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QUALITY STUDY AND PROCESS CONTROL OF

GMP PLASMID PROCESS DEVELOPMENT

White Paper

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Content

INTRODUCTION

Gene and cell therapy has potential to transform medicine, providing long term and potentially curative treatment options for a wide range of diseases. The first two CAR-T therapies emerged as new pillars for B-Cell ALL in 2017, achieved FDA approvals in USA and recently EMA in Europe. It is a landmark point for pharmaceutical industry, and gene and cell therapy is expected to dominate the market with over 500 clinical trials, billion dollars of investments and a long list of pharma collaborations, mergers and acquisitions.

To accelerate the development path of gene and cell therapy, GenScript is dedicated to produce plasmid DNA for gene and cell therapy with advanced technologies and experienced team. It enabled us to support Legend Biotech not only for plasmid and viral vectors production, but also supported its successful IND clearance from both FDA and NMPA. Our team has capability and confidence to provide you with best CDMO service from preclinical plasmid preparation through IND application to commercial plasmid manufacture. GMP plasmid service has well optimized manufacture process and best in class plasmids can meet your requirements in different stages with rapid turn-around time.

Regulations

FDA requires investigational new drug (IND) filing for all products from sponsors and CMC information for the drug substance are needed. The new draft guidance, Chemistry, Manufacturing, and Control (CMC) Information for Human Gene Therapy Investigational New Drug Applications (INDs), issued in July 2018, is organized to follow the structure of the FDA guidance on the Common Technical Document (CTD). Module 1 should contain administrative information, including administrative documents, product labels, environmental analyses, and references to previously submitted information. Module 2 should contain a summary of the quality information that will be presented in greater detail in Module 3. And in Module 3, detailed recommendations are addressed concerning both drug substance (DS) and drug products (DP) from general information, manufacturing process, characterization to quality controls. Another part of module 3 has manufacturers, description of manufacturing process and process controls (batch and scale, manufacturing process, cell culture, vector production, genetically modified cell production, irritated cells and shipping information) and control of materials.

The emphasis for CMC review in all phases of development is product safety and manufacturing control. The FDA approaches certain aspects of gene therapy products to regulate and control the product quality, and the following parts will summarize the critical and significant aspects that need attention.

Manufacturing Process

- The description of your manufacturing process should include a flow diagram(s) and a detailed narrative. Your description should clearly identify any process controls and in-process testing (e.g., titer, bioburden, viability, impurities) as well as acceptable operating parameters (e.g., process times, temperature ranges, cell passage number, pH, CO_2, dissolved O_2, glucose level).
- Plasmid Production

  For the manufacture of bacterial plasmids, you should provide a description of all production and purification procedures. Production procedures should include a description of the cell substrate, cell culture and expansion steps, transfection or infection procedures, harvest steps, hold times, purification (e.g., centrifugation, column purification, density gradients), concentration or buffer exchange steps, and the reagents/components used during these processes.
- As an active ingredient, the same level of control should be applied to each DS, and each DS should be manufactured under appropriate Good Manufacturing Practice (GMP) conditions.

Control of Materials

- You must provide a list of all materials used in manufacturing (21 CFR 312.23(a)(7)(iv)(b)) and a description of the quality and control of these materials. This information may be provided in tabular format and include the identity of the material, the supplier, the quality (e.g., clinical-grade, FDA-approved), the source of material (e.g., animal, human, insect), and the stage at which each material is used in the manufacturing process (e.g., culture media, vector purification).
- Reagent

  Animal-derived materials increase the risk of introducing adventitious agents. Certain animal-derived materials, such as sera, are complex mixtures that are difficult to standardize, and such materials may have significant batch-to-batch variations that may affect the reproducibility of your manufacturing process or the quality of your final product. We recommend that you use non-animal-derived reagents whenever possible (for example, serum-free tissue culture media and recombinant proteases).
- Banking System

  Banking assures an adequate supply of equivalent, well-characterized material for production over the expected lifetime of production. For these reasons, banked materials are a common starting point for many routine production applications. We recommend that you provide a summary of the testing and COAs in this section.

For all bacterial or microbial (e.g., yeast) MCBs, you should describe the genotype and source of the microbial cells. You should provide a detailed description of the history and derivation of the materials used to generate the cell bank, including information on how plasmid vectors were designed and constructed.

For the bank material, itself, you should provide information on how the material was generated and how the bank is stored and maintained as well as detailed information on qualification of the bank (including cell bank COAs) to adequately establish the safety, identity, purity, and stability of the microbial cell preparation used in the manufacturing process.

