Dynamic changes in gene expression of the cyanobacterium Synechocystis sp. PCC 6803 in response to nitrogen starvation

Krasikov, V.

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Appendices*

*Note:
Supporting information for Chapter 5 is available for download from the website of the journal: http://www.plantphysiol.org/content/155/3/1445/suppl/DC1

Appendix 2A. List of up-regulated ORFs.
Numbers represent the log ratio of gene expression, which is defined as $2\log(I_{\text{treatment}}/I_{\text{control}})$. Up-regulations are highlighted in dark gray, and down-regulations in light gray. The column ‘Category’ is the functional category number assigned as in Table 3. Columns ‘S’, ‘N’, and ‘P’ represent ‘salt stress’, ‘nitrogen starvation’, and ‘phosphorus starvation’, respectively. The column ‘other studies’ indicates regulation of the corresponding ORF in other *Synechocystis* microarray studies; reference list is as in Table 2.

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<th>P</th>
<th>Other studies</th>
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Appendix 2A (continued). List of up-regulated ORFs.

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### Appendix 2A (continued). List of up-regulated ORFs.

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Appendix 2B. List of down-regulated ORFs.
Numbers represent the log ratio of gene expression, which is defined as $2^{\log(I_{\text{treatment}}/I_{\text{control}})}$. Up-regulations are highlighted in dark gray, and down-regulations in light gray. The column ‘Category’ is the functional category number assigned as in Table 3. Columns ‘S’, ‘N’, and ‘P’ represent ‘salt stress’, ‘nitrogen starvation’, and ‘phosphorus starvation’, respectively. The column ‘other studies’ indicates regulation of the corresponding ORF in other *Synechocystis* microarray studies; reference list is as in Table 2.

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### Appendix 2B (continued). List of down-regulated ORFs.

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Appendix 2B (continued). List of down-regulated ORFs.

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Appendix 3.
### R-script used to assess differential expression patterns
### in the nitrogen starvation experiments

> "Command in R"
+ denotes commands longer than one string

*Output of the command in R-shell is in italic*

# Comments

```r
>library(limma)  # Main library
>library(vsn)  # Library for vsn-normalization
>library(gplots)
# Library with advanced heatmap function used for data
# visualisation and clustering.
```

### Data import:

```r
>loadPath <- "D:/R/Data/DataNNt"
# Path for the folder containing raw hybridization data
>savePath <- "D:/R/Data/Out"
# Path to the output folder
>targetsFile <- "ExpDescr.txt"
# Text file describing experiment; contains file names for all
# hybridizations and targets applied in red and green
# channels.
>targets <- readTargets(file=targetsFile, path=loadPath, sep="\t")
# targets – variable collecting information about two nitrogen
# starvation experiments: 12 hours starvation and time seria
# starvation-recovery experiment.
>targets.Nt <- targets[9:26,]
# variable selecting data related only to the time seria experiment
>show(targets.Nt)
```

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data.columns <- list(R="rMeanSignal", G="gMeanSignal",
+ Rb="rBGMedianSignal", Gb="gBGMedianSignal")

# Variable specifying column names in raw data file;
# this columns contains raw probe intensities and
# background intensities in red and green channels

anno.columns <- c("ControlType", "ProbeName")

# Variable specifying column names with annotation data

RG.Nt <- read.maimages(targets.Nt$fileName, path=loadPath,
+ source="generic", quote="", columns = data.columns,
+ annotation=anno.columns)

# read.maimages - Generic function for data import

dim(RG.Nt)
[1] 8635 18

# Dimensions of the imported data matrix; denotes number of
# probes in each hybridization and number of loaded hybridizations

### SpotTypes attachment:

spottypes <- readSpotTypes(file = "SpotTypeFile.txt", path = "D:/R/DataV")

# Import of the text file describing types of probes spotted on
# the microarray (e.g. positive and negative controls and probes)

show(spottypes)

SpotType ControlType color cex
1     Gene           * black 0.2
2 PosContr           1   red 0.2
3 NegContr          -1  blue 0.2

RG.Nt$genes$Status <- controlStatus(spottypes, RG.Nt)

Matching patterns for: ControlType
Found 8635 Gene
Found 465 PosContr
Found 79 NegContr

