

GENE SYNTHESIS SERVICES

invitrogen™  
by *life* technologies™

build your next  
**breakthrough**

GeneArt® Gene Synthesis Services

*life*  
technologies™

# GeneArt® services—your partner for gene synthesis through protein production

Whether you need industry-leading gene synthesis services or optimized protein expression, or want to outsource the entire process from gene synthesis to protein production, this brochure outlines GeneArt® services to help you succeed.

## Gene synthesis

An increasingly cost-effective method for obtaining DNA constructs with 100% sequence accuracy, GeneArt® services offer:

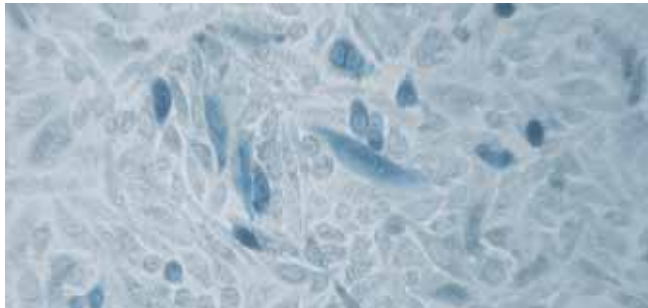
- Largest capacity and fastest production processes
- Outstanding quality—ISO 9001:2008 certification
- Gene optimization for maximum protein expression



## 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 to custom cell line and protein production
- ISO 9001:2008 certification and responsive project management



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# GeneArt® services by Life Technologies

Considered one of the foremost global specialists in the area of synthetic biology, the GeneArt® facility is the world's leading manufacturer of synthetic genes.

## History

In 1999, Dr. Ralf Wagner, Dr. Marcus Graf, and Dr. Hans Wolf founded the GeneArt® company after leaving the University of Regensburg. Dr. Wagner needed custom-built genes to develop vaccines. They were not commercially available at the time and thus had to be synthesized *de novo*. The synthetic genes performed exceptionally well, and the scientists recognized their potential benefit for research and industry. They launched GeneArt® services with the vision to synthesize artificial genes on a large scale at affordable rates, thus enabling access to these new tools for scientists worldwide.

The GeneArt® company was awarded one of the largest gene synthesis contracts for completion of the "Mammalian Gene Collection Program" by the US National Institutes of Health. In addition, they produced subgenomic elements for the construction of the first synthetic bacterial genome by the J. Craig Venter Institute.

## Customers

The world's largest pharmaceutical companies, many international biotechnology companies, and major universities/research institutes benefit from using the GeneArt® technology platform. Customers rely on the expertise and experience of GeneArt® scientists to improve enzymes, construct genetically altered bacteria, and develop and produce new therapeutics and vaccines, in addition to providing the highest-quality synthetic genes.



# Gene optimization to maximize protein expression

Production of recombinant human proteins in human cells for biomedical research and product development can be hampered by low expression yields. These expression issues can limit researchers' ability 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 pain points associated with protein expression—yield, solubility, and functionality—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 1). Optimization has been experimentally proven to increase protein expression rates up to 100-fold in a variety of host systems.

## Multigene study of optimized mammalian genes

In a first-of-its-kind study<sup>1</sup>, five important, biologically relevant protein classes were selected for study—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 sliding window GeneOptimizer® algorithm.<sup>2</sup> The corresponding wild type genes were subcloned using native sequences available from the NCBI database. Each gene was then prepped and expressed in triplicate in HEK293T cells.

## Summary

Following optimization, the 50 genes all showed reliable expression and 86% exhibited elevated expression (Figure 2, page 4). Further analysis showed no detrimental effect on protein solubility and unaltered functionality was demonstrated for JNK1, JNK3, and CDC2 using optimized constructs (data not shown).

