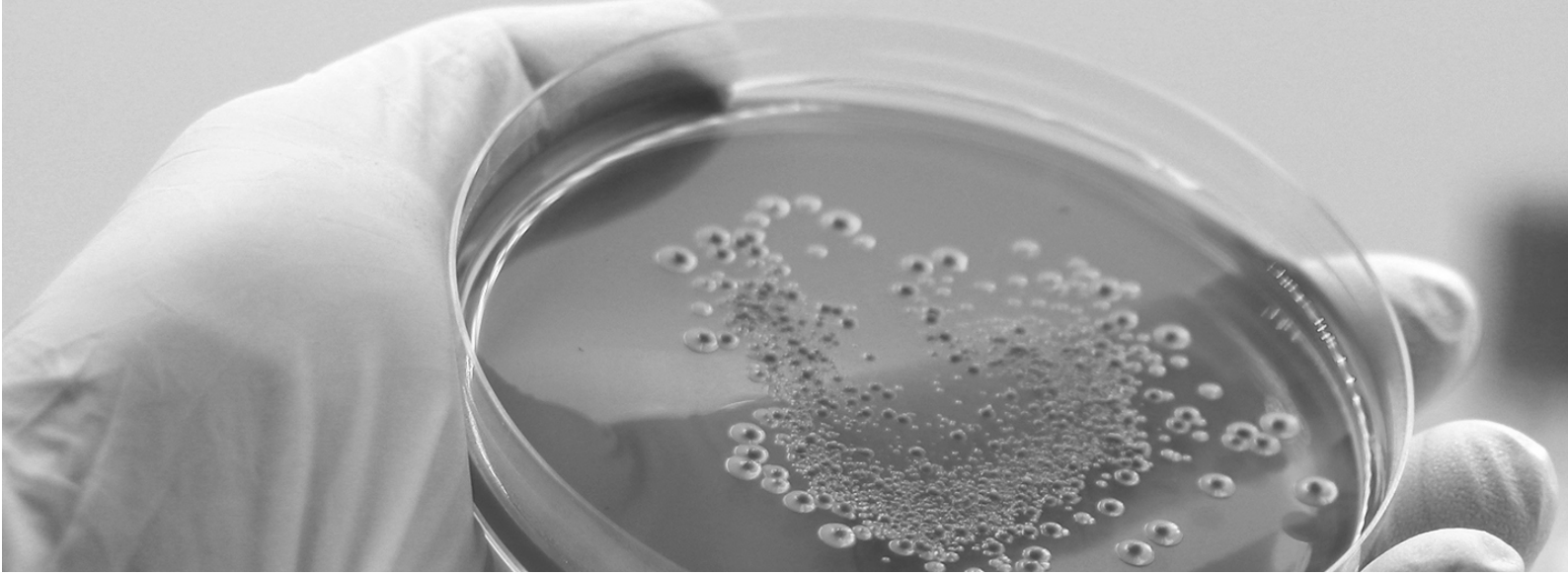
YAOHAIBIO

Strain Bank Construction Service

Escherichia coli

Yeast

YAIHAI BIO-PHARMA



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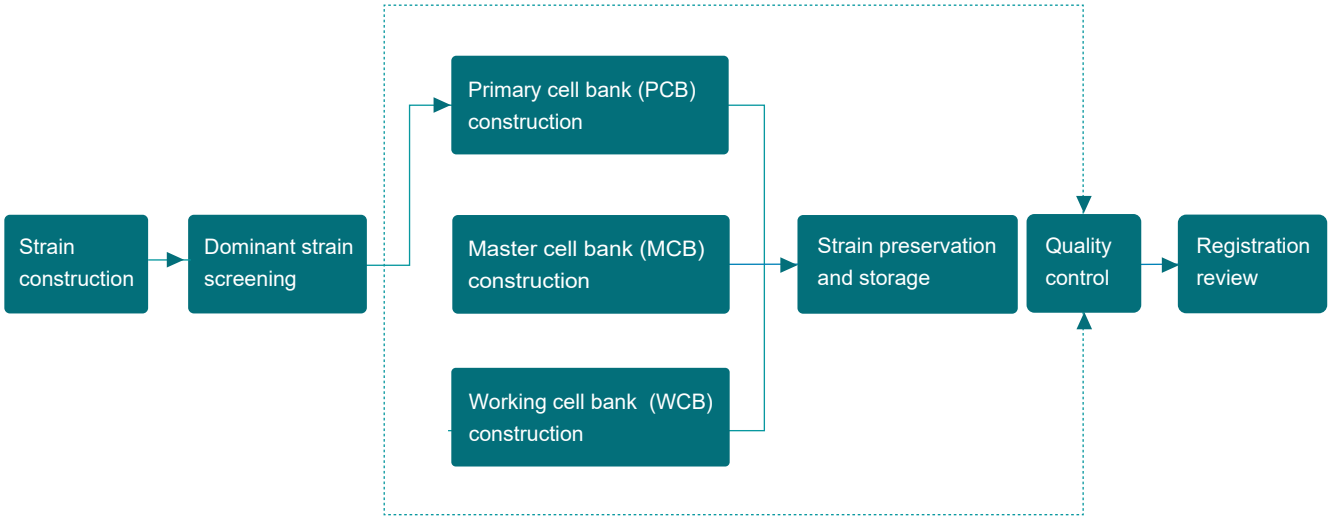


The management of cells used in the manufacturing of biological products should meet the requirements in Chinese Pharmacopoeia (2020 edition)-Management and Quality Control of Bacterial and Viral Strains Used for Production and Testing of Biological Products in ChP 2020, so as to provide strains that 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.

