

Assessing the Cold Shock Response of Δ HAP4 and Wild-type Transcription Factor

Lauren Kelly and Cameron Rehmani Seraji

**Department of Biology
Loyola Marymount University**

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Outline

- Background on the cold shock experimental design
- Significant expression levels in various genes were found by doing statistical analysis of microarray data
- YEASTRACT results yielded 12 significant gene profiles
- Profile 45 gene ontology terms indicate a strong effect on tRNA processes
 - Deletions of HAP4 and SWI5 led to changes in production rates, threshold b parameters, and weight parameters of transcription factors
 - Deletions of HAP4 and SWI5 led to changes in expression of MSN2 and SOK2
- Profile 9 gene ontology terms indicate a strong effect on cytoplasm and its components
 - Deletions of GLN3 and SPT23 led to changes in production rates, weight parameters, and threshold b parameters of transcription factors
 - GLN3 and SPT23 are important in regulating the cold shock response
- Tai et al. (2007) and up/down-regulation of transcription factors

Understanding How Cells Respond to Cold Shock

- Cold Shock is the response to a sudden decrease in temperature and it has not been well studied in the past.
- DNA microarray analysis was performed on data for Δ HAP4 that was generated in the Dahlquist Lab.
 - Red spots represent an increase in expression and green spots represent a decrease in expression.
- The experiment was conducted at 13°C for a total of 60 minutes followed by a recovery at 30°C.
- The experiment was run using ANOVA, STEM, YEASTRACT, GRNsight, and GRNmap

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According to the ANOVA Results of Δ HAP4, the Amount of Significant Genes Ranges from 75 to 2,479

ANOVA	dHAP4
$p < 0.05$	2479 genes 40.05%
$p < 0.01$	1583 genes 25.58%
$p < 0.001$	739 genes 11.94%
$p < 0.0001$	280 genes 4.52%
B & H $p < 0.05$	1735 genes 28.03%
Bonferroni $p < 0.05$	75 genes 1.21%

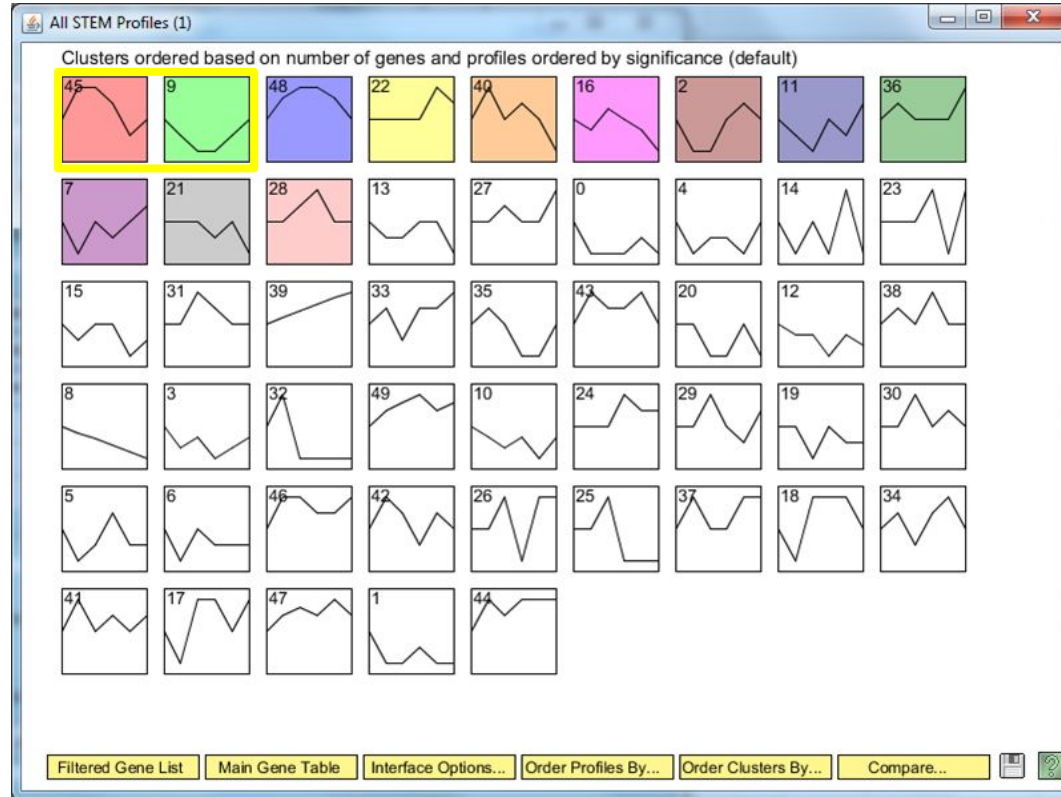
There are More Significant Genes at t30 than Any Other Time Point

	Cold Shock			Recovery	
t test	t₁₅	t₃₀	t₆₀	t₉₀	t₁₂₀
Average Log Fold Change > 0.25 and p < 0.05	1182 genes 19.10%	1242 genes 20.07%	1207 genes 19.50%	918 genes 14.83%	894 genes 14.44%
Average Log Fold Change < -0.25 and p < 0.05	1048 genes 16.93%	1067 genes 17.24%	1057 genes 17.08%	852 genes 13.77%	1011 genes 16.34%
Total p < 0.05	2479 genes 40.05%	2479 genes 40.05%	2479 genes 40.05%	2479 genes 40.05%	2479 genes 40.05%
Total B & H p < 0.05	1735 genes 28.03%	1735 genes 28.03%	1735 genes 28.03%	1735 genes 28.03%	1735 genes 28.03%
Total Bonferroni p < 0.05	75 genes 1.21%	75 genes 1.21%	75 genes 1.21%	75 genes 1.21%	75 genes 1.21%

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Profile #9 and #45 Were Chosen for the Analysis of Significant Transcription Factors

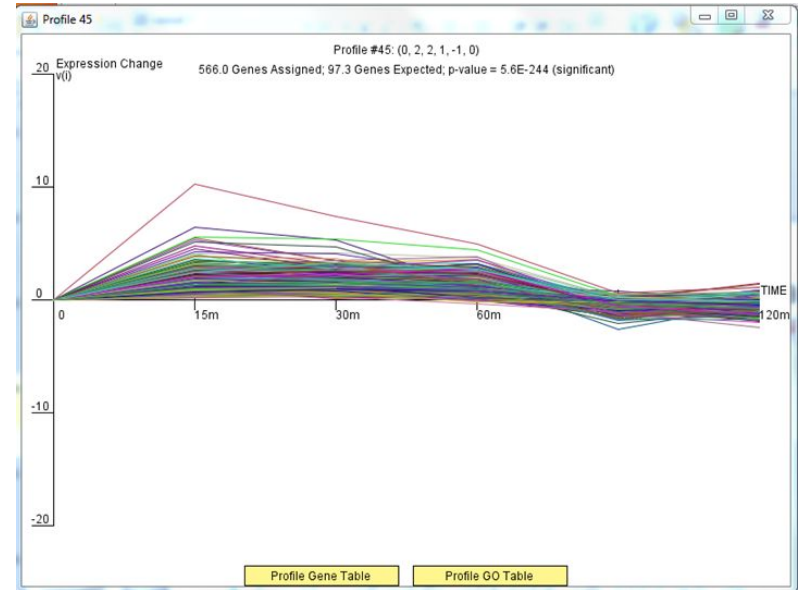


Outline

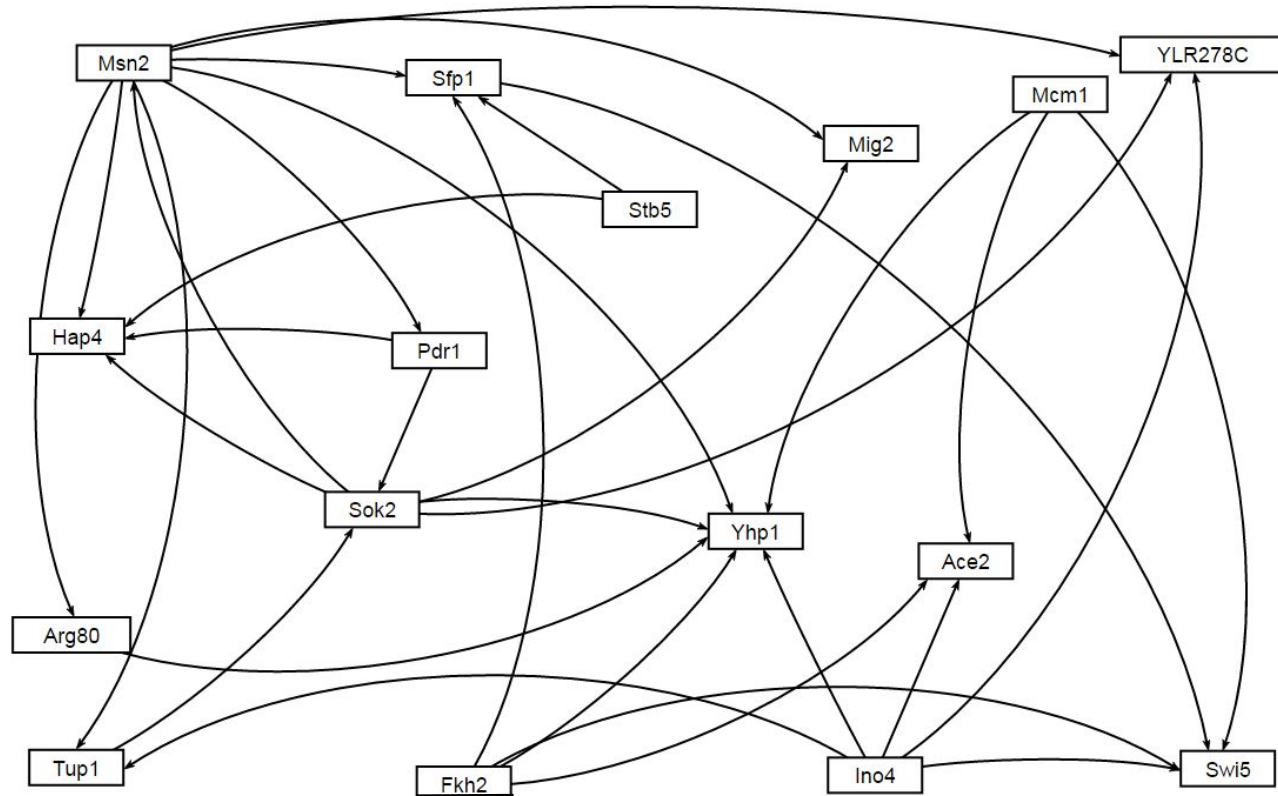
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Gene Ontology Terms of Profile 45 Demonstrate Strong Effect on tRNA Processes

GO number	Category Name	#Genes Assigned	Corrected p-value
GO:0006400	tRNA modification	22	0.002
GO:0030488	tRNA methylation	11	0.002
GO:0008033	tRNA processing	29	0.004
GO:0008175	tRNA methyltransferase activity	10	0.004
GO:0006399	tRNA metabolic process	41	0.001



