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Module 4
More Depth on Pathway & Network Analysis

Lincoln Stein

Pathway and Network Analysis of –Omics Data

June 26, 2024

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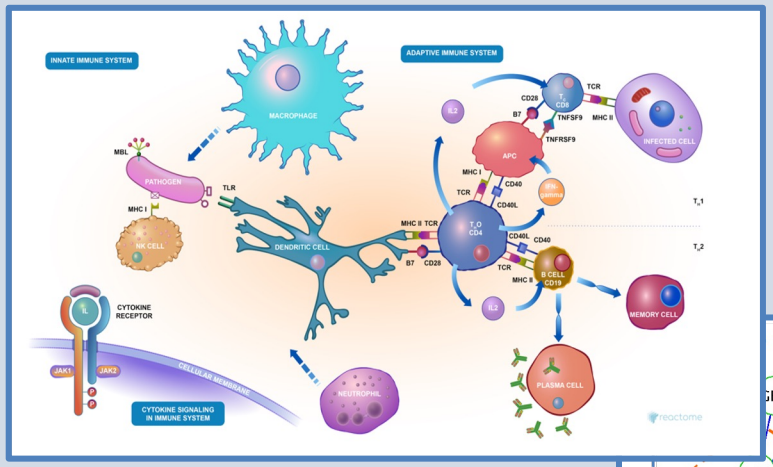
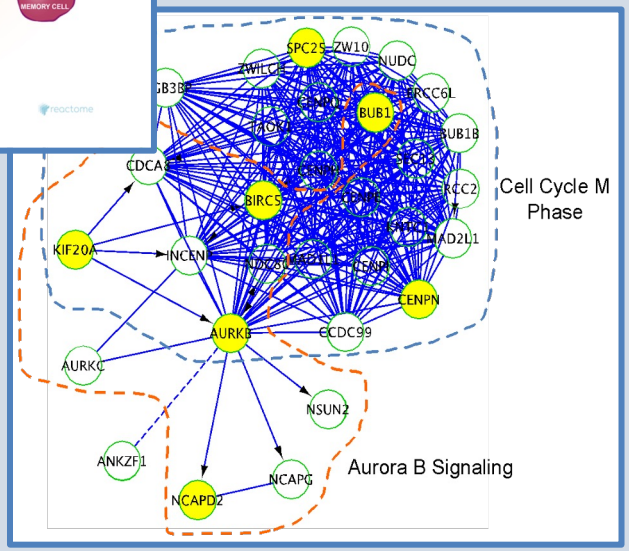




OICR
Ontario Institute for Cancer Research

Learning Objectives of Module

- Understand the principles of pathway and network analysis.
  - Sources of pathway and network data.
  - Analytical approaches to data analysis, visualization and integration.
  - Applications of pathway enrichment analysis.

Why Pathway Analysis?

- Dramatic data size reduction: 1000's of genes => dozens of pathways.
- Increase statistical power by reducing multiple hypotheses.
- Find meaning in the "long tail" of rare cancer mutations.
- Tell biological stories:
  - Identifying hidden patterns in gene lists.
  - Creating mechanistic models to explain experimental observations.
  - Predicting the function of unannotated genes.
  - Establishing the framework for quantitative modeling.
  - Assisting in the development of molecular signatures.

What is Pathway/Network Analysis?

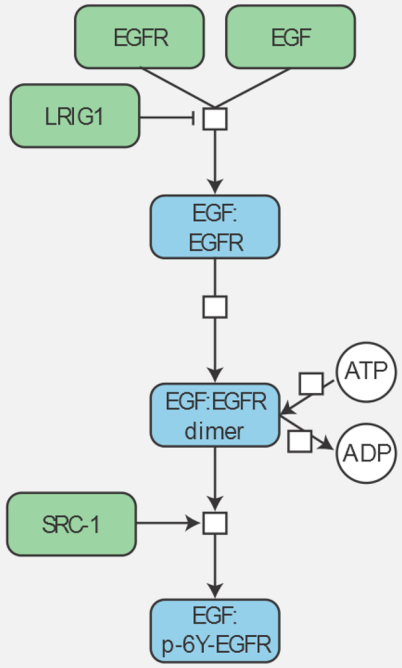
- Any analytic technique that makes use of biological pathway or molecular network information to gain insights into a tumor or other biological system.
- A rapidly evolving field.
- Many approaches.

Ingredients you will Need

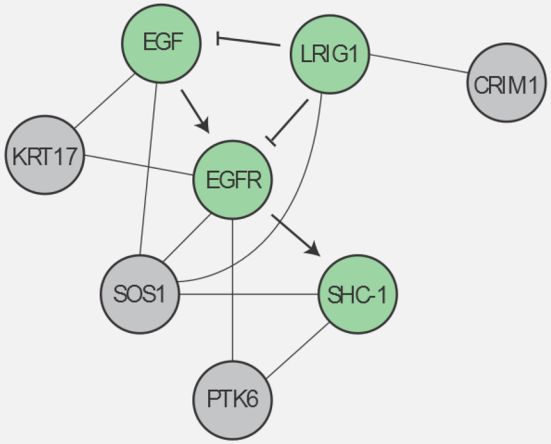
1. High-throughput biological data: A list of altered genes, proteins, RNAs, etc.
2. A source of pathways or networks.

Pathways vs Networks

EGFR-centered Pathway



EGFR-centered Network

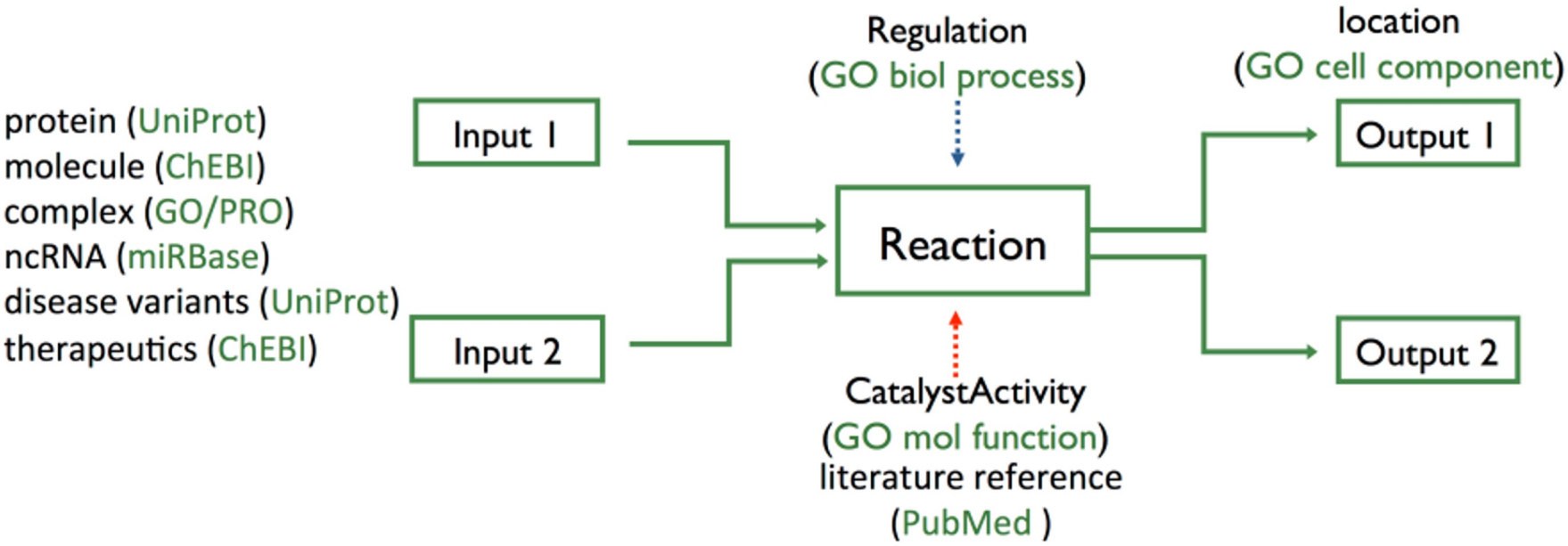


Pathway Databases

- Advantages:
  - Usually curated.
  - Biochemical view of biological processes.
  - Cause and effect captured.
  - Human-interpretable visualizations.
- Disadvantages:
  - Sparse coverage of genome.
  - Different databases disagree on boundaries of pathways.

Reaction-Network Databases

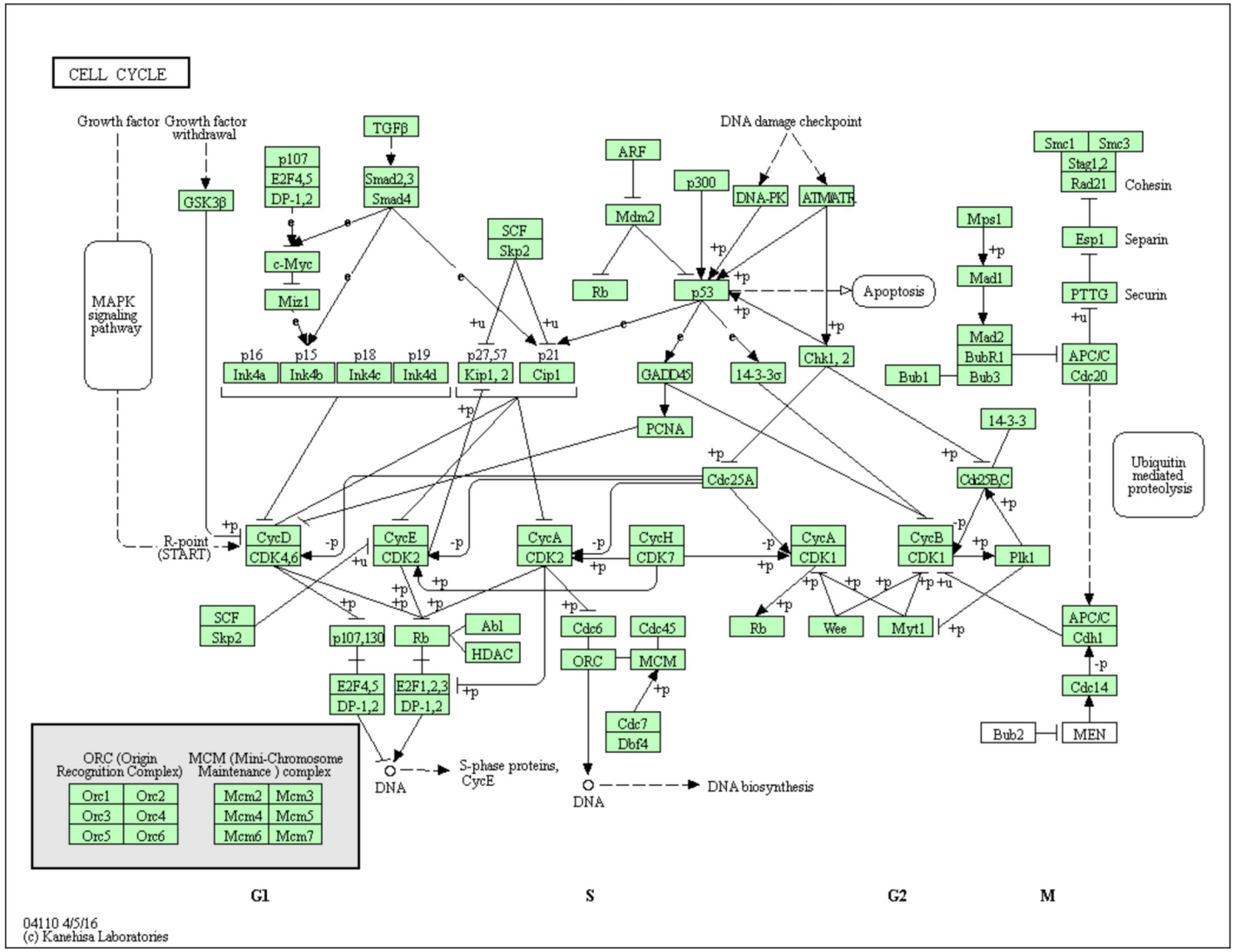
- Reactome & KEGG
  - explicitly describe biological processes as a series of biochemical reactions.
  - represents many events and states found in biology.



