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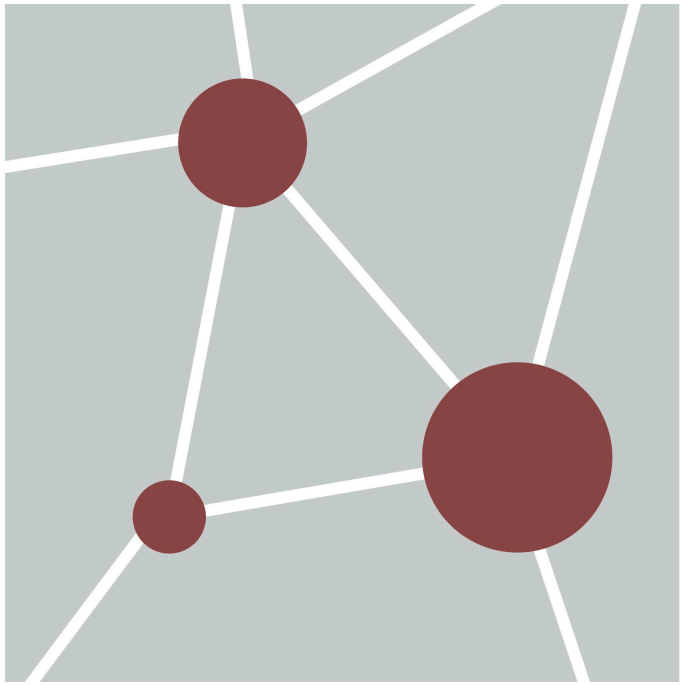
# Finding over-represented pathways in gene lists

