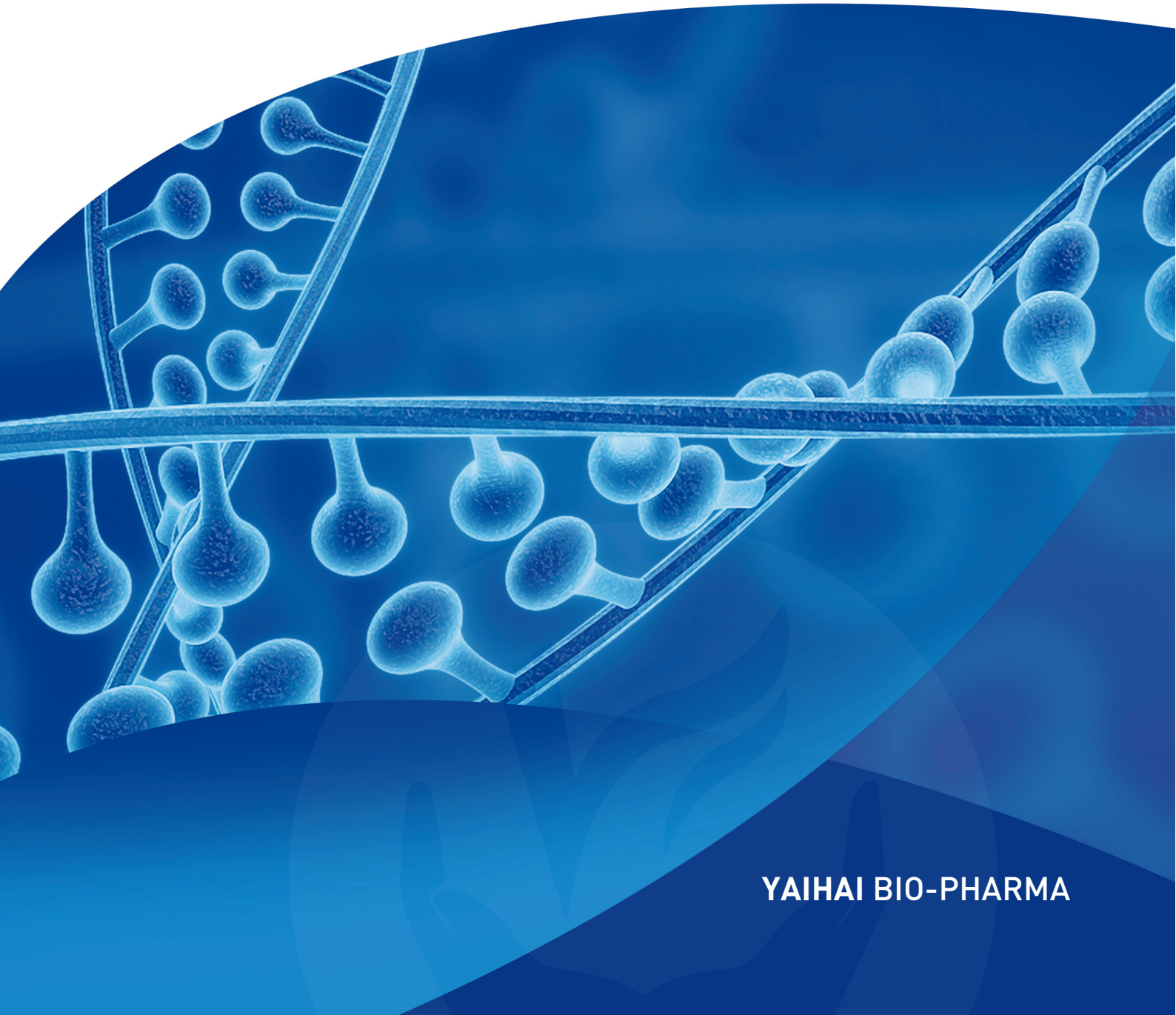
Yaohabio
耀海生物

YAOHAIBIO

mRNA

ONE-STOP SERVICE PLATFORM



YAIHAI BIO-PHARMA

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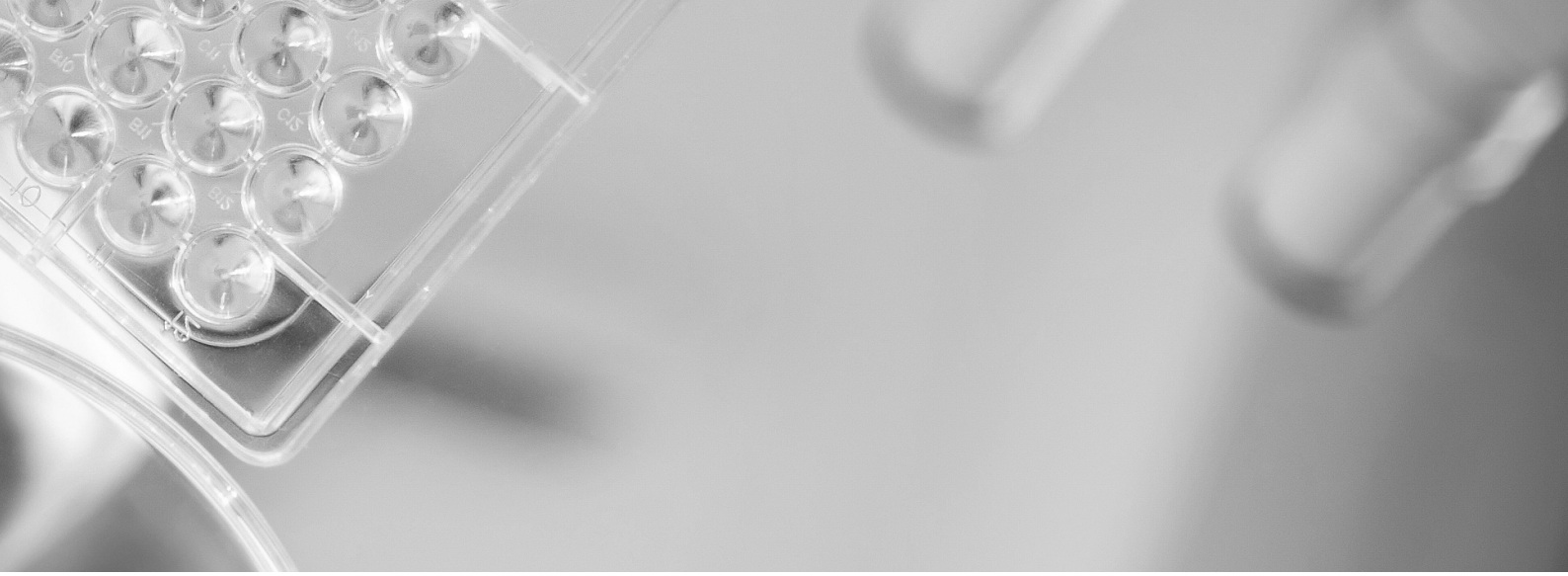

ABOUT
YAOHAI BIO-PHARMA

Founded in August 2010, Jiangsu Yaohai Biopharmaceutical Co., Ltd. is a national high-tech enterprise based in China Pharmaceutical City Park, Taizhou, Jiangsu Province, China. It is a CDMO service provider specializing in microbial expression systems, focusing on "recombinant proteins/peptides, nucleic acid drugs, Nano-antibodies, cell & gene therapy, novel recombinant vaccines and other fields", and is committed to building an open and integrated CRO/CD-MO/MAH service platform. The company's business covers one-stop CMC services such as engineering bacteria construction, strain library establishment, lab-scale process development and optimization, pilot process scale-up production, clinical sample equipment, specification establishment, analytical method development and validation, GMP compliance, and registration, etc.

The Company adheres to the service concept of "Service with heart and create the future", with the mission of "Create global standards, facilitate the process of new drugs and achieve a healthy life", and continues to empower the creation of new drugs worldwide.

End-to-end Microbial Expression Systems CRDMO / MAH

One-stop service platform

- Recombinant Proteins/peptides
- Nucleic Acid Drug
- Nanobodies
- Cell & Gene Therapy
- Novel Recombinant Vaccines

Strain Development & Library Building Services	Process Development & Optimization Services	Method Development & Quality Control Services	Clinical & Commercial GMP production	Registration For Application Services

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Overview of mRNA research-grade

sample preparation services

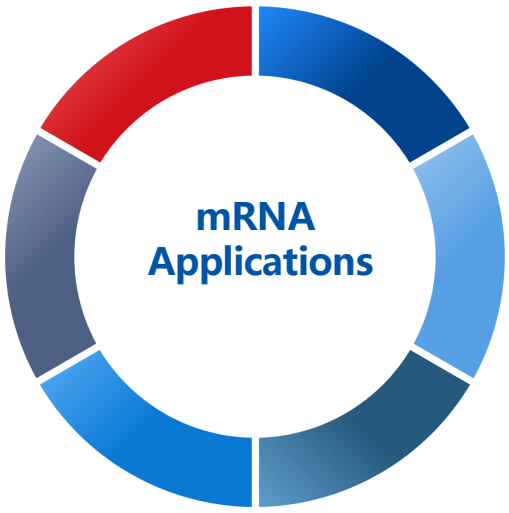
The outbreak of the COVID-19 pandemic in 2020 pushed mRNA technology to center stage, with unprecedented heat for related research and rapid development in multiple fields such as infectious disease prevention, tumor therapy, protein replacement therapy, regenerative medicine, and cell and gene therapy.

mRNA Applications

01 Prophylactic vaccines for infectious diseases

02 Therapeutic Oncology Vaccine

03 Therapeutic Oncology Drugs



06 Protein Replacement Therapy

05 Regenerative Medicine

04 Cell and Gene Therapy

Yaohai Bio has built a mature and perfect "RNASci" mRNA research-grade sample preparation service platform, which consists of four counting modules, and provides one-stop services for sequence design and optimization, gene synthesis, recombinant plasmid equipment, linearized template preparation, IVT and purification, and mRNA quality control, etc., throughout the whole lifecycle of mRNA design to sample generation, and comprehensively empower the process of mRNA vaccine and drug development.

"RNASci"mRNA service platform

PLATFORM TECHNICAL CONTENT	Platform for mRNA structural design and optimization	Platform for mRNA synthesis and modification	mRNA purification platform	mRNA quality analysis and control platform
mRNA SERVICE PLATFORM	• 5' UTR and 3' UTR optimization design • CDS zone optimization design • PolyA tail optimization design	• mRNA template plasmid design and construction • Conventional mRNA (capping structure, PolyA tail structure) • Nucleotide modification (e.g. Φ, N1ψ, 5mC) mRNA synthesis	• LiCl precipitation and purification • Magnetic bead purification • Self-developed chromatography column process purification	• Purity detection system [AGE (agarose gel electrophoresis), CE] • (WB,ELISA)Translation expression detection system (WB,ELISA) • Capping rate detection system (cooperative detection) • PolyA distribution assay system (collaborative assay)
RNASci	RNADes	RNASyn	RNAPur	RNAQua

Features of "RNASci" mRNA service platform

Highly Expressed Natural & Modified Utr

- Establishment of natural UTR library, and diversified UTR source selection can match the appropriate UTR sequence for different products;
- 5'UTR optimization for more efficient transcription of templates;
- Internationalized PolyA tail structural design strategy;
- Well-developed codon optimization methods and special optimization needs can be performed in cooperation with professional AI algorithm team.

Superior Capping Process For Efficient Transcription And Improvement Of Application Activity

- Highly productive and stable capping process with a capping efficiency of >95%;
- PolyA tail integrated transcription formation, with more uniform distribution;
- Diversified mRNA modified nucleotides effectively reduce the adverse immune response of mRNA in human;
- Flexible plasmid template design scheme to meet customer's specific needs.

