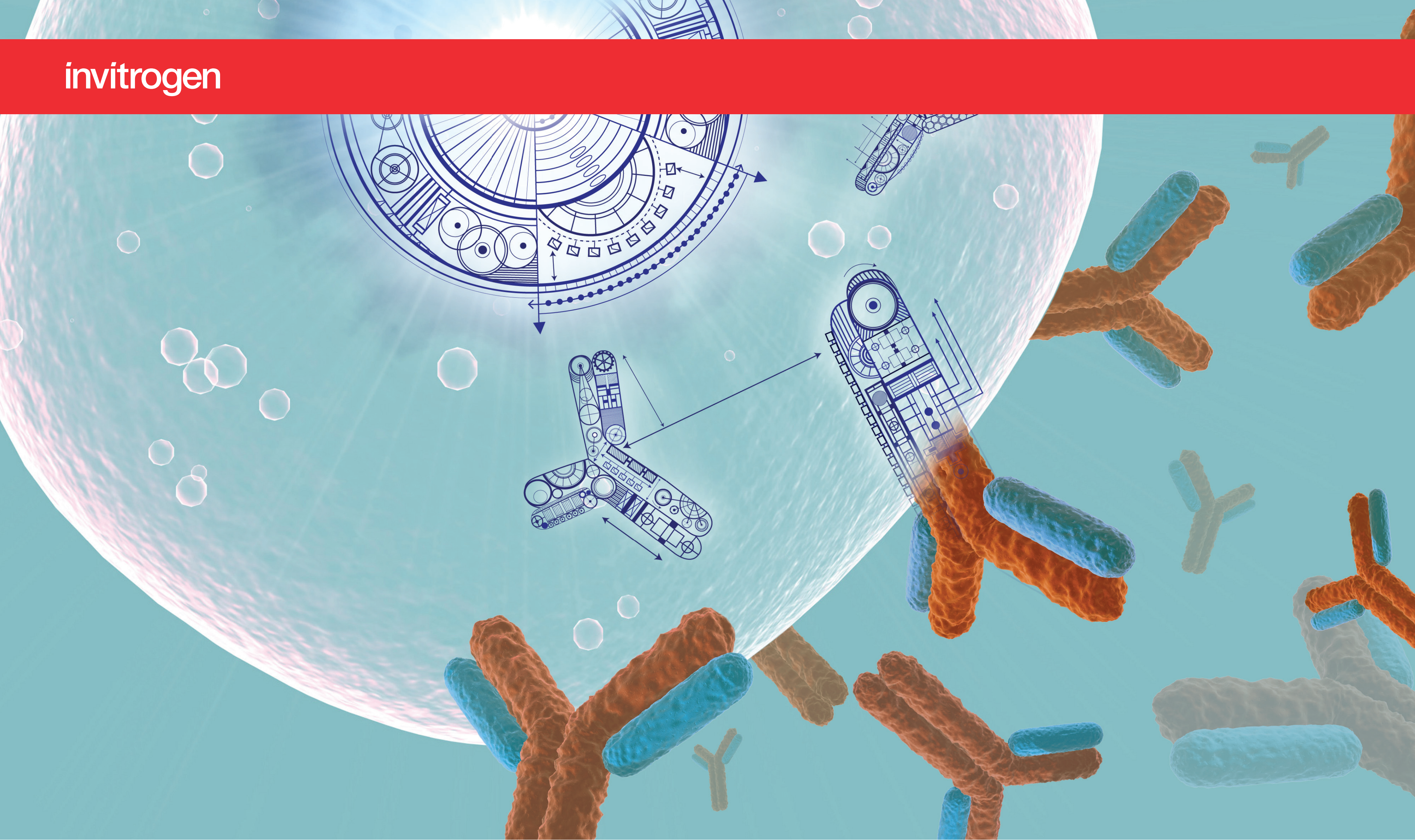

GeneArt Gene to Protein

Your gene, your way.

ThermoFisher SCIENTIFIC

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Find out more at **thermofisher.com/geneartproducts**

Gene to protein

From creating your gene to expressing your protein, we offer 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 our recommended products and services for your research needs, which can help save you weeks of hands-on time compared to using traditional methods. We also offer a complete service for the entire gene-to-protein workflow (page 18).