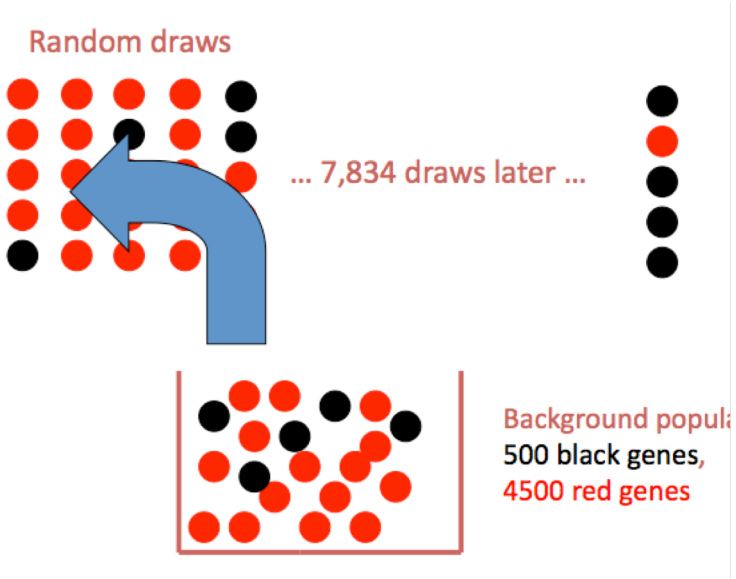
Veronique Voisin

Pathway and Network Analysis of –omics Data

May, 10-12, 2021

bioinformatics.ca

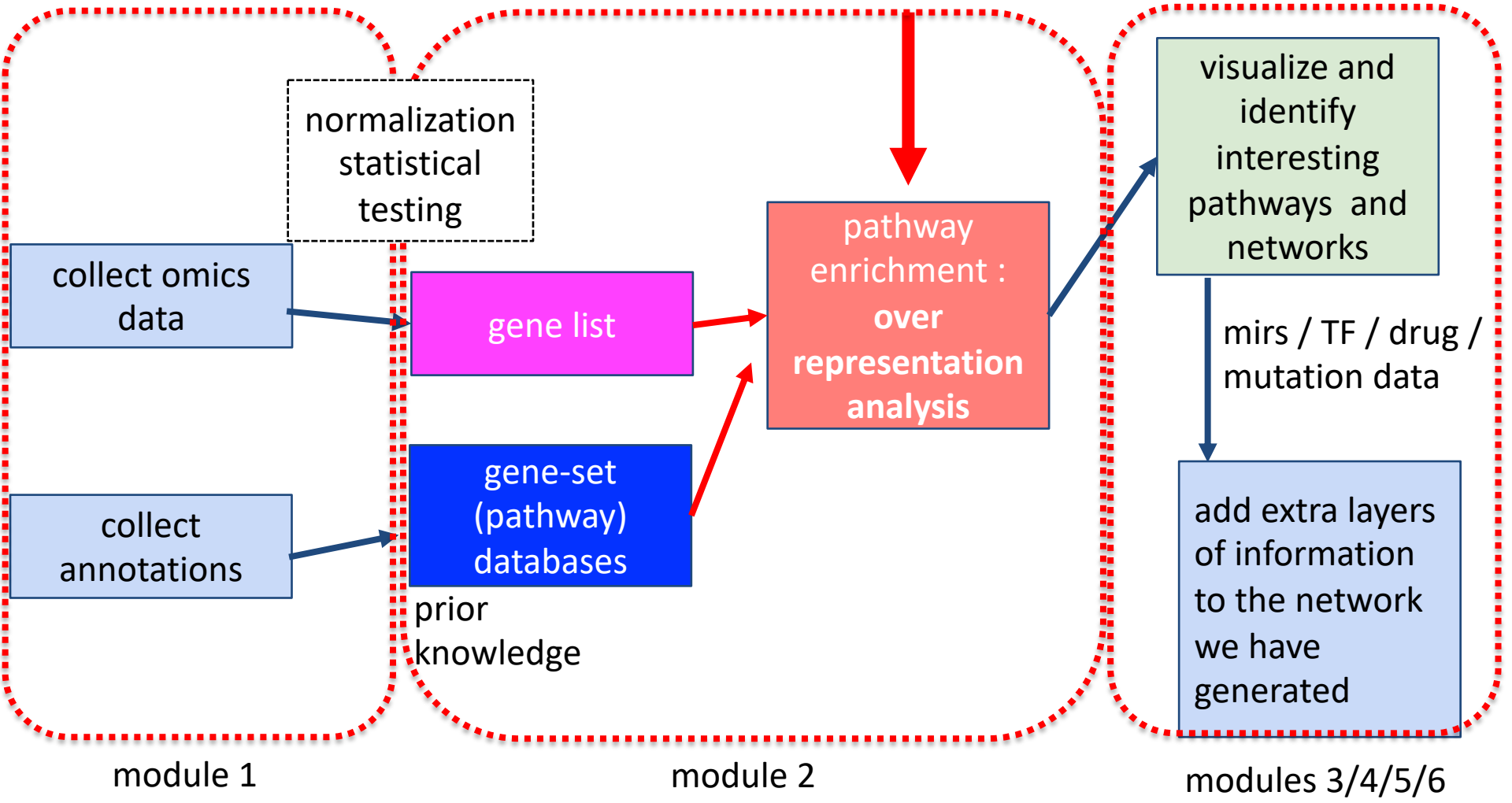




# Learning Objectives

- 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**

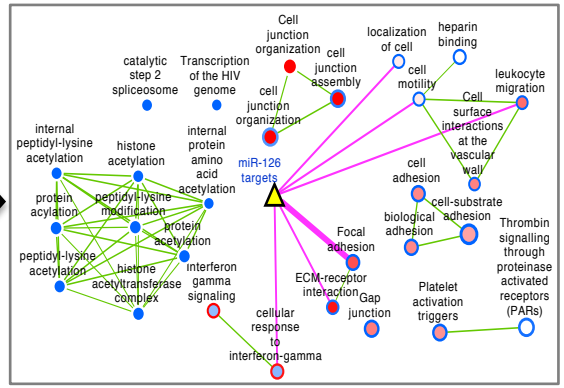
↓

**GENE LIST**

enrichment analysis

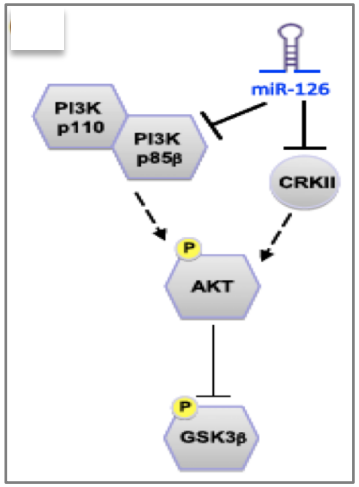
↓

Focus of this module

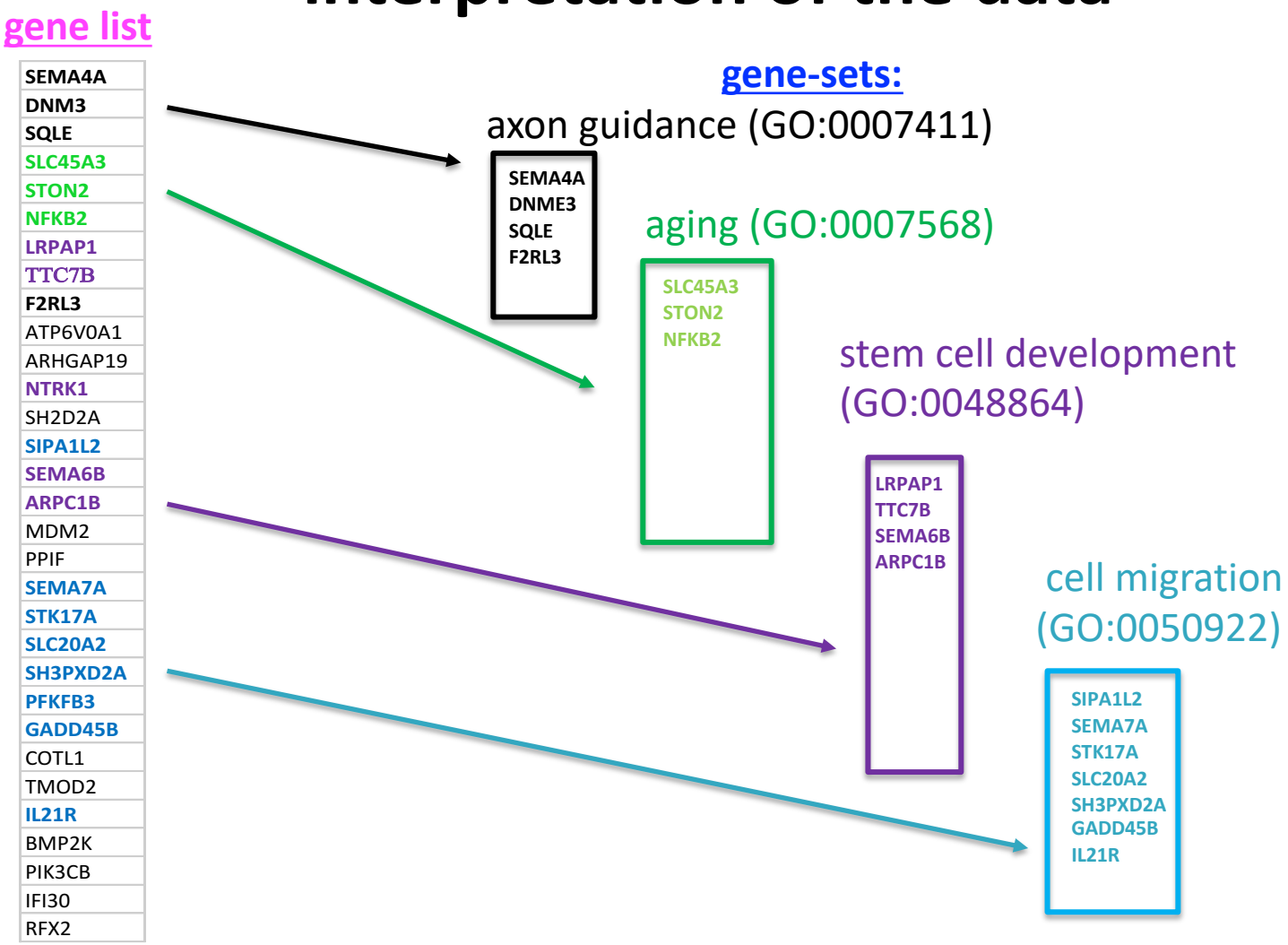



enrichment map

"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



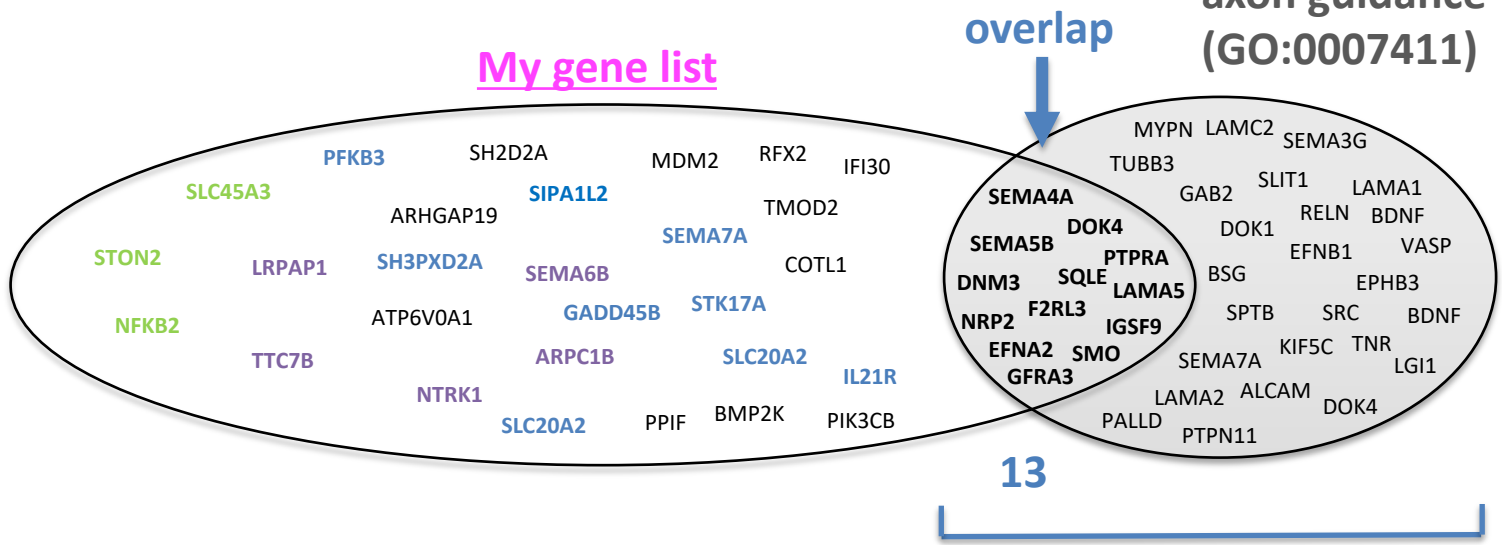
# Pathway enrichment analysis calculates the overlap between our gene list and a pathway

