Canadian Bioinformatics Workshops

www.bioinformatics.ca

bioinformaticsdotca.github.io

Supported by McGill University

Creative Commons

This page is available in the following languages:
Afrikaans български Català Dansk Deutsch Ελληνικά English English (CA) English (GB) English (US) Esperanto
Castellano Castellano (AR) Español (CL) Castellano (CO) Español (Ecuador) Castellano (MX) Castellano (PE)
Euskara Suomeksi français français (CA) Galego עברית hrvatski Magyar Italiano 日本語 한국어 Macedonian Melayu
Nederlands Norsk Sesotho sa Leboa polski Português română slovenski jezik српски srpski (latinica) Sotho svenska
中文 華語 (台灣) isiZulu

Attribution-Share Alike 2.5 Canada

You are free:

to Share — to copy, distribute and transmit the work

to Remix — to adapt the work

Under the following conditions:

Attribution. You must attribute the work in the manner specified by the author or licensor (but not in any way that suggests that they endorse you or your use of the work).

Share Alike. If you alter, transform, or build upon this work, you may distribute the resulting work only under the same or similar licence to this one.

- For any reuse or distribution, you must make clear to others the licence terms of this work.
- Any of the above conditions can be waived if you get permission from the copyright holder.
- The author's moral rights are retained in this licence.

Disclaimer

Your fair dealing and other rights are in no way affected by the above.
This is a human-readable summary of the Legal Code (the full licence) available in the following languages:
English French

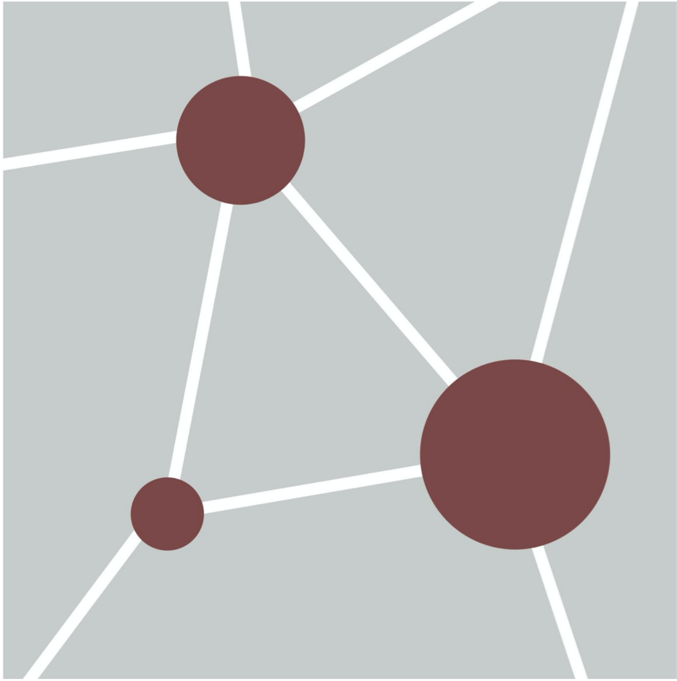
Learn how to distribute your work using this licence

Module 3 Enrichment Maps

Ruth Isserlin

Pathway and Network Analysis of –omics Data

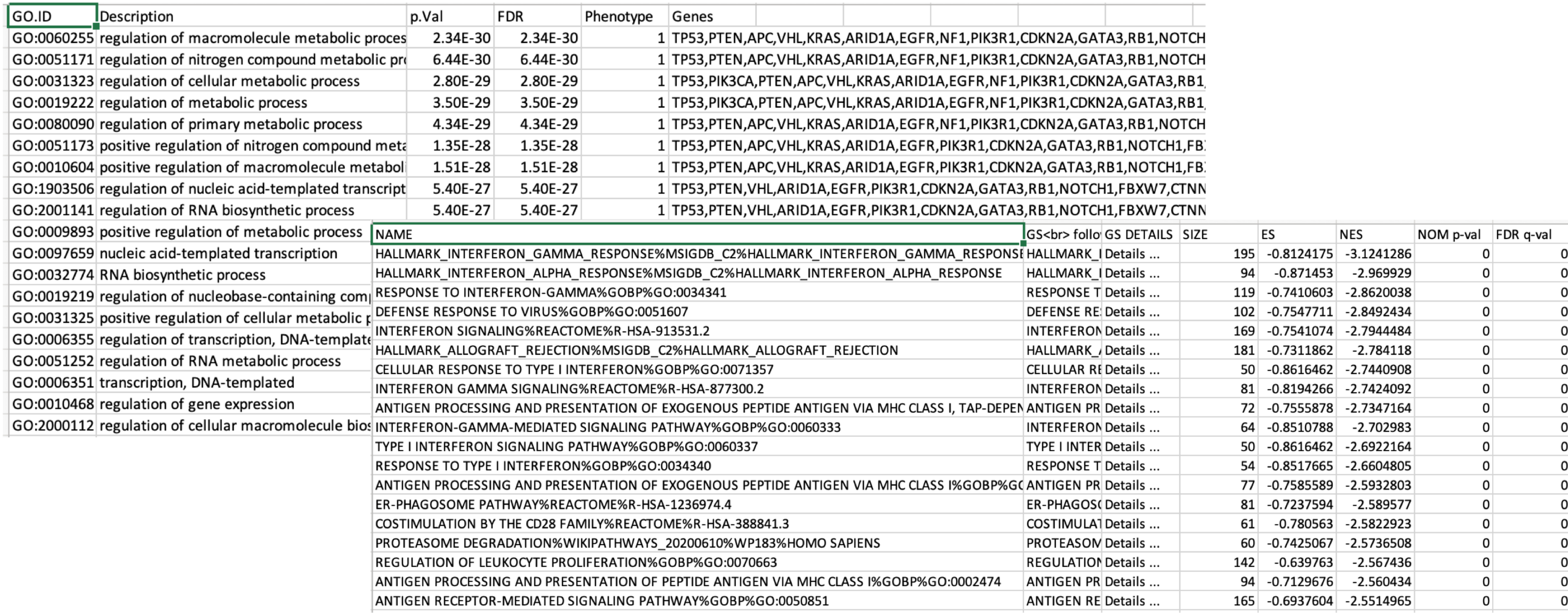
May, 10-12, 2021



Learning Objectives

- By the end of this lecture, you will:
  - Understand how to transform your enrichment results from g:profiler, gsea or other enrichment algorithms to a Network
  - Understand the differences between a network and an Enrichment Map
  - Be able to summarize your enrichment results with annotation using the Autoannotate App.

Results of Lab 2 – Enrichment Results

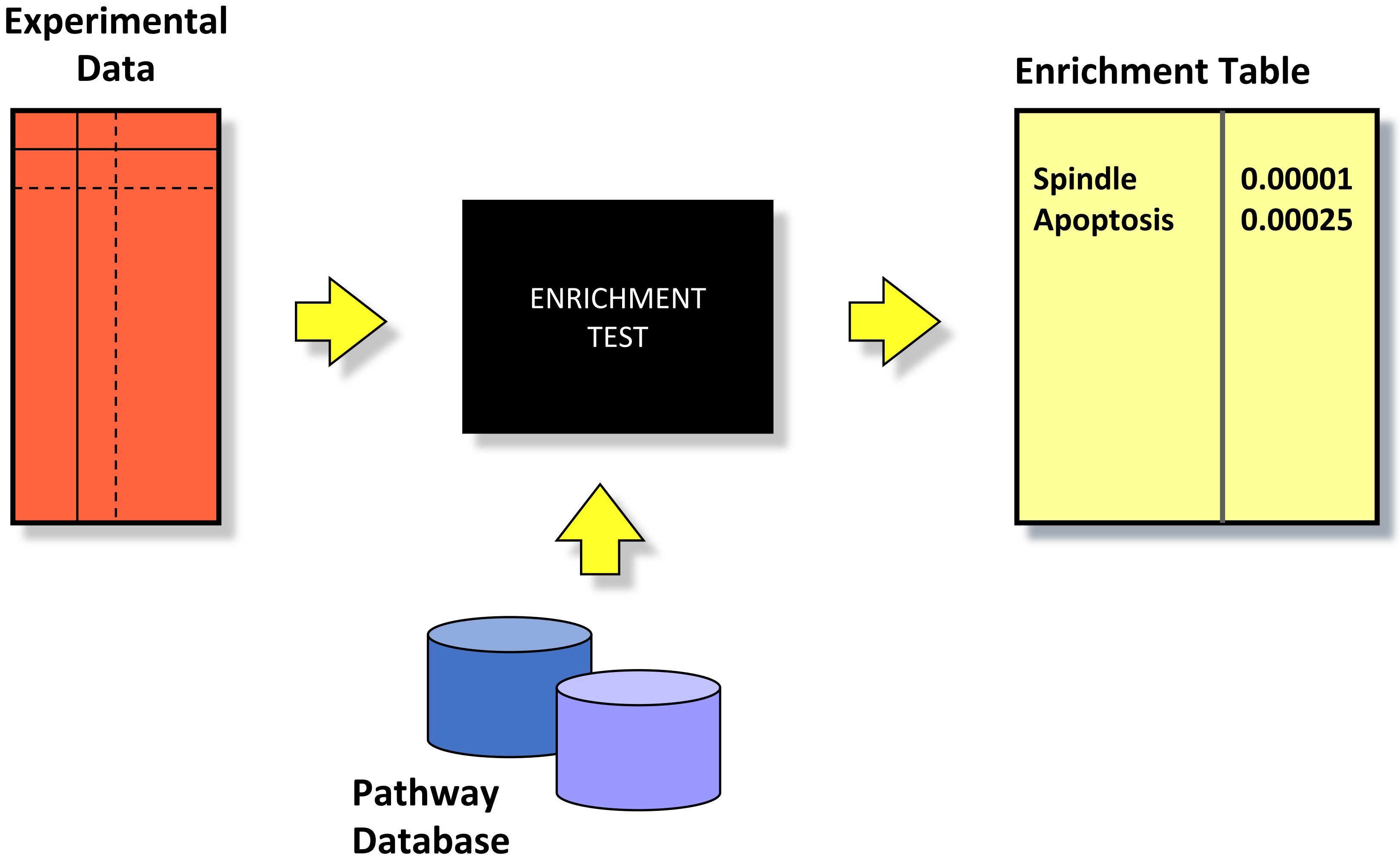


GO.ID	Description	p.Val	FDR	Phenotype	Genes
GO:0060255	regulation of macromolecule metabolic proces	2.34E-30	2.34E-30	1	TP53,PTEN,APC,VHL,KRAS,ARID1A,EGFR,NF1,PIK3R1,CDKN2A,GATA3,RB1,NOTCH
GO:0051171	regulation of nitrogen compound metabolic pr	6.44E-30	6.44E-30	1	TP53,PTEN,APC,VHL,KRAS,ARID1A,EGFR,NF1,PIK3R1,CDKN2A,GATA3,RB1,NOTCH
GO:0031323	regulation of cellular metabolic process	2.80E-29	2.80E-29	1	TP53,PIK3CA,PTEN,APC,VHL,KRAS,ARID1A,EGFR,NF1,PIK3R1,CDKN2A,GATA3,RB1,
GO:0019222	regulation of metabolic process	3.50E-29	3.50E-29	1	TP53,PIK3CA,PTEN,APC,VHL,KRAS,ARID1A,EGFR,NF1,PIK3R1,CDKN2A,GATA3,RB1,
GO:0080090	regulation of primary metabolic process	4.34E-29	4.34E-29	1	TP53,PTEN,APC,VHL,KRAS,ARID1A,EGFR,NF1,PIK3R1,CDKN2A,GATA3,RB1,NOTCH
GO:0051173	positive regulation of nitrogen compound meta	1.35E-28	1.35E-28	1	TP53,PTEN,APC,VHL,KRAS,ARID1A,EGFR,PIK3R1,CDKN2A,GATA3,RB1,NOTCH1,FB
GO:0010604	positive regulation of macromolecule metaboli	1.51E-28	1.51E-28	1	TP53,PTEN,APC,VHL,KRAS,ARID1A,EGFR,PIK3R1,CDKN2A,GATA3,RB1,NOTCH1,FB
GO:1903506	regulation of nucleic acid-templated transcript	5.40E-27	5.40E-27	1	TP53,PTEN,VHL,ARID1A,EGFR,PIK3R1,CDKN2A,GATA3,RB1,NOTCH1,FBXW7,CTNN
GO:2001141	regulation of RNA biosynthetic process	5.40E-27	5.40E-27	1	TP53,PTEN,VHL,ARID1A,EGFR,PIK3R1,CDKN2A,GATA3,RB1,NOTCH1,FBXW7,CTNN
GO:0009893	positive regulation of metabolic process				
GO:0097659	nucleic acid-templated transcription				
GO:0032774	RNA biosynthetic process				
GO:0019219	regulation of nucleobase-containing comp				
GO:0031325	positive regulation of cellular metabolic p				
GO:0006355	regulation of transcription, DNA-template				
GO:0051252	regulation of RNA metabolic process				
GO:0006351	transcription, DNA-templated				
GO:0010468	regulation of gene expression				
GO:2000112	regulation of cellular macromolecule bios				