Unweighted Transcription Factor Network of 16 Significant Edges and 32 Nodes



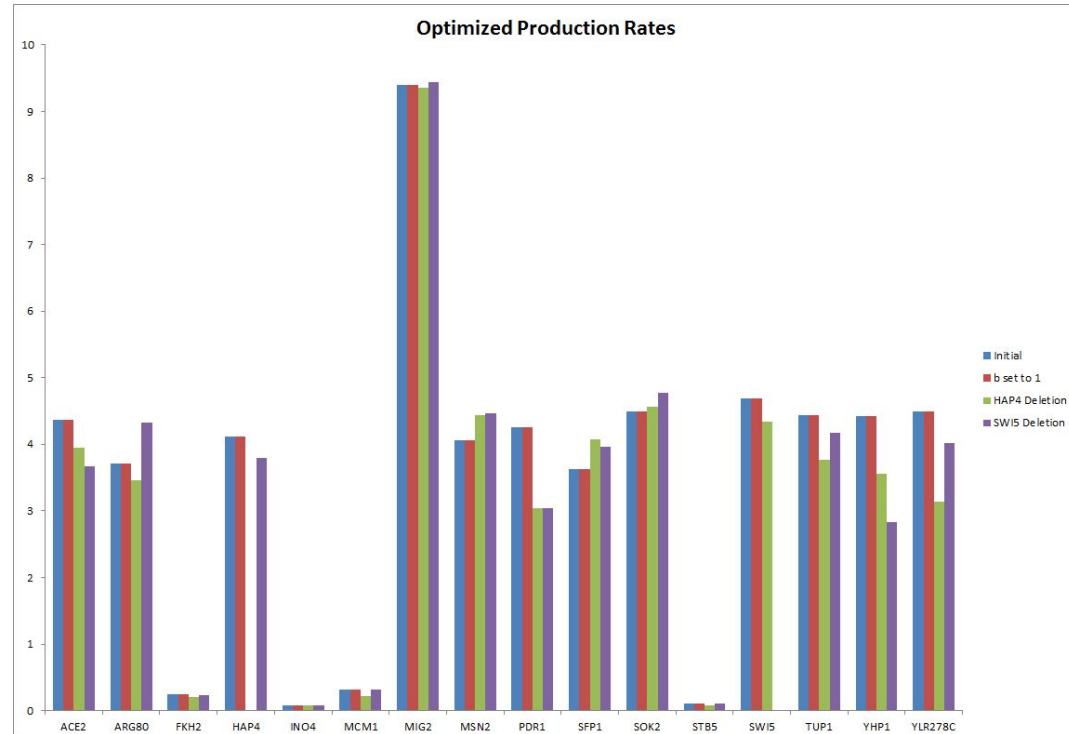
20 Significant Transcription Factors Found in YEASTRACT

- In total, 20 transcription factors were significant.
- Initially deleted all transcription factors that had no regulators or only 1 input.

Transcription Factor	p-value from YEASTRACT	Transcription Factor	p-value from YEASTRACT
MSN2	1.78E-08	ARG80	8.58E-06
HAP4	0.998	TUP1	9.1E-13
SFP1	0	PDR1	1.69E-05
SOK2	1.1E-05	FKH2	7.36E--10
STB5	1.2E-12	YHP1	0
MIG2	1.85E-07	INO4	4.86E-05
ACE2	5E-15	MCM1	1.88E-05
YLR278C	2.18E-06	SWI5	4.03E-09

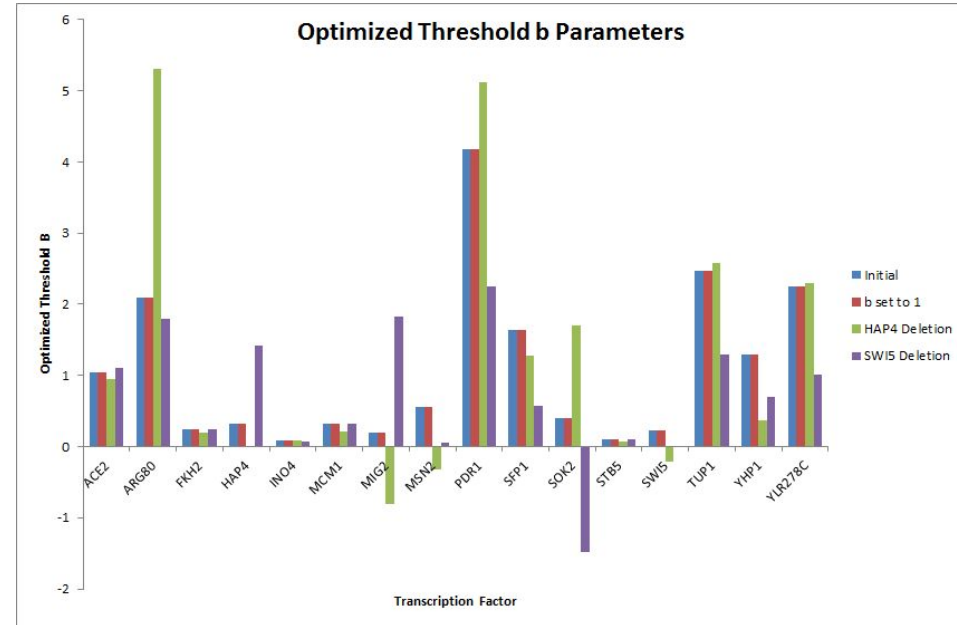
Minor Changes in Optimized Production Rate Across the 4 Trials

- MIG2 had the highest production rate in all 4 trials.
- INO4 had the lowest production rate in all 4 trials.
- The initial trial and trial where b was set to 1 have the same value for all genes.
- Deletions of HAP4 and SWI5 caused the production rates to change in all genes.



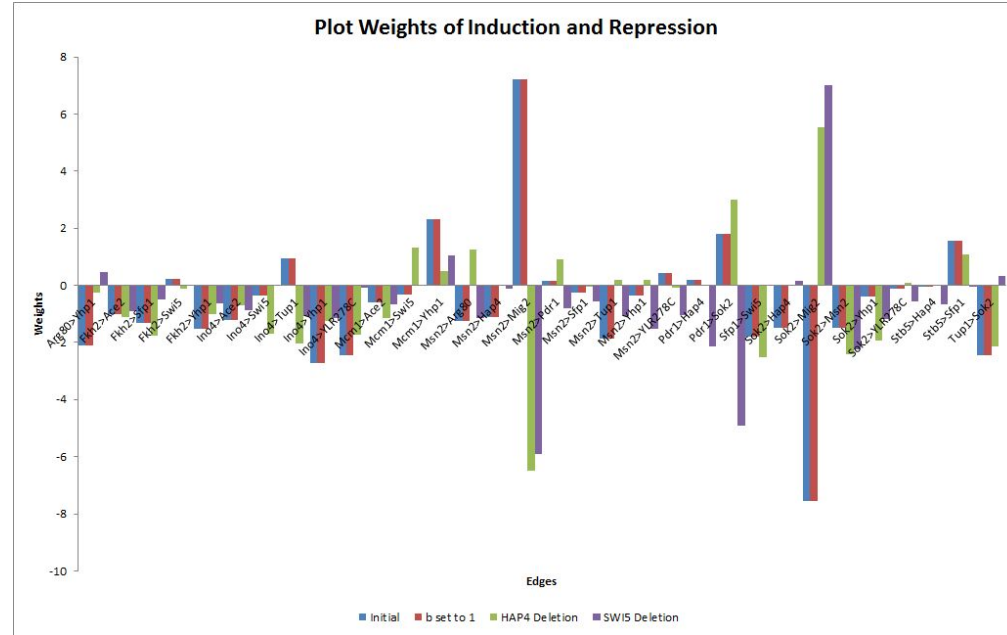
Deletions of HAP4 and SWI5 Affected the Optimized Threshold b Parameters

- The initial trial and trial where b was set to 1 have the same value for all genes.
- Large difference between initial trial and HAP4 and SWI5 deletion for 11 different transcription factors.
- INO4 had the lowest optimized threshold b parameters for all trials.

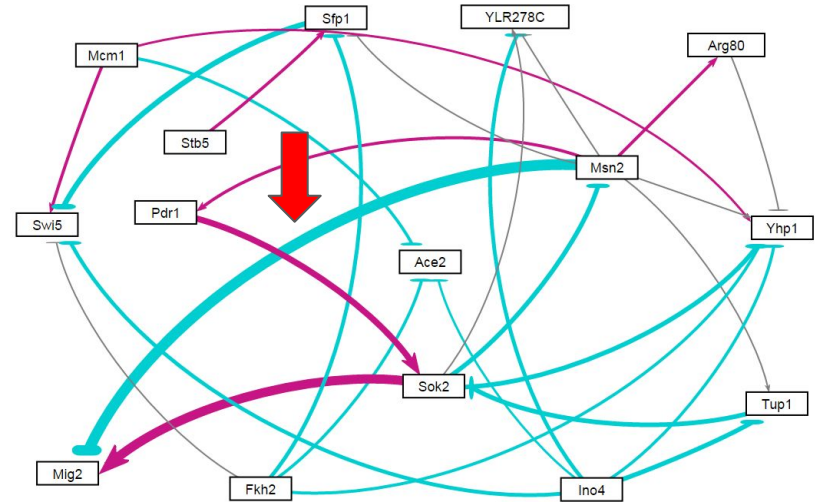
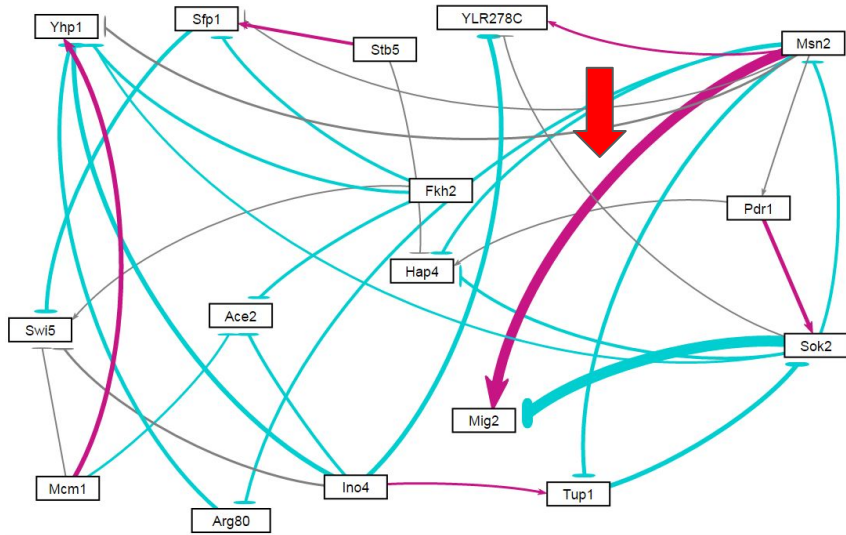


