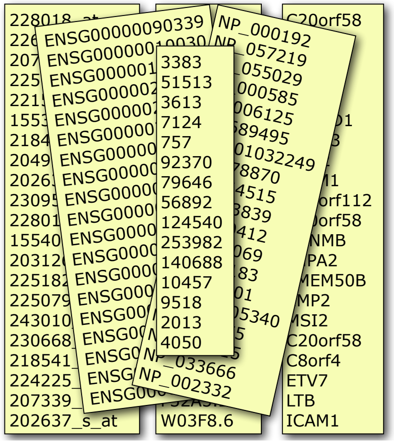
- Variations of the math: overlap, ranking, networks... ➢ *Not critical, different algorithms show similar performances*
- DEGs come from your experiment ➢ *Critical, needs to be as clean as possible*
- Pathway genes ("geneset") come from annotations ➢ *Important, but typically not a competitive advantage*

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

- **Post-transcriptional regulation** is neglected
- **Directionality** is hard to capture sensibly
  - e.g. IκBα/NF-κB
- **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
- **Geneset annotation bias**: can only discover what is already known
- **Non-model organisms**: no high-quality genesets available
- Many pathways/receptors **converge** to few regulators
  - e.g. tens of innate immune receptors activate 4 TFs: NF-kB, 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.
- Sometimes you 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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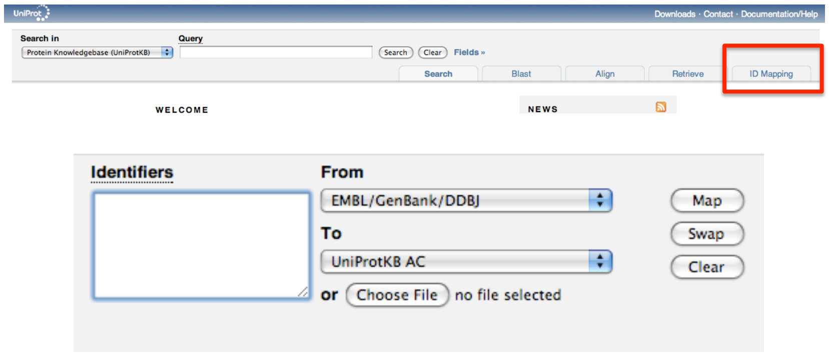
  - **UniProt < www.uniprot.org>; IDConverter < idconverter.bioinfo.cnio.es >**

Translating between identifiers: UniProt < www.uniprot.org >



Identifiers

From: EMBL/GenBank/DDBJ

To: UniProtKB AC

or Choose File no file selected

Translating between identifiers

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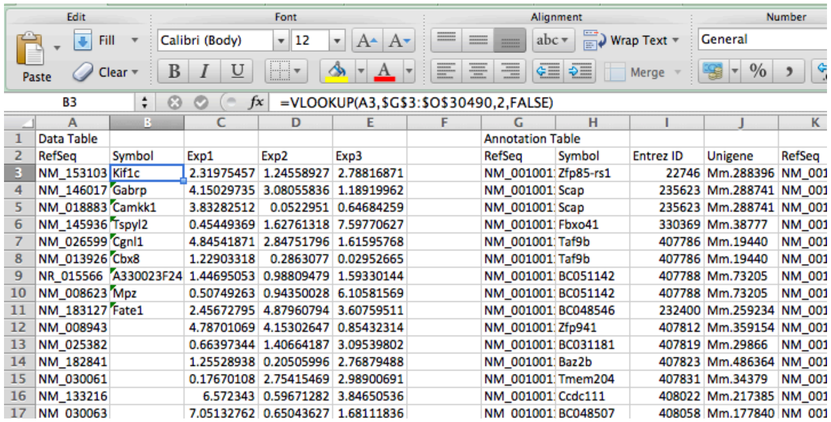
- Various web sites translate ids -> *best for small lists*
  - UniProt < www.uniprot.org>; IDConverter < idconverter.bioinfo.cnio.es >
- **VLOOKUP in Excel - *good if you are an excel whizz - I am not!***
  - **Download flat file from Entrez, Uniprot, etc; Open in Excel; Find columns that correspond to the 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)



