Yaohabio
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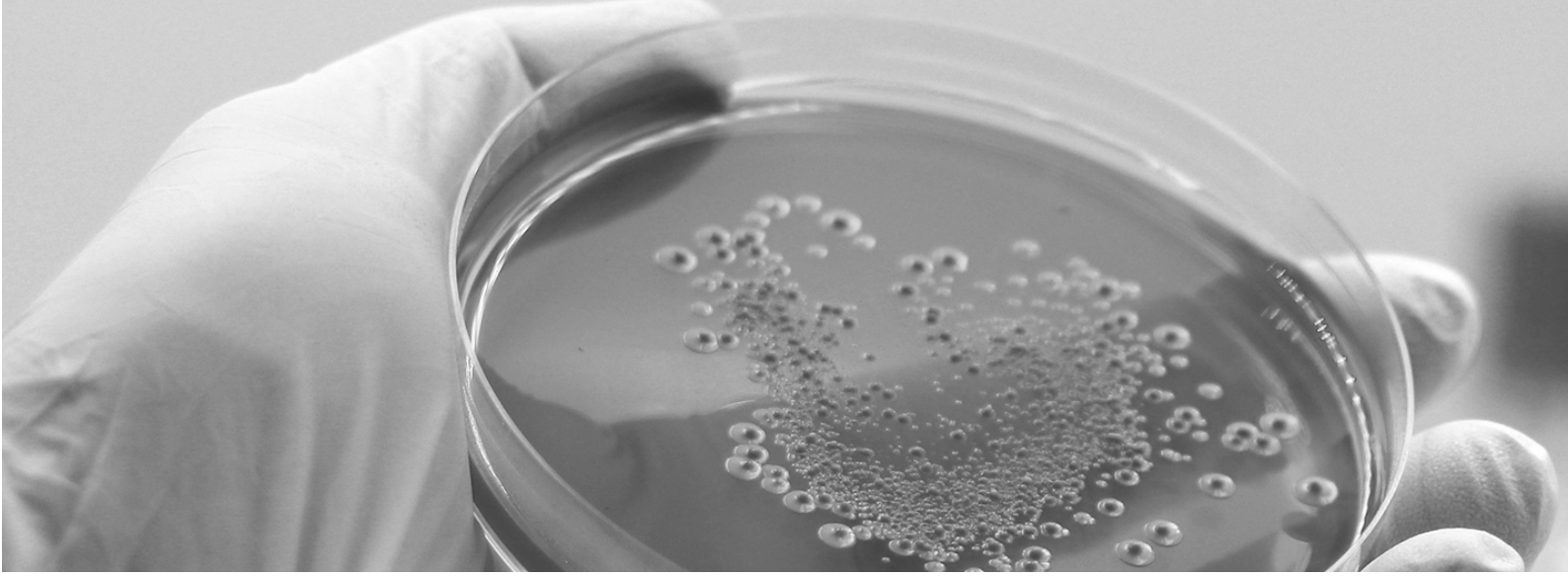
Recombinant Plasmids CMO Services of Yaohai Bio-Pharma

YAOHAI BIO-PHARMA



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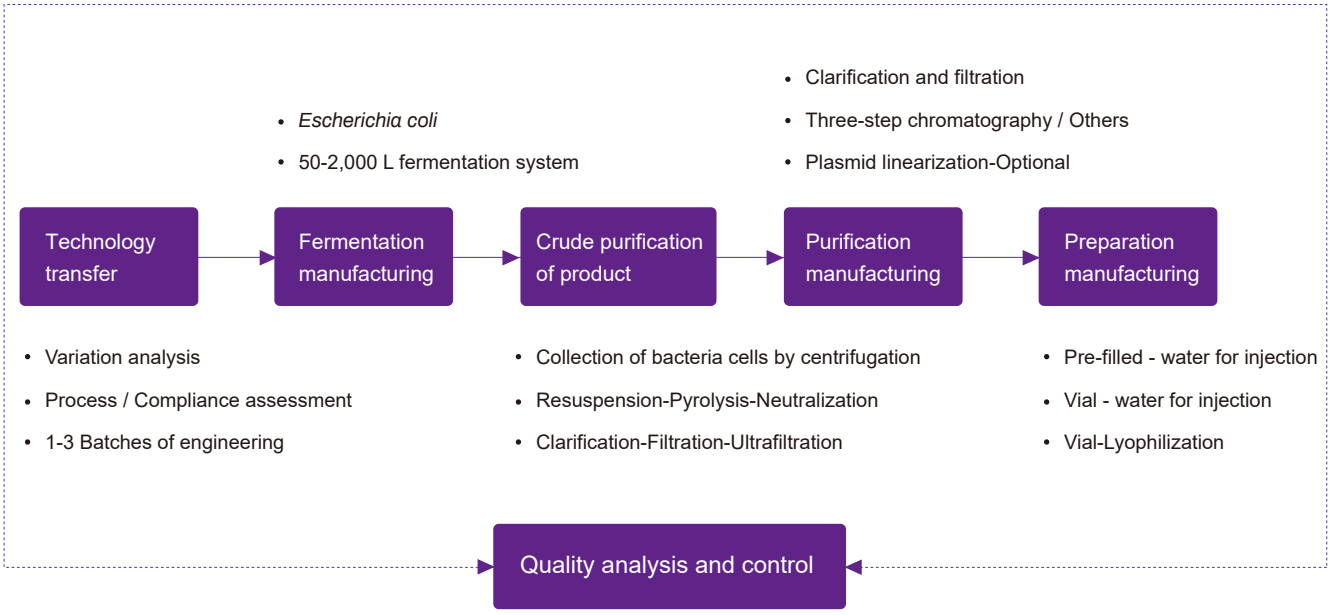
Overview of recombinant plasmids CMO services



