Integrative Network Analysis using R and RCytoscape

Ignatius Pang, Apurv Goel, Marc Wilkins

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Sydney Users of R (SURF) Meetup
SMSA Townhall

i.pang (at) unsw.edu.au

UNSW AUSTRALIA

SYSTEMS BIOLOGY INITIATIVE

https://github.com/IgnatiusPang/Network_SURF

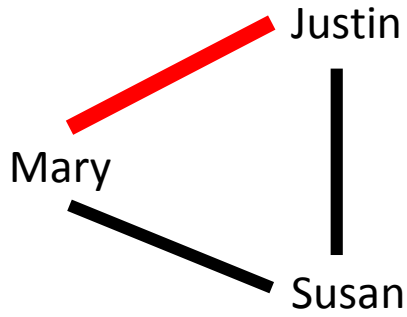
Outline

- Why is network being used?
- R and RCytoscape
- What are negative genetic interactions?
- Applications of negative genetic interactions
- Investigating the network basis of genetic interactions
- Results & Code Examples
- Summary

https://github.com/IgnatiusPang/Network_SURF

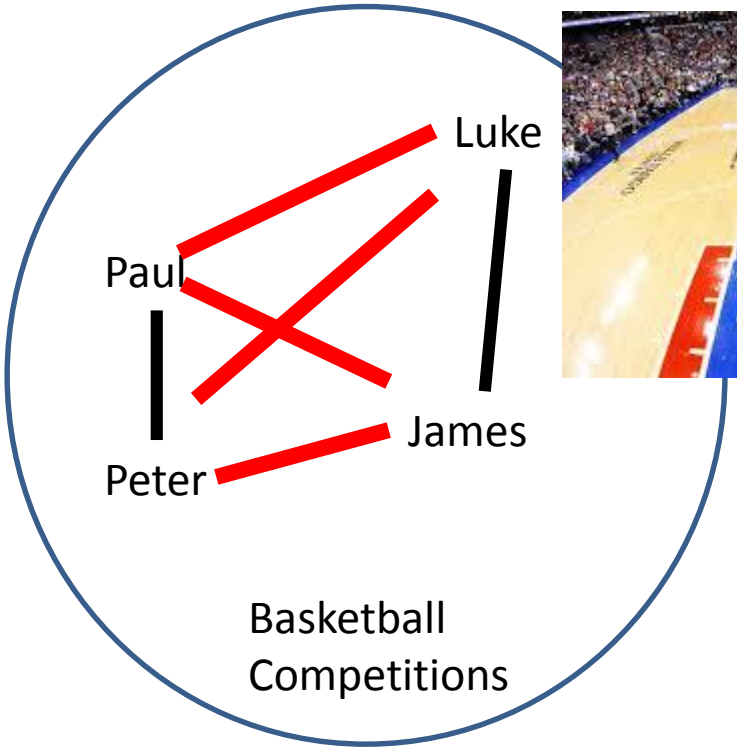
Where is network being used?

- Friends recommendation on facebook



Friends of Friends

Red = relationships predicted/suggested by Facebook



Same Social Group

Also suggest advertisement for Basketball match tickets / memorabilia

https://github.com/IgnatiusPang/Network_SURF

Modelling the Spread of Viruses in an Epidemic

EPIDEMIC RAPID TRANSIT MAP

http://www.mobs-lab.org/epi-rail.html

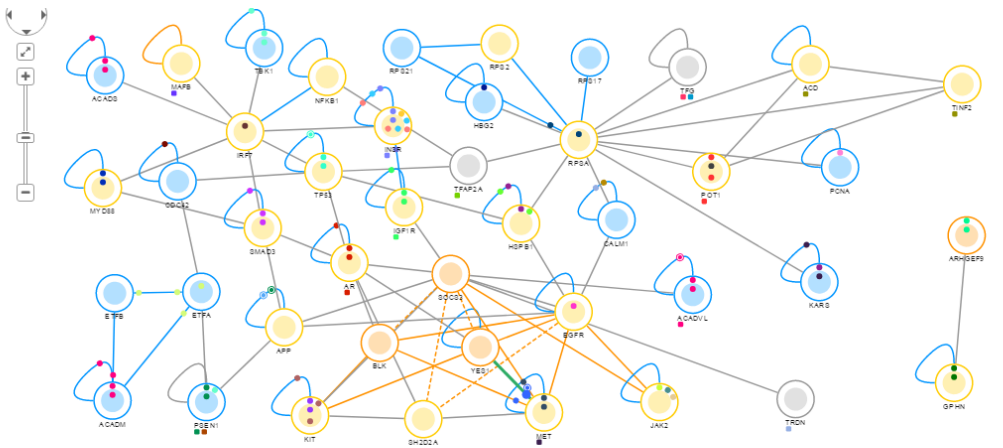
https://github.com/IgnatiusPang/Network_SURF

dsysmap

Help

Network of disease related mutations for proteins ACADM, ACADS, ACADVL, ETFA, ETFB... [43 proteins and 98 interactions]

Reload the network including all neighbors

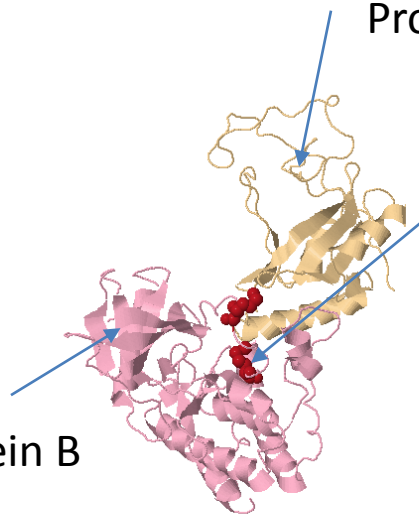


Diseases Legend Options

Protein A

Protein B

Red dots = mutation at protein-protein interaction surface



Mutated residues >> Buried Surface Interface

Acyl-CoA dehydrogenase deficiency

An autosomal recessive metabolic disorder associated with mitochondrial complex I deficiency, resulting in multisystemic and variable manifestations. Clinical features include infantile onset of acute metabolic acidosis, Reye-like episodes (brain edema and vomiting that may rapidly progress to seizures, coma and death), exercise intolerance, hypertrophic cardiomyopathy, liver failure, muscle weakness, and neurologic dysfunction.

