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Introduction to the gene synthesis workflow

Why choose GeneArt Gene Synthesis?

Have you ever lacked the time to clone your favorite gene? Conventional PCR and cloning techniques require optimization and troubleshooting, which take up valuable lab time and resources. What if you could have your favorite gene made for you, analogous to an optimized, error-free PCR reaction? This is where gene synthesis, sometimes referred to as synthetic biology, comes in. It’s simply molecular cloning made easy.

Here’s a step-by-step guide to a typical Invitrogen™ GeneArt™ Gene Synthesis workflow:

**Step 1—Analyze**
Utilize sequencing platforms to generate knowledge and analyze cellular data (using Invitrogen™ Vector NTI™ sequence analysis and design software, and Ion Torrent™ or Applied Biosystems™ SOLiD™ Systems)

**Step 2—Design**
Apply rational design software to optimize gene expression (using Invitrogen™ GeneArt™ GeneOptimizer software)

**Step 3—Construct**
Whether you outsource to us or do it yourself, select from our leading line of GeneArt products (such as gene synthesis tools) and services (such as subcloning and plasmid preparation) for optimal speed, quality, and performance

**Step 4—Express**
From small-scale research to industrial production in applied markets, put it all together with a complete toolbox of products and services for superior cell line development and growth, as well as protein expression and production

Which technology is right for you?
To view our online gene synthesis product selection guide, please go to thermofisher.com/genesynthesisproductselection
GeneArt It

We are your partner for gene synthesis through protein and cell production

**Invitrogen™ GeneArt™ Gene Synthesis**
A reliable and cost-effective method for obtaining customized DNA constructs with 100% sequence accuracy, GeneArt Gene Synthesis offers:

- The largest capacity and fastest production processes
- Outstanding quality—ISO 9001:2008 certification
- Gene optimization for reliable maximum protein expression

**Invitrogen™ GeneArt™ Strings™ DNA Fragments**
A time-saving alternative to PCR, GeneArt Strings DNA Fragments are available up to 3 kb and are compatible with any downstream cloning method of choice, providing:

- An economical solution that maintains the gene synthesis benefits of both flexibility and performance
- Easy ordering—you can directly enter, edit, optimize, and order your sequence through the online Invitrogen™ GeneArt™ portal

**Invitrogen™ GeneArt™ custom services**
Whether you’re looking to save time or improve upon existing processes, GeneArt services provide:

- A single resource for all of your outsourcing needs
- Gene synthesis, custom cell lines, and mammalian/baculovirus protein production
- Cloning and plasmid prep services
- ISO 9001:2008 and ISO 13485:2003 certification and responsive project management

**Invitrogen™ GeneArt™ Directed Evolution**
Directed evolution can be used to create biomolecules for basic research, medical science, and industrial production. Results can be achieved quickly, with just a few rounds of mutagenesis and selection. GeneArt Directed Evolution services include:

- Invitrogen™ GeneArt™ combinatorial libraries
- Invitrogen™ GeneArt™ site-directed mutagenesis
Invitrogen™ GeneArt™ facilities
Our Global Centers of Excellence for gene synthesis services are located in Regensburg, Germany and Pleasanton, California, USA. Since 1999, GeneArt products and services have provided superior gene synthesis solutions for researchers around the globe.

While the Regensburg site provides the market with a monthly capacity of ~7.5 million bp, the newer Pleasanton site currently has an output of ~2 million bp per month. Both sites are certified by the International Organization for Standardization.

We’re constantly expanding the capacities of both sites to meet the growing market demand for synthetic genes. More than 300 employees worldwide are currently working to provide you with the best possible products and services.
GeneArt Gene Synthesis services offer ease, flexibility, and reliability for your daily DNA work. Gene synthesis is a cost-effective, time- and resource-saving method for obtaining your desired DNA construct with 100% accuracy (see Figure 1 on page 9). It is a true alternative to conventional molecular biology techniques, while enabling better, more reliable protein expression and quality. GeneArt Gene Synthesis tools go beyond traditional gene synthesis by enabling expression optimization for maximum performance.