### Using the GeneOptimizer® algorithm:

- 96% of optimized genes showed equal or higher protein expression
- Protein yields increased up to 15-fold with optimized genes
- 100% of optimized genes expressed versus 88% of wild type genes

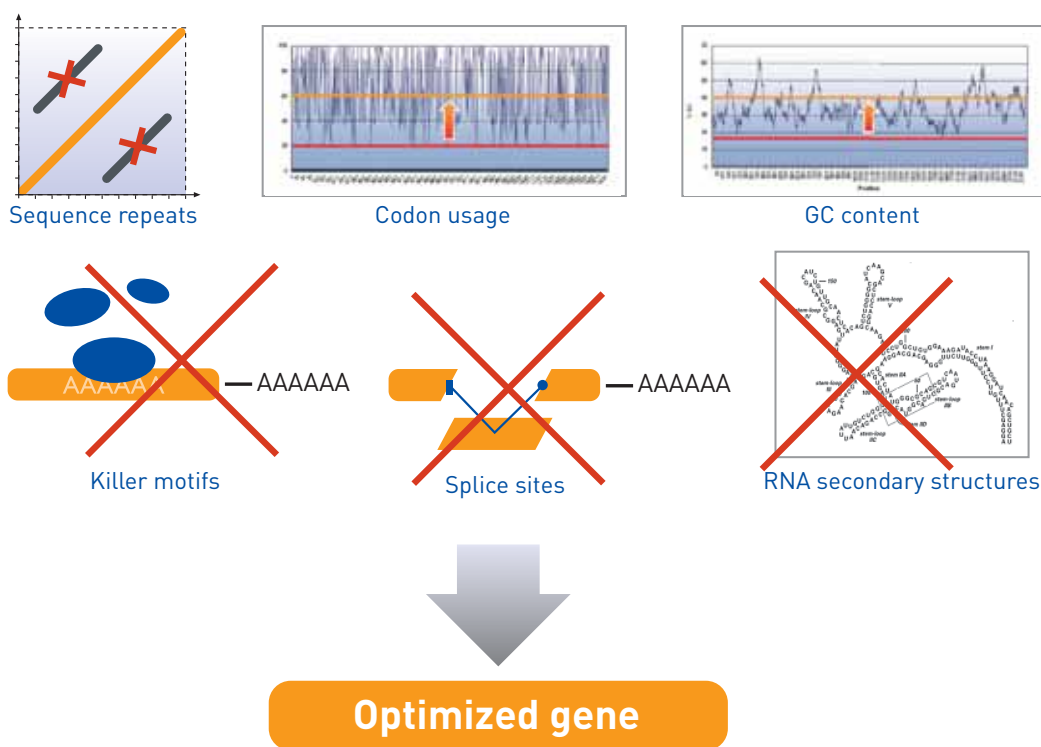
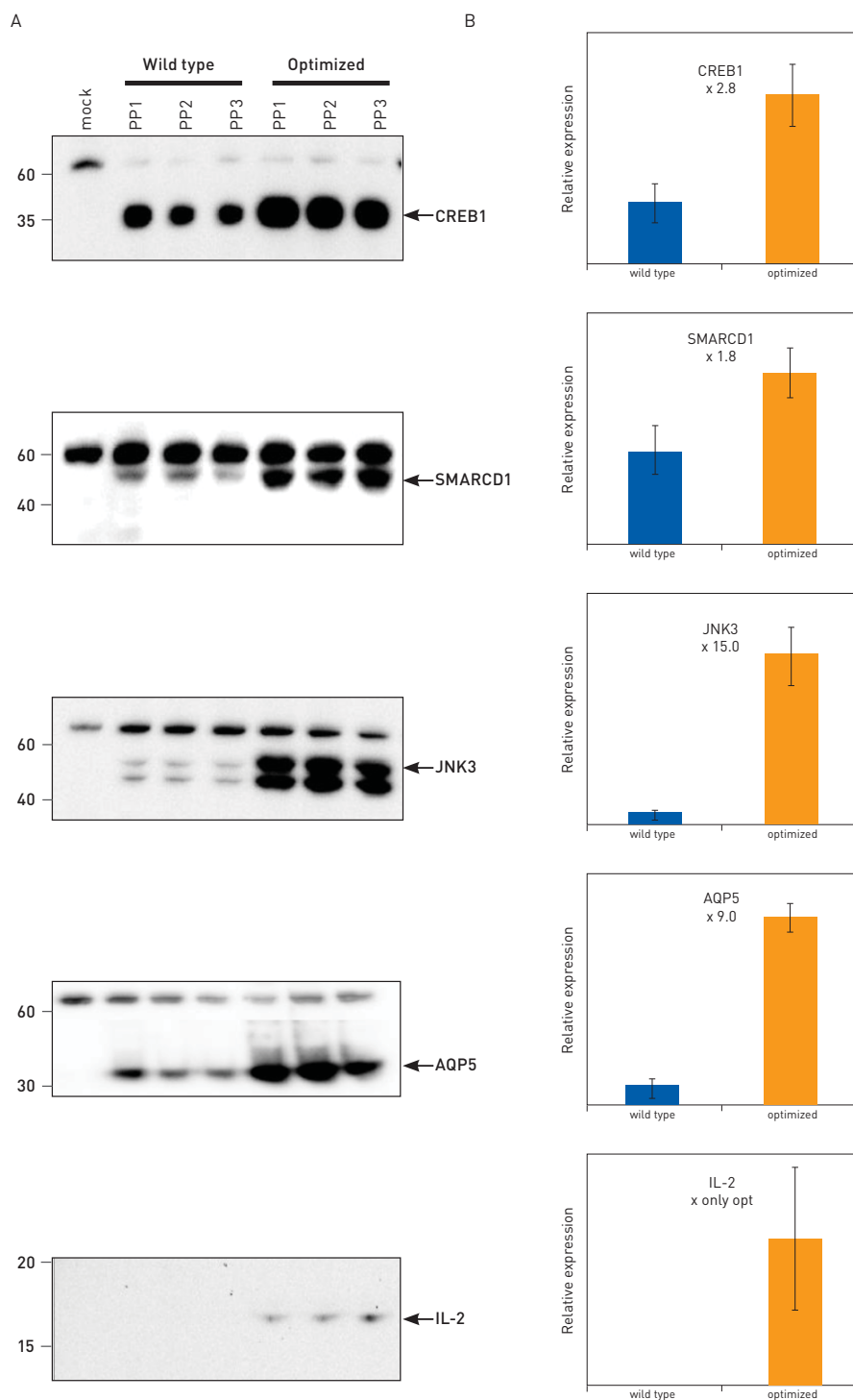