B3 =VLOOKUP(A3,$G$3:$O$30490,2,FALSE)

	A	B	C	D	E	F	G	H	I	J	K
1	Data Table						Annotation Table				
2	RefSeq	Symbol	Exp1	Exp2	Exp3		RefSeq	Symbol	Entrez ID	Unigene	RefSeq
3	NM_153103	Kif1c	2.31975457	1.24558927	2.78816871		NM_001001	Zfp85-rs1	22746	Mm.288396	NM_001
4	NM_146017	Gabrp	4.15029735	3.08055836	1.18919962		NM_001001	Scap	235623	Mm.288741	NM_001
5	NM_018883	Camkk1	3.83282512	0.0522951	0.64684259		NM_001001	Scap	235623	Mm.288741	NM_001
6	NM_145936	Tspyl2	0.45449369	1.62761318	7.59770627		NM_001001	Fbxo41	330369	Mm.38777	NM_001
7	NM_026599	Cgnl1	4.84541871	2.84751796	1.61595768		NM_001001	Taf9b	407786	Mm.19440	NM_001
8	NM_013926	Cbx8	1.22903318	0.2863077	0.02952665		NM_001001	Taf9b	407786	Mm.19440	NM_001
9	NR_015566	A330023F24	1.44695053	0.98809479	1.59330144		NM_001001	BC051142	407788	Mm.73205	NM_001
10	NM_008623	Mpz	0.50749263	0.94350028	6.10581569		NM_001001	BC051142	407788	Mm.73205	NM_001
11	NM_183127	Fate1	2.45672795	4.87960794	3.60759511		NM_001001	BC048546	232400	Mm.259234	NM_001
12	NM_008943		4.78701069	4.15302647	0.85432314		NM_001001	Zfp941	407812	Mm.359154	NM_001
13	NM_025382		0.66397344	1.40664187	3.09539802		NM_001001	BC031181	407819	Mm.29866	NM_001
14	NM_182841		1.25528938	0.20505996	2.76879488		NM_001001	Baz2b	407823	Mm.486364	NM_001
15	NM_030061		0.17670108	2.75415469	2.98900691		NM_001001	Tmem204	407831	Mm.34379	NM_001
16	NM_133216		6.572343	0.59671282	3.84650536		NM_001001	Ccdc111	408022	Mm.217385	NM_001
17	NM_030063		7.05132762	0.65043627	1.68111836		NM_001001	BC048507	408058	Mm.177840	NM_001

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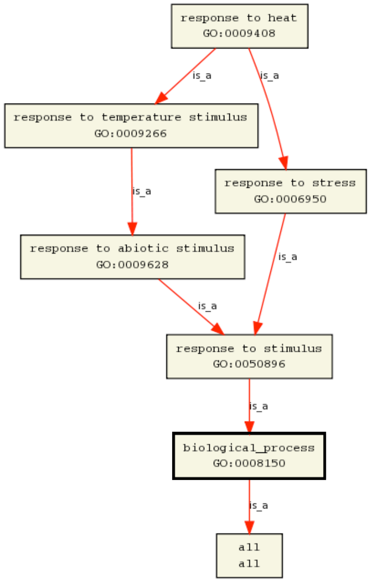
- VLOOKUP in Excel -> *good if you are an excel whizz - I am not!*
  - Download flat file from Entrez, Uniprot, etc; Open in Excel; Find columns that correspond to the 2 ids you want to convert between; Use vlookup to translate your list
- Use the **merge()** or **mapIDs()** functions in **R** - fast, *versatile* & reproducible!
  - Also **clusterProfiler::bitr()** function and many others… [Link to clusterProfiler vignette]

What functional set databases do you want?

