USER GUIDE

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GeneArt® *Chlamydomonas* Engineering Kits

For expression of recombinant proteins in *Chlamydomonas reinhardtii*

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Contents

Product Information

Contents and Storage

Types of kits

This manual is supplied with the products listed below. For a list of components supplied with each catalog number, see below.

Product	Catalog number
GeneArt® *Chlamydomonas* Engineering Kit	A14258
GeneArt® *Chlamydomonas* Engineering Kit with 6L media	A14262

Kit components

Each GeneArt® *Chlamydomonas* Engineering Kit contains the components listed below. See page 3 for a detailed description of each of the components.

Box	Component	Catalog number A14258	Catalog number A14262
1	GeneArt® *Chlamydomonas reinhardtii* cells	✓	✓
2	GeneArt® *Chlamydomonas* Vector Set	✓	✓
3	Gibco® TAP Growth Media		✓

Shipping/Storage

The GeneArt® *Chlamydomonas* Engineering Kits are shipped in separate boxes as described below. Upon receipt, store each box as detailed below. All reagents are guaranteed for six months if stored properly.

Box	Component	Shipping	Storage
1	GeneArt® *Chlamydomonas reinhardtii* cells	Dry ice	–80°C
2	GeneArt® *Chlamydomonas* Vector Set	Dry ice	–20°C
3	Gibco® TAP Growth Media	Gel ice	4°C

Continued on next page

GeneArt® *Chlamydomonas reinhardtii* cells

Each GeneArt® *Chlamydomonas* Engineering Kit is supplied with 10 vials of GeneArt® *Chlamydomonas reinhardtii* 137c cells, with each vial containing 240 µL of frozen cells. Store the cells at –80°C upon receipt. Avoid repeated freeze/thaw cycles and temperature fluctuations.

GeneArt® *Chlamydomonas* Vector Set

The table below lists the components of the GeneArt® *Chlamydomonas* Vector Set (Box 2). Store the contents of Box 2 at –20°C.

Component	Concentration	Amount
pChlamy_3 Vector	20 µL of vector at 0.5 µg/µL in TE buffer, pH 8.0*	10 µg
pChlamy_2/Control Vector	80 µL of vector at 0.5 µg/µL in TE buffer, pH 8.0	40 µg

***TE buffer, pH 8.0:** 10 mM Tris–HCl, 1 mM EDTA, pH 8.0

Gibco® TAP Growth Media

Gibco® TAP Growth Media, included in the GeneArt® *Chlamydomonas* Engineering Kit with 6 L media (Cat. no. A14262), is supplied in 6 × 1 L bottles and is optimized for the growth and maintenance of *Chlamydomonas reinhardtii* cells. Store the Gibco® TAP Growth Medium at 4°C.

Note: Gibco® TAP Growth Media (Cat. nos. A13798-01, A13798-02) are also available separately from Life Technologies. See page 22 for ordering information.

Description of the System

GeneArt® *Chlamydomonas* Engineering Kit

The GeneArt® *Chlamydomonas* Engineering Kit is a eukaryotic genetic engineering system based on the unicellular green alga *Chlamydomonas reinhardtii* 137c (Proschold *et al.*, 2005), offering a simplified approach for metabolic engineering of algae for downstream applications such as biofuels, specialty chemicals, and industrial enzymes. This system is designed for nuclear integration of your gene of interest. The integration is random and the number of integrated copies depends on various factors such as cell age, the sequence content, and the size of the gene of interest.

Chlamydomonas reinhardtii

The green algae *Chlamydomonas reinhardtii* has served as a genetic workhorse and model organism for understanding everything from the mechanisms of light and nutrient regulated gene expression to the assembly and function of flagella (Harris, 2001; Hippler *et al.*, 1998; Merchant *et al.*, 2007; Miller *et al.*, ; Molnar *et al.*, 2007). Recently, green algae have started to be used as a platform for the production of biofuel and bio-products, due mainly to their rapid growth and ability to use sunlight and CO_2 as their main inputs (Radakovits *et al.*, 2010; Wang *et al.*, 2012). Green algae also offer a variety of beneficial attributes including:

- the ease of transformation and the relatively short time between the generation of initial transformants and their scale up to production volumes
- the ability to induce gametogenesis and carry out genetic crosses between haploid cells of opposite mating types
- the ability to grow phototrophically or heterotrophically
- the ability to grow cultures on scales ranging from a few milliliters to 500,000 liters, in a cost effective manner

These attributes, and the fact that green algae fall into the GRAS category (i.e., generally recognized as safe by FDA), make *C. reinhardtii* a particularly attractive system for the expression of recombinant proteins.

Growth characteristics of *C. reinhardtii*

Compared to land plants, *C. reinhardtii* grows at a much faster rate, doubling cell numbers in approximately 8 hours under heterotrophic growth and 12 hours under photosynthetic growth. As *C. reinhardtii* propagates by vegetative division, the time from initial transformation to product production is significantly reduced relative to plants, requiring as little as six weeks to evaluate production at flask scale, with the potential to scale up to 64,000 liters in another four to six weeks. *C. reinhardtii* also possesses a well characterized mating system, making it possible to carry our classical breeding through matings between various transgenic algal lines, again in a very short period of time (3–4 weeks) (Harris, 2001).

Continued on next page

Expressing heterologous genes in *C. reinhardtii*

In *C. reinhardtii*, expression of heterologous proteins presentsseveral difficulties. The first problem is represented by the unusual codon bias of the *C. reinhardtii* nuclear genes that is highly G-C rich (62%), so codon optimization must be performed on any gene for which high levels of protein expression are desired (Fuhrmann *et al.*, 2004; Fuhrmann *et al.*, 1999; Heitzer *et al.*, 2007). Additionally, expression levels of optimized foreign genes may vary considerably due to position effect that is driven by random integration of the gene of interest and strong silencing mechanism that drives by epigenetic phenomena similar to those in land plants (Schroda, 2006). In *C. reinhardtii* and other algae, as in land plants, silenced multiple-copy transgenes exhibit high levels of DNA methylation (Babinger *et al.*, 2001; Cerutti *et al.*, 1997). In contrast, single-copy transgenes are subject to transgene silencing without detectable cytosine methylation (Cerutti *et al.*, 1997). Another feature of most *C. reinhardtii* nuclear genes is the presence of several small introns in their coding sequences that exert a positive role in gene expression.

pChlamy_3 Vector

pChlamy_3 vector is designed to facilitate rapid cloning of your gene of interest for expression in *C. reinhardtii*. This vector is a nuclear integrative vector; the integration is a random event across the genome. However, depending on the context of the gene of interest, the copy number of the integrated gene will be varied. Some of the features of the vector are listed below. For a map of the vector, see page 19.

