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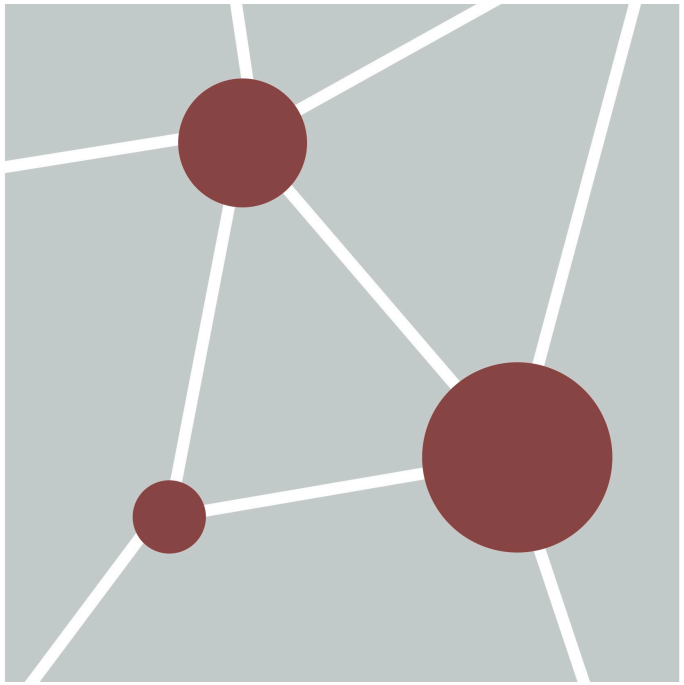
Finding over-represented pathways in gene lists

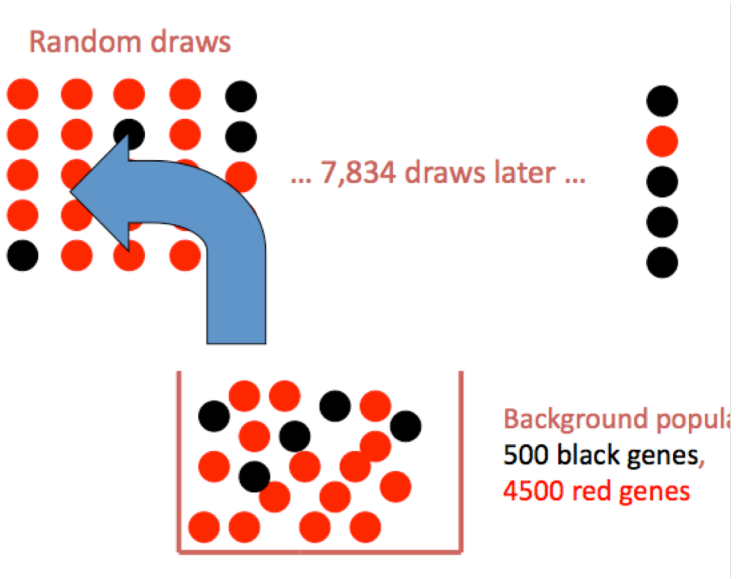
Veronique Voisin

Pathway and Network Analysis of –omics Data

May, 10-12, 2021

bioinformatics.ca

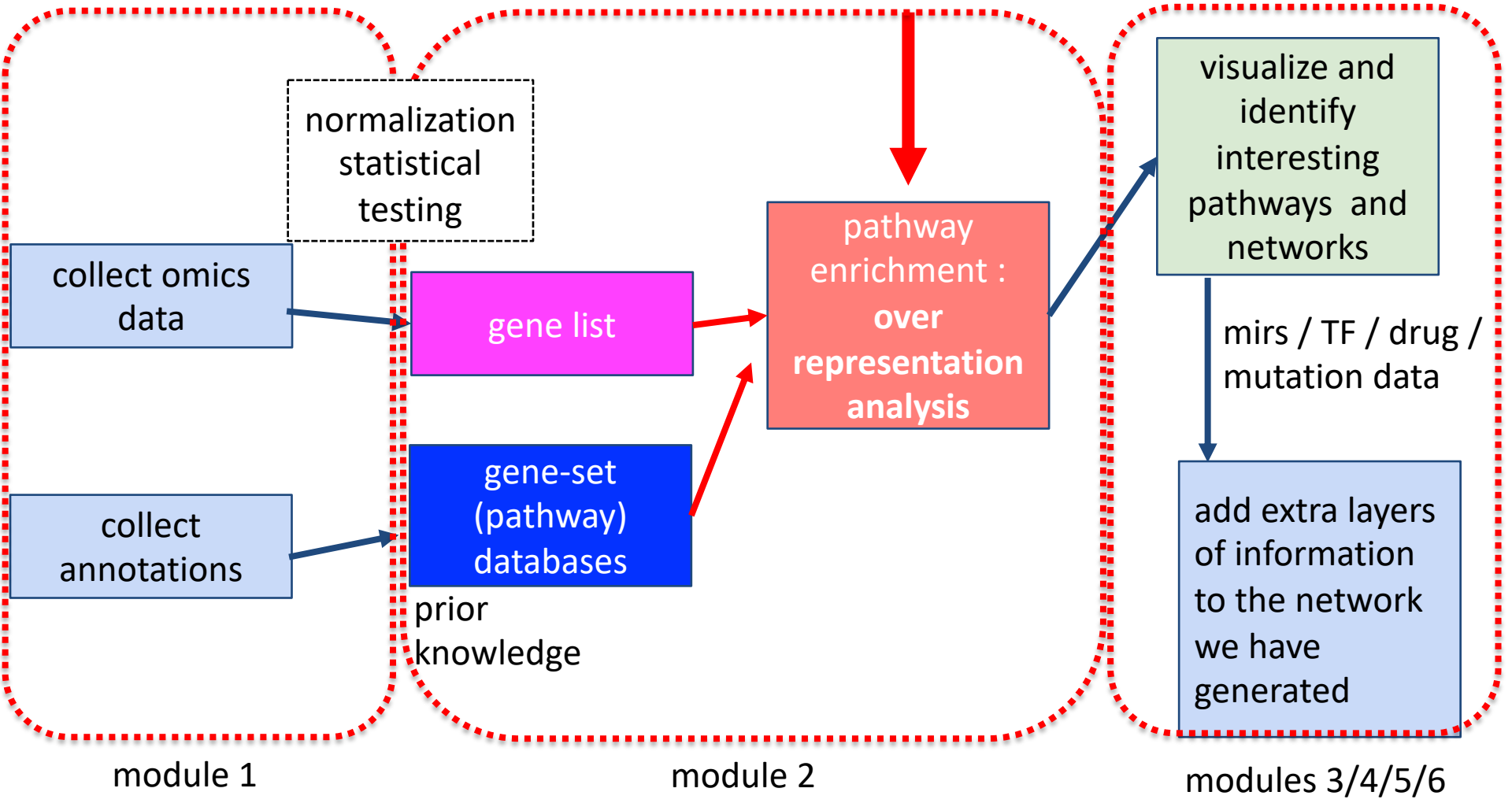




Learning Objectives

- Be able to understand the differences between a **defined gene list** and a **ranked gene list** and which enrichment test to apply.
- Be able to understand the concept of **pvalue** and **corrected pvalue (FDR)** in the context of enrichment analysis.
- Be able to understand the **result of an enrichment test** and how to interpret it
- Presentation of 2 enrichment tools

Analysis workflow



Pathway Analysis Workflow

RNA OR DNA EXTRACTION

↓

HIGH THROUGPUT SEQUENCING

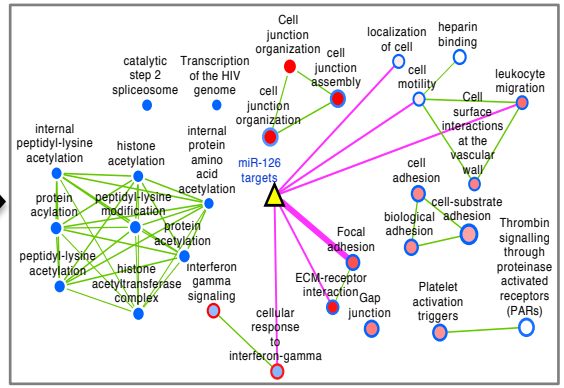
↓

GENE LIST

enrichment analysis

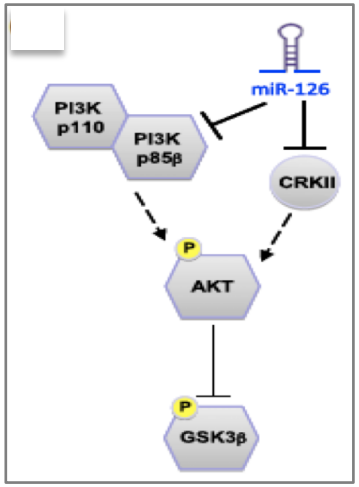
↓

Focus of this module

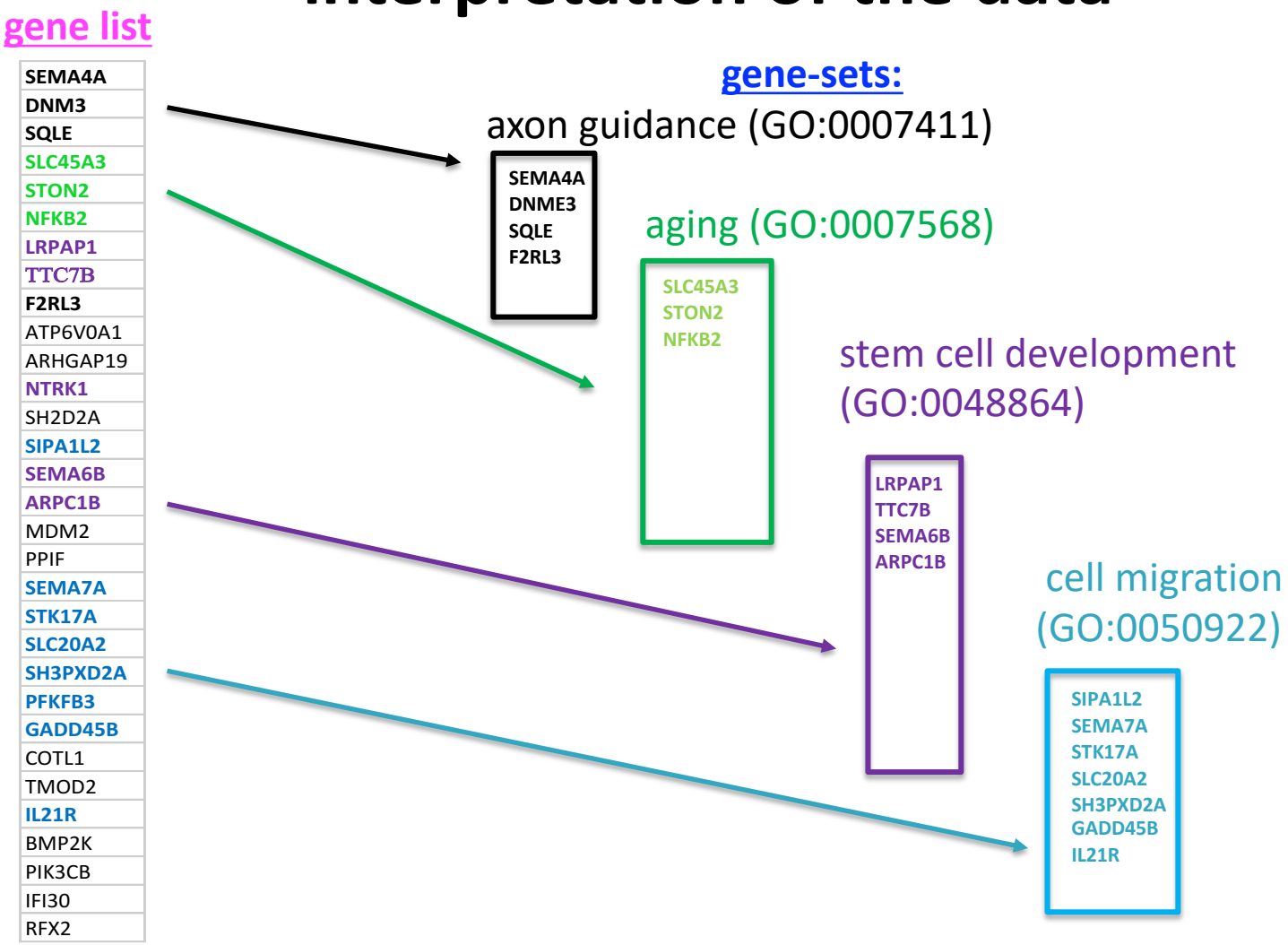


enrichment map

"In HSC/early progenitors, miR-126 regulates multiple targets within the PI3K/AKT/GSK3β pathway, attenuating signal transduction in response to extrinsic signals. "



