

# Canadian Bioinformatics Workshops

[www.bioinformatics.ca](http://www.bioinformatics.ca)

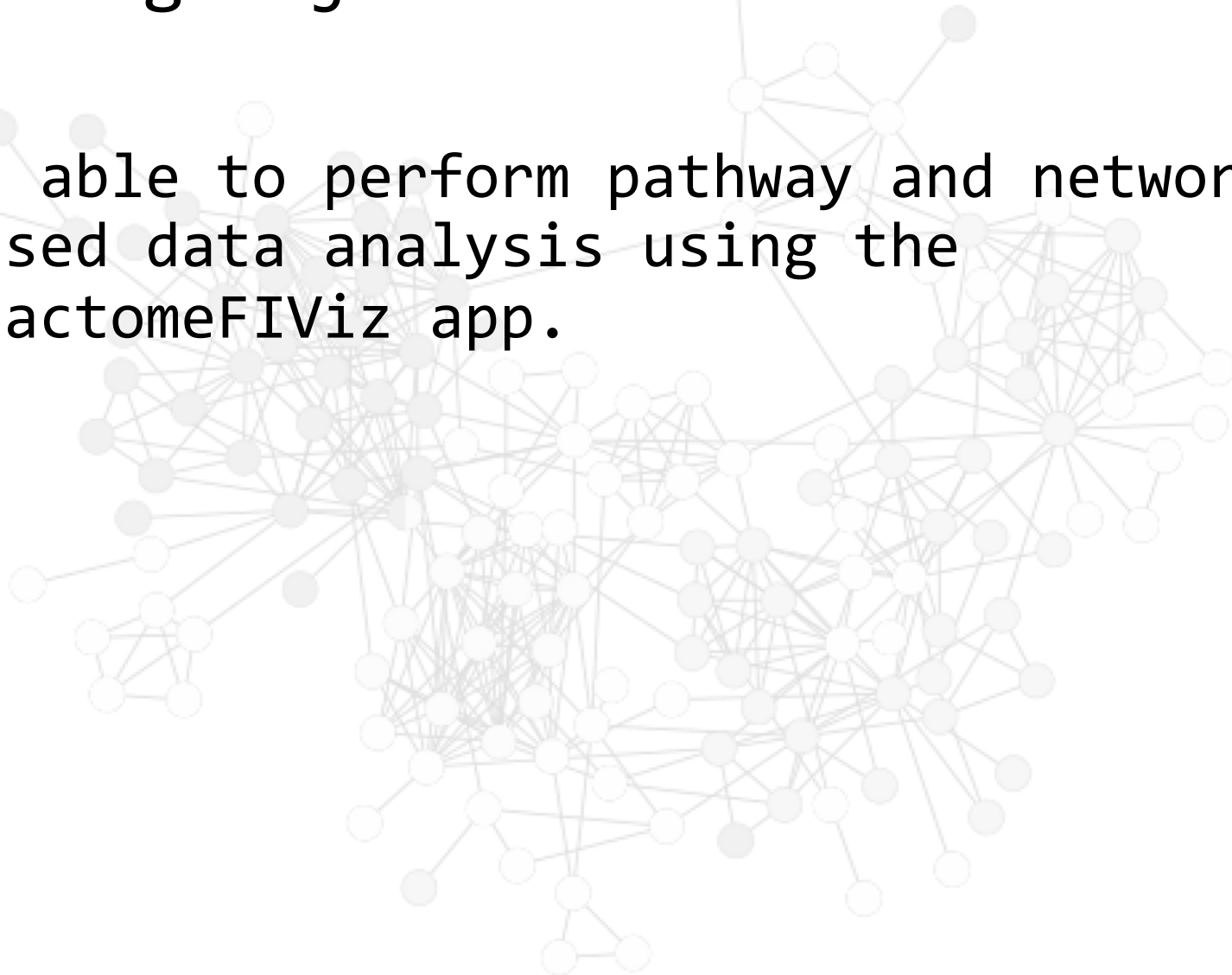
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Supported by



# Learning Objectives of Module

- Be able to perform pathway and network-based data analysis using the ReactomeFIViz app.



# Major Features in ReactomeFIViz

- Pathway and Network Analysis Features:
  - Support pathway enrichment analysis: Binomial test and GSEA.
  - Integration of Reactome Voronoi map for pathway holistic view.
  - Perform Boolean network and PGM-based pathway modeling
  - Construct FI subnetwork.
  - Support for HotNet analysis.
  - Perform network clustering.
  - Overlay cancer gene index, gene and cancer drug annotations.
  - Support to mouse pathways and FI network.
  - scRNA-seq data analysis and visualization.
  - Perform survival analysis.

# A1) Reactome Pathway Enrichment Analysis

The screenshot displays the Reactome software interface. The main window, titled "Diagram of Cell Cycle Checkpoints", shows a hierarchical pathway diagram. The nodes are represented as boxes: "MCC:APC/C complex" at the top, followed by "phosphorylated anaphase promoting complex (APC/C)", "hBUBR1:hBUB3:MAD2\*:CDC20 complex", "BUB1B", "BUB3", "CDC20", "MAD1L1", and "Kinetochores Complex". A "Slide to Zoom" slider is located below the diagram. A context menu is open over the "Cell Cycle Checkpoint" node in the left-hand "Control Panel". The menu options are:

- View Reactome Source
- View in Reactome
- Show Diagram
- Search
- Analyze Pathway Enrichment
- Perform GSEA Analysis
- Run Graphical Model Analysis
- Load Graphical Model Results
- View Cancer Drugs
- View DrugCentral Drugs
- Expand Pathway
- Collapse Pathway
- Open Reactome Reactoam

The interface also includes a "Control Panel" with tabs for "Network", "Style", "Select", and "Reactome". At the bottom, there are tabs for "Node Table", "Edge Table", and "Network Table", along with a "Memory" indicator.



# Displaying Reactome Pathways in the FI Network View

The screenshot displays the FI Network View interface for a Reactome pathway. The main window shows a network diagram with nodes representing proteins and their interactions. A control panel on the left allows navigation through the pathway hierarchy, with 'Mitotic Spindle Checkpoint' selected. Below the control panel is a zoomed-in view of a specific sub-network. A table panel at the bottom lists the entities in the network. A context menu on the right provides various analysis and visualization options.

**Control Panel - Selected Event Branch:**

- Cell Cycle
  - Cell Cycle Checkpoints
    - G1/S DNA Damage Checkpoints
    - G2/M Checkpoints
    - Mitotic Spindle Checkpoint**
  - Cell Cycle, Mitotic
  - Chromosome Maintenance
  - Meiosis
  - Cell-Cell communication
  - Cellular responses to stress
  - Chromatin organization
  - Circadian Clock
  - Developmental Biology
  - DNA Repair
  - DNA Replication
  - Extracellular matrix organization

**Table Panel:**

shared name	name	module	commonName	nodeToolTip	nodeLabel
BABAM1	BABAM1	BABAM1	BABAM1	BABAM1	BABAM1
BRCA1	BRCA1	BRCA1	BRCA1	BRCA1	BRCA1
PSMB1	PSMB1	PSMB1	PSMB1	PSMB1	PSMB1
PSME2	PSME2	PSME2	PSME2	PSME2	PSME2
HERC2	HERC2	HERC2	HERC2	HERC2	HERC2
HIST3H2BB	HIST3H2BB	HIST3H2BB	HIST3H2BB	HIST3H2BB	HIST3H2BB
PSME1	PSME1	PSME1	PSME1	PSME1	PSME1
HIST1H2BO	HIST1H2BO	HIST1H2BO	HIST1H2BO	HIST1H2BO	HIST1H2BO
KDM4B	KDM4B	KDM4B	KDM4B	KDM4B	KDM4B