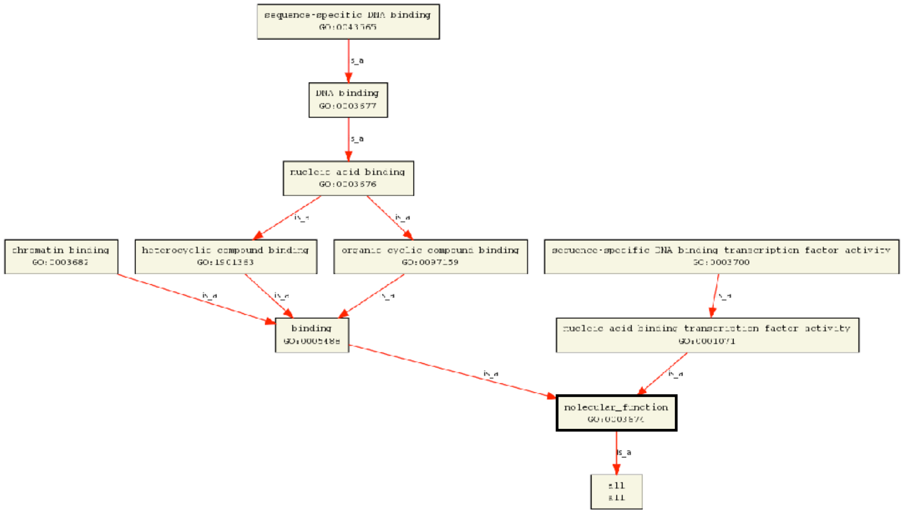
- Commonly used
  - **Gene Ontology (GO)**
  - **KEGG Pathways** (mostly metabolic)
  - **GeneGO MetaBase**
  - **Ingenuity Pathway Analysis (IPA)**
  - **MSigDB** (gene sets based on chromosomal position, cis-regulatory motifs, GO terms, etc)
- Many others…
  - Enzyme Classification, Pfam families
  - Open Biomedical Ontologies (OBO, www.obofoundry.org)

GO database < 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**

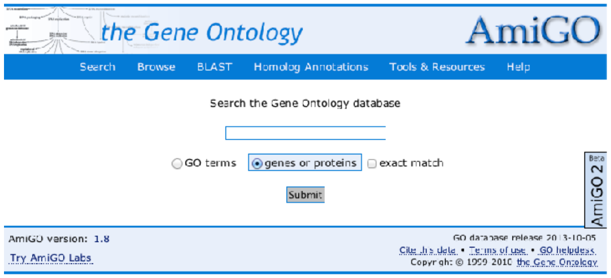


- Terms are nodes
- Relationships are edges
- Parent terms are more general
- Terms can have multiple parents



GO Annotations

- GO is not a database of genes/proteins or sequences
- Gene products get annotated with GO terms by organism specific databases, such as Flybase, Wormbase, MGI, ZFIN, UniProt, etc
- Annotations are available through AmiGO < amigo.geneontology.org >



