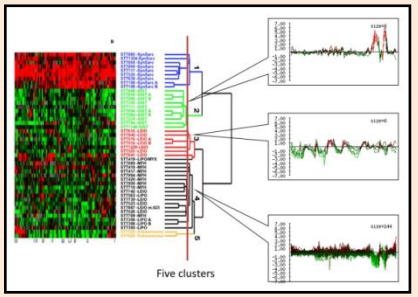
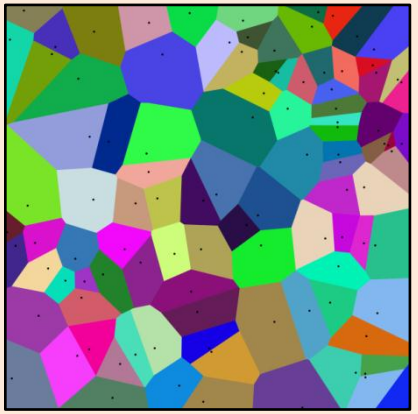
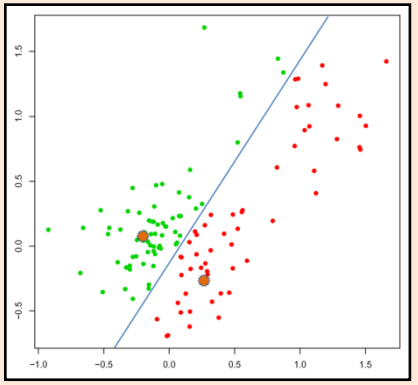
# A quick review

- **The clustering problem:**
  - partition genes into distinct sets with high homogeneity and high separation
- **Hierarchical clustering algorithm:**
  1. Assign each object to a separate cluster.
  2. Regroup the pair of clusters with shortest distance.
  3. Repeat 2 until there is a single cluster.
- Many possible distance metrics
- **K-mean clustering algorithm:**
  1. Arbitrarily select k initial centers
  2. Assign each element to the closest center
     - **Voronoi** diagram
  3. Re-calculate centers (i.e., means)
  4. Repeat 2 and 3 until termination condition reached





