

LIFE TECHNOLOGIES™

SYNTHETIC BIOLOGY



CO₂ sequestration



Nutraceuticals



Fuel

Defined, consistent quality

The only all-in-one solution to simplify algae engineering:
GeneArt® Algae Engineering Kits for rapid production

life
technologies™

Enter a new era of quality research—the first of many synthetic biology innovations

Previously, algae research and production labs relied on poorly characterized, nonoptimized cell stocks and cloning tools for their work. Preparing growth medium was convoluted and time-consuming, and growth rates and yields from the transformed cells were disappointing.

New GeneArt® Algae Engineering Kits for *Chlamydomonas reinhardtii* and *Synechococcus elongatus* are the first commercially available genetic modification and expression systems for photosynthetic microalgae. These kits are designed for rapid scale-up and production and consistent, defined quality.

Our GeneArt® products and solutions are at the forefront of research, bringing you the predictability, adaptability, scalability, and confidence required to unleash the full potential of this promising field.

Discover our optimized specialty systems for *Chlamydomonas reinhardtii* and *Synechococcus elongatus*, offering:

- A simplified approach for genetic engineering and rapid scale-up to production
- Defined quality control and reliable results





Quality algae engineering for reliable, meaningful results

GeneArt® Algae Engineering Kits are designed and manufactured to help ensure that the starting materials are always consistent and the results are reproducible.

- Algal cells arrive ready to resuscitate, grow, and transform or store at -80°C until ready to use
- Every cell lot is manufactured using a standardized manufacturing protocol, so every experiment is controlled from the start
- Optimized media, vectors, and protocols ensure robust selection and expression

Get transformed cells more quickly

GeneArt® Algae Engineering Kits save time required for strain optimization and transitioning to downstream applications such as bioproduction (Figure 1).

- Cells are ready for transformation in less than 5 days
- GeneArt® vectors facilitate rapid directional cloning of synthetic genes or PCR products
- Gibco® media are provided at 1X concentration, eliminating time-consuming media preparation

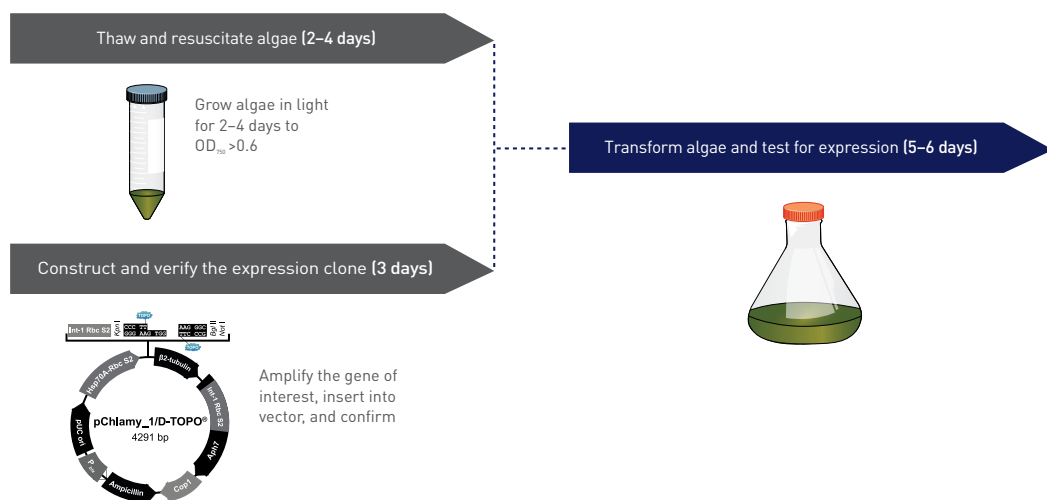
Take control of the quality of your reagents

Don't wonder about the condition and characteristics of your cultures and expression clones. With GeneArt® Algae Engineering Kits, you have access to a quality-controlled supply of algae cells and cloning tools. GeneArt® Algae Engineering Kits:

- Are shipped and stored at -80°C
- Contain viable, pure, and standardized cell stocks
- Are supplied with fully characterized GeneArt[®] vectors that are optimized for engineering and expression in each strain

GeneArt® Algae Engineering workflow is complete in 7–10 days

(compared to 2–3 weeks with nonoptimized lab strains and procedures)



Advantages of the optimized solution for engineered algae

- Cells have high viability, and resuscitate and grow rapidly
- Optimized medium is supplied sterile and ready to use
- Vectors are sequence verified and deliver high-level expression
- Protocols are streamlined for faster results and reliable performance

Figure 1. GeneArt® Algae Engineering Kits offer quality-controlled components and significant process efficiencies for both research and bioproduction environments. Following the optimized workflow, you can typically see transformed cells in less than 10 days.

