Pathway analysis

Urmo Võsa

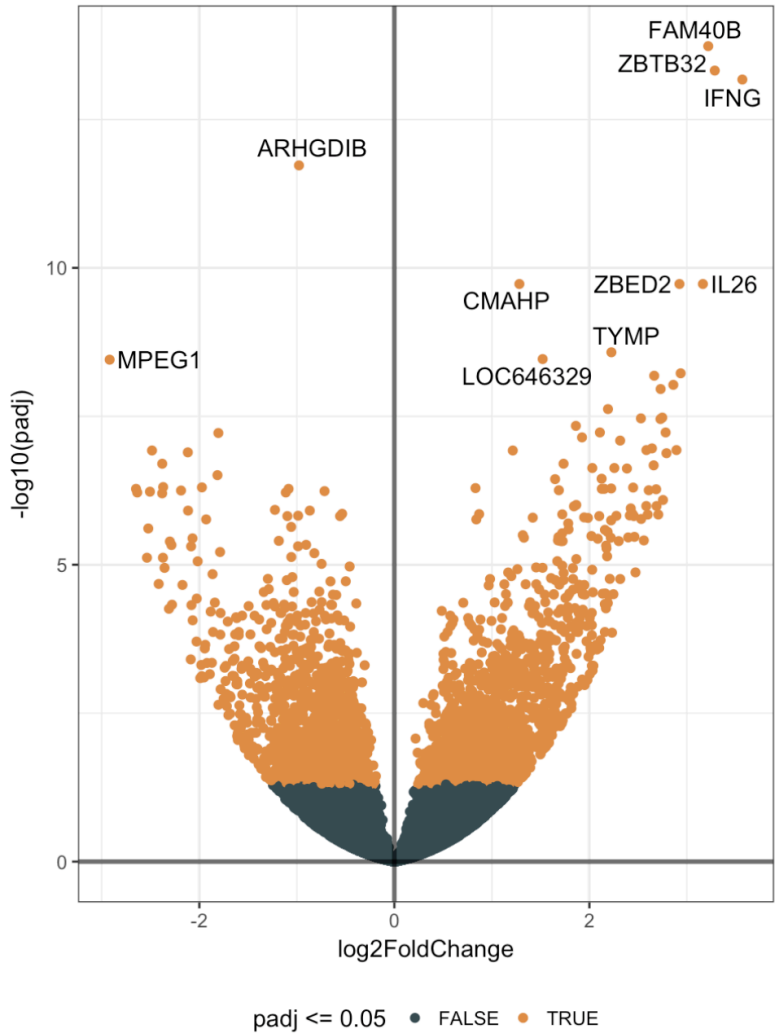
iBMS course 2017

FrankeSwertzLab

Department of Genetics

UMCG

What next?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IFNG
ARHGDIB
CMAHP
ZBED2
IL26
TYMP
MPEG1
LOC646329
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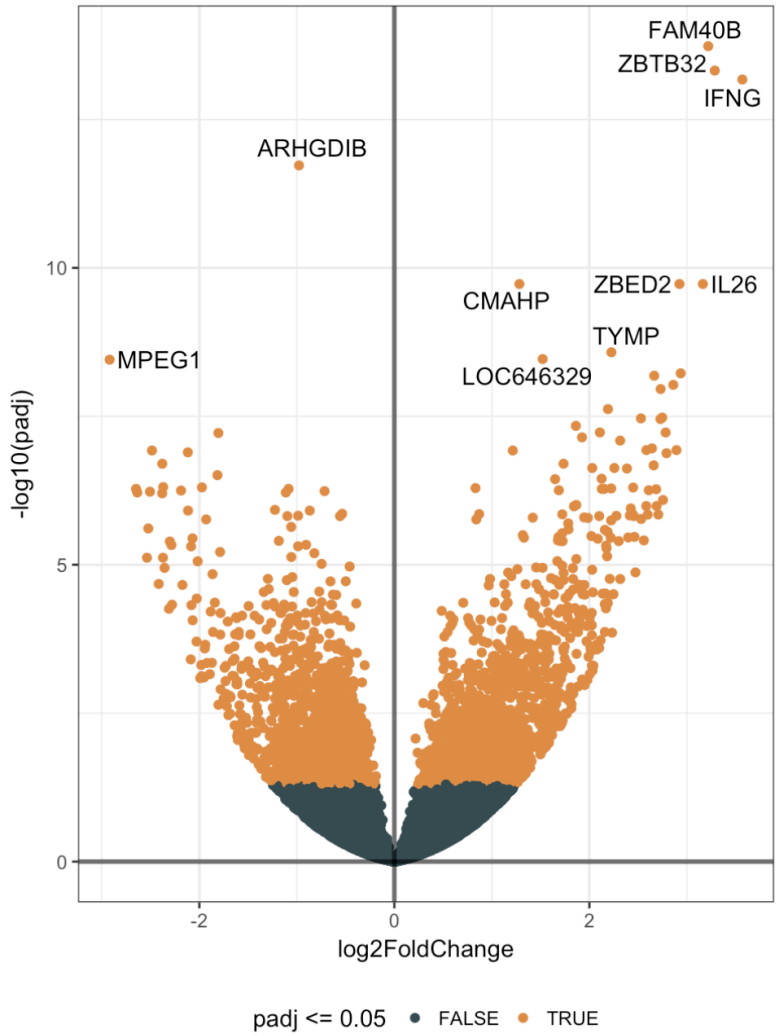
5
0
-2
0
2
-log10(padj)
log2FoldChange
padj <= 0.05
FALSE
TRUE

What next?



Basic science:

- New knowledge about biological mechanisms of …

Practical aspects:

- Diagnostic/prognostic biomarkers for the disease
- Potential drug targets

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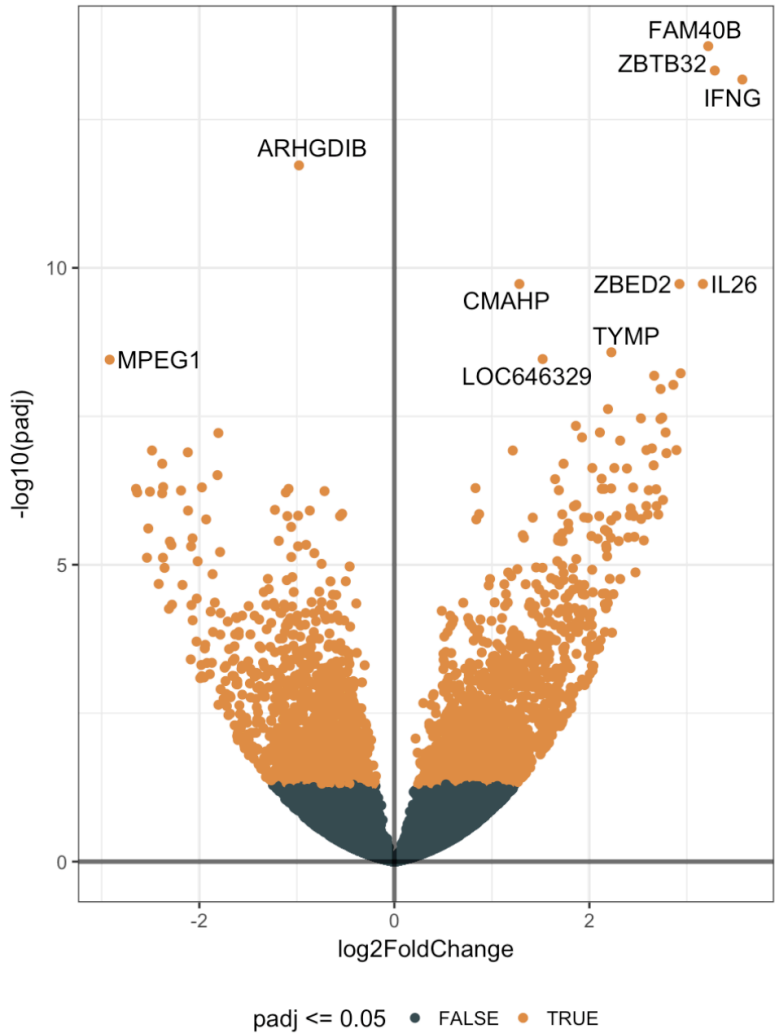