Setting attributes: values color cex

# function attaching to each probe on the array its status:
# whether it belongs to the control type or not

### Removal from the data-set probes related to the control:

isGene.Nt <- RG.Nt$genes$Status == "Gene"

# isGene.Nt – list of indexes of probes,
# which are not of the control type

RG.Nt <- RG.Nt[isGene.Nt,]

dim(RG.Nt)
[1] 8091 18
### Normalisation:

```r
> RG.non <- RG.Nt
  # not normalized intensity data set
> MA.non <- MA.RG(RG.non)
  # conversion of the intensities into the not normalized MA data set,
  # where M is the ratio of the intensities in red and green channels
  # in log-scale and A is the average intensity in both channels
> RG.b <- backgroundCorrect(RG.non, method="minimum")
  # Correction for the background;
  # option "method" selects method for background correction
> MA.lo <- normalizeWithinArrays(RG.b, method="loess")
  # Data normalized within each array with LOESS normalization
> MA.aq <- normalizeBetweenArrays(MA.lo, method="Aq")
  # MA normalized by “Aquantile” method between all arrays
> RG.aq <- RG.MA(MA.aq)
  # conversion of the normalized MA data into the normalized
  # intensity data set
```

### Diagnostic plots:

```r
> arrayNo <- 3
  # array Number to plot
> plot(0:12, 0:12, xlab = "Green", ylab = "Red", type = "n")
> abline(0,1)
> points(log(RG.non[,arrayNo]$R), log(RG.non[,arrayNo]$G), cex = 0.3)
  # plot(…) function sets scale; abline(0,1) sets diagonal;
  # Intensity RG-plot of not normalized data of the array 3;
  # intensities are log-trasformed
> plot(0:12, 0:12, xlab = "Green", ylab = "Red", type = "n")
> abline(0,1)
> points(log(RG.aq[,arrayNo]$R), log(RG.aq[,arrayNo]$G), cex = 0.3)
  # plot(…) sets scale; abline(0,1) sets diagonal;
  # Intensity RG-plot of normalized data of the array 3;
  # intensities are log-trasformed
> plotMA(MA.non[,arrayNo])
> abline(0,0)
  # MA-plot of not normalized data of the array 3
> plotMA(MA.aq[,arrayNo])
> abline(0,0)
  # MA-plot of normalized data of the array 3
> plotDensities(MA.non)
  # Density-plot of not normalized complete data set
```
>plotDensities(MA.aq)
  # Density-plot of normalized complete data set
>boxplot(MA.non$M~col(MA.non$M), names=colnames(MA.non$M))
  # Box-plot of not normalized complete data set
>boxplot(MA.aq$M~col(MA.aq$M), names=colnames(MA.aq$M))
  # Box-plot of normalized complete data set

### Cluster analysis and Intensity Heatmap of the complete data set:

>RG.R <- RG.aq$R
>RG.G <- RG.aq$G

  # Extraction of the separate R (Red) and G (Green) channel intensities
>colnames(RG.R) <- paste(colnames(RG.R), "R", sep=".")
>colnames(RG.G) <- paste(colnames(RG.G), "G", sep=".")

  # Assignment of the column names to separate channels
>RG.only <- cbind(RG.R, RG.G)

  # Combining separate Red and Green channels into one data matrix
>DataCol = colorpanel(256, 'green', 'black', 'red')

  # Sets up colour-range represented on the image varying from green to red
>heatmap.2(heatdata, col=DataCol, Rowv=NA, scale="non", dendrogram="col",
  + cexCol=0.7, mar=c(7,5), symkey=FALSE, trace="none", density.info="none")

  # heatmap.2() function performs visualisation and cluster analysis
  # in search for similarities and dissimilarities in the data;
  # Rowv=NA defines that there would be no clustering on rows
  # of the data matrix (representing 8091 probes);
  # Clustering is performed on columns which represent separate
  # normalized intensities in red and green channels of all
  # hybridizations; # heatmap.2() function utilises functions
dist() for calculation of distances between data columns with
# “Euclidian” distance measure and hclust() to perform
# “hierarchical” cluster analysis.

