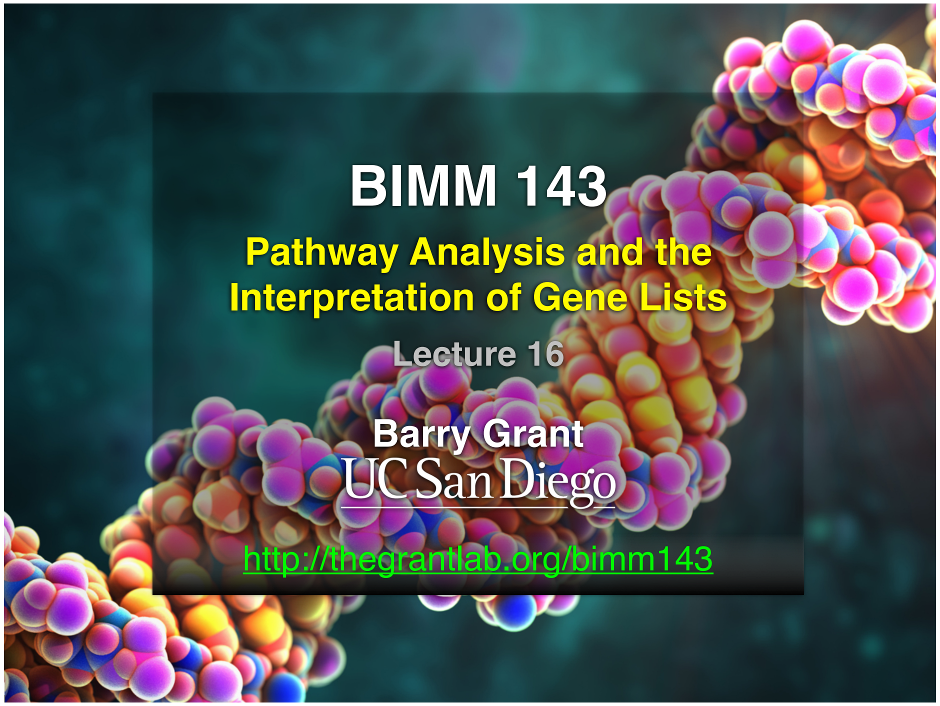
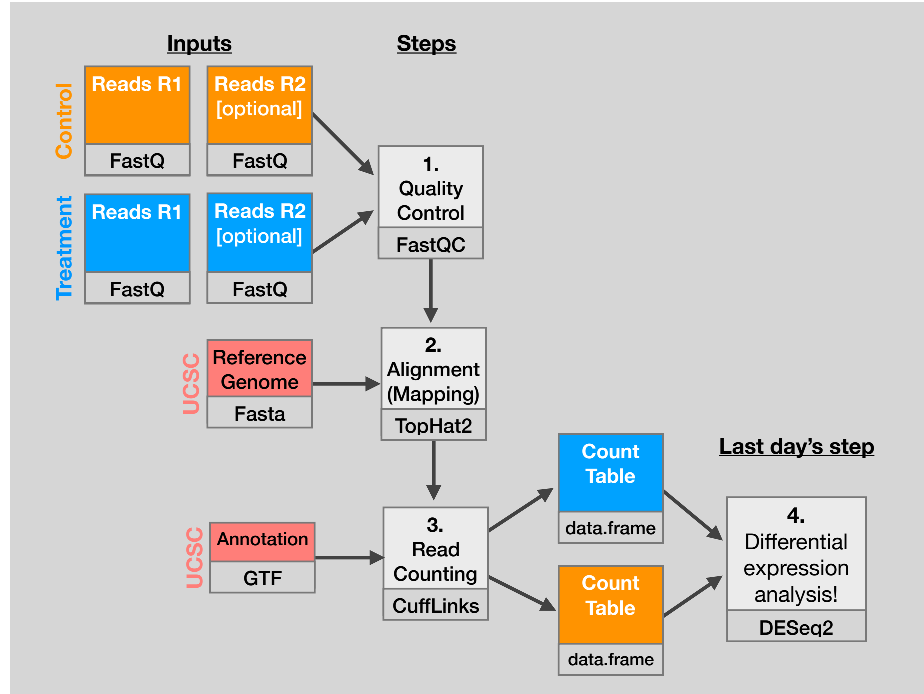
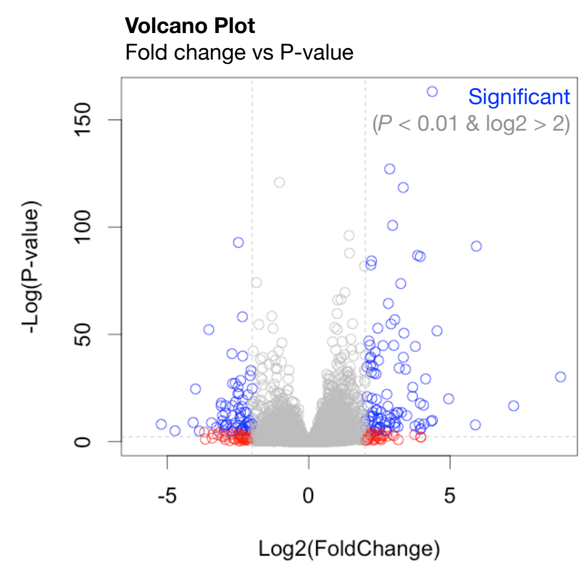