**Context Menu:**

- Convert to FI Network
- Run Logic Model Analysis
- Remove Analysis Results
- Run Graphical Model Analysis
- Show Gene Level Analysis Results
- Show Observation
- Save Analysis Results
- Open Analysis Results
- Overlay Gene Scores
- Remove Gene Scores
- Fetch Cancer Drugs
- Fetch DrugCentral Drugs
- Filter Drugs
- Load Mechismo Results
- Remove Mechismo Results
- Search Entities
- Search Reactions
- Export Diagram

# Pathway Enrichment Analysis

Session: New Session

Control Panel

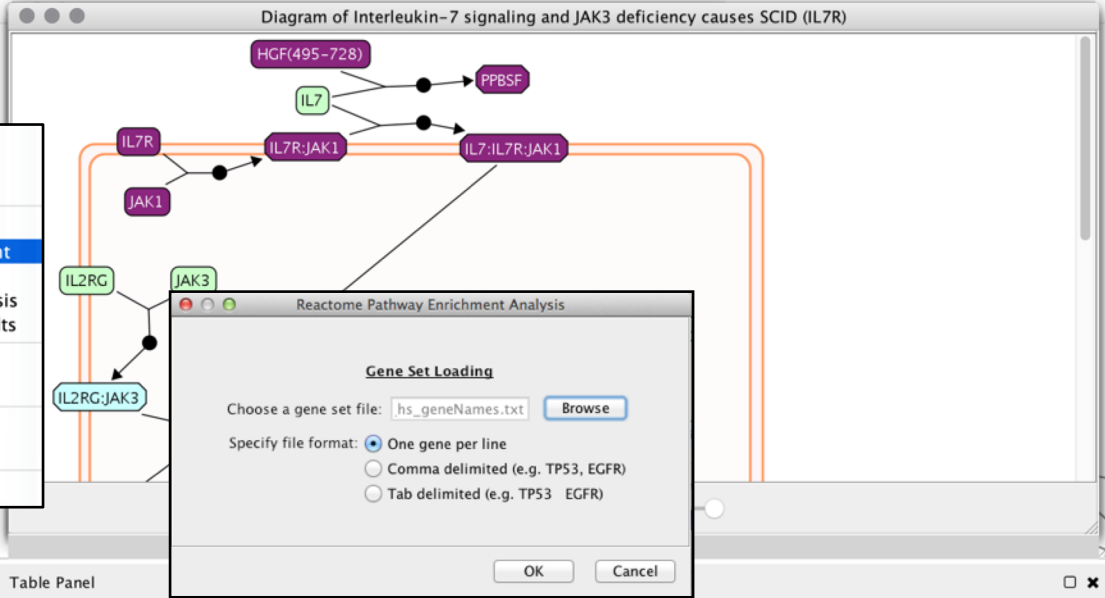
Network Style Select Reactome

FDR:  $\geq 0.1$   $\geq 0.01$   $\geq 0.001$   $< 0.001$

- Interleukin-7 signaling (FDR: 0.091)
  - IL7 binds HGF(495-728)
  - IL7R binds JAK1
  - IL2RG binds JAK3
  - IL7 binds IL7R:JAK1
  - IL7:IL7R:JAK1 binds IL7
  - IL7 is phosphorylated
  - IL7-p-Y449-IL7R:JAK1
  - IL7-p-Y449-IL7R:JAK1
  - IL7-p-Y449-IL7R:JAK1
  - Interleukin-11 receptor a
  - Interleukin-11 receptor a
  - IL27 is a dimer of IL27A a
  - IL27 binds IL27RA:gp130

Selected Event Branch

- Immune System (FDR: 0.291)
  - Cytokine Signaling in Immune system (FDR: 0.089)
    - Signaling by Interleukins (FDR: 0.045)
      - Interleukin-7 signaling (FDR: 0.091)



Reactome Pathway Enrichment Analysis

**Gene Set Loading**

Choose a gene set file:

Specify file format:

- One gene per line
- Comma delimited (e.g. TP53, EGFR)
- Tab delimited (e.g. TP53 EGFR)

Table Panel

Apply Filters: FDR 1.0

ReactomePathway	RatioOfProteinInPat...	NumberOfProteinIn...	ProteinFromGeneSet	P-value	FDR	HitGenes
Laminin interactions	0.0032	23	5	0.0111	0.0890	ITGA6,LAMC1,LAMB...
Activation of Rac	0.0011	8	3	0.0114	0.0911	ROBO1,SOS1,SLIT2
Interleukin-7 signali...	0.0011	8	3	0.0114	0.0911	IL7R,HGF,JAK1
CREB phosphorylati...	0.0021	15	4	0.0115	0.0917	GRIN2B,GRIN2A,AKA...
Mismatch Repair	0.0021	15	4	0.0115	0.0917	RPA1,POLD3,MLH1,...
M Phase	0.0315	225	22	0.0117	0.0937	KNTC1,NDE1,UBC,A...
GABA receptor activ...	0.0074	53	8	0.0124	0.0993	GABBR2,GNAT3,GAB...
Mitotic Prometaphase	0.0139	99	12	0.0131	0.1016	KNTC1,NDE1,PD55B,...
Digestion of dietary ...	0.0004	3	2	0.0132	0.1016	MGAM,LT
Mitotic Anaphase	0.0227	162	17	0.0136	0.1016	KNTC1,NDE1,UBC,A...
TGF-beta receptor s...	0.0022	16	4	0.0142	0.1016	UBC.PARD3,TCFBR2...

# Gene Set Enrichment Analysis (GSEA)

Diagram of Cell Cycle Checkpoints

Results Panel

Diffusion Output **Gene Scores**

**Gene scores and ranks:**

Gene	Score	Rank
CDK2	3.358	5
WEE1	2.743	29
CENPT	2.343	103
CCNA2	2.224	130
PCBP4	2.087	204
MCM3	2.032	235
CLASP1	1.969	271
NSD2	1.751	442
RPA1	1.677	540
ANAPC4	1.668	553
YWHAH	1.424	938
CDC20	1.338	1125
CKAP5	1.329	1136
SGO1	1.324	1147
CDC23	1.277	1263
KIF2A	1.265	1295
ATR	1.129	1633
KDM4A	1.123	1657
MCM5	1.092	1744
CDC7	1.061	1824
XPO1	1.008	1961
RAD17	1.003	1976
YWHAE	0.992	2013
EXO1	0.989	2025
CDC16	0.972	2078
CCNA1	0.905	2317
KNTC1	0.903	2323
HERC2	0.897	2347
ANAPC5	0.883	2398
ITGB3BP	0.867	2458
NUP133	0.849	2520
CENPK	0.822	2630
ORC2	0.777	2774
ZNF385A	0.769	2802

Filter genes to diagram

Table View Plot View

Reactome GSEA Analysis

Data

Choose a gene score file:  Browse

*Note: The gene score file should contain at least two tab-delimited columns, first for human gene symbols and second for scores. The first row should be for column headers.*

Configuration

Choose pathways having sizes: Minimum:  Maximum:

Set number of permutations:

OK Cancel

# Visualize Cancer Targetome in Reactome Pathways

Diagram of Cell Cycle Checkpoints

Control Panel

- Cell Cycle
  - Cell Cycle Checkpoints
    - G1/S DNA Damage Checkpoints
    - G2/M Checkpoints
      - G2/M DNA damage checkpoint
      - G2/M DNA replication checkpoint
    - Activation of ATR in response to replication stress
      - Stalling of DNA replication fork and RPA binding
      - Binding of ATR-ATRIP to the RPA-ssDNA complex
      - Recruitment of Rad17-RFC complex to DNA
      - Recruitment of the Rad9-Hus1-Rad1 complex to C
      - Loading of claspin onto DNA during replication or
      - Activation of claspin
      - Recruitment and activation of Chk1
      - Phosphorylation of Cdc25A at Ser-123 by Chk1
      - Phosphorylation of Cdc25C at Ser 216 by Chk1
    - TP53 binds TP53
    - TP53 promotes translocation of TP53 to the cytosol
    - TP53 facilitates proteasome-mediated degradation of
  - Mitotic Spindle Checkpoint
  - Cell Cycle, Mitotic
  - Chromosome Maintenance
  - Meiosis
  - Cell-Cell communication
  - Cellular responses to stress
  - Chromatin organization
  - Circadian Clock
  - Developmental Biology
  - DNA Repair
  - DNA Replication
  - Extracellular matrix organization
  - Gene Expression
  - Hemostasis
  - Immune System
  - Mitophagy
  - Metabolism