NAME	GS follo	GS DETAILS	SIZE	ES	NES	NOM p-val	FDR q-val
HALLMARK_INTERFERON_GAMMA_RESPONSE%MSIGDB_C2%HALLMARK_INTERFERON_GAMMA_RESPONSE	HALLMARK_I	Details ...	195	-0.8124175	-3.1241286	0	0
HALLMARK_INTERFERON_ALPHA_RESPONSE%MSIGDB_C2%HALLMARK_INTERFERON_ALPHA_RESPONSE	HALLMARK_I	Details ...	94	-0.871453	-2.969929	0	0
RESPONSE TO INTERFERON-GAMMA%GOBP%GO:0034341	RESPONSE T	Details ...	119	-0.7410603	-2.8620038	0	0
DEFENSE RESPONSE TO VIRUS%GOBP%GO:0051607	DEFENSE RE	Details ...	102	-0.7547711	-2.8492434	0	0
INTERFERON SIGNALING%REACTOME%R-HSA-913531.2	INTERFERON	Details ...	169	-0.7541074	-2.7944484	0	0
HALLMARK_ALLOGRAFT_REJECTION%MSIGDB_C2%HALLMARK_ALLOGRAFT_REJECTION	HALLMARK_A	Details ...	181	-0.7311862	-2.784118	0	0
CELLULAR RESPONSE TO TYPE I INTERFERON%GOBP%GO:0071357	CELLULAR RE	Details ...	50	-0.8616462	-2.7440908	0	0
INTERFERON GAMMA SIGNALING%REACTOME%R-HSA-877300.2	INTERFERON	Details ...	81	-0.8194266	-2.7424092	0	0
ANTIGEN PROCESSING AND PRESENTATION OF EXOGENOUS PEPTIDE ANTIGEN VIA MHC CLASS I, TAP-DEPEN	ANTIGEN PR	Details ...	72	-0.7555878	-2.7347164	0	0
INTERFERON-GAMMA-MEDIATED SIGNALING PATHWAY%GOBP%GO:0060333	INTERFERON	Details ...	64	-0.8510788	-2.702983	0	0
TYPE I INTERFERON SIGNALING PATHWAY%GOBP%GO:0060337	TYPE I INTER	Details ...	50	-0.8616462	-2.6922164	0	0
RESPONSE TO TYPE I INTERFERON%GOBP%GO:0034340	RESPONSE T	Details ...	54	-0.8517665	-2.6604805	0	0
ANTIGEN PROCESSING AND PRESENTATION OF EXOGENOUS PEPTIDE ANTIGEN VIA MHC CLASS I%GOBP%GC	ANTIGEN PR	Details ...	77	-0.7585589	-2.5932803	0	0
ER-PHAGOSOME PATHWAY%REACTOME%R-HSA-1236974.4	ER-PHAGOS(	Details ...	81	-0.7237594	-2.589577	0	0
COSTIMULATION BY THE CD28 FAMILY%REACTOME%R-HSA-388841.3	COSTIMULAT	Details ...	61	-0.780563	-2.5822923	0	0
PROTEASOME DEGRADATION%WIKIPATHWAYS_20200610%WP183%HOMO SAPIENS	PROTEASOM	Details ...	60	-0.7425067	-2.5736508	0	0
REGULATION OF LEUKOCYTE PROLIFERATION%GOBP%GO:0070663	REGULATION	Details ...	142	-0.639763	-2.567436	0	0
ANTIGEN PROCESSING AND PRESENTATION OF PEPTIDE ANTIGEN VIA MHC CLASS I%GOBP%GO:0002474	ANTIGEN PR	Details ...	94	-0.7129676	-2.560434	0	0
ANTIGEN RECEPTOR-MEDIATED SIGNALING PATHWAY%GOBP%GO:0050851	ANTIGEN RE	Details ...	165	-0.6937604	-2.5514965	0	0

- Over the years thousands of papers have been translated into digital representations as pathways or functional annotation.
- Enrichment analysis allows us to interpret data from these papers
- But …. Major cognitive burden relating overlapping information

Pathway Enrichment Test: General Framework



Experimental Data

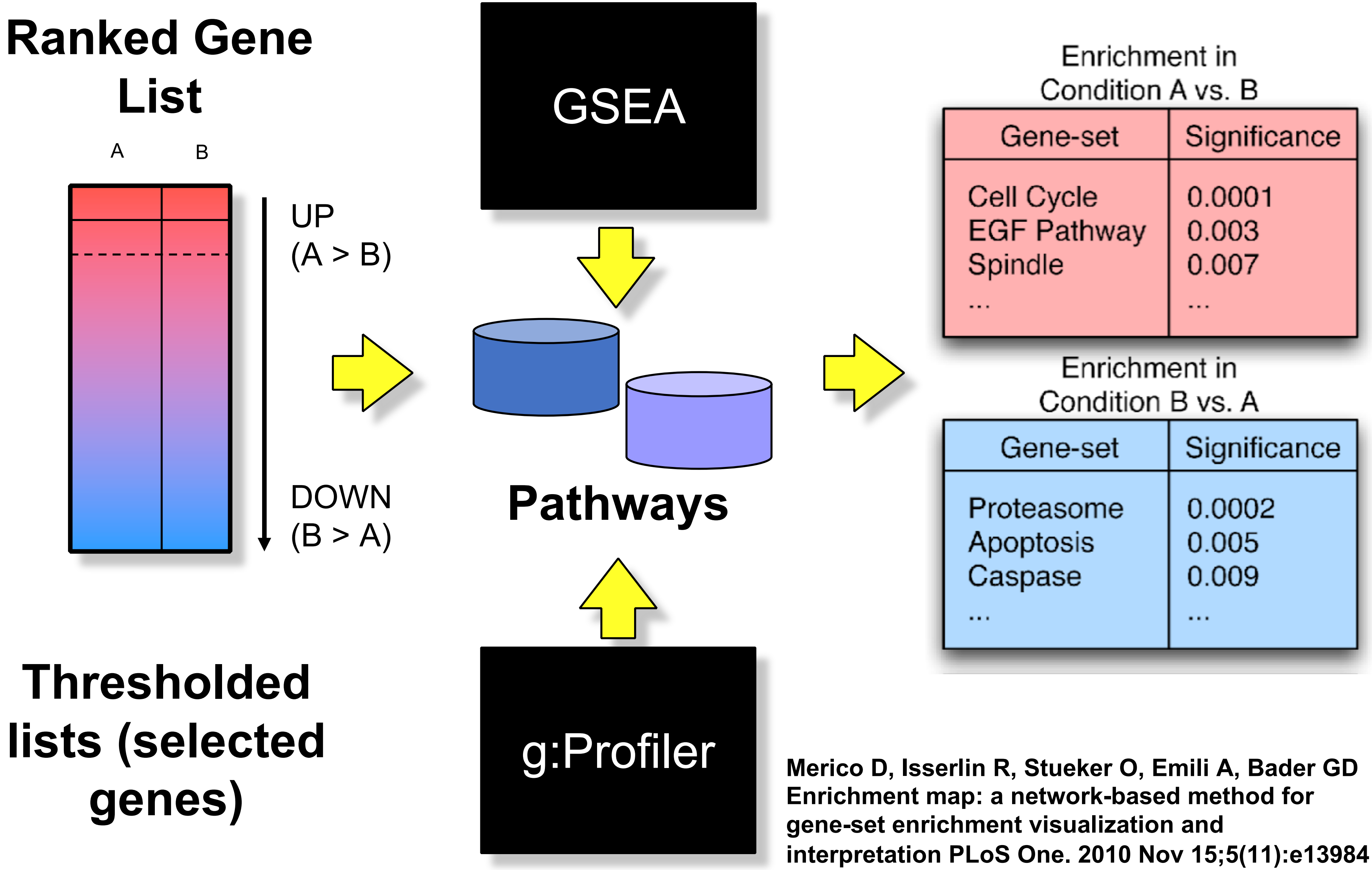
ENRICHMENT TEST

Pathway Database

Enrichment Table

Spindle	**0.00001**
Apoptosis	**0.00025**

Pathway Enrichment Analysis



Ranked Gene List

Thresholded lists (selected genes)

Enrichment in Condition A vs. B

Gene-set	Significance
Cell Cycle	0.0001
EGF Pathway	0.003
Spindle	0.007
...	...

Enrichment in Condition B vs. A

Gene-set	Significance
Proteasome	0.0002
Apoptosis	0.005
Caspase	0.009
...	...

Merico D, Isserlin R, Stueker O, Emili A, Bader GD Enrichment map: a network-based method for gene-set enrichment visualization and interpretation PLoS One. 2010 Nov 15;5(11):e13984

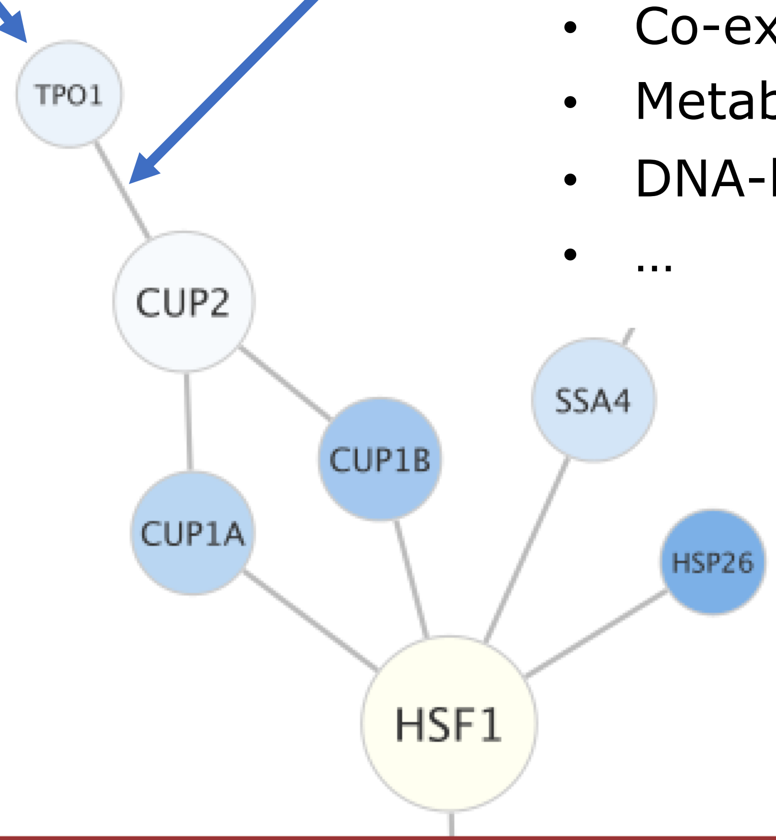
Network Basics

Node (molecule/entity)

