BIMM 143

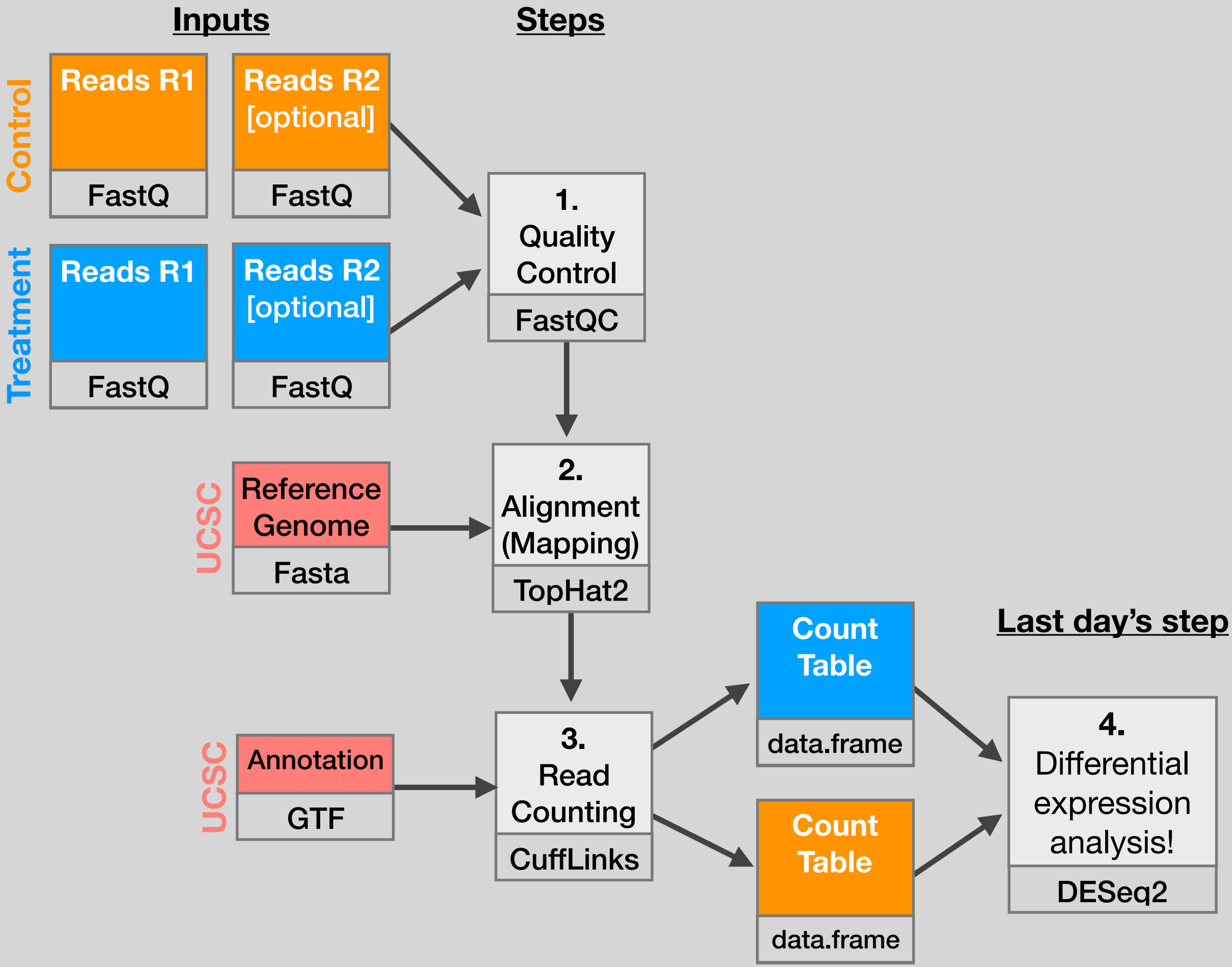
Pathway Analysis and the Interpretation of Gene Lists

Lecture 15

Barry Grant

UC San Diego

http://thegrantlab.org/bimm143


Inputs
Steps
Control
Reads R1
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FastQ
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FastQ
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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…

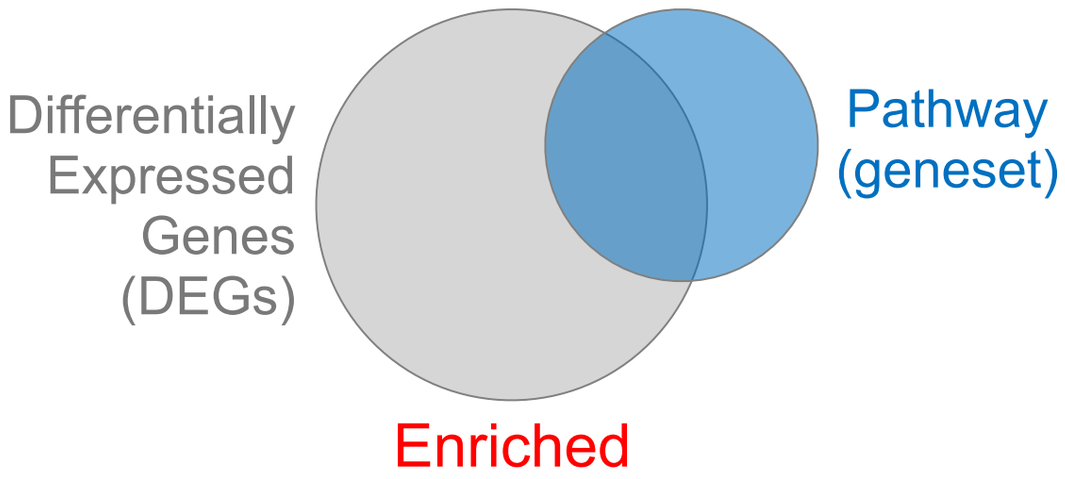
What do I do now?

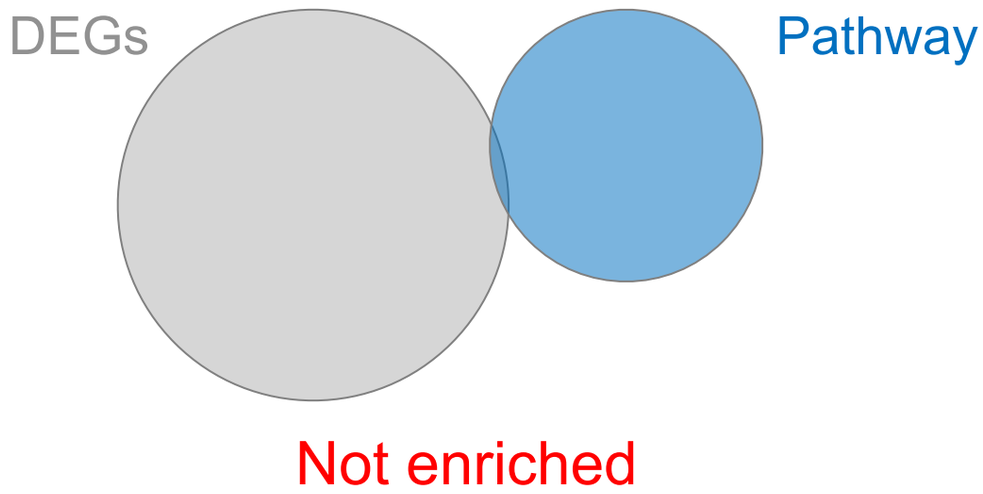
Pathway analysis!

