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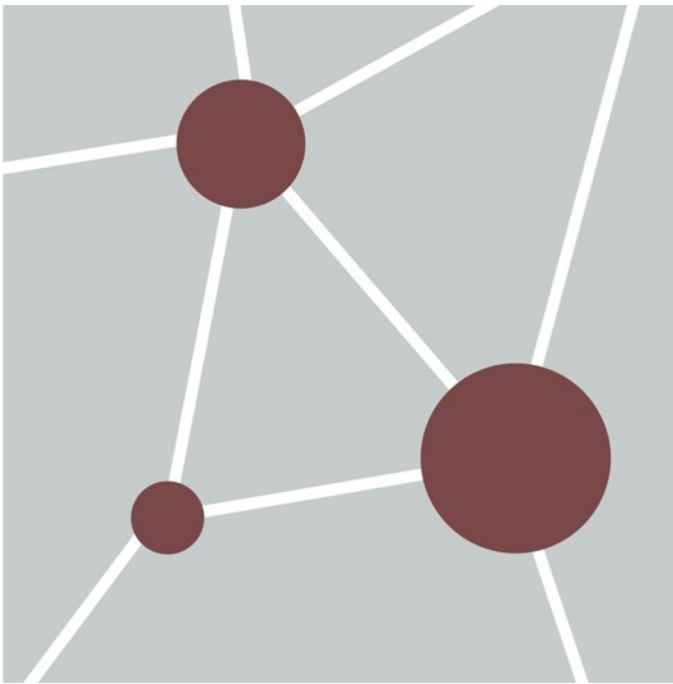
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More Depth on Pathway & Network Analysis Lab

Robin Haw

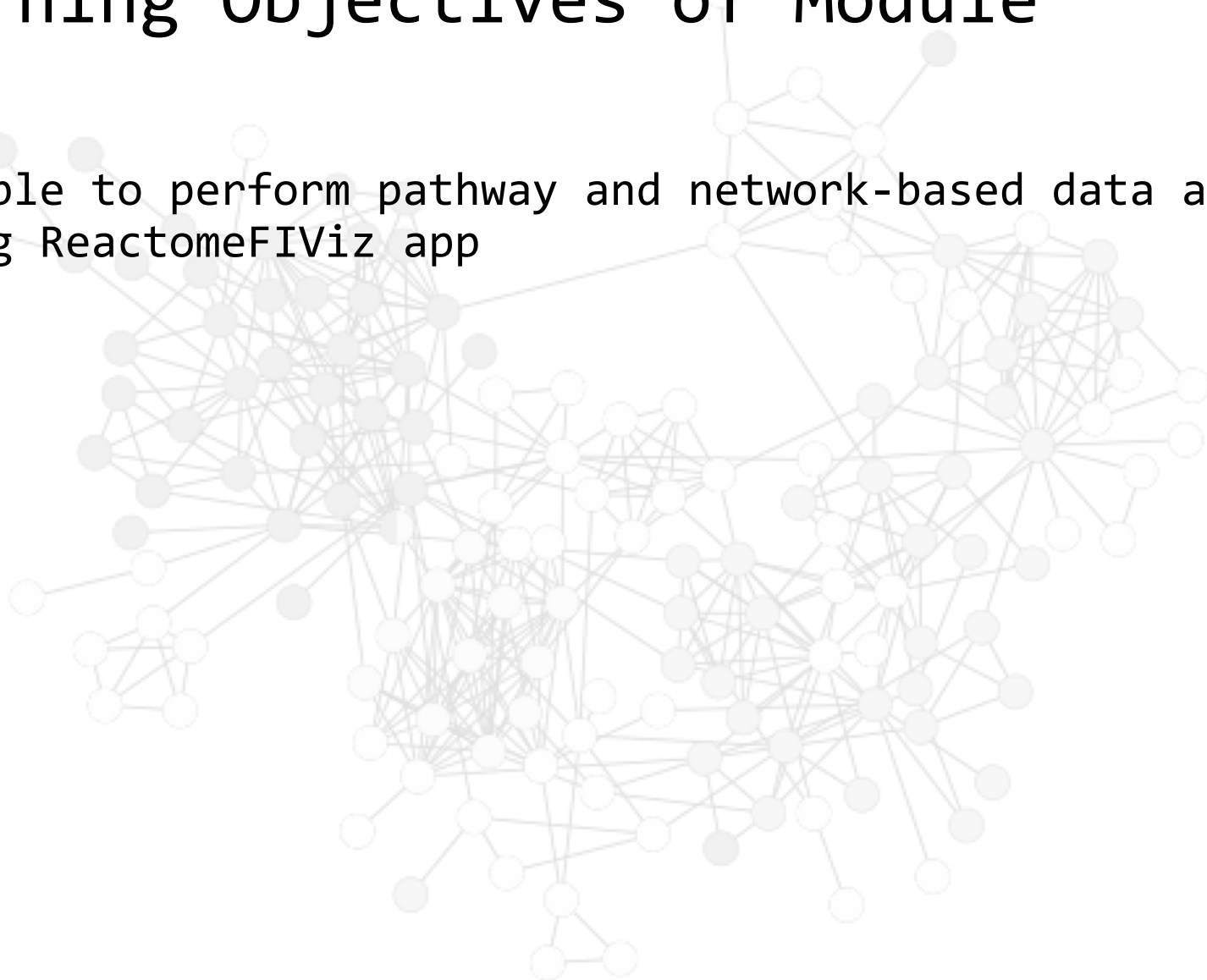
Pathway and Network Analysis of -Omics Data

July 27-29, 2020

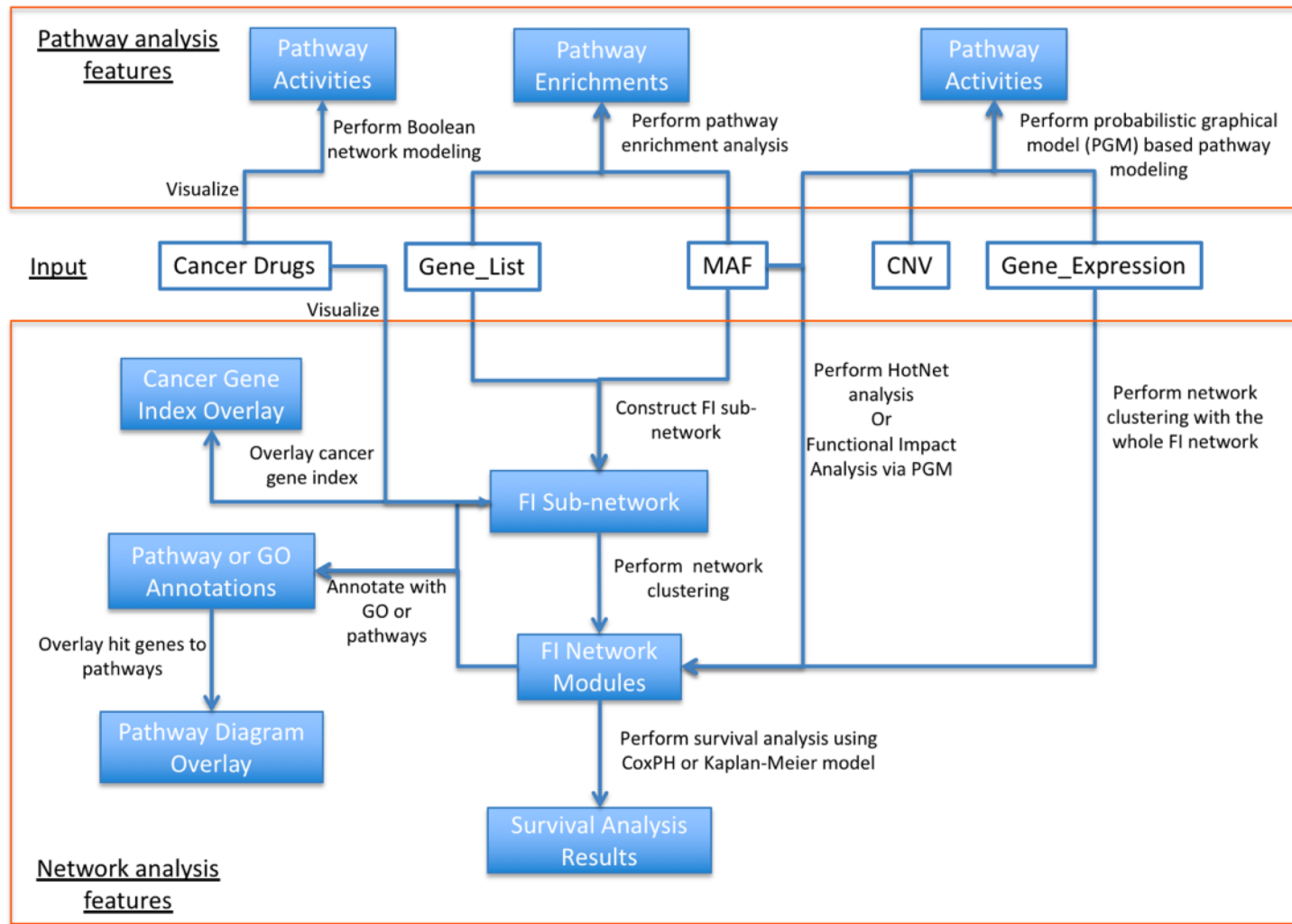


Learning Objectives of Module

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



Major Features in ReactomeFIViz



A1) Reactome Pathway Enrichment Analysis

The screenshot displays the Reactome web application interface. At the top, a toolbar contains icons for file operations and navigation. Below the toolbar is a 'Control Panel' with tabs for 'Network', 'Style', 'Select', and 'Reactome'. The 'Reactome' tab is active, showing a hierarchical tree of pathways. The 'Cell Cycle' pathway is expanded, and the 'Cell Cycle Checkpoint' sub-pathway is selected. A context menu is open over the 'Cell Cycle Checkpoint' entry, listing various actions:

