USER GUIDE

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# GeneArt® *Chlamydomonas* TOPO® Engineering Kits

For directional TOPO® cloning and expression of recombinant proteins in *Chlamydomonas reinhardtii*

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# Product Information

## Contents and Storage

### Types of kits

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

| Product | Catalog number |
|---|---|
| GeneArt® *Chlamydomonas* TOPO® Engineering Kit | A14260 |
| GeneArt® *Chlamydomonas* TOPO® Engineering Kit with 6L media | A14264 |

### Kit components

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

| Box | Component | Catalog number A14260 | Catalog number A14264 |
|---|---|---|---|
| 1 | GeneArt® *Chlamydomonas reinhardtii* cells | ✓ | ✓ |
| 2 | GeneArt® *Chlamydomonas* TOPO® Vector Set | ✓ | ✓ |
| 3 | One Shot® TOP10 Chemically Competent *E. coli* | ✓ | ✓ |
| 4 | Gibco® TAP Growth Media | | ✓ |

### Shipping/Storage

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

| Box | Component | Shipping | Storage |
|---|---|---|---|
| 1 | GeneArt® *Chlamydomonas reinhardtii* cells | Dry ice | –80°C |
| 2 | GeneArt® *Chlamydomonas* TOPO® Vector Set | Dry ice | –20°C |
| 3 | One Shot® TOP10 Chemically Competent *E. coli* | Dry ice | –80°C |
| 4 | Gibco® TAP Growth Media | Gel ice | 4°C |

*Continued on next page*

### GeneArt® *Chlamydomonas reinhardtii* cells

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

### GeneArt® *Chlamydomonas* TOPO® Vector Set

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

| Component | Concentration | Amount |
|---|---|---|
| pChlamy_3/D-TOPO® Vector | 10 µL at 20 ng/µL in:<br>50% glycerol<br>50 mM Tris-HCl, pH 7.4 (at 25°C)<br>1 mM EDTA<br>2 mM DTT<br>0.1% Triton X-100<br>100 µg/mL BSA<br>30 µM bromophenol blue | 10 µL |
| pChlamy_2/Control Vector | 0.5 µg/µL in TE buffer, pH 8.0* | 80 µL |
| Salt Solution | 1.2 M NaCl<br>0.06 M $MgCl_2$ | 50 µL |
| Sterile Water | — | 1 mL |
| Forward Sequencing Primer pChlamy_3 vector | 100 ng/ µL in TE buffer, pH 8.0 | 20 µL |
| Reverse Sequencing Primer pChlamy_3 vector | 100 ng/ µL in TE buffer, pH 8.0 | 20 µL |
| Control PCR primers, Directional (for TOPO® reaction insert control) | 100 ng/ µL **each** in TE buffer, pH 8.0 | 10 µL |
| dNTP Mix | 12.5 mM each dATP, dCTP, dGTP, and dTTP; neutralized at pH 8.0 in water | 10 µL |

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

*Continued on next page*

### Primer sequences

"Forward Sequencing Primer pChlamy_3 vector" and "Reverse Sequencing Primer pChlamy_3 vector" are used for verifying the sequence of your gene of interest after the *E. coli* transformation step (page 15). The sequences of these primers are provided below.

| | |
|---|---|
| **Forward Sequencing Primer pChlamy_3 vector** | 5′-GCA AGC AGT TCG CAT GCA G-3′ |
| **Reverse Sequencing Primer pChlamy_3 vector** | 5′-GCT CGC CCT GGA GCG GCA TCG G-3′ |

### One Shot® TOP10 Chemically Competent *E. coli*

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.

| Component | Concentration | Amount |
|---|---|---|
| TOP10 Cells | — | 11 × 50 µL |
| S.O.C. Medium<br>(may be stored at room temperature or 4°C) | 2% Tryptone<br>0.5% Yeast Extract<br>10 mM NaCl<br>2.5 mM KCl<br>10 mM $MgCl_2$<br>10 mM $MgSO_4$<br>20 mM glucose | 7 mL |
| pUC19 Transformation Control DNA | 10 pg/µL in 5 mM Tris-HCl, 0.5 mM EDTA, pH 8.0 | 20 µL |

### Genotype of TOP10

Use this strain to clone the PCR product into the pChlamy_3/D-TOPO® vector.

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

### Gibco® TAP Growth Media

Gibco® TAP Growth Media, included in the GeneArt® *Chlamydomonas* TOPO® Engineering Kit with 6 L media (Cat. no. A14264), is supplied in 6 × 1 L bottles and is optimized for the growth and maintenance of *Chlamydomonas reinhardtii* cells. Store the Gibco® TAP Growth Medium at 4°C.

**Note:** Gibco® TAP Growth Media (Cat. nos. A13798-01, A13798-02) is also available separately from Life Technologies. See page 27 for ordering information.

# Description of the System

## Introduction

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

## *Chlamydomonas reinhardtii*

The green algae *Chlamydomonas reinhardtii* has served as a genetic workhorse and model organism for understanding everything from the mechanisms of light and nutrient regulated gene expression to the assembly and function of flagella (Harris, 2001; Hippler *et al.*, 1998; Merchant *et al.*, 2007; Miller *et al.*, 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 a variety of beneficial attributes including:

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

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

## Growth characteristics of *C. reinhardtii*

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

*Continued on next page*

### Expressing heterologous genes in *C. reinhardtii*

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

### pChlamy_3/ D-TOPO® Vector

pChlamy_3/D-TOPO® Vector is designed to facilitate rapid, directional TOPO® Cloning of blunt-end PCR products for expression in *C. reinhardtii*. This vector is a nuclear integrative vector; the integration is a random event across the genome. However, depending on the context of the gene of interest, the copy number of the integrated gene will be varied. Some of the features of the vector are listed below. For a map and additional features of the vector, see page 24.

