OLIGO

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Tsingke Oligo Synthesis

2025 EDITION

TSINGKE

CONTENTS

ABOUT US

01

Chapter 1

About Us

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 300,000 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.

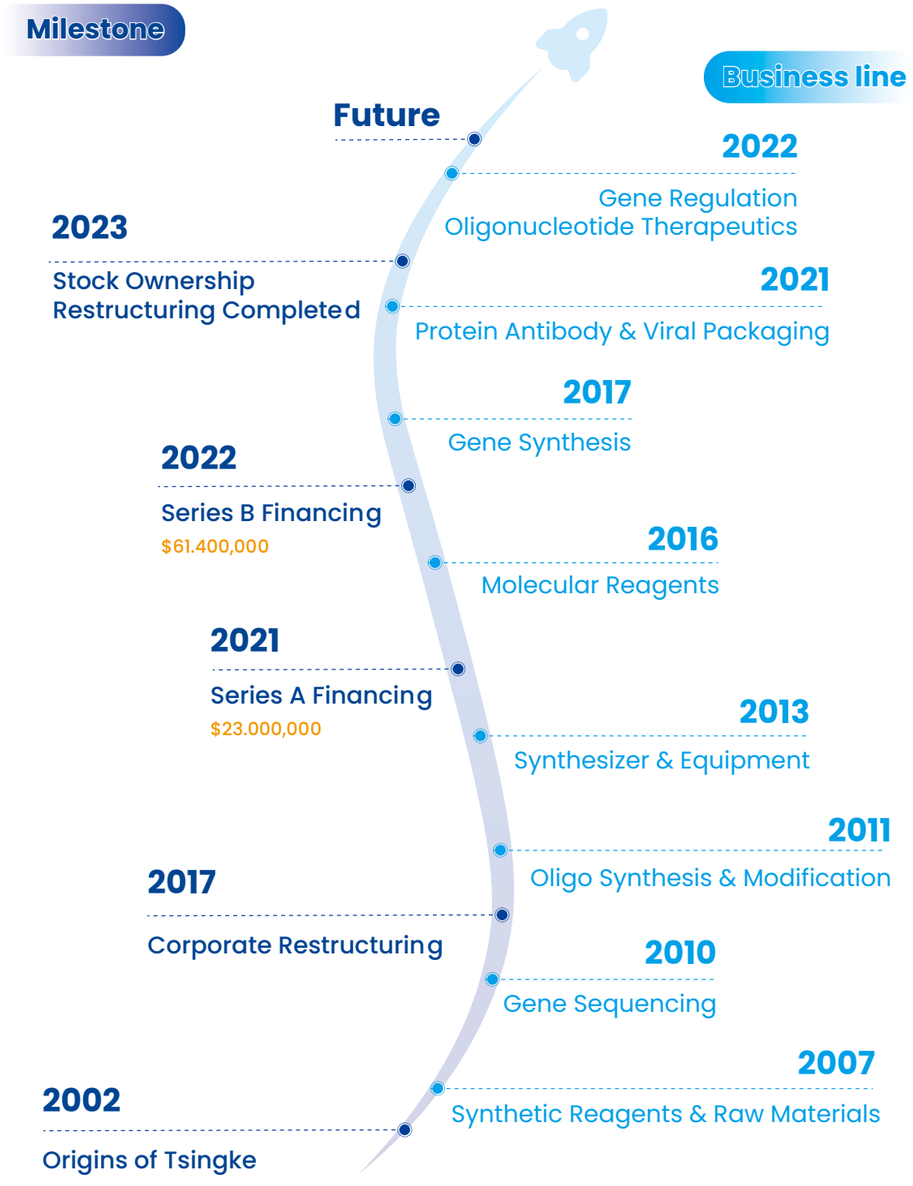
Our Mission
Biotech for a Better World

Our Vision
The great Tsingke gene factory

Our Values
Quality
Innovation
Striving
Win-win

Chapter 1

History & Milestones of Tsingke



Chapter 1

Core Facilities Overview

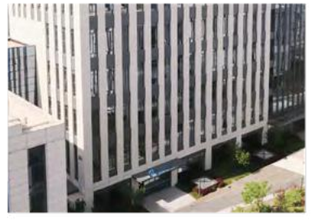
Localization of Branches in China
- Over 20 localized branches covering key cities such as Shanghai and Guangzhou
- Equipped with around-the-clock oligonucleotide synthesis and Sanger sequencing laboratories

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



RNA Synthesis · Beijing

Gene Synthesis · Nanjing

GMP Oligos · Suzhou

Molecular Biology Reagents · Wuhan

Synthetic Reagents & Consumables · Cangzhou

Gene Synthesis/Automated Equipment · Tianjing

THE WHOLE INDUSTRIAL CHAIN

02

Chapter 2

The Whole Industrial Chain

01. Synthetic Reagents & Raw Materials

Synthetic reagents and raw materials play a crucial role in chemical oligo synthesis. Tsingke has a comprehensive raw material production and supply system that achieved 100% fully autonomous production of essential **chemical reagents, biological reagents, and raw materials** such as synthesis columns, synthesis plates, monomers, modified dyes CPG, molecular sieves, resins, etc.

Tsingke implements stringent replacement and testing systems, as well as precise limitations on the use-by dates after reagent unpacking and dissolution, to ensure coupling efficiency, prevent the introduction of impurities or contaminants, and achieve consistency and stability of oligo products.

02. Oligo Synthesizer

Tsingke possesses a comprehensive synthesis platform and develops proprietary oligo synthesis equipment such as Single-stranded DNA Synthesizer, Modified Oligos Synthesizer, and High-Capacity Synthesizer, realizing synthesis from **12 to 768 oligos per run**, with a single-channel synthesis capacity reaching the **millimolar level yield** and promising coupling efficiency exceeding 99%. Tsingke also develops other advanced equipment such as Ammonia analyzer, Dissolution instrument, Oligo Dispenser to provide high-quality oligos according to different throughput and capacity requirements.

We carry out precise control of temperature, mixing of reactants, and handling of gases and other processes to ensure the accuracy and stability of synthesis conditions for oligos, achieving synthesis scales ranging from **micrograms to kilograms**.

TISKER Syn-HCY-192P/B

Synthesize 192 oligonucleotides at one time, with a single-channel yield of 10 nmol to 3 μmol.

TISKER-Syn-HCY-768P

Synthesize 768 oligonucleotides at one time, with a single-channel yield of 2 nmol to 50 nmol.

TISKER Syn-HCY-12P

Synthesize 12 oligonucleotides at one time, with a single-channel yield of 10 nmol to 100 μmol.

TISKER Syn-HCY-24P

Synthesize 24 oligonucleotides at one time, with a single-channel yield of 25 nmol to 3 μmol.

TISKER Syn-HL12

High load capacity, with a single-channel yield of 2 μmol to 12 mmol.

03. Intelligent Production Line

(1) Production Environment

Tsingke has obtained ISO 13485 certification and operates a GMP-like manufacturing facility with a cleanliness level of up to 100,000. We comprehensively consider factors influencing the production environment by monitoring parameters such as temperature, humidity, pressure differentials, and dust particles, thus preventing contamination and ensuring stable, high-quality oligo production.

(2) Process Control

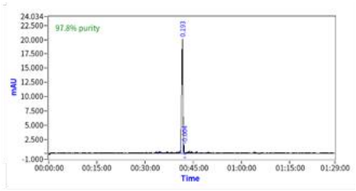
Tsingke has standardized key parameters such as temperature, reaction time, imput reagent quantity, solvent selection in our laboratories.

We strictly adhere to ISO 13485 standards and implement Lean Six Sigma management systems, established over 400 controlled documents, including quality manuals, procedural documents, management specifications and forms, though effective risk management plans and analysis, we promise the stability of oligo products.

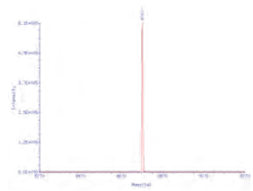
Order Input → Oligo Synthesis → Ammonia Deprotection and Purification → QC and Packaging → Delivery

(3) QC

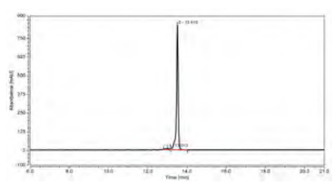
Tsingke Biotech provides various customized quality inspection methods, including MS, HPLC, OD detection, and NTC. Capillary electrophoresis (CE) detection achieves single-base resolution and is particularly suitable for high-throughput oligo purity testing. We monitor all key raw materials-intermediates-final products throughout the entire process for effective traceability; Our stringent quality control ensures the accuracy of oligo sequences, preventing potential impurities or contaminants from affecting downstream applications.



CE



MS



HPLC

OLIGO SYNTHESIS SERVICE

03

Chapter 3

Custom DNA Oligos

· Overview

DNA Oligos are short, single-stranded DNA molecules, usually consisting of 20 to 30 base pairs. They play a key role in PCR, sequencing and other molecular biology experiments.

Tsingke integrates synthetic raw materials, synthetic equipment, synthetic technology and services to form a complete industrial chain. Tsingke provides safe, reliable and economical customized DNA oligos for scientific research and R&D production to universities, scientific research institutes, hospitals, government agencies and pharmaceutical diagnostic companies.

