Potassium Starvation in Yeast Triggers Changes in the Expression of Genes Related to Different Metabolic and Biosynthetic Pathways

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Outline

- Potassium crucial to cell
- Raw Data Required Manual Input
- Multiple significant changes found in genes
- Eight Significant Gene Clusters Observed
- Profile 39 was most significant
- Top Transcription Factor Genes Serve Importance in Cell
- 12 Gene Network
- Database
- Network Discovered

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Potassium is extremely important to normal cell function

- Potassium is the most abundant cation
- Without potassium balance life threatening conditions might occur
- Potassium homeostasis is required to regulate the cell
- Potassium helps to establish membrane potential
- Potassium limits growth when ammonium is the nitrogen source
- Ammonium toxicity is increased under high-potassium conditions
    - Caused by over-expression of ammonium transporters

Potassium is extremely important to normal cell function

- “**The Short-Term Response of Yeast to Potassium Starvation**” by Barreto, L., Canadell, D., Valverde-Saubí, D., Casamayor, A., & Ariño, J.
  - Study looked at potassium starvation in yeast cells for 10, 20, 40, 60, and 120 minutes in replicates of four
    - Found changes in gene expression impacted by potassium starvation
      - Oxidative Stress
      - Methionine/Cysteine Biosynthesis
      - Cyclin Levels
      - Septin Rings
      - Retrograde Pathway
      - Methylglyoxal production
      - Trehalose metabolism
  - No studies that directly correlated to work on potassium starvation prior to this study

Potassium is extremely important to normal cell function

- GRNmap and GRNsight help visualize the relationship between transcription factors during potassium starvation
- MS Access database will be used for present and future use of understanding and exploring new information from microarray data

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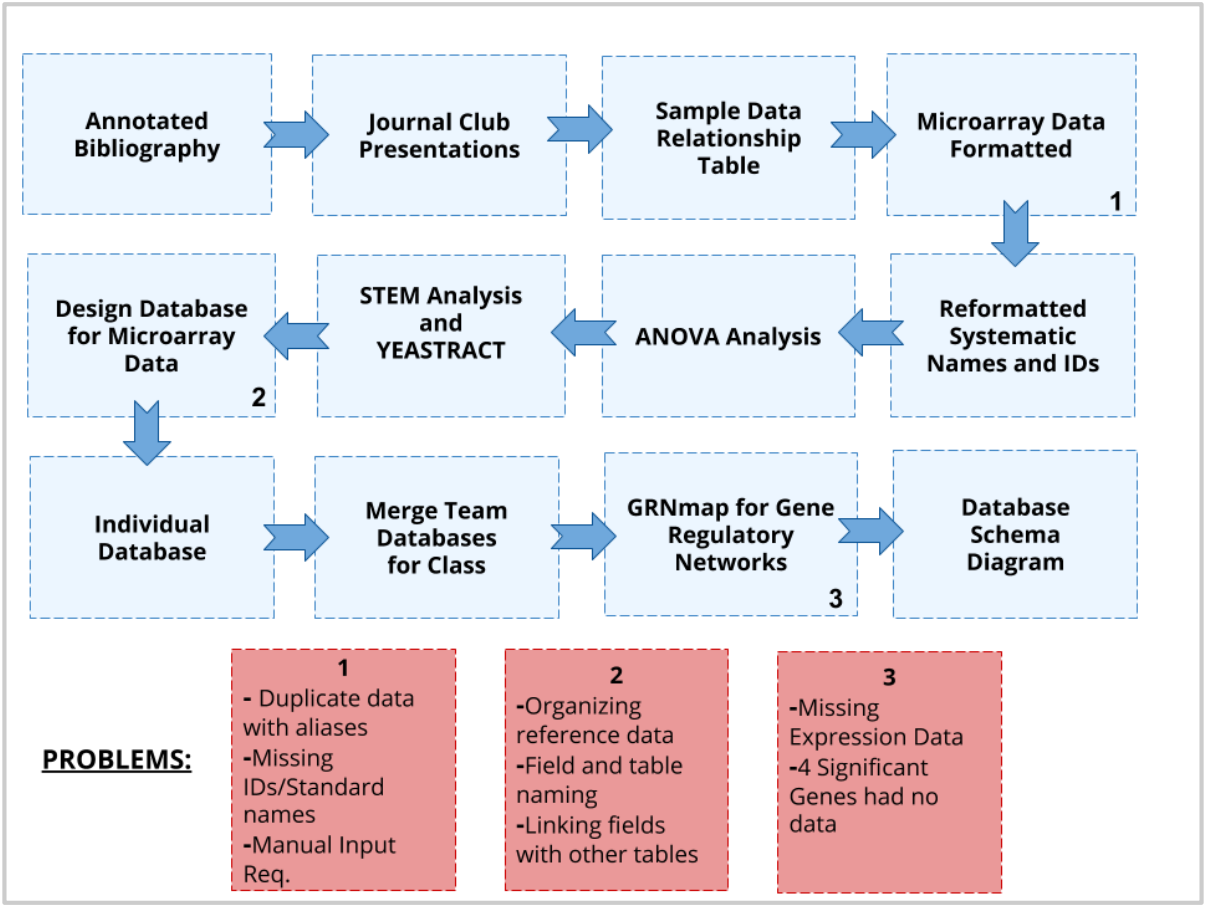
Complications with the Raw Data



