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in the green alga *Chlamydomonas Reinhardtii***

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Gene expression analysis by arylsulfatase assays in the green alga *Chlamydomonas reinhardtii*

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Summary:

Chlamydomonas reinhardtii, a single-celled green alga, is a powerful microbial experimental system for understanding gene function. As a consequence of a high-quality genome sequence, community-wide efforts for gene model refinement and annotation, resources for strain collections and robust molecular techniques, research with this organism has significantly expanded in the past few decades. In two companion articles, we outline colorimetric and fluorescence-based methodologies for genetic reporter systems in *Chlamydomonas*, which can be used to investigate and delineate gene expression and regulatory mechanisms. Here, we describe protocols for arylsulfatase activity assays using *ARS2*, activity of which can be measured either quantitatively or qualitatively, and in low (individual sample) or high (96-well format) throughput.

Chlamydomonas

1. Introduction

Chlamydomonas reinhardtii (Chlamydomonas) is a soil-dwelling unicellular alga of the Chlorophyte lineage. The organism was initially isolated over half a century ago, and a rich body of literature is available on all aspects of its biology **(1)**. As a consequence of its unique phylogenetic position among characterized organisms **(2)**, and aided by a comprehensive repertoire of molecular and genetic techniques **(3, 4)**, Chlamydomonas is often an organism of choice for addressing fundamental biological questions as they relate to both the plant and animal lineages (for example, photosynthesis and cilia, respectively). In recent years many studies have exploited the advanced genomics capabilities of the organism, enabling questions to be addressed at the -omics scale. Numerous illuminating systems-level and trans-omic studies have resulted from these approaches (recent examples: **(5-7)**). However, the cost (both in terms of time and finances) associated with these large-scale studies can limit their ability to rapidly assess individual genes under many conditions where genome-scale data is not required. Another factor to consider is that most cDNA sequencing (i.e. RNA-Seq) techniques, for which robust methods have been described in Chlamydomonas, does not discriminate between transcriptional and post-transcriptional regulation. Furthermore, mutational analyses, in which the upstream regions of genes can be scrutinized at the nucleotide level, are more suited to experiments on the individual gene scale.

Gene reporter systems can be used to interrogate genetic regulatory elements as well as discriminating conditions of gene expression. Moreover, if one assumes that the signal output is proportional to the transcript level of the target gene, the extent of gene expression can be extrapolated. Several reporter systems have been developed in Chlamydomonas that can be used for expression studies, employing fluorescence **(8, 9)**, luminescence **(10, 11)** and colorimetric outputs. Arylsulfatase assays, first described in Chlamydomonas by de Hostos et al, rely on genetic fusions between the native Chlamydomonas *ARS2* gene and the upstream region of a gene of interest **(12)**. *ARS2* encodes a periplasmic arylsulfatase, which in its native genomic context is induced only under conditions of sulfur deprivation **(13)**, enables the scavenging of sulfur from the natural environment by catalyzing the cleavage of sulfate groups from aromatic substrates. The activity of *ARS2* can be detected in the presence of a chromogenic substrate

(14), such as *para*-nitrophenylsulfate, 5-bromo-4-chloro-3-indolyl sulfate (X-SO₄) and α naphthylsulfate (15). By fusing the promoter-less *ARS2* gene to a promoter of interest, both quantitative and qualitative assays can be performed using cleavage of the chromogenic substrate as a proxy for promoter activity (14, 15). A major advantage of these colorimetric methods over the alternative fluorescence or luciferase-based systems is that, at least for qualitative assays, no specialist apparatus such as a plate reader or spectrophotometer is required. The unicellular nature of *Chlamydomonas* lends itself to experimental scale-up using 96- and 384-well plate formats. This has the benefits of increasing throughput of experimental conditions and replicate number whilst simultaneously reducing individual sample processing/analysis time and costs of generating large volumes of growth medium and the required incubator capacity. Additionally, analyzing dozens of independent transformants of a single construct provides robust statistical power to overcome inherent noise in expression level resulting from genome insertion positional effects. Herein, we outline considerations, experimental designs and protocols for the deployment of this organism and utilization of gene expression studies using arylsulfatase assays. Readers are referred to the next article for protocols utilizing fluorescent proteins in *Chlamydomonas* reporter assays.