Two algae for maximum versatility

Chlamydomonas reinhardtii and *Synechococcus elongatus* are model algal organisms for the study of photosynthesis, nutrient regulation, pesticide resistance, lipid metabolism, and more. They also serve as bioproduction platforms for biofuels, nutraceuticals, and specialty chemicals.



Chlamydomonas reinhardtii

- Model alga (strain 137C) for studying photosynthesis, nutrient-related gene expression, flagellar motility, protein expression, and lipid metabolism
- Eukaryote, larger genome size (121 Mb)
- Exhibits rapid growth



Synechococcus elongatus

- Model cyanobacterium (strain PCC 7942) for studying photosynthesis, prokaryotic circadian rhythms, nutrient regulation, environmental response, and lipid metabolism
- Prokaryote, small genome size (2.7 Mb)
- Easy to manipulate

GeneArt® kits include products from brands you know and trust

TOPO® cloning for simplified vector construction

Directional TOPO® cloning enables you to clone your blunt-end PCR products in the correct orientation for expression using a 5-minute ligation reaction.

- 5-minute TOPO® cloning of PCR product
- Clone directly into the pSyn_1/D-TOPO® or pChlamy_1/D-TOPO® vector
- 90% of recombinant clones in the correct orientation for expression
- Screen fewer colonies

lifetechnologies.com/topo



Gibco® media for consistent, high-quality cell culture

Rely on the superior quality of Gibco® media to help you achieve better consistency and efficiency and more reproducible and robust data.

- Gibco® BG-11 and TAP media are conveniently supplied as ready-to-use 1X formulations
- No need to add water and risk contamination or inaccurate concentrations
- Award-winning bottle design is easier to use, helps to reduce the risk of contamination, and improves consistency in your cell culture

lifetechnologies.com/gibco



GeneArt® *Synechococcus* Engineering Kits

The GeneArt® *Synechococcus* Engineering Kits (Figure 2) employ a cyanobacterium-based model system that offers a simplified approach for studying circadian rhythms, nutrient regulation, environmental response, and lipid metabolism. Using the optimized cloning and expression vectors, frozen cells, specially formulated Gibco® medium, and easy-to-follow protocols, growth and transformation of the cyanobacterium *Synechococcus elongatus* is straightforward (Figure 3), which helps to ensure reliable results and faster scale-up.

Characteristics of *S. elongatus*

Synechococcus elongatus is a cyanobacterium—one of the unicellular photosynthetic prokaryotes sometimes referred to as blue-green algae. *S. elongatus* is an excellent model system with the following features:

- Fully sequenced and annotated genome (strain PCC 7942)
- Easily manipulated by transformation or conjugation from *E. coli*
- Small genome size, ~2.7 Mb
- Neutral cloning loci can be disrupted with no aberrant phenotype, allowing homologous recombination in the cell's chromosome
- Can be engineered to produce and secrete high-value products (*Appl Environ Microbiol* 76:3462 (2010))

Transformation of *S. elongatus*

Transformation of *S. elongatus* relies on homologous recombination between the cell's chromosome and exogenous, nonautonomously replicating DNA containing sequences homologous to the chromosome. Sites on the *S. elongatus* chromosome have been developed as cloning loci, called “neutral sites.” They can be disrupted without causing any aberrant phenotype, thus allowing the homologous recombination of ectopic sequences. When *S. elongatus* is transformed with a vector containing an antibiotic resistance cassette and neutral site sequences, homologous recombination occurs between the neutral site vector and the *S. elongatus* chromosome; the transgene and the selective marker are inserted into the neutral site and the vector sequence is lost, allowing the expression of the introduced genes in *S. elongatus*.