X	baseMean	log2FoldChange	lfcSE	stat	pvalue	padj	symbol
ENSG00000152583	954.77093	4.3683590	0.23713648	18.421286	8.867079e-76	1.342919e-71	SPARCL1
ENSG00000179094	743.25269	2.8638885	0.17555825	16.313039	7.972621e-60	6.037267e-56	PER1
ENSG00000116584	2277.91345	-1.0347000	0.06505273	-15.905557	5.798513e-57	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	1.029569e-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

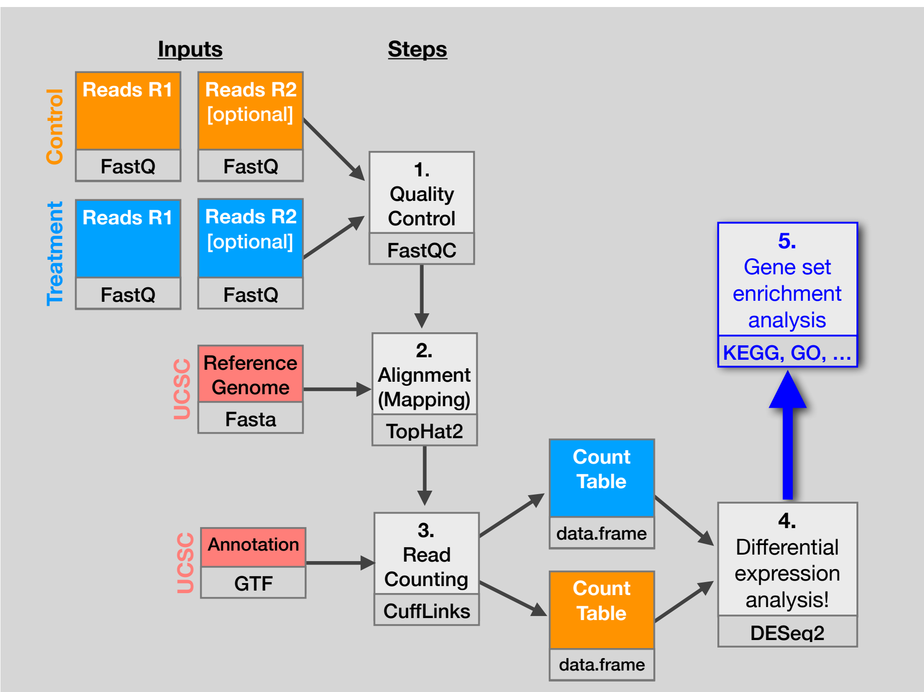


My high-throughput experiment generated a long list of genes/proteins…

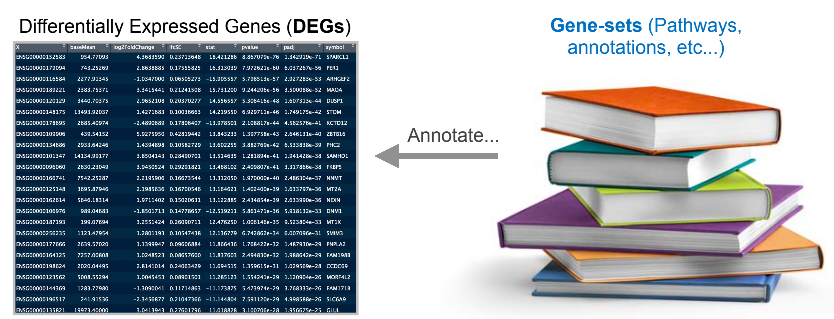
What do I do now?

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

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



Basic idea



Basic idea

Differentially Expressed Genes (**DEGs**)

X	baseMean	log2FoldChange	lfcSE	stat	pvalue	padj	symbol
ENSG00000152583	954.77093	4.3683590	0.23713648	18.421286	8.867079e-76	1.342919e-71	SPARCL1
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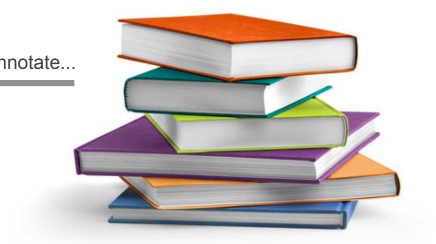
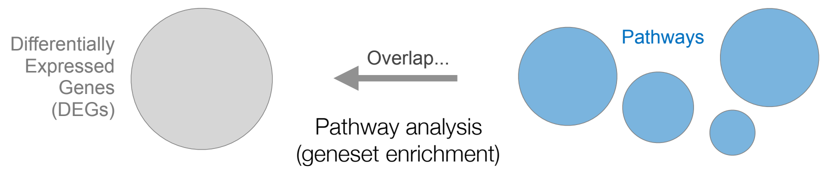
Annotate...

