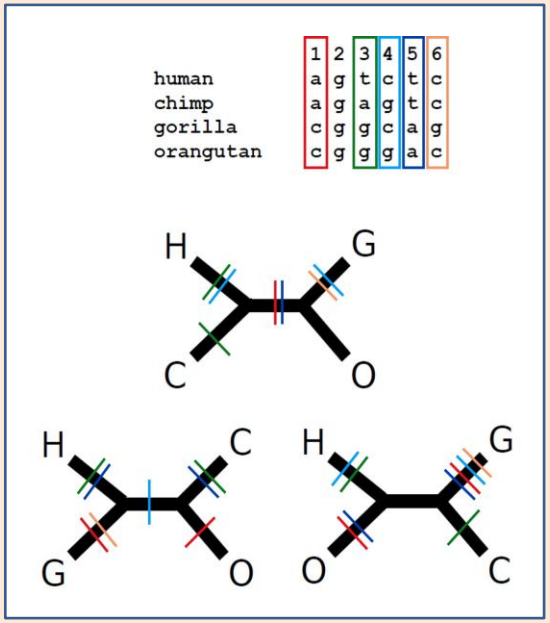
Gene Ontology and Functional Enrichment

Genome 559: Introduction to Statistical and Computational Genomics

Elhanan Borenstein

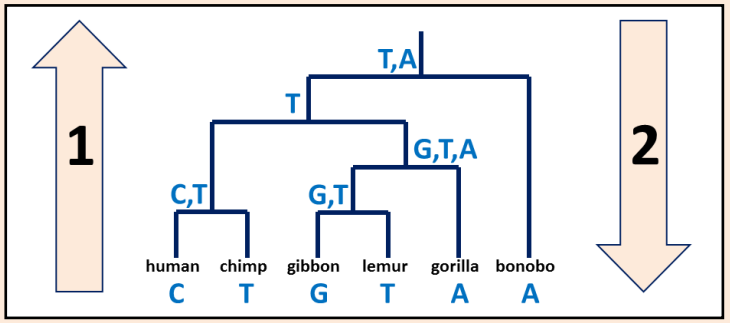
A quick review

- The parsimony principle:
  - Find the tree that requires the fewest evolutionary changes!
- A fundamentally different method:
  - Search rather than reconstruct
- Parsimony algorithm
  1. Construct all possible trees
  2. For each site in the alignment and for each tree count the minimal number of changes required
  3. Add sites to obtain the total number of changes required for each tree
  4. Pick the tree with the lowest score



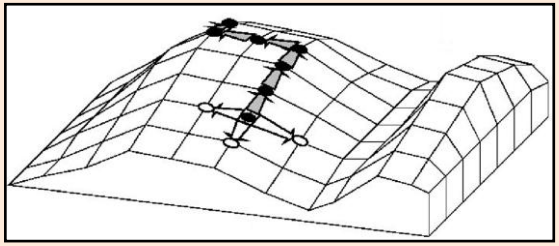
A quick review – cont'

- Small vs. large parsimony



- Fitch's algorithm:
  1. **Bottom-up phase**: Determine the set of possible states
  2. **Top-down phase**: Pick a state for each internal node

- Searching the tree space:
  - Exhaustive search, branch and bound
  - Hill climbing with Nearest-Neighbor Interchange



- Branch confidence and bootstrap support

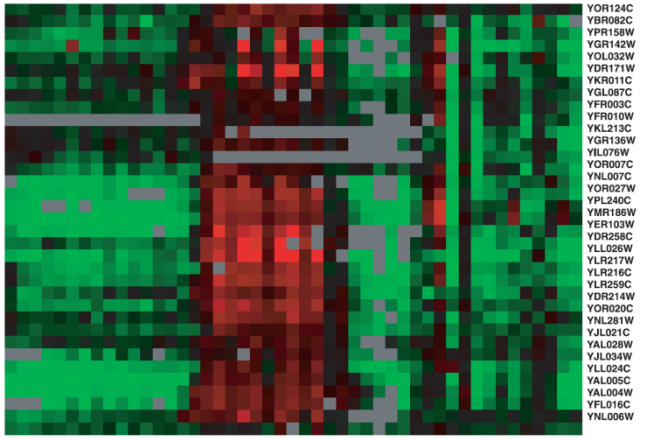
From sequence to function

Which molecular processes/functions are involved in a certain phenotype - disease, response, development, etc.

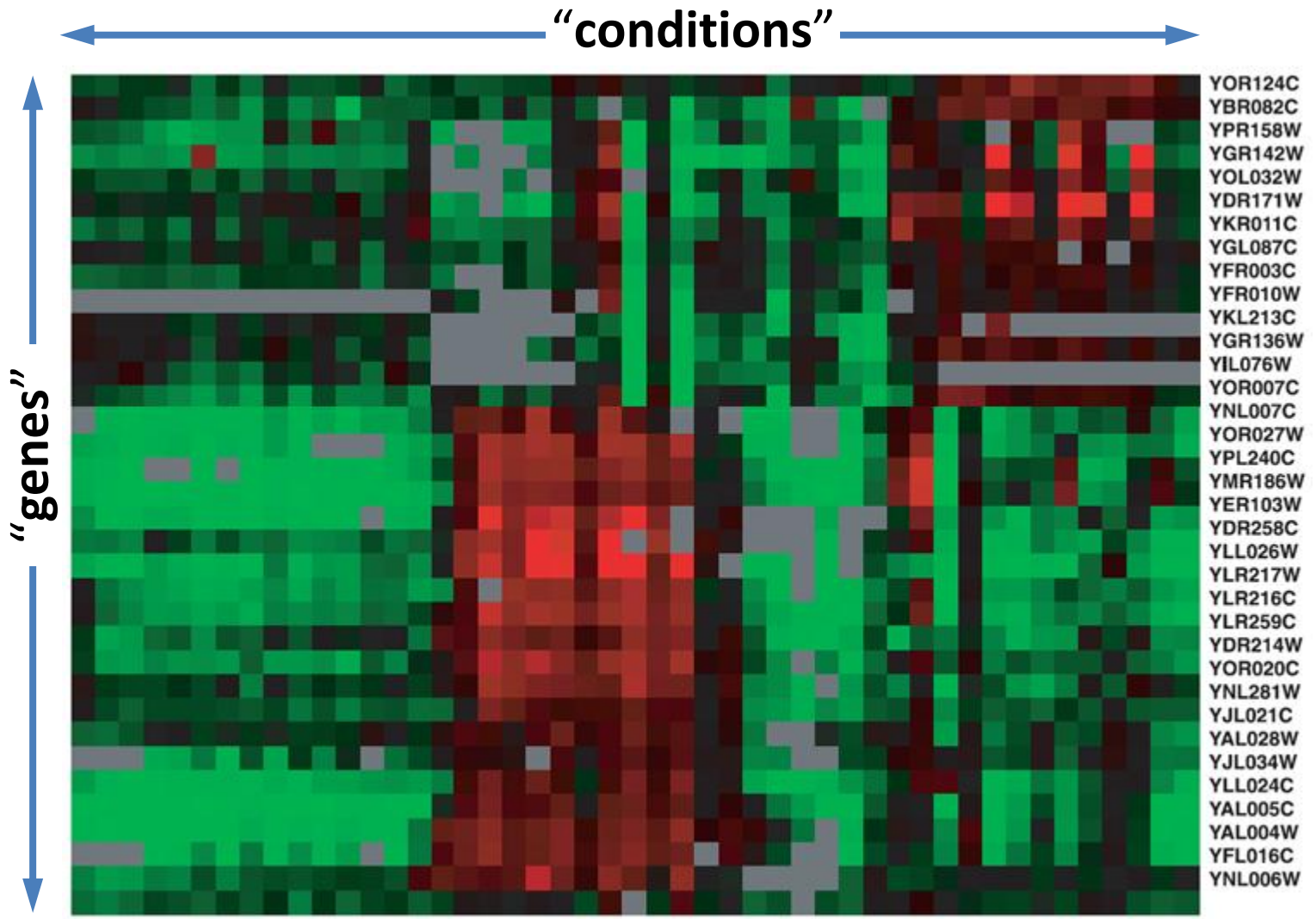
(what is the cell doing vs. what it could possibly do)

