



Canadian Bioinformatics Workshops

www.bioinformatics.ca

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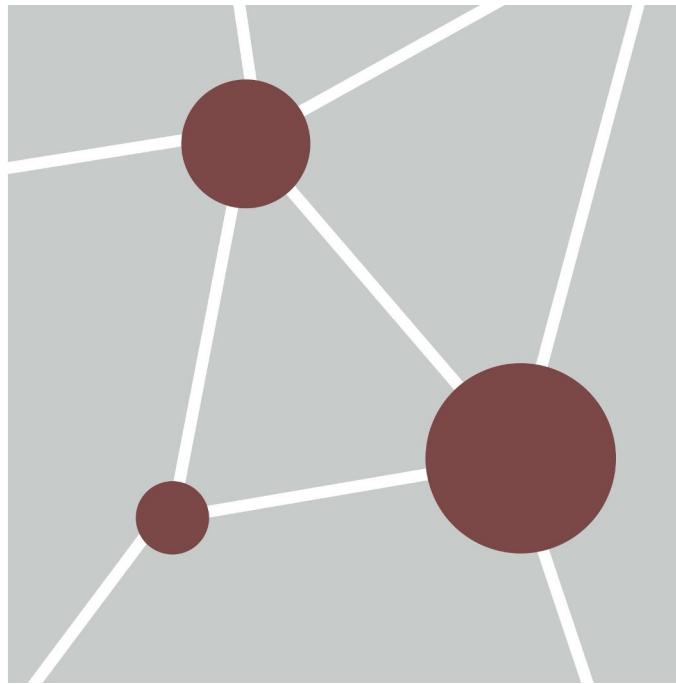
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Final Slides