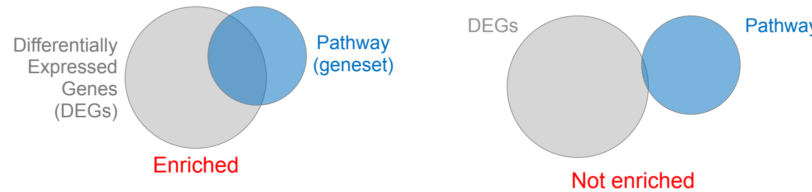
Gene-sets (Pathways, annotations, etc...)





Pathway analysis (geneset enrichment)

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*

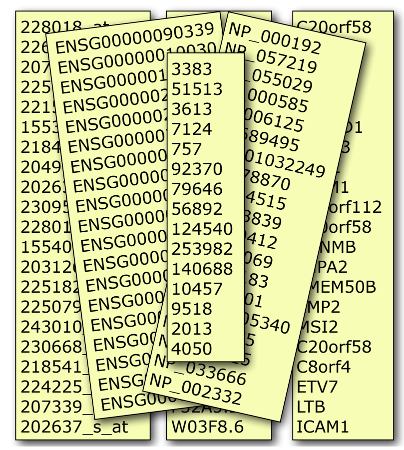
Pathway analysis (a.k.a. geneset enrichment) **Limitations**

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-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.
- Often you will have to translate one set of ids into another
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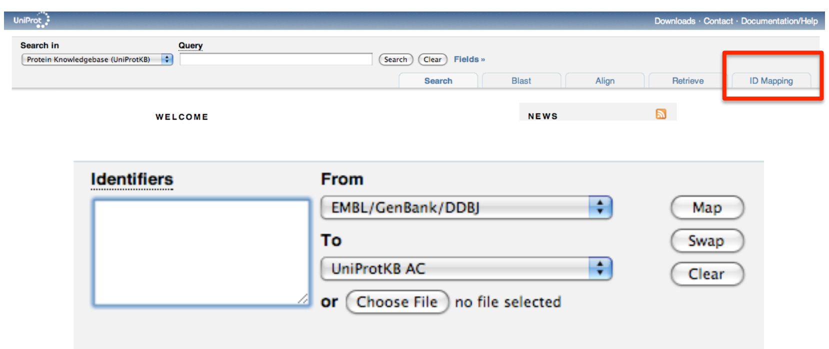
Translating between identifiers: UniProt < www.uniprot.org >



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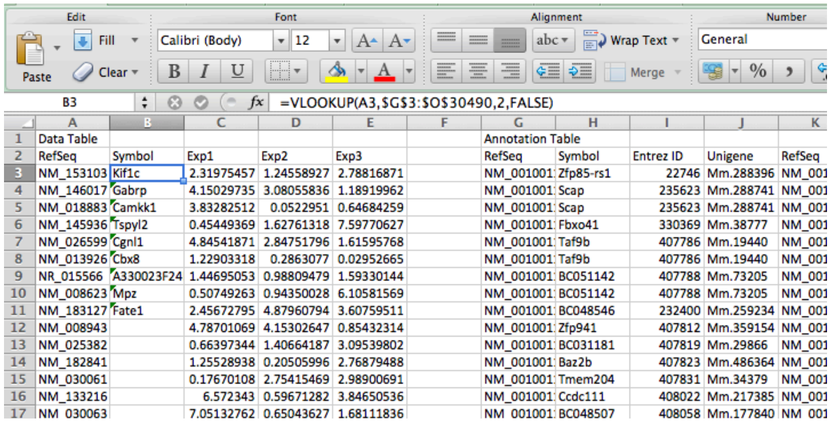
- **VLOOKUP in Excel - *good if you are an excel whizz - I am not!***
  - **Download flat file from Entrez, Uniprot, etc; Open in Excel; Find columns that correspond to the 2 IDs you want to convert between; Sort by ID; Use vlookup to translate your list**

Translating between identifiers: Excel VLOOKUP

VLOOKUP(lookup_value, table_array, col_index_num)



