

Reactome Functional Interaction Network and ReactomeFIViz app

Contact: help@reactome.org



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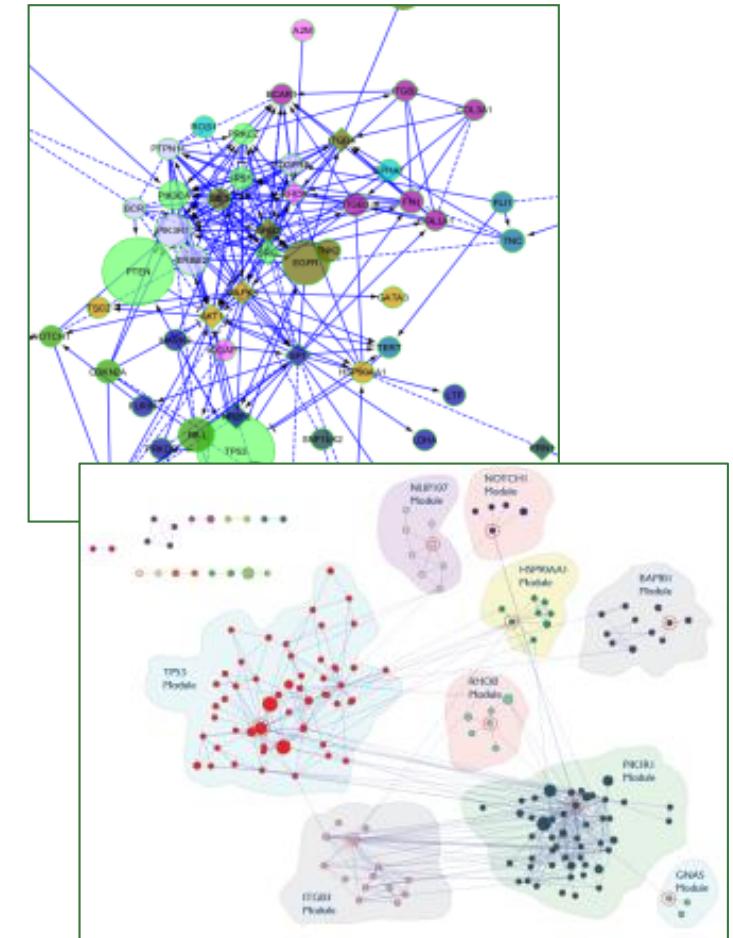
Learning Objectives of Module

- Be able to perform pathway and network-based data analysis using ReactomeFlViz app



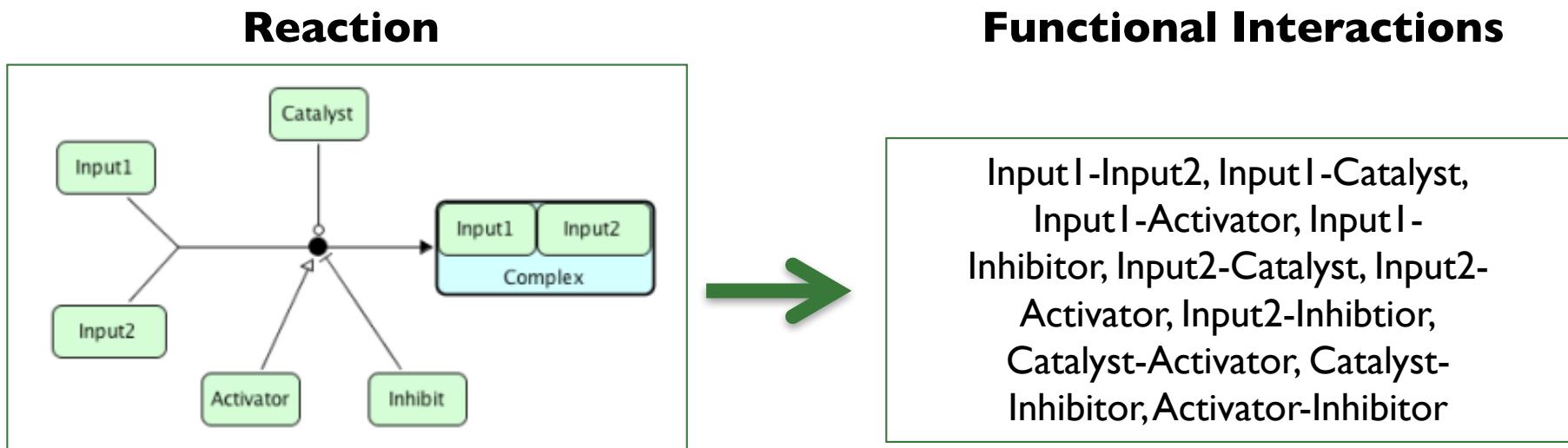
Reactome Functional Interaction (FI) Network and ReactomeFlViz app

- No single mutated gene is necessary and sufficient to cause cancer.
 - Typically one or two common mutations (e.g. TP53) plus rare mutations.
- Analyzing mutated genes in a network context:
 - reveals relationships among these genes.
 - can elucidate mechanism of action of drivers.
 - facilitates hypothesis generation on roles of these genes in disease phenotype.
- Network analysis reduces hundreds of mutated genes to < dozen mutated pathways.



What is a Functional Interaction?

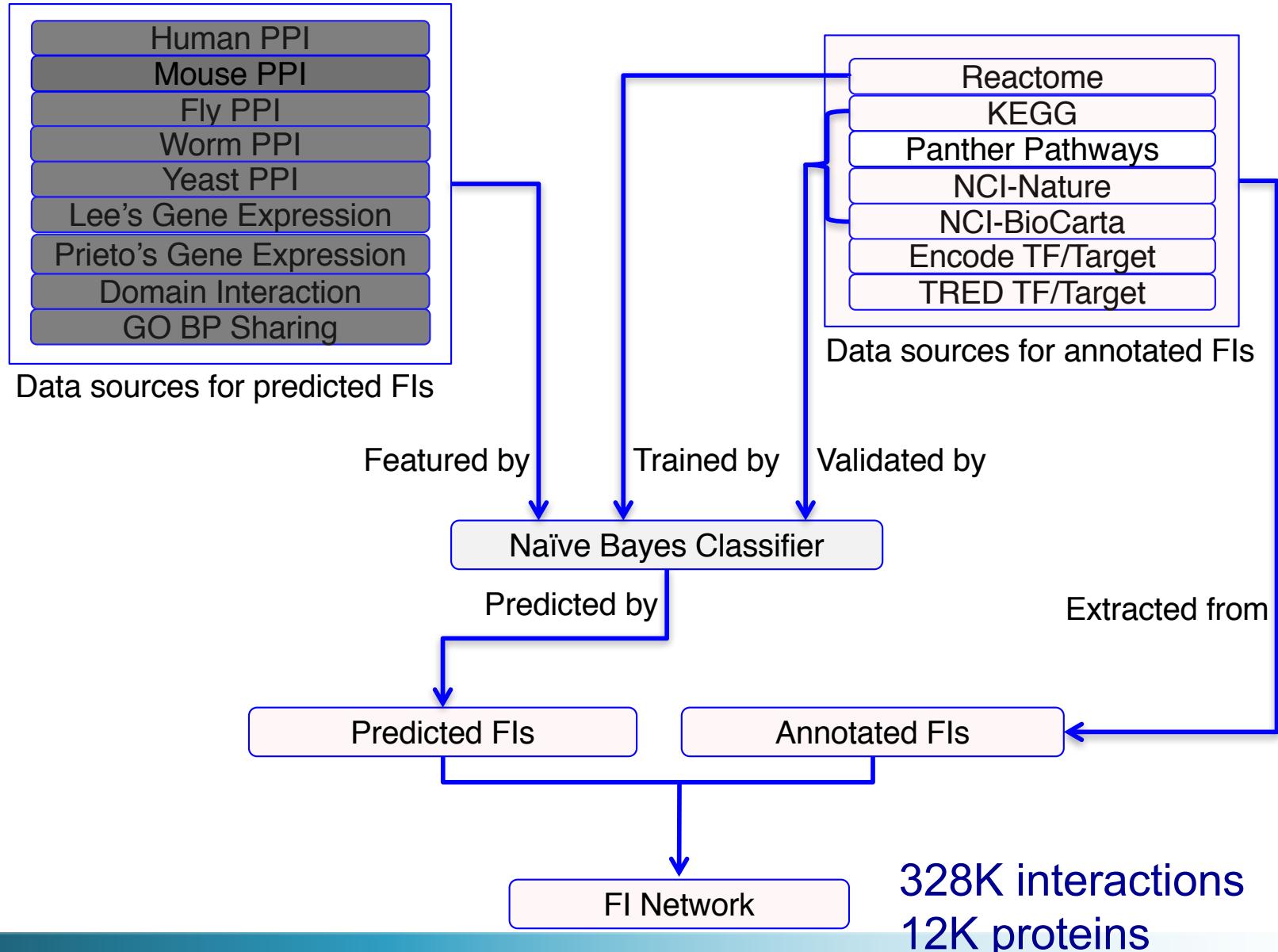
- Convert reactions in pathways into pair-wise relationships
 - **Functional Interaction:** an interaction in which two proteins are involved in the same reaction as input, catalyst, activator and/or inhibitor, or as components in a complex



Method and practical application: A human functional protein interaction network and its application to cancer data analysis, [Wu et al. 2010 Genome Biology](#).