**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

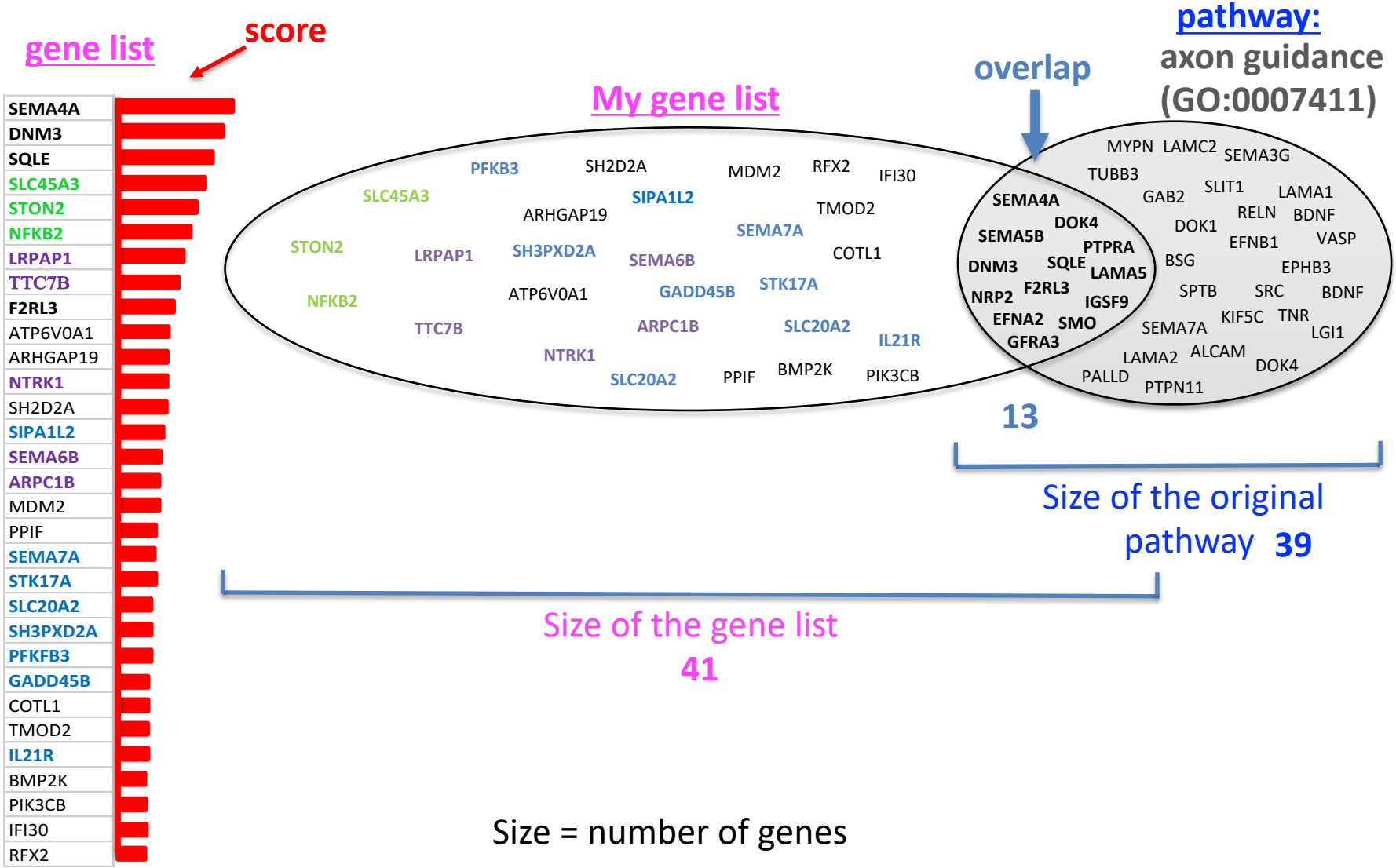



13/41 → 1/4

13/39 → 1/4

Size = number of genes

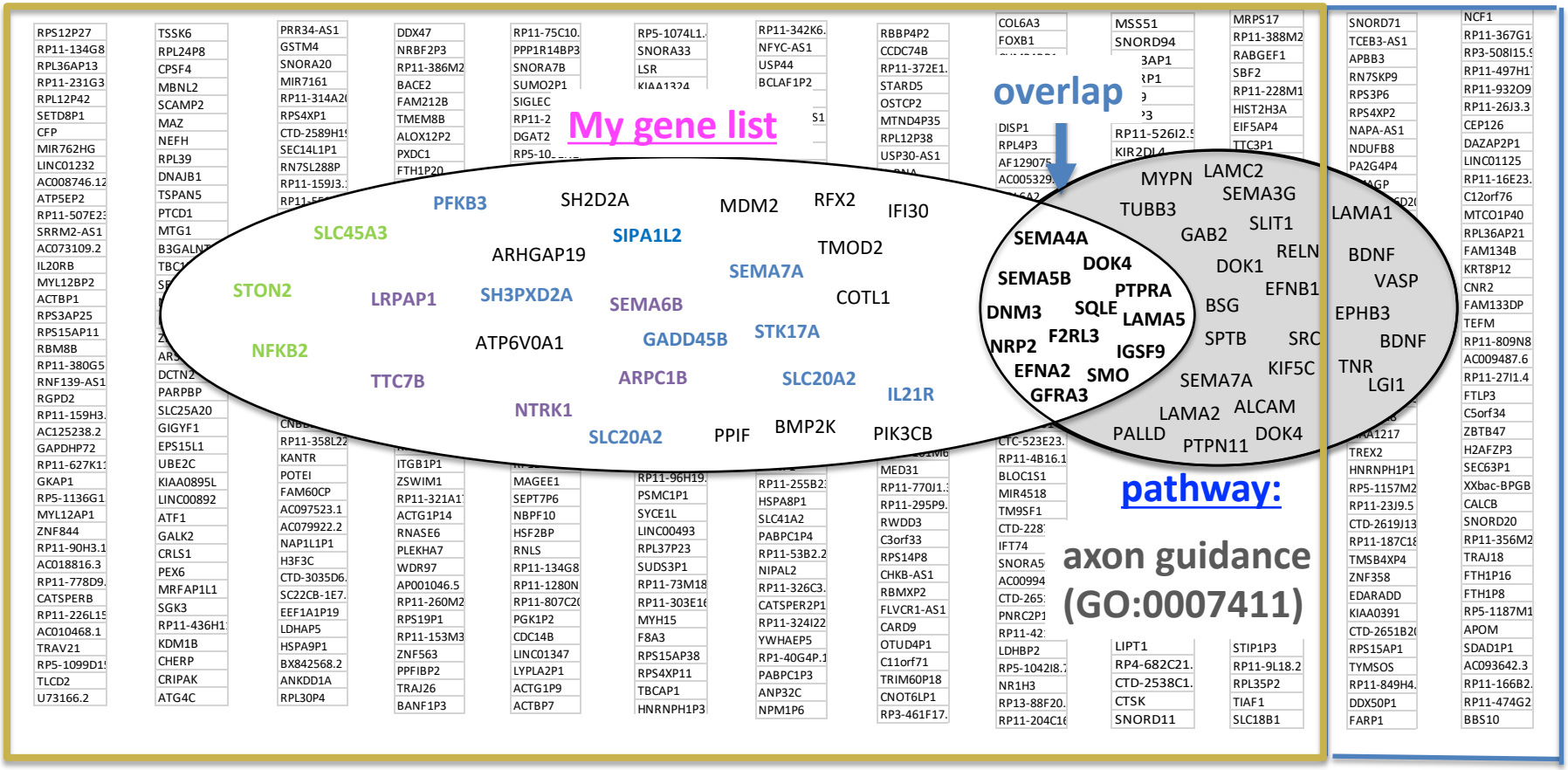
# Can we add a score associated with the genes when calculating the enrichment score?