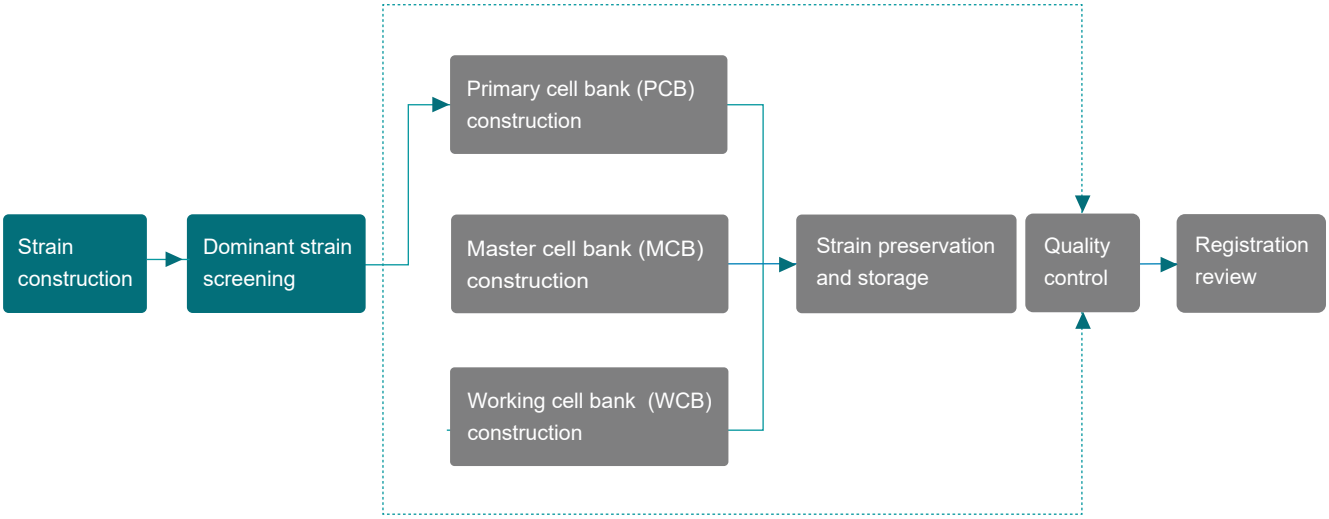
Usage	Plasmid	Type Of Promoter	Type Of Signal Peptide	Resistance/ Screening Marker	Copy Type
Manufacturing of recombinant protein	pPIC9K	AOX1	α-Factor	Ampicillin/kanamycin/His4	High copy
	Pinkα-HC	AOX1	α-Factor	Ampicillin/Ade2	High copy
	pPICZαA	AOX1	α-Factor (containing ATG)	Zeocin	High copy
	pPICZαB	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 intracellular 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.