Figure 1. Proprietary GeneOptimizer® algorithm determines optimal gene sequence for your experiments.



**Figure 2. Comparative expression analysis.** Comparative expression analysis of wild type versus optimized genes representing different protein classes. **(A)** Cell culture supernatants (immunomodulators, IM) or cell lysates (all other protein classes) were analyzed by western blots using the  $\alpha$ -Penta-His antibody. One example from each protein class is shown. A cross-reactive 60 kD protein used to standardize protein amounts is visible, including in the empty-vector negative controls (mock). Left: molecular weight (kDa) markers, right: arrows indicating specific protein bands. **(B)** Relative expression levels were derived by comparing mean expression (three independent transfections) of wild type or optimized constructs, with wild type set to 1. The X-fold expression increase following gene optimization is indicated for each protein (only opt = no detectable wild type expression).

## 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(3):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(3):215–225.



# Gene synthesis

Gene synthesis has become a cost-effective, time- and resource-saving method for obtaining nearly any desired DNA construct with 100% accuracy. It outperforms conventional molecular biology techniques in terms of time and cost, while providing equivalent or better expression performance, and construct stability and quality. GeneArt® gene synthesis tools go beyond traditional synthesis and enable expression optimization and maximum performance.

## Benefits

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

## Quality

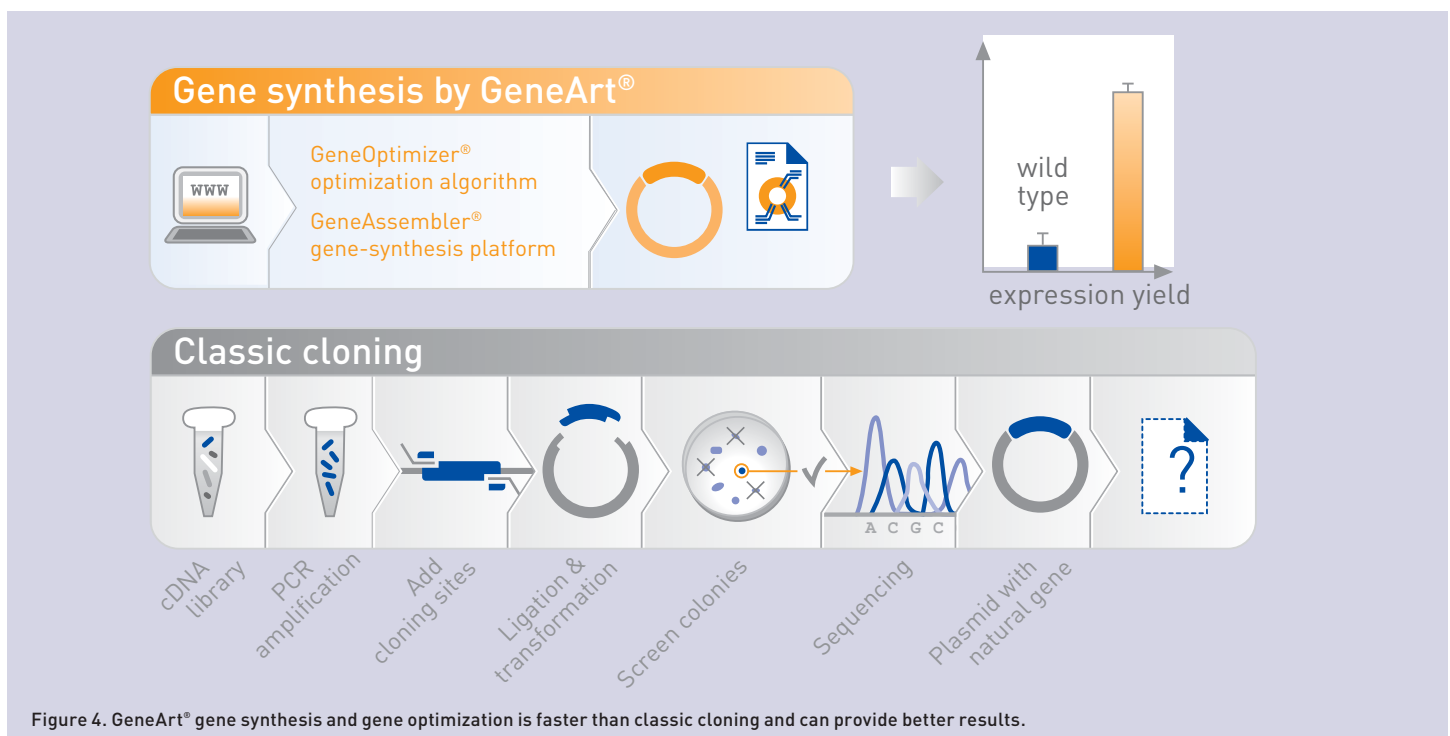
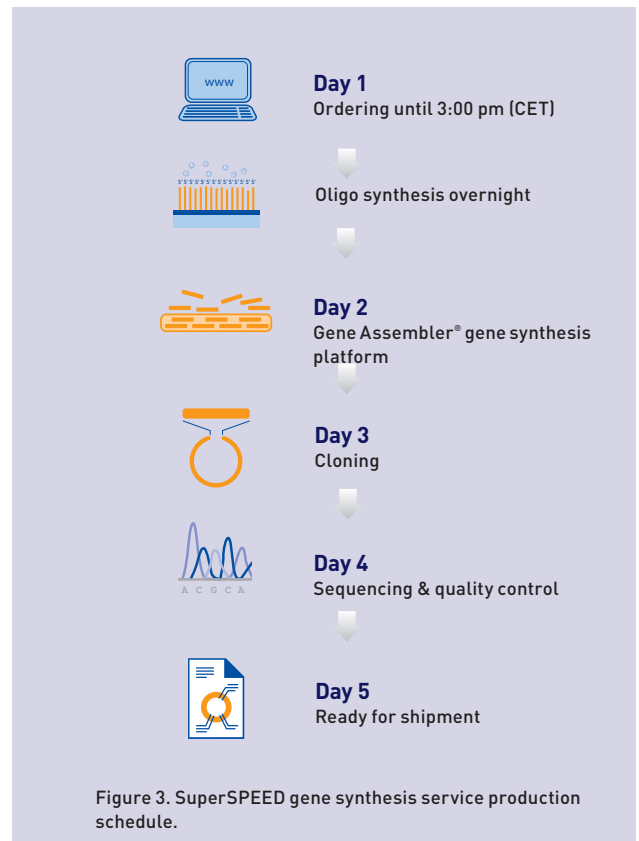
- All processes are ISO 9001:2008 quality certified
- Comprehensive quality documentation included
- Automated production processes

## SuperSPEED

- Up to 1,200 bp in 5 business days (Figure 3)
- Up to 1,800 bp in 7 business days
- Emergency genes on request

