

# ***Saccharomyces cerevisiae* Acclimation to Cold Shock Revealed Change in Expression of Genes Involved With Ribosome Biogenesis, Mitosis, and Nitrogen Catabolism**

**Acclimation of *Saccharomyces cerevisiae* to Low Temperature:  
A Chemostat-based Transcriptome Analysis. Tai, S. L.,  
Daran-Lapujade, P., Walsh, M. C., Pronk, J. T., & Daran, J. M.  
(2007) *Molecular Biology of the Cell*, 18, 5100-5112.**

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BIOL 368: Bioinformatics Laboratory  
December 13, 2016

# Outline

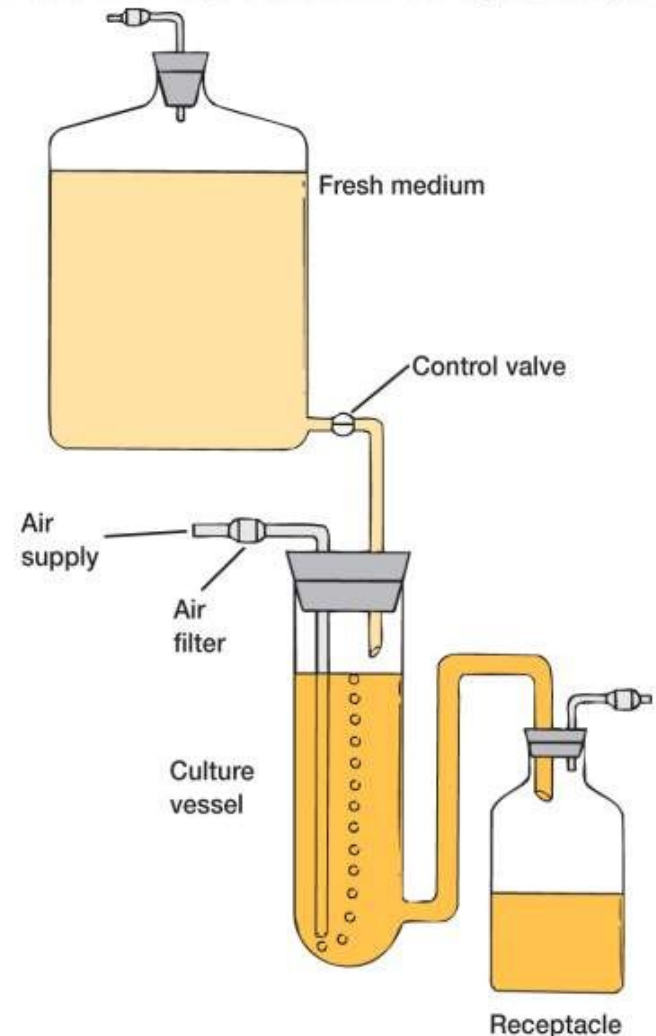
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# Chemostat Culture Allows For Fixed Experimental Conditions

- Chemostat culture involves the continuous addition of fresh medium.
- Allows for control of growth factors.
- Specific growth rate can be measured accurately.
- Batch culture is a closed system which does not allow for accurate measurement of specific growth rate.



# **Low-temperature Acclimation and Cold Shock Have Differing Results**

- **Cold shock entails a rapid decrease to near freezing temperatures.**
- **Low-temperature acclimation is the biological response of cells to steady decreases to near freezing temperatures.**
- **Trehalose is required for transcription in low temperature situations.**
  - **Not present in low temperature acclimation results.**

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# *S. Cerevisiae* Grown In Varying Conditions of Temperature and Nutrient Content

| Limiting nutrient | Growth temperature (°C) | $Y_{\text{Glu}/X}$<br>( $\text{g}_{\text{DW}} \cdot \text{g}_{\text{glucose}}^{-1}$ ) | $q_{\text{Glu}}^a$ | $q_{\text{EtOH}}^a$ | $q_{\text{CO}_2}^a$ | Carbon recovery (%) | Residual glucose (mM) | Residual ammonia (mM) |
|-------------------|-------------------------|---|--------------------|---------------------|---------------------|---------------------|-----------------------|-----------------------|
| Glucose           | 12                      | $0.07 \pm 0.01$   | $-2.5 \pm 0.2$     | $3.8 \pm 0.3$       | $4.4 \pm 0.3$       | $100 \pm 3$         | $2.8 \pm 1.1$         | $65.2 \pm 2.2$        |
| Glucose           | 30                      | $0.07 \pm 0.00$   | $-2.3 \pm 0.0$     | $3.5 \pm 0.0$       | $3.8 \pm 0.2$       | $95 \pm 1$          | $0.3 \pm 0.1$         | $61.3 \pm 4.5$        |
| Ammonium          | 12                      | $0.05 \pm 0.00$   | $-3.6 \pm 0.2$     | $6.1 \pm 0.3$       | $6.0 \pm 0.6$       | $97 \pm 4$          | $90.0 \pm 9.8$        | $1.5 \pm 0.2$         |
| Ammonium          | 30                      | $0.04 \pm 0.00$   | $-4.0 \pm 0.1$     | $6.8 \pm 0.2$       | $7.4 \pm 0.2$       | $97 \pm 2$          | $85.1 \pm 8.2$        | $0.2 \pm 0.1$         |

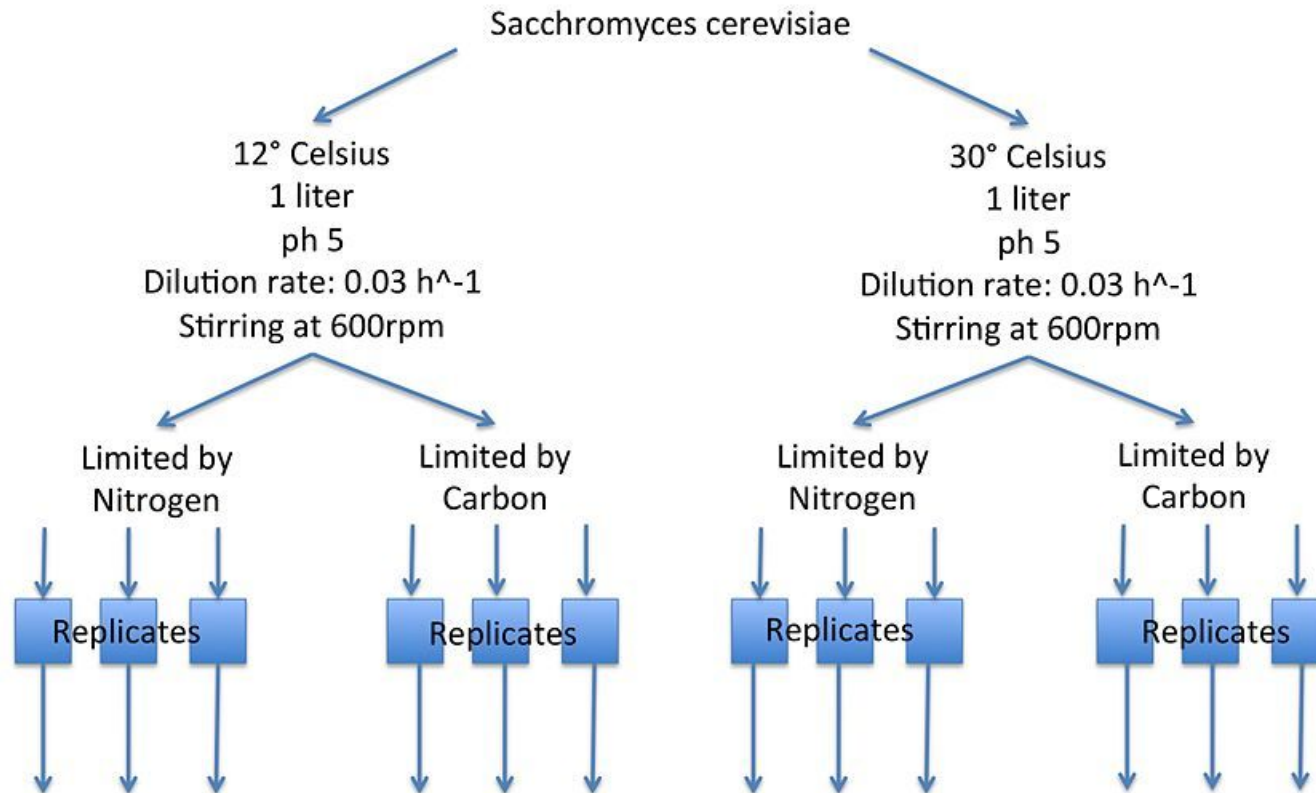
Cultures were grown at 30 and 12°C ( $D = 0.03 \text{ h}^{-1}$ ). Values represent the mean  $\pm$  SD of data from three independent steady-state chemostat cultivations.  $Y_{\text{Glu}/X}$ , biomass yield on glucose; DW, dry weight.

