
Yaohabio
耀海生物
PROFESSIONAL MICROBIAL
EXPRESSION SYSTEM
CRDMO SERVICES PROVIDER
RECOMBINANT PROTEINS/PEPTIDES
NANO-ANTIBODYS
RNA DRUGS
RECOMBINANT NOVEL VACCINES
RECOMBINANT PLASMIDS

CONTENTS

Yaohabio 雅海生物

ABOUT YAOHAI BIO-PHARMA

Yaohai Bio-Pharma was founded in August 2010, based in China Medical City (CMC), Taizhou, Jiangsu Province, China, and received the Drug Production License in 2012. It is a biologics CRDMO focusing on microbial expression system, with business focalizing the recombinant proteins/polypeptides, nanobodies, gene therapies and nucleic acid drugs, novel recombinant vaccines, and other areas. The company is committed to build an open and integrated production and research service platform for CRO/CDMO. The scope of business covers one-stop CMC services throughout the entire drug lifecycle, such as strain construction, cell bank services, lab scale process development and optimization, pilot process scale-up and production, clinical sample preparation, quality specification establishment, analytical method development and validation, manufacturing in GMP or non-GMP level, quality management system establishment and registration application, etc.

Adhering to the service concept of “Serve with heart, create the future together”, we persevere in empowering the global new drugs development with the mission of “Establish global standards, boost new drug development process, and achieve healthy life”.

Yaohai Bio-Pharma

THE LEADING CRDMO, EMPOWERING AND ACCELERATING NEW DRUG DEVELOPMENT PROCESS

12 years +

Of Diligent Development

as a pionner in microbial expression systems CRDMO services, national high-tech enterprise

100+

Successful Audits

successfully passed NMPA inspection

100+

Project Experience

100+ CRDMO projects successfully delivered

300+

Project Reserves

with 100+ clinical projects
200+ commercial project
under negotiation

300+

Global Customers

and many world-renowned companies as strategic partners

CRDMO SERVICE PLATFORM

C

- Contract customization services

R

- Nanobody recombinant expression and purification
- mRNA UTR/IRES sequence-based screening
- CircRNA trial sample preparation and activity evaluation
- Establishment of analytical method
- Recombinant protein trial sample preparation
- Host bacteria screening
- LNP preparation

D

- Strain construction
- Original strain bank construction
- Fermentation process development
- Purification process development
- Formulation process development
- Pilot scale-up
- Pilot production
- Quality specification establishment
- Analytical method development
- Process sample testing
- IND application

M

- MCB/WCB strain bank construction
- Strain stability study
- API and excipients testing
- Analytical method transfer, verification, validation
- GMP production of drug substance (Phase I, II, and III)
- GMP production of drug product (Phase I, II, and III)
- Release testing of intermediates, drug substance, semi-finished product and finished product
- Preparation of standard substance/reference substance and structure characterization
- Industrialized production
- Stability study
- BLA application support

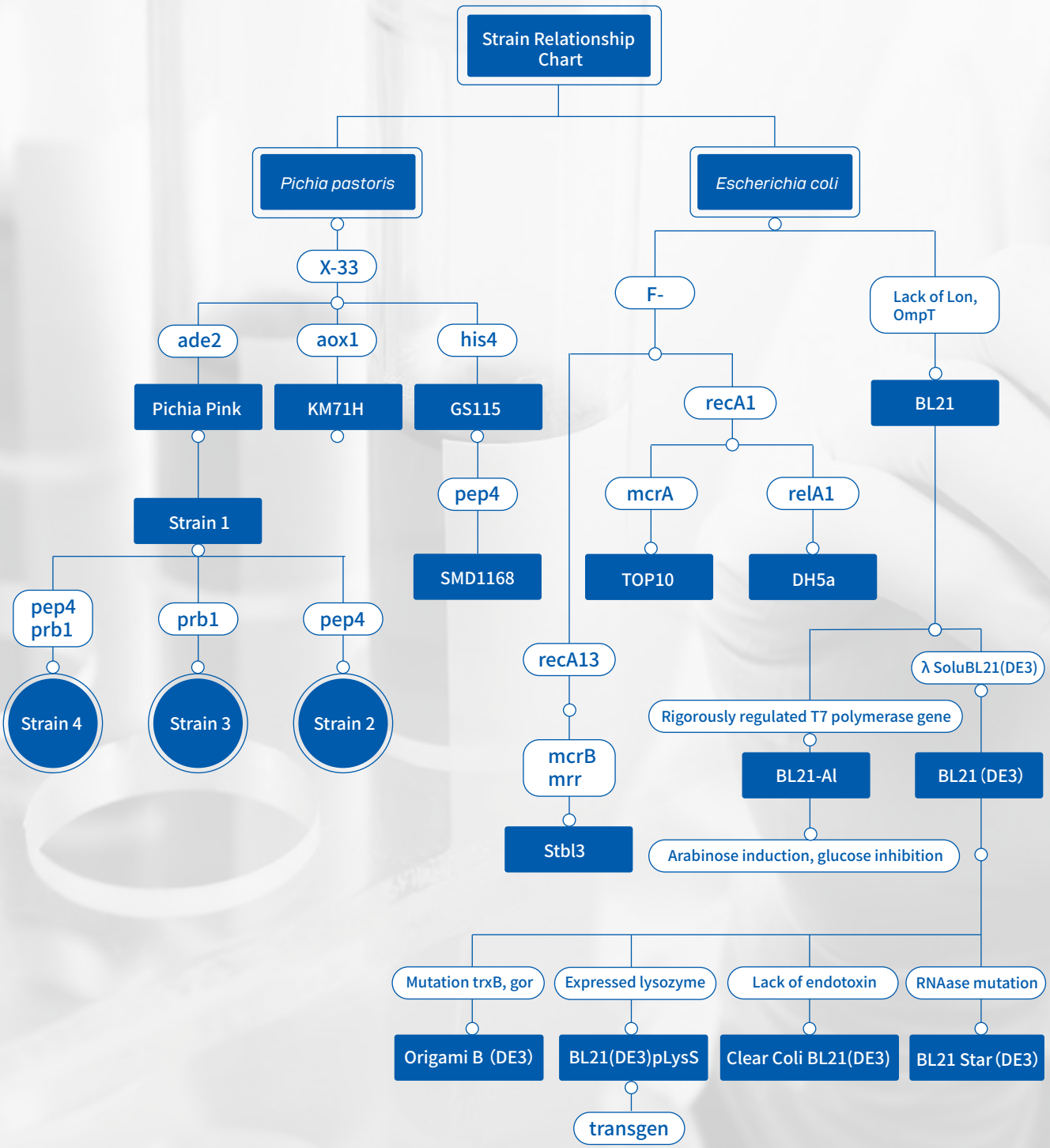
O

- Omni-directional end-to-end services

Scientific Sample Customization → **Customized R&D**

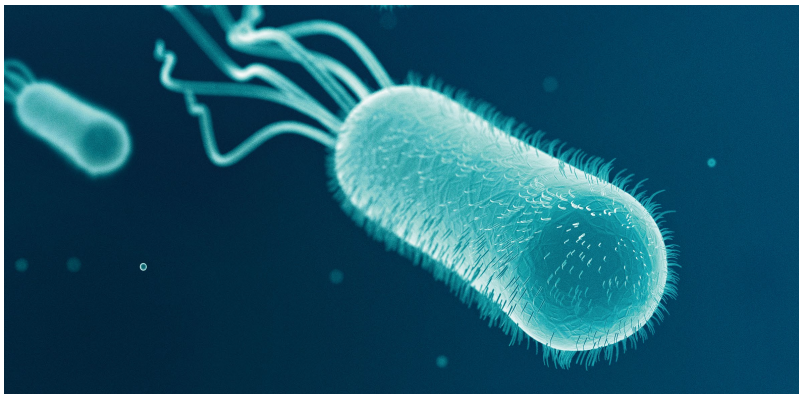
Commercialized / Customized Production Lines → **Customized Production Services**

STRAIN RELATIONSHIP



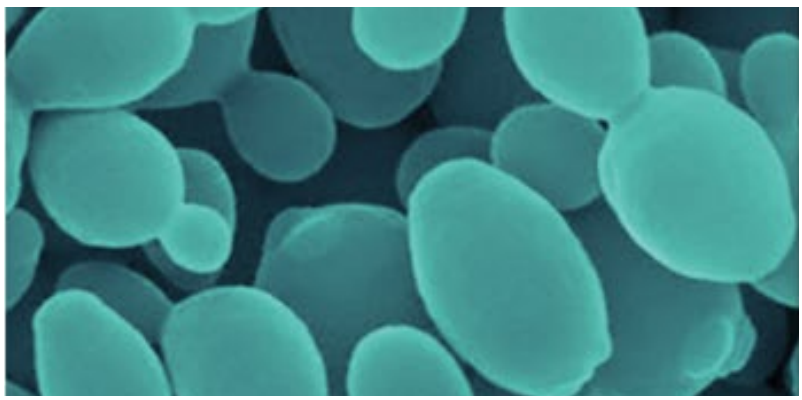
E. coli

K-12 strains & derivatives (DH1.DH5a. RV308,W3110,MG1655,JM109,BW25113...)
B strains(BL21,BL21(DE3)，BL21(DE3) pLysS,BL21(DE3) Rosetta...)



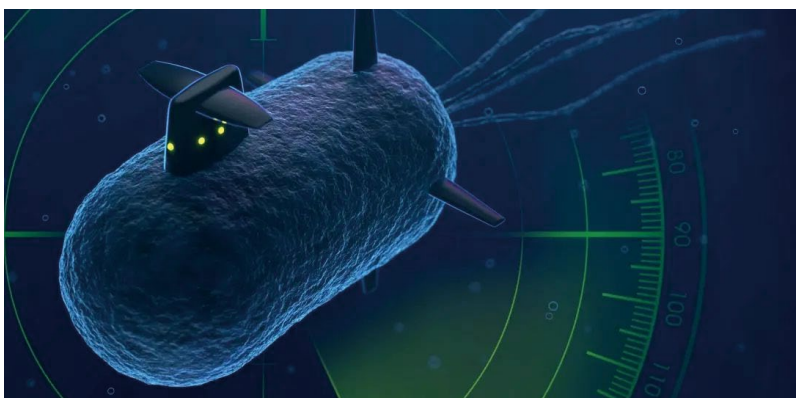
Yeast

*Pichia pastoris, Hansenula polymorpha, Saccharomyces cerevisiae,*etc.

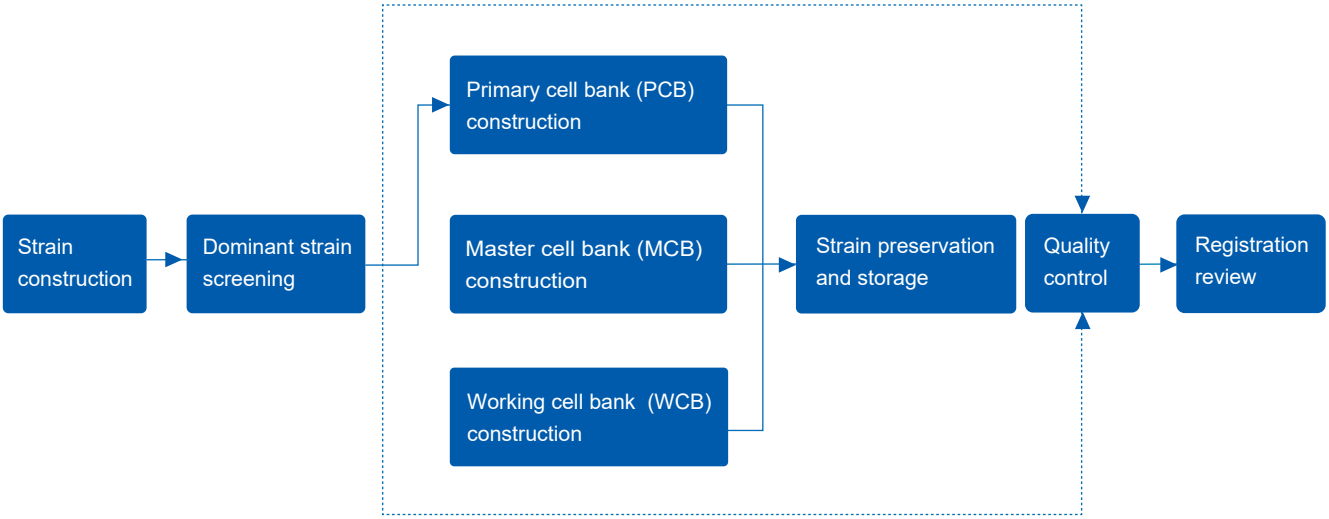


Tailor constructed Strains

Other microbe/microbiota/microbiome provided by clients
Customized strains



Overview of cell bank
construction services



The management of cells used in the manufacturing of biological products should meet the requirements in Chinese Pharmacopoeia (2020 edition)-Management and Quality Control of Bacterial and Viral Strains Used for Production and Testing of Biological Products in ChP 2020, so as to provide strains that are tested qualified, of the same quality and capable of sustained and stable passage. According to ChP, the seed batch system should be used for the bacterial and virus strains used in the manufacture of biological products to minimize passage times to reduce the risk of genetic variation. The history, source and biological characteristics of the original strain should be verified. The master cell bank is stored after the passage and amplification of the original strains, and the working cell bank which is used for the manufacturing of biological products is stored after the passage and amplification of the master cell bank.

Leveraging the ***Escherichia coli* and *yeast*** expression system, Yaohai BioPharma's recombinant protein/recombinant plasmid CDMO service has established a **one-stop service platform for quality test and registration review**, including **strain construction, primary cell bank (PCB) construction, master cell bank (MCB) construction, working cell bank (WCB) construction, strain storage, passage stability study, storage stability study, and etc.**, with the capability of undertaking services of secondary or tertiary cell bank construction. Note: MCB and WCB secondary cell bank construction services are carried out in an independent GMP workshop (Class C+A: Class A biosafety cabinet in Class C clean area).

Service Details

Service Name	Service Items-optional	Service Details	Minimum Manufacturing Cycle (working days)	Deliverables
Strain construction service	Construction of recombinant *Escherichia coli* strains[1]	Plasmid transformation (multi-host)	5	Strain construction report
		PCR verification		
		Strain purification and preservation		
	Construction of recombinant *yeast* strains [2]	Preparation of *yeast* competent cells	10	
		Plasmid linearization and electro transformation		
		Screening of resistant/defective plates		
		Strain purification and preservation		
Dominant strain screening service	Dominant strain screening	Plasmid extraction[1]/Genome extraction[2]	15-20	
		PCR/enzyme-digesting identification[1]/ sequencing		
		Screening of high-expression strains		
		Screening of strains of stable passage		
Primary cell bank construction service PCB	PCB bank construction	Growth curve determination	5	• Construction report of cell bank • PCB strains • MCB strains • WCB strains
		Passage culture and strain preservation		
	PCB release test	See the quality control section for details.	TBD	
Master cell bank (MCB) construction service	MCB construction*	Passage culture and strain preservation	4	
	MCB release test	See the quality control section for details.	TBD	
Working cell bank (WCB) construction service	WCB construction*	Passage culture and strain preservation	4	
	WCB release test	See the quality control section for details.	TBD	
Strain preservation and storage service	-	-	-	The strains can be stored for free
Registration review service ★	Compliance review	-	-	

Note: [1] is the specific item of Escherichia coli expression system, and [2] is the specific item of yeast strain expression system;
* marked items -MCB/WCB bank construction conducted in GMP workshop.
Delivery cycle: Except for long-term stability test, the average period of bank construction is 4-6 months.

Quality Control of *Escherichia Coli* Strains

Service Name	Service Items	Service Details	Minimum Manufacturing Cycle (working days)	Deliverables
***E. coli* strain quality control service**	Stability study	Passage stability	TBD	• Passage record • Test record • Test report COA
		Storage stability		
	Strain streaking LB agar plate	Culture methods	3	
	Staining microscopy	Gram staining method		
	Viable count determination	Culture methods		
	Resistance to antibiotics	Culture methods		
	Biochemical reaction	Biochemical characteristic reaction detection method		
	Plasmid copy number	qPCR method	30	
	Expression amount of target product	SDS-PAGE method	5	
	Plasmid enzyme digestion profile	Agarose gel electrophoresis		
	Plasmid loss rate	Culture methods		
	Bacteriophages assay	Plague or proliferation method	30	
	Target gene nucleotide sequence	Sanger sequencing method	7	
	Conserved 16SrRNA region sequence	Sanger sequencing method		
	Electron microscopy	Electron microscopy method	30	

Yeast Strain Quality Control

Service Name	Service Items	Service Details	Minimum Manufacturing Cycle (working days)	Deliverables
***Yeast* strain quality control service**	Stability study	Passage stability	TBD	• Passage record • Test record • Test report COA
		Storage stability		
	Colony characteristics on plate	Culture methods	3	
	Microscopy morphology	Staining method		
	Viable count determination	Culture methods		
	Resistance	Culture methods		
	Expression amount of target product	SDS-PAGE method		
	Detection of exogenous gene integrated into host chromosome	PCR method	30	
	Identification	WB	5	
	Biochemical characterization	Biochemical assay		
	Copy number determination	qPCR method		
	Target gene sequencing	Sanger sequencing method	30	
	ITS identification	Sanger sequencing method	7	
	18S rRNA identification	Sanger sequencing method		
	Electron microscopy	Transmission electron microscopy method	30	

Service Features

One-stop service platform

One-stop service for strain construction, dominant strains screening, tertiary cell bank construction and strain testing is provided.

Independent GMP cell bank construction workshop

MCB and WCB constructions are carried out in an independent workshop in line with GMP specifications to effectively avoid cross-contamination, ensure quality of strains and meet the regulatory requirements.

Diversified strain preservation platform

Multiple strain preservation methods, such as glycerol freezing/liquid nitrogen/freeze-drying, are provided to meet the needs of off-site storage .

Comprehensive quality inspection platform

Testing items such as strain passage stability, storage stability and others under the guidance of pharmacopoeia and other regulations are provided.

Compliance review platform

A registration team shall participate in project review to meet the requirements of biological product registration.

GMP system online audit platform

Online audit ports are opened to share the VR videos of GMP workshop.

Periodic communication platform

Periodic communication meetings are scheduled to share project progress in a timely manner according to client needs and project characteristics.

E. coli Expression System

E. coli expression system is the earliest developed, most widely used and most economical classical expression system in gene expression technology. Its features include: clear genetic background, fast cell proliferation, high expression amount, excellent stability and strong anti-pollution ability, which is the preferred system for exogenous recombinant protein expression, and it has great advantages especially for the manufacturing of uncomplicated glycosylated proteins such as plasmids, polypeptide hormones, or cytokines:

Plasmid DNA

Naked plasmid, viral vector raw material or mRNA raw material applied in the field of cell and gene therapy (CGT).

Recombinant peptides

Growth hormone (GH), glucagon-like peptide analogs (GLP-1 analogs), parathyroid hormone (PTH).

Cytokines

Interleukin-2 (IL-2), IL-15, IL-21.

Enzyme formulations

Cas9 nuclease, other nucleases, proteases.

Recombinant protein vaccines

Recombinant subunit vaccines, virus-like particle vectors (VLP vectors).

Nanobodies

Nanobodies of different potencies (monovalent/bivalent/ trivalent).

Collagen

Type I collagen, type III collagen.

Commonly Used Plasmids and Characteristics

As a DNA vector that can be independently replicated, plasmid has made a crucial contribution in the process of rapid development of gene recombination technology. Generally, plasmids are composed of the following functional elements: origin of replication (ORI), multiple cloning site (MCS), promoter region and resistance screening markers. The plasmid vectors commonly used in Yaohai BioPharma *E.coli* molecular construction platform include pET family vectors and other applicable plasmids.

Usage	Plasmid	Type Of Promoter	Type Of Operon	Resistance/ Screening Marker	Copy Type
Manufacturing of recombinant protein	pET28a	T7	lac	Kanamycin	High copy
Manufacturing of recombinant protein	pET200/D-TOPO	T7	lac	Neomycin, kanamycin	Low copy
Manufacturing of recombinant protein or plasmids	Other applicable plasmids	-	-	-	-

Commonly Used Host Strains and Characteristics

Due to the difference of functional elements (mutation or missing), different Escherichia *coli* host strains have different expression characteristics, which are suitable for the production of specific products. The host strains commonly used in Yaohai BioPharma Escherichia coli molecular construction technological platform and their expression characteristics are shown in the following table:

Usage	Host Strains	Resistance	Suitable Promoter	Characteristics Of Strains
Manufacturing of plasmid	DH5α	-	-	Suitable for plasmid amplification; RecA1 and endA1 mutations increase the stability of foreign DNA.
	TOP10	Streptomycin	-	
	Trans10	Streptomycin	-	
	JM108/JM109	-	-	
	Stbl3	Streptomycin	-	RecA13 mutation type, and is suitable for the amplification of lentiviral vectors.
Manufacturing of recombinant protein	BL21 (DE3)	-	T7, non-T7	Proteinase-deficient type, used for the expression of non-toxic proteins.
	BL21 star (DE3)	-	T7, non-T7	RNaseE deficiency, used for the expression of non-toxic proteins.
	BL21 AI	Tetracycline	T7	Protease deficiency type, and can be used for the expression of toxic proteins.
	BL21 (DE3) pLysS	Chloromycetin	T7	T7 lysozyme inhibits background expression, and can be used for the expression of toxic proteins.
	BL21 (DE3) pLysE	Chloromycetin	T7	T7 lysozyme inhibits background expression, and can be used for the expression of toxic proteins.
	C41(DE3)	-	T7	Low RNAP activity inhibits background expression, and can be used for toxic protein expression.
	Tuner(DE3)	-	T7, non-T7	LacY inactivation, IPTG concentration-dependent and protease-deficient type.
	Origami B(DE3)	Kanamycin/ tetracycline	T7, non-T7	LacY inactivation; expression of reductase increasing the solubility of protein.
	Shuffle T7-B	Spectinomycin/ streptomycin	T7	Constitutive expression of disulfide isomerase contributing to the correct folding of protein.
	Clearcoli BL21(DE3) Duos	-	T7	Endotoxin-deficient strains for expression of recombinant protein or plasmid.
	JM108/109	-	T7, non-T7	Used for the expression of non-toxic proteins.
Other	Other Escherichia *coli*	-	-	-

Yeast Strain Expression System

Yeast strain expression system is a widely used system in industrial manufacturing. Its genetic background is clear and safe, and it has the advantages of both prokaryotic and eukaryotic expression systems: rapid proliferation, high-density fermentation in cheap culture medium, glycosylation mode, strong secretion and expression ability effectively reducing the cost of downstream purification. Recombinant biological products that can be produced with yeast include but are not limited to:

Recombinant protein vaccine

Recombinant subunit vaccine (including virus-like particle vaccine and VLP vaccine).

Recombinant polypeptides

Growth hormone (GH), glucagon-like peptide analogs (GLP-1 analogs).

Cytokines

Interleukin-2 (IL-2), IL-15, IL-21.

Nanobodies

Nanobodies of different potencies (monovalent/bivalent/ trivalent).

Collagen

type I collagen, type III collagen.

Commonly Used Plasmids and Characteristics

As a DNA vector that can be independently replicated, plasmid has made a crucial contribution in the process of rapid development of gene recombination technology. Generally, plasmids are composed of the following functional elements: origin of replication (ORI), multiple cloning site (MCS), promoter region and resistance screening markers. The plasmid vectors commonly used in the Yaohai BioPharma yeast strain molecular construction technological platform include pPIC9k, Pinka-HC and pPICZa family vectors, etc.

Usage	Plasmid	Type Of Promoter	Type Of Signal Peptide	Resistance/ Screening Marker	Copy Type
Manufacturing of recombinant protein	pPIC9K	AOX1	α-Factor	Ampicillin/kanamycin/His4	High copy
	Pinkα-HC	AOX1	α-Factor	Ampicillin/Ade2	High copy
	pPICZαA	AOX1	α-Factor (containing ATG)	Zeocin	High copy
	pPICZαB	AOX1	α-Factor	Zeocin/His4	High copy
	pPICZαC	AOX1	α-Factor	Zeocin	High copy
	pGAPZαA/B/C	GAP	α-Factor	Zeocin/His4	High copy
	Other applicable plasmids	-	-	-	-

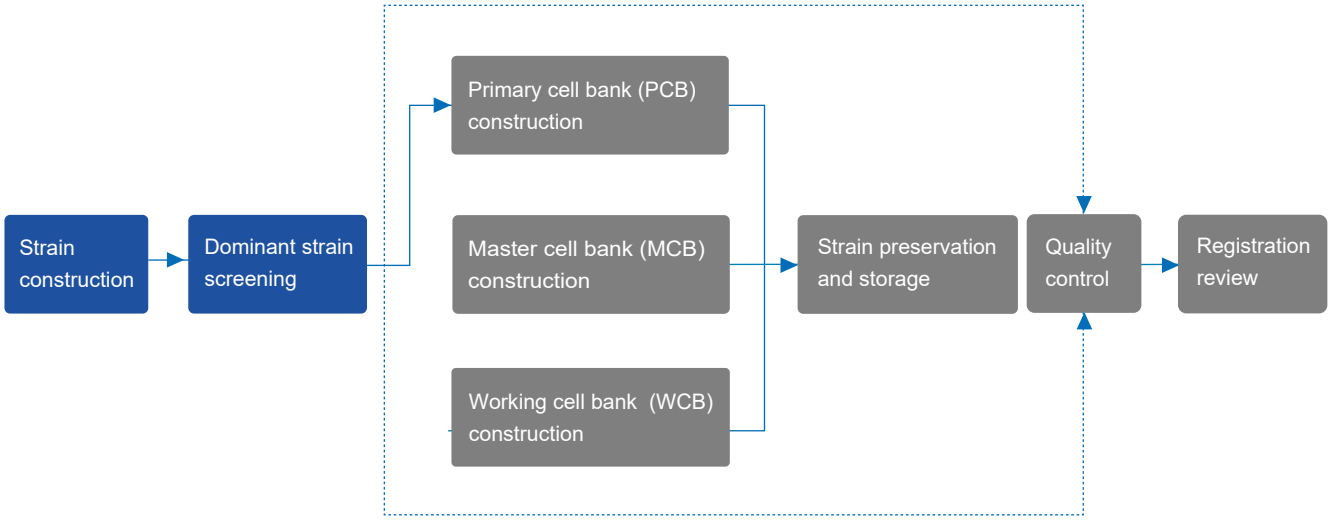
Commonly Used Host Strains and Characteristics

Due to the differences of functional elements (mutation or deletion), different *yeast* host strains have different expression characteristics, which are suitable for producing specific products.

The *yeast* host strains commonly used in the Yaohai BioPharma technological platform and their expression characteristics are shown in the following table.

Usage	Host Strains	Characteristics Of Strains
Manufacturing of recombinant proteins	X-33	Methanol type: Mut+; Genotype: wild type.
	SMD1168H	Methanol type: Mut+; Genotype: Pep4- Protease deficient type.
	GS115	Methanol type: Mut+; Genotype: His4- histidine-deficient type.
	PichiaPink strain/2/3/4	Methanol type: Mut+; genotype: Ade2- Adenine-deficient type; 2/3/4 are different protease-deficient types: strain2: Pep4-; strain3: Prb1-; strain4: Pep4-, Prb1-.
	Other *yeast* strains	-

Strain construction & Dominant strain screening service



The construction of expression strains and screening of dominant strains are at the upstream of the primary cell bank construction, aiming to provide primary strains with high expression and high stability (passage stability and storage stability). According to the pharmacopoeia, the history, source (including the construction process of recombinant engineered bacterial and viral strains) and biological characteristics of the primary strains should be verified.

Relying on the ***E. coli*** and ***Yeast*** molecular construction technological platform, Yaohai BioPharma provides recombinant engineered strain construction and dominant strain screening services and provides plasmids and host strains with clarified source and standard COA report.

The Yaohai BioPharma platform's ***E.coli*** expression system includes the commonly used pET family vectors, and the commonly used host strains, including DH5α, TOP10, BL21(DE3), BL21 star(DE3), BL21 AI and other widely used expression strains. ***Yeast*** expression system includes pPIC9k, Pinka-HC & pPICZa family vectors, and the widely used expression strains includes SMD1168H, X-33, GS115, PichiaPink cell1/2/3/4 and other commonly used host strains.

Service Details

Service Name	Service Items-optional	Service Details	Minimum Manufacturing Cycle (working days)	Deliverables
***Escherichia coli* strain construction service** [1]	Plasmid transformation	Plasmid transformation (multi-host)	5	Strain construction report • Strain construction Process • High-expression strain screening process • Strain of stable passage screening process
		Resistance or defective type screening		
	Strain verification	PCR verification		
	Strain purification	Monoclonal purification		
		Strain spread culture and preservation		
***Yeast* strain construction service**[2]	Plasmid transformation	Preparation of yeast competent cells	10	
		Plasmid linearization		
		Electro transformation		
	High-copy strain screening	Resistance/nutritional deficiency screening		
	Strain purification	Monoclonal purification		
		Strain spread culture and preservation		
Dominant strain screening service	Strain verification	Plasmid extraction[1]/Genome extraction[2]		
		PCR/enzyme digestion verification[1]	15-20	
		Target gene sequencing		
	Screening of high expression strain	Expression inducing* and product analysis		
	Screening of strains of stable passage	Plasmid loss rate test[1]		
		Exogenous gene test[2]		
		Screening of strains of stable passage		
	Strain preservation	Strain preservation		

Note: [1] is the specific item in E. coli expression system, [2] is the specific item in yeast expression system.
The * marked items are specific processes for recombinant protein projects.

Service Features

Multi-host strain transformation platform

DH5α, TOP10, Trans10 and BL21 derived strains are included for *E. coli*;
SMD1168H, X-33, GS115, and PichiaPink strain1/2/3/4 are included for *yeast*, with clear source of host strain and standard CoA report; host strains with patent overdue are selected preferentially for platform process to avoid patent restrictions.

Diversified resistance markers

No antibiotics or antibiotic selected under regulatory guidance to meet regulatory requirements.

Dominant strain screening platform

The platform has a certain screening throughput, and 50+ samples can be tested in a single run with SDS-PAGE.

Good record writing standard

Ensuring that the construction process of recombinant engineered strains is traceable and meet regulatory requirements.

Service Case

Project background

A recombinant protein project using *E. coli* expression system to recombine protein to express in the form of intra-cellular soluble protein.

Strain construction and screening process

The recombinant plasmids and initial expression strains are provided by clients, and then the recombinant plasmids are transformed into three different BL21-derived strains for dominant host strain screening. Targeting the proportion of target protein, the dominant strains with high protein yield and high passage stability are obtained after the dominant host strain screening, screening of dominant strains for dominant host strains, and monoclonal purification of dominant strains.

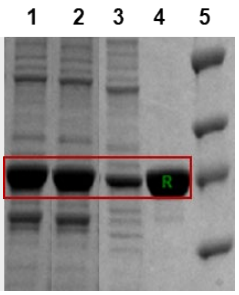
Protein Expression Amount

The dominant host strains were identified by shaking flask culture, inducing of expression and SDS-PAGE analysis, and then the dominant strain S1 was screened out; the expression products of two monoclonal S1-1 and S1-2 of the dominant strain S1 were analyzed with SDS-PAGE.

The results showed that the expression amount of target proteins was significantly higher in strain S1-1 and S1-2 compared with the client's initial strains.

Screening of high expression strains (SDS-PAGE)

1: S1-1 (dominant strain - monoclonal 1)
2: S1-2 (dominant strain - monoclonal 2)
3: Clients' initial strains
4: Control-quantitative reference
5: Marker



Strain Stability

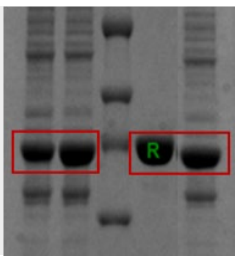
The retention rates of plasmids of the passaged strains P5 and P10 of S1-1 were not less than 95%.

SDS-PAGE analysis conducted on the products of the passaged strains P5~P10 of S1-1 showed that the expression amount of its target proteins was basically the same as the original strains.

The above results suggest that the plasmid loss rate was low, the expression level of recombinant protein did not change significantly, and the strain passage was stable during the 10 successive passages of strain S1-1.

Screening of passage-stable strains (SDS-PAGE)

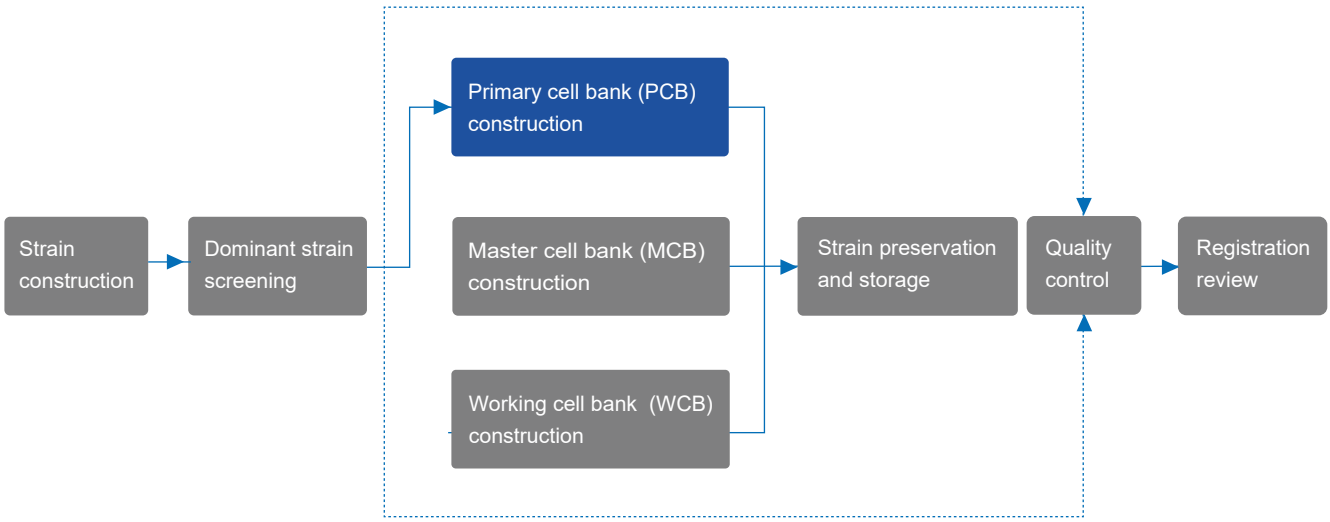
1: S1-1 P0 (10 passages)
2: S1-1 P5 (5 passages)
3: Marker
4: Control-quantitative reference
5: S1-1 P10 (10 passages)



Other Services

- Strain construction
- Dominant strain screening
- Strain preservation and storage
- Strain quality control
- Primary cell bank (PCB) construction
- Master cell bank (MCB) construction
- Working cell bank (WCB) construction
- Registration review service

Primary cell bank (PCB) construction service



The primary cell bank (PCB) is derived from the dominant strains with high expression and high stability screened in the early stage, and the release of PCB shall be subject to quality standards of compliance established.

Relying on Escherichia coli and yeast molecules, Yaohai BioPharma established the technological platform and quality control platform to provide services for primary cell bank (PCB) construction, and developed complete quality control and release criteria according to the pharmacopoeia and other regulations to ensure the compliance of the PCB bank construction process and quality study methods, and fully meet the requirements of registration applications.

Service Details

Service Name	Service Items	Service Details	Minimum Manufacturing Cycle (working days)	Deliverables
Primary cell bank (PCB) construction	PCB bank construction	Growth curve determination	5	Report of cell bank construction PCB strains
		Passage culture		
		Strain preservation		
	PCB release test	See the quality control section for details.	TBD	

Note: The table shows the shortest service cycle with *E. coli* as an example, and *yeast* items are increased as appropriate.

Service Features

Perfect quality control platform

Test items include but are not limited to plasmid loss rate, passage stability, storage stability, and etc.;

Diversified strain storage platform

Freeze-dried/liquid nitrogen/glycerol freeze-storage and other kinds of bacteria storage methods, meeting the needs of off-site storage;

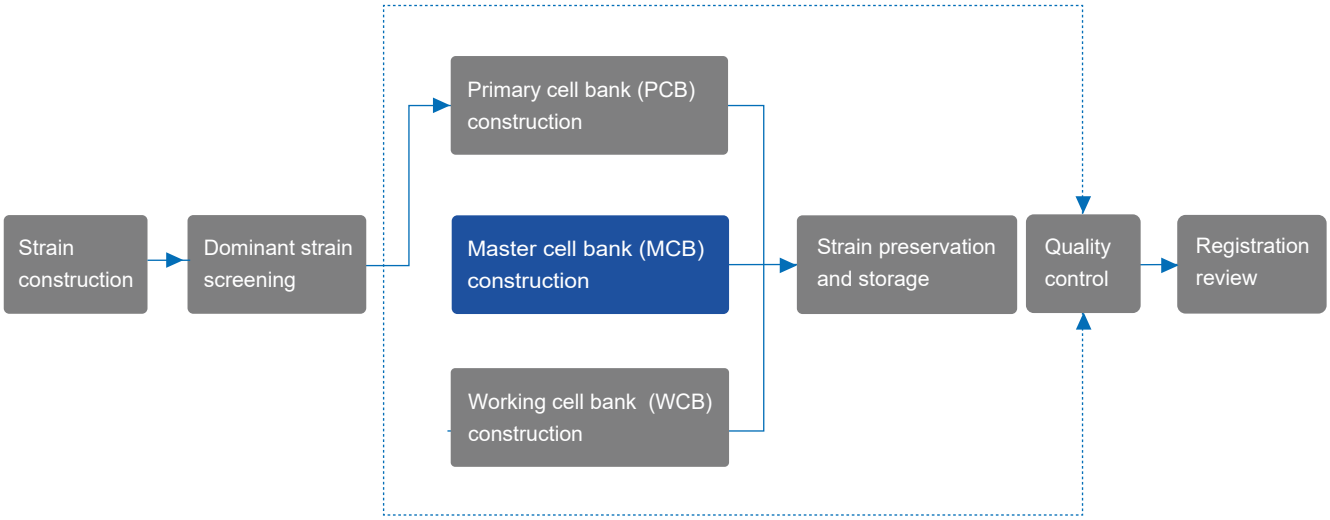
Good record writing specification

Ensure that the experimental processes can be traced and meet the audit requirements.

Other Services

- Strain construction
- Dominant strain screening
- Strain preservation and storage
- Strain quality control
- Primary cell bank (PCB) construction
- Master cell bank (MCB) construction
- Working cell bank (WCB) construction
- Registration review service

Master Cell Bank (MCB)
construction service



Master cell bank (MCB): A homogenous cell suspension that reaches a specific multiplication level or passage level after the strains from the primary cell bank (PCB) are passaged and multiplied in a specified way, and then stored for use through appropriate preservation methods. The passed strains must be fully tested according to its specific quality control requirements, and will be used as MCB after passing the test. MCB's compliance is mainly reflected in the GMP workshop and the more stringent quality release criteria.

Relying on the independent GMP-level cell bank construction workshop and quality control platform, Yaohai BioPharma provides the master cell bank (MCB) construction service. The quality standard of MCB is strictly higher than that of PCB, and the passages of MCB is strictly limited, so as to fully meet the requirements of registration application.

Service Details

Service Name	Service Items	Service Details	Minimum Manufacturing Cycle (working days)	Deliverables
Primary cell bank (MCB) construction	MCB bank construction	Passage culture	4	Report of cell bank construction MCB strains
		Strain preservation		
	MCB release test	See the quality control section for details.	TBD	

Note: The table shows the shortest service cycle with *E. coli* as an example, and *yeast* items are increased as appropriate.

Service Features

Independent GMP bank construction workshop

The constructions of the master cell bank (MCB) and the working cell bank (WCB) are carried out in an independent workshop in compliance with GMP specifications, effectively avoiding cross contamination, ensuring the quality of strains, and meeting regulatory requirements;

Diversified strain storage platform

Freeze-dried/liquid nitrogen/glycerol freeze-storage and other kinds of strain preservation methods to meet the needs of off-site storage;

Compliant quality test platform

Many of the test items such as strain passage stability and storage stability under the guidance of pharmacopoeia and other regulations have obtained clinical approval documents, including China, the United States and Australia;

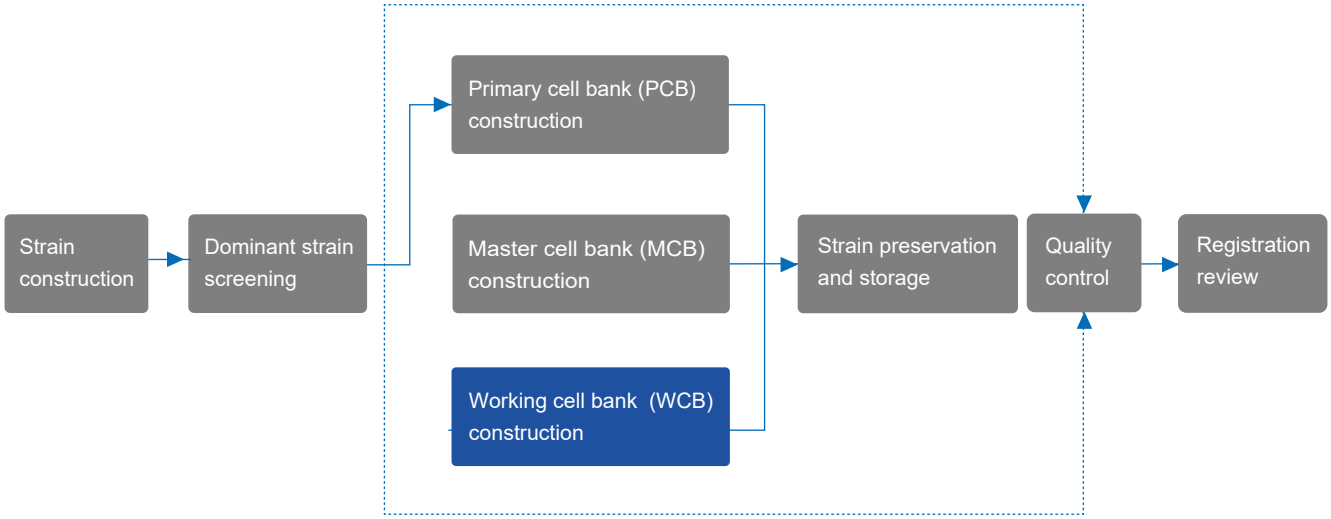
GMP system online audit platform

Online audit port is opened to share VR videos of GMP workshop.

Other Services

- Strain construction
- Dominant strain screening
- Strain preservation and storage
- Strain quality control
- Primary cell bank (PCB) construction
- Master cell bank (MCB) construction
- Working cell bank (WCB) construction
- Registration review service

Working cell bank (WCB)
construction service



Working cell bank (WCB): a homogenous cell suspension that has reached a specific level of passage times after the strains from the master cell bank (MCB) are passed and multiplied, and then stored for use through appropriate preservation methods. The prepared WCB can be used for manufacturing after being tested qualified. The quality criteria of WCB should be established on the basis of the quality of MCB.

Based on the independent GMP-level bank construction workshop and quality control platform, Yaohai BioPharma provides working cell bank (WCB) construction services. The quality release criteria are based on the passage capacity of different strains, and the passage times of WCB strains are strictly limited to meet the needs of registration application in all aspects.

Service Details

Service Name	Service Items	Service Details	Minimum Manufacturing Cycle (working days)	Deliverables
Primary cell bank (MCB) construction	WCB construction	Passage culture	4	Report of cell bank construction WCB strains
		Strain preservation		
	MCB release test	See the quality control section for details.	TBD	

Note: The table shows the shortest service cycle with *E. coli* as an example, and *yeast* items are increased as appropriate.

Service Features

Independent GMP bank construction workshop

The constructions of the master cell bank (MCB) and the working cell bank (WCB) are carried out in an independent workshop in compliance with GMP specifications, which effectively avoids cross contamination, ensures the quality of strains, and meets regulatory requirements;

Diversified strain preservation platform

freeze-dried/liquid nitrogen/glycerol freeze-storage and other kinds of strain preservation methods, meeting the needs of off-site storage;

Compliance quality inspection platform

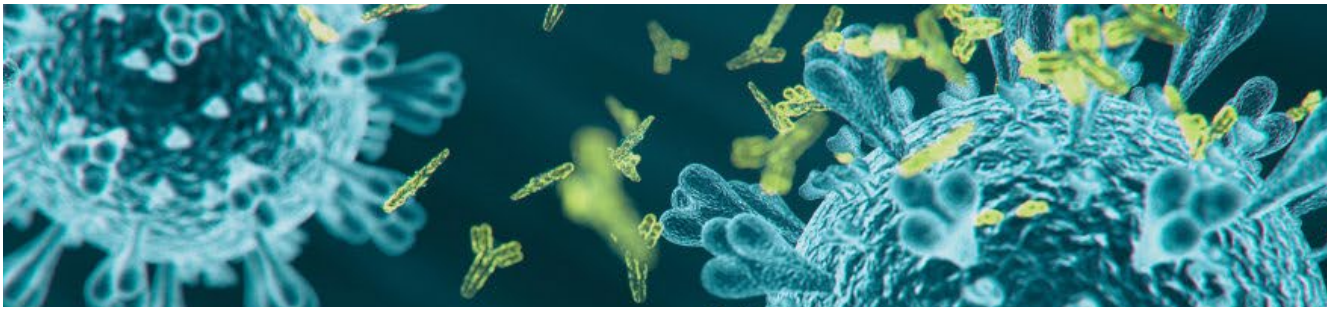
Cell passage stability, storage stability and other testing projects under the guidance of pharmacopoeia and other regulations, and several projects have been declared clinical (China, US and Australia).

GMP system online audit platform

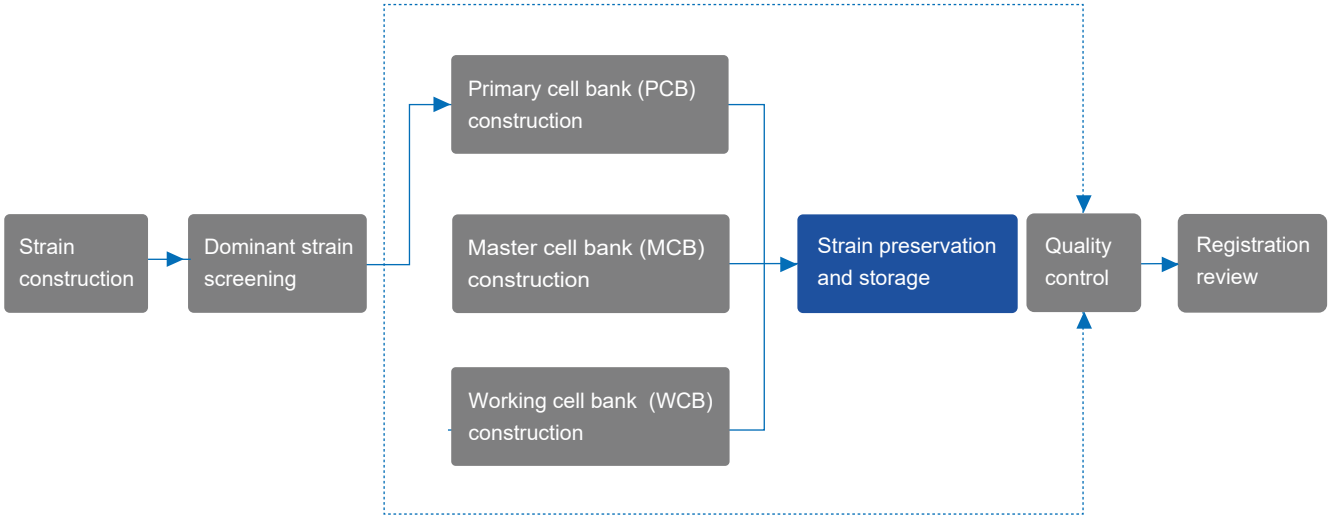
online audit port is opened to share VR videos of GMP workshop.

Other Services

- Primary cell bank (PCB) construction
- Master cell bank (MCB) construction
- Working cell bank (WCB) construction
- Registration review service
- Strain construction
- Dominant strain screening
- Strain preservation and storage
- Strain quality control

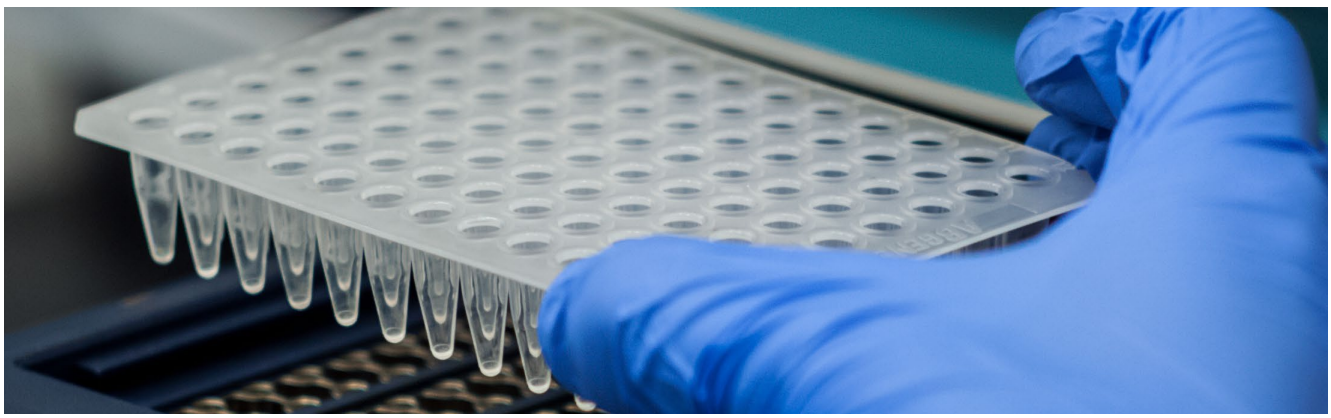


Strain preservation & storage service



After the strain has been tested, it should be preserved in time according to its characteristics by choosing glycerol tube method, lyophilization method, liquid nitrogen method or other appropriate methods to reduce the rate of metabolism of the strains, so that the strains are in a semi-permanent dormant state to guarantee the qualified condition of the strains. The common preservation methods of E. coli include: freeze-drying method, liquid nitrogen method and glycerol freezing method.

Yaohai BioPharma has established an independent GMP strain preservation and storage workshop to improve the service process of secondary cell bank construction and cell storage, and can undertake the service items of strain lyophilization or glycerol freezing and strain storage, meeting the needs of off-site storage and backup for important strains.



Service Details

Service Name	Service Items	Service Details	Minimum Manufacturing Cycle (working days)	Deliverables
Strain preservation and storage	Strain preservation	Cryopreservation (-70°C)	TBD	- Free storage for 6 months - For off-site storage needs
		Liquid Nitrogen preservation		
		Freeze-dried preservation		
	Strain storage	Off-site strain storage and backup		

Service Features

Diversified strain storage solutions

provide customized strain storage services according to strain characteristics.

Independent GMP-level lyophilization workshop

Independent workshop operation, avoiding cross-contamination of strains and effectively guaranteeing the quality of strains.