- Gene
- Protein
- Transcript
- Drug
- MicroRNA
- …

Edge (interaction/relationship)

- Genetic interaction
- Physical protein interaction
- Co-expression
- Metabolic reaction
- DNA-binding
- …



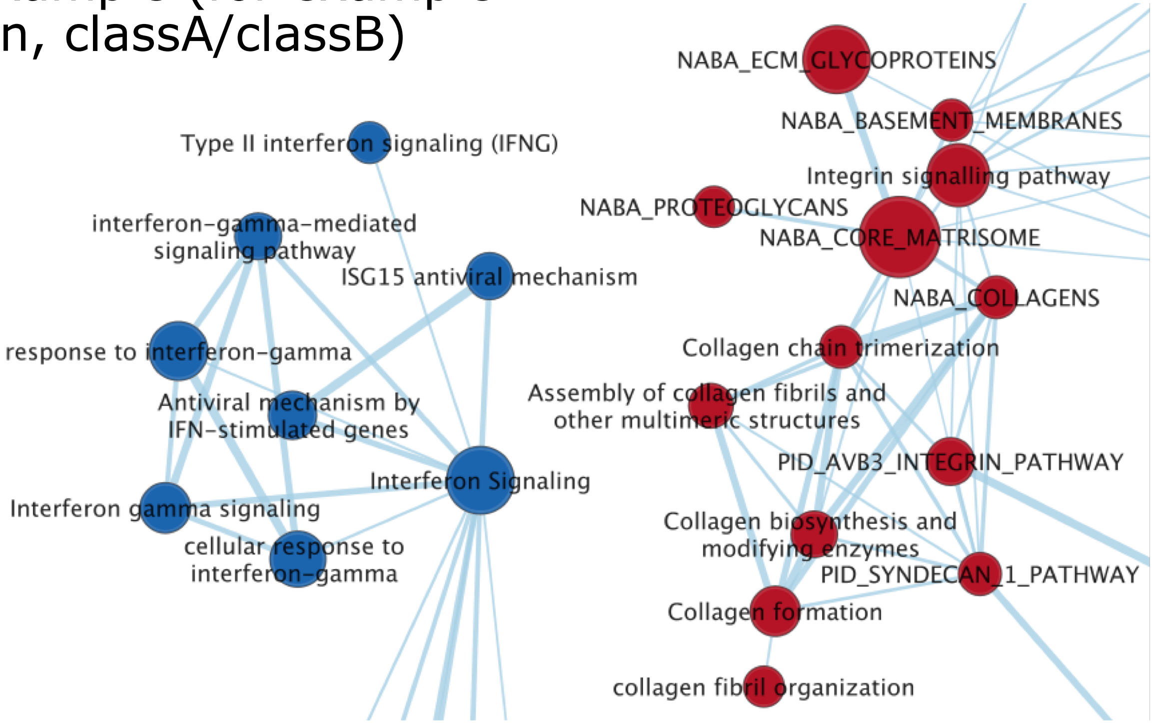
Enrichment Map Basics

Node (molecule/entity)

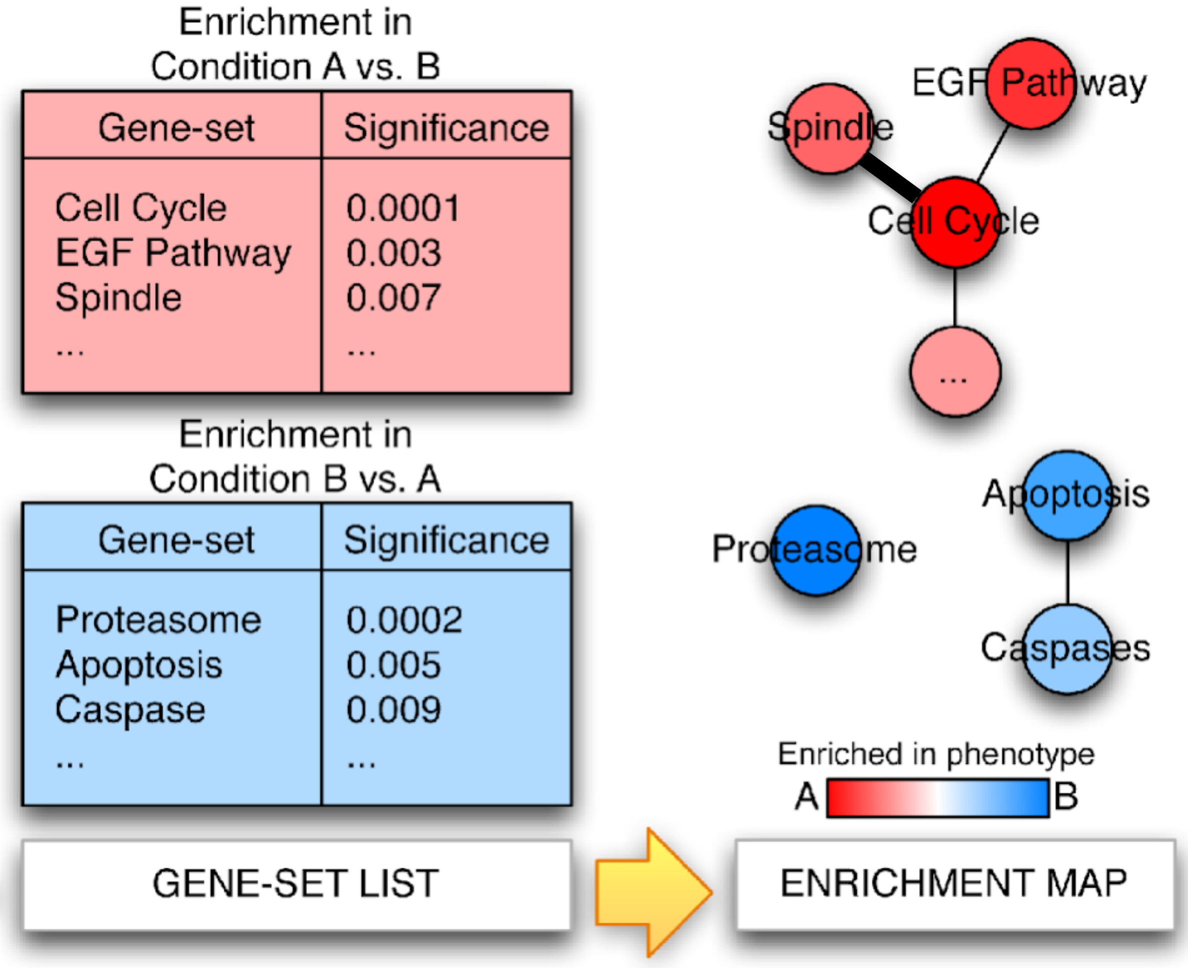
- Pathway or geneset
- Size is correlated to number of genes in set
- Color indicates class in below example (for example Up/Down, classA/classB)

Edge (interaction/relationship)

- Degree of overlap between two genesets
- The more genes two pathways have in common the thicker/stronger the connection



Enrichment Map



Enrichment in Condition A vs. B

Gene-set	Significance
Cell Cycle	0.0001
EGF Pathway	0.003
Spindle	0.007
...	...

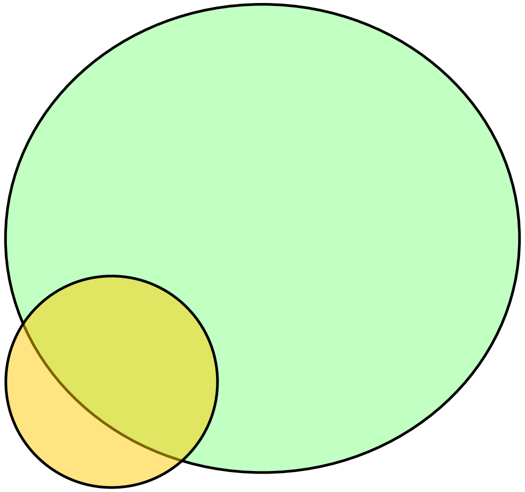
Enrichment in Condition B vs. A

Gene-set	Significance
Proteasome	0.0002
Apoptosis	0.005
Caspase	0.009
...	...

