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GenScript ProBio

VIRAL VECTOR

GENE AND CELL THERAPY

Lentivirus Process Development and Manufacturing

GenScript ProBio

GENE AND CELL THERAPY

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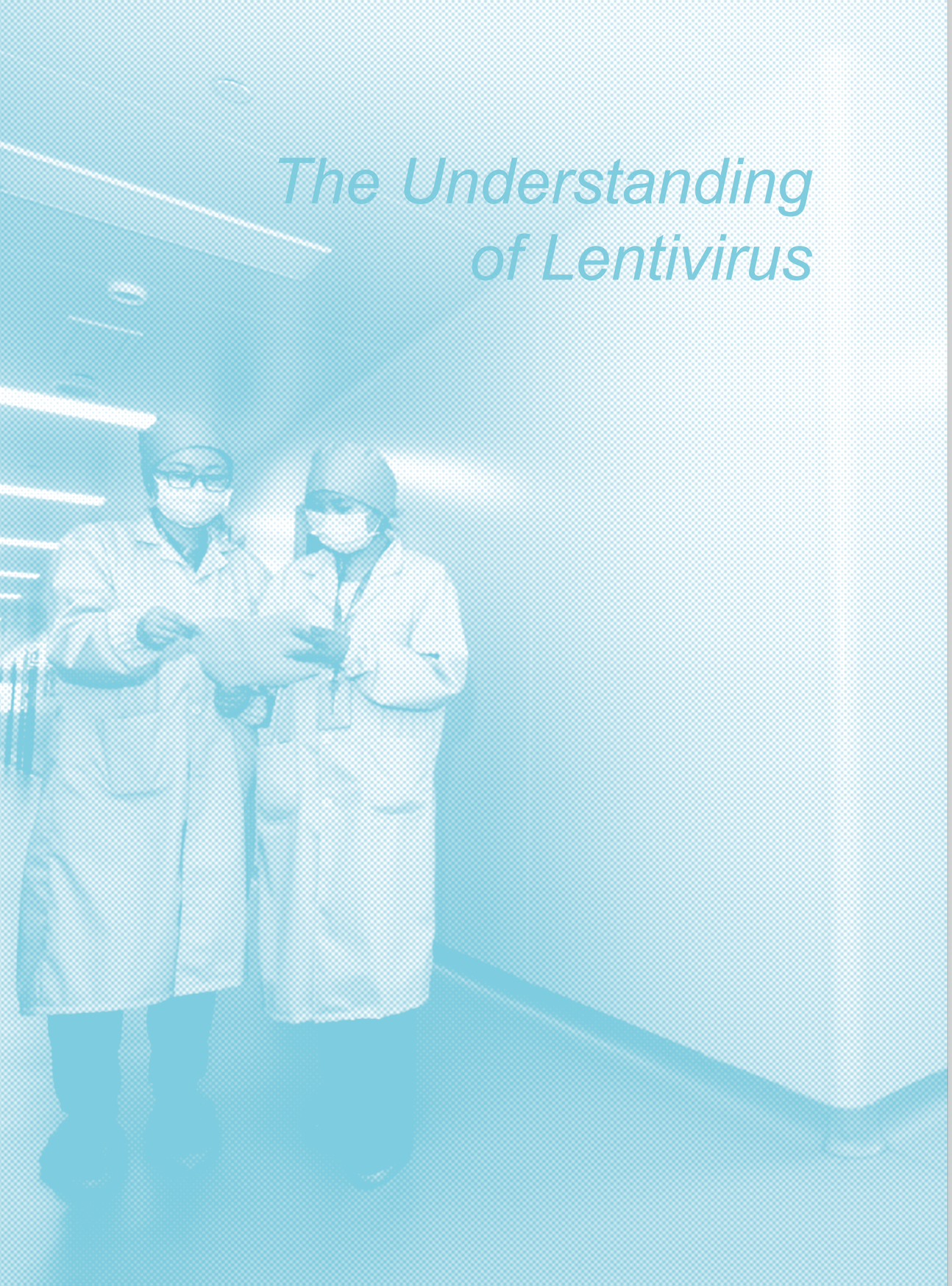
About GenScript ProBio

About GenScript ProBio

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.