- HSP70A/RBCS2 chimeric constitutive promoter for strong expression of gene of interest
- Directional TOPO® Cloning site for rapid and efficient directional cloning of a blunt-end PCR product (see page 7 for more information on Directional TOPO® Cloning)
- A 3′-UTR fragment from RbcS2 (Ribulose Bisphosphate Carboxylase/ Oxygenase Small Subunit 2 ) gene downstream of the TOPO® Cloning site for ensuring the proper termination of transcript

  **Note:** 3' UTR may contain sequences that regulate translation efficiency, mRNA stability, and polyadenylation signals.
- Hygromycin resistance gene (aph7) driven by the β2-tubulin promoter for selection in *C. reinhardtii*
- Ampicillin resistance gene for selection in *E. coli*
- pUC origin for maintenance in *E. coli*

# How Directional TOPO® Cloning Works

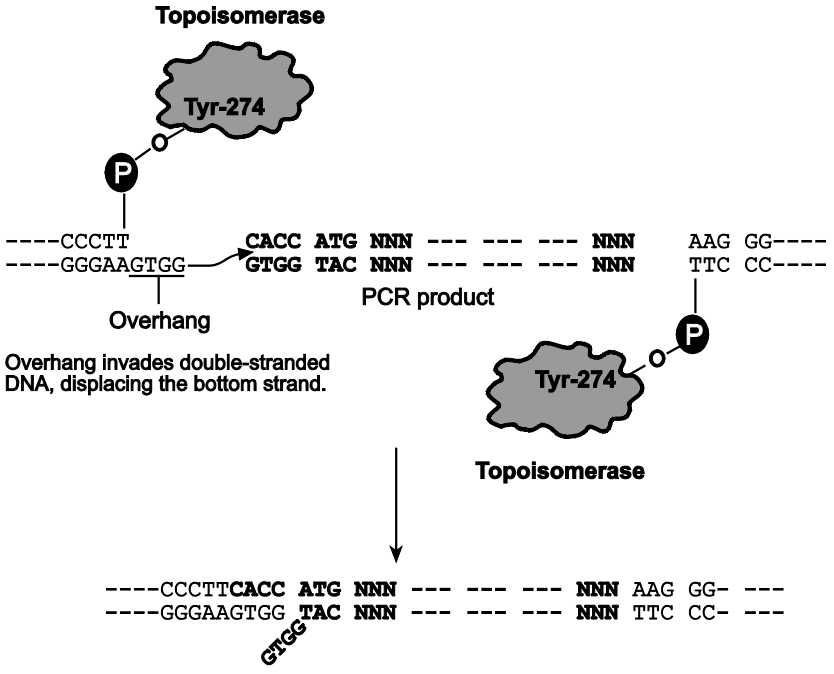
## How Topoisomerase I works

Topoisomerase I from *Vaccinia* virus binds to duplex DNA at specific sites and cleaves the phosphodiester backbone after 5′-CCCTT in one strand (Shuman, 1991). The energy from the broken phosphodiester backbone is conserved by formation of a covalent bond between the 3′ phosphate of the cleaved strand and a tyrosyl residue (Tyr-274) of topoisomerase I. The phospho-tyrosyl bond between the DNA and enzyme can subsequently be attacked by the 5′ hydroxyl of the original cleaved strand, reversing the reaction and releasing topoisomerase (Shuman, 1994). TOPO® Cloning exploits this reaction to efficiently clone PCR products.

## Directional TOPO® Cloning

Directional joining of double-strand DNA using TOPO®-charged oligonucleotides occurs by adding a 3′ single-stranded end (overhang) to the incoming DNA (Cheng & Shuman, 2000). This single-stranded overhang is identical to the 5′ end of the TOPO®-charged DNA fragment. At Life Technologies, this idea has been modified by adding a 4 nucleotide overhang sequence to the TOPO®-charged DNA and adapting it to a 'whole vector' format.

In this system, PCR products are directionally cloned by adding four bases to the forward primer (CACC). The overhang in the cloning vector (GTGG) invades the 5′ end of the PCR product, anneals to the added bases, and stabilizes the PCR product in the correct orientation. Inserts can be cloned in the correct orientation with efficiencies equal to or greater than 90%.



# Experiment Outline

## Workflow

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

| Step | Action | Page |
|---|---|---|
| 1 | PCR amplify your codon optimized gene of interest using the appropriate primers | 9 |
| 2 | TOPO® clone your codon optimized gene of interest into pChlamy_3/D-TOPO® Vector | 12 |
| 3 | Transform One Shot® TOP10 *E. coli* with pChlamy_3/D-TOPO® Vector containing your gene of interest and select the transformants on LB plates containing Ampicillin | 14 |
| 4 | Analyze *E. coli* transformants by restriction digestion or PCR | 15 |
| 5 | Thaw and resuscitate *C. reinhardtii* cells | 17 |
| 6 | Transform *C. reinhardtii* cells by electroporation and select transformants | 18 |
| 7 | Screen *C. reinhardtii* transformants by colony PCR for full integration of your gene of interest | 21 |

# PCR Amplifying the Gene of Interest

## Design PCR primers

The pChlamy_3/D-TOPO® is a directional TOPO® cloning vector. Therefore, it is critical that the PCR primers to amplify your gene of interest contain the sequences required for directional cloning and expression. Consider the following when designing your PCR primers:

- You do not need to add the initiation ATG to your PCR primer, because the pChlamy_3/D-TOPO® vector contains the initiation ATG codon at position 4351–4353 (Vector ATG), which is properly spaced to ensure optimal translation.
- To enable directional cloning, the forward PCR primer **must** contain the sequence CACC at the 5′ end of the primer (see **Example 1** below). The 4 nucleotides, CACC, base pair with the overhang sequence, GTGG, in the pChlamy_3/D-TOPO® vector.