KEGG

- Kyoto Encyclopedia of Genes and Genomes(KEGG):
  - A vast library of information = fully sequenced genomes, genes, proteins, pathways, and chemical compounds pertaining to over a hundred different species of both prokaryotes and eukaryotes.
  - KEGG PATHWAY is a collection of manually drawn pathway maps representing knowledge on the molecular interaction and reaction networks for Metabolism, Cellular Processes, Organismal Systems, Human Diseases and Drug Development
- Subscription required for access to underlying data for analysis use.

KEGG Cell Cycle



Reactome

- Open source and open access pathway database
- Curated human pathways encompassing metabolism, signaling, and other biological processes.
- Rigorous curation standards – every pathway is traceable to primary literature.
- Cross-reference to many other bioinformatics databases.
- Provides data visualization and analysis tools
  - Google-map style reaction diagrams and textbook-style illustrations with overlays;
  - Find pathways containing your gene list;
  - Calculate gene overrepresentation in pathways;
  - Find corresponding pathways in other species.

Reactome Cell Cycle

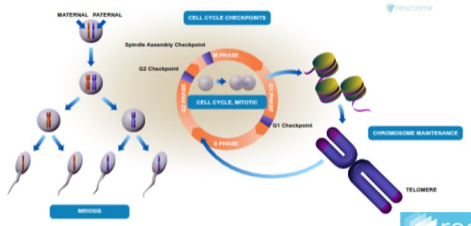
Locations in the PathwayBrowser

Cell Cycle (Homo sapiens)

General

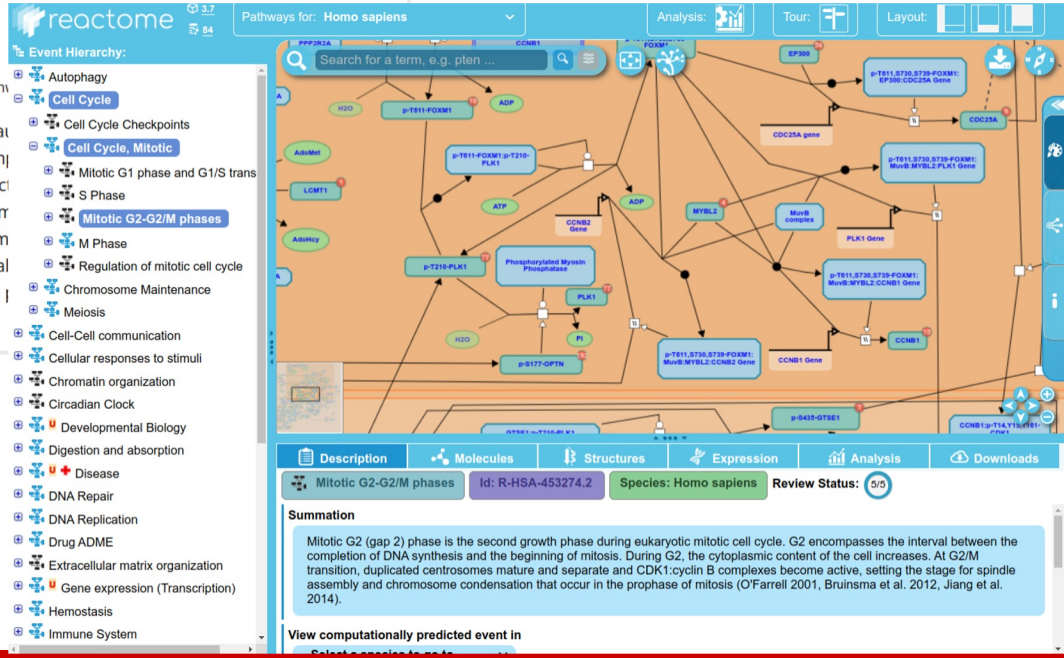
SBML | BioPAX | PDF

SVG | PNG



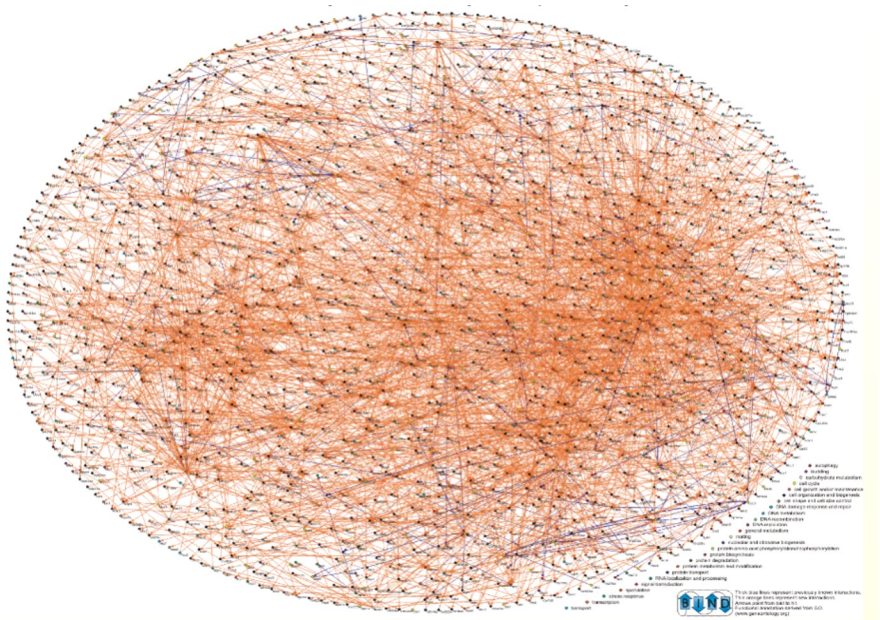
Click the image above or here to open this pathway in the Pathv

The replication of the genome and the subsequent segregation of chromosomes into dau
collectively known as the cell cycle. DNA replication is carried out during a discrete temp
and chromosome segregation occurs during a massive reorganization to cellular architec
major cell cycle events: G1 between mitosis and S-phase, and G2 between S-phase and m
cells can exit the cell cycle for a period and enter a quiescent state known as G0, or term
again, but undergo morphological development to carry out the wide variety of special
protein serine/threonine kinases known as the cyclin-dependent kinases (CDKs) controls
suggests, the activity of the catalytic subunit is dependent... Read more



Networks

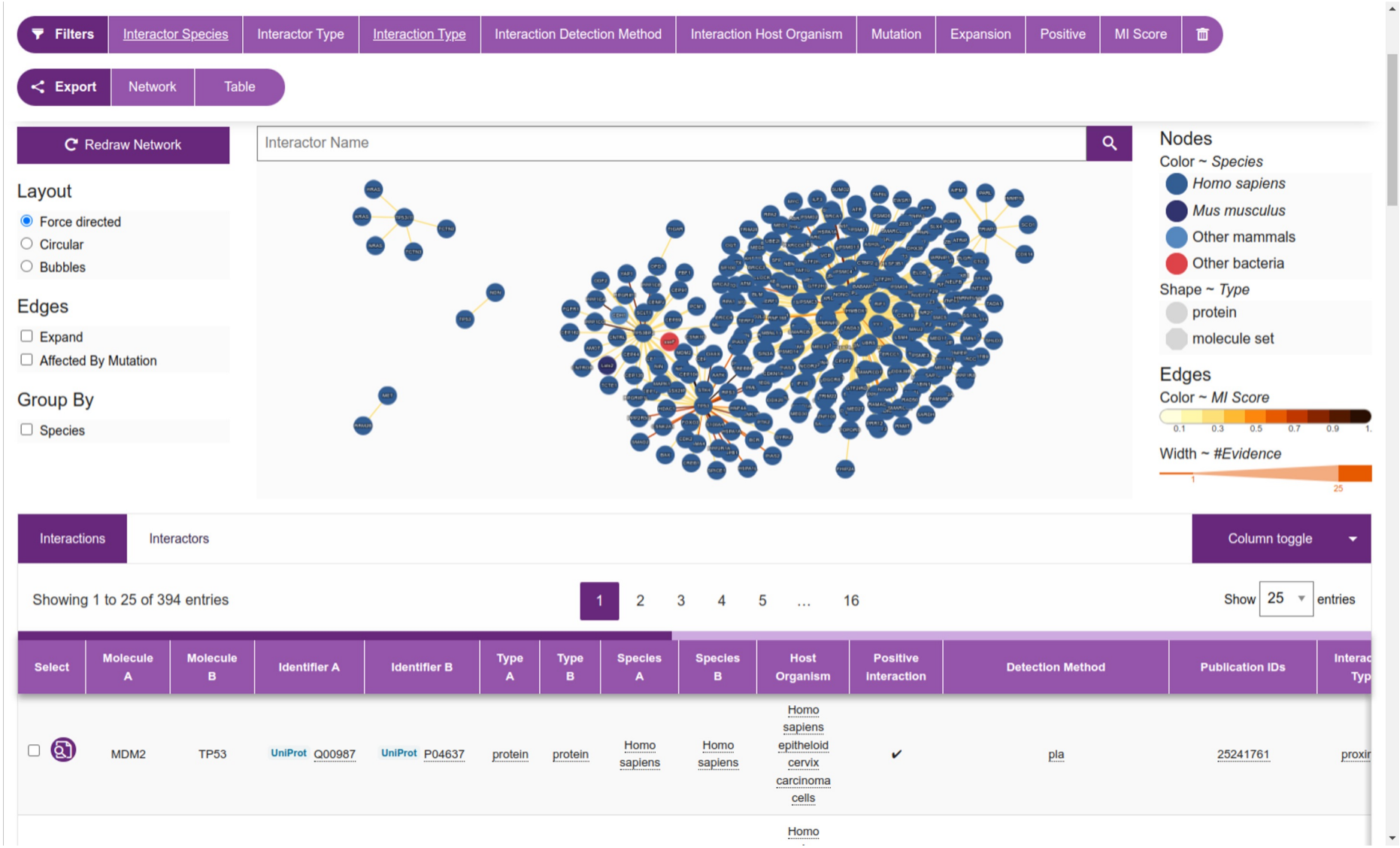
- Pathways capture only the “well understood” portion of biology.
- Networks cover less well understood relationships:
  - Genetic interactions
  - Physical interaction
  - Coexpression
  - GO term sharing
  - Adjacency in pathways