The Understanding of Lentivirus

Introduction

Lentiviruses (LV) are RNA viruses that belong to the *Retroviridae* family. The best known lentivirus is the Human Immunodeficiency Virus (HIV), which causes AIDS. Lentiviruses can integrate a significant amount of viral cDNA into the DNA of the host cell and can efficiently infect non-dividing cells, so they are one of the most efficient methods of gene delivery (Cockrell, Adam S., 2007).

The lentivirus genome usually comprises three open reading frames (ORFs):

- **Group-specific antigen (gag):** Encodes structural proteins that form the viral capsid.
- **Polymerase (pol):** Encodes the reverse transcriptase, protease and integrase enzymes.
- **Envelope (env):** Encodes the viral envelope (surface and transmembrane glycoprotein) proteins.

Further, these viruses have additional genes, called accessory genes, which encode proteins that are responsible for various replicative functions.

Advantages and Limitations

In the past, many clinical trials based on the use of another kind of retroviral vectors, murine leukemia viruses (MLV), were successful. Although these vectors are still used, the use of LV vectors is generally preferred for the following reasons:

- LVs are able to transduce non-dividing cells because they can translocate across the nuclear membrane.
- Their integration patterns seem to be less risky: these vectors stably integrate the transgene into the target cell without transferring the sequences that encode for proteins that are derived from the packaging virus. This reduces the risk of setting off an adverse immune response inside the patient's body.
- Lentiviral vectors can be pseudotyped to broaden their tropism.
- They can be produced at high vector titer (Merten, Hebben, & Bovolenta, 2016).

There are also some limitations in the use of lentiviral vectors, as follows:

- Lentiviral vectors may induce oncogenesis through insertional mutagenesis.
- These vectors have the potential of generating replication competent lentivirus (RCL).

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LV Vector System (s)

The lentiviruses that have been developed as gene transfer vectors include mainly HIV-1 and HIV-2, but HIV-1 is the one that is very well-studied. Most of the others have not yet reached the clinical study stage.

Considering the safety issue of the pathogenicity of HIV-1 in humans, different generations of LV vector systems have been developed by modification of the helper plasmids and the existing genes.

Two-plasmid systems

In two-plasmid systems, all helper functions (gag-pol, rev, tat and VSV-g) are on one plasmid. Though such a production system is easier to produce and less expensive in its application and leads to higher vector titer than the three or four plasmid systems, the presence of all helper genes located on one plasmid might be a concern with respect to the formation of replication-competent lentivirus (RCLs).

Three-plasmid systems

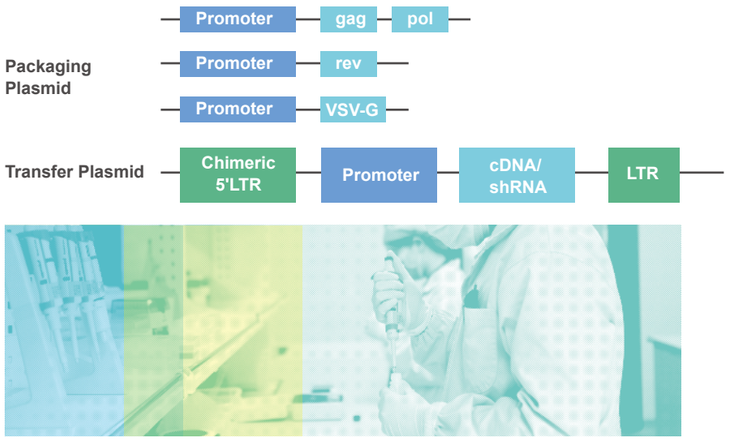
Two helper plasmids coding for the gag-pol and the env functions and one transfer plasmid comprise three-plasmid systems. All the accessory genes are removed to improve the safety of the virus.

