



Time Course study of Gene Expression in *Chlamydomonas reinhardtii* during a State 1 to State2 Transition using Microarray technology

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Chlamydomonas reinhardtii

Genome size: 120 MB, 17 haploid chromosomes

Model Organism for studies related to:

- Photosynthesis
- Mutation
- Hydrogen – renewable source of energy

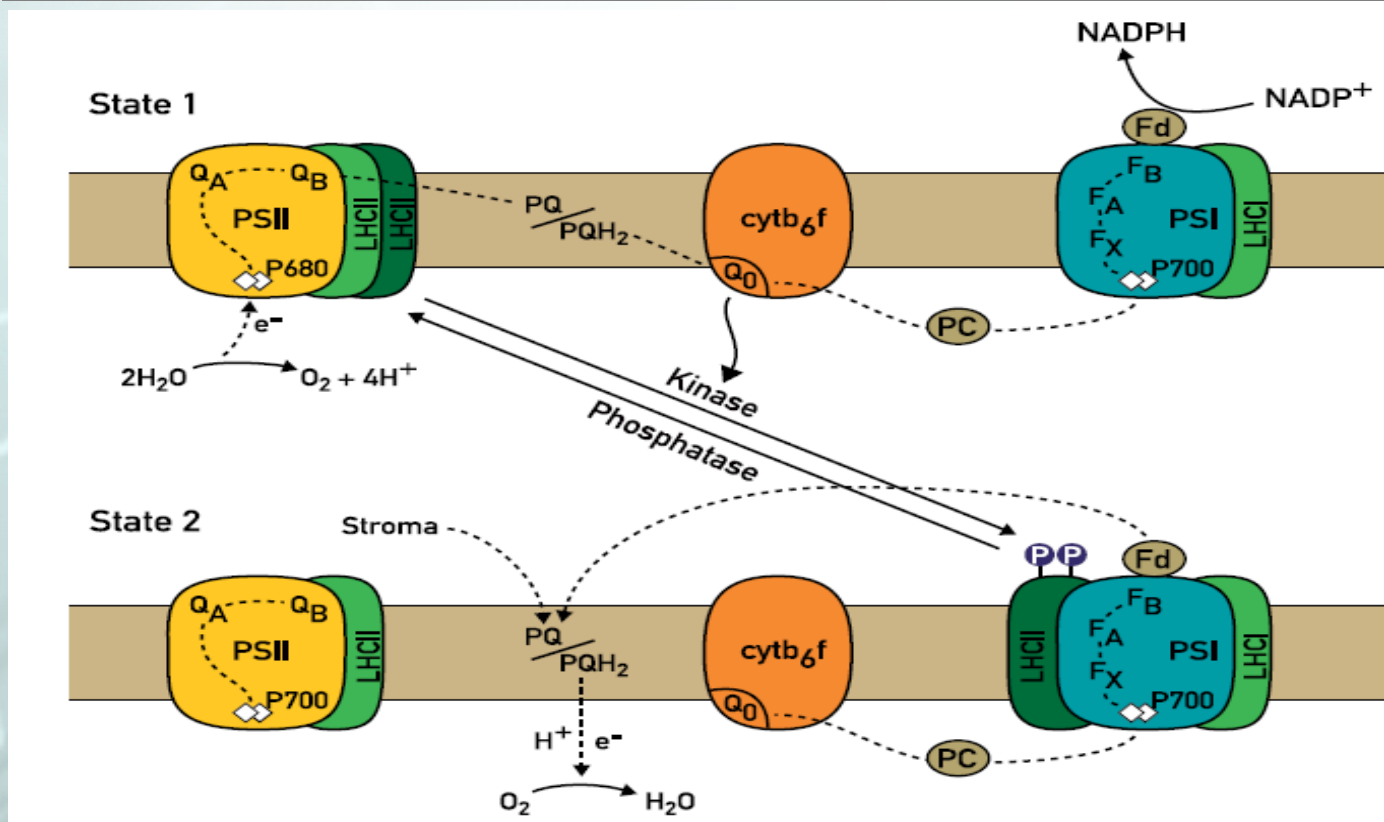
Reasons:

- Easy to handle
- Non-pathogenic
- Life cycle (generation time = 5 hours)





State Transition Phenomenon



- Ref: Rochaix, J. D. (2007) Role of thylakoid protein kinases in photosynthetic acclimation. FEBS Letters 581,:2768-2775.