  **Note:** The first three base pairs of the PCR product following the 5′ CACC overhang will constitute a functional codon.
- To ensure that your PCR product is cloned directionally with high efficiency, the reverse PCR primer **must not** be complementary to the overhang sequence GTGG at the 5′ end. A one base pair mismatch can reduce the directional cloning efficiency from 90% to 50% and increase the likelihood of your ORF cloning in the opposite orientation (see **Example 2** on page 10). We have not observed evidence of PCR products cloning in the opposite orientation from a two base pair mismatch.
- 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.
- When synthesizing PCR primers, **do not** add 5′ phosphates to the primers, because 5′ phosphates prevent the synthesized PCR product from ligating into the pChlamy_3/D-TOPO® vector.
- We recommend that you gel-purify your oligonucleotides, especially if they are long (>30 nucleotides).

For a diagram of the TOPO® Cloning site of the pChlamy_3/D-TOPO® vector, refer to page 10. For more information on directional TOPO® cloning, refer to our website at **www.lifetechnologies.com**.

## Example 1: forward primer design

Below is the DNA sequence of the N-terminus of a theoretical protein and the proposed sequence for your forward PCR primer:

**DNA sequence:** `5′-GTA GGA TCT GAT AAA`

**Proposed Forward PCR primer:** `5′-C ACC GTA GGA TCT GAT AAA`

*Continued on next page*

### Example 2: reverse primer design

Below is the sequence of the C-terminus of a theoretical protein. You want to fuse the protein in frame with a C-terminal tag. The stop codon is underlined.

**DNA sequence:** **AAG TCG GAG CAC TCG ACG ACG GTG TAG-3′**

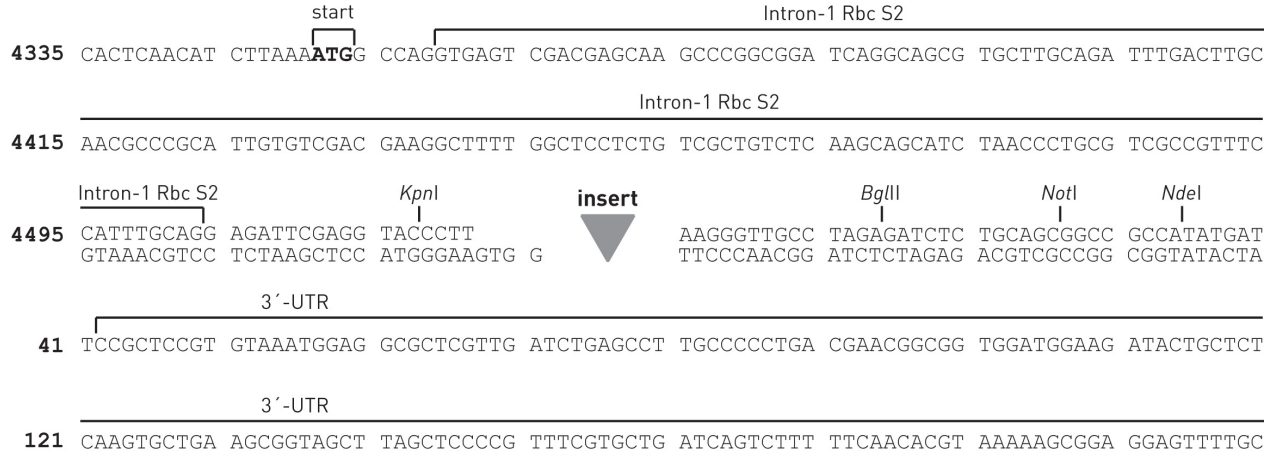
One solution is to design the reverse PCR primer to start with the codon just upstream of the stop codon, but the last two codons contain GTGG (underlined below), which is identical to the 4-bp overhang sequence. As a result, the reverse primer will be complementary to the 4-bp overhang sequence, increasing the probability that the PCR product will clone in the opposite orientation. You want to avoid this situation.

**DNA sequence:** **AAG TCG GAG CAC TCG ACG ACG GTG TAG-3′**
**Proposed Reverse PCR primer sequence:** **TG AGC TGC TGC CAC AAA-5′**

Another solution is to design the reverse primer so that it hybridizes just downstream of the stop codon, but still includes the C-terminus of the ORF. Note that you need to replace the stop codon with a codon for an innocuous amino acid such as glycine, alanine, or lysine.

### TOPO® Cloning Site

The diagram below depicts the TOPO® cloning site of pChlamy_3/D-TOPO® and adjacent sequences. Restriction sites are labeled to indicate the cleavage site. The ATG initiation codon is shown in bold. Use this diagram to design suitable PCR primers to clone and express your PCR product in pChlamy_3/D-TOPO®. The vector sequence of pChlamy_3/D-TOPO® is available for downloading at **www.lifetechnologies.com** or by contacting Technical Support (page 29).