Four-plasmid systems

The third generation of the LV vector system is widely used guided by safety considerations of the potential generating of RCLs.

All accessory genes of HIV-1 (vif, vpr, vpu, and nef) have also been removed because they are not necessary. The rev gene is placed on a helper plasmid independently to improve the safety of the system. The regulatory tat gene, present in the second-generation LV, has been eliminated because its transacting fuction is dispensable.

The whole system with three helper plasmids and one transfer plasmid reduces the possibility of formation of RCLs and improves safety.



Production of Lentivirus

Production of Lentivirus

Cell Lines

Due to the difficulties in establishing stable LV producer cell lines, transient transfection of HEK293 or HEK293-derived cells with vector and helper or packaging plasmids is the most widely used method to generate lentiviral vectors.

Compared with HEK293 cells, HEK293T cells may be preferred in producing LV because the presence of SV40 T-antigen in the producer cells can lead to more efficient production of vectors. Besides, HEK293T cells show increased cell growth and transfection efficiency in comparison to HEK293 cells (Merten et al., 2016). In spite of this, the parental HEK-293 cell line may present an advantage in terms of safety since SV40 large T antigen is an oncogene.

GCT Key Points in Selecting Cell Lines

- **Virus titer:** Select high-yield cell line; developing cell strains on your own will be a time-consuming and labor-intensive process;
- **Cell lines with clear source:** Traceability is a critical issue for regulatory compliance. Therefore, the cell line used should have a clear source.

GCT GenScript ProBio's Solutions

- Tested and compared the virus yield of several cell lines from different vendors and selected the one with optimal performance in adherent culture system.
- Obtained license from the cell line owner for CDMO services and solved the issue of cell line traceability.
- GenScript ProBio has established and certified cell banks (PCB, MCB, WCB) under GMP for adherent processes. Cell bank sterility inspection, mycoplasma inspection, in vitro adventitious viruses contamination inspection, and cell identification have been completed. Stability test of cell banks have been conducted in the meatime, and the cell banks have been fully characterized by a third party.

GCT GenScript ProBio's Case Studies

GenScript ProBio compared several cell lines' performance and chose one with high expression level. Below presents an example that compares the performance of two cell lines. GenScript ProBio chose cell line B due to its high titer in all projects.

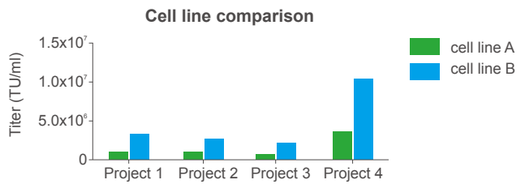


Figure 1 Cell line comparison through four batches of manufacturing.

Comparing the performance of cell line A and cell line B in 4 projects of manufacturing, cell line B always led to a much higher titer than cell line A.

Cell Culture Systems

Currently used cell culture systems are predominantly adherent or suspension-cell-based systems. The key difference between these two kinds of cell culture systems are the way of passaging the cells. In case of suspension system, the cells can be directly diluted into the fresh medium, so that to easily scale-up the process. It can also be as complicated as detaching the adherent cells from a surface and plating them onto a new surface in a fresh medium. And in this system, the scale-up is limited due to the surface available for the cell growth.

Vector production using adherent cultures

Adherent cells are usually cultured in multilayer tissue culture flasks such as CFs or CellSTACKS for research or development purposes, because they are easy to manipulate and cost-effective.

With respect to larger-scale LV production in clinical trials, the production process need to be scaled up. With the use of adherent cells in CF systems, a scale-out approach should be performed by adding supplementary production units. Generally the 10-stack CF and the largest 40-stack CF or 36 HYPERStack are the most widely used. The 40-stack device is a semi-closed system, which provides improved safety for the operator, the environment as well as the final product. However, it also requires a specific handling system due to its semi-closed architecture and the elevated weight of the CF-40 (Merten et al., 2016). Another method for larger-scale LV production that is easier for scale up is the use of suspension cultures in large bioreactors.