- HSP70A/RBCS2 chimeric constitutive promoter for strong expression of gene of interest
- A versatile multiple cloning site for simplified cloning of your gene of interest
- Hygromycin resistance gene (aph7) driven by the β2-tubulin promoter for selection in *C. reinhardtii*
- A 3′-UTR fragment from RbcS2 (Ribulose Bisphosphate Carboxylase/ Oxygenase Small Subunit 2) gene downstream of the multiple cloning site for ensuring the proper termination of transcript

  Note: 3' UTR may contain sequences that regulate translation efficiency, mRNA stability, and polyadenylation signals.
- Ampicillin resistance gene for selection in *E. coli*
- pUC origin for maintenance in *E. coli*

Experiment Outline

Workflow

The table below describes the major steps needed to clone and express your gene of interest in *C. reinhardtii*. For more details, refer to the pages indicated.

Step	Action	Page
1	Clone your codon optimized gene of interest into pChlamy_3 vector	7
2	Transform *E. coli* with the pChlamy_3 construct containing your gene of interest and select the transformants on LB plates containing Ampicillin	9
3	Analyze transformants by restriction digestion or PCR	10
4	Thaw and resuscitate *C. reinhardtii* cells	12
5	Transform *C. reinhardtii* cells by electroporation and select transformants	13
6	Screen *C. reinhardtii* transformants by colony PCR for full integration of your gene of interest	16

Methods

Cloning into pChlamy_3 Vector

General molecular biology techniques

For help with PCR amplification, DNA ligations, *E. coli* transformations, restriction enzyme analysis, DNA sequencing, and DNA biochemistry, refer to *Molecular Cloning: A Laboratory Manual* (Sambrook *et al.*, 1989) or *Current Protocols in Molecular Biology* (Ausubel *et al.*, 1994).

E. coli host

For cloning and transformation, we recommend using a recombination deficient (*rec*A) and endonuclease A-deficient (*end*A) strain such as TOP10 (available separately; see page 22). Note that other *rec*A, *end*A *E. coli* strains are also suitable.

Genotype of TOP10:

F$^-$ *mcr*A Δ(*mrr-hsd*RMS-*mcr*BC) Φ80*lac*Z M15 Δ*lac*74 *rec*A1 *ara*D139 Δ(*ara-leu*)7697 *gal*U *gal*K *rps*L (Str^R) *end*A1 *nup*G

Maintaining pChlamy_3

To propagate and maintain the pChlamy_3 vector, use 10 ng of the vector to transform a *rec*A, *end*A *E. coli* strain like TOP10, DH5α™, JM109, or equivalent. Select transformants on LB plates containing 50–100 µg/ml ampicillin. Be sure to prepare a glycerol stock of the plasmid for long-term storage (see page 10 for a protocol).

Cloning considerations

- Since the *C. reinhardtii* genome has a very high GC content (~62% GC), the expression levels of recombinant genes are significantly improved if the gene of interest is adapted to the preferred codon usage of highly expressed *C. reinhardtii* genes.
- Note that the Intron-1 Rbc S2 (bases 574–718, see page 8) is spliced out from the mature RNA and does not constitute actual codons. The reading frame after the removal of Intron-1 Rbc S2 is:

```
                start                                KpnI        XbaI    BglII       PstI  NotI      NdeI
                 ⌐¬                                   |           |       |           |     |         |
ACTCAACATC TTAAAATGGC CAGGAGATTC GAGGTACCAT ACAGTTCTAG AGATCTCTGCA GCGGCCGCCA TATGATTCGAA
```

- pChlamy_3 vector contains the ATG initiation codon (Vector ATG) for proper initiation of translation at position 566–568. Be sure to clone your gene of interest in frame with the ATG initiation codon (Vector ATG) using the sequence with the intron spliced out (see above).
- Your insert must contain a stop codon for proper termination of your mRNA. You can either use the native sequence containing the stop codon in the reverse primer or make sure that the stop codon is upstream from the reverse PCR primer binding site. **Note that the *Xba* I site contains an internal stop codon (TCTAGA).**

Continued on next page

Cloning into pChlamy_3 Vector, continued

Multiple cloning site of pChlamy_3

Below is the multiple cloning site for pChlamy_3 before and after splicing. Restriction sites are labeled to indicate the cleavage site. The ATG initiation codon (Vector ATG) is shown in bold and potential stop codons are underlined. If your gene of interest (GOI) contains an ATG initiation codon, it must be cloned in frame to the Vector ATG after splicing, so use the after splicing sequence below as a guide; otherwise, your GOI will not be properly expressed. Make sure that your insert also contains a stop codon. Use the diagram below to design suitable PCR primers to clone and express your PCR product in pChlamy_3.

The vector sequence of pChlamy_3 is available for downloading at **www.lifetechnologies.com** or by contacting Technical Support (page 24).

Before splicing:

```
                        start                                   Intron-1 Rbc S2
551 ACTCAACATC TTAAAATGGC CAGGTGAGTC GACGAGCAAG CCCGGCGGAT CAGGCAGCGT GCTTGCAGAT TTGACTTGCA

                                                Intron-1 Rbc S2
631 ACGCCCGCAT TGTGTCGACG AAGGCTTTTG GCTCCTCTGT CGCTGTCTCA AGCAGCATCT AACCCTGCGT CGCCGTTTCC

    Intron-1 Rbc S2              KpnI      XbaI  BglII       PstI  NotI      NdeI          3´-UTR
711 ATTTGCAGGA GATTCGAGGT ACCATACAGT TCTAGAGATC TCTGCAGCGG CCGCCATATG ATTCCGCTCC GTGTAAATGG

                                                        3´-UTR
791 AGGCGCTCGT TGATCTGAGC CTTGCCCCCT GACGAACGGC GGTGGATGGA AGATACTGCT CTCAAGTGCT GAAGCGGTAG
```

After splicing:

```
                    start                   KpnI        XbaI   BglII       PstI  NotI      NdeI
ACTCAACATC TTAAAATGGC CAGGAGATTC GAGGTACCAT ACAGTTCTAG AGATCTCTGCA GCGGCCGCCA TATGATTCGAA
```

Ligation

Once you have determined a cloning strategy and PCR amplified your gene of interest, digest pChlamy_3 with the appropriate restriction enzyme and ligate your insert containing your gene of interest using standard molecular biology techniques.

