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GeneArt™ *Chlamydomonas* Protein Expression Vector

For expression of recombinant proteins in *Chlamydomonas reinhardtii*

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Revision history

Revision	Date	Description
C.0	26 April 2017	Updated kit contents, MCS and vector maps, reorganized protocols, and rebranded.
B.0	20 May 2014	Basis for the current revision.

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Contents

Product information

Contents and storage

Kit contents

The GeneArt™ *Chlamydomonas* Protein Expression Vector kit contains the following components.

Component	Concentration	Amount
pChlamy_4 vector	20 µL of vector at 0.5 µg/µL in TE buffer, pH 8.0*	10 reactions

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

Shipping and storage

The GeneArt™ *Chlamydomonas* Protein Expression Vector is shipped on dry ice. Upon receipt, store the vector at –20°C. When stored as directed, the vector is guaranteed for six months.

Description of the system

GeneArt™ *Chlamydomonas* Protein Expression System

The GeneArt™ *Chlamydomonas* Protein Expression System is a eukaryotic genetic engineering system based on the unicellular green alga *Chlamydomonas reinhardtii* 137c (mt+) (Pröschold et al., 2005) and the pChlamy_4 vector. The system offers a simplified approach for protein expression in algae for downstream applications such as biofuels, specialty chemicals, and industrial enzymes. The pChlamy_4 vector (page 4) is designed for high-level expression of your gene of interest (up to 1% of total soluble protein) and provides selection against gene silencing. The vector contains dual protein tags for detection and/or purification of your gene of interest. Note that the expression level 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.*, 2010; 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 various beneficial attributes including:

- The ease of transformation and the relatively short time between the generation of first 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., recognized as safe by FDA), make *C. reinhardtii* an 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 the 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, which makes it possible to carry our classical breeding through matings between transgenic algal lines in a short period of time (3–4 weeks) (Harris, 2001).

Heterologous gene expression in *C. reinhardtii*

In *C. reinhardtii*, expression of heterologous proteins presents several difficulties. The first problem is represented by the unusual codon bias of the *C. reinhardtii* nuclear genes that are highly GC 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). Also, 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 multicopy 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 (Rasala *et al.*, 2012).

pChlamy_4 vector

pChlamy_4 vector is designed for rapid cloning of your gene of interest for expression in *C. reinhardtii*. Like its predecessor, pChlamy_3, this vector is a nuclear integrative vector; its integration across the genome is a random event and the copy number of the integrated gene varies depending on the context of the gene of interest. However, several advancements have been developed for improved nuclear transgene expression on the newest version of pChlamy_4 since the launch of our pChlamy vector series. Some of the features of the vector are listed below. For a map of the vector, see page 24.

- Hsp70A-Rbc S2 chimeric constitutive promoter enables strong expression of the gene of interest.
- Antibiotic resistance gene for bleomycin/Zeocin™ is introduced into the vector as an effective selection marker. The *Sh ble* (*Streptoalloteichus hindustanus* bleomycin gene) gene product confers resistance to the DNA double strand break-inducing bleomycin family of antibiotics through binding and sequestration, therefore antibiotic resistance is proportional to *Sh ble* expression levels. Compared to other selection markers, higher level of expression is observed for the protein of interest when *Sh ble* gene is used as a selection marker.
- Two copies of the native *C. reinhardtii* Intron-1 from Rbc S2 have been inserted into the *Sh ble* gene for mRNA stability and efficient expression. These introns are at vector positions 505–649 and 831–975 and they are spliced out from the *Sh ble* mature mRNA (Lumbreras *et al.*, 1998).
- Hsp70A-Rbc S2 hybrid promoter fusion to the bleomycin/Zeocin™-resistance gene forms a DNA element counteracting the silencing of Hsp70A-Rbc S2-*ble* gene, allowing the positive transformants to maintain protein expression levels for multiple passages with or without selection pressure.

pChlamy_4 vector continued

- Foot-and-mouth disease-virus (FMDV) 2A peptide encoding a 20 amino acid sequence that mediates a self-cleavage reaction is linked to transgene expression (Ryan *et al.*, 1991). During translation elongation of the 2A sequence, the last amino acid of the 2A sequence, a proline, is fused to the N-terminal of the first protein of interest or the N-terminal of the protein tag.

  VKQTLNFDLLKLAGDVESNPG▲P

- The 5′-UTR from the *C. reinhardtii* RbcS2 (Ribulose Bisphosphate Carboxylase/Oxygenase Small Subunit 2) gene is directly upstream from the translational start site of the Bleomycin gene (Goldschmidt-Clermont and Rahire, 1986).
- A 3′-UTR fragment from RbcS2 gene downstream of the multiple cloning site ensures the proper termination of transcript.

  Note: The 5′ and 3′ UTR may contain sequences that regulate translation efficiency, mRNA stability, and polyadenylation signals (Anthonisen *et al.*, 2001; Rasala *et al.*, 2011).
- The versatile multiple cloning site facilitates simplified cloning of your gene of interest by seamless, Type IIs, or restriction enzyme digestion-based cloning.
- Dual protein tags provide the flexibility to express your protein of interest fused to either or both or none of the N-terminal and C-terminal tags.
- Ampicillin resistance gene allows selection in *E. coli.*
- Bleomycin/Zeocin™-resistance gene allows selection in *C. reinhardtii.*
- pUC origin allows the maintenance of the vector in *E. coli.*

Experiment outline

Workflow

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

Step	Action	Page
1	Clone your codon optimized gene of interest into pChlamy_4 vector.	7
2	Transform *E. coli* with the pChlamy_4 construct containing your gene of interest and select the transformants on LB plates containing Ampicillin.	11
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Methods

Clone into pChlamy_4 vector

General molecular biology techniques

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

For help with seamless cloning, see **www.thermofisher.com/geneart** and our free, web-based GeneArt™ Primer and Construct Design Tool, which is available at **www.thermofisher.com/order/oligoDesigner**.

E. coli host

For cloning and transformation, use a recombination deficient (*rec*A) and endonuclease A-deficient (*end*A) strain such as TOP10. Note that other *rec*A, *end*A *E. coli* strains are also suitable.