GCT Key Points in Adherent Systems

- Difficulties in large-scale manufacturing.
- Stable and well-established manufacturing process.

GCT GenScript ProBio's Solutions

- Staff training: All the staff members are well trained with OJT, and follow operation rules in a stringent manner.
- Control of materials: Establish risk-based material management system, and control the materials according to risk assessment and classification.
- GMP grade reagents are used for key reagents such as transfection reagents. GenScript ProBio can provide PEI residual risk assessment reports, and a third-party can provide residual PEI assays and reports.
- Instrument: Use instruments from well-known brands, and performs 3Qs for qualification. All qualification documents are available.
- Well-established process for large-scale cell expansion and transfection, satisfies up to 50L vector manufacturing, which meets current requirements in LV manufacturing.
- Essential tests of adventitious factors and RCL for lentivirus supernatant and EOPC (end of production cell).

GCT GenScript ProBio's Case Studies

During adherent culture process development, different parameters are assessed, in order to contribute to an optimal manufacturing process which can lead to higher yield and virus titer and is more stable compared to platform process. Below are two cases from GenScript ProBio's platform. The left one is to find the optimal cell density, with other parameters defined, and the right one is to find the optimal total DNA.

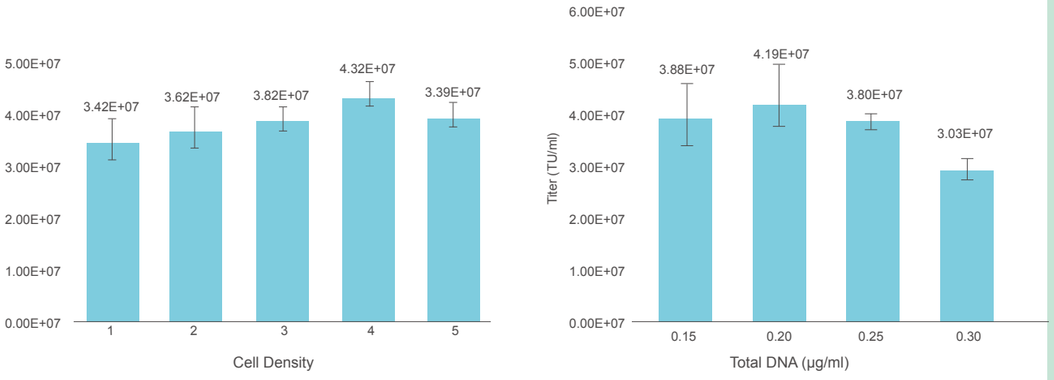


Figure 2 Assessment of cell density and total DNA to find the optimal value. Left: 5 cell densities were tested, and after comparison, cell density 4 led to a higher titer; **Right:** 4 levels of total DNA were investigated and option 2 is the optimal value.

Suspension cell culture system

Expansion of suspension cell in large-scale bioreactors is the first step in the suspension culture system. Adaptation procedures will be needed for choosing cell lines. Several cell lines used for lentivirus production (293T, 293FT, and 293SF-3F6) have been described to grow readily in suspension with no need for microcarriers (Merten et al., 2016), which means expansion of suspension cells is easier than that of adherent cells. In addition, unlike in the/an adherent culture system, the suspension cell culture system usually uses serum-free media. The absence of bovine serum and animal origin components in the culture media is the most suitable situation for clinical manufacturing as this decreases the risk of contamination by adventitious agents.

