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GenScript ProBio

PLASMID DNA

GENE AND CELL THERAPY

GMP Plasmid DNA
Process Development and Manufacturing

GenScript ProBio

GENE AND CELL THERAPY

Content

GenScript ProBio | GENE AND CELL THERAPY

About GenScript ProBio

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GenScript ProBio is the CDMO segment of the world's leading biotechnology company, GenScript, providing a one-stop biological drug research and development platform. GenScript ProBio's one-stop antibody drug development solutions include antibody drug discovery (hybridoma, single B-cell, phage display, full human and bispecific antibodies technologies), antibody engineering (antibody humanization, evaluation and optimization of drug compounds and affinity maturation) and other development services. GenScript ProBio's total cell therapy solution covers investigational new drug (IND) preparation as well as clinical sample and commercial production. Process development controls ensure compliance, data integrity ensures traceability, and all test deviations are strictly studied and documented.

Following the principle of "providing best-in-class quality and customer service" GenScript ProBio is committed to helping customers shorten the timeline for biological drugs from development to clinical use. In doing so, we are significantly reducing R&D costs, accelerating the commercialization of medicines, and building a healthy future, while at the same time making contributions to the development of the pharmaceutical industry.

Our professional plasmid team has developed a well-established plasmid manufacturing process, which not only guarantees the high quality and high purity of clients' products, but also enables the clients to obtain lower cost and shorter turn-around-time. GenScript ProBio's GCT platform provides plasmid manufacturing & process development services for regulatory filing as well as GMP plasmid manufacturing services from mg to g level to meet various expectations of the projects. Our team has collaborated several clients to support their projects and has assisted several clients to receive IND clearance from NMPA from 2018.

Our mission is to to be Professional CDMO partner to the global gene and cell therapy industry, by delivering best in class Product plasmid and viral vectors to Promote life changing gene and cell therapy Program.

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Plasmid Manufacturing Technology and Platform

Strains for Plasmid Manufacturing

Because of its long history of the laboratory culture and ease of manipulation, E. coli plays an important role in modern biological engineering and industrial microbiology (Sang YupLee, n.d.). Large-scale production using E.coli is rather economic and it is therefore the first choice for plasmid production. The most widely used cell lines for plasmid DNA manufacture are derivatives of K12, for example, DH5α, DH10B, JM108, Stbl3 and Stable-NEB, etc.

GCT Key Points in Selecting Strains

- To select a K12 strain with clear source;
- To solve the IP issue when submitting BLA/NDA;
- Screening for strains with high yield.

GCT GenScript ProBio's Solutions

- Create document to record history of each strains;
- Reached agreement with strain owner on the commercial use of the strain and also the sub-license authority. Solved the IP issue of strains in BLA/NDA and commercialization;
- The strains we adopted is efficient in producing plasmids with high purity and high supercoiled plasmid content.

Typical GMP Plasmid Manufacturing Process

The typical GMP plasmid manufacturing process can be summarized in the flowchart shown below (Figure 1). The whole process procedure begins from master cell bank (MCB) or working cell bank (WCB), continues to upstream fermentation and downstream purification, end ends up with fill and finish. Each step is involved with specific process control to ensure the final product quality (Macro et al., 2017).