### Ratio Heatmap:

>MA.M <- MA.aq$M

  # Extraction of M-values (Ratio) only from the data
>DataCol = colorpanel(256, 'green', 'black', 'red')

  # Sets up colour-range represented on the image varying from
  # green (down-regulation) to black (no-regulation) and to red (up-regulation)
>heatmap.2(heatdata, col=DataCol, Rowv=NA, scale="non", dendrogram="col",
  + cexCol=0.8, mar=c(7,5), symkey=FALSE, trace="none", density.info="none")

  # Here columns represents ratios of individual arrays,
so the cluster analysis highlights similarities between biological replicates and dissimilarities between time-points.

### Fit linear model for each probe on the array, application of empirical Bayes statistics, band adjustment for multiple testing in search for differentially expressed genes.

```r
> design.Nt <- modelMatrix(targets.Nt, ref="Nt.0")
Found unique target names:
Nt.0 Nt.102 Nt.108 Nt.12 Nt.24 Nt.6 Nt.96
> show(design.Nt)
   Nt.102 Nt.108 Nt.12 Nt.24 Nt.6 Nt.96
      9      0      0     0    0   -1     0
     10      0      0     0    0   -1     0
     11      0      0     0    0   -1     0
     12      0     -1     0    0     0   -1
     13      0     -1     0    0     0   -1
     14      0     -1     0    0     0   -1
     15      0      0     0    0   -1     0
     16      0      0     0    0   -1     0
     17      0      0     0    0   -1     0
     18      0      0     0    0   -1     0
     19      0      0     0    0   -1     0
     20      0      0     0    0   -1     0
     21     -1      0     0    0     0   -1
     22     -1      0     0    0     0   -1
     23     -1      0     0    0     0   -1
     24      0     -1     0    0     0   -1
     25      0     -1     0    0     0   -1
     26      0     -1     0    0     0   -1

# Sets design matrix with zero time point as a reference
> fit.Nt <- lmFit(MA.aq, design.Nt)
# This function fits multiple linear models. It accepts data from
# a experiment involving a series of microarrays with the same set
# of probes. A linear model is fitted to the expression data
# for each probe. The coefficients of the fitted models describe
# the differences between the RNA sources hybridized to the arrays.
> cont.matrix.Nt <- makeContrasts(Nt.6, Nt.12, Nt.24, Nt.96, Nt.102, Nt.108,
+ Nt.6.rec=Nt.102-Nt.96, Nt.12.rec=Nt.108-Nt.96, levels=design.Nt)
# Construct the contrasts matrix; as contrasts we define here
# comparisons of interests, e.g. Nt.6 is the comparison of expression
# of Nt.6 (which is 6 hours time point) against reference (which is 0
# hours time point), Nt.6.rec is the 6 hours recovery time point
> fit.Nt <- contrasts.fit(fit.Nt, cont.matrix.Nt)
# Given a linear model fit to microarray data, compute estimated
# coefficients and standard errors for a given set of contrasts.
> fit.Nt <- eBayes(fit.Nt)
```
These functions are used to rank genes in order of evidence for differential expression. It uses an empirical Bayes method to shrink the gene-wise sample variances towards a common values and, in so doing, augmenting the degrees of freedom for the individual variances. It computes moderated t-statistics and log-odds of differential expression.

```r
>d.Nt <- decideTests(fit.Nt, method="separate", adjust.method="fdr", p.value=0.01)
```

Classify a series of related t-statistics as up, down or not significant. A number of different multiple testing schemes are offered which adjust for multiple testing down the genes as well as across contrasts for each gene. It produce essentially a numeric matrix with elements `-1`, `0` or `1` depending on whether each t-statistic is classified as significantly negative, not significant or significantly positive respectively.

```r
>summary(d.Nt)
```

<table>
<thead>
<tr>
<th>Nt.6</th>
<th>Nt.12</th>
<th>Nt.24</th>
<th>Nt.96</th>
<th>Nt.102</th>
<th>Nt.108</th>
<th>Nt.6.rec</th>
<th>Nt.12.rec</th>
</tr>
</thead>
<tbody>
<tr>
<td>-1</td>
<td>1473</td>
<td>1902</td>
<td>1859</td>
<td>2186</td>
<td>1825</td>
<td>1855</td>
<td>2262</td>
</tr>
<tr>
<td>0</td>
<td>5131</td>
<td>4279</td>
<td>4421</td>
<td>3683</td>
<td>4476</td>
<td>4480</td>
<td>3951</td>
</tr>
<tr>
<td>1</td>
<td>1487</td>
<td>1910</td>
<td>1811</td>
<td>2222</td>
<td>1790</td>
<td>1756</td>
<td>1878</td>
</tr>
</tbody>
</table>