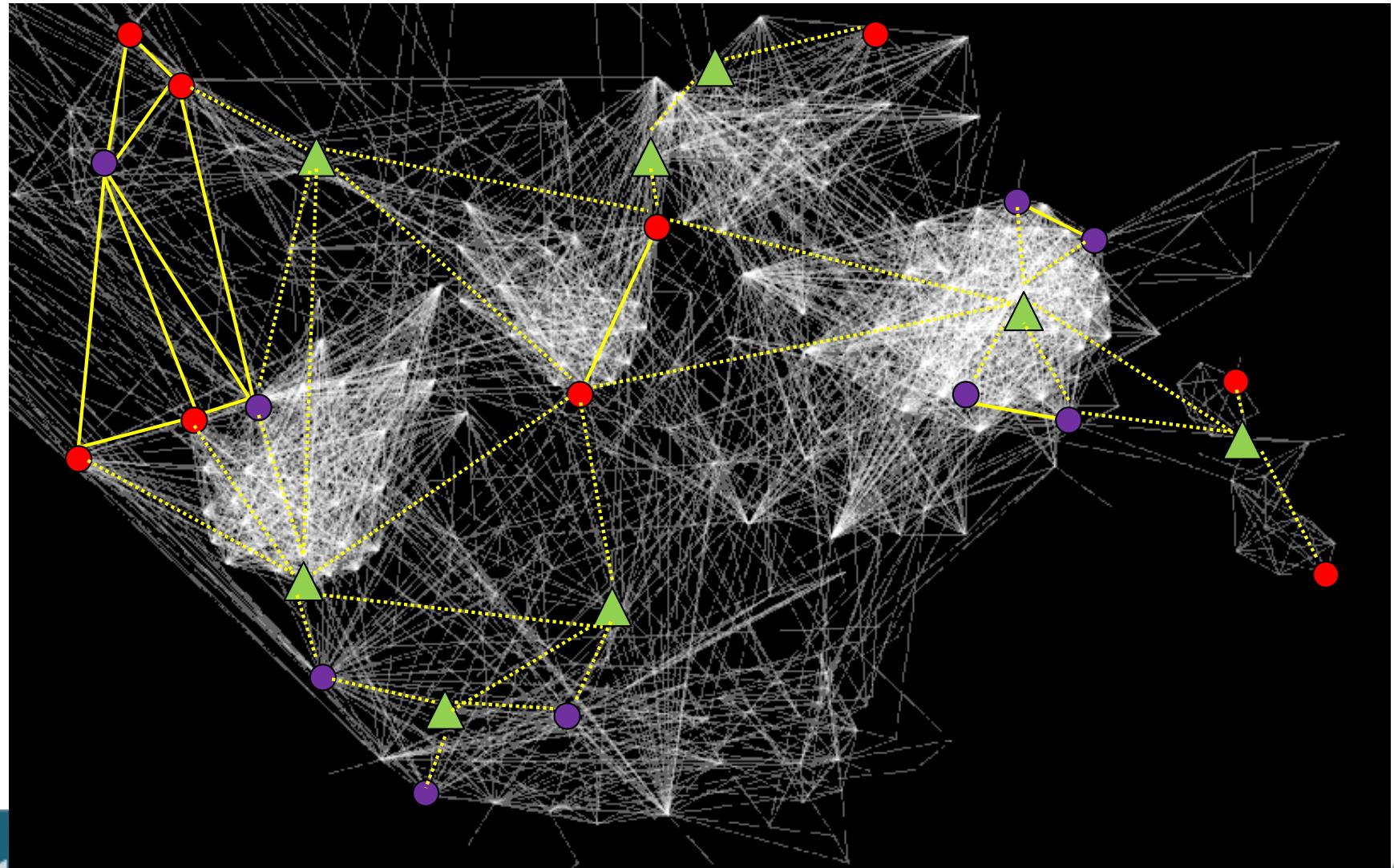


Construction of the FI Network



328K interactions
12K proteins

Projecting Experimental Data onto FI Network



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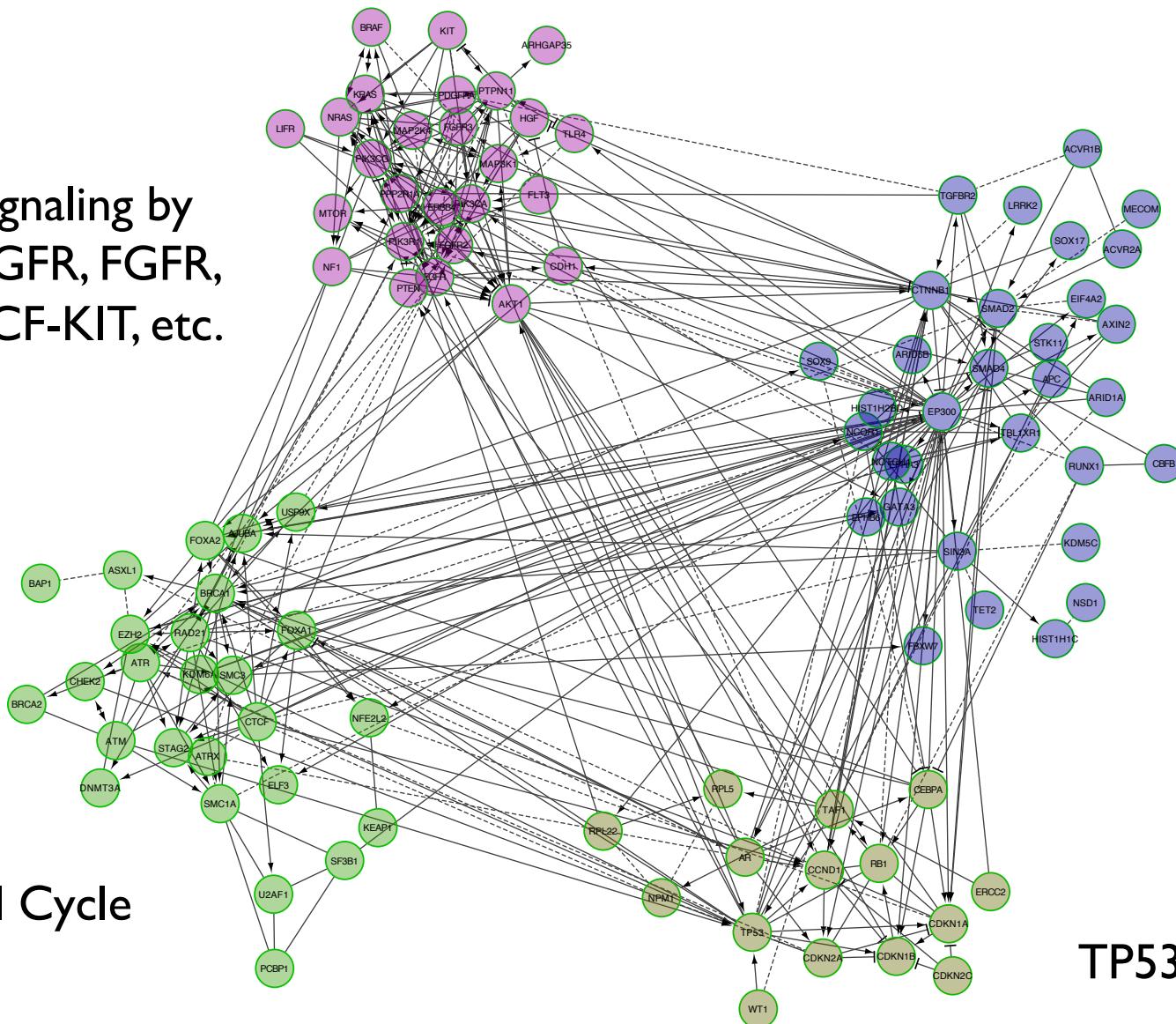
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127 Cancer Driver Genes

Signaling by
EGFR, FGFR,
SCF-KIT, etc.

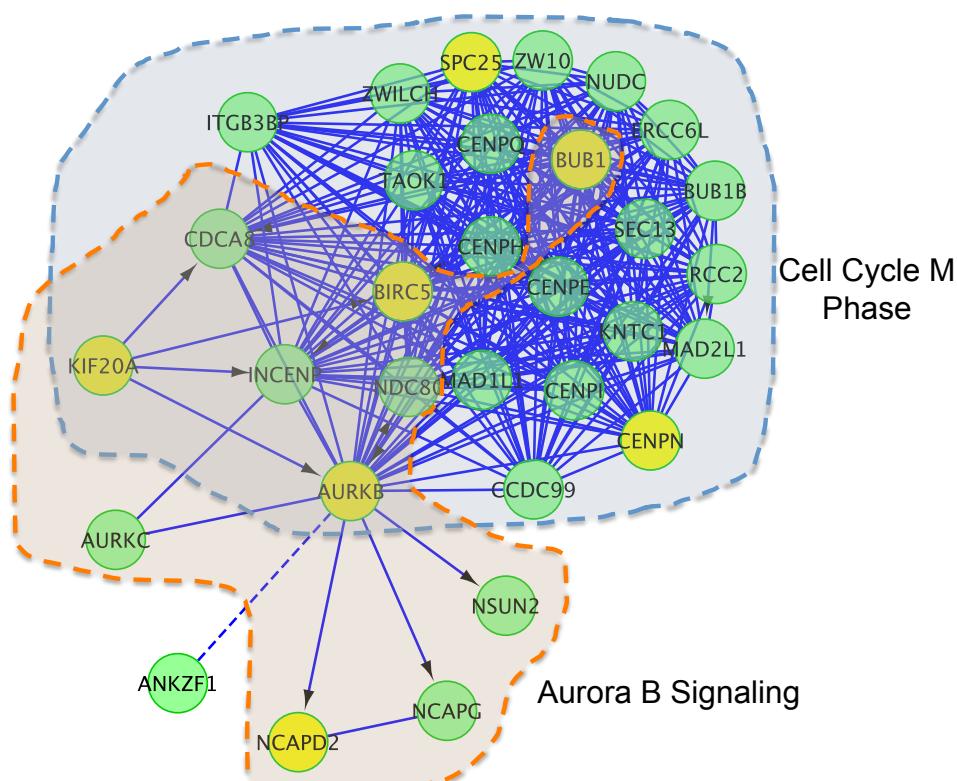


Signaling by
Notch, Wnt,
TGF-beta,
SMAD2/3, etc.