E. coli transformation method

You may use any method of your choice for *E. coli* transformation. Chemical transformation is the most convenient for most researchers. Electroporation is the most efficient and the method of choice for large plasmids. For your convenience, a protocol for chemical transformation using One Shot® TOP10 Chemically Competent *E. coli* is provided on page 9; however, you may also transform electrocompetent cells.

Transforming One Shot® TOP10 Competent *E. coli* Cells

Introduction

Once you have performed the cloning reaction, you will transform your pChlamy_3 construct into competent *E. coli*. The following protocol for transforming One Shot® TOP10 Chemically Competent *E. coli* (available separately; see page 22) is included for your convenience. Note that you may also transform electrocompetent cells using the protocol supplied with the electrocompetent cells.

Materials needed

- pChlamy_3 construct containing your gene of interest
- One Shot® TOP10 Chemically Competent *E. coli* (Cat. no. C4040; see page 22)
- S.O.C. Medium (supplied with Cat. no. C4040)
- pUC19 positive control (supplied with Cat. no. C4040; recommended for verifying transformation efficiency)
- 42°C water bath
- LB plates containing 100 µg/mL ampicillin (two for each transformation)
- 37°C shaking and non-shaking incubator

Preparing for transformation

For each transformation, you will need one vial of competent cells and two selective plates.

1. Equilibrate a water bath to 42°C.
2. Warm the vial of S.O.C. medium to room temperature.
3. Warm LB plates containing 100 µg/mL ampicillin at 37°C for 30 minutes.
4. Thaw **on ice** 1 vial of One Shot® TOP10 for each transformation.

One Shot® chemical transformation protocol

1. Add 1–5 µL of the DNA (10 pg to 100 ng) into a vial of One Shot® Chemically Competent *E. coli* and mix gently. **Do not mix by pipetting up and down**.
   Note: If you are transforming the pUC19 control plasmid, use 10 pg (1 µL).
2. Incubate on ice for 5 to 30 minutes.
   Note: Longer incubations on ice seem to have a minimal effect on transformation efficiency.
3. Heat-shock the cells for 30 seconds at 42°C without shaking.
4. Immediately transfer the tubes to ice.
5. Add 250 µL of room temperature S.O.C. Medium.
6. Cap the tube tightly and shake the tube horizontally (200 rpm) at 37°C for 1 hour.
7. Spread 50–200 µL from each transformation on a prewarmed selective plate and incubate overnight at 37°C. We recommend that you plate two different volumes to ensure that at least one plate will have well-spaced colonies.
8. Pick 5–10 colonies for analysis (see **Analyzing *E. coli* Transformants**, page 10).

Analyzing *E. coli* Transformants

Picking positive *E. coli* clones

1. Pick 5–10 colonies and culture them overnight in LB medium containing 100 µg/mL ampicillin.
2. Isolate plasmid DNA using your method of choice. If you need ultra-pure plasmid DNA for automated or manual sequencing, we recommend using the PureLink® HQ Mini Plasmid Purification Kit (Cat. no. K2100-01; see page 22).
3. Analyze the plasmids by restriction analysis or PCR (see below) to confirm the presence and correct orientation of the insert.

Analyzing *E. coli* transformants by PCR

Use the protocol below (or any other suitable protocol) to analyze positive *E. coli* transformants using PCR. You will have to determine the primer sequences and amplification conditions based on your gene of interest. Design a forward primer to hybridize to the vector backbone flanking your insert and a reverse primer to hybridize within your insert. You can also perform restriction analysis in parallel.

Materials Needed:

- PCR Super Mix High Fidelity (Cat. no. 10790-020)
- Appropriate forward and reverse PCR primers (20 µM each)

Procedure:

1. For each sample, aliquot 48 µL of PCR SuperMix High Fidelity into a 0.5 mL microcentrifuge tube. Add 1 µL each of the forward and reverse PCR primer.
2. Pick 5–10 colonies and resuspend them individually in 50 µL of the PCR SuperMix containing PCR primers (remember to make a patch plate to preserve the colonies for further analysis).
3. Incubate reaction for 10 minutes at 94°C to lyse cells and inactivate nucleases.
4. Amplify for 20 to 30 cycles.
5. For the final extension, incubate at 72°C for 10 minutes. Store at 4°C.
6. Visualize by agarose gel electrophoresis.

Analyzing *E. coli* transformants by sequencing

Once you have identified the correct clone(s), you may sequence your construct to confirm that your gene is cloned in the correct orientation. Design a primer that hybridizes to the vector backbone flanking your insert to help you sequence your insert. For the complete sequence of the pChlamy_3 vector, refer to our website (**www.lifetechnologies.com**) or contact Technical Support (see page 24).

Long-term storage

Once you have identified the correct clone, make a glycerol stock for long-term storage. We recommend that you store a stock of plasmid DNA at –20°C.

Guidelines for Culturing *Chlamydomonas reinhardtii*

General guidelines for *C. reinhardtii* culture

- *C. reinhardtii* is easy and inexpensive to grow. Routine maintenance is usually done at room temperature on 1.5% agar, while growth for individual experiments is typically done in liquid culture in shake flasks or bottles.
- *C. reinhardtii* has a short generation time of less than 8 hours under optimum conditions.
- All solutions and equipment that may contact cells must be sterile. Always use proper sterile technique and work in a laminar flow hood.
- Grow the cells using Gibco® TAP medium, which is specifically formulated for optimal growth and maintenance of *C. reinhardtii* cells.
- *C. reinhardtii* laboratory and wild type strains grow well in the range of 20–25°C and can tolerate temperatures as low as 15°C and as high as 35°C. The strain in this kit (*C. reinhardtii* 137c) should be grown at 26°C under continuous illumination using moderate light intensities of cool fluorescent white light (50 ± 10 µE m^{-2} s^{-1}) with constant agitation on a gyrotary shaker set to 100–150 rpm.
- The optimal equipment for culturing *C. reinhardtii* is an algal growth chamber (e.g., Percival Algal Chamber from Geneva Scientific) with regulatable light supply and a light meter (e.g., LI-250A Light Meter from LI-COR®) to guide adjustments. If an algal growth chamber is not available, the cells can be grown in a standard cell culture incubator illuminated with cool fluorescent lights placed within 12 inches of the culture plates. Standard room lights provide sub-optimal growth conditions.
- Phototrophic cultures should be supplied with CO_2 at 5% for maximal growth, although the *C. reinhardtii* 137c strain included in the kit can grow in the incubator without the need of additional CO_2 supply.
- Flasks for liquid culture can be stoppered with sterile foam plugs, polypropylene caps, alminium foil, cotton, or any cap that allows air exchange.
- After transformation and plating, do not stack the culture plates to allow continuous uniform illumination.
- *C. reinhardtii* is classified as a GRAS (generally regarded as safe) organism with no known viral or bacterial pathogens. However, we recommend following general safety guidelines under Biosafety Level 1 (BL-1) containment, similar to working with *E. coli* or yeast. For more information on BL-1 guidelines, refer to *Biosafety in Microbiological and Biomedical Laboratories,* 5th ed., published by the Centers for Disease Control, which isavailable for downloading at: **www.cdc.gov/od/ohs/biosfty/bmbl5/bmbl5toc.htm**.