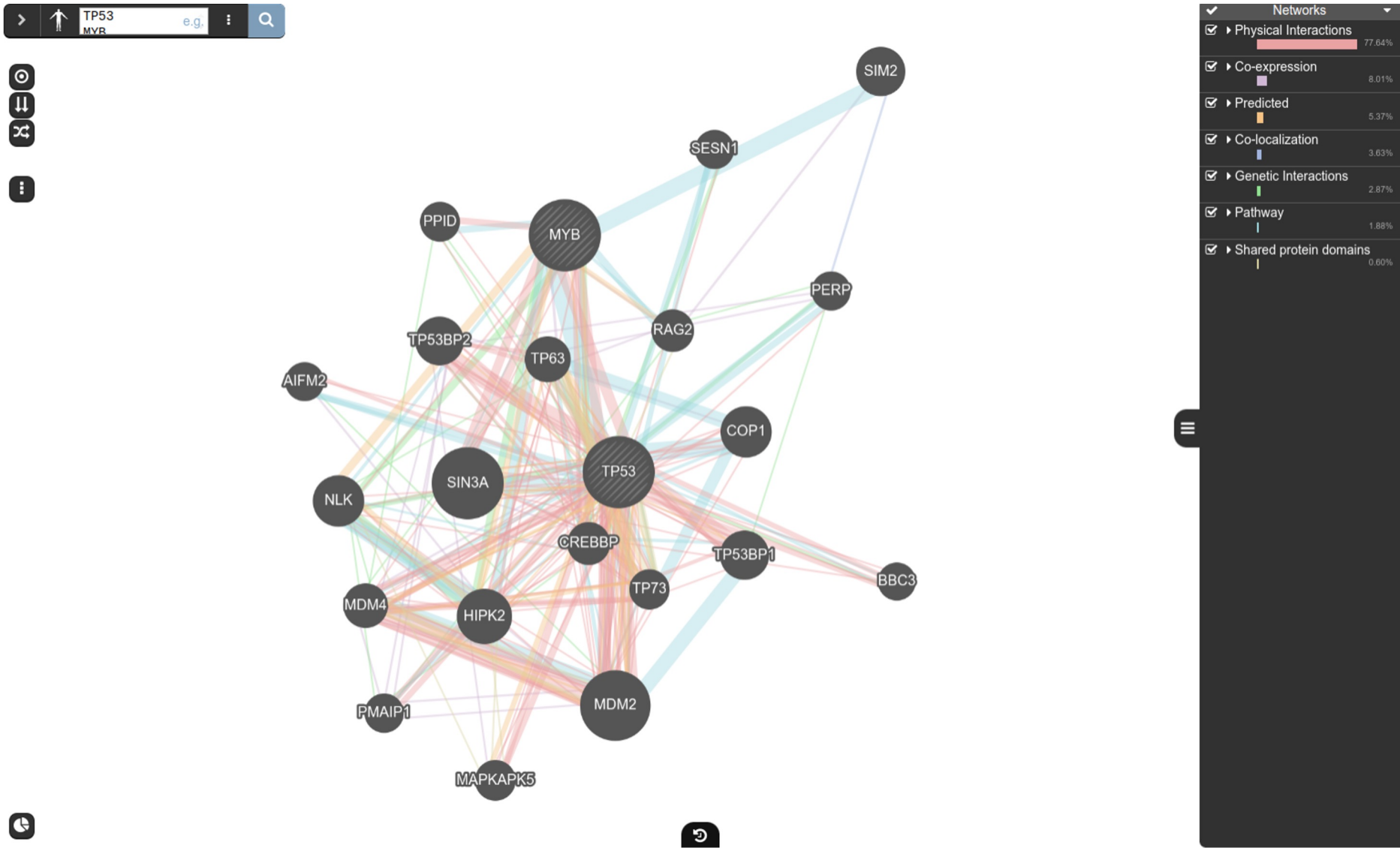
Network Databases

- Can be built automatically or via curation.
- More extensive coverage of biological systems.
- Relationships and underlying evidence more tentative.
- Popular sources of curated networks:
  - BioGRID – Curated physical and genetic interactions from literature; 89K genes & 2.1M interactions from 80 species (https://thebiogrid.org/)
  - IntAct – Curated interactions from literature; 143K interactors & 1.5M interactions from 9000 species. (https://www.ebi.ac.uk/intact/home)
  - GeneMANIA - Compendium of 2.8K gene association networks representing 167K genes and 660M interactions from 9 species

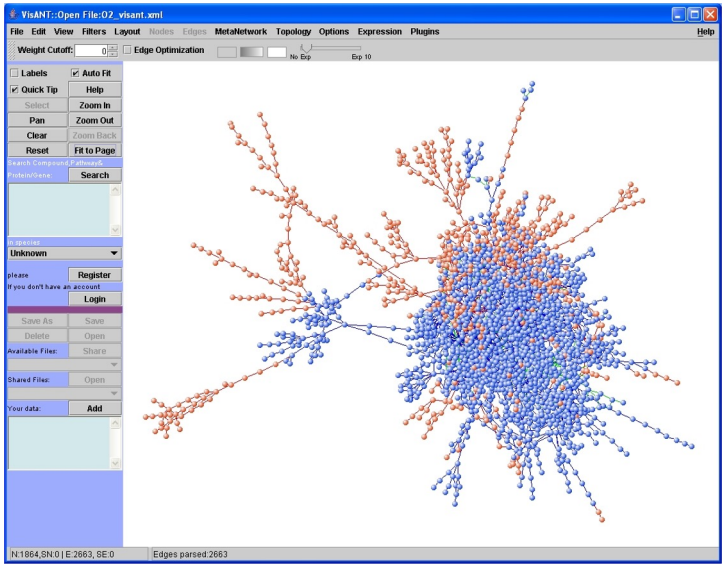
IntAct - Search for TP53



GeneMANIA

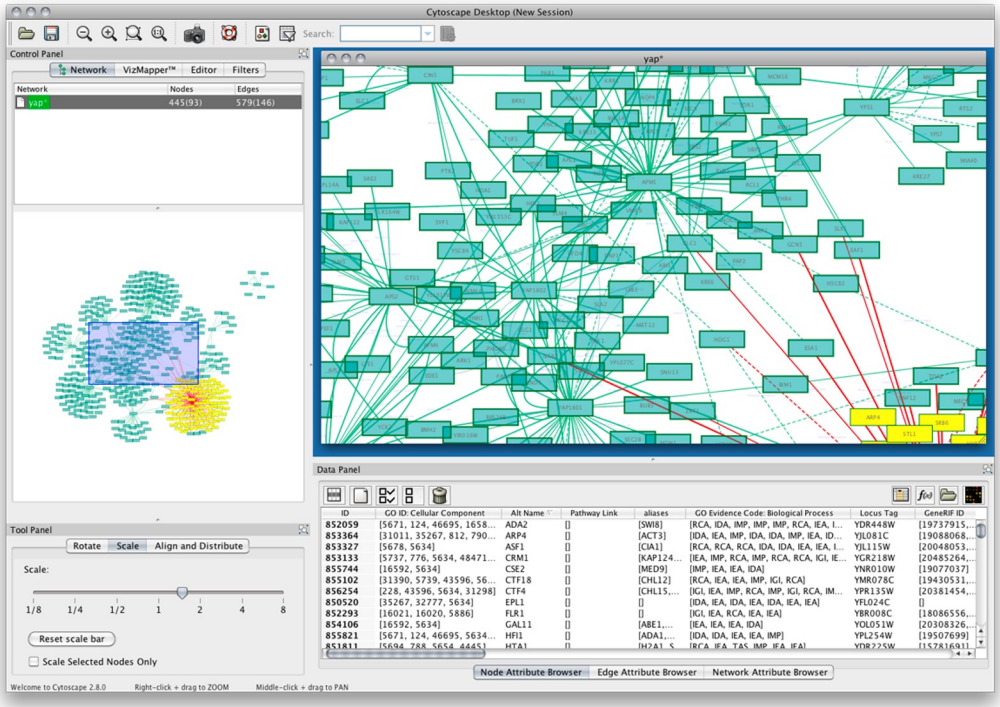

TP53
MYB
e.g.
SIM2
SESN1
PPID
MYB
PERP
TP53BP2
RAG2
TP63
AIFM2
COP1
SIN3A
TP53
NLK
CREBBP
TP53BP1
BBC3
TP73
MDM4
HIPK2
MDM2
PMAIP1
MAPKAPK5
Networks
Physical Interactions
77.64%
Co-expression
8.01%
Predicted
5.37%
Co-localization
3.63%
Genetic Interactions
2.87%
Pathway
1.88%
Shared protein domains
0.60%

Visualization and Analysis Tools for Biological Networks



VisANT

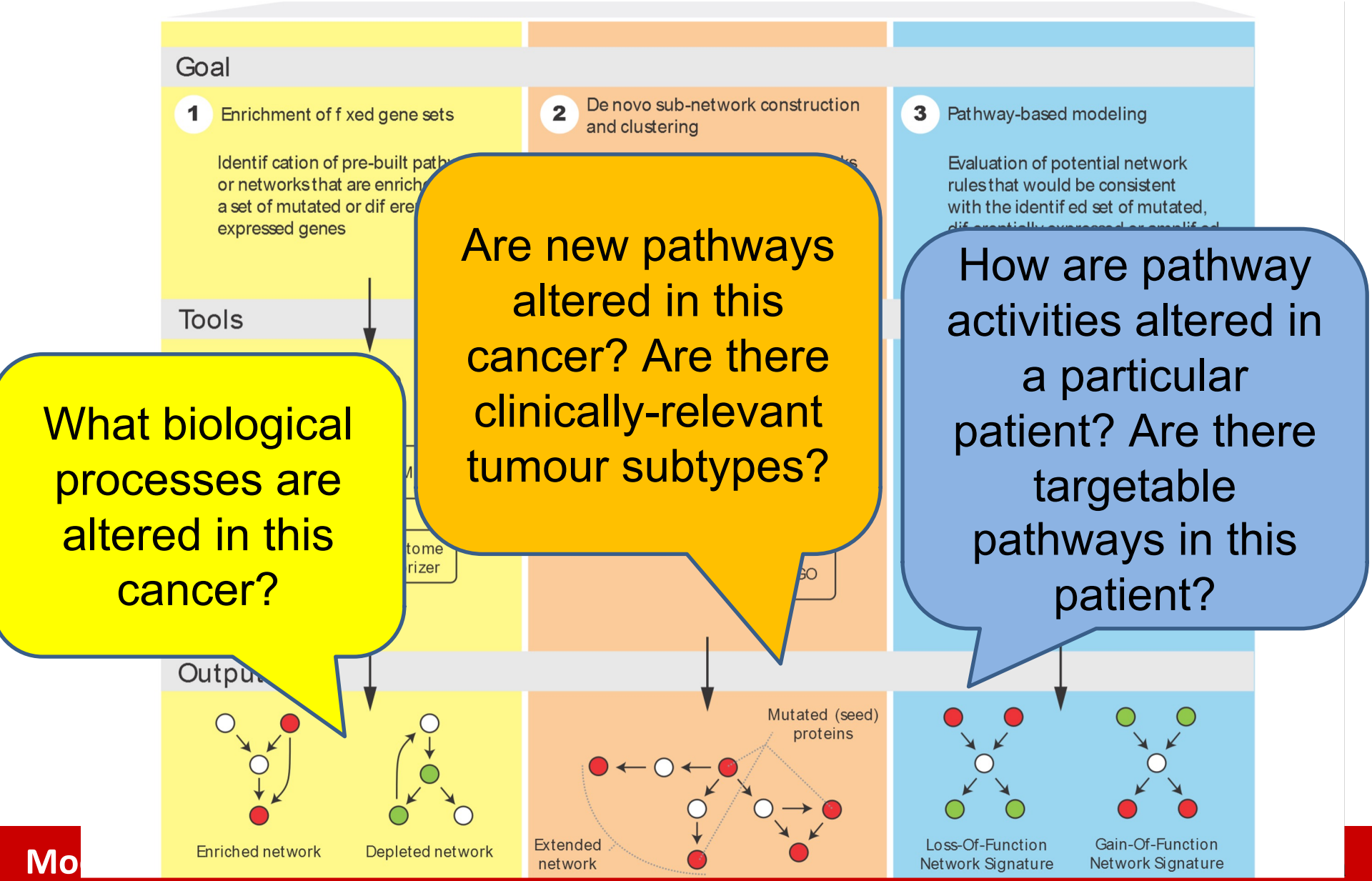
Web & desktop versions
(offline since Jan 2024)