Basic science:

- New knowledge about biological mechanisms of …

Practical aspects:

- Diagnostic/prognostic biomarkers for the disease
- Potential drug targets

- Find common themes from the results
- Pinpoint potentially relevant genes
- Put results into biological context

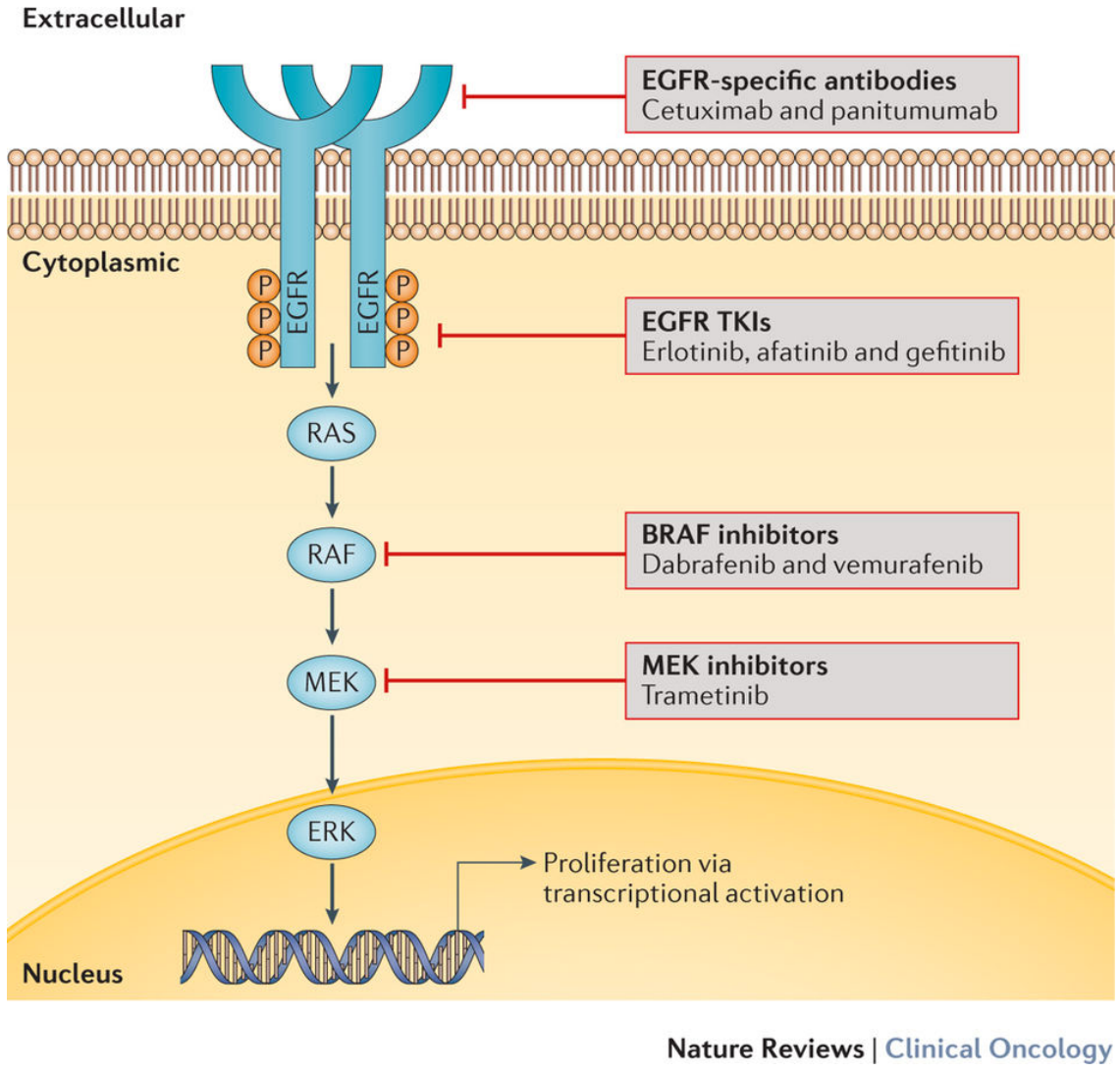
Pathway analysis

What is molecular pathway?

*A **molecular pathway** is a series of interactions that occurs between the molecules and proteins within a cell and between cells.*

http://www.geneticalliance.org/

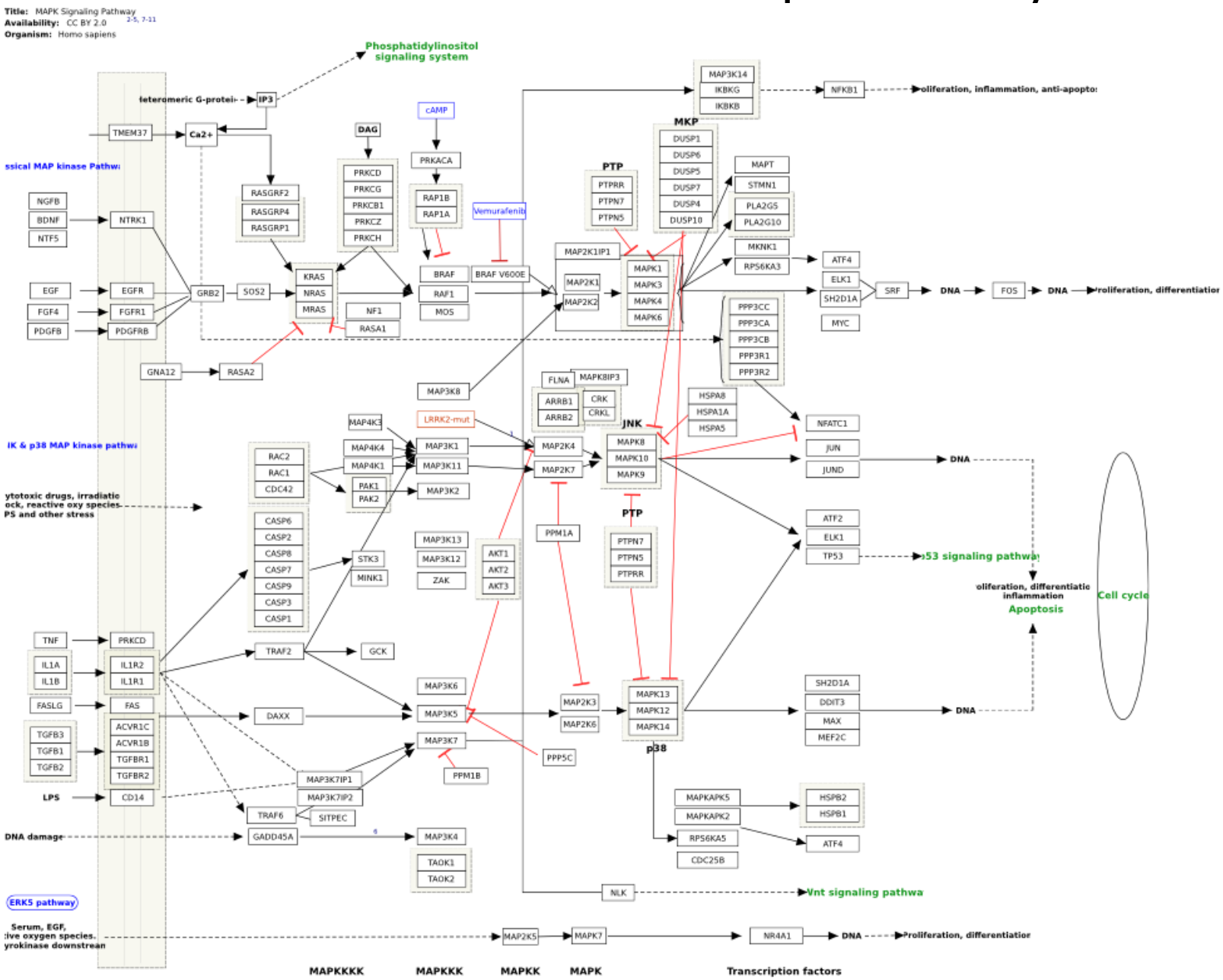
What is molecular pathway?