Independent GMP-level storage workshop

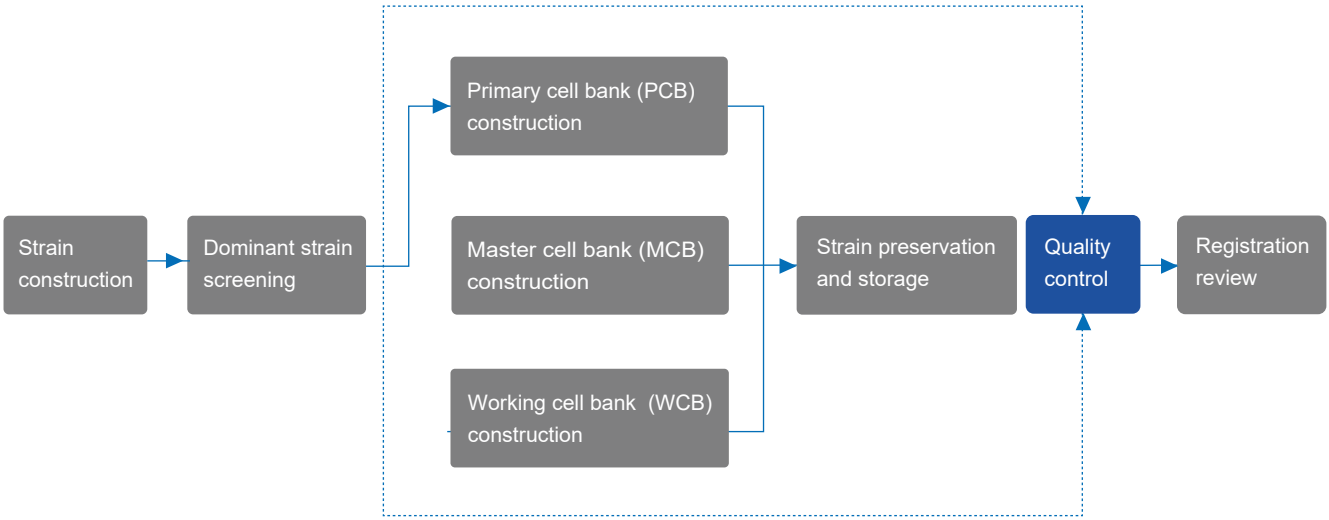
Undertake strain storage services, meeting the needs of off-site storage and backup for important strains.

Other Services

- Strain construction
- Dominant strain screening
- Strain preservation and storage
- Strain quality control
- Primary cell bank (PCB) construction
- Master cell bank (MCB) construction
- Working cell bank (WCB) construction
- Registration review service

Strain Quality

Control Service - *E. coli*



The pharmacopoeia stipulates the testing items of strains for manufacturing, including the biological characteristics, biochemical characteristics and genetic characteristics of strains, and requires the testing of the passage stability of cell banks at all levels to limit the passage times of cell banks, so as to provide strains or cells tested qualified, with the same quality and with the capability of continuous and stable passage for the manufacturing of biological products.

Relying on the quality study and control platform, Yaohai BioPharma has established a complete criterion for quality control and release for three-level cell banks, covering PCB, MCB and WCB. Oriented by registration application, Yaohai BioPharma is able to establish compliant quality control and release criteria for clients according to the characteristics of different strains based on the experience of a number of declared projects to meet the requirements of registration application.

Service Details

Service Name	Service Items	Service Details	Minimum Manufacturing Cycle (working days)	Deliverables
Strain quality control	Stability study	Passage stability	TBD	• Passage record • Test record • Test report COA
		Storage stability		
	Strain streaking LB agar plate	Culture methods	3	
	Staining microscopy	Gram staining method		
	viable count determination	Culture methods		
	Resistance to antibiotics	Culture methods		
	Biochemical reaction	Biochemical characteristic reaction detection method		
	Plasmid copy number	qPCR method	30	
	Expression amount of target product	SDS-PAGE method	5	
	Plasmid enzyme digestion profile	Agarose gel electrophoresis		
	Plasmid loss rate	Culture methods		
	Bacteriophages assay	Plague or proliferation method	30	
	Target gene nucleotide sequence	Sanger sequencing method	7	
	Conserved 16SrRNA region sequence	Sanger sequencing method		
	Electron microscopy	Electron microscopy method	30	

Service Features

High-standard quality control principles

Quality control and release criteria that meet regulatory needs are established based on strain specificities.

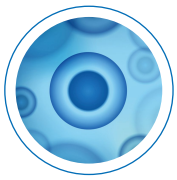
Diversified strain storage platform

Testing items are provided, such as strain passage stability,
storage stability and others under the guidance of pharmacopoeia and other regulations.

Professional QC team

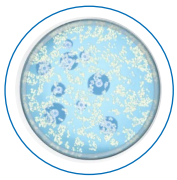
Oriented by registration application, the team is sophisticated in the study of pharmacopoeia and other regulations, facilitating the speed of the approval of the applications.

Other Services

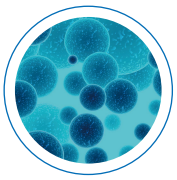


Strain construction

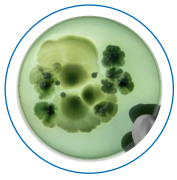
Dominant strain screening



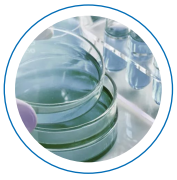
Primary cell bank (PCB) construction



Master cell bank (MCB) construction



Working cell bank (WCB) construction

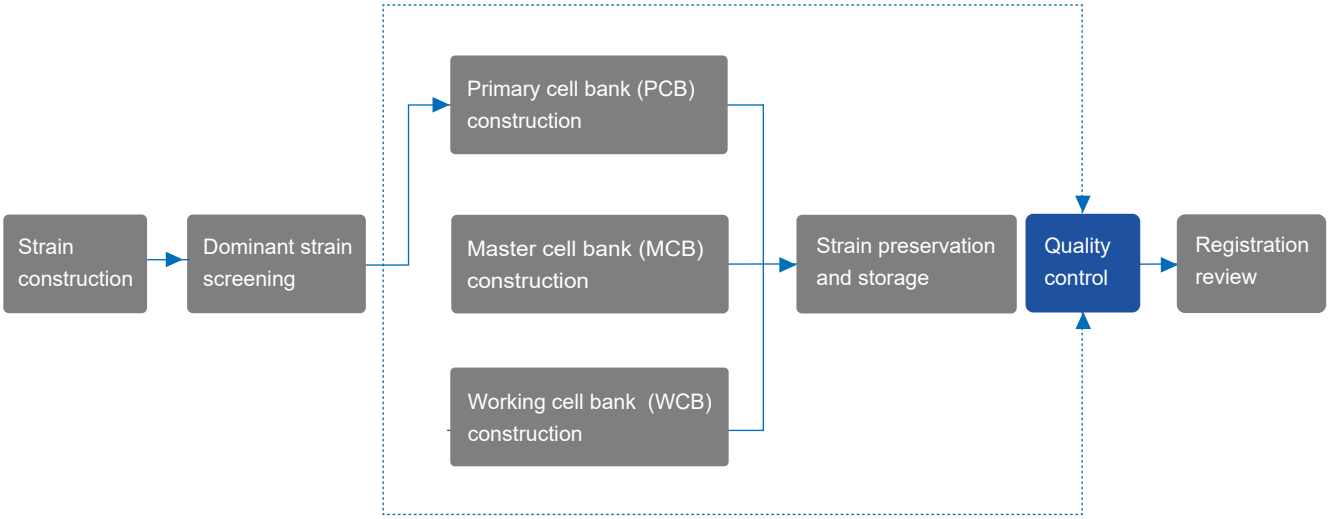


Strain preservation and storage

Strain quality control

Registration review service

Cell Quality Control Service - *Yeast*



The pharmacopoeia stipulates the testing items of strains for manufacturing, including the biological characteristics, biochemical characteristics and genetic characteristics of strains, and requires the testing of the passage stability of cell banks at all levels to limit the passage times of cell banks, so as to provide strains or cells tested qualified, with the same quality and with the capability of continuous and stable passage for the manufacturing of biological products.

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Service Details

Service Name	Service Items	Service Details	Minimum Manufacturing Cycle (working days)	Deliverables
Strain quality control	Stability study	Passage stability	TBD	• Passage record • Test record • Test report COA
		Storage stability		
	Colony characteristics on plate	Culture methods	5	
	Microscopy morphology	Staining method		
	viable count determination	Culture methods		
	Resistance	Culture methods		
	Expression amount of target product	SDS-PAGE method	7	
	Detection of exogenous gene integrated into host chromosome	PCR method		
	Identification	WB		
	Biochemical characterization	Biochemical assay	18	
	Copy number determination	qPCR method	40	
	Target gene sequencing	Sanger sequencing method	14	
	ITS identification	Sanger sequencing method	7	
	18S rRNA identification	Sanger sequencing method	14	
	Electron microscopy	Transmission electron microscopy method	35	

Service Features

Cutting-edge quality control programs

Compliant quality control and release criteria are established based on strain specificities.

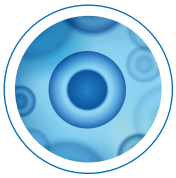
Compliant QC platform

Testing items such as strain passage stability, storage stability and others are provided under the guidance of pharmacopoeia and other regulations.

Professional QC team

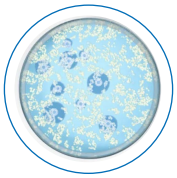
Oriented by registration application, the team is sophisticated in study of pharmacopoeia and other regulations, facilitating the speed of the approval of the applications.

Other Services

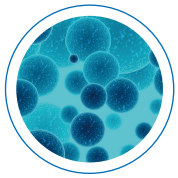


Strain construction

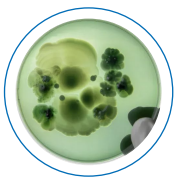
Dominant strain screening



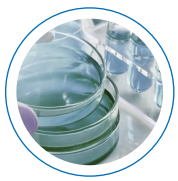
Primary cell bank (PCB) construction



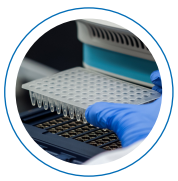
Master cell bank (MCB) construction



Working cell bank (WCB) construction



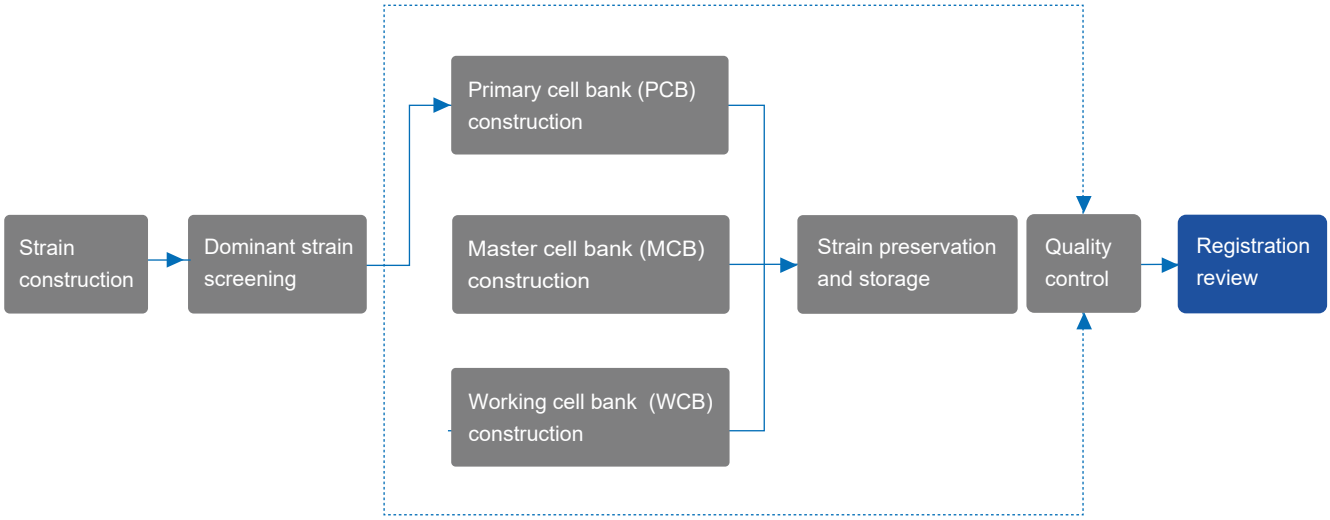
Strain preservation and storage



Strain quality control

Registration review service

Registration Review Service



Yaohai BioPharma registration team will participate in the whole process of cell bank construction service, providing regulatory support for the release criteria for cell bank construction process, passage stability and storage stability and ect., reviewing the bank construction report and giving feedback on compliance and meeting the future IND application needs of our clients in all aspects.

Strain construction and screening process

The traceability of the source of host strains should be clear, avoid patent restrictions as much as possible and be helpful for the commercial manufacturing of client projects.
The source of the original strain should clear, and the construction process should be clarified and traceable.

Cell bank construction and preservation conditions

Secondary cell bank construction should be strictly conducted in GMP workshop.
Suitable strain storage conditions and storage period should be established.

Strain release criteria

Quality standards for strain biological characteristics, phage and others should not be lower than the regulatory requirements.
Testing times and key times for the passage stability identification should meet the regulatory requirements.
Sampling time and long-term stability study for the storage stability identification should meet the regulatory requirements.

Service Features

Extensive project experience

We have served more than 200 clients, covering a wide range of project types with a accurate command of the regulatory guidelines, review requirements and key points of drug registration, and can predict the important and difficult points of the project in advance, greatly enhancing the efficiency of the project.

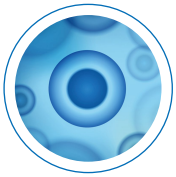
Professional registration team

Our core members have rich experience in registration applications, have conducted a in-depth study of domestic and international registration-related regulations with a profound understanding, and can provide comprehensive guidance to clients on regulatory strategies throughout the life cycle of product research and development.

Comprehensive regulatory study

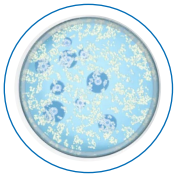
Regulatory study, content interpretation and project implementation from global drug regulatory agencies are fully covered.

Other Services

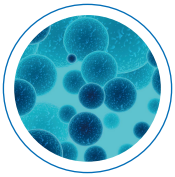


Strain construction

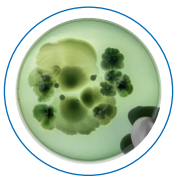
Dominant strain screening



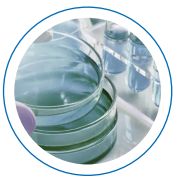
Primary cell bank (PCB) construction



Master cell bank (MCB) construction



Working cell bank (WCB) construction



Strain preservation and storage

Strain quality control

Registration review service

Frequently Asked
Questions & Answers

01 ***Is a screening process for host strain necessary?***

TIPs: Screening of host organisms is an important step and is recommended except in special cases. If the client has a preliminary research foundation, screening of the host strain may not be performed after evaluation.

---

02 ***Is it necessary to conduct sequencing verification before screening the dominant strain after the recombinant plasmid is transformed into the host strains?***

TIPs: After the plasmid transformation, simple verification is recommended, such as PCR validation and enzyme digestion verification, to confirm that the recombinant strains carries the plasmid or target gene, to avoid incorrect subsequent sequencing results and wasting time. However, the work of PCB bank construction and sequencing should be completed before the construction of the master cell bank and working seed bank as well as passage and storage stability testing.

---

03 ***Is it necessary to evaluate the passage stability during PCB bank construction?***

TIPs: The purpose of PCB bank construction is to screen out strains with stable passage ability and high expression, while the passage stability of PCB strains and the passage stability of screening are two concepts. It is recommended to evaluate the passage stability during strain construction and screening.

---

04 ***Is it also fine to consider the passage stability after the PCB bank is constructed?***

TIPs: If the strain passage is stable, this method can reduce the workload of construction; however, there is also a risk that if the strain passage is not stable, then it is required to re-screen the dominant strain and the workload will increase accordingly.

---

05 ***What material/document should we provide if we want to delegate Yaohai BioPharma to build the MCB and WCB secondary cell bank with a PCB bank that has been already constructed?***

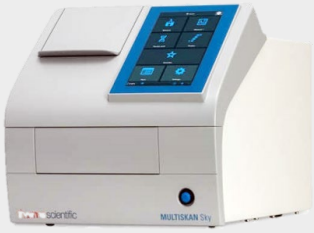
TIPs: Our client need to provide a test report, including live bacteria count, plasmid copy number, antibiotic resistance, expression of target product, and etc. when handing over the PCB to avoid to the maximum extent the application barriers in the future.

Cell Bank
Construction Platform

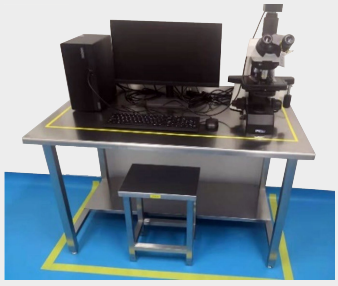
Bio-Rad Gel Imagers

Thermo qPCR instrument

Bio-Rad PCR Instrument



Thermo Full Wavelength
Enzyme Labeler



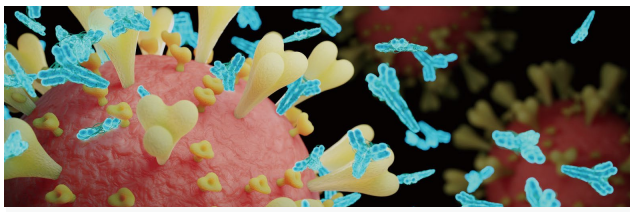
Microscope
(GMP construction workshop)

Class A Biological Safety Cabinet
GMP construction environment: Class A biosafety cabinet in Class C clean area

CRMDO SERVICES OVERVIEW

CRDMO Services of Recombinant Proteins/Polypeptides

- Provide services from strain bank construction, process and analytical method development, cGMP production to aseptic filling of drug product
- A production scale of 2-2000L
- Support recombinant polypeptides/proteins, recombinant antibodies (antibody fragments), and recombinant vaccines (VLP), etc.



CRDMO Services of Nanobodies

- *E.coli* prokaryotic expression system, eukaryotic expression system, and mammalian cell expression system
- Monovalent, bivalent and trivalent diversely nanobodies
- Expression level from μg to kg
- GMP production capacity of drug substance at a scale of 7-2000L

CRDMO Services of Polypeptides Nucleic Acid Drug

- Process development from sequence design and optimization, gene synthesis, IVT, purification and mRNA quality control
- Provide pre-made/customized RNA products
- Support mRNA, CircRNA, etc.

CRDMO Services of Cell And Gene Therapy Polypeptides

- Provide different levels of plasmids such as nonGMP, GMP-like and GMP according to customer's requirements, to meet the needs of different phases of pre-research, IIT, IND registration and application, clinical research and commercial production.

CRO SERVICES

R&D Direction

Raw material enzymes, plasmids, mRNA, CircRNA long chain nucleic acid drugs, nanobodies, recombinant proteins/polypeptides and many other categories

Service Contents

- Biological raw material development
- Drug screening
- Basic technology research in the field of gene therapy
- Quality research of biological products
- Preparation process development of microbial expression drugs
- Preparation process development of cell-related drugs (biosafety at P2 level) and related quality research

mRNA Research-Grade

sample preparation services

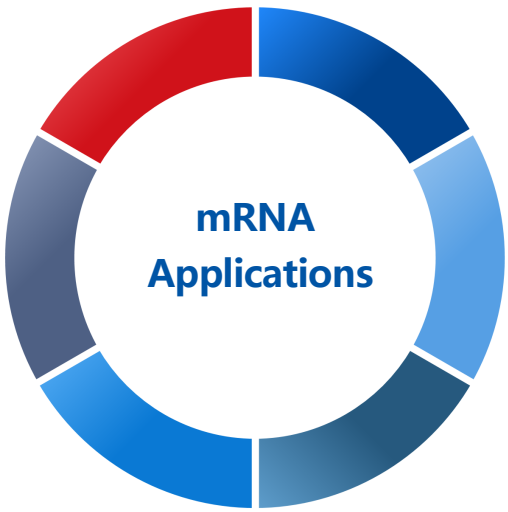
The outbreak of the COVID-19 pandemic in 2020 pushed mRNA technology to center stage, with unprecedented heat for related research and rapid development in multiple fields such as infectious disease prevention, tumor therapy, protein replacement therapy, regenerative medicine, and cell and gene therapy

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	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)
mRNA SERVICE PLATFORM	Platform for mRNA structural design and optimization	Platform for mRNA synthesis and modification	mRNA purification platform	mRNA quality analysis and control platform
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.

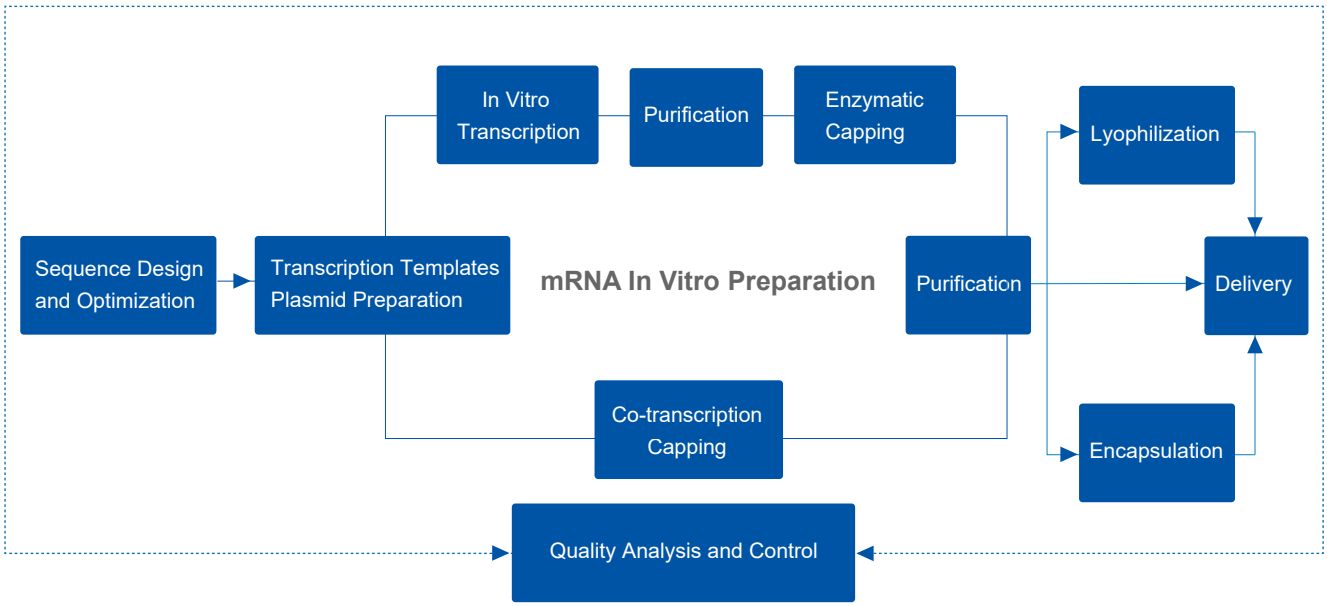
Comprehensive Quality Control Platform to Meet The Quality Control Needs of Each Research Phase

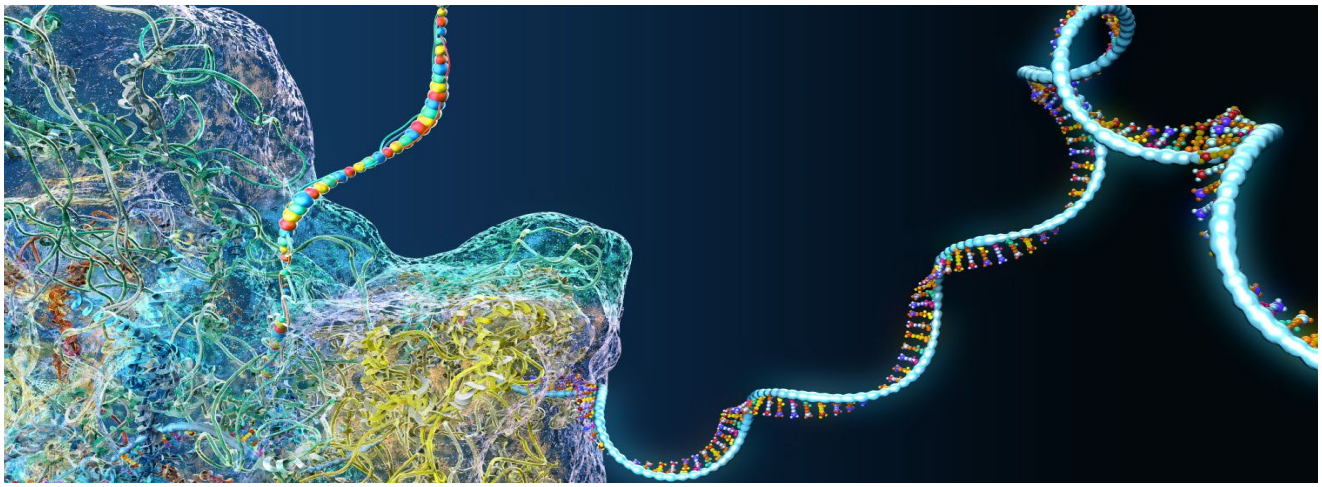
- Meet the general QC requirements for scientific -grade concentration and purity;
- Meet the special QC needs such as mRNA translation test, capping rate, and tail distribution, etc.

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
Reported genes	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			
Viral antigens	mRNA_Spike protein (COVID-19)			
Cytokines	mRNA_IL-2			
	mRNA_IL-4			
	mRNA_IL-22			
Immunogen	mRNA_OVA			
Nucleases	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.

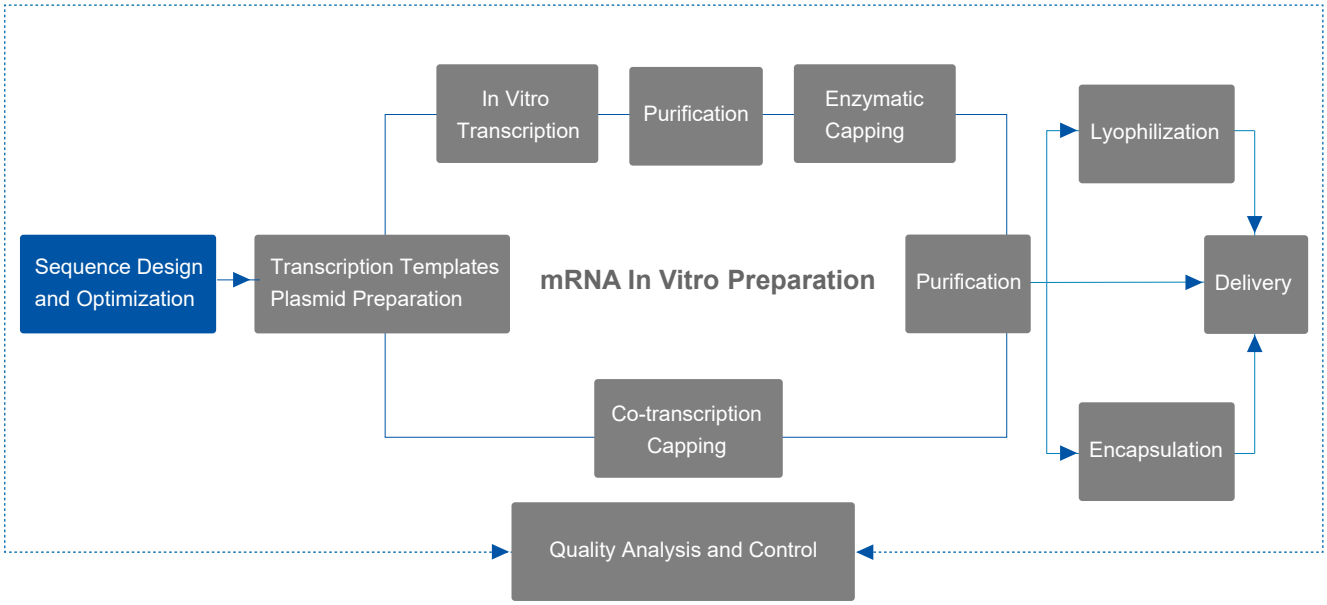
Mature purification platform

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.

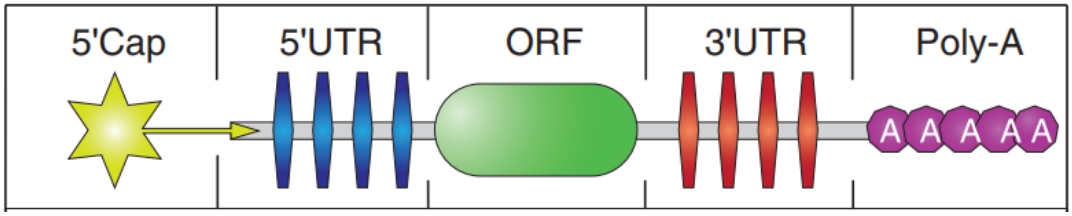
Fast delivery

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

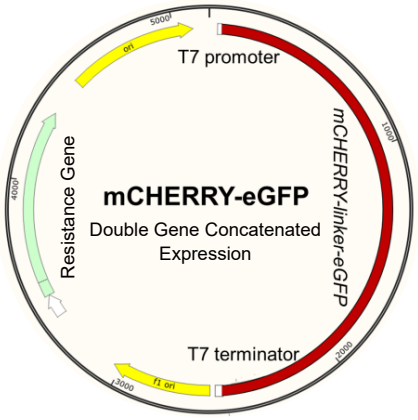
Integrated transcription formation of PolyA tail, with more homogeneous distribution.

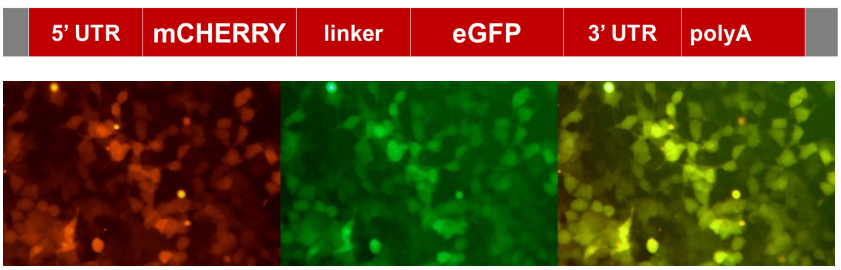
Diversified optimization combination

Achieve efficient expression of mRNA, with low immunogenicity.

Case Study

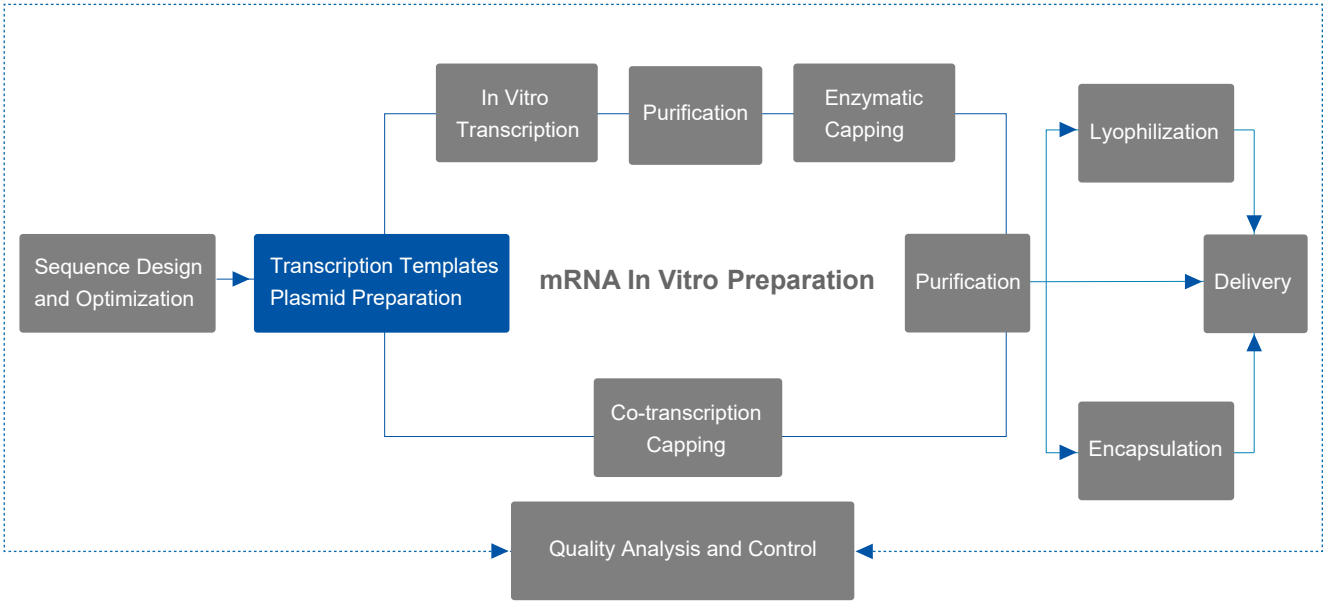
Yaohai Bio's mRNA service continues to be upgraded with the design and optimization of a double reporter gene tandem sequence, which allows co-expression of dual genes. Using a conventional transfection reagent, the double gene tandem sequence mRNA_mCherry-eGFP is transfected into 293T cells, and two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



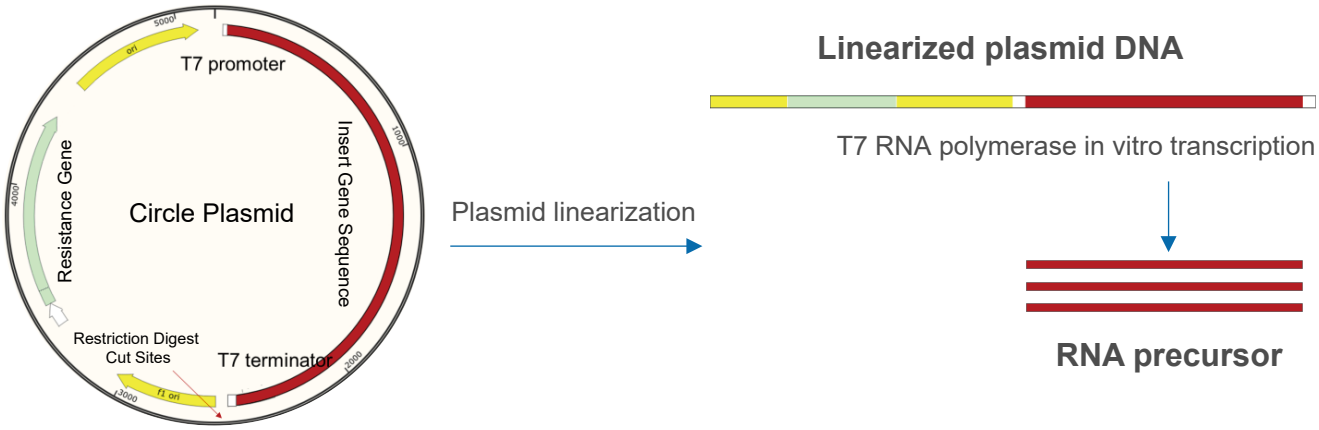


Sequence design and in vitro expression validation of circRNA_eGFP

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.

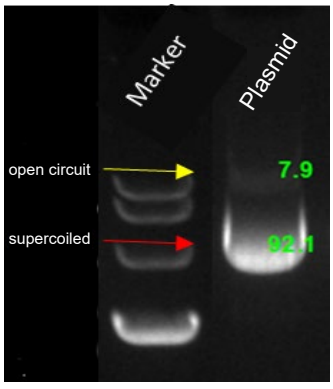
Stringent specification

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

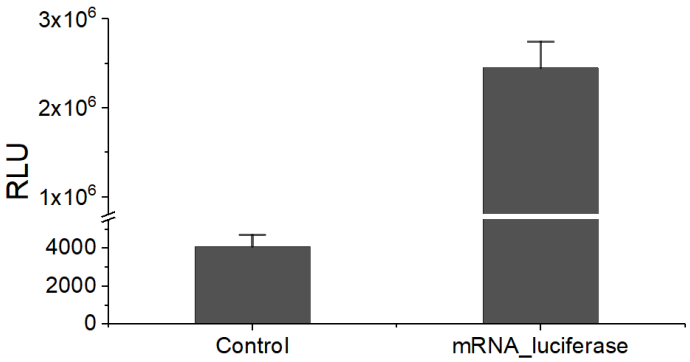
Case Study

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.

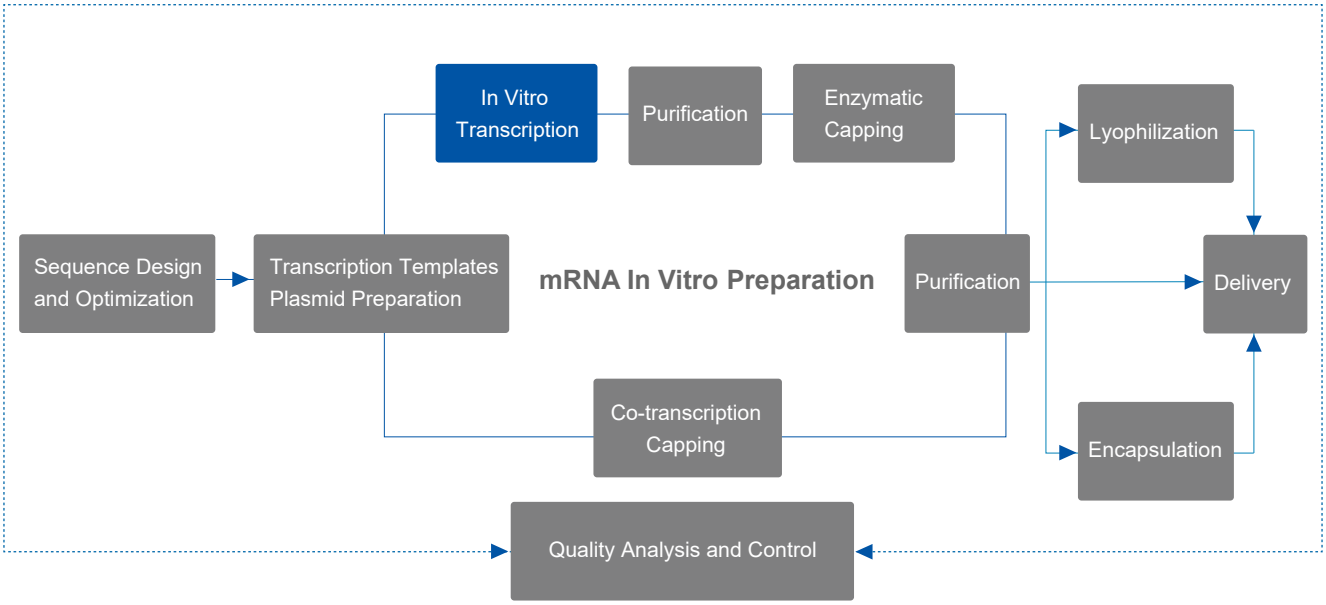


Plasmid Superhelix Ratio Assay



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

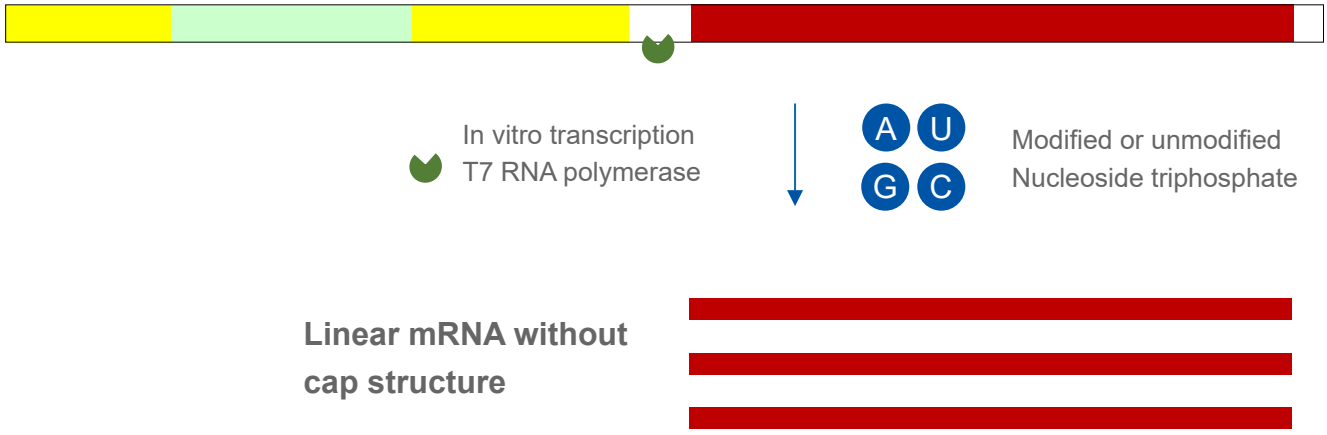


Diagram: IVT reaction diagram

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

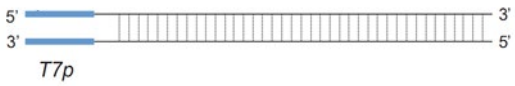
Stringent enzyme control through experimental environment and consumables can effectively prevent mRNA degradation

Stringent enzyme specificatio

Case Study

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.

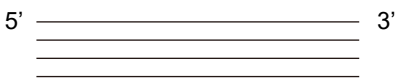
In order to prepare customized mRNA sequences with a length of about 10 kb, Yaohai Bio has successfully prepared high-quality samples with a high transcription ratio of 1:135 and obtained 135 μg of crude and pure mRNA products after 1 μg of linearized plasmid was transcribed in vitro through rigorous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



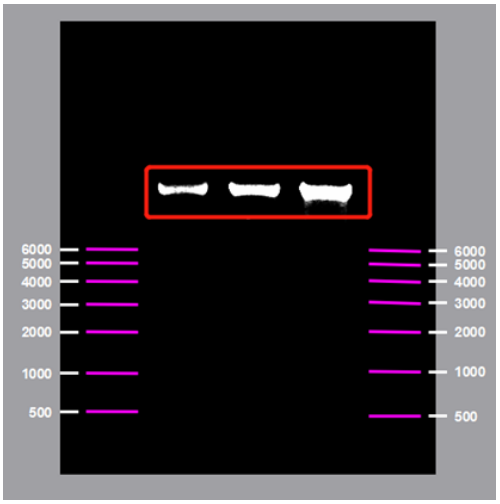
DNA template **1 μg**

High transcription ratio

IVT reaction
Lithium chloride crude purification



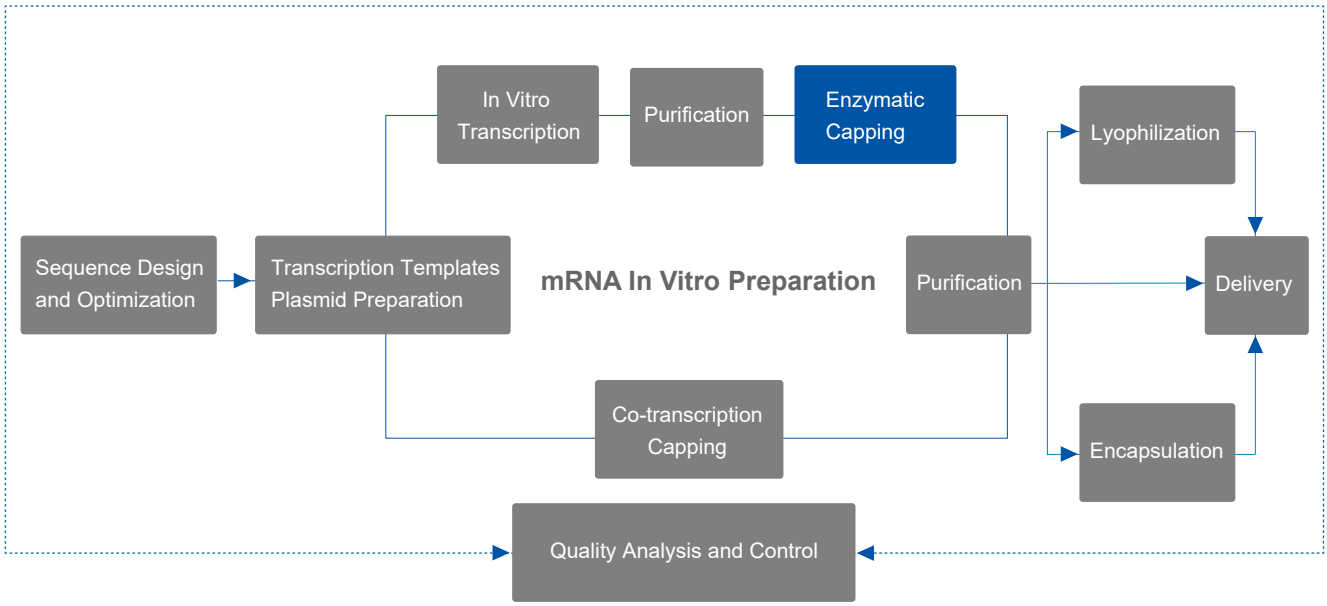
ImRNA product after crude purification **135 μg**



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).

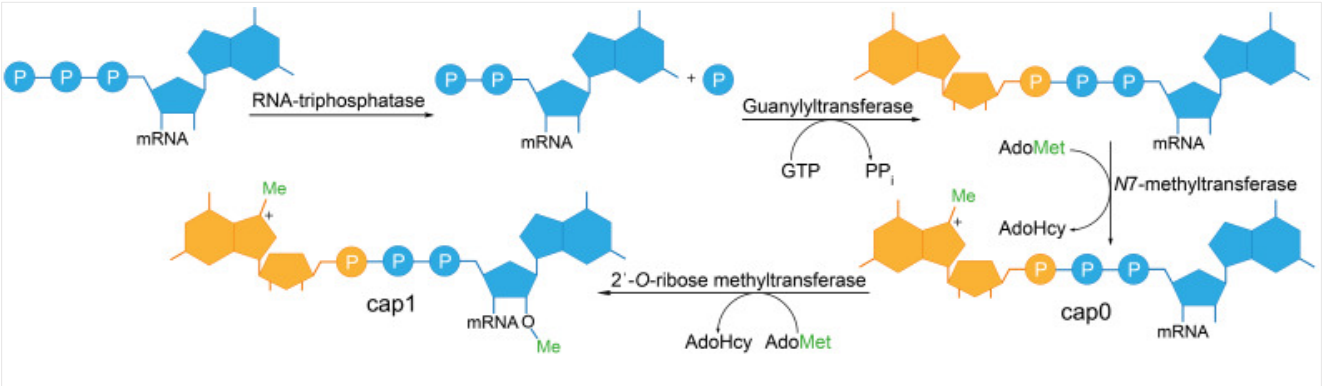


Figure: Diagram of natural cap structure formation

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.

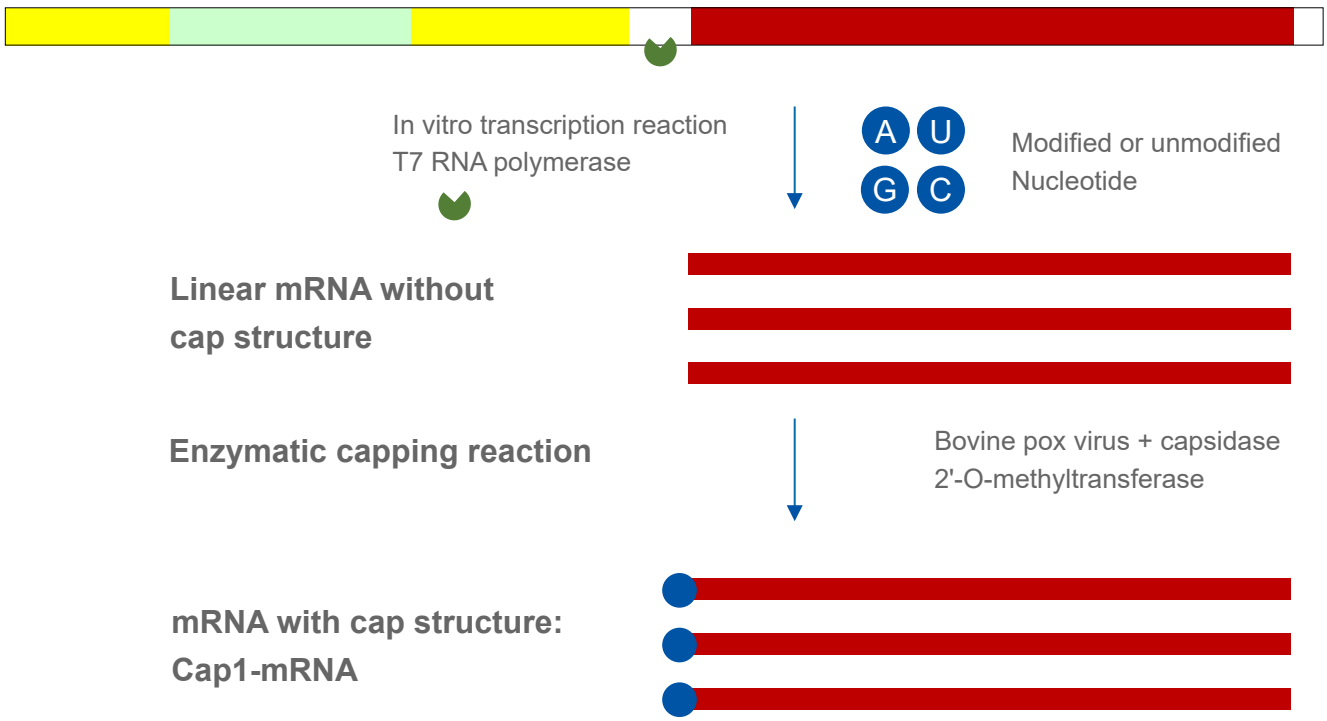


Figure: Diagram of mRNA enzymatic capping reaction

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**

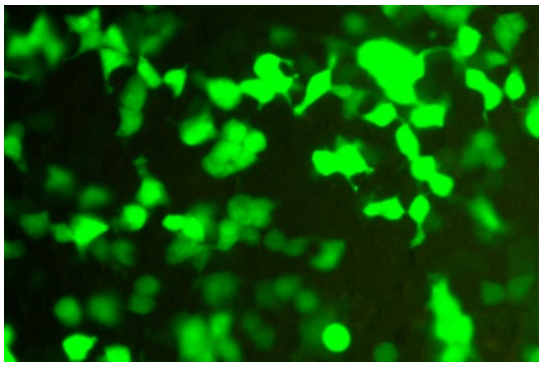
The capped mRNA is transfected into 293T cells, and the expression of the target protein can be detected.

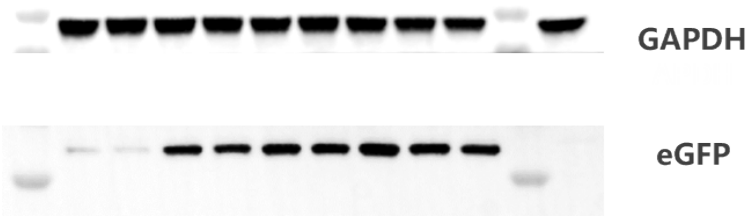
Stringent enzyme specification

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

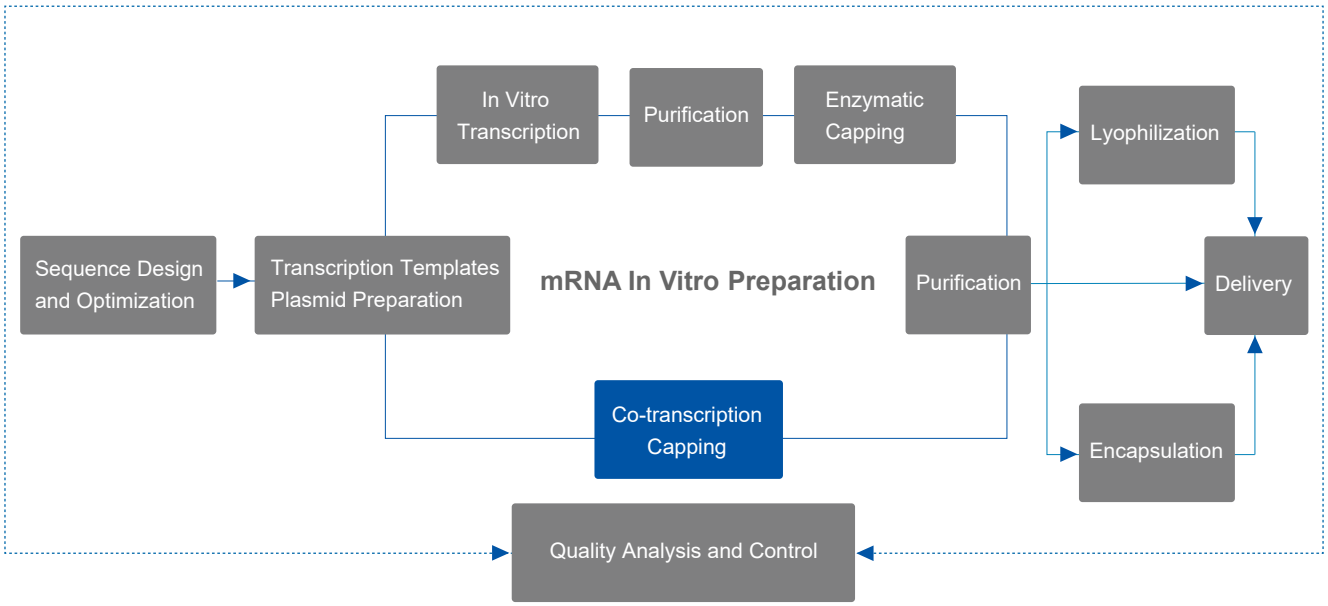
Case Study

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).

