GENCEFE

Gene Synthesis and Related Services

GenCefe Biotech's experienced R&D and production team has established an advanced gene synthesis technology platform, standardized operating procedures, and a strict quality control system. We provide high-quality customized services including gene synthesis, codon optimization, PCR cloning, subcloning, plasmid preparation, site-directed mutagenesis, and synthetic DNA library construction.

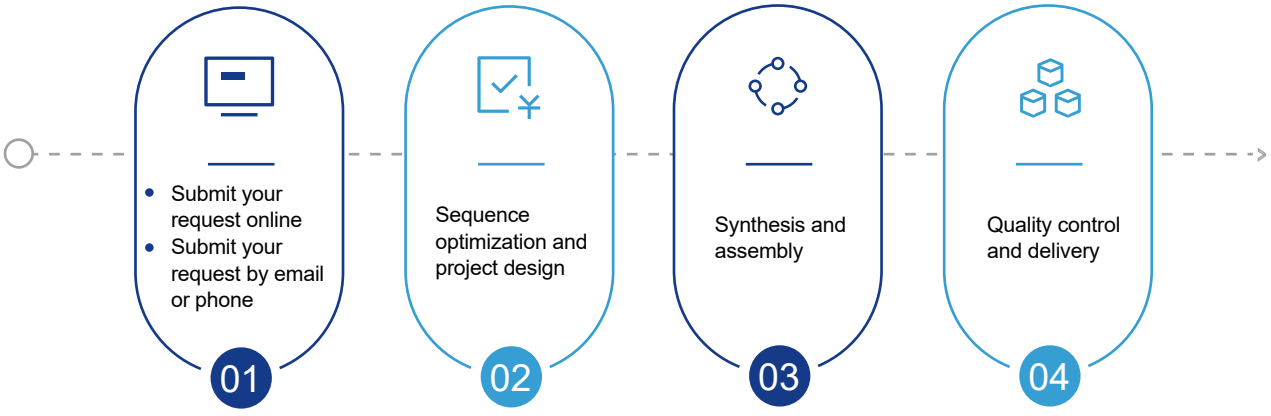
Just submit the gene sequence you need, and GenCefe will deliver the desired plasmid to you on time!

GenCefe Biotech Gene Synthesis Platform

Key Features

- **Advanced technology platform:** our team has successfully synthesized various complex gene sequences, such as repetitive sequences, GC/AT-rich sequences, etc., and delivered the plasmids according to customer-specific requirements
- **Professional technical support:** considerate pre-sales and after-sales services, free codon optimization and project design, timely project updates, and free technical consulting
- **On-time delivery:** Experienced production and R&D teams ensure the on-time delivery rate of over 95%
- **Intellectual property protection:** the nucleic acid/amino acid sequence provided by the customer is kept strictly confidential and will not be distributed to third parties in any form

How to Order



Gene Synthesis Services

GenCefe provides synthesis services for natural sequences, codon-optimized sequences, gene libraries, complex sequences, long sequences, and other customized gene sequences, which can be cloned into any site of the specified vector, and the successful delivery rate can reach 99.9%. Our professional technical support team can communicate with you and solve technical problems quickly and efficiently.

All you need to do is enter the sequence and your requirements, and we will deliver the ideal plasmid containing the target gene on time.

Service Process

Sequence Optimization & Project Design → Oligo Synthesis → Gene Assembly → Cloning → Quality Control → Delivery