(a.k.a. geneset enrichment)

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

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





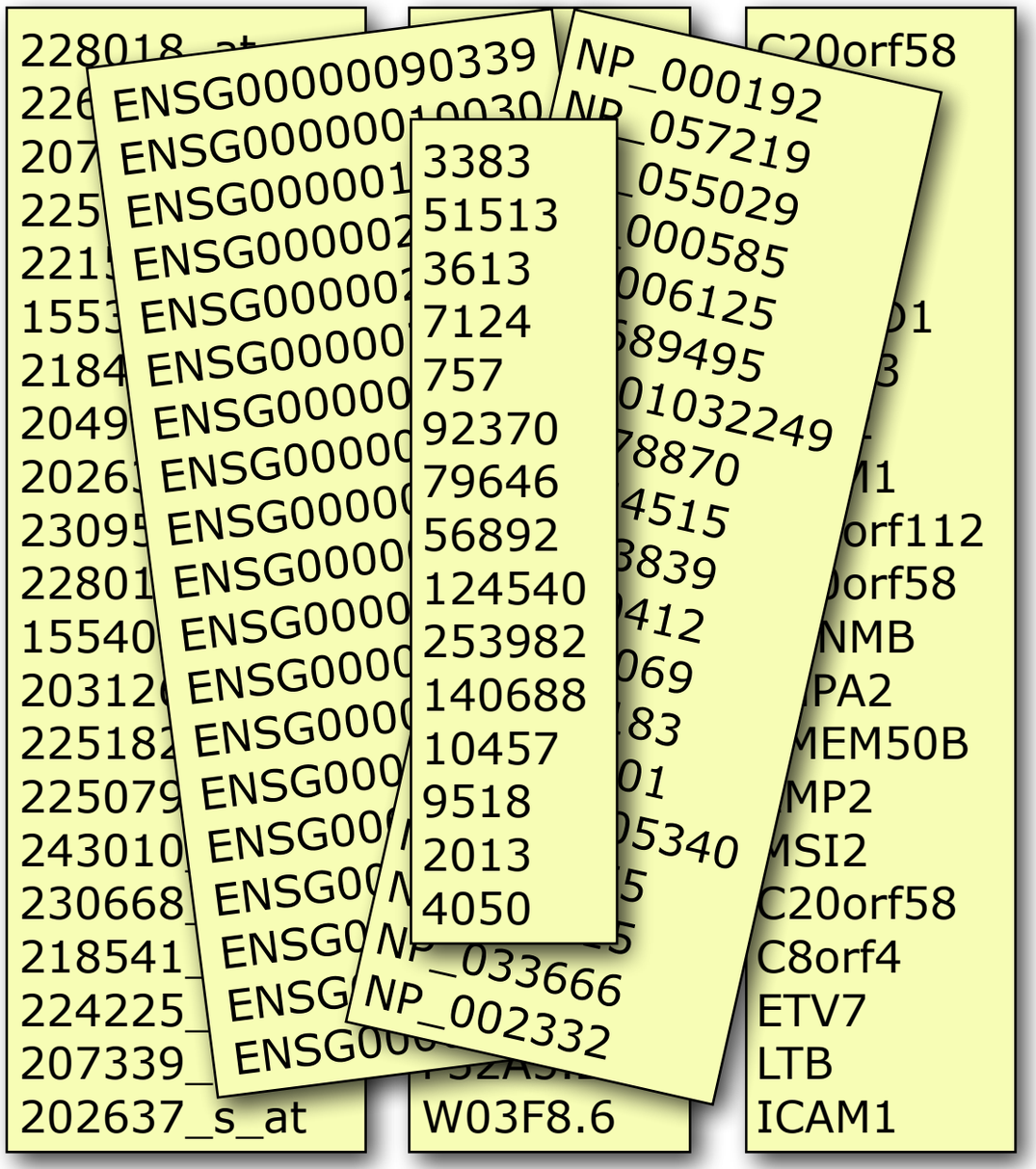
- Variations of the math: overlap, ranking, networks... ➢ *Not critical, different algorithms show similar performances*
- DEGs come from your experiment ➢ *Critical, needs to be as clean as possible*
- Pathway genes (“geneset”) come from annotations ➢ *Important, but typically not a competitive advantage*

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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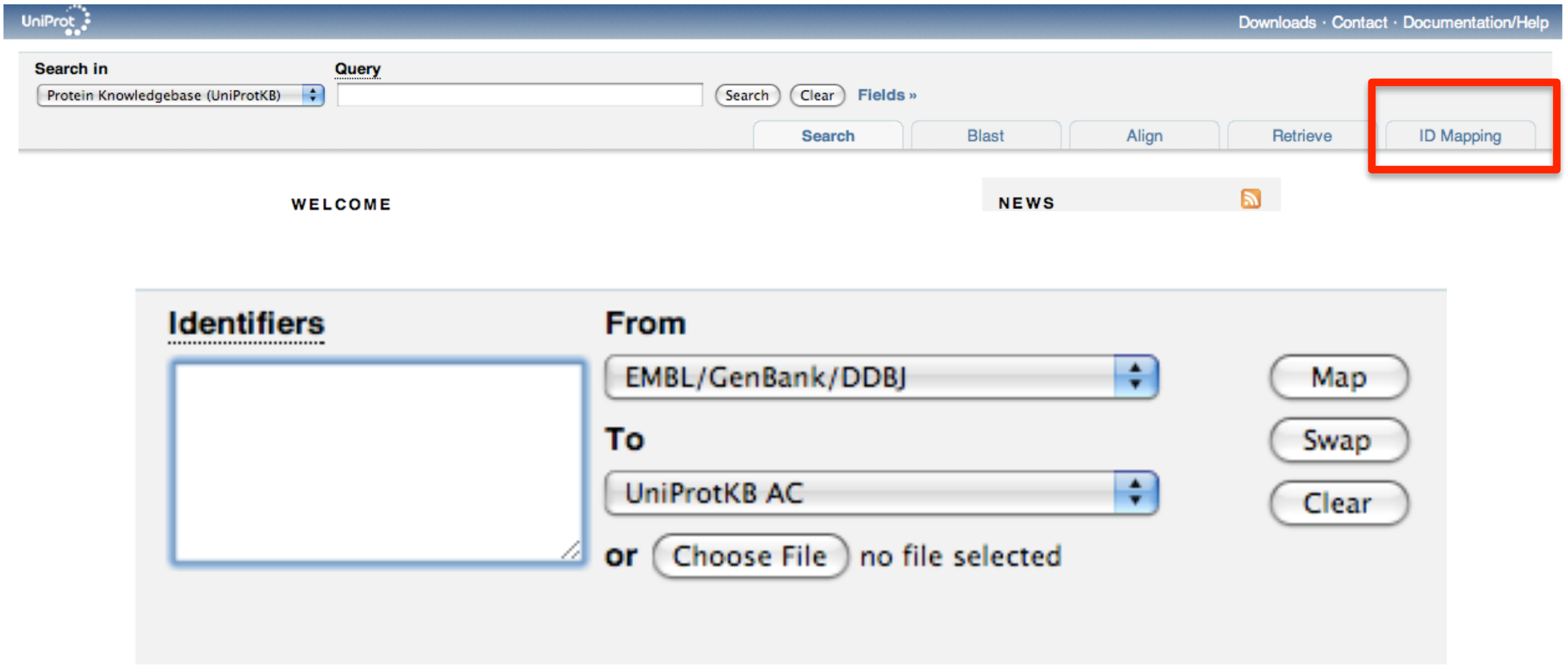
- **Various web sites translate ids -> *best for small lists***
  - **UniProt < www.uniprot.org>; IDConverter < idconverter.bioinfo.cnio.es >**