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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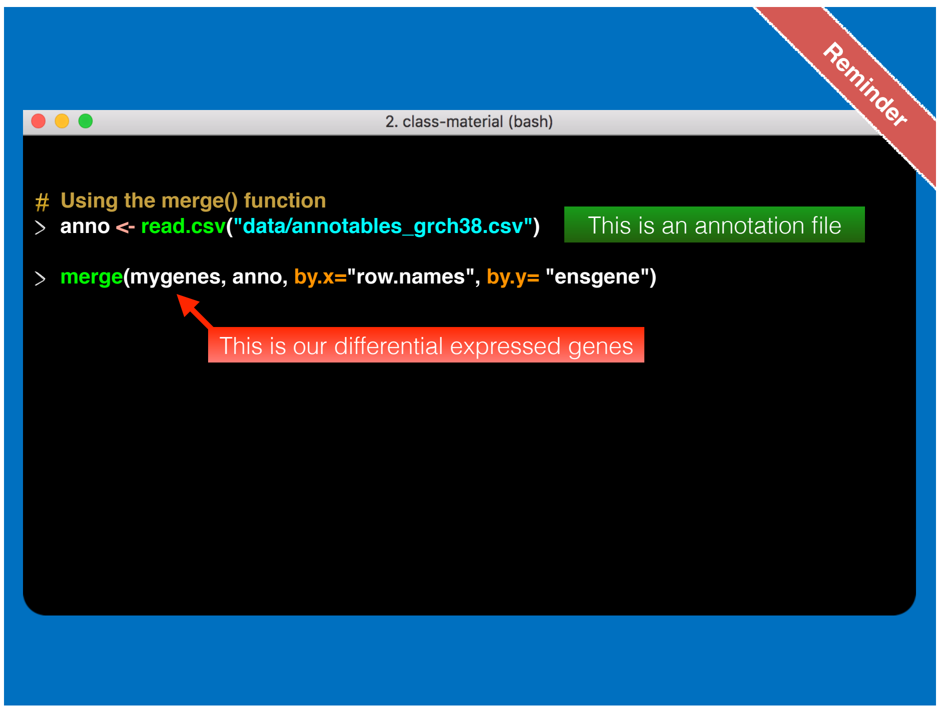
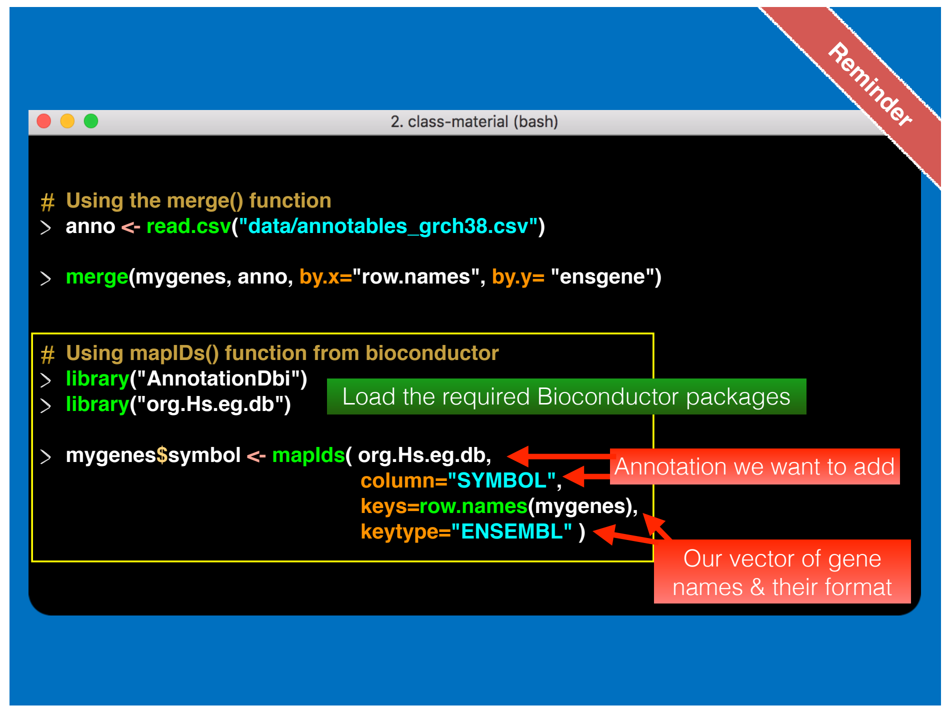
- Use the **merge()** or **mapIDs()** functions in **R** - fast, *versatile* & reproducible!
  - Also **clusterProfiler::bitr()** function and many others… [Link to clusterProfiler vignette]





`bitr`: Biological Id TranslatoR

Alternative...

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.