- Measuring gene expression:
  - (Northern blots and RT-qPCR)
  - **Microarray**
  - RNA-Seq

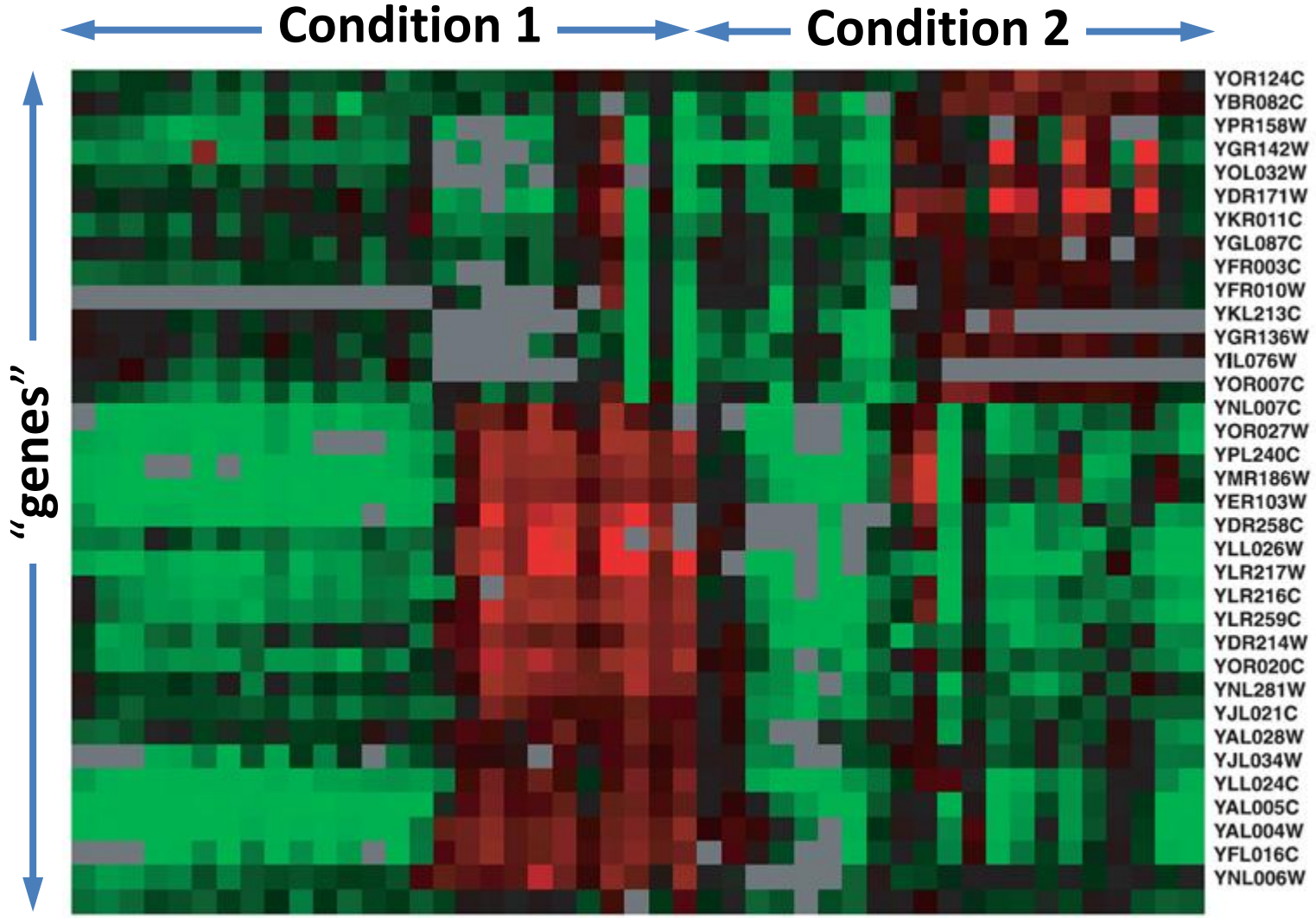
Gene expression profiling



Gene expression profiling

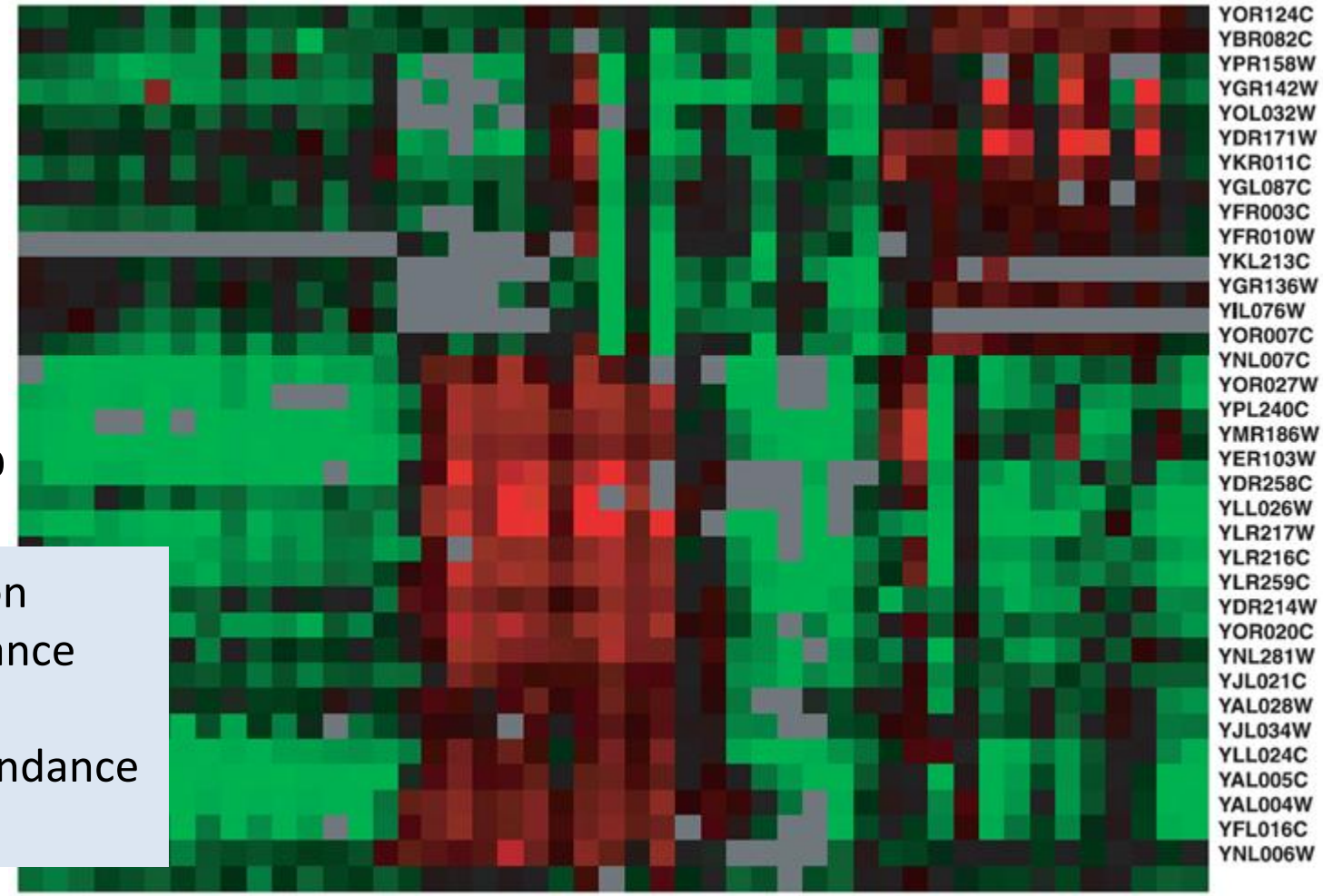


Comparative analysis



Different techniques, same structure

- Disease vs. control
- Across tissues
- Across time
- Across environments
- Many more …



- Gene expression
- Protein abundance
- Mutation rate
- Short read abundance
- Many more …

Back in the good old days …

1. Find the set of differentially expressed genes.
2. Survey the literature to obtain insights about the functions that differentially expressed genes are involved in.
3. Group together genes with similar functions.
4. Identify functional categories with many differentially expressed genes.