Selected Event Branch

- Cell Cycle
  - Cell Cycle Checkpoints

Table Panel

Drag table files here

Node Table Edge Table

Memory

Convert to FI Network

Run Logic Model Analysis

Remove Analysis Results

Run Graphical Model Analysis

Show Gene Level Analysis Results

Show Observation

Save Analysis Results

Open Analysis Results

Overlay Gene Scores

Remove Gene Scores

Fetch Cancer Drugs

Fetch DrugCentral Drugs

Filter Drugs

Load Mechismo Results

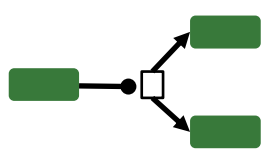
Remove Mechismo Results

Search Entities

Search Reactions

Export Diagram

# A2) De Novo Subnetwork Construction & Clustering



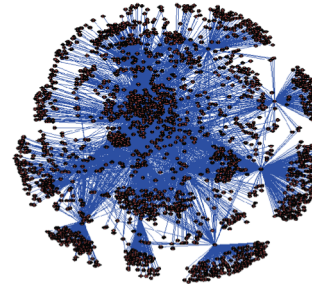
Curated Pathway DBs

+



Uncurated Interaction Evidence

Machine Learning



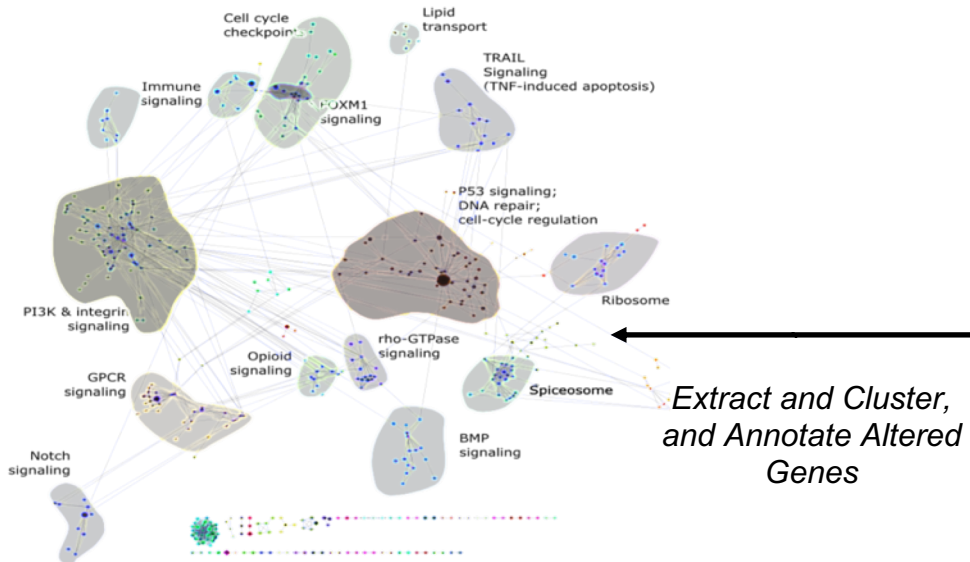
Reactome Functional Interaction Network  
(~13K proteins; ~436K interactions)

+



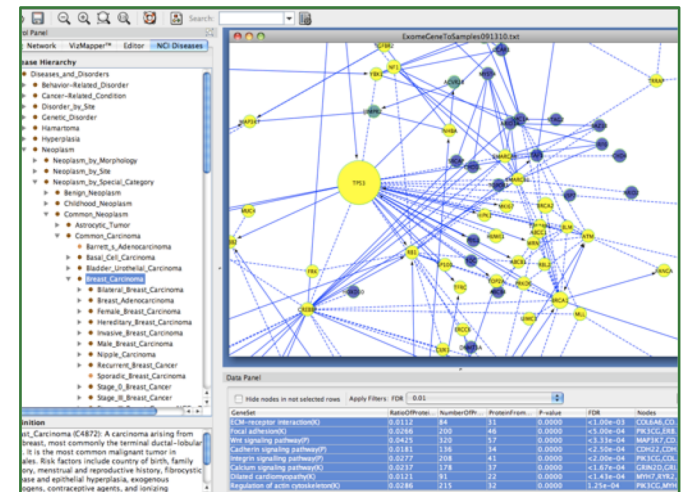
DATA

Project your data into Reactome FI Network



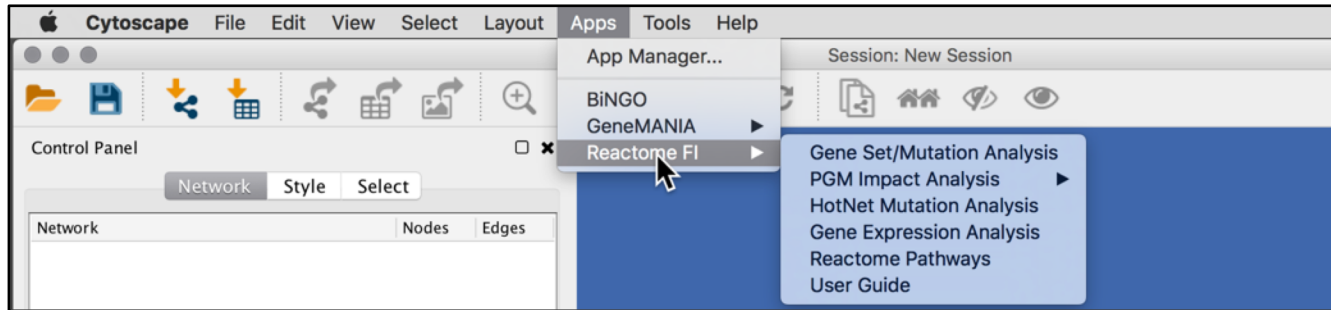
Extract and Cluster, and Annotate Altered Genes

Disease "modules" (10-30)