Search the Gene Ontology database

○ GO terms ● genes or proteins □ exact match

AmiGO version: 1.8

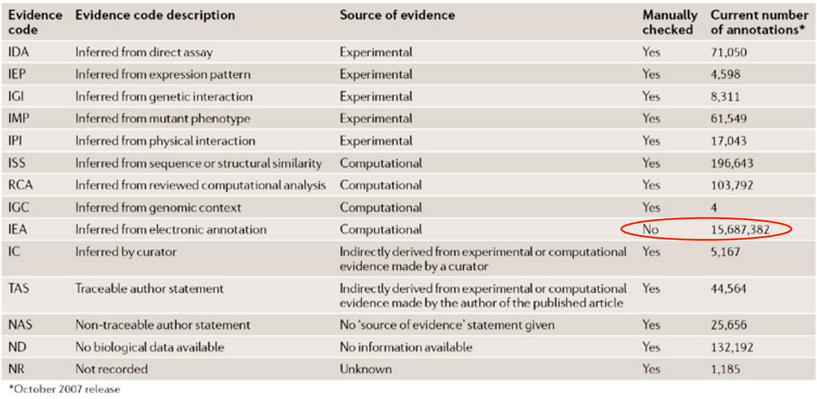
Try AmiGO Labs

GO database release 2013-10-05

Cite this data • Terms of use • GO helpdesk

Copyright © 1999 2010 the Gene Ontology

GO evidence codes

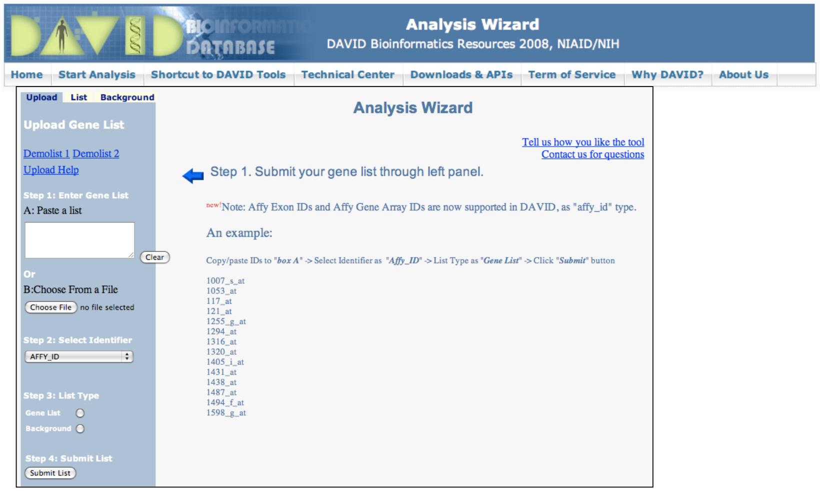


Evidence code	Evidence code description	Source of evidence	Manually checked	Current number of annotations*
IDA	Inferred from direct assay	Experimental	Yes	71,050
IEP	Inferred from expression pattern	Experimental	Yes	4,598
IGI	Inferred from genetic interaction	Experimental	Yes	8,311
IMP	Inferred from 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

Use and misuse of the gene ontology annotations
Seung Yon Rhee, Valerie Wood, Kara Dolinski & Sorin Draghici
Nature Reviews Genetics **9**, 509-515 (2008)

DAVID at NIAID < david.abcc.ncifcrf.gov >



Upload Gene List

Demolist 1 Demolist 2

Upload Help

Step 1: Enter Gene List

A: Paste a list

Or

B:Choose From a File

no file selected

Step 2: Select Identifier

AFFY_ID

Step 3: List Type

Gene List ○

Background ○

Step 4: Submit List

Analysis Wizard

Tell us how you like the tool
Contact us for questions

Step 1. Submit your gene list through left panel.

new!Note: Affy Exon IDs and Affy Gene Array IDs are now supported in DAVID, as "affy_id" type.

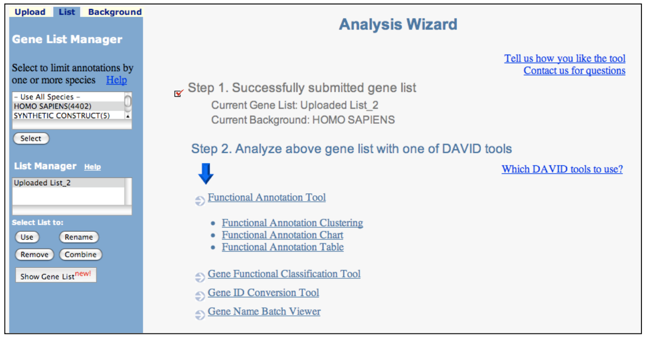
An example:

Copy/paste IDs to "*box A*" -> Select Identifier as "*Affy_ID*" -> List Type as "*Gene List*" -> Click "*Submit*" button

1007_s_at
1053_at
117_at
121_at
1255_g_at
1294_at
1316_at
1320_at
1405_i_at
1431_at
1438_at
1487_at
1494_f_at
1598_g_at

DAVID

- Notice that you can pick a *Background* (Universe)



Gene List Manager

Select to limit annotations by one or more species Help

- Use All Species -
HOMO SAPIENS(4402)
SYNTHETIC CONSTRUCT(5)

List Manager Help

Uploaded List_2

Select List to:

Analysis Wizard

Tell us how you like the tool
Contact us for questions

☑ Step 1. Successfully submitted gene list
Current Gene List: Uploaded List_2
Current Background: HOMO SAPIENS

Step 2. Analyze above gene list with one of DAVID tools

Which DAVID tools to use?

