



**XiaLab Analytics**

Empowering researchers through trainings, tools and AI

<https://www.xialab.ca> • [contact@xialab.ca](mailto:contact@xialab.ca)

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# **Omics Data Science Training Course**

Winter 2024

# XiaLab Team



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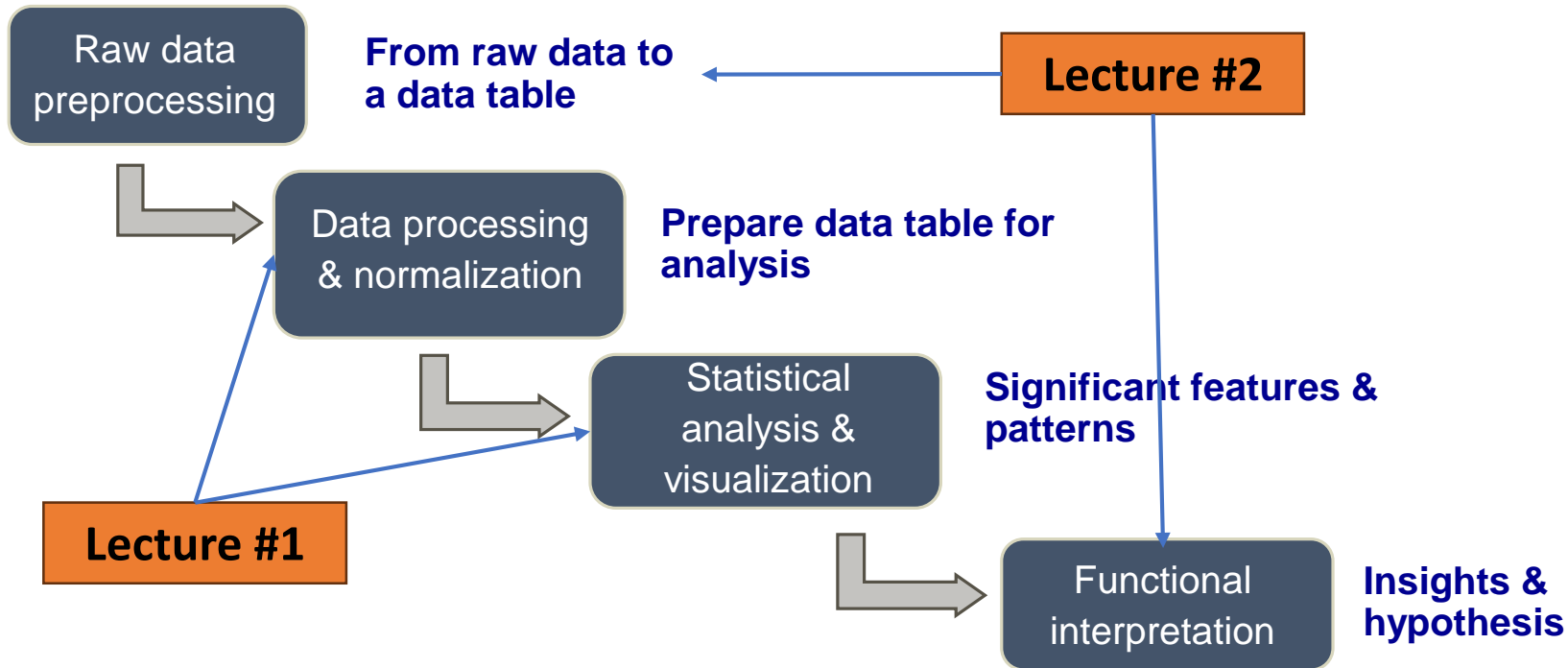
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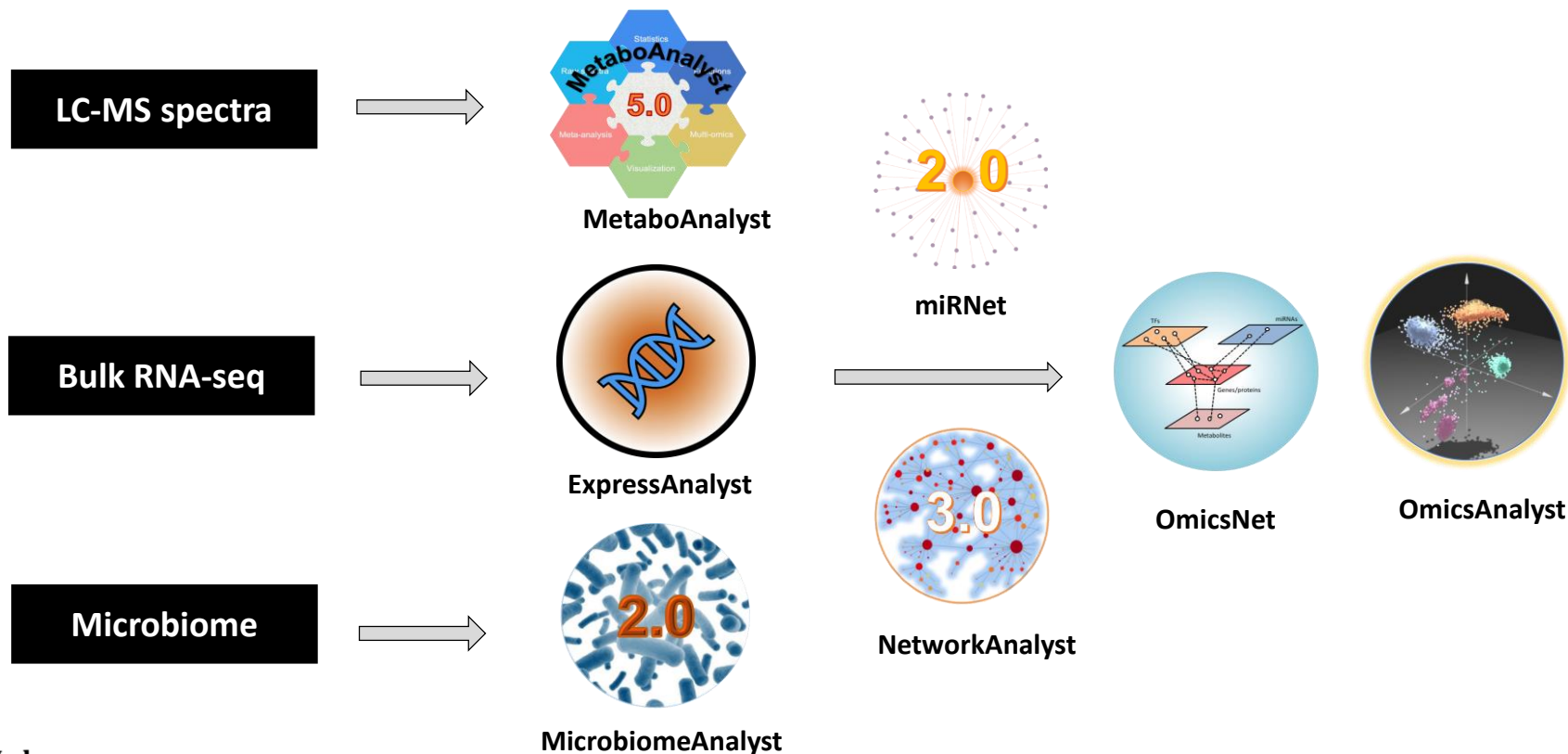
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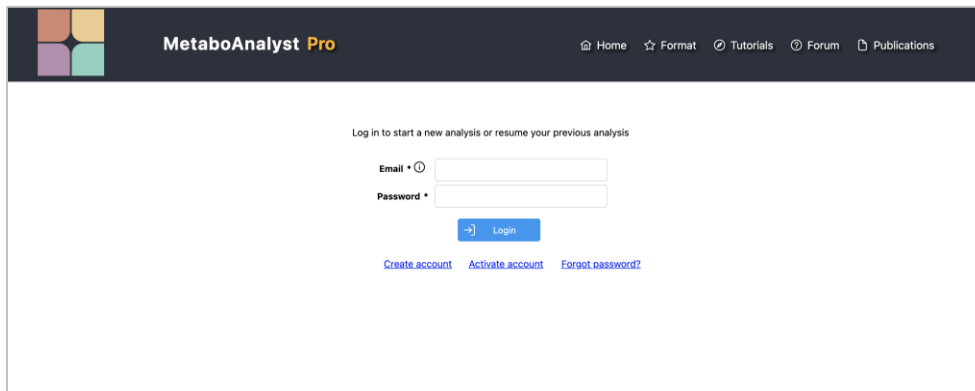
# Two foundation lectures (**prerequisite**)



# Raw data → statistics → networks → functions



# More on the “pro” tools



MetaboAnalyst Pro

Home Format Tutorials Forum Publications

Log in to start a new analysis or resume your previous analysis

Email •

Password •

[Create account](#) [Activate account](#) [Forgot password?](#)

Community version  
~ 5000 users / day  
~ 100s concurrent users

A total of six tools have their “pro” versions









- <https://pro.metaboanalyst.ca>
- <https://pro.microbiomeanalyst.ca>
- <https://pro.expressanalyst.ca>
- <https://pro.omicsnet.ca>
- <https://pro.omicsanalyst.ca>
- <https://pro.mirnet.ca>

1. Dedicated computing
2. Live report & project management
3. More stable
4. Prioritized support



# Live Report & Project Management

[Home](#) > [Upload](#) > [Data check](#) > [Normalization](#) > [Statistics](#) > [Correlations](#)



[<< Project Home](#)

[Edit](#) [HTML](#) [PDF](#)

[>> Slide Deck](#)

## Metabolomic Data Analysis with MetaboAnalyst Pro

**Completed:** Thu Jan 4 12:01:48 2024

### 1. Overview

The *Statistics [one factor]* module is designed for general-purpose exploratory analysis of metabolomics data with simple study design with one experimental factor (two group or multiple group). A variety of statistics, visualization and machine learning methods are supported. It accepts a data table from targeted peak list files from NMR or complex study design with

Coming: PDF report, Slide deck, AI co-pilot

### 2. Data Processing

Data processing includes four steps: *data integrity check*, *missing value estimation*, *data filtering* and *data normalization*. Together, these steps enable comprehensive data treatments to produce a clean, high-quality table suitable for downstream statistical or machine learning algorithms. Some steps are optional depending on your data type. For instance, missing value estimation and data filtering are primarily for untargeted metabolomics data which typically contain a lot of missing values and noises.



# Our Resources

## Recordings & slides

- <https://www.xialab.ca> under the “Training” tab within 3 days after lecture

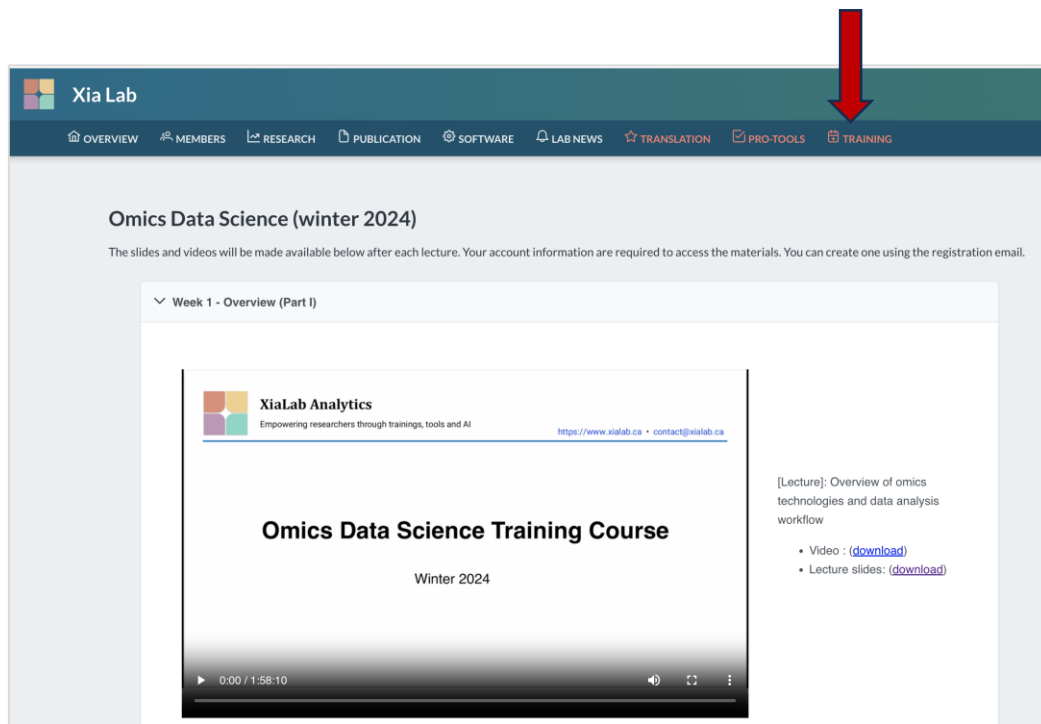
- **Faster with Firefox**

## Community tool:

- <https://www.####.ca>

## Pro Tools:

- <https://pro.####.ca>
  - ❖ You will be assigned to one of the cloud nodes



# Our Syllabus

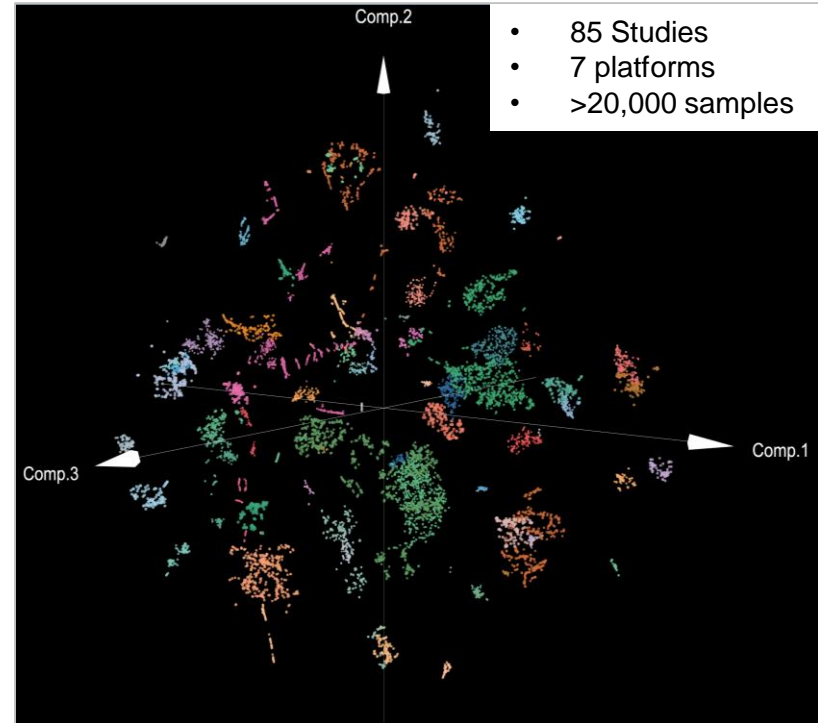
Topic	Date	Lecture	Lab
Omics Data Science Foundations	Jan. 6	Omics data processing, statistics and visualization	--
	Jan. 13	From raw data to functional insights	--
Transcriptomics	Jan. 20	Gene expression data analysis (part I)	ExpressAnalyst & NetworkAnalyst
	Jan. 27	Gene expression data analysis (part II)	ExpressAnalyst & Seq2Fun
Biological Networks	Feb. 3	Biological network analysis & gene regulatory networks	NetworkAnalyst & miRNet
Proteomics & Biomarker	Feb. 10	Proteomics & biomarker analysis	ExpressAnalyst & MetaboAnalyst
Metabolomics	Feb. 17	Targeted metabolomics data analysis	MetaboAnalyst
	Feb. 24	LC-MS untargeted metabolomics data analysis	MetaboAnalyst
Microbiomics	Mar. 2	Marker gene data analysis	MicrobiomeAnalyst
	Mar. 9	Shotgun metagenomics data analysis	MicrobiomeAnalyst
Multi-omics	Mar. 16	Knowledge-driven multi-omics integration	OmicsNet
	Mar. 23	Data-driven multi-omics integration	OmicsAnalyst