GENE-SET LIST

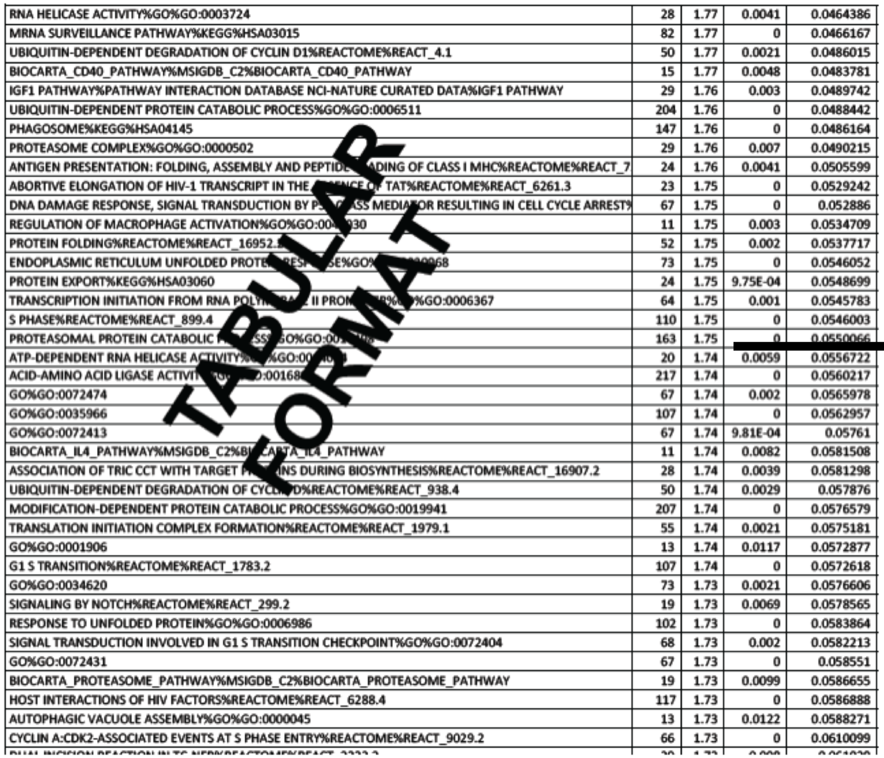
ENRICHMENT MAP

Overlap



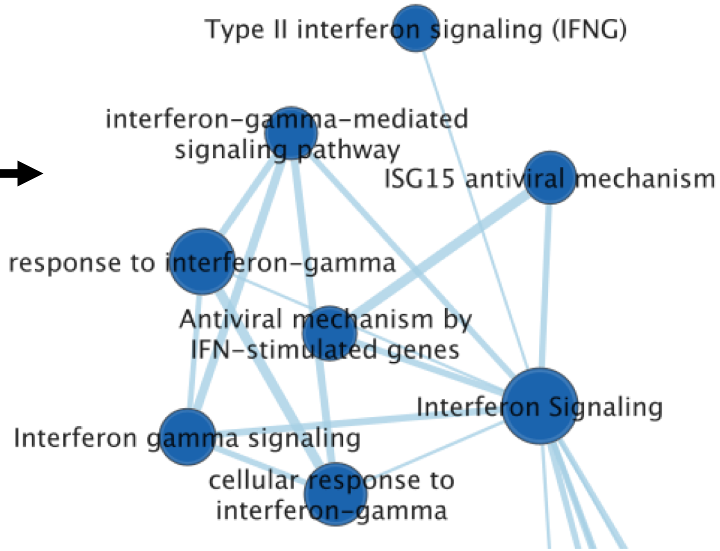
$$\frac{|A \cap B|}{\min(|A|,|B|)}$$

Typical Output



RNA HELICASE ACTIVITY%GO%GO:0003724	28	1.77	0.0041	0.0464386
MRNA SURVEILLANCE PATHWAY%KEGG%HSA03015	82	1.77	0	0.0466167
UBIQUITIN-DEPENDENT DEGRADATION OF CYCLIN D1%REACTOME%REACT_4.1	50	1.77	0.0021	0.0486015
BIOCARTA_CD40_PATHWAY%MSIGDB_C2%BIOCARTA_CD40_PATHWAY	15	1.77	0.0048	0.0483781
IGF1 PATHWAY%PATHWAY INTERACTION DATABASE NCI-NATURE CURATED DATA%IGF1 PATHWAY	29	1.76	0.003	0.0489742
UBIQUITIN-DEPENDENT PROTEIN CATABOLIC PROCESS%GO%GO:0006511	204	1.76	0	0.0488442
PHAGOSOME%KEGG%HSA04145	147	1.76	0	0.0486164
PROTEASOME COMPLEX%GO%GO:0000502	29	1.76	0.007	0.0490215
ANTIGEN PRESENTATION: FOLDING, ASSEMBLY AND PEPTIDE [illegible]ADING OF CLASS I MHC%REACTOME%REACT_7	24	1.76	0.0041	0.0505599
ABORTIVE ELONGATION OF HIV-1 TRANSCRIPT IN THE [illegible] OF TAT%REACTOME%REACT_6261.3	23	1.75	0	0.0529242
DNA DAMAGE RESPONSE, SIGNAL TRANSDUCTION BY P[illegible]ASS MEDIA[illegible]OR RESULTING IN CELL CYCLE ARREST%	67	1.75	0	0.052886
REGULATION OF MACROPHAGE ACTIVATION%GO%GO:00[illegible]930	11	1.75	0.003	0.0534709
PROTEIN FOLDING%REACTOME%REACT_16952.[illegible]	52	1.75	0.002	0.0537717
ENDOPLASMIC RETICULUM UNFOLDED PROTE[illegible]RESP[illegible]SE%GO%[illegible]968	73	1.75	0	0.0546052
PROTEIN EXPORT%KEGG%HSA03060	24	1.75	9.75E-04	0.0548699
TRANSCRIPTION INITIATION FROM RNA POLY[illegible] II PROM[illegible]%GO:0006367	64	1.75	0.001	0.0545783
S PHASE%REACTOME%REACT_899.4	110	1.75	0	0.0546003
PROTEASOMAL PROTEIN CATABOLIC [illegible]SS%GO%GO:00[illegible]	163	1.75	0	0.0550066
ATP-DEPENDENT RNA HELICASE ACTIVITY%[illegible]%GO:00[illegible]	20	1.74	0.0059	0.0556722
ACID-AMINO ACID LIGASE ACTIVIT[illegible]:00168[illegible]	217	1.74	0	0.0560217
GO%GO:0072474	67	1.74	0.002	0.0565978
GO%GO:0035966	107	1.74	0	0.0562957
GO%GO:0072413	67	1.74	9.81E-04	0.05761
BIOCARTA_IL4_PATHWAY%MSIGDB_C2%BI[illegible]CARTA_IL4_PATHWAY	11	1.74	0.0082	0.0581508
ASSOCIATION OF TRIC CCT WITH TARGET P[illegible]INS DURING BIOSYNTHESIS%REACTOME%REACT_16907.2	28	1.74	0.0039	0.0581298
UBIQUITIN-DEPENDENT DEGRADATION OF CYC[illegible]D%REACTOME%REACT_938.4	50	1.74	0.0029	0.057876
MODIFICATION-DEPENDENT PROTEIN CATABOLIC PROCESS%GO%GO:0019941	207	1.74	0	0.0576579
TRANSLATION INITIATION COMPLEX FORMATION%REACTOME%REACT_1979.1	55	1.74	0.0021	0.0575181
GO%GO:0001906	13	1.74	0.0117	0.0572877
G1 S TRANSITION%REACTOME%REACT_1783.2	107	1.74	0	0.0572618
GO%GO:0034620	73	1.73	0.0021	0.0576606
SIGNALING BY NOTCH%REACTOME%REACT_299.2	19	1.73	0.0069	0.0578565
RESPONSE TO UNFOLDED PROTEIN%GO%GO:0006986	102	1.73	0	0.0583864
SIGNAL TRANSDUCTION INVOLVED IN G1 S TRANSITION CHECKPOINT%GO%GO:0072404	68	1.73	0.002	0.0582213
GO%GO:0072431	67	1.73	0	0.058551
BIOCARTA_PROTEASOME_PATHWAY%MSIGDB_C2%BIOCARTA_PROTEASOME_PATHWAY	19	1.73	0.0099	0.0586655
HOST INTERACTIONS OF HIV FACTORS%REACTOME%REACT_6288.4	117	1.73	0	0.0586888
AUTOPHAGIC VACUOLE ASSEMBLY%GO%GO:0000045	13	1.73	0.0122	0.0588271
CYCLIN A:CDK2-ASSOCIATED EVENTS AT S PHASE ENTRY%REACTOME%REACT_9029.2	66	1.73	0	0.0610099

Network Visualization



Each row is a gene-set (pathway).
It displays:

- a score associated with the magnitude of overlap between gene-set and gene list.
- a pvalue that estimates the significance of the enrichment (by chance or not).
- a corrected pvalue (FDR) that corrects for multiple hypothesis testing.

Enrichment Map: use case I
Single enrichment

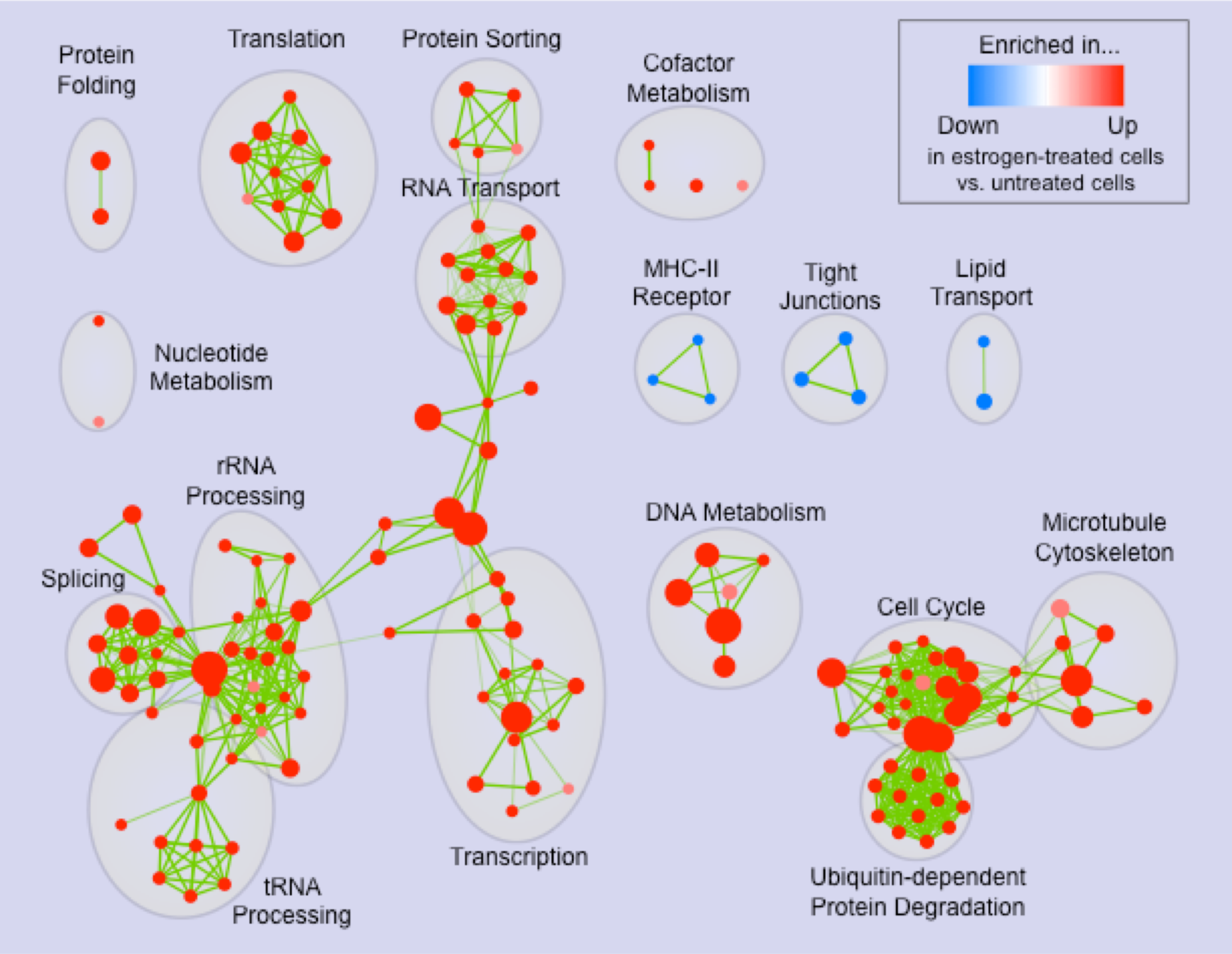
Estrogen treatment of breast cancer cells

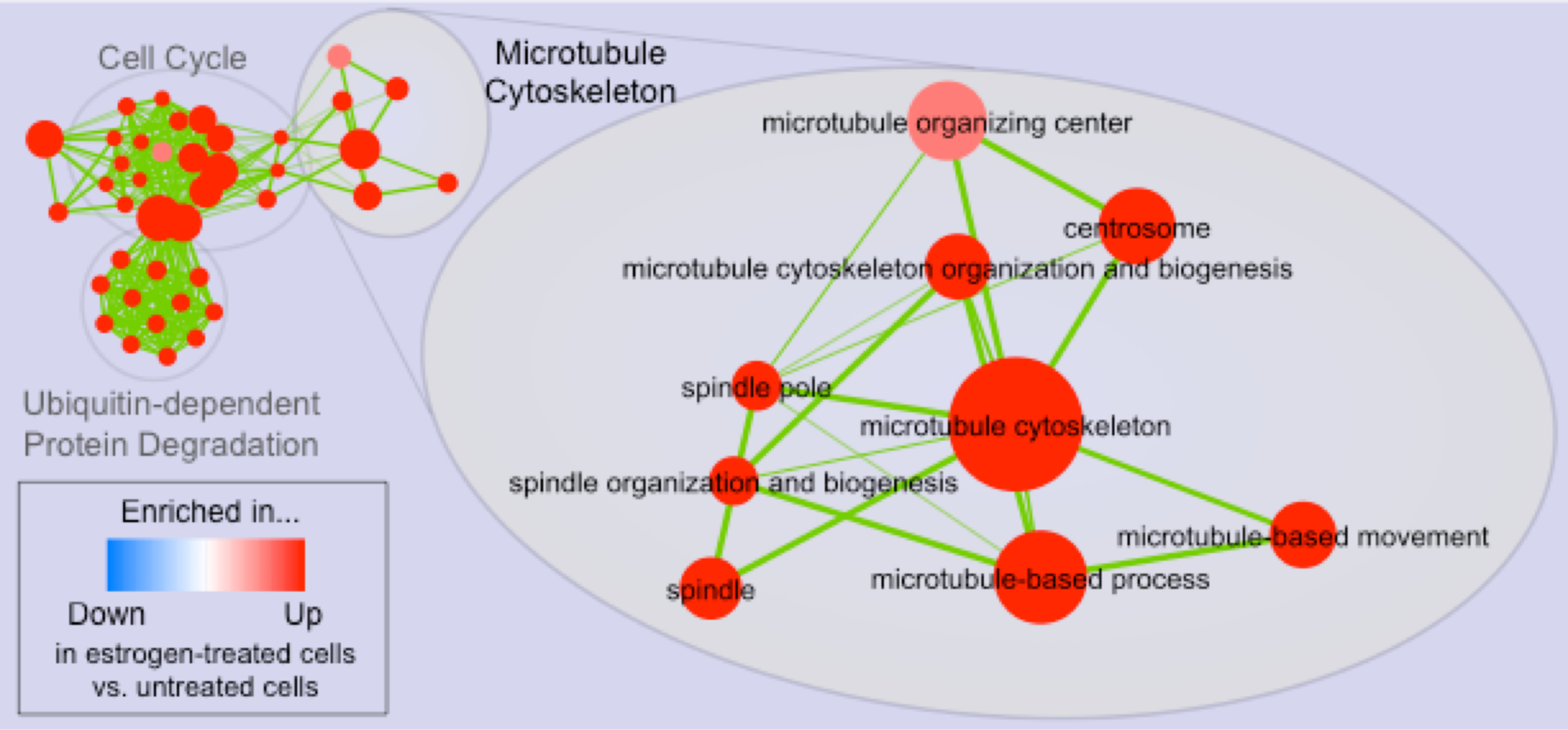
- Design:
  2-time points, **two-class**