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

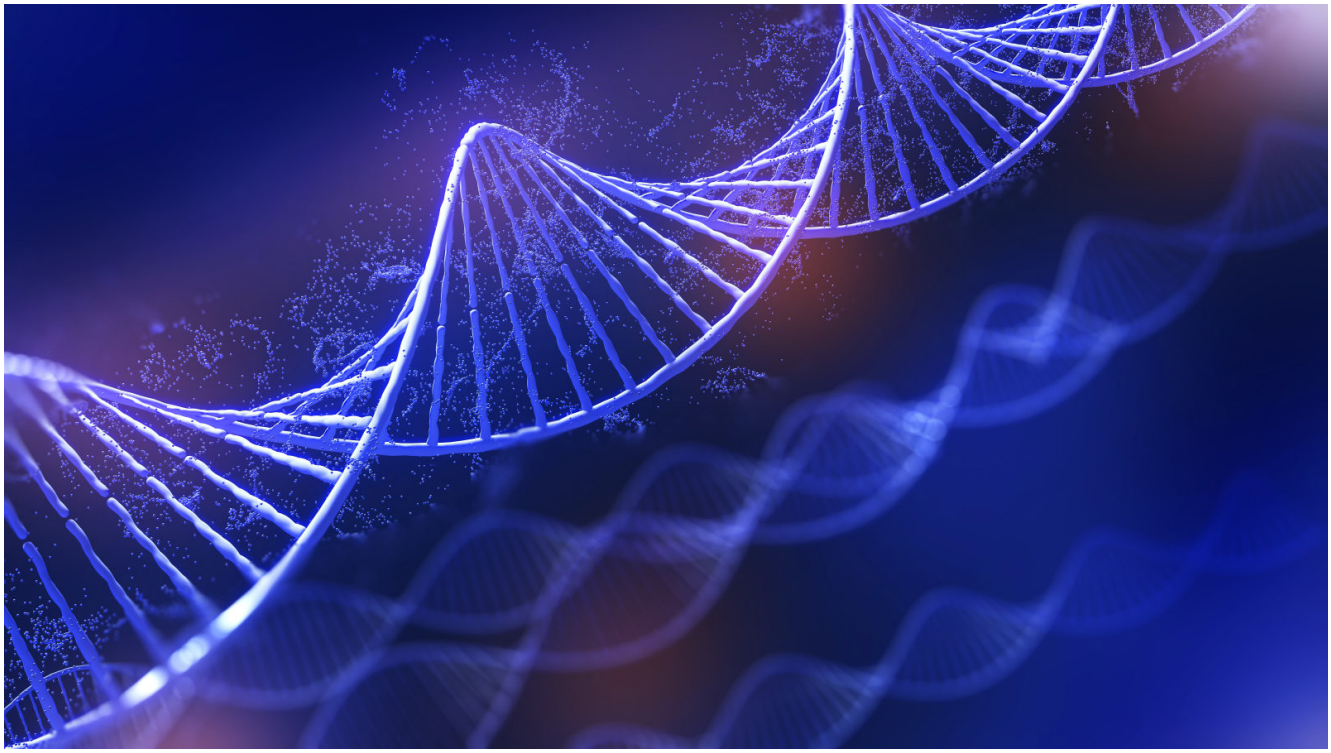
Recombinant plasmids may be used to assemble lentivirus (LV) and adeno-associated virus (AAV) for DNA vaccine, gene therapy or gene editing;

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.

