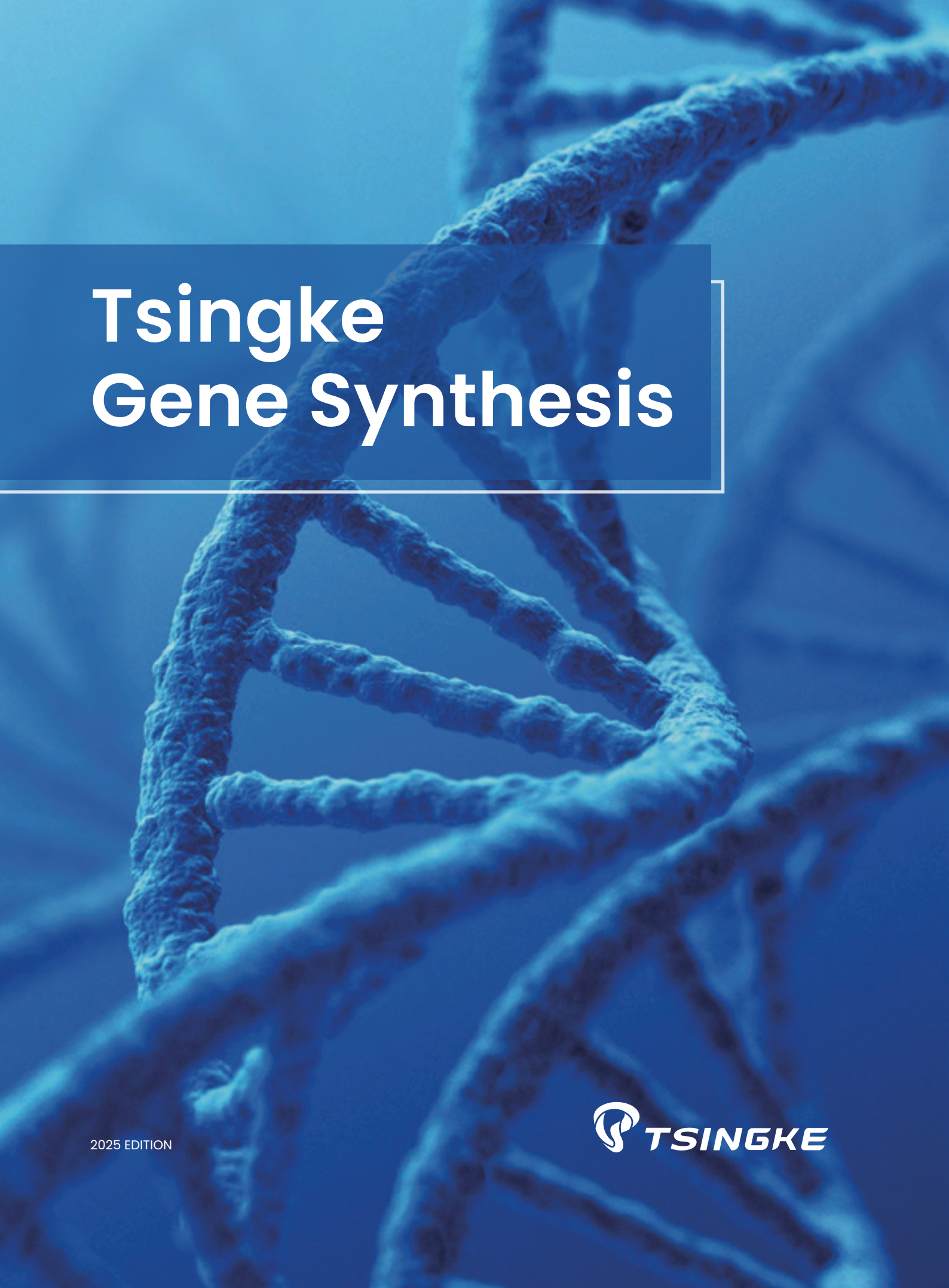


Tsingke Gene Synthesis

2025 EDITION

TSINGKE

CONTENTS

ABOUT US

01

Chapter 1

About Us

Our Mission

Biotech for a Better World

Our Vision

The great Tsingke gene factory

Our Values

Quality, Innovation, Striving, Win-win

Tsingke has achieved significant milestones in synthetic biology, including the development of state-of-the-art synthesis columns and oligo synthesizers capable of handling pmol to kg-level production. With the establishment of our independent facilities, we can offer comprehensive solutions for DNA/RNA manufacturing.

