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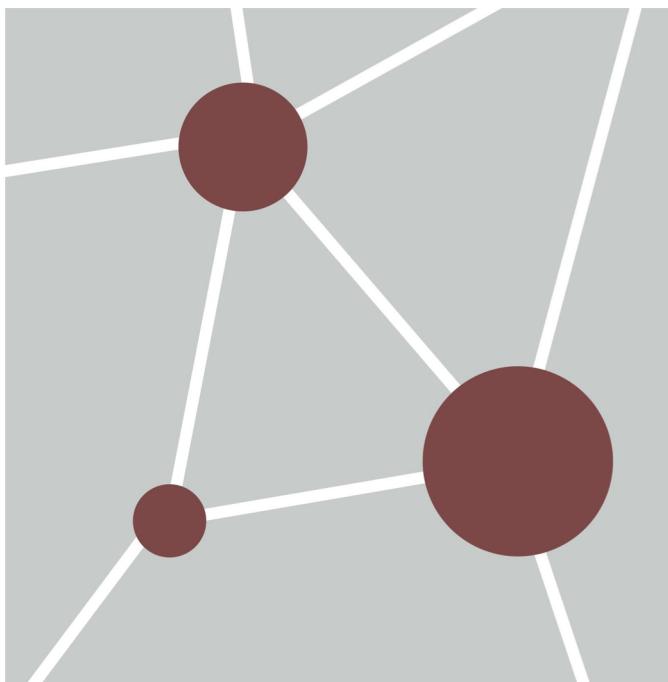
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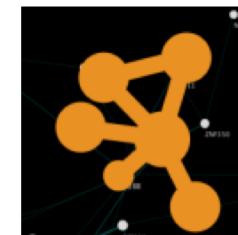
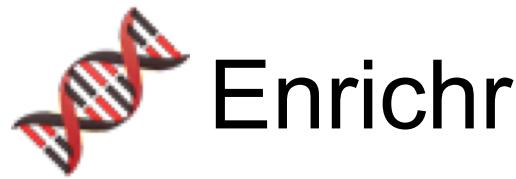
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Gene Regulation and Motif Analysis Practical Lab / iRegulon



Veronique Voisin
Pathway and Network Analysis of –omics Data

May, 10-12, 2021



Learning Objectives

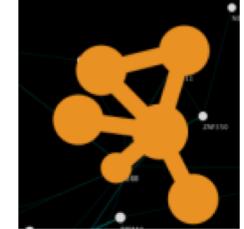
This practical lab focuses on finding potential transcription factors that would regulate our gene list.

We are using 2 tools

- EnrichR which is a web app doing enrichment analysis.
- IRegulon which is a Cytoscape app applying a GSEA like algorithm.



iRegulon

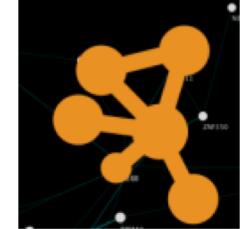


<http://iregulon.aertslab.org/>

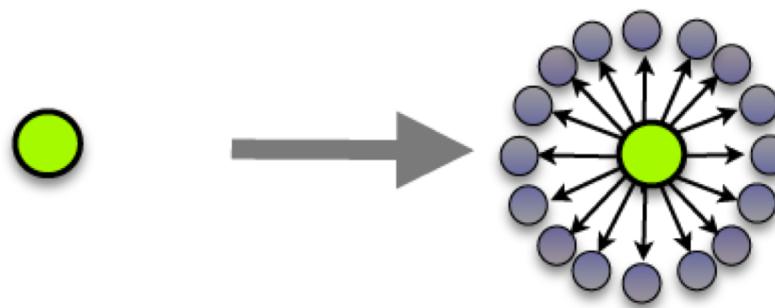
- Cytoscape app
- Developed to find transcription factor targets in a set of co-regulated genes
- Use precomputed results:
 - * precomputed occurrence of transcription motifs and chip-Seq peaks in promoter of all genes in genome
 - * rank all genes in genomes based on this result for each known transcription factor



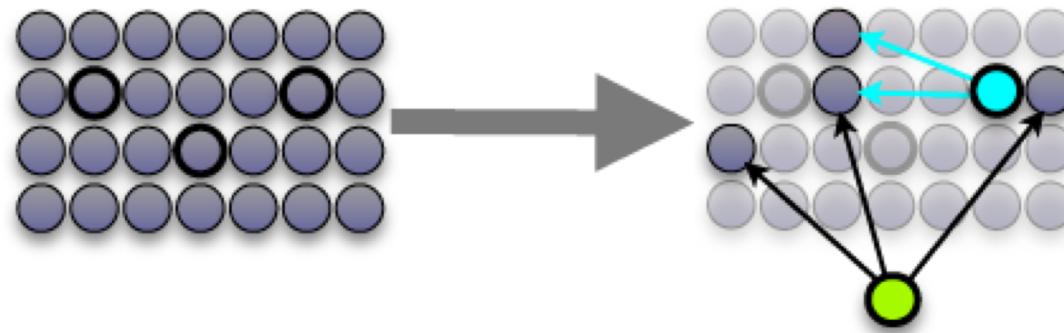
We are using iRegulon
to answer 2 questions:



- 1) Find the targets of a transcription factor of interest
 - Advantages: Targets are displayed as a network, we can overlay extra information of the created network like node colors.