2. Materials, equipment and experimental considerations

1. *C. reinhardtii* str. CC-425 (genotype: *cw15 arg2 sr-u-2-60 mt⁺*); catalogued strains of *Chlamydomonas* can be obtained from the *Chlamydomonas* resource center (<http://chlamycollection.org/>)

2. Growth conditions:

Due to the slow growth rate of *Chlamydomonas* relative to other microorganisms, it is strongly advised that transfer and inoculation of media with *Chlamydomonas* be performed under sterile conditions (ideally in a laminar flow hood). Aseptic technique and clean, organized working practices should be applied at all stages. All *Chlamydomonas* liquid cultures are grown at 23°C, $\sim 80 \mu\text{mol m}^{-2} \text{s}^{-1}$, with rotational shaking at 180 RPM, e.g. in Innova incubators (Eppendorf), or similar. Light is supplied by fluorescent tubes in a 1:2 ratio of warm (3,000K) to cool (4,100K). To ensure adequate aeration, flasks should contain no more than 20% medium of total flask volume.

Static growth, either on petri dishes, Omni-trays or 96-well plates are grown at 23°C, $\sim 80 \mu\text{mol m}^{-2} \text{s}^{-1}$ in growth chambers (Percival CU-36L6 or similar). Prior to selection of transformation of vectors encoding ARG7, growth medium for str. CC-425 should be supplemented with 100 $\mu\text{g/ml}$ arginine (filter sterilized if added subsequent to autoclaving).

3. Chlamydomonas growth medium:

Tris-acetate phosphate (TAP) medium **(16)**: each liter contains 10mL TAP salts solution (15 g NH_4Cl , 4g $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 2 g $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, final volume of 1 L H_2O), 8.3 mL phosphate solution (14.34 g K_2HPO_4 , 7.26 g KH_2PO_4 , final volume of 1 L H_2O), 10 mL Tris-acetate solution (242 g Tris, 100 mL glacial acetic acid, final volume of 1 L H_2O) and 1 mL each component of Kropat trace metal solutions **(17)**.

4. Select agar (Thermo Fisher 30391-023); washed three times in purified H_2O . Use 15 g washed agar per L TAP.

5. Plasmids:

pJD100GW (a Gateway-adapted version of pJD100 **(18)**; note that pJD100 was referred to as ptubB2~~B~~,2,1/ars in the original publication **(19)**). pJD100 (and pJD100GW) contains a minimal β tubulin promoter directly upstream of ARS2. pARG7.8 (for co-selection of pJD100GW, see below). Both vectors are available from the Chlamydomonas resource center. pDONR221 is available from ThermoScientific, cat #12536-017.

6. *Escherichia coli* transformants are selected on agar-solidified LB medium containing 50 $\mu\text{g/ml}$ kanamycin (for pDONR221 derivatives) or 100 $\mu\text{g/ml}$ ampicillin (for pJD100GW derivatives).

7. TAPS medium:

TAP medium containing sorbitol at a final concentration of 50 μM . Autoclave in 50 mL aliquots.

8. Plastics:

Sterile, lidded, 96-well plates (eg Nunc cat# 267313 for colorimetric assays, transparent plates are adequate). Omni-trays (ThermoScientific, cat# 140156). Deep dish petri dishes (ThermoScientific, cat# 4031) and 50mL Falcon tubes are conveniently sized for culture centrifugation prior to transforming.

