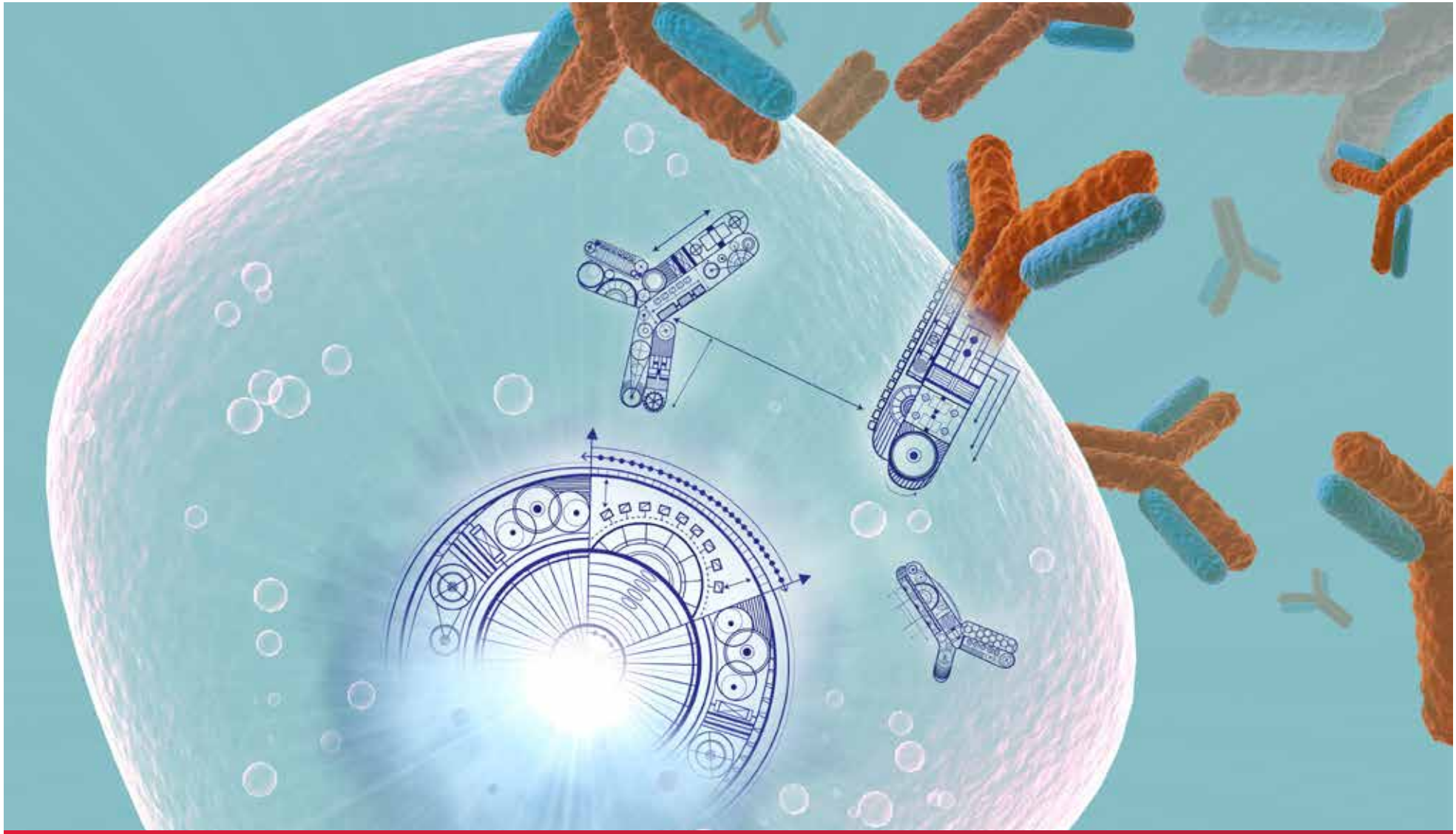


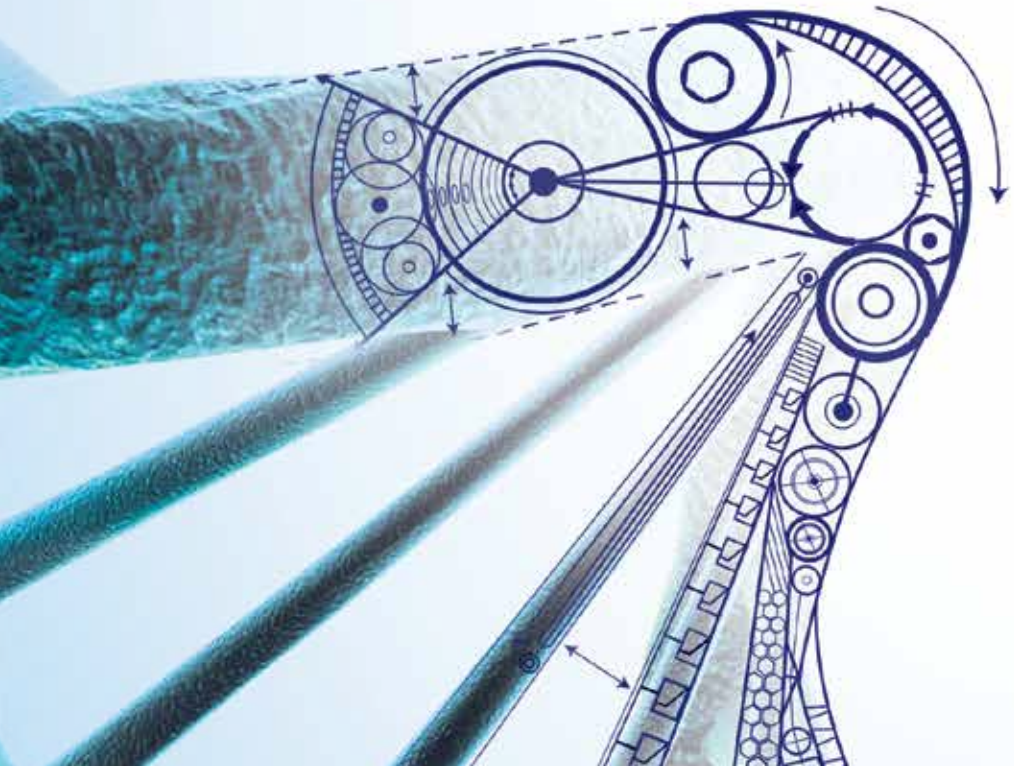
Invitrogen™

Gene to protein

Engineer innovation

life technologies™

Contents



Find out more at **lifetechnologies.com/proteinexpress**

life technologies™

Gene to protein

From creating your gene to expressing your protein, Life Technologies offers a wide range of tools and expression systems that are easy to use, enable rapid results, and offer high protein yields for every downstream application (Figure 1). This brochure outlines the best products and services for your research needs, which can help save you weeks of hands-on time compared to using traditional methods. We even offer a complete service for the entire gene-to-protein workflow (page 18).