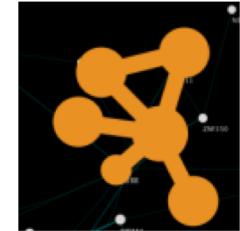


- 2) Given a set of genes, found if these genes are co-regulated by some transcription factors listed in the iRegulon database.

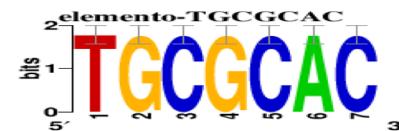




iRegulon uses:



- Position Weight Matrix (PWM) (+transcription factor predicted binding site)



and

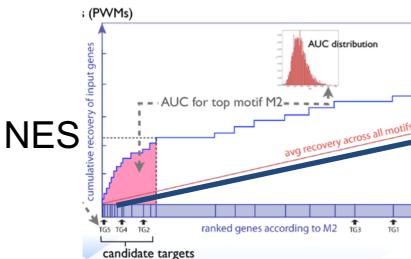
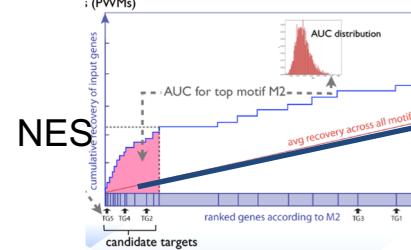
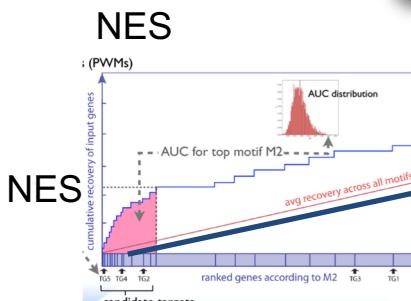
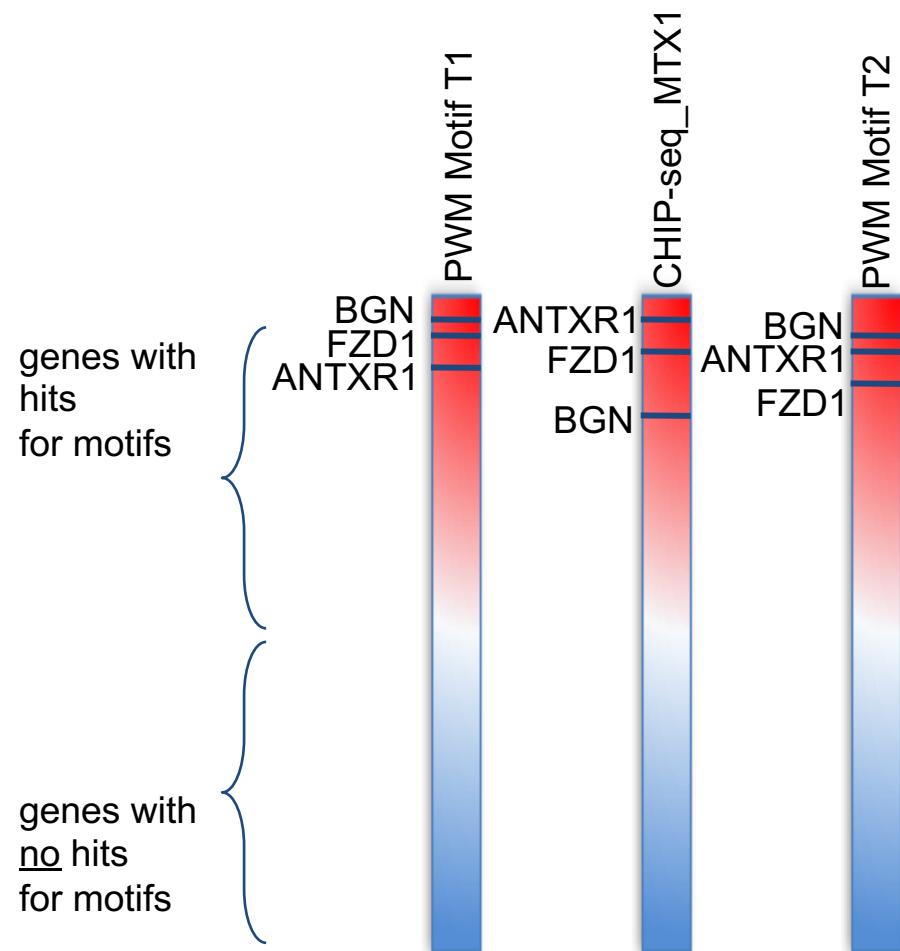
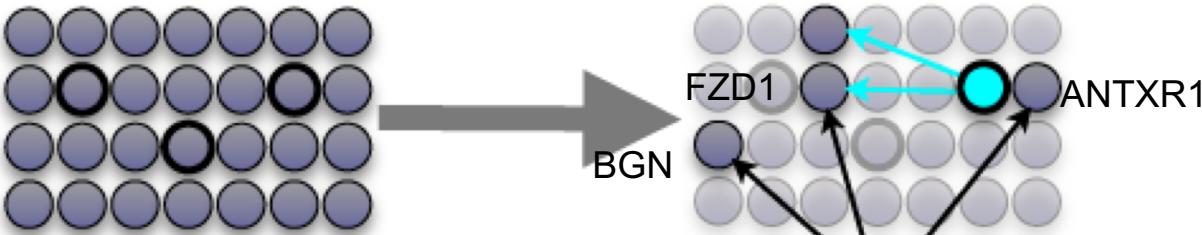
- CHIP-Seq data (immunoprecipitation of chromating using an antibody specific to a TF of interest + DNA sequencing)

To estimate transcription factor binding on promoter of genes of interest

Description of the motif and track collections used.