Comparison of Plot Weights Shows Contrasting Values for Multiple Gene Pathways

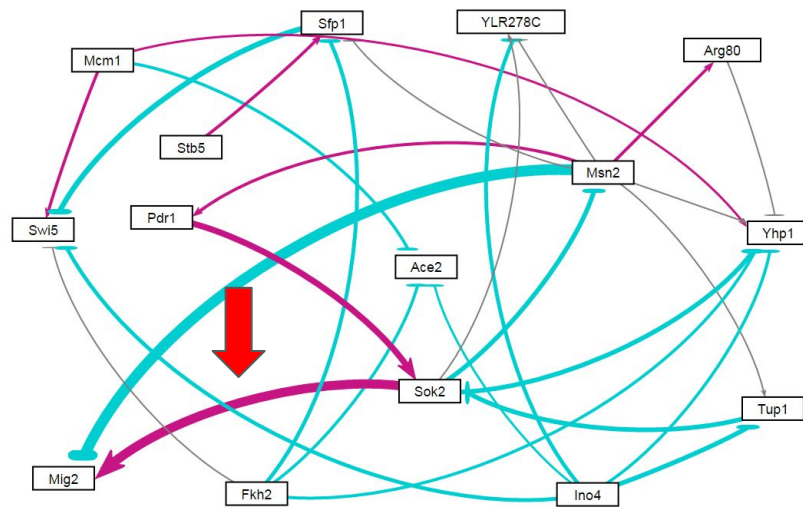
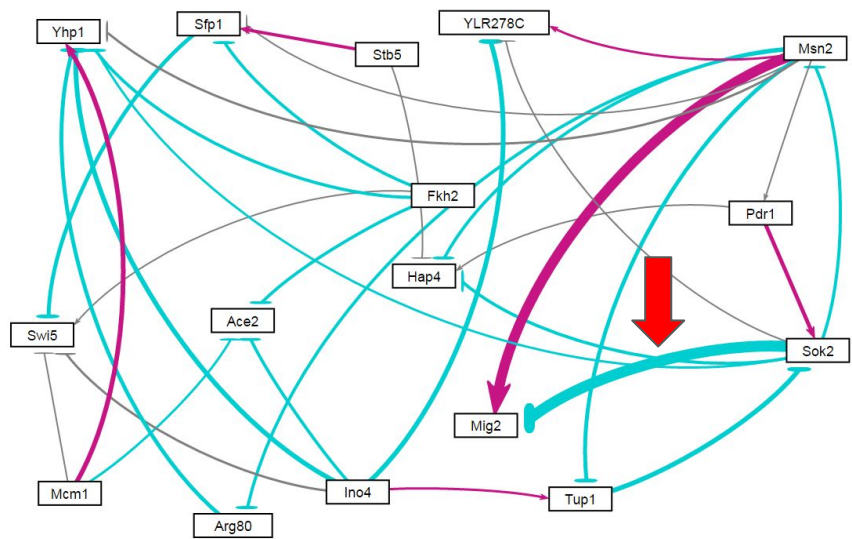
- The initial trial and trial where b was set to 1 have the same value for all genes.
- Two biggest pathways that stand out on the graph are **MSN2→MIG2** and **SOK2→MIG2**.
- **MSN2→MIG2** changed from induction to repression when HAP4 and SWI5 were deleted.
- **SOK2→MIG2** changed from repression to induction when HAP4 and SWI5 were deleted.



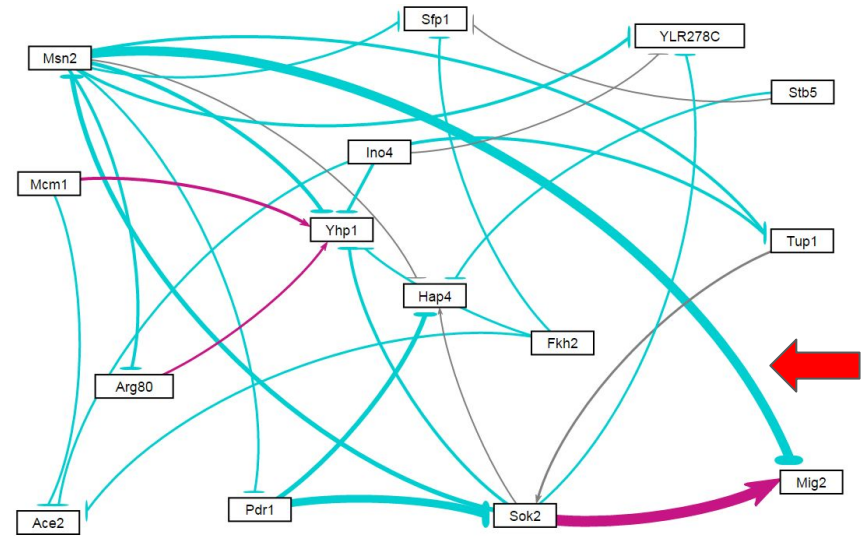
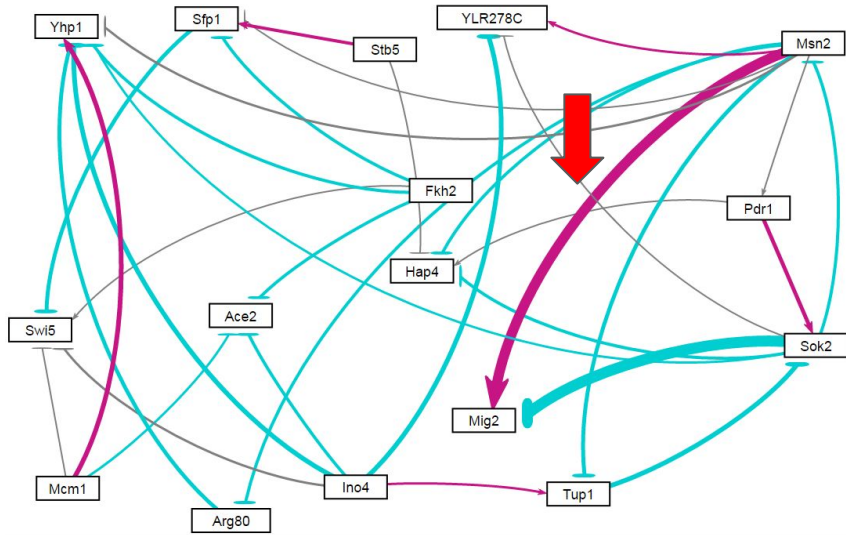
MSN2→MIG2 Plot Weight Changed from 7.202137 to -6.47552 when HAP4 was Deleted



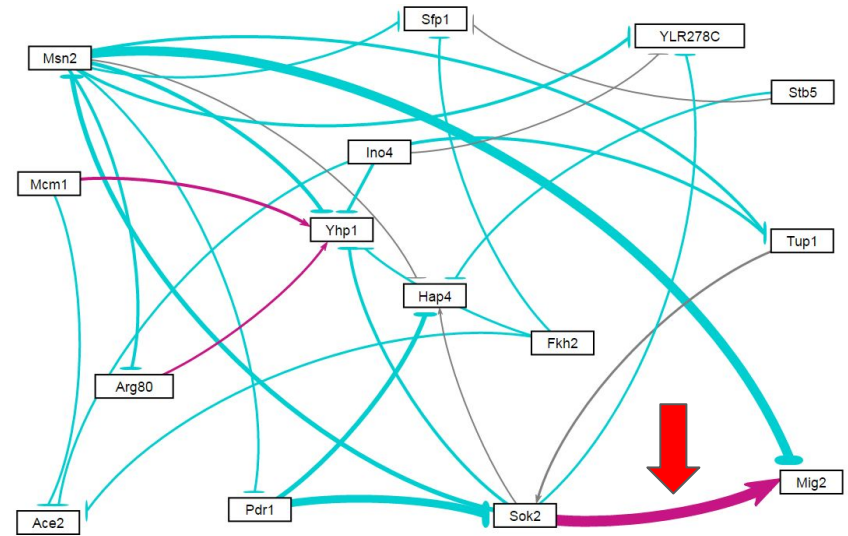
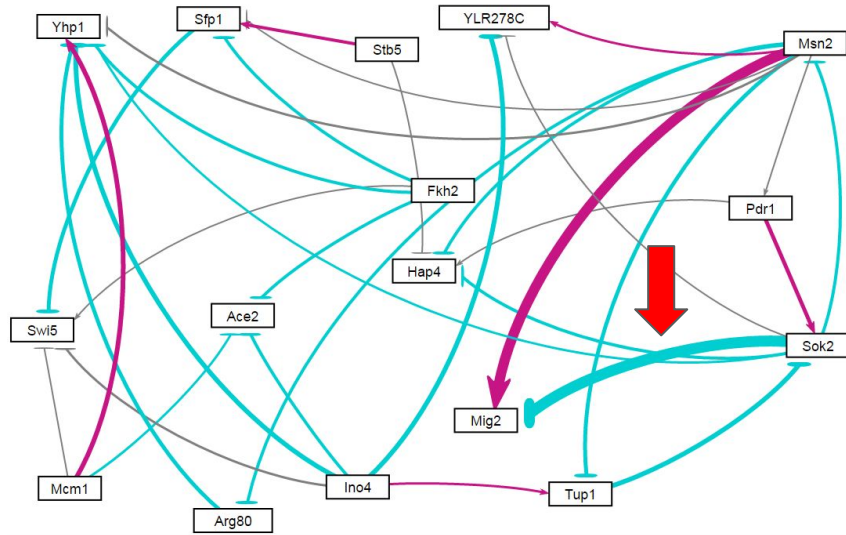
SOK2→MIG2 Plot Weight Changed from -7.55775 to 5.522197 when HAP4 was Deleted



MSN2→MIG2 Plot Weight Changed from 7.202137 to -5.89503 when SWI5 was Deleted

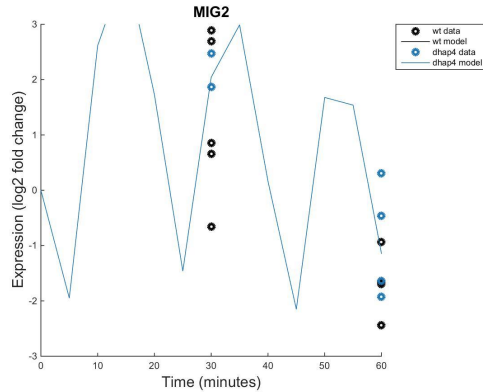


SOK2→MIG2 Plot Weight Changed from -7.55775 to 7.002023 when SWI5 was Deleted

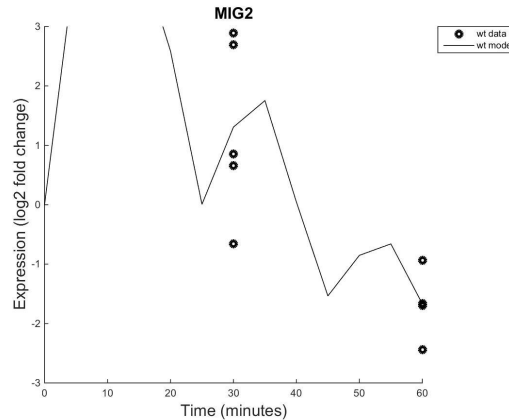


Line of Best Fit Changes from Repression to Induction Multiple Times for MIG2 in All Trials