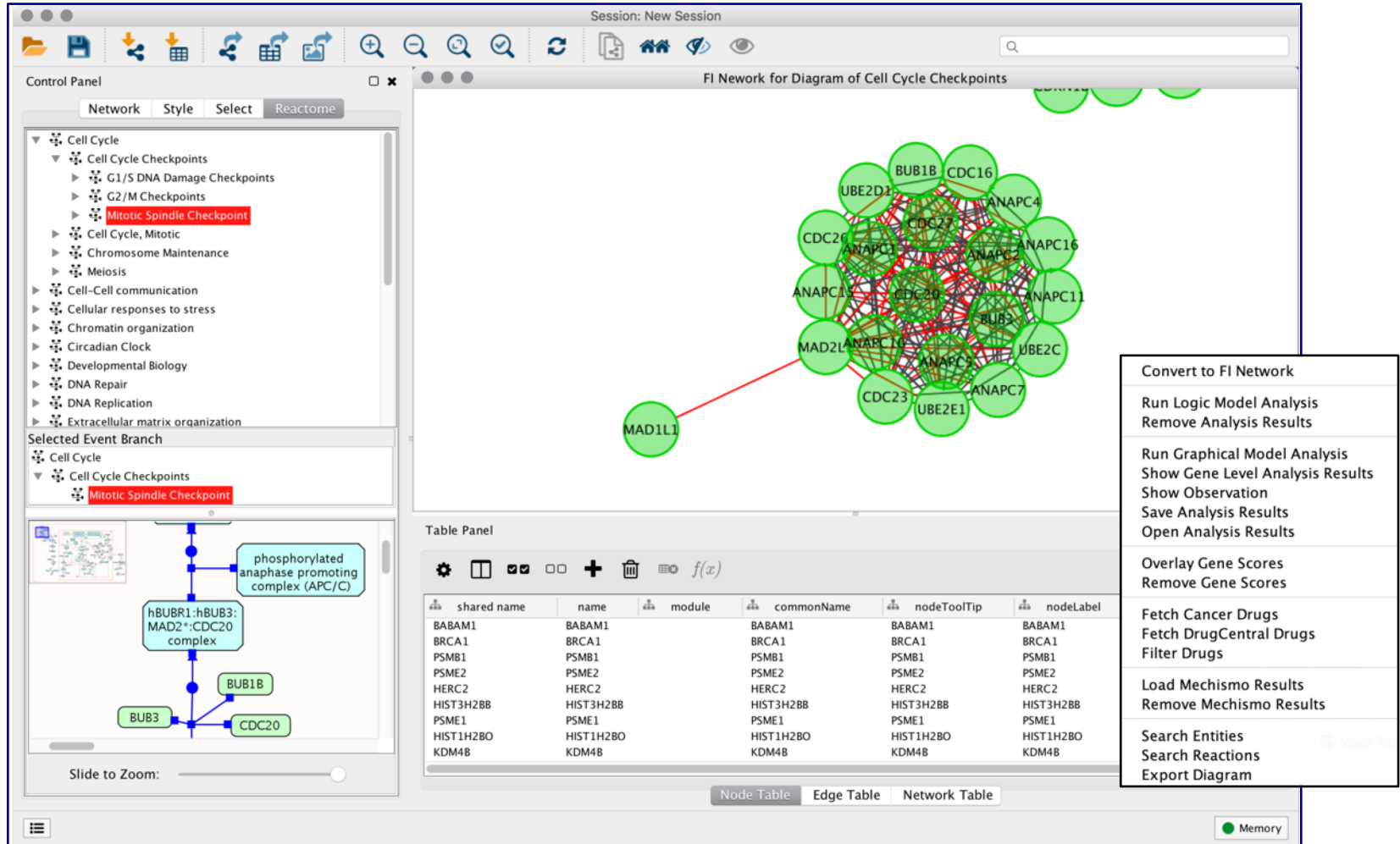
- View Reactome Source
- View in Reactome
- Show Diagram
- Search
- Analyze Pathway Enrichment
- Perform GSEA Analysis
- Run Graphical Model Analysis
- Load Graphical Model Results
- View Cancer Drugs
- View DrugCentral Drugs
- Expand Pathway
- Collapse Pathway
- Open Reactome Reactoam

The main window displays a 'Diagram of Cell Cycle Checkpoints'. The diagram shows a regulatory network involving several complexes and proteins:

- MCC:APC/C complex** (top)
- phosphorylated anaphase promoting complex (APC/C)** (top right)
- hBUBR1:hBUB3:MAD2+:CDC20 complex** (middle)
- BUB3** (bottom left)
- BUB1B** (bottom left)
- CDC20** (bottom left)
- MAD1L1** (bottom right)
- Kinetochore Complex** (bottom right)

A 'Slide to Zoom' slider is located below the diagram. At the bottom of the interface, there are tabs for 'Node Table', 'Edge Table', and 'Network Table', and a 'Memory' indicator.

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)
- IL7R binds JAK1
- IL2RG binds JAK3
- IL7 binds IL7R:JAK1
- IL7:IL7R:JAK1 binds IL7
- IL7R 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)

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

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

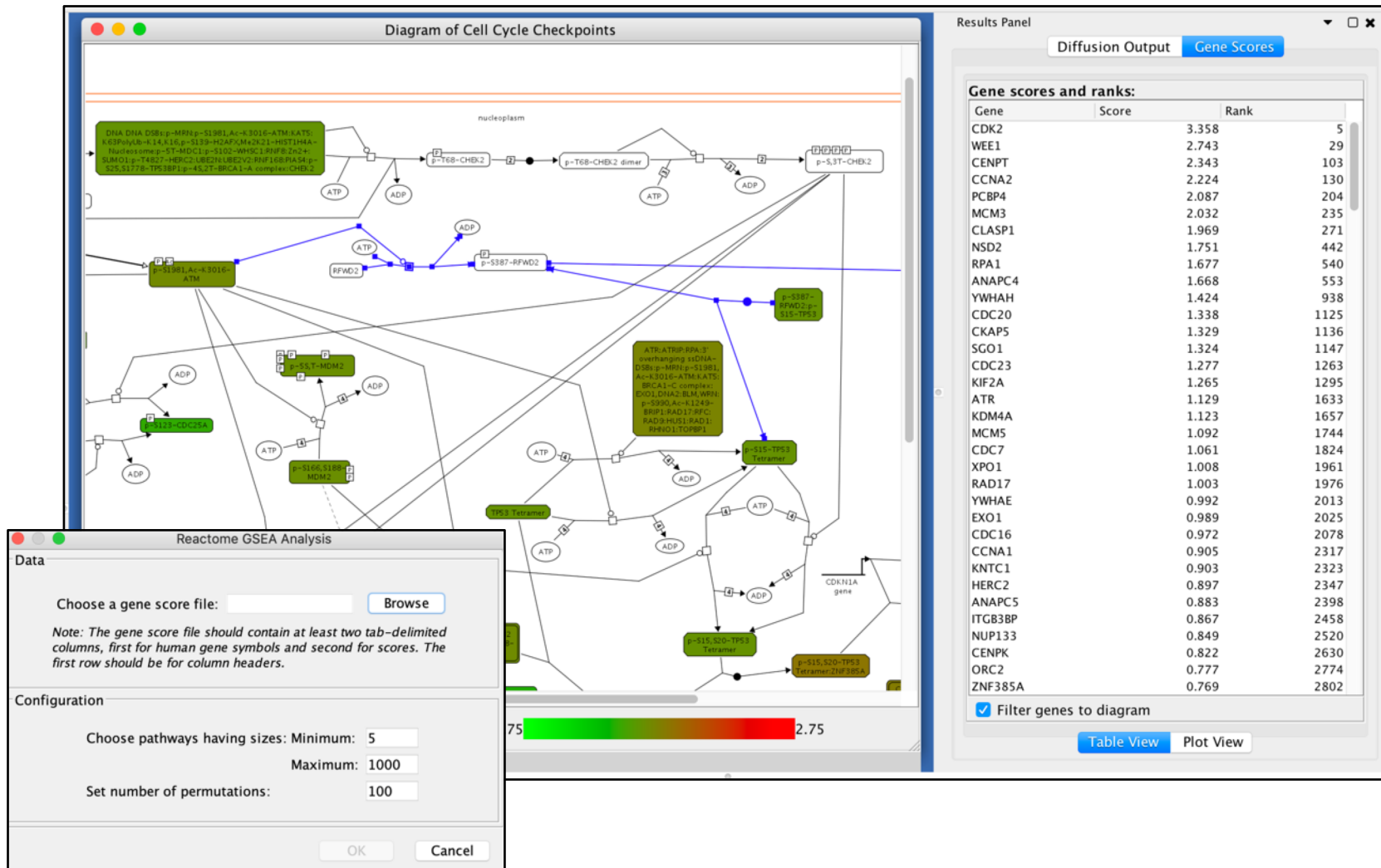
Apply Filters: FDR 1.0

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,PDS5B,...
Digestion of dietary ...	0.0004	3	2	0.0132	0.1016	MGAM,LCT
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...