ISO 13485 and 100,000-level clean room

Over 200 chemical modifications available for your selection

Flexible synthesis specifications, μg~kg delivery ability

100% mass spectrometry with a variety of additional detection options

· Application

PCR

DNA library

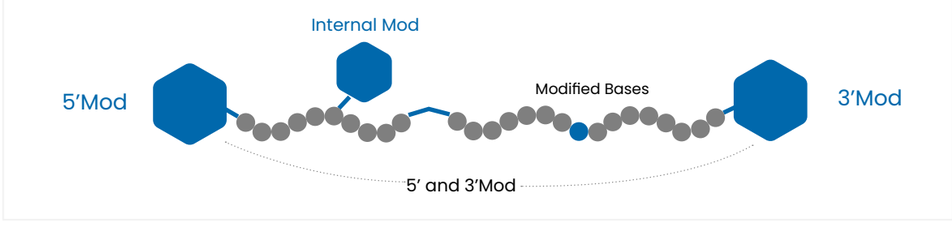
Sequencing

· Service Details

Service name	Length (nt)	Purification	Price/ turnaround time	Deliverable	Application
Common Oligos	5~60	DSL/OPC/ PAGE/HPLC	Inquire	· Tube or customized lyophilized DNA · COA report (electronic)	PCR, DNA sequencing
Long Oligos	60+	PAGE/HPLC/ Dual PAGE & HPLC			NGS, genomic research
Large-scale Oligos	Customized	Customized			New drug screening, drug production
Modified Oligos	10~120	DSL/OPC/ PAGE/HPLC/ Dual PAGE & HPLC			Various molecular biology research

*Note: In addition to the recommended content, Oligo length and purification methods can also be customized.

· Modification Type



· Oligo Modification List

5' Modifications	3' Modification	Double Label Modifications	Internal Modifications	
5'Alexa Flour 350	3'Alexa Flour 350	CY3,BHQ2	(dT-Alexa Flour 350)	(dC-Alexa Flour 350)
5'Alexa Flour 405	3'Alexa Flour 405	CY3,MGB	(dT-Alexa Flour 405)	(dC-Alexa Flour 405)
5'Alexa Flour 488	3'Alexa Flour 488	CY5,BHQ2	(dT-Alexa Flour 488)	(dC-Alexa Flour 488)
5'Alexa Flour 532	3'Alexa Flour 532	CY5,BHQ3	(dT-Alexa Flour 532)	(dC-Alexa Flour 532)
5'Alexa Flour 546	3'Alexa Flour 546	CY5,MGB	(dT-Alexa Flour 546)	(dC-Alexa Flour 546)
5'Alexa Flour 555	3'Alexa Flour 555	CY5.5,BHQ2	(dT-Alexa Flour 555)	(dC-Alexa Flour 555)
5'Alexa Flour 568	3'Alexa Flour 568	CY7,BHQ2	(dT-Alexa Flour 568)	(dC-Alexa Flour 568)
5'Alexa Flour 594	3'Alexa Flour 594	CY7,BHQ3	(dT-Alexa Flour 594)	(dC-Alexa Flour 594)
5'Alexa Fluor 610	3'Alexa Fluor 610	FAM,BHQ1	(dT-Alexa Flour 647)	(dC-Alexa Flour 647)
5'Alexa Fluor 633	3'Alexa Fluor 633	FAM,BHQ2	(dT-Alexa Flour 680)	(dC-Alexa Flour 680)
5'Alexa Flour 647	3'Alexa Flour 647	FAM,DAB	(dT-Alexa Fluor 610)	(dC-Alexa Fluor 610)
5'Alexa Flour 680	3'Alexa Flour 680	FAM,ECL	(dT-Alexa Fluor 620)	(dC-Alexa Fluor 620)
5'Alexa Fluor 620	3'Alexa Fluor 620	FAM,MGB	(dT-Alexa Fluor 633)	(dC-Alexa Fluor 633)
5'Alexa Fluor 700	3'Alexa Fluor 700	FAM,TAM	(dT-Alexa Fluor 700)	(dC-Alexa Fluor 700)
5'Alexa Fluor 750	3'Alexa Fluor 750	HEX,BHQ1	(dT-Alexa Fluor 750)	(dC-Alexa Fluor 750)
5'ATTO 425	3'ATTO 425	HEX,BHQ2	(dT-ATTO 425)	(dC-ATTO 425)
5'ATTO 550	3'ATTO 550	HEX,ECL	(dT-ATTO 550)	(dC-ATTO 550)
5'ATTO 565	3'ATTO 565	HEX,MGB	(dT-ATTO 565)	(dC-ATTO 565)
5'ATTO Rho11	3'ATTO Rho11	HEX,TAM	(dT-ATTO 594)	(dC-ATTO 594)
5'ATTO 594	3'ATTO 594	JOE,BHQ1	(dT-ATTO 700)	(dC-ATTO 700)
5'ATTO 700	3'ATTO 700	JOE,BHQ2	(dT-ATTO Rho11)	(dC-ATTO Rho11)
5'Acrydite	3'Azide(N3)	JOE,DAB	(dT-Azide(N3))	(dC-Azide(N3))
5'Azide(N3)	3'AMCA	JOE,MGB	(dT-BHQ1)	(dC-BHQ1)
5'BHQ1	3'BHQ0	JOE,TAM	(dT-BHQ2)	(dC-BHQ2)
5'BHQ2	3'BHQ1	JOE.ECL	(dT-Biotin)	(dC-Biotin)
5'BIO	3'BHQ2	PHO,FAM	(dT-Chromeo 494)	(dC-Chromeo 494)
5'BIO-TEG	3'BHQ3	Quasar 670,BHQ3	(dT-CY3)	(dC-CY3)
5'C6-NH2	3'BBQ-650	Quasar570,BHQ2	(dT-CY5)	(dC-CY5)
5'C12-NH2	3'BKHFQ	ROX,BHQ2	(dT-CY5-M)	(dC-CY5-M)
5'C7-NH2	3'BIO	ROX,ECL	(dT-Digoxin)	(dC-Digoxin)
5'CHCH	3'BIO-TEG	ROX,MGB	(dT-FAM)	(dC-FAM)
5'CHO	3'C3-FAM	TAM,BHQ2	(dT-FITC)	(dC-FITC)
5'Cholesteryl	3'C7-NH2	TAM,DAB	(dT-HEX)	(dC-HEX)
5'COOH	3'CHCH	TAM,ECL	(dT-Methylene Blue)	(dC-Methylene Blue)
5'CY3	3'Cholesteryl	TAM,MGB	(dT-NH2)	(dC-NH2)
5'CY5	3'CY3	TET,BHQ1	(dT-Quasar 570)	(dC-Quasar 570)
5'CY5-M	3'CY5	TET,BHQ2	(dT-Quasar 670)	(dC-Quasar 670)

5' Modifications	3' Modification	Double Label Modifications	Internal Modifications	
5'CY5.5	3'CY5-M	TET,DAB	(dT-Quasar 705)	(dC-Quasar 705)
5'CY7	3'CY5.5	TET,ECL	(dT-ROX)	(dC-ROX)
5'DAB	3'CY7	TET,MGB	(dT-SF670)	(dC-SF670)
5'DBCO	3'DAB	TET,TAM	(dT-TAM)	(dC-TAM)
5'DIG	3'DBCO	TXR,BHQ2	(C7-NH2)	
5'ET-ROX	3'ddC	TXR,DAB	(dI)	
5'ET-TAMRA	3'DIG	TXR,MGB	(dSpacer)	
5'ET-B (Blue)	3'ECL	VIC,BHQ1	(dT-CY7)	
5'ET-G (Green)	3'Ferrocene	VIC,BHQ2	(dU)	
5'ET-Y (Yellow)	3'HEX	VIC,DAB	(HMC)	
5'ET-R (Red)	3'InvdT	VIC,MGB	(Me-dC)	
5'ET-P (Purple)	3'Joe	VIC,TAM	(PC Linker)	
5'ET-0 (Orange)	3'MGB		(Spacer 18)	
5'FAM	3'Methylene Blue		(Spacer 9)	
5'FITC	3'PHO		(Spacer C12)	
5'Ferrocene	3'QSY7		(Spacer C3)	
5'HEX	3'ROX		(Spacer C6)	
5'HMC	3'RHO 101		(SS)	
5'JOE	3'SF670		(TAO)	
5'Me-DC	3'-CT+Signal		(XEN)	
5'Methylene Blue	3'SH		-CT+Signal	
5'NED	3'Spacer(C3)		LNA-A	
5'PET	3'SS		LNA-C	
5'PHO	3'TAM		LNA-G	
5'Quasar 570	3'TXR		LNA-T	
5'Quasar 670				
5'Quasar 705				
5'ROX				
5'SF670				
5'SH				
5'SS				
5'TAM				
5'TET				
5'TXR				
5'triple Biotin				
5'triple SH				
5'VIC				
5'YakYel				

· Degenerate Bases

B=G,C,T	D=A,G,T	H=A,C,T	K=G,T	M=A,C	N=A,G,C,T
R=A,G	S=G,C	V=A,G,C	W=A,T	Y=C,T	

· Purification Method

In order to improve the purification efficiency and improve the quality of Oligo synthesis, Tsingke provides a variety of purification methods: DSL purification, OPC purification, PAGE purification, HPLC purification and Dual PAGE & HPLC.