# Gene Ontology and Functional Enrichment

Genome 373

Genomic Informatics

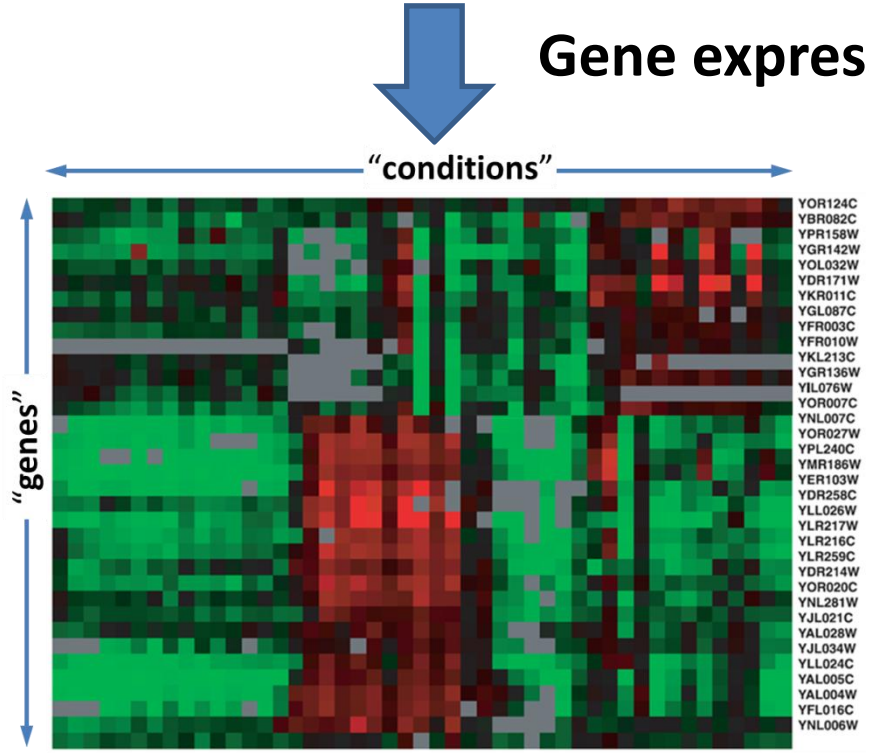
Elhanan Borenstein

# 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)

**Gene expression profiling**

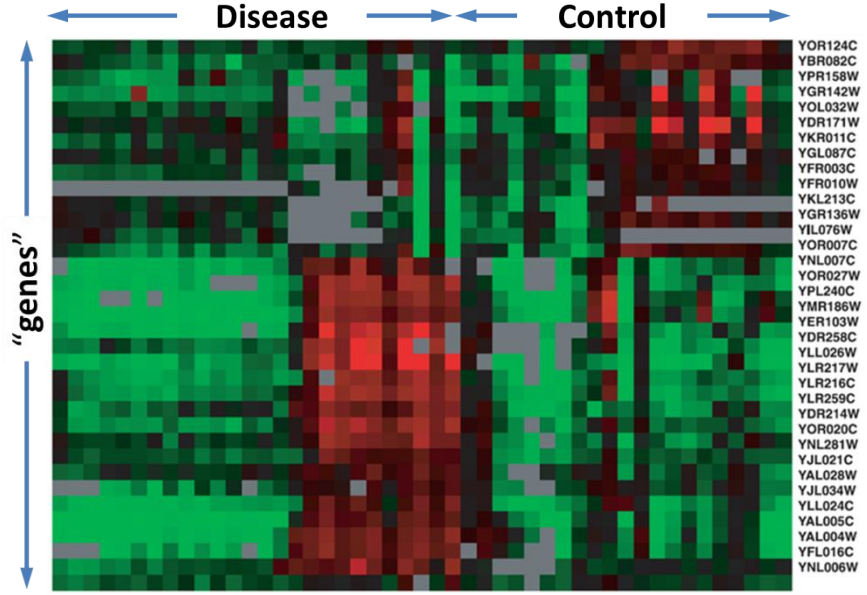


# From sequence to function

**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)

**Gene expression profiling**



# 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***

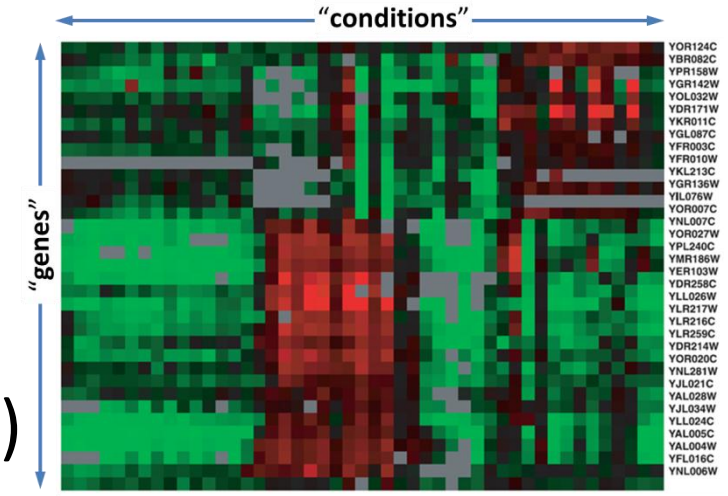
***Not systematic***

***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**

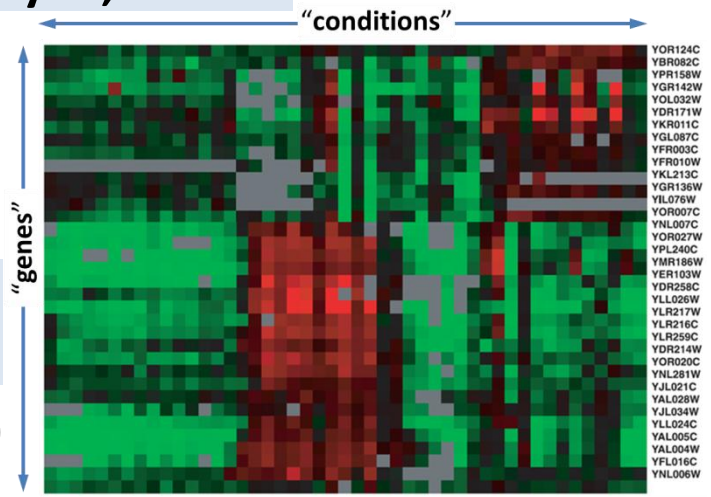
- Systematic linkage between genes and functions

- 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**




**Clustering, classification**

- (A way to identify "related" genes)

# 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.

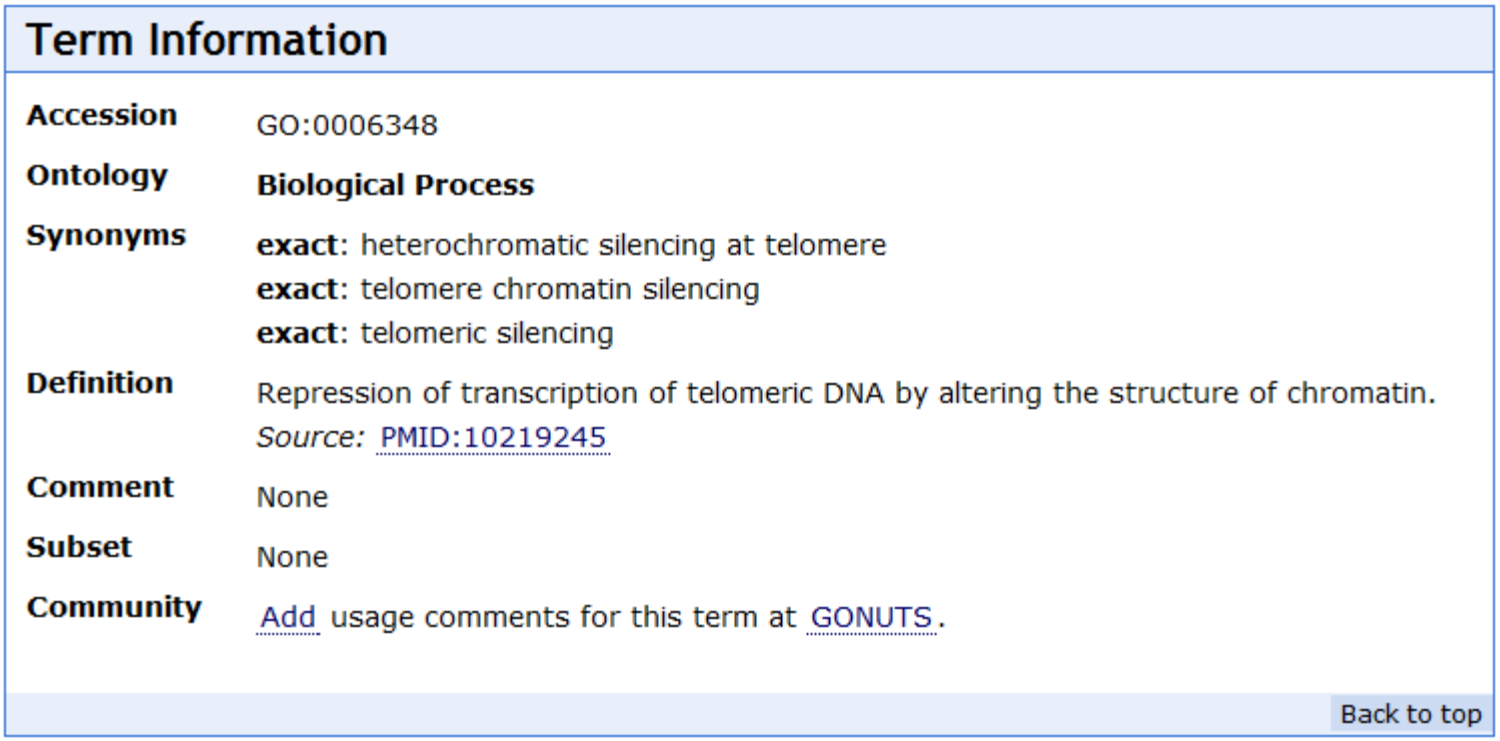
- Three goals:
  1. *Maintain and further develop its controlled **vocabulary** of gene and gene product attributes*

  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.



