WORKFLOWS TO FOLLOW UP ON RNA-SEQ ANALYSIS

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7 NOVEMBER 2016

TOPICS TO BE COVERED TODAY

- High-throughput sequencing basics
- Pattern recognition by hierarchical clustering
- Gene Ontology mapping
- Canonical pathway mapping

NEXT-GENERATION GAP

JOHN D. MCPHERSON
NATURE METHODS **6**, S2 - S5 (2009)

http://www.urmc.rochester.edu/libraries/miner/mdl.aspx?U=http://dx.doi.org/doi:10.1038/nmeth.f.268

- Discusses high-throughput sequencing platforms with references to more information on each technology
  - Roche 454
  - Illumina GAIIx
  - Applied Biosystems SOLID
  - Helicos HeliScope

SENSE FROM SEQUENCE READS: METHODS FOR ALIGNMENT AND ASSEMBLY

PAUL FLICEK & EWAN BIRNEY

NATURE METHODS **6**, S6 - S12 (2009)

http://www.urmc.rochester.edu/libraries/miner/mdl.aspx?U=http://dx.doi.org/doi:10.1038/nmeth.1376

- Discusses alignment methods
  - Hash-based methods
  - Burrows-Wheeler transform methods
- Explains assembly based on those alignments

COMPUTATION FOR CHIP-SEQ AND RNA-SEQ STUDIES

SHIRLEY PEPKE, BARBARA WOLD & ALI MORTAZAVI

NATURE METHODS **6**, S22 - S32 (2009)

http://www.urmc.rochester.edu/libraries/miner/mdl.aspx?U=http://dx.doi.org/doi:10.1038/nmeth.1371

- Provides overview of ChIP-seq and RNA-seq analyses
- ChIP-seq:
  - Classes of ChIP-seq signals
  - Peak-finders, regions, summits and sources
  - Publicly available ChIP-seq software
- RNA-seq:
  - Approaches to handle spliced reads
  - Quantifying gene expression
  - Publicly available RNA-seq software

VISUALIZATION OF OMICS DATA FOR SYSTEMS BIOLOGY

N. GEHLENBORG, S.I. O'DONOGHUE, N.S. BALIGA, A. GOESMANN, M.A. HIBBS, H. KITANO, O. KOHLBACHER, H. NEUWEGER, R. SCHNEIDER, D. TENENBAUM & A. GAVIN

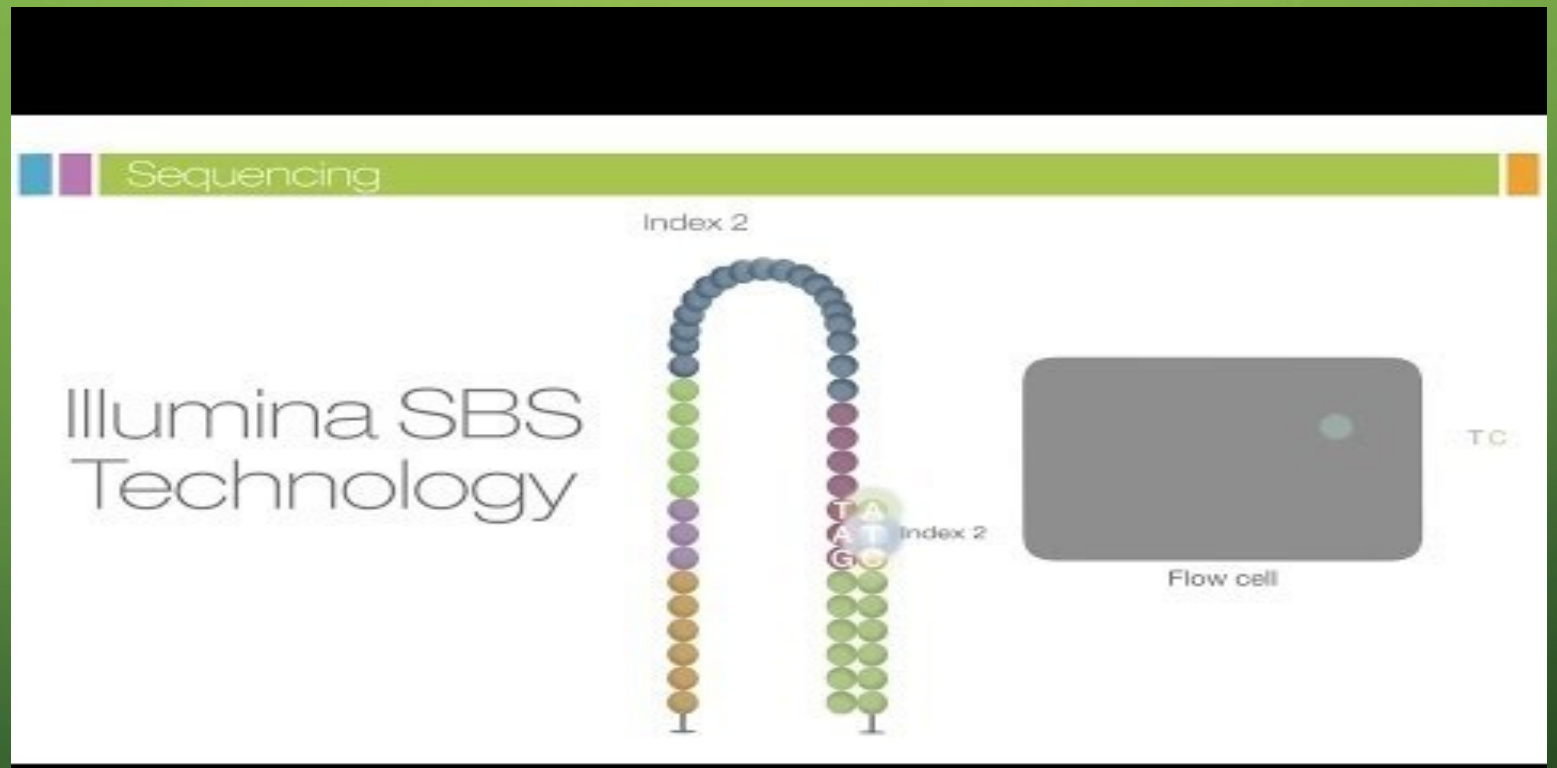
NATURE METHODS **7**, S56 - S68 (2010)

http://www.urmc.rochester.edu/libraries/miner/mdl.aspx?U=http://dx.doi.org/doi:10.1038/nmeth.1436

- Visualization based on networks vs. pathways
  - Protein interaction networks
  - Spatial information
  - Expression profile data
  - Multivariate –omics analysis
  - Metabolic network visualization
  - Pathway editing

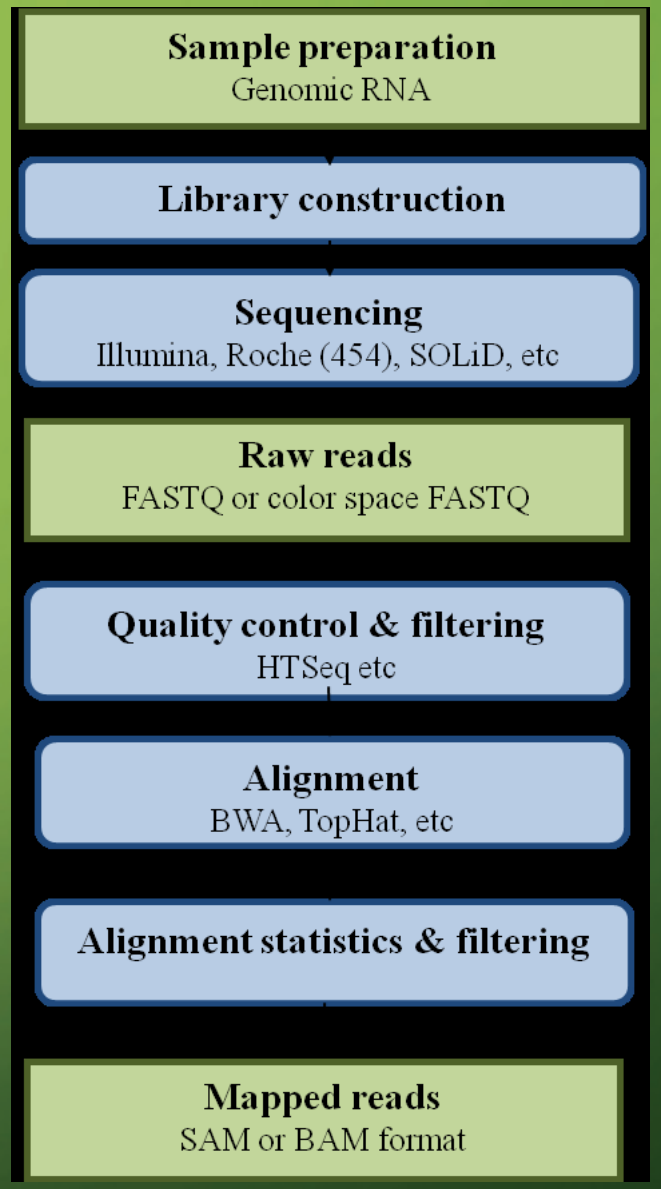
HIGH-THROUGHPUT SEQUENCING WORKFLOW

HOW DOES ILLUMINA SEQUENCING BY SYNTHESIS WORK?