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

*genes measured in the experiment*

*genes not measured*

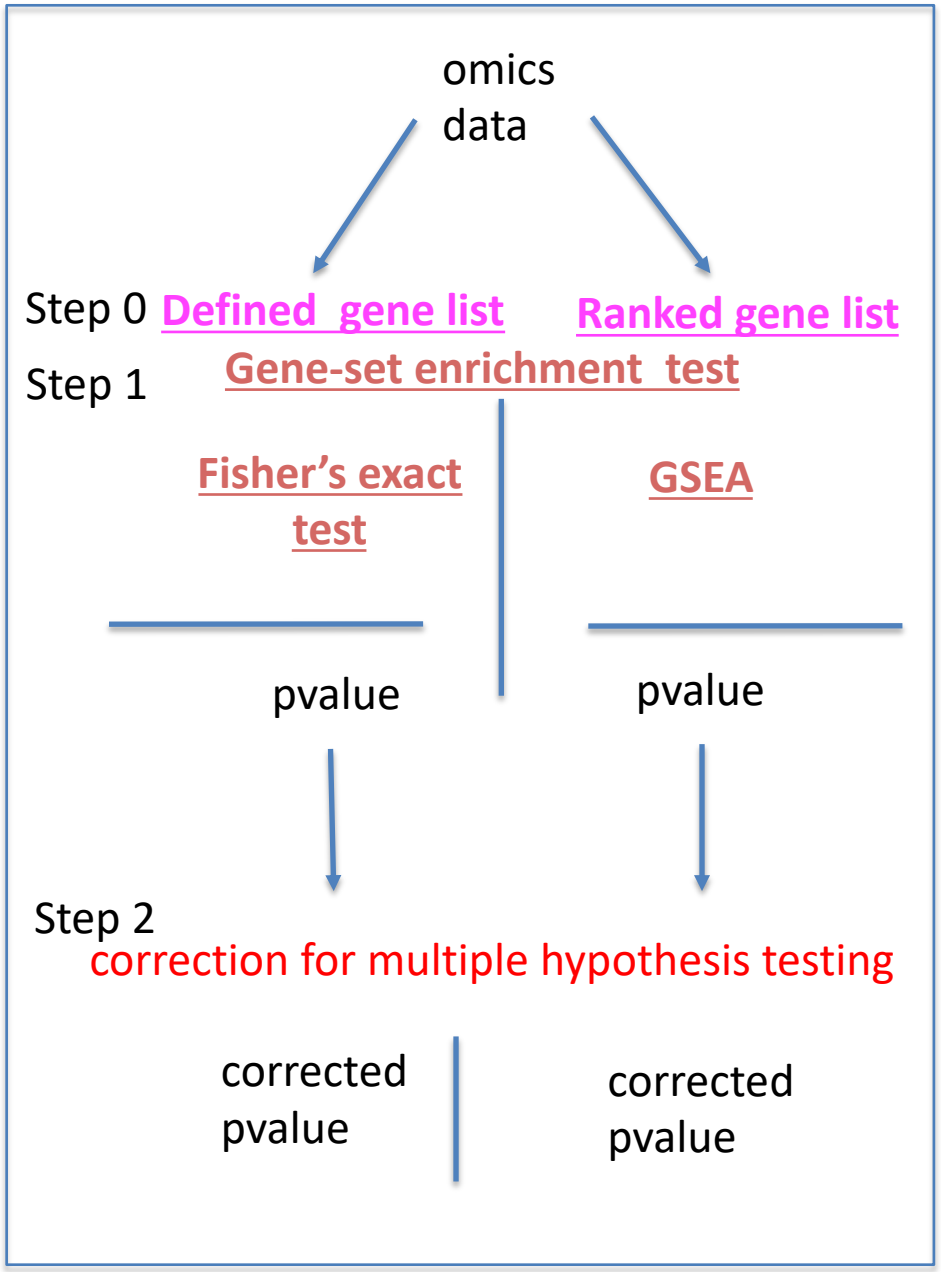



estimated 20,000-25,000 **human** protein-**coding genes**

**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

↓

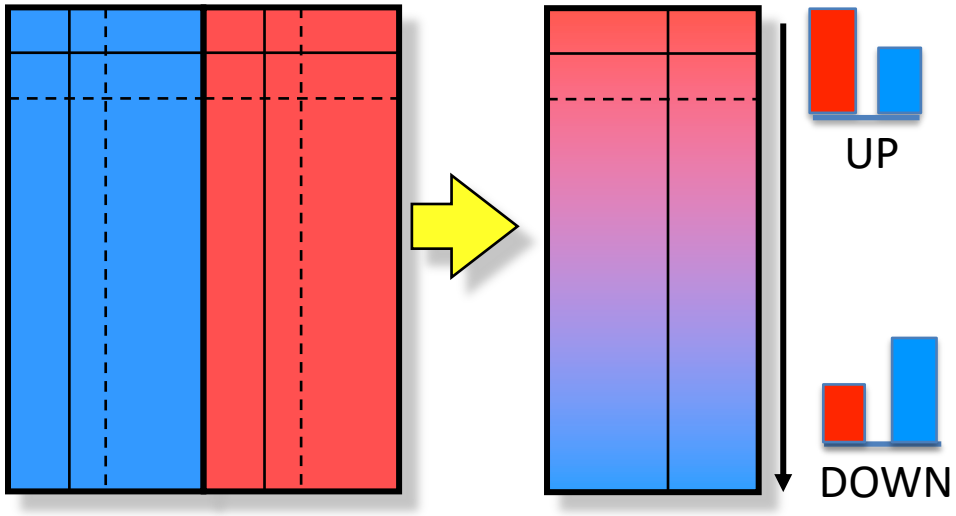
Differential expression between treated and control

↓

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

# Two-class design : ranked gene list

**Expression Matrix**

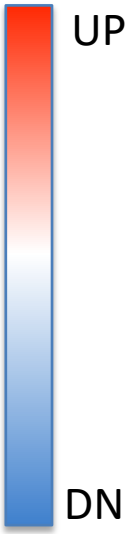



**Genes Ranked by Differential Statistic**

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

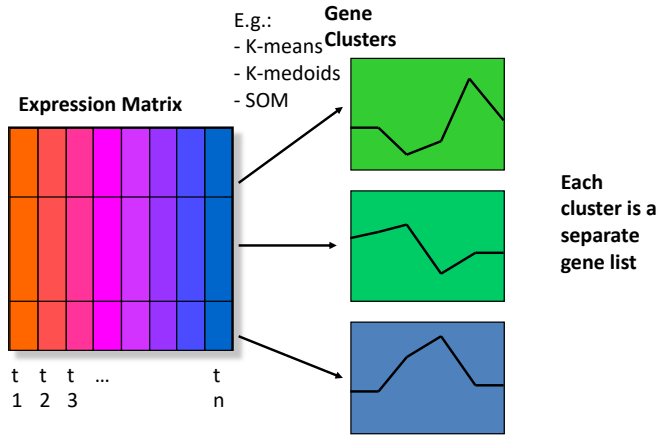
↓

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

↓

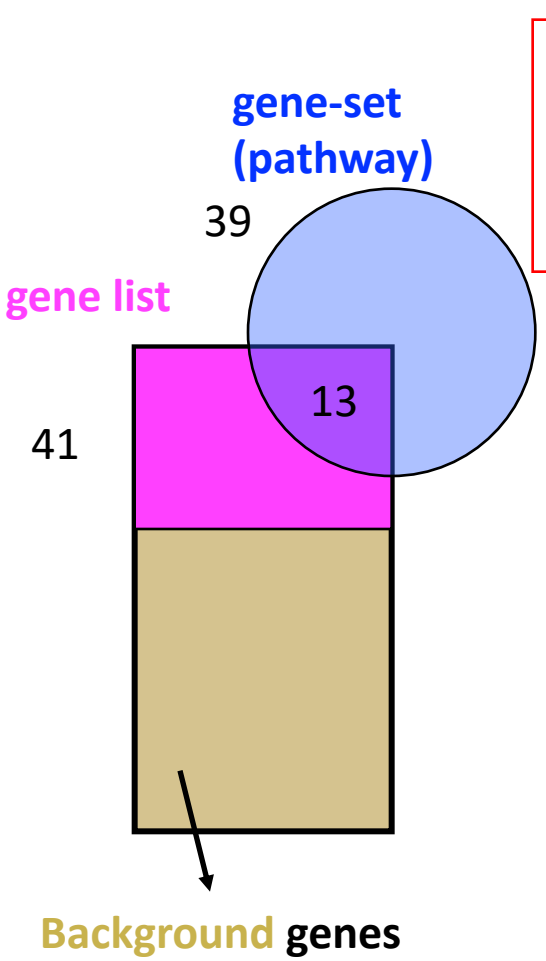
Gene list of associated with peaks of interest

# 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)

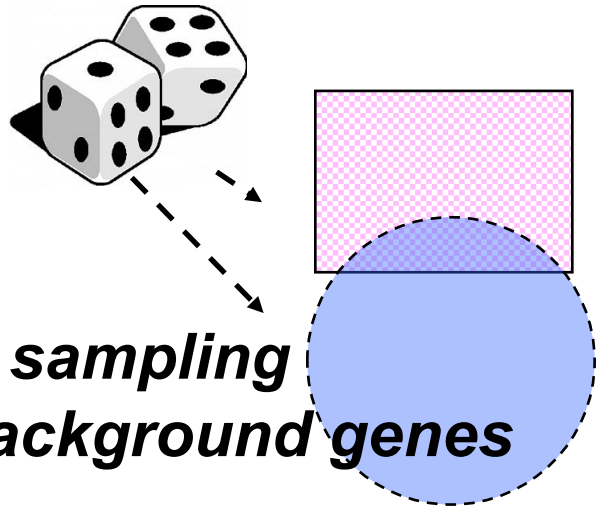
1

Enrichment Table

| Gene-set | p-value |
|---|---|
| Spindle | 0.0001 |
| Apoptosis | 0.025 |

2

***Is this overlap larger than expected by chance?***



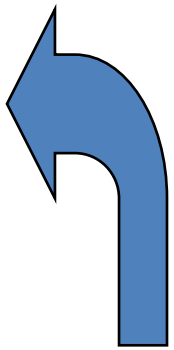
***random sampling using background genes***

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

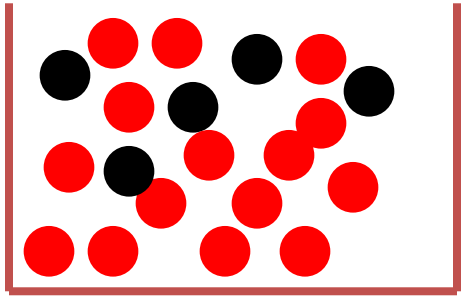
# The Fisher's exact test

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

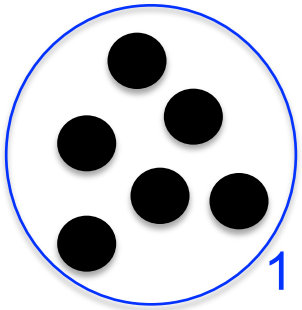
# The Fisher's exact test

## a.k.a., hypergeometric test

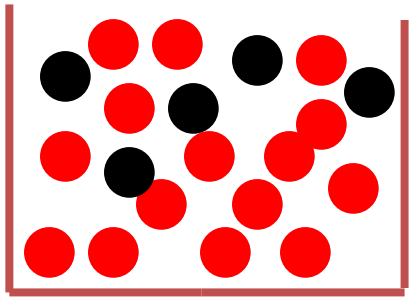
Gene list

- RRP6
- MRD1
- RRP7
- RRP43
- RRP42

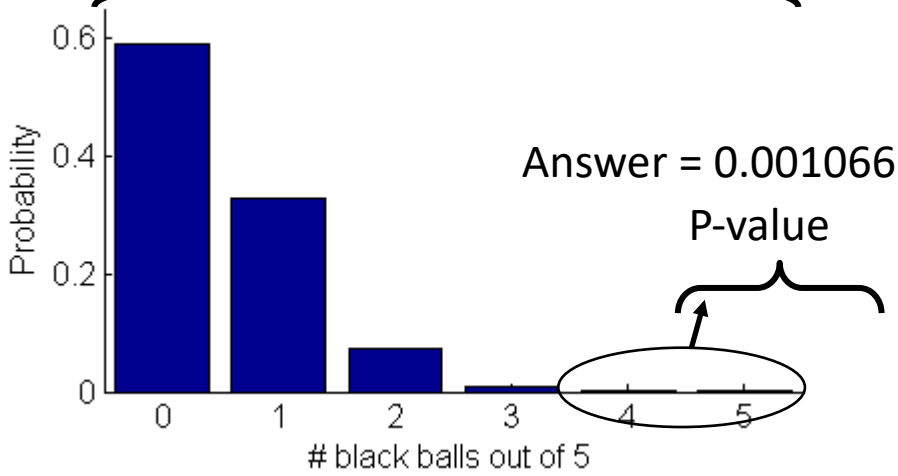
Background population:
500 black genes,
4500 red genes