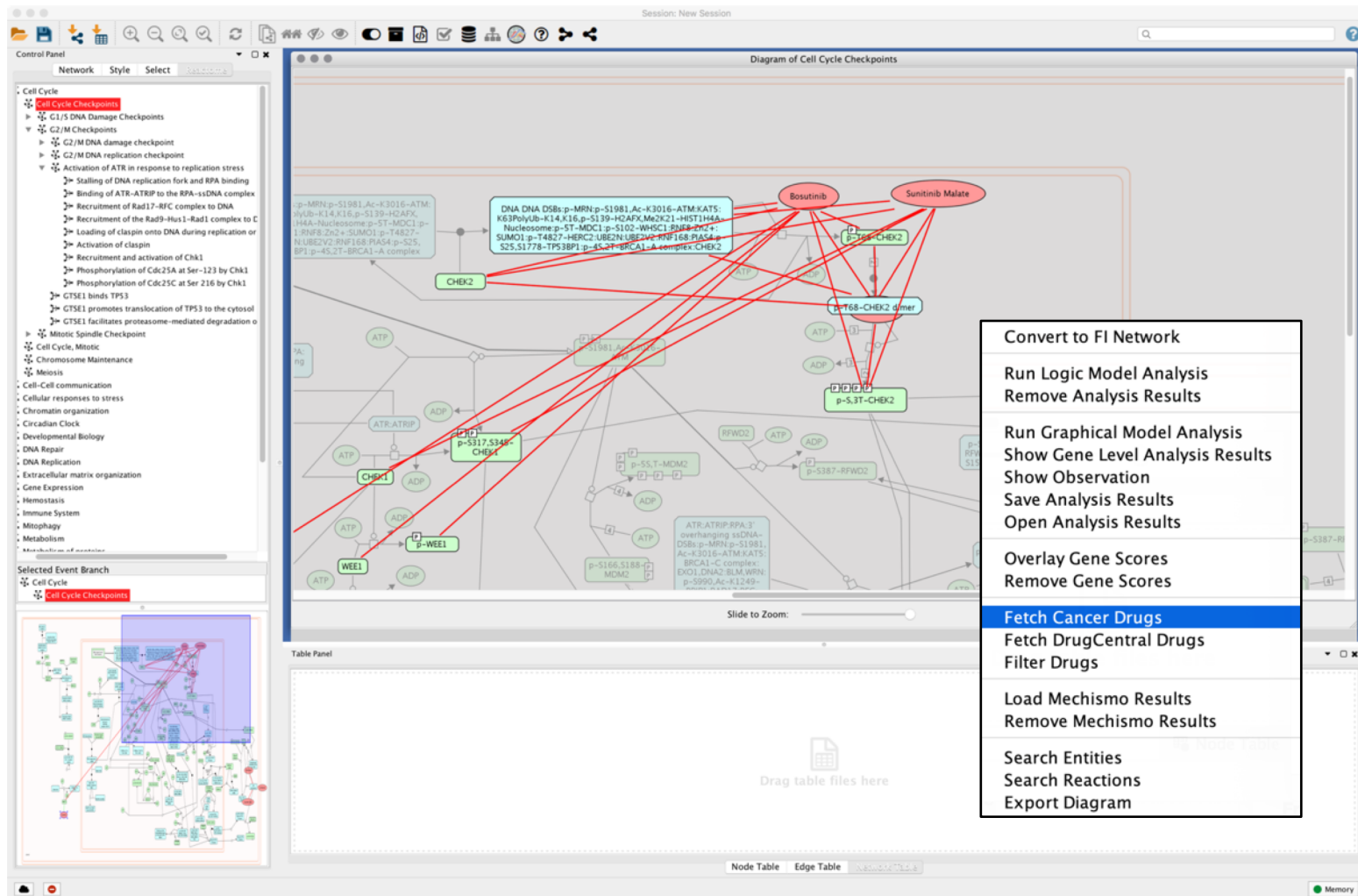
Node Table Edge Table Network Table Reactome Pathway Enrichment

Memory

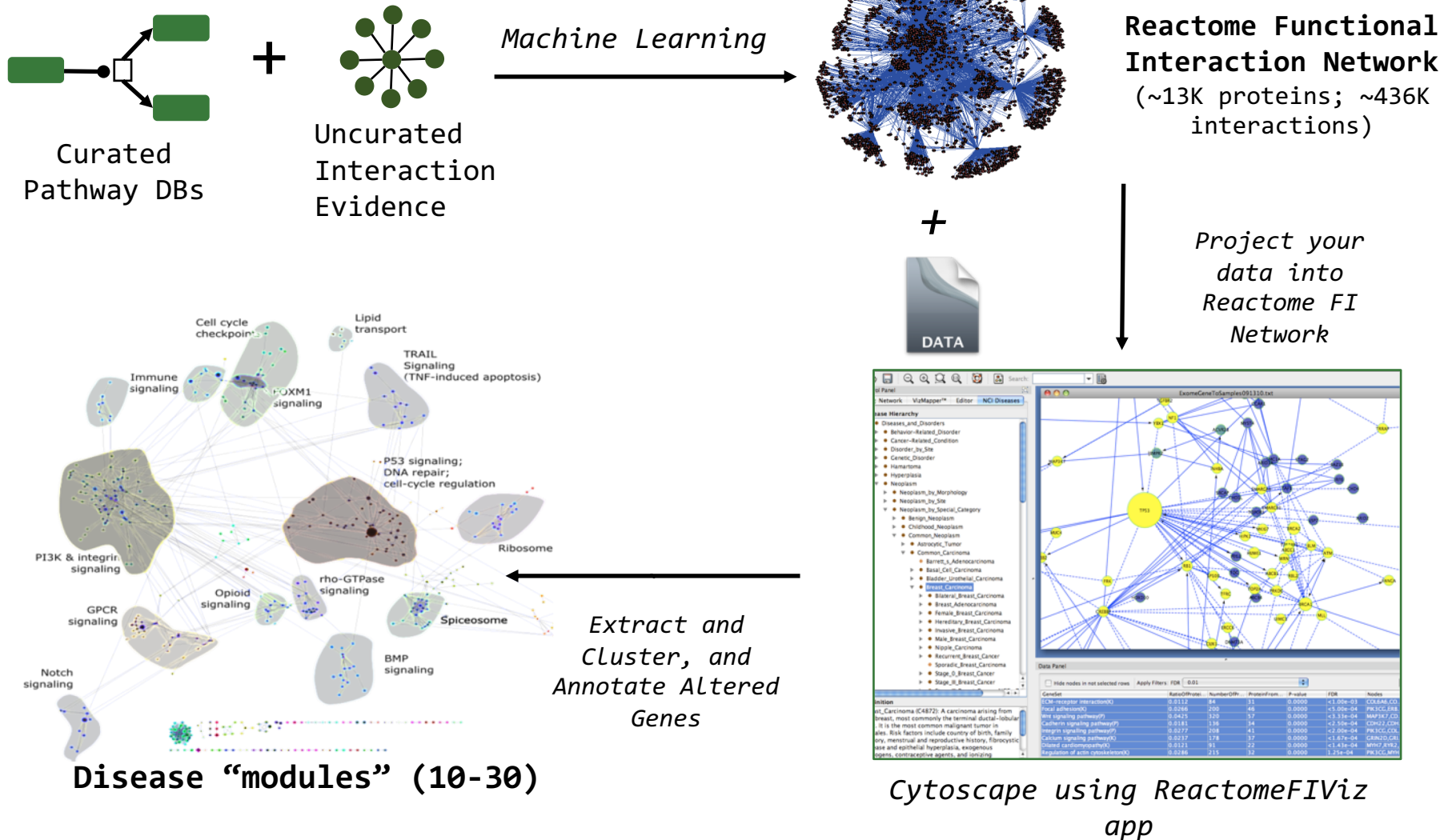
Gene Set Enrichment Analysis (GSEA)



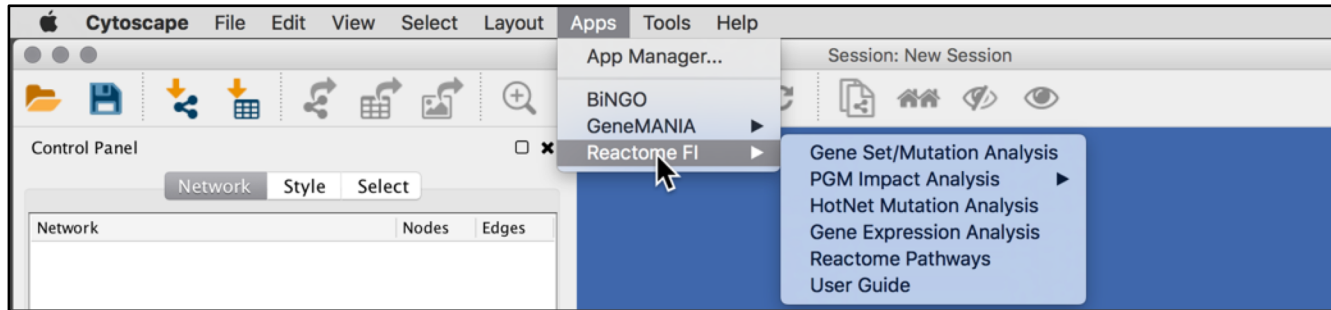
Visualize Cancer Targetome in Reactome Pathways



A2) De Novo Subnetwork Construction & Clustering



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 FIs.