9. 2 mm gap electroporation cuvettes (e.g. FisherScientific cat# FB102)

10. Electroporation System (such as a Bio-Rad Gene Pulser)
11. LR clonase II and BP clonase II reaction kits (Life Technologies, cat# 12538-120 and 11789-020).
12. Small spray bottle (e.g. FisherScientific, cat# S06755)
13. Colorimetric reaction solution A:
 10 mM 5-Bromo-4-chloro-3-indolyl sulfate potassium salt (X-SO₄; Sigma, Cat# B1379) in 0.1 M Tris-HCl, pH 7.5
14. Colorimetric reaction solution B:
 20 mM alpha-Naphthyl sulfate potassium salt (MP Biomedicals, cat# 0210025801)
15. 4% SDS (w/v) in 0.2 M Na acetate buffer, pH 4.8
16. Colorimetric reaction solution C:
 0.1 M Glycine– NaOH (pH 9.0), 10 mM imidazole, 4.5 mM p-nitrophenyl sulfate
17. 2.5 M NaOH
18. All protocol steps using 96-well plates can be significantly expedited by using an 8- or 12-channel pipette (e.g. Eppendorf 10-100 µL 12-channel pipette). Additionally, the reduced number of liquid transfers minimizes risk of missed wells and pipetting errors.
19. Plate reader capable of absorbance measurements at 750 nm and 540 nm, e.g. Tecan M1000 PRO.
20. Bench-top centrifuge with tube and 96-well plate format capabilities. e.g. Eppendorf 5810

3. Methods

3.2. Construction of reporter (See Figure 1)

In general, a 500bp fragment downstream of the gene (+500 to +1; where transcription start site is position 0) is sufficient to capture the promoter. Use the most accurate and up-to-date version of the *Chlamydomonas* genome sequence and gene models, presently v5 and v5.5 respectively, available on Phytozome (<http://phytozome.jgi.doe.gov/pz/portal.html>) to determine the transcription start site. The accuracy of the gene model can be confirmed by comparison to publicly available expressed sequence tags (ESTs), which can be viewed in the genome browser hosted on Phytozome. To clone the promoter fragment into pJD100GW, amplify purified genomic

DNA by PCR. The forward primer needs to contain four guanines (G) followed by the 25-nucleotide *attB1* sequence (5' ACAAGTTTGTACAAAAAGCAGGCT 3') and 18- to 25-nucleotide target-specific sequence. The reverse primer needs to contain four guanines followed by the 25-nucleotide *attB2* sequence (5' ACCACTTTGTACAAGAAAGCTGGGT 3') and 18- to 25-nucleotide target-specific sequence.

We have found Primer3Plus (<http://www.bioinformatics.nl/cgi-bin/primer3plus/primer3plus.cgi/> (20)) to be a convenient and robust tool for selecting the target-specific oligonucleotide sequences, with the following changes to default parameters for amplification from the *Chlamydomonas* genome: primer size Min: 21, Opt: 24, Max: 27 and primer Tm Min 59, Opt: 62, Max: 65. We also routinely use Phusion High-Fidelity DNA Polymerase with Phusion GC buffer (ThermoScientific F-530S) according to the manufacture's protocol, and add DMSO to a final concentration of 3% (v/v). The PCR product is then cloned using Gateway® Technology with pDONR™221 as the entry vector for the BP reaction (ThermoFisher Scientific 12535-029) and pJD100GW as the destination vector in the LR reaction (ThermoFisher Scientific 11791-020). Alternatively, traditional cloning techniques can be employed to insert the region of interest into the KpnI site of pJD100. Once the region of interest has been successfully cloned, regulatory sites can be identified and/or validated by introducing nucleotide mutations by a variety of techniques (see (21) for an efficient method).

Recent advances in chemical gene synthesis offer new capabilities in terms of throughput, experimental design and time/cost savings. DNA promoter fragments can be synthesized containing pre-designed point mutations or deletions, and flanked by *attB* sites for direct cloning by Gateway® methods (without the need for a PCR step). In particular, G-blocks (IDT, IA) are convenient, rapidly synthesized and cost-effective for this purpose.

3.3.1. Transformation by Electroporation

Expected efficiency: ~1 colony (i.e. a single transformant) per ng linearized DNA.

