

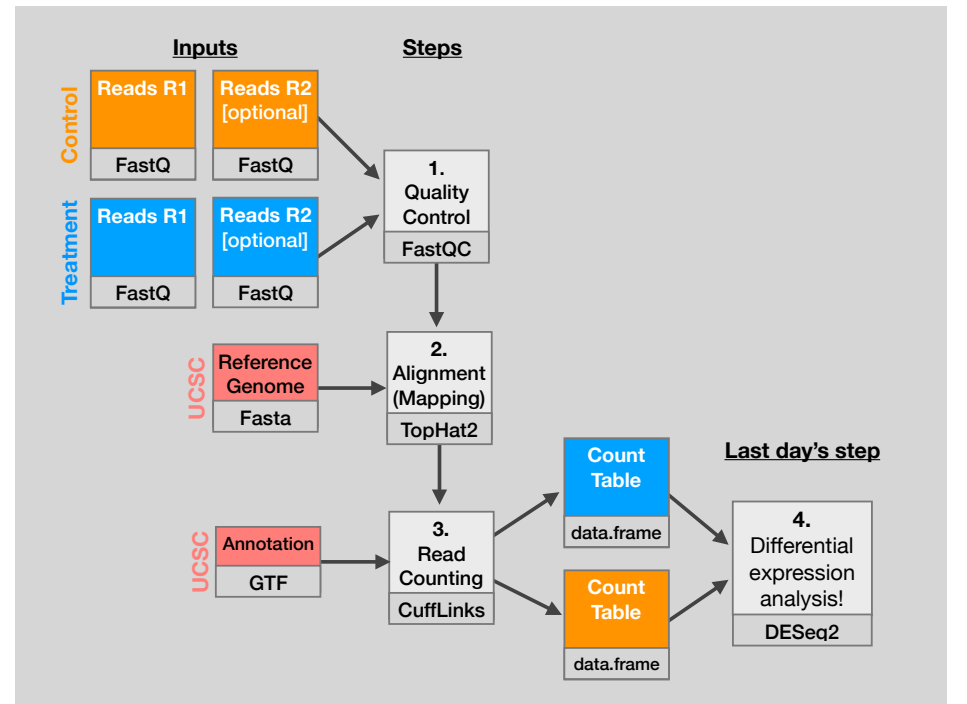
BIMM 143

Pathway Analysis and the Interpretation of Gene Lists

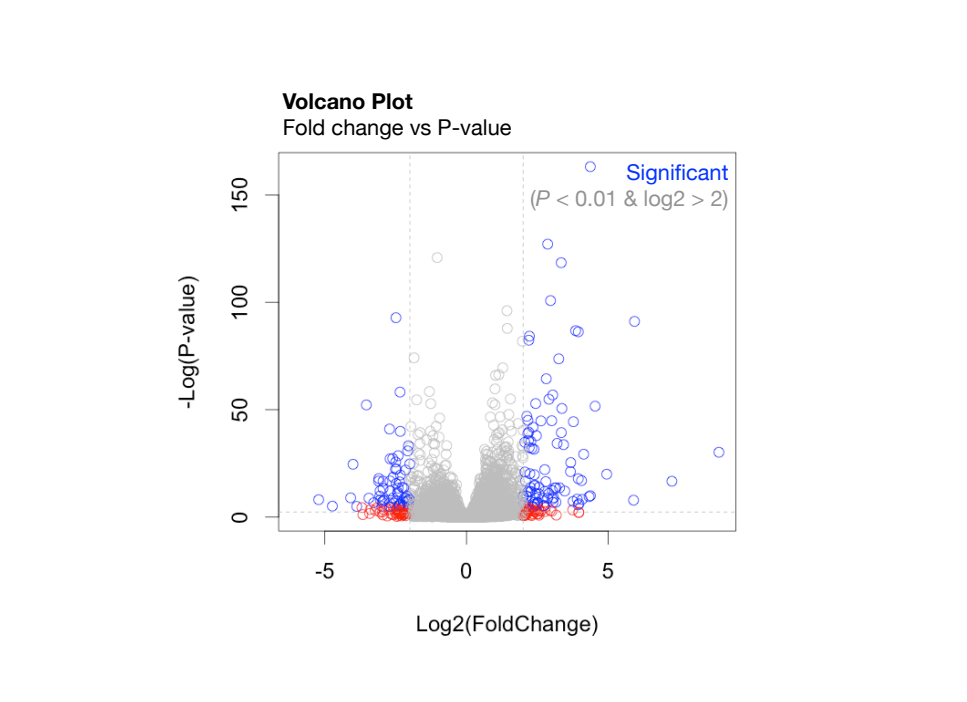
Lecture 16

Barry Grant
UC San Diego


<http://thegrantlab.org/bimm143>



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

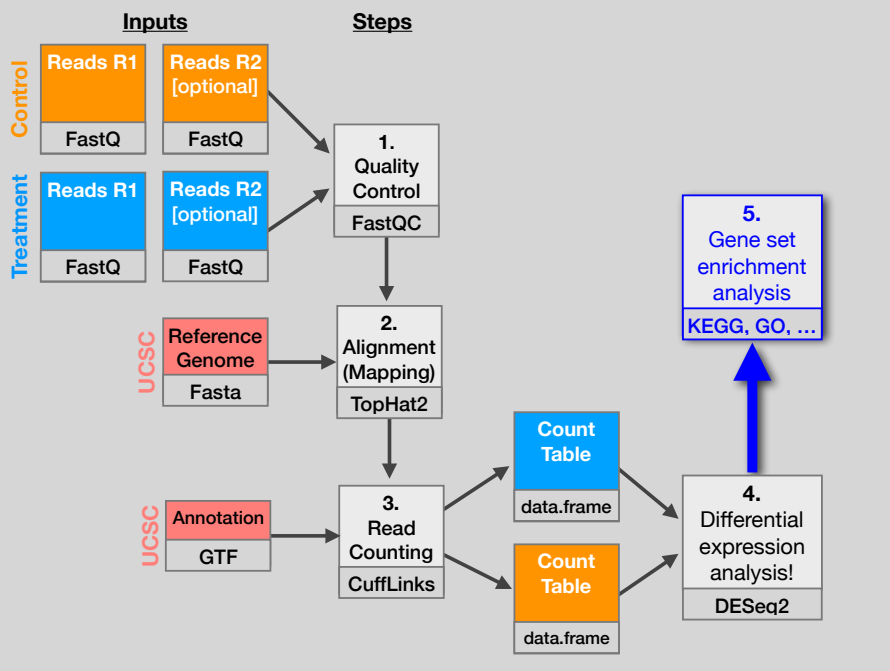


My high-throughput experiment generated a long list of genes/proteins...

What do I do now? 

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

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

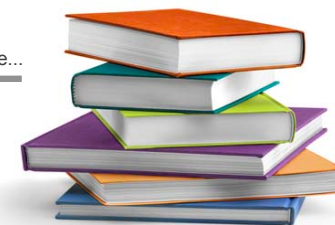


Basic idea

Differentially Expressed Genes (DEGs)