Simplified MAPK pathway

Schmitt et al., 2016

What is molecular pathway?



http://www.wikipathways.org/

Gene set

Any set of genes which shares some common theme:

- **on the shared pathway**
- share biological process/theme
- share cellular localization
- share common disease annotation
- share common regulating transcription factor/microRNA
- share common genomic location (chromosome band)

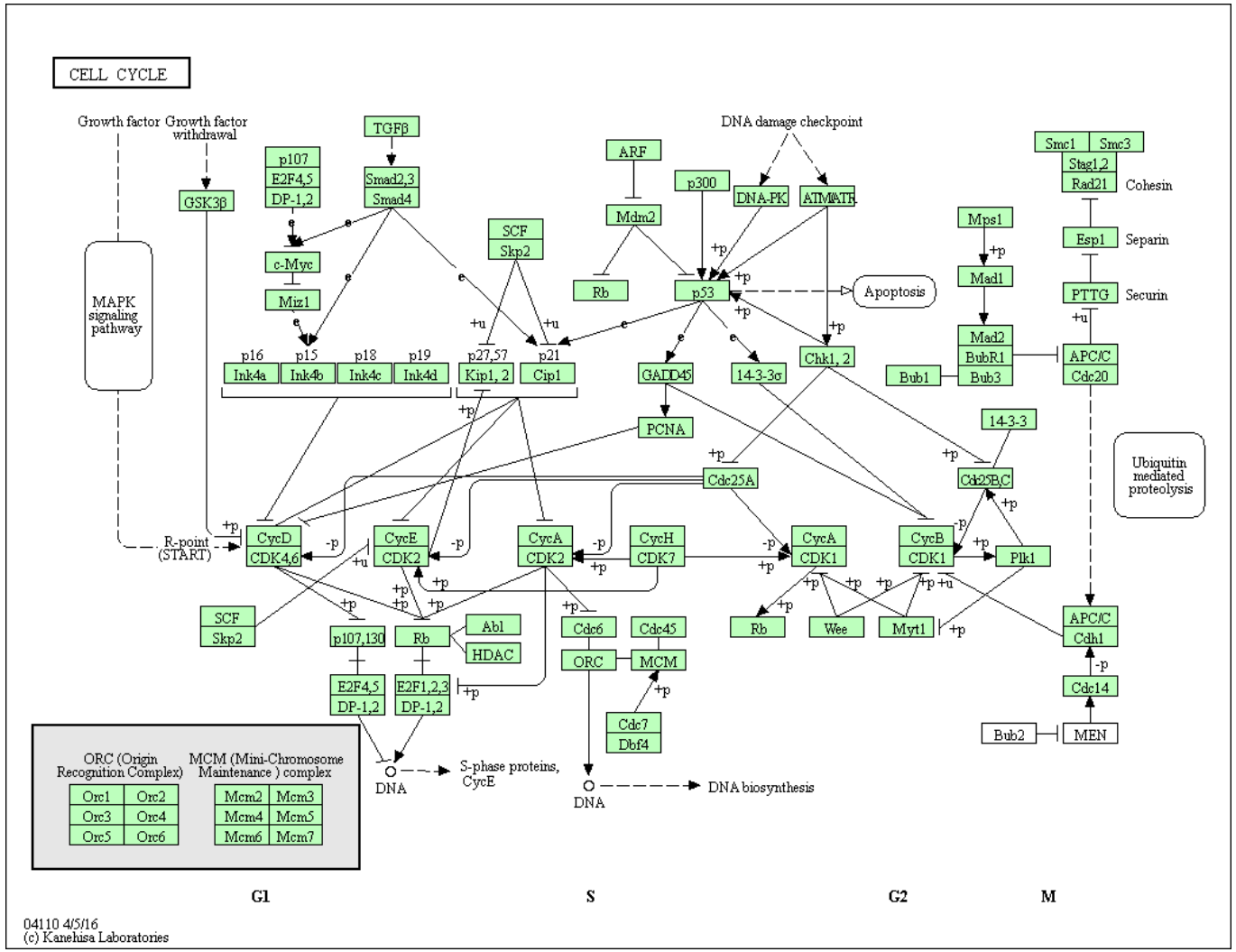
- “genes associated with NIH grants”
- …

Gene set is not pathway!