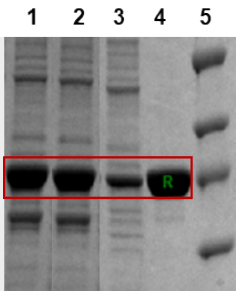
Protein Expression Amount

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

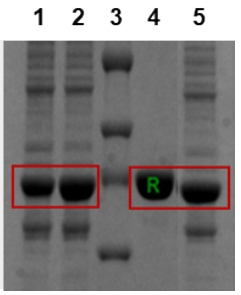
The retention rates of plasmids of the passaged strains P5 and P10 of S1-1 were not less than 95%.

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)

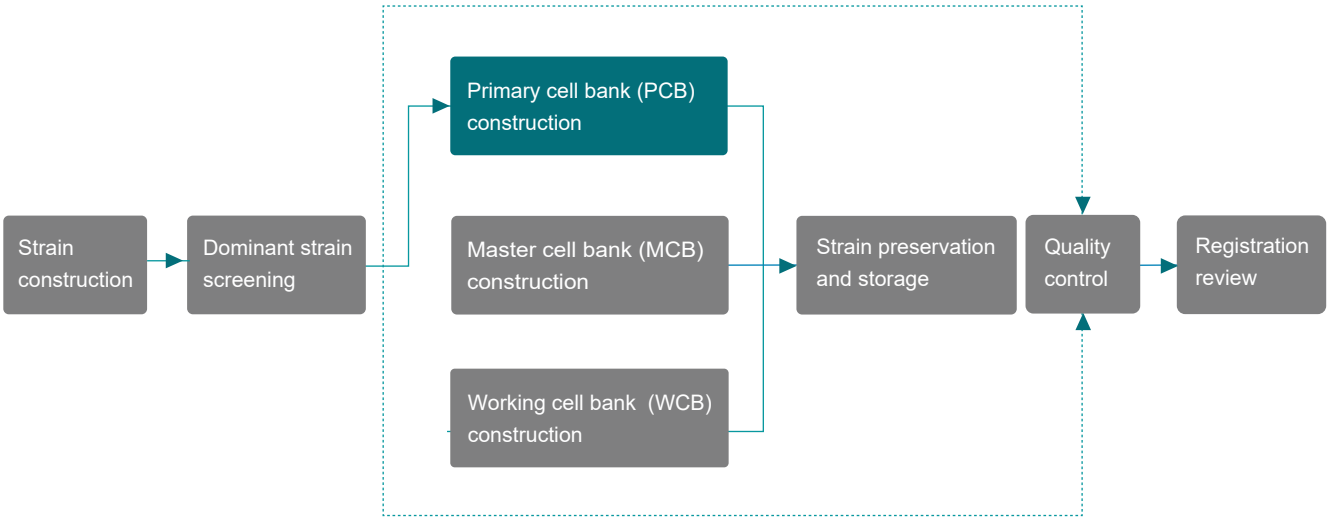
1: S1-1 P0 (10 passages)
2: S1-1 P5 (5 passages)
3: Marker
4: Control-quantitative reference
5: S1-1 P10 (10 passages)



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;