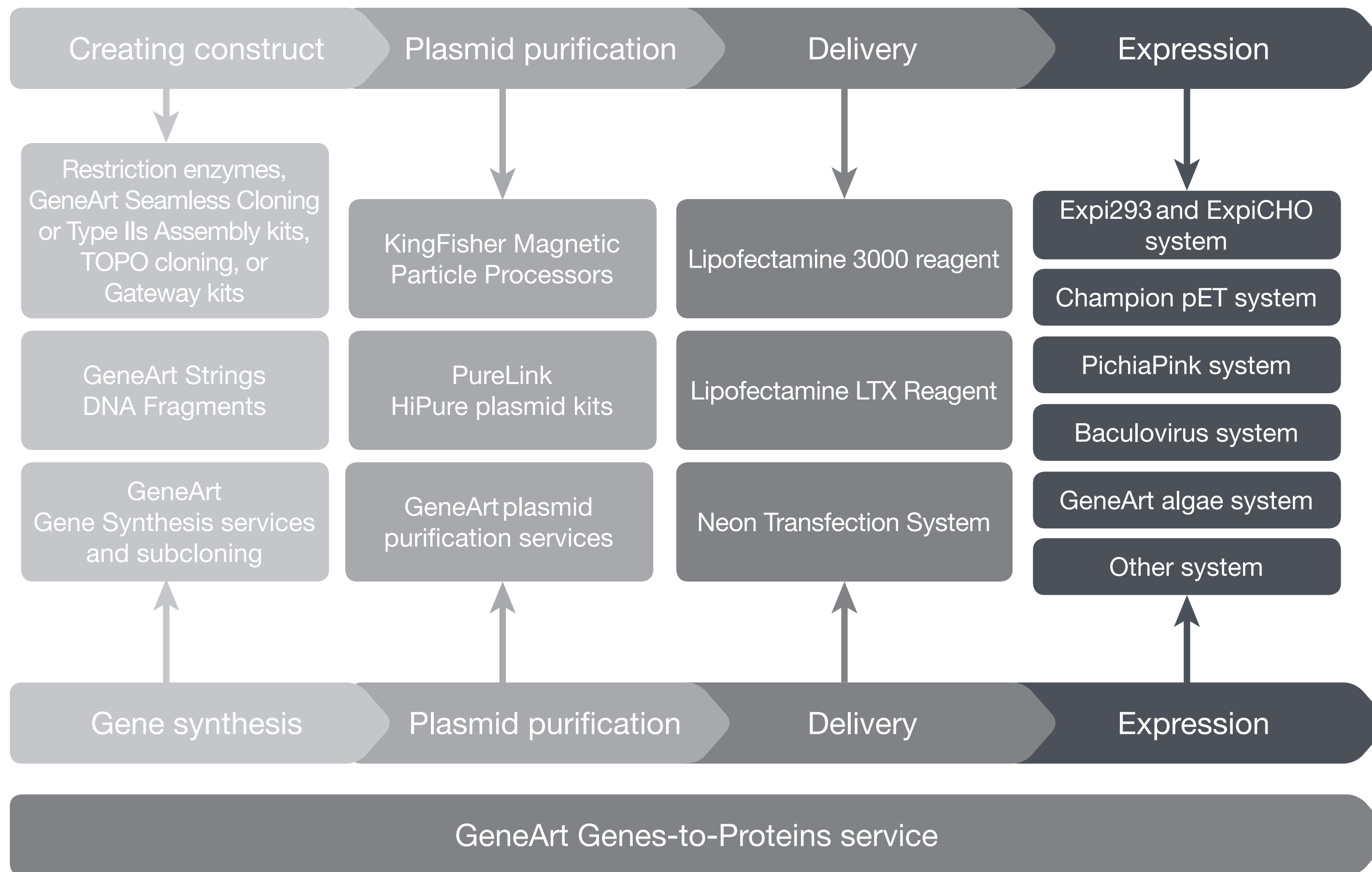
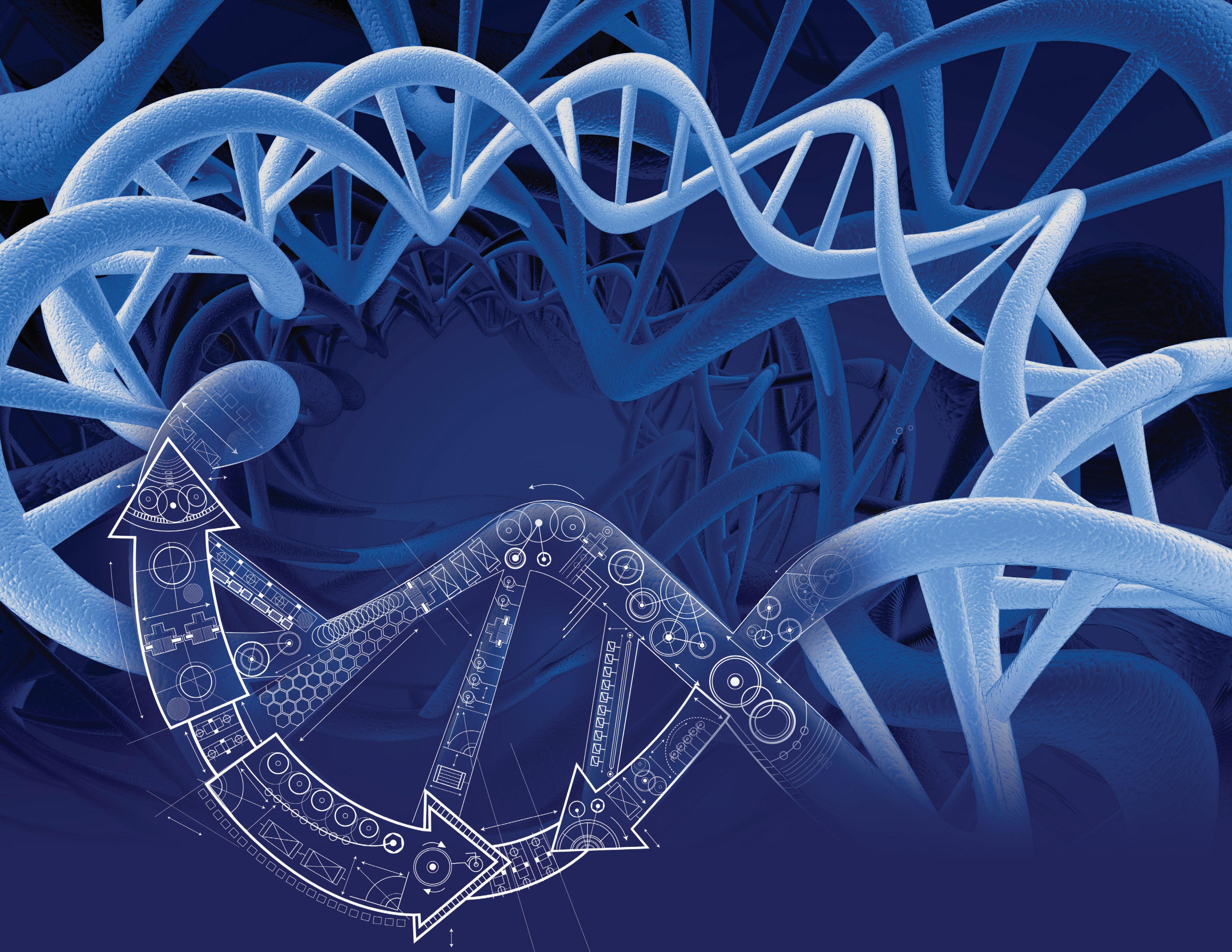


Figure 1. General workflow for protein expression.



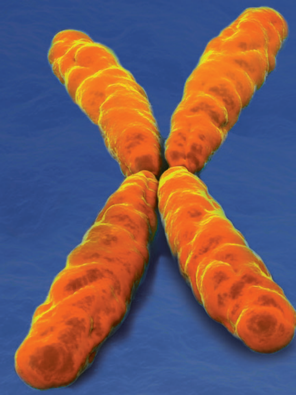
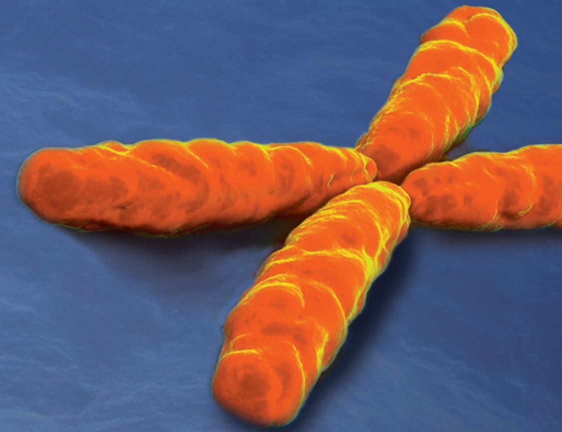
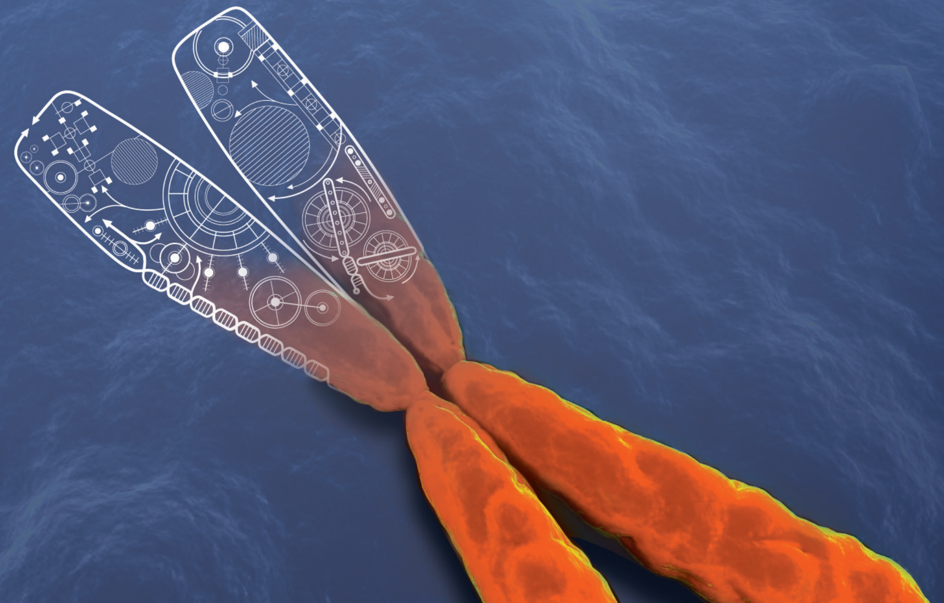
Choosing an expression system