However, choosing a transfection reagent for a/the suspension cell culture system is quite complex. Due to continuous culture stirring, DNA precipitation using calcium phosphate is expected to be less efficient. Thus, other transfection agents like cationic polymers (eg. PEI) are used mostly. But the drawback of PEI is also obvious. To transfect suspension cells, enormous amounts of plasmids are needed at large scale; this leads to a high cost of raw materials and residual elimination in downstream processing. Another problem associated with PEI is the absence of an analytical method to detect and quantify the molecule. It is therefore unable to evaluate the risk that residual PEI may carry.

Finally, the harvesting cell in suspension is another issue compared to adherent cells. The LV culture supernatant can be harvested at least twice a day, which is efficient in reducing costs, while in suspension, this approach is hard to implement because the culture medium cannot be collected independently from the cells. A perfusion system has been introduced and has suggested the feasibility of multiple harvest in suspension culture, though the system was also proved to be complicated and costly in large scale (Ansorge et al., 2009).

Downstream Process (DSP)

Downstream process aims to remove impurities from the product to ensure its safety, purity, identity and quality. And purification methods are required to remove any contaminants of potential adverse effect when vector preparations are used in vivo. In addition, when used for clinical or industrial applications, the purification methods involved are supposed to be scalable in large-scale processes (Merten & Chahal, 2014). In small-scale purification, centrifugation methods are usually applied. But these methods lack scalability and may lead to partial vector inactivation due to long process time. The purity of the final products are insufficient for an in vivo application. Therefore, other methods have been developed in large-scale purification.

Generally, four phases can be distinguished in large-scale purification. Firstly, a capture step is the initial purification process for eliminating major contaminants. In this step, membrane filtration is usually applied for clarification purpose. A concentration step based either on TFF/ultracentrifugation or ion-exchange chromatography follows.

To eliminate residual cellular contamination and in particular, plasmid-derived DNA contaminants, a benzonase step is often applied, either in the capture step or afterwards. In different protocols, there are different considerations in the benzonase step. When it takes place in the capture step, large DNA pieces are easily reduced in their size and following purification steps may elminate residual benzonases; however, the disadvantage is that large quantities of benzonases are used. On the other hand, the late use of the benzonase step can reduce the amount of benzonases that have to be applied, but residual purification steps must ensure that residual benzonases are reduced to acceptable levels. What's more, if the benzonase step is not applied in the early capture step, the large size DNA pieces might lead to the formation of aggregates which may capture vector particles and therefore result in vector loss (Merten et al., 2016).

In the next phase of the downstream process, an intermediate purification step may be taken to remove specific impurities. In most cases, diafiltration/concentration is applied, which is followed by diafiltration or size exclusive chromatography steps as polishing steps, to remove trace contaminants and impurities.

Finally, the vector is sterile filtered with 0.2 μm membranes. This is a standard regulatory requirement to mitigate the risk of microbial contamination of the final product in GMP conditions. Although sterile filtration is strongly recommended, it is possible to skip it provided that the process can be certified as being fully aseptic.The table below summarizes several large-scale downstream process protocols from different companies and institutes, which also offer some examples of downstream process design.

Table 1 Principle process steps of large-scale downstream processing protocols for the purification of VSV-g (glycoprotein of the vesicular stomatitis virus) pseudotyped lentiviral vectors (for clinical purposes) (Merten & Chahal, 2014).

Company/Institute	Process steps in chronological order					
Beckman Research Institute, City of Hope	Clarification (0.45μm)	Benzonase	Ultrafiltration (500 kDa)	High-speed centrifugation	Resuspension and removal of particulate material	No sterile filtration Sterile filtration
Virxsys	Clarification (?)	Q IEX chromatography (Capsule)	Concentration /diafiltration	Benzonase	Diafiltration	Sterile filtration
Genethon/MolMed	Clarification (0.45μm)	Benzonase	DEAE-IEX chromatography	Concentration/ diafiltration	Formulation (SEC)	Aseptic hollow-fiber ultrafiltration/ concentration
Oxford BioMedica/ Henogen	Clarification (?)	Benzonase	Anion EX chromatography	Concentration/ diafiltration	Sterile filtration	
BlueBird Bio	Clarification	IEC chromatography	Concentration /diafiltration	Sterile filtration		

DEAE: Diethylaminoethanol; EX: Exchange; IEC: Ion-exchange chromatography; SEC: Size-exclusion chromatography.

