BMEG3102 Bioinformatics

Lecture 9. Functional Annotations

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BMEG3102 Bioinformatics

The Chinese University of Hong Kong

Lecture outline

1. Genome annotations

2. Gene ontology and biological pathways

3. Functional enrichment analysis

Part 1

Genome Annotations

Genomic elements

- Genome annotation is the process to catalog all functional elements in a genome and characterize their properties
- Types of functional genomic elements:
  - Protein coding genes
    - Transcripts, exons, introns, coding sequences (CDSs), untranslated regions (UTRs), ...
  - Non-coding RNAs
  - ...
- They are also called “biotypes”

Genomic annotations

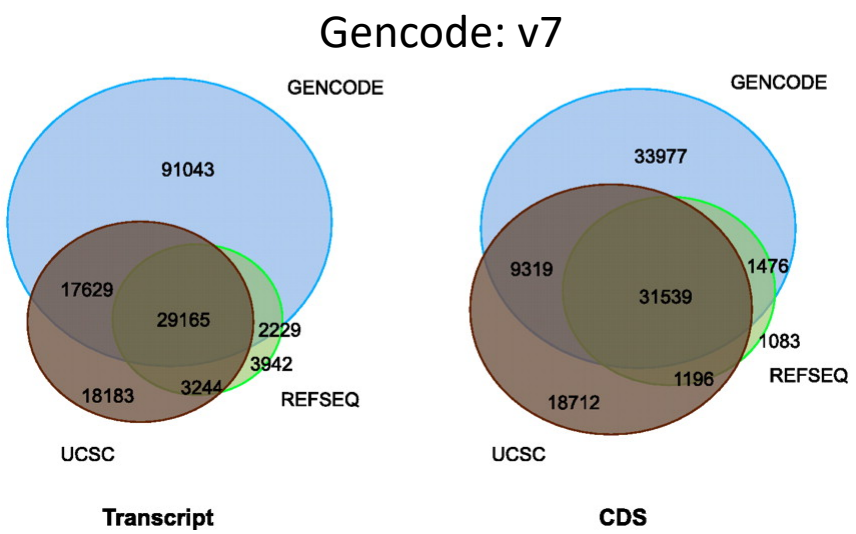
- For each element, what do we record?
  - Its location
    - Chromosome, start, end (strand)
    - Reference-specific (e.g., hg19 for human)
  - Its name
    - Standard IDs (linking to other databases)
    - Official name
    - Aliases
  - Its sequence
  - ...
  - Confidence of annotation

Human lists of genomic elements

- Where to find the whole list of genomic elements in human?
  - NCBI Reference Sequence (RefSeq)
    - Integrated
  - Ensembl
    - Automatic annotation
  - UCSC
  - Gencode
    - HAVANA manual annotation + Ensembl automatic annotation
    - Multiple levels of confidence
      - Level 1: verified
      - Level 2: manually annotated
      - Level 3: automatically annotated

Comparisons

- Comparing different sets and different versions of a set:
  - A lot more in recent versions of Gencode due to large amount of high-throughput experimental data produced



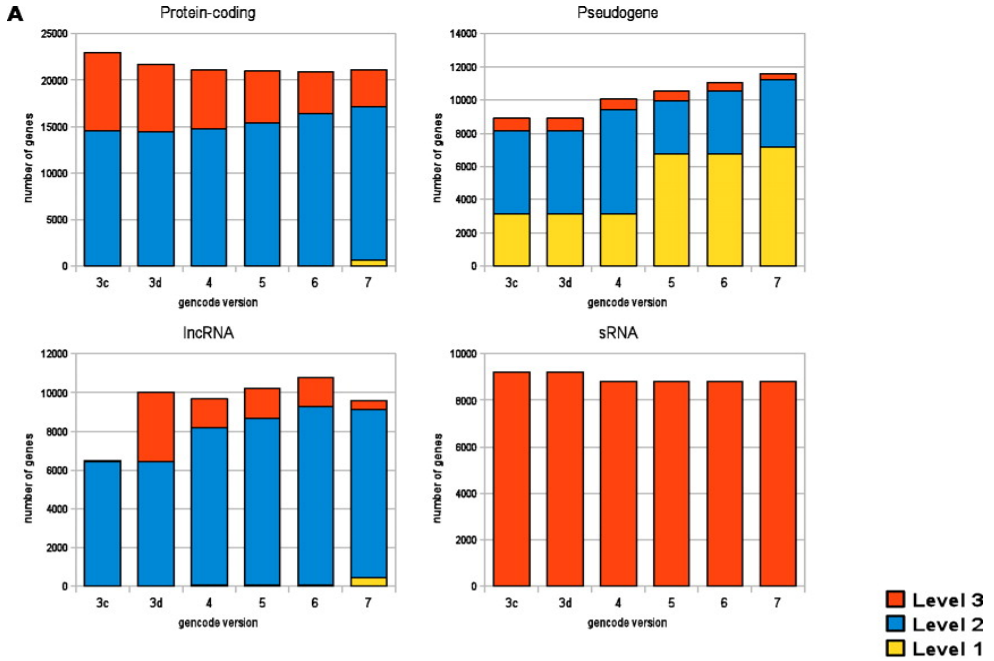


Image credit: Harrow et al., *Genome Research* 22(9):1760-1774, (2012)

Annotation files

- GFF format (from http://genome.ucsc.edu/FAQ/FAQformat.html): tab-delimited. Fields:
  1. seqname - The name of the sequence. Must be a chromosome or scaffold.
  2. source - The program that generated this feature.
  3. feature - The name of this type of feature. Some examples of standard feature types are "CDS", "start_codon", "stop_codon", and "exon".
  4. start - The starting position of the feature in the sequence. The first base is numbered 1.
  5. end - The ending position of the feature (inclusive).
  6. score - A score between 0 and 1000. If the track line useScore attribute is set to 1 for this annotation data set, the score value will determine the level of gray in which this feature is displayed (higher numbers = darker gray). If there is no score value, enter ".".
  7. strand - Valid entries include '+', '-', or '.' (for don't know/don't care).
  8. frame - If the feature is a coding exon, frame should be a number between 0-2 that represents the reading frame of the first base. If the feature is not a coding exon, the value should be '.'.
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- GTF format: Similar to GFF, except that the group field is replaced by a list of attributes in <name>, <value> pairs

Example

- Gencode v12 GTF file:

chr1 ENSEMBL exon 17021 17055 . - . gene_id "ENSG00000227232.3"; transcript_id "ENST00000430492.2"; gene_type "pseudogene"; gene_status "KNOWN"; gene_name "WASH7P"; transcript_type "unprocessed_pseudogene"; transcript_status "KNOWN"; transcript_name "WASH7P-202"; level 3; havana_gene "OTTHUMG00000000958.1";
chr1 HAVANA gene 29554 31109 . + . gene_id "ENSG00000243485.1"; transcript_id "ENSG00000243485.1"; gene_type "antisense"; gene_status "NOVEL"; gene_name "MIR1302-11"; transcript_type "antisense"; transcript_status "NOVEL"; transcript_name "MIR1302-11"; level 2; tag "ncRNA_host"; havana_gene "OTTHUMG00000000959.2";
...
chr1 HAVANA gene 34554 36081 . - . gene_id "ENSG00000237613.2"; transcript_id "ENSG00000237613.2"; gene_type "protein_coding"; gene_status "KNOWN"; gene_name "FAM138A"; transcript_type "protein_coding"; transcript_status "KNOWN"; transcript_name "FAM138A"; level 2; havana_gene "OTTHUMG00000000960.1";
chr1 HAVANA transcript 34554 36081 . - . gene_id "ENSG00000237613.2"; transcript_id "ENST00000417324.1"; gene_type "protein_coding"; gene_status "KNOWN"; gene_name "FAM138A"; transcript_type "protein_coding"; transcript_status "KNOWN"; transcript_name "FAM138A-001"; level 2; havana_gene "OTTHUMG00000000960.1"; havana_transcript "OTTHUMT00000002842.1";
chr1 HAVANA exon 35721 36081 . - . gene_id "ENSG00000237613.2"; transcript_id "ENST00000417324.1"; gene_type "protein_coding"; gene_status "KNOWN"; gene_name "FAM138A"; transcript_type "protein_coding"; transcript_status "KNOWN"; transcript_name "FAM138A-001"; level 2; havana_gene "OTTHUMG00000000960.1"; havana_transcript "OTTHUMT00000002842.1";
chr1 HAVANA CDS 35721 35736 . - 0 gene_id "ENSG00000237613.2"; transcript_id "ENST00000417324.1"; gene_type "protein_coding"; gene_status "KNOWN"; gene_name "FAM138A"; transcript_type "protein_coding"; transcript_status "KNOWN"; transcript_name "FAM138A-001"; level 2; havana_gene "OTTHUMG00000000960.1"; havana_transcript "OTTHUMT00000002842.1";
chr1 HAVANA start_codon 35734 35736 . - 0 gene_id "ENSG00000237613.2"; transcript_id "ENST00000417324.1"; gene_type "protein_coding"; gene_status "KNOWN"; gene_name "FAM138A"; transcript_type "protein_coding"; transcript_status "KNOWN"; transcript_name "FAM138A-001"; level 2; havana_gene "OTTHUMG00000000960.1"; havana_transcript "OTTHUMT00000002842.1";

Additional information

- The information in GFF/GTF files seems to be very basic
- What additional information do we know about genes?
  - Functional annotation: gene ontology, biological pathways, etc.
- Where to find the information?
  - Some useful sites:
    - The three main sequence databases:
      - DDBJ
      - EMBL
      - GenBank
    - GeneCards
    - UCSC Genome Browser
    - UniProt

Part 2

Gene Ontology and Biological Pathways

Knowledge representation

- How to systematically organize knowledge about genomic elements?
  - Define the concepts associated with standard terms
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    - E.g., An exon is part of a gene

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- We want to define an "ontology":
  - "The philosophical study of the nature of being, existence, or reality as such, as well as the basic categories of being and their relations" (Wikipedia)

Gene Ontology (GO)

- An ontology for gene products produced by the Gene Ontology Consortium
  - Most frequently-used biological ontology
- 3 sub-ontologies:
  - Molecular function
    - Low-level functions of a gene product
  - Biological process
    - High-level processes that a gene product is involved
  - Cellular component
    - Which compartment can the gene product be found

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    - Which compartment can the gene product be found
- 2 parts:
  - The ontologies
    - Directed acyclic graphs (DAG) – Some terms have multiple parents
    - Edges indicate three types of relationships: is-a, part-of, regulates
  - Organism-specific instances (each gene can have 0, 1 or more annotated terms)