1. Inoculate str. CC-425 into TAP medium plus 100 µg/ml arginine. If inoculation is directly from a slant or plate that has been grown under day/night cycle conditions, subculture several times to lose synchronicity. Cells are competent within the range of 6×10^5 – 6×10^6 cells/mL, with a peak efficiency at $\sim 8 \times 10^5$ cells/mL.
2. To prevent vector DNA strand breakage in regions of specific interest (such as the cloned region, or the selection marker (*aphVIII*)), supercoiled DNA should be linearized using appropriate endonuclease enzyme(s), which cleave outside of the fusion construct and gene used to confer selection in *Chlamydomonas*. To subsequently remove salts introduced in the enzyme buffer prior to electroporation, the linearized DNA should be purified, for example by column-based methods (e.g. Qiagen Qiaquick PCR purification).
3. Pre-chill electroporation cuvettes (gap size 2 mm) on ice.
4. Once the culture has achieved a density of $\sim 8 \times 10^5$ cells/mL, transfer to 50 mL sterile tubes. Add 12.5 µL 20% (v/v) Tween 20 (dilution of 1:4000). Gently mix by inverting 5 times then incubate statically for 5min.
5. Collect the cells by centrifugation 1900 x g, 5 min, room temperature. Pour off the supernatant.
6. Gently resuspend the pellet in 10 mL TAPS by slow pipette action.
7. Collect the cells by centrifugation 1900 x g, 5 min, room temperature. Pour off the supernatant.
8. Gently resuspend the pellet by slow pipette action in TAPS to a final cell count of 4×10^8 cells/mL.
9. Add 300ng of linearized pARG7.8 to 3 separate electroporation cuvettes. Label the three cuvettes as negative control, positive control and test. Then, add a 3-fold molar excess of linearized i) negative control, such as pJD100GW; ii) positive control (pJD100GW containing a promoter fragment that leads to inducible *ARS2* expression during growth in the test condition) and iii) test construct to each cuvette respectively. Use additional cuvettes as required for the number of test constructs.
10. Transfer 150 µL concentrated *Chlamydomonas* cells to each electroporation cuvette. Gently tap to mix cells and DNA. Place on ice for 5 min.
11. Wipe off any melted ice from cuvette, and electroporate using the settings: 800 V, 1000 Ω 25 µF (settings optimized for a BioRad Gene Pulser). As quickly as possible add 100 µL TAPS. Having

a pipette tip pre-loaded can save a few seconds. Time constants (as collected in a BioRad Gene Pulser) should be in the range of 9-11.

12. Incubate the cells in the electroporation cuvettes for 30 min room temperature, static.
13. Gently collect the cells using a pipette, transfer to a 15 mL sterile tube pre-loaded with 5 mL TAPS. Place tubes on a benchtop tube rotator and agitate for 8 hr, room temp, $20\text{-}40\ \mu\text{mol m}^{-2}\text{ s}^{-1}$ (a lower light intensity is advised for this step since division of cells will result in non-independent transformants, and is undesirable for assay statistical robustness).
14. Collect the cells by centrifugation $300 \times g$, 5 min, room temperature. Pour off the supernatant and gently resuspend the pellet in the remaining $\sim 200\ \mu\text{l}$ TAPS. Pipette cells onto TAP agar (without arginine) and gently spread using a sterilized (e.g. flamed, and subsequently cooled) loop.
15. Incubate plates in growth chamber. Colonies should be visible in 7-12 days.

Researchers should also note the excellent protocols for *Chlamydomonas* nucleus genome transformations by Neupert et al (**22**).

3.3.2. Re-array transformants

Transformed DNA near-randomly inserts primarily by illegitimate recombination into the *Chlamydomonas* genome, resulting in position-specific effects on the expression level of the reporter construct.

