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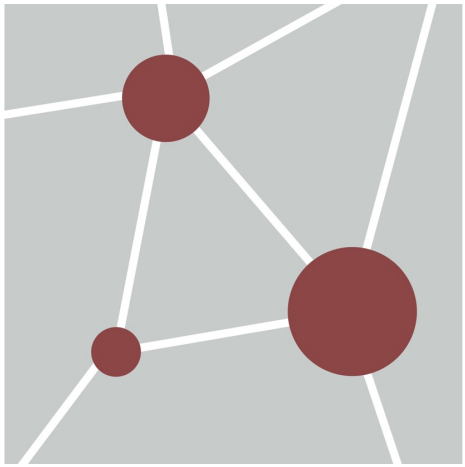
Introduction to Pathway and Network Analysis of Gene Lists

Gary Bader

Pathway and Network Analysis

June 26-28, 2024

bioinformatics.ca



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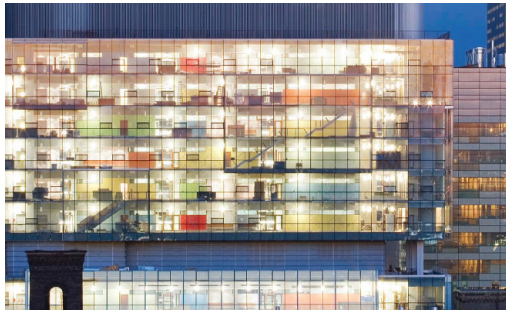
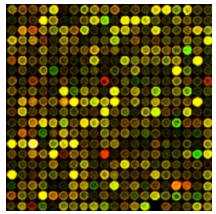


Interpreting gene lists

- My cool new screen worked and produced 1000 hits! …Now what?
- Genome-Scale Analysis (Omics)
  - Genomics, Proteomics
- Tell me what's interesting about these genes



Ranking or clustering

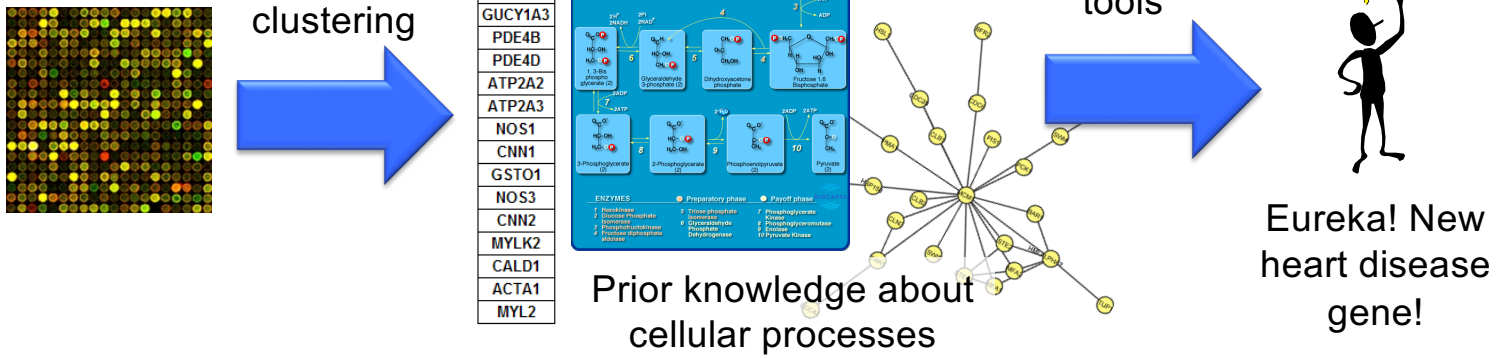
GNAQ
GNAS
DGKZ
GUCY1A3
PDE4B
PDE4D
ATP2A2
ATP2A3
NOS1
CNN1
GSTO1
NOS3
CNN2
MYLK2
CALD1
ACTA1
MYL2

?