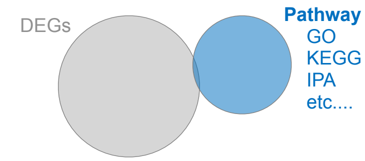
```
x <- c("GPX3",  "GLRX",   "LBP",   "CRYAB", "DEFB1", "HCLS1",   "SOD2",   "HSPA2",
       "ORM1",  "IGFBP1", "PTHLH", "GPC3",  "IGFBP3","TOB1",    "MITF",   "NDRG1",
       "NR1H4", "FGFR3",  "PVR",   "IL6",   "PTPRM", "ERBB2",   "NID2",   "LAMB1",
       "COMP",  "PLS3",   "MCAM",  "SPP1",  "LAMC1", "COL4A2",  "COL4A1", "MYOC",
       "ANXA4", "TFPI2",  "CST6",  "SLPI",  "TIMP2", "CPM",     "GGT1",   "NNMT",
       "MAL",   "EEF1A2", "HGD",   "TCN2",  "CDA",   "PCCA",    "CRYM",   "PDXK",
       "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

What functional set databases do you want?

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



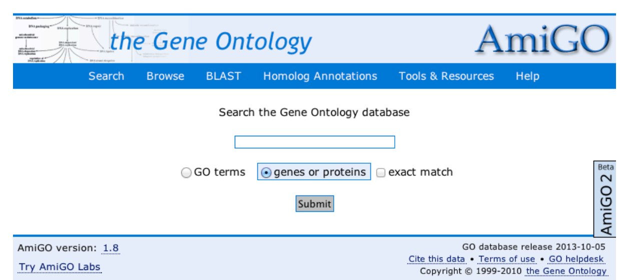
- Many others...
  - **Enzyme Classification**, **PFAM**, **Reactome**,
  - Disease Ontology, MSigDB, Chemical Entities of Biological Interest, Network of Cancer Genes etc…
  - See: Open Biomedical Ontologies (www.obofoundry.org)

GO < www.geneontology.org >

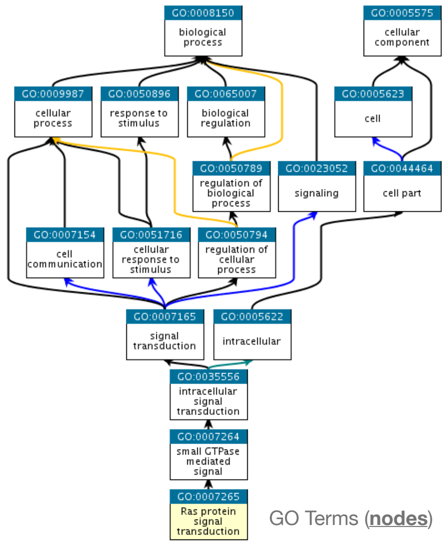
- **What function does HSF1 perform?**
  - *response to heat; sequence-specific DNA binding; transcription; etc*
