Manipulation of Gene Regulatory Network Dynamics Identifies Several Important Transcription Factors Involved in Regulating the Response to Cold Shock in *Saccharomyces Cerevisiae*

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Outline

- **Goal: To identify which transcription factors control the early response to cold shock**
- **The systems biology approach to studying cold shock**
- **Genes with significant change in gene expression identified through statistical analysis of DNA microarray data**
- **Profiles 9 and 45 chosen due to large sizes and clear cold shock/recovery patterns and used to generate gene regulatory networks**
  - **Profile 9: Changes in parameter specifications has significant effect on estimations of relative influence of transcription factors**
  - **Profile 45: Deletion of SWI5 and ACE2 identifies significant effects of ACE2 on optimized weight parameters**
- **Implications of experiment and future direction**

Yeast respond to cold shock by changing their gene expression

- *Saccharomyces cerevisiae* is an ideal model for systems biology.
- Yeast respond to environmental stresses (cold shock) by changing their gene expression.
- Cold shock is not well studied in comparison to heat shock.
- The response to cold shock can be divided into an early and late response.
- The complete set of transcription factors responsible for the early response remain unknown.

Goal: Identify which transcription factors control the early response to cold shock

- What are the relative levels of influence of these transcription factors?

- What are the indirect effects of other transcription factors in the gene regulatory network?

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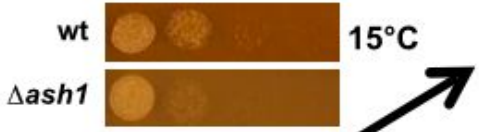
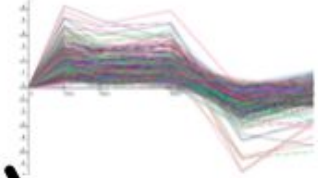
Systems Biology Approach to Understanding the Regulation of the Cold Shock Response in Yeast



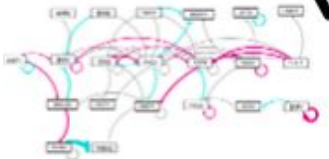
Cold shock microarray data from GLN3 deletion strains



Normalization, statistical analysis, clustering

Derivation of gene regulatory networks from YEASTRACT

Dynamical systems modeling using GRNmap



Visualization of modeling results using GRNsight

Interpretation, new questions, new experiments

Dahlquist, 2017

GRNmap Estimates Parameters With Least Squares Minimization

- Model similar to the version constructed by Vu and Vohradsky.
- Uses sigmoidal production function to model dynamics
- The main difference in models is the inclusion of a penalty term in the mean square error function.
- Penalized least squares is popular in genetics and genomics research where the number of potential predictor variables is very large.
- Inclusion of penalty term ensures there is always a unique solution for the parameters, different from OLS.

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WT S. cerevisiae displays higher percentage of genes with significant change in gene expression

ANOVA	*WT*	*dGLN3*
p < 0.05	*2528 genes, 40.8%*	*2088 genes, 33.74%*
p < 0.01	*1652 genes, 26.7%*	*1232 genes, 19.91%*
p < 0.001	*919, genes, 14.8%*	*594 genes, 9.60%*
p < 0.0001	*496 genes, 8%*	*236 genes, 3.81%*
B-H p < 0.05	*1822 genes, 29.4%*	*1231 genes, 19.89%*
Bonferroni p < 0.05	*248 genes, 4%*	*72 genes, 1.16%%*

Highest percentage of significant genes identified in t60

	Cold Shock			Recovery	
t test	t_{15}	t_{30}	t_{60}	t_{90}	t_{120}
Average Log Fold Change > 0.25 and p < 0.05	*1017 genes (16.43%)*	*1060 genes (17.13%)*	*1019 genes (16.46%)*	*681 genes (11.00%)*	*780 genes (12.60%)*
Average Log Fold Change < -0.25 and p < 0.05	*834 genes (13.48%)*	*862 genes (13.93%)*	*809 genes (23.00%)*	*903 genes (14.59%)*	*817 genes (13.20%)*
Total p < 0.05	*2088 genes (33.74%)*	*2088 genes (33.74%)*	*2088 genes (33.74%)*	*2088 genes (33.74%)*	*2088 genes (33.74%)*
Total B & H p < 0.05	*1231 genes (19.89%)*	*1231 genes (19.89%)*	*1231 genes (19.89%)*	*1231 genes (19.89%)*	*1231 genes (19.89%)*
Total Bonferroni p < 0.05	*72 genes (1.16%)*	*72 genes (1.16%)*	*72 genes (1.16%)*	*72 genes (1.16%)*	*72 genes (1.16%)*