**Features**
- Proprietary expression and mRNA stability optimization
- Nearly unlimited flexibility in gene and vector design
- Empirically proven increases in expression
- Ready-to-use constructs for expression and transfection
- Easy online ordering

**Quality**
- All processes are ISO quality certified
- Comprehensive quality documentation included
- Automated production processes
- 100% sequence validation

**Performance**
- Project setup assistance and individual project support
- Maximum performance available using the GeneArt GeneOptimizer algorithm, with its scientifically proven track record of expression optimization
- Maximum production speed and worldwide delivery; capacity and reliability supported by a fully automated, industrial-scale gene-processing platform

**Timely production**
Visit our website for the latest updates on turnaround time

**Standard**
- Up to 1,200 bp in 9 business days*
- Up to 3,000 bp in 14 business days

**Express**
- Up to 1,200 bp in 7 business days
- Up to 3,000 bp in 12 business days

**SuperSPEED**
- Up to 1,200 bp in 5 business days
- Up to 1,800 bp in 7 business days

*Production time is the number of business days required to synthesize GeneArt genes in our manufacturing facility. Delivery time is in addition to production time and depends on the destination of the shipment.

**There is an upgrade fee for Express and SuperSPEED deliveries.

For more information, please contact geneartsupport@thermofisher.com or go to thermofisher.com/genesynthesis
**GeneArt Gene Synthesis**

- GeneOptimizer optimization algorithm
- GeneAssembler gene synthesis platform

**Classic cloning**

- cDNA library
- PCR amplification
- Addition of cloning sites
- Ligation & transformation
- Colony screening
- Sequencing
- Plasmid with natural gene

**Figure 1.** GeneArt Gene Synthesis and optimization are typically faster than classic cloning methods and can provide better results.

**Solutions from gene fragments to genomes (long genes)**

- High-throughput gene synthesis with a capacity of >8 Mbp per month (for fast and reliable production of DNA fragments for assembly)
- Expert design and assembly technologies and process expertise for the assembly of constructs from ten to several hundred kilobases, with timelines starting from 25 business days

- Robust technologies to build DNA constructs with highest complexity
- Flexible sequencing solutions for sequence verification and final QC
- Proven track record of reliable and timely production for long and complex constructs
- Assembly kits that enable do-it-yourself construction and assembly

For more information, please go to [thermofisher.com/longgenes](https://thermofisher.com/longgenes)
GeneArt Strings DNA Fragments

GeneArt Strings DNA Fragments are custom-made, uncloned, double-stranded linear DNA fragments up to 3,000 bp in length, assembled from synthetic oligonucleotides using the same high-quality process developed for GeneArt Gene Synthesis (Figure 2). Strings DNA Fragments are delivered dried and ready for resuspension, cloning, and screening to enable identification of the correct clone. GeneArt Strings DNA Fragments are a fast and smart alternative for getting your synthetic gene and clone into your expression plasmid (see Figure 3 for an application example).

Affordable
GeneArt Strings DNA Fragments are a cost-effective alternative to gene synthesis

Flexible
Full gene design and cloning flexibility; clone with any downstream method of choice such as restriction enzymes, Invitrogen™ TOPO™ cloning, Invitrogen™ Gateway™ cloning, Invitrogen™ GeneArt™ Seamless Cloning and Assembly, or Invitrogen™ GeneArt™ Type IIs Assembly

Fast
At least 200 ng of GeneArt Strings DNA Fragments are produced within 5 (for up to 1,000 bp) or 8 (for 1,000–3,000 bp) business days*

Streamlined
Enter your sequence and directly edit, optimize, and order through our online GeneArt portal

*Depending on the nature of the sequence, production time can vary. Delivery times are in addition to the specified production times and depend on location.