Conclude that these functions are important in disease/condition under study

The good old days were not so good!

Time-consuming

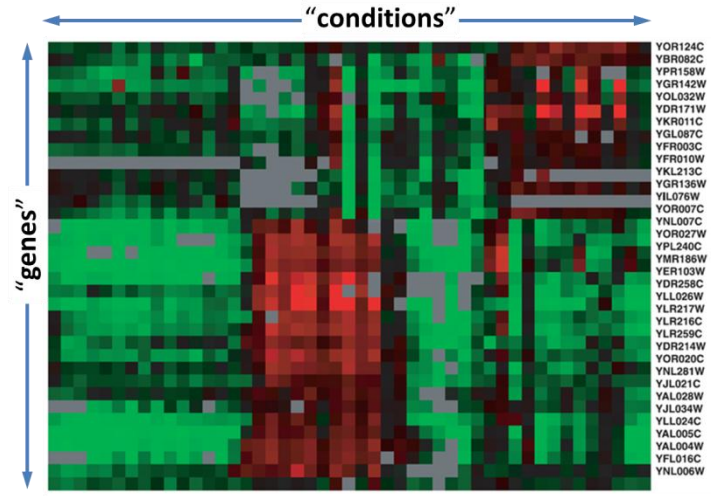
Not systematic

Extremely subjective

No statistical validation

What do we need?

- A shared functional vocabulary
- Systematic linkage between genes and functions
- A way to identify genes relevant to the condition under study
- Statistical analysis
  (combining all of the above to identify cellular functions that contributed to the disease or condition under study)
- A way to identify “related” genes



What do we need?

- A shared functional vocabulary

Gene Ontology

Annotation

- Systematic linkage between genes and functions

- A way to identify genes relevant to the condition under study

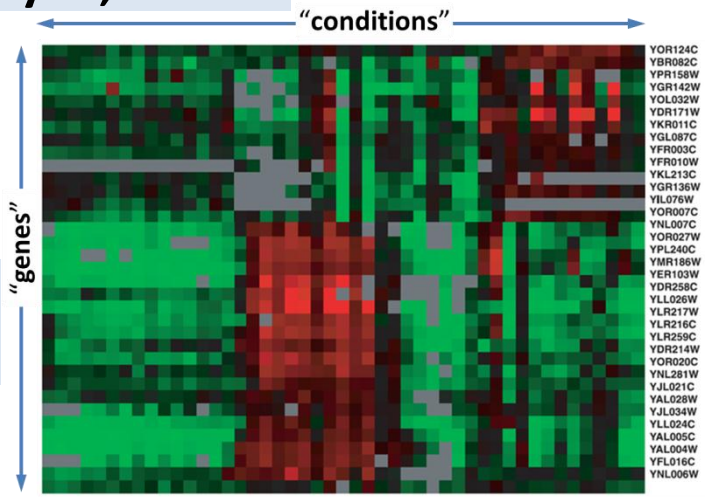
Fold change, Ranking, ANOVA

- Statistical analysis
(combining all of the above to identify cellular functions that contributed to the disease or condition under study)

Enrichment analysis, GSEA

- A way to identify “related” genes

Clustering, classification



The Gene Ontology (GO) Project

- A major bioinformatics initiative with the aim of standardizing the representation of gene and gene product attributes across species and databases.

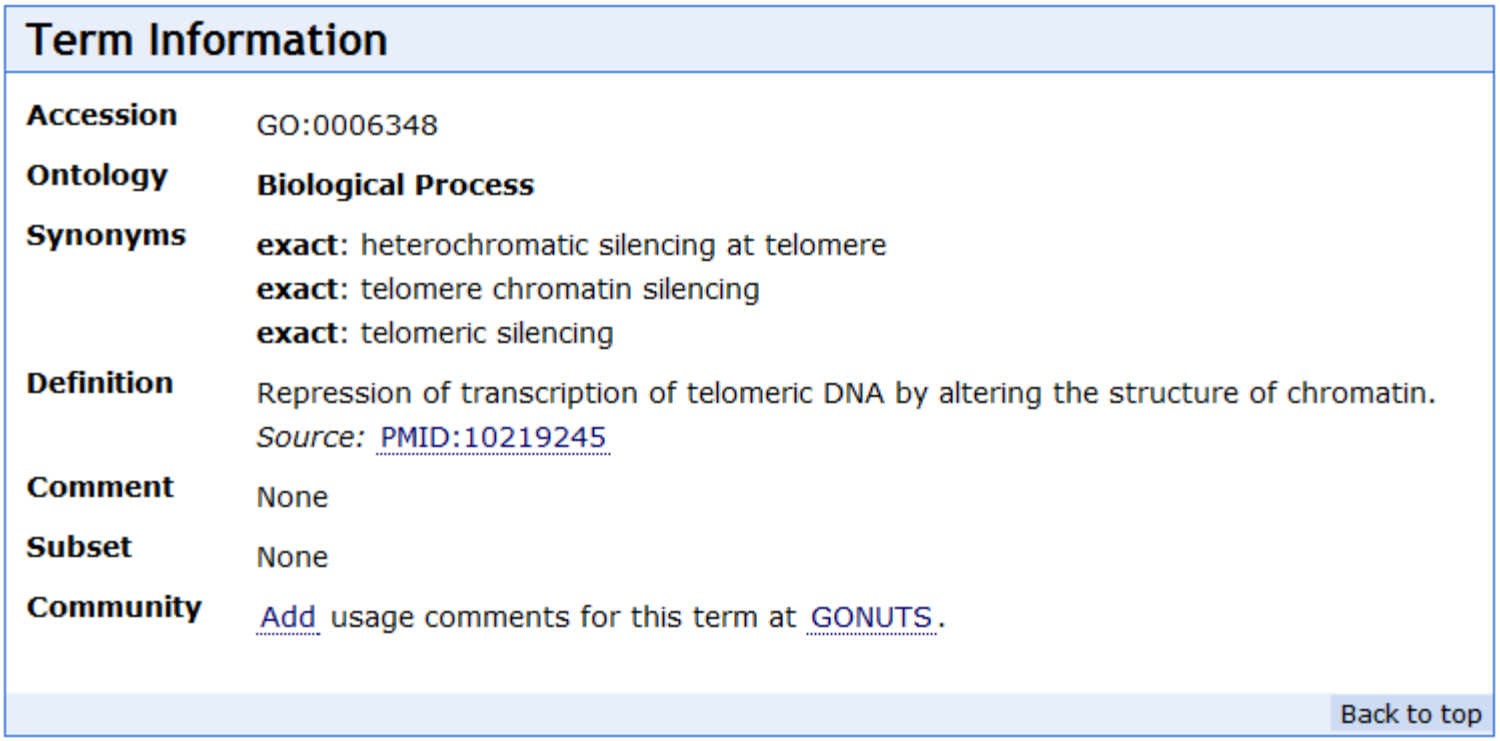
- Three goals:
  1. *Maintain and further develop its controlled **vocabulary** of gene and gene product attributes*

  2. ***Annotate** genes and gene products, and assimilate and disseminate annotation data*

  3. *Provide **tools** to facilitate access to all aspects of the data provided by the Gene Ontology project*

GO terms

- The Gene Ontology (GO) is a **controlled vocabulary**, a set of standard **terms** (words and phrases) used for indexing and retrieving information.



Term Information

Accession	GO:0006348
Ontology	**Biological Process**
Synonyms	**exact**: heterochromatic silencing at telomere **exact**: telomere chromatin silencing **exact**: telomeric silencing
Definition	Repression of transcription of telomeric DNA by altering the structure of chromatin. *Source:* PMID:10219245
Comment	None
Subset	None
Community	Add usage comments for this term at GONUTS.

