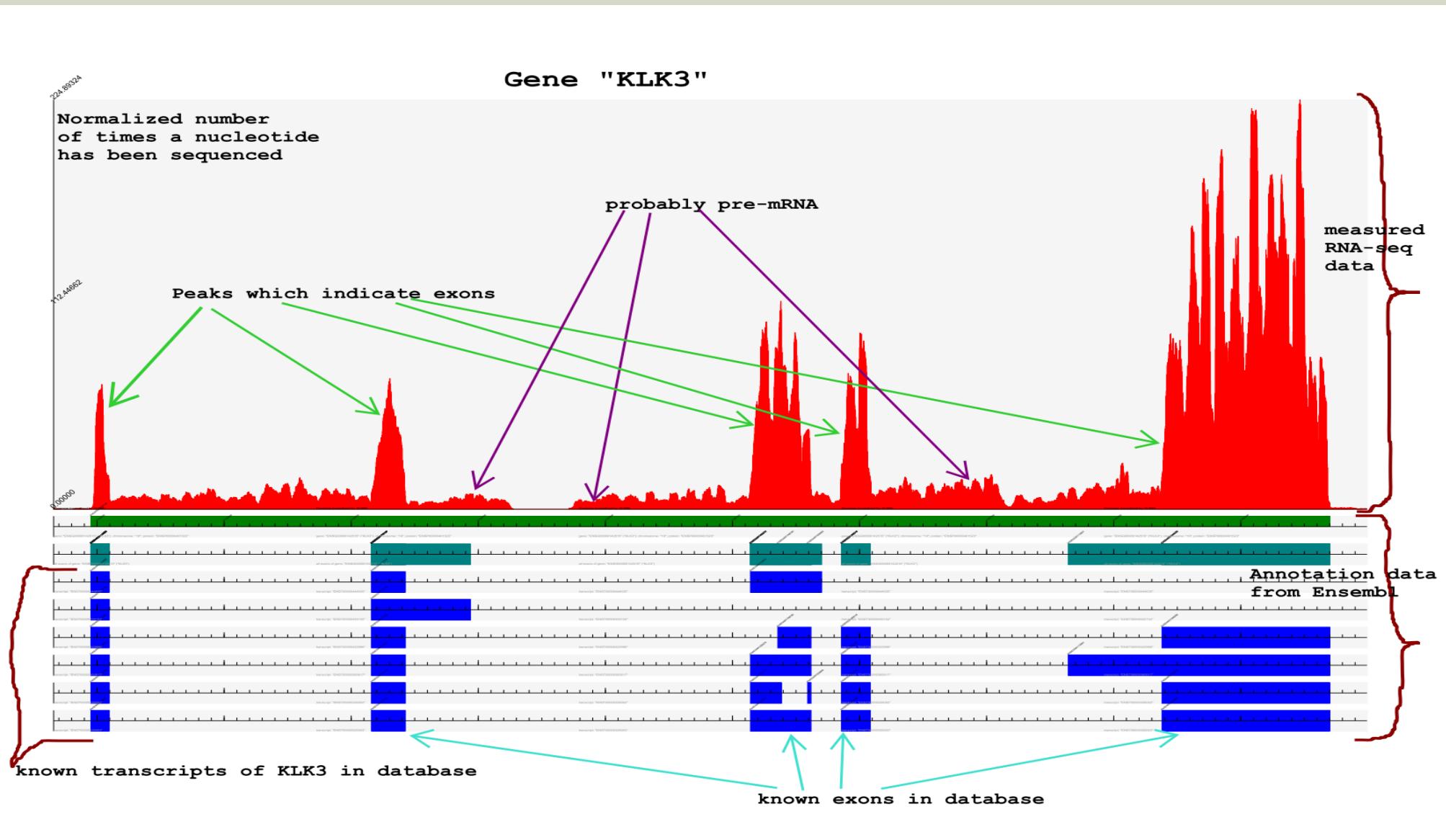
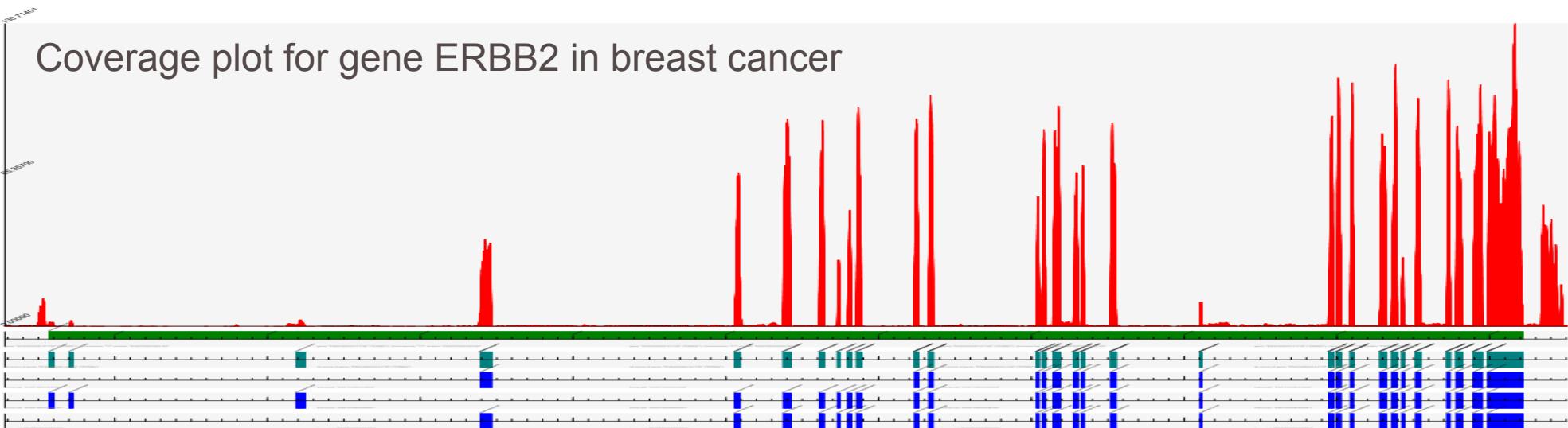


RNA-SEQ DATA ANALYSIS PIPELINE: EXPRESSION ESTIMATION

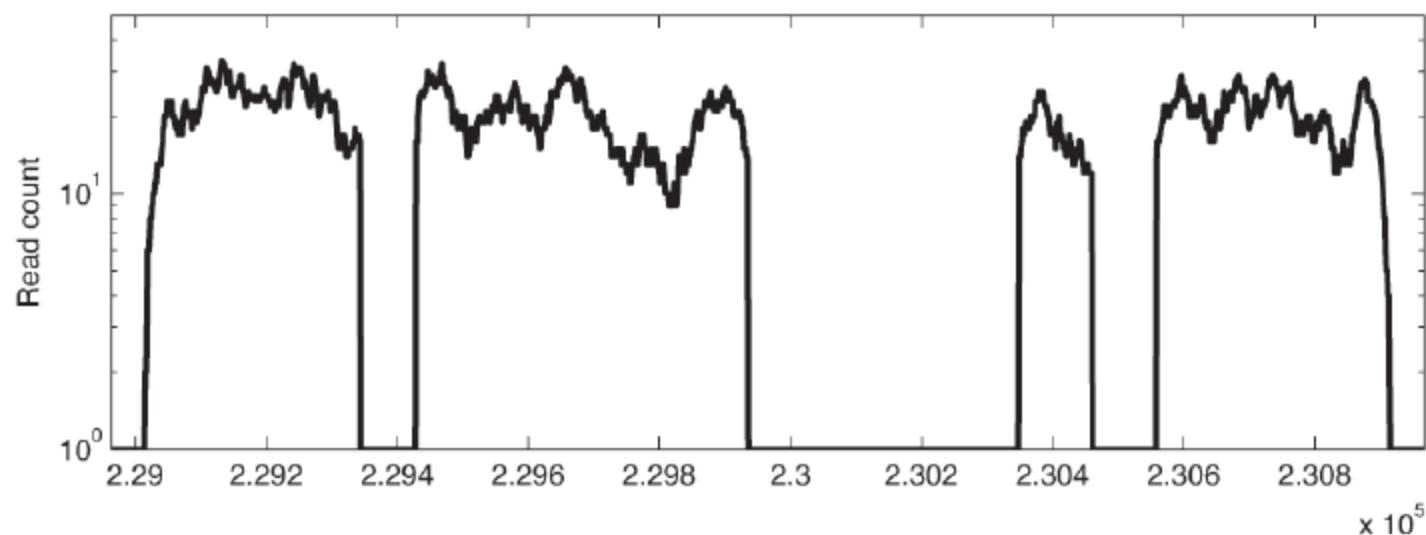
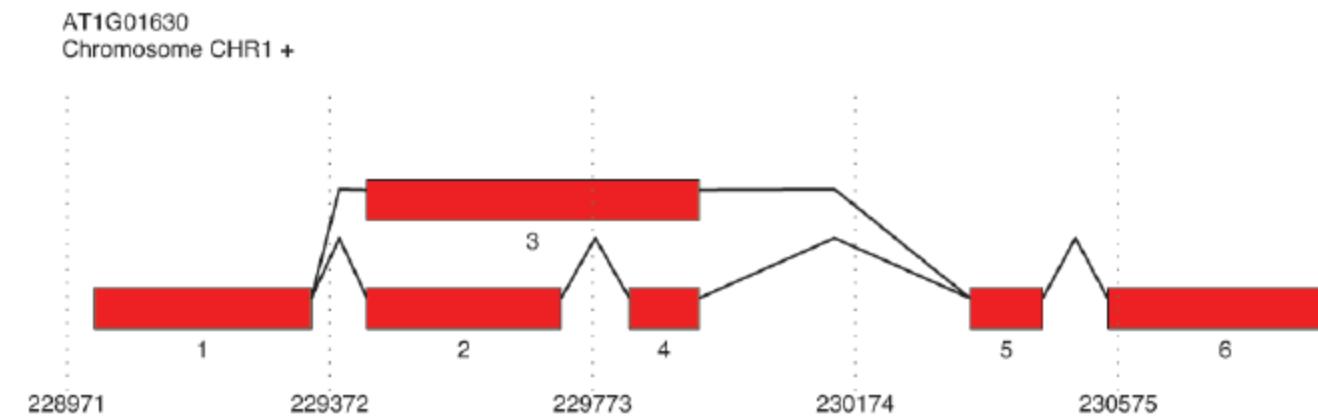
TRANSCRIPTOME RECONSTRUCTION



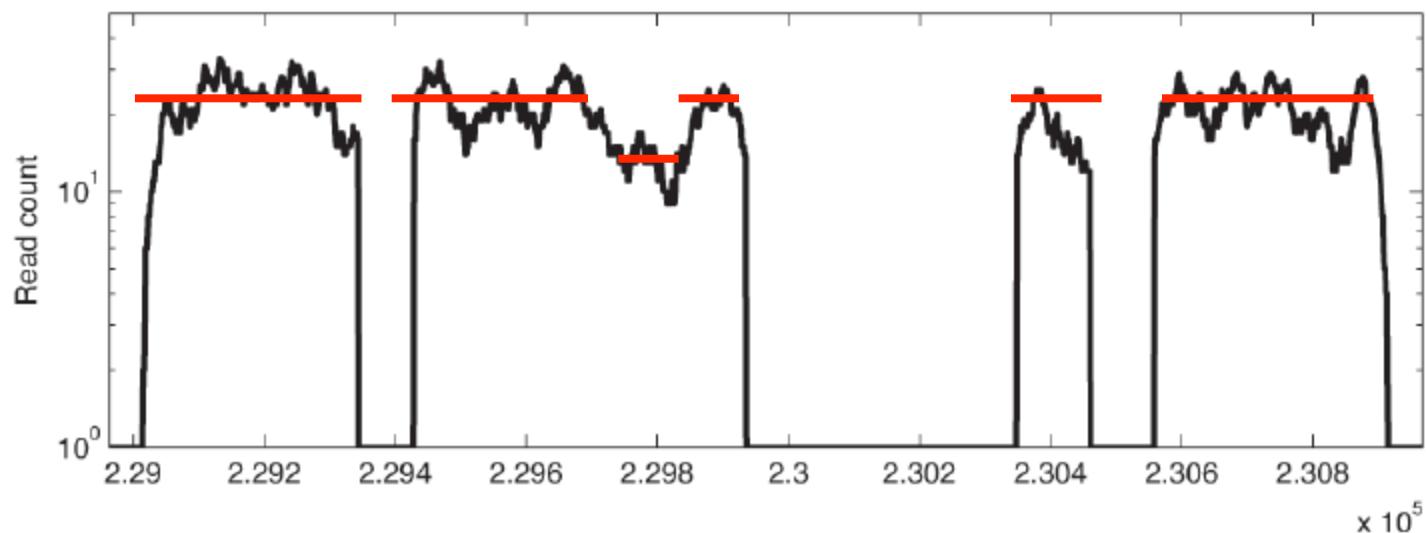
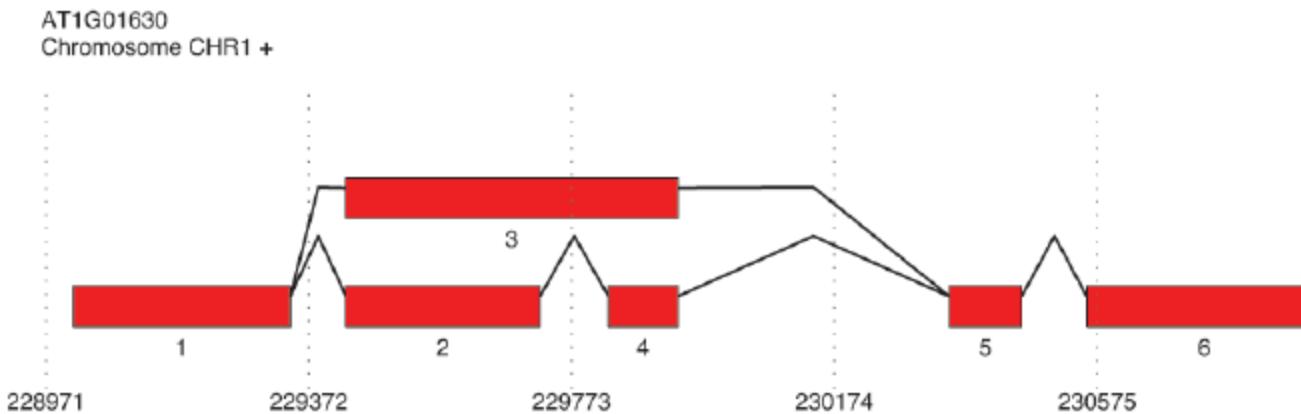
TRANSCRIPTOME RECONSTRUCTION