~~Pathway analysis~~
Gene enrichment analysis

Some most widely used databases

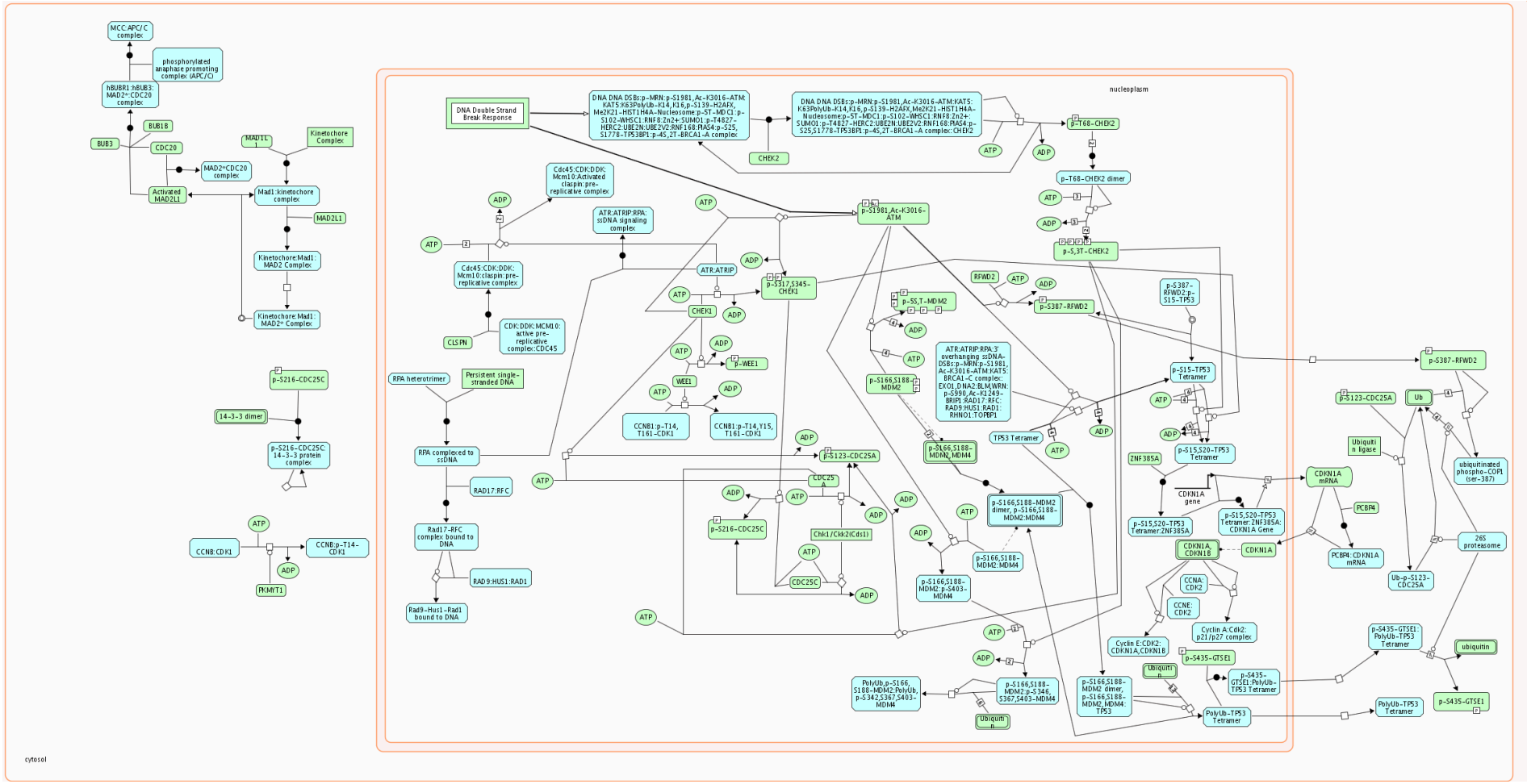
- Kyoto Encyclopedia of Genes and Genomes (**KEGG**)



www.genome.jp/kegg/

Some most widely used databases

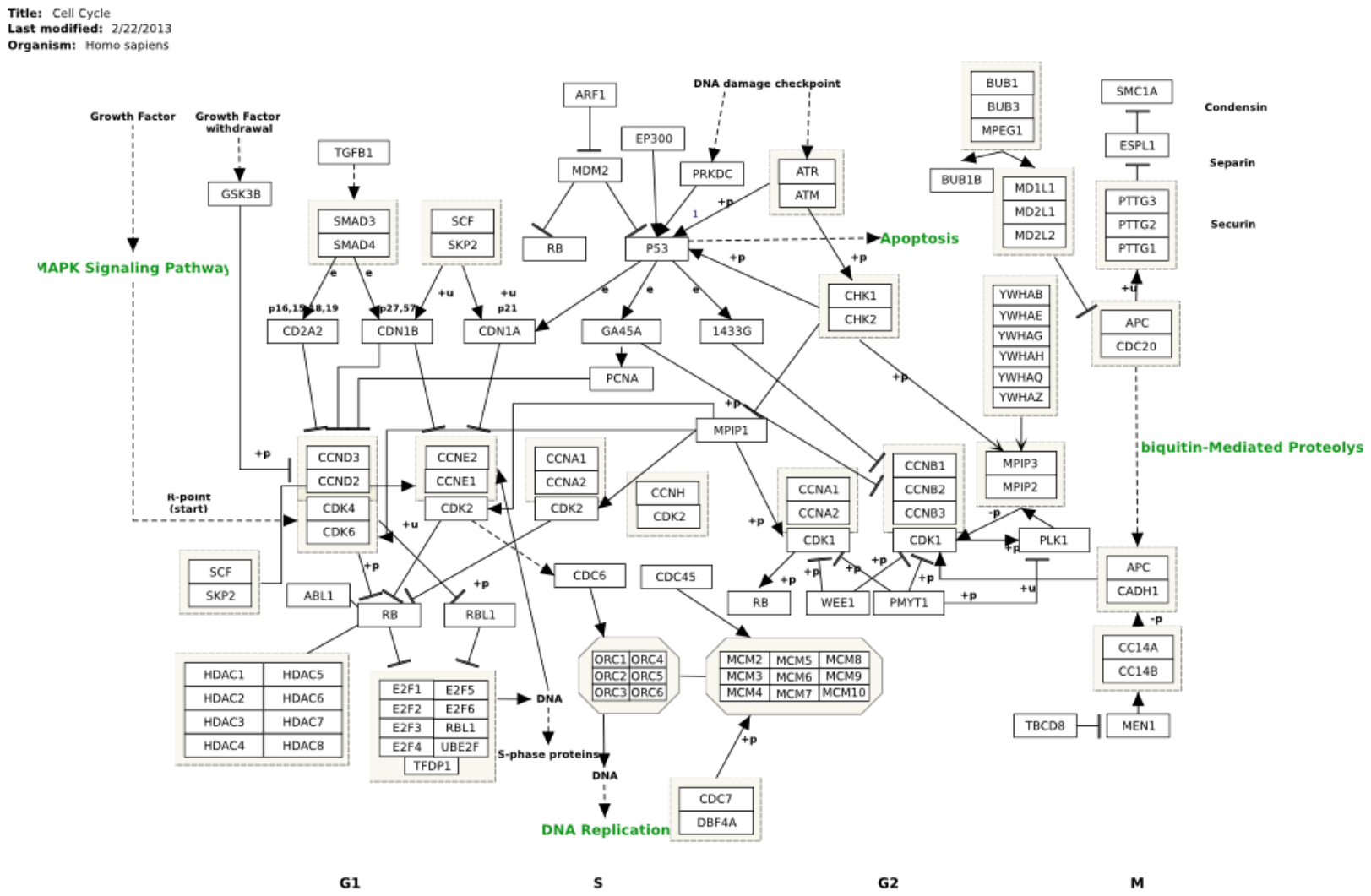
- REACTOME



www.reactome.org

Some most widely used databases

- WikiPathways



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Some most widely used databases

- Gene Ontology (GO):
  - Biological processes
  - Molecular function
  - Cellular composition

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http://cbl-gorilla.cs.technion.ac.il/

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Not exactly the pathway but gene set!

