GeneArt® *Chlamydomonas* Protein Expression Kit

For expression of recombinant proteins in *Chlamydomonas reinhardtii*

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Product information

Contents and storage

Kit components

The GeneArt® Chlamydomonas Protein Expression Kit contains the components listed below, which are shipped in separate boxes as described. Upon receipt, store each box as detailed below. All reagents are guaranteed for six months if stored properly.

<table>
<thead>
<tr>
<th>Box</th>
<th>Component</th>
<th>Shipping</th>
<th>Storage</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>GeneArt® Chlamydomonas reinhardtii cells</td>
<td>Dry ice</td>
<td>–80°C</td>
</tr>
<tr>
<td>2</td>
<td>GeneArt® Chlamydomonas vector set</td>
<td>Gel ice</td>
<td>–20°C</td>
</tr>
<tr>
<td>3</td>
<td>One Shot® TOP10 Chemically Competent E. coli</td>
<td>Dry ice</td>
<td>–80°C</td>
</tr>
</tbody>
</table>

GeneArt® Chlamydomonas reinhardtii cells

Each GeneArt® Chlamydomonas Protein Expression 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). Upon receipt, store the entire box at –20°C. The GeneArt® MAX Efficiency® Transformation Reagent can also be stored at 2–8°C.

<table>
<thead>
<tr>
<th>Component</th>
<th>Concentration</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>pChlamy_.4 vector</td>
<td>20 µL of vector at 0.5 µg/µL in TE buffer, pH 8.0*</td>
<td>20 µL</td>
</tr>
<tr>
<td>GeneArt® MAX Efficiency® Transformation Reagent</td>
<td>1×</td>
<td>55 mL</td>
</tr>
</tbody>
</table>

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

Continued on next page
The table below describes the items included in the One Shot® TOP10 Chemically Competent E. coli kit (Box 3). Store the contents of Box 3 at –80°C.

The transformation efficiency of One Shot® TOP10 Chemically Competent E. coli is $1 \times 10^9$ cfu/µg DNA.

<table>
<thead>
<tr>
<th>Component</th>
<th>Concentration</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>TOP10 Cells</td>
<td>—</td>
<td>$11 \times 50 \mu L$</td>
</tr>
<tr>
<td>S.O.C. Medium (may be stored at room temperature or 2–8°C)</td>
<td>2% Tryptone, 0.5% Yeast Extract, 10 mM NaCl, 2.5 mM KCl, 10 mM MgCl₂, 10 mM MgSO₄, 20 mM glucose</td>
<td>7 mL</td>
</tr>
<tr>
<td>pUC19 Transformation Control DNA</td>
<td>10 pg/µL in 5 mM Tris-HCl, 0.5 mM EDTA, pH 8.0</td>
<td>20 µL</td>
</tr>
</tbody>
</table>

**Genotype of TOP10**

Use this strain to clone the PCR product into the pChlamy_4 vector.

Genotype: F⁻ mcrA Δ(mrr-hsdRMS-mcrBC) F80lacZ M15 Δlac74 recA1 araD139 Δ(ara-leu)7697 galU galK rpsL (Str^R) endA1 nupG
Description of the system

GeneArt®
Chlamydomonas
Protein Expression
Kit

The GeneArt® Chlamydomonas Protein Expression Kit is a eukaryotic genetic engineering system based on the unicellular green alga Chlamydomonas reinhardtii 137c ((Rasala et al., 2012), offering a simplified approach for protein expression in algae for downstream applications such as biofuels, specialty chemicals, and industrial enzymes. This system is designed for high level expression of your gene of interest, accounting for up to 1% of total soluble protein. 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 CO₂ 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
Description of the system, continued

Expressing heterologous genes 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 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_4 vector

pChlamy_4 vector is designed to facilitate 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 25.