Gene	log2(foldChange)	adjP	negLog10P	negLog10Padj	negLog10Padj	negLog10Padj	negLog10Padj	negLog10Padj	negLog10Padj
UCSC0000011513	0.7417028	0.002330	0.2171644	14.411288	8.802796e-78	3.242179e-71	0.000000	0.000000	0.000000
UCSC0000017094	741.21269	2.861885	0.1751825	16.313059	7.972014e-40	6.072079e-36	0.000000	0.000000	0.000000
UCSC0000011834	2277.81345	-1.024700	0.0802173	-15.903557	5.788131e-37	2.027238e-33	0.000000	0.000000	0.000000
UCSC0000018121	2382.72373	2.914541	0.2124108	15.723200	2.948000e-36	3.000000e-29	0.000000	0.000000	0.000000
UCSC0000012019	1440.70379	2.065208	0.3057027	14.555555	5.386456e-46	1.607713e-44	0.000000	0.000000	0.000000
UCSC00000148175	13493.80037	1.427163	0.1006663	14.218950	6.929711e-46	1.748175e-42	0.000000	0.000000	0.000000
UCSC0000017990	1085.80979	-2.480889	0.1788407	-13.970310	2.188810e-44	6.025724e-41	0.000000	0.000000	0.000000
UCSC0000015096	439.54152	9.879950	0.4281942	13.803213	1.327758e-43	6.646131e-40	0.000000	0.000000	0.000000
UCSC00000114026	2093.64246	1.439488	0.1058279	13.602253	3.882769e-42	6.533818e-39	0.000000	0.000000	0.000000
UCSC00000101307	1414.99172	3.850413	0.2848701	13.514833	3.241689e-41	1.914201e-38	0.000000	0.000000	0.000000
UCSC0000019660	1070.32489	3.910514	0.2928182	13.461820	2.403020e-41	3.132764e-38	0.000000	0.000000	0.000000
UCSC00000166741	7542.25287	2.219906	0.1667354	13.312050	1.970000e-40	2.488304e-37	0.000000	0.000000	0.000000
UCSC00000121148	3695.87346	2.138636	0.1870546	13.164621	1.402400e-39	1.033737e-36	0.000000	0.000000	0.000000
UCSC00000126215	1646.18116	1.971182	0.1500821	13.120885	2.438400e-39	6.033399e-36	0.000000	0.000000	0.000000
UCSC00000160676	989.04683	-1.851713	0.1477867	-12.510211	5.861471e-36	5.918132e-33	0.000000	0.000000	0.000000
UCSC00000187133	199.07968	3.255124	0.2600711	12.478250	1.098460e-35	3.523804e-33	0.000000	0.000000	0.000000
UCSC00000124233	1122.47924	1.801533	0.1505418	12.238770	5.838000e-34	6.007700e-31	0.000000	0.000000	0.000000
UCSC0000017786	2053.17020	1.139947	0.0960884	11.866458	1.768424e-32	1.497030e-29	0.000000	0.000000	0.000000
UCSC00000164121	7257.00858	1.024823	0.0887900	11.837923	2.444800e-32	1.588647e-29	0.000000	0.000000	0.000000
UCSC00000189624	1025.04919	2.814104	0.2408142	11.694110	1.570100e-31	1.020900e-28	0.000000	0.000000	0.000000
UCSC00000123582	1008.52324	1.045453	0.0890105	11.581123	1.544542e-29	1.120004e-26	0.000000	0.000000	0.000000
UCSC00000144389	1283.77980	-1.300041	0.1171486	-11.173875	5.473974e-29	3.788331e-26	0.000000	0.000000	0.000000
UCSC00000188317	241.81138	-2.458357	0.2107708	-11.144800	3.931100e-29	4.988388e-26	0.000000	0.000000	0.000000
UCSC00000115811	1073.40000	3.451811	0.2520708	11.114824	3.100100e-27	4.951912e-24	0.000000	0.000000	0.000000

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



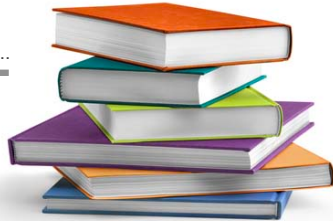
Annotate...

Basic idea

Differentially Expressed Genes (DEGs)