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Gene set:
e.g. all genes part of BP GO:0000075
Cell cycle checkpoint

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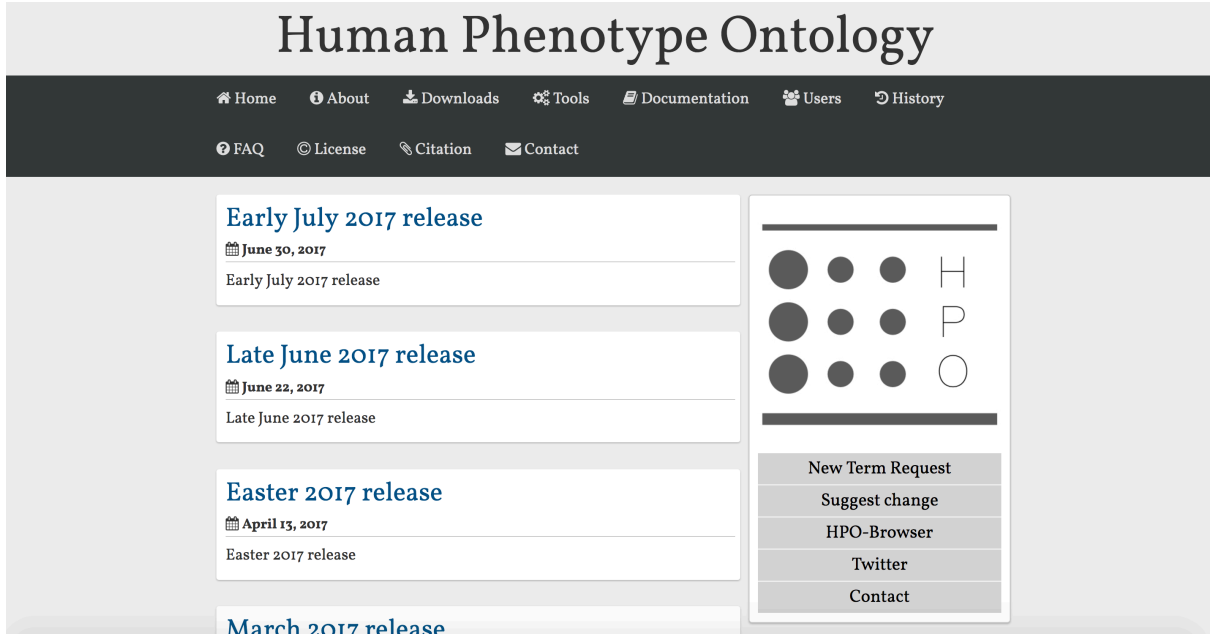
Some most widely used databases

- Human Phenotype Ontology (HPO):
  - 11,000 terms
  - 115,000 hereditary diseases
  - 4,000 common diseases



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Köhler et al., 2017
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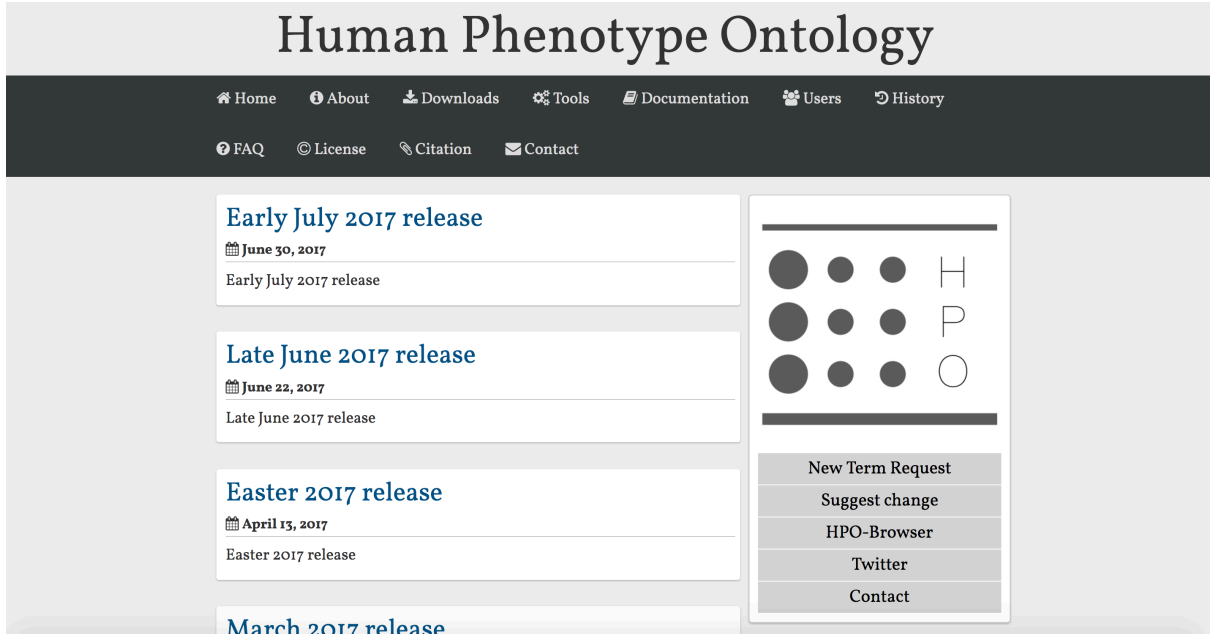

Not exactly the pathway but gene set!

Gene set:
e.g. all genes part of HP:0030359
Squamous cell lung carcinoma

Köhler et al., 2017
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Gene enrichment analysis

Question: are genes of interest significantly enriched by some gene set (pathway/process/…)?

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In our case: are the genes differentially expressed between Coeliac disease patients and control individuals over-represented by some specific biological themes?

Classification of pathway analysis methods

Overrepresentation analysis (ORA)

- Hypergeometric test
- Fisher's exact test
- Chi-squared test
- Bayesian statistics
- …

Functional class scoring (FCS)

- GSEA
- CAMERA
- …

Topology-based methods (PT)

- SPIA
- …

Semantic similarity analyses

- GoSemSim
- …

Miscallaneous

- GeneNetwork
- …

Khatri et al., 2012 (Plos Comp. Biol.)

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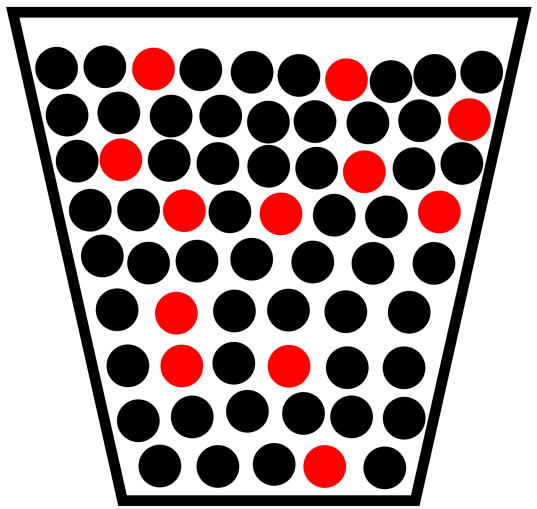
Urn analogy:



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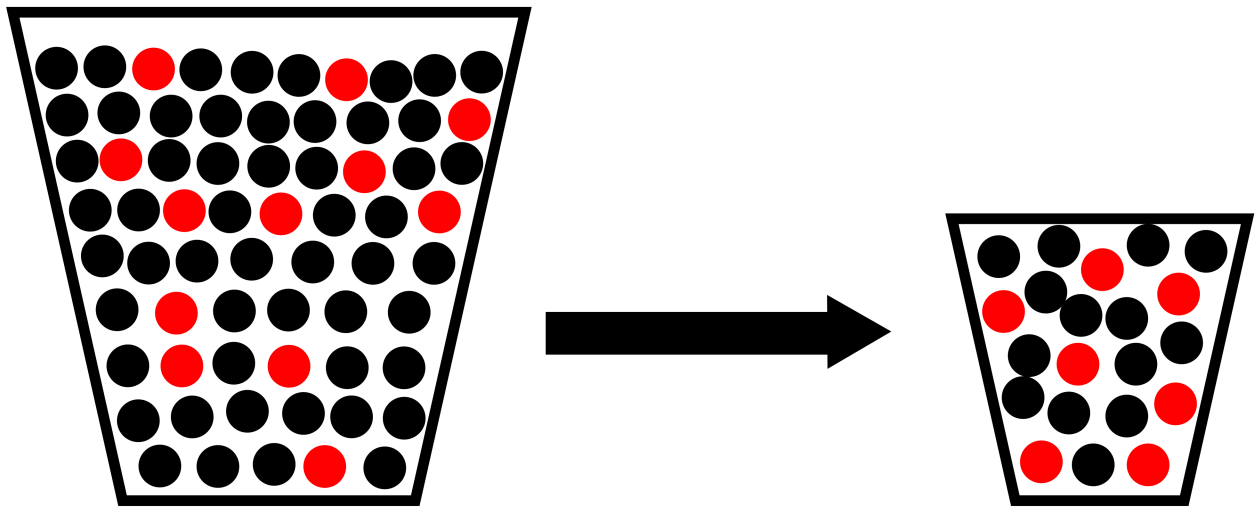