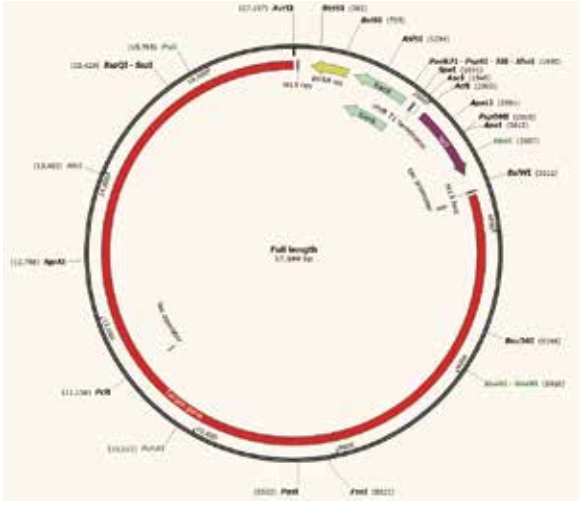
Service Specifications

Service Type	Gene Length	Turnaround Time (Business Day)	List Price	Deliverables
Clonal Gene	<=526 bp	5-7	$79/gene	4 μg of lyophilized plasmid Construct map of the plasmid (e-version) Sequence chromatograms covering the gene (e-version) Quality assurance certificate
	527~1500 bp	6-9	$0.15/bp	
	1501~3000 bp	10-13	$0.15/bp	
	3001~5000 bp	14-18	Get a Quote	
	5001~8000 bp	20-25		
	>8000 bp	Get a Quote		
Gene Fragment	≤500 bp	2~4	$69	Deliver 400 ng RCR Product by default Quality assurance certificate
	501~1000 bp	2~4	$99	
	1001~1500 bp	3~5	Get a Quote	

Gene Synthesis Case Studies

Case Study 1—13.7 kb gene synthesis and plasmid modification (~6 weeks)

The 13.7 kb gene sequence was synthesized, assembled, and cloned into the designated vector site specified by the customer. The sequence was verified to be 100% correct by sequencing, and high-quality plasmids were successfully delivered by GenCefe.



Difficulties of the Project

- The sequence length reaches 13.7 kb, and it is prone to deletions during assembly
- The vector is provided by the customer, and there is no suitable restriction site for the insertion of the target gene on the vector
- Low copy vector, difficult to prepare

GenCefe Solutions

- The proposal was designed by experienced technical experts; using our proprietary assembly technology to ensure the correct full-length sequence
- Carried out vector modification, and successfully clone the target sequence into the designated position of the modified vector
- Self-developed competent cells and formula medium were used to increase the number of plasmid copies.

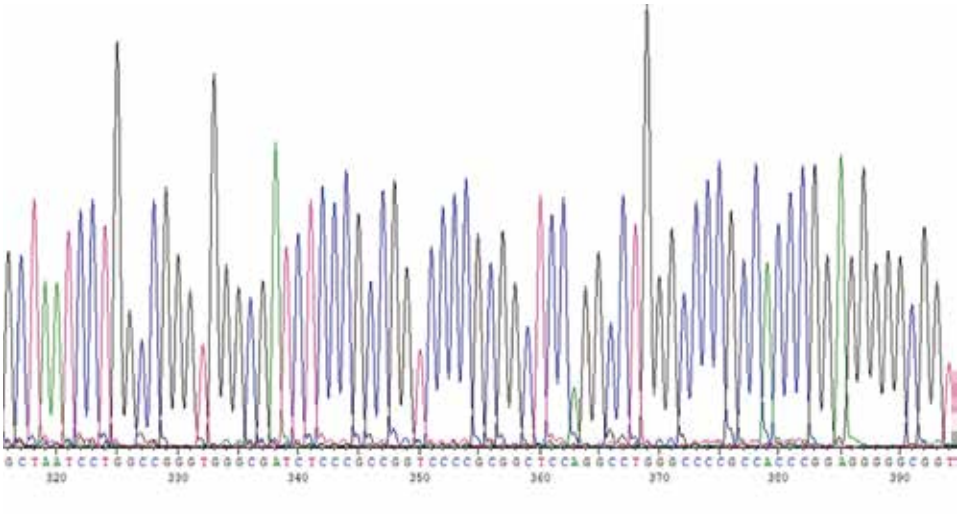
Case Study 2—difficult-to-synthesis gene sequence (PolyA-rich)

The gene sequence containing 110 consecutive PolyA has been successfully synthesized and verified by sequencing. And the 100% sequence correct plasmids were delivered to customers.