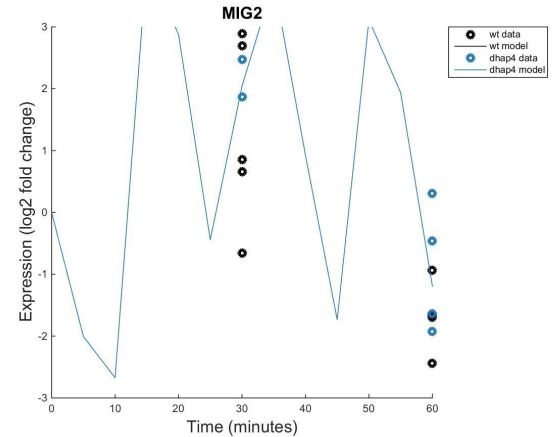
Initial



HAP4 Deletion

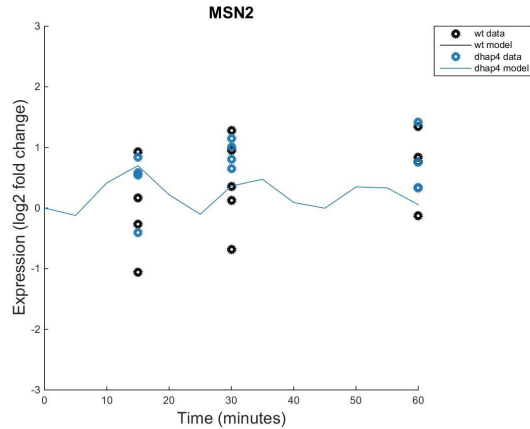


SWI5 Deletion

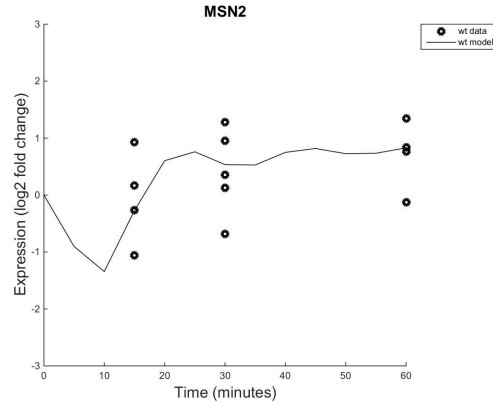


The Expression of MSN2 is Very Similar for the Initial and SWI5 Deletion

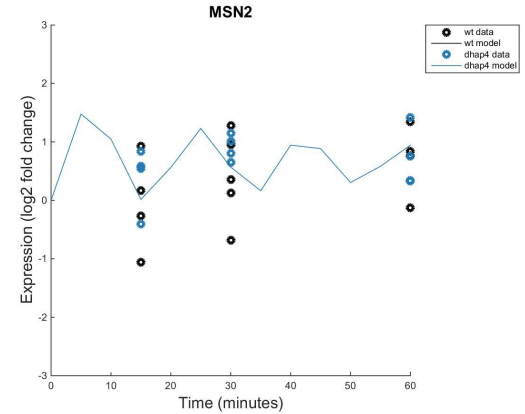
Initial



HAP4 Deletion

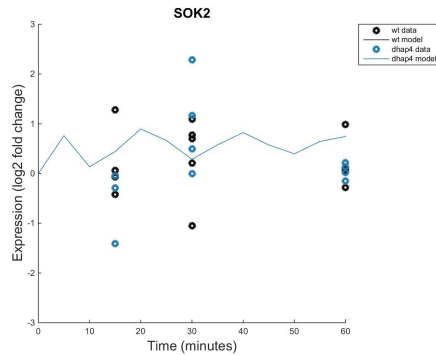


SWI5 Deletion

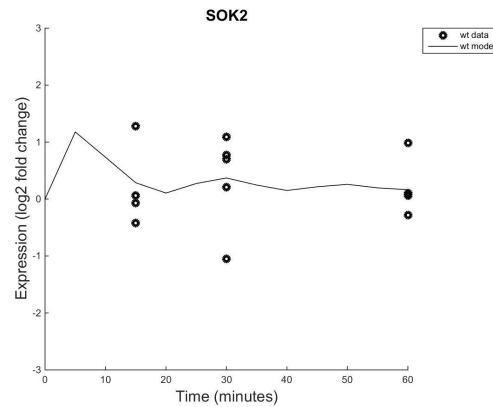


The Expression of SOK2 is Relatively Similar for the Three Trials

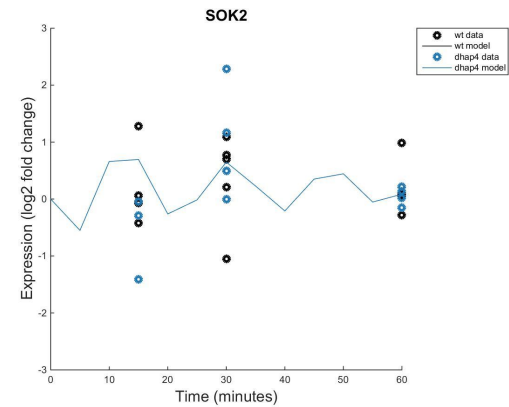
Initial



HAP4 Deletion



SWI5 Deletion



From the Visualized GRN, There are Two Major Regulators in the Network for Profile 45

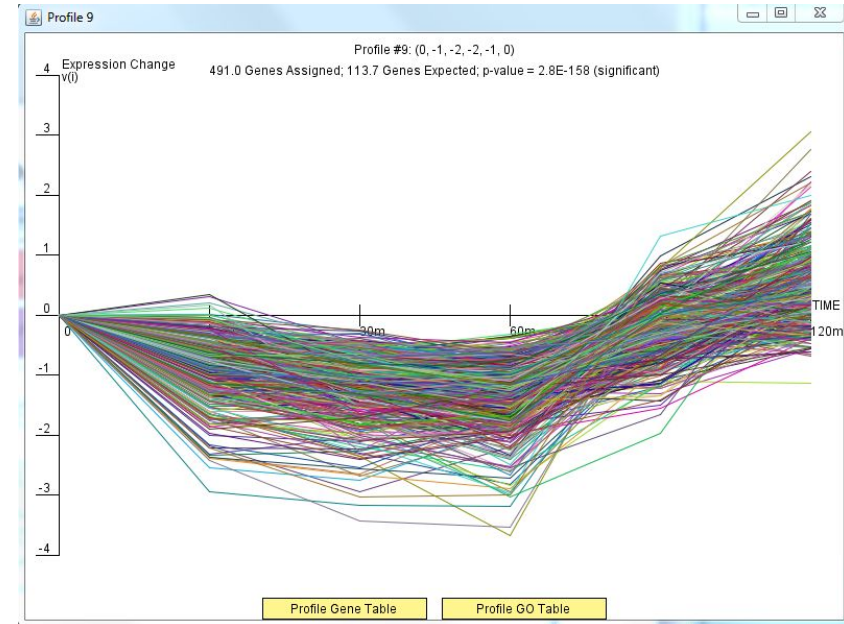
- **The two major regulators are MSN2 and SOK2 and both of these regulators directly regulate MIG2.**
- **The regulation of MSN2 and SOK2 may be due to the presence of HAP4 and SWI5 in the model.**
- **Future Studies:**
 - **Determine why deleting HAP4 and SWI5 causes the regulation of MSN2 and SOK2 to MIG2 to switch to the opposite of its original expression value.**
 - **Look at the effect of eliminating the transcription factors that have gray arrows from the constructed GRN.**

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Gene Ontology Terms from Profile 9 Suggest Strong Effect on the Cytoplasm and its Components

Category ID	Category Name	#Genes Assigned	Corrected p-value
GO:0005737	cytoplasm	393	0.001
GO:0044444	cytoplasmic part	310	0.001
GO:0022626	cytosolic ribosome	36	0.001
GO:0002181	cytoplasmic translation	35	0.002
GO:0044445	cytosolic part	42	0.002
GO:0005829	cytosol	104	0.006



P-value = 2.8E-158

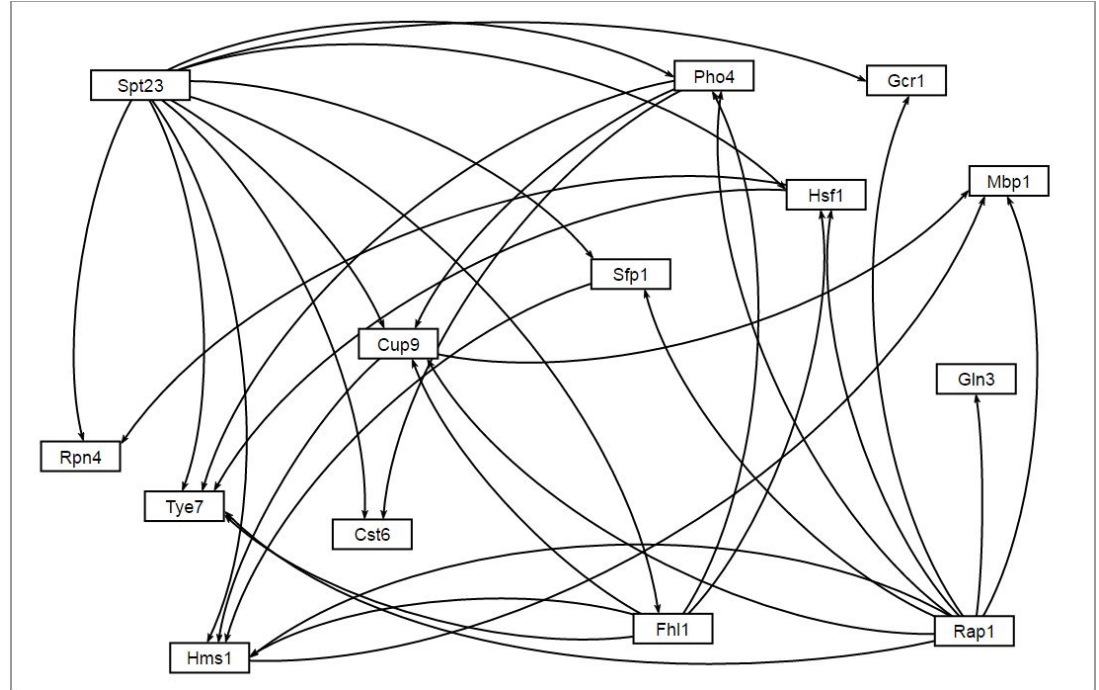
33 Significant Transcription Factors Found in YEASTRACT

- In total, 33 transcription factors were significant
 - GLN3 and HAP4 showed no significance according to YEASTRACT
- Initially deleted all transcription factors that had no regulators or only one input