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



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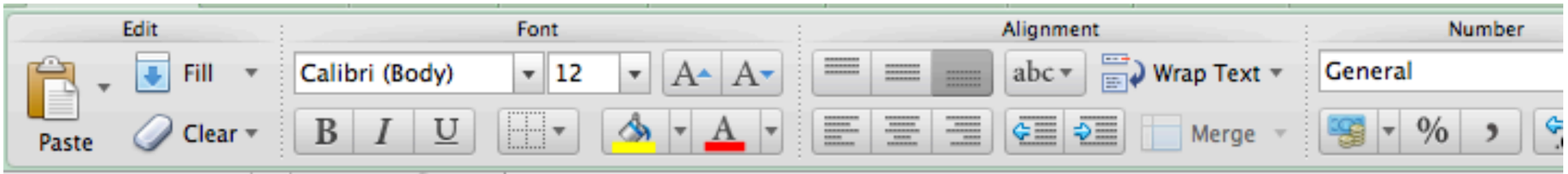
- **VLOOKUP in Excel - *good if you are an excel whizz - I am not!***
  - **Download flat file from Entrez, Uniprot, etc; Open in Excel; Find columns that correspond to the 2 IDs you want to convert between; Sort by ID; Use vlookup to translate your list**

Translating between identifiers: Excel VLOOKUP

VLOOKUP(lookup_value, table_array, col_index_num)



	A	B	C	D	E	F	G	H	I	J	K
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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  - 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?

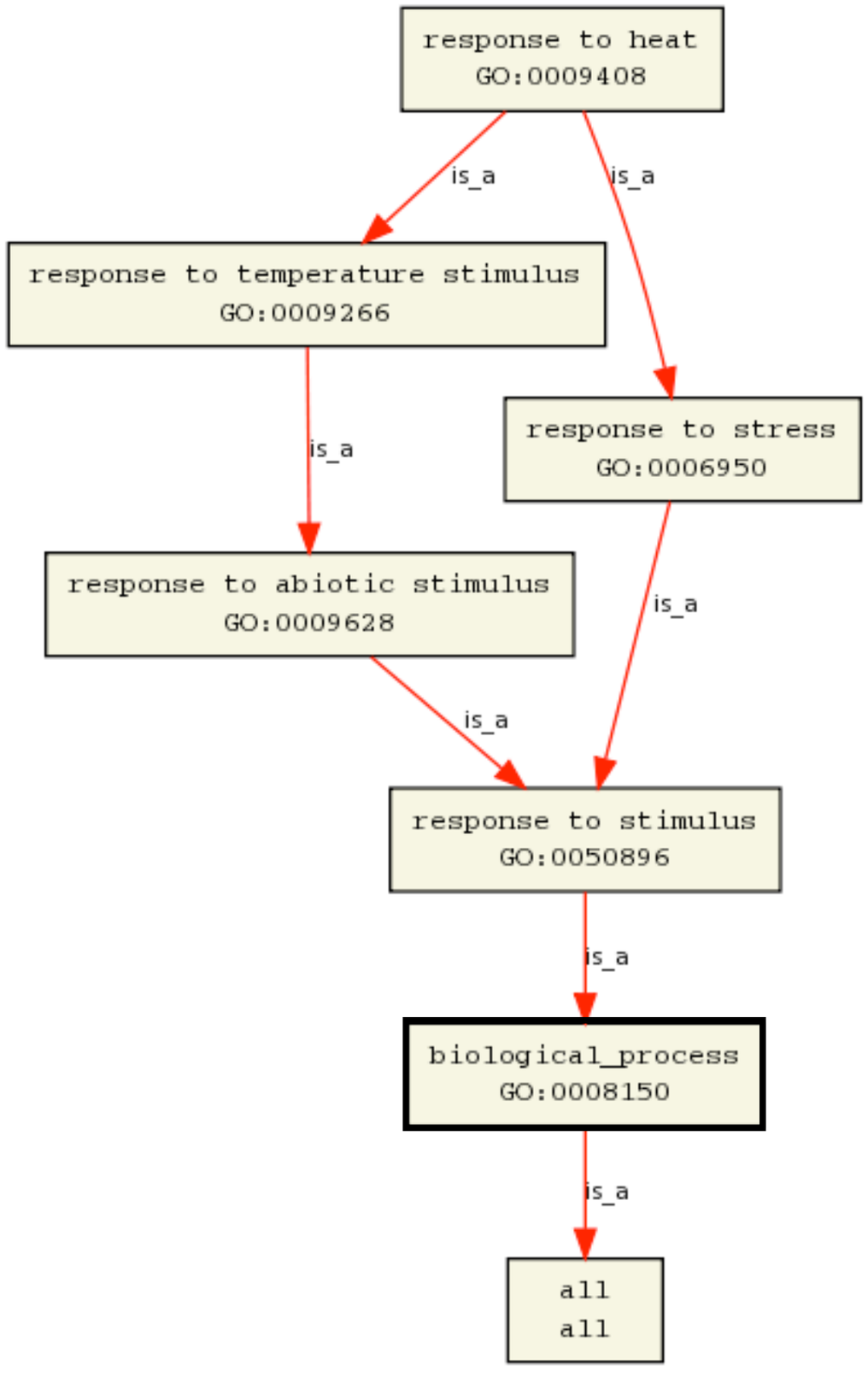
- Commonly used
  - **Gene Ontology (GO)**
  - **KEGG Pathways** (mostly metabolic)
  - **GeneGO MetaBase**
  - **Ingenuity Pathway Analysis (IPA)**
  - **MSigDB** (gene sets based on chromosomal position, cis-regulatory motifs, GO terms, etc)
- Many others...
  - Enzyme Classification, Pfam families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