Difficulties of the Project	GenCefe Solutions
The sequence contains a large repeat structure, including a continuous PolyA structure of up to 110 bases. Such consecutive single-base repeats can lead to plasmidinstability and abnormal sequencing signals.	• A specific synthetic scheme was designed for this sequence. • Optimized construction and cloning protocols to reduce mutations. • Optimized sequencing protocols to ensure accurate validation.

Case Study 3—difficult-to-synthesis gene sequence (GC-rich)

We have successfully synthesized and verified the gene sequence containing high GC contains, direct repeats, and polymer structures. And the 100% sequence correct plasmids were delivered to customers.



Difficulties of the Project	GenCefe Solutions
• The GC-rich region accounts for >35% of the total length, and the sequence GC content fluctuates notably. Such structure often leads to non-specific amplification and causes failure to amplify the target gene.	• A specific synthesis scheme is designed for this sequence, and the gene sequence is synthesized in segments. • Assembled using proprietary assembly technology to ensure the correct full-length sequence. • Optimize construction and cloning protocols to reduce mutations. • Optimize sequencing protocols to ensure accurate validation.
• Direct repeats plus polymer structures account for >30% of the total sequence. Repetitive sequences cause difficulties in assembly, while polymer structures cause sequencing difficulties.	

Codon Optimization

GenCefe has introduced advanced artificial intelligence (AI) technology into codon optimization and independently developed the Codon Optimization Tool to assist your research and application with more optimized algorithms.

Key Features

- **Custom codon optimization:** sequence optimization based on the host's codon preference to improve protein translation efficiency
- **Advanced algorithms:** significantly improve protein yield, stability and activity, and reduce toxicity
- **Multiple species options:** we can provide codon optimization for a variety of different species, including bacteria, yeast, plants, mammalian cells, etc.

Case Study: 7.4-Fold increase in eukaryotic protein expression in E. coli

Objective : To evaluate the expression level of an optimized eukaryotic gene in E. coli.

Method : The wild-type SKN-1 gene was analyzed and optimized by our Codon Optimization software and procedure. The optimized gene was then cloned into an E. coli expression vector. The bacteria containing this gene were cultured and protein expression was induced, with a wild-type gene as comparison. The protein expression was checked by Western Blot. Three clones of each optimized or wild-type gene were examined and the result was shown in the Figure below.

Result : The three clones containing optimized SKN-1 gene showed a 6-fold, 7.2-fold, and 9-fold increase separately, with an average of 7.4-fold increase, over the clones containing wild-type.