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 our wide variety of expression systems, you're sure to find one that meets your needs (Table 1). The following pages focus on building and cloning options for your gene and provide details on mammalian systems 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
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	thermofisher.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	thermofisher.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	thermofisher.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	thermofisher.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	thermofisher.com/algaeexpression

For a complete list of our expression systems, please go to **thermofisher.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. Invitrogen™ GeneArt™ Gene Synthesis tools go beyond traditional cloning and enable you to:

- Improve protein expression with Invitrogen™ 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, we also offer Invitrogen™ 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 Invitrogen™ GeneArt™ Gene Synthesis Kit, which provides all of the high-quality reagents necessary for successful production of synthetic fragments. Table 2 gives a summary of our gene synthesis options.

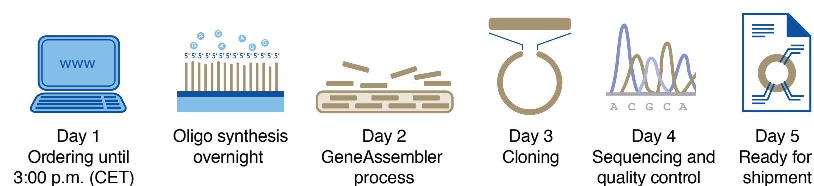


Figure 2. Invitrogen™ GeneArt™ SuperSPEED production schedule. Genes can be synthesized, cloned, and shipped in as few as 5 business days.

Table 2. Gene synthesis options.

	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 physical template required • No synthesis and cloning hands-on time needed • Optional service 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 and 8 business days for fragments up to 3,000 bp	From 9 business days for genes up to 1,200 bp

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

Optimizing expression

Optimization of your gene sequence can be beneficial from several perspectives. 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.

Secondly, if you consider total costs for your expression experiments, an optimized gene with higher expression rates can save you money. With higher expression rates, you might consider to scale down experimental setup and save on costs for materials (e.g., media, vessels).

Thirdly, gene optimization can help you to also obtain genes, which, due to instability elements in the wild type sequence, might not be efficiently cloned by other cloning methods.

How does it work?

The Invitrogen™ GeneOptimizer™ algorithm determines the optimal gene sequence for your expression system as part of a real multiparametric approach, which is not just simply a codon optimization process (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.

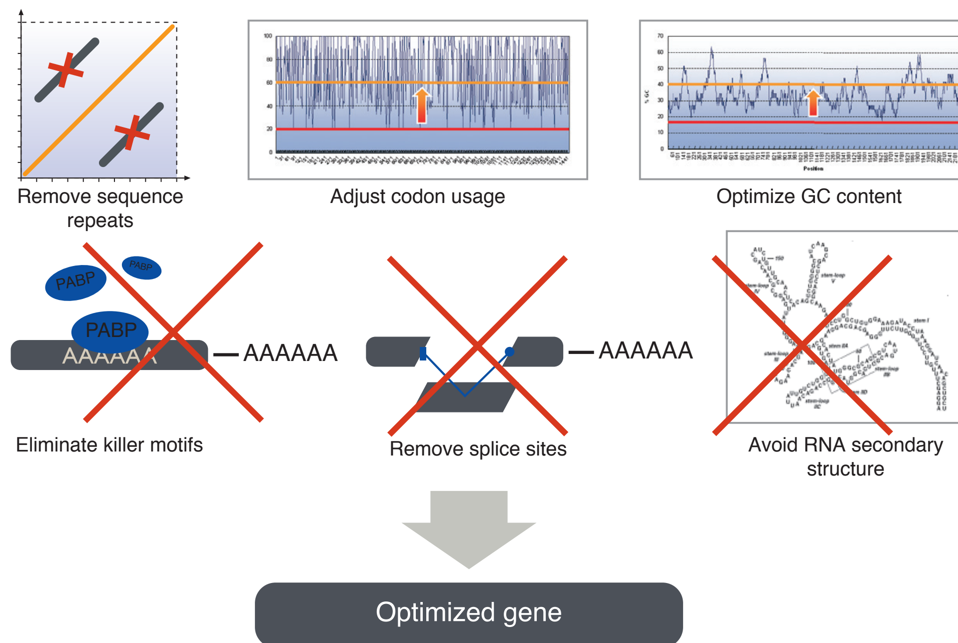


Figure 3. The GeneOptimizer algorithm determines the optimal gene sequence for your experiments. The algorithm takes many factors into account—for example, it removes DNA sequence repeats, optimizes codon usage and GC content, and minimizes the formation of RNA secondary structures that may reduce protein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

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 (example in Figure 4). 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

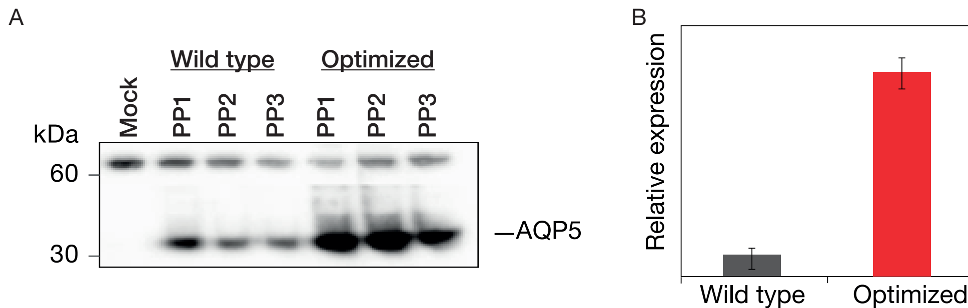


Figure 4. Comparative expression analysis of wild type vs. the optimized gene for aquaporin 5, an integral membrane protein. (A) Cell lysate was analyzed by western blot using an anti-His antibody. A 60 kDa protein used to standardize protein amount is visible. **(B)** Expression levels were derived for the wild type and optimized construct (mean of 3 independent transfections) and the relative expression from each construct is indicated. Figure adapted from Fath et al., 2011 [1].

