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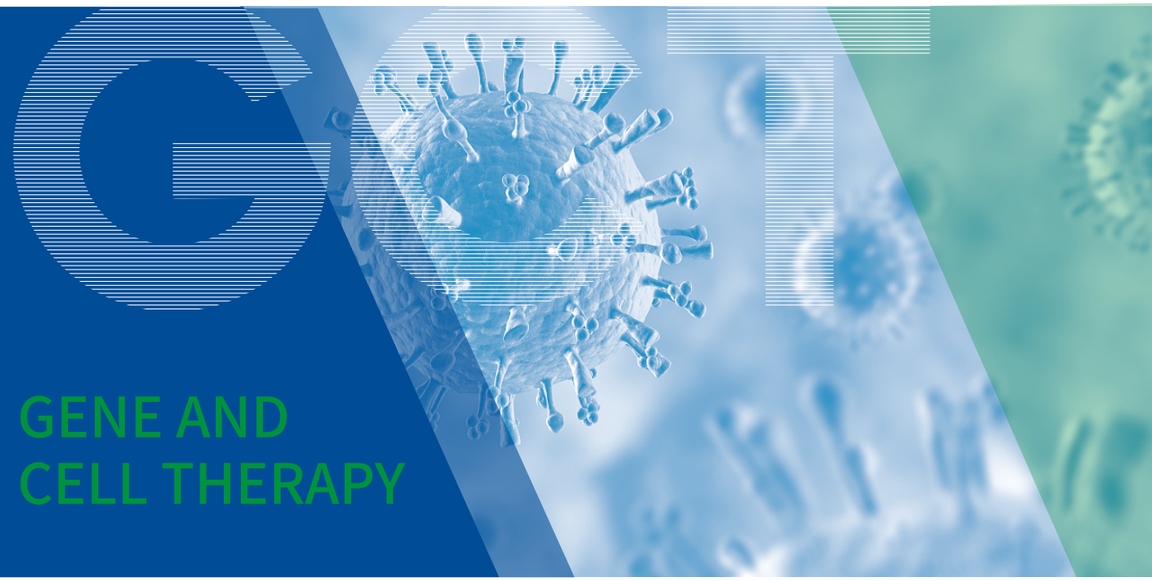