<sup>a</sup> Values expressed as  $\text{mmol} \cdot \text{g}_{\text{DW}}^{-1} \cdot \text{h}^{-1}$ .

Table 1

- *S. Cerevisiae* is minimally affected by variation in temperature.
- Higher temperatures correlated with lesser amounts of a limiting nutrient corresponding residual residue.
  - Larger amounts of residual glucose at 12°C connected to possible contamination of gene sets.

# *S. Cerevisiae* Grown In Varying Conditions of Temperature and Nutrient Content



Microarrays



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# Temperature and Nutrient Limitations Were Analyzed Using DNA Microarray Analysis

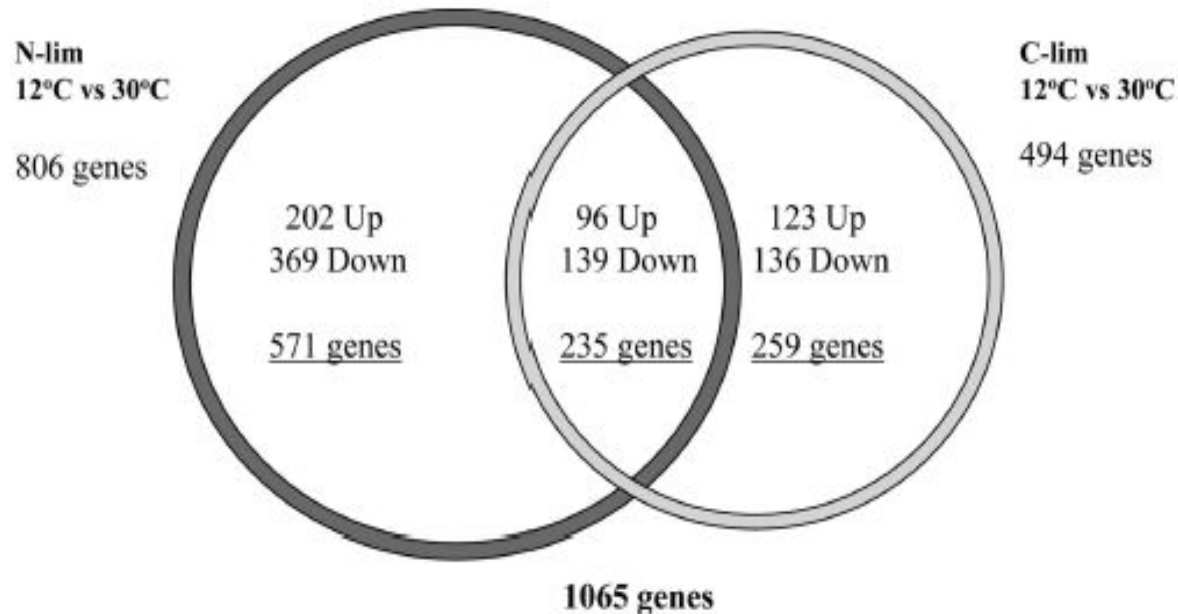
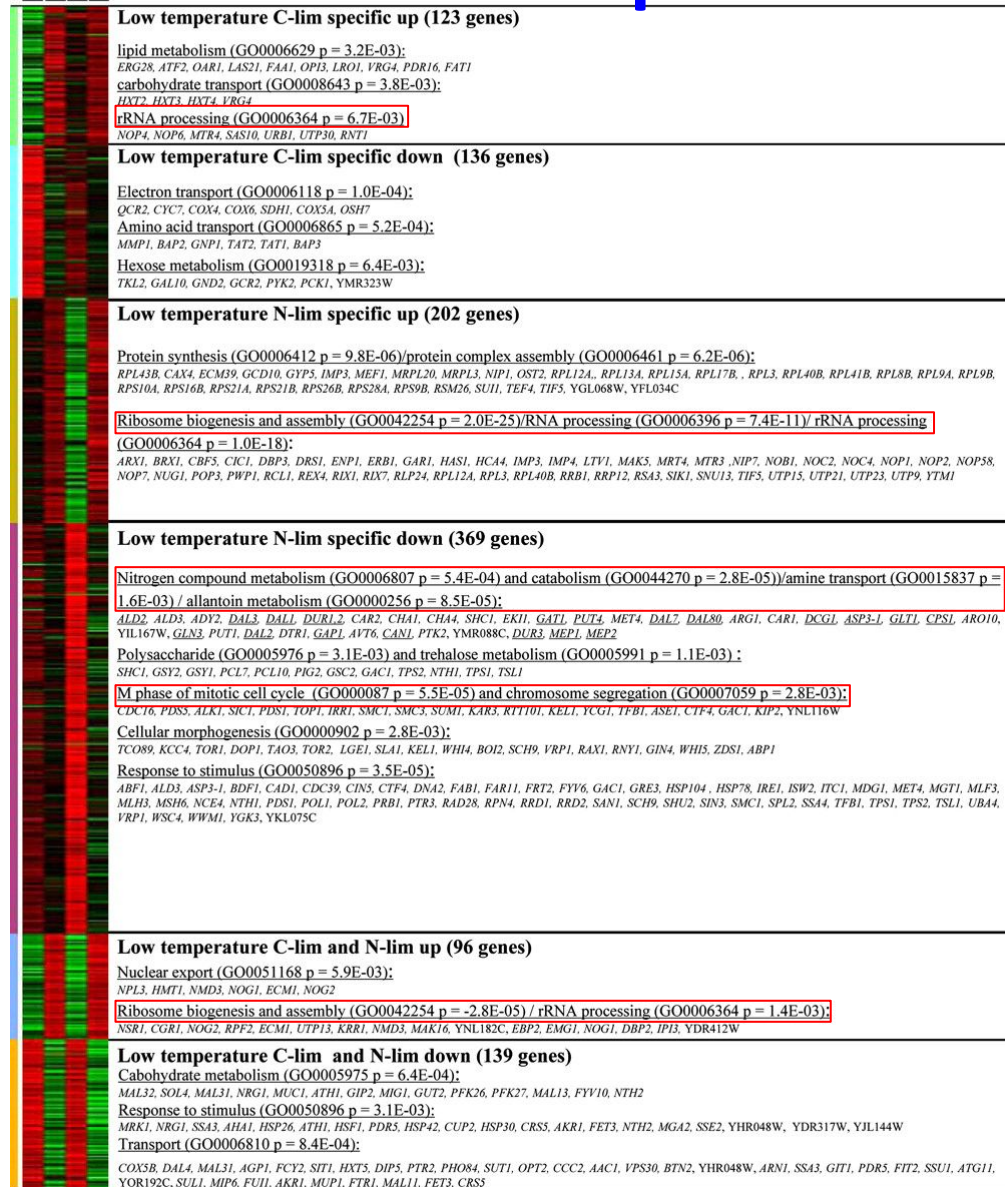


Figure 1

- 1065 represents the number of temperature responsive genes in *S. cerevisiae* genome.