Cytoscape

(Web & desktop versions)

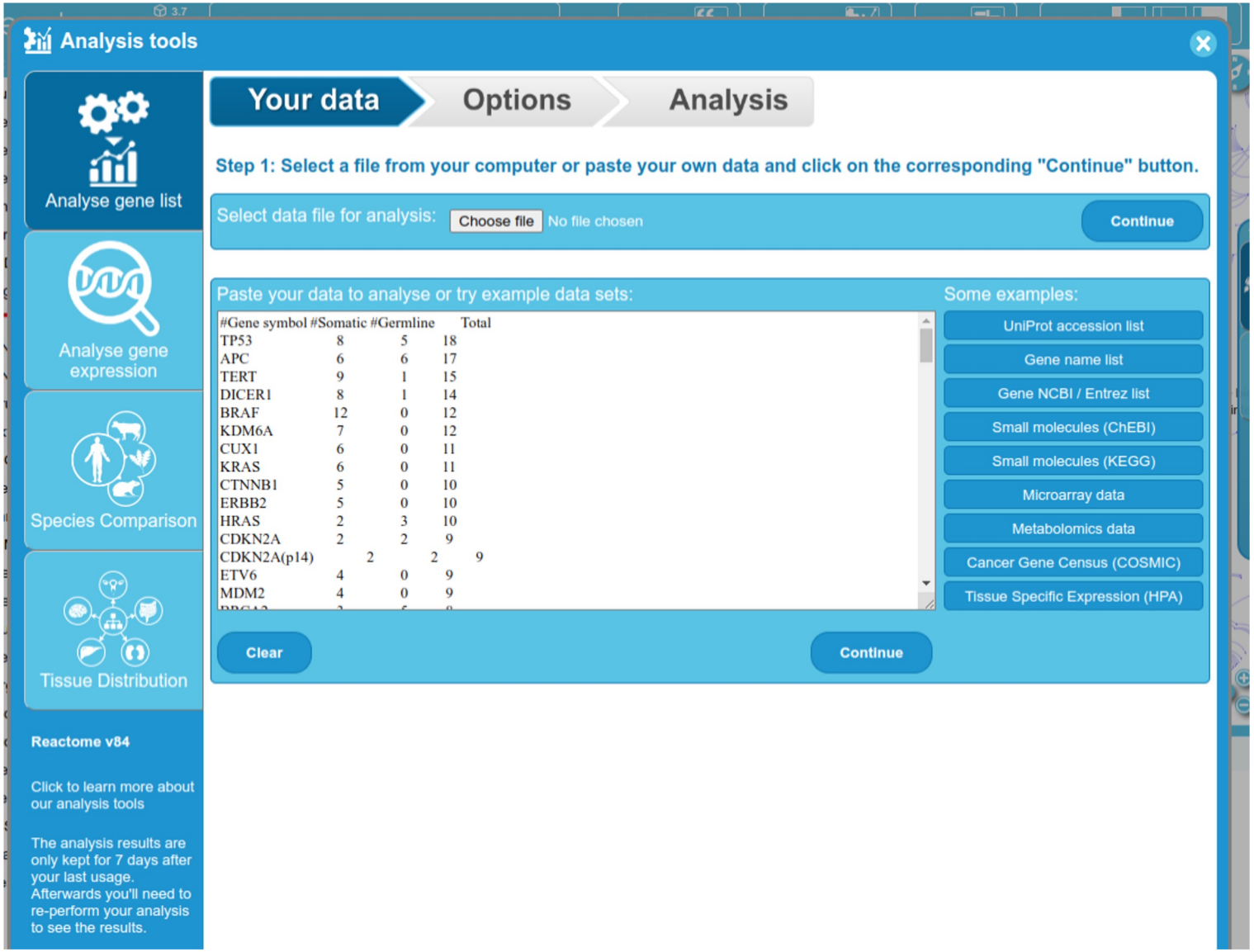
Pathway/Network Analysis



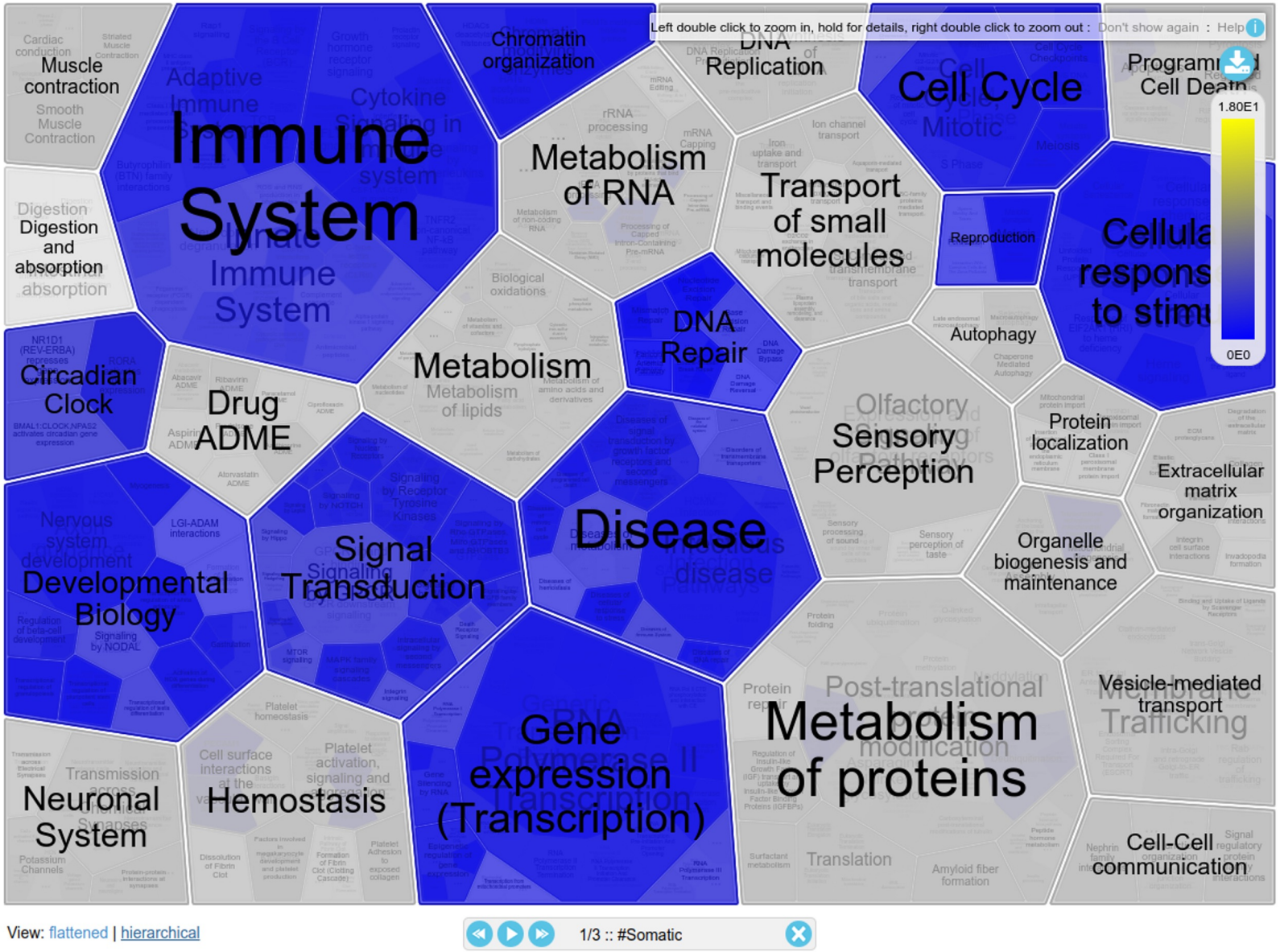
1) Enrichment of Fixed Gene Sets

- Covered in Module 2.
- Most popular form of pathway/network analysis.
  - Overrepresentation analysis vs functional class scoring.
- Advantages:
  - Easy to perform.
  - Many good end-user tools.
  - Statistical model well worked out.
- Disadvantages:
  - Many possible gene sets;
  - Gene sets are heavily overlapping;
  - “Bags of genes” obscure regulatory relationships among them.