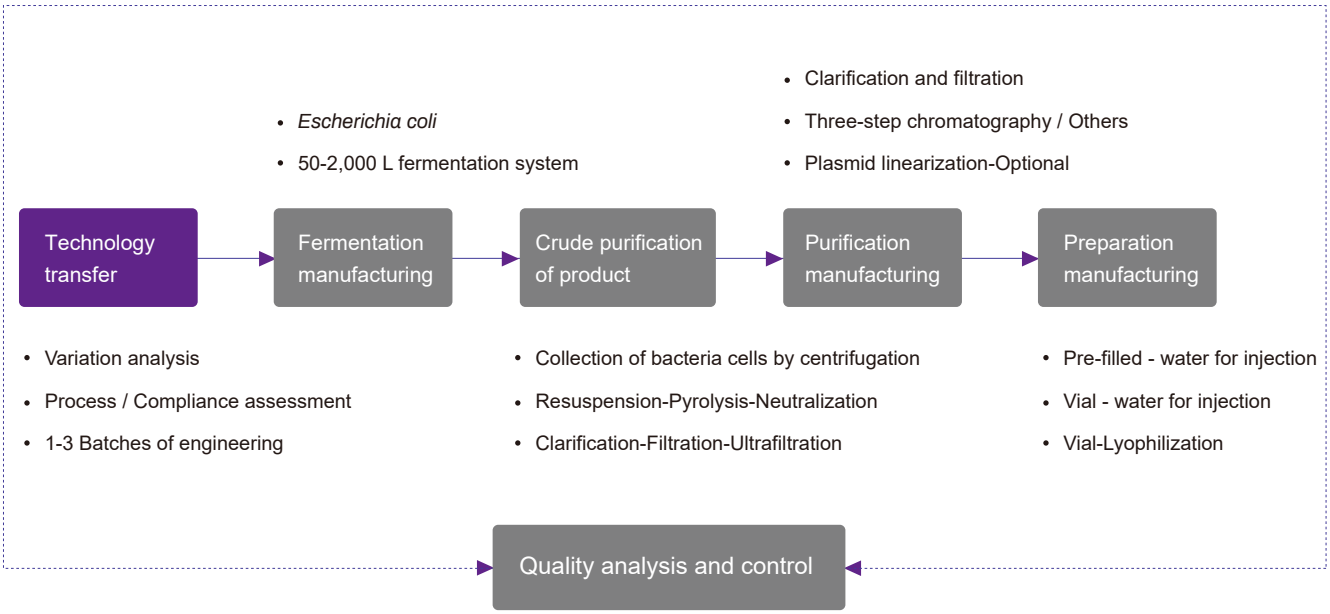
Extensive experience in technology transfer/scaling up

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.

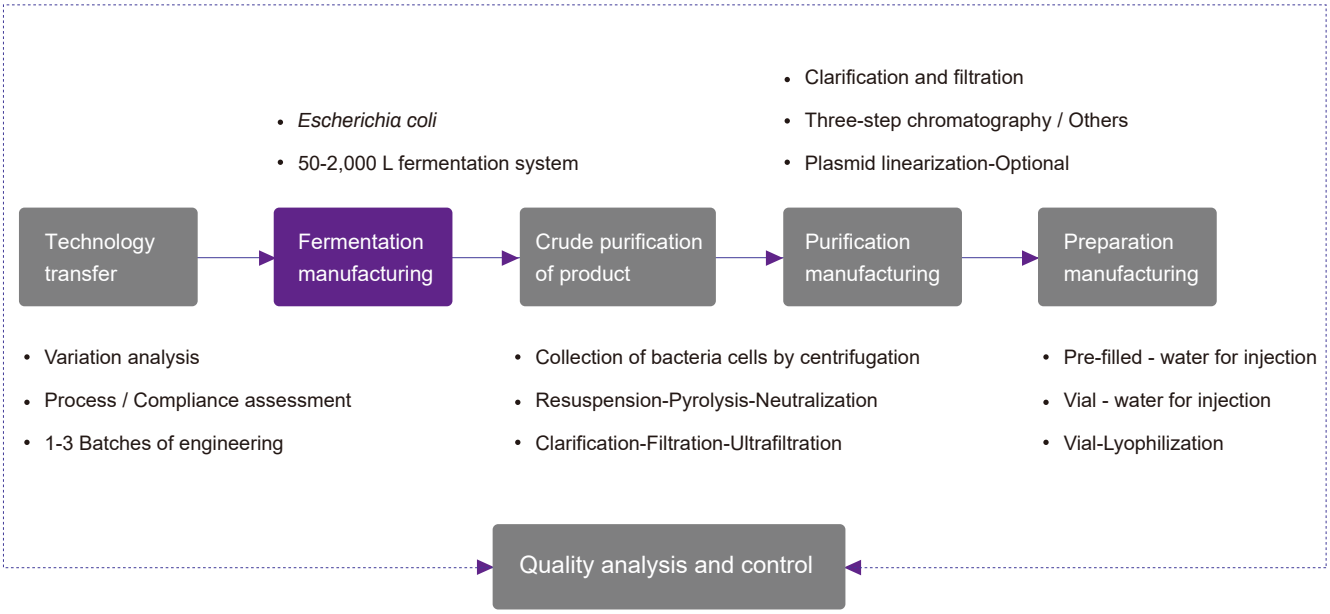
Based on the extensive CMO service experience of recombinant proteins/peptides/plasmids, Yaohai Bio-Pharma has accumulated experience in equipment-related process transfer of various brands, performances and models. We can quickly identify and adjust key equipment parameters to facilitate our customers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