Consequently, each transformant will have different levels of arylsulfatase activity, dependent on the position of insertion. Additionally, because pJD100GW lacks a selection marker for *Chlamydomonas*, transformants are selected by co-transformation with a marker (pARG7.8 in protocols above). As a result, not every arginine prototroph may have been successfully transformed with the reporter. To overcome these limitations, we routinely screen 96 independent transformants per construct, by inoculating a 96-well plate, which can then be used as a “master plate” (Figure 1), which is a starting point for assay steps 3.4.1 and 3.4.2. For the same reasons, 96 independent transformants of the pJD100GW negative control should also be checked for co-transformation efficiency, and should be carried forwards through subsequent steps.

1. Under aseptic conditions, fill a standard 96-well plate with $200\ \mu\text{L}$ TAP per well.

2. Inoculate each well with an independent transformant colony. Autoclaved pipette tips are useful for this purpose. Ensure transfer of cells by swirling tips in the well.
3. Incubate plates in growth chamber. Inoculated wells should have grown sufficiently to be visibly green in ~7 days (forming the master plate for steps 3.4.1 and 3.4.2).
4. To determine whether the reporter construct was successfully co-transformed with pARG7.8, transfer 10 μ L cell culture from each well to a new 96-well plate. Confirm presence of pJD100GW by PCR using a primer pair designed to anneal uniquely to pJD100GW and amplify a product of roughly 250 – 500 bp. Colony PCR methods, as described by Cao et al, avoid the need to prepare genomic DNA, and can be easily scaled to 96-well format **(23)**. We have found that approaching 100% of transformants contain both plasmids. Should the researcher find that the co-transformation efficiency is lower, he/she may wish to array additional transformants, validated to contain the reporter construct, to maximize the plate capacity in downstream assays.

3.4. Arylsulfatase assay (see Figure 2)

3.4.1. Qualitative (i.e. end-point) assay: This assay is used to quickly screen independent promoter constructs and identify regions of the promoter fragment that are required for condition-specific expression. Because of the site-of-insertion position effects (see above, and next article for further discussion), this assay can also be used to screen transformants for expression level, and accordingly identify clones to carry forwards for low-throughput kinetic assay (3.4.3 below), or to quickly screen constructs on an express/does not express basis.

1. Fill two Omni-trays (55 mL per tray), one with regular TAP agar and the second with agar-solidified medium that will lead to induction of your promoter.
2. Ensure cells in master plate, growing in liquid media, are uniformly distributed in the well. If they are not, gently resuspend by pipetting with sterile tips.
3. Using a multi-channel pipette, transfer 5 μ L of cells from the 96-well master plate to both Omni-trays. Take care to ensure the drops to not flood into each other, and that the agar surface is dry before moving.
4. Incubate the two Omni-trays in growth chamber for 7 days.

5. Uniformly spray surface of the agar with Colorimetric solution A until all colonies are lightly covered. Allow the plates to sit on a benchtop for 6 – 24 hours. Cells that are expressing the arylsulfatase will have a blue halo around them in the agar. If the blue halo is difficult to see after 24 hours, the cells can be removed from the agar by gently applying H₂O (e.g. from either a serological pipette or squeeze-bottle).

3.4.2. Quantitative assay (end-point) – high-throughput.

In addition to providing data acquired by the qualitative assay above, this assay is also used to screen for promoter constructs that no longer regulate arylsulfatase activity under the test condition, can quantify the difference between different constructs, or pick transformants for the low-throughput kinetic assay (3.4.3 below). The largest difference between the two assays is that the level of arylsulfatase activity can be quantified. This assay can also be used to screen activity of a promoter fragment under various growth conditions or stresses.

For each construct or master plate perform the following steps:

1. Using a multi-channel pipette and basin, fill one sterile 96-well plate with 200 µl TAP per well and a second 96-well plate with liquid medium that will lead to absence of repression of your promoter.
2. Transfer 10 µl of culture per well from the master plate to the corresponding wells in the two fresh plates.
3. Incubate the plates in a static incubator until the OD_{750nm} reaches ~0.8 (~3-6 days, depending on the inoculum density).
4. Record absorbance at 750 nm, for normalization purposes
5. Add 10 µl Colorimetric reaction solution B.
6. Incubate at 37°C for 1 hr.
7. Collect the supernatant by centrifugation of the plates at 200 x g for 2 min, room temperature, in a bench-top swinging bucket centrifuge. Transfer 100 µl supernatant to clean 96-well plate.
8. Add 100 µl 4% (w/v) SDS / 0.4 M Na-acetate, pH 4.8 to supernatant.