Pathway enrichment analysis is a way to summarize your gene list into pathways to ease biological interpretation of the data



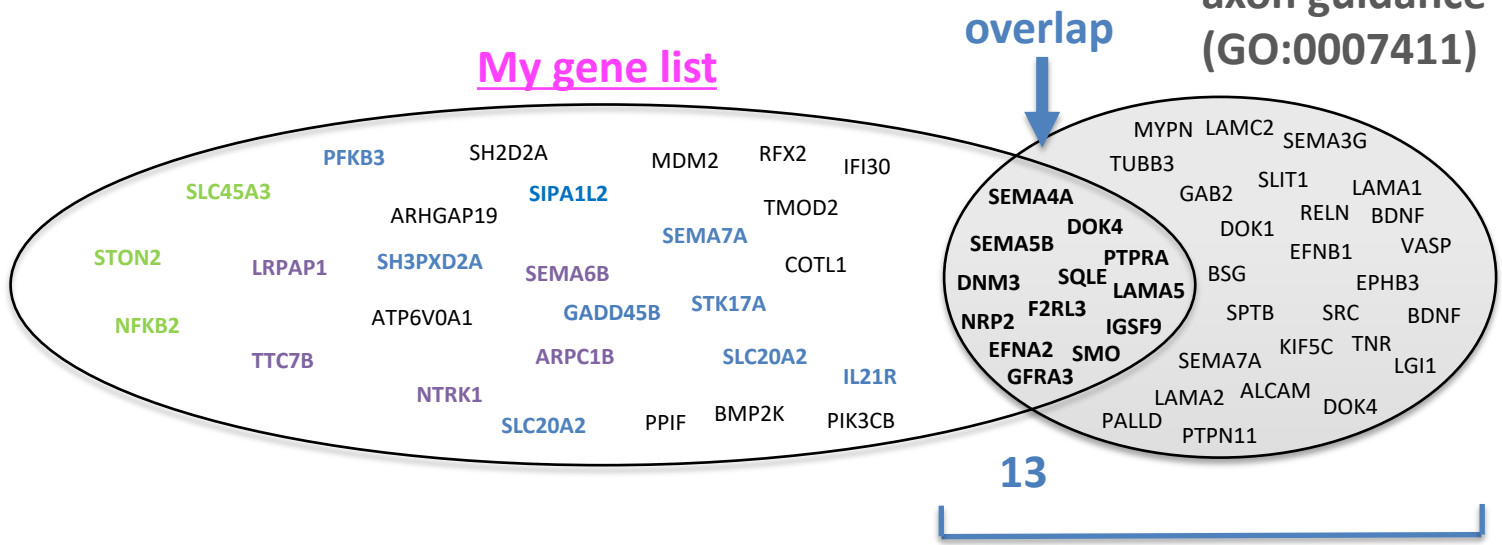
Pathway enrichment analysis calculates the overlap between our gene list and a pathway

gene list

SEMA4A
DNM3
SQLE
SLC45A3
STON2
NFKB2
LRPAP1
TTC7B
F2RL3
ATP6V0A1
ARHGAP19
NTRK1
SH2D2A
SIPA1L2
SEMA6B
ARPC1B
MDM2
PPIF
SEMA7A
STK17A
SLC20A2
SH3PXD2A
PFKFB3
GADD45B
COTL1
TMOD2
IL21R
BMP2K
PIK3CB
IFI30
RFX2

• • •

FDR<0.05

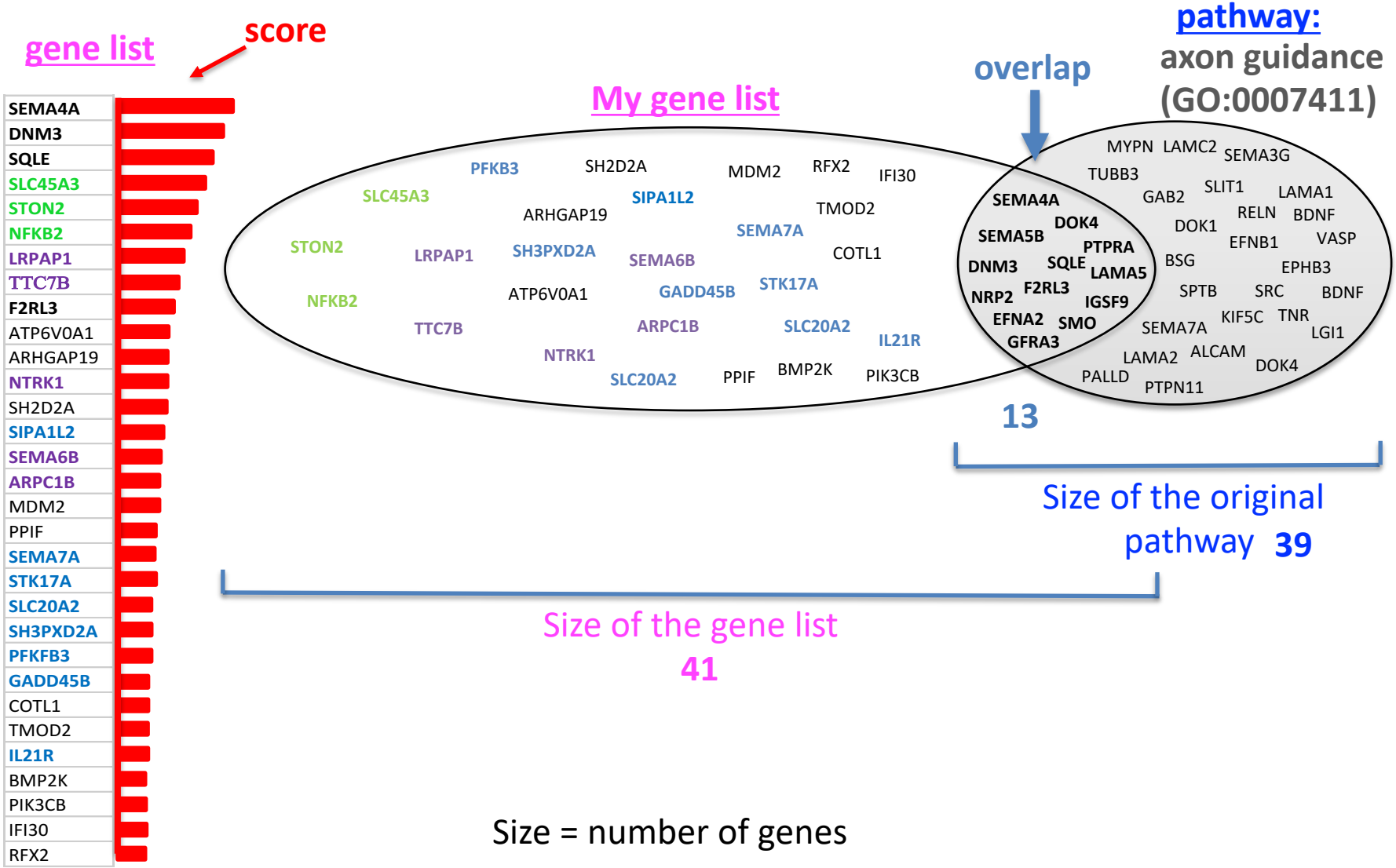


13/41 → 1/4

13/39 → 1/4

Size = number of genes

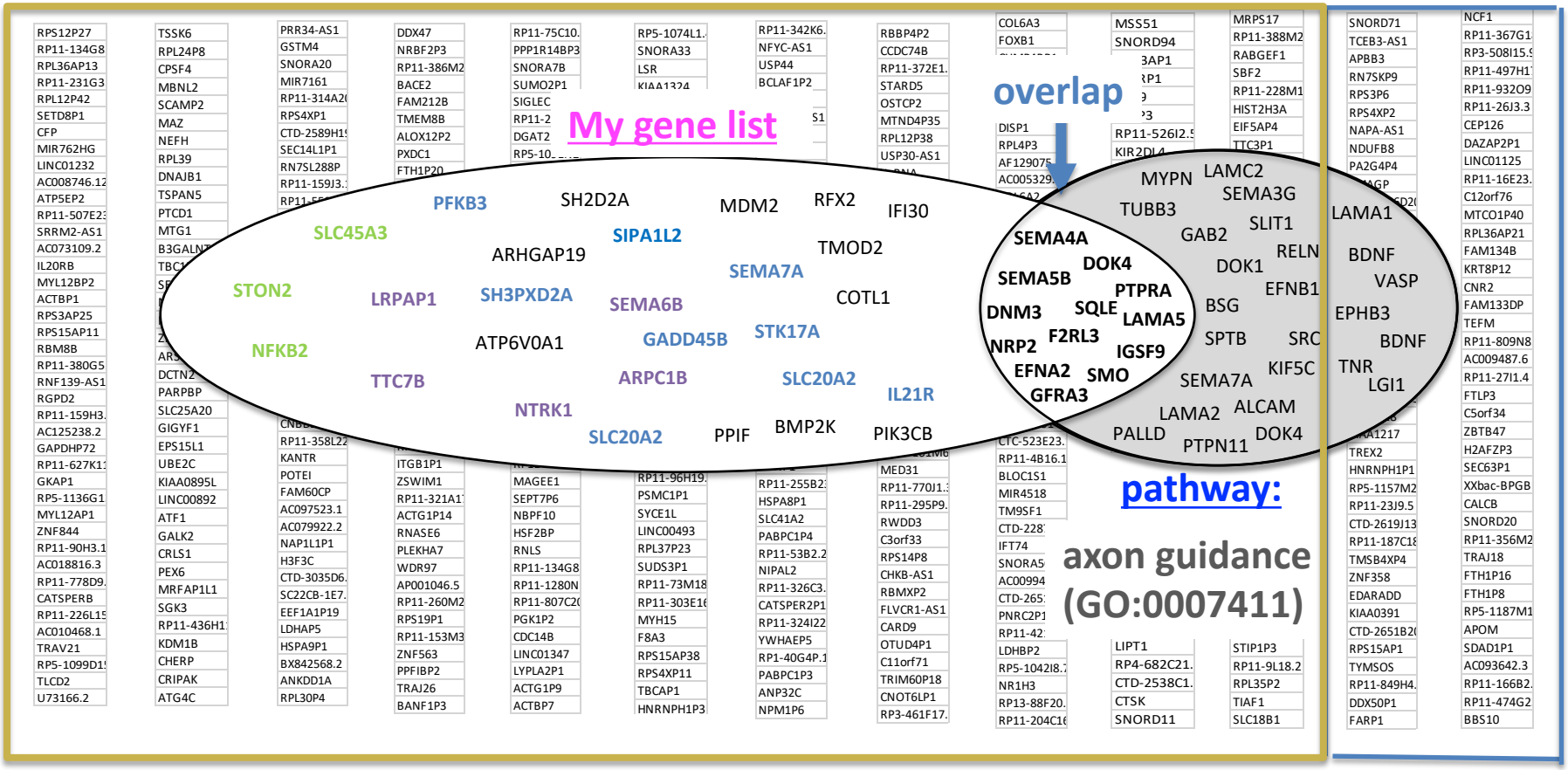
Can we add a score associated with the genes when calculating the enrichment score?