Simple Gene List

MSI2
PTPRT
PELO
SLC18A1
TACC2
FAM148B
PRC1
MSTN
ATP6V1G2
APOE
IMPA2
AGER
XPO5
MEST
RREB1
BAT1
WIPI1

NCI MAF (mutation annotation file)

Hugo_Symbol	Entrez_Gene_Center	NCBI_Build	Chromosome	Start_position	End_position	Strand								
BAI2	576 broad.mit.edu	36	1	31974808	31974808	+								
LRRCL4	10489 broad.mit.edu	36	1	46524584	46524584	+								
C1orf173	127254 broad.mit.edu	36	1	74828082	74828082	+								
SSX2IP	117178 broad.mit.edu	36	1	84900740	84900740	+								
NTNG1	22854 broad.mit.edu	36	1	107668441	107668441	+								
PGLYRP3	114771 broad.mit.edu	36	1	151541551	151541551	+								
FCRL5	83416 broad.mit.edu	36	1	155783467	155783467	+								
FBXO28	23219 broad.mit.edu	36	1	222388418	222388418	+								
OR2M2	391194 broad.mit.edu	36	1	246410342	246410342	+								
MKX	283078 broad.mit.edu	36	10	28063690	28063690	+								
.....														
Variant_Classification	Variant_Type	Reference_Allele	Tumor_Seq_Allele1	Tumor_Seq_dbsNP_RS	dbSNP_Val_1	Tumor_Sampl								
Missense_Mutation	SNP	C	G	novel	none	TCGA-04-1331								
Missense_Mutation	SNP	C	G	novel	none	TCGA-04-1331								
Missense_Mutation	SNP	T	T	G	novel	TCGA-04-1331								
Silent	SNP	A	A	G	novel	TCGA-04-1331								
Missense_Mutation	SNP	G	G	A	novel	TCGA-04-1331								
Missense_Mutation	SNP	G	G	T	novel	TCGA-04-1331								
Missense_Mutation	SNP	A	A	C	novel	TCGA-04-1331								
Missense_Mutation	SNP	A	T	novel	none	TCGA-04-1331								
Missense_Mutation	SNP	G	A	novel	none	TCGA-04-1331								
Missense_Mutation	SNP	C	C	A	novel	TCGA-04-1331								
.....														
Matched_No	Match_Norm	Match_Norm	Tumor_Valid	Match_Norm	Match_Norm	Verification	Validation_S	Mutation_Si	Sequencing	Sequence_Si	Validation_M	Score	BAM_file	Sequencer
TCGA-04-1331-10A-01W						Unknown	Unknown	Unknown	x	x	---		x	
TCGA-04-1331-10A-01W						Unknown	Unknown	Unknown	x	x	---		x	
TCGA-04-1331-10A-01W						Unknown	Unknown	Unknown	x	x	---		x	
TCGA-04-1331-10A-01W						Unknown	Unknown	Unknown	x	x	---		x	
TCGA-04-1331-10A-01W						Unknown	Unknown	Unknown	x	x	---		x	
TCGA-04-1331-10A-01W						Unknown	Unknown	Unknown	x	x	---		x	
TCGA-04-1331-10A-01W						Unknown	Unknown	Unknown	x	x	---		x	
TCGA-04-1331-10A-01W						Unknown	Unknown	Unknown	x	x	---		x	
TCGA-04-1331-10A-01W						Unknown	Unknown	Unknown	x	x	---		x	
TCGA-04-1331-10A-01W						Unknown	Unknown	Unknown	x	x	---		x	

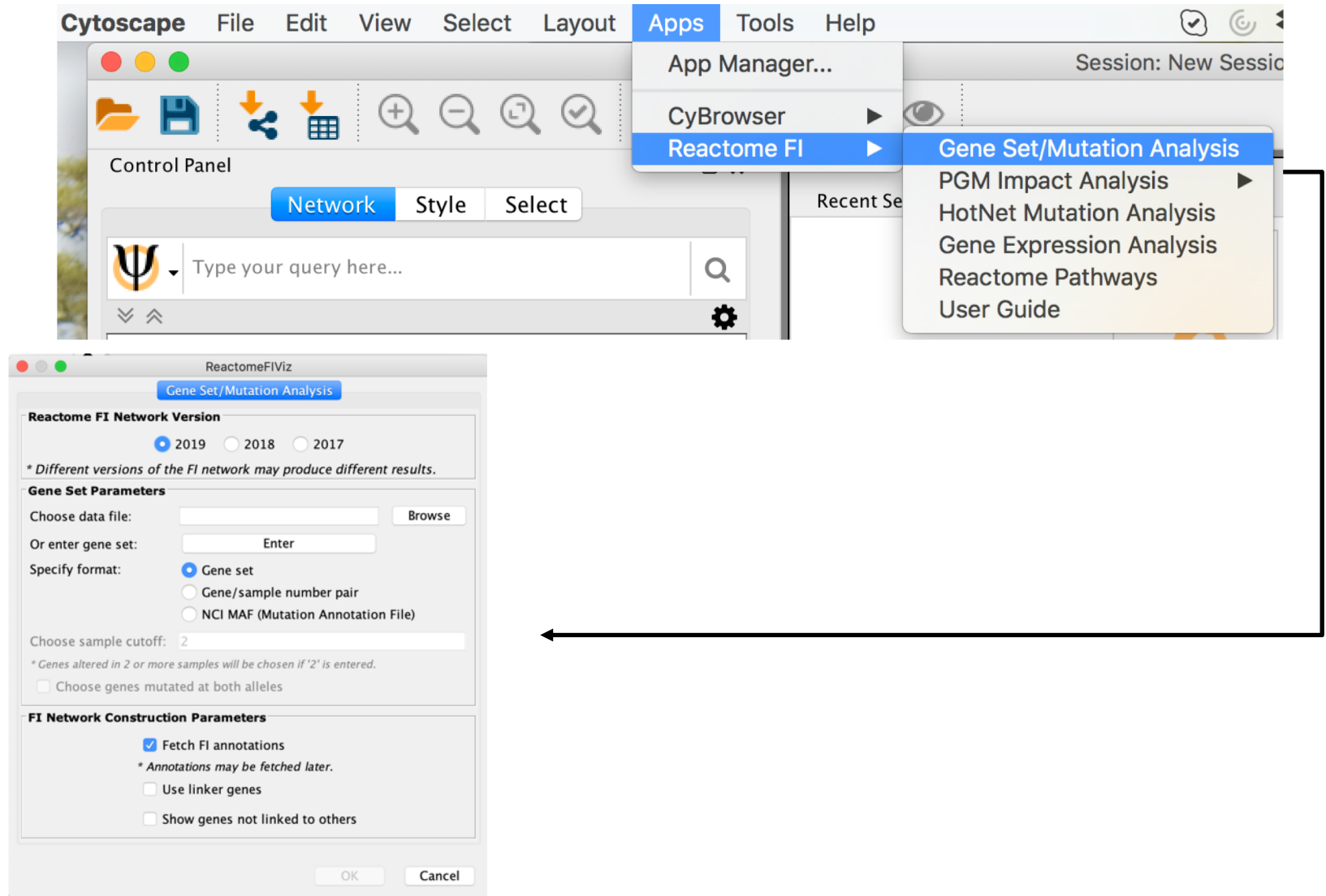
Gene/Sample Number Pairs

Gene	Sample_Number	Samples
IFT88	1	TCGA-10-0937
TACC2	5	TCGA-24-2288; TCGA-09-1662; TCGA-13-0755; TCGA-13-0920; TCGA-24-2035
ADAD2	1	TCGA-24-1419
PODXL2	1	TCGA-24-2290
RTN3	1	TCGA-13-0726
TADA2B	1	TCGA-25-1313
CD274	1	TCGA-25-1627
SULF1	2	TCGA-24-2262; TCGA-09-2056
SYCP2	3	TCGA-24-1563; TCGA-61-2008; TCGA-24-1427
CD200R1L	1	TCGA-13-1488
RCBTB2	1	TCGA-61-2113
TOP2B	1	TCGA-13-0923
C1orf50	1	TCGA-25-2392
ATP9A	5	TCGA-25-1329; TCGA-04-1530; TCGA-24-2262; TCGA-10-0933; TCGA-13-0795
NUDT5	1	TCGA-61-2003
ZNF189	1	TCGA-24-2254
KCNB1	1	TCGA-23-1028
FERMT3	1	TCGA-24-1464
ZCCHC2	1	TCGA-61-1998
DDX18	1	TCGA-13-1509
TGM1	2	TCGA-24-0975; TCGA-25-1321
SMAD7	1	TCGA-09-2051
OR2F2	3	TCGA-13-0723; TCGA-24-2267; TCGA-25-2392
ZSWIM3	2	TCGA-24-1466; TCGA-23-1118
EIF2AK2	1	TCGA-59-2355; TCGA-24-1463
KIAA2018	4	TCGA-23-1024; TCGA-04-1367; TCGA-24-2288; TCGA-24-2289
.....		