General & Self-developed Chromatography Process, Providing Diversified Purification Methods

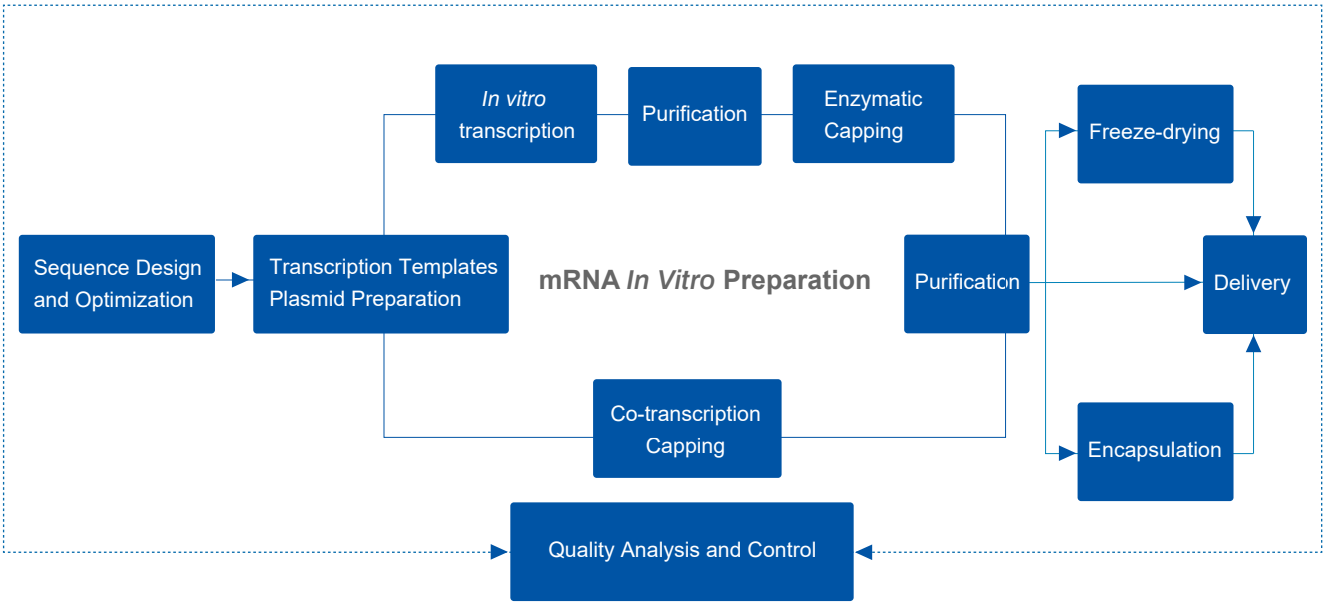
- **Diversification:**
  A comprehensive purification solution consisting of tangential flow filtration + multiple chromatography packing can effectively remove impurities from mRNA crude products for high quality applications;
- **General & self-developed purification process:**
  Well-developed and perfect LiCl precipitation + magnetic bead purification + chromatography purification solution;
  Completely self-developed, chromatography purification solution can effectively remove impurities in mRNA preparation.

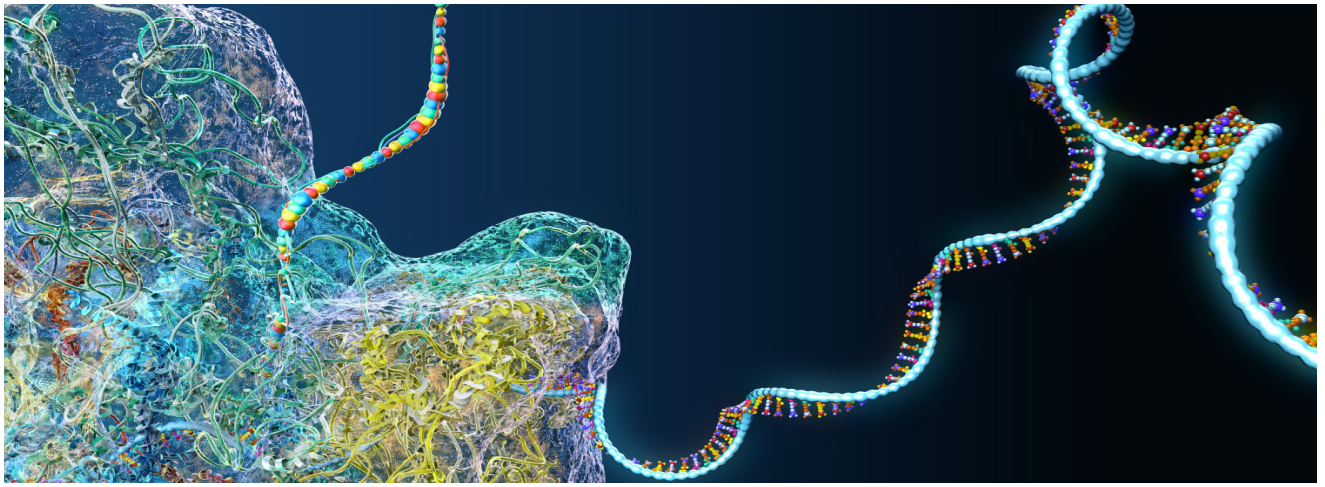
Comprehensive Quality Control Platform To Meet The Quality Control Needs Of Each Research Phase

- Meet the general QC requirements for scientific -grade concentration and purity;
- Meet the special QC needs such as mRNA translation test, capping rate, and tail distribution, etc.

Overview of mRNA
research-grade sample preparation services

Process Development Flow





Service Details

Service items	Optional items	Service Details	Delivery Period (days)	Delivery
mRNA sequence design and optimization	Design and optimization of coding sequences	CDS sequence design and codon optimization	1-3	Sequence file
	Design and optimization of non-coding sequences	Design and optimization of UTR, polyA sequences		
Transcription template plasmid preparation	Recombinant plasmid preparation	Gene synthesis	7-10	
		Plasmid amplificationand extraction	4	
		Plasmid linearization and purification		
mRNA *in vitro* transcription	Co-transcription and capping (one-step method)	*In vitro* transcription (Clean Cap analog)	1-2	N/A
		Nucleotide modifications (UTP/CTP modifications)		
		DNA template removal (DNase I)		
	Enzymatic capping (two-step process)	*In vitro* transcription	2-3	
		Nucleotide modifications (UTP/CTP modifications)		
		DNA template removal (DNase I)		
		mRNA purification (lithium chloride/magnetic beads)		
		Enzymatic capping		
mRNA purification	Conventional purification solutions	Lithium chloride precipitation	1	mRNA drug substance
		Magnetic bead purification		
	Chromatography column purification solution	Combination of multiple chromatography methods	1-2	
	Solution exchange	Ultrafiltration and liquid exchange	1	
mRNA lyophilization	Lyophilization	Pre-freezing	2-3	mRNA lyophilized powder
		Primary sublimation		
		Secondary Sublimation		
mRNA encapsulation	LNP encapsulation	LNP encapsulation	2-3	mRNA-LNP Drug product
		Concentration and liquid exchange		
mRNA quality analysis	mRNA drug substance/ lyophilized powder	Concentration, purity	1	CoAs
		Integrity, capping rate, polyA tail distribution	2-5	
	mRNA-LNP preparation	Encapsulation rate	1	
		Particle size and distribution detection		
		Surface charge detection		
mRNA expression validation	293T cell evaluation	Cell plating	4	
		Transient transfection of cells		
		Fluorescence signal observation	1-3	
		Western blot/ELISA		

Pre-Products Cataloge

Classification of coded proteins	Product Name	Optional Modified Nucleotides	Delivery Form	Product Specification
circRNA purification	mRNA_mCherry-eGFP	• No modification • Pseudouracil (Ψ) • N1methyl pseudouracil (N1Ψ) • N5methylcytosine (5mC) • Other modifications	• Lyophilized powder • Drug substance (500 ng/μL)	• 10μg • 50μg • 100 μg • 1 mg • 10 mg
	mRNA_eGFP			
	mRNA_mCherry			
	mRNA_luciferase			
	mRNA_Spike protein (COVID-19)			
	mRNA_IL-2			
	mRNA_IL-4			
circRNA quality control	mRNA_IL-22			
	mRNA_OVA			
	mRNA_Cas9			

Service Advantages

Integrated service flow

Provide a series of services from front-end sequence design to back-end mRNA preparation, quality control and expression validation.

International cutting-edge sequence design and optimization

Professional mRNA sequence design and optimization facilitates efficient mRNA expression.

Diversified nucleotide modifications

Effectively increase mRNA expression and reduce mRNA adverse immune responses.

Mature purification platform

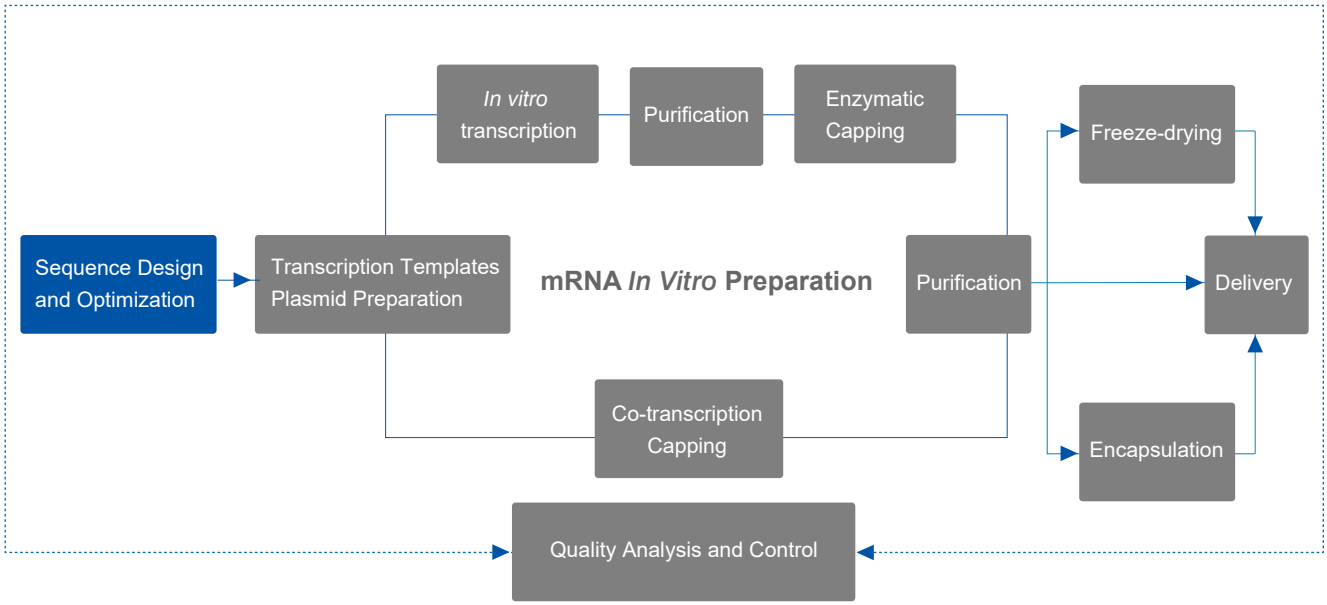
A combination of general & self-developed purification process provides high purity mRNA samples. Complete QC platform: Enrich QC options to meet the requirements of routine tests, such as concentration, A260/280 purity, and integrity, as well as high quality controlrequirements, such as capping rate/polyA distribution.

Fast delivery

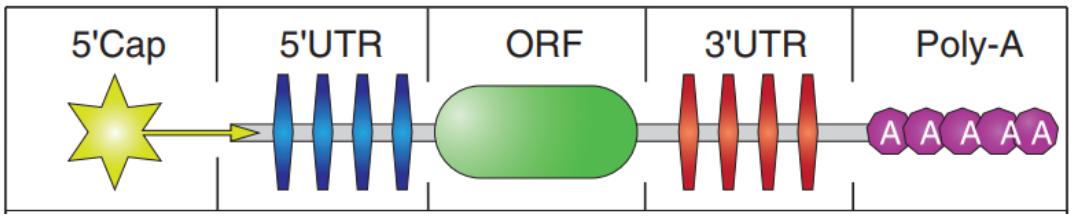
Same-day shipment of mRNA pre-products.Customized mRNA can be delivered in as fast as 7 days except for outsourced sequence synthesis.

mRNA

Sequence Design And Optimization Services



According to the central dogma, messenger RNA (mRNA) is the bridge for the transmission of genetic material from DNA to proteins. mRNA plays a biological role by encoding proteins in vivo, and mature mRNA in eukaryotic organisms consists of **five components**: 5' Cap (cap structure), 5' UTR (non-coding region), the ORF (open reading frame), 3' UTR, and 3' polyA tail (polyadenylate tail).