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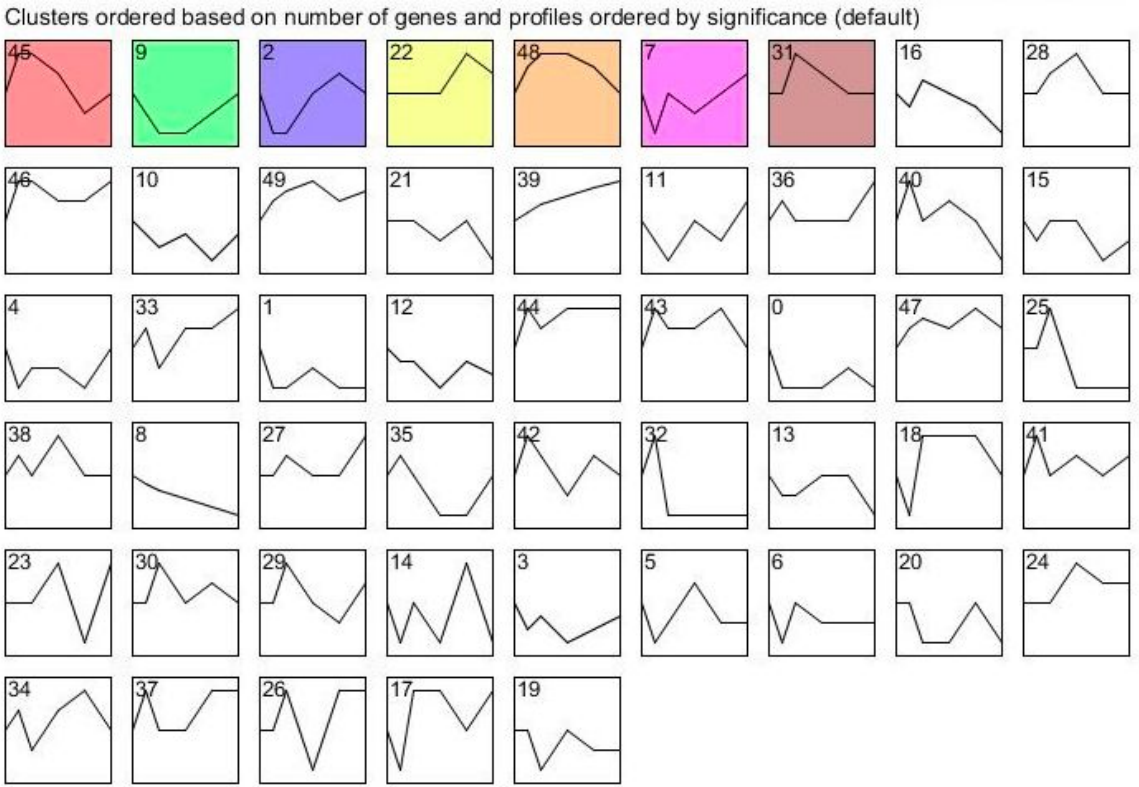
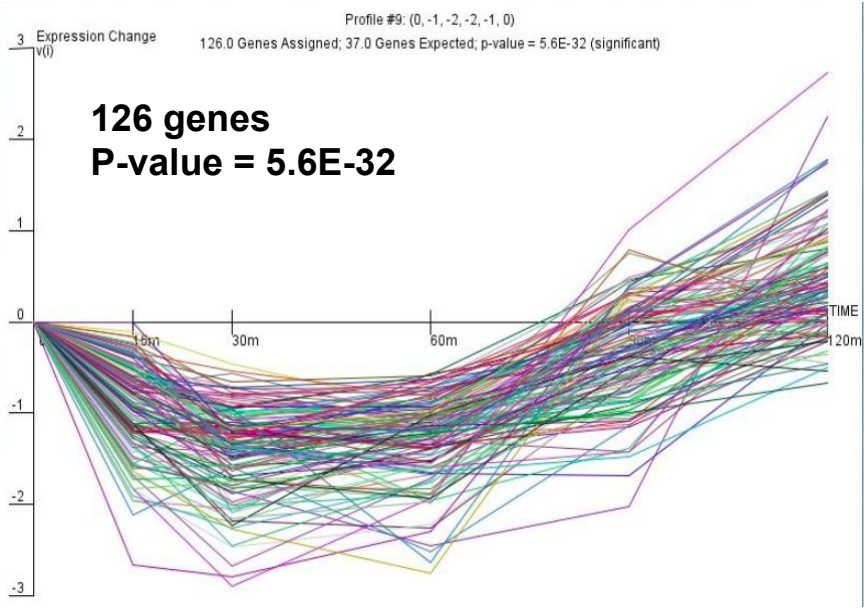
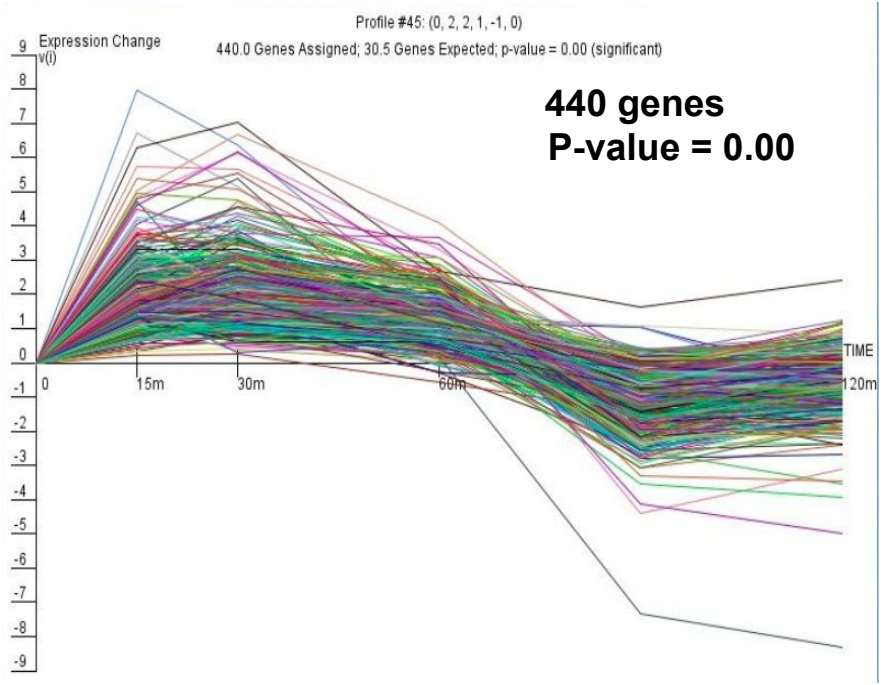
Stem Analysis Identifies 7 Significant Profiles



Profiles 9 and 45 chosen due to large sizes and clear cold shock and recovery patterns





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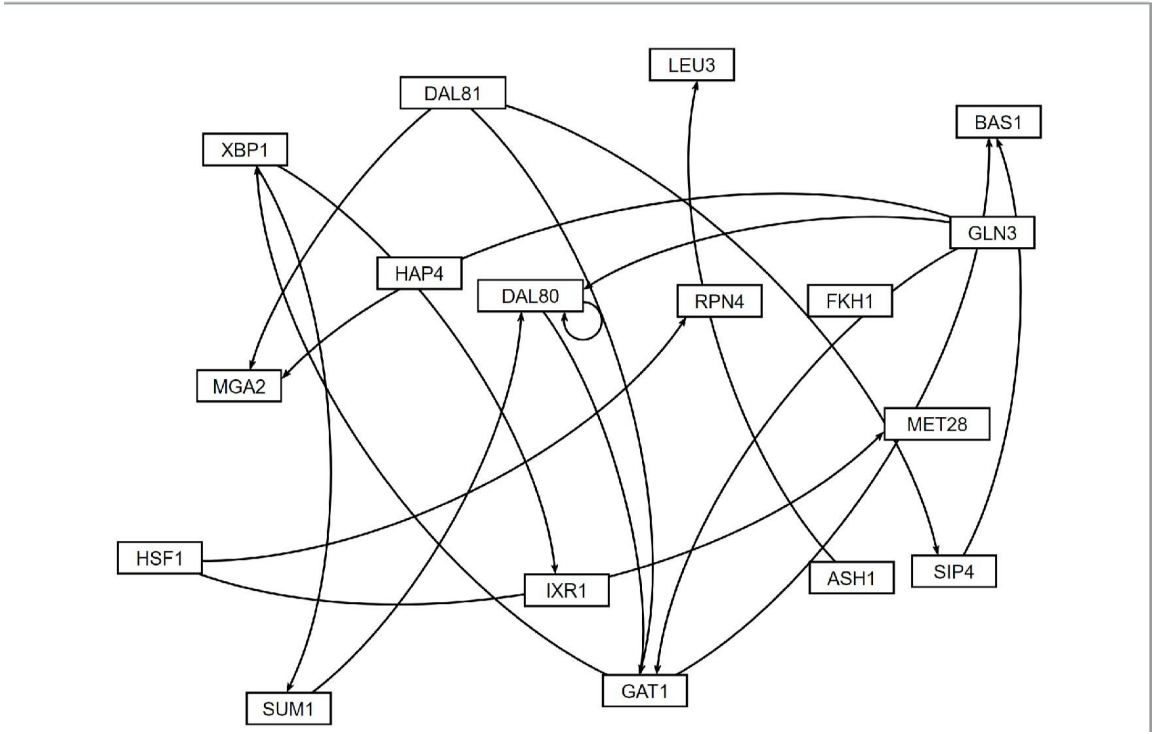
Profile 9 gene ontology terms primarily concerned with metabolic processes

GO Term	Definition
single-organism metabolic process	A metabolic process - chemical reactions and pathways, including anabolism and catabolism, by which living organisms transform chemical substances - which involves a single organism.
small-molecule metabolic process	The chemical reactions and pathways involving small molecules, any low molecular weight, monomeric, non-encoded molecule.
carboxylic acid metabolic process	The chemical reactions and pathways involving carboxylic acids, any organic acid containing one or more carboxyl (COOH) groups or anions (COO-).
oxoacid metabolic process	The chemical reactions and pathways involving any oxoacid; an oxoacid is a compound which contains oxygen, at least one other element, and at least one hydrogen bound to oxygen, and which produces a conjugate base by loss of positive hydrogen ion(s) (hydrons).
organic acid metabolic process	The chemical reactions and pathways involving organic acids, any acidic compound containing carbon in covalent linkage.
alpha-amino acid metabolic process	The chemical reactions and pathways involving an alpha-amino acid.