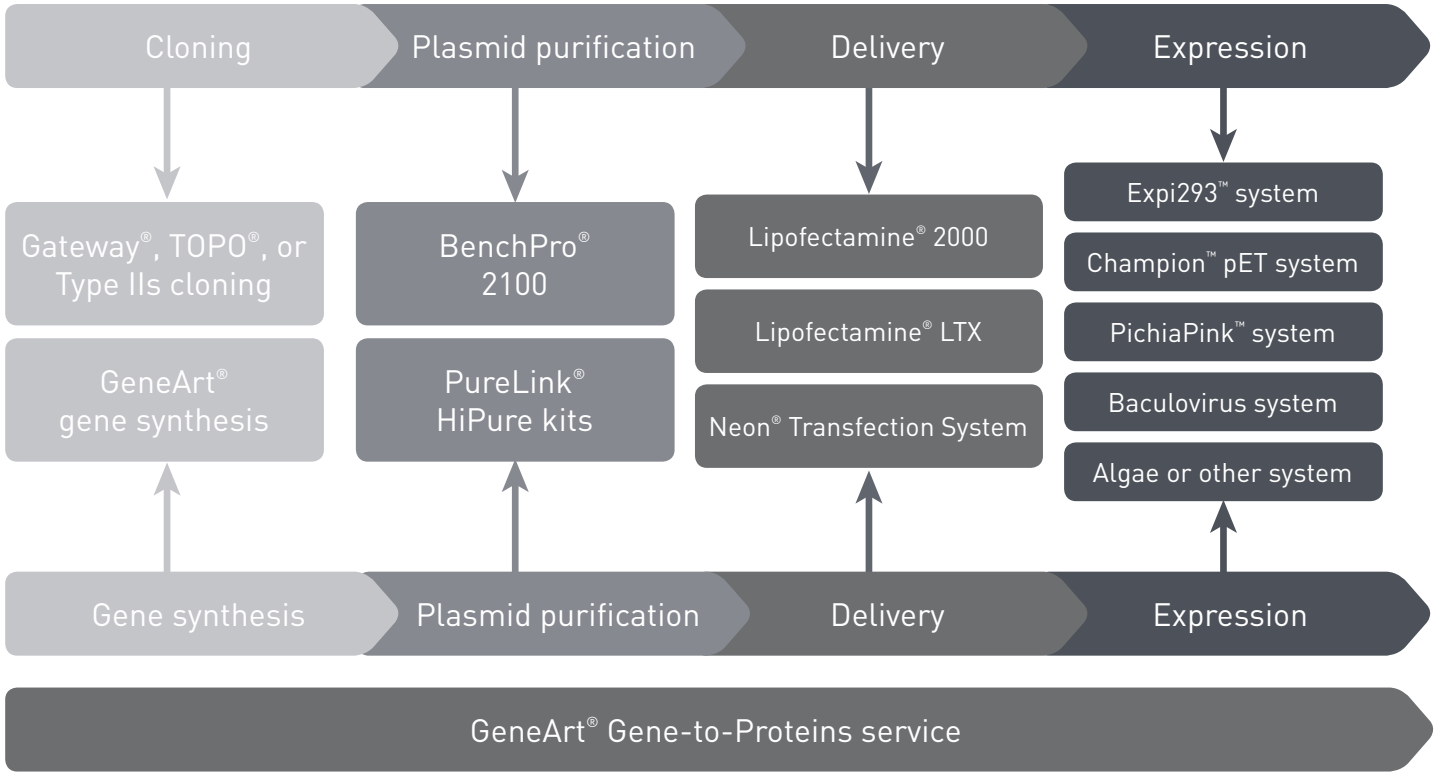


Figure 1. General workflow for protein expression.

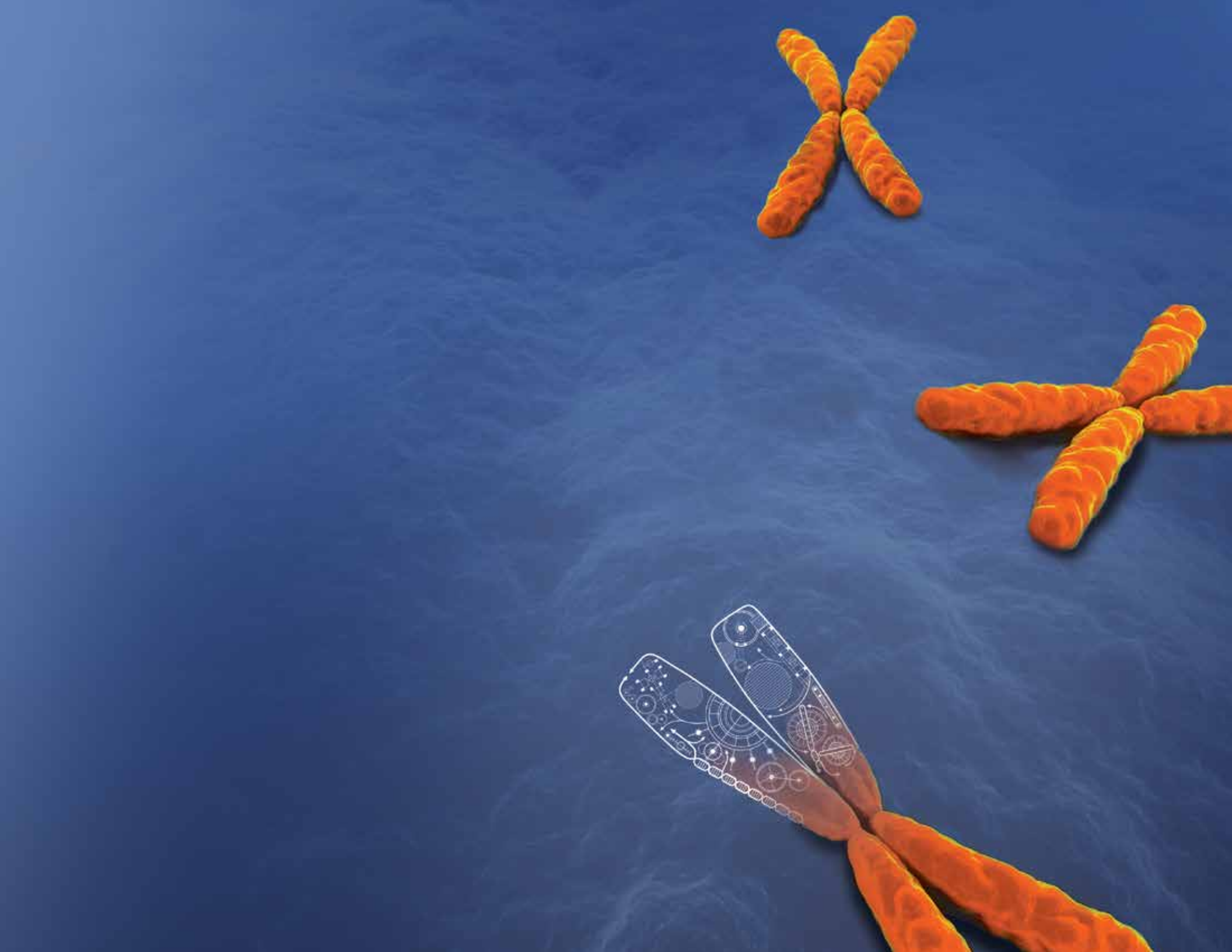
Choosing an expression system

Recombinant protein expression technology is essential for a wide variety of applications. From the study of protein function to the large-scale production of proteins for drug discovery and development, using the right expression system for your application is important for success. Protein solubility, functionality, purification speed, and yield are often crucial factors to consider when choosing an expression system. With the wide variety of expression systems available from Life Technologies, you're sure to find one that meets your needs (Table 1). The following pages detail mammalian and algal tools to enhance your expression.

Table 1. Protein expression systems and their applications. Each system has its own strengths and challenges, which are important to consider when choosing an expression system.