The background represents the genes that could have been captured in my omics experiment

genes measured in the experiment

genes not measured

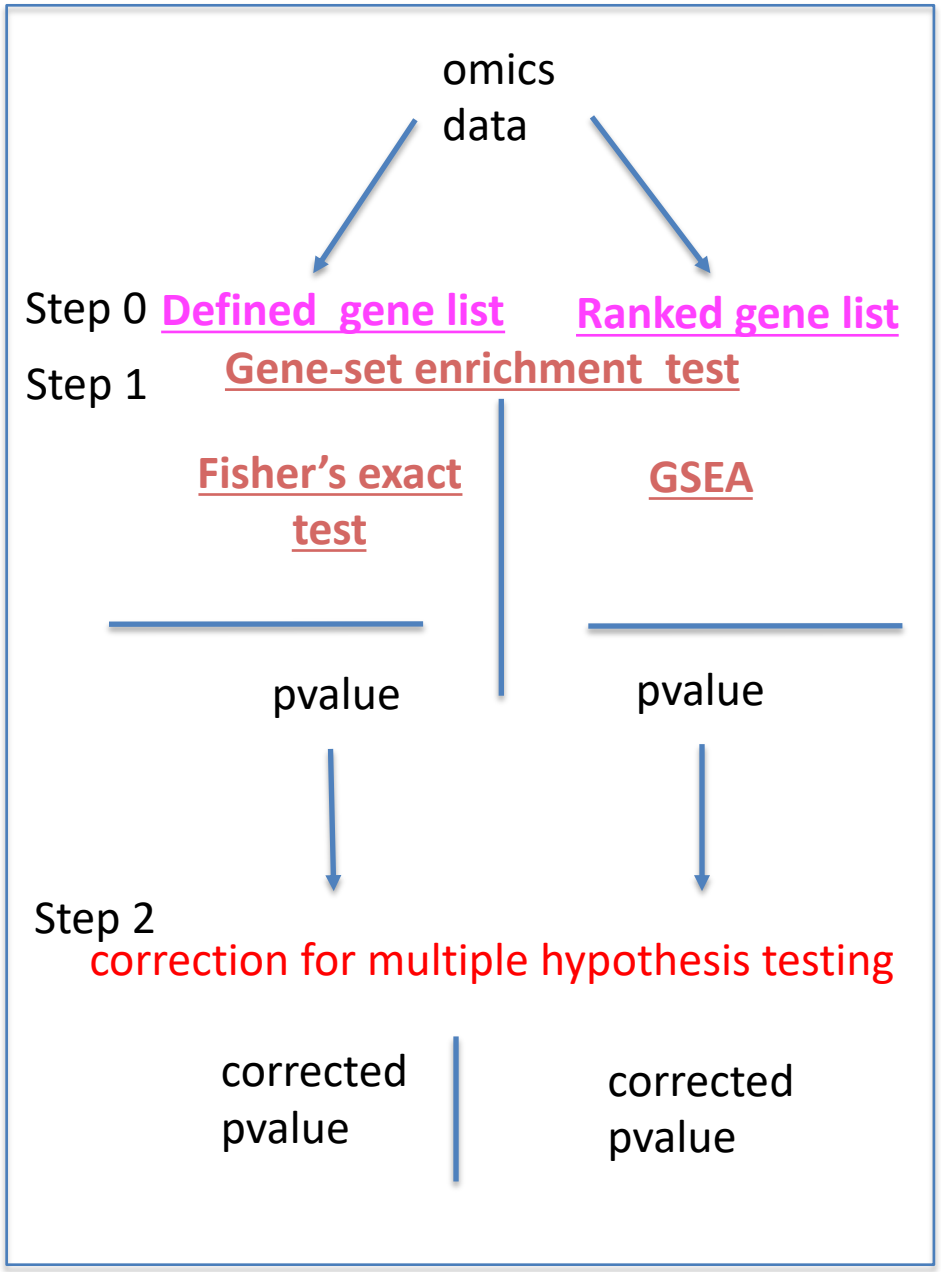


estimated 20,000-25,000 **human** protein-**coding genes**

How many genes could have been captured in your experiment?

Outline

- Two types of gene lists (ranked or not)
- Introduction to enrichment analysis
- Fisher's Exact Test, aka Hypergeometric Test
- GSEA for ranked lists.
- Multiple test corrections:
  - Bonferroni correction
  - False Discovery Rate computation using Benjamini-Hochberg procedure



Types of enrichment analysis

- Defined gene list (e.g. expression change > 2-fold)
  - Answers the question: **Are any pathways (gene sets) surprisingly enriched in my gene list?**
  - Statistical test: Fisher's Exact Test (aka Hypergeometric test)
- Ranked gene list (e.g. by differential expression)
  - Answers the question: **Are any pathways (gene sets) ranked surprisingly high or low in my ranked list of genes?**
  - Statistical test: **GSEA**, Wilcoxon rank sum test (+ others we won't discuss)

Why test enrichment in ranked gene lists?

- Possible problems with gene list test
  - No “natural” value for the threshold
  - Different results at different threshold settings
  - Possible loss of statistical power due to thresholding
    - No resolution between significant signals with different strengths
    - Weak signals neglected

OMICS gene lists: ranked or not ranked?

a few examples

Experimental design: 2 class-design, treated versus control

Starting point:

Bulk RNAseq

RNA

↓

Differential expression between treated and control

↓

Ranked list of **all genes** by differential expression score

Single cell RNA seq

RNA

↓

Cell clusters

↓

Differential expression between cluster 1 and cluster 2

↓

Ranked list of **all genes** by differential expression score

Label free proteomics

proteins

> 5,000 proteins

↓

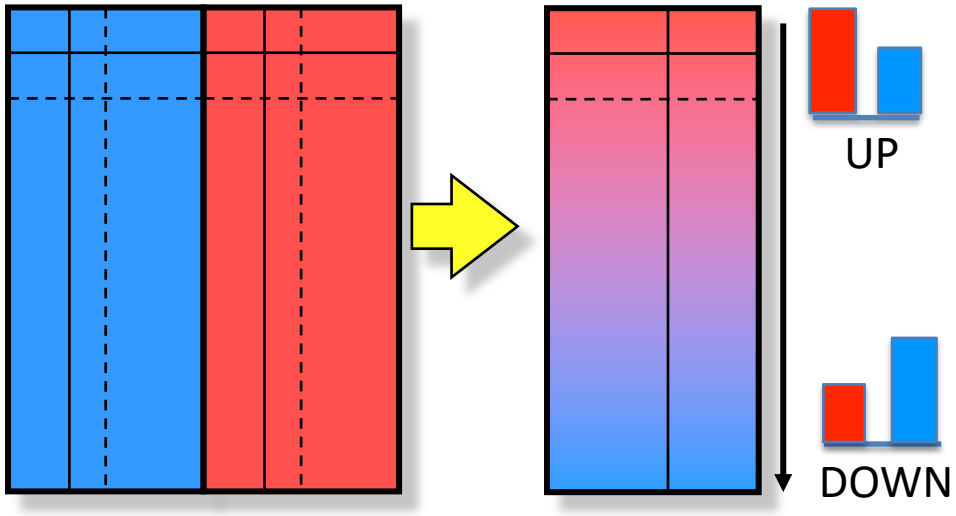
Differential expression between treated and control

↓

Ranked list of **all proteins** by differential expression score

Two-class design : ranked gene list

Expression Matrix

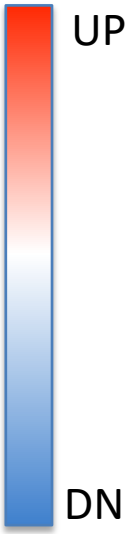


Genes Ranked by Differential Statistic

E.g.:
Fold change
Log (ratio)
t values from t-test

Ranking score = sign(logFC)*-log10(pvalue)

	LogFC	Pvalue	score
BGN	+1	1.73E-33	32.76
ANTXR1	+1	4.39E-31	30.36
FZD1	+1	4.41E-30	29.36
COL16A1	+1	1.33E-29	28.88
KLF3	+1	8.32E-02	1.08
RASEF	+1	9.01E-01	0.05
ISOC1	+1	9.01E-01	0.05
ANO1	+1	9.01E-01	0.04
CBWD3	-1	8.18E-02	-1.09
GBP4	-1	2.45E-16	-15.61
TAP1	-1	1.04E-19	-18.98
PSMB9	-1	1.84E-20	-19.73



OMICS gene lists: ranked or not ranked?

a few examples, cont.

Start point is: DNA

Looking for somatic mutations or CNV

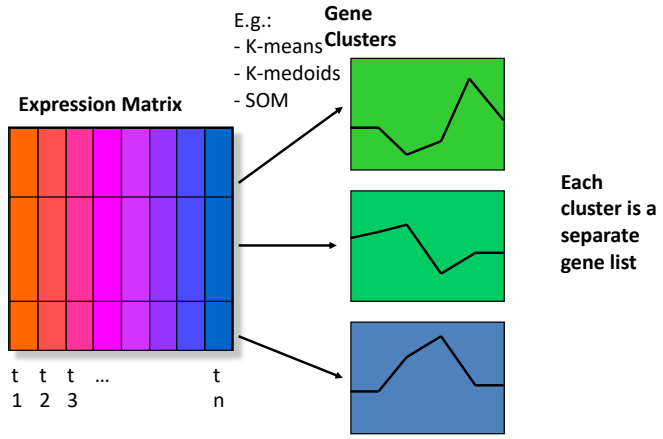
↓

Variant calling

gene list

(eg list of frequently mutated genes)

RNA: Time course or cluster analysis



ATAC-seq

Chip-seq

↘ ↙

Peaks regions (BED FILE)

↓

Need to associate peak regions with genes

↓

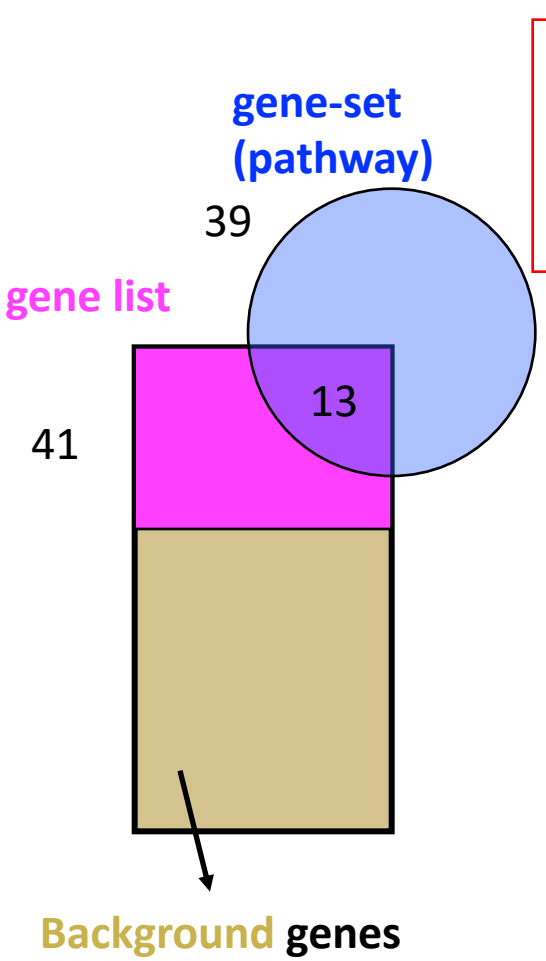
Gene list of associated with peaks of interest

Gene list enrichment test

Gene list enrichment analysis

- Given:
  1. **Gene list**: e.g. RRP6, MRD1, RRP7, RRP43, RRP42 (yeast)
  2. **Gene sets (pathways)** or annotations: e.g. The Gene Ontology, transcription factor binding sites in promoter
- Question: ***Are any of the gene sets (pathways) surprisingly enriched in the gene list?***
- Details:
  - Where do the **gene lists** come from?
  - How to assess "surprisingly" (statistics)
  - How to correct for repeating the tests

How do simple enrichment tests work?