Veronique Voisin
Pathway and Network Analysis of -omics Data
June 26-28, 2024



Summary

Cytoscape apps are organized by categories

cytoscape app store Submit an App ▾ Search the App Store Sign In

Wall of Apps 184 total

http://apps.cytoscape.org

network generation

online data import

graph analysis

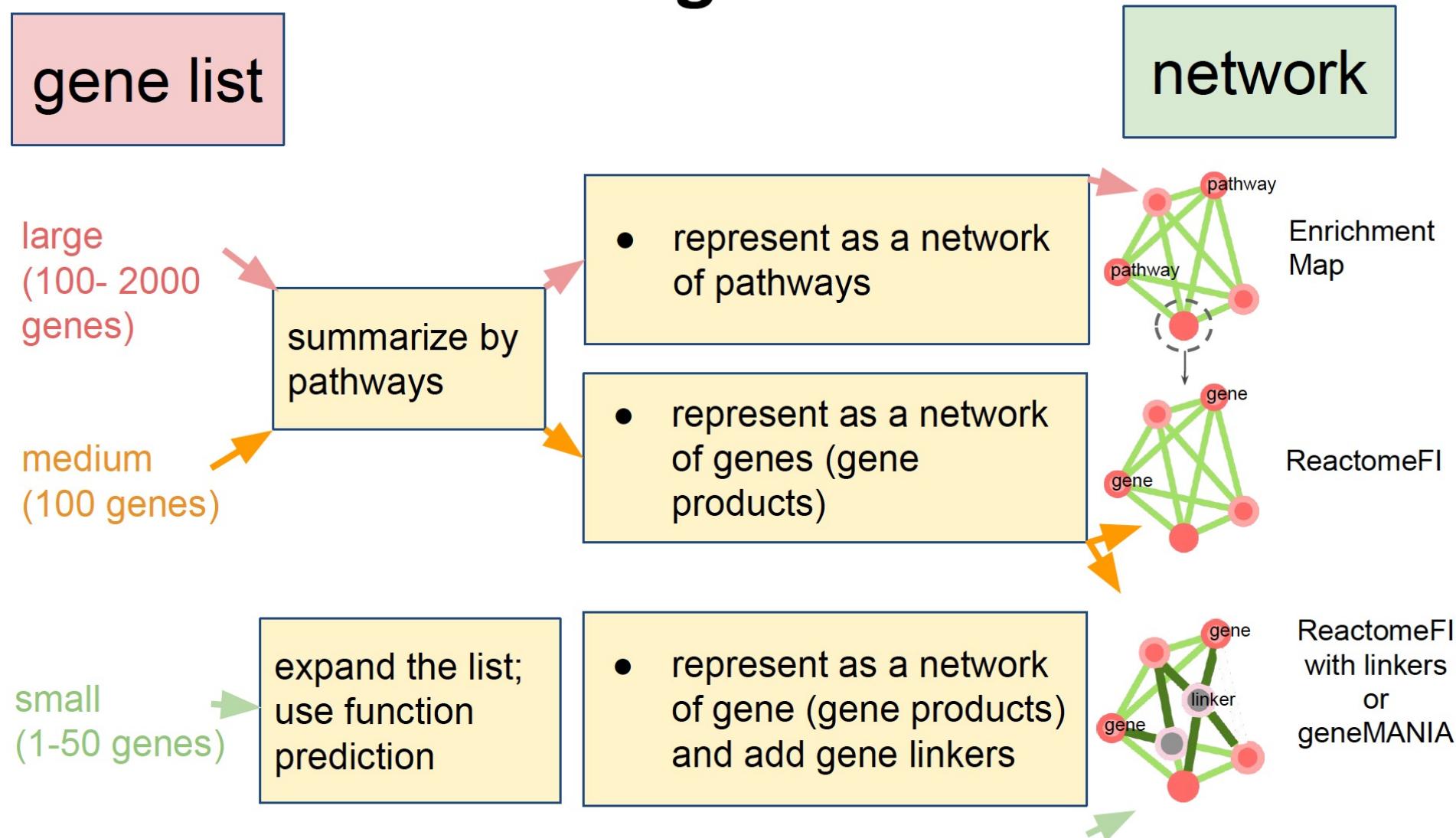
All Apps

Categories

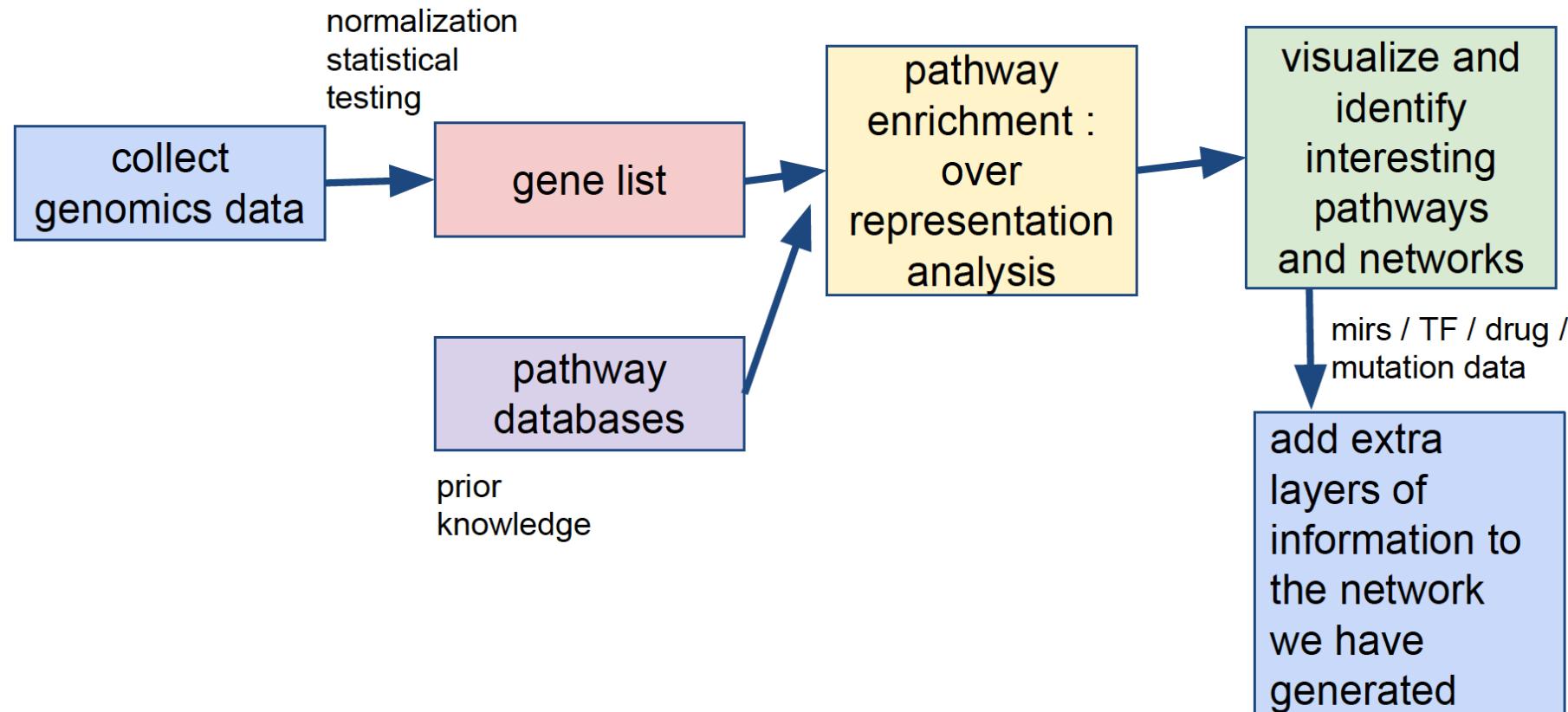
- data visualization**
- network generation
- graph analysis
- network analysis
- online data import
- automation
- integrated analysis
- clustering
- utility
- systems biology
- enrichment analysis
- visualization
- data integration
- core app
- layout
- annotation
- pathway database
- ontology analysis
- import
- gene expression

more »