To continue our GeneOptimizer gene optimization success story and demonstrate its true value, we performed a large-scale competitor study. Genes for 3 different human kinases were optimized and synthesized in-house or by 5 different competitors. Triplicate expression studies in HEK 293 cells not only show that the GeneArt optimization raises expression over the wild type genes, but also performs better than any competitor's optimization algorithm in every case (Figure 5).

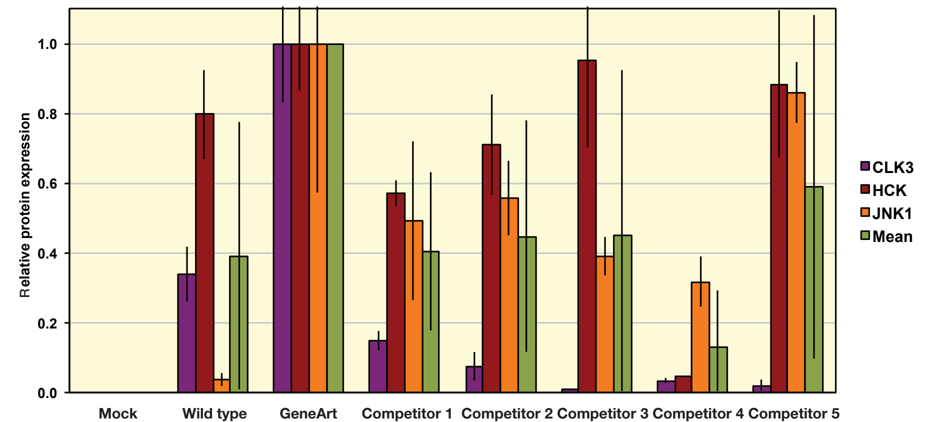


Figure 5. Expression levels from wild type genes and the same genes optimized by GeneArt technology and five competitors. Relative protein expression values are normalized to the respective GeneArt™ sequence.

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 **thermofisher.com/geneoptimization**

GeneArt Strings DNA Fragments

GeneArt Strings DNA Fragments are custom-made linear double-stranded DNA fragments up to 3 kb. A quantity of at least 200 ng of GeneArt Strings DNA Fragments is produced within 5 business days, ready for cloning in your lab. GeneArt Strings DNA Fragments can be ordered online and offer a fast, convenient, and cost-effective 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 (Figure 6) show a high probability of finding a correct clone if you sequence:

- 2–4 full-length clones for Strings DNA Fragments ≤1 kb
- 3–5 full-length clones for Strings DNA Fragments 1–2 kb
- 4–8 full-length clones for Strings DNA Fragments 2–3 kb

We offer several reliable and efficient Invitrogen™ cloning systems, such as restriction enzymes, TOPO™ cloning, Gateway™ cloning, GeneArt™ Seamless Cloning, and GeneArt™ Type IIs Assembly Kits (see page 10). We recommend that you first choose a suitable cloning system and then design your Strings DNA Fragments according to the respective requirements.

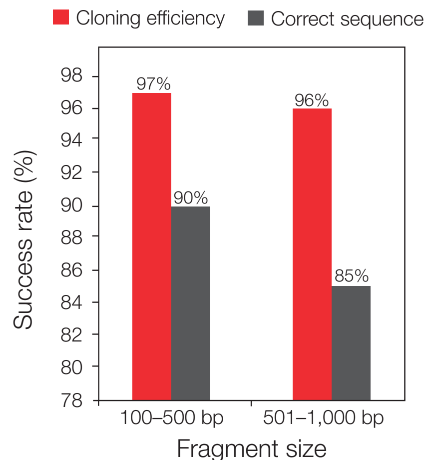
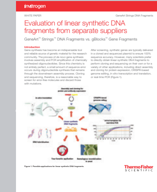


Figure 6. Cloning Strings DNA Fragments using restriction enzyme cloning. The success rate of conventional restriction enzyme cloning was evaluated with more than 1,000 fragments directly taken from our standard gene synthesis manufacturing process. Fragments of different size ranges, 100–500 bp and 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.

GeneArt Strings DNA white paper

See how GeneArt Strings DNA Fragments outperformed IDT gBlocks™ Gene Fragments.



Download the white paper at **thermofisher.com/strings**

Did you know?

GeneArt Strings DNA Fragments are also available as GeneArt Strings DNA Libraries (see page 21 for more details).

Choice of your vector

GeneArt Elements Vector Construction

The choice of the appropriate expression vector is essential for the success of your protein expression experiments. We offer a broad variety of expression vectors for all common expression systems, which can be found using the Vector Selection Tool at **thermofisher.com/vectors**

If your required vector is not commercially available, you can design and create your individual vector using our Invitrogen™ GeneArt™ Elements™ Vector Construction services. We offer a growing collection of biologically well-characterized components, such as promoters, terminators, enhancers, operators, and open reading frames, with exactly defined sequences and functionalities (Table 3). These predefined parts can be individually combined with custom components to build your favorite vector. Our online portal features *in vitro* assembly of predefined and custom components without any limitations from the DNA sequences at the junctions. The designed vector can be directly ordered via our online order portal.

Table 3. Examples of elements that can be used to construct customized vectors.

Part number	Public name	Part family/class	Part category
GA-pro-00016.1	CMV promoter	regulation	promoter
GA-pro-00015.1	EF-1alpha promoter	regulation	promoter
GA-cut-00012.1	AscI	construction	restriction site
GA-rep-00001.1	emerald GFP	CDS	reporter
GA-rep-00002.1	YFP TOPAZ	CDS	reporter
GA-cut-00094.1	KpnI	construction	restriction site
GA-clv-00002.1	TEV recognition site	CDS	protease cleavage site
GA-tag-00002.1	6x His tag	CDS	tag (purification, detection)
GA-cod-00063.1	Stop codon TAA	CDS	codon
GA-pla-00001.1	BGH polyA site	regulation	polyA site
GA-ori-00002.1	f1 origin	regulation	replication origin
GA-pro-00020.1	SV40 promoter	regulation	promoter
GA-cod-00001.1	Start codon	CDS	codon
GA-tag-00006.1	V5 tag	CDS	tag (purification, detection)
GA-tag-00001.1	c-myc tag	CDS	tag (purification, detection)
GA-rca-00003.1	Neomycin resistance cassette for mammalian cells	CDS	resistance cassette
GA-res-00004.1	Neomycin resistance ORF; npt2	CDS	resistance gene
GA-tag-00007.1	HA tag	CDS	tag (purification, detection)

Additional parts can be found at **thermofisher.com/geneartelementsvc**

GeneArt online portal

Use our online portal for easy and convenient ordering.

Get started at:
thermofisher.com/genearttutorials

Choice of cloning kits

There is broad range of cloning technologies available to clone your GeneArt Strings DNA Fragment or gene synthesis product into your favorite vector yourself. We have developed a variety of easy-to-use cloning kits to meet your needs (Table 4).