Maintaining pChlamy_4

To propagate and maintain the pChlamy_4 vector, use 10 ng of the vector to transform a *rec*A, *end*A *E. coli* strain like TOP10, DH5α™, or equivalent. Select transformants on LB plates containing 50–100 µg/mL of ampicillin. Make sure to prepare a glycerol stock of the plasmid for long-term storage (page 12).

Cloning considerations

- Since the *Synechococcus elongatus* PCC 7942 genome has a 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. You can order synthesized codon-optimized *C. reinhardtii* genes from Invitrogen™ GeneArt™ Gene Synthesis services (**www.thermofisher.com/geneartgenesynthesis**).
- Note that two copies of the Intron-1 Rbc S2 (bases 505–649 and 831–977) are spliced out from the mature RNA and do not constitute actual codons. The reading frame before and after the removal of both copies of Intron-1 Rbc S2 is shown on page 8.
- pChlamy_4 vector contains the ATG initiation codon (vector ATG) for proper initiation of translation at position 497–499, found at the beginning of the *Sh ble* gene after the removal of Intron-1 Rbc S2 (see Figure 1, page 8). You do not need to add an ATG start codon to your insert.
- The FMDV 2A peptide gene flanking the Multiple Cloning Site 1 (MCS1) is in frame with the *Sh ble* gene. Make sure to clone your gene of interest in frame with the FMDV 2A gene using the sequence information for the MCS (Figure 2, page 9).
- **N-terminal tag:** To use the N-terminal 6× His-V5-TEV tag, clone your insert in-frame after the TEV site using your preferred cloning method. Ensure that your insert includes a stop codon for proper translation termination.

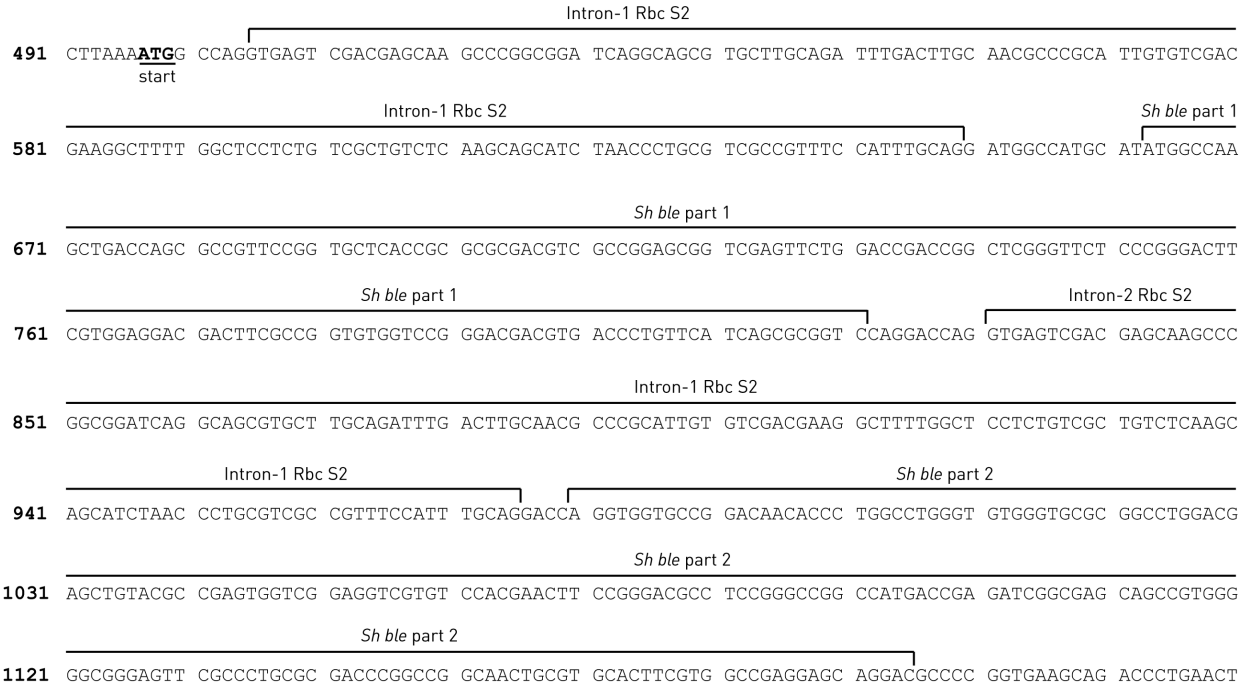
  IMPORTANT! If you want to use the C-terminal tag, ensure that your insert does **not** contain a stop codon.

  If you do not need to use the C-terminal tag, 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 ensure that the stop codon is upstream from the reverse PCR primer binding site.

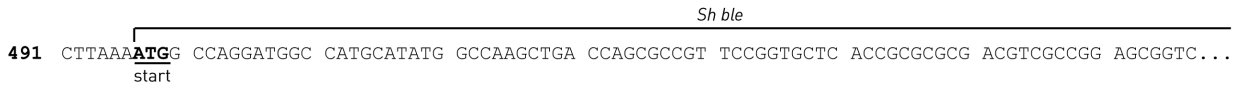
  Note that the *Xba* I site contains an internal stop codon (TCTAGA).
- **C-terminal tag:** To use the C-terminal V5-6× His tag, ensure that your insert does **not** contain a stop codon and is in-frame with the C-terminal tag.

Figure 1. The region around the *Sh ble* gene before and after the splicing of Intron-1 Rbc S2.