Host organism	Most common applications	Advantages	Challenges	For more information, go to:
Prokaryotic	• Structural analysis • Antibody generation • Functional assays • Protein interactions	• Scalable • Low cost • Simple culture conditions • Compatible with Gateway® cloning	• Protein solubility • Minimal posttranslational modifications • May be difficult to express functional mammalian proteins	lifetechnologies.com/bacterialexpression
Yeast	• Structural analysis • Antibody generation • Functional assays • Protein interactions	• Eukaryotic protein processing • Scalable up to fermentation (grams/liter) • Simple media requirements	• Fermentation required for very high yields • Growth conditions may require optimization	lifetechnologies.com/yeastexpression
Insect	• Functional assays • Structural analysis • Antibody generation	• Posttranslational modifications similar to mammalian systems • Usually greater yields than from mammalian systems • Compatible with Gateway® cloning	• More demanding culture conditions	lifetechnologies.com/insectexpression
Mammalian	• Functional assays • Protein interactions • Antibody generation	• Highest level of correct posttranslational modifications • Highest probability of obtaining fully functional human proteins • Compatible with Gateway® cloning	• Multimilligram/liter yields only possible in suspension cultures • More demanding culture conditions	lifetechnologies.com/mammalianexpression
Algal	• Basic algal research • Plant sciences • Protein production	• Fast-growing, photosynthetic model organism • Superb experimental control for biofuels, nutraceuticals, and specialty chemical production • Optimized system for robust selection and expression	• Difficulty preserving and reviving cells • Silencing of expression of gene of interest • Long growth cycle of photosynthetic land plants	lifetechnologies.com/algaeexpression

For a complete list of expression systems from Life Technologies, please go to **lifetechnologies.com/proteinexpress**



Building your gene

GeneArt® Gene Synthesis

Gene synthesis has become the most cost-effective and time-saving method for obtaining nearly any desired DNA construct. Simply provide the sequence you want, and the gene will be synthesized and cloned for you (Figure 2). When combined with gene optimization, gene synthesis produces clones that outperform constructs made using conventional molecular biology techniques in expression performance. GeneArt® Gene Synthesis tools go beyond traditional cloning and enable you to:

- Improve protein expression with GeneOptimizer® technology
- Gain access to hard-to-clone constructs with long, complex DNA
- Overcome gene or vector design limitations
- Create unlimited numbers of mutants for screening experiments
- Engineer proteins to improve enzyme activity and increase binding affinities of antibodies

Beyond gene synthesis, Life Technologies also offers GeneArt® Strings™ DNA Fragments, which are delivered as linear, double-stranded DNA fragments. If you prefer to synthesize your own genes, you can use the GeneArt® Gene Synthesis Kit, which provides all of the high-quality reagents necessary for successful production of synthetic fragments. Table 2 provides a summary of gene synthesis options available from Life Technologies.

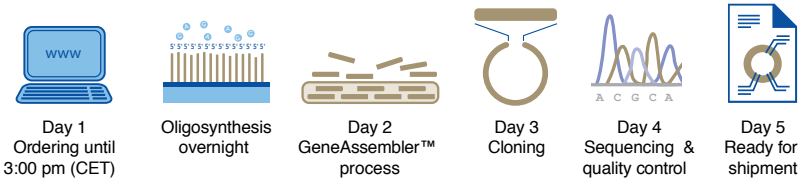


Figure 2. GeneArt® SuperSPEED production schedule. Genes can be synthesized, cloned, and shipped in as few as 5 business days.

Table 2. Gene synthesis options from Life Technologies.

	Do-it-yourself gene synthesis	Custom DNA fragments	Custom gene synthesis
Product or service	GeneArt® Gene Synthesis Kit	GeneArt® Strings™ DNA Fragments	GeneArt® Gene Synthesis and Subcloning Service
Advantage	• Full cost control • You control every step	• Fast and affordable • Design flexibility • Gene optimization • No physical template required • Reliable technology available to assemble your complete gene (e.g., GeneArt® Seamless PLUS Cloning and Assembly Kit)	• 100% sequence verified • Convenient ordering • Design flexibility • Gene optimization • No synthesis and cloning hands-on time needed • Optional speed upgrades (e.g., SuperSPEED (see Figure 2))
Lab work	High	Medium	Low
Standard processing time	NA	5 business days for fragments up to 1,000 bp	9 business days for genes up to 1,200 bp

Order genes on our easy-to-use portal at **lifetechnologies.com/genesynthesis**

Optimizing expression

Production of recombinant proteins for biomedical research and product development can be hampered by low expression yields. These expression issues can limit the ability of researchers to conduct structural and functional analyses, delaying and in some cases halting the discovery process. Gene optimization is the solution to traditional protein expression limitations. The common challenges associated with protein expression—yield, solubility, and functionality—can now be addressed in a rational and systematic way.

The GeneOptimizer® algorithm determines the optimal gene sequence for your expression system as part of a real multiparametric approach (Figure 3). By evaluating the relevant expression parameters in parallel, GeneOptimizer® technology generates a plethora of variants of your target sequence in an evolutionary approach and selects the best match for your specific requirements. Sequence optimization using the GeneOptimizer® software process is included as an optional step with all GeneArt® Gene Synthesis Services and with GeneArt® Strings™ DNA Fragments.

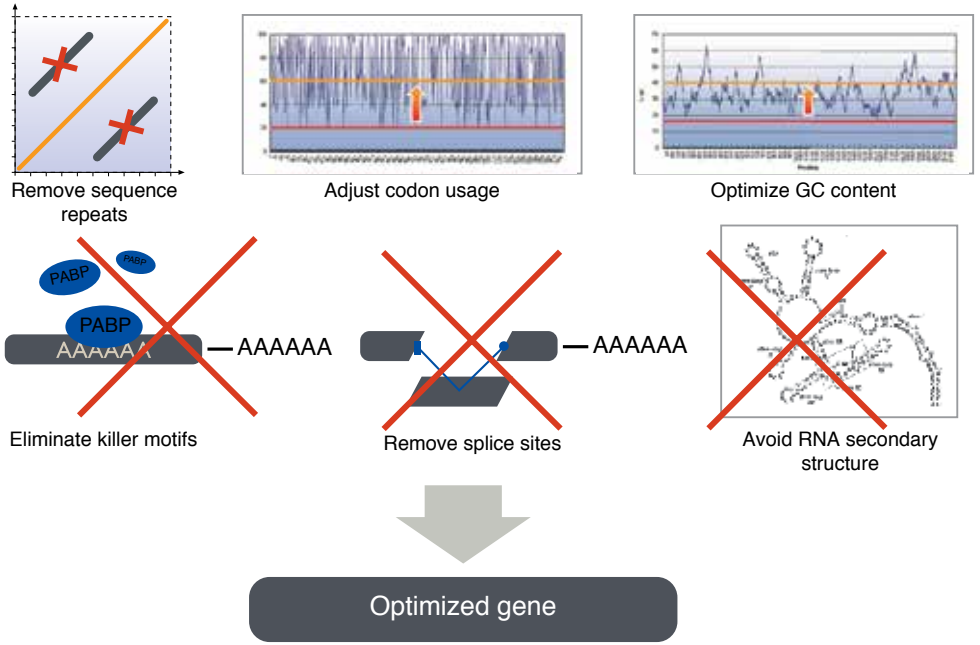


Figure 3. The GeneOptimizer® algorithm determines the optimal gene sequence for your experiments. The algorithm removes DNA sequence repeats, optimizes codon usage and GC content, and minimizes the formation of RNA secondary structures that may reduce protein yield. Protein sequence is not affected by the optimization process.

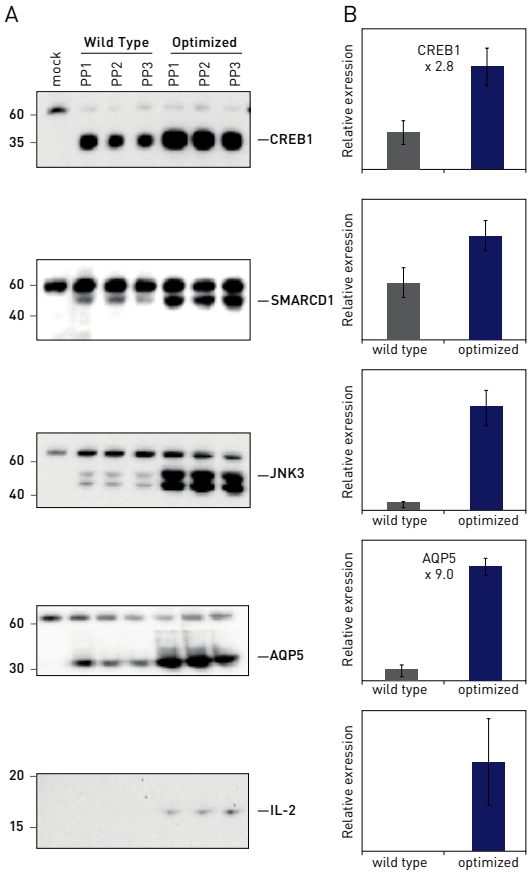


Figure 4. Comparative expression analysis of wild type versus optimized genes representing different protein classes. (A) Cell culture supernatants (for secreted proteins) or cell lysates (all other proteins) were analyzed by western blots using an anti-His antibody. One example of each protein class is shown. A 60 kDa protein used to standardize protein amount is visible, including in the empty vector negative controls. Left of each image: molecular mass values in kDa. Right of each image: identifiers for specific protein bands. (B) Relative expression levels were derived for wild type or optimized constructs (mean of three independent transfections). The fold increase in expression for the optimized construct is indicated for each protein. There was no detectable expression for IL-2 using the wild type construct.≠ Figure adapted from Fath et al., 2011 [1].