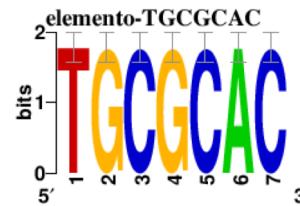
Source	Organism(s)	Type of motif	# motifs "6K"	# motifs "10K"	# tracks "1K ChIP"
Elemento [73]	Drosophila	Predicted (conserved) ^a	371	371	-
FlyFactorSurvey [75]	Drosophila	B-1H, others (e.g., FlyReg)	614	652	-
hPDI [77]	Human	Experimental	437	437	-
Jaspar [21]	Multiple species	Curated	1315	1315	-
SelexConsensus [76]	Drosophila	Curated (FlyReg)	38	38	-
Stark [74]	Drosophila	Predicted (conserved) ^a	228	228	-
Tiffin [76]	Drosophila	Predicted (gene sets) ^a	120	120	-
TRANSFAC PUBLIC [5]	Multiple species	Curated, ChIP-chip	398	398	-
TRANSFAC PRO [5]	Multiple species	Curated, ChIP-chip	1153	1850	-
YetFasco [78]	Yeast	Uniprobe, Curated, ChIP-chip	1709	1709	-
ENCODE [79]	Human	Predicted (from DHS) ^a	-	683	-
Factorbook [46]	Human	ENCODE ChIP-Seq motifs	-	79	-
Taipale [132]	Human, Mouse	HT-Selex	-	820	-
iDMMPPMM [133]	Human	footprints, Selex, b1h, peaks	-	39	-
SwissRegulon [134]	Human	Curated	-	190	-
Wolfe [135]	Drosophila	ZFP motifs	-	36	-
HOMER [116]	Multiple species	ChIP-Seq Motifs, others (e.g. ENCODE)	-	1865	-
Dimers [136]	Human	Predicted dimers	-	603	-
ENCODE ChIP-Seq [23]	Human	-	-	-	999
Taipale ChIP-Seq [24]	Human	-	-	-	117
p53 and control ChIP-Seq (this study)	Human	-	-	-	2
Total			6383	11611 (9713 nr)	1118

How does iRegulon find if a set of co-expressed genes is the target of a TF?

