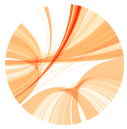
June 2021

BIO634: making sense of gene lists

Adapted from **Stefan Wyder**
class on BIO634



Universität
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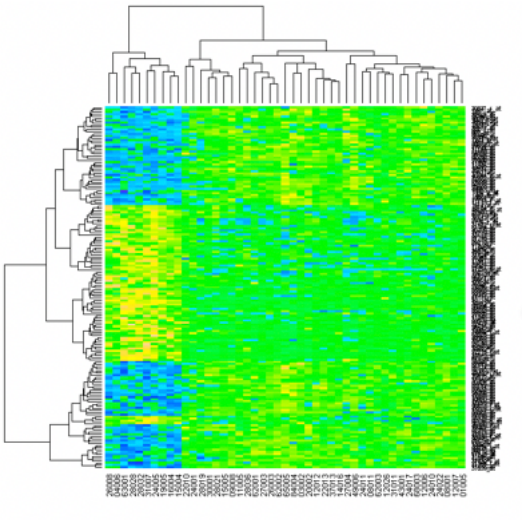
Gene list annotation

you performed a genomic experiment and obtained a gene list
hundreds of genes is too much, you would like to pinpoint interesting gene candidates



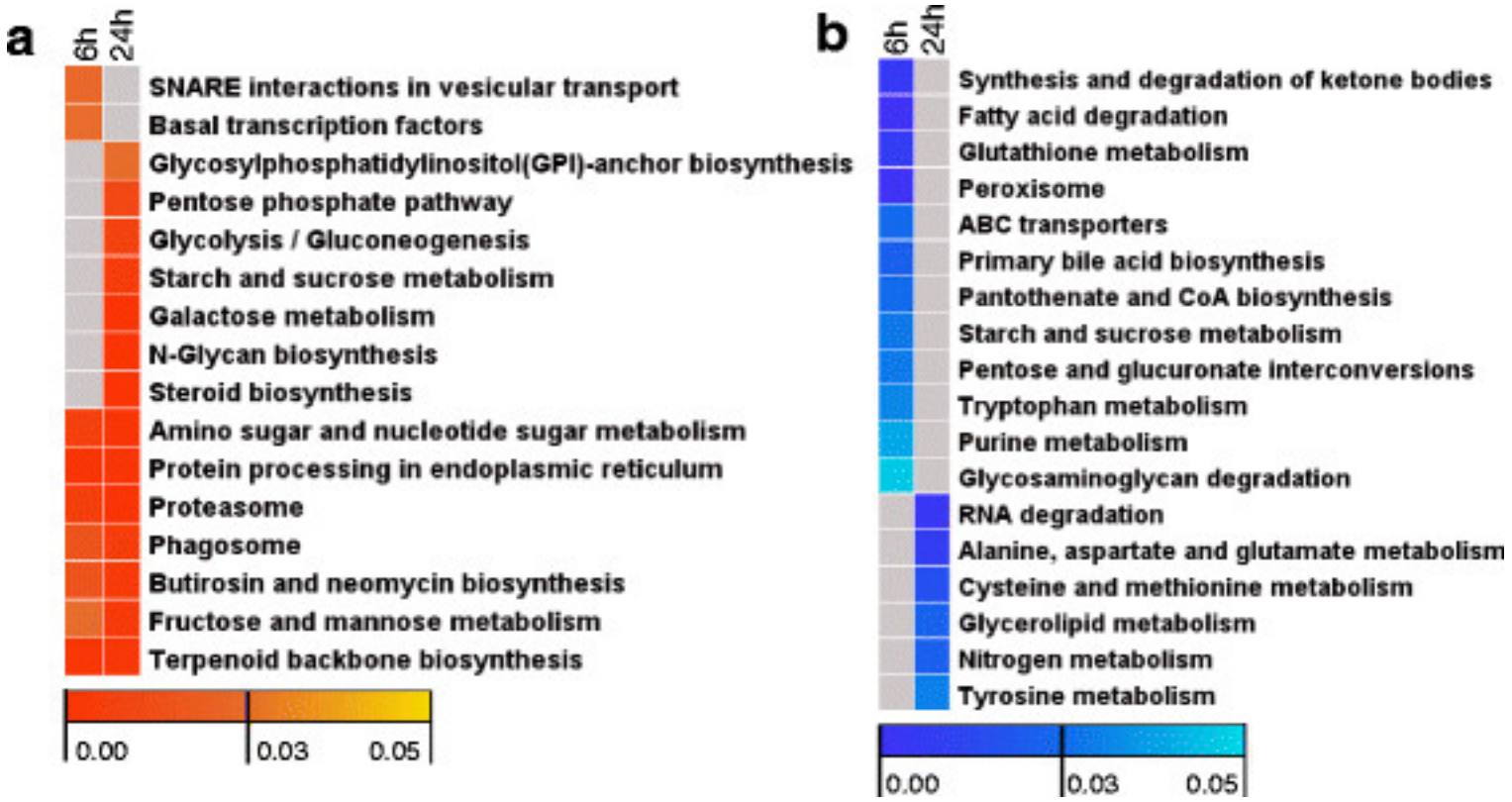
experimental data
RNA-seq, ChIP-seq, CLIP