Table 4. Suitable cloning systems for GeneArt Strings DNA Fragments and gene synthesis products.

Cloning system	Description	Technical resources
Restriction enzymes	Cloning by restriction enzyme digestion and ligation is a simple and easy way of moving a fragment of double-stranded DNA from one plasmid to another. GeneArt Strings DNA Fragments or gene synthesis products must have the restriction enzyme sites incorporated at the ends of the sequence, plus stuffer nucleotides. Continue cloning via standard restriction enzyme and DNA ligase technology.	**thermofisher.com/restriction**
GeneArt Type IIs Assembly kits	Assembly with Type IIs restriction enzymes and DNA ligase, also known as Golden Gate cloning, can be used to assemble up to eight DNA fragments. It is highly efficient and is not based on homologous recombination. Consequently, it is less prone to unwanted rearrangements due to repetitive or homologous sequences and minimizes the need for sequence confirmation of your final construct. GeneArt Strings DNA Fragments or gene synthesis products must have appropriate restriction enzyme sites incorporated at the ends (either *Aar*I, *Bsa*I, or *Bbs*I, depending on the kit), plus stuffer nucleotides. For more detailed information, refer to the GeneArt Type IIs Assembly Kit manual, “Guidelines for Generating DNA Inserts” (Cat. Nos. A15916, A15917, and A15918).	**thermofisher.com/typeiis**
GeneArt Seamless Cloning and Assembly kits	GeneArt Seamless Cloning and Assembly technology is a highly efficient, vector-independent system for the simultaneous and seamless assembly of up to four DNA inserts between 100 bp and 5 kb (recommended) and any vector, totaling up to 13–40 kb in length (depending on the kit). This technology relies on homologous recombination to assemble adjacent DNA inserts sharing end-terminal homology. GeneArt Strings DNA Fragments or gene sythesis products must have a 15–80 bp sequence overlap with the adjacent insert (including the cloning vector), and the length of the overlap depends on the size of the insert and the total size of the final construct. For detailed information, refer to the GeneArt Seamless Cloning and Assembly Kit manual, “Prepare DNA inserts by PCR” (Cat. No. A13288), or the GeneArt Seamless PLUS Cloning and Assembly Kit manual, “Guidelines for Successful Cloning and Assembly” (Cat. No. A14603).	**thermofisher.com/seamless**

Table 4. continued

Cloning system	Description	Technical resources
Gateway technology	Gateway technology is a universal cloning method based on the site-specific recombination properties of bacteriophage lambda. It provides a rapid and highly efficient way to move DNA sequences into multiple vector systems for functional analysis and protein expression. The most typical method of Gateway vector construction is to first create an entry clone by recombining your DNA insert with an Invitrogen Gateway pDONR vector. To do so, Strings DNA Fragments or gene synthesis products must have *att*B sites, and then are recombined with pDONR in a recombination reaction mediated by Invitrogen Gateway BP Clonase enzyme. For more detailed information, refer to the Gateway Technology with Clonase II manual, "Designing *att*B PCR Primers" (Cat. Nos. 12535029 and 12535037).	**thermofisher.com/gateway**
Zero Blunt TOPO PCR Cloning kits	Invitrogen™ Zero Blunt™ TOPO PCR Cloning provides a highly efficient 5-minute, one-step cloning strategy for the direct insertion of blunt-ended DNA fragments into a plasmid vector. Primers containing specific sequences are not required. Strings DNA Fragments are blunt-ended, and therefore no further manipulation is required. However, we recommend that you add an additional 5–10 nucleotides of random stuffer DNA to both ends of the DNA fragment because small terminal truncations can occur on linear DNA fragments. For more detailed information, refer to the Zero Blunt PCR Cloning Kit manual, "Clone into pCR-Blunt" (Cat. No. K270020).	**thermofisher.com/topo**
TA Cloning and TOPO TA Cloning methods	Invitrogen TA Cloning and TOPO TA Cloning methods provide a quick, one-step cloning strategy for the direct insertion of *Taq* polymerase–amplified PCR products (or DNA fragments with A-overhangs) into a plasmid vector. Primers containing specific sequences are not required. Strings DNA Fragments are blunt-ended, and therefore further manipulation is required to add end-terminal A-overhangs. For more detailed information, refer to the TA Cloning Kit manual, "Add 3´ A-Overhangs" (Cat. No. K200001), or the TOPO TA Cloning Kit manual, "Adding 3´ A-Overhangs Post-Amplification" (Cat. No. K450001).	**thermofisher.com/tacloning** **thermofisher.com/topo**

Subcloning options

If you have chosen or created your favorite vector, but don't want to do the cloning yourself, your gene can be inserted into the vector using one of our subcloning services (Table 5).

Table 5. Gene cloning and subcloning options.

	GeneArt Express Cloning service	Standard subcloning service
Production time in addition to gene synthesis	1–2 business days	4–5 business days
Vector choice	Growing list of commercially available expression vectors	All vectors possible
Deliverable	5 µg subcloned plasmid DNA	5 µg subcloned plasmid DNA and 5 µg pMx cloning vector with gene
Insert length	Up to 5 kb	No limit
Service description	Your gene will be subcloned directly into a growing choice of commercially available vectors for all major expression systems without subcloning in one of our pMx series standard cloning vectors first.	This service gives you full vector flexibility and your gene will be delivered in both our standard vector and the vector of your choice.

Vectors available for Invitrogen™ GeneArt™ Express cloning services

- pcDNA3.1(+)
- pcDNA3.3-TOPO
- pcDNA3.4-TOPO
- pFastBac1
- pDONR221
- pET100/D-TOPO
- pET151/D-TOPO
- pRSET A
- pYes2.1V5-His TOPO

Plasmid isolation

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

Go to thermofisher.com/plasmidprep

For more information on subcloning options and vector availability for express cloning service, go to **thermofisher.com/expressgenes**

Mammalian expression systems

Selecting a mammalian expression system

We offer a wide variety of mammalian expression systems for your needs. Ask yourself the three following questions, and then use Table 6 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 Invitrogen™ Lipofectamine™ LTX or Lipofectamine™ 3000 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 Gibco™ Expi™ expression systems for HEK293 and CHO cells, which are used for producing large amounts of protein, are 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 Gibco FreeStyle™ and Expi systems use suspension-adapted HEK293 or CHO cells at culture scales from 1 mL to 100 L to generate large amounts of protein. The Gibco Expi293™ Expression System has been demonstrated to produce up to 1 gram of protein per liter of cell culture.

A stable cell line that can be used over a long experiment or multiple experiments can be produced by various technologies. For targeted integration, we offer products and services including CRISPR and TAL effector-mediated genome editing tools as well as our Invitrogen™ Jump-In™ technology. If you don't want to produce your stable cell line on your own, you can benefit from our pre-engineered cell lines or custom cell line generation services. We have the in-house expertise to support your cell engineering projects whether your research requires engineered cells using CRISPR-Cas9 or TAL effector technology; stable isogenic cell lines using the Jump-In™ system; or large-scale, validated screening-ready cells for high-throughput studies. For randomly integrated stable cell lines, any Invitrogen™ Gateway™ pcDNA™ vector delivered by a cationic lipid–based transfection reagent such as Lipofectamine LTX Reagent may be used. For difficult-to-transfect cells, the Invitrogen™ 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 Invitrogen™ T-REx™ expression system for tetracycline-induced expression or the Invitrogen™ Flp-In™ T-REx™ system for tetracycline-induced expression with targeted genomic integration.