GenMAPP.org

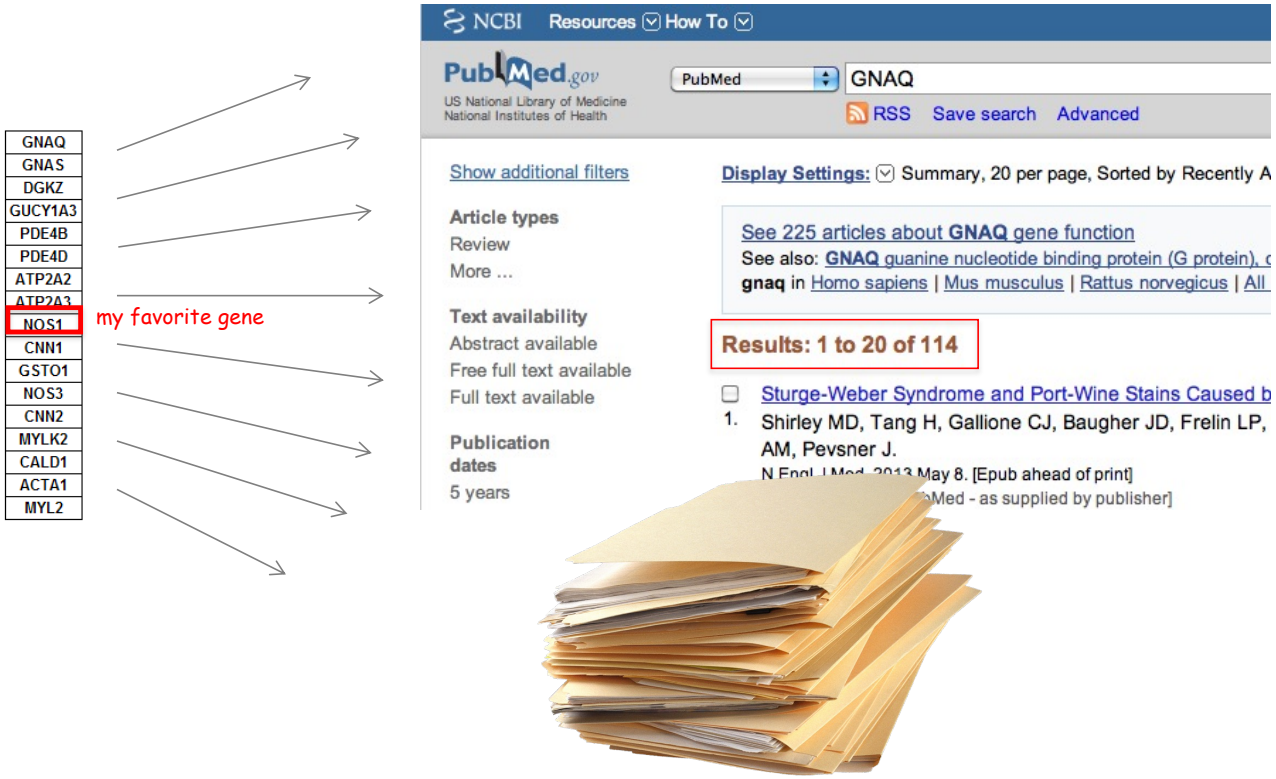
Interpreting gene lists

- My cool new screen worked and produced 1000 hits! …Now what?
- Genome-Scale Analysis (Omics)
  - Genomics, Proteomics
- Tell me what's interesting about these genes
  - Are they enriched in known pathways, complexes, functions



Pathway and network analysis

- Save time compared to traditional approach



Pathway and network analysis

- Helps gain mechanistic insight into ‘omics data
  - Identifying a master regulator, drug targets, characterizing pathways active in a sample
- Any type of analysis that involves pathway or network information
- Most commonly applied to help interpret lists of genes
- Most popular type is pathway enrichment analysis, but many others are useful

Pathway analysis example 1

Autism spectrum disorder (ASD)

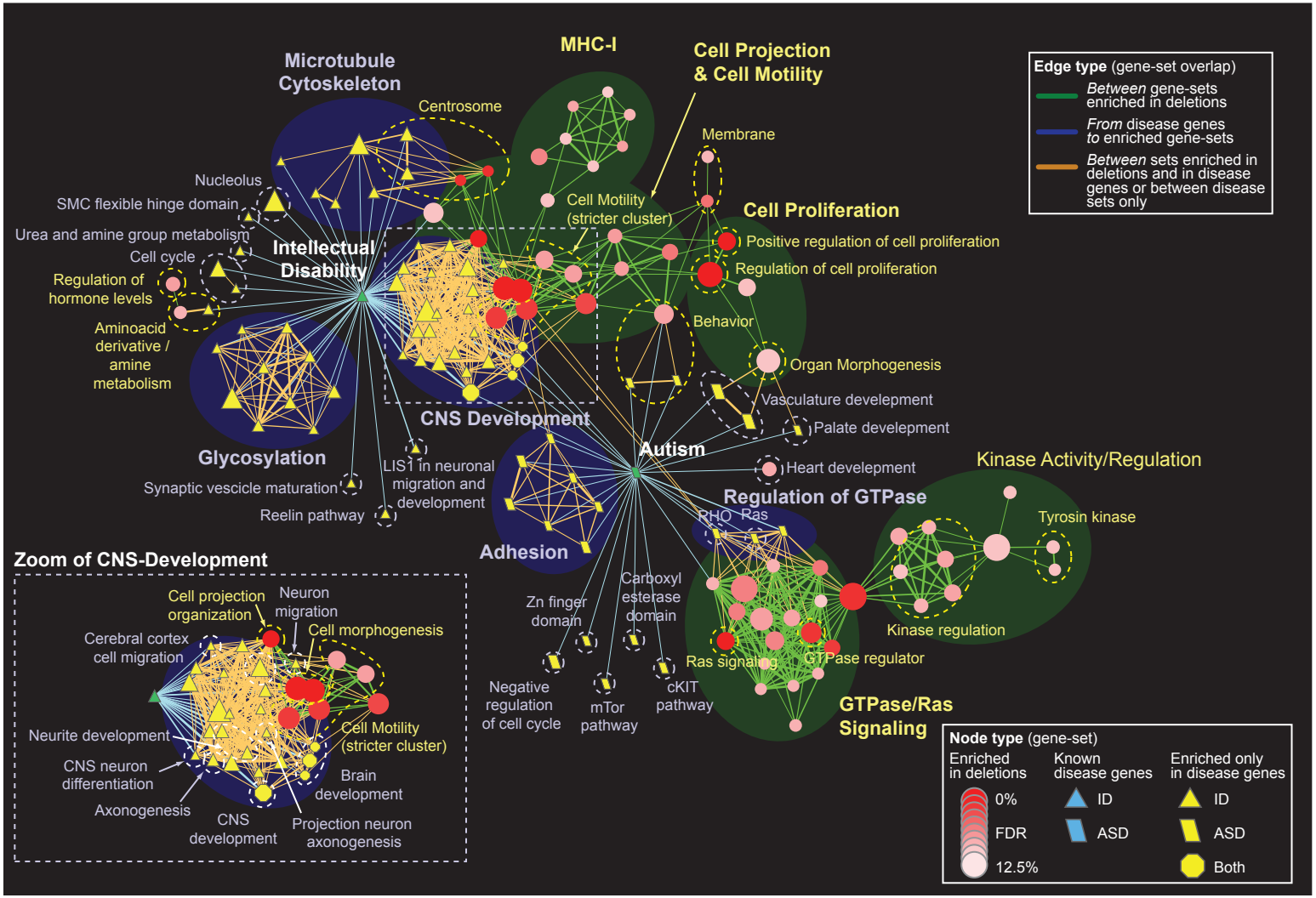
- Genetics
  - highly heritable
    - monozygotic twin concordance 60-90%
    - dizygotic twin concordance 0-10% (depending on the stringency of diagnosis)
  - known genetics:
    - 5-15% rare single-gene disorders and chromosomal re-arrangements
    - de-novo CNV previously reported in 5-10% of ASD cases
    - GWA (Genome-wide Association Studies) have been able to explain only a small amount of heritability

