

# GeneArt® MAX Efficiency® Transformation Reagent for Algae

## Description

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 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.

Product	Catalog No.	Amount	Storage	Shelf Life*
GeneArt® MAX Efficiency® Transformation Reagent	A24229	250 mL	Store at 2°C to 8°C.	6 months

\* Shelf Life duration is determined from Date of Manufacture.

## Product Use

For Research Use Only. Not for use in diagnostic procedures.

## Safety Information

Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

## Culture Conditions for *C. reinhardtii*

**Media:** Gibco® TAP medium (Cat. no. A13798)

**Culture Type:** 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.

**Temperature Range:** Optimum temperature for the growth of *C. reinhardtii* 137c is 26°C, but *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.

**Incubation Conditions:** Phototrophic cultures should be supplied with 5% CO<sub>2</sub> for maximal growth and incubated under continuous illumination using moderate light intensities of cool fluorescent white light (50 ± 10 µE m<sup>-2</sup> s<sup>-1</sup>) with constant agitation on a gyrotary shaker set to 100–150 rpm. However, the *C. reinhardtii* 137c strain can grow in the incubator without the need of additional CO<sub>2</sub> supply. Ensure that proper gas exchange is achieved in culture vessels. After transformation and plating, do not stack the culture plates to allow continuous uniform illumination.

**Recommended Equipment:** 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.

## Guidelines for transforming *C. reinhardtii*

- Nuclear transformation of *C. reinhardtii* can be achieved with circular DNA; however, transformation with linearized DNA is much more efficient. To get the maximum number of colonies, we recommend linearizing your vector. If you are transforming with pChlmy vectors from Life Technologies, we recommend using *ScaI* restriction enzyme for linearization, provided that the insert does not contain the recognition sequence for *ScaI*. Otherwise, you can choose from *PvuI*, *SspI*, or *FspI*.
- 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.

- For best results, grow the cells to 1 × 10<sup>6</sup>–2 × 10<sup>6</sup> cells/mL before proceeding with electroporation. You may use <1 × 10<sup>6</sup> cells/mL, but the concentration should not exceed 3 × 10<sup>6</sup> cells/mL.
- Insertion of the plasmid DNA into the genome occurs randomly. As an optional step, you may first screen the colonies by colony PCR 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

- pChlmy vector construct containing your gene of interest and linearized with the appropriate restriction enzyme
- Gibco® TAP medium (Cat. no. A13798), pre-warmed to room temperature
- TAP-40 mM sucrose solution, pre-warmed to room temperature
- Selective TAP-Agar plates: TAP-Agar-Hygromycin plates (10 µg/mL) or TAP-Agar-Zeocin™ plates (5 µg/mL)
- Sterile 15-mL and 50-mL centrifugation tubes
- 0.4-cm electroporation cuvettes (Cat. no. P460-50), chilled on ice
- Electroporation device such as 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)

## Prepare Media and Reagents

### TAP-40 mM sucrose solution

- 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.
- To prepare the TAP-40 mM sucrose solution, add 44 mL of 1 M sucrose to 1 L of Gibco® TAP medium.

### Selective TAP-Agar plates

- Add 15 g of agar to 200 mL of Gibco® TAP medium and autoclave on liquid cycle for 20 minutes.
- Warm 800 mL of Gibco® TAP medium to 55–60°C in a water bath.
- After autoclaving, cool the agar containing flask to ~55°C.
- Combine the agar containing flask with 800 mL of Gibco® TAP medium, add hygromycin B to a final concentration of 10 µg/mL or Zeocin™ to a final concentration of 5 µg/mL, and pour into 10 cm plates.
- Let the plates harden (do **not** overdry), invert them, and store at 4°C in the dark. Final agar concentration will be 1.5%.

## Transform *C. reinhardtii* using BioRad® Gene Pulser® II

- Revive 1 vial of frozen *C. reinhardtii* cells and inoculate into 200 mL of TAP medium. Culture the cells under standard conditions and keep monitoring their concentration for 3 days.
- When cell concentration reaches  $1 \times 10^6$ – $2 \times 10^6$  cells/mL, 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  $<1 \times 10^6$  cells/mL, you may still harvest the cells without significantly affecting the transformation efficiency.
- 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.
- Resuspend the pellet again in 10 mL of GeneArt® MAX Efficiency® Transformation Reagent and centrifuge once more at 2500 rpm for 5 minutes.
- Resuspend the cells in GeneArt® MAX Efficiency® Transformation Reagent to a final concentration of  $2 \times 10^8$ – $3 \times 10^8$  cells/mL.
- Add 2–4 µg of linearized DNA per 250 µL of cell suspension and incubate at 4°C for 5 minutes.
- Set electroporation parameters on the Gene Pulser® II as follows:

Voltage	Capacity	Resistance
500 V	50 µF	800 Ω

- Transfer 250 µL of the cell-DNA mix into an ice-cold cuvette just before electroporation.
- Electroporate the cells using the appropriate settings. Usually, the electro pulse duration is about 30 ms.
- After electroporation, allow the cells to recover on the bench for 15 minutes.
- Transfer the cells into a 50-mL conical tube or flask containing 10 mL of TAP-40 mM sucrose solution at room temperature.
- Place the cells in the algal chamber and incubate for 14–16 hours.
- Harvest the cells centrifugation at 2500 rpm for 5 minutes, discard the supernatant, and resuspend the pellet in 200 µL TAP medium.
- Plate the cells on selective TAP-agar plate, and incubate in the algal chamber for 5–7 days.