For bacterial cell banks used to manufacture a DNA plasmid, we recommend MCB testing include:

- 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.

Control of Plasmid DNA

We recommend that DNA plasmid intermediates be derived from qualified banks.In addition, we recommend that you provide information on the plasmid manufacturing procedures, reagents, and plasmid specifications for use. In general, we recommend that this testing include assays to ensure the identity, purity, potency, and safety of the final product. For a DNA plasmid, this may include 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). A COA documenting plasmid quality testing should be included in the IND.

Process Validation and/or Evaluation

INDs at all stages of development should have established written standard operating procedures (SOPs) to ensure proper manufacturing control and oversight. Your IND should include a description of your Quality Unit, including the manner in which quality control testing and oversight are separated from the manufacturing unit.

Manufacturing Process Development

You should provide a description and discussion of the developmental history of the manufacturing process.

We recommend that you describe how manufacturing differences are expected to impact product performance. If you make significant manufacturing changes, then comparability studies may be necessary to determine the impact of these changes on the identity, purity, potency, and safety of the product.

Stability

We recommend that you describe in your original IND submission the types of stability studies (either conducted or planned) to demonstrate that the DS is within acceptable limits. Your stability analysis may include measures of product sterility (or container integrity), identity, purity, quality, and activity or potency. We recommend that you provide justification for the test methods and acceptance criteria used in the stability analysis and we recommend that you provide the results of your stability studies in your IND and update this information on a regular basis (e.g., annual reports).
A post-approval stability protocol and stability commitment in the IND is not recommended to provide, but at your late phase IND meetings, the discussion of these items is recommended.

GMP Plasmid Quality Control

FDA drafted specific guidance on CMC study for gene therapy IND filings, which emphasizes product safety and manufacturing control in CMC review in all phases of development. FDA requires an IND submission containing the overview of manufacture process control and quality control(Ich et al., 2018). As a critical intermediate in the manufacturing process for preparation of desired viral vectors, the quality of the plasmids are highly related and therefore, manufacture process and quality of the plasmids should also be well controlled.

GenScript monitors whole production process, establishes strict quality control system and sets different specifications. Using high-sensitivity and high-precision test methods, the quality of plasmids are controlled in real time, and release standards are set accordingly.

Along with the process development, the release standards in different stages: small-scale release, pilot-plant release, batch release and long-term stability, are set which can represent the process and process controls accurately.

▼ **Table 1 GenScript QC Items and Release Standards**

Category		QC Item	Small-scale/ Pilot Release	Batch Release	Long-term Stability
Normal	1	Appearance	Clarify	Clarify	Clarify
Purity	2	PH	7.0-8.5	7.0-8.5	-
	3	A260/A280	1.8-2.0	1.8-2.0	-
	4	Plasmid DNA Concentration	-	-	1±0.1mg/mL
	5	Supercoiled Content	≥ 95%	≥ 95%	≥ 90%
	6	Host Protein	≤ 0.1%	≤ 0.1%	Correspond to the standard
	7	Residual *E. coli* DNA	<< 0.1%	<< 0.1%	Correspond to the standard
	8	Residual *E. coli* RNA	undetectable (gel)	undetectable (gel)	Correspond to the standard
Contamination	9	Endotoxin	≤ 10 EU/mg	≤ 10 EU/mg	≤ 10 EU/mg
	10	Sterility	-	No growth	Negative
Plasmid Identity	11	Sequencing	Pass	Pass	-
	12	Restriction Digest	Match the expected	Match the expected	-

GMP Plasmid Process Control

A. Plasmid DNA Construction

From the DNA sequence provided by the customer, stable monoclonal strain is produced and selected by cloning, transformation and screening.

B. GMP Cell Banking

After a stable cell line is selected, primary cell bank (PCB), main cell bank (MCB) and working cell bank (WCB) are built, and the quality of each cell bank is comprehensively controlled. The test items and release standards of PCB, MCB and WCB are listed as follows.