- 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, thus 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.
- 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.
- 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. 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.

\[
\text{APVKQLNFDLLKLAGDVE} \text{SNPG} \uparrow
\]

Continued on next page
### Description of the system, continued

<table>
<thead>
<tr>
<th>pChlamy_4 vector continued</th>
</tr>
</thead>
<tbody>
<tr>
<td>• A 3’-UTR fragment from RbcS2 (Ribulose Bisphosphate Carboxylase/Oxygenase Small Subunit 2) gene downstream of the multiple cloning site ensures the proper termination of transcript.</td>
</tr>
<tr>
<td>• <strong>Note:</strong> 3’ UTR may contain sequences that regulate translation efficiency, mRNA stability, and polyadenylation signals.</td>
</tr>
<tr>
<td>• The versatile multiple cloning site facilitates simplified cloning of your gene of interest by restriction enzyme digestion or seamless cloning.</td>
</tr>
<tr>
<td>• 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.</td>
</tr>
<tr>
<td>• Ampicillin resistance gene allows selection in <em>E. coli</em>.</td>
</tr>
<tr>
<td>• Bleomycin/Zeocin™-resistance gene permits selection in <em>C. reinhardtii</em>.</td>
</tr>
<tr>
<td>• pUC origin allows the maintenance of the vector in <em>E. coli</em>.</td>
</tr>
</tbody>
</table>
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.

<table>
<thead>
<tr>
<th>Step</th>
<th>Action</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Clone your codon optimized gene of interest into pChlamy_4 vector</td>
<td>8</td>
</tr>
<tr>
<td>2</td>
<td>Transform <em>E. coli</em> with the pChlamy_4 construct containing your gene of interest and select the transformants on LB plates containing Ampicillin</td>
<td>11</td>
</tr>
<tr>
<td>3</td>
<td>Analyze transformants by restriction digestion or PCR</td>
<td>12</td>
</tr>
<tr>
<td>4</td>
<td>Thaw and resuscitate <em>C. reinhardtii</em> cells</td>
<td>14</td>
</tr>
<tr>
<td>5</td>
<td>Transform <em>C. reinhardtii</em> cells by electroporation and select transformants</td>
<td>15</td>
</tr>
<tr>
<td>6</td>
<td>Screen <em>C. reinhardtii</em> transformants by colony PCR for full integration of your gene of interest, or by an appropriate enzymatic assay</td>
<td>19</td>
</tr>
</tbody>
</table>
Methods

Cloning 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, 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, use a recombination deficient (recA) and endonuclease A-deficient (endA) strain such as TOP10 (included in the kit; see page 3 for genotype). Note that other recA, endA 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 recA, endA E. coli strain like TOP10, DH5α™, JM109, or equivalent. Select transformants on LB plates containing 50–100 μg/mL of ampicillin. Be sure to prepare a glycerol stock of the plasmid for long-term storage (see page 12).

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 505–649, see page 9) is spliced out from the mature RNA and does not constitute actual codons. The reading frame before and after the removal of Intron-1 Rbc S2 is shown below.

Before splicing

<table>
<thead>
<tr>
<th>Intron-1 Rbc S2</th>
<th>Sh ble</th>
</tr>
</thead>
<tbody>
<tr>
<td>GAGGCTTTT TTGCCTCCTTG TAGCCCACATC TAAACCTGCTC CATTTGCAGG AAGCACCAGG CTCGGTTCTC CCGGGGACCTT</td>
<td></td>
</tr>
</tbody>
</table>

After splicing

<table>
<thead>
<tr>
<th>Sh ble</th>
</tr>
</thead>
<tbody>
<tr>
<td>CTTAAAAATGG CCAAGTGGAG TGGCTCAGGTC GCGGGACGTC GCCGGAGGGG TCGAGTTCTG GACCCAGCGG CTCGGTTCTC CCGGGGACCTT</td>
</tr>
</tbody>
</table>

pChlamy_4 vector contains the ATG initiation codon (vector ATG) for proper initiation of translation at position 497–499, located at the beginning of the Sh ble gene after the removal of Intron-1 Rbc S2 (see above). You do not need to add an ATG start codon to your insert.

Continued on next page
Cloning into pChlamy_4 vector, continued

Cloning considerations, continued

- 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 below.
- If you wish to use the C-terminal tag, make sure 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 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).**

Multiple cloning site of pChlamy_4

Below is the multiple cloning site for pChlamy_4. The three sets of restriction sites are labeled, where the black triangles indicate the cleavage site. The ATG initiation codon (vector ATG) is located immediately upstream of Sh ble gene (not shown here; see graph on page 8), and the potential stop codons are shown in bold. 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. Use the diagram below to design suitable PCR primers to clone and express your PCR product in pChlamy_4.

The vector sequence of pChlamy_4 is available at [www.lifetechnologies.com](http://www.lifetechnologies.com) or by contacting Technical Support (page 29).