Phenotype	Protein		Mutations (Buried, Surf, Int., Not Classified)
Acyl-CoA dehydrogenase medium-chain deficiency	ACADM	Medium-chain specific acyl-CoA dehydrogenase... P11310	I78T C116Y M149I G195R C244R M326T Y67H T121I T193A R206L S245L G267R R281T R53C G310R K329E S336R Y352C I375T
Acyl-CoA dehydrogenase short-chain deficiency	ACADS	Short-chain specific acyl-CoA dehydrogenase, m... P16219	S353L R46W G90S G92C R107C W177R A192V R325W R380W R383C
Acyl-CoA dehydrogenase very long-chain deficiency	ACADVL	Very long-chain specific acyl-CoA dehydrogenas... P49748	Q159R V174M G185S A213P L243R K247E K247T T260M A281D V283A V317A M352V G441D F458L G290D G294E D405H R450H

SOCS3 :: O14543 **MET :: P08581**

Source	Detection method	Publication	Ext. Ref.
Intact	Fluorescence polarization spe...	24728074	EBI-9473568

Structure	Protein	Interface mutations
View Model from 4gl9_H-B	MET	K1244R M1250I M1250T

Protein	Templ.	Seq. id.	Cov
SOCS3	4gl9_H	76.7%	72.4% (22-184)
MET	4gl9_B	35%	18.9% (1078-1340)

Download Interactome3D

Disease	Protein	Interface mutations
Hepatocellular carcinoma	MET	K1244R M1250I
Renal cell carcinoma papillary	MET	M1250T

http://dsysmap.irbbarcelona.org/

https://github.com/IgnatiusPang/Network_SURF

Why would you want to drive Cytoscape using R scripts?

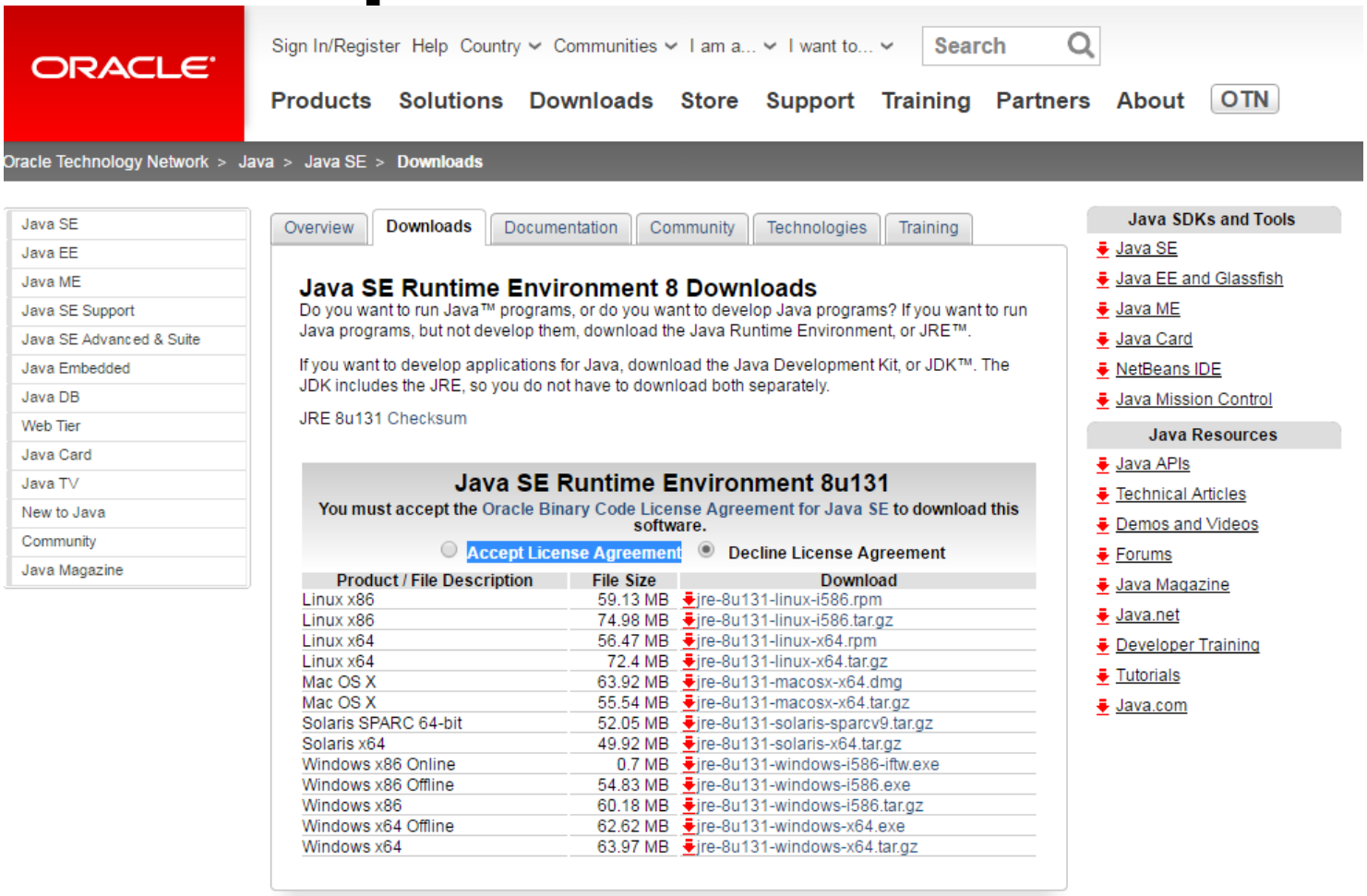
- Animate your network, make the network change dynamically, visualize time series data

- Support reproducibility, sharing of methods via code
- Another way of developing applications, without the difficulty of developing Cytoscape apps in Java