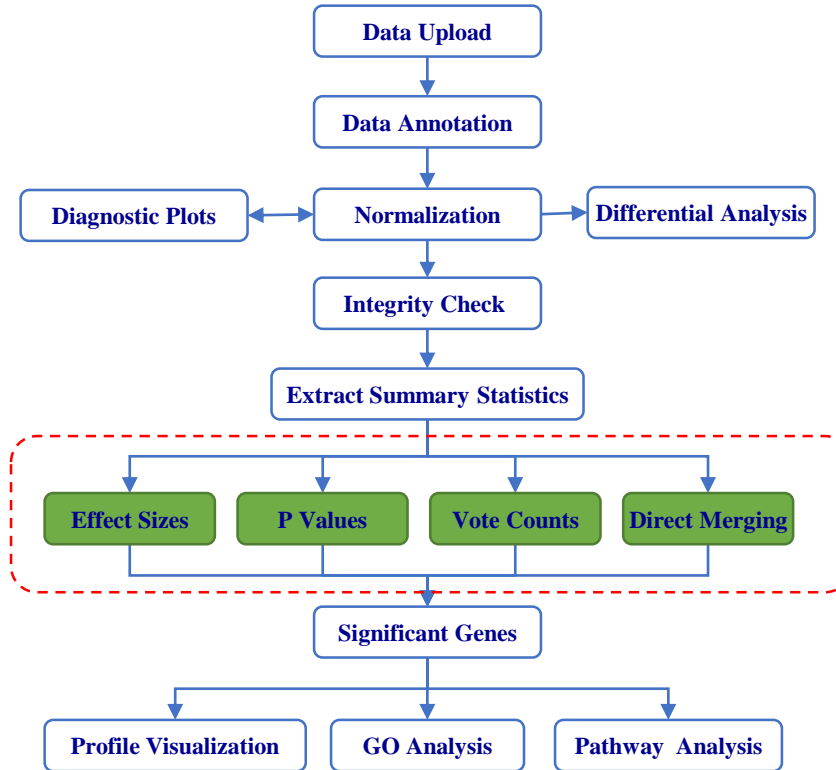
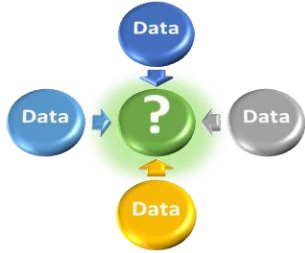


# Recap: meta-analysis

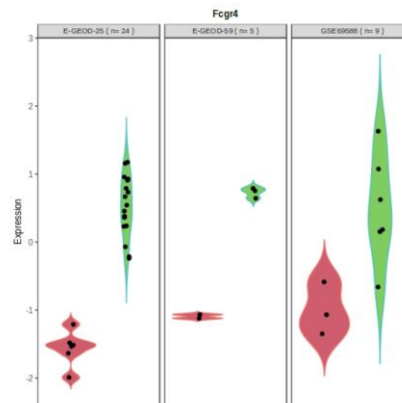
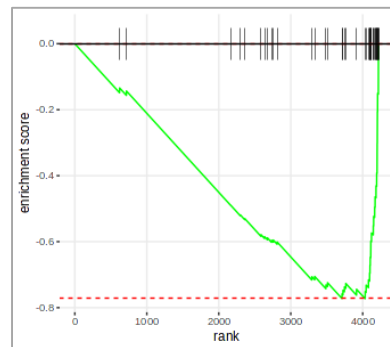
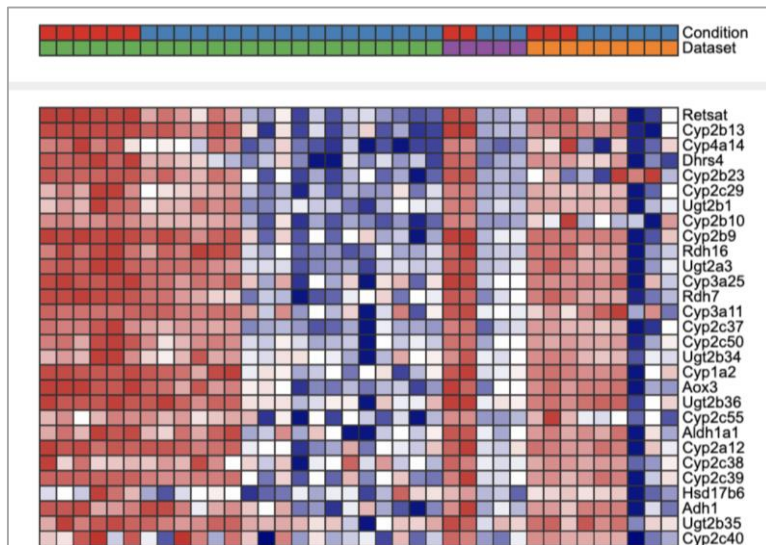
- Integrating results from multiple independent studies
- Based on **summary statistics** that are more “integrable”
  - P-values
  - Effect sizes
- Apply “batch effect” adjustment to reduce study specific effects



# Feature-level meta-analysis

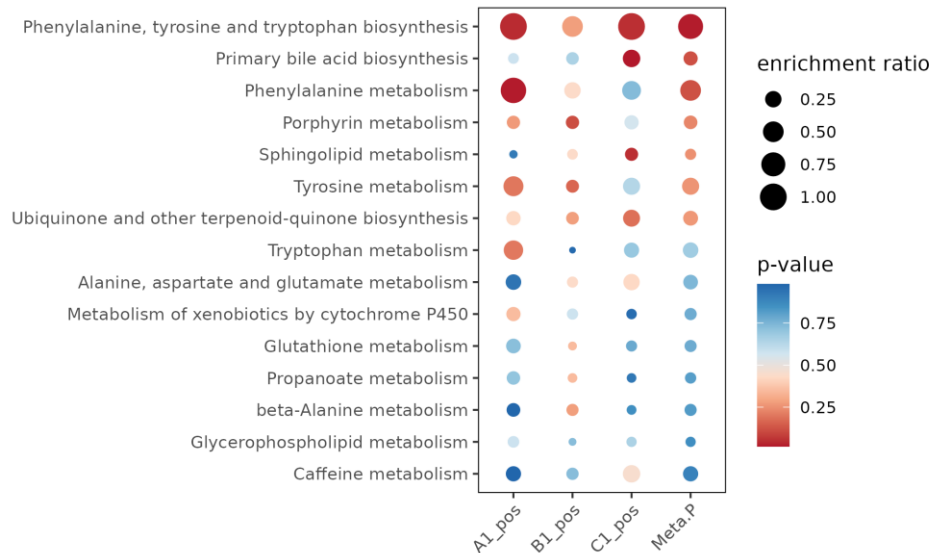


# Visualization across different studies



# Meta-analysis at pathway level

- Summary statistics can be computed at different levels such as pathway enrichment p-values
- Treat pathways as operational units (instead of features)
  - Useful when features are similar but not identical. Such as in multi-omics, untargeted metabolomics based on MS peaks

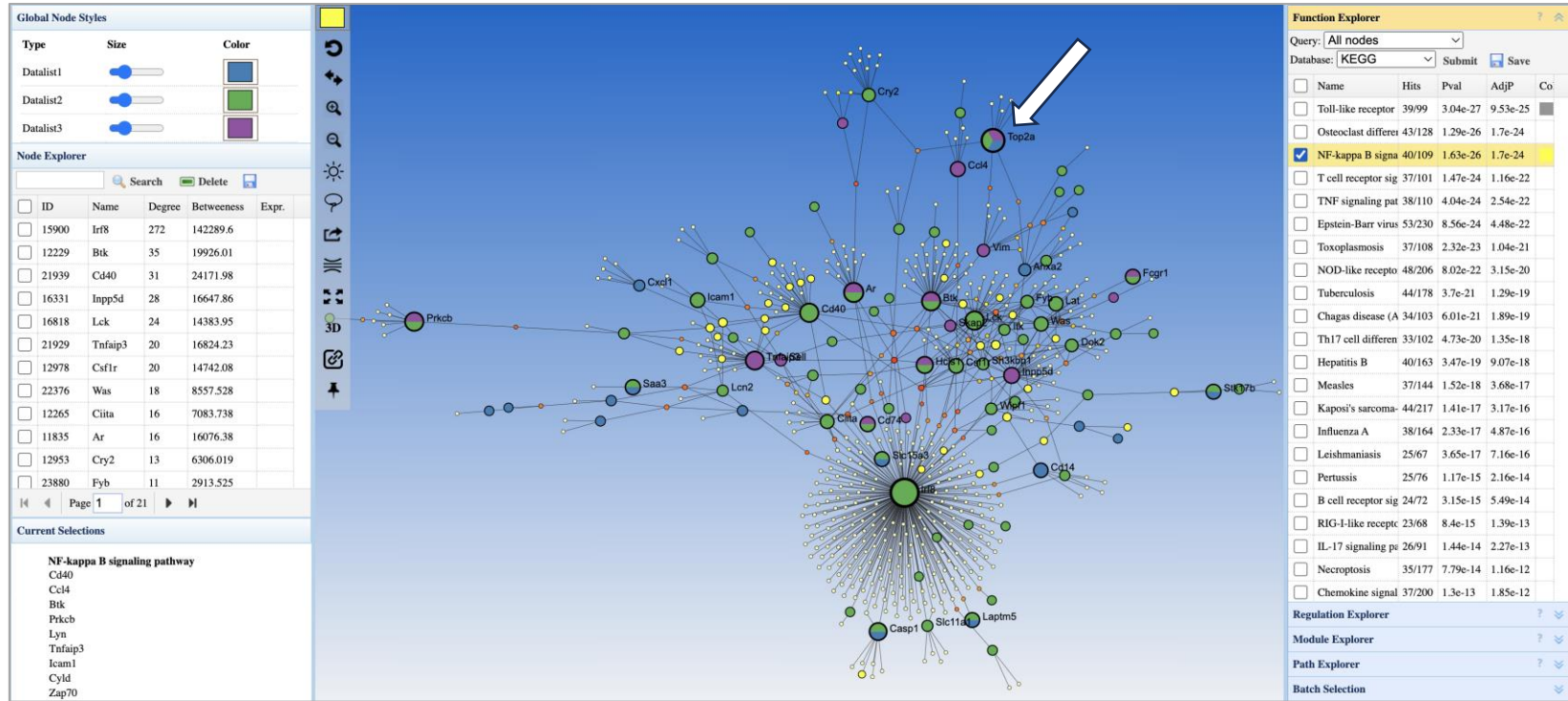


# Network is “naturally” integrative

1. Obtain gene signatures from different studies
2. Enter them into NetworkAnalyst
  - Signatures separated by “//”
3. View their direct relationships & shared context in networks
  - Shared genes will be shown as **pie chart** indicating source
  - Similar genes will be close to each other
    - They will share the same context (i.e. interaction partners)
4. Detect enriched functions or common themes

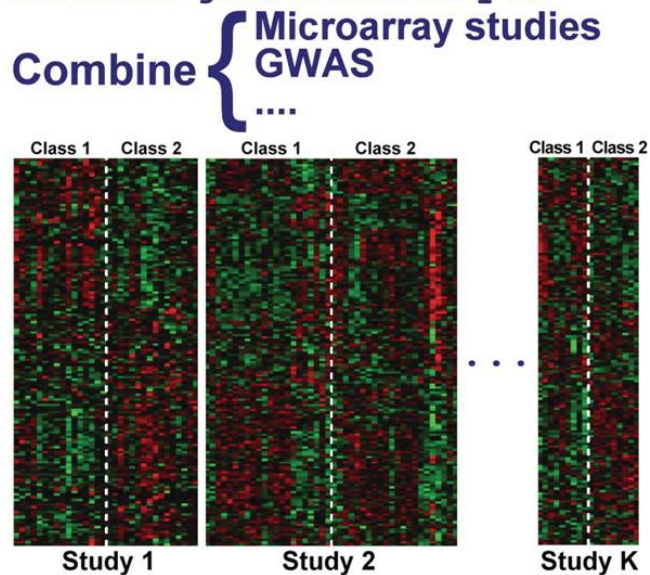


# Using networks to integrate gene signatures

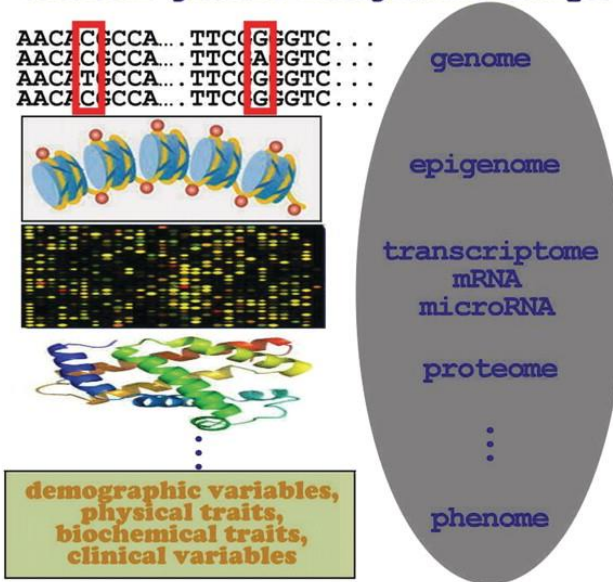


# Horizontal & vertical integration

## A Horizontal genomic meta-analysis



## B Vertical genomic integrative analysis



<https://doi.org/10.1093/nar/gkr1265>



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# Schedule for today



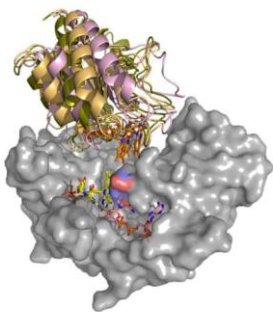
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9:00 – 9:15	General introduction & recap
9:15 – 9:40	Network analysis and visualization
9:45 – 10:15	NetworkAnalyst live demo & hands on
10:20 – 10:50	Gene regulatory networks
10:55 – 11:25	miRNet live demo & hands on
Summary and discussion	





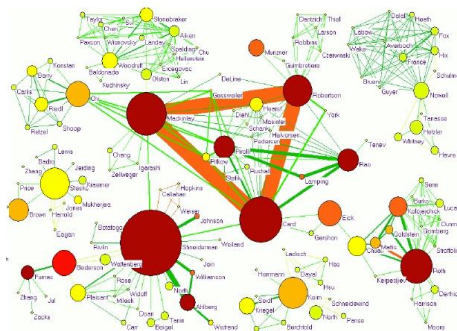
# Goals of omics data analytics

## Individual Molecules



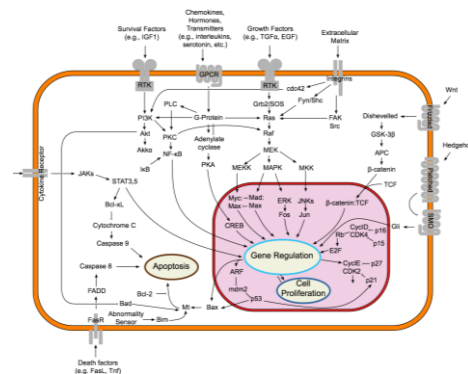
Mechanism

## Omics & Multi-omics



Patterns, functions,  
hypothesis

## Systems Biology



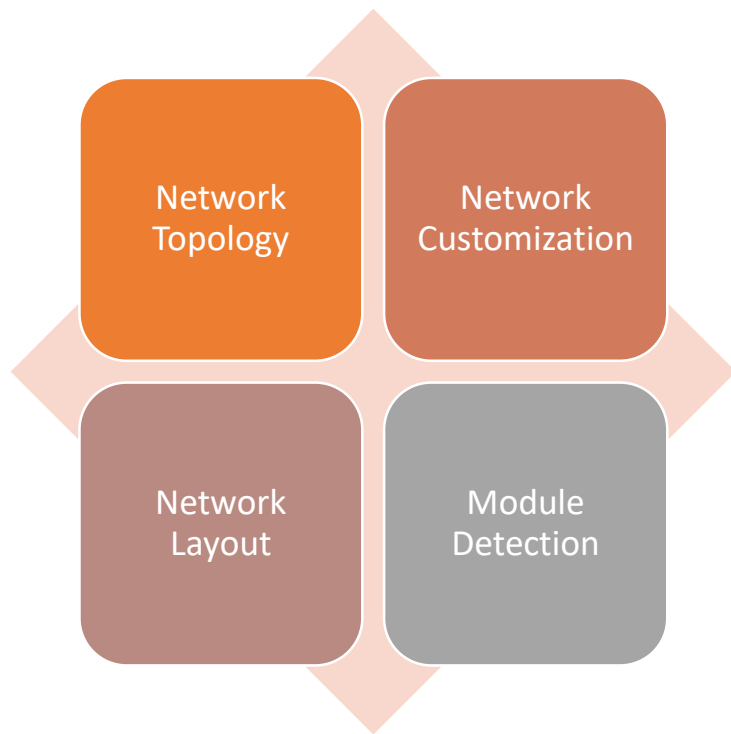
Knowledge



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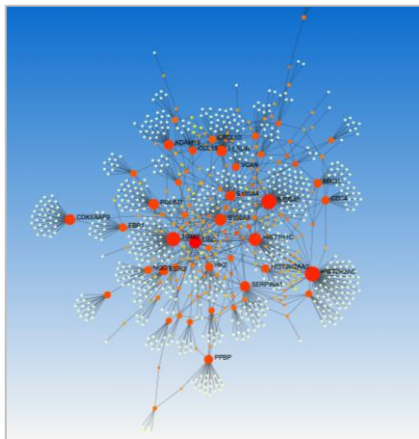
# Network Analysis