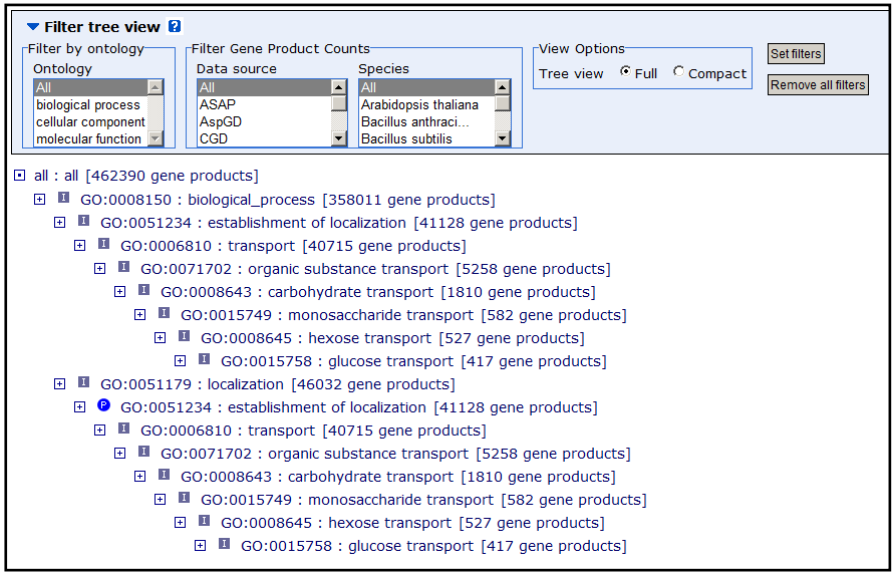
**Term Information**

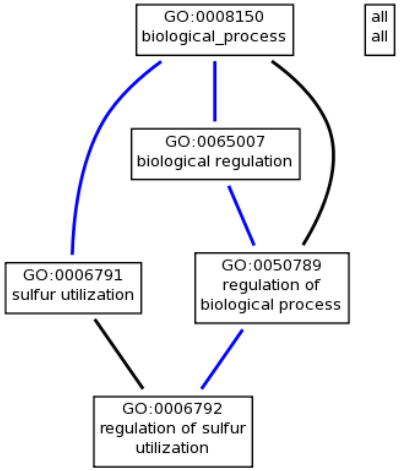
| | |
|---|---|
| **Accession** | GO:0006348 |
| **Ontology** | **Biological Process** |
| **Synonyms** | **exact**: heterochromatic silencing at telomere<br>**exact**: telomere chromatin silencing<br>**exact**: telomeric silencing |
| **Definition** | Repression of transcription of telomeric DNA by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