Schematic diagram of mRNA structure

Please refer to the following for the functions and optimization strategies of each component of mRNA:

mRNA components	Biological Functions	Optimization Strategies
5’ Cap	Protect mRNA from degradation by exonucleases and act in concert with the polyA tail at the 3' end, polyA binding protein and translation initiation factor protein to initiate protein translation.	The natural Cap1 structure avoids pattern recognition receptor and thus reduces the natural immune response, which can be achieved by one-step co-transcription capping or two-step enzymatic capping [see mRNA enzymatic capping and co-transcription capping for details].
5’ UTR	The 5’ UTR can be recognized by ribosomes, regulate the translation of mRNA and affect the stability of mRNA	Contain Kozak sequences without a very stable secondary structure. Natural UTRs of highly expressed genes are preferred for synthetic mRNAs such as α- and β-bead protein gene sources.
CDS	Protein-coding regions, and coding sequences for antigens, antibodies or other functional proteins.	Codon optimization increase the level of translation, noting that certain non-optimal codons may play a role in protein folding.
3’ UTR	Regulate mRNA translation and stability.	Natural UTRs of highly expressed genes are preferred for synthetic mRNAs, such as α- and β-bead protein gene sources.
3’ polyA tail	Regulate protein expression and protect cap structure from degradation.	Adequate length (100-150 bp) is required; encoding poly(A) tail on the transcription template plasmid ensures a more defined polyA tail length.

[1] Linares-Fernández S, et al. Trends Mol Med. 2020;26(3):311-323.

Service Details

Service Items	Optional Items	Detailed Steps	Delivery Period (Days)
mRNA sequence design and optimization	Design and optimization of coding sequences	• CDS sequence matching • CDS codon optimization	1
	Design and optimization of non-coding sequences	• 5' UTR sequence design and optimization • 3' UTR sequence design and optimization • polyA sequence design and optimization	1-2

Service Advantages

Diversified TR source selection

Multiple sources of highly expressed natural & modified UTR libraries, and mature UTR modification strategy;

Cutting-edge CDS optimization team

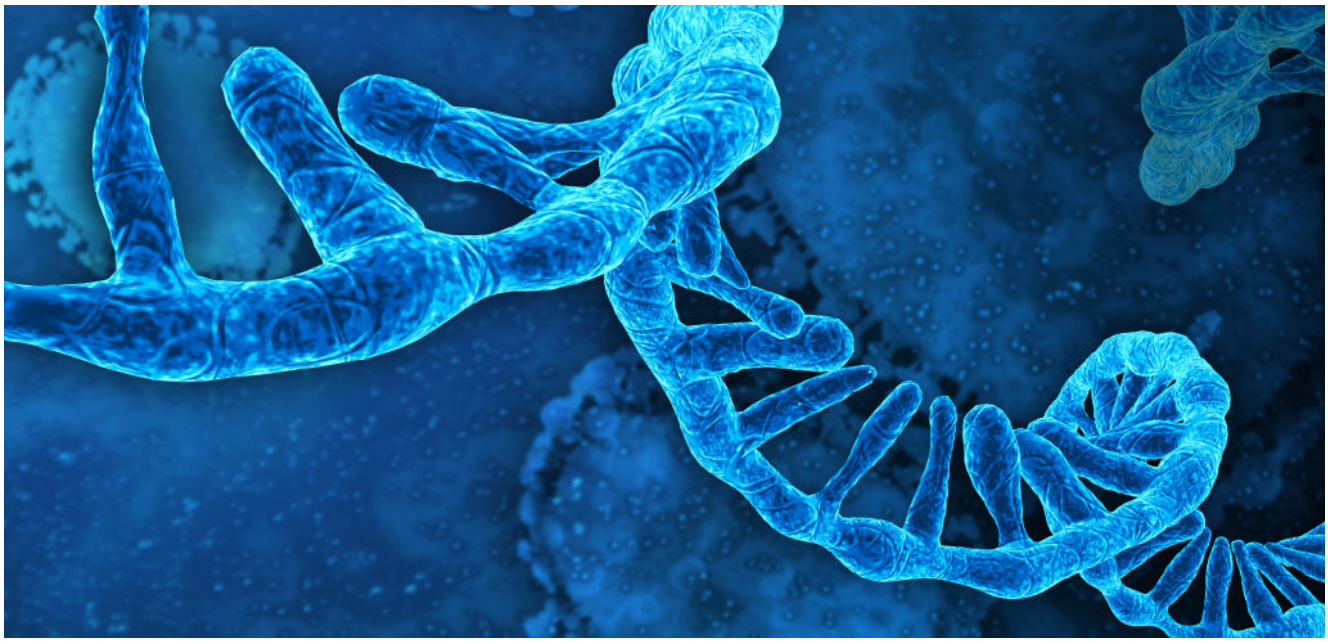
Cooperate with professional AI algorithm team to complete the optimization of codons.

Homogeneous polyA tail distribution

Integrated transcription formation of PolyA tail, with more homogeneous distribution.

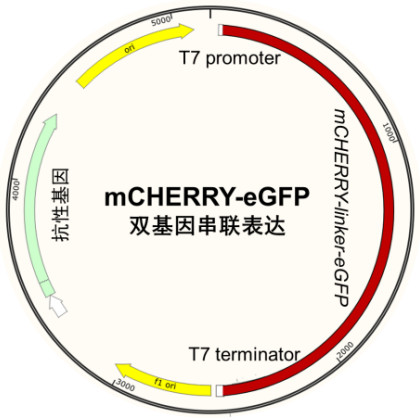
Diversified optimization combination

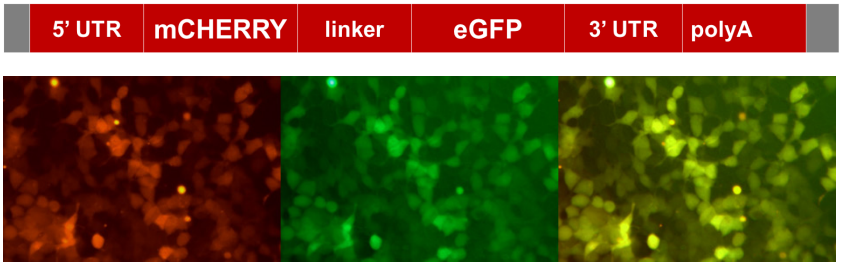
Achieve efficient expression of mRNA, with low immunogenicity.



Case Studies

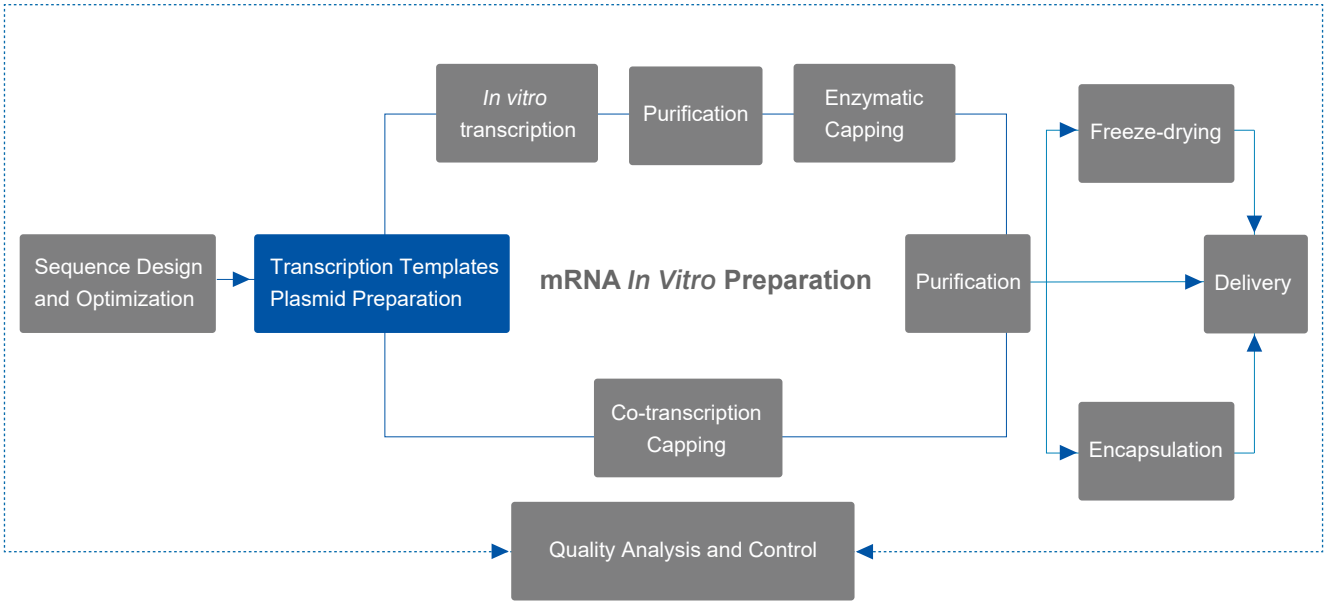
Yaohai Bio's mRNA service continues to be upgraded with the design and optimization of a double reporter gene tandem sequence, which allows co-expression of dual genes. Using a conventional transfection reagent, the double gene tandem sequence mRNA_mCherry-eGFP is transfected into 293T cells, and two fluorescent signals of mCherry (red) and eGFP (green) are detected with simultaneous expression after 48 hours, and the stacked graph is highlighted in yellow.



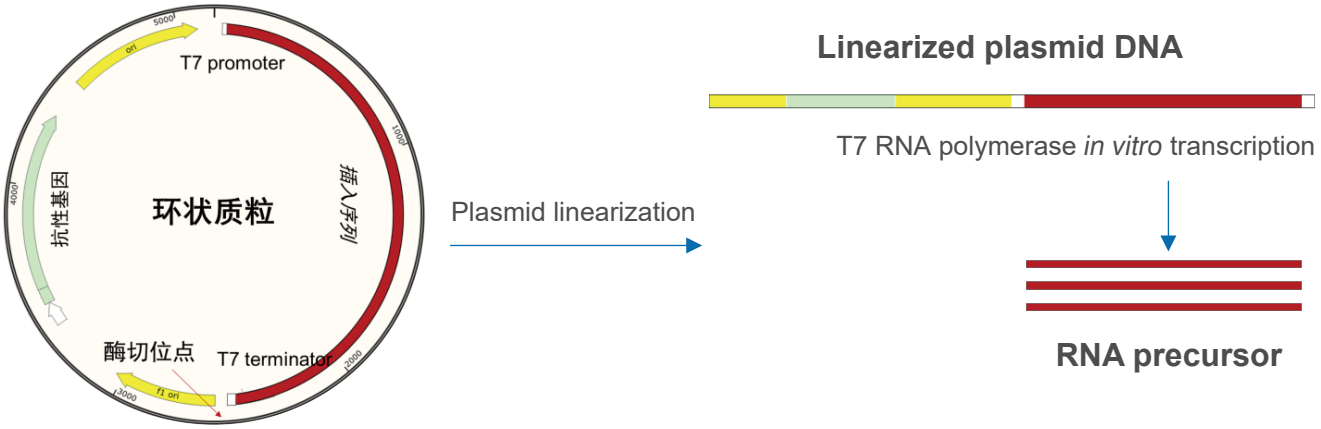


Sequence design and in vitro expression validation of circRNA_eGFP

mRNA
Transcription Template Plasmid Service



In the process of in vitro mRNA preparation, linearized plasmid DNA is required as the transcription template for in vitro transcription with the help of T7 RNA polymerase. High quality plasmid DNA is crucial for downstream in vitro transcription (IVT). Based on the mature plasmid preparation service platform, linearized plasmid DNA preparation service of high purity and high standard can be provided to achieve efficient downstream IVT transcription.



Schematic diagram of in vitro transcription using linearized plasmid DNA as template

Service Details

Service Items	Optional Services	Service Details	Delivery Period (Days)
Cyclic plasmid preparation	Gene synthesis	Gene synthesis (outsourced)	7-10
	Plasmid amplification	Plasmid amplification	2
		Plasmid extraction	
Linearized plasmid preparation	Plasmid linearization and purification	Plasmid linearization	1
		Linearization product purification	
Plasmid DNA quality control	Concentration purity	Ultraviolet spectrophotometry (UV)	1-2
	Plasmid conformation	Agarose gel electrophoresis (AGE)	
		Capillary electrophoresis (CE)-Ooptional	
	Plasmid integrity	Restriction enzyme identification (AGE)	

Service Advantages

Freecut Template Plasmids

Flexible plasmid template design options can satisfy the specific customization needs.

