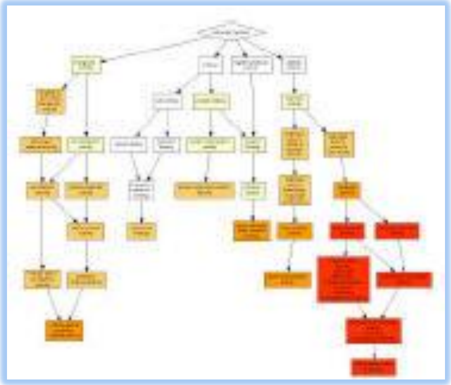
מכון ויצמן למדע
WEIZMANN INSTITUTE OF SCIENCE

LIFE SCIENCE
CORE FACILITIES

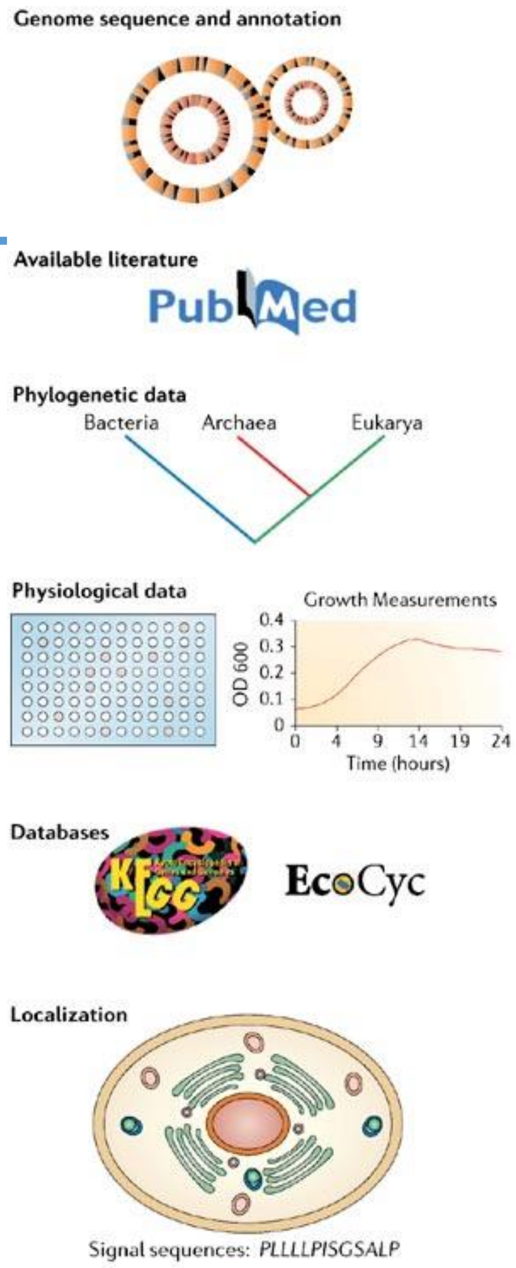
Functional analysis of gene lists using Gene Ontology (GO)



Noa Wigoda
17.12.19
An Introduction to deep-sequencing analysis for biologists

OUTLINE

- Single gene analysis / information
- Analysis of group of genes
- Gene ontology (GO)
- Enrichment analysis
  - Hypergeometric Test and Fisher exact test
  - GO Independence Assumption



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20 Questions

Ask question which can be answered with a simple "Yes" or "No."

20 Questions

Is it part of a complex?

Is it a protein coding gene?

Is it a regulator – transcription factor?

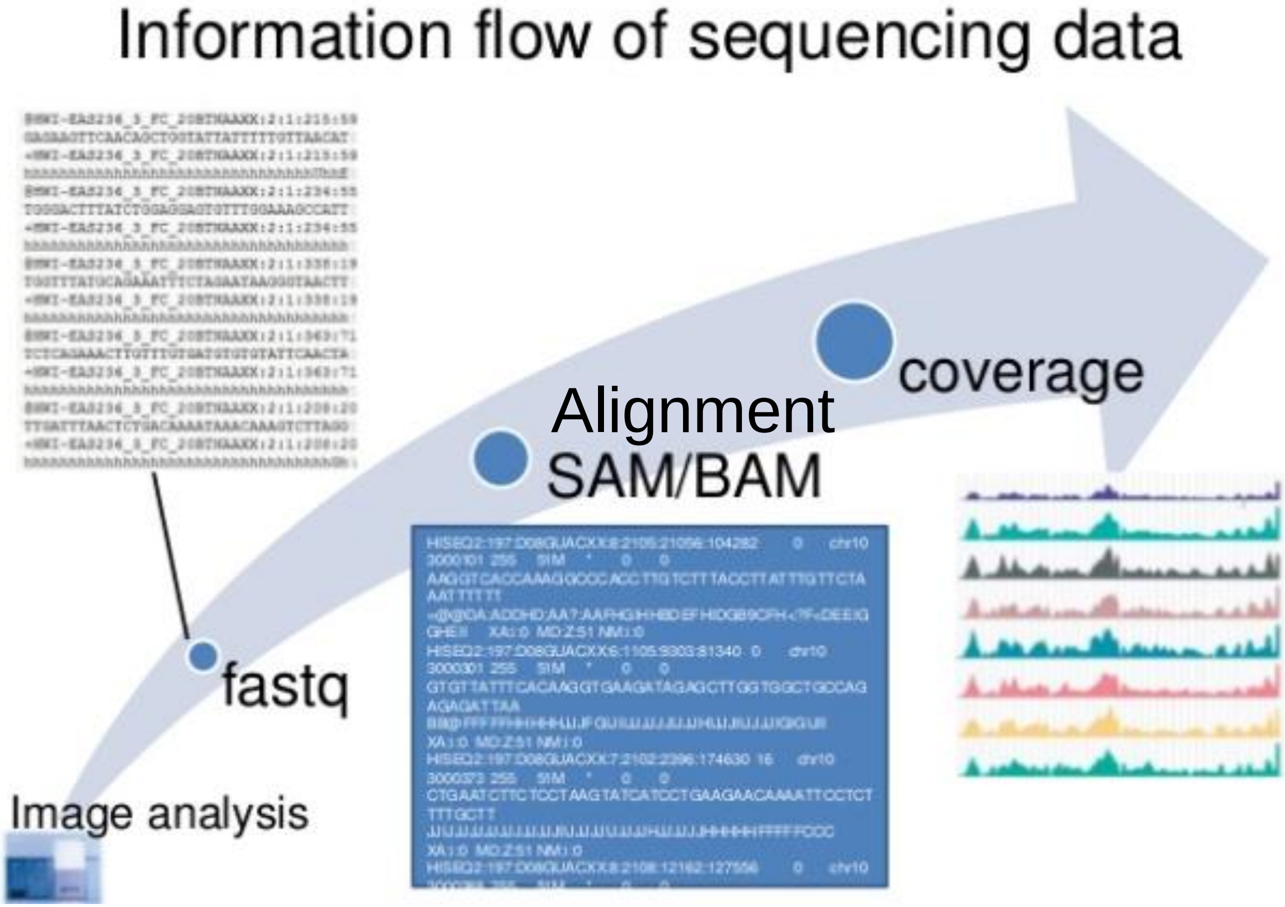
Is it in the nucleus?

Is it an enzyme?

Is it related to a disease?

All the answers are “attributes” or characteristics of the item (gene).

What have we done until now?



Information flow of sequencing data

Image analysis

fastq

```
@HWI-EAS236_3_FC_20BTNAAXX:2:1:215:59
GAGAAGTTCAACAGCTGGTATTATTTTTTGTTAACAT
+HWI-EAS236_3_FC_20BTNAAXX:2:1:215:59
hhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhUhhE
@HWI-EAS236_3_FC_20BTNAAXX:2:1:234:55
TGGGACTTTATCTGGAGGAGTGTTTGGAAAGCCATT
+HWI-EAS236_3_FC_20BTNAAXX:2:1:234:55
hhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhh
@HWI-EAS236_3_FC_20BTNAAXX:2:1:338:19
TGGTTTATGCAGAAATTTCTAGAATAAGGGTAACTT
+HWI-EAS236_3_FC_20BTNAAXX:2:1:338:19
hhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhh
@HWI-EAS236_3_FC_20BTNAAXX:2:1:363:71
TCTCAGAAACTTGTTTTGTGATGTGTGTATTCAACTA
+HWI-EAS236_3_FC_20BTNAAXX:2:1:363:71
hhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhh
@HWI-EAS236_3_FC_20BTNAAXX:2:1:208:20
TTGATTTAACTCTGACAAAATAAACAAAGTCTTAGG
+HWI-EAS236_3_FC_20BTNAAXX:2:1:208:20
hhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhG8h
```

Alignment
SAM/BAM

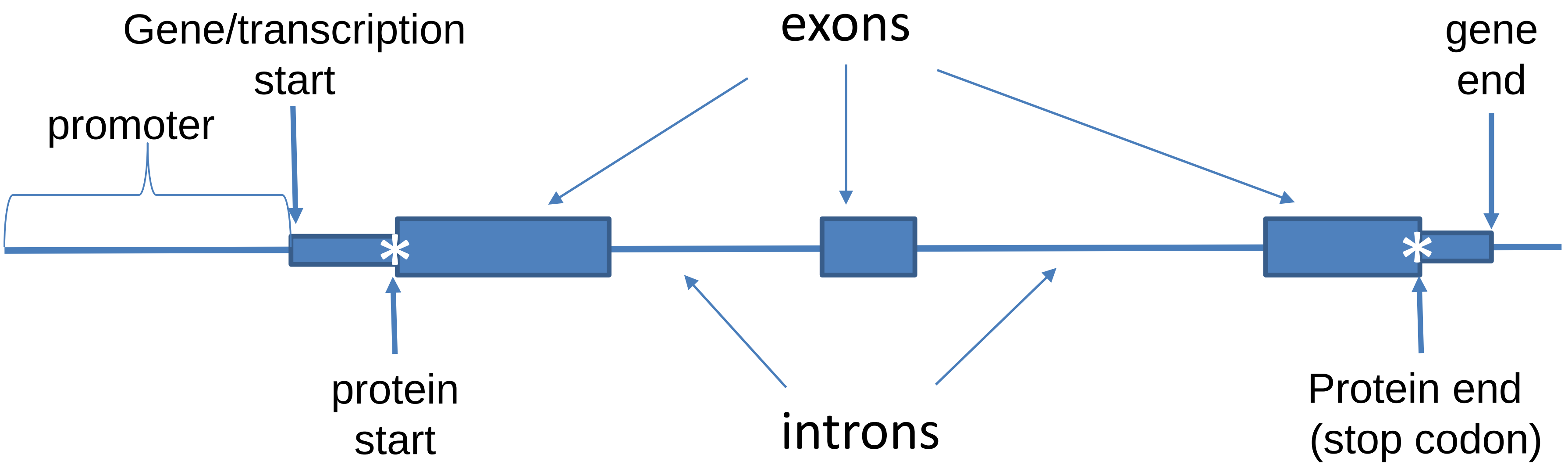
```
HISEQ2:197:D08GUACXX:8:2105:21056:104282      0     chr10
3000101  255     51M     *     0     0
AAGGTCACCAAAGGCCCACCTTGTCTTTACCTTATTTGTTCTA
AATTTTTT
=@@DA:ADDHD:AA?:AAFHGIHHBDEFHIDGB9CFH<?F<DEEIG
GHEII     XA:i:0  MD:Z:51 NM:i:0
HISEQ2:197:D08GUACXX:6:1105:9303:81340  0      chr10
3000301  255     51M     *     0     0
GTGTTATTTCACAAGGTGAAGATAGAGCTTGGTGGCTGCCAG
AGAGATTAA
BB@FFFFFHHHHHJJJFGIJIJJJJJJJJJJHJJJIJJJJJIGGIJII
XA:i:0  MD:Z:51 NM:i:0
HISEQ2:197:D08GUACXX:7:2102:2396:174630 16     chr10
3000373  255     51M     *     0     0
CTGAATCTTCTCCTAAGTATCATCCTGAAGAACAAAATTCCTCT
TTTGCTT
JJJJJJJJJJJJJJJJJJJJIJJJJJJJJJJJJHJJJJJJJHHHHHHFFFFFCCC
XA:i:0  MD:Z:51 NM:i:0
HISEQ2:197:D08GUACXX:8:2108:12162:127556      0     chr10
3000388  255     51M     *     0     0
```

coverage

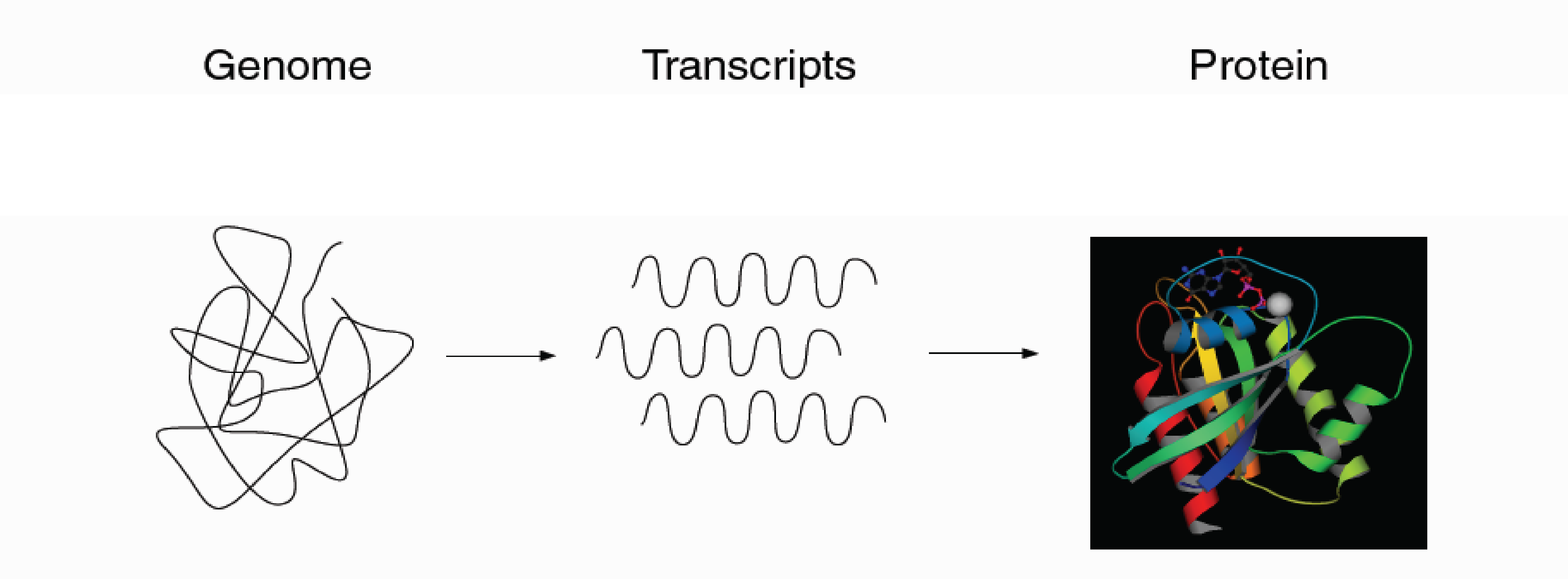
What is a Gene ?