- **Ontology** => a structured and controlled vocabulary that allows us to annotate gene products consistently, interpret the relationships among annotations, and can easily be *handled by 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

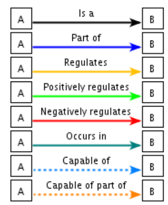
- GO is not a stand-alone database of genes/proteins or sequences
- Rather gene products get annotated with **GO terms** by UniProt and other organism specific databases, such as Flybase, Wormbase, MGI, ZFIN, etc.
- Annotations are available through AmiGO < amigo.geneontology.org >



GO is structured as a “directed graph”



GO Terms (**nodes**)



Relationships (**edges**)

Parent terms are more general & child terms more specific

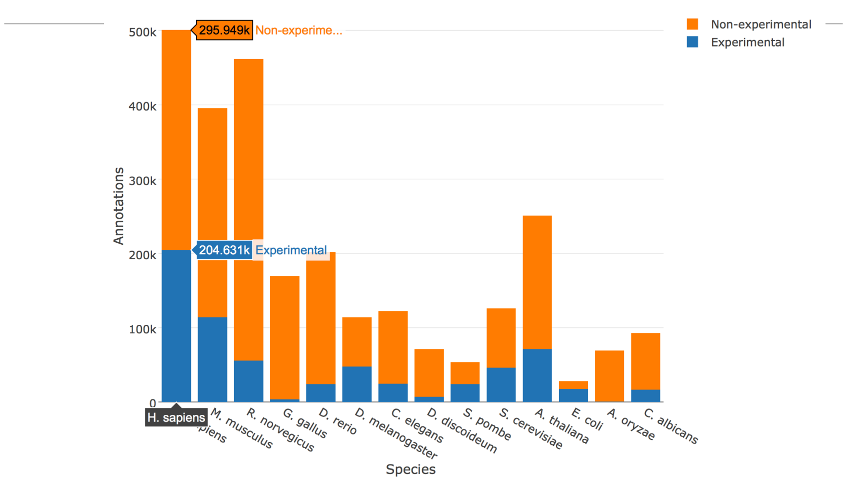
GO evidence codes

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

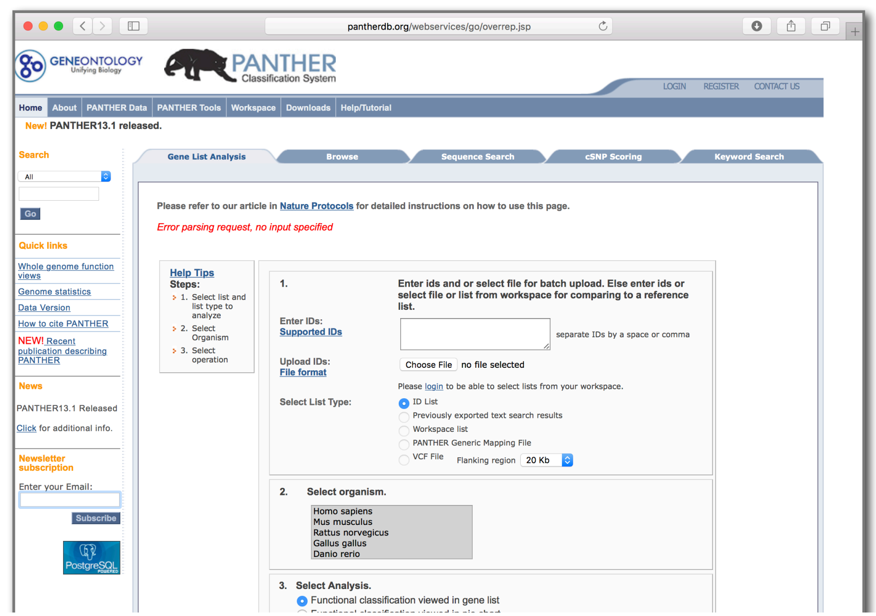
Use and misuse of the gene ontology annotations
Seung Yon Rhee, Valerie Wood, Kara Dolinski & Sorin Draghici
Nature Reviews Genetics **9**, 509-515 (2008)

Experimental annotations by species

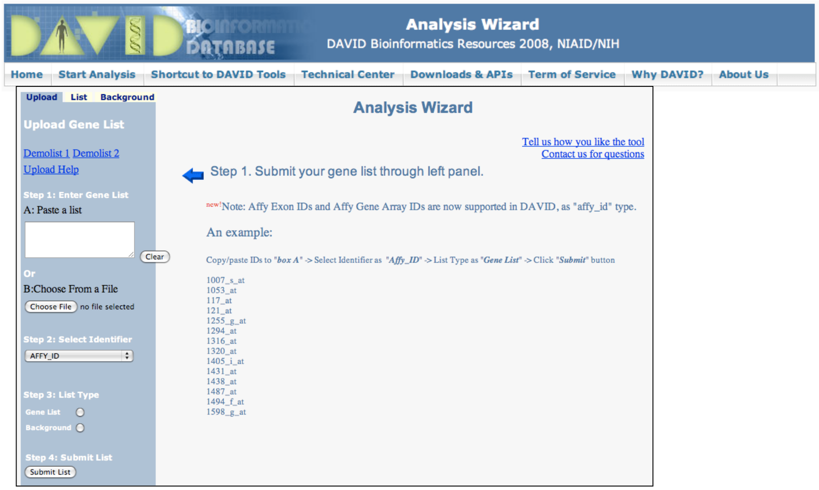