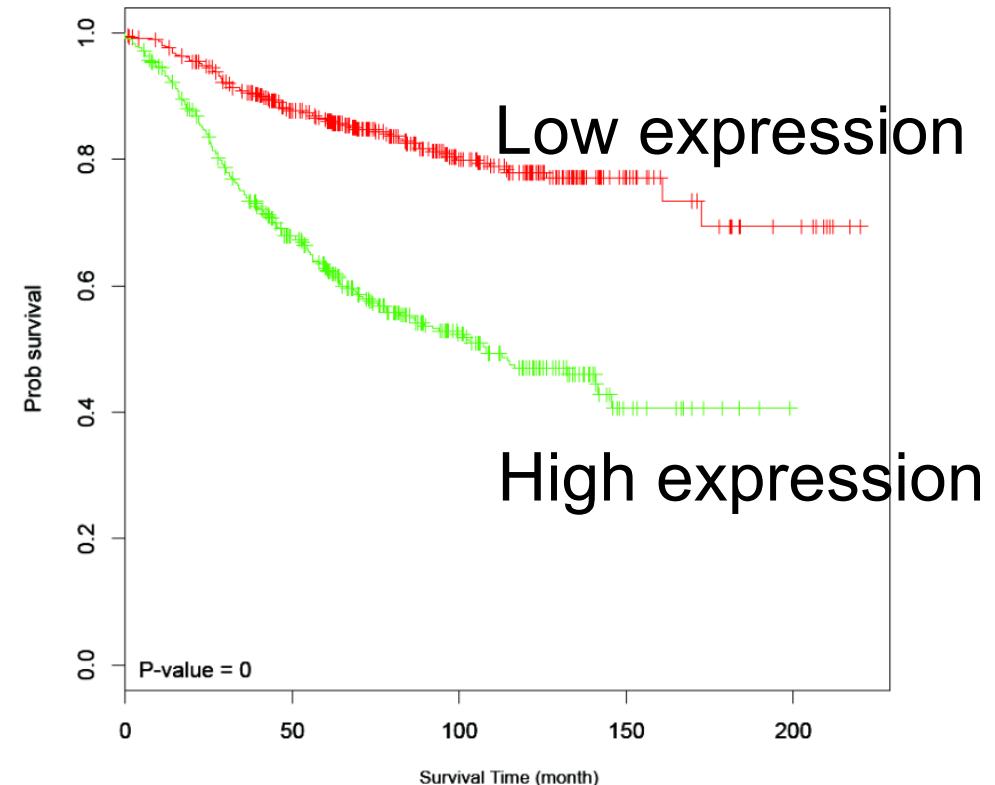
Cell Cycle

TP53 Pathway

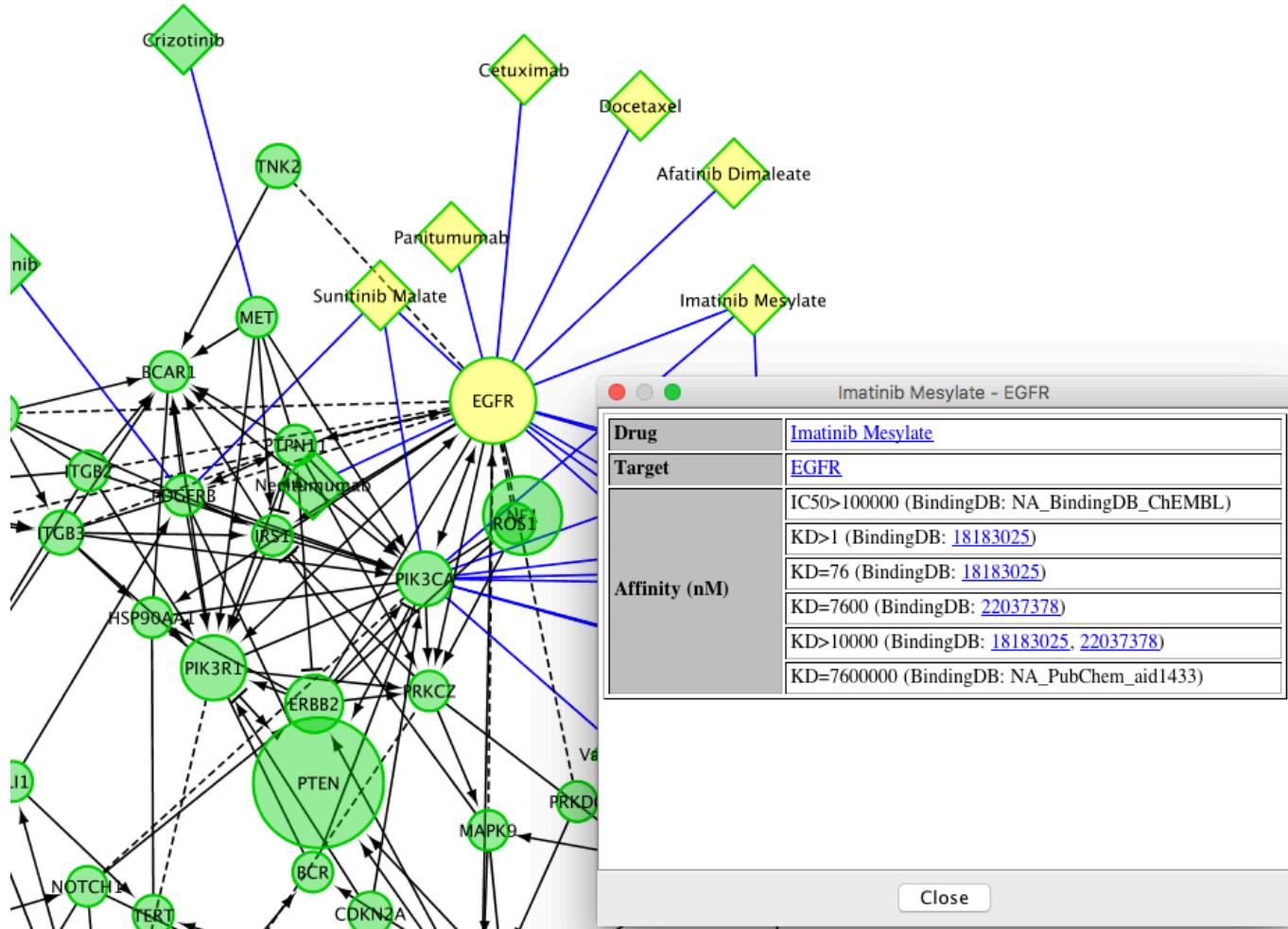
Module-Based Prognostic Biomarker in ER+ Breast Cancer



Measure levels of expression
of the genes in this network
module



Visualize Cancer Targetome in the Reactome FI Network



TCGA GBM Mutation Profile



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

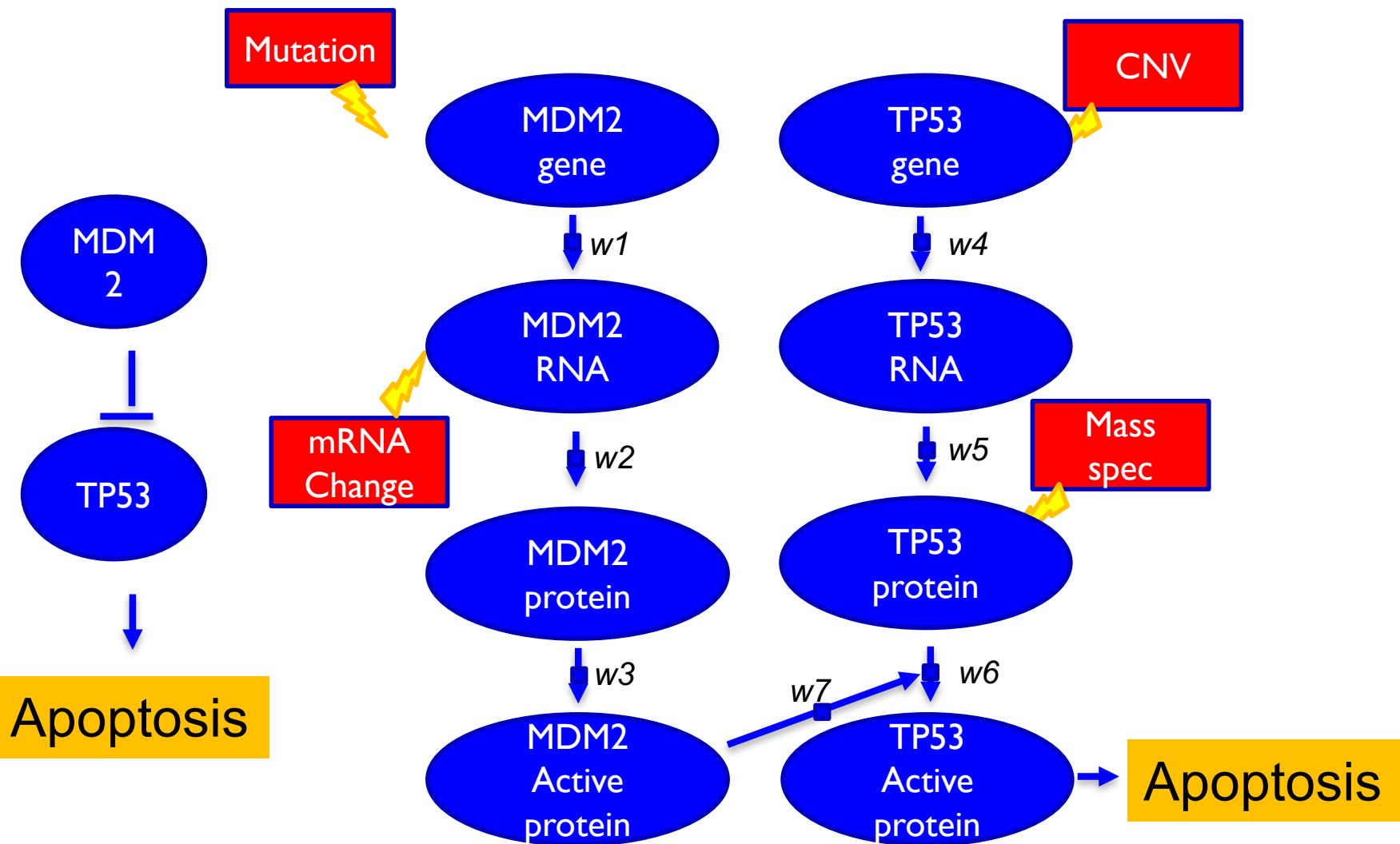
Types of Pathway-Based Modeling

- Partial differential equations/boolean models, e.g. CellNetAnalyzer
 - Mostly suited for biochemical systems (metabolomics)
- Network flow models, e.g. NetPhorest, NetworKIN
 - Mostly suited for kinase cascades (phosphorylation info)
- Transcriptional regulatory network-based reconstruction methods, e.g. ARACNe (expression arrays)
- Probabilistic graph models (PGMs), e.g. PARADIGM
 - Most general form of pathway modeling for cancer analysis at this time.

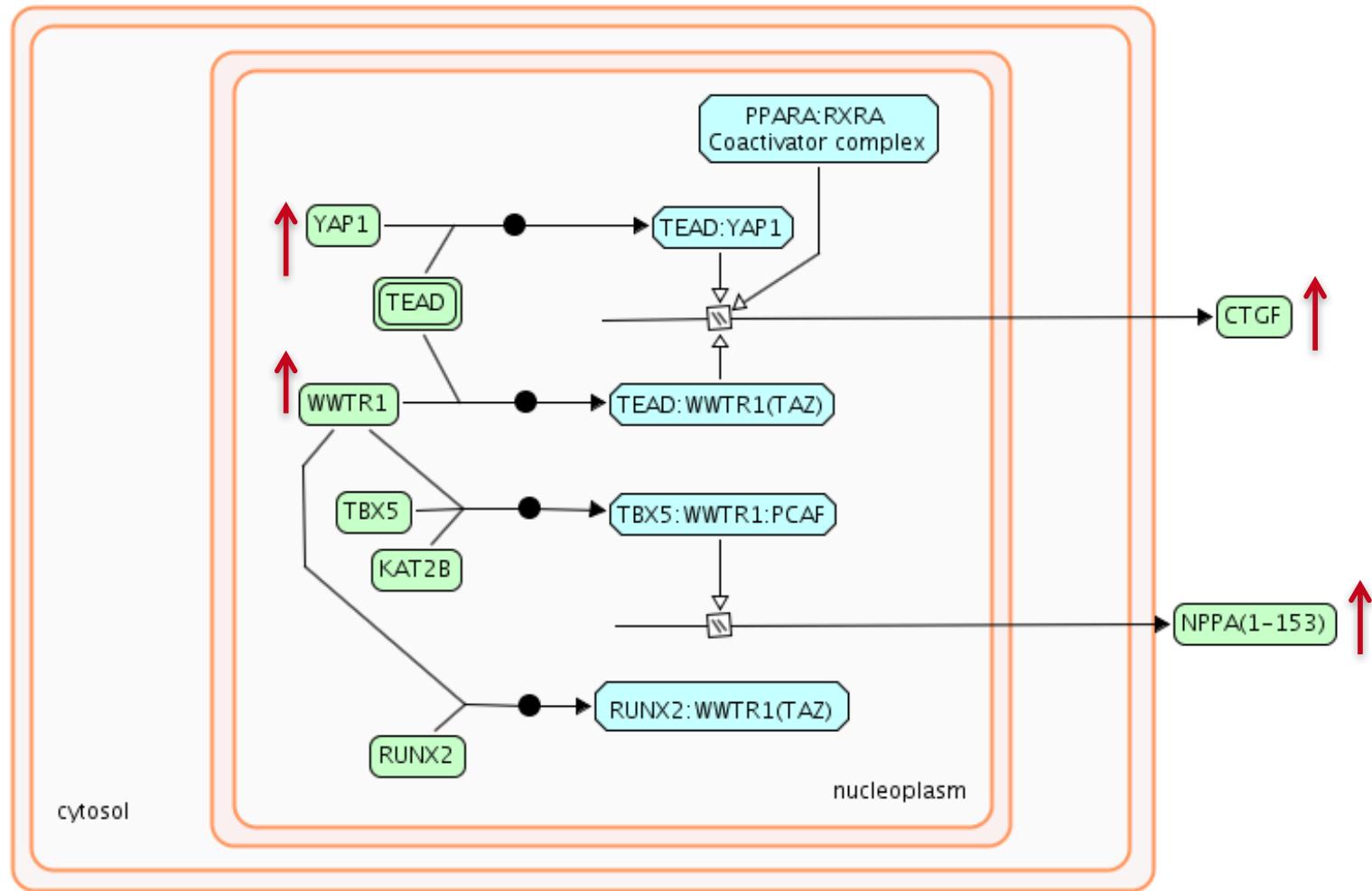
Probabilistic Graphical Model (PGM) based Pathway Analysis

- Attempt to integrate multiple molecular alterations together to yield lists of altered pathway activities.
 - Many omics data types available for one single patient
 - CNV, gene expression, methylation, somatic mutations, etc.
- Pathway and network-based Simulation
 - Find significantly impacted pathways for diseases
 - Link pathway activities to patient phenotypes
 - Predict drug effects: one drug or multiple drugs together?