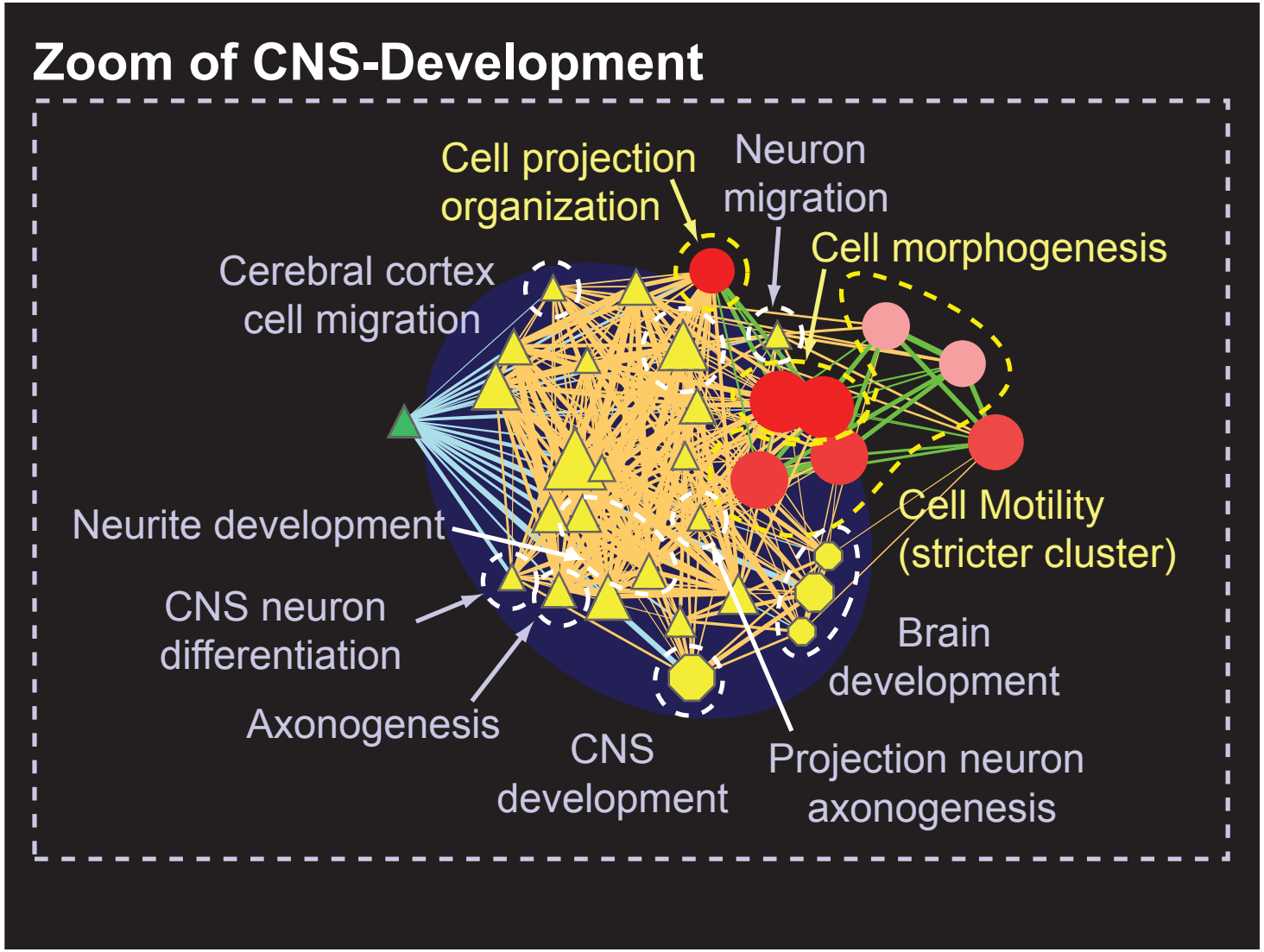
Pinto et al. Functional impact of global rare copy number variation in autism spectrum disorders. Nature. 2010 Jun 9.

Rare copy number variants in ASD

- Rare Copy Number Variation screening (Del, Dup)
  - 889 Case and 1146 Ctrl (European Ancestry)
  - Illumina Infinium 1M-single SNP
  - high quality rare CNV (90% PCR validation)
    - identification by three algorithms required for detection
      - QuantiSNP, iPattern, PennCNV
    - frequency < 1%, length > 30 kb
- Results
  - average CNV size: 182.7 kb, median CNVs per individual: 2
  - > 5.7% ASD individuals carry at least one de-novo CNV
  - Top ~10 genes in CNVs associated to ASD

Pathways enriched in autism spectrum




Zoom of CNS-Development
Cell projection organization
Neuron migration
Cell morphogenesis
Cerebral cortex cell migration
Neurite development
Cell Motility (stricter cluster)
CNS neuron differentiation
Brain development
Axonogenesis
CNS development
Projection neuron axonogenesis