▼ **Table 2 Test Items and Release Standards of PCB**

Objects of study	Items	Standards
PCB bacterial glycerol stock	OD600 during bacteria retention	0.6~1.2
	Coefficient of variation of OD600	≤ 30%
Stability Test of Plasmid	Coefficient of variation of plasmid yield	≤ 25%
	Plasmid retention rate	≥ 80%
	Restriction Digestion	Fragment size is identical with theory
	Sequencing	Sequence is identical with theory
	Supercoiled Plasmid Content	≥ 90%
	Colonial Morphology	Neat edge, smooth surface, translucent small bumps

▼ **Table 3 Test Items and Release Standards of MCB/WCB**

Objects of study	Items	Release Standards
MCB/WCB Bacterial Glycerol Stock	Cell Activity	>1.0×10^7 CFU/mL
	Bacteria and Fungus	Negative
	Gram Stain	Gram-negative, red and straight rod
	Phage	Negative
Stability Test of Plasmid	Purity Test	no other bacteria grow on TSA and SDA
	Coefficient of Variation of Plasmid Yield	≤25%
	Plasmid Retention Rate	≥80%
	Restriction Digest	Fragment size is identical with theory
	Sequencing	Sequence is identical with theory
	Supercoiled Plasmid Content	>90%

C. Process Development

Plasmid manufacture process development includes fermentation process development, purification process development and small-scale production.

Upstream process development standards

After the cell bank is well built, strains are first expanded in shake flask and then transferred to fermenter for further fermentation. The shake flask fermentation and high-density fermentation are supposed to meet the following standards:

▼ **Table 4 Upstream process development standards**

Criteria of Shake Flask Fermentation	Verify cell growth curves
	Determine inoculation time window
	Determine OD600 range for inoculation
Criteria of High-density Fermentation Process	OD600 reaches over 30
	The yield reaches over 200 mg/L
	Determine feeding strategy
	Determine oxygen uptake rate and pressure adjustment scheme.
	3 batches of small-scale production.
	Parameters remain stable and the deviation keep lower than 20%.
	Process development report, completion of process development

By manipulating pH, temperature, oxygen uptake rate, optimizing fermentation time period, an optimal fermentation condition with high yield and high stability can be developed.Only when the parameters, yield and quality of 3 batches of small-scale production keep lower than 20%, the process development comes to the end and the process development report can be written.

Downstream process development standards

The plasmid obtained after fermentation and cell lysis need to be purified through several steps of purification to reach high-level purity. Downstream process aims at removing host cell residuals and other adventitious contaminants and is to be developed until optimal purification methods are found to make the final products qualify the quality standards.

The downstream process is regarded to be up to standard when the following criteria are met:

- The quality of the plasmids meet the QC standards
- The parameters, yield and quality of three batches of small-scale production remain stable and the deviation are less than 20%
- The process is proved to be feasible for large scale production, and the results remain consistent with that of small scale production (yield and QC standards)

Small scale quality control and release standards

When the best optimized process is developed, it should be verified by three batches of small scale production. The criteria for a well-established plasmid manufacture process is listed as follows:

- The yield of purification remain consistent
- The final plasmids meet the QC standards
- The indications remain stable and consistent
- Deviation is less than 20%

Consistency of three batches of small scale production is shown through these criteria and the final plasmid manufacture process is completed and determined.

D. Quality Study

As an important intermediate, it is recommended that the plasmids be comprehensively tested to ensure the identity, purity, potency and safety(Ich et al., 2018). GenScript sets totally 11 QC items to examine the plasmids from different aspects and refers to USP for an applicable assay method. The detailed information of the QC items are listed in **Table 5**.

▼ **Table 5 Quality Control Items**

	QC Items	Stages of test	Standards
1	Appearance	Intermediate control batch release	USP 631 Color and achromicity
2	pH	Intermediate control batch release	USP 791 pH
3	A260/A280	Intermediate control batch release	USP 851 Spectrophotometry and light-scattering
4	Supercoiled plasmid	Intermediate control batch release	USP 621 Chromatography
5	Host Protein	Intermediate control batch release	USP 1132 Residual Host Cell Protein Measurement in Biopharmaceuticals
6	Residual *E.coli* DNA	Intermediate control batch release	USP 509：RESIDUAL DNA TESTING
7	Residual *E.coli* RNA	Intermediate control batch release	USP 726 Electrophoresis
8	Endotoxin	Intermediate control batch release	USP Bacterial Endotoxins
9	Sterility	Batch Release	USP 71 Sterility Test
10	Sequencing	Intermediate control batch release	-
11	Restriction Digest	Intermediate control batch release	USP 726 Electrophoresis

E. Pilot Production and Batch Release

After verifying the stability and consistency of the manufacture process through three batches of small-scale production, pilot production is also needed for further verification of the feasibility of the manufacture process to scale up.

Three batches of pilot production are carried out in GMP facilities using stirred bioreactors and verified purification methods. The final products are tested. Only when all the three batches exhibit consistent yield, purity and quality specifications. Meanwhile, all the indicators are in line with that of small scale production, is the manufacture process proved to be stable suitable for large scale production.