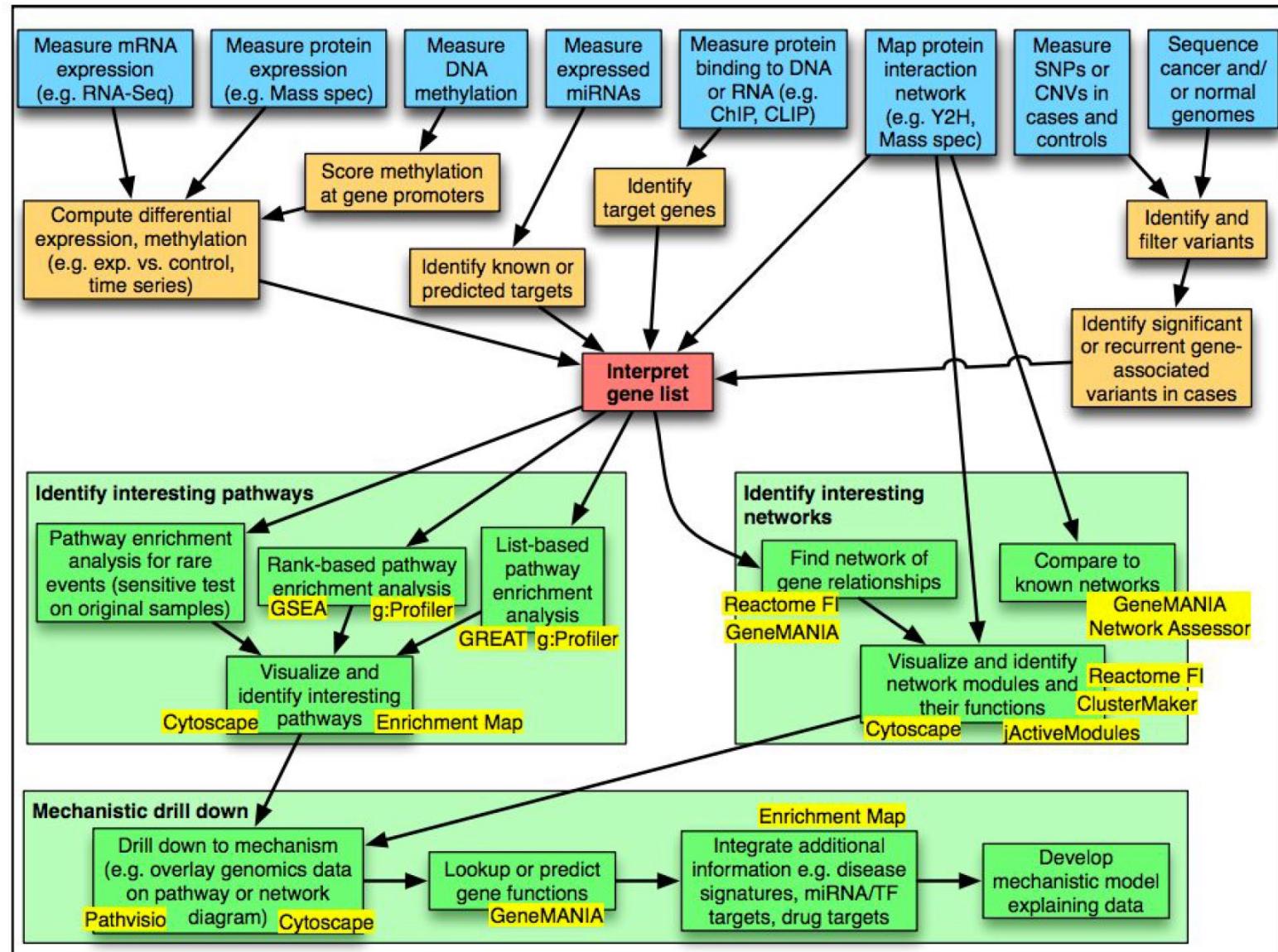
Creating Networks



WORKFLOW SUMMARY



ROADMAP



Mirs, pathways and targets

miRPathDB v2.0 Home About Documentation Download



miRPathDB 2.0

Search

Enter a miRNA or pathway name:

Fc gamma R-mediated phagocytosis

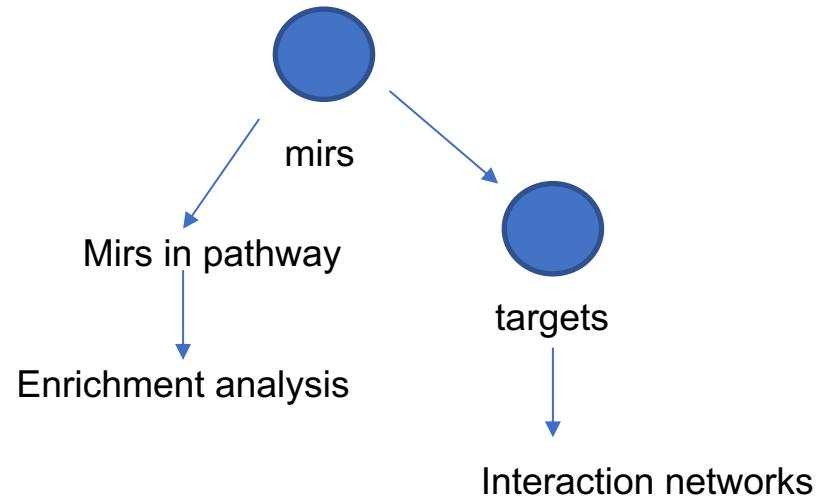
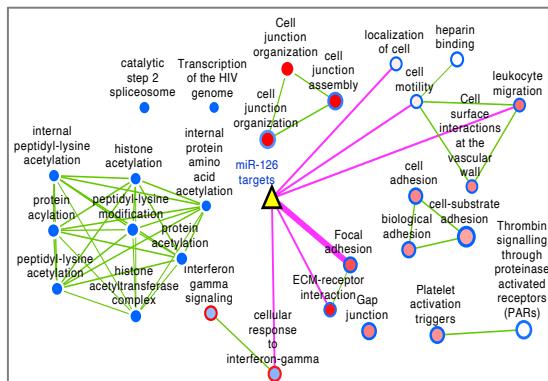
miRNAs that are significantly enriched for this pathway

In this table miRNAs are depicted that have significantly more targets in this pathway than expected by chance.

Show	10	entries	Search:			
Excel	CSV	Column visibility				
Database	miRNA	Evidence	Hits	Expected hits	P-value	Targets
miRBase	hsa-miR-126-3p	experimental (any)	7	0.281231	3.34e-8	AKT1,AKT2,CRK,ORKL,PK3CG,PKC
miRBase	hsa-miR-184	experimental (any)	5	0.297456	1.61e-4	AKT1,AKT2,INPL1,LPLP3,PRKCB
miRCarta	m-5765	predicted (union)	56	36.0553	3.01e-4	AKT2,ANPH,ARPC2,ARPC3,ARPC4
miRCarta	m-17942	predicted (Intersection)	12	2.26763	3.89e-4	ARPC2,CRK,LAT,MARK1,NCFL1,PIP5
miRCarta	m-152	predicted (union)	57	38.8709	4.14e-4	AKT2,ANPH,ARPC2,ARPC3,ARPC4
miRCarta	m-12614	predicted (union)	55	38.7072	4.47e-4	AKT1,AKT2,ARPC1B,ARPC2,ARPC4,ARP
miRBase	hsa-miR-184	experimental (strong)	5	0.336283	4.49e-4	AKT1,AKT2,INPL1,LPLP3,PRKCB
miRBase	hsa-miR-550a-3p	experimental (strong)	2	0.0053082	6.66e-4	MARK1,MARK3