- No need to click buttons

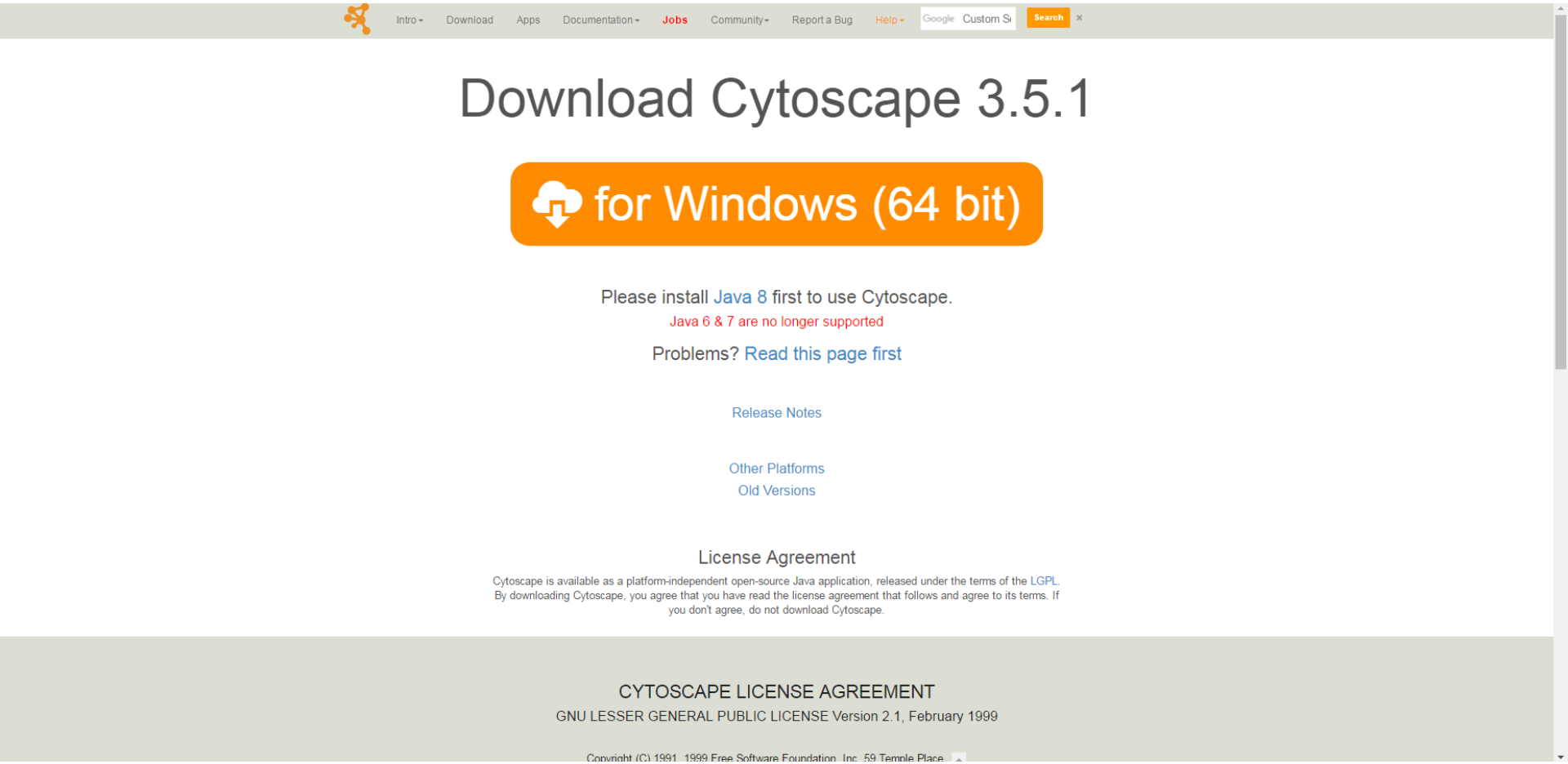
Installation Guide – How to get R and Cytoscape to talk to each other?

Step 1: Install Java 8



- http://www.oracle.com/technetwork/java/javase/downloads/jre8-downloads-2133155.html

Step 2: Install Cytoscape



- http://www.cytoscape.org/download.php

Step 3: Understand what the Cytoscape app CyREST is for?

- **A Cytoscape Core app for driving Cytoscape from R/Python/Julia/Node.js/etc.**

- **IMPORTANT: Now this app is a part of the Cytoscape Core!**

- **Ono, Keiichiro, et al. "CyREST: Turbocharging Cytoscape Access for External Tools via a RESTful API." F1000Research 4 (2015).**

Step 4: Rstudio and libraries

- Install R and/or Rstudio
- Install igraph
- install.packages("igraph")