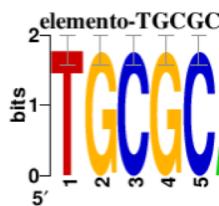


From Motifs, Tracks to Transcription Factor

Motif2Fmapping

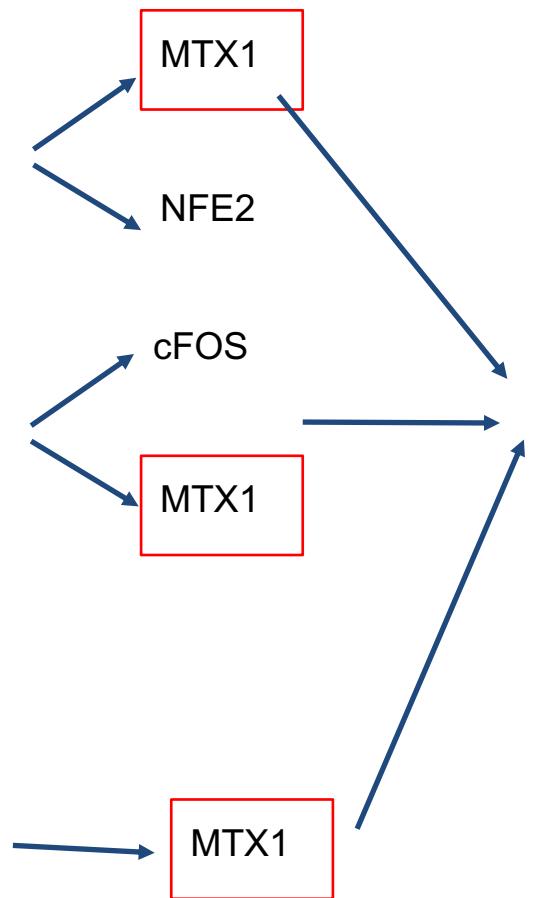


PWM Motif T1



PWM Motif T2

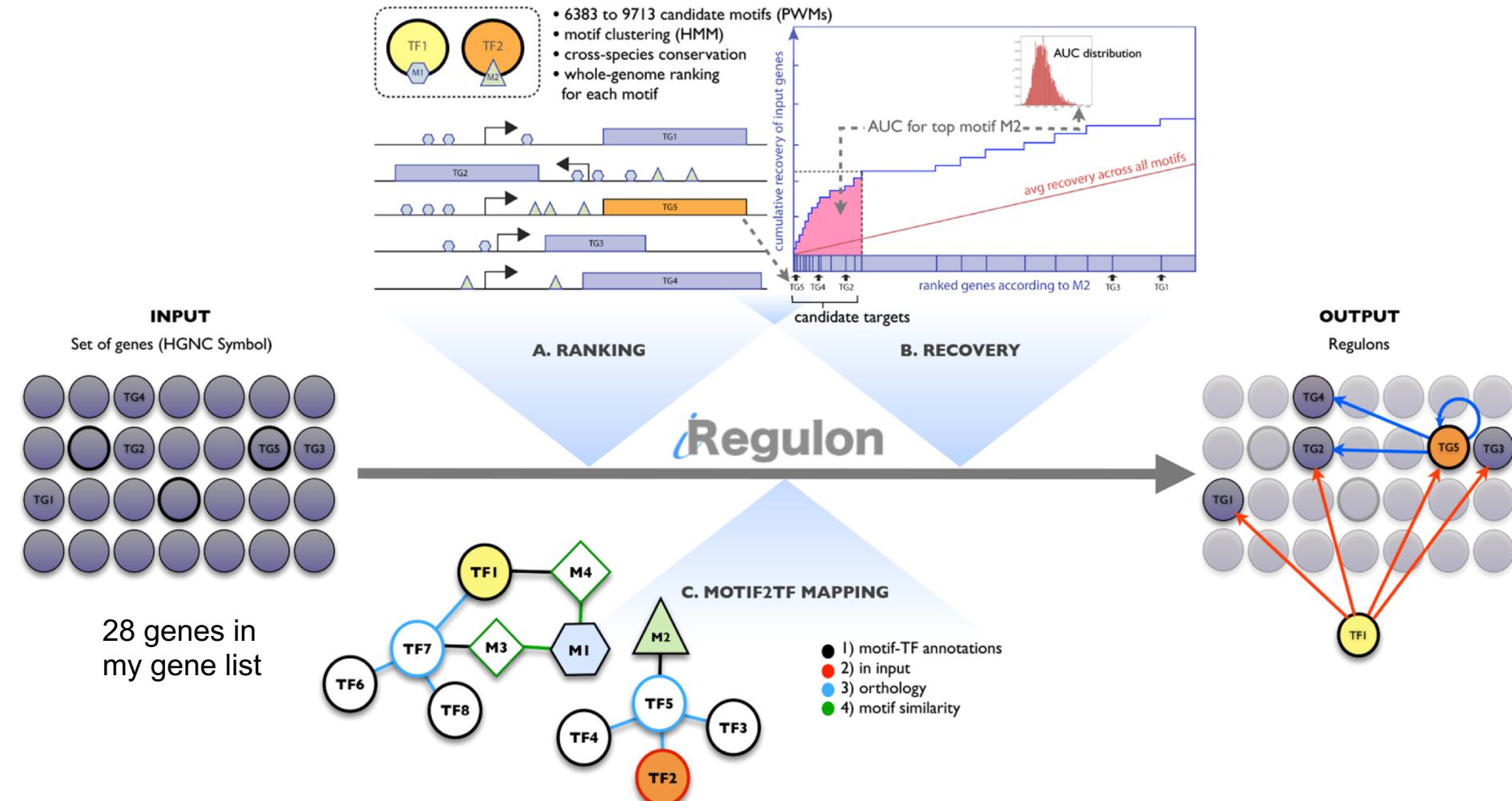
TRACK CHIP-
seq_MTX1



Predicted TF
coregulating
our list of genes