Note that the Intron-1 Rbc S2 (bases 4359–4503) is spliced out from the mature RNA and does not constitute actual codons. The reading frame after the removal of Intron-1 Rbc S2 is:


*Continued on next page*

### Producing blunt-end PCR products

After you have decided on a PCR strategy and have synthesized the primers, produce your blunt-end PCR product using any thermostable, proofreading polymerase. We recommend Platinum® *Pfx* DNA Polymerase, AccuPrime™ *Pfx* DNA Polymerase, or *Pfx50*™ DNA Polymerase, available separately from Life Technologies (see page 27 for ordering information).

Follow the guidelines below to set up a 25 µL or 50 µL PCR for producing your blunt-end PCR product.

- Follow the instructions and recommendations provided by the manufacturer of your thermostable, proofreading polymerase to produce blunt-end PCR products.
- Use the cycling parameters suitable for your primers and template. Make sure to optimize PCR conditions to produce a single, discrete PCR product.
- Use a 7 to 30 minute final extension to ensure that all PCR products are completely extended.
- After cycling, place the tube on ice or store at –20ºC for up to 2 weeks. Proceed to **Checking the PCR Product**, below.

### Checking the PCR product

After you have produced your blunt-end PCR product, use agarose gel electrophoresis to verify the quality and quantity of your PCR product. Check for the following outcomes below.

- Be sure you have a single, discrete band of the correct size. If you do not have a single, discrete band, follow the manufacturer's recommendations to optimize your PCR with the polymerase of your choice. Alternatively, gel-purify the desired product.
- Estimate the concentration of your PCR product. Use this information when setting up your TOPO® Cloning reaction (see **Amount of PCR Product to Use in the TOPO® Cloning Reaction**, page 12 for details).

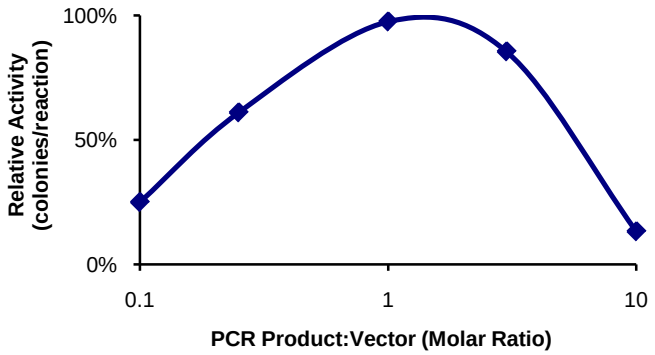
# Performing the TOPO® Cloning Reaction

## Introduction

Once you have produced the desired PCR product, you are ready to TOPO® clone it into the pChlamy_3/D-TOPO® vector and transform the recombinant vector into One Shot® TOP10 *E. coli*. It is important to have everything you need set up and ready to use to ensure best results. We suggest that you read this section and the section entitled **Transforming One Shot® TOP10 Competent *E. coli* Cells** (page 14) before beginning. If this is the first time you have TOPO® cloned, perform the control reactions in parallel with your samples.

## Amount of PCR product to use in the TOPO® cloning reaction

When performing directional TOPO® cloning, we have found that the molar ratio of PCR product:TOPO® vector used in the reaction is critical to its success. **To obtain the highest TOPO® cloning efficiency, use a 0.5:1 to 2:1 molar ratio of PCR product:TOPO® vector** (see figure below). Note that the TOPO® cloning efficiency decreases significantly if the ratio of PCR product: TOPO® vector is <0.1:1 or >5:1. These results are generally obtained if too little PCR product is used (i.e., PCR product is too dilute) or if too much PCR product is used in the TOPO® cloning reaction. If you have quantitated the yield of your PCR product, you may need to adjust the concentration of your PCR product before proceeding to TOPO® cloning.




## Using Salt Solution in the TOPO® cloning reaction

Perform TOPO® cloning in a reaction buffer containing salt (i.e., using the stock salt solution provided in the kit). Note that the amount of salt added to the TOPO® cloning reaction varies depending on whether you plan to transform chemically competent cells (provided) or electrocompetent cells (see page 27 for ordering information).

- If you are transforming chemically competent *E. coli*, use the stock Salt Solution as supplied and set up the TOPO® cloning reaction as directed below.
- If you are transforming electrocompetent *E. coli*, **reduce** the amount of salt in the TOPO® cloning reaction to 50 mM NaCl, 2.5 mM $MgCl_2$ to prevent arcing during electroporation. Dilute the stock Salt Solution 4-fold with water to prepare a 300 mM NaCl, 15 mM $MgCl_2$ Dilute Salt Solution. Use the Dilute Salt Solution to set up the TOPO® cloning reaction as directed on the page 13.

*Continued on next page*

## TOPO® cloning reaction

Use the procedure below to perform the TOPO® cloning reaction. Set up the TOPO® cloning reaction depending on whether you plan to transform chemically competent *E. coli* or electrocompetent *E. coli.* **For optimal results, use a 0.5:1 to 2:1 molar ratio of PCR product:TOPO® vector in your TOPO® Cloning reaction.**

**Note:** The blue color of the TOPO® vector solution is normal and is used to visualize the solution.

| Reagents* | Chemically Competent *E. coli* | Electrocompetent *E. coli* |
|---|---|---|
| Fresh PCR product | 0.5 to 4 µL | 0.5 to 4 µL |
| Salt Solution | 1 µL | — |
| Dilute Salt Solution (1:4) | — | 1 µL |
| Sterile Water | add to a final volume of 5 µL | add to a final volume of 5 µL |
| TOPO® vector | 1 µL | 1 µL |
| Final volume | 6 µL | 6 µL |

*Store all reagents at –20°C when finished. Store salt solutions and water at room temperature or 4°C.