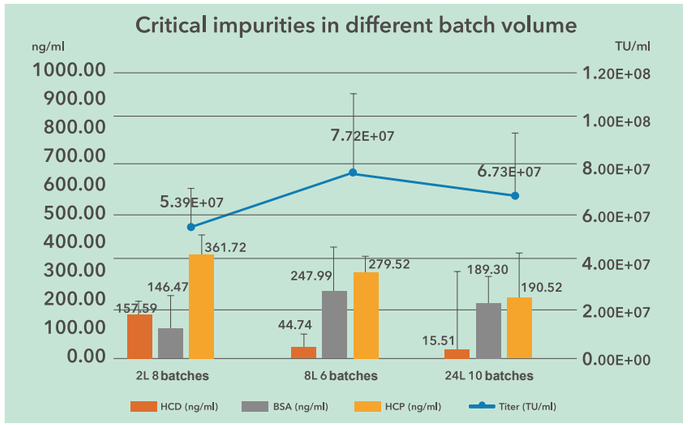
GCT Key Points in Large-scale Purification

- Scalability of the purification methods.
- The effect of sterilization on the yield is large, and the entire downstream purification process should have a high efficiency of removing impurities to improve the safety of the final product.

GCT GenScript ProBio's Solution in DSP of Adherent System

- Reagents in purification process: All reagents applied in the downstream process are pharmaceutical grade and compliant with regulatory requirements.
- Key consumables: Chromatography resin and hollow fiber applied for each single project are disposable, eliminating the risk of cross contamination.
- Fill/finish: After aseptic filtration, all processes are performed in a sterile isolator. Closed semi-automatic dispensing equipment is used to ensure the aseptic filling process and aseptic filling verification.

GCT GenScript ProBio's Case Studies



HIGH PURITY

- BSA remain between 150~300 ng/ml
- Endotoxin <10 EU/ml
- HCP between 200~400 ng/ml
- HCD <30 ng/ml in larger scale (24L)

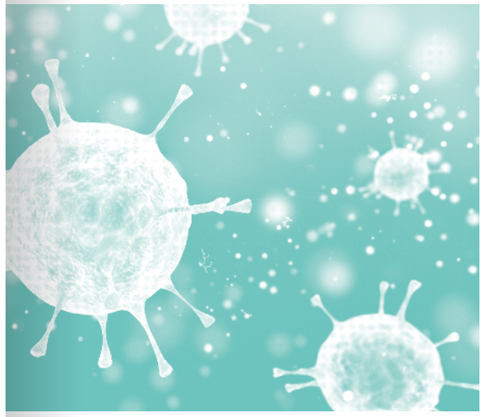
HIGH TITER

- Titer >5x10^7 TU/ml

HIGH STABILITY: STABLE FROM BATCH TO BATCH SCALABILITY

- Performance remains stable during scaling up

Figure 3 Critical impurities and virus titer in different batch volume



Product Quality Control

In the process of manufacturing LV, contaminants from the production medium, producer cells or the process may constitute a risk when using the product in patients. Therefore, the downstream process is extremely important to improving product safety. Characterization of the specific vector contaminants and product identification also guarantee the quality of the final product.

The LV products may be characterized in terms of identity, potency, purity and safety. Identity assays are applied to identify the product and distinguish it from other products. Purity assays may include a variety of characterizations of impurities, including proteins, DNA, cell debris, etc. One of the process by-products is Benzonase, which is supposed to be eliminated in the late steps of purification and should also be controlled at product release. Another constituent, bovine serum albumin, which usually exists in viral production medium, should be controlled in the final product to keep it at a low value. The SV40 large T antigen, which is a specific product of the producer cells, is oncogenic for many cell types. And in the product that is to be applied in patients, such kind of impurities must be controlled in the final product as well. In addition to all of these safety measures, the absence of RCL must be demonstrated under permissive conditions using a sensitive assay.