A gene is a region of DNA that encodes instructions for how the cell can make a gene product, which can be:

- a protein
- a noncoding RNA.



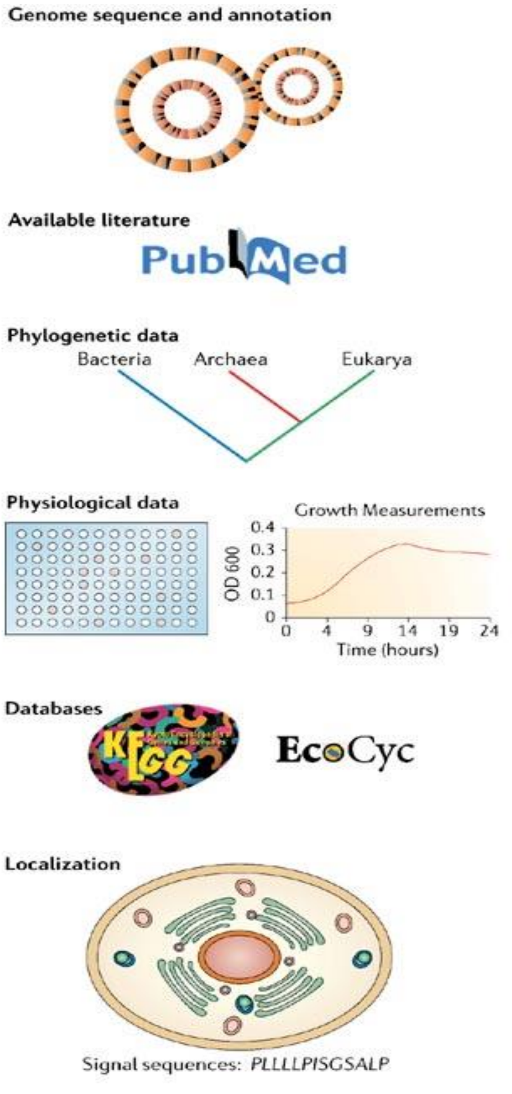
Data sources



- There are several kinds of databases, looking at the genome, transcriptome or proteome level
- The mapping of the different names is not trivial

Levels of annotation per gene

Level	Database
Sequence	GenBank SwissProt (curated)
Metabolic pathways	Kegg Transpath MetaCyc
Literature	PubMed
Gene ontology (GO)	Biological process Molecular function Cell compartment
Integrated – Meta databases	GeneCards Entrez Gene OMIM InterPro



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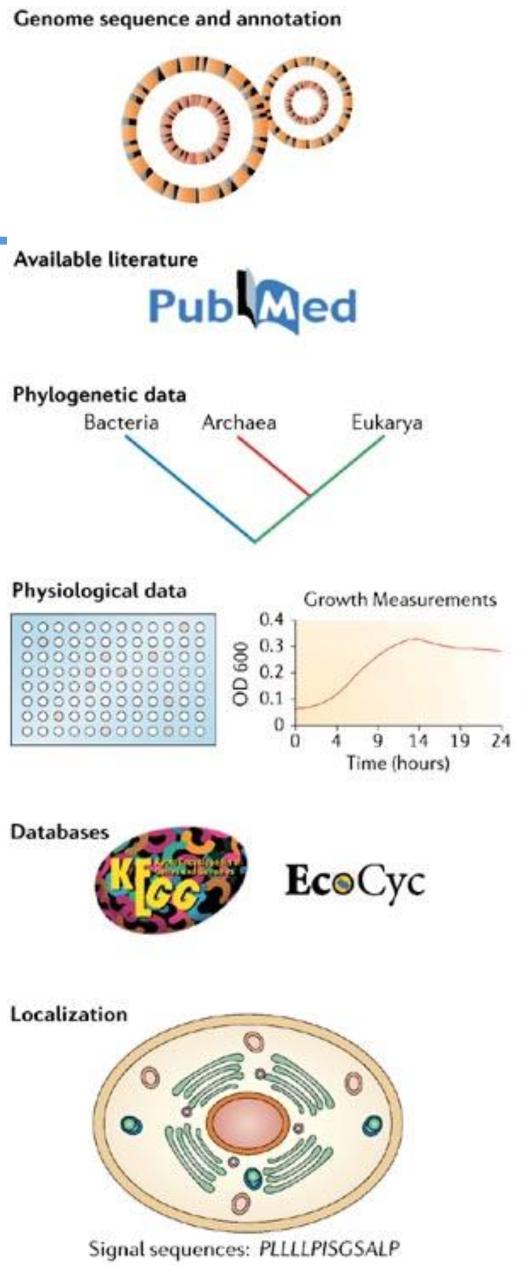

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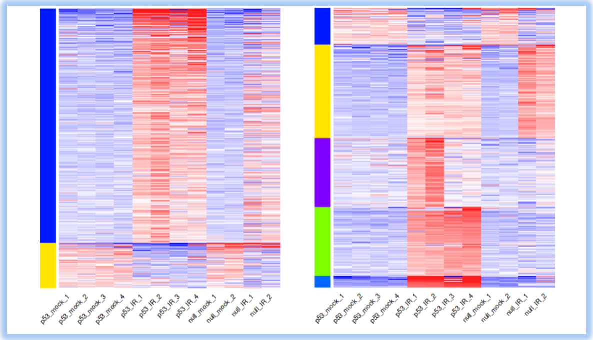
What have we done until now?

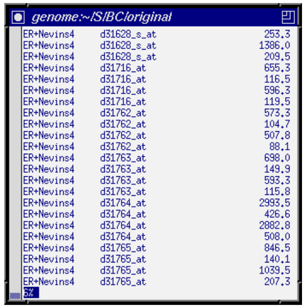
A complex high-throughput experiment:
Deep Sequencing
Proteomics
Microarrays
...

What did we get?

Lists of genes

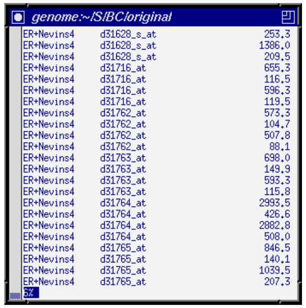


Clusters of differential genes



```
ER+Nevins4    d31628_s_at        253.3
ER+Nevins4    d31628_s_at       1386.0
ER+Nevins4    d31628_s_at        209.5
ER+Nevins4    d31716_at          655.3
ER+Nevins4    d31716_at          116.5
ER+Nevins4    d31716_at          596.3
ER+Nevins4    d31716_at          119.5
ER+Nevins4    d31762_at          573.3
ER+Nevins4    d31762_at          104.7
ER+Nevins4    d31762_at          507.8
ER+Nevins4    d31762_at           88.1
ER+Nevins4    d31763_at          698.0
ER+Nevins4    d31763_at          149.9
ER+Nevins4    d31763_at          593.3
ER+Nevins4    d31763_at          115.8
ER+Nevins4    d31764_at         2993.5
ER+Nevins4    d31764_at          426.6
ER+Nevins4    d31764_at         2882.8
ER+Nevins4    d31764_at          508.0
ER+Nevins4    d31765_at          846.5
ER+Nevins4    d31765_at          140.1
ER+Nevins4    d31765_at         1039.5
ER+Nevins4    d31765_at          207.3
S%
```

Up regulated



```
ER+Nevins4    d31628_s_at        253.3
ER+Nevins4    d31628_s_at       1386.0
ER+Nevins4    d31628_s_at        209.5
ER+Nevins4    d31716_at          655.3
ER+Nevins4    d31716_at          116.5
ER+Nevins4    d31716_at          596.3
ER+Nevins4    d31716_at          119.5
ER+Nevins4    d31762_at          573.3
ER+Nevins4    d31762_at          104.7
ER+Nevins4    d31762_at          507.8
ER+Nevins4    d31762_at           88.1
ER+Nevins4    d31763_at          698.0
ER+Nevins4    d31763_at          149.9
ER+Nevins4    d31763_at          593.3
ER+Nevins4    d31763_at          115.8
ER+Nevins4    d31764_at         2993.5
ER+Nevins4    d31764_at          426.6
ER+Nevins4    d31764_at         2882.8
ER+Nevins4    d31764_at          508.0
ER+Nevins4    d31765_at          846.5
ER+Nevins4    d31765_at          140.1
ER+Nevins4    d31765_at         1039.5
ER+Nevins4    d31765_at          207.3
S%
```

Down regulated

Functional Genomics:
Find the biological meaning

- Take a list of "interesting" genes and find their biological meaning
- Requires a reference set of "biological knowledge“
- Linking between genes and biological function:
  - ❖ Gene ontology: GO
  - ❖ Pathways databases

The problem

- Vast amounts of biological data
- Different names/terms for the same concepts

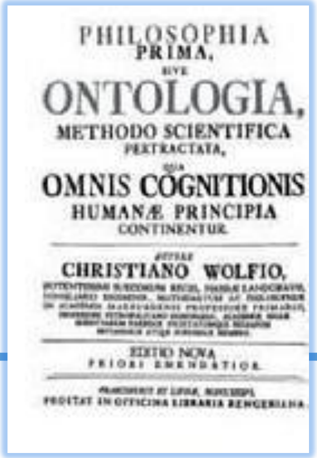
  For example: the same function can be called

  translation or protein synthesis.
- Cross-species comparison is difficult

Part of the solution

Gene Ontology

What is Ontology?



PHILOSOPHIA
PRIMA,
SIVE
ONTOLOGIA,
METHODO SCIENTIFICA
PERTRACTATA,
QUA
OMNIS COGNITIONIS
HUMANÆ PRINCIPIA
CONTINENTUR.

AUCTORE
CHRISTIANO WOLFIO,
[illegible]

EDITIO NOVA
PRIORI EMENDATIOR.

FRANCOFURTI ET LIPSIAE, [illegible]
PROSTAT IN OFFICINA LIBRARIA RENGERIANA.

1700s

Ontology (from the Greek…) is the philosophical study of the nature of being, existence or reality in general, as well as of the basic categories of being and their relations.

Traditionally listed as a part of the major branch of philosophy known as metaphysics, ontology deals with questions concerning what entities exist or can be said to exist, and how such entities can be grouped, related within a hierarchy, and subdivided according to similarities and differences.

GENEONTOLOGY
Unifying Biology

- The Gene Ontology (GO) project is a collaborative effort to address the need for consistent descriptions of gene products in different databases.
- Gene ontology is an annotation system
- The project provides the controlled and consistent vocabulary of terms and gene product annotations, i.e. terms occur only once, and there is a dictionary of allowed words

http://geneontology.org/

Why use GO?

- The goal of the GeneOntology (GO) project is to provide a uniform way to describe the functions of gene products from organisms across all kingdoms of life and thereby enable analysis of genomic data.

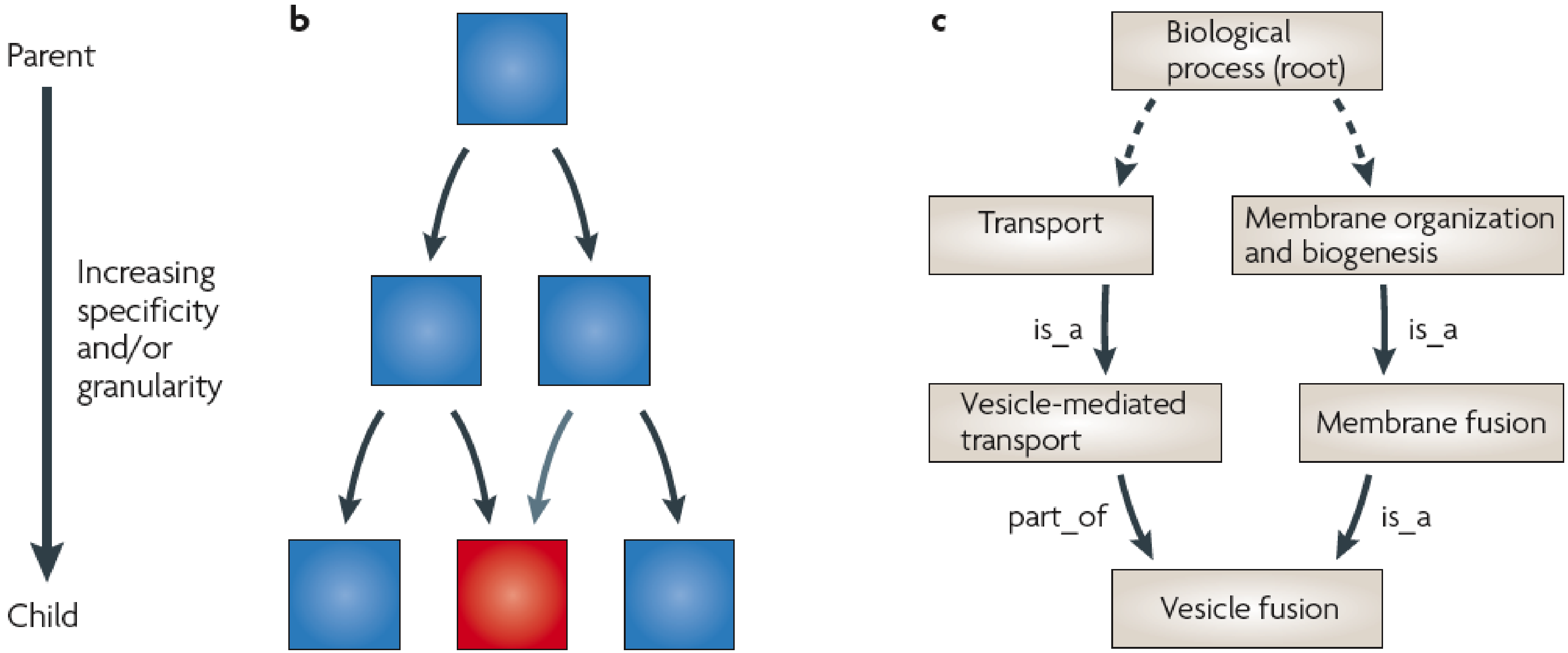
- bio-ontologies such as GO make domain knowledge available to both humans and computers.

- GO provides the ability to group gene products to some high level term.