1. Mix reaction gently and incubate for 5 minutes at room temperature (22–23°C).

   **Note:** For most applications, 5 minutes yields plenty of colonies for analysis. Depending on your needs, you can vary the length of the TOPO® cloning reaction from 30 seconds to 30 minutes. For routine subcloning of PCR products, 30 seconds may be sufficient. For large PCR products (> 1 kb) or if you are TOPO® cloning a pool of PCR products, increasing the reaction time may yield more colonies.

2. Place the reaction on ice and proceed to **Transforming One Shot® TOP10 Competent *E. coli* Cells**, page 14.

   **Note:** You may store the TOPO® cloning reaction at –20°C overnight.

## *E. coli* transformation method

You may use any method of your choice for *E. coli* transformation. Chemical transformation is the most convenient for most researchers. Electroporation is the most efficient and the method of choice for large plasmids. For your convenience, a protocol for chemical transformation using One Shot® TOP10 Chemically Competent *E. coli* is provided on page 14; however, you may also transform electrocompetent cells.

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

## Introduction

Once you have performed the TOPO® cloning reaction, you will transform your pChlamy_3/D-TOPO® construct into competent *E. coli*. One Shot® TOP10 Chemically Competent *E. coli* are included with the kit to facilitate transformation; however, you may also transform electrocompetent cells. This section provides protocols to transform chemically competent *E. coli*.

## Materials needed

- TOPO® Cloning reaction (from Step 2, page 13)
- One Shot® TOP10 Chemically Competent *E. coli* (supplied with the kit, Box 3)
- S.O.C. Medium (supplied with the kit, Box 3)
- pUC19 positive control (supplied with the kit, Box 3; recommended for verifying transformation efficiency)
- 42°C water bath
- LB plates containing 100 µg/mL ampicillin (two for each transformation)
- 37°C shaking and non-shaking incubator

## Preparing for transformation

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

1. Equilibrate a water bath to 42°C (for chemical transformation) or set up your electroporator if you are using electrocompetent *E. coli*.
2. Warm the vial of S.O.C. medium from Box 3 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 cells from Box 3 for each transformation.

## One Shot® chemical transformation protocol

1. Add 2 µL of the TOPO® cloning reaction (from Step 2, page 13) 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. An efficient TOPO® Cloning reaction may produce several hundred colonies. Pick 5–10 colonies for analysis (see **Analyzing *E. coli* Transformants**, page 15).

# Analyzing *E. coli* Transformants

## Picking positive *E. coli* clones

1. Pick 5–10 colonies and culture them overnight in LB medium containing 100 µg/mL ampicillin.
2. Isolate plasmid DNA using your method of choice. If you need ultra-pure plasmid DNA for automated or manual sequencing, we recommend using the PureLink® HQ Mini Plasmid Purification Kit (Cat. no. K2100-01; see page 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 transformants using PCR. For PCR primers, use a combination of the "Forward Sequencing Primer pChlamy_3 vector" or the "Reverse Sequencing Primer pChlamy_3 vector" (from Box 2) and a primer that hybridizes within your insert. You will have to determine the amplification conditions. If you are using this technique for the first time, we recommend performing restriction analysis in parallel. Artifacts may be obtained because of mispriming or contaminating template.

**Materials Needed:**

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

**Procedure:**

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

## Analyzing *E. coli* transformants by sequencing

Once you have identified the correct clone(s), you may sequence your construct to confirm that your gene is cloned in the correct orientation. Use the "Forward Sequencing Primer pChlamy_3 vector" or the "Reverse Sequencing Primer pChlamy_3 vector" (from Box 2) to help you sequence your insert. For the complete sequence of the pChlamy_3/D-TOPO® 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 20–25°C and can tolerate temperatures as low as 15°C and as high as 35°C. The strain in this kit (*C. reinhardtii* 137c) should be grown at 26°C under continuous illumination using moderate light intensities of cool fluorescent white light (50 ± 10 µE $m^{-2}$ $s^{-1}$) with constant agitation on a gyrotary shaker set to 100–150 rpm.
- The optimal equipment for culturing *C. reinhardtii* is an algal growth chamber (e.g., Percival Algal Chamber from Geneva Scientific) with regulatable light supply and a light meter (e.g., LI-250A Light Meter from LI-COR®) to guide adjustments. If an algal growth chamber is not available, the cells can be grown in a standard cell culture incubator illuminated with cool fluorescent lights placed within 12 inches of the culture plates. Standard room lights provide sub-optimal growth conditions.
- Phototrophic cultures should be supplied with $CO_2$ (5% in air) for maximal growth, although the *C. reinhardtii* 137c strain included in the kit can grow in the incubator without the need of additional $CO_2$ supply.
- Flasks for liquid culture can be stoppered with sterile foam plugs, polypropylene caps, alminium foil, cotton, or any cap that allows air exchange.
- After transformation and plating, do not stack the culture plates to allow continuous uniform illumination.
- *C. reinhardtii* is classified as a GRAS (generally regarded as safe) organism with no known viral or bacterial pathogens. However, we recommend following general safety guidelines under Biosafety Level 1 (BL-1) containment, similar to working with *E. coli* or yeast. For more information on BL-1 guidelines, refer to *Biosafety in Microbiological and Biomedical Laboratories,* 5th ed., published by the Centers for Disease Control, which isavailable for downloading at: **www.cdc.gov/od/ohs/biosfty/bmbl5/bmbl5toc.htm**.