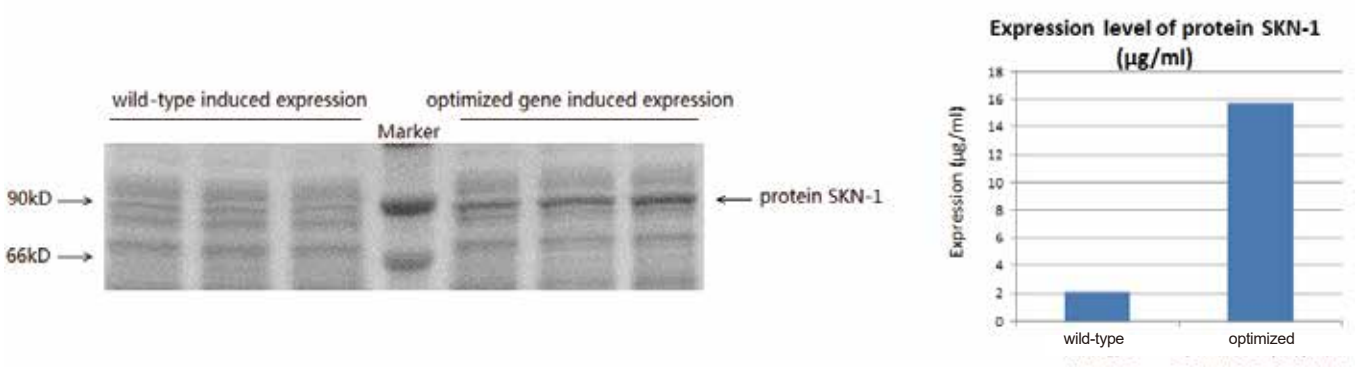


Figure: Left: Protein electrophoresis of bacteria lysate expressing wild-type and optimized SKN-1 gene. Right: protein quantitation by Western Blot.

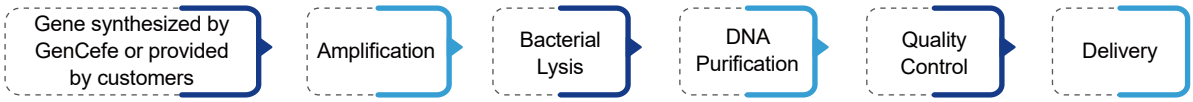
Gene-to-Protein Solutions

Sequence analysis and codon optimization → Gene synthesis and subcloning → Protein expression with selected expression system → Protein purification and QC

Plasmid Preparation

GenCefe provides you with high-quality plasmid preparation services, flexible production scale from micrograms, milligrams to grams; strict quality control to ensure batch-to-batch stability. We can perform endotoxin removal according to customer requirements to meet different plasmid production needs of research level and transfection level.

Service Process



Key Features

- **Flexible production scale:** microgram to gram level plasmid preparation
- **Strict quality control methods:** multiple QC standards and customized QC items are available upon request
- **Fast turnaround time:** deliver the plasmid DNA in as soon as 3 business days
- **Comprehensive services:** from gene synthesis to custom cloning and plasmid preparation

Service Specifications

	Research Level	Transfection Level
Endotoxin Level	NA	<0.05EU/μg or <0.005EU/μg
Scale	μg to g level	
Turnaround Time	Starting from 3 business days	
QC Method	Standard QC Items Research-level QC standards	Standard QC Items Transfection-level QC standards
Recommended Applications	Molecular biology research: gene cloning, mutagenesis, DNA library construction, transient protein expression, etc.	Industrial applications: mammalian cell transfection, viral packaging, protein expression, antibody production, GCT, vaccine development, etc.
Deliverables	Plasmid DNA, QC Report	

Plasmid Preparation QC Standards

	QC Items	Detection Method	Standards	
			Research Level	Transfection Level
Standard Items	Appearance	Visual inspection	Transparent without impurities	Transparent without impurities
	A260/280	Micro-spectrophotometer	1.8-2.0	1.8-2.0
	Concentration		1μg/μL	1μg/μL
	Supercoil ratio analysis	Agarose electrophoresis grayscale analysis	>50%	>90%
	Genomic DNA		< 15% of total plasmid DNA	< 15% of total plasmid DNA
	RNA residue	Agarose electrophoresis analysis	Undetectable	Undetectable
	Restriction Enzyme Validation	Agarose gel electrophoresis analysis of digested products	No stray bands	No stray bands
	Sequencing verification	Sanger sequencing	100% correct	100% correct
	Endotoxin Residue Analysis	Endotoxin Analysis Kit	NA	≤50EU/mg
Custom Items	Bioburden	Plate coating after filtration	No colonies after 48 hours	No colonies after 48 hours
	Others	Customizable according to customer's needs		

PCR Cloning & Subcloning

GenCefe can help customers clone the target gene into any site of the specified vector to meet different needs. Our professional technical team has experience in cloning more than 100 commercially available vectors and is also confident in designing customized cloning solutions for customers' vectors.