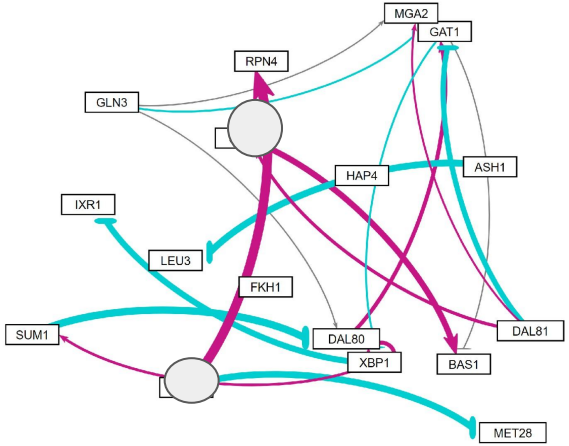
6 Out of 17 Transcription Factors in Network Are Statistically Significant

Transcription Factor	p-valuc	Transcription Factor	p-valuc
GAT1	0.6741	ASH1	0.011
HAP4	0.9066	LEU3	0.9066
SIP4	0.042	GLN3	0.019
RPN4	0.2545	FKH1	0.066
IXR1	0.1532	DAL80	0.069
DAL81	0.1971	MGA2	0.0016
MET28	0.0425	SUM1	0.0341
BAS1	0.1136	XBP1	0.2824
HSF1	0.1704		

Unweighted Network Consists of 17 Nodes and 17 Edges

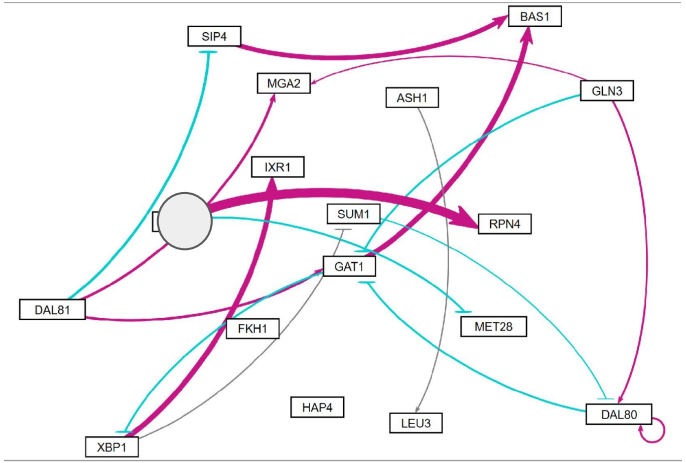


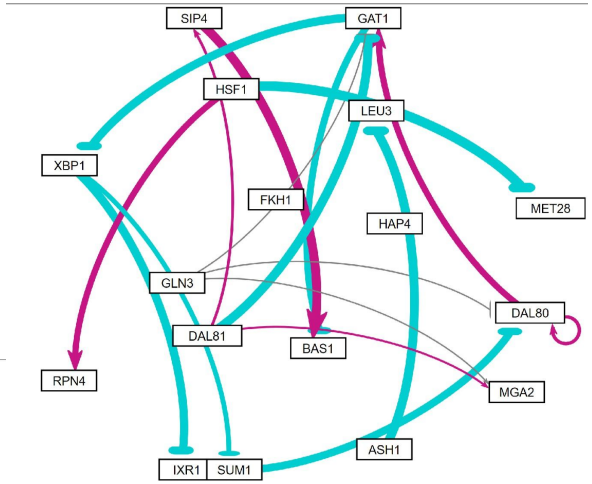
Changes in Weight and Direction of Transcription Factor Activity



Initial Run

P and b fixed

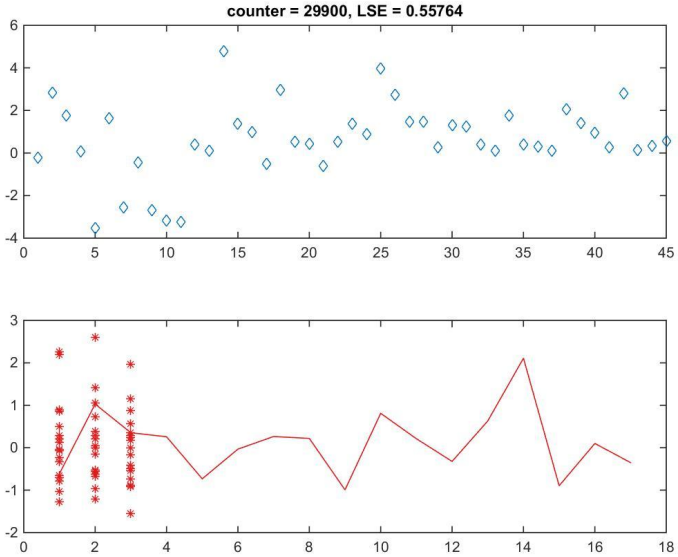




P fixed

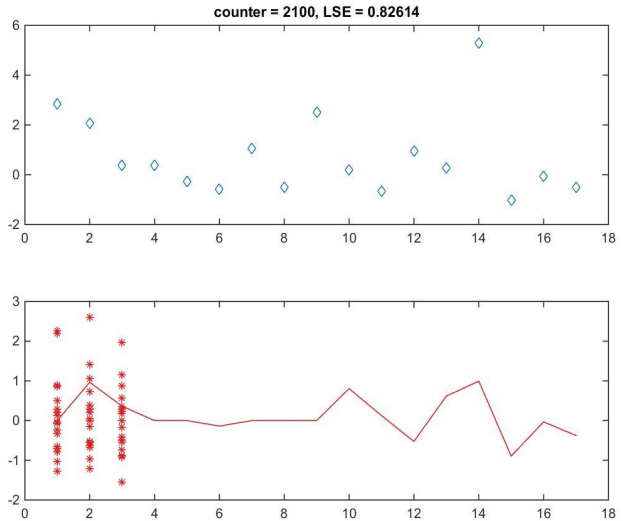
Significant Increase in LSE/minLSE Ratio with Fixed Parameters P and b