Thawing *Chlamydomonas reinhardtii*

Materials needed

- 35°C water bath
- Algal Growth Chamber (e.g., Percival Algal Chamber from Geneva Scientific) set to 26°C, 50 µE m^{-2} s^{-1}

  Note: If an Algal Chamber is not available, you can use a standard cell culture incubator under continuous illumination using moderate intensities of cool fluorescent white light (50 µE m^{-2} s^{-1}).
- Rotary shaking platform set to 110 rpm
- 6-well clear-bottom culture plates
- Gibco® TAP medium (Cat. no. A13798-01 or A13798-02), pre-warmed to room temperature
- 70% ethanol
- Dry ice

Thawing procedure

1. Remove the frozen cells from –80°C storage and immediately place them in a dry ice container. Bury the vial(s) containing the cells in dry ice to minimize temperature fluctuations before thawing.
2. Add 4 mL of Gibco® TAP medium, pre-warmed to room temperature, into each well of a 6-well plate.
3. Remove the cryovial containing the frozen cells from the dry ice storage and **immediately** place it into a 35°C water bath.
4. Quickly thaw the cells by gently swirling the vial in the 35°C water bath until the cell have completely thawed (1–2 minutes).
5. Before opening, wipe the outside of the vial with 70% ethanol.
6. Transfer 230 µL of thawed cells from the vial into each well of the 6-well plate containing 4 mL of Gibco® TAP medium.
7. Place the 6-well plate(s) in the algal growth chamber set to 26°C and 50 µE m^{-2} s^{-1}. **Do not stack the plates.**
8. Incubate the cells for 3–6 days with agitation on a rotary shaker set to 110 rpm.
9. On Day 3, measure the optical density of the cells at 750 nm (OD_{750}) in Gibco® TAP medium. If the culture has not yet reached OD_{750} 0.6, return it to the algal growth chamber and continue the incubation. Check the OD_{750} of the culture daily until it reaches OD_{750} 0.6. Once the OD_{750} has reached 0.6, proceed to step 10.
10. Add 40 mL of fresh Gibco® TAP medium, pre-warmed to room temperature, in a 125-mL flask.
11. Dilute the cells from the 6-well plate(s) into the flask containing the Gibco® TAP medium to obtain a **final OD_{750} of 0.06.**
12. Place the culture(s) in the algal growth chamber set to 26°C and 50 µE m^{-2} s^{-1}.
13. Grow the cultures for 20–24 hours with agitation on a gyrotary shaker set to 110 rpm and proceed to transformation (page 13). Do not exceed a culture time of 24 hours because transformation efficiency drops rapidly as cells reach saturating densities (OD_{750}>1).

Transforming *Chlamydomonas reinhardtii* by Electroporation

Guidelines for transforming *C. reinhardtii*

- Perform all steps of the electroporation procedure at room temperature.
- Nuclear transformation of *C. reinhardtii* can be achieved with circular DNA; however, transformation with linearized DNA is much more efficient (~70%). We recommend using *Sca*I restriction enzyme for linearization, provided that the insert does not contain the recognition sequence for *Sca*I.
- The number of insertions into the *C. reinhardtii* genome is also influenced by the amount of DNA used. We recommend using 2 µg of linearized plasmid DNA per electroporation.
- The quality and the concentration of DNA used play a central role for the efficiency of transformation. Use a commercial kit such as the PureLink® HQ Mini Plasmid Purification or the PureLink® HiPure Plasmid Miniprep kits that deliver pure DNA.
- For best results, grow the cells to OD_{750} 0.3–0.5 before proceeding with electroporation.
- Insertion of the plasmid DNA into the genome occurs randomly. On average only 20% of transformants will express the gene of interest at appreciable levels. We recommend first screening the colonies by colony PCR (see page 16) to ensure full integration of the promoter and the gene of interest, followed by the screening of several positive clones for the expression of the gene of interest to pick the highest expressing clone.
- Because the *C. reinhardtii* genome has a very high GC content (~62% GC), the expression levels of recombinant genes are significantly improved if the gene of interest is adapted to the preferred codon usage of highly expressed *C. reinhardtii* genes.

Materials needed

- pChlamy_3 construct containing your gene of interest and linearized with the appropriate restriction enzyme
  Note: We recommend using *Sca*I restriction enzyme for linearization, provided that the insert does not contain the recognition sequence for *Sca*I.
- pChlamy_2/Control Vector, linearized with *Sca*I restriction enzyme
- Gibco® TAP medium (Cat. no. A13798-01 or A13798-02), pre-warmed to room temperature
- TAP-40 mM sucrose solution, pre-warmed to room temperature (see page 18 for recipe)
- TAP-Agar-Hygromycin plates (10 µg/mL) (see page 18 for recipe)
- Sterile 15-mL and 50-mL centrifugation tubes
- 0.4-cm electroporation cuvettes (Cat. no. P460-50)
- Electroporation device such the Bio-Rad® Gene Pulser® II
  Optional: Alternatively, you can use the Neon® Transfection System (Cat. no. MPK5000) or the Neon® Transfection System 100 µL Kit (Cat. no. MPK10025).
- ColiRollers™ plating glass beads (Novagen, Cat. no. 71013)

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Transforming *Chlamydomonas reinhardtii* by Electroporation, continued

Electroporation procedure

If using an electroporation device such as the Bio-Rad® Gene Pulser® II, follow the protocol below. If using the Neon® Transfection System, first read the **Notes for using the Neon® Transfection System**, page 15, and adjust the electroporation conditions accordingly.