OVERLAP BETWEEN GENE LIST AND GENE_SETS (PATHWAYS)

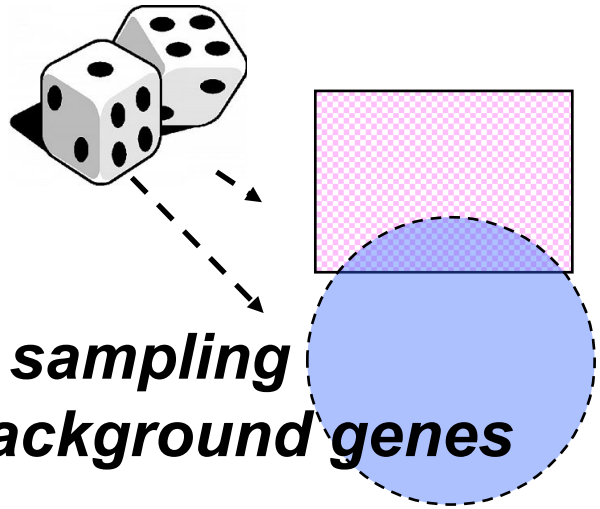
1

Enrichment Table

Gene-set	p-value
Spindle	0.0001
Apoptosis	0.025

2

Is this overlap larger than expected by chance?



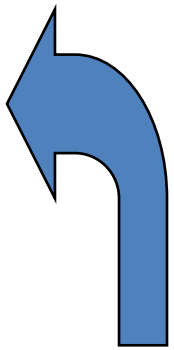
random sampling using background genes

Empirical pval =(#obs_overlap > random_overlap) +1) / (number of tests + 1)

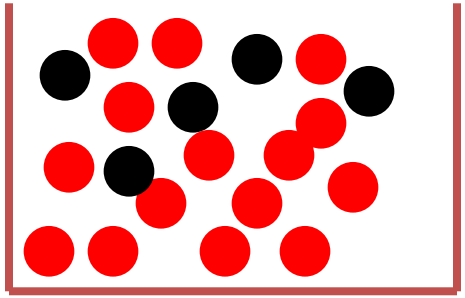
The Fisher's exact test

Gene list

- RRP6
- MRD1
- RRP7
- RRP43
- RRP42



Null hypothesis: List is a random sample from population
Alternative hypothesis: More black genes than expected in my list



Background population:
500 black genes,
4500 red genes

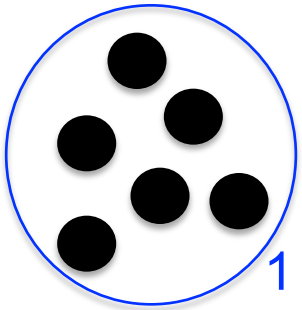
The Fisher's exact test

a.k.a., hypergeometric test

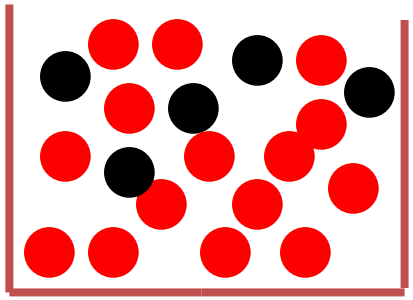
Gene list

- RRP6
- MRD1
- RRP7
- RRP43
- RRP42

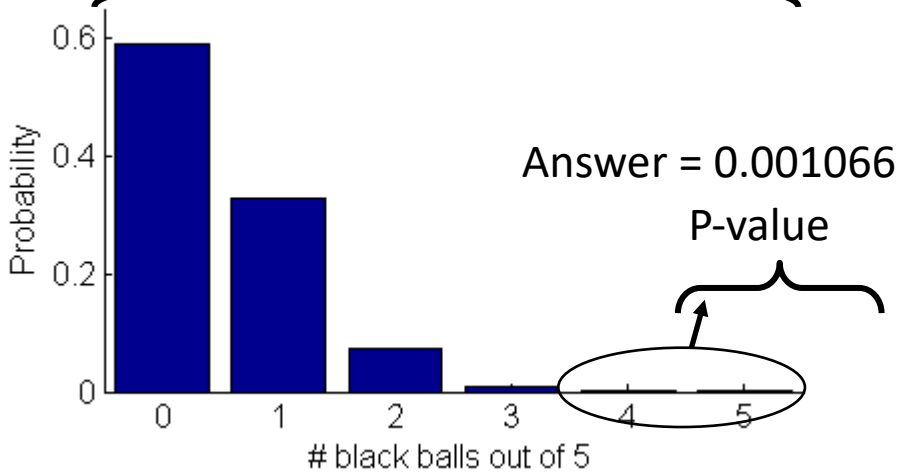
Background population:
500 black genes,
4500 red genes



1 gene-set/pathway

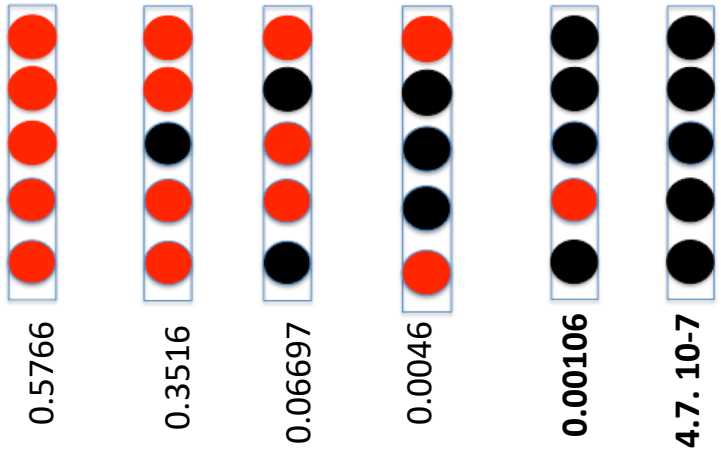


Null distribution



Answer = 0.001066662

P-value



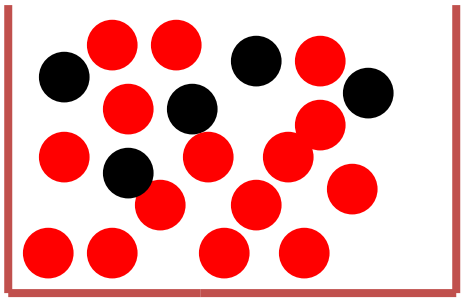
2x2 contingency table for Fisher's Exact Test

Gene list

- RRP6
- MRD1
- RRP7
- RRP43
- RRP42

	In gene list	Not in gene list	
In pathway	x = 4	496	m = 500
Not in pathway	k-x = 1	4499	t – m = 4500
	k= 5	4995	t =5000

$$P(X = x > q) = \sum_{x=q}^{m} \frac{\binom{m}{x}\binom{t-m}{k-x}}{\binom{t}{k}}.$$



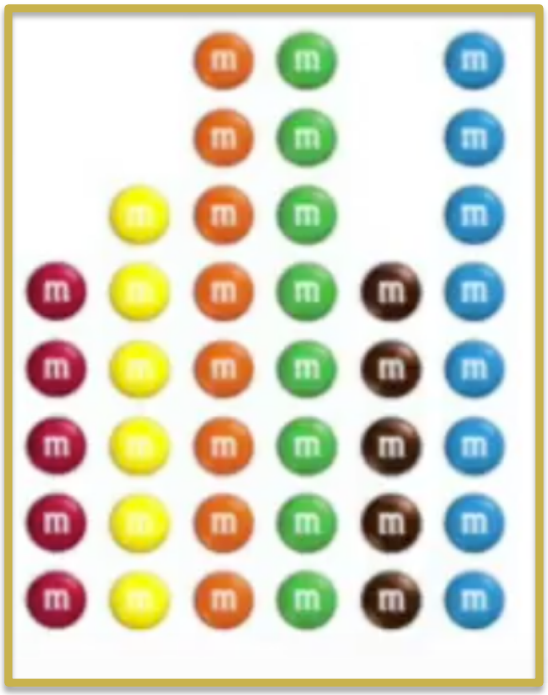
Background population:
500 black genes,
4500 red genes

Do you need to learn more about Fisher's exact test?

VIDEO the M&M's examples:
https://www.youtube.com/watch?v=udyAvvaMjfM

[StatQuest with Josh Starmer]

gene sets



gene list

I'm going to use the histogram of the "ideal" bag of m&m's, based on proportions I got off the internet, and my "sample", my handful of m&m's, to determine if my bag is special

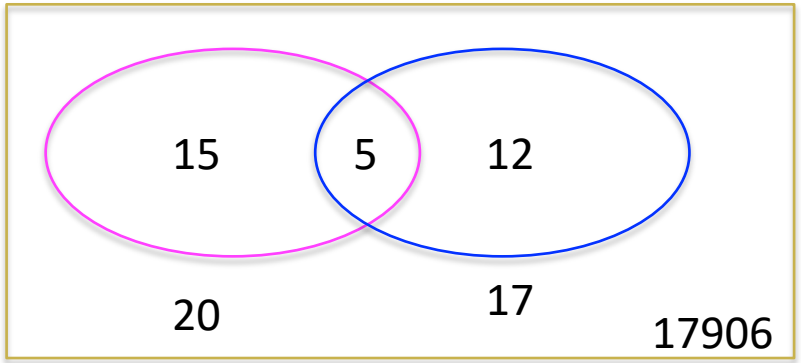
Background

And
Pathway Commons Guide:
https://www.pathwaycommons.org/guide/primers/statistics/fishers_exact_test/

g:Profiler

GO:BP		stats					
Term name	Term ID	padj	−log10(padj) (0 … ≤16)	T	Q	TnQ	U ↑
pulmonary valve morphogenesis	GO:0003184	1.034×10^{-8}		17	20	5	17906
pulmonary valve development	GO:0003177	3.392×10^{-8}		21	20	5	17906
regulation of myeloid leukocyte differentiation	GO:0002761	6.876×10^{-8}		122	20	7	17906
regulation of osteoclast differentiation	GO:0045670	1.353×10^{-7}		67	20	6	17906

T (term): pathway that is being tested
Q (query): my gene list
TnQ: overlap between pathway and gene list
U (universe): background