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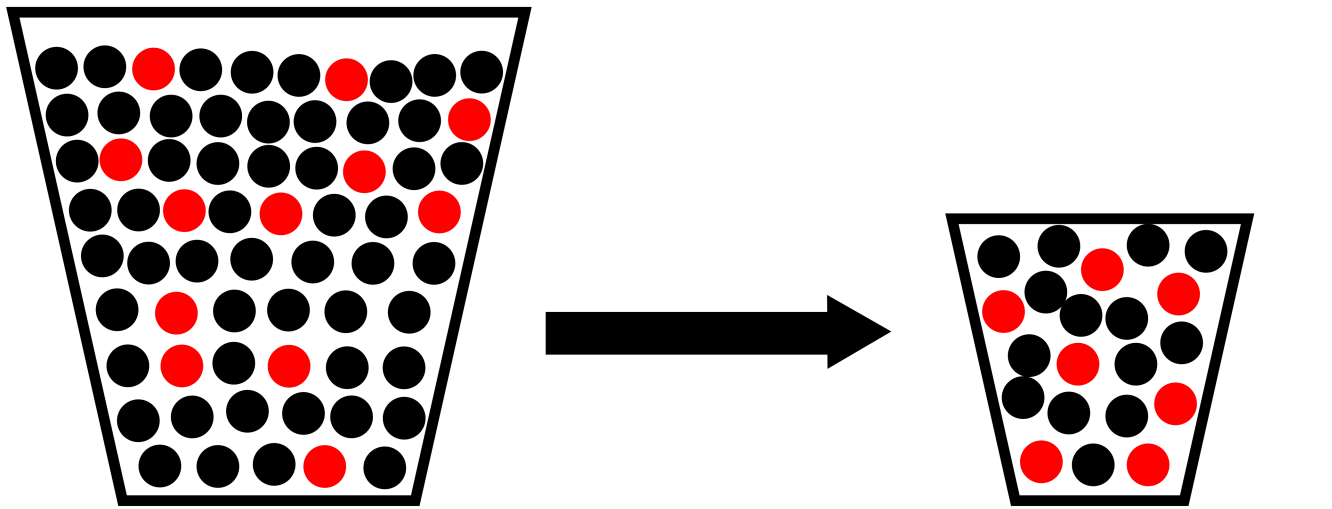

12/66 (18.2%)

7/21 (33.3%)

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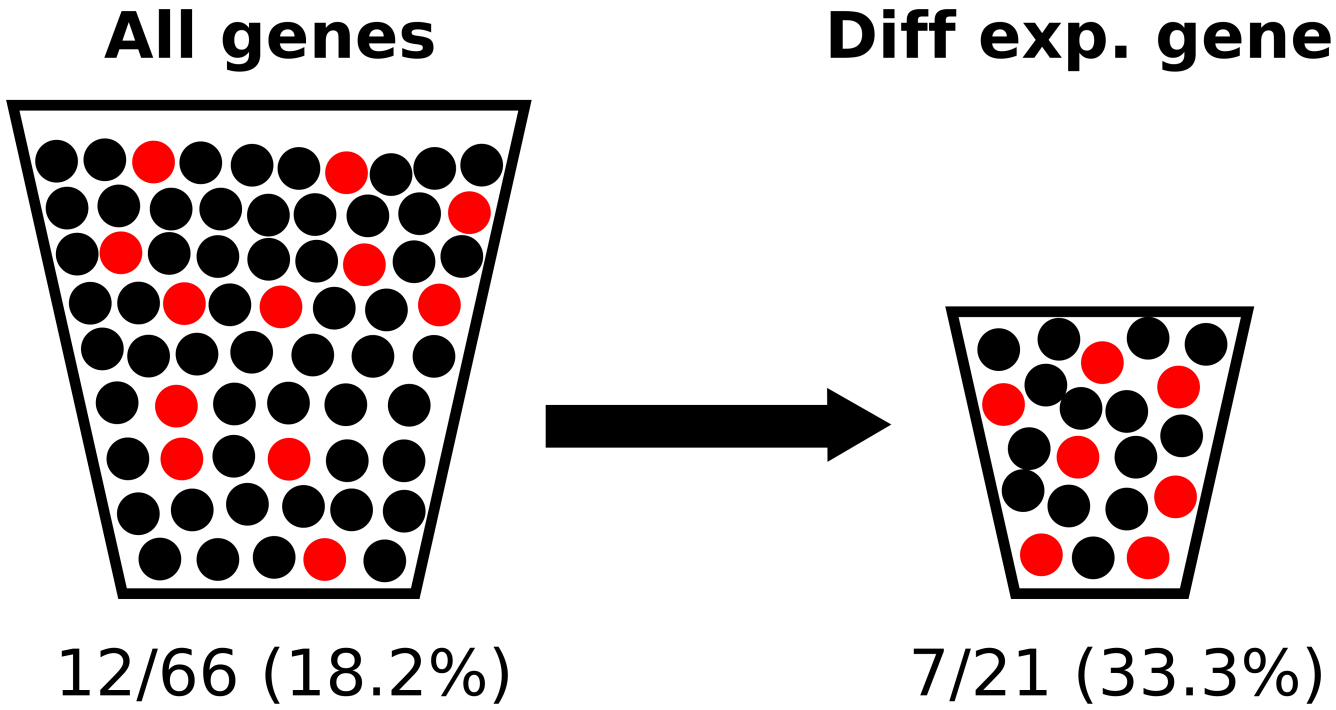
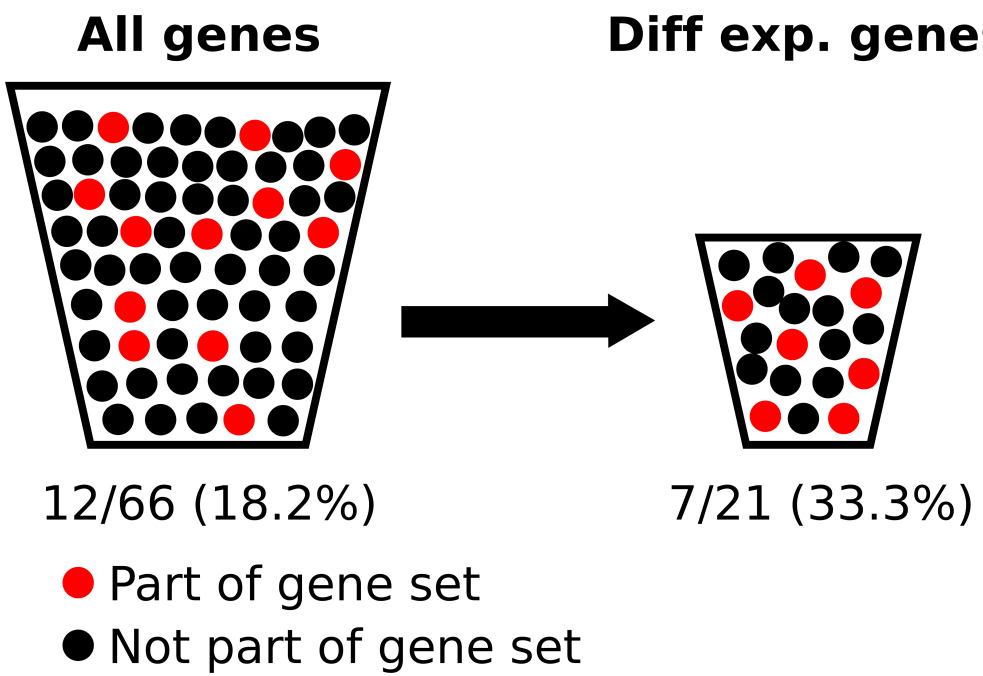