geneA
geneB
geneC
geneD
…
geneX

annotation via previous knowledge
gene ontology, pathway analysis

biological insight

Gene list annotation



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Biological insight

interpretation of an experiment

find regulated processes / pathways

find involved regulatory elements, TF, RNA-binding proteins

identify new members of a pathway

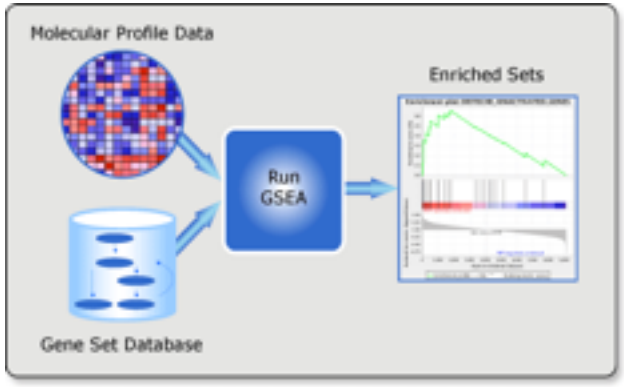
find similar experiments

Analysis based on gene lists expected to be more robust and reproducible compared to single gene analysis.

Enrichment analysis

1 Over-representation analysis

hypergeometric / Fisher's exact test
setting a cutoff a priori
different results at different thresholds

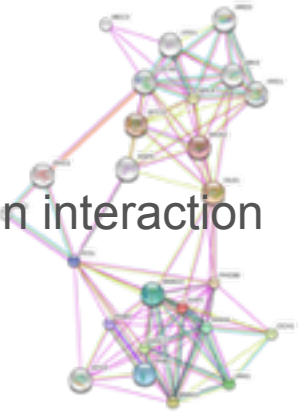


2 GSEA, gene set enrichment analysis

bypasses the need for a cutoff
input: list of all measured genes ranked by some measure / effect size
weak but consistent regulation of several members of a gene set can be detected

3 Network analysis

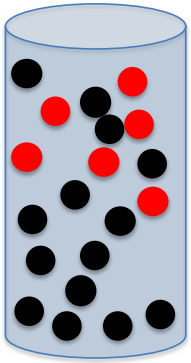
also covers less understood parts of gene interactions
often inferred from co-expression data
string-db.org, combines co-expression, co-citation, protein-protein interaction



1 Over-representation analysis

5.000 black and 10 red “genes”

10 red “genes” are cytochromes



our list of differentially expressed genes

- CYP4F11
- CYP1A
- MEP1A
- CYP26B
- CYP3A43

what is the probability?

	selected	not-selected
red	4	6
black	1	4989

one sided Fisher’s exact test
p-value: 4.03e-11

Gene Ontology

describes how gene products behave in a cellular context:
biological process, cellular component, molecular function