	12 hrs	24 hrs
Estrogen-treated	3	3
Untreated	3	3

- Gene set Database:
  Gene Ontology

Lin C-Y, Vega VB, Thomsen JS, Zhang T, Kong SL, et al. (2007) Whole-genome cartography of estrogen receptor alpha binding sites. PLoS Genetics 3:e87


Protein Folding
Translation
Protein Sorting
Cofactor Metabolism
Enriched in...
Down
Up
in estrogen-treated cells vs. untreated cells
RNA Transport
MHC-II Receptor
Tight Junctions
Lipid Transport
Nucleotide Metabolism
rRNA Processing
Splicing
DNA Metabolism
Microtubule Cytoskeleton
Cell Cycle
Transcription
tRNA Processing
Ubiquitin-dependent Protein Degradation


Cell Cycle
Microtubule Cytoskeleton
microtubule organizing center
centrosome
microtubule cytoskeleton organization and biogenesis
spindle pole
microtubule cytoskeleton
spindle organization and biogenesis
microtubule-based movement
microtubule-based process
spindle
Ubiquitin-dependent Protein Degradation
Enriched in...
Down
Up
in estrogen-treated cells vs. untreated cells

Enrichment Map: use case II
Comparison of two enrichments

Estrogen treatment of breast cancer cells

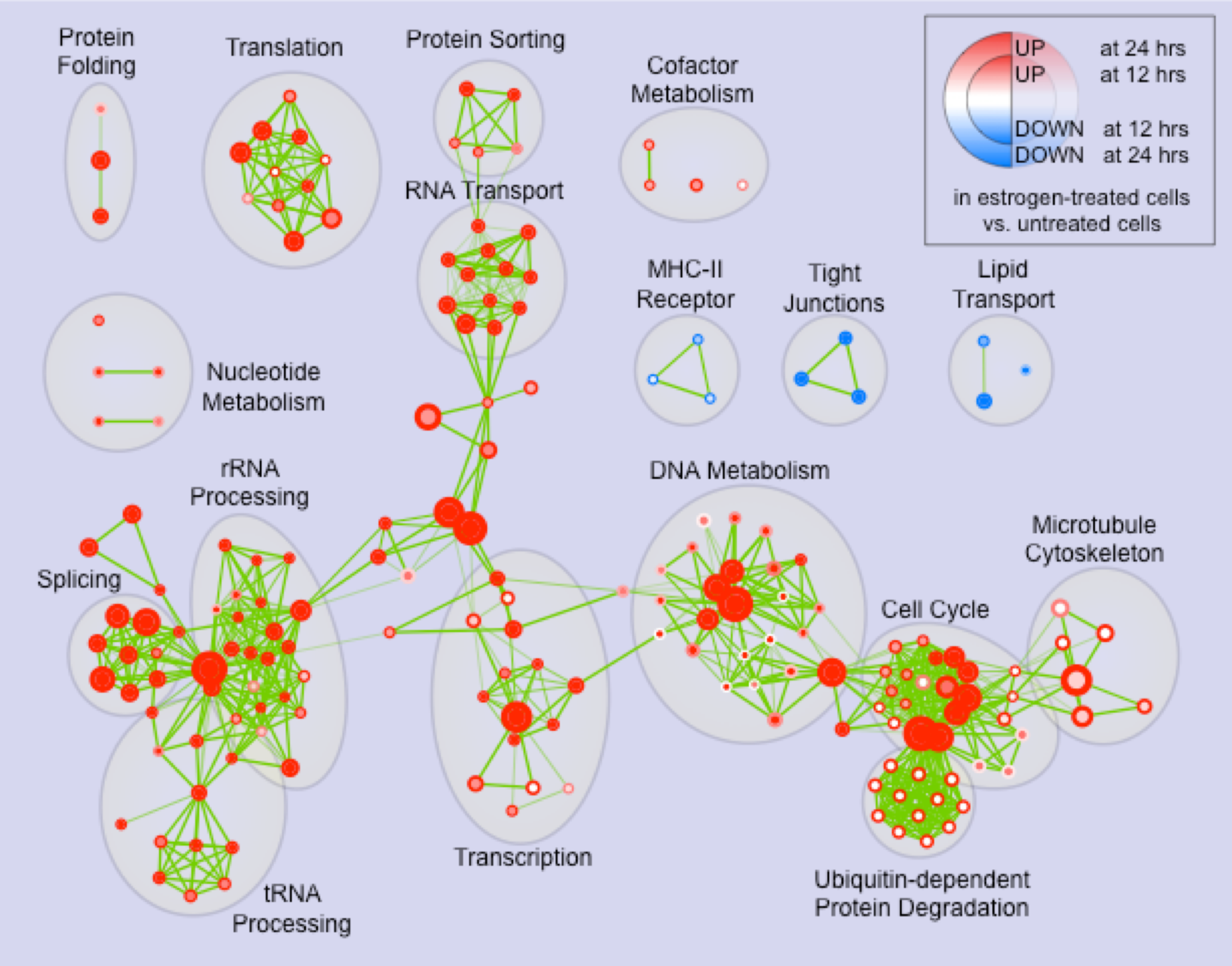
- Design:

  2-time points, **two-class**

	12 hrs	**24 hrs**
Estrogen-treated	3	3
Untreated	3	3

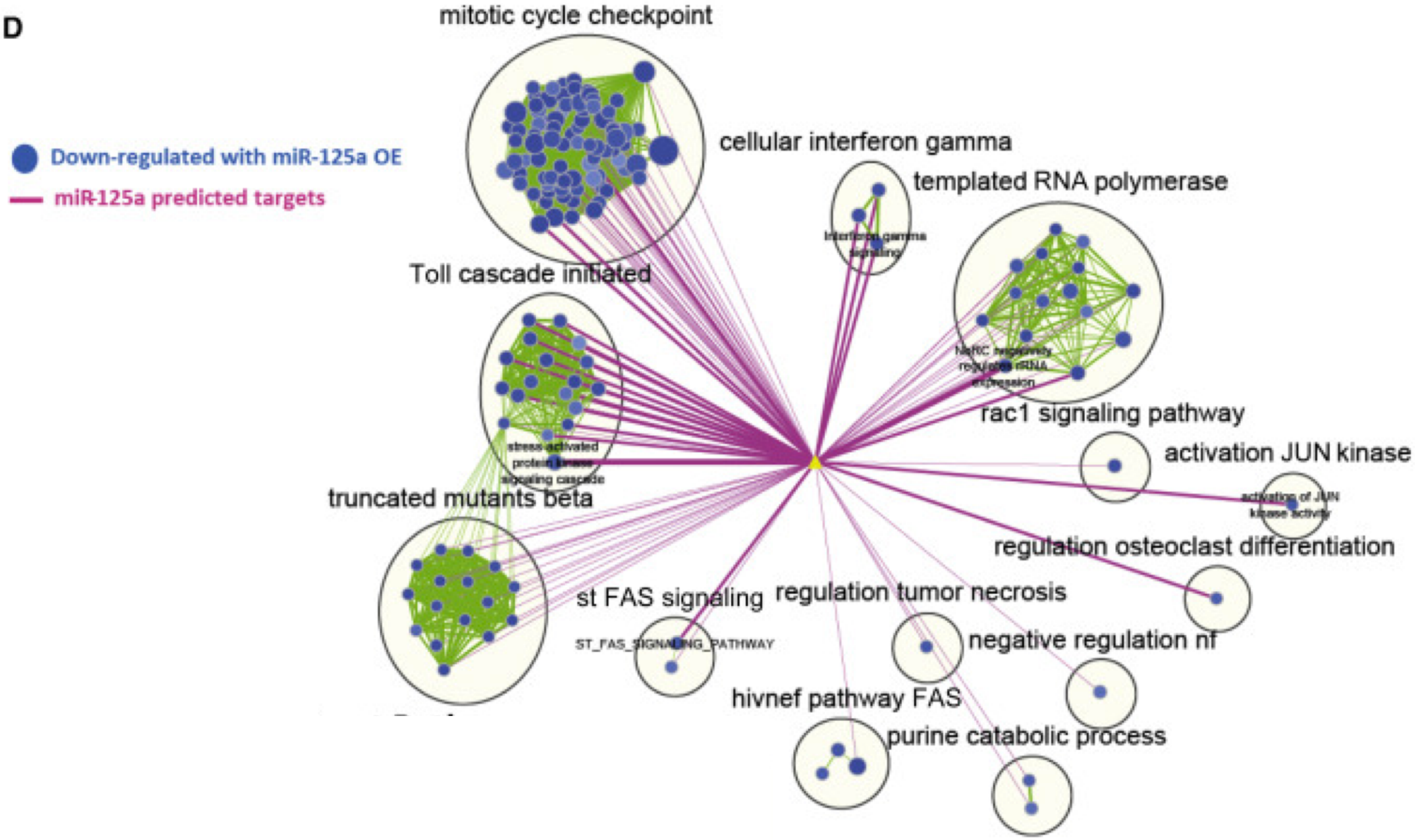
- Gene set Database:

  Gene Ontology


Protein Folding
Translation
Protein Sorting
Cofactor Metabolism
UP at 24 hrs
UP at 12 hrs
DOWN at 12 hrs
DOWN at 24 hrs
in estrogen-treated cells vs. untreated cells
RNA Transport
MHC-II Receptor
Tight Junctions
Lipid Transport
Nucleotide Metabolism
rRNA Processing
DNA Metabolism
Microtubule Cytoskeleton
Splicing
Cell Cycle
Transcription
tRNA Processing
Ubiquitin-dependent Protein Degradation