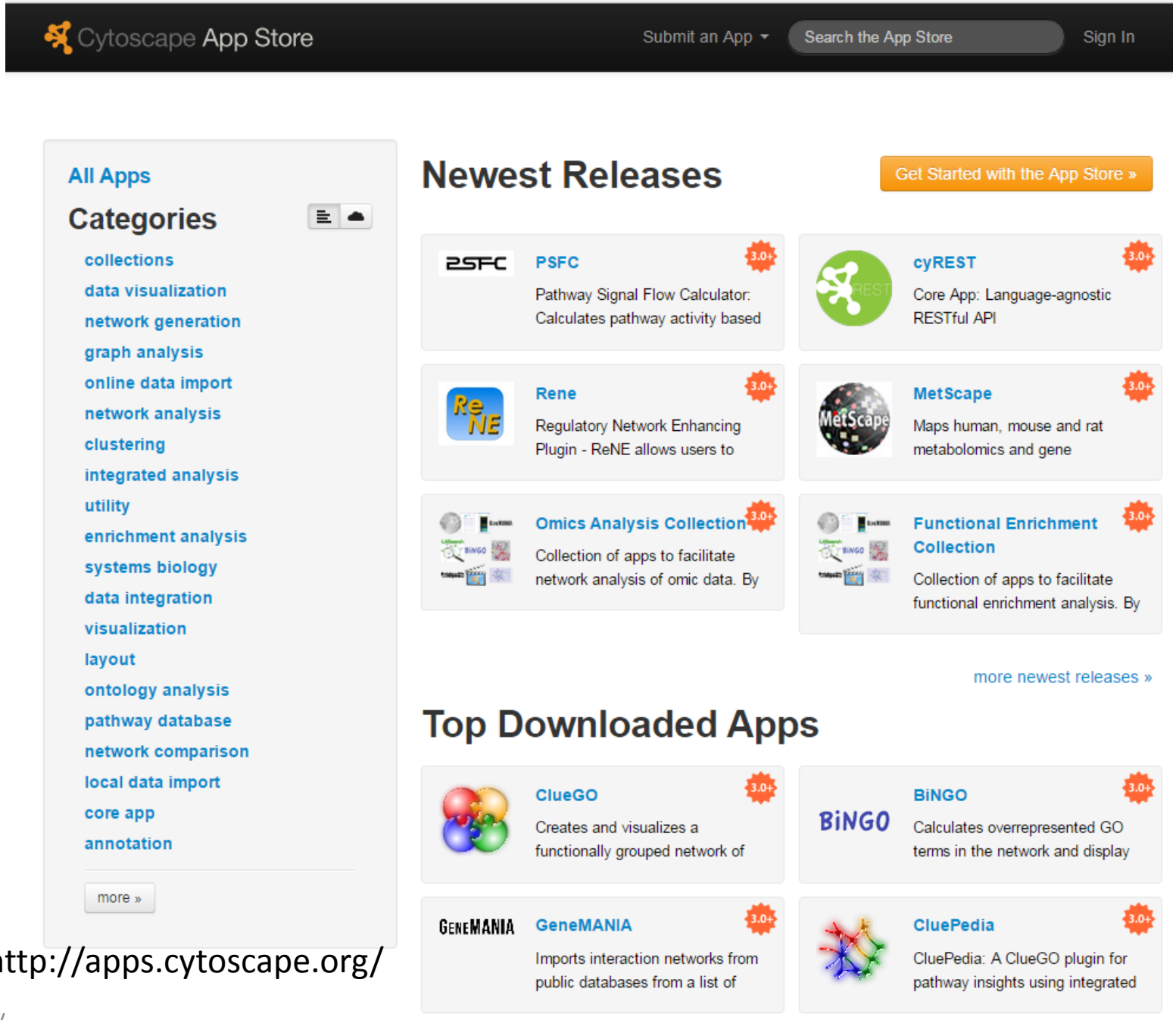
- Install Bioconductor download manager (lite version)
- source("https://bioconductor.org/biocLite.R")

- Install RCy3 in R (which connects R to Cytoscape, R side application).
- biocLite("RCy3") # previously called RCytoscape

Step 5: Running R and Cytoscape together

- In the same desktop environment
- Open Rstudio and load the following packages:
- library (igraph)
- library(RCy3)

- Open Cytoscape and have it side by side. CyREST is automatically loaded once you start Cytoscape. Now you're ready to draw some networks!



http://apps.cytoscape.org/

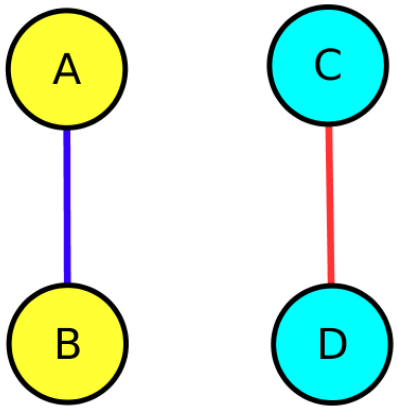
Code example 1 – Drawing a triplet in Cytoscape

Notes:

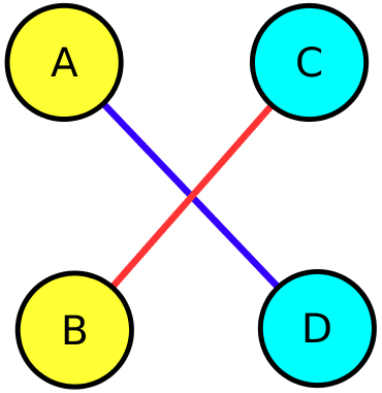
- Please refer to the script ‘draw_a_single_triplet.Rmd’ in the Github page.
- I've used the Bioconductor RCy3 vignette extensively for this example. Here is the reference : https://bioconductor.org/packages/release/bioc/vignettes/RCy3/inst/doc/RCy3.R

Generating Randomized Networks but Keeping the Degree Distribution

Before Edge Swap



After Edge Swap



- The node 'degree' is the number of interaction partners each node has
- Establishing the background level of triplets
- The number of times the edges were swapped was equal to the size of the network.
- Each type of biological network (e.g. protein-protein interaction network) was randomized independently of other types of networks.
- These were combined to form the randomized integrated network.

https://github.com/IgnatiusPang/Network_SURF

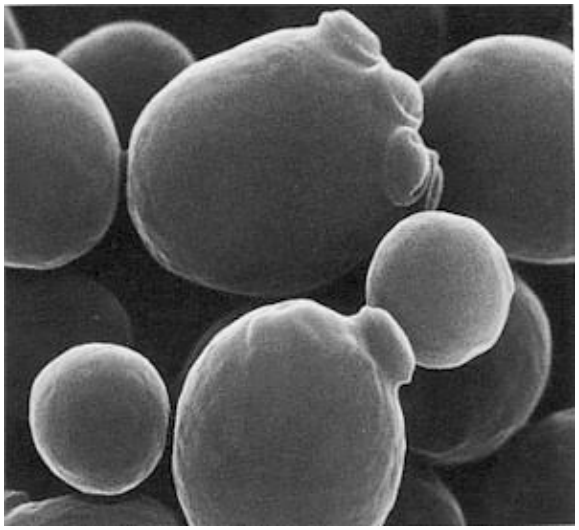
Code example 2 – Performing Network Randomization

Notes:

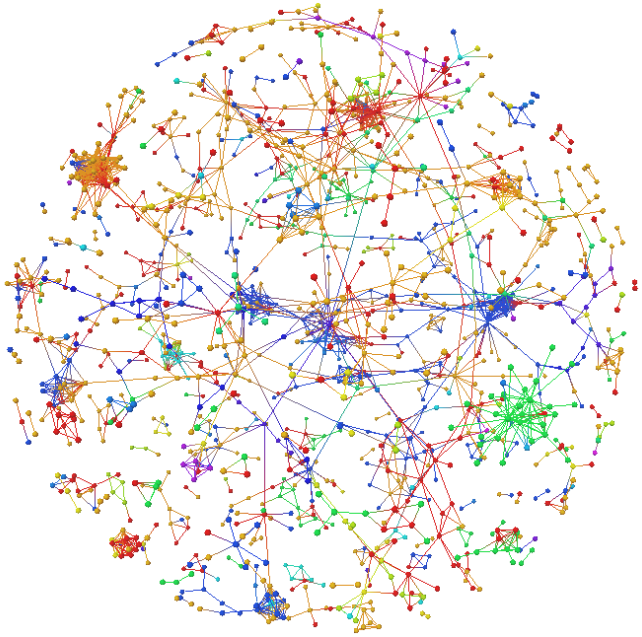
- Please refer to the script ‘rewire_network.Rmd’ in the Github page.
- This script show you how to perform network randomization, by edge swapping, while keeping the degree distribution the same. The number of interaction partners for every node should remain the same after this type of network randomization. The script also show you what happens if we randomize the network without conserving the degree distribution.

Saccharomyces cerevisiae

- **The world's most characterized eukaryote**
  - Vast genome, transcriptome and proteome resources
  - Knock out and over-expression mutants available for many genes
  - Databases for protein-protein, proteir DNA, protein-RNA interactions

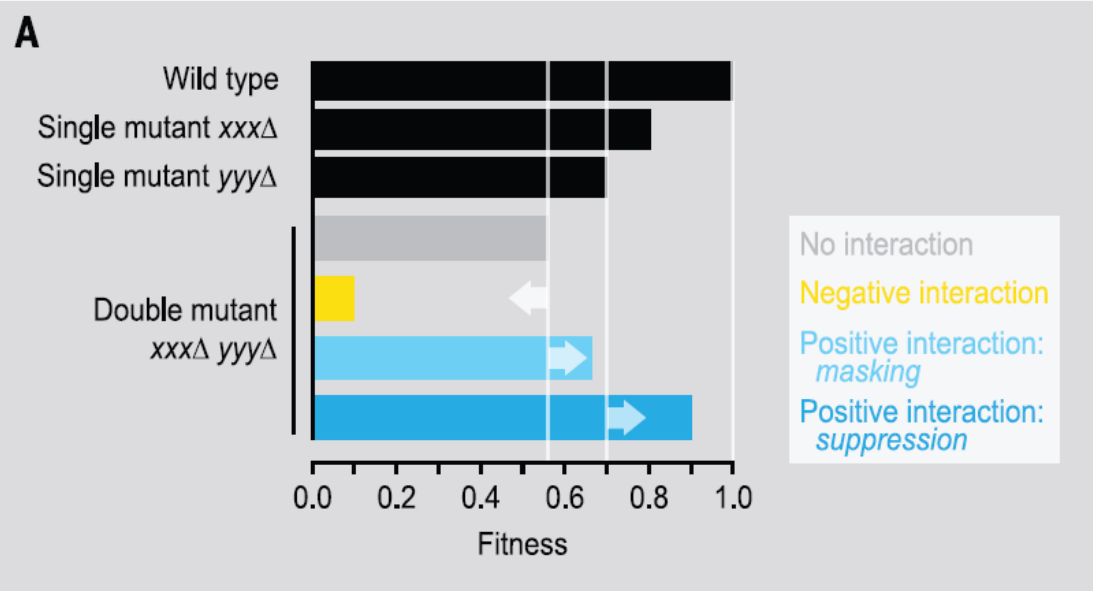


http://tinyurl.com/q36n6dh



→ Start with *Saccharomyces*
→ Testing grounds for algorithms
→ Map results to genes that are evolutionarily conserved in humans (e.g. essential genes, drug targets)

Genetic Interactions



Expected Score If there is No Interaction
= F(wild type) – F(*xxxΔ*) * F(*yyyΔ*)

van Leeuwen et al. (2016) *Science 354*(6312):aag0839

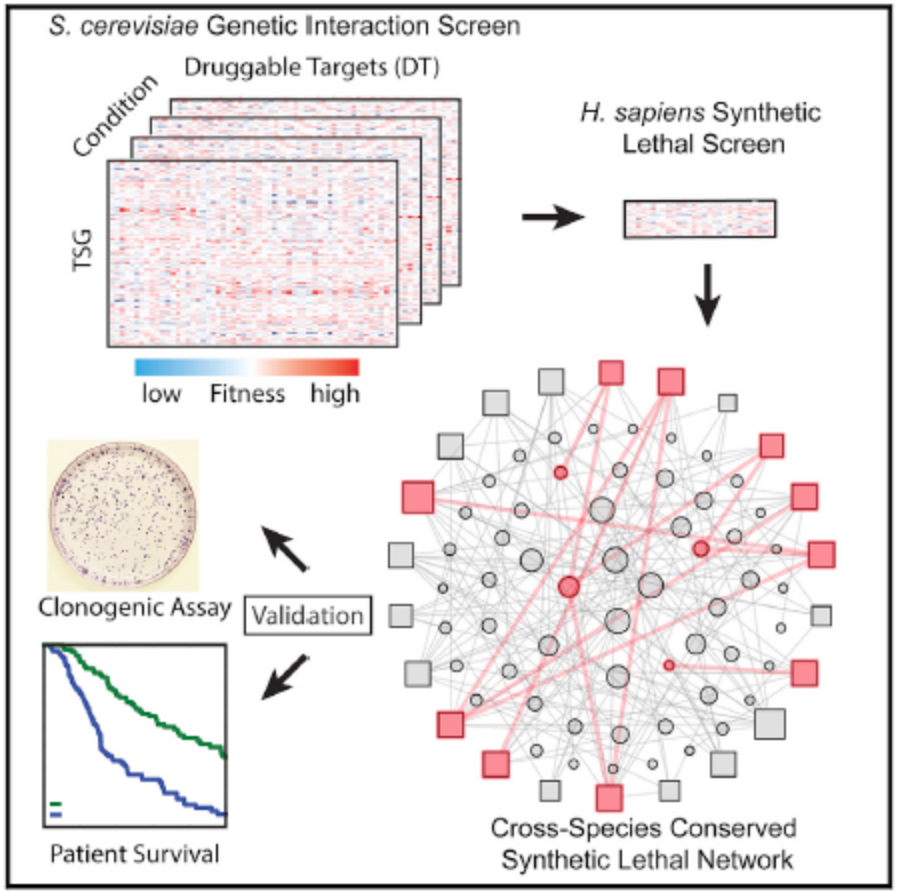
https://github.com/IgnatiusPang/Network_SURF