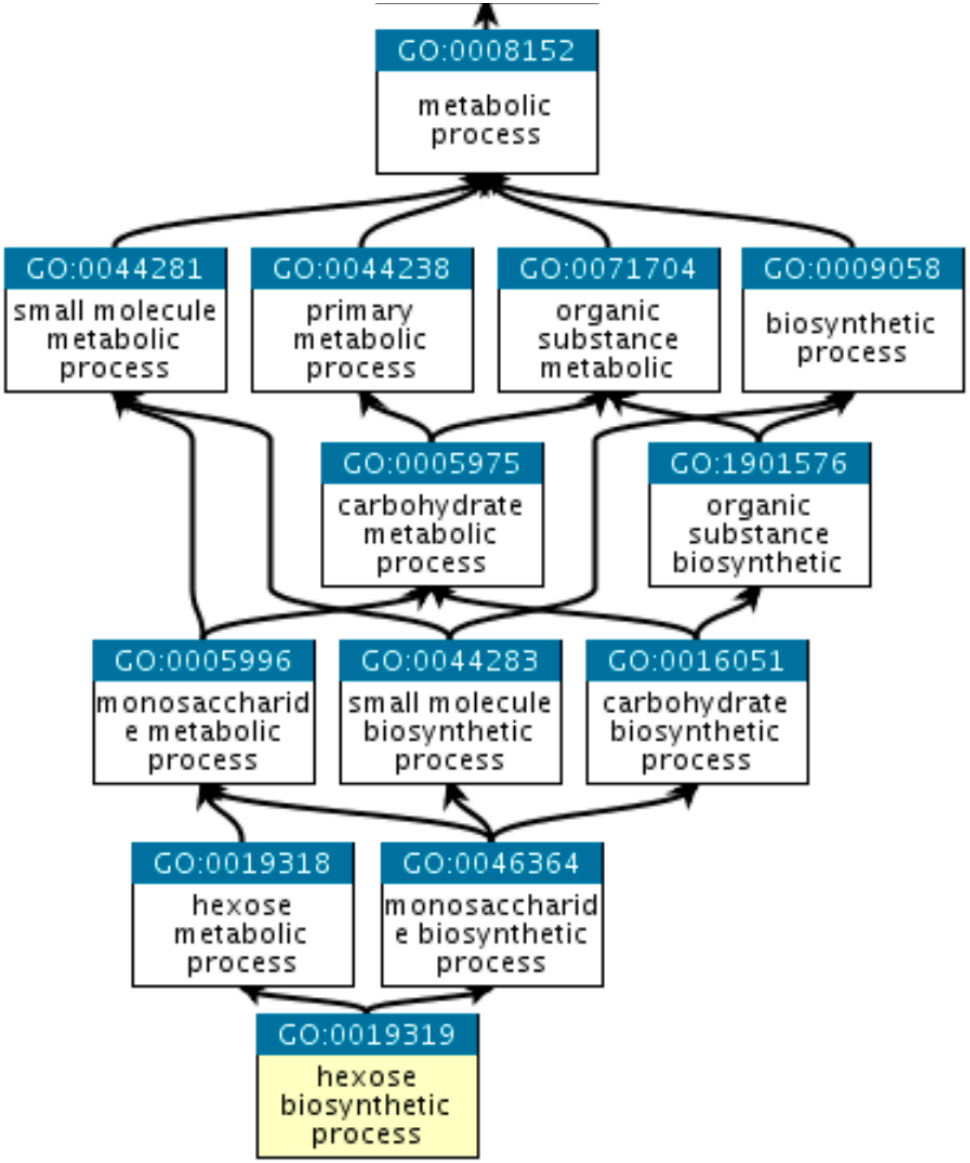
controlled vocabulary of terms

transparent (sources)