Estimated Parameters



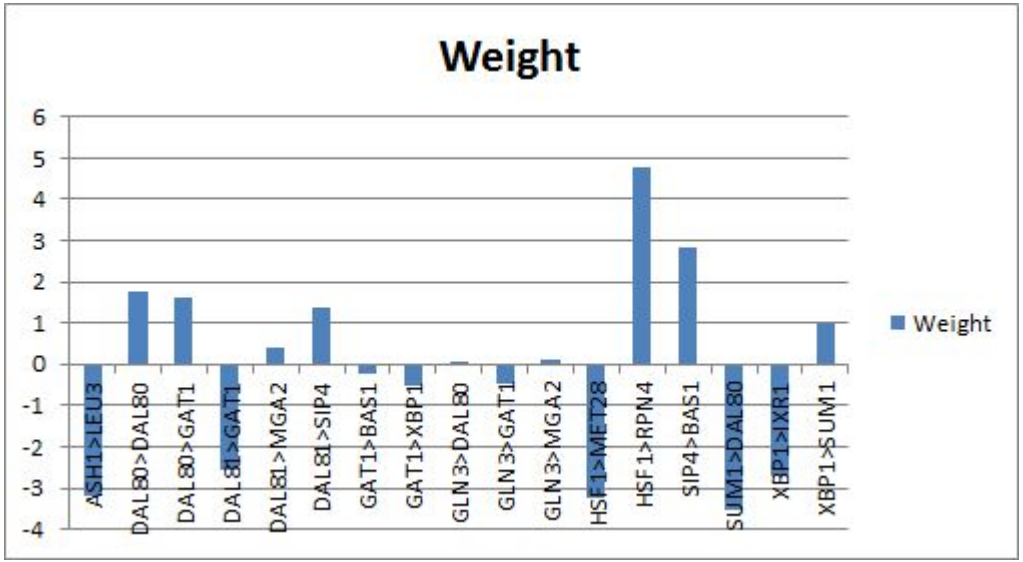
LSE/minLSE = 1.364

Fixed Parameters

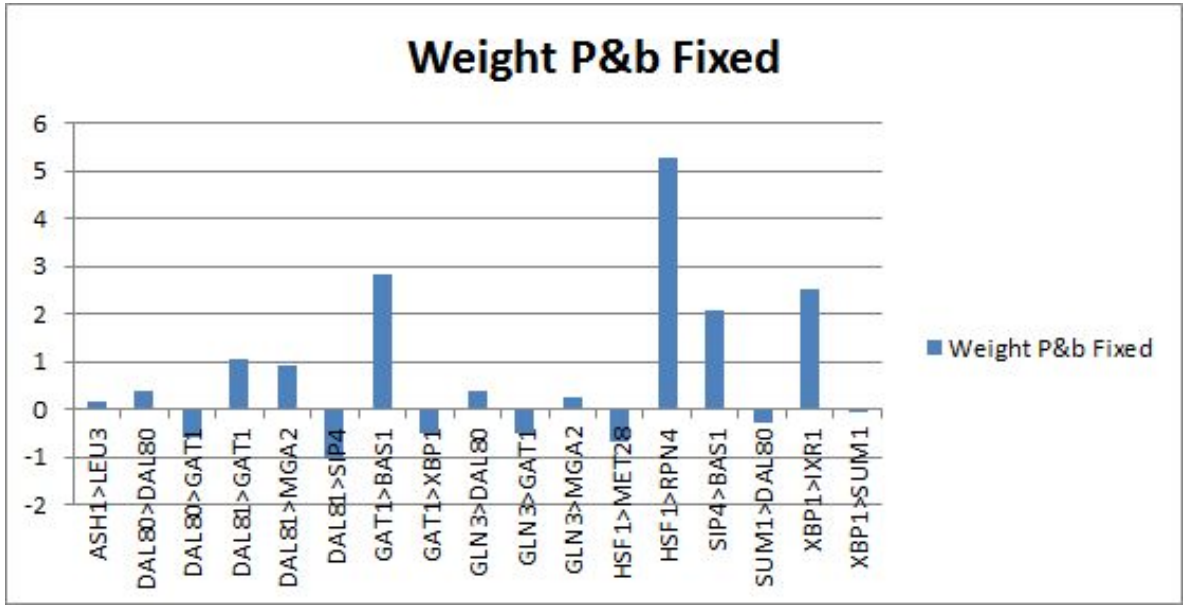


LSE/minLSE = 2.021

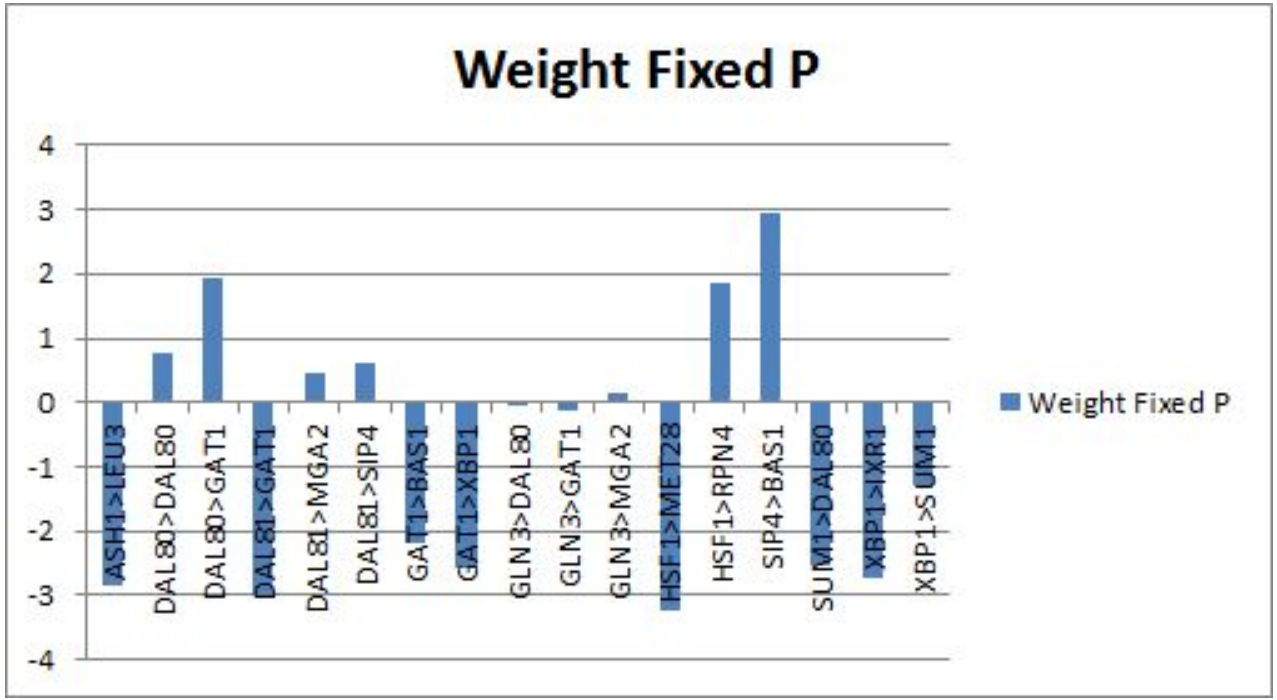
Weights and Direction of Influence for 17 Transcription Factors