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More than 5% of the Genes Had A Significant Change in Gene Expression

ANOVA	Percent of Genes
p < 0.05	2,985 (71.9 %)
p < 0.01	2,403 (57.9 %)
p < 0.001	1,703 (41 %)
p < 0.0001	1,198 (28.9 %)
B & H p < 0.05	2,839 (68.4 %)
Bonferroni p < 0.05	776 (18.7 %)

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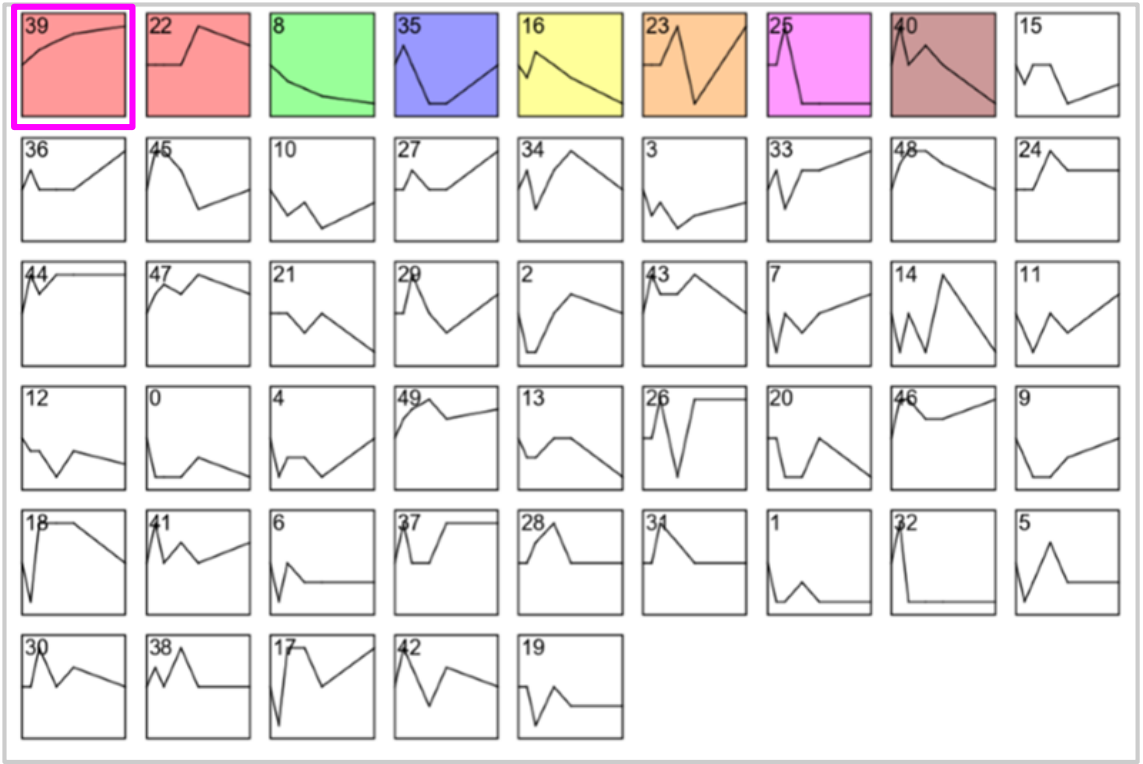
Eight Significant Gene Clusters Observed