Tree view

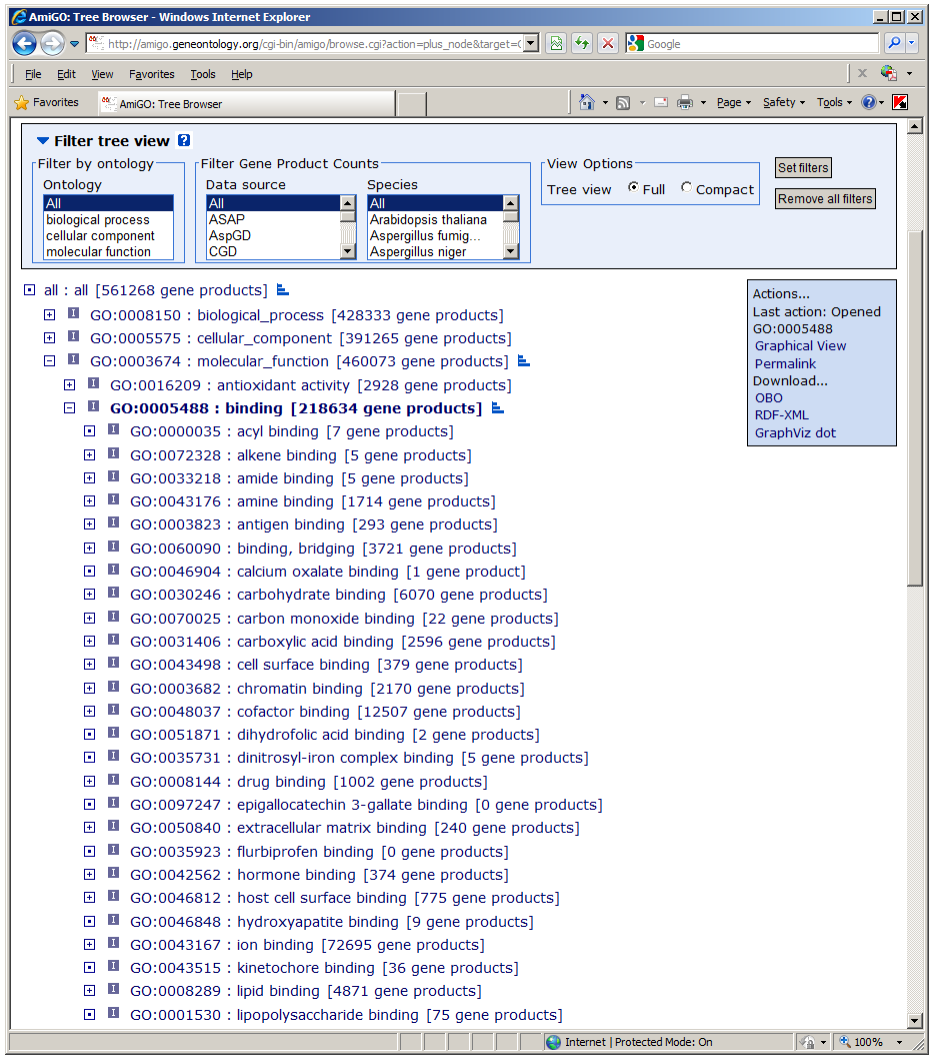


Image source: http://amigo.geneontology.org/

Directed acyclic graph view

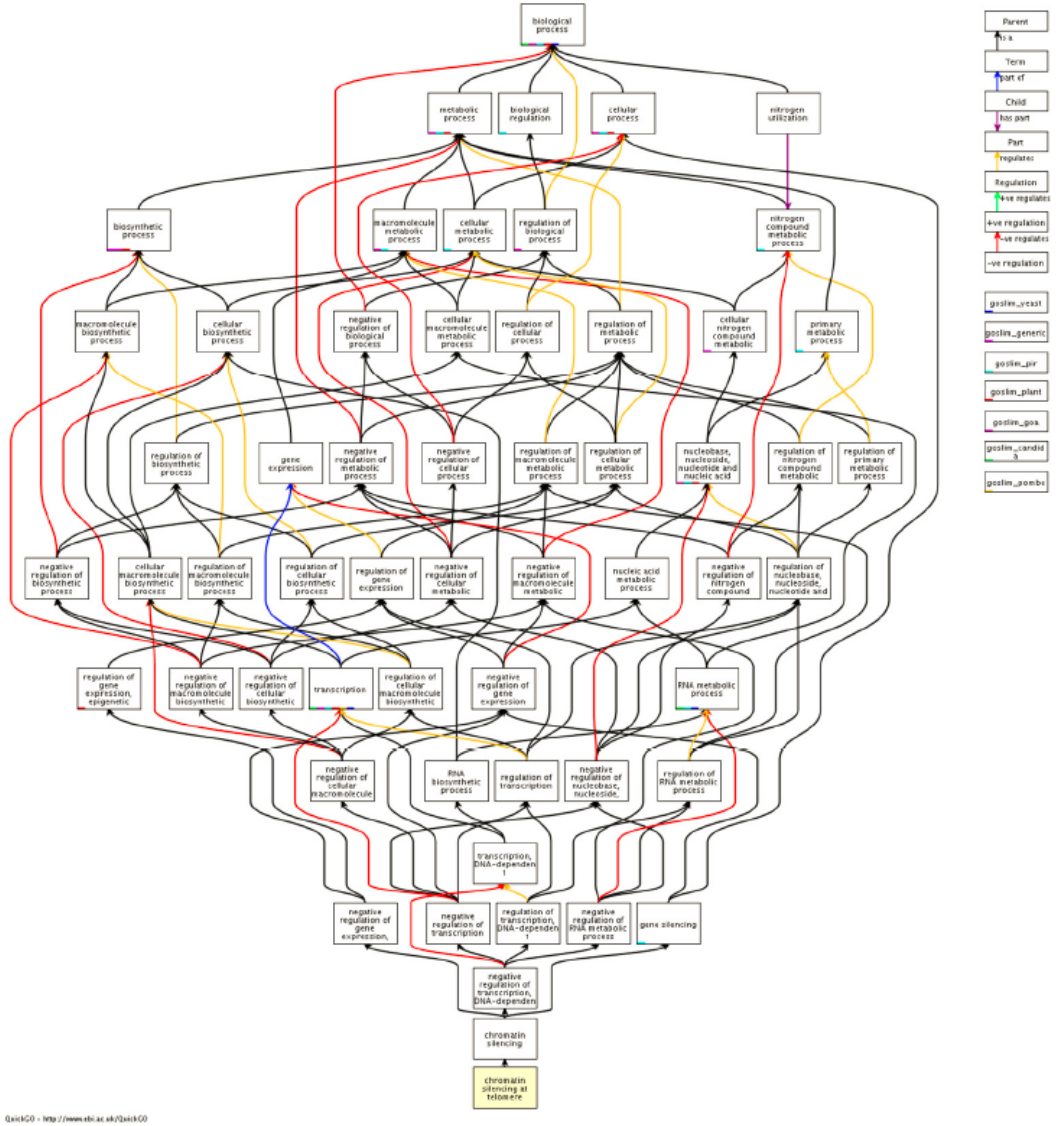


Image source: http://elbo.gs.washington.edu/courses/GS_559_11_wi/slides/11A-GeneAnnotation.pdf

Evidence codes

- Specifying how terms are used to annotate particular instances (http://www.geneontology.org/GO.evidence.shtml)
  - Very important, but usually neglected

Experimental Evidence Codes
- EXP: Inferred from Experiment
- IDA: Inferred from Direct Assay
- IPI: Inferred from Physical Interaction
- IMP: Inferred from Mutant Phenotype
- IGI: Inferred from Genetic Interaction
- IEP: Inferred from Expression Pattern

Computational Analysis Evidence Codes
- ISS: Inferred from Sequence or Structural Similarity
- ISO: Inferred from Sequence Orthology
- ISA: Inferred from Sequence Alignment
- ISM: Inferred from Sequence Model
- IGC: Inferred from Genomic Context
- IBA: Inferred from Biological aspect of Ancestor
- IBD: Inferred from Biological aspect of Descendant
- IKR: Inferred from Key Residues
- IRD: Inferred from Rapid Divergence
- RCA: inferred from Reviewed Computational Analysis

Author Statement Evidence Codes
- TAS: Traceable Author Statement
- NAS: Non-traceable Author Statement

Curator Statement Evidence Codes
- IC: Inferred by Curator
- ND: No biological Data available

Automatically-assigned Evidence Codes
- IEA: Inferred from Electronic Annotation

Obsolete Evidence Codes
- NR: Not Recorded

Pathways

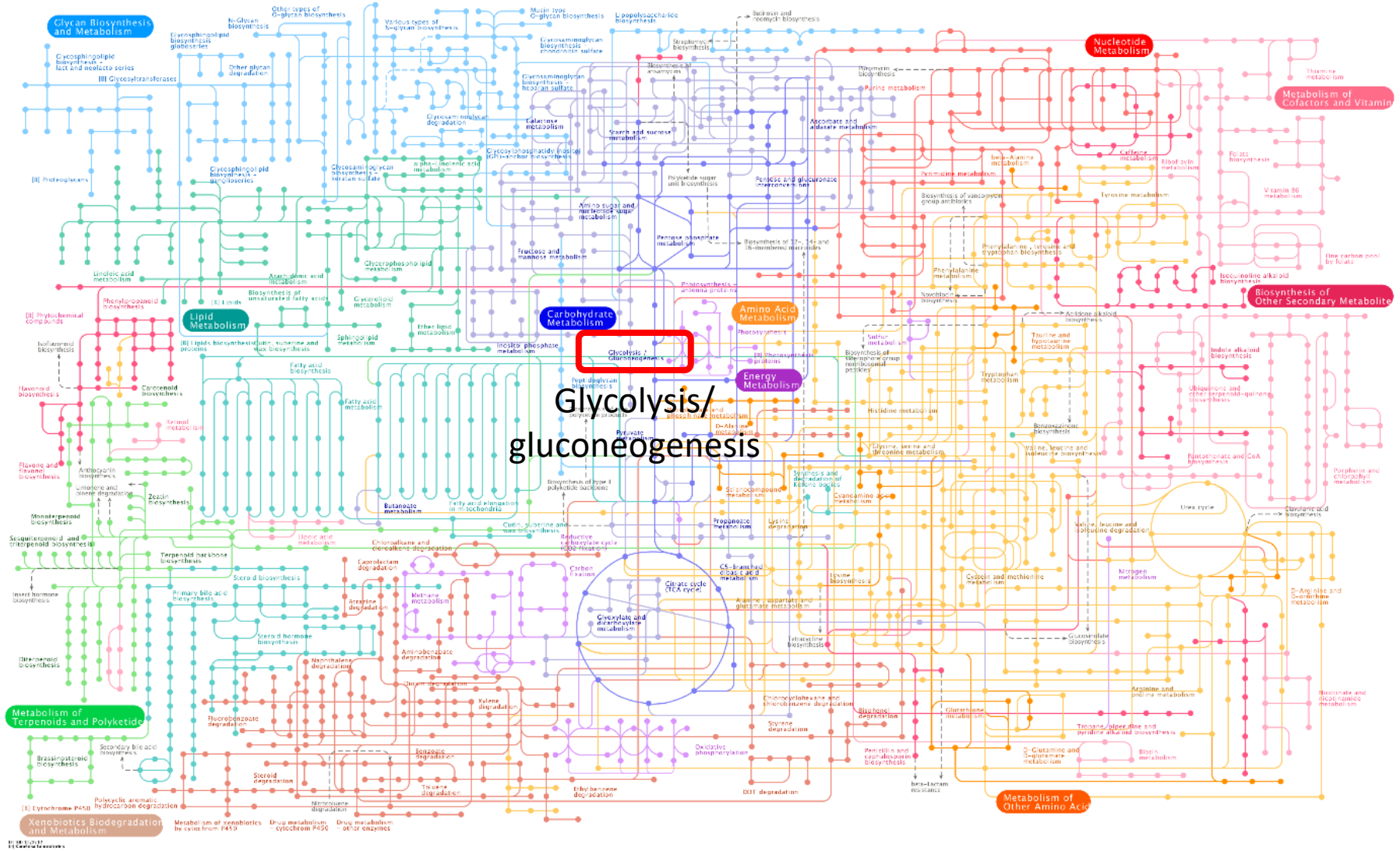
- Gene ontology provides a simple relationship between different objects: they are both annotated with a common term
- Pathways describe detailed mechanistic relationships between the objects
  - E.g., a metabolic pathway records how metabolites are converted to other metabolites through the actions of enzymes