TRANSCRIPTOME RECONSTRUCTION

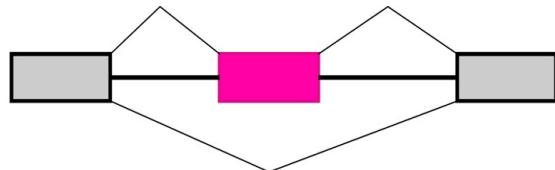


TRANSCRIPTOME RECONSTRUCTION

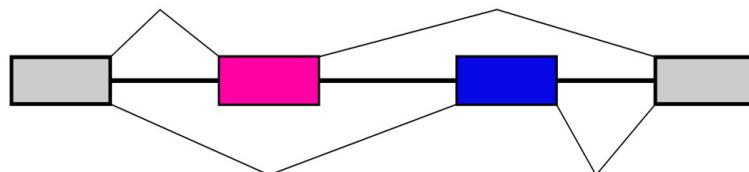


TRANSCRIPTOME RECONSTRUCTION

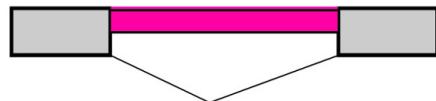
Cassette Exon



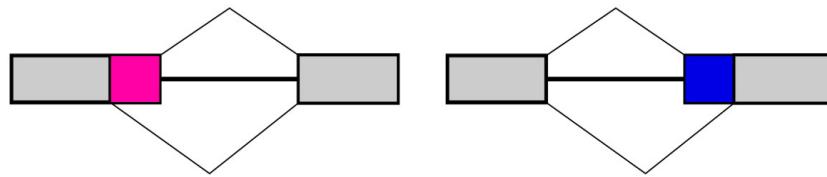
Mutually Exclusive Exons



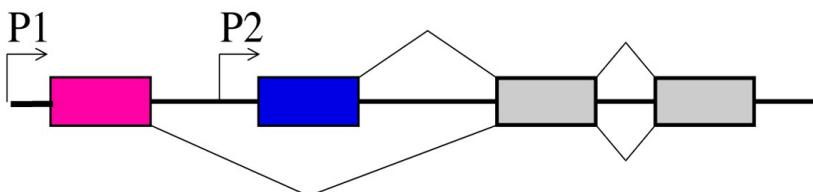
Intron Retention



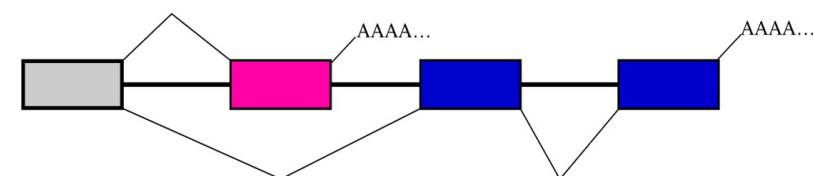
Alternative 5' or 3' Splice Sites



Alternative Promoters



Alternative Splicing and Polyadenylation

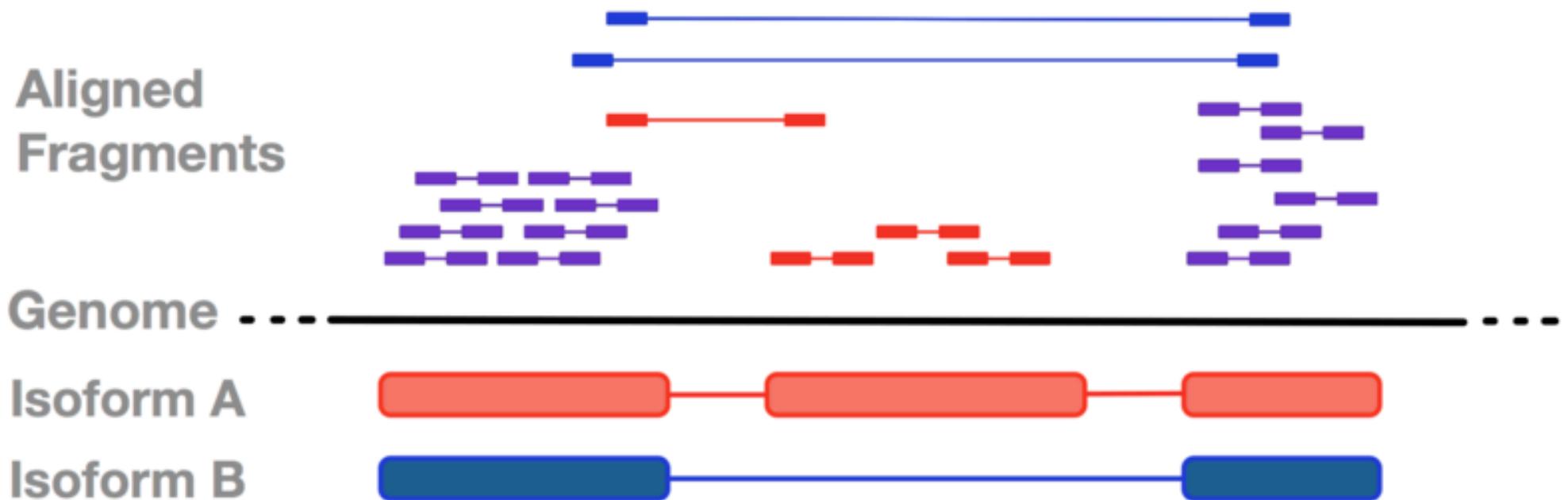


There are different forms of Alternative Splicing:

- Whole exons can be included or excluded (cassette exons)
- Exons can be mutually exclusive
- Intron segments can be retained in the mature transcript
- Exons can be partially included through an internal donor/acceptor site
- Transcription can start from different promoters and/or end at different sites

TRANSCRIPTOME RECONSTRUCTION

Connectivity information from paired end reads and from spliced reads helps in understanding which splicing variants a gene encodes in the analysed sample.



TRANSCRIPTOME RECONSTRUCTION



— Exon 1 — Exon 2 — Exon 3 —

— Exon 1 — Exon 3 —

— Exon 1 — Exon 2 — Exon 3 —

— Exon 1 — Exon 2 — Exon 3 —

— Exon 1 — Exon 2 — Exon 3 —

— Exon 1 — Exon 2 — Exon 3 —

— Exon 1 — Exon 2 — Exon 3 —

— Exon 1 — Exon 2 — Exon 3 —

— Exon 1 — Exon 2 — Exon 3 —

— Exon 1 — Exon 2 — Exon 3 —

— Exon 1 — Exon 2 — Exon 3 —

— Exon 1 — Exon 2 — Exon 3 —

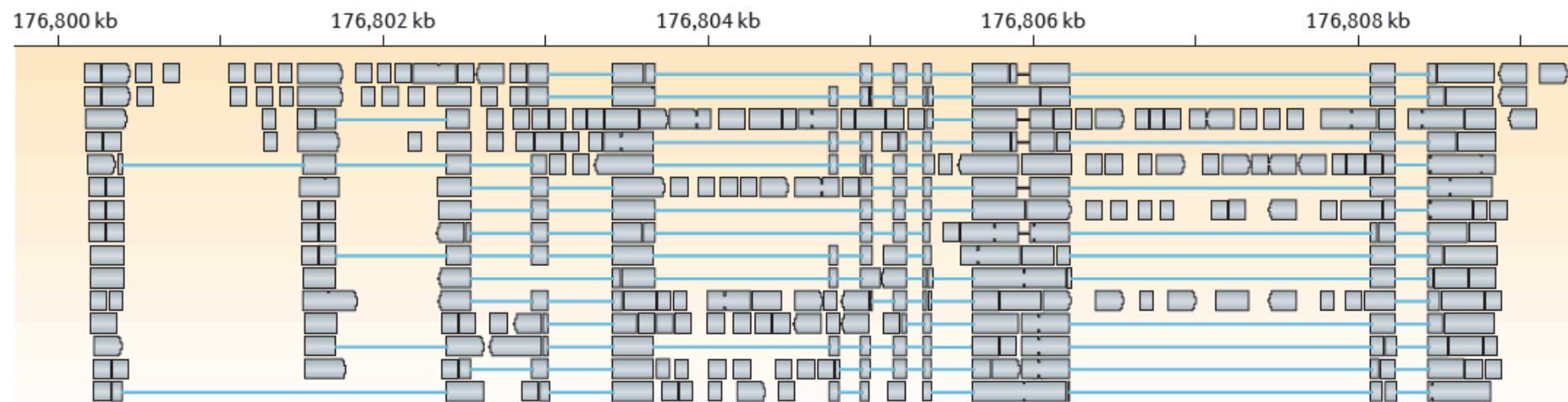
TRANSCRIPTOME RECONSTRUCTION

- Given two splicing variants (isoforms) encoded by a gene, and all reads mapping onto them:

	Exon 1	Exon 2	Exon 3	abundance
Isoform 1				x_1
Isoform 2				x_2
Isoform 3				x_3
Length	l_1	l_2	l_3	
#reads	n_1	n_2	n_3	

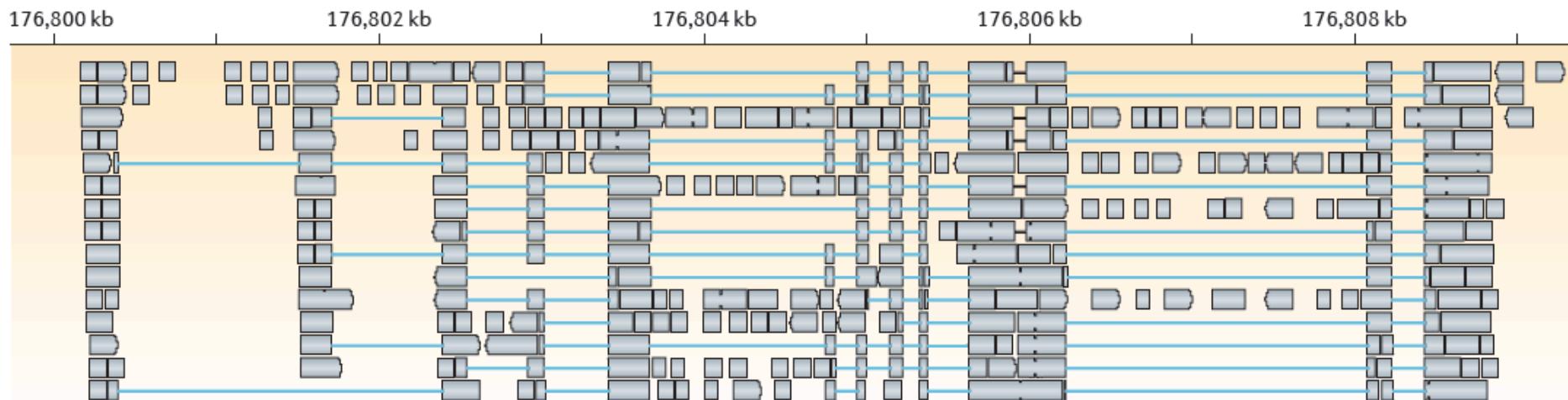
TRANSCRIPTOME RECONSTRUCTION

a Splice-align reads to the genome

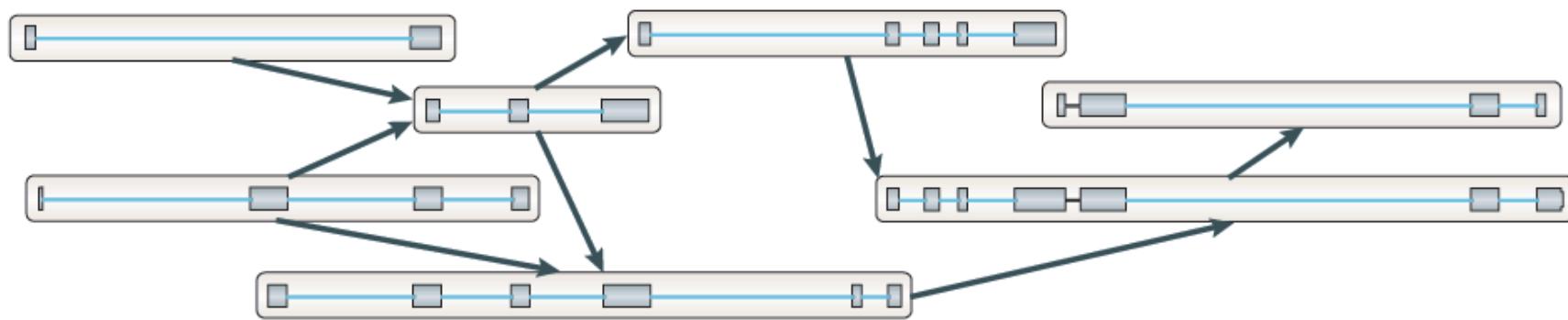


TRANSCRIPTOME RECONSTRUCTION

a Splice-align reads to the genome

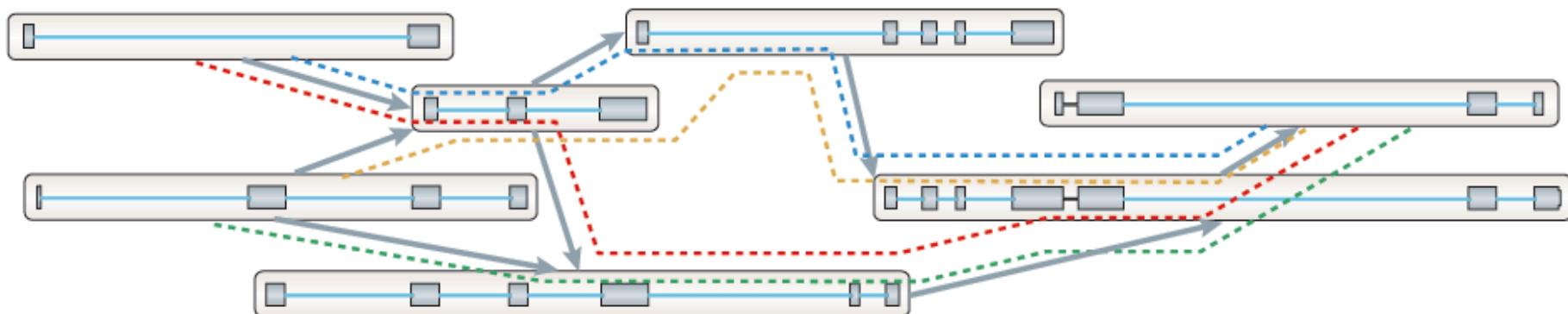


b Build a graph representing alternative splicing events

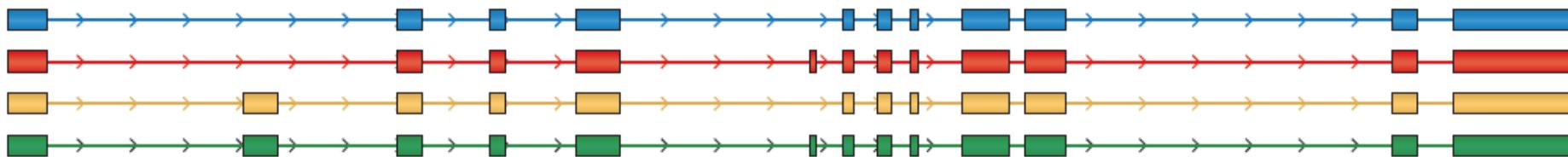


TRANSCRIPTOME RECONSTRUCTION

c Traverse the graph to assemble variants



d Assembled isoforms



TRANSCRIPTOME RECONSTRUCTION

GTF File Format

Seqid	source	type	start	end	score	strand	phase	attributes
Chr1	Snap	exon	234	1543	.	+	.	gene_id "gene1"; transcript_id "transcript1";
Chr1	Snap	CDS	577	1543	.	+	0	gene_id "gene1"; transcript_id "transcript1";
Chr1	Snap	exon	1822	2674	.	+	.	gene_id "gene1"; transcript_id "transcript1";
Chr1	Snap	CDS	1822	2674	.	+	2	gene_id "gene1"; transcript_id "transcript1";
		start_codon						
		stop_codon						

TRANSCRIPTOME RECONSTRUCTION

The **GTF** format: Gene transfer format, for the storage of gene annotations

1. seqname – name of chromosome or scaffold
2. source – name of the algorithm that predicted the gene or of database from which the gene was taken
3. Feature annotation class (e.g. gene, polymorphism)
4. start – genomic coordinate of the annotation start
5. end – end coordinate
6. score – score of the prediction algorithm
7. strand - + (forward) or - (reverse)
8. frame – ':', '0', '1' o '2'. '0' indicates that the start position is the start of a codon, '1' that is the second nucleotide of codone, etc.
9. attribute – List of tag:values pairs describing the annotation

TRANSCRIPTOME RECONSTRUCTION

Single species data

Popular species are listed first. You can customise this list via our [home page](#).

★	Species	Show/hide columns														Filter
		DNA (FASTA)	cDNA (FASTA)	CDS (FASTA)	ncRNA (FASTA)	Protein sequence (FASTA)	Annotated sequence (EMBL)	Annotated sequence (GenBank)	Gene sets	Whole databases	Variation (GVF)	Variation (VCF)	Variation (VEP)	Regulation (GFF)	Data files	BAM
Y	Human <i>Homo sapiens</i>	FASTA	EMBL	GenBank	GTF	MySQL	GVF	VCF	VEP	Regulation (GFF)	Regulation data files	BAM				
Y	Mouse <i>Mus musculus</i>	FASTA	EMBL	GenBank	GTF	MySQL	GVF	VCF	VEP	Regulation (GFF)	Regulation data files	BAM				
Y	Zebrafish <i>Danio rerio</i>	FASTA	EMBL	GenBank	GTF	MySQL	GVF	VCF	VEP	-	-	BAM				
	Alpaca <i>Vicugna pacos</i>	FASTA	EMBL	GenBank	GTF	MySQL	-	-	VEP	-	-	-				
	Anole lizard <i>Anolis carolinensis</i>	FASTA	EMBL	GenBank	GTF	MySQL	-	-	VEP	-	-	BAM				
	Armadillo <i>Dasypus novemcinctus</i>	FASTA	EMBL	GenBank	GTF	MySQL	-	-	VEP	-	-	BAM				
	Bushbaby <i>Otolemur garnettii</i>	FASTA	EMBL	GenBank	GTF	MySQL	-	-	VEP	-	-	-				
	<i>C.intestinalis</i> <i>Ciona intestinalis</i>	FASTA	EMBL	GenBank	GTF	MySQL	-	-	VEP	-	-	-				
	<i>C.savignyi</i> <i>Ciona savignyi</i>	FASTA	EMBL	GenBank	GTF	MySQL	-	-	VEP	-	-	-				
	<i>Caenorhabditis elegans</i> <i>Caenorhabditis elegans</i>	FASTA	EMBL	GenBank	GTF	MySQL	-	-	VEP	-	-	-				

TRANSCRIPTOME RECONSTRUCTION

Cufflinks

Transcript assembly, differential expression, and differential regulation for RNA-Seq



Cufflinks assembles transcripts, estimates their abundances, and tests for **differential expression** and **regulation** in RNA-Seq samples. It accepts aligned RNA-Seq reads and assembles the alignments into a parsimonious set of transcripts. Cufflinks then estimates the relative abundances of these transcripts based on how many reads support each one, taking into account biases in library preparation protocols.