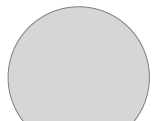
Gene	log2FC	negLog10P	adjP	negLog10Q	adjQ	negLog10R
ENSG0000015213	0.7417028	4.082330	0.2171644	18.41238	8.807276	1.242121
ENSG0000017094	743.21268	2.861885	0.1751825	16.31309	7.972624	0.6172476
ENSG0000018584	2277.81345	-1.024700	0.0510273	-15.50157	5.788134	-7.207238
ENSG0000018921	2381.72121	3.241641	0.2124108	12.31202	2.482006	3.300084
ENSG00000120139	1440.70375	2.965208	0.2057027	14.55555	5.386458	1.0071136
ENSG00000148175	1493.80037	1.427583	0.1003663	14.21959	6.927114	1.748175
ENSG0000017690	1085.80976	-2.489088	0.3788407	-13.70101	3.188116	-4.025294
ENSG0000010996	419.54120	9.075950	0.4261842	13.82521	1.977758	3.246113
ENSG00000134036	2031.64248	1.439488	0.1052729	13.60225	3.882708	4.513818
ENSG00000101307	4114.90177	3.826424	0.2384970	13.51403	1.261884	1.911209
ENSG00000169000	7076.20489	3.910524	0.2928421	13.46102	2.448021	3.312165
ENSG00000166741	7542.25287	2.212096	0.1667354	13.31209	1.970004	2.488204
ENSG00000121248	3058.87946	2.189536	0.1870546	13.16462	1.402400	1.833787
ENSG00000126215	1044.18114	1.971182	0.1500521	13.12085	2.438104	3.023399
ENSG00000160976	989.04683	-1.851713	0.1477867	-12.51921	5.861474	3.518132
ENSG00000187183	139.07084	3.253124	0.2400711	12.47820	1.091460	3.512804
ENSG00000130193	1121.47924	1.811153	0.1204718	12.28778	0.928026	4.007094
ENSG00000177846	2035.17020	1.139947	0.0960484	11.86448	1.768424	1.487930
ENSG00000164121	7237.00808	1.824822	0.0887600	11.81703	2.444824	1.888424
ENSG00000188824	2025.94495	2.814124	0.2404929	11.68418	1.378104	1.820904
ENSG00000123582	1008.15294	1.804543	0.0890101	11.58123	1.544424	1.220904
ENSG00000144389	1283.77980	-1.300041	0.1171480	-11.17875	5.473974	3.788134
ENSG00000188137	241.91338	-2.385887	0.2107768	-11.14480	3.931100	4.988184
ENSG00000115821	1073.40000	-0.415181	0.2020792	-11.01828	3.190700	2.955724

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



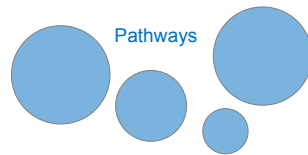
Annotate...

Differentially Expressed Genes (DEGs)



Overlap...

Pathway analysis (geneset enrichment)



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

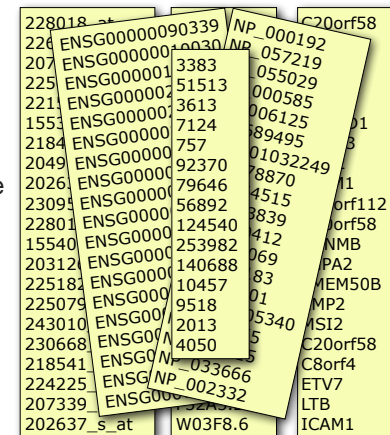


- DEGs come from your experiment ➤ *Critical, needs to be as clean as possible*
- Pathway genes ("geneset") come from annotations ➤ *Important, but typically not a competitive advantage*
- Variations of the math: overlap, ranking, networks... ➤ *Not critical, different algorithms show similar performances*

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>; IDConverter < idconverter.bioinfo.cnio.es >

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

The screenshot shows the UniProt website's ID Mapping tool. At the top, there is a navigation bar with 'UniProt' and links for 'Downloads', 'Contact', 'Documentation/Help'. Below this is a search bar with 'Search in' and 'Query' fields, and a dropdown menu set to 'Protein Knowledgebase (UniProtKB)'. A red box highlights the 'ID Mapping' button in the top navigation bar. Below the search bar, there are buttons for 'Search', 'Blast', 'Align', 'Retrieve', and 'ID Mapping'. The main content area is titled 'Identifiers' and contains a large empty text box for input. To the right of the text box are two dropdown menus: 'From' (set to 'EMBL/GenBank/DDBJ') and 'To' (set to 'UniProtKB AC'). Below these are 'Map', 'Swap', and 'Clear' buttons. At the bottom, there is a 'Choose File' button and the text 'no file selected'.