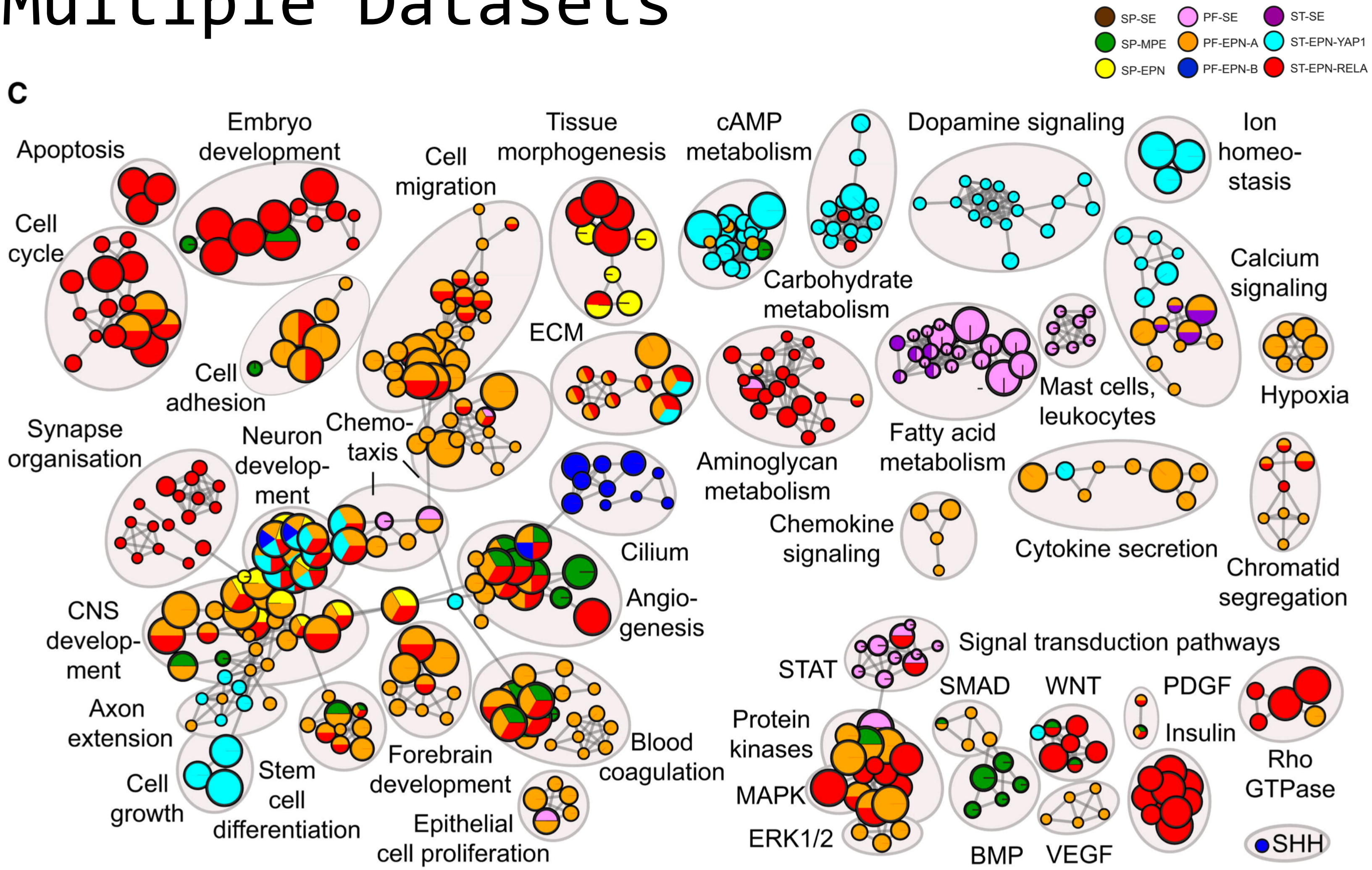

UP at 24 hrs
UP at 12 hrs
DOWN at 12 hrs
DOWN at 24 hrs
in estrogen-treated cells vs. untreated cells
Estrogen-treated vs untreated:
🟩 Up
🟪 Down
Replication Fork
treated
untreated
treated
untreated
12 hrs
24 hrs
APC-dependent Protein Degradation
treated
untreated
treated
untreated
12 hrs
24 hrs
DNA Metabolism
Cell Cycle
Microtubule Cytoskeleton
Ubiquitin-dependent Protein Degradation

Enrichment Map: use case III
Query Set Analysis



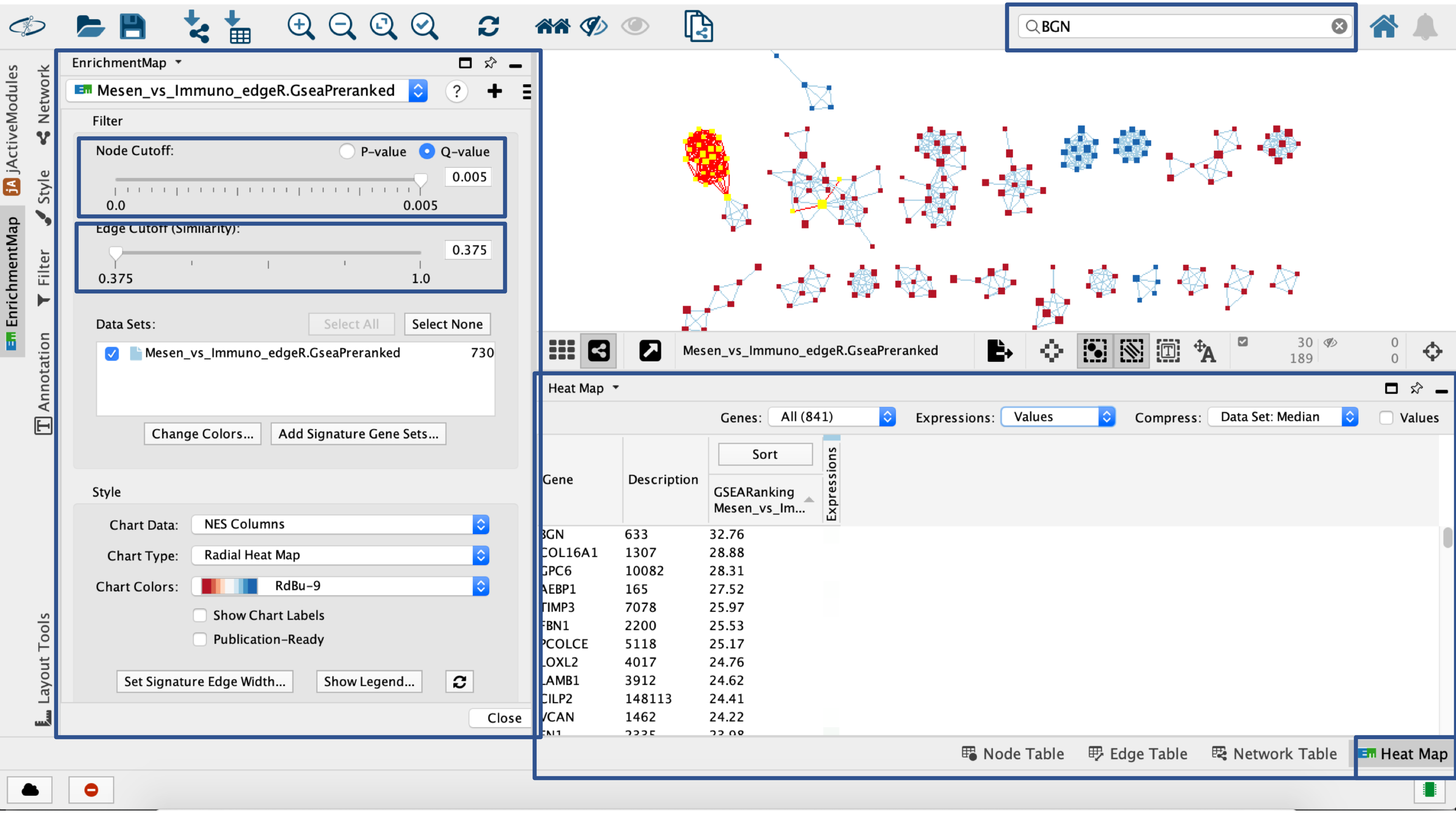
Wojtowicz EE, Lechman ER, Hermans KG, et al. Ectopic miR-125a Expression Induces Long-Term Repopulating Stem Cell Capacity in Mouse and Human Hematopoietic Progenitors. *Cell Stem Cell*. 2016;19(3):383-396

Enrichment Map: use case IV Multiple Datasets



Pajtler KW, Witt H, Sill M, et al. Molecular Classification of Ependymal Tumors across All CNS Compartments, Histopathological Grades, and Age Groups. *Cancer Cell*. 2015;27(5):728-743

Enrichment Map Features



EnrichmentMap

Mesen_vs_Immuno_edgeR.GseaPreranked

Filter

Node Cutoff: ○ P-value ● Q-value 0.005

0.0 0.005

Edge Cutoff (Similarity): 0.375

0.375 1.0

Data Sets: Select All Select None

☑ Mesen_vs_Immuno_edgeR.GseaPreranked 730

Change Colors... Add Signature Gene Sets...

Style

Chart Data: NES Columns

Chart Type: Radial Heat Map

Chart Colors: RdBu-9

☐ Show Chart Labels

☐ Publication-Ready

Set Signature Edge Width... Show Legend...

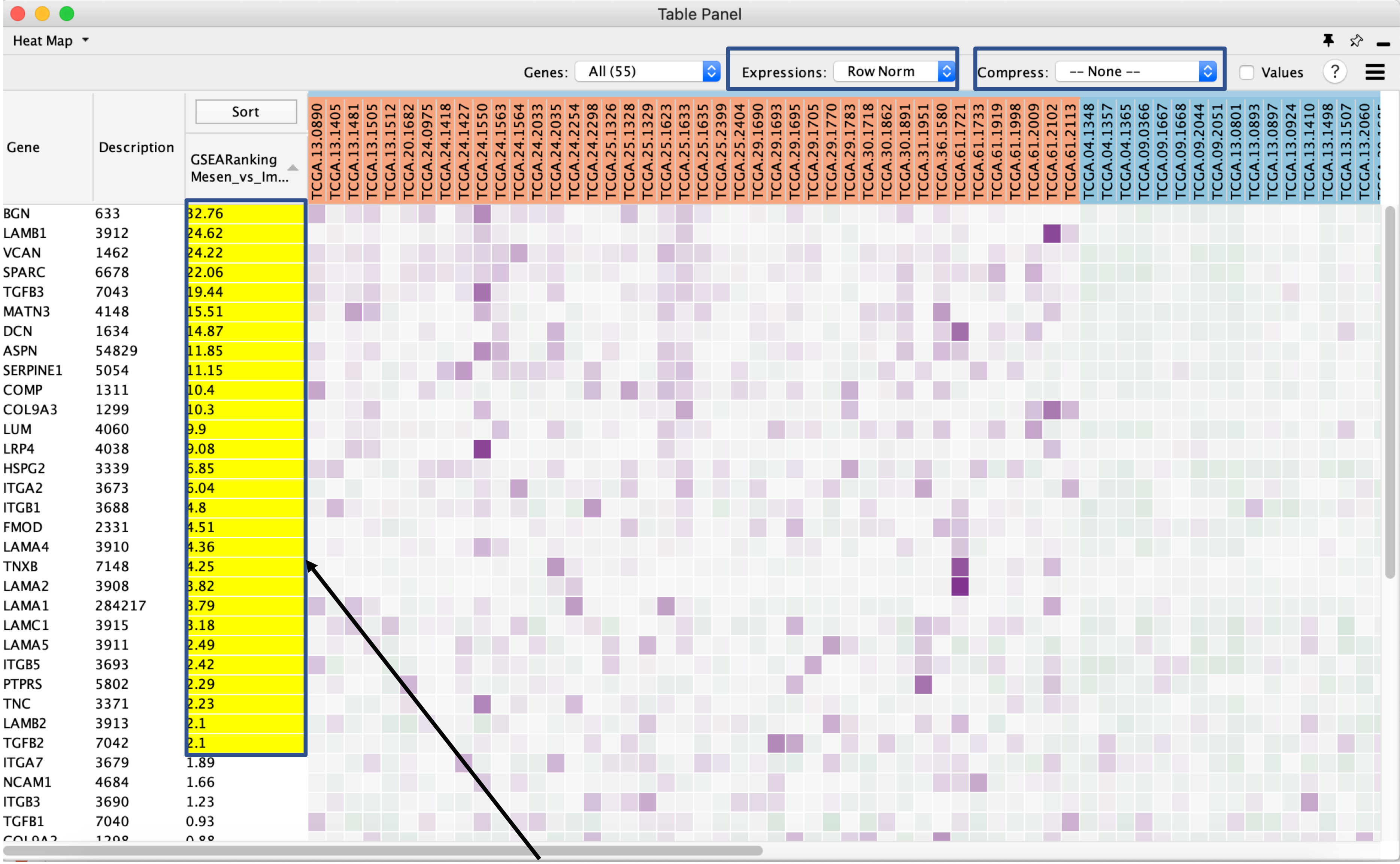
Close

Heat Map

Genes: All (841) Expressions: Values Compress: Data Set: Median ☐ Values

Gene	Description	GSEARanking Mesen_vs_Im...
BGN	633	32.76
COL16A1	1307	28.88
GPC6	10082	28.31
AEBP1	165	27.52
TIMP3	7078	25.97
FBN1	2200	25.53
PCOLCE	5118	25.17
LOXL2	4017	24.76
LAMB1	3912	24.62
CILP2	148113	24.41
VCAN	1462	24.22