# Heat Mapping Was Used To Identify Transcription Level of Temperature Responsive Genes

C-lim 30°C  
C-lim 12°C  
N-lim 30°C  
N-lim 12°C



- Goal was to identify regulatory networks involved in acclimation of *S. cerevisiae* to low temperature.
- Observed changes in
  - Transporters of limiting nutrient
  - Translational machinery
- NCR genes and catabolite repression underlined

Figure 2

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# *S. cerevisiae* Stores Protein and Carbohydrate In Chemostat Culture

| Limiting nutrient | Growth temperature (°C) | Biomass dry weight (g <sub>DW</sub> · l <sup>-1</sup> ) | Whole cell protein (g <sub>protein</sub> · g <sub>DW</sub> <sup>-1</sup> ) | Biomass nitrogen content (mg <sub>nitrogen</sub> · g <sub>DW</sub> <sup>-1</sup> ) | Trehalose (g <sub>equivalent glucose</sub> · g <sub>DW</sub> <sup>-1</sup> ) | Glycogen (g <sub>equivalent glucose</sub> · g <sub>DW</sub> <sup>-1</sup> ) |
|-------------------|-------------------------|---|--|--|--|---|
| Glucose           | 12                      | 1.71 ± 0.09   | 0.40 ± 0.01  | nd   | <0.005   | 0.06 ± 0.01   |
| Glucose           | 30                      | 1.89 ± 0.06   | 0.43 ± 0.01  | nd   | 0.02 ± 0.00  | 0.04 ± 0.00   |
| Ammonium          | 12                      | 2.27 ± 0.05   | 0.47 ± 0.03  | 63 ± 3   | <0.005   | 0.02 ± 0.00   |
| Ammonium          | 30                      | 3.53 ± 0.01   | 0.34 ± 0.01  | 41 ± 2   | 0.04 ± 0.00  | 0.05 ± 0.01   |

Cultures were grown at 30 and 12°C (D = 0.03 h<sup>-1</sup>). Values represent the mean ± SD of data from three independent steady-state chemostat cultivations. DW, dry weight; nd, not determined.

Table 2

- Trehalose and glycogen biosynthesis occurs under stress conditions.
  - Biosynthesis varies with temperature.
- Reason for storage of carbohydrates in nonfreezing temperature is unclear.
  - Trehalose was not produced in tps1 and tps2 strains, yet did not affect viability.

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# Promoter Analysis Reveals Regulation Trends in Gene Regulation

(A) 5' upstream cis-regulatory motif

| Regulatory cluster                | Motif name | Putative-binding protein | Promoter element | occ <sup>a</sup> | Expected occ <sup>b</sup> | occ E <sup>c</sup> |
|-----------------------------------|------------|--------------------------|------------------|------------------|---------------------------|--------------------|
| Low Temperature C-lim Up          | —          | —                        | —                | —                | —                         | —                  |
| Low Temperature C-lim Up          | —          | —                        | —                | —                | —                         | —                  |
| Low Temperature N-lim Up          | —          | —                        | —                | —                | —                         | —                  |
|                                   | PAC        | —                        | TGAAAAA          | 206              | 113.04                    | 2.30E-11           |
|                                   | —          | —                        | CGATGAG          | 57               | 17.49                     | 6.1E-14            |
|                                   | —          | —                        | TGAGATG          | 49               | 16.3                      | 4.1E-07            |
| Low Temperature N-lim Down        | GATAA      | Gln3/Gat1/Dal80/Gzf3     | AGATAAG          | 203              | 102.57                    | 3.1E-15            |
|                                   | STRE       | Msn2/Msn4                | ACCCCTT          | 29               | 8.73                      | 1.6E-03            |
| Low Temperature C- and N-lim Up   | PAC        | —                        | CGATGAG          | 30               | 8.39                      | 5.0E-05            |
| Low Temperature C- and N-lim Down | —          | —                        | CGTCCAC          | 13               | 2.85                      | 7.8E-03            |

(B) Overrepresentation of transcription factors (TF) binding targets

| Regulatory cluster                | Factor     | p value | K <sup>d</sup> | P <sup>e</sup> |
|-----------------------------------|------------|---------|----------------|----------------|
| Low Temperature C-lim Up          | Mbp1p      | 1.6E-03 | 10             | 65             |
| Low Temperature C-lim Down        | Hap2-Hap1  | 3.9E-05 | 3              | 4              |
|                                   | Hap3-Hap1  | 9.9E-06 | 3              | 3              |
| Low Temperature N-lim Up          | Phl1p      | 3.4E-05 | 19             | 203            |
|                                   | Sfp1p      | 1.3E-03 | 7              | 51             |
| Low Temperature N-lim Down        | Gln3p      | 2.1E-07 | 20             | 92             |
|                                   | Gln3-Dal82 | 5.5E-07 | 8              | 15             |
|                                   | Hap2-Dal82 | 6.8E-05 | 5              | 9              |
| Low Temperature C- and N-lim Up   | Aft2p      | 7.5E-04 | 10             | 34             |
| Low Temperature C- and N-lim Down | Hsf1p      | 3.0E-08 | 16             | 133            |
|                                   | Nrg1p      | 7.6E-07 | 14             | 128            |
|                                   | Phd1p      | 3.3E-04 | 9              | 99             |
|                                   | Rcs1p      | 1.1E-04 | 9              | 86             |
|                                   | Rox1p      | 6.0E-05 | 8              | 62             |
|                                   | Sok2p      | 5.7E-05 | 9              | 79             |
|                                   | Nrg1-Aft2  | 6.0E-05 | 5              | 20             |
|                                   | Phd1-Nrg1  | 1.4E-05 | 7              | 37             |
|                                   | Rox1-Phd1  | 7.8E-05 | 5              | 21             |
|                                   | Sok2-Nrg1  | 4.3E-07 | 8              | 33             |

(A) Significantly overrepresented *cis*-regulatory binding motifs in 5' upstream regions. (B) Significantly overrepresented promoter elements that bind known transcription factors (TF) or TF pairs according to ChiP-on-chip analysis (Harbison *et al.*, 2004). C-Lim, glucose-limited; N-Lim, ammonium-limited.

<sup>a</sup> The number of occurrences of promoter element in the regulatory cluster.

<sup>b</sup> Expected number of occurrences of the promoter element in a randomly chosen cluster of genes of the same cluster size.

<sup>c</sup> The probability of finding the number of patterns with the same level of overrepresentation, which would be expected by chance alone.

<sup>d</sup> Number of genes in category in cluster.

<sup>e</sup> Number of genes in category in genome.

- STRE elements exist in promoters of downregulated genes in N-limited cultures
- Upregulation in both conditions show PAC regulatory motifs in promoters
- Common promoter sequences show possible regulation relationships