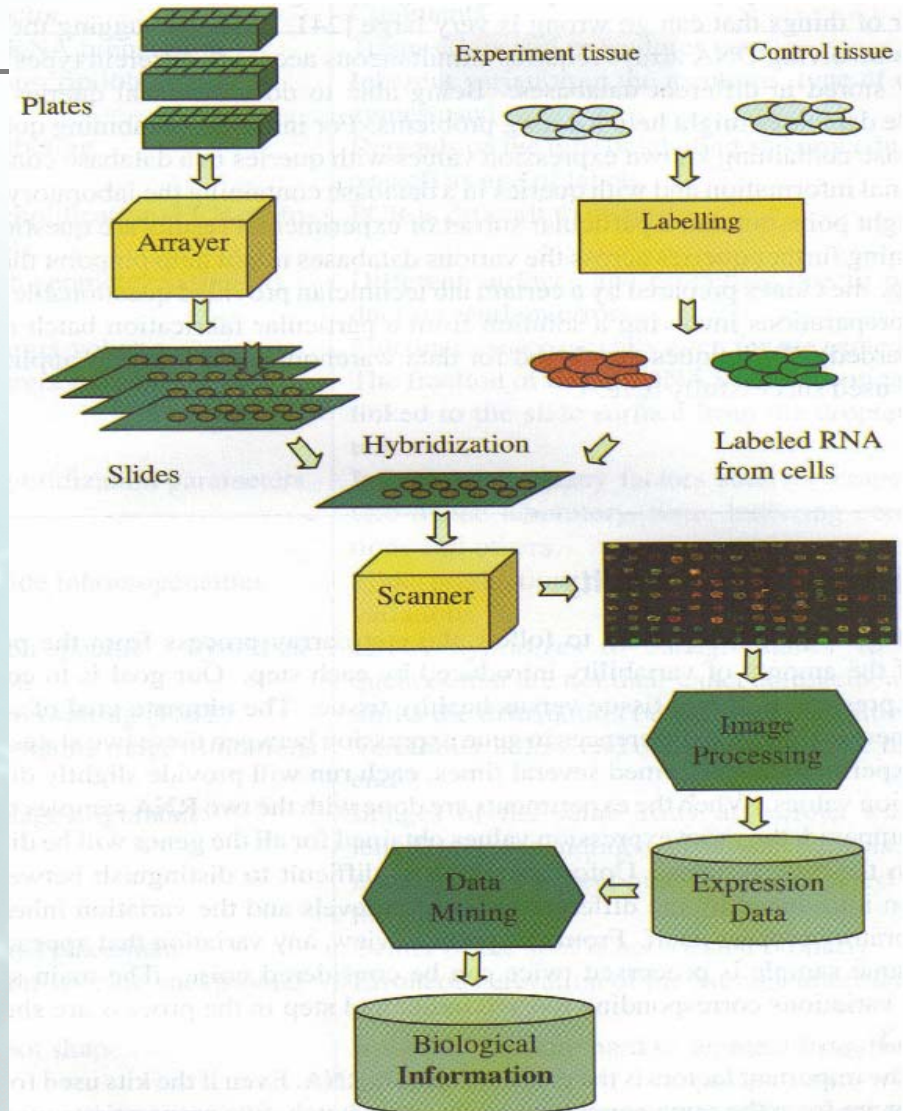
Project Workflow



Stage I: Experimental Design (Complete)

- **Framing the Biological Question:** Gene Expression changes occurring at three time points (30, 60, 120 min), in the dark, in the presence and absence of oxygen within the *Chlamydomonas reinhardtii* CC-125 strain.
- **Choosing a Microarray Platform:** 70mer oligonucleotide, unique genes, printed twice = total 20,000 spots per slide.
- **Data Replicates:** 24 slides (original+validation experiments) = 48 (10,001) replicates total.
 - Normal: Red (Treated N₂) / Green (Control O₂)
 - Dye Swap: Green (Treated N₂) / Red (Control O₂)
- **Design the series of hybridizations:** The order of hybridizations was not specifically decided, they were performed serially to test protocol.