Heatmap Panel



Genes: All (55)

Expressions: Row Norm

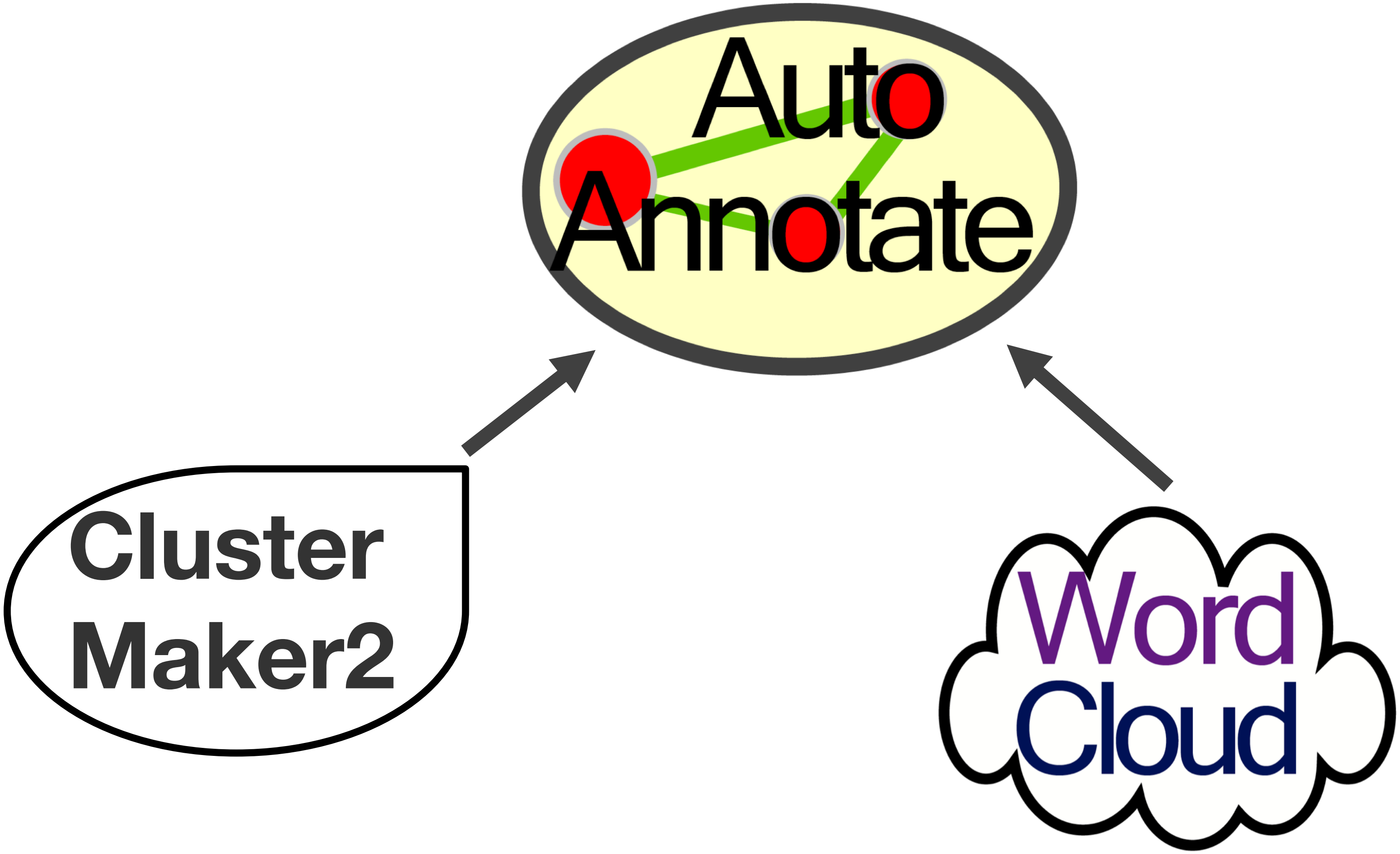
Compress: -- None --

☐ Values

Gene	Description	GSEARanking Mesen_vs_Im...
BGN	633	32.76
LAMB1	3912	24.62
VCAN	1462	24.22
SPARC	6678	22.06
TGFB3	7043	19.44
MATN3	4148	15.51
DCN	1634	14.87
ASPN	54829	11.85
SERPINE1	5054	11.15
COMP	1311	10.4
COL9A3	1299	10.3
LUM	4060	9.9
LRP4	4038	9.08
HSPG2	3339	6.85
ITGA2	3673	6.04
ITGB1	3688	4.8
FMOD	2331	4.51
LAMA4	3910	4.36
TNXB	7148	4.25
LAMA2	3908	3.82
LAMA1	284217	3.79
LAMC1	3915	3.18
LAMA5	3911	2.49
ITGB5	3693	2.42
PTPRS	5802	2.29
TNC	3371	2.23
LAMB2	3913	2.1
TGFB2	7042	2.1
ITGA7	3679	1.89
NCAM1	4684	1.66
ITGB3	3690	1.23
TGFB1	7040	0.93
COL9A2	1298	0.88

Leading Edge

Enrichment Map Features



Auto Annotate

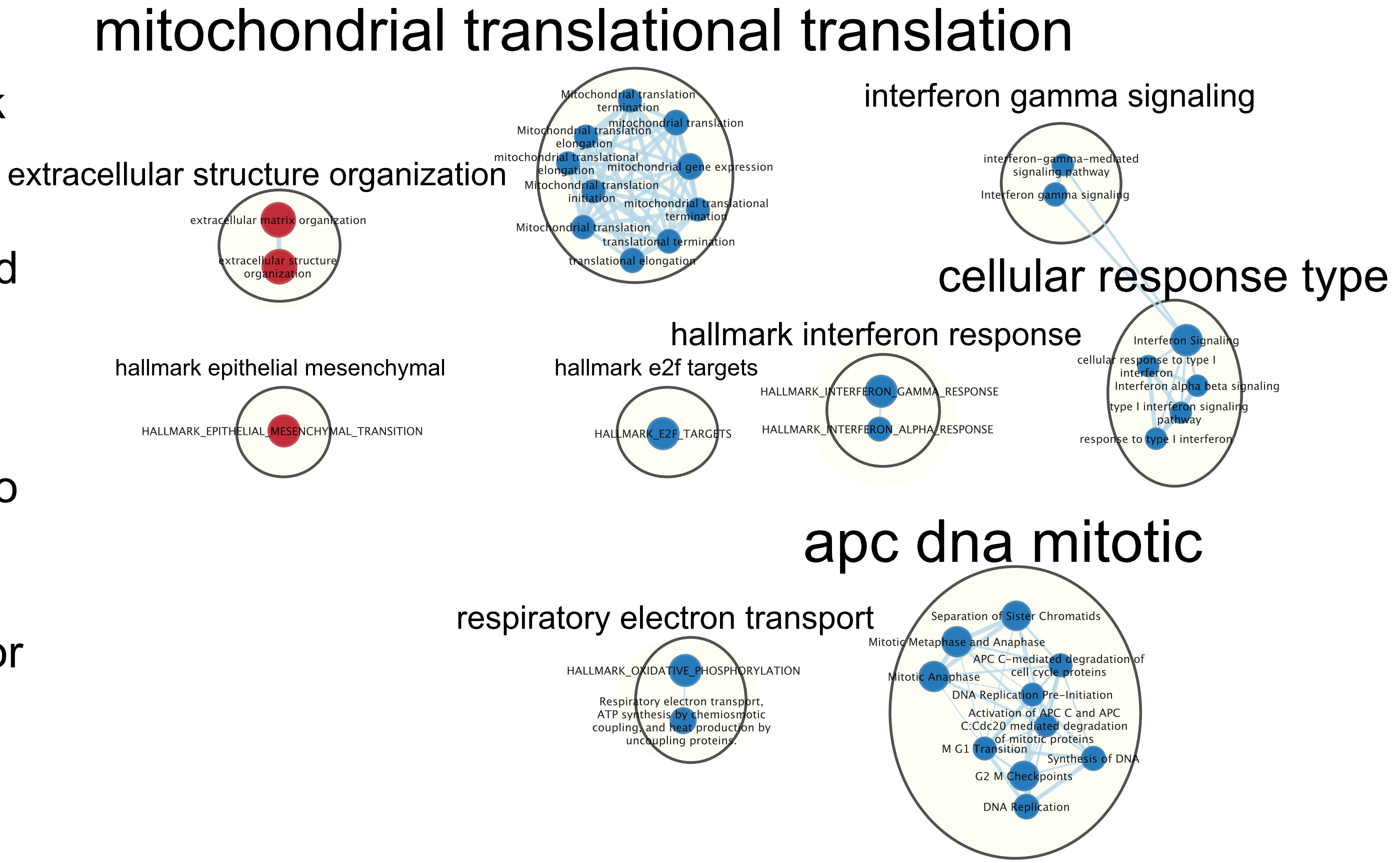
Cluster Maker2

Word Cloud

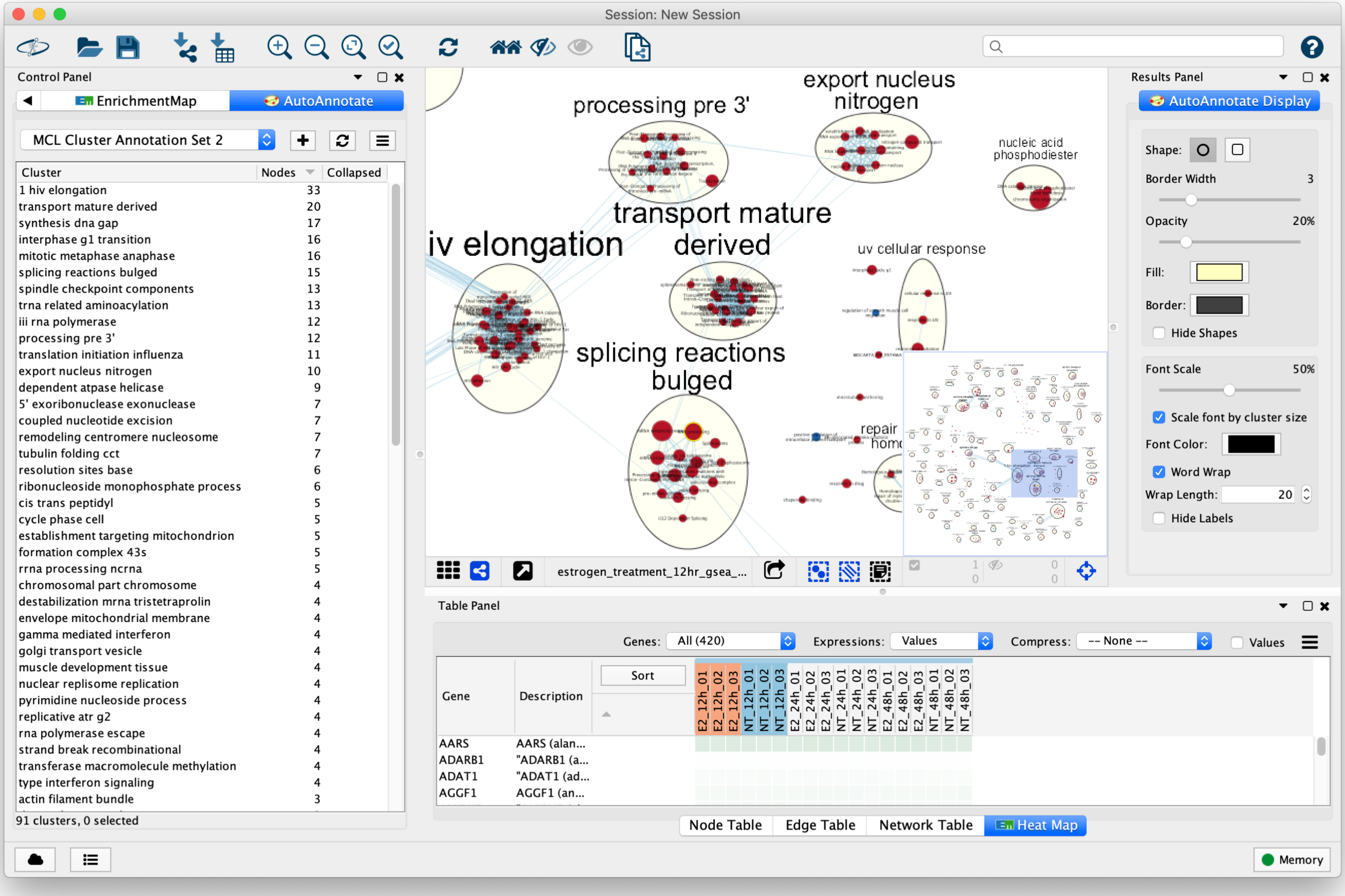
AutoAnnotate

Steps:

1. Cluster the. Network
2. For each cluster, find frequent words in node labels
3. Select top 3 words to create labels
4. Possibility to move or edit labels to make figure publication ready.