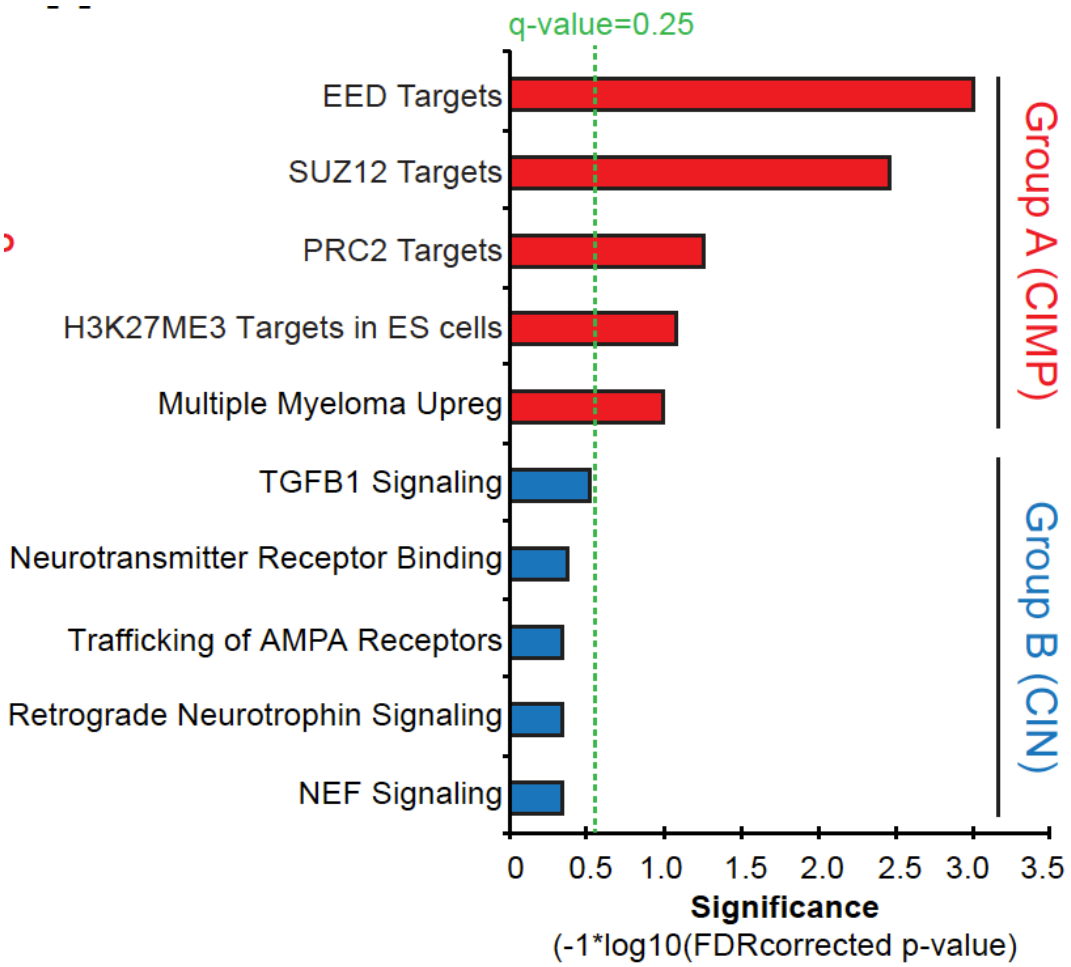
Pathway analysis example 2

Ependymoma pathway analysis

- Ependymoma brain cancer - most common and morbid location for childhood is the posterior fossa (PF = brainstem + cerebellum)
- Two classes: PFA - young, dismal prognosis, PFB - older, excellent prognosis. Determined by gene expression clustering.
- Exome sequencing (42 samples), WGS (5 samples) showed almost no mutations, however methylation arrays showed clear clustering into PFA and PFB (79 samples)
- PFA more transcriptionally silenced by CpG methylation

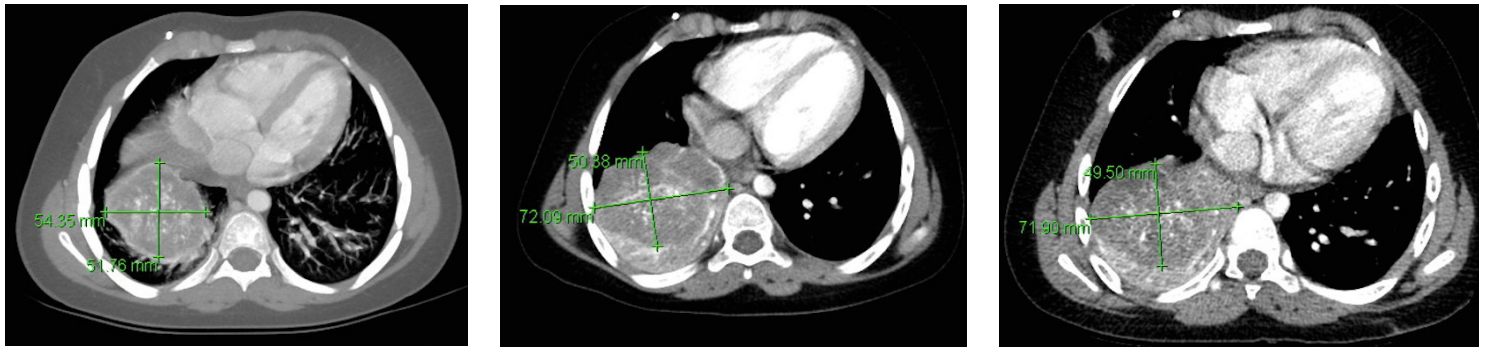
Witt et al., Cancer Cell 2011 **Nature. 2014 Feb 27;506(7489):445-50**