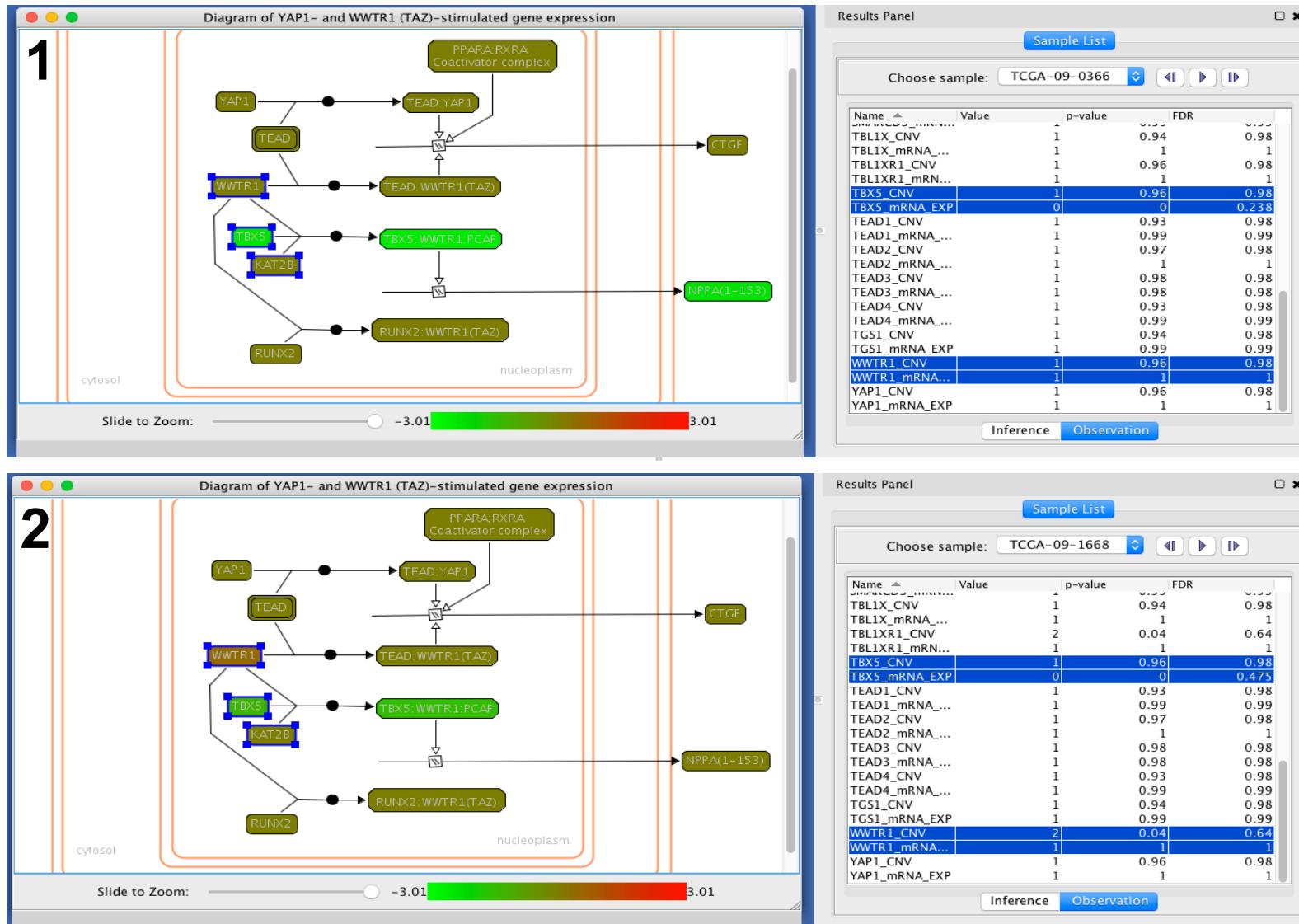
PARADIGM



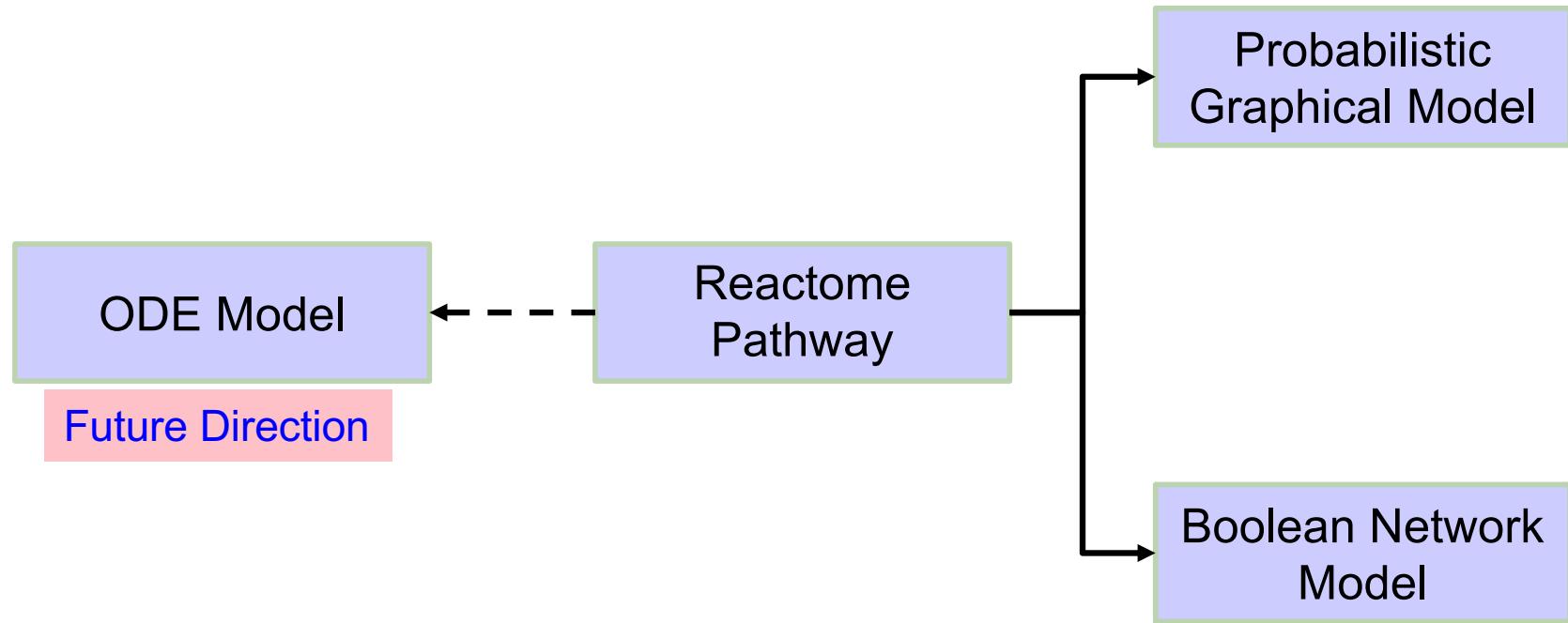
Probabilistic Graphical Models (PGMs) for Reactome Pathways



PGM-based Single Patient Pathway View



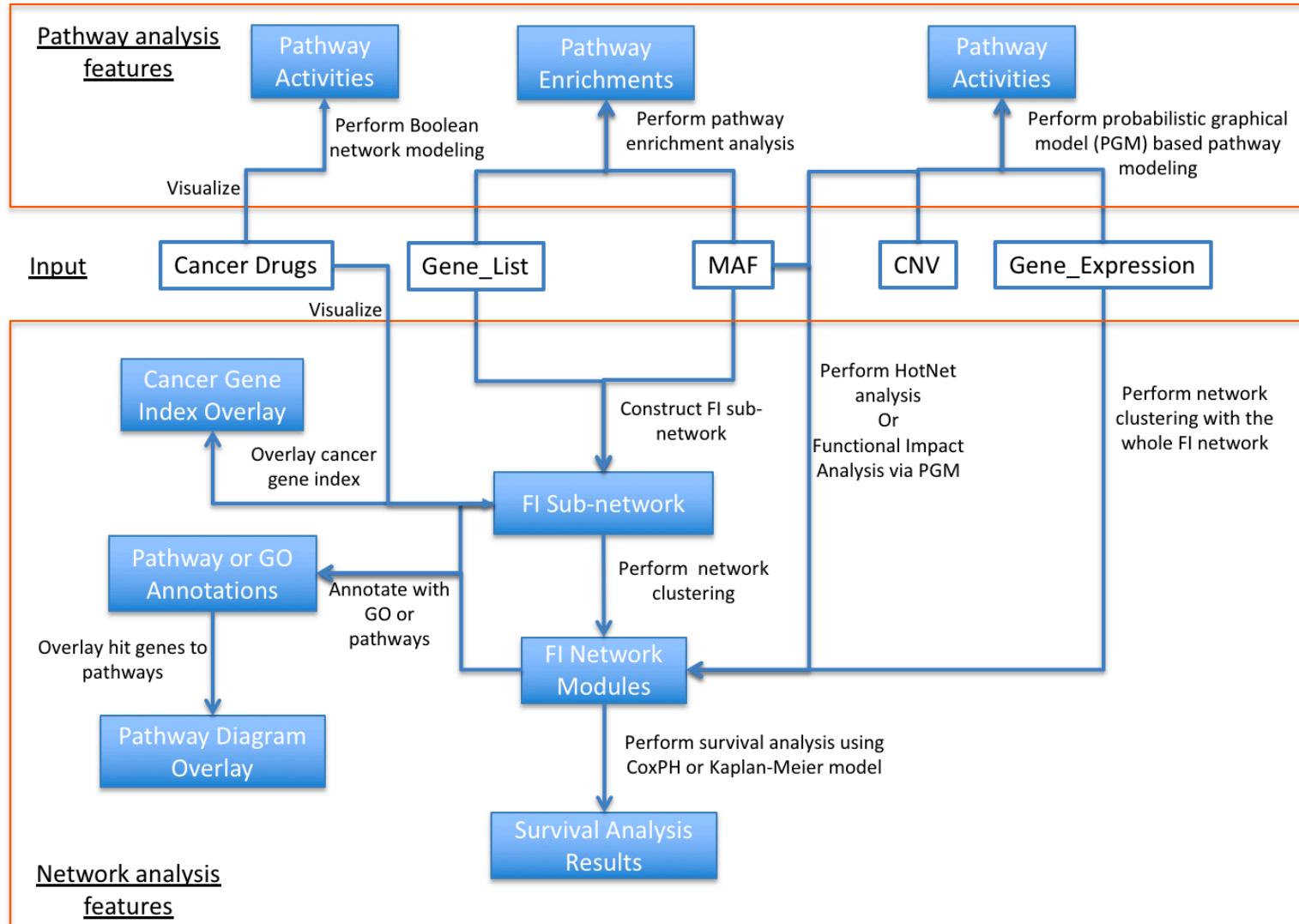
Building Quantitative Models for Reactome Pathways



Tutorial



Major Features in ReactomeFIViz



A) Reactome Pathway Enrichment Analysis

Session: New Session

Control Panel

Network Style Select Reactome

Cell Cycle

Cell Cycle Checkpoints

- View Reactome Source
- View in Reactome
- Show Diagram

Search

Analyze Pathway Enrichment

Run Graphical Model Analysis

Load Graphical Model Results

Expand Pathway

Collapse Pathway

Selected Event Branch

Cell Cycle

Cell Cycle Checkpoints

Diagram of Cell Cycle Checkpoints

The diagram illustrates the molecular components and interactions of the G1/S DNA Damage Checkpoint. At the top, the MCC:APC/C complex is shown. Below it, the phosphorylated anaphase promoting complex (APC/C) is connected to the hBUBR1:hBUB3:MAD2*:CDC20 complex. This complex is further connected to BUB1B and BUB3. BUB3 is also connected to CDC20. To the right, MAD1L1 is connected to the Kinetochore Complex. A zoom slider is present at the bottom of the diagram panel.