High Recovery

Continuous optimization of DNA extraction and purification methods can achieve high recovery.

Mature platform process

Provide plasmid preparation and quality control services of high standard and high efficiency to meet the needs of downstream tests.

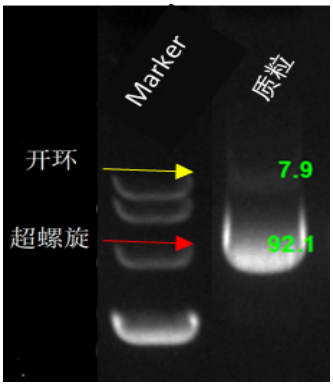
Stringent specification

Plasmid samples for research with a superhelical conformation ratio of >70%.

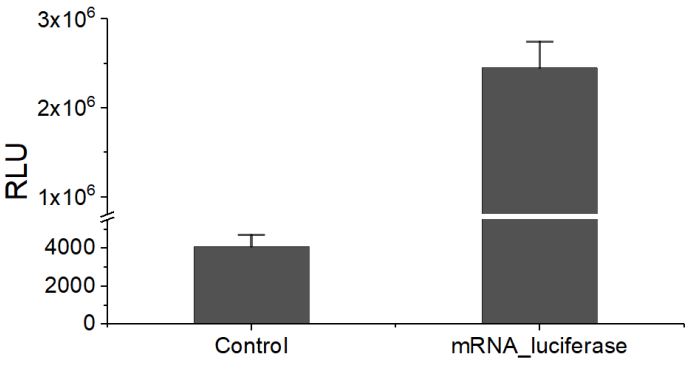
Case Studies

Taking YaoHai pre-product mRNA_luciferase as an example, the transcription template plasmid sample (research grade) has a superhelical ratio of more than 90%, a linearization ratio close to 100%, and a subsequent transcription ratio up to 1:200 (linearized plasmid DNA:mRNA).

The mRNA_luciferase obtained through the preparation of linearized plasmid as template is transfected into 293T cells, and the enzyme-substrate reaction activity is evaluated 24 h after transfection, and an obvious strong luciferase activity signal can be detected, i.e. luciferase protein is expressed efficiently, suggesting the purity of the transcription template, which can fully satisfy the requirement of high-quality mRNA preparation.

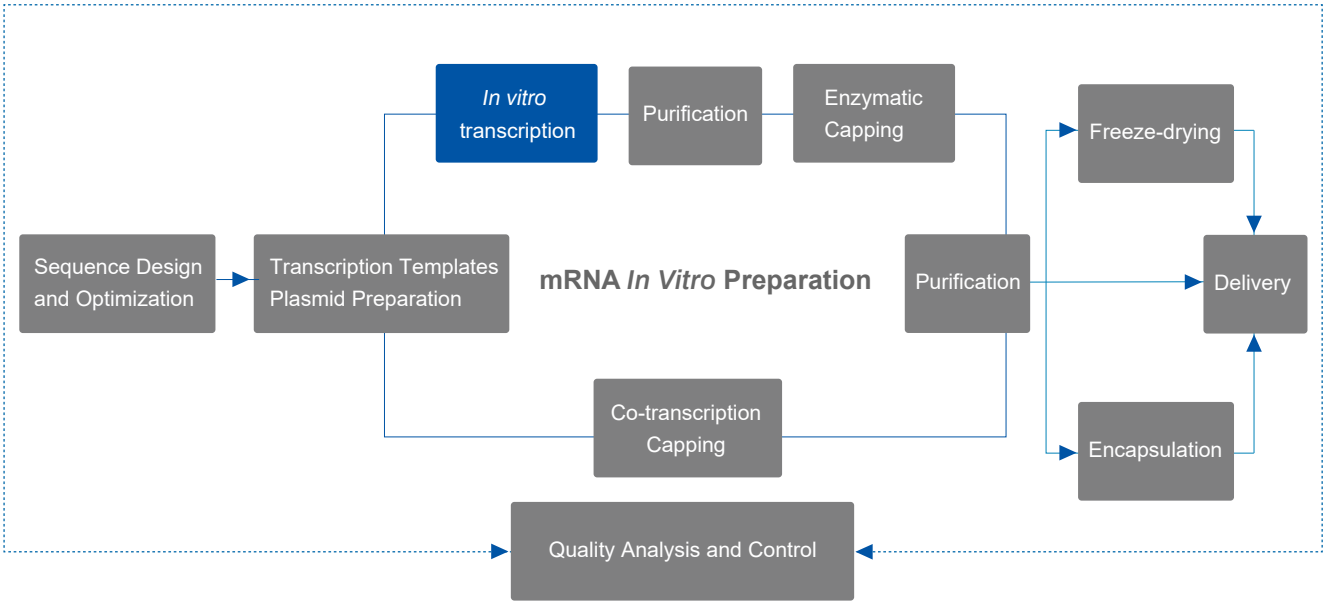


Plasmid Superhelix Ratio Assay



Validation of mRNA-mCherry expression *in vitro*

mRNA
in vitro transcription services



Regarding the preparation of mRNA in batches, in vitro transcription (IVT, In Vitro Transcription) is a more efficient and mature method. The reaction of IVT reaction adopts linearized plasmid DNA containing T7 promoter as template and mRNA is synthesized with nucleoside triphosphates (NTPs) as substrate in the presence of T7 RNA polymerase.

Nucleotide modification is a major breakthrough in the exploration of drug formulation of mRNA , where unmodified mRNA molecules are recognized by intracellular RNA sensors to activate innate immunity. For considerations of mRNA in vivo immunogenicity and translation efficiency, the IVT process usually employs certain kind of modified NTPs, and common modified nucleotides are pseudouridine (Ψ), N1-methyl-pseudouridine (N1Ψ), and 5-methylcytosine (5mC).

In vitro transcription process of mRNA

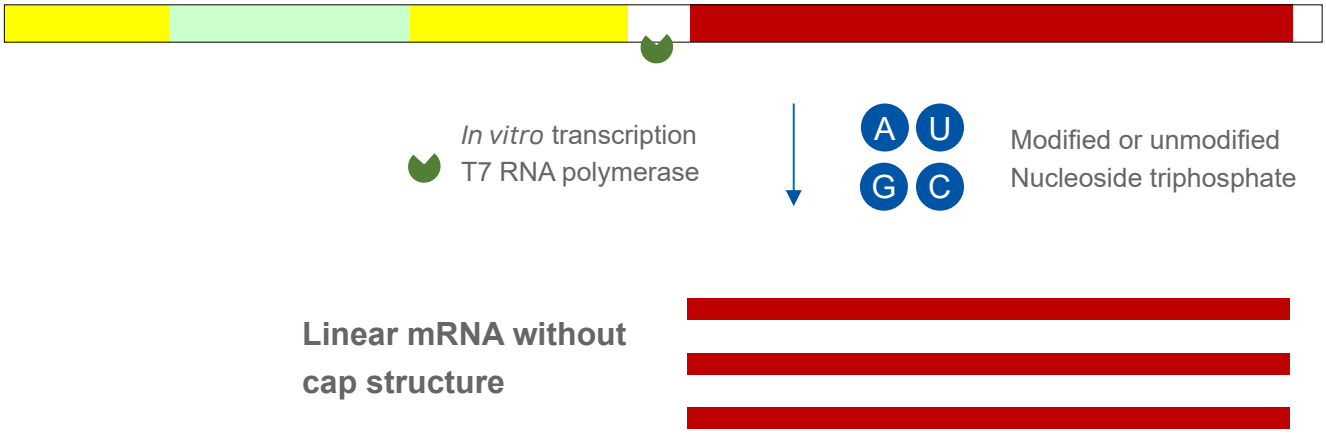


Diagram: IVT reaction diagram

Service Details

Service Items	Service Details	Delivery Period (Days)
***In vitro* transcription (IVT)**	Reaction system confirmation	1
	In vitro transcription (IVT)	
	Nucleotide modifications (Ψ/N1Ψ/5mC)	
	DNA template removal (DNase I)	
IVT condition optimization - optional	Reaction system design and optimization	2-5

Service Advantages

Improve mRNA stability and protein expression levels in vivo.

Diversified nucleotide modification strategies

mRNA fragment preparation up to 10kb can be achieved.

Rigorous test design and optimization

By optimizing IVT reaction conditions, high efficiency transcription is achieved with a transcription ratio of up to 1:200.

Efficient transcription

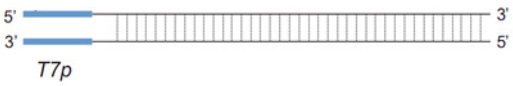
Stringent enzyme control through experimental environment and consumables can effectively prevent mRNA degradation

Stringent enzyme specificatio

Case Studies

The current IVT reaction system is roughly optimized for synthetic systems in a length of about 100 nt, not for mRNAs of arbitrary length. The longer the mRNA sequence, the more difficult it is to transcribe and the more prone to degradation.

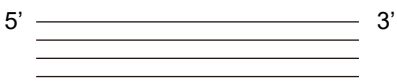
In order to prepare customized mRNA sequences with a length of about 10 kb, Yaohai Bio has successfully prepared high-quality samples with a high transcription ratio of 1:135 and obtained 135 μg of crude and pure mRNA products after 1 μg of linearized plasmid was transcribed in vitro through rigorous experimental design and continuous optimization of reaction conditions and strict control of RNase.



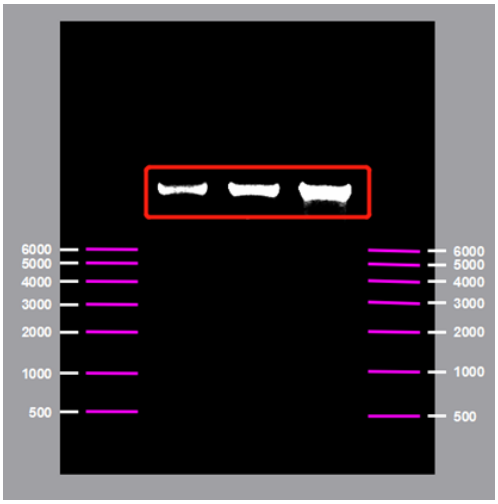
DNA template **1 μg**

High transcription ratio

IVT reaction
Lithium chloride crude purification



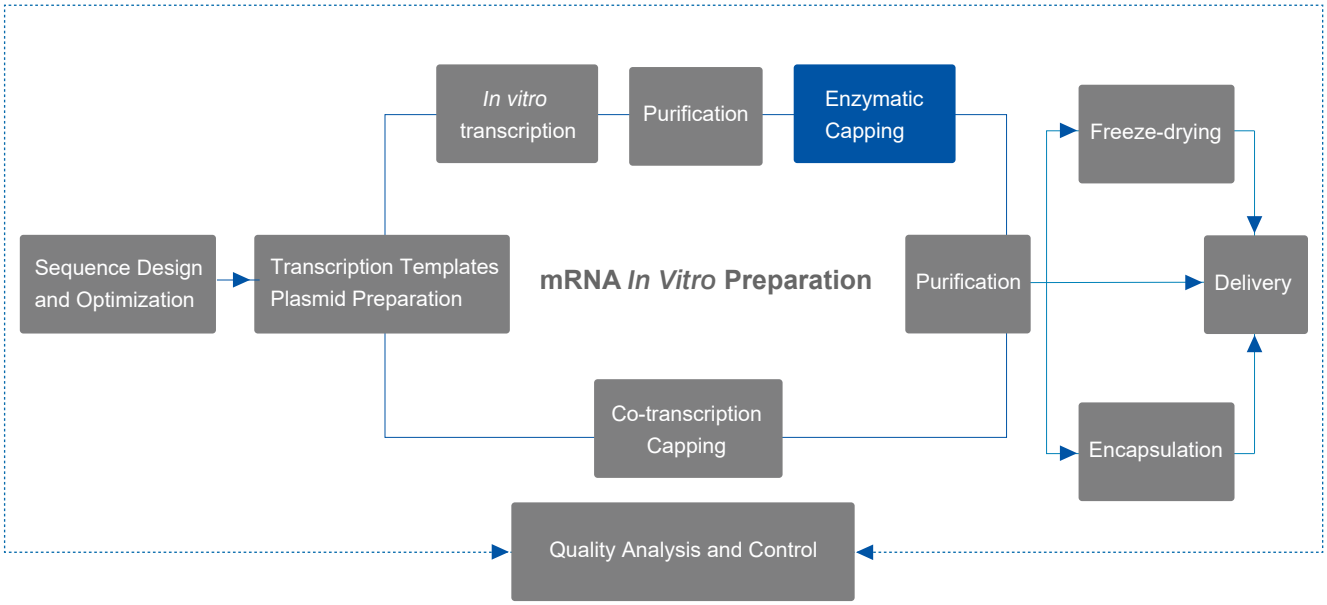
ImRNA product after crude purification **135 μg**



mRNA length and purity assay

mRNA

Enzymatic Capping Service



5'-end capping is an essential modification of mRNA. mRNAs with cap structures, especially Cap1 cap structures, facilitate mRNAs evade innate immune responses in vivo, resulting in efficient protein translation.