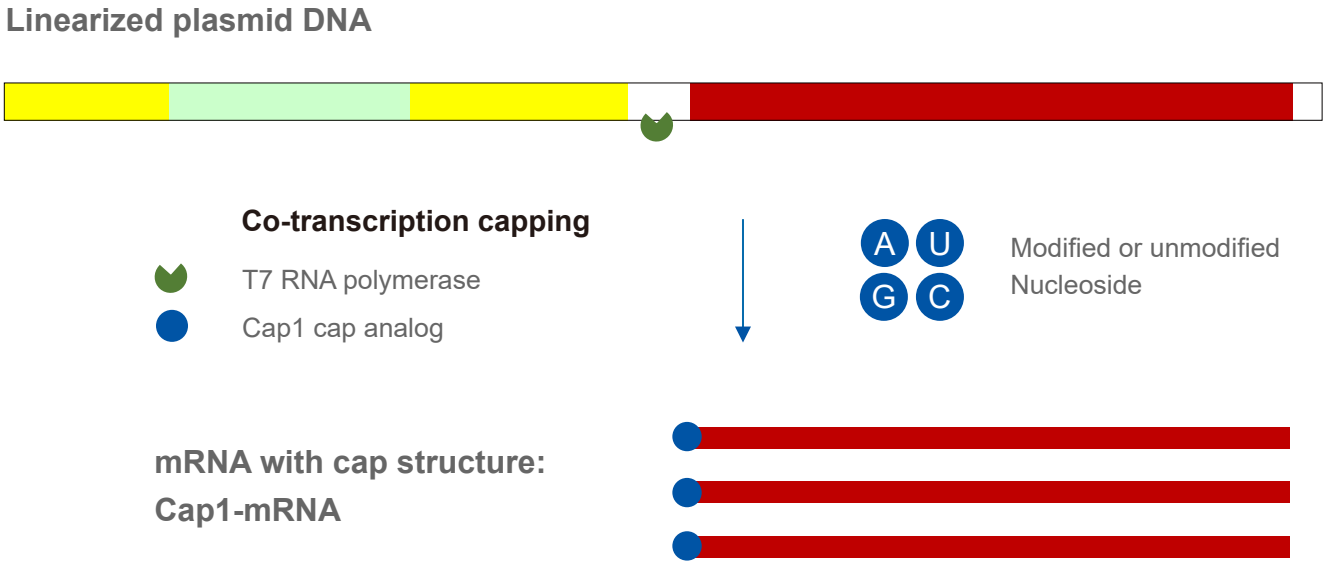


Figure: Diagram of mRNA co-transcription and capping reaction

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%.

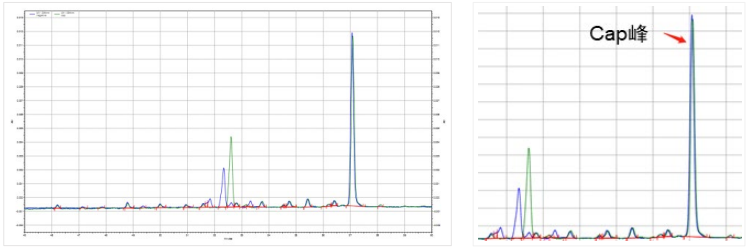
Stable capping process

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

Stringent enzyme specification

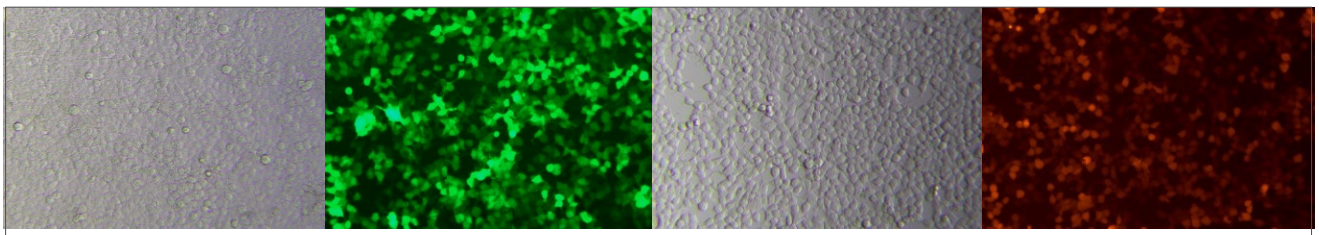
Case Study

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%.



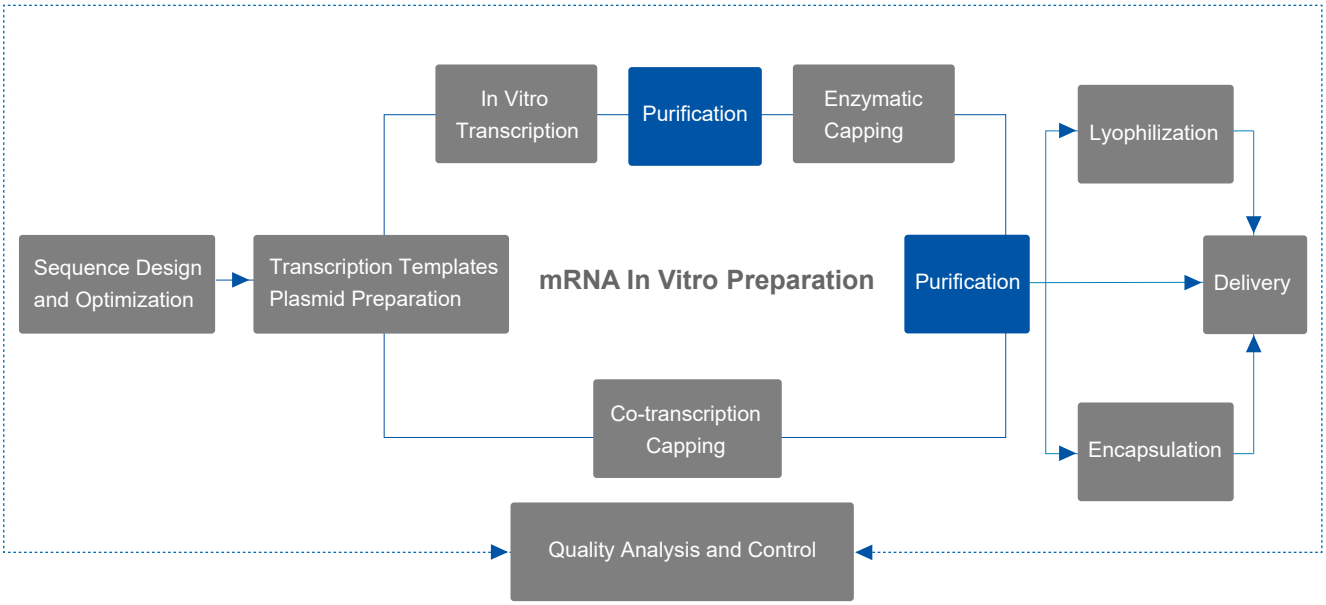
A mRNA_eGFP capping rate of more than 95%

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

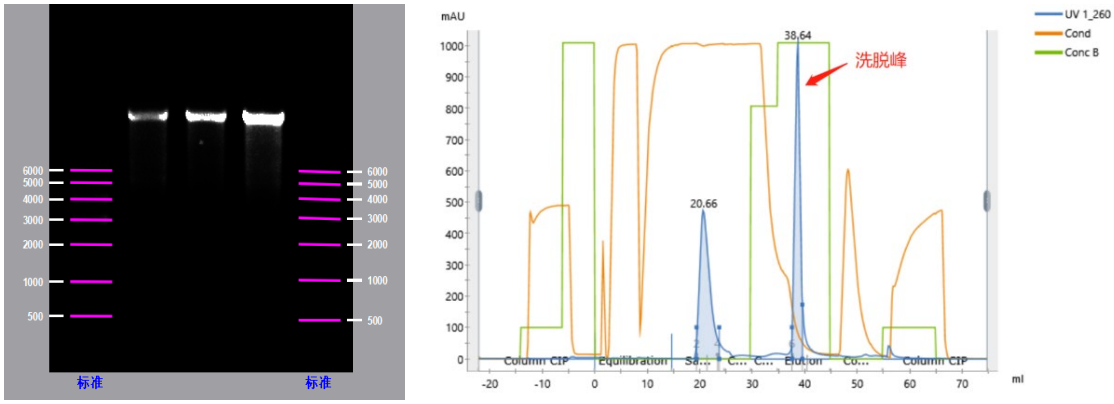
A variety of optional purification solutions can meet different downstream application scenarios.

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

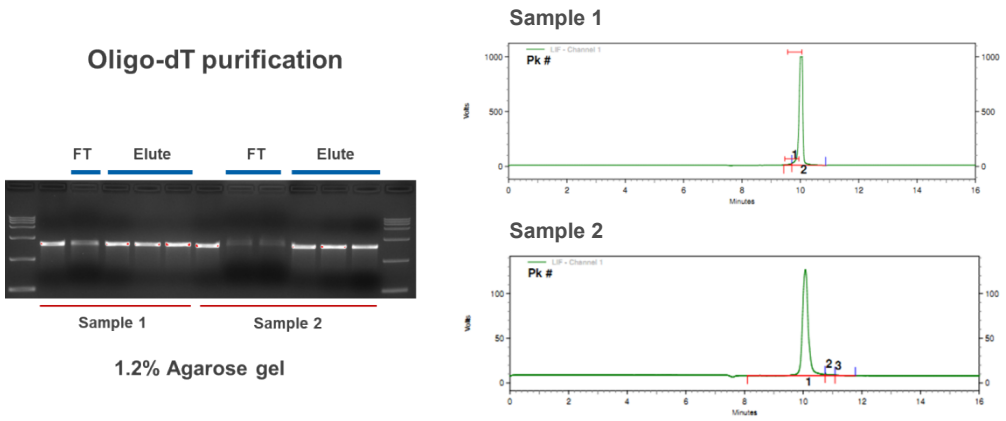
Stringent enzyme specification can prevent mRNA degradation through stringent enzyme control of experimental environment and consumables.

Case Study

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.

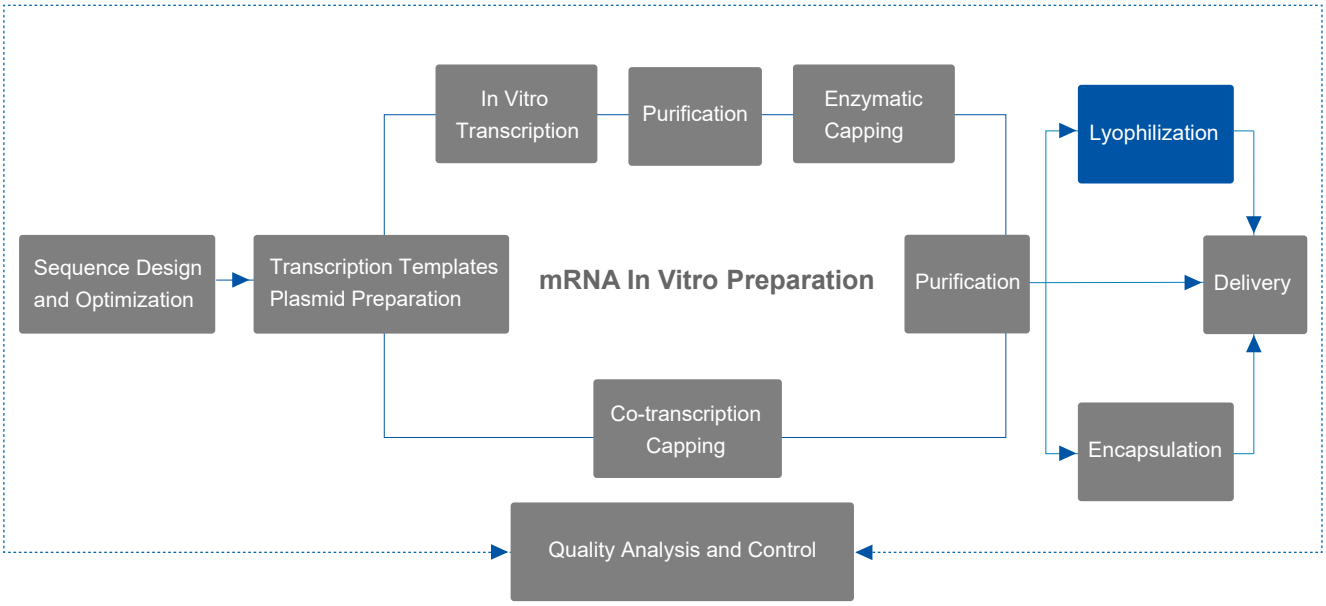


Customized purification of mRNA (>9kb)

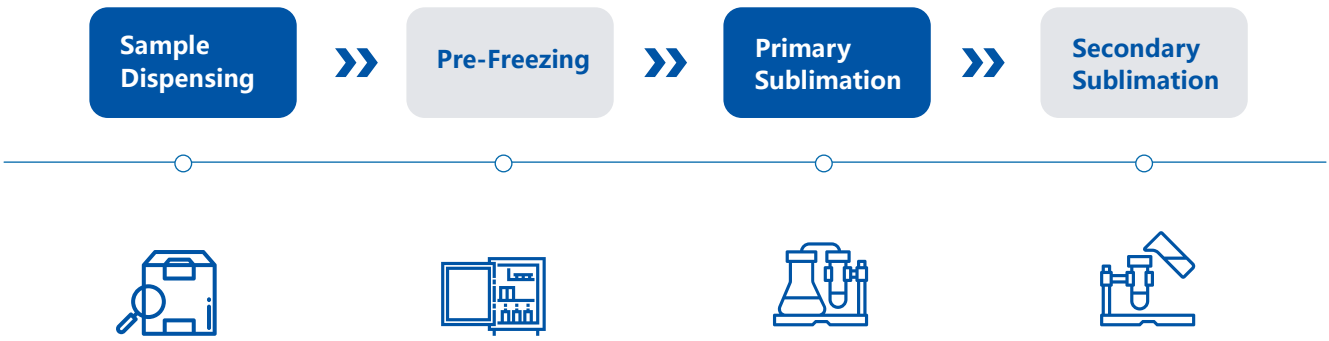


The purity of pre-product mRNA_spike protein (new coronavirus) is more than 95%.

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

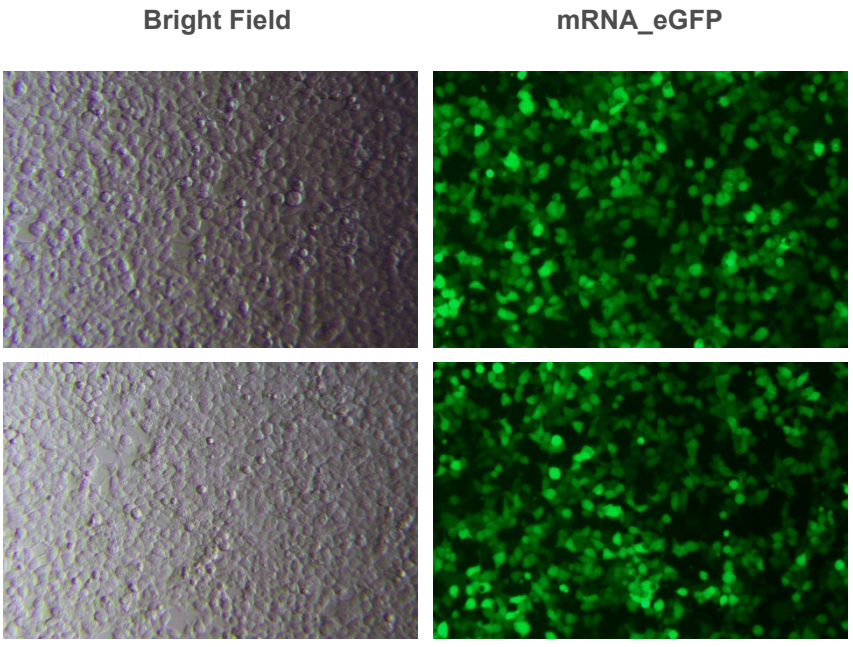
mRNA samples before and after lyophilization can successfully express the target protein.

High stability

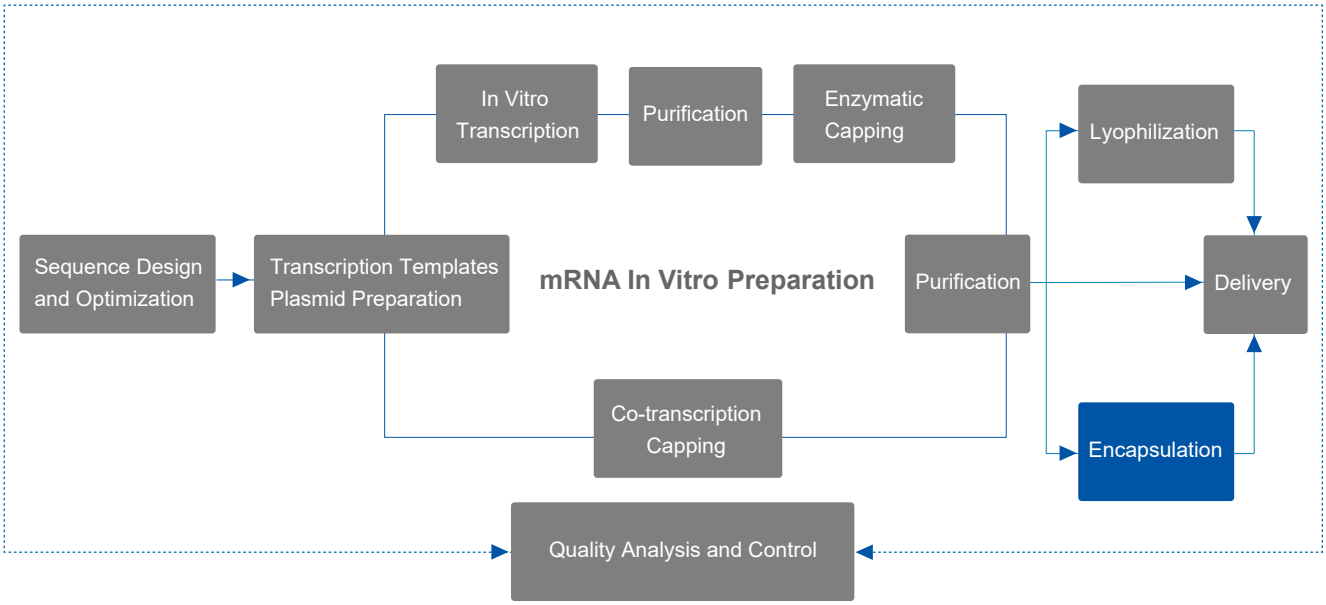
mRNA lyophilized powder is easy to store and transport.

Case Study

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*.

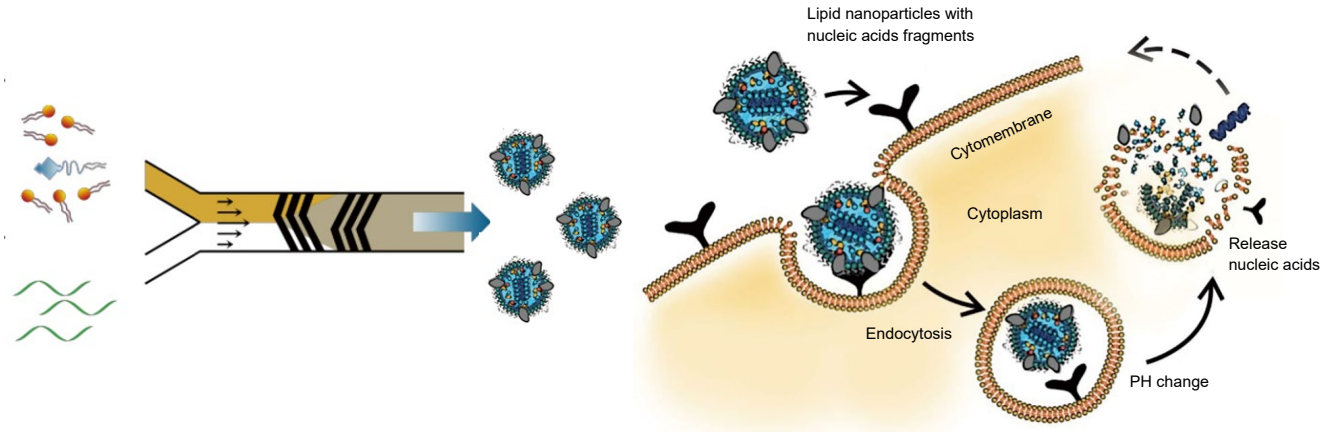


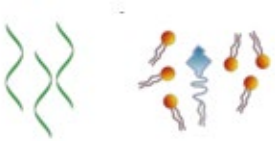
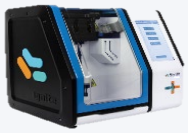
mRNA-LNP
encapsulation Service



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%.

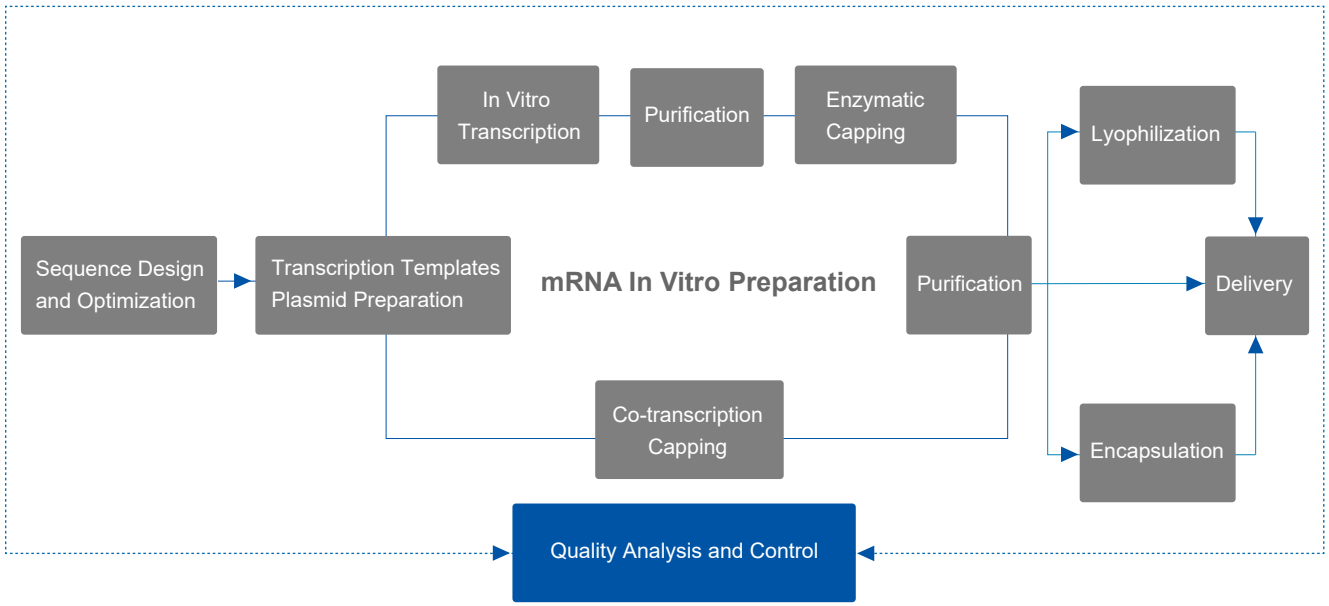
Nanoparticle size

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.

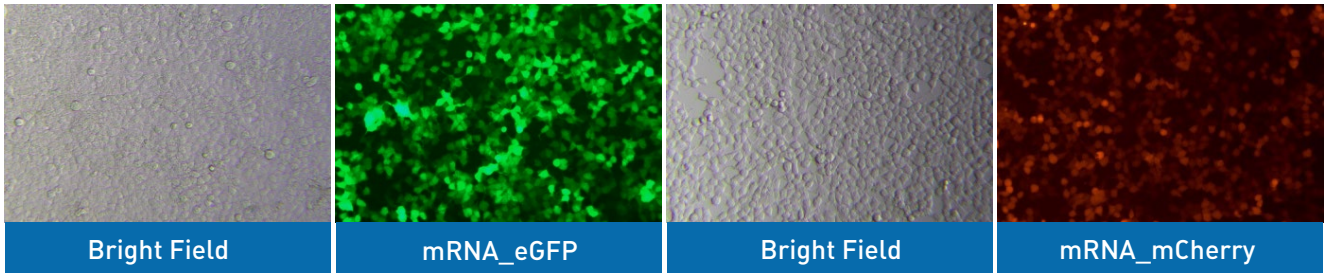
Cell Plating	Sample Preparation	Cell Transfection	Target Protein Detection
Recording cell generations Observation of cell morphology Cell pavement	mRNA + Transfection reagents Or mRNA-LNP	mRNA Transfected to 293T cells	Fluorescent photo shoot Western blot ELISA

Service Details

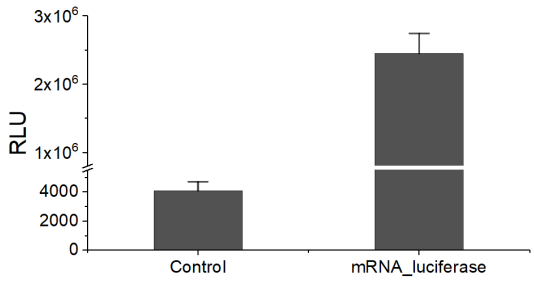
Samples	Test Items	Testing Method	Delivery Period (Days)	Delivery
mRNA expression validation	293T cell evaluation	Cell plating	4	CoAs
		Transient transfection of cells		
		Fluorescence signal observation	1-3	
		Western blot (WB)		
		ELISA		

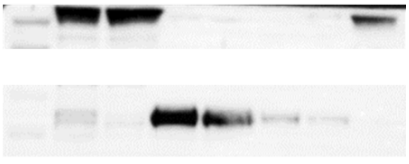
Case Study

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





GAPDH

The full length of the novel coronavirus sprotein

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

CircRNA Innovative Therapeutic

CRO Service Platform

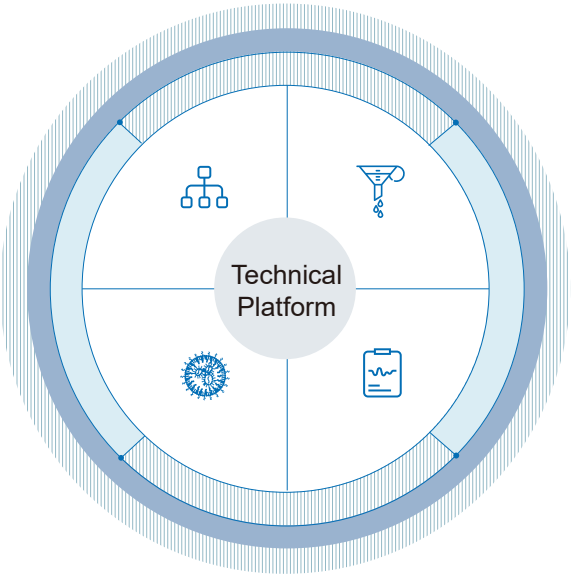
Technical Platform

RNADes

circRNA structure design and optimization Platform

RNASyn

circRNA synthesis and modification platform



RNAPua

circRNA purification platform

RNAQua

circRNA quality analysis and control platform

Yaohai Biopharmaceutical has built a mature CRO service platform for circRNA innovative therapeutics, which can realize efficient preparation and purification of circRNA, and is committed to providing the whole process and in vitro preparation technology service of circRNA with high quality for universities and research institutions.

Platform Features

Structure Design And Optimization Platform

Cutting-edge "PIE" loop-forming technology, efficient intron and exon combination

CDS, IRES optimization design

CircRNA Synthesis And Modification Platform

circRNA template plasmid design and construction

circRNA synthesis solution with a loop formation rate of >80%

CircRNA Purification Platform

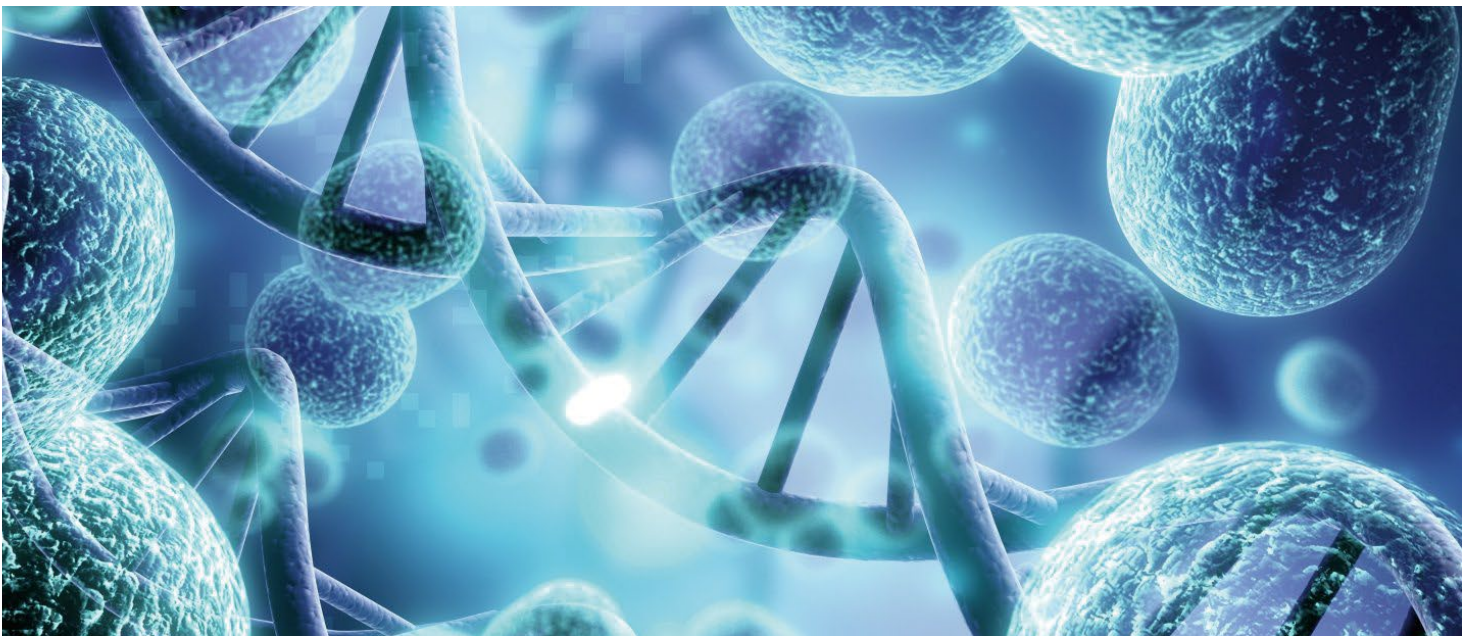
Conventional purification solution of trial grade

Self-developed chromatography column purification process

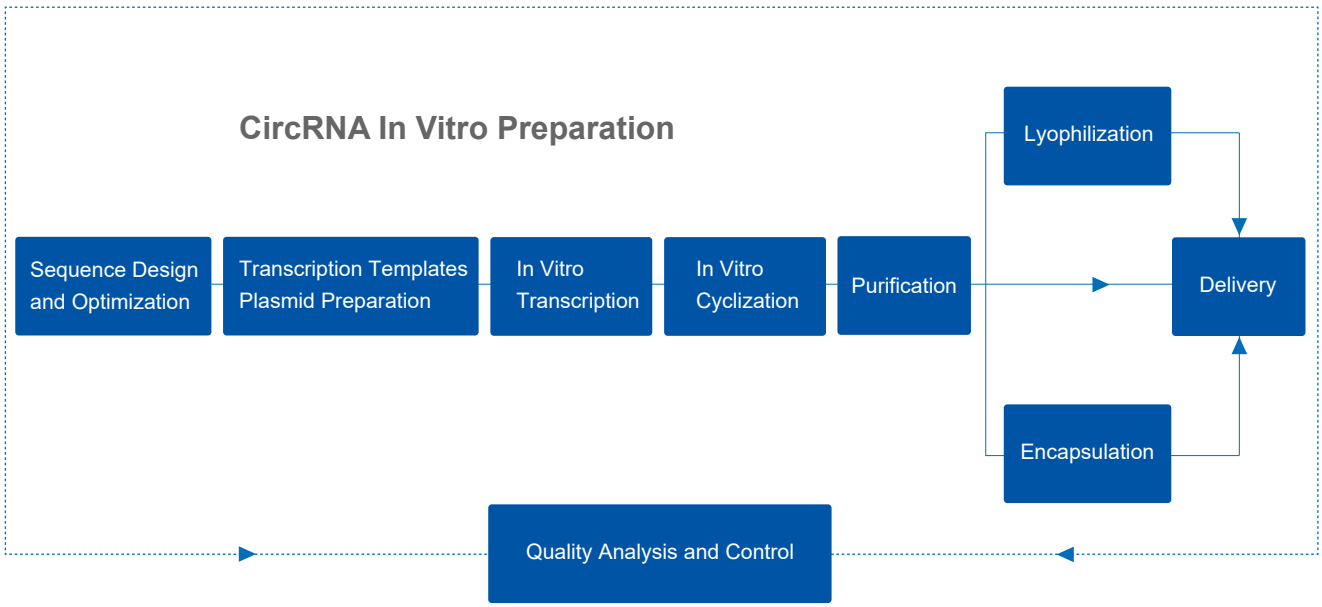
CircRNA Quality Analysis And Control Platform

Multiple purity assays

High-performance loop formation rate assay



Process Development Flow



Service Details

Service items	Optional items	Service Details	Delivery Period (days)	Delivery Content
circRNA sequence design and optimization	Design and optimization of coding sequences	CDS codon optimization	1-3	Sequence file
	Design and optimization of non-coding sequences	Design and optimization of IRES, intron		
circRNA transcription template plasmid service	Plasmid DNA preparation	Gene synthesis	7-10	
		Plasmid amplificationand extraction	4	
		Plasmid linearization and purification		
circRNA in vitro transcription and cyclization	*In vitro* transcription	In vitro transcription (IVT)	2-3	N/A
	In vitro cyclization	RNA cyclization based on PIE system		
		circRNA enrichment		
circRNA purification	Conventional purification solutions	Lithium chloride precipitation	1	circRNA drug substance
		Magnetic bead purification		
	Self-developed purification solutions	Self-developed purification solutions	1-2	
	Solution exchange	Ultrafiltration liquid exchange	1	
circRNA lyophilization	Lyophilization	Pre-freezing	2-3	circRNA lyophilized powder
		Primary sublimation		
		Secondary Sublimation		
circRNA encapsulation	LNP encapsulation	LNP encapsulation	2-3	circRNA-LNP Drug product
		Concentration liquid exchange and filtration		
circRNA quality analysis	circRNA drug substance/ lyophilized powder	Concentration, purity	1	CoAs
		Cyclization rate	2-3	
	circRNA-LNP drug product	Encapsulation rate	1	
		Particle size and distributiondetection		
		Surface charge detection		
circRNA expression validation	293T cell evaluation	Cell plating	4	CoAs
		Transient transfection of cells		
		Fluorescence signal observation	1-3	
		Western blot/ELISA		

Pre-Products Catalog

Product Name	Test Uses	Delivery Form	Product Specification
circRNA_eGFP circRNA_mCherry circRNA_luciferase circRNA_OVA circRNA_IL-2 circRNA_Cas9	• Reference standard • In vitro or in vivo tests	• Lyophilized powder • Drug substance (500 ng/μL)	• 100 μg • 1 mg • 10 mg

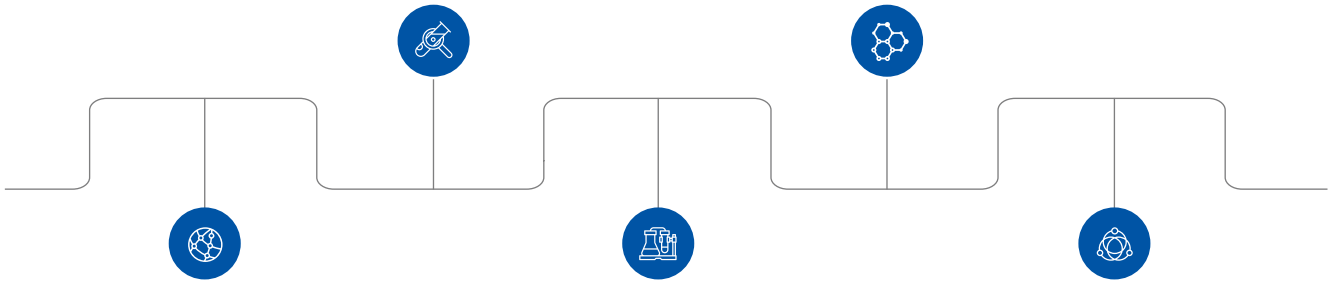
Service Advantages

Process Robustness

Cyclization of RNAs from 50-4000 nt in length;

Stability

Rigorous quality control methods, with successful in vitro expression of products in cells;



All-round

from front-end sequence design to back-end circRNA cyclization, purification, quality control and expression validation;

High Efficiency

With a validated cyclization rate of NLT 80%;

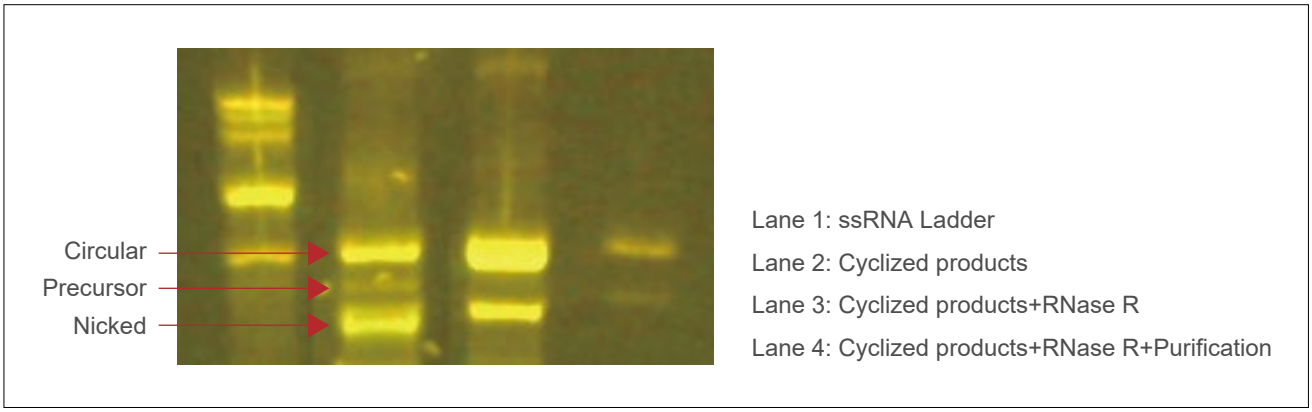
Flexibility

Diversified purification methods meeting different downstream test needs;

Case Study

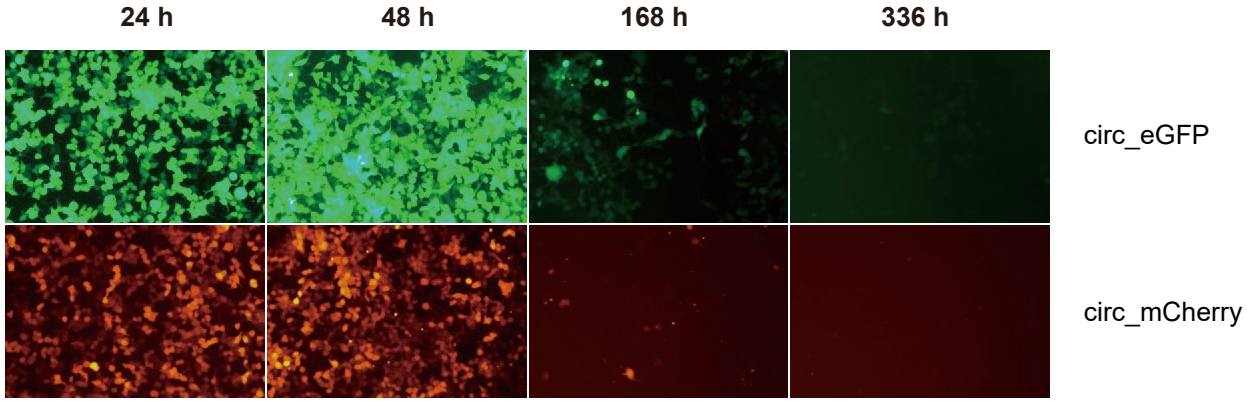
circRNA enrichment and purification

To enrich circRNA, PIE cyclized products are treated with RNase R. The electrophoresis results show that the linear RNA precursors are digested; and after further purification*, most of the nicked circRNA can be removed.* The purification solution is self-developed by YAOHAI circRNA platform.



circRNA expression validation

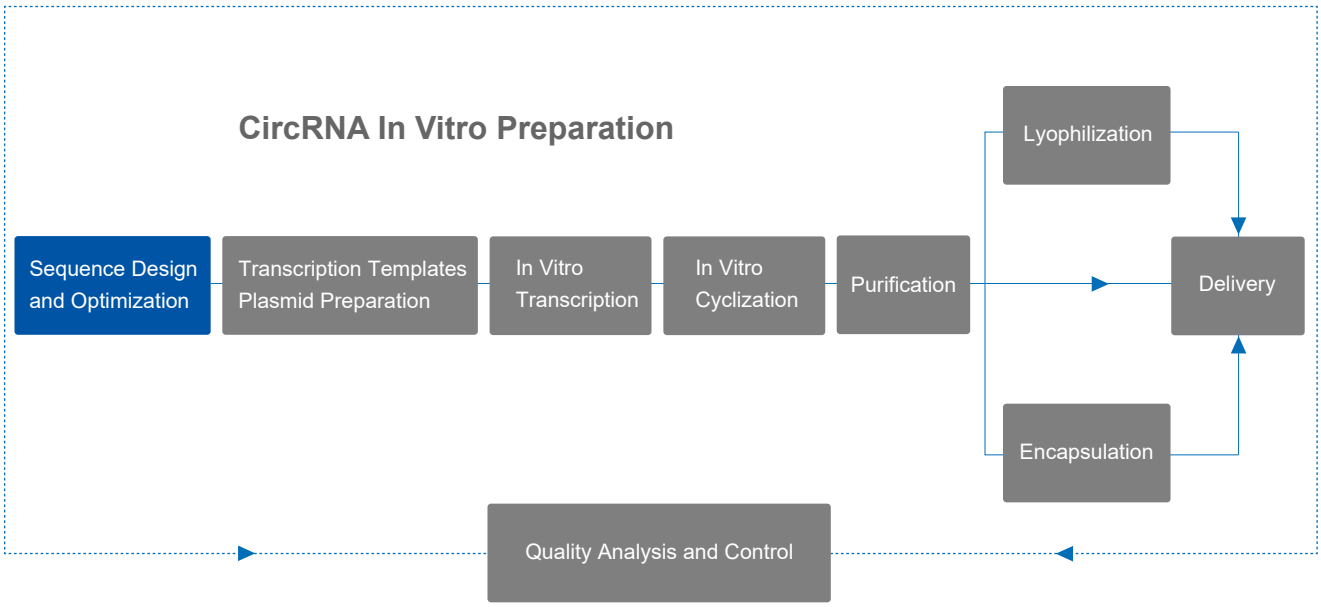
The purified circRNA eGFP are transfected with circRNA Cherry into 293T cells, and fluorescence signal can be observed after 24 h, which will continuously enhanced after 48 h. The fluorescence signal can still be observed after 7 days and 14 days of transfection.



CircRNA

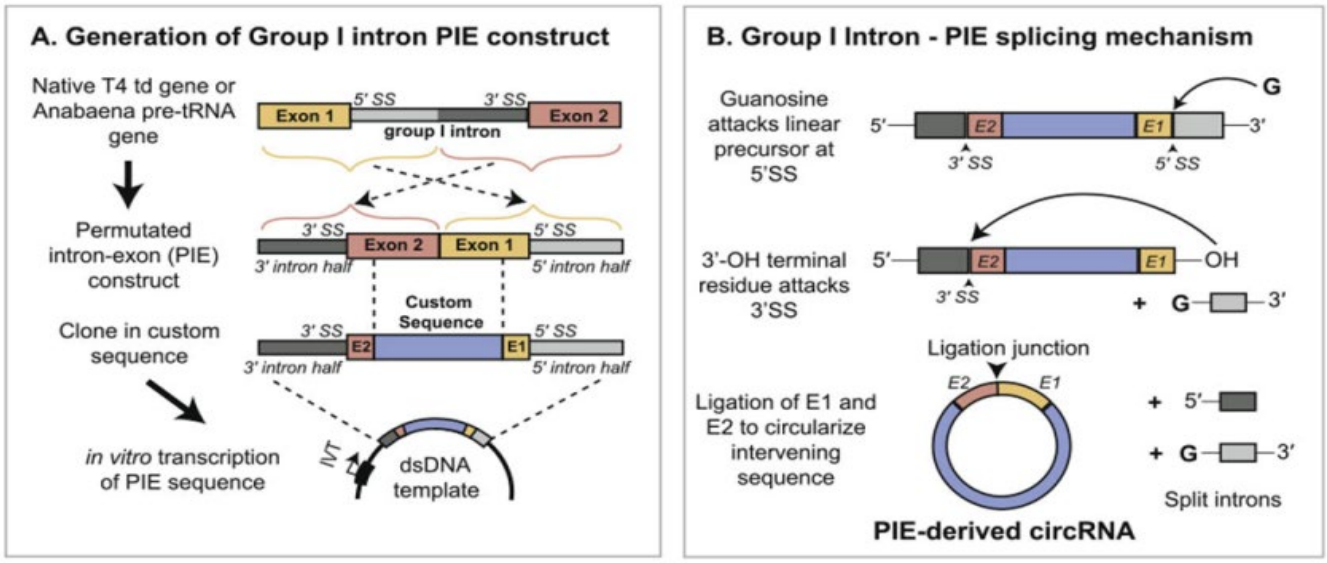
Sequence Design And Optimization Services

Process Development Flow



Yaohai Biopharmaceutical prepares circRNA based on the PIE system (alignment of exons and introns), which relies on the self-splicing function of type I introns to achieve RNA cyclization. The PIE structure is designed using the T4 td gene or fishy tRNA precursor gene, and the arrangement is as follows: The RNA intron and supporting exon fragment are divided into two parts (5' terminal and 3' terminal), where the 5' terminal sequence is transferred to the tail of the target sequence, the 3 ' terminal sequence is inserted into the front of the target sequence, and the target gene sequence is inserted in the middle.

Under GTP catalysis, the PIE structure leads to the cyclization of sequences other than introns. Combined with a reasonable enhancement strategy of cyclization rate, YAOHAI can achieve cyclization of sequences up to 4 kb with a cyclization rate of more than 80%.



The functions and design strategies of each component of circRNA are referenced below

CircRNA Components		Biological Functions	Design Strategy
Two-terminal intron and exon sequences		GTP-catalyzed intron self-splicing for cyclization of sequences outside the intron.	Designed according to the T4 td gene or fishoil tRNA precursor gene.
Coding	IRES	Internal ribosome recognition site that regulates the translation of circRNA.	Screening of IRES sequences from different viral sources, e.g. EMCV, CVB3 sources.
	CDS	Protein-coding regions, sequences coding for antigens, antibodies or other functional proteins.	Codon optimization increases the level of translation; note that certain non-optimal codons may play a role in protein folding.
Non-coding	Non-coding sequences	Target miRNAs or proteins to exert gene or protein regulation.	Targeting specific binding sites for miRNAs or proteins can repeat the sequences of the binding site.

[1] Chen X, Lu Y. Front Bioeng Biotechnol. 2021 Nov 30;9:787881..

Service Details

Service Items	Optional Items	Detailed Steps	Delivery Period (Days)
circRNA sequence design and optimization	Design and optimization of coding sequences	• CDS sequence matching • CDS codon optimization	1
	Design and optimization of non-coding sequences	• Intron and exon sequence design and optimization • Homologous arm sequence design and optimization • Interval sequence design and optimization	1-2

Service Advantages

Optimized PIE cyclization system

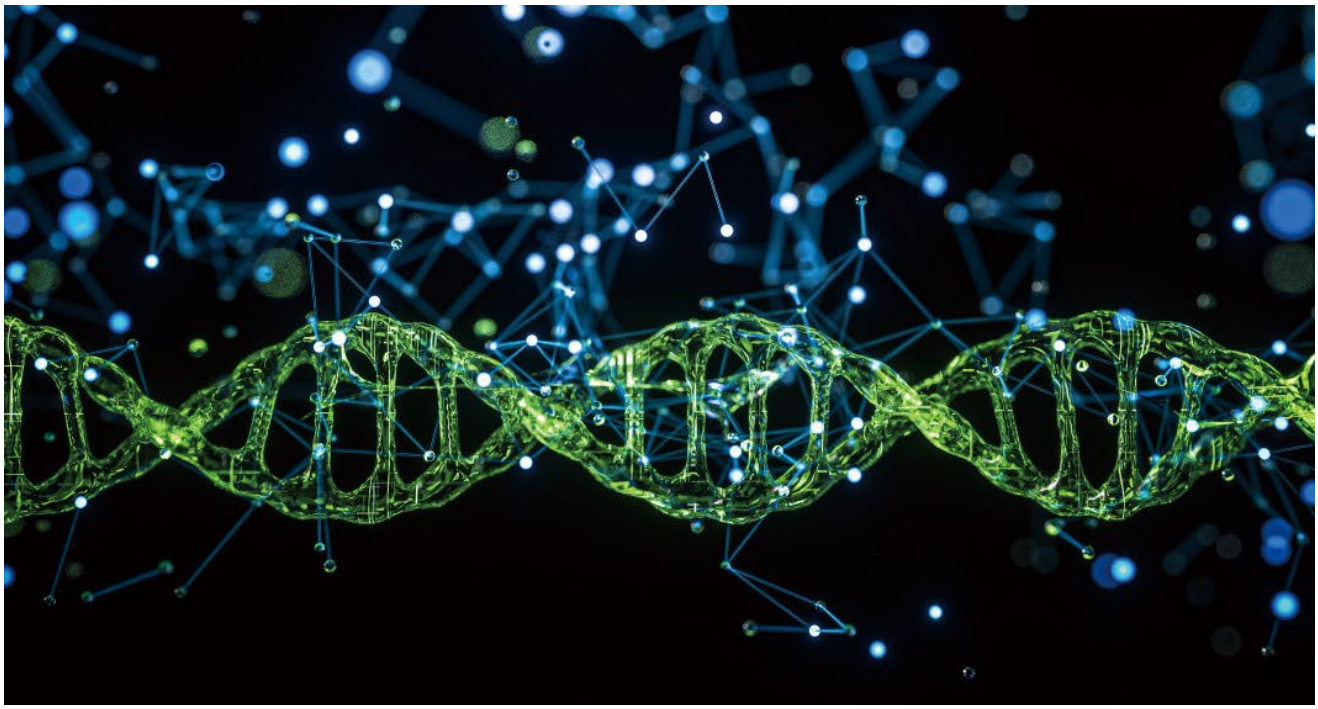
Combined with reasonable optimization strategies to achieve a cyclization rate of NLT 80%;

Cutting-edge CDS optimization team

Cooperation with professional AI algorithm team to complete the optimization of CDS region codons;

Mature and perfect process

circRNA can be achieved with a high loop formation rate, high stability and high translation efficiency.