F. Plasmid Stability Study

The stability study of the viral vector is a significant part in IND submission, which calls for plasmid long-term stability for designing the storage condition and assessing the period of validity of viral vectors. To test the plasmid stability thoroughly, following items are examined and monitored, and the acceptable limits are also exhibited **(Table 6)**.

▼ **Table 6 Plasmid Long-term Stability Study Criteria**

Items	Acceptable criteria
Appearance	Clear, transparent, no particles
DNA concentration	1±0.1mg/mL
DNA Purity (AGE)	Corresponds the standard
Supercoiled plasmid	>90%
Endotoxin	<10EU/mg
Sterility	Negative

We emphasize that, our predetermined criteria for long-term stability of stability shows failure of any specifications.

G. Documentation

- The source and the batch description of the plasmids are all recorded in detail to ensure traceability.
- The process development, manufacture process, test record and all the deviations are well documented to ensure traceability.

GMP Plasmid Manufacture Process

GenScript plasmid manufacture process is well developed through several times of optimization and validation. Together with the advanced technologies, GMP facilities and experienced team, GenScript provide high yield and best in class plasmid for gene and cell therapy. We are able to produce plasmid of different quantities and our plasmids can satisfy different needs.

A. Fermentation Process

Equipped with 5L to 200L stainless steel bio-fermenter, GenScript has the capability to manufacture plasmids of various quantities with rapid turnaround time. By manipulating the parameters repeatedly, an optimal condition is validated and final products with best quality and highest yield is guaranteed.

GenScript fermentation process is characterized with:

- High-density fermentation
- Rapid fermentation within 16~24 hours
- High quality, high titer: 600~800 mg/L
- Animal-free, antibiotic-free, low risk to animal and human health

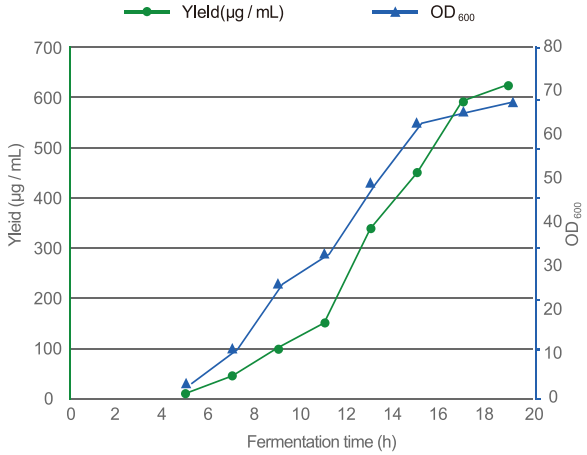


Figure 1 GenScript High Density Fermentation. Left: stainless steel Fermenter; **Right:** Fermentation process. The Fermentation room is equipped with Bio-fermenters from 2L to 150L. And GenScript high density fermentation can already reach a plasmid titer of 600 ~ 800 mg/L.

B. Purification Process

ombined with three steps of chromatographic methods, GenScript purification process eliminates hazardous host cell residuals and other contaminants efficiently and improved purity of plasmids greatly. Other undesired plasmid forms are removed as well, and the content of the active ingredient, supercoiled plasmid, is greatly improved. GenScript three-step purification process has following advantages:

- Three steps of **chromatographic** purifications
- Step 1: Aims at removing host cell residuals, including host cell proteins, host cell DNA and host cell RNA, etc.
- Step 2: High efficiency in removing undesired plasmid forms, such as open plasmid and plasmid dimer. Supercoiled plasmid content can be increased to over 95%
- Step 3: further purification of all the impurities and contaminants
- Free of enzymes (eg. RNase), detergents and organic solvents

Figure 2 is a purification case that proves high purification efficiency. In sample 1, a series of strong RNA bands can be seen which represents a high concentration of the residual RNA, while in sample 2, there are almost no bands observed in RNA area. This is a powerful evidence of the efficiency of first step for removing the residual RNA. Another proof is that in the waste samples (3, 4, 5), intensive RNA bands are obvious to be seen, which indicates that large amount of RNA are removed.