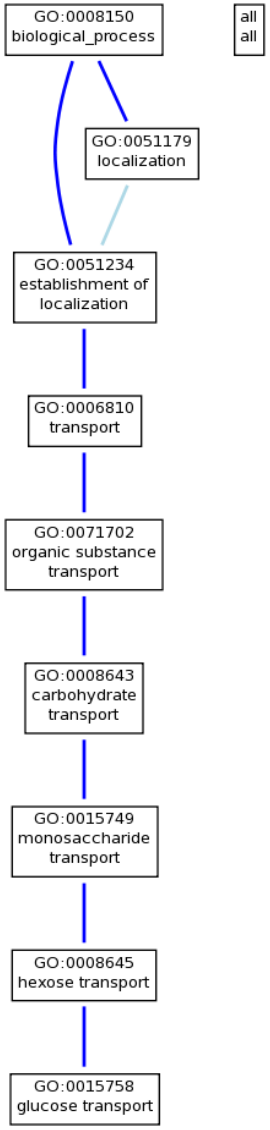
Back to top

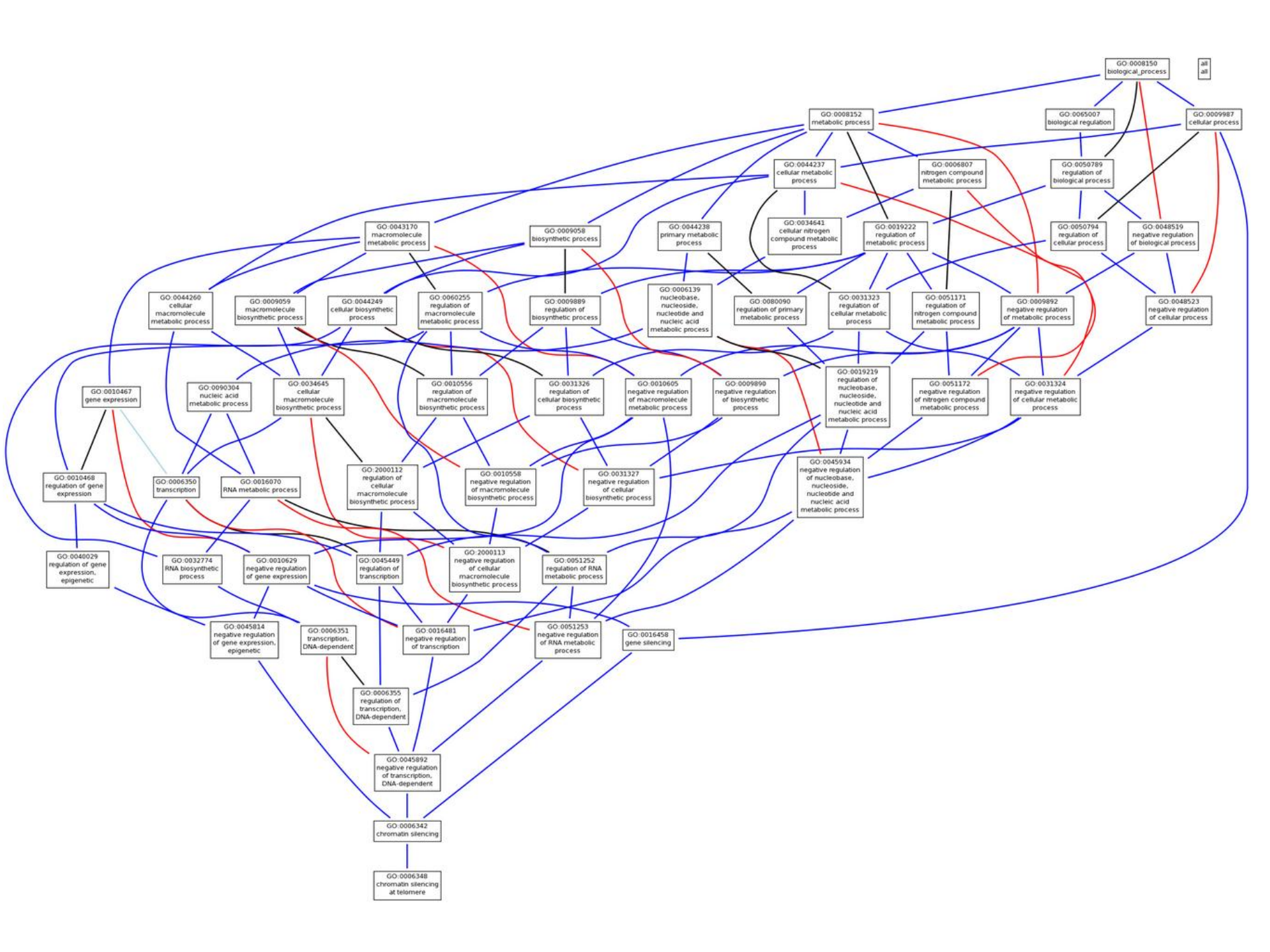
# Ontology structure

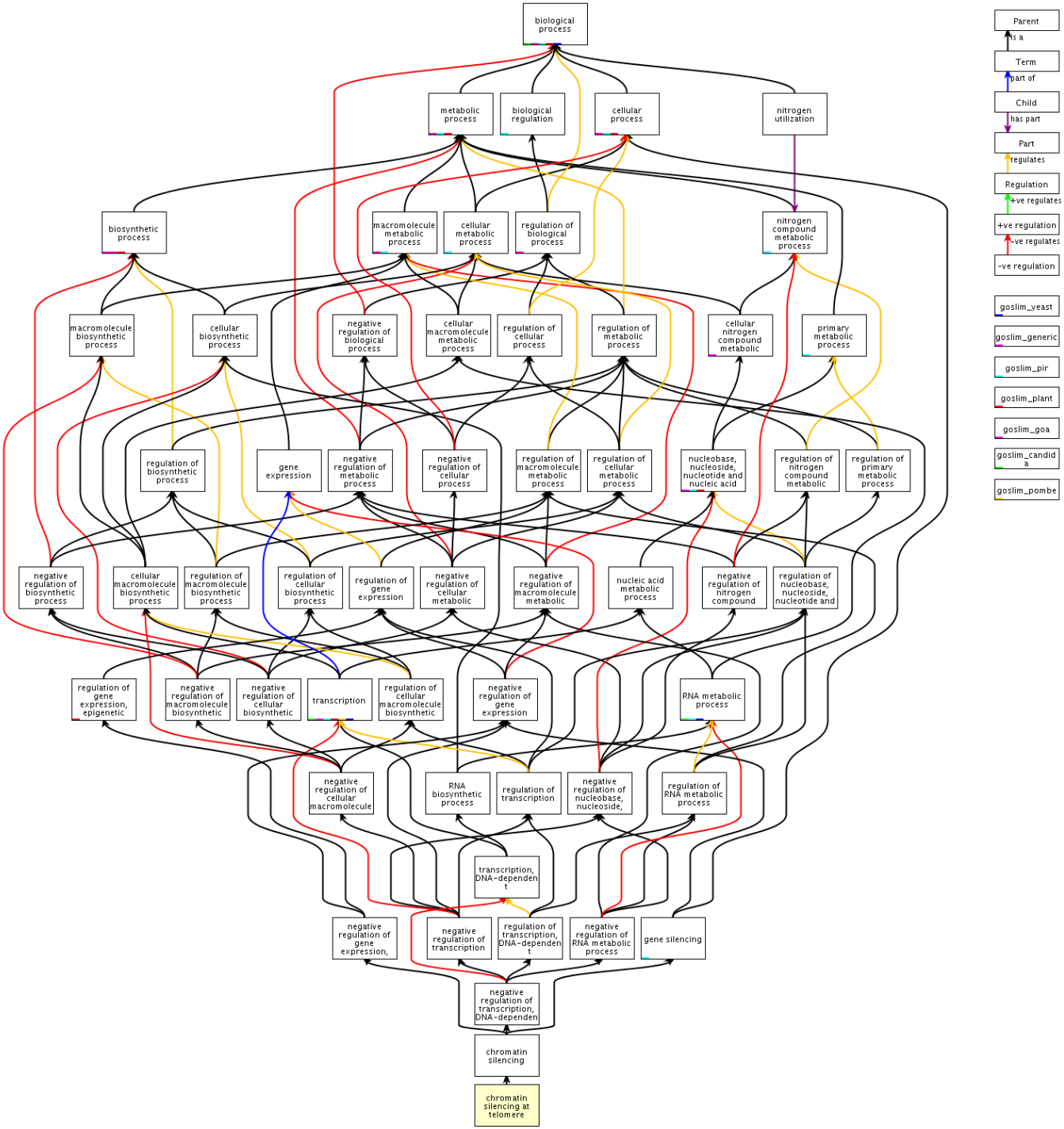
- GO also defines the **relationships** between the terms, making it a **structured** vocabulary.
- GO is structured as a **directed acyclic graph**, and each term has defined relationships to one or more other terms.