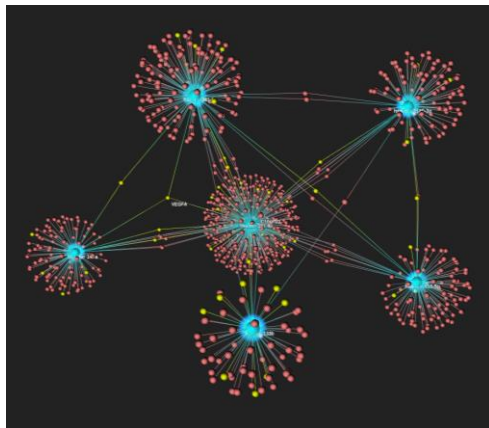
# Network Topology



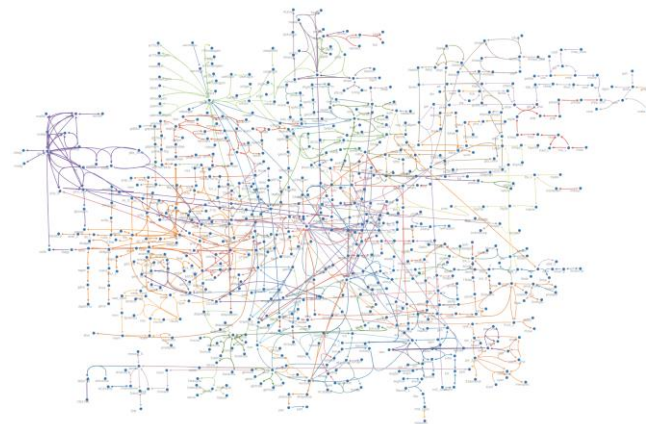
# Common types of networks



**PPI network**



**miRNA-gene target**



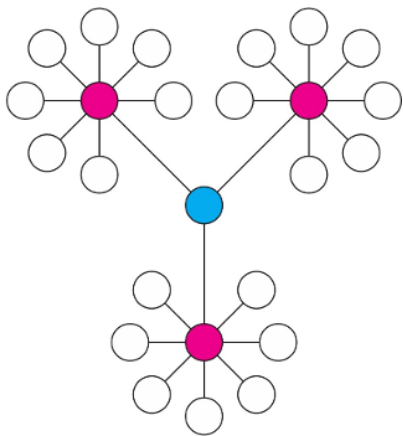
**Metabolic networks**

# Network topology – centrality measures

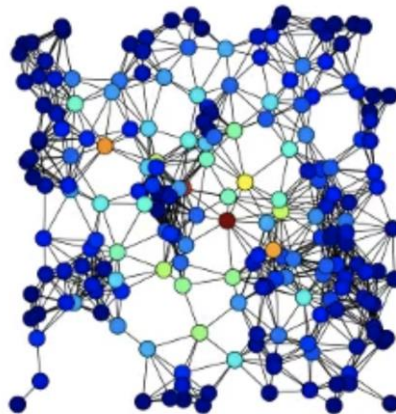
- Degree centrality
  - Number of links to other nodes
  - Popular or densely connected nodes
- Betweenness centrality
  - How many times shortest paths cross a node
  - Nodes influence the information flow around a system
- Closeness centrality
  - Average farness (inverse of shortest distance) to all other nodes. Nodes with a high closeness score have the shortest distances to all other nodes
  - Nodes that can influence the entire network most quickly



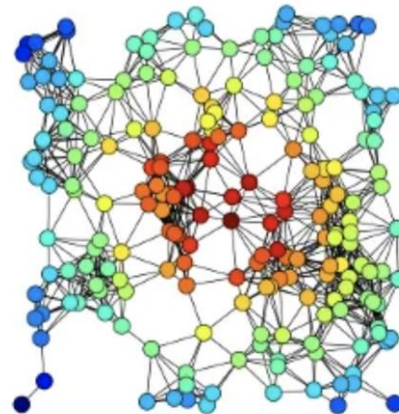
# Illustrations



**High degree  
centrality**



**High betweenness  
centrality**

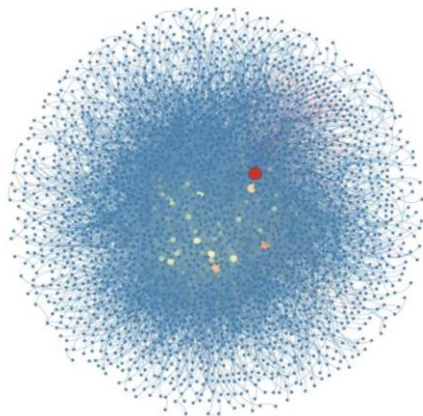


**High closeness  
centrality**

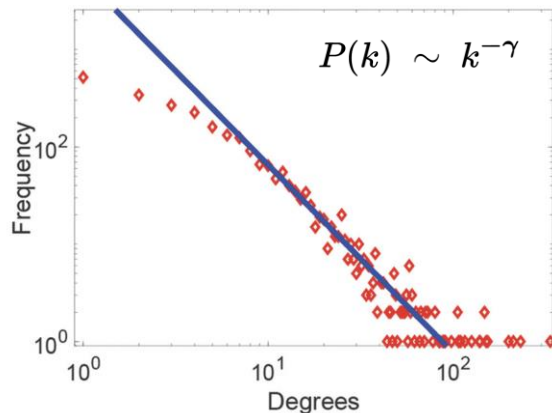
# Scale-free network

A network whose degree distribution follows a power law

- Scale-free networks are more robust against failure (random node failure).
- Scale-free networks are more vulnerable against non-random attacks.
- Scale-free networks have short average path lengths.



PPI network



# Network customization

Large networks are difficult to view and interpret. How can we reduce the network size while keep its main structure and main features?

- **Zero-order Network:** keep only seed proteins that directly interact with each other - suitable when there are a large number of seeds;
- **Minimum Network:** compute pair-wise shortest paths between all seed nodes and remove the nodes that are not on the shortest paths.
- **Steiner Forest Network:** this is similar to minimum network, but based on a fast heuristic Prize-collecting Steiner Forest (PCSF) algorithm
- **Batch Exclusion:** Enter a list of genes that you want to exclude from the resulting network.
- **Tissue Filter:** filter genes according to gene expression measured in specific tissues.

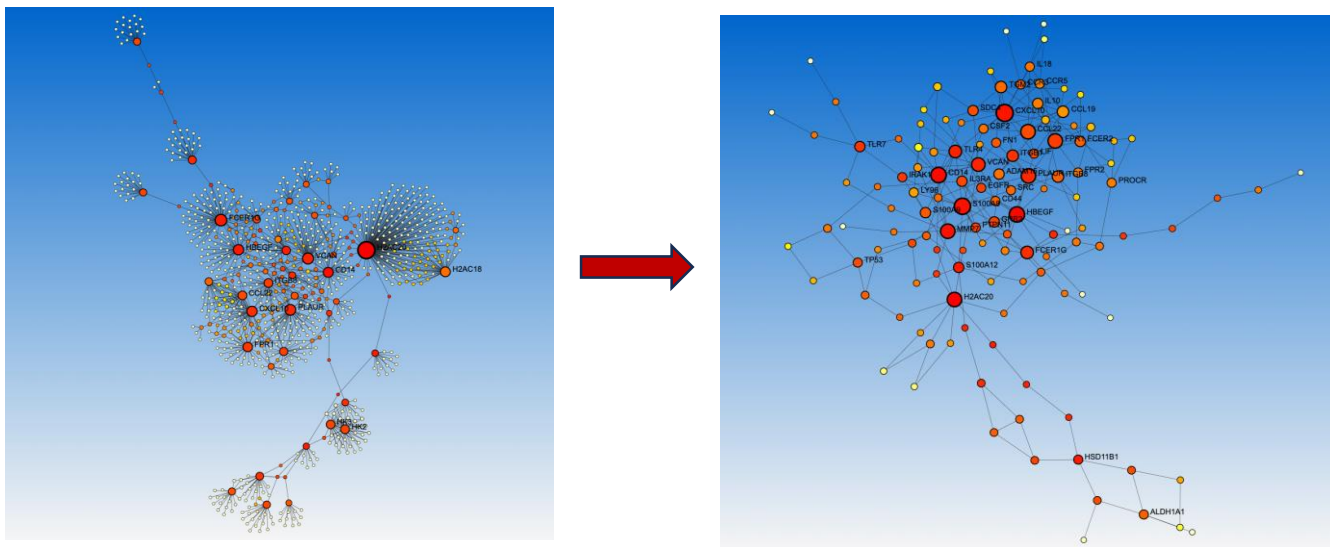
**Aiming for 200 – 2000 nodes**





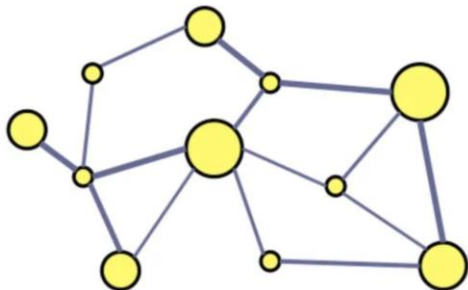
# Minimum network

- Based on pair-wise shortest paths to compute the minimum nodes to be introduced in order to connect all seeds (queries)?

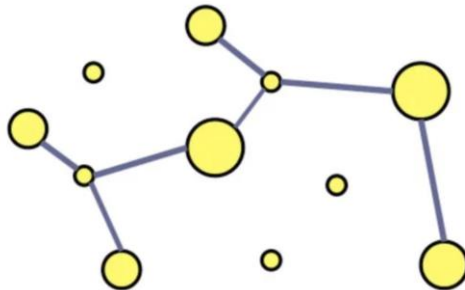


# Prize-collecting Steiner Forest (PCSF) method

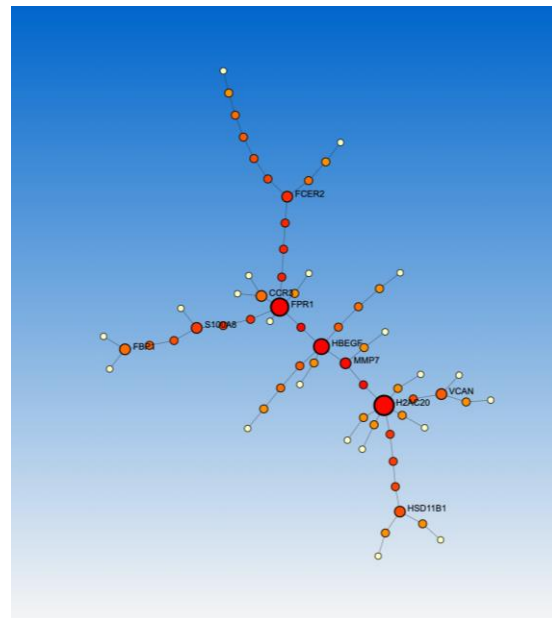
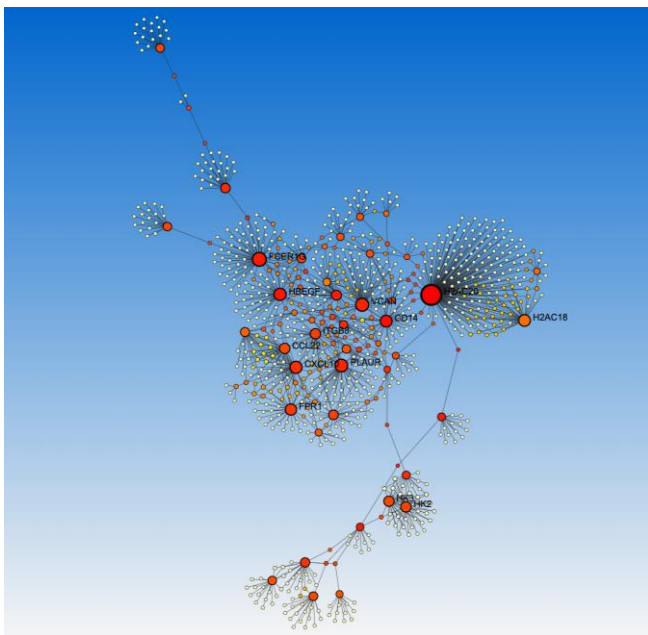
- The goal is to establish connections between pairs of nodes in a given network at minimum **cost**.
- The cost is customizable based on different context
  - ➔ We can adjust weights to encourage traveling through “seeds”
- A “global” optimization problem



Input



# PCSF network

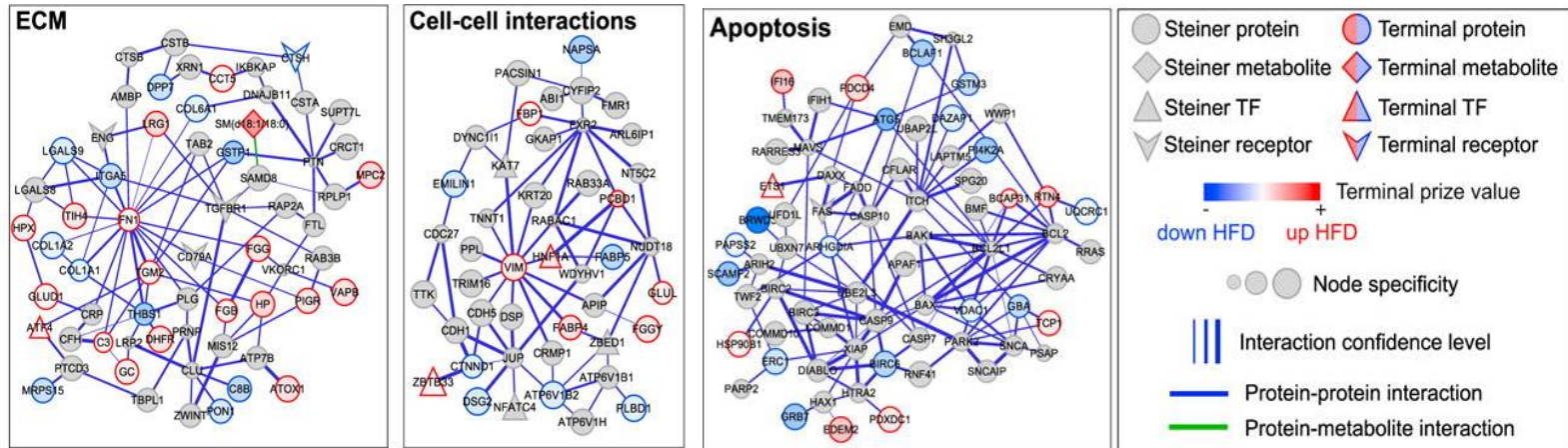


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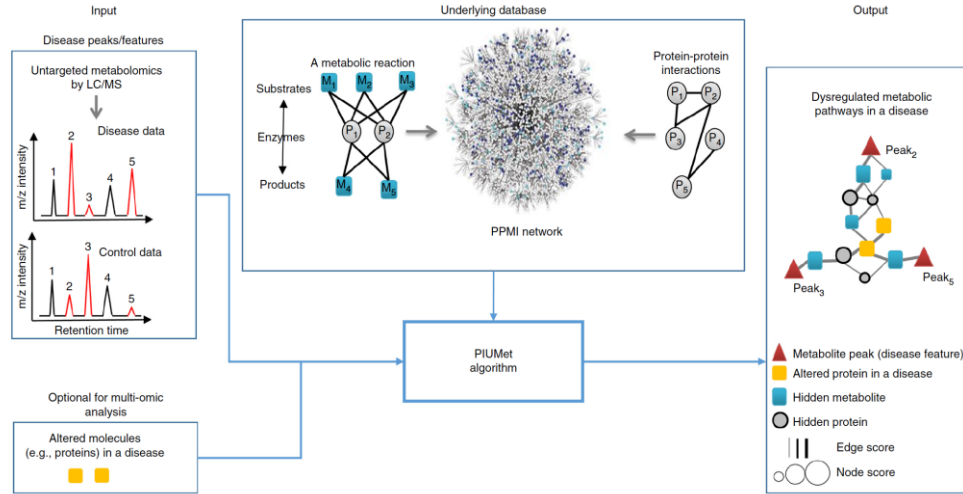
# PCSF applications in biology