GENE AND
CELL THERAPY

Lentiviral Vector Process Development and Manufacturing

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Gene and
Cell Therapy

Content

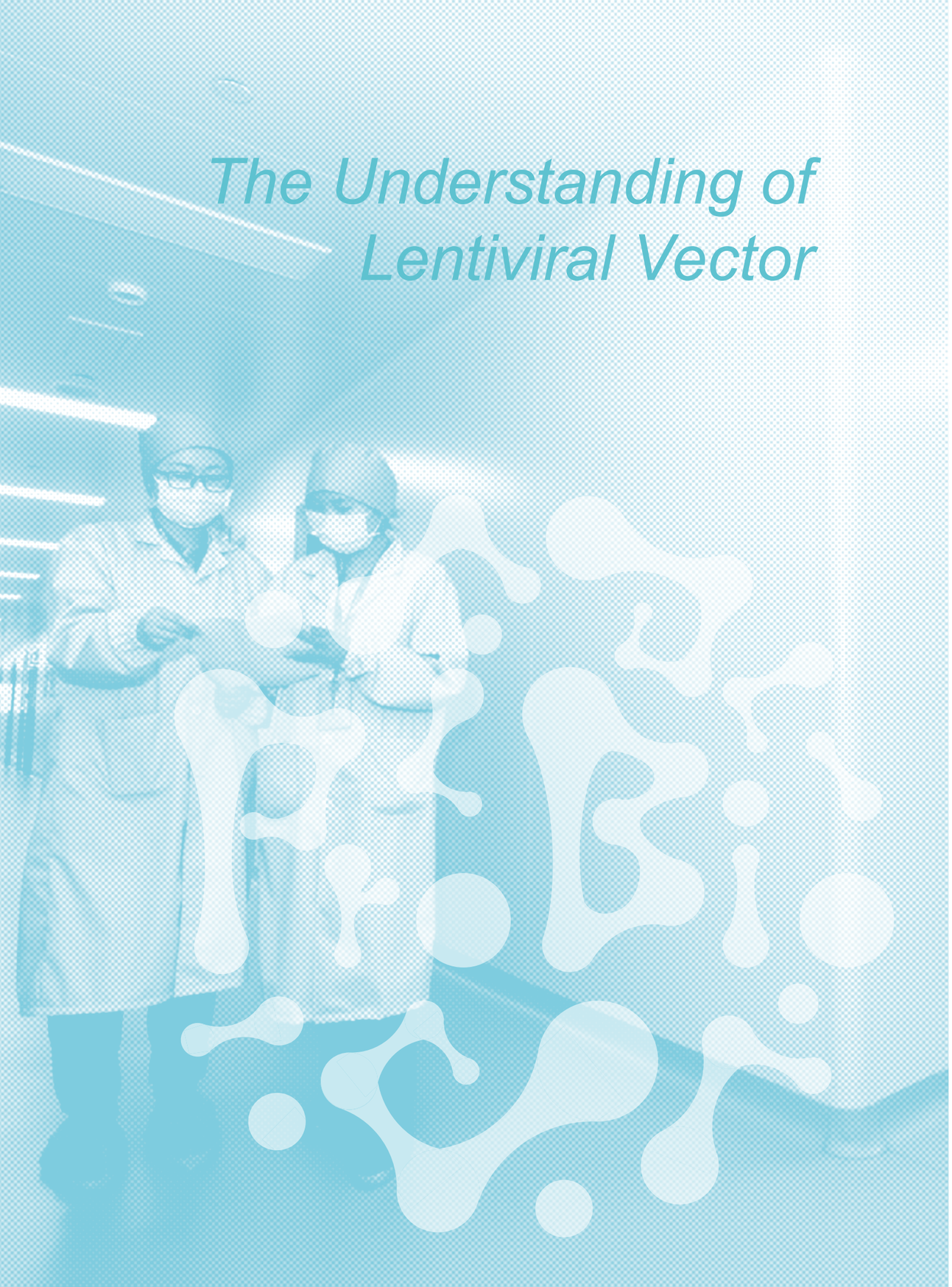
About GenScript ProBio

About GenScript ProBio

GenScript ProBio is the bio-pharmaceutical CDMO segment of world's leading biotechnology company, GenScript Biotech Corporation. GenScript ProBio's one-stop antibody drug development solutions include antibody drug discovery, antibody engineering and other development services. GenScript ProBio's total gene and cell therapy (GCT) solution covers process development for IND application, plasmid DNA production as well as viral vectors production for clinical or commercial supply.

The platform has over 10 years of experience in plasmid DNA manufacturing, and has successfully delivered products for global clients in pre-clinical studies, IND filing and clinical trials. For the production of lentiviral vector (LVV), GenScript ProBio is able to apply either adherent or suspension culture, with a manufacturing scale up to 200L. Furthermore, GenScript ProBio is capable of producing AAV vector for gene therapy products as well.

Currently, 1,200 m^2 plasmid process development facility and 2,500 m^2 GMP viral vector manufacturing facility is in full operation. Both facilities can cover the needs for preclinical, IND and early phase clinical applications. By September 2021, a new plasmid GMP facility will be in full operation to enhance cGMP plasmid production for late phase clinical trials and commercialization. And in 2022, a Commercial Manufacturing Center will be in operation to complement commercialization capability. By then, GenScript ProBio will cover all the needs from pre-clinical studies through clinical trials to commercialization.



The Understanding of Lentiviral Vector

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:

- LVVs 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.
- LVVs can be pseudotyped to broaden their tropism.
- They can be produced at high vector titer (Merten, Hebben, & Bovolenta, 2016).

But there are also limitations, for example:

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

GenScript PROBIO GENE AND CELL THERAPY

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 LVV systems are developed by modification of helper plasmids and 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

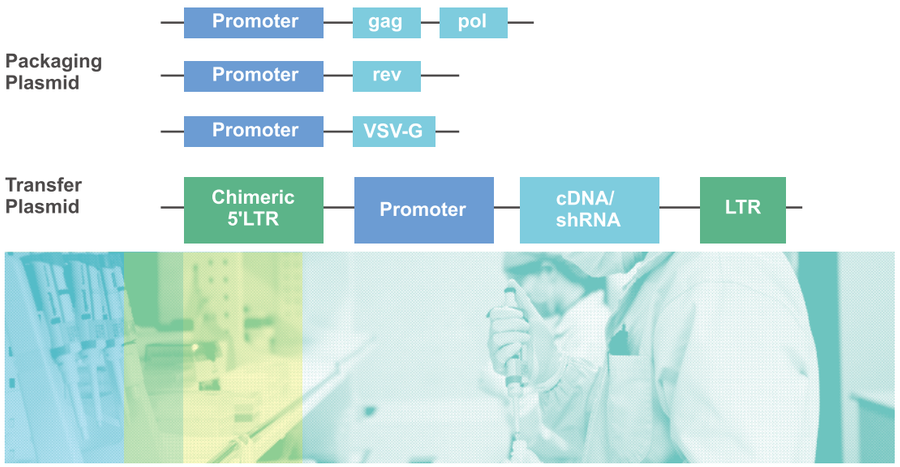
Two helper plasmids coding for the gag-pol and the env functions and one transfer plasmid comprise three-plasmid systems. All the accessory genes are removed to improve virus safety.