GO database < www.geneontology.org >

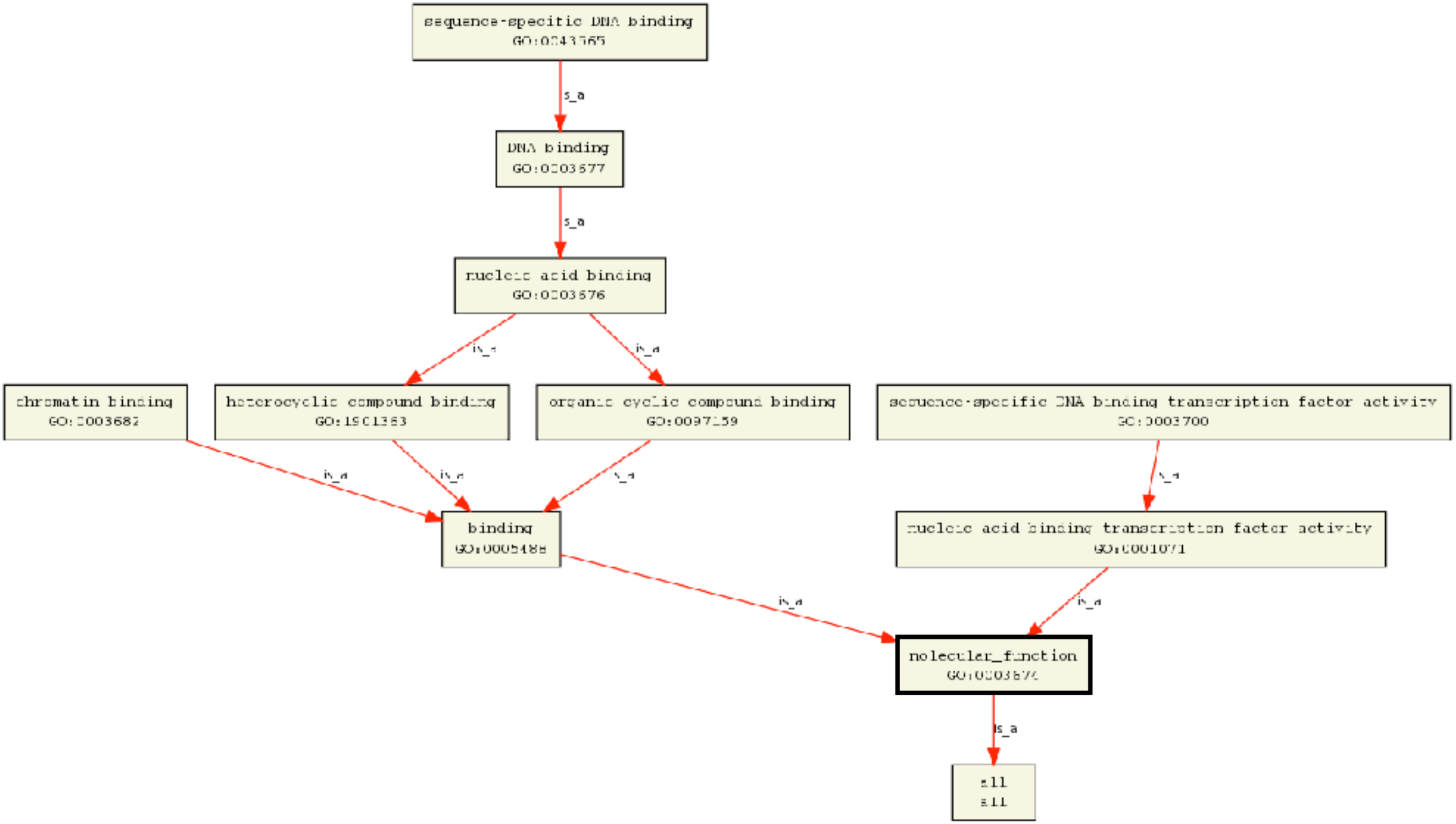
- **What function does HSF1 perform?**
  - *response to heat; sequence-specific DNA binding; transcription; etc*

- **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**

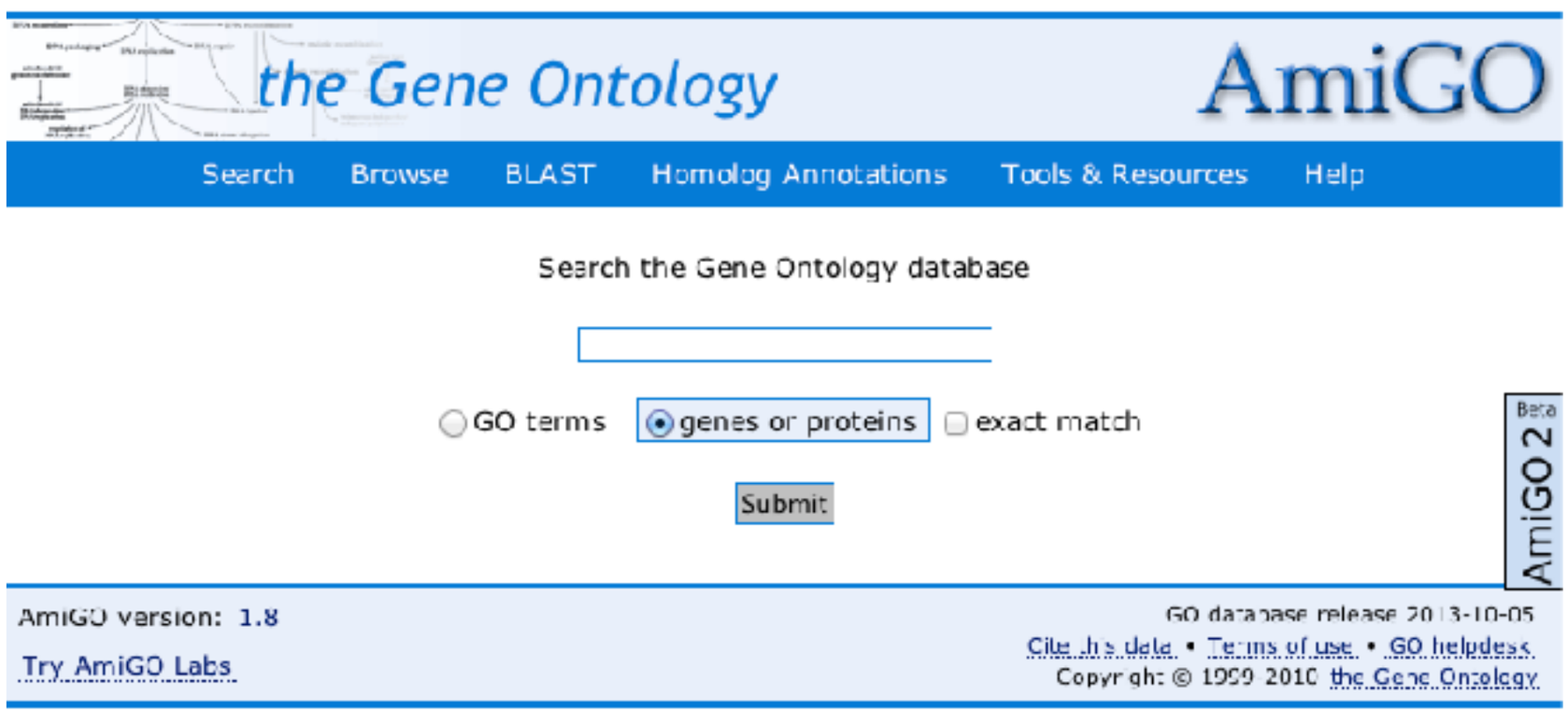


- Terms are nodes
- Relationships are edges
- Parent terms are more general
- Terms can have multiple parents


sequence-specific DNA binding
GO:0043565
is_a
DNA binding
GO:0003677
is_a
nucleic acid binding
GO:0003676
is_a
is_a
chromatin binding
GO:0003682
heterocyclic compound binding
GO:1901363
organic cyclic compound binding
GO:0097159
sequence-specific DNA binding transcription factor activity
GO:0003700
is_a
is_a
is_a
is_a
binding
GO:0005488
nucleic acid binding transcription factor activity
GO:0001071
is_a
is_a
molecular_function
GO:0003674
is_a
all
all