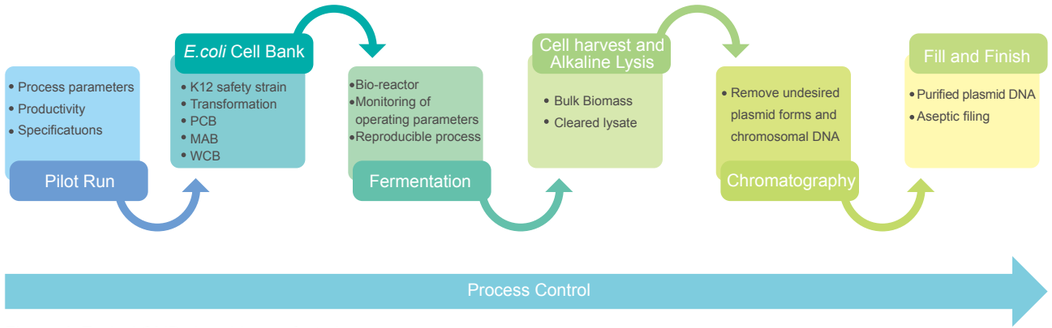


Figure 1. Typical GMP plasmid manufacturing process

Cell Banking

The viral vectors are generated using two, three or four plasmid constructs for each vector with two or three helper plasmids and a single therapeutic transgene. In the case of lentivirus system, for high safety standards, 4-plasmid system is the most widely used currently. And in Adeno-associated virus system, 2 packaging plasmids are required.

MCB is regarded as the starting point of a GMP plasmid DNA by the regulators, yet the initial plasmid construct is used to generate MCB. The plasmid DNA is transformed in *E.coli* and expanded to generate PCB, MCB, and WCB. To guarantee the quality of plasmid DNA and to manufacture an intended viral vector for the intended therapeutic benefit, the MCB is supposed to be thoroughly tested to remain homogeneous. Besides, the cell bank should be large enough to provide a stable source for a long-term large-scale production to ensure the consistency of the plasmid DNA.

For manufacturing of DNA plasmid from bacterial cell banks, FDA recommends MCB testing to include (ICH et al., 2018):

- Bacterial host strain identity;
- Plasmid presence, confirmed by bacterial growth on selective medium, restriction digest, or DNA sequencing;
- Bacterial cell count;
- Bacterial host strain purity (no inappropriate organisms, negative for bacteriophage);
- Plasmid identity by restriction enzyme (RE) analysis;
- Full plasmid sequencing. We recommend that you fully sequence plasmid vectors and submit an annotated sequence for the vector, as described in more detail in the section below on viral vector banks; and
- Transgene expression and/or activity.

Fermentation Process

Fermentation is usually run in shaking flasks or bio-reactors. When comparing to the shake flask fermentation, fermentation in bio-reactors usually shows advantages in high yield and feasibility for scaling-up, because Up-scaling is not just a question of using bigger devices but of carefully adjusting each process step in a way that can be reproducibly handled. The most commonly adopted fermentation process is fed-batch, and they are likely to be antibiotic free to reduce the risk of the viral vectors to animals or human. By adjusting and monitoring the operating parameters, such as pH, oxygen uptake rate, specific growth rate, etc., the fermentation rate and yield can be optimized to find the best fermentation conditions (Macro et al., 2017).

After the fermentation process, the crude product is obtained through cell lysis. The plasmid DNA stays in the solution and can be separated from genomic DNA and other floccules by filtration or centrifugation.

GCT Key Points in Fermentation

- The fermentation process is supposed to lead to high plasmid yield;
- The fermentation process is supposed to be easy to scale up;
- The fermentation process is supposed to have high stability.

GCT GenScript ProBio's Solutions

- High density fermentation;
- Animal-free, antibiotic free during fermentation process;
- Manufacturing scale from 5L to 50L, feasible to scale up;
- 3 batches of lab-scale production and pilot-scale production to ensure the stability of manufacturing process.