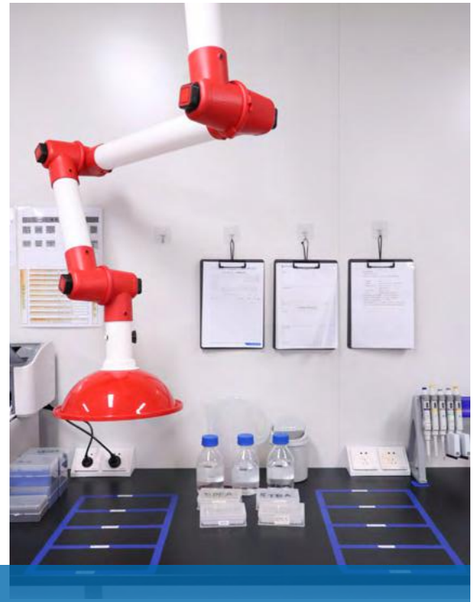
Purification method selection						
Method	Oligo length(mer)					Application
	5~10	11~40	41~60	61~90	91~140	
DSL	✓	✓	✓	✓	✓	There are requirements for preventing contamination of oligos, reducing personnel operations, and directly desalting and packaging after ammonia hydrolysis.
OPC	✓	✓	✓	N/A	N/A	Mainly aimed at purifying oligos below 60 mer, the purity is better than DSL.
PAGE	N/A	✓	✓	✓	✓	Commonly used for purification of oligos above 60 mer. It is mainly used in experiments such as site-directed mutagenesis, cloning, protein-binding gel shift electrophoresis analysis, and research fields such as treatment and diagnosis.
HPLC	✓	✓	✓	✓	N/A	Commonly used for purification of 15~60 mer primers and probes.It is mainly used in experiments such as site-directed mutagenesis, cloning, and protein-binding gel shift electrophoresis analysis, as well as commercial diagnostic primers or probes for treatment and diagnosis.
Dual PAGE & HPLC	N/A	N/A	N/A	✓	✓	Commonly used for purification of primers and probes with mers above 90 mer.It is mainly used in experiments such as site-directed mutagenesis, cloning, and protein-binding gel shift electrophoresis analysis, as well as commercial diagnostic primers or probes for treatment and diagnosis.

Chapter 3

Custom RNA Oligos

· Overview

Tsingke provides high quality RNA oligonucleotides of various lengths and modifications. The quality of each RNA oligonucleotide is ensured by advanced mass spectrometry (MS) and chromatography (HPLC) detection methods. Services include: common RNA synthesis, siRNA/miRNA synthesis, modified RNA synthesis, sgRNA synthesis and ASO synthesis.



Powerful synthesis capabilities support kg-level production

Stable process enables precise and efficient chemical modification

Supports multiple modification types such as LNA/2'-OMe

· Application

Gene editing

Functional research

Nucleic acid drugs

· Service Details

Service name	Length (nt)	Purification	Price/ turnaround time	Deliverable	Application
Common RNA	3~180	OPC/HPLC	Inquire	· Tube or customized lyophilized RNA · COA report (electronic)	Gene function research
siRNA	20~25				RNAi
miRNA	20~25				Pathological research
sgRNA	97~103				CRISPR/CGT
crRNA	42				
tracrRNA	64				
ASO	16~20				Nucleic acid drug

*Note: In addition to the recommended content, Oligo length and purification methods can also be customized.

Chapter 3

qPCR Probes

· Overview

qPCR Probes are important raw materials in qPCR. This technology has been widely used in scientific research and production practice, especially in medical testing and diagnosis.

Tsingke has a 100,000-level clean room that meets biomedical diagnostic standards, using internationally advanced synthesis technology, high-quality synthesis reagents, advanced automated production equipment, and strict ISO 13485 quality control system. Tsingke provides customized qPCR probes and oligo primers for qPCR experiments and kit, such as: qPCR Probes, MGB Probes, Double-Quenched Probes and Molecular Beacons etc.

ISO 13485 and 100,000-level clean room

High-quality synthetic raw materials and technology , the probes have high purity and low background noise

Strictly control contamination from *E.coli* and human sources to avoid NTC peaks

Trusted by customers, chosen by more than 500 IVD companies

· Application

IVD

FISH/STR research

Genetic testing

· Service Details

Service name	Length (nt)	Purification	Price/ turnaround time	Deliverable	Application
qPCR probes	15~30	PAGE/HPLC/ Dual PAGE & HPLC	Inquire	· Tube or customized lyophilized DNA · COA report (electronic)	The most commonly used types of qPCR experiments
MGB probes	13~25				For qPCR experiments with higher TM
Double-Quenched probes	15~45				For qPCR experiments with longer probes
Molecular beacons	25~40				For qPCR experiments with extremely high sensitivity requirements
Other probes	Customized	Customized			Special application directions

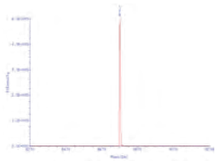
*Note: In addition to the recommended content, Oligo length and purification methods can also be customized.

· Common Fluorescent Dyes and Quenchers Types

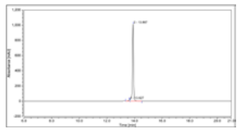
Fluorescent Dyes	Max Abs	Max Em	Quenchers	Quenching range	Quenching Max
FAM	494 nm	518 nm	Dabcyl	380 nm~530 nm	452 nm
TET	521 nm	536 nm	Eclipse	390 nm~625 nm	522 nm
JOE	520 nm	548 nm	MGB	390 nm~625 nm	522 nm
VIC	538 nm	554 nm	TAMRA	470 nm~560 nm	544 nm
HEX	535 nm	556 nm	BHQ1	480 nm~580 nm	534 nm
Quasar 570	547 nm	570 nm	BHQ2	550 nm~650 nm	579 nm
Cy3	552 nm	570 nm	BHQ3	620 nm~730 nm	672 nm
TAMRA	565 nm	580 nm			
ROX	585 nm	605 nm			
Texas Red	595 nm	615 nm			
Alexa Fluor 633	632 nm	647 nm			
Cy5	643 nm	667 nm			
Quasar 670	647 nm	667 nm			
Cy5.5	684 nm	710 nm			
Cy7	750 nm	773 nm			

· Case

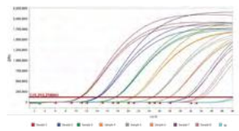
Name	Modification	Theoretical MW	Actual MW	MW error (%)	HPLC purity
Probe-01	5'FAM,3'MGB	8770.1	8770.7	0.01%	98.59%



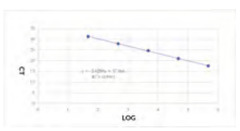
MS



HPLC



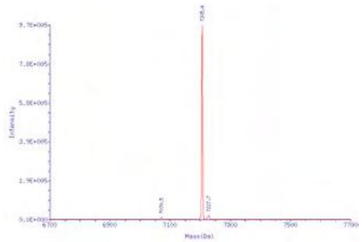
Amplification curve



Standard curve

Name	Modification	Theoretical MW	Actual MW	MW error (%)	HPLC purity
SNP-01	5'FAM,3'MGB	7204.6	7205.4	0.01%	98.75%

SNP-01



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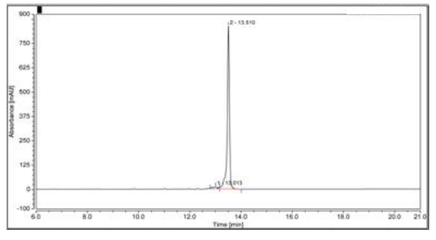
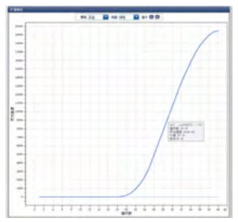
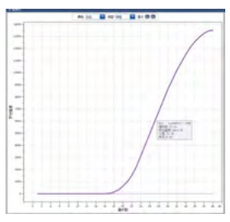


HPLC

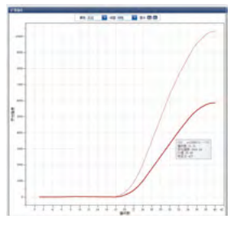
MGB Probe for SNP detection



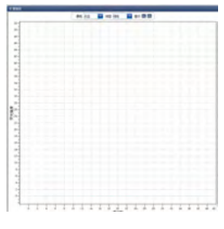
Genotype: TT



Genotype: CC

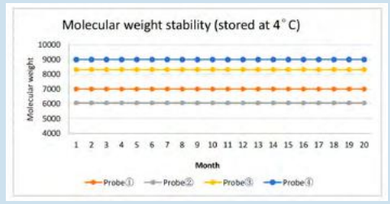


Genotype: CT

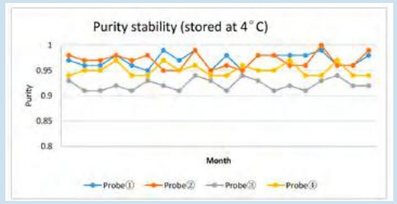


Negative control

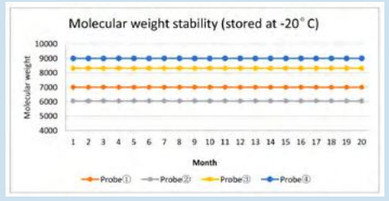
Long-term stability



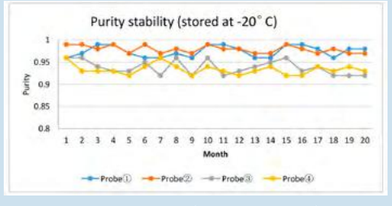
Molecular weight stability (stored at 4ºC)



Purity stability (stored at 4ºC)