Table 3

# Genes Common to All Researchers Were Categorized According to Regulation

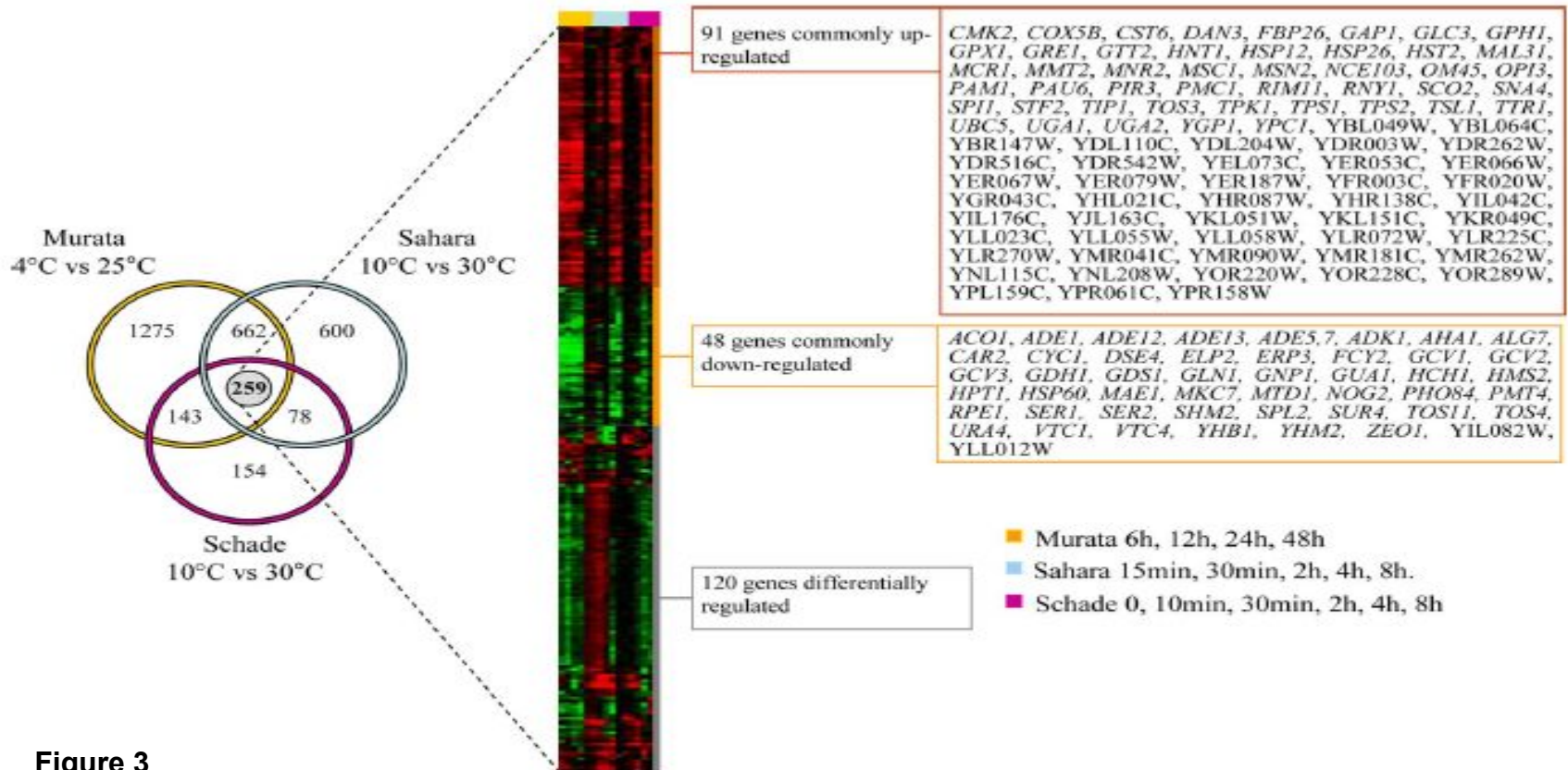


Figure 3

- Sahara and Schade tested cold shock, dropping temperature to between 10 and 20 degrees Celsius
- Murata tested cold shock at temperatures below 10 degrees Celsius, which caused cell growth to cease.



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# Overlapping Genes Showing the Same Temperature Response

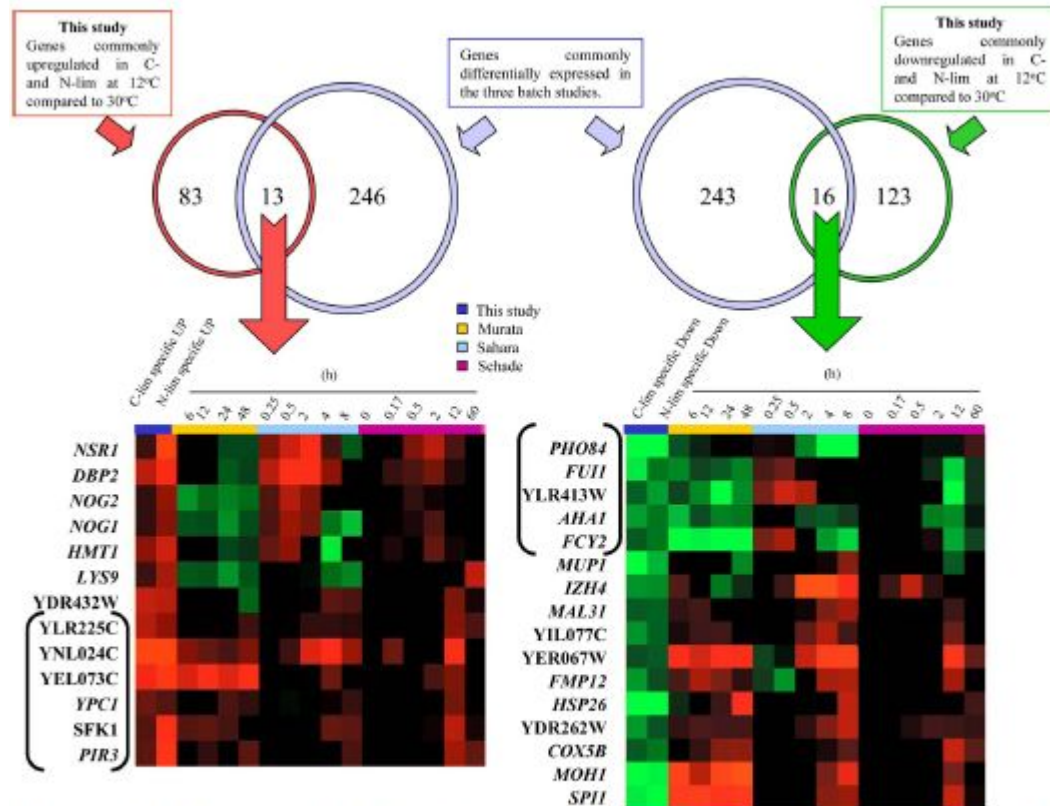


Figure 4

- Chemostat study compared with Sahara et al. 2002, Schade et al. 2004, and Murata et al. 2006
- 29 genes total were in common, only 11 showed consistency

# Few Genes Simultaneously Respond to Temperature and Growth Rate

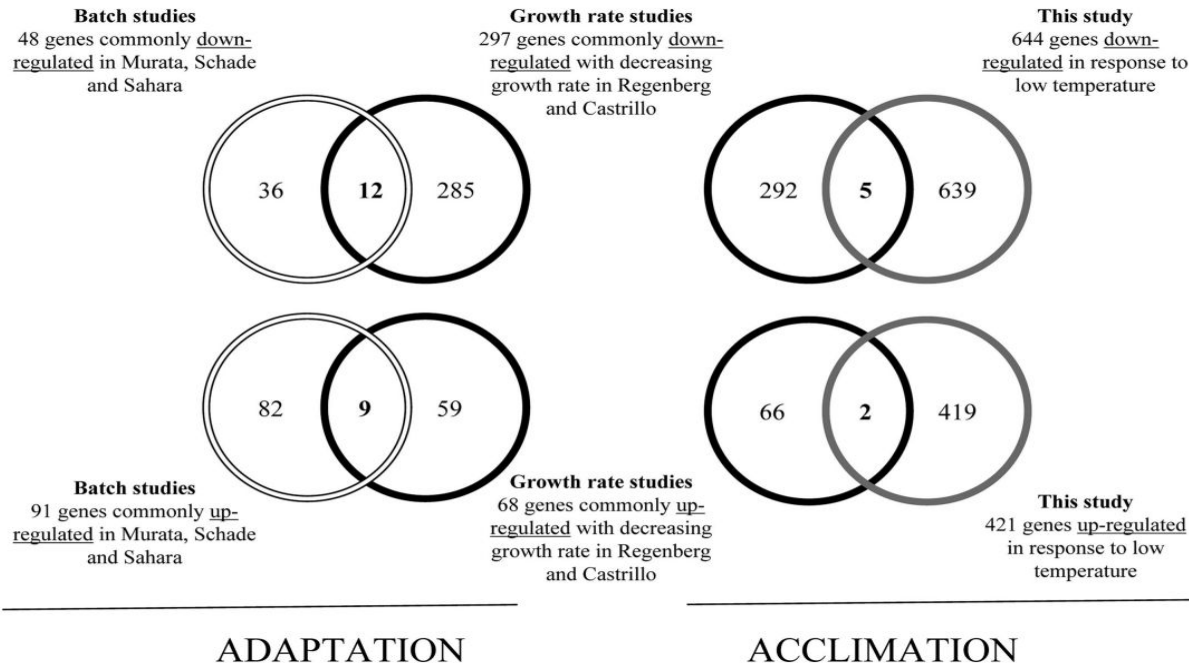


Figure 5

- Studies examined changes in gene regulation in response to a decrease in growth rate compared to both the batch studies and study data for overlap in gene regulation.
- A very small number of genes overlap in response to both temperature decrease and growth rate decrease.