EnrichmentMap
Post analysis
Mir targets

Post analysis:
Good for drug target
and transcription
factors



http://www.ccb.uni-saarland.de/mieaa_tool/

<http://www.lirmed.com/tam2/>

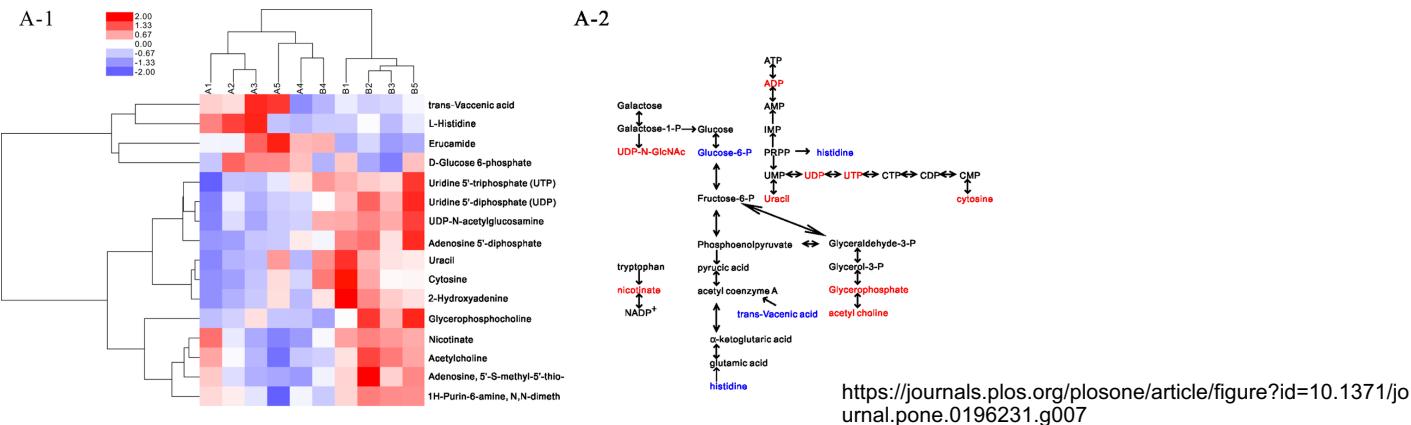
Result

Enrichment analysis results

Category	Count	Percent	Fold	P-value	Bonferroni	FDR
Cluster (4 Items)	1	0.33333	33.63889	0.0295	1	0.3755
Cluster (4 Items)	2	0.33333	33.63889	1.32e-3	0.3569	0.08
Cluster (4 Items)	1	0.5	50.45833	0.0197	1	0.3365
Cluster (4 Items)	1	0.2	20.18333	0.0487	1	0.479
Disease (194 Items)	1	0.16667	16.81944	0.0581	1	0.5292
Disease (194 Items)	2	0.14286	14.41667	7.67e-3	1	0.1858
Disease (194 Items)	2	0.04348	4.38768	0.0731	1	0.5944
Disease (194 Items)	1	0.14286	14.41667	0.0675	1	0.5676
Adenocarcinoma, Colon (details)	2	0.08696	8.77536	0.0203	1	0.2926
Adenocarcinoma, Esophageal (details)	1	0.04545	4.58712	0.1983	1	1
Adenocarcinoma, Gastric (details)	1	0.02632	2.6557	0.3191	1	1
Adenocarcinoma, Lung (details)	2	0.0198	1.99835	0.2642	1	1
Adrenal Cortex Neoplasms (details)	1	0.08333	8.40972	0.1131	1	0.7828

Metabolomics Data Analysis

A) list of metabolites from your experiment



B) pathway: a set of metabolites known to be involved in metabolic pathways

The colored compounds indicate potential matches from the user's input, with red colors indicating significant hits and blue colors indicating non-significant hits.

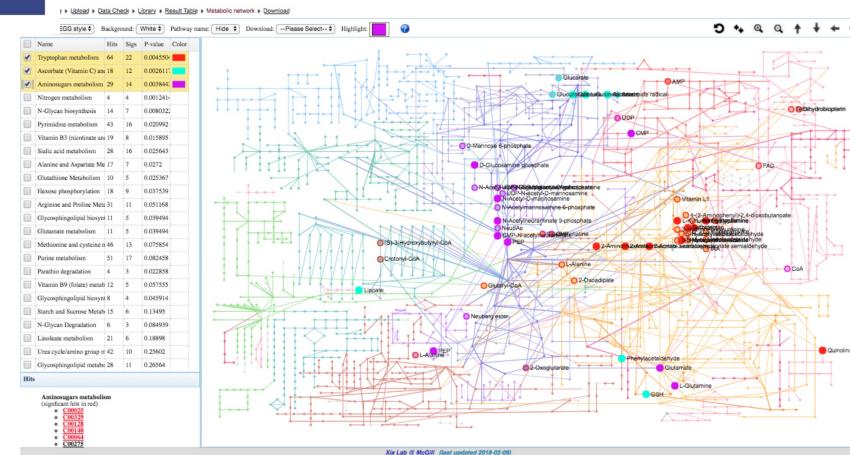
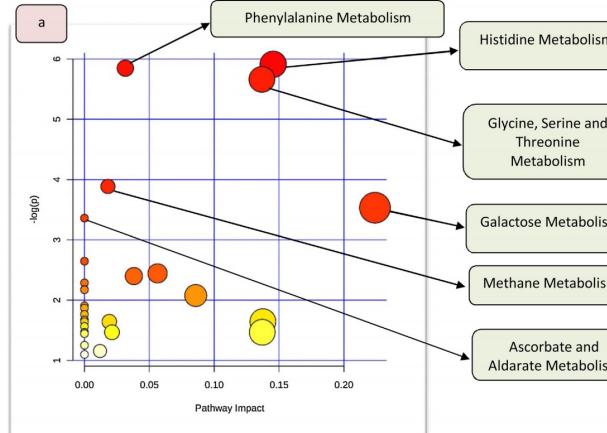
Pathway	Metabolites
Tryptophan metabolism	C00025; C00024; C00027; C00026 ; C00021; C00020 ; CE2119 ; C00028; C03722 ; C05647 ; C05645 ; CE1395 ; C05643 ; C05640 ; C00780 ; C05648 ; C00704; C01342; C00010 ; C00014; C00016 ; C00019; C15605 ; C00067; thbpt4acam; C05651 ; C03512; C05653 ; C02693; C05660 ; C00398 ; CE5982; C00643 ; C02220 ; C00078 ; C00978 ; C00877; C05642 ; CE5860; C01252 ; C05637 ; C02406 ; C00108 ; C00272 ; CE1918 ; C01652; C02470 ; C01144 ; C06212 ; C06213 ; CE2949; CE2948; CE3140 ; CE2122 ; C00479; CE2947 ; C01717; CE3092; CE6205 ; CE5899; C03161; C00268 ; C00632 ; CE2152 ; CE2153; C02700 ; CE2095; C00328 ; CE3087; CE3086; C00322 ; C04409 ; C01352; C00051 ; C00058; C00030; C00331 ; C00332; C00936 ; C03824; C05636 ; C05635 ; C05634 ; C05639; C05638 ; C10164 ; C00637 ; C03227 ; C01598 ; C00525 ; C00527 ; C00041; C03024; C00954