2x2 contingency table

	In gene list	Not in gene list
In pathway	5	12
Not in pathway	15	17894
	20	17906

Enrichr output table

Fisher's exact test

GO Biological Process

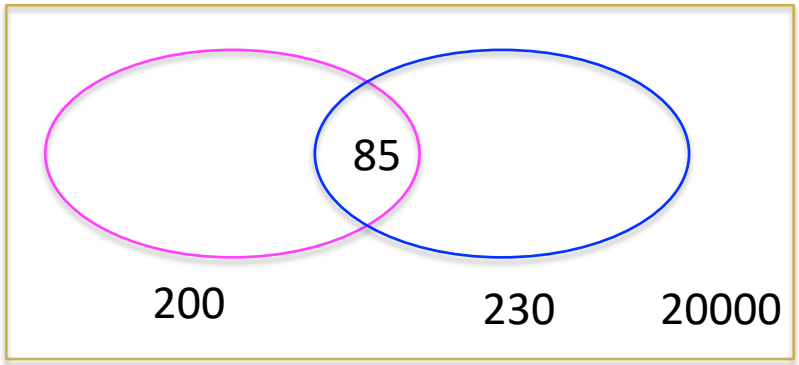
Term	Overlap	P-value	Adjusted P-value	Old P-value	Old Adjusted P-value	Z-score	Combined Score	Genes
extracellular matrix organization (GO:0030198)	85/230	2.1E-50	6.4E-47	4.3E-39	1.3E-35	-1.64651	188.3195	ITGB1;APP;COL16A1;SPARC;COL14A1;
negative regulation of signal transduction (GO:0009968)	58/284	7.2E-20	1.1E-16	2.4E-16	3.6E-13	-1.31194	57.83351	PID1;IRS1;FLT4;PEAR1;GLI3;CYP26B1;
skeletal system development (GO:0001501)	38/147	4.9E-17	4.9E-14	8.3E-14	6.2E-11	-1.47253	55.30609	DLX5;COL12A1;CHRD;AEBP1;PCSK5;PT
regulation of cell migration (GO:0030334)	57/317	7.2E-17	5.5E-14	5.4E-14	5.5E-11	-1.27044	47.21385	ROBO4;SERPINE1;LDB2;FGF1;RND3;CY
collagen fibril organization (GO:0030199)	18/30	2.4E-16	1.5E-13	6.5E-12	3.6E-09	-1.57943	56.77949	LUM;COL14A1;COL11A1;COL12A1;DP
glycosaminoglycan biosynthetic process (GO:0006024)	29/100	9.5E-15	4.8E-12	7.1E-12	3.6E-09	-1.2711	41.04479	CHPF;SDC2;XYLT1;HS2ST1;ACAN;NDST
regulation of angiogenesis (GO:0045765)	38/178	4.1E-14	1.8E-11	1.3E-11	5.4E-09	-1.77078	54.58956	SEMA5A;ITGB1;ECM1;SPARC;SERPINE
positive regulation of cell motility (GO:2000147)	36/180	1.5E-12	5.7E-10	2.1E-10	8E-08	-1.22301	33.29297	LRRC15;SEMA7A;SEMA3C;SEMA3D;TV
protein complex subunit organization (GO:0071822)	18/46	3.6E-12	1.2E-09	1.5E-09	3.6E-07	-1.44324	38.01215	LUM;COL14A1;COL11A1;COL12A1;DP

Pathways (gene-sets)

Overlap:
Numerator -> genes in my gene list and tested pathway

Denominator -> Genes in the original pathway

List of genes in the overlap



PANTHER output

of genes in original pathway

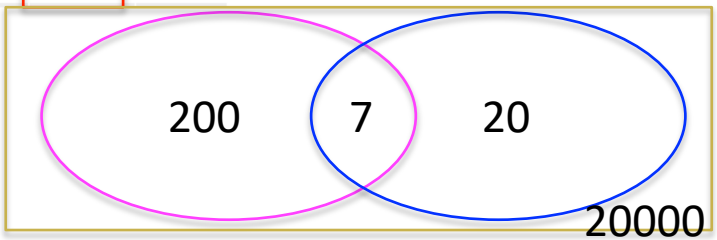
Overlap:# of genes in my gene list and tested pathway

Significance of the enrichment.

Pathway (gene-sets)

Displaying only results for FDR P < 0.05, click here to display all results

	Homo sapiens (REF)		Client Text Box Input (Hierarchy) NEW!				
PANTHER GO-Slim Biological Process	#	#	expected	Fold Enrichment	Δ +/-	raw P value	FDR
tissue morphogenesis	27	7	1.31	5.33	+	8.09E-04	1.75E-02
regulation of phosphorus metabolic process	250	25	12.16	2.06	+	1.29E-03	2.66E-02
actin filament bundle organization	39	8	1.90	4.22	+	1.31E-03	2.64E-02
regulation of phosphate metabolic process	250	25	12.16	2.06	+	1.29E-03	2.63E-02
regulation of cell communication	359	47	17.46	2.69	+	1.17E-08	1.61E-06
ameboidal-type cell migration	25	8	1.22	6.58	+	1.02E-04	3.17E-03
glycoprotein biosynthetic process	101	13	4.91	2.65	+	2.41E-03	4.33E-02
response to growth factor	75	16	3.65	4.39	+	4.01E-06	1.80E-04
regulation of cell size	28	7	1.36	5.14	+	9.71E-04	2.05E-02
multicellular organism development	609	84	29.61	2.84	+	6.18E-16	2.78E-13
cell-cell signaling	523	47	25.43	1.85	+	1.58E-04	4.37E-03
extracellular matrix organization	69	31	3.36	9.24	+	8.89E-18	1.60E-14
neuron differentiation	224	29	10.89	2.66	+	7.03E-06	2.87E-04
vasculature development	38	13	1.85	7.04	+	3.92E-07	3.20E-05
carbohydrate derivative metabolic process	282	27	13.71	1.97	+	1.54E-03	3.03E-02
cell differentiation	302	38	14.69	2.59	+	6.17E-07	4.26E-05
cellular response to stimulus	1977	140	96.14	1.46	+	1.62E-05	5.83E-04
cell-substrate adhesion	54	10	2.63	3.81	+	6.83E-04	1.51E-02
response to endogenous stimulus	116	16	5.64	2.84	+	4.11E-04	9.84E-03
regulation of Wnt signaling pathway	40	9	1.95	4.63	+	3.69E-04	8.95E-03
regulation of intracellular signal transduction	293	31	14.25	2.18	+	1.44E-04	4.05E-03

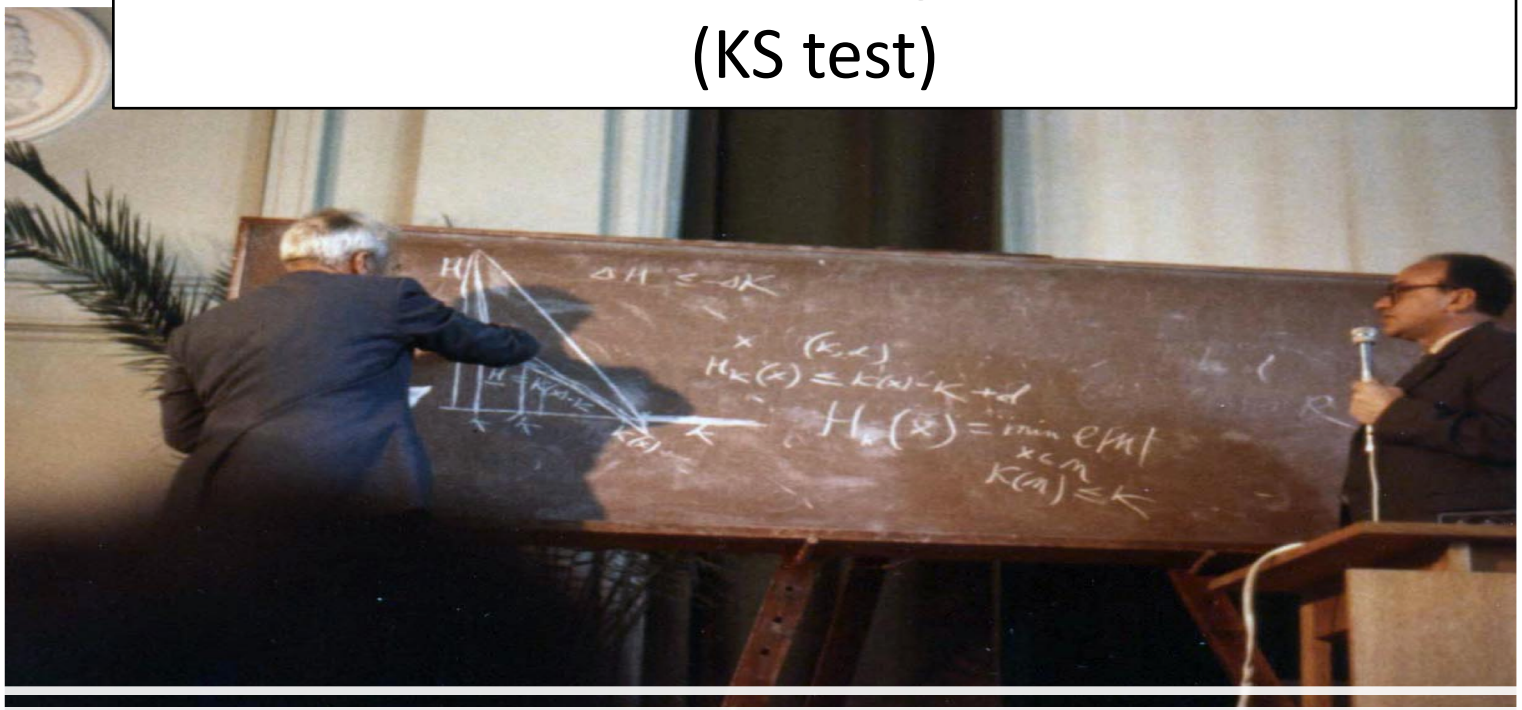


Notes

- We usually test **over-enrichment** of “black”. To test for ***under-enrichment*** of “black”, test for *over-enrichment* of “red”.
- **Fisher’s Exact Test** is often called the **hypergeometric test**
- **Other enrichment tests** for defined gene lists (not covered in this lecture):
  - Approximation of the Fisher’s Exact Test (Monte Carlo simulation)
  - Binomial test
  - Chi-squared test