- Wikipedia: “A biological pathway is a series of actions among molecules in a cell that leads to a certain product or a change in a cell”

KEGG

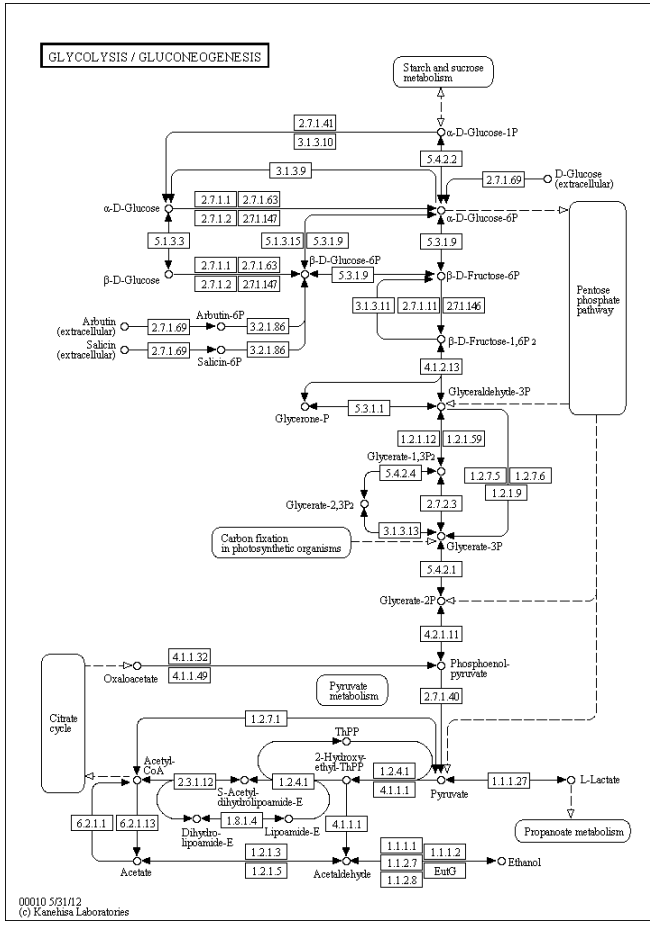
- KEGG: Kyoto Encyclopedia of Genes and Genomes Pathway Database
- One of the most commonly used pathway databases
- Provides non-species-specific reference pathways, as well as species-specific versions
- Different types of pathways:
  - Metabolic pathways
  - Genetic information processing
  - Environmental information processing
  - Cellular processes
  - Organismal systems
  - human diseases
  - Drug development

The global map for metabolic pathways



Glycolysis/gluconeogenesis

- The reference pathway:



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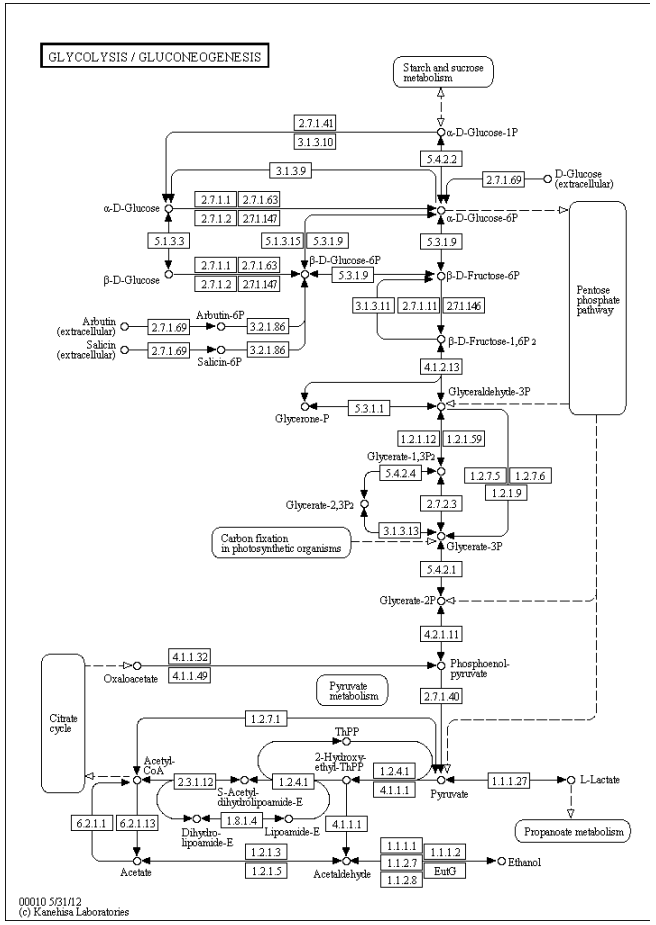
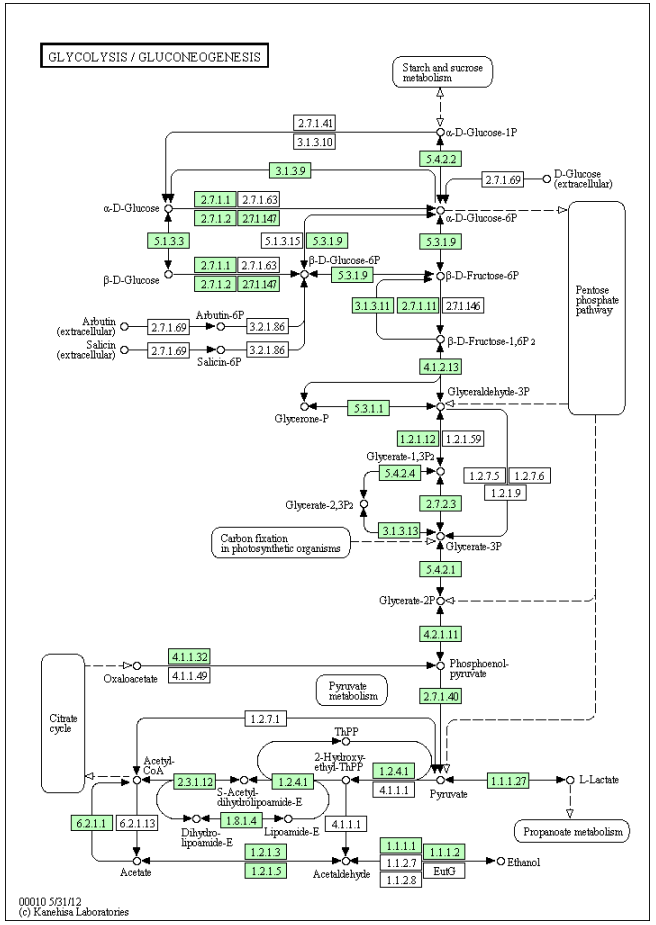