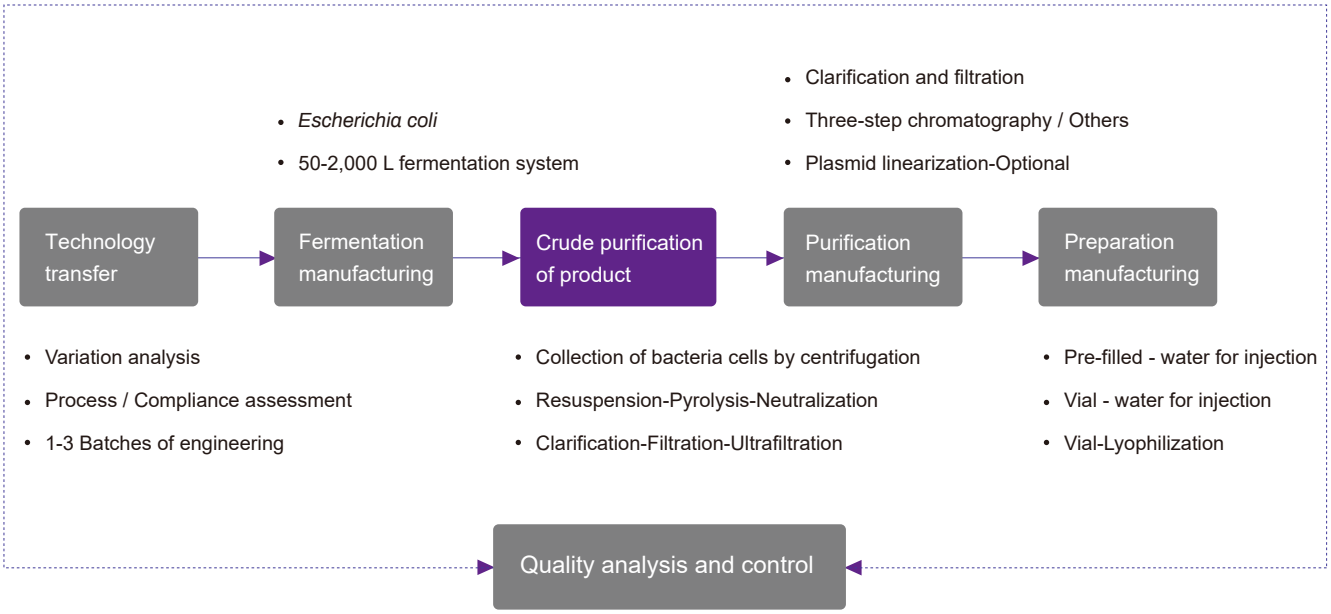
Parameter types	Related parameters	Frequently asked questions	Prevention or solutions
Questions related to culture conditions	Temperature and pH	[Volume-independent parameters, consistent]	The temperature, pH sensor, pump and other equipment are calibrated and tested under GMP quality standards.
	Feeding 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

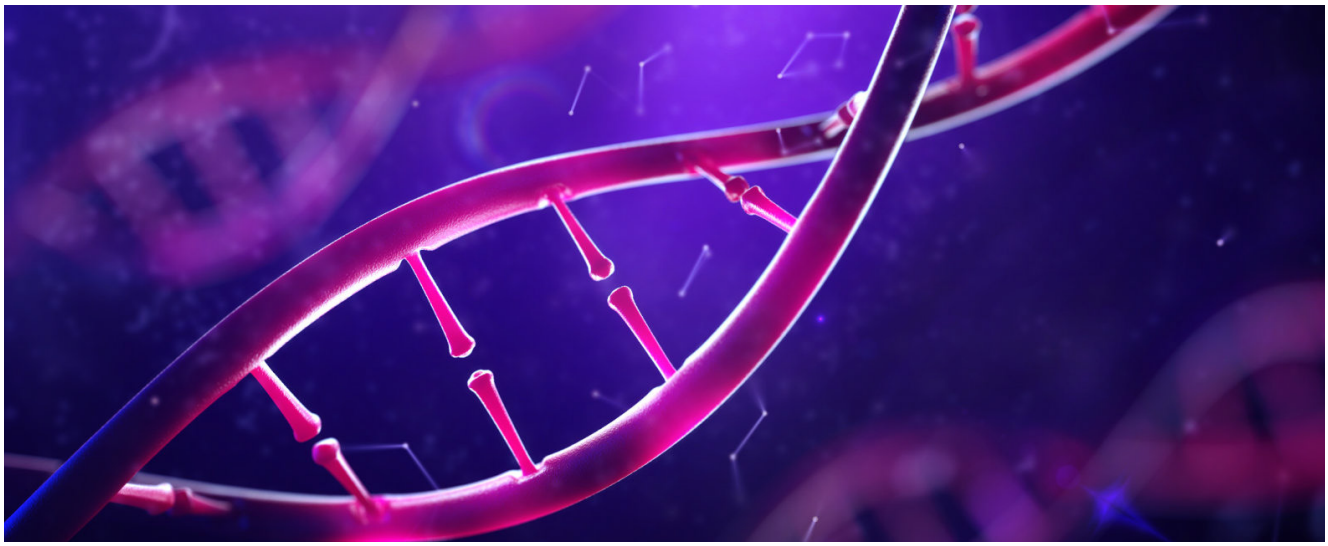
- Solution clarification
  Solids removed by filtration