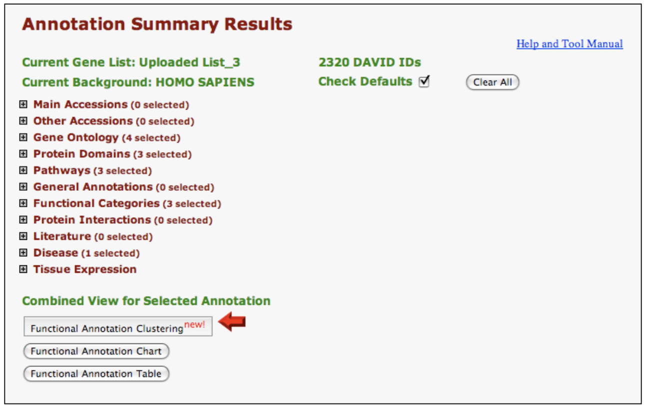
Functional Annotation Tool

- Functional Annotation Clustering
- Functional Annotation Chart
- Functional Annotation Table

Gene Functional Classification Tool
Gene ID Conversion Tool
Gene Name Batch Viewer

DAVID

- *Functional Annotation Tool*


Clear All
Functional Annotation Clustering new!
Functional Annotation Chart
Functional Annotation Table

Annotation Summary Results

Help and Tool Manual

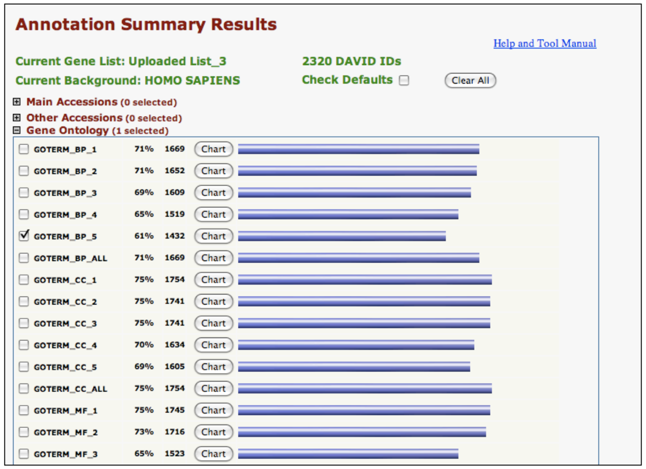
Current Gene List: Uploaded List_3 2320 DAVID IDs
Current Background: HOMO SAPIENS Check Defaults ☑

⊞ Main Accessions (0 selected)
⊞ Other Accessions (0 selected)
⊞ Gene Ontology (4 selected)
⊞ Protein Domains (3 selected)
⊞ Pathways (3 selected)
⊞ General Annotations (0 selected)
⊞ Functional Categories (3 selected)
⊞ Protein Interactions (0 selected)
⊞ Literature (0 selected)
⊞ Disease (1 selected)
⊞ Tissue Expression

Combined View for Selected Annotation

DAVID

- Specify functional sets



Annotation Summary Results

Help and Tool Manual

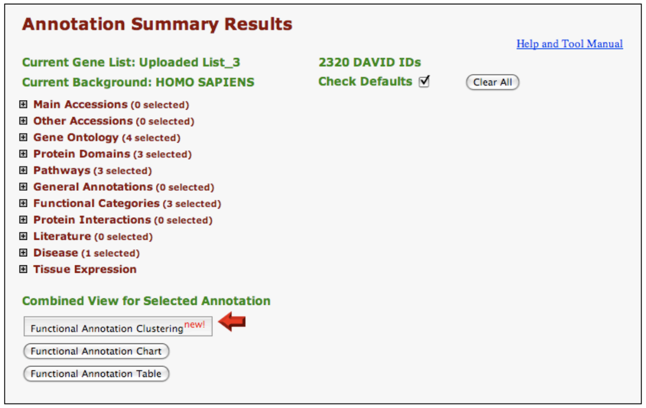
Current Gene List: Uploaded List_3 2320 DAVID IDs
Current Background: HOMO SAPIENS Check Defaults □

⊞ Main Accessions (0 selected)
⊞ Other Accessions (0 selected)
⊟ Gene Ontology (1 selected)