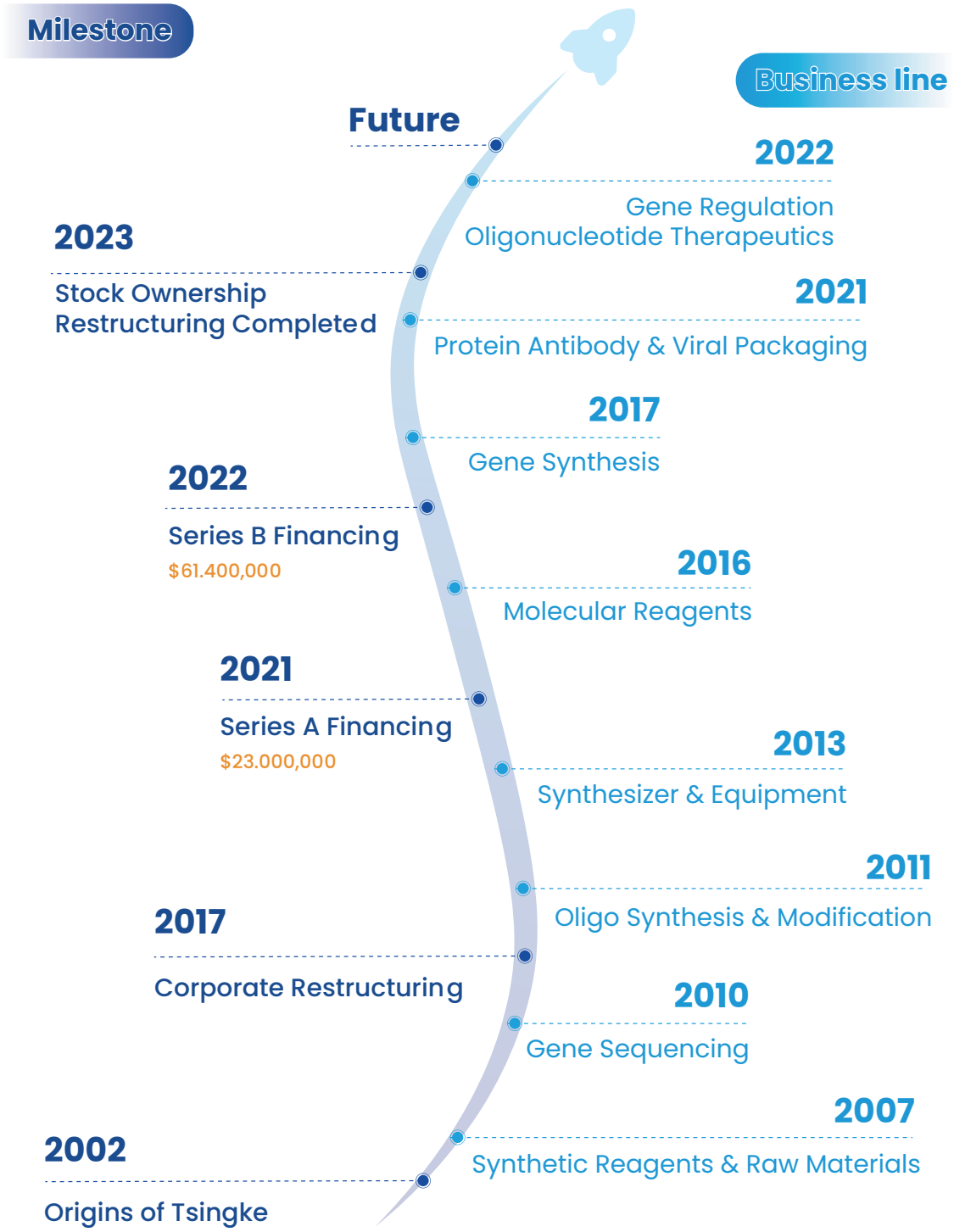
Tsingke has served over 200,000 customers worldwide with a diverse range of products and services, including Oligo and Gene Synthesis, Antibody and Protein Expression, DNA Sequencing, and Bioreagents, ensuring comprehensive support across various technical needs.

Tsingke will persistently focus on advancing the synthetic biology industry chain. We are committed to delivering superior production platforms and service offerings, aimed at expediting the research discoveries of scientists.

02 | Abouts Us

Chapter 1

History & Milestones of Tsingke



Chapter 1

Core Facilities Overview

Localization of Branches in China

· Over 20 localized branches covering key cities such as Shanghai and Guangzhou
· Equipped with around-the-clock oligonucleotide synthesis and Sanger sequencing laboratories

Large-scale Oligonucleotide Production Centers (Beijing, Suzhou)

· Equipped with Class 100,000 cleanrooms
· ISO 13485 certified
· Support GMP-level production capabilities

Intelligent Gene Synthesis Production Lines (Nanjing, Tianjin)

· Delivery capability as fast as 3 days
· Able to synthesize genes up to 200 kb in length

Nationwide Specialized Bases

· Ezhou: GMP-grade molecular reagent R&D and manufacturing
· Cangzhou: Chemical plant covering approximately 53 acres, supplying reagents and consumables

Annual output of 1 billion bases supported by

· Over 100 active 192-well synthesis instruments
· Multiple 768-well high-throughput synthesis systems
· Kilogram-scale synthesis platforms

Chapter 1

Tsingke Gene Factory

TSINGKE GENE FACTORY is a gathering of synthetic elements in gene synthesis, including synthetic raw materials, equipment, and processes. It constructs an automated synthesis production platform and is equipped with an independently developed intelligent gene synthesis production system to achieve stable and efficient production.