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# One Third of Low Temperature Response Genes in the Batch Studies are Attributed to ESR

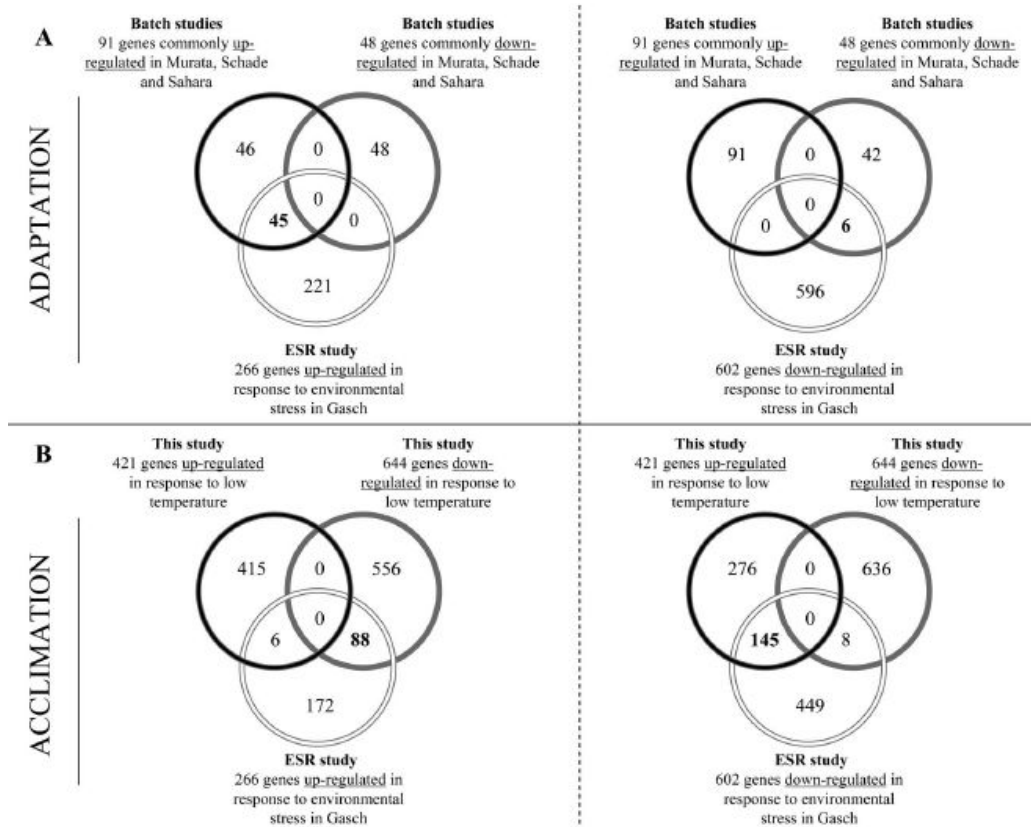


Figure 6

- ESR: environmental stress response
  - General mechanism responds to multiple stimuli
- Batch and chemostat genes compared with Gasch et al. 2000
- Some genes showed opposite results in response to low temperatures

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# Experimental Design Differences Distinguish Dahlquist Lab Data from Tai et al. Data

|                    | Dahlquist Lab                        | Tai et al.                                       |
|--------------------|--------------------------------------|--|
| <b>Temperature</b> | Cold Shock: 13°C<br>Recovery: 30°C   | Cold Shock: 12°C<br>Recovery: 30°C               |
| <b>Time Points</b> | 15, 30, 60, 90, 120 min.             | “Random Sampling”                                |
| <b>Media</b>       | YPD rich media                       | Synthetic media limited by carbon or by nitrogen |
| <b>Replicates</b>  | 3-5 replicates                       | 3 replicates                                     |
| <b>Strain</b>      | <i>S. cerevisiae</i> , $\Delta$ gln3 | <i>S. cerevisiae</i> strain CEN.PK113-7D         |

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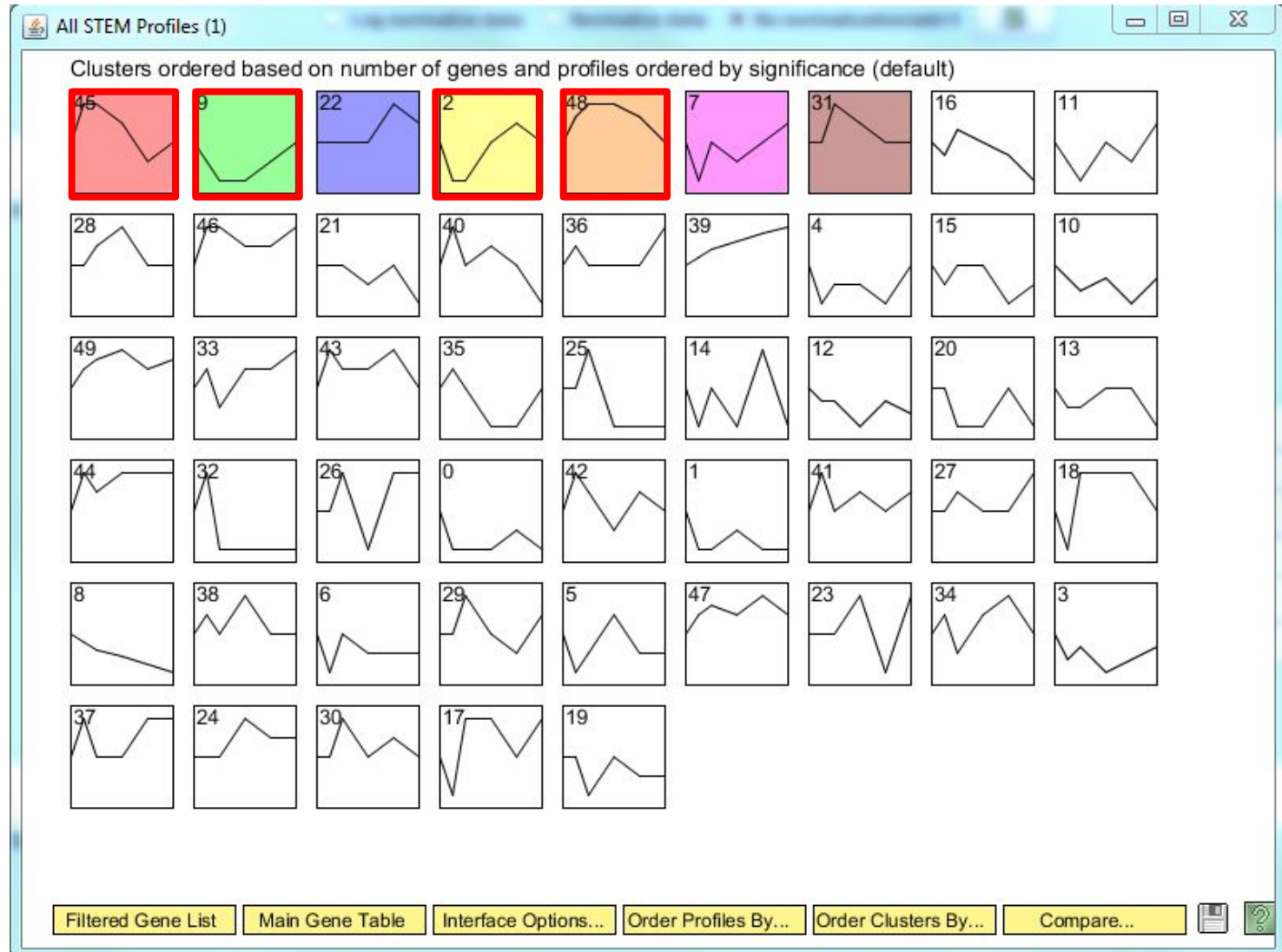
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## 25% of $\Delta$ gln3 Genes Showed Significant Difference In Expression Over Time

| ANOVA                 | <i>dGLN3</i> |
|-----------------------|--------------|
| $p < 0.05$            | 2393 (38.7%) |
| $p < 0.01$            | 1453 (23.5%) |
| $p < 0.001$           | 696 (11.2%)  |
| $p < 0.0001$          | 320 (5.2%)   |
| B & H $p < 0.05$      | 1549 (25.0%) |
| Bonferroni $p < 0.05$ | 155 (2.5%)   |