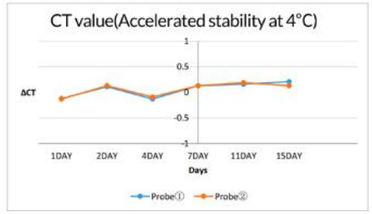


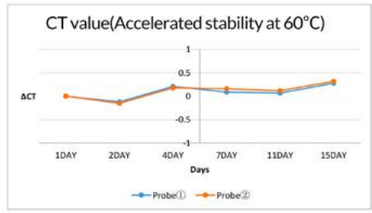
Molecular weight stability (stored at -20ºC)

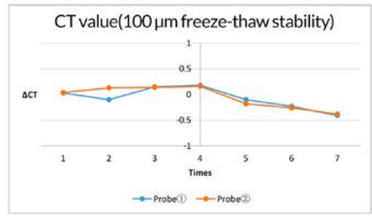


Purity stability (stored at -20ºC)

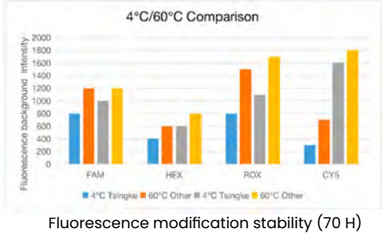
Accelerated stability







Fluorescence modification stability



Fluorescence modification stability (70 H)

The product has successfully passed a series of rigorous tests including long-term stability, accelerated stability, and repeated freeze-thaw stability tests. These results demonstrate its excellent stability and tolerance, making it suitable for various demanding applications.

Chapter 3

NGS Oligos

· Overview

NGS Oligos are used in Next-Generation Sequencing (NGS) technology. These Oligos have different functions such as library construction, amplification and capture, and are important raw materials for NGS.

Tsingke has rich experience in oligonucleotide synthesis, provides NGS products that support two platforms, including hybridization-based enrichment and amplicon-based enrichment, with efficient sealing effects and excellent capture efficiency.Tsingke brings customers high-quality Oligo products with high purity, high quality, and low cross-contamination rate to ensure the accuracy of downstream experiments.

ISO 13485 and 100,000-level clean room

High coupling efficiency with dedicated NGS team

Low batch-to-batch difference to ensure high homogeneity of multiple target fragments obtained

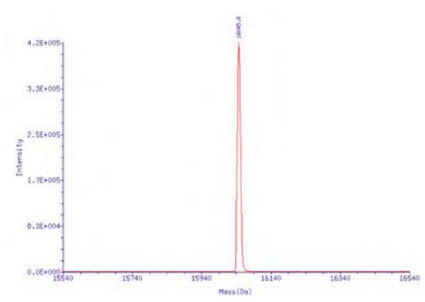
Stringent purification standards produce higher quality products with less than 0.05% cross-contamination rate

· Service Details

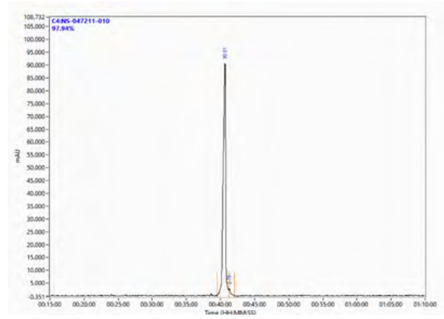
Service name	Length (nt)	Purification	Price/ turnaround time	Deliverable	Application
Adapter	15~80	HPLC/PAGE	Inquire	· Tube or customized lyophilized DNA · COA report (electronic)	NGS library construction
Blocker	Customized	HPLC			Blocked sample adapter sequence
Capture probes	80~120	HPLC/PAGE			Target area capture
Multiplex PCR oligos	15~120	HPLC/PAGE			Sequencing of specific target regions

*Note: In addition to the recommended content, Oligo length and purification methods can also be customized.

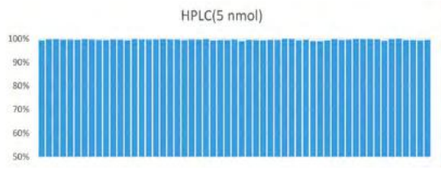
· Case



MS



HPLC



CV (HPLC):0.28%

CV(OD260):1.69%

Chapter 3

siRNA

· Overview

Tsingke has a comprehensive oligonucleotides synthesis platform and strictly adheres to ISO 13485:2016 quality control standards, ensuring the delivery of high-quality siRNA.

We offer custom synthesis of siRNA oligonucleotides in quantities ranging from micrograms to kilograms and provide free assistance with sequence design, guaranteeing silencing effect if needed. Additionally, we supply ready-to-use negative and positive siRNA controls to monitor your experimental conditions.

ÄKTA pure™: Support gram-scale purification

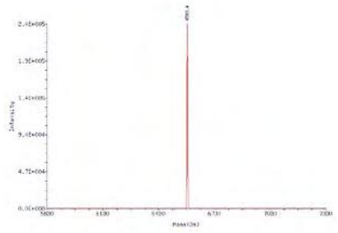
· Service Details

Service	Details	Length(nt)	Quantity	Purification	QC	Deliverable
siRNA	Custom synthesis	20~25	5 nmol or 2 OD	HPLC	HPLC	· Tube or customized lyophilized RNA · COA report (electronic)
siRNA NC	Various types available: negative/positive control siRNA, transfection control siRNA, etc.		2.5 nmol/ 1 OD			
3 siRNA, 1 Guaranteed	With a transfection efficiency of 90%, at least one out of the three siRNAs should achieve an mRNA interference efficiency of over 70%		5 nmol or 2 OD			
4 siRNA, 1 Guaranteed	With a transfection efficiency of 90%, at least one out of the four siRNAs should achieve an mRNA interference efficiency of over 70%		5 nmol or 2 OD			
Large-scale siRNA	Up to kg level		≥20 nmol or 8 OD			

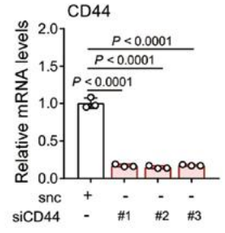
*Note: In addition to the recommended content, Oligo length and purification methods can also be customized.

· Case

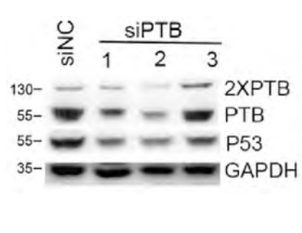
siRNA synthesis



MS



siRNA transfected MRC-5 cells and interfered with CD44



siRNA transfected HEK-293 cells to interfere with PTB

MS molecular weight detection with an error margin of less than 0.1% HPLC purity detection ensures purity levels greater than 90%

Achieving a high modification rate and incorporating stable groups such as PS, 2′-F, 2′-OMe, and FAM to ensure suitability for downstream applications

Leverage Tsingke's comprehensive industry chain advantages to provide fast and cost-effective services

Multi-channel synthesizer with μg to kg level synthesis capabilities, synthesizing over 100,000 oligos annually

Chapter 3

ASO

· Overview

Tsingke provides comprehensive ASO synthesis services with strict adherence to ISO 13485:2016 quality standards to ensure the delivery of high-quality products. We offer a wide range of modifications to enhance oligo stability, as well as flexibility in synthesis scale from micrograms to kilograms. Our purification options, including ESI mass spectrometry and HPLC, are designed to meet your specific experimental requirements.

During the drug production phase, we rigorously adhere to GMP requirements to guarantee the high standards of quality and safety. We provide technical support encompasses the production process, ensuring robust compliance with regulatory and standard protocols, make the entire drug development pipeline compliant, safe and efficacious.

Comprehensive modifications

LNA, cEt, 2′-O-C16, 2′-MOE, 2′-Ome, psiU, N1-psiU, PS, FAM, Fluorophores modifications.

Flexible

Synthesis scale from micrograms to kilograms, flexible packaging options, and precise quantification

High quality

Customizable QC standards, ISO 13485:2016, from RUO to GMP compliance, HPLC purity up to 99%

· Service Details

Product	Modification	Purification	QC	Deliverable
Custom ASO Synthesis	LNA cEt 2′-O-C16 2′-MOE 2′-OMe psiU N1-psiU PS FAM Fluorophores modifications	HPLC	MS optional: HPLC, SEC-HPLC	· Tube or customized lyophilized RNA · COA report (electronic)

· Customizable Testing

RUO

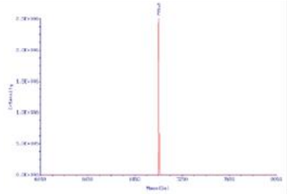
· MW
· Purity

GMP

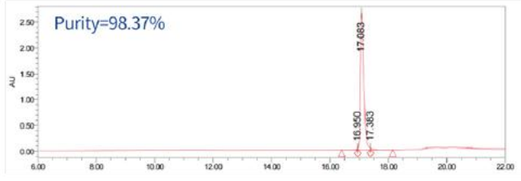
· Oligo content
· Purity and impurities
· Sequence
· Water content
· Residual solvents
· Elemental impuruties
· Endotoxin
· Bioburden
·····

· Case

2′-MOE and PS Modification

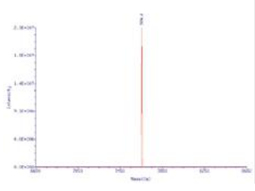


MS

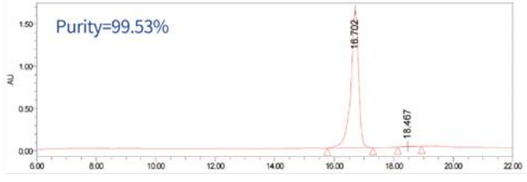


HPLC

LNA and PS Modification



MS



HPLC

Chapter 3

sgRNA

· Overview

Small guide RNA (sgRNA) is essential for the CRISPR gene knockout and knock-in system. It combines with the Cas9 protein to target and cut genomic DNA. Commonly, sgRNA is transfected into cells via plasmids, either on the same or separate plasmids as the Cas protein. However, this method often results in low editing efficiency and higher off-target effects.