· The Whole Industrial Chain

Tsingke has a comprehensive synthetic reagents and raw materials production and supply system, achieving 100% fully autonomous production of essential raw materials and key reagents, including synthesis columns, monomers, CPG, molecular sieves, magnetic beads, kit, etc.

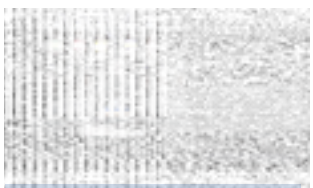
Equipments

Tsingke possesses a comprehensive synthesis platform and develops proprietary oligonucleotide Synthesizesr capable of accommodating 12 to 768 oligos per run, achieves coupling efficiency that consistently exceeds 99%.

We also develope other advanced equipment such as Ammonia Analyzer, Dissolution Instrument, Purification Instrument, Automated Dispenser and Automated Diluters to provide high-quality and stable DNA fragments.

TISKER-Syn-HCY-768P
Synthesis Scale:2~10 nmol

TISKER Syn-HCY-192P/B
Synthesis Scale:10 nmol~3 μmol



TISKER Syn-HCY-12P
Synthesis Scale:3~100 μmol

TISKER Syn-HCY-24P
Synthesis Scale:25 nmol~3 μmol

Purification Instrument-768

Dissolution Instrument

Automated Gene Synthesis Production Platform

Tsingke has developed an automated production platform, achieved automated production and significantly enhances production efficiency.

This platform is furnished with various devices, including single-strand nucleic acid synthesizers, dilution workstations, pipetting robot arms, rotating storage bins, PCR amplifiers, automatic colony pickers, automatic coating machines, DNA gel cutting workstations, and more. Additionally, it strategically aligns with independent process-oriented workshops based on the production process, ensuring the segregation of plasmids and bacterial liquids to mitigate contamination risks.

Tsingke Gene Synthesis Automated Production Platform can achieve automated production of short fragments within 1.5 kb. This not only reduces the risk of human error but also effectively increases production efficiency. It meets the demands of biopharmaceutical researchers for timely and high-quality gene synthesis delivery.

· TSINGKE HELIXTECH Digitally Empowering Gene Factory

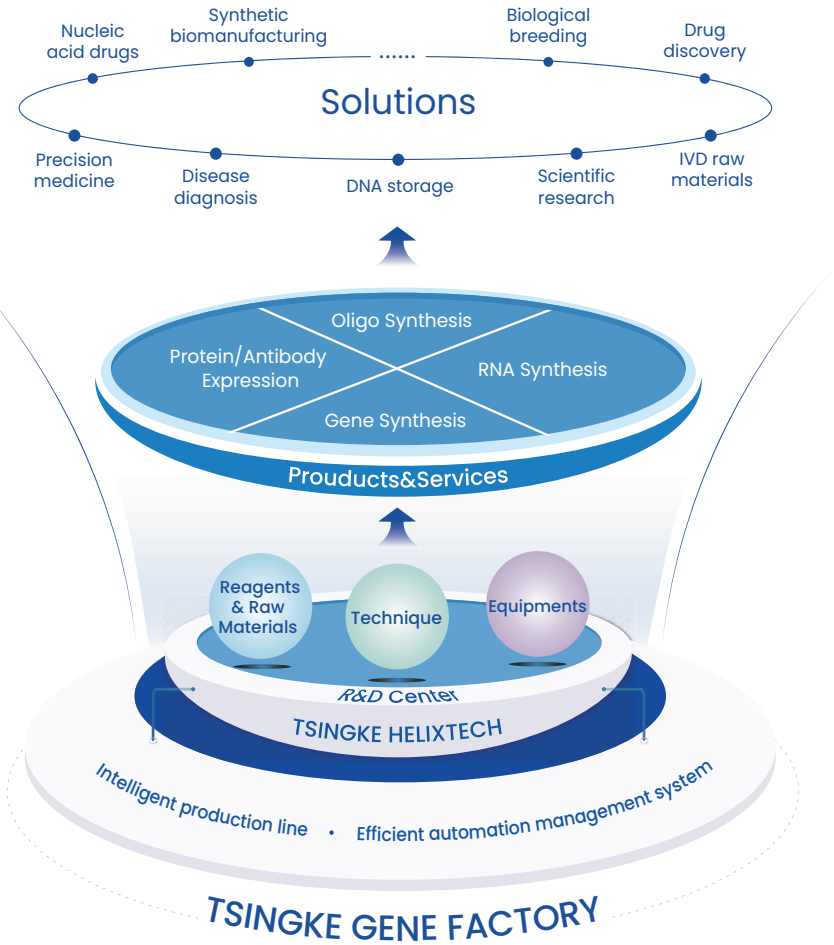
Stable

Automation

Efficient

The TSINGKE HELIXTECH system is an integrated platform that represents the full life cycle management system for gene synthesis at the production level. It encompasses customer service systems, production management systems, lean management systems, and more. This system is characterized by the integration of intelligent AI algorithms, online management platforms, and offline automated production platforms, creating a seamless connection throughout the gene synthesis process.