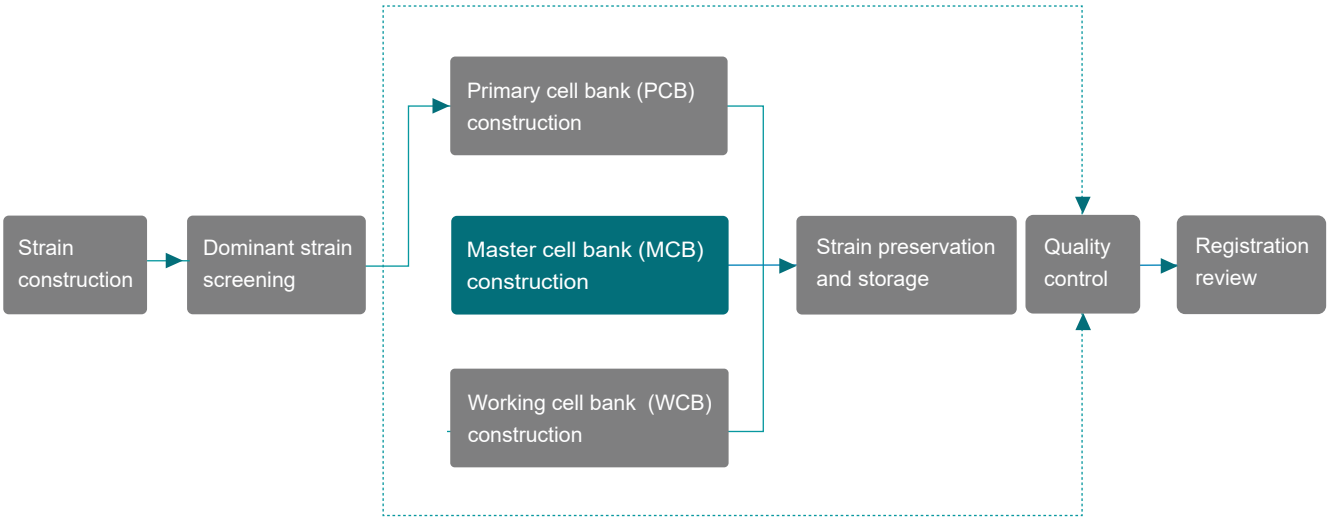
Good record writing specification

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;