Steve Mack, Michael Taylor, Scott Zuyderduyn



polycomb repressor complex 2 – inhibited by SAHA, DZNep, GSK343 – killed PFA cells
No known treatment. Now in clinical trial

9 yo with metastatic PF ependymoma to lung treated with azacytidine



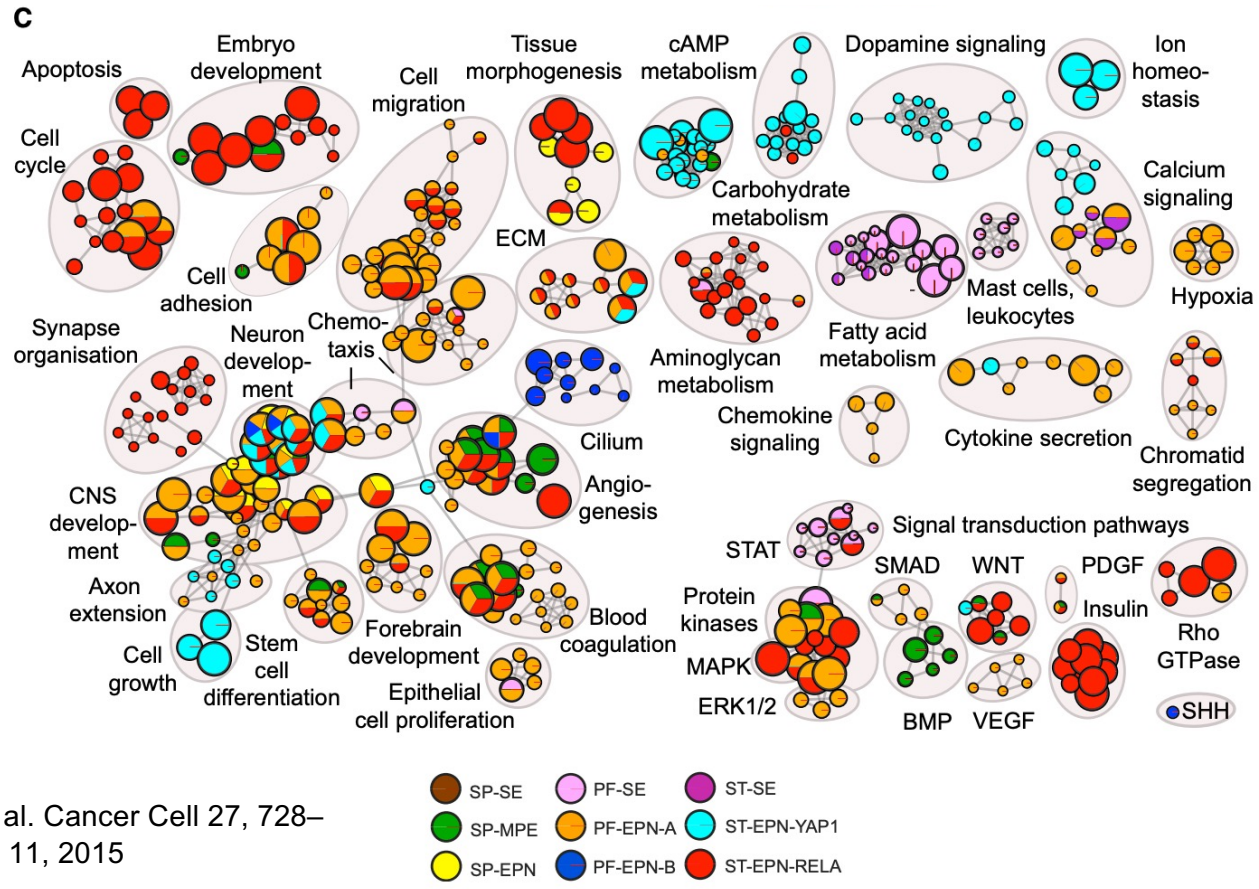
2 months

3 months
3 cycles
Vidaza

Effect lasted 15 months

Pathway analysis example 3

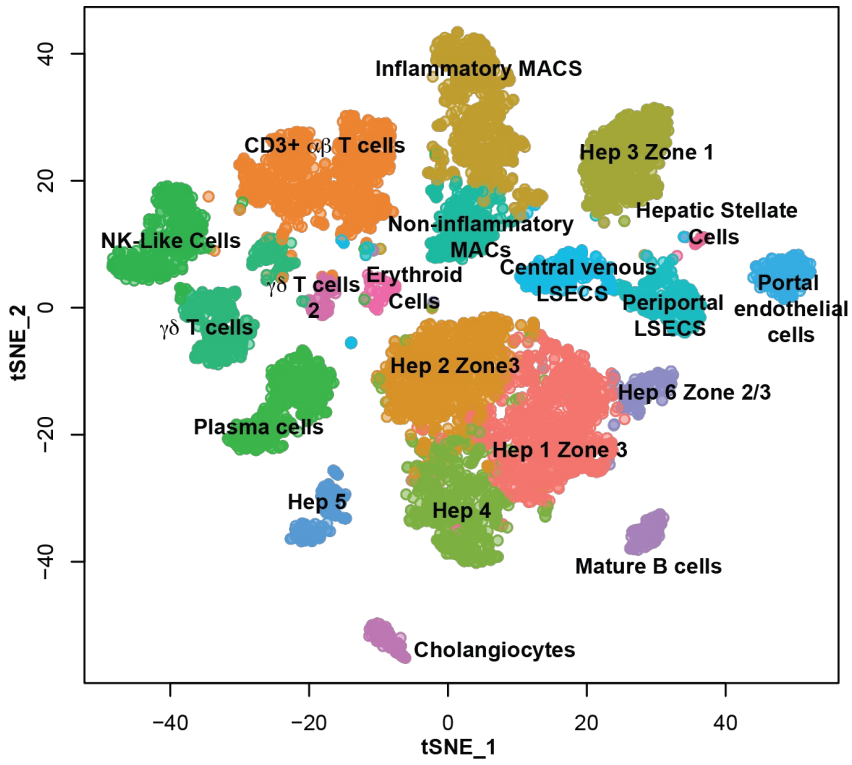
Molecular classification of ependymal tumors