For more information, please go to thermofisher.com/strings

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

- Up to three blocks of degenerate nucleotides with randomized distribution
- Each block can consist of up to 30 bp using full IUPAC code of DNA nucleotides
- Ready for cloning and screening
- Full GeneArt order portal support
- At least 500 ng are produced within 10–15 business days

For more information, please go to thermofisher.com/strings
Production of DNA fragments using GeneArt technology

Design

Oligonucleotide synthesis

Gene assembly

Direct cloning

Direct assembly

Downstream assembly

Figure 2. Producing a synthetic gene using GeneArt Strings DNA Fragments.

Figure 3. Ten Strings DNA Fragments up to 3,000 bp were cloned using the GeneArt Seamless Cloning and Assembly Kit. The figure shows the percentages of clones with correct length as identified by colony PCR (cloning efficiency), and the percentages of correct-sequence clones (fidelity) based on the number of full-length clones.
Gene optimization

The value of GeneArt technology: maximize protein expression

Experience enhanced protein expression based on gene optimization

Production of recombinant human proteins in human cells for biomedical research and product development can be hampered by low expression yields. Similarly, transient protein expression in any research lab is at risk of being insufficient, especially (but not exclusively) for heterologous expression. These expression issues can limit researchers’ ability to conduct their structural and functional analyses, delaying (and in some cases halting) the discovery process or the entire research project.

Gene optimization is the solution to traditional protein expression limitations. Common pain points associated with protein expression, such as yield, can now be addressed in a rational and systematic way. Using data available from published literature in combination with proprietary data, the GeneOptimizer algorithm determines the optimal gene sequence for your expression experiments (Figure 4). Optimization has been experimentally proven to increase protein expression rates up to 100-fold in a variety of host systems (Fath et al. 2011).†

GeneArt GeneOptimizer sequence processing includes the following:

- Identification of the best way to incorporate your requested sequence elements
- Elimination of cryptic splice sites and mRNA-destabilizing sequence elements for increased mRNA stability
- Codon optimization and GC content adaptation for your expression system
- Avoidance of undesirable mRNA secondary structures
- Elimination of repetitive sequences
- siRNA-resistant forms of wild type genes that can be used in RNAi rescue experiments

For more information, please go to thermofisher.com/geneoptimization

Figure 4. The GeneOptimizer software tool optimizes sequences for maximum transcription and translation efficiency.

Up to a 100-fold increase in expression
The GeneOptimizer process has been shown to help deliver large increases in protein yield via a combination of factors that stabilize mRNA and maximize translation efficacy (Figure 5). Efficacy has been proven in a large comparative gene expression study (Fath et al. 2011).†

Figure 5. Comparative expression analysis of wild type vs. an optimized human gene. Western blot analysis using α-His antibody. Expression levels of 2 independent transfections per wild type and optimized construct are displayed, showing a 3-fold difference in expression in this particular analysis. Standardization is based on endogenous protein.
Expression-ready genes

GeneArt Express cloning service
You can save 4–5 days of turnaround time and receive expression-ready genes faster by choosing express cloning into selected Invitrogen™ vectors (Figure 6). Simply order via the online GeneArt portal and add the Express cloning service to your gene synthesis request. You will receive your synthesized gene in the selected expression vector, without the intermediate pMX vector that is delivered with subcloning services.

Turnaround time for gene synthesis and Express cloning starts at 11 business days (depending on the length of your gene). Adding gene synthesis Express delivery in addition to Express cloning saves an additional 2 days, so it is possible to realize a turnaround time of 9 business days for your cloned, expression-ready gene. Genes qualifying for Express cloning must be <5 kb and must not contain complex sequences (optimization of your sequence with GeneOptimizer software in the GeneArt portal can reduce complexity).

For more information, please go to thermofisher.com/expressgenes

Figure 6. Express cloning directly into an expression vector (top) compared with classic subcloning from pMX into an expression vector (bottom).