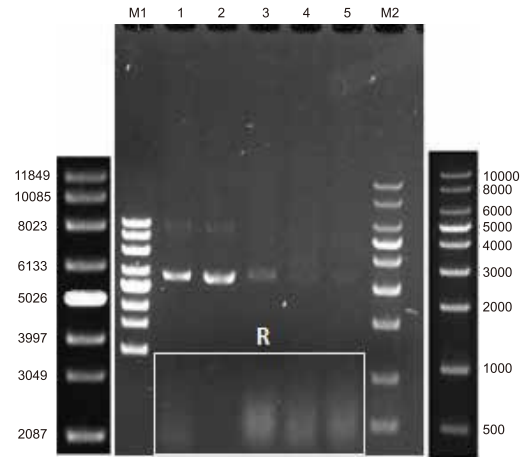
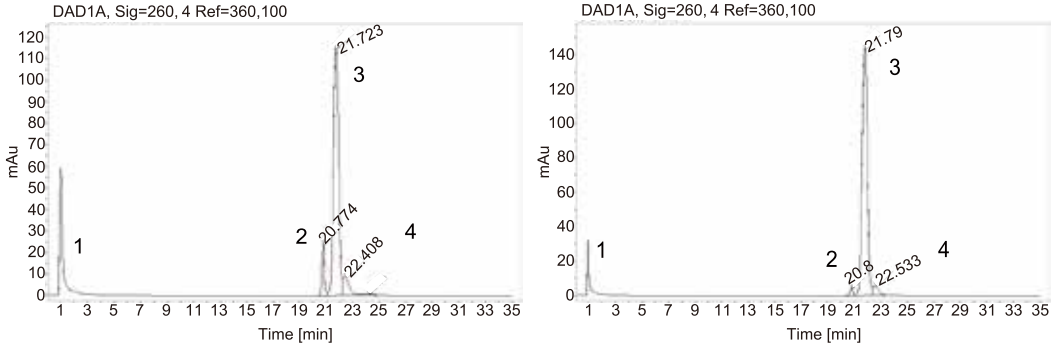


Figure 2 Agarose gel electrophoresis (AGE) of the samples and the wastes from first step of chromatographic purification. M1: Supercoiled DNA Ladder Marker; **M2:** 1 kb DNA Ladder Marker; **1:** Lysate; **2:** Sample after GF elution; **3:** Waste of salt elution; **4:** Waste of salt elution; **5:** Waste of water elution; **R**: RNA bands.

Another case indicating the high quality and high purity of GenScript's plasmid is shown below **(Figure 3)**. After first step of purification, the supercoiled plasmid content reaches already 83% and after step 2 which aims at removing the undesired plasmid forms, the supercoiled plasmid content reaches over 95%.



	Sample	Time	Area(%)
Step1	OC-Plasmid	20.774	8.02
	SC-Plasmid	21.723	83.17
	dimer-Plasmid	22.408	7.47

	Sample	Time	Area(%)
Step2	OC-Plasmid	20.800	1.62
	SC-Plasmid	21.790	94.07
	dimer-Plasmid	22.533	4.31

Figure 3 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.

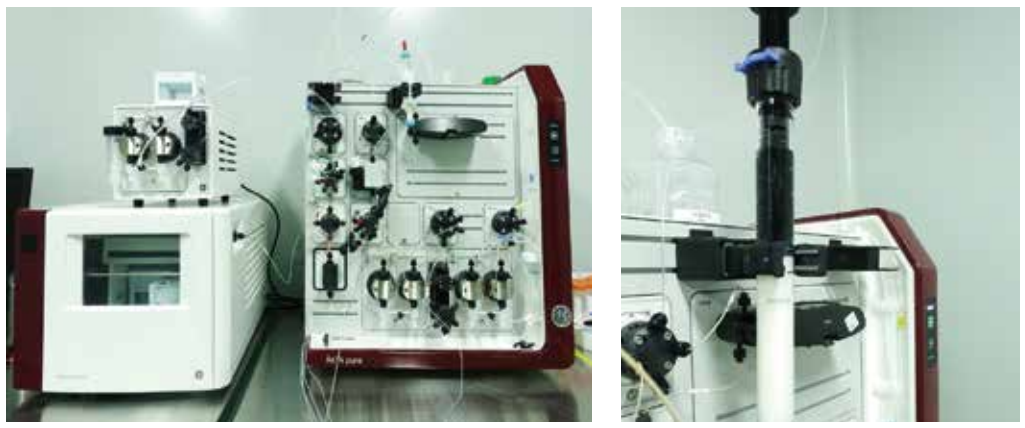


Figure 4 AKTA system for purification

References

Ich, Cber, Fda, FDA/CBER, Wang, Y., Bergelson, S., … Engelhardt, J. F. (2018). Chemistry, Manufacturing, and Control (CMC) Information for Human Gene Therapy Investigational New Drug Applications -Draft Guidance for Industry. *Journal of Clinical Investigation, 1994*(July), 10371–10376.

GenScript GCT Vector Platform

4 Core Services

- ✓ Plasmid Manufacturing
- ✓ Plasmid Process Development
- ✓ Virus Manufacturing
- ✓ Virus Process Development