Computing PCSF sub-networks to select meaningful biological processes

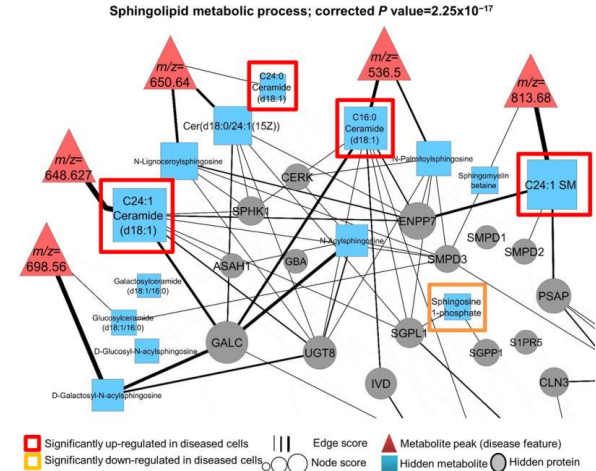


*Cell Rep.* 2017;21(11):3317-3328.

# Revealing disease-associated pathways by PCSF



Integrating metabolic network with PPI



Pirhaji, et al 2017. Nature Methods **13**, pages770–776



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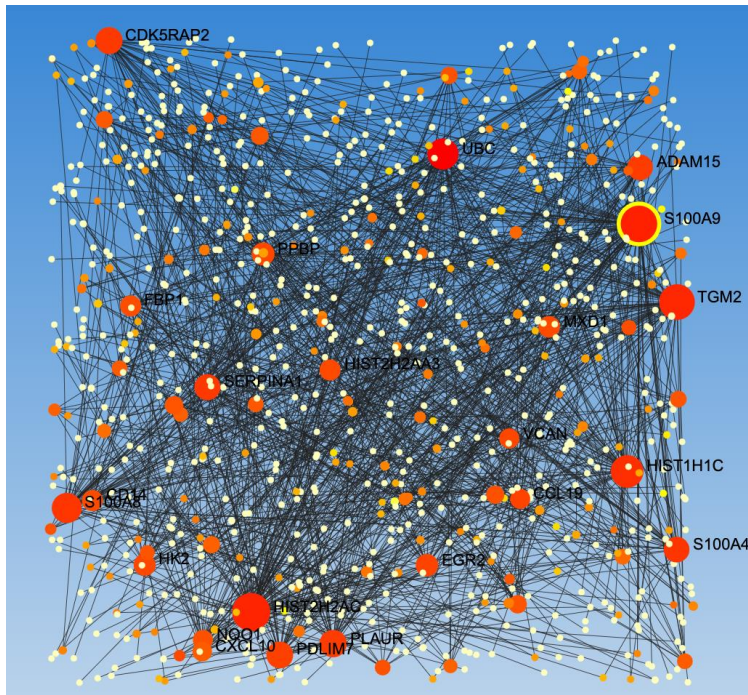
Empowering researchers through trainings, tools, and AI

# Network Layout

# Mix art with science

A layout algorithm takes as input a set of nodes and edges along with a set of options and return their locations

- Heuristic
- Subjective
- Often used in combination with
  - Color
  - Size
  - ...



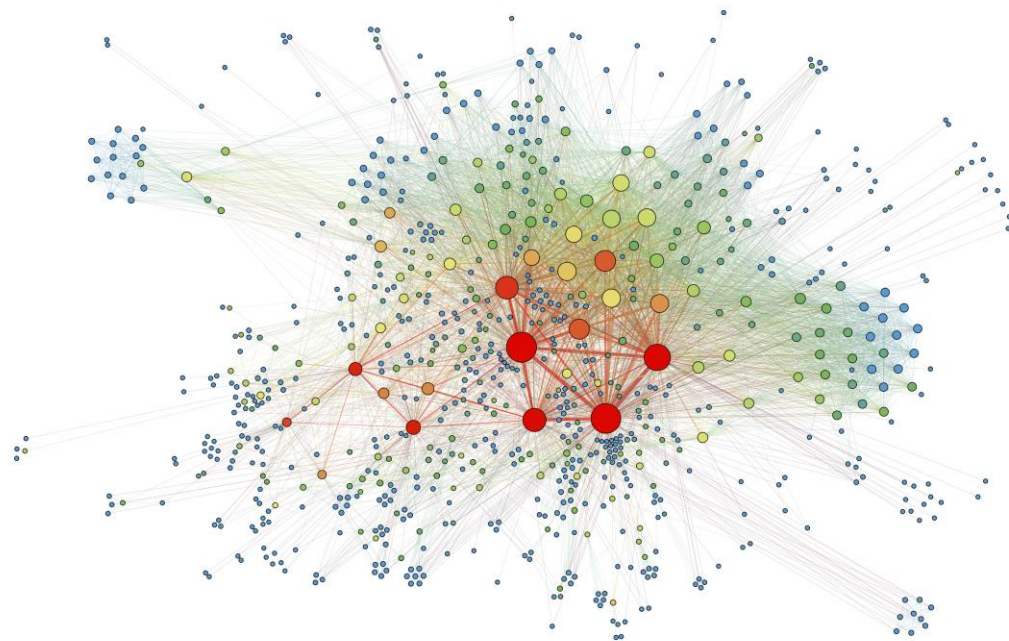
**Random Layout**



# Network layout: force-directed layout

- The connections between nodes are treated like metal springs attached to the pair of nodes.
- These springs repel or attract their end points according to a force function.

Highlights the underlying topology of the graph. We can visually identify clusters, cliques, and bridges.

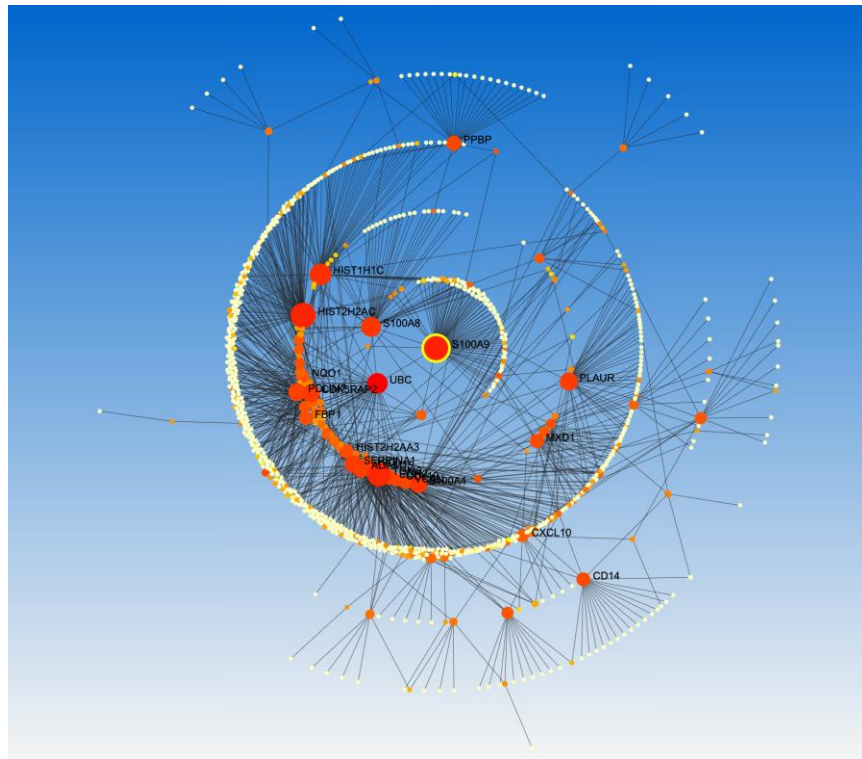




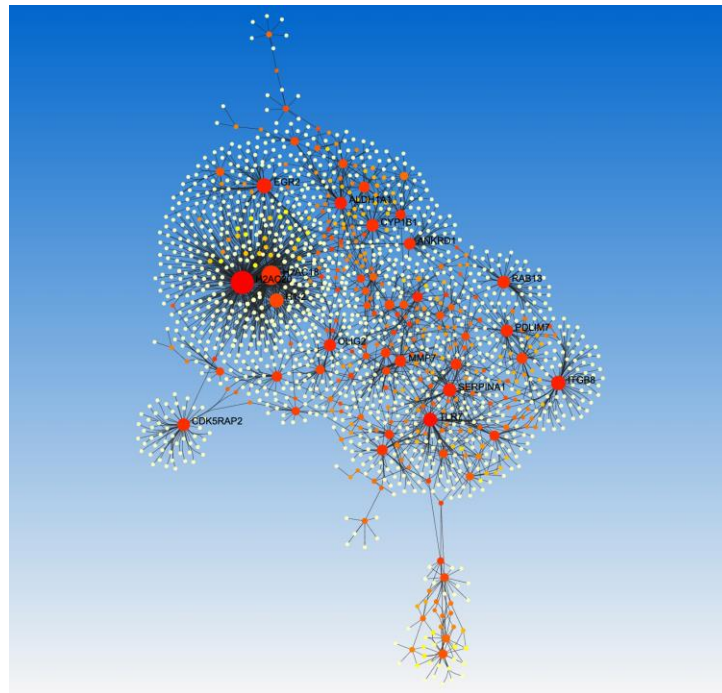
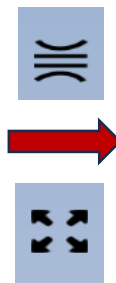
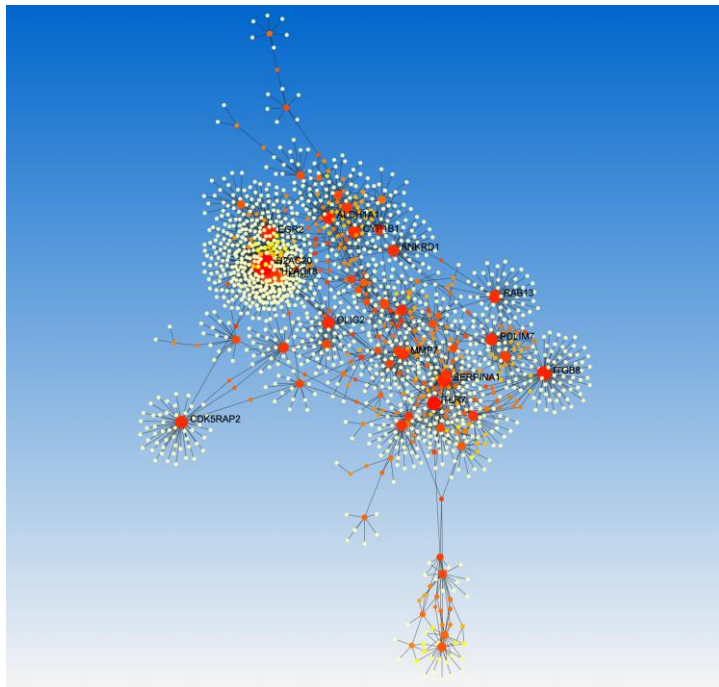
# Network layout: concentric circle

- Organize the nodes into concentric circles, based on the specified metric.
- The nodes with the highest metric values are placed in the innermost circle, and the metric values of the nodes descend for each outward circle.
- Each circle has nodes with metric values between a specified range, with nodes within a circle sorted accordingly.

Useful for highlighting relative importance of the nodes

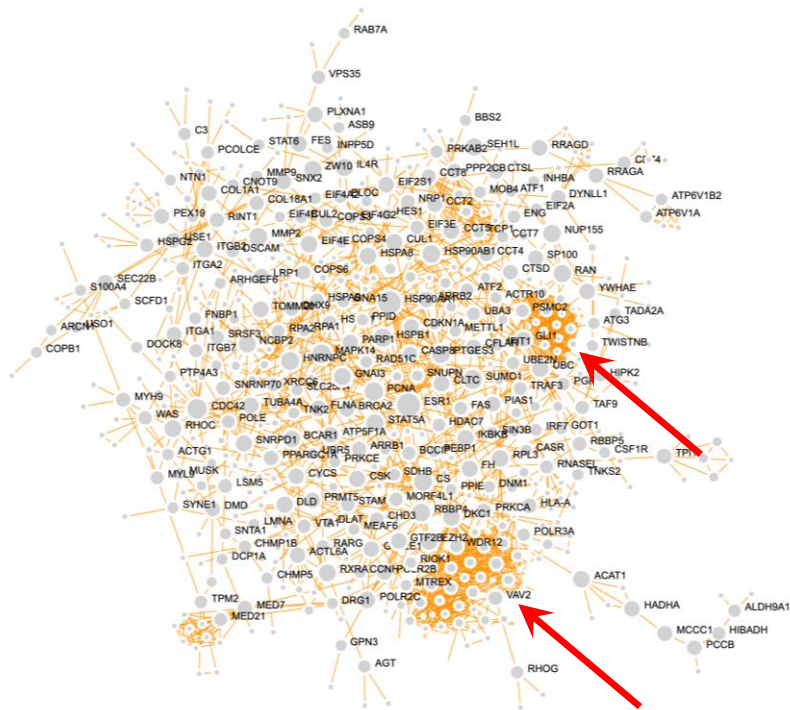


# Tips: Edge Bundling & Reduce Overlap



## Network modules (communities)

- Community: having dense connections between the nodes within modules but sparse connections between nodes in different modules
- Could have biological or functional implications
- **Structural module  $\approx$  Functional module**



# Common module detection methods

## Label propagation

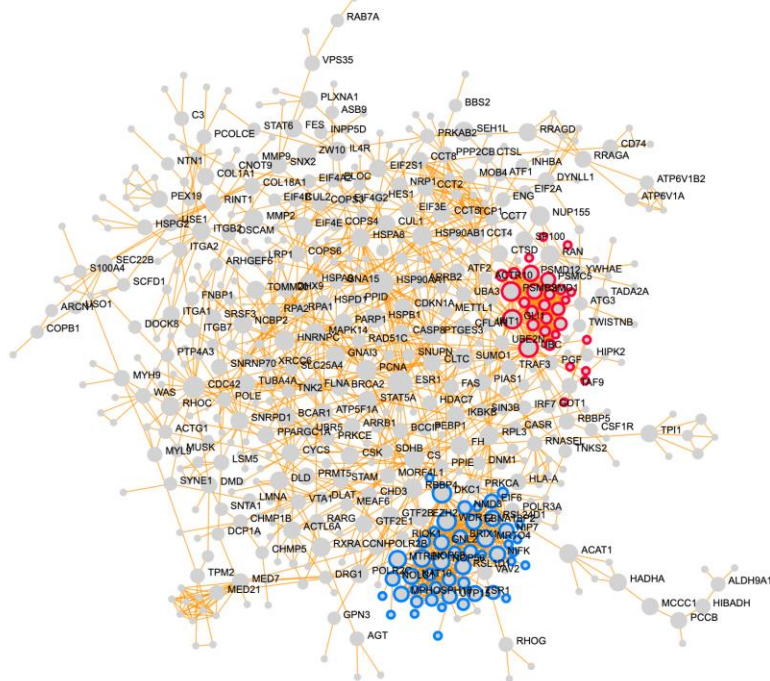
- ❖ Each node in the network is assigned to the same community as the majority of its neighbours (through iterative process)