# Thawing *Chlamydomonas reinhardtii*

## Materials needed

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

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

## Thawing procedure

1. Remove the frozen cells from –80°C storage and immediately place them in a dry ice container. Bury the vial(s) containing the cells in dry ice to minimize temperature fluctuations before thawing.
2. Add 4 mL of Gibco® TAP medium, pre-warmed to room temperature, into each well of a 6-well plate.
3. Remove the cryovial containing the frozen cells from the dry ice storage and **immediately** place it into a 35°C water bath.
4. Quickly thaw the cells by gently swirling the vial in the 35°C water bath until the cell have completely thawed (1–2 minutes).
5. Before opening, wipe the outside of the vial with 70% ethanol.
6. Transfer 230 µL of thawed cells from the vial into each well of the 6-well plate containing 4 mL of Gibco® TAP medium.
7. Place the 6-well plate(s) in the algal growth chamber set to 26°C and 50 µE $m^{-2}$ $s^{-1}$. **Do not stack the plates.**
8. Incubate the cells for 3–6 days with agitation on a rotary shaker set to 110 rpm.
9. On Day 3, measure the optical density of the cells at 750 nm ($OD_{750}$) in Gibco® TAP medium. If the culture has not yet reached $OD_{750}$ 0.6, return it to the algal growth chamber and continue the incubation. Check the $OD_{750}$ of the culture daily until it reaches $OD_{750}$ 0.6. Once the $OD_{750}$ has reached 0.6, proceed to step 10.
10. Add 40 mL of fresh Gibco® TAP medium, pre-warmed to room temperature, in a 125-mL flask.
11. Dilute the cells from the 6-well plate(s) into the flask containing the Gibco® TAP medium to obtain a **final $OD_{750}$ of 0.06.**
12. Place the culture(s) in the algal growth chamber set to 26°C and 50 µE $m^{-2}$ $s^{-1}$.
13. Grow the cultures for 20–24 hours with agitation on a gyrotary shaker set to 110 rpm and proceed to transformation (page 18). Do not exceed a culture time of 24 hours because transformation efficiency drops rapidly as cells reach saturating densities ($OD_{750}$>1).

# Transforming *Chlamydomonas reinhardtii* by Electroporation

## Guidelines for transforming *C. reinhardtii*

- Perform all steps of the electroporation procedure at room temperature.
- Nuclear transformation of *C. reinhardtii* can be achieved with circular DNA; however, transformation with linearized DNA is much more efficient (~70%). We recommend using *Sca*I restriction enzyme for linearization, provided that the insert does not contain the recognition sequence for *Sca*I.
- The number of insertions into the *C. reinhardtii* genome is also influenced by the amount of DNA used. We recommend using 2 µg of linearized plasmid DNA per electroporation.
- The quality and the concentration of DNA used play a central role for the efficiency of transformation. Use a commercial kit such as the PureLink® HQ Mini Plasmid Purification or the PureLink® HiPure Plasmid Miniprep kit that delivers pure DNA.
- For best results, grow the cells to $OD_{750}$ 0.3–0.5 before proceeding with electroporation.
- Insertion of the plasmid DNA into the genome occurs randomly. On average only 20% of transformants will express the gene of interest at appreciable levels. We recommend first screening the colonies by colony PCR (see page 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_3/D-TOPO® construct containing your gene of interest and linearized with the appropriate restriction enzyme

  **Note:** We recommend using *Sca*I restriction enzyme for linearization, provided that the insert does not contain the recognition sequence for *Sca*I.
- pChlamy_2/Control Vector, linearized with *Sca*I restriction enzyme
- Gibco® TAP medium (Cat. no. A13798-01 or A13798-02), pre-warmed to room temperature
- TAP-40 mM sucrose solution, pre-warmed to room temperature (see page 23 for recipe)
- TAP-Agar-Hygromycin plates (10 µg/mL) (see page 23 for recipe)
- Sterile 15-mL and 50-mL centrifugation tubes
- 0.4-cm electroporation cuvettes (Cat. no. P460-50)
- Electroporation device such the Bio-Rad® Gene Pulser® II

  *Optional:* Alternatively, you can use the Neon® Transfection System (Cat. no. MPK5000) or the Neon® Transfection System 100 µL Kit (Cat. no. MPK10025).
- ColiRollers™ plating glass beads (Novagen, Cat. no. 71013)

*Continued on next page*

# Transforming *Chlamydomonas reinhardtii* by Electroporation, continued

## Electroporation procedure

If using an electroporation device such as the Bio-Rad® Gene Pulser® II, follow the protocol below. If using the Neon® Transfection System, first read the **Notes for using the Neon® Transfection System**, page 20, and adjust the electroporation conditions accordingly.