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
GO:0031323
regulation of cellular metabolic process
GO:0051171
regulation of nitrogen compound metabolic process
GO:0009892
negative regulation of metabolic process
GO:0048523
negative regulation of cellular process
GO:0010467
gene expression
GO:0090304
nucleic acid metabolic process
GO:0034645
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
regulation of nucleobase, nucleoside, nucleotide and nucleic acid metabolic process
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

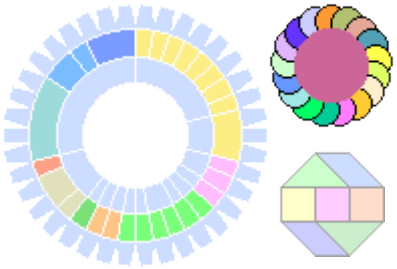

biological process
metabolic process
biological regulation
cellular process
nitrogen utilization
biosynthetic process
macromolecule metabolic process
cellular metabolic process
regulation of biological process
nitrogen compound metabolic process
macromolecule biosynthetic process
cellular biosynthetic 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
negative regulation of metabolic process
negative regulation of cellular process
regulation of macromolecule metabolic process
regulation of cellular metabolic process
nucleobase, nucleoside, nucleotide and nucleic acid
regulation of nitrogen compound metabolic
regulation of primary metabolic process
negative regulation of biosynthetic process
cellular macromolecule biosynthetic process
regulation of macromolecule biosynthetic process
regulation of cellular biosynthetic process
regulation of gene expression
negative regulation of cellular metabolic
negative regulation of macromolecule metabolic
nucleic acid metabolic process
negative regulation of nitrogen compound
regulation of nucleobase, nucleoside, nucleotide and
regulation of gene expression, epigenetic
negative regulation of macromolecule biosynthetic
negative regulation of cellular biosynthetic
transcription
regulation of cellular macromolecule biosynthetic
negative regulation of gene expression
RNA metabolic process
negative regulation of cellular macromolecule
RNA biosynthetic process
regulation of transcription
negative regulation of nucleobase, nucleoside,
regulation of RNA metabolic process
transcription, DNA-dependent
negative regulation of gene expression,
negative regulation of transcription
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**



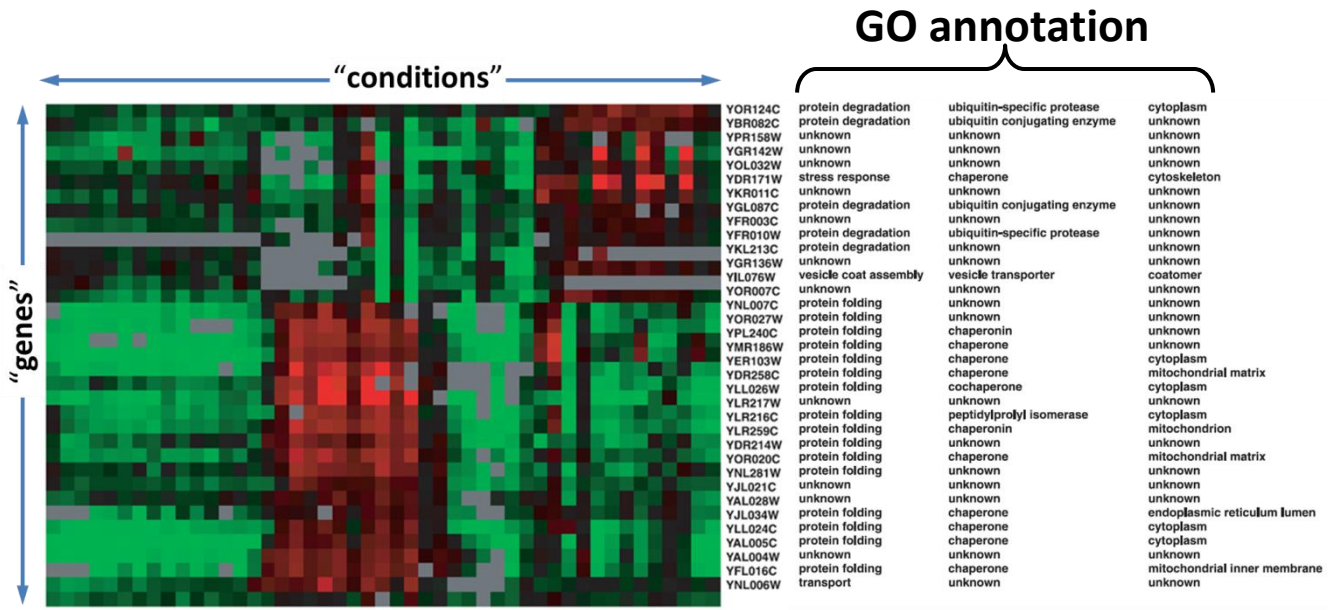
**Clusters of Orthologous Groups (COG)**


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

Andrew S. Tanenbaum

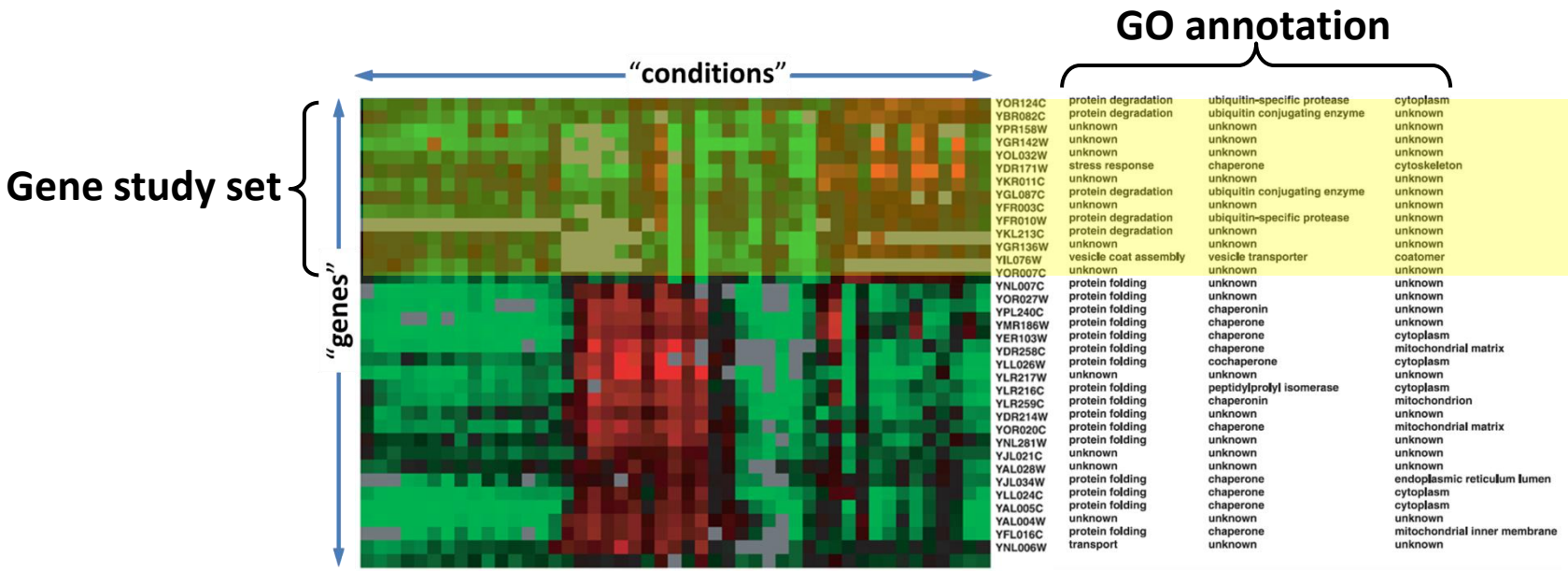
# What do we need?