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

Table 6. Mammalian protein expression systems.

Application	System	Key features
Constitutive expression (CMV promoter)	pcDNA vectors	Constitutive 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
	ExpiCHO Expression System	Fully optimized system for transient production of recombinant protein in CHO cells
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

Genome editing

Do you know our genome editing tools for efficient cell engineering?

Learn more at:
thermofisher.com/geneedit

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

Expi expression systems

Mammalian transient expression systems enable flexible and rapid production of proteins. They are ideal for generating large amounts of protein within 1 to 2 weeks. With the Gibco™ ExpiCHO™ and Expi293™ Expression systems, we offer two fully optimized mammalian transient expression systems for ultrahigh protein yields in CHO and HEK293 cells. Both Expi293 and ExpiCHO systems comprise high-density, high-expressing cell lines, media specifically matched to the growth properties of the cells with expression enhancer and feed supplements, and high-efficiency transfection reagents. Both systems are able to generate g/L protein yields, allowing researchers to choose either a human HEK293 cell line or a CHO cell line for protein expression depending on the requirements of their research.

These systems enable expression of human or other mammalian proteins with more native folding and posttranslational modifications—including glycosylation—than expression systems based on hosts such as *E. coli*, yeast, or insect cells.

ExpiCHO Expression System

The Gibco™ ExpiCHO™ mammalian transient expression system marks a revolutionary leap forward in the transient production of recombinant proteins in CHO cells. This fully optimized system has been designed to deliver protein yields up to 3 g/L, which is higher than the best HEK293–based systems. The ExpiCHO system enables you to rapidly and cost-effectively access CHO cell–expressed proteins early in the drug development process, providing you the highest confidence that transiently expressed drug candidates will mimic downstream biotherapeutics manufactured in CHO.

The Gibco™ ExpiCHO™ Expression System Kit brings together a high-expressing CHO cell line, a chemically defined animal origin–free culture medium, an optimized culture feed, and a high-efficiency transfection reagent that synergistically act to provide titers as much as 160x higher than the Gibco™ FreeStyle™ CHO Expression System and 3x higher than the Gibco™ Expi293™ Expression System. Expression levels of up to 3 g/L were achieved for human IgG proteins (Figure 7).

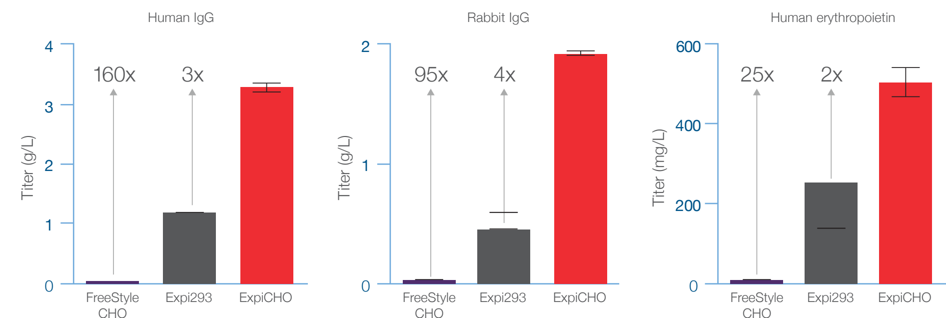


Figure 7. Recombinant protein titers in FreeStyle CHO, Expi293, and ExpiCHO systems. Expression levels of human IgG, rabbit IgG, and erythropoietin in FreeStyle CHO, Expi293, and ExpiCHO transient expression systems are shown. ExpiCHO titers range from 25x to 160x those of FreeStyle CHO, and 2x to 4x those obtained using the Expi293 system.

Go straight to CHO cells

The ExpiCHO Expression System will revolutionize the use of CHO cells for transient protein expression during early phase drug candidate screening. The glycosylation patterns of recombinant IgG produced by the Expi293 and ExpiCHO transient expression systems were compared to the same protein expressed in stable CHO cells. It is clear that glycosylation of recombinant IgG produced in the ExpiCHO system is much more like glycosylation of the stable CHO cell system (Figure 8) which provides a very strong correlation between transiently expressed drug candidates and downstream biotherapeutics manufactured in CHO.

Literature tip

Download the new protein expression handbook

Find more information at: **thermofisher.com/expressionguide**

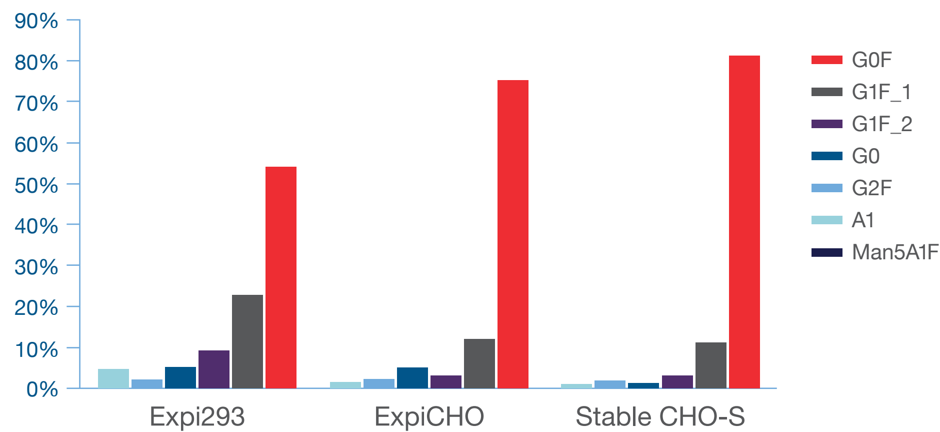


Figure 8. Glycosylation patterns in proteins expressed by CHO cells (transient and stable) and HEK 293 cells. Human IgG supernatant samples were collected and purified using Applied Biosystems™ POROS™ MabCapture™ A resin. Following PNGase digestion and APTS labeling, glycan profiles were analyzed on an Applied Biosystems™ 3500 Series Genetic Analyzer by capillary electrophoresis.

For more information, go to **thermofisher.com/expicho**

Expi293 Expression System

The Expi293 Expression System is a major advance in transient expression technology for rapid and ultrahigh-yield protein production in human cells. It is based on high-density culture of Gibco™ Expi293F™ Cells in Gibco™ Expi293™ Expression Medium. Transient expression is powered by the cationic lipid-based Gibco™ 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 are attained with previous 293-transient expression systems. Expression levels of greater than 1 g/L can be achieved for IgG and non-IgG proteins (Figure 9).