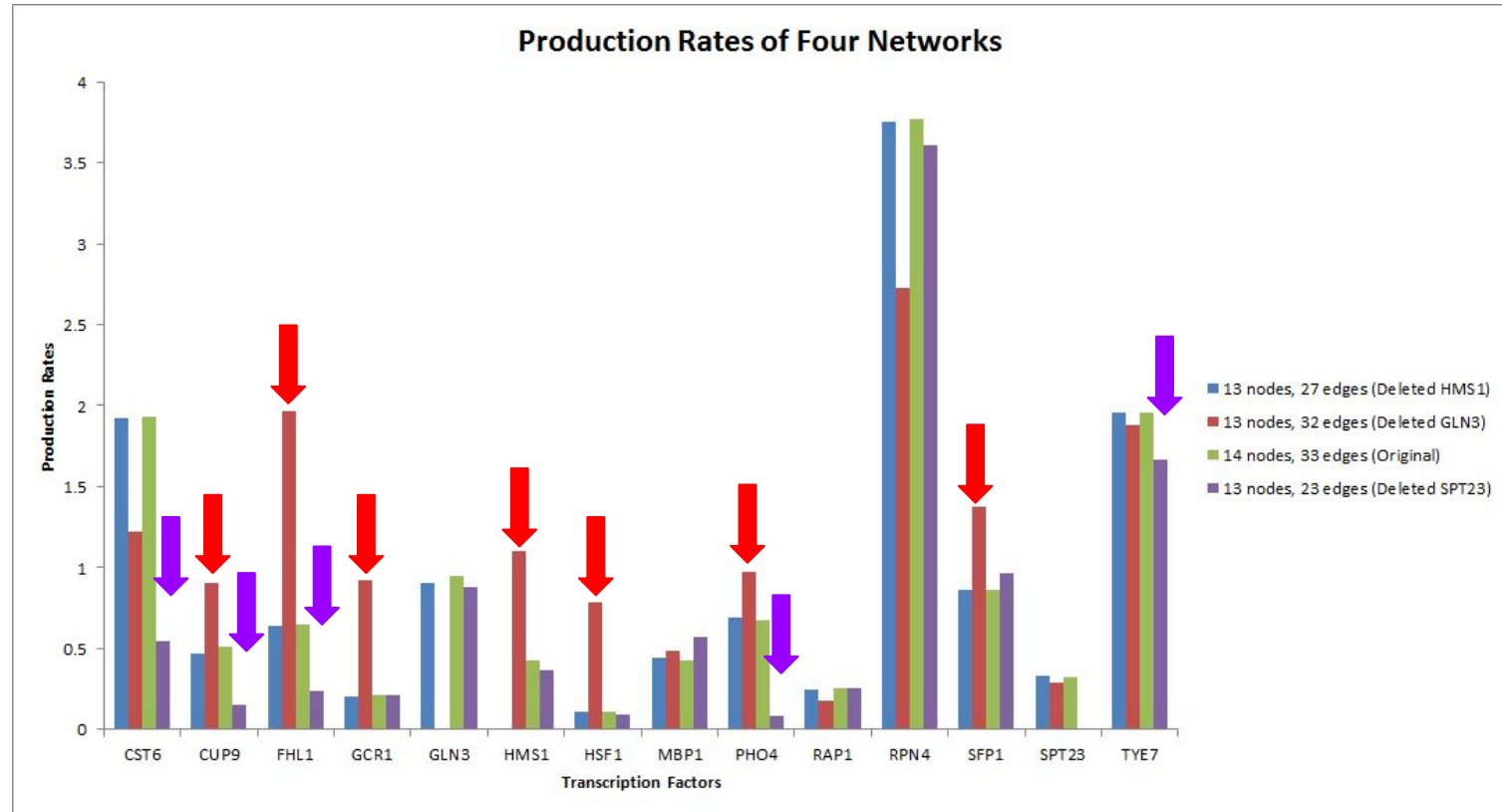
Transcription Factor	p-value from YEASTRACT	Transcription Factor	p-value from YEASTRACT
CST6	2.56441E-05	MBP1	1.20671E-06
CUP9	1.27643E-06	PHO4	1.12068E-05
FHL1	1.23921E-05	RAP1	2.81392E-05
GCR1	1.63413E-05	RPN4	1.96342E-05
GLN3	0.009729	SFP1	4.21512E-05
HMS1	1.84972E-06	SPT23	1.6982E-05
HSF1	2.45662E-05	TYE7	1.02978E-05

Initial Network Has 14 Nodes and 33 Edges

- This network was used as the starting point for future experimental deletions
- 3 additional deletions: GLN3, SPT23, and HMS1