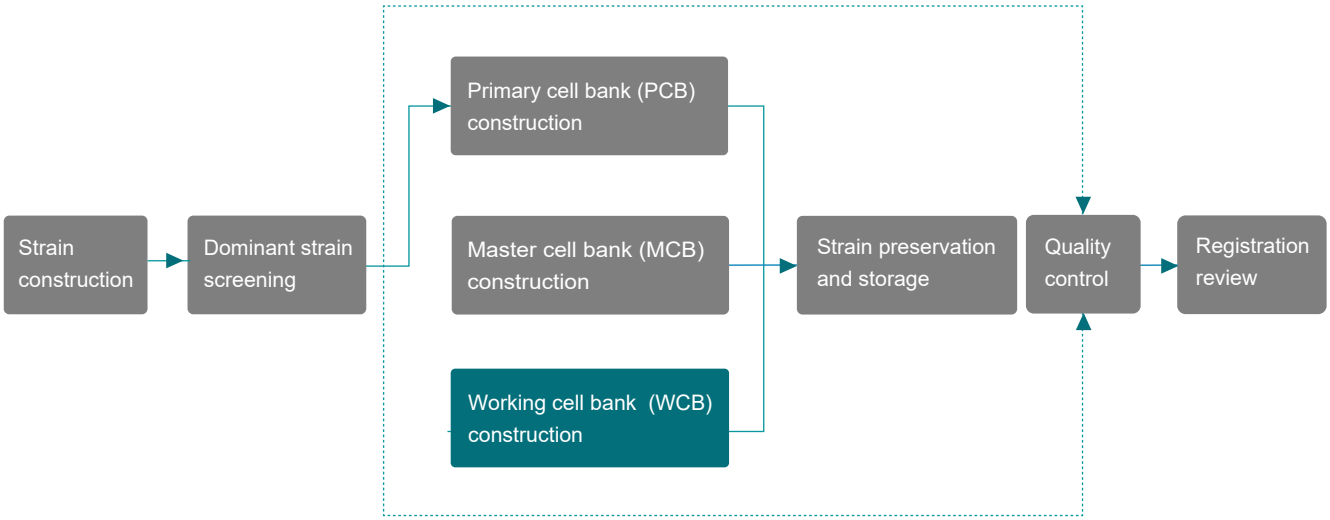
GMP system online audit platform

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

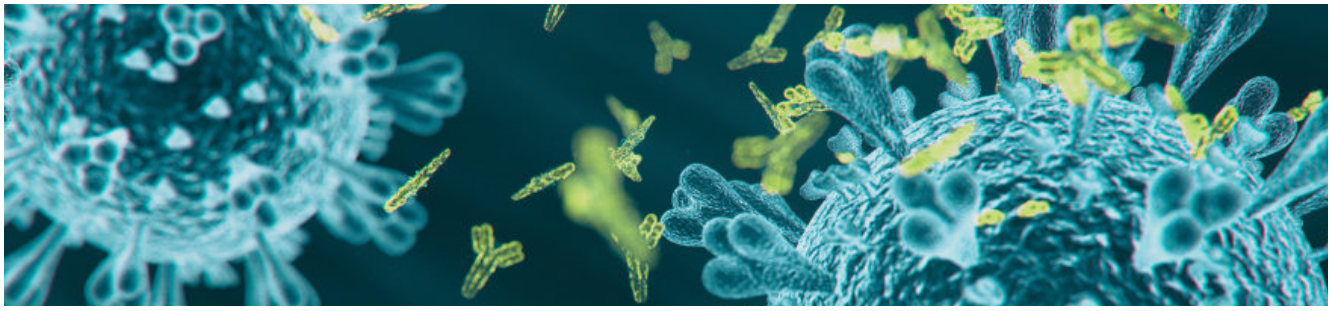
Cell passage stability, storage stability and other testing projects under the guidance of pharmacopoeia and other regulations, and several projects have been declared clinical (China, US and Australia).

GMP system online audit platform

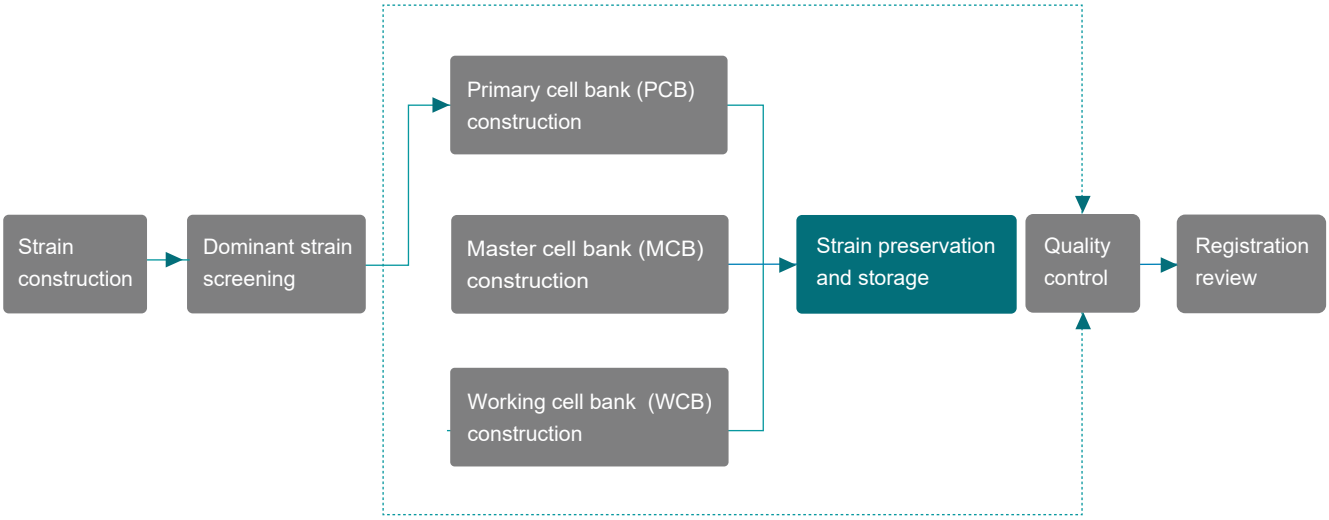
online audit port is opened to share VR videos of GMP workshop.