Cytoscape using ReactomeFIViz app

# Upload your data

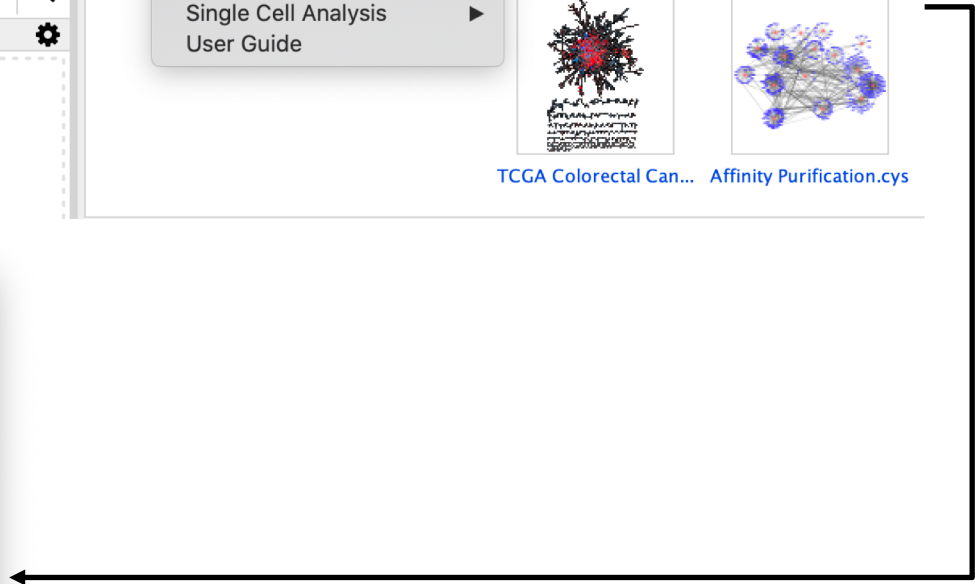
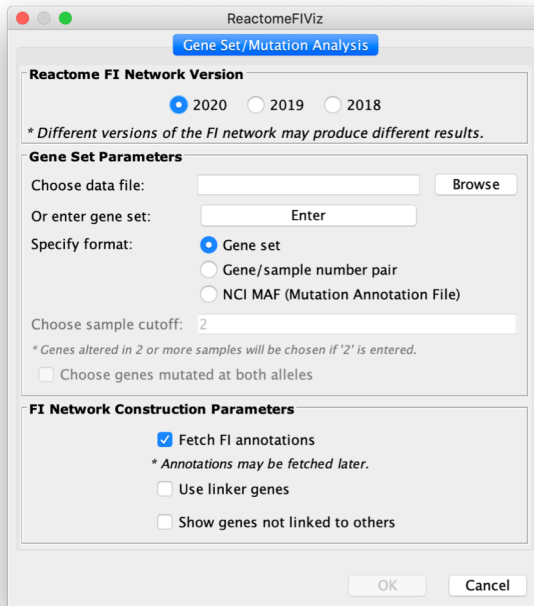
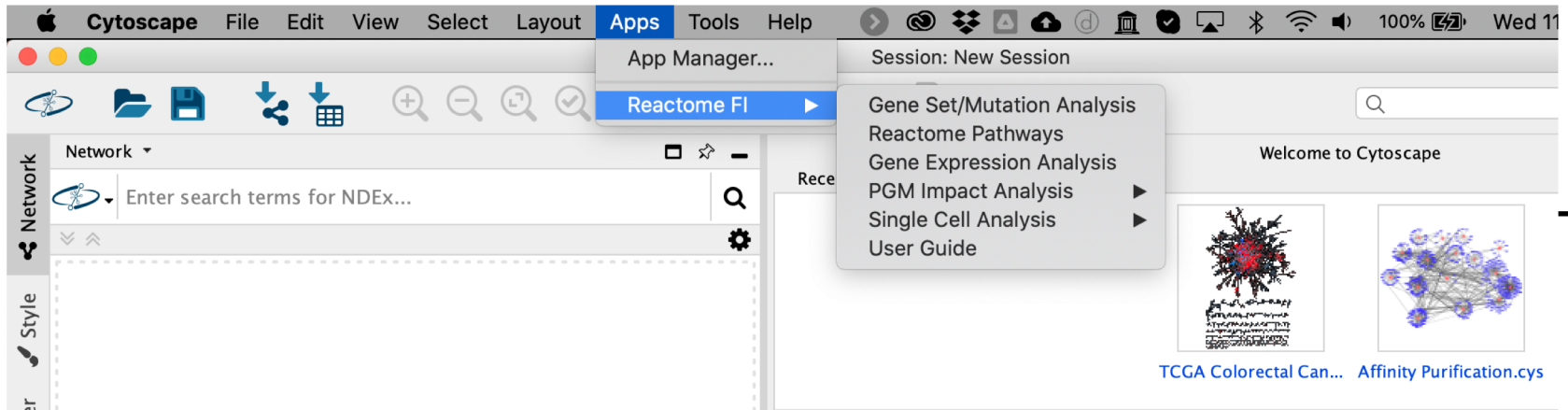


- FI plug-in supports four file formats:
  - Simple gene set: one line per gene
  - Gene/sample number pair. Contains two required columns, gene and number of samples having gene mutated, and an optional third column listing sample names (delimited by semi-colon ;)
  - NCI MAF (mutation annotation file)
  - Sample Gene Expression data file





# Gene Set-based Analysis





# FI Results Display

The screenshot displays a software interface for network visualization. The main window, titled "LAC\_genelist.txt", shows a dense network graph with numerous green circular nodes and black edges. A context menu is open over a node, with the "Apps" option selected, leading to a sub-menu where "Reactome FI" is chosen. This sub-menu includes options like "Fetch FI Annotations", "Analyze Network Functions", "Cluster FI Network", "Analyze Module Functions", and "Load Cancer Gene Index".

In the bottom-left corner, there is a smaller, semi-transparent view of the network graph. Below the main graph is a "Table Panel" with a table of node data. The table has columns for shared name, name, module, commonName, nodeToolTip, nodeLabel, and nodeType. The data rows are as follows:

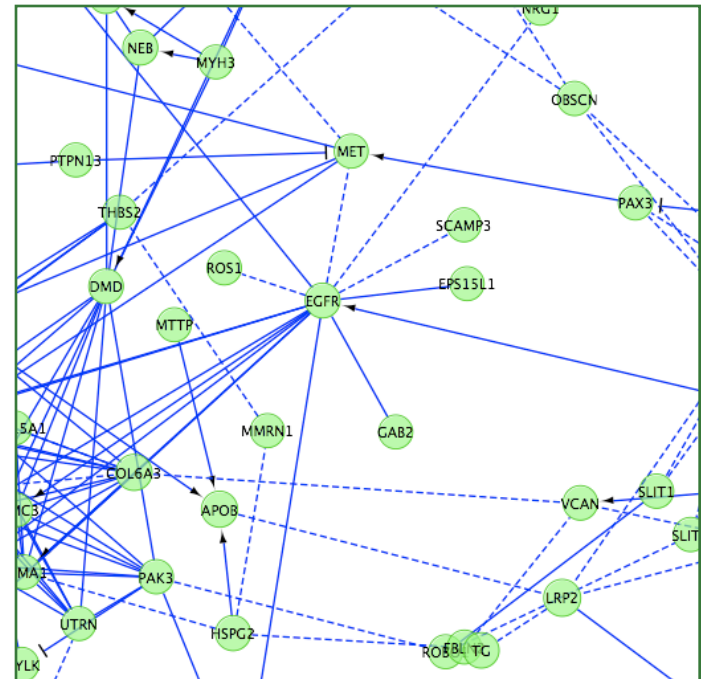
shared name	name	module	commonName	nodeToolTip	nodeLabel	nodeType
ACTB	ACTB		ACTB	ACTB	ACTB	Gene
ITGAV	ITGAV		ITGAV	ITGAV	ITGAV	Gene
RAD21	RAD21		RAD21	RAD21	RAD21	Gene
YAP1	YAP1		YAP1	YAP1	YAP1	Gene
JUN	JUN		JUN	JUN	JUN	Gene
MAP2K7	MAP2K7		MAP2K7	MAP2K7	MAP2K7	Gene
FOS	FOS		FOS	FOS	FOS	Gene
UBC	UBC		UBC	UBC	UBC	Gene
USF1	USF1		USF1	USF1	USF1	Gene

At the bottom of the interface, there are tabs for "Node Table", "Edge Table", and "Network Table", with "Node Table" currently selected. A "Memory" indicator is visible in the bottom right corner.