## InfoMap

- ❖ Based on information flow through a network using random walk

## WalkTrap

- ❖ Short distance random walks tend to stay in the same community

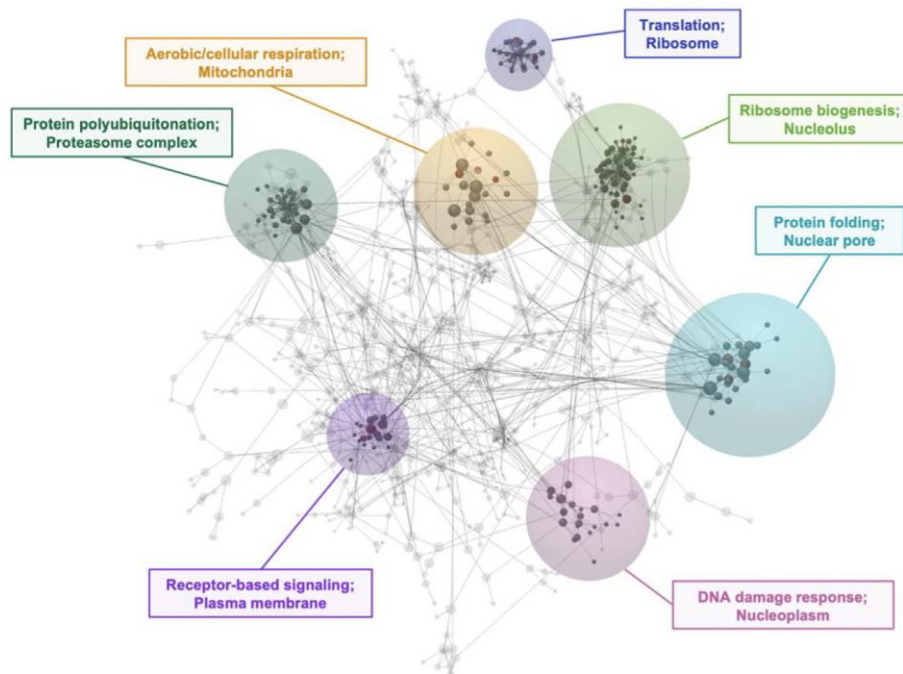


All Heuristic Methods



# Understand & view modules

- We can use Wilcoxon rank-sum test to compare the number of connections of each node with that of other nodes within the module and with that for nodes outside the module.
- We can test whether certain biological functions are enriched in these modules.
- We can also extract the modules for more focused visualization

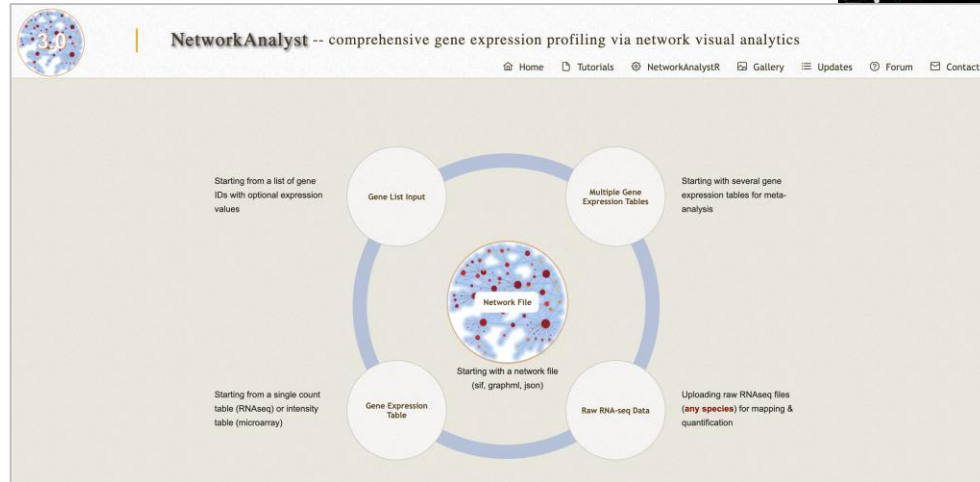




# Cytoscape vs. NetworkAnalyst

## Cytoscape:

- Standalone platform for network visualization
- Need to install plug-ins for other analysis



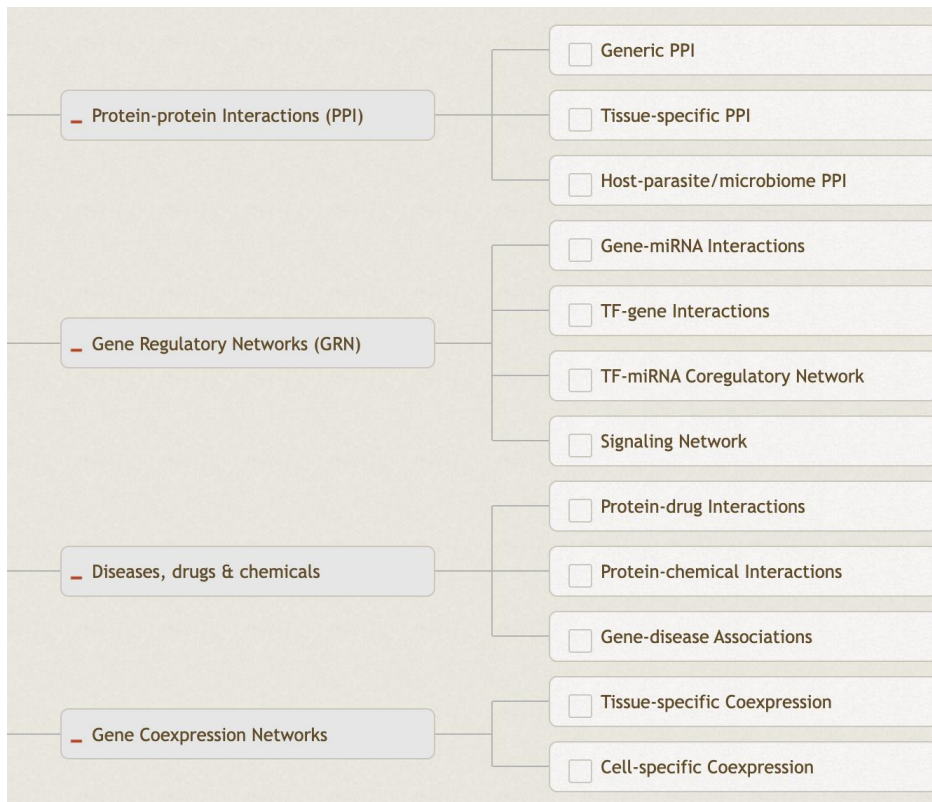
## NetworkAnalyst:

- Web-based platform for network visualization
- Built-in databases for network creation & functional analysis

# NetworkAnalyst – network creation

Built-in databases:

- ❖ PPI network
- ❖ miRNA-gene
- ❖ TF-gene
- ❖ Signaling network
- ❖ Protein-drugs
- ❖ .....



# NetworkAnalyst – Network Refinement

Comprehensive support for automatic filtering (topology and biology), as well as manual network filtering

## Mapping Overview

The genes of interest (seeds) from previous analysis are mapped to the corresponding molecular interaction database.

The procedure typically produces one big subnetwork ("continent") with several smaller ones ("islands"). Please note:

- Subnetworks with **at least 3 nodes** are listed below. You can visually explore them in the next step.
- The common **network topology measures** are also computed based on well-established [the igraph R package](#)
- These subnetworks can be downloaded as **SIF (simple interaction format)** files to be explored in other tools (i.e. Cytoscape).

Networks	Nodes	Edges	Seeds	Topology	Interactions
subnetwork1	916	1118	85	 View	 SIF
subnetwork2	3	2	1	 View	 SIF
subnetwork3	3	2	1	 View	 SIF

## Network Tools: ?

First Order Network

Zero-order Network

Second-order Network

Minimum Network

Steiner Forest Network

Degree Filter

Betweenness Filter

Tissue Filter

Batch Exclusion

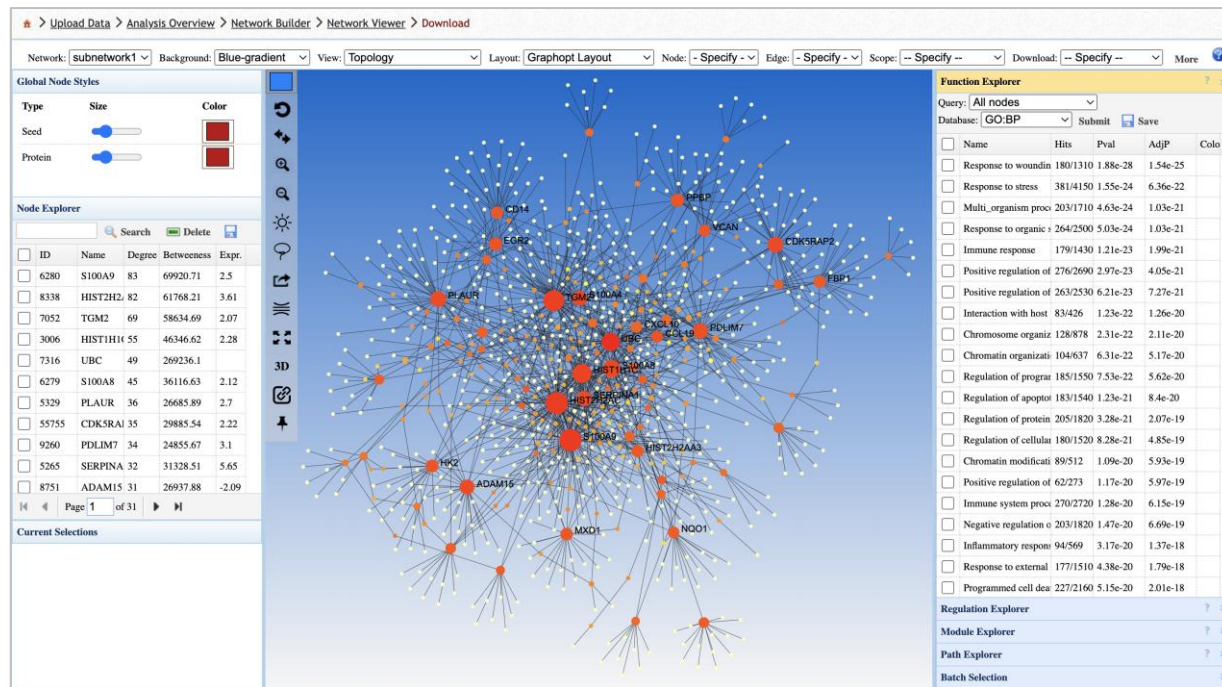




# NetworkAnalyst – Network Visualization

Build-in network  
visual analytics  
system

- ❖ Multiple algorithms for layout & module detection
- ❖ Enrichment analysis
- ❖ 2D and 3D support!



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# Schedule for today

Time	Topics
9:00 – 9:15	General introduction & recap
9:15 – 9:40	Network analysis and visualization
9:45 – 10:15	NetworkAnalyst live demo & hands on
10:20 – 10:50	Gene regulatory networks
10:55 – 11:25	miRNet live demo & hands on
Summary and discussion	



# Live Demo & Hands On



# Tasks

1. Multi-list upload and analysis.
2. Targeted functional analysis - extract module/subnetworks
3. Save a network state



# Task 1: Multi-list


- For multiple lists, insert "//" in a line to indicate the start of new list.

Specify organism

Set ID type

Copy-and-paste one or more gene lists (Insert a "//" line to indicate the start of a new gene list, or [click here](#) to upload multiple gene list files)

Dram1  
C1qc  
Tlr2  
Casp1  
Laptn5  
Hist1h2ak  
Ifi27l2a  
Top2a  
Tmsb4x  
//  
Cd3g  
Slc11a1  
Ciita  
Eif4e3  
Rnase6  
H2-DMb2  
Csf1r  
Ccr5  
Gimap7  
Wipf1  
Dennd1c

 Upload

# Task 1: Multi-list

- Allow you to explore relationships between different lists:

Building option: ☐ Zero order ☒ First order

Seed option:

Database selection:

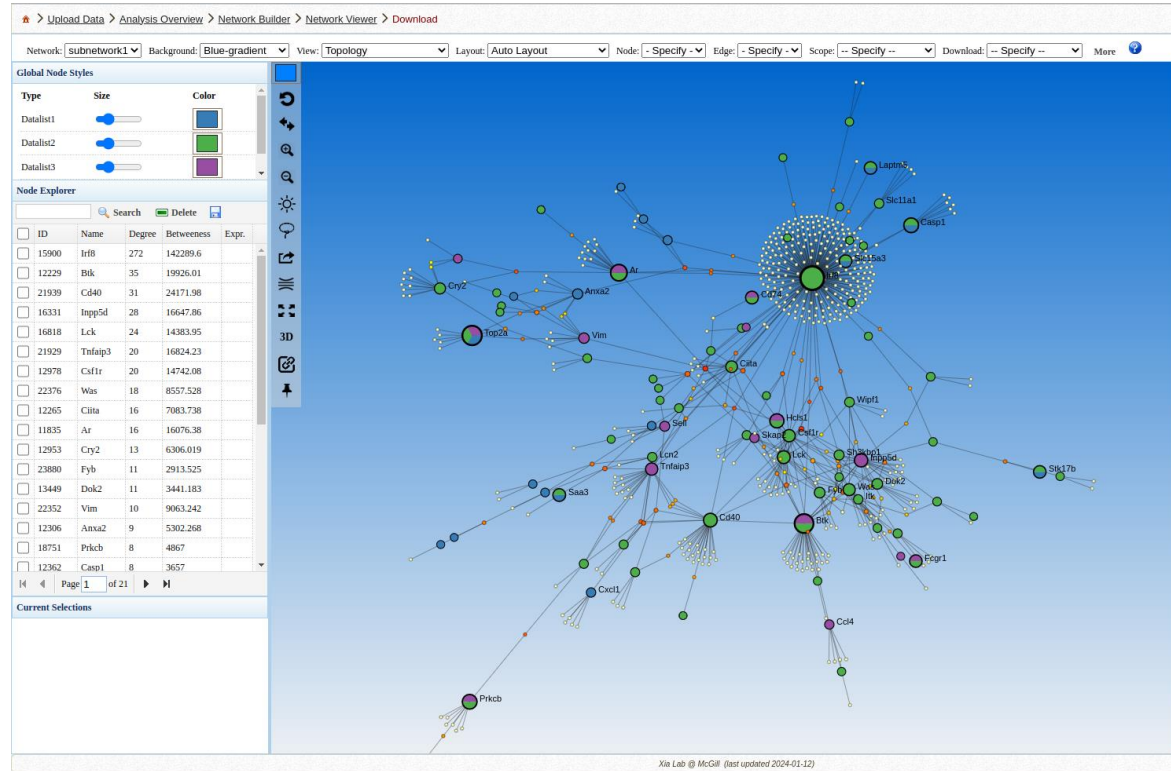
Database	Interactome	Confidence score
IMEx	<input checked="" type="radio"/>	None
STRING	<input type="radio"/>	medium (400) - high (1000) confidence score ( <a href="#">Szklarczyk D et al</a> )

Confidence score cutoff:

Require experimental evidence: ☒

# Task 1: Multi-list

- Visualize intersections in network view --> **Pie nodes**
- Color legend on top right corner
- Genes coming from any of the list(s) are larger in size