Case Study

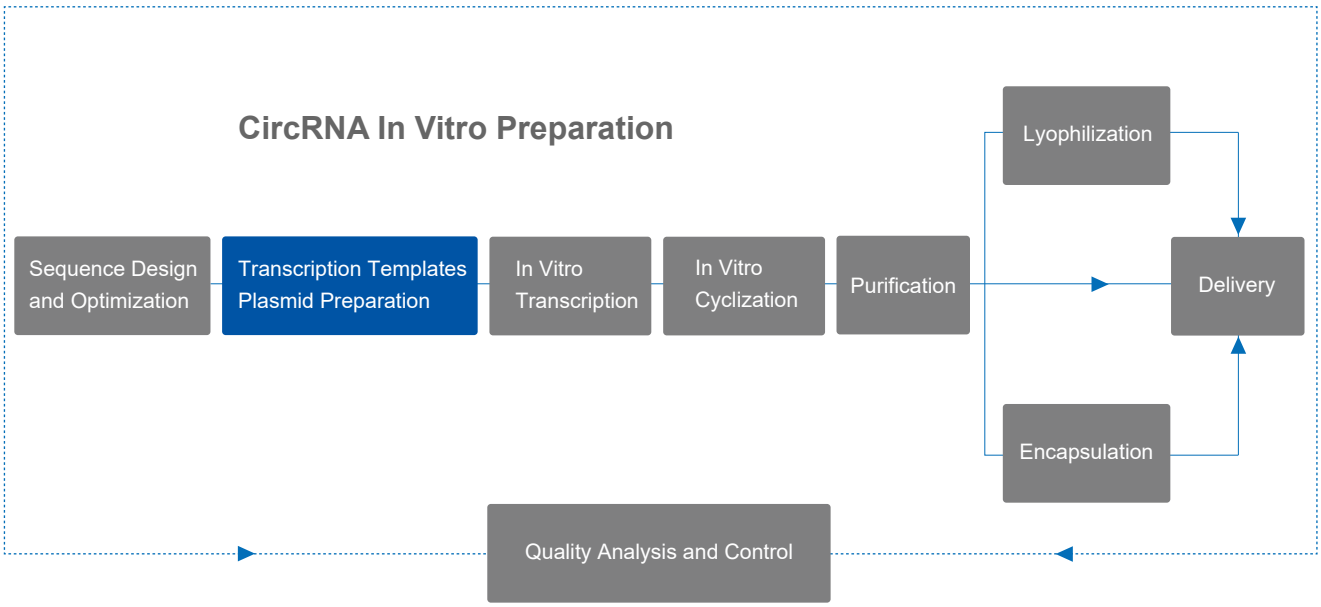
Yaohai Biopharmaceutical launched the control product circRNA_eGFP, which is based on the PIE system to achieve the cyclization of RNA. Using a conventional transfection reagent, circRNA_eGFP is transfected with 293T cells, and eGFP (green) fluorescent signal can be detected after 24h, which will be enhanced after 48h, and the fluorescent signal can still be detected after 7 days and 14 days of transfection.

Sequence design and in vitro expression validation of circRNA_eGFP

CircRNA

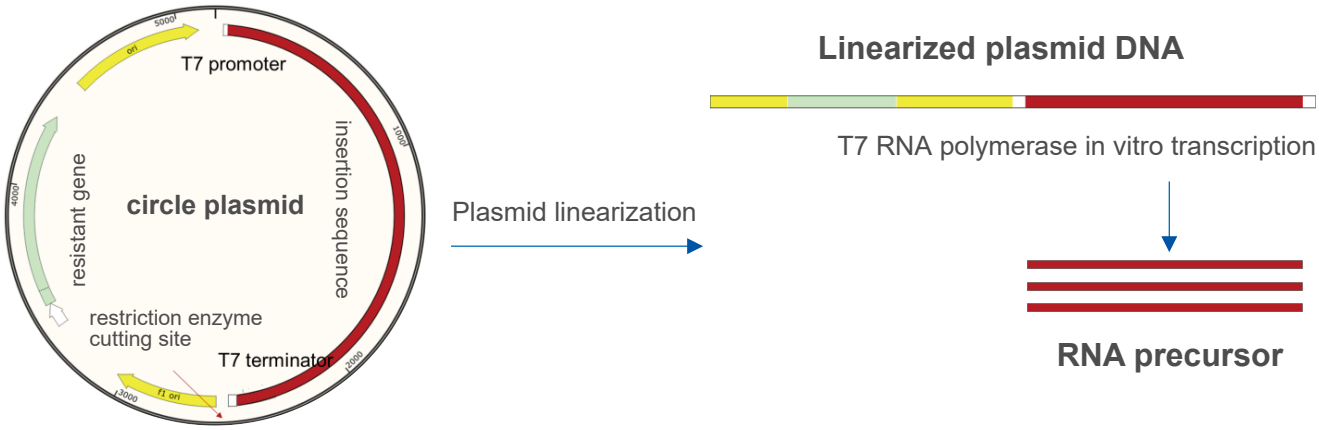
Transcription Template Plasmid Service

Process Development Flow



CircRNA preparation requires linear RNA as precursor material (RNA precursor) for cyclization, where the RNA precursor is usually prepared using linearized plasmid DNA as transcription template and transcribed in vitro with the help of T7 RNA polymerase.

High quality plasmid DNA is essential for downstream in vitro transcription (IVT). Based on the mature plasmid preparation service platform, YAOHAI BIO can provide high purity and standard linearized plasmid DNA preparation service to ensure the integrity of downstream IVT products.



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)	

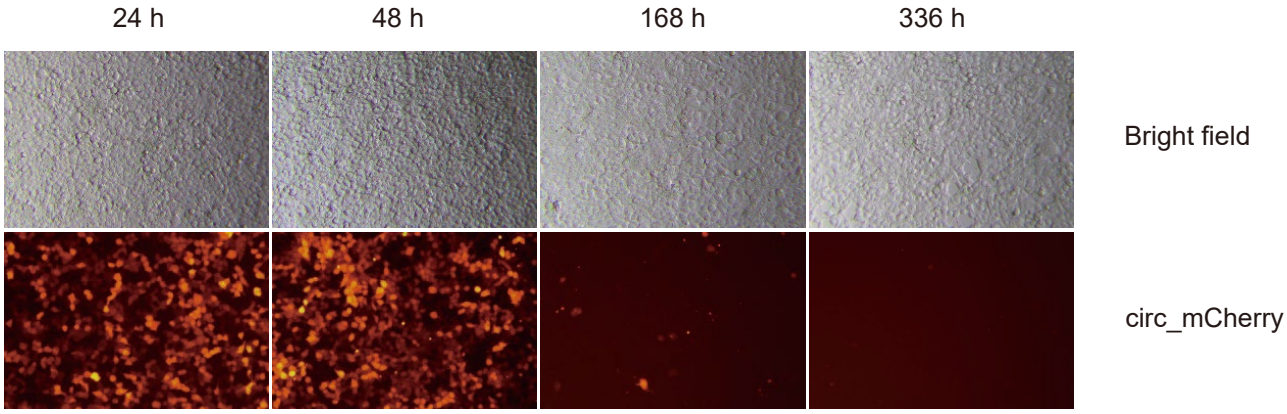
Service Advantages

Freecut Template Plasmid	Flexible selection of linearization methods
High Recovery Rate	Continuous optimization of DNA extraction and purification methods to achieve high recovery rates.
Stringent Quality Control	Stringent process control specifications, with a superhelical conformation rate of plasmid samples for research of >80%.
Mature Platform Process	High standard and high efficiency plasmid preparation and quality control services to meet downstream test needs.

Case Study

Take YAOHAI's pre-product circRNA_mCherry as an example, the superhelix rate of transcription template plasmid samples (research grade) is more than 70%, with a linearization rate of close to 100%.

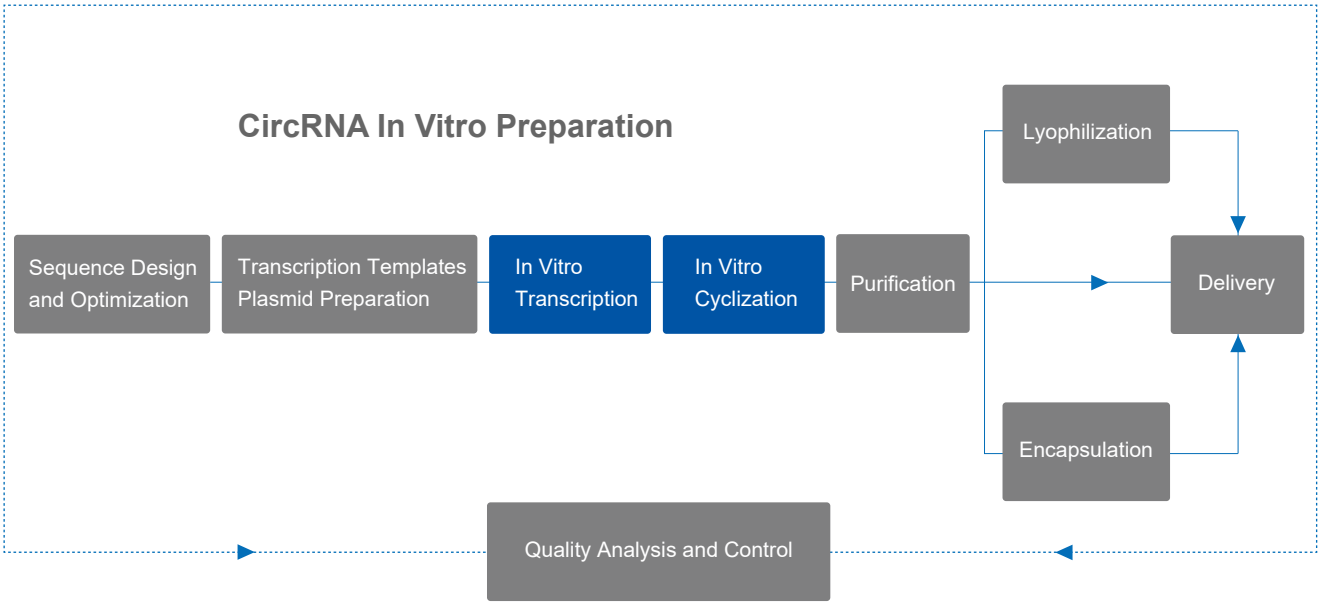
CircRNA_mCherry is prepared with linearized plasmid as template and transfected into 293T cells. High level of fluorescence expression (red fluorescence) is detected after 24 hours of transfection, which will be continuously enhanced after 48 hours, and can still be detected after 7 and 14 days of transfection. mCherry protein is stably and efficiently expressed, and the transcriptional template purity can meet the requirement of circRNA drug product with high quality.



In vitro expression validation of circRNA-mCherry

CircRNA

in vitro transcription and in vitro cyclization services



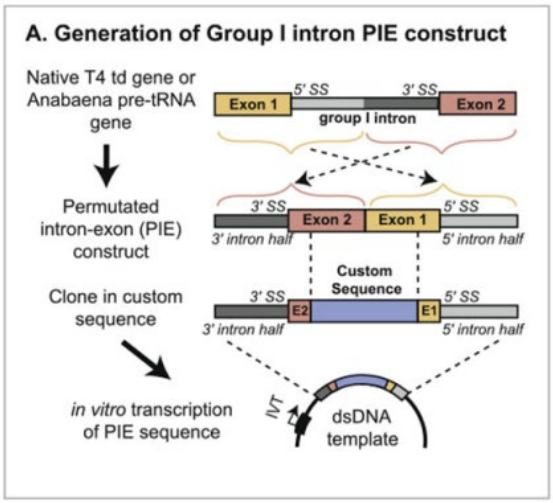


Figure: PIE sequence diagram

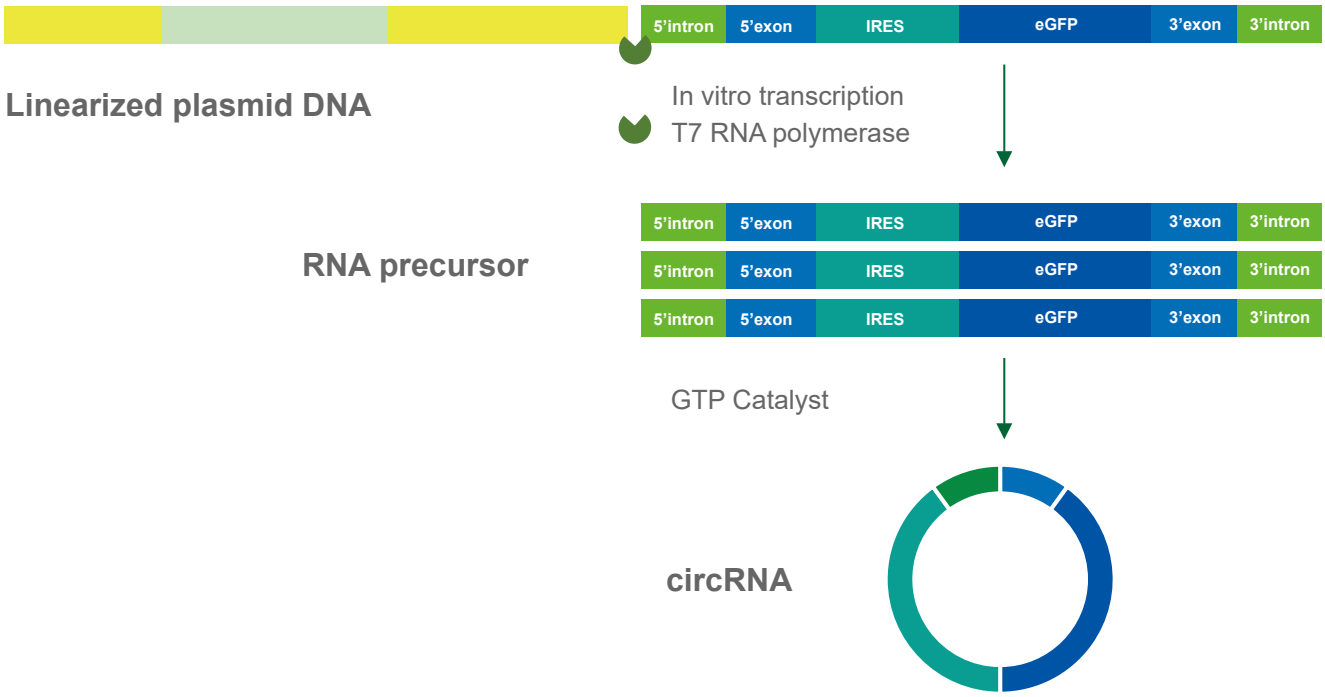
Regarding the batch preparation of RNA precursor, the commonly used method is in vitro transcription (IVT, *In Vitro* Transcription). IVT reaction uses linearized plasmid DNA containing T7 promoter as a template and synthesizes RNA precursor with nucleoside triphosphates (NTPs) as a substrate in the presence of T7 RNA polymerase.

In vitro cyclization methods include chemical ligation, enzymatic ligation and PIE nuclease method. Chemical ligation and enzymatic ligation are suitable for the cyclization of shorter RNAs, and the cyclization rate decreases significantly for fragments larger than 100 nt, while the nuclease method based on the PIE system can achieve cyclization of 5 kb sequences.

Under GTP catalysis, the PIE structure undergoes cyclization of extra-intron sequences. Combined with a reasonable enhancement strategy of cyclization rate, YAOHAI can achieve cyclization of up to 3 kb sequences with a cyclization rate of more than 80%.

The in vitro cyclization reaction of RNA flows as follows:

RNA precursor is synthesized by in vitro transcription, and the PIE component completes self-splicing under GTP catalysis to form circRNA.



Service Details

Service Items	Detail Steps	Delivery Period (Days)
circRNA in vitro transcription and in vitro cyclization	Reaction system confirmation	1-2
	In vitro transcription and cyclization reactions	
	RNase R enrichment	
Conditions optimization	Reaction system design and optimization	2-5

Service Advantages

Rigorous test design and optimization

Up to 4 kb RNA cyclization can be achieved.

High cyclization efficiency

A cyclization rate of more than 80% can be achieved through a rational sequence optimization strategy.

Stringent enzyme control specification

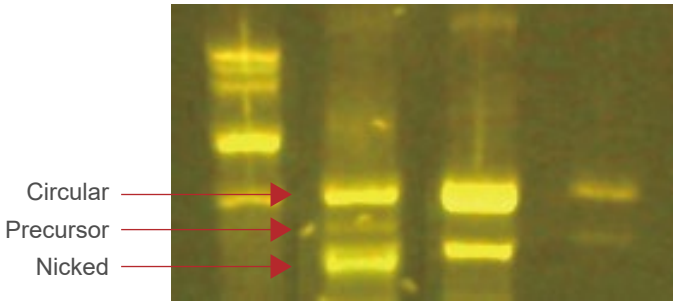
Stringent enzyme control on test environment and consumables to effectively prevent RNA degradation.

Case Study

In vitro cyclization and enrichment of circRNA

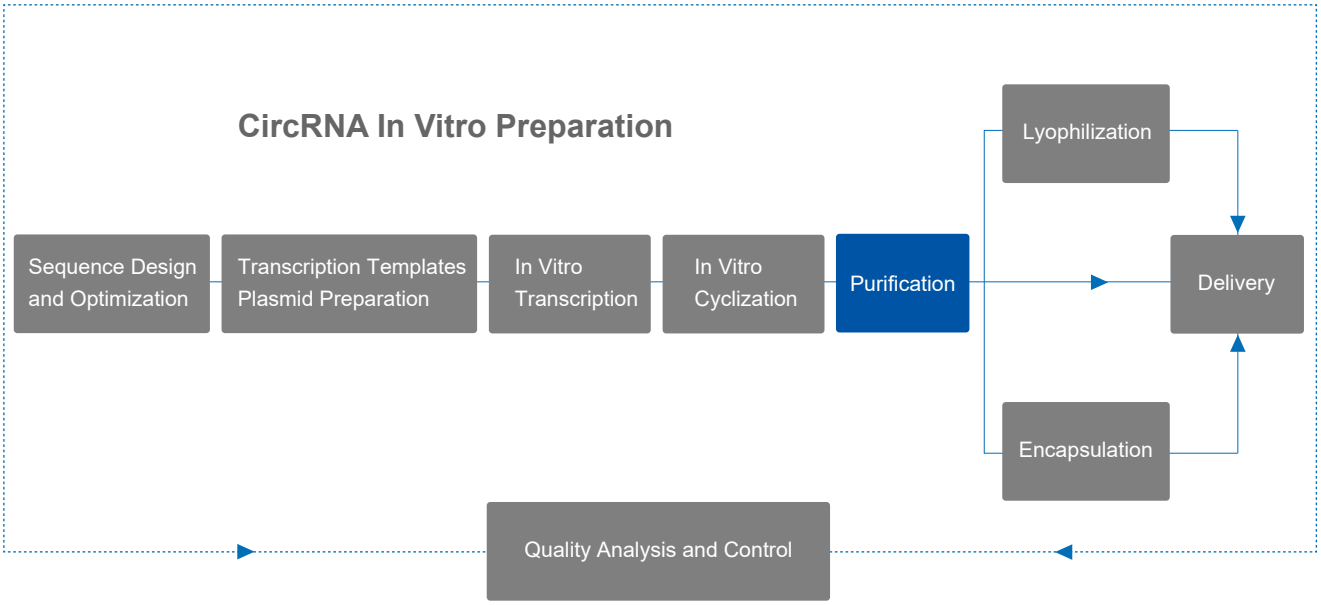
Based on the PIE system, Yaohai Biopharmaceutical has optimized the sequence of circRNA, with a cyclization rate of more than 80% by agarose gel electrophoresis.

Using RNase R to enrich circRNA, E-gel electrophoresis results show that the linear RNA precursor is digested, and after further purification*, most of the nicked circRNA can be removed.* The purification solution is self-developed by the circRNA platform of Yaohai Biopharmaceutical.



Lane 1: ssRNA Ladder
Lane 2: Cyclized products
Lane 3: Cyclized products +RNase R
Lane 4: Cyclized products +RNase R+ Purify

CircRNA
Purification Services



The products obtained by in vitro cyclization and RNase R enrichment require to be further removed from IVT, unconsumed substrates in cyclization reaction, reaction by-products and nicked RNA to meet the downstream test requirements.

Yaohai Biopharmaceutical has mature LiCl precipitation, magnetic bead purification, self-developed purification and other diversified purification solutions to effectively remove various impurities and prepare high purity circRNA.

LiCl precipitation method

Simplified purification solution for small amounts of circRNA for cell transfection and part of animal experiments.

Magnetic bead purification method

Small amount of circRNA purification solution for cell transfection, and part of animal experiments.

Yaohai purification solution

Meeting downstream application scenarios with higher quality requirements;

Stable and scalable purification process to meet downstream GMP production requirements ☆ ☆ ☆ ☆ ☆

Service Details

Service Items	Optional Items	Detailed steps	Delivery Period (Days)	Delivery
circRNA purification	Conventional purification solution	Lithium chloride precipitation	1	circRNA drug substance
		Magnetic bead purification		
	Purification solution with high purity	Yaohai purification solution	1-2	
	Solution substitution	Ultrafiltration liquid exchange	1	
circRNA quality control	Concentration determination	Ultraviolet spectrophotometry (UV)	0.5	CoAs
	Purity testing	Agarose gel electrophoresis (AGE/E-gel)		
		HPLC-Optional	1	

Service Advantages

A variety of optional purification options

to meet different downstream application scenarios;.

High purity

circRNA with a purity of more than 90%;

Stringent enzyme contr ol specification

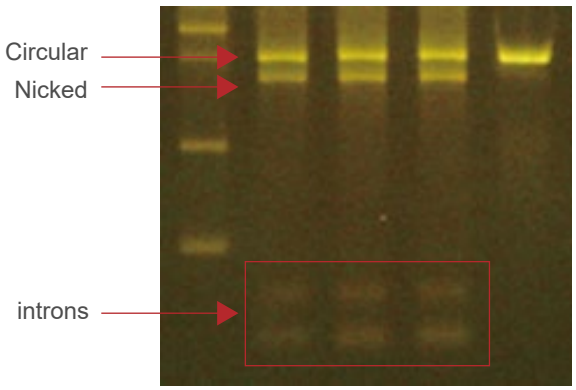
Stringent enzyme control on the test environment and consumables to effectively prevent the degradation of circRNA.

Case Study

Yaohai Biopharmaceutical has established a mature circRNA purification solution, which can effectively remove various process-related impurities.

The circular RNA products purified by the conventional solution are still obviously mixed with nicked RNA and introns.

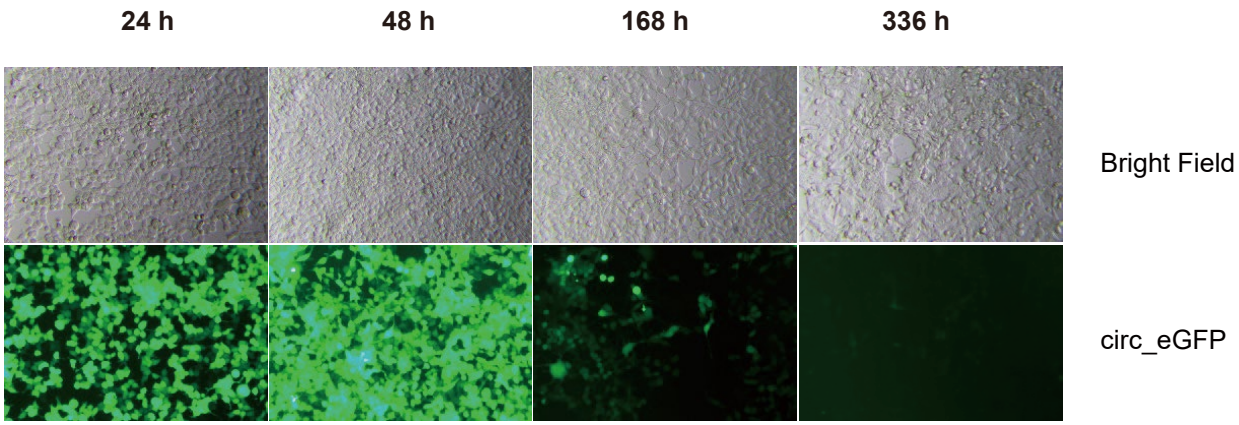
After the purification solution developed by Yaohai Biopharmaceutical, various linear RNA impurities, such as nicked RNA and introns, can be successfully removed.



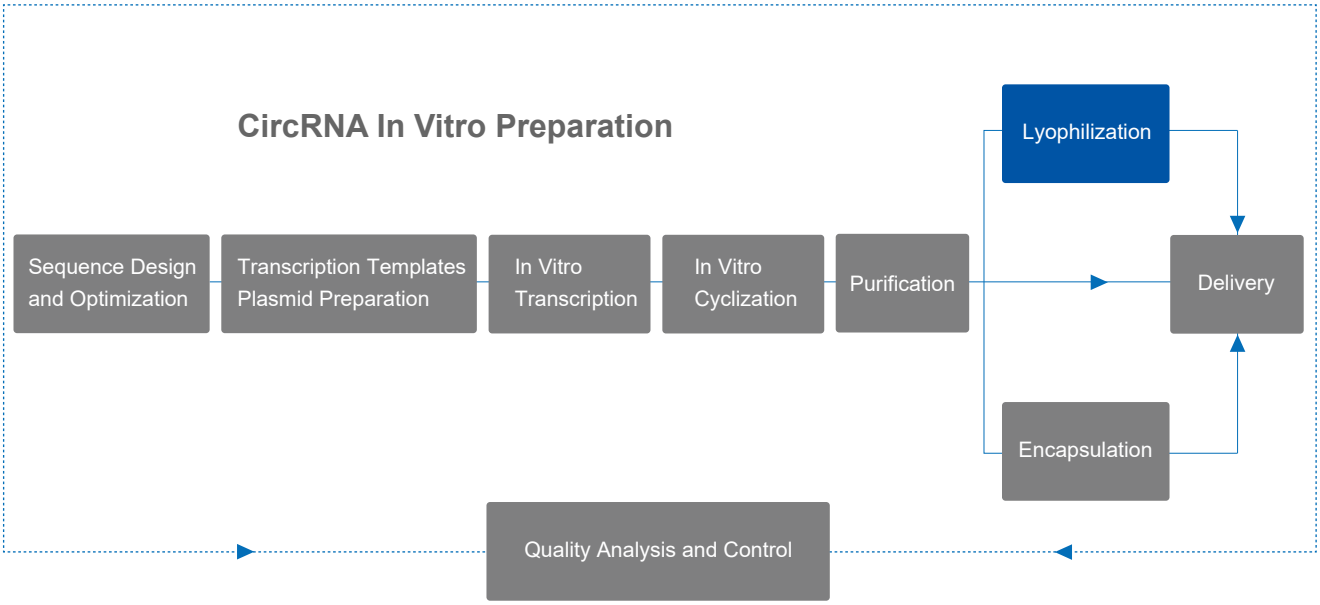
Lane 1: ssRNA Ladder
Lane 2: Cyclized products
Lane 3: Cyclized product + conventional purification protocol 1
Lane 4: Cyclized product + conventional purification protocol 2
Lane 5: Cyclized product + YAOHAI's own purification solution

circRNA expression validation

Using a conventional liposome, purified circRNA_eGFP is transfected into 293T cells, and fluorescence signal is observed after 24 h and continued to be enhanced at 48 h. Fluorescence signal can still be detected after 7 and 14 days of transfection.



CircRNA
Lyophilization Service



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

Sample Dispensing » **Pre-Freezing** » **Primary sublimation** » **Secondary Sublimation**

Service Details

Service Items	Optional Items	Detailed steps	Delivery Period (Days)	Delivery
circRNA lyophilization	Sample dispensing	Dispensing	2-3	circRNA lyophilized powder
	Lyophilization	Pre-freezing		
		Primary sublimation		
		Secondary sublimation		
circRNA quality control	Reconstitution of lyophilized powder	Reconstitution/resuspension	-	CoAs
	Solubility of lyophilized powder	Appearance inspection	-	
	Concentration determination	Ultraviolet spectrophotometry (UV)	0.5	
	Integrity and purity testing	Agarose gel electrophoresis (AGE/E-gel)		
		HPLC-optional	1	

Service Advantages

Mature lyophilization process

The quality indicators of samples before and after lyophilization are consistent, with good reproducible results.

Homogeneous quality properties

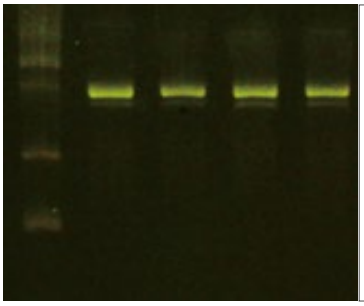
circRNA samples before and after lyophilization successfully express the target protein.

High stability

circRNA lyophilized powder is easy to store and transport.

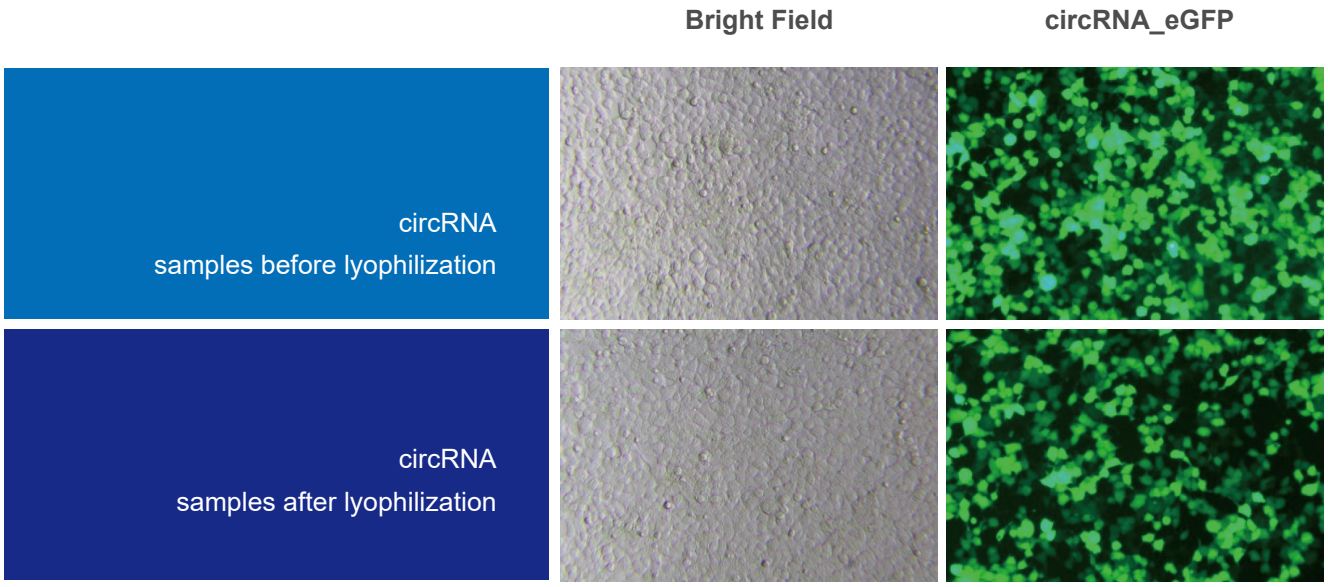
Case Study

E-gel electrophoresis assay is performed for circRNA before lyophilization and after reconstituted lyophilized powder to analyze the integrity and purity, respectively. The results show that there is no significant difference between the circRNA bands before and after lyophilization, and the test results after lyophilization show good reproducible.



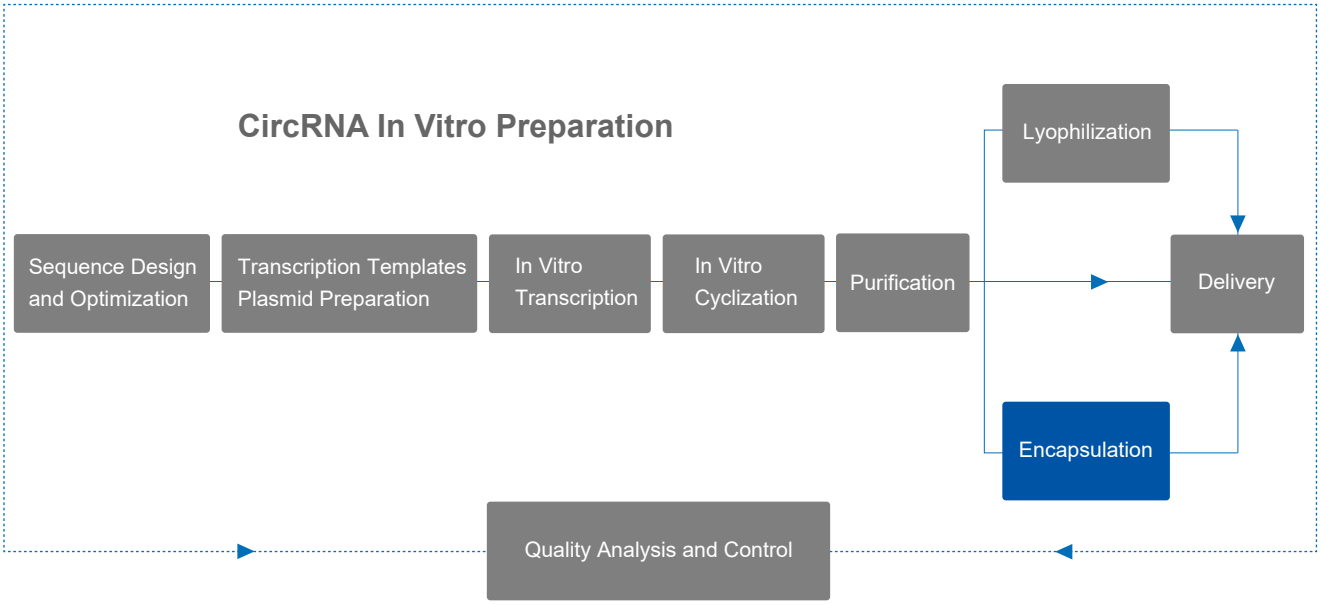
Lane 1: ssRNA Ladder
Lane 2: circRNA before lyophilization
Lane 3: circRNA after lyophilization
Lane 4: circRNA after lyophilization
Lane 5: circRNA after lyophilization

The 293T cell evaluation of circRNA samples before and after lyophilization show that strong fluorescent signals aer observed in the pre-product circRNA_eGFP samples before and after lyophilization, and the target protein is expressed efficiently *in vitro*.



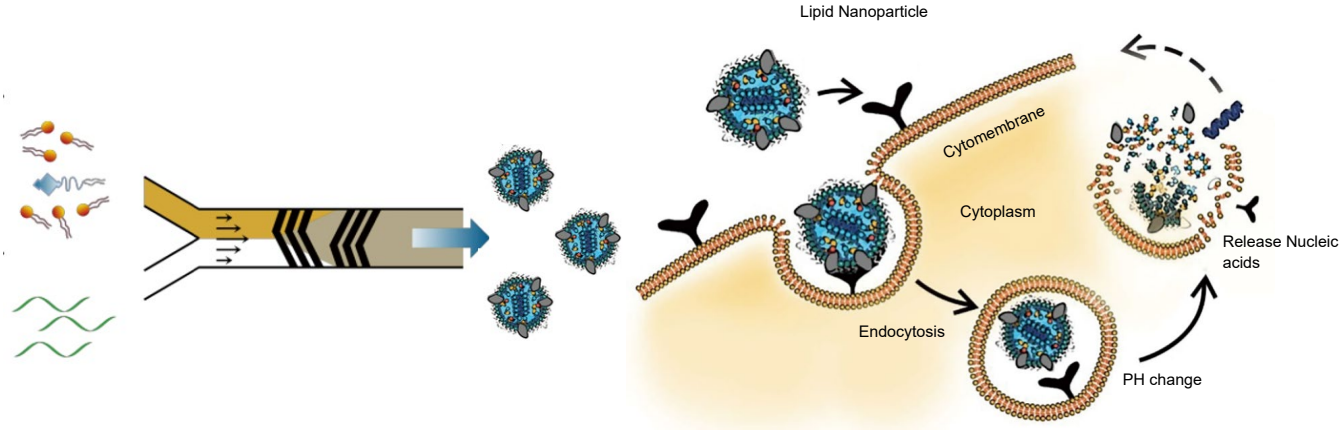
CircRNA-LNP

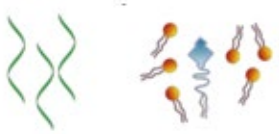
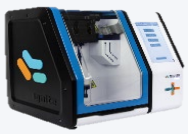
Encapsulation Service



The basis of encapsulation is the design and development of the delivery system. A well-designed delivery system allows circRNA molecules to enter the body without being degraded by RNA enzymes, and then be effectively delivered to the target site, cross the cell membrane, and be released intracellularly. Lipid nanoparticles (LNPs) are the best delivery system available, with advantages in 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 entering the cell, the acidic environment of the intracellular body protonates and positively charges the head of the ionized lipid, thus fusing with the inner membrane of the intracellular body and releasing the target nucleic acid into the cell for action.

YAOHAI circRNA service continues to improve, and can provide circRNA-LNP encapsulation service to optimize relevant critical process parameters and improve the consistency and reproducibility of circRNA drug production.



Pre-treatment of material and liquid	Microfluidics	Tangential Flow Filtration	Sterilization filtration
			
Two drug substances are prepared: one for circRNA in aqueous buffer and one for lipids dissolved in ethanol.	Rapid mixing of lipid, circRNA two-phase solutions using microfluidic devices, resulting in uniform LNP and high efficiency encapsulation.	The drug substance is concentrated to the target concentration using a tangential flow technique and the buffer is replaced with a neutral storage solution to remove unencapsulated circRNA, excess lipids and acetic acid.	Comply with sterility regulatory requirements, select terminal sterilizing filtration system and pass the validation of bacterial challenge test.

Service Details

Service Items	Detailed Steps	Delivery Period (Days)	Delivery
circRNA-LNP encapsulation	**Material and liquid pretreatment**	2	circRNA-LNP drug product
	Microfluidic device mixing		
	Tangential flow filtration	1	
	Sterilizing filtration		
circRNA quality control	Encapsulation rate	1	CoAs
	Particle size and distribution detection		
	Surface charge detection		

Service Advantages

Formulation screening of drug product

Fast synthesis, high R&D efficiency and pre-optimized solutions;

High encapsulation rate

Encapsulation effect of up to 90% or more;

Moderate particle size

The size of lipid nanoparticles can be controlled by changing the fluid injection rate and ratio.

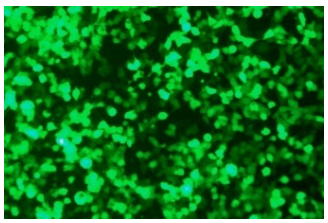
Efficient expression

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

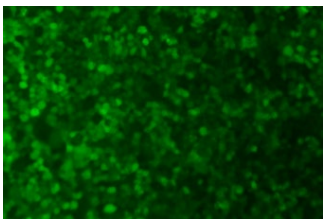
Case Study

LNP-circRNA_eGFP samples are prepared with different levels (1ug-2ug-4ug) and directly transfected 293T cells to verify whether they can express the target protein. After transfection for 48h, a clear fluorescent signal can be observed, and there is a dose-escalation effect of fluorescence intensity.

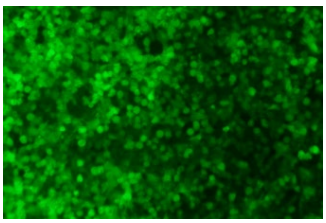
[Note: Lipo-circ_eGFP is liposome + unencapsulated circRNA_eGFP, as transfection control]



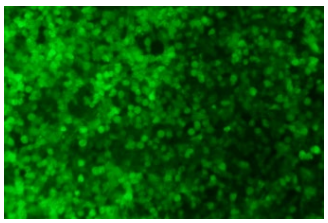
Lipo-circ_eGFP



LNP-circ_eGFP-1ug



LNP-circ_eGFP-2ug



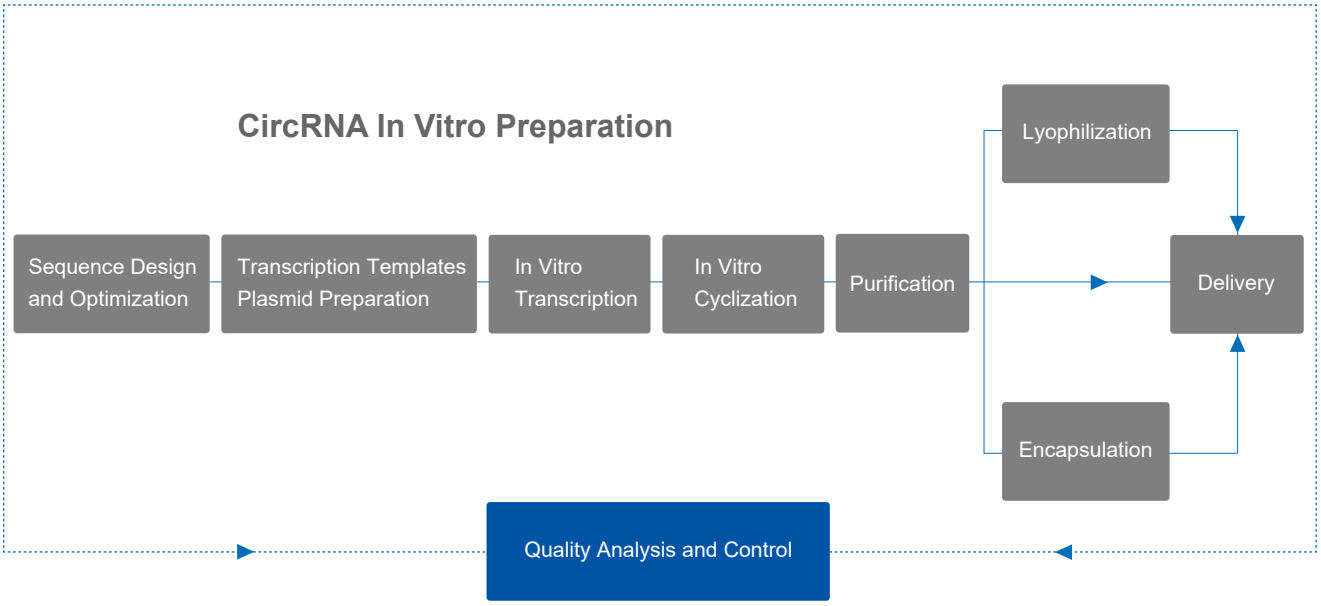
LNP-circ_eGFP-4ug

Other Services

circRNA Sequence Design And Optimization	Transcription Template Plasmid Preparation	In Vitro Transcription Of Linear RNA
circRNA *in vitro* cyclization	circRNA purification	circRNA-LNP encapsulation
circRNA lyophilization	cricRNA quality analysis and control	*In vitro* expression validation of circRNA

CircRNA
Quality Analysis and Control Services

Process Development Flow



Combined with the requirements of mRNA quality analysis guidelines, YAOHAI has developed specifications for circRNA drug product and can provide quality analysis services for cyclic and linearized plasmid templates, circRNA drug substance and finished product of circRNA-LNP, and the service details are shown below:

Samples	Test Items	Testing Method	Delivery Period (Days)	Delivery Content
Cyclic plasmid DNA	Concentration and purity	Ultraviolet spectrophotometry (UV)	N/A	CoAs
	Super Spiral rate	Agarose gel electrophoresis (AGE)	0.5	
		Capillary electrophoresis (CE)	1	
Linearized plasmid DNA	Concentration and purity	Ultraviolet spectrophotometry (UV)	N/A	
	Linearization ratio	Agarose gel electrophoresis	0.5	
		Capillary electrophoresis (CE)	1	
circRNA drug substance	Concentration	Ultraviolet spectrophotometry (UV)	N/A	
	Purity	Agarose gel electrophoresis (AGE/E-gel)	0.5	
		HPLC	1	
	Cyclization rate	HPLC、qPCR	1-2	
circRNA-LNP drug product	Encapsulation rate	RiboGreen Method	1	
	Particle size and distribution	Particle size meter	1	
	Surface charge	Particle size meter	1	
Validation of circRNA expression	293T cell evaluation	Cell transfection	4	
		Fluorescence observation	1-3	
		Western Blot/ELISA		

CircRNA in vitro expression validation service

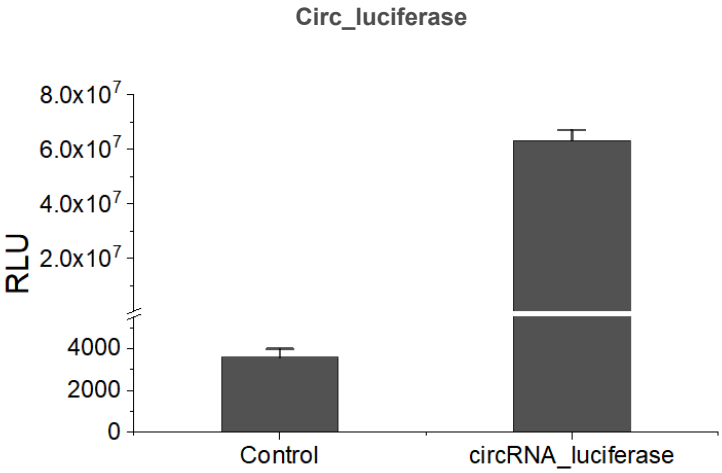
In addition to circRNA-related quality attributes, YAOHAI can provide customers with circRNA cell transfection and target protein specificity assay services based on its well-established cell culture platform. By transiently transfecting 293T cells with circRNA, we can confirm whether circRNA can successfully express the target protein in cells in vitro. The range of samples available for testing includes circRNA drug substance and finished product of circRNA-LNP.

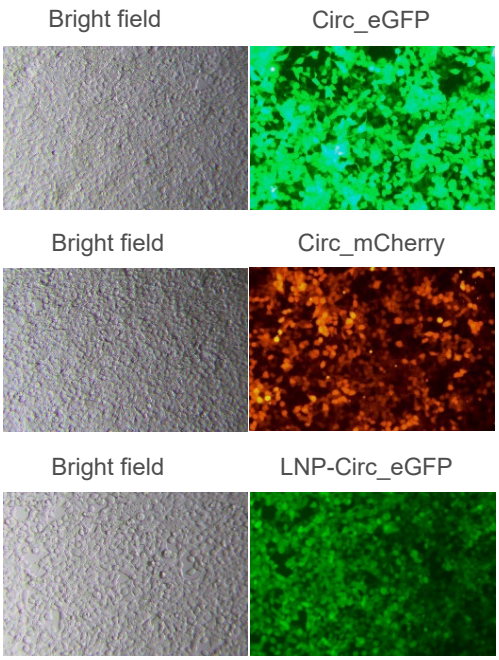
Cell pavement	Sample Preparation	Cell transfection	Target protein detection
Recording cell generations Observation of cell morphology Cell pavement	circRNA＋Transfection reagents or circRNA-LNP	circRNA transfection To293Tcells	Fluorescent photo shoot Western blot ELISA

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

Case Study

Yaohai Biopharmaceutical has built a mature cell transfection platform, and the transfection samples include circRNA drug substance and finished product of circRNA-LNP; based on fluorescent signal, and enzyme-substrate reaction, strong specific signal of target protein can be detected.

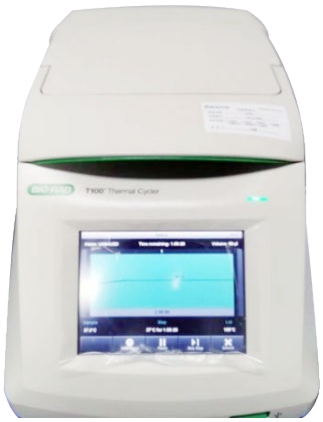




CircRNA Platform

Equipment

Fluorescence Microscope



Bio-Rad PCR Instrument

Thermo Full Wavelength Enzyme Labeler

Bio-Rad Gel Imagers

Thermo qPCR instrument

Waters HPLC

PNI Microfluidic Nanoparticle Preparation System

SCIEX Capillary Electrophoresis Instrument

Cytiva AKTA Purification System

Nano-antibodies CRO Service Platform

Yaohai Bio-Pharma's nanobodies CRO service platform is dedicated to provide customers with one-stop nanobodies R&D and production services from strain construction, multifunctional nanobodies expression and purification to large-scale production, which are efficient and flexible to meet customers' different experimental or project needs.

Full ecological recombinant expression system

E.coli prokaryotic expression system
Yeast eukaryotic expression system
Mammalian cell expression system

Diversified nanobodies types

Monodomain nanobodies
Bivalent nanobodies
Multivalent nanobodies

Well-developed and perfect purification platform

Complete purification platforms
Combination purification methods
Highly efficient and flexible

From μg to kg

Nanobodies with a high expression of 10g/L
Production at a scale of 7-2000L

Full ecological recombinant expression system

At present, Yaohai Bio-Pharma has established a full ecological recombinant expression system for nanobodies. The existing expression systems include: *E.coli* prokaryotic expression system, yeast expression system (*pichia pastoris*), and mammalian cell expression system, and are skilled in using a variety of expression host strains to provide nanobodies with high quality according to the actual needs of customers.

E.coli prokaryotic expression system

- Development experiences of 20+ products
- Flexible selection of different *E. coli* hosts
- Efficient selection of different expression vectors

Yeast expression system *(pichia pastoris)*

- Development experiences of 20+ products
- Flexible selection of different *pichia pastoris* hosts
- PAOX1 methanol induced expression system
- PGAP constitutive expression system

Mammalian cell expression system

- Rich experience in nanobodies development of 5+ products
- Transient expression nanobodies
- Stable transformation strain expression nanobodies

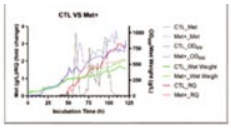
Nano-antibodies Expression CRO Services

The customer provides the gene sequence (or amino acid sequence) of the nanobodies, selects the expression host cells, and Yaohai Bio-Pharma provides one-stop gene synthesis to nanobodies expression, purification and production of a full range of customized nanobodies services.