Profile 39 was the most Significant Cluster

Profile 39 was used for the rest of the study



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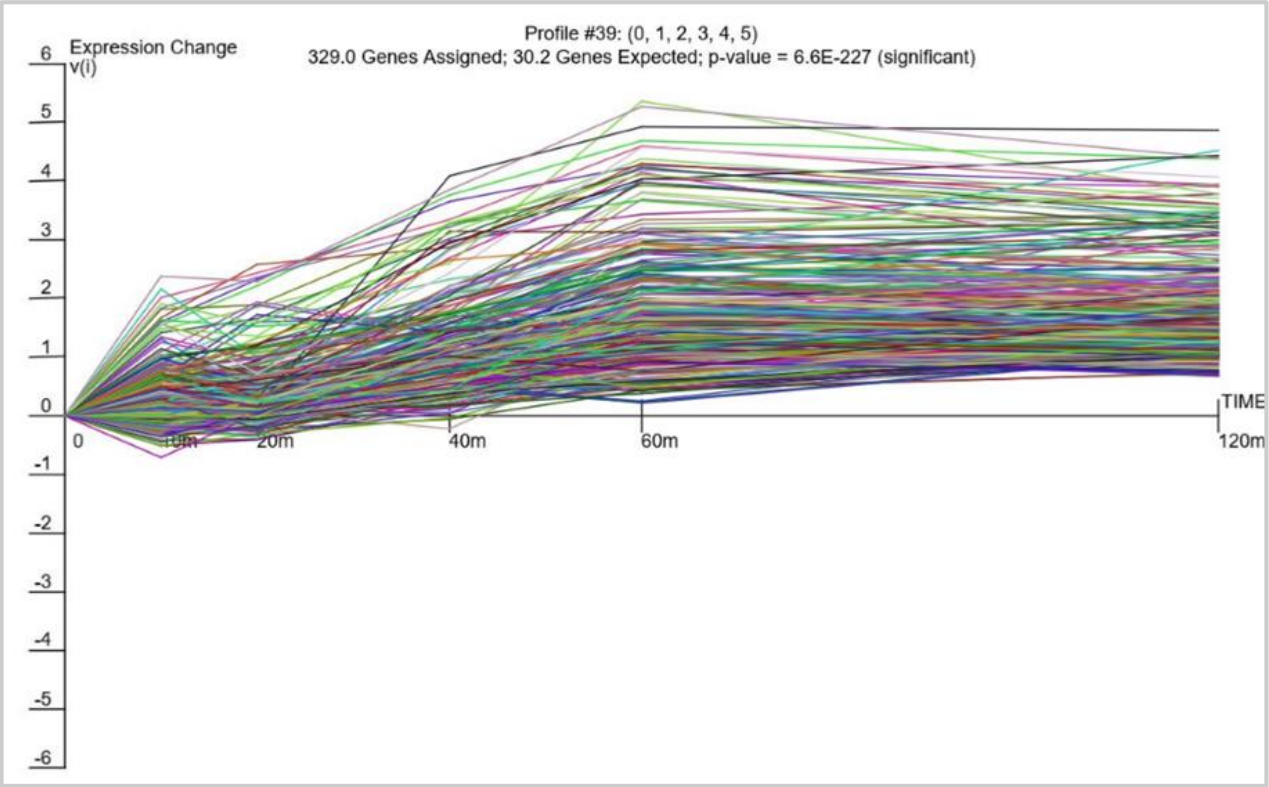
Gene Plot of Profile 39 Displayed Significant Changes at Every Time-Point



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F/C in Genes Associated with Cellular Functions

Category Name	#Genes Category	#Genes Assigned	#Genes Expected	#Genes Enriched	p-value	Corrected p-value	Fold
cysteine biosynthetic process	10	9	1.2	7.8	3.10E-08	<0.001	7.8
cellular amino acid biosynthetic process	67	24	7.8	16.2	1.40E-07	<0.001	3.1
methionine biosynthetic process	26	13	3	10	1.40E-06	<0.001	4.3
hydrogen sulfide biosynthetic process	6	6	0.7	5.3	2.30E-06	<0.001	8.6
molecular function	694	114	80.4	33.6	6.20E-06	<0.001	1.4
oxidation-reduction process	211	45	24.5	20.5	1.80E-05	0.004	1.8