Resource

Molecular Cell

A Network of Conserved Synthetic Lethal Interactions for Exploration of Precision Cancer Therapy

Graphical Abstract



Authors

Rohith Srivas, John Paul Shen, Chih Cheng Yang, ..., Pedro Aza-Blanc, Robert W. Sobol, Trey Ideker

Correspondence

tideker@ucsd.edu

In Brief

An emerging strategy for precision cancer therapy is to induce synthetic lethality in a tumor based on its pattern of genetic mutations. Here we map a large network of conserved synthetic lethal interactions between selective drugs and tumor suppressor genes somatically mutated in human cancer. All of these interactions are mirrored in budding yeast, an accessible model for researching drug mode of action.

Srivas et al., 2016, Molecular Cell 63, 514–525
August 4, 2016
http://dx.doi.org/10.1016/j.molcel.2016.06.022

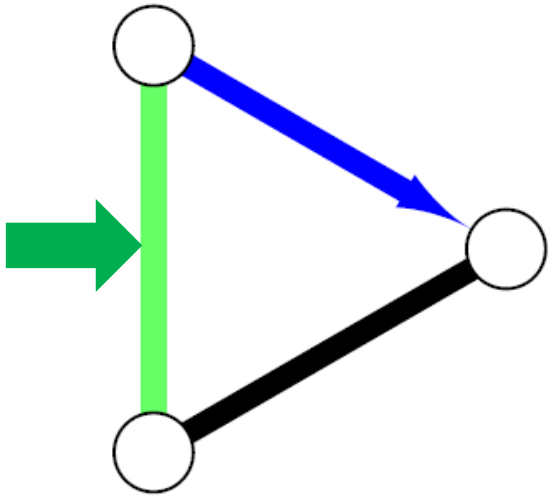
Applications

- Searching for Synergistic drug targets

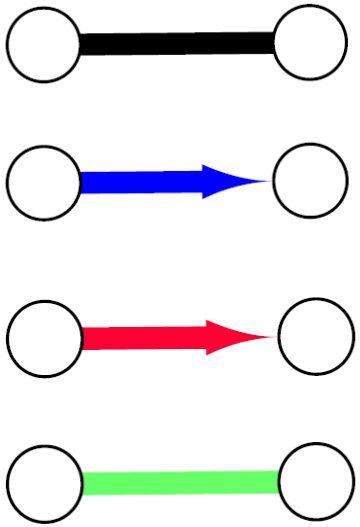
- One drug target Protein A
- Another drug target Protein B
- Their combined effect is greater than expected (i.e. kills cancer cells or microbes quicker)
- Limit the chance of drug resistance developing
- New drugs are hard to develop, use pairs of existing drugs

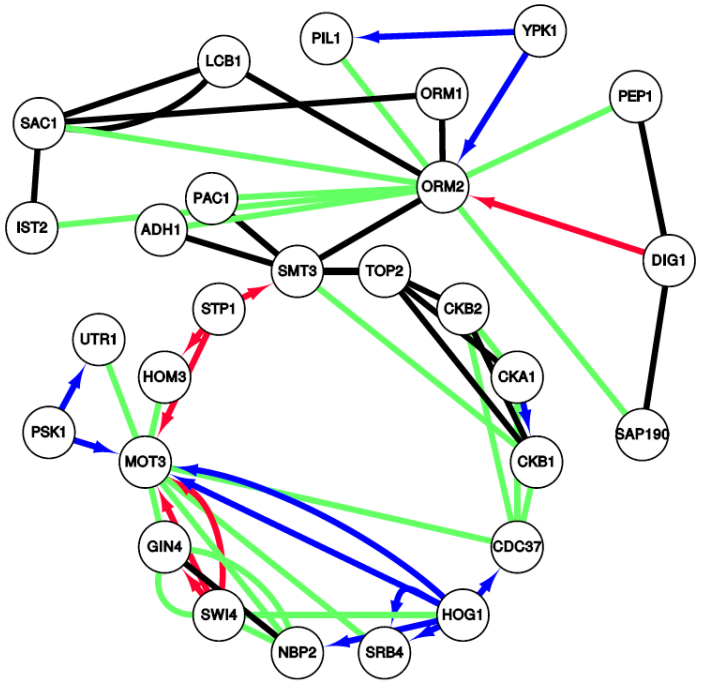
https://github.com/IgnatiusPang/Network_SURF

A triplet consists of one negative genetic interaction and two other interactions.



- **Protein-protein interactions**
- **Kinase-substrate interactions**
- **Transcription factor-target gene interactions**
- **Negative genetic interactions**