1. Measure the optical density of the *C. reinhardtii* cultures (from Step 14, page 12) at 750 nm (i.e., OD_{750}).
   Note: For best performance, the OD_{750} of cultures should be between 0.3–0.5. If the OD_{750} does not reach 0.3 within 24 hours of incubation after dilution (Steps 11–13, page 12), incubate the cells for an extra 3–5 hours to allow for an additional cell division.
2. Harvest 15 mL of the cells (per transformation) by centrifugation at 2,500 rpm for 10 minutes at room temperature. Centrifuge the cells in 15-mL conical tubes to obtain tight pellets.
3. Discard the supernatant by decanting. Remove the remaining supernatant using a pipette.
4. Resuspend the cells in 250 µL of TAP-40 mM sucrose solution at room temperature by gently pipetting up and down.
5. Add 2 µg linearized plasmid DNA (i.e., pChlamy_3 construct containing your gene of interest) into the resuspended cells. Mix the DNA-cell suspension gently by flicking the tube. In a separate tube, prepare a control transformation with the pChlamy_2/Control Vector, linearized using *Sca*I restriction enzyme.
6. Transfer 250 µL of the transformation mixture into an electroporation cuvette and incubate at room temperature for 5 minutes.
7. While the transformation mixtures are incubating, add 5 mL of TAP-40 mM sucrose solution at room temperature into each well of a 6-well plate.
   Note: You will divide each transformation mixture between two wells of a 6-well plate after electroporation, so that the cells in each transformation mixture will recover in 10 mL of TAP-40 mM sucrose solution total.
8. Set the electroporation parameters as follows:

Voltage	Capacity	Resistance
600 V	50 µF	infinity

9. Gently tap the electroporation cuvette to mix the contents and resuspend the settled cells, and place the cuvette in the cuvette chamber.
10. Electroporate the cells with the above parameters.
11. Split the transformation mixture into two aliquots of 125 µL each and transfer each aliquot into one well of the 6-well plate containing 5 mL/well of TAP-40 mM sucrose solution at room temperature. Wash the cuvette with 1 mL of TAP-40 mM sucrose solution to get most cells out of the cuvette and split and add the wash into the same two wells.

Continued on next page

Transforming *Chlamydomonas reinhardtii* by Electroporation, continued

Electroporation procedure, continued

12. Place the 6-well plate in the algal growth chamber set to 26°C and 50 µE m^{-2} s^{-1}.
13. Incubate the cells for 24 hours with gentle agitation (100–150 rpm) to let them recover.
14. Centrifuge the cells at 2,500 rpm for 10 minutes at room temperature.
15. Discard the supernatant by decanting. Remove the remaining supernatant with a pipette.
16. Resuspend the cells with gentle pipetting in 150 µL of TAP-40 mM sucrose solution at room temperature.
17. Plate the entire cell solution from each transformation on one TAP-agar-Hygromycin plate using disposable cell spreaders or glass plating beads to spread the cells evenly. Make sure the plates do not have condensation on them.
18. Place the plates agar side at the bottom in the algal growth chamber set to 26°C and 50 µE m^{-2} s^{-1}. Do not stack the plates to ensure continuous and even illumination.
19. Incubate the plates for 5 days or until *C. reinhardtii* colonies are clearly visible. Control vector should produce a minimum of 30 transformants per electroporation reaction. The transformation efficiency with the pChlamy_3 construct will depend on the nature, size, and codon content of the gene of interest, and the physiological state of the cells.
20. Proceed to determination of integration by colony PCR (see page 16) before selecting clones for further scale-up.

    About 20% of the colonies should be positive for the gene of interest. Due to random integration and silencing events in *C. reinhardtii*, we recommend picking at least 10 positive colonies and testing them for the expression level of the gene of interest by RT-PCR (or Western blotting, if you have the antibody to detect it).

Notes for using the Neon® Transfection System

If using the Neon® Transfection System, follow the guidelines below.

- For high transformation efficiencies, the OD_{750} of the *C. reinhardtii* culture should be >0.8 before electroporation.
- Carry out all transformation steps at 4°C using solutions pre-equilibrated solutions at 4°C.
- Electroporate the cells in TAP-40 mM sucrose solution at 4°C.
- Use the following Neon® electroporation parameters:

Voltage	Pulse width	Pulse number
2300 V	13 ms	3

- For detailed instructions on using the Neon® Transfection System, refer to the Neon® Transfection System user guide, available for downloading at **www.lifetechnologies.com**.

Screening for Integration by Colony PCR

Introduction

Use the protocols below to prepare cell lysates and perform colony PCR to screen the transformed *C. reinhardtii* colonies for full integration of the promoter and the gene of interest. You will have to design the forward and reverse PCR primers appropriate for your insert and determine the amplification conditions. We recommend using the AccuPrime™ *Pfx* Polymerase SuperMix for best results.

Materials needed

- AccuPrime™ *Pfx* SuperMix (Cat. no. 12344-040)
- Appropriate forward and reverse primers (10 µM each)

Preparing cell lysates

1. Pick half of a colony for analysis using a P-20 pipette tip and drop it into the PCR tube containing 10 µL of water. Repeat for up to 20 additional colonies.
   Note: Remember to make a patch plate to preserve the colonies for further experiments.
2. Boil the tubes at 95 °C for 10 minutes (a thermocycler can also be used).
3. After 10 minutes, resuspend each colony in water by pipetting up and down. This is the cell lysate that you will use as a template for PCR in the next step.

Colony PCR procedure

1. Prepare the following PCR mix for each cell lysate:

Reagent	Amount
AccuPrime™ *Pfx* SuperMix	47 µL
Cell lysate	1 µL
Forward primer (10 µM)	1 µL
Reverse primer (10 µM)	1 µL
Total volume:	**50 µL**

2. Mix the contents of the tubes and load into a thermal cycler.
3. Use the following PCR program as a starting point for your template and primers:

   95°C for 5 minutes
   35 cycles of:
      95°C for 15 seconds
      55–65°C for 30 seconds
      68°C for 1 minute per kb
4. Maintain reaction at 4°C after cycling. Samples can be stored at –20°C.
5. Analyze the results by agarose gel electrophoresis. Approximately 20% of the colonies should be positive for full integration of the promoter and the gene of interest.