- A shared functional vocabulary
- Systematic linkage between genes and functions
- A way to identify genes relevant to the condition under study
- Statistica
  (combining a
  functions tha
  condition un
- A way to

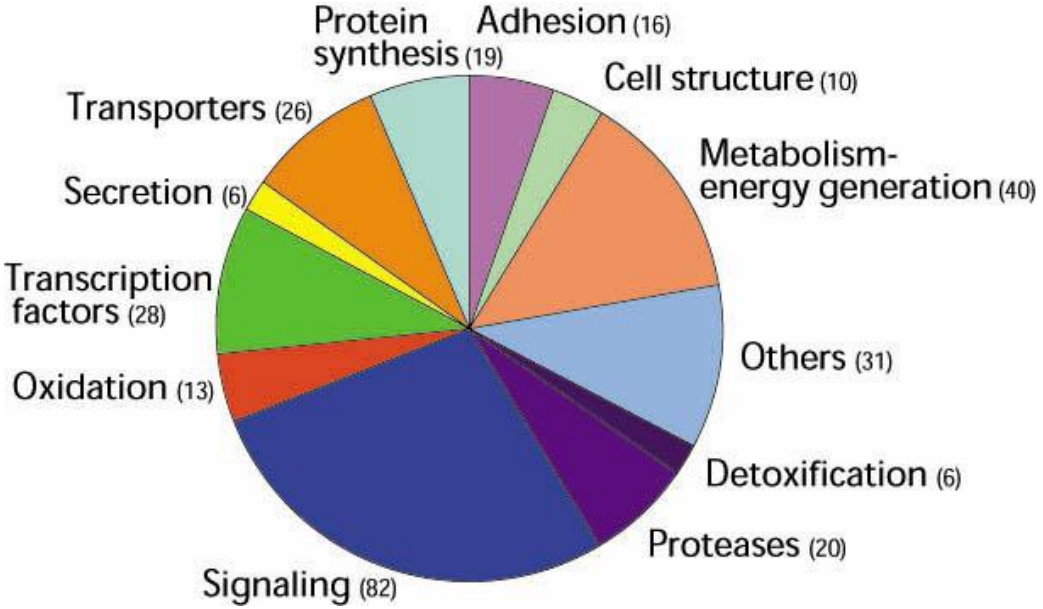


# Picking “relevant” genes

- 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)