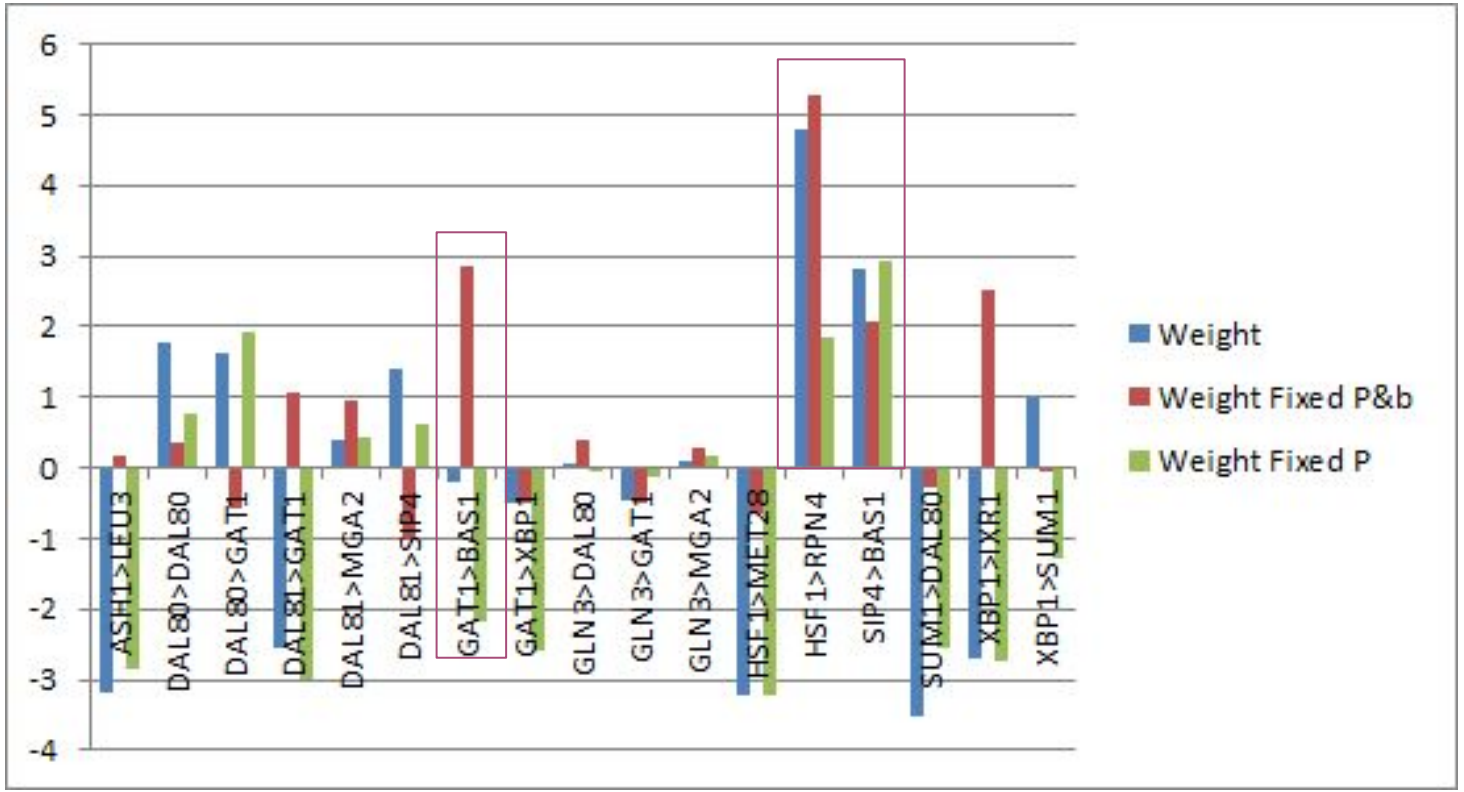
Holding Parameters Constant Results in Underestimation of Repression Activity



Holding P Constant Results in Overestimation of of Repression Activity



HSF1 and SIP4 Weights Most Consistently Estimated



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894 Gene Ontology (GO) terms associated with genes that fit profile 45

- 262 significant GO terms identified
  - P-value < 0.05 = 262 (29.31%)
  - Corrected p-value < 0.05 = 33 (3.69%) ‘

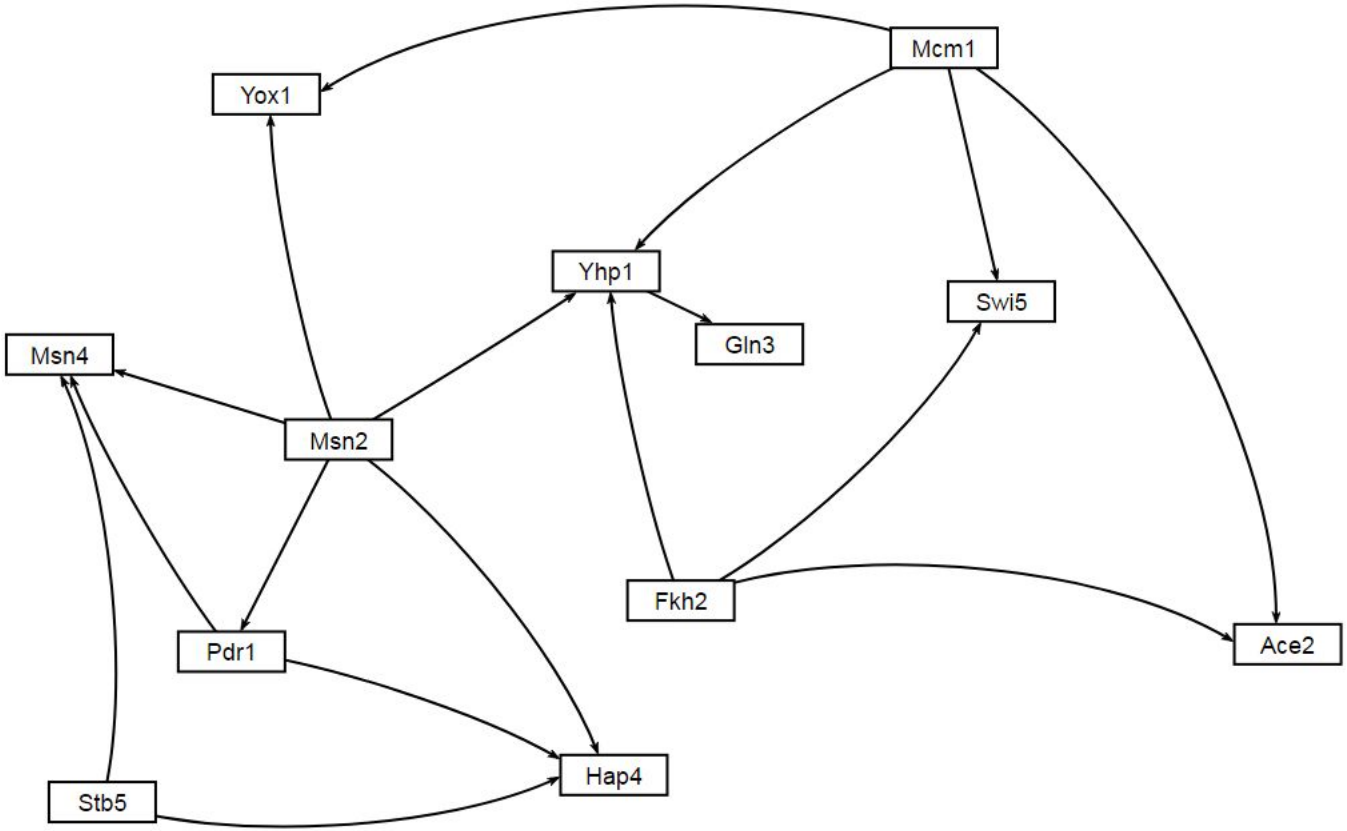
Profile 45 gene ontology terms primarily concerned with RNA synthesis and processing