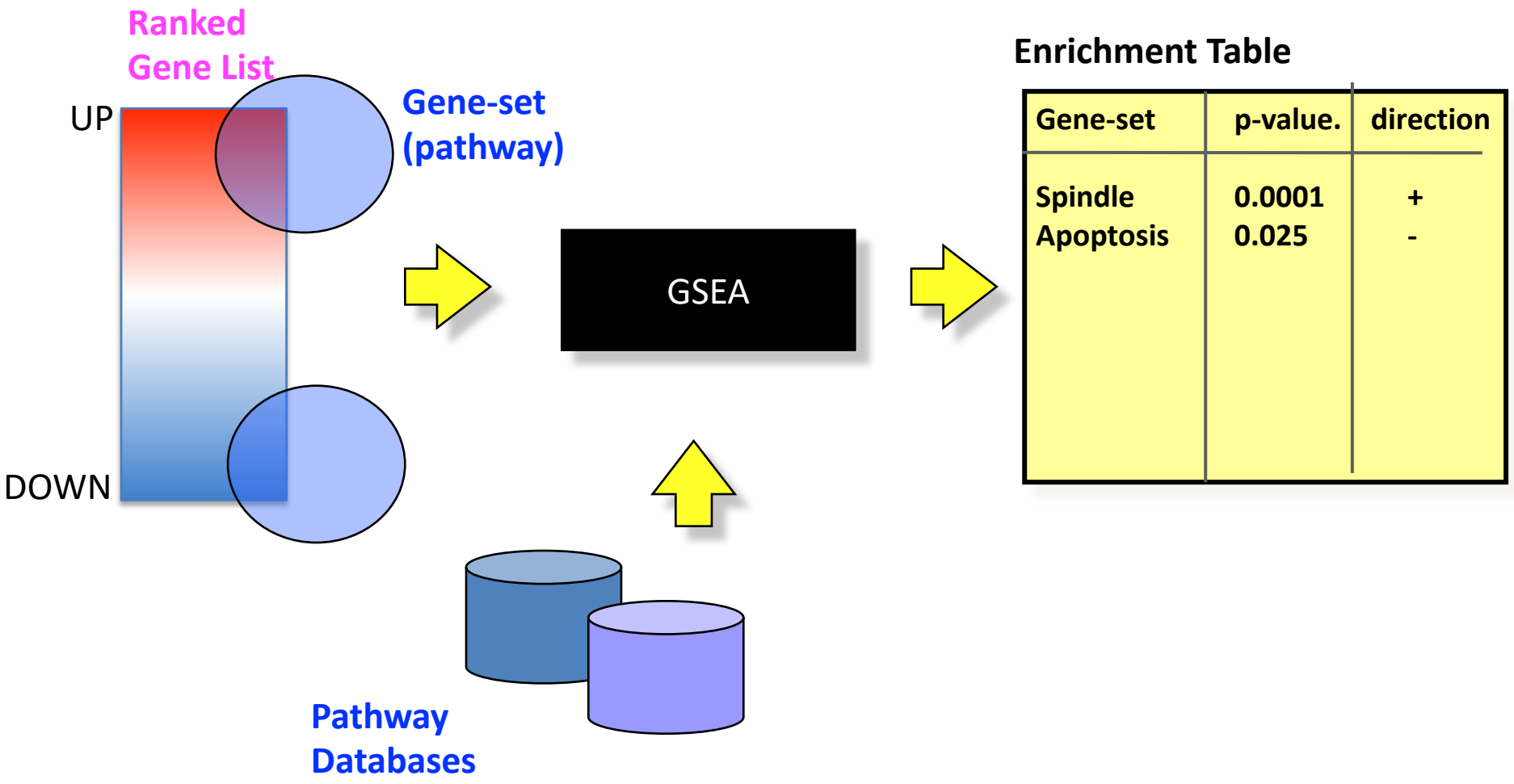
Ranked gene list enrichment test

GSEA → modified Kolmogorov Smirnov test (KS test)



https://en.wikipedia.org/wiki/**Andrey_Kolmogorov**#/media/File:Kolm_complexity_lect.jpg

Example of a ranked list enrichment test



- In their original paper, Mootha et al (2003) studied diabetes and identified that their gene list was significantly enriched in a pathway called “oxidative phosphorylation”.
- The particularity of this finding was that individual genes in this pathway were only down-regulated by a small amount but the addition of all these subtle decreases had a great impact on the pathway.
- They validated their finding experimentally.

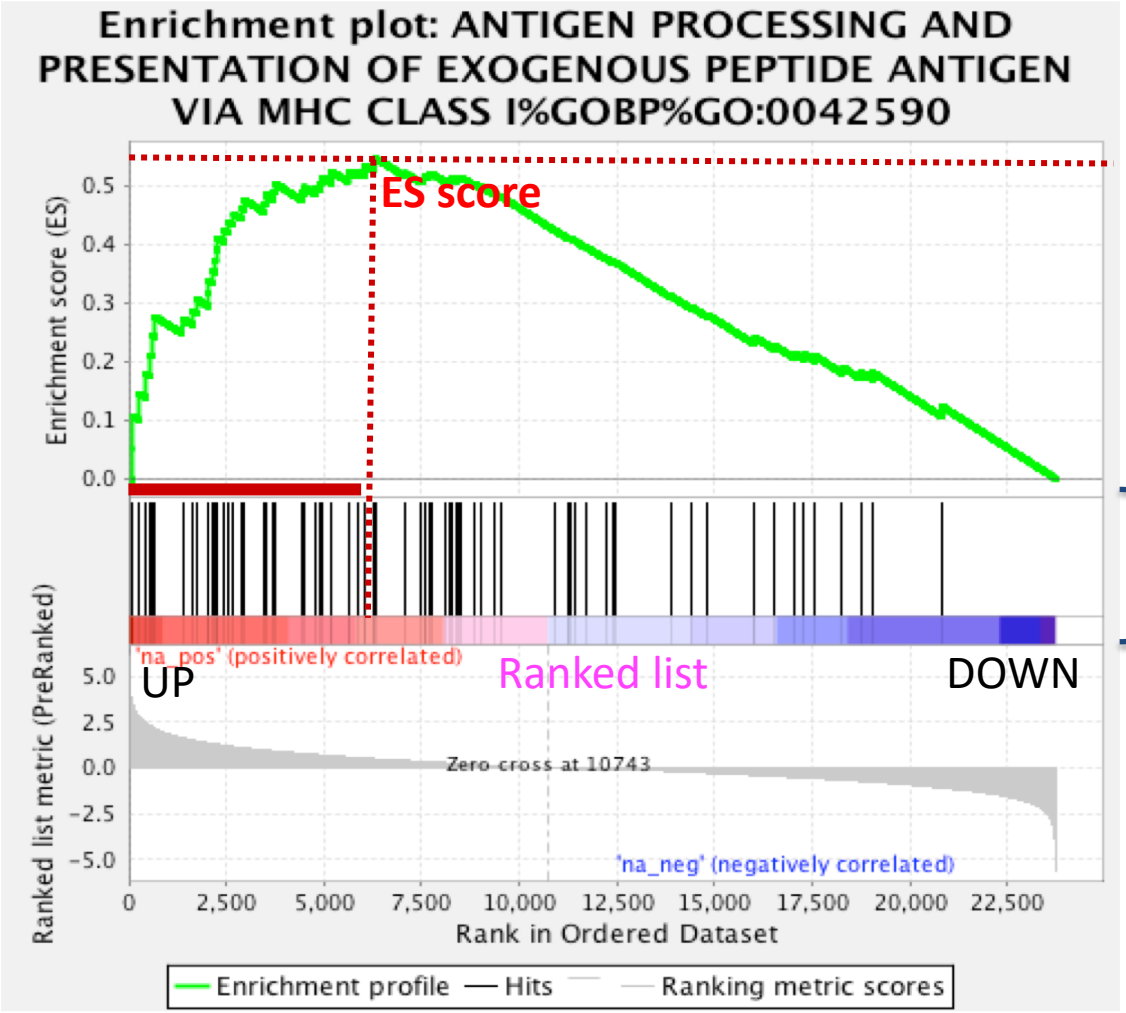
http://www.people.vcu.edu/~mreimers/HTDA/Mootha%20-%20GSEA.pdf

GSEA score calculation

Ranked gene list

	UP
BGN	32.76
ANTXR1	30.36
FZD1	29.36
COL16A1	28.88
KLF3	1.08
RASEF	0.05
…	…
…	…
ISOC1	0.05
ANO1	0.04
CBWD3	-1.09
GBP4	-15.6
TAP1	-19
PSMB9	-19.7

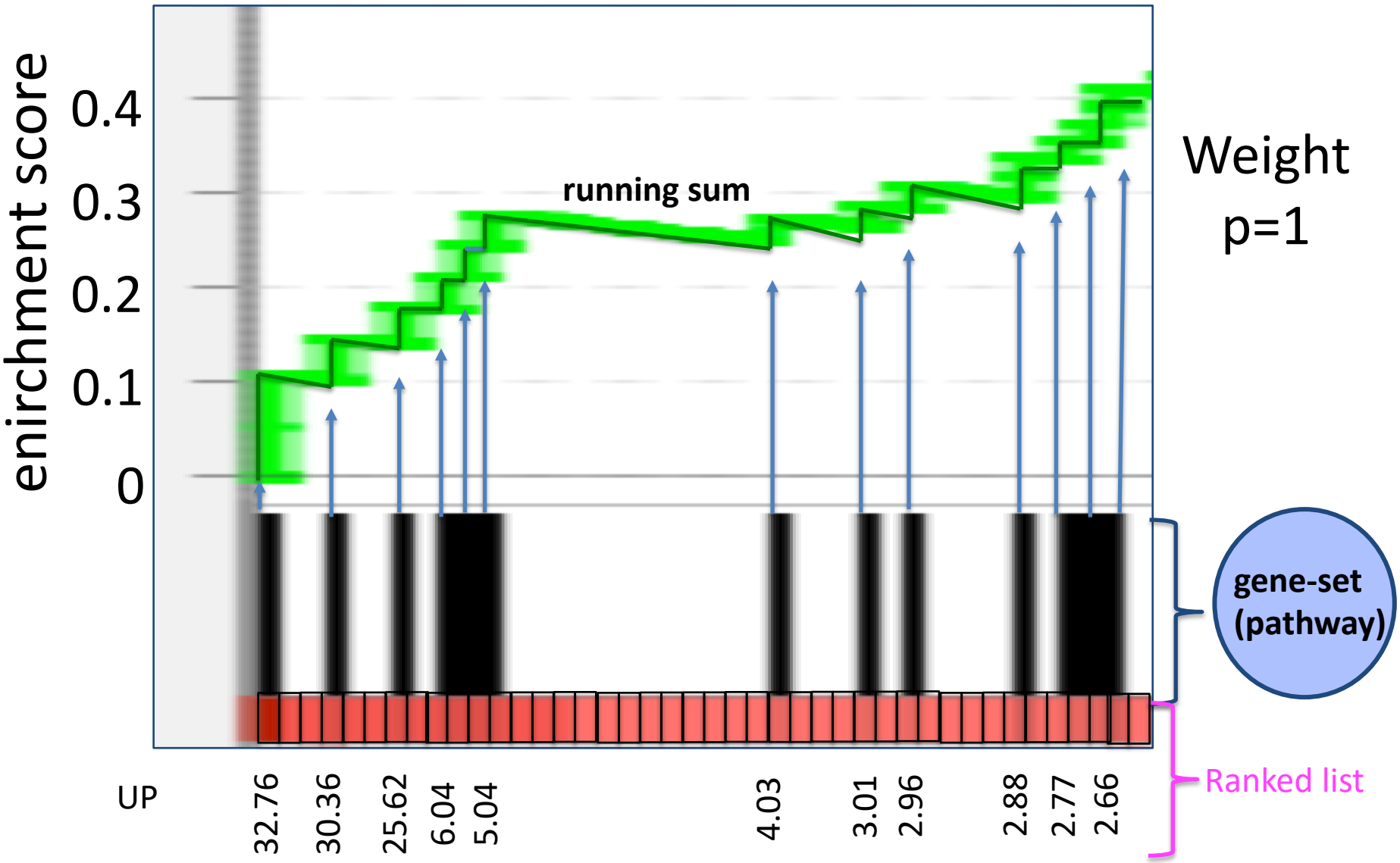
DOWN



gene-set (pathway)

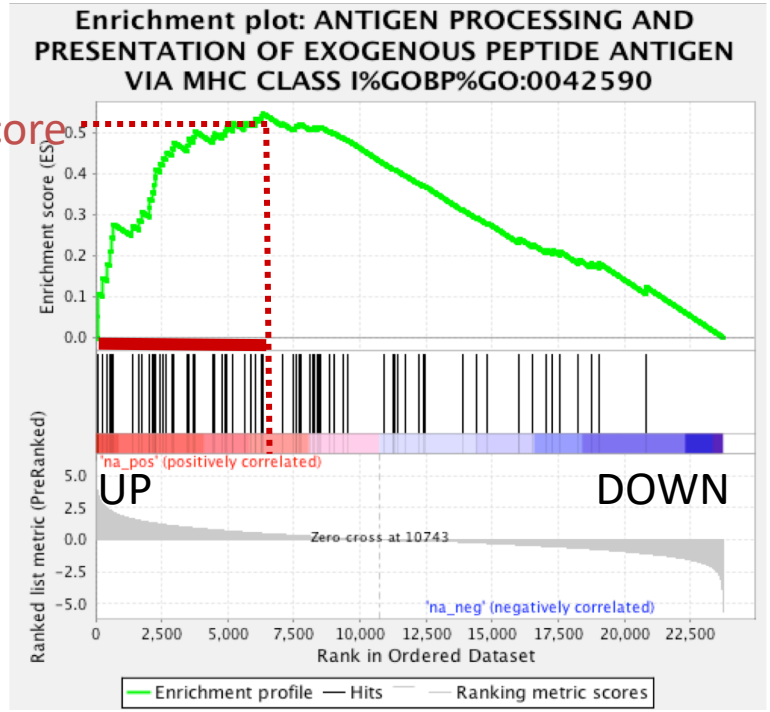
1. Maximum (or minimum) ES score is the final **ES score** for the gene set
2. Can define “leading edge subset” as all those genes ranked as least as high as the enriched set.