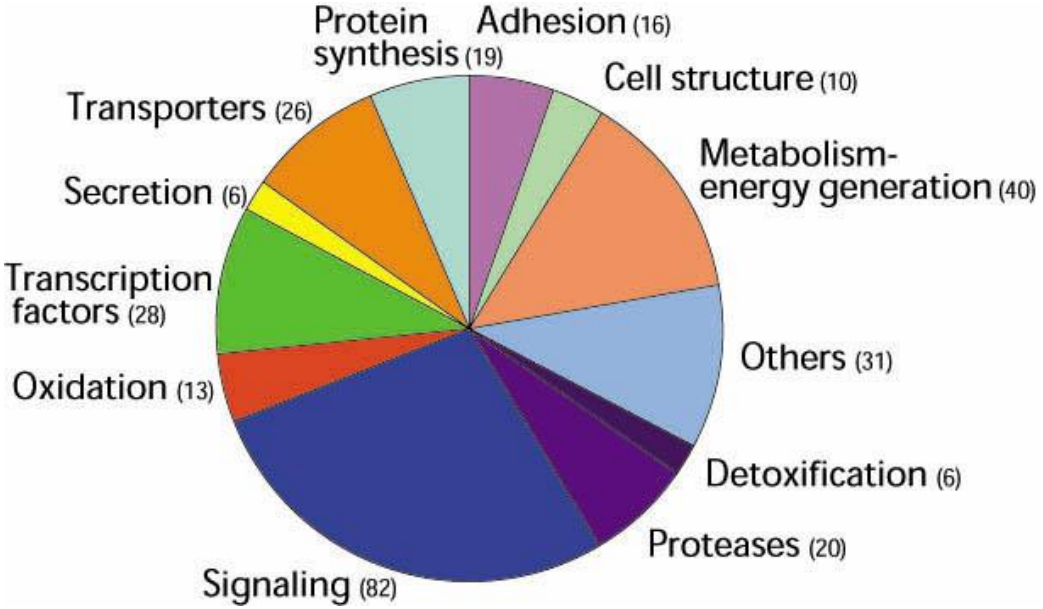
# Enrichment analysis


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


Signaling 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

- 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

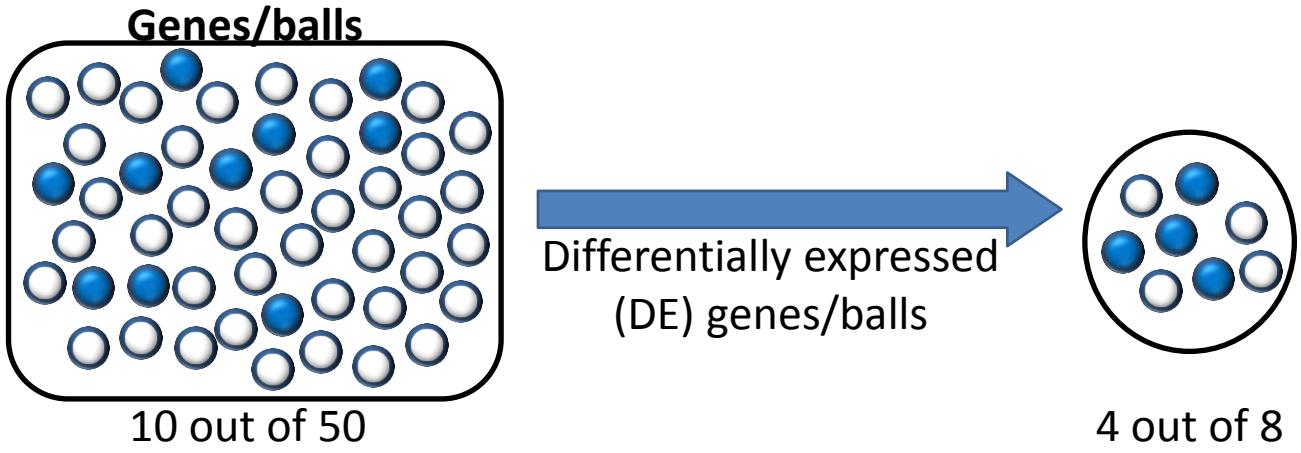
*Say, the microarray contains 50 genes, 10 of which are annotated as 'signaling'. Your expression analysis reveals 8 differentially expressed genes, 4 of which are annotated as 'signaling'.*
*Is this significant?*

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?*

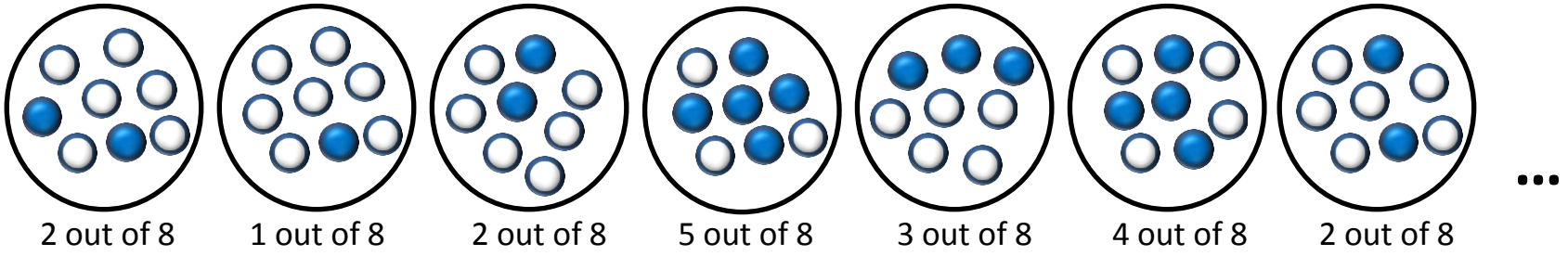
# A quick review: Modified Fisher's exact test




**Do I have a surprisingly high number of blue genes?**

---

**Null model: the 8 genes/balls are selected randomly**



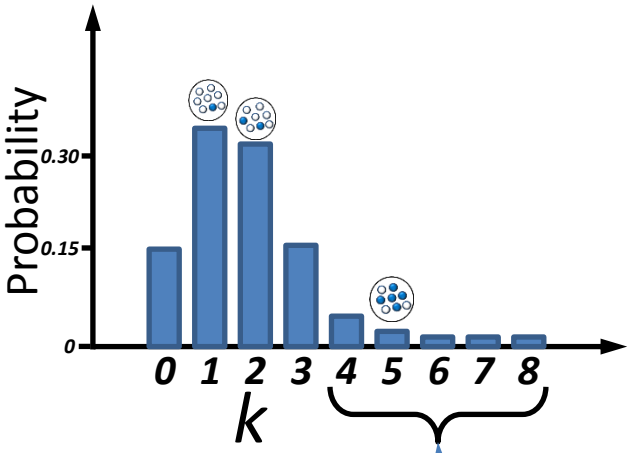

So, if you have 50 balls, 10 of them are blue, and you pick 8 balls randomly, what is the probability that *k* of them are blue?

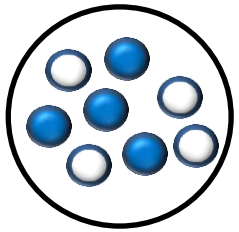
# A quick review: Modified Fisher's exact test

*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?**

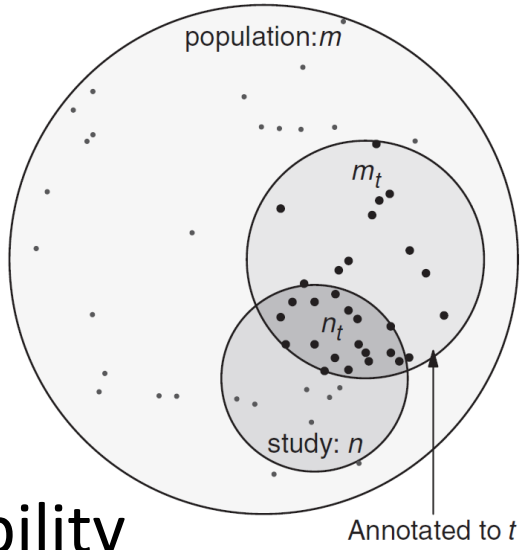
What is the probability of getting at least 4 blue genes in the null model?