Storage and Scale-Up

Storing *C. reinhardtii* transformants

Plates containing transformed cells can be wrapped in Parafilm® laboratory film and stored at room temperature for at least one month. Longer term storage of cells can be achieved by streaking the colonies onto selective plates, sealing the plates with Parafilm® laboratory film, and placing them in dim light at 10–15°C.

Growing and scaling-up *C. reinhardtii* transformants

- For downstream biochemical applications and/or scale-up, liquid *C. reinhardtii* cultures should not be inoculated directly from agar plates. Instead, you can start a seed culture by inoculating a single large colony into 250 mL of Gibco® TAP medium, growing the cells until they reach the mid-log phase of growth (1×10^6–5×10^6 cells/mL), and then taking an appropriate aliquote to inoculate the experimental cultures at a starting density of 1×10^5 cells/mL in 300 mL of culture.
- We recommend using the Gibco® TAP medium for shake flask or fermentation experiments. Large scale phototrophic cultures should be bubbled with CO_2 (5% in air) for maximal growth. Smaller cultures such as shake flasks do not need bubbling, but the flask should be sealed by airpore tapes or aluminum foil to allow air exchange.

Appendix A: Support Protocols

Preparing Reagents and Media

Handling Hygromycin B

When added to cultured eukaryotic cells, hygromycin B acts as an aminocyclitol to inhibit protein synthesis by disrupting translocation and promoting mistranslation. When handling hygromycin B, follow the guidelines below:

- Hygromycin B is light sensitive. Store the liquid stock solution at 4°C protected from exposure to light.
- Hygromycin B is toxic. Do not ingest solutions containing the drug.
- Wear gloves, a laboratory coat, and safety glasses or goggles when handling hygromycin B and hygromycin B-containing solutions.

TAP-40 mM sucrose solution

1. Prepare 1 M sucrose stock solution by dissolving 342.3 g of sucrose in 800 mL of deionized water and adding water to bring the final volume to 1 L. Filter sterilize the 1 M sucrose solution through a 0.22 µm filter.
   Note: You can prepare this solution several days before performing the electroporation.
2. To prepare the TAP-40 mM sucrose solution, add 40 mL of 1 M sucrose to 1 L of Gibco® TAP medium.

TAP-Hygromycin B solution

1. Add hygromycin B stock solution (Cat. no. 10687-010; at 100 mg/mL) to Gibco® TAP medium to a final concentration of 10 µg/mL.
2. Filter-sterilize through a 0.22 µm filter and store at 4°C in the dark.

TAP-Agar plates

1. Add 15 g of agar to 200 mL of Gibco® TAP medium in an autoclaveable flask.
2. Autoclave on liquid cycle for 20 minutes.
3. Warm 800 mL of Gibco® TAP medium to 55–60°C in a water bath
4. After autoclaving, cool the agar containing flask to ~55°C.
5. Combine the agar containing flask with 800 mL of Gibco® TAP medium and pour into 10 cm plates.
6. Let the plates harden (do **not** overdry), invert them, and store at 4°C in the dark. Final agar concentration will be 1.5%.
   Note: Overdrying the plates drastically reduces the transformation efficiency.

TAP-Agar-Hygromycin B plates

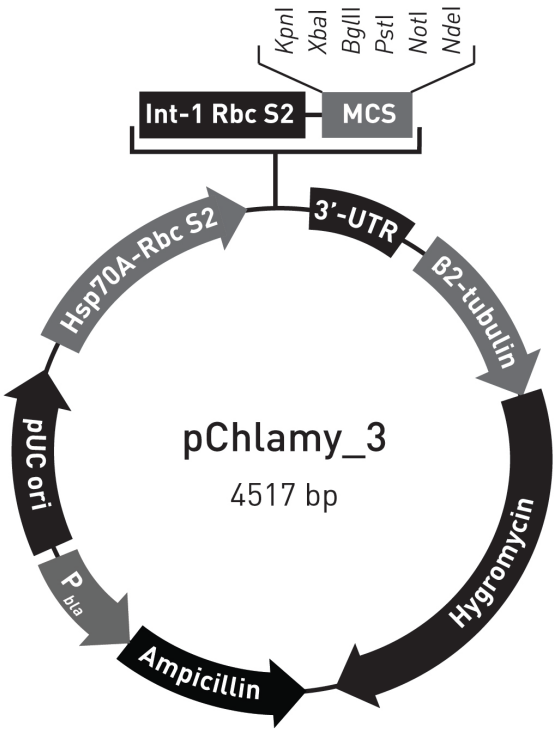
1. Add 15 g of agar to 200 mL of Gibco® TAP medium in an autoclaveable flask.
2. Autoclave on liquid cycle for 20 minutes.
3. Warm 800 mL of Gibco® TAP medium to 55–60°C in a water bath
4. After autoclaving, cool the agar containing flask to ~55°C.
5. Combine the agar containing flask with 800 mL of Gibco® TAP medium
6. Add hygromycin B to a final concentration of 10 µg/mL (i.e., 100 µL of 100 mg/mL stock solution), and pour into 10 cm plates.
7. Let the plates harden (do **not** overdry), invert them, and store at 4°C in the dark. Final agar concentration will be 1.5%.

Appendix B: Vectors

Map and Features of pChlamy_3 Vector

Map of pChlamy_3 vector

The map below shows the features of pChlamy_3 vector. The complete sequence of the vector is available for downloading at **www.lifetechnologies.com** or from Technical Support (page 24).