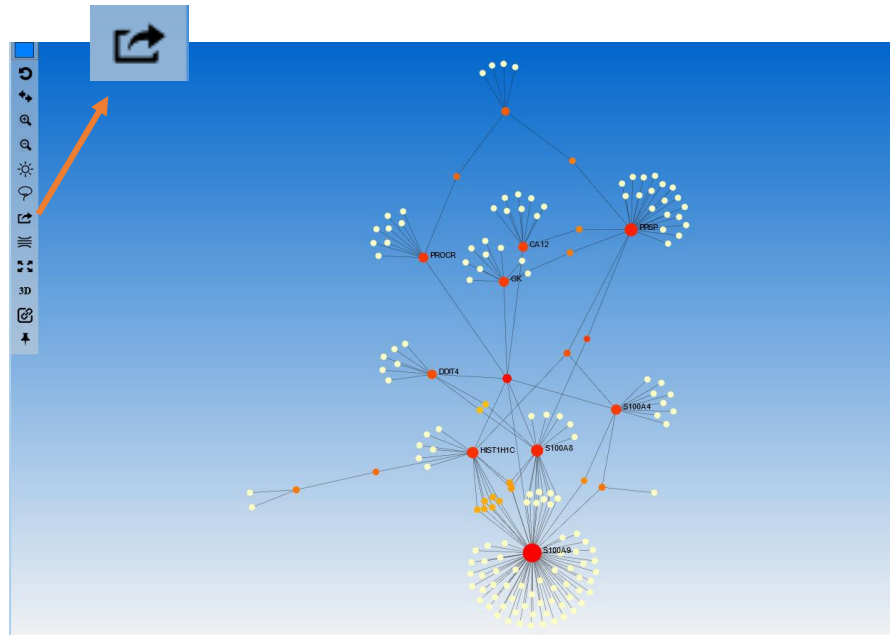




# Task 2: Targeted analysis

## Analyze the nodes of interest:

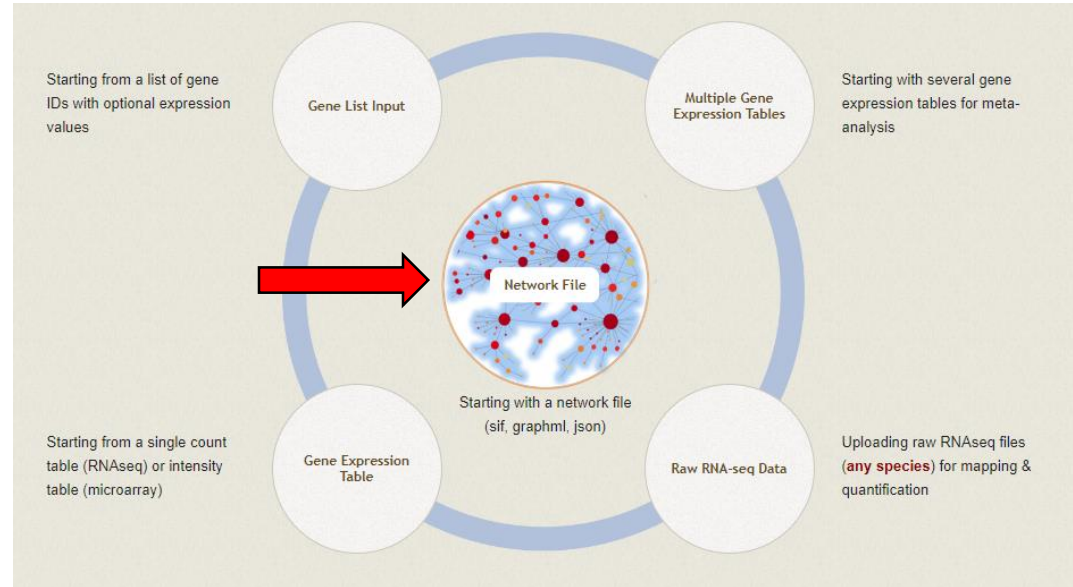
- Enrichment analysis on **highlighted nodes** in the current network
- **Extract** the nodes as a connected subnetwork
  - Use "**Minimum Network**" approach --> pairwise shortest paths and remove those that are not traversed



**Extract a subnetwork**

# Task 3: Save and load subnetwork

- You can save your network as a .json file that you can reload later.
- NetworkAnalyst can take **graph file** as input.



# Task 3: Save and load subnetwork

ID mapping (optional)  
- allows functional enrichment analysis

**Upload a network file**

Please use the panel below to upload a network file for visual exploration. The detailed descriptions on how to prepare different network files can be found from our [FAQ](#)

Submit your data

Data Type:

Specify organism:

ID type:

Data file:  No file chosen

Try our example data

<input type="radio"/> A small network (Cytoscape .json format)	Human gene symbols
<input type="radio"/> A small network (NetworkAnalyst .json format)	Human gene symbols
<input type="radio"/> A medium network (.txt)	Unspecified organism, gene symbol
<input checked="" type="radio"/> A large network (.graphml)	Mouse gene symbols

# Schedule for today

Time	Topics
9:00 – 9:10	General introduction & recap
9:15 – 9:40	Network analysis and visualization
9:45 – 10:15	NetworkAnalyst live demo & hands on
10:20 – 10:50	Gene regulatory networks
10:55 – 11:25	miRNet live demo & hands on
Summary and discussion	



# Biological Networks

- **Protein-protein interaction network:** proteins that are connected in physical interactions
  - Proteins and their interaction partners
- **Gene regulatory network:** a collection of molecular regulators that interact with each other and with other substances in the cell to govern the gene expression levels of mRNA and protein
  - miRNA, transcription factors, non-coding genes, etc
- **Metabolic network:** metabolic products and substrates that participate in biochemical reactions
  - Metabolites, enzymes, co-factors



# Protein-protein interaction (PPI) network

## Experimental

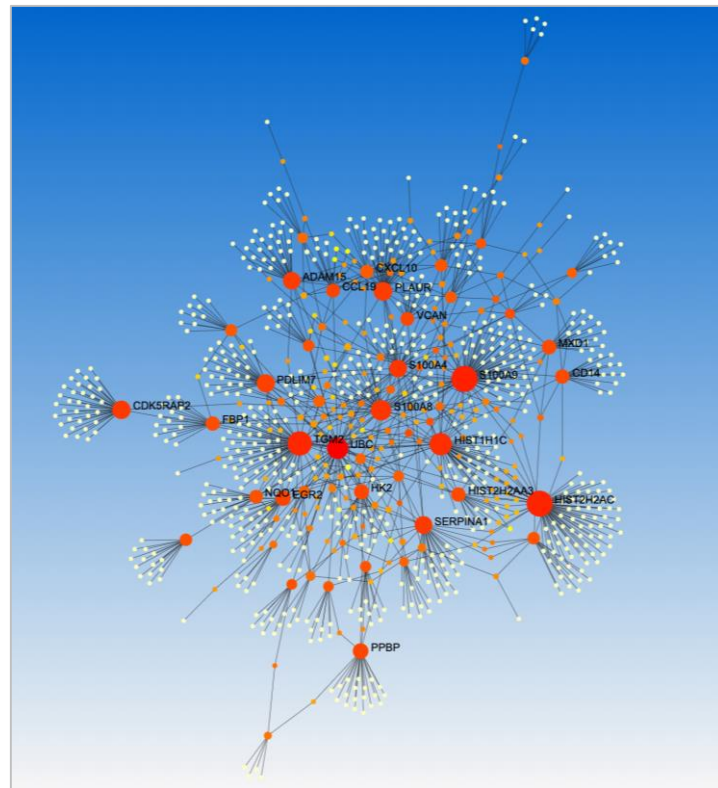
- InnataDB/IMEx Interactome
- Rolland Interactome (2014)
- HuRi (human reference interactome, 2020):  
Pairwise combinations of human protein-coding genes are tested systematically using high throughput yeast two-hybrid screens to detect protein-protein interactions.

## Predicted

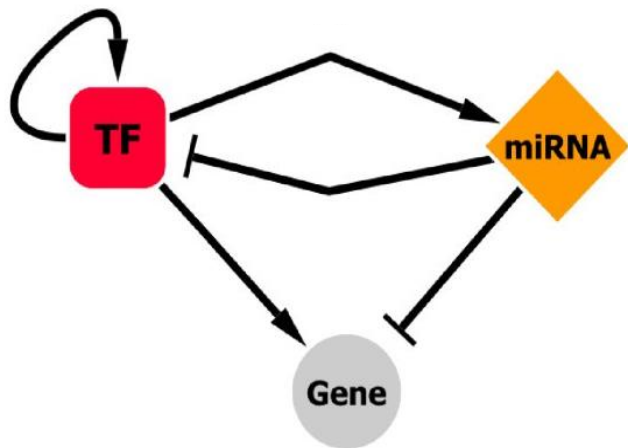
- Binding motifs, evolution, etc

## Both:

- STRING:
  - Experimentally derived interactions through literature curation;
  - Computationally predicted interactions through text mining and inference from other organisms
  - Supporting > 10,000 organisms

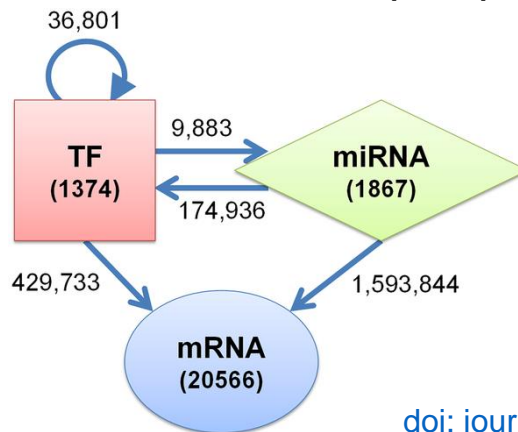


# Core Gene Regulatory Networks



**TF:** a protein that controls the rate of transcription from DNA to mRNA, by binding to a specific DNA sequence.

GRN in human (2015)

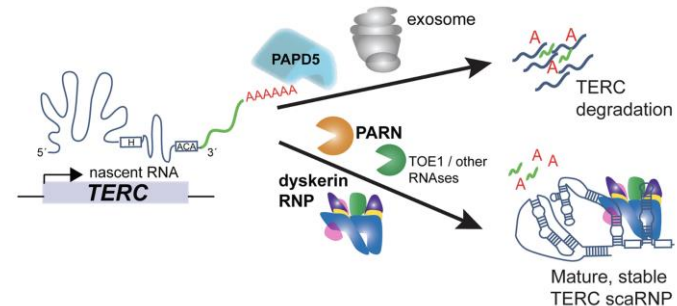
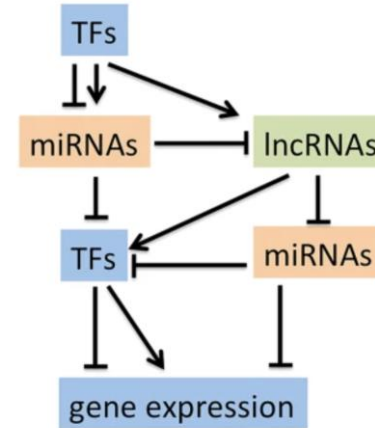


[doi: journal.pcbi.1004504](https://doi.org/journal.pcbi.1004504)

**miRNA:** small, single-stranded, non-coding RNA molecules (21-23 nt), involved in post-transcriptional regulation of gene expression

# Long noncoding RNAs (lncRNAs)

- Transcripts that are over 500 nucleotides and don't encode proteins
- Over 20,000 lncRNAs have been reported, most of which have no known functions
- Known functions
  - Gene transcriptional regulation
    - Interact with miRNAs
    - Interact with TFs
  - Function as scaffolds
    - Assembles telomerase





# How to obtain GRN?

## Infer network from gene expression data

- Data driven, suitable for non-model species without good database
  - Weight Gene Correlation Network Analysis (WGCNA)
  - GENE Network Inference with Ensemble of trees (GENIE3)
- **Issues:**  
require a lot of data, computing intensive, high false positives

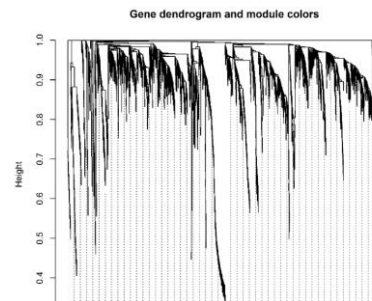
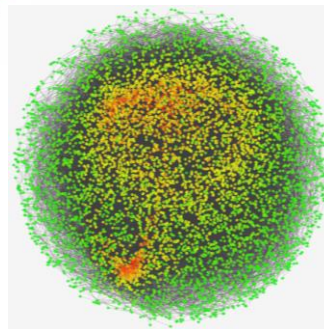
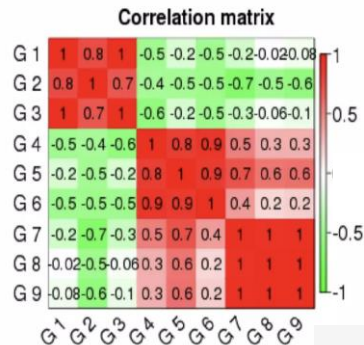
## Search from the databases

- Suitable for model species with high-quality knowledge & coverage
  - miRNA-target gene database
  - TF-target databases
- **Issues:**  
tend to be generic, need to add more context specific information



# WGCNA

1. Compute the gene **co-expression matrix**
2. Create a **weighted co-expression network** by selecting the threshold for constructing the network to reflect small world, power law distribution
3. Compute topological overlap measure (TOM) adjacency matrix based on overlap of network neighbors for **module detections**.



# DREAM Challenges

## DREAM (Dialogue for Reverse Engineering Assessment and Methods)

DREAM5: network inference challenge on genome-scale transcriptional regulatory network from gene expression data

- Community driven;
- Synthetic data (ground truth known);
- Time series experimental data

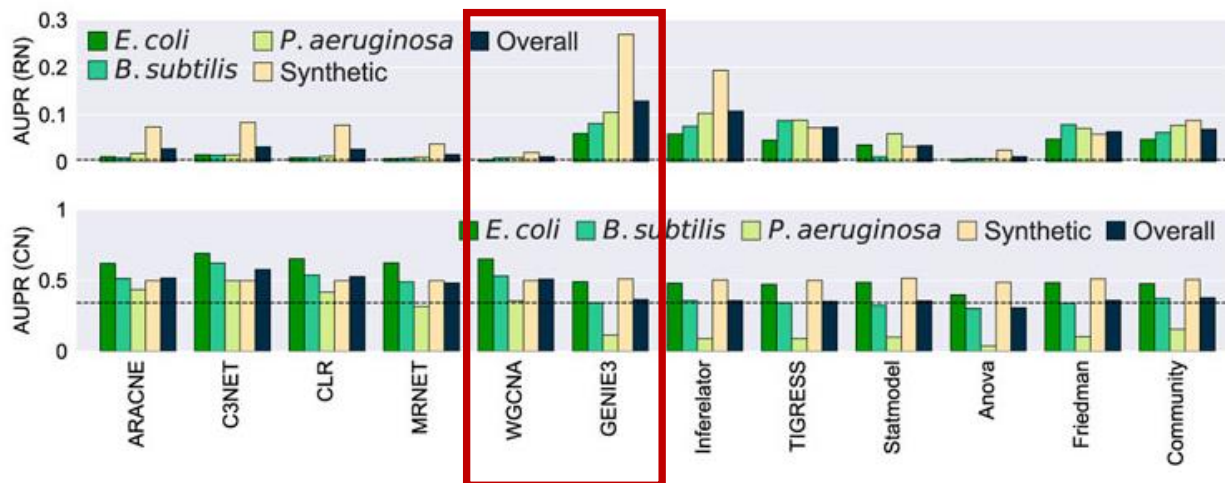
Method	Network type	Directed network	Main References
ARACNE	COEX	FALSE	Margolin et al. (2006)
C3NET	COEX	FALSE	Altay and Emmert-Streib (2010)
CLR	COEX	FALSE	Faith et al. (2007)
MRNET	COEX	FALSE	Meyer et al. (2007)
LSTrAP	COEX	FALSE	Proost et al. (2017)
RNA-seqNet	COEX	FALSE	Proost et al. (2017)
WGCNA	COEX	FALSE	Zhang and Horvath (2005)
GENIE3	CAUS	TRUE	Huynh-Thu et al. (2010)
INFERELATOR	CAUS	TRUE	Bonneau et al. (2006)
TIGRESS	CAUS	TRUE	Haury et al. (2012)
StatModel	CAUS	TRUE	Zorro-Aranda et al. (2022)
iBMA	CAUS	TRUE	Annest et al. (2009)
ScanBMA	CAUS	TRUE	Young et al. (2014)
ANOVA	HYBR	TRUE	Zorro-Aranda et al. (2022)
FRIEDMAN	HYBR	TRUE	Zorro-Aranda et al. (2022)