- The human version (in green):



Part 3

Functional Enrichment Analysis

Gene set analysis

- A lot of times we obtain a certain set of genes
  - –Genes with co-expression (or simultaneous differential expression)
  - –Genes of which the promoters are bound by a common transcription factor
  - –Genes that harbor some mutations in patients of a certain disease
- We want to study if these genes have any relationships

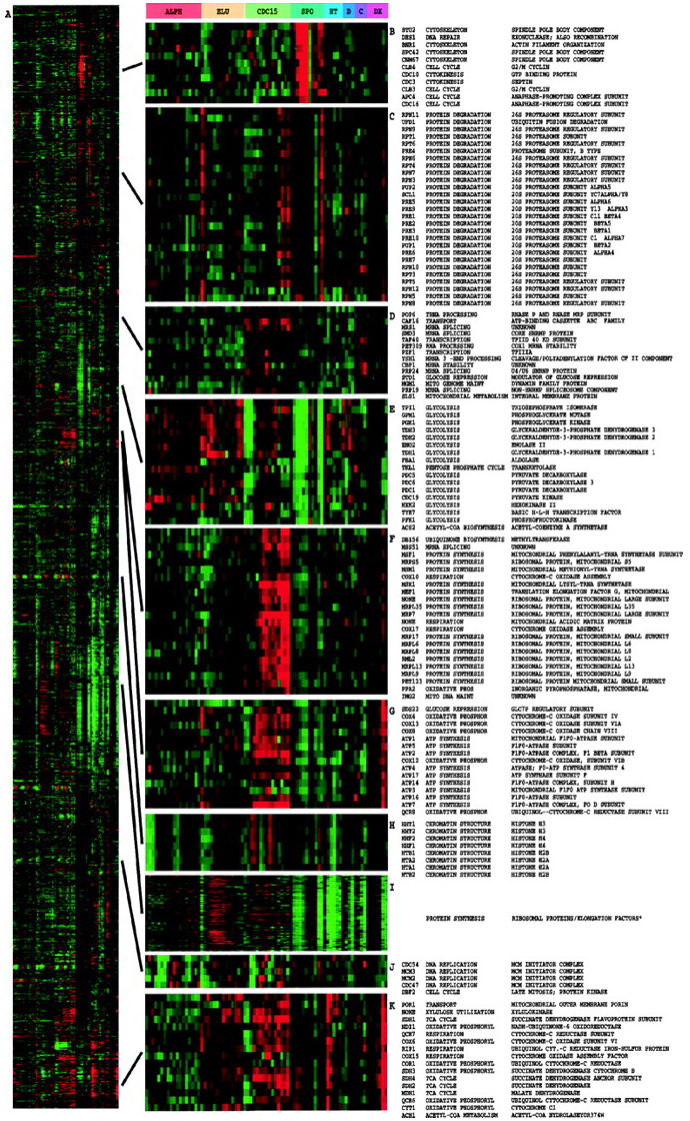


Image credit: Eisen et al., *PNAS* 95(25):14863-14868, (1998)

Functional enrichment

- If each gene is annotated with some standard terms (e.g., from GO), we can look for enriched terms
  - Enrichment: statistically significant
  - Contingency table for a term (e.g., binding):

	Genes in target set	Genes not in target set	Total
Genes annotated with a term	m_1	m_2	m_1+m_2
Genes not annotated with a term	n_1-m_1	n_2-m_2	$n_1+n_2-m_1-m_2$
Total	n_1	n_2	n_1+n_2

  - Null hypothesis H_0: the term and gene set are independent

$$\Pr(m_1|H_0) = \frac{\binom{n_1}{m_1}\binom{n_2}{m_2}}{\binom{n_1+n_2}{m_1+m_2}}$$

  - Compute one-sided p-value: $\Pr(m \geq m_1 \mid H_0)$; Define cutoff (usually $p<0.05$ or $p<0.01$ is considered statistically significant)

Interpreting analysis results

- Generating hypotheses based on enrichment results:
  - If many genes in the target set are annotated with a certain term, the term may be related to the phenotypic observation
  - If many genes in the target set are annotated with a certain term, the other genes in the set may also be annotated with this term -- “guilt by association”
  - If the genes are annotated with two terms, the terms may be related
  - If no statistically significant terms can be found, the phenotypic observation may be non-genetic, largely affected by other (e.g., environmental) factors, or is affected by many loci

Problems

- Choosing a proper background
  - Whole genome vs. all genes studied in the experiment
    - Suppose your experiment involves 100 genes, 20 of which (i.e., 20%) are annotated with a certain GO term, but overall only 10% of all human genes are annotated with that term. Using a different background to test your gene set could give very different results.
- Different evidence codes
  - Filtering
- Hierarchical relationships between different terms
  - Problems:
    - Redundancy
    - Different levels of detail/number of annotated genes – affecting p-values
    - Different curators used terms at different levels: deeper if more is known
  - Some proposed solutions:
    - GO slims
    - Fixed level (e.g., level 3)
    - More complex analysis involving term distances, information content, etc.
- Multiple hypothesis testing

Graph-based methods

- If a gene is annotated with a term, and another gene is annotated with a sub-term (child node in the DAG), they do not share terms but are clearly related
- Some ideas for tackling the problem based on the graph:
  - Use shortest-path in the graph as distance. Consider distance instead of common terms
    - Add edge weights based on number of instances annotated with the two terms
  - Consider the lowest common ancestor

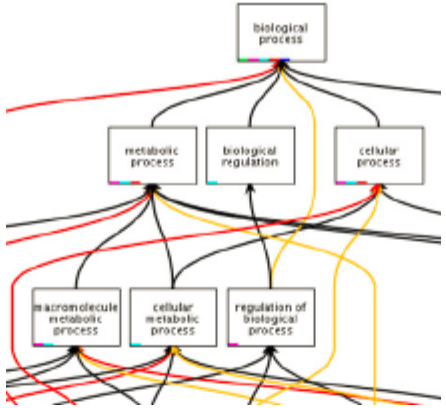


Image source: http://elbo.gs.washington.edu/courses/GS_559_11_wi/slides/11A-GeneAnnotation.pdf

Multiple hypothesis testing

- Suppose you have a set of genes from a certain experiment (e.g., those with a 2-fold increase of expression in cancer samples)
- You perform enrichment analysis using GO terms, KEGG biological pathways, OMIM disease annotations, etc., and find a term with p-value 0.001
  - Recap of the meaning: Say you have n_1 genes in your set and m_1 of them are annotated with this term, if the term is randomly assigned to m_1+m_2 genes among all n_1+n_2 genes, the probability of having m_1 or more genes annotated with the term in a random set of n_1 genes is 0.001
  - Is it statistically significant?
  - Is it biologically significant?

Multiple hypothesis testing

- The famous “stock prediction email” metaphor:
  - On day 1, you receive an email predicting the price of a stock will go up on day 2, and it really happens
  - On day 2, you receive an email again predicting the price of the stock will go down on day 3, and it really happens
  - Similar things happen for 10 days. If every day the probability for the stock price to go up or down is independent of the other days and equals 0.5, then the p-value, i.e., the probability that in 10 random guesses there are 10 or more correct is $0.5^{10} \approx 0.001$
  - Is it statistically significant?
  - Is it financially significant?