1. Measure the optical density of the *C. reinhardtii* cultures (from Step 14, page 17) at 750 nm (i.e., $OD_{750}$).
   **Note:** For best performance, the $OD_{750}$ of cultures should be between 0.3–0.5. If the $OD_{750}$ does not reach 0.3 within 24 hours of incubation after dilution (Steps 12–14, page 17), incubate the cells for an extra 3–5 hours to allow for an additional cell division.
2. Harvest 15 mL of the cells (per transformation) by centrifugation at 2,500 rpm for 10 minutes at room temperature. Centrifuge the cells in 15-mL conical tubes to obtain tight pellets.
3. Discard the supernatant by decanting. Remove the remaining supernatant using a pipette.
4. Resuspend the cells in 250 µL of TAP-40 mM sucrose solution at room temperature by gently pipetting up and down.
5. Add 2 µg linearized plasmid DNA (i.e., pChlamy_3/D-TOPO® construct containing your gene of interest) into the resuspended cells. Mix the DNA-cell suspension gently by flicking the tube. In a separate tube, prepare a control transformation with the pChlamy_2/Control Vector, linearized using *Sca*I restriction enzyme.
6. Transfer 250 µL of the transformation mixture into an electroporation cuvette and incubate at room temperature for 5 minutes.
7. While the transformation mixtures are incubating, add 5 mL of TAP-40 mM sucrose solution at room temperature into each well of a 6-well plate.
   **Note:** You will divide each transformation mixture between two wells of a 6-well plate after electroporation, so that the cells in each transformation mixture will recover in 10 mL of TAP-40 mM sucrose solution total.
8. Set the electroporation parameters as follows:

| Voltage | Capacity | Resistance |
|---|---|---|
| 600 V | 50 µF | infinity |

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

*Continued on next page*

# Transforming *Chlamydomonas reinhardtii* by Electroporation, continued

## Electroporation procedure, continued

12. Place the 6-well plate in the algal growth chamber set to 26°C and 50 µE $m^{-2}$ $s^{-1}$.
13. Incubate the cells for 24 hours with gentle agitation (100–150 rpm) to let them recover.
14. Centrifuge the cells at 2,500 rpm for 10 minutes at room temperature.
15. Discard the supernatant by decanting. Remove the remaining supernatant with a pipette.
16. Resuspend the cells with gentle pipetting in 150 µL of TAP-40 mM sucrose solution at room temperature.
17. Plate the entire cell solution from each transformation on one TAP-agar-Hygromycin plate using disposable cell spreaders or glass plating beads to spread the cells evenly. Make sure the plates do not have condensation on them.
18. Place the plates agar side at the bottom in the algal growth chamber set to 26°C and 50 µE $m^{-2}$ $s^{-1}$. Do not stack the plates to ensure continuous and even illumination.
19. Incubate the plates for 5 days or until *C. reinhardtii* colonies are clearly visible. Control vector should produce a minimum of 30 transformants per electroporation reaction. The transformation efficiency with the pChlamy_3/D-TOPO® construct will depend on the nature, size, and codon content of the gene of interest, and the physiological state of the cells.
20. Proceed to determination of integration by colony PCR (see page 21) before selecting clones for further scale-up.
21. About 20% of the colonies should be positive for the gene of interest. Due to random integration and silencing events in *C. reinhardtii*, we recommend picking at least 10 positive colonies and testing them for the expression level of the gene of interest by RT-PCR (or Western blotting, if you have the antibody to detect it).

## Notes for using the Neon® Transfection System

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

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

| Voltage | Pulse width | Pulse number |
|---|---|---|
| 2300 V | 13 ms | 3 |

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

# Screening for Integration by Colony PCR

## Introduction

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

## Materials needed

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

## Preparing cell lysates

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

## Colony PCR procedure

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

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

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

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

# Storage and Scale-Up

## Storing *C. reinhardtii* transformants

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

## Growing and scaling-up *C. reinhardtii* transformants

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

# Appendix A: Support Protocols

## Preparing Reagents and Media

### Handling Hygromycin B

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

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

### TAP-40 mM sucrose solution

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

### TAP-Hygromycin B solution

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

### TAP-Agar plates

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

### TAP-Agar-Hygromycin B plates

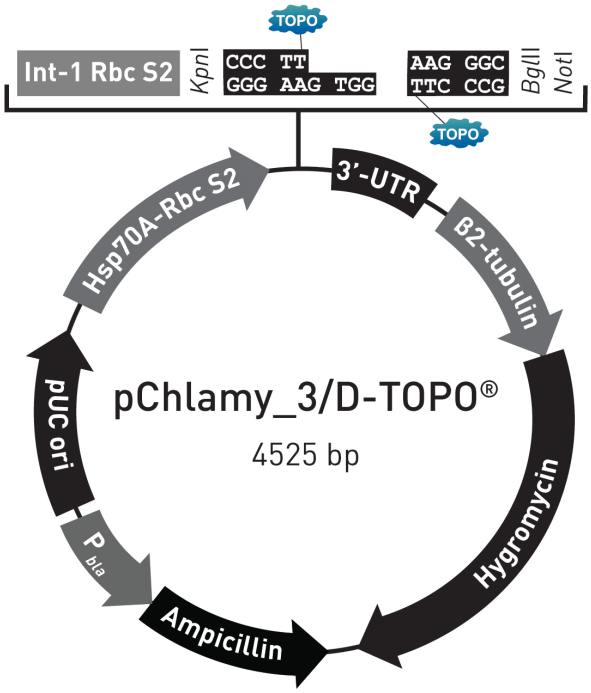
1. Add 15 g of agar to 200 mL of Gibco® TAP medium in an autoclaveable flask.
2. Autoclave on liquid cycle for 20 minutes.
3. Warm 800 mL of Gibco® TAP medium to 55–60°C in a water bath
4. After autoclaving, cool the agar containing flask to ~55°C.
5. Combine the agar containing flask with 800 mL of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

# Appendix B: Vectors

## Map and Features of pChlamy_3/D-TOPO® Vector

### Map of pChlamy_3/D-TOPO® Vector

The map below shows the features of pChlamy_3/D-TOPO®. The complete sequence of the vector is available for downloading at **www.lifetechnologies.com** or from Technical Support (page 29).