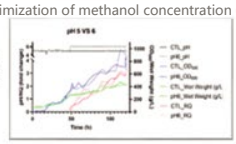
Steps	Service content	Period	Delivery
Construction of nanobodies-expressed engineering strains (selection from *B. aeruginosa*, *E. coli* or mammalian cells)	*E. coli* expression system (cytoplasmic or periplasmic space expression) *Pichia pastoris* expression systems (either methanol-induced expression system PAOX1 or constitutive expression system PGAP) Mammalian cell expression system (nanobodies expression by transient or stable transfection)	1 week - 2 weeks for *Escherichia coli* (excluding gene synthesis period) 2 weeks-3 weeks for *Pichia pastoris* 1 week - 2 weeks for transient mammalian cells, and about 2 months for sstable transformation strains	Purification of the obtained nanobodies, purification antibody assay report SDS-PAGE, SEC-HPLC (optional), RP-HPLC (optional) and CE-SDS (optional) purity analysis methods
Lab scale expression purification (labeling)	For the nanobodies expression of the constructed engineering strains, purify the protein for an expression volume of 1 L (labeling is recommended).	2 weeks - 3 weeks	
Large-scale expression purification	Large-scale fermentation of nanobodies samples, along with the expression purification of the fermented nanobodies.	5 weeks - 8 weeks	

Production process development » **Large-scale production** » **Purification system** » **Quality analysis system**

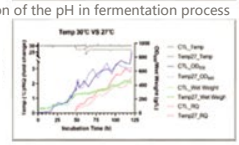
- To achieve the optimal fermentation conditions by single-factor optimization of methanol concentration and fermentation temperature and pH in the fermenter
- To achieve higher yields of expressed nanobodies fermentation liquid by optimizing the combination of optimal conditions for nanobodies fermentation.
- Rich experience in purification of nanobodies
- Flexible combination of various purification methods, such as affinity chromatography, ion chromatography, hydrophobic chromatography, etc.
- Quality analysis system assurance
- Diversified quality analysis methods
- Rich experience and purity assurance



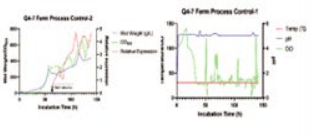
Optimization of methanol concentration



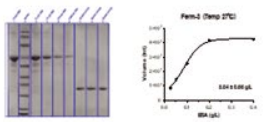
Optimization of the pH in fermentation process



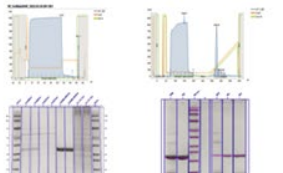
Optimization of the temperature in fermentation process



Fermentation process optimization

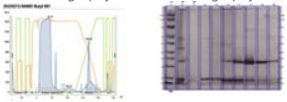


Nanobodies fermentation yield



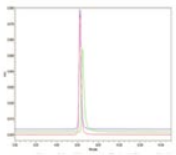
Affinity chromatography

Ion exchange chromatography

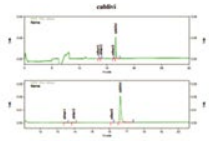


Hydrophobic chromatography

SDS-PAGE gel analysis



SEC-HPLC analysis

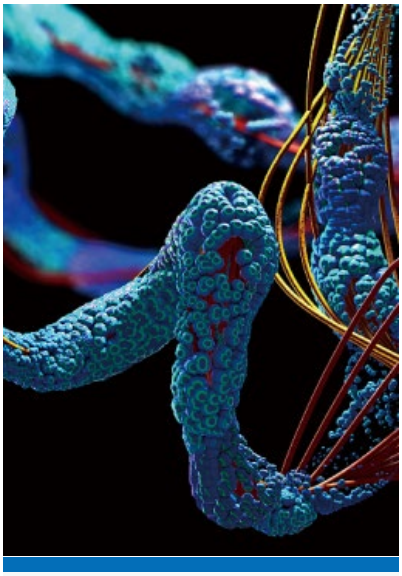


CE-SDS analysis

Yaohabio 耀海生物

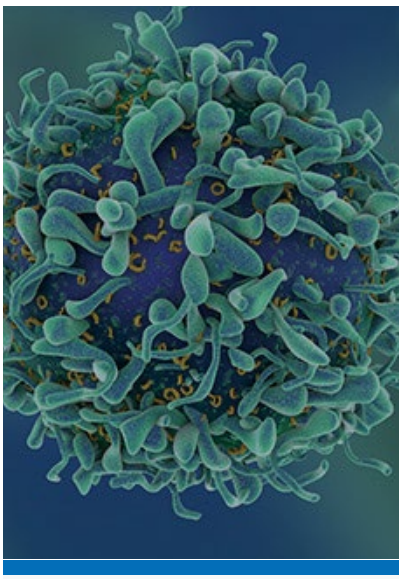
Yaohai Bio-Pharma
CDMO SERVICES

Yaohai Bio-Pharma, a CDMO services provider focusing on microbial expression system, can provide integrated one-stop biopharmaceutical end-to-end services, focalizing three major technology areas of recombinant proteins, nucleic acid drugs and nanobodies, with high efficiency and flexibility, provide CDMO services such as process development, IND-CMC pharmacological research, GMP production of clinical samples and registration application for global biotechnology companies, help customers to resolve the whole process from DNA to commercial production and jointly boost the process of new drug development.



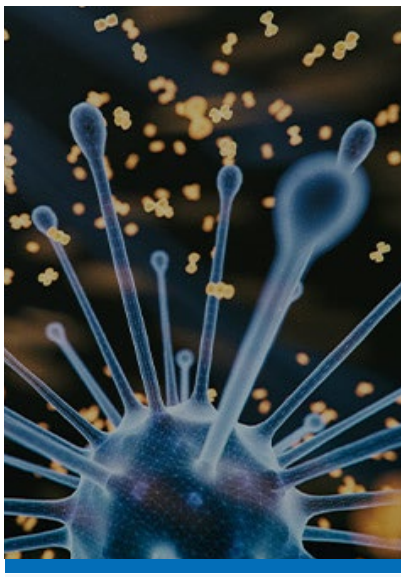
Recombinant Proteins

One stop service platform for CDMO of comprehensive recombinant proteins and peptides



Nucleic Acid Drugs

Focus on plasmids, mRNA/CircRNA and other long chain nucleic acid drugs to accelerate the process from basic scientific research to clinical application



Nano-antibodies

Full domain recombinant expression system providing integrated and end-to-end nano-antibody CDMO services

One-stop CRDMO services platform

DRUG DISCOVERY	PRECLINICAL RESEARCH PHASE	CLINICAL RESEARCH PHASE	COMMERCIAL PHASE
Trial sample preparation services (mRNA、CircRNA、nanobodies)	Strain construction/ Strains bank construction Process development Process transfer Formulation development Analytical method development Preclinical sample preparation Registration application and consulting services Stability study Regulatory support	Process transfer Process scale-up Clinical sample production Stability studies Release testing Regulatory support	Process characterization Process validation Product production Stability studies Release testing Regulatory support
	· 2L · 50L · 500L · 10L · 100L · 1000L · 30L · 200L · 2000L	· 50L GMP · 500L GMP · 100L GMP · 1000L GMP · 200L GMP · 2000L GMP	· 50L GMP · 500L GMP · 100L GMP · 1000L GMP · 200L GMP · 2000L GMP

Rich project experience

More than 100 projects have been served, covering preclinical research, clinical phase I, II and III, including multiple registration projects for China, US FDA, and Australia.

Professional team guarantee

With an experienced CDMO execution team, supported by gradient professionals, the entrusted project can be efficiently and collaboratively boosted.

One-stop service

Provide one-stop service from process development to commercial production

Compliance service guarantee

With a professional, standardized and regulated service guarantee system, and the whole life cycle can comply with the requirements of the new edition of pharmacopoeia, GMP and other related guidelines.

Comprehensive production line guarantee

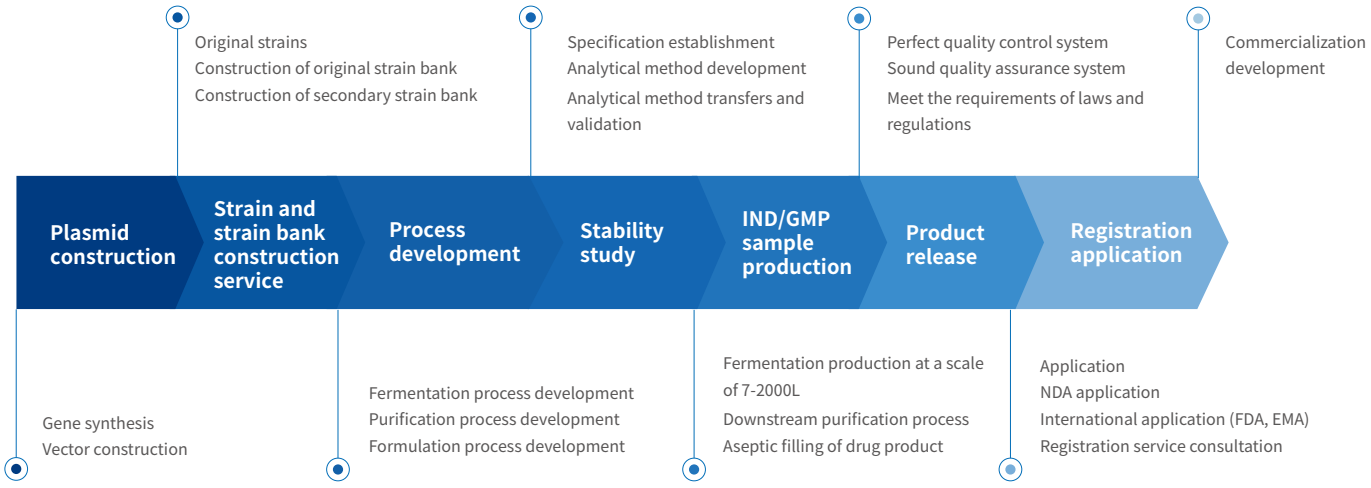
With an automatic fermentation system with a multi-scale of 2-2000L, high-quality and diversified fermentation purification services can be provided.

MICROBIAL FERMENTATION

RECOMBINANT PROTEIN CDMO SERVICES OVERVIEW

In the field of recombinant protein services, Yaohai Bio-Pharma can provide one-stop services of CMC for many types of recombinant proteins, including cytokines, vector proteins, recombinant polypeptides, enzymes, allergens, VLPs, vaccines and other types of recombinant proteins.

Recombinant protein CDMO services cover the full cycle of product development



Recombinant Protein Expression Services

Yaohai Bio-Pharma has an integrated CMC development and cGMP production process platform to produce recombinant proteins, plasmids, and DNA fragments using *E. coli* and yeast expression systems.

STRAIN CONSTRUCTION	LAB SCALE PROCESS DEVELOPMENT	PILOT SCALE UP AND PRODUCTION	QUALITY ANALYSIS AND CONTROL
Services	**Services**	**Services**	**Services**
• Gene synthesis • Plasmid construction • Strain construction • Vial target proteins and assays • Strain preservation and testing • Strain bank construction	• Optimization and verification of fermentation conditions • Scale-up and verification of 30L fermentation process • Purification process development, optimization and verification • Scale-up and verification of 30L purification process	• Analytical method development, validation and verification • Analysis and release testing of intermediate products and finished drug substances obtained during lab -scale • development and scale-up production Stability study • Provide release testing of strain bank and testing of raw materials and excipients	• Pilot-scale process optimization and scale-up production • Registration application batch production • Clinical phase I-III sample production • Industrialized production • Standard substance preparation
Service features	**Service features**	**Service features**	**Service features**
• Highly efficient screening of high expression strains within four weeks at the earliest • Selection of a variety of host strains and expression vectors, with codon analysis and optimization • One-stop service from gene sequencing to stable strain delivery by experienced technical team	• Provide optimization and screening of more than 10 parameters and complete fermentation process development within 1.5 month at the earliest • With various types of fermenters and bioreactors, fermentation of different vectors with high-density fermentation can be satisfied • Establish the evaluation, optimization and control strategies of fermentation and purification process parameters based on the concept of Quality by Design (QbD) • Build a high-throughput chromatography media screening platform and introduce DOE design for rapid process optimization	• With fermentation processes at a scale of 30L-50L-200L-1000L-2000L, matched by purification and drug product scale, the needs of different projects can be satisfied • 20+ pilot-scale up and production projects have been completed, including pilot-scale up, IND sample preparation, clinical phase I & II sample preparation, with extensive project experiences	• Rich experience in quality research, with several projects successfully passing on-site inspections by NMPA • The laboratory is equipped with a variety of chromatography techniques and assays to meet different types of compounds • With a perfect quality management system, the quality management and risk management throughout the whole process of experimental projects, as well as the compliance with the correspond-ing requirements of NMPA and FDA can be ensured

Recombinant Protein Service Platform Advantages

01 Integrated recombinant protein process development capability

With comprehensive and diversified recombinant protein process development experience, including: recombinant polypeptides, cytokines, carrier proteins, recombinant enzymes, allergens, VLPs, vaccines, and other types of recombinant proteins.
Advanced process development concept. The critical quality attributes (CQA) of the product are studied to establish the critical process parameters (CPP) through DoE based on the requirements of QbD (Quality by Design), which are robust and meet the product quality requirements.
Well-developed platforming process. Well-developed label-free protein process development capability reduces the process steps, improves protein purity, and ensures the process impurities and residual product impurity conforming to the requirements. Platform-based process can rapidly response to the project needs and shorten the process development time.

02 Rich project experience

Rich experience in recombinant protein CRDMO services

More than 5 IND clinical approval letter.

More than 100 successfully serviced recombinant protein CMC projects

Professional team guarantee

Support by experienced and stable CRDMO services team, with extensive service experience and accumulated technical experience in multiple types of recombinant protein projects, and focus on process route innovation, quickly resolve process difficulties, and reduce R&D costs.

Professional PM project management team proficiently masters the project management of the whole life cycle of biologics development, can identify and manage the project critical path, identify, control and manage the project risks.

03 Comprehensive production capacity guarantee

Large-scale preparation service at a scale of 50L-100L, 200L, 500L, 1000L and 2000L, etc.
2 production lines of drug products (vial lyophilized powder/injection, pre-filled cartridge).

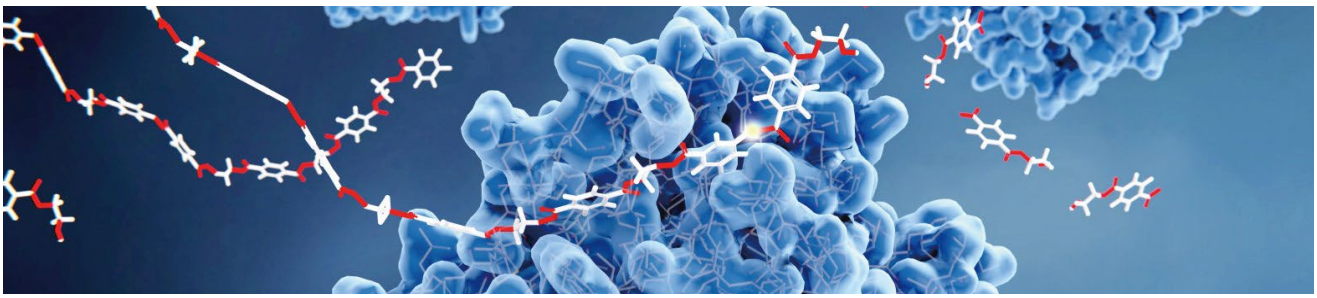
04 Perfect quality management system

Provide a full range of quality management service, with professional and standardized service guarantee system, and the whole cycle complies with the requirements of the new edition of pharmacopoeia and GMP related guidelines.

05 One-stop CRDMO services

Provide one-stop service from strain construction to commercial production, covering all stages of preclinical, clinical phase I, II, III and biologics production.

Recombinant Protein CDMO Service Case



Recombinant human interleukin-2 services

Target product: Recombinant human interleukin-2

Expression system: *E.coli*

Before process optimization

Before optimization, there were process problems such as low expression of the target protein, poor purification, and the result of bacterial endotoxin exceeding the requirement of pharmacopoeia (pharmacopoeia standard: it should be less than 10 EU per 1 million IU), and the in-house process was adjusted several times by the client, but still could not reach the expected target.

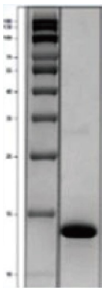
After process optimization

Yaohai Bio-Pharma adopted the *E.coli* prokaryotic expression system for the expression and purification of the target protein. After process optimization such as fermentation and purification:

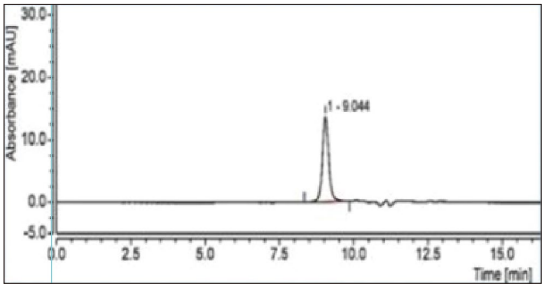
- Bacterial endotoxin <1EU/mg
- Purity>98%
- Yield of target protein>10mg/g cell

The process optimization was finally completed and successfully delivered according to the customer's requirements.

Relevant SEC-HPLC quality analysis
The results of SDS-PAGE analysis are shown in the figure



SDS-PAGE analysis



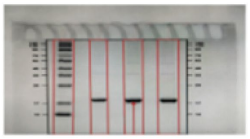
SEC-HPLC quality analysis

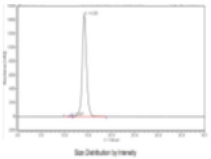
VLPs services

After process optimization

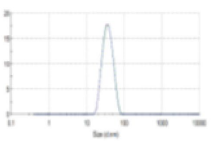
Based on Yaohai Bio-Pharma's well-developed recombinant protein platform-based process technology, the process optimization was completed quickly, which greatly shortened the R&D cycle and accelerated the project R&D progress, which was beyond the customer's expectation.

- Short process development cycle: process optimization can be completed within 2-4 months
- High success rate: platform-based process, with a success rate of 100%
- Bacterial endotoxin<1EU/mg
- Purity >99%

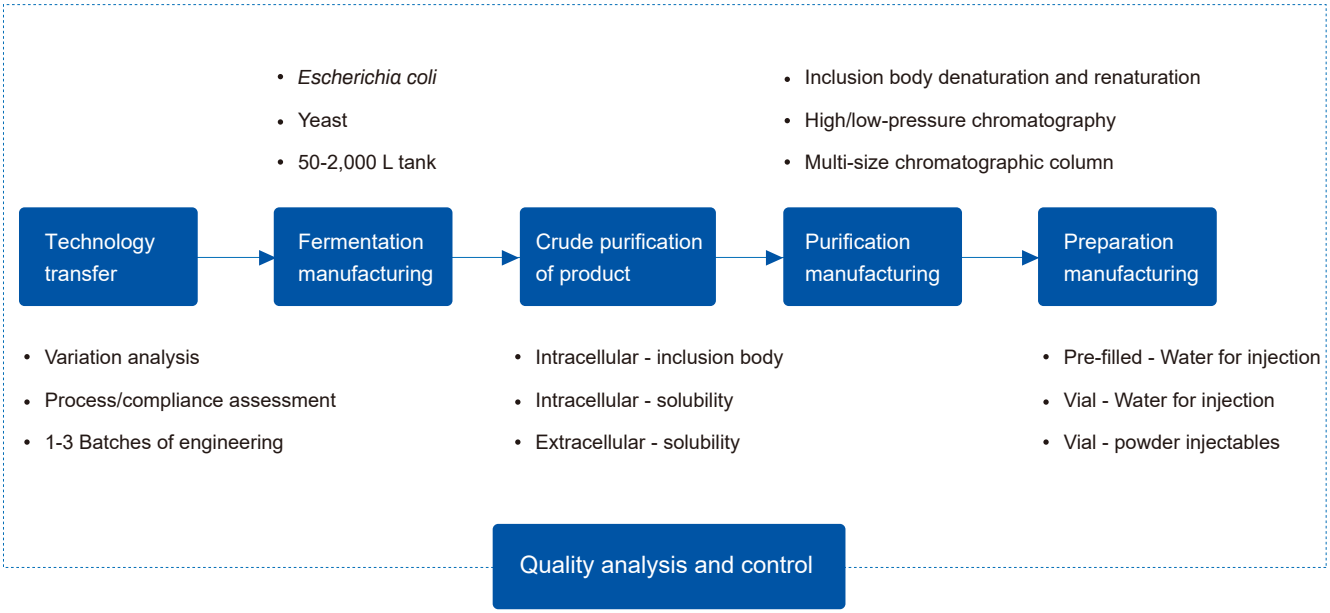




SEC-HPLC
PURITY99.6%



Overview of recombinant proteins CMO services



Yaohai Bio-Pharma is the preferred partner for customers in the field of microbial expression systems in China. We have extensive experience in recombinant proteins and recombinant plasmids manufacturing services. We focus on *Escherichia coli* and yeast expression systems, relying on GMP-level production workshops and the comprehensive quality management system. We take strict control of the process and the release criteria of raw materials, intermediates and final products of recombinant biologics enabling us to ensure batch-to-batch consistency. The BLA reporting strategy is adjusted according to the regulations of different countries to meet the requirements of our customers in both China and other regions.

Yaohai Bio-Pharma can provide GMP-level recombinant proteins manufacturing services, with multi-scale fermentation platforms of 50 L-100 L-200 L-500 L-1,000 L-2,000 L. The platform is equipped with multiple sizes of low/medium/high-pressure chromatography systems, and the automatically aseptic filling system for water for injection vials/lyophilization, pre-filled syringe/cartridge. Yaohai Bio-Pharma can provide the manufacturing services for IND application samples and phase I-III clinical samples, as well as the commercialization manufacturing service. Our service tenet is to help customers comprehensively accelerate the drug development process.

The CMO platform of Yaohai Bio-Pharma can serve the following recombinant protein products:

Recombination vaccines

Prophylactic/therapeutic recombinant protein-based vaccines such as virus-like particle vaccine (VLP) and recombinant subunit vaccine.

Recombinant peptides

Glucagon-like peptide (GLP-1) analogue, growth hormone (GH), insulin, parathyroid hormone (PTH 1 -34, teriparatide) and other polypeptide hormones.

Cytokines

Interleukin-2 (IL-2), IL-15, IL-21, Interferon (IFN), Granulocyte Colony Stimulating Factor (G-CSF), Osteocyte Factor (OF), and etc.Growth factors: fibroblast growth factor (FGF), epidermal growth factor (EGF), keratinocyte growth factor (KGF), platelet-derived growth factor (PDGF), and etc.

Growth factors

Fibroblast growth factor (FGF), epidermal growth factor (EGF), keratinocyte growth factor (KGF), platelet-derived growth factor (PDGF), and etc.

Enzyme preparations

Cas9 nuclease (gene editing enzyme), other nucleases, tool protease, target protease, etc.

Nano-Antibodies

Nano-antibodies with different potencies (monovalency/bivalency/trivalency).

Collagens

Type III collagen, type I collagen.

Other proteins

Cas protein family, tuberculosis allergen (allergen), antigen, carrier protein, ferritin, human serum albumin fusion protein, MEPE, protein A affinity chromatography ligand protein and other recombinant proteins or peptides expressed with Escherichia coli/yeast

Service details

Service Name	Service Items	Service Details	Minimum Delivery Cycle (working days)	Deliverables
Technology transfer	Document transfer	Manufacturing process/analytical methods/quality specification	TBD	Process transfer report
	Assessment of technical and regulatory compliance	Variation analysis of man, machine, material, method and environment	1	
		Assessment of formulation and process	1	
		Assessment of analytical methods	3	
	Protocol transfer	Determination of overall transfer protocol	7	
	Process validation	Manufacturing of 1-3 batches of engineering	TBD Subject to customer' s process	
Recombinant proteins Fermentation manufacturing services	Confirmation before fermentation	Man, machine, material, method and environment	1	Intermediates
	Preparation of fermentation system	Preparation of culture medium and solution	2-3	
		Seed tank-fermentor sterilization		
	Fermentation manufacturing	Seed propagation-fermentation-induction	2-4	
		Lowering tank in cooling		
Recombinant proteins Crude purification manufacturing services	Confirmation before production	Man, machine, material, method and environment	1	
	Manufacturing preparation	Solution preparation	1-2	
	Crude purification of product	Collection and concentration of culture supernatant - optional	2	
		Collection and crushing of bacterial cells -optional	1	
		Collection and washing of inclusion body - optional	2	

Service Name	Service Items	Service Details	Minimum Manufacturing Cycle (working days)	Deliverables
Recombinant proteins Purification manufacturing services	Confirmation before purification	Man, machine, material, method and environment	1	Protein stock solution
	Preparation of chromatography system	Buffer solution preparation	2-3	
		Filler preconditioning		
	Purification manufacturing	Inclusion body denaturation and renaturation-optional	TBD Subject to customer' s process	
		According to the process: Ultrafiltration, chromatography, enzyme digestion, modification, coupling		
Recombinant protein preparation manufacturing service	Confirmation before preparation production	Man, machine, material, method and environment	1	Vial-water for injection vials Vial-filing lyophilization Prefilled syringe-water for injection vials Cartridge-water for injection vials
	Pre-production preparation	Apparatus cleaning and sterilization	1-2	
	Preparation manufacturing	Filling of sterilized preparation	TBD Subject to customer' s process	
		Lyophilization-optional		
		Capping and visual inspection	2	
		Labeling or blind coding	-	

Note:
the mentioned “recombinant protein” generally refers to recombinant protein or recombinant polypeptide; TBD: to be determined (subject to the customer' s process); Multiple testing items can be carried out at the same time.
For CMO project of recombinant protein stock solution + preparation, Yaohai BioPharma' s average delivery cycle is 3-5 months (including engineering batch, cycle for reference), and the actual delivery cycle is subject to the customer' s process.

Continued table - quality analysis and control of recombinant proteins

Service items	Test items	Test methods	Minimum Delivery Cycle (working days)
Raw materials and excipients/ packaging materials test and release	Raw materials and excipients-critical items	Conducted in accordance to the specific test items	2
	Raw materials and excipients - full tests		11
	Packaging materials		60
Recombinant protein quality analysis and control	Appearance, visible foreign material	visual	1
	Insoluble particle	Light obscuration method	1
	Particle diameter	Zeta potential method	2
	pH	Potential method	1
	Total organic carbon (TOC)	UV method	1
	Electrical conductivity	Electrode method	1
	Osmotic pressure molar concentration	Freezing point titration method	1
	Moisture content	Titration method	1
	Loss on drying	Atmospheric pressure/ Vacuum drying method	2
	Residue on ignition	Burning method	2
	Deviation of deliverable volume	Volumetric/gravimetric method	1

Service items	Test items	Test methods	Minimum Delivery Cycle (working days)
Recombinant proteins Quality analysis and control	Target protein expression validation	SDS-PAGE, WB, ELISA	2-3
	Target protein expression amount	Non-reducing SDS-PAGE, HPLC, CE	1-3
	Purity of target protein		
	Molecular weight of target protein	Reduced SDS-PAGE	1
	Protein concentration	UV, BCA, Bradford, Lowry	1-2
	Enzyme activity-optional	UV and etc., depending on the characteristics of the enzyme	TBD
	PI isoelectric point	CE	3
	Peptide mapping	HPLC	4
	Bacterial endotoxin residue	Gel method, chromogenic method	3
	Host protein residue-HCP	ELISA	2
	Host DNA residue-HCD	qPCR	1
	Host RNA residue	RT-qPCR	1
	Other customized test items	-	TBD
	Antibiotic residue	ELISA, culture method	5
	Microbial limit test	Plate method, membrane filtration method	10
	Aseptic test	Direct culture method, membrane filtration method	18
	Investigation of sample stability	High-temperature test	40
		Photostability test	40
		Repeated freeze-thaw test	40
		Accelerated stability test	Sampling: 0, 1, 2, 3 and 6 months
		Long-term stability test	Sampling: 0, 3, 6, 9, 12, 18 and 24 months
GMP workshop environmental monitoring	Non-host strain monitoring	Plate method	5
	Settling microbe monitoring	Culture method	8
	Surface microbial monitoring	Culture method	8
	Planktonic bacteria monitoring	Culture method	8
	Compressed air monitoring	-	10

Note:
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CMO service features

Multi-scale CMO service platform

The stock solution workshop contains GMP-grade 50 L-100 L-200 L-500 L-1,000 L-2,000 L multi-scale fermentation platform, which is matched with centrifugal, high-pressure homogenization and low-pressure/high-pressure chromatography equipment of corresponding scale. The preparation workshop is accommodated with GMP-level automatic filling systems, covering 1-25 mL water for injection vials (60,000 vials/batch), powder injectables (37,800 vials/batch) and 1-3 mL prefilled syringes/cartridges (20,000 vials/batch).

Standard GMP-level explosion-proof workshop

The explosion-proof solution dispensing system adheres to the explosion-proof requirements. The workshop is equipped with electrostatic discharge instrument and combustible gas alarm devices, which can meet the solution dispensing needs for special processes, such as reverse phase chromatography.

Compliance ensuring platform

Comprehensively evaluate the compliance of products and quality standards, such as host source, antibiotic type, toxicity or sensitization, to meet the requirements of registration application.

Quality control and analysis services

Quality control services driven by the latest edition of Pharmacopoeia and the guiding principles of pharmaceutical manufacturing in China and at abroad, involving the release of raw materials and excipients/packaging materials, intermediate products and final products.

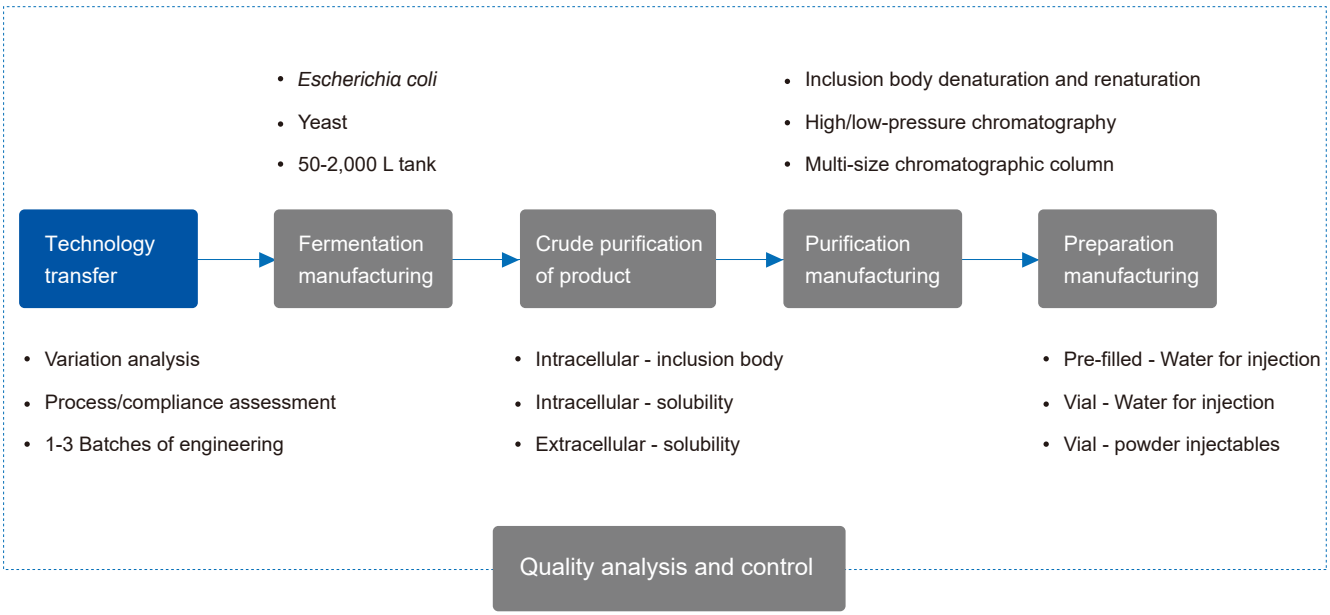
Extensive experience in technology transfer/scaling up

Conversion and scaling up parameters can be adjusted for fermentation and chromatography systems with different scales. More than 100+ recombinant protein-polypeptide-plasmid CMC projects and >5 IND clinical approvals have been successfully delivered, including several China-US dual applications and Australian registered projects.

Open online audit platform

Open online audit port, sharing VR videos of GMP workshop.

Recombinant proteins technology transfer services



According to the ICH Q10 guidelines, the life cycle of a drug product is divided into four stages: drug development, technology transfer, commercial manufacturing, and product discontinuation. Technology transfer is an important part of the drug life cycle and is the key connecting link between drug R&D and commercial manufacturing. Technology transfer mainly includes manufacturing processes, intermediates control, quality specification of raw materials and excipients, testing methods and other technologies and methods related to product quality. The main goal of technology transfer is to realize the transfer of products and related knowledge between R&D and manufacturing or between different manufacturing sites. We will facilitate our customers to realize the transfer between commercial production and CDMO, CMO, CRO enterprises, to ensure the continuous and stable production of products.

Yaohai Bio-Pharma has established technology transfer management measures from small test process development, medium test production to GMP production stage (stock solution and preparation) in accordance with the Chinese Pharmacopoeia 2020 edition, ICH Q10, WHO, PDA TR65, ISPE and other technology transfer guidelines. We are always clear that the technology transfer process is based on the concept of Quality by Design (QbD). A comprehensive risk assessment has been conducted on the transfer process in terms of regulations and quality management, and the management of whole life cycles of drugs is strengthened, to ensure the success of technology transfer. And fully guarantee the safety, efficacy and quality control of drugs to our customers.

01 **Project launch**

- Team building
- Personnel training

02 **Document transfer**

- Manufacturing process
- Quality standard
- Analytical method

03 **Technical assessment Regulatory compliance assessment**

- Man-machine-material-method environment
- Process/formulation assessment
- Test method assessment

04 **Scheme determination**

- Manufacturing conditions
- Sampling plan
- Release criteria

05 **Process validation**

- 1-3 batches
- Engineering batch verification

Service details

Service name	Service items	Service details	Minimum Delivery Cycle (working days)	Deliverables
Recombinant protein manufacturing technology transfer	Document transfer	Manufacturing process	TBD Subject to customer' s process	Process transfer report
		Quality specification		
		Analysis method		
	Evaluation of technical and regulatory compliance	Man, machine, material, method and environment variation analysis	1	
		Evaluation of formulation and process	1	
		Evaluation of analysis methods	3	
	Protocol determination	Transfer protocol determination	7	
	Process verification	Manufacturing of 1-3 batches of engineering	TBD Subject to customer' s process	

Note:
TBD: to be determined (subject to the customer' s process).
Reference regulations: *Chinese Pharmacopoeia* 2020 edition; ICH Q10. Guidance for Industry Q10 Pharmaceutical Quality System; WHO Guidelines on the Transfer of Technology in Pharmaceutical Manufacturing;
PDA Technical Report 65: Technology transfer; ISPE Good Practice Guide: Technology Transfer.

Service features

Extensive experience in process transfer

Fully assess the completeness and feasibility of process flow and the test methods, and provide customers with comprehensive process transfer solutions.

Compliance ensuring platform

Comprehensively assess the compliance of products and quality specification, such as host source, antibiotic type, toxicity or sensitization, to meet the requirements of registration application. Establish the release criteria for raw materials and excipients, packaging materials, intermediates and final products that are compliant, with the whole process complying with the latest version of pharmacopoeia and GMP related guidelines.

Professional project management team

Professional PMs are specialized in fermentation, purification and preparation process transfer and manufacturing process, able to identify and control project risks and drive project operation in whole cycle.

Technology transfer key parameters

Critical equipment	Main reasons affecting process parameters	Key parameters	Yaohai BioPharma equipment
Fermenter	Culture volume, diameter-to-height ratio, mixing blade, maximum rotation speed	Aeration, rotation speed, dissolved oxygen	Tofflon
Centrifuge	Sample size, type of equipment (benchtop type, floor type, drum type, disc stack type)	Rotating speed, feeding, residue discharge time	GEA, Beckman, Junmiao
Homogenizer	Equipment brand variation and performance variation	Flow rate, pressure, number of times	GEA, ATS
Chromatography system	UV detector, maximum flow rate	Retention time, sample collection time	Hanbaon, Rongjie
Chromatography columns	Processing batch, column volume	Column volume, loading/buffer solution volume	GE, Hanbon, Rongjie
Filtration/Ultrafiltration system	Processing batch, membrane area	Membrane area, flow rate	PALL, Sartorius

Note: The Yaohai Bio-Pharma Equipment column lists some equipment brands we have. Please consult our staff for more information.

During the process of technology transfer, Yaohai Bio-Pharma will perform parameter conversions for process transfer or scale up based on the inconsistent equipment models (e.g. fermenters, centrifuges and homogenizers). We will face differences in diameter-to-height ratio, stirring blade distribution and maximum speed of different brands of fermenters. Process validation and scale-up can be completed by controlling key parameters such as ventilation, rotational speed and dissolved oxygen. Different centrifugation equipment are available (benchtop type, floor type, drum type or disc stack type), and homogenizers with different capabilities are applicable for different volumes of samples. Therefore, during scaling-up process, the processes of some projects require conversion of centrifugation and homogenization equipment. The data that needs to be converted includes: centrifugation process parameters, including speed, feeding and residue discharging times (disc stack type), and the key homogenization parameters, including flow rate, pressure and times.

A conversion is required only for scale-up parameter under the condition that the Yaohai Bio-Pharma's equipment models are basically the same. During chromatography purification, with the column height and column efficiency being maintained within a controlled range, we maintain the retention time, loading capacity and elution conditions (linear flow rate) of the original process. Only the column volume and loading volume are required to be changed according to the actual scale. During filtration or ultrafiltration, we need to change the membrane area and control the flow rate according to the actual scale-ups.

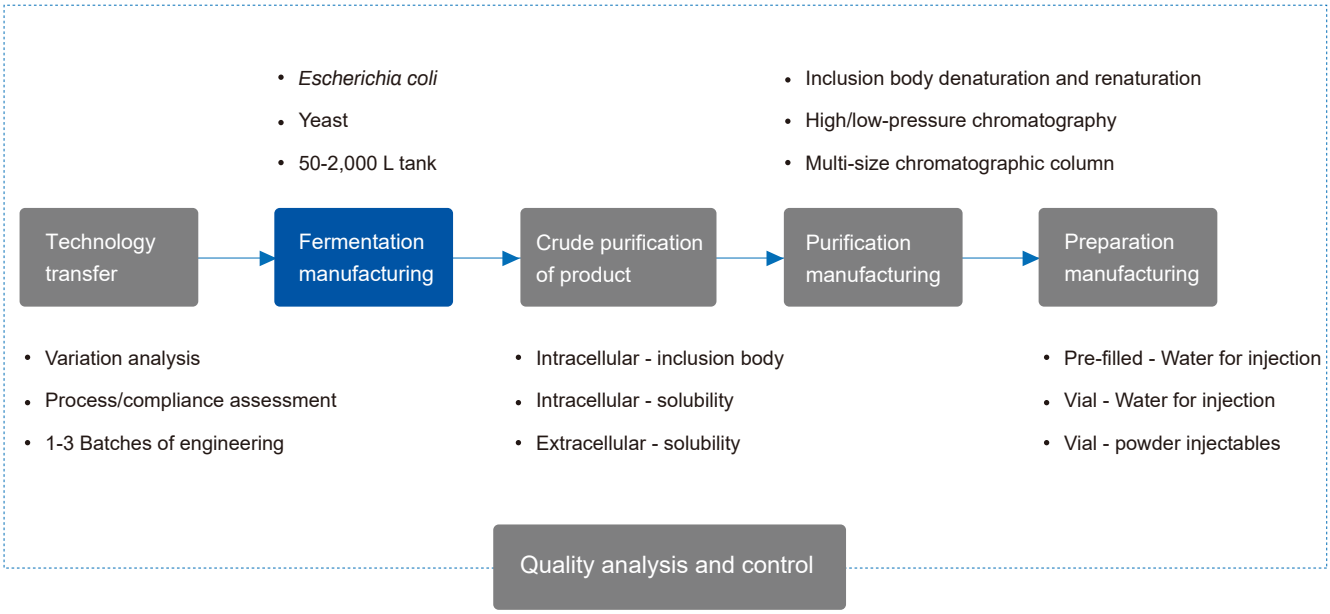
Based on the extensive CMO service experience of recombinant proteins/peptides/plasmids, Yaohai Bio-Pharma has accumulated experience in equipment-related process transfer of various brands, performances and models. We can quickly identify and adjust key equipment parameters to facilitate our customers to achieve fast delivery while maintaining the original quality of their products.

Yaohai Bio-Pharma TIP: Times of process scale-up is recommended to be within 10 times, and 1--3 batches of engineering batch are recommended to be used to control the risk of process scale-up.

Other Services

- Technology transfer
- Fermentation manufacturing services
- Crude purification manufacturing services
- Purification manufacturing services
- Recombinant protein preparation manufacturing service
- Quality analysis and control service

Fermentation manufacturing services



Yaohai Bio-Pharma, in the field of microbial expression system, has extensive experience in the manufacturing services of recombinant proteins and recombinant plasmids. Relying on five independently operating GMP-level customized production lines and 50 L-140 L-200 L-500 L-1,000 L-2,000 L fermentation scales, we can meet different project needs of customers. Currently, Yaohai Bio-Pharma has served more than 100 customers at home and abroad, with extensive experience in industrial manufacturing of fermentation.

During the scale-up of fermentation process, process control parameters unrelated to scale are also kept consistent, including the culture and induction conditions (such as the basal medium, fed-batch medium, induction agent, temperature and pH), the process parameters of inoculation, feed supplement and induction. For scale-related parameters, including culture volume, aeration and agitation rate, it is required to control the key parameters during process transfer.

Based on the extensive experience in CMO services, Yaohai Bio-Pharma can perform the appropriate process transfer and scale-up for different size/brand of fermenters, control key parameters, successfully achieve scale-up manufacturing of upstream processes and transfer to downstream processes with high-quality.

01 Preparation of fermentation system

- Empty elimination of fermentation tank
  Medium - real elimination of fermentation tank
  Preparation and sterilization of other solutions

02 Shake flask culture

- Inoculate working seeds to shake flask for cultivation (Primary or secondary)

03 Seed tank culture

- Seed tank inoculation
  Culture process control

04 Fermentation culture

- Feeding control
  Fermentation process control

05 Induced expression

- Induced expression
  Fermentation process control

Service details

Service items	Service details	Detailed procedures	Minimum lead time (working days)	Deliverables
Recombinant proteins Fermentation manufacturing services	Confirmation before fermentation	Confirmation of man, machine, material, method and environment	1	Intermediates
	Preparation before fermentation manufacturing	Receipt of documents and materials		
		Reconfirmation of conditions before production in GMP workshop		
	Preparation of fermentation system	Seed tank empty elimination, culture medium preparation and real elimination	2-3	
		Fermenter empty elimination, culture medium preparation and real elimination		
		Feeding tank empty elimination, culture medium preparation and real elimination		
		Preparation of induction agent and antifoam solution		
	Fermentation manufacturing	Seed culture in shake flask	2-4	
		Seed tank culture		
		Fermentation culture		
		Induced expression		
		Lowering tank in cooling		
Line clearance	Line clearance of fermentation workshop	Equipment cleaning and sterilization and environmental disinfection	-	-

Note: the table shows the shortest service period by taking E.coli as an example, and the yeast is increased as appropriate according to the fermentation process.

Service features

Mature GMP management system

The workshop staffs and QA/QC personnel have been strictly trained and instructed under GMP, and comply with all specifications of the latest GMP requirements.

Multi-size fermentation system

There are five production lines for stock solution, which are built in accordance with international GMP requirements, and can provide mixing and ventilating fermenters with sizes of 50 L-140 L-200 L-500 L-1,000 L-2,000 L, to support the production needs at different development stages.

Diversified fermentation platform

To meet the needs of customer projects for high-density fermentation processes, customized fed-batch and induction processes of Escherichia coli and yeast with or without antibodies.

Compliant testing and releasing specification

The brand and batch number of materials (raw materials and excipients) are verified, and the key materials are tested for releasing to ensure consistency and effectiveness of the materials.

Single project operation system

Only one project is allowed to be operated in each workshop during each time period to effectively prevent contamination and mix-ups, and the subsequent project shall be carried out only after the line clearance passes the requirements.

Experience sharing of fermentation process scale-up

The key parameters of the fermentation process include dissolved oxygen (DO), temperature and pH. Dissolved oxygen is an valid feedback parameter of growth state of strains. Temperature and pH directly affects the growth, proliferation and product expression of strains.

Based on extensive experience in CMO production services, Yaohai Bio-Pharma has summarized the issues frequently occurred during fermentation process transfer or scale-up process and the scale-up strategies:

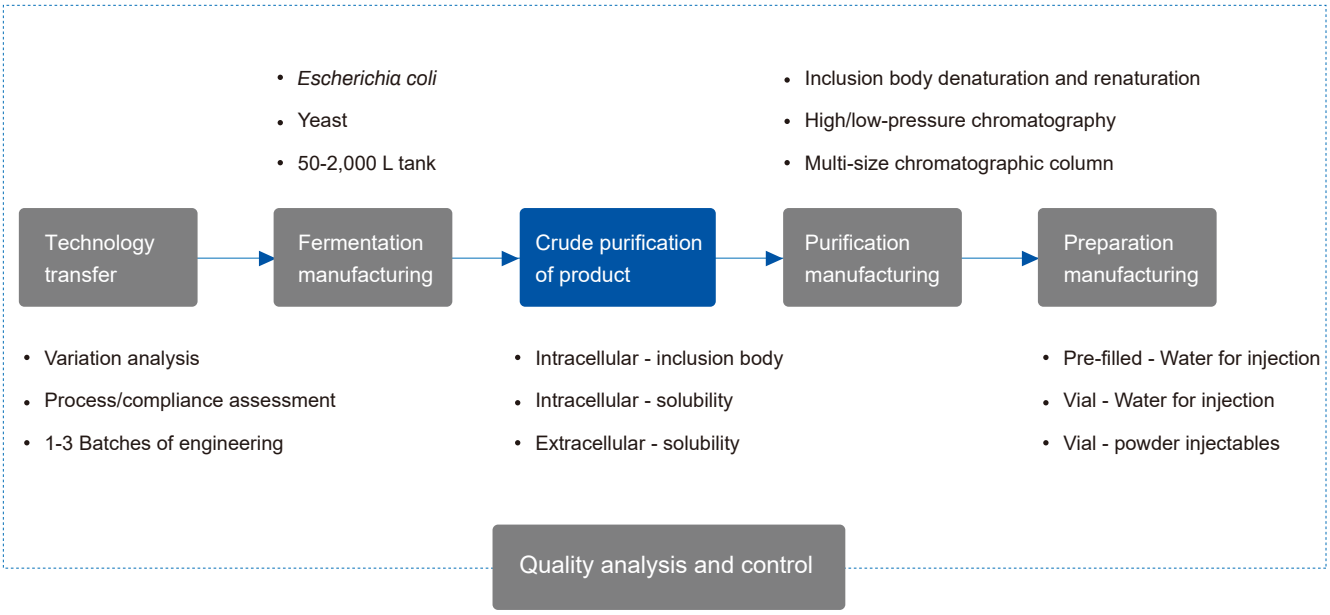
Parameter types	Related parameters	Frequently asked questions	Prevention or solutions
Questions related to culture condition	Temperature and pH	[Volume-independent parameters, consistent]	The temperature, pH sensor, pump and other equipment are calibrated and tested under GMP standards.
	Feeding strategy		
	Induction time		
Bacterial cell volume related questions	Rotation speed	How to conduct process transfer and scale-up if the maximum rotation speed of the fermenter is lower than the original process?	The function of agitation is to mix materials and improve the oxygen transfer coefficient, which is generally adjusted according to **dissolved oxygen**. A certain range of rotate speed is recommended during the process development, which may facilitate process transfer and scale-up.
	Ventilation	How to determine the aeration amount of the fermentation process during process transfer or scale-up?	The purpose of aeration is to provide oxygen for bacterial cells, improve oxygen transfer coefficient, and discharge exhaust gas at the same time, and the amount can be set to a fixed value or adjusted according to **dissolved oxygen**. It is recommended that a certain range of aeration amount should be validated during process development to facilitate process transfer and scale-up
	Dissolved oxygen	The influencing factors of dissolved oxygen include: fermentation liquor volume, viscosity, rotational speed, aeration amount, etc.	The process in which dissolved oxygen can be automatically controlled: after the parameter range of dissolved oxygen is set, it is controlled by adjusting agitation and aeration amount.
	$OD_{600\ nm}$	There is a significant variation between $OD_{600\ nm}$ value and the value of original process	The variation of instruments should be considered. As the principle and sensitivity of different spectrophotometers are different, it is not recommended to limit OD value excessively.
	Solid content of bacterial solution	-	It is recommended to use wet/dry weight of bacteria cells (weighing method) as the valid parameter of bacteria cell amount in reference to the solid content of bacterial solution (visual method).
	Bacterial cell weight	-	

Tips: it is not recommended to establish quality standards for intermediate products when there are only few running batches. A collection of relevant data is recommended, and then the quality standards and error range can be set by using statistical methods when there are enough data.

Other Services

- Technology transfer
- Fermentation manufacturing services
- Crude purification manufacturing services
- Purification manufacturing services
- Recombinant protein preparation manufacturing service
- Quality analysis and control service

Crude purification manufacturing services



The function of crude purification is to separate substances with large differences, such as solid-liquid separation, intracellular or extracellular substance separation. The crude purification process with *Escherichia coli* or yeast as the expression platform includes the separation of culture supernatant, intracellular soluble substances or inclusion bodies, which is usually realized by centrifugation and crushing. Due to the different scale of small tests and manufacturing, the adapted centrifugation and homogenization equipment also varies, and the conversion of centrifugation and homogenization parameters is especially important, which largely affects the quality and yield of the product.

In the field of microbial expression systems, Yaohai Bio-Pharma has extensive experience in the manufacturing services of recombinant proteins and recombinant plasmids. We rely on five independently operating GMP-level customized production lines, which are equipped with centrifuges and homogenization equipment of different processing batches. There are fermentation tanks of 50 L-140 L-200 L-500 L-1,000 L-2,000 L, which can meet the needs of different customers. Based on the extensive experience in CMO services, corresponding parameter conversions for centrifuges and homogenizers of different scales/performance can be performed. The key parameters can be controlled, which can successfully realize the scale-up production of crude purification process and the effective removal of some impurities.

01 Solution preparation

- Buffer solution preparation

02 Bacterial cell collection

- Centrifuge to collect bacteria cells
  Rotation speed/feeding residue discharge time

03 Bacterial cell crushing

- Cell heavy suspension and crushing
  Flow rate/number of pressure

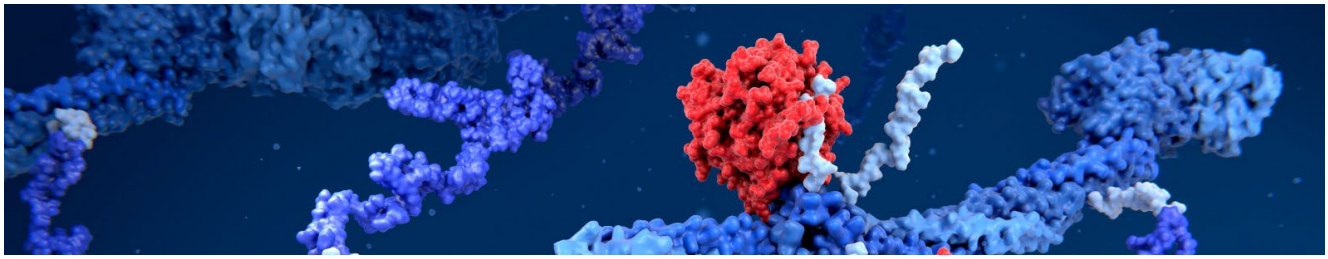
04 Inclusion body collection

- Collection of inclusion bodies by centrifugation
  Rotation speed/feeding/residue discharge time

05 Inclusion body washing

- Washing, centrifuging and dispensing
  Rotation speed/feeding/residue discharge time

Note:
The figure shows the crude purification process of intracellular -inclusion body. The crude purification process of the other two expression forms is described as follows:
Extracellular solubility: centrifuge to collect supernatant ⟶ concentration and solution replacement
Intracelular solublity: centrifuge to collect bacterial cells ⟶ high-pressure homogenizaton and crushing ⟶ centrifuge to remove debris of bacterial cells



Service details

Service items	Service details	Detailed procedures	Minimum Delivery Cycle (working days)	Deliverables
Recombinant proteins Crude purification manufacturing services	Confirmation before production	Confirmation of man, machine, material, method and environment	1	Intermediates
	Preparation before production	Buffer solution preparation	1-2	
	Extracellular soluble form-optional	Supernatant collection by centrifugation	2	
		Concentration and solution replacement		
	Intracellular soluble form-optional	Collection of bacteria cells by centrifugation	2	
		High pressure homogenizing and crushing		
		Centrifugal removing of bacterial debris		
	Intracellular inclusion bodies-optional	Collection of bacteria cells by centrifugation	3	
		High pressure homogenizing and crushing		
		Collection of inclusion bodies by centrifugation		
		Inclusion body washing and subpackage		
Line clearance	Workshop line clearance	Equipment cleaning, sterilization and environmental disinfection	-	-

Note:
centrifuge and homogenize equipment with high adaptability shall be selected according to the batch size of fermentation liquor/process sample

Service features

Mature GMP management system

The workshop staffs and QA/QC personnel have been strictly trained and instructed under GMP, and comply with all specifications of the latest GMP requirements.