This function counts number of regulated genes in each specified contrast.

```r
>vennCounts(d.Nt[,1:3], include="up")
```

<table>
<thead>
<tr>
<th>Nt.6</th>
<th>Nt.12</th>
<th>Nt.24</th>
<th>Counts</th>
</tr>
</thead>
<tbody>
<tr>
<td>[1,]</td>
<td>0</td>
<td>0</td>
<td>5490</td>
</tr>
<tr>
<td>[2,]</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>[3,]</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>[4,]</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>[5,]</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>[6,]</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>[7,]</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>[8,]</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

This function produce classification counts for any number of contrasts specified; in this particular case it highlights up-regulated genes in 6, 12 and 24 hours of time-seria experiment, and calculates number of specifically or commonly regulated probes.

```r
>plotMA(fit.Nt, status=d.Nt[,1], cex=0.2, legend=TRUE)
```

plotMA() function graphically represents here up- and down-regulated probes for one of the contrasts; Here we used as example 6 hours time point.

```r
>vennDiagram(d.Nt[,1:3], include="up")
```
# This function is analogous to the previous one, but it creates
# Venn diagram; number of contrasts is limited to three
# parameter include="down" will plot commonly down-regulated probes.

### Export of the resulting matrix:
```r
> write.fit(fit.Nt, results=d.Nt, file="D:/R/Data/Out/fit.Nt.txt", digits=3, adjust="fdr", sep="\t")
```
# Data exported as text tab-delimited file with Ratios for each
# contrast specified by cont.matrix and with the decision whether
# probe is differentially expressed or not in particular contrast.

### Analysis of the expression behaviour of selected genes of interest
```r
> fit.Nt.File <- "D:/R/Data/Out/fit.Nt.txt"
# filename and the path to the file storing the results of the
# the specified contrasts and decisions about differential probe
# expression

> geneList.File <- "D:/R/Data/GeneList.PSI.txt"
# filename and the path to the file storing the list of probes
# corresponding to genes of interest; here we used as an example
# list of structural genes encoding subunits of Photosystem I (PSI)

> Nt.complete <- read.table(file=fit.Nt.File, header=TRUE, sep="\t", quote=""
# Import of the complete linear fit in text format

> g.list <- read.table(file = geneList.File, header = TRUE, sep = "\t", quote=""
# Import of the list of genes of interest

> g.Probe <- g.list$Probe.ID
# Extraction from the list of genes probe identifiers only

> g.Name <- g.list$GeneName
# Extraction from the list of genes gene names only

> i.names.Nt <- match(g.Probe, Nt.complete$Genes.ProbeName)
# Extracting indexes from complete fit table matching
# the identifiers from the list of genes of interest

> write.table(Nt.complete[i.names.Nt,], file="D:/R/Data/Out/fit.Nt.PSI.txt",
+ row.names = TRUE, sep="\t")
# Save of the fit information for the PSI genes only in text file

> M.list.fit <- as.matrix(Nt.complete[i.names.Nt,2:7])
# Extracting from the complete fit-object only ratios related to
# PSI genes and converting it into the matrix format

> min(M.list.fit)
> max(M.list.fit)
# Check for the min and max of the matrix

> rownames(M.list.fit) <- paste(g.Name,"-",g.Probe)
# Assignment of the row names for the PSI genes in form:# “gene name – probe ID”

> heatdata.fit <- M.list.fit
```

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> heatmap.2(heatdata.fit, col=DataCol, Colv=FALSE, scale="non",
+ dendrogram="row", cexCol=0.7, cexRow=0.7, mar=c(8,9),
+ symkey=FALSE, trace="none", density.info="none", breaks=seq(-2,2,0.015625))
    # This function performs hierarchical clustering and visualisation of
    # the expression profile of the photosystem I related genes
**Appendix 4A. List of functional categories of *Synechocystis* genes.**  
Category and subcategory annotation are as in Cyanobase (genome.kazusa.or.jp/cyanobase).  
Category numbers are assigned in alphabetical order.