Before splicing



After splicing

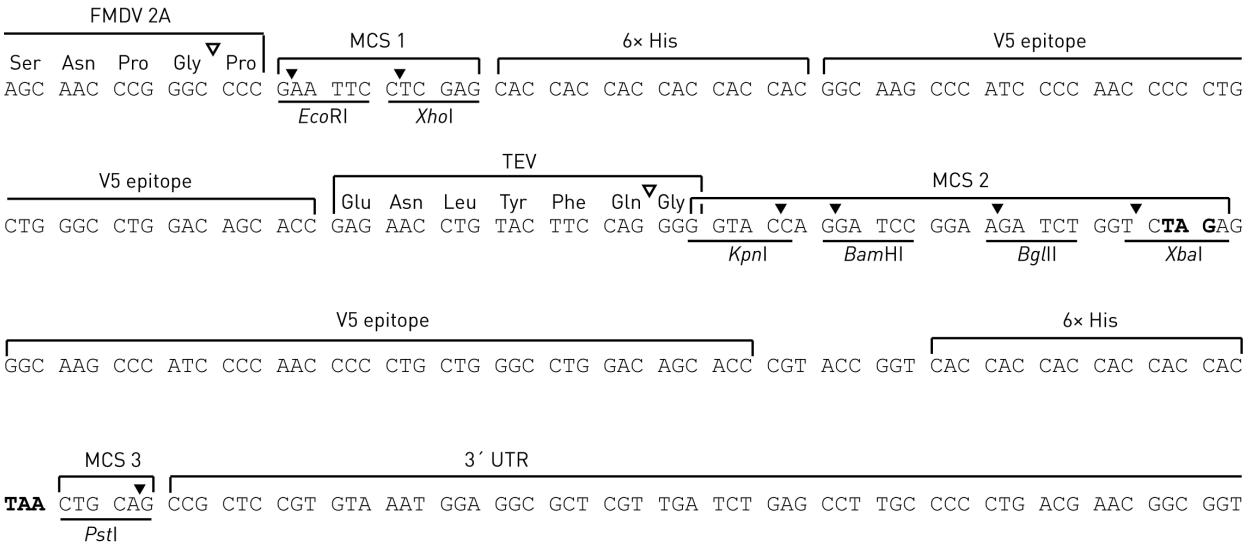


Multiple cloning site of pChlamy_4

Figure 2 below depicts the multiple cloning sites for pChlamy_4. With the choice of three multiple cloning sites (MCS1, MCS2, MCS3), you can express your protein as a native, an N-terminal, or a C-terminal fusion protein. Use Figure 2 to design appropriate PCR primers to clone and express your PCR product in pChlamy_4.

- The three sets of restriction sites are labeled, where the black triangles indicate the cleavage sites. The ATG initiation codon (vector ATG) is found immediately upstream of *Sh ble* gene (not shown here; see Figure 1, page 8), and the potential stop codons are shown in bold.
- The white rectangles denote the cleave sites for the FMDV 2A peptide and TEV protease cleavage sites.
- Whether or not your gene of interest (GOI) contains an ATG initiation codon, it must be cloned in frame to the *Sh ble* and FMDV 2A peptide genes, and the tags. Otherwise, your GOI will not be properly expressed.
- The vector sequence of pChlamy_4 is available at **www.thermofisher.com** or by contacting Technical Support (page 30).

Figure 2. Multiple cloning site of the pChlamy_4 vector.



Ligation

After you have determined a cloning strategy and PCR amplified your gene of interest, digest pChlamy_4 with the appropriate restriction enzyme and ligate your insert containing your gene of interest using standard molecular biology techniques. We recommend using high concentration T4 ligase (Cat. No. 15224041).

Seamless cloning with GeneArt™ Seamless and Seamless PLUS Cloning and Assembly Kits

The pChlamy_4 vector is compatible with the GeneArt™ Seamless Cloning and Assembly technology, which allows the simultaneous and seamless assembly of up to 4 DNA inserts between 100 bp and 10 kb and a linearized pChlamy_4 vector, totaling up to 13 kb in length.

After you have determined where to position your gene of interest in the pChlamy_4 vector, use the diagram depicting the MCS of pChlamy_4 (page 9) to design appropriate PCR primers to clone and express your PCR product in the pChlamy_4 vector. The primers should have at least 15-nt homology to the pChlamy_4 vector at their 5′ end.

After digesting the pChlamy_4 vector with the appropriate restriction enzymes, PCR amplify your gene(s) of interest, then use the GeneArt™ Seamless Cloning and Assembly Enzyme Mix (Cat. No. A14606) or the GeneArt™ Seamless PLUS Cloning and Assembly Kit (Cat. No. A14603) to seamlessly assemble into the digested pChlamy_4 vector.

For detailed instructions on how to use the GeneArt™ Seamless Cloning and Assembly Enzyme Mix or the GeneArt™ Seamless PLUS Cloning and Assembly Kit, see the relevant user guide, which is available for download at **www.thermofisher.com**.

For help with primer design for seamless cloning, use our free, web-based GeneArt™ Primer and Construct Design Tool, available at **www.thermofisher.com/order/oligoDesigner**.

For more information on seamless cloning and assembly technologies, see our website at **www.thermofisher.com/geneart**.

Seamless cloning with GeneArt™ Type IIs Assembly Kits

You can also use the GeneArt™ Type IIs Assembly Kit, *Aar* I (Cat. No. A15916) or the GeneArt™ Type IIs Assembly Kit, *Bbs* I (Cat. No. A15918) to seamlessly clone and assemble up to 8 DNA fragments and the pChlamy_4 vector by simultaneous cleavage and ligation in a single reaction.

However, the GeneArt™ Type IIs Assembly Kit, *Bsa* I (Cat. No. A15917) **cannot** be used with the pChlamy_4 vector, because the vector contains a *Bsa* I restriction site in the Ampicillin resistance gene.

For more information, see the relevant user guide, which is available for download at **www.thermofisher.com**.

E. coli transformation method

Chemical transformation is the most convenient for most researchers and a protocol for the chemical transformation of One Shot™ TOP10 Chemically Competent *E. coli* cells is provided on page 11. You can use the chemical transformation method for seamless, Type IIs, and restriction enzyme digestion-based cloning.

You can also transform electrocompetent *E. coli* cells by electroporation, which is more efficient and the method of choice for large plasmids. You can use electroporation for Type IIs and restriction enzyme digestion-based cloning. Do **not** use electroporation for seamless cloning.

Transform One Shot™ TOP10 Competent *E. coli* cells

Introduction

After you have performed the cloning reaction, transform your pChlamy_4 construct into competent *E. coli*. The following protocol describes the transformation of OneShot™ TOP10 Chemically Competent *E. coli* cells, but you can also use other suitable cells. If you are performing Type IIs or restriction enzyme digestion-based cloning, you can also transform electrocompetent cells using the protocol supplied with the electrocompetent cells.