Top 16 Significant Transcription Factor Genes From Yeastract

RPN4, PDR1, PDR3, HSF1 Removed

- Fold change data for these genes was not measured in the study
- Empty query results

Transcription Factor	% in s. cerevisiae
Gat1p	0.6079
Gcn4p	0.9878
Met4p	0.2827
Rpn4p	0.9635
Yrr1p	0.535
Pdr1p	0.9149
Pdr3p	0.9726
Hsf1p	0.5015

Transcription Factor	% in s. cerevisiae
Bas1p	0.7964
Hac1p	0.3951
Mga2p	0.3587
Rlm1p	0.2766
Sok2p	0.5927
Fhl1p	0.5137
Arr1p	0.3313
Gis1p	0.2462

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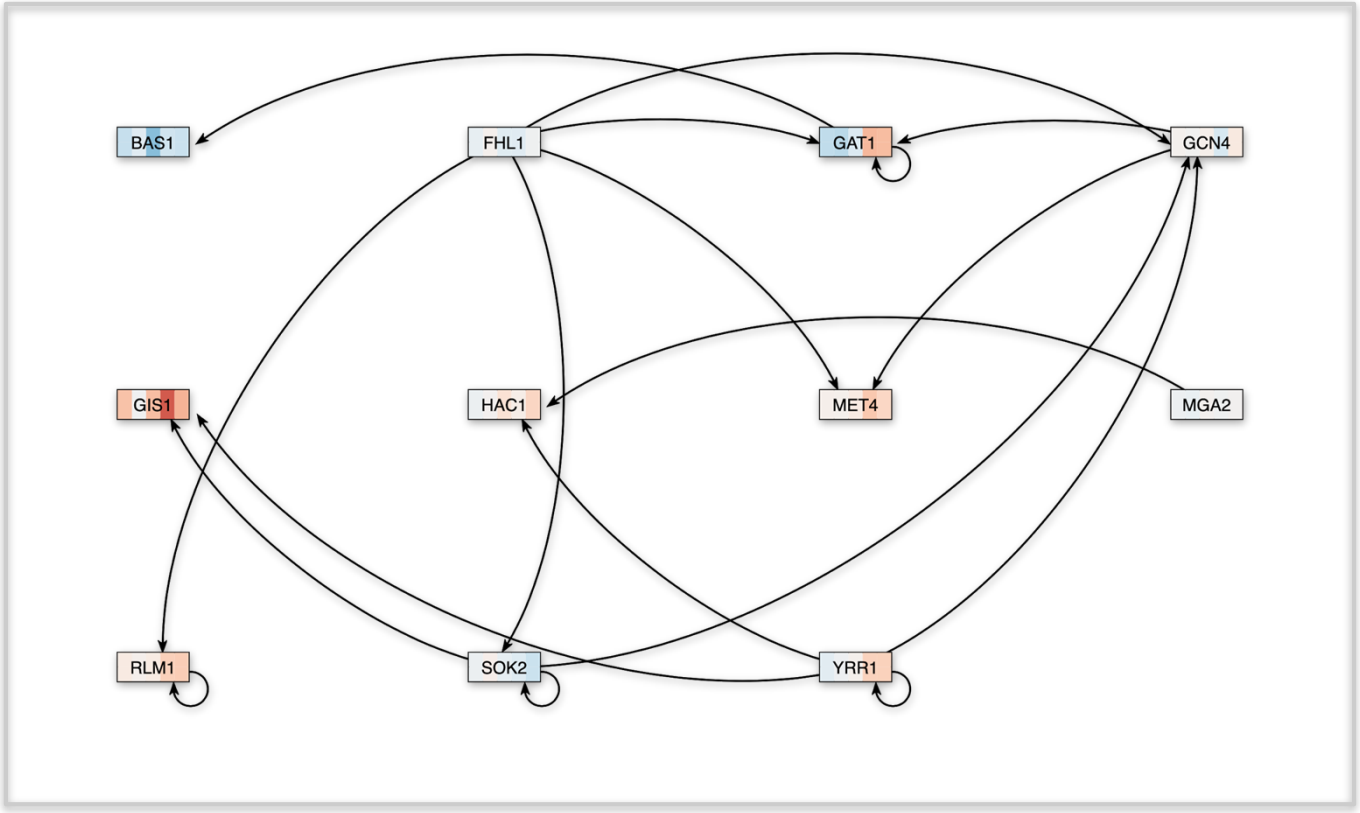
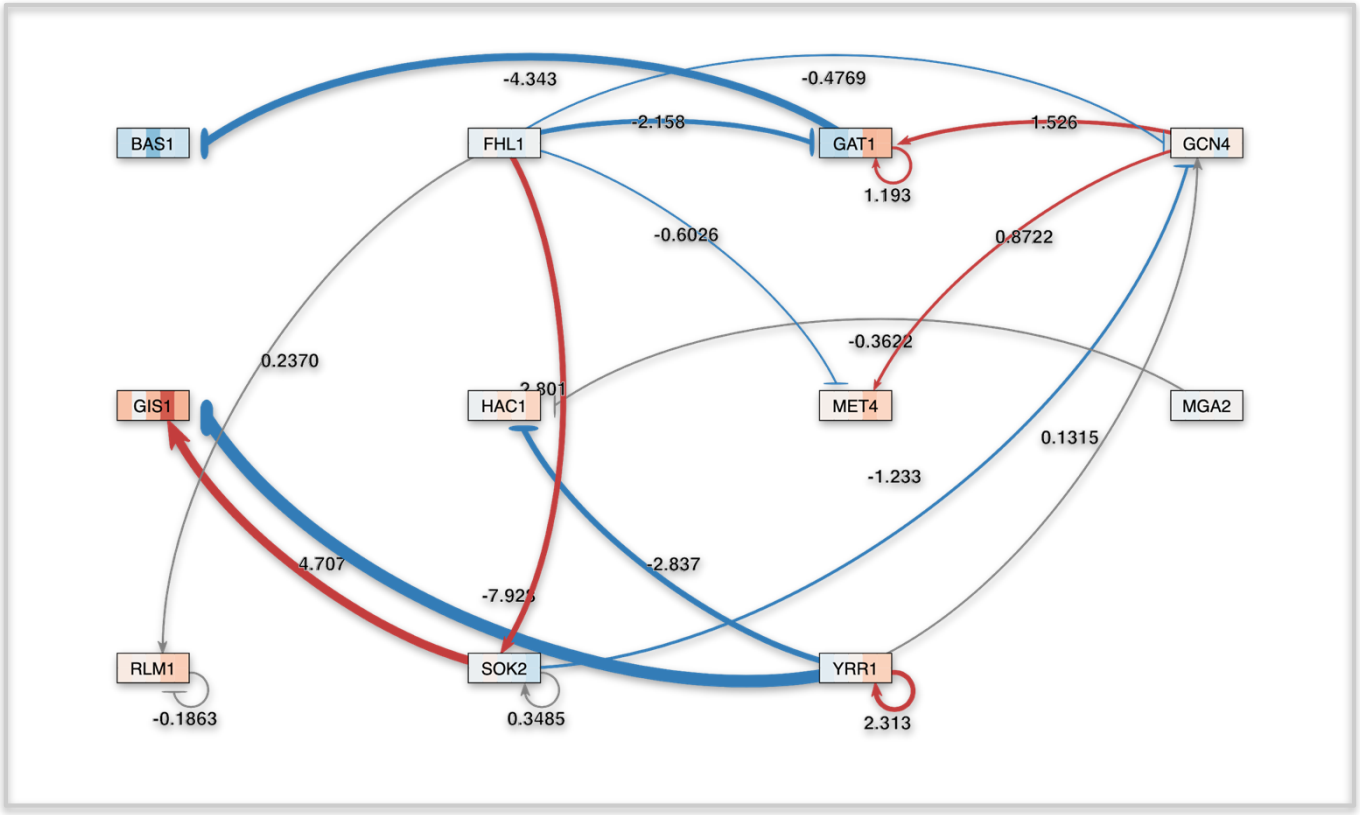
Regulatory Network Established Relationship between 12 Genes