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 >



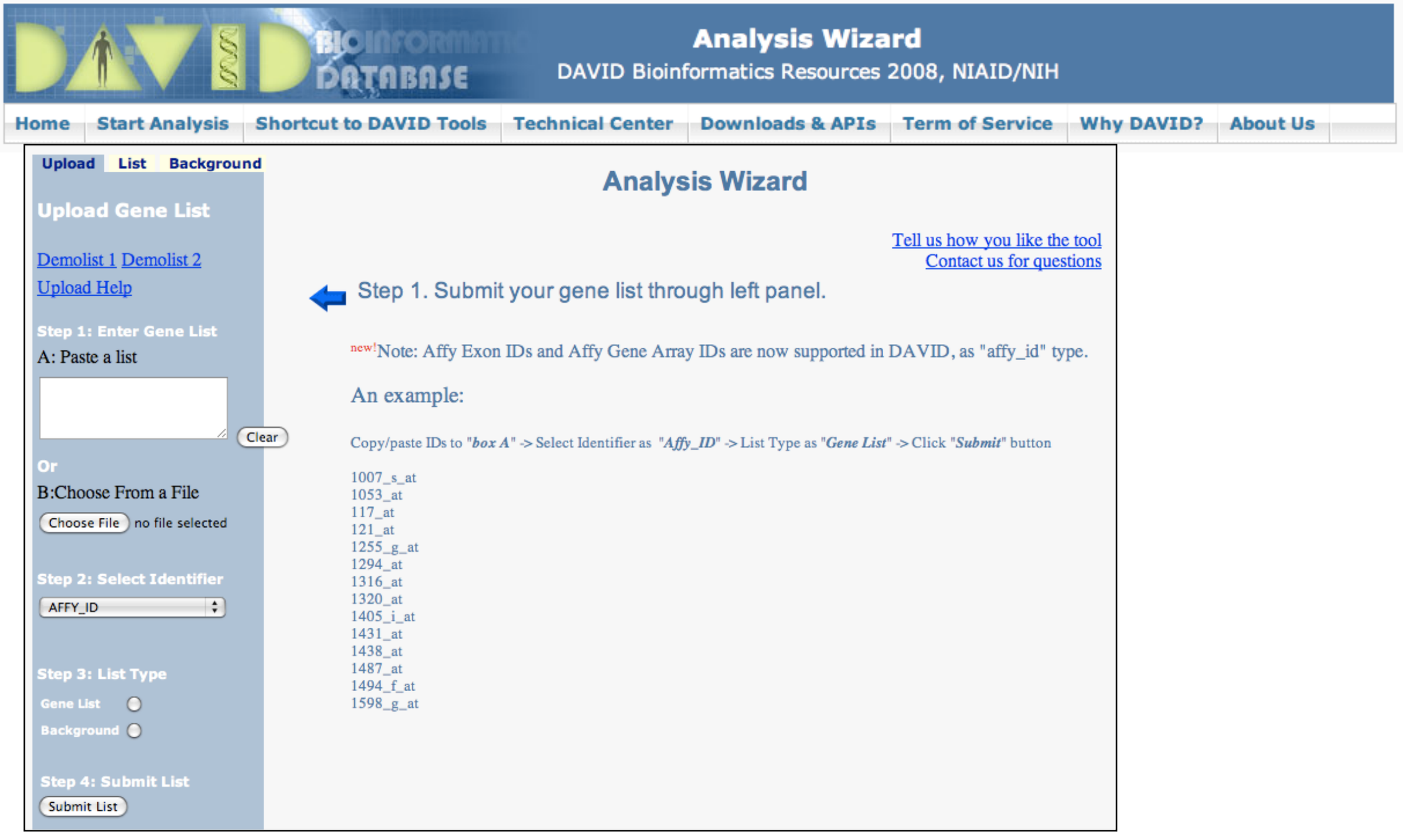
GO evidence codes

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

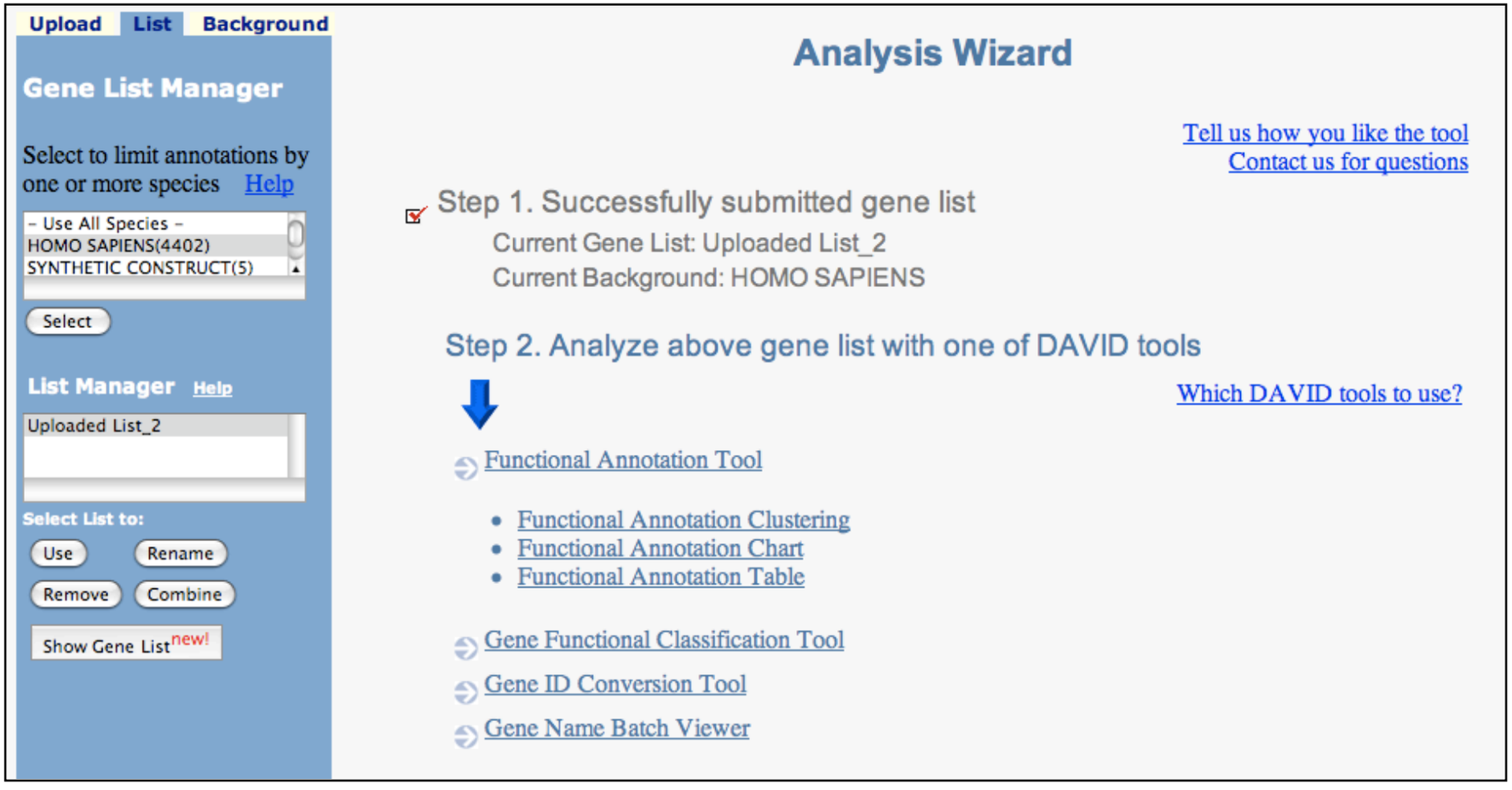
Use and misuse of the gene ontology annotations
Seung Yon Rhee, Valerie Wood, Kara Dolinski & Sorin Draghici
Nature Reviews Genetics **9**, 509-515 (2008)