1 gene-set/pathway

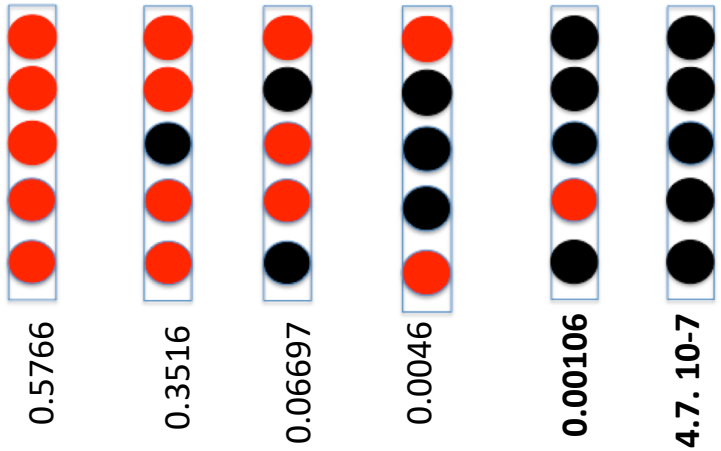


Null distribution




Answer = 0.001066662

P-value



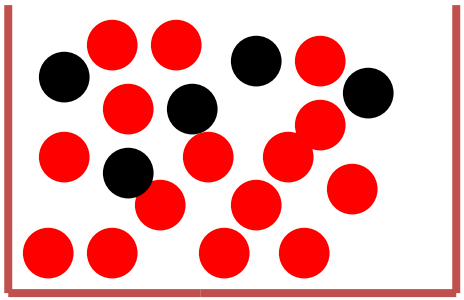
# 2x2 contingency table for Fisher's Exact Test

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 |

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



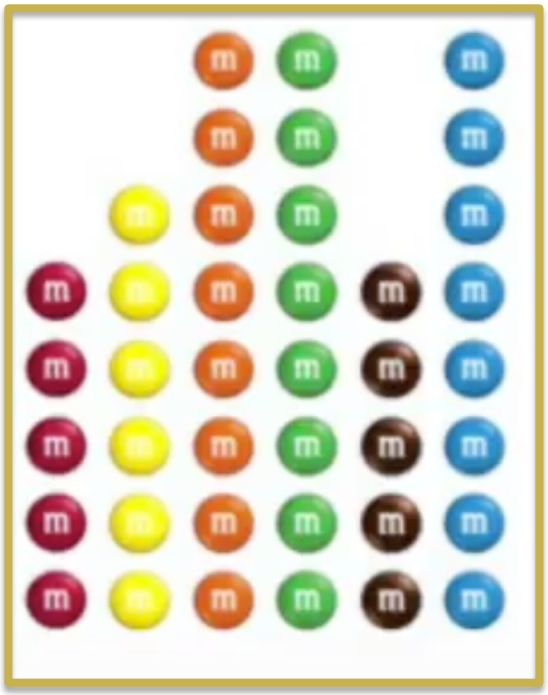
Background population:
500 black genes,
4500 red genes

# 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

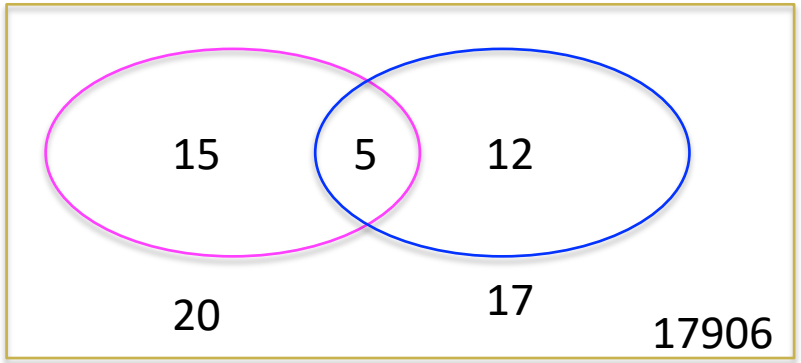

**Background**

**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 | $1.034\times10^{-8}$ | | 17 | 20 | 5 | 17906 |
| pulmonary valve development | GO:0003177 | $3.392\times10^{-8}$ | | 21 | 20 | 5 | 17906 |
| regulation of myeloid leukocyte differentiation | GO:0002761 | $6.876\times10^{-8}$ | | 122 | 20 | 7 | 17906 |
| regulation of osteoclast differentiation | GO:0045670 | $1.353\times10^{-7}$ | | 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**

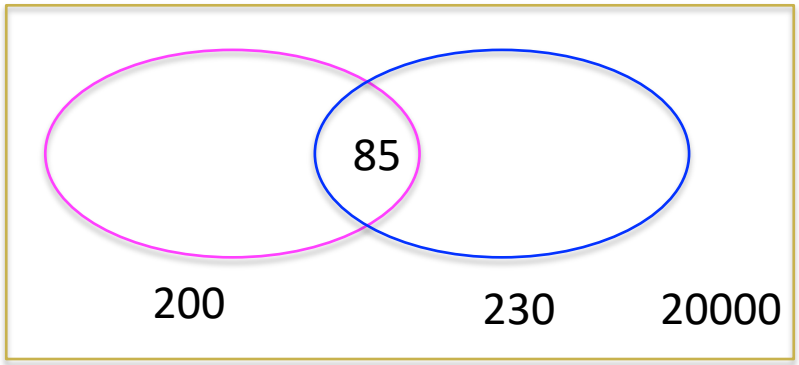
| Term | Overlap | P-value | Adjusted P-value | Old P-value | Old Adjusted P-value | Z-score | Combined Score | Genes |
|---|---|---|---|---|---|---|---|---|
| extracellular matrix organization (GO:0030198) | 85/230 | 2.1E-50 | 6.4E-47 | 4.3E-39 | 1.3E-35 | -1.64651 | 188.3195 | ITGB1;APP;COL16A1;SPARC;COL14A1; |
| 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

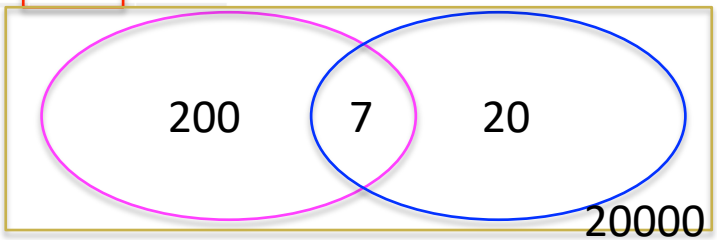
Overlap:# of genes in my gene list and tested pathway