Regulatory Network Established Relationship between 12 Genes



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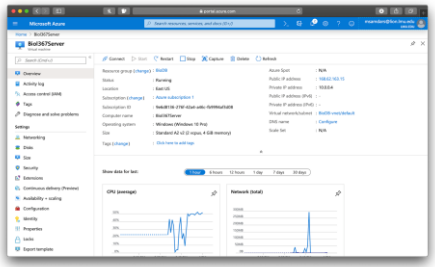
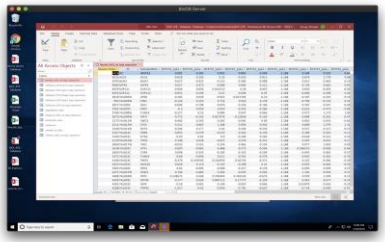
Database of DNA microarray data was created in Microsoft Access using existing database

Windows Virtual Machine - Microsoft Azure





Existing database utilized as starting point

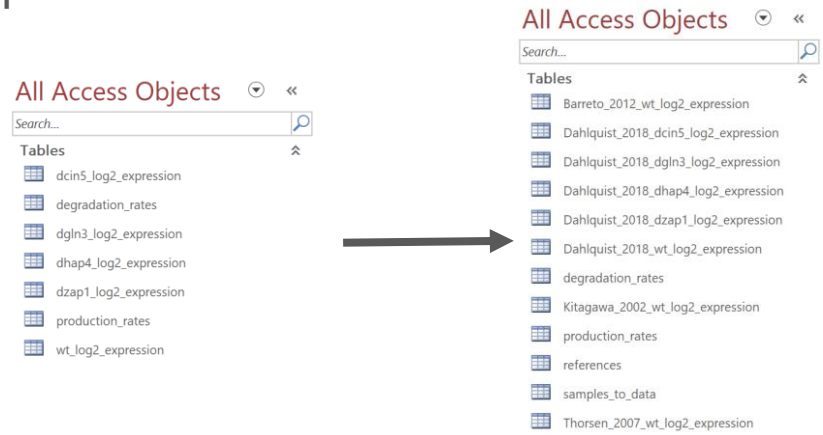
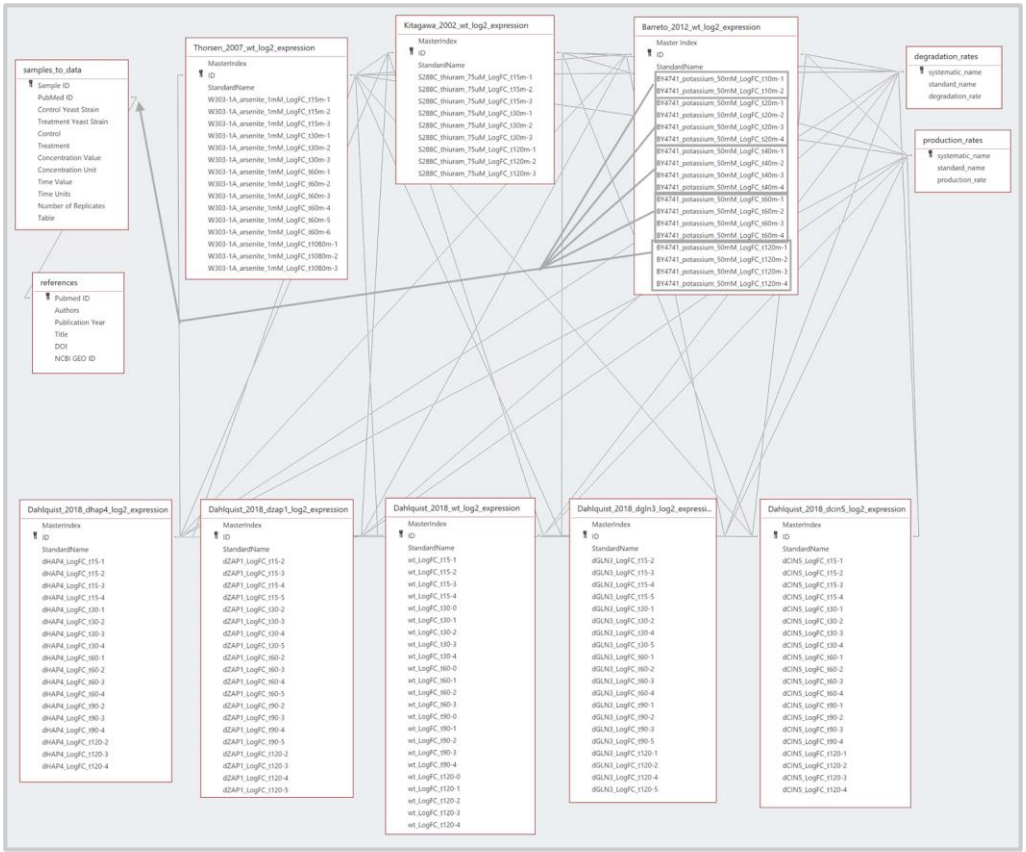