Continued on next page
Once 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. 15224-041).

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. Since the technology relies on homologous recombination, each DNA insert must have a 15-bp overlap with the adjacent insert, including the cloning vector. This homology may be split between adjacent fragments in any combination (e.g., 7+8, 6+9, 5+10 etc.).

Once 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 suitable 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 enzyme(s), PCR amplify your gene(s) of interest, and 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. Because there are two similar tag sequences in the vector, you must remove one of the two tag sequence fragments from the vector before performing the seamless cloning reaction for optimal cloning efficiency.


You may use any method of your choice for E. coli transformation. Chemical transformation is the most convenient for most researchers. One Shot® TOP10 Chemically Competent E. coli cells are included in the GeneArt® Chlamydomonas Protein Expression Kit for this purpose and a protocol for chemical transformation is provided on page 11. However, you may also transform electrocompetent cells by electroporation, which is more efficient and the method of choice for large plasmids.
Transforming One Shot® TOP10 Competent *E. coli* cells

Introduction

Once you have performed the cloning reaction, you will transform your pChlamy_4 construct into competent *E. coli*. The GeneArt® Chlamydomonas Protein Expression Kit includes One Shot® TOP10 Chemically Competent *E. coli* cells for this purpose. However, you may 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

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 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 will have well-spaced colonies.
8. Pick 5–10 colonies for analysis (see Analyzing *E. coli* transformants, page 12).
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 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. K2100-01; see page 27).
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 92–98°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 2°C–8°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_4 vector, refer to our website (www.lifetechnologies.com) or contact Technical support (see page 29).

### 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 20–28°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 sub-optimal growth conditions.
- Phototrophic cultures should be supplied with CO₂ 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 CO₂ 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, refer to *Biosafety in Microbiological and Biomedical Laboratories*, 5th ed., published by the Centers for Disease Control, which is available for downloading at: [www.cdc.gov/od/ohs/biosfty/bmbl5/bmbl5toc.htm](http://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
- 500-mL glass culture flask
- 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 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 flask(s) 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 × 10⁶ 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 × 10⁶ cells/mL. Once the culture has reached 1 × 10⁶ cells/mL, proceed to the transformation step (page 15).
Transforming *Chlamydomonas reinhardtii* 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 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. We recommend using *ScaI* restriction enzyme for linearization, provided that the insert does not contain the recognition sequence for *ScaI*. Otherwise, you may choose from *PvuI*, *SspI*, or *FspI*.
- 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. K2100-01) or the PureLink® HiPure Plasmid Miniprep Kit (Cat. no. K2100-02) that delivers pure DNA, and elute the purified DNA from the purification column using pure water rather than TE or E1 buffer.
- For best results, grow the cells to 1 × 10⁶–2 × 10⁶ cells/mL before proceeding with electroporation. You may use <1 × 10⁶ cells/mL, but the concentration should not exceed 3 × 10⁶ 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 19) 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.

*Continued on next page*
Transforming *Chlamydomonas reinhardtii* by electroporation, continued

**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.
- GeneArt® MAX Efficiency® Transformation Reagent (Cat. no. A24229)
- 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 21 for recipe)
- TAP-Agar-Zeocin™ plates (5 µg/mL) (see page 22 for recipe)
- Sterile 15-mL and 50-mL centrifugation tubes
- 0.4-cm electroporation cuvettes (Cat. no. P460-50), chilled on ice
- 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)

**Electroporation 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, refer to **Electroporation using the Neon® Transfection System**, page 18.

1. When cell concentration reaches 1 × 10⁶–2 × 10⁹ cells/mL (see page 14), harvest them by centrifugation at 2500 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 × 10⁶ cells/mL, you may still harvest the cells without significantly affecting the transformation efficiency. If the cell concentration exceeds 3 × 10⁶ 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 2500 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 2500 rpm for 5 minutes.
4. Resuspend the cell pellet in GeneArt® MAX Efficiency® Transformation Reagent to a final concentration of 2 × 10⁵–3 × 10⁶ cells/mL.
5. Add 2–4 µg of linearized DNA per 250 µL of cell suspension and incubate at 2°C–8°C for 5 minutes.
6. Set electroporation parameters on the Gene Pulser® II as follows:

<table>
<thead>
<tr>
<th>Voltage</th>
<th>Capacity</th>
<th>Resistance</th>
</tr>
</thead>
<tbody>
<tr>
<td>500 V</td>
<td>50 µF</td>
<td>800 Ω</td>
</tr>
</tbody>
</table>

*Continued on next page*
Transforming *Chlamydomonas reinhardtii* by electroporation, continued

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**Electroporation using the Bio-Rad® Gene Pulser® II device, continued**

7. Transfer 250 µL of the cell-DNA mix into an ice-cold cuvette (pre-chilled on ice) just before electroporation.
8. Electroporate the cells using the appropriate settings (500 V, 50 µF, 800 Ω). Usually, the electro pulse duration is about 30 ms.
9. After electroporation, allow the cells to recover on the bench for 15 minutes.
10. Transfer the cells into a 50-mL conical tube or flask containing 10 mL of TAP-40 mM sucrose solution at room temperature.
11. 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.
12. 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.

7. 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.
8. 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.
9. Incubate the plates for 5–7 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_4 construct will depend on the nature, size, and codon content of the gene of interest, and the physiological state of the cells.
10. Proceed to determination of integration by colony PCR (see page 19) 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).

*Continued on next page*
Transforming *Chlamydomonas reinhardtii* by electroporation, continued

**Electroporation using the Neon® Transfection System**

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

1. Harvest the cells as described in Steps 1–3 of the GenePulser® II protocol (page 16), and resuspend them in GeneArt® MAX Efficiency® Transformation Reagent to a final concentration of $1 \times 10^8$–$3 \times 10^8$ cells/mL.

2. Add 1 µg of linearized DNA per 100 µL of cell suspension and incubate at 2°C–8°C for 5 minutes.

3. 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.

4. Set electroporation parameters on the Neon® device as follows:

<table>
<thead>
<tr>
<th>Voltage</th>
<th>Pulse width</th>
<th>Pulse number</th>
</tr>
</thead>
<tbody>
<tr>
<td>2300V</td>
<td>13 ms</td>
<td>3</td>
</tr>
</tbody>
</table>

5. 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.

6. Press **Start** on the touchscreen to deliver the electric pulse.

7. 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.

8. Add 4 mL of TAP-40 mM sucrose solution at room temperature to the cells and incubate them in the algal chamber overnight.

9. The next day, centrifuge the cells at 2500 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.

10. 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.

11. 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.

12. Incubate the plates for 5–7 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_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 19) before selecting clones for further scale-up.

14. 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).
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:

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>AccuPrime™ Pfx SuperMix</td>
<td>47 µL</td>
</tr>
<tr>
<td>Cell lysate</td>
<td>1 µL</td>
</tr>
<tr>
<td>Forward primer (10 µM)</td>
<td>1 µL</td>
</tr>
<tr>
<td>Reverse primer (10 µM)</td>
<td>1 µL</td>
</tr>
<tr>
<td><strong>Total volume:</strong></td>
<td>50 µL</td>
</tr>
</tbody>
</table>

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 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. The best method for long-term storage and preservation of algae is cryopreservation, which dramatically reduces genetic drift, lowers labor and cost associated with the maintenance of algae plates, and facilitates strain and clone exchange between laboratories. For cryopreservation, we recommend using the GeneArt® Cryopreservation Kit for Algae, available separately from Life Technologies (Cat. no. A24228), which allows algae to be frozen and stored in a –80°C freezer for at least 2 years. For a cryopreservation protocol using the GeneArt® Cryopreservation Kit for Algae, see page 23.

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 or a vial of frozen cells into 250 mL of Gibco® TAP medium, growing the cells until they reach the mid-log phase of growth (1 × 10⁶–5 × 10⁶ cells/mL), and then taking an appropriate aliquot to inoculate the experimental cultures at a starting density of 1 × 10⁵ 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 CO₂ (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

Preparation of reagents and media

**Handling Zeocin™ selection reagent**

Zeocin™ selection reagent (Cat. no. R250-01) 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 Cu²⁺ to Cu⁺ 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 hygromycin B, 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.
- Be sure to 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 44 mL of 1 M sucrose to 1 L of Gibco® TAP medium.