**Note:** The colony yield depends on the size of the plasmid DNA, the selection marker, and other factors.

## Transform *C. reinhardtii* using 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](http://www.lifetechnologies.com).

- Harvest the cells as described in Steps 1–3 of the GenePulser® II protocol, and resuspend them in GeneArt® MAX Efficiency® Transformation Reagent to a final concentration of  $1 \times 10^8$ – $3 \times 10^8$  cells/mL.
- Add 1 µg of linearized DNA per 100 µL of cell suspension and incubate at 4°C for 5 minutes.
- 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.

- Set electroporation parameters on the Neon® device as follows:

Voltage	Pulse width	Pulse number
2300V	13 ms	3










- 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.
- Press the **Start** button on the touchscreen to deliver the electric pulse.
- 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.
- Add 4 mL of TAP-40 mM sucrose solution at room temperature to the cells and incubate them in the algal chamber overnight.
- 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.
- Spread 200 µL of the cell suspension on selective TAP-agar plates, and incubate in the algal chamber for 5–7 days.

## Related Products

Product	Cat. No.
GeneArt® <i>Chlamydomonas</i> Protein Expression Kit	A24244
GeneArt® <i>Chlamydomonas</i> Engineering Kit	A14258
GeneArt® <i>Chlamydomonas</i> Engineering Kit with 6L media	A14262
GeneArt® <i>Chlamydomonas</i> TOPO® Engineering Kit	A14260
GeneArt® <i>Chlamydomonas</i> TOPO® Engineering Kit with 6 L media	A14264
Gibco® TAP medium	A13798
Electroporation cuvettes, 0.4 cm	P460-50
PureLink® HQ Mini Plasmid Purification Kit	K2100-01
PureLink® HiPure Plasmid Miniprep Kit	K2100-02
Neon® Transfection System	MPK5000
Neon® Transfection System 100 µL Kit	MPK10025
Hygromycin B Selective Antibiotic	10687-010
Zeocin™ Selection Reagent	R250-01

## Explanation of Symbols and Warnings

The symbols present on the product label are explained below:

				
Batch Code	Catalog number	Manufacturer	Use By:	Read Safety Data Sheet
				
Caution, consult accompanying documents	Temperature Limitation	Sterilized using aseptic processing techniques	Consult instructions for use	

## 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](http://www.lifetechnologies.com/termsandconditions). If you have any questions, please contact Life Technologies at [www.lifetechnologies.com/support](http://www.lifetechnologies.com/support).

## Important Licensing Information

This product may be covered by one or more Limited Use Label Licenses. By use of this product, you accept the terms and conditions of all applicable Limited Use Label Licenses.

For additional technical information such as Safety Data Sheets (SDS), Certificates of Analysis, visit [www.lifetechnologies.com/support](http://www.lifetechnologies.com/support).

For further assistance, email [techsupport@lifetech.com](mailto:techsupport@lifetech.com)

© 2013 Life Technologies Corporation. All rights reserved. The trademarks mentioned herein are the property of Life Technologies Corporation and/or their affiliate(s) or their respective owners.

**DISCLAIMER:** LIFE TECHNOLOGIES CORPORATION AND/OR ITS AFFILIATE(S) DISCLAIM ALL WARRANTIES WITH RESPECT TO THIS DOCUMENT, EXPRESSED OR IMPLIED, INCLUDING BUT NOT LIMITED TO THOSE OF MERCHANTABILITY, FITNESS FOR A PARTICULAR PURPOSE, OR NON-INFRINGEMENT. TO THE EXTENT ALLOWED BY LAW, IN NO EVENT SHALL LIFE TECHNOLOGIES AND/OR ITS AFFILIATE(S) BE LIABLE, WHETHER IN CONTRACT, TORT, WARRANTY, OR UNDER ANY STATUTE OR ON ANY OTHER BASIS FOR SPECIAL, INCIDENTAL, INDIRECT, PUNITIVE, MULTIPLE OR CONSEQUENTIAL DAMAGES IN CONNECTION WITH OR ARISING FROM THIS DOCUMENT, INCLUDING BUT NOT LIMITED TO THE USE THEREOF.

[www.lifetechnologies.com](http://www.lifetechnologies.com)

20 November 2013

**life**  
technologies™