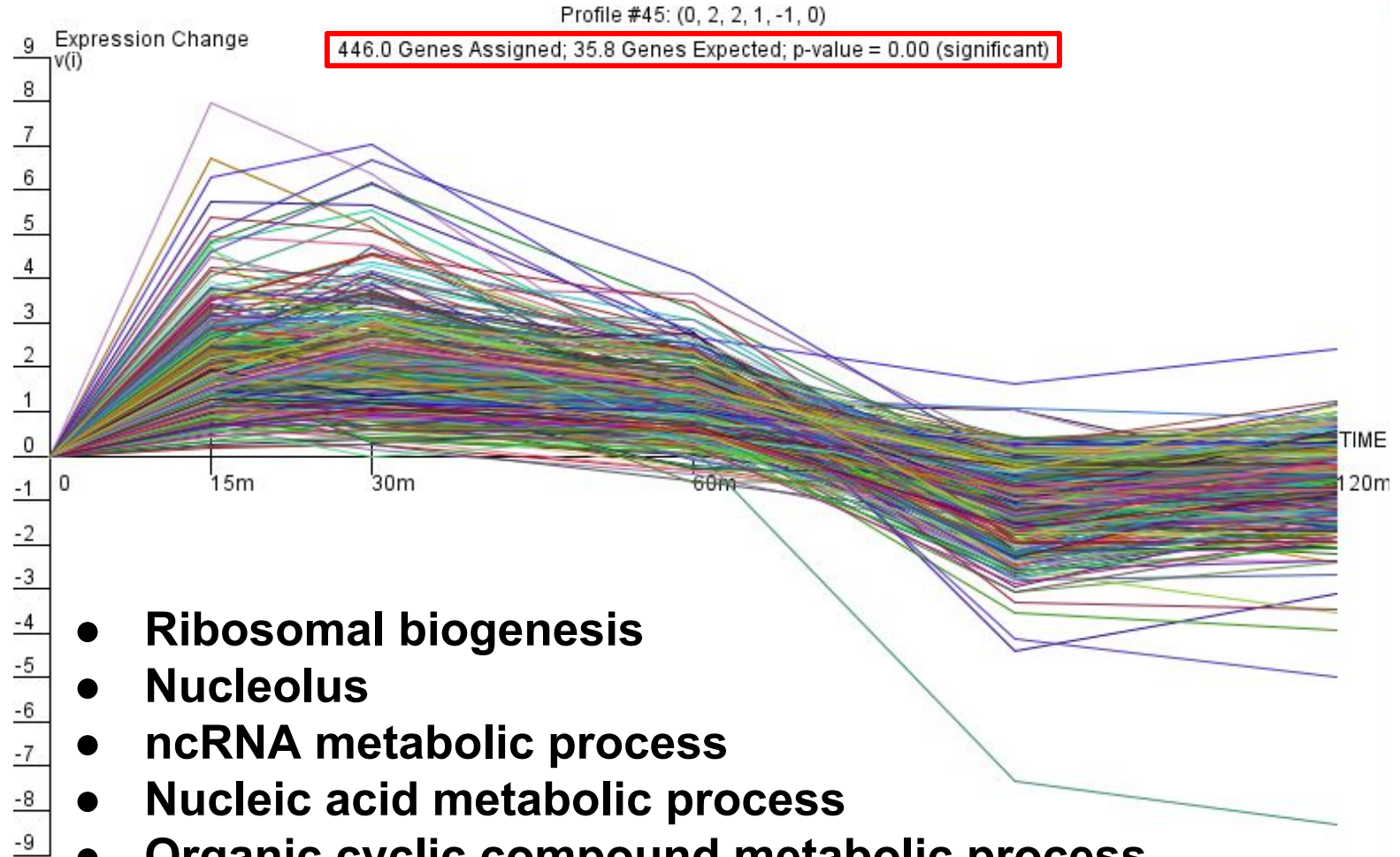
# Genes Were Clustered According to Expression Pattern Using STEM Software



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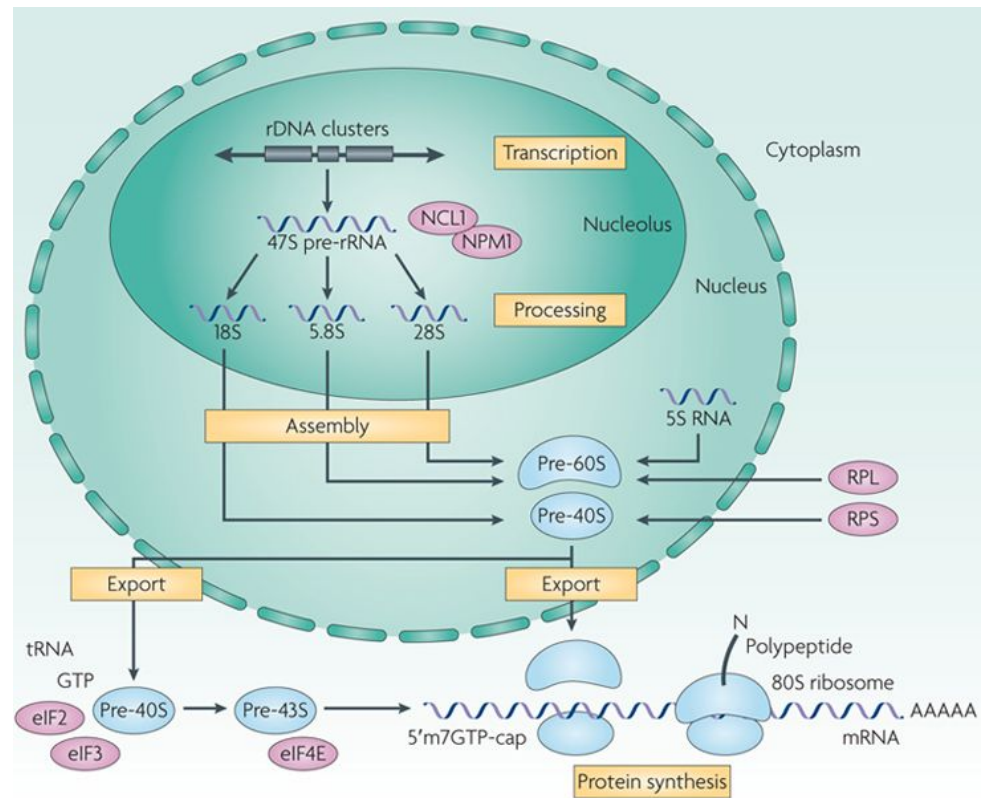
# Profile 45 Indicated Upregulation During Cold Shock and Down Regulation During Recovery



- Ribosomal biogenesis
- Nucleolus
- ncRNA metabolic process
- Nucleic acid metabolic process
- Organic cyclic compound metabolic process
- Maturation of 5.8 rRNA from tricistronic rRNA transcript

# Ribosomal Biogenesis is Upregulated During Cold Shock in dGLN3

- **Nucleolus:** prime function is to transcribe nuclear DNA into 45S ribosomal precursor RNA and process RNA into 5.8S, 18S and 28S components
- **ncRNA metabolic process:** chemical reactions and pathways involving ncRNA transcripts
- **Maturation of 5.8 rRNA from tricistronic rRNA transcript:** processing of transcripts that contain small, large and 5.8S rRNA