□ GOTERM_BP_1	71%	1669	Chart	
□ GOTERM_BP_2	71%	1652	Chart	
□ GOTERM_BP_3	69%	1609	Chart	
□ GOTERM_BP_4	65%	1519	Chart	
☑ GOTERM_BP_5	61%	1432	Chart	
□ GOTERM_BP_ALL	71%	1669	Chart	
□ GOTERM_CC_1	75%	1754	Chart	
□ GOTERM_CC_2	75%	1741	Chart	
□ GOTERM_CC_3	75%	1741	Chart	
□ GOTERM_CC_4	70%	1634	Chart	
□ GOTERM_CC_5	69%	1605	Chart	
□ GOTERM_CC_ALL	75%	1754	Chart	
□ GOTERM_MF_1	75%	1745	Chart	
□ GOTERM_MF_2	73%	1716	Chart	
□ GOTERM_MF_3	65%	1523	Chart	

DAVID

- Let's look at the *Functional Annotation Chart*


Clear All
Functional Annotation Clustering new!
Functional Annotation Chart
Functional Annotation Table

Annotation Summary Results

Help and Tool Manual

Current Gene List: Uploaded List_3 2320 DAVID IDs
Current Background: HOMO SAPIENS Check Defaults ☑

⊞ Main Accessions (0 selected)
⊞ Other Accessions (0 selected)
⊞ Gene Ontology (4 selected)
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⊞ Pathways (3 selected)
⊞ General Annotations (0 selected)
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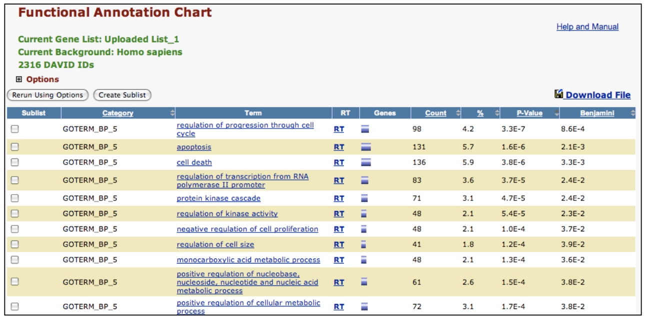
⊞ Protein Interactions (0 selected)
⊞ Literature (0 selected)
⊞ Disease (1 selected)
⊞ Tissue Expression

Combined View for Selected Annotation

DAVID

- *Functional Annotation Chart*



Functional Annotation Chart

Help and Manual

Current Gene List: Uploaded List_1
Current Background: Homo sapiens
2316 DAVID IDs

⊞ **Options**

(Rerun Using Options) (Create Sublist)