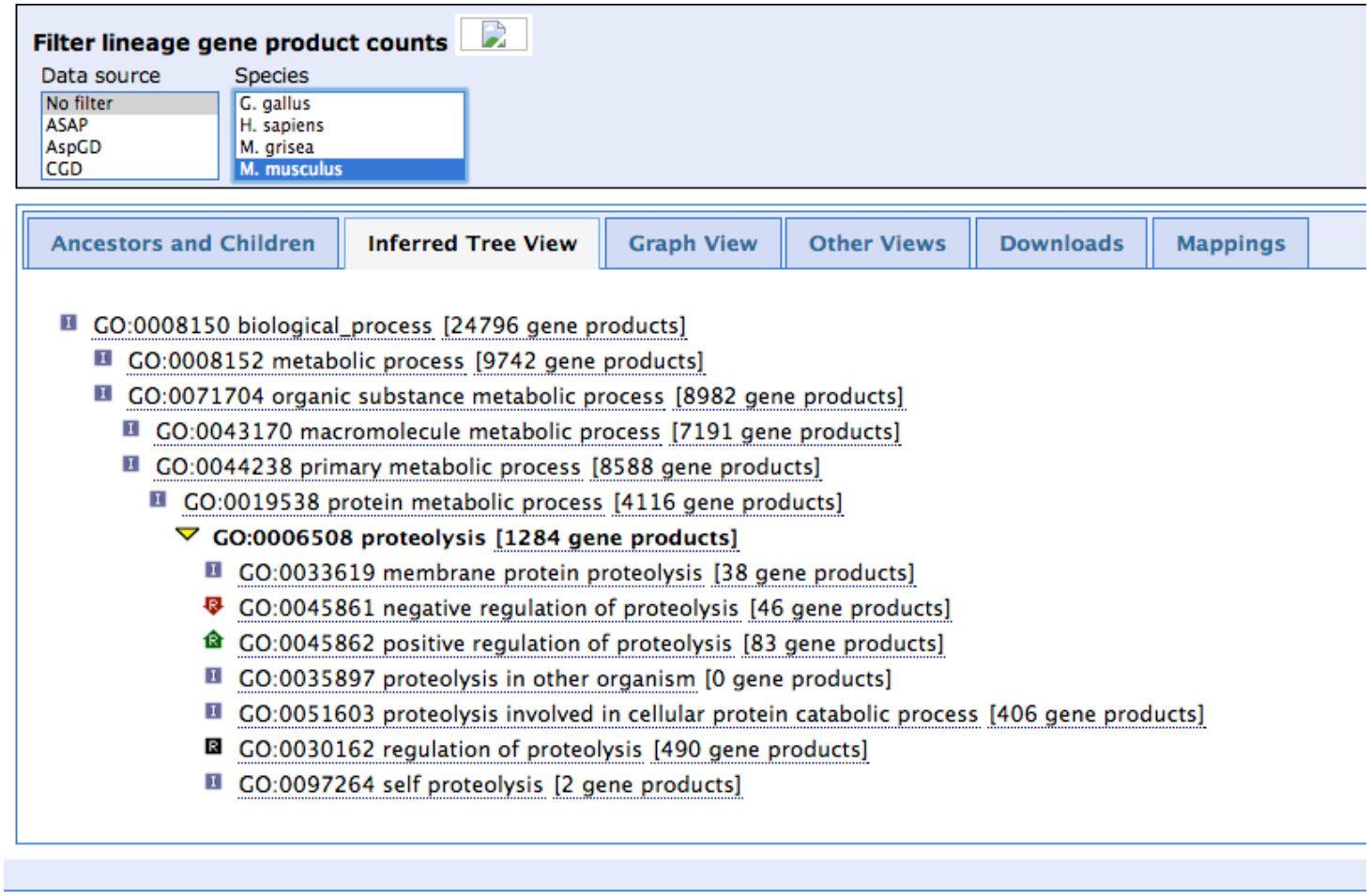
manually curated lists for model species

transfer to orthologs in other species (inferred annotation)