In a first-of-its-kind study [1], five important protein classes were selected for optimization—protein kinases, transcription factors, ribosomal proteins, cytokines, and membrane proteins. Then, 50 human genes were chosen from the NCBI database to represent the five protein classes. The selected genes were individually optimized using the GeneOptimizer® algorithm [2]. For comparison, the corresponding wild type genes were subcloned using native sequences available from the NCBI database. Each gene was then expressed in triplicate in HEK 293T cells. Following optimization, the 50 genes all showed reliable expression and 86% exhibited elevated expression. Further analysis showed no detrimental effect on protein solubility, and unaltered functionality was demonstrated for *JNK1*, *JNK3*, and *CDC2* (data not shown). Using the GeneOptimizer® algorithm, in this study:

- 86% of optimized genes showed significantly increased protein expression
- Protein yields increased up to 15-fold with optimized genes
- 100% of optimized genes were expressed, versus 88% of wild type genes

References

1. Fath S, Bauer AP, Liss M et al. (2011) Multiparameter RNA and codon optimization: a standardized tool to assess and enhance autologous mammalian gene expression. *PLoS One* 6:e17596.
2. Raab D, Graf M, Notka F et al. (2010) The GeneOptimizer® algorithm: using a sliding window approach to cope with the vast sequence space in multiparameter DNA sequence optimization. *Syst Synth Biol* 4:215–225.

Find out more about gene optimization at
lifetechnologies.com/genesynthesis

GeneArt® Strings™ DNA Fragments

GeneArt® Strings™ DNA Fragments are custom-made linear double-stranded DNA fragments. A quantity of at least 200 ng of GeneArt® Strings™ DNA Fragments is shipped within 5 business days, ready for cloning in your lab. GeneArt® Strings™ DNA Fragments can be ordered online and offer a fast, convenient, and affordable alternative to traditional PCR-based cloning.

GeneArt® Strings™ DNA Fragments are produced with the same technology used for GeneArt® Gene Synthesis. DNA fragments are assembled from high-quality synthetic oligonucleotides and bulk sequenced to verify that your desired gene is highly represented in the fragment pool.

Experimental data show a high probability of finding a correct clone if you sequence at least 4 colonies containing full-length inserts (Figure 5). Life Technologies offers several reliable and efficient cloning systems, such as TOPO® cloning, Gateway® cloning, GeneArt® Seamless Cloning, and GeneArt® Type IIs Assembly Kits. We recommend that you first choose a suitable cloning system and then design your Strings™ DNA Fragments according to the respective requirements.

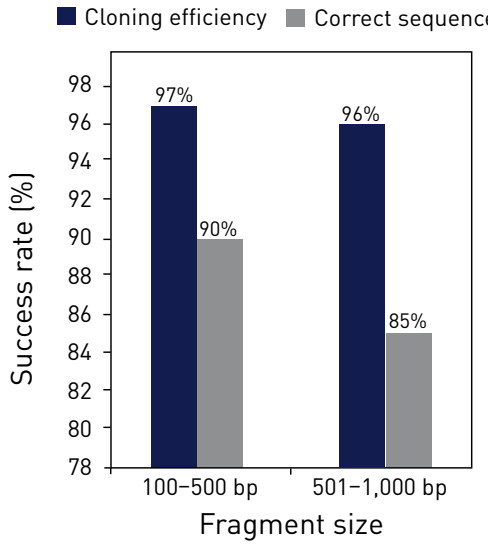


Figure 5. Cloning Strings™ DNA Fragments using restriction enzyme cloning. The success rate of conventional restriction enzyme cloning was evaluated with more than 1,000 fragments directly taken from our standard gene synthesis manufacturing process. Fragments of different size ranges, 100–500 bp and 501–1,000 bp, were subjected to restriction enzyme cloning. Bacterial colonies were analyzed by colony PCR to determine cloning efficiency and then sequenced. Sequencing of up to 4 full-length fragments between 100 bp and 500 bp and up to 6 full-length clones between 501 bp and 1,000 bp resulted in success rates of 90% and 85%, respectively, to find correct clones.

Plasmid isolation

Looking for the right kit to isolate plasmid DNA at the purity and scale you need?

Go to lifetechnologies.com/plasmidprep

Find out more at lifetechnologies.com/strings

Gene assembly

GeneArt® Type IIs Assembly Kits

GeneArt® Type IIs Assembly Kits provide seamless cloning and assembly of up to 8 DNA fragments by simultaneous cleavage and ligation in a single reaction (Figure 6). The kits use a technology similar to Golden Gate cloning with Type IIs restriction enzymes, and can be used to assemble multiple fragments in a predetermined order into any compatible vector. Since Type IIs assembly is not based upon homologous recombination, there is minimal risk of rearrangements, and minimal sequence confirmation of your final construct is required. Life Technologies offers three kits, each with one of three Type IIs restriction enzymes: AarI, BsaI, and BbsI, all of which come with an all-in-one enzyme mix, cloning vector, and cloning controls. Our new and improved GeneArt® Primer and Construct Design Tool recommends the appropriate GeneArt® assembly kit for your fragments and provides an easy-to-use interface to design your construct and create and order primers (should they be required). With GeneArt® Type IIs Assembly Kits, you can:

- Assemble multiple DNA fragments in any order, into any compatible vector, without scars
- Avoid homologous recombination and associated rearrangements when cloning homologous or repetitive sequences
- Assemble GeneArt® Strings™ DNA Fragments, GeneArt® Precision TALs, gene variants, and repetitive or small sequences
- Create your own cloning and expression vectors with custom vector elements
- Minimize sequence confirmation of final constructs
- Utilize the free online GeneArt® Primer and Construct Design Tool to facilitate the design of up to 8 DNA fragments for assembly

Cloning efficiency, flexibility, and precision

The main factors affecting cloning efficiency are the size of the DNA elements, the total size of the final molecule, and the quality and specificity of the fragment. Typical cloning efficiencies for different numbers of fragments cloned into the pType IIs vector are:

- >95% for five fragments of 1 kb each
- >60% for eight fragments of 1 kb each
- >85% for two identical fragments of 1 kb each

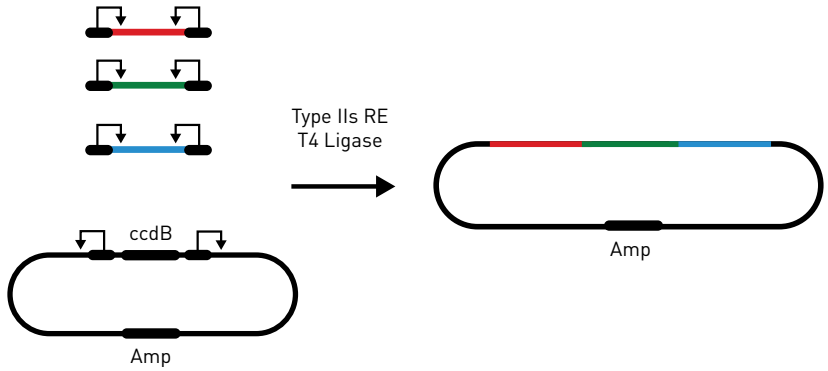


Figure 6. Type IIs cloning. Type IIs restriction endonucleases recognize asymmetric sequences and cleave these sequences at a defined distance from the recognition site. DNA ends can be designed to be flanked by a Type IIs restriction site such that digestion of the fragments removes the enzyme recognition sites and generates complementary overhangs. Such ends can be ligated seamlessly, creating a junction that lacks the original site.