There are three structured, controlled vocabularies (ontologies) that use terms to describe gene products in a species-independent manner:

- Biological processes
  - A recognized series of events, must have more than one distinct steps
  - Examples: cell division, pyrimidine metabolic process
- Cellular components
  - Where a gene product is located (an anatomical structure)
  - Examples: nucleus, proteasome
- Molecular functions
  - describes activities, such as catalytic or binding activities

Gene ontology is represented as a directed acyclic graph (DAG)

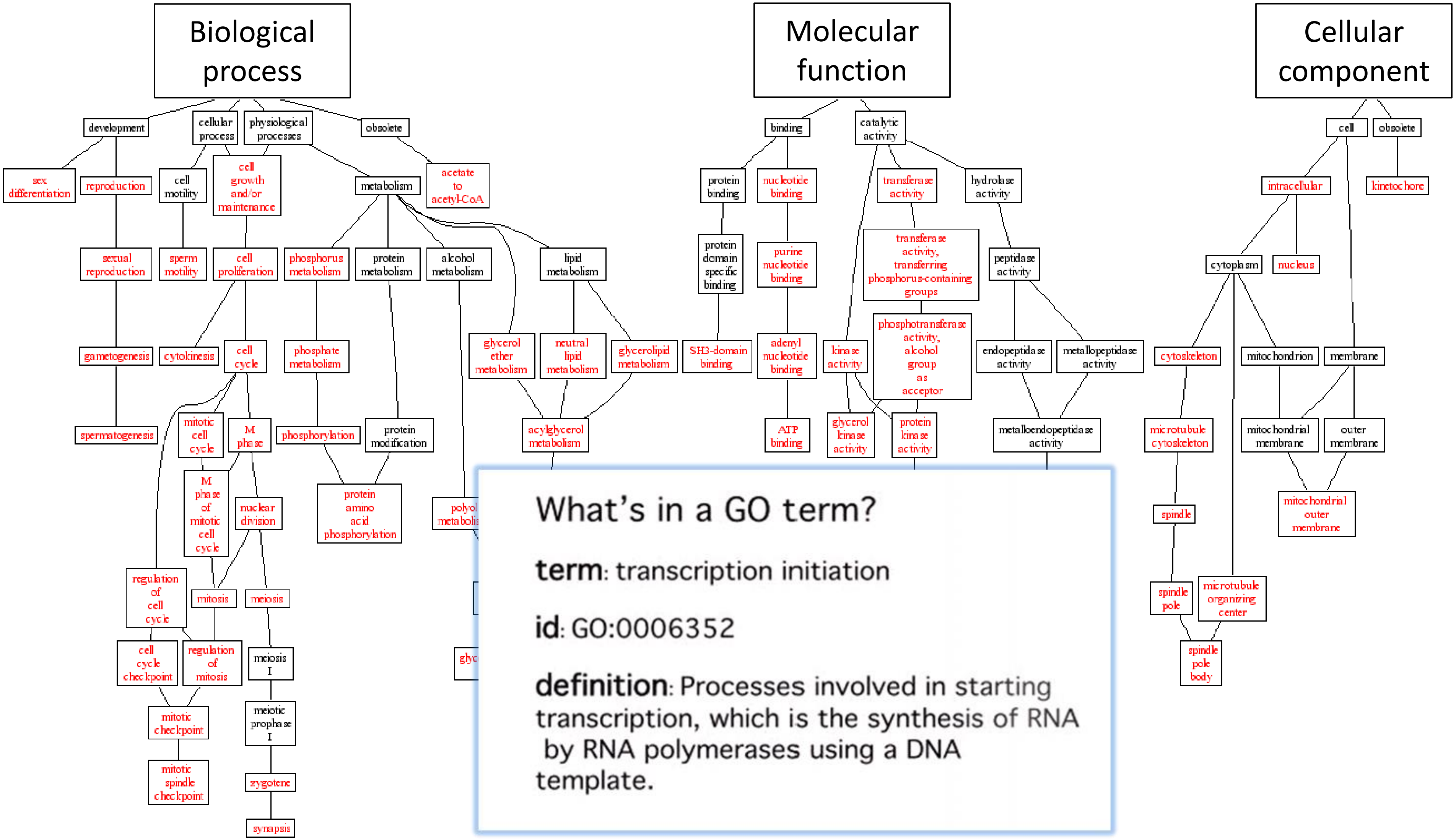

Parent
Increasing specificity and/or granularity
Child
b
c
Biological process (root)
Transport
Membrane organization and biogenesis
is_a
is_a
Vesicle-mediated transport
Membrane fusion
part_of
is_a
Vesicle fusion

Taken from: Nature Reviews Genetics 9:509-515 (2008)

Directed Acyclic Graph (DAG)

- A child can have more than one parent
  - parents are closer to the root and are more general
  - children are further from the root and more specific
- There are no cycles - there is a root
- It is a directed graph
- You can skip levels in the graph

Example



What's in a GO term?

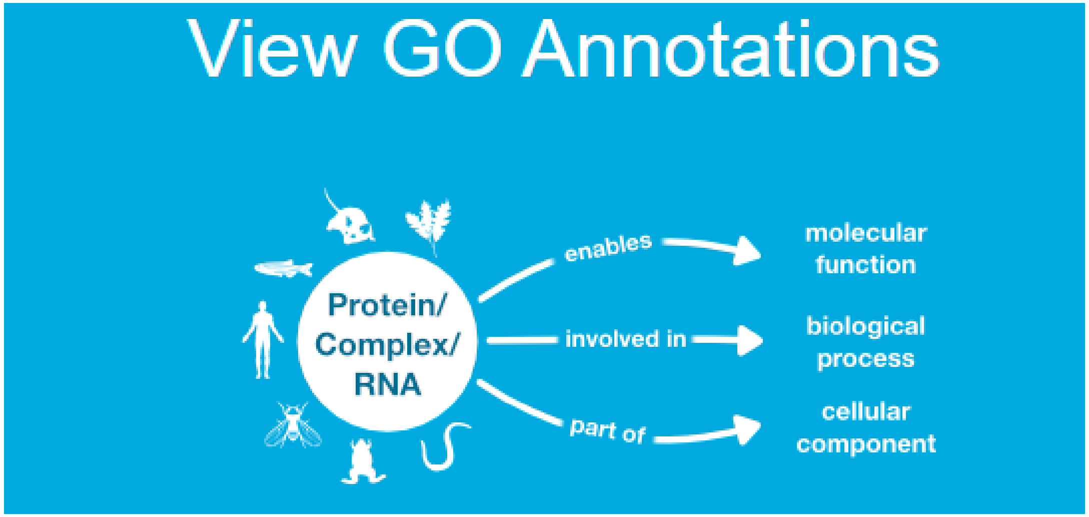
term: transcription initiation

id: GO:0006352

definition: Processes involved in starting transcription, which is the synthesis of RNA by RNA polymerases using a DNA template.

GO annotation

- A GO annotation is a statement about the function of a particular gene.
- GO annotations are associations made between gene products or protein complexes and the GO terms that describe them.
  - attributed to a source
  - indicate the evidence upon which it is based.



https://www.ebi.ac.uk/QuickGO/

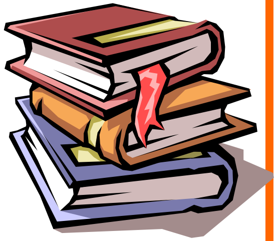
Evidence codes

not all annotations are created equal

BLAST

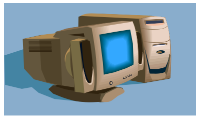
	EXP	Inferred from Experiment
HTP		Inferred from High Throughput Experiment

HDA	IDA	Inferred from Direct Assay
	IPI	Inferred from Physical Interaction
HMP	IMP	Inferred from Mutant Phenotype
HGI	IGI	Inferred from Genetic Interaction
HEP	IEP	Inferred from Expression Pattern



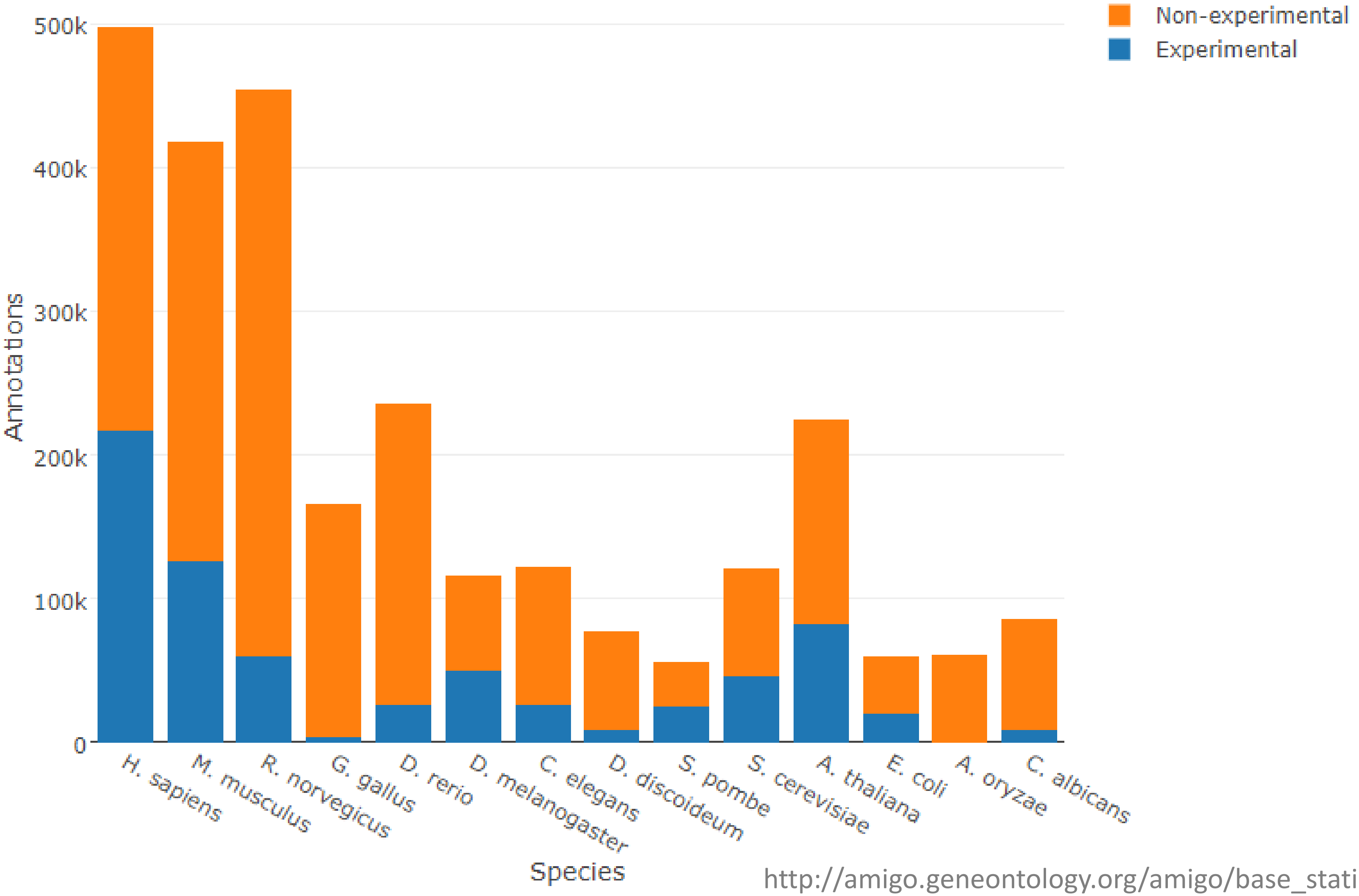
ISS	Inferred from Sequence/Structural Similarity
TAS	Traceable Author Statement
NAS	Non-traceable Author Statement
IC	Inferred by Curator
ND	No Data available

IEA	Inferred from electronic annotation



http://geneontology.org/docs/guide-go-evidence-codes/

Type of annotation per species



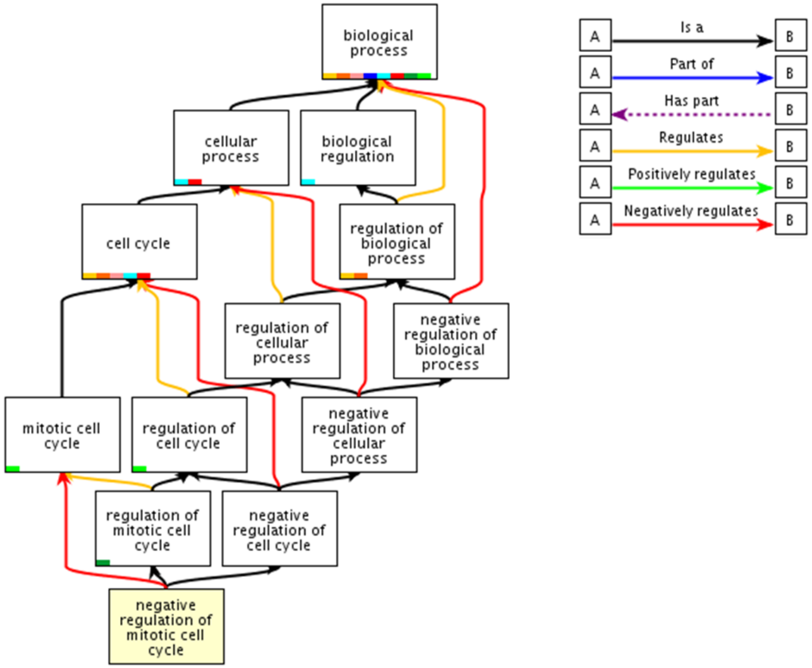
http://amigo.geneontology.org/amigo/base_statistics

Ontology Relations

Defines the relationships (the arrows) between the ontology terms.

There are three types of relationships:

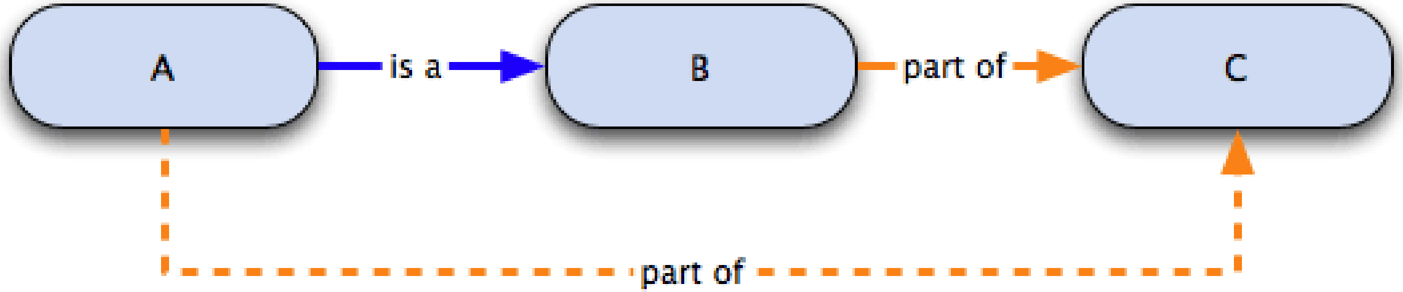
- is_a
- part_of
- regulates:
  - positively regulates
  - negatively regulates


biological process
cellular process
biological regulation
cell cycle
regulation of biological process
regulation of cellular process
negative regulation of biological process
mitotic cell cycle
regulation of cell cycle
negative regulation of cellular process
regulation of mitotic cell cycle
negative regulation of cell cycle
negative regulation of mitotic cell cycle
A Is a B
A Part of B
A Has part B
A Regulates B
A Positively regulates B
A Negatively regulates B

Ontology Relations

- is_a is a simple class-subclass relationship

Example: nuclear chromosome is_a chromosome.