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



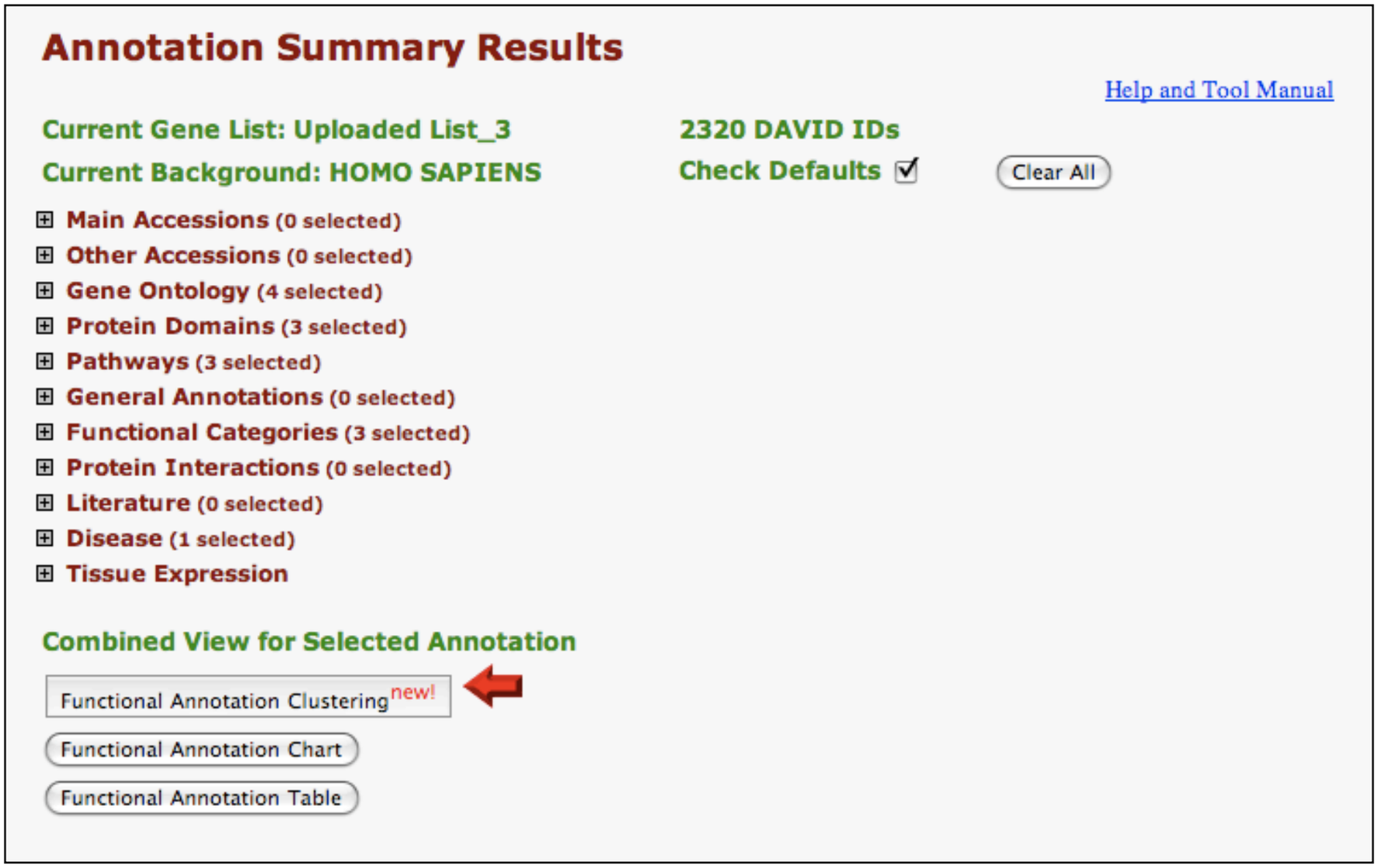
DAVID

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



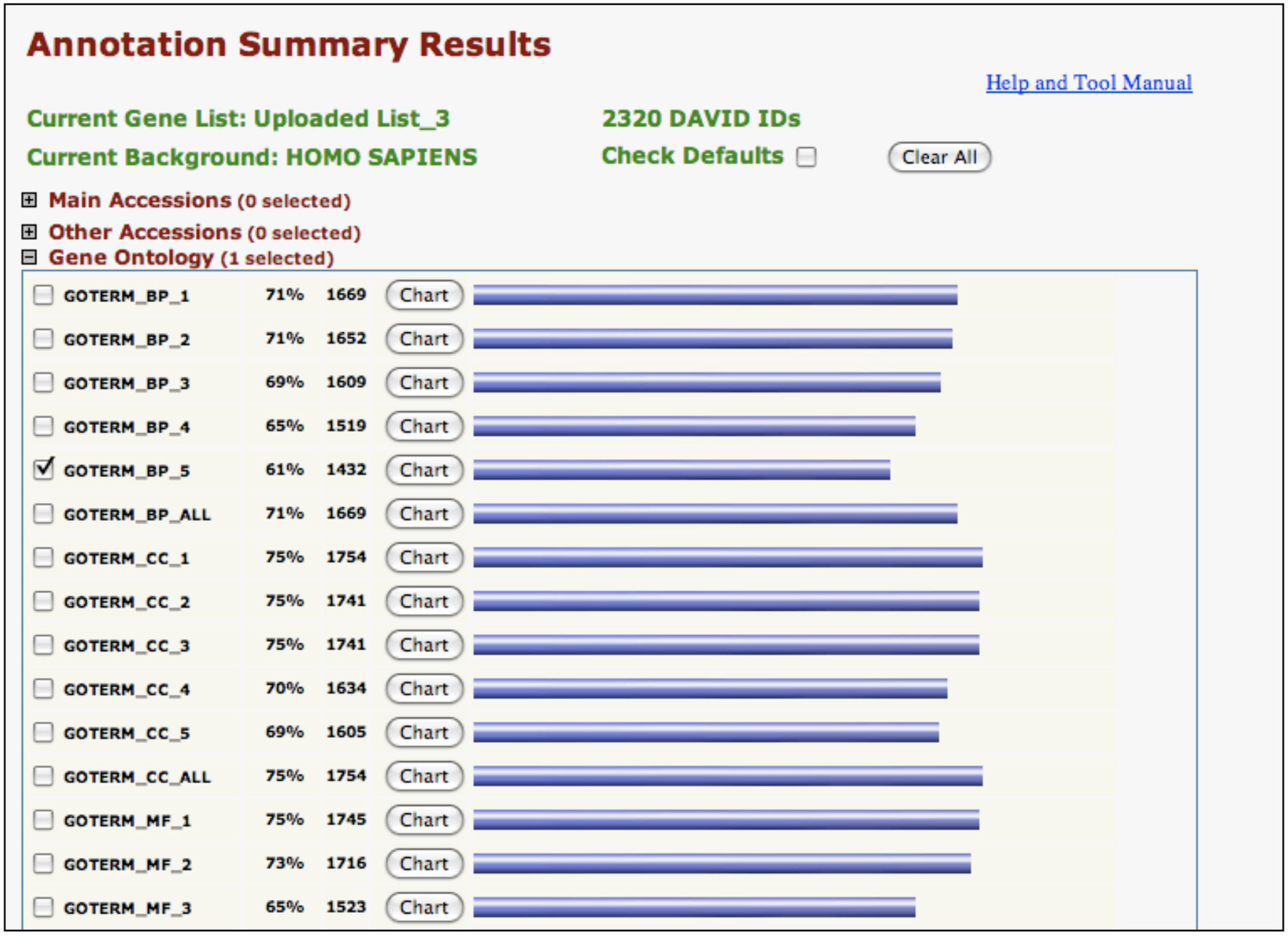
DAVID

- *Functional Annotation Tool*



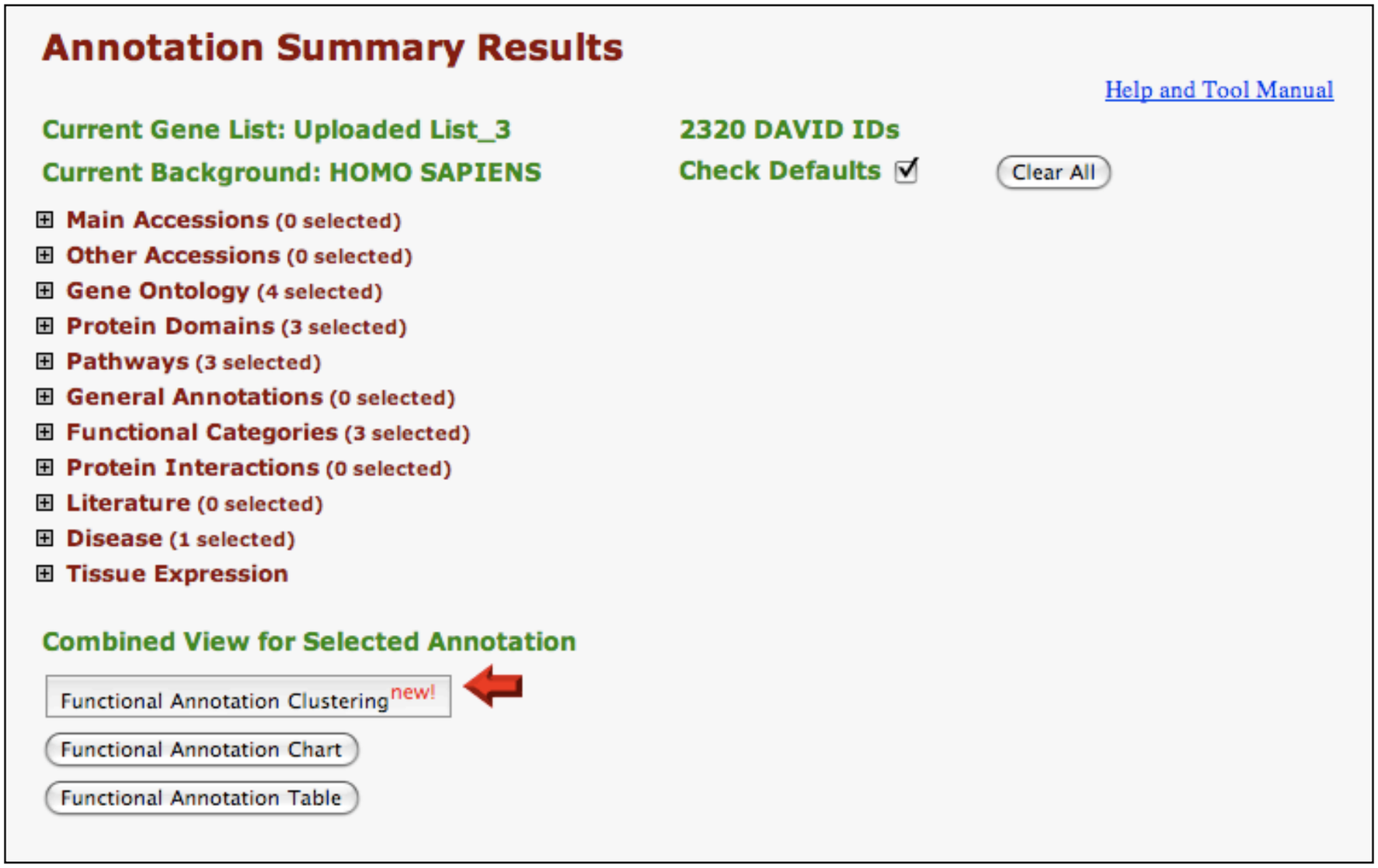
DAVID

- Specify functional sets



DAVID

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



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

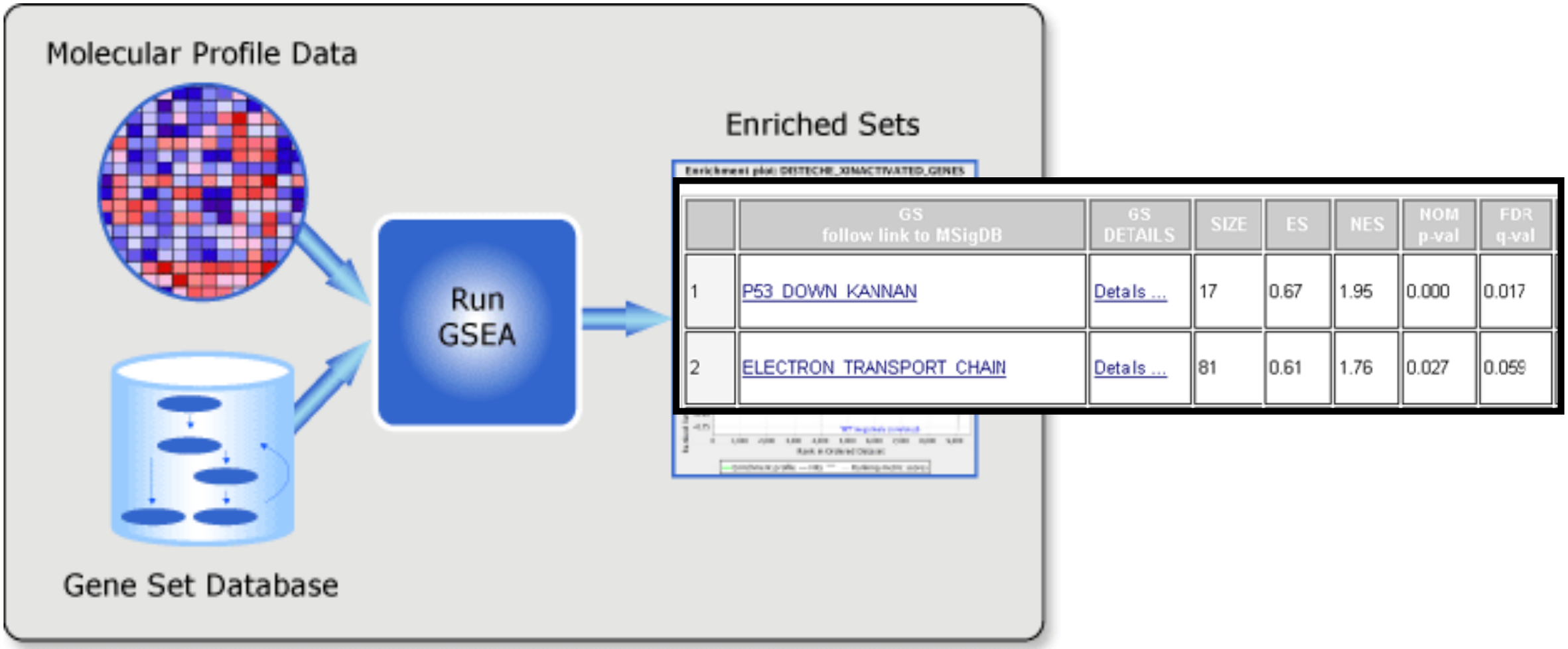
Download File

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

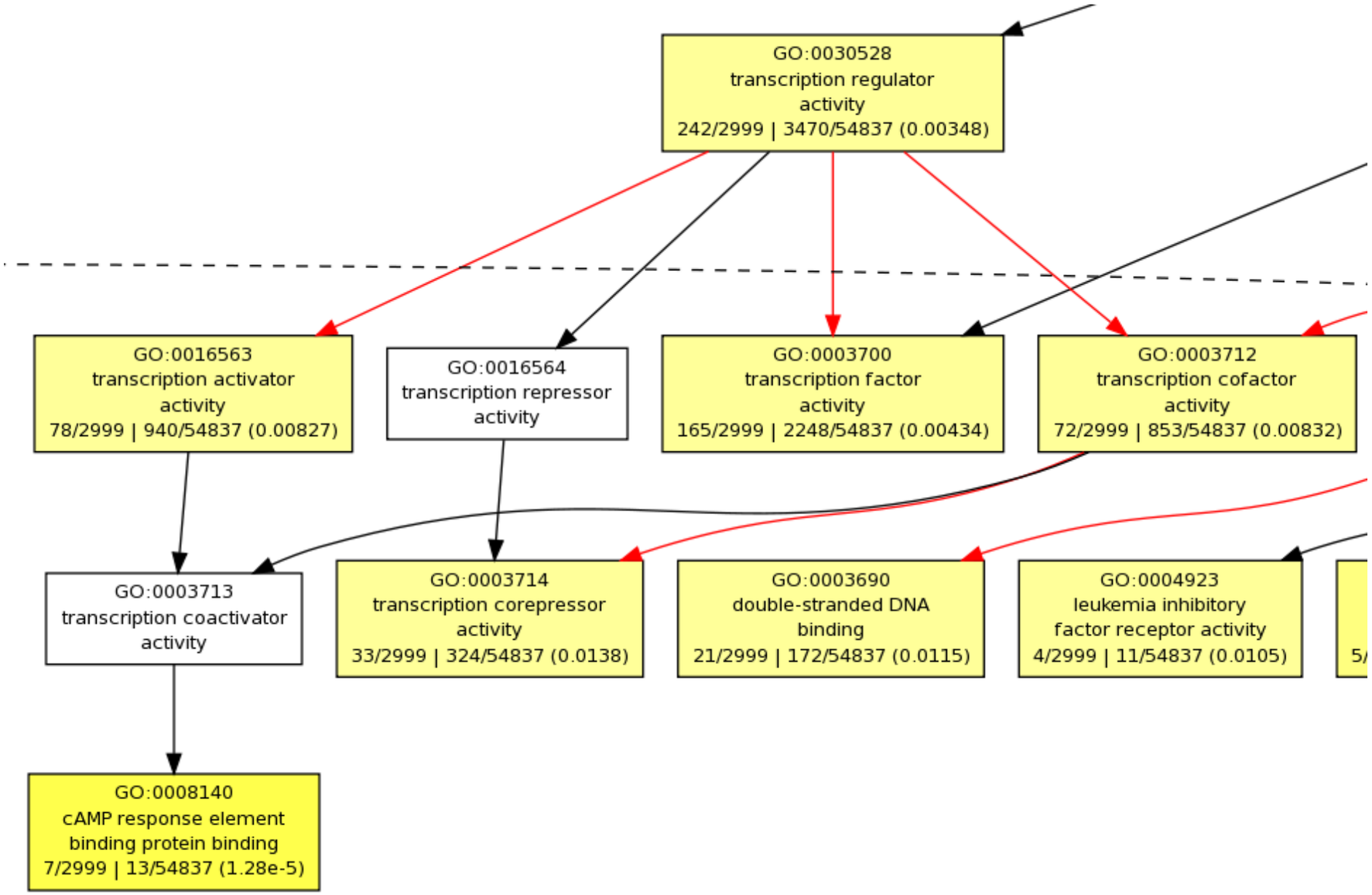