Based on TSINGKE HELIXTECH, we integrate synthetic materials, synthetic equipment, and synthesis technologies in the gene synthesis process, achieving a highly intelligent production system.



GENE SYNTHESIS SERVICE

02

Chapter 2

Gene Synthesis Service

· Overview

Tsingke is dedicated to leading gene synthesis technology. We have established the autonomous research and development, automated self-aggregation TSINGKE Gene Factory synthesis platform, equipped with advanced **Tsynth® synthesis** technology. Based on TSINGKE HELIXTECH, we integrate synthetic materials, synthetic equipment, and synthesis processes in the gene synthesis elements, achieving a highly intelligent production system.

By continuously researching and upgrading our synthesizers and synthetic materials for oligo production, our gene factory ensures the production of highly accurate oligos. This, in turn, guarantees stable and efficient gene synthesis with timely delivery. The plasmids Tsingke construct undergo NGS and Sanger sequencing validation to ensure 100% sequence accuracy.

200 kb

Achieved construction of 200 kb plasmid

Proficient in synthesizing large sequence

Free

Additional free services

Choose from 160+ commercial vectors at no extra cost, and enjoy free codon optimization

Unique intelligent splitting algorithm

Rapidly and scientifically designs oligos for each gene

5

Fast turnaround time

As fast as 5 days for standard gene synthesis

Standard Gene Synthesis	- Highly Customized: From simple sequences to complex sequences - Gene Length: Up to 10 kb - Standard delivery 1~4 μg plasmid - Fast Turnaround: As fast as 5 days
ProLongGene	- Up to 200 kb - Standard delivery 1~4 μg plasmid - One-step assembly of DNA fragments
DNA Fragment	- From 100 bp to 1.2 kb - Deliver 500 ng or 1 μg PCR products - Efficient turnaround time of 2 to 3 days
Single-Stranded DNA (ssDNA)	- From 100 nt to 6000 nt - Deliver lyophilized product powder

· Workflow

Chapter 2

Standard Gene Synthesis

· Overview

Compared to conventional molecular cloning methods, gene synthesis saves time and effort in obtaining target genes. Our standard gene synthesis service offers personalized synthesis plans and services tailored to different lengths and application requirements. Simply provide the DNA sequence you need to synthesize, and we assure prompt delivery of the ideal plasmid containing your target gene.

The Cost-effective solution through Tsingke's comprehensive nucleic acid synthesis industrial chain

Free 160+ vectors

100% sequence accuracy guaranteed with Sanger sequencing and NGS

Free codon optimization

· Service Details

Length	Turnaround time (Calendar day)*	Vector
< 1.5 kb	5~8	Any vector; If not specified otherwise, default to using pUC57.
1.5 kb~3 kb	7~11	
3 kb~6 kb	10~15	
6 kb~8 kb	15~22	
> 8 kb	Evaluation	

*Turnaround Time for simple sequences only and may change with the complexity of the gene sequence. Get your accurate estimated turnaround time by emailing info@tsingke.com.cn

· Deliverables

(1)1 tube of lyophilized plasmid DNA (about 1-4 μg/ tube);

(2)QC files: Sequencing map (.ab1 file); Target sequence (.seq file); COA Report (electronic);

(3)Additional identification by restriction endonuclease digestion is available (please specify your request before placing your order).

· Value Added Services

(1)Gene cloning: We possess the capability to clone sequences into any vector, offering over 160 common commercial vectors free of charge.

(2)Plasmid preparation: Our services extend from microgram to gram-scale plasmid DNA preparation, catering to scientific research as well as industrial requirements.

Chapter 2

ProLongGene

· Overview

Tsingke leads in addressing challenges in genetic synthesis, focusing on advancing long-fragment synthesis and assembly technologies. In gene synthesis, where chemical methods limit lengths to 200 nucleotides, we excel in achieving kilobase to megabase-level genes, even entire genomes, through advanced in vitro assembly technologies. With extensive expertise and ongoing innovation, we've surpassed genetic synthesis boundaries. Our breakthroughs include mastering yeast assembly for multiple segments and large fragments, enabling precise and efficient one-step assembly of DNA fragments in Saccharomyces cerevisiae. We proficiently produce industrial-scale 50 kb large fragment DNA, demonstrating the capability to deliver fragments up to 200 kb.