Pajtler et al. Cancer Cell 27, 728–743, May 11, 2015

Pathway analysis example 4

ScRNA-seq of 5 healthy livers reveals 20 cell types



8444 single cells from five human liver samples

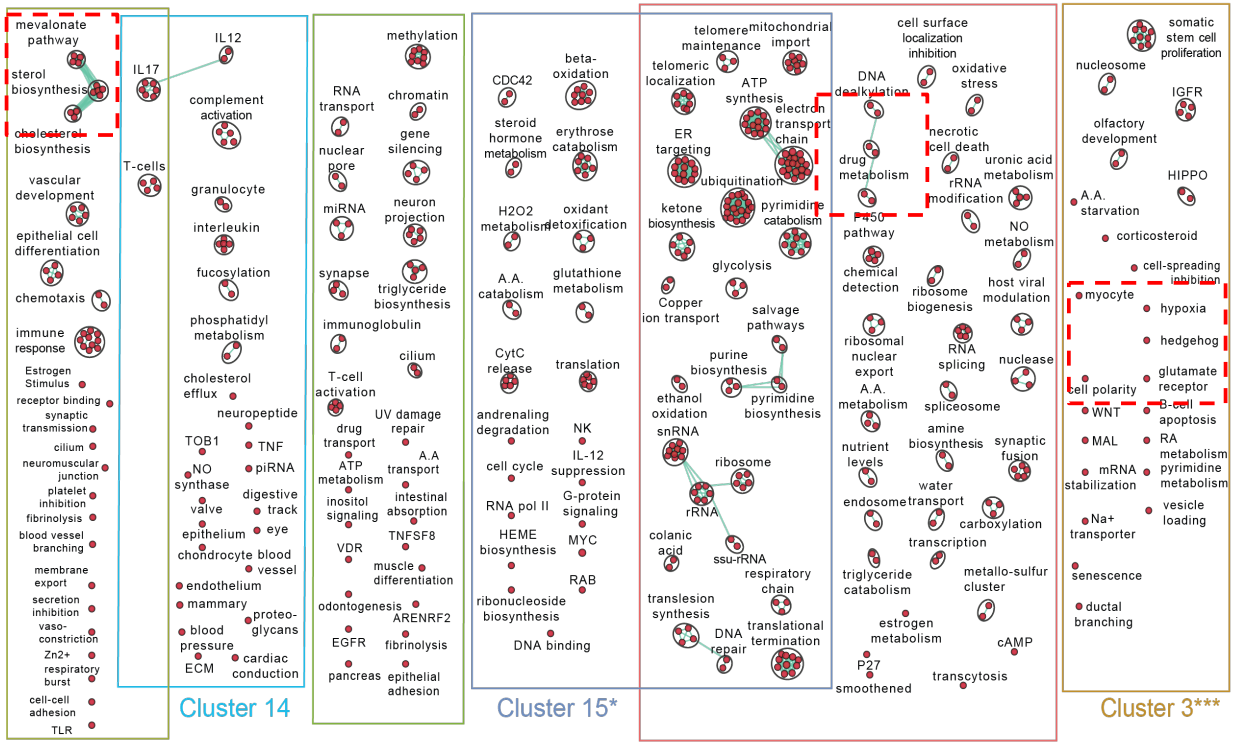
HUMAN CELL ATLAS

Public data, contributed to Human Cell Atlas (https://www.humancellatlas.org)

shiny.baderlab.org/HumanLiverAtlas/

MacParland et al., Oct.22.2018, Nature Communications

Pathway analysis identifies the division of labour among hepatocyte populations



Pathway analysis by GSVA

Periportal — Pericentral

L9 — L1

Mouse Hepatocyte Layers (Halpern et al., 2017)

Example: Genome Wide Association Study (GWAS)

- Genotypes for 5 cases and 5 controls – ideal situation

	Cases					Controls				
SNP	1	2	3	4	5	6	7	8	9	10
A	**1**	**1**	**1**	**1**	**1**	0	0	0	0	0
B	0	0	0	0	0	0	0	0	0	0
C	0	0	0	0	0	0	0	0	0	0
D	0	0	0	0	0	**1**	**1**	**1**	**1**	**1**
E	0	0	0	0	0	0	0	0	0	0
F	0	0	0	0	0	0	0	0	0	0

Mutation A is perfectly associated with cases (5/5)
Mutation D is perfectly associated with controls (5/5)

Example: Genome Wide Association Study (GWAS)

- More realistic situation:

	Cases					Controls				
SNP	1	2	3	4	5	6	7	8	9	10
A	**1**	0	0	0	0	0	0	0	0	0
B	0	**1**	0	0	0	0	0	0	0	0
C	0	0	**1**	0	0	0	0	0	0	0
D	0	0	0	**1**	0	0	0	0	0	0
E	0	0	0	0	**1**	0	0	0	0	0
F	0	0	0	0	0	**1**	0	0	0	0

No mutation is associated with cases or controls

Example: Genome Wide Association Study (GWAS)