would be

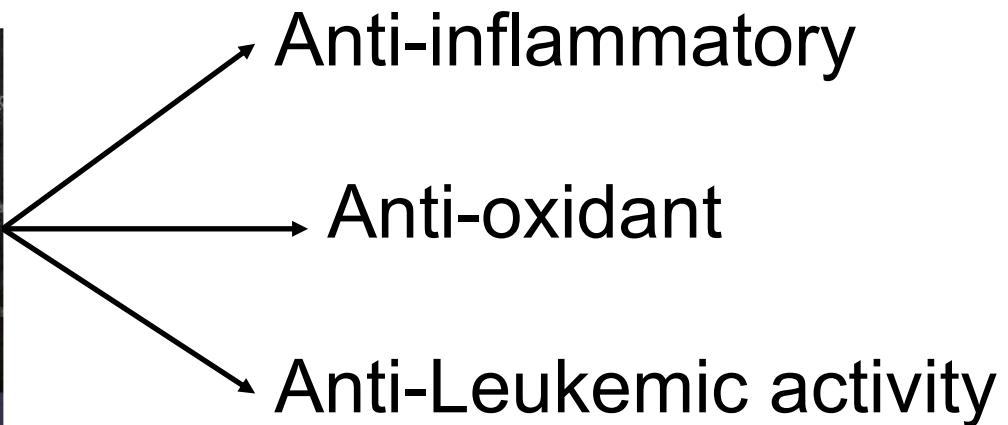
MTX1

Motif2Fmapping



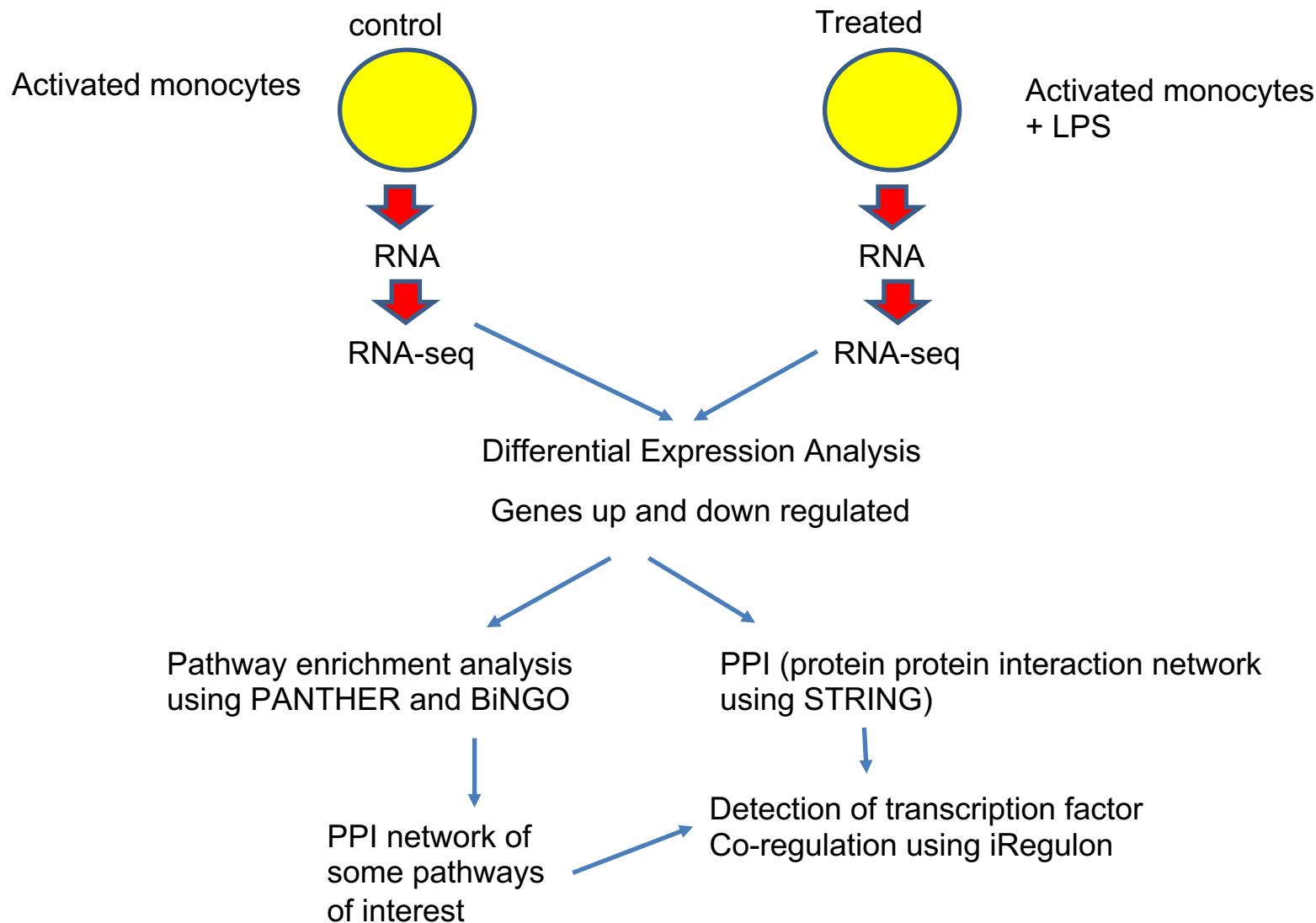
- Example of a published paper using iRegulon

Resveratrol decreases the expression of genes involved in inflammation through transcriptional regulation