Cufflinks is a collaborative effort between the [Laboratory for Mathematical and Computational Biology](#), led by Lior Pachter at UC Berkeley, Steven Salzberg's [computational genomics group](#) at the Institute of Genetic Medicine at Johns Hopkins University, and [Barbara Wold's lab](#) at Caltech.



Cufflinks is provided under the OSI-approved [Boost License](#)

» 2.2.0 release - 3/25/2014

This release introduces some new features designed to simplify and speed up Cufflinks workflows. Release version 2.2.0 includes two new programs, [cuffquant](#) and [cuffnorm](#) that make it easier to quantify gene expression in experiments with many samples. These are particularly helpful for single cell RNA-Seq experiments, where the reads for each cell are provided as a separate FASTQ file or pair of files.

Release 2.2.0 also introduces [sample sheets](#) and [contrast files](#). These facilities make it easier to work with large analyses involving many samples.

Cuffquant quantifies gene and transcript expression levels for a single BAM file. These levels are stored in a new binary file type, the CXB file. You can then provide CXB files for your samples directly to Cuffdiff instead of BAMs. Mixing BAM and CXB files is not yet supported. Because expression levels for each sample are quantified by Cuffquant, Cuffdiff doesn't have to perform this step, which speeds up Cuffdiff runs substantially and lowers their memory footprints. We recommend that most users switch to this new Cuffdiff workflow for all experiments that involve more than a few samples. However, note that running Cuffquant prior to running Cuffdiff is [optional](#) - you can still directly supply BAM files to Cuffdiff.

Cuffquant files can also be passed to Cuffnorm, which simply computes a normalized table of expression values for genes and transcripts. Unlike Cuffdiff, Cuffnorm performs no differential expression testing. Cuffnorm also does not calculate

Site Map

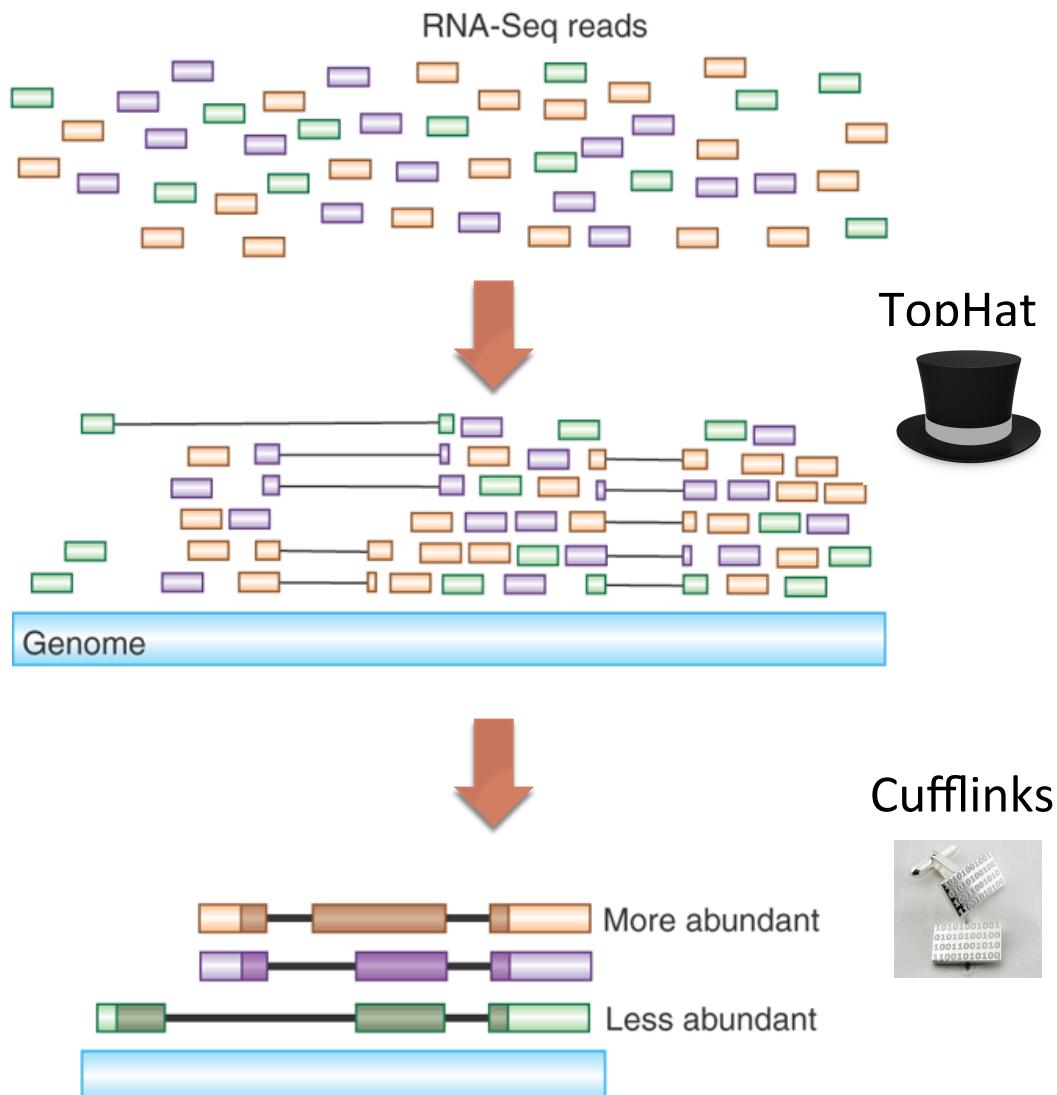
[Home](#)
[Getting started](#)
[Manual](#)
[How Cufflinks works](#)
[Index and annotation downloads](#)
[FAQ](#)
[Protocol](#)
[Benchmarking](#)

News and updates

New releases and related tools will be announced through the [mailing list](#)

Getting Help

TRANSCRIPTOME RECONSTRUCTION



The Tuxedo Suite:
End-to-end Genome-based
RNA-Seq Analysis
Software Package

NATURE PROTOCOLS | PROTOCOL

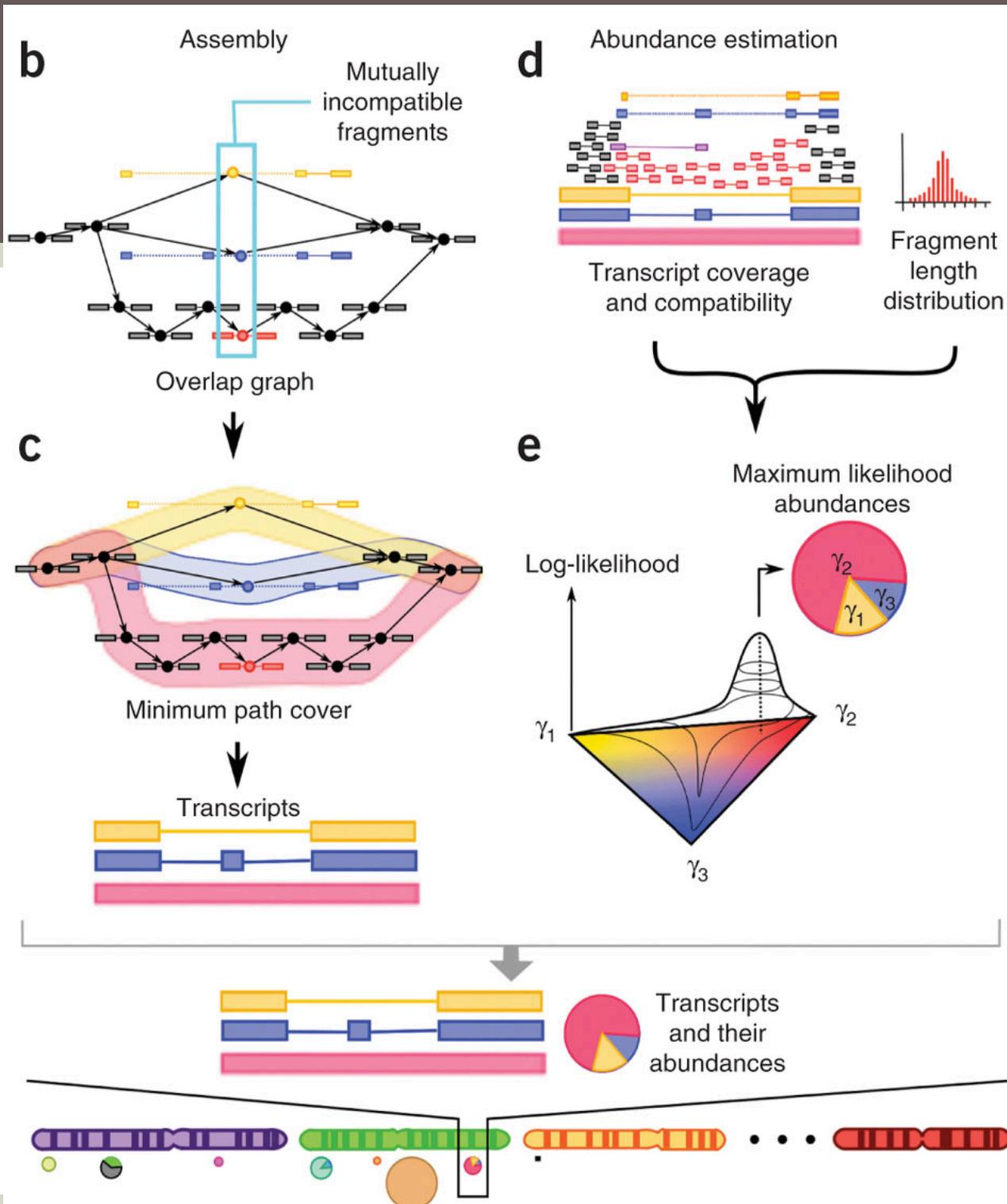


Differential gene and transcript expression analysis of RNA-seq experiments with TopHat and Cufflinks

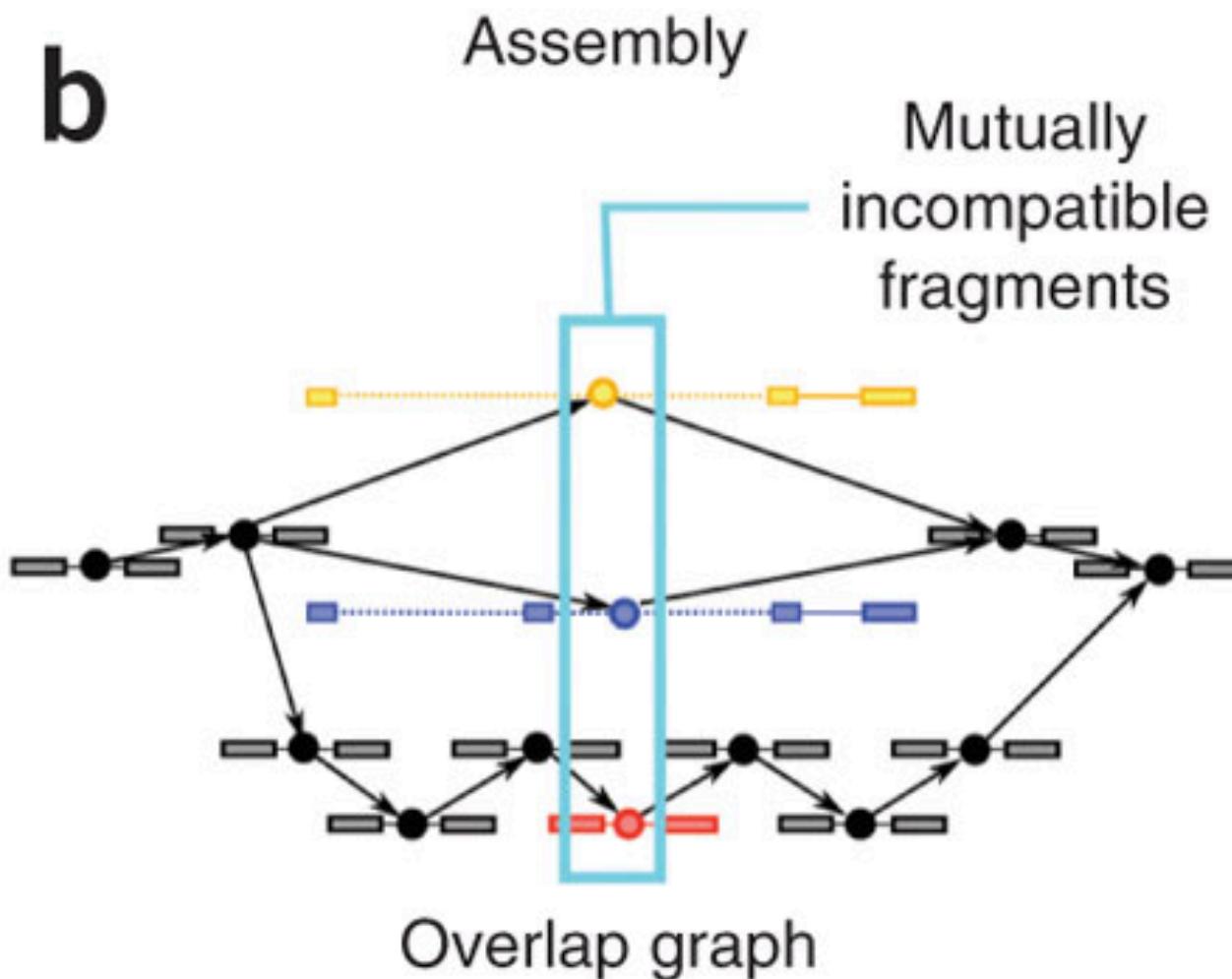
Cole Trapnell, Adam Roberts, Loyal Goff, Geo Pertea, Daehwan Kim, David R Kelley, Harold Pimentel, Steven L Salzberg, John L Rinn & Lior Pachter

Affiliations | Contributions | Corresponding author

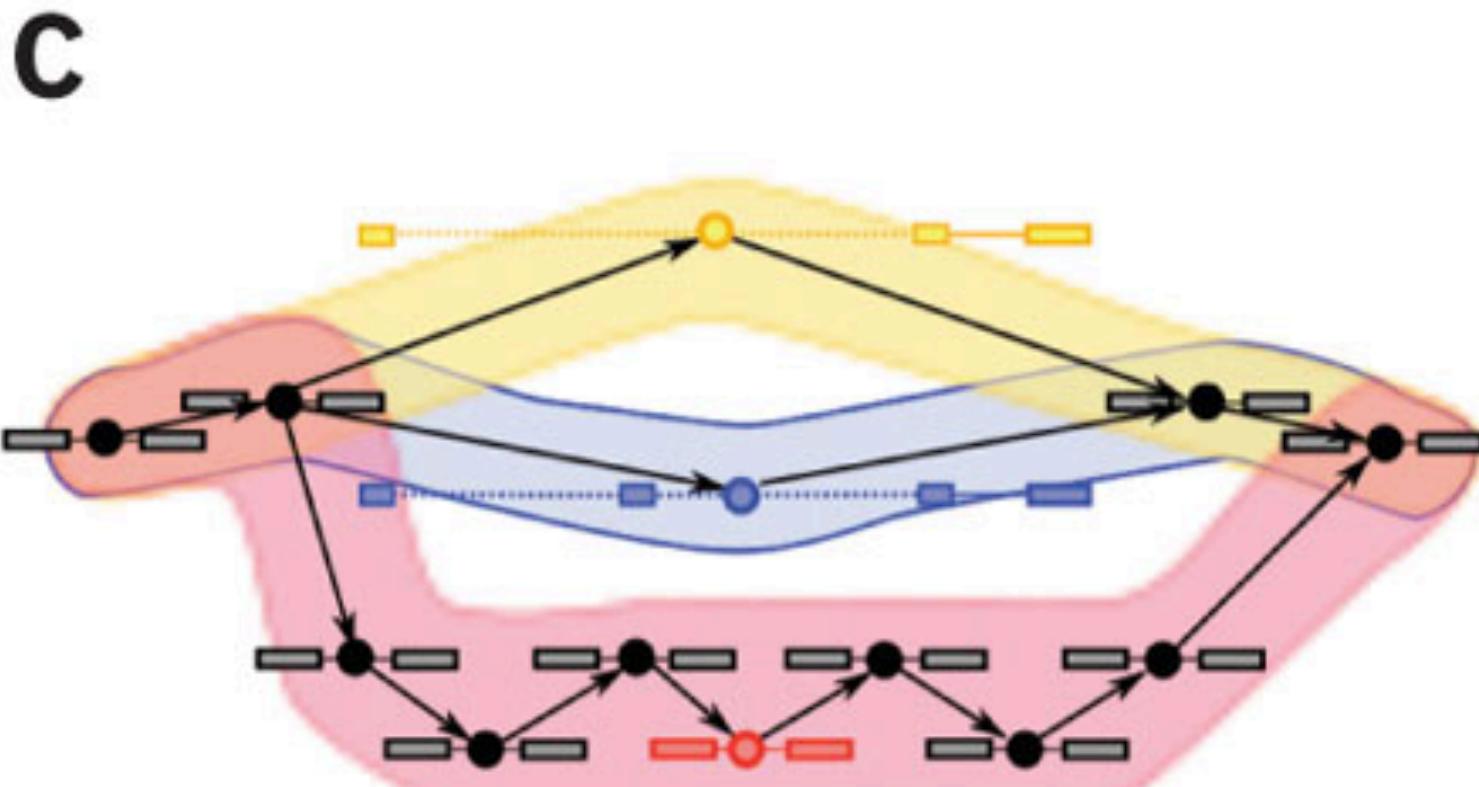
Nature Protocols 7, 562–578 (2012) | doi:10.1038/nprot.2012.016
Published online 01 March 2012