Find out more about GeneArt® Type IIs cloning at **lifetechnologies.com/typeiis**

Mammalian expression systems

Selecting a mammalian expression system

Life Technologies offers a wide variety of mammalian expression systems for your needs. Ask yourself the three following questions, and then use Table 3 to select the best system to meet your needs.

1. How much protein do I need?

Protein yields are highly variable and depend largely on the specific protein to be expressed. In general, small amounts of protein (nanograms to micrograms) can be readily generated using transient transfection of plasmid DNA into a wide variety of cells. It is very important to achieve the highest transfection efficiency possible using effective transfection reagents such as Lipofectamine® LTX or Lipofectamine® 2000 reagents.

Producing larger amounts of protein (milligrams to grams) requires more cells. Thus, stable cell lines are often employed to produce a large, selected population of cells that is stably expressing your protein of interest. Alternatively, large-scale transient transfection of suspension-adapted cells such as HEK 293 or CHO cells can generate large amounts of protein in a much shorter time period than can be achieved with stable cells. The Expi293™ Expression System, which is used for producing large amounts of protein, is discussed in the next section of this brochure.

2. How quickly do I need to obtain my protein?

Expression by transient transfection results in high levels of expression within a few days to a week. Therefore, transient expression is ideal for rapid protein production and quick data generation. Transient expression systems such as the FreeStyle™ 293, FreeStyle™ MAX CHO, and Expi293™ systems use suspension-adapted 293 or CHO cells at culture scales from 1 mL to 100 L to generate large amounts of protein. The new Expi293™ Expression System has been demonstrated to produce up to 1 gram of protein per liter of cell culture.

To produce a cell line that can be used over a long experiment time or over many experiments, it is necessary to generate a stable cell line in which your expression construct is integrated into the host genome. A selection agent, added to the medium, is used to select the cells that have the integrated construct. If you want to make cell lines with targeted integration into an expression hotspot, the Jump-In™ system is recommended. For randomly integrated stable cell lines, any pcDNA™ vector delivered by a cationic lipid–based transfection reagent such as Lipofectamine® LTX reagent may be used. For difficult-to-transfect cells, the Neon® Transfection System or ViraPower™ Lentiviral Expression System may be used.

3. Is it important to control when expression begins?

Expression vectors with constitutive promoters do not allow you to control expression. If you are working with a nontoxic gene and the timing of expression is not important, choose a constitutively expressing vector such as a pcDNA™ vector.

Expression systems with inducible promoters require you to add an inducer to begin expression, which allows you to control the timing of gene expression. In the absence of an inducer, your gene is not expressed. This option is ideal for expressing toxic proteins. Choose from the T-REx™ Expression System for tetracycline-induced expression or the Flp-In™ T-REx™ System for tetracycline-induced expression with targeted genomic integration.

For detailed selection guides and tools, please go to **lifetechnologies.com/mammalianexpression**

Table 3. Mammalian protein expression systems.

Application	System	Key features
Constitutive expression (CMV promoter)	pcDNA™ vectors	Constitutive CMV expression with your choice of several epitope tags and selection markers
Transient expression	pcDNA™ vectors and cationic transfection reagents Adenoviral expression systems	High-level gene expression in any dividing or nondividing mammalian cell type
Scalable, transient expression	FreeStyle™ 293 Expression System	Scalable suspension culture transfections from 1 mL to 100 L
	FreeStyle™ MAX CHO Expression System	Obtain protein in several days to 1 week
	Expi293™ Expression System	Production of up to 1 gram of protein per liter of culture
Stable cell lines, targeted integration	Jump-In™ Fast Gateway® System	Rapid generation of stable cell lines with integration into expression hotspots
	Jump-In™ TI™ Gateway® System	Generation of stable cell lines with integration into preselected genomic sites
	Flp-In™ T-REx™ System	Regulated expression from CMV promoter
Inducible expression	T-REx™ System, Flp-In™ T-REx™ System	Rapid generation of regulated stable expression cell lines
	ViraPower™ Lentiviral T-REx™ System	Regulated expression in any dividing or nondividing mammalian cell type

Transfection

Looking for the right transfection reagent to deliver your plasmid?

Choose from:
Lipofectamine® 2000 Transfection Reagent
Lipofectamine® LTX with PLUS™ Reagent

Go to lifetechnologies.com/transfection

Find out more about mammalian expression systems at
lifetechnologies.com/mammalianexpression

Expi293™ Expression System

High-density mammalian transient protein expression

The Expi293™ Expression System is a major advancement in transient expression technology for rapid and high-yield protein production in mammalian cells. It is based on high-density culture of Expi293F™ cells in Expi293™ Expression Medium. Transient expression is powered by the new cationic lipid–based ExpiFectamine™ 293 transfection reagent in combination with optimized transfection enhancers designed to work specifically with this transfection reagent.

All components work in concert to generate 2- to 10-fold higher protein yields than conventional transient systems such as the FreeStyle™ 293 Expression System. Expression levels of greater than 1 g/L have been achieved for IgG and non-IgG proteins (Figure 7). For the expression of human or other mammalian proteins, expression in the human-derived 293 cells offers the benefit of more native folding and posttranslational modifications, such as glycosylation, when compared to expression systems based on hosts such as *E. coli*, yeast, or insect cells.

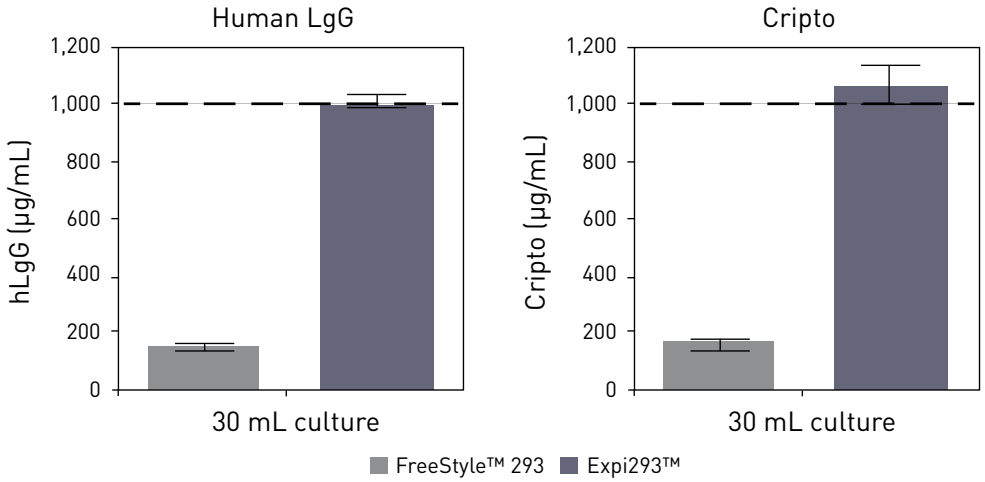


Figure 7. Expression of human IgG and Fc-tagged Cripto protein is achieved at levels of > 1 g/L in the Expi293™ Expression System.

What's in the system?

The components included in the kit are:

- 2 vials of frozen Expi293F™ cells
- 1 L of Expi293™ Expression Medium
- 1 ExpiFectamine™ 293 Transfection Kit, sufficient to transfect 1 L of culture
- OptiMEM® Reduced Serum Medium
- An antibody-expressing positive control

The ExpiFectamine™ 293 Reagent and ExpiFectamine™ 293 Transfection Enhancers 1 and 2 are sold together as the ExpiFectamine™ 293 Transfection Kit. All other components of the system are available separately.

What makes the Expi293™ Expression System unique?

Expi293™ Expression Medium is a chemically defined, serum-free, protein-free medium. Expi293™ Expression Medium enables high-density cell culture (Figure 8) and transfection, allowing for significantly higher volumetric protein yields than traditional culture systems.