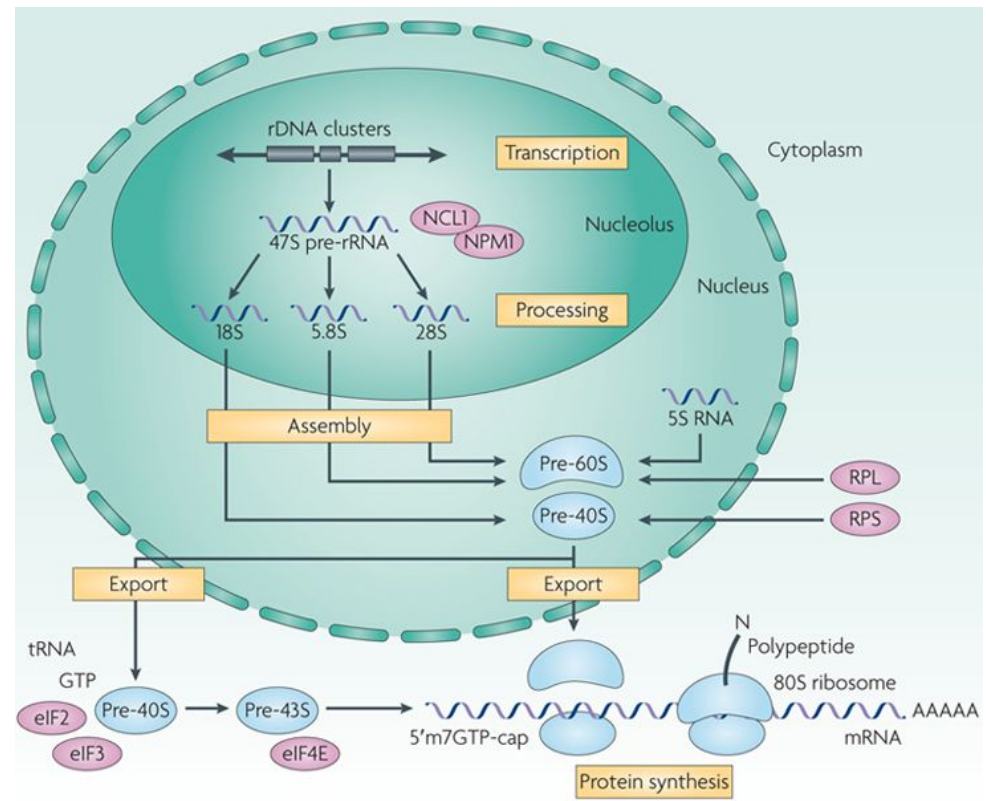
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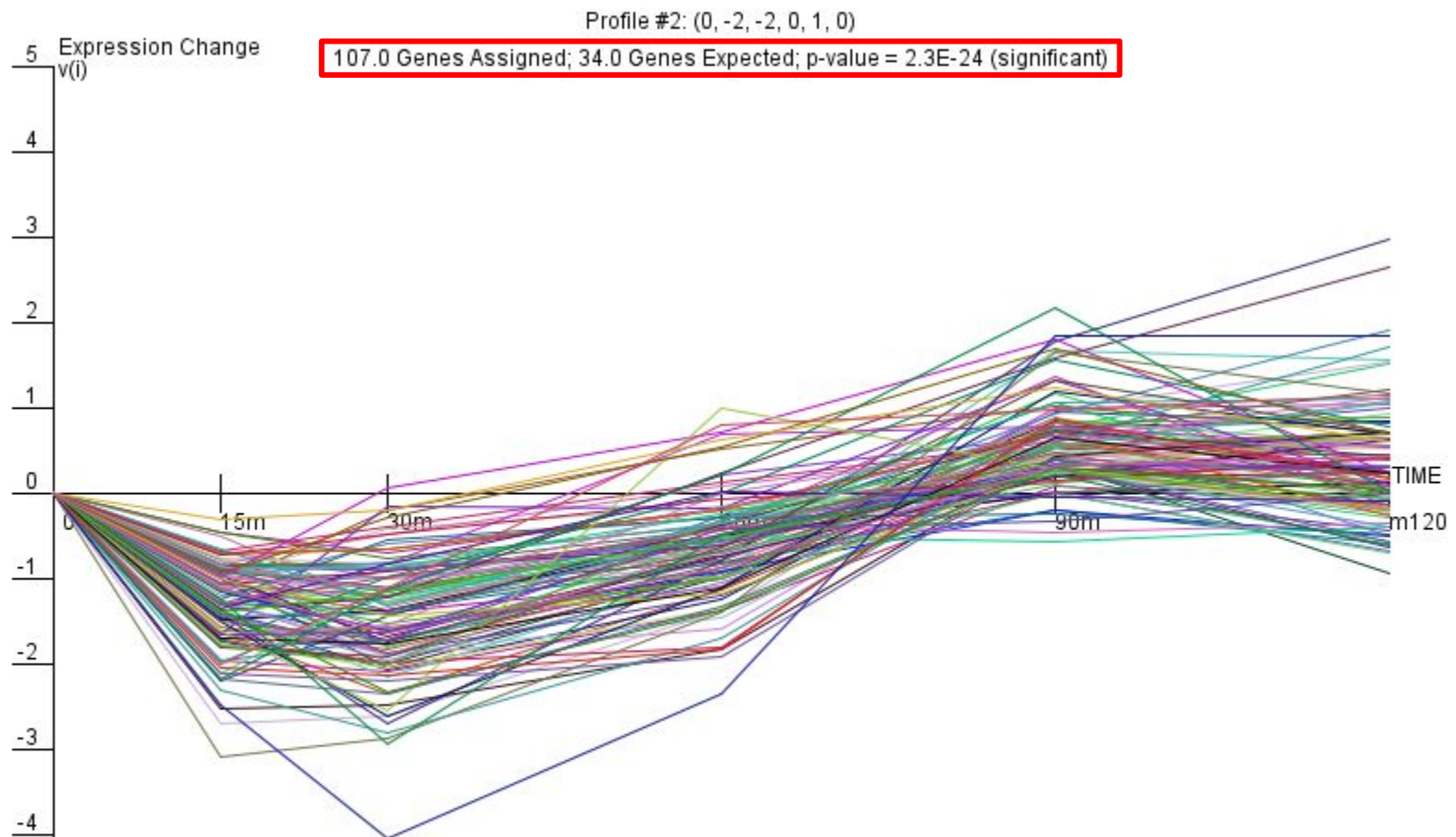
- Nucleic acid metabolic process
- Organic cyclic compound metabolic process
- Ribosomal biogenesis

- Increased ribosome genesis allows for increased protein expression
- Allows for cell to produce proteins that may protect it from cold shock
- Infanzon et al. (2010) found similar results



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# Profile 2 Exhibits a Variance in DNA Replication During Onset of Cold Shock through Revcovery



- Cellular Protein Modification Process
- Cytoplasmic Vesicle
- Mitotic Cell Cycle Phase Transition
- Phosphorylation
- DNA Replication
- Kinase Activity

# **DNA Synthesis is Initially Affected by Cold Shock then is Upregulated Through Recovery**

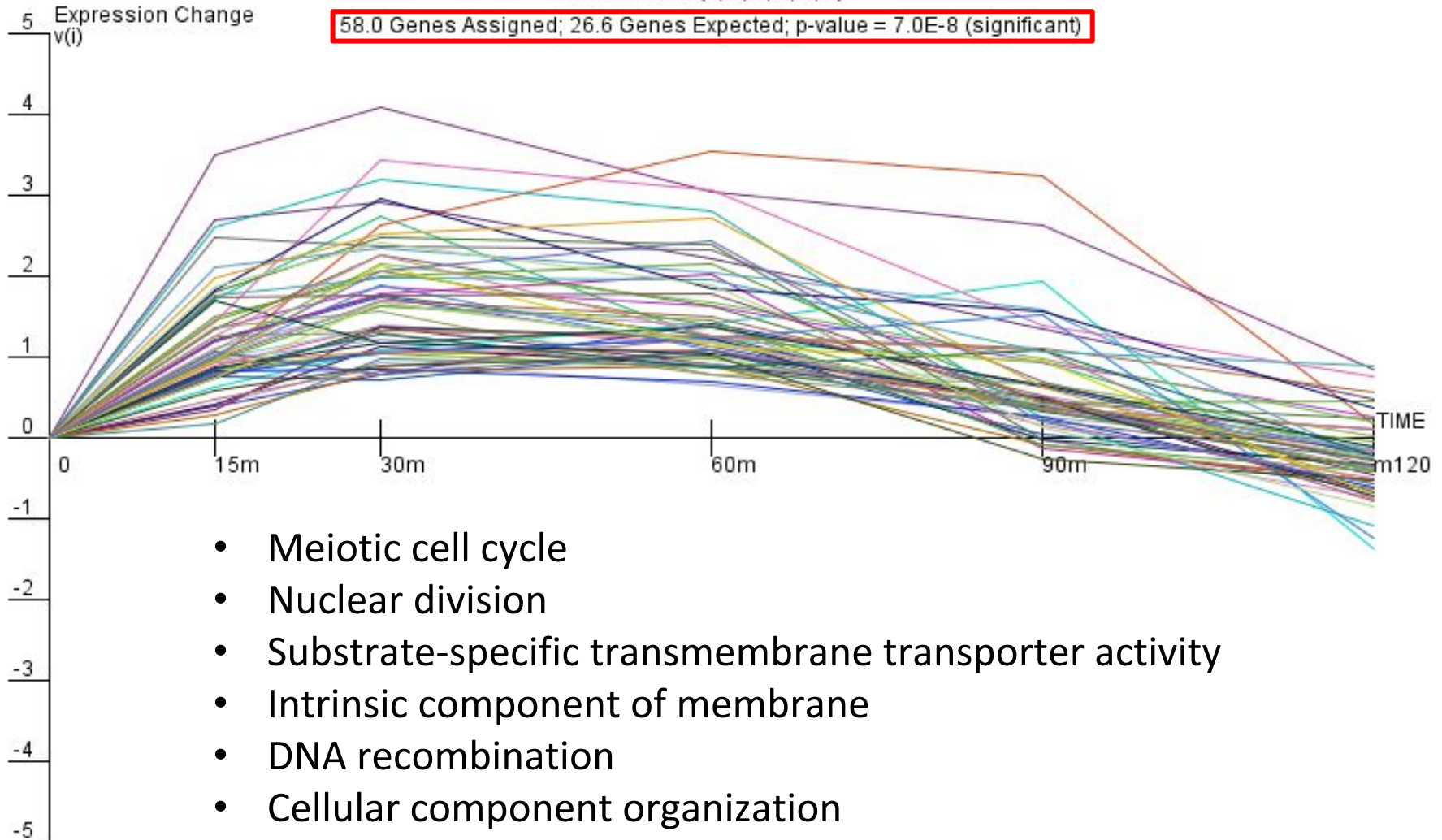
- **Down regulation followed by an upregulation**
- **DNA synthesis and related processes are repressed during the period of cold shock.**
- **The other processes seem to inform the overall theme of *DNA replication*.**
  - **This suggests that once the cells cleared the cold shock the cells were able to reactivate pathways of DNA synthesis in the cell cycle, indicating that the cells do not allocate resources for growth amidst the environmental stress.**
  - **During cold stress proteins bind to single-stranded regions of the replication fork and blocks DNA replication (Ermolenko 2002).**