<table>
<thead>
<tr>
<th>Category</th>
<th>SubCategory</th>
</tr>
</thead>
</table>
| 1 - Amino acid biosynthesis | 1a - Aromatic amino acid family  
1b - Aspartate family  
1c - Branched chain family  
1d - Glutamate family / Nitrogen assimilation  
1e - Serine family / Sulfur assimilation |
| 2 - Biosynthesis of cofactors, prosthetic groups, and carriers | 2a - Carotenoid  
2b - Cobalamin, heme, phycobilin and porphyrin  
2c - Folic acid  
2d - Menaquinone and ubiquinone  
2e - Molybdenopterin  
2f - Thiamin  
2g - Thioredoxin, glutaredoxin, and glutathione |
| 3 - Cell envelope | 3a - Membranes, lipoproteins, and porins  
3b - Murein sacculus and peptidoglycan  
3c - Surface polysaccharides, lipopolysaccharides |
| 4 - Cellular processes | 4a - Chaperones  
4b - Chemotaxis  
4c - Detoxification  
4d - Protein and peptide secretion  
4e - Transformation |
| 5 - Central intermediary metabolism | 5a - Other  
5b - Polysaccharides and glycoproteins |
| 6 - Energy metabolism | 6a - Amino acids and amines  
6b - Glycolysis  
6c - Pentose phosphate pathway  
6d - Pyruvate and acetyl-CoA metabolism  
6e - Pyruvate dehydrogenase  
6f - Sugars  
6g - TCA cycle |
| 7 - Fatty acid, phospholipid and sterol metabolism | |
| 8 - Photosynthesis and respiration | 8a - ATP synthase  
8b - CO2 fixation  
8c - Cytochrome b6f complex  
8d - NADH dehydrogenase  
8e - Photosystem I  
8f - Photosystem II  
8g - Phycobilisome  
8h - Respiratory terminal oxidases  
8i - Soluble electron carriers |
| 9 - Purines, pyrimidines, nucleosides, and nucleotides | 9a - Purine ribonucleotide biosynthesis  
9b - Pyrimidine ribonucleotide biosynthesis |
| 10 - Regulatory functions | |
| 11 - DNA replication, restriction, modification, recombination, and repair | |
| 12 - Transcription | 12a - RNA synthesis, modification, and DNA transcription |
| 13 - Translation | 13a - Aminoacyl tRNA synthetases and tRNA modification  
13b - Degradation of proteins, peptides, and glycopeptides |
Appendix 4A (continued). List of functional categories of *Synechocystis* genes.

<table>
<thead>
<tr>
<th>Category</th>
<th>SubCategory</th>
</tr>
</thead>
<tbody>
<tr>
<td>13c - Nucleoproteins</td>
<td>13d - Protein modification and translation factors</td>
</tr>
<tr>
<td>13e - Ribosomal proteins: synthesis and modification</td>
<td></td>
</tr>
<tr>
<td>14 - Transport and binding proteins</td>
<td>15a - Adaptations and atypical conditions</td>
</tr>
<tr>
<td>15 - Other categories</td>
<td>15b - Drug and analog sensitivity</td>
</tr>
<tr>
<td></td>
<td>15c - Hydrogenase</td>
</tr>
<tr>
<td></td>
<td>15d - Other</td>
</tr>
<tr>
<td></td>
<td>15e - Transposon-related functions</td>
</tr>
<tr>
<td></td>
<td>15f - WD repeat proteins</td>
</tr>
<tr>
<td>16 - Hypothetical</td>
<td></td>
</tr>
<tr>
<td>17 - Unknown</td>
<td></td>
</tr>
</tbody>
</table>
**Appendix 4B. List of significantly down-regulated genes.**

ORF, gene, gene product, category and subcategory and functional annotation are as in Cyanobase (genome.kazusa.or.jp/cyanobase). Category numbers are assigned in alphabetical order. Categories description is listed in Appendix 4A. p-value - significance of differential expression, adjusted for multiple hypothesis testing using the false discovery rate; FC - fold change; only genes with p < 0.01 and FC > 1.5 are listed. Top 50 of most strongly down-regulated genes are highlighted in bold.