- Many functional sets overlap, in particular those from databases that are hierarchical in nature (e.g. GO)

- 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 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			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**			**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**			**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

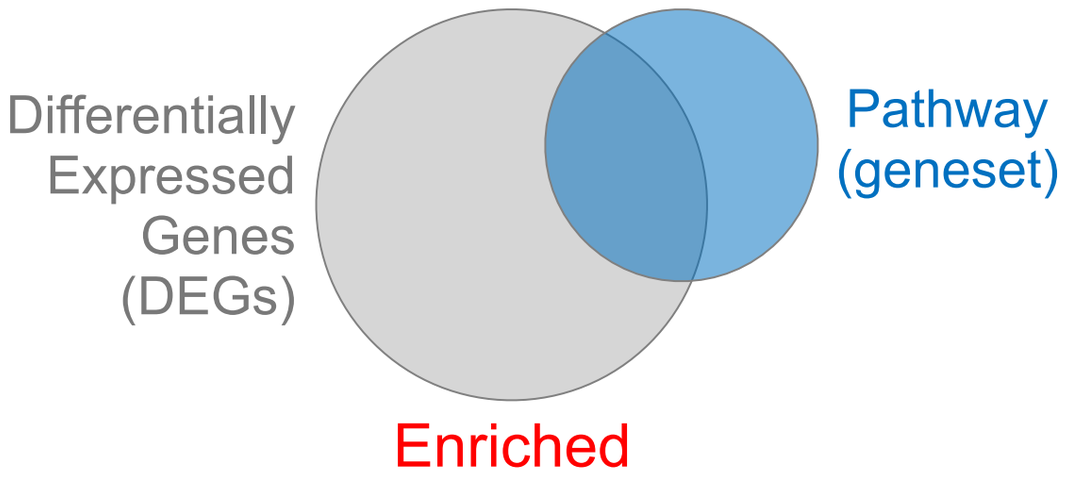
Advice:
Figure out “**What do I want to do with my list?**”

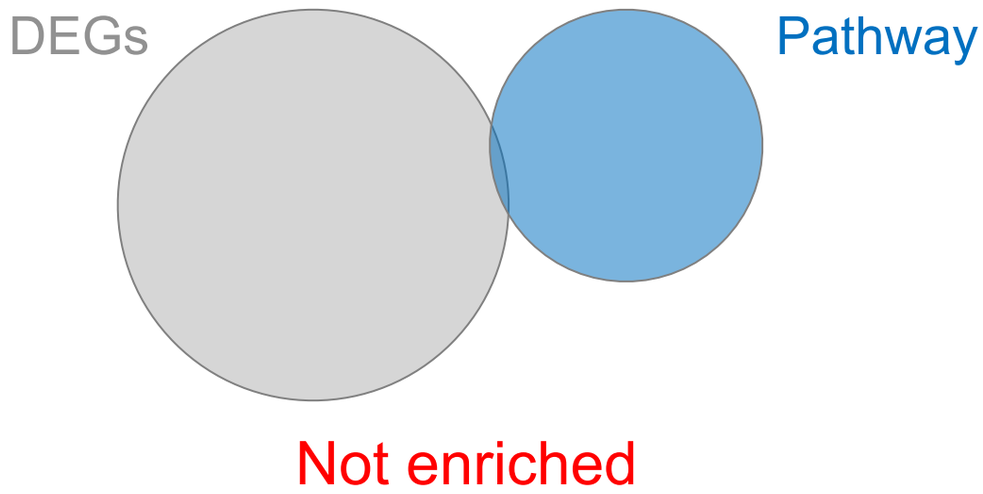
- Organize/summarize data for presentation or manuscript
  - DAVID: GO_FAT -> Functional Annotation Clustering -> Pick threshold

- 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





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- Pathway genes (“geneset”) come from annotations ➢ *Important, but typically not a competitive advantage*

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- **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
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- **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
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