Chemically synthesized sgRNA oligos, forming ribonucleoprotein complexes (RNPs) with Cas9 protein in vitro, avoid these issues. RNPs offer higher editing efficiency, lower off-target effects, and minimal immunogenicity compared to plasmid-based methods.

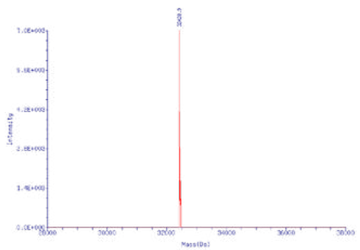
· Service Details

Service	Modifications	length(nt)	Quantity	Turnaround Time*	Purification	QC	Deliverable
Cas9 sgRNA-OPC	PS and 2'-OMe modifications at the first 3 and last 3 nucleotides	97~103	1.5 nmol	5	OPC	UV& MS	· Tube or customized lyophilized RNA · COA report (electronic)
			3 nmol				
			5 nmol				
Cas9 sgRNA-HPLC			1.5 nmol	10~12			
			3 nmol				
			5 nmol				
Cas9 crRNA		~36	5 nmol	5~7	HPLC	UV & MS & HPLC	
Cas9 tracrRNA		~67	3 nmol				
LbaCas12a crRNA	One PS modification at the 5'end, one OMe modification and two PS modifications at the 3' end	~40	5 nmol				
AsCas12a crRNA		~40	5 nmol				
LwCas13a crRNA	None	~55	5 nmol				
PspCas13b crRNA		~56	5 nmol				
RxCas13d crRNA		~43	5 nmol				

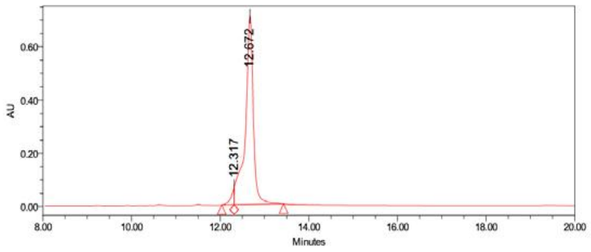
*Business Day.

· Case

The sample is a 100 nt sgRNA with three 2'-OMe modifications at both ends. Mass spectrometry and chromatography analyses confirm its sequence accuracy and a high purity of 96.54%.



MS



HPLC

Low cytotoxicity

Low impurity (residual substance) content

High editing efficiency

Increased experimental success rate

Stable quality

Strict quality control between batches

No DNA interference

No unnecessary gene fragment insertion

Chapter 3

piRNA

· Overview

Tsingke offers precision piRNA synthesis tailored for RNA-mediated gene regulation research, with a wide range of chemical modifications to enhance stability, target specificity, and delivery, supporting advanced studies in RNA biology.

Piwi-interacting RNAs (piRNAs) are essential small RNAs involved in transposon silencing, germline development, and epigenetic regulation. By guiding PIWI proteins to form piRNA-induced silencing complexes (piRISCs) that mediate post-transcriptional cleavage of target RNAs and transcriptional silencing via epigenetic modifications.

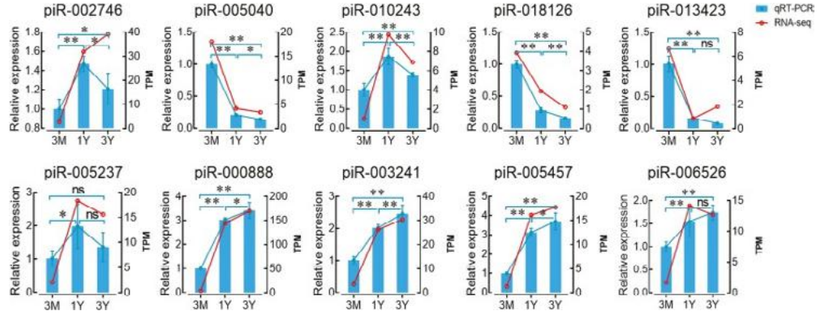
· Service Details

Modifications	Function	length(nt)	Turnaround time*	Purification	Deliverable
2'-O-Methyl (2'-OMe)	Mimics endogenous piRNA 3'-end, stabilizes against exonucleases	30~40	3~5	HPLC	· Tube or customized lyophilized RNA · COA report (electronic)
2'-Fluoro (2'-F)	Increases duplex rigidity; enhances binding affinity				
Phosphorothioate (PS)	Confers resistance to RNase H and plasma nucleases				
Unlocked Nucleic Acids (UNA)	Reduces off-target effects by destabilizing non-specific interactions				
Fluorescent Dye(FAM/Cy5/Cy3)	Real-time tracking of piRNA				
Inverted dT	Prevents extension and degradation, locks structure for conformational studies				
Biotin	For interaction studies,enables RNA pull-down, piRNA-protein interaction mapping				

*Business Day.

· Case

A application of the piRNAs, synthesized by Tsingke, in the qRT-PCR validation.



Highly Customized

Synthesized under strict RNase control, with a wide range of modification and packaging options

High Purity

HPLC purification and mass spectrometry QC ensure consistency and data transparency

Reliable Reordering

Batch consistency and sequence archiving for seamless project continuity

Scalable Delivery

From nmol to mmol scale, supporting both pilot and expanded studies with fast turnaround

Chapter 3

Large Scale Capability

· Overview

Tsingke intergrates a professional large-scale Oligo synthesis platform, including raw materials, synthetic instrumentation and equipment, synthesis techniques, and services.

Our DNA and RNA oligonucleotide synthesis services ranging from μg to kg level, conducted within ISO 13485 certified and GMP-like manufacturing facility boasting a cleanliness level of up to 100,000.

We offer customizable oligo synthesis of different lengths, comprehensive modifications & labeling and synthesis scales, include small nucleic acid drugs (ASO, siRNA, etc.), diagnostics (qPCR & NGS), CpG-ODN, DEL, sgRNA libraries, etc

Our products can address development and production requirements across various fields, including scientific research, drug development, diagnostic reagents, and more.

· Service Details

Service name	Length (nt)	Quantity	Turnaround Time (Business day)	Deliverable	QC
DNA Oligo	≤60 nt	1000 μg~1000 g	≤100 g:10~15 100 g~1000 g:60	Lyophilized DNA/RNA	· MS: Molecular weight deviation ≤0.05% · HPLC: Purity ≥90% · Customized quality control
	61~200 nt	1000 μg~10 g	≤1 g:10~15 1 g~10 g:30		
RNA Oligo	≤60 nt	1000 μg~1000 g	Inquire		
	61~120 nt	1000 μg~10 g	Inquire		

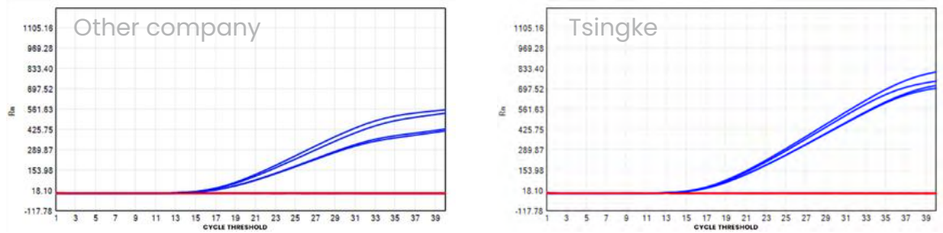
*We offer purification methods such as DSL and HPLC.
*For other customized options, regarding length, delivery scale, purity, etc., please feel free to inquire for more details.

· Case

1. Tsingke synthesized three 500 OD strands for use as raw materials in three diagnostic assay kits: 5'FAM with 3'BHQ1, 5'HEX with 3'BHQ1, and 5'CY5 with 3'BHQ2. For one of the strands, measured results are as follows:

At the same concentration, Tsinkge has lower Ct value, and the higher fluorescence intensity.

Wild-type plasmid



ISO 13485 and 100,000-level clean room

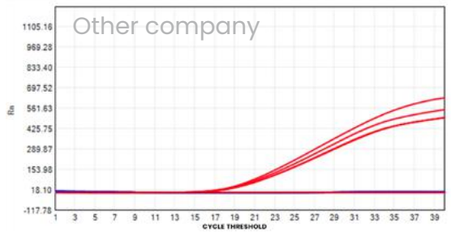
State-of-the-art oligo-synthesizer, purification, and testing equipment, including TsiKer Syn HL-12, Waters 2767, Labconco freeze dryer, Agilent Oligo Pro II, etc.

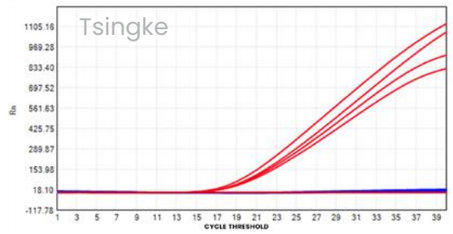
Up to 250 nt DNA & 180 nt RNA, kg delivery ability

Comprehensive 200+ modifications (LNA, PS, GalNac, 2'-OMe, etc.)