Subcloning Service Specifications

Service Type	Gene Length	Turnaround Time (Business Day)	Price
Subcloning bundled with gene synthesis	<3000 bp	5 Business Days	$29/gene
	3001 bp - 5000 bp	8-10 Business Days	Get a Quote
	>5000 bp	Get a Quote	Get a Quote
Subcloning Only	<3000 bp	6-10 Business Days	$59/gene
	3001 bp - 5000 bp	10-12 Business Days	Get a Quote
	>5000 bp	Get a Quote	Get a Quote

PCR Cloning Service Specifications

Gene Length	Turnaround Time (Business Day)	Price
< 1000 bp	5-8 Business Days	$79/gene
1000 bp - 3000 bp	8-10 Business Days	Get a Quote
3001-5000 bp	10-14 Business Days	Get a Quote
> 5000 bp	Get a Quote	Get a Quote

Site-directed Mutagenesis

GenCefe provides various site-directed mutagenesis services, including insertions, deletions, and substitutions. Relying on our extensive experience in molecular biology techniques, we can precisely introduce mutations into DNA sequences and guarantee the delivery of 100% sequence-accurate mutant sequences.

Gene Length	Mutated Site(s)	Turnaround Time (Business Day)
< 1000 bp	1	5-8 Business Days
	2	10-14 Business Days
1000～2000bp	1	5-8 Business Days
	2	10-14 Business Days
	3	15-20 Business Days
2001～3000bp	1	5-8 Business Days
	2	10-14 Business Days
	3	15-20 Business Days
3001～5000bp	1	5-8 Business Days
	2	10-14 Business Days
	3	15-20 Business Days

Synthetic DNA Libraries

A synthetic DNA library, also known as a gene mutation library, is a collection of numerous DNA mutant sequences assembled for screening for mutated proteins with specific functions. It has been widely used in protein-directed evolution, antibody screening, and other research fields. Leveraging an advanced gene synthesis technology platform, GenCefe provides tailored gene mutation library services, which can mutate amino acid or nucleotide sequences specified by customers, or design and synthesize mutation libraries according to customer requirements. Our offerings encompass various library construction services, including site-directed saturation mutation libraries, combinatorial libraries, alanine scanning libraries, and other types of customized gene libraries.

Service Features

- **Advanced technology platform:** Experienced R&D and production teams, leading bioinformatics and gene synthesis technology platforms, provide you with high-quality library design and synthesis services;
- **Comprehensive library types:** site-directed mutagenesis libraries, random mutant libraries, degenerate mutation libraries, controlled libraries, sgRNA libraries and other types of library construction services;
- **Professional technical support:** provide considerate pre-sales technical consultation and after-sales service, and timely update project progress.

Service Specifications

Library Type	Recommended Applications
Site-directed mutagenesis libraries	1) Research on protein structure and function; 2) Study of the enzyme active sites; 3) Study on the antibodies binding domains; 4) Protein property modifications, such as changing thermal stability, substrate binding specificity, etc.
Random mutant libraries	1) Research on protein structural functional domains; 2) Antibody humanization; 3) Protein property modifications, such as the determination of the catalytic domain of the enzyme, the improvement of catalytic properties, etc.
Degenerate mutation libraries	1) Research on protein structural functional domains; 2) Directed evolution of proteins, enzymes, antibodies; 3) Determination and modification of key amino acids in enzyme active sites; 4) Antibody humanization, fine-tuning of antibody affinity and binding properties.
Controlled libraries	1) Directed evolution of proteins, enzymes, antibodies; 2) New drug target screening; 3) In-depth study of the effect of a few amino acids around the catalytic site of the enzyme on the catalytic properties of the enzyme, etc.
sgRNA Libraries	1) Large-scale gene knockout, activation and suppression; 2) Identification and verification of new drug targets; 3) Identification of cancer treatment targets; 4) Applications in agricultural field, such as development of disease-resistant crops.

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