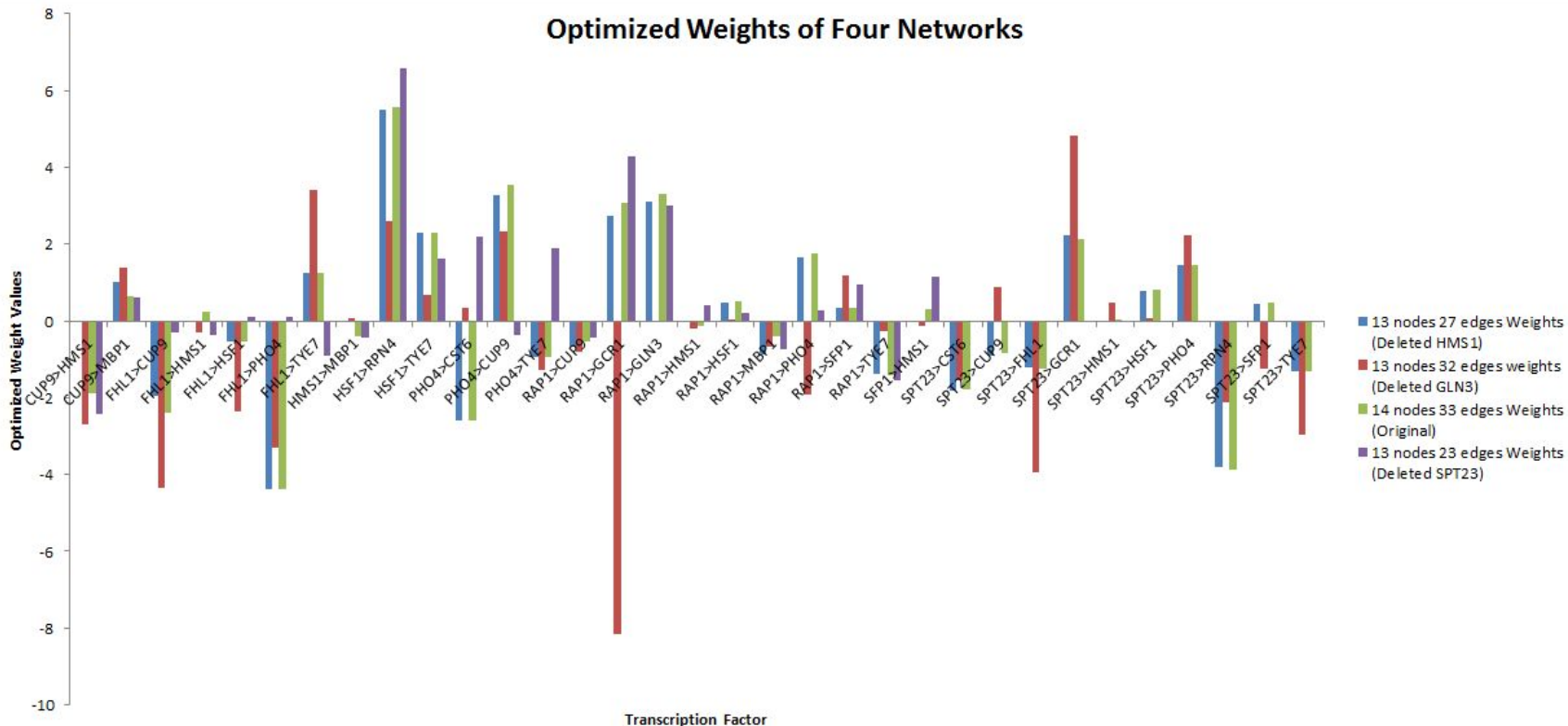


Deletion of GLN3 and SPT23 has Effects on Production rates

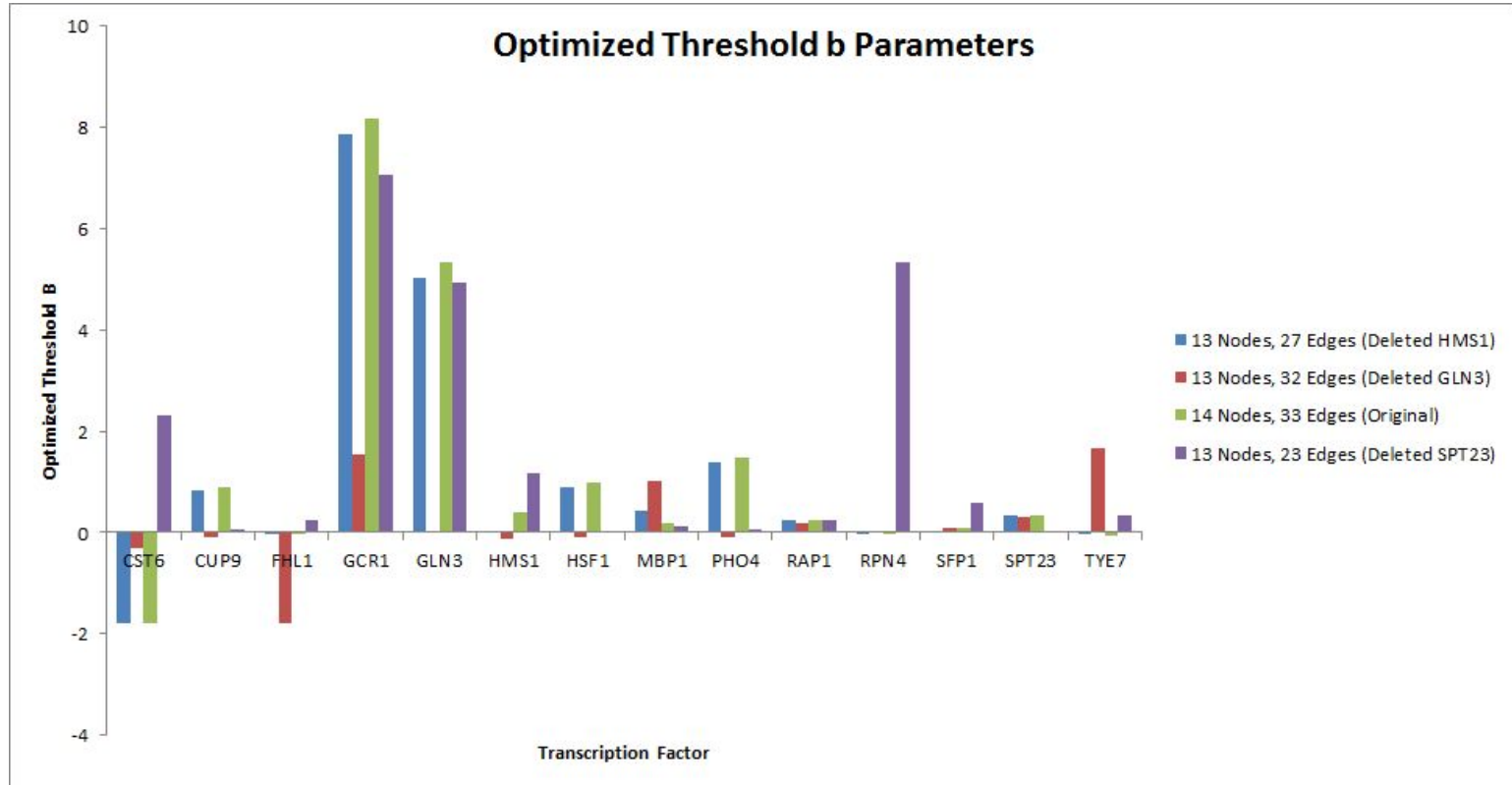


Deletion of GLN3 and SPT23 has Effects on Optimized Weights

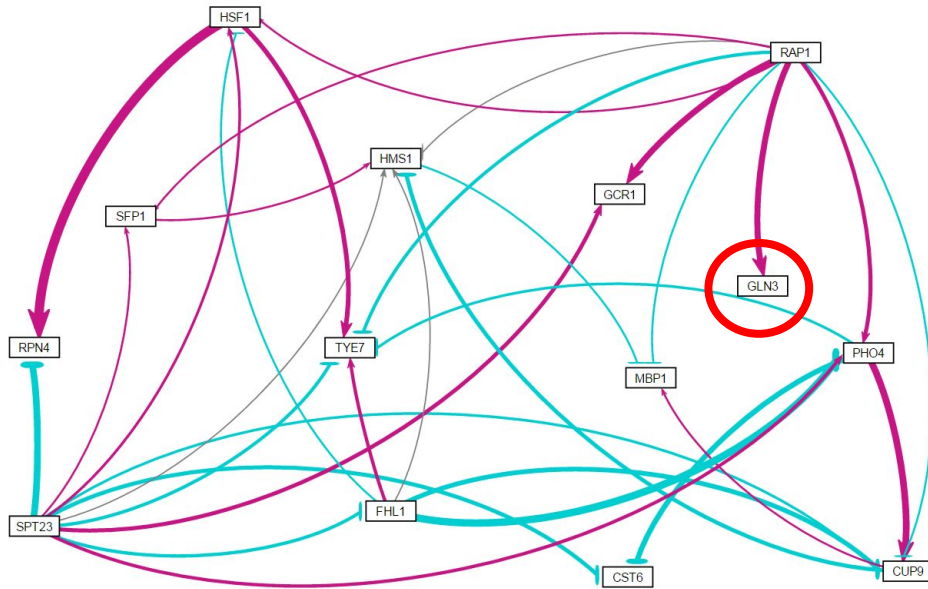
Optimized Weights of Four Networks



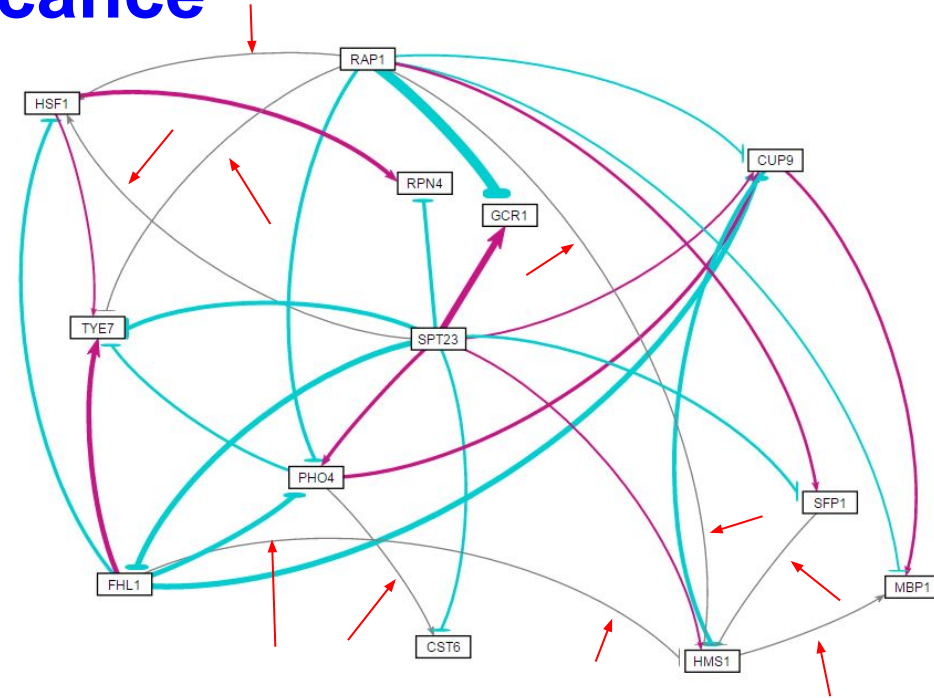
Deletions of GLN3 and SPT23 Affected the Optimized Threshold b Parameters