AutoAnnotate



MCL Cluster Annotation Set 2

Cluster	Nodes	Collapsed
1 hiv elongation	33	
transport mature derived	20	
synthesis dna gap	17	
interphase g1 transition	16	
mitotic metaphase anaphase	16	
splicing reactions bulged	15	
spindle checkpoint components	13	
trna related aminoacylation	13	
iii rna polymerase	12	
processing pre 3'	12	
translation initiation influenza	11	
export nucleus nitrogen	10	
dependent atpase helicase	9	
5' exoribonuclease exonuclease	7	
coupled nucleotide excision	7	
remodeling centromere nucleosome	7	
tubulin folding cct	7	
resolution sites base	6	
ribonucleoside monophosphate process	6	
cis trans peptidyl	5	
cycle phase cell	5	
establishment targeting mitochondrion	5	
formation complex 43s	5	
rrna processing ncrna	5	
chromosomal part chromosome	4	
destabilization mrna tristetraprolin	4	
envelope mitochondrial membrane	4	
gamma mediated interferon	4	
golgi transport vesicle	4	
muscle development tissue	4	
nuclear replisome replication	4	
pyrimidine nucleoside process	4	
replicative atr g2	4	
rna polymerase escape	4	
strand break recombinational	4	
transferase macromolecule methylation	4	
type interferon signaling	4	
actin filament bundle	3	

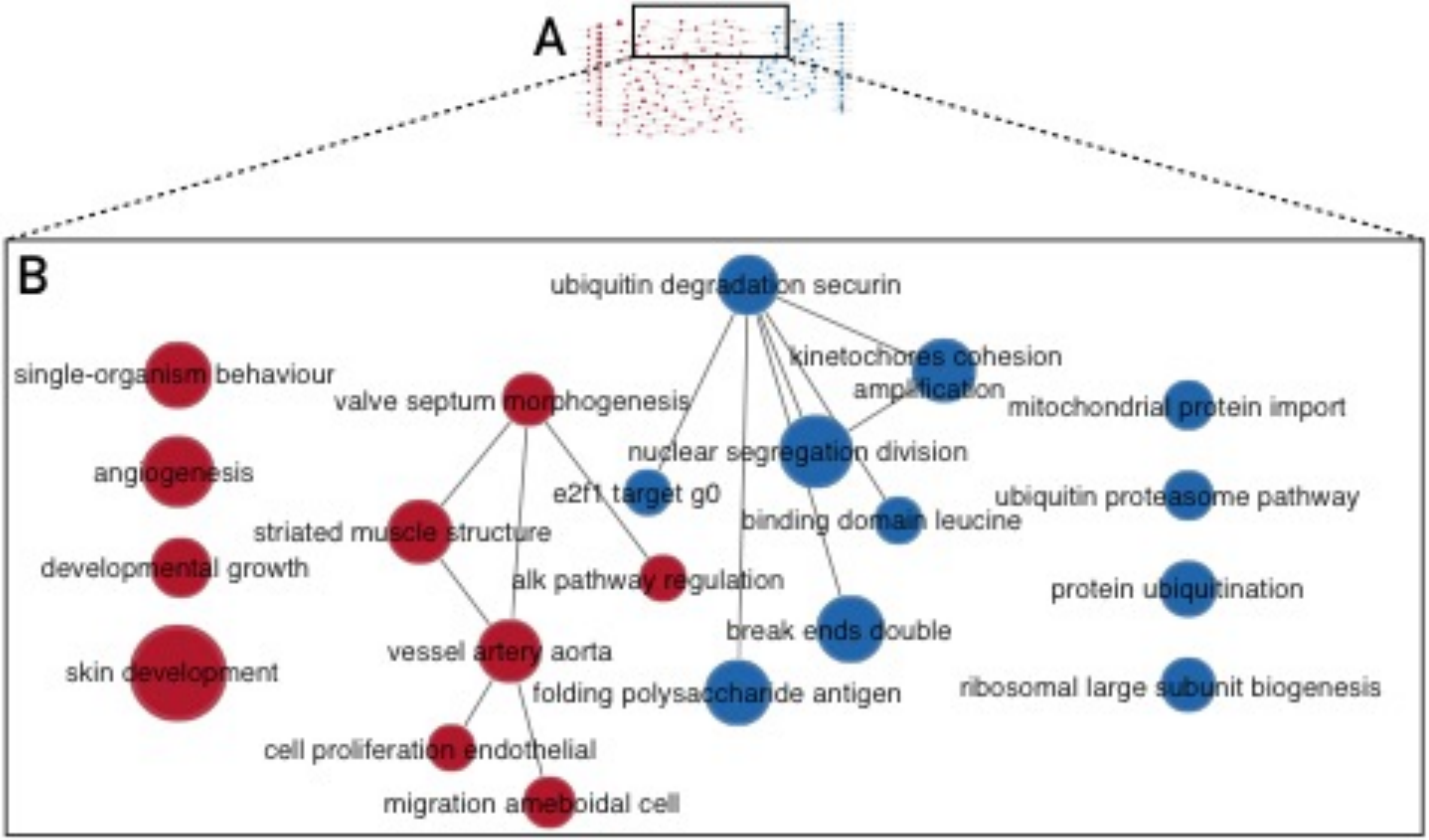
91 clusters, 0 selected

Shape: ○ ☐
Border Width 3
Opacity 20%
Fill:
Border:
☐ Hide Shapes
Font Scale 50%
☑ Scale font by cluster size
Font Color:
☑ Word Wrap
Wrap Length: 20
☐ Hide Labels

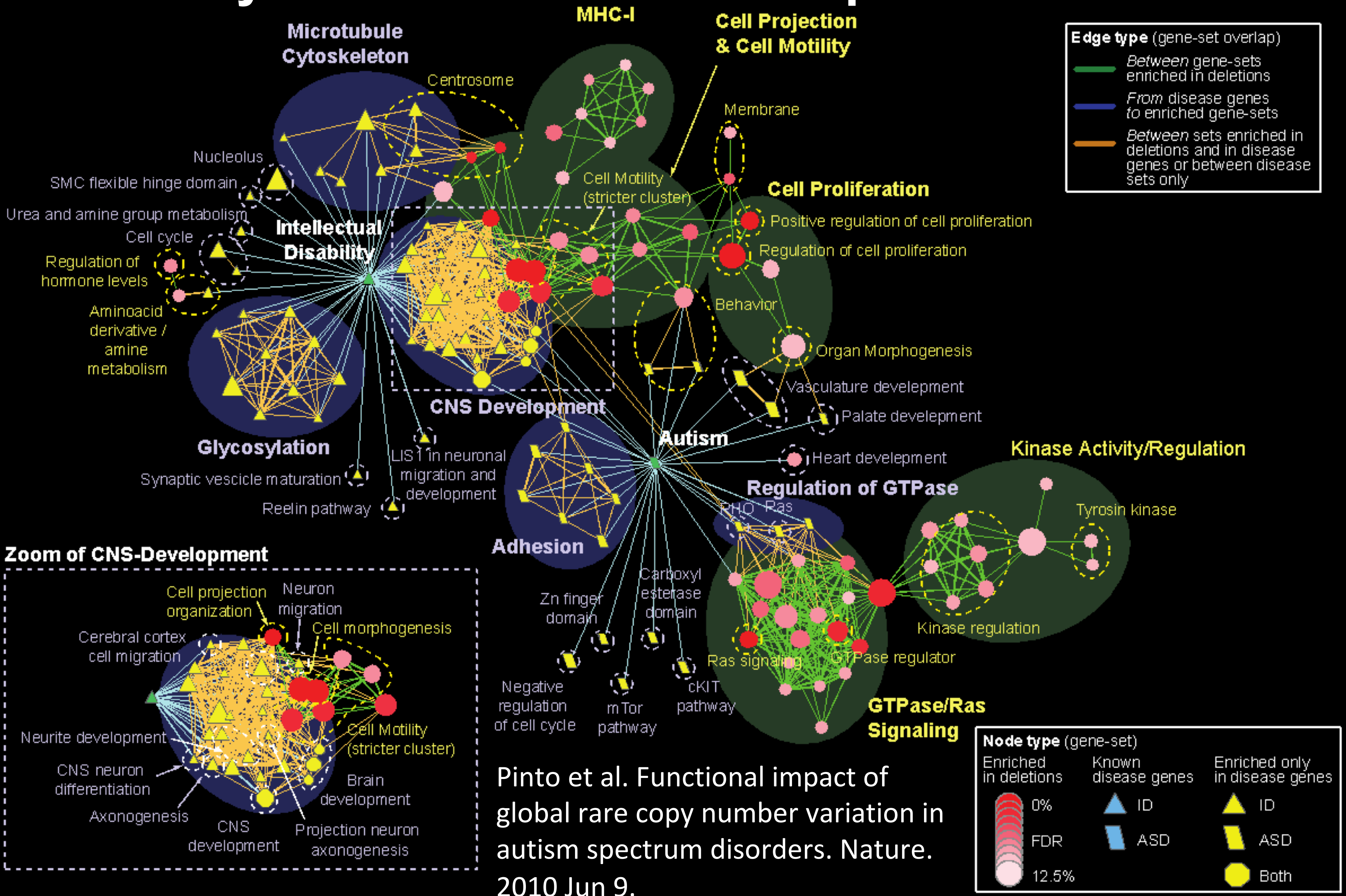
Genes: All (420)
Expressions: Values
Compress: -- None --
☐ Values

Gene	Description	E2_12h_01	E2_12h_02	E2_12h_03	NT_12h_01	NT_12h_02	NT_12h_03	E2_24h_01	E2_24h_02	E2_24h_03	NT_24h_01	NT_24h_02	NT_24h_03	E2_48h_01	E2_48h_02	E2_48h_03	NT_48h_01	NT_48h_02	NT_48h_03
AARS	AARS (alan...																		
ADARB1	"ADARB1 (a...																		
ADAT1	"ADAT1 (ad...																		
AGGF1	AGGF1 (an...																		

Collapsed network



Pathways Enriched in Autism Spectrum Disorder



Pinto et al. Functional impact of global rare copy number variation in autism spectrum disorders. Nature. 2010 Jun 9.

Practical Lab

- Experiment with Cytoscape
- Try out Enrichment Map
- Load your g:Profiler results – and - load the GSEA enrichment analysis files that you generated in Module 2 lab
- Try out all the different Enrichment Map features
- Try AutoAnnotate to annotate your networks.

We are on a Coffee Break & Networking Session

Workshop Sponsors:

Canadian Centre for Computational Genomics

HPC4Health

OICR Ontario Institute for Cancer Research

MiCM McGill initiative in Computational Medicine