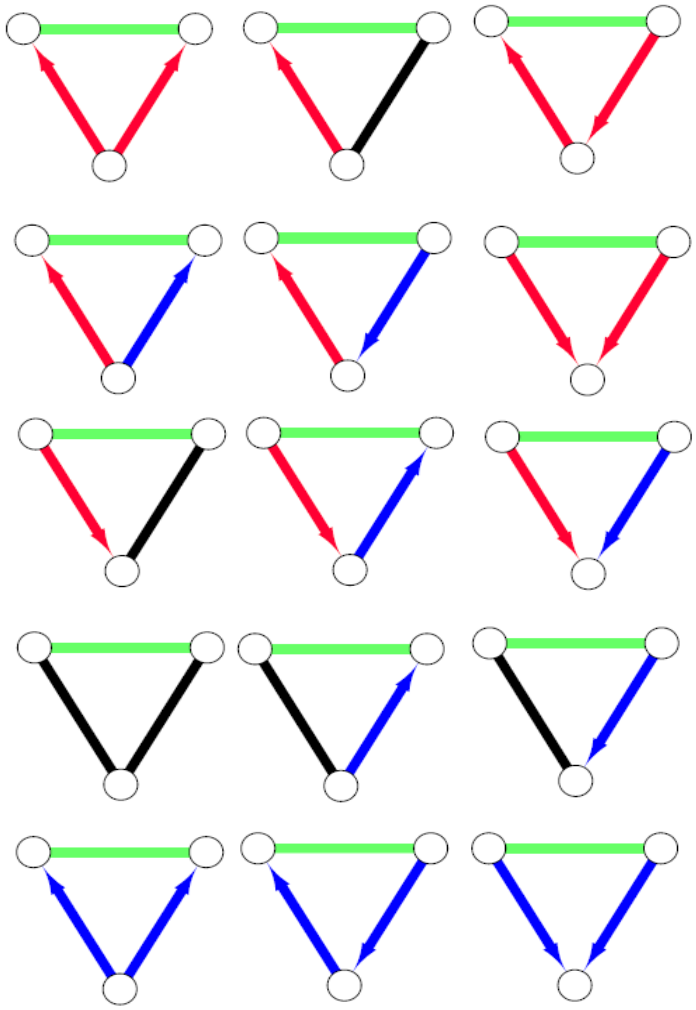




https://github.com/IgnatiusPang/Network_SURF

Identification of Triplet Motifs

- There are 15 possible types of motifs that could occur, from the different combinations of the edges.
- We have identified all 15 possible types of motifs, with 30,850 triplets, 3,293 proteins and 23,225 interactions in total.



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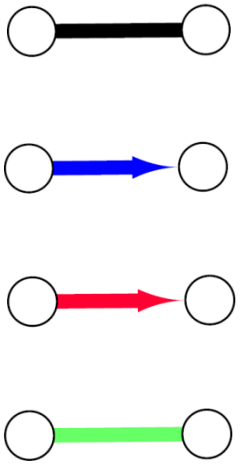


https://github.com/IgnatiusPang/Network_SURF

Edge list table - caveat

Node A	Node B
Adam	Xavier
Xavier	Adam

* The edges can be entered into the edge list table in two different directions. Sometimes you need to filter / clean the table so that you only have one of these entry (e.g. Node A string > Node B string, “Adam” is alphabetically before “Xavier” etc…). Other times you need to keep both entries. I’ve done these filtering several times in the script ‘form_triplet_motifs.Rmd’.

Code example 3 – Setting random number seeds and distributing tasks to multiple cores

Notes:

- Please refer to the script 'random_number_generator.Rmd' in the Github page.
- This script show you how to set the seed so that the results are replicable each time you run it. It also show you how to do this in a setting where you send the execution to multicores (in a Linux / Unix environment).
- I did not show this example in the actual talk, mainly due to time constraint, but it is included here as a useful resource.

Code example 4 – counting the number of each type of triplet motif

Notes:

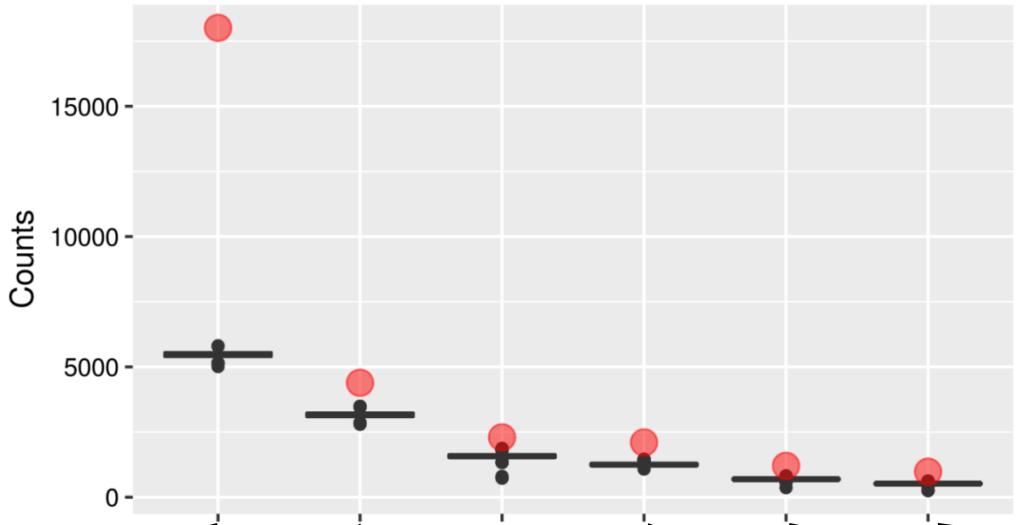
- Please refer to the script ‘form_triplet_motifs.Rmd’ in the Github page.
- It counts the number of times each type of triplet motif has been identified in the network.

Calculating the Significance of Triplet Motifs

- p-value calculated by the number of times 2000 randomized networks has triplet motif counts greater than counts of the same motif in the actual network
- The Bonferroni method was used for multiple testing correction.
- A triplet motif is significant if:
  - The adjusted p-value is < 0.05, and
  - Triplet motifs with observed counts less 2% of the total number of triplets is deemed un-reliable under a 2% false discovery rate.

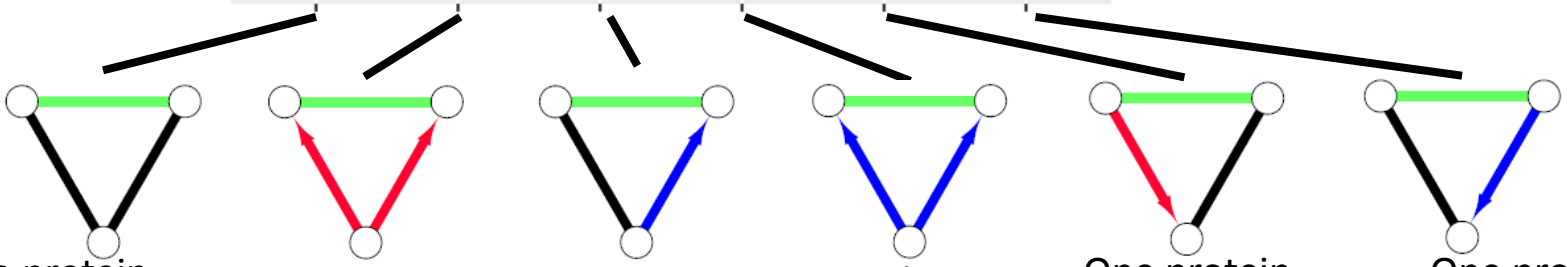
https://github.com/IgnatiusPang/Network_SURF