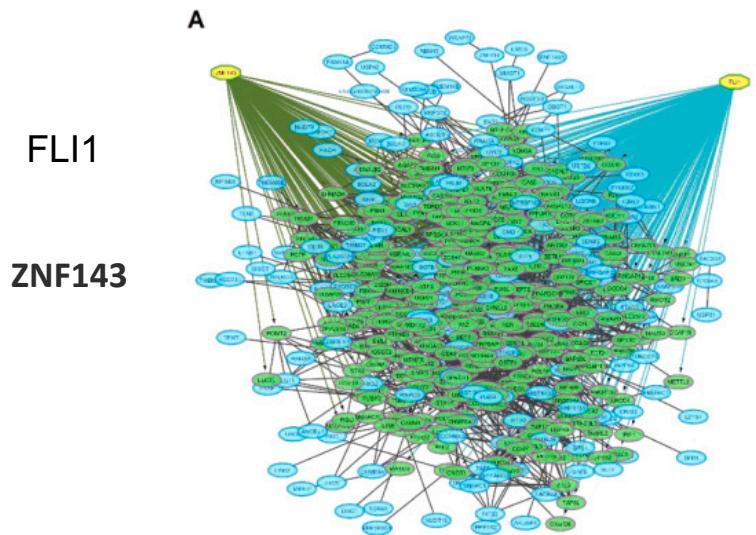


<https://www.sciencedirect.com/science/article/pii/S0891584918314886?via%3Dhub>

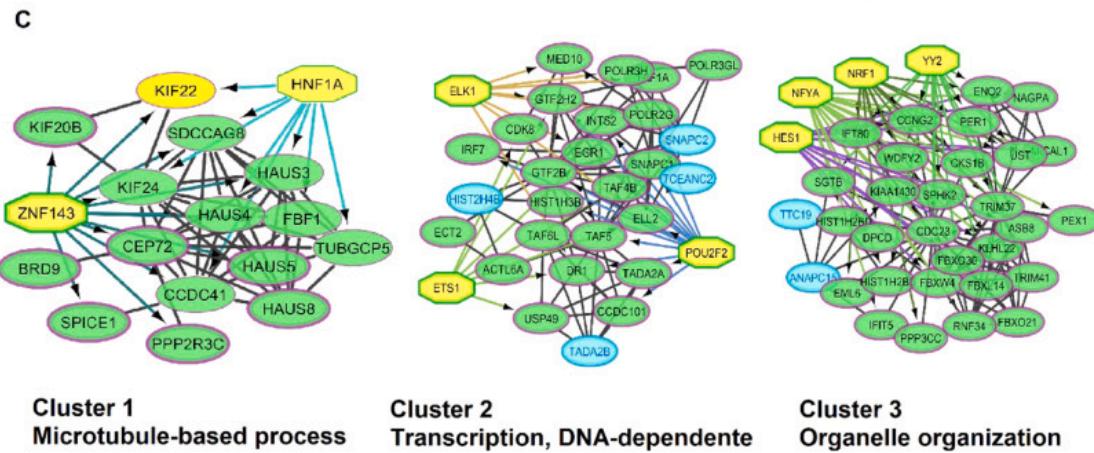
Resveratrol decreases the expression of genes involved in inflammation through transcriptional regulation



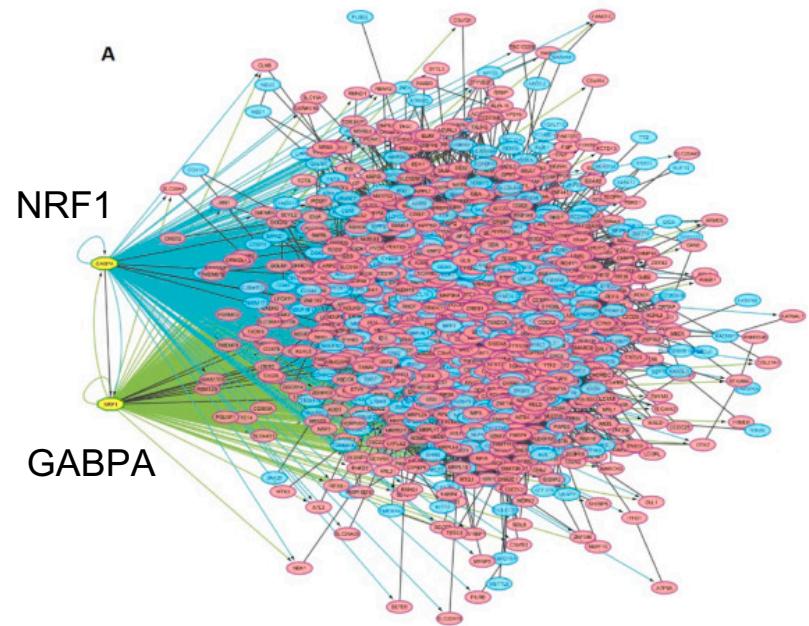
Resveratrol decreases the expression of genes involved in inflammation through transcriptional regulation



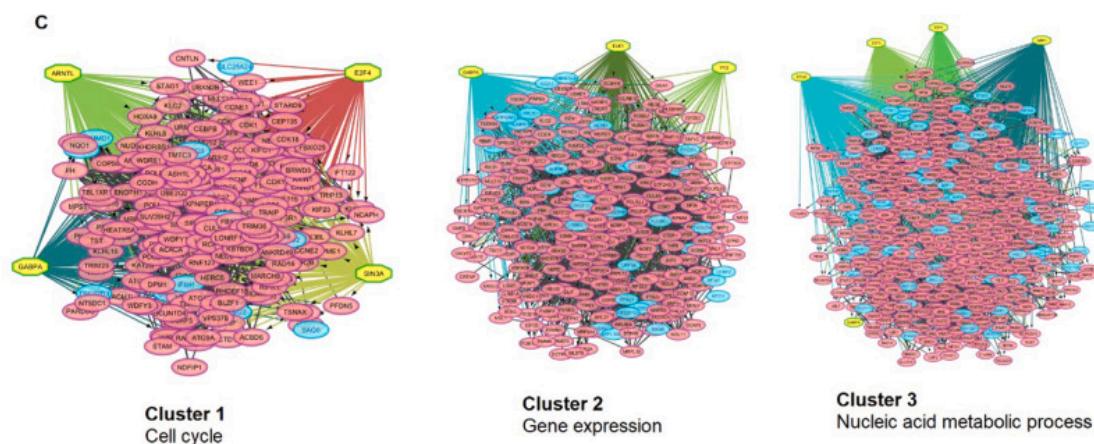
iRegulon on
GENES UP
REGULATED



Resveratrol decreases the expression of genes involved in inflammation through transcriptional regulation