Materials needed

- pChlamy_4 construct containing your gene of interest
- One Shot™ TOP10 Chemically Competent *E. coli*
- S.O.C. Medium
- pUC19 positive control (recommended for verifying transformation efficiency)
- 42°C water bath
- LB plates containing 100 µg/mL of ampicillin (two for each transformation)
- 37°C shaking and non-shaking incubator

Prepare for transformation

For each transformation, you 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 of 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 has well-spaced colonies.
8. Pick 5–10 colonies for analysis (see "Analyze *E. coli* transformants", page 12).

Analyze *E. coli* transformants

Pick positive *E. coli* clones

1. Pick 5–10 colonies and culture them overnight in LB medium containing 100 µg/mL of 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. K210001; see page 27).
3. Analyze the plasmids by restriction analysis or PCR (see below) to confirm the presence and correct orientation of the insert.

Analyze *E. coli* transformants by PCR

Use the following protocol (or any other appropriate protocol) to analyze positive *E. coli* transformants using PCR. You 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. 10790020)
- 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 92–98°C to lyse cells and inactivate nucleases.
4. Amplify for 20–30 cycles.
5. For the final extension, incubate at 72°C for 10 minutes. Store at 2°C–8°C.
6. Visualize by agarose gel electrophoresis.

Analyze *E. coli* transformants by sequencing

After you have identified one or more correct clones, 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_4 vector, see our website (**www.thermofisher.com**) or contact Technical support (see page 30).

Long-term storage

After 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.

Appendix A: Support protocols

Prepare reagents and media

How to handle Zeocin™ selection reagent

Zeocin™ selection reagent (Cat. No. R25001) is a basic, water-soluble, copper-chelated glycopeptides that is a member of the bleomycin/phleomycin family of antibiotics isolated from *Streptomyces verticillus*. It shows strong toxicity against bacteria, fungi (including yeast), plants, and mammalian cell lines (Calmels *et al.*, 1991; Drocourt *et al.*, 1990; Gatignol *et al.*, 1987; Mulsant *et al.*, 1988; Perez *et al.*, 1989). The copper-chelated form of Zeocin™ selection reagent is inactive. When the antibiotic enters the cell, the copper cation is reduced from Cu^{2+} to Cu^{1+} and removed by sulfhydryl compounds in the cell. Upon removal of the copper, Zeocin™ becomes activated and binds and cleaves DNA, causing cell death. When handling Zeocin™ reagent, follow the guidelines below:

- High ionic strength, acidity, and basicity inhibit the activity of Zeocin™.
- Store Zeocin™ at –20°C and thaw on ice before use.
- Zeocin™ is light sensitive. Store the drug and plates or medium containing the drug in the dark.
- Wear gloves, a laboratory coat, and safety glasses when handling Zeocin™ containing solutions.
- Do not ingest or inhale solutions containing the drug.
- Bandage any cuts on your fingers to avoid exposure to the drug.

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 42 mL of 1 M sucrose to 1 L of Gibco™ TAP medium.

TAP-Zeocin™ solution

1. Add Zeocin™ stock solution (Cat. No. R25001; at 100 mg/mL) to Gibco™ TAP medium to a final concentration of 2.5 µg/mL.
2. Filter-sterilize through a 0.22-µm filter and store at 2°C–8°C in the dark.

TAP-Agar plates

1. Add 15 g of agar to 200 mL of Gibco™ TAP medium in an autoclavable flask.
2. Autoclave on liquid cycle for 20 minutes.
3. Warm 800 mL of Gibco™ TAP medium to 55°C–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 2°C–8°C in the dark. Final agar concentration will be 1.5%.

   Note: Overdrying the plates drastically reduces the transformation efficiency.

TAP-Agar-Zeocin™ plates

1. Add 15 g of agar to 200 mL of Gibco™ TAP medium in an autoclavable 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 Zeocin™ stock solution to a final concentration of 5 µg/mL (i.e., 50 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 2°C–8°C in the dark. Final agar concentration will be 1.5%.

Guidelines for *Chlamydomonas reinhardtii* 137c (mt+) culture

General guidelines for *C. reinhardtii* culture

The following culture guidelines are for *Chlamydomonas reinhardtii* 137c (mt+). For more information, see Pröschold *et al.* (2005).

- *C. reinhardtii* is easy and inexpensive to grow. Routine maintenance is 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 can 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 formulated for optimal growth and maintenance of *C. reinhardtii* cells.
- *C. reinhardtii* laboratory and wild type strains grow well between 20–28°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 gyratory 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 suboptimal 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, aluminum 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, see *Biosafety in Microbiological and Biomedical Laboratories,* 5th ed., published by the Centers for Disease Control (**www.cdc.gov**).

Thaw *Chlamydomonas reinhardtii* 137c (mt+)

Materials needed

- *C. reinhardtii* 137c (mt+) cells

  Note: You can order *C. reinhardtii* strains from the *Chlamydomonas* Resource Center at **www.chlamycollection.org**.
- 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
- 500-mL glass culture flask
- Gibco™ TAP medium (Cat. No. A1379801 or A1379802), pre-warmed to room temperature
- 70% ethanol
- Dry ice
- Disposable cell spreaders
- Reagents and equipment to determine viable and total cell counts (such as counting chamber/hemocytometer, Countess™ II Automated Cell Counter, or similar)

Thaw *C. reinhardtii* cells

1. Remove the frozen cells from –80°C storage and immediately place them in a dry ice container. Bury the vials containing the cells in dry ice to minimize temperature fluctuations before thawing.
2. Add 200 mL of Gibco™ TAP medium, pre-warmed to room temperature, into a 500-mL glass culture flask.
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 the glass culture flask containing 200 mL of Gibco™ TAP medium.
7. Place the flasks in the algal growth chamber set to 26°C and 50 µE m^{-2} s^{-1}.
8. Incubate the cells for 3–6 days with agitation on a rotary shaker set to 110 rpm.
9. On Day 3, count the cell number. If the culture has not yet reached 1×10^6 cells/mL, return it to the algal growth chamber and continue the incubation. Check the cell concentration of the culture daily until it reaches 1×10^6 cells/mL. Once the culture has reached 1×10^6 cells/mL, proceed to the transformation step (page 17).