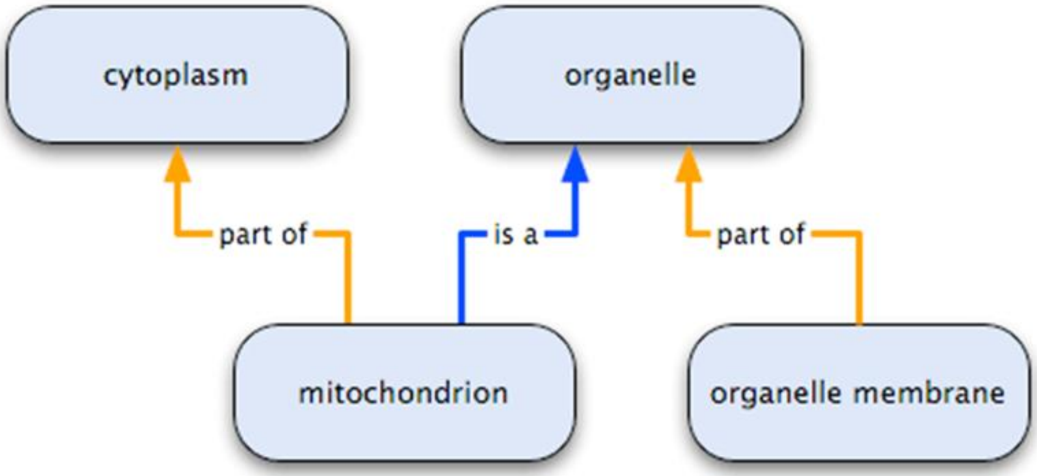


A dotted line means an inferred relationship, e.g. one that has not been expressly stated

http://geneontology.org/docs/ontology-relations/

Ontology Relations

- part_of represent part-whole relationships; C part_of D means that whenever C is present, it is always a part of D.



Example: nucleus part_of cell; nuclei are always part of a cell, but not all cells have nuclei.

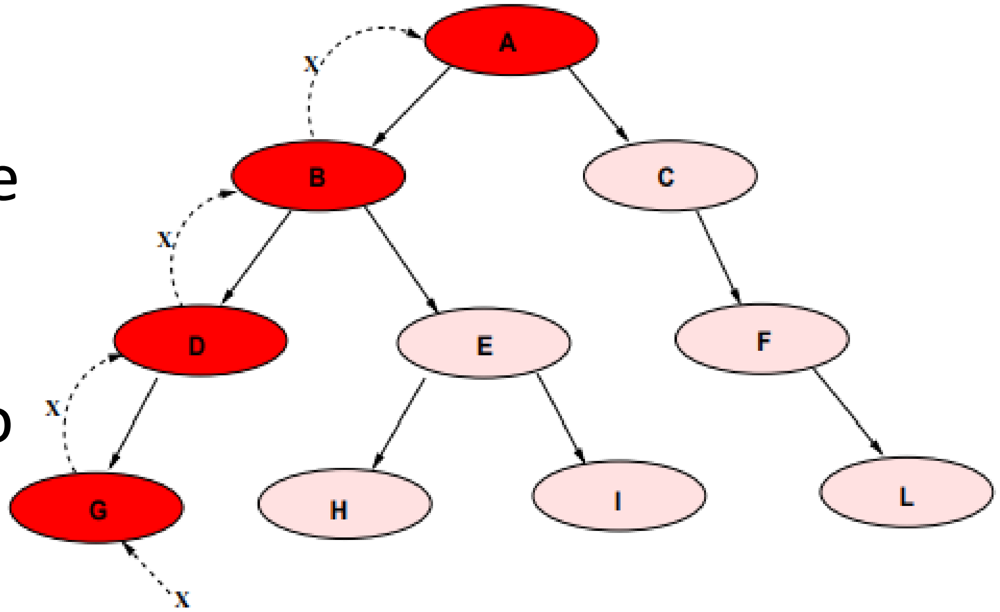
mitochondrion has two parents: it *is an* organelle and it is *part of* the cytoplasm;
organelle has two children: mitochondrion *is an* organelle, and organelle membrane is *part of* organelle

Ontology Structure

Every GO term obeys “the true path rule”:

- If a child term describes the gene product, then all its ancestors (parent) terms must also apply to that gene product.

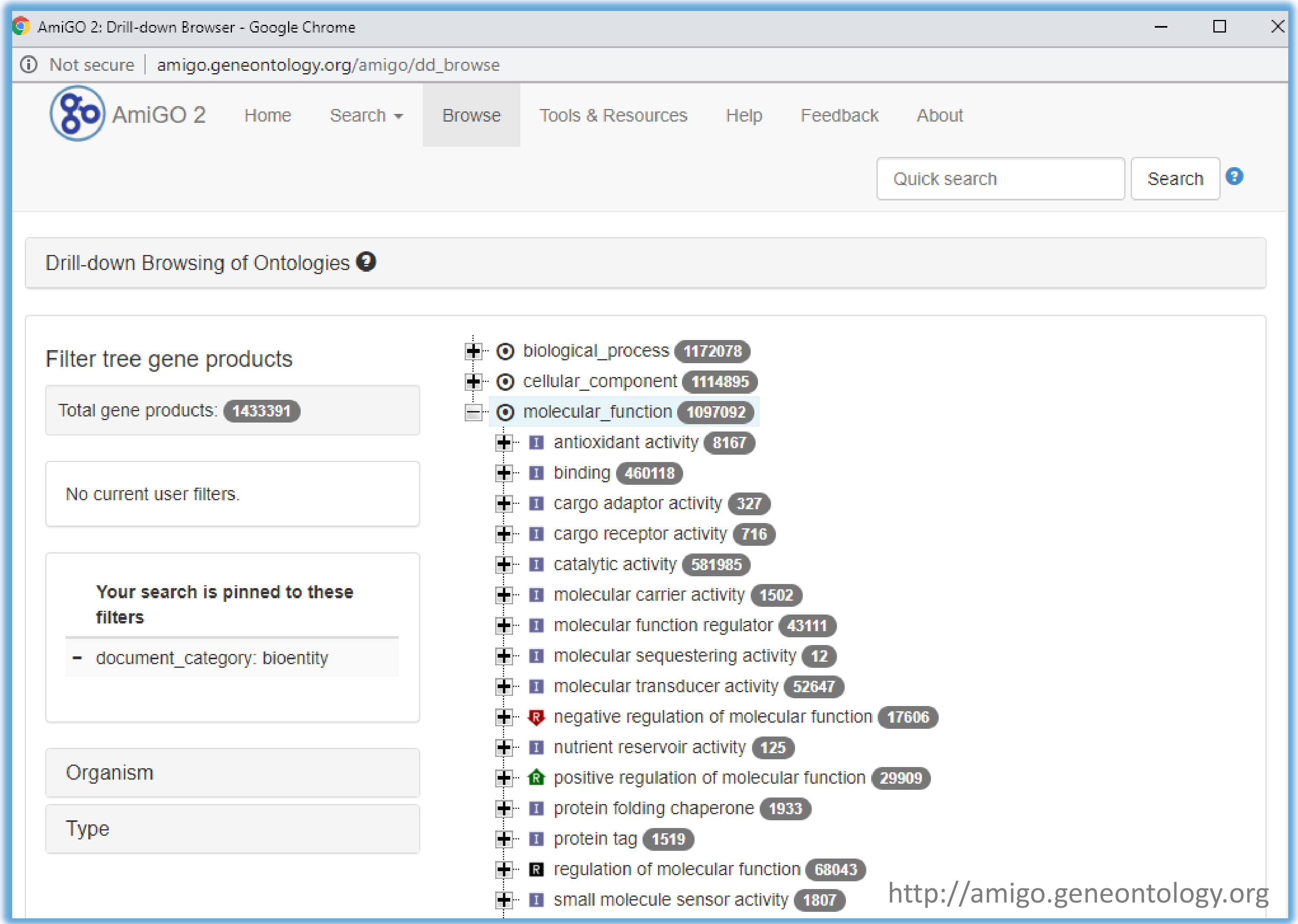
- If a gene is not annotated to a term, it cannot be annotated to its offsprings.



https://homes.di.unimi.it/~valentini/papers/vale.TPR.hier.revised.pdf

AmiGO

a web application to query, browse and visualize ontologies



Drill-down Browsing of Ontologies

Filter tree gene products

Total gene products: 1433391

No current user filters.

Your search is pinned to these filters

- document_category: bioentity

- biological_process 1172078
- cellular_component 1114895
- molecular_function 1097092
  - antioxidant activity 8167
  - binding 460118
  - cargo adaptor activity 327
  - cargo receptor activity 716
  - catalytic activity 581985
  - molecular carrier activity 1502
  - molecular function regulator 43111
  - molecular sequestering activity 12
  - molecular transducer activity 52647
  - negative regulation of molecular function 17606
  - nutrient reservoir activity 125
  - positive regulation of molecular function 29909
  - protein folding chaperone 1933
  - protein tag 1519
  - regulation of molecular function 68043
  - small molecule sensor activity 1807

http://amigo.geneontology.org

Available GO Information

Current ontology statistics: as of Dec, 2019:

44674 terms, 100.0% defined

- 29,380 Biological process terms
- 11,113 Molecular function terms
- 4,181 Cellular component terms
- 2711 obsolete terms (not included in figures above)

http://geneontology.org/stats.html

20 Questions

Which attribute is not a GO term?

Is it part of a complex?

Is it a protein coding gene?

Is it a regulator – transcription factor?

Is it in the nucleus?

Is it an enzyme?

Is it related to a disease?

All the answers are “attributes” or characteristics of the item (gene).

Reminder

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Unifying Biology

There are three structured, controlled vocabularies (ontologies) that use terms to describe gene products in a species-independent manner:

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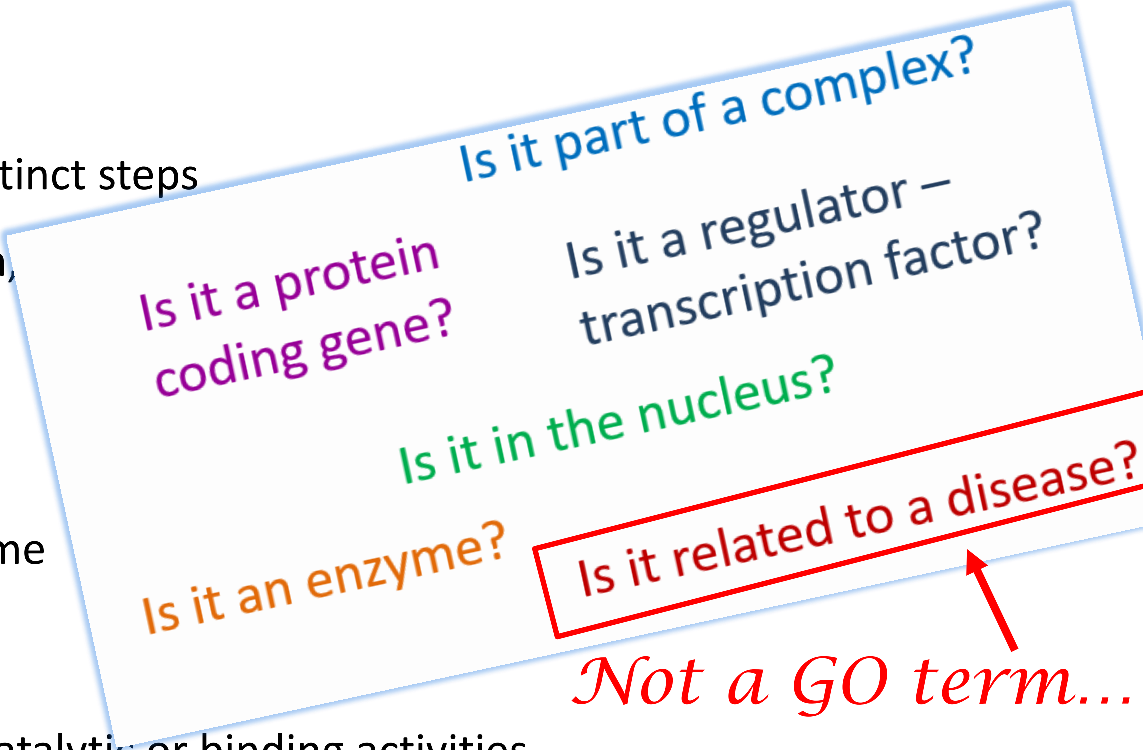
  - must have more than one distinct steps
  - Examples: signal transduction,
- Cellular components
  - an anatomical structure
  - Examples: nucleus, proteasome
- Molecular functions
  - describes activities, such as catalytic or binding activities



Is it part of a complex?

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Not a GO term…

What is not GO?