GCT Key Points in Quality Control

- Difficulties in development and qualification of analytical methods.

GCT GenScript ProBio's Solution

- 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, functional titer testing, 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 for lentivirus.
- 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.

Production of Lentivirus

GenScript ProBio Lentivirus Services

GenScript ProBio is dedicated to providing the best solutions for overcoming industrial bottlenecks in lentivirus manufacturing. By bringing together state-of-the-art manufacturing facilities, advanced equipment and technological innovations, as well as experienced team members and well-established quality systems, GenScript ProBio is providing one-stop lentivirus services that help clients advance gene and cell therapy products smoothly from research to commercialization.

State-of-the-art Facility

GMP Virus Manufacturing Center

GenScript ProBio currently operates a 1200 m^2 GMP facility for virus manufacturing. The facility is equipped with 4 segregated manufacturing suites, and a parallel production line in a clean room with a Grade A in C environment, avoiding the possibility of cross contamination from batch to batch.

GenScript ProBio is expanding capacity with the construction of a Commercial Manufacturing Center that will add an aseptic fill/finish capability and commercial capabilities. The 30,000 m^2 new center is expected to be in full operation from 2022.

Dedicated Process Development and Analytical Laboratories

A dedicated process development (PD) and analytical laboratory is in use, separate from the GMP manufacturing center. The laboratory is equipped with advanced analytical instruments and an independent physical and chemical lab, microbial lab, and FACS test room, etc., ensuring all the analytical development and testing is well controlled.

Lentivirus One-stop Solution

Process Development

GenScrip ProBio has developed an adherent production system in a dedicated PD lab to satisfy most clients' lentivirus process development needs. Successful deliveries over the past year have enabled us to reduce development cycle and streamline processes.

Lentivirus process development services include:

- Upstream process development
- Downstream process development
- Lab-scale lentivirus manufacturing for process validation
- Process scale-up

Cell Banking

GenScript ProBio has screened cell lines and selected a clone with a high expression level. With the selected cell line, GenScript ProBio has established a cell bank that is adapted to adherent cultures. Ready for regulatory submissions, the cell bank has a clear source and is free of IP issues.

Working within GMP regulations, GenScript ProBio is also available to expedite cell banking for clients, ensuring manufacturing that is fully compliant with GMP and with full characterization.

- Ready to establish cell banks under GMP
- Established cell banks with full characterization
- Appropriate for regulatory submissions with clear source and free of IP issues

Manufacturing Capabilities

The lentivirus is manufactured in a GMP facility under comprehensive quality oversight. Each batch of production is conducted in independent suites, removing the risk of cross contamination. The entire process, manufacturing environment and documents are well controlled to meet regulatory requirements.

GenScript ProBio is experienced in manufacturing of lentiviral vectors, adopting an adherent culture system, and providing customers with quality products for preclinical and clinical supply.

- GMP facility with comprehensive quality assurance process
- Well-established adherent culture system, ensuring high titer and high quality
- In-process control for whole monitoring

Analytics

Analytical development, qualification and validation services, and stability test are available to serve clients' varied requirements, including preparing for regulatory filings. Various testing capabilities applying highly sensitive methods are accessible for characterization and quality controls of lentiviral products, including:

- Benzonase
- SV40
- Host cell protein
- Residual plasmid
- Sterility
- Bioburden
- Endotoxin
- FACS
- P24 ELISA
- ……

Value-added Offerings

GenScript ProBio is always prepared to provide clients regulatory support. Our highly experienced teams in process, quality and regulatory can help clients with technical transfer, document filing, regulatory consulting, etc. to help their projects go smoothly from early stage development through clinical trials to commercialization.