Transform *Chlamydomonas reinhardtii* 137c (mt+) by electroporation

Introduction

Introduction of exogenous DNA into the unicellular, green alga *Chlamydomonas reinhardtii* is hindered by the organism's rigid cell wall. Although various methods, such as glass beads agitation, electroporation, and microparticle bombardment, have been successfully used to transform *C. reinhardtii*, they provide very low transformation efficiency. The GeneArt™ MAX Efficiency™ Transformation Reagent (Cat. No. A24229) facilitates the delivery of DNA into the cell during electroporation, providing 2 to 3 orders of magnitude increase in transformation efficiency compared to conventional electroporation methods.

Guidelines for *C. reinhardtii* transformation

- 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. We recommend using *Sca*I restriction enzyme for linearization, provided that the insert does not contain the recognition sequence for *Sca*I. Otherwise, you may select from *Pvu*I, *Ssp*I, or *Fsp*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 Kit (Cat. No. K210001), PureLink™ HiPure Plasmid Miniprep Kit (Cat. No. K210002), or the PureLink™ Expi Endotoxin-Free Mega Plasmid Purification Kit (Cat. No. A31232) that delivers pure DNA, and elute the purified DNA from the purification column using pure water instead of TE or E1 buffer.
- For best results, grow the cells to 1×10^6–2×10^6 cells/mL before proceeding with electroporation. You may use $<1 \times 10^6$ cells/mL, but the concentration should not exceed 3×10^6 cells/mL.
- Insertion of the plasmid DNA into the genome occurs randomly. On average only 50% of transformants will express the gene of interest at appreciable levels. We recommend first screening the colonies by colony PCR (see page 21) 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_4 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. Other restriction enzymes you can use as an alternative to *Sca*I are *Pvu*I, *Ssp*I, or *Fsp*I.
- GeneArt™ MAX Efficiency™ Transformation Reagent (Cat. No. A24229)
- Gibco™ TAP medium (Cat. No. A1379801 or A1379802), pre-warmed to room temperature
- TAP-40 mM sucrose solution, pre-warmed to room temperature (see page 13 for recipe)
- TAP-Agar-Zeocin™ plates (5 µg/mL) (see page 14 for recipe)
- Sterile 15-mL and 50-mL centrifugation tubes
- 0.4-cm red electroporation cuvettes (Cat. No. P46050), chilled on ice
- Electroporation device such as the Neon™ Transfection System (Cat. No. MPK5000), Neon™ Transfection System 100 µL Kit (Cat. No. MPK10025), or the Bio-Rad™ Gene Pulser™ II
- ColiRollers™ plating glass beads (Novagen, Cat. No. 71013) or disposable cell spreaders

Electroporate using the Neon™ Transfection System

For detailed instructions on using the Neon™ Transfection System, see the Neon™ Transfection System user guide, available for download at **www.thermofisher.com**.

1. When cell concentration reaches 1×10^6–2×10^6 cells/mL (see page 16), harvest them by centrifugation at 2,500 rpm for 5 minutes. Discard the supernatant and carefully remove all liquid as much as possible.

   Note: Cells must be in early log phase and harvested gently. If the cell concentration is $<1 \times 10^6$ cells/mL, you can still harvest the cells without significantly affecting the transformation efficiency. If the cell concentration exceeds 3×10^6 cells/mL, discard the cells and start a new culture.
2. Resuspend the cell pellet in 10 mL of GeneArt™ MAX Efficiency™ Transformation Reagent and centrifuge at 2,500 rpm for 5 minutes. Discard the supernatant and carefully remove all liquid as much as possible.
3. Resuspend the cell pellet again in 10 mL of GeneArt™ MAX Efficiency™ Transformation Reagent, and centrifuge the cells once more at 2,500 rpm for 5 minutes.
4. Resuspend the cell pellet in GeneArt™ MAX Efficiency™ Transformation Reagent to a final concentration of 1×10^8–3×10^8 cells/mL.
5. Add 1 µg of linearized DNA per 100 µL of cell suspension and incubate at 2°C–8°C for 5 minutes.
6. Fill the Neon™ Tube with 3 mL of ice-cold E2 buffer and insert it into the Neon™ Pipette Station until you hear a click.

   Note: After 2–3 shocks, E2 buffer needs to be chilled on ice again.
7. Set electroporation parameters on the Neon™ device as follows:

Voltage	Pulse width	Pulse number
2300V	13 ms	3

8. Pipette up 100 µL of the DNA-cell mix in the 100-µL Neon™ Tip and insert the tip into the Neon™ Tube in the pipette station until you hear a click.

9. Press **Start** on the touchscreen to deliver the electric pulse.
10. Eject the electroporated cells into a 15-mL centrifuge tube (chilled on ice) and allow the cells to recover on the bench for 15 minutes.
11. Add 4 mL of TAP-40 mM sucrose solution at room temperature to the cells and incubate them in the algal chamber overnight.
12. The next day, centrifuge the cells at 2,500 rpm for 5 minutes, discard 3.8 mL of the supernatant, and resuspend the cells in the remaining 200 µL of TAP-40 mM sucrose solution.
13. Spread 200 µL of the cell suspension on a TAP-agar-Zeocin™ plate using disposable cell spreaders or glass plating beads to spread the cells evenly. Make sure the plates do not have condensation on them.
14. Place the plates agar side down 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.
15. Incubate the plates for 5–7 days or until *C. reinhardtii* colonies are clearly visible. The transformation efficiency with the pChlamy_4 construct depends on the nature, size, and codon content of the gene of interest, and the physiological state of the cells.
16. Proceed to colony PCR to determine colony integration (see page 21) before selecting clones for further scale-up.
17. About 50% of the colonies should be positive for the geneof interestof. 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).

Electroporate using the Bio-Rad™ Gene Pulser™ II device

If using an electroporation device such as the Bio-Rad™ Gene Pulser™ II, follow the protocol below. If using the Neon™ Transfection System, see **Electroporation using the Neon™ Transfection System**, page 18.