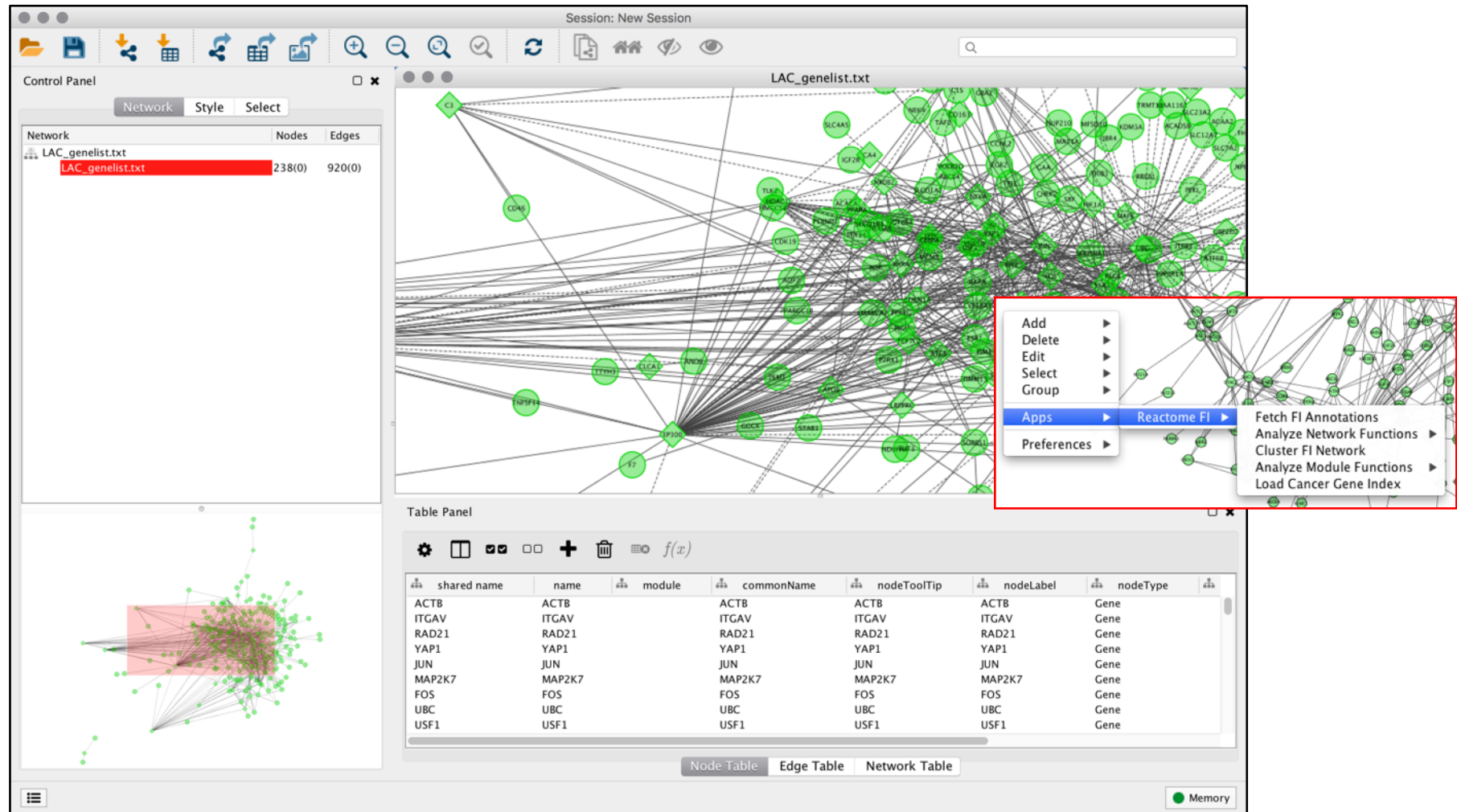
Microarray (array) data file

gene	Sample 122	Sample 123	Sample 124	Sample 125	Sample 126	Sample 127	Sample 128	Sample 129	Sample 130
IFT88	1.433357937	0.749571365	0.204170171	1.376375723	0.049504161	0.912377692	0.464660294	0.106486375	0.40767808
KIF9	0.348692336	-0.093947168	-0.568902295	0.177455762	-0.30793794	-0.574121582	-0.772454492	0.850743799	0.19832391
CP5F3	-0.628846878	-0.519774777	0.200101088	-0.388888256	-0.094395584	0.549113111	0.440059971	-0.247094525	1.421708617
TACC2	0.985299156	-0.73255355	0.103466591	-0.057886422	0.730172249	0.554418157	1.280112472	0.463706367	0.956951722
C14orf132	1.249528811	0.104876045	0.524096007	-0.43464182	-1.528259113	-0.467450338	-0.715336925	-0.525776594	-1.06893985
RTN3	0.10643433	-0.286920286	-0.329065423	0.548958273	1.054699921	-0.827782882	-0.462525025	1.398885211	-0.588960437
PODXL2	-0.031403138	-0.390413822	-0.075387324	-0.412392415	0.085789024	-0.097365917	-0.053408731	-0.104692114	0.134380939
CORO1A	-0.247037235	0.664838693	-0.423713196	-0.001970579	0.488162732	-0.435111645	0.596447999	-0.463607768	0.055021666
RBMI17	-0.360070659	-0.142165087	-0.233914802	0.018396914	-0.130696373	-0.394476802	-0.818819233	-0.692663375	0.993237633
WTF1	-0.768498156	-0.669419285	-0.763665041	-0.674252401	-0.618671571	-0.393931693	-0.906241952	-0.35285021	-0.538925163
SULF1	-0.863622291	1.588947769	0.272263522	-0.419487008	0.401966747	-0.081472544	-1.17805435	-0.32789133	-0.21903657
SYCP2	-0.365259803	1.246319117	-0.107066702	0.891658263	0.914356558	0.091543376	-1.037696782	0.727095627	0.009262058
PHYH	-0.252934465	-0.340129465	0.084219531	-0.613340462	-1.85732245	0.479503528	0.22954453	-1.130697457	1.037551523
RCBTB2	-1.216044791	1.137367735	-0.741461602	0.636779988	-0.695953625	-0.46841374	-0.344892088	-0.130354482	-0.572431973
TOP2B	1.329376011	1.272684609	0.636481097	0.611284918	0.466406891	0.982928554	-0.749308731	0.397117399	0.050669942
SEC22B	-0.712050988	0.661526326	-0.33943161	0.917245507	0.427725933	0.413113408	-0.638988365	0.632301277	0.486176031
ATP9A	1.547427859	0.444111328	1.415509578	-0.14352465	0.540051896	0.510070468	-0.233460968	-0.149520936	-0.047584083
TGFB1	-0.782212802	-0.264862477	-0.123008356	0.027190126	-0.239829397	0.427719409	-0.713866842	0.110613726	1.270499777
NUDT5	-0.1616643202	-0.117594702	0.072831699	-0.025664715	0.37488737	0.433985219	-0.89899595	0.020300278	0.112230265
VTI18	1.252187159	0.515526231	0.073529674	0.770902019	-0.368466883	-1.684634408	0.191395423	0.967344934	2.116535981
RP56KB1	-0.387163384	0.639591396	-0.597648114	-0.392297158	0.932216509	1.563670699	-0.782463975	0.465043084	-0.674654723
RAPGEF4	0.202545341	0.240920292	0.106607963	1.564856103	0.557513638	-0.027704365	-0.051297319	-0.612922368	-1.178952896
PRO1596	-0.010044297	0.228155239	0.228155239	0.445509484	0.148635904	0.079718194	-0.376195587	0.138032748	0.03730761
KCNB1	0.093447081	-0.061401218	1.135678435	0.628377569	0.656531805	1.185927337	1.318156356	-0.469637643	0.346835207
ALDH9A1	0.702468807	-0.1692273	0.963272942	-1.181038168	-1.083824907	1.698904754	1.844724649	-0.038783225	-1.54559605
ZCCHC2	0.53864339	0.120590563	0.845270548	-0.4759704052	0.477522406	-1.144895382	-0.398583158	0.53233533	-0.09571821
LOC51274	-0.291416641	-0.264217345	0.134705668	-0.527143876	-0.073822271	-0.699406086	-0.971399049	-0.218885185	-0.146353728
DDX18	0.34967191	0.294984327	-1.00840307	1.015037505	0.1400336175	0.076233995	-0.160745532	0.51373466	1.069725088