- See AmiGO for details: http://amigo.geneontology.org/amigo/base_statistics

Can now do gene list analysis with GeneGO online!

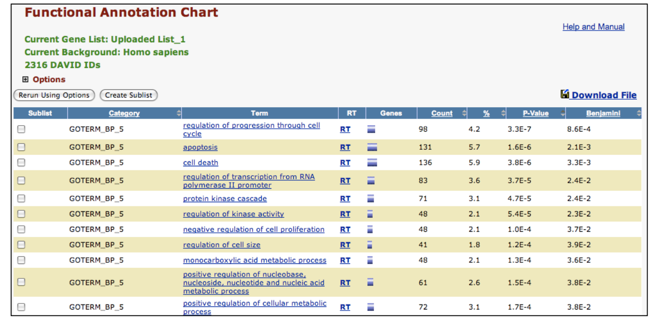


Another popular online tool: **DAVID** at NIAID < david.abcc.ncifcrf.gov >



DAVID

- *Functional Annotation Chart*



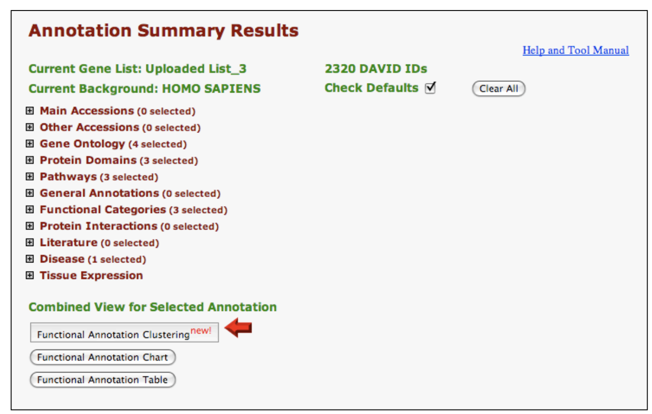
Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources
Da Wei Huang, Brad T Sherman & Richard A Lempicki
Nature Protocols **4**, *44 - 57 (2009)*

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

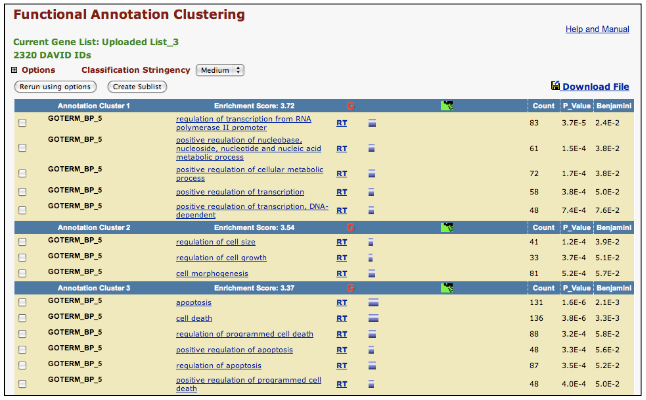
DAVID

- DAVID now offers functional annotation clustering:



DAVID Functional Annotation Clustering

- Based on shared genes between functional sets



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?

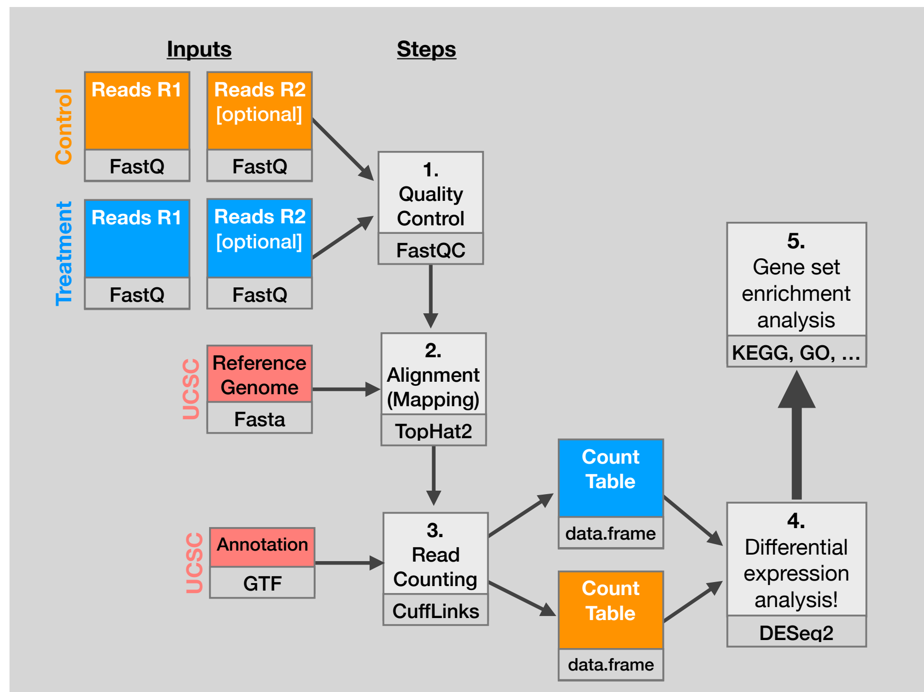
- **GeneGO** < portal.genego.com >
  - MD/PhD curated annotations, great for certain domains (eg, Cystic Fibrosis)
  - Nice network analysis tools
  - Email us for access
- **Oncomine** < www.oncomine.org >
  - Extensive cancer related expression datasets
  - Nice concept analysis tools