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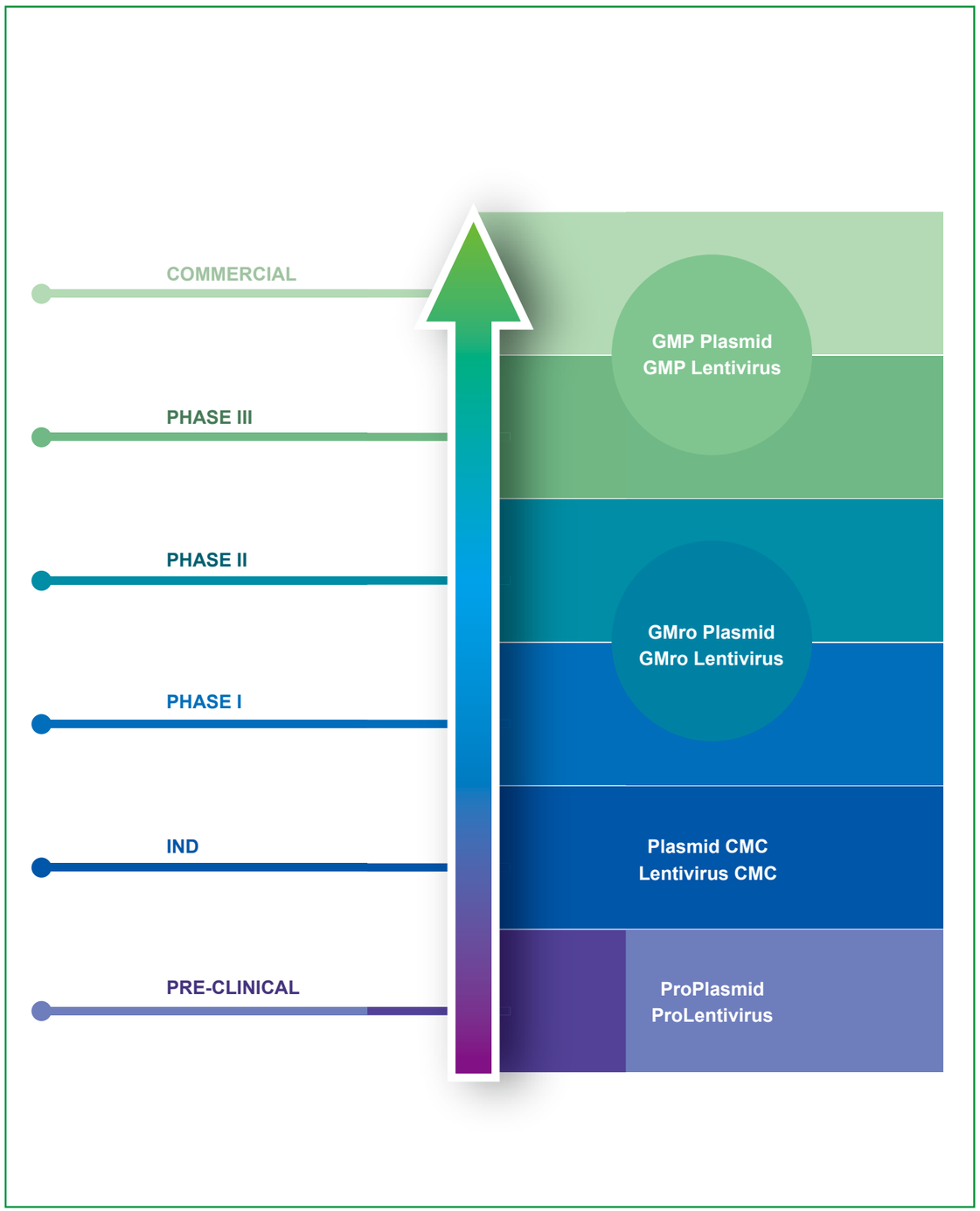

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