Gene Set-based Analysis



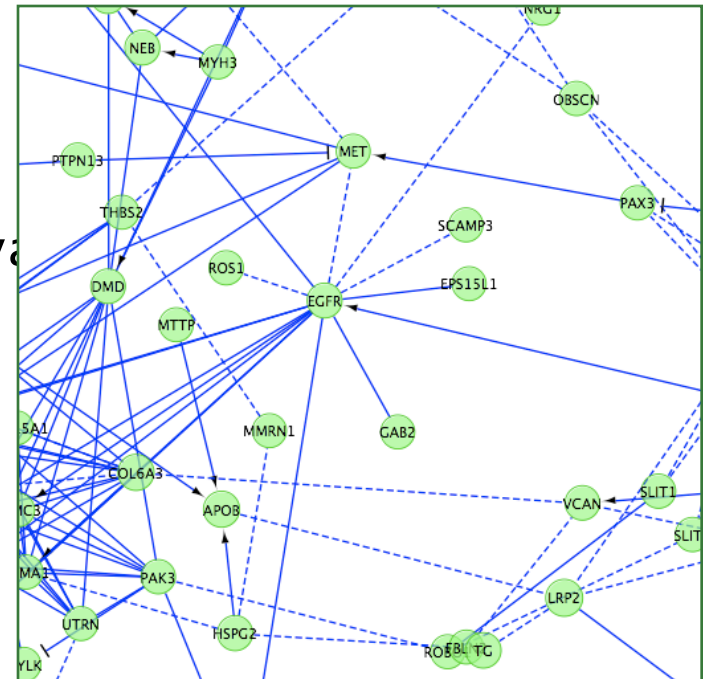
FI Results Display



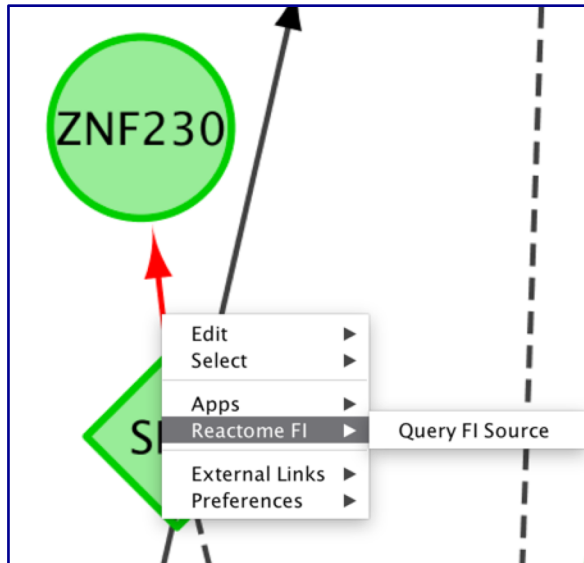
- Constructed network is displayed in the Network View panel using an FI specific visual style

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 via
 - --> 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	ENCODE
definition	ENCODE proximal_filtered TF/target interaction; supported by GO BP sharing
factor	SIX5
species	Homo sapiens
target	ZNF230

Close

Predicted FIs

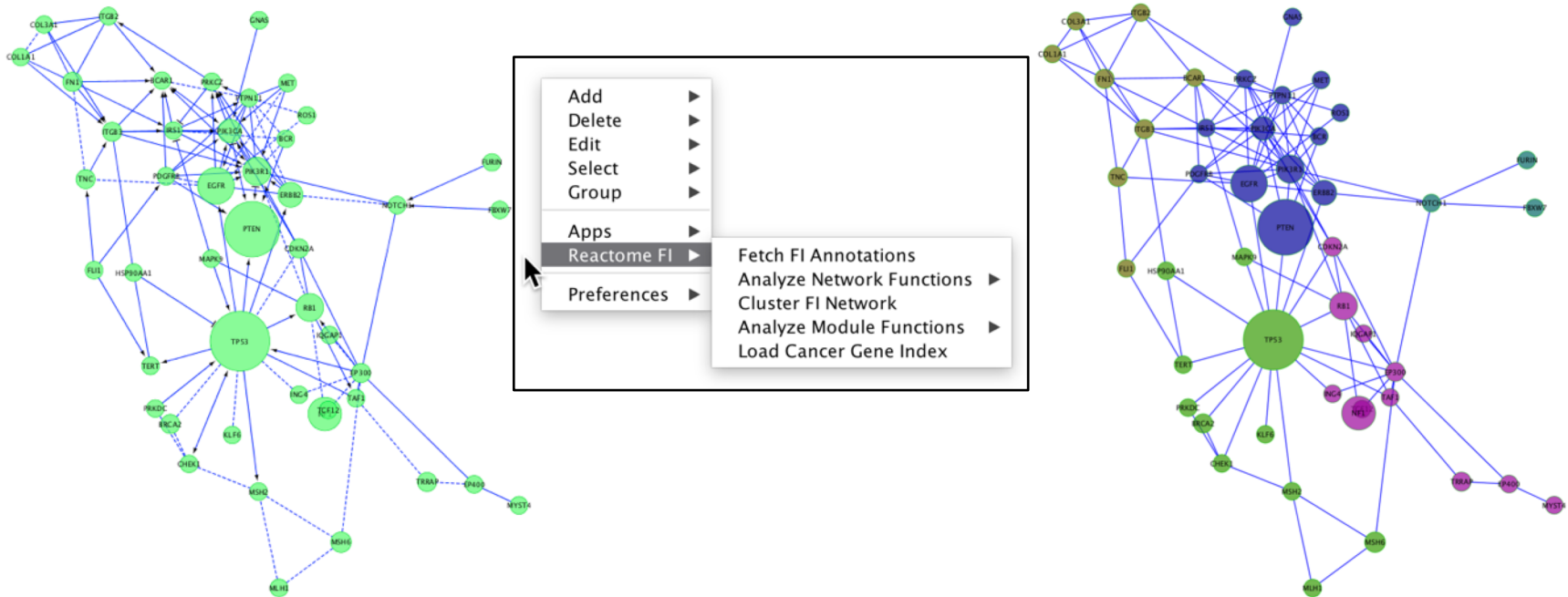
Interaction Info
Interaction: SNX4 - SNX6

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

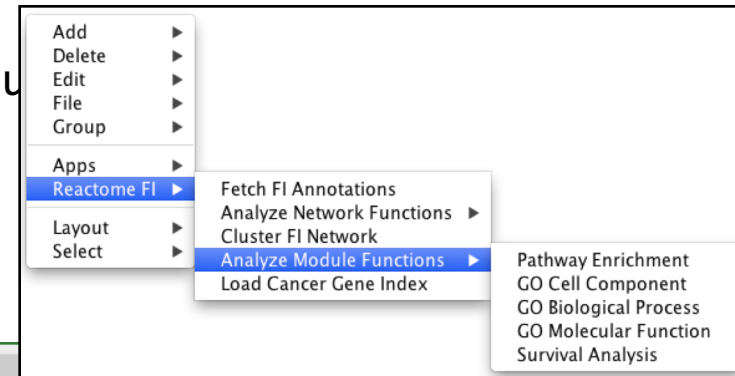
Cluster FI Network

- 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	0.0010	mModule	0.0010	FDR	Nodes
0	Translation(R)	0.0158	0.0050	13	0.0000	<2.50e-04	RPL18,RPL17,RPL36...
0	Influenza Life Cycle(R)	0.0249	✓ 0.01	12	0.0000	<2.50e-04	RPL18,RPL17,RPL36...
0	Ribosome(K)	0.0117	0.05	11	0.0000	<2.50e-04	RPL18,RPL17,RPL36...
0	Metabolism of protei...	0.0267	0.25	✓ 10	0.0000	<2.50e-04	RPL18,RPL17,RPL36...
1	M Phase(R)	0.0128	0.5	9	0.0000	<5.00e-04	ITGB3BP,MAD1L1,CC...
1	Aurora B signaling(N)	0.0052	1.0	8	0.0000	<5.00e-04	AURKC,AURKB,CDCA...
1	Signaling by Aurora k...	0.0125		7	0.0000	<3.33e-04	AURKC,AURKB,CDCA...
2	Glucose Regulation of...	0.0197	148	16	0.0000	<1.67e-04	NDUFB4,NDUFA2,ND...
2	Parkinson's disease(K)	0.0176	132	15	0.0000	<1.67e-04	NDUFB4,NDUFA2,ND...
2	Alzheimer's disease(K)	0.0223	168	15	0.0000	<1.67e-04	NDUFB4,NDUFA2,ND...
2	Huntington's disease(K)	0.0246	185	15	0.0000	<1.67e-04	NDUFB4,NDUFA2,ND...
2	Oxidative phosphoryl...	0.0178	134	15	0.0000	<1.67e-04	NDUFB4,NDUFA2,ND...
2	Electron Transport C...	0.0101	76	16	0.0000	<1.67e-04	NDUFB4,NDUFA2,ND...
2	Metabolic pathways(K)	0.1489	1120	16	0.0000	<1.43e-04	NDUFB4,NDUFA2,ND...
3	Antigen processing a...	0.0104	78	9	0.0000	<1.00e-03	KLRC2,KIR2DS1,HLA...
3	Immunoregulatory int...	0.0153	115	8	0.0000	<5.00e-04	KIR2DS1,HLA-B,HLA...
3	Natural killer cell me...	0.0182	137	7	0.0000	<3.33e-04	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