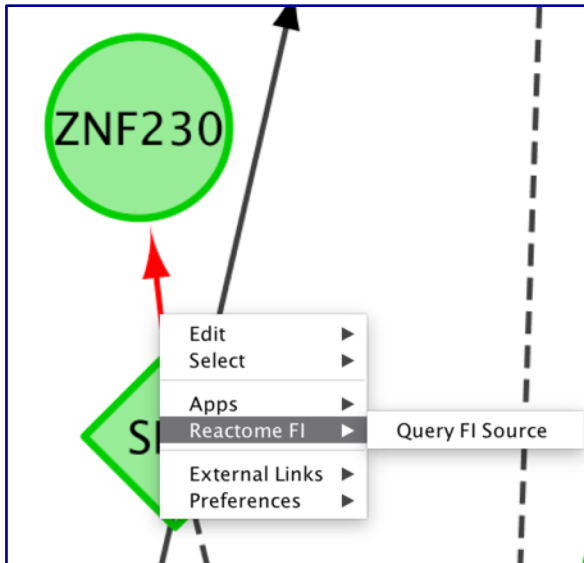
- Constructed network is displayed in the Network View panel using an FI specific visual style

# FI Annotations

- Provides detailed information on selected FIs.
- Three edge attributes are created:
  - FI Annotation.
  - FI Direction.
  - FI Score (for predicted FI).
- Edges display direction attribute values.
  - --> for activating/catalyzing.
  - --| for inhibition.
  - solid line for complexes or inputs.
  - --- for predicted FIs.



# Query FI Source



## Annotated FIs

Interaction Info  
Interaction: SIX5 - ZNF230

Reactome Sources

Reactome ID	Type	Data Source
6951674	TARGETED_INTERAC	ENCODE

View Reactome Source

Reactome Instance View

classType	TargetedInteraction
dbId	6951674
displayName	SIX5 ZNF230
dataSource	<a href="#">ENCODE</a>
definition	ENCODE proximal_filtered TF/target interaction; supported by GO BP sharing
factor	<a href="#">SIX5</a>
species	<a href="#">Homo sapiens</a>
target	<a href="#">ZNF230</a>

Close

## Predicted FIs

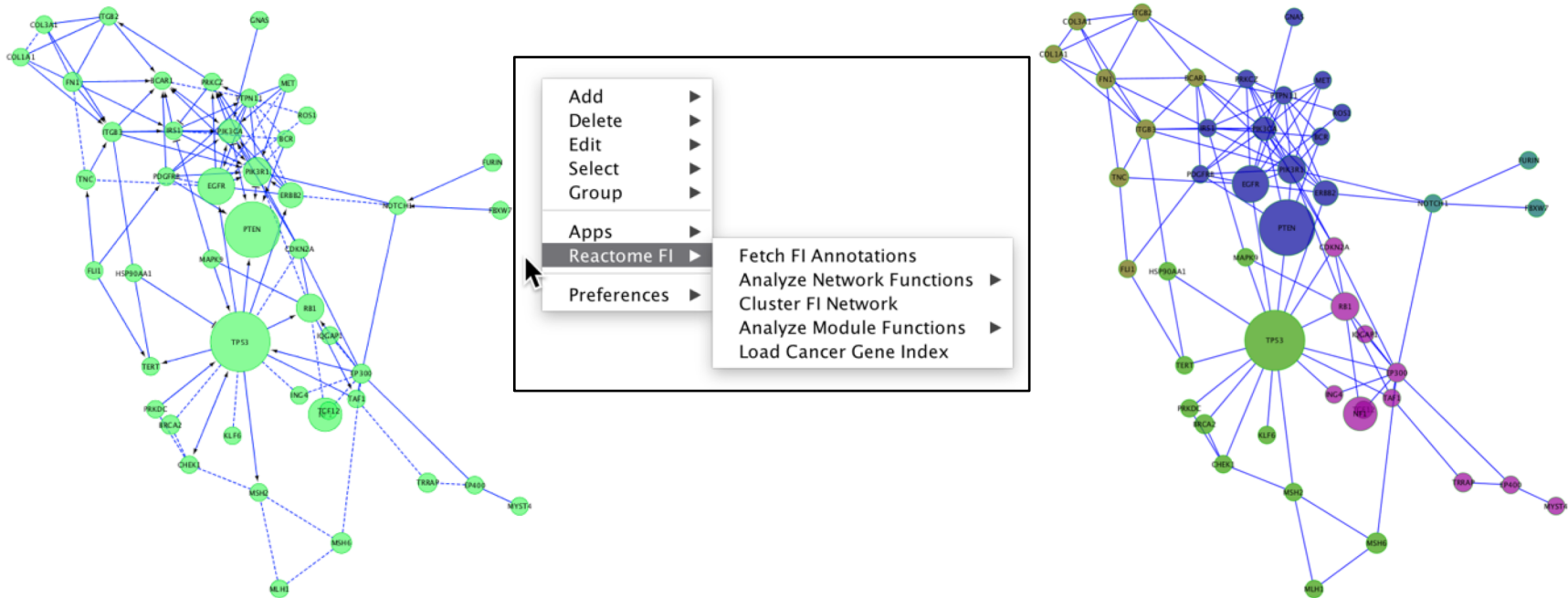
Interaction Info  
Interaction: SNX4 - SNX6

Support Evidence

Predictor	Value
Human Interaction	true
Yeast Interaction	false
Fly Interaction	false
Worm Interaction	false
Mouse Interaction	false
Pavlidis Gene Exp	false
Carlos Gene Exp	false
PfamDomain Interaction	true
GO BP Sharing	true
Score	0.9642255584531738

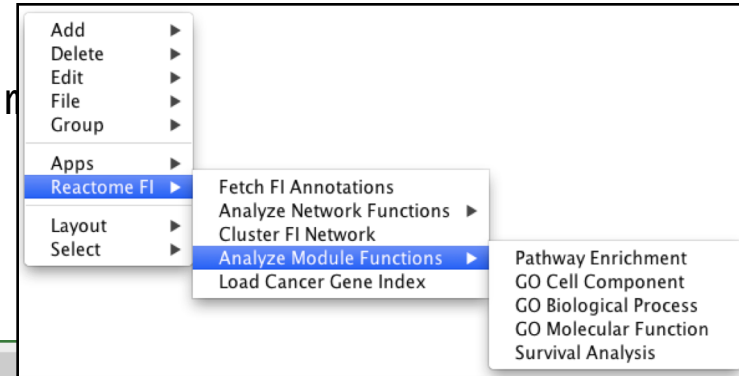
# Cluster FI Network

- Runs spectral partition based network clustering ([Newman, 2006](#)) on the displayed FI network.
- Nodes in different network modules will be shown in different colours (max 15 colours).
- Analyze cancer mutation data with HotNet algorithm ([Vandin, 2012](#))



# Analyze Module Functions

- Pathway or GO term enrichment analysis on individual network modules.
  - Use filter to remove small network modules
  - Filter by FDR



Data Panel

Hide nodes in not selected rows    Apply Filters: FDR 0.01    Module Size 10

Module	GeneSet	RatioOfProteinIn	NumberOfProteinCe...	Protein	mModule	FDR	Nodes
0	Translation(R)	0.0158	0.0010		0		RPL18,RPL17,RPL36...
0	Influenza Life Cycle(R)	0.0249	0.0050		0	<2.50e-04	RPL18,RPL17,RPL36...
0	Ribosome(K)	0.0117	✓ 0.01		0	<2.50e-04	RPL18,RPL17,RPL36...
0	Metabolism of protei...	0.0267	0.05		0	<2.50e-04	RPL18,RPL17,RPL36...
1	M Phase(R)	0.0128	0.25	119	0	<5.00e-04	ITGB3BP,MAD1L1,CC...
1	Aurora B signaling(N)	0.0052	0.5	187	0	<5.00e-04	AURKC,AURKB,CDCA...
1	Signaling by Aurora k...	0.0125	1.0	88	0	<3.33e-04	AURKC,AURKB,CDCA...
2	Glucose Regulation of...	0.0197		148	16	0.0000	NDUFB4,NDUFA2,ND...
2	Parkinson's disease(K)	0.0176		132	15	0.0000	NDUFB4,NDUFA2,ND...
2	Alzheimer's disease(K)	0.0223		168	15	0.0000	NDUFB4,NDUFA2,ND...
2	Huntington's disease(K)	0.0246		185	15	0.0000	NDUFB4,NDUFA2,ND...
2	Oxidative phosphoryl...	0.0178		134	15	0.0000	NDUFB4,NDUFA2,ND...
2	Electron Transport C...	0.0101		76	16	0.0000	NDUFB4,NDUFA2,ND...
2	Metabolic pathways(K)	0.1489		1120	16	0.0000	NDUFB4,NDUFA2,ND...
3	Antigen processing a...	0.0104		78	9	0.0000	KLRC2,KIR2DS1,HLA...
3	Immunoregulatory int...	0.0153		115	8	0.0000	KIR2DS1,HLA-B,HLA...
3	Natural killer cell me...	0.0182		137	7	0.0000	KLRC2,KIR2DS1,HLA...