Ref: Draghici, (2003) Pg. 41 Fig. 3.6



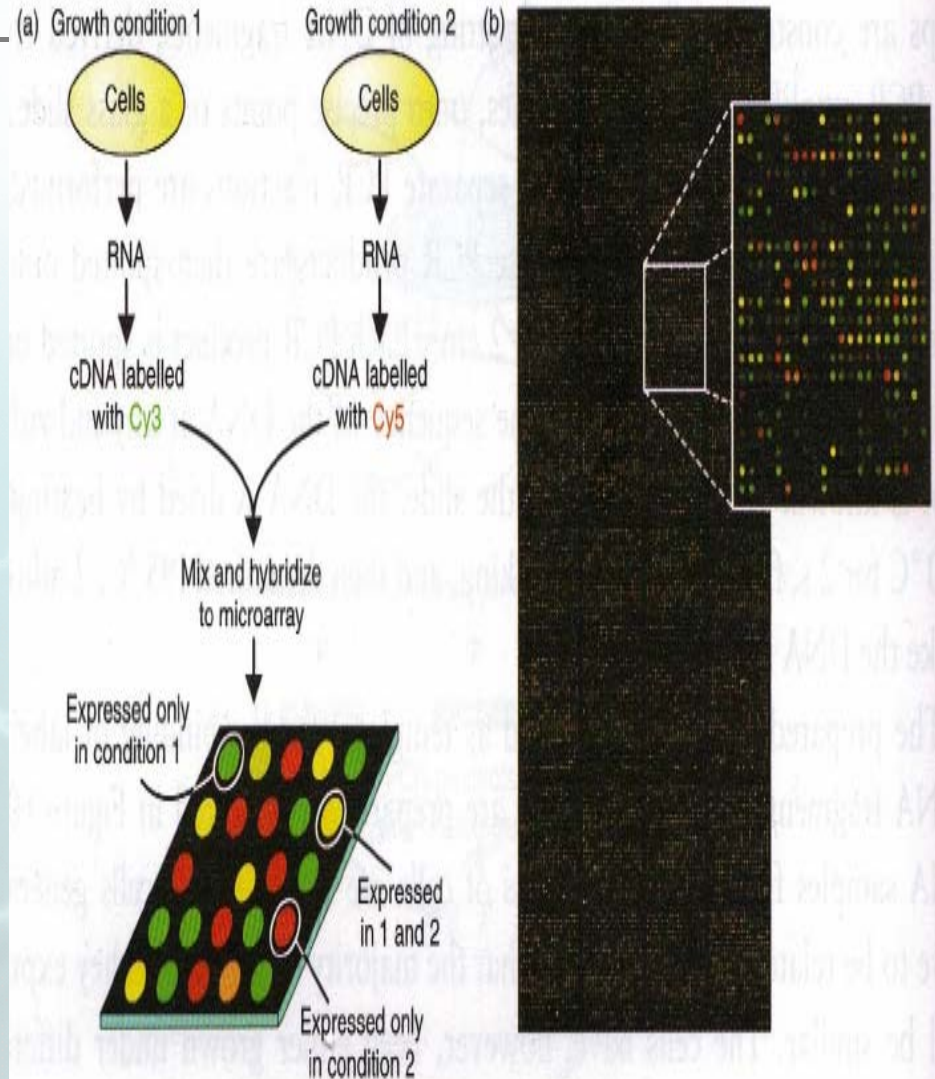
Project Workflow



Stage II: Technical Performance (Complete)

- Growth of Cells: wild type CC-125 *Chlamydomonas reinhardtii* strain grown on Cox liquid medium
- Isolation and purification of total RNA
- Cleanup of RNA for contaminating DNA using DNase-I.
- Reverse transcription to synthesize cDNA
- Purify cDNA and transcribe to amino allyl aRNA
- Purify, quantify and label aRNA.
- Perform the hybridizations: As per Oligo-array Protocol by Stephan Eberhard (2005)

Ref: Reece, 2004, Pg. 320 Fig. 10.3



Project Workflow



Stage III: Statistical Analysis

- **Extract fluorescence intensities:** Agilent Technologies Microarray Scanner G2565BA. The original scanned image is collected by the Agilent Feature Extraction software in the form of .tiff file.
- **Primary Image Analysis** is conducted using the automatic feature finding algorithm of Axon Instruments/ Molecular Devices Corp., GenePix Pro 6.0 software. Manual feature editing is also done wherever necessary using the same and the files are saved in .GPR format.
- **Secondary Analysis:** Normalize data (lowess, log of ration mode: geometric mean) to remove biases using Agilent GeneSpring GX 7.3.1 and 9.0
 - Gene X normalized value =
$$\frac{\text{Cy5 signal [F635 Median-B635 Median]}}{\text{Lowess adjusted Cy3 Ref [F532 Median-B532 Median]}}$$
 - Comparative Conditions:
 - Treated vs. Control
 - Normal vs. Dye Swap
- **Statistical Tests:** *t*-tests for pairwise comparisons and one way ANOVA.

Project Workflow



Stage IV: Data Mining (To be continued using GeneSpring GX 7.3.1 and 9.0)

- **Cluster analysis** and **expression pattern** recognition.
- Study lists from **Gene Ontology** classification (i.e. Gene lists from the experiment hierarchically classified based on their structural, functional and molecular properties).