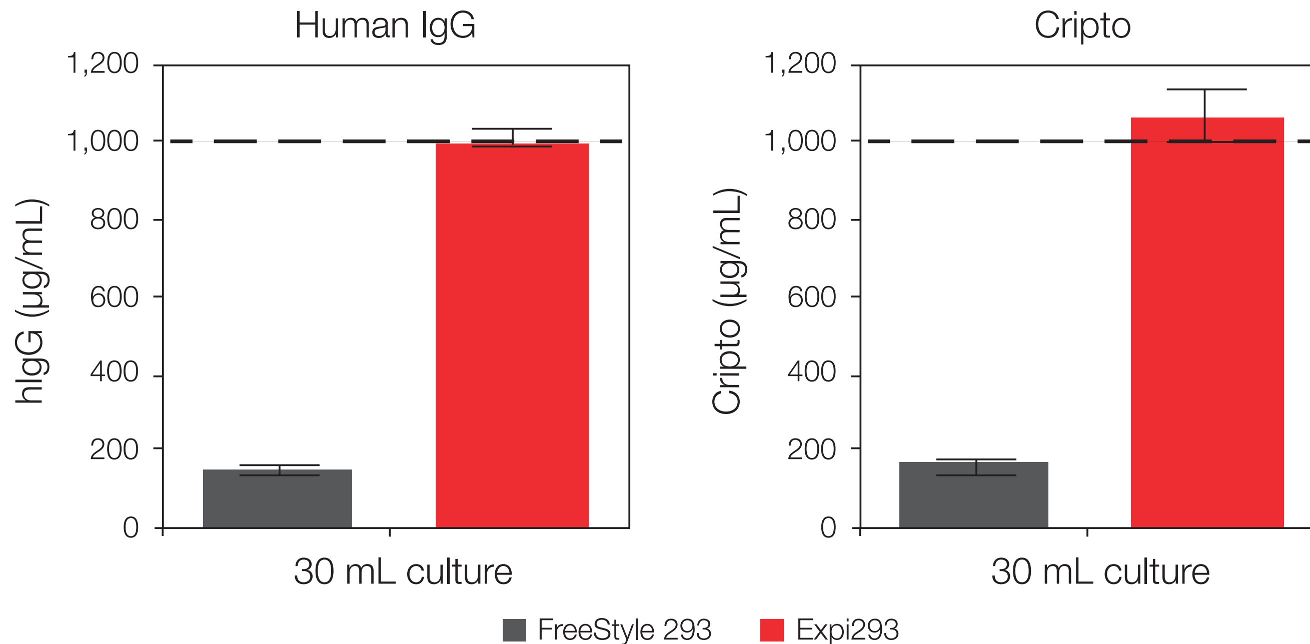


Figure 9. Expression of human IgG and Fc-tagged Cripto protein is achieved at levels of >1 g/L in the Expi293 Expression System.

For more information go to **thermofisher.com/expi293**

Protein production services

Fast, reliable protein production from mammalian cells

The Invitrogen™ 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 10). 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 7 for more information on the service.

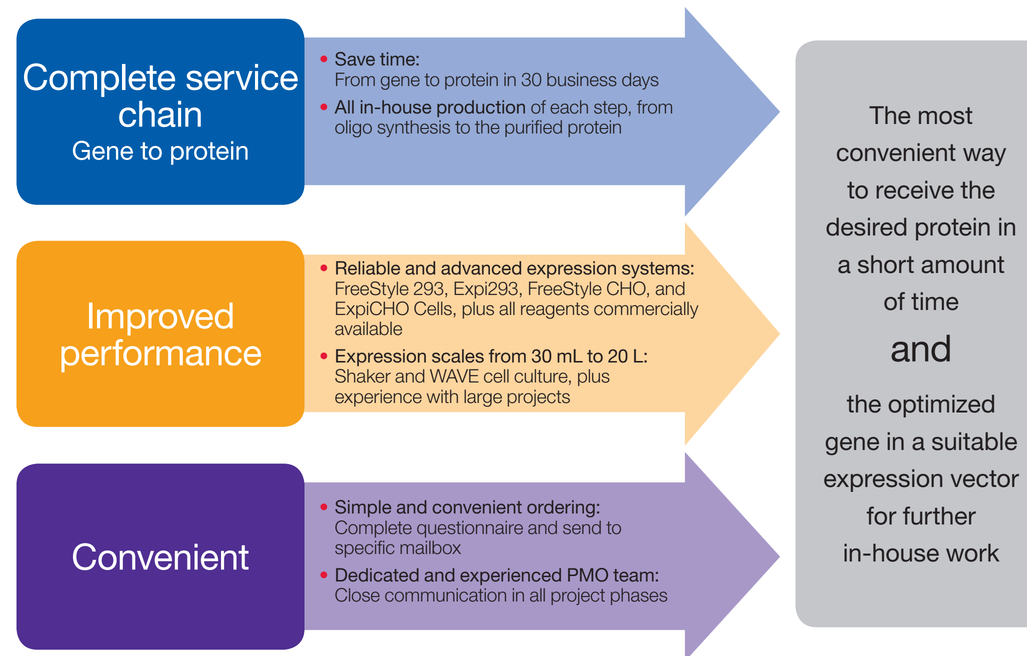


Figure 10. Advantages of the GeneArt Genes-to-Proteins service.

For more information, go to **thermofisher.com/g2pservice**

Table 7. Summary of protein production services.

Service	Description	Deliverables
Gene to protein **specified culture volume**	Protein expression and purification from **customer specified culture volume** of transiently transfected HEK293 or CHO cells	• All protein purified from specified culture volume (alternatively, culture supernatant or cells) • Documentation including Coomassie-stained gel and western blot
Gene to protein **specified protein amount**	Protein expression and purification of **customer specified guaranteed protein amount** using transiently transfected HEK293 or CHO cells **Pilot expression service mandatory**	• Purified protein amount as specified by customer • Documentation including Coomassie-stained gel and western blot
Gene to protein **pilot**	Feasibility study for determination of production yield from transiently transfected HEK293 or CHO cells	• Price quote for production of a customer specified protein amount • Documentation including Coomassie gel and western blot • Purified protein

The combination of GeneArt expression optimization and our advanced expression systems (e.g., Expi293) can lead to higher overall project reliability and expression yields than obtained with nonoptimized genes.