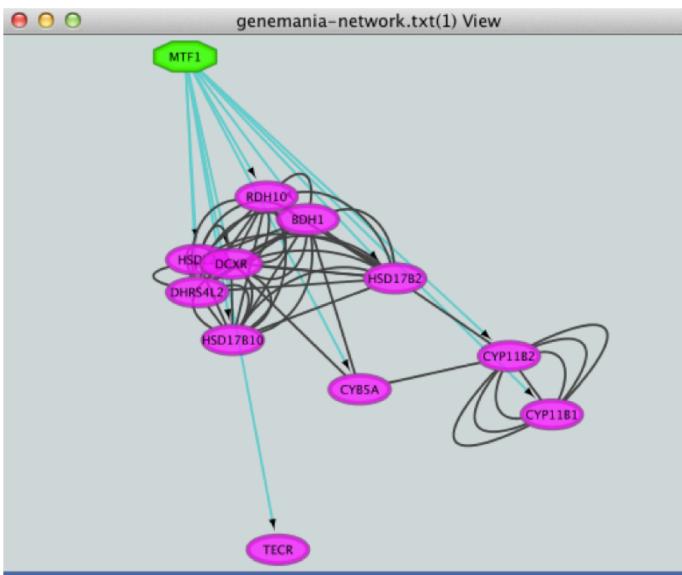


iRegulon on
GENES
DOWN
REGULATED

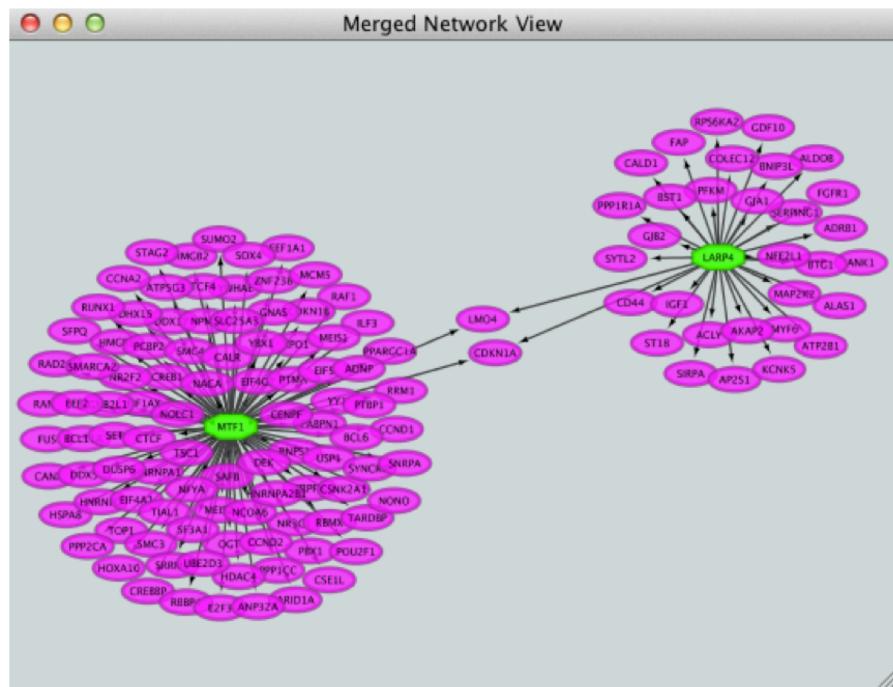
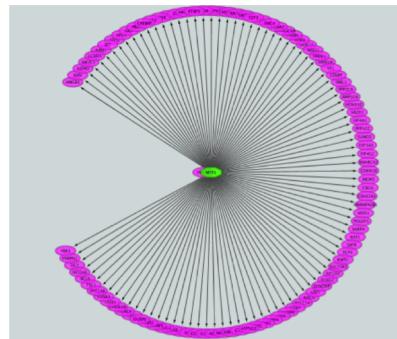


The prediction of master regulators using the iRegulon tool showed **nuclear respiratory factor 1 (NRF1)** and **GA-binding protein alpha subunit (GABPA)** as the main regulators of the downregulated genes

Exercise 1. Detect regulons from co-expressed genes



Exercise 2. Create a metatargetome using iRegulon and merge 2 networks in Cytoscape.





Enrichr

- Input can be a gene list or a bed file

Note: a bed file contains chromosome coordinates of the peaks identified for example in ATACseq or ChipSeq experiments. Consider filtering peaks in regions proximal to associated genes like in promoter regions.

Input data

Choose an input file to upload. Either in BED format or a list of genes.

Try an example [BED file](#).