*Expression vector example. Multiple choices for vectors are available.
Cloning and plasmid services

**Invitrogen™ GeneArt™ Subcloning service**
Get your gene ready to use in your downstream applications. After gene synthesis, we can subclone your gene into any vector you send us, and we will store the plasmid for future subcloning projects. We also have our own vectors in stock for you to choose from. Even complex cloning projects with multiple open reading frames are no problem (e.g., large double-gene vectors expressing monoclonal antibodies).

The GeneArt Subcloning service can be used to move your synthetic gene into any vector you choose, including novel vectors built using Invitrogen™ GeneArt™ Elements™ vector construction. If desired, we deliver a customized production report detailing the reagents we used and the lot numbers. Focus on your results and leave the subcloning to GeneArt services.

**Benefits**
- Competitive price
- Convenient—tell us what you need and receive your ready-to-use clone quickly
- Reliable—inserts are 100% sequence-verified and documented
- Sole provider of Gateway recombination cloning technology
- Confidential—no data or material are provided to third parties
- Extendable—order a plasmid prep in addition to receiving your subcloned gene, ready to transfec

**Invitrogen™ GeneArt™ Plasmid service**
GeneArt Plasmid DNA preparations have consistently high quality and can be used for research applications and preclinical studies. From vector construction to the production of plasmid DNA, GeneArt Plasmid services make the development and execution of your project easy.

**High-quality, scalable plasmid DNA preparation service**
- Highly pure and homogeneous plasmid DNA
- Low levels of endotoxin (down to 0.01 EU/μg DNA)
- Milligram to gram scale
- Fill-and-finish service (receive your DNA aliquotted and labeled for immediate use, per your specifications)

**Project documentation**
A Certificate of Analysis (CoA) is provided with every plasmid order. Premium documentation of all DIN ISO–certified production processes can be provided upon request.

For more information, please go to [thermofisher.com/geneartplasmid](thermofisher.com/geneartplasmid)
GeneArt Elements vector construction

Design your own individualized vector

The GeneArt Elements DNA parts collection is comprised of a growing subset of biologically well-characterized parts such as promoters, terminators, enhancers, operators, and open reading frames with defined sequences and functionalities. GeneArt Elements parts can be combined with your defined custom sequences/parts.

The online GeneArt portal features an application that allows for in vitro assembly of parts (Figure 7). The intuitive drag-and-drop functionality of the portal facilitates the combination of parts symbolically, without any limitations from the nucleic acid sequence at the junctions. The designed vector can be directly ordered via the portal.

Figure 7. The GeneArt Elements vector construction template within the online GeneArt portal.
• Flexible—GeneArt Elements DNA can be seamlessly assembled with minimal design restrictions*

• Reliable—all offered parts are 100% sequence-verified

• Easy to use—intuitive CAD-like software within the GeneArt portal assists you with vector design

To demonstrate the flexibility of the offering, vectors were made that contain GFP with either an IgK or tPA secretion leader or an Fc-Tag affinity tag. HEK 293 cells were transiently transfected with the indicated constructs, and the conditioned media from the transfected cells was subjected to western analysis with an anti-GFP antibody (Figure 8). All GeneArt Elements vector construction results were functional, generating a variety of positive controls for protein secretion.

Figure 8. Western blot showing proteins expressed from pCMV-GFP vectors constructed using GeneArt Elements vector construction. The proteins in conditioned media were probed with an anti-GFP antibody.

*Functionality in the final vector requires that you choose a resistance marker and an {E.coli} origin of replication from the GeneArt Elements repository.

For more information, please go to thermofisher.com/elementsvc
GeneArt Elements combinatorial parts assembly

GeneArt Elements combinatorial parts assembly (CPA) is a way to combine predefined DNA parts (e.g., promoters, terminators, enhancers, operators, open reading frames) to build a diverse set of larger constructs (Figure 9). Users simply provide individual part sequences and progression of the parts within the order form. The final construct is synthesized, with the reading frame and sequence junctions managed seamlessly by the assembly.