S. elongatus cloning vectors

We have developed two *S. elongatus* expression vectors: pSyn_1, which contains a standard multiple cloning site (MCS), and pSyn_1/D-TOPO®, which allows you to carry out rapid and efficient directional cloning of a blunt-end PCR product using our proven TOPO® cloning technology. Our *S. elongatus* vectors contain:

- Multiple cloning site (standard MCS or TOPO® cloning site)
- Nickel promoter for regulated expression of the gene of interest
- NS1 (neutral site 1) for integration of the vector into the *S. elongatus* genome
- Spectinomycin resistance gene for selection in *E. coli* and *S. elongatus*
- pUC origin for maintenance in *E. coli*

Engineered *S. elongatus* strains deliver improved production

Our manufactured *S. elongatus* cell lots have very good transformation efficiencies, typically around 5×10^4 per microgram of DNA. To test the expression obtained using the GeneArt® *Synechococcus* Engineering Kit, we created a lactate-producing strain of *S. elongatus*. After 5 days, lactate production in the engineered strain was dramatically improved compared to the untransformed strain (Figure 4).

Figure 2. GeneArt® Synechococcus Engineering Kits. All kits come with algal cells and an expression vector (either pSyn_1/D-TOPO® or pSyn_1). You can also choose kits that include One Shot® TOP10 *E. coli* competent cells and Gibco® BG-11 Growth Medium. See the ordering information for details.

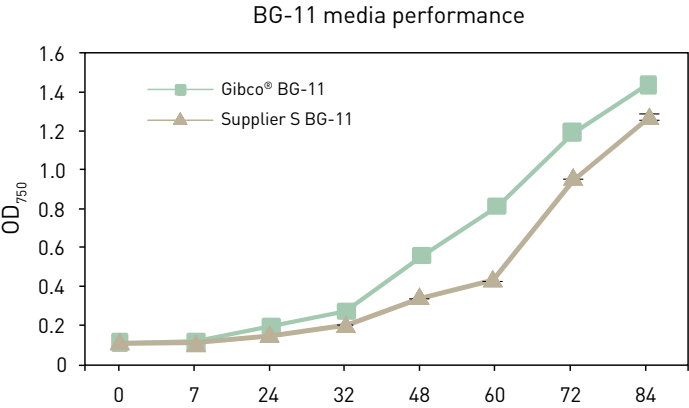


Figure 3. Faster growth of *S. elongatus* cells in Gibco® BG-11 Growth Medium compared to the competition. Frozen *S. elongatus* cells were resuscitated in Gibco® BG-11 Growth Medium and medium from competitor S in triplicate samples. Growth was monitored by OD₇₅₀ measurements taken periodically during the study.

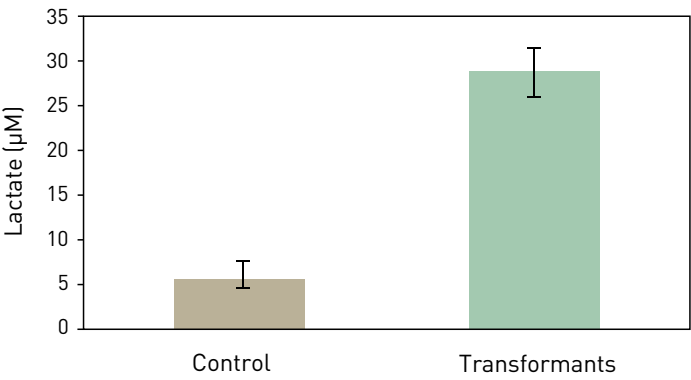


Figure 4. Lactate production in *S. elongatus* engineered with GeneArt® Algae Engineering Kits. A strain of *S. elongatus* already expressing the *ldh1* gene was transformed with the pSyn_1/D-TOPO® vector containing the *ldhA* gene. Transformants were selected on spectinomycin and grown in 4 mL of Gibco® BG-11 Growth Medium for 6 days. Lactate production was measured by an enzymatic assay.

GeneArt® *Chlamydomonas* Engineering Kits

The GeneArt® *Chlamydomonas* Engineering Kits (Figure 5) employ a eukaryotic protein expression system, based on the green alga *Chlamydomonas reinhardtii*, that offers a simplified approach for metabolic engineering, protein expression, and downstream applications such as production of biofuels, specialty chemicals, and nutraceuticals. Using the optimized cloning and expression vectors, frozen cells, specially formulated Gibco® medium, and easy-to-follow protocols, growth and transformation of *C. reinhardtii* is straightforward (Figure 6), which helps to ensure reliable results and faster scale-up.