- Gene products: e.g. cytochrome c is not in the ontologies, but attributes of cytochrome c, such as oxidoreductase activity, are
- Processes, functions or components that are unique to mutants or diseases: e.g. oncogenesis
- Attributes of sequence such as intron/exon parameters
- Protein domains or structural features
- Protein-protein interactions
- Environment, evolution and expression
- It is not **complete,** it is done “by hand” by curators
- A pathway

GO Pitfalls

- Not complete
- Computational annotations
- NOT qualifier
- Identifier flagged as ‘obsolete’, some tools do not update their databases

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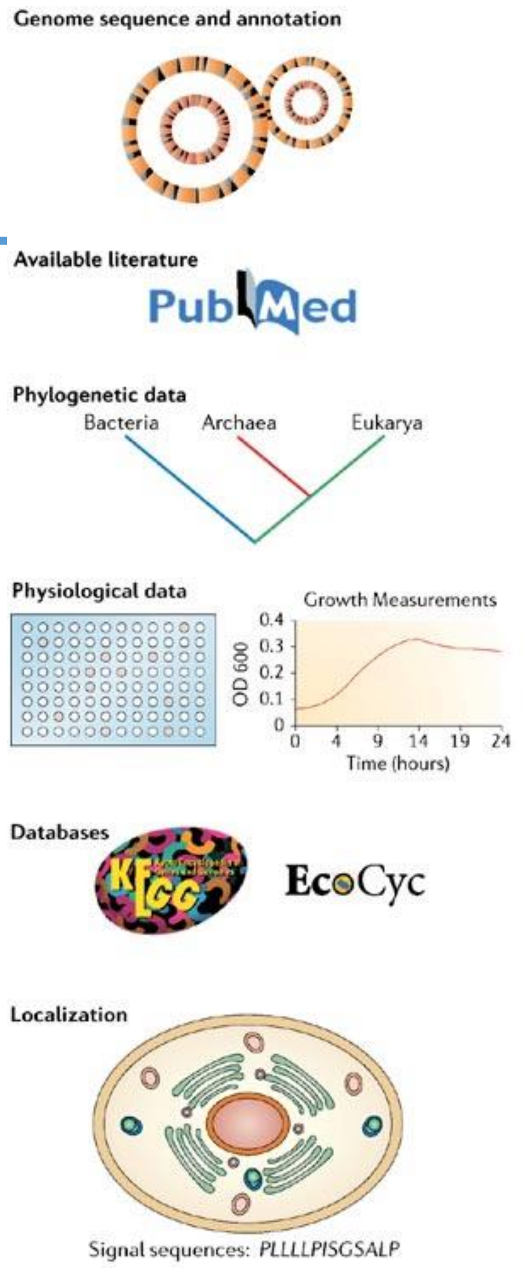

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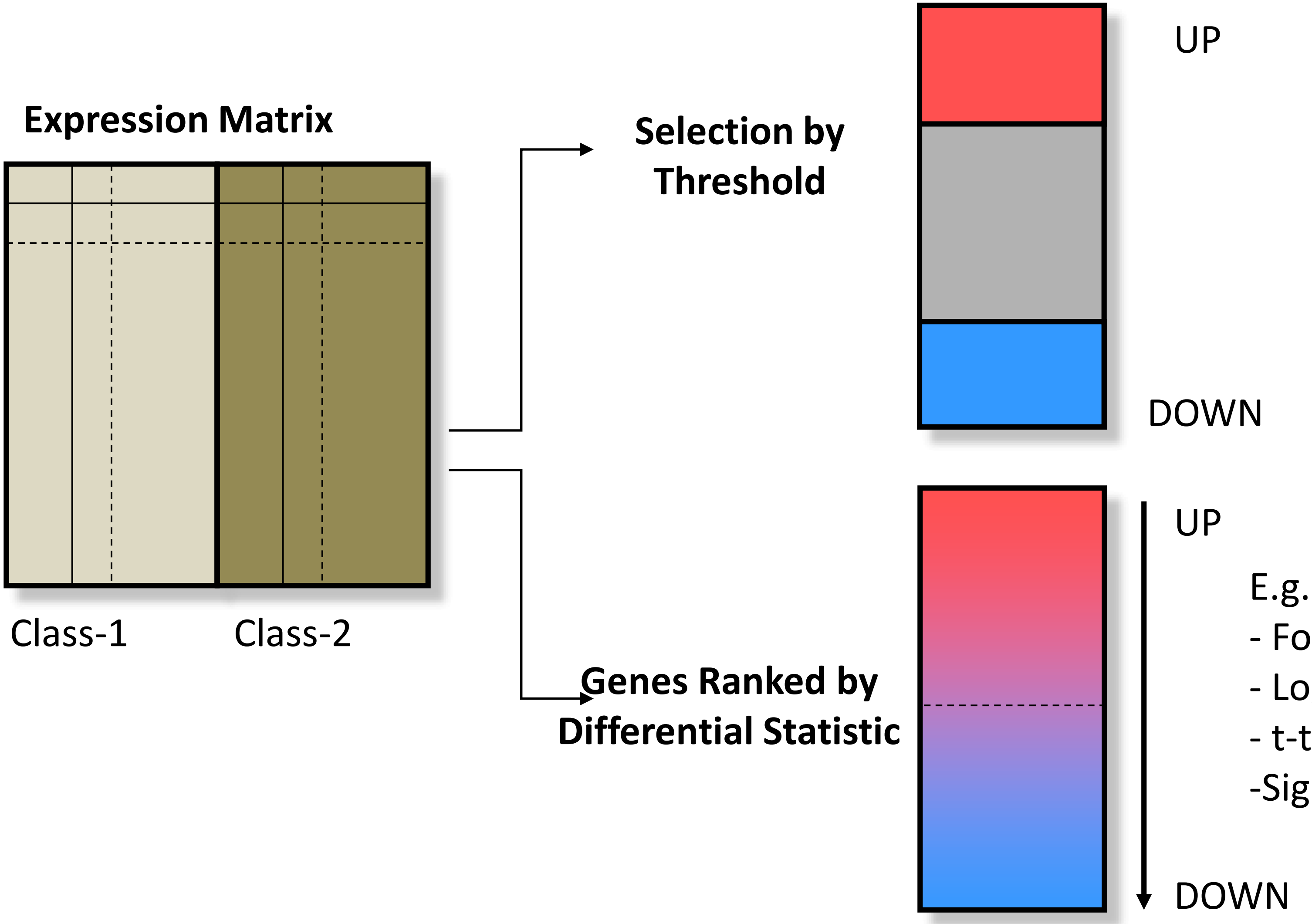
Two-class Design



Expression Matrix

Class-1

Class-2

Selection by Threshold

UP

DOWN

Genes Ranked by Differential Statistic

UP

DOWN

E.g.:

- Fold change
- Log (ratio)
- t-test

-Significance analysis

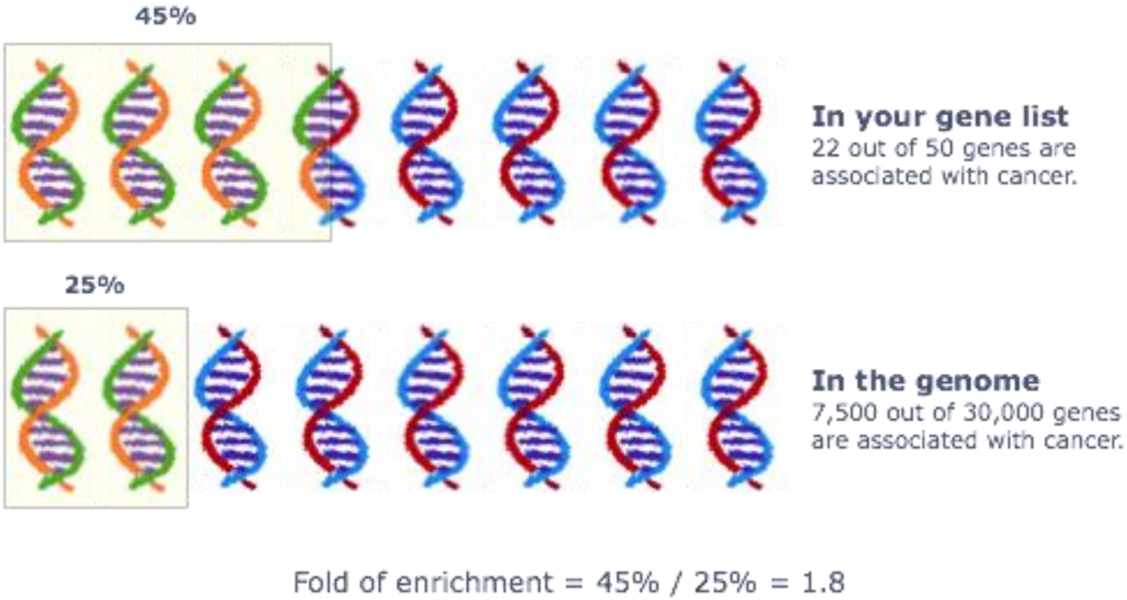
(modified) bioinformatics.ca

What is functional enrichment?

- It is a measure of how much a group of gene products is found in our data set
- It requires some type of background measure, as a basis for comparison
- What we look at is how many we have (observed) as opposed to how many we would expect to see at random, given our background.

Background

The choice of an appropriate background is critical to get meaningful results



In your gene list
22 out of 50 genes are associated with cancer.

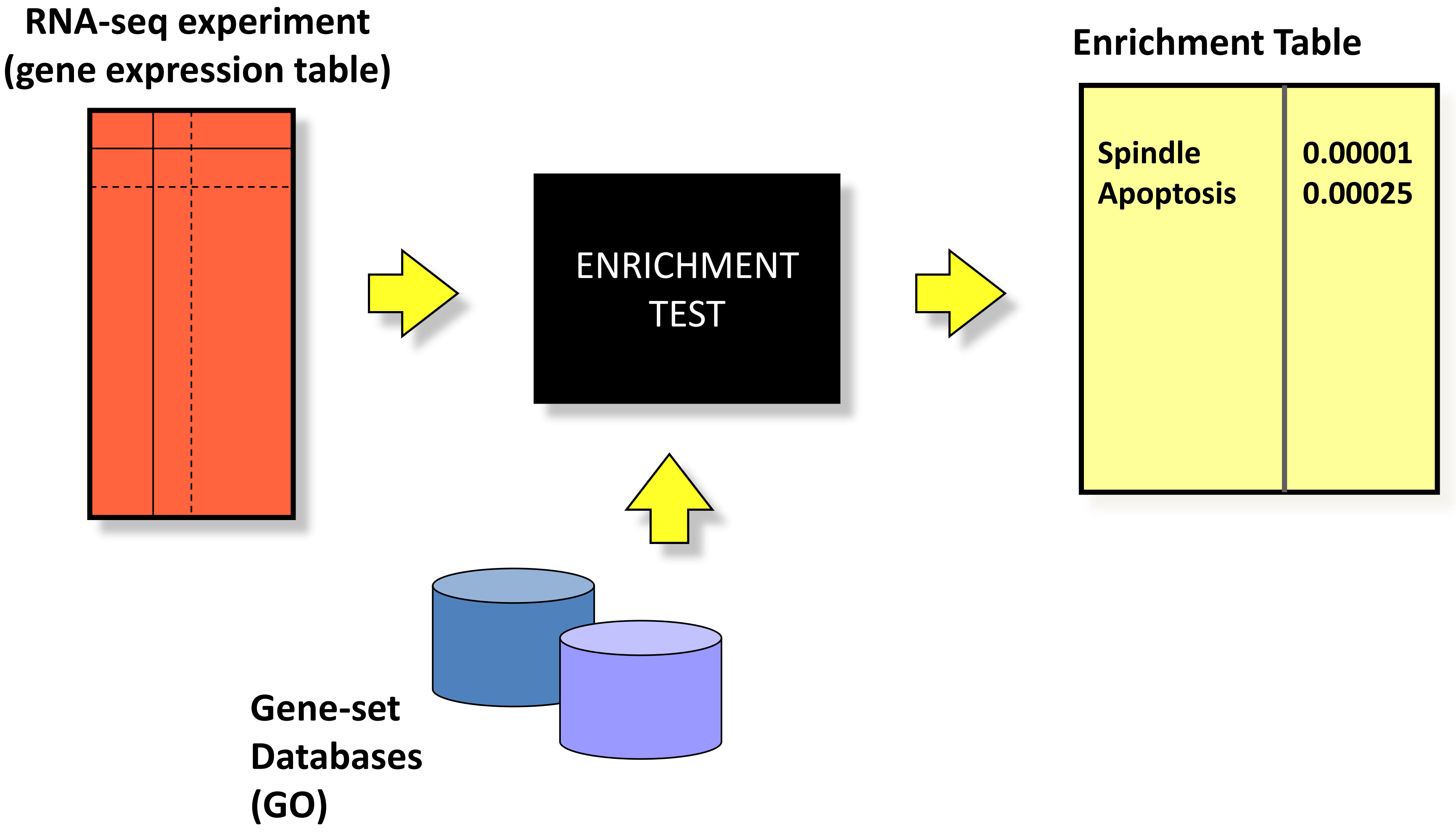
In the genome
7,500 out of 30,000 genes are associated with cancer.

Fold of enrichment = 45% / 25% = 1.8

You should use all the genes detected by the method used in your experiment,

not all the genes in the genom, if possible.

Enrichment test



RNA-seq experiment
(gene expression table)

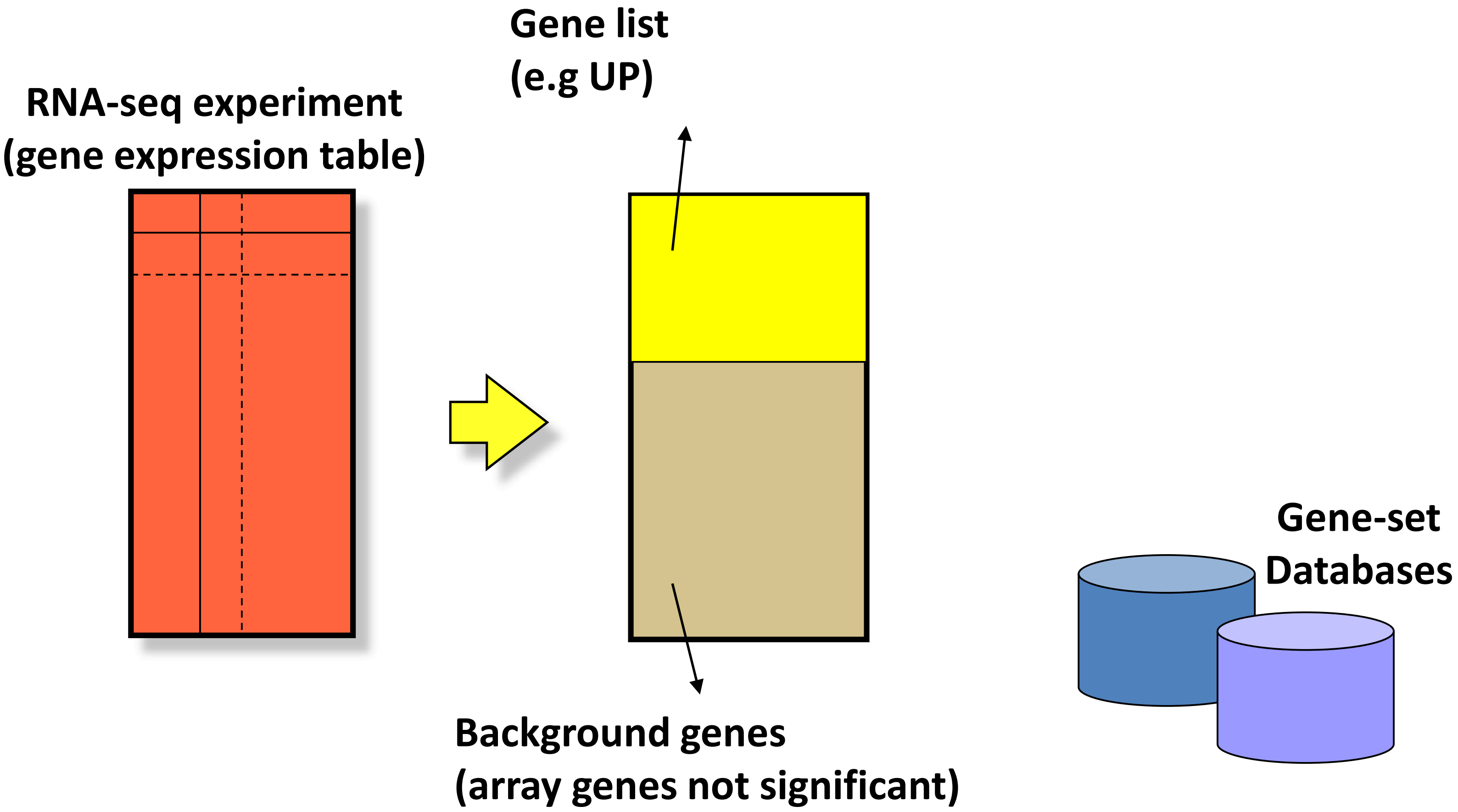
ENRICHMENT TEST

Enrichment Table

Spindle	0.00001
Apoptosis	0.00025

Gene-set Databases (GO)