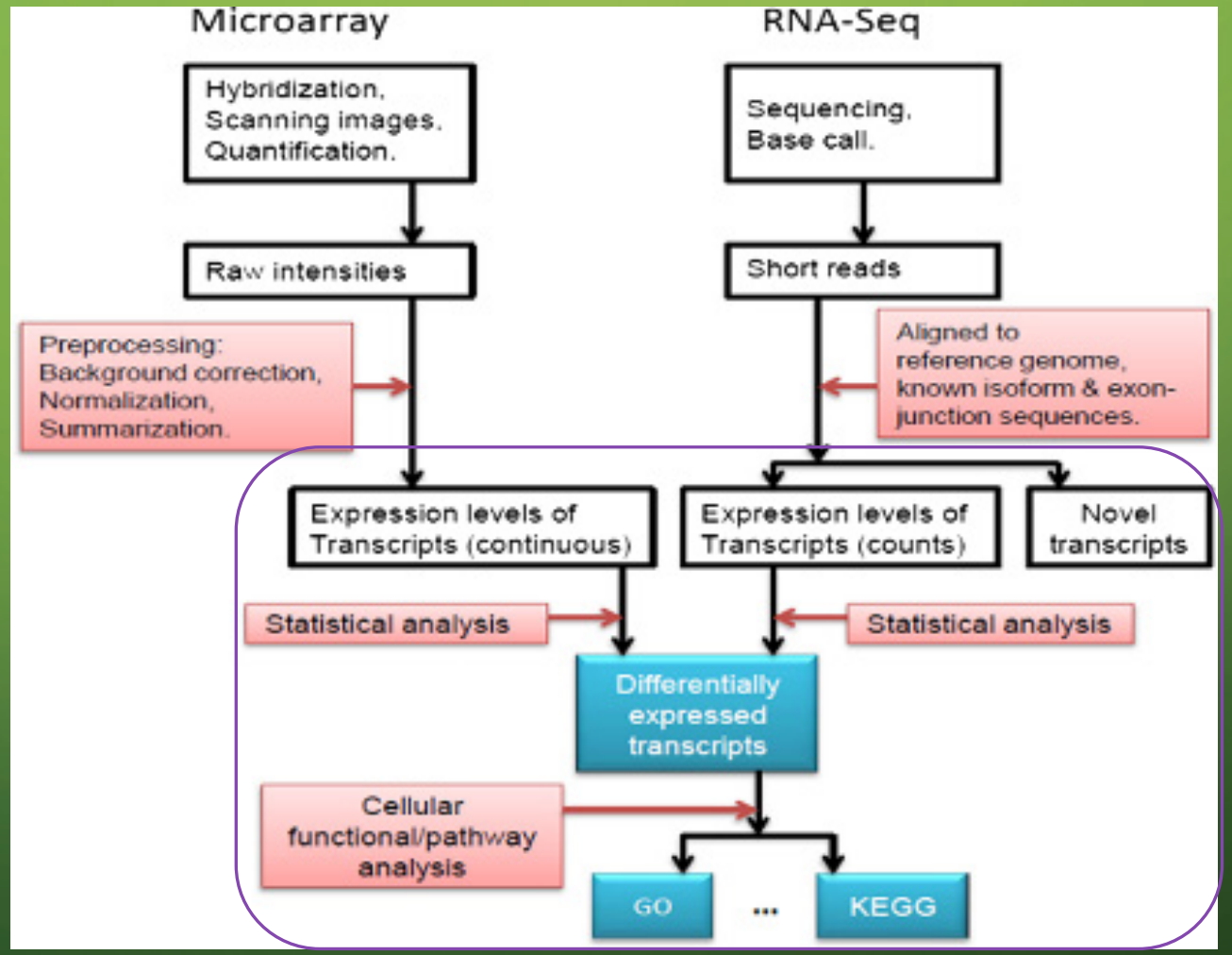


BASIC HIGH-THROUGHPUT SEQUENCING WORKFLOW: UPSTREAM

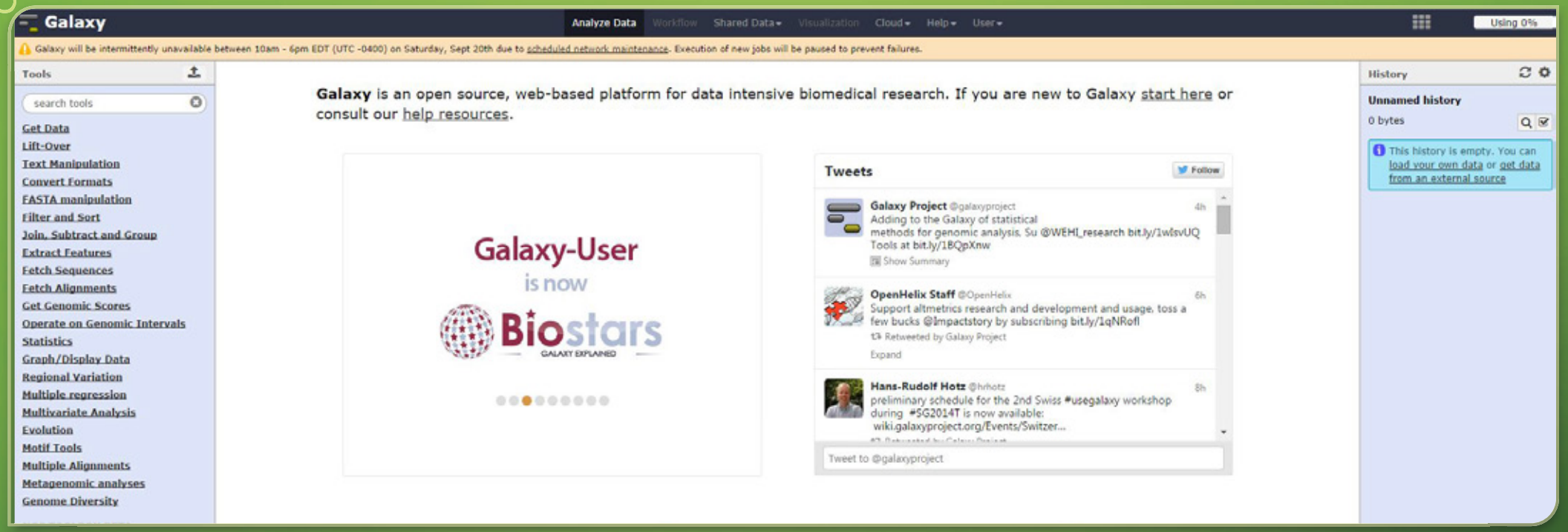
http://en.wikipedia.org/wiki/List_of_sequence_alignment_software