Independently developed yeast assembly system

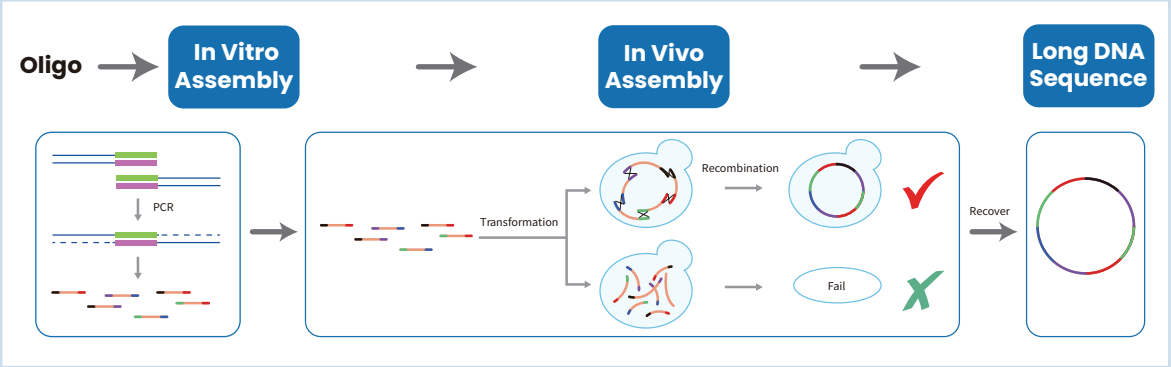
One-step assembly of DNA fragments in saccharomyces cerevisiae

100%

100% sequence accuracy guaranteed

ATCG TACG 200 kb

Achieved construction as long as 200 kb



Long Fragment and Genome Synthesis Based on Saccharomyces Cerevisiae System.

· Service Details

Length	Turnaround time (Calendar day)	Vector
> 10 kb	Evaluation	pCC1413

· Deliverables

(1)1 tube of lyophilized plasmid DNA (about 1-4 μg/ tube);

(2)QC files: Sequencing map (.ab1 file); Target sequence (.seq file); COA Report (electronic).

· Application

(1)Genome Synthesis.

(2)Gene Storage.

(3)Microbial Strain Modification (Primarily used for microbial cells).

(4)Metabolic Engineering (Used for host cells).

(5)Stem Cell Therapy.

Chapter 2

DNA Fragment

· Overview

Tsingke's DNA Fragment Service provides double-stranded DNA fragments, accommodating lengths of up to 1,200 base pairs. This service caters to the requirements of straightforward gene construction or modification, empowering you to accelerate your research and development activities for various applications such as antibody development, CRISPR-mediated genome editing, and NGS controls.

High yield

Low error rate
(column-based)

High delivery rate

Free codon optimization

High coupling efficiencies

· Service Details

Length	Turnaround time (Calendar day)
100 bp~1.2 kb	2~3

· Deliverables

(1)1 tube of lyophilized DNA (about 500 ng/ tube);

(2)QC files: COA Report (electronic);

(3)Additional identification by Sanger sequencing is available (please specify your request before placing your order).

· Application

(1)Gene Cloning: Insert specific DNA sequences into a target vector for expression.

(2)Molecular Labeling: Be employed as molecular labels, for instance, in experiments like Southern blot, Northern blot, and Western blot.

(3)Gene Mutation Research: By introducing specific DNA fragments, gene knock-in, knock-out, or mutation studies can be conducted to understand gene functions.

(4)Molecular Diagnosis: Detecting the presence or absence of specific genes through PCR.

(5)Genome Editing: CRISPR-Cas9 for genome editing and modification.

Chapter 2

Single-stranded DNA

· Overview

Recent research highlights the effectiveness of single-stranded DNA (ssDNA) as a homology-directed repair donor template in CRISPR gene editing. Compared to double-stranded DNA (dsDNA), using ssDNA as a template for homologous recombination will achieve a 9-fold higher CRISPR/Cas9 gene knock-in efficiency compared to dsDNA. and ssDNA is versatile in various biological reactions, especially in DNA nanotechnology.

Tsingke now provides top-notch,sequence-validated ssDNA to enhance the efficiency of your CRISPR experiments.

HIGH

High accuracy

9

High editing efficiency

Low cytotoxicity

6000

Wide synthesis range

· Service Details

Length(nt)	Turnaround time (Business Day)
101~240	10
241~1000	15
1001~3000	22
3001~4000	28
4001~5000	32
＞5000	Evaluation

· Deliverables

(1)Lyophilized Product Powder;

(2)QC files: Sequencing map (.ab1 file); Target sequence (.seq file); COA Report (electronic).

· Application

In CRISPR and CRISPR-Cas9 genome editing experiments, long ssDNA serves as a potent donor template, enhancing both insertion and gene replacement efficiency. Its applications extend to single-strand conformation polymorphism, in vitro transcription studies, nucleic acid enzyme S1 mapping, probe preparation, labeling, and differential hybridization.

Beyond experiments, ssDNA plays a role in DNA nanotechnology, serving as a scaffold for drug delivery, molecular diagnostics, DNA-based data storage, and diverse nanoscale applications. With a lower risk of random integration, ssDNA is particularly suitable for gene editing in primary cells, stem cells, and the creation of genetically modified animal models.

GENE CLONING SERVICE

03

Chapter 3

Cloning Service

· Overview

PCR (Polymerase Chain Reaction) cloning and subcloning are techniques commonly used in molecular biology to amplify and manipulate DNA fragments for various purposes, including gene cloning, sequencing, and functional analysis.

Site-directed mutagenesis serves as a powerful tool to efficiently enhance the expression, traits, and characterization of the targeted protein.