Enzymatic capping (two-step method) is the conventional method of mRNA capping, similar to the capping process in eukaryotic organisms. Under the action of a series of enzymes, 7-methylguanine (m7G) is linked to the 5'-end of mRNA through a 5'-5' triphosphate bond and undergoes methylation modification to form the cap structure Cap 1 (m7GpppN)

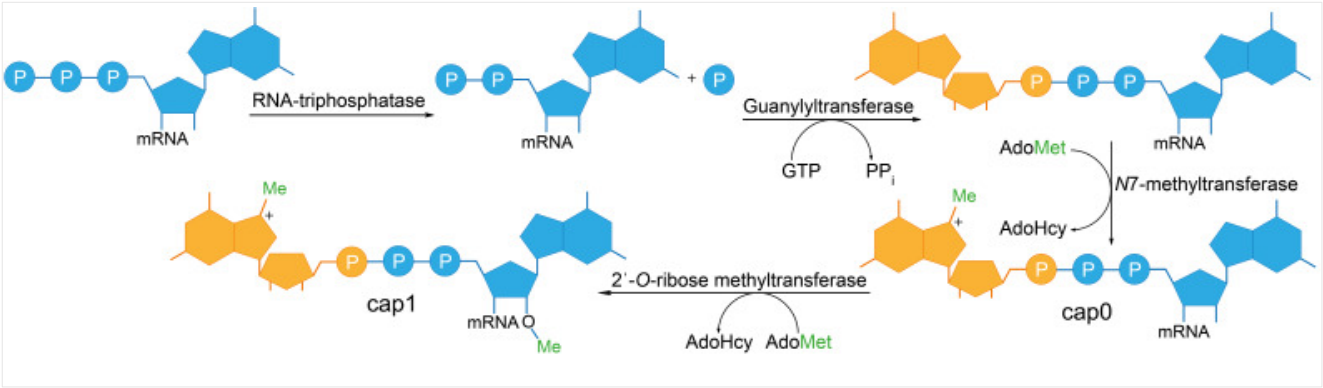


Figure: Diagram of natural cap structure formation

The enzymatic capping reaction flow is as follows: Linearized plasmid DNA is used as a template for in vitro transcription (IVT) in the presence of T7 polymerase, and mRNA with a 5' end-cap structure is formed after a one-step purification using cowpox virus capping enzyme and 2'-O-methyltransferase.

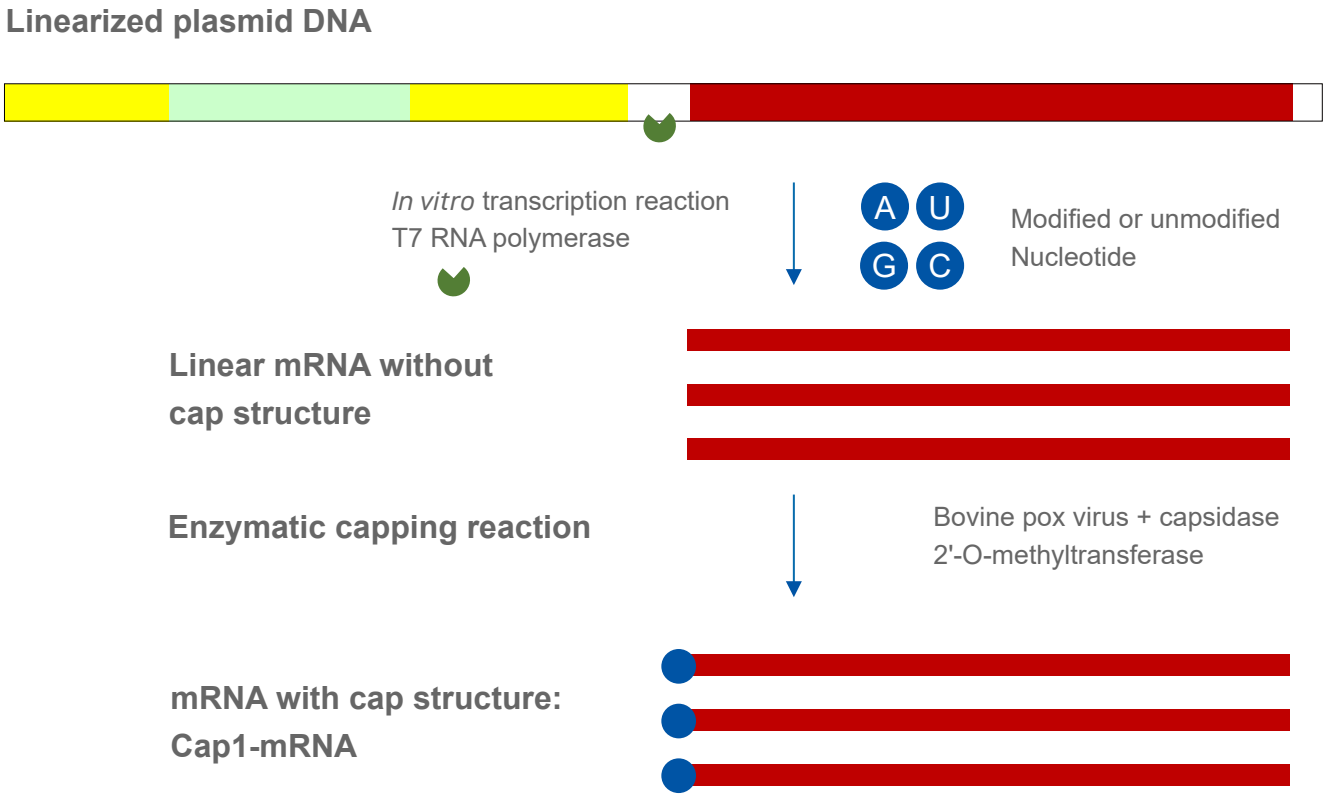


Figure: Diagram of mRNA enzymatic capping reaction

Service Details

Service Items	Service Details	Delivery Period (Days)
mRNA enzymatic capping	Reaction system verification	1
	Enzymatic capping reaction	
Capping response optimization - optional	Reaction system design and optimization	3-7

Service Advantages

Design and optimization of the capping reaction system

The IVT reaction system is adjusted and the mRNA transcription product is greatly enhanced.

***In vitro* expression verification**

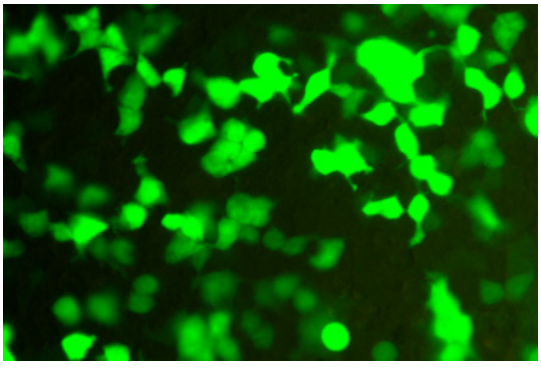
The capped mRNA is transfected into 293T cells, and the expression of the target protein can be detected.

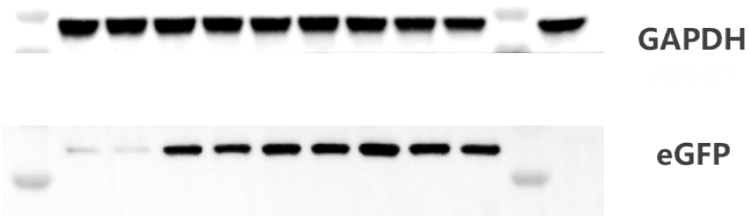
Stringent enzyme specification

Through stringent enzyme control on experimental environment and consumables, mRNA degradation is effectively prevented.

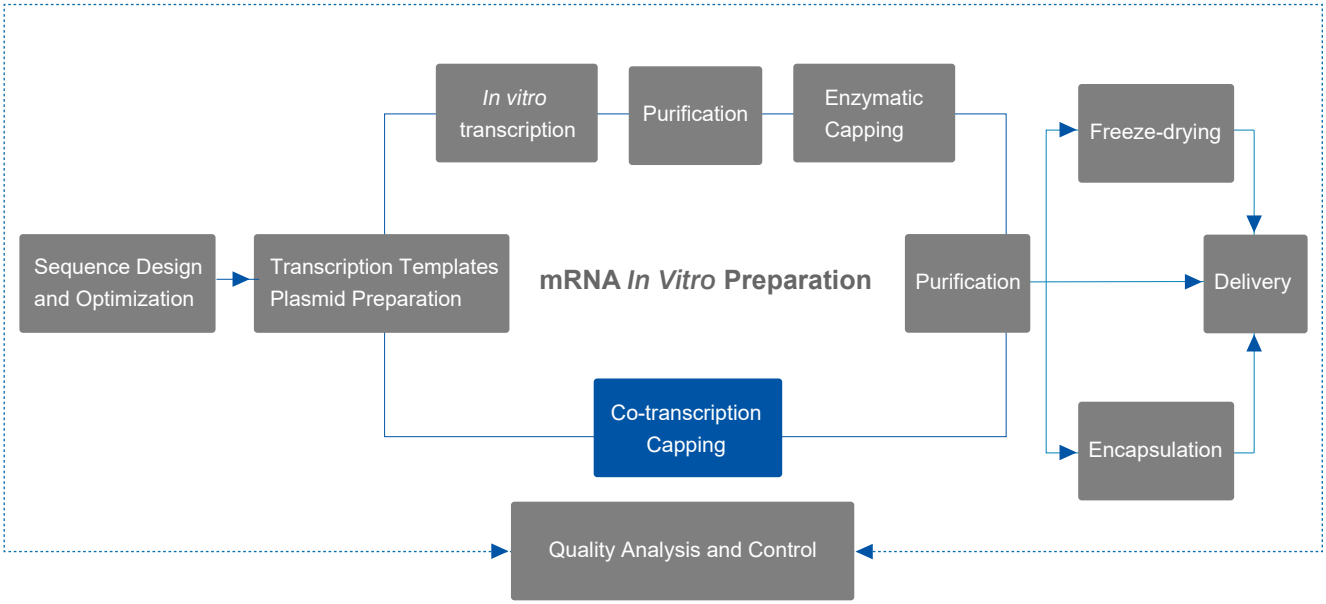
Case Studies

Yaohai Bio's mRNA platform has built a perfect capping reaction system. For mRNA_eGFP, an mRNA pre-product prepared by enzymatic capping, eGFP fluorescence signal (green fluorescence) at a high level can be observed after transfecting 293T cells for 24 hours, which is detected by Western Blot, demonstrating that the target protein eGFP can be efficiently expressed *in vitro*.





mRNA
co-transcription capping service



Compared with the two-step enzymatic capping method, the one-step co-transcription capping method can significantly reduce the process flow. The method is result-oriented, and by the addition of cap analogs to the in vitro transcription reaction system, cap analogs can be introduced at the start of transcription, and mRNA with cap structure can be obtained upon completion of transcription. Current third generation cap analogs can avoid reverse capping and directly add Cap 1 cap structure to the transcription product.