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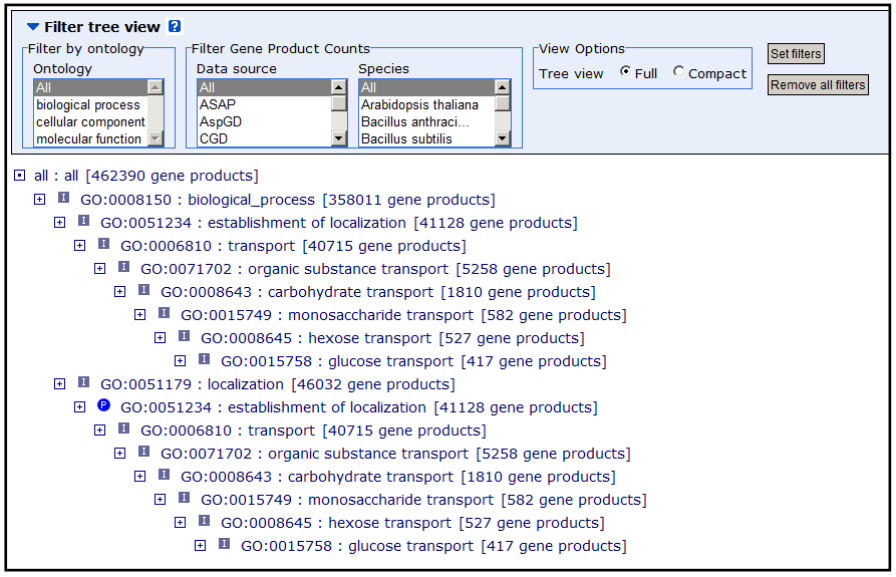
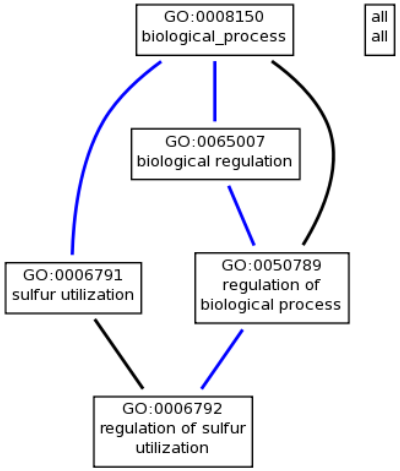
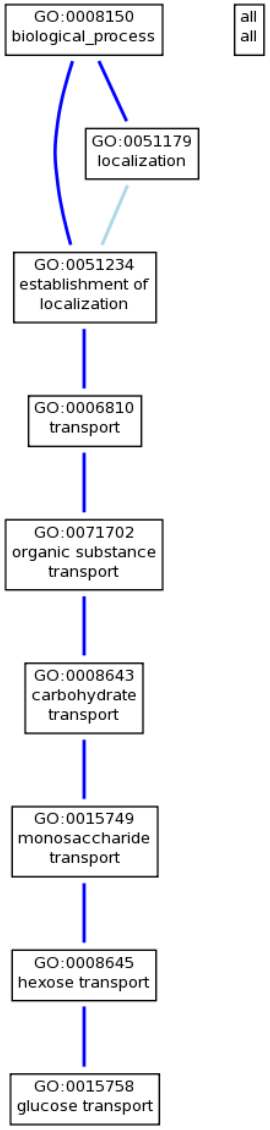
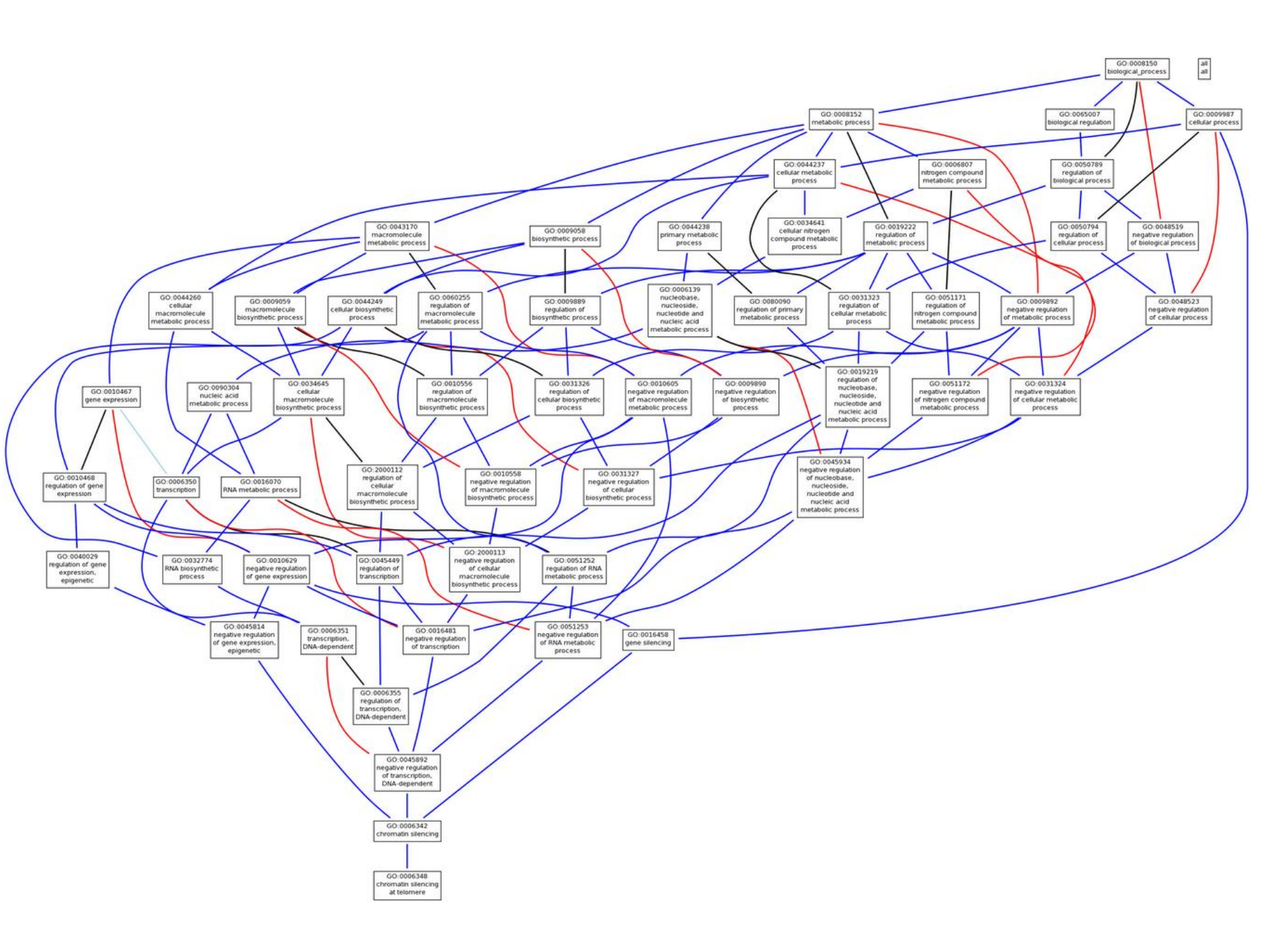
Ontology structure

- GO also defines the **relationships** between the terms, making it a **structured** vocabulary.
- GO is structured as a **directed acyclic graph**, and each term has defined relationships to one or more other terms.