ExpiFectamine™ 293 transfection reagent is capable of high-efficiency transfection of high-density 293 cultures, and is coupled with transfection enhancers that further boost transfection performance and expression levels (Figure 9).

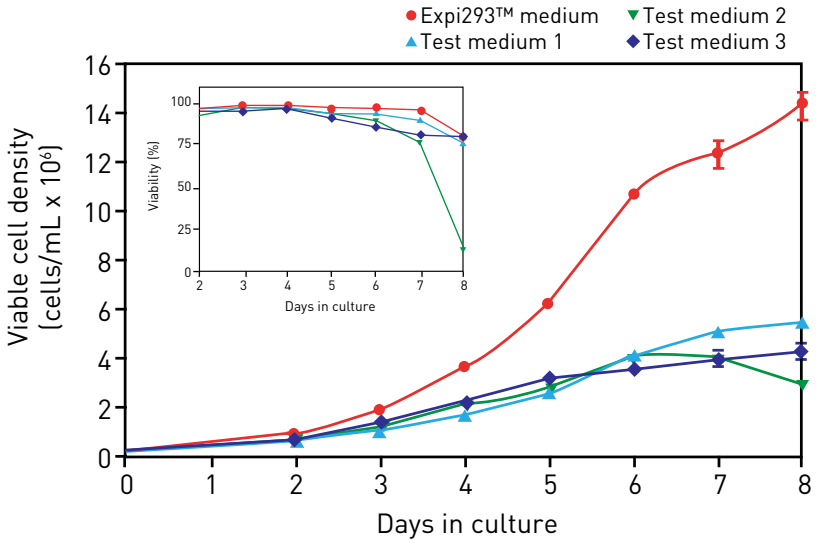


Figure 8. Viable cell density and cell viability over time for cells grown in Expi293™ Expression Medium compared to other 293 culture media.

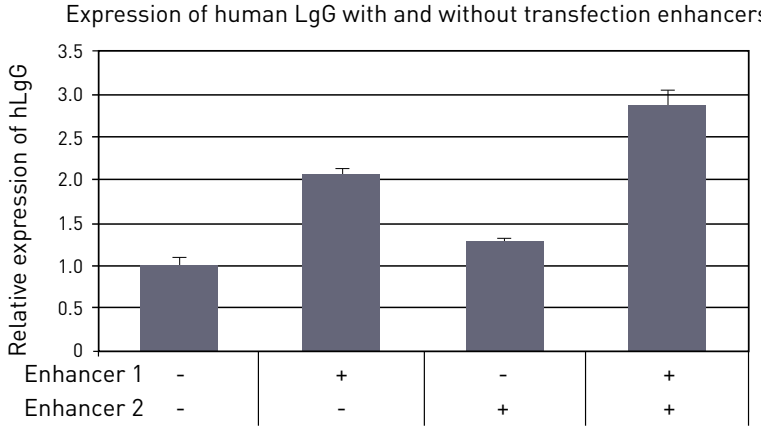


Figure 9. Expression of human IgG, with and without transfection enhancers.

How does the system work?

The Expi293™ Expression System contains all of the components needed to easily produce high levels of recombinant protein in your laboratory. The only additional component required is a mammalian expression vector containing the DNA for your protein of interest.

Expi293F™ cells are thawed and grown in suspension culture in Expi293™ Expression Medium. Cells are typically grown in flasks on a shaker platform inside a CO_2 cell culture incubator or in bioreactors. The cells are transfected using ExpiFectamine™ 293 Reagent. Transfection enhancers 1 and 2 are then added to the culture 16–18 hours post-transfection. There is no need to change culture medium or otherwise supplement the cultures during protein production. Protein harvest can be performed from 2 to 7 days post-transfection, depending on the expression profiles of the protein of interest. For expression of recombinant antibodies, maximum yields are often seen from 5 to 7 days posttransfection.

Expi293F™ cells are specifically adapted to exhibit higher productivity than typical 293 cells and produce more protein than Freestyle™ 293F cells when used in the Expi293™ Expression System (Figure 10). The system is highly scalable and should produce similar volumetric protein yields in transfection formats ranging from 1 mL cultures in a 24-well plate up to a 1 L culture in a shake flask. The 2- to 10-fold higher protein yields possible with the Expi293™ Expression System offer greater value when compared to conventional methods (Table 4).

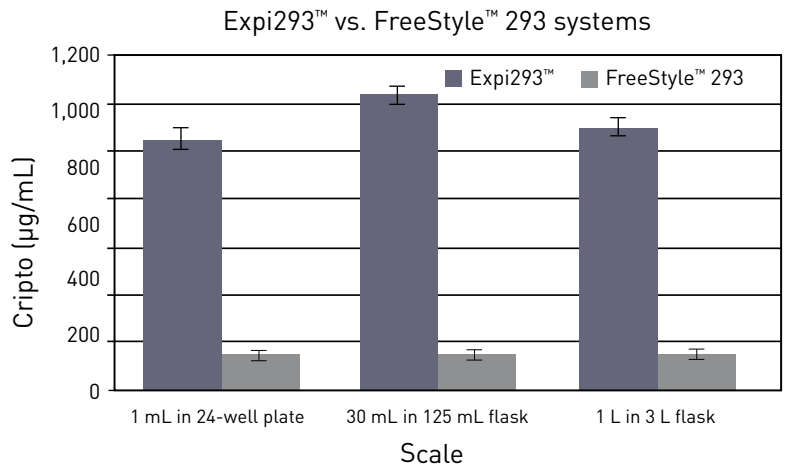


Figure 10. Expression of Fc-tagged Cripto protein achieves similar volumetric protein yields at three different scales.

Table 4. Expi293™ Expression System cost comparison.

	PEI with FreeStyle™ Expression Medium (1 L culture)	Expi293™ system (335 mL culture)	Expi293™ system (200 mL culture)
Total cost	$548	$1,531	$1,531
Media cost	$458	$893	$893
Transfection reagent cost	$0	$548	$548
Estimated plasmid cost	$90	$90	$90
Protein yield differential*	1x	3x	5x
Culture volume for equivalent protein yield	1 L	335 mL	200 mL
Approximate culture vessel cost†	$59	$26	$18
Total cost for equivalent protein yield	$607	$536	$324

*Protein yields in each culture are expressed as normalized values, based on the amount obtained using 1 L of FreeStyle™ Expression Medium and polyethylenimine (PEI) as the transfection reagent.
†Disposable Erlenmeyer shake flasks, cost/unit:
500 mL, nonbaffled, vent cap: $18.00
1,000 mL, nonbaffled, vent cap: $25.81
3,000 mL, Fernbach-style, nonbaffled, vent cap: $58.51

Find out more about the Expi293™ Expression System at **lifetechnologies.com/expi293**

GeneArt® Algae Engineering Kits

Genetic modification and expression systems for photosynthetic microalgae

GeneArt® Algae Expression and Engineering Kits for *Chlamydomonas reinhardtii* 137c and *Synechococcus elongatus* PCC 7942 are the first commercially available genetic modification and expression systems for photosynthetic microalgae. Our most recent GeneArt® kits for algae are optimized for high levels of protein expression, with dual protein tags for detection and purification as well as selection against the gene silencing often seen in *Chlamydomonas* (Figure 11). In contrast, our first-generation algal kits are designed to deliver flexible genetic engineering options to produce algae for applications that are hindered by strong expression, such as pathway engineering or complementation of mutant genes normally expressed at low levels.