**TAP-Zeocin™ solution**

1. Add Zeocin™ stock solution (Cat. no. R250-01; 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.

*Continued on next page*
Preparing reagents and media, continued

**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°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 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 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%.
**Cryopreserving *Chlamydomonas reinhardtii***

**GeneArt® Cryopreservation Kit for Algae**

The best method for the preservation and long-term storage of *C. reinhardtii* is cryopreservation, which dramatically reduces genetic drift, lowers labor and cost associated with the maintenance of algae plates, and facilitates strain and clone exchange between laboratories. In contrast to most cryopreservation methods that require liquid nitrogen storage, the GeneArt® Cryopreservation Kit for Algae (Cat. no. A24228) 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)
- Mr. Frosty® freezing container (Nalgene, Cat. no. 5100-0001)
- Benchtop centrifuge (e.g., Sorvall)
- Cryovials (Sarstaeedt, Cat. no. 72.685.701).
- 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

**Freezing *C. reinhardtii* cells**

1. Grow *C. reinhardtii* 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 OD₇₅₀ of 0.1 (usually, 2–5 mL of seed culture). Do not exceed OD₇₅₀ 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 may 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 OD₇₅₀ of the culture and calculate the cell concentration using the equation below.

\[
\text{Cell concentration (cells/mL)} = \frac{(\text{OD}_{750} - 0.088)}{(9 \times 10^{-8})}
\]

*Continued on next page*
Cryopreserving *Chlamydomonas reinhardtii*, continued

6. **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.

7. Harvest the cells by centrifugation at 2500 rpm for 5 minutes and carefully remove as much of the supernatant as possible.

8. Resuspend the cells to a final concentration of $2.5 \times 10^7$ 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 $5 \times 10^7$ cells/mL (cell viability will be dramatically reduced at higher concentrations).

9. Aliquot exactly 240 µL of cell suspension into each cryovial and incubate at room temperature for 30–45 minutes.

10. 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 of the slots of the vial holder, fill the rest of slots with similar liquid-filled cryovials to ensure a proper cooling profile. Do not fill the container with 100% isopropyl alcohol or any other freezing liquid.

11. 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.

12. 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 may result in decreased cell viability.

13. 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.

14. The cells can be stored at −80°C for at least 2 years. Note that this freezing protocol may also be suitable for other species of *Chlamydomonas*. 
Appendix B: Vectors

Map and features of pChlamy_4 vector

The map below shows the features of pChlamy_4 vector. The complete sequence of the vector is available for downloading at www.lifetechnologies.com or from Technical support (page 29).

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Features of pChlamy_4 Vector
3640 nucleotides

- Hap70A--Rbc S2 promoter: 1–661
- 5′-UTR: 462–496
- ATG start codon: 497–499
- Intron-1 Rbc S2: 505–649*
- Zeocin resistance gene (S. biei): 650–1185
- FMDV 2A peptide sequence: 1186–1287
- MCS 1: 1288–1289
- 6x His tag: 1270–1280
- V5 epitope: 1289–1329
- TEV recognition site: 1330–1360
- MCS 2: 1350–1379
- V5 epitope: 1381–1422
- 6x His tag: 1432–1449
- MCS 3: 1453–1458
- 3′-UTR: 1459–1692
- AmpCillin resistance gene (bla): 1690–2750 [c]**
- bba promoter (P_bba): 2751–2802 [c]
- pUC origin: 2848–3251

* spliced out from the mature RNA
** [c]: complementary strand

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Continued on next page
The pChlamy_4 vector contains the following elements. All features have been functionally tested.