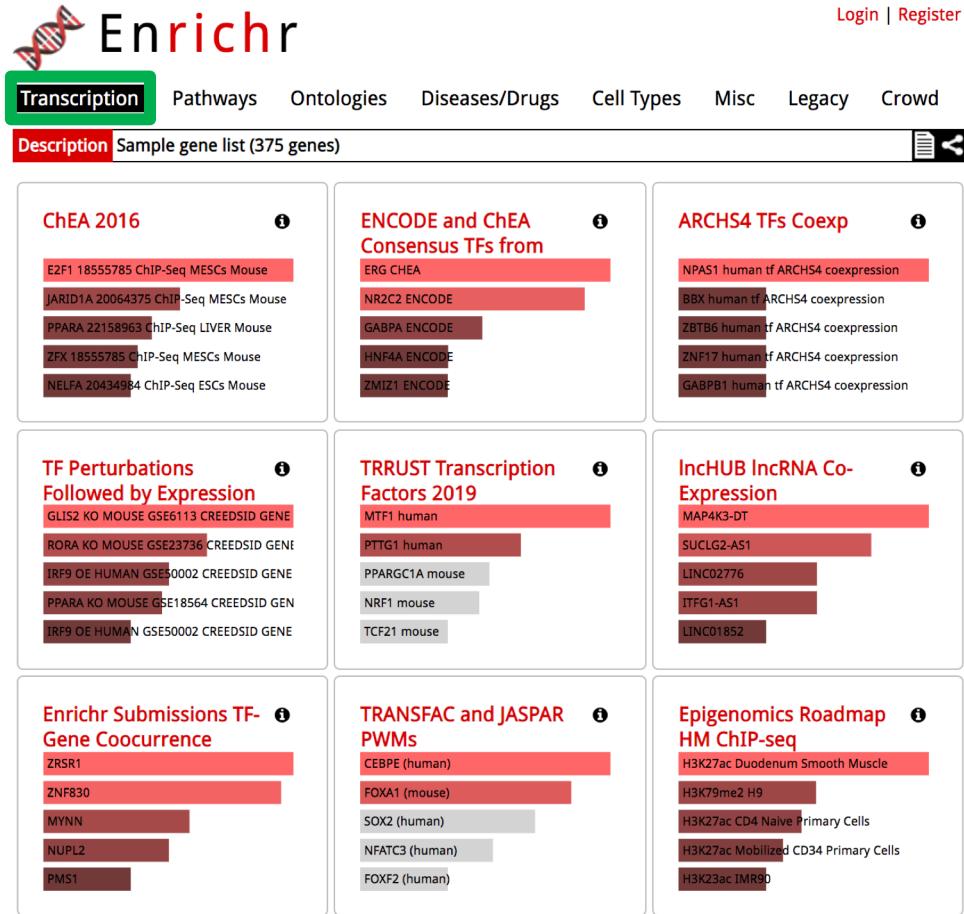
No file chosen

Paste a list of valid Entrez gene symbols on each row in the text-box below. [Try a gene set example](#).

0 gene(s) entered



- Test: Fisher's exact test, with a pvalue corrected for multiple hypothesis testing
- Output: a downloadable table or graphs
- No option to put a reference background: use only if you are doing a whole genome study!
- Enrichr has a vast variety of gene-set sources.
- In this practical lab, we are only going to focus on “**Transcription**”.
- Possibility to download a result of interest and create an enrichment map in Cytoscape using the Generic/gProfiler/Enrichr format



Enrichr output table

Fisher's exact test

Term	Overlap	P-value	Adjusted P-value	Old P-value	Old Adjusted	Odds Ratio	Combined Score	Genes
NR5A1 human	8/22	1.86E-16	1.06E-13	0	0	151.515152	5488.395484	HSD3B2;STAR;CYP11A1;CYP11B2;CYP11B1;HSD3B1;CYP19A1;CYP17A1
NR5A2 human	5/12	5.02E-11	1.43E-08	0	0	173.611111	4117.076135	HSD3B2;STAR;CYP11A1;CYP11B1;CYP19A1
NR5A1 mouse	5/23	2.09E-09	3.98E-07	0	0	90.5797101	1810.220371	STAR;CYP11A1;CYP11B2;CYP19A1;CYP17A1
NR4A1 human	4/15	3.91E-08	5.58E-06	0	0	111.111111	1895.289459	HSD3B2;STAR;CYP11A1;CYP17A1
CREB1 human	6/90	7.39E-08	8.44E-06	0	0	27.7777778	456.1178554	AR;CYP11A1;CYP11B2;CYP11B1;CYP19A1;CYP17A1
NR0B1 human	3/10	1.54E-06	1.46E-04	0	0	125	1673.092298	STAR;CYP19A1;CYP17A1
NR0B1 mouse	3/11	2.11E-06	1.72E-04	0	0	113.636364	1484.996478	STAR;CYP11A1;CYP19A1
SF1 human	3/12	2.81E-06	2.01E-04	0	0	104.166667	1331.455379	STAR;HSD3B2;CYP11A1
SF1 mouse	3/15	5.78E-06	3.67E-04	0	0	83.3333333	1005.029762	STAR;CYP11A1;CYP17A1
SP1 human	8/472	1.49E-05	8.53E-04	0	0	7.06214689	78.4727961	AR;STAR;HSD17B1;HSD17B2;HSD3B1;HSD17B11;CYP19A1;CYP17A1
NR2F1 human	2/9	2.01E-04	0.010427214	0	0	92.5925926	788.2250101	CYP19A1;CYP17A1
GATA6 human	2/9	2.01E-04	0.009558279	0	0	92.5925926	788.2250101	CYP11A1;CYP17A1
NR4A1 mouse	2/10	2.51E-04	0.011011909	0	0	83.3333333	690.9348177	AR;HSD3B1
JUN human	4/149	4.46E-04	0.018198668	0	0	11.1856823	86.2946171	AR;STAR;CYP11A1;CYP19A1

gene-set
(pathway)

Overlap:
Numerator -> # of genes in my gene list and tested gene-set

Denominator ->
of genes in the original gene-set

FDR:
Correction for multiple hypothesis testing

List of genes in the overlap

We are on a Coffee Break & Networking Session

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