- Express >10% of total soluble protein as your protein of interest
- Select against gene silencing in *Chlamydomonas*, even over multiple passages
- Detect and purify your gene of interest with 6xHis-TEV or 6xHis-V5 epitope tags

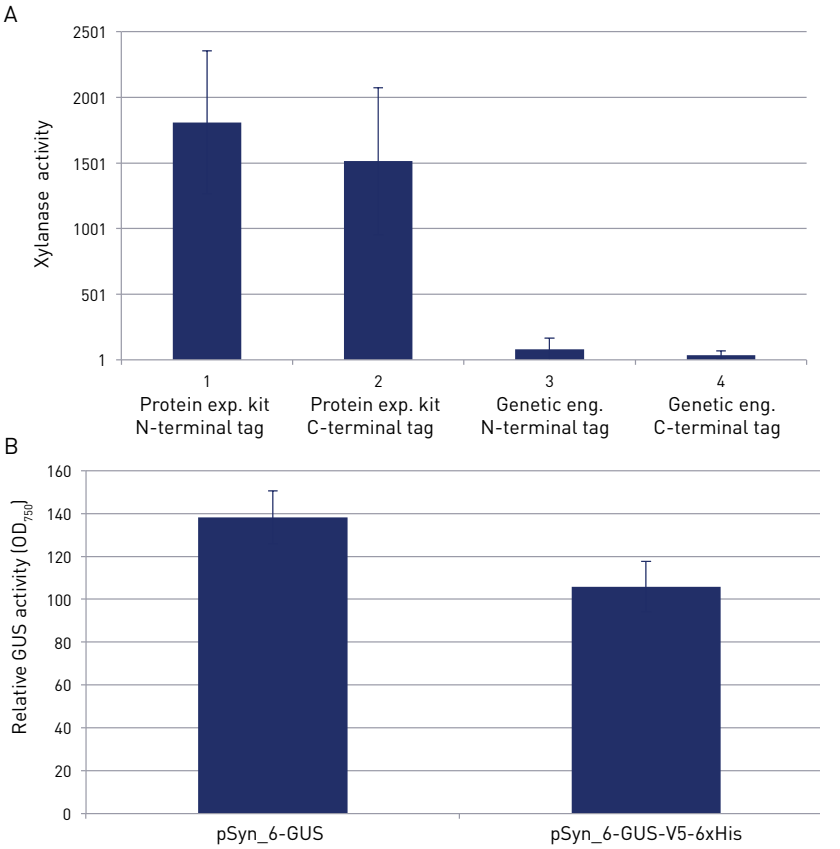


Figure 11. Protein expression levels. (A) The gene for the hydrolytic enzyme xylanase (Xyn1) from *Trichoderma reesei* was cloned into vector pChlamy_4 and transformed into *Chlamydomonas reinhardtii* 137C. Xylanase activity was measured by EnzChek® Ultra Xylanase Assay Kit (Cat. No. E33650) and were 17.6-fold over that observed with previous systems. **(B)** The gene for ß-glucuronidase (GUS) was cloned into vector pSyn_6 and transformed into *Synechococcus elongatus* (negative for GUS activity) following our recommended protocols. GUS activity levels were >100-fold over those seen in our previous system.

Two algal strains for maximum versatility

Chlamydomonas reinhardtii 137c and *Synechococcus elongatus* PCC 7942 are model algal organisms for the study of photosynthesis, plant biology, lipid metabolism, and more. They also serve as bioproduction platforms for biofuels, nutraceuticals, and specialty chemicals.

Chlamydomonas reinhardtii

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

Optimized solutions for algal culture storage and transformation

The GeneArt® Cryopreservation Kit for Algae can be used to preserve algal strains and clones for storage at –80°C for years, thus allowing you to avoid liquid nitrogen storage and continuous cultures as a way to maintain your clones. In addition, our MAX Efficiency® Transformation Reagent for Algae is designed to deliver enhanced transformation efficiency for multiple strains of *Chlamydomonas* species.

- Preserve *Chlamydomonas* and *Chlorella* strains and clones for –80°C storage
- Avoid liquid nitrogen storage
- Avoid contamination and genetic drift that typically occur in continuous cultures
- Obtain ≥100-fold increases in transformation efficiencies with *Chlamydomonas* strains

Find out more about algae expression systems at **lifetechnologies.com/algaeexpression**

Gateway® technology for multiple expression systems

The typical restriction enzyme cloning workflow involves many steps that may limit your success. For example, certain restriction enzymes cannot be used because they might cut within your gene of interest, truncating the insert and making the gene useless for downstream expression. Additional DNA cleanup steps are also needed with restriction enzyme cloning, there is typically low recovery of recombinants from cloning large fragments, and more time is needed to find the clone you need.

Gateway® cloning technology circumvents these limitations, enabling you to access virtually any expression system (Figure 12). Gateway® recombination-based cloning uses a 1-hour, 99% efficient, reversible recombination reaction without using restriction enzymes, ligase, additional subcloning steps, or screening of multiple colonies. Widely adopted in the research community with more than 1,500 references since its launch, Gateway® technology makes collaboration across research disciplines easy and convenient, and enables access to a multitude of vectors from these research groups for truly multidisciplinary scientific studies. Additional advantages of Gateway® technology include:

- Orientation and reading frame of insert are maintained after the reaction, for expression-ready clones
- Resequencing is not required after the Gateway® reaction, saving time and helping to ensure consistent results
- Shuttling insert DNA from one expression vector to another affords flexibility while simplifying your cloning workflow

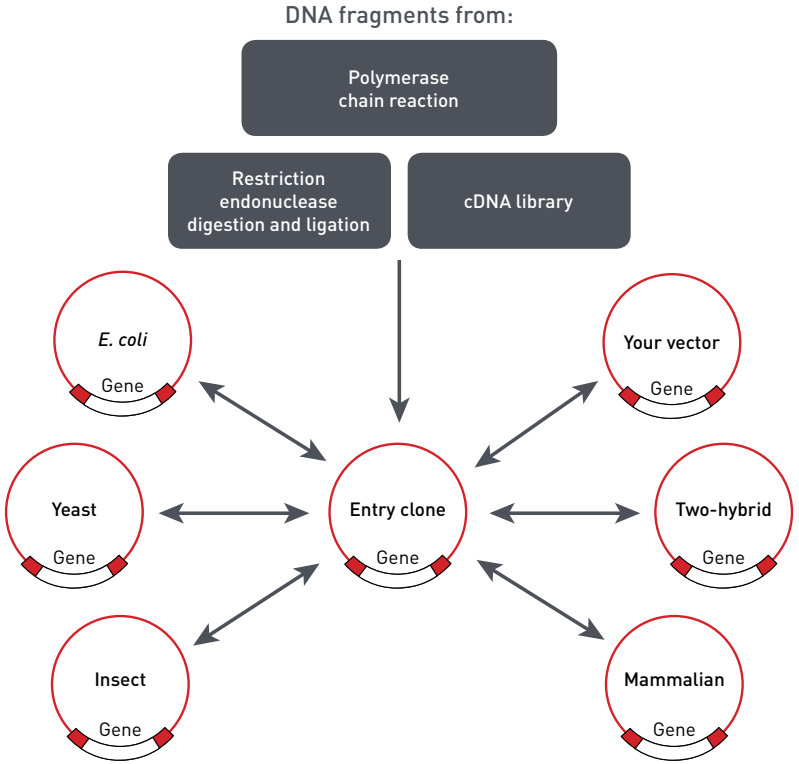


Figure 12. Overview of Gateway® cloning technology. Gateway® technology facilitates cloning of genes into multiple vectors via site-specific recombination. Once a gene is cloned into an entry clone, you can then move the DNA fragment into one or more destination vectors simultaneously.

Find out more at **lifetechnologies.com/gateway**

Protein production services

Fast, reliable protein production from mammalian cells

The GeneArt® Genes-to-Proteins service is an extremely fast way to obtain correctly folded, native protein from transiently transfected mammalian cells. Starting with only the nucleotide sequence, we can provide purified protein typically within 30 business days (Figure 13). We clone your expression-optimized gene into one of our expression vectors, produce transfection-grade plasmid DNA, and then use one of our advanced expression systems to obtain high expression yields. Secreted or intracellular protein is then purified using affinity chromatography (e.g., Fc tag, His tag). Further purification steps are available if highly purified protein is needed. Detailed documentation, including Coomassie-stained PAGE gel and western blot, is provided with every purified protein. Project deliverables are the protein of interest and the expression vector used for transfection. Please refer to Table 5 for more information on the service.