Multi-scale crude purification equipment

There are five GMP-level production lines for stock solution, equipped with bench top type/drum type/disc stack type centrifuges and high-pressure homogenizers of different performance to meet the crude purification needs of fermentation liquor of different scales.

Diversified crude purification platform

We can provide crude purification service for extracellular soluble products, intracellular soluble products, and inclusion bodies according to customer's specific process.

Compliant testing and releasing specification

The brand and batch number of materials (raw materials and excipients) are verified, and the key materials are tested for releasing to ensure consistency and effectiveness of the materials.

Single project operation system

Only one project is allowed to be operated in each workshop during each time period to effectively prevent contamination and mix-ups, and the subsequent project shall be carried out only after the line clearance passes the requirements

Experience sharing of scale-up of crude purification process

The purpose of centrifugation is to achieve solid-liquid separation. Application scenarios include collection of bacterial cells or supernatant, removal of bacterial cells debris and collection of inclusion body, and etc. Key parameters include rotational speed, feeding rate and residue discharge time. Centrifugation that does not meet the criteria may result in poor solid-liquid separation, which may lead to the decrease in product yield or increasing of the burden of downstream purification.

High-pressure homogenizer can be used to crush cells and release intracellular products. Key parameters include flow rate, homogenization pressure and number of times, and the control indicator is the crushing degree of bacterial cells. Insufficient crushing of bacterial cells will lead to the decrease in the product yield; while excessive crushing will result in the inability to remove the debris of bacterial cells, releasing of too many impurities and increase of the pressure of purification.

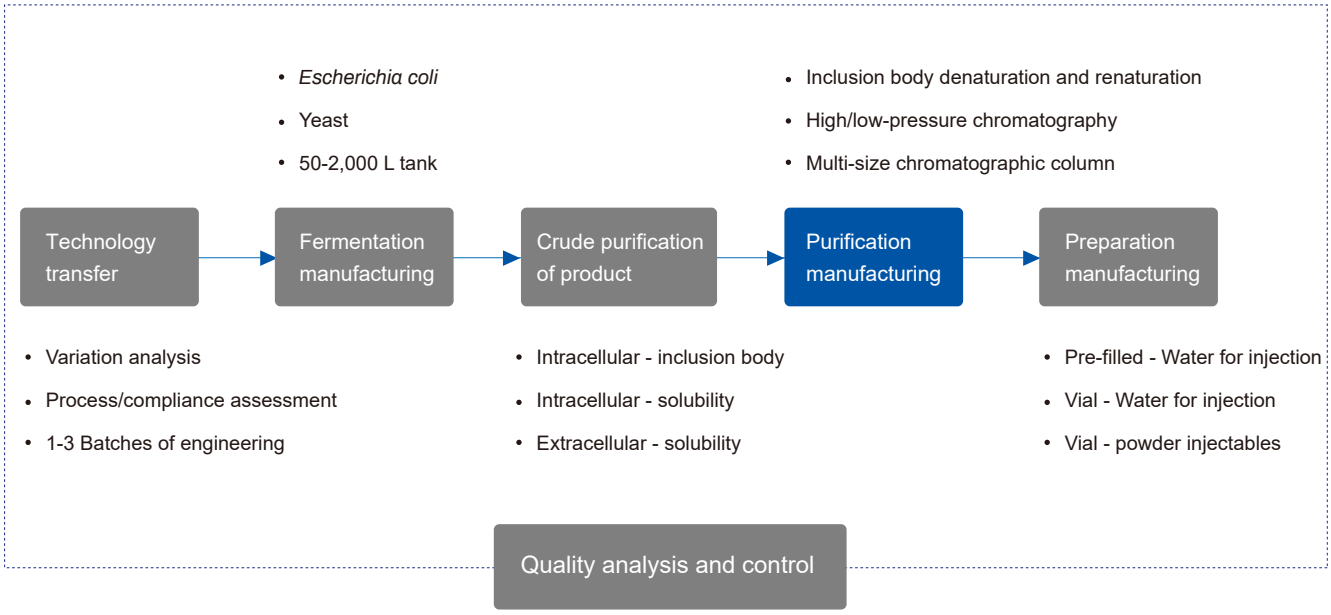
Based on our extensive experience in crude purification service of products, Yaohai Bio-Pharma has summarized the questions frequently occurred during the transfer of centrifugation and high pressure homogenization process and the solutions:

Crude purification process	Frequently asked questions	Question analysis	Solutions
Centrifugation	Turbid supernatant	Poor solid-liquid separation Decrease in yield Increase of purification pressure	• Too much feed: reduce the feeding rate • Uneven feed: fully stirring before feeding • Too low rotate speed: increase the rotate speed • Improper residue discharge time (disc-stack type): adjust the residue discharge time
High pressure crushing	Inadequate crushing of the bacterial cells	Decrease in product yield Increasing of manufacturing cost	• Too much feed: reduce the feeding rate • Low pressure: increase homogenization pressure • Less number of times of homogenization: increase number of times of homogenization
	Excessive crushing of the bacterial cells	Unable to separate the debris of bacterial cells effectively Increasing of the downstream purification pressure May lead to poor product quality	• Higher pressure: lower homogenization pressure • More number of times of homogenization: reduce the number of times of homogenization *Note*: the performance of different brands of homogenizer is inconsistent, so the relevant parameters can not be directly transferred, requiring adjusting of the key parameters. It is recommended to explore a larger parameter range for the small test process to facilitate process transfer.

Other Services

- Technology transfer
- Fermentation manufacturing services
- Crude purification manufacturing services
- Purification manufacturing services
- Recombinant protein preparation manufacturing service
- Quality analysis and control service

Purification manufacturing services



In the field of microbial expression systems, Yaohai Bio-Pharma has extensive experience in manufacturing services of recombinant proteins. The purification workshop is equipped with different sizes of automatic or manual membrane filtration systems and low/medium/high-pressure chromatography systems. The following purification services are available: filtration and clarification, gel filtration chromatography (molecular sieve), affinity chromatography (AC), ion exchange chromatography (IEX), hydrophobic interaction chromatography (HIC), reverse phase chromatography (RPC, explosion-proof solution dispensing system), composite chromatography, and ultrafiltration solution replacement. Currently, Yaohai Bio-Pharma has served more than 100 customers at home and abroad and has rich experience in industrial manufacturing of purification.

01 Preparation of purification system

- Buffer solution preparation
- Filler preconditoning

02 Inclusion body denaturation and renaturation

- Form of inclusion body
- Unique process

03 Filtration clarification

- Deep filtration
- Remove particles

04 Chromatography purification

- High/low-pressure chromatographic column
- Combined purification by multiple chromatography

05 Concentration and solution replacement

- Concentration by ultrafiltration
- Concentration by precipitation

Service details

Service items	Service details	Detailed procedures	Minimum lead time (working days)	Deliverables
Recombinant proteins Purification manufacturing services	Confirmation before purification	Confirmation of man, machine, material, method and environment	1	Recombinant proteins-stock solution
	Preparation before purification	Receipt of documents and materials		
		Reconfirmation of conditions before production in GMP workshop		
	Purification system preparation	Buffer solution preparation	2	
		Filler preconditioning		
	Purification manufacturing	Inclusion body denaturation and renaturation- optional	TBD (subject to customer's process)	
		Clarification/concentration		
		High/low-pressure chromatography		
		Enzyme digestion, modification, and coupling-optional		
		Concentration and solution replacement		
		Filtering sterilization		
Line clearance	Workshop line clearance	Equipment cleaning and sterilization and environmental disinfection	-	-

Note:
TBD: to be determined (subject to the customer's process).
The protocol for chromatography are determined based on the process, including but not limited to: gel filtration chromatography (molecular sieve), affinity chromatography (AC), ion exchange chromatography (IEX), hydrophobic interaction chromatography (HIC), reverse phase chromatography (RPC, explosion-proof dispensing system), and composite chromatography.

Service features

Mature GMP management system

The workshop staffs and QA/QC personnel have been strictly trained and instructed under GMP, and comply with all specifications of the latest GMP requirements.

Multi-size fermentation system

There are five production lines for stock solution, which are built in accordance with international GMP requirements, and can provide mixing and ventilating fermenters with sizes of 50 L-140 L-200 L-500 L-1,000 L-2,000 L, to support the production needs at different development stages.

Standard GMP-level explosion-proof worksho

The explosion-proof solution dispensing system meets the requirements of explosion-proof, and the workshop is equipped with electrostatic discharge instruments and flammable gas alarm devices, which satisfies the needs of solution dispensing in special process, such as reversed-phase chromatography.

Compliant testing and releasing specification

The brand and batch number of materials (raw materials and excipients) are verified, and the key materials are tested for releasing to ensure consistency and effectiveness of the materials.

Single project operation system

Only one project is allowed to be operated in each workshop during each time period to effectively prevent contamination and mix-ups, and the subsequent project shall be carried out only after the line clearance passes the requirements.

★★★ To meet the needs of solution dispensing of organic solvent in special processes such as reversed-phase chromatography, Yaohai Bio-Pharma purification workshops are equipped with explosion-proof solution dispensing systems, which meet the requirements of explosion-proof, and are installed with electrostatic discharge instruments and equipped with flammable gas alarm devices.

Experience sharing of purification process scale-up

Filtration and clarification are essential for the manufacturing of chromatography purification. Clarification is designed to further remove particulate substances to avoid posing negative impacts on the purification process in the downstream, which is usually completed using hollow fiber or membrane cassette. Key parameters in the process transfer or scale-up include the processing batch size, membrane area, and flow rate.

Chromatographic purification is the procedure of removing impurities of different sizes, charges, polarities and specificities using different chromatographic fillers to obtain a high purity target product. The manual/automatic chromatography systems, chromatographic columns and fillers are usually chosen to complete chromatographic purification in manufacturing workshop. Key parameters in process transfer include: processed batch size, column volume, loading volume and flow rate.

Based on our extensive experience in purification manufacturing services, Yaohai Bio-Pharma has summarized the questions frequently occurred during the transfer of purification process and the scale-up strategies:

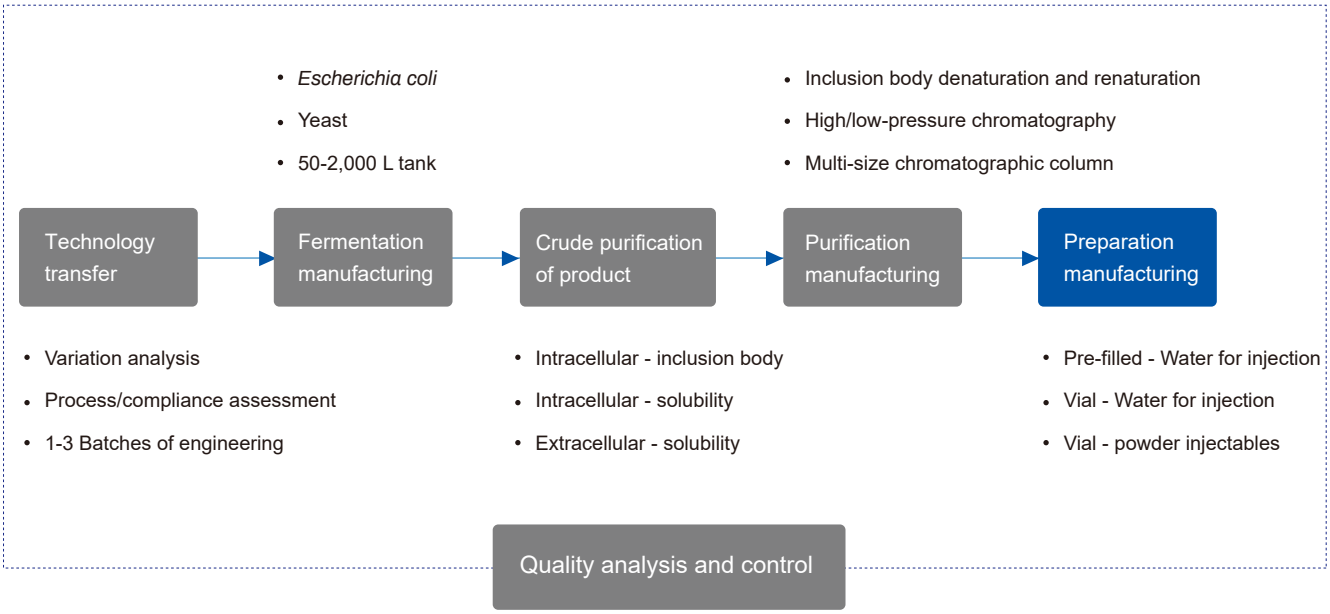
Purification process	Frequently asked questions	Process scale-up strategies
Filtration and clarification	What if there is no clarification process, or this process step is omitted in the small tests or medium tests?	*Suggestion*: The samples should be clarified during the process scale-up to remove the solid substances, so as not to increase the burden in the downstream purification.
Chromatographic process	How to scale up the chromatographic process?	*Consistent parameters*: sample concentration and composition, buffer solution composition, filler, column height, linear flow rate, loading volume/ column volume; *Scale-up* parameters: sample volume, column diameter, buffer solution volume, volume flow rate.
Membrane filtration	How to scale up the membrane filtration process?	*Consistent parameters:* sample concentration and composition, membrane aperture, linear flow rate; *Scale-up parameters*: sample volume, membrane area, volume flow rate.
Temperature control of sample-special requirements	If the temperature control of the glass tank is poor, how to improve it?	*Suggestion:* replace with a conforming stainless steel tank.

Note: the above table lists some simple and general scale-up strategies of purification process. If there are special process needs, you can also communicate with Yaohai Bio-Pharma technical team to solve them.

Other Services

- Technology transfer
- Fermentation manufacturing services
- Crude purification manufacturing services
- Purification manufacturing services
- Recombinant protein preparation manufacturing service
- Quality analysis and control service

Preparation manufacturing services



Yaohai Bio-Pharma relies on the GMP-level high-tech automatic production lines for sterile biopharmaceutical preparations, including multiple processes of vial washing, drying, sterilizing, aseptic filling, lyophilization, capping, and etc. We can provide services for different dosage forms of preparations, such as Water for injection vials, vials filling lyophilization, pre-filled water for injection vials (pre-filled syringe/cartridge).

The sterile preparation production lines of Yaohai Bio-Pharma conform to the manufacturing requirements for sterile preparations of US FDA, EU EMA, China NMPA and Australia TGA. We can provide the services of formulation preparation and aseptic filling of drugs and placebos, to meet the needs of different customers for IND application, phase I-III clinical research, and MAH commercialization.

Yields / Dosage form	Water for injection vials 1 mL-25 mL	Vial powder injectables 1 mL-25 mL	Pre-filled syringe/cartridge water for injection vials 1 mL-3 mL
Batch manufacturing	60,000 vials/batch (1-10 mL)	37,800 vials/batch (2 mL/4 mL) 20,043 vials/batch (7 mL/10 mL)	20,000 vials/batch
Annual yields	10 million vials/year	5 million vials/year	10 million vials/year

01 Preparation for formulation manufacturing

- Preparation and sterilization of formullation
  Sterilization of rubber stopper-aluminum cap

02 Vial sorting and vial washing

- Vial sorting - vial washing
  Drying sterilization

03 Filling and stoppering

- Normal/nitrogen filling/vacuum
  Partial stoppering/full stoppering

04 Freeze-drying

- Freeze-drying - full stoppering
  Unique process of powder injectables

05 Capping and visual inspection

- Capping - light inspection - warehousing

Service Details

Service items	Service details	Detailed procedures	Minimum delivery cycle (working days)	Deliverables
Recombinant proteins Preparation manufacturing services	Confirmation before preparation production	Confirmation of man, machine, material, method and environment	1	Vial-water for injection vials Vial-filing lyophilization Prefilled syringe-water for injection vials Cartridge-water for injection vials
	Preparation before production	Receipt of documents and materials		
		Reconfirmation of conditions before production in GMP workshop		
	Apparatus preparation	Apparatus cleaning and sterilization	1	
	Preparation manufacturing	Vial sorting and vial washing	1	
		Formulation preparation-optional	1	
		Sample sterilization and filtration		
		Filling and stoppering (normal/nitrogen filling/vacuum)	1	
		Lyophilization-optional (normal/nitrogen filling/vacuum)	TBD (subject to customer' s process)	
		Capping	1-2	
		Visual inspection		
		Labeling and blind coding	-	
Line clearance	Workshop line clearance	Equipment cleaning and sterilization and environmental disinfection	-	

Note: TBD: to be determined (subject to customer's process and batch size);
The current preparation workshop can provide the production of water for injection vials/filling lyophilization, pre-filled water for injection vials (pre-filled syringe and cartridge), and communication on other dosage forms are also welcomed.

Service features

Mature GMP training system

The workshop staff and QA/QC personnel have been strictly trained and instructed under GMP, and comply with all specifications of the latest GMP requirements.

Diversified preparation types

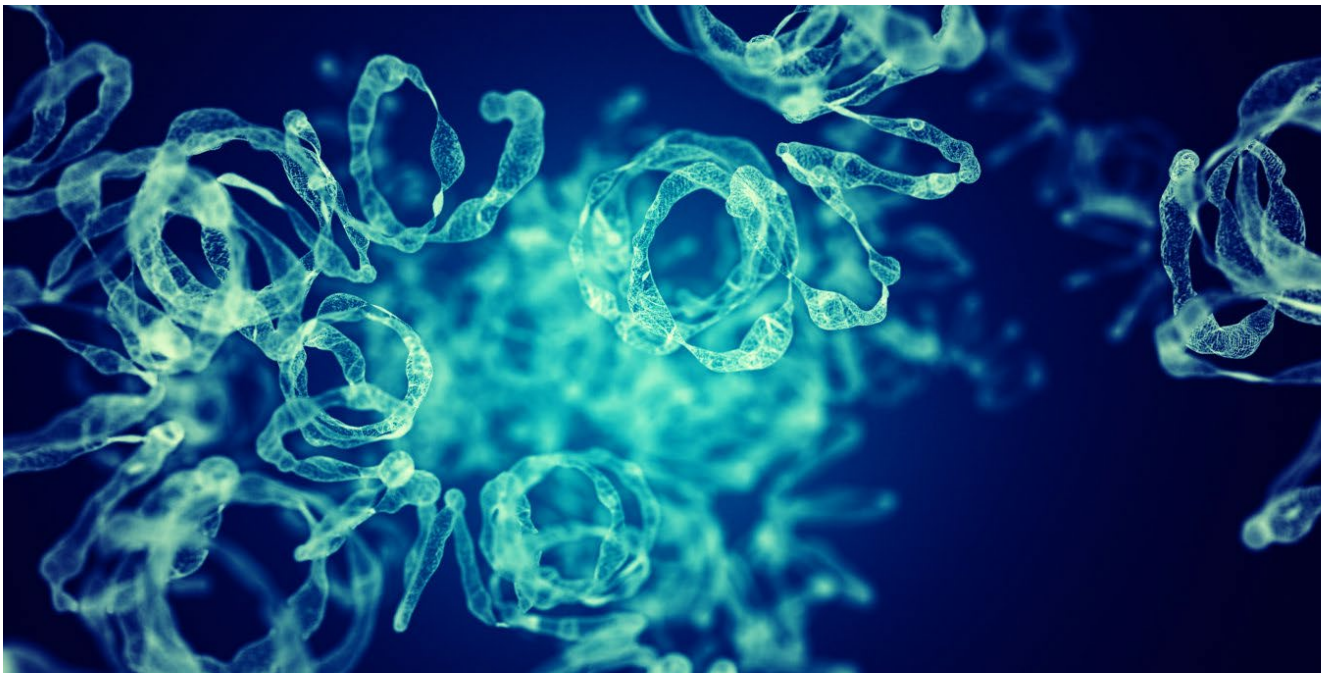
GMP-compliant automated sterile preparation production lines can serve the following products: 1-25 mL vial water injectables/filling lyophilization, 1-3 mL pre-filled syringe/cartridge water injectables.

Aseptic preparation filling production line

Conforming to aseptic preparation manufacturing requirements of US FDA, EU EMA, China NMPA and Australia TGA. O-rabs system (Open Restricted Access Barrier System) are used to protect the exposure areas of products (and the packaging materials), providing grade A environmental protection under grade B background.

Extensive project experience

100+ CMO project experience; the professional PMs are proficient in the scale-up manufacturing of preparation process, and can provide professional advice for multiple types of protein drugs, including the compatibility of packaging materials with active substances of drug and excipients.



Experience sharing of aseptic preparation process scale-up

With extensive experience in preparation filling service, Yaohai Bio-Pharma can help customers to develop compatible and suitable strategies for packaging materials based on the characteristics of drug solutions and excipients of various types of biological products, and fully promote the manufacturing process of products.

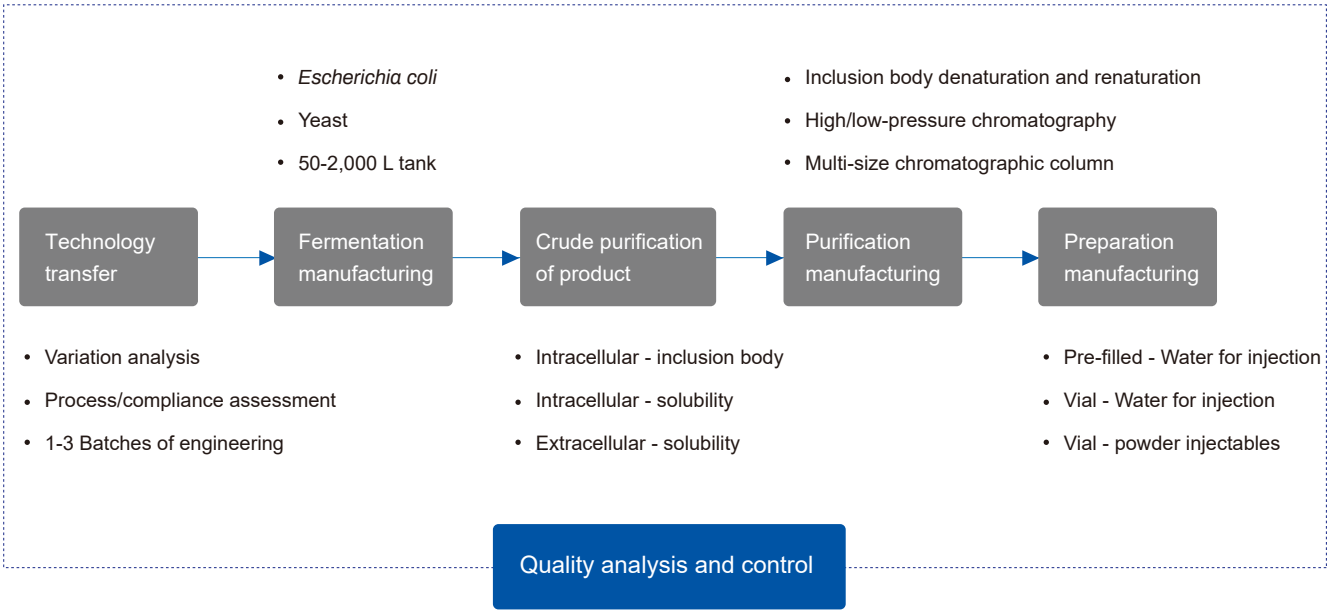
Purification process	Frequently asked questions	Yaohai BioPharma’ s experience
Lyophilization	Why does the freeze-dried powder appear the phenomenon of wall climbing traces of drug solutions?	The phenomenon of wall climbing traces is related to the characteristics of the drug solution (active ingredient of drug and formulation excipients), such as surface activity, surface tension, viscosity, etc.; the different adsorption properties of packaging materials, such as the inner surface of glass vials, may also lead to the wall climbing traces of drug solutions.
	How to improve if there is the phenomenon of wall climbing traces of lyophilized powder?	It is recommended to change to glass bottle with lamination without changing the formulation to reduce the adsorbability of glass bottle for drug, so that the phenomenon of wall climbing traces of drug solutions can be improved.

Note:
the phenomenon of wall climbing traces of drug solutions refers to the obvious traces left on the inner wall of the bottle after the lyophilization of the drug. In normal circumstances, the wettability/contact angle between aqueous solution and low borosilicate glass vial is small, so it is not easy for most varieties of solutions to leave wall climbing traces. Such phenomenon may appear in only a few varieties and surfactant is contained in excipients.

Other Services

- Technology transfer
- Fermentation manufacturing services
- Crude purification manufacturing services
- Purification manufacturing services
- Recombinant protein preparation manufacturing service
- Quality analysis and control service

Quality analysis and control services



According to the pharmacopoeia, the quality control system of recombinant DNA protein products mainly includes raw materials and excipients, package materials, manufacturing process and process control, tests of products. Quality control involves assessment of known/potential products and process-related substances by using standard substances and validated methods, and analysis of test items of product appearance identification, activity, purity and impurities.

Yaohai Bio-Pharm has a comprehensive quality analysis and control system. Our team members have thoroughly proficient in pharmacopoeia and other regulatory specifications. They have own extensive experience in quality testing and analysis. We are able to implement sample tests in conformity with the specifications, guarantee the release criteria of raw materials and excipients, intermediates and stock solutions/preparations, and deliver complete COA reports to customers.

Service details

Service items	Test items	Test methods	Minimum Delivery Cycle (working days)
Raw materials and excipients/ packaging materials Test and release	Raw materials and excipients - critical items	Conducted in accordance to the specific test items	2
	Raw materials and excipients - full tests		11
	Packaging materials		60
Recombinant proteins Quality analysis and control	Appearance, visible foreign material	Visual	1
	Insoluble particle	Light obscuration method	1
	Particle diameter	Zeta potential method	2
	pH	Potential method	1
	Total organic carbon (TOC)	UV method	1
	Electrical conductivity	Electrode method	1
	Osmotic pressure molar concentration	Freezing point titration method	1
	Moisture content	Titration method	1
	Loss on drying	Atmospheric pressure/ Vacuum drying method	2
	Residue on ignition	Burning method	2
	Deviation of deliverable volume	Volumetric/gravimetric method	1
	Target protein expression validation	SDS-PAGE, WB, ELISA	2-3
	Protein expression amount	Non-reducing SDS-PAGE, HPLC, CE	1-3
	Purity of protein		
	Protein molecular weight	Reduced SDS-PAGE	1
	Protein concentration	UV, BCA, Bradford, Lowry	1-2
	Enzyme activity-optional	UV and etc., depending on the characteristics of the enzyme	TBD
	Isoelectric point (pI)	CE	3
	Peptide mapping	HPLC	4
	Bacterial endotoxin residue	Gel method, chromogenic method	3
	Host protein residue-HCP	ELISA	2
	Host DNA residue-HCD	qPCR	1
	Host RNA residue	RT-qPCR	1
	Other customized test items	-	TBD

Service items	Test items	Test methods	Minimum Delivery Cycle (working days)
Recombinant proteins Quality analysis and control	Antibiotic residue	ELISA, culture method	5
	Microbial limit test	Plate method, membrane filtration method	10
	Sterility test	Direct culture method, membrane filtration method	18
	Investigation of sample stability	High-temperature test	40
		Photostability test	40
		Repeated freeze-thaw test	40
		Accelerated stability test	Sampling: 0, 1, 2, 3 and 6 months
		Long-term stability test	Sampling: 0, 3, 6, 9, 12, 18 and 24 months
GMP workshop environmental monitoring	Non-host strain monitoring	Plate method	5
	Settling microbe monitoring	Culture method	8
	Surface microbial monitoring	Culture method	8
	Planktonic bacteria monitoring	Culture method	8
	Compressed air monitoring	-	10

Note:
The mentioned "recombinant proteins" generally refers to recombinant proteins or recombinant peptides; TBD: to be determined (subject to the customer's process). Multiple test items can be carried out at the same time.
For CMO project of recombinant proteins stock solution + preparation, the average delivery cycle of Yaohai Bio-Pharma is 3-5 months (including engineering batch, cycle for reference), and the actual delivery cycle is subject to the customer's process.

CMO service features

Mature GMP training system

The QA/QC personnel have been strictly trained and instructed under GMP comply with all specifications of the latest GMP requirements.

Compliant QC testing process

Being able to reasonably assess the compliance of analytical methods and quality release specifications, and can quickly complete the transfer and validation of the analytical methods.

Whole-process quality control

The raw materials and excipients, package materials, intermediates, stock solution and preparations of recombinant proteins are tested for releasing, with the quality specification of materials and samples strictly controlled.

Complete quality analysis platform

Based on our extensive experience in CMO services, the quality control team of Yaohai Bio-Pharma has established a highly applicable, robust and reliable analysis platform that can meet the requirements of physiological, biochemical and microbiological testing.

BSL-2 microbiology laboratory certification

Meeting the needs of special projects such as pathogen tests.

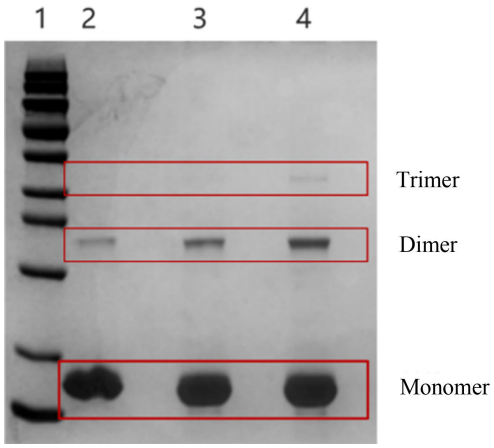
Quality analysis case sharing

Improper pretreatment method of the test samples can pose certain impacts on the quality test results.

In a purity test of the lyophilized powder of recombinant proteins, different volumes of re-suspension solution were used by Yaohai Bio-Pharma for re-dissolution to obtain protein samples at different concentrations: 1 mg/mL, 5 mg/mL and 10 mg/mL, and the non-reduced SDS-PAGE was selected to determine the purity of protein monomer.

The results of electrophoresis showed that the content of protein monomers at different protein concentrations varied significantly, so the pretreatment method directly affected the quality index of the product.

Lane 1: Marker;
Lane 2: protein concentration is 1 mg/mL;
Lane 3: protein concentration is 5 mg/mL;
Lane 4: protein concentration is 10 mg/mL
Result analysis: the target protein is known to be hydrophobic and the target product is monomeric form. The high protein concentration after re-dissolution promotes the formation of protein aggregates, resulting in the reduction of the purity of monomer protein.
[Note: protein loading volume >10 μg (Coomassie Brilliant Blue Staining Method), in accordance with the provisions of the Chinese Pharmacopoeia]



The quality control team of Yaohai Bio-Pharma has established a highly applicable, robust and reliable analysis platform, by which the compliance assessment, method transfer and validation of quality test methods can be accomplished, to match against the product quality requirements in a high-standard way.

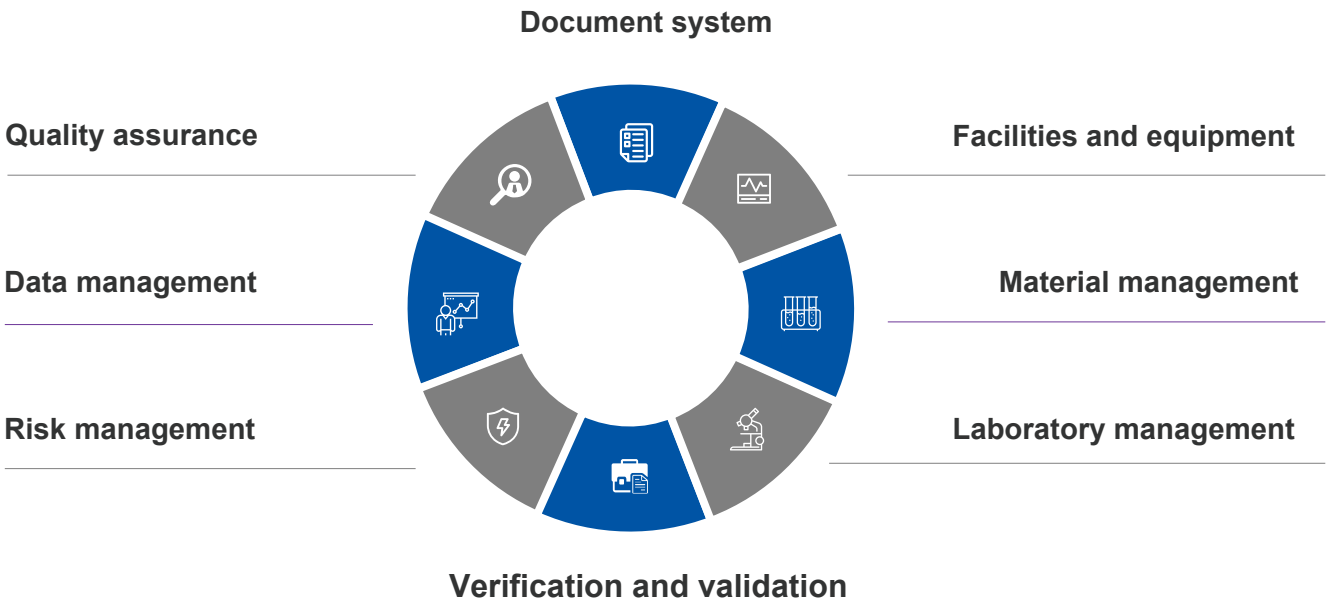
Other Services

- Technology transfer
- Fermentation manufacturing services
- Crude purification manufacturing services
- Purification manufacturing services
- Recombinant protein preparation manufacturing service
- Quality analysis and control service

GMP quality assurance system

Good Manufacturing Practice (GMP) is the basic guideline for drug manufacturing and quality management, which applies to the whole process of drug preparation manufacturing and the key processes affecting the quality of finished products in API manufacturing. The vigorous implementation of GMP is to avoid contamination and cross-contamination in the drug manufacturing process to the maximum extent possible and to reduce the occurrence of various errors, which is an important measure to improve the quality of drug products.

The bio-quality system management personnel in Yaohai Bio-Pharma have GMP certification experience, and the executive team has GMP work experience. Our team members are proficient in studying, interpreting and translating global regulations. We have developed a compliant quality management system by combining different life cycle stages of drugs. We also manage and control the whole process of man-machine-material-method-environment in the production stage.



Document system

- Policies of management (POL), standard operation procedures (SOPs)
- Process procedures/quality specification/standard test procedures (STP)
- Form records: adhere to SOP and STP, with independent approval

Quality assurance

• System management: Document/record, training, change/deviation/CA PA/complaints, self-test, material/supplier manage
ment
• Site management: Manufacturing site, QC site, material control, utility system, record review, product release

Data management

• Computerized system management
• Laboratory raw data management
• Data audit, data reliability management

Risk management

• Line confluence risk control: stage manufacturing/dedicated apparatus
• Sterile contamination risk control: facility/equipment/material control
• Compliance risk control: self-test/audit/regulation translating
• Quality system risk control: change/deviation/CAPA

Verification and validation

• Verification of plant and facilities
• Equipment verification
• Computerized system validation
• Process validation
• Metrology management
• Cleaning verification
• Aseptic process simulation
• Validity period verification, etc.

Laboratory management

• Management of samples/references, reagents and consumables
• Verification and validation of analytical methods, management of entrusted testing
• Data, record and report management, quality information management

Material management

• 1,400 m2 storage area, conforming to GMP and FDA specifications
• For storage of raw materials and excipients, packaging materials, intermediate products, finished products, and etc.
• Storage conditions include freezing, refrigerating or ambient/room temperature

Facilities and equipment

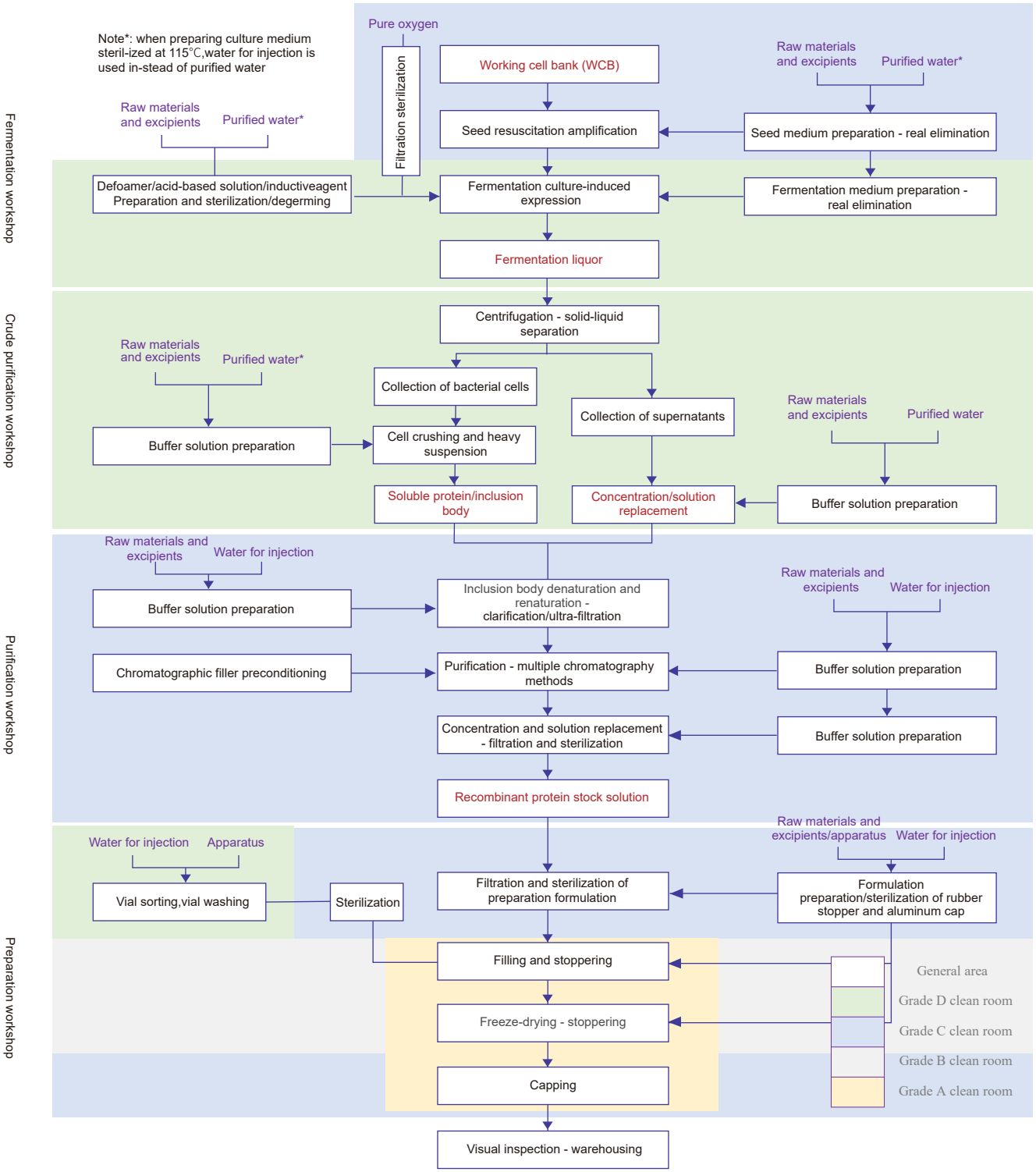
• Management of functional areas of different cleanliness classes: air conditioners are independently formulated to control differential pressure, temperature and humidity and suspended particles
• Safeguard of medium: water for injection, purified water, pure steam, and etc.
• Equipment: authority setting, on-line monitoring, validation and measurement

Management and control of clean room in GMP workshop

Maximum allowable number of suspended particles/m^3

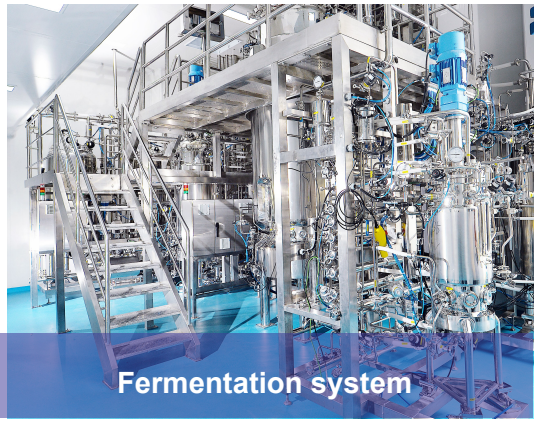
Cleanliness level	Static		Dynamic	
	≥0.5 μm	≥5.0 μm	≥0.5 μm	≥5.0 μm
Grade A	3,520	20	3,520	20
Grade B	3,520	29	352,000	2,900
Grade C	352,000	2,900	3,520,000	29,000
Grade D	3,520,000	29,000	No provision	No provision

Functional area of GMP workshop



Presentation of GMP workshop and equipment

Solution dispensing system



Fermentation system

Disc stack type centrifuge

Cartridge centrifuge

Homogenizer

Hollow fiber system

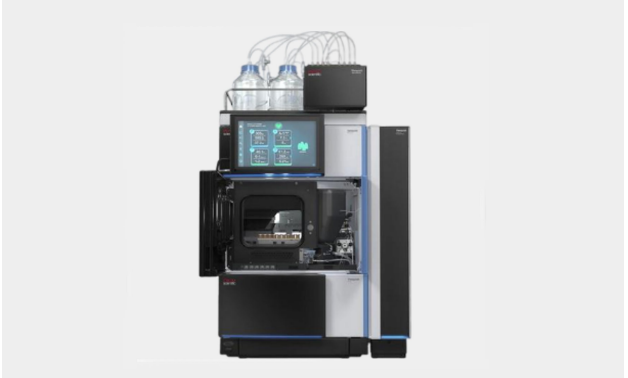
Low-pressure chromatography system

High-pressure chromatography system

Aseptic filling system

Capillary electrophoresis instrument

Gas Chromatograph



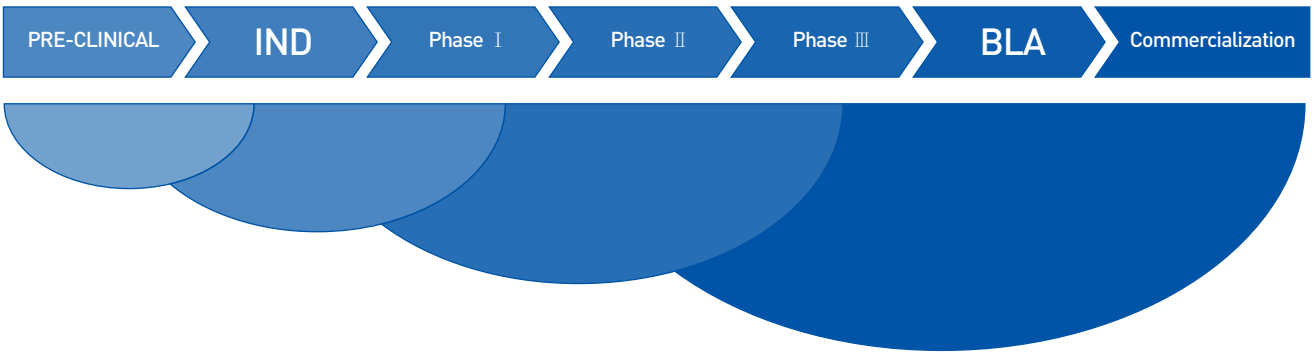
Liquid chromatograph

NUCLEIC ACID DRUGS

PLASMID CDMO SERVICES

Plasmid CDMO Services Overview

Yaohai Bio-Pharma commits to provide one-stop plasmid CDMO services, has established a GMP-compliant circular plasmid production platform and a linearized plasmid production platform, with well-developed process development and GMP production experience, and can provide customers with integrated CDMO services from plasmid construction, strain bank construction, process development, quality methodology study, stability study, non-clinical research plasmid production to clinical plasmid GMP production and registration application, meeting the needs of plasmid services at different stages from preclinical research, IND application, clinical trial and commercial production.



SERVICES

- PLASMID CONSTRUCTION
- STUDY ON STORAGE AND STORAGE STABILITY OF STRAIN
- DEVELOPMENT AND VALIDATION OF ANALYTICAL METHODS
- STRAIN CONSTRUCTION AND SCREENING
- DEVELOPMENT AND OPTIMIZATION OF FERMENTATION PROCESS
- HUNDRED-MILLIGRAM PLASMID PRODUCTION PREPARATION AND DETECTION[GMP-LIKE]
- ESTABLISHMENT OF STRAIN BANK [PCB/MCB/WCB]
- DEVELOPMENT AND OPTIMIZATION OF PURIFICATION PROCESS
- PRODUCTION AND RELEASE OF GMP PLASMID
- TEST AND PASSAGE STABILITY STUDY OF STRAIN BANK
- PROCESS VERIFICATION
- STUDY ON PLASMID STABILITY
- WRITING OF REGISTRATION MATERIALS

Different Levels of Plasmids

Yaohai Bio-Pharma can provide plasmids at different levels to meet the needs of different stages of pre-research, IIT, IND application, clinical research and commercial production

Plasmid production of non-registration clinical research level (IIT)

Development and production of plasmids for non-registration clinical research level

Overall solution for plasmid clinical application (IND)

Plasmid development and production of gene cell therapy and nucleic acid drug for clinical registration and application

GMP production of plasmids at clinical level

Clinical samples and commercial GMP production for gene cell therapy and nucleic acid drugs

Plasmid level	Scale	Applications	Preparation conditions
Plasmids at research level	1-500mg	Preclinical research	Process development laboratory
GMP-like plasmids	100mg-5g	Non-registration clinical/preclinical research	GMP workshop
GMP plasmids	100mg-5g	IND application/phase I-III/commercial production	GMP workshop

Plasmid Process Development Platform

The plasmid process development platform of Yaohai Bio-Pharma adopts the concept of “Quality by Design (QbD)” and is equipped with comprehensive CMC process development and optimization, analytical method development and quality control capabilities, supporting the preparation of plasmid at research level under non-GMP and GMP-like conditions and providing plasmid vector services to meet various needs.

Fermentation purification systems at different scales to meet the needs of different scales from laboratory development to GMP production.

		Laboratory	Pilot scale up	GMP production
Fermentation system	Equipment	Quadruple fermenter	Fermentation system*2	Tofflon fermentation system*5
	Scale	2L/7L*4 sets	20L/30L fermentation system*1 50L/69L fermentation system*1	50L-100L-200L-500L-1000L-2000L
Ultrafiltration system	Equipment	Fluxs tangential flow membrane filtration system	Hollow fiber/film package	Fully automated ultrafiltration system
	Scale	50ml-5L	100ml-30L	5L-60L
Chromatography system	Equipment	AKTA(pure/Avant)	RJBIO LPLC 180G	Gradient chromatography system
	Scale	9L/H	3L/H-180L/H	60L/H、180L/H、600L/H

Plasmid Process Development Platform

With GMP plasmid production and process development workshops, Yaohai Bio-Pharma can provide plasmid production services at different stages of non-registration clinical research, IND application, clinical research and commercial production.