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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)

Data Table					Annotation Table				
RefSeq	Symbol	Exp1	Exp2	Exp3	RefSeq	Symbol	Entrez ID	Unigene	RefSeq
NM_153103	Kif1c	2.31975457	1.24558927	2.78816871	NM_001001	Zfp85-rs1	22746	Mm.288396	NM_001
NM_146017	Gabrp	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	1.44699053	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	232400	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

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

```

# 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

```

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

> mygenes$symbol <- mapIDs(org.Hs.eg.db,
  column="SYMBOL", Annotation we want to add
  keys=row.names(mygenes),
  keytype="ENSEMBL") 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", "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", "FXRD2", "RBP4", "SLC6A12", "KDELRL3", "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:

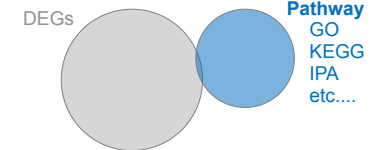
<https://bioconductor.org/packages/release/bioc/html/clusterProfiler.html>

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)

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*

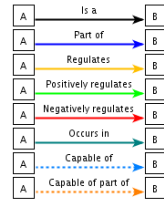
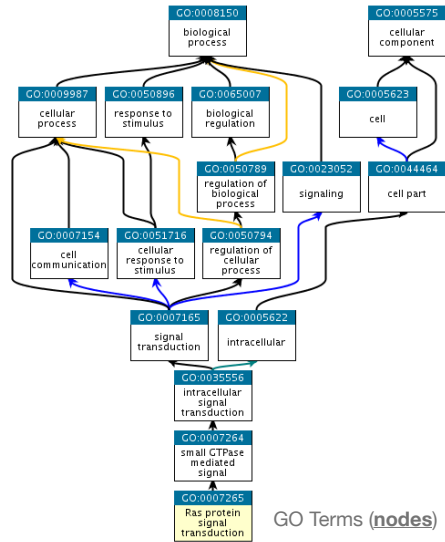
• GO database consists of 3 ontologies that describe gene products in terms of their associated **biological processes, cellular components and molecular functions**

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 >

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

GO is structured as a “directed graph”



Relationships (edges)

Parent terms are more general & child terms more specific

GO Terms (nodes)

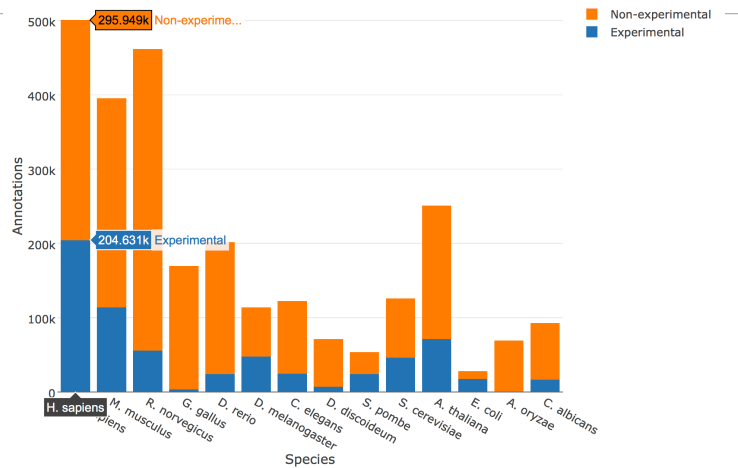
GO evidence codes

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*

Functional Annotation Chart [Help and Manual](#)

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

Options [Download File](#)

[Rerun Using Options](#) [Create Sublist](#)

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

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:

Annotation Summary Results [Help and Tool Manual](#)

Current Gene List: Uploaded List_3
Current Background: HOMO SAPIENS
2320 DAVID IDs
Check Defaults [Clear All](#)

- 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

Functional Annotation Clustering ^{new!} [←](#)

Functional Annotation Chart

Functional Annotation Table

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

[Download File](#)

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

Want more?