TRANSCRIPTOME RECONSTRUCTION



TRANSCRIPTOME RECONSTRUCTION

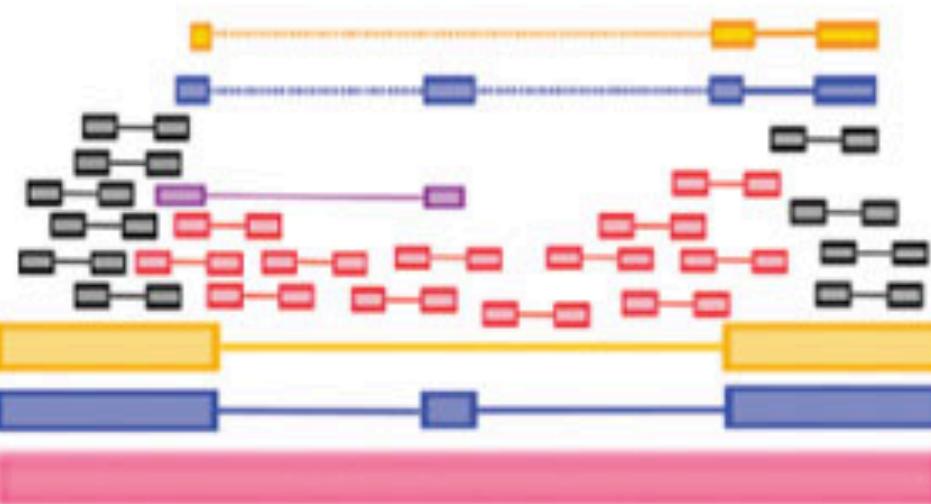


Minimum path cover

TRANSCRIPTOME RECONSTRUCTION

d

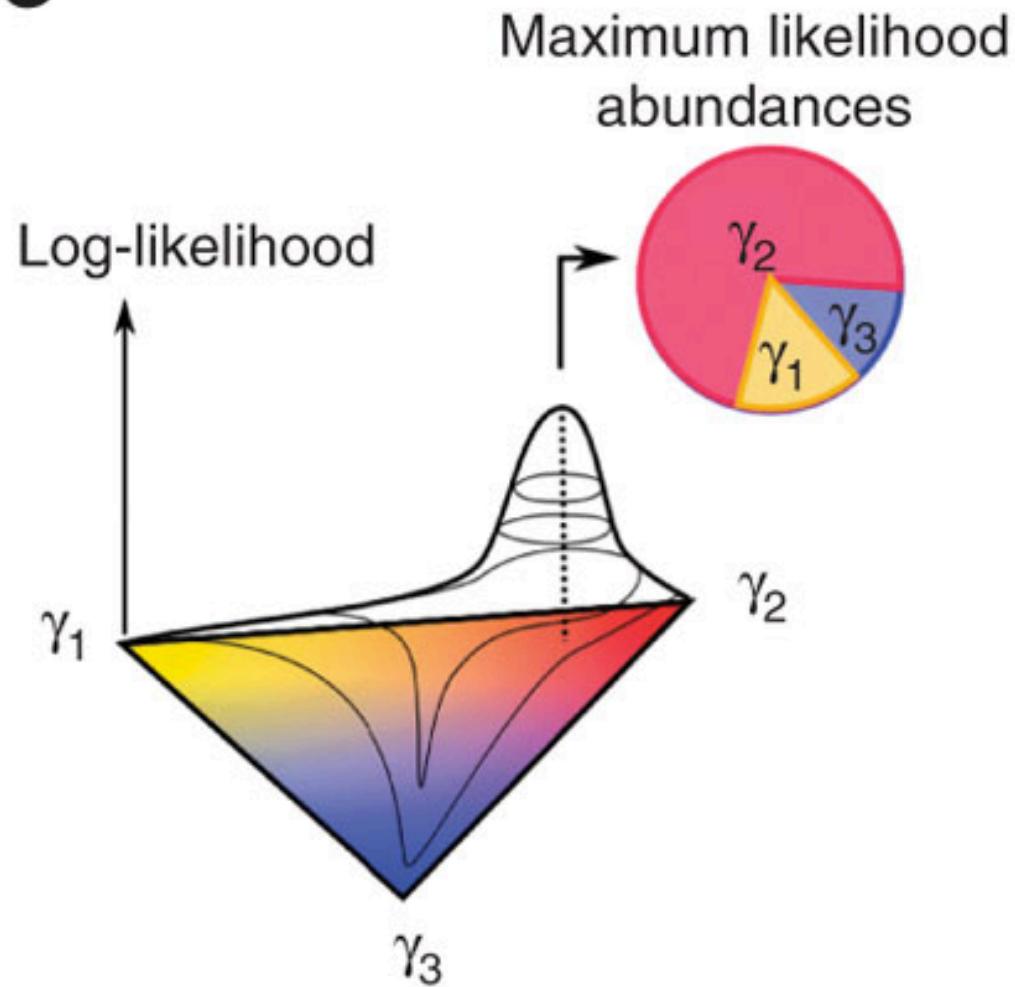
Abundance estimation



Transcript coverage
and compatibility

TRANSCRIPTOME RECONSTRUCTION

e

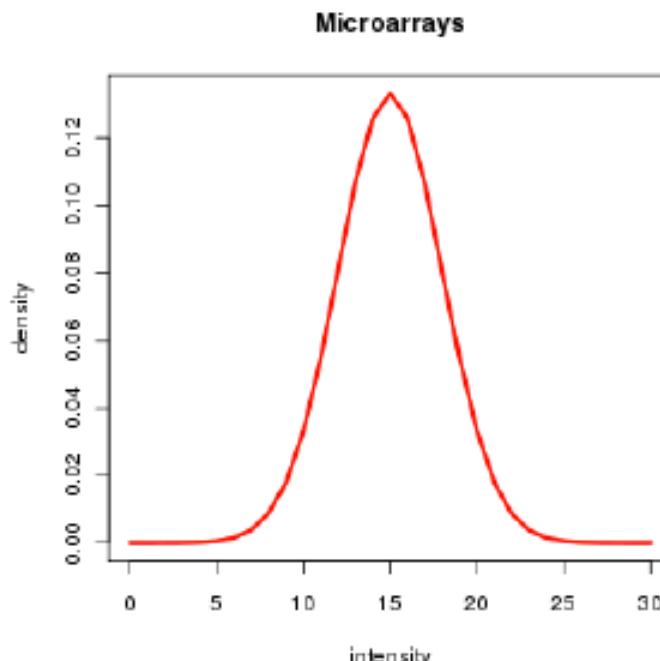
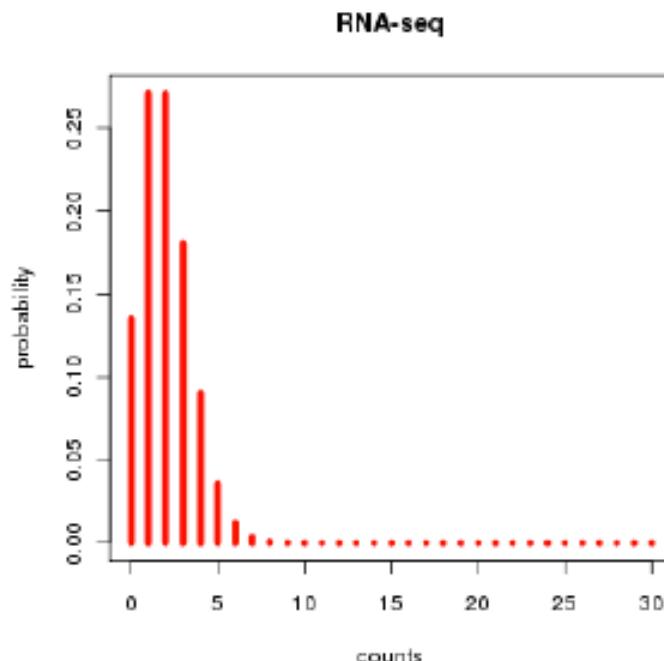


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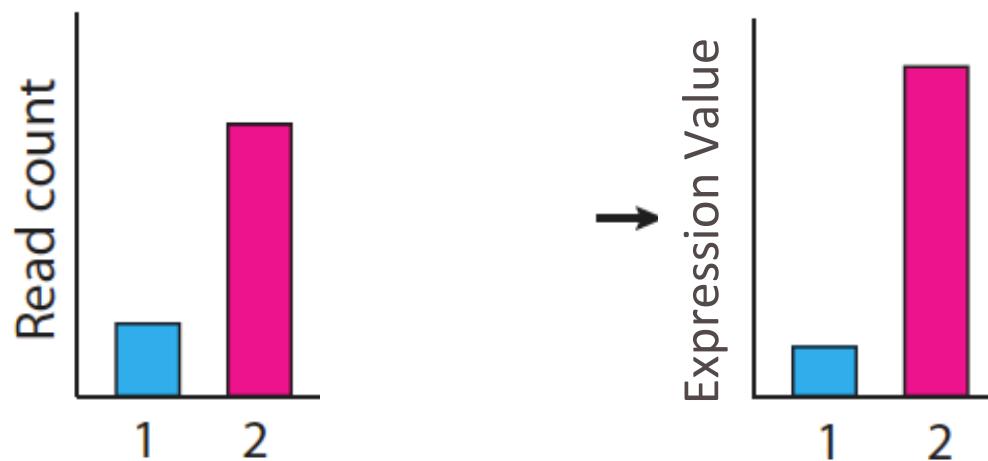
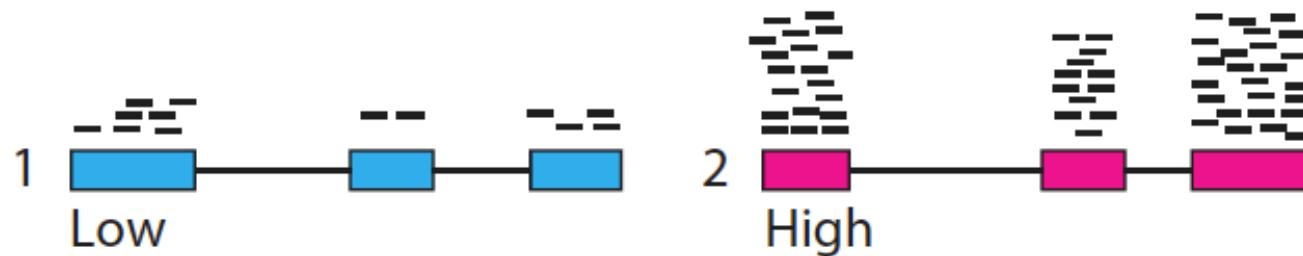
Expression level

RNA-Seq: The number of reads (counts) mapping to the biological feature of interest (**gene**, transcript, exon, etc.) is considered to be linearly related to the abundance of the target feature.

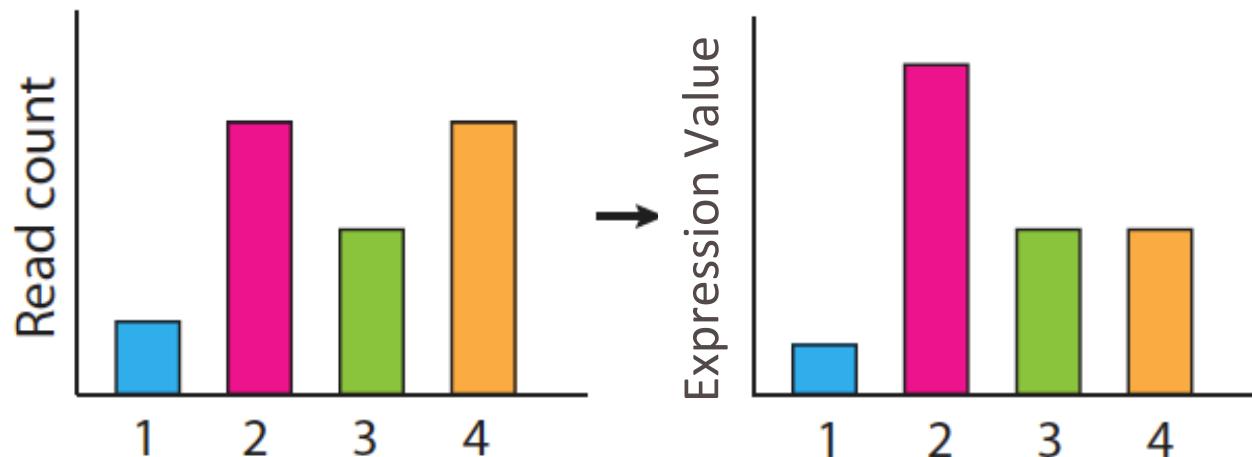
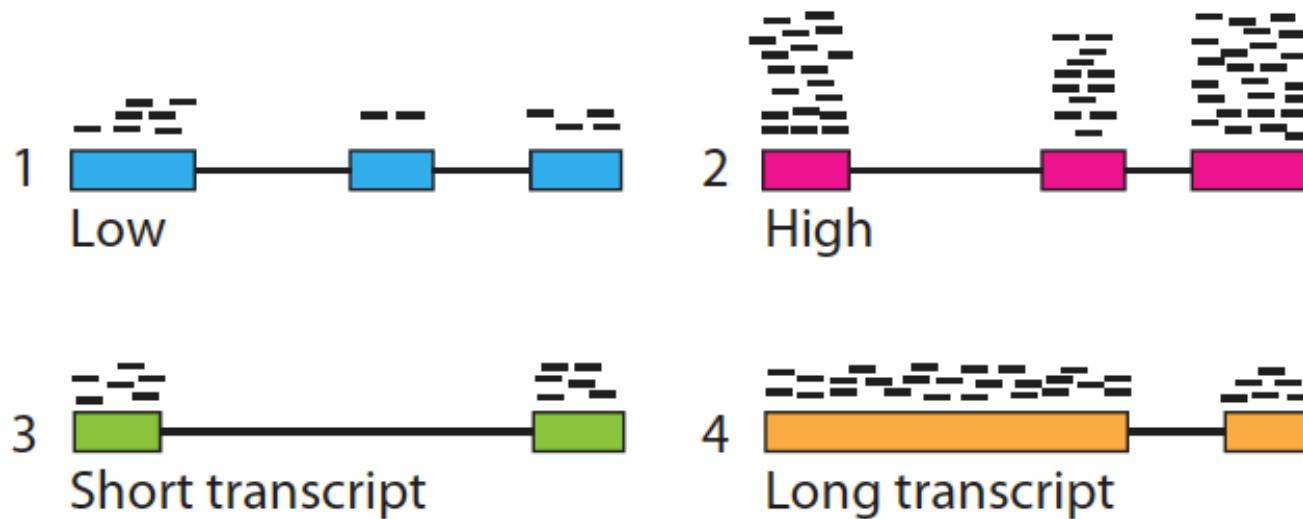
Microarrays: The abundance of each sequence is a function of the fluorescence level recovered after the hybridization process.