GenScript ProBio's Case Studies

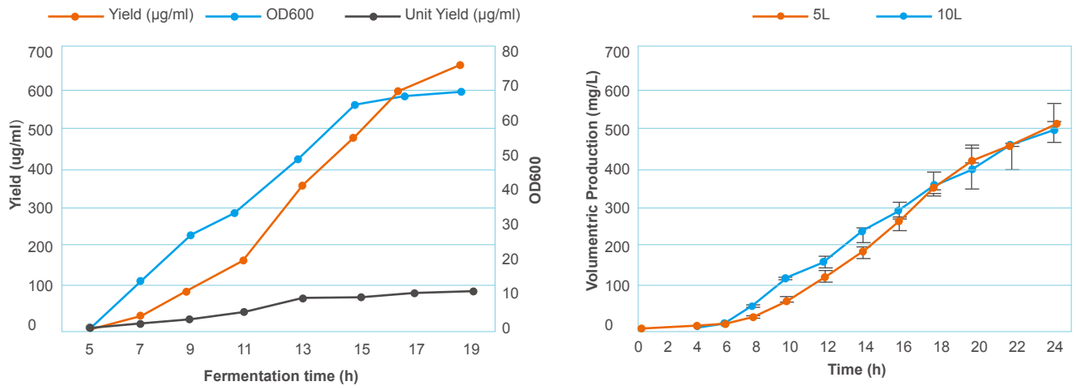


Figure 2. High density fermentation process. Left: yield, OD600 and unit yield of one batch of plasmid DNA during fermentation process. **Right:** yield (volumetric production) of 3 batches at 5 L scale and 3 batches at 10 L scale during the whole fermentation period.

Purification Process of Plasmid DNA

The purity of the plasmid DNA is one of the most relevant specifications regarding the quality of the plasmids and even final efficacy and toxicity of the final cell products. For this reason, the purification process plays an important role in removing the impurities and contaminants. Chromatographic methods are widely applied for plasmid purification for their high efficiency in removing undesired components. Another reason for the application of chromatographic purification methods is the feasibility in large-scale production. Chromatography methods are usually stable, reproducible and easy to scale up.

Certain chromatographic step is responsible for removing host cell residuals, including host cell proteins, host cell DNA and host cell RNA, and certain chromatographic steps can eliminate endotoxin and other contaminants. Some steps such as hydrophobic-interaction chromatography (HIC) work well for separating open and closed-circle plasmid forms (Hitchcock, 2016). By reasonably combining various purification steps, the purity and the quality of the plasmid DNA can be improved significantly.

GCT Key Points in Purification

- Improve the stability of the process, especially in lysis;
- Improve recovery rate;
- Improve the efficiency in removing impurities, especially the critical quality attributes, such as HCP (host cell proteins) and HCD (host cell DNA), so as to improve content of supercoiled plasmid.

GCT GenScript ProBio's High Quality Solutions

- Recovery rate has reached between 20%~50% to get higher yield of plasmid after purification;
- Established 3-step purification methods based on experience with several projects;
- Adopted proprietary automated closed continuous lysis system to improve the stability of the process.

GCT GenScript ProBio's Case Studies

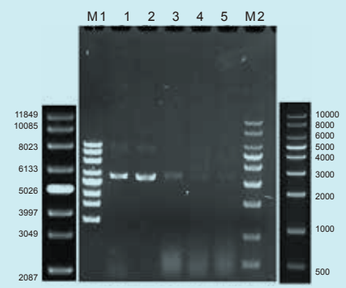
A series of chromatographic methods are adopted by GenScript ProBio, and it is sufficient in obtaining high purity plasmid DNA.

Low content of impurities

Project	HCD (%)	IEC (%)	HCP (%)
1	0.04	96.00	<0.0003
2	0.02	97.05	<0.0003
3	0.02	93.00	<0.0003
4	0.03	93.80	<0.0003

Table 1 Data of 4 projects, all including 3 batches of production.

Critical quality attributes: HCD as low as 0.02%; HCP lower than the minimum detection value 0.0003%; Supercoil content (IEX) exceeds 93%.



Separating the host cell RNA efficiently

Figure 3. Agarose gel electrophoresis (AGE) of the samples and the wastes from first step of chromatographic purification.

M1: Supercoiled DNA Ladder Marker;

M2: 1 kb DNA Ladder Marker;

1: Lysate; **2:** Sample after first purification step;

3: Waste of salt elution; **4:** Waste of salt elution;

5: Waste of water elution; **R:** RNA bands.

Quality Control

Through purification process, the impurities are removed and controlled for obtaining products in high purity and quality. As a core part in the whole manufacturing process, the quality control of a batch release should be carefully studied and the best suitable assay methods are validated to ensure the final plasmid quality. Due to the specialty of plasmid DNA, it can either be used to produce viral vectors, or be drug substance or drug product itself. With respect to different uses of plasmid DNA, the critical quality attributes and the release standards are different. However, the basic rules remain the same. The quality of the plasmids is highly related to the final products, either viral vector, cells or plasmid, the quality of the plasmid DNA should be strictly controlled in terms of identity, purity, potency, and safety. The following quality items may be listed in QC: sterility, endotoxin, purity (including percent of supercoiled form and residual cell DNA, RNA, and protein levels), and identity testing (restriction digest and sequencing if sequencing was not performed on the bacterial bank). In addition, reasonable assay methods and release standards should be studied as well (ICH et al., 2018).