<table>
<thead>
<tr>
<th>ORF</th>
<th>Gene</th>
<th>p-value</th>
<th>FC</th>
<th>Gene Product</th>
<th>Category</th>
</tr>
</thead>
<tbody>
<tr>
<td>slr0608</td>
<td>hisIE</td>
<td>6.10E-04</td>
<td>-1.78</td>
<td>histidine biosynthesis bifunctional protein HisIE</td>
<td>1a</td>
</tr>
<tr>
<td>slr0966</td>
<td>trpA</td>
<td>4.22E-03</td>
<td>-1.65</td>
<td>tryptophan synthase alpha chain</td>
<td>1a</td>
</tr>
<tr>
<td>slr1867</td>
<td>trpD</td>
<td>1.32E-03</td>
<td>-1.96</td>
<td>anthranilate phosphoribosyltransferase</td>
<td>1a</td>
</tr>
<tr>
<td>slr1662</td>
<td></td>
<td>1.92E-04</td>
<td>-2.01</td>
<td>probable prephenate dehydratase</td>
<td>1a</td>
</tr>
<tr>
<td>slr1058</td>
<td>dapB</td>
<td>1.70E-04</td>
<td>-2.13</td>
<td>dihydrodipicolinate reductase</td>
<td>1b</td>
</tr>
<tr>
<td>slr1172</td>
<td>thrC</td>
<td>3.75E-03</td>
<td>-1.74</td>
<td>threonine synthase</td>
<td>1b</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2.01E-04</td>
<td>-2.26</td>
<td>putative aminotransferase</td>
<td>1b</td>
</tr>
<tr>
<td>slr2072</td>
<td>ilvA</td>
<td>5.29E-04</td>
<td>-2.61</td>
<td>L-threonine deaminase</td>
<td>1c</td>
</tr>
<tr>
<td>slr0452</td>
<td>ilvD</td>
<td>1.14E-03</td>
<td>-3.55</td>
<td>dihydroxyacid dehydratase</td>
<td>1c</td>
</tr>
<tr>
<td>slr0065</td>
<td>ilvN</td>
<td>2.17E-03</td>
<td>-2.02</td>
<td>acetolactate synthase small subunit</td>
<td>1c</td>
</tr>
<tr>
<td>slr1517</td>
<td></td>
<td>1.95E-03</td>
<td>-1.81</td>
<td>3-isopropylmalate dehydrogenase</td>
<td>1c</td>
</tr>
<tr>
<td>slr0504</td>
<td>lysA</td>
<td>6.06E-06</td>
<td>-2.20</td>
<td>diaminopimelate decarboxylase</td>
<td>1c</td>
</tr>
<tr>
<td>slr0710</td>
<td>gdhA</td>
<td>9.07E-05</td>
<td>-2.91</td>
<td>glutamate dehydrogenase (NADP+)</td>
<td>1d</td>
</tr>
<tr>
<td>slr0450</td>
<td>norB</td>
<td>8.43E-04</td>
<td>-1.65</td>
<td>cytochrome b subunit of nitric oxide reductase</td>
<td>1d</td>
</tr>
<tr>
<td>slr0461</td>
<td>proA</td>
<td>1.19E-03</td>
<td>-1.53</td>
<td>gamma-glutamyl phosphate reductase</td>
<td>1d</td>
</tr>
<tr>
<td>slr0601</td>
<td></td>
<td>9.15E-04</td>
<td>-1.99</td>
<td>nitrlase homolog</td>
<td>1d</td>
</tr>
<tr>
<td>slr0644</td>
<td></td>
<td>7.07E-03</td>
<td>-1.64</td>
<td>nitrogen regulation protein NifR3 homolog</td>
<td>1d</td>
</tr>
<tr>
<td>slr1931</td>
<td>glyA</td>
<td>4.13E-04</td>
<td>-2.11</td>
<td>serine hydroxymethyltransferase</td>
<td>1e</td>
</tr>
<tr>
<td>slr0739</td>
<td></td>
<td>5.99E-03</td>
<td>-1.52</td>
<td>geranylgeranyl pyrophosphate synthase</td>
<td>2a</td>
</tr>
<tr>
<td>slr0772</td>
<td>chlB</td>
<td>1.61E-04</td>
<td>-2.07</td>
<td>light-independent protoclorophyllide reductase subunit ChlB</td>
<td>2b</td>
</tr>
<tr>
<td>slr1777</td>
<td>chlD</td>
<td>5.09E-03</td>
<td>-1.66</td>
<td>magnesium protoporphyrin IX chelatase subunit D</td>
<td>2b</td>
</tr>
<tr>
<td>slr0749</td>
<td>chlL</td>
<td>5.52E-04</td>
<td>-4.56</td>
<td>light-independent protoclorophyllide reductase iron protein subunit ChlL</td>
<td>2b</td>
</tr>
<tr>
<td>slr0750</td>
<td>chlN</td>
<td>3.00E-05</td>
<td>-3.93</td>
<td>light-independent protoclorophyllide reductase subunit ChlN</td>
<td>2b</td>
</tr>
<tr>
<td>slr1091</td>
<td>chlP</td>
<td>1.28E-04</td>
<td>-1.65</td>
<td>geranylgeranyl hydrogenase</td>
<td>2b</td>
</tr>
<tr>
<td>slr1184</td>
<td>ho1</td>
<td>3.41E-03</td>
<td>-2.02</td>
<td>heme oxygenase</td>
<td>2b</td>
</tr>