For considerations of mRNA in vivo immunogenicity and translation efficiency, the IVT process often adopts certain kind of modified NTPs, and common modified nucleotides are pseudouridine (Ψ), N1-methyl-pseudouridine (N1Ψ), and 5-methylcytosine (5mC).

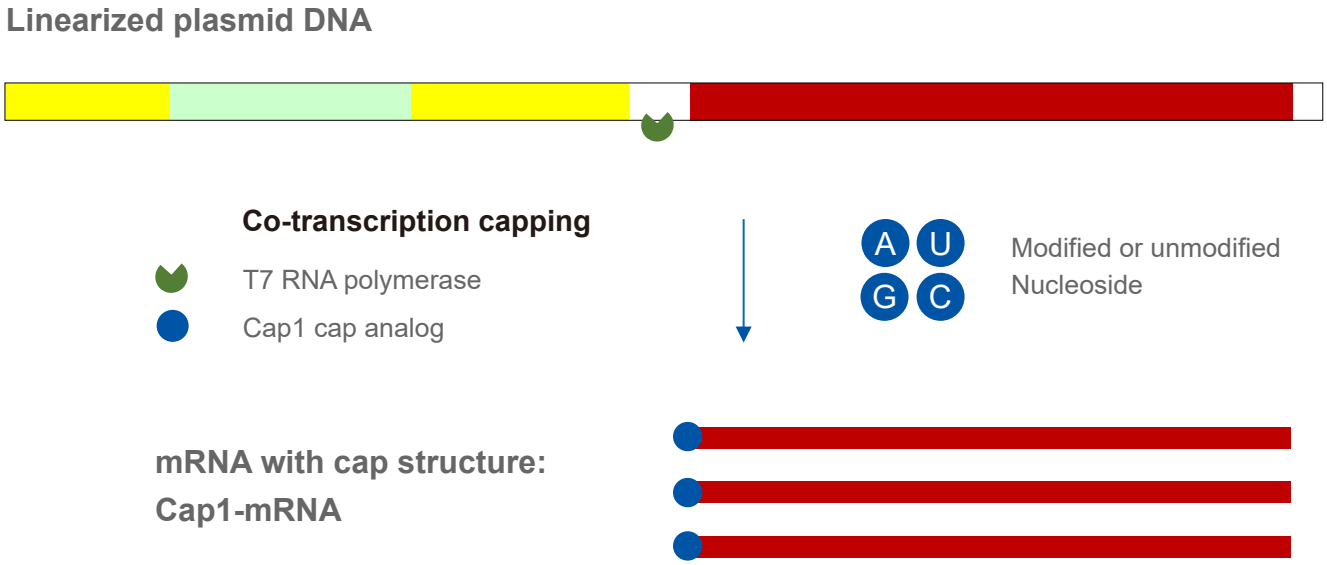


Figure: Diagram of mRNA co-transcription and capping reaction

Service Details

Service Items	Service Details	Delivery Period (Days)
Co-transcription capping	Reaction system verification	1-2
	In vitro transcriptional response (Clean Cap analog)	
	Nucleotide modifications (Ψ/N1Ψ/5mC)	
	DNA template removal (DNase I)	
IVT condition optimization - optional	Reaction system design and optimization	3-7

Service Advantages

Multiple optional nucleotide modification strategies can improve protein expression.

Diversified nucleotide modification strategies

Achieve high transcription ratio and high capping rate.

Optimized reaction system

Achieve a capping rate of more than 95%.

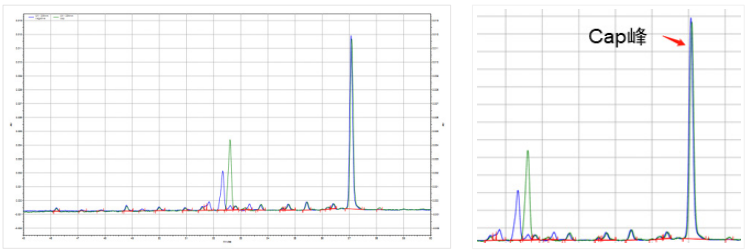
Stable capping process

Prevent mRNA degradation effectively by stringent enzyme control on experimental environment and consumables.

Stringent enzyme specification

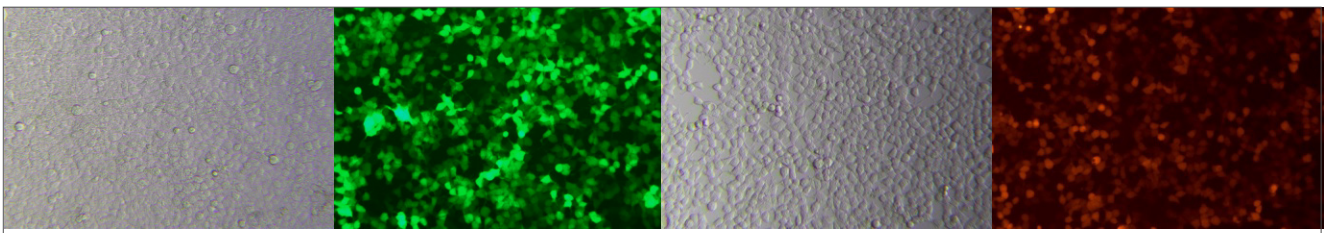
Case Studies

YaoHai has built a mature co-transcription capping process platform, using Clean Cap analogs to directly add Cap1 cap structure while avoiding reverse capping. After standardized sample pre-treatment and capillary electrophoresis detection, the capping rate of pre-product mRNA_eGFP can reach more than 95%.



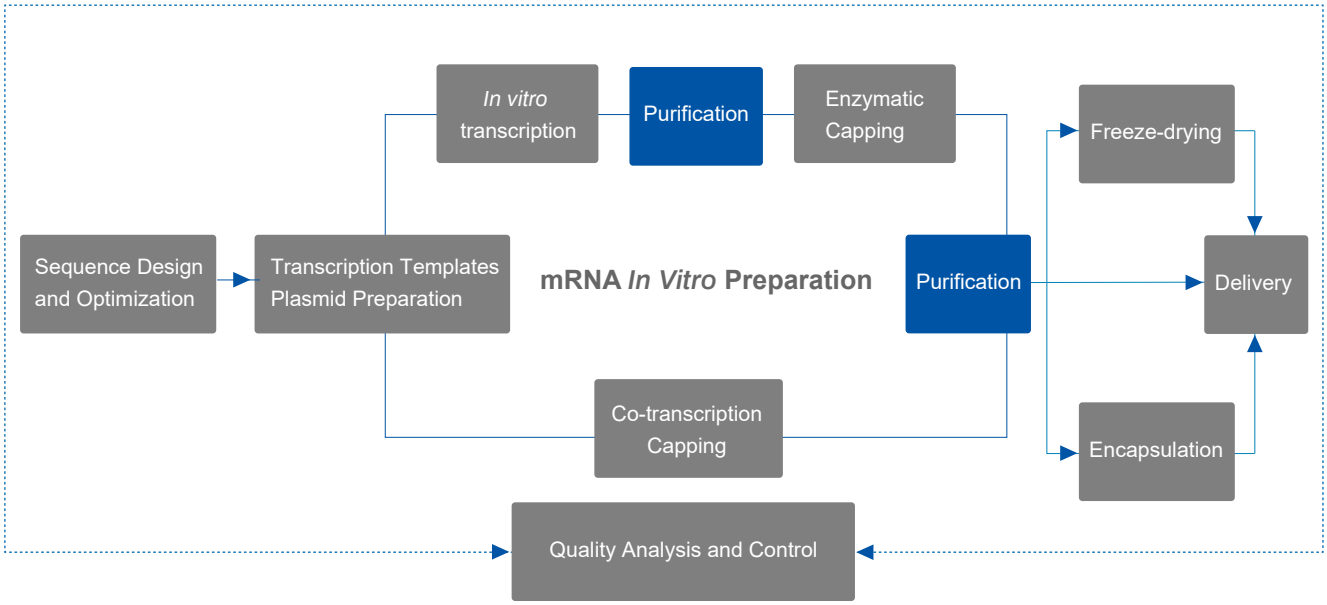
A mRNA_eGFP capping rate of more than 95%

The pre-products mRNA_eGFP and mRNA_mCherry prepared by co-transcription capping are transfected into 293T cells, respectively, and a strong fluorescent signal is observed after 48h, suggesting that the mRNA is efficiently expressed in 293T cells.



Bright Field	mRNA_eGFP	Bright Field	mRNA_mCherry

mRNA
purification services



The mRNA prepared by in vitro transcription (IVT) and capping reaction requires to be further purified to remove the immunogenic unconsumed substrates and reaction by-products from IVT and capping reaction to ensure the efficacy and safety of mRNA drug.
Yaohai Bio can provide mature solutions for LiCl precipitation, magnetic bead purification and chromatography purification, which can effectively remove multiple impurities and prepare high-purity mRNA.

LiCl precipitation method

Simplified purification solution of small amounts of mRNA for cell transfection, and some animal experiments;
For the purification of pre-capped samples after in vitro transcription.

Oligo dT magnetic bead purification method

Purification solutions of small amounts of mRNA for cell transfection, and some animal experiments;
For the purification of pre-capped samples after in vitro transcription.

Chromatography purification method

Purification solutions with multiple chromatographycompositions such as affinity, ion exchange and hydrophobic chromatography;
Meet the downstream application scenarios with higher quality requirements, such as cell transfection, and LNP encapsulation, etc.

Service Details

Service Items	Optional Items	Detailed steps	Delivery Period (Days)	Delivery
mRNA purification	Conventional purification solution	Lithium chloride precipitation	1	mRNA drug substance
		Magnetic bead purification		
	High purity purification solutions	Affinity chromatography or multiple chromatography combinations	2	
	Buffer exchange	Ultrafiltration and buffer exchange	1	
mRNA quality control	Concentration measurement	Ultraviolet spectrophotometry (UV)	0.5	CoAs
	Integrity and purity testing	Agarose Gel Electrophoresis (AGE)		
		Capillary Electrophoresis (CE)-Optional	1	

Service Advantages

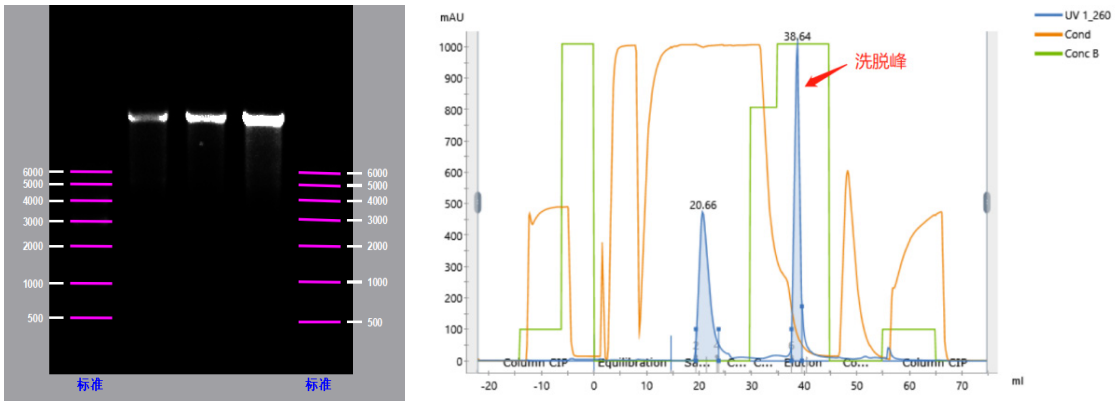
A variety of optional purification solutions can meet different downstream application scenarios.

The purity of mRNA can routinely reach more than 95%, with the highest purity of reaching 100%.