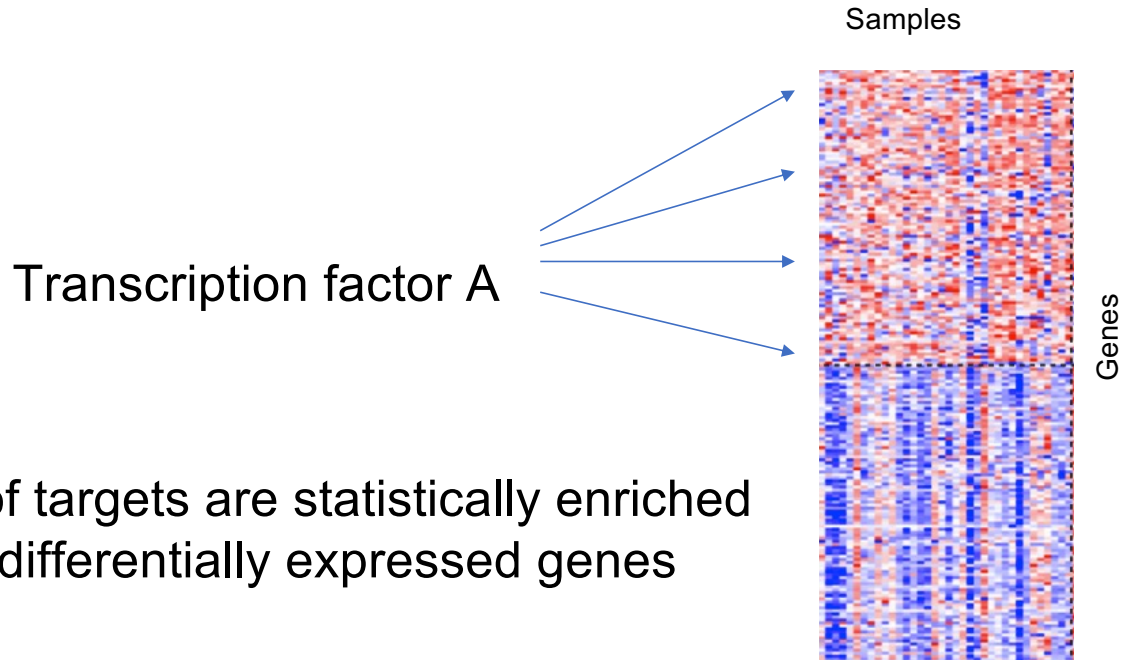
- Pathway analysis view: SNPs A-F are part of one pathway

	Cases					Controls				
Pathway	1	2	3	4	5	6	7	8	9	10
Apoptosis	**1**	**1**	**1**	**1**	**1**	0	0	0	0	0

- Mutations in 'apoptosis' pathway are perfectly associated with cases (5/5)
- Increased statistical power via **aggregating counts** and **reducing multiple testing**
- Generating mechanistic hypotheses (i.e. apoptosis is related to the case phenotype)

Example: Master regulator analysis

- What do 1000 differentially expressed genes have in common?



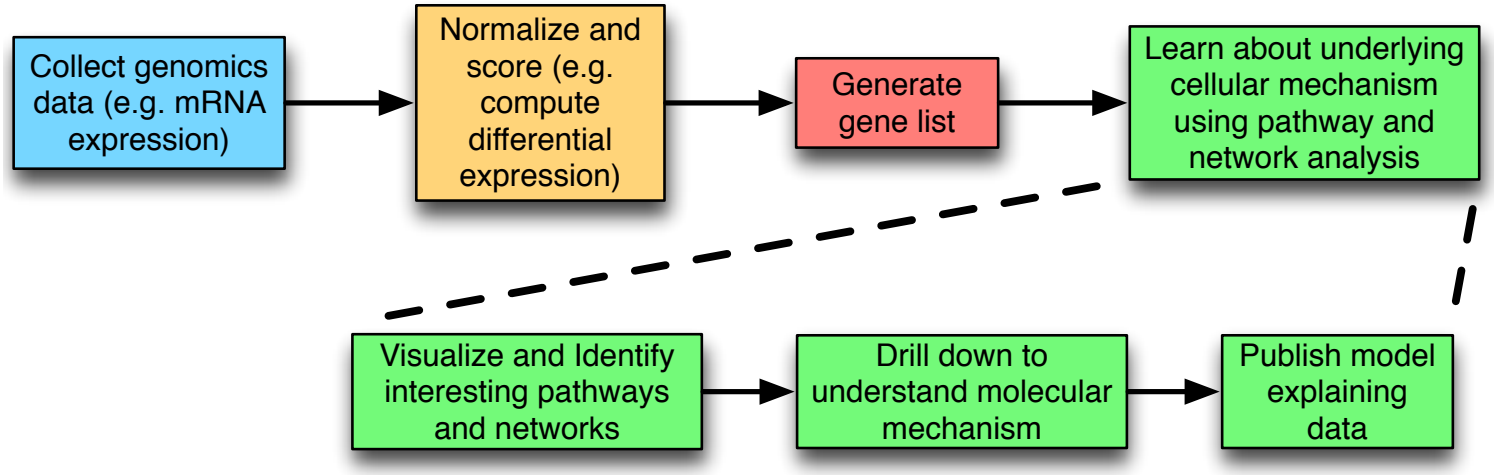
Known set of targets are statistically enriched in the list of differentially expressed genes