***$P(\sigma_t >= 4)$***

# 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.



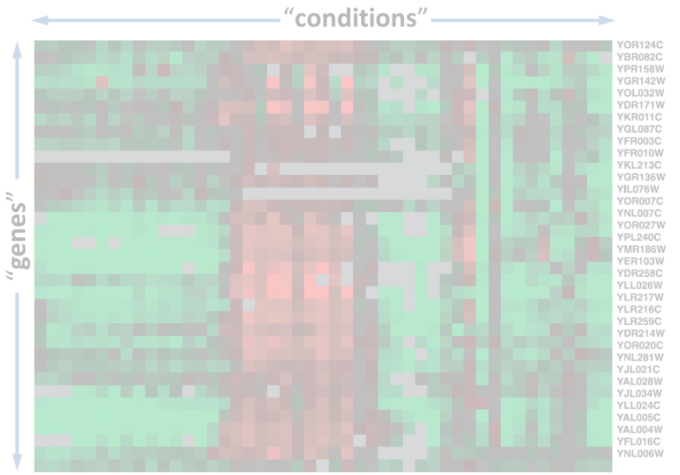

- We are interested in knowing the probability of seeing $n_t$ or more annotated genes!

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

(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**

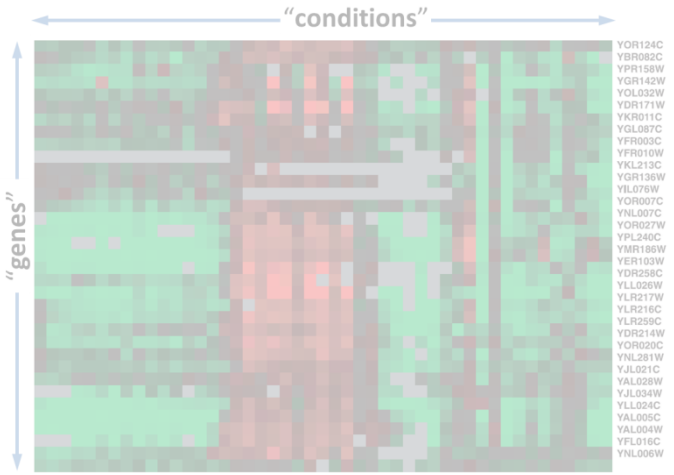
- A way to identify genes relevant to the condition under study

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

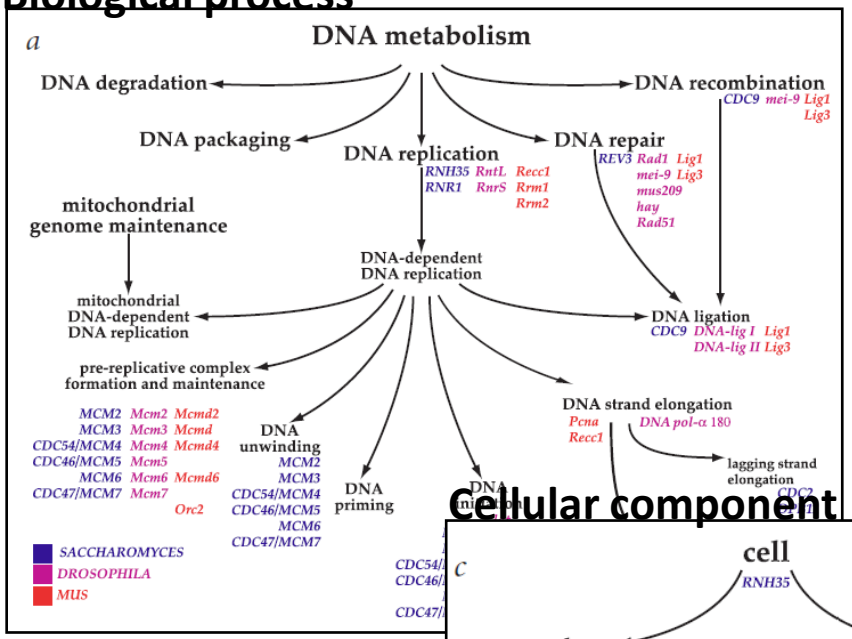
- Three ontology domains:
  1. **Molecular function:** basic activity or task
     *e.g. catalytic activity, calcium ion binding*
  2. **Biological process:** broad objective or goal
     *e.g. signal transduction, immune response*
  3. **Cellular component:** location or complex
     *e.g. nucleus,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

- Genes can have multiple annotations:

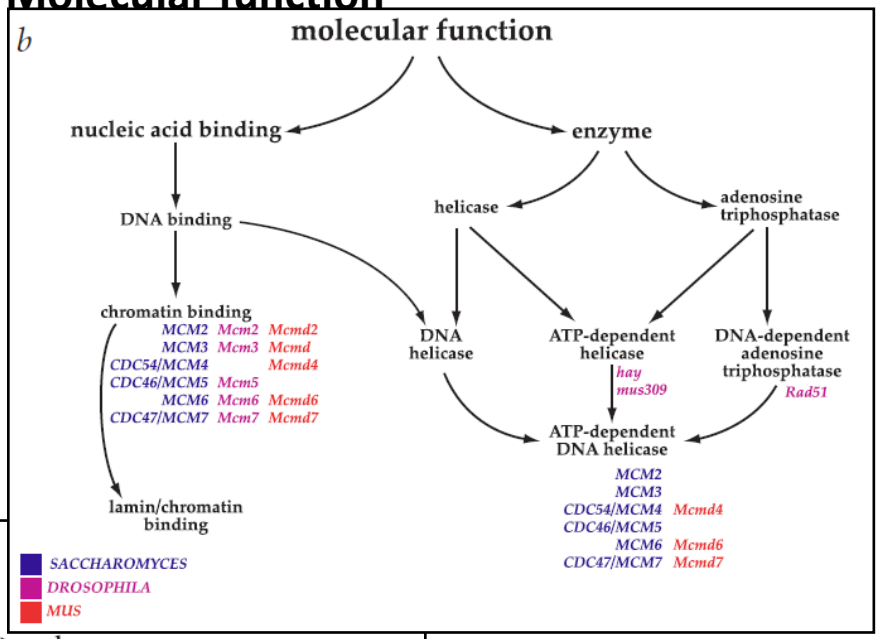
*For example, the gene product cytochrome c can be described by the molecular function term oxidoreductase activity, the biological process termsoxidative phosphorylation and induction of cell death, and the cellular component terms mitochondrial matrix and mitochondrial inner membrane.*

# Go domains

## Biological process




## Molecular function




## Cellular component