TRANSCRIPTOME RECONSTRUCTION



TRANSCRIPTOME RECONSTRUCTION



TRANSCRIPTOME RECONSTRUCTION

Gene	Treatment 1			Treatment 2		
1	14	18	10	47	13	24
2	10	3	15	1	11	5
3	1	0	10	80	21	34
4	0	0	0	0	2	0
5	4	3	3	5	33	29
.
.
.
53256	47	29	11	71	278	339
Total	22910173	30701031	18897029	20546299	28491272	27082148

TRANSCRIPTOME RECONSTRUCTION

FPKM: Fragments Per Kilobase of exon model per Million mapped fragments

$$FPKM = 10^9 \times \frac{C}{NL}$$

C = number of reads mapped on the exons of a gene

N = total number of reads produced by the sequencing

L = Total length of the exon of a gene

TRANSCRIPTOME RECONSTRUCTION

- Even normalizing by library size, the sum of all RPKMs of all genes detected in a sample might not be the same in all analyzed samples
- To overcome this, one can perform the two normalizations (by length and by library size) separately
- First, one can divide the read count C for a gene by its length, obtaining C' . Then, instead of dividing by the total number of reads, one can divide by the sum all C' values
- Then one multiplies this value by 10^9 to obtain the so-called **TPM** (*transcripts per million*)

$$TPM(g) = \frac{c'_g}{\sum_g c'_g} \cdot 10^6 \cdot 10^3$$

TRANSCRIPTOME RECONSTRUCTION

- **RPKM** (Mortazavi et al., 2008): Counts are divided by the transcript length (kb) times the total number of millions of mapped reads.

$$RPKM = \frac{\text{number of reads of the region}}{\frac{\text{total reads}}{1000000} \times \frac{\text{region length}}{1000}}$$

- **FPKM** (Trapnell et al., 2010): Fragments Per Kilobase of exon per Million fragments mapped (analogous to RPKM, generated by Cufflinks).
- **Upper-quartile** (Bullard et al., 2010): Counts are divided by upper-quartile of counts for transcripts with at least one read.
- **TMM** (Robinson and Oshlack, 2010): Trimmed Mean of M values.
- **EDASeq** (Risso et al., 2011): Loess robust local regression (within lane normalization) and global-scaling (median, upper quartile, full-quantile) for both within and between lane normalization.
- **CQN** (Hansen and Irizarry, 2012): Based on Poisson model where length or GC effects are incorporated as smooth functions using natural cubic splines and estimated using robust quantile regression, together with full-quantile between lanes normalization.

TRANSCRIPTOME RECONSTRUCTION

	Sequencing depth	Gene length	Count distribution	GC content
RPKM	✓	✓		
Upper Quartile	✓			
TMM			✓	
EDASeq	✓		✓	✓
CQN	✓	✓	✓	✓

HOW TO CHARACTERIZE A LIST OF GENES?

BIOLOGICAL INTERPRETATION

What you need

- A standardized method to represent functional characteristics of gene products
- An accurate mapping to associate functions with genes
- A statistical method to evaluate whether a particular functional characteristic is found more than expected in the gene list

THE GENE ONTOLOGY

The Gene Ontology (GO) Consortium:



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commentary

Gene Ontology: tool for the unification of biology

The Gene Ontology Consortium*

Genomic sequencing has made it clear that a large fraction of the genes specifying the core biological functions are shared by all eukaryotes. Knowledge of the biological role of such shared proteins in one organism can often be transferred to other organisms. The goal of the Gene Ontology Consortium is to produce a dynamic, controlled vocabulary that can be applied to all eukaryotes even as knowledge of gene and protein roles in cells is accumulating and changing. To this end, three independent ontologies accessible on the World-Wide Web (<http://www.geneontology.org>) are being constructed: biological process, molecular function and cellular component.

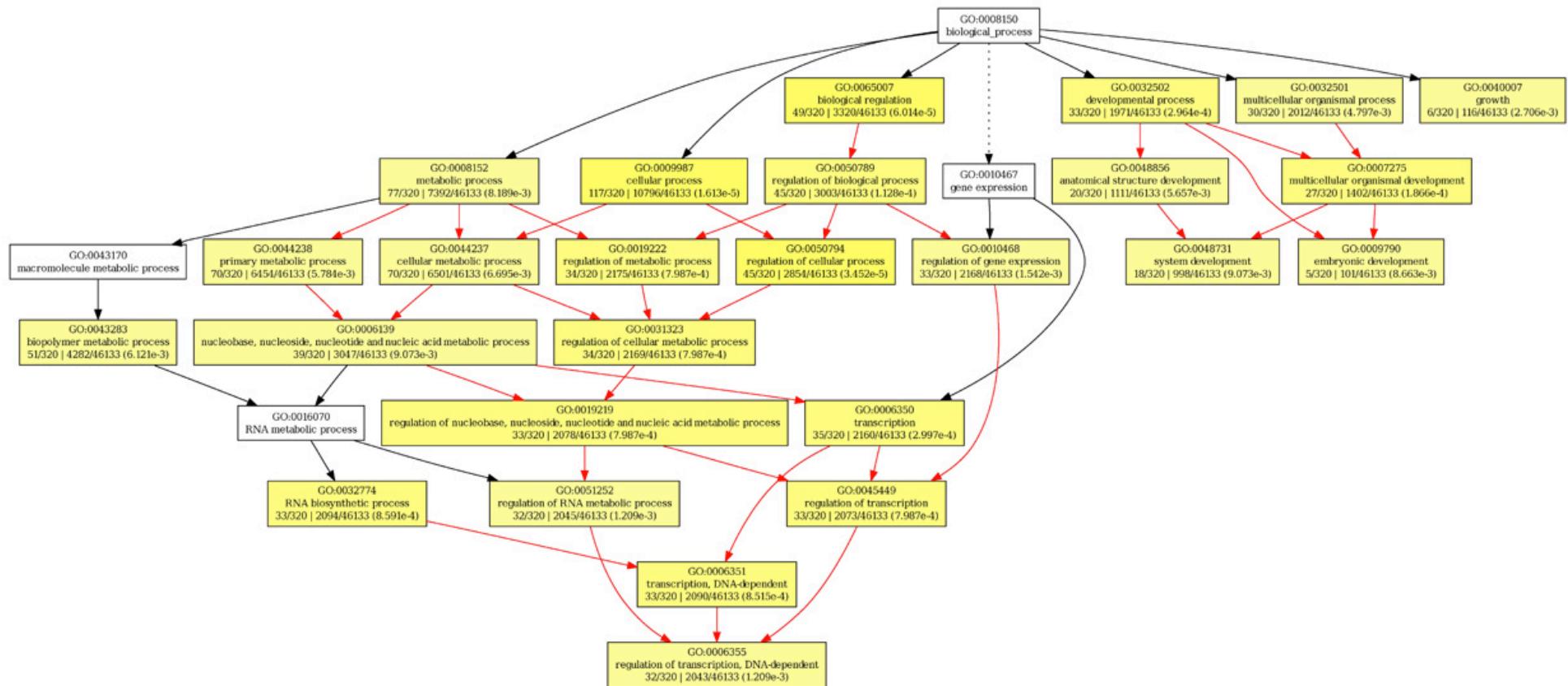
THE GENE ONTOLOGY

- The Gene Ontology Consortium is a collaborative effort from a number of consortia specialized in functional annotation of model organisms (FlyBase, SGD, MGD).
- Ontology: A formal representation of a series of concepts and the relations among them
- Aim: to get a stable way to annotate the functions of gene products in an organism and between different organisms

THE GENE ONTOLOGY

- In GO there are three controlled vocabularies (the main ontologies) composed by **terms** describing gene products by the **biological processes** in which they participate, their **molecular functions** and their **sub-cellular or extra-cellular localization**
- These terms are organized hierarchically, going from more generic terms to more detailed ones

THE GENE ONTOLOGY



THE GENE ONTOLOGY

biological_process

[Term information](#) [Term neighborhood](#) [External references](#) 665024 gene product associations

Term Information

Accession	GO:0008150
Ontology	Biological Process
Synonyms	exact: biological process narrow: biological process unknown alt_id: GO:0000004 alt_id: GO:0007582 exact: physiological process
Definition	Any process specifically pertinent to the functioning of integrated living units: cells, tissues, organs, and organisms. A process is a collection of molecular events with a defined beginning and end. Source: GOC:go_curators, GOC:isa_complete
Comment	Note that, in addition to forming the root of the biological process ontology, this term is recommended for use for the annotation of gene products whose biological process is unknown. Note that when this term is used for annotation, it indicates that no information was available about the biological process of the gene product annotated as of the date the annotation was made; the evidence code ND, no data, is used to indicate this.
Subset	Aspergillus GO slim Candida GO slim Generic GO slim Metagenomics GO slim PIR GO slim Plant GO slim Fission yeast GO slim Yeast GO slim Prokaryotic GO subset
Community	View or edit usage comments for this term on the GONUTS wiki.

Beta
AmiGO 2

Childrens of Biological Process

subject ↗	relation ↗
biological adhesion (GO:0022610)	I is_a
biological regulation (GO:0065007)	I is_a
cell killing (GO:0001906)	I is_a
cellular component organization or biogenesis (GO:0071840)	I is_a
cellular process (GO:0009987)	I is_a
developmental process (GO:0032502)	I is_a
establishment of localization (GO:0051234)	I is_a
growth (GO:0040007)	I is_a
immune system process (GO:0002376)	I is_a
localization (GO:0051179)	I is_a
locomotion (GO:0040011)	I is_a
metabolic process (GO:0008152)	I is_a
multi-organism process (GO:0051704)	I is_a
multicellular organismal process (GO:0032501)	I is_a
negative regulation of biological process (GO:0048519)	R negatively_regulates
positive regulation of biological process (GO:0048518)	R positively_regulates
regulation of biological process (GO:0050789)	R regulates
reproduction (GO:0000003)	I is_a
reproductive process (GO:0022414)	I is_a
response to stimulus (GO:0050896)	I is_a
rhythmic process (GO:0048511)	I is_a
signaling (GO:0023052)	I is_a
single-organism process (GO:0044699)	I is_a

THE GENE ONTOLOGY

- GO ID: GO:0007268
- GO term: synaptic transmission
- Ontology: biological process
- Definition: The process of communication from a neuron to a target (neuron, muscle, or secretory cell) across a synapse

Search GO Terms Genes or proteins Exact Match[Submit Query](#)

synaptic transmission

[Term information](#) [Term lineage](#) [External references](#) [Term associations](#)

Term Information

Accession	GO:0007268
Ontology	biological process
Synonyms	related: neurotransmission related: regulation of synapse
Definition	The process of communication from a neuron to a target (neuron, muscle, or secretory cell) across a synapse. [source: GOC:jl, MeSH:D009435]
Comment	None

[Back to top](#)

Term Lineage

Filter tree view [?](#)

Filter Gene Product Counts

Data source:

- All
- CGD
- dictyBase
- FlyBase

Term View Options

Term ancestors Term parents, siblings and children

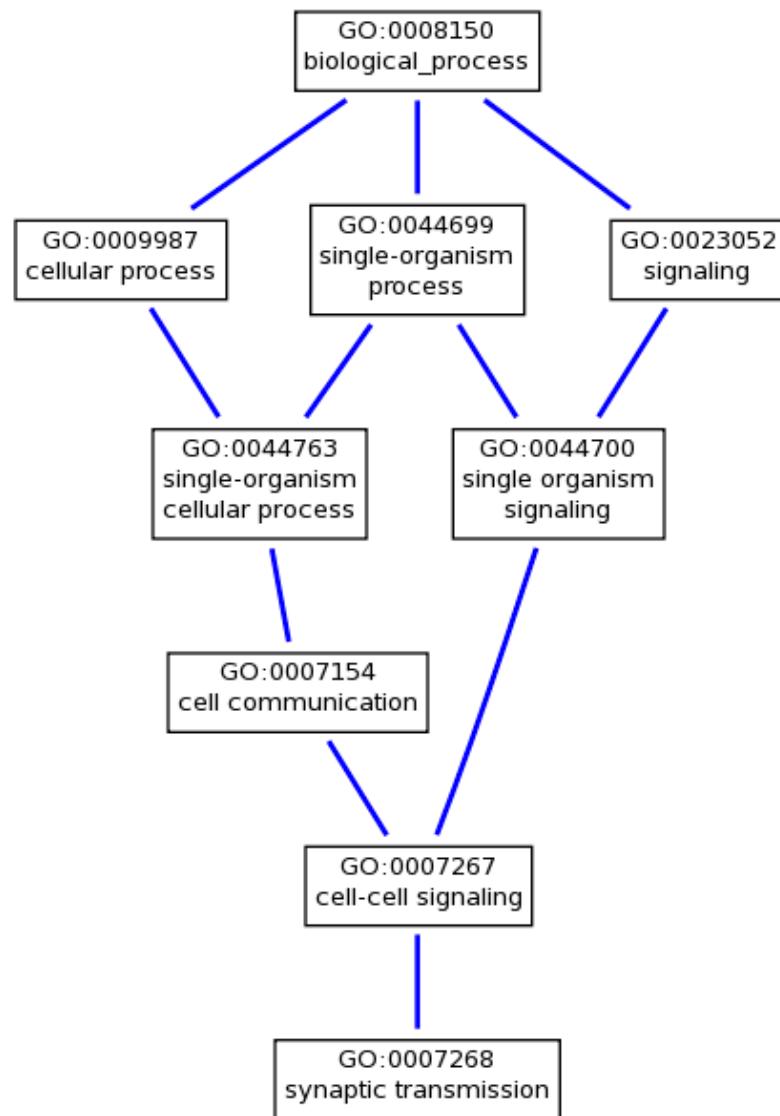
[Set filters](#) [Remove all filters](#)

- + all : all [239263]
 - + [i](#) GO:0008150 : biological_process [159403]
 - + [i](#) GO:0009987 : cellular process [78963]
 - + [i](#) GO:0007154 : cell communication [14536]
 - + [i](#) GO:0007267 : cell-cell signaling [2034]
 - + [i](#) **GO:0007268 : synaptic transmission [1159]**
 - + [i](#) GO:0019226 : transmission of nerve impulse [1308]
 - + [**i**](#) **GO:0007268 : synaptic transmission [1159]**
 - + [i](#) GO:0032501 : multicellular organismal process [19141]
 - + [i](#) GO:0003008 : system process [5599]
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 - + [**i**](#) **GO:0007268 : synaptic transmission [1159]**

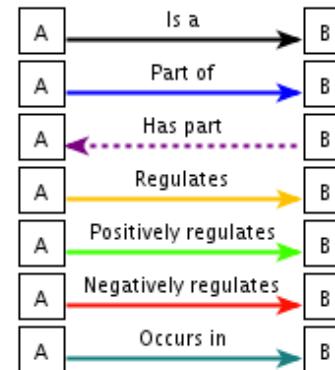
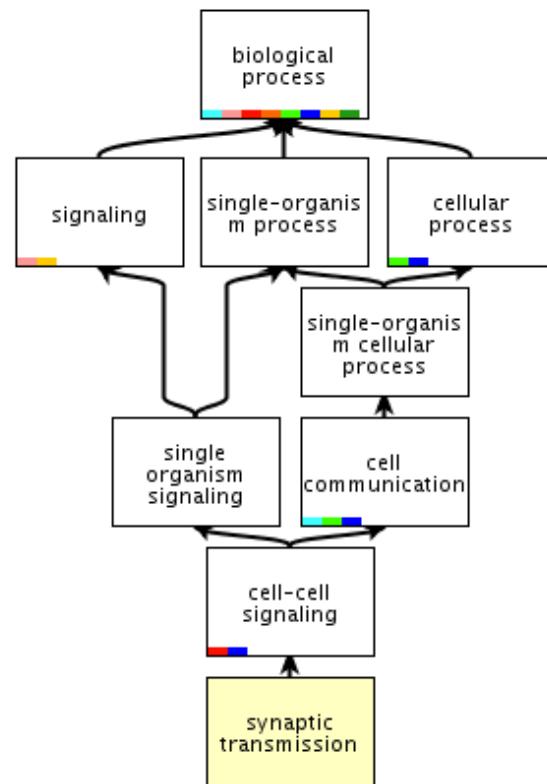
[Graphical View](#)
[View in tree browser](#)

[Back to top](#)

THE GENE ONTOLOGY



THE GENE ONTOLOGY



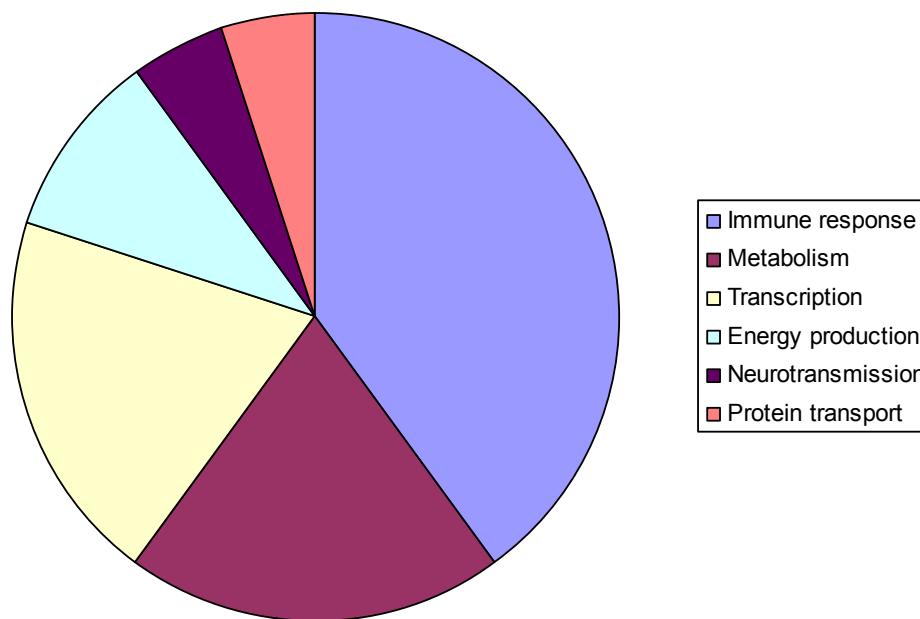
THE GENE ONTOLOGY

Association between a term and a gene product is based on experimental and computational evidences:

- 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
 - RCA: inferred from Reviewed Computational Analysis

FUNCTIONAL ENRICHMENT

Categoria Funzionale	Numero di geni
Immune response	40
Metabolism	20
Transcription	20
Energy production	10
Neurotransmission	5
Protein transport	5
TOTALE	100



FUNCTIONAL ENRICHMENT

- Observing that a certain fraction of genes in a gene list share the same function or participate in the same biological process, is not enough: one must estimate its statistical significance, that is how much this fraction is different from what one can expect. This is called **functional enrichment**
- The gene list must be compared to a background gene set, which might be the whole proteome, to verify whether a given functional annotation is present more (or less) than expected from a random sampling of the background set.
- One test that is often used for this kind of analyses is the **hypergeometric distribution test**

FUNCTIONAL ENRICHMENT

Hypergeometric distribution test

- Consider the following example:
 - In a drawer there are N socks.
 - There are exactly B blue socks, and the remaining $N-B$ socks are pink.
 - Let's take n socks (without putting them back in) from the drawer; of these, b are blue
- Is the number of blue socks taken from the drawer significantly higher or smaller than what one can expect from the socks distribution in the drawer?
- If this is true, then the extraction method preferred the blue socks over the pink ones, or the other way around



FUNCTIONAL ENRICHMENT

- The probability of finding **exactly** b blue socks in the n sampled socks is given by the hypergeometric function:

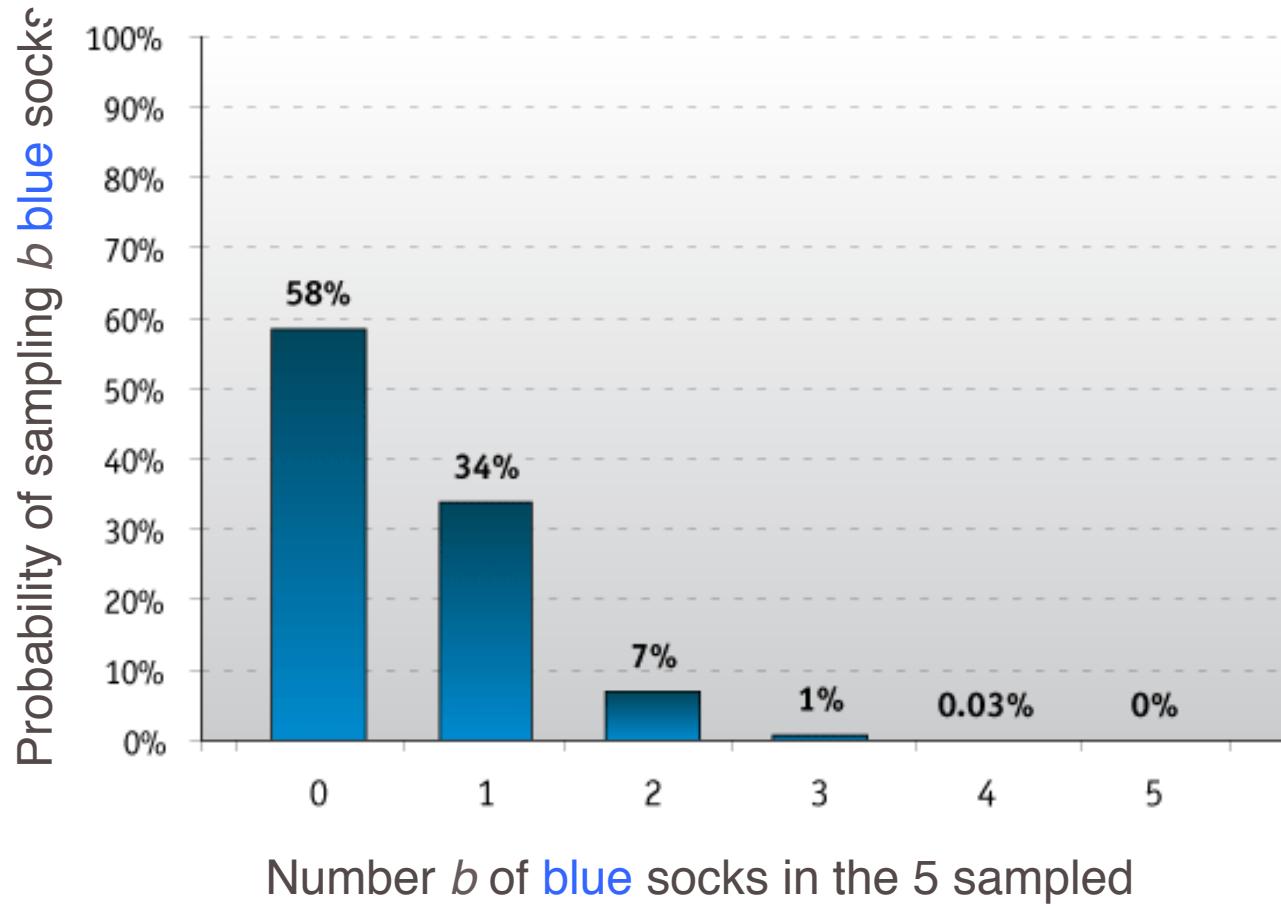
$$HG(N, B, n, b) = \frac{\binom{n}{b} \binom{N-n}{B-b}}{\binom{N}{B}}$$

- The probability of finding **at least** b blue socks is:

$$HGT(N, B, n, b) = \sum_{i=b}^{\min(n, B)} HG(N, B, n, i)$$

FUNCTIONAL ENRICHMENT

In the drawer there are 100 socks, 90 pink and 10 blue, and I sampled 5 randomly



FUNCTIONAL ENRICHMENT

- In our case, we have a list of N genes in the genome, of which B genes can be associated to a given function described by a given GO term, and $N-B$ genes that do not have that function
- Imagine that, among the n differentially expressed genes detected in an RNA-Seq experiment, b genes are associated to that GO term, while $n-b$ genes are not
- If this number b is significantly higher or smaller than expected, then this means that the gene sampling method (that is the selection of differentially expressed genes among all tested genes) enriched the gene list in genes having that function

Enrichment tool name	Year of release	Key statistical method	Category
FunSpec	2002	Hypergeometric	Class I
Onto-express	2002	Fisher's exact; hypergeometric; binomial; chi-square	Class I
EASE	2003	Fisher's exact (modified as EASE score)	Class I
FatiGO/FatiWise/FatiGO +	2003	Fisher's exact	Class I
FuncAssociate	2003	Fisher's exact	Class I
GARBAN	2003	Hypergeometric	Class I
GeneMerge	2003	Hypergeometric	Class I
GoMiner	2003	Fisher's exact	Class I
MAPPFinder	2003	Z-score; hypergeometric	Class I
CLENCH	2004	Hypergeometric; chi-square; binomial	Class I
GO::TermFinder	2004	hypergeometric	Class I
GOAL	2004	Permutation	Class I
GOArray	2004	Hypergeometric; Z-score; permutation	Class I
GOStat	2004	Fisher's exact; chi-square	Class I
GoSurfer	2004	Chi-square	Class I
OntologyTraverser	2004	Hypergeometric; Fisher's exact	Class I
THEA	2004	Hypergeometric	Class I
BINGO	2005	Hypergeometric; binomial	Class I
FACT	2005	Adopt GeneMerge and GO::TermFinder statistical modules	Class I
gfinder	2005	Fisher's exact	Class I
Gobar	2005	Hypergeometric	Class I
GOCluster	2005	Hypergeometric	Class I
GOSSIP	2005	Fisher's exact	Class I
L2L	2005	Binomial; hypergeometric	Class I
WebGestalt	2005	Hypergeometric	Class I
BayGO	2006	Bayesian; Goodman and Kruskal's gamma factor	Class I
eGOn/GeneTools	2006	Fisher's exact	Class I
Gene Class Expression	2006	Z-statistics	Class I
GOALIE	2006	Hidden Kripke model	Class I
GOFFA	2006	Fisher's inverse chi-square	Class I
GOLEM	2006	Hypergeometric	Class I
JProGO	2006	Fisher's exact; Kolmogorov-Smirnov test; student's t-test; Wilcoxon's test; hypergeometric	Class I
PageMan	2006	Fisher's exact; chi-square; Wilcoxon	Class I
STEM	2006	Hypergeometric	Class I
WEGO	2006	Chi-square	Class I
EasyGO	2007	Hypergeometric; chi-square; binomial	Class I
g-Profiler	2007	Hypergeometric	Class I
ProBCD	2007	Yule's Q; Goodman-Kruskal's gamma; Cramer's T	Class I
GOEAST	2008	Hypergeometric	Class I
GOHyperGAll	2008	Hypergeometric	Class I
CatMap	2004	Permutations	Class II
Godist	2004	Kolmogorov-Smirnov test	Class II
GO-Mapper	2004	Gaussian distribution; EQ-score	Class II
iGA	2004	Permutations; hypergeometric; t-test; Z-score	Class II
GSEA	2005	Kolmogorov-Smirnov-like statistic	Class II
MEGO	2005	Z-score	Class II
PAGE	2005	Z-score	Class II
T-profiler	2005	t-Test	Class II
FuncCluster	2006	Fisher's exact	Class II
FunScan	2007	Fisher's Exact	Class II
FINA	2007	Fisher's exact	Class II
GAzer	2007	Z-statistics; permutation	Class II
GeneTrail	2007	Hypergeometric; Kolmogorov-Smirnov	Class II
MetaGP	2007	Z-score	Class II
Ontologizer	2004	Fisher's exact	Class III
POSOC	2004	POSET (a discrete math: finite partially ordered set)	Class III
topGO	2006	Fisher's exact	Class III
GO-2D	2007	Hypergeometric; binomial	Class III
GENECODIS	2007	Hypergeometric; chi-square	Class III
GOSim	2007	Rosnik's similarity	Class III
PalS	2008	Percent	Class III
ProfCom	2008	Greedy heuristics	Class III
GOTM	2004	Hypergeometric	Class I,II
ermineJ	2005	Permutations; Wilcoxon rank-sum test	Class I,II
DAVID	2003	Fisher's Exact (modified as EASE score)	Class I,III
GOToolBox	2004	Hypergeometric; Fisher's exact; Binomial	Class I,III
ADGO	2006	Z-statistic	Class II,III
FunNet	2008	Unclear	Unclear

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SURVEY AND SUMMARY

Bioinformatics enrichment tools: paths toward the comprehensive functional analysis of large gene lists

Da Wei Huang, Brad T. Sherman and Richard A. Lempicki*

Laboratory of Immunopathogenesis and Bioinformatics, Clinical Services Program, SAIC-Frederick, Inc., National Cancer Institute at Frederick, Frederick, MD 21702, USA

FUNCTIONAL ENRICHMENT

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

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Shortcut to DAVID Tools

Functional Annotation
Gene-annotation enrichment analysis, functional annotation clustering , BioCarta & KEGG pathway mapping, gene-disease association, homologue match, ID translation, literature match and [more](#)

Gene Functional Classification
Provide a rapid means to reduce large lists of genes into functionally related groups of genes to help unravel the biological content captured by high throughput technologies. [More](#)

Gene ID Conversion
Convert list of gene ID/accessions to others of your choice with the most comprehensive gene ID mapping repository. The ambiguous accessions in the list can also be determined semi-automatically. [More](#)

Gene Name Batch Viewer
Display gene names for a given gene list; Search functionally related genes within your list or not in your list; Deep links to enriched detailed information. [More](#)

Recommending: A paper published in *Nature Protocols* describes step-by-step procedure to use DAVID!

Welcome to DAVID 6.7

2003 - 2014

Search

What's Important in DAVID?

- [Current \(v 6.7\) release note](#)
- [New requirement to cite DAVID](#)
- [IDs of Affy Exon and Gene arrays supported](#)
- [Novel Classification Algorithms](#)
- [Pre-built Affymetrix and Illumina backgrounds](#)
- [User's customized gene background](#)
- [Enhanced calculating speed](#)

Statistics of DAVID

DAVID Bioinformatic Resources Citations

Year	Citations
2004	~100
2005	~200
2006	~300
2007	~400
2008	~500
2009	~600
2010	~800
2011	~1000
2012	~1200
2013	3182

> 10,000 Citations

FUNCTIONAL ENRICHMENT

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

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Shortcut to DAVID Tools

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2007	~400
2008	~500
2009	~600
2010	~800
2011	~1000
2012	~1200
2013	3182

> 10,000 Citations

FUNCTIONAL ENRICHMENT

DAVID BIOINFORMATICS DATABASE

Analysis Wizard
DAVID Bioinformatics Resources 6.7, NIAID/NIH

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Upload List Background

Upload Gene List

[Demolist 1](#) [Demolist 2](#)
[Upload Help](#)

Step 1: Enter Gene List

A: Paste a list

1007_s_at
1053_at
117_at
121_at
1255_g_at
1294_at
1316_at
1320_at
1405_i_at
1431_at
1438_at
1487_at
1494_f_at
1598_g_at

Clear

Or

B: Choose From a File

No file chosen
 Multi-List File ?

Step 2: Select Identifier

Step 3: List Type

Gene List
Background

Step 4: Submit List

Analysis Wizard

← Step 1. Submit your gene list through left panel.

[Tell us how you like the tool](#)
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FUNCTIONAL ENRICHMENT

DAVID BIOINFORMATICS DATABASE

Analysis Wizard
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Upload List Background

Upload Gene List

[Demolist 1](#) [Demolist 2](#)
[Upload Help](#)

Step 1: Enter Gene List

A. Paste a list

B. Copy and Paste
C. Choose From a File

D. Choose From a File
 No file chosen
 Multi-List File ?

Step 2: Select Identifier

AFFYMETRIX_3PRIME_IVT_ID

Step 3: List Type

Gene List
Background

Step 4: Submit List

Analysis Wizard

[Tell us how you like the tool](#)
[Contact us for questions](#)

← Step 1. Submit your gene list through left panel.

An example:

Copy/paste IDs to "box A" -> Select Identifier as "Affy_ID" -> List Type as "Gene List" -> Click "Submit" button

1007_s_at
1053_at
117_at
121_at
1255_g_at
1294_at
1316_at
1320_at
1405_i_at
1431_at
1438_at
1487_at
1494_f_at
1598_g_at



Upload List
Background

Upload Gene List

[Demolist 1](#) [Demolist 2](#)

[Upload Help](#)

Step 1: Enter Gene List

A: Paste a list

ENSG00000169474
ENSG00000137203
ENSG00000159674
ENSG00000148600

← Step 1. Submit your gene list through left panel.

An example:

Copy/paste IDs to "box A" -> Select Identifier as "Affy_ID" -> List Type as "Gene List" -> Click "Submit" button

1007_s_at
1053_at
117_at
121_at
1255_g_at
1294_at
1316_at
1320_at
1405_i_at
1431_at
1438_at
1487_at
1494_f_at
1598_g_at

Or

B: Choose From a File

No file chosen

Multi-List File ?