GO Term	Definition
RNA 3'-end processing	Any process involved in forming the mature 3' end of an RNA molecule
rRNA binding	Interacting selectively and noncovalently with ribosomal RNA
snRNA processing	Any process involved in the conversion of a primary small nuclear RNA (snRNA) transcript into a mature snRNA molecule
exonucleolytic trimming involved in rRNA processing	Exonucleolytic digestion of a pre-rRNA molecule in the process to generate a mature rRNA molecule
ribosomal large subunit export from nucleus	The directed movement of a ribosomal large subunit from the nucleus into the cytoplasm
Polyadenylation-dependent RNA catabolic process	Chemical reactions/pathways resulting in breakdown of RNA molecule, initiated by polyadenylation at the 3'-end of target RNA

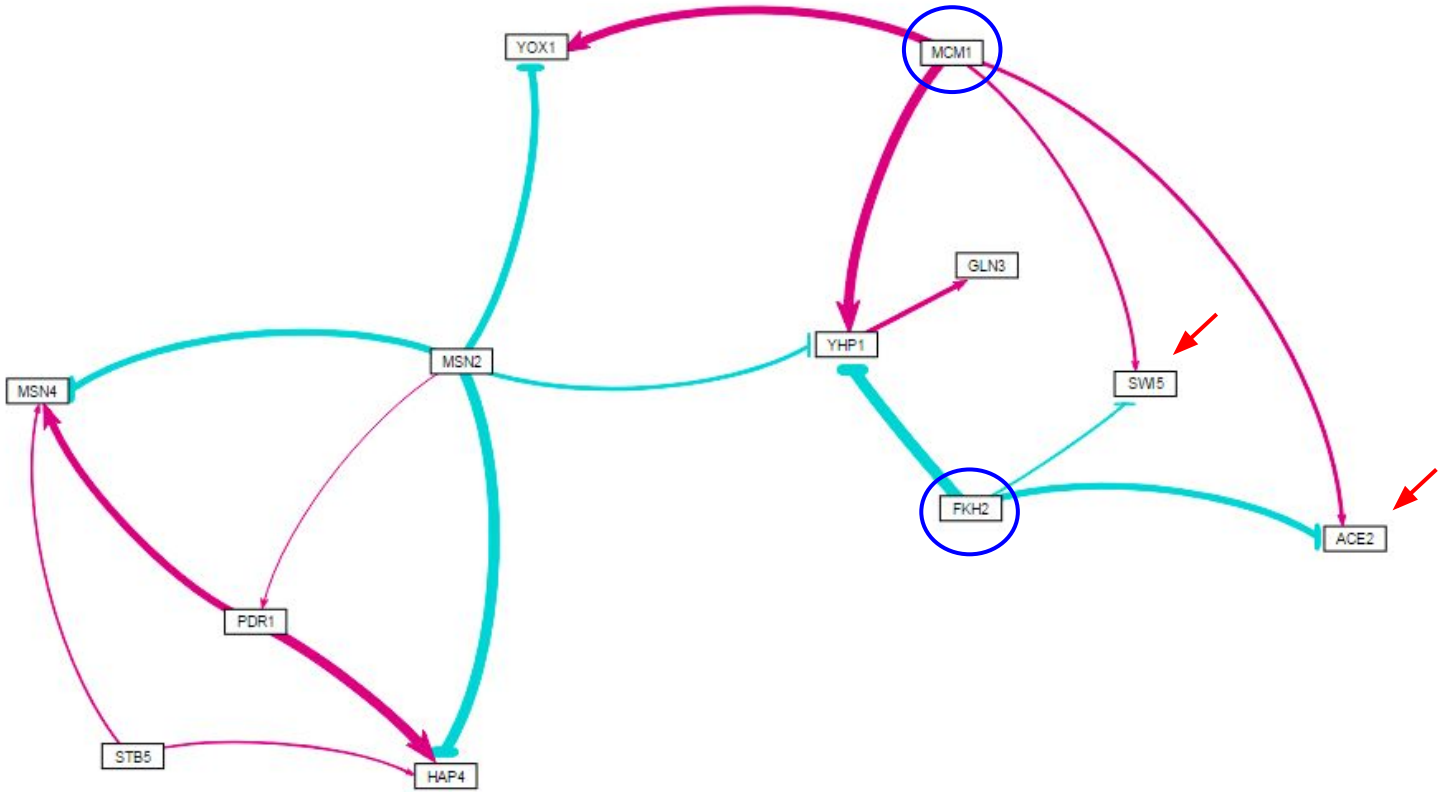
22 significant genes initially identified in YEASTRACT

Transcription Factor	*P-value*		Transcription Factor	*P-value*
ACE2	5E-15		MSN4	1.51E-07
FKH2	0		PDR1	3.45E-10
GLN3	1.94E-2		STB5	2.22E-11
HAP4	9.07E-2		SWI5	1.18E-09
MCM1	1.39E-08		YHP1	0
MSN2	3E-15		YOX1	0

Unweighted network comprised of 12 nodes and 17 edges

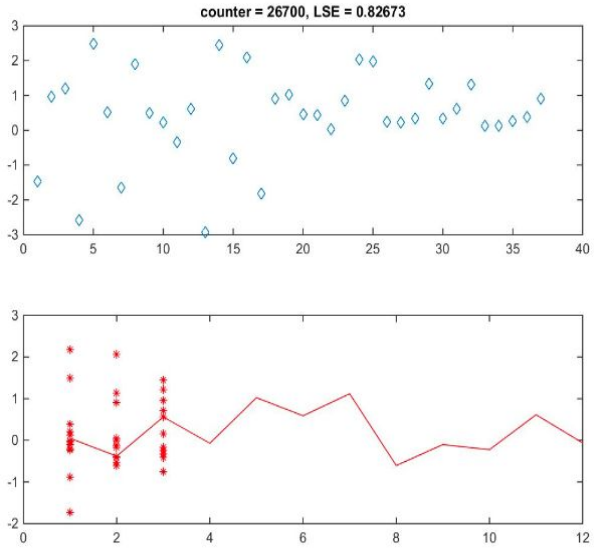


SWI5 and ACE2 selected for deletion



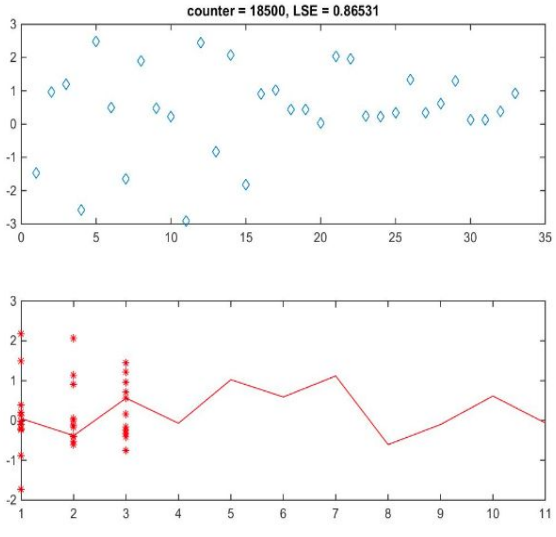
LSE/minLSE ratio remains constant with deletion of SWI5 and ACE 2