Enrichment test



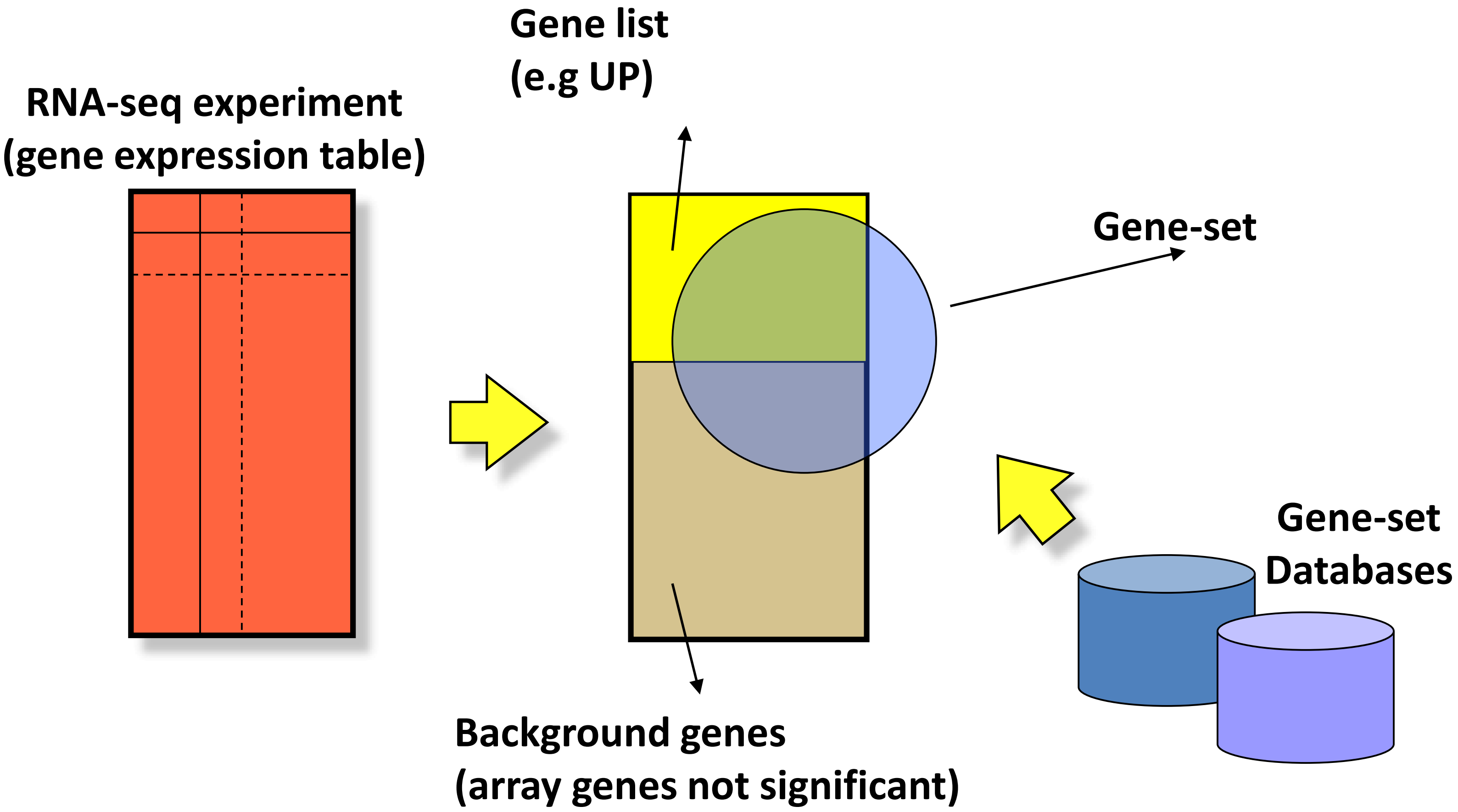
RNA-seq experiment
(gene expression table)

Gene list
(e.g UP)

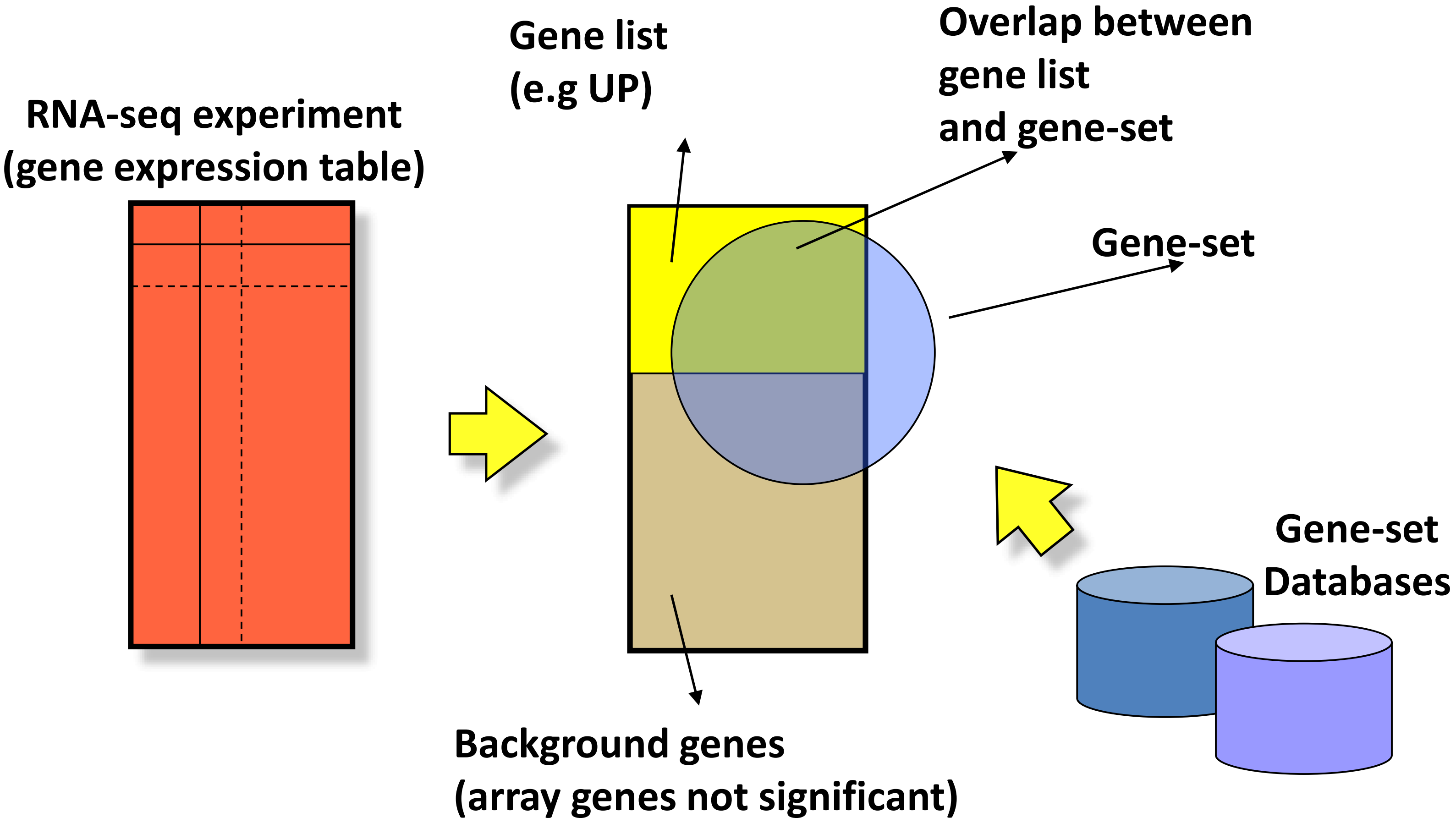
Background genes
(array genes not significant)

Gene-set
Databases

Enrichment test



Enrichment test



RNA-seq experiment
(gene expression table)

Gene list
(e.g UP)

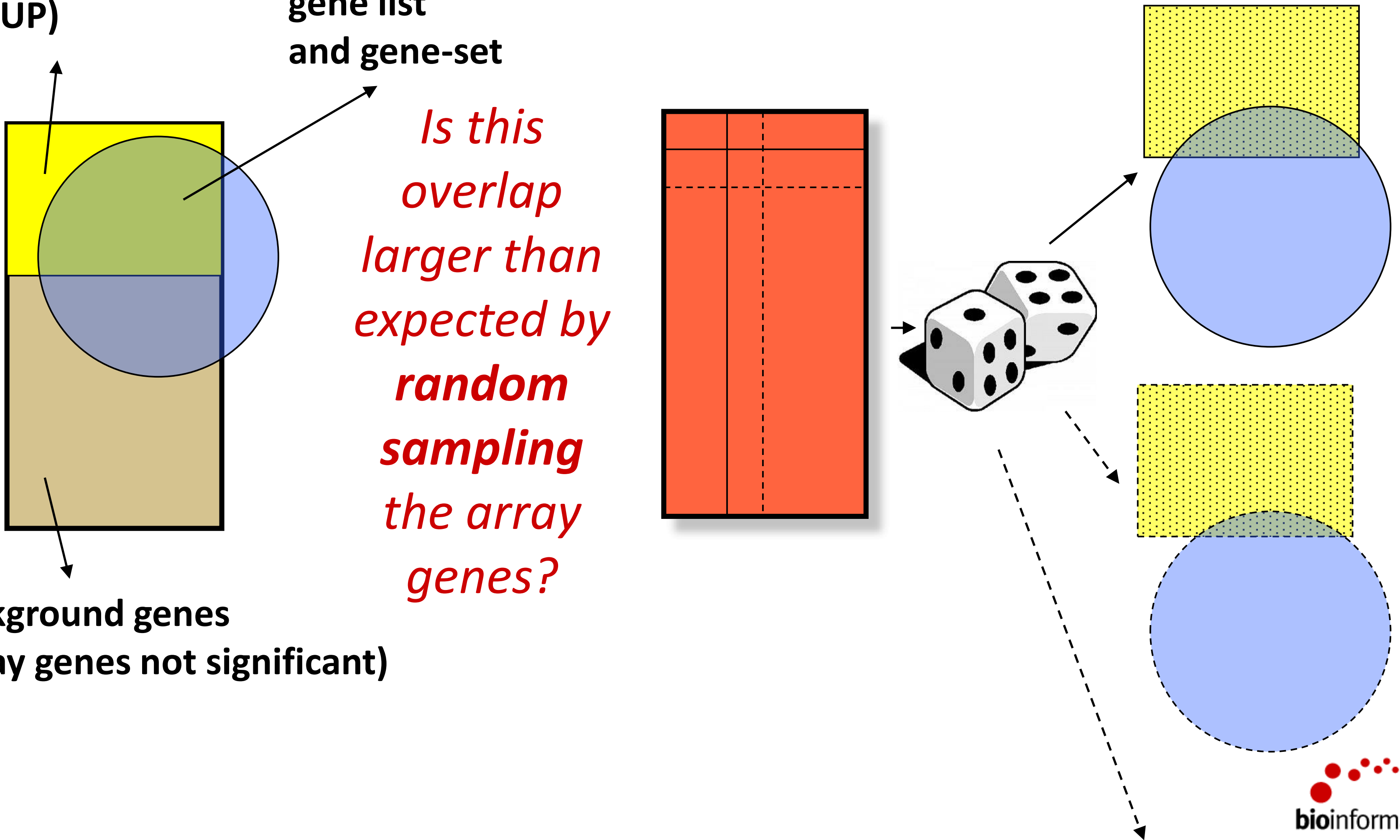
Overlap between
gene list
and gene-set

Gene-set

Gene-set
Databases

Background genes
(array genes not significant)

Enrichment test



Significant genes (e.g UP)

Overlap between gene list and gene-set

Is this overlap larger than expected by ***random sampling*** *the array genes?*

Random samples of array genes

Background genes (array genes not significant)

Enrichment analysis

- Given:

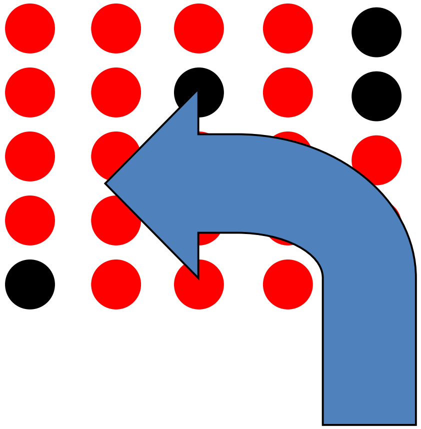
1. Gene list: e.g. RRP6, MRD1, RRP7, RRP43, RRP42
2. Gene sets or annotations: e.g. Gene ontology, transcription factor binding sites in promoter

- Question: *Are any of the gene annotations surprisingly enriched in the gene list?*

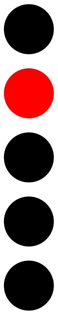
- Details:
  - Where do the gene lists come from?
  - How to assess "surprisingly" (statistics)
  - How to correct for repeating the tests