Gene Ontology



GO table view



GO:0006508 Proteolysis

Gene Ontology example

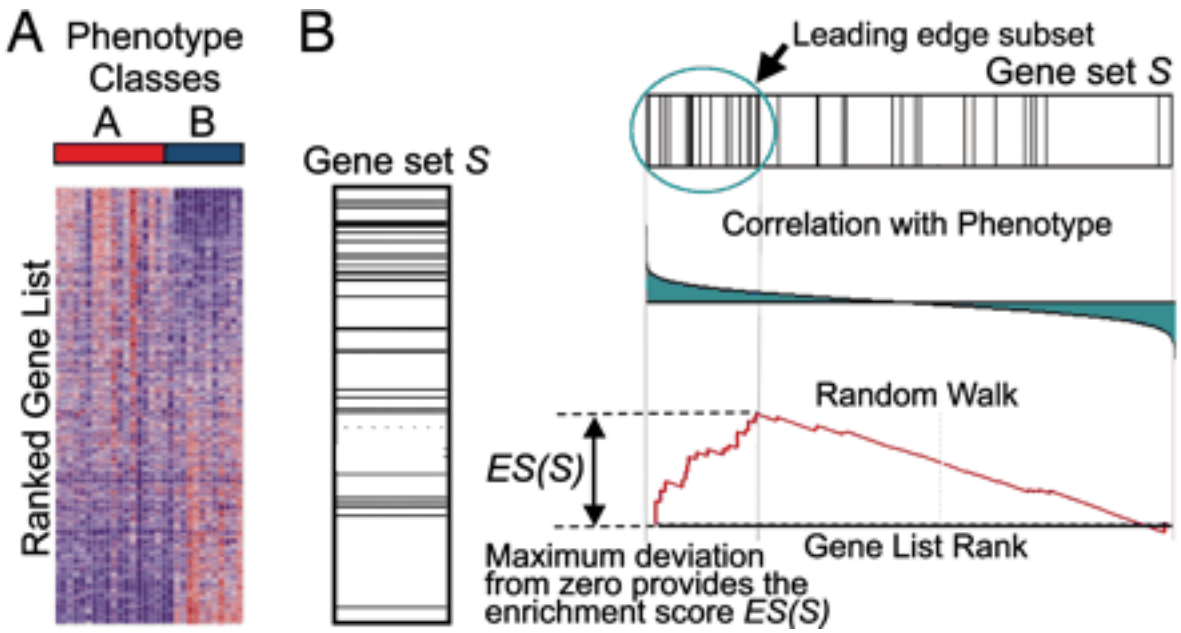
murine ADAM10

Molecular Function
GO:0008237 metallopeptidase activity
GO:0042169 SH2 domain binding
..

Biological Process
GO:0007220 Notch receptor processing
GO:0001701 in utero embryonic development
GO:0008284 positive regulation of cell proliferation
..

Cellular Compartment
GO:0005794 Golgi apparatus
GO:0009986 cell surface

2 Gene Set Enrichment Analysis



Subramanian et al. (2005) PNAS

Pathways

pathway maps (aka reaction networks / wiring diagrams) represent experimental knowledge on metabolism and various other functions of the cell and the organism