Slide to Zoom:

Table Panel

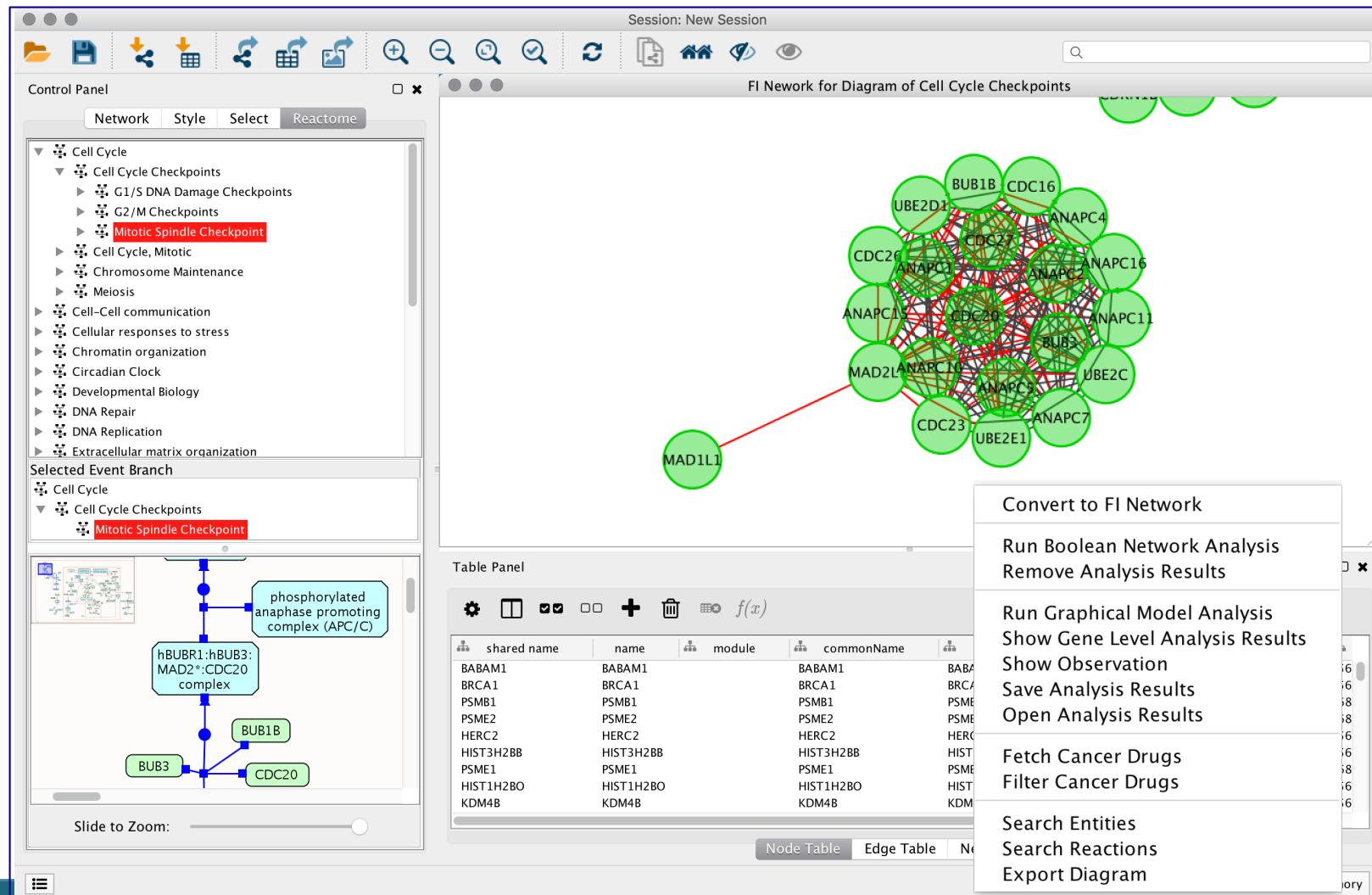
f(x)

Node Table Edge Table Network Table

Memory



Displaying Reactome Pathways in the FI Network View



Pathway Enrichment Analysis

Session: New Session

Control Panel

Network Style Select Reactome

FDR: $>=0.1$ $>=0.01$ $>=0.001$ <0.001

Interleukin-7 signaling (FDR: 0.091)

- IL7 binds HGF(495-728) to form PPBSF
- IL7R binds JAK1
- IL2RG binds JAK3
- IL7 binds IL7R:JAK1
- IL7:IL7R:JAK1 binds IL7R
- IL7R is phosphorylated
- IL7:p-Y449-IL7R:JAK1
- IL7:p-Y449-IL7R:JAK1
- IL7:p-Y449-IL7R:JAK1
- Interleukin-11 receptor alpha
- Interleukin-11 receptor alpha
- IL27 is a dimer of IL27A and IL27B
- 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)

Diagram of Interleukin-7 signaling and JAK3 deficiency causes SCID (IL7R)

Reactome Pathway Enrichment Analysis

Gene Set Loading

Choose a gene set file: hs_geneNames.txt

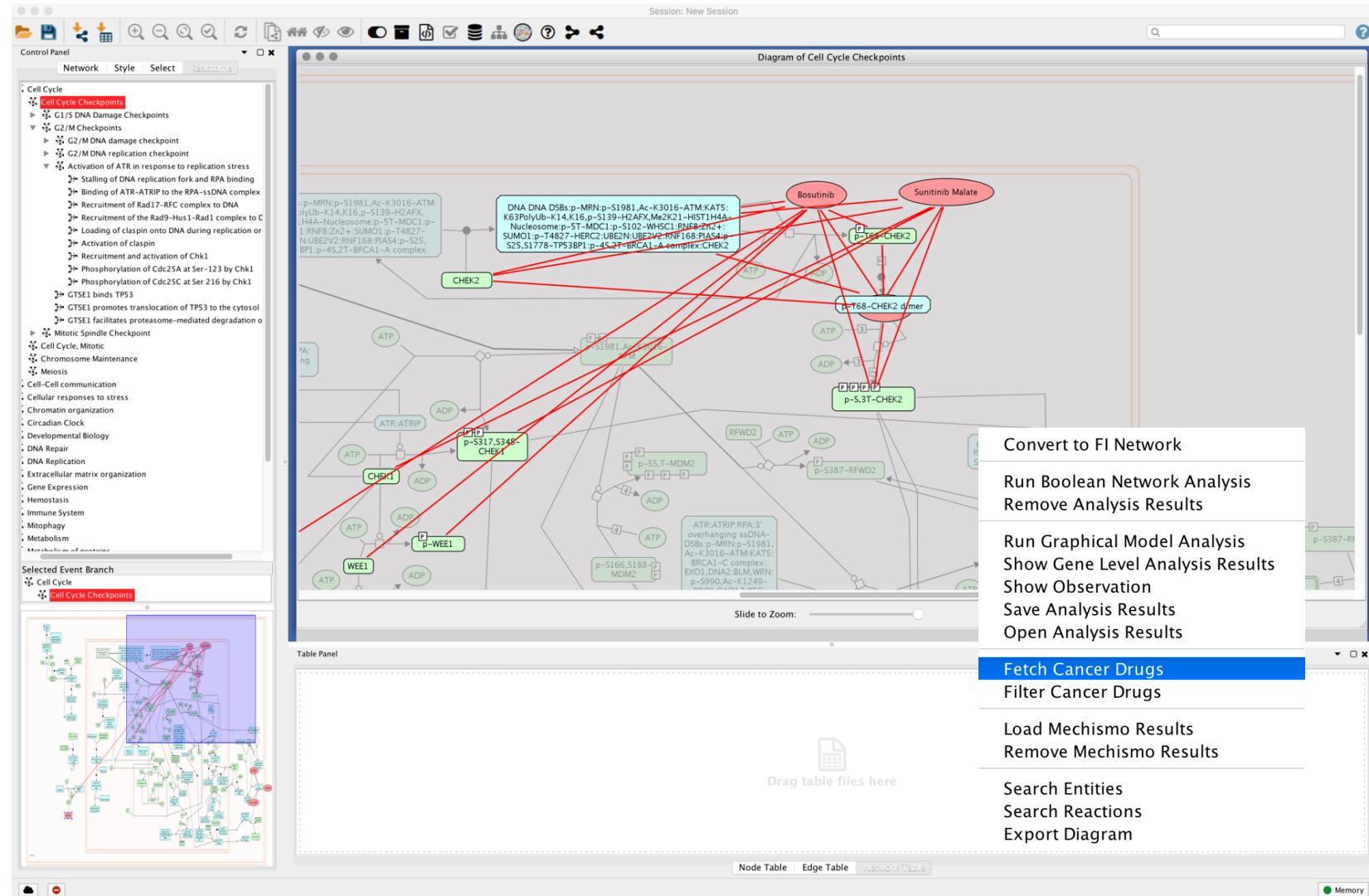
Specify file format: One gene per line
 Comma delimited (e.g. TP53, EGFR)
 Tab delimited (e.g. TP53 EGFR)

ReactomePathway	RatioOfProteinInPathway	NumberOfProteinInPathway	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 signaling	0.0011	8	3	0.0114	0.0911	IL7R,HGF,JAK1
CREB phosphorylation	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 activation	0.0074	53	8	0.0124	0.0993	GABBR2,GNAT3,GAB...
Mitotic Prometaphase	0.0139	99	12	0.0131	0.1016	KNTC1,NDE1,PDSSB...
Digestion of dietary ...	0.0004	3	2	0.0132	0.1016	MGAMLCT
Mitotic Anaphase	0.0227	162	17	0.0136	0.1016	KNTC1,NDE1,UBC,A...
TGF-beta receptor signaling	0.0022	16	4	0.0142	0.1016	UBC,PARD3,TGFBR2...

Node Table Edge Table Network Table Reactome Pathway Enrichment

Memory

Visualize Cancer Targetome in Reactome Pathways



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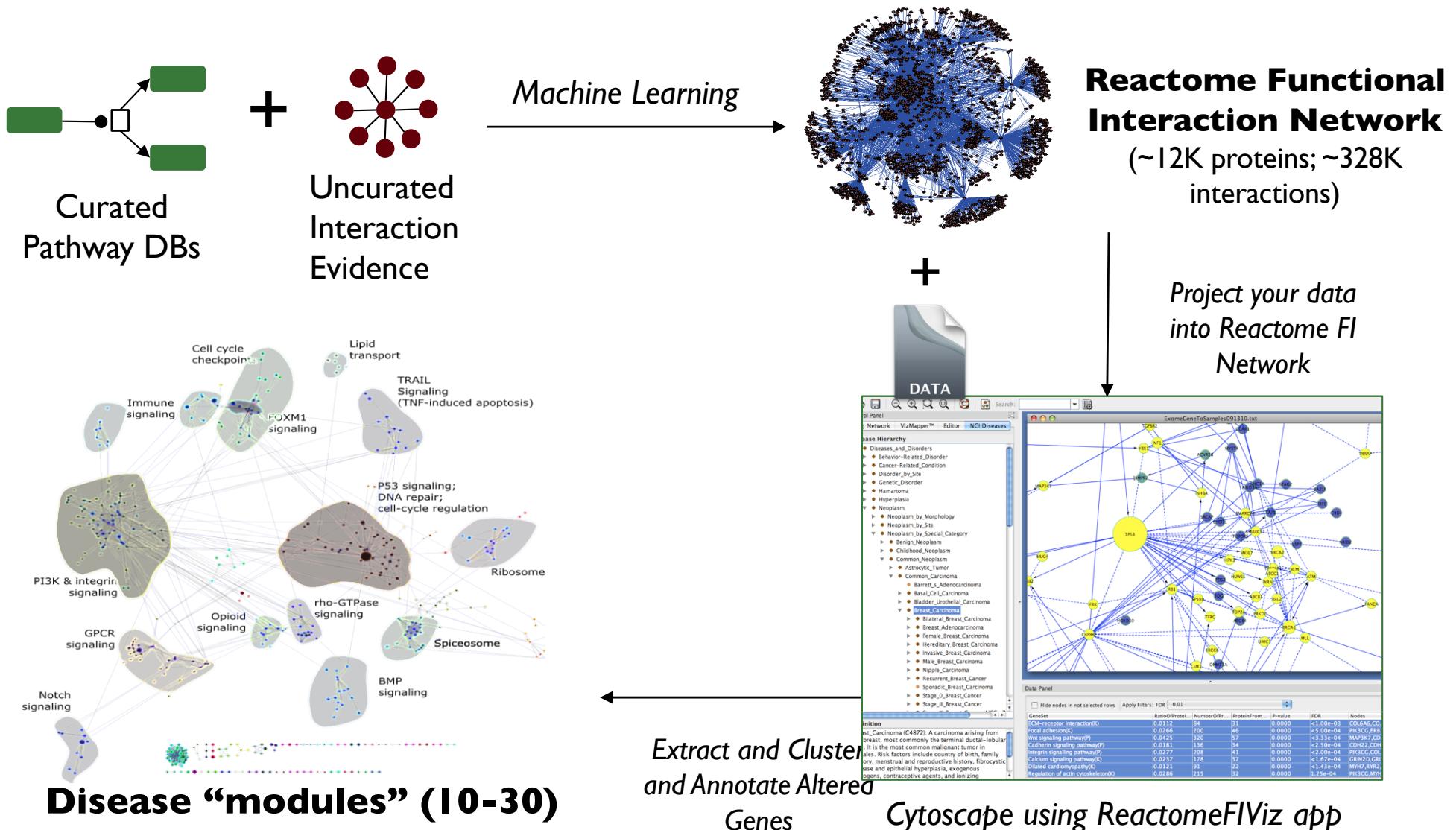
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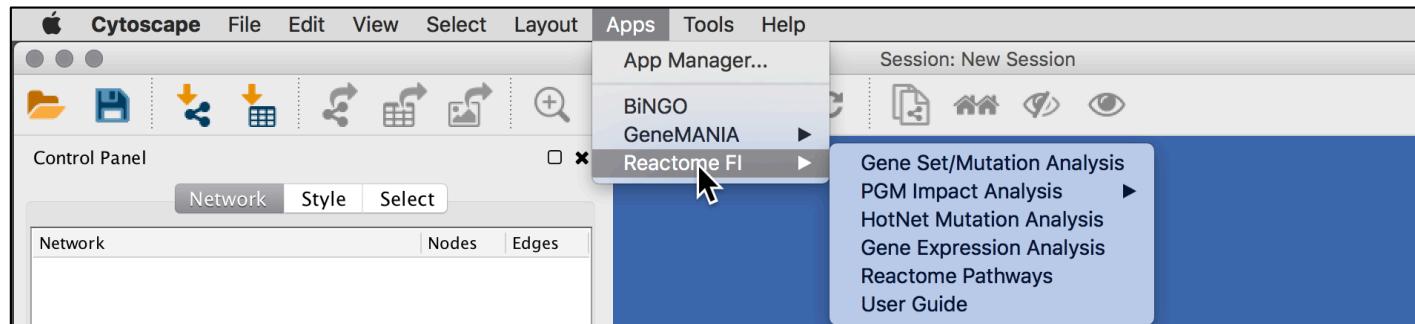
A2) De Novo Subnetwork Construction & Clustering