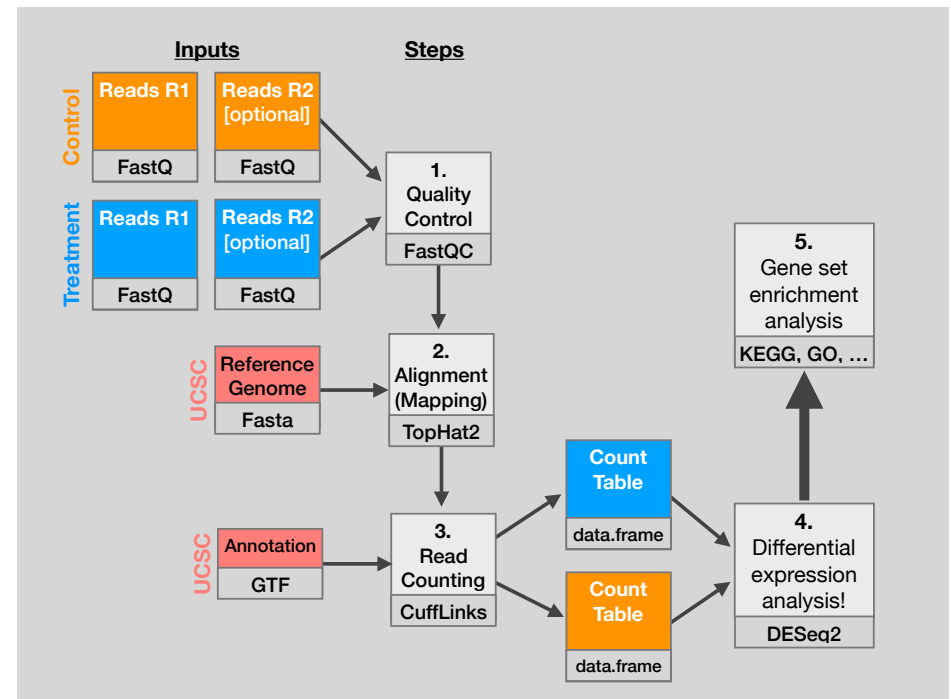


- GeneGO** < portal.genego.com >
 - MD/PhD curated annotations, great for certain domains (eg, Cystic Fibrosis)
 - Nice network analysis tools
 - Email us for access
- OncoPrint** < www.oncoPrint.org >
 - Extensive cancer related expression datasets
 - Nice concept analysis tools
 - Research edition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

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_2
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	...

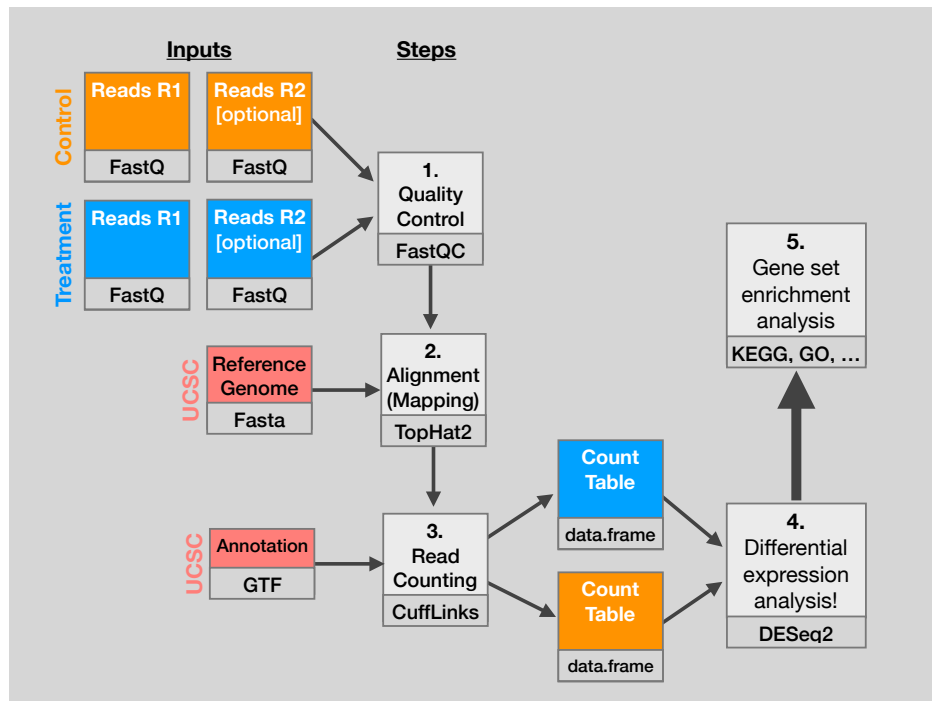
Sample names:
ctrl_1, ctrl_2, exp_1, exp_2