BASIC HIGH-THROUGHPUT SEQUENCING WORKFLOW: DOWNSTREAM

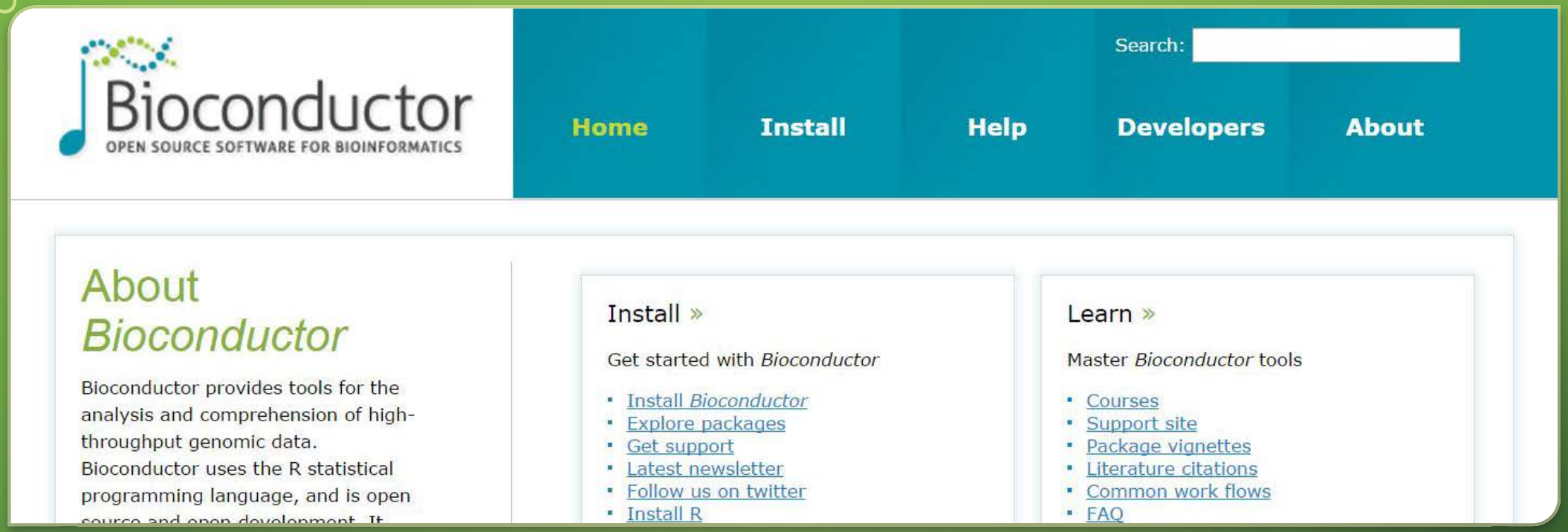


From: Fang Z, et al. Cell Biosci. 2012;2:26



GALAXY SUITE OF TOOLS

http://galaxyproject.org/



BIOCONDUCTOR AND R

https://www.bioconductor.org/

https://www.r-project.org/

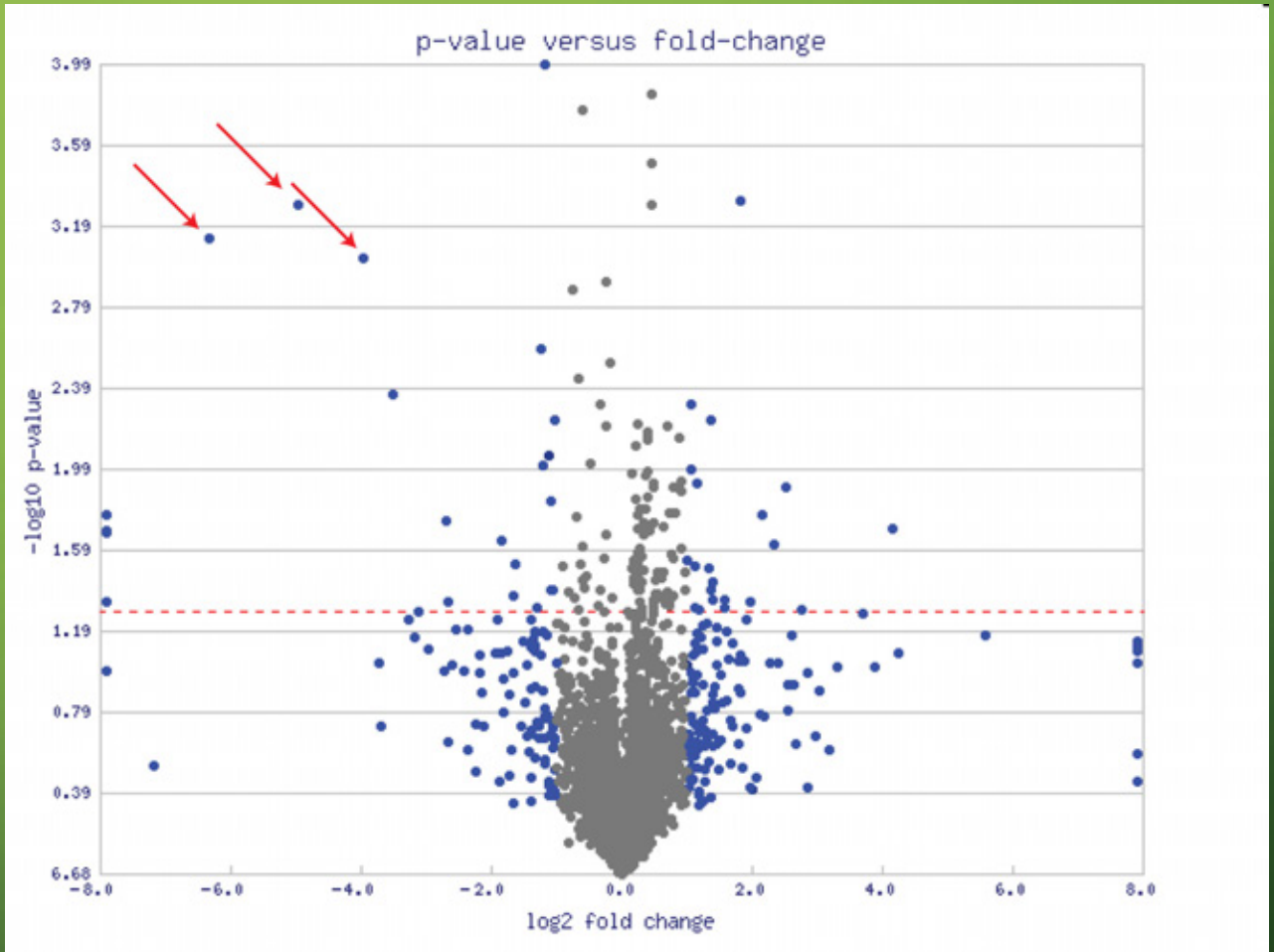
IDENTIFICATION OF PATTERNS IN THE DATA

COMMON WAYS TO MAKE SENSE OF –OMICS DATA

- Identification of patterns in the data
  - Advantage: Unbiased; Disadvantage: Can be hard to interpret, easy to over-interpret
  - Hierarchical clustering
  - Principle Components Analysis
  - Other graphical representations
- Mapping new information on to prior knowledge
  - Gene Ontology mapping
  - Canonical pathway mapping
  - Set-level analysis (GSEA, e.g.)
  - Protein interaction analysis
  - Common functions of compounds

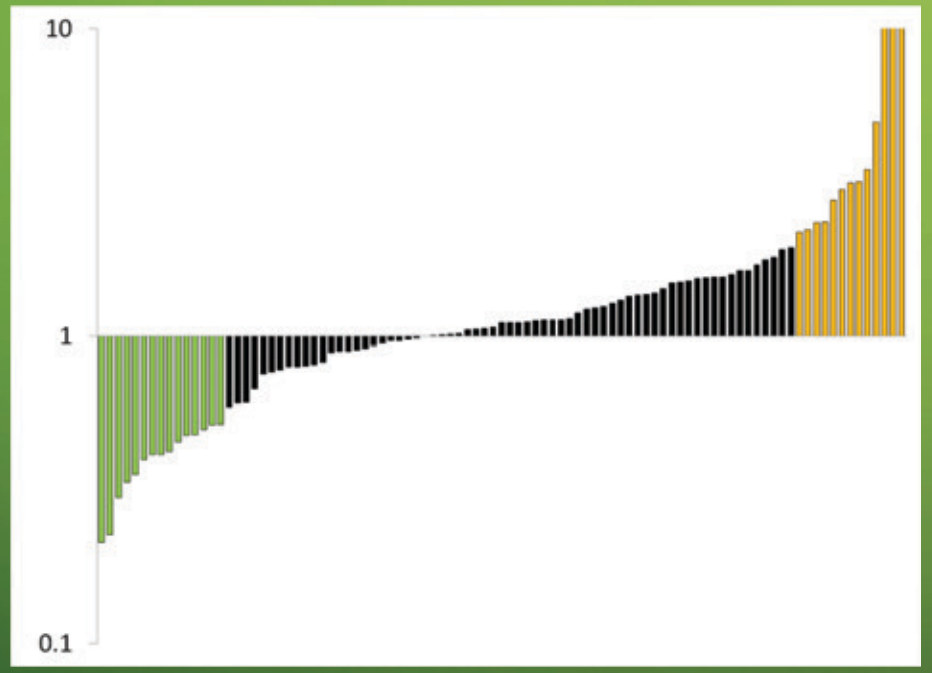
VOLCANO PLOT

By Roadnottaken - Own work, Public Domain, https://commons.wikimedia.org/w/index.php?curid=8901192