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

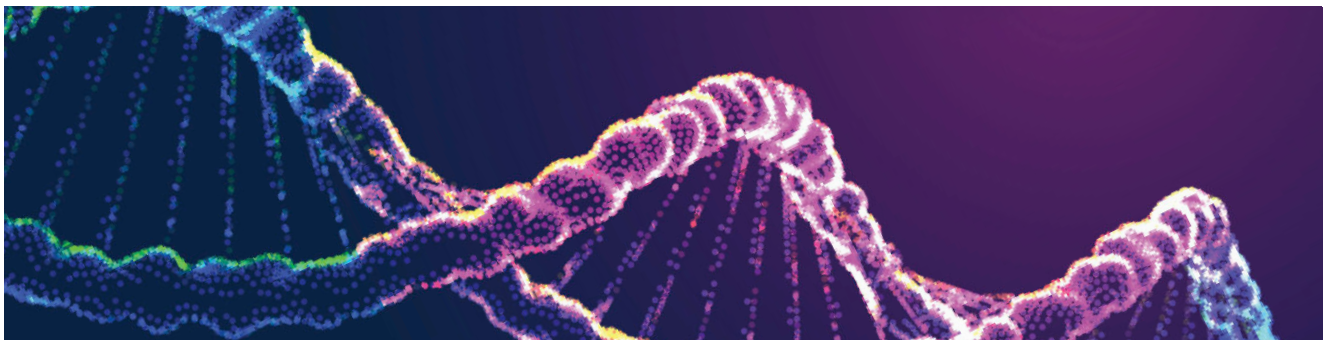
Key materials (raw materials and excipients) are tested for releasing to ensure effectiveness of the materials.

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.



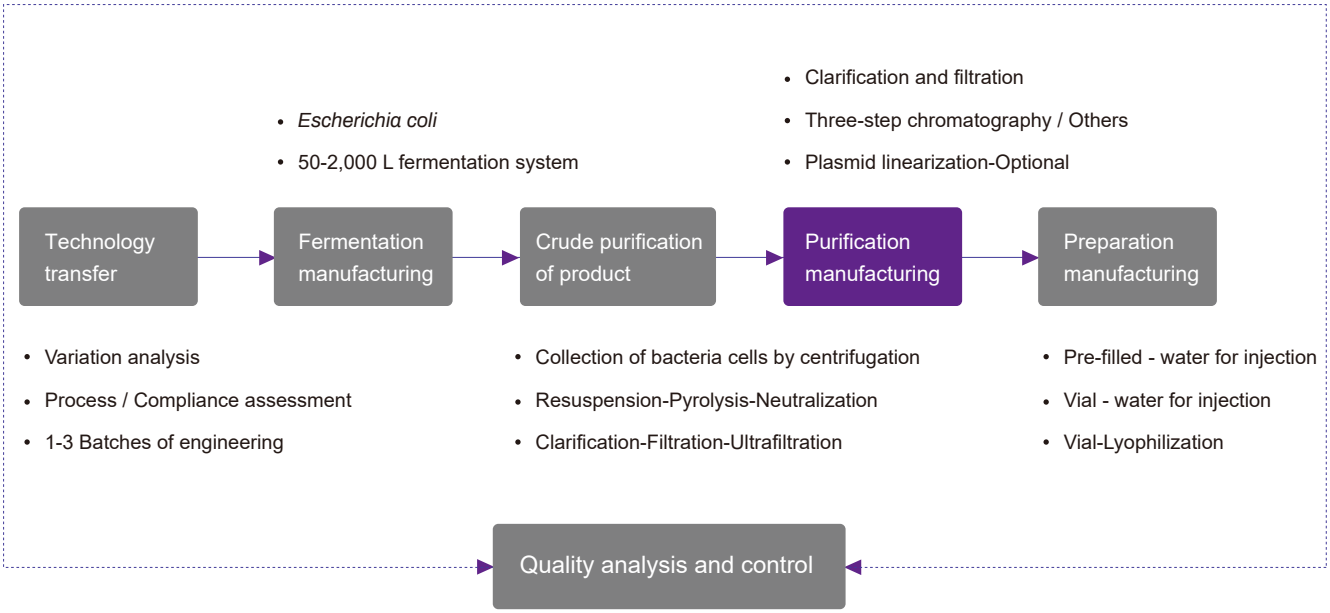
Based on the extensive experience in production, Yaohai Bio-Pharma has summarized the questions frequently occurred in the transfer process of centrifugation and clarification and filtration and the solutions:

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 Chromatography 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 specifica

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:

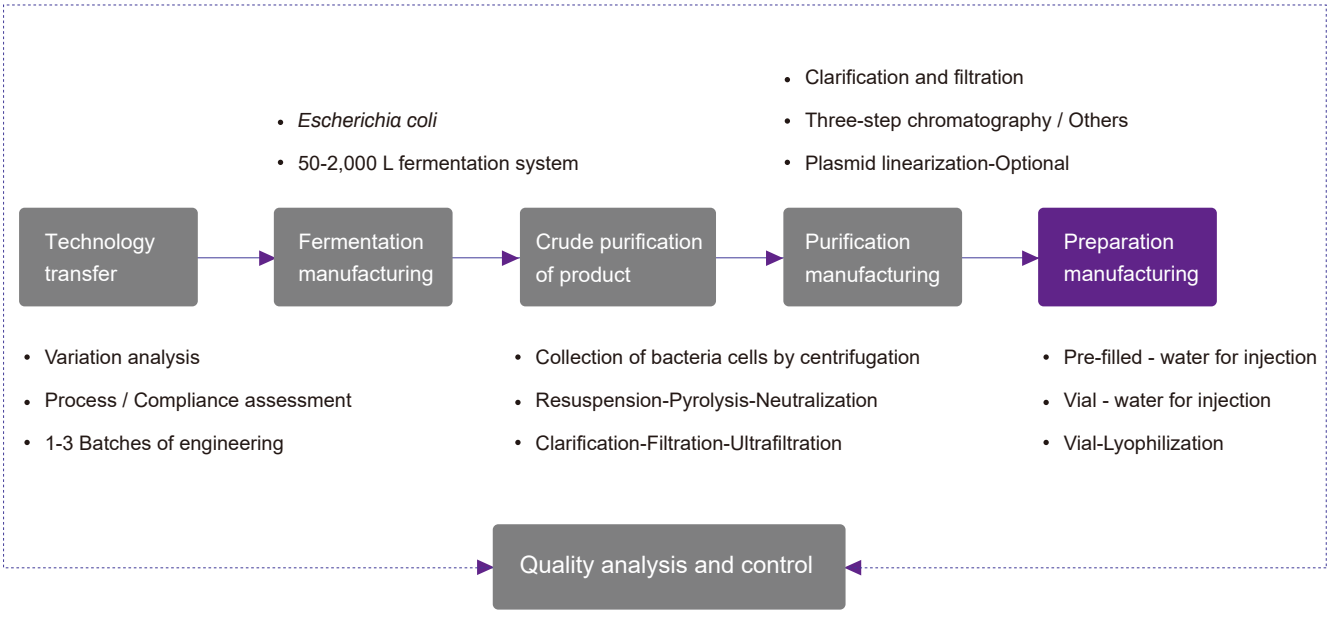
Purification process	Frequently asked questions	Process scale-up strategies
Filtration and clarification	No clarification process This process step is omitted in the small tests or medium tests	*Suggestion*: clarify the samples during the process scale-up to remove the solid-shaped substances, so as 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

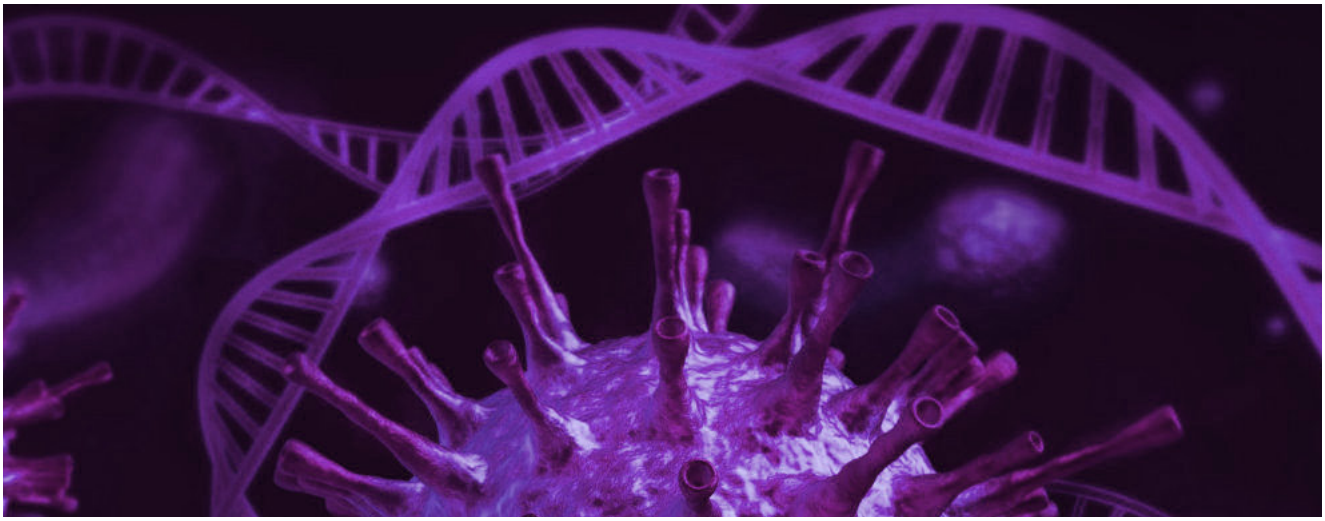
GMP-compliant automated sterile preparation production lines can serve the following products: 1-25 mL water for injection vials/lyophilization, 1-3 mL pre-filled syringe/cartridge water injectables.

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 experienc

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.