Other Services

- 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

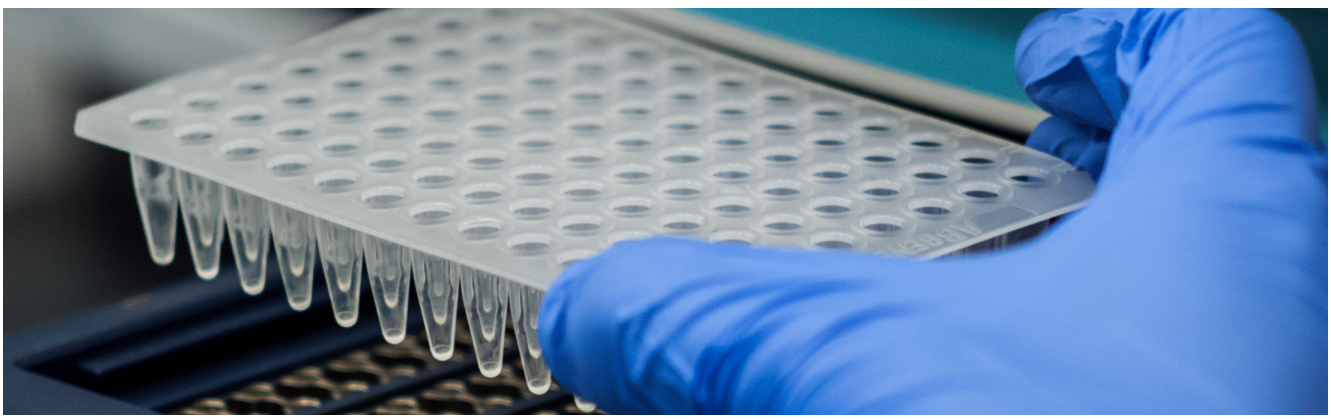


Strain preservation & storage service



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

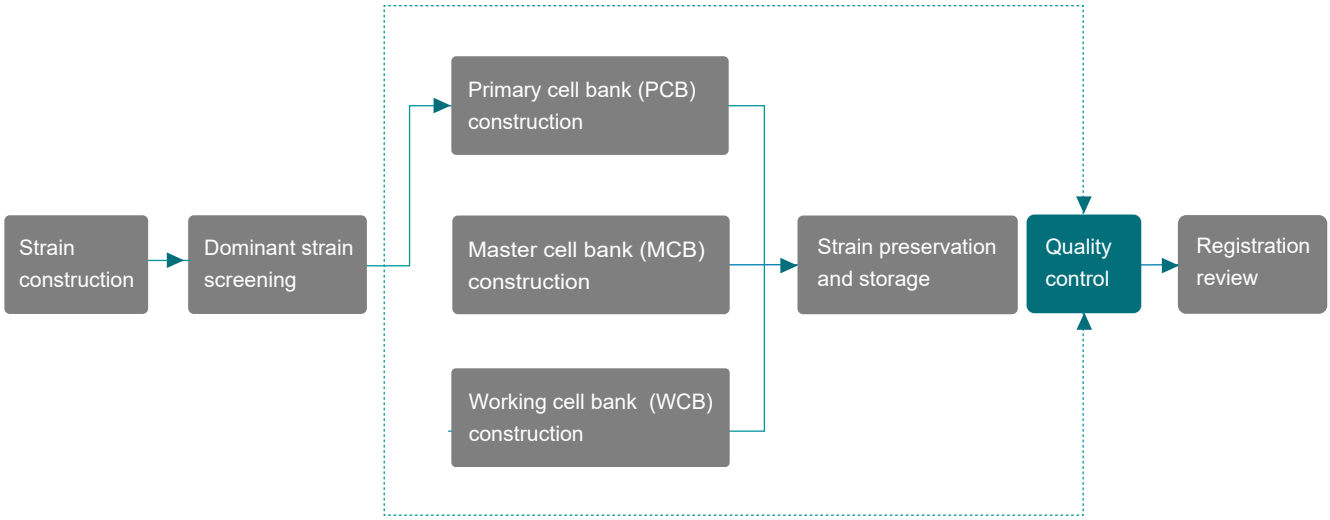
Undertake strain storage services, meeting the needs of off-site storage and backup for important strains.

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.