Deletion of GLN3 Changed the Network Significance

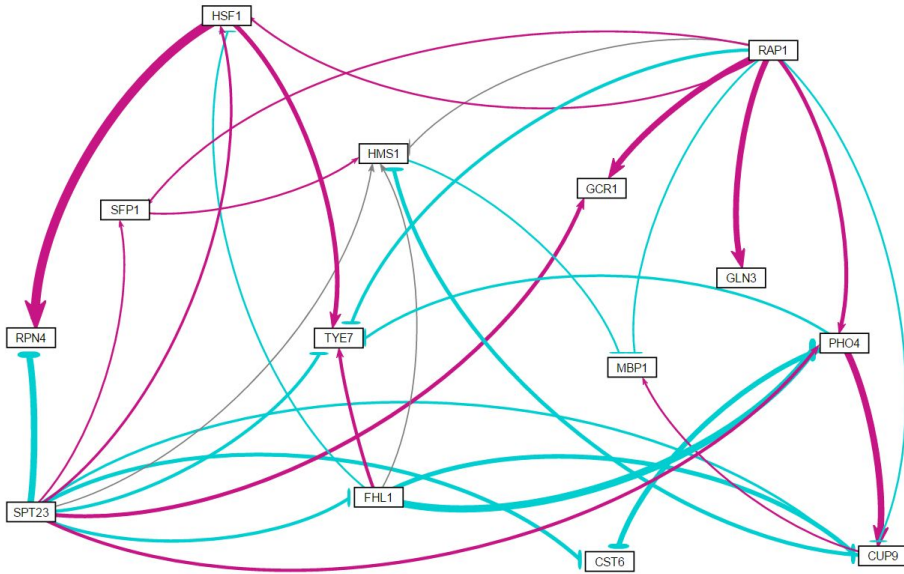


14 nodes, 33 edges

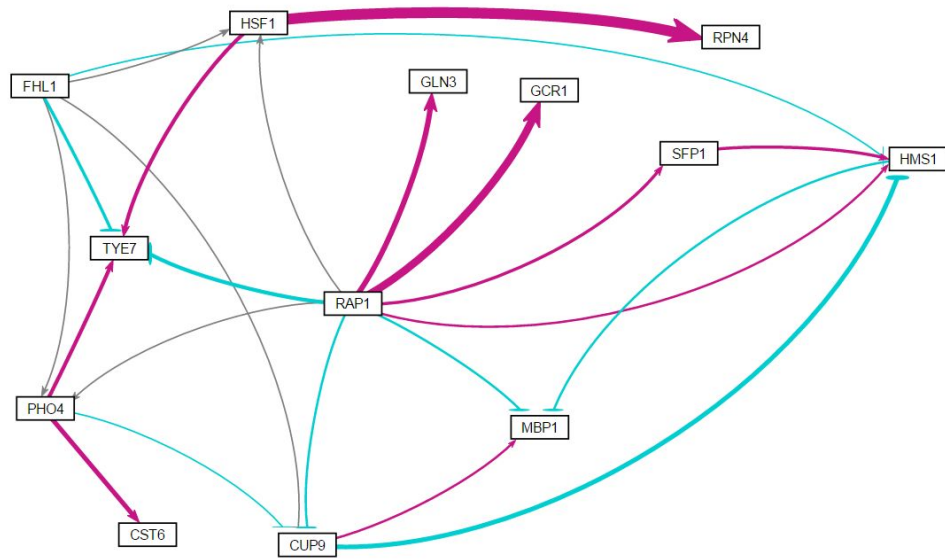


13 nodes, 32 edges

Deletion of SPT23 Led To Various Changes in the Network

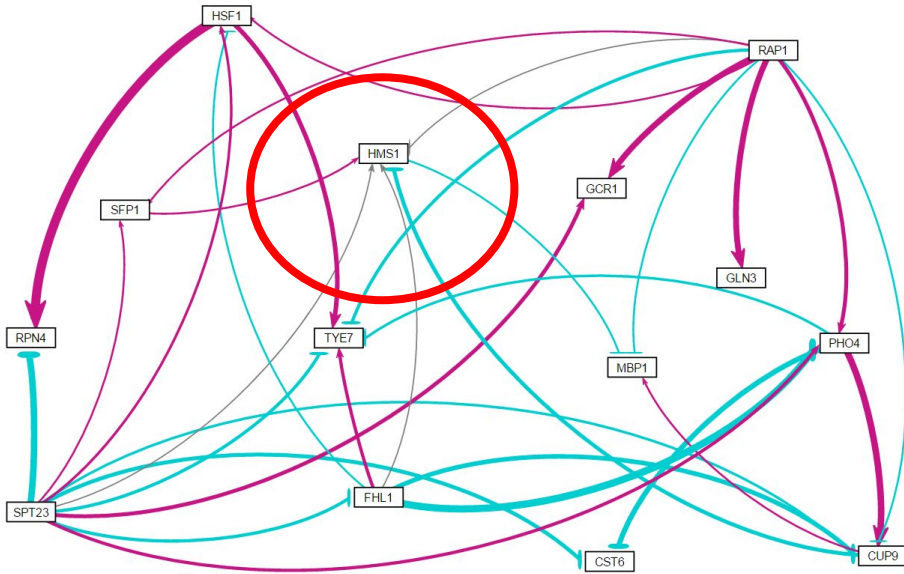


14 nodes, 33 edges

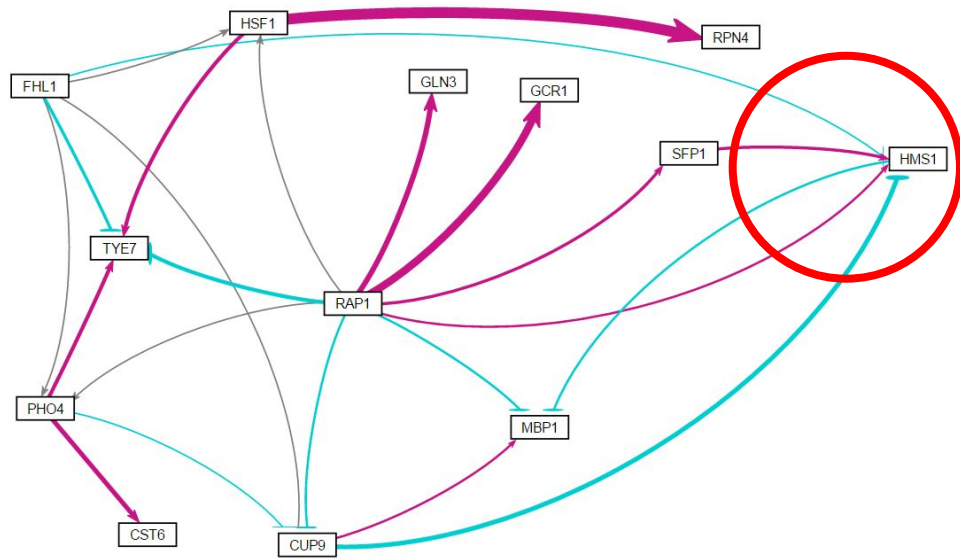


13 nodes 23 edges

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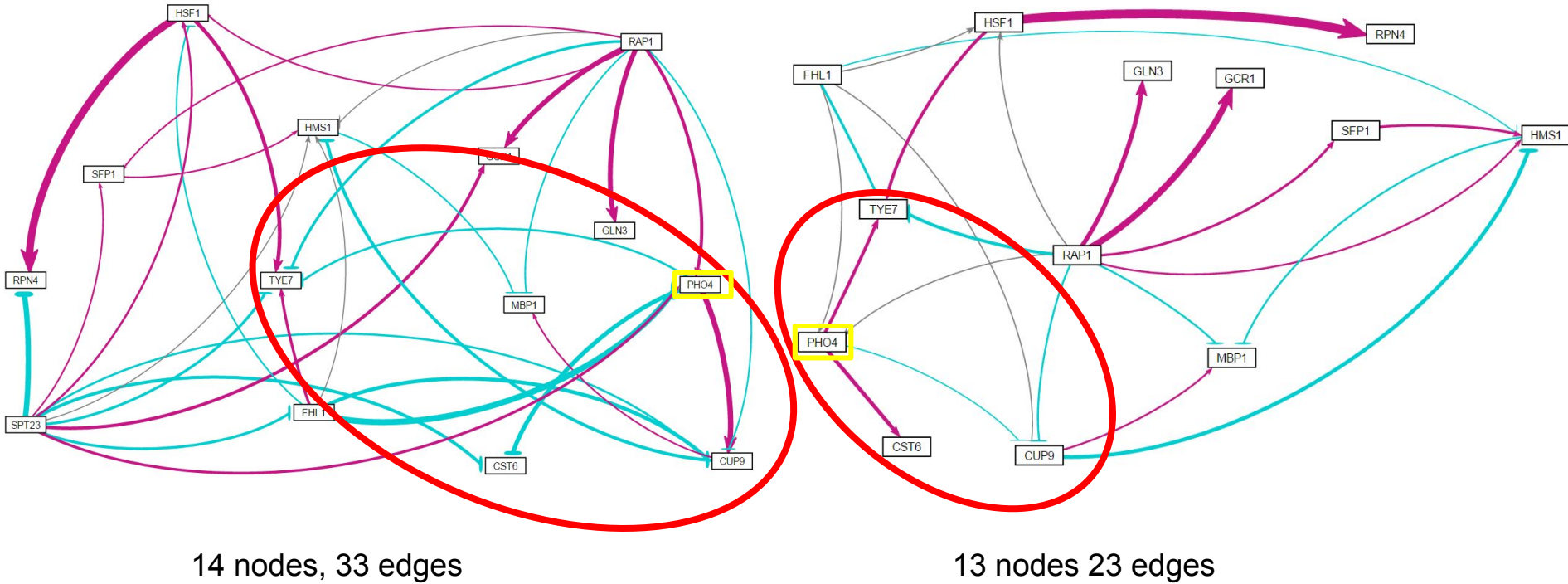


14 nodes, 33 edges

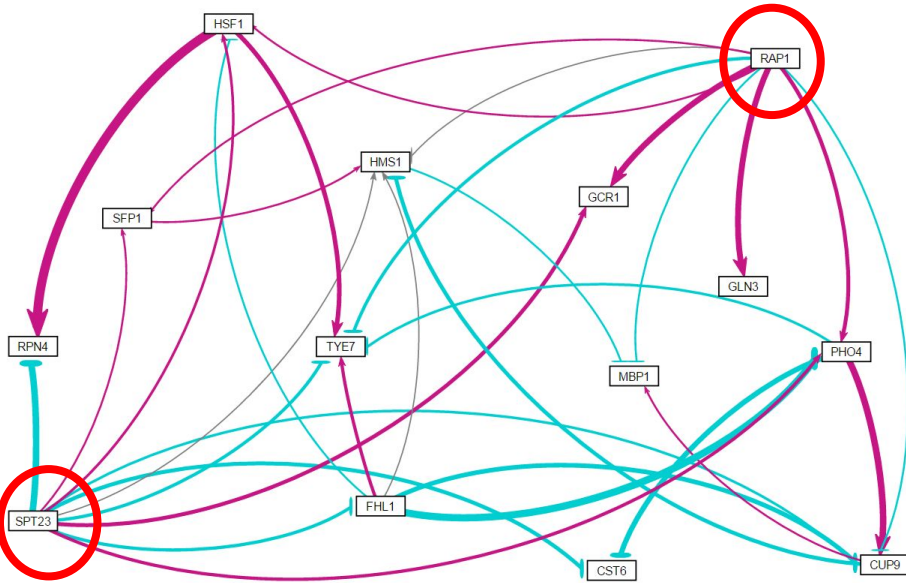


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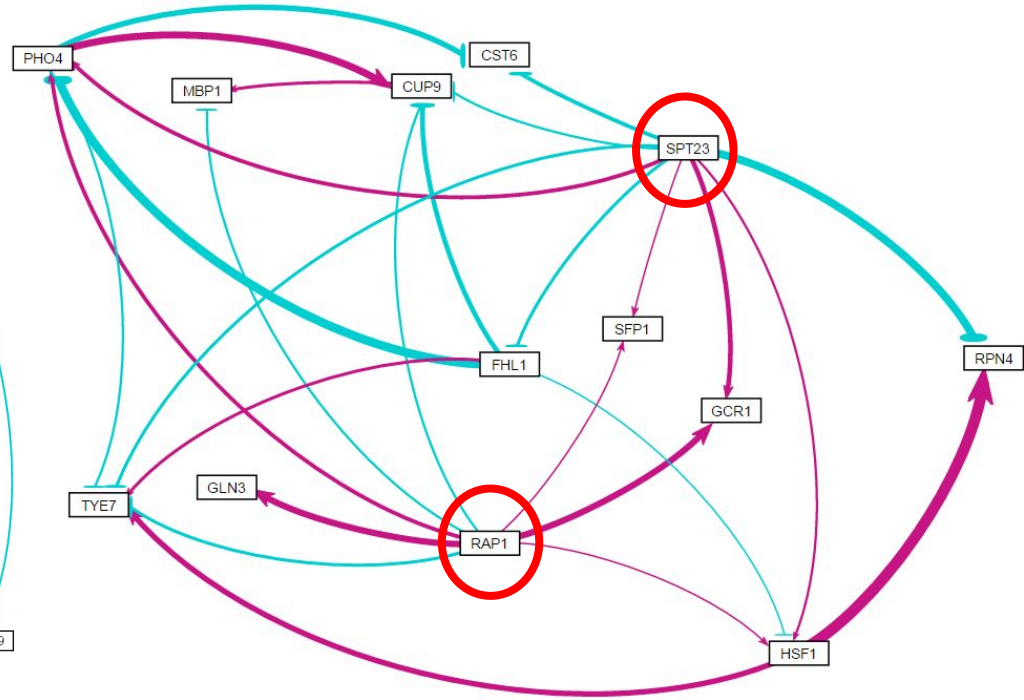
Deletion of SPT23 Led To Various Changes in the Network