1. Harvest the cells as described in Steps 1–3 of the Neon™ II protocol (page 18), and resuspend them in GeneArt™ MAX Efficiency™ Transformation Reagent to a final concentration of 2×10^8–3×10^8 cells/mL.
2. Add 2–4 µg of linearized DNA per 250 µL of cell suspension and incubate at 2°C–8°C for 5 minutes.
3. Set electroporation parameters on the Gene Pulser™ II as follows:

Voltage	Capacity	Resistance
500 V	50 µF	800 Ω

4. Transfer 250 µL of the cell-DNA mix into an ice-cold cuvette (pre-chilled on ice) just before electroporation.
5. Electroporate the cells using the appropriate settings (500 V, 50 µF, 800 Ω). Usually, the electro pulse duration is about 30 ms.
6. After electroporation, allow the cells to recover on the bench for 15 minutes.
7. Transfer the cells into a 50-mL conical tube or flask containing 10 mL of TAP-40 mM sucrose solution at room temperature.
8. Place the cells in the algal chamber algal growth chamber set to 26°C and 50 µE m^{-2} s^{-1} and incubate for 14–16 hours.
9. Harvest the cells centrifugation at 2500 rpm for 5 minutes, discard the supernatant, and resuspend the pellet in 200 µL TAP medium at room temperature.
10. Plate the entire cell solution from each transformation on one TAP-agar-Zeocin™ plate using disposable cell spreaders or glass plating beads to spread the cells evenly. Make sure the plates do not have condensation on them.
11. Place the plates with agar side at the bottom in the algal growth chamber that is set to 26°C and 50 µE m^{-2} s^{-1}. Do not stack the plates to ensure continuous and even illumination.
12. Incubate the plates for 5–7 days or until *C. reinhardtii* colonies are clearly visible. The transformation efficiency with the pChlamy_4 construct will depend on the nature, size, and codon content of the gene of interest, and the physiological state of the cells.
13. Proceed to determination of integration by colony PCR (see page 21) before selecting clones for further scale-up.

    About 50% 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).

Screen 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. 12344040)
- Appropriate forward and reverse primers (10 µM each)

Prepare 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.

Perform colony PCR

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 2°C–8°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 (short-term)

Store *C. reinhardtii* transformants

Plates containing transformed cells can be wrapped in Parafilm™ laboratory film and stored at room temperature for at least one month.

The best method for the preservation and long-term storage of *C. reinhardtii* is cryopreservation (see below), which dramatically reduces genetic drift, lowers labor and cost associated with the maintenance of algae plates, and facilitates strain and clone exchange between laboratories.

Cryopreserve *Chlamydomonas reinhardtii* 137c (mt+) (long-term)

GeneArt™ Cryopreservation Kit for Algae

For cryopreservation, we recommend using the GeneArt™ Cryopreservation Kit for Algae, available separately from Thermo Fisher Scientific (Cat. No. A24228), which allows algae to be frozen and stored in a –80°C freezer for at least 2 years.

Materials needed

- *C. reinhardtii* cells (wild type or transformants) to cryopreserve

  Note: Cells should be in mid- to late-logarithmic phase for cryopreservation.
- GeneArt™ Cryopreservation Kit for Algae (Cat. No. A24228)
- Reagents and equipment to determine viable and total cell counts (such as a counting chamber/hemocytometer or Countess™ II Automated Cell Counter)
- Mr. Frosty™ freezing container (Cat. No. 5100-0001)
- Benchtop centrifuge (e.g., Sorvall)
- Nalgene™ General Long-Term Storage Cryogenic Tubes (Cat. No. 5000-0020)
- 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 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.
- Rotary shaking platform set to 110 rpm
- 250-mL clear-glass culture flask
- Gibco™ TAP medium (Cat. No. A13798), pre-warmed to room temperature
- 70% ethanol
- Dry ice

Freeze *C. reinhardtii* cells

1. Grow *C. reinhardtii* 137c (mt+) cells (wild type or transformants) into mid- to late-logarithmic phase under standard culture conditions.
2. Prepare pre-conditioning medium in a 250-mL clear-glass culture flask by adding 1 mL of Cryopreservation Reagent A into 45 mL of fresh Gibco™ TAP medium.
3. Inoculate the pre-conditioning medium with *C. reinhardtii* cells from step 1 to a final OD_{750} of 0.1 (usually, 2–5 mL of seed culture). Do not exceed OD_{750} of 0.4.
4. Place the culture flask on a rotary shaking platform set to 110 rpm in an algal growth chamber at 26°C and 50 µE m^{-2} s^{-1}, and incubate for 3 days. You can let the cells grow in pre-conditioning medium for 2–5 days, but the optimal time is 3 days.
5. After 3 days of growth, measure the OD_{750} of the culture and calculate the cell concentration using the equation below.
6. Cell concentration (cells/mL) = $(OD_{750} - 0.088)/(9 \times 10^{-8})$
7. *Optional:* After 3 days growth under lighted conditions, the culture can be moved to dim light condition for overnight incubation before harvest (step 7, below). This optional step could increase cell viability during freezing.
8. Harvest the cells by centrifugation at 2,500 rpm for 5 minutes and carefully remove as much of the supernatant as possible.
9. Resuspend the cells to a final concentration of 2.5×10^7 cells/mL in Cryopreservation Reagent B. Start counting the incubation time at this point (30–45 minutes at room temperature; see step 9, below).