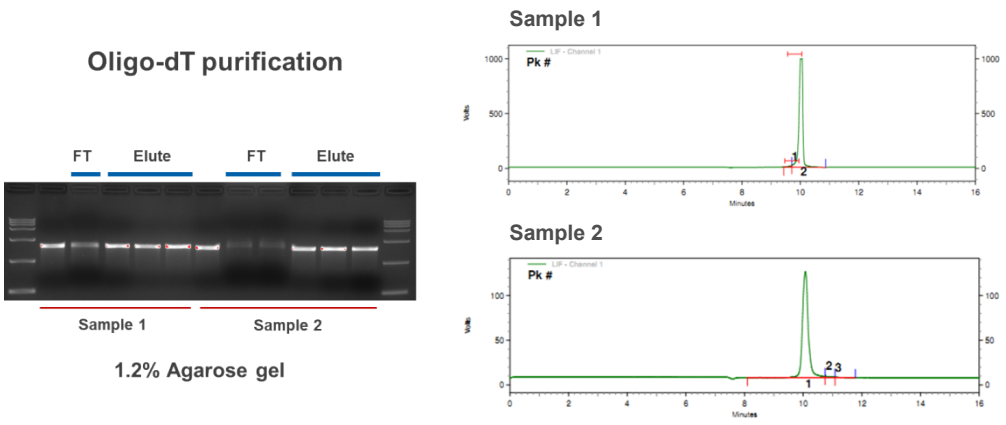
Stringent enzyme specification can prevent mRNA degradation through stringent enzyme control of experimental environment and consumables.

Case Studies

Yaohai Bio can provide mature mRNA purification solutions, which can effectively remove various small molecule process-related impurities.
The purity of mRNA samples prepared by chromatography purification can reach more than 95% as detected by capillary electrophoresis, and the content of dsRNA is less than 0.06% as detected by ELISA kit, which meets the demand of downstream application of mRNA with high quality.

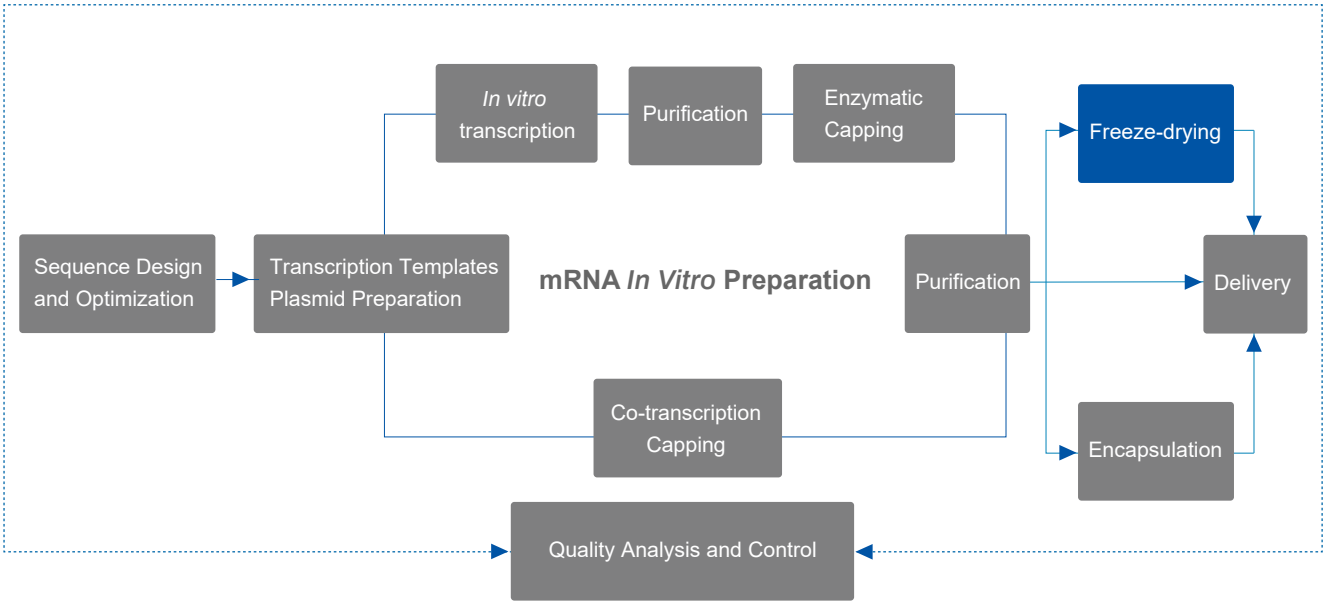


Customized purification of mRNA (>9kb)

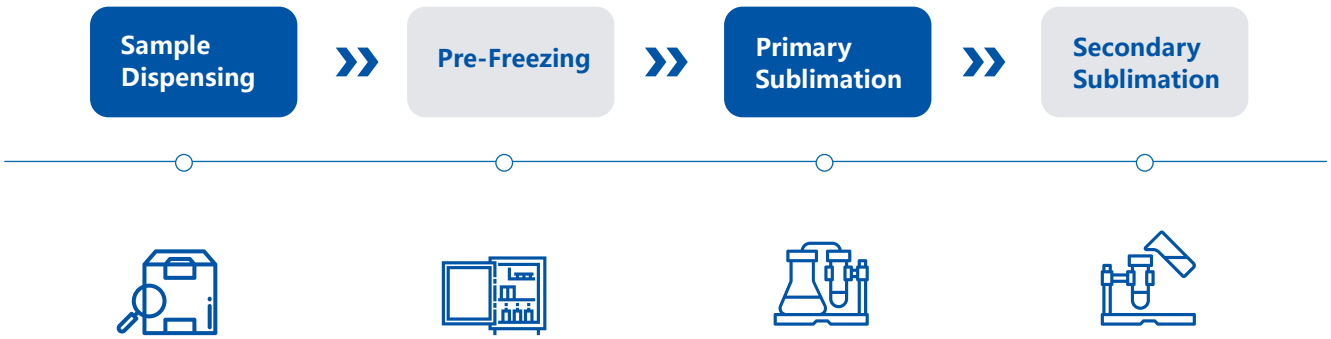


The purity of pre-product mRNA_spike protein (new coronavirus) is more than 95%.

mRNA
lyophilization services



In order to improve the stability of mRNA and avoid the loss in storage and transportation, YaoHai can provide mRNA lyophilization service for customers to freeze-dry the mRNA drug substance and store or transport in the form of lyophilized powder, which can significantly reduce the degradation and loss of mRNA during storage and transportation.



Service Details

Service Items	Optional Items	Detailed steps	Delivery Period (Days)	Delivery
mRNA lyophilization	Sample dispensing	Dispensing	2-3	mRNA lyophilized powder
	Lyophilization	Pre-freezing		
		Primary sublimation		
		Secondary sublimation		
mRNA quality control	Reconstitution of lyophilized powder	Reconstitution / Resuspension	-	CoAs
	Solubility of lyophilized powder	Appearance inspection	-	
		Ultraviolet spectrophotometry (UV)	0.5	
	Concentration measurement	Agarose Gel Electrophoresis (AGE)		
	Integrity and purity testing	Capillary Electrophoresis (CE)-Optional	1	

Service Advantages

Mature lyophilization process

Lyophilization has no effect on mRNA integrity.

Homogeneous quality properties

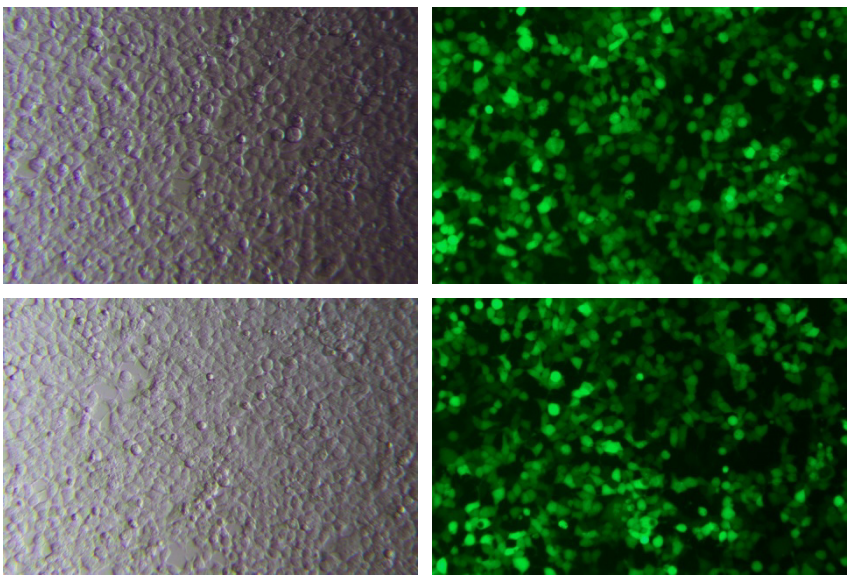
mRNA samples before and after lyophilization can successfully express the target protein.

High stability

mRNA lyophilized powder is easy to store and transport.

Case Studies

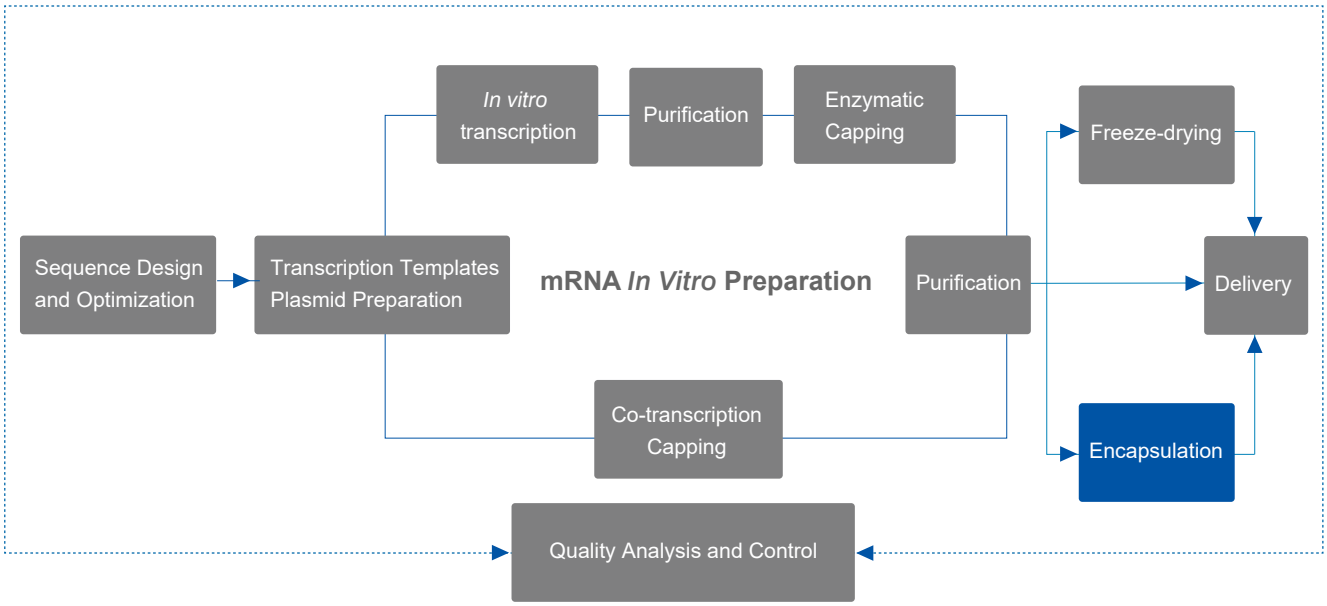
Using conventional liposomes, mRNA samples before and after lyophilization are transfected with 293T cells for cellular evaluation. The results show that strong fluorescence signals are observed before and after the lyophilization of pre-product mRNA_eGFP samples, which can express the target protein efficiently *in vitro*.



mRNA samples
before lyophilization

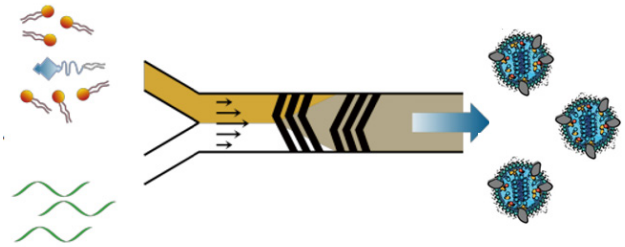
mRNA samples
after lyophilization

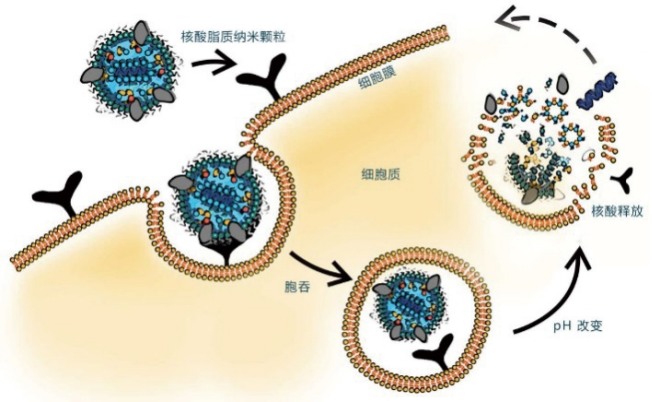
mRNA-LNP
encapsulation Service

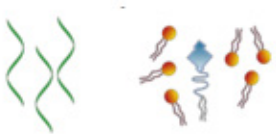
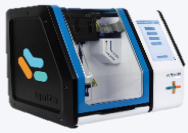
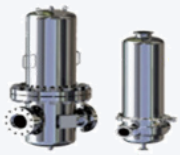


The basis of encapsulation is the design and development of the delivery system. A well-designed delivery system allows mRNA molecules to enter the body without being degraded by RNA enzymes, and then to be effectively delivered to the target site, cross the cell membrane and be released intracellularly. Lipid nanoparticles (LNPs) are the optimal delivery systems available, with advantages in terms of encapsulation, in vivo expression, and in vivo safety compared to other delivery systems. Lipid nanoparticles with nucleic acid fragments are easily swallowed into cells and form intracellular bodies. Once inside the cell, the acidic environment of the intracellular body protonates and positively charges the head of the ionized lipid, which fuses with the inner membrane of the intracellular body and releases the target nucleic acid into the cell for action.