Characteristics of *C. reinhardtii*

The unicellular green alga *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. Green algae are used as platforms for the production of biofuels and other bioproducts, mainly because of their rapid growth and ability to convert sunlight and CO₂ to energy. In addition, *C. reinhardtii* is categorized as GRAS (generally recognized as safe) by the USDA. That, along with the following features, makes it a particularly attractive system for the expression of recombinant proteins:

- Fully sequenced and annotated genome (strain 137C)
- Short time between the generation of initial transformants and scale-up to production volumes
- Ability to grow phototrophically or heterotrophically
- Ability to grow cultures at scales ranging from a few milliliters to 500,000 L, in a cost-effective manner
- Well-characterized mating system, making it possible to carry out classical breeding

Achieve production targets sooner

Compared to land plants, *C. reinhardtii* grows at a much faster rate, doubling cell numbers in approximately 8 hours under heterotrophic growth conditions and 12 hours under photosynthetic growth conditions. As *C. reinhardtii* propagates vegetatively, the time from initial transformation to product production is significantly reduced relative to plants, requiring as little as 6 weeks to evaluate production at flask scale, with the potential to scale up to 64,000 L in 4 to 6 weeks.

C. reinhardtii cloning vectors

We have developed two *C. reinhardtii* expression vectors: pChlamy_1, which contains a standard multiple cloning site (MCS), and pChlamy_1/D-TOPO®, which allows you to carry out rapid and efficient directional cloning of a blunt-end PCR product using our proven TOPO® cloning technology. Our *C. reinhardtii* vectors contain:

- Hsp70A-RbcS2 constitutive promoter for expression of the gene of interest
- Cop1 3' UTR
- Directional TOPO® cloning site for rapid and efficient directional cloning of a blunt-end PCR product
- Hygromycin resistance gene driven by the beta-tubulin promoter for selection in *C. reinhardtii*
- Ampicillin resistance gene for selection in *E. coli*
- pUC origin for maintenance in *E. coli*

Figure 5. GeneArt® *Chlamydomonas* Engineering Kits. All kits come with algal cells and an expression vector (either pChlamy_1/D-TOPO® or pChlamy_1). We also offer kits that include One Shot® TOP10 *E. coli* competent cells and Gibco® TAP Growth Medium. See the ordering information for details.



To test the expression obtained using the GeneArt® *Chlamydomonas* Engineering Kit, an ethanol production system was used to transform *C. reinhardtii*. After 5 days, ethanol production in the engineered strain was dramatically improved compared to the untransformed strain (Figure 7).

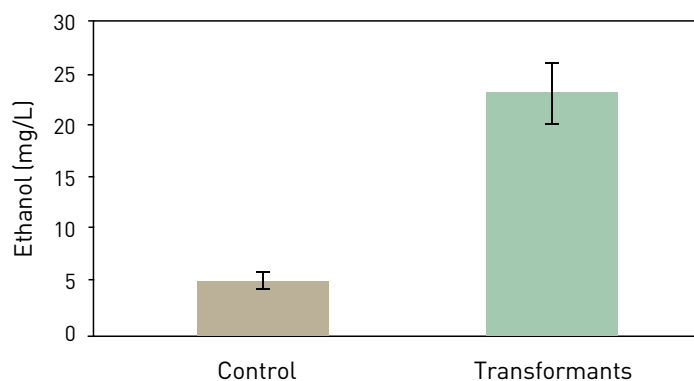


Figure 7. Ethanol production in *C. reinhardtii* engineered with GeneArt® Algae Engineering Kits. The *adh1* alcohol dehydrogenase gene was PCR-amplified from *Saccharomyces cerevisiae*, cloned into the pChlamy_1/D-TOPO® vector, and propagated in One Shot® TOP10 *E. coli* competent cells. The plasmid was used to transform GeneArt® *C. reinhardtii* cells that were grown and prepared for transformation using GeneArt® Algae Engineering Kit protocols. Transformants were selected on Gibco® TAP Growth Medium plates containing hygromycin. Colonies were inoculated into 4 mL of Gibco® TAP Growth Medium containing hygromycin and grown for 5 days. Ethanol production was measured by enzymatic assay.