Overrepresentation of 6 out of 15 Types of Triplet Motifs



Observed counts of one type of triplet motif

Counts among 2,000 randomized networks



Two protein-protein interactions (*PP*)

Two transcription factor-target gene interactions (*TUTU*)

One protein-protein and one kinase-substrate interactions (*PKU*)

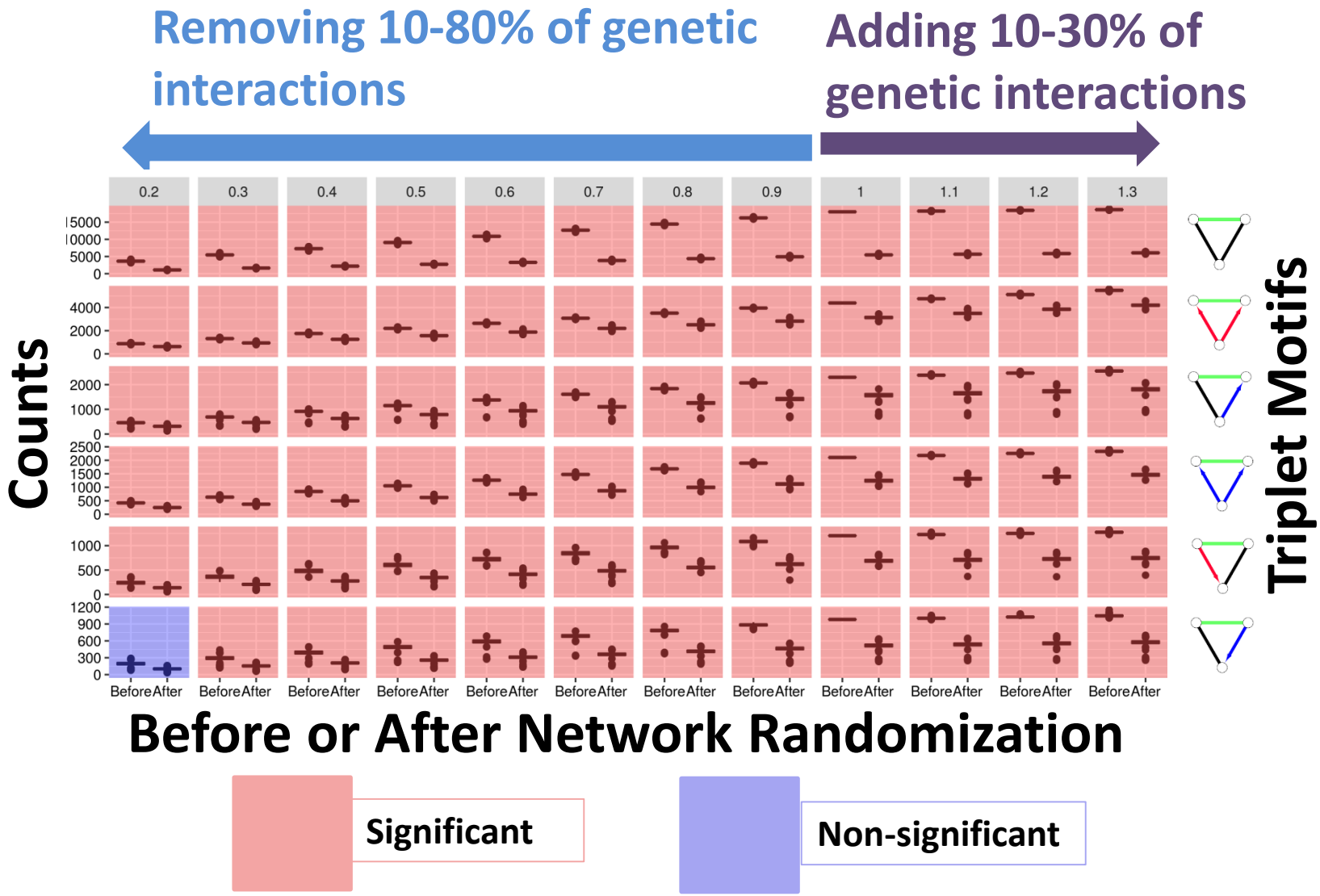
Two kinase-substrate interactions (*KUKU*)

One protein-protein and one transcription factor- target gene interactions (*TDP*)

One protein-protein and one kinase-substrate interactions (*PKD*)

P= protein-protein, *T*= transcription factor-target gene, *K*=kinase-substrate, *U*=up, *D*=down

Triplet Motifs are Robust



Known Limitations

- Network is only a reference map, static not dynamic
- Lacking information on whether interaction is present or absent in a specific condition
- Condition-specific interactions need to be tested for under various stresses, drug treatments, genetic backgrounds etc…
- False positive or false negative interactions

Summary

- Cytoscape can be used to visualize networks
- RCytoscape can be used to drive network analyses from R, providing a programmable workflow
- Network randomization can be used to generate many randomized networks. For these background set, we can calculate the 'null' or expected distribution. We can then compared our observed data with the expected distribution and infer the significance of the differences.

https://github.com/IgnatiusPang/Network_SURF

Resources and Databases

- Github page for today's talk
  - https://github.com/IgnatiusPang/Network_SURF

- Biological Networks Databases
  - https://thebiogrid.org/
  - http://string-db.org/
  - http://www.ebi.ac.uk/intact/

- Cytoscape, Rcytoscape, igraph
  - http://www.cytoscape.org
  - https://bioconductor.org/packages/release/bioc/html/RCy3.html
  - http://igraph.org/r/
  - Igraph tutorial: http://kateto.net/networks-r-igraph

References

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Acknowledgements

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