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 |

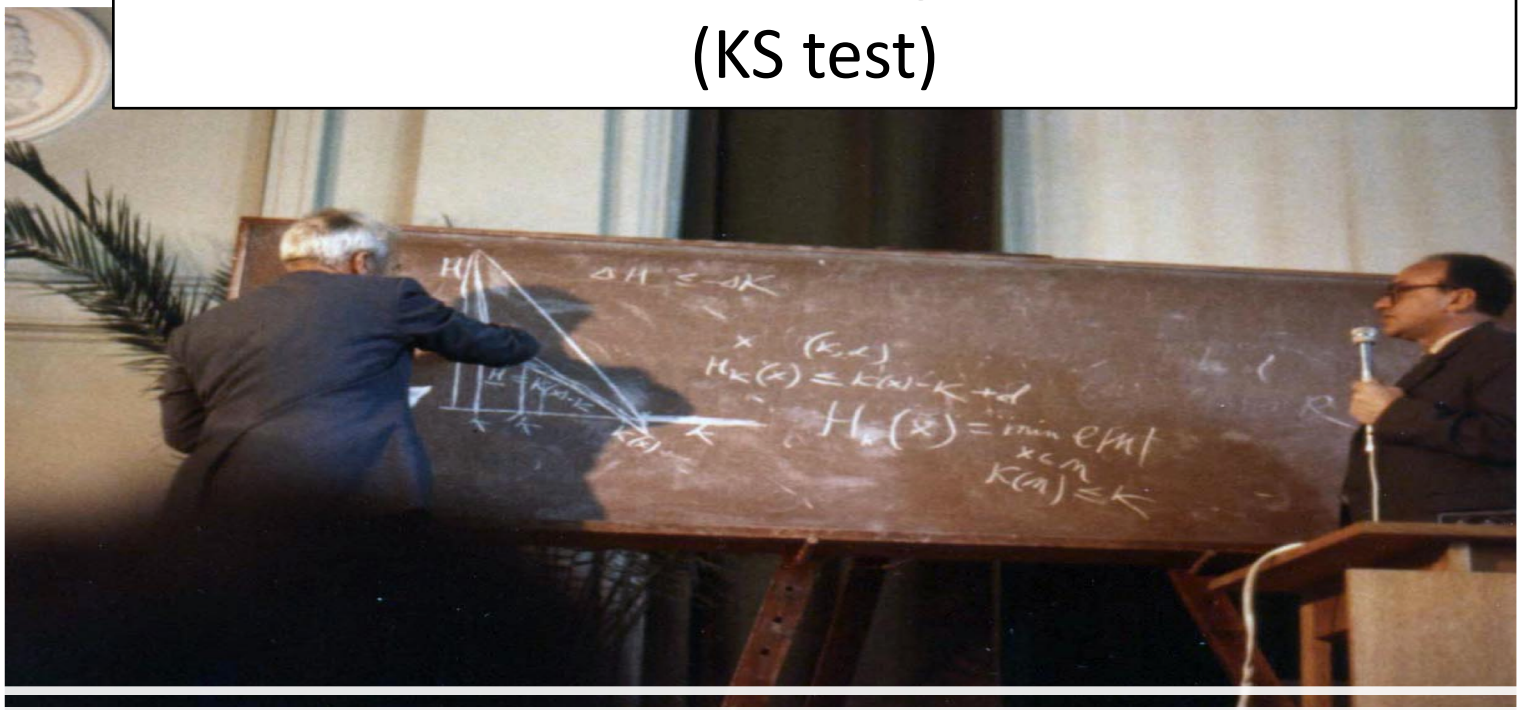


# Notes

- 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

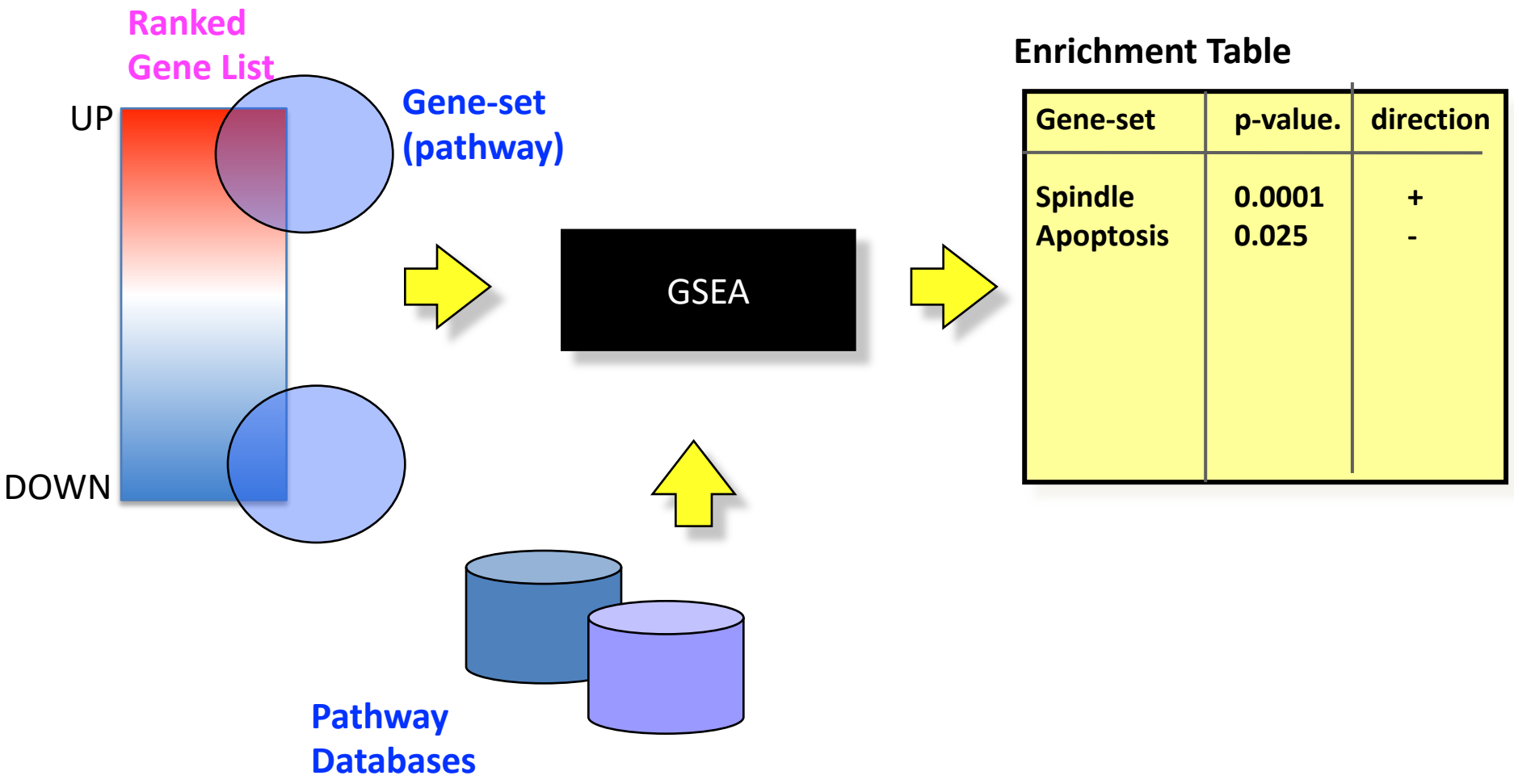
# Ranked gene list enrichment test

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.

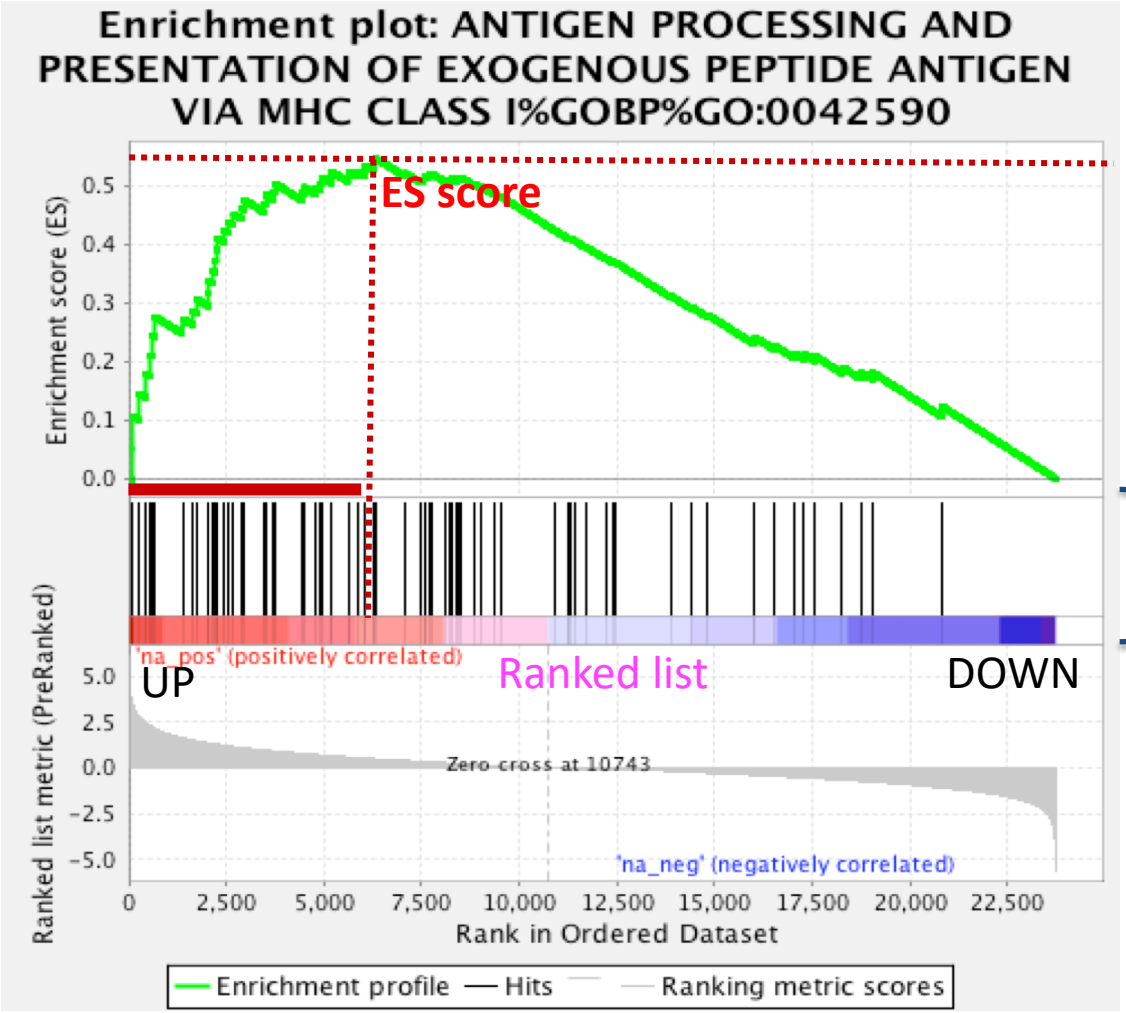
http://www.people.vcu.edu/~mreimers/HTDA/Mootha%20-%20GSEA.pdf