Tsingke offers a comprehensive service encompassing gene synthesis, vector construction, and cloning service. This integrated approach ensures heightened efficiency throughout the entire process.

100% sequence accuracy
guaranteed with Sanger sequencing and NGS

No limit
Not limited by restriction enzyme cutting sites

Clonable
Clone target gene fragment at any site in any vector system

PCR Cloning & Subcloning	-100% sequence accuracy: Guaranteed with Sanger sequencing and NGS -Not limited by restriction enzyme cutting sites -Customizable: Clone target gene fragment at any site in any vector system
Mutagenesis	- 100% Sequence Accuracy: Guaranteed with Sanger sequencing and NGS -Unlimited Sites: Introduce your mutations at any site -Cost-effective: All mutations found within a 30-base region will be defined as one whole mutation

Chapter 3

PCR Cloning & Subcloning

· Overview

PCR cloning and subcloning are common molecular biology techniques for amplifying and manipulating DNA fragments, including gene cloning, sequencing, and functional analysis. PCR cloning uses the polymerase chain reaction to amplify a specific DNA fragment without requiring specific restriction enzyme sites. Subcloning involves transferring a DNA fragment from one vector to another.

Tsingke offers a one-stop service ranging from gene synthesis and vector construction to cloning and subcloning. Our advanced technology and extensive experience ensure high-quality cloning services.

100% sequence accuracy
Sanger sequencing and NGS

One-stop solutions

Customizable
Any site, any vector

· Service Details

Length	Turnaround time (Calendar day)
< 1.5 kb	7~10
1.5 kb~3 kb	7~11
3 kb~6 kb	10~15
6 kb~8 kb	13~18
> 8kb	Evaluation

· Deliverables

(1)1 tube of lyophilized plasmid DNA (about 1~4 μg/ tube);

(2)QC files: Sequencing map (.ab1 file); Target sequence (.seq file); COA Report (electronic).

· Application

(1)Introduction of Mutations.

(2)Gene Library Construction.

(3)DNA Labeling.

(4)Gene Construction for Protein Expression.

Chapter 3

Mutagenesis

· Overview

Site-directed mutagenesis, a molecular biology technique, could deliberately introduce precise changes or mutations into a specific DNA sequence. This includes base **insertions, deletions, and point mutations**. The technology of site-directed mutagenesis is now extensively employed in the investigation of protein functional site structures, optimization of enzyme activity, understanding of DNA component functions and interactions, as well as applications in gene therapy and other research areas.

Tsingke provides specialized and cost-effective site-directed mutagenesis services, precisely introducing your requirement mutations for any gene template and any target number of mutations. This facilitates the synthesis of increased experimental efficiency and cost-effectiveness for your research endeavors.

100% sequence accuracy
Sanger sequencing and NGS

Cost-effective
All mutations found within a 30-base region will be defined as one whole mutation

Unlimited sites
Introduce your mutations at any site

· Service Details

Length	Turnaround time (Calendar day)
< 1.5 kb	7~10
1.5 kb~3 kb	7~11
3 kb~6 kb	10~15
6 kb~8 kb	13~18
> 8kb	Evaluation

· Deliverables

(1)1 tube of lyophilized plasmid DNA (about 1~4 μg/ tube);

(2)QC files: Sequencing map (.ab1 file); Target sequence (.seq file); COA Report (electronic);

(3)Any mutations identified within a 30-base region will be considered as a single comprehensive mutation

· Application

(1)Disease Research.

(2)Gene Function Studies.

(3)Drug Development.

(4)Genetic Engineering.

PLASMID PREPARATION

04

Chapter 4

Plasmid Preparation

· Overview

Plasmids are small, circular DNA molecules used as vectors in DNA recombination. In molecular biology, the target gene is inserted into a plasmid's multiple cloning sites, forming a recombinant plasmid. This can enter recipient cells, undergo replication and expression, and transfer to progeny cells during host cell division. Plasmid quantity and purity may vary, emphasizing the importance of obtaining high-quality plasmids for successful experiments.

Tsingke offers research grade and transfection grade plasmids preparation services to satisfy different downstream applications' requirement.

·Research Grade: Obtain increased quantities of plasmid (default concentration 1000 ng/μL);

·Transfection Grade: Removes endotoxin, increases supercoil ratio, and achieves highly efficient cell transfection.

One-stop solution
From gene synthesis, vector construction, to plasmid preparation

High Industrial standard
High supercoil content, low endotoxin level

Flexible customized
Produce plasmid DNA from microgram to gram level

· Service Details

(1) Research Grade

Service Details

Volume	Turnaround time(Calendar Day)*	Deliverables
100 μg	5	Prepared lyophilized plasmid DNA; Sequencing map (.abl file); COA Report(electronic).
200 μg	6	
500 μg	8	
1 mg	8	
2 mg	9	
3 mg	10	
4 mg	11	
5 mg	12	
10 mg	15	
20 mg	16	
50 mg	17	
100 mg	21	

*Opt for Gene Synthesis Alongside Plasmid Preparation and Save 2 Days on Turnaround Time.