Four-plasmid systems

The third generation of the LVV 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 LVV, has been eliminated due to its dispensable transacting function.

The whole system with 3 helper plasmids and 1 transfer plasmid reduces the possibility of formation of RCLs and improves safety.



Production of Lentiviral Vector

Production of Lentiviral Vector

Cell Lines

Due to the difficulties in establishing stable LVV 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 vector.

Compared with HEK293 cells, HEK293T cells may be preferred in producing LVV 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

- **Viral vector titer:** Develop and select a 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

Adherent cell line

- Selected the cell line with optimal performance among various cell lines in adherent culture system.
- Obtained license from the cell line owner for CDMO services and solved cell line traceability issue.
- Established and certified cell banks (PCB, MCB, WCB) under GMP for adherent processes.

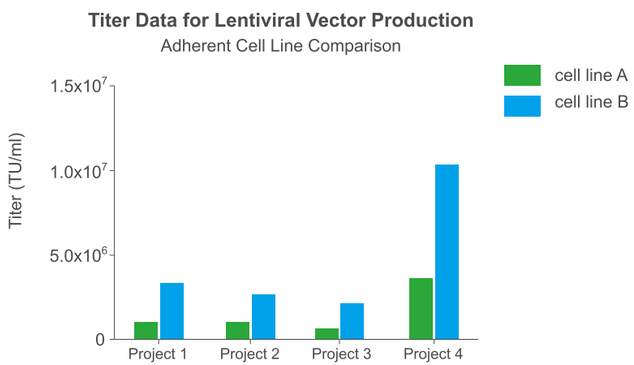


Figure 1 Adherent cell line comparison through four batches of manufacturing.
Comparing the performance of cell line A and cell line B in 4 projects, cell line B always led to a much higher titer than cell line A.

Suspension cell line – Proprietary PowerS™-293T

- In-house developed proprietary suspension cell line; adapted from HEK 293T cell line
- Cost-effective license for IND, clinical and commercial
- Royalty free, maintenance free
- Similar performance with top vendor's commercial cell line

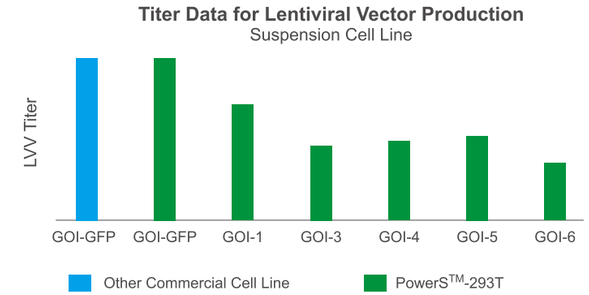


Figure 2 Lentiviral vector titer comparison with different GOI in PowerS™-293T relative to commercially available suspension cell line.

Cell Culture Systems

Currently used cell culture systems are predominantly adherent or suspension-cell-based systems. The key difference between these 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. In such system, the scale-up is limited due to the surface available for 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 LVV 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 LVV production that is easier for scale up is the use of suspension cultures in large bioreactors.

GCT Key Points in Adherent Systems

- Feasibility of large-scale manufacturing and manipulation.
- Stable and well-established manufacturing process.

GCT GenScript ProBio's Solutions

Closed adherent culture system – mitigate risk & improve stability

- Avoid contamination which may result from open operating
- Full-automatic operation in sampling & harvesting; reproducibility for stable process improved
- Easy to manipulate
- Feasible to scale up

Before cell seeding Transfection & packaging

Well-developed adherent manufacturing process

Different parameters are assessed during upstream process, in order to improve the stability and applicability of the process. Below are two case studies in developing manufacturing process.