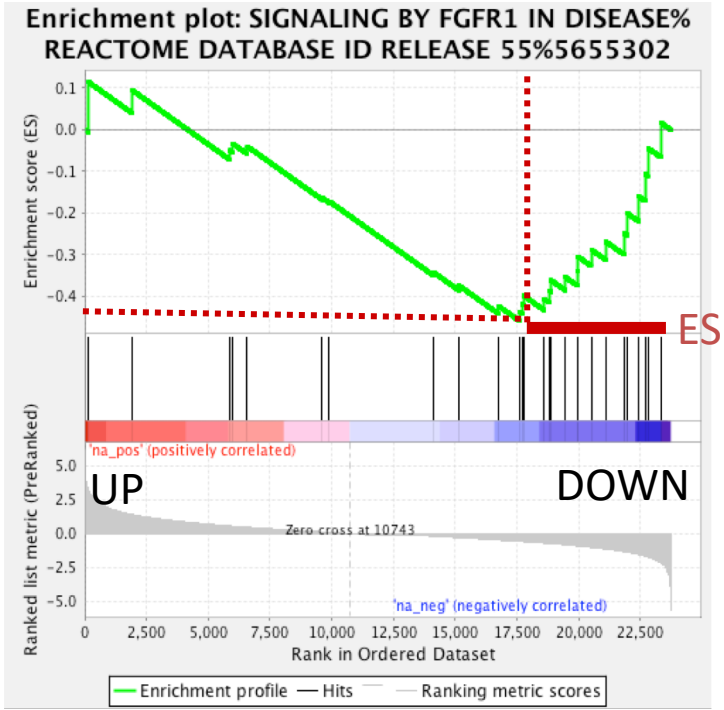
GSEA running sum



Positive and negative enrichment scores

ES score

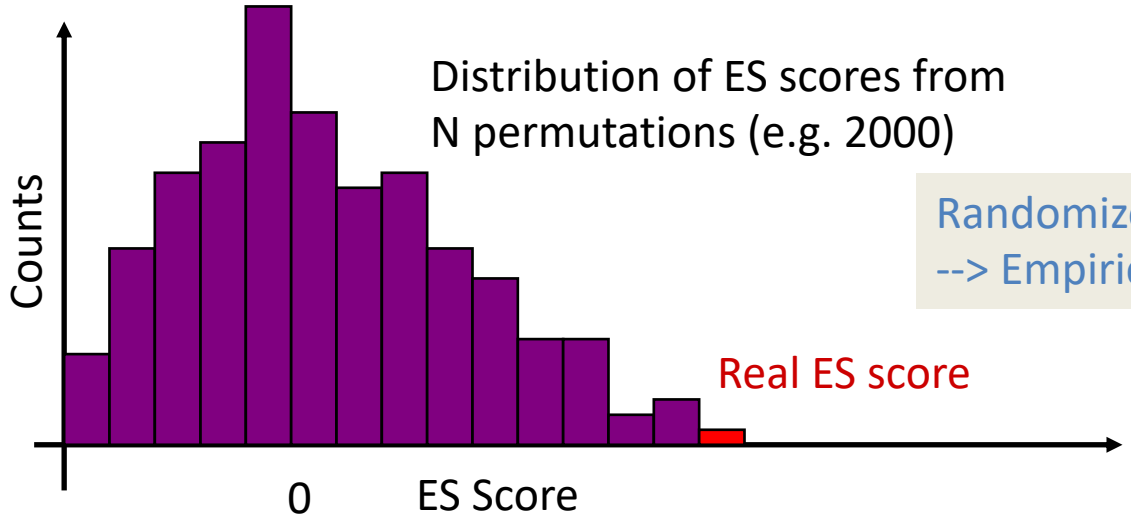




ES score

Going from ES score → P-value

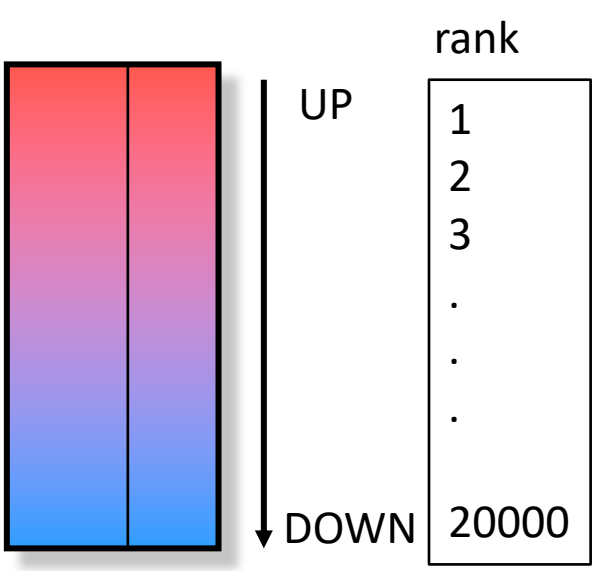
1. Generate null-hypothesis distribution from randomized data (see permutation settings)
2. Estimate empirical p-value by comparing observed ES score to null-hypothesis distribution from randomized data (for every gene-set)

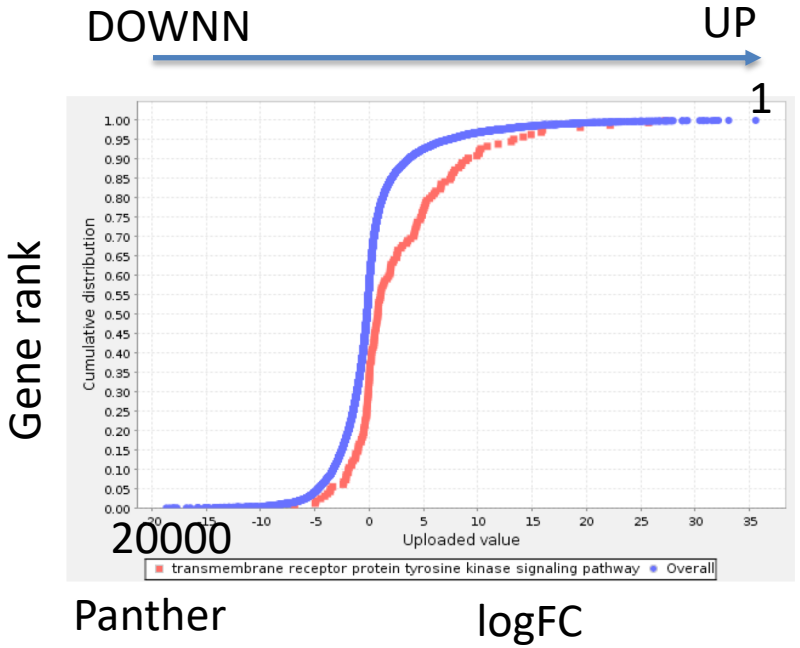


Randomized with ES score ≥ real: 4 / 2,000
--> Empirical p-value = 0.002

Other enrichment tests for a ranked gene list

Wilcoxon ranksum test





Outline of theory component

- Fisher's exact test (or binomial) for calculating enrichment P-values for defined gene lists
- GSEA, wilcoxon rank sum test for computing enrichment P-values for ranked gene lists

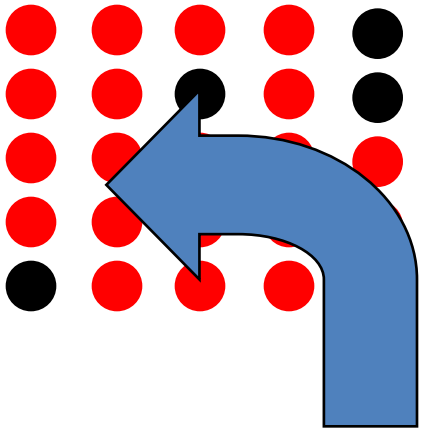
Multiple test corrections

We are testing many pathways at the same time

correction for multiple hypothesis testing

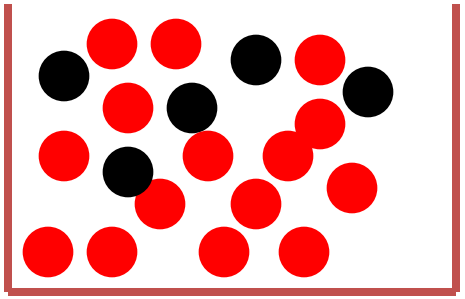
How to win the p-value lottery

Random draws

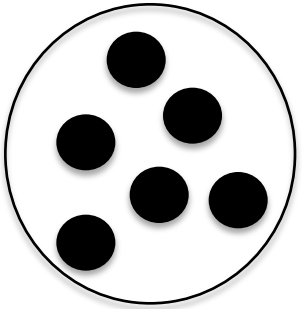


… 7,834 draws later …

Expect a random draw with observed enrichment once every 1 / P-value draws



Background population:
500 black genes,
4500 red genes



1 gene-set
(apoptosis)

Simple P-value correction: Bonferroni

If *M* = # of gene-sets (pathways) tested:

Corrected P-value = *M* x original P-value

Corrected P-value is greater than or equal to the probability that **one or more** of the observed enrichments could be due to random draws. The jargon for this correction is **"controlling for the *Family-Wise Error Rate (FWER)*"**

False discovery rate (FDR)

- FDR is *the expected **proportion** of the observed enrichments due to random chance.*
- Compare to Bonferroni correction which is a bound on *the probability that **any one** of the observed enrichments could be due to random chance.*
- Typically FDR corrections are calculated using the Benjamini-Hochberg procedure.
- FDR threshold is often called the "q-value"

False discovery rate (FDR)

1. **Sort P-values** of all tests in increasing order

2. **Adjusted P-value** is “nominal” P-value times # of tests divided by the rank of the P-value in sorted list: **P-value x [# of tests] / Rank**

3. **Q-value** (or FDR) corresponding to a nominal P-value **is the smallest adjusted P-value** assigned to P-values **with the same or larger ranks**.

4. Look at which gene-sets have a **FDR of 0.05 or less** and report them as **significantly enriched.**

Benjamini-Hochberg example

Rank	Category	(Nominal) P-value	Adjusted P-value	FDR / Q-value
1	*Transcriptional regulation*	0.001	0.001 x 53/1 = 0.053	0.040
2	*Transcription factor*	0.002	0.002 x 53/2 = 0.053	0.040
3	*Initiation of transcription*	0.003	0.003 x 53/3 = 0.053	0.040
4	*Nuclear localization*	0.0031	0.0031 x 53/4 = 0.040	0.040
5	*Chromatin modification*	0.005	0.005 x 53/5 = 0.053	0.053
…	…	…	…	…
52	*Cytoplasmic localization*	0.97	0.985 x 53/52 = 1.004	0.99
53	*Translation*	0.99	0.99 x 53/53 = 0.99	0.99

Q-value (or FDR) corresponding to a nominal P-value is the smallest adjusted P-value assigned to P-values with the same or larger ranks.