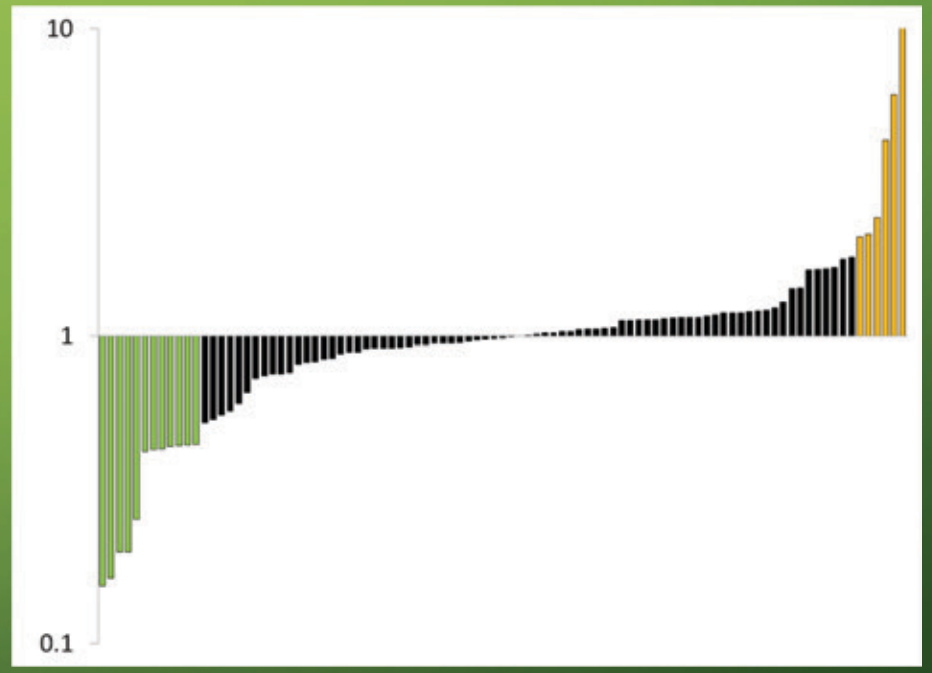


WATERFALL PLOTS

Cell Type 1



Cell Type 2



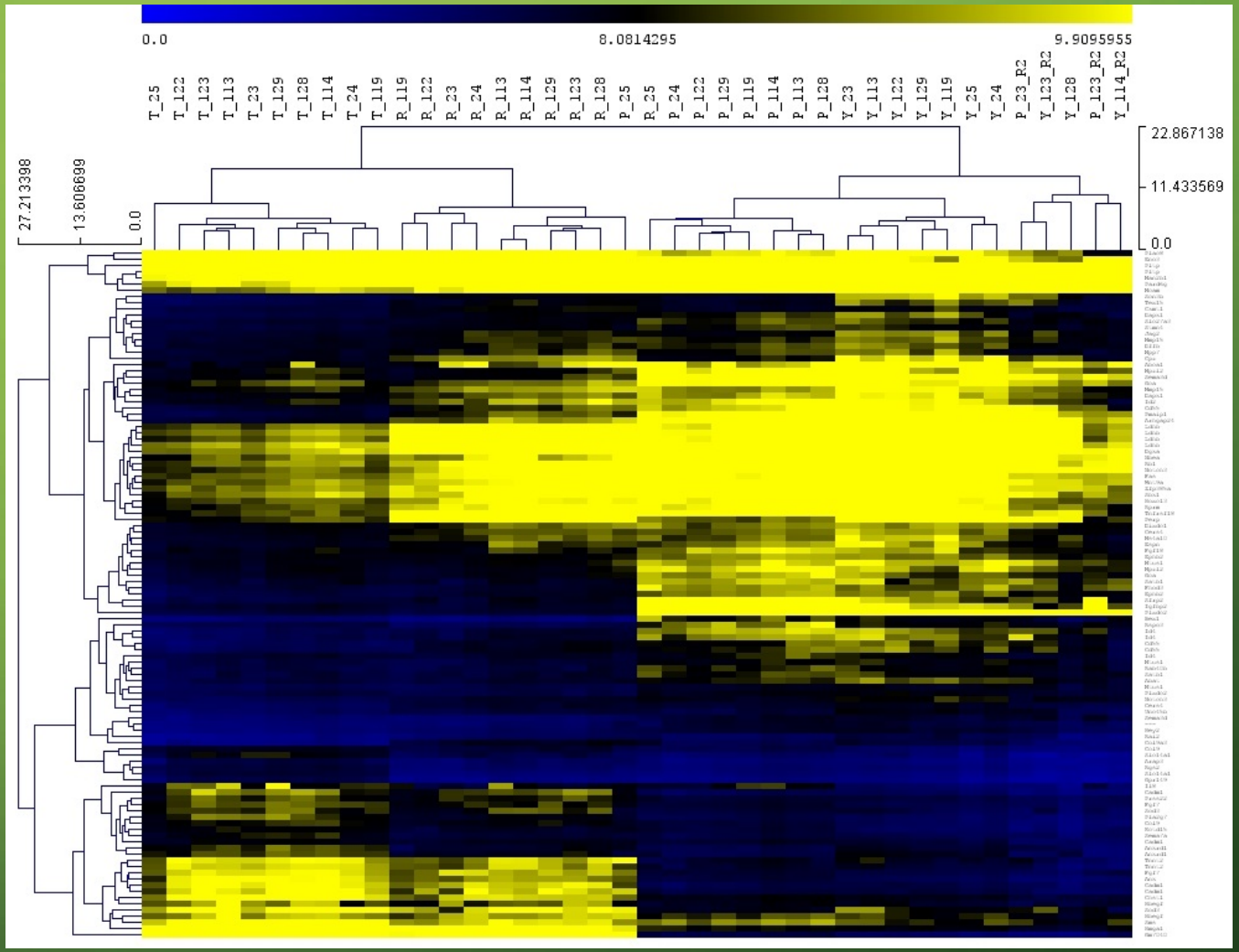
Gene order on these graphs is different!

HIERARCHICAL CLUSTERING

- Hierarchical clustering relates the samples or elements to one another in hierarchical fashion
  - Based on various metrics (correlation, ranking or distance between samples)
- There are other types of clustering, used less commonly but may be important in your project
  - k-means clustering, self-organizing maps (SOM), etc.

HIERARCHICAL CLUSTERING

Clustering and visualization via TM4 MeV software, Version 4.9.0



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As the advanced analytics version of our software, JMP Pro contains everything users know and love about JMP – and more. With the release of this latest version – JMP Pro 12 – users will enjoy new capabilities and see a leap in performance in almost all of its earlier platforms.

› Learn how to get JMP Pro 12

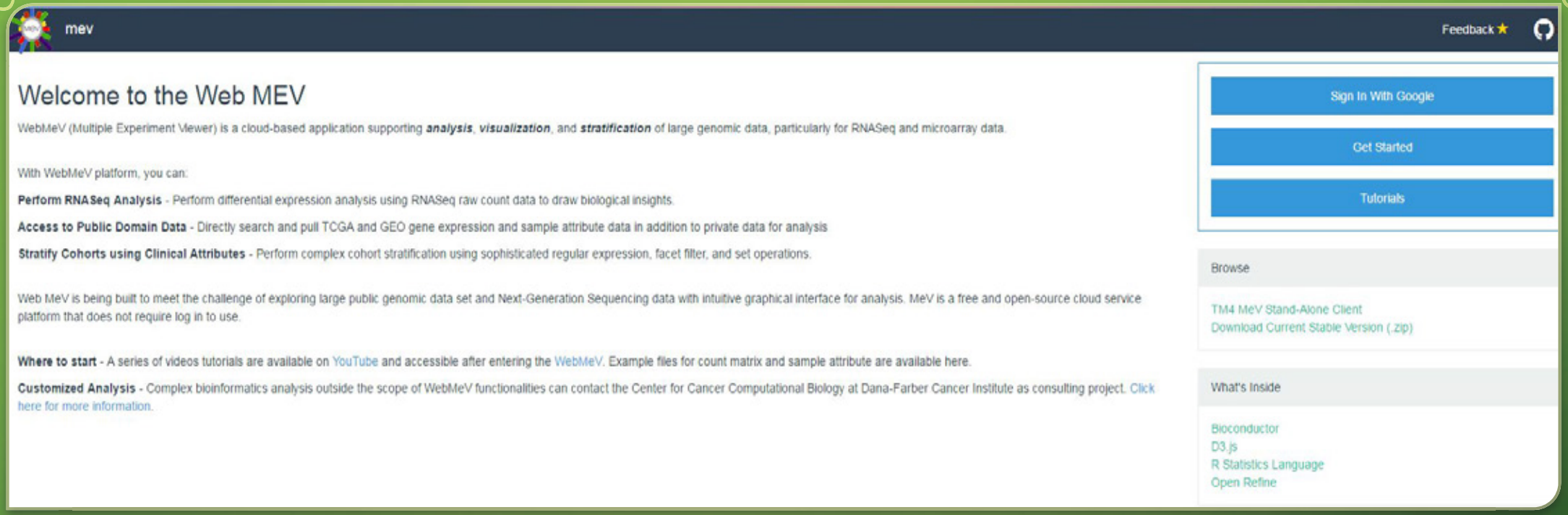
JMP: BETTER STATISTICAL ANALYSIS

http://www.jmp.com/en_us/home.html

http://tech.rochester.edu/services/software-site-licensing/

MORPHEUS

https://software.broadinstitute.org/morpheus/



MULTI-EXPERIMENT VIEWER (MEV)