Node Attribute Browser    Edge Attribute Browser    Network Attribute Browser    MCL Module Browser    Pathways in Modules

# Show Pathway Diagrams

- Select a pathway in "Pathways in Network/Modules" tabs, right click, select "Show Pathway Diagram"

Data Panel

Hide nodes in not selected rows Apply Filters:

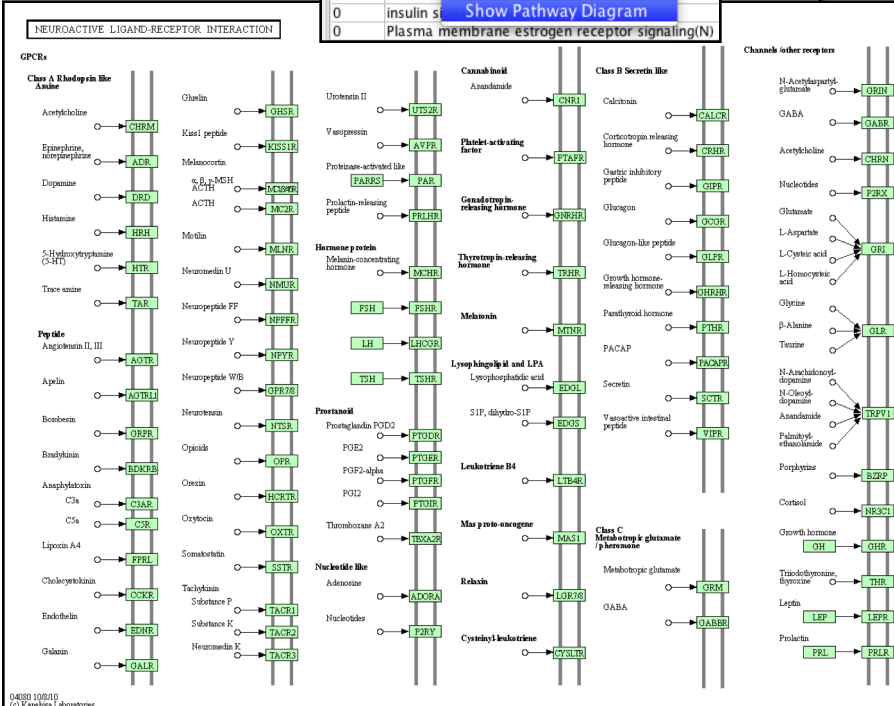
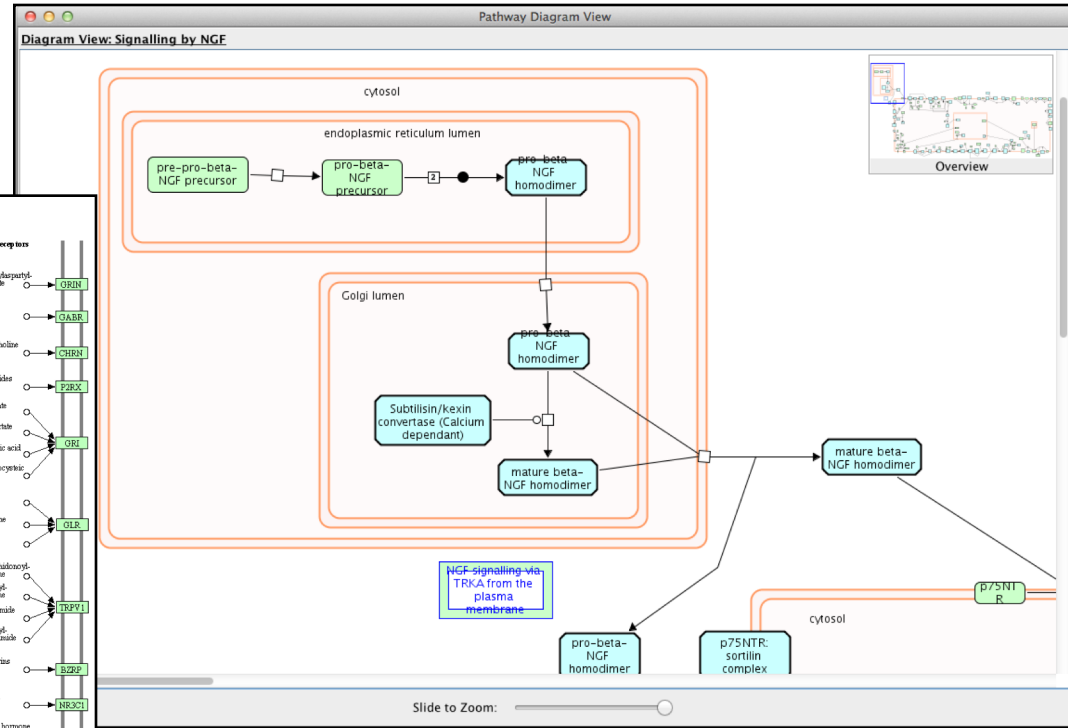
Module	GeneSet
0	Arf6 signaling events(N)
0	Insulin Pathway(N)
0	Melanin signaling(N)
0	IGF1 pathway(N)
0	Endothelin signaling(N)
0	Prostate cancer(N)
0	insulin signaling(N)
0	Plasma membrane estrogen receptor signaling(N)

Export Annotations

Show Pathway Detail

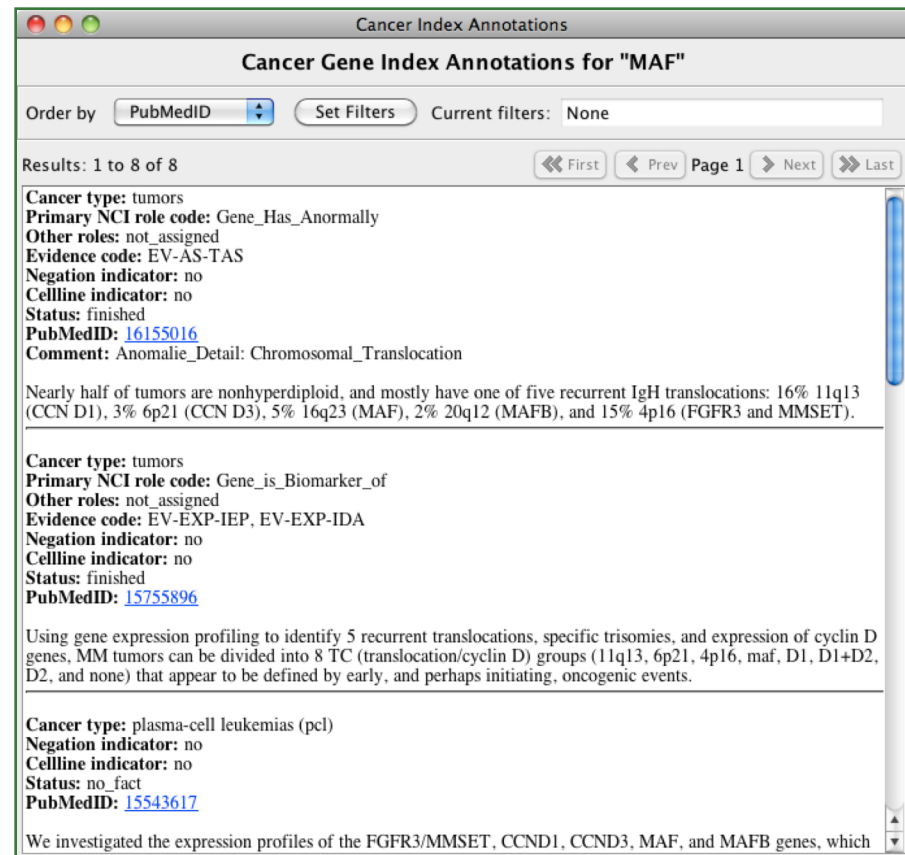
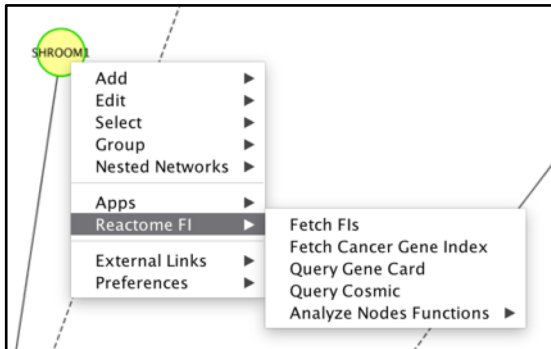
Show Pathway Diagram