Input Data Type	Available Modules (click on a module to proceed, or scroll down for more details)																																																																																																																																																																													
Raw Spectra (mzML, mzXML or mzData)	LC-MS Spectra Processing																																																																																																																																																																													
MS Peaks (peak list or intensity table)	Functional Analysis Functional Meta-analysis																																																																																																																																																																													
Annotated Features (compound list or table)	Enrichment Analysis Pathway Analysis Joint-Pathway Analysis Network Analysis																																																																																																																																																																													
Generic Format (.csv or .txt table files)	Statistical Analysis [one factor]	Statistical Analysis [metadata table]	Biomarker Analysis	Statistical Meta-analysis	Power Analysis	Other Utilities																																																																																																																																																																								
	<table border="1"> <thead> <tr> <th>Pathway Name</th> <th>Total</th> <th>Hits (all)</th> <th>Hits (sig.)</th> <th>Fisher's P-value</th> <th>EASE Score</th> <th>Gamma Pvalue</th> <th>Match Details</th> </tr> </thead> <tbody> <tr><td>Tryptophan metabolism</td><td>94</td><td>64</td><td>21</td><td>0.0045504</td><td>0.0098086</td><td>0.0046682</td><td>View</td></tr> <tr><td>Ascorbate (Vitamin C) and Aldarate Metabolism</td><td>29</td><td>18</td><td>9</td><td>0.0026117</td><td>0.010691</td><td>0.0046835</td><td>View</td></tr> <tr><td>Aminosugar metabolism</td><td>69</td><td>29</td><td>12</td><td>0.0038443</td><td>0.011655</td><td>0.0047003</td><td>View</td></tr> <tr><td>Nitrogen metabolism</td><td>6</td><td>4</td><td>4</td><td>0.0012414</td><td>0.022604</td><td>0.0048951</td><td>View</td></tr> <tr><td>N-Glycan biosynthesis</td><td>48</td><td>14</td><td>7</td><td>0.0080322</td><td>0.032406</td><td>0.0050767</td><td>View</td></tr> <tr><td>Pyrimidine metabolism</td><td>70</td><td>43</td><td>14</td><td>0.020992</td><td>0.045203</td><td>0.0053243</td><td>View</td></tr> <tr><td>Vitamin B3 (nicotinate and nicotinamide) metabolism</td><td>28</td><td>19</td><td>8</td><td>0.015895</td><td>0.049225</td><td>0.0054047</td><td>View</td></tr> <tr><td>Sialic acid metabolism</td><td>107</td><td>28</td><td>10</td><td>0.025643</td><td>0.062907</td><td>0.0056878</td><td>View</td></tr> <tr><td>Alanine and Aspartate Metabolism</td><td>30</td><td>17</td><td>7</td><td>0.0272</td><td>0.08133</td><td>0.0060936</td><td>View</td></tr> <tr><td>Glutathione Metabolism</td><td>19</td><td>10</td><td>5</td><td>0.025367</td><td>0.099714</td><td>0.0065288</td><td>View</td></tr> <tr><td>Hexose