No Deletion



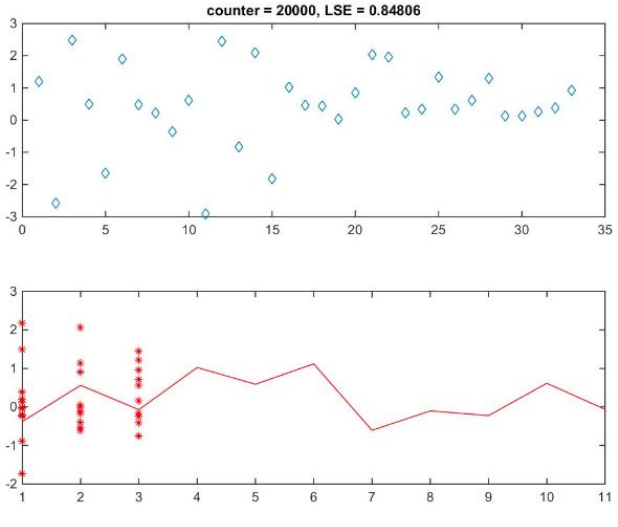
LSE/minLSE =1.448

SWI5 Deletion



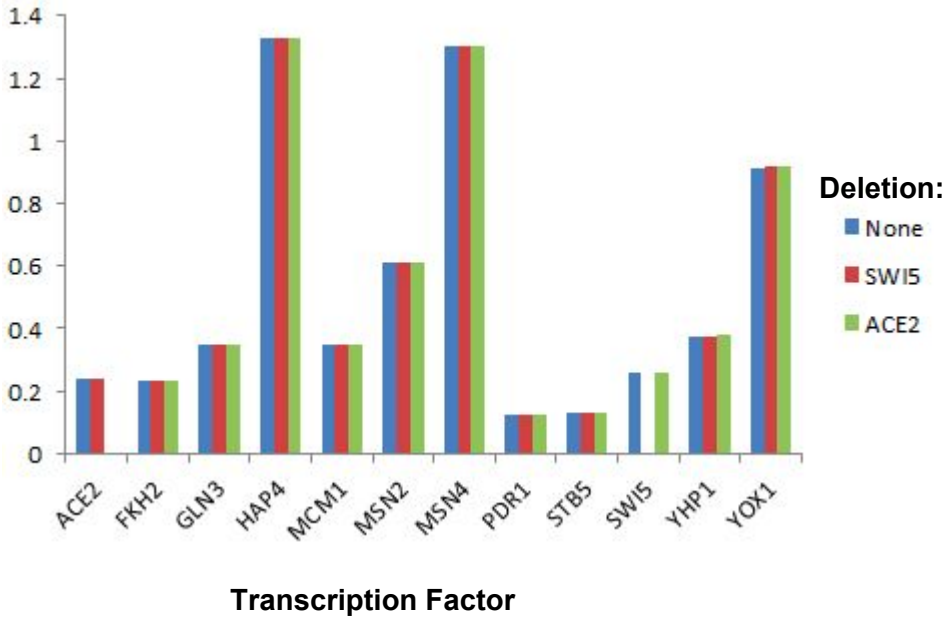
=1.462

ACE2 Deletion

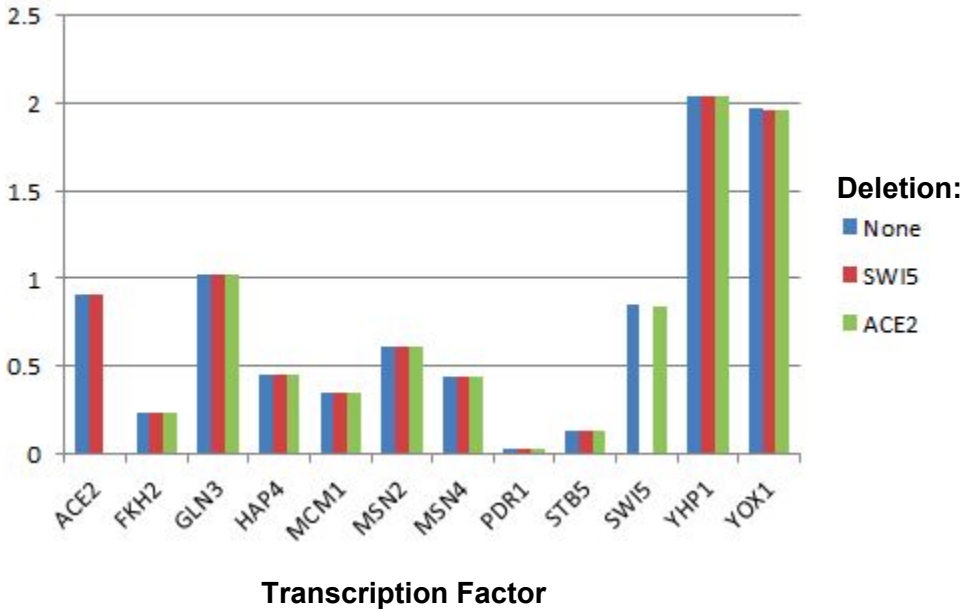


=1.462

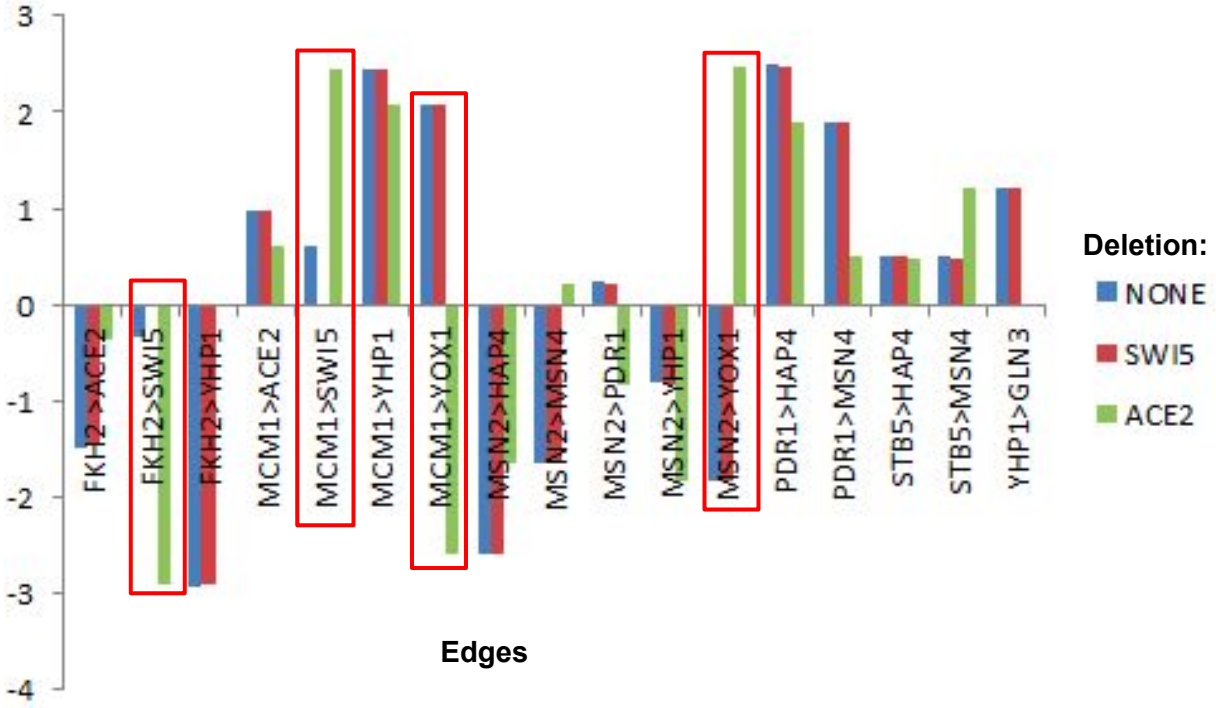
Deletion of SWI5 and ACE2 has no effect on optimized production rates