GO:0008150
biological_process
all
all
GO:0008152
metabolic process
GO:0065007
biological regulation
GO:0009987
cellular process
GO:0044237
cellular metabolic process
GO:0006807
nitrogen compound metabolic process
GO:0050789
regulation of biological process
GO:0043170
macromolecule metabolic process
GO:0009058
biosynthetic process
GO:0044238
primary metabolic process
GO:0034641
cellular nitrogen compound metabolic process
GO:0019222
regulation of metabolic process
GO:0050794
regulation of cellular process
GO:0048519
negative regulation of biological process
GO:0044260
cellular macromolecule metabolic process
GO:0009059
macromolecule biosynthetic process
GO:0044249
cellular biosynthetic process
GO:0060255
regulation of macromolecule metabolic process
GO:0009889
regulation of biosynthetic process
GO:0006139
nucleobase, nucleoside, nucleotide and nucleic acid metabolic process
GO:0080090
regulation of primary metabolic process
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regulation of nitrogen compound metabolic process
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negative regulation of metabolic process
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gene expression
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nucleic acid metabolic process
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cellular macromolecule biosynthetic process
GO:0010556
regulation of macromolecule biosynthetic process
GO:0031326
regulation of cellular biosynthetic process
GO:0010605
negative regulation of macromolecule metabolic process
GO:0009890
negative regulation of biosynthetic process
GO:0019219
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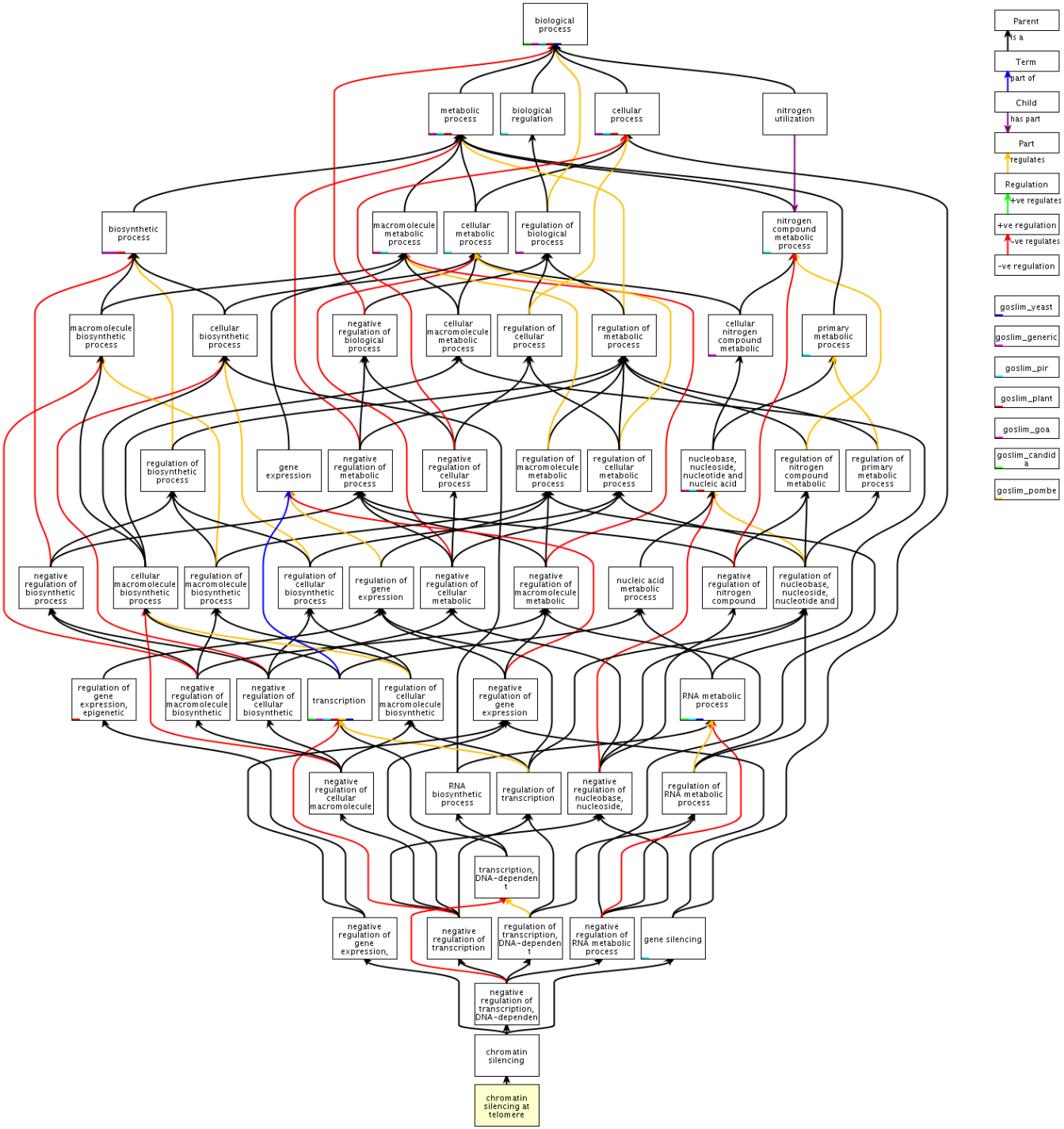
GO:0051172
negative regulation of nitrogen compound metabolic process
GO:0031324
negative regulation of cellular metabolic process
GO:0010468
regulation of gene expression
GO:0006350
transcription
GO:0016070
RNA metabolic process
GO:2000112
regulation of cellular macromolecule biosynthetic process
GO:0010558
negative regulation of macromolecule biosynthetic process
GO:0031327
negative regulation of cellular biosynthetic process
GO:0045934
negative regulation of nucleobase, nucleoside, nucleotide and nucleic acid metabolic process
GO:0040029
regulation of gene expression, epigenetic
GO:0032774
RNA biosynthetic process
GO:0010629
negative regulation of gene expression
GO:0045449
regulation of transcription
GO:2000113
negative regulation of cellular macromolecule biosynthetic process
GO:0051252
regulation of RNA metabolic process
GO:0045814
negative regulation of gene expression, epigenetic
GO:0006351
transcription, DNA-dependent
GO:0016481
negative regulation of transcription
GO:0051253
negative regulation of RNA metabolic process
GO:0016458
gene silencing
GO:0006355
regulation of transcription, DNA-dependent
GO:0045892
negative regulation of transcription, DNA-dependent
GO:0006342
chromatin silencing
GO:0006348
chromatin silencing at telomere


biological process
metabolic process
biological regulation
cellular process
nitrogen utilization
biosynthetic process
macromolecule metabolic process
cellular metabolic process
regulation of biological process
nitrogen compound metabolic process
macromolecule biosynthetic process
cellular biosynthetic process
negative regulation of biological process
cellular macromolecule metabolic process
regulation of cellular process
regulation of metabolic process
cellular nitrogen compound metabolic
primary metabolic process
regulation of biosynthetic process
gene expression
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negative regulation of cellular process
regulation of macromolecule metabolic process
regulation of cellular metabolic process
nucleobase, nucleoside, nucleotide and nucleic acid
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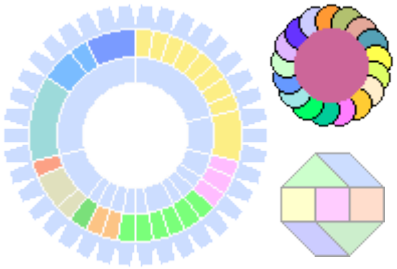
regulation of transcription, DNA-dependent
negative regulation of RNA metabolic process
gene silencing
negative regulation of transcription, DNA-dependen
chromatin silencing
chromatin silencing at telomere
Parent
is a
Term
part of
Child
has part
Part
regulates
Regulation
+ve regulates
+ve regulation
-ve regulates
-ve regulation
goslim_yeast
goslim_generic
goslim_pir
goslim_plant
goslim_goa
goslim_candida
goslim_pombe

Ontology and annotation databases

eggNOG



Clusters of Orthologous Groups (COG)

“The nice thing about standards is that there are so many to choose from”

Andrew S. Tanenbaum

What do we need?