phosphorylation</td><td>20</td><td>18</td><td>7</td><td>0.037539</td><td>0.10359</td><td>0.0066246</td><td>View</td></tr> <tr><td>Arginine and Proline Metabolism</td><td>45</td><td>31</td><td>10</td><td>0.051168</td><td>0.10984</td><td>0.0067823</td><td>View</td></tr> <tr><td>Glycosphingolipid biosynthesis - ganglosides</td><td>62</td><td>11</td><td>5</td><td>0.039494</td><td>0.13447</td><td>0.0074426</td><td>View</td></tr> <tr><td>Glutamate metabolism</td><td>15</td><td>11</td><td>5</td><td>0.039494</td><td>0.13447</td><td>0.0074426</td><td>View</td></tr> <tr><td>Methionine and cysteine metabolism</td><td>94</td><td>46</td><td>13</td><td>0.076564</td><td>0.13715</td><td>0.0075184</td><td>View</td></tr> <tr><td>Purine metabolism</td><td>80</td><td>51</td><td>14</td><td>0.082458</td><td>0.1436</td><td>0.007704</td><td>View</td></tr> <tr><td>Parathio degradation</td><td>6</td><td>4</td><td>3</td><td>0.022858</td><td>0.16222</td><td>0.0082678</td><td>View</td></tr> <tr><td>Vitamin B9 (folate) metabolism</td><td>33</td><td>12</td><td>5</td><td>0.057555</td><td>0.17336</td><td>0.0086255</td><td>View</td></tr> <tr><td>Glycosphingolipid biosynthesis - globosides</td><td>16</td><td>8</td><td>4</td><td>0.045914</td><td>0.17647</td><td>0.0087285</td><td>View</td></tr> <tr><td>Starch and Sucrose Metabolism</td><td>33</td><td>15</td><td>5</td><td>0.13495</td><td>0.30626</td><td>0.014409</td><td>View</td></tr> </tbody> </table>						Pathway Name	Total	Hits (all)	Hits (sig.)	Fisher's P-value	EASE Score	Gamma Pvalue	Match Details	Tryptophan metabolism	94	64	21	0.0045504	0.0098086	0.0046682	View	Ascorbate (Vitamin C) and Aldarate Metabolism	29	18	9	0.0026117	0.010691	0.0046835	View	Aminosugar metabolism	69	29	12	0.0038443	0.011655	0.0047003	View	Nitrogen metabolism	6	4	4	0.0012414	0.022604	0.0048951	View	N-Glycan biosynthesis	48	14	7	0.0080322	0.032406	0.0050767	View	Pyrimidine metabolism	70	43	14	0.020992	0.045203	0.0053243	View	Vitamin B3 (nicotinate and nicotinamide) metabolism	28	19	8	0.015895	0.049225	0.0054047	View	Sialic acid metabolism	107	28	10	0.025643	0.062907	0.0056878	View	Alanine and Aspartate Metabolism	30	17	7	0.0272	0.08133	0.0060936	View	Glutathione Metabolism	19	10	5	0.025367	0.099714	0.0065288	View	Hexose phosphorylation	20	18	7	0.037539	0.10359	0.0066246	View	Arginine and Proline Metabolism	45	31	10	0.051168	0.10984	0.0067823	View	Glycosphingolipid biosynthesis - ganglosides	62	11	5	0.039494	0.13447	0.0074426	View	Glutamate metabolism	15	11	5	0.039494	0.13447	0.0074426	View	Methionine and cysteine metabolism	94	46	13	0.076564	0.13715	0.0075184	View	Purine metabolism	80	51	14	0.082458	0.1436	0.007704	View	Parathio degradation	6	4	3	0.022858	0.16222	0.0082678	View	Vitamin B9 (folate) metabolism	33	12	5	0.057555	0.17336	0.0086255	View	Glycosphingolipid biosynthesis - globosides	16	8	4	0.045914	0.17647	0.0087285	View	Starch and Sucrose Metabolism	33	15	5	0.13495	0.30626	0.014409	View
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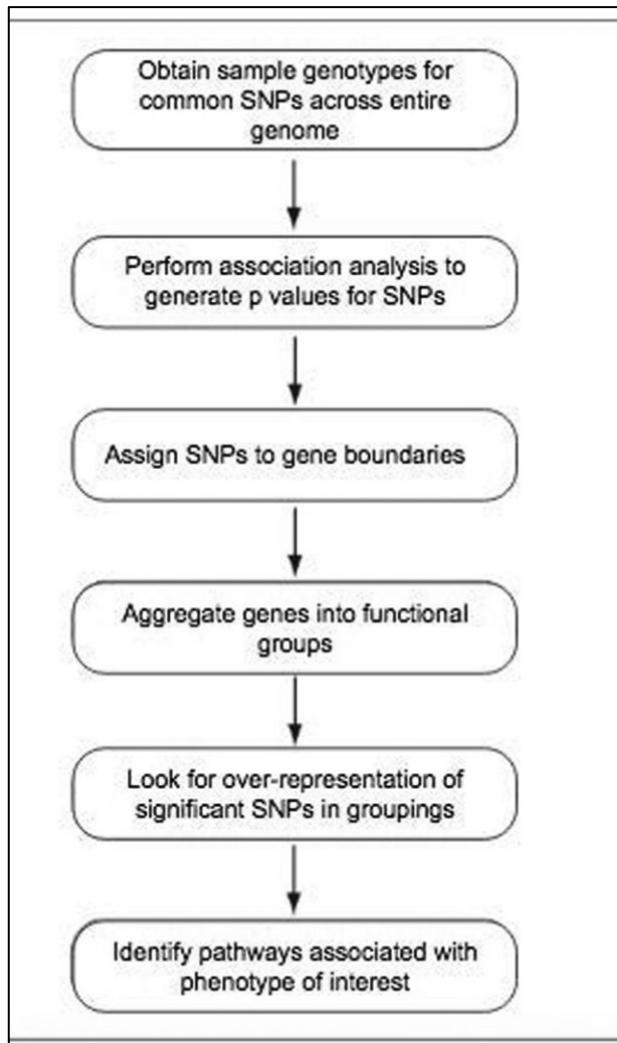
Download Result Tables: [Pathway Hits](#) [Compound Hits](#)