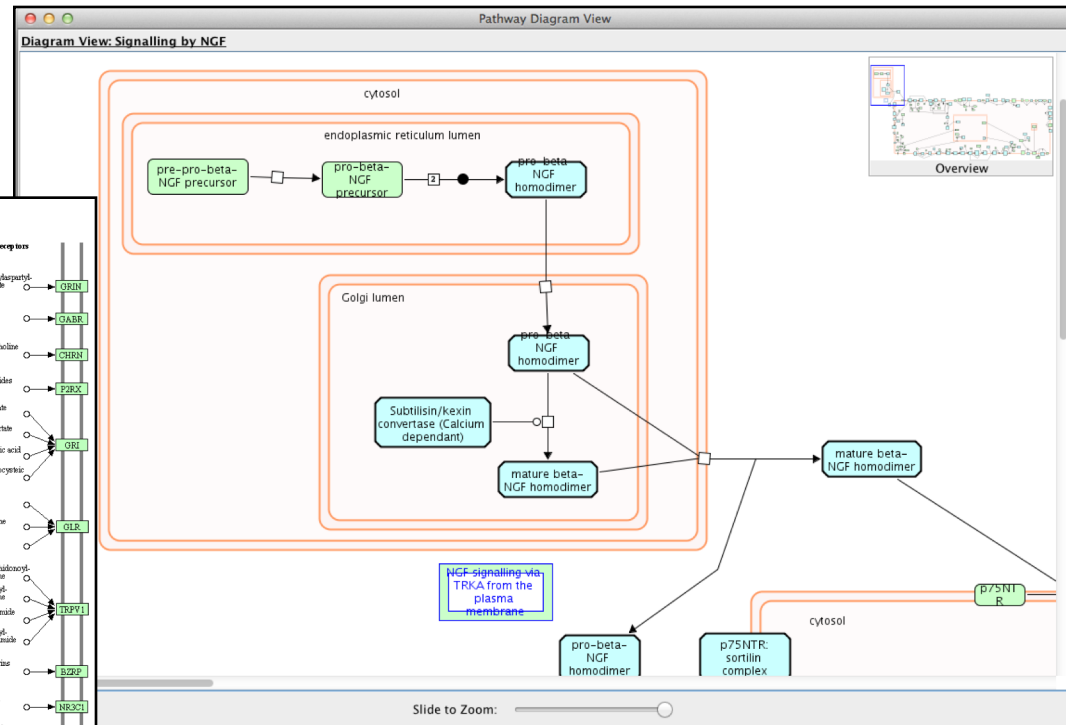
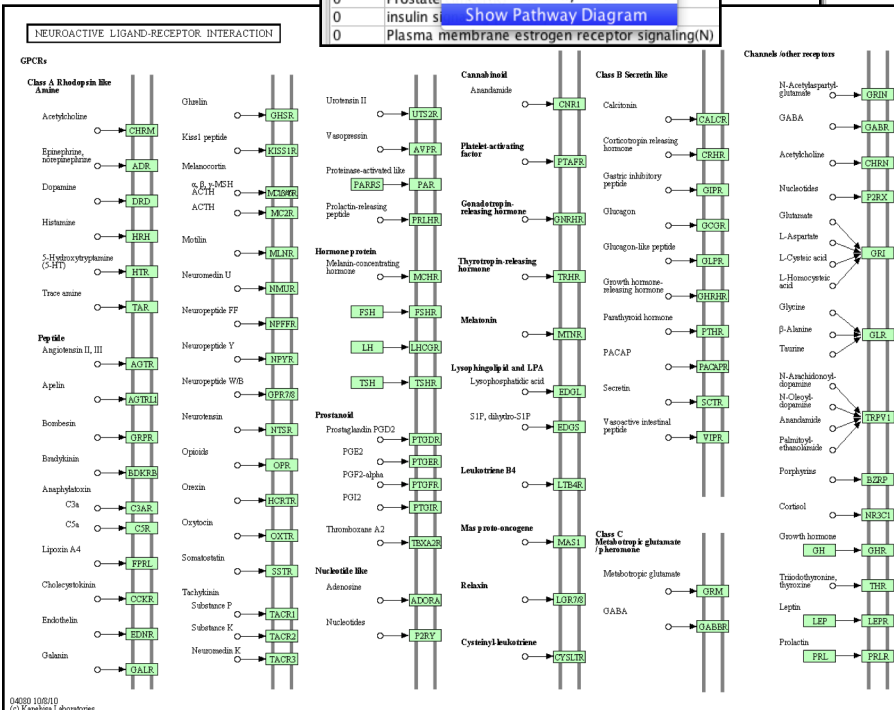
☐ Hide nodes in not selected rows Apply Filters:

Module	GeneSet
0	Arf6 signaling events(N)
0	Insulin Pathway(N)
0	Melanoma
0	IGF1 pat
0	Endothe
0	Prostate
0	insulin s
0	Plasma membrane estrogen receptor signaling(N)

Export Annotations

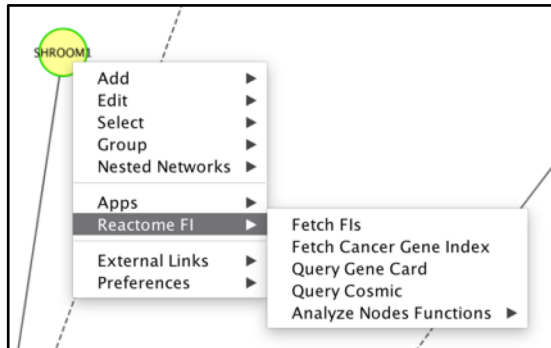
Show Pathway Detail

Show Pathway Diagram



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 [dropdown] 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](#)

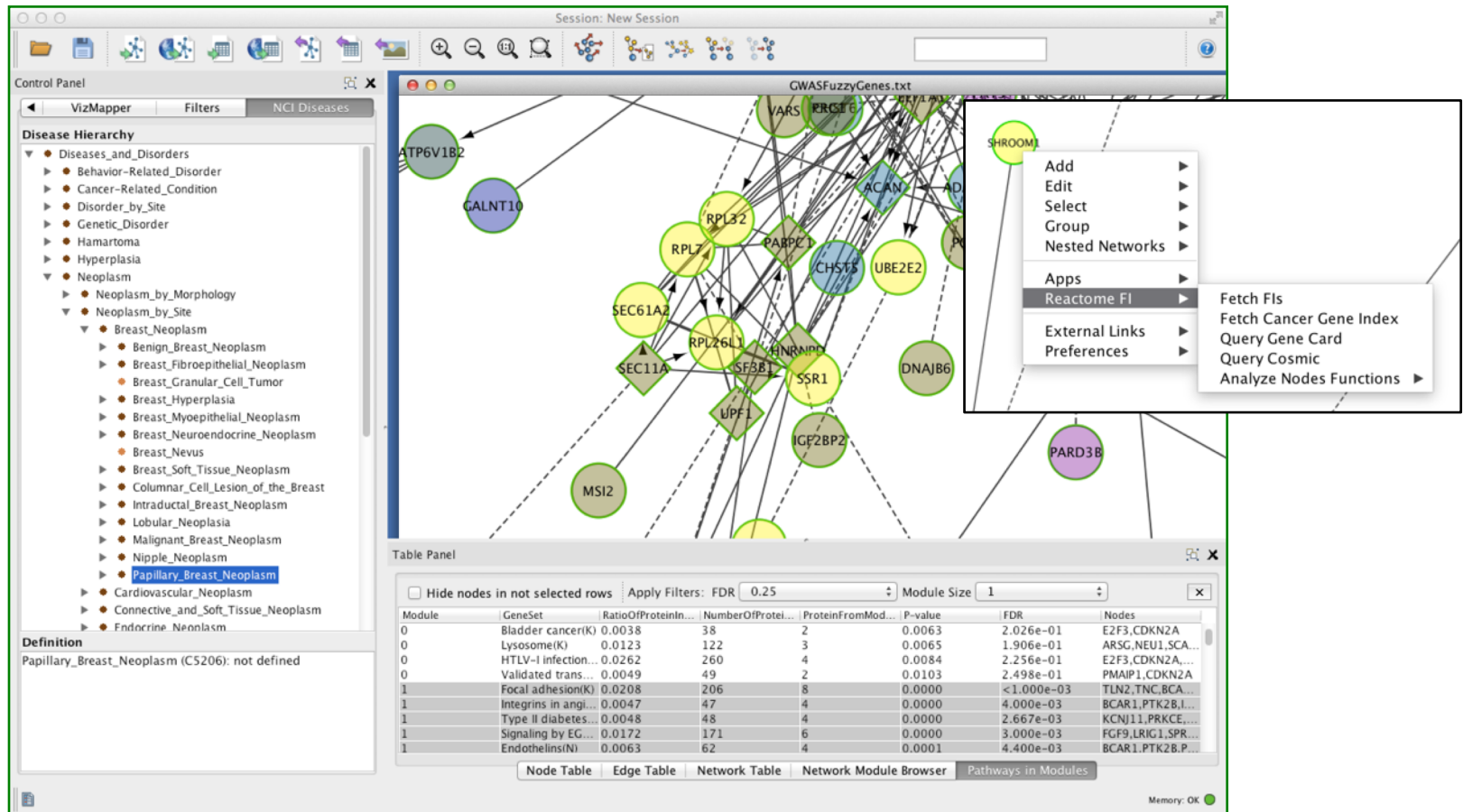
Using gene expression profiling to identify 5 recurrent translocations, specific trisomies, and expression of cyclin D genes, MM tumors can be divided into 8 TC (translocation/cyclin D) groups (11q13, 6p21, 4p16, maf, D1, D1+D2, D2, and none) that appear to be defined by early, and perhaps initiating,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