- A shared functional vocabulary ✓
- Systematic linkage between genes and functions ✓
- A way to identify genes relevant to the condition under study
- Statistica
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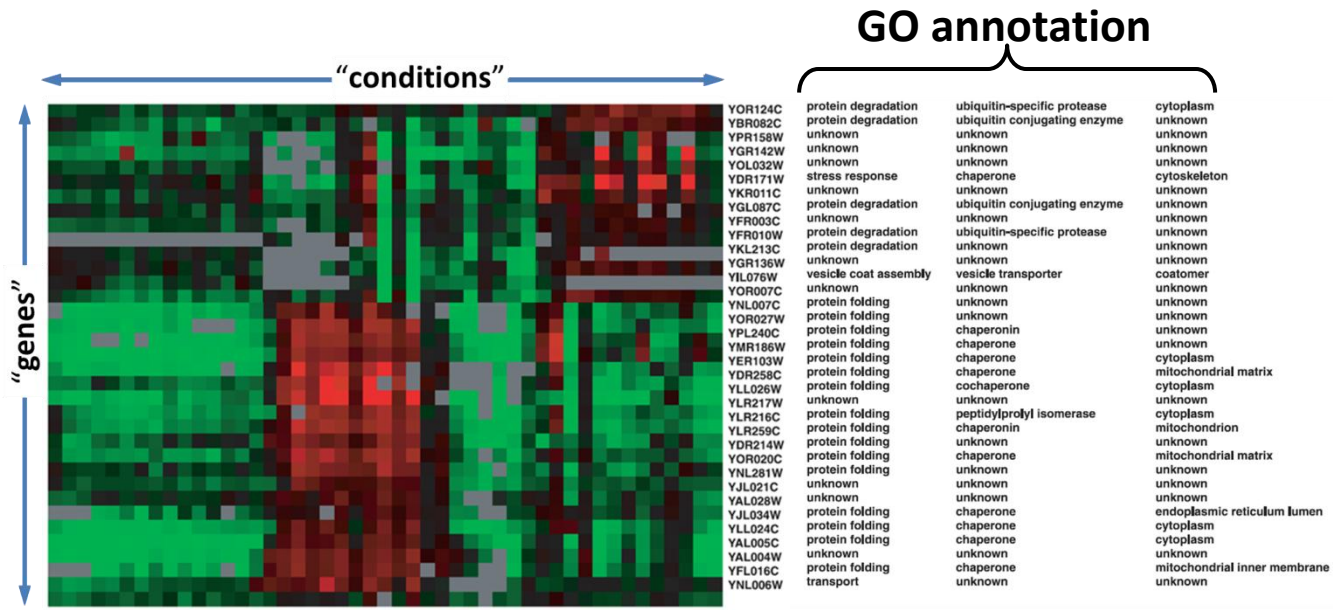
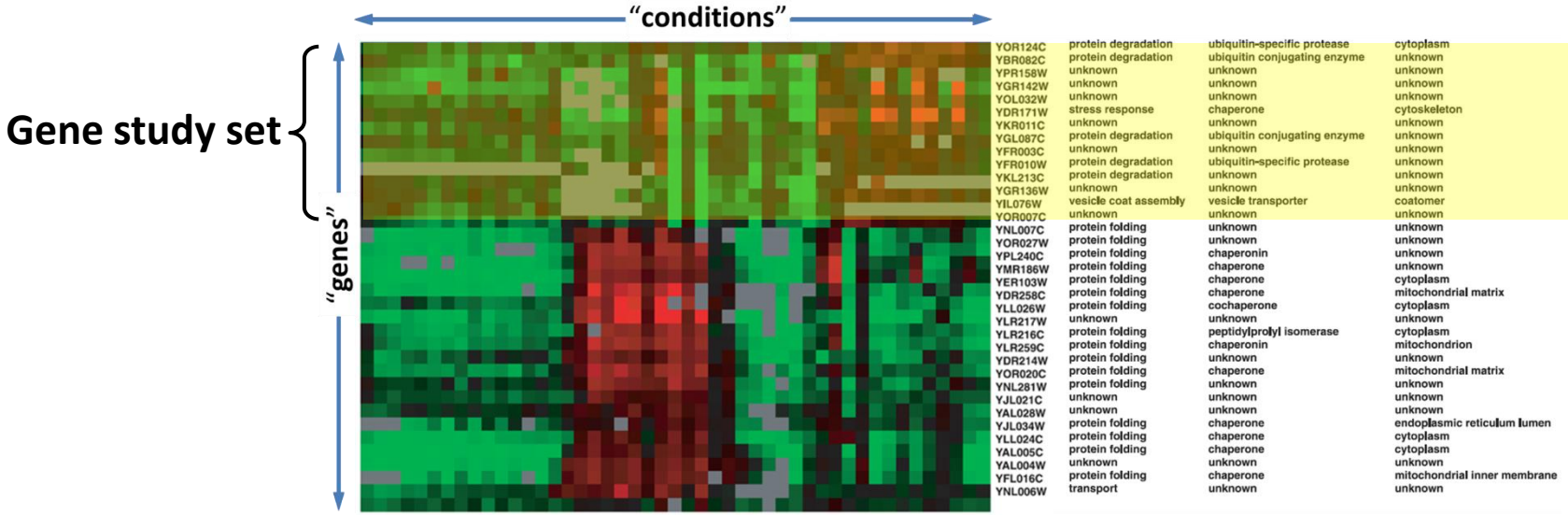
- A way to

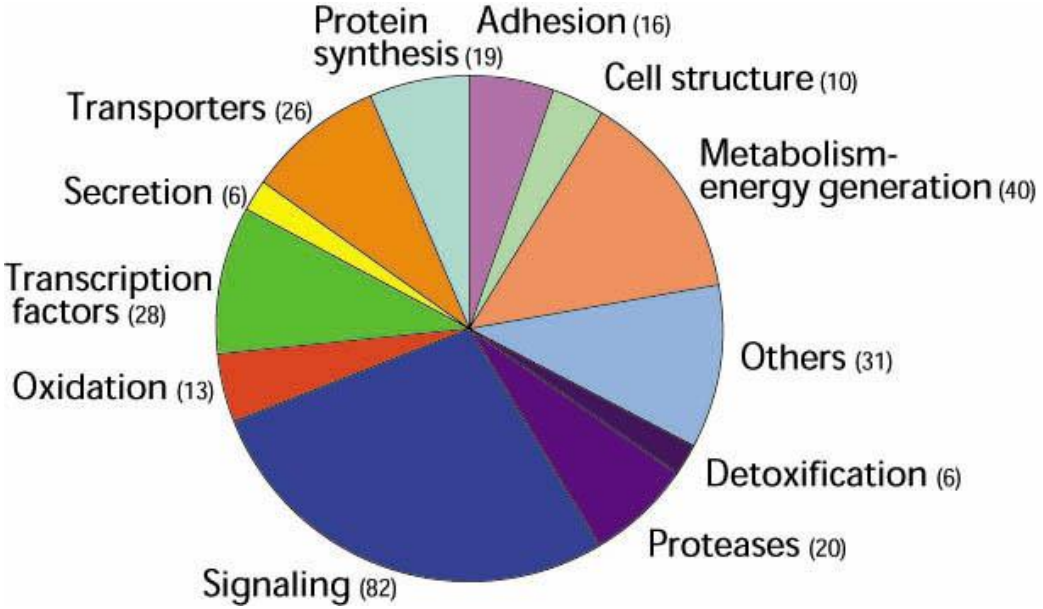


Picking “relevant” genes

- In most cases, we will consider differential expression as a marker:
  - Fold change cutoff (e.g., > two fold change)
  - Fold change rank (e.g., top 10%)
  - Significant differential expression (e.g., ANOVA)
    (don’t forget to correct for multiple testing, e.g., Bonferroni or FDR)



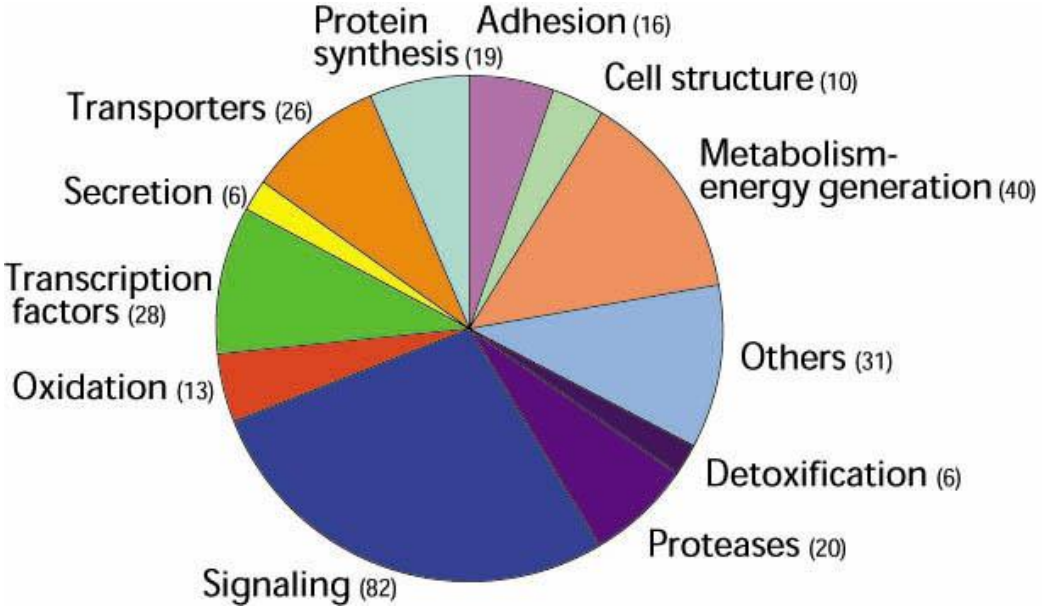
Enrichment analysis



Signalling category contains 27.6% of all genes in the study set - **by far the largest category.** Reasonable to conclude that signaling may be important in the condition under study