9. Add 20 μ l 10 mg/ml tetrazotized o-dianisidine and immediately read absorbance at 540 nm in plate reader. Save data.

3.4.3. Quantitative assay (time course) – low-throughput (See Figure 3)

1. Inoculate three independent transformants from the master plate into TAP-containing flasks to a density of $\sim 1 \times 10^5$ cells/mL.
2. Once the cultures reach 1×10^6 cells/mL, transfer half of each culture into 50mL Falcon tubes. Collect cells by centrifugation (1900 x g, 5min, room temp). Resuspend half the culture in control medium and half the culture in test medium (6 cultures total: 3 biological replicates in control medium and 3 biological replicates in test medium).
3. Incubate culture flasks in shaking incubator for 24 hours.
4. Transfer 50 μ l of cell culture to a new 15 mL tube and add 500 μ l Colorimetric reaction solution C. Repeat for each of the six cultures. At this stage, also take a sample from each culture to determine cell density (cells/mL) for normalization of arylsulfatase activity between samples.
5. Incubate tube for 30 min at 27°C.
6. Quench the reaction by addition 2 ml of 2.5 M NaOH to each tube 15 mL tube.
7. Pellet the cells by centrifugation of the reaction mixture at 1900 x g for 5 min, room temp.
8. Transfer supernatant to spectrophotometer cuvette, and measure the absorbance at 410 nm.
9. Repeat steps 4–8 after 2, 4, 8, 12, 24, 48, and 72 hours. Note that these times may be gene-dependent, and may require some optimization. Save all data.
10. Normalize OD₄₁₀ values to cell density. Generate a plot of normalized absorbance by time.

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REFERENCES

1. Harris E (2009) The *Chlamydomonas* Sourcebook, 2nd edition, (San Diego, CA: Academic Press).
2. Merchant SS, Prochnik SE, Vallon O, et al (2007) The *Chlamydomonas* genome reveals the evolution of key animal and plant functions. *Science* (New York, NY) 318:245-250.
3. Jinkerson RE, Jonikas MC (2015) Molecular techniques to interrogate and edit the *Chlamydomonas* nuclear genome. *Plant J* 82:393-412.
4. Mussnug JH (2015) Genetic tools and techniques for *Chlamydomonas reinhardtii*. *Appl Microbiol Biotechnol* 99:5407-5418.
5. Zones J, Blaby I, Merchant S, Umen J. (2015) High-Resolution Profiling of a Synchronized Diurnal Transcriptome from *Chlamydomonas reinhardtii* Reveals Continuous Cell and Metabolic Differentiation. *Plant Cell* 27:2743-2769.
6. Mettler T, Mühlhaus T, Hemme D, et al (2014) Systems Analysis of the Response of Photosynthesis, Metabolism, and Growth to an Increase in Irradiance in the Photosynthetic Model Organism *Chlamydomonas reinhardtii*. *Plant Cell* 26:2310-2350.
7. Ngan CY, Wong C-H, Choi C, et al (2015) Lineage-specific chromatin signatures reveal a regulator of lipid metabolism in microalgae. *Nature Plants* 1(8) doi:10.1038/nplants.2015.107
8. Fuhrmann M, Oertel W, Hegemann P (1999) A synthetic gene coding for the green fluorescent protein (GFP) is a versatile reporter in *Chlamydomonas reinhardtii*. *Plant J* 19:353-361.
9. Rasala BA, Barrera DJ, Ng J, et al (2013) Expanding the spectral palette of fluorescent proteins for the green microalga *Chlamydomonas reinhardtii*. *Plant J* 74:545-556.
10. Shao N, Bock R (2008) A codon-optimized luciferase from *Gaussia princeps* facilitates the in vivo monitoring of gene expression in the model alga *Chlamydomonas reinhardtii*. *Curr Genet* 53:381-388.
11. Fuhrmann M, Hausherr A, Ferbitz L, et al (2004) Monitoring dynamic expression of nuclear genes in *Chlamydomonas reinhardtii* by using a synthetic luciferase reporter gene. *Plant Mol Biol* 55:869-881.