Gene set enrichment significant at FDR < 0.05

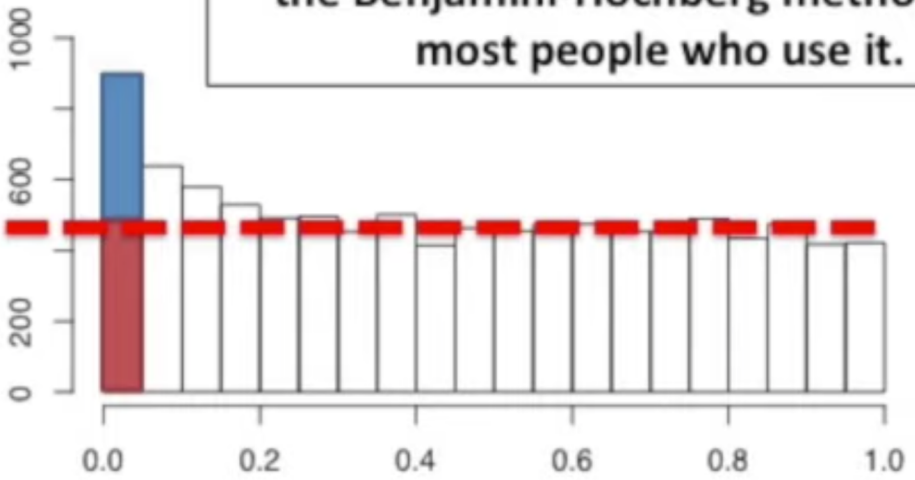
Reducing multiple test correction stringency

- The correction to the P-value threshold α depends on the # of tests that you do, so, no matter what, the more tests you do, the more sensitive the test needs to be
- Can control the stringency by reducing the number of tests: e.g. use GO slim; restrict testing to the appropriate GO annotations; or filter gene sets by size.

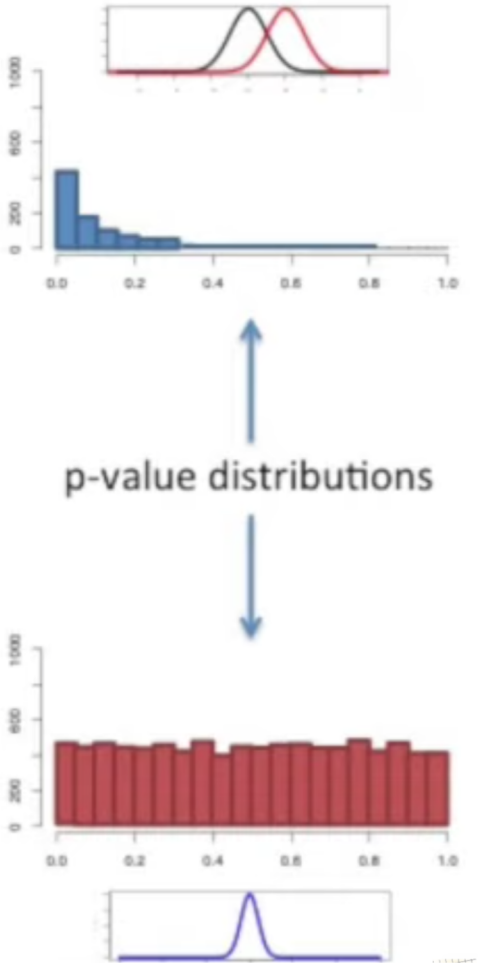
How to win the p-value lottery, part 2

Keep the gene list the same, evaluate different gene-sets(pathways)

If you can understand these concepts, then you understand more about FDR and the Benjamini-Hochberg method than most people who use it.



P value



https://www.youtube.com/watch?v=K8LQSvtjcEo

What Have We Learned? Typical output

gene-set name (pathway)

number of overlapping genes

… corrected for gene-set size

p-value

… corrected for multiple hypothesi

RNA HELICASE ACTIVITY%GO%GO:0003724	28	1.77	0.0041	0.0464386
MRNA SURVEILLANCE PATHWAY%KEGG%HSA03015	82	1.77	0	0.0466167
UBIQUITIN-DEPENDENT DEGRADATION OF CYCLIN D1%REACTOME%REACT_4.1	50	1.77	0.0021	0.0486015
BIOCARTA_CD40_PATHWAY%MSIGDB_C2%BIOCARTA_CD40_PATHWAY	15	1.77	0.0048	0.0483781
IGF1 PATHWAY%PATHWAY INTERACTION DATABASE NCI-NATURE CURATED DATA%IGF1 PATHWAY	29	1.76	0.003	0.0489742
UBIQUITIN-DEPENDENT PROTEIN CATABOLIC PROCESS%GO%GO:0006511	204	1.76	0	0.0488442
PHAGOSOME%KEGG%HSA04145	147	1.76	0	0.0486164
PROTEASOME COMPLEX%GO%GO:0000502	29	1.76	0.007	0.0490215
ANTIGEN PRESENTATION: FOLDING, ASSEMBLY AND PEPTIDE LOADING OF CLASS I MHC%REACTOME%REACT_7	24	1.76	0.0041	0.0505599
ABORTIVE ELONGATION OF HIV-1 TRANSCRIPT IN THE ABSENCE OF TAT%REACTOME%REACT_6261.3	23	1.75	0	0.0529242
DNA DAMAGE RESPONSE, SIGNAL TRANSDUCTION BY P[illegible]CLASS MEDIATOR RESULTING IN CELL CYCLE ARREST%	67	1.75	0	0.052886
REGULATION OF MACROPHAGE ACTIVATION%GO%GO:0043030	11	1.75	0.003	0.0534709
PROTEIN FOLDING%REACTOME%REACT_16952.2	52	1.75	0.002	0.0537717
ENDOPLASMIC RETICULUM UNFOLDED PROTEIN RESPONSE%GO%GO:0030968	73	1.75	0	0.0546052
PROTEIN EXPORT%KEGG%HSA03060	24	1.75	9.75E-04	0.0548699
TRANSCRIPTION INITIATION FROM RNA POLYMERASE I PROMOTER%GO%GO:0006367	64	1.75	0.001	0.0545783
S PHASE%REACTOME%REACT_899.4	110	1.75	0	0.0546003
PROTEASOMAL PROTEIN CATABOLIC PROCESS%GO%GO:0010	163	1.75	0	0.0550066
ATP-DEPENDENT RNA HELICASE ACTIVITY%GO%GO:0004004	20	1.74	0.0059	0.0556722
ACID-AMINO ACID LIGASE ACTIVITY%GO%GO:0016881	217	1.74	0	0.0560217
GO%GO:0072474	67	1.74	0.002	0.0565978
GO%GO:0035966	107	1.74	0	0.0562957
GO%GO:0072413	67	1.74	9.81E-04	0.05761
BIOCARTA_IL4_PATHWAY%MSIGDB_C2%BIOCARTA_IL4_PATHWAY	11	1.74	0.0082	0.0581508
ASSOCIATION OF TRIC CCT WITH TARGET PROTEINS DURING BIOSYNTHESIS%REACTOME%REACT_16907.2	28	1.74	0.0039	0.0581298
UBIQUITIN-DEPENDENT DEGRADATION OF CYCLIN D1%REACTOME%REACT_938.4	50	1.74	0.0029	0.057876
MODIFICATION-DEPENDENT PROTEIN CATABOLIC PROCESS%GO%GO:0019941	207	1.74	0	0.0576579
TRANSLATION INITIATION COMPLEX FORMATION%REACTOME%REACT_1979.1	55	1.74	0.0021	0.0575181
GO%GO:0001906	13	1.74	0.0117	0.0572877
G1 S TRANSITION%REACTOME%REACT_1[illegible]2	107	1.74	0	0.0572618
GO%GO:0034620	73	1.73	0.0021	0.0576606
SIGNALING BY NOTCH%REACTOME%REACT_299.2	19	1.73	0.0069	0.0578565
RESPONSE TO UNFOLDED PROTEIN%GO%GO:0006986	102	1.73	0	0.0583864
SIGNAL TRANSDUCTION INVOLVED IN G1 S TRANSITION CHECKPOINT%GO%GO:0072404	68	1.73	0.002	0.0582213
GO%GO:0072431	67	1.73	0	0.058551
BIOCARTA_PROTEASOME_PATHWAY%MSIGDB_C2%BIOCARTA_PROTEASOME_PATHWAY	19	1.73	0.0099	0.0586655
HOST INTERACTIONS OF HIV FACTORS%REACTOME%REACT_6288.4	117	1.73	0	0.0586888
AUTOPHAGIC VACUOLE ASSEMBLY%GO%GO:0000045	13	1.73	0.0122	0.0588271
CYCLIN A:CDK2-ASSOCIATED EVENTS AT S PHASE ENTRY%REACTOME%REACT_9029.2	66	1.73	0	0.0610099

TABULAR FORMAT

NETWORK VISUALIZATION

Many available enrichment analysis tools

web-based

Cytoscape app

Standalone

R package

How to choose a tool?

- Does it cover your model organism?
- Is there a good choice of gene-sets (pathway database)
- Are the pathway databases up to date?
- Which statistics (for gene list or ranked gene list)?
- Is the description of statistics clear enough ?
- Do you like the output style?
- Can you connect it with network visualization tools like Cytoscape?

Defined gene list (Fisher's exact test)

	g:Profiler	PANTHER	biNGO	Cluego
Updated database	yes	yes	no? *1	yes
Choice of database (more than 1)	yes	yes	no (GO) *1	yes
Do we test database individually or together	together	individually	individually	together
Multiple model organisms?	yes	yes	yes	yes
Possibility to upload your own custom database	yes	no?	yes	no?
Statistics: possibility to use the Fisher's exact test (ORA) (thresholded gene list)	yes	yes	yes	yes
Multiple hypothesis correction; possibility to use B-H FDR	yes	yes	yes	yes
Possibility to upload reference genes (background)	yes	yes	yes	yes
Website (Web) or Cytoscape App (App)	Web	Web	App	App
Possibility to visualize with Cytoscape EnrichmentMap	YES	no	YES	Cytoscape

*1: can still be used with custom database ;

Ranked list

	GSEA	PANTHER
Rank test	Modified KS test	Wilcoxon Rank Sum test
Correction for multiple hypothesis testing	yes	yes
Choice of gene-sets + able to custom pathway database , can therefore be use for different model organisms	yes	no
Possibility to visualize results with Cytoscape enrichment map	yes	no

Recipe for defined gene list enrichment test

- **Step 1:** Define your gene list and your background list,
- **Step 2:** Select your gene sets (pathways) to test for enrichment,
- **Step 3:** Run enrichment tests using the Fisher's exact test and correct for multiple testing if you test more than one gene set (pathway)
- **Step 4:** Interpret your enrichments
- **Step 5:** Publish! ;)

Recipe for ranked list enrichment test

- **Step 1:** Rank your genes,
- **Step 2:** Select your gene sets (pathways) to test for enrichment,
- **Step 3:** Run enrichment tests (rank based sum test) and correct for multiple testing
- **Step 4:** Interpret your enrichments
- **Step 5:** Publish! ;)

Advanced topics (not covered in this lecture)

- Issues with tests: correlation between gene-sets, dependency of genes.
- Other types of tools: topology aware.

Go to: Pathway enrichment analysis and visualization of omics data using g:Profiler, GSEA, Cytoscape and EnrichmentMap

https://www.nature.com/articles/s41596-018-0103-9

Final Tips

- Be precise at each step of your analysis
- Try to answer one biological question at a time

We are on a Coffee Break & Networking Session

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