<https://doi.org/10.3389/fgene.2023.1143382>



# DREAM5 result

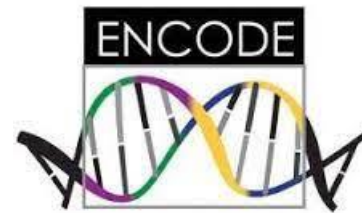
- ❖ GENIE3 is the best tool for bulk RNA-seq network inference
- ❖ WGCNA is the most widely used tool for co-expression network inference



# TF - target gene databases

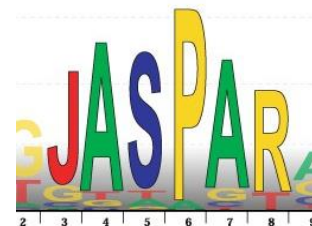
## ➤ ENCODE

- ✓ Transcription factor and gene target data derived from the ENCODE ChIP-seq data
- ✓ Integrates ChIP-seq of TFs or chromatin regulators with differential gene expression data to infer direct target genes
- ✓ 379 TFs, ~400k interactions total



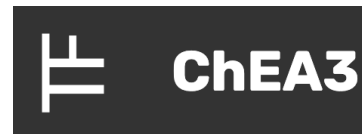
## ➤ JASPAR

- ✓ Manually curated high-quality and non-redundant DNA-binding profiles for transcription factors (TFs) across taxa.
- ✓ <https://jaspar.elixir.no/>
- ✓ 111 TFs, ~150k interactions total



## ➤ CHEA

- ✓ TF-gene co-expression from RNA-seq studies, TF-target associations from ChIP-seq experiments, and TF-gene co-occurrence
- ✓ <https://maayanlab.cloud/chea3/>
- ✓ 199 TFs, ~386k interactions



# TRRUST TF-target gene database

- Transcriptional Regulatory Relationships Unraveled by Sentence-based Text mining
- Text mining, followed by manual curation

**Table 1.**

Summary of databases of transcription factor (TF)–target interactions


	Human					Mouse				
	TF	TG	Interaction	MoR	PMID	TF	TG	Interaction	MoR	PMID
TRRUST v2	800	2521	8444	4866	6562	828	2187	6552	4106	5069
TRRUST v1	748	2375	8015	4861	6175					
PAZAR	173	2747	4918		489	162	1726	2329		328
TFactS	275	1873	4209	1468	776	238	1080	2149	1176	472
TRED	119	1621	3275		1542	75	236	508		745
TFe	76	623	1058	134	510	147	388	673	90	353

TF, the number of TF; TG, the number of target genes; Interaction, the number of TF–target interactions;

<https://doi.org/10.1093/nar/gkx1013>



# miRBase



The screenshot shows the miRBase website's 'Browse' page. The header is dark blue with the 'miRBase' logo and navigation links: Home, Search, Browse (active), Help, and Downloads. A search bar is on the right. The main content area is white and titled 'Browse miRBase by species (271 organisms)'. Below the title, it says 'Jump to:' followed by buttons for 'human', 'mouse', 'rat', 'fly', 'worm', and 'Arabidopsis'. A paragraph explains that clicking taxa expands/collapses the tree and clicking species names lists microRNAs. A key indicates the format: 'species name (miRNA count) [assembly version]'. There are 'Expand all' and 'Collapse all' buttons. A partial taxonomic tree is visible, listing: Alveolata, Chromalveolata, Metazoa, Mycetozoa, Viridiplantae, and Viruses.

miRbase: the microRNA database  
the archive for microRNA sequences  
and annotations

<https://mirbase.org/>



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# miRNA - target gene databases

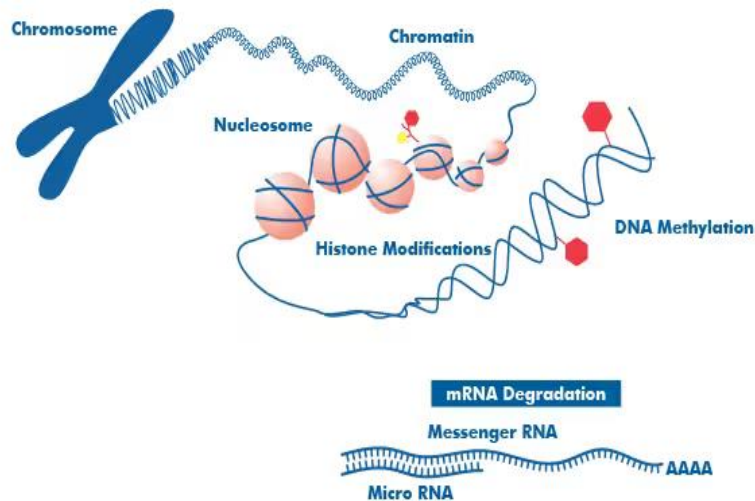
	Database	Number of miRs	Number of target genes	Number of MTIs	Number of citations (WoS)	Experimental validation methods
➡	MiRTarBase (10, 16–20)	2599	15 064	380 639	3217	CLIP-Seq, Luciferase assay, microarray, NGS, pSILAC, western blot
➡	starBase/ENCORI (11, 21, 38)	155	687	1286	2605	CLIP-Seq
➡	DIANA-TarBase (3, 22, 23–25)	1084	20 820	422 662	1894	AGO-IP, biotin miRNA tagging, CLASH, CLEAR-CLIP, CLIP-Seq, ELISA, IMPACT-seq, microarrays, PAR-CLIP, proteomics, qPCR, Reporter genes, RIP-seq, RNA-seq, RPF-seq, TRAP, western blot, other
	miRWalk (26–28)	2656	19 128	31 235 408	1737	CLIP-Seq, Luciferase assay, microarray, NGS, pSILAC, western blot
	miRecords (29)	303	1112	1748	986	ELISA, immunocytochemistry, northern blot, qRT-PCR, reporter assay, western blot, other
	miRGator (30–32)	N/A	N/A	N/A	266	qRT-PCR, reporter assay, western blot





# miRNA-Epigenetics modifier

- Regulation between epigenetics and miRNA:
  - Methylation of CpG island --> reduced transcription of corresponding miRNAs
  - Histone modifications --> up or down regulation of miRNAs expression
  - miRNA --> methylation and histone modifications by targeting genes encoding enzymes
- EpimiR --> database of experimentally validated regulations collected from 2000 papers
  - 19 types of epigenetic modifications
  - 617 miRNAs across 7 species
  - 1974 regulatory relationships



<http://www.jianglab.cn/EpimiR/index.jsp>

# Xeno-miRNA regulation

- Exogenous miRNAs have been detected to interact with host – Cross-species interactions
- miRNet contains both experimentally detected and computational predicted interactions

Hosts	Tissue/ sources	Xeno- species	Xeno- miRNAs (detected/ predicted)	Potential targets
Human	18	40	296/625	20,791
Mouse	18	27	83/418	19,430
Pig	4	14	20/116	12,537
Chicken	2	15	23/10	16,459
Fruit fly	6	6	16/44	12,445
<i>C. elegans</i>	8	6	15/36	16,391
Total	49	54 (unique)	1702	98,053

# miRNA target prediction

- Algorithms to predict interactions between miRNAs and target mRNA sequences
- i.e. miRanda
  - **Complementarity** of nucleotides, especially at seed region
  - **Stability** of interactions by measuring free energy of miRNA-mRNA
  - **Conservation** of target site across species (More conserved --> more likely to be functionally relevant)



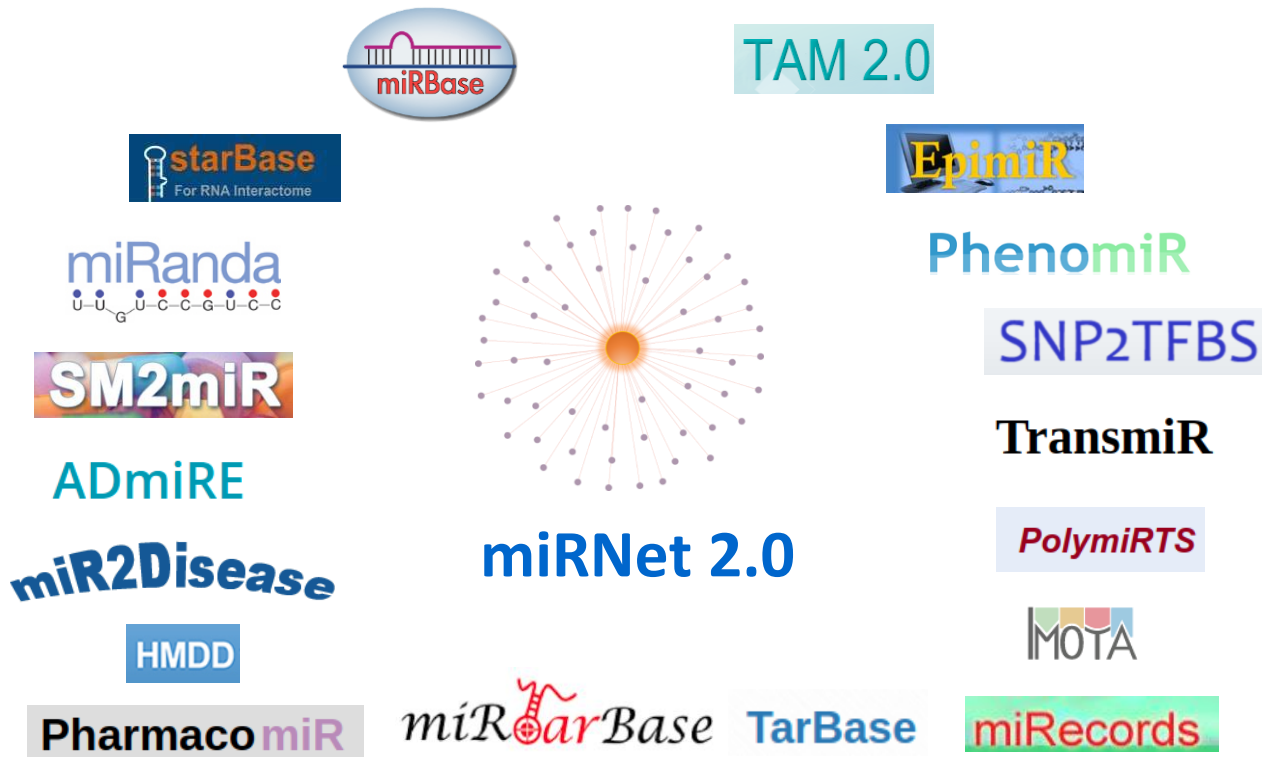
```

Query:      3' cgucucugaAUCGGUGACACUu 5'
           |||||
Ref:        5' cactcttcTGGAAACTGTGAg 3'

Energy:     -14.890000 kCal/Mol

```

# miRNet & miRNA-centric knowledgebase



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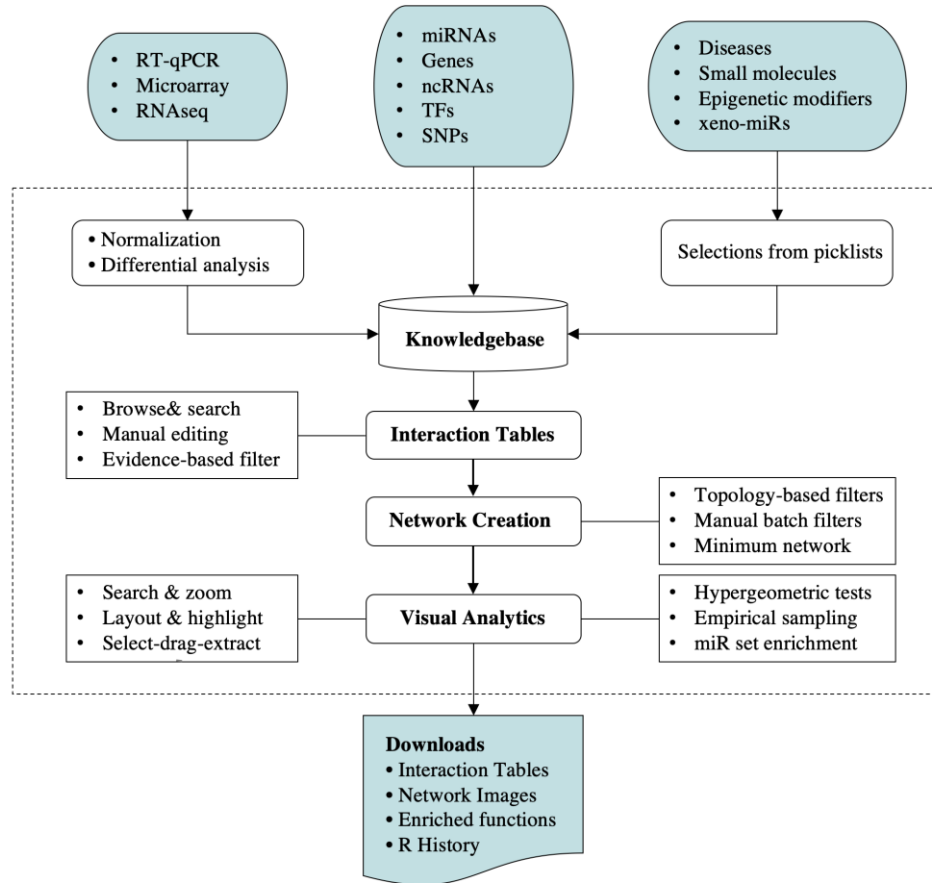
# Comparison with other tools

Tool name	miRNet		miRTargetLink	MIENTURNET	Arena- Idb	starBase
	2.0	1.0				
Data input and processing						
Species #	11	8	1	6	1	23
Target genes						
Experimental	+++	++	++	+	++	+++
Predicted	√	√	√	√	√	√
Other targets & associations						
miR-SNP	√	-	-	-	-	-
TF	√	-	-	-	-	-
ncRNA	+++	+	-	-	+	++++
xeno-miRNA	√	-	-	-	-	-
Disease	+++	++	-	-	++	++
Epigenetic modifier	√	√	-	-	-	-
Small molecule	√	√	-	-	-	-
Expression profiling	√	√	-	-	-	-
Enrichment analysis						
Hypergeometric tests	√	√	√	√	-	√
Empirical sampling	√	√	-	-	-	-
miR-set enrichment	√	-	-	-	-	-
Network visual analytics						
Multiple query types	√	-	-	-	-	-
Integration with PPI network	√	-	-	-	-	-
Multipartite network visualization	√	-	-	-	-	-
Subnetwork extraction	√	-	-	-	-	-