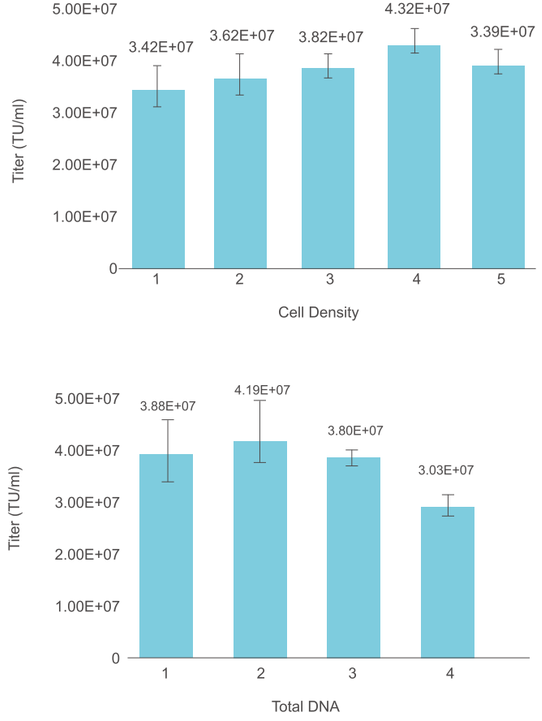


Figure 3 Assessment of cell density and total DNA for the optimal range.

Suspension cell culture system

Although transfection of adherent cells is the gold standard methodology for producing LVV, this system is rather limited in scalability. For large-scale manufacturing, cell culture in bioreactors is usually the most convenient approach.

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 lentiviral vector 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.

In conclusion, large-scale production of LVV using transient transfection of suspension cells is feasible and shows promising productivity. **And as the market demand on lentiviral vector is increasing, the suspension process is an inevitable tendency in the near future.**

GCT Key Points in Suspension Systems

- High performance cell line with clear history
- Robust manufacturing process applicable for large-scale production
- Cost-effectiveness

GCT GenScript ProBio's Solutions

- **Proprietary suspension cell line PowerS™-293T**
- Robust and scalable suspension process verified by different projects
- Superior yield improvement
- **50%** unit cost reduction

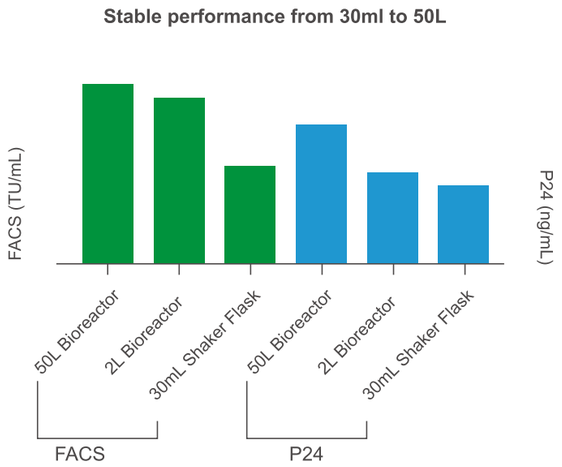


Figure 4 Titer data of lentiviral vector in 30ml shaker flask, 2L bioreactor and 50L bioreactor.

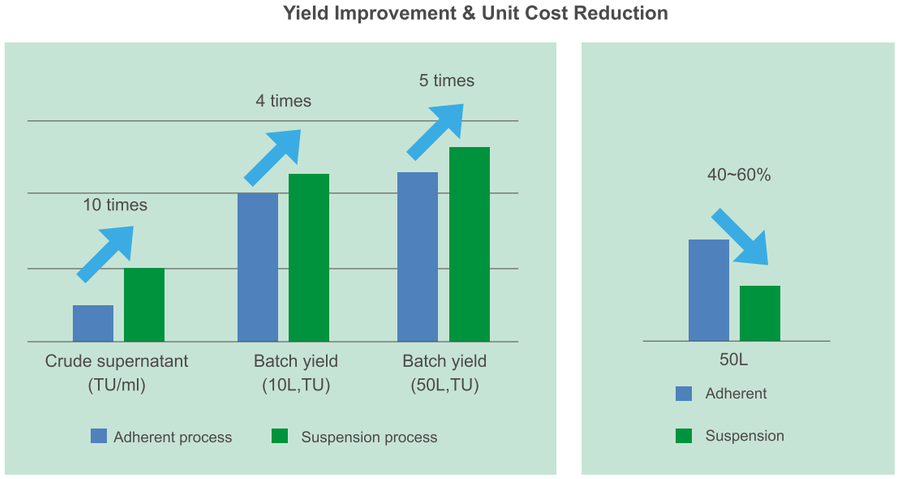