<table>
<thead>
<tr>
<th>Feature</th>
<th>Benefit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hsp70A-Rbc S2 promoter</td>
<td>A hybrid constitutive promoter consisting of Hsp70 and RbcS2 promoters for strong expression of the gene of interest</td>
</tr>
<tr>
<td>Intron-1 Rbc S2</td>
<td>First intron of the small subunit of the ribulose bisphosphate carboxylase (rbcS2); necessary to maintain the high expression of your gene of interest.</td>
</tr>
<tr>
<td>Zeocin™ resistance gene (She ble)</td>
<td><em>Streptoballochus hindustanus</em> bleomycin-Zeocin™ resistance gene (Sh ble) permits selection in <em>C. reinhardtii</em></td>
</tr>
<tr>
<td>FMDV 2A peptide sequence</td>
<td>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</td>
</tr>
<tr>
<td>3 multiple cloning sites with 7 unique restriction enzyme recognition sequences (<em>EcoRI, XhoI, KpnI, BamHI, BglII, XbaI, PstI</em>)</td>
<td>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</td>
</tr>
<tr>
<td>V5 epitopes (Gly-Lys-Pro-Ile-Pro-Asn-Pro-Leu-Gly-Leu-Asp-Ser-Thr)</td>
<td>Allows detection of the fusion protein with the Anti-V5 Antibody or the Anti-V5-HRP Antibody (Southern et al., 1991)</td>
</tr>
<tr>
<td>N-terminal and C-terminal polyhistidine (6× His) tags</td>
<td>Permits purification of your fusion protein on metal-chelating resins (i.e., ProBond™)</td>
</tr>
<tr>
<td>TEV recognition site</td>
<td>Allows TEV protease-dependent cleavage of the N-terminal 6× His tag from your recombinant protein upon purification</td>
</tr>
<tr>
<td>3′ UTR from RbcS2 gene</td>
<td>Assures the proper termination of transcript; 3′ UTR may contain sequences that regulate translation efficiency, mRNA stability, and polyadenylation signals.</td>
</tr>
<tr>
<td>Ampicillin resistance gene (<em>bla</em>)</td>
<td>Allows selection of the plasmid in <em>E. coli.</em></td>
</tr>
<tr>
<td><em>bla</em> promoter</td>
<td>Allows expression of the Ampicillin resistance gene.</td>
</tr>
<tr>
<td>pUC origin</td>
<td>Allows high-copy replication and growth in <em>E. coli.</em></td>
</tr>
</tbody>
</table>
Appendix C: Ordering information

Accessory products

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

<table>
<thead>
<tr>
<th>Product</th>
<th>Quantity</th>
<th>Cat. no.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Platinum® Pfx DNA Polymerase</td>
<td>100 units</td>
<td>11708-013</td>
</tr>
<tr>
<td>AccuPrime™ Pfx DNA Polymerase</td>
<td>200 reactions</td>
<td>12344-024</td>
</tr>
<tr>
<td>Pfx50™ DNA Polymerase</td>
<td>100 reactions</td>
<td>12355-012</td>
</tr>
</tbody>
</table>

Competent cells

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

<table>
<thead>
<tr>
<th>Product</th>
<th>Quantity</th>
<th>Cat. no.</th>
</tr>
</thead>
<tbody>
<tr>
<td>One Shot® TOP10 Chemically Competent Cells</td>
<td>10 reactions</td>
<td>C4040-10</td>
</tr>
<tr>
<td></td>
<td>20 reactions</td>
<td>C4040-03</td>
</tr>
<tr>
<td>One Shot® TOP10 Electrocomp™ E. coli</td>
<td>10 reactions</td>
<td>C4040-50</td>
</tr>
<tr>
<td></td>
<td>20 reactions</td>
<td>C4040-52</td>
</tr>
<tr>
<td>TOP10 Electrocomp™ Kits</td>
<td>20 reactions</td>
<td>C664-55</td>
</tr>
<tr>
<td></td>
<td>40 reactions</td>
<td>C664-11</td>
</tr>
<tr>
<td></td>
<td>120 reactions</td>
<td>C664-24</td>
</tr>
</tbody>
</table>

Additional products

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

<table>
<thead>
<tr>
<th>Product</th>
<th>Quantity</th>
<th>Cat. no.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gibco® TAP Growth Media: Optimized for Chlamydomonas</td>
<td>1 L</td>
<td>A13798-01</td>
</tr>
<tr>
<td></td>
<td>6 x 1 L</td>
<td>A13798-02</td>
</tr>
<tr>
<td>Electroporation cuvettes, 0.4 cm</td>
<td>50/bag</td>
<td>P460-50</td>
</tr>
<tr>
<td>PureLink® Growth Block</td>
<td>50 blocks</td>
<td>12256-020</td>
</tr>
<tr>
<td>PureLink® HQ Mini Plasmid Purification Kit</td>
<td>100 preps</td>
<td>K2100-01</td>
</tr>
<tr>
<td>PureLink® HiPure Plasmid Miniprep Kit</td>
<td>25 preps</td>
<td>K2100-02</td>
</tr>
<tr>
<td></td>
<td>100 preps</td>
<td>K2100-03</td>
</tr>
<tr>
<td>Zeocin™ Selection Reagent</td>
<td>8 x 1.25 mL</td>
<td>R250-01</td>
</tr>
<tr>
<td>Ampicillin Sodium Salt, irradiated</td>
<td>200 mg</td>
<td>11593-027</td>
</tr>
<tr>
<td>Hygromycin B</td>
<td>20 mL</td>
<td>10687-010</td>
</tr>
<tr>
<td>LB Broth (1X), liquid</td>
<td>500 mL</td>
<td>10855-021</td>
</tr>
</tbody>
</table>