Yaohai Bio mRNA service continues to improve, and now can provide mRNA-LNP encapsulation service, optimize relevant critical process parameters, and improve the consistency and reproducibility of mRNA drug production.





Material and liquid pretreatment	Microfluidics	Tangential Flow Filtration	Sterilization filtration
			
Two strands of bulk are prepared: one for mRNA in aqueous buffer and one for lipids dissolved in ethanol.	Rapid mixing of lipid, mRNA two-phase solutions using microfluidic devices, resulting in uniform LNP and high efficiency encapsulation.	The bulk is concentrated to the target concentration of the drug product using ultrafiltration and the buffer is exchanged with a neutral storage solution to remove unencapsulated mRNA, and the extra lipids and acetic acid.	Comply with the sterility regulations, the terminal sterilizing filtration system is selected, and the bacterial challenge test passes.

Service Details

Service Items	Detailed Steps	Delivery Period (Days)	Delivery
mRNA-LNP encapsulation	Material and liquid pretreatment	2	**mRNA-LNP drug product**
	Microfluidic device mixing		
	Ultrafiltration concentration	1	
	Sterilizing filtration		
mRNA-LNP quality control	Encapsulation rate	1	**CoAs**
	Particle size and distribution detection		
	Surface charge detection		
	mRNA-LNP expression validation	5-7	

Service Advantages

Mature Process

Fast synthesis speed, high R&D efficiency, pre-optimized solutions available.

High encapsulation rate

mRNA-LNP encapsulation rate can reach more than 90%.

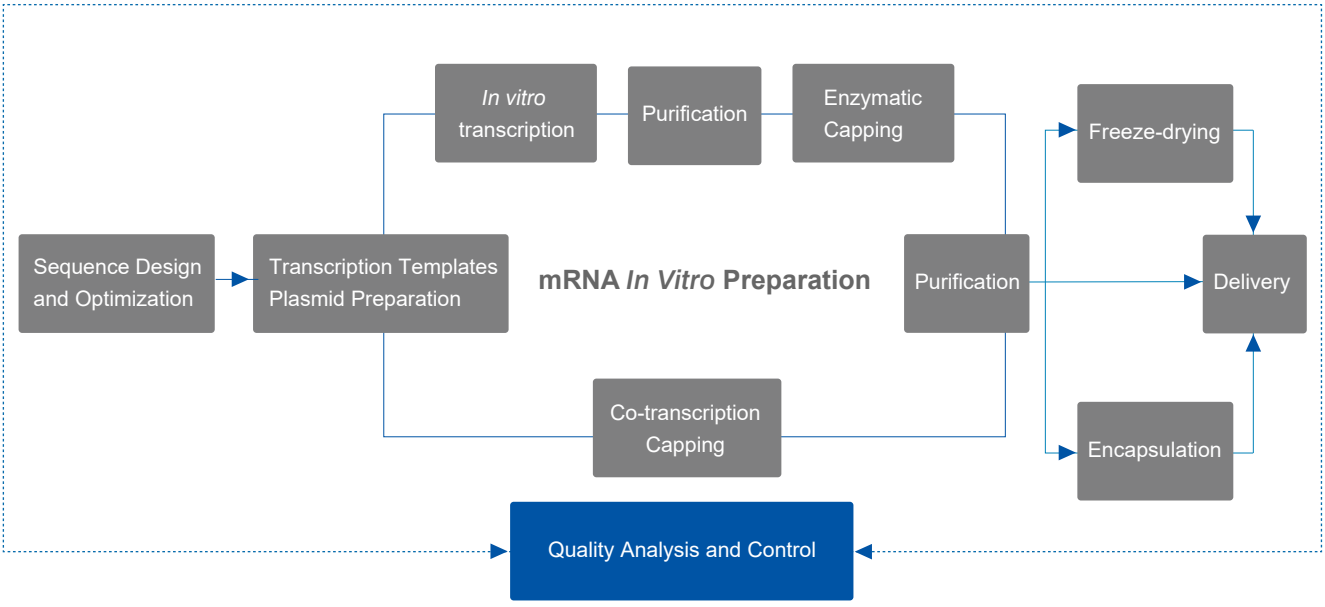
Nanoparticle size

Lipid nanoparticle size can be effectively controlled by changing the fluid injection rate and ratio.

Efficient expression

mRNA-LNP pre-products are validated by in vitro cell expression and can express the target protein efficiently.

mRNA quality analysis and control services



According to the Technical Guidelines for Pharmacological Studies of Novel Coronavirus Prophylactic mRNA Vaccines issued by NMPA in 2020, quality control of DNA template, mRNA drug substance and finished mRNA-LNP is recommended.

Yaohai Bio can provide quality analysis services for cyclic and linearized plasmids, mRNA drug substance and finished LNP-mRNA to meet customer project needs in all aspects.

Service Details

Samples	Test Items	Testing Method	Delivery Period (Days)	Delivery
Cyclic plasmid DNA	Concentration/Purity	Ultraviolet spectrophotometry (UV)	N/A	CoAs
	Superhelix ratio	Agarose Gel Electrophoresis (AGE)	0.5	
		Capillary Electrophoresis (CE)	1	
Linearized plasmid DNA	Concentration/Purity	Ultraviolet spectrophotometry (UV)	N/A	
	Linearized rate and integrity	Agarose Gel Electrophoresis (AGE)	0.5	
		Capillary Electrophoresis (CE)	1	
mRNA drug substance	Concentration/Purity	Ultraviolet spectrophotometry (UV)	N/A	
	Integrity	Agarose Gel Electrophoresis (AGE)	0.5	
		Capillary electrophoresis (CE)	1	
	Capping rate	Capillary electrophoresis (CE)	3	
	PolyA distribution	Capillary electrophoresis (CE)	3	
mRNA-LNP drug product	dsRNA	ELISA	1	
	Encapsulation rate	RiboGreen method	1	
	Particle size and distribution	Particle size meter	1	
	Surface charge	Particle size meter	1	
mRNA expression validation	293T cell evaluation	Cell transfection	4	
		Fluorescence observation	1-3	
		Western Blot/ELISA		

mRNA

in vitro expression validation service

In addition to mRNA-related quality attributes, based on the perfect cell culture platform, YaoHai can provide customers with specificity assay services of mRNA cell transfection and target protein to transiently transfect 293T cells with mRNA to verify whether mRNA can successfully express the target protein in cells in vitro. The range of samples that can be tested includes mRNA drug substancec and finished mRNA-LNP.

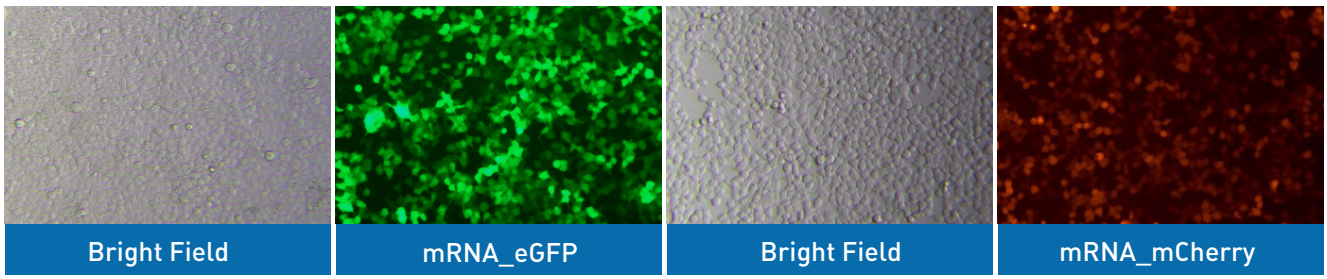
Cell Plating	Sample Preparation	Cell Transfection	Target Protein Detection
Recording cell generations Observation of cell morphology Cell pavement	mRNA + Transfection reagents Or mRNA-LNP	mRNA Transfected to 293T cells	Fluorescent photo shoot Western blot ELISA

Service Details

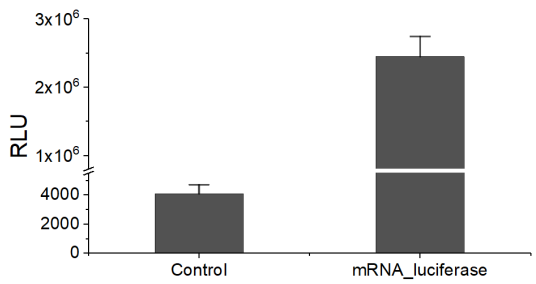
Samples	Test Items	Testing Method	Delivery Period (Days)	Delivery
mRNA expression validation	293T cell evaluation	Cell plating	4	CoAs
		Transient transfection of cells		
		Fluorescence signal observation	1-3	
		Western blot (WB)		
		ELISA		

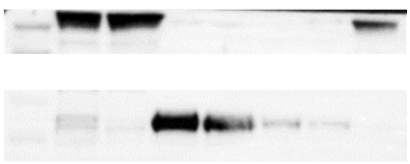
Case Studies

Yaohai Bio has built a perfect platform for cell culture, cell transfection and protein specificity assay, which can verify the in vitro expression of target proteins based on fluorescence signal, Western blot/ELISA or substrate - enzyme reaction signal.



Bright Field | mRNA_eGFP | Bright Field | mRNA_mCherry





mRNA_luciferase | mRNA_Spike protein(Newcrest)

mRNA Platform

Equipment

Bio-Rad Gel Imagers

Cytiva AKTA Purification System

Bio-Rad PCR Instrument

Thermo qPCR instrument

SCIEX Capillary Electrophoresis Instrument

Waters HPLC

Thermo Full Wavelength Enzyme Labeler

Fluorescence Microscope

PNI Microfluidic Nanoparticle Preparation System

Yaohabio 耀海生物

Cooperative

Customer Presentation

君实生物 TopAlliance

甲贝医药 Shell BioTech

清華大學 Tsinghua University

澳門科技大學
UNIVERSIDADE DE CIÊNCIA E TECNOLOGIA DE MACAU
MACAU UNIVERSITY OF SCIENCE AND TECHNOLOGY

復旦大學

中国科学院北京生命科学研究院
Beijing Institutes of Life Science, Chinese Academy of Sciences

上海交通大學
SHANGHAI JIAO TONG UNIVERSITY

北京市卫生健康委员会
Beijing Municipal Health Commission

西安交通大學
XI'AN JIAOTONG UNIVERSITY

中国医学科学院 北京协和医学院 药物研究所
Institute of Materia Medica,Chinese Academy of Medical Sciences & Peking Union Medical College

浙江大學醫學院
ZHEJIANG UNIVERSITY SCHOOL OF MEDICINE

SERVE
WITH HEART &
CREATE
THE FUTURE TOGETHER

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