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

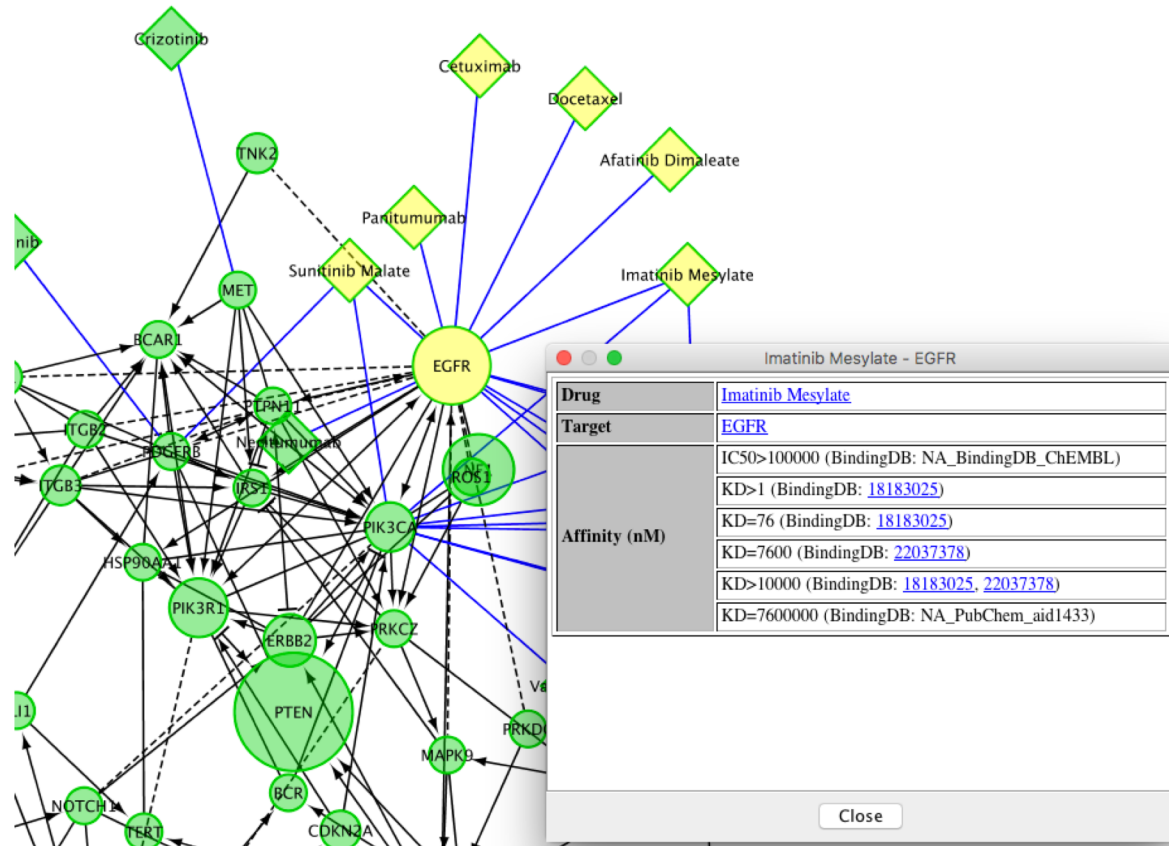
We investigated the expression profiles of the FGFR3/MMSET, CCND1, CCND3, MAF, and MAFB genes, which

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.



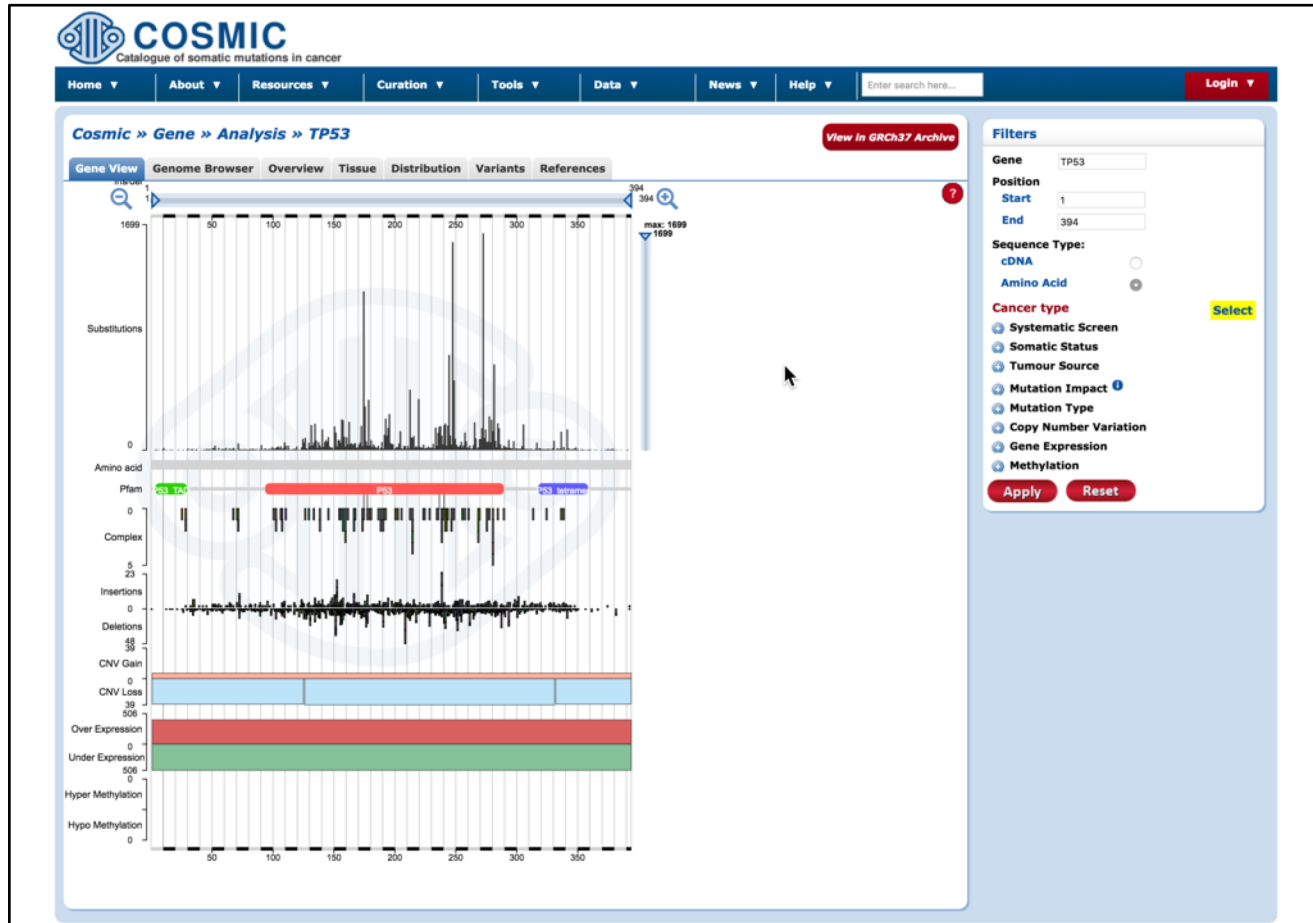
Visualize Cancer Targetome in the Reactome FI Network



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.

Results Panel

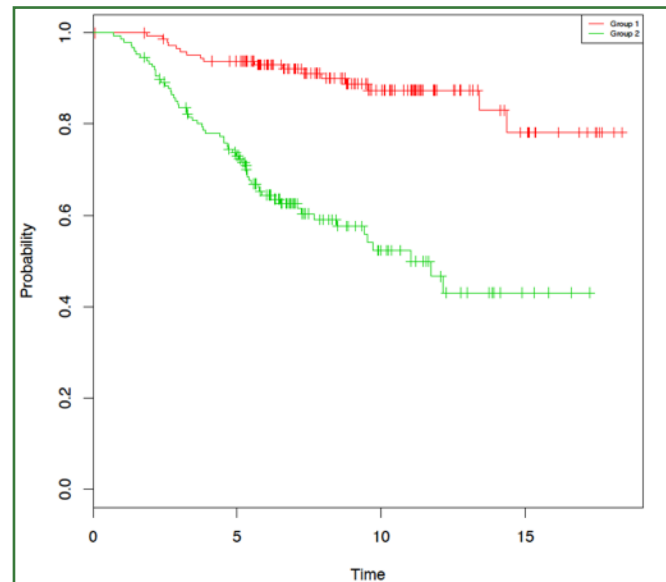
Survival Analysis

Analysis: Coxph (all modules)

----Output----

Note: Click underlined modules in blue for single module-based analysis. You may not see any underlined module if all p-values > 0.05.

Module	Coefficient	P-value
<u>0</u>	<u>-0.6142421</u>	<u>0.0015</u>
<u>1</u>	<u>1.308675</u>	<u>2.7e-10</u>
<u>2</u>	<u>0.4908374</u>	<u>0.015</u>
3	0.3854999	0.071
4	0.1916897	0.38
<u>5</u>	<u>1.015691</u>	<u>4.4e-07</u>
6	-0.06630745	0.71
7	-0.01376751	0.94
8	0.4978022	0.053
<u>9</u>	<u>0.948809</u>	<u>3.8e-06</u>
<u>10</u>	<u>0.6773846</u>	<u>0.024</u>
11	0.09736692	0.61
<u>12</u>	<u>-0.6155788</u>	<u>0.00033</u>



REACTOMEFIVIZ APP DEMO

We are on a Coffee Break & Networking Session

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