01

With five independent production lines of drug substances and two automatic aseptic production lines of drug products, automatic aseptic production of injection (vial), lyophilized powder and pre-filled cartridge can be achieved

02

Provide plasmid production at different scales from 30L-2,000L to meet the production needs of research, lab-scale and pilot-scale production

03

GMP production workshop, meeting the standards of FDA, EMA, and NMPA

04

International mainstream automated fermentation, ultrafiltration and purification system

05

Unidirectional design of human flow, material flow and sample flow to avoid cross-contamination

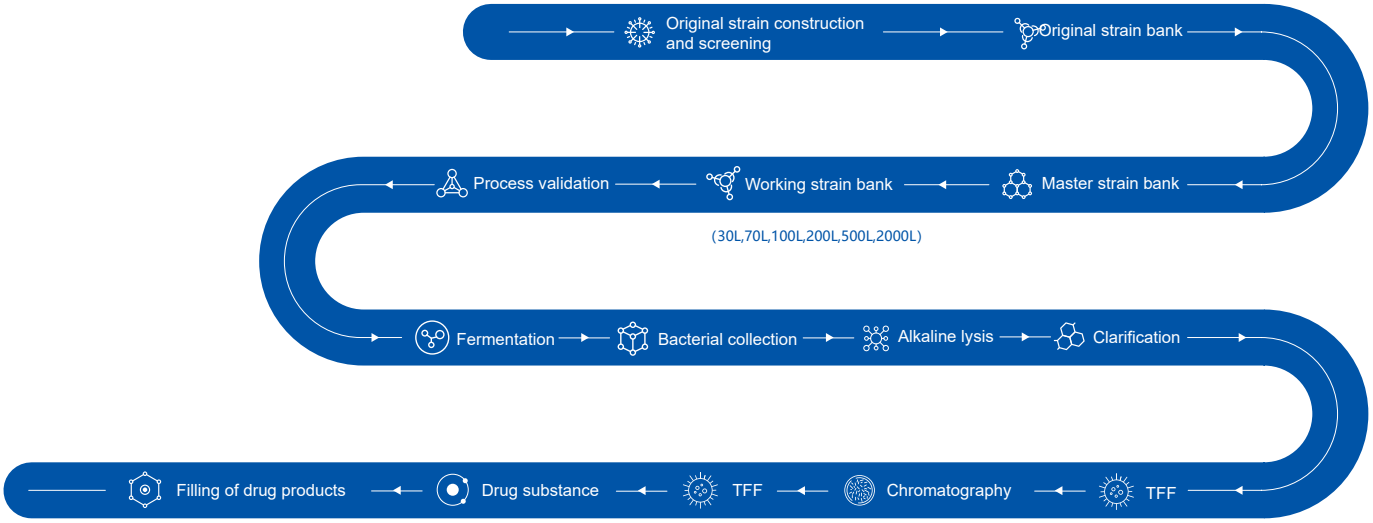
Plasmind GMP Production Process

Supercoiled plasmid process development flow

- Recombinant plasmids
- Genetically stable strain screening
- Tertiary strain bank construction
- Strain bank passaging and storage stability study
- Fermentation process development/optimization
- Purification process development/optimization
- Process scale-up study and validation

Application types	bare plasmid products, DNA vaccines/DNA drugs, viral vector constructs (LV/AAV), viral vaccines, LcDNA
Preparation conditions	Lab scale plasmid preparation under non-GMP/GMP-like conditions
Scale	GMP-like plasmid sample preparation at a scale of 100 mg

Supercoiled Plasmid Production Process



High-density fermentation

In-line lysis process

Two/three step chromatography

IND Project Progress Overview

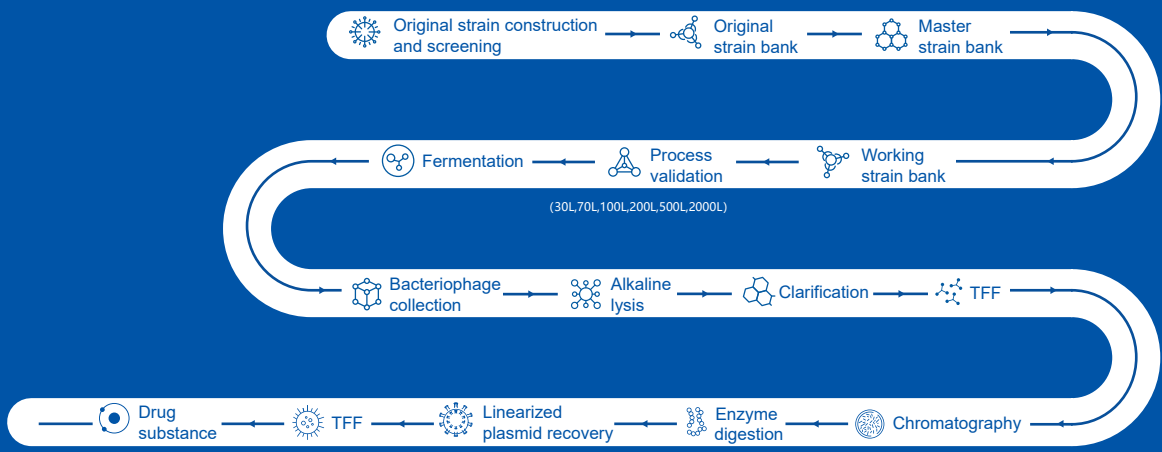
Development of plasmid project cycle	Month	1				2				3				4			
Milestones	Week	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
Recombinant strain construction	4	●	●	●	●												
Tertiary strain bank construction and passaged stability	5					●	●	●	●	●							
Lab-scale plasmid development and verification	4						●	●	●	●							
Analytical method validation	4								●	●	●	●					
GMP plasmid production, testing and release	4												●	●	●	●	
Long-term stability study (as per protocol)	N / A																→

Linearized Plasmid Process Development Flow

- Recombinant plasmids
- Genetically stable strain screening
- Tertiary strain bank construction
- Strain bank passaging and storage stability study
- Fermentation process development/optimization
- Supercoiled plasmid purification process development/optimization
- Enzyme digestion and linearization plasmid purification process study
- Process scale-up study and validation

Provide GMP-like linearized plasmid sample preparation at a scale of 100 mg

Linearized Plasmid Generation Process Flow



High-density fermentation | In-line lysis process | Two/three step chromatography

IND Project Progress Overview

Development of plasmid project cycle	Month	1				2				3				4			
Milestones	Week	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
Recombinant strain construction	4	●	●	●	●												
Tertiary strain bank construction and passaged stability	5					●	●	●	●	●							
Lab-scale plasmid process development and validation	5						●	●	●	●	●						
Analytical method validation	4								●	●	●	●					
GMP plasmid production, testing and release	5												●	●	●	●	●
Long-term stability study (as per protocol)	N / A																→

Testing Standards

Supercoiled Plasmid

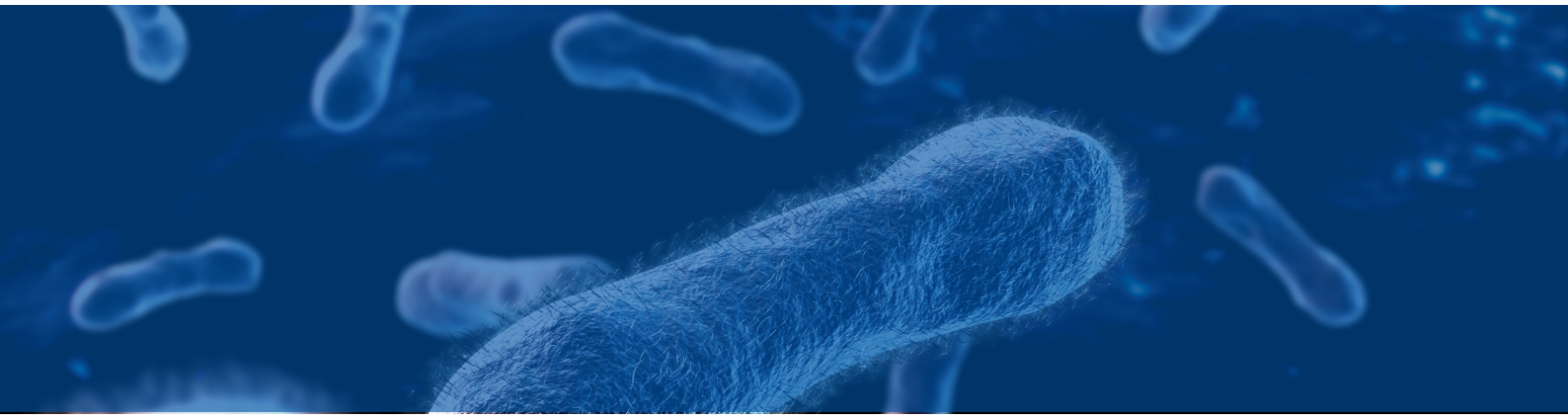
Test Items	Test Method	Specification
pH	pH determination method	7.2±0.5
Appearance	Visual method	Colorless clear liquid
Plasmid concentration	UV method	N/A
Plasmid identification	Sanger sequencing	Consistent with theoretical sequence
Plasmid assay	Restriction nuclease method	Consistent with the theoretical chromatogram
Plasmid purity	UV260/UV280	1.8~2.0
Supercoil ratio	CE	＞80%

Test Items	Test Method	Specification
Residual host genomic DNA	Q-PCR	＜0.2%
Host pre-white residue	ELISA	＜0.1%
Residual host genomic RNA	qRT-PCR	＜50μg/mg
Endotoxin	Gel method	＜10EU/mg
Antibiotic residues	ELISA	＜50ng/mg
Sterility	Direct inoculation/ film filtration	Meet the requirements

Linearized plasmids

Test Items	Test Method	Specification
pH	pH determination method	N/A
Appearance	Visual method	Colorless clear liquid
Plasmid concentration	UV method	N/A
Plasmid identification	Sanger sequencing	Consistent with theoretical sequence
Plasmid purity	UV260/UV280	1.8~2.0
Linearized plasmid ratio	CE	＞80%

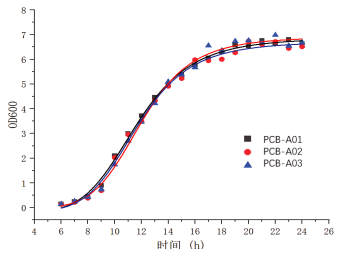
Test Items	Test Method	Specification
Residual host genomic DNA	Q-PCR	＜0.2%
Host pre-white residue	ELISA	＜0.1%
Residual host genomic RNA	qRT-PCR	＜50μg/mg
Endotoxin	v	＜10EU/mg
Antibiotic residues	ELISA	＜50ng/mg
Microbial limits	Direct inoculation method/film filtration method	Conformity
Poly A length(Optional)	LC-MS	N/A



Plasmid Service Cases

Good strain stability

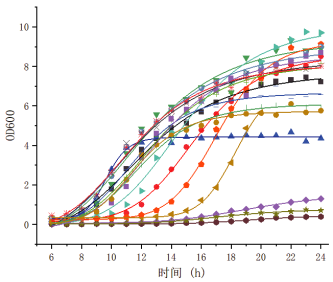
Primary Cell Bank (PCB) growth curve



PCB growth curve

Achievable DoE design of fermentation process

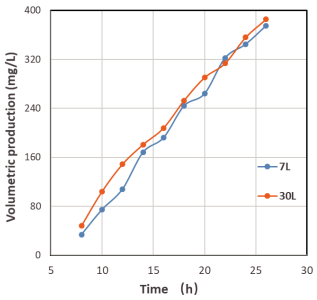
DoE design of medium screening



fermentation strain growth curve

Good stability and scalability

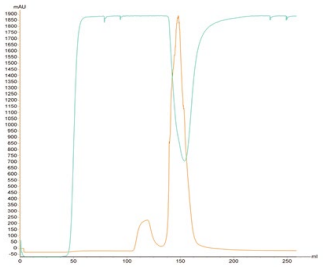
3 batches of plasmid yield at different fermentation scale of 7L and 30L



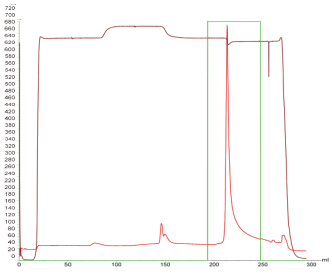
fermentation plasmid yield

Three-step / two-step purification process

Purification chromatogram



plasmid purification:
Bastarsose 6FF chromatogram



plasmid purification:
PlasmidCap HP chromatogram

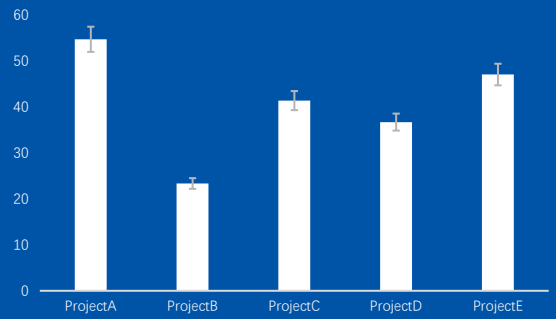
Plasmid purification platform

Recovery up to 54.67%

supercoil ratio up to 97.20%

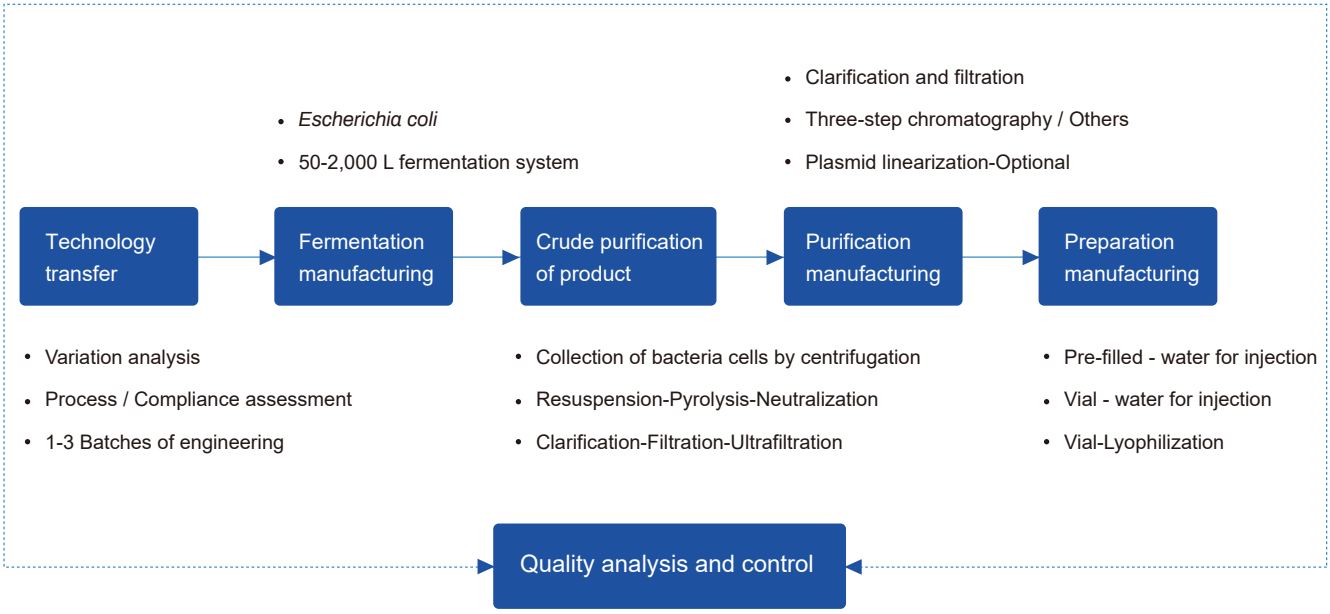
Critical residues:

HCP < 0.01%，HCD < 0.2%。



	Project A	Project B	Project C	Project D	Project E
IEC(%)	96.09	97.20	92.46	93.15	96.04
HCP(%)	< 0.1	< 0.1	< 0.01	< 0.1	< 0.1
HCD(%)	< 0.2	< 0.2	< 0.2	< 0.2	< 0.2

Overview of recombinant plasmids CMO services



Recombinant plasmids are an important vector in the field of cell and gene therapy (CGT) and gene editing, which can be used for:

Nude plasmid therapy drugs

Nude plasmid as a gene expression vector, as an alternative to protein therapy;

Raw materials of viral vector

Recombinant plasmids may be used to assemble lentivirus (LV) and adeno-associated virus (AAV) for DNA vaccine, gene therapy or gene editing;

Raw materials of mRNA/circRNA

linearized plasmid, as mRNA transcription templates in vitro, are important upstream raw materials for mRNA/circRNA vaccines or drugs.

Yaohai Bio-Pharma, Yaohai Bio-Pharma is the preferred partner for customers in the field of microbial expression systems in China. We have extensive experience in recombinant plasmids and recombinant proteins manufacturing services. We focus on *Escherichia coli* and yeast expression systems, relying on GMP-level production workshops and the comprehensive quality management system. The process flow will be strictly controlled, the releasing specification of the raw materials and excipients, intermediates and final products of recombinant biologics will also be controlled, thus to ensure an inter-batch consistency. The BLA reporting strategy is adjusted according to the regulations of different countries to meet the requirements of our customers in both China and other regions. We have served more than 100 domestic and international customers, including four plasmid projects in Phase II/III clinical stage and several projects in IND application stage.

Yaohai Bio-Pharma can provide GMP-level recombinant plasmids manufacturing services for customers, including cyclic plasmids and linearized plasmids. Our platform covers multi-scale fermentations of 50 L-100 L-200 L-500 L-1,000 L-2,000 L. The platform is equipped with multiple sizes of low/medium/high-pressure chromatography systems, and also the automatically aseptic filling system, such as water for injection vials, pre-filled syringe/-cartridge. Guaranteed by diversified production line scales, Yaohai Bio-Pharma can provide the manufacturing services for IND application samples and phase I-III clinical samples, as well as the MAH commercialization manufacturing services. We can accelerate the drug development process for our customers in a comprehensive manner.

Service details

Service Names	Service Items	Service Details	Minimum Delivery Cycle (working days)	Deliverables
Technology transfer	Document transfer	Manufacturing process/analysis methods/quality specification	TBD	Process transfer report
	Assessment of technical and regulatory compliance	Man, machine, material, method and environment variation analysis	1	
		Assessment of formulation and process	1	
		Assessment of analysis methods	3	
	Protocol transfer	Determination of overall transfer protocol	7	
	Process validation	Manufacturing of 1-3 batches of engineering	TBD Subject to customer's process	
Recombinant plasmids Fermentation manufacturing services	Confirmation before fermentation	Man, machine, material, method and environment	1	Intermediates
	Preparation of fermentation system	Preparation of culture medium and solution	2-3	
		Seed tank-fermentor sterilization		
	Fermentation manufacturing	Seed propagation-fermentation	2-3	
		Lowering tank in cooling		
Recombinant plasmid crude product purification manufacturing service	Confirmation before production	Man, machine, material, method and environment	1	
	Manufacturing preparation	Solution preparation	1-2	
	Crude purification of product	Bacterial cell collection	3	
		Bacteria suspension - alkali lysis - acid neutralization		
		Flocculation - filtration clarification and concentration - solution replacement		

Service Names	Service Items	Service Details	Minimum Manufacturing Cycle (working days)	Deliverables
Recombinant plasmids Purification manufacturing services	Confirmation before purification	Man, machine, material, method and environment	1	Plasmid stock solution
	Preparation of chromatography system	Buffer solution preparation	2-3	
		Filler preconditioning		
	Purification manufacturing	Filtration clarification	TBD Subject to customer' s process	
		Two-step / three-step chromatography		
		Plasmid linearization and purification - optional		
		Concentration and solution replacement		
Recombinant plasmids Preparation manufacturing services	Confirmation before preparation production	Man, machine, material, method and environment	1	Vial-water for injection Vial-lyophilization Prefilled syringe-water for injection Cartridge-water for injection
	Preparation before preparation production	Apparatus cleaning and sterilization	1-2	
	Preparation manufacturing (Containing placebo)	Filling of sterilized preparation	TBD Subject to customer' s process	
		Lyophilization-optional		
		Capping and visual inspection	2	
		Labeling or blind coding	-	

Note:
TBD: to be determined (subject to the customer's process); multiple testing items can be carried out at the same time.
For CMO project of recombinant plasmids stock solution + preparation, Yaohai Bio-Pharma's average delivery cycle is 1-3 months (including engineering batch, cycle for reference), and the actual delivery cycle is subject to the customer's process.

Continued table - quality analysis and control of recombinant plasmids

Service items	Test items	Test methods	Minimum Delivery Cycle (working days)
Raw materials and excipients/ packaging materials Test and release	Raw materials and excipients-critical items	Conducted in accordance to the specific test items	2
	Raw materials and excipients - full tests		11
	Packaging materials		60
Recombinant plasmid quality analysis and control	Appearance, visible foreign material	Visual	1
	Insoluble particle	Light obscuration method	1
	Particle diameter	Zeta potential method	2
	pH	Potential method	1
	Electrical conductivity	Electrode method	1
	Osmotic pressure molar concentration	Cryoscopic method	1
	Moisture content	Titration method	1
	Loss on drying	Atmospheric pressure/ Vacuum drying method	2
	Residue on ignition	Burning method	2
	Deviation of deliverable volume	Volumetric/gravimetric method	1
	Supercoiled plasmid purity or linearity plasmid purity	AGE, HPLC, CE	1-3
	Plasmid DNA concentration	UV	
	Restriction enzymes analysis spectrum	AGE	

Service items	Test items	Test methods	Minimum Delivery Cycle (working days)
Recombinant plasmid quality analysis and control	Nucleotide sequence examination of target gene	Sequencing-alignment	20-30
	Whole plasmid DNA sequencing	Sequencing-alignment	20-30
	Whole genome sequencing	Whole genome sequencing	20-30
	Host protein residue-HCP	ELISA	2
	Host DNA residue-HCD	qPCR	1
	Host RNA residue	RT-qPCR	1
	Other customized test items	-	TBD
	Bacterial endotoxin residue	Gel method, chromogenic method	3
	Antibiotic residue	ELISA, culture method	5
	Microbial limit test	Plate method, membrane filtration method	10
	Sterility test	Direct culture method, membrane filtration method	18
	Investigation of sample stability	High-temperature test	40
		Photostability test	40
		Repeated freeze-thaw test	40
		Accelerated stability test	Sampling: 0, 1, 2, 3 and 6 months
		Long-term stability test	Sampling: 0, 3, 6, 9, 12, 18 and 24 months
GMP workshop environmental monitoring	Non-host strain monitoring	Plate method	5
	Settling microbe monitoring	Culture method	8
	Surface microbial monitoring	Culture method	8
	Planktonic bacteria monitoring	Culture method	8
	Compressed air monitoring	-	10

Note:
TBD: to be determined (subject to the customer's process); Multiple testing items can be carried out at the same time.
For CMO project of recombinant plasmid stock solution + preparation, Yaohai BioPharma's average delivery cycle is 1--3 months (including engineering batch, cycle for reference), and the actual delivery cycle is subject to the customer's process.

CMO service features

Multi-scale CMO service platform

50 L-100 L-200 L-500 L-1,000 L-2,000 L multi-scale fermentation platform match with centrifugal, hollow fiber and low-pressure/medium-pressure/high-pressure chromatography equipment of corresponding scale. The preparation workshop is accommodated with GMP-level automatic filling systems, covering 1-25 mL water for injection vials (60,000 vials/batch), lyophilization (37,800 vials/batch) and 1-3 mL prefilled syringes/cartridges (20,000 vials/batch).

Standard GMP-level explosion-proof workshop

The explosion-proof solution dispensing system meets the requirements of explosion-proof. The workshop is equipped with electrostatic discharge instruments and flammable gas alarm devices, which can meet the needs of explosion-proof solution dispensing for special process.

Compliance ensuring platform

Comprehensively assess the compliance of products and quality specification, such as host source, antibiotic type, toxicity or sensitization, to meet the requirements of registration application.

Quality control and analysis services

Quality control services driven by the latest edition of Pharmacopoeia and the guiding principles of pharmaceutical manufacturing in China and at abroad, involving the release of raw materials and excipients/packaging materials, intermediates and final products.

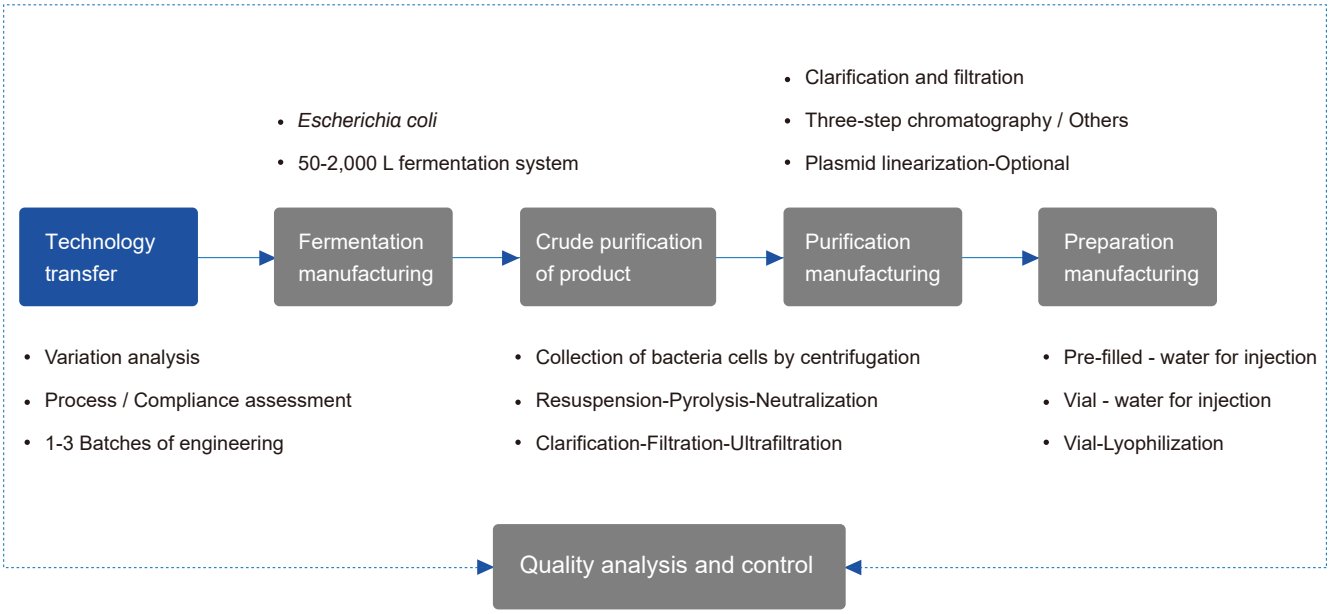
Extensive experience in technology transfer/scaling up

Conversion and scaling up parameters can be adjusted for fermentation and chromatography systems with different scales. We have successfully finished 100+ recombinant proteins & peptides & plasmids CMC projects, four of which are in phase II-III clinical stage and several of which are in IND stage.

Open online audit platform

Open online audit port, sharing VR videos of GMP workshop.

Recombinant plasmids technology transfer services



According to the ICH Q10 guidelines, the life cycle of a drug product is divided into four stages: drug R&D, technology transfer, commercial manufacturing, and product discontinuation. Technology transfer is an important part of the drug life cycle and is the key connecting link between drug R&D and commercial manufacturing. Technology transfer mainly includes manufacturing processes, intermediates control, quality specification of raw materials and excipients, testing methods and other technologies and methods related to product quality. The main goal of technology transfer is to realize the transfer of products and related knowledge between R&D and manufacturing or between different manufacturing sites, including the transfer between MAH and CDMO, CMO as well as CRO enterprises, to achieve the sustained and stable manufacturing of products.

Yaohai Bio-Pharma has established technology transfer management measures from small test process development, medium test production to GMP manufacturing stage (stock solution and preparation), and specified the technology transfer procedures in accordance with the Chinese Pharmacopoeia 2020 edition, ICH Q10, WHO, PDA TR65, ISPE and other technology transfer guidelines. Based on the concept of Quality by Design (QbD), a comprehensive risk assessment has been conducted on the transfer process in terms of regulations and quality management, and the management of whole life cycles of drugs is strengthened, to ensure the success of technology transfer and fully guarantee the safety, efficacy and quality control of drugs.

01 **Project launch**

- Team building
- Personnel training

02 **Document transfer**

- Manufacturing process
- Quality standards
- Analysis method

03 **Technical assessment Regulatory compliance assessment**

- Man，machine，material，method and environment
- Process/formulation assessment
- Test method assessment

04 **Protocol determination**

- Manufacturing conditions
- Sampling plan
- Release criteria

05 **Process verification**

- 1-3 batches
- Engineering batch verification

Service details

Service names	Service items	Service details	Minimum Delivery Cycle (working days)	Deliverables
Recombinant plasmids Manufacturing technology transfer	Document transfer	Manufacturing process	TBD (subject to customer's needs)	Process transfer report
		Quality specification		
		Analysis method		
	Assessment of technical and regulatory compliance	Man, machine, material, method and environment variation analysis	1	
		Assessment of formulation and process	1	
		Assessment of analysis methods	3	
	Protocol determination	Transfer protocol determination	7	
	Process validation	Manufacturing of 1-3 batches of engineering	TBD (subject to customer's needs)	

Note:
TBD: to be determined (subject to the customer's process).
Referred Regulations: Chinese Pharmacopoeia 2020; ICH Q10. Guidance for Industry Q10 Pharmaceutical Quality System; WHO Guidelines on the Transfer of Technology in Pharmaceutical Manufacturing; PDA Technical Report 65: Technology Transfer; ISPE Good Practice Guide: Technology Transfer.

Service features

Extensive experience in process transfer

Fully assess the completeness and feasibility of process flow and the test methods, and provide customers with comprehensive process transfer solutions.

Compliance ensuring platform

Comprehensively assess the compliance of products and quality specification, such as host source, antibiotic type, toxicity or sensitization, to meet the requirements of registration application. Establish the release criteria for raw materials and excipients, packaging materials, intermediates and final products that are compliant, with the whole process complying with the latest version of pharmacopoeia and GMP related guidelines.

Professional project management team

Professional PMs are specialized in fermentation, purification and preparation process transfer and manufacturing process, able to identify and control project risks and drive project operation in whole cycle.

Extensive experience in technology transfer/scaling up

Key parameters can be fast identified and adjusted for fermentation and chromatography systems with different scales. We have successfully finished 100+ recombinant protein & Peptide & plasmid CMC projects, four of which are in phase II-III clinical stage and several are in IND stage.

Technology transfer key parameters

Critical equipment	Main reasons affecting process parameters	Key parameters	Yaohai Bio-Pharma equipment
Fermenter	Culture volume, diameter-to-height ratio, mixing blade, maximum rotation speed	Aeration, rotation speed, dissolved oxygen	Tofflon
Centrifuge	Sample size, type of equipment (benchtop type, floor type, drum type, disc stack type)	Rotating speed, feeding, residue discharge time	GEA, Beckman, Junmiao
Chromatography system	UV detector, maximum flow rate	Retention time, sample collection time	Hanbaon, Rongjie
Chromatography columns	Processing batch, column volume	Column volume, loading/buffer solution volume	GE, Hanbon, Rongjie
Filtration/Ultrafiltration system	Processing batch, membrane area	Membrane area, flow rate	PALL, Sartorius

Note: The Yaohai Bio-Pharma Equipment column lists some equipment brands we have. Please consult our staff for more information.

During the process of technology transfer, Yaohai Bio-Pharma will perform parameter conversions for process transfer or scale up based on the inconsistent equipment models (e.g. fermenters, centrifuges and homogenizers). We will face differences in diameter-to-height ratio, stirring blade distribution and maximum speed of different brands of fermenters. Process validation and scale-up can be completed by controlling key parameters such as ventilation, rotational speed and dissolved oxygen. Different centrifugation equipment are available (benchtop type, floor type, drum type or disc stack type), and homogenizers with different capabilities are applicable for different volumes of samples. Therefore, during scaling-up process, the processes of some projects require conversion of centrifugation and homogenization equipment. The data that needs to be converted includes: centrifugation process parameters, including speed, feeding and residue discharging times (disc stack type), and the key homogenization parameters, including flow rate, pressure and times.

A conversion is required only for scale-up parameter under the condition that the Yaohai Bio-Pharma's equipment models are basically the same. During chromatographic purification, with the column height and column efficiency being maintained within a controlled range, we maintain the retention time, loading capacity and elution conditions (linear flow rate) of the original process. Only the column volume and loading volume are required to be changed according to the actual scale. During filtration or ultrafiltration, we need to change the membrane area and control the flow rate according to the actual scale-ups.

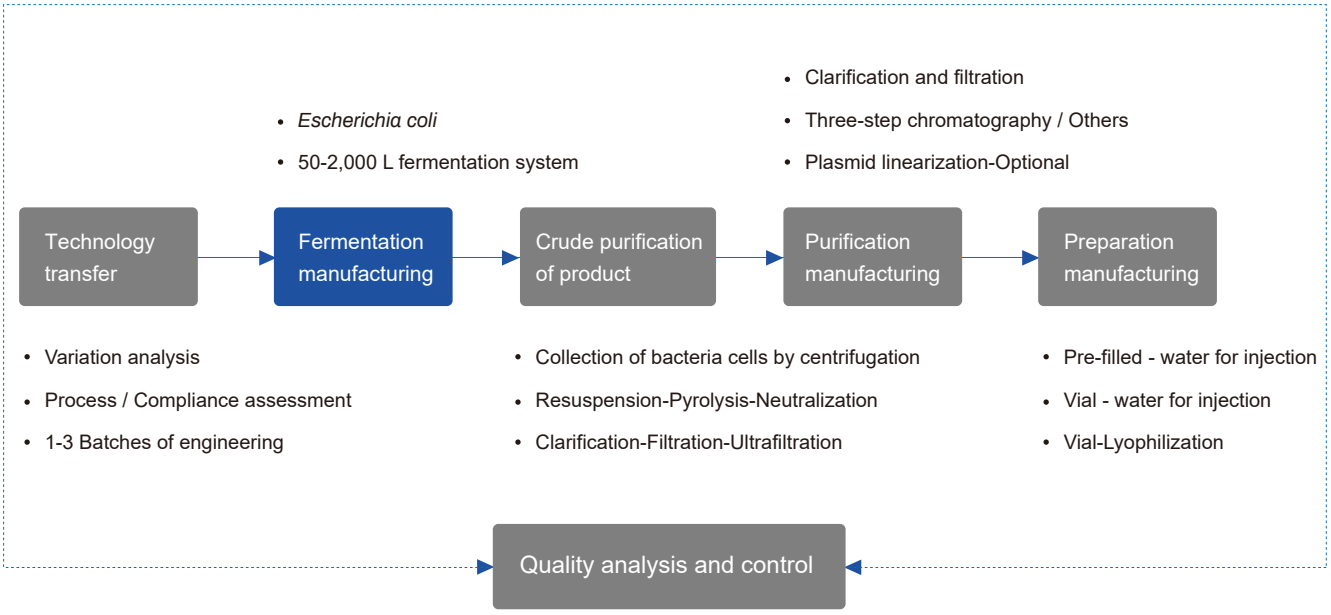
Based on the extensive CMO service experience of recombinant proteins/peptides/plasmids, Yaohai Bio-Pharma has accumulated experience in equipment-related process transfer of various brands, performances and models. We can quickly identify and adjust key equipment parameters to facilitate our customers to achieve fast delivery while maintaining the original quality of their products.

Yaohai Bio-Pharma TIPs: Times of process scale-up is recommended to be within 10 times, and 1-3 batches of engineering are recommended to be used to control the risk of process scale-up.

Other Services

- Technology transfer
- Fermentation manufacturing services
- Crude purification manufacturing services
- Purification manufacturing services
- Preparation manufacturing services
- Quality analysis and control services

Fermentation manufacturing services



Yaohai Bio-Pharma, in the field of microbial expression system, has extensive experience in the manufacturing services of recombinant plasmids and recombinant proteins. Relying on five independently operating GMP-level customized production lines with fermentation scales of 50 L-140 L-200 L-500 L-1,000 L-2,000 L, we can meet different project needs of customers, and have served more than 100 customers at home and abroad, with extensive experience in industrial manufacturing of fermentation.

During the fermentation process scale-up, process control parameters unrelated to scale are also kept consistent, including culture conditions, such as basal medium, fed-batch medium, temperature, and pH, as well as the inoculation and feeding supplement. For scale-related parameters, including culture volume, aeration and agitation rate, it is required to control the key parameters during process transfer.

Based on the extensive experience in CMO service, Yaohai Bio-Pharma can perform the appropriate process transfer and scale-up for different size/brand of fermenters, control key parameters, successfully achieve scale-up manufacturing of upstream processes and transfer to downstream processes with high-quality.

01 Preparation of fermentation system

- Empty elimination of fermentation tank
  Medium - real elimination of fermentation tank
  Preparation and sterilization of other solutions

02 Shake flask culture

- Inoculate working seeds to shake flask for cultivation (Primary or secondary)

03 Seed tank culture

- Seed tank inoculation
  Culture process control

04 Fermentation culture

- Feeding control
  Fermentation process control

05 Lowering tank in cooling

- Lowering tank in cooling
  Workshop line clearance

Service details

Service items	Service details	Detailed procedures	Minimum delivery cycle (working days)	Deliverables
Recombinant plasmids Fermentation manufacturing services	Confirmation before fermentation	Confirmation of man, machine, material, method and environment	1	Intermediates
	Preparation before fermentation manufacturing	Receipt of document and material		
		Reconfirmation of conditions before production in GMP workshop		
	Preparation of fermentation system	Seed tank empty elimination, culture medium preparation and real elimination	2-3	
		Fermenter empty elimination, culture medium preparation and real elimination		
		Feeding tank empty elimination, culture medium preparation and real elimination		
		Preparation of acid and base and defoamer solution		
	Fermentation manufacturing	Seed culture in shake flask	2-3	
		Seed tank culture		
		Fermentation culture		
		Lowering tank in cooling		
Line clearance procedure	Line clearance procedure	Equipment Cleaning, Sterilization and Environmental Disinfection	-	-

Note: the table shows the shortest service period by taking *Escherichia coli* as an example, and the yeast is increased as appropriate according to the fermentation process.

Service features

Mature GMP management system

The workshop staffs and QA/QC personnel have been strictly trained and instructed under GMP, and comply with all specifications of the latest GMP requirements.

Multi-size fermentation system

There are five manufacturing lines for stock solution, which are built in accordance with international GMP requirements, and can provide fermentation scales of 50 L-140 L-200 L-500 L-1,000 L-2,000 L and support manufacturing needs at different development stages.

Diversified fermentation platform

According to the needs of customers' process, meet the needs of the high-density fermentation process, customized feeding process of *Escherichia coli* with or without resistance.

Compliant testing and releasing specification

The brand and batch number of materials (raw materials and excipients) are verified, and the key materials are tested for releasing to ensure consistency and effectiveness of the materials.

Single project operation system

Only one project is allowed to be operated in each workshop during each time period to effectively prevent contamination and mix-ups, and the subsequent project shall be carried out only after Line clearance procedure the requirements.

Experience sharing of fermentation process transfer

The key parameters of the fermentation process include dissolved oxygen (DO), temperature and pH. Dissolved oxygen is an valid feedback parameter of growth state of strains. Temperature and pH directly affects the growth, proliferation and product expression of strains.
Based on the extensive experience in CMO manufacturing services, Yaohai Bio-Pharma has summarized the questions frequently occurred during fermentation process transfer or scale-up and the solutions:

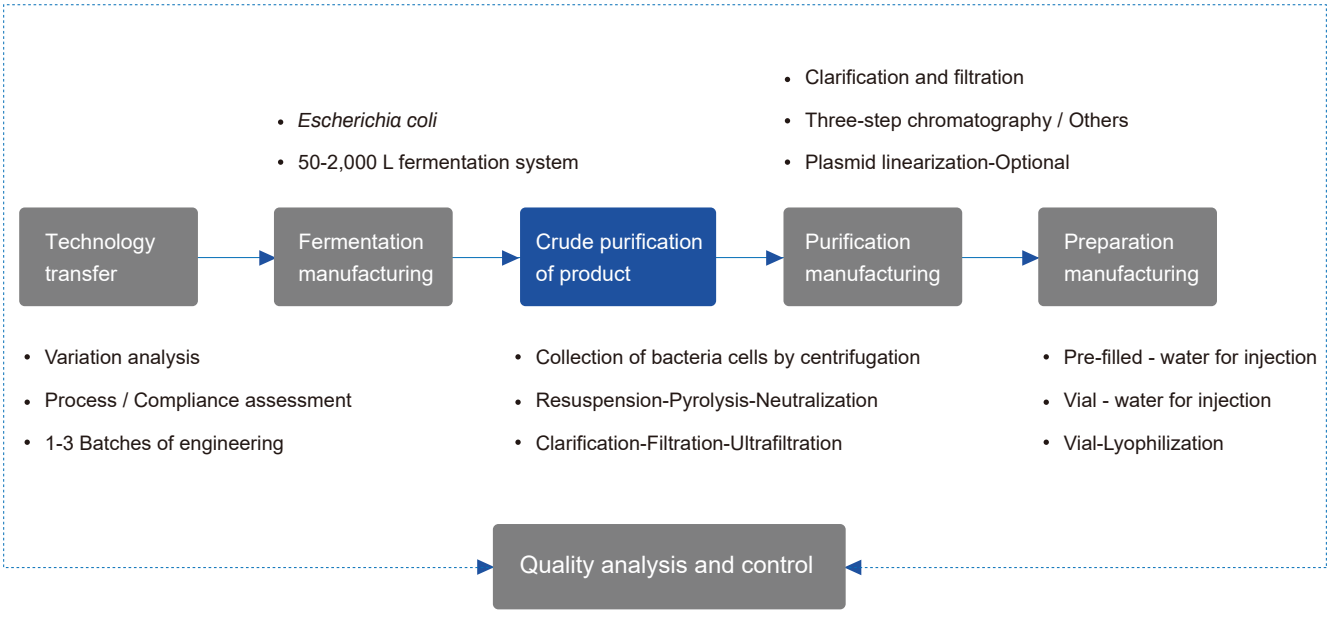
Parameter types	Related parameters	Frequently asked questions	Prevention or solutions
Questions related to culture conditions	Temperature and pH	[Volume-independent parameters, consistent]	The temperature, pH sensor, pump and other equipment are calibrated and tested under GMP quality standards.
	Feeding strategy		
	Rotation speed	How to conduct process transfer and scale-up if the maximum rotation speed of the fermenter is lower than the original process?	The function of agitation is to mix materials and improve the oxygen transfer coefficient, which is generally adjusted according to dissolved oxygen. A certain range of rotate speed is recommended during the process development, which may facilitate process transfer and scale-up.
	Ventilation	How to determine the aeration volume of the fermentation process during process transfer or scale-up?	The purpose of aeration is to provide oxygen for bacterial cell, improve oxygen transfer coefficient, and discharge exhaust gas at the same time, and the amount can be set to a fixed value or adjusted according to **dissolved oxygen**. It is recommended that a certain range of aeration amount should be verified during process development to facilitate process transfer and scale-up.
	Dissolved oxygen	The influencing factors of dissolved oxygen include: Fermentation liquor volume, viscosity, rotational speed, aeration volume, etc.	The process in which dissolved oxygen can be automatically controlled: after the parameter range of dissolved oxygen is set, it is controlled by adjusting agitation and aeration volume.
Bacterial cell volume related questions	$OD_{600\ nm}$	There is a significant variation between $OD_{600\ nm}$ value and the value of original process	The variation of instruments should be considered. As the principle and sensitivity of different spectrophotometers are different, so it is not recommended to limit OD value excessively.
	Solid content of bacterial solution	-	It is recommended to use wet/dry weight of bacteria cells (weighing method) as the valid parameter of bacteria cell amount in reference to the solid content of bacterial solution (visual method).
	Bacterial cell weight	-	

Yaohai BioPharma TIPs: It is not recommended to establish quality standards for intermediate products when there are only few running batches. A collection of relevant data is recommended, and then the quality standards and error ranges can be set by using statistical methods when there are enough data.

Other Services

- Technology transfer
- Fermentation manufacturing services
- Crude purification manufacturing services
- Purification manufacturing services
- Preparation manufacturing services
- Quality analysis and control services

Crude purification manufacturing services



In the field of microbial expression systems, Yaohai Bio-Pharma has extensive experience in the manufacturing services of recombinant plasmids and recombinant proteins. Five independently operating GMP-level production lines are available, which are equipped with centrifugation equipment with different processing batches and different types. It can match the crude purification manufacturing for fermentation batches of 50 L-140 L-200 L-500 L-1,000 L-2,000 L fermentation tank. Yaohai Bio-Pharma can meet the needs of different projects of our customers. Currently, Yaohai Bio-Pharma has served more than 100 customers at home and abroad and has extensive experience in industrial manufacturing of crude purification.

The function of crude purification is to separate substances with large differences, such as solid-liquid separation, intracellular or extracellular substance separation. The crude purification process of plasmids includes centrifugation, alkali lysis and membrane filtration. Collection of bacterial cells is performed by solid-liquid separation, and the plasmid DNA is released using alkaline lysis, then the suspended substances are finally removed through filtration. Due to the different scales of small tests and manufacturing, the adapted centrifugation equipment also varies. The conversion of centrifugation parameters is particularly important during the manufacturing process, which can greatly affect the quality and yield of the product.

01 Solution dispensing

- Resuspension preparation
  Lysate preparation
  Neutralization solution preparation

02 Bacterial cell collection

- Centrifugal collection of bacteria cells
  Rotation speed/feeding residue discharge time

03 Bacterial lysis

- Suspension - lysis - neutralization
  Duration of lysis / neutralization

04 Clarification-filtration

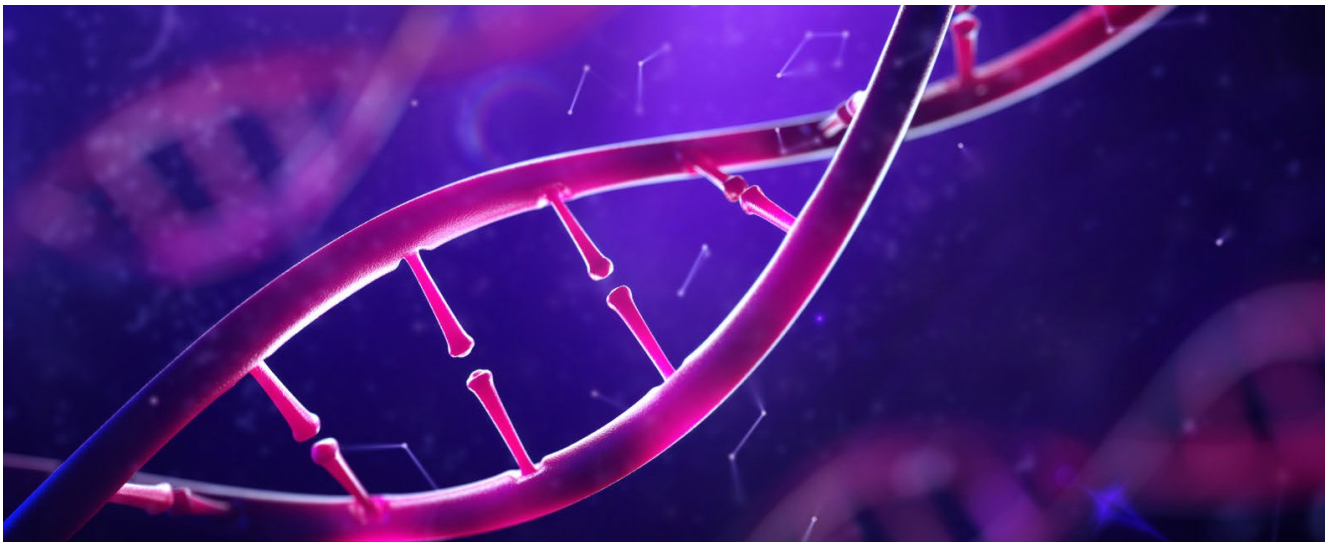
- Solution clarification
  Solids removed by filtration

05 Concentration and solution replacement

- Hollow fiber
  Concentration/solution replacement

Service details

Service items	Service details	Detailed procedures	Minimum Delivery Cycle (working days)	Deliverables
Recombinant protein crude product purification manufacturing services	Confirmation before production	Confirmation of man, machine, material, method and environment	1	Intermediates
	Preparation before production	Buffer solution preparation	1-2	
	Crude purification manufacturing	Collection of bacteria cells by centrifugation	1	
		Cell resuspension	1	
		Alkali lysis		
		Acid neutralization		
		Filtration and clarification	2	
		Concentration and solution replacement by ultrafiltration		
Line clearance	Workshop line clearance	equipment cleaning, sterilization and environmental disinfection	-	-



Service features

Mature GMP management system

The workshop staffs and QA/QC personnel have been strictly trained and instructed under GMP, and comply with all specifications of the latest GMP requirements.

Multi-scale crude purification equipment

Five GMP-level production lines for stock solution are available, which are equipped with benchtop type/floor type/drum type/disc stack type centrifuges and membrane cassettes with different pore sizes, areas and flow rates to meet the crude purification needs of fermentation liquor product with different sizes.

Compliant testing and release criteria

Key materials (raw materials and excipients) are tested for releasing to ensure effectiveness of the materials.

Single project operation system

Only one project is allowed to be operated in each workshop during each time period to effectively prevent contamination and mix-ups, and the subsequent project shall be carried out only after the line clearance passes the requirements.

Experience sharing of crude purification process transfer

The purpose of centrifugation and filtration is to achieve solid-liquid separation, and the application scenarios include: collection of bacterial cells and the removal of solid-shaped substances, etc. The key parameters of centrifugation equipment include: rotation speed, feeding speed and residue discharging time. The key parameters of filtration include: aperture size of filter membrane, flow rate and filter pressure. Centrifugation or filtration that does not meet the criteria may result in poor solid-liquid separation, which may lead to the decrease in product yield, increase of the burden of downstream purification and pose impacts on product quality.

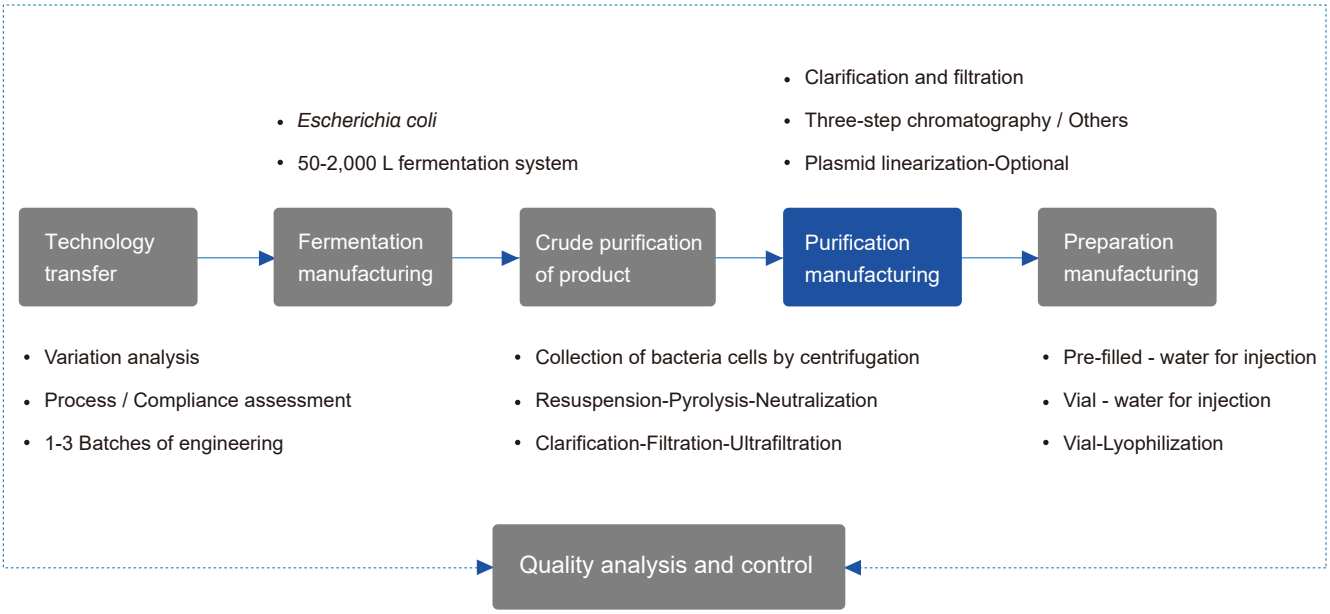
Based on the extensive experience in production, Yaohai Bio-Pharma has summarized the questions frequently occurred in the transfer process of centrifugation and clarification and filtration and the solutions:

Crude purification process	Frequently asked questions	Question analysis	Solutions
Centrifugation	Turbid supernatant	Poor solid-liquid separation Reduced yield (intracellular products) Have negative effect on product quality	• Too much feed: reduce the feeding rate • Uneven feed: fully stirring before feeding • Low rotate speed: increase the rotate speed • Improper residue discharge time: adjust the residue discharge time
Filtration	How to ensure the effectiveness of filtration after clarification?	If obvious suspended matter exists after clarification, direct use of small aperture filter may lead to clogging.	• It is recommended to adopt at least two-step filtration, filter membrane aperture shall be from large to small. • Real-time monitoring shall be conducted for filter pressure to control the pressure below 0.2 Mpa.

Other Services

- Technology transfer
- Fermentation manufacturing services
- Crude purification manufacturing services
- Purification manufacturing services
- Preparation manufacturing services
- Quality analysis and control services

Purification manufacturing services



In the field of microbial expression systems, Yaohai Bio-Pharma has extensive experience in the manufacturing services of recombinant plasmids and recombinant proteins. The purification workshop is equipped with different sizes of automatic or manual membrane filtration systems and low/medium/high-pressure chromatography systems. It can meet the requirements of classical three-step chromatography (molecular sieve/gel filtration chromatography [SEC], sulfurophilic affinity chromatography [AC], anion exchange chromatography [IEX]) and GMP manufacturing requirements for other customized chromatography processes.

01 Purification system preparation

- Buffer solution preparation
  Filler preconditoning

02 Filtration clarification

- Filtration clarification of samples
  Solids removing

03 Chromatograp hy purification

- Classic three-step process
  Two-step process
  Other chromatographic process

04 Linear purification -purification

- Linearity plasmid
  -Optional steps

05 Concentration and solution replacement

- Hollow fiber
  Concentration/solution replacement

Service details

Service items	Service details	Detailed procedures	Minimum lead time (working days)	Deliverables
Recombinant plasmids Purification manufacturing services	Confirmation before purification	Confirmation of man, machine, material, method and environment	1	Plasmid stock solution
	Preparation before purification	Receipt of documents and materials		
		Reconfirmation of conditions before production in GMP workshop		
	Purification system preparation	Buffer solution preparation	2	
		Filler preconditioning		
	Purification manufacturing	Filtration and clarification	TBD subject to customer's process	
		Classic three-step process Gel filtration chromatography → Sulfurophilic affinity chromatography → Ion Exchange chromatography		
		Other purification process-optional		
		Plasmid linearization and purification - optional		
		Concentration/solution replacement		
		Filtration sterilization		
Line clearance	Workshop line clearance	Equipment cleaning, sterilization and environmental disinfection	-	-

Note:
TBD: to be determined (subject to the customer's process).
It can meet the requirements of classical three-step processes (molecular sieve/gel filtration chromatography [SEC], sulfurophilic affinity chromatography [AC], anion exchange chromatography [IEX]) and other customized processes requirements, including composite chromatography.

Service features

Mature GMP management system

The workshop staffs and QA/QC personnel have been strictly trained and instructed under GMP, and comply with all specifications of the latest GMP requirements.

Multi-scale purification system

There are five independent purification production lines, which are equipped with low-pressure chromatography systems with flow rates of 6-600 L/h, multi-size solution dispensing tanks and chromatography columns, high-pressure chromatography for industrial preparation, and 5-60 L ultrafiltration system.

Standard GMP-level explosion-proof workshop

The explosion-proof solution dispensing system meets the requirements of explosion-proof, and the workshop is equipped with electrostatic discharge instruments and flammable gas alarm devices, which satisfies the needs of solution dispensing in special process, such as reversed-phase chromatography.

Compliant testing and releasing specification

The brand and batch number of materials (raw materials and excipients) are verified, and the key materials are tested for releasing to ensure consistency and effectiveness of the materials.

Single project operation system

Only one project is allowed to be operated in each workshop during each time period to effectively prevent contamination and mix-ups, and the subsequent project shall be carried out only after the line clearance passes the requirements.

★★★ To meet the needs of solution dispensing of organic solvent in special processes such as reversed-phase chromatography, purification workshops are equipped with explosion-proof solution dispensing systems complying with the requirements of explosion-proof, and are installed with electrostatic discharge instruments and equipped with combustible gas alarm devices.

Experience sharing of purification process transfer

In the process of purification and scale-up production, filtration and clarification of the first step are essential. Clarification is designed to further remove particulate substances to avoid negative effects on downstream purification, which is usually completed using hollow fiber or membrane packs. Key parameters during process transfer or scale-up include processed batch size, membrane area, and flow rate.

Chromatographic purification is the procedure of removing impurities of different sizes, charges, polarities and specificities using different chromatographic fillers to obtain a high purity target product. The classical three-step plasmid purification chromatography is: 1. RNA removal by molecular sieve/gel filtration chromatography (SEC); 2. superhelical plasmids capture by affinity chromatography (AC); and 3. dotoxin removal by anion exchange chromatography (ICX). Key parameters in chromatographic process transfer include: processed batch size, column volume, loading volume and flow rate.