## Performance

- Project setup assistance and individual project support
- Maximum performance is available using the GeneOptimizer® optimization algorithm—the industry-preferred optimization algorithm
- Maximum production speed and worldwide delivery; capacity and reliability is supported by the world's only industrial gene-processing platform



# Directed evolution

Directed evolution strategies are the most efficient method for creating proteins with improved or novel properties. The directed evolution technologies from GeneArt® synthesis 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, alanine scans, etc.

```

      50      60      70      80
.....|.....|.....|.....|.....|
wild type GVVPILVLDGVDVNGHKFSVSGEGEGDATYGKLTLLKFICT
F58A      GVVPILVLDGVDVNGHKASVSSGEGEGDATYGKLTLLKFICT
F59A      GVVPILVLDGVDVNGHKFAVVSSGEGEGDATYGKLTLLKFICT
F60A      GVVPILVLDGVDVNGHKFSAVSSGEGEGDATYGKLTLLKFICT
F61A      GVVPILVLDGVDVNGHKFSVAAGEGEGDATYGKLTLLKFICT
.....

```

## 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, alienation of proteins from patented sequences, etc.

```

      50      60      70      80
.....|.....|.....|.....|.....|
wild type GVVPILVLDGVDVNGHKFSVSSGEGEGDATYGKLTLLKFICT
F58A      GVVPILVLDGVDVNGHKASVSSGEGEGDATYGKLTLLKFICT
F58C      GVVPILVLDGVDVNGHKCVSSGEGEGDATYGKLTLLKFICT
F58D      GVVPILVLDGVDVNGHKDVSSGEGEGDATYGKLTLLKFICT
F58E      GVVPILVLDGVDVNGHKEVSSGEGEGDATYGKLTLLKFICT
.....

```

## 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 combination of substitutions identified by site-directed mutagenesis.

```

      50      60      70      80
.....|.....|.....|.....|.....|
wild type GVVPILVLDGVDVNGHKXXVSGXXXATYGKLTLLKFICT
peer A01  GVVPILVLDGVDVNGHKRQVSGGGGDATYGKLTLLKFICT
peer A02  GVVPILVLDGVDVNGHKAGVSGGGGDATYGKLTLLKFICT
peer A03  GVVPILVLDGVDVNGHKTYVSGLGGDATYGKLTLLKFICT
peer A04  GVVPILVLDGVDVNGHKLKVSGPGGDATYGKLTLLKFICT
.....

```

## 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, modification of enantioselectivity of enzymes, etc.

```

      50      60      70      80
.....|.....|.....|.....|.....|
wild type GVVPILVLDGVDVNGHKFSVSSGEGGDATYGKLTLLKFICT
peer A01  GVVPILVLDGVDVNGHKFTVSSGEGGDATYGKLTLLKFICT
peer A02  GVVPILVLDGVDVNGHKTVSSSGEGGDDATYGKLTLLKFICT
peer A03  GVVPILVLDGVDVNGHKFSVSGAEGGDATYGKLTLLQDICT
peer A04  GVVPILVLDGVDVNGHKFSVSSGEGGVATDGKLTLLPICT
.....

```

## Truncation libraries

Create custom-defined populations of up to 40,000 in-frame truncated constructs.

**Benefits:** Highest quality by avoiding out-of-frame mutations.

**Applications:** solubility screen, minimal functional-size evaluation, domain identification, inhibitory screenings, epitope mapping, etc.

```

      50      60      270      280
.....|.....|.....|.....|.....|
wild type GVVPILVLDGVDVNGHKFSVSSGEGDATYGKLTLLKFICT
peer A01  GVVPILVLDGVDVNGHKFSVSSGEGDATYGKLTLLKFICT
peer A02  GVVPILVLDGVDVNGHKFSVSSGEGDATYGKLTLLKFICT
peer A03  GVVPILVLDGVDVNGHKFSVSSGEGDATYGKLTLLKFICT
peer A04  GVVPILVLDGVDVNGHKFSVSSGEGDATYGKLTLLKFICT
.....

```

# Cell lines and proteins

Starting with only the nucleotide sequence, GeneArt® services can provide purified protein within 30 business days. Protein purification from transiently transfected mammalian cells assures correct folding and processing. Usage of optimized genes for stable cell-line generation leads to production of high levels of active protein.