**Features of pChlamy_3/D-TOPO® Vector**

4525 nucleotides

| | |
|---|---|
| TOPO® binding site 1: | 1–5 |
| 3´-UTR: | 42–275 |
| β2-tubulin promoter: | 282–594 |
| Hygromycin resistance gene: | 595–1905 |
| Ampicillin resistance gene: | 2104–2964 (c)* |
| *bla* promoter ($P_{bla}$): | 2965–3016 (c) |
| pUC origin: | 3062–3735 |
| Hsp70A-Rbc S2 promoter: | 3855–4350 |
| Intron-1 Rbc S2: | 4359–4503 |
| TOPO® binding site 2: | 4517–4521 |
| GTGG overhang: | 4522–4525 |

*(c): complementary strand

*Continued on next page*

# Map and Features of pChlamy_3/D-TOPO®, continued

## Features of pChlamy_3/ D-TOPO® Vector

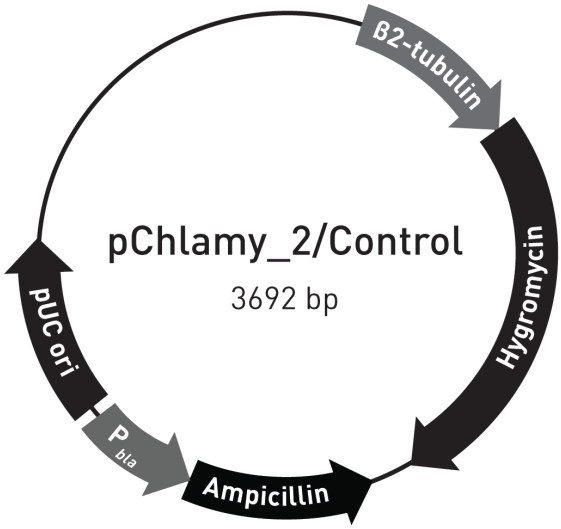
The pChlamy_3/D-TOPO® vector contains the following elements. All features have been functionally tested.

| Feature | Benefit |
|---|---|
| TOPO® binding sites (directional) | Allows rapid cloning of your PCR product for expression in *C. reinhardtii.* |
| 3′ UTR from RbcS2 gene | Assures the proper termination of transcript; 3′ UTR may contain sequences that regulate translation efficiency, mRNA stability, and polyadenylation signals. |
| β2-tubulin promoter | Strong native *C. reinhardtii* promoter driving the expression of Aph7 gene (Berthold *et al.*, 2002; Davies & Grossman, 1994). |
| Hygromycin resistance gene (*aph7*) | *Streptomyces hygroscopicus* aminoglycoside phosphotransferase gene; confers resistance to hygromycin and allows selection in *C. reinhardtii* (Berthold *et al.*, 2002). |
| Ampicillin resistance gene (*bla*) | Allows selection of the plasmid in *E. coli*. |
| *bla* promoter | Allows expression of the Ampicillin resistance gene. |
| pUC origin | Allows high-copy replication and growth in *E. coli*. |
| Hsp70A-Rbc S2 promoter | A hybrid constitutive promoter consisting of Hsp70 and RbcS2 promoters. |
| Intron-1 Rbc S2 | First intron of the small subunit of the ribulose bisphosphate carboxylase (rbcS2), necessary to maintain the high expression of your gene of interest. |

# Map of pChlamy_2/Control Vector

## pChlamy_2/Control Vector

The map below shows the features of pChlamy_2/Control Vector. The complete sequence of the vector is available for downloading at **www.lifetechnologies.com** or from Technical Support (page 29).




**Features of pChlamy_2/Control Vector**

3692 nucleotides

| | |
|---|---|
| Ampicillin resistance gene (*bla*): | 298–1158 (c)* |
| *bla* promoter ($P_{bla}$): | 1159–1203 (c) |
| pUC origin: | 1256–1929 |
| ß2-tubulin promoter: | 2069–2382 |
| Hygromycin resistance (*aph*7): | 2383–3528 |

*(c): complementary strand

# Appendix C: Ordering Information

## Accessory Products

### Proofreading DNA polymerases

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

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

### Competent cells

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

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

### Additional products

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

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

*Continued on next page*

## Other GeneArt® Algal Kits

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

| Product | Quantity | Cat. no. |
|---|---|---|
| GeneArt® *Chlamydomonas* Engineering Kit | 1 kit | A14258 |
| GeneArt® *Chlamydomonas* Engineering Kit with 6 L media | 1 kit | A14262 |
| GeneArt® Synechococcus Engineering Kit | 1 kit | A14259 |
| GeneArt® Synechococcus Engineering Kit with 6 L media | 1 kit | A14263 |
| GeneArt® Synechococcus TOPO® Engineering Kit | 1 kit | A14261 |
| GeneArt® Synechococcus TOPO® Engineering Kit with 6 L media | 1 kit | A14265 |

# Documentation and Support

## Obtaining Support

### Technical Support

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

At the website, you can:

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

### Safety Data Sheets (SDS)

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

### Limited Product Warranty

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

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