Quality Control

	Items	Method	Specifications
Research Grade	Appearance	Visual inspection	Colorless Liquid of Pure Transparency
	A260/280 ratio	UV Absorbance	1.8~2.0
	Supercoil content	Agarose gel electrophoresis	＞ 50%
	Concentration	UV Absorbance	90%~110%
	Residual DNA	Agarose gel electrophoresis	Not visible
	Residual RNA	Agarose gel electrophoresis	Not visible
	Restriction enzyme analysis	Agarose gel electrophoresis	Adjustable according to requirement
	Sequence verification	Sanger sequencing	Consistent with the confirmed plasmid sequence

(2) Transfection Grade

Service Details

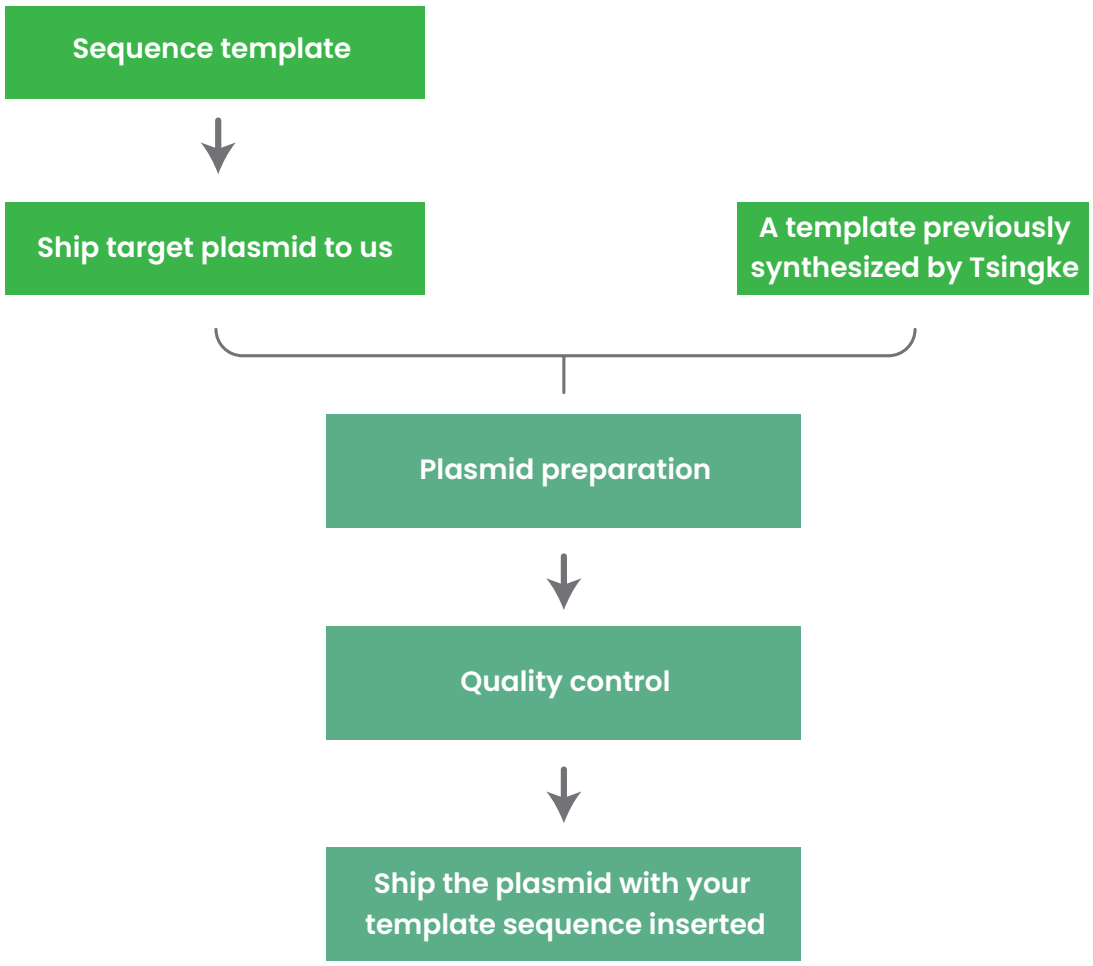
Endotoxin	Volume	Turnaround time(Calendar Day)*	Deliverables
< 0.1 Eu/ μg < 0.01 Eu/ μg < 0.005 Eu/ μg	100 μg	5	Prepared lyophilized plasmid DNA; Sequencing map (.abl file); COA Report(electronic).
	200 μg	6	
	500 μg	8	
	1 mg	8	
	2 mg	9	
	3 mg	10	
	4 mg	11	
	5 mg	12	
	10 mg	15	
	20 mg	17	
	50 mg	19	
	100 mg	22	

*Opt for Gene Synthesis Alongside Plasmid Preparation and Save 2 Days on Turnaround Time.