Disease “modules” (10-30)



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

File Formats

- Choose Plugins, Reactome Fls.
- Fl plug-in supports four file formats:
 - Gene list
 - Gene-Sample gene
 - NCI Mutation Annotation File
 - Microarray Expression Data



Gene Set-based Analysis

Cytoscape File Edit View Select Layout Apps Tools Help

Session: New Session

Control Panel

Gene Set/Mutation Analysis

Reactome FI Network Version

2016 2015 2014

* Different versions of the FI network may produce different results.

Gene Set Parameters

Choose data file: Browse

Or enter gene set: 127 Genes Entered

Specify format: Gene set
 Gene/sample number pair
 NCI MAF (Mutation Annotation File)

Choose sample cutoff: 2

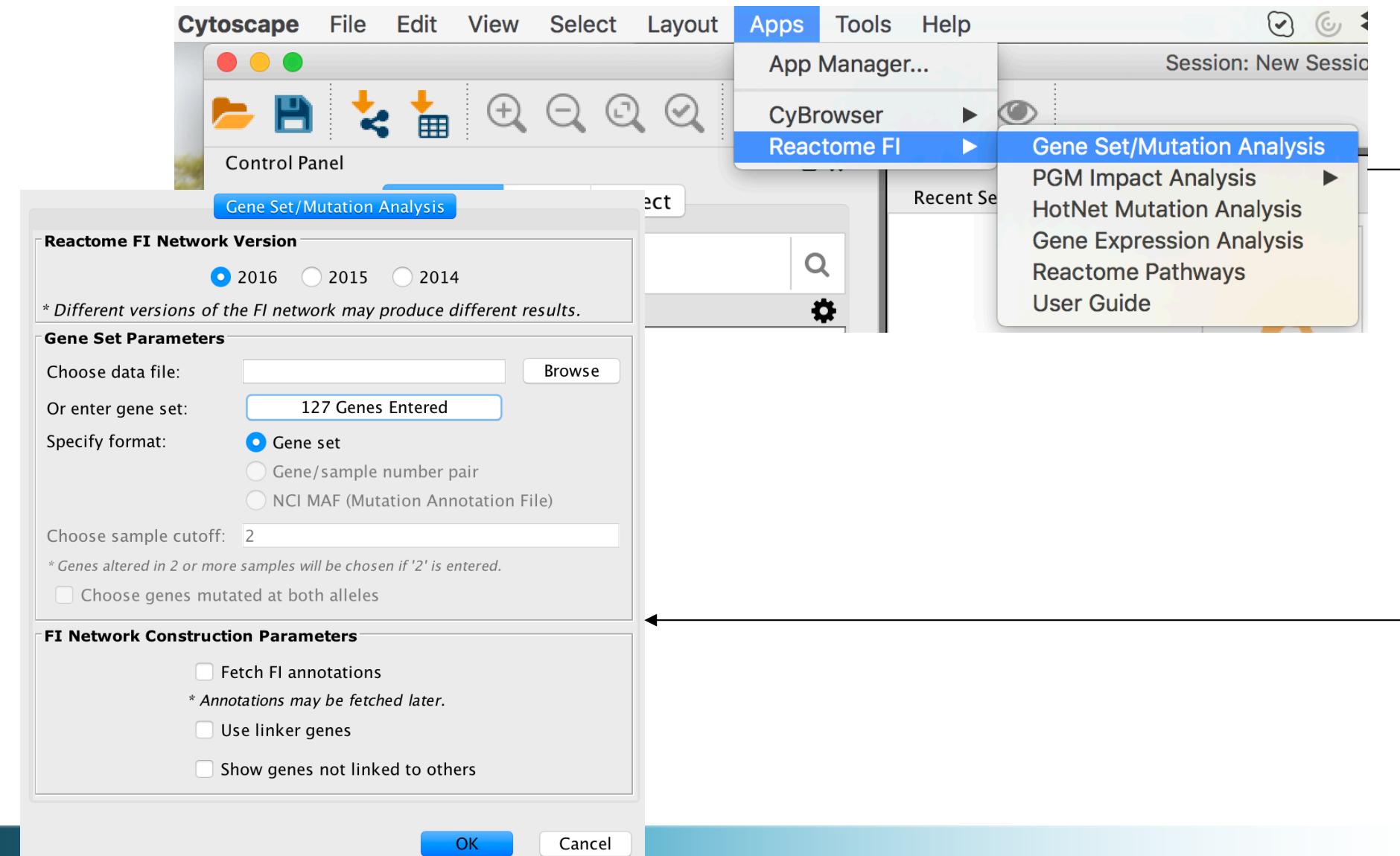
* Genes altered in 2 or more samples will be chosen if '2' is entered.

Choose genes mutated at both alleles

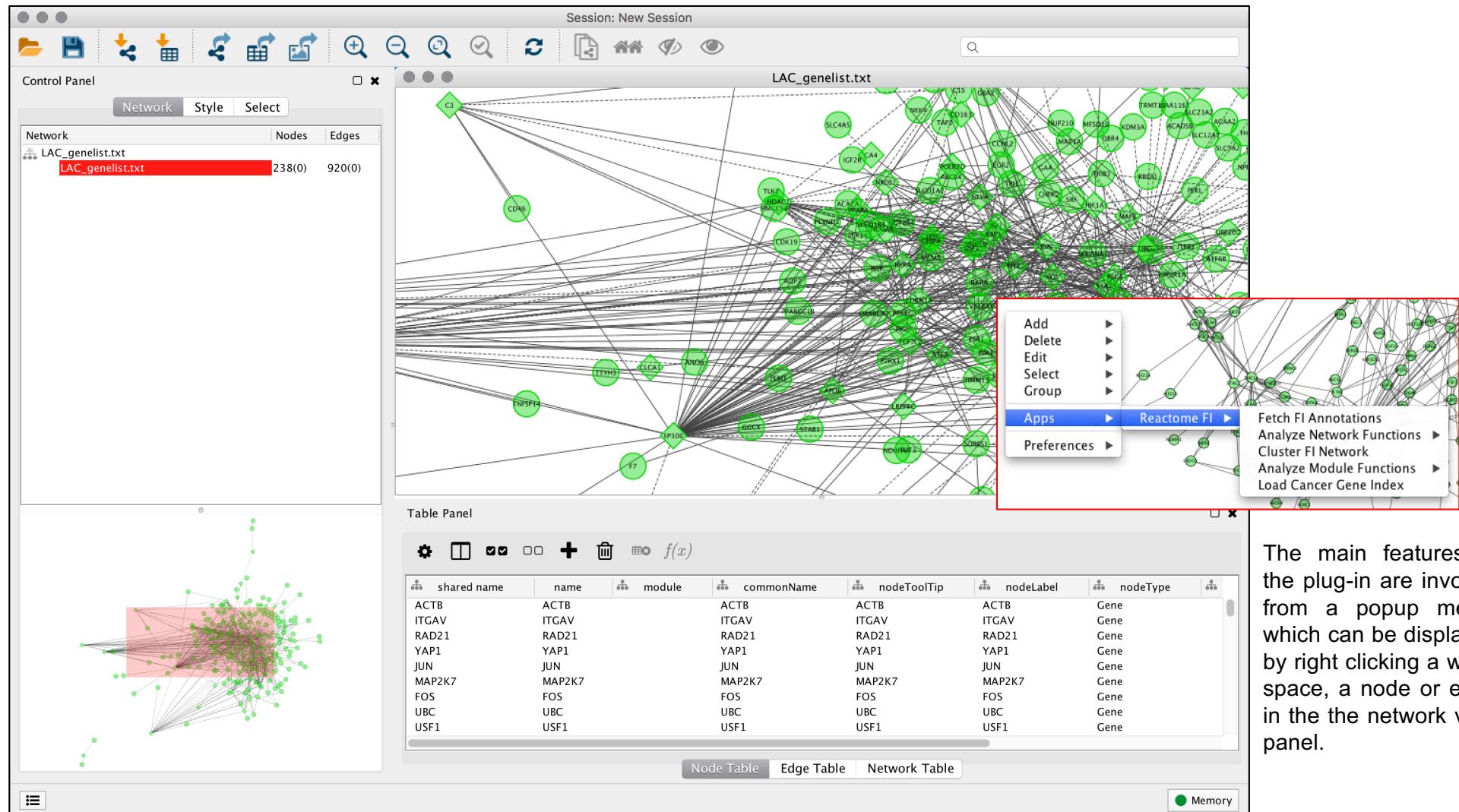
FI Network Construction Parameters

Fetch FI annotations
* Annotations may be fetched later.
 Use linker genes
 Show genes not linked to others

OK Cancel



FI Results Display

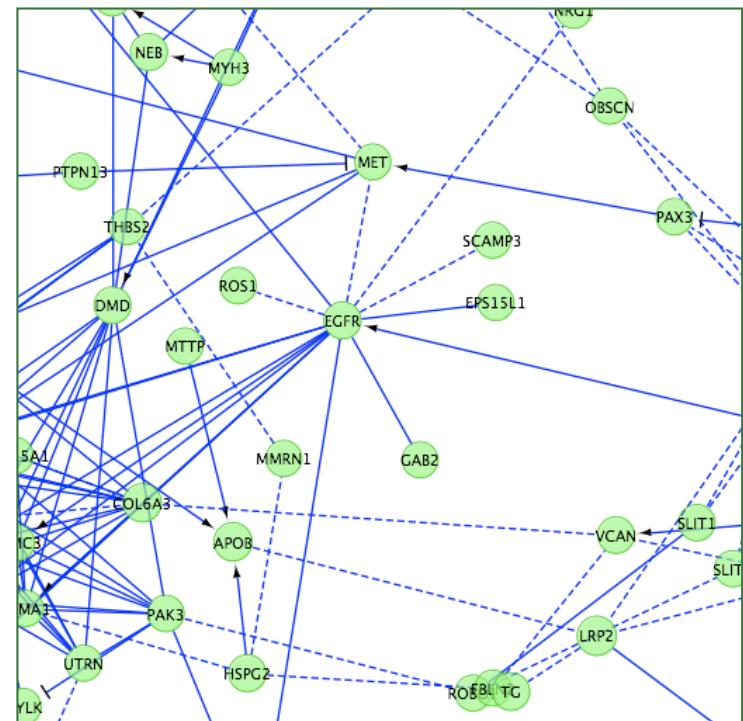


- Constructed network is displayed in the Network View panel using an FI specific visual style

The main features of the plug-in are invoked from a popup menu, which can be displayed by right clicking a white space, a node or edge in the the network view panel.