12. de Hostos EL, Schilling J, Grossman AR (1989) Structure and expression of the gene encoding the periplasmic arylsulfatase of *Chlamydomonas reinhardtii*. *Mol Gen Genet* 218:229-239.
13. de Hostos EL, Togasaki RK, Grossman A (1988) Purification and biosynthesis of a derepressible periplasmic arylsulfatase from *Chlamydomonas reinhardtii*. *J Cell Biol* 106:29-37.
14. Davies JP, Weeks DP, Grossman AR (1992) Expression of the arylsulfatase gene from the beta 2-tubulin promoter in *Chlamydomonas reinhardtii*. *Nucleic Acids Res* 20:2959-2965.
15. Ohresser M, Matagne RF, Loppes R (1997) Expression of the arylsulphatase reporter gene under the control of the *nit1* promoter in *Chlamydomonas reinhardtii*. *Curr Genet* 31:264-271.
16. D. S. Gorman, R. P. Levine, Cytochrome *f* and plastocyanin: their sequence in the photosynthetic electron transport chain of *Chlamydomonas reinhardtii*. *Proc Natl Acad Sci U S A* **54**, 1665-1669 (1965).
17. J. Kropat *et al.*, A revised mineral nutrient supplement increases biomass and growth rate in *Chlamydomonas reinhardtii*. *The Plant journal : for cell and molecular biology* **66**, 770-780 (2011).
18. Allen MD, Kropat J, Merchant SS (2008) Regulation and localization of isoforms of the aerobic oxidative cyclase in *Chlamydomonas reinhardtii*. *Photochem Photobiol* 84:1336-1342.
19. Davies JP, Yildiz F, Grossman AR (1994) Mutants of *Chlamydomonas* with Aberrant Responses to Sulfur Deprivation. *Plant Cell* 6:53-63.
20. Rozen S, Skaletsky H. 2000. Primer3 on the WWW for general users and for biologist programmers. *Methods Mol Biol* 132:365-386.
21. Hemsley A, Arnheim N, Toney MD, et al (1989) A simple method for site-directed mutagenesis using the polymerase chain reaction. *Nucleic Acids Res* 17:6545-6551.
22. Neupert J, Shao N, Lu Y, Bock R (2012) Genetic transformation of the model green alga *Chlamydomonas reinhardtii*. *Methods Mol Biol* 847:35-47.
23. Cao M, Fu Y, Guo Y, Pan J (2009) *Chlamydomonas* (Chlorophyceae) colony PCR. *Protoplasma* 235:107-110.

FIGURE LEGENDS

Figure 1 Workflow and schematic of protocol steps 3.2–3.3.2. Panels 1 – 3 refer to protocol 3.2.

Panel 4 refers to section 3.3.1 and panel 5 refers to section 3.3.2. Reaction is abbreviated to rxn.

Figure 2 Workflow and schematic of protocols 3.4.1 and 3.4.2. Starting with a master plate containing 96 individual *C. reinhardtii* arginine prototrophs, transformants can be transferred with a multi-channel pipette to agar-solidified plates containing control and test media to perform a qualitative assay (A) or transferred to 96 well plates containing control and test liquid media to perform a quantitative assay (B).

Figure 3 Workflow and schematic of protocol step 3.4.3. Starting with a master plate containing 96 individual *C. reinhardtii* arginine prototrophs, three independent transformants, whose condition-specific arylsulfatase activity was confirmed as in Figure 2, are used to start three separate cultures. Cells from these three cultures are subsequently grown in test and control media. Samples are collected at regular intervals, and arylsulfatase activity is measured in a low-throughput quantitative assay.