Plasma membrane estrogen receptor signaling(N)



# NCI Cancer Gene Index

- View detailed annotations for the selected gene or protein.
- Annotations are sortable by PubMed ID, Cancer type, status, and other criteria.



Cancer Index Annotations

Cancer Gene Index Annotations for "MAF"

Order by PubMedID Set Filters Current filters: None

Results: 1 to 8 of 8

« First < Prev Page 1 > Next » Last

**Cancer type:** tumors  
**Primary NCI role code:** Gene\_Has\_Anormally  
**Other roles:** not\_assigned  
**Evidence code:** EV-AS-TAS  
**Negation indicator:** no  
**Cellline indicator:** no  
**Status:** finished  
**PubMedID:** [16155016](#)  
**Comment:** Anomalie\_Detail: Chromosomal\_Translocation

Nearly half of tumors are nonhyperdiploid, and mostly have one of five recurrent IgH translocations: 16% 11q13 (CCN D1), 3% 6p21 (CCN D3), 5% 16q23 (MAF), 2% 20q12 (MAFB), and 15% 4p16 (FGFR3 and MMSET).

**Cancer type:** tumors  
**Primary NCI role code:** Gene\_is\_Biomarker\_of  
**Other roles:** not\_assigned  
**Evidence code:** EV-EXP-IEP, EV-EXP-IDA  
**Negation indicator:** no  
**Cellline indicator:** no  
**Status:** finished  
**PubMedID:** [15755896](#)

Using gene expression profiling to identify 5 recurrent translocations, specific trisomies, and expression of cyclin D genes, MM tumors can be divided into 8 TC (translocation/cyclin D) groups (11q13, 6p21, 4p16, maf, D1, D1+D2, D2, and none) that appear to be defined by early, and perhaps initiating, oncogenic events.

**Cancer type:** plasma-cell leukemias (pcl)  
**Negation indicator:** no  
**Cellline indicator:** no  
**Status:** no\_fact  
**PubMedID:** [15543617](#)

We investigated the expression profiles of the FGFR3/MMSET, CCND1, CCND3, MAF, and MAFB genes, which



# Overlay Cancer Gene Index

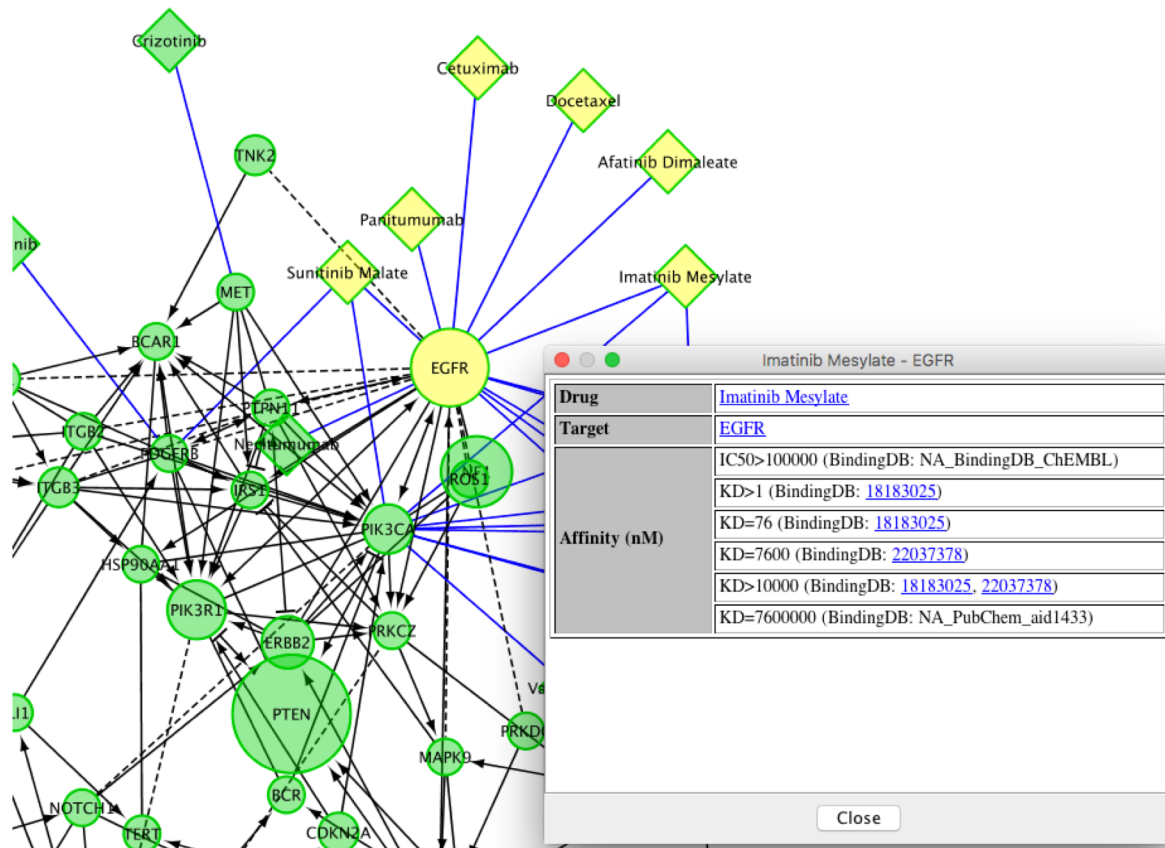
- Load the NCI disease terms hierarchy in the left panel.
- Select a disease term in the tree to select all nodes that have this annotation or one of its sub-terms.

The screenshot displays the VizMapper software interface. On the left, the 'Control Panel' shows a 'Disease Hierarchy' tree with 'Papillary\_Breast\_Neoplasm' selected. The main window shows a network diagram with nodes representing genes and edges representing interactions. A context menu is open over the network, showing options like 'Add', 'Edit', 'Select', 'Group', 'Nested Networks', 'Apps', 'Reactome FI', 'External Links', and 'Preferences'. The 'Reactome FI' option is expanded, showing sub-options: 'Fetch Fis', 'Fetch Cancer Gene Index', 'Query Gene Card', 'Query Cosmic', and 'Analyze Nodes Functions'. At the bottom, the 'Table Panel' displays a table of gene sets with columns for Module, GeneSet, RatioOfProteinIn..., NumberOfProtei..., ProteinFromMod..., P-value, FDR, and Nodes.