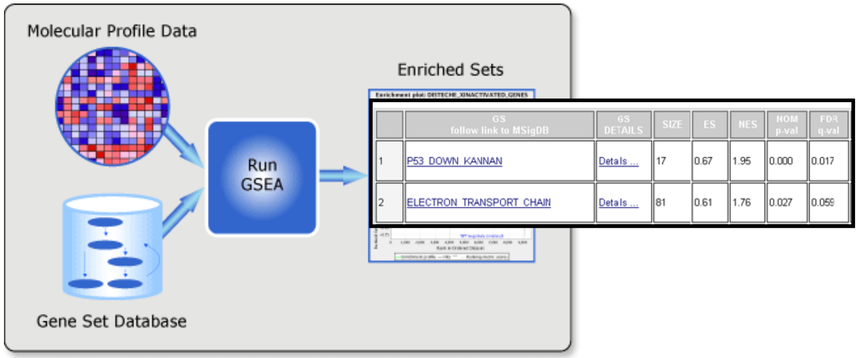
Download File

Sublist	Category	Term	RT	Genes	Count	%	P-Value	Benjamini
□	GOTERM_BP_5	regulation of progression through cell cycle	RT	▬	98	4.2	3.3E-7	8.6E-4
□	GOTERM_BP_5	apoptosis	RT	▬	131	5.7	1.6E-6	2.1E-3
□	GOTERM_BP_5	cell death	RT	▬	136	5.9	3.8E-6	3.3E-3
□	GOTERM_BP_5	regulation of transcription from RNA polymerase II promoter	RT	▬	83	3.6	3.7E-5	2.4E-2
□	GOTERM_BP_5	protein kinase cascade	RT	▬	71	3.1	4.7E-5	2.4E-2
□	GOTERM_BP_5	regulation of kinase activity	RT	▬	48	2.1	5.4E-5	2.3E-2
□	GOTERM_BP_5	negative regulation of cell proliferation	RT	▬	48	2.1	1.0E-4	3.7E-2
□	GOTERM_BP_5	regulation of cell size	RT	▬	41	1.8	1.2E-4	3.9E-2
□	GOTERM_BP_5	monocarboxylic acid metabolic process	RT	▬	48	2.1	1.3E-4	3.6E-2
□	GOTERM_BP_5	positive regulation of nucleobase, nucleoside, nucleotide and nucleic acid metabolic process	RT	▬	61	2.6	1.5E-4	3.8E-2
□	GOTERM_BP_5	positive regulation of cellular metabolic process	RT	▬	72	3.1	1.7E-4	3.8E-2

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)*

GSEA < www.broadinstitute.org/gsea >

- Download GSEA desktop application



	GS follow link to MSigDB	GS DETAILS	SIZE	ES	NES	NOM p-val	FDR q-val
1	P53_DOWN_KANNAN	Details ...	17	0.67	1.95	0.000	0.017
2	ELECTRON_TRANSPORT_CHAIN	Details ...	81	0.61	1.76	0.027	0.059

- Excellent tutorial, user's guide and example datasets to work through

Gene set enrichment analysis: A knowledge-based approach for interpreting genome-wide expression profiles
Aravind Subramanian, Pablo Tamayo, Vamsi K. Mootha, Sayan Mukherjee, Benjamin L. Ebert, Michael A. Gillette, ...
PNAS ***102****, 15545-15550 (2005)*

Overlapping functional sets

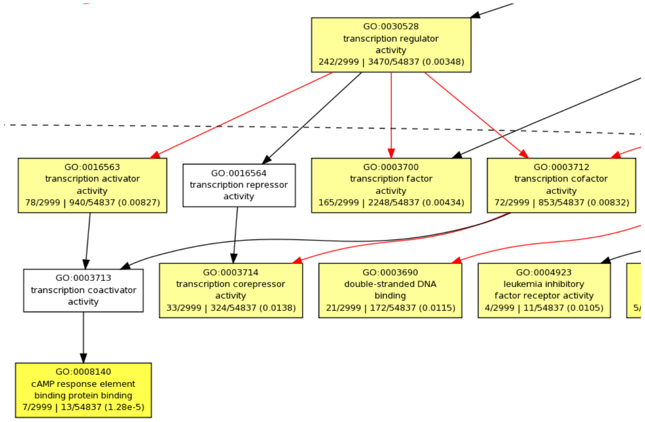
- Many functional sets overlap, in particular those from databases that are hierarchical in nature (e.g. GO)

- 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

GOEast < omicslab.genetics.ac.cn/GOEAST >

- Graphical view of enriched GO terms and their relationships

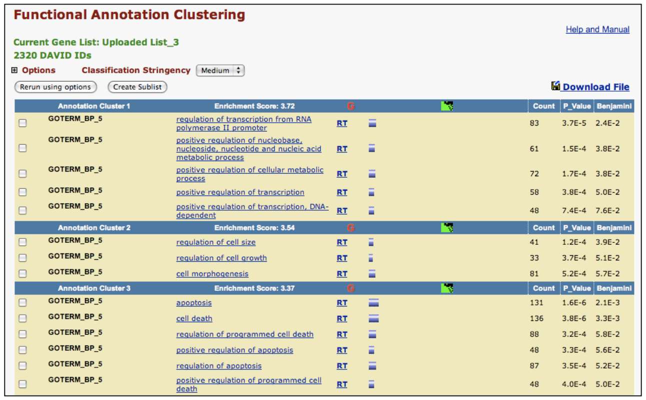

GO:0030528
transcription regulator activity
242/2999 | 3470/54837 (0.00348)
GO:0016563
transcription activator activity
78/2999 | 940/54837 (0.00827)
GO:0016564
transcription repressor activity
GO:0003700
transcription factor activity
165/2999 | 2248/54837 (0.00434)
GO:0003712
transcription cofactor activity
72/2999 | 853/54837 (0.00832)
GO:0003713
transcription coactivator activity
GO:0003714
transcription corepressor activity
33/2999 | 324/54837 (0.0138)
GO:0003690
double-stranded DNA binding
21/2999 | 172/54837 (0.0115)
GO:0004923
leukemia inhibitory factor receptor activity
4/2999 | 11/54837 (0.0105)
5/
GO:0008140
cAMP response element binding protein binding
7/2999 | 13/54837 (1.28e-5)