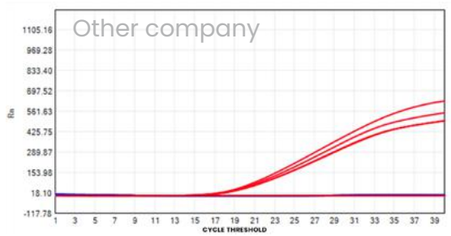
Experienced synthesis team provides tailored services covering synthesis scale, purification, QC and packaging.

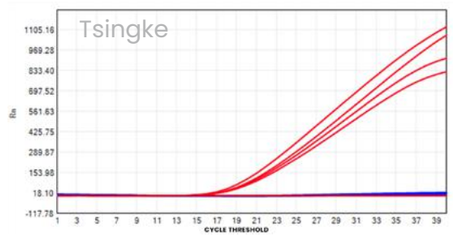
Mutant plasmids



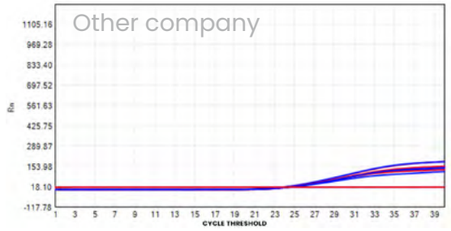


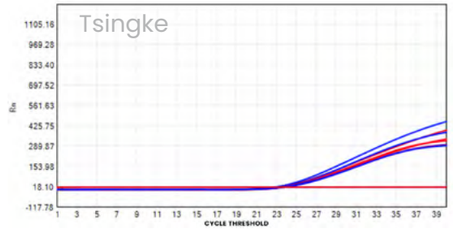
(Sample1)10 ng/μL DNA



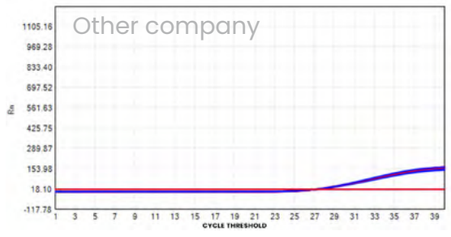


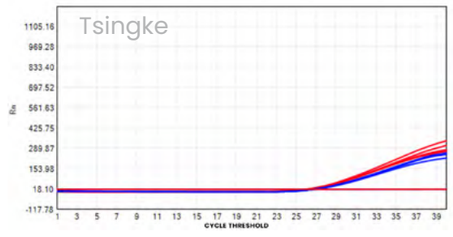
(Sample 2)3 ng/μL DNA



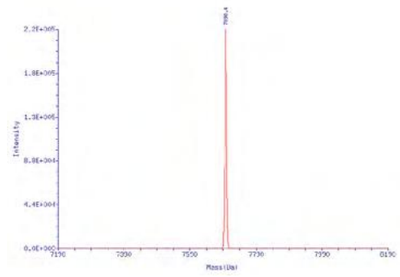


(Sample 3)0.5 ng/μL DNA

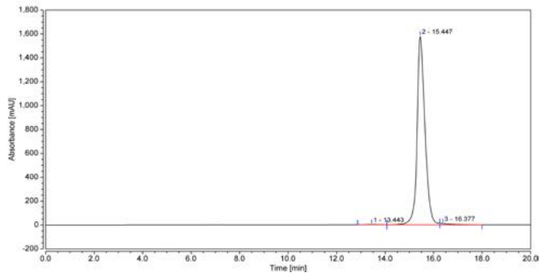




2. Tsingke synthesized three 3000 OD strands of CpG-ODN, each with a single modification. The mass spectrometry(MS) and HPLC chromatograms for one of the strands are provided below. The measured molecular weight deviates from the theoretical molecular weight by ≤±0.05%. Additionally, the main peak area of the HPLC purification is 98.76%, meeting the qualification standard of over 90%.

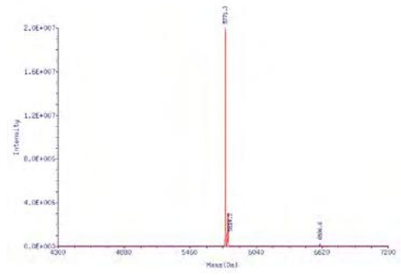


MS

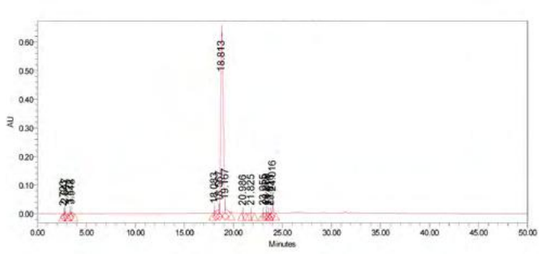


HPLC

3. Tsingke Biotech synthesized a single strand of ASO product at 20,000 OD (approximately 800 mg). The crude purity of the product reached 71%. After purification, the HPLC purity exceeded 90%.



MS



HPLC

COMMON MODIFICATIONS

04

Chapter 4

Common Modifications

· Amino Modifications (AMN, AMB)

(1)Internal Amino Modification

Tsingke uses mainly C6-dT Aminolinker to add to thymine residues for internal modification. The modified amino group is 10 atoms away from the main chain and can be used for further labeling and enzymatic ligation. dT-Dabcyl, dT-Biotin and dT-Digoxingenin are currently available for internal amino modification.

(2)5' Amino modification

can be easily used to prepare functionalized oligonucleotides for a wide range of applications in DNA microarrays and multi-labeling diagnostic systems. C6 amino modifications are currently available for ligating compounds that do not affect the function of the oligonucleotide even in close proximity to it.

(3)3' Amino Modification

Tsingke currently offers C7 amino modifications which can be used to design new diagnostic probes and antisense nucleotides, for example the 5' can be labeled with the highly sensitive 32P or luciferin while the 3' can be amino modified for additional ligation. In addition the 3' modification is resistant to 3' exonuclease digestion and thus can be used for antisense experiments.

· Biotin

The avidin-biotin technology has a wide range of applications, including non-radioactive immunoassays for protein detection, intracellular chemical staining, cell separation, nucleic acid isolation, hybridization to detect specific DNA/RNA sequences, and probes for ion channel conformational changes. Functionalized biotin can also be used for immobilization on encapsulated solid-phase surfaces, and the use of this technology for photodegradable biotin products has recently been explored.

Biotin-modified oligonucleotides bind tightly to streptavidin proteins, which can be labeled with fluorescent dyes and enzymes or attached as intermediate conjugates on the surface of solid organisms, and different molecular biology and purification methods include biotin modifications. Biotin modifications can be added to the 5' or terminal end of the oligonucleotide using either C6 or TEG spacer, biotin TEG requires purification, and intermediate biotin modifications can be added via dT bases, a form that requires additional purification steps. Primer biotin labeling for non-radioactive immunoassays to detect proteins, intracellular chemical staining, cell isolation, nucleic acid isolation, hybridization to detect specific DNA/RNA sequences, ion channel conformational changes, etc.

· Thiol (THS)

The 5'-sulfhydryl group is similar to amino modifications in many ways. The sulfhydryl group can be used to attach various modifications such as fluorescent markers and biotin. For example, thiol-linked fluorescent probes can be made in the presence of iodoacetic acid and maleimide derivatives. 5' thiol modifications are mainly done with 5' thiol-modifier monomers (5'-Thiol-Modifier C6-CE Phosphoramidite or the Thiol-Modifier C6 S-S CE Phosphoramidite). After modification with 5'-Thiol-Modifier C6-CE monomer, silver nitrate must be oxidized to remove the protecting group (trityl), while the Thiol-Modifier C6 S-S CE monomer must be modified with DTT to reduce the disulfide bond to a sulfhydryl group. However, it is recommended that customers perform the reduction prior to use. This modification can be used for a variety of applications such as cross-linking or hybridization experiments, increasing DNA double-strand stability and nuclease protection.

· DeoxyUridine (dU)

Deoxyuracil can be inserted into oligonucleotides to increase the melting point temperature of the double strand and thus increase the stability of the double strand. Each deoxythymidine substituted by deoxyuracil can increase the melting point temperature of the double strand by 1.7°C.

· Deoxylnosine (dI)

Although deoxy hypoxanthine is a naturally occurring base, it is not really a universal base but when combined with other bases it is relatively more stable than other base mismatches. dI:dC>dI:dA>dI:dG>dI:dT is the binding ability of deoxy hypoxanthine with other bases. dC is the preferred base for deoxy hypoxanthine when catalyzed by DNA polymerase.

· 5 Methylated Me-dC (MedC)

The substitution of MedC for dC increases the stability of the DNA duplex and improves the Tm value by pairing with dG. The melting temperature of each dC replaced by 5-Me-Dc will increase by 0.5°C. In addition, the presence of 5-methyl deoxycytidine in the CpG modification will prevent or limit unwanted immune reactions when oligonucleotide fragments are implanted in vivo, which is an important aspect in antisense nucleic acid applications. This is a very important aspect in antisense nucleic acid applications. In addition, it can be used for applications such as: enhanced PCR primer binding; antisense oligonucleotides and DNA methylation studies.