Functional category	# of genes in the study set	%
Signaling	82	27.6
Metabolism	40	13.5
Others	31	10.4
Trans factors	28	9.4
Transporters	26	8.8
Proteases	20	6.7
Protein synthesis	19	6.4
Adhesion	16	5.4
Oxidation	13	4.4
Cell structure	10	3.4
Secretion	6	2.0
Detoxification	6	2.0

Enrichment analysis – the wrong way



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Secretion	6	2.0
Detoxification	6	2.0

Enrichment analysis – the wrong way

- What if ~27% of the genes on the array are involved in signaling?
  - The number of signaling genes in the set is what expected by chance.
  - We need to consider not only the number of genes in the set for each category, but also the total number on the array.
- We want to know which category is **over-represented** (occurs more times than expected by chance).

Functional category	# of genes in the study set	%	% on array
Signaling	82	27.6%	26%
Metabolism	40	13.5%	15%
Others	31	10.4%	11%
Trans factors	28	9.4%	10%
Transporters	**26**	**8.8%**	**2%**
Proteases	20	6.7%	7%
Protein synthesis	19	6.4%	7%
Adhesion	16	5.4%	6%
Oxidation	13	4.4%	4%
Cell structure	**10**	**3.4%**	**8%**
Secretion	6	2.0%	2%
Detoxification	6	2.0%	2%

Enrichment analysis – the right way

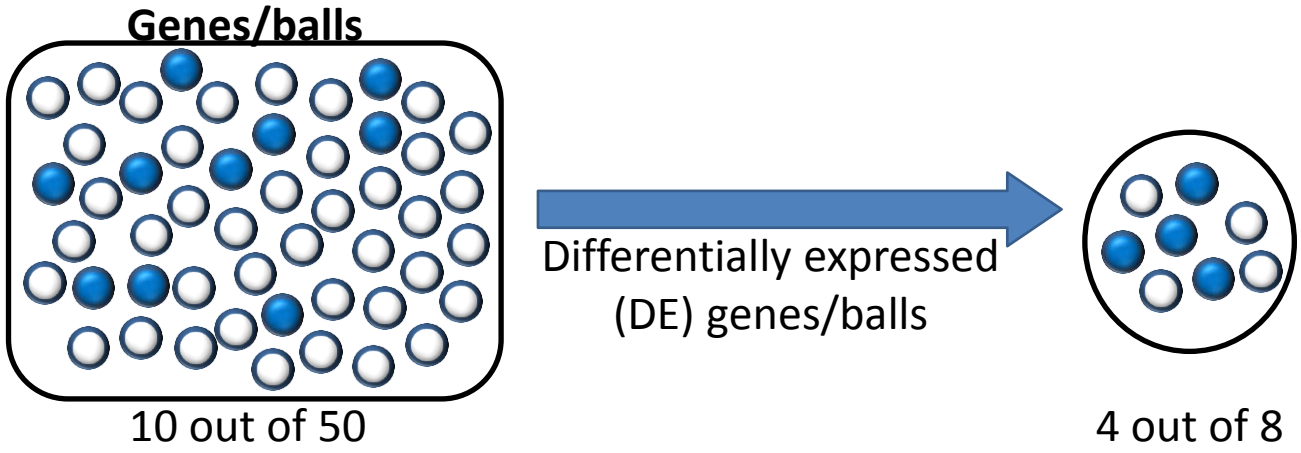
Say, the microarray contains 50 genes, 10 of which are annotated as 'signaling'. Your expression analysis reveals 8 differentially expressed genes, 4 of which are annotated as 'signaling'.
Is this significant?

A statistical test, based on a **null model**

Assume the study set has nothing to do with the specific function at hand and was selected randomly, would we be surprised to see this number of genes annotated with this function in the study set?

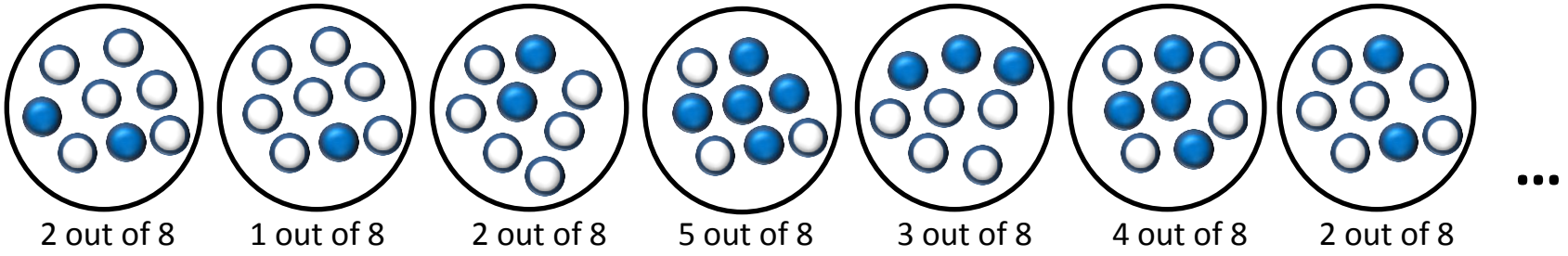
The "urn" version: You pick a ranndon set of 8 balls from an urn that contains 50 balls: 40 white and 10 blue. How surprised will you be to find that 4 of the balls you picked are blue?

A quick review: Modified Fisher's exact test



Do I have a surprisingly high number of blue genes?

Null model: the 8 genes/balls are selected randomly



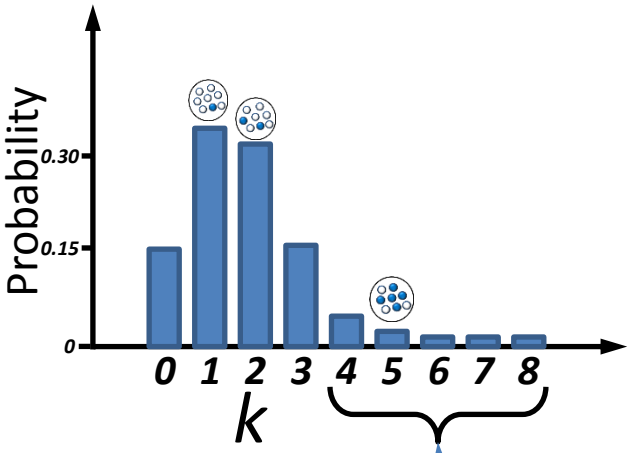
So, if you have 50 balls, 10 of them are blue, and you pick 8 balls randomly, what is the probability that *k* of them are blue?

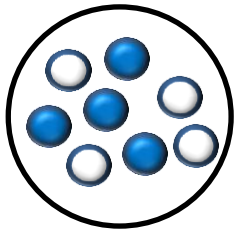
A quick review: Modified Fisher's exact test

Hypergeometric distribution

$$\mathbb{P}(\sigma_t = k) = \frac{\binom{m_t}{k}\binom{m-m_t}{n-k}}{\binom{m}{n}}$$

m=50, m_t=10, n=8





So … do I have a surprisingly high number of blue genes?