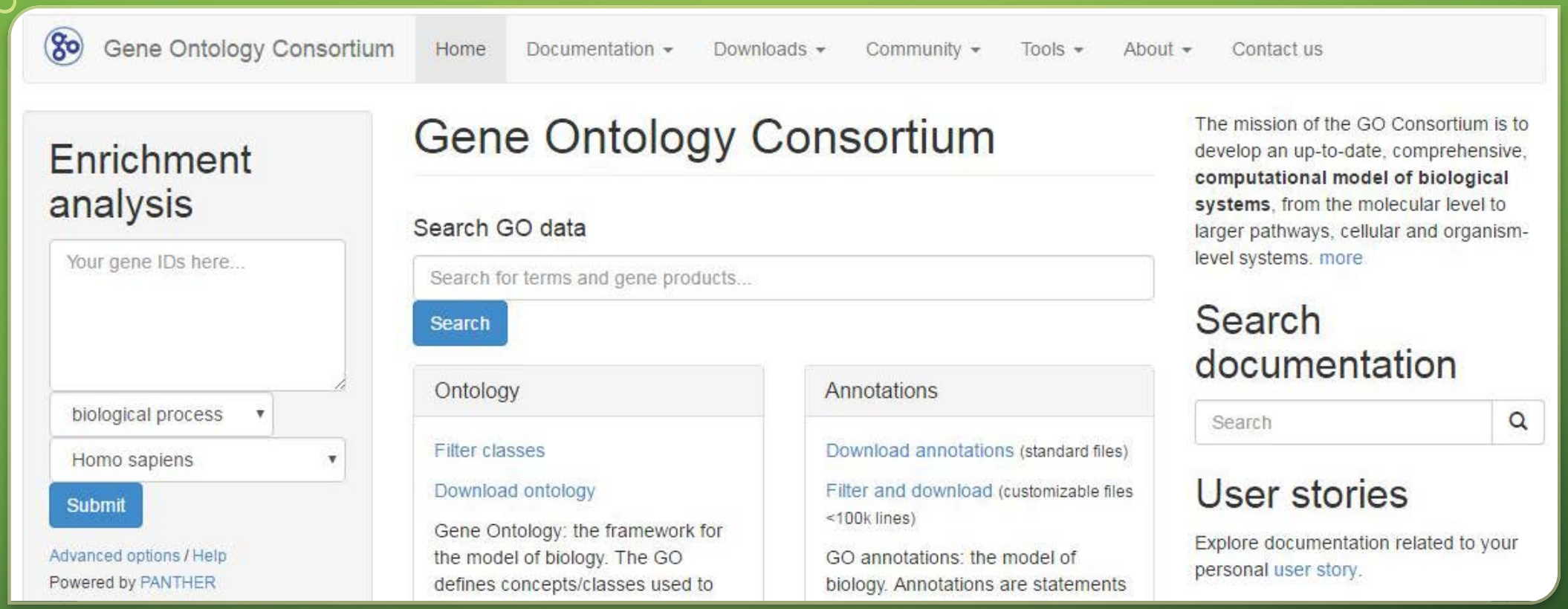
http://www.tm4.org/#/welcome

MAPPING NEW INFORMATION ONTO PRIOR KNOWLEDGE

SET-LEVEL / ENRICHMENT ANALYSIS

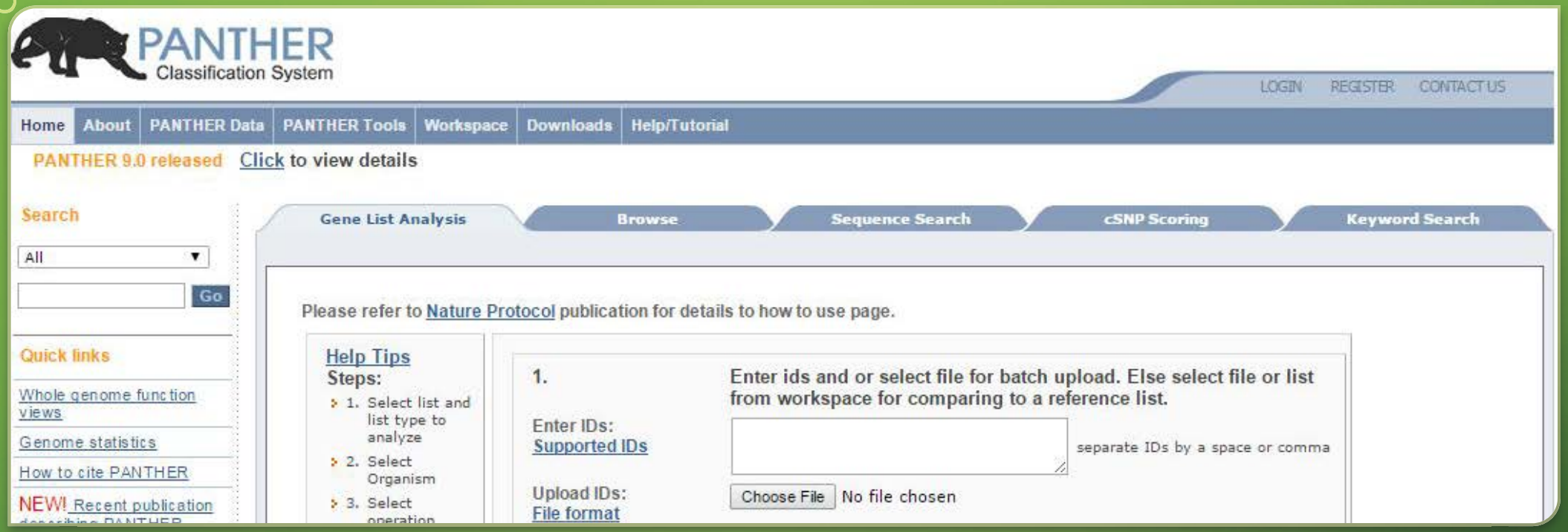
- Tests for over-representation of elements of a given set in a larger set
  - Gene ontology analysis
    - Hierarchical system of categorizing genes
    - Controlled vocabulary
    - Assess over- or under-represented ontology terms
  - Pathway analysis
    - Allows integration of novel experimental data with known biological function
      - Relies on accurate mapping of pathways in public domain
      - Assumes that pathways are the same in all contexts

GENE ONTOLOGY ANALYSIS



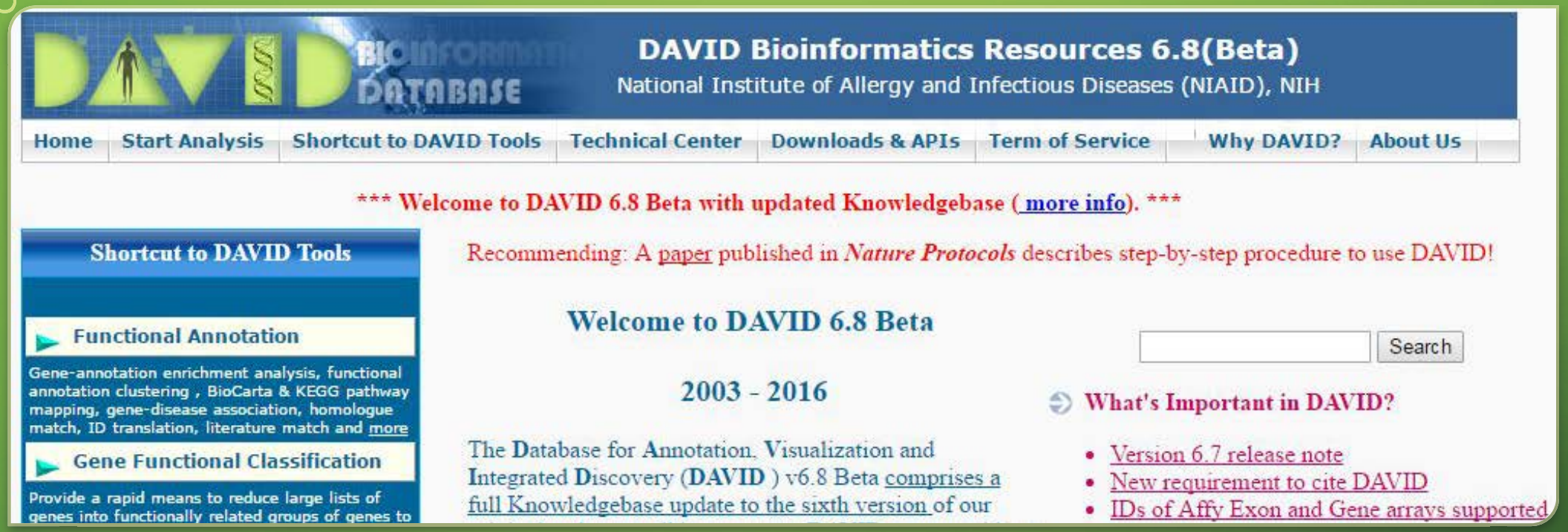
THE GENE ONTOLOGY CONSORTIUM

http://geneontology.org/



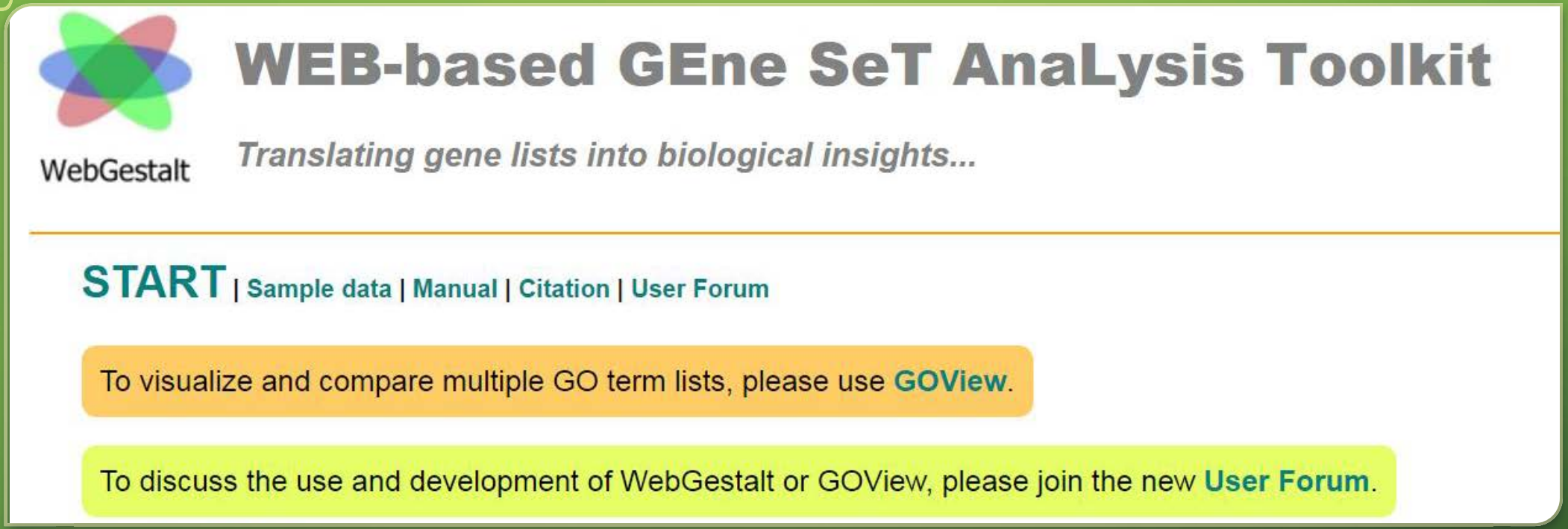
PROTEIN ANALYSIS THROUGH EVOLUTIONARY RELATIONSHIPS (PANTHER)

http://www.pantherdb.org/



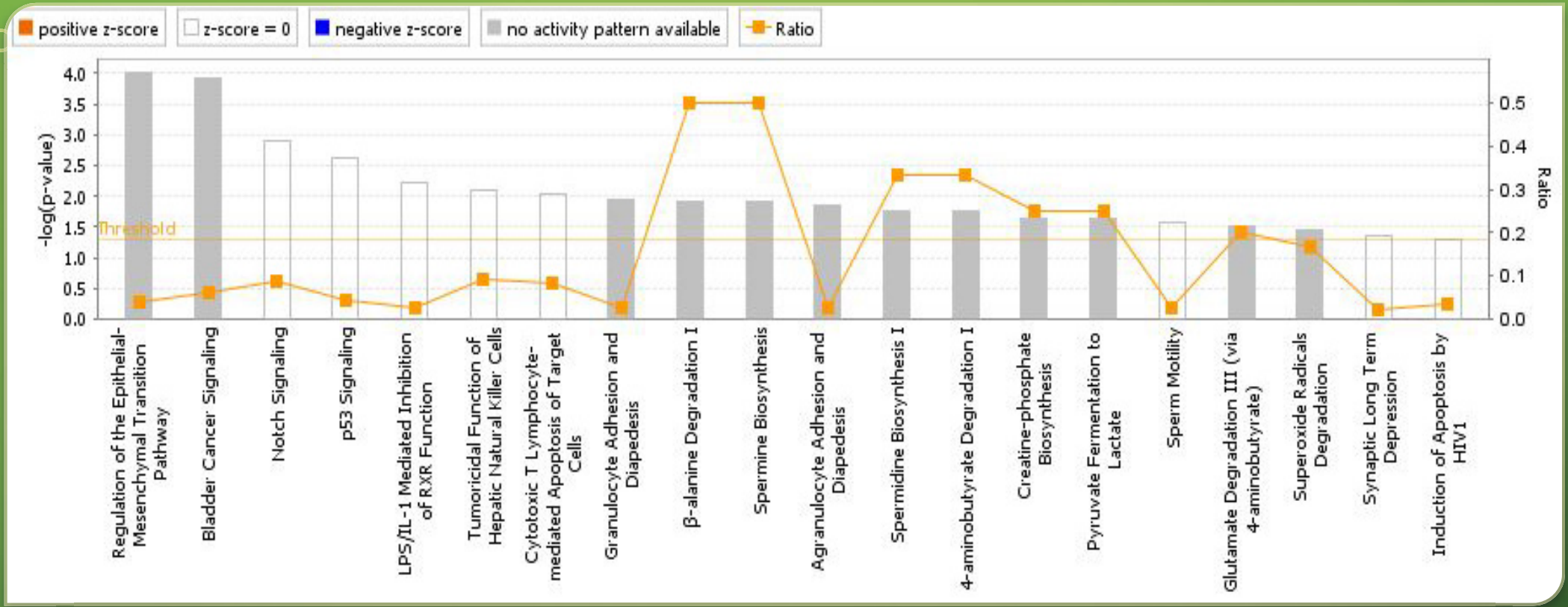
DATABASE FOR ANNOTATION, VISUALIZATION AND INTEGRATED DISCOVERY (DAVID)