Diagram describes MS Access database schema



Two new tables were added containing data source metadata

`samples_to_data` and `references` Tables

samples_to_data

Field Name	Data Type
Sample ID	Number
PubMed ID	Number
Control Yeast Strain	Short Text
Treatment Yeast Strain	Short Text
Control	Short Text
Treatment	Short Text
Concentration Value	Number
Concentration Unit	Short Text
Time Value	Number
Time Units	Short Text
Number of Replicates	Number
Table	Short Text

references

Field Name	Data Type
Pubmed ID	Number
Authors	Short Text
Publication Year	Number
Title	Short Text
DOI	Short Text
NCBI GEO ID	Short Text

Field and table names were standardized across gene expression data tables

Gene Expression Data Table Name

Format: `[Author 1 Surname]_[Year]_[wt or mutant]_log2_expression`
Example: `Barreto_2012_wt_log2_expression`

Gene Expression Data Field Name (in Table)

Format: `[Yeast Strain]_LogFC_t[Time Point]-[Replicate Number]`
Example: `Barreto_2012_wt_log2_expression`

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A Gene Regulatory Network Was Induced in the Yeast by Potassium Starvation

- Potassium is proven to play crucial role in cell function
- Raw data needed multiple edits including deletion of aliases
- More than 5% of the genes had a significant change in gene expression
- Eight significant gene clusters
  - Profile 39 → most significant
  - Significant time points for every change
- Important relationship observed between 12 genes
  - 4/16 original top transcription factors had no data
  - Genes related to roles in cellular function

Genes Highlighted in Our Regulatory Network Are Absent in the Article

- Genes in the Article are associated with:
  - Oxidative Stress
  - Methionine/Cysteine Biosynthesis
  - Cyclin Levels
  - Septin Rings
  - Retrograde Pathway
  - Methylglyoxal production
  - Trehalose metabolism
- Our Network highlights genes associated with:
  - Amino Acid Biosynthesis
  - Methionine/Cysteine Biosynthesis
  - Hydrogen Sulfide Biosynthesis
  - Molecular Function
  - Oxidation-Reduction Processes

Network and GO Definitions Are Substantiated by Previous Studies

- Bas1 is a regulator of the Histidine Pathway and Purine Biosynthetic Pathway (Daignan-Fornier, et al., 19920)
- Fhl1 functions as a suppressor of mutations in RNA Polymerase III (Hermann-Le Denmat, et al., 1994)
- Gat1 and Fhl1 involved in similar metabolic pathways (Bandhakavi, 2008)
- Gcn4 regulates gene expression during amino acid starvation in yeast (Natarajan, et al., 2010)
  - Activator of Gat1, Met4, Bas1
- Sok2 and Gis1 cooperate in regulating the promoter of TPK1 (Pautasso, Rossi, 2014)

Network and GO Definitions Are Substantiated by Previous Studies

- Met4 related to sulfur metabolism and binding factor both needed for transcriptional activation (Thomas et al., 2004)
- Hac1 and Mga2 found to be related in cellular function but exact relationship unknown (Covino et al. 2018)
- Rlm1 delays the transition from G1 to S in cell cycle (Piccirillo et al., 2017)
- Yrr1 responds to oxidative stress (Nadai et al., 2016)
- Gls1 induces other metabolic genes in catabolic pathways (Wang et al., 2011)

Discovering More About Network

- Finding the 4 missing gene expressions
- How the 12 significant genes affect:
  - Other cations and the effects on the cell
  - Gene expression
  - Cellular Function in relation to homeostasis and membrane potential

Acknowledgments

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