Deletion of SWI5 and ACE2 has no effect on optimized threshold b parameters

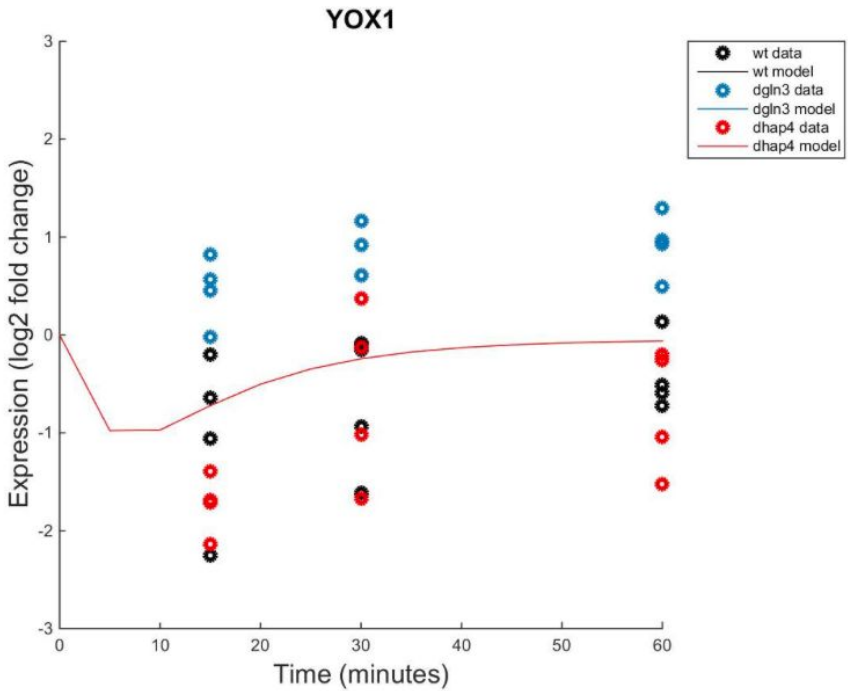


ACE2 deletion results in changes in optimized weight parameters for multiple connections

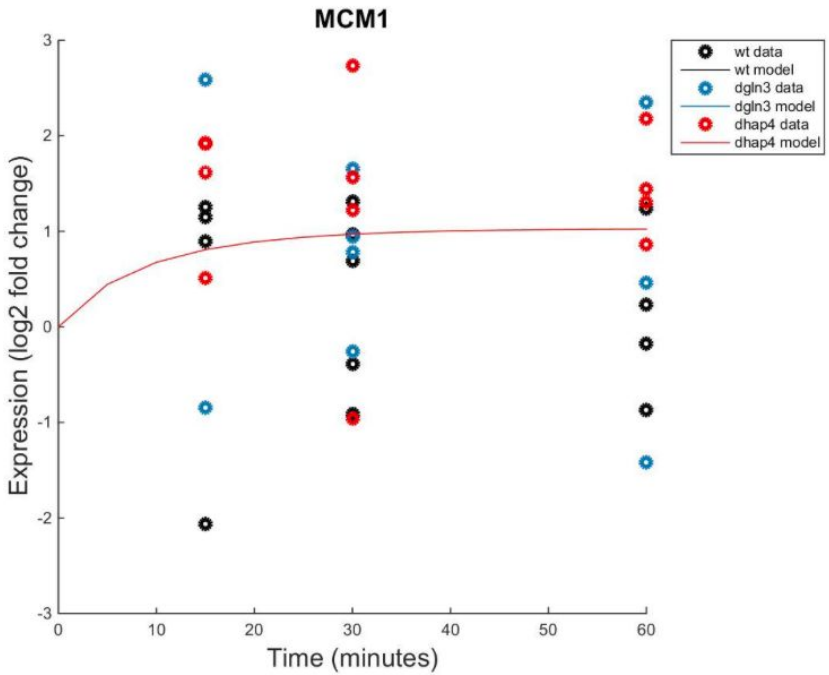


- **Increased drastically for edges including SWI5**
- **Changed sign for edges including YOX1**

YOX1 and MCM1 expression varies distinctly between WT, dGLN3 and dHAP4 data



-Activated by MCM1
-Repressed by MSN2 (represses 4 target genes)



Activates 4 target genes: YHP1, YOX1, SWI5, ACE2

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Several transcription factors identified as key players in regulating the response to cold shock

- Manipulation of parameters
  - HSF1: Consistently estimated as a strong repressor in all three trials
  - SIP4: Consistently estimated as a repressor in all three trials
- Deletion of SWI5 and ACE2
  - ACE 2: Drastic changes in optimized weight parameters
  - YOX1: Distinct changes in gene expression between WT, dGLN3 and dHAP4
  - MCM1: Central activator
- Manipulation of the gene regulatory network dynamics allows for analysis of
  - the relative levels of influence of specific transcription factors
  - indirect effects of other transcription factors in the gene regulatory network
- Future experimentation could focus on deleting other target genes, such as YOX1, in order to examine indirect relationships with other TFs

Acknowledgements

LMU | LA
Loyola Marymount University

- **We would like to thank Dr. Dahlquist, Dr. Fitzpatrick and the rest of our classmates.**

References

- **Dahlquist, Kam D. (2017). GRNmap and GRNsight: Open Source Software for Dynamical Systems Modeling and Visualization of Medium-Scale Gene Regulatory Networks [PowerPoint slides]. Retrieved from http://www.openwetware.org/wiki/Dahlquist.**
- **Vu, T. T., & Vohradsky, J. (2007). Nonlinear differential equation model for quantification of transcriptional regulation applied to microarray data of Saccharomyces cerevisiae. *Nucleic acids research, 35*(1), 279-287. doi: 10.1093/nar/gkl1001**