Multiple hypothesis testing: The problem

- If you are the only one receiving emails, the predictions are indeed surprisingly accurate
- But if many have receive the emails with different predictions, together the results may not be that surprising
  - If 1,024 people have received the emails, each with a different sequence of predictions, then one of them must be correct for all 10 days (p-value=1)
  - If 1,024 people have received the emails and their contents are independently generated, one person is expected to have got 10 correct predictions
- Similarly, if you have tested your gene set with many terms, it would be not surprising if a term has p-value 0.001.

Multiple hypothesis testing: The solution

- Need to adjust the way to calculate or interpret the p-value
- Many ways:
  - Bonferroni
    - Multiply the p-value by the number of terms tested
    - If you have tested 100 terms and you want to use a cutoff of p=0.01 to define statistical significance, then a term is significantly enriched in your gene set only if it gets a p-value of 0.01/100 = 0.0001
  - Benjamini-Hochberg
    - Less conservative
  - ...
- Beware of cherry picking: shrinking the set of tested terms *a posteriori*
  - Scientifically and ethically wrong

Tools for enrichment analysis

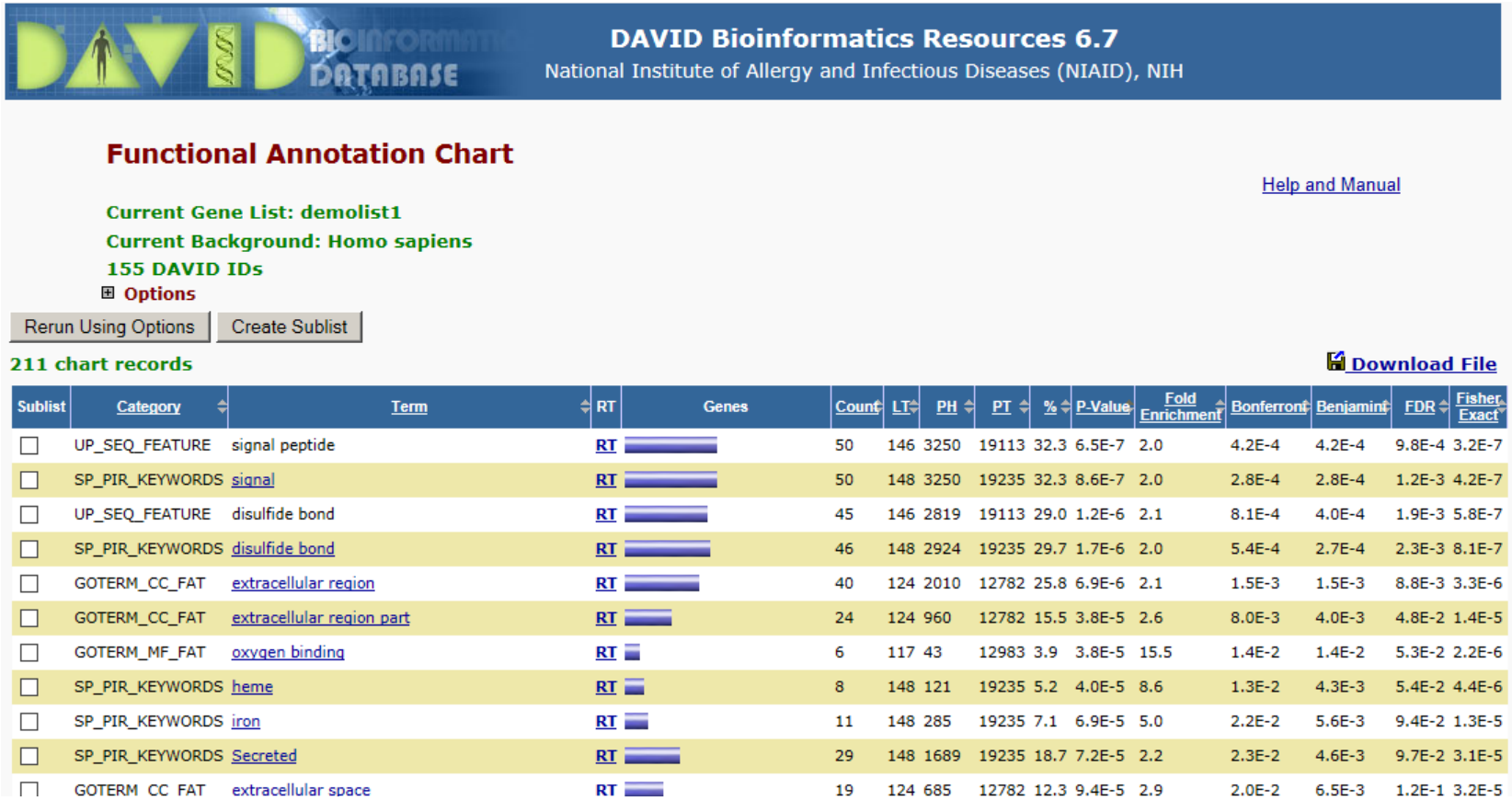
- AmiGO
  - Default software on the Gene Ontology web site
- BiNGO (A Biological Network Gene Ontology tool)
  - Flanders Interuniversitary Institute for Biotechnology
  - Plugin for the Cytoscape network viewer (next lecture)
- DAVID (Database for Annotation, Visualization and Integrated Discovery)
  - National Cancer Institute
  - One of the most cited tool for functional enrichment analysis
- GSEA (Gene Set Enrichment Analysis)
  - Broad Institute
- ... (see http://www.geneontology.org/GO.tools_by_type.term_enrichment.shtml)

DAVID

- Functionality:
  - –ID conversion
  - –Enrichment analysis
    - Disease
    - Functional categories
    - Gene ontology
    - Protein domains
    - Pathways
    - …
  - –Correction for multiple hypothesis testing

Sample results

- Functional annotation chart:



DAVID Bioinformatics Resources 6.7
National Institute of Allergy and Infectious Diseases (NIAID), NIH

Functional Annotation Chart

Help and Manual

Current Gene List: demolist1
Current Background: Homo sapiens
155 DAVID IDs
Options

Rerun Using Options | Create Sublist

211 chart records

Download File

Sublist	Category	Term	RT	Genes	Count	LT	PH	PT	%	P-Value	Fold Enrichment	Bonferroni	Benjamini	FDR	Fisher Exact
☐	UP_SEQ_FEATURE	signal peptide	RT		50	146	3250	19113	32.3	6.5E-7	2.0	4.2E-4	4.2E-4	9.8E-4	3.2E-7
☐	SP_PIR_KEYWORDS	signal	RT		50	148	3250	19235	32.3	8.6E-7	2.0	2.8E-4	2.8E-4	1.2E-3	4.2E-7
☐	UP_SEQ_FEATURE	disulfide bond	RT		45	146	2819	19113	29.0	1.2E-6	2.1	8.1E-4	4.0E-4	1.9E-3	5.8E-7
☐	SP_PIR_KEYWORDS	disulfide bond	RT		46	148	2924	19235	29.7	1.7E-6	2.0	5.4E-4	2.7E-4	2.3E-3	8.1E-7
☐	GOTERM_CC_FAT	extracellular region	RT		40	124	2010	12782	25.8	6.9E-6	2.1	1.5E-3	1.5E-3	8.8E-3	3.3E-6
☐	GOTERM_CC_FAT	extracellular region part	RT		24	124	960	12782	15.5	3.8E-5	2.6	8.0E-3	4.0E-3	4.8E-2	1.4E-5
☐	GOTERM_MF_FAT	oxygen binding	RT		6	117	43	12983	3.9	3.8E-5	15.5	1.4E-2	1.4E-2	5.3E-2	2.2E-6
☐	SP_PIR_KEYWORDS	heme	RT		8	148	121	19235	5.2	4.0E-5	8.6	1.3E-2	4.3E-3	5.4E-2	4.4E-6
☐	SP_PIR_KEYWORDS	iron	RT		11	148	285	19235	7.1	6.9E-5	5.0	2.2E-2	5.6E-3	9.4E-2	1.3E-5
☐	SP_PIR_KEYWORDS	Secreted	RT		29	148	1689	19235	18.7	7.2E-5	2.2	2.3E-2	4.6E-3	9.7E-2	3.1E-5
☐	GOTERM_CC_FAT	extracellular space	RT		19	124	685	12782	12.3	9.4E-5	2.9	2.0E-2	6.5E-3	1.2E-1	3.2E-5