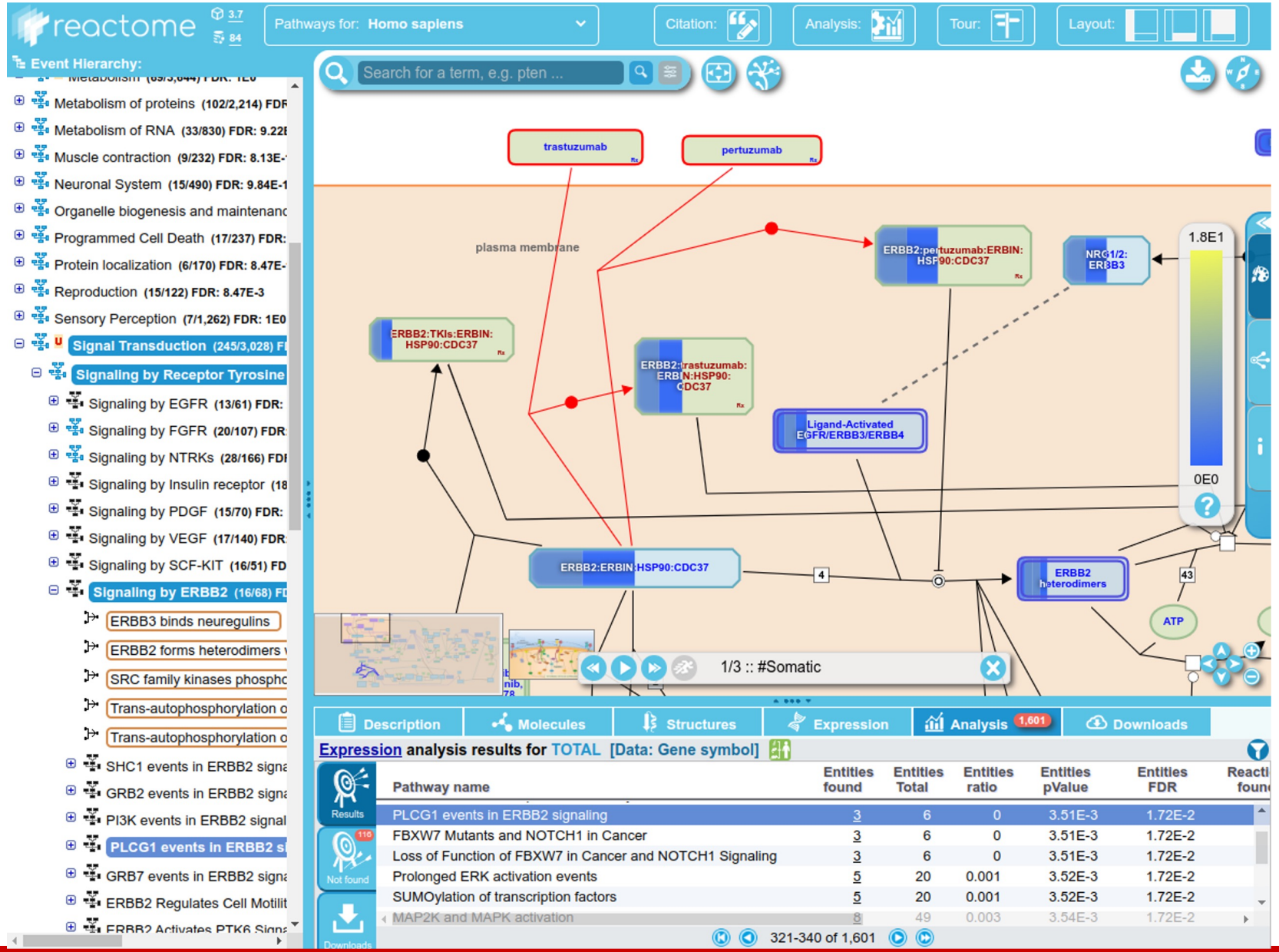
Reactome: Pathway Enrichment Analysis



Reactome: Pathway Enrichment Analysis



Reactome: Pathway Enrichment Analysis

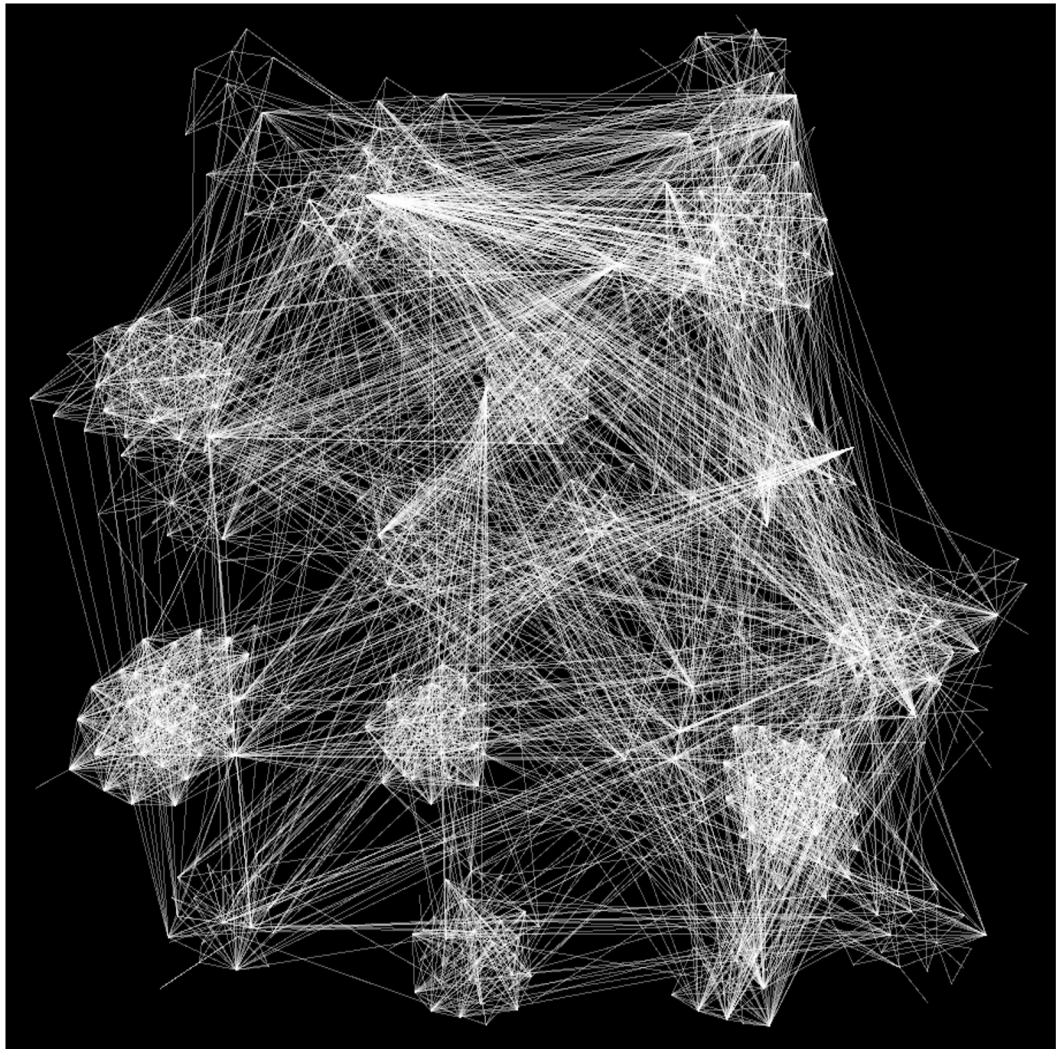


2) De Novo Subnetwork Construction & Clustering

- Apply list of altered {genes,proteins,RNAs} to a biological network.
- Identify “topologically unlikely” configurations.
  - E.g. a subset of the altered genes are closer to each other on the network than you would expect by chance.
- Extract clusters of these unlikely configurations.
- Annotate the clusters.

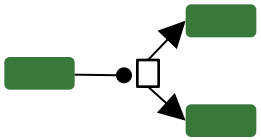
Reactome FI Network

- 12,441 Genes
- 291,172 FIs
- ~61% coverage of genome.
- False (+) rate < 1%
- False (-) rate ~80%



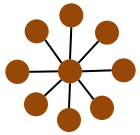
5% of network shown here

Reactome FI Network



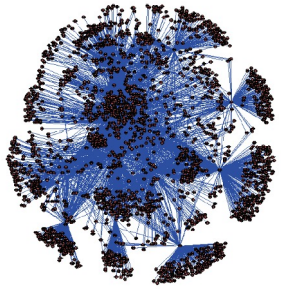
Curated Pathway Dbs

+



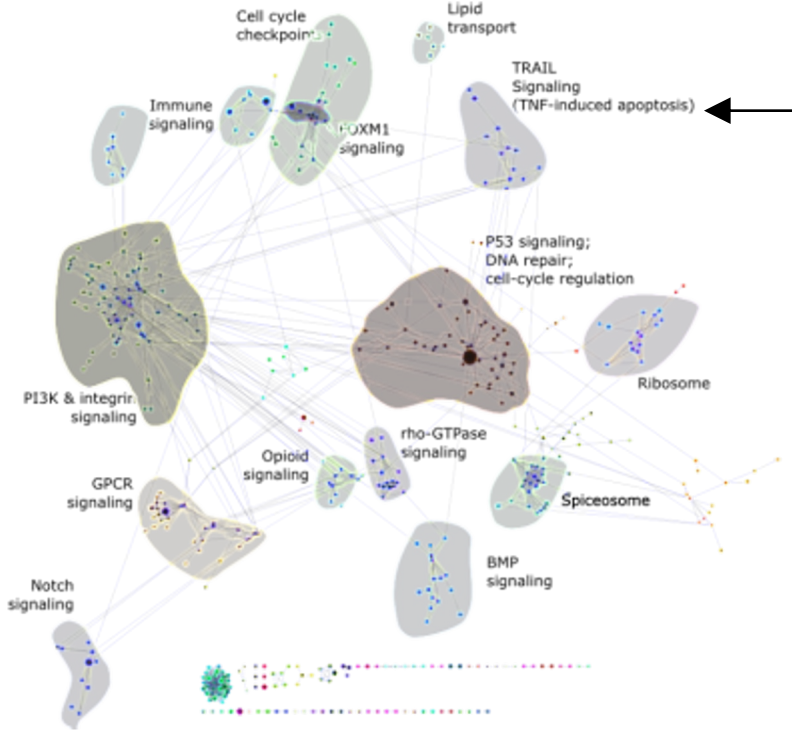
Uncurated Interaction Evidence

Machine Learning →

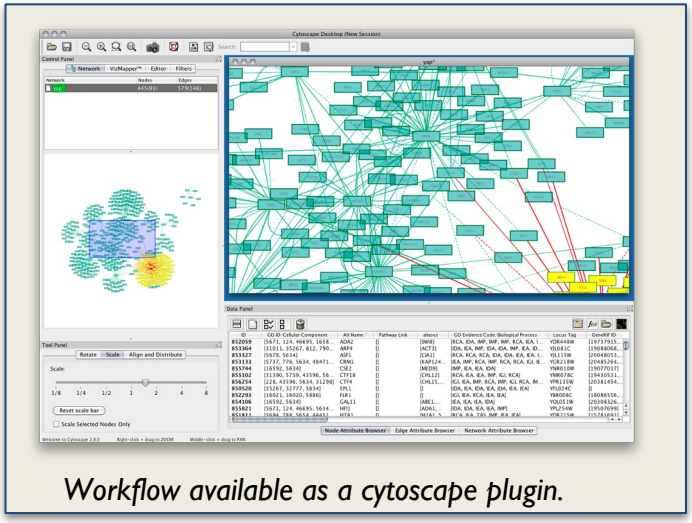


Reactome Functional Interaction Network
(~11,000 proteins; 270,000 interactions)

Extract and Cluster "Disease Genes"



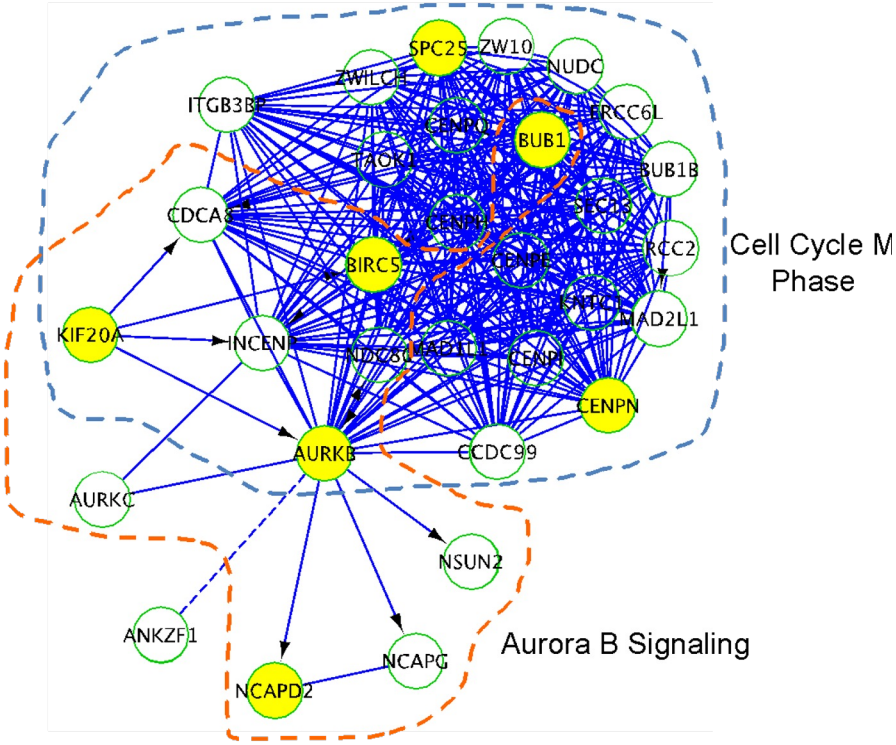
Disease "modules" (10-30)