## Benefits

- Seamless project processing—gene synthesis and protein purification from one source
- Speed—from gene to protein within 30 business days

## Services

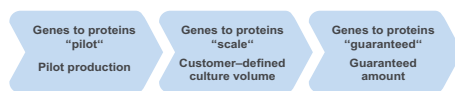
### Expression analysis

Verification of your expression construct or evaluation of the best variant of your protein



### Protein production

Protein production in mammalian cells using advanced transient transfection protocols



### Cell line development

Generation of cloned or uncloned stable cell lines



## Examples

- Improved expression reliability—optimized genes show expression of otherwise non-expressible proteins (Figure 4)
- Protein production by transient transfection—natural or approved artificial leader-peptides yield excellent secretion of engineered protein into the culture supernatant (Figure 5)
- Improved transgene expression—productivity of antibodies from stable cell lines is improved with optimized expression constructs (Figure 6)

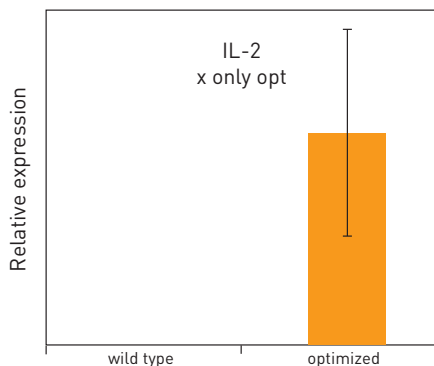
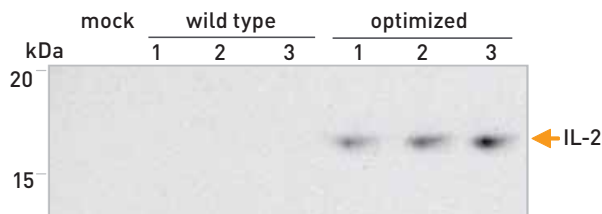


Figure 4. Three independent transfections of each wild type and optimized IL-2 gene were analyzed by western blot and densitometric analysis of the resulting bands.

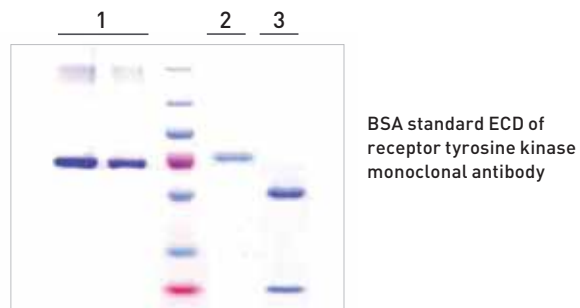


Figure 5. 10 mg of the extracellular domain of a receptor tyrosine kinase and a monoclonal antibody (heavy and light chain) were purified from the supernatant of transiently transfected HEK293/CHO cells via affinity tag/ProteinA.

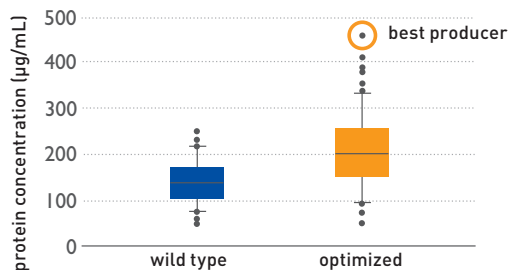


Figure 6. Stable cell lines expressing different combinations of HC and LC of a human antibody were generated with wild type and optimized sequences and antibody production yield was compared.



# Plasmid services

GeneArt® plasmid DNA purification protocols help ensure consistent high quality for research applications and preclinical studies. From vector construction to the production of plasmid DNA for preclinical trials, GeneArt® plasmid services make the development and execution of your project easy.

## High-quality, scalable plasmid DNA for all applications

- Highly pure and homogeneous plasmid DNA
- Low levels of endotoxin (down to 0.01 EU/μg pDNA)
- Milligram to gram scale
- Fill-and-finish service

## Applications

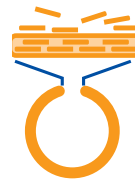
- Cell transfection
- Immunization studies
- Preclinical studies
- Toxicological studies
- DNA vaccine research

## Vector construction

Using expression-optimized, exchangeable genetic elements, GeneArt® plasmid services manage individual vector design and complex subcloning projects, delivering streamlined plasmid production and optimal gene expression.

## 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 on request.



Gene synthesis



Subcloning



Plasmid production



Vector construction



Production documentation





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