# Profile 48 Shows Upregulation of Meiosis Related Processes

Profile #48: (0, 2, 3, 3, 2, 0)

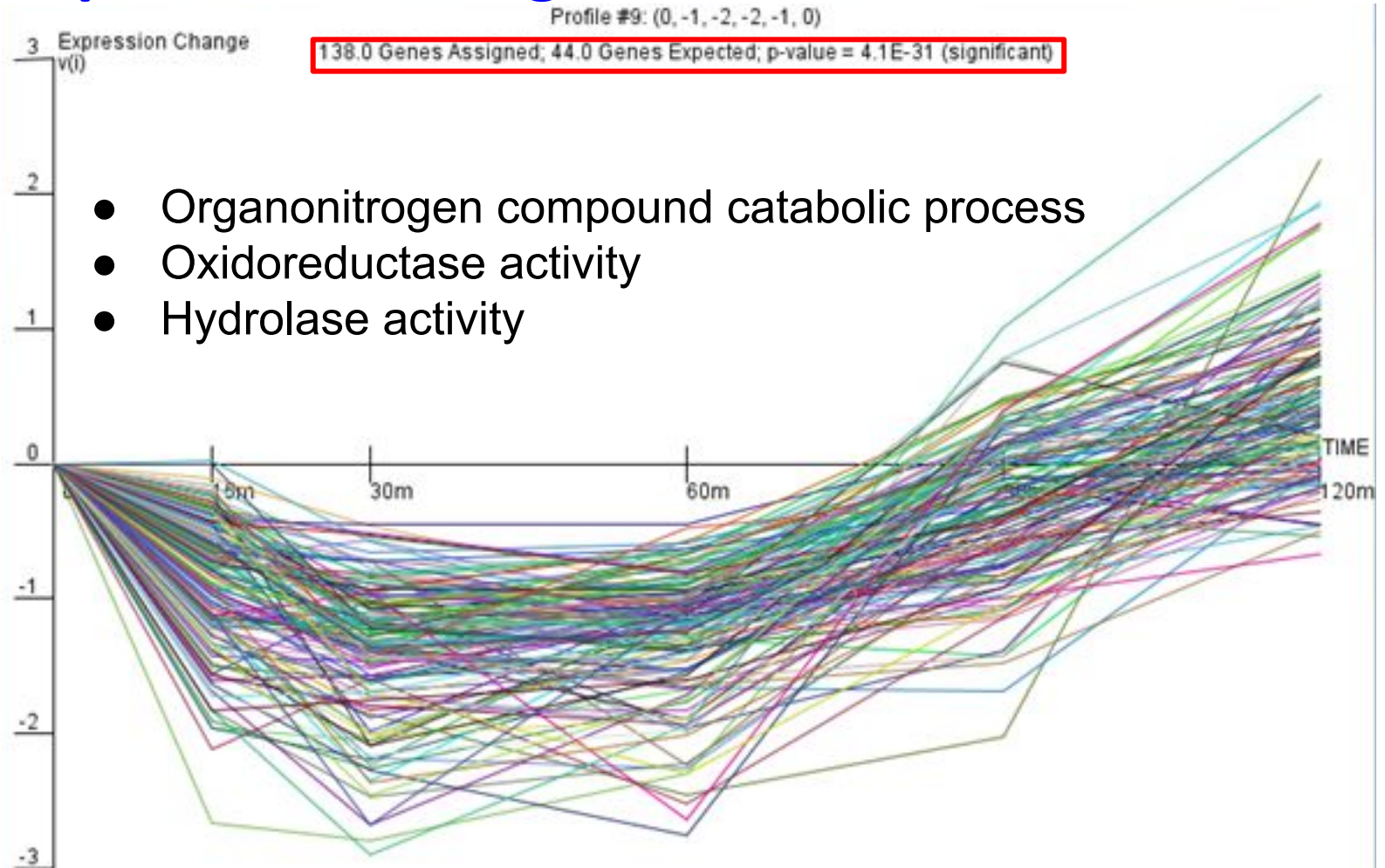
58.0 Genes Assigned; 26.6 Genes Expected; p-value = 7.0E-8 (significant)



# **Cold Stress Promoted Up-regulation of Meiosis Inhibiting Genes**

- **Overall Theme: Meiosis**
- **This profile's graph contains slight upregulation followed by a slight downregulation of genes associated with meiosis.**
  - **Down regulation occurs after cold shock.**
- **Cold stress may positively regulate meiosis, while it is known that heat stress will denature proteins and prevent meiosis.**
  - **Process has greater tolerance for colder temperatures.**
- **It is likely that cold stress causes inhibition of meiosis and related functions.**
  - **Research by Xiang-Ping Zhu et. al. suggests inhibition of meiosis during cold shock and then a return to normal functioning after temperature is readjusted.**

# Profile 9 Shows a Downregulation of Gene Expression During Cold Shock Time Points



- Organonitrogen compound catabolic process
- Oxidoreductase activity
- Hydrolase activity

- Carboxylic acid biosynthetic process
- Glutamine family amino acid metabolic process
- Transferase activity, transferring nitrogenous groups

# **Glutamine family amino acid biosynthesis was downregulated during cold shock**

- **Organonitrogen compound catabolic process: The chemical reactions and pathways resulting in the breakdown of organonitrogen compound**
- **Glutamine family amino acid biosynthesis: The chemical reactions and pathways resulting in the formation of amino acids of the glutamine family, comprising arginine, glutamate, glutamine and proline**
- **Glutamine based amino acids contain nitrogen**
  - **Decreased nitrogenous organic compound catalysis results in less nitrogen available for biosynthesis (Cai 2012)**

# Outline

- Limitations of previous studies led to development of experiment using chemostat culture and low temperature acclimation.
- *S. cerevisiae* was grown in four different experimental conditions.
- DNA microarray analysis was used to analyze growth limitations applied to *S. cerevisiae*.
- *S. cerevisiae* stores carbohydrates during chemostat culture for unknown reasons.
- Promoter analysis reveals regulation trends in gene regulation.
- Temperature response genes overlap in expression.
- Environmental stimulation responses differed in batch vs. chemostat cultures.
- Experimental design differences distinguish Dahlquist Lab data from Tai et al.
- Analysis of  $\Delta\text{gln3}$  revealed significant difference in gene expression over time.
- Model expression profiles show that many biological processes are affected by cold shock.
- **Ribosome biogenesis, mitosis, and nitrogen catabolism genes were affected in Tai et al. and Dahlquist Lab data analysis**

# **Ribosome Biogenesis, Mitosis, and Nitrogen Catabolism Genes Were Affected in Tai et al. and Dahlquist Lab**

| <b>Tai et al.</b>  | <b>Similarities</b>  | <b>Dahlquist Lab</b>  |
|--|--|---|
| <b>Down regulation of target genes for glucose catabolite repression in cold shock conditions.</b> | <b>Genes involved with ribosome biogenesis and assembly upregulated in cold shock, but not in recovery.</b><br><br><b>Down regulation of M-phase and chromosome segregation in cell cycle.</b><br><br><b>Specific downregulation of genes responsible for nitrogen compound catabolism</b> | <b>Upregulation of genes related to meiosis inhibition.</b> |

# Summary

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Good luck with finals!