GCT Key Points in quality control

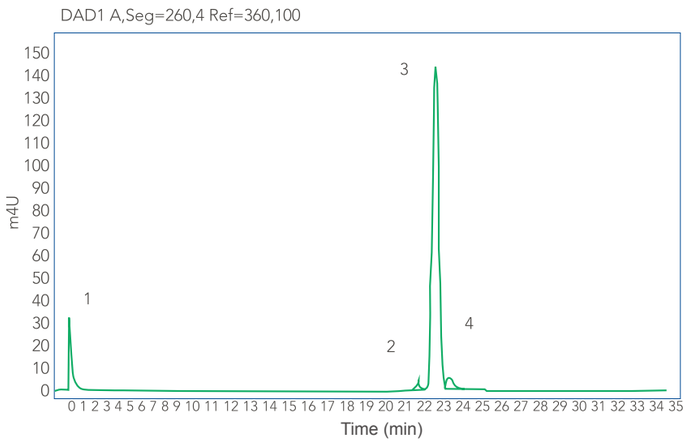
Difficulties in development and qualification of analytical methods.

GCT GenScript ProBio's Solutions

- GenScript ProBio recognizes the critical quality attributes of products based on industrial experience and regulation information. The safety and efficacy properties of products, such as the product characterization, process-related impurity detection and safety related items will be assessed.
- GenScript ProBio designs and develops assay procedures scientifically and systematically to meet the varied analytical requirements. The assay performance applied in GenScript ProBio can meet or exceed the industry standards in the field of quality characterization.
- GenScript ProBio follows the guidelines of the Chinese Pharmacopoeia (ChP) Technical Guideline 9101, ICH Q2 and the United States Pharmacopoeia (USP) General Rules 1225/1226 to confirm the performance of the quality analysis method and prove that the method is suitable for testing requirements.

GCT GenScript ProBio's Case Studies

Through the first and the second purification processes, most of the undesired plasmid forms can be eliminated and the active ingredient content, supercoiled plasmid content reaches around 95% after the second purification process.



Sample	Time	Area (%)
OC-plasmid	20.800	1.62
SC-Plasmid	21.790	94.07
dimer-plasmid	22.533	4.31

Figure 4. High Performance Liquid Chromatography (HPLC) of the purified samples after step 1 and step 2 to detect the content of supercoiled plasmid.

1: Solvent; **2:** Open circular plasmid; **3:** Supercoiled plasmid; **4:** Dimer plasmid.

GenScript ProBio's Plasmid DNA Services

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GenScript ProBio's Plasmid DNA Services

GenScript ProBio has FDA standard manufacturing technologies for plasmid DNA production and has sufficient scales to meet all the requirements from milligrams to gram level scale. Requirements for bulk production of plasmid DNA can be achieved in a single batch with high purification standards and strict batch release standards.

Facilities

GenScript ProBio's plasmid process development facility has 2 segregated manufacturing suites, and is capable of providing manufacturing scales ranging from 5L to 100L, to satisfy needs from pre-clinical to early stage clinical trials.

GenScript ProBio is expanding its capability and capacity at a new facility, which will more than double the footprint and capability of the current one. The new facility will be in full operation from 2021.

In addition, a US partner of GenScript ProBio, who has a GMP facility and is capable of providing GMP plasmid manufacturing for clients, is ready to provide services from the US.

Plasmid DNA Manufacturing

GenScript ProBio provides a series of plasmid manufacturing services for various applications depends on clients need and to meet demands in different stages. In different applications, the requirements on manufacturing process, quality control and quality assurance process are quite different. Recognized the essential conditions for each degree of plasmids, GenScript ProBio offers plasmids with the degree of ProPlasmid, GMPro plasmid, and GMP plasmid, all with the most appropriate manufacturing process and quality oversight that are compliant to regulatory.