  - Research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

Do it Yourself!

Hands-on time!

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)

colData

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

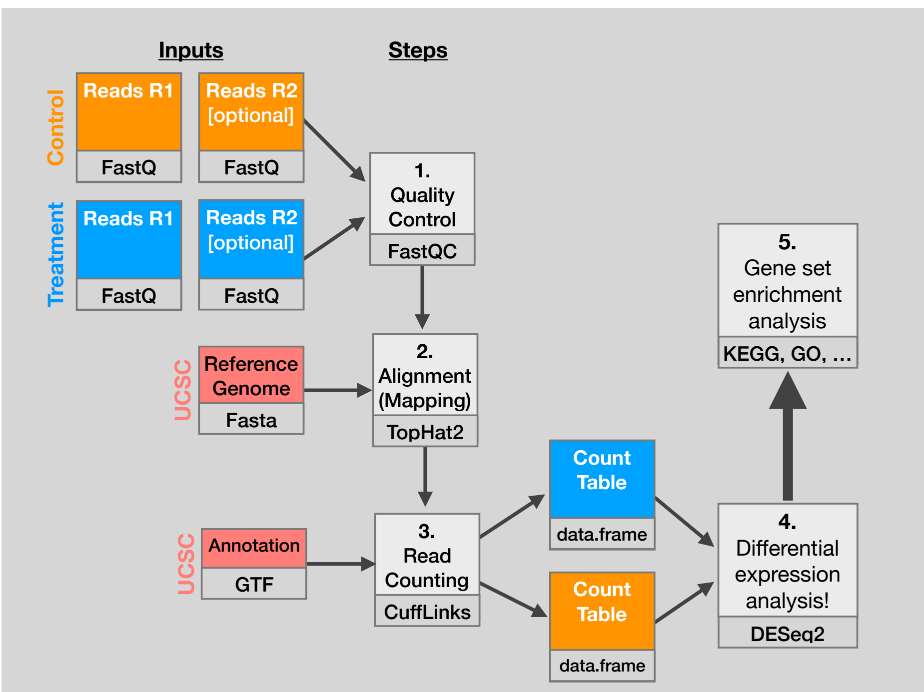
Sample names:
ctrl_1, ctrl_2, exp_1, exp_2

colData describes metadata about the *columns* of countData

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

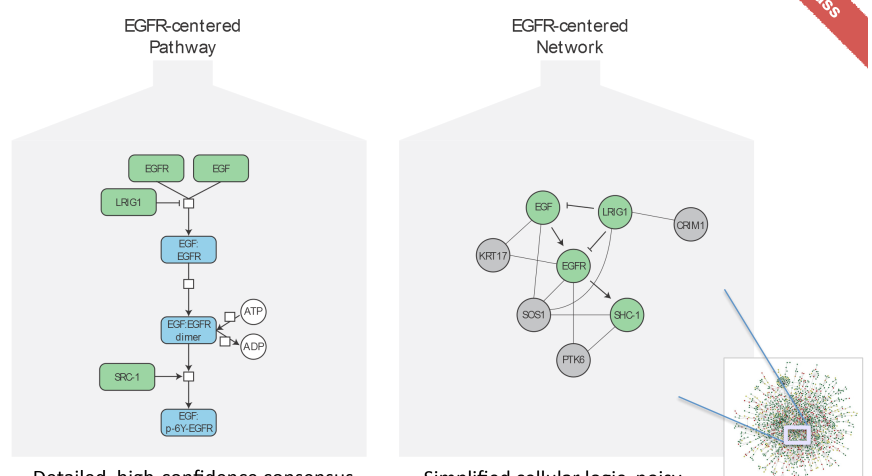
Advice:
Figure out "**What do I want to do with my list?**"

- 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

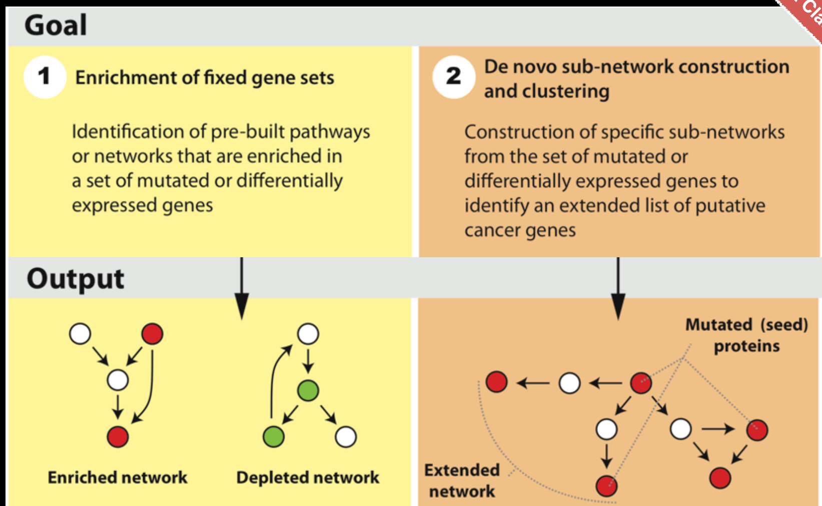
Next Class



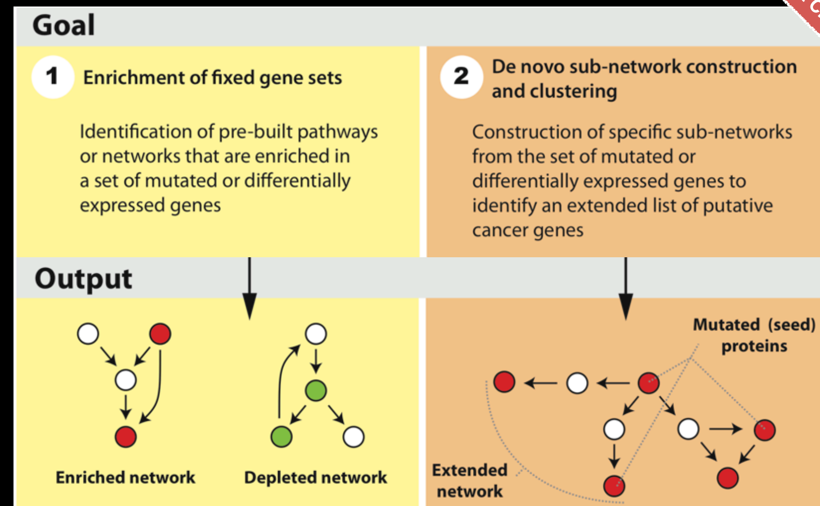
- Detailed, high-confidence consensus
- Biochemical reactions
- Small-scale, fewer genes
- Concentrated from decades of literature

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

Next Class



Next Class



What biological process is altered in this cancer?

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

Side-note:

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

- **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-kB, AP-1, IRF3/7, NFAT

Network Analysis Overview