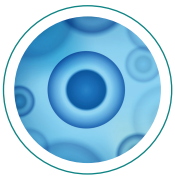
Diversified strain storage platform

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

Professional QC team

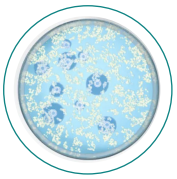
Oriented by registration application, the team is sophisticated in the study of pharmacopoeia and other regulations, facilitating the speed of the approval of the applications.

Other Services

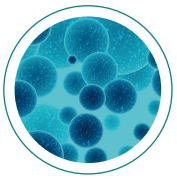


Strain construction

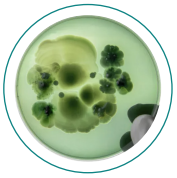
Dominant strain screening



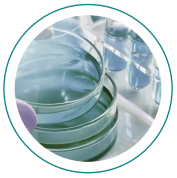
Primary cell bank (PCB) construction



Master cell bank (MCB) construction



Working cell bank (WCB) construction

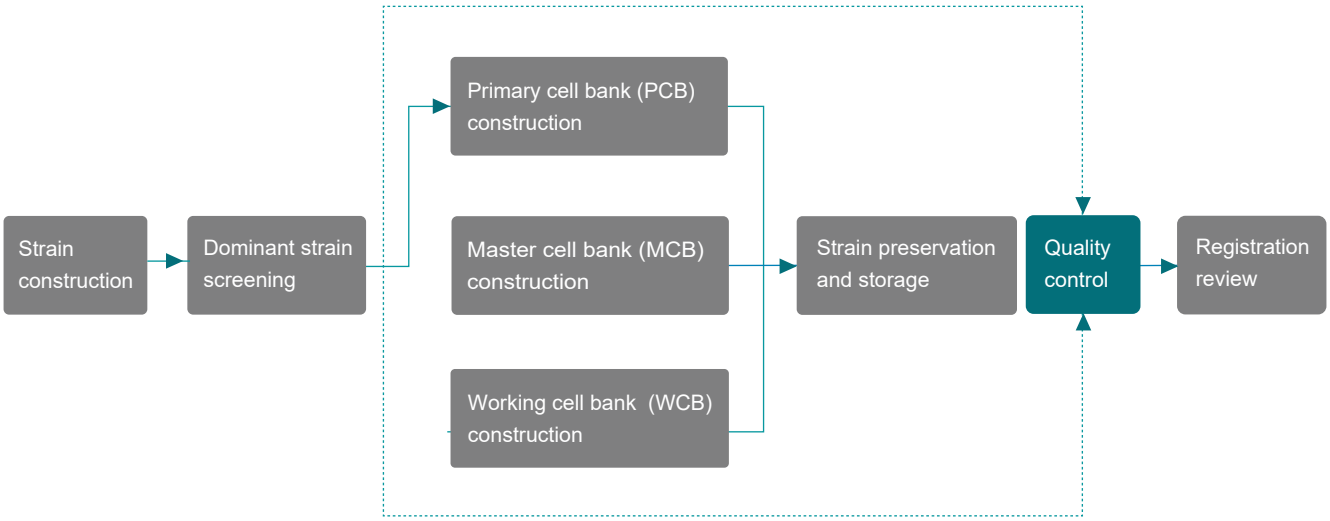


Strain preservation and storage

Strain quality control

Registration review service

Cell Quality Control Service - *Yeast*



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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		
	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.

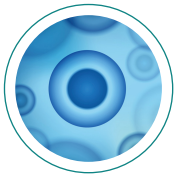
Compliant QC platform

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

Professional QC team

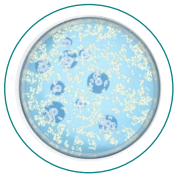
Oriented by registration application, the team is sophisticated in study of pharmacopoeia and other regulations, facilitating the speed of the approval of the applications.