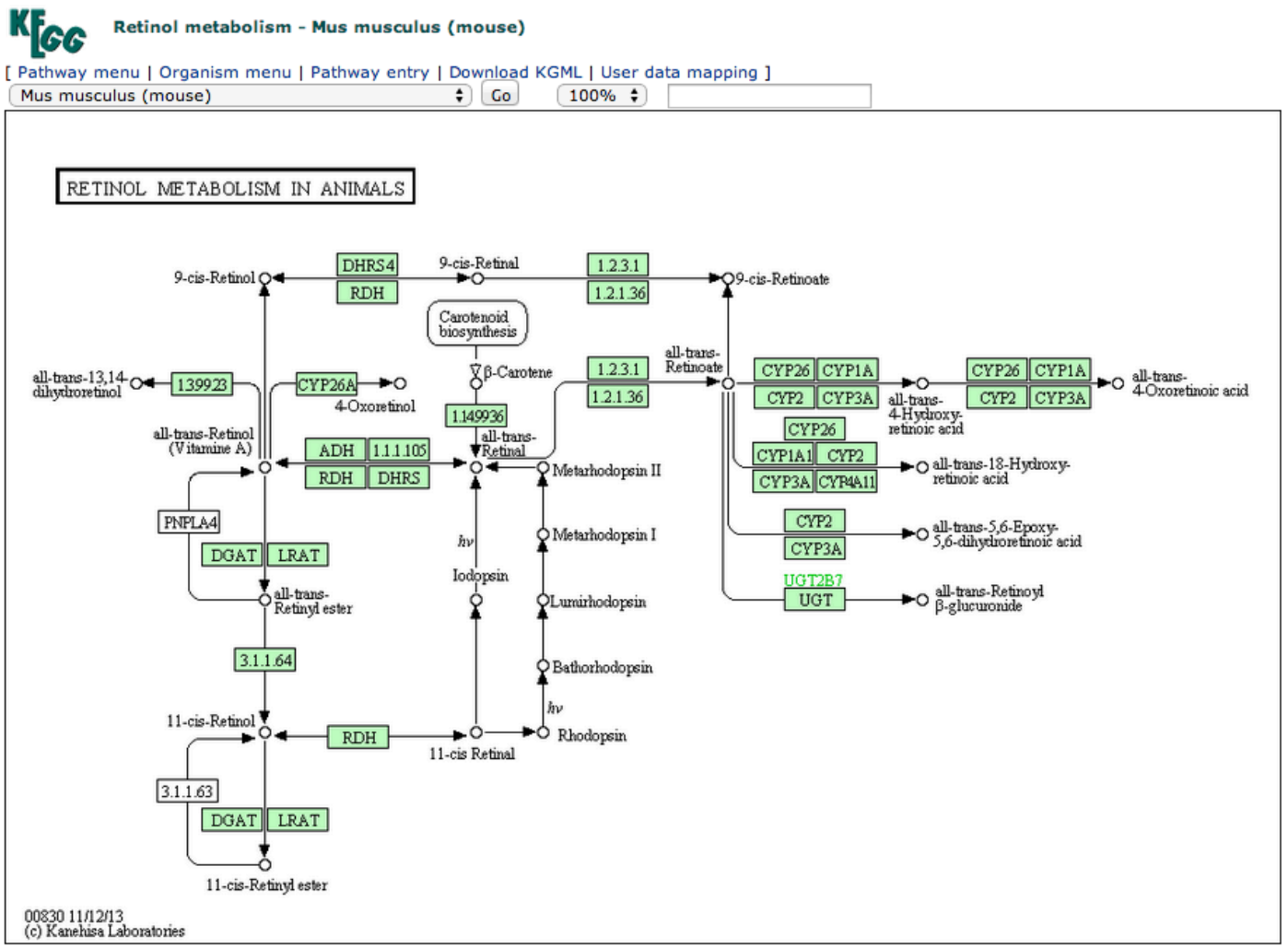
manually curated

the main databases are **KEGG** and **Reactome**

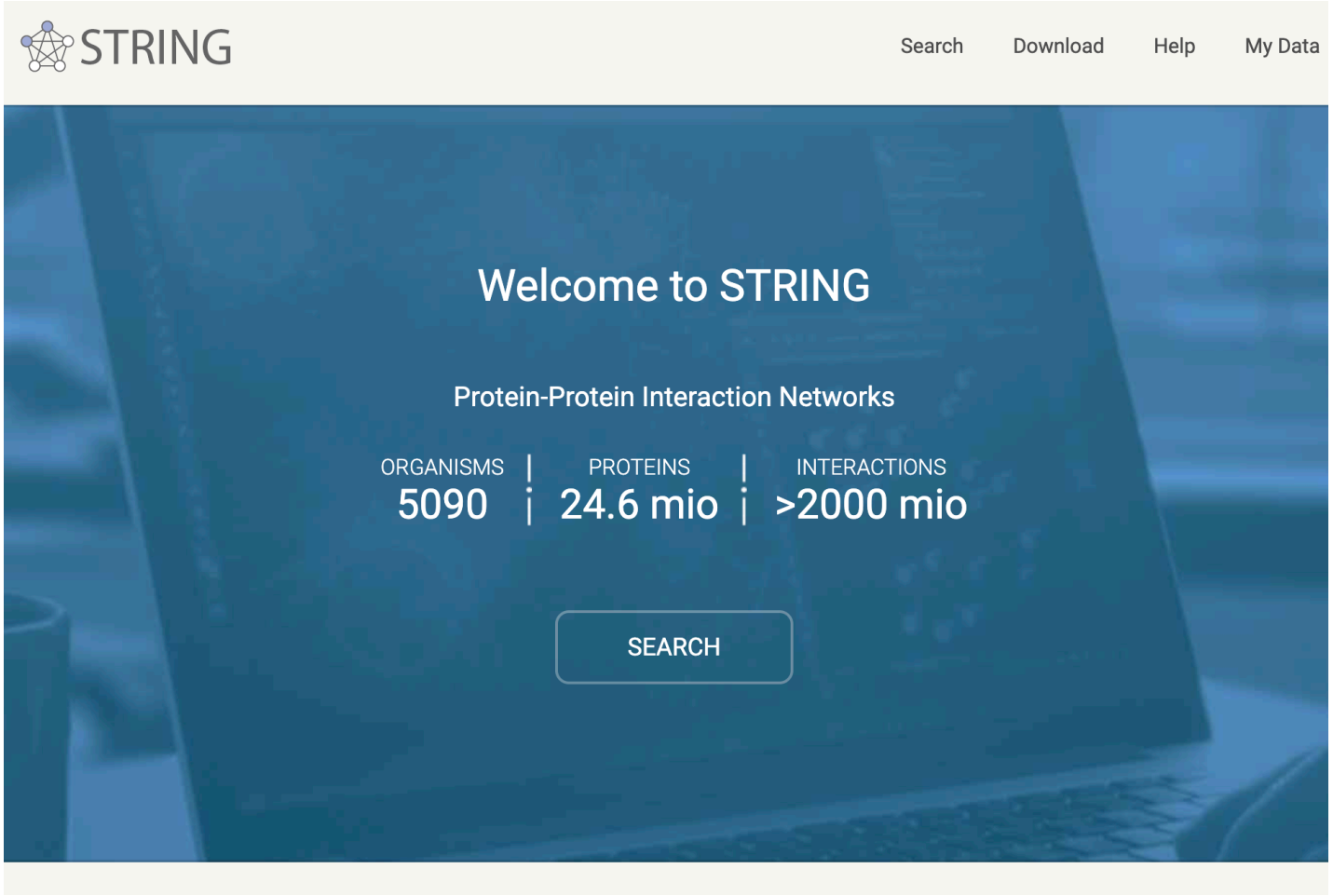
KEGG is free to use over the web but file download requires subscription

KEGG covers > 3.800 species (Archae, Bacteria, Plants, Animals) and Reactome covers 20 species (mostly mammals + fly + plants + E.coli)

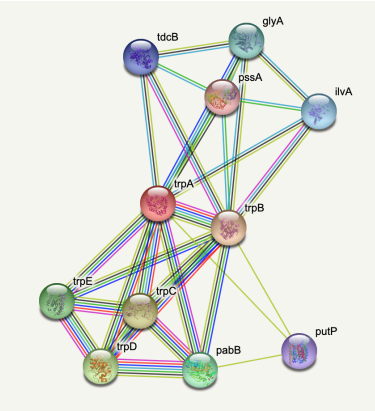
KEGG: example



3 Network analysis: string-db.org



string-db.org



functional association networks (physical or functional interactions)

focus on useability and speed

integrated scoring scheme (each interaction has confidence score)

information transfer between species
(>5000 species: Animals, Bacteria, Plants)

Network currently showing
Summary view: shows current interactions. Nodes can be moved; popups provide information on nodes & edges.

Neighborhood
Groups of genes that are frequently observed in each other's genomic neighborhood.

Experiments
Co-purification, co-crystallization, Yeast2Hybrid, Genetic Interactions, etc ... as imported from primary sources.

Fusion
Genes that are sometimes fused into single open reading frames.

Databases
Known metabolic pathways, protein complexes, signal transduction pathways, etc ... from curated databases.

Cooccurrence
Gene families whose occurrence patterns across genomes show similarities.

Textmining
Automated, unsupervised textmining - searching for proteins that are frequently mentioned together.