# 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 |

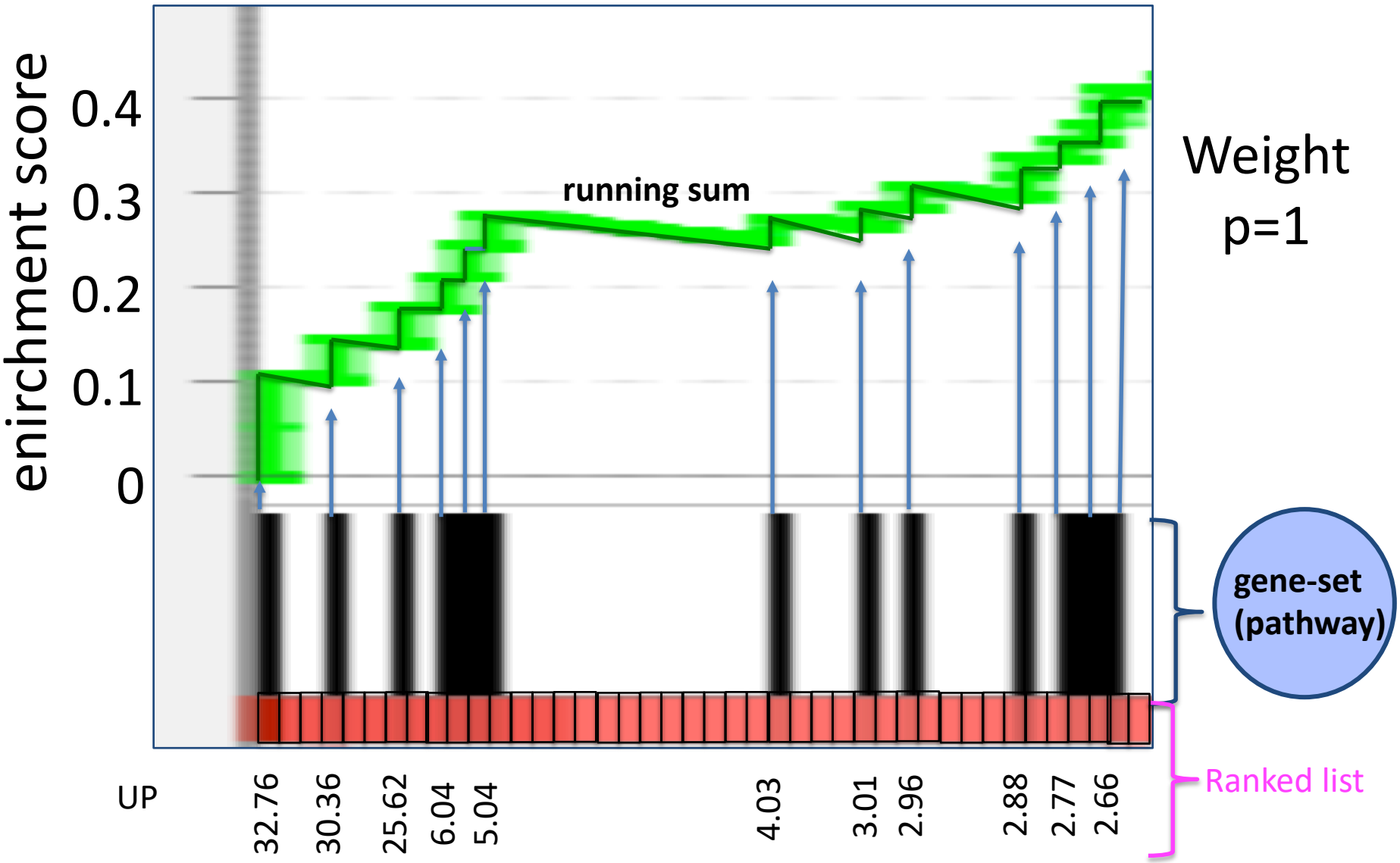
DOWN




gene-set (pathway)

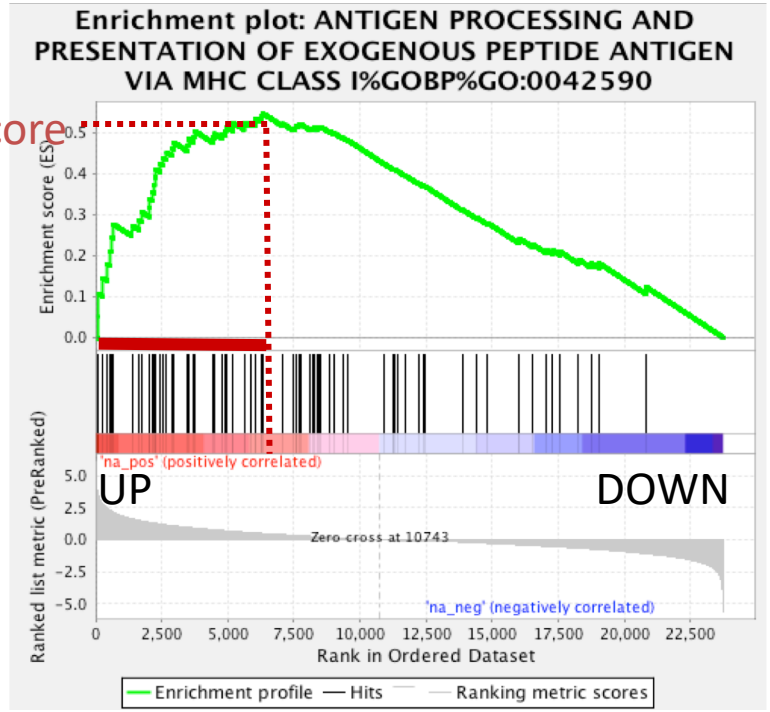
1. Maximum (or minimum) ES score is the final **ES score** for the gene set
2. Can define “leading edge subset” as all those genes ranked as least as high as the enriched set.