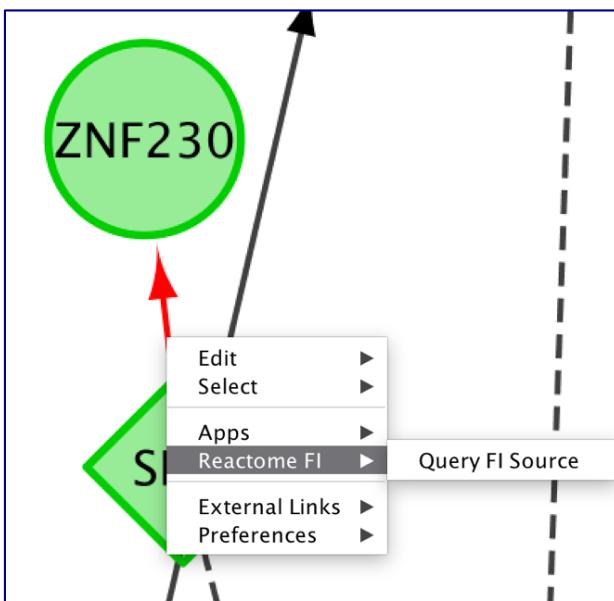
FI Annotations

- 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.



The main features of the plug-in are invoked from a popup menu, which can be displayed by right clicking a node or edge in the the network view panel.

Query FI Source



Annotated FIs

The "Interaction Info" window shows details for interaction "SIX5 - ZNF230".
Reactome Sources:

Reactome ID	Type	Data Source
6951674	TARGETED_INTERACTION	ENCODE

View Reactome Source button is visible.

Reactome Instance View:

classType	TargetedInteraction
dbId	6951674
displayName	SIX5 ZNF230
dataSource	ENCODE
definition	ENCODE proximal_filtered TF/target interaction; supported by GO BP sharing
factor	SIX5
species	Homo sapiens
target	ZNF230

Close button at the bottom.

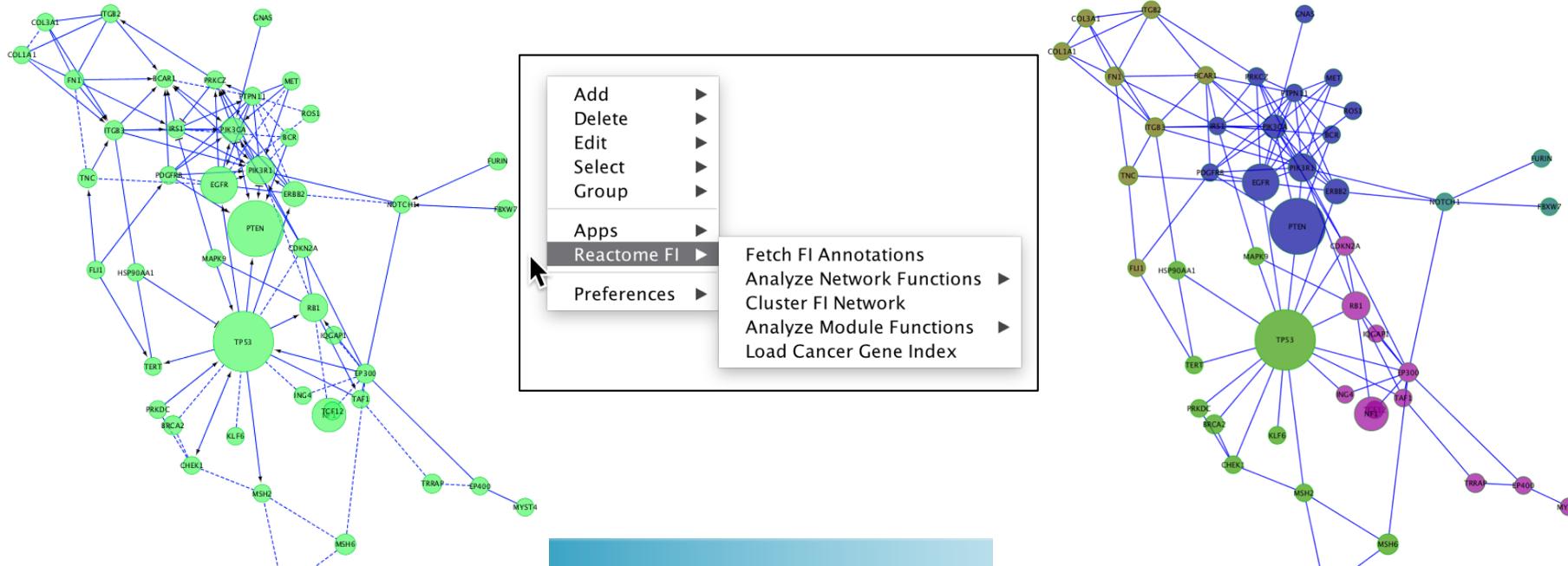
Predicted FIs

The "Interaction Info" window shows details for 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](#))



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Analyze Module Functions

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

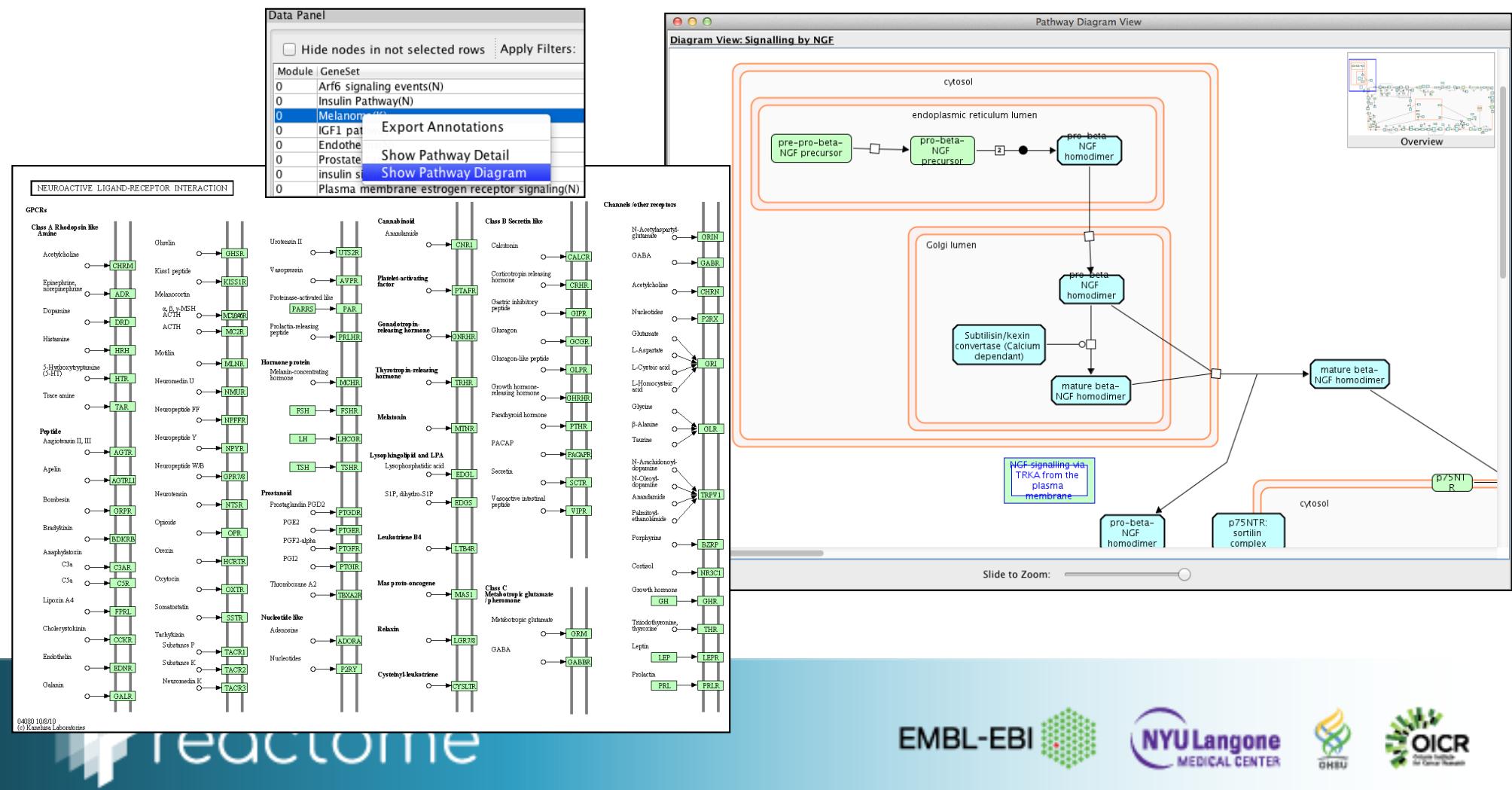
The screenshot shows the Reactome software interface. At the top right, a context menu is open from the 'Reactome FI' option in the 'Apps' menu. The path 'Reactome FI > Analyze Module Functions' is highlighted. The menu also includes options like 'Fetch FI Annotations', 'Analyze Network Functions', 'Cluster FI Network', and 'Load Cancer Gene Index'. To the left, a 'Data Panel' window is visible, displaying a table of network module data. The table has columns for 'Module', 'GeneSet', 'RatioOfProteinInModule', 'mModule', 'P', 'FDR', and 'Nodes'. A filter dialog is overlaid on the table, showing 'Apply Filters: FDR' set to '0.01'. The 'Module Size' column is also filtered to show only rows where the value is '10'. The bottom of the window contains tabs for 'Node Attribute Browser', 'Edge Attribute Browser', 'Network Attribute Browser', 'MCL Module Browser', and 'Pathways in Modules'.

Module	GeneSet	RatioOfProteinInModule	mModule	P	FDR	Nodes
0	Translation(R)	0.0158	0.0010	15	<2.30e-04	RPL18,RPL17,RPL36...
0	Influenza Life Cycle(R)	0.0249	0.0050	13	<2.30e-04	RPL18,RPL17,RPL36...
0	Ribosome(K)	0.0117	✓ 0.01	12	<2.30e-04	RPL18,RPL17,RPL36...
0	Metabolism of protein...	0.0267	0.05	11	<2.50e-04	RPL18,RPL17,RPL36...
1	M Phase(R)	0.0128	0.25	10	<1.07e-04	ITGB3BP,MAD1L1,CC...
1	Aurora B signaling(N)	0.0052	0.5	9	<1.67e-04	AURKC,AURKB,CDCA...
1	Signaling by Aurora k...	0.0125	1.0	8	<1.67e-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	<1.67e-04	NDUFB4,NDUFA2,ND...
2	Alzheimer's disease(K)	0.0223	168	15	<1.67e-04	NDUFB4,NDUFA2,ND...
2	Huntington's disease(K)	0.0246	185	15	<1.67e-04	NDUFB4,NDUFA2,ND...
2	Oxidative phosphoryl...	0.0178	134	15	<1.67e-04	NDUFB4,NDUFA2,ND...
2	Electron Transport C...	0.0101	76	16	<1.67e-04	NDUFB4,NDUFA2,ND...
2	Metabolic pathways(K)	0.1489	1120	16	<1.43e-04	NDUFB4,NDUFA2,ND...
3	Antigen processing a...	0.0104	78	9	<1.00e-03	KLRC2,KIR2DS1,HLA...
3	Immunoregulatory int...	0.0153	115	8	<5.00e-04	KIR2DS1,HLA-B,HLA...
3	Natural killer cell me...	0.0182	137	7	<3.33e-04	KLRC2,KIR2DS1,HLA...