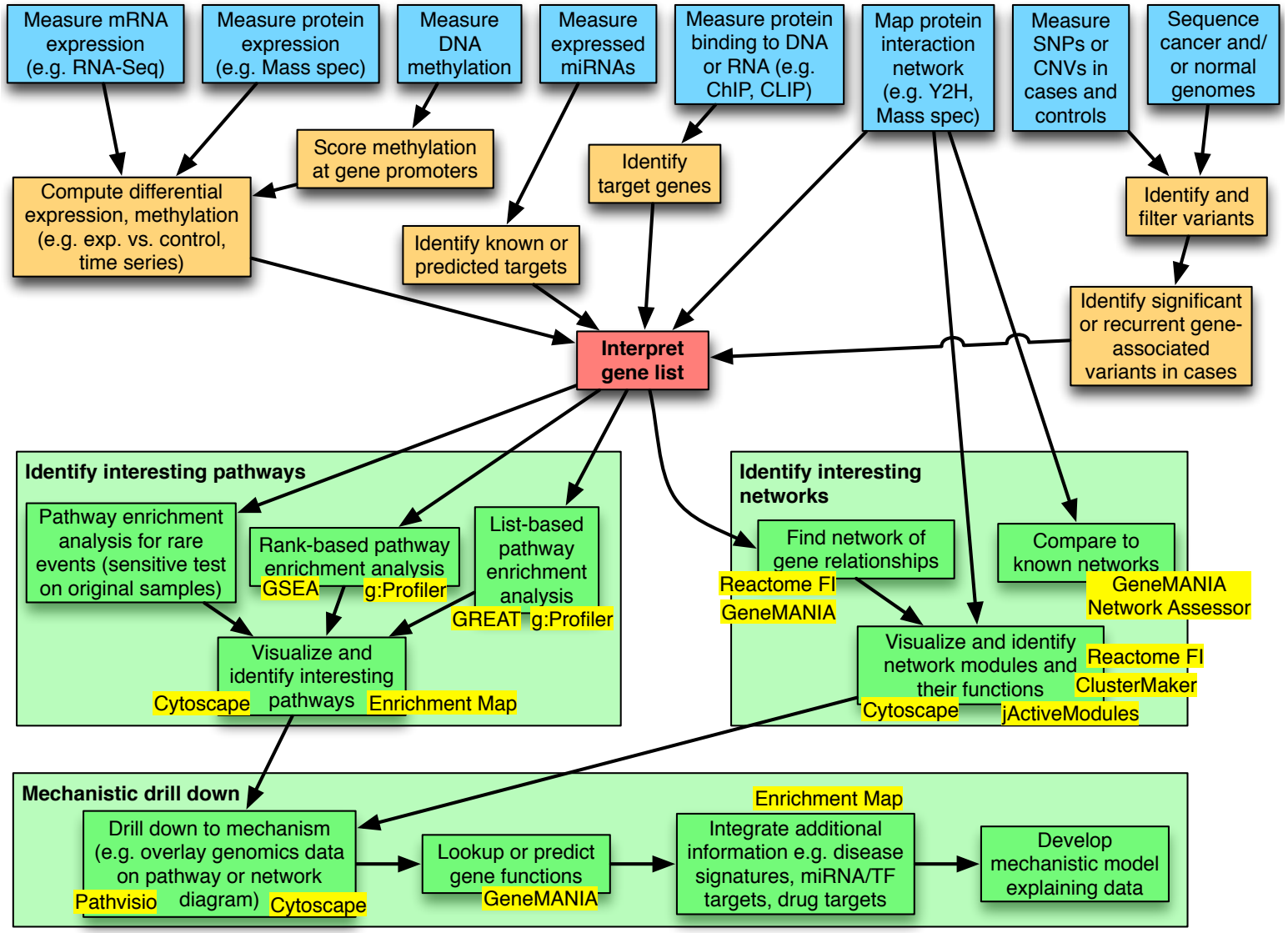
Benefits of pathway analysis

vs. transcripts, proteins, SNPs…

- Easier to interpret
  - Familiar concepts e.g. cell cycle
- Identifies possible causal mechanisms
- Predicts new roles for genes
- Improves statistical power
  - Fewer tests, aggregates data from multiple genes into one pathway
- More reproducible
  - E.g. gene expression signatures
- Facilitates integration of multiple data types

Pathway analysis workflow overview




Measure mRNA expression (e.g. RNA-Seq)
Measure protein expression (e.g. Mass spec)
Measure DNA methylation
Measure expressed miRNAs
Measure protein binding to DNA or RNA (e.g. ChIP, CLIP)
Map protein interaction network (e.g. Y2H, Mass spec)
Measure SNPs or CNVs in cases and controls
Sequence cancer and/ or normal genomes
Score methylation at gene promoters
Identify target genes
Compute differential expression, methylation (e.g. exp. vs. control, time series)
Identify known or predicted targets
Identify and filter variants
Identify significant or recurrent gene-associated variants in cases
Interpret gene list
Identify interesting pathways
Pathway enrichment analysis for rare events (sensitive test on original samples)
Rank-based pathway enrichment analysis
GSEA
g:Profiler
List-based pathway enrichment analysis
GREAT
g:Profiler
Visualize and identify interesting pathways
Cytoscape
Enrichment Map
Identify interesting networks
Find network of gene relationships
Reactome FI
GeneMANIA
Compare to known networks
GeneMANIA
Network Assessor
Visualize and identify network modules and their functions
Cytoscape
Reactome FI
ClusterMaker
jActiveModules
Mechanistic drill down
Drill down to mechanism (e.g. overlay genomics data on pathway or network diagram)
Pathvisio
Cytoscape
Lookup or predict gene functions
GeneMANIA
Integrate additional information e.g. disease signatures, miRNA/TF targets, drug targets
Enrichment Map
Develop mechanistic model explaining data

Workshop outline

Analysis and concepts we will cover:

1) Pathway enrichment analysis:

- Starting with a gene list, summarize and compare
- Visualization of the pathway enrichment results

2) Create different types of networks:

- Nodes represent a molecular entities like genes or proteins
- Edges represent relationships between the entities
- Create a network using an app or create a custom network

3) Network analysis:

- predict gene function, find new pathway members, identify functional modules (new pathways)

4) Cell-cell communication networks:

- Predict another type of network with cells as nodes and edge represent ligand-receptor relationships

We are on a Coffee Break & Networking Session

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