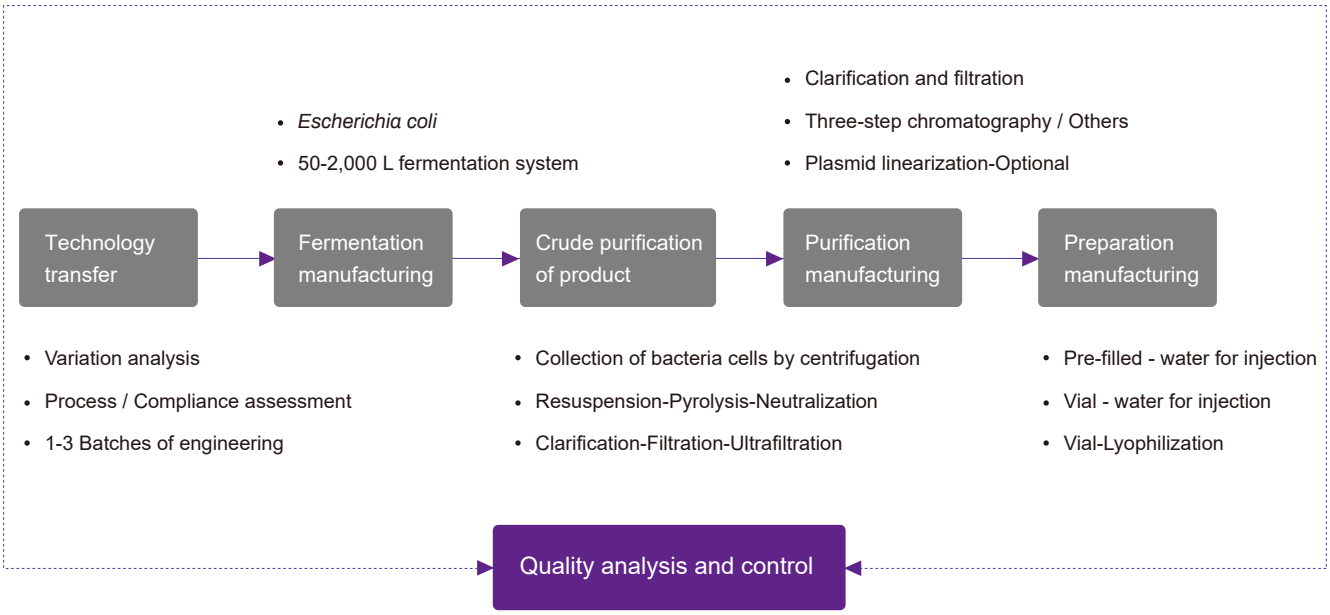
Preparation process	Critical equipment type	Equipment features
Aseptic filling	Ceramic plunger pump	• High filling precision, stable control of filling volume • Robust and corrosion resistant • Higher cost • Pump body is in direct contact with liquid medicine, with a certain cleaning difficulty
	Peristaltic 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

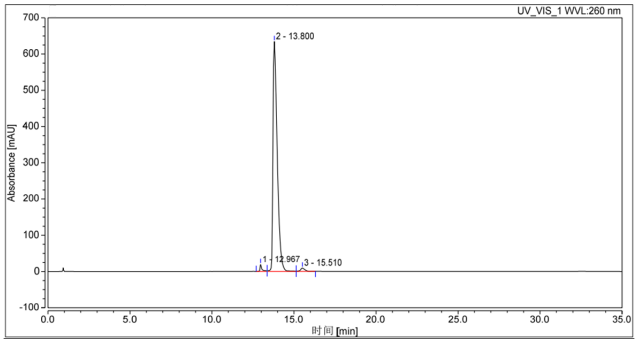
Based on our extensive experience in CMO services, the quality control team of Yaohai Bio-Pharma has established a highly applicable, robust and reliable analysis platform that can meet the requirements of physiological, biochemical and microbiological testing with stringent specification.

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.