Metabolomics Data Analysis



List of metabolites: Fishers' exact test
If it is possible to rank all the metabolites: GSEA

Overall protocol for pathway-based GWAS / WGS analysis



GWAS --> MAGENTA
(Meta-Analysis Geneset Enrichment of variaNT Associations)
<https://software.broadinstitute.org/mpg/magenta/>

The only **input** required is a table with variant association p-values and their chromosome positions taken from a genome-wide association study or meta-analysis. **Optional:** pathway/s or gene set/s of interest. Otherwise, we provide a set of pathways from public databases (see below).

The main **output** of MAGENTA is a nominal **gene set enrichment analysis (GSEA) p-value** and a **false discovery rate** for each gene set or pathway tested. There are several options, including running MAGENTA in the absence of a subset of genes, such as a predefined set of disease or trait genes. Additional information is provided, such as the expected and observed number of genes above the enrichment cutoff, and the number and name of genes in each tested gene set that lie near validated disease or trait SNPs if inputted by the user.

Ref: <https://pubmed.ncbi.nlm.nih.gov/30387919/>

GWAS and pathway analysis

- **BridGE: a pathway-based analysis tool for detecting genetic interactions from GWAS.**
Published: 21 March 2024. Nature Protocol

ATACseq / CHIPseq

- GREAT (Stanford) is a recommended tool (from chromosomal position to gene + enrichment analysis)
- All tools compatible with EnrichmentMap

The screenshot shows the Great website homepage. At the top, there is a dark header bar with the 'GREAT' logo and links for Overview, News, Use GREAT, Demo, Video, How to Cite, Help, and Forum. Below the header, a dropdown menu shows 'GREAT version 4.0.4 current (08/19/2019 to now)'. The main content area has a title 'GREAT predicts functions of cis-regulatory regions.' followed by a detailed paragraph about the tool's purpose and methods. Below this, there is a 'News' section with a list of recent updates, each accompanied by a small sunburst icon. At the bottom of the news section, there is a link to 'More news items...'

Tips:

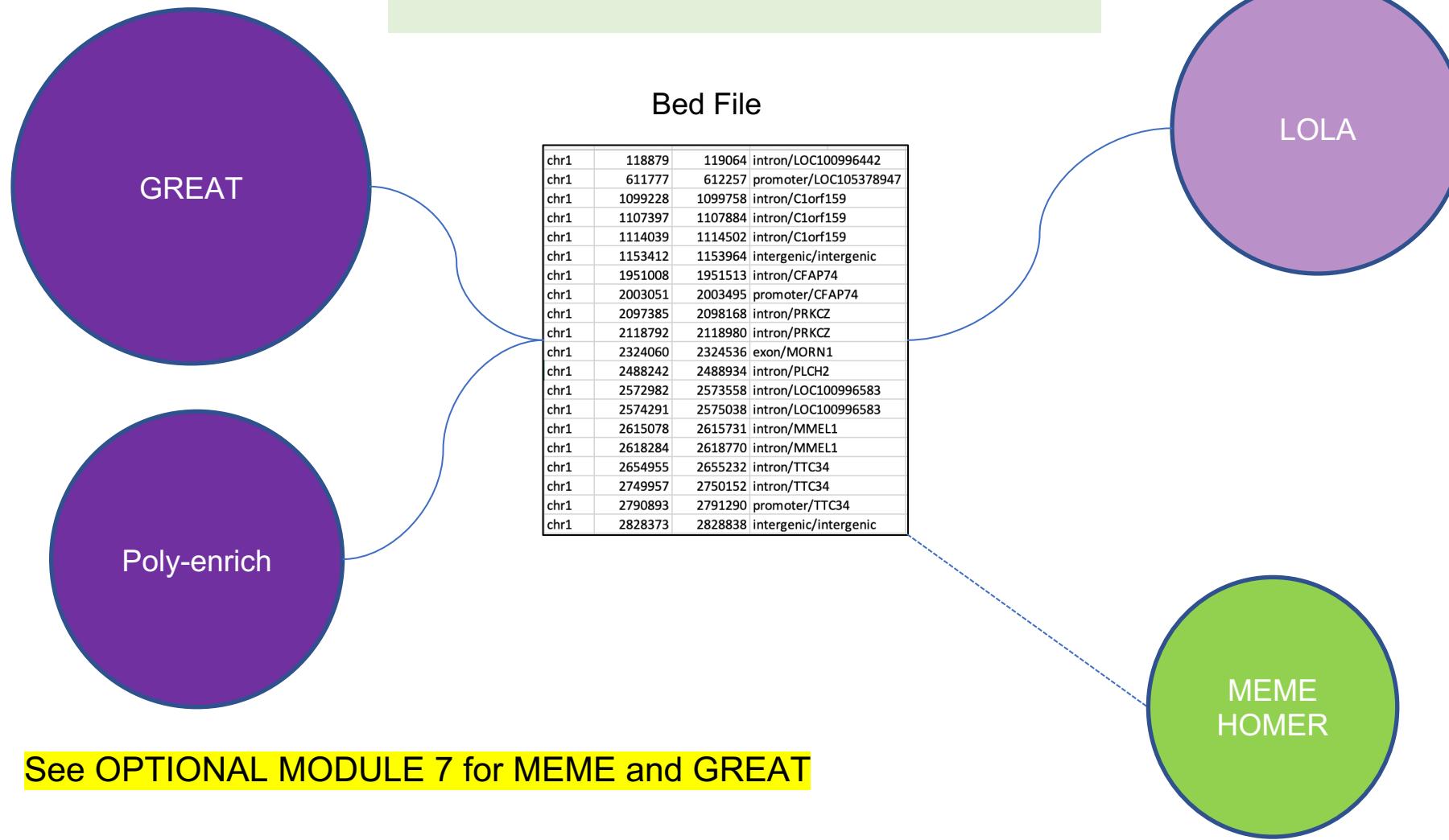
Do

- 1) Proximal analysis (+-2kb around TSS of genes)

And

- 2) Distal analysis (+-50kb around genes, filter genomic regions using tools like Segway or BEHST)