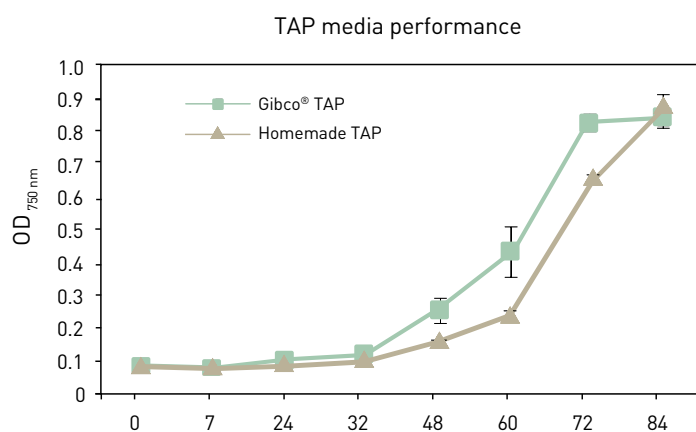


Figure 6. Rapid growth of *C. reinhardtii* cells in Gibco® TAP Growth Medium. Frozen *C. reinhardtii* cells were resuscitated in Gibco® TAP Growth Medium in triplicate samples. Growth was monitored by OD₇₅₀ measurements taken periodically during the study.

The potential of engineered algae



Develop and produce sustainable, renewable fuels to help meet global demands for energy.



Capture and store CO₂ and other greenhouse gases to reduce our impact on the environment.



Manufacture nutraceuticals and additives for value-added processed foods



Utilize algae as a model organism for photosynthesis and plant research

Ordering information

The GeneArt® Algae Engineering Kit product line

We have developed algae engineering kits in various configurations. Whether you're performing basic research or embarking on a new bioproduction strategy, you can rely on the optimized and quality-controlled reagents and protocols in our GeneArt® Algae Engineering Kits.

Product name	Expression vector	<i>E. coli</i> competent cells	Algae cells (10 vials)	Growth medium	Cat. No.
GeneArt® <i>Synechococcus</i> TOPO® Engineering Kit	pSyn_1/D-TOPO® Vector	One Shot® TOP10 cells (10 vials)	<i>S. elongatus</i>	None	A14261
GeneArt® <i>Synechococcus</i> TOPO® Engineering Kit with media	pSyn_1/D-TOPO® Vector	One Shot® TOP10 cells (10 vials)	<i>S. elongatus</i>	Gibco® BG-11 Medium (6 L)	A14265
GeneArt® <i>Synechococcus</i> Engineering Kit	pSyn_1 Vector	None	<i>S. elongatus</i>	None	A14259
GeneArt® <i>Synechococcus</i> Engineering Kit with media	pSyn_1 Vector	None	<i>S. elongatus</i>	Gibco® BG-11 Medium (6 L)	A14263
GeneArt® <i>Chlamydomonas</i> TOPO® Engineering Kit	pChlamy_1/D-TOPO® Vector	One Shot® TOP10 cells (10 vials)	<i>C. reinhardtii</i>	None	A14260
GeneArt® <i>Chlamydomonas</i> TOPO® Engineering Kit with media	pChlamy_1/D-TOPO® Vector	One Shot® TOP10 cells (10 vials)	<i>C. reinhardtii</i>	Gibco® TAP Medium (6 L)	A14264
GeneArt® <i>Chlamydomonas</i> Engineering Kit	pChlamy_1 Vector	None	<i>C. reinhardtii</i>	None	A14258
GeneArt® <i>Chlamydomonas</i> Engineering Kit with media	pChlamy_1 Vector	None	<i>C. reinhardtii</i>	Gibco® TAP Medium (6 L)	A14262
Gibco® BG-11 Medium: optimized for cyanobacteria (including <i>Synechococcus elongatus</i>), 1 L					A1379901
Gibco® BG-11 Medium: optimized for cyanobacteria (including <i>Synechococcus elongatus</i>), 6 L					A1379902
Gibco® TAP Growth Medium: optimized for <i>Chlamydomonas</i> , 1 L					A1379801
Gibco® TAP Growth Medium: optimized for <i>Chlamydomonas</i> , 6 L					A1379802

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