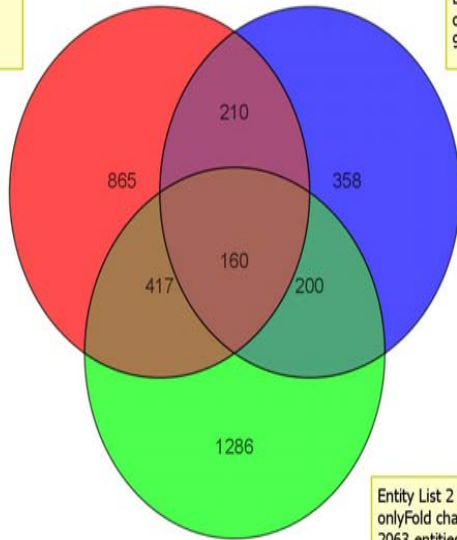
Some lists that we are working on:

- 2 fold change lists at all 3 time points
- 2 fold change list of genes Commonly occurring within the 3 time points.
- Gene Ontology lists for:
 - Photosynthetic genes: Chlorophyll, chloroplast and photosystem related genes.
 - Serine Threonine Kinase genes
 - Cell death related genes etc.
- **Design validation** using Validation Experiment and Quantitative PCR.

Results: Venn Diagrams



Entity List 3 : (GX9)120
onlyFold change>= 2.0
1652 entities



Entity List 1 : (GX9)30
onlyFold change >= 2.0
928 entities

Entity List 3 : T-Test 120 only
2-Fold Change
79 entities



Entity List 1 : T-Test 30 only
2-Fold Change
80 entities

Entity List 2 : T-Test 60 only
2-Fold Change
85 entities

Venn

Venn2

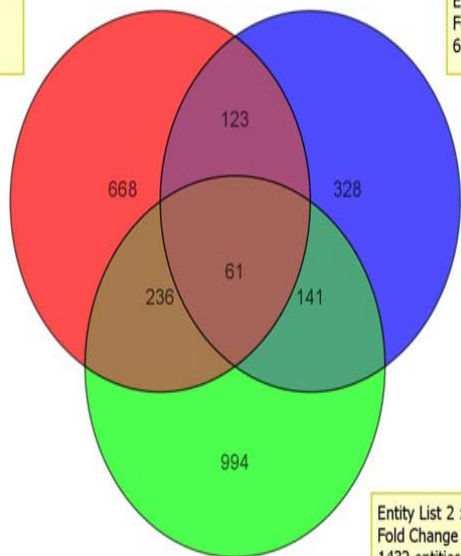
GeneSpring GX 9.0 Experiment: Chlamy 30,60,120 Corr Summary Data

Time Point	No. of Genes (Venn)	No. of Genes (Venn2)
30 min	928	80
60 min	2063	85
120 min	1652	79

Results: Validation Experiment



Entity List 3 : 120 only on 2-Fold Change
1088 entities



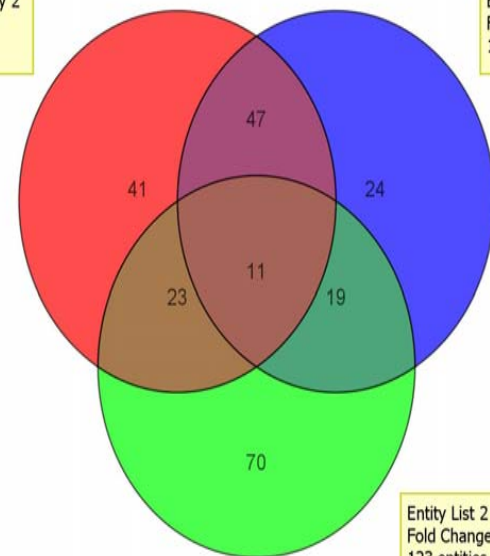
Entity List 1 : 30 only on 2-Fold Change
653 entities

Entity List 3 : T-test 120 only 2 Fold Change
122 entities

Entity List 2 : 60 only on 2-Fold Change
1432 entities

Venn

Entity List 1 : T-test 30 only 2 Fold Change
101 entities



Entity List 2 : T-test 60 only 2 Fold Change
123 entities

Venn2

GeneSpring GX 9.0 Experiment: Chlamy 30, 60, 120+Validate Summary Data

Time Point	No. of Genes (Venn)	No. of Genes (Venn2)
30 min	653	101
60 min	1432	123
120 min	1088	122