Randomization test

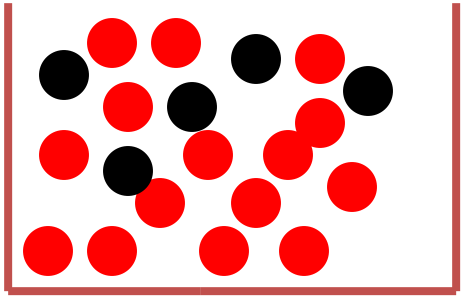
Random draws



... 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

Fisher’s exact test

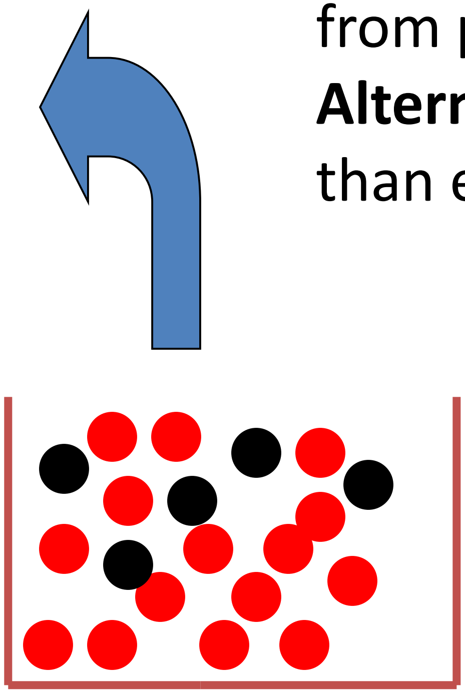
a.k.a., the hypergeometric test

Gene list

- ● RRP6
- 🔴 MRD1
- ● RRP7
- ● RRP43
- ● RRP42

Null hypothesis: List is a random sample from population

Alternative hypothesis: More black genes than expected



Background population:
500 black genes
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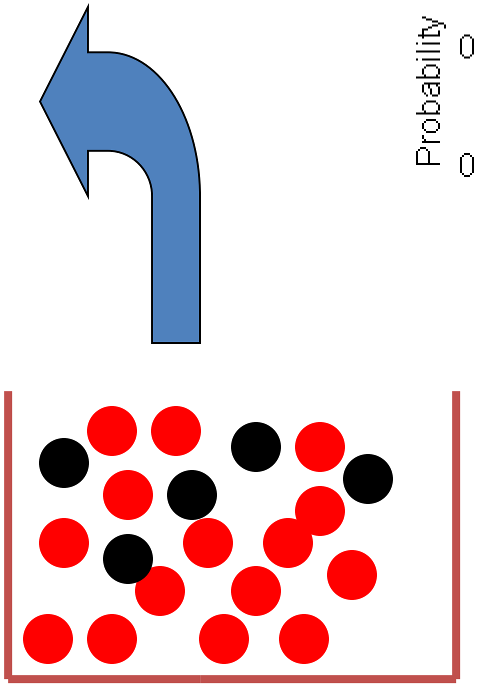
Fisher’s exact test

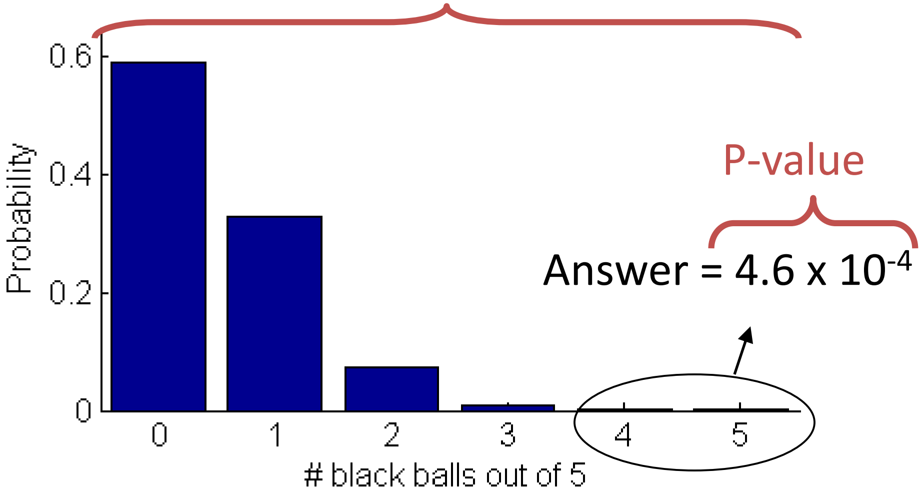
a.k.a., the hypergeometric test

Gene list

● RRP6
● MRD1
● RRP7
● RRP43
● RRP42



Null distribution



P-value

Answer = 4.6 x 10^{-4}

Background population:
500 black genes
4500 red genes

Problems working with large data sets

- The more comparisons we make, the more there is a chance that we will get random hits

- We need to correct for multiple tests, using statistical methods such as Bonferroni, FDR (Benjamini)

- Statistical significance doesn’t necessarily mean biological significance

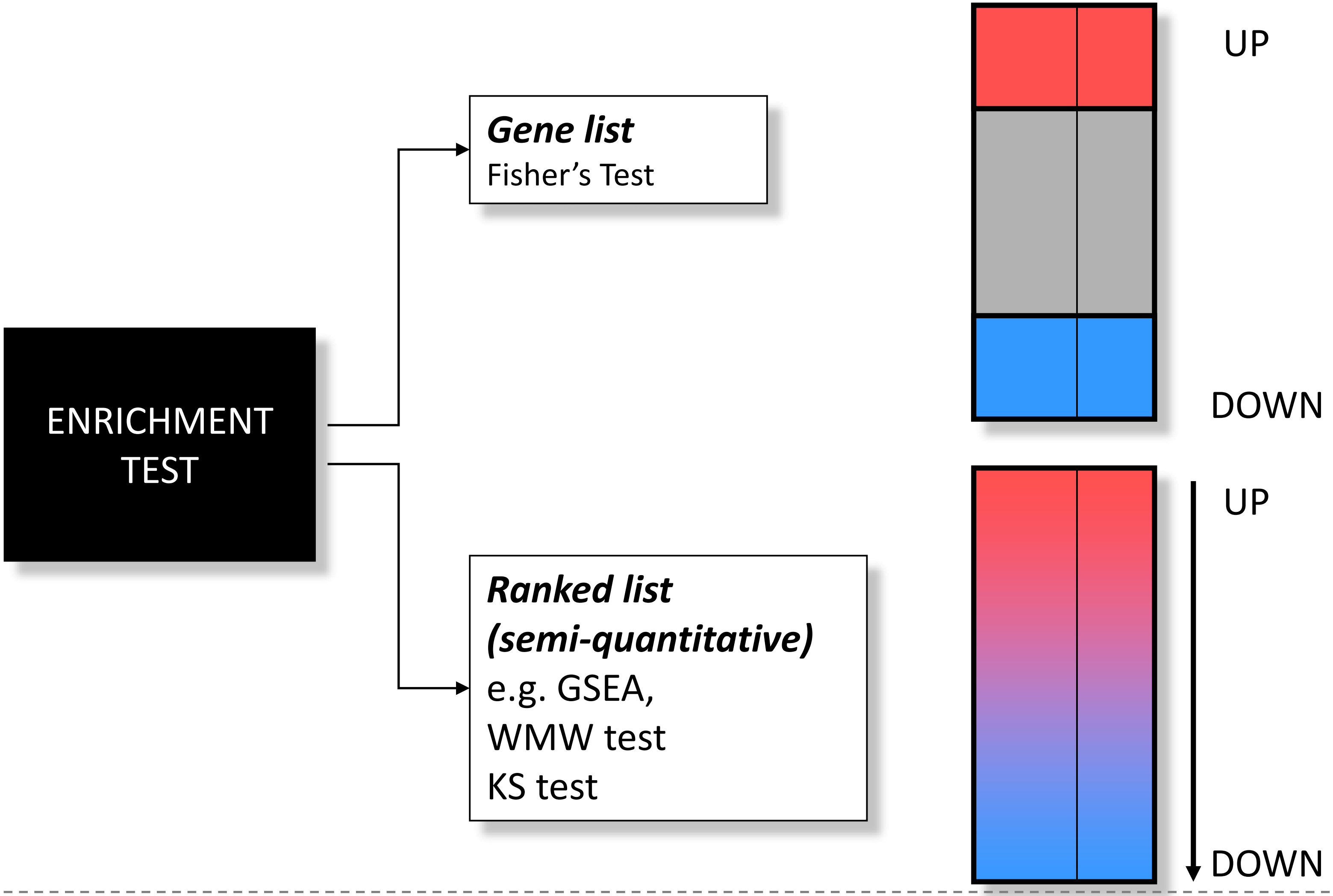
Beyond Fisher’s Exact Test

Possible problems with Fisher’s Exact 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

Solution: enrichment tests based on ranked lists

Beyond Fisher’s Exact Test



ENRICHMENT TEST

Gene list
Fisher’s Test

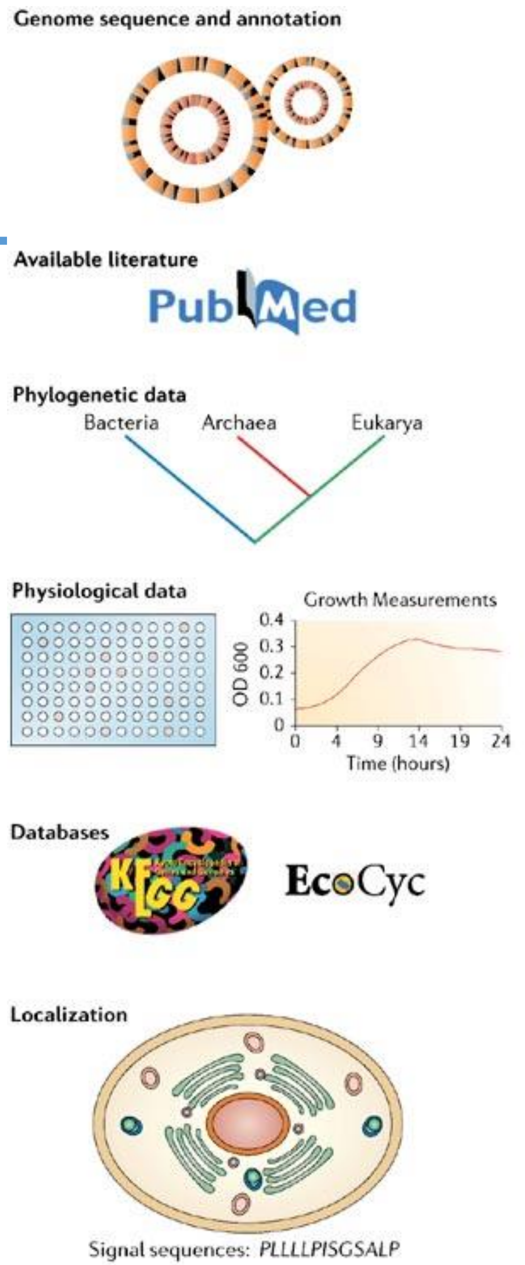
Ranked list (semi-quantitative)
e.g. GSEA,
WMW test
KS test

WMW - the Wilcoxon-Mann-Whitney test

KS - the Kolmogorov–Smirnov test

OUTLINE

- Single gene analysis / information
- Analysis of group of genes
- Gene ontology (GO)
- Enrichment analysis
  - Hypergeometric Test and Fisher exact test
  - GO Independence Assumption



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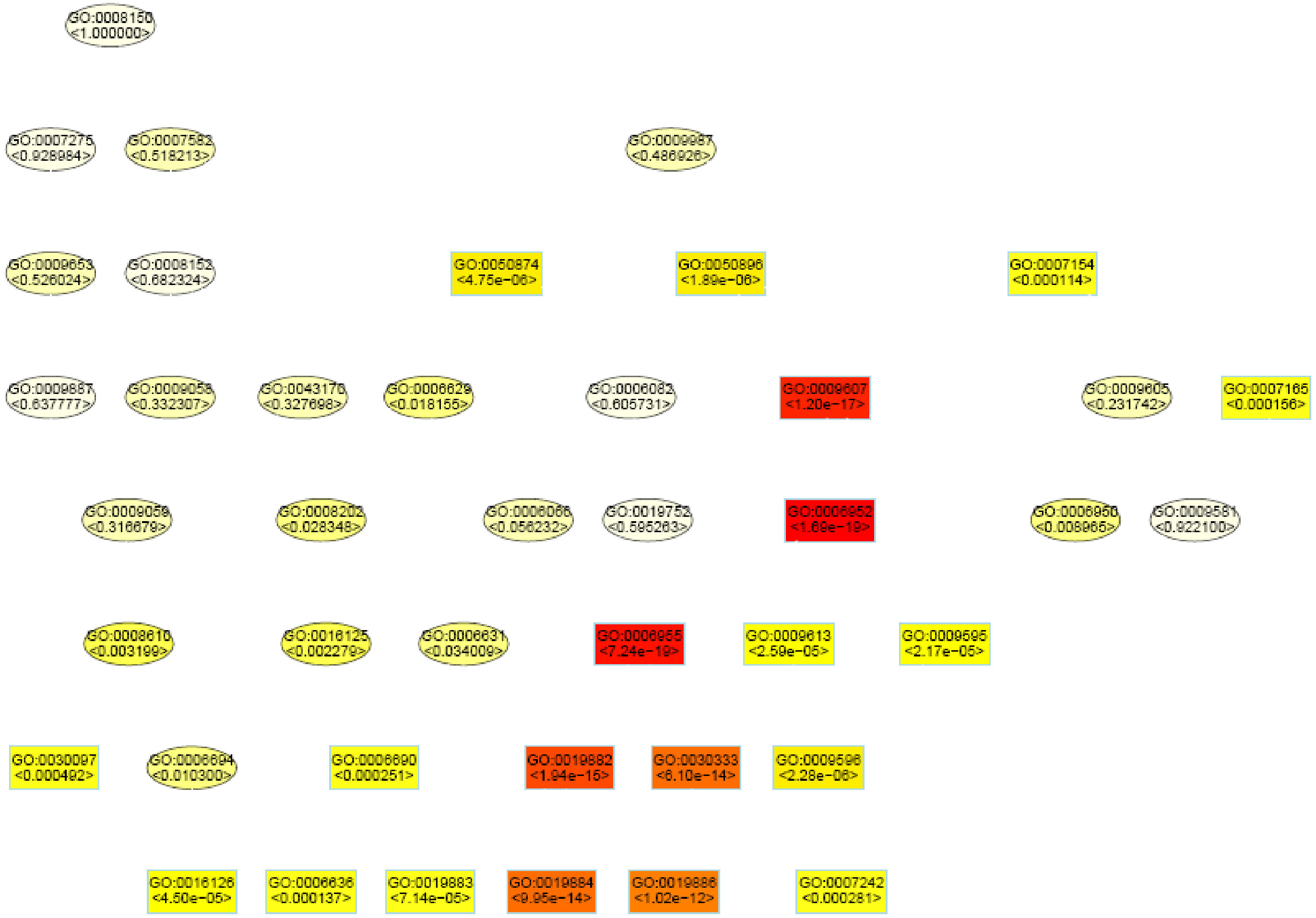
Term-for-term