Sample results

- Functional annotation chart:

Sublist	Category	Term	RT	Genes	Count	LT	PH	PT	%	P-Value	Fold Enrichment	Bonferroni	Benjamini	FDR	Fisher Exact
☐	UP_SEQ_FEATURE	signal peptide	RT		50	146	3250	19113	32.3	6.5E-7	2.0	4.2E-4	4.2E-4	9.8E-4	3.2E-7

  - RT: related terms
  - Count: number of genes on your list annotated with the term
  - LT: number of genes on your list
  - PH: number of genes in the whole genome annotated with the term
  - PT: number of genes in the whole genome
  - %: Count / LT
  - P-Value: uncorrected p-value based on a procedure similar to Fisher's exact test (more conservative)
  - Fold Enrichment: (Count / LT) / (PH / PT)
  - Bonferroni: p-value corrected for multiple hypothesis testing based on the Bonferroni procedure
  - Benjamini: p-value corrected for multiple hypothesis testing based on the Benjamini-Hochberg procedure
  - FDR: False discovery rate (highly related to the Benjamini-adjusted p-value)
  - Fish Exact: uncorrected p-value based on Fisher's exact test

Thresholding issue

- Sometimes the way to define the gene set is quite arbitrary
  - Differential expression – Should we use 1.5-fold or 2-fold?
  - TF binding – How far away from a gene should we still consider a site as a promoter of a gene?
- An illustration:
  - 2-fold over-expressed:

Over-expressed "binding"	Yes	No
Yes	1	2
No	1	6

One-sided p-value: 0.5333

  - 1.5-fold over-expressed:

Over-expressed "binding"	Yes	No
Yes	2	1
No	1	6

One-sided p-value: 0.1833

Gene	Expression fold change in cancer	Annotated with term "binding"
1	2.7	Yes
2	2.5	No
3	1.9	Yes
4	1.2	No
5	1.1	No
6	0.9	No
7	0.7	Yes
8	0.6	No
9	0.4	No
10	0.2	No

GSEA

- GSEA tries to avoid using arbitrary thresholds to call gene sets
- Ideas:
  - For each term, find the set of genes annotated with it
  - Check the ranks of these genes based on a phenotypic measure for calling gene sets (e.g., expression fold-change). Compute a statistic (enrichment score) based on these ranks: increase score when a gene with the term is encountered, decrease score otherwise
    - If the genes annotated with the term is randomly distributed across the whole list of genes, this process is essentially a random walk. The point with maximum distance can be used for evaluating the deviation from this random case.
  - Evaluate statistical significance of the score by permuting phenotypic measure values

GSEA (cont'd)

- Example: Are genes highly expressed in cancer related to binding?

		Actual data		Random set 1		Random set 2		Random set 3		Random set 4	
Gene	Expression fold change in cancer	Annotated with term "binding"	Enrichment score	Annotated with term "binding"	Enrichment score	Annotated with term "binding"	Enrichment score	Annotated with term "binding"	Enrichment score	Annotated with term "binding"	Enrichment score
1	2.7	Yes	1	No	-1	No	-1	No	-1	Yes	1
2	2.5	No	0	No	-2	Yes	0	No	-2	Yes	2
3	1.9	Yes	1	Yes	-1	No	-1	Yes	-1	No	1
4	1.2	No	0	No	-2	No	-2	Yes	0	No	0
5	1.1	No	-1	Yes	-1	Yes	-1	No	-1	Yes	1
6	0.9	No	-2	No	-2	No	-2	No	-2	No	0
7	0.7	Yes	-1	No	-3	No	-3	No	-3	No	-1
8	0.6	No	-2	No	-4	No	-4	No	-4	No	-2
9	0.4	No	-3	Yes	-3	Yes	-3	Yes	-3	No	-3
10	0.2	No	-4	No	-4	No	-4	No	-4	No	-4

Random set 5		Random set 6		Random set 7		Random set 8		Random set 9		Random set 10	
Annotated with term "binding"	Enrichment score	Annotated with term "binding"	Enrichment score	Annotated with term "binding"	Enrichment score	Annotated with term "binding"	Enrichment score	Annotated with term "binding"	Enrichment score	Annotated with term "binding"	Enrichment score
No	-1	Yes	1	No	-1	Yes	1	No	-1	No	-1
No	-2	No	0	Yes	0	No	0	No	-2	Yes	0
Yes	-1	No	-1	No	-1	No	-1	Yes	-1	No	-1
No	-2	Yes	0	No	-2	No	-2	Yes	0	No	-2
Yes	-1	No	-1	No	-3	No	-3	No	-1	Yes	-1
No	-2	No	-2	Yes	-2	Yes	-2	Yes	0	No	-2
No	-3	Yes	-1	No	-3	No	-3	No	-1	Yes	-1
No	-4	No	-2	No	-4	Yes	-2	No	-2	No	-2
No	-5	No	-3	Yes	-3	No	-3	No	-3	No	-3
Yes	-4	No	-4	No	-4	No	-4	No	-4	No	-4

GSEA (cont'd)

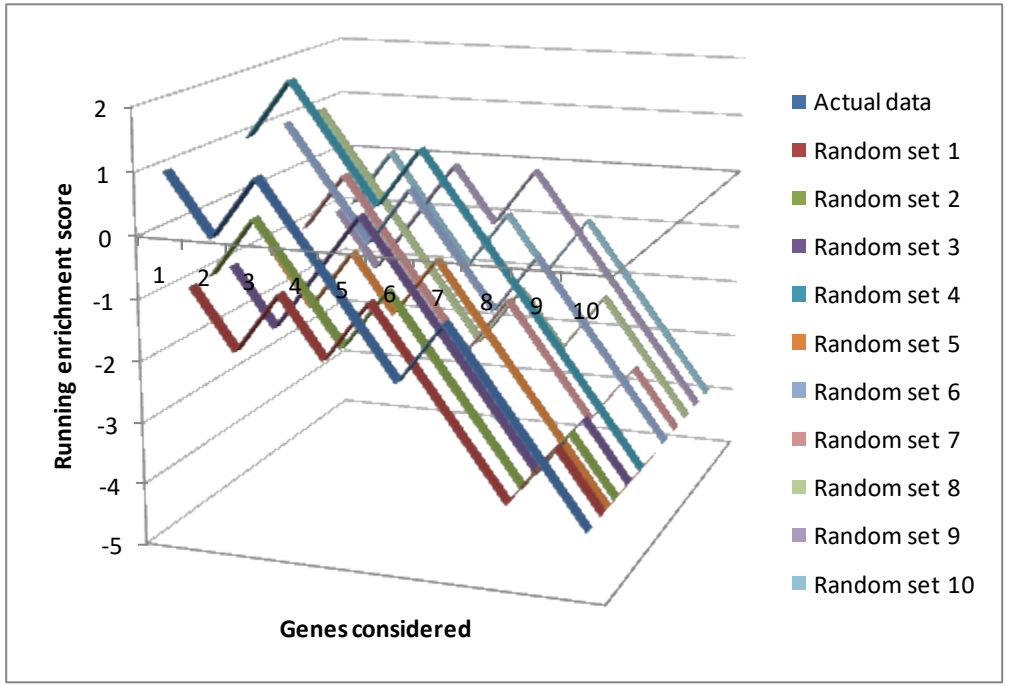
- Maximum values highlighted in blue:

		Actual data		Random set 1		Random set 2		Random set 3		Random set 4	
Gene	Expression fold change in cancer	Annotated with term "binding"	Enrichment score	Annotated with term "binding"	Enrichment score	Annotated with term "binding"	Enrichment score	Annotated with term "binding"	Enrichment score	Annotated with term "binding"	Enrichment score
1	2.7	Yes	1	No	-1	No	-1	No	-1	Yes	1
2	2.5	No	0	No	-2	Yes	0	No	-2	Yes	2
3	1.9	Yes	1	Yes	-1	No	-1	Yes	-1	No	1
4	1.2	No	0	No	-2	No	-2	Yes	0	No	0
5	1.1	No	-1	Yes	-1	Yes	-1	No	-1	Yes	1
6	0.9	No	-2	No	-2	No	-2	No	-2	No	0
7	0.7	Yes	-1	No	-3	No	-3	No	-3	No	-1
8	0.6	No	-2	No	-4	No	-4	No	-4	No	-2
9	0.4	No	-3	Yes	-3	Yes	-3	Yes	-3	No	-3
10	0.2	No	-4	No	-4	No	-4	No	-4	No	-4

Random set 5		Random set 6		Random set 7		Random set 8		Random set 9		Random set 10	
Annotated with term "binding"	Enrichment score	Annotated with term "binding"	Enrichment score	Annotated with term "binding"	Enrichment score	Annotated with term "binding"	Enrichment score	Annotated with term "binding"	Enrichment score	Annotated with term "binding"	Enrichment score
No	-1	Yes	1	No	-1	Yes	1	No	-1	No	-1
No	-2	No	0	Yes	0	No	0	No	-2	Yes	0
Yes	-1	No	-1	No	-1	No	-1	Yes	-1	No	-1
No	-2	Yes	0	No	-2	No	-2	Yes	0	No	-2
Yes	-1	No	-1	No	-3	No	-3	No	-1	Yes	-1
No	-2	No	-2	Yes	-2	Yes	-2	Yes	0	No	-2
No	-3	Yes	-1	No	-3	No	-3	No	-1	Yes	-1
No	-4	No	-2	No	-4	Yes	-2	No	-2	No	-2
No	-5	No	-3	Yes	-3	No	-3	No	-3	No	-3
Yes	-4	No	-4	No	-4	No	-4	No	-4	No	-4

GSEA (cont'd)

- Example:
  - Since in 3 of the 10 random sets, the maximum score ≥ 1, the observed maximum score for the real data is not significant (p=0.3)
- It is possible to compute the p-value without performing the simulation



GSEA (cont'd)

- Illustration and example:

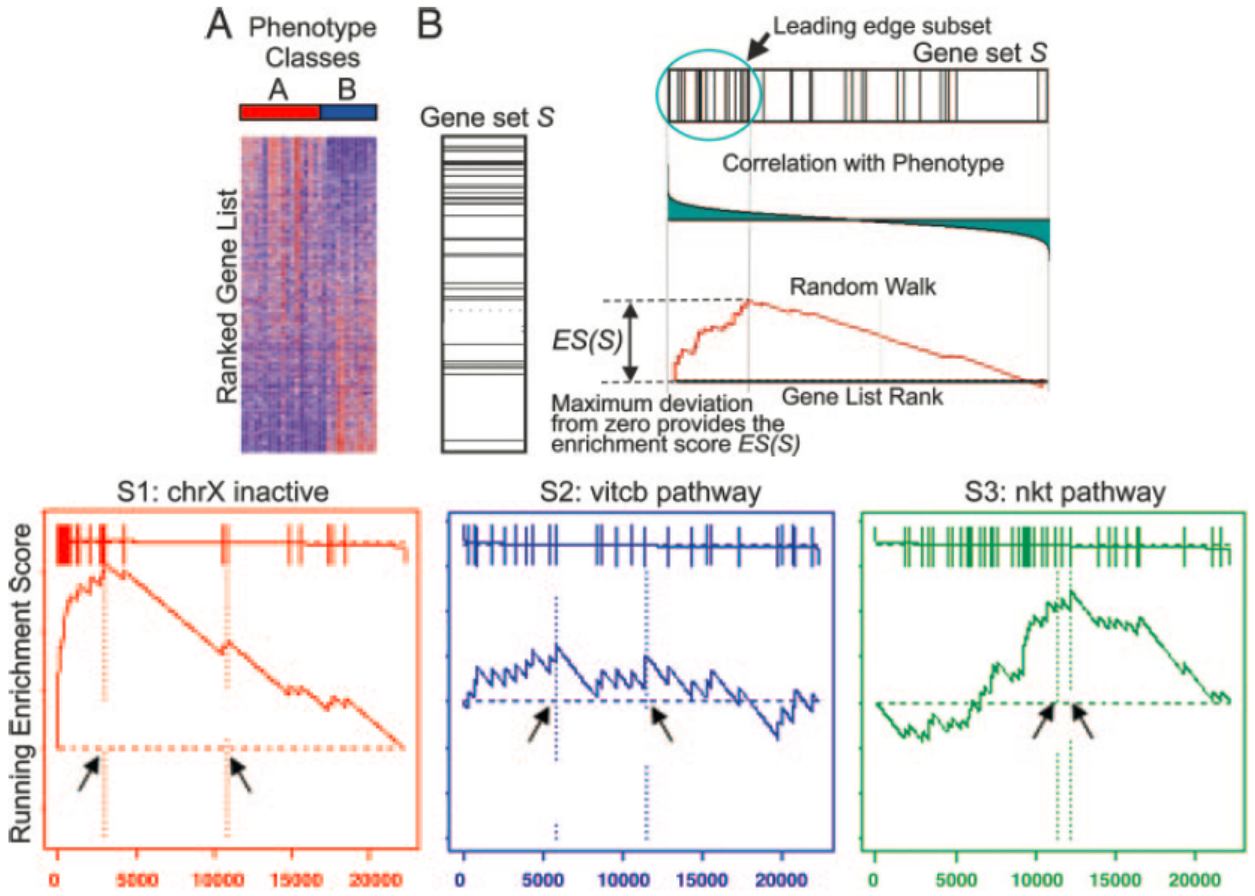


Image credit: Subramanian et al., *PNAS* 102(43):15545-15550, (2005)

Epilogue

Summary and Further Readings

Summary

- Gene annotations
  - Location (Chromosome, start, end, strand)
  - Biotype
  - Attributes
- Gene ontology
  - Molecular function
  - Biological process
  - Cellular component
- Biological pathways
- Functional enrichment analysis for gene sets

Further readings

- Very good review papers on the topics covered in this lecture:
  - Rhee et al., Use and Misuse of the Gene Ontology Annotations. *Nature Reviews Genetics* 9(7):509-515, (2008)
  - Noble, How Does Multiple Hypothesis Testing Correction Work? *Nature Biotechnology* 27(12):1135-1137, (2009)