All conceivable part combinations can be created to build and test new metabolic pathways or a variety of expression cassettes to identify the most valuable combination for your research needs.

• Tested for accuracy—all CPAs that are delivered as separate constructs are sequenced as part of our ISO 9001:2008–certified quality management system; we only ship constructs with exact sequence agreement

• Cost-effective—parts of the final constructs have the potential to be used multiple times

• Comprehensive—all permutations of the available genetic elements are possible

Figure 9. CPA construct set up with 5 different yeast promoters in combination with 5 different yeast terminators, in order to find the optimal combination for protein expression.
To demonstrate the CPA process, we generated a combinatorial library composed of a small set of yeast promoters and corresponding terminators from the GeneArt Elements part collection to analyze relative luciferase expression levels (Figure 10). In this experiment, the highest firefly luciferase expression was shown using a TEF1 promoter/TEF1 terminator combination. This demonstrates the proof of concept and can be extrapolated to test other functional elements, such as in metabolic pathways.

Figure 10. Analysis of a luciferase expression CPA experiment, comparing 25 combinations of the promoters and terminators shown in Figure 9.
Gene-to-protein and Genes-to-cell lines

Genes-to-proteins
Starting with only a nucleotide sequence, GeneArt services can provide purified protein typically within 30 business days. Protein purified from mammalian suspension cells (Gibco™ FreeStyle™ 293, FreeStyle™ CHO, Exp293™, and ExpiCHO™ cells), or produced by the baculovirus expression system (e.g., in Sf9, Sf21) helps ensure correct protein folding and processing. The combination of optimized genes with our advanced expression systems usually leads to higher protein yields than achievable with other expression systems and wild type genes.

Service types
Culture volume–based service:
• Protein expression and affinity purification from customer-specified culture volume

• Deliverables are the purified protein and a comprehensive report, including Coomassie-stained gel and western blot with affinity tag–detected protein

Guaranteed protein amount service:
• Protein expression and affinity purification of customer-specified protein amount guaranteed

• Pilot project with culture volume–based service is required to evaluate the expression yield per liter

• Deliverables are the purified protein and a comprehensive report, including Coomassie-stained gel and western blot with affinity tag–detected protein

• Additional purification steps and analytics are possible

Benefits
• Seamless project processing—gene synthesis and protein purification from one source

• Speed—from gene to protein typically within 20–30 business days

• Increased protein yield—combination of optimized genes and advanced expression systems routinely leads to increased expression

• Improved protein expression—optimized genes can show expression of otherwise nonexpressible proteins

• Full process transparency and transferability—usage of our commercially available reagents and protocols; delivery of optimized gene in expression vector together with the purified protein

• Comprehensive documentation—every protein comes with a detailed report; analytical SEC or other advanced analytics optionally available

• Experienced team—routine handling of multiple large projects with tight timelines

For more information, please contact geneartprotein@thermofisher.com or go to thermofisher.com/G2Pservice

Genes-to-cell lines
We use the Gibco™ Freedom™ CHO DG44 system to generate cell lines with high levels of protein expression (e.g., antibodies in the milligram to gram per liter range).

• Generation of Invitrogen™ Flp-In™ expression cell lines based on existing host cell lines using expression-optimized genes

• Generation of monoclonal CHO cell lines for high-level protein expression

Mammalian cell line engineering services
We’ll apply our expertise in creating stable cell lines to design, develop, and validate a custom stable cell line using validated GeneArt TALs or GeneArt CRiSPR and a customer-supplied mammalian cell line.

For more information, contact us at custom.services@thermofisher.com

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GeneArt Directed Evolution

Protein engineering

GeneArt 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.