Continued on next page
Accessory products, continued

**Other GeneArt® products for algae**

In addition to the GeneArt® *Chlamydomonas* Protein Expression Kits, we offer the following products as model algal hosts. Ordering information is provided below. For details, visit [www.lifetechnologies.com](http://www.lifetechnologies.com).

<table>
<thead>
<tr>
<th>Product</th>
<th>Quantity</th>
<th>Cat. no.</th>
</tr>
</thead>
<tbody>
<tr>
<td>GeneArt® MAX Efficiency® Transformation Reagent</td>
<td>250 mL</td>
<td>A24229</td>
</tr>
<tr>
<td>GeneArt® Cryopreservation Kit for Algae</td>
<td>1 kit</td>
<td>A24228</td>
</tr>
<tr>
<td>GeneArt® <em>Chlamydomonas</em> Engineering Kit</td>
<td>1 kit</td>
<td>A14258</td>
</tr>
<tr>
<td>GeneArt® <em>Chlamydomonas</em> Engineering Kit with 6 L media</td>
<td>1 kit</td>
<td>A14262</td>
</tr>
<tr>
<td>GeneArt® <em>Chlamydomonas</em> TOPO® Engineering Kit</td>
<td>1 kit</td>
<td>A14260</td>
</tr>
<tr>
<td>GeneArt® <em>Chlamydomonas</em> TOPO® Engineering Kit with 6 L media</td>
<td>1 kit</td>
<td>A14264</td>
</tr>
<tr>
<td>GeneArt® <em>Synechococcus</em> Protein Expression Kit</td>
<td>1 kit</td>
<td>A24243</td>
</tr>
<tr>
<td>GeneArt® <em>Synechococcus</em> Engineering Kit</td>
<td>1 kit</td>
<td>A14259</td>
</tr>
<tr>
<td>GeneArt® <em>Synechococcus</em> Engineering Kit with 6 L media</td>
<td>1 kit</td>
<td>A14263</td>
</tr>
<tr>
<td>GeneArt® <em>Synechococcus</em> TOPO® Engineering Kit</td>
<td>1 kit</td>
<td>A14261</td>
</tr>
<tr>
<td>GeneArt® <em>Synechococcus</em> TOPO® Engineering Kit with 6 L media</td>
<td>1 kit</td>
<td>A14265</td>
</tr>
</tbody>
</table>

**GeneArt® Seamless Assembly products**

We also offer GeneArt® products that can be used for seamless assembly of up to 10 DNA inserts and vector. For more information, refer to [www.lifetechnologies.com](http://www.lifetechnologies.com) or contact Technical Support (see page 29).

<table>
<thead>
<tr>
<th>Product</th>
<th>Quantity</th>
<th>Cat. no.</th>
</tr>
</thead>
<tbody>
<tr>
<td>GeneArt® Type IIs Assembly Kit, AarI</td>
<td>1 kit</td>
<td>A15916</td>
</tr>
<tr>
<td>GeneArt® Type IIs Assembly Kit, BsaI</td>
<td>1 kit</td>
<td>A15917</td>
</tr>
<tr>
<td>GeneArt® Type IIs Assembly Kit, BbsI</td>
<td>1 kit</td>
<td>A15918</td>
</tr>
<tr>
<td>GeneArt® Seamless PLUS Cloning and Assembly Kit</td>
<td>1 kit</td>
<td>A14603</td>
</tr>
<tr>
<td>GeneArt® Seamless Cloning and Assembly Enzyme Mix</td>
<td>20 reactions</td>
<td>A14606</td>
</tr>
<tr>
<td>GeneArt® Linear pUC19L Vector for Seamless Cloning</td>
<td>20 reactions</td>
<td>A13289</td>
</tr>
<tr>
<td>GeneArt® Seamless Cloning and Assembly Kit</td>
<td>1 kit</td>
<td>A13288</td>
</tr>
</tbody>
</table>
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.

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References


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