https://david.ncifcrf.gov/



WEB-BASED GENE SET ANALYSIS TOOLKIT (WEBGESTALT)

http://www.webgestalt.org/



GENE ONTOLOGY ANALYSIS

Created with Ingenuity Pathway Analysis software, March 2016

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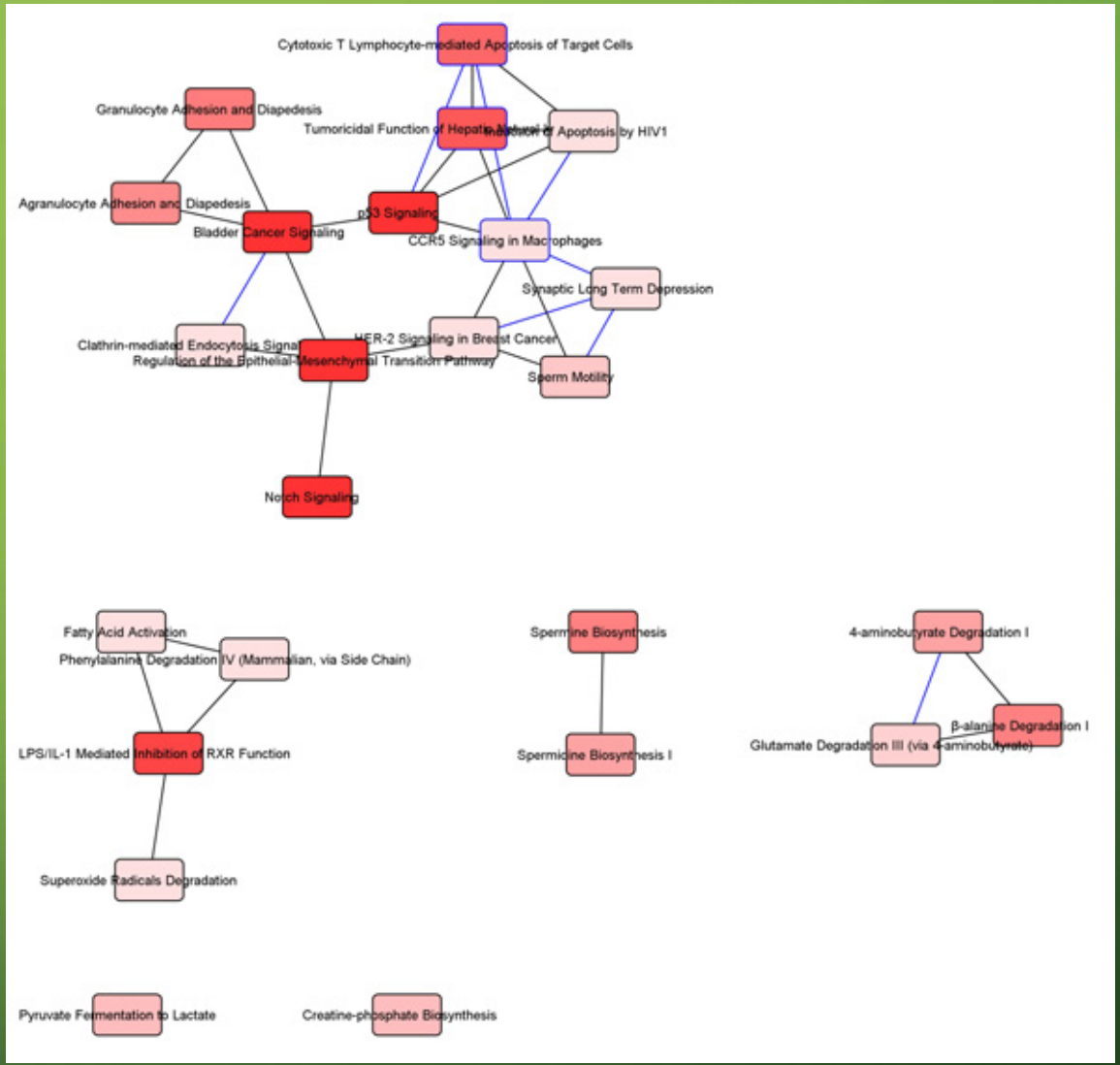
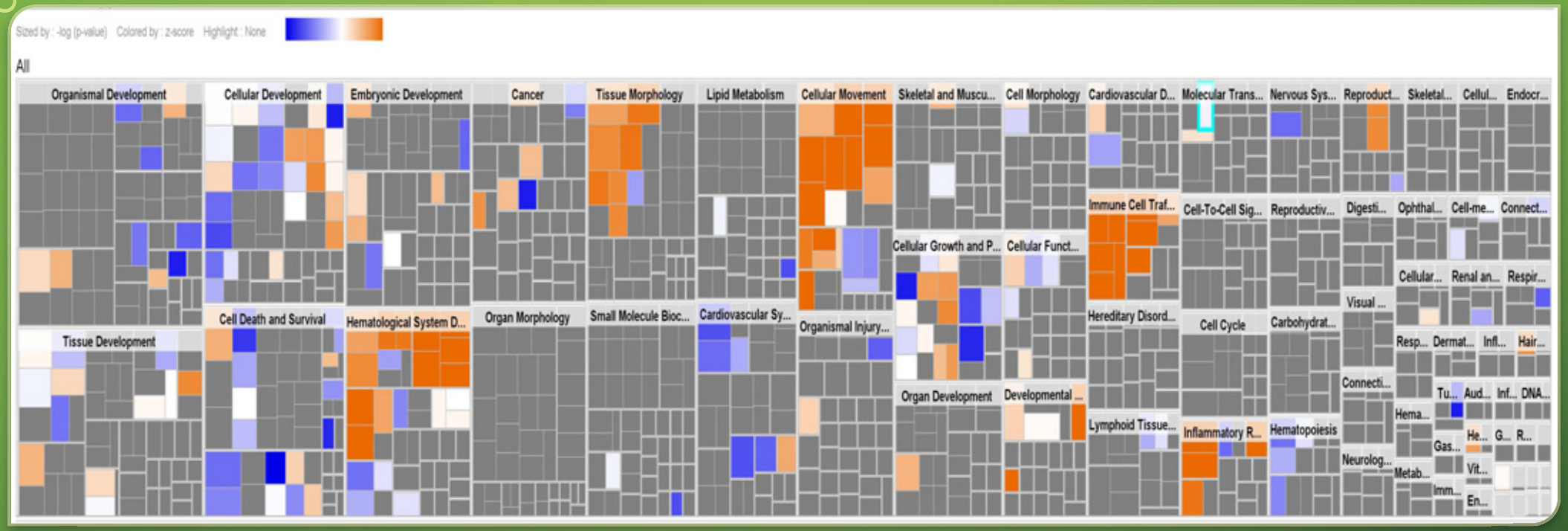
Created with Ingenuity Pathway Analysis software, March 2016





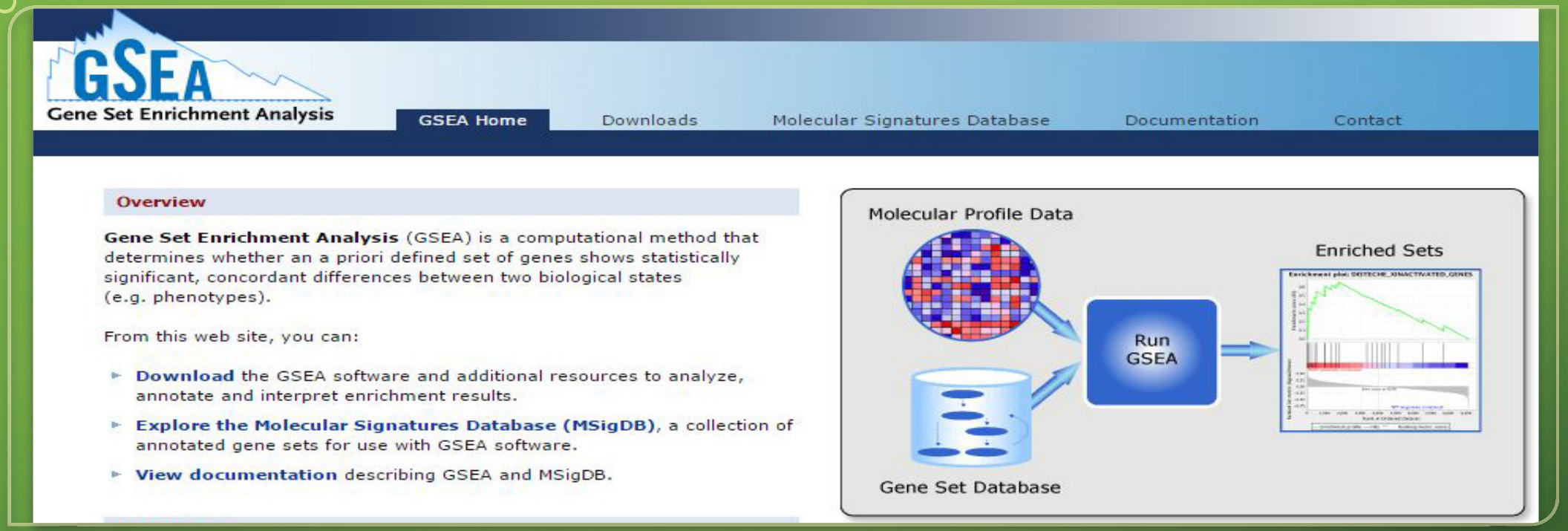
GENE ONTOLOGY ANALYSIS

Created with Ingenuity Pathway Analysis software, March 2016

PATHWAY ANALYSIS

THE REACTOME PROJECT

http://www.reactome.org/



GENE SET ENRICHMENT ANALYSIS (GSEA) & THE MOLECULAR SIGNATURES DATABASE (MSIGDB)