ATACseq / CHIPseq



See OPTIONAL MODULE 7 for MEME and GREAT

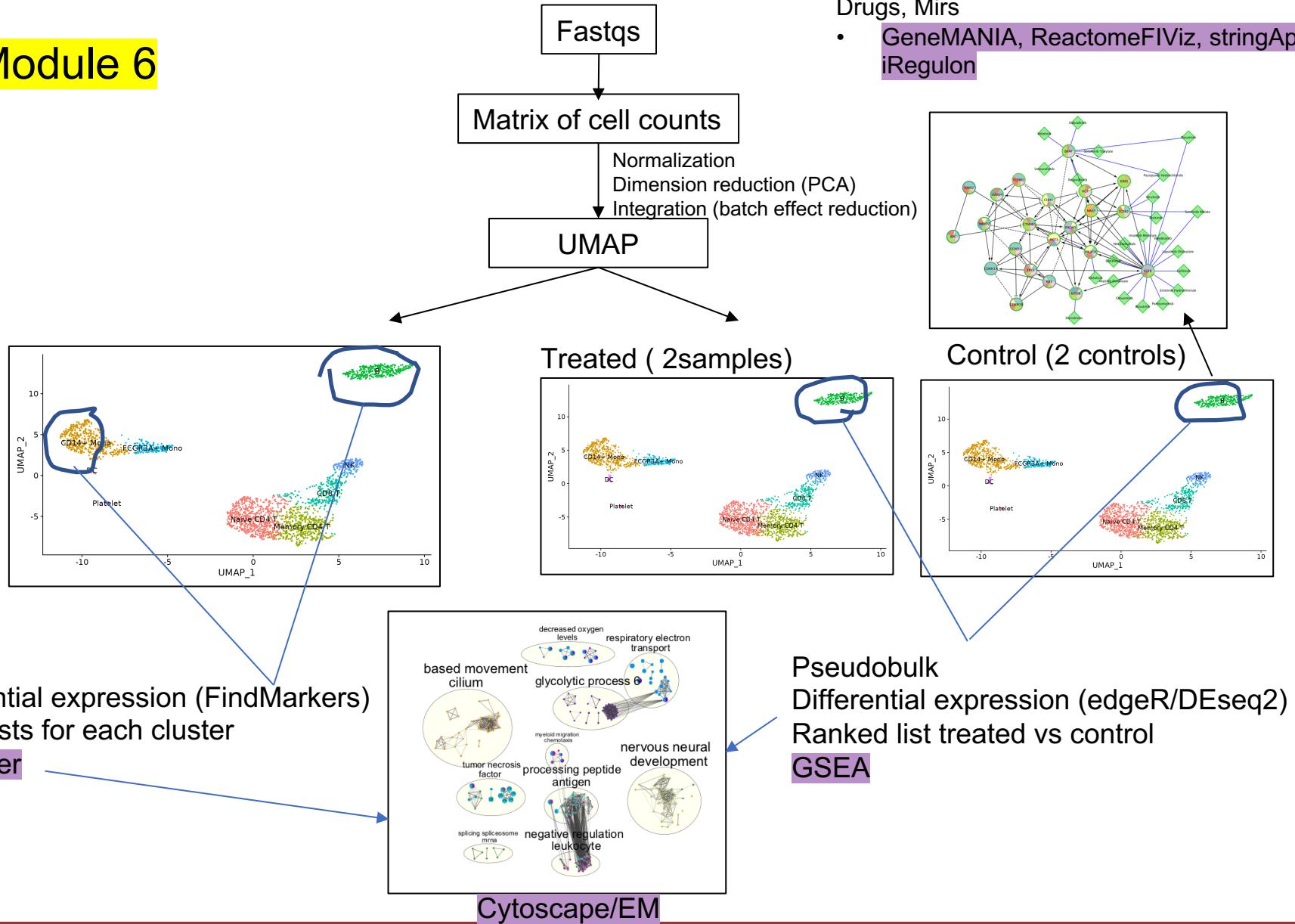
Included in module 7: iRegulon

Transcriptomics: bulk RNA-seq 2 class design

- Module 2 and 3:
- GSEA
- Enrichment Map

Module 6

scRNA-seq



Non model organisms

1. Find a pathway database/ gmt file which is the closest to your organism
2. Convert your gene identifier to the gene identifiers used in the gmt file that you found using g:Convert and g:Orth
3. Both GSEA and g:Profiler accept custom gmt file.
4. GeneMANIA offers several organisms and the option to build your interaction networks.

The screenshot shows the g:GOST functional profiling tool. At the top, there are four tabs: g:GOST (Functional profiling), g:Convert (Gene ID conversion), g:Orth (Orthology search), and g:SNPense (SNP id to gene name). Below the tabs, there are three input options: 'Query' (selected), 'Upload query', and 'Upload bed file'. A note says 'Input is whitespace-separated list of genes'. The main area has a large text input field and a 'Run query' button. Below the input field are links for 'random example' and 'mixed query example'. At the bottom, a note states: 'g:GOST performs functional enrichment analysis, also known as over-representation analysis'.

g:GOST
Functional profiling

g:Convert
Gene ID conversion

g:Orth
Orthology search

g:SNPense
SNP id to gene name

Query Upload query Upload bed file

Input is whitespace-separated list of genes

Options

Organism: Homo sapiens (Human)

Favourites (14)

- Arabidopsis thaliana
- Aspergillus fumigatus A1163
- Bos taurus (Cow)
- Caenorhabditis elegans
- Danio rerio (Zebrafish)
- Drosophila melanogaster
- Gallus gallus (Chicken)
- Homo sapiens (Human)
- Mus musculus (Mouse)
- Oryza sativa Japonica Group
- Pyrenophora teres f. teres 0-1 (Pyrenophora teres)
- Rattus norvegicus (Rat)
- Saccharomyces cerevisiae

g:GOST performs functional enrichment analysis, also known as over-representation analysis

Script to create a gmt file from the GO ontology:

<https://www.dropbox.com/s/wm3kq4lsdlfwcoq/creategmt.R?dl=0>

Module 7 (Review of the tools)

- Work with your own data
- Integrated Assignment
- Q and A with instructors
- Automation (R coding for advanced users)
- clusterProfiler (R coding for advanced users)

Module 7 (optional)

- Chipseq and ATAC data: MEME and GREAT, EnrichmentMap
- iregulon: TF network

We are on a Coffee Break & Networking Session

Workshop Sponsors:



Canadian Centre for
Computational
Genomics



HPC4Health



OICR
Ontario Institute
for Cancer Research



Ontario
Genomics



GenomeCanada