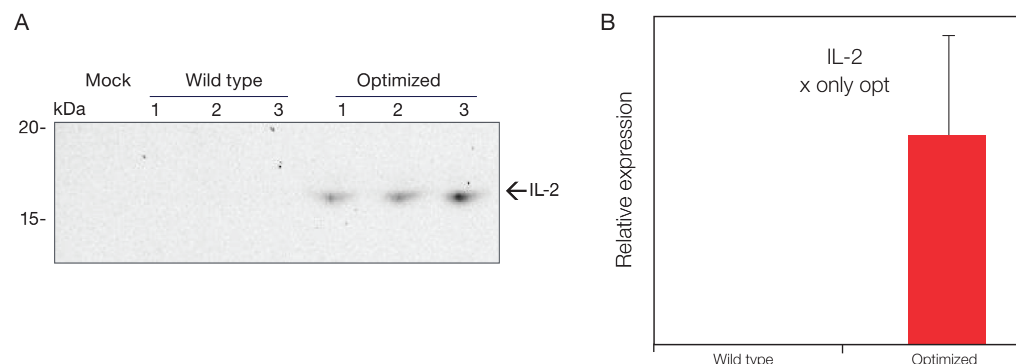


Figure 11. Improved protein expression with gene optimization. (A) Three independent transfections of wild type and optimized IL-2 constructs were analyzed by western blot. **(B)** The resulting bands were analyzed by densitometry. From Fath et al., 2011; see page 7, Figure 4.

Please email **geneartsupport@thermofisher.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

```
                 50      60      70         80
wild type  GVVPILVELDGDVNGHKFSVSGEGEGDATYGKLTLKFIC   T
F58A       GVVPILVELDGDVNGH KASVSGEGEGDATYGKLTLKFICT
S59A       GVVPILVELDGDVNGHK FAVSGEGEGDATYGKLTLKFIC T
V60A       GVVPILVELDGDVNGHKF SASGEGEGDATYGKLTLKFIC T
S61A       GVVPILVELDGDVNGHKFSV  AGEGEGDATYGKLTLKFIC T
- - -      - - -
```

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

```
                 50      60      70      80
wild type  GVVPILVELDGDVNG HKFSVSGEGEGDATYGKLTLKF  ICT
F58A       GVVPILVELDGDVNG HKASVSGEGEGDATYGKLTLKF  ICT
F58C       GVVPILVELDGDVNGH KCSVSGEGEGDATYGKLTLKFI  CT
F58D       GVVPILVELDGDVNGH KDSVSGEGEGDATYGKLTLKF  ICT
F58 E      GVVPILVELDGDVNGHK ESVSGEGEGDATYGKLTLKF  ICT
- - -      - - -
```

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, quality control by next-generation sequencing available
Applications: construction of recombinant antibody libraries, promoter libraries, and combining of substitutions identified by site-directed mutagenesis

```
                 50      60      70      80
wild type  GVVPILVELDGDVNGH KXXVSGXGEXXATYGKLTLKFIC T
peer A01   GVVPILVELDGDVNGH KRQVSGGGEGDATYGKLTLKFIC T
peer A02   GVVPILVELDGDVNGH KAGVSGEGEGDATYGKLTLKFIC T
peer A03   GVVPILVELDGDVNGH KIYVSGLGEGDATYGKLTLKFIC T
peer A04   GVVPILVELDGDVNGH KLKVSGPGEGDATYGKLTLKFIC T
- - -      - - -
```

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

```
                 50    60      70         80
wild type  GVVPILVELDGDVNGHKFSVSGEGEGDATYGKLTLXXIC   T
peer A01   GVVPILVELDGDVNGHK FTVSGEGEGDATYGKLTLLKIC T
peer A02   GVVPILVELDGDVNGH KTSVSGEGE DDATYGKLTLIGICT
peer A03   GVVPILVELDGDVNGHKFSVSG  AGEGDATYGKLTLQDIC T
peer A04   GVVPILVELDGDVNGHKFSVSGEGEG  YATDGKLTLLPIC T
- - -      - - -
```

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

```
                 50     60  //   2702
wild type  GVVPILVELDGDVNGHKFSVSGEGEGD  ATYGKLTLKFICT
peer A01   .VVPILVELDGDVNGHKFSVSGEGEGDATYGKLTLKFI   CT
peer A02   GVVPILVELDGDVNGHKFSVSGEGEGDATYGKLT   LK_ _ _
peer A03   _ _ _ILVELDGDVNGHKFSVSGEGEGDATYGKLTLK   FI_ _
peer A04   _ _ _ _ _ _ _DGDVNGHKFSVSGEGEGDATYGKLTLK   _ _ _
```

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

GeneArt Strings DNA Libraries

GeneArt Strings DNA Libraries are our cost effective alternative to complete combinatorial libraries with also a shorter turn-around time. They consist of pools of custom-made GeneArt Strings DNA Fragments of 200-2,000 bp and can be used for antibody and protein engineering or other applications.

- Up to three blocks of degenerated nucleotides with randomized distribution
- Each block can consist of up to 30 bp using full IUPAC code (Table 8) of DNA nucleotides
- Ready for cloning (see cloning options at page 10)
- At least 500 ng are produced within 10-15 business days
- Full GeneArt order portal support

Table 8. IUPAC code.

Symbol	Description	Bases represented				
A	Adenine	A				1
C	Cytosine		C			1
G	Guanine			G		1
T	Thymine				T	1
W	Weak	A			T	2
S	Strong		C	G		2
M	aMino	A	C			2
K	Keto			G	T	2
R	puRine	A		G		2
Y	pYrimidine		C		T	2
B	not A (B comes after A)		C	G	T	3
D	not C (D comes after C)	A		G	T	3
H	not G (H comes after G)	A	C		T	3
V	not T (V comes after T and U)	A	C	G		3
N or -	any Nucleotide (not a gap)	A	C	G	T	4

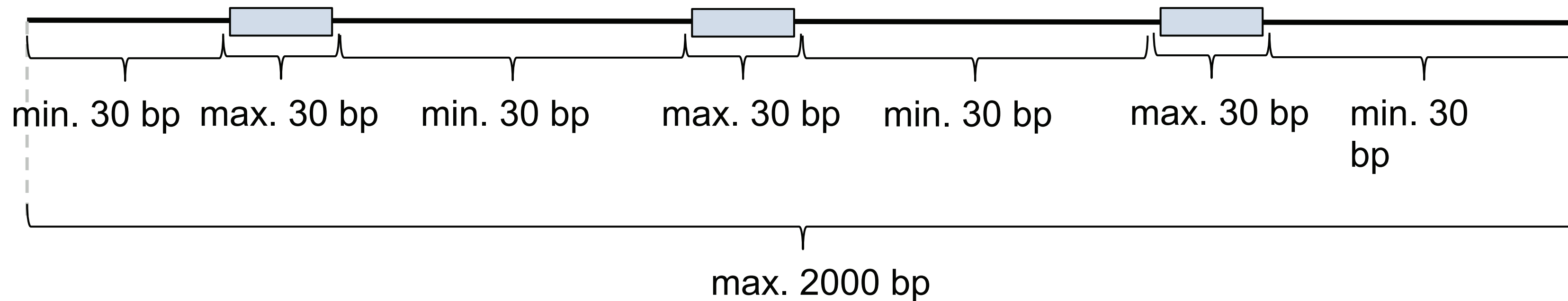


Figure 12. Schematic example for the design of a GeneArt Strings DNA Library. Three degenerated nucleotide blocks are shown in grey.

Find more information about GeneArt Strings DNA Libraries at **thermofisher.com/strings**

invitrogen

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

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