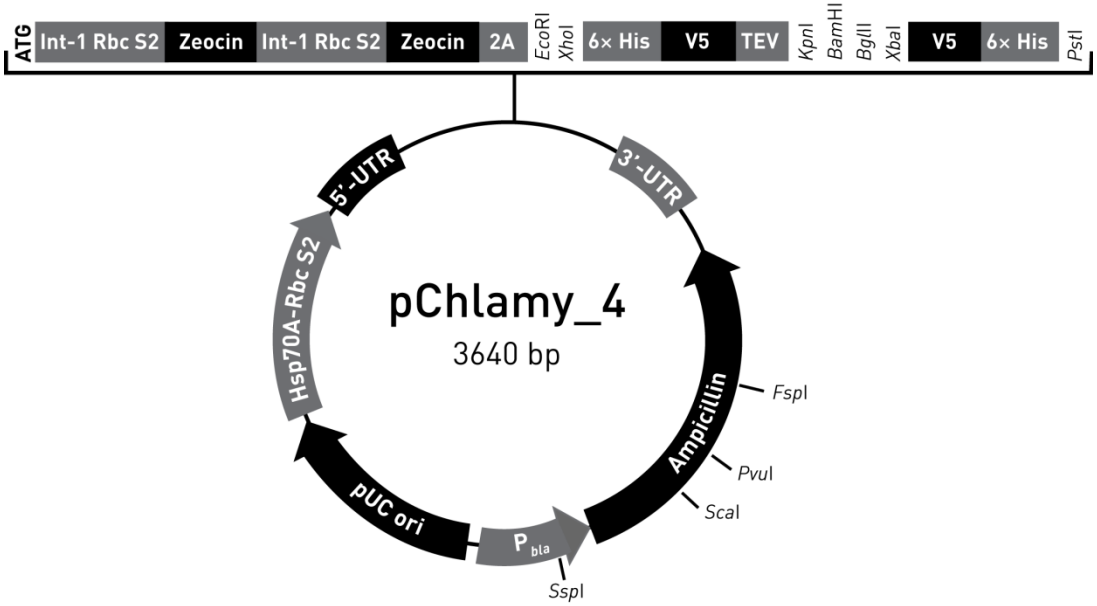
   Note: Do not exceed more than 5×10^7 cells/mL (cell viability is dramatically reduced at higher concentrations).
10. Aliquot exactly 240 µL of cell suspension into each cryovial and incubate at room temperature for 30–45 minutes.
11. Remove the sponge insert from the Mr. Frosty™ freezing container and directly insert the gray high-density polyethylene vial holder in its place. Transfer the cryovials containing the cells into the Mr. Frosty™ freezing container. If you do not have 18 vials to occupy all the slots of the vial holder, fill the remainder of slots with similar liquid-filled cryovials to ensure a proper cooling profile. Do not fill the container with 100% isopropanol or any other freezing liquid.
12. Move Mr. Frosty™ freezing container with the cryovials to –80°C. Place the Mr. Frosty™ freezing container on an open space in the freezer to ensure that no other objects block the cooling process.
13. In the next 2 hours, make sure that the –80°C freezer remains unopened. Opening the freezer door during this period changes the cells' cooling profile and can result in decreased cell viability.
14. After 4 hours, the cryovials can be transferred to another container for longer term storage at –80°C or remain in the Mr. Frosty™ freezing container.
15. The cells can be stored at –80°C for at least 2 years. Note that this freezing protocol may also be appropriate for other species of *Chlamydomonas*.

Appendix B: pChlamy_4 vector

Map and features of pChlamy_4 vector

Map of pChlamy_4 vector

The following map shows the features of pChlamy_4 vector. The complete sequence of the vector is available for download at **www.thermofisher.com** or from Technical support (page 30).



Features of pChlamy_4 Vector

3640 nucleotides

Hsp70A-Rbc S2 promoter:	1–461
5´-UTR:	462–496
ATG start codon:	497–499
Intron-1 Rbc S2 (copy 1):	505–649*
Zeocin resistance gene (*Sh ble*):	663–1185
Intron-1 Rbc S2 (copy 2):	831–975*
FMDV 2A peptide sequence:	1186–1257
MCS 1:	1258–1269
6× His tag:	1270–1287
V5 epitope:	1288–1329
TEV recognition site:	1330–1351
MCS 2:	1350–1379
V5 epitope:	1381–1422
6× His tag:	1432–1449
MCS 3:	1453–1458
3´-UTR:	1459–1692
Ampicillin resistance gene (*bla*):	1890–2750 (c)**
bla promoter (P_{bla}):	2751–2802 (c)
pUC origin:	2848–3251

* spliced out from the *She ble* mature mRNA
** (c): complementary strand

Features of pChlamy_4 vector

The pChlamy_4 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 for strong expression of the gene of interest
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. This is the endogenous (i.e., native) *C. reinhardtii* Rbc S2.
Zeocin™ resistance gene (*Sh ble*)	*Streptoalloteichus hindustanus* bleomycin-Zeocin™ resistance gene (*Sh ble*) allows selection in *C. reinhardtii.* The *Sh ble* gene contains two copies of the Intron-1 RbcS2 (at positions 505–649 and 831–975).
FMDV 2A peptide sequence	Foot-and-mouth disease-virus (FMDV) 2A peptide linked to transgene expression mediates a self-cleavage reaction. During translation elongation of the 2A sequence, the last amino acid of the 2A sequence, a proline, is fused to the N-terminal of the first protein of interest or the N-terminal of the protein tag.
Multiple cloning sites with 7 unique restriction enzyme recognition sequences (*Eco*RI, *Xho*I, *Kpn*I, *Bam*HI, *Bgl*II, *Xba*I, *Pst*I)	Allows insertion of your gene into pChlamy_4 vector with the flexibility to include either or both or none of the N-terminal and C-terminal tags using seamless, Type IIs, or restriction enzyme-based cloning methods.
V5 epitopes (Gly-Lys-Pro-Ile-Pro-Asn-Pro-Leu-Leu-Gly-Leu-Asp-Ser-Thr)	Allows detection of the fusion protein with the Anti-V5 Antibody or the Anti-V5-HRP Antibody (Southern *et al.*, 1991).
N-terminal and C-terminal polyhistidine (6× His) tags	Allows purification of your fusion protein on metal-chelating resins (i.e., ProBond™).
TEV recognition site	Allows TEV protease-dependent cleavage of the N-terminal 6× His tag from your recombinant protein upon purification.
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.
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*.

Appendix C: Ordering information

Accessory products

Proofreading DNA polymerases

We offer various proofreading, thermostable DNA polymerases for generating blunt-end PCR products. For more information, visit **www.thermofisher.com**.

Product	Quantity	Cat. No.
Platinum™ *Pfx* DNA Polymerase	100 units	11708013
AccuPrime™ *Pfx* DNA Polymerase	200 reactions	12344024
Pfx50™ DNA Polymerase	100 reactions	12355012

Competent cells

Chemically competent and electrocompetent cells that can be used with GeneArt™ *Chlamydomonas* Protein Expression Vector are also available separately. For more information, visit **www.thermofisher.com**.