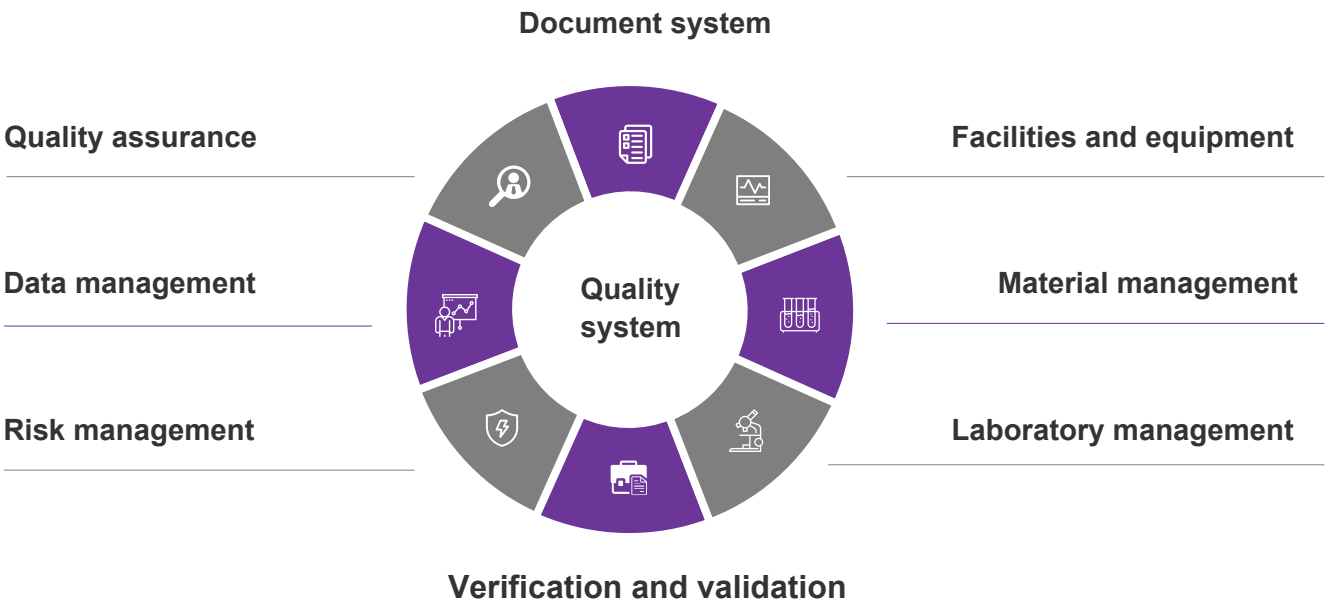
Other Services

- 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

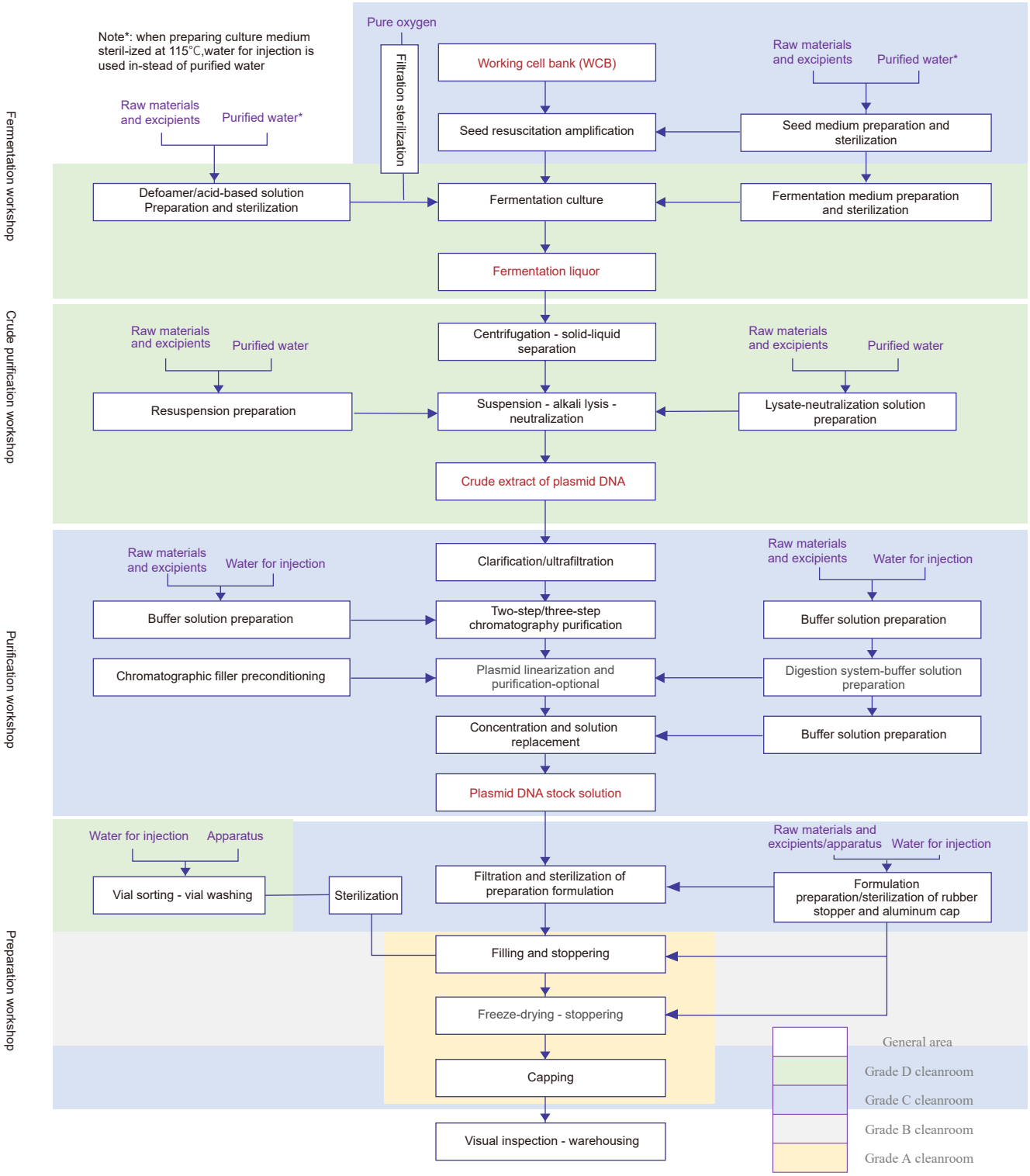
• Management of functional areas of different cleanliness classes: air conditioners are independently formulated to control differential pressure, temperature and humidity and suspended particles
• Safeguard of medium: water for injection, purified water, pure steam, and etc.
• Equipment: authority setting,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

SERVE
WITH HEART &
CREATE
THE FUTURE TOGETHER

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