Quality Control

	Items	Method	Specifications
Transfection Grade	Appearance	Visual inspection	Colorless Liquid of Pure Transparency
	A260/280 ratio	UV Absorbance	1.8~2.0
	Supercoil content	Agarose gel electrophoresis	> 85%
	Concentration	UV Absorbance	90%~110%
	Residual DNA	Agarose gel electrophoresis	Not visible
	Residual RNA	Agarose gel electrophoresis	Not visible
	Restriction enzyme analysis	Agarose gel electrophoresis	Adjustable according to requirement
	Sequence verification	Sanger sequencing	Consistent with the confirmed plasmid sequence
	Endotoxin	Limulus amebocyte lysate (LAL) test	Endotoxin ⩽ Standard
	Exogenous Contamination Detection	NGS(Depth > 30×)	Genomic DNA < 1% Other DNA Contamination < 0.1%

· Workflow


Sequence template
Ship target plasmid to us
A template previously synthesized by Tsingke
Plasmid preparation
Quality control
Ship the plasmid with your template sequence inserted

RESOURCE CENTER

05

Chapter 5

Codon Optimization

· Overview

Drawing on diverse backgrounds in life science and computer science, Tsingke has developed a sequence deep optimization technology based on the Genetic and Evolutionary Algorithm, also known as codon optimization, that optimizes gene sequences extensively without altering the protein-coding properties. Through specific algorithms, it adjusts around 200 factors affecting gene synthesis and protein expression, such as overall GC content and special structures in the gene sequence. This optimization aims to achieve Pareto optimality, enhancing gene expression while keeping other factors unaffected. The algorithm is currently adaptable to various hosts, providing personalized adaptations for each, making it applicable to a wide range. Genes sequences optimized through Tsingke codon optimization significantly reduce synthesis difficulty and markedly improve subsequent protein expression levels.

· Host Adaptation

-Arabidopsis_thaliana
-Bacillus_subtilis
-Corynebacterium_glutamicum
-Cricetulus_griseus
-Escherichia_coli
-Homo_sapiens
-Lactobacillus_acidophilus
-Macaca_fascicularis
-Mus_musculus
-Nicotiana_tabacum
-Oryctolagus_cuniculus
-Oryza_sativa
-Pichia_pastoris
-Rattus_norvegicus
-Saccharomyces_cerevisiae

· Optimize Parameters

-GC Content
-Cutting Sites
-Codon Preference
-Inhibition Sites
-Poly(A) Signal
-Ribosome Binding Sites
-Repeat Sequences

· Case

(1) Enhanced protein expression after cryptographic optimization

The target protein 16.8kDa was not expressed before codon optimization, but was highly expressed after codon optimization.

(2) Cryptographic optimization enhances protein soluble expression

The target protein 100 kDa was expressed in inclusion bodies before codon optimization, and the supernant was soluble in 75% after codon optimization.

Chapter 5
Citation

Tsingke has been cited in numerous journals such as Nature, Cell, Science, and others, totaling thousands of citations.

Below are some publication details from customers who have placed gene synthesis service orders.

Serial Number	Journal	Impact Factor	Title	DOI
1	Nature Biotechnology	68.164	Strand-selective base editing of human mitochondrial DNA using mitoBEs	10.1038/s41587-023-01791-y
2	Science	56.9	Structure of the human PKD1-PKD2 complex	10.1126/science.aat9819
3	Science	56.9	Enhancing rice panicle branching and grain yield through tissue-specific brassinosteroid inhibition	10.1126/science.adk8838
4	Mol Cancer	41.44	A novel polypeptide encoded by the circular RNA ZKSCAN1 suppresses HCC via degradation of mTOR	10.1186/s12943-023-01719-9
5	Circulation	39.9	Cannabinoid Receptor 2-Centric Molecular Feedback Loop Drives Necroptosis in Diabetic Heart Injuries	10.1161/CIRCULATIONAHA.122.059304
6	JOURNAL OF INFECTION	38.637	MAMDC2, a gene highly expressed in microglia in experimental models of Alzheimers Disease, positively regulates the innate antiviral response during neurotropic virus infection	10.1016/j.jinf.2021.12.004
7	Signal Transduction and Targeted Therapy	38.1196	UBQLN1 mediates sorafenib resistance through regulating mitochondrial biogenesis and ROS homeostasis by targeting PGC1β in hepatocellular carcinoma.	10.1038/s41392-021-00594-4
8	Signal Transduction and Targeted Therapy	38.1196	O-GlcNAcylation of YTHDF2 promotes HBV-related hepatocellular carcinoma progression in an N6-methyladenosine-dependent manner	10.1038/s41392-023-01316-8
9	Signal Transduction and Targeted Therapy	38.1196	Excessive branched-chain amino acid accumulation restricts mesenchymal stem cell-based therapy efficacy in myocardial infarction	10.1038/s41587-023-01791-y
10	Signal Transduction and Targeted Therapy	38.1196	Targeting carnitine palmitoyl transferase 1A (CPT1A) induces ferroptosis and synergizes with immunotherapy in lung cancer	10.1126/science.aat9819

Chapter 6

Contact Information

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