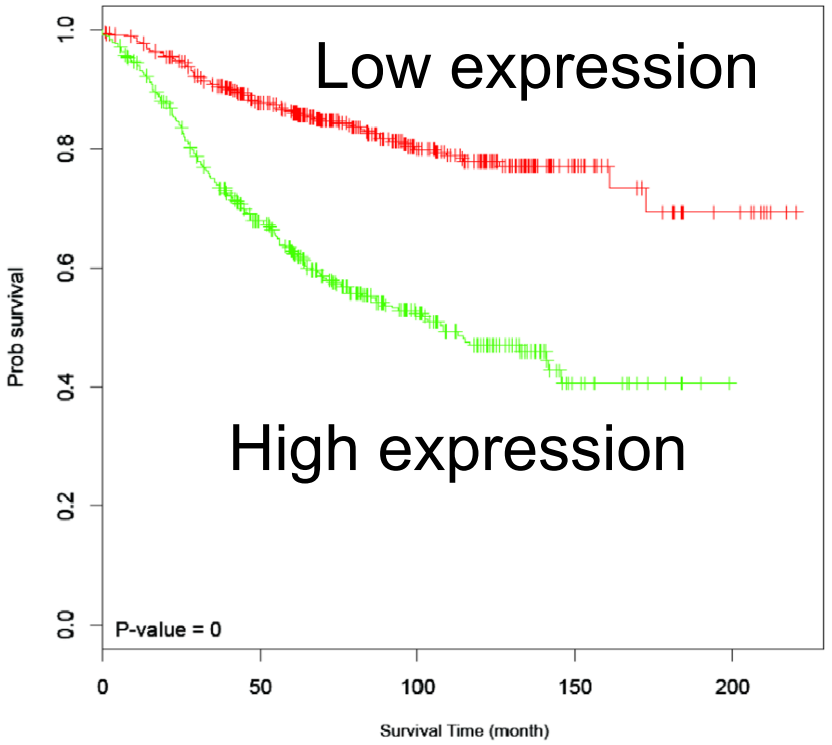
Workflow available as a cytoscape plugin.

A human functional protein interaction network and its application to cancer data analysis, [*Wu et al. 2010 Genome Biology*]

Module-Based Prognostic Biomarker in ER+ Breast Cancer



Measure levels of expression of the genes in this network module



A human functional protein interaction network and its application to cancer data analysis, [*Wu et al. 2010 Genome Biology*]

Popular Network Clustering Algorithms

- GeneMANIA
  - "Birds of a feather" principle.
  - Very useful for finding genes that are related to an experimentally defined set.
- HotNet
  - Finds "hot" clusters based on propagation of heat across metallic lattice.
  - Avoids ascertainment bias on unusually well-annotated genes.
- HyperModules Cytoscape App
  - Find network clusters that correlate with clinical characteristics.
- Reactome FI Network Cytoscape App
  - Offers multiple clustering and correlation algorithms (including HotNet, and survival correlation analysis)

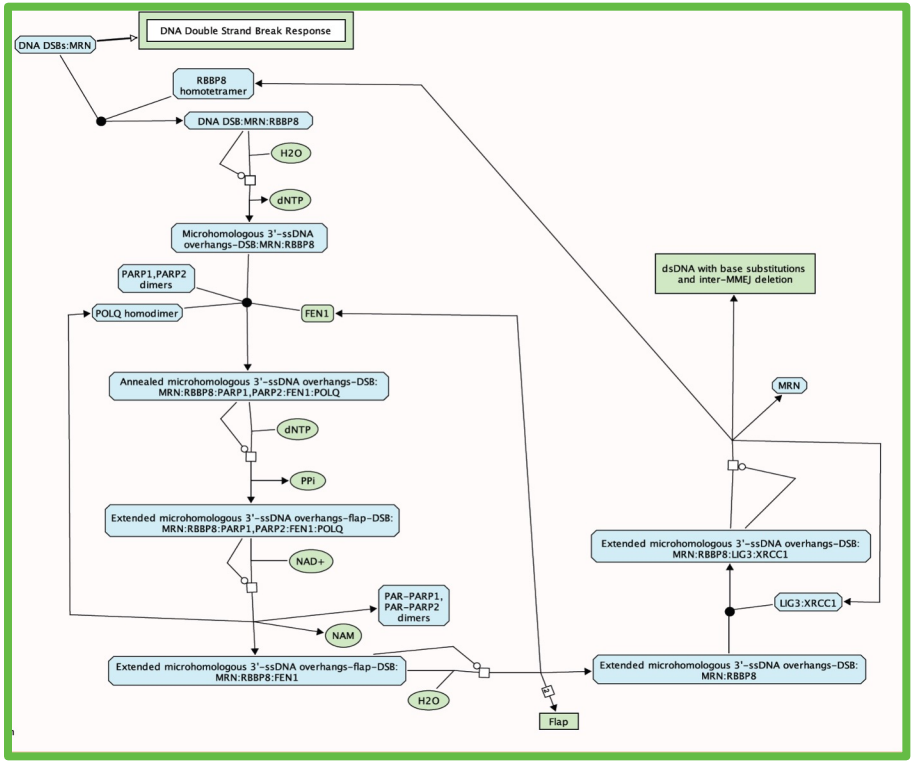
3) Pathway-Based Modeling

- Apply list of altered {genes,proteins,RNAs} to biological pathways.
- Preserve detailed biological relationships.
- Attempt to integrate multiple molecular alterations together to yield lists of altered pathway activities.
- Pathway modeling shades into Systems Biology

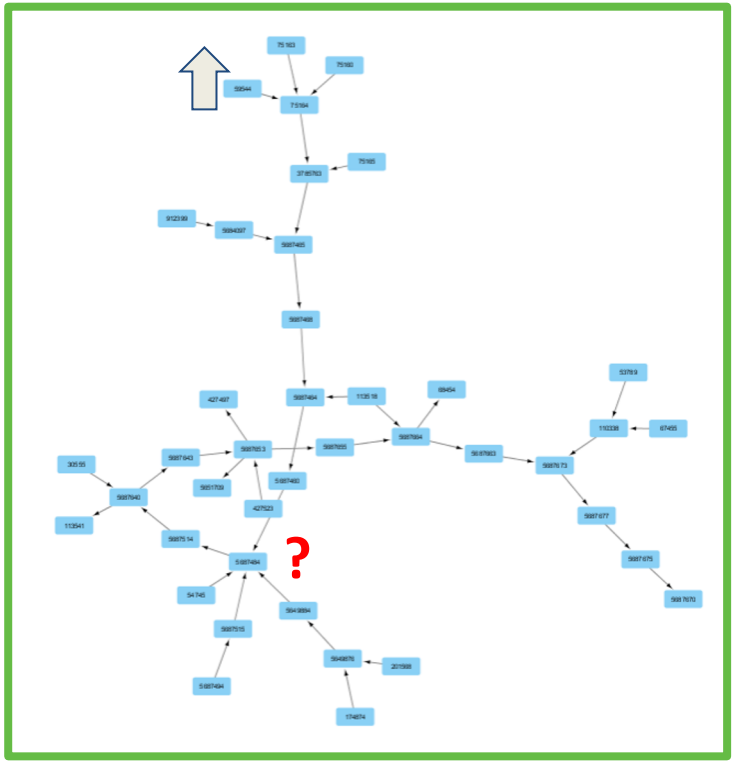
Types of Pathway-Based Modeling

- Partial differential equations, e.g. CellNetAnalyzer
  - Mostly suited for biochemical systems (metabolomics)
- Network flow models, e.g. NetPhorest
  - Mostly suited for kinase cascades (phosphorylation info)
- Transcriptional regulatory network-based reconstruction methods, e.g. ARACNe
- Logic Graphs and probabilistic graph models (PGMs)
  - Capture the logic of a pathway without needing rate/binding constants.
- Generative AI Models

Boolean Network Inference



Pathway View



Logic Graph View

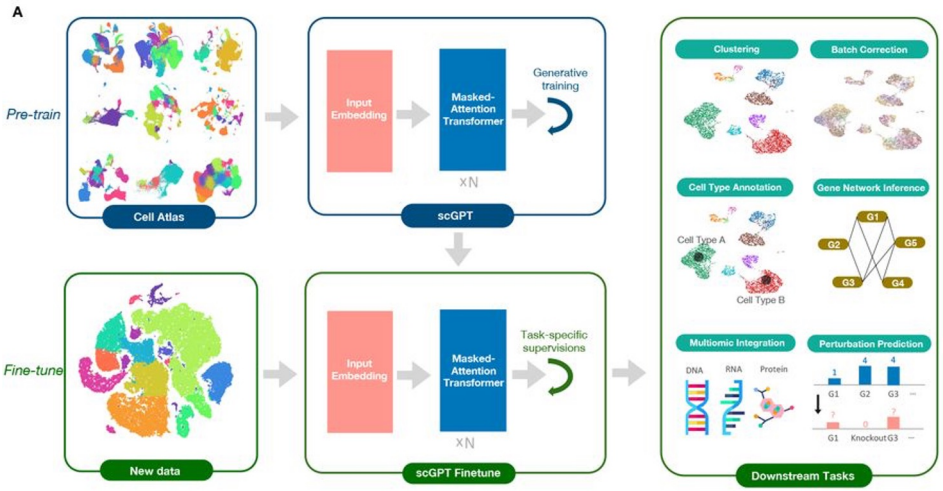
Generative AI Network Models

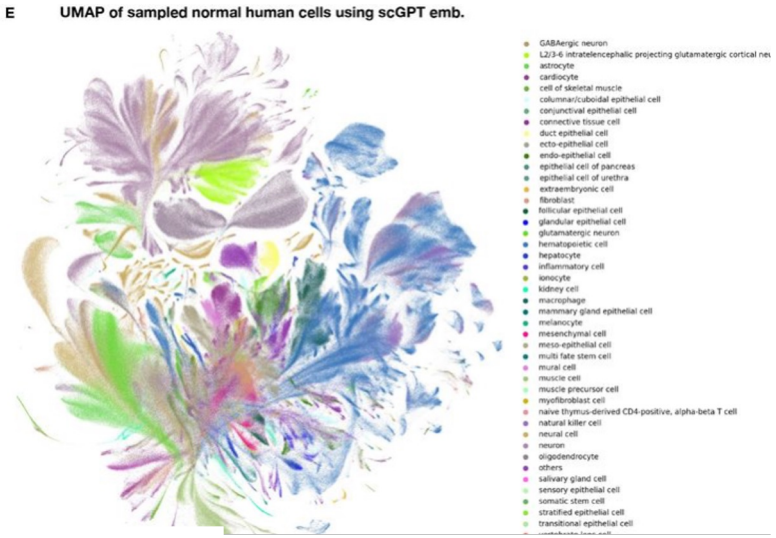
Large Language Models (GPT-3) are trained to predict masked text:

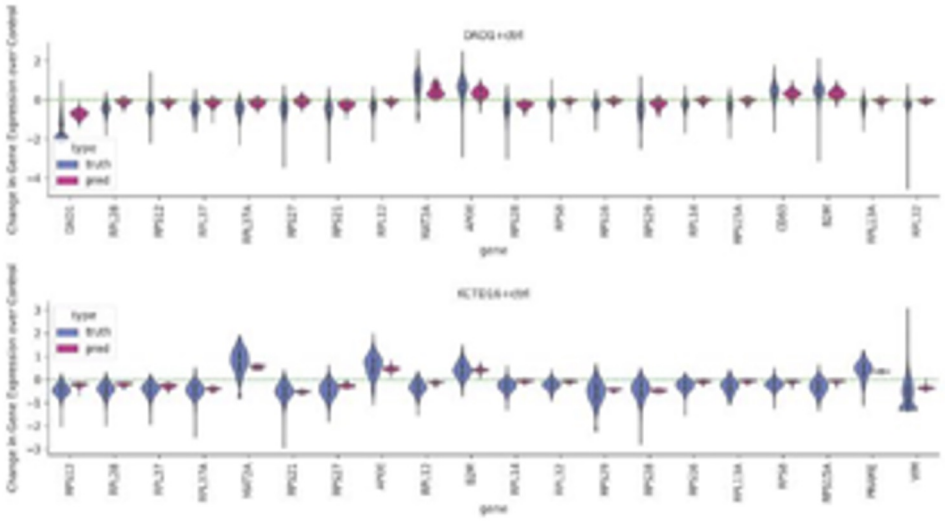
`The quick brown` **?** `jumped over the lazy dog.`

Generative pathway models are trained to predict gene network perturbations:

scGPT - Trained on scRNAseq of 33M Cells

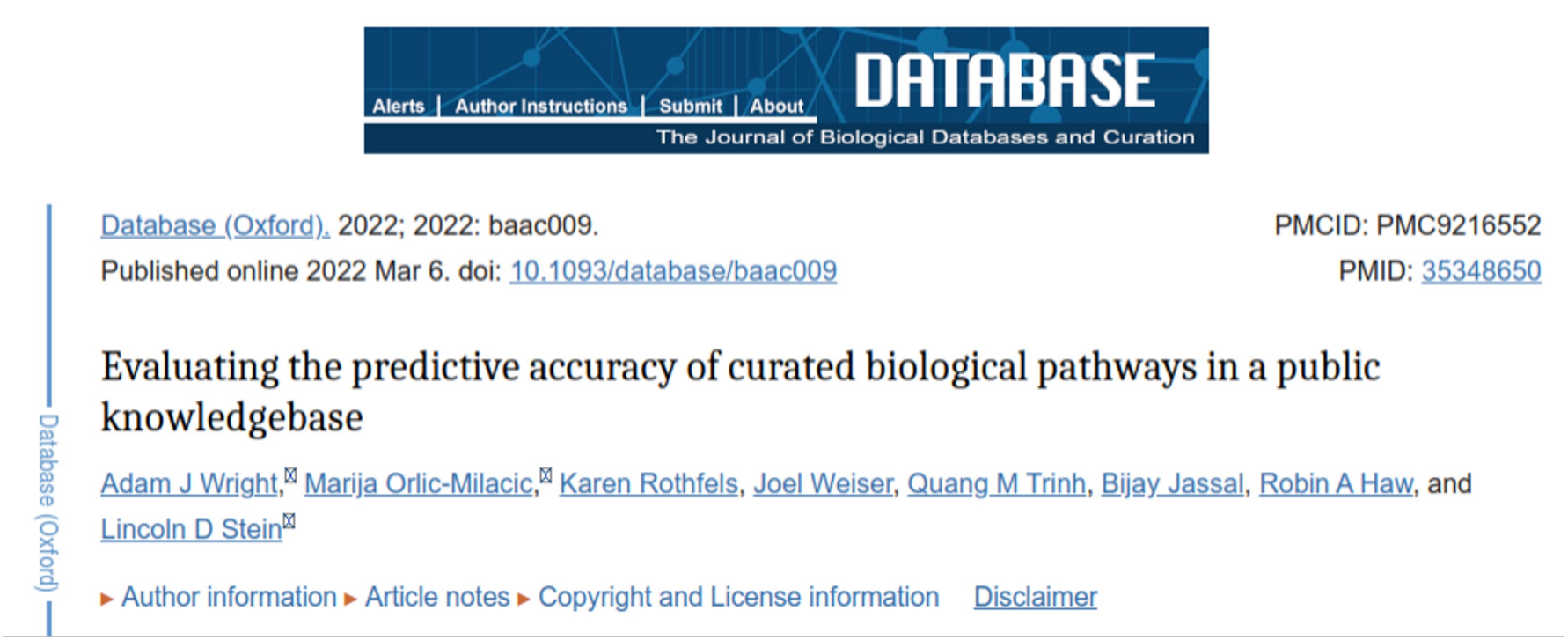






Cui et al 2023
Nature Methods 10.1038/s41592-024-02201-0

Can you use pathways to predict biology?



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DATABASE
The Journal of Biological Databases and Curation

Database (Oxford), 2022; 2022: baac009.
Published online 2022 Mar 6. doi: 10.1093/database/baac009
PMCID: PMC9216552
PMID: 35348650

Evaluating the predictive accuracy of curated biological pathways in a public knowledgebase

Adam J Wright,[✉] Marija Orlic-Milacic,[✉] Karen Rothfels, Joel Weiser, Quang M Trinh, Bijay Jassal, Robin A Haw, and Lincoln D Stein[✉]

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Database (Oxford)

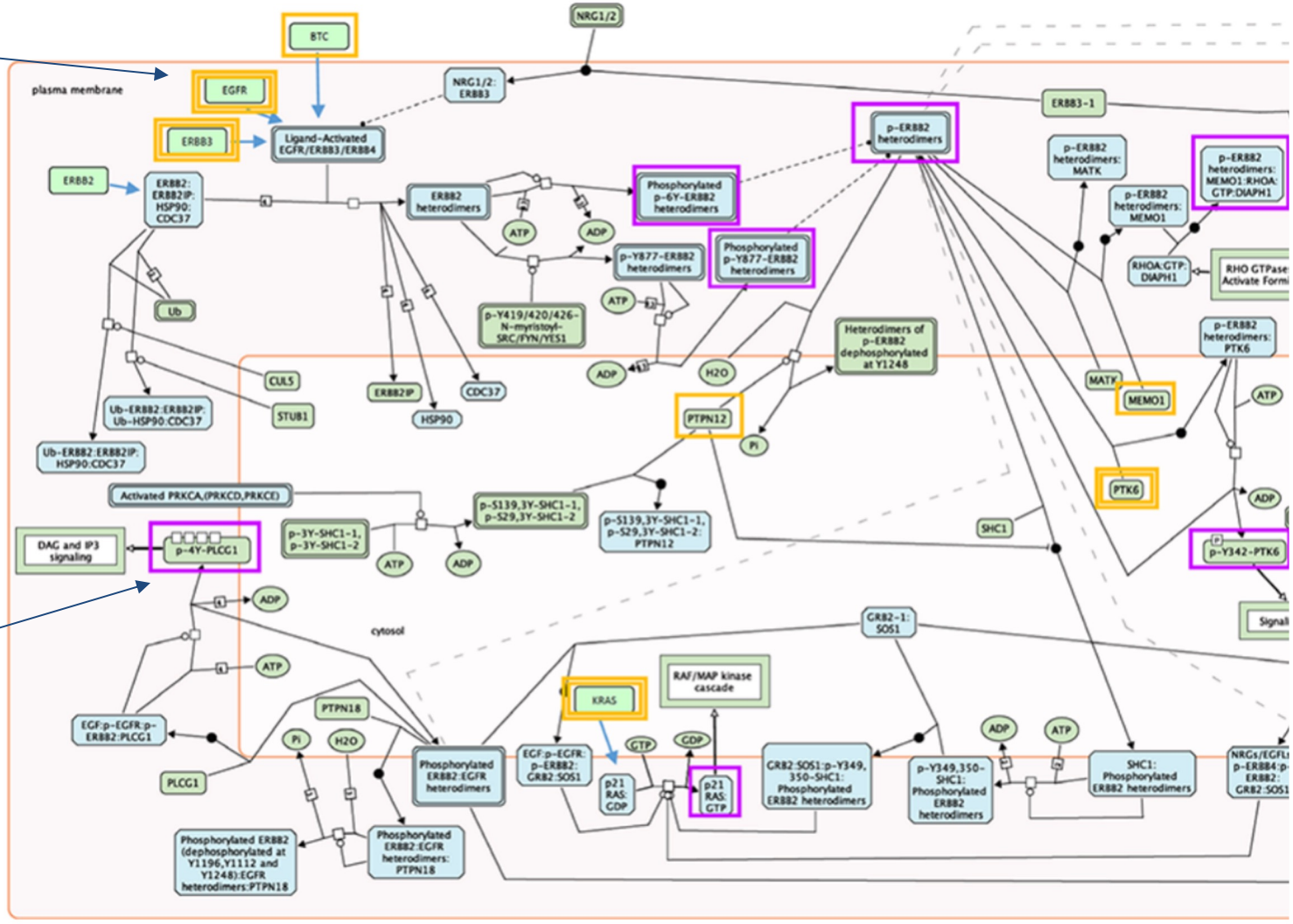
- How well can we predict the downstream effects of knocking up/down a gene using:
  - Experts gazing at pathway diagrams?
  - A graph-based inference algorithm?