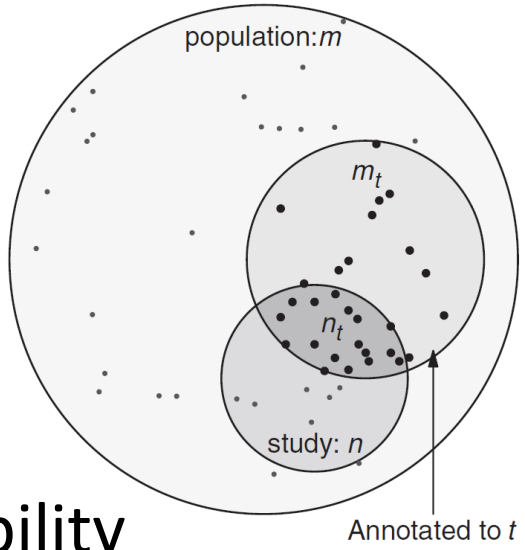
What is the probability of getting at least 4 blue genes in the null model?

$P(\sigma_t >= 4)$

Modified Fisher's Exact Test

- Let ***m*** denote the total number of genes in the array and ***n*** the number of genes in the study set.

- Let m_t denote the total number of genes annotated with function ***t*** and n_t the number of genes in the study set annotated with this function.



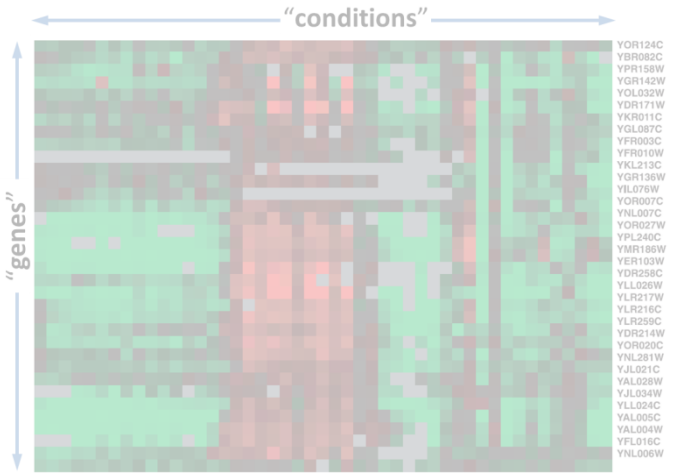
- We are interested in knowing the probability of seeing n_t or more annotated genes!

$$\mathbb{P}(\sigma_t \geq n_t) = \sum_{k=n_t}^{\min(m_t,n)} \frac{\binom{m_t}{k}\binom{m-m_t}{n-k}}{\binom{m}{n}}$$

(This is equivalent to a one-sided Fisher exact test)

So … what do we have so far?

- A shared functional vocabulary ✓
- Systematic linkage between genes and functions ✓
- A way to identify genes relevant to the condition under study ✓
- Statistical analysis ✓
  (combining all of the above to identify cellular functions that contributed to the disease or condition under study)
- A way to identify “related” genes



Still far from being perfect!

- A shared functional vocabulary
- Systematic linkage between genes and functions

Arbitrary!

Considers only a few genes

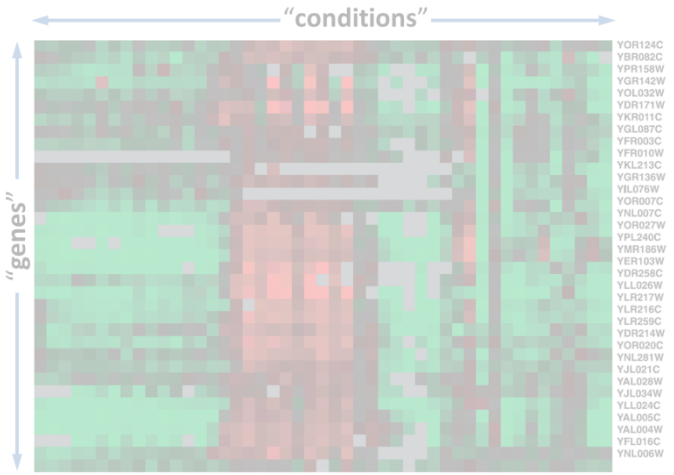
- A way to identify genes relevant to the condition under study

Ignores links between GO categories

Limited hypotheses

Simplistic null model!

- Statistical analysis
  (combining all of the above to identify cellular functions that contributed to the disease or condition under study)



- A way to identify "related" genes

GO domains

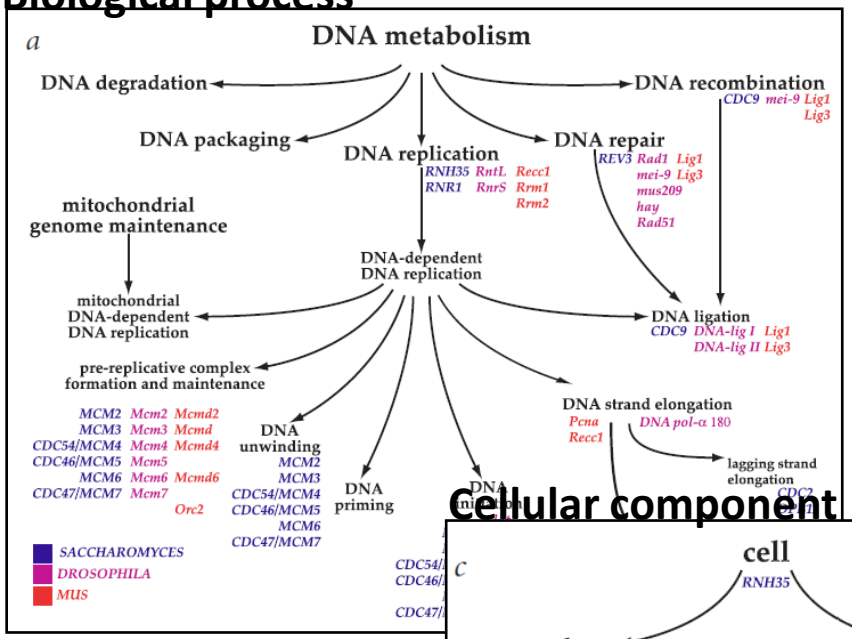
- Three ontology domains:
  1. **Molecular function:** basic activity or task
     e.g. catalytic activity, calcium ion binding
  2. **Biological process:** broad objective or goal
     e.g. signal transduction, immune response
  3. **Cellular component:** location or complex
     e.g. nucleus, mitochondrion

- Genes can have multiple annotations:

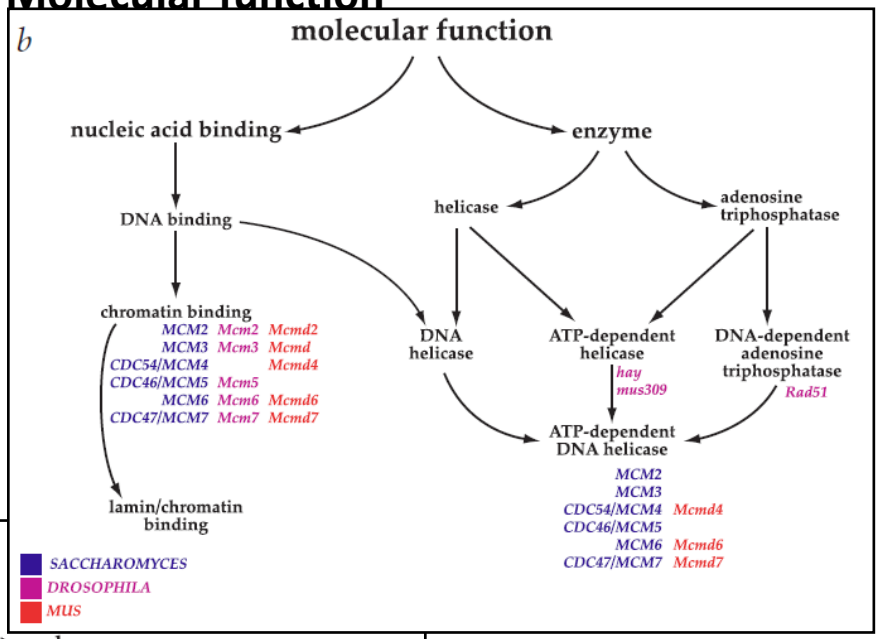
For example, the gene product cytochrome c can be described by the molecular function term oxidoreductase activity, the biological process termsoxidative phosphorylation and induction of cell death, and the cellular component terms mitochondrial matrix and mitochondrial inner membrane.

Go domains

Biological process



Molecular function



Cellular component