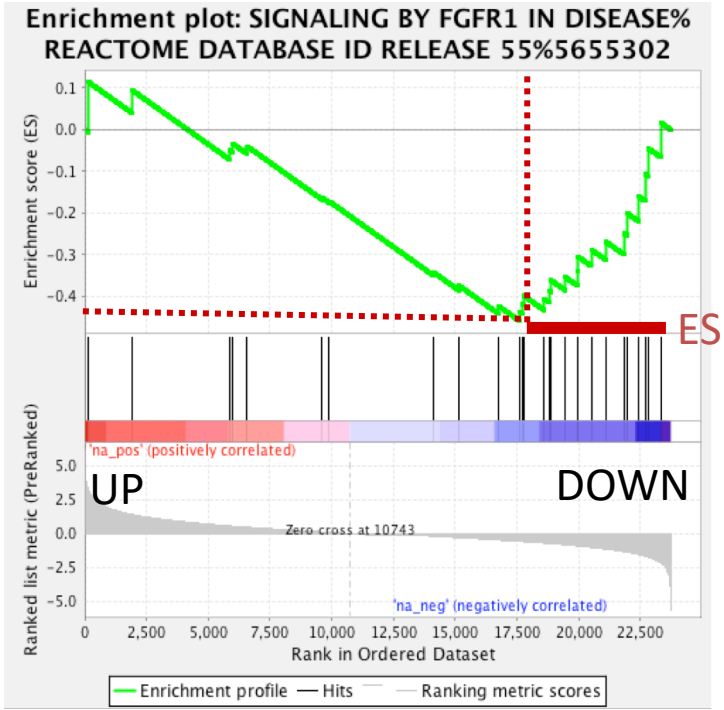
# GSEA running sum



# Positive and negative enrichment scores

ES score

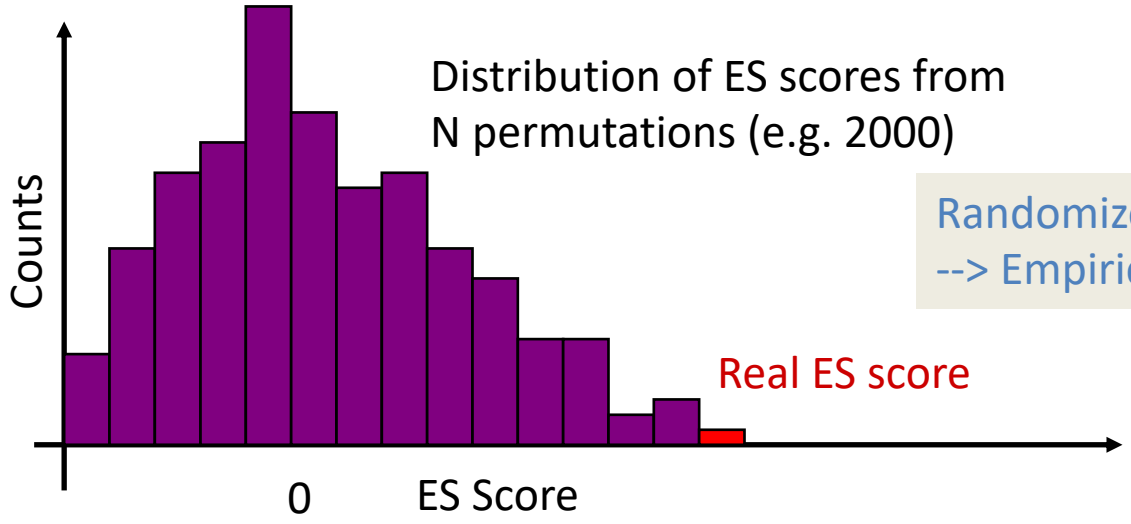



ES score

# Going from ES score → P-value

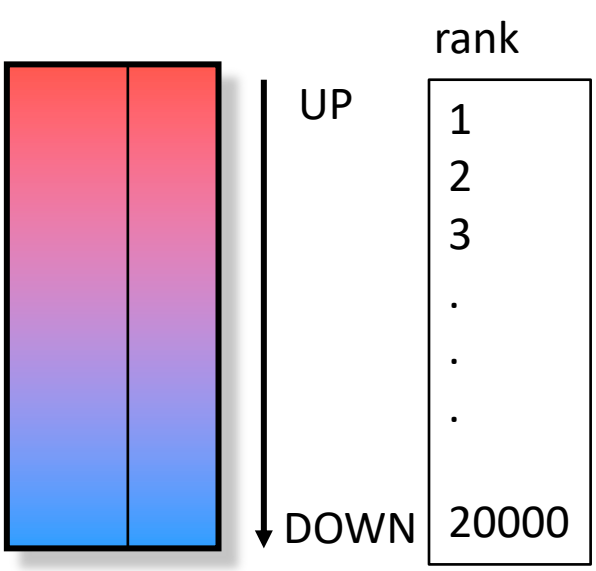
1. Generate null-hypothesis distribution from randomized data (see permutation settings)
2. Estimate empirical p-value by comparing observed ES score to null-hypothesis distribution from randomized data (for every gene-set)

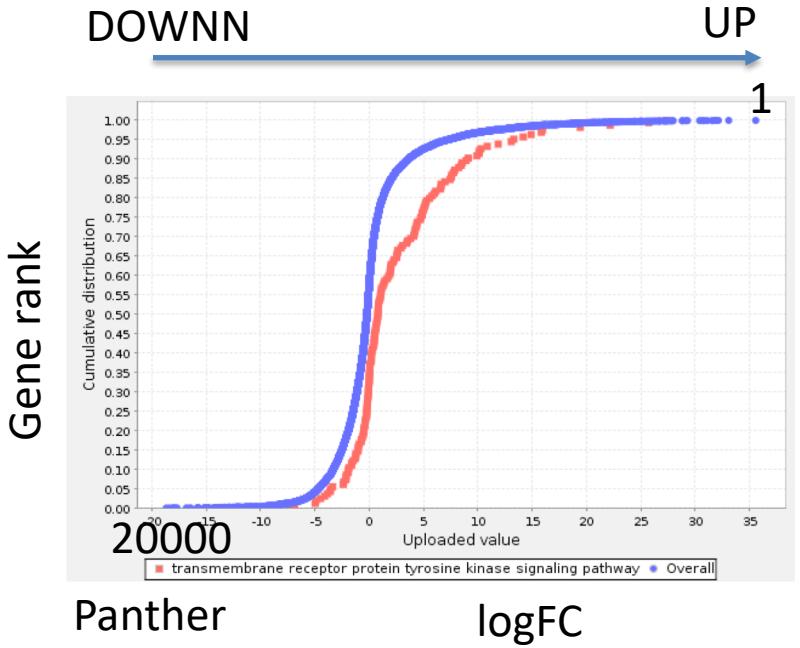



Randomized with ES score ≥ real: 4 / 2,000
--> Empirical p-value = 0.002

# 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

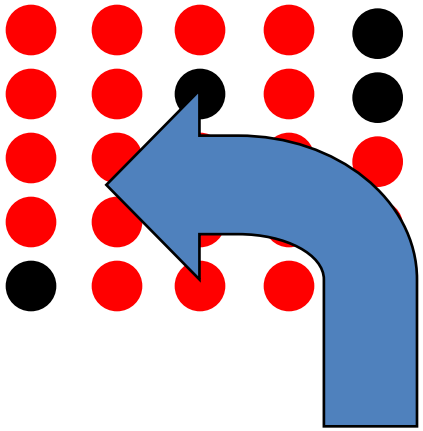
# Multiple test corrections

# We are testing many pathways at the same time

correction for multiple hypothesis testing

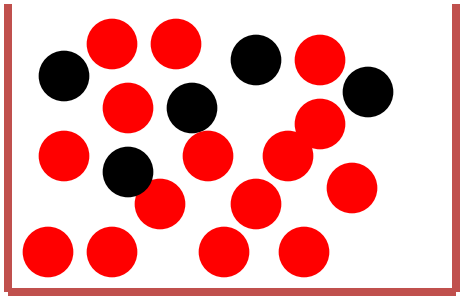
# How to win the p-value lottery

Random draws

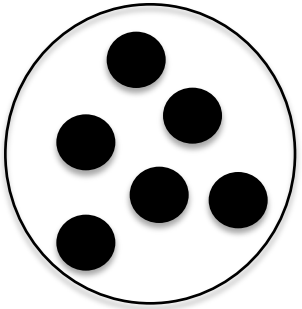


… 7,834 draws later …

*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

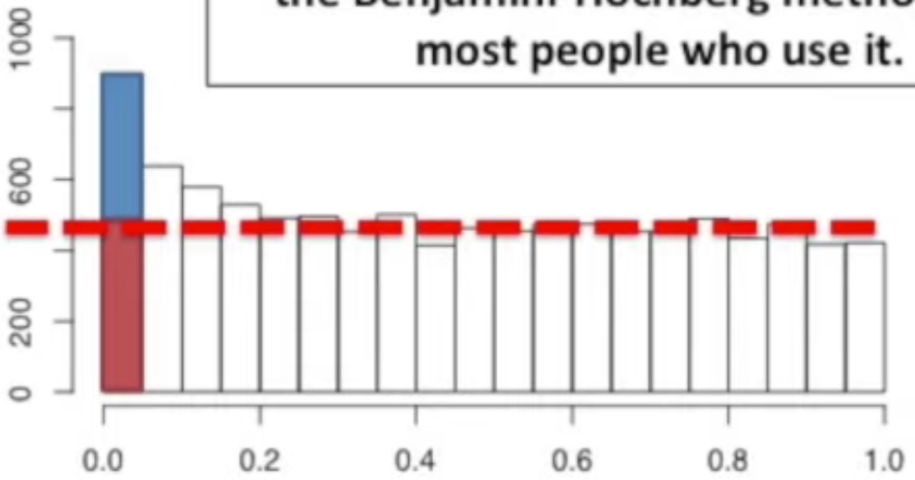
# Reducing multiple test correction stringency

- The correction to the P-value threshold $\alpha$ 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.

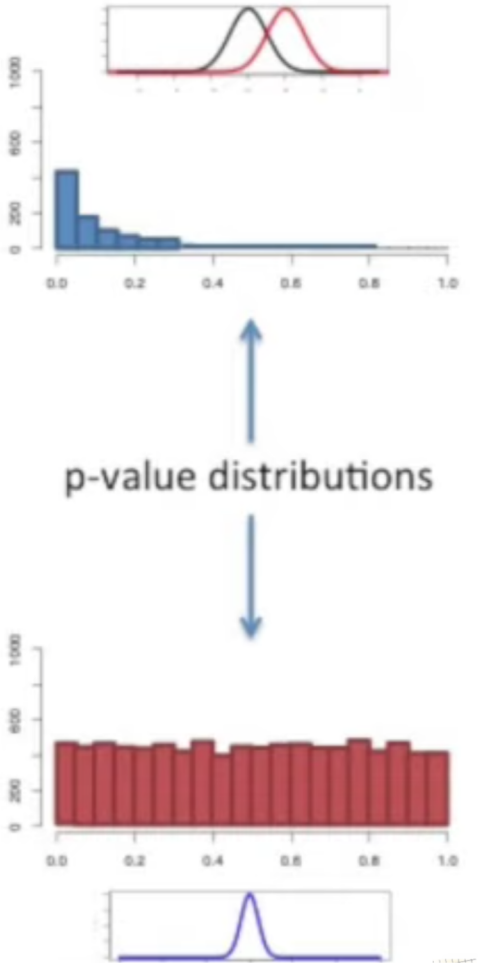
# How to win the p-value lottery, part 2

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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