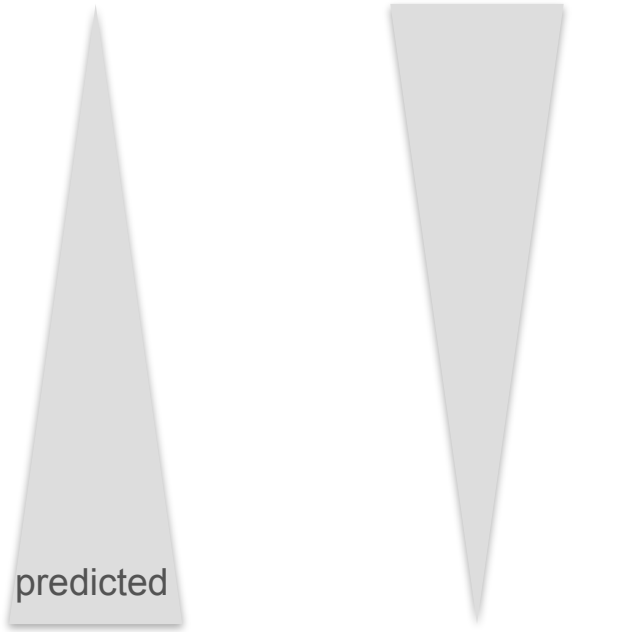
Coexpression
Proteins whose genes are observed to be correlated in expression, across a large number of experiments.

Annotation sources

Pathways
KEGG, Reactome, BioCyc

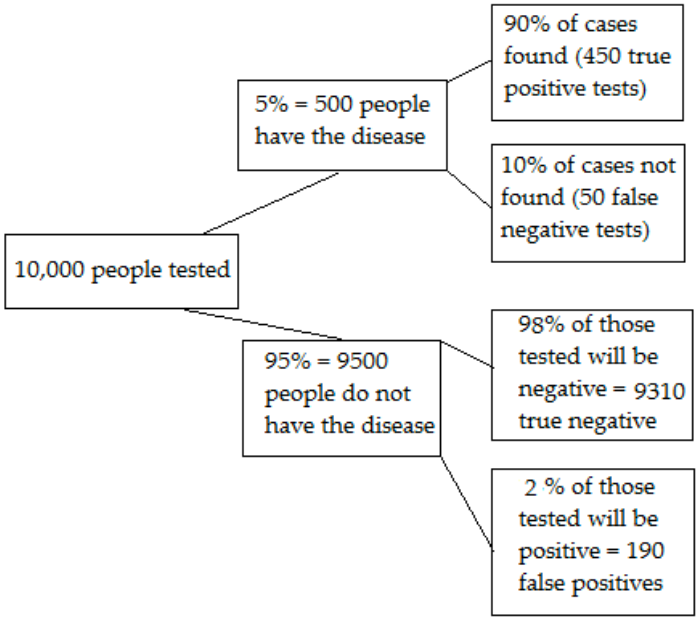
Gene Ontology

Networks
STRING



FDR: false discovery rate

"if you repeat a test enough times, you're going to find an effect…but that effect may not actually exist"



The FDR approach adjusts the p-value for a series of tests. A p-value gives you the probability of a false positive on a single test; If you're running a large number of tests from small samples (which are common in fields like genomics and protoemics), you should use q-values instead.

- A p-value of 5% means that 5% of all tests will result in false positives.
- A q-value of 5% means that 5% of *significant* results will be false positives.

The procedure to control the FDR, using q-values, is called the Benjamini-Hochberg procedure, named after Benjamini and Hochberg (1995), who first described it.

Summary

Gene list annotation with Pathways and Gene Ontology can help to obtain biological insight

A Over-Representation Analysis
B Gene Set Enrichment Analysis, GSEA
C Network Analysis

Biological interpretation requires broad knowledge of physiology & biochemistry and is often the most difficult and time-consuming step of an experiment

Even experts can usually not make sense of all the significantly enriched processes/ pathways in well understood biological systems

Good experiments start with good experimental design, think of possible confounders