Deleting HMS1 Did Not Affect Network



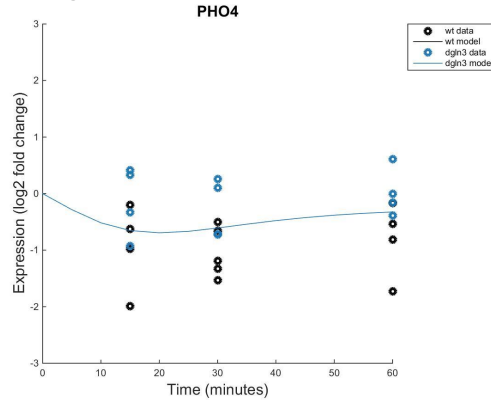
14 nodes 33 edges



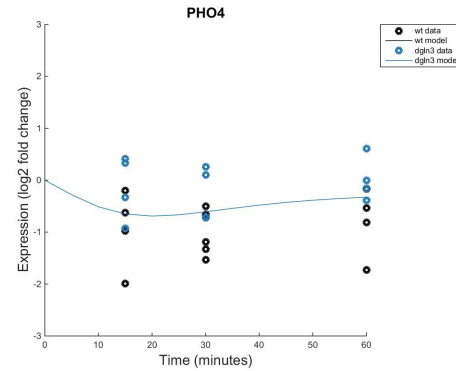
13 nodes 27 edges

PHO4 Expression and the SPT23 Deletion

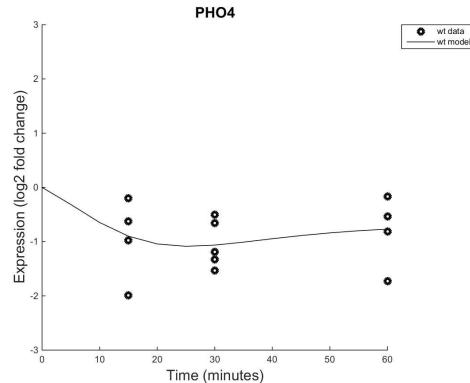
Original



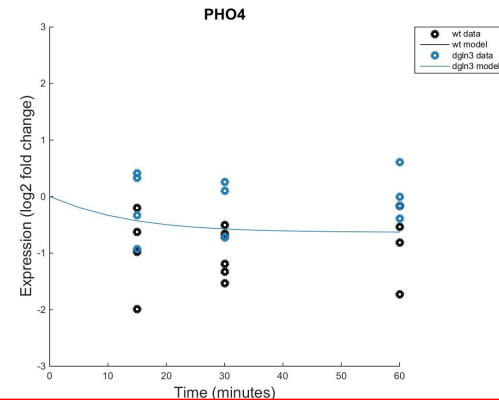
Deleted HMS1



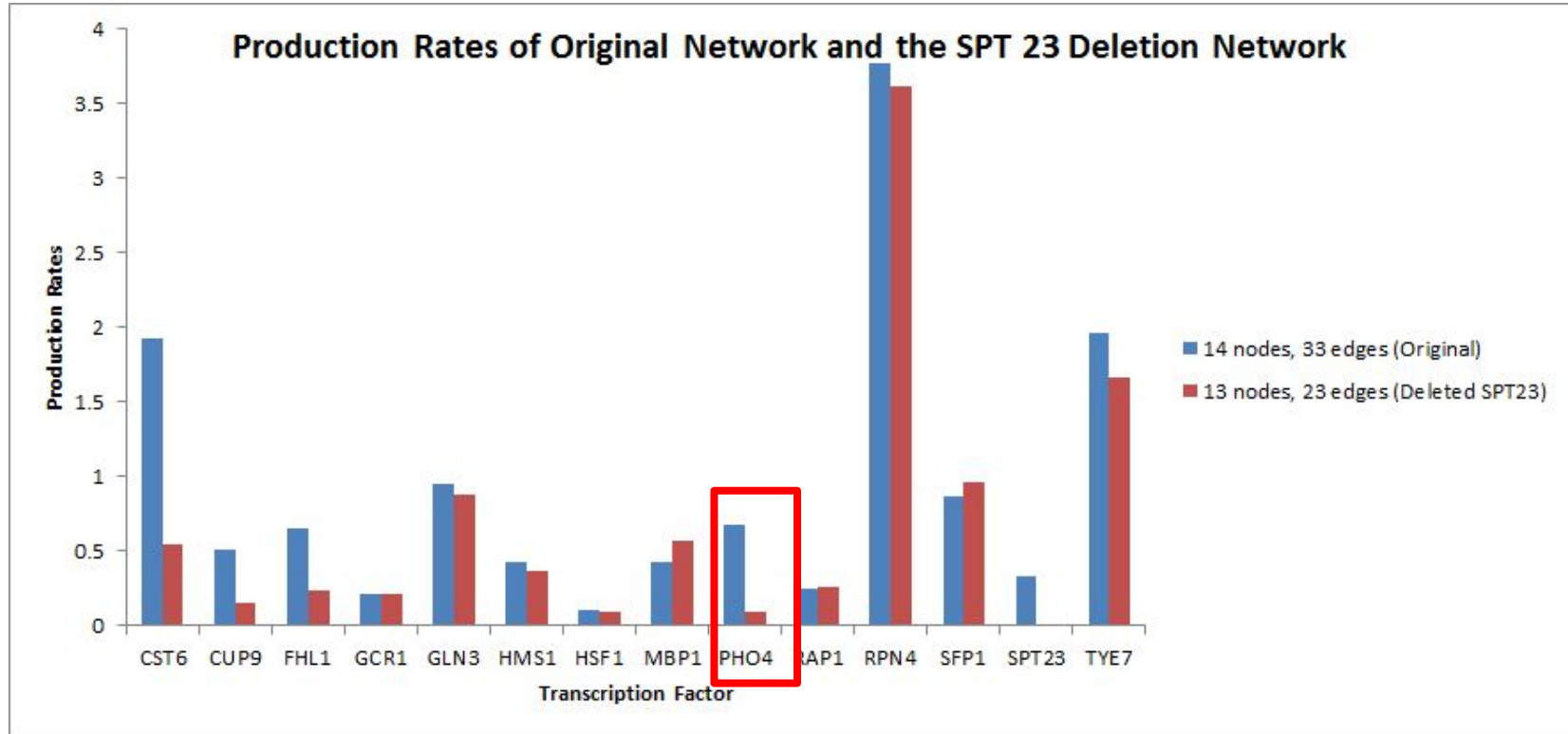
Deleted GLN3



Deleted SPT23



Production Rate of PHO4 is Reduced



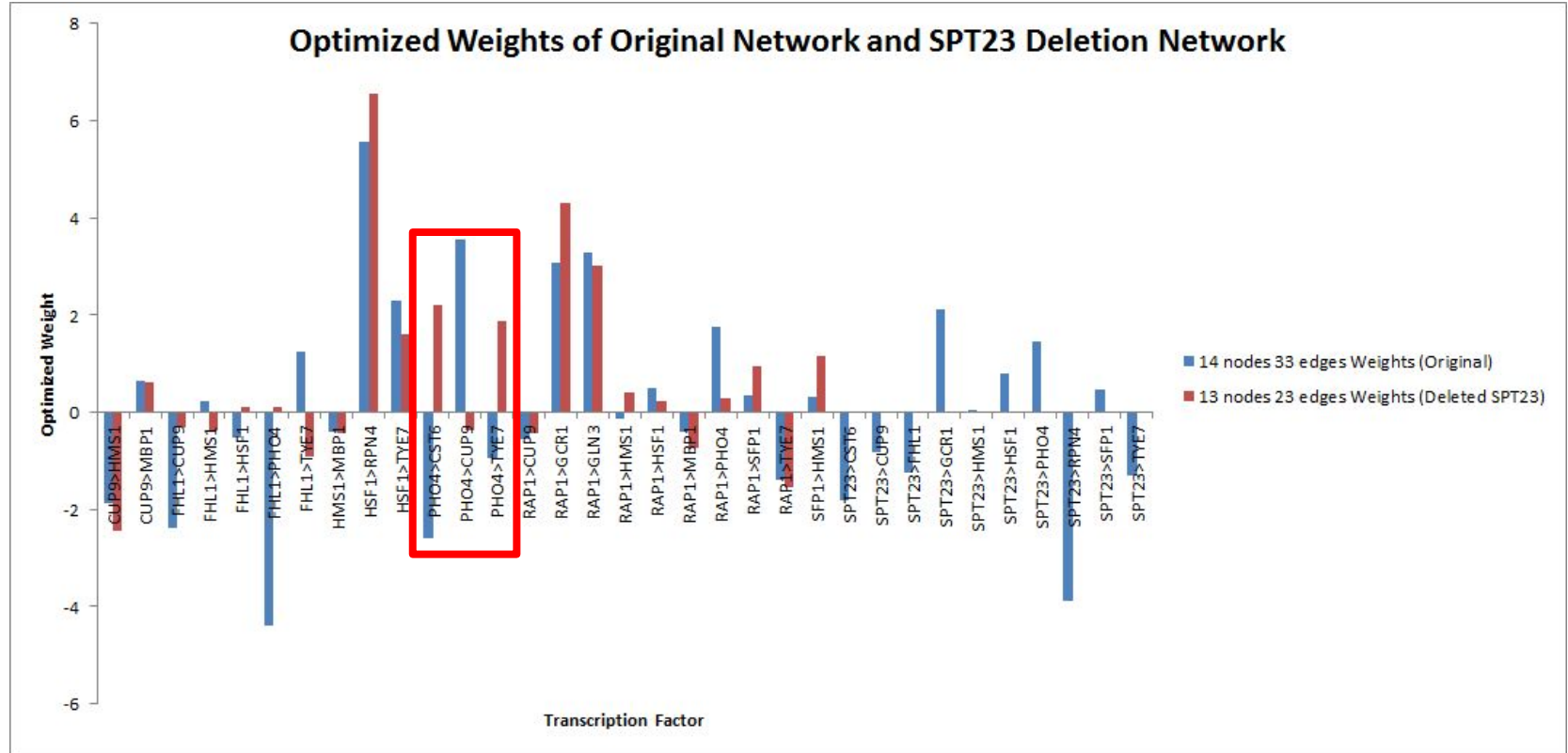
Original Rate: 0.676131091

Rate with SPT23 Deletion: 0.08752916



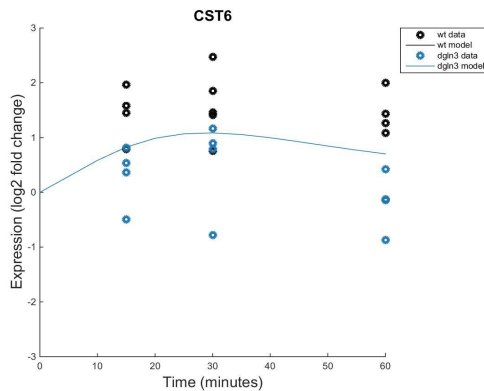
Production rate decreased by ~0.6

Weights Display the Change of Regulation of PHO4

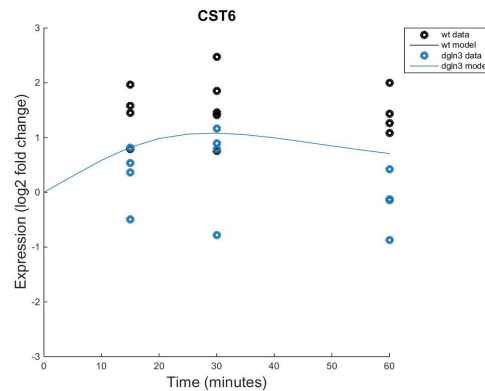


CST6 Affected By GLN3 Deletion

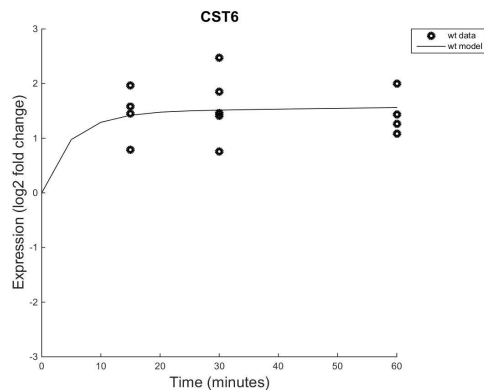
Original



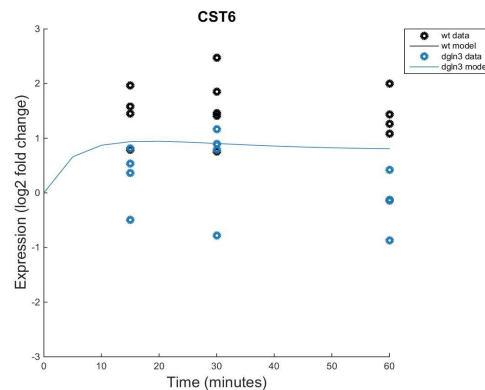
Deleted HMS1



Deleted GLN3



Deleted SPT23



SPT23 and GLN3 Appear to be Important in Regulating Cold Shock Response

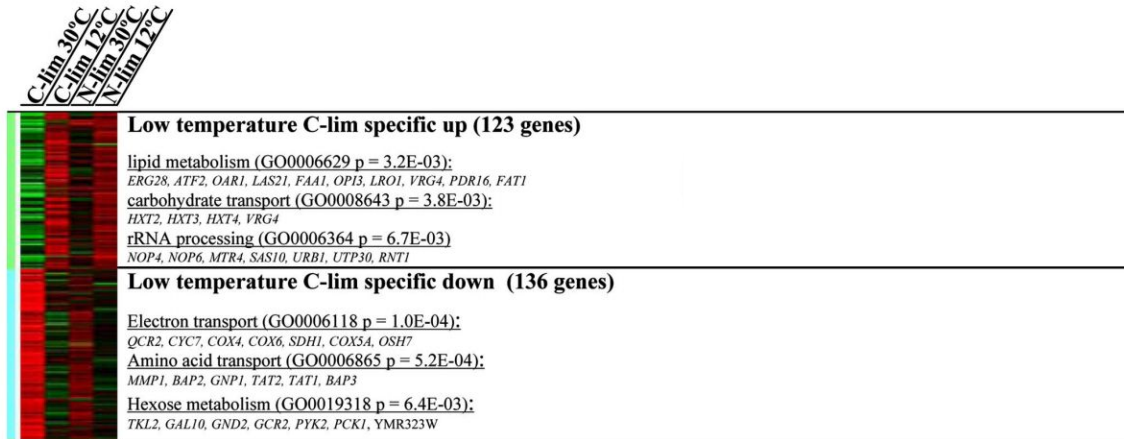
- While GLN3 was not significant according to YEASTRACT, the production rates/optimized weights bar charts and the GRNsight visualizations indicate that removing GLN3 changes the network.
- SPT23 consistently regulated many of the transcription factors and was a central part of the GRNsight visualization
 - The deletion of SPT23 changed various things about the network
- Future studies would include why GLN3 appears to have an effect despite the insignificant p-value from YEASTRACT and continuing to manipulate the model to see if other transcription factors play an important role in cold shock response

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Connection to Tai et al. (2007)

- Both Tai et al. (2007) and this project looked at how transcription factors related to specific gene ontology terms react to cold shock.
 - No overlap in transcription factors or GO terms
- Heat maps are another way to visualize the up-regulation and down-regulation that is seen in the GRNsight networks.
- Tai et al. (2007) did not include deletions of transcription factors.



Acknowledgments

Dr. Kam Dahlquist

Dr. Ben Fitzpatrick

LMU Department of Biology

LMU Department of Mathematics



References

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