Step 2: Select Identifier

AFFYMETRIX_3PRIME_IVT_ID
 AFFYMETRIX_EXON_GENE_ID
 AFFYMETRIX_SNP_ID
 AGILENT_CHIP_ID
 AGILENT_ID
 ACUCHEM_ID
 ENSEMBL_GENE_ID
 ENSEMBL_TRANSCRIPT_ID
 ENTREZ_GENE_ID
 FLYBASE_GENE_ID
 FLYBASE_TRANSCRIPT_ID
 GENBANK_ACCESSION
 GENOMIC_GI_ACCESSION
 GENPEPT_ACCESSION
 ILLUMINA_ID

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Analysis Wizard

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[Background](#)

Upload Gene List

[Demolist 1](#) [Demolist 2](#)

[Upload Help](#)

Step 1: Enter Gene List

A: Paste a list

```
ENSG00000169474  
ENSG00000137203  
ENSG00000159674  
ENSG00000148600
```

Or

B: Choose From a File

No file chosen

Multi-List File [?](#)

Step 2: Select Identifier

Step 3: List Type

Gene List

Background

Step 4: Submit List

Analysis Wizard

[Tell us how you like the tool](#)
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1007_s_at
1053_at
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1316_at
1320_at
1405_i_at
1431_at
1438_at
1487_at
1494_f_at
1598_g_at



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Upload [List](#)
Background

Gene List Manager

Select to limit annotations by one or more species [Help](#)

- Use All Species -

Homo sapiens(167)

Unknown(22)

[Select Species](#)

List Manager [Help](#)

List_1

Select List to:

[Use](#) [Rename](#)

[Remove](#) [Combine](#)

[Show Gene List](#)

[View Unmapped Ids](#)

Analysis Wizard

[Tell us how you like the tool](#)
[Contact us for questions](#)

Step 1. Successfully submitted gene list

Current Gene List: List_1

Current Background: Homo sapiens

Step 2. Analyze above gene list with one of DAVID tools



Functional Annotation Tool

- [Functional Annotation Clustering](#)
- [Functional Annotation Chart](#)
- [Functional Annotation Table](#)

⊕ [Gene Functional Classification Tool](#)

⊕ [Gene ID Conversion Tool](#)

⊕ [Gene Name Batch Viewer](#)

[Which DAVID tools to use?](#)

Upload **List**
Background

Gene List Manager

Select to limit annotations by one or more species [Help](#)

- Use All Species -
Homo sapiens(167)
Unknown(22)

[Select Species](#)

List Manager [Help](#)

List_1

Select List to:

[Use](#) [Rename](#)
[Remove](#) [Combine](#)

[Show Gene List](#)

[View Unmapped Ids](#)

Annotation Summary Results

[Help and Tool Manual](#)

Current Gene List: List_1

Current Background: Homo sapiens

- Disease** (1 selected)
- Functional Categories** (3 selected)
- Gene_Ontology** (3 selected)
- General Annotations** (0 selected)
- Literature** (0 selected)
- Main_Accessions** (0 selected)
- Pathways** (3 selected)
- Protein_Domains** (3 selected)
- Protein_Interactions** (0 selected)
- Tissue_Expression** (0 selected)

164 DAVID IDs

Check Defaults

[Clear All](#)

Red annotation categories denote DAVID defined defaults

Combined View for Selected Annotation

- [Functional Annotation Clustering](#)
- [Functional Annotation Chart](#)
- [Functional Annotation Table](#)

[Upload](#) [List](#)
[Background](#)**Gene List Manager**

Select to limit annotations by one or more species [Help](#)

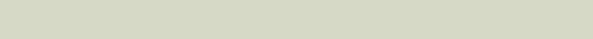
- Use All Species -
Homo sapiens(167)
Unknown(22)

[Select Species](#)**List Manager** [Help](#)

List_1

Select List to:

[Use](#) [Rename](#)
[Remove](#) [Combine](#)
[Show Gene List](#)[View Unmapped Ids](#)**Annotation Summary Results**[Help and Tool Manual](#)**Current Gene List:** List_1**Current Background:** Homo sapiens**164 DAVID IDs****Check Defaults** [Clear All](#) **Disease** (1 selected) **Functional_Categories** (3 selected) **Gene_Ontology** (3 selected)

<input type="checkbox"/> GOTERM_BP_1	79.3%	130	Chart	
<input type="checkbox"/> GOTERM_BP_2	78.7%	129	Chart	
<input type="checkbox"/> GOTERM_BP_3	75.0%	123	Chart	
<input type="checkbox"/> GOTERM_BP_4	74.4%	122	Chart	
<input type="checkbox"/> GOTERM_BP_5	70.7%	116	Chart	
<input type="checkbox"/> GOTERM_BP_ALL	79.3%	130	Chart	
<input checked="" type="checkbox"/> GOTERM_BP_FAT	76.8%	126	Chart	
<input type="checkbox"/> GOTERM_CC_1	81.1%	133	Chart	
<input type="checkbox"/> GOTERM_CC_2	74.4%	122	Chart	
<input type="checkbox"/> GOTERM_CC_3	73.8%	121	Chart	
<input type="checkbox"/> GOTERM_CC_4	66.5%	109	Chart	
<input type="checkbox"/> GOTERM_CC_5	63.4%	104	Chart	
<input type="checkbox"/> GOTERM_CC_ALL	81.1%	133	Chart	
<input checked="" type="checkbox"/> GOTERM_CC_FAT	67.7%	111	Chart	
<input type="checkbox"/> GOTERM_MF_1	82.3%	135	Chart	
<input type="checkbox"/> GOTERM_MF_2	80.5%	132	Chart	
<input type="checkbox"/> GOTERM_MF_3	68.3%	112	Chart	
<input type="checkbox"/> GOTERM_MF_4	61.0%	100	Chart	
<input type="checkbox"/> GOTERM_MF_5	50.6%	83	Chart	
<input type="checkbox"/> GOTERM_MF_ALL	82.3%	135	Chart	
<input checked="" type="checkbox"/> GOTERM_MF_FAT	71.3%	117	Chart	
<input type="checkbox"/> PANTHER_BP_ALL	70.7%	116	Chart	
<input type="checkbox"/> PANTHER_MF_ALL	74.4%	122	Chart	

Functional Annotation Chart

[Help and Manual](#)
Current Gene List: List_1

Current Background: Homo sapiens

164 DAVID IDs
 Options

[Rerun Using Options](#) [Create Sublist](#)
81 chart records
 [Download File](#)

Sublist	Category	Term	RT	Genes	Count	%	P-Value	Benjamini
	GOTERM_BP_FAT	tube morphogenesis			8	4.9	1.7E-4	1.8E-1
	GOTERM_BP_FAT	immune response			18	11.0	2.0E-4	1.1E-1
	GOTERM_BP_FAT	blood vessel development			10	6.1	4.5E-4	1.6E-1
	GOTERM_BP_FAT	vasculature development			10	6.1	5.3E-4	1.4E-1
	GOTERM_BP_FAT	chemotaxis			8	4.9	7.0E-4	1.5E-1
	GOTERM_BP_FAT	taxis			8	4.9	7.0E-4	1.5E-1
	GOTERM_BP_FAT	blood vessel morphogenesis			9	5.5	7.6E-4	1.4E-1
	GOTERM_BP_FAT	tube development			9	5.5	9.9E-4	1.5E-1
	GOTERM_BP_FAT	angiogenesis			7	4.3	2.5E-3	3.1E-1
	GOTERM_BP_FAT	extracellular matrix organization			6	3.7	2.8E-3	3.0E-1
	GOTERM_BP_FAT	regeneration			5	3.0	3.8E-3	3.6E-1
	GOTERM_BP_FAT	extracellular structure organization			7	4.3	4.0E-3	3.5E-1
	GOTERM_BP_FAT	ossification			6	3.7	4.2E-3	3.4E-1
	GOTERM_BP_FAT	bone development			6	3.7	5.6E-3	4.0E-1
	GOTERM_BP_FAT	response to hypoxia			6	3.7	8.0E-3	4.9E-1
	GOTERM_BP_FAT	wound healing			7	4.3	8.6E-3	4.9E-1
	GOTERM_BP_FAT	regulation of cell adhesion			6	3.7	8.8E-3	4.8E-1
	GOTERM_BP_FAT	skeletal system development			9	5.5	9.5E-3	4.8E-1
	GOTERM_BP_FAT	response to oxygen levels			6	3.7	9.9E-3	4.8E-1
	GOTERM_BP_FAT	response to wounding			12	7.3	1.0E-2	4.6E-1
	GOTERM_BP_FAT	locomotory behavior			8	4.9	1.4E-2	5.5E-1
	GOTERM_BP_FAT	enzyme linked receptor protein signaling pathway			9	5.5	1.4E-2	5.5E-1
	GOTERM_BP_FAT	response to oxidative stress			6	3.7	1.8E-2	6.2E-1

Functional Annotation Chart

[Help and Manual](#)

Current Gene List: List_1

Current Background: Homo sapiens

164 DAVID IDs

Options

[Rerun Using Options](#) [Create Sublist](#)

17 chart records

[Download File](#)

Sublist	Category	Term	RT	Genes	Count	%	P-Value	Benjamini
	GOTERM_MF_FAT	cytokine activity	RT		10	6.1	6.2E-5	1.8E-2
	GOTERM_MF_FAT	extracellular matrix structural constituent	RT		7	4.3	1.2E-4	1.7E-2
	GOTERM_MF_FAT	chemokine activity	RT		5	3.0	7.4E-4	6.9E-2
	GOTERM_MF_FAT	chemokine receptor binding	RT		5	3.0	9.4E-4	6.6E-2
	GOTERM_MF_FAT	antigen binding	RT		5	3.0	1.6E-3	8.7E-2
	GOTERM_MF_FAT	structural molecule activity	RT		13	7.9	1.1E-2	4.2E-1
	GOTERM_MF_FAT	growth factor activity	RT		6	3.7	1.5E-2	4.6E-1
	GOTERM_MF_FAT	sugar binding	RT		6	3.7	3.1E-2	6.8E-1
	GOTERM_MF_FAT	carbohydrate binding	RT		8	4.9	3.9E-2	7.3E-1
	GOTERM_MF_FAT	protein dimerization activity	RT		10	6.1	5.4E-2	8.0E-1
	GOTERM_MF_FAT	identical protein binding	RT		11	6.7	6.0E-2	8.1E-1
	GOTERM_MF_FAT	calcium ion binding	RT		14	8.5	6.6E-2	8.1E-1
	GOTERM_MF_FAT	glycine hydroxymethyltransferase activity	RT		2	1.2	6.9E-2	8.0E-1
	GOTERM_MF_FAT	dipeptidase activity	RT		2	1.2	6.9E-2	8.0E-1
	GOTERM_MF_FAT	dipeptidyl-peptidase activity	RT		2	1.2	9.4E-2	8.7E-1
	GOTERM_MF_FAT	heme binding	RT		4	2.4	9.4E-2	8.5E-1
	GOTERM_MF_FAT	amino acid transmembrane transporter activity	RT		3	1.8	9.8E-2	8.5E-1

Functional Annotation Chart

[Help and Manual](#)

Current Gene List: List_1

Current Background: Homo sapiens

164 DAVID IDs

Options

[Rerun Using Options](#) [Create Sublist](#)

10 chart records

[Download File](#)

Sublist	Category	Term	RT	Genes	Count	%	P-Value	Benjamini
	GOTERM_CC_FAT	extracellular region	RT		42	25.6	2.9E-8	5.6E-6
	GOTERM_CC_FAT	extracellular region part	RT		27	16.5	1.1E-7	1.1E-5
	GOTERM_CC_FAT	extracellular matrix	RT		14	8.5	8.1E-6	5.2E-4
	GOTERM_CC_FAT	proteinaceous extracellular matrix	RT		13	7.9	1.9E-5	9.3E-4
	GOTERM_CC_FAT	extracellular space	RT		17	10.4	2.4E-4	9.3E-3
	GOTERM_CC_FAT	collagen	RT		4	2.4	3.3E-3	1.0E-1
	GOTERM_CC_FAT	extracellular matrix part	RT		6	3.7	3.4E-3	8.9E-2
	GOTERM_CC_FAT	chromosome passenger complex	RT		2	1.2	2.6E-2	4.7E-1
	GOTERM_CC_FAT	smooth muscle contractile fiber	RT		2	1.2	3.4E-2	5.2E-1
	GOTERM_CC_FAT	fibrillar collagen	RT		2	1.2	9.9E-2	8.7E-1

Upload List
Background

Gene List Manager

Select to limit annotations by one or more species [Help](#)

- Use All Species -
Homo sapiens(167)
Unknown(22)

[Select Species](#)

List Manager [Help](#)

List_1

Select List to:

[Use](#) [Rename](#)
[Remove](#) [Combine](#)

[Show Gene List](#)

[View Unmapped Ids](#)

Annotation Summary Results

[Help and Tool Manual](#)

Current Gene List: List_1

Current Background: Homo sapiens

164 DAVID IDs

Check Defaults

[Clear All](#)

- Disease** (1 selected)
- Functional_Categories** (3 selected)
- Gene_Ontology** (3 selected)
- General Annotations** (0 selected)
- Literature** (0 selected)
- Main_Accessions** (0 selected)
- Pathways** (3 selected) *
- Protein_Domains** (3 selected)
- Protein_Interactions** (0 selected)
- Tissue_Expression** (0 selected)

Red annotation categories denote DAVID defined defaults

Combined View for Selected Annotation

- [Functional Annotation Clustering](#)
- [Functional Annotation Chart](#)
- [Functional Annotation Table](#)



Functional Annotation Tool

DAVID Bioinformatics Resources 6.7, NIAID/NIH

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Upload [List](#)
Background

Gene List Manager

Select to limit annotations by one or more species [Help](#)

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Annotation Summary Results

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Current Gene List: List_1

Current Background: Homo sapiens

164 DAVID IDs

Check Defaults

[Clear All](#)

- Disease (1 selected)
- Functional_Categories (3 selected)
- Gene_Ontology (3 selected)
- General Annotations (0 selected)
- Literature (0 selected)
- Main_Accessions (0 selected)
- Pathways (3 selected)

<input checked="" type="checkbox"/> BBID	6.7%	11	Chart	
<input checked="" type="checkbox"/> BIOCARTE	12.2%	20	Chart	
<input type="checkbox"/> EC_NUMBER	18.3%	30	Chart	
<input checked="" type="checkbox"/> KEGG_PATHWAY	29.9%	49	Chart	
<input type="checkbox"/> PANTHER_PATHWAY	23.2%	38	Chart	
<input type="checkbox"/> REACTOME_PATHWAY	23.8%	39	Chart	

- Protein_Domains (3 selected)
- Protein_Interactions (0 selected)
- Tissue_Expression (0 selected)

Red annotation categories denote DAVID defined defaults

Combined View for Selected Annotation

[Functional Annotation Clustering](#)
[Functional Annotation Chart](#)
[Functional Annotation Table](#)



KEGG

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[Plea from KEGG](#)**KEGG Database**[KEGG overview](#)
[Searching KEGG](#)
[KEGG mapping](#)
[Color codes](#)**KEGG Objects**[Pathway maps](#)
[Brite hierarchies](#)**KEGG Software**[KegTools](#)
[KEGG API](#)
[KGML](#)**KEGG FTP**[Subscription](#)**GenomeNet****DBGET/LinkDB****Feedback****Kanehisa Labs**

KEGG: Kyoto Encyclopedia of Genes and Genomes

KEGG is a database resource for understanding high-level functions and utilities of the biological system, such as the cell, the organism and the ecosystem, from molecular-level information, especially large-scale molecular datasets generated by genome sequencing and other high-throughput experimental technologies (See [Release notes](#) for new and updated features).

Please see: [Renewed plea to support KEGG](#) *New!*

Main entry point to the KEGG web service

[KEGG2](#) [KEGG Table of Contents](#) [Update notes](#)

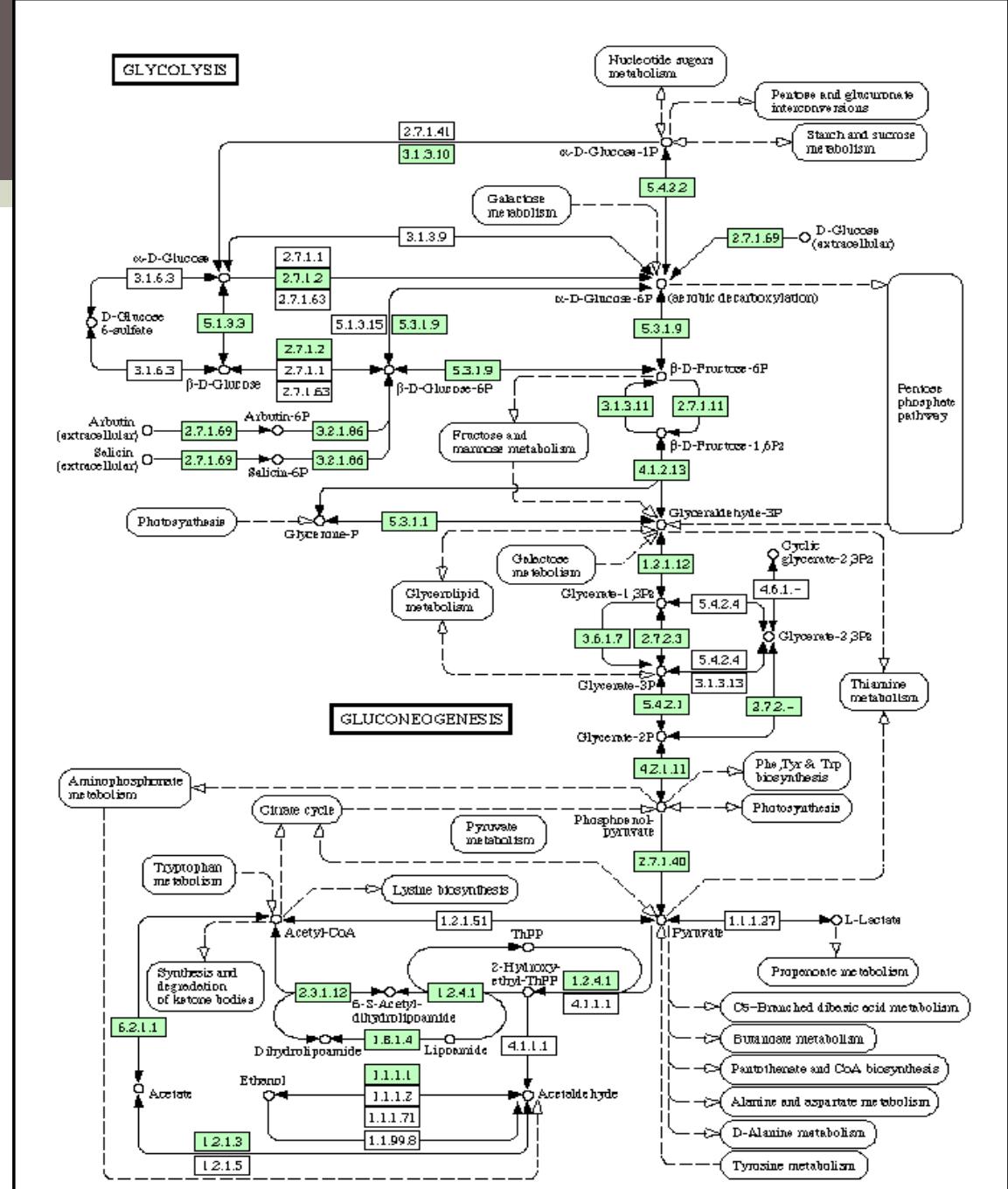
Data-oriented entry points

KEGG PATHWAY	KEGG pathway maps	[Pathway list]
KEGG BRITE	BRITE functional hierarchies	[Brite list]
KEGG MODULE	KEGG modules	[Module list]
KEGG ORTHOLOGY	Ortholog groups	[KO system]
KEGG GENOME	Genomes	[KEGG organisms]
KEGG GENES	Genes and proteins	Release history
KEGG COMPOUND	Small molecules	[Compound classification]
KEGG REACTION	Biochemical reactions	[Reaction modules]
KEGG DISEASE	Human diseases	[Cancer Infectious disease]
KEGG DRUG	Drugs	[ATC drug classification]
KEGG MEDICUS	Health information resource	[Drug labels search]