Based on the extensive experience in plasmid manufacturing services, Yaohai Bio-Pharma has summarized the questions frequently occurred during purification process transfer and scale-up and the solutions:

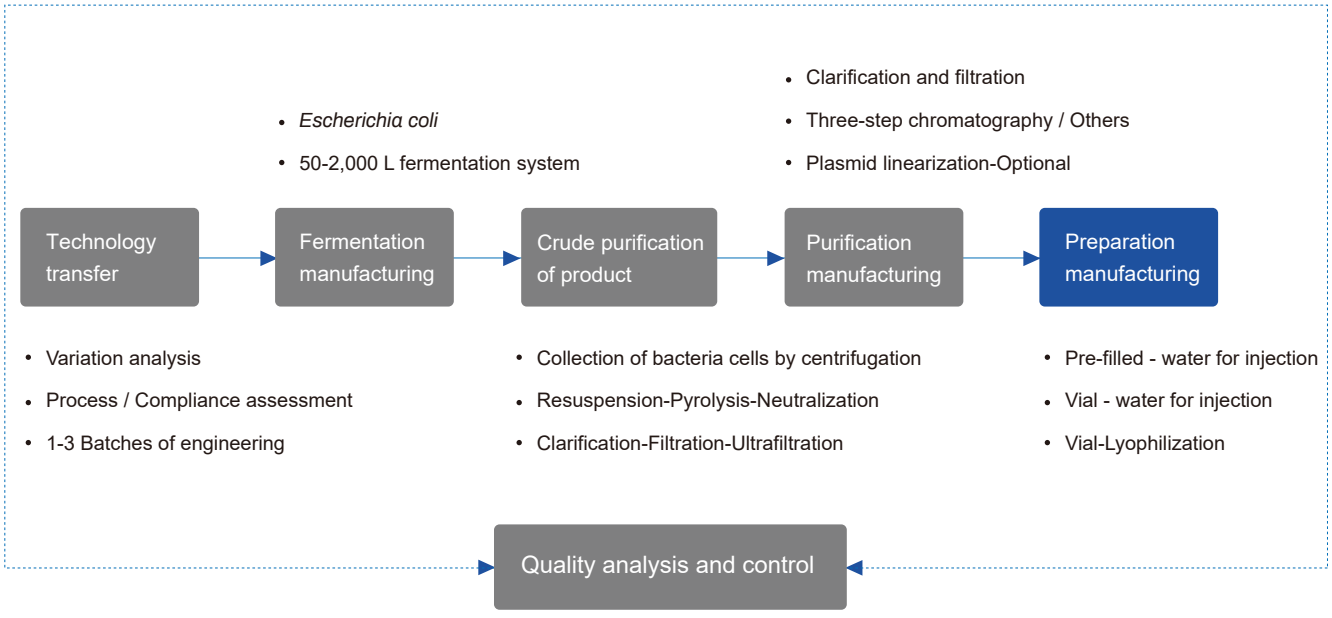
Purification process	Frequently asked questions	Process scale-up strategies
Filtration and clarification	No clarification process This process step is omitted in the small tests or medium tests	*Suggestion*: clarify the samples during the process scale-up to remove the solid-shaped substances, so as not to increase the burden in the downstream purification.
Chromatographic process	How to calculate column volume and loading amount when scaling up the chromatography process?	*Consistent parameters*: sample concentration and composition, buffer solution composition, column height, linear flow rate, ratio of loading volume/ column volume; *Scale-up* parameters: sample volume, column diameter, buffer solution volume, volume flow rate.
Membrane filtration	How to calculate membrane area and flow rate when membrane filtration process is scaled up?	*Consistent parameters*: sample concentration and composition, membrane aperture, linear flow rate; *Scale-up parameters*: sample volume, membrane area, volume flow rate.

Note: the above table lists the simple and general purification process scale-up strategies. If there are special process needs, you can also communicate with the technical team from Yaohai Bio-Pharma to solve them.

Other Services

- Technology transfer
- Fermentation manufacturing services
- Crude purification manufacturing services
- Purification manufacturing services
- Preparation manufacturing services
- Quality analysis and control services

Preparation manufacturing services



Relying on the GMP-level high-tech automatic manufacturing lines with multiple processes (including vial washing, drying, sterilizing, aseptic filling, freeze-drying, capping, and etc.) integrated together, Yaohai BioPharma provides the manufacturing services of sterile biopharmaceutical preparations. The types of preparations include vial water injectables 10 million vials/year, vial powder injectables 5 million vials/year, and pre-filled water injectables (prefilled syringes/cartridges 8 million vials/year.

Yaohai BioPharma's sterile preparation manufacturing lines conform to the manufacturing specifications for sterile preparations of FDA, EU EMA, China NMPA and Australia TGA, and can be used for the formulation and aseptic filling of drugs and placebos, meeting the needs for IND application, Phase I-III clinical research, and MAH commercialization.

Yields / Dosage form	Water for injection vials 1 mL-25 mL	Lyophilized vials 1 mL-25 mL	Pre-filled syringe/cartridge water for Injection 1 mL-3 mL
Batch manufacturing	60,000 vials/batch (1-10 mL)	37,800 vials/batch (2 mL/4 mL) 20,043 vials/batch (7 mL/10 mL)	20,000 vials/batch
Annual yields	10 million vials/year	5 million vials/year	10 million vials/year

01 **Preparation for formulation manufacturing**

- Preparation and sterilization of formullation
  Sterilization of rubber stopper-aluminum cap

02 **Vial sorting and vial washing**

- Vial sorting - vial washing
  Drying sterilization

03 **Filling and stoppering**

- Normal/nitrogen filling/ vacuum
  Partial stoppering/full stoppering

04 **Freeze-drying**

- Freeze-drying - full stoppering
  Unique process of powder injectables

05 **Capping and visual inspection**

- Capping - light inspection - warehousing

Service Details

Service items	Service details	Detailed procedures	Minimum delivery cycle (working days)	Deliverables
Recombinant plasmids Preparation manufacturing services	Confirmation before preparation production	Confirmation of man, machine, material, method and environment	1	Vial-water for injection Vial-powder injectables Prefilled syringe-water for injection Cartridge-water for injection
	Preparation before production	Receipt of documents and materials		
		Reconfirmation of conditions before production in GMP workshop		
	Apparatus preparation	Apparatus cleaning and sterilization	1	
	Preparation manufacturing	Vial sorting and vial washing	1	
		Formulation preparation-optional	1	
		Filtration sterilization of samples		
		Filling and stoppering (normal/nitrogen filling/vacuum)	1	
		Lyophilization-optional (normal/nitrogen filling/vacuum)	TBD subject to customer' s process	
		Capping	1-2	
		visual inspection		
		Labeling and blind coding	-	
Line clearance	Workshop line clearance	Equipment Cleaning, Sterilization and Environmental Disinfection	-	-

Note: TBD: TBD: to be determined (subject to customer's process and batch size);
The current preparation workshop can provide the productions of water for injection vials/lyophilization, pre-filled water for Injection (pre-filled syringe and cartridge), and communication on other dosage forms are also welcomed.

Service features

Mature GMP training system

The workshop staff and QA/QC personnel have been strictly trained and instructed under GMP, and comply with all specifications of the latest GMP requirements.

Diversified preparation service

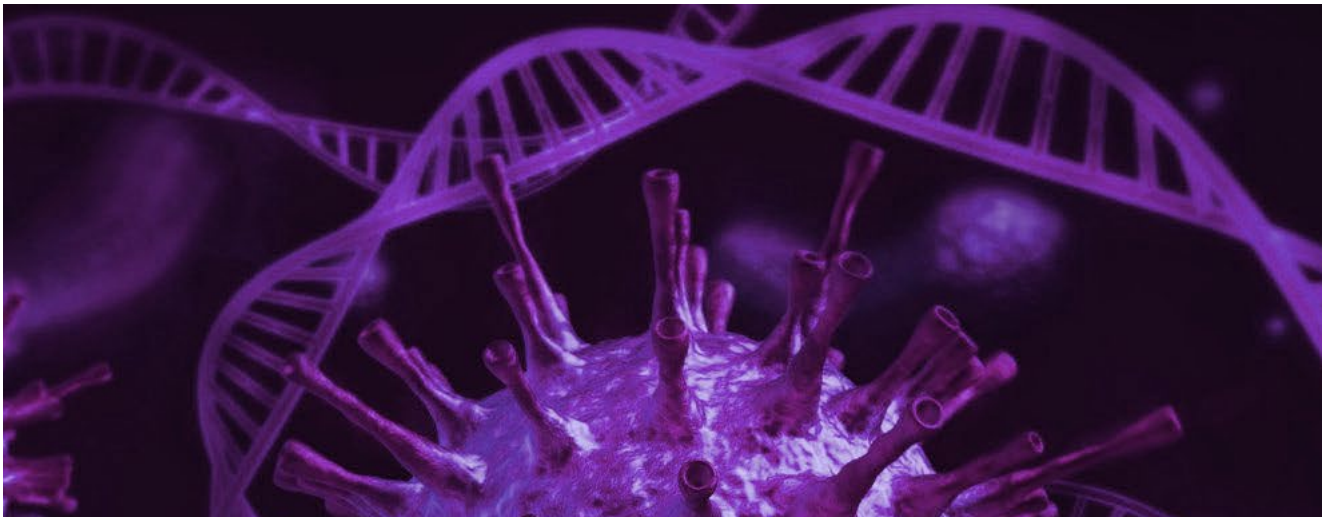
GMP-compliant automated sterile preparation production lines can serve the following products: 1-25 mL water for injection vials/lyophilization, 1-3 mL pre-filled syringe/cartridge water injectables.

Aseptic preparation production line

Conforming to aseptic preparation manufacturing requirements of US FDA, EU EMA, China NMPA and Australia TGA, O-rabs system (Open Restricted Access Barrier System) are used to protect the exposure areas of products (and the packaging materials), providing grade A environmental protection under grade B background.

Extensive project experience

The professional PMs have 100+ CMO project experience and are proficient in preparation process scale-up manufacturing, and can provide professional advice for a variety of plasmid drugs based on the products of customer.



Experience sharing of aseptic preparation process scale-up

The DNA purity of superhelical plasmids is an important index of plasmid products, which directly affects the release of products. However, some plasmids are sensitive to shear force and are easily affected by technological factors, resulting in fracture and notch formation, which affects the drug quality.

Therefore, in the process of plasmid production, we should control the process in all aspects to reduce the loss of supercoiled plasmids and ensure the high quality delivery of products. We strictly control the adverse effects of our manufacturing equipment and processes on plasmid DNA. For shear-sensitive plasmid products, we select equipment with high adaptability to minimize shear effects.

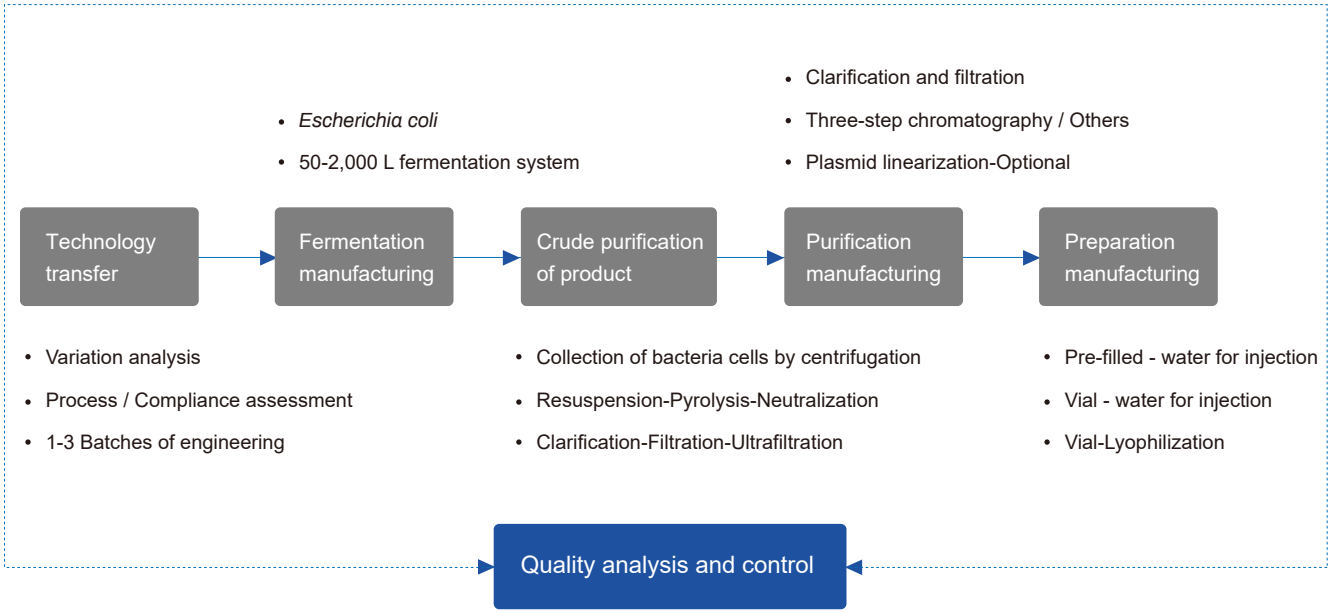
Preparation process	Critical equipment type	Equipment features
Aseptic filling	Ceramic plunger pump	• High filling precision, stable control of filling volume • Robust and corrosion resistant • Higher cost • Pump body is in direct contact with liquid medicine, with a certain cleaning difficulty
	Peristaltic Pump	• Lower filling precision • The liquid in the pump only contacts the silicone tube • Low cost, only need to replace the silicone tube, facilitating exclusive use • Weak shear force, suitable for shear sensitive biomacromolecule drugs

Yaohai BioPharma TIPs: some plasmid DNA is sensitive to shear force, so it is recommended to use peristaltic pump for filling control to avoid the impact of shear force on the quality of plasmid products. In addition, considering the cost of the equipment use, the liquid in the peristaltic pump only contacts the pipeline, not other pump body. Replacing the silicone tube will realize the exclusive use of a single variety, saving the cost.

Other Services

- Technology transfer
- Fermentation manufacturing services
- Crude purification manufacturing services
- Purification manufacturing services
- Preparation manufacturing services
- Quality analysis and control services

Quality analysis and control services



Human gene therapy products include plasmid vector, viral vector or bacterial vector. According to the pharmacopoeia, the quality control system of gene therapy products mainly includes raw materials and excipients, package materials, manufacturing process and process control and tests of products. Quality control involves assessment of known/potential products and process-related substances by using standard substances and validated methods, and analysis of test items of product appearance identification, activity, purity and impurities.

Yaohai Bio-Pharm has a comprehensive quality analysis and control system. Our team members have thoroughly proficient in pharmacopoeia and other regulatory specifications. They have own extensive experience in quality testing and analysis. We can quickly complete the transfer and validation of analytical method and quality specification, and effectively guarantee the release specification of raw materials and excipients, intermediates, stock solution of recombinant plasmids and preparations.

Service details

Service items	Test items	Test methods	Minimum Delivery Cycle (working days)
Raw materials and excipients /packaging materials Test and release	Raw materials and excipients-critical items	Conducted in accordance to the specific test items	2
	Raw materials and excipients-full inspection		11
	Packaging materials		60
Recombinant plasmid Quality analysis and control	Appearance, visible foreign material	visual	1
	Insoluble particle	Light obscuration method	1
	Particle diameter	Zeta potential method	2
	pH	Potential method	1
	Electrical conductivity	Electrode method	1
	Osmotic pressure molar concentration	Cryoscopic method	1
	Moisture content	Titration method	1
	Loss on drying	Atmospheric pressure/ Vacuum drying method	2
	Residue on ignition	Burning method	2
	Deviation of deliverable volume	Volumetric/gravimetric method	1
	supercoiled plasmid purity or linearity plasmid purity	AGE, HPLC, CE	1-3
	Plasmid DNA concentration	UV	
	Restriction enzymes analysis spectrum	AGE	
	Nucleotide sequence examination of target gene	Sequencing-alignment	20-30
	Whole plasmid DNA sequencing	Sequencing-alignment	20-30
	Whole genome sequencing	Whole genome sequencing	20-30
	Host protein residue-HCP	ELISA	2
	Host DNA residue-HCD	qPCR	1
	Host RNA residue	RT-qPCR	1
	Other customized test items	-	TBD

Service items	Test items	Test methods	Minimum Delivery Cycle (working days)
Recombinant plasmid Quality analysis and control	Bacterial endotoxin residue	Gel method, chromogenic method	3
	Antibiotic residue	ELISA, culture method	5
	Microbial limit test	Plate method, membrane filtration method	10
	Sterility test	Direct culture method, membrane filtration method	18
	Investigation of sample stability	High-temperature test	40
		Photostability test	40
		Repeated freeze-thaw test	40
		Accelerated stability test	Sampling: 0, 1, 2, 3 and 6 months
		Long-term stability test	Sampling: 0, 3, 6, 9, 12, 18 and 24 months
GMP workshop environmental monitoring	Non-host strain monitoring	Plate method	5
	Settling microbe monitoring	Culture method	8
	Surface microbial monitoring	Culture method	8
	Planktonic bacteria monitoring	Culture method	8
	Compressed air monitoring	-	10

Note: Multiple test items can be carried out at the same time.

CMO service features

Mature GMP training system

The QA/QC personnel have been strictly trained and instructed under GMP comply with all specifications of the latest GMP requirements.

Compliant QC testing process

Being able to reasonably assess the compliance of analytical methods and quality release specifications, and can quickly complete the transfer and validation of the analytical methods.

Whole-process quality control

The raw materials and excipients, intermediates, stock solution of plasmid DNA and preparations are tested for releasing, with the releasing quality specification of materials and samples strictly controlled.

Complete quality analysis platform

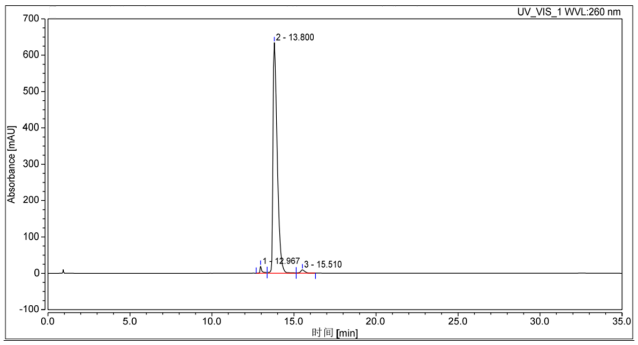
Based on our extensive experience in CMO services, the quality control team of Yaohai Bio-Pharma has established a highly applicable, robust and reliable analysis platform that can meet the requirements of physiological, biochemical and microbiological testing with stringent specification.

Quality analysis case sharing

In a project of testing the long-term stability of AAV raw plasmid samples, the QC team of Yaohai Bio-Pharma tested long-term stored GMP-level plasmid products after successfully transferring the analytical method, including: supercoiled plasmid purity, residual endotoxins, residual host proteins (HCP), residual host DNA (HCD), residual host RNA, and residual antibiotics, etc, all of the results met the quality acceptance specification, and a complete COA report was delivered to the customer.

Purity of supercoiled plasmid:

According to HPLC analysis, the proportion of superhelix plasmids could reach more than 97% (Figure-Peak 2).



Critical residual items	
Endotoxin residue	＜40 EU/mg
Kanamycin residue	＜0.1 ng/mg
Host protein residue(HCP)	＜0.003 μg/mg
Host DNA residue(HCD)	0.93 μg/mg
Host RNA residue	＜0.0004 μg/mg

Note: All the above test results are with good repeatability.

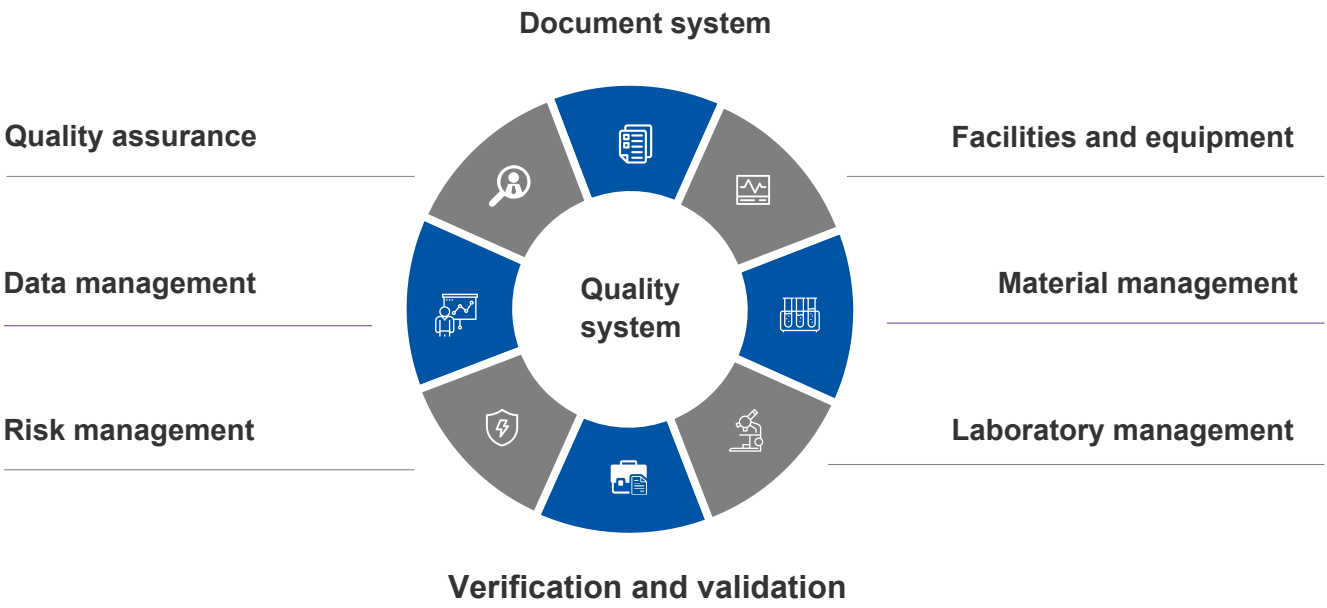
Other Services

- Technology transfer
- Fermentation manufacturing services
- Crude purification manufacturing services
- Purification manufacturing services
- Preparation manufacturing services
- Quality analysis and control services

GMP quality assurance system

Good Manufacturing Practice (GMP) is the basic guideline for drug manufacturing and quality management, which applies to the whole process of drug preparation manufacturing and the key processes affecting the quality of finished products in API manufacturing. The vigorous implementation of GMP is to avoid contamination and cross-contamination in the drug manufacturing process to the maximum extent possible and to reduce the occurrence of various errors, which is an important measure to improve the quality of drug products.

The bio-quality system management personnel in Yaohai Bio-Pharma have GMP certification experience, and the executive team has extensive GMP work experience. Our team members are proficient in studying, interpreting and translating global regulations. We have developed a compliant quality management system by combining different life cycle stages of drugs. We also manage and control the whole process of man-machine-material-method-environment in the production stage.



Document system

- Policies of management (POL), standard operation procedures (SOPs)
- Process procedures/quality specification/standard test procedures (STP)
- Form records: adhere to SOP and STP, with independent approval

Quality assurance

• System management: document/record, training, change/deviation/CAPA/complaints, self-test, material/supplier management

• Site management: manufacturing site, QC site, material control, utility system, record review, product release

Data management

- Computerized system management
- Laboratory raw data management
- Data audit, data reliability management

Risk management

- Line confluence risk control: stage manufacturing/dedicated apparatus
- Sterile contamination risk control: facility/equipment/material control
- Compliance risk control: self-test/audit/regulation translating
- Quality system risk control: change/deviation/CAPA

Verification and validation

- Verification of plant and facilities
- Equipment verification
- Computerized system validation
- Process validation
- Metrology management
- Cleaning verification
- Aseptic process simulation
- Validity period validation, etc.

Laboratory management

• Management of samples/references, reagents and consumables
• Verification and validation of analytical methods, management of entrusted testing
• Data, record and report management, quality information management

Material management

• 1,400 m^2 storage area, conforming to GMP and FDA specifications
• For storage of raw materials and excipients, packaging materials, intermediates, finished products, and etc.
• Storage conditions include freezing, refrigerating or ambient/room temperature

Facilities and equipment

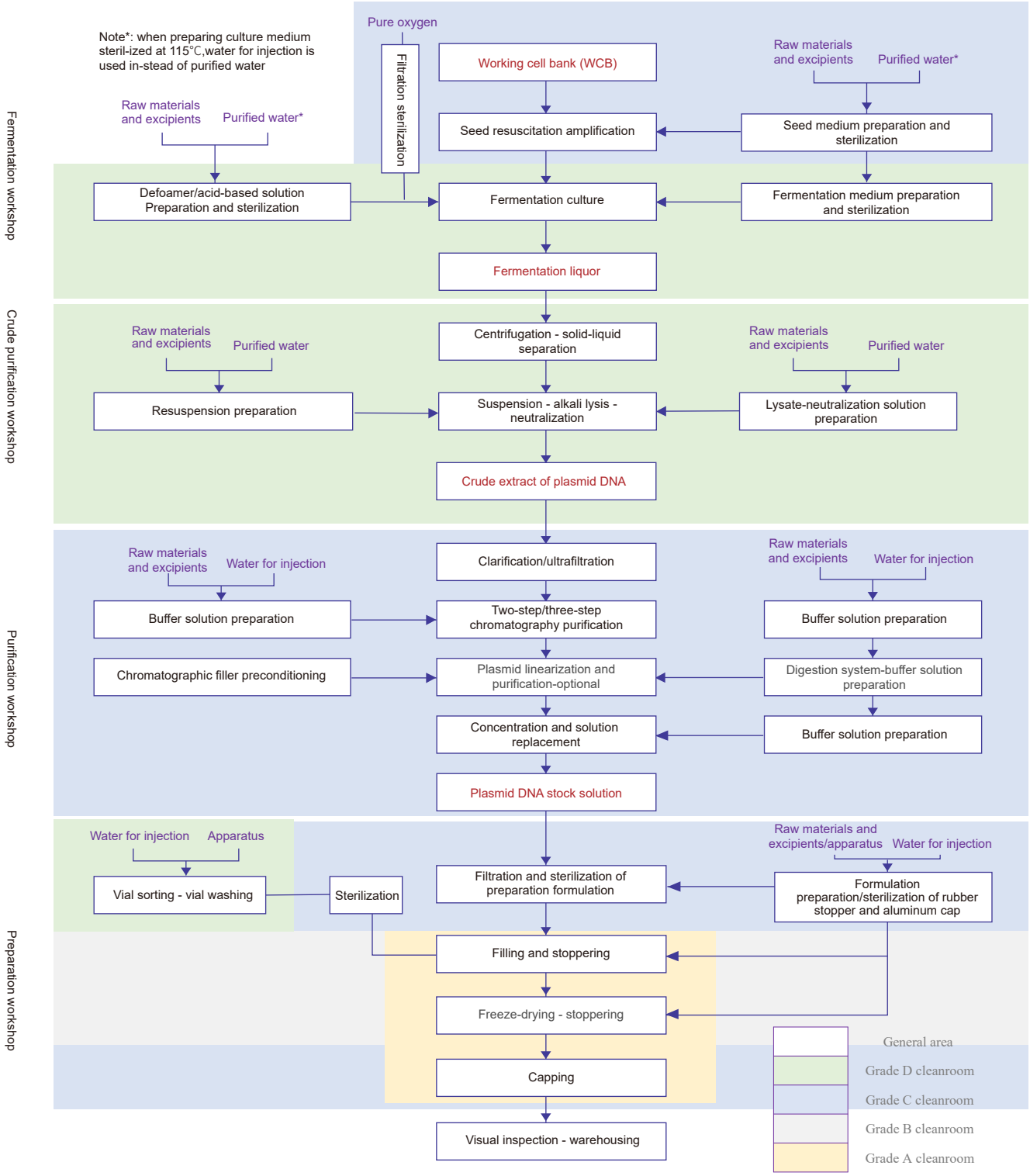
• Management of functional areas of different cleanliness classes: air conditioners are independently formulated to control differential pressure, temperature and humidity and suspended particles
• Safeguard of medium: water for injection, purified water, pure steam, and etc.
• Equipment: authority setting, on-line monitoring, verification and measurement

Management and control of clean room in GMP workshop

Maximum allowable number of suspended particles/m^3

Cleanliness level	Static		Dynamic	
	≥0.5 μm	≥5.0 μm	≥0.5 μm	≥5.0 μm
Grade A	3,520	20	3,520	20
Grade B	3,520	29	352,000	2,900
Grade C	352,000	2,900	3,520,000	29,000
Grade D	3,520,000	29,000	No provision	No provision

Functional areas of GMP workshop



Presentation of GMP workshop and equipment

Solution dispensing system

Fermentation system

Disc stack type centrifuge

Cartridge centrifuge

Homogenizer

Hollow fiber system

Low-pressure chromatography system

High-pressure chromatography system

Aseptic filling system

Capillary electrophoresis instrument

Gas Chromatograph

Liquid chromatograph

NANO-ANTIBODY

CDMO SERVICES

Nano-antibody Full Ecological Recombinant Expression CDMO Services Platform

Yaohai Bio-Pharma has a complete one-stop technology platform and CDMO overall solution for nanobodies, which can provide customers with the whole life cycle services from genetically engineered strain construction, establishment of strain bank, lab-scale process development/optimization, pilot process scale-up, IND application and clinical sample preparation, quality specification establishment, analytical method development/verification, quality management system establishment, NDA registration application and commercial production. Life cycle services, supported by the production platform from lab-scale, pilot-scale to large-scale, with a series of services such as process and method development and verification, equipment verification and quality control, and quality research, etc., can meet the cooperation needs of customers from early drug findings, clinical research to marketing at commercial scale.




Plasmid construction

Gene synthesis
Vector construction

Strain and strain establishment services

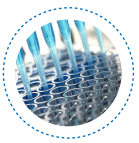
Original strains
Original strain bank construction
Secondary strain bank constructio

Process developmen

Fermentation process development
Purification process development
Drug product process development

Stability study

Specification establishment
Analytical method development
Analytical method transfers and verification




Registration and application

IND application
NDA application
International application* (FDA, EMA)
Registration service consultation

Product release

Perfect quality control system
Sound quality assurance system
Meet the requirements of laws and regulations

IND/GMP sample production

Fermentation production at a scale of 7-2000L
Downstream purification process
Aseptic filling of drug product

Commercial production

Nano-antibody Service Advantages

Advanced Process Development Concept

- A stable process with high output and yield can be achieved by determining the critical process parameters (CPP) with CQA (critical quality attribute) as the starting point obtained through DoE based on the concept of quality by design (QbD).

Rich project experience

- More than 100 projects have been successfully served, covering the preclinical research, clinical phase I, II and III, including several registration projects filed for China, US FDA and Australia.

Professional team guarantee

- Support by experienced and stable CDMO services team, with rich service and accumulated technical experiences in multiple categories of recombinant protein projects, and focus on process route innovation, quickly resolve process difficulties and reduce the R&D costs
- Professional PM project management team proficiently masters the project management of the whole life cycle of biologics development, can identify and manage the project critical path, identify, control and manage the project risks

Comprehensive production capacity guarantee

- Large-scale preparation services at a scale of 50L-100L, 200L, 500L, 1,000L and 2,000L
- 2 production lines for drug products (vial lyophilized powder/injection, pre-filled cartridge)

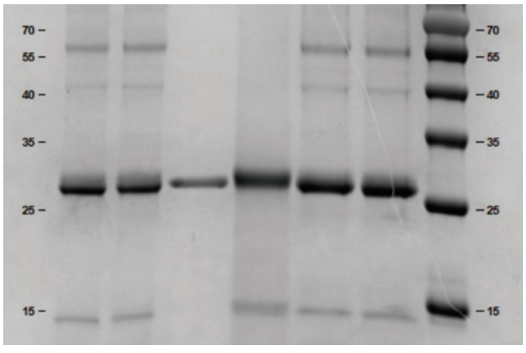
Perfect quality management system

- Provide a full range of quality management service, with professional and standardized service guarantee system, and the whole cycle can comply with the requirements of the new edition of the pharmacopoeia and the GMP related guidelines, to continue to deliver products with stable quality for customers.

One-stop CDMO services

- Deliver one-stop service from strain construction to commercial production, covering all stages of preclinical research, clinical phase I, phase II, phase III and biologics production.

Service Cases



Objective

Purity ≥95%; endotoxin <50EU/mg protein.

Developed a two-step chromatography method

Affinity chromatography: affinity using A3, with a purity of up to 94.1%;
Anion exchange chromatography: using 50HQ, with a yield of 73.9%, and a purity of 98.1%

The process target requirements were met after testing the endotoxin.

DRUG SUBSTANCE PRODUCTION

Service Capacity Guarantee

Industrial scale guarantee

Production services of drug substances at a scale of 50L-100L, 200L, 500L, 1,000L, and 2,000L to meet the needs of different projects

Rich technology transfer experience

Comprehensive and perfect technology transfer process and risk control system

Compliance assurance

Well-established quality management system in compliance with the requirements of NMPA/FDA and EMA, and experienced quality management team

Powerful data management

More than 80% of production lines are intelligently operated

GMP Production and Quality Control Service Platform
—— Overview of Drug Substance Production

With the capability of one-stop entrust manufacturing service, Yaohai Bio-Pharma can provide customers with the services of preclinical, clinical and marketed drug production. There are five production lines of drug substance designed based on QbD, and in compliance the GMP requirements of NMPA, FDA and EMA, which can provide bioreactors at various sizes of 50 L-100 L, 200 L, 500 L, 1,000 L and 2,000 L to support customers' production needs at different stages of development. Relying on the international advanced production equipment, flexible production line configuration, and high standard quality system, the new drug development process of the customers can be efficiently promoted.

Service Items

Strain bank establishment under GMP system	Pilot process optimization and scale-up production
Preparation batch production ofsamples for IND registration and application	Production of samples at clinical phase I-III
Industrialized production	Preparation of standard substances

GMP production of drug substances with high productivity and flexibility

- GMP-compliant production area for drug substances at an area of more than 10,000 square meters
- GMP-compliant fermentation service platform at a various scale of 50L-100L, 200L, 500L, 1,000L and 2,000L
- Independent upstream and downstream production areas, supported by fully functional upstream and downstream process equipment
- Upstream: 5 production lines for drug substances, with a production capacity of 7,500L, and equipped with fermenters at different specifications
- Downstream: 5 purification production lines, equipped with low, medium and high chromatography and ultrafiltration systems, covering a wide process scale
- The plant has been reasonably designed, with qualified air conditioning system and water system (4Q) to deliver a GMP-compliant production workshop
- Advanced equipment (sourced globally) is all qualified (3Q), with PQ available; the instruments and gauges are all completely calibrated
- Supported with compliance QA, operation QA and verification team to ensure efficient implementation of quality system

DRUG PRODUCT PRODUCTION

GMP Production and Quality Control Services Platform
—— drug product production

Sterile Drug Products Production Services

For the production of sterile drug products (DP, Drug Product), Yaohai Bio-Pharma has built a production workshop for drug product at an area of 2000 ㎡ , which can provide automated production services for sterile drug products in accordance with GMP requirements, and is a high-tech automatic production line integrating multiple processes such as vial washing, oven, sterilization (depyrogenation), filling, lyophilization and capping. The production line meets the requirements of sterile drug products for China NMPA, EU EMA and US FDA. Yaohai Bio-Pharma is experienced in the production of sterile biological drug products and delivers high quality production services from clinical sample production to commercial production of vials and pre-filled cartridges.

For vial injections, the maximum annual output is 10 million
For vial lyophilized powder, the maximum annual output is 5 million

For pre-filled vials and cartridges, the maximum annual output is 8 million

IND, clinical phase I/II/II and commercial production

Comprehensively designed production line for injections, lyophilized powder and pre-filled vials

Category of products to be filled: recombinant proteins, polypeptides, plasmids, antibodies, vaccines and other mainstream biological products

Production line of vial injection and sterile lyophilized powder drug product

Filling Range

- 1ml-25ml

Aseptic Production Line

- Product (and package material) exposure area are equipped with O-rabs system under Grade A environment protection
- Fully automatic loading and unloading system
- Fully automatic SIP/CIP system for the lyophilizer
- Equipped with online ammonia applying system for nitrogen protection
- PMS online monitoring system

Filling Accuracy

- Filling with a very high accuracy: (the filling medium is water for injection) ±0.25%

Filling Speed

- Take an example of 2 mL of vials, the maximum production speed is 300 vials/min, and the maximum lyophilized powder batch size is 37,800 vials/batch

Production line of pre-filled sterile drug product

Filling Process

- Equipped with plunger pump, peristaltic pump, and double pump system, different process needs can be satisfied.

Filling Range

- Pre-filled vials, 1 mL and 3 mL, cartridge, 3 mL

Filling Accuracy

- The filling accuracy is within ±3% for 0.2 mL to 0.5 mL
- TThe filling accuracy is within ±2% for 0.5 mL to 3 mL

Aseptic Filling

- A variety of filling methods (typical filling, filling by applying nitrogen, vacuum filling)
- Vacuum stoppering method, suitable for multiple types of stoppering process requirements
- O-rabs system is applied in the product exposure areas (and packaging materials) with Grade A environment protection
- Ergonomic glove port settings can minimize the impact of interventions on the product
- With PMS online monitoring system, the production environment status can be real-time monitored and the environmental abnormalities can be rapidly detected.

Production Capacity

10 Million Vials

(annual production)

Specification 1ml-25ml

Vial Injection

5 Million Vials

(annual production)

Specification 1ml-25ml

Vial lyophilized powder

8 Million Vials

(annual production)

1ml-3ml

Pre-filled Vial/cartridges

QUALITY RESEARCH PLATFORM-QC

QC-Quality Control System (GMP)

Based on the rich experience in GMP quality management, Yaohai Bio-Pharma provides customers with continuous and stable quality services through a close cooperation among the quality control (QC) team, the production and quality assurance (QA) team in the areas of testing of raw materials and excipients, intermediate process control, stability study and product release testing of biological drug product. Meanwhile, Yaohai Bio-Pharma established a sound quality control system, in compliance with the regulatory requirements, with certified quality system throughout all phases of QC testing

Service Content

Analytical method transfer/ verification/validation

Strain bank release testing, passaging stability, and storage stability

Raw materials and excipients release testing

Testing and standardization of self-made standard substances

Releasing testing of intermediate products, drug substances, semi-finished products, and finished products

Stability study

Service Features

- Equipped with advanced quality analysis testing instruments
- The QC team has undergone strict GMP training and guidance and is familiar with the newly revised GMP requirements
- Skilled in physical, chemical, biological and microbiological quality control testing methods
- Rich experience in project execution
- In addition to the current scope of testing, the testing capabilities is continuing to be expanded

Bioanalytical Testing Services

At present, Yaohai Bio-Pharma has established a perfect quality testing platform in terms of physicochemical, microbiological and biochemical testing, with well-established quality control methods for different products according to their physicochemical characteristics, which can meet the release testing of biological products (recombinant proteins, polypeptides and plasmid products) and support the analysis and quality control needs of the life cycle of biological drug products.

Classification	Biochemical Testing Items	Physical And Chemical Testing Items	Microbial Testing Items
Testing items	Expression of target product	Appearance	Plasmid loss rate
	Plasmid restriction digestion mapping	PH	Seeding LB plate
	Protein content	Visible foreign matter	Staining microscopy
	Purity	Loading	Viable bacteriocins
	Molecular weight	Particulate matters	Antibiotic resistance
	Activity test	Osmolality	Biochemical reaction
	Exogenous DNA residue	Water	Residual antibiotic
	Residual host bacterial proteins	Density	Bacterial endotoxin
	Isoelectric point	Residual organic solvent	Microbial limits
	UV spectroscopy	Optical rotation	Sterility
	Polypeptides mapping		
	Identification		

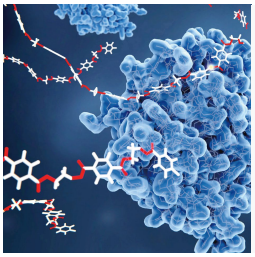
Testing Capability

With approximately 30 testing items and 50 testing methods accessible, various regular items can be tested, and testing services capabilities keep improving.

30+ Test Items

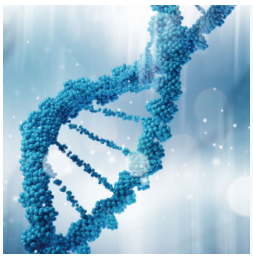
50+ Test Method

Service Capacity Guarantee



Recombinant Protein Project Service Experience

100+ recombinant protein projects have been successfully served, including several PEG-modified protein projects and enzyme-based product projects, with extensive experience in the full testing of recombinant protein projects.



VLP Vaccine Project Experience

VLP vaccine testing on multiple projects have been successfully implemented, with proficiency in the quality specification and test items of VLP particles.

Stability Study

Dozens of individual stability study projects have been successfully conducted.

Experience in Plasmid Projects

Many projects with therapeutic plasmids and viral vector products have been served, with accumulated extensive experience in HCD and HCR assays for critical projects.

Analytical Method Verification/Validation

150+ analytical method transfer/verification/validation activities have been completed.

YAOHAI BIO-PHARMA

QUALITY RESEARCH PLATFORM - QA

Service Capability Guarantee

Quality management is the lifeline of Yaohai Bio-Pharma. Yaohai Bio-Pharma provides a full range of quality management service, adheres to the goal of customer satisfaction, establishes a quality policy of “quality-oriented, perfect compliance, simple efficiency, unity and cooperation” , and is committed to providing sample preparation of IND and clinical phases to meet the requirements of FDA, EMA, and NMPA, and satisfies the comprehensive quality management services for the commercial production of drug products required by NMPA.

Establishment of Comprehensive Documentation System for Ten Systems

Quality management	organization and personnel	premises and facilities	equipment management	materials and products
Validation and verification	document management	production management	quality assurance	laboratory management

Establishment Principles of Quality System

The quality system covers the whole life cycle of a drug product from development to commercial production.

The quality system is based on the existing laws and regulations at home and abroad, and complies with the laws and regulations requirements of CMC (chemistry, production and control) activities.

It is combined with CDMO business characteristics to preserve flexibility and meet customers' high expectations for contract manufacturing.

Document Assurance System

Yaohai Bio-Pharma establishes a comprehensive document assurance system, which is based on GMP requirements and closely follows the company's business model to ensure that all GMP activities of the Company are covered.

Management Policy	POL
Standard Operating Procedure	SOP
Process flow, specification, inspection procedure	STP
Record, the numbering follows therequirements of SOPs and STPs, with independent review and approval process	Form

Regulatory Support

The Company engages a well-known third-party GMP consulting company at home and abroad at irregular intervals according to the business needs, to perform consulting and improvement activities on the Company' s quality system to ensure continuous improvement. In addition, the Company has employed experts with FDA background as consultants to assist in resolving issues arising during the operation of the quality system in a timely manner.

Global

Measures for Administrative of Drug Registration, Good Laboratory Practice for Pharmaceuticals, ICHQ5, Q8, Q9, Q10, Q12, Good Clinical Laboratory Practice, and Good Manufacturing Practice

Yaohabio 耀海生物

Globalized

REGISTRATION & APPLICATION SERVICES

Overview of Services

01 With an extensive drug registration and application team, high-quality, efficient and accurate registration support can be provided, including domestic and international IND/BLA application services.

02 The comprehensive registration and application services include CMC consulting services, guidance on registration and application strategy, assistance in completing the writing and submission of CMC-related CTD documents, assistance in communication with official agencies, guidance on site verification for development and research, organization of drug registration regulations training and conference guidance, etc.

03 With in-depth research and understanding of domestic and foreign registration-related regulations, comprehensive guidance on regulatory strategies for clients throughout the product development lifecycle can be provided.

Service Content

Registration Services

- Dedicated to CMC regulatory consulting services
- Provide guidance on CMC strategy development and gap analysis for domestic and international registration applications
- Assist in communication with regulatory agencies, assist in response to approval comments and submission of supplemental information
- Convene scientific consultation meetings

Regulatory Support Matrix

- Global regulatory research for drug regulatory agencies
- Regulatory strategy & implementation guidance
- Sorting and interpretation of general regulations and special regulations
- Routine regulatory consultation throughout the year
- One-on-one regulatory consulting
- Project management

Writing of CMC registration dossier

- Writing of IND and NDA registration dossier
- Flexible and customized writing services of registration dossier

On-site verification

- Guidance on preparation of verification materials
- Guidance on the development of on-site verification

Other value-added and special services

- Project demonstration in the process of technology development or transfer
- Process analysis on IND/NDA registration strategy
- Research and evaluation of case-by-case drug product

Service Advantages

Professional Team Guarantee

Core members have more than ten years of experience in drug registration and project management, with multi-module expertise, rich professional operation experience, and strong professional support guarantee from domestic and foreign experts.

Rich Project Operation Experience

More than 200 clients have been served, covering a wide range of project types, with rich project experience, accurate grasp of regulatory guidelines, review requirements and critical points of drug registration, to predict the important and difficult points of the project in advance, which greatly enhances the project efficiency.

Real-time Information Sharing

Through familiarity with the perfect communication channels with official authorities, grasping the latest regulatory trends in real time, and fully understanding the laws and regulations of regulatory agencies, the real-time information sharing can be realized with customers based on sufficient information integration and analysis with a powerful database of regulations and document templates.

Full Life-cycle Service Management

With a one-stop service chain advantages of establishment of R&D system, registration and application of IND and NDA projects, and project management, the management concept of the whole life cycle of drug products is applied throughout the project.

Perfect project management services

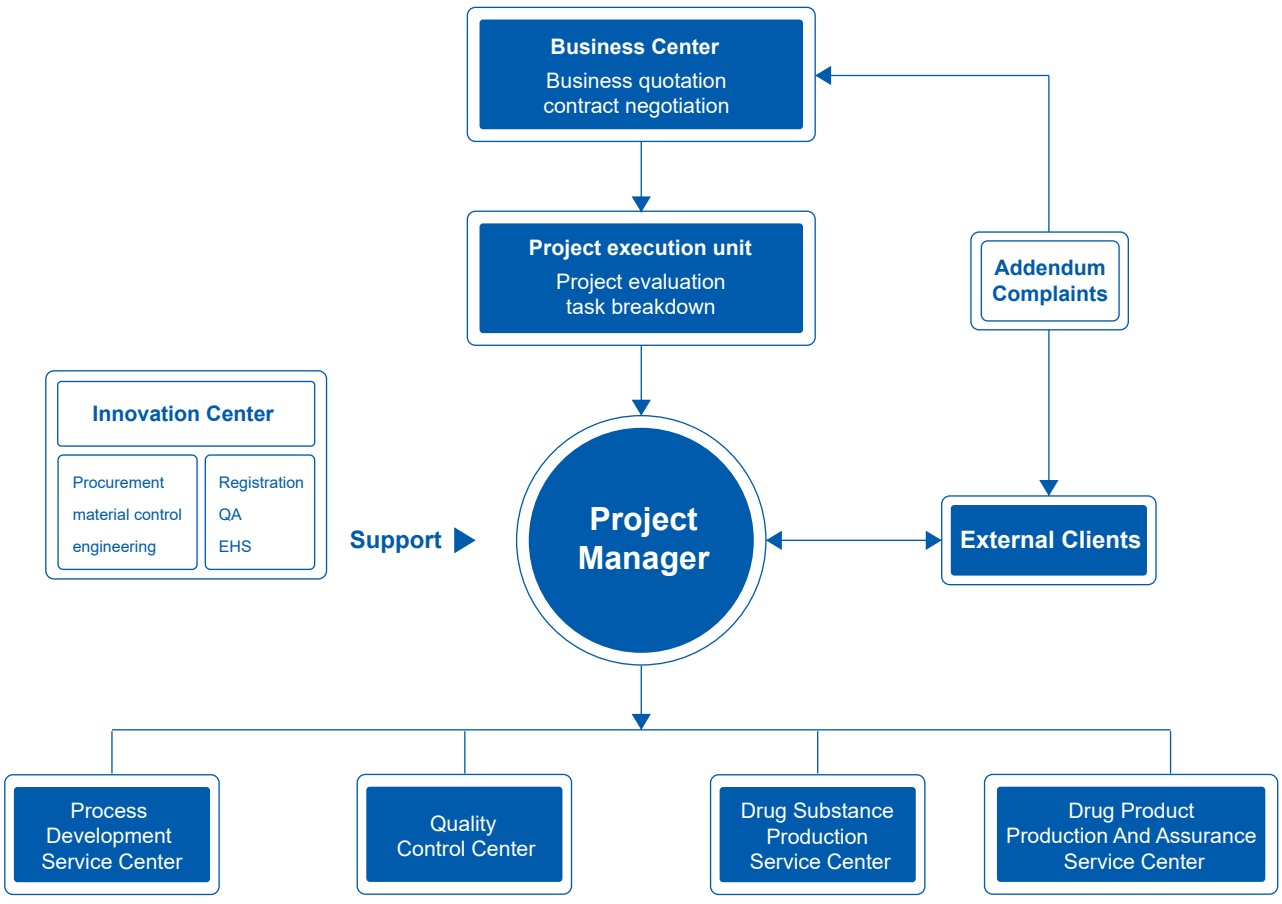
Provide planning and guidance services for the whole life cycle of each overall project, put forward feasible suggestions, focus on risk management and control budget, closely integrate with the actual situation of the project, develop implementable solutions and ensure the quality of the project.

Project Management & Service Process

313 SERVICE SUPPORT MODEL

“313 service support model” is adopted to provide strong implementation guarantee for project operation
Implement three-cycle supply chain guarantee based on procurement center, material control center, and engineering center
Strengthen the innovation center to support technical guarantee
Three-cycle compliance guarantee based on registration department, QA and EHS
Thus to jointly maintain the project with high quality.

Service Guarantee



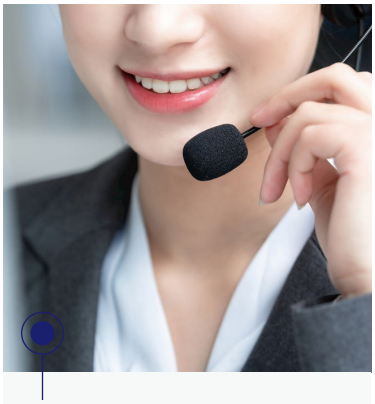
Service Process

PROJECT CONTACT

Project Communication
Confidentiality agreement /
Needs analysis

READY START UP

Contracting
Gap analysis /
quality agreements



POST-SALE SERVICE

Follow Up Services
Assistance in official verification /
technical consultation

ACCEPTANCE DELIVERY

Project Delivery
Deliverables management /
cost settlement

EXECUTIVE CONTROL

Project Implementation
GWBS promotion /
process control

SERVE **WITH HEART** & CREATE **THE FUTURE TOGETHER**

CHOOSE YAOHAI BIO-PHARMA

Rich Project Experience

More than 100 projects have been successfully served, covering the preclinical research, and clinical phase I, II and III, including several registration projects filed for China, US FDA and Australia.

Professional Team Guarantee

With experienced CRDMO services execution team supported by gradient professionals, the contracting services can be efficiently and collaboratively boosted.

Comprehensive Production Line Protection

High quality and diversified fermentation purification services can be provided with the fully automated fermentation systems at a scale of 2-2000 L.

Compliance Service Guarantee

With professional, standardized and regulated service guarantee system, the whole life cycle complies with the requirements of the new edition of pharmacopoeia, GMP and other related guidelines.

Flexible Cooperation Mode

Provide customized services to meet the needs of different types of projects and provide quality and efficient services to clients.

One-stop Whole-course Service

Provide one-stop service from process development to commercial production.

CORPORATE CULTURE

Vision

To be a sustainable leader in the CDMO industry for microbial expression systems

Mission

To create global standards, facilitate the process of new drugs, and achieve a healthy life

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CRDMO Services

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[CMC development service platform] https://www.yaohai-bio.com.cn/strain

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