GenScript ProBio's plasmid manufacturing services are generally featured with:

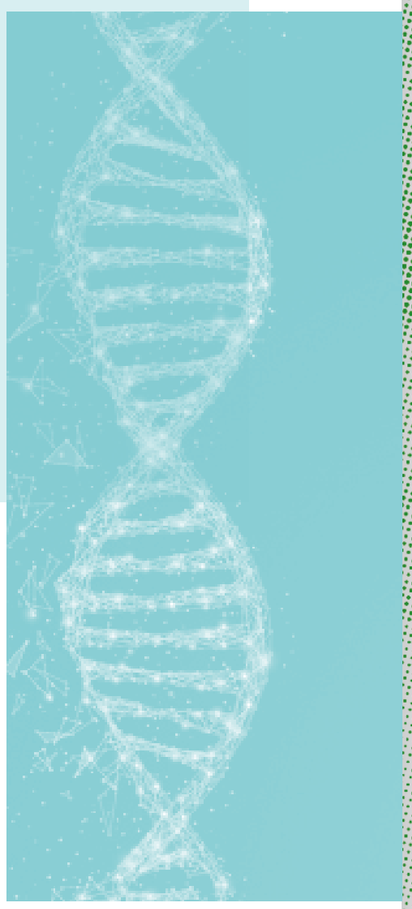
- **Animal free, antibiotic free,** low risk to animal health
- High yield with **600~800 mg/L** in helper plasmids from GenScript ProBio
- High supercoiled plasmid content**: >90%**
- Strict quality controls
- Appropriate quality assurance process
- Cost-effective with short lead time

ProPlasmid is an option suitable for the use in non-clinical stages. It is manufactured in well controlled environment and under critical quality assurance.

GMPro Plasmid, which is manufactured from WCB (working cell bank) under comprehensive quality oversight, is applicable in early stage clinical trials. It is a cost-effective alternative that enables faster transition to clinic.

GMP Plasmid we provide can meet all needs through the development cycle from clients. No matter it is used as raw materials for production of viral vectors or it is used as the drug product, GMP plasmid from GenScript ProBio can meet all these requirements.

Besides, all the necessary work, such as cell banking, quality controls, stability test are available to support clients' projects.



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Track Record of GenScript ProBio

11.2018

Opening ceremony of Plasmid Process Development Facility in ZJ, CN

11.2018

1st CN Order & 1st strategic collaborator

- 1st ProPlasmid & lentivirus manufacturing order
- 1st Plasmid & lentivirus CMC order

01.2019

2nd CN Order & **2nd strategic collaborator**

- ProPlasmid & lentivirus manufacturing
- Plasmid & lentivirus CMC

03.2019

Strategic collaboration with **Merck** for platform construction

03.2019

CN Order

- ProPlasmid & lentivirus manufacturing

03.2019

CN Order & **3rd strategic collaborator**

- ProPlasmid & lentivirus manufacturing
- Plasmid & lentivirus CMC

04.2019

Ground-breaking ceremony of **Commercial Manufacturing Center** in ZJ, CN

04.2019

CN Order

- Plasmid CMC

06.2019

CN Order

- Plasmid CMC

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08.2019

1st US Order

- 1st ProPlasmid manufacturing order

12.2019

Opening ceremony of **GMP Virus Facility** in ZJ, CN

12.2019

CN Order

- Plasmid cell banking

01.2020

2nd US Order

- ProPlasmid manufacturing

01.2020

3nd US Order

- ProPlasmid manufacturing

01.2020

3nd US Order

- ProPlasmid manufacturing

03.2020

CN Order

- 1st GMPro Plasmid manufacturing order for clinical supply

04.2020

1st Korean order

- Plasmid & virus CMC projects

2021

Opening of GMP Plasmid Facility in ZJ, CN

2022

Opening of Commercial Manufacturing Center in ZJ, CN

GMP Plasmid DNA Process Development and Manufacturing