http://www.broadinstitute.org/gsea/index.jsp

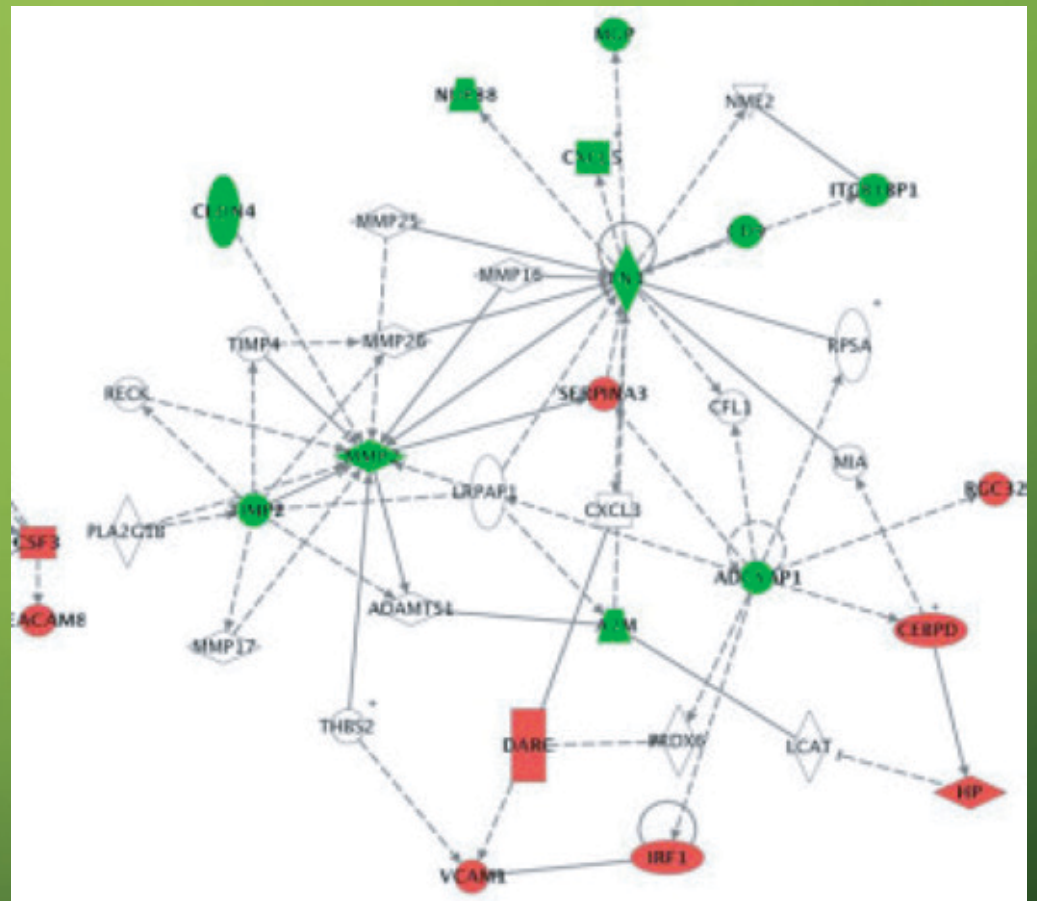
INGENUITY PATHWAY ANALYSIS

http://www.ingenuity.com/products/ipa

https://www.urmc.rochester.edu/libraries/Miner/research/MolecularBiologyTools.cfm

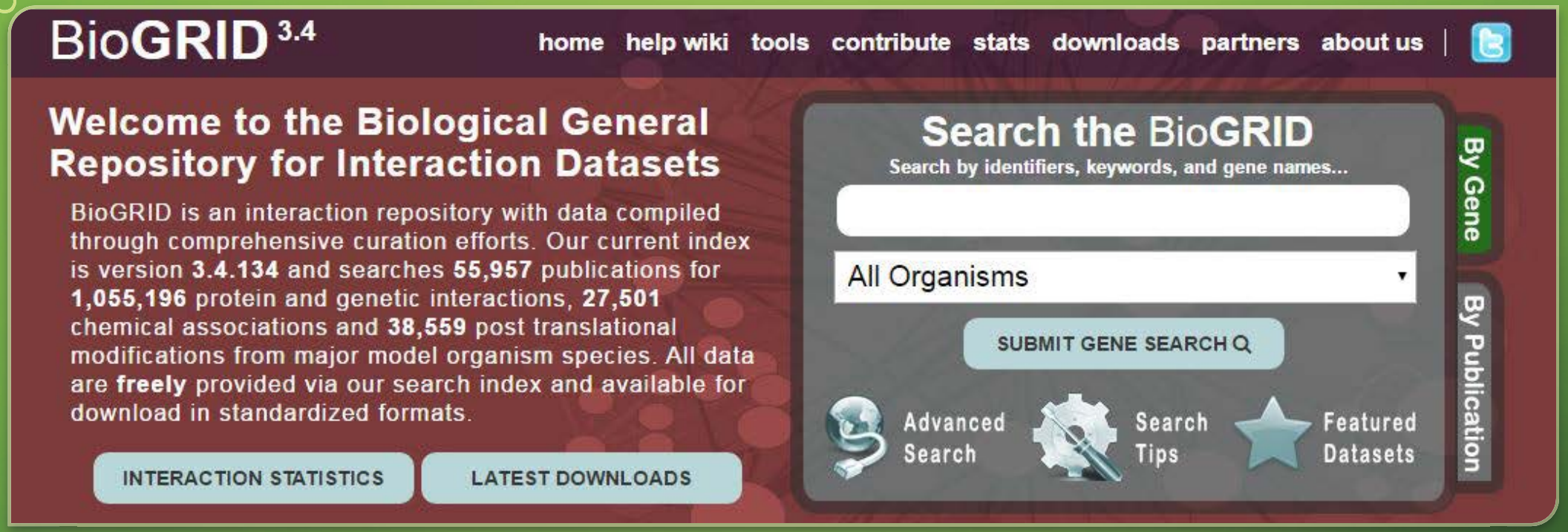
INGENUITY PATHWAY ANALYSIS OUTPUT

Hypothetical “network” generated by Ingenuity Pathway Analysis



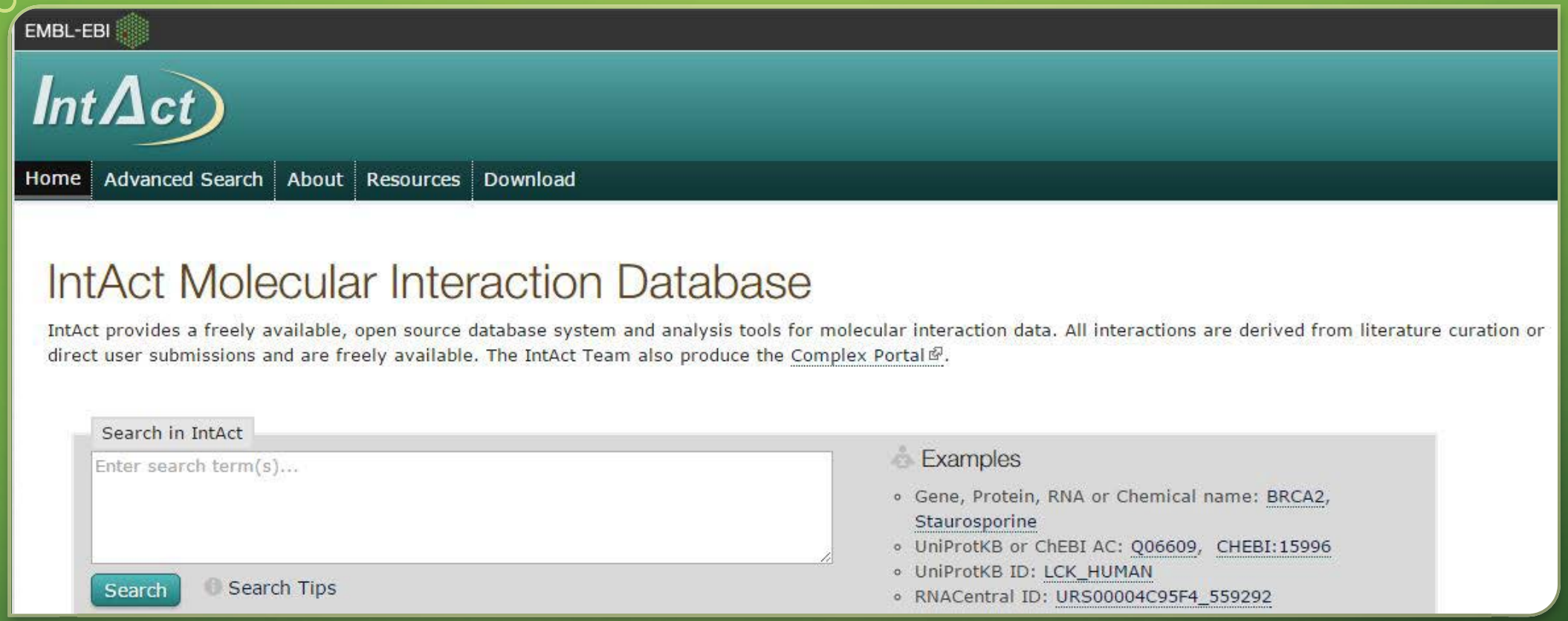
b: 8 hrs; IPA Network 1

PROTEIN INTERACTION ANALYSIS



BIOLOGICAL GENERAL REPOSITORY FOR INTERACTION DATASETS (BIOGRID)