Other Services

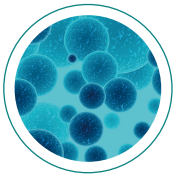


Strain construction

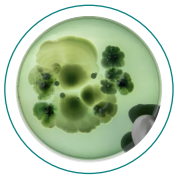
Dominant strain screening



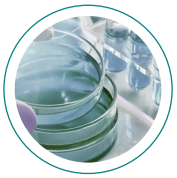
Primary cell bank (PCB) construction



Master cell bank (MCB) construction



Working cell bank (WCB) construction

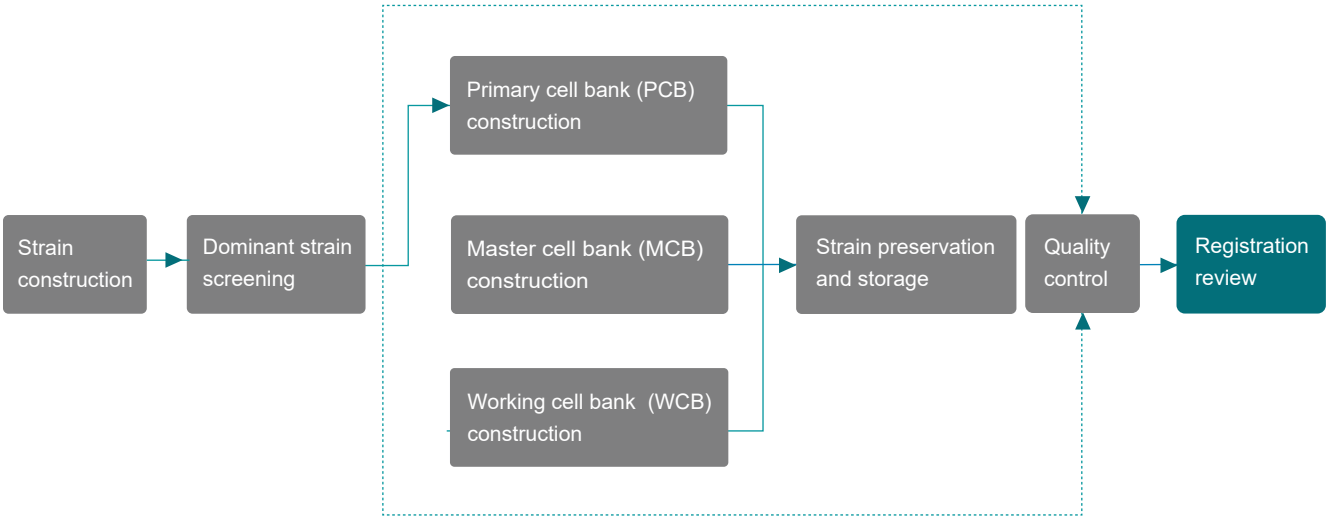


Strain preservation and storage

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.

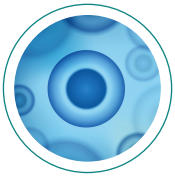
Professional registration team

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

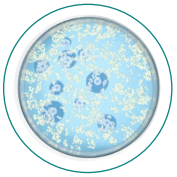
Regulatory study, content interpretation and project implementation from global drug regulatory agencies are fully covered.

Other Services



Strain construction

Dominant strain screening



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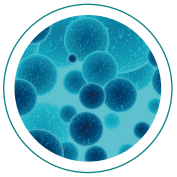
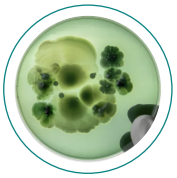
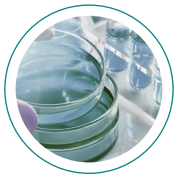


Master cell bank (MCB) construction



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.

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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.

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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.

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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.

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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.

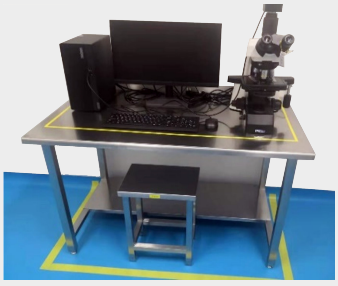
Cell Bank
Construction Platform

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

SERVE
WITH HEART &
CREATE
THE FUTURE TOGETHER

CONTACT US

www.yaohai-bio.com.cn/

Enterprise mailbox:BD@yaohaibio.cn
Link： https://www.linkedin.com/company/yaohaibio/
Address: Building 29, No. 801, Jiankang Dadao, Taizhou, Jiangsu