GO SLIMs

- Cut-down versions of the GO ontologies containing a subset of the terms in the whole GO
- GO FAT (DAVID):
  - filters out very broad GO terms based on a measured specificity of each term

DAVID Functional Annotation Clustering

- Based on shared genes between functional sets



Functional Annotation Clustering

Help and Manual

Current Gene List: Uploaded List_3
2320 DAVID IDs

⊞ Options Classification Stringency [Medium]

[Rerun using options] [Create Sublist] Download File

Annotation Cluster 1		Enrichment Score: 3.72	G	Count	P_Value	Benjamini
□	GOTERM_BP_5	regulation of transcription from RNA polymerase II promoter	RT	83	3.7E-5	2.4E-2
□	GOTERM_BP_5	positive regulation of nucleobase, nucleoside, nucleotide and nucleic acid metabolic process	RT	61	1.5E-4	3.8E-2
□	GOTERM_BP_5	positive regulation of cellular metabolic process	RT	72	1.7E-4	3.8E-2
□	GOTERM_BP_5	positive regulation of transcription	RT	58	3.8E-4	5.0E-2
□	GOTERM_BP_5	positive regulation of transcription, DNA-dependent	RT	48	7.4E-4	7.6E-2
Annotation Cluster 2		Enrichment Score: 3.54	G	Count	P_Value	Benjamini
□	GOTERM_BP_5	regulation of cell size	RT	41	1.2E-4	3.9E-2
□	GOTERM_BP_5	regulation of cell growth	RT	33	3.7E-4	5.1E-2
□	GOTERM_BP_5	cell morphogenesis	RT	81	5.2E-4	5.7E-2
Annotation Cluster 3		Enrichment Score: 3.37	G	Count	P_Value	Benjamini
□	GOTERM_BP_5	apoptosis	RT	131	1.6E-6	2.1E-3
□	GOTERM_BP_5	cell death	RT	136	3.8E-6	3.3E-3
□	GOTERM_BP_5	regulation of programmed cell death	RT	88	3.2E-4	5.8E-2
□	GOTERM_BP_5	positive regulation of apoptosis	RT	48	3.3E-4	5.6E-2
□	GOTERM_BP_5	regulation of apoptosis	RT	87	3.5E-4	5.2E-2
□	GOTERM_BP_5	positive regulation of programmed cell death	RT	48	4.0E-4	5.0E-2

Want more?

- **GeneGO** < 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 >
  - Extensive cancer related expression datasets
  - Nice concept analysis tools
  - Research edition is free for academics, Premium edition $$$
- **Lots of other Bioconductor packages in this area!**

Do it Yourself!

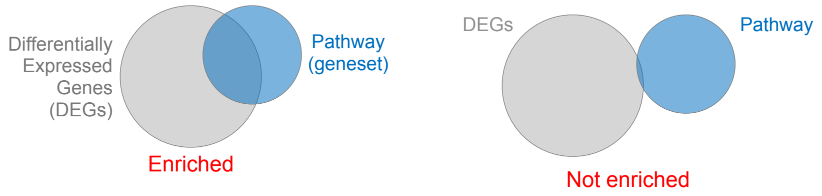
Hands-on time!

https://bioboot.github.io/bimm143_W18/lectures/#15

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

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



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

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

- **Post-transcriptional regulation** is neglected
- **Directionality** is hard to capture sensibly
  - e.g. IκBα/NF-κB
- **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
- **Geneset annotation bias**: can only discover what is already known
- **Non-model organisms**: no high-quality genesets available
- Many pathways/receptors **converge** to few regulators
  - e.g. tens of innate immune receptors activate 4 TFs: NF-kB, AP-1, IRF3/7, NFAT