Step 1: Gather Input/Output Pairs

Root Input

Key Output

4,968 pairs collected from 10 cancer-related pathways

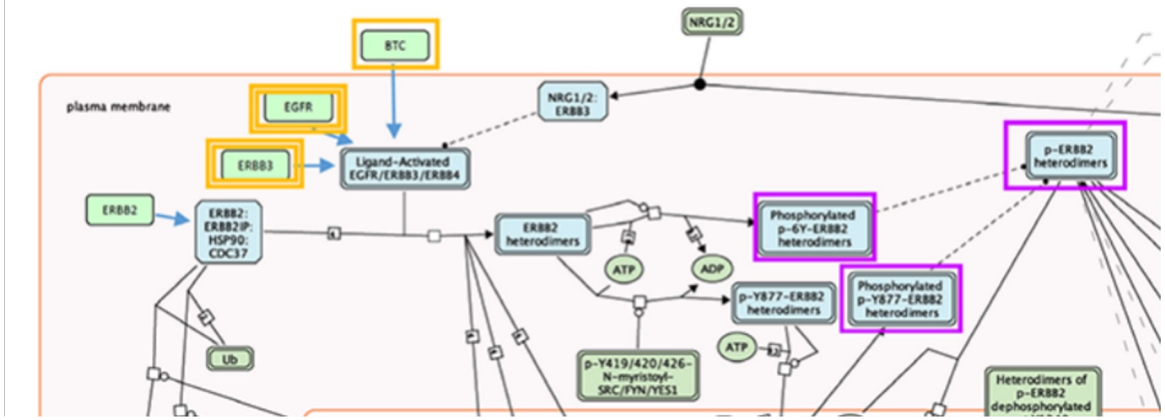


Step 2: Collect Empirical Results

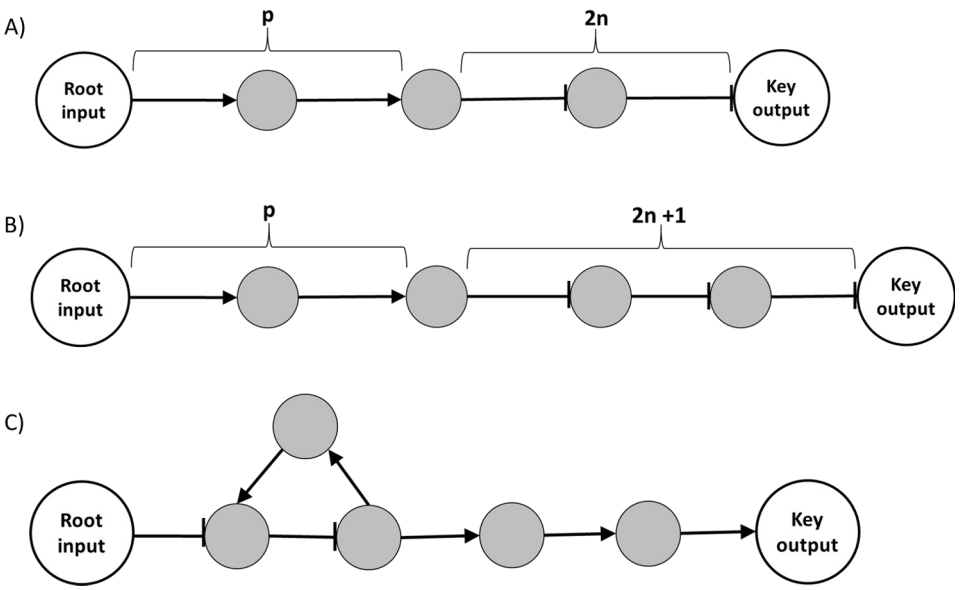
- Literature searches for functional genomics experiments in which key input was perturbed and effect on key output measured.
- 531 papers found, reporting 847 tested cases.

Step 3: Predict Downstream Effects

1. Curators gaze at pathway diagram and apply logic rules to predict effect of perturbation.



2. Apply a boolean inference algorithm, MP-BioPath, to predict effect of perturbation.

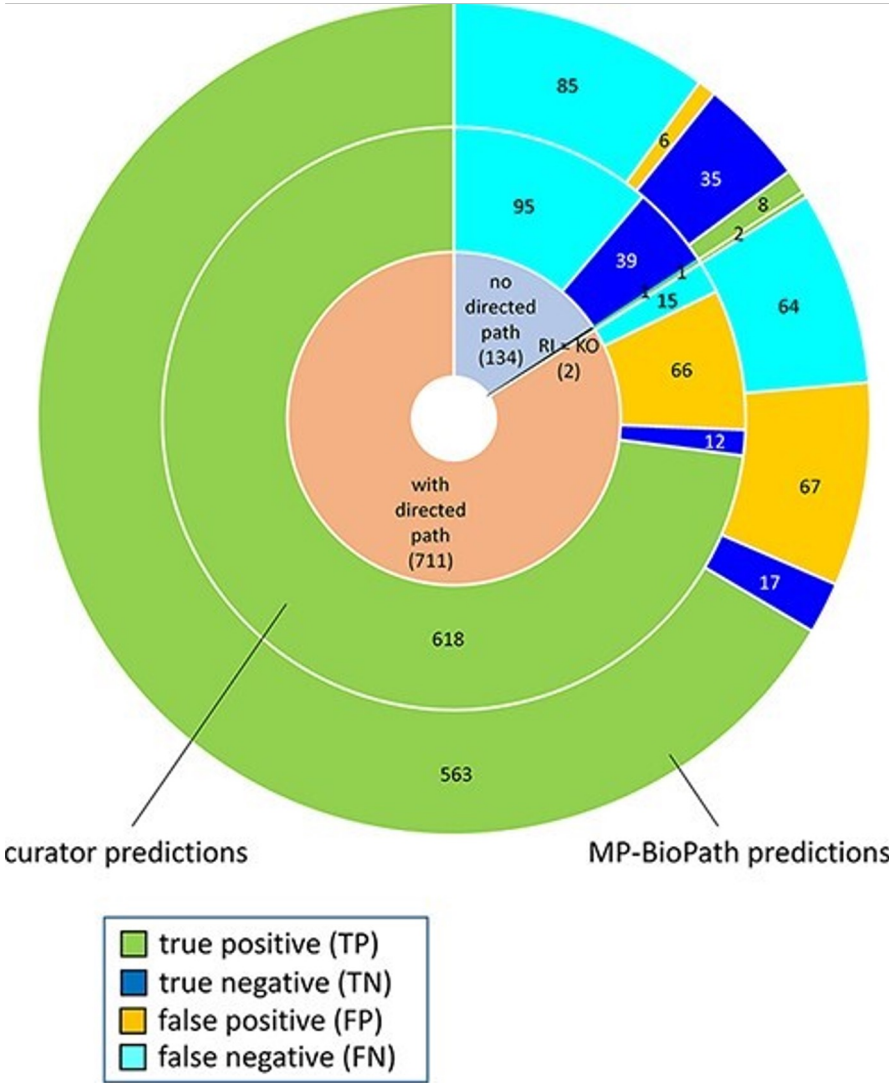


Step 4: Compare Predictions to Empirical

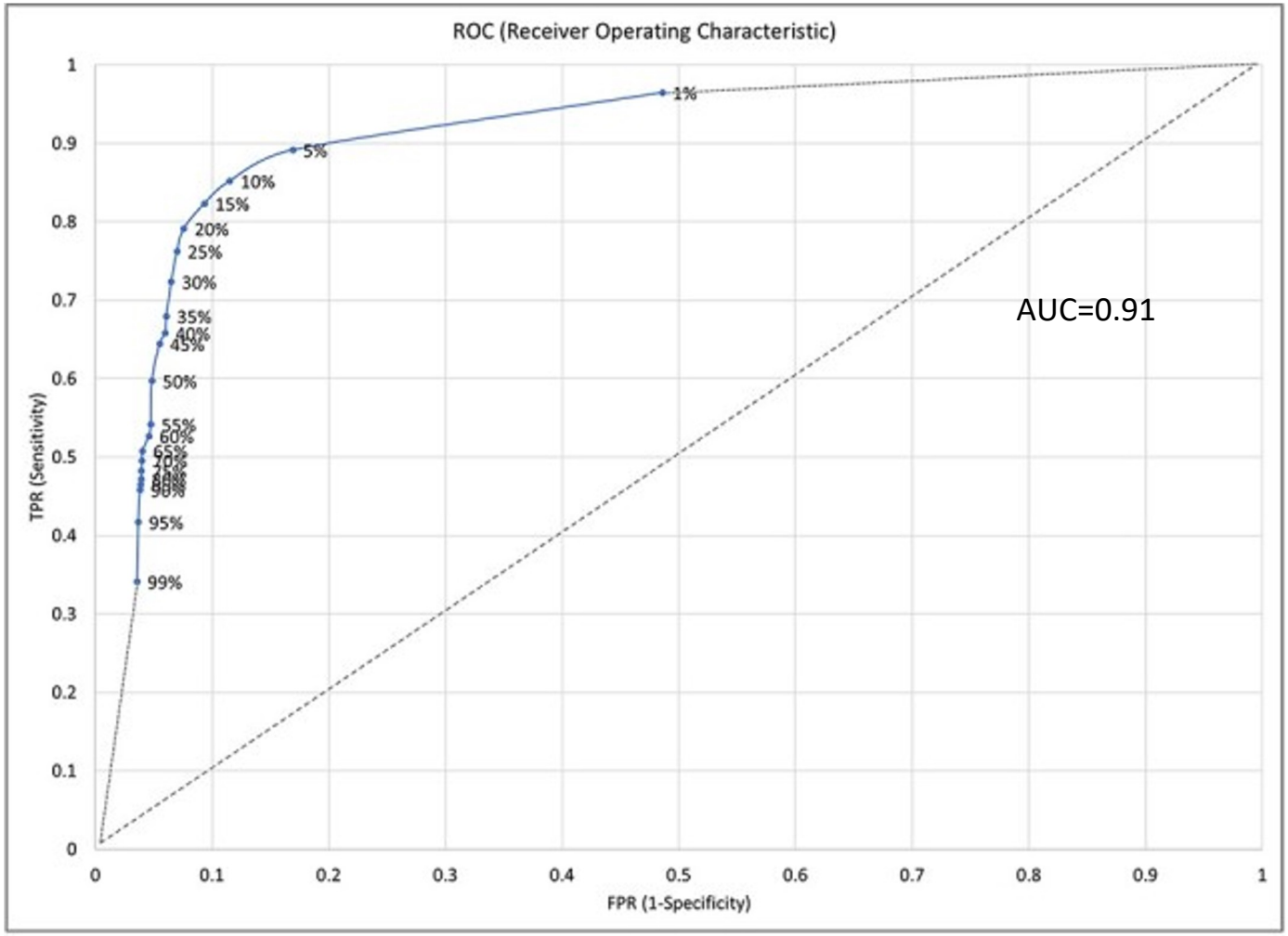
Curator Predictive Accuracy: **81%**
MP-BioPath Predictive Accuracy: **75%**
Random Guessing: **33%**

Largest source of error were false negatives due to missing elements of the pathway.

Largest source of false positives was direction of perturbation predicted incorrectly, also related to missing elements.



Excellent Concordance between Algorithm's & Humans' Predictions



Conclusions & Takeaways

- Pathway analysis allows discovery of biological processes hidden in large-scale data sets.
- Many databases and tools to choose from.
- Curated pathway databases now reaching levels of completeness that allow for accurate prediction of perturbations.
- Field is ripe for machine learning approaches.

Pathway/Network Database URLs

- BioGRID
  - http:// www.thebiogrid.org
- IntAct
  - http://www.ebi.ac.uk/intact/
- KEGG
  - http:// www.genome.jp/kegg
- Reactome
  - http:// www.reactome.org

De novo network construction & clustering

- GeneMANIA
  - http://www.genemania.org
- HotNet
  - http://compbio.cs.brown.edu/projects/hotnet/
- HyperModules
  - http://apps.cytoscape.org/apps/hypermodules
- Reactome Cytoscape FIViz App
  - http://apps.cytoscape.org/apps/reactomefis

Pathway Modeling

- CellNetAnalyzer
  - http://www.ebi.ac.uk/research/saez-rodriguez/software
- NetPhorest/NetworKIN
  - http://netphorest.info, http://networkin.info
- ARACNe
  - http://wiki.c2b2.columbia.edu/califanolab/index.php/Software/ARACNE
- scGPT
  - https://github.com/bowang-lab/scGPT
- Pathway Prediction Evaluation Paper
    - https://www.ncbi.nlm.nih.gov/pmc/articles/PMC9216552/

We are on a Coffee Break & Networking Session