· Dideoxy ddC

This base is mainly used in two types of experiments: 3-terminal blocking of strand extension in PCR reactions, such as microarray experiments. The other is to prevent the 3-terminal 5'-adenylated oligos from self-associating or linking to each other as a junction during miRNA library construction.

· Phosphorylation

5' phosphorylation is used for splicing, cloning and gene construction as well as ligase-catalyzed ligation reactions. 3' phosphorylation is used in experiments related to resistance to 3' exonuclease digestion and to block DNA strand extension reactions catalyzed by DNA polymerases.

· Phosphorthioate

Phosphorothioate modified oligonucleotides are mainly used in antisense experiments to prevent degradation by nucleases. You can choose full Phosphorthioate, but as the number of phospho bases increases, the Tm value of the oligonucleotide decreases. To reduce this effect, Phosphorthioate modification can be applied to 2~5 bases at each end of the primer, usually 3 bases each of 5' and 3' can be chosen for Phosphorthioate modification.

· FAM

Carboxyfluorescein, a fluorescein derivative, reacts more rapidly with amino groups and has a more stable product than FITC, but the amount of FITC-bound protein is greater and the process is more controlled.

FAM is also suitable for the 488 nm spectral line of Argon-ion Laser, Abs/Em=492/518 nm (pH=9.0), and has the general properties of fluorescein derivatives and is stable in water.

· TET

Tetrachloro fluorescein, a fluorescein derivative, is a modification of FAM, where the chlorine atom causes a redshift in both Abs and Em values of FAM and reduces pH sensitivity to some extent. TET, HEX, FAM and TAMRA can be used together for automated DNA sequencing, where TET is used to label d/ddATP.

· 5'Cy3 & 5'Cy5

Cy3 and Cy5 are new fluorescent molecules with good photostability, high water solubility and high fluorescence efficiency.

The peak excitation and emission spectra of Cy3 and Cy5 are 548/562 nm and 646/664 nm, respectively, and the molecular structures and molecular weights of Cy3 and Cy5 are very similar, but the spectra of Cy3 and Cy5 are well separated from each other, therefore, Cy3 and Cy5 are commonly used in many two-color experiments, such as in gene chips and protein chips.

· 5'TAMRA & 3'TAMRA

Compared to fluorescein, which has the disadvantage of high photoquenching rate and high pH sensitivity, TAMRA has better photostability and the spectrum of TAMRA is not affected at pH between 4~10.

The fluorescence yield of TAMRA couples is about 1/4 of that of fluorescein couples, however, TAMRA is easily excited by the 546 nm spectral line of mercury-arc lamps and has better photostability than fluorescein, therefore, TAMRA couples often show stronger fluorescence than the corresponding fluorescein couples.

TAMRA-labeled oligonucleotides are also commonly used for quantitative and real-time quantitative PCR, DNA sequencing, etc. The TAMRA-labeled oligonucleotides can be excited by the 543 nm spectral line of the He-Ne laser, and are widely used in analytical instruments.

· Spacer

Spacer provides the necessary spacing and distance between oligonucleotide markers to reduce the interaction between the marker group and the oligonucleotide, and is mainly used in studies of DNA hairpin structures and double-stranded structures. dSpacer is mainly used to insert a base that has lost its ability to pair (deacylation site) into the DNA sequence. 5' and 3'-Spacer introduces a spacer arm that increases the distance between the modification group and the DNA sequence.

· 3'Dabcyl

When Dabcyl is spatially adjacent to a fluorescent group, it can absorb energy to quench the fluorescence, so it is widely used in molecular beacons and molecular quenching probes, etc.

· 2’-F-dU

A and G are difficult to synthesize because of their complex chemical structures, and C and U are commonly used as 2-fluorosubstitutes.

· 2'-O-methyl-ribonucleic acid

Among RNA modifications, the 2'-O-methyl-ribonucleic acid (2'-O-methyl-RNA) modification is commonly found in tRNA and other small post-transcriptionally regulated RNAs, and oligonucleotide fragments containing 2'-O-methyl-ribonucleic acid can be synthesized directly, this modification increases the Tm value of RNA:RNA duplexes, but will have a minor change on the stability of RNA:DNA. This modification can increase the Tm value of RNA:RNA duplexes, but there will be a small change in the stability of RNA:DNA, which is stable if expected to be attacked as a single-stranded nucleic acid and is typically 5~10 folded and less likely to bind to DNases than DNA. 2'-O-methyl ribonucleic acid modification is commonly used in antisense oligonucleotides as an effective tool to stably increase the binding capacity to the target message.

· Inverted dT

Inverted dT can be synthesized at the-end of an oligonucleotide, resulting in a crosslink that inhibits degradation by exonucleases during DNA polymerase extension.

· Digoxigenin

Digoxigenin is a steroidal substance isolated from the plant Trichoderma viride because the flowers and leaves of Trichoderma viride are the only natural sources of this substance, so anti-digoxigenin antibodies do not bind to other biological substances. Digoxin is attached to the C5 position of uracil via an 11-atom intercalary arm. Hybridized digoxin probes can be detected by anti-digoxin antibodies, which are typically coupled to alkaline phosphatase, peroxidase, luciferin or colloidal gold. Or anti-digoxin antibodies without coupling but coupled antibodies. Digoxin-labeled probes can be used in various hybridization reactions such as DNA-DNA hybridization (Southern blotting), DNA-RNA hybridization (Northern blotting), dot blotting, clonal hybridization, in situ hybridization, and enzyme-linked immunoassays (ELISA).

· Nucleic acid locking

LNA bases modify the backbone of the ribose pair, locking the bases at the C3 medial position and favoring the RNA A-helical double-stranded structure. This modification significantly increases the Tm value and is highly resistant to nucleases.

· 5' Acrydite

The modified oligonucleotides enable the copolymerization of oligonucleotides and polypropylene gels.

FAQ

05

Chapter 5

FAQ about DNA Oligos

1. What are the basic principles of primer design?

(1) Primer length generally between 15~30 bases.

(2) Primer GC content between 40% and 60%.

(3) Bases should be randomly distributed.

(4) There should be no complementary sequences in the primers themselves and between the primers

(5) The primers should have relatively high ΔG values at the 5'end and middle ΔG values, and low ΔG values at the 3' end.

(6) The single strand of the amplification product should not form a secondary structure.

(7) The primers should have specificity.

2. Why the yield of modified primers is lower than the general primers and the price is higher?

The main reason is that the stability of the modified monomer is poor, the coupling time is long and the efficiency is low, and the final yield obtained is naturally lower than that of the general primers. Modified primers usually need to be purified by PAGE or HPLC, and the purification process has a large loss, and the raw materials used in modified primers are hundreds of times more than the raw materials used in general primers, so the price of the product is naturally high.

3. What impurities are contained in the crude product of DNA synthesis?

It is mainly the failed fragments produced during the synthesis reaction and the ammonium salts produced during the deprotection group.

4. What are the ways of primer purification?

The common purification methods are DSL, OPC, PAGE, HPLC and Dual PAGE & HPLC.

5. Is there phosphorylation at the 5' end of the synthesized primer?

The synthetic primers have hydroxyl groups at the 5' end and no phosphate groups. If needed, you can use polynucleotide kinase to phosphorylate the 5' end, or ask us to phosphorylate the 5' or 3' end directly when synthesizing for an additional charge.

6. What is the longest primer that can be synthesized?

The longer the primer, the higher the probability of problems. Unless there is a special need, we recommend not to synthesize fragments longer than 80 mer. According to the current primer synthesis efficiency, the percentage of full-length primers will not exceed 40% for a crude product of 80 mer, and a lot will be lost in subsequent processing, so the final yield is very low. The maximum length of primers that can be synthesized is 120 bases.

7. Why do I charge more for long-chain primers than short-chain primers?

When synthesizing long-chain primers, more reagents are required than short-chain primers, especially for primers longer than 90 bases. This results in a higher price due to the increased cost.

8. What happened when sequencing found mutations in the primers?

Primer synthesis is a multi-step chemical reaction, and the efficiency of each step of synthesis is 99% at most, and by-products cannot be avoided. The longer the chain, the higher the cumulative frequency of mutations. When you clone and sequence after PCR amplification, in order to save time and improve the success rate, we have the following suggestions.

(1) Please prepare 2~3 positive clones of the bacteriophage after you detect the positive clone, and try to send 2 or more clones for sequencing, so that the success rate will be greatly improved and a lot of time will be saved.

(2) You can also send one clone for testing first, and store the rest two clones of the bacteriophage in the refrigerator at 4 degrees, and send the remaining two clones for testing as soon as individual point mutations or deletions occur.

(3) This will have a very high probability of getting the correct sequence and will eliminate the need for a series of experimental operations such as re-PCR, ligation, cloning and screening, saving even more time.

If you find more than 2~3 clones with mutations in the primer region, and it is confirmed that it is caused by the primer, we will arrange for the expedited free recombination immediately and send it to you as soon as possible.

9. How to determine how many OD primers need to be synthesized?

Determine according to the purpose of the experiment. For general PCR amplification, 2 OD for primers of about 20 bases can do 400 times 50 μL standard PCR reactions. If you are doing gene splicing or ligation after annealing, 1 OD is enough.