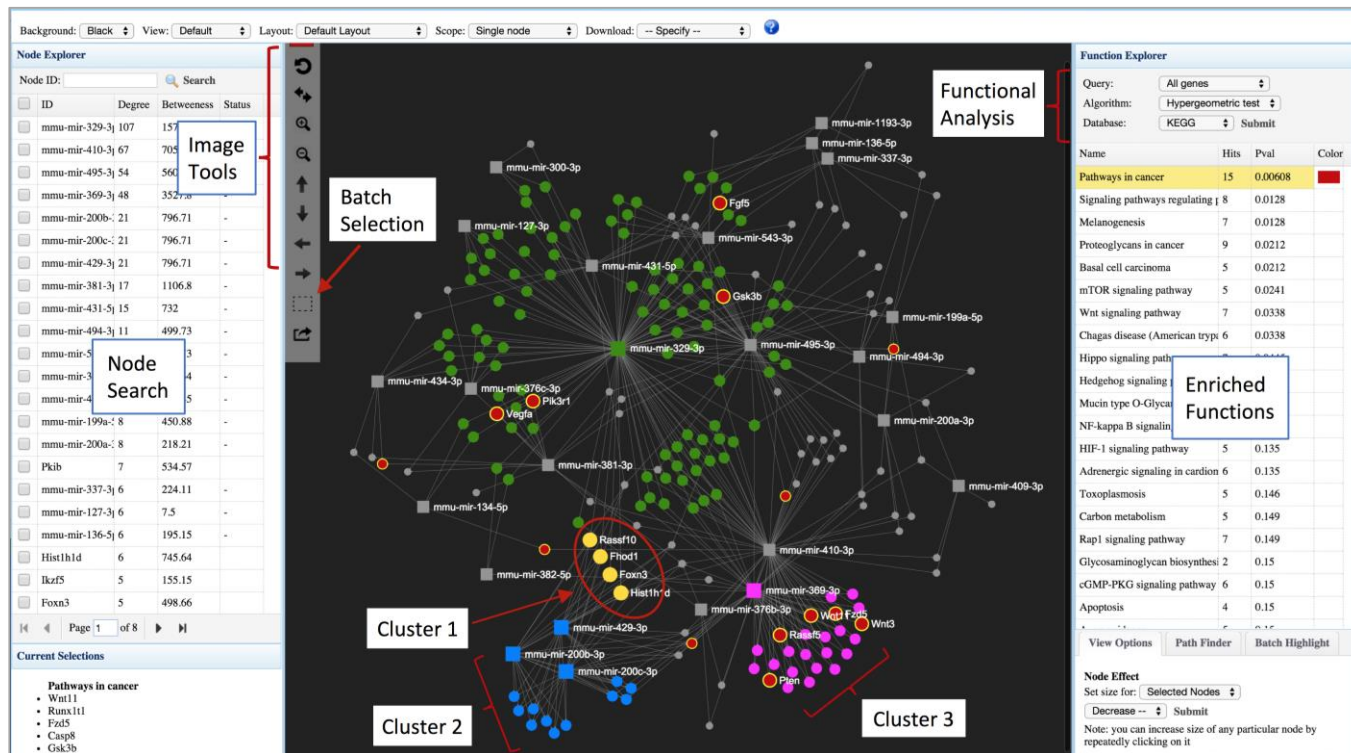
- miRTargetLink: <https://ccb-web.cs.uni-saarland.de/mirtargetlink/>
- MIENTURNET: <http://userver.bio.uniroma1.it/apps/mienturnet/>
- Arena-Idb: <http://ncrnadb.scienze.univr.it/sites/arenaidb/>
- starBase: <http://starbase.sysu.edu.cn/index.php>



# miRNet 2.0 Workflow



# Comprehensive miRNA functional analysis



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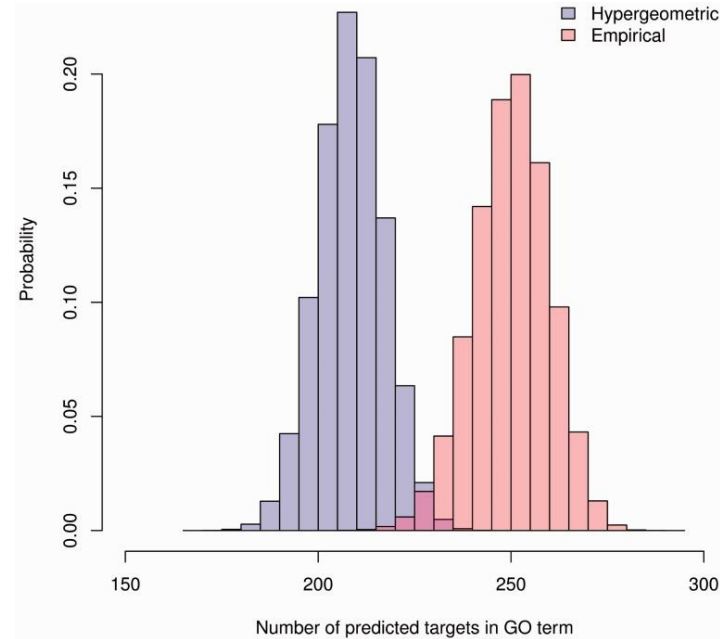
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# How to understand the functions of miRNAs?

**Standard approach:** apply hypergeometric test to identify enriched functions based on the target genes.

**Potential issue:** hypergeometric distribution describes the situation where samples are picked uniformly at random from a finite population which contains a labelled subset.

But miRNA target gene set is not random



<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4426843/>





# Using empirical sampling to address bias

The method is used to estimate the null distribution of the target genes as selected based on the input miRNAs. The procedures can be divided into the following steps:

1. A list of miRNAs of the same size are randomly selected from all the miRNAs with known targets in the database;
2. The functional annotations (i.e. KEGG) are then performed for the list;
3. The process is repeated 1000 times (default);
4. Compare the KEGG pathway hits. The empirical p values are calculated as the proportion of overlaps from 1000 random process that equal or larger than the original. A p value  $< 0.001$  will be reported if no results from random process are better than the original.

Function Explorer			
Query:	<input type="text" value="All genes"/>		
Algorithm:	<input type="text" value="Empirical sampling"/>		
Database:	<input type="text" value="KEGG"/>	<input type="button" value="Submit"/>	
Name	Hits	Pval	adj.Pval
Pentose and glucuronate interconvers	3	0.0595	0.401
Carbohydrate digestion and absorptic	5	0.0612	0.401
Rheumatoid arthritis	4	0.0748	0.401
Type I diabetes mellitus	1	0.0775	0.401
Drug metabolism - cytochrome P450	3	0.089	0.401
Steroid hormone biosynthesis	4	0.092	0.401
Autoimmune thyroid disease	1	0.0992	0.401
Drug metabolism - other enzymes	4	0.106	0.401
Taste transduction	3	0.125	0.401
Folate biosynthesis	1	0.128	0.401
Metabolism of xenobiotics by cytoch	4	0.128	0.401
Pancreatic cancer	19	0.145	0.401
Phototransduction	3	0.146	0.401

# Schedule for today

Time	Topics
9:00 – 9:10	General introduction & recap
9:15 – 9:40	Network analysis and visualization
9:45 – 10:15	NetworkAnalyst live demo & hands on
10:20 – 10:50	Gene regulatory networks
10:55 – 11:25	miRNet live demo & hands on
Summary and discussion	



# Live Demo & Hands On



# Tasks

- Understand the network building process in miRNet
  - Single input upload
  - Multi-input upload
- Explore different layouts for network visualization



# Single input

Upload

Organism

H. sapiens (human)

ID type

miRBase ID

Tissue (human only)

Kidney [551]

Targets

Selections

Include PPI (gene only)

☐ Database selection

Include tf2gene

☐ ?

miRNA list (one entry per line)

hsa-mir-101-3p  
hsa-mir-133b  
hsa-mir-147a  
hsa-mir-3140-3p  
hsa-mir-361-5p  
hsa-mir-510-5p

Submit

Targets

Selections

Include PPI (gene only)

☐

Include tf2gene

☒ Genes (miRTarBase v8.0)  
☐ Genes (TarBase v8.0)  
☐ Genes (miRecords)  
☐ lncRNAs

Try Examples

Proceed

You can select miRNA targets



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# Multi-input upload

The screenshot displays the miRNet Pro web application interface. At the top, the miRNet Pro logo is visible on the left, and navigation links for Home, Tutorials, Forum, Gallery, and Publications are on the right. The main content area features a table with input options and a modal window for selecting items.

Input Type	Click on a module below to start
Enter mixed query types	
Select queries from database	
Upload a query list	miRNAs

**Choose items**

Please choose multiple items below to proceed

Organism: H. sapiens (human)

Tissue (human): ----Not specified----

Select data types:

<input checked="" type="checkbox"/> miRNAs	<input checked="" type="checkbox"/> Genes
<input type="checkbox"/> lncRNAs	<input type="checkbox"/> circRNAs
<input type="checkbox"/> Pseudogene	<input type="checkbox"/> sncRNAs
<input type="checkbox"/> Transcription factors	<input type="checkbox"/> Diseases
<input type="checkbox"/> Small compounds	<input type="checkbox"/> Epigenetic modifiers

OK Cancel

Epigenetic modifiers

ncRNAs

Transcription factors

Xeno-miRs: [Search](#) [Browse](#)










miRNet Pro-2024.1



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# Multi-input upload

 > Upload        

### Upload multiple lists

Please use the tabs below to prepare your input for: **H. sapiens (human)**. Make sure to click **Submit** button before switching to the next tab.

Input tabs 

miRNAs

Genes

ID type

miRBase ID

Target type

Genes (miRtarBase v8.0)

miRNA list  
(one entry per line)

hsa-mir-101-3p  
hsa-mir-133b  
hsa-mir-147a  
hsa-mir-3140-3p  
hsa-mir-361-5p  
hsa-mir-510-5p

Submit

[Try example](#)

Data uploaded:


☐ mimu

☐ gene

Include PPI: ☐

Database selection

Proceed

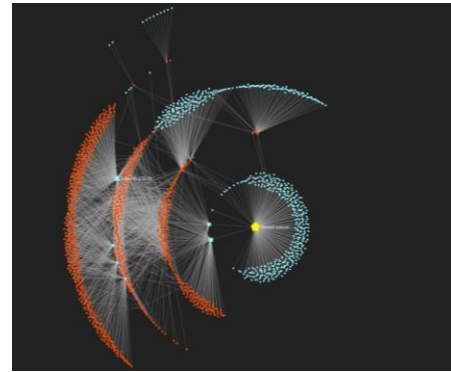
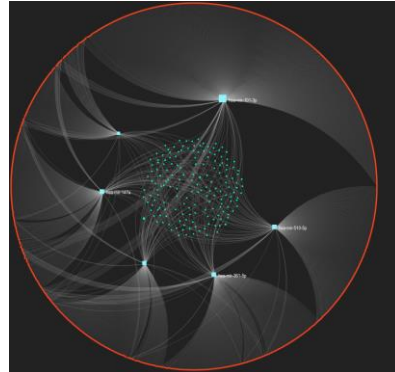
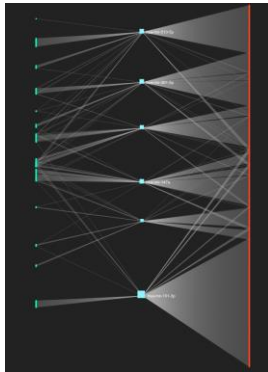
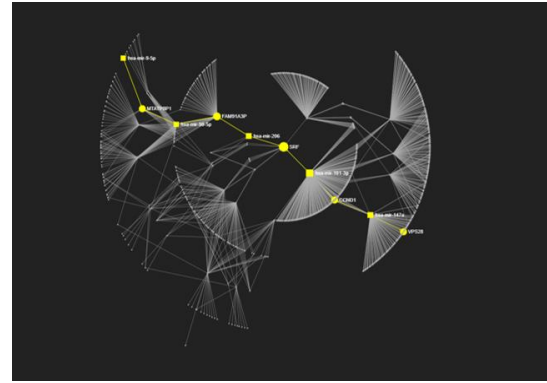
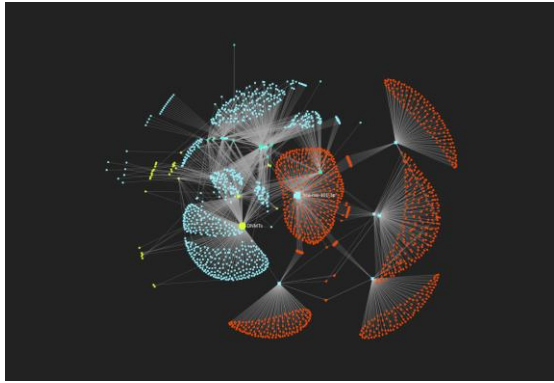
Upload progress 



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# Network Layout




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# Next Lecture

Topic	Date	Lecture	Lab
Omics Data Science Foundations	Jan. 6	Omics data processing, statistics and visualization	--
	Jan. 13	From raw data to functional insights	--
Transcriptomics	Jan. 20	Gene expression data analysis (part I)	ExpressAnalyst
	Jan. 27	Gene expression data analysis (part II)	ExpressAnalyst
miRNAs & non-coding RNAs	Feb. 3	MicroRNAs, noncoding RNAs and biological networks	miRNet & NetworkAnalyst
 Proteomics	Feb. 10	Proteomics data analysis and integration	ExpressAnalyst & NetworkAnalyst
Metabolomics	Feb. 17	Targeted metabolomics data analysis	MetaboAnalyst
	Feb. 24	LC-MS untargeted metabolomics data analysis	MetaboAnalyst
Microbiomics	Mar. 2	Marker gene data analysis	MicrobiomeAnalyst
	Mar. 9	Shotgun metagenomics data analysis	MicrobiomeAnalyst
Multi-omics	Mar. 16	Knowledge-driven multi-omics integration	OmicsNet
	Mar. 23	Data-driven multi-omics integration	OmicsAnalyst



# Comprehensive protocols (76 pages)


**Basic Protocol 1:** RNA-seq count table uploading, processing, and normalization

**Basic Protocol 2:** Differential expression analysis with linear models

**Basic Protocol 3:** Functional analysis with volcano plot, enrichment network, and ridgeline visualization

**Basic Protocol 4:** Hierarchical clustering analysis of transcriptomics data using interactive heatmaps

**Basic Protocol 5:** Cross-species gene expression analysis based on ortholog mapping results

 **Basic Protocol 6:** Proteomics and microarray data processing and normalization

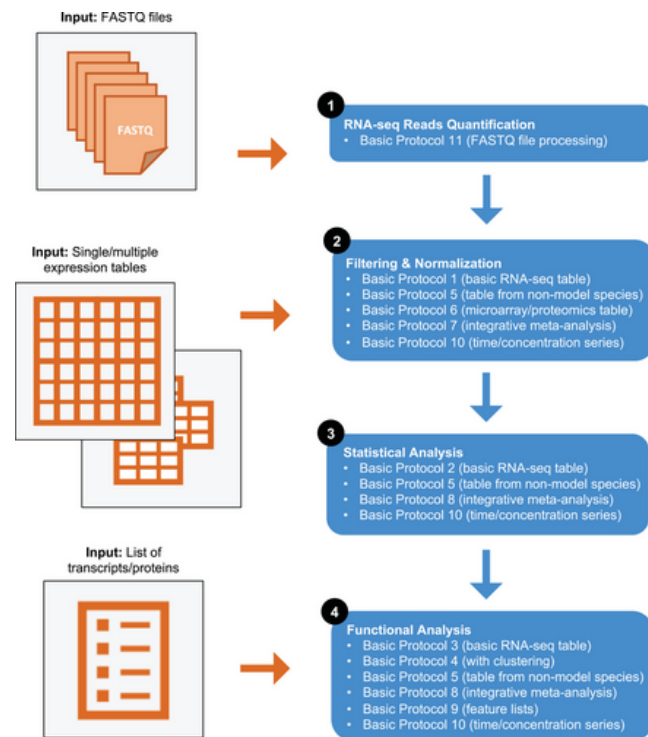
**Basic Protocol 7:** Preparing multiple gene expression tables for meta-analysis

**Basic Protocol 8:** Statistical and functional meta-analysis of gene expression data

**Basic Protocol 9:** Functional analysis of transcriptomics signatures

**Basic Protocol 10:** Dose-response and time-series data analysis

**Basic Protocol 11:** RNA-seq reads processing and quantification with and without reference transcriptomes



# Tutorials



PROTOCOL | Open Access |

## Using ExpressAnalyst for Comprehensive Gene Expression Analysis in Model and Non-Model Organisms

Jessica Ewald, Guangyan Zhou, Yao Lu, Jianguo Xia

First published: 06 November 2013

Metabolomics (2013) 9:280–299  
DOI 10.1007/s11306-012-0482-9

REVIEW ARTICLE

## Translational biomarker discovery in clinical metabolomics: an introductory tutorial

Jianguo Xia · David I. Broadhurst ·  
Michael Wilson · David S. Wishart

**Basic Protocol 6: Proteomics data processing and normalization**

**biomarker analysis**



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**We would like to hear your  
comment & feedback**

[contact@xialab.ca](mailto:contact@xialab.ca)

**See (most of) you next week!**