Features of pChlamy_3 Vector

4517 nucleotides

Hsp70A-Rbc S2 promoter:	70–565
Intron-1 Rbc S2:	574–718
Multiple cloning site:	728–770
3´-UTR:	774–1007
ß2-tubulin promoter:	1014–1326
Hygromycin resistance (*aph*7):	1327–2637
Ampicillin resistance gene (*bla*):	2836–3696 (c)*
bla promoter (P_{bla}):	3697–3748 (c)
pUC origin:	3794–4467

*(c): complementary strand

Continued on next page

Map and Features of pChlamy_3 Vector, continued

Features of pChlamy_3 vector

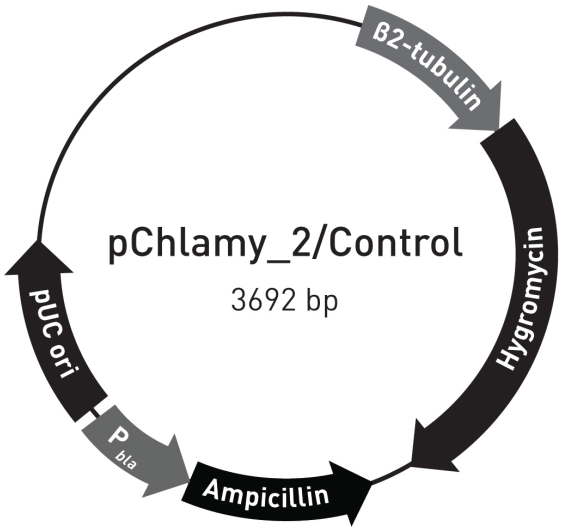
The pChlamy_3 vector contains the following elements. All features have been functionally tested.

Feature	Benefit
Hsp70A-Rbc S2 promoter	A hybrid constitutive promoter consisting of Hsp70 and RbcS2 promoters.
Intron-1 Rbc S2	First intron of the small subunit of the ribulose bisphosphate carboxylase (rbcS2); necessary to maintain the high expression of your gene of interest.
Multiple cloning site with 6 unique restriction sites (*Kpn*I, *Xba*I, *Bgl*II, *Pst*I, *Not*I, *Nde*I)	Allows insertion of your gene into pChlamy_3.
3′ UTR from RbcS2 gene	Assures the proper termination of transcript; 3′ UTR may contain sequences that regulate translation efficiency, mRNA stability, and polyadenylation signals.
β2-tubulin promoter	Strong native *C. reinhardtii* promoter driving the expression of Aph7 gene (Berthold *et al.*, 2002; Davies & Grossman, 1994).
Aph7 (Hygromycin resistance gene)	*Streptomyces hygroscopicus* aminoglycoside phosphotransferase gene; confers resistance to hygromycin (Berthold *et al.*, 2002).
Ampicillin resistance gene (*bla*)	Allows selection of the plasmid in *E. coli*.
bla promoter	Allows expression of the Ampicillin resistance gene.
pUC origin	Allows high-copy replication and growth in *E. coli*.

Map of pChlamy_2/Control Vector

pChlamy_2/Control Vector

The map below shows the features of pChlamy_2/Control Vector. The complete sequence of the vector is available for downloading at **www.lifetechnologies.com** or from Technical Support (page 24).



Features of pChlamy_2/Control Vector

3692 nucleotides

Ampicillin resistance gene (*bla*):	298–1158 (c)*
bla promoter (P_{bla}):	1159–1203 (c)
pUC origin:	1256–1929
B2-tubulin promoter:	2069–2382
Hygromycin resistance (*aph*7):	2383–3528

*(c): complementary strand

Appendix C: Ordering Information

Accessory Products

Proofreading DNA polymerases

Life Technologies offers a variety of proofreading, thermostable DNA polymerases for generating blunt-end PCR products. Ordering information is provided below. For details, visit **www.lifetechnologies.com**.

Product	Quantity	Cat. no.
Platinum® *Pfx* DNA Polymerase	100 units	11708-013
AccuPrime™ *Pfx* DNA Polymerase	200 reactions	12344-024
Pfx50™ DNA Polymerase	100 reactions	12355-012

Competent cells

Chemically competent and electrocompetent cells that can be used with GeneArt® *Chlamydomonas* Engineering Kits are also available separately from Life Technologies. Ordering information is provided below. For details, visit **www.lifetechnologies.com**.

Product	Quantity	Cat. no.
One Shot® TOP10 Chemically Competent Cells	10 reactions 20 reactions	C4040-10 C4040-03
One Shot® TOP10 Electrocomp™ *E. coli*	10 reactions 20 reactions	C4040-50 C4040-52
TOP10 Electrocomp™ Kits	20 reactions 40 reactions 120 reactions	C664-55 C664-11 C664-24

Additional products

The following reagents are recommended for use with the GeneArt® *Chlamydomonas* Engineering Kits. Ordering information for these reagents is provided below. For details, visit **www.lifetechnologies.com**.

Product	Quantity	Cat. no.
Gibco® TAP Growth Media: Optimized for *Chlamydomonas*	1 L 6 × 1 L	A13798-01 A13798-02
Electroporation cuvettes, 0.4 cm	50/bag	P460-50
PureLink® Growth Block	50 blocks	12256-020
PureLink® HQ Mini Plasmid Purification Kit	100 preps	K2100-01
PureLink® HiPure Plasmid Miniprep Kit	25 preps 100 preps	K2100-02 K2100-03
Coelenterazine	250 µg	C2944
Ampicillin Sodium Salt, irradiated	200 mg	11593-027
Hygromycin B	20 mL	10687-010
LB Broth (1X), liquid	500 mL	10855-021

Continued on next page

Other GeneArt® Algal Kits

In addition to the GeneArt® *Chlamydomonas* Engineering Kits, Life Technologies offers the following products as model algal hosts. Ordering information is provided below. For details, visit **www.lifetechnologies.com**.

Product	Quantity	Cat. no.
GeneArt® *Chlamydomonas* TOPO® Engineering Kit	1 kit	A14260
GeneArt® *Chlamydomonas* TOPO® Engineering Kit with 6 L media	1 kit	A14264
GeneArt® *Synechococcus* Engineering Kit	1 kit	A14259
GeneArt® *Synechococcus* Engineering Kit with 6 L media	1 kit	A14263
GeneArt® *Synechococcus* TOPO® Engineering Kit	1 kit	A14261
GeneArt® *Synechococcus* TOPO® Engineering Kit with 6 L media	1 kit	A14265

Documentation and Support

Obtaining Support

Technical Support

For the latest services and support information for all locations, go to **www.lifetechnologies.com**.

At the website, you can:

- Access worldwide telephone and fax numbers to contact Technical Support and Sales facilities
- Search through frequently asked questions (FAQs)
- Submit a question directly to Technical Support (**techsupport@lifetech.com**)
- Search for user documents, SDSs, vector maps and sequences, application notes, formulations, handbooks, certificates of analysis, citations, and other product support documents
- Obtain information about customer training
- Download software updates and patches

Safety Data Sheets (SDS)

Safety Data Sheets (SDSs) are available at **www.lifetechnologies.com/sds**.