colData describes metadata about the *columns* of countData

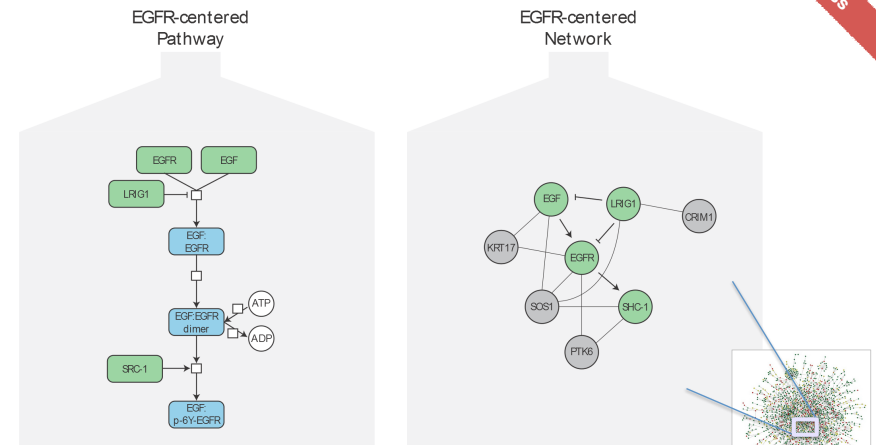
First column of **colData** must match column names of **countData** (-1st)

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



- 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

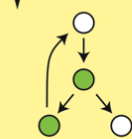
2 De novo sub-network construction and clustering

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

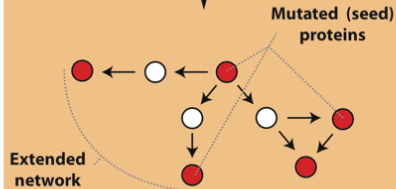
Output



Enriched network



Depleted network



Extended network

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

Next Class

Goal

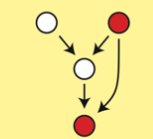
1 Enrichment of fixed gene sets

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

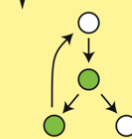
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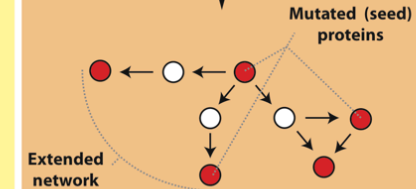
Output



Enriched network



Depleted network

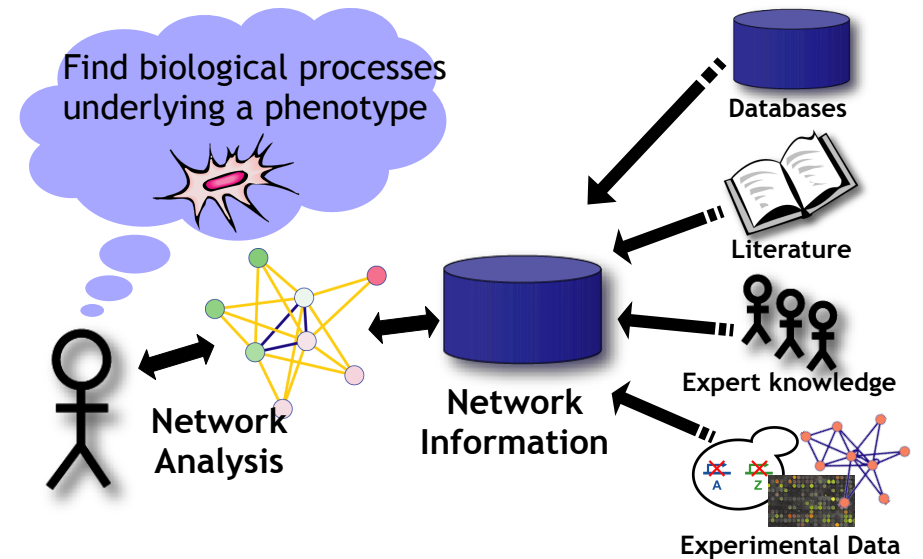


Extended network

What biological process is altered in this cancer?

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

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)