http://thebiogrid.org/



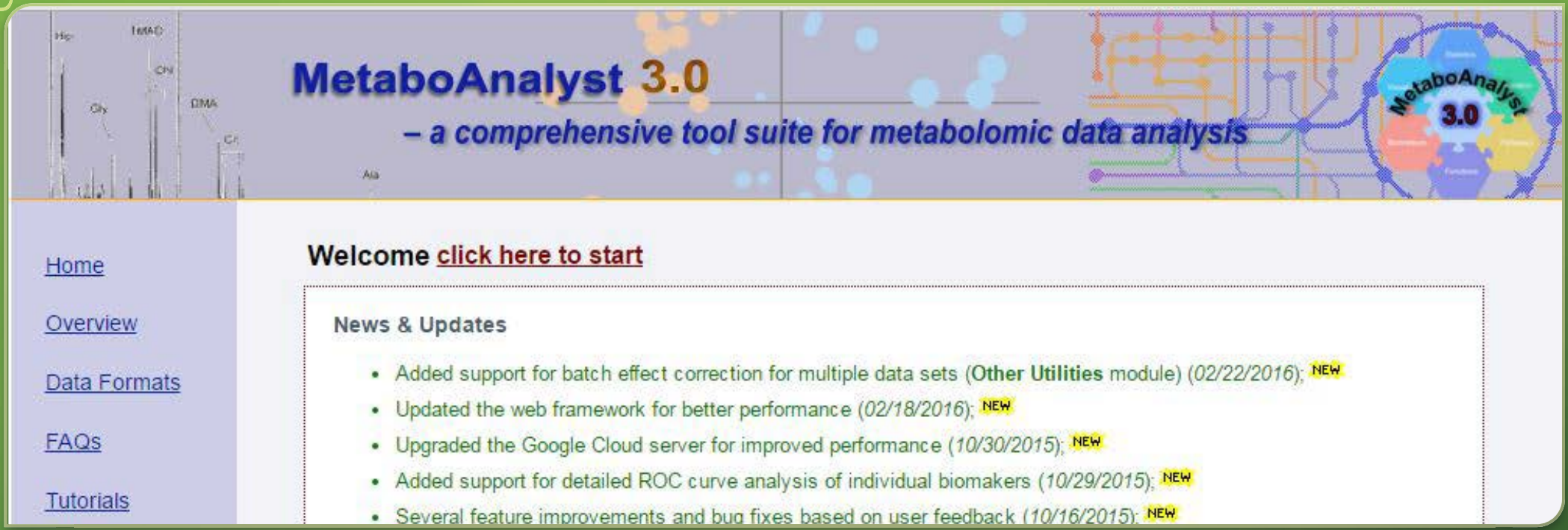
INTACT MOLECULAR INTERACTION DATABASE

http://www.ebi.ac.uk/intact/

SEARCH TOOL FOR THE RETRIEVAL OF INTERACTING GENES/PROTEINS (STRING)

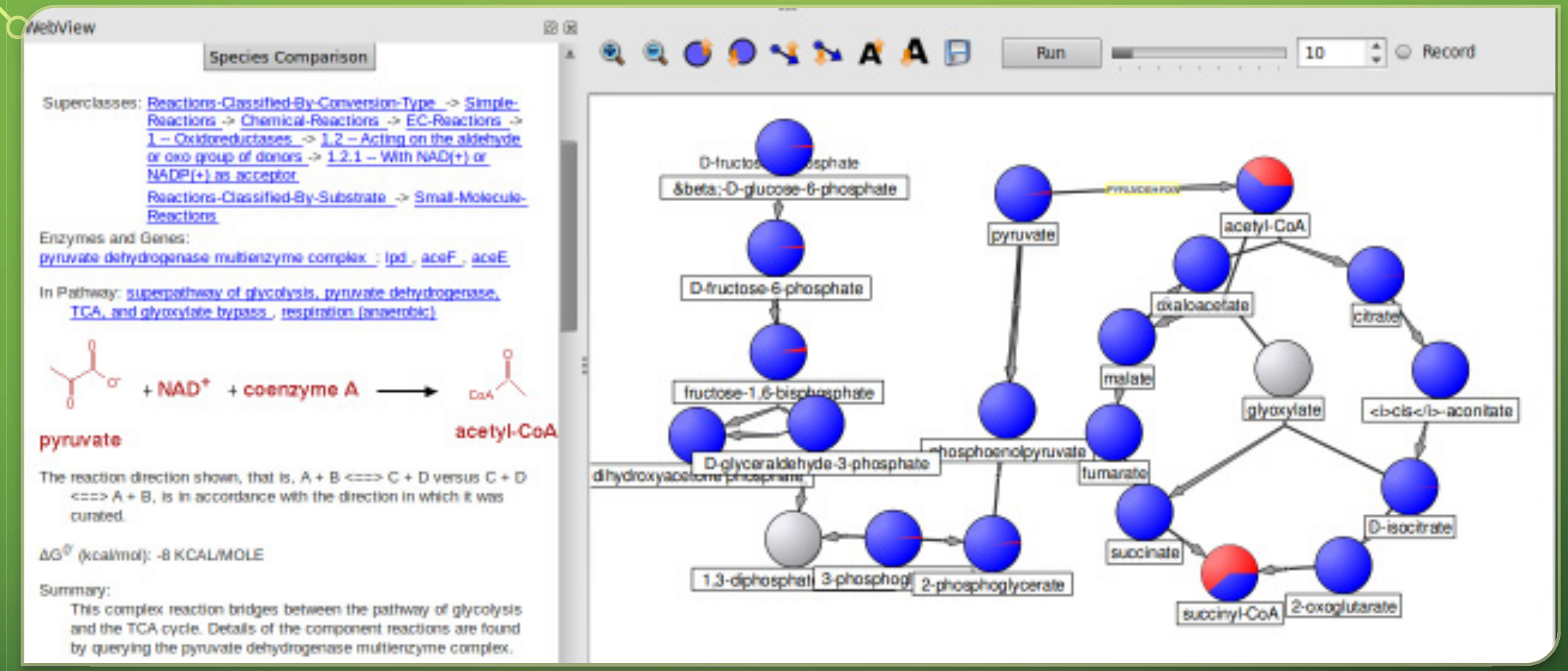
http://string-db.org/

METABOLOMICS DATA ANALYSIS



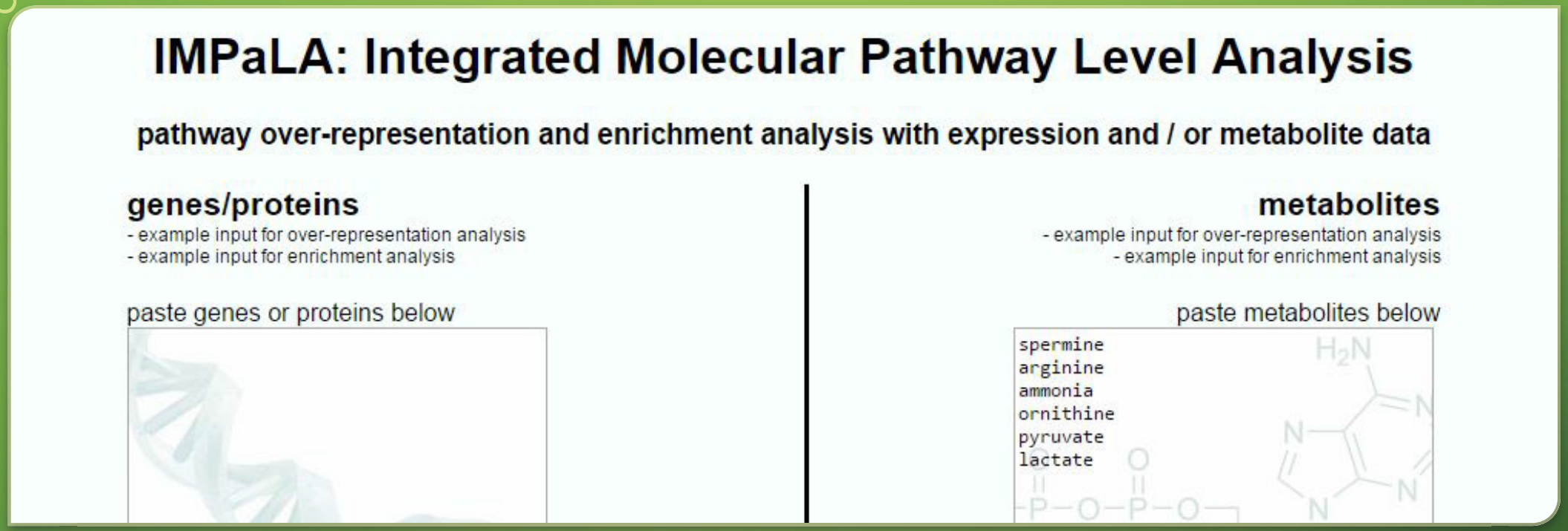
METABOANALYST 3.0

http://www.metaboanalyst.ca/



MAVEN: OPEN-SOURCE METABOLOMICS DATA ANALYZER

http://genomics-pubs.princeton.edu/mzroll/index.php?show=index



INTEGRATED MOLECULAR PATHWAY LEVEL ANALYSIS (IMPALA)

http://impala.molgen.mpg.de/

HIGH-THROUGHPUT CHEMICAL SCREENING

PUBCHEM

https://pubchem.ncbi.nlm.nih.gov/