Results: Serine Threonine Protein Kinases

30 Reg	Regulation	7.3.1 FC	Description
244.A	up	2.668	(+) (P74745) Probable serine/threonine-protein kinase C (EC 2.7.1.-) [Schizosaccharomyces pombe], 10.7% id
60 Reg	Regulation	7.3.1 FC	Description
201.A	down	0.395	(+) putative serine/threonine kinase [Chlamydomonas reinhardtii] [Chlamydomonas reinhardtii], 82.3% id
244.A	down	0.301	(+) (P74745) Probable serine/threonine-protein kinase C (EC 2.7.1.-) [Schizosaccharomyces pombe], 10.7% id
2454.C	down	0.234	(+) (P43293) Probable serine/threonine-protein kinase NAK (EC 2.7.1.-) [Arabidopsis thaliana], 15.9% id
4320.C	down	0.13	(+) (O65672) Putative serine/threonine protein kinase [Arabidopsis thaliana], 21.4% id
4788.C	down	0.0987	(+) (O82754) Putative serine/threonine kinase [Arabidopsis thaliana], 36.4% id
582.C	down	0.385	(-) putative serine/threonine-protein kinase ctr1 [Oryza sativa (japonica cultivar-group)] [Oryza sativa (japonica cultivar-group)], 93.2% id
7405.C	down	0.387	(+) putative serine/threonine-protein kinase ctr1 [Oryza sativa (japonica cultivar-group)] [Oryza sativa (japonica cultivar-group)], 92.2% id
8776.D	down	0.338	(+) serine/threonine protein kinase [Oryza sativa (japonica cultivar-group)] [Oryza sativa (japonica cultivar-group)], 41.0% id
120 Reg	Regulation	7.3.1 FC	Description
4320.C	down	0.381	(+) (O65672) Putative serine/threonine protein kinase [Arabidopsis thaliana], 21.4% id
9938.E	down	0.201	(-) (Q11179) Putative serine/threonine-protein kinase C05D10.2 in chromosome III (EC 2.7.1.37) [Caenorhabditis elegans], 63.4% id

Results: Gene Lists....



Gene Name	30 FC	30 Reg	60 FC	60 Reg	120 FC	120 Reg	Description
159.A	4.758	up	0.0424	down	3.185	up	(+) light-harvesting chlorophyll-a/b binding protein Lhcb4 [Chlamydomonas reinhardtii] [Chlamydomonas reinhardtii], 100.0% id
262.A	2.171	up	0.133	down	2.161	up	(+) light-harvesting complex I protein [Chlamydomonas reinhardtii] [Chlamydomonas reinhardtii], 100.0% id
345.A	2.19	up	2.447	up	0.403	down	(-) PHOTOSYSTEM I REACTION CENTRE SUBUNIT III PRECURSOR (LIGHT-HARVESTING COMPLEX I 17 KDA PROTEIN) (PSI-F) (P21 PROTEIN) [Chlamydomonas reinhardtii], 100.0% id
371.A	2.838	up	15.6	up	0.156	down	(+) light harvesting complex I protein precursor [Chlamydomonas reinhardtii] [Chlamydomonas reinhardtii], 100.0% id
442.A	2.068	up	0.0526	down	2.709	up	(-) Photosystem I reaction center subunit IV, chloroplast precursor (PSI-E) (Photosystem I 8.1 kDa protein) (P30 protein) [Chlamydomonas reinhardtii], 66.0% id
69.A	2.296	up	0.269	down	2.516	up	(+) Photosystem I reaction center subunit VI, chloroplast precursor (PSI-H) (Light-harvesting complex I 11 kDa protein) (P28 protein) [Chlamydomonas reinhardtii], 100.0% id
8255.D	0.253	down	2.123	up	9.344	up	(+) CALK protein [Chlamydomonas reinhardtii] [Chlamydomonas reinhardtii], 100.0% id
9538.E	2.142	up	0.286	down	2.924	up	(-) chlorophyll a/b-binding protein [Chlamydomonas reinhardtii] [Chlamydomonas reinhardtii], 92.3% id
9853.E	3.031	up	0.291	down	3.791	up	(-) 50S RIBOSOMAL PROTEIN L13, CHLOROPLAST PRECURSOR (CL13) [Spinacia oleracea], 58.4% id



Future Work – Recommendations

- Validation using quantitative polymerase chain reaction
- Updating annotation related information
- Updating Gene Ontology related information
- Automating the gene list sorting process
- Cluster analysis for expression pattern detection
- Adding more time points
- Clubbing this data together with structural studies

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