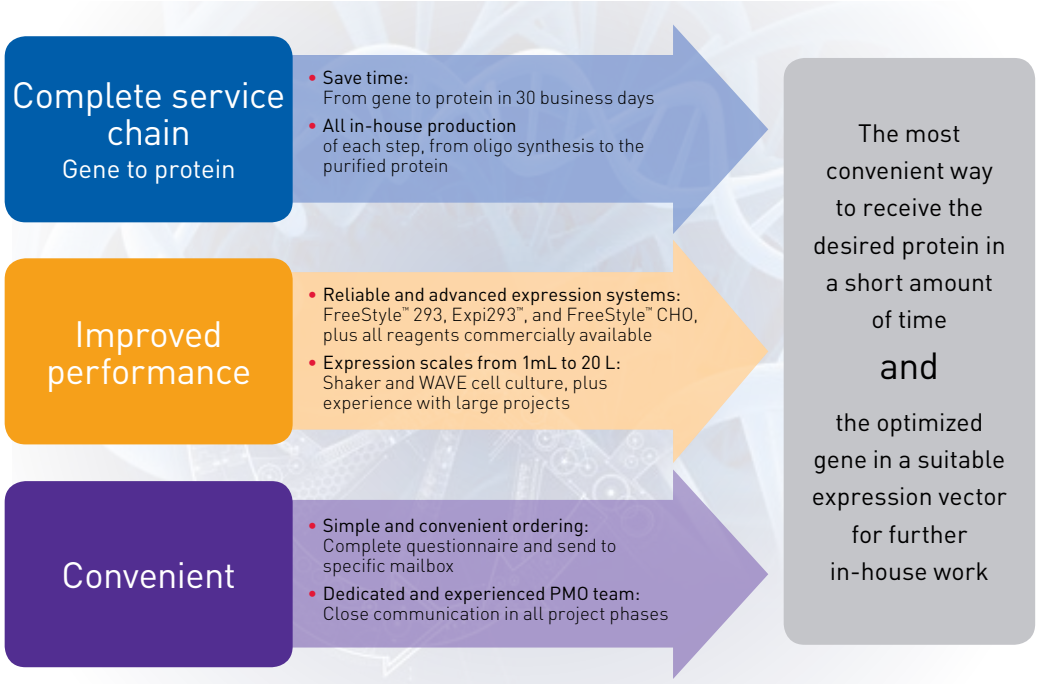


Figure 13. Advantages of the GeneArt® Genes-to-Proteins service.

For more information, go to **lifetechnologies.com/G2Pservice**

Table 5. Summary of protein production services.

Service	Description	Deliverables
Genes-to-proteins pilot	Feasibility study for determination of production yield from transiently transfected 293 or CHO cells	• Price quote for production of a customer-specified protein amount • Documentation including Coomassie gel and western blot • Purified protein
Genes-to-proteins purification	Protein expression and purification from customer-specified culture volume of transiently transfected 293 or CHO cells	• All protein purified from specified culture volume (alternatively, culture supernatant or cells) • Documentation including Coomassie gel and western blot
Genes-to-proteins complete	Protein expression and purification of customer-specified protein amount using transiently transfected 293 or CHO cells Pilot service mandatory	• Purified protein amount as specified • Documentation including Coomassie gel and western blot

The combination of GeneArt® expression optimization and advanced expression systems (e.g., Expi293™) from Life Technologies usually leads to higher overall project reliability and expression yields than obtained with nonoptimized genes (Figure 14).

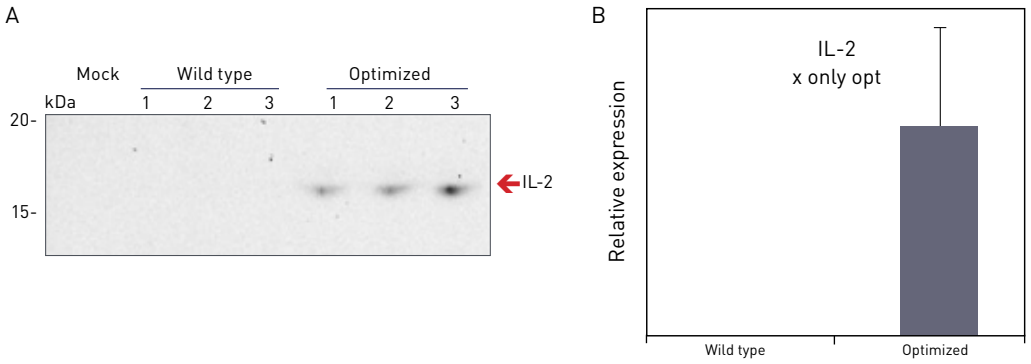


Figure 14. Improved protein expression with gene optimization. (A) Three independent transfections of wild type and optimized IL-2 constructs were analyzed by western blot. **(B)** The resulting bands were analyzed by densitometry. From Fath et al., 2011; see Figure 4 on page 7.

Please email **geneartprotein@lifetech.com** for more information or a quote

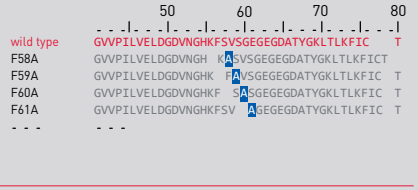
Creating improved proteins

GeneArt® Directed Evolution

Directed evolution strategies are the most efficient method for creating proteins with improved or novel properties. GeneArt® Directed Evolution technologies help to evolve proteins in a goal-oriented, systematic process.

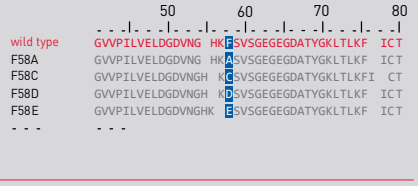
Site-directed mutagenesis

Introduce single or multiple mutations (substitutions, insertions, or deletions) into existing DNA sequences.
Benefits: fully sequence-verified clones, no unwanted backbone mutants, and the fastest turnaround times
Applications: construction of fusion proteins, tagged proteins, alternative splice forms, and alanine scans



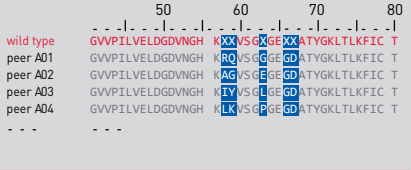
Site-saturation mutagenesis

Scanning a protein region by site-saturation mutagenesis identifies all beneficial substitutions for enhanced function.
Benefits: best cost efficiency, with no structural data needed for protein improvement
Applications: improvement of industrial proteins and alienation of proteins from patented sequences



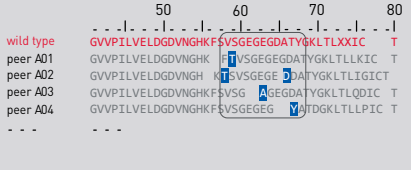
Combinatorial libraries

True rational design for defined randomization of selected sites only, while providing maximum framework integrity.
Benefits: lowest ancillary mutation rates and highest diversities
Applications: construction of recombinant antibody libraries, promoter libraries, and combining of substitutions identified by site-directed mutagenesis



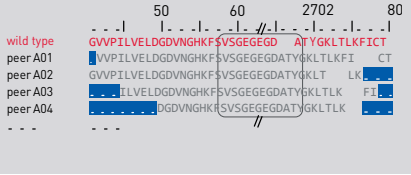
Controlled randomization libraries

Substitute any amino acid in a gene with a defined probability.
Benefits: accurate fine-tuning of mutation rate, and randomization of the entire open reading frame
Applications: affinity maturation of antibodies, improvement of industrial enzymes, and modification of enantioselectivity of enzymes



Truncation libraries

Create custom-defined populations of up to 40,000 in-frame truncated constructs.
Benefits: high quality by avoiding out-of-frame mutations
Applications: solubility screen, minimal functional-size evaluation, domain identification, inhibitory screenings, and epitope mapping



For more information, go to **lifetechnologies.com/directedevolution**

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Find out more at **lifetechnologies.com/geneart**

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