Limited Product Warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale found on Life Technologies' website at **www.lifetechnologies.com/termsandconditions**. If you have any questions, please contact Life Technologies at **www.lifetechnologies.com/support.**

References

Ausubel, F. M., Brent, R., Kingston, R. E., Moore, D. D., Seidman, J. G., Smith, J. A., and Struhl, K. (1994) *Current Protocols in Molecular Biology*, Greene Publishing Associates and Wiley-Interscience, New York

Babinger, P., Kobl, I., Mages, W., and Schmitt, R. (2001) A link between DNA methylation and epigenetic silencing in transgenic Volvox carteri. Nucleic Acids Res *29*, 1261-1271

Berthold, P., Schmitt, R., and Mages, W. (2002) An engineered Streptomyces hygroscopicus aph 7" gene mediates dominant resistance against hygromycin B in Chlamydomonas reinhardtii. Protist *153*, 401-412

Cerutti, H., Johnson, A. M., Gillham, N. W., and Boynton, J. E. (1997) Epigenetic silencing of a foreign gene in nuclear transformants of Chlamydomonas. Plant Cell *9*, 925-945

Davies, J. P., and Grossman, A. R. (1994) Sequences controlling transcription of the Chlamydomonas reinhardtii beta 2-tubulin gene after deflagellation and during the cell cycle. Mol Cell Biol *14*, 5165-5174

Fuhrmann, M., Hausherr, A., Ferbitz, L., Schodl, T., Heitzer, M., and Hegemann, P. (2004) Monitoring dynamic expression of nuclear genes in Chlamydomonas reinhardtii by using a synthetic luciferase reporter gene. Plant Mol Biol *55*, 869-881

Fuhrmann, M., Oertel, W., and 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

Harris, E. H. (2001) Chlamydomonas as a Model Organism. Annu Rev Plant Physiol Plant Mol Biol *52*, 363-406

Heitzer, M., Eckert, A., Fuhrmann, M., and Griesbeck, C. (2007) Influence of codon bias on the expression of foreign genes in microalgae. Adv Exp Med Biol *616*, 46-53

Hippler, M., Redding, K., and Rochaix, J. D. (1998) Chlamydomonas genetics, a tool for the study of bioenergetic pathways. Biochim Biophys Acta *1367*, 1-62

Merchant, S. S., Prochnik, S. E., Vallon, O., Harris, E. H., Karpowicz, S. J., Witman, G. B., Terry, A., Salamov, A., Fritz-Laylin, L. K., Marechal-Drouard, L., Marshall, W. F., Qu, L. H., Nelson, D. R., Sanderfoot, A. A., Spalding, M. H., Kapitonov, V. V., Ren, Q., Ferris, P., Lindquist, E., Shapiro, H., Lucas, S. M., Grimwood, J., Schmutz, J., Cardol, P., Cerutti, H., Chanfreau, G., Chen, C. L., Cognat, V., Croft, M. T., Dent, R., Dutcher, S., Fernandez, E., Fukuzawa, H., Gonzalez-Ballester, D., Gonzalez-Halphen, D., Hallmann, A., Hanikenne, M., Hippler, M., Inwood, W., Jabbari, K., Kalanon, M., Kuras, R., Lefebvre, P. A., Lemaire, S. D., Lobanov, A. V., Lohr, M., Manuell, A., Meier, I., Mets, L., Mittag, M., Mittelmeier, T., Moroney, J. V., Moseley, J., Napoli, C., Nedelcu, A. M., Niyogi, K., Novoselov, S. V., Paulsen, I. T., Pazour, G., Purton, S., Ral, J. P., Riano-Pachon, D. M., Riekhof, W., Rymarquis, L., Schroda, M., Stern, D., Umen, J., Willows, R., Wilson, N., Zimmer, S. L., Allmer, J., Balk, J., Bisova, K., Chen, C. J., Elias, M., Gendler, K., Hauser, C., Lamb, M. R., Ledford, H., Long, J. C., Minagawa, J., Page, M. D., Pan, J., Pootakham, W., Roje, S., Rose, A., Stahlberg, E., Terauchi, A. M., Yang, P., Ball, S., Bowler, C., Dieckmann, C. L., Gladyshev, V. N., Green, P., Jorgensen, R., Mayfield, S., Mueller-Roeber, B., Rajamani, S., Sayre, R. T., Brokstein, P., Dubchak, I., Goodstein, D., Hornick, L., Huang, Y. W., Jhaveri, J., Luo, Y., Martinez, D., Ngau, W. C., Otillar, B., Poliakov, A., Porter, A., Szajkowski, L., Werner, G., Zhou, K., Grigoriev, I. V., Rokhsar, D. S., and Grossman, A. R. (2007) The Chlamydomonas genome reveals the evolution of key animal and plant functions. Science *318*, 245-250

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References, continued

Miller, R., Wu, G., Deshpande, R. R., Vieler, A., Gartner, K., Li, X., Moellering, E. R., Zauner, S., Cornish, A. J., Liu, B., Bullard, B., Sears, B. B., Kuo, M. H., Hegg, E. L., Shachar-Hill, Y., Shiu, S. H., and Benning, C. Changes in transcript abundance in Chlamydomonas reinhardtii following nitrogen deprivation predict diversion of metabolism. Plant Physiol *154*, 1737-1752

Molnar, A., Schwach, F., Studholme, D. J., Thuenemann, E. C., and Baulcombe, D. C. (2007) miRNAs control gene expression in the single-cell alga Chlamydomonas reinhardtii. Nature *447*, 1126-1129

Proschold, T., Harris, E. H., and Coleman, A. W. (2005) Portrait of a species: Chlamydomonas reinhardtii. Genetics *170*, 1601-1610

Radakovits, R., Jinkerson, R. E., Darzins, A., and Posewitz, M. C. (2010) Genetic engineering of algae for enhanced biofuel production. Eukaryot Cell *9*, 486-501

Sambrook, J., Fritsch, E. F., and Maniatis, T. (1989) *Molecular Cloning: A Laboratory Manual*, Second Ed., Cold Spring Harbor Laboratory Press, Plainview, New York

Schroda, M. (2006) RNA silencing in Chlamydomonas: mechanisms and tools. Curr Genet *49*, 69-84

Wang, B., Wang, J., Zhang, W., and Meldrum, D. R. (2012) Application of synthetic biology in cyanobacteria and algae. Front Microbiol *3*, 344

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