Product	Quantity	Cat. No.
One Shot™ TOP10 Chemically Competent Cells	10 reactions 20 reactions	C404010 C404003
One Shot™ TOP10 Electrocomp™ *E. coli*	10 reactions 20 reactions	C404050 C404052
TOP10 Electrocomp™ Kits	20 reactions 40 reactions 120 reactions	C66455 C66411 C66424

Other GeneArt™ products for algae

The following GeneArt™ products for algal expression, culture, and maintenance are available from Thermo Fisher Scientific. For more information, visit **www.thermofisher.com**.

Product	Quantity	Cat. No.
GeneArt™ MAX Efficiency™ Transformation Reagent	250 mL	A24229
GeneArt™ Cryopreservation Kit for Algae	1 kit	A24228
GeneArt™ *Synechococcus* Protein Expression Vector	10 reactions	A24240
Gibco™ TAP Growth Media, optimized for *Chlamydomonas* culture	1 L 6 × 1 L	A1379801 A1379802
Gibco™ BG-11 Media, optimized for cyanobacteria	1 L 6 L	A1379901 A1379902

GeneArt™ Seamless Assembly products

The following GeneArt™ products can be used for seamless assembly of up to 10 DNA inserts and vector. For more information, visit **www.thermofisher.com** or contact Technical Support (see page 30).

Product	Quantity	Cat. No.
GeneArt™ Type IIs Assembly Kit, *Aar*I	1 kit	A15916
GeneArt™ Type IIs Assembly Kit, *Bbs*I	1 kit	A15918
GeneArt™ Seamless PLUS Cloning and Assembly Kit	1 kit	A14603
GeneArt™ Seamless Cloning and Assembly Enzyme Mix	20 reactions	A14606
GeneArt™ Linear pUC19L Vector for Seamless Cloning	20 reactions	A13289
GeneArt™ Seamless Cloning and Assembly Kit	1 kit	A13288

Additional products

The following products are recommended for use with the GeneArt™ *Chlamydomonas* Protein Expression Vector. For more information, visit **www.thermofisher.com**.

Product	Quantity	Cat. No.
Neon™ Transfection System	1 each	MPK5000
Neon™ Transfection System 100 µL Kit	25 × 2 reactions	MPK10025
Electroporation cuvettes, 0.4 cm	50/bag	P46050
PureLink™ Growth Block	50 blocks	12256020
PureLink™ HQ Mini Plasmid Purification Kit	100 preps	K210001
PureLink™ HiPure Plasmid Miniprep Kit	25 preps 100 preps	K210002 K210003
PureLink™ Expi Endotoxin-Free Mega Plasmid Purification Kit	2 preps 4 preps	A31233 A31232
Zeocin™ Selection Reagent	8 × 1.25 mL	R25001
Ampicillin Sodium Salt, irradiated	200 mg	11593027
LB Broth (1X), liquid	500 mL	10855021
V5 Epitope Tag Antibody	50 µL	R96025
AcTEV™ Protease	1000 units	12575015
EnzChek™ *Ultra* Xylanase Assay Kit	1 kit	E33650

Appendix D: Safety

Chemical safety

WARNING! GENERAL CHEMICAL HANDLING. To minimize hazards, ensure laboratory personnel read and practice the general safety guidelines for chemical usage, storage, and waste provided below, and consult the relevant SDS for specific precautions and instructions:

- Read and understand the Safety Data Sheets (SDSs) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials. To obtain SDSs, see the "Documentation and Support" section in this document.
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing).
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with adequate ventilation (for example, fume hood).
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer's cleanup procedures as recommended in the SDS.
- Handle chemical wastes in a fume hood.
- Ensure use of primary and secondary waste containers. (A primary waste container holds the immediate waste. A secondary container contains spills or leaks from the primary container. Both containers must be compatible with the waste material and meet federal, state, and local requirements for container storage.)
- After emptying a waste container, seal it with the cap provided.
- Characterize (by analysis if necessary) the waste generated by the particular applications, reagents, and substrates used in your laboratory.
- Ensure that the waste is stored, transferred, transported, and disposed of according to all local, state/provincial, and/or national regulations.
- **IMPORTANT!** Radioactive or biohazardous materials may require special handling, and disposal limitations may apply.

Biological hazard safety

WARNING! BIOHAZARD. Biological samples such as tissues, body fluids, infectious agents, and blood of humans and other animals have the potential to transmit infectious diseases. Conduct all work in properly equipped facilities with the appropriate safety equipment (for example, physical containment devices). Safety equipment can also include items for personal protection, such as gloves, coats, gowns, shoe covers, boots, respirators, face shields, safety glasses, or goggles. Individuals should be trained according to applicable regulatory and company/ institution requirements before working with potentially biohazardous materials. Follow all applicable local, state/provincial, and/or national regulations. The following references provide general guidelines when handling biological samples in laboratory environment.

In the US:

- U.S. Department of Health and Human Services guidelines published in Biosafety in Microbiological and Biomedical Laboratories found at:

  www.cdc.gov/biosafety

- Occupational Safety and Health Standards, Bloodborne Pathogens (29 CFR§1910.1030), found at:

  www.access.gpo.gov/nara/cfr/waisidx_01/29cfr1910a_01.html

- Your company's/institution's Biosafety Program protocols for working with/handling potentially infectious materials.

- Additional information about biohazard guidelines is available at:

  www.cdc.gov

In the EU:

Check local guidelines and legislation on biohazard and biosafety precaution and refer to the best practices published in the World Health Organization (WHO) Laboratory Biosafety Manual, 3rd edition, found at:

www.who.int/csr/resources/publications/biosafety/WHO_CDS_CSR_LYO_2004_11/en/

Documentation and support

Obtaining support

Technical support

For the latest services and support information for all locations, go to **www.thermofisher.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 (**thermofisher.com/support)**
- 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.thermofisher.com/sds**.

IMPORTANT! For the SDSs of chemicals not distributed by Thermo Fisher Scientific contact the chemical manufacturer.

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.thermofisher.com/us/en/home/global/terms-and-conditions.html**. If you have any questions, please contact Life Technologies at **www.thermofisher.com/support**.

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