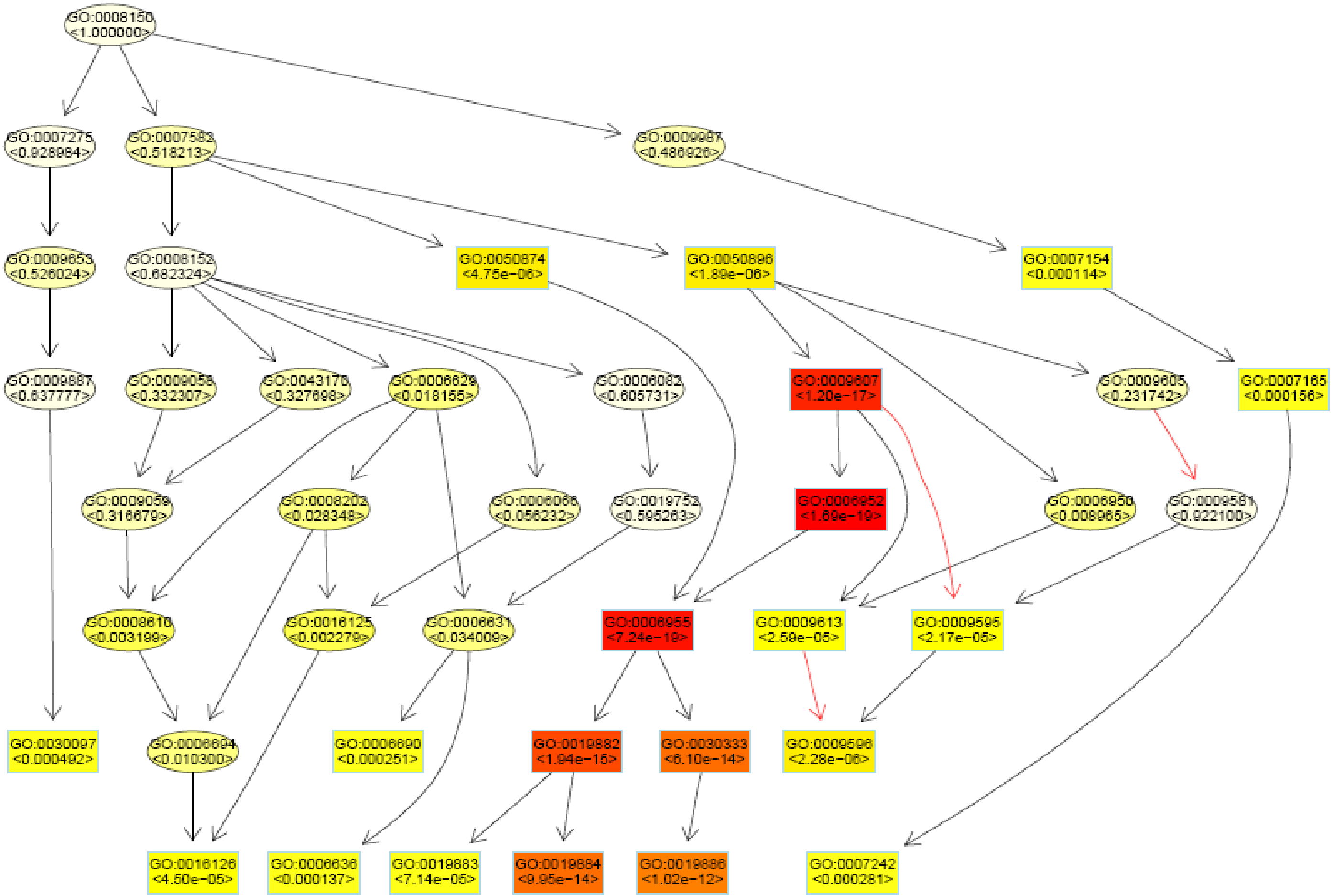
- The most common type of analysis
- Each term is considered independently of its neighbors in the GO tree
- Compares observed to expected and calculates significance

GO Independence Assumption



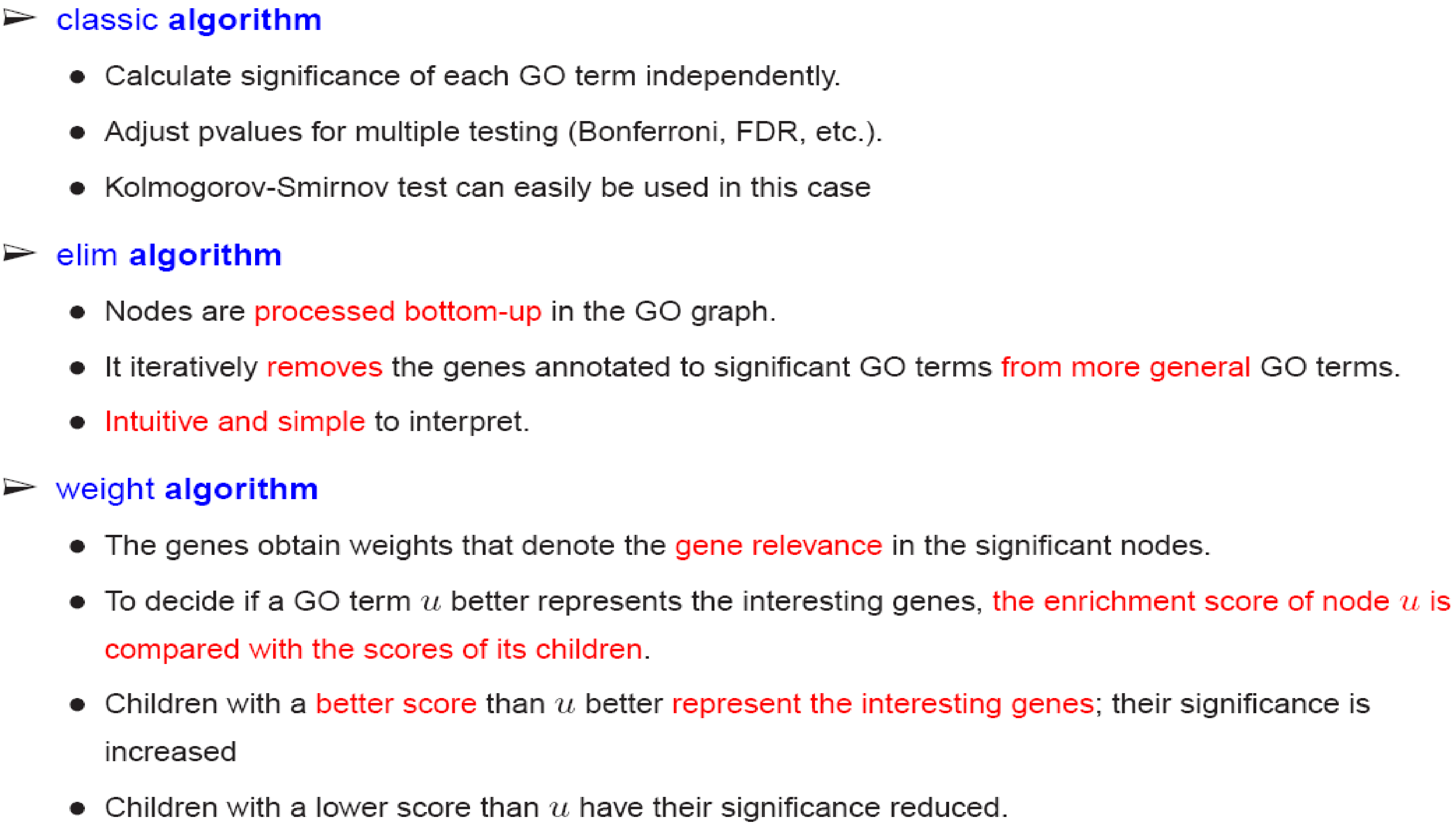
Note: The coloring of the nodes represent the *relative* significance of the GO terms: dark red is the most significant, light yellow is the least significant from the graph

GO Independence Assumption



Note: The coloring of the nodes represent the *relative* significance of the GO terms: dark red is the most significant, light yellow is the least significant from the graph

Algorithms review

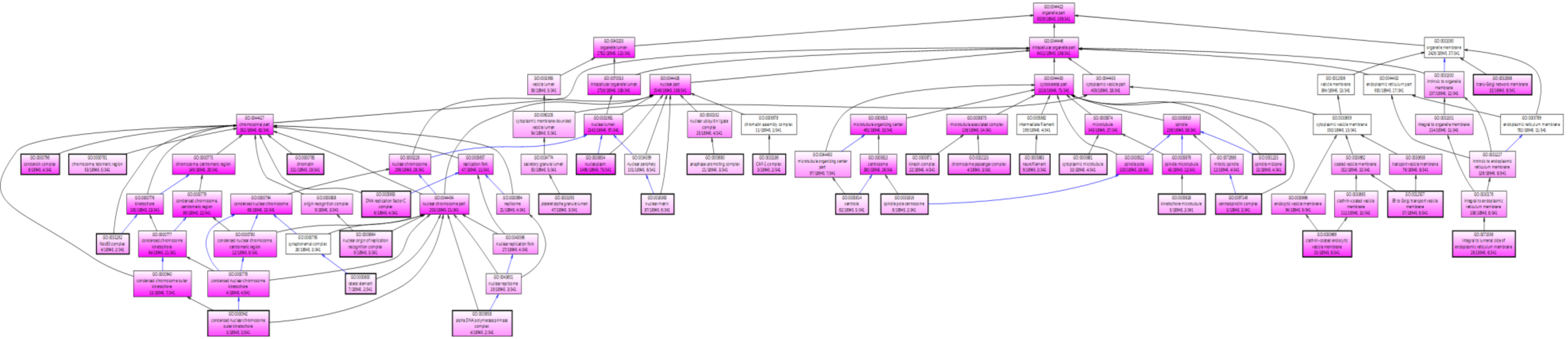


- classic **algorithm**
  - Calculate significance of each GO term independently.
  - Adjust pvalues for multiple testing (Bonferroni, FDR, etc.).
  - Kolmogorov-Smirnov test can easily be used in this case
- elim **algorithm**
  - Nodes are processed bottom-up in the GO graph.
  - It iteratively removes the genes annotated to significant GO terms from more general GO terms.
  - Intuitive and simple to interpret.
- weight **algorithm**
  - The genes obtain weights that denote the gene relevance in the significant nodes.
  - To decide if a GO term u better represents the interesting genes, the enrichment score of node u is compared with the scores of its children.
  - Children with a better score than u better represent the interesting genes; their significance is increased
  - Children with a lower score than u have their significance reduced.

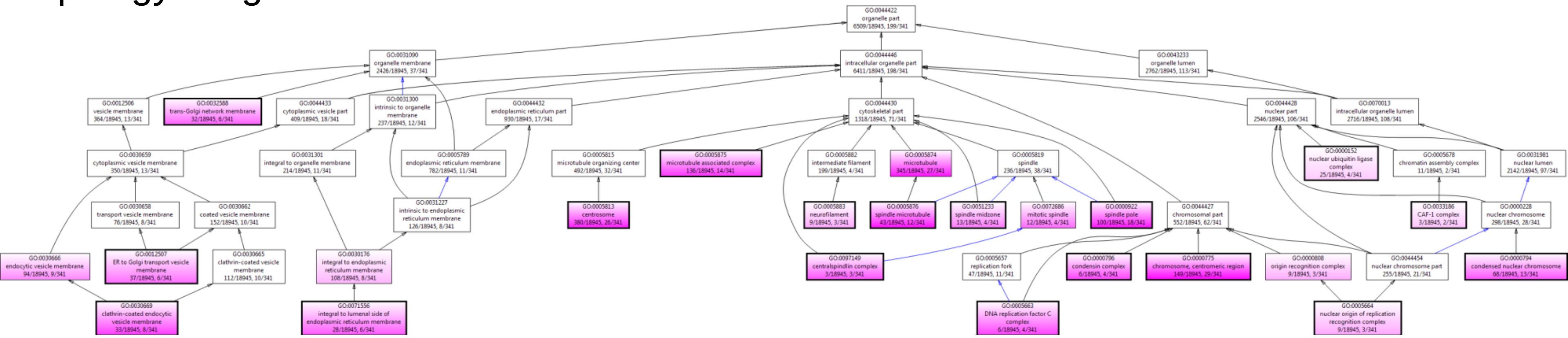
Alexa A, Rahnenführer J, Lengauer T. Bioinformatics. 2006 Jul 1;22(13):1600-7

Same input data – different results….

Term for term



Topology weighted


GO:0044422 organelle part 6509/18945, 199/341
GO:0031090 organelle membrane 2426/18945, 37/341
GO:0044446 intracellular organelle part 6411/18945, 198/341
GO:0043233 organelle lumen 2762/18945, 113/341
GO:0012506 vesicle membrane 364/18945, 13/341
GO:0032588 trans-Golgi network membrane 32/18945, 6/341
GO:0044433 cytoplasmic vesicle part 409/18945, 18/341
GO:0031300 intrinsic to organelle membrane 237/18945, 12/341
GO:0044432 endoplasmic reticulum part 930/18945, 17/341
GO:0044430 cytoskeletal part 1318/18945, 71/341
GO:0044428 nuclear part 2546/18945, 106/341
GO:0070013 intracellular organelle lumen 2716/18945, 108/341
GO:0030659 cytoplasmic vesicle membrane 350/18945, 13/341
GO:0031301 integral to organelle membrane 214/18945, 11/341
GO:0005789 endoplasmic reticulum membrane 782/18945, 11/341
GO:0005815 microtubule organizing center 492/18945, 32/341
GO:0005875 microtubule associated complex 136/18945, 14/341
GO:0005882 intermediate filament 199/18945, 4/341
GO:0005874 microtubule 345/18945, 27/341
GO:0005819 spindle 236/18945, 38/341
GO:0000152 nuclear ubiquitin ligase complex 25/18945, 4/341
GO:0005678 chromatin assembly complex 11/18945, 2/341
GO:0031981 nuclear lumen 2142/18945, 97/341
GO:0030658 transport vesicle membrane 76/18945, 8/341
GO:0030662 coated vesicle membrane 152/18945, 10/341
GO:0031227 intrinsic to endoplasmic reticulum membrane 126/18945, 8/341
GO:0005813 centrosome 380/18945, 26/341
GO:0005883 neurofilament 9/18945, 3/341
GO:0005876 spindle microtubule 43/18945, 12/341
GO:0051233 spindle midzone 13/18945, 4/341
GO:0072686 mitotic spindle 12/18945, 4/341
GO:0000922 spindle pole 100/18945, 18/341
GO:0044427 chromosomal part 552/18945, 62/341
GO:0033186 CAF-1 complex 3/18945, 2/341
GO:0000228 nuclear chromosome 298/18945, 28/341
GO:0030666 endocytic vesicle membrane 94/18945, 9/341
GO:0012507 ER to Golgi transport vesicle membrane 37/18945, 6/341
GO:0030665 clathrin-coated vesicle membrane 112/18945, 10/341
GO:0030176 integral to endoplasmic reticulum membrane 108/18945, 8/341
GO:0097149 centralspindlin complex 3/18945, 3/341
GO:0005657 replication fork 47/18945, 11/341
GO:0000796 condensin complex 6/18945, 4/341
GO:0000775 chromosome, centromeric region 149/18945, 29/341
GO:0000808 origin recognition complex 9/18945, 3/341
GO:0044454 nuclear chromosome part 255/18945, 21/341
GO:0000794 condensed nuclear chromosome 68/18945, 13/341
GO:0030669 clathrin-coated endocytic vesicle membrane 33/18945, 8/341
GO:0071556 integral to lumenal side of endoplasmic reticulum membrane 28/18945, 6/341
GO:0005663 DNA replication factor C complex 6/18945, 4/341
GO:0005664 nuclear origin of replication recognition complex 9/18945, 3/341

Thanks to:

Dr. Esti Feldmeser & Dr. Shifra Ben Dor for interchanging and improving slides

for
your attention
Questions?