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

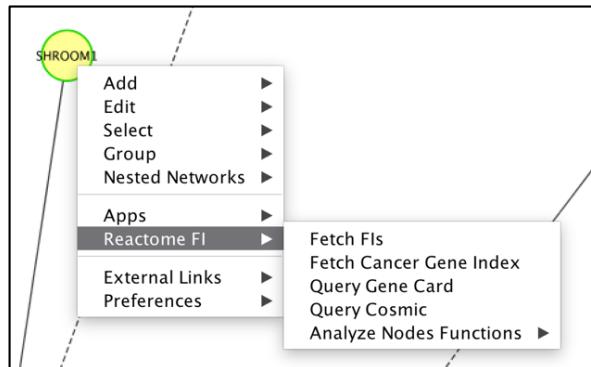
Other Features – Show Pathway Diagrams

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



Other Features – 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.



The screenshot shows the Reactome interface. A context menu is open over the gene 'SHROOM1'. The menu items include: Add, Edit, Select, Group, Nested Networks, Apps, Reactome FI, External Links, and Preferences. The 'Reactome FI' item is highlighted. A dashed line connects this menu to the main application window.

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

MAF

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).

MAF

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.

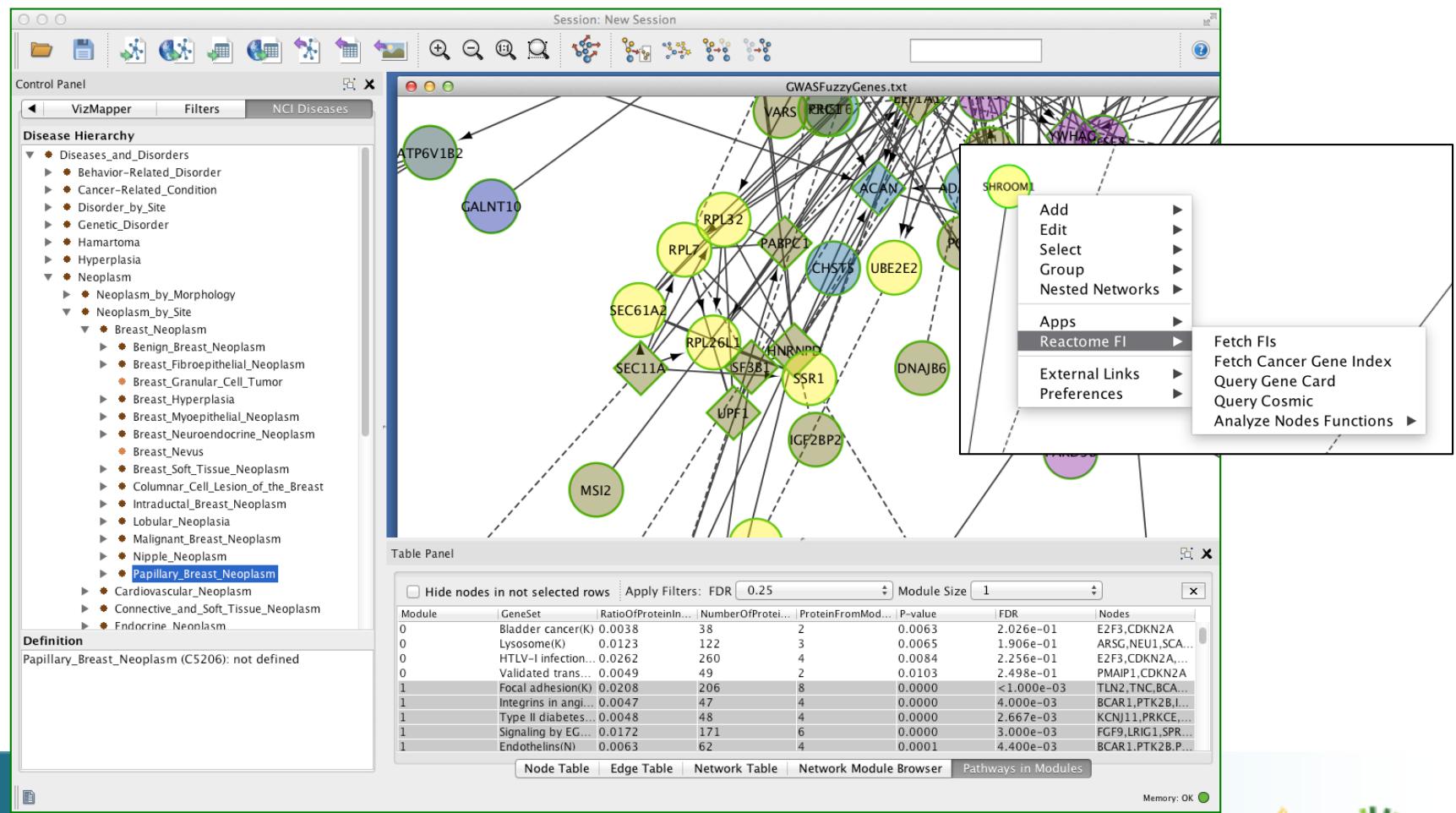
MAF

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

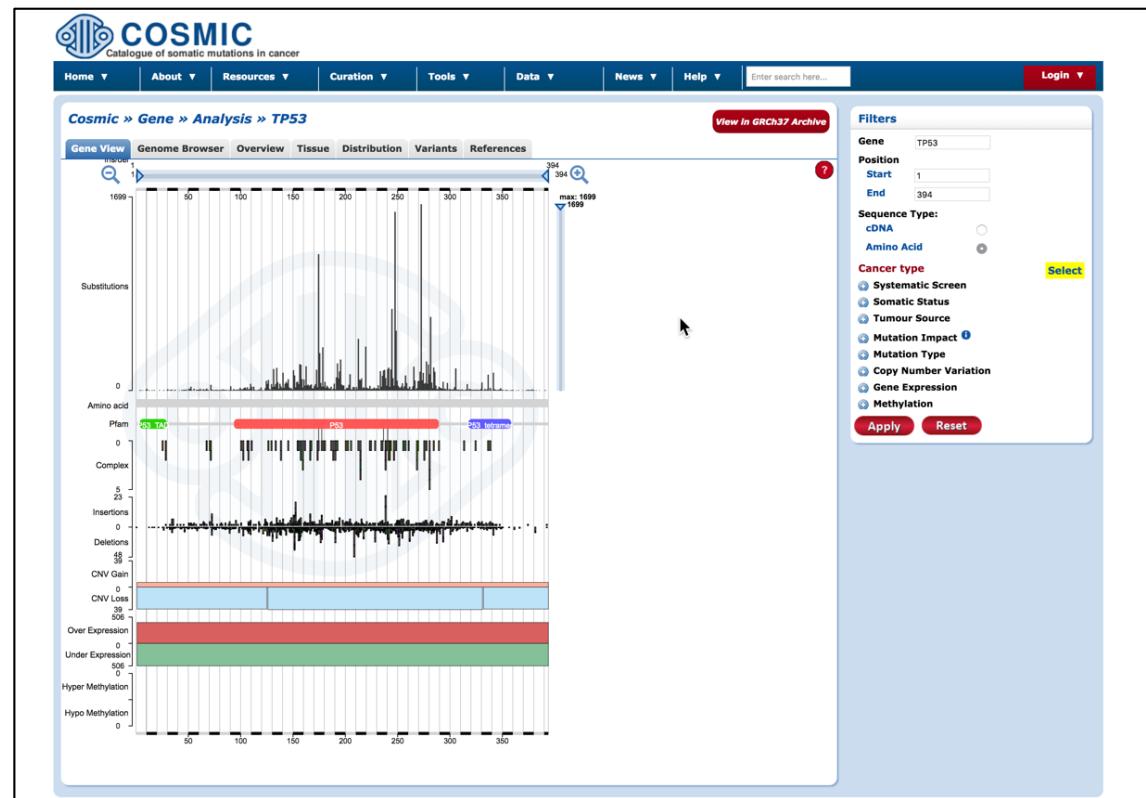
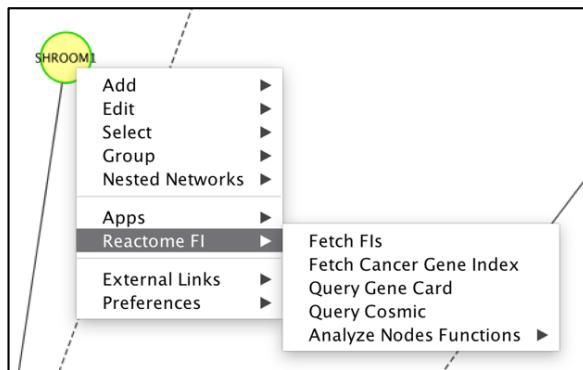
Other Features – Overlay Cancer Gene Index

- 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.



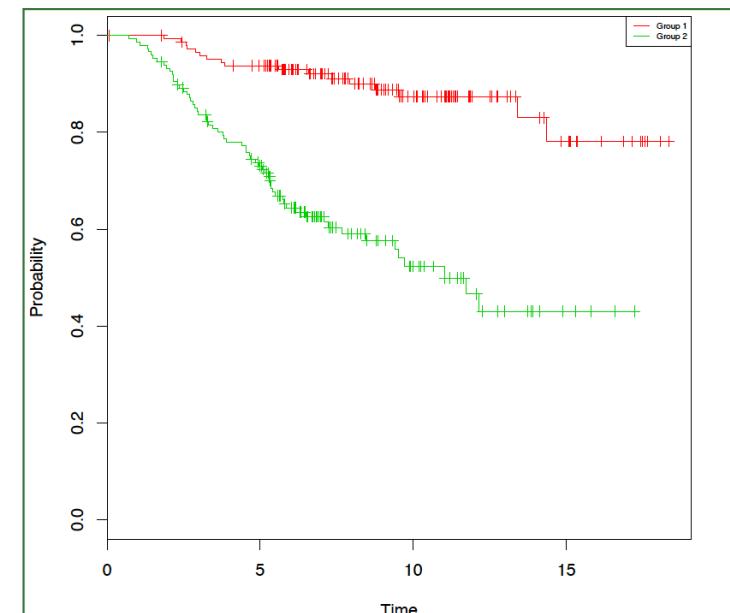
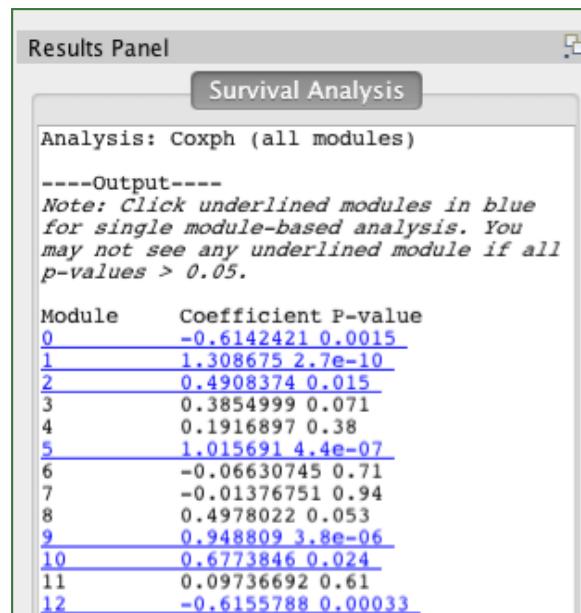
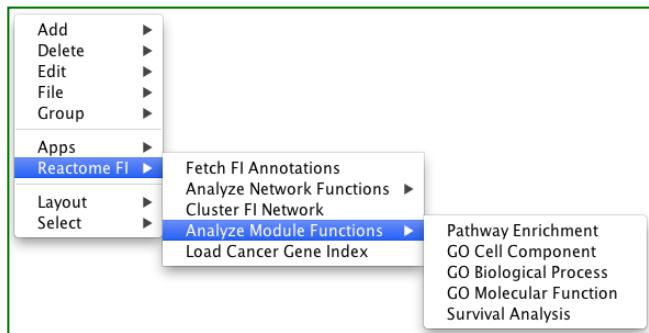
Other Features – COSMIC

- 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-Meyer survival analysis.
- Requires appropriate clinical data file.



For any questions or feedback, please contact
help@reactome.org