Most widely used method

Hypergeometric test (one-sided Fisher's Exact Test)



	Part of gene set	Not part of gene set
Diff. expressed	**7**	21-7=**14**
Not. diff expressed	12-7=**5**	66-21-(12-7)=**40**

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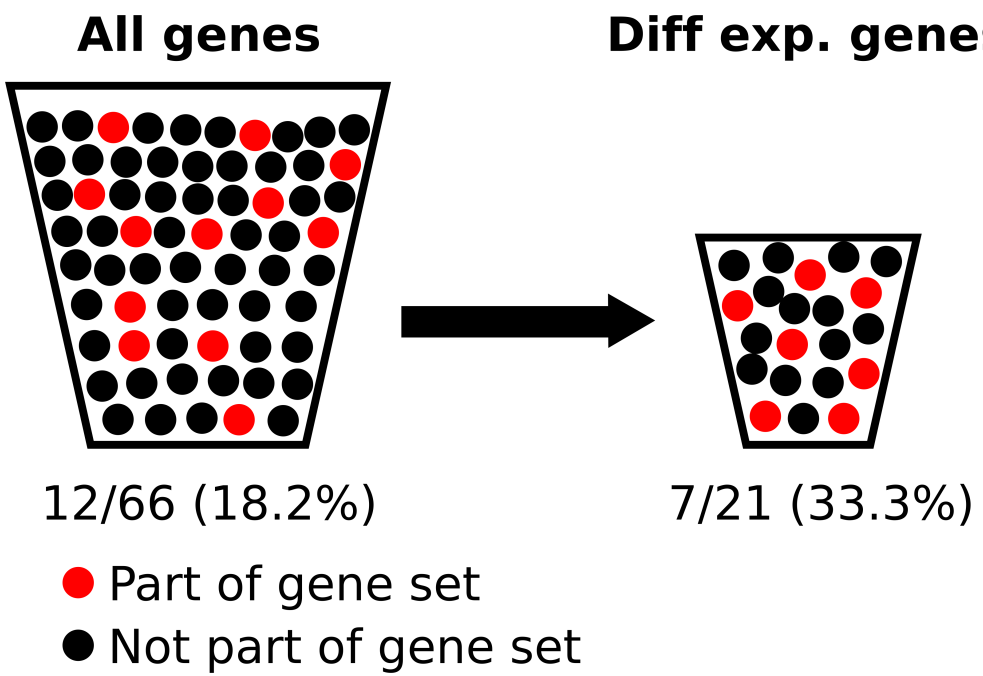

	Part of gene set	Not part of gene set	∑
Diff. expressed	**7**	21-7=**14**	21
Not. diff expressed	12-7=**5**	66-21-(12-7)=**40**	45
∑	12	54	66

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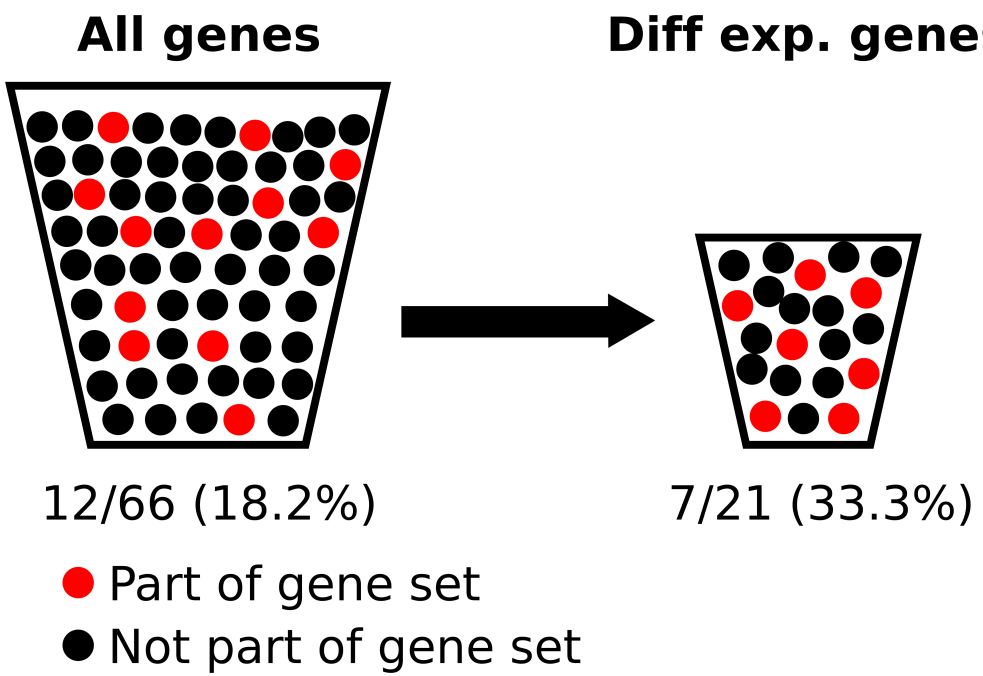

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∑	12	54	66

One-sided FET: **P=0.036**

Odds ratio: **OR=3.9**

Multiple testing!

Usually many gene sets tests are done in parallel.

Multiple testing correction is needed to limit false positive results!

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- Bonferroni
- FDR

Gene Universe

"*Gene Universe*" AKA "*Background*"

The set of genes which could be identified by your analysis.

- Often used: all annotated genes for investigated organism.
- For diff. exp. analysis: all genes which were tested by your method.
- It is not trivial to choose suitable background for more complex designs.

Incorrect selection of background may bias your analysis and lead to wrong conclusions!

Limitations of ORA

- Defining significance of the diff. expressed genes is often arbitrary.
- All genes are treated with equal weight in the analysis.
- Assumes the independence of the genes.

GSEA

- Uses the full information from genes in the analysis
- Small, non-significant but consistent expression changes are picked up by this analysis.
- Ranks genes based on their effect size (e.g. strength of expression change)
- Analyses whether genes in the top/bottom of the ranked list contain more pathway members as you would expect by chance.