Combinatorial libraries

DNA sequences will be diversified using preassembled trinucleotides as building blocks (trinucleotide mutagenesis, or TRIM, technology) for the chemical synthesis process. This allows for the complete customization of the amino acid composition at randomized sites and thus avoids the occurrence of unwanted stop codons or amino acids.

The deliverable is a pool of clones for your screening assay (e.g., phage display, mRNA display). Receive the library as linear double-stranded PCR product (at least 2 μg of DNA provided), or have the library cloned into the E. coli strain of your choice with transformation rates of $10^9$ or higher. With the cloned library, at least 30 μg of plasmid DNA and 12 x 0.5 mL glycerol stocks will be provided. Also available with optional next-generation sequencing quality control.

Site-directed mutagenesis

Introduce single or multiple mutations (substitutions, insertions, or deletions) into existing DNA sequences. Up to 5 regions covering 40 bp each can be modified within the template sequence.

Receive separate constructs made from a template sequence—all variants with 100% insert verification—as 5 μg of plasmid preparation.

For other directed evolution services or benefits and applications, please go to thermofisher.com/directedevolution
The online GeneArt portal

Online ordering
The GeneArt Gene Synthesis sequence design and order portal ("GeneArt portal") on our website offers convenient and simple ordering of your gene synthesis projects. This automated portal helps you enter, edit, and optimize your sequences, and offers immediate price quote information and ordering capabilities. Nearly all of the GeneArt services can be ordered using this intuitive ordering tool. You can even perform in silico cloning and store your gene sequences, projects, and personal vectors for future design. Save time and have full control and flexibility with this one-stop shopping feature.

Instructions on using the GeneArt portal:
Start and organize your projects in the Project Manager (Figure 11). Its smart design leads to efficient management of your projects. Then set up your individual project requirements with the icon-based Project Configurator (Figure 12).

For more information, please go to thermofisher.com/genesynthesis
Ordering GeneArt Gene Synthesis: an example (Figure 13)

- Immediate ordering of wild type genes in the starting screen—just copy and paste your sequence
- Choice of standard pMX vector or expression-ready vectors for Express cloning
- Quick procedure for sequence editing and full gene optimization (optional, if wild type is not desired)
- Editing: include 5’ or 3’ cloning sites; add/delete/exchange sequences
- Optimization: choose expression organism, sequences to protect, and motifs to avoid
- Downloadable sequence summary containing all customer requirements
- Add to cart and obtain price quote for easy ordering

The GeneArt portal can be used to order many GeneArt products and services, in addition to gene synthesis. These include GeneArt Strings DNA Fragments, GeneArt Elements, GeneArt™ Precision TALs, and services such as subcloning, plasmid preps, directed evolution, and custom proteins and cell lines. Moreover, you can check the status of your web order(s) in the manufacturing process—simply use the GeneArt™ GeneObserver™ module within the portal, available 24 hours per day.

Please see our library of videos on GeneArt portal usage, including overview, setup, quick order, optimization, and subcloning services, at thermofisher.com/genearttutorials
Related product information

Invitrogen products
Synthetic Biology
Cloning reagents and tools
GeneArt Type II Assembly kits
GeneArt Seamless Cloning and Assembly kits
Gateway Cloning technology
Genome editing
GeneArt CRISPR products and services
GeneArt TALs products and services
Vector NTI software
Transfection reagents
Plasmid isolation
Gibco Expi293 Expression system
Gibco ExpiCHO Expression system
Ion Torrent next-generation sequencing
GeneArt Gene to Protein brochure

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thermoFisher.com/gateway
thermoFisher.com/genomeediting
thermoFisher.com/crispr
thermoFisher.com/tals
thermoFisher.com/vectornti
thermoFisher.com/transfection
thermoFisher.com/plasmidpreP
thermoFisher.com/expi293
thermoFisher.com/expicho
thermoFisher.com/lonTorrent
thermoFisher.com/genetoprotein