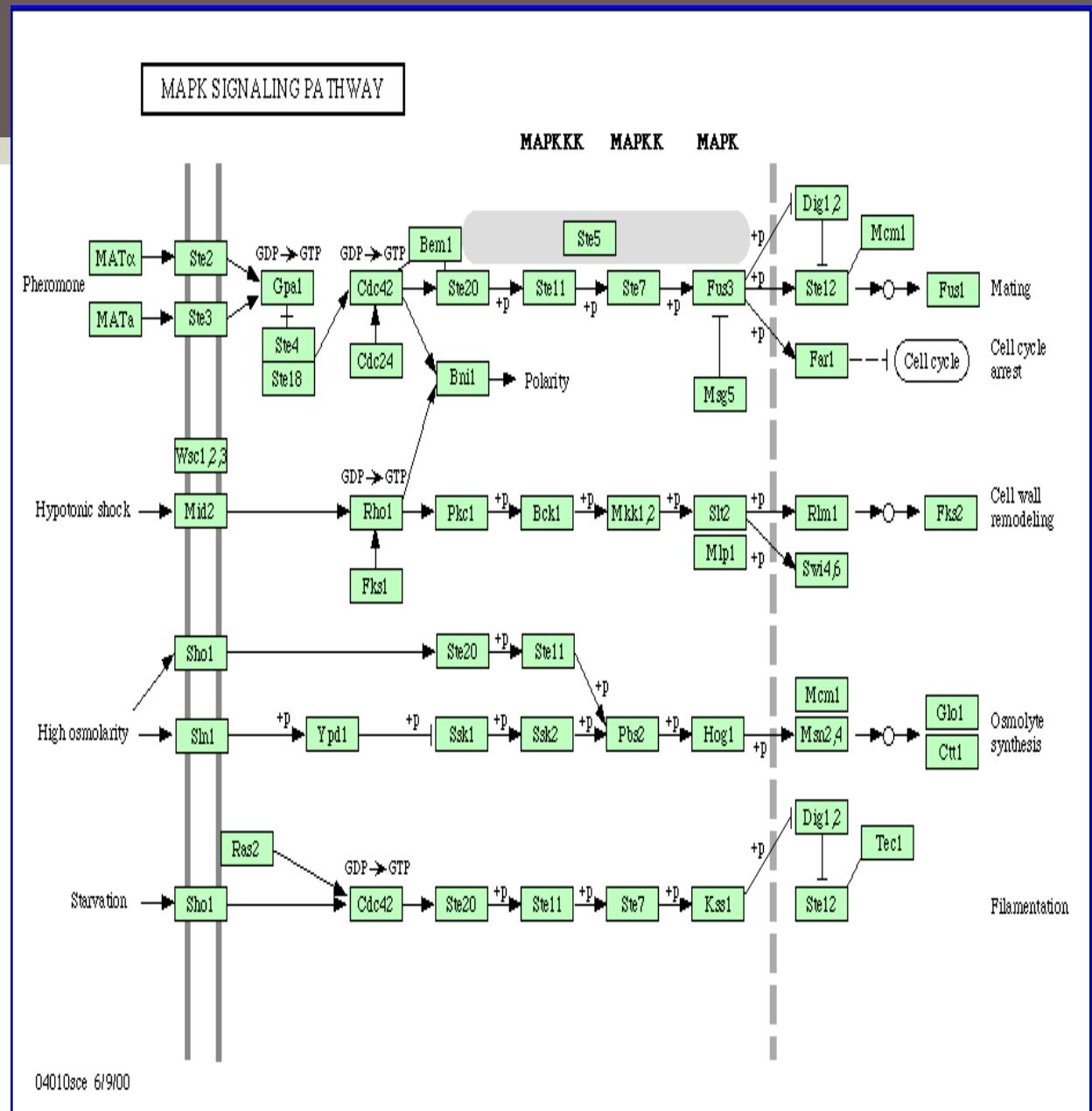
Organism-specific entry points

[KEGG Organisms](#) Enter org code(s) hsa hsa eco

- KEGG stores metabolic pathways
- Example: Glycolysis
- Substrates and products are represented by circles
- Enzymes are represented by dashed rectangles



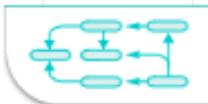
- KEGG contains signal transduction and regulatory pathways as well
- Example: MAPK signaling pathway





Interactive graphic models of molecular and cellular pathways

PATHWAYS ▶ MAIN CATEGORIES



Observe how genes interact in dynamic graphical models. Our online maps depict molecular relationships from areas of active research. In an "open source" approach, this community-fed forum constantly integrates emerging proteomic information from the scientific community. It also catalogs and summarizes important resources providing information for over 120,000 genes from multiple species. Find both classical pathways as well as current suggestions for new pathways.



BROWSE PATHWAYS BY CATEGORY

[▶ New Pathways](#)[▶ Browse all Pathways](#)[▶ Adhesion](#)[▶ Developmental Biology](#)[▶ Apoptosis](#)[▶ Hematopoiesis](#)[▶ Cell Activation](#)[▶ Immunology](#)[▶ Cell Cycle Regulation](#)[▶ Metabolism](#)[▶ Cell Signalling](#)[▶ Neuroscience](#)[▶ Cytokines/Chemokines](#)

SEARCH PATHWAYS BY TITLE

Pathway Name



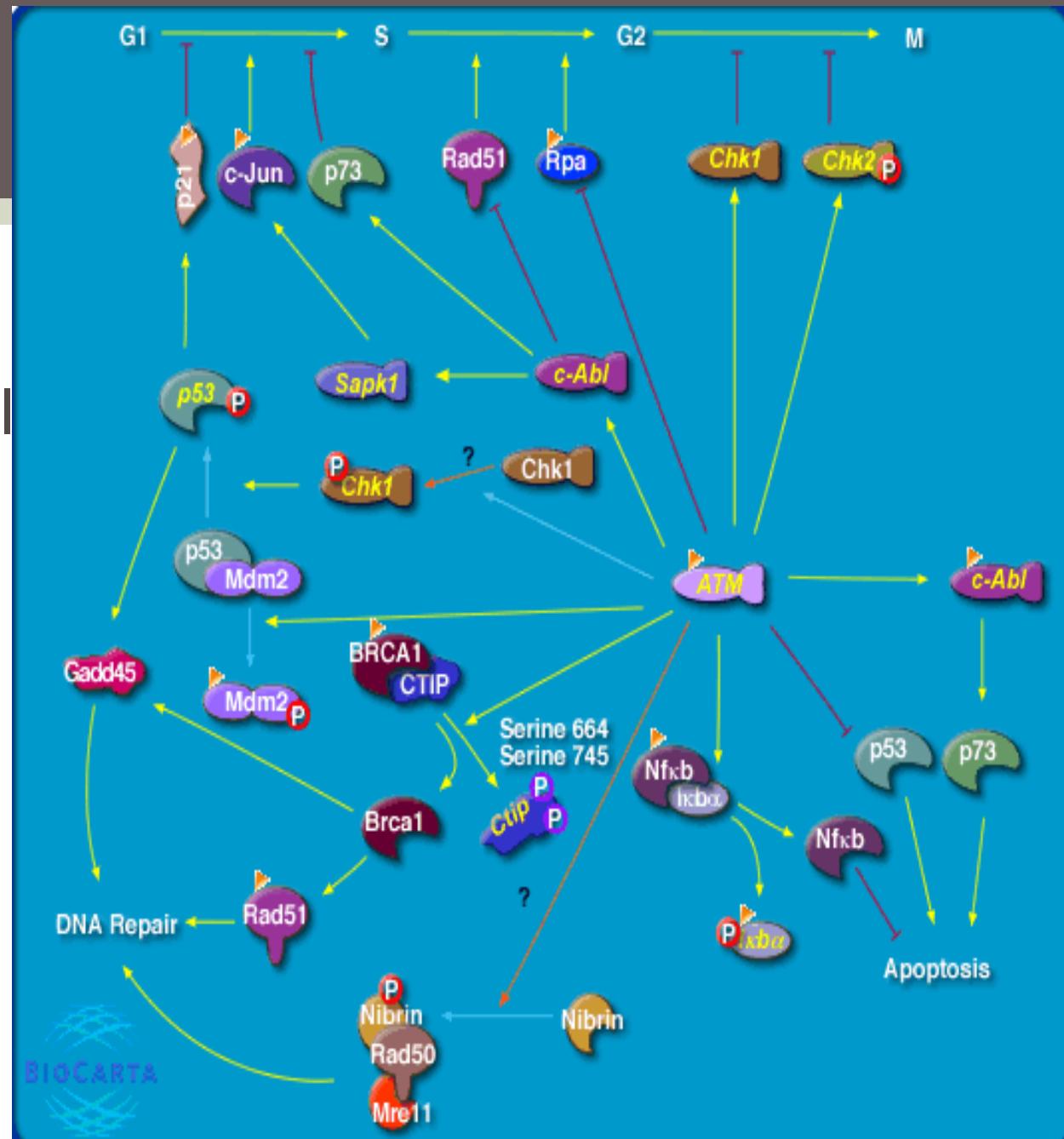
SEARCH

Gene Name

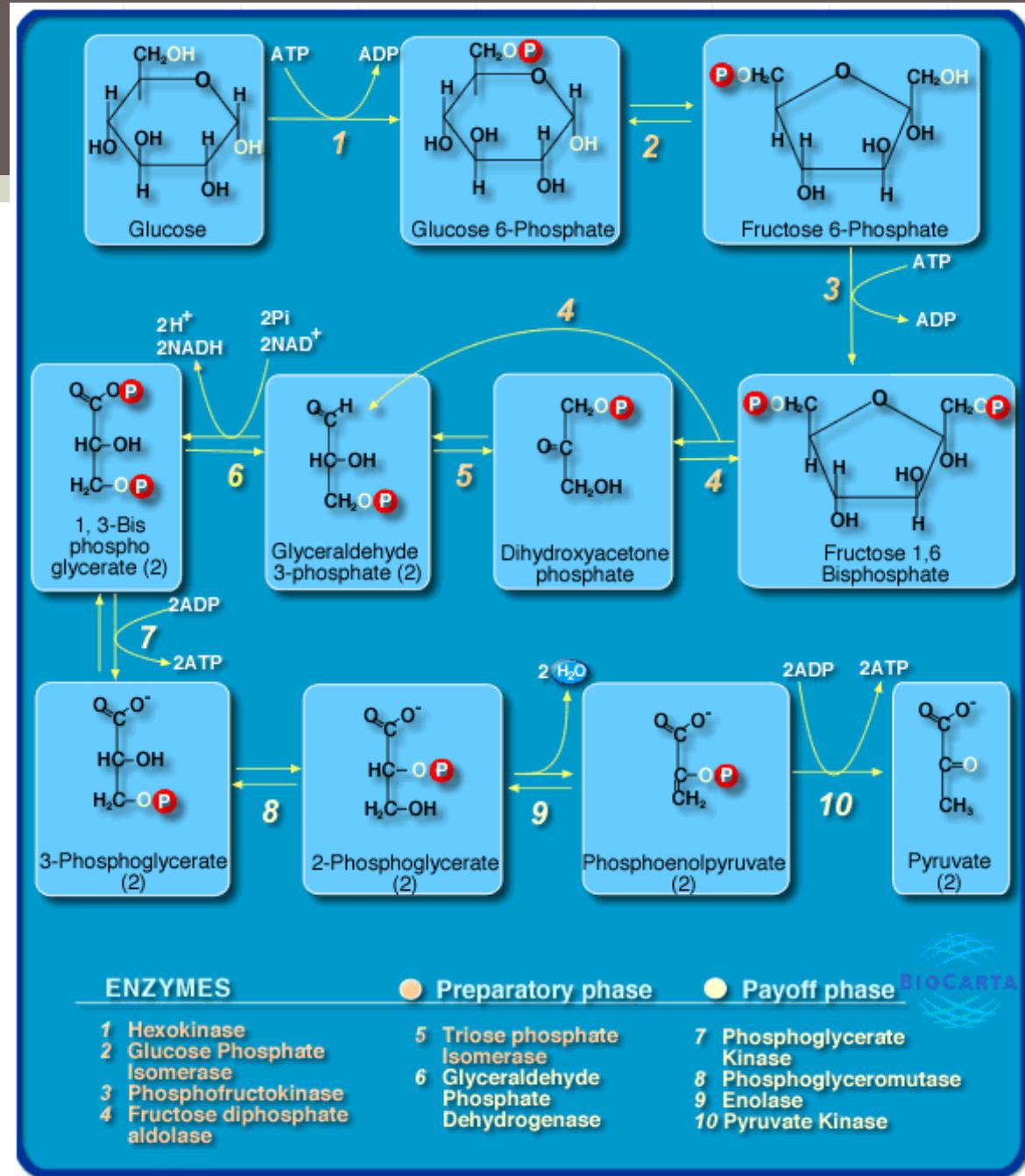


SEARCH

- BioCarta is specialized in signal transduction pathways
 - Example: ATM signalling pathway



- It contains metabolic pathways as well
- Example: Glycolysis
- Compounds are shown as chemical structures and formulas
- Enzymes mark the conversion from substrate to products





Functional Annotation Chart

[Help and Manual](#)

Current Gene List: List_1

Current Background: Homo sapiens

164 DAVID IDs

Options

[Rerun Using Options](#) [Create Sublist](#)

10 chart records

[Download File](#)

Sublist	Category	Term	RT	Genes	Count	%	P-Value	Benjamini
	REACTOME_PATHWAY	REACT_18266:Axon guidance	RT	■	5	3.0	2.0E-3	5.0E-2
	KEGG_PATHWAY	Chemokine signaling pathway	RT	■	7	4.3	7.7E-3	4.7E-1
	KEGG_PATHWAY	ECM-receptor interaction	RT	■	5	3.0	7.8E-3	2.7E-1
	KEGG_PATHWAY	Cytokine-cytokine receptor interaction	RT	■	8	4.9	1.1E-2	2.5E-1
	PANTHER_PATHWAY	P00005:Angiogenesis	RT	■	8	4.9	2.1E-2	6.1E-1
	KEGG_PATHWAY	Pathways in cancer	RT	■	8	4.9	3.3E-2	4.9E-1
	REACTOME_PATHWAY	REACT_16888:Signaling by PDGF	RT	■	4	2.4	3.4E-2	3.6E-1
	REACTOME_PATHWAY	REACT_15518:Transmembrane transport of small molecules	RT	■	3	1.8	7.9E-2	5.1E-1
	PANTHER_PATHWAY	P00004:Alzheimer disease-presenilin pathway	RT	■	5	3.0	8.9E-2	8.7E-1
	REACTOME_PATHWAY	REACT_6900:Signaling in Immune system	RT	■	7	4.3	9.5E-2	4.8E-1