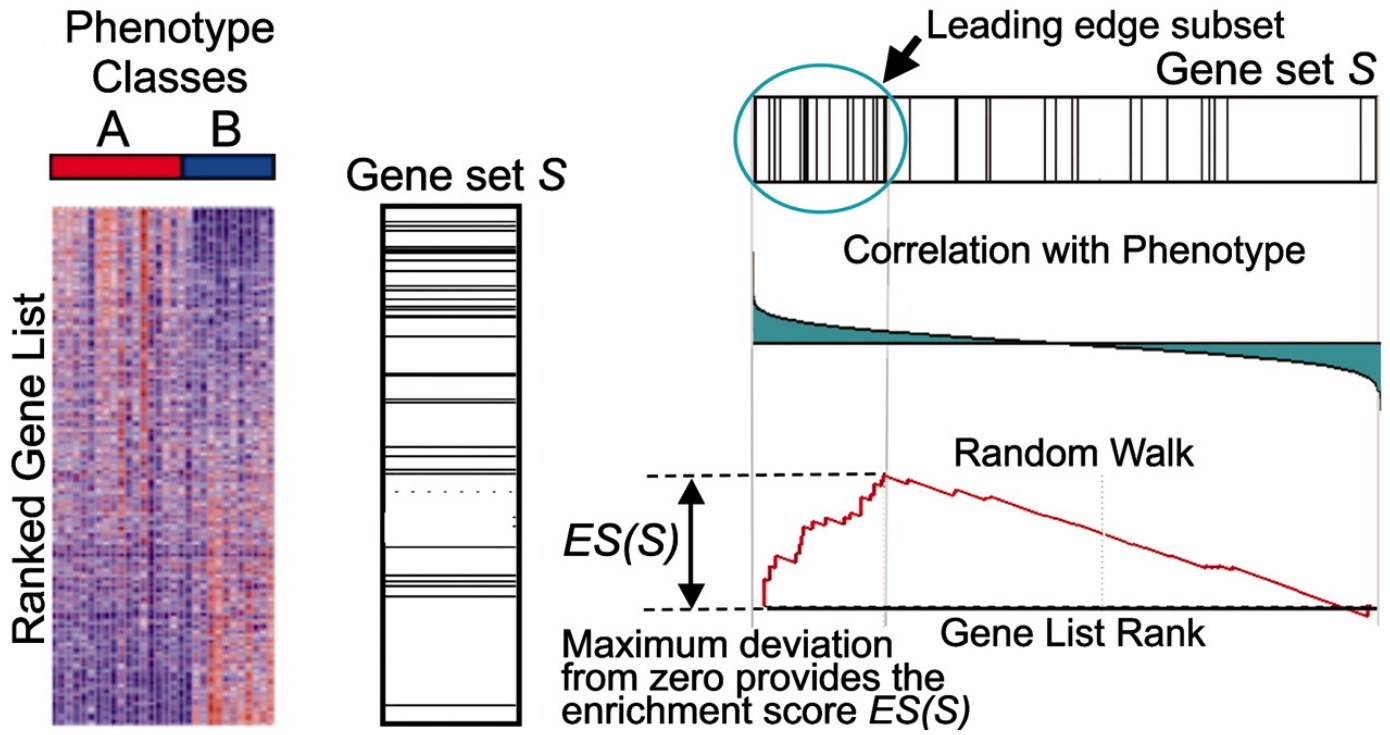
NB! The term is often used interchangeably to designate ORA (*confusing!*)

Subramanian et al., 2005

GSEA algorithm

For each Gene Set:

- Walk down from the ranked list of diff. exp. genes
  - Each time the gene is part of Gene Set: add value to the running sum. The increment is weighted by the correlation with phenotype (or fold change of diff expression).
  - Each time the gene is not part of Gene Set: subtract the weighted value from the running sum.
  - Enrichment score (**ES**) is the max. deviation of running sum from 0.



Subramanian et al., 2005

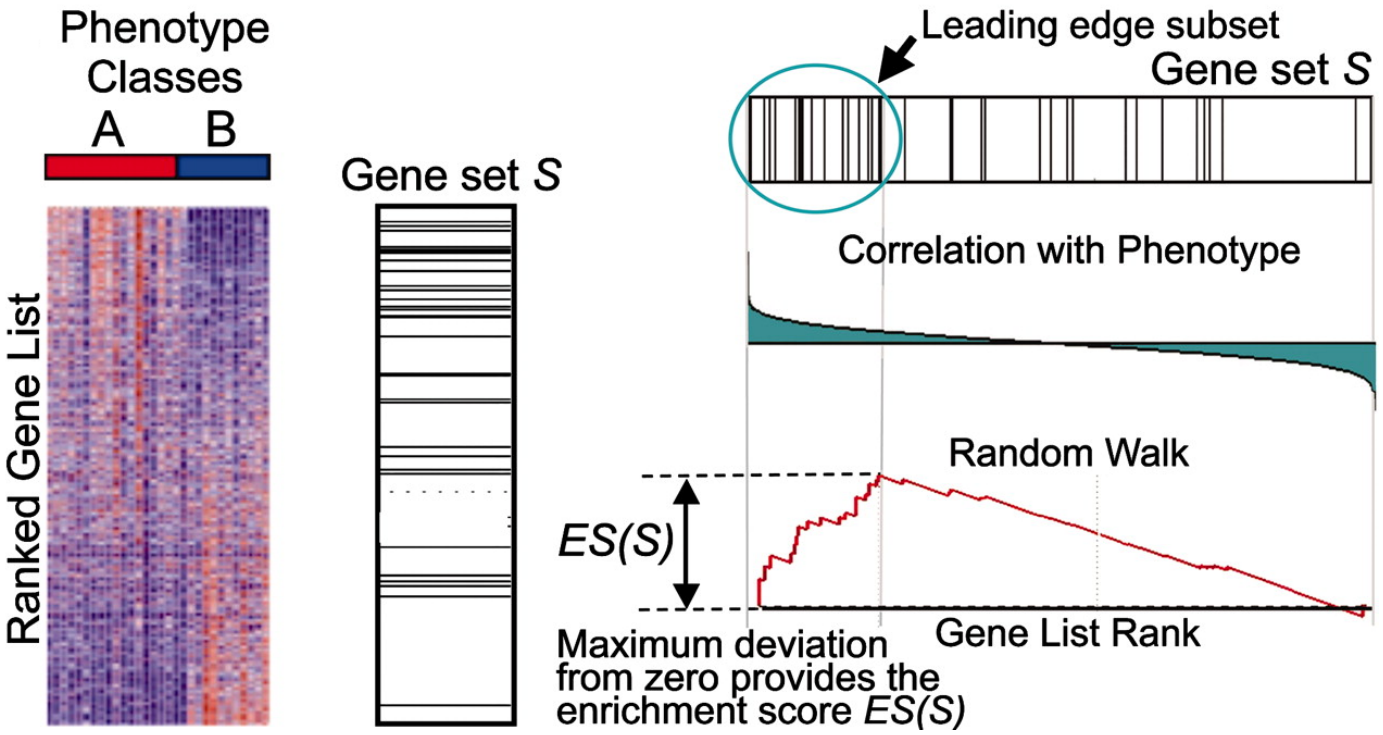
GSEA algorithm

For each Gene Set:

- Calculate P-value for ES
  - Permute phenotypes or gene labels to get null distribution of ES
- Correct for multiple testing

- Normalized enrichment score (**NES**) is more proper comparison metric between gene sets. It is defined as follows:

$$NES = \frac{actual\ ES}{mean\ ES\ over\ permutations}$$



Subramanian et al., 2005

Some recommendations

- Gene sets which are too small are usually not very trustworthy in ORA and GSEA.
- Likewise, gene sets which are very big are difficult to interpret.

Web- or command-line tool?

Web-based tools

+ Easy to use
+ Often gives comprehensive report

- Not replicable
- Often out of date
- Often “black box”
- Often not flexible
- Prone to mistakes

Command-line tools

+ Replicable
+ Very flexible
+ Parallelizable
+ Integratable to the pipelines
+ Smaller possibility to make mistakes

- Steep learning curve