Module	GeneSet	RatioOfProteinIn...	NumberOfProtei...	ProteinFromMod...	P-value	FDR	Nodes
0	Bladder cancer(K)	0.0038	38	2	0.0063	2.026e-01	E2F3,CDKN2A
0	Lysosome(K)	0.0123	122	3	0.0065	1.906e-01	ARSG,NEU1,SCA...
0	HTLV-1 infection...	0.0262	260	4	0.0084	2.256e-01	E2F3,CDKN2A,...
0	Validated trans...	0.0049	49	2	0.0103	2.498e-01	PMAIP1,CDKN2A
1	Focal adhesion(K)	0.0208	206	8	0.0000	<1.000e-03	TLN2,TNC,BCA...
1	Integrins in angi...	0.0047	47	4	0.0000	4.000e-03	BCAR1,PTK2B,I...
1	Type II diabetes...	0.0048	48	4	0.0000	2.667e-03	KCNJ11,PRKCE,...
1	Signaling by EG...	0.0172	171	6	0.0000	3.000e-03	FGF9,LRIG1,SPR...
1	Endothelins(NI)	0.0063	62	4	0.0001	4.400e-03	BCAR1,PTK2B,P...



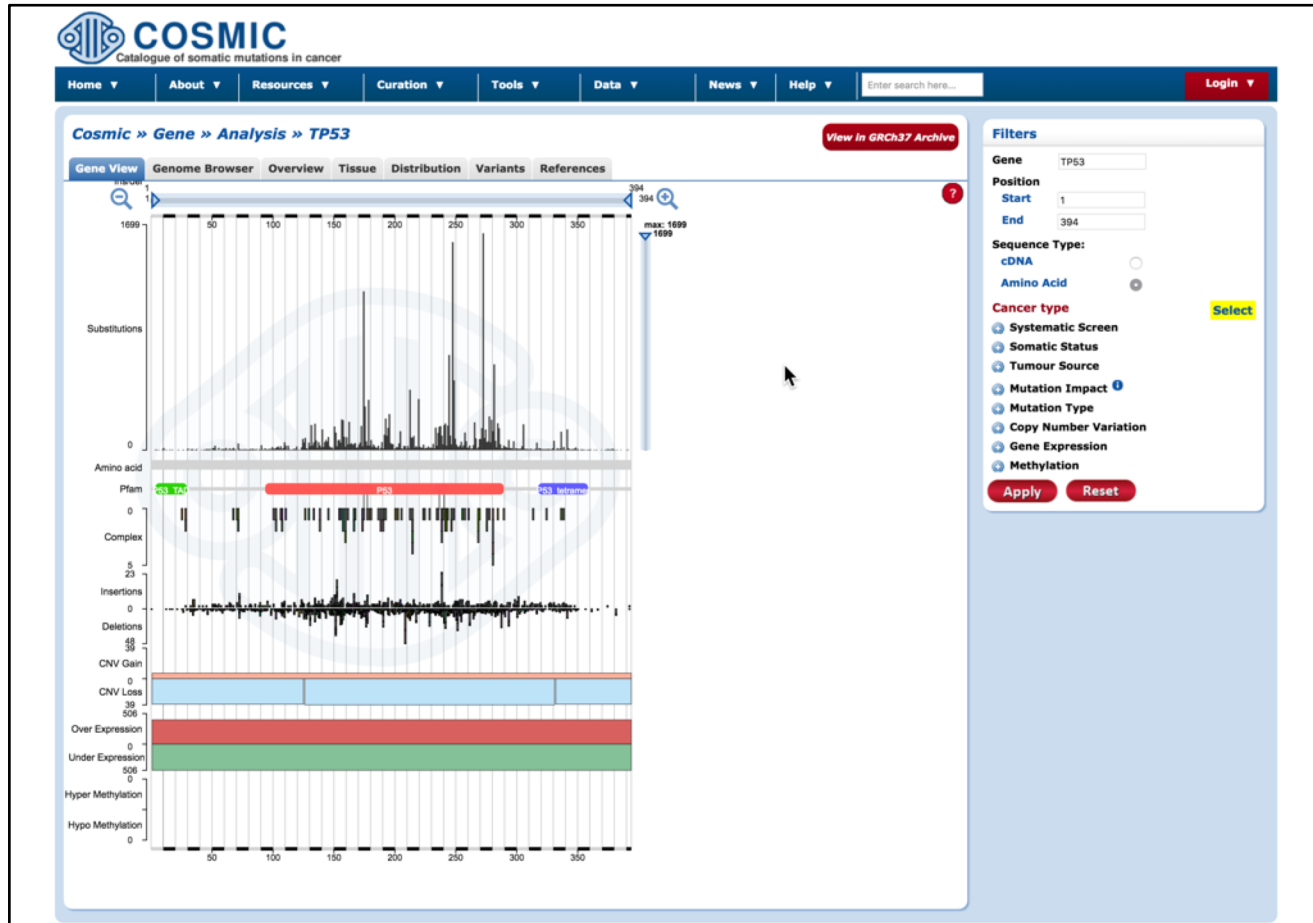
# Visualize Cancer Targetome in the Reactome FI Network



TCGA GBM Mutation Profile

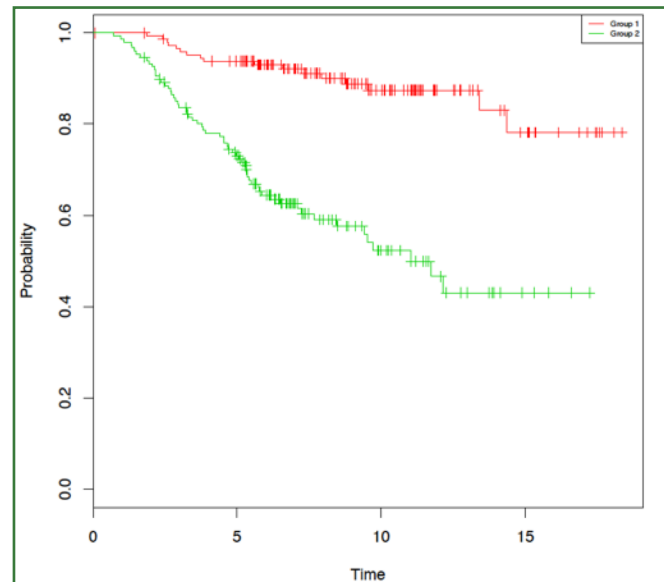
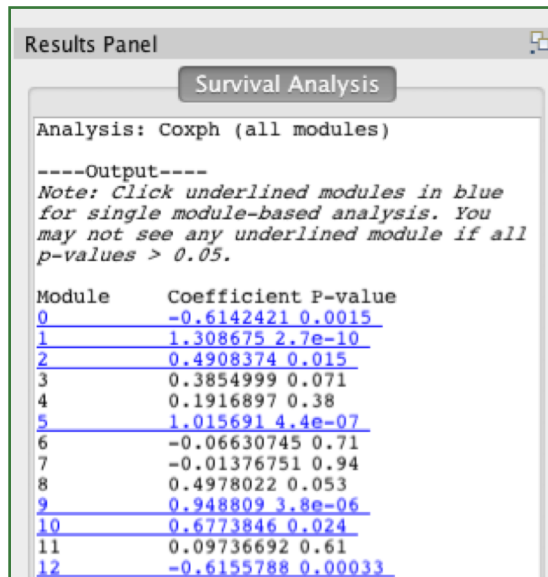
# COSMIC

- View detailed variant annotations for the selected gene or protein from COSMIC database.



# Module Based Survival Analysis

- Discover Prognostic Signatures in Disease Module Datasets.
- Based on a server-side R script that runs either CoxPH or Kaplan-Meier survival analysis.
- Requires appropriate clinical data file.



# Demos

REACTOMEFIVIZ APP

# We are on a Coffee Break & Networking Session

Workshop Sponsors:

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