<tr>
<td>slr0506</td>
<td>por</td>
<td>9.73E-04</td>
<td>-2.73</td>
<td>light-dependent NADPH-protoclorophyllide oxidoreductase</td>
<td>2b</td>
</tr>
<tr>
<td>slr1457</td>
<td></td>
<td>2.09E-04</td>
<td>-1.59</td>
<td>precorrin isomerase</td>
<td>2b</td>
</tr>
<tr>
<td>slr0426</td>
<td>fdlE</td>
<td>1.11E-03</td>
<td>-1.89</td>
<td>GTP cyclodrolase I</td>
<td>2c</td>
</tr>
<tr>
<td>slr1093</td>
<td>fdlK</td>
<td>2.39E-04</td>
<td>-1.50</td>
<td>2-amino-4-hydroxy-6-hydroxymethylidihydropyridine pyrophosphokinase</td>
<td>2c</td>
</tr>
<tr>
<td>slr0409</td>
<td></td>
<td>3.09E-03</td>
<td>-1.57</td>
<td>similar to O-succinylbenzoate-CoA synthase</td>
<td>2d</td>
</tr>
<tr>
<td>slr0635</td>
<td>thiE</td>
<td>2.77E-03</td>
<td>-1.70</td>
<td>probable thiamine-phosphate pyrophosphorylase</td>
<td>2f</td>
</tr>
<tr>
<td>slr1787</td>
<td></td>
<td>2.32E-04</td>
<td>-1.91</td>
<td>thiamine-monophosphate kinase</td>
<td>2f</td>
</tr>
<tr>
<td>slr0623</td>
<td>trxA</td>
<td>2.75E-05</td>
<td>-3.12</td>
<td>thioredoxin</td>
<td>2g</td>
</tr>
<tr>
<td>slr1057</td>
<td>trnM2</td>
<td>2.98E-04</td>
<td>-1.79</td>
<td>thioredoxin M</td>
<td>2g</td>
</tr>
<tr>
<td>slr2326</td>
<td></td>
<td>1.64E-03</td>
<td>-1.52</td>
<td>similar to glutathione S-transferase</td>
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Appendix 4B (continued). List of significantly down-regulated genes.

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Appendix 4B (continued). List of significantly down-regulated genes.

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Appendix 4B (continued). List of significantly down-regulated genes.

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Appendix 4B (continued). List of significantly down-regulated genes.

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Appendices

Appendix 4B (continued). List of significantly down-regulated genes.

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### Appendix 4B (continued). List of significantly down-regulated genes.

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Appendix 4B (continued). List of significantly down-regulated genes.

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Appendix 4C. List of significantly up-regulated genes.

ORF, gene, gene product, category and subcategory and functional annotation are as in Cyanobase (genome.kazusa.or.jp/cyanobase). Category numbers are assigned in alphabetical order. Categories description is listed in Appendix 4A. p-value - significance of differential expression, adjusted for multiple hypothesis testing using the false discovery rate; FC - fold change; only genes with p < 0.01 and FC > 1.5 are listed. Top 50 of most strongly down-regulated genes are highlighted in bold.

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Appendix 4C (continued). List of significantly up-regulated genes.

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### Appendix 4C (continued). List of significantly up-regulated genes.

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## Appendix 4C (continued). List of significantly up-regulated genes.

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### Appendix 4C (continued). List of significantly up-regulated genes.

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Appendix 4C (continued). List of significantly up-regulated genes.

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