10. How to determine the OD value of primers?

The OD of synthetic primers is determined by using a UV spectrophotometer with a wavelength of 260 nm and a quartz colorimeter with an optical range of 1 cm to determine the optical density of the solution. The optical density of the solution is best diluted to between 0.2~0.8. After the DNA dry powder is dissolved with a certain volume of water, the OD value is measured by diluting it with 1 ml of water. It is necessary to convert the OD value of the master solution according to the dilution times. For example, to verify whether the amount of 2 OD primer is accurate, it is simple to add 1ml of water, dissolve and mix thoroughly, take 100 μL, add 900 μL of water, and use a quartz colorimetric cup with an optical diameter of 1 cm and a wavelength of 260 nm, at which time the optical absorption reads 0.2.

11. Will the primers degrade when transported at room temperature?

No, the dried primers can be stored stably at room temperature for at least 2 weeks. The general shipping time is usually 1~3 days, so the primers you receive will not degrade.

12. Why the primers that have been lysed are not good when they were used normally and then used again after a period of time?

If the pH of the water you lysed the primers in is too low or contaminated with bacteria or nucleases, it will degrade the primers. Insufficient thawing and mixing of the liquid when using may also cause inaccurate primer addition. It is recommended to split the primers, avoid repeated freeze-thawing, and use 10 mM Tris pH 7.5 buffer to dissolve the primers. There is also a possibility that the primers are not faulty, but that the quality of the materials used for PCR, especially the template, is not exactly the same as previously used.

13. How to test the purity of primers?

A common laboratory method is to use PAGE method. Use polyacrylamide gels spiked with 7 M urea for electrophoresis. Use 20% gel for primers with less than 12 bases, 16% gel for primers with 12~60 bases, and 12% gel for primers with more than 60 bases. The primers of 0.2~0.5 OD were taken and dissolved with urea saturation solution or the primer solution was added with dry urea powder until saturation, and denatured by heating (95°C, 2 min) before sampling. The purpose of adding urea is one is denaturation, the other is to increase the specific gravity of the sample, easy to add samples. 600 V voltage for electrophoresis, after a certain time (about 2~3 hours), peel the gel, detect the band type with fluorescent TLC plate under UV lamp, there is no stray band under the main band, which means the purity is good. (Sometimes, due to insufficient denaturation, there may be bands above the main band, which are primer secondary structure bands.)

14. Why does the EB staining vary in shade for the same OD detected by PAGE?

The amount of double-stranded DNA (e.g. plasmid DNA) can usually be judged by EB staining, because EB stains nucleic acids by embedding them between the double helix. Synthetic single-stranded DNA, on the other hand, can only be stained by EB by forming a local hairpin loop structure by folding back on itself or by forming a partial double helix structure between the strands. Due to the different base composition, the possibility of forming secondary structure differs from primer to primer, and the degree of EB staining will also vary, for example, Oligo(dT), etc. does not form secondary structure, and the EB staining effect is very poor. Therefore, EB staining cannot be used for quantification, and UV spectrophotometry should be applied for detection.

15. When PAGE electrophoresis is performed, why are the swim bands of Oligo DNA of exactly the same length not in the same position?

This situation is more likely to happen when the Oligo DNA is shorter, and the difference between long-stranded Oligo DNA is smaller. There are two main reasons.

(1) different components of A, G, C and T and different electrophoresis speeds.

(2) The steric structure of DNA is different and the electrophoresis speed is different.

16. Can I use Agarose gel electrophoresis to analyze the synthetic primers?

It is important to use denaturing PAGE electrophoresis for primers. Since the primers are single-stranded DNA, it is easy to form a complex steric structure, so when Agarose electrophoresis is performed, it is easy to have multiple bands or no bands, and it is even more impossible to quantify by Agarose electrophoresis.

17. Sometimes the dried primers are yellowish-brown, is this the color of DNA itself?

Synthetic primers may be yellowish brown, white or transparent, which is related to the base composition of the primers and the preparation process of synthesis, primers with high A and G content in the sequence and large OD value are usually yellowish brown, so the yellowish brown primers will not have any effect on the experiment.

18. Is it related to the primers that PCR amplification does not work?

Basically not. Nowadays, various PCR amplification techniques and high-temperature polymerases have been developed to solve the problems of amplification failure and low amplification efficiency encountered in PCR amplification. For example, slot PCR amplifies gene fragments with very low copy number. Some repeat fragments, fragments with high GC content amplification, must use special amplification means to amplify.

Amplification does not come out, mainly the following two situations are more common.

(1) RT-PCR. many genes are difficult to amplify by conventional RT-PCR methods. the key to successful RT-PCR lies in the quality of the RNA of the RT reaction and the content of the target gene in the particular tissue and cell.

(2) Amplification from the genome. Generally, genes are single copies in the genome, and the genome needs to be used as a template in strictly controlled amounts. Too much genomic DNA can affect the Mg2+ concentration and pH in the reaction system.

19. PCR amplification has strong non-specific bands, does it indicate that the primers are contaminated?

No. We have analyzed some non-specific bands and sequencing found that at least one primer sequence can be found at both ends of these non-specific fragments. Therefore non-specific amplification is usually the result of template contamination (e.g. contaminated genome in RNA) or unsuitable amplification conditions.

Chapter 5

FAQ about RNA Oligos

1. Precautions for using siRNA

(1) The product is shipped in the form of lyophilized powder, the dry powder is attached to the wall of the tube and is easily dispersed when opened, so please centrifuge the tube for a few seconds before dilution to make the product gather at the bottom of the tube and open the cap carefully, cover the tube after dissolution, vortex and shake to mix to make full dissolution. It is recommended to store at -20°C or below, avoid repeated freezing and thawing, and keep in separate containers. Centrifuge instantly before use and prepare 20~100 μM storage solution with RNase-free Water or ddH_2O, the average molecular weight of siRNA is 13300.

(2) RNA handling rules should be strictly followed and RNase-free lab supplies should be used for experimental operations to avoid degradation of RNA. Please keep on ice when using this product.

(3) For RNA with fluorescent marker, we use brown centrifuge tube to pack, because the fluorescent marker is sensitive to light, it must be stored away from light.

2. Low efficiency of cell transfection?

siRNA purity is too low, pay attention to lysis, avoid RNA enzyme contamination, use consumables and reagents treated by DEPC; siRNA transfection reagent complex concentration is low, need to optimize transfection efficiency, use appropriate transfection reagent dose; serum affects transfection efficiency, when preparing the mixture, it should not contain serum; culture can have serum, but do not have Antibiotics; improper storage of liposome transfection reagent, reagent failure, should be stored at 4°C.

3. A lot of cell death after transfection?

Poor cell status, need to adjust cell growth status: generally cells in good growth status have better tolerance to transfection reagents; siRNA or transfection reagent concentration is too high; transfection reagent is more toxic, replace transfection reagent; siRNA purity is low, impurities affect cell status; transfection time is too long, not timely replacement of fresh medium after transfection.

4. After transfection, no fluorescence is observed?

Usually, FAM shows green fluorescence after transfection and CY3 shows red fluorescence after transfection. When observing, first confirm whether the fluorescence excitation wavelength is correct, the green light wavelength is 495 nm and the red light is 550 nm.

After transfection, you need to wash immediately to observe the fluorescence and avoid too long time.

If the product is not stored properly, siRNA will be degraded and FAM will be quenched. FAM is easily decomposed by light, so please store it away from light.

When transfecting, operate away from light and avoid exposing FAM to white light for a long time.

Transfection time is too long, and fresh medium is not replaced in time after transfection.

5. If the interference effect of the target gene is only about 60%, is the package considered invalid?

Usually, the package products are guaranteed to interfere with about 70% of the target gene, if the interference effect is about 60%, we also consider the interference to be meaningful. If there is a significant change in the phenotype and function of the cells, it is also recognized in the literature. Generally, the interference efficiency can be improved by increasing the transfection efficiency and optimizing the experimental system.

6. Why is it important to emphasize mRNA level detection? Can we directly detect protein and function?

siRNA acts directly on mRNA, so mRNA level detection is the most direct detection indicator. Many clients believe that the direct result of mRNA degradation should correspond to a decrease in protein content, and therefore protein level assay results can also be used as an indicator of effectiveness. In fact, in many cases there is often a mRNA decline level that does not correspond to the protein decline level. The possible reasons for this are:

Related to the time chosen for the assay. It may be that the decline in mRNA has not yet been reflected in changes in protein amounts or has not reached a level sufficient for detection, so it is generally recommended that protein and functional assays be pushed back slightly.

The translation process of mRNA is very complex, and the expression of genes in the cell must always maintain a balance, and after a certain level of expression of some proteins is sufficient to maintain their cellular function, the expression may be temporarily "turned off" and the transcribed Some mRNAs may not be involved in the process of protein translation, therefore, the down-regulation of mRNA level is not exactly positively correlated with the down-regulation of protein level.

7. How long can siRNA act in the cell? When is the best time for detection?

siRNA-mediated RNAi is a transient phenomenon and cannot be stably transmitted. Generally, its action time cannot be maintained for a long time, and it is usually recommended to finish the detection within 3~4 d after transfection. The optimal detection time varies by cell and target gene, and is mostly detected between 24~48 h after transfection. It is generally recommended to detect mRNA level at 24~48 h and protein level at 48~72 h.

8. Is the transfection efficiency of cells related to siRNA sequence?

The transfection efficiency depends on the cells themselves and the transfection method, and is not directly related to the sequence of siRNA. Therefore, the transfection efficiency of siRNA in different cells may be different.

9. Why is the same siRNA very effective in cell A but not in cell B?

Different cells have different transfection efficiency and different gene expression levels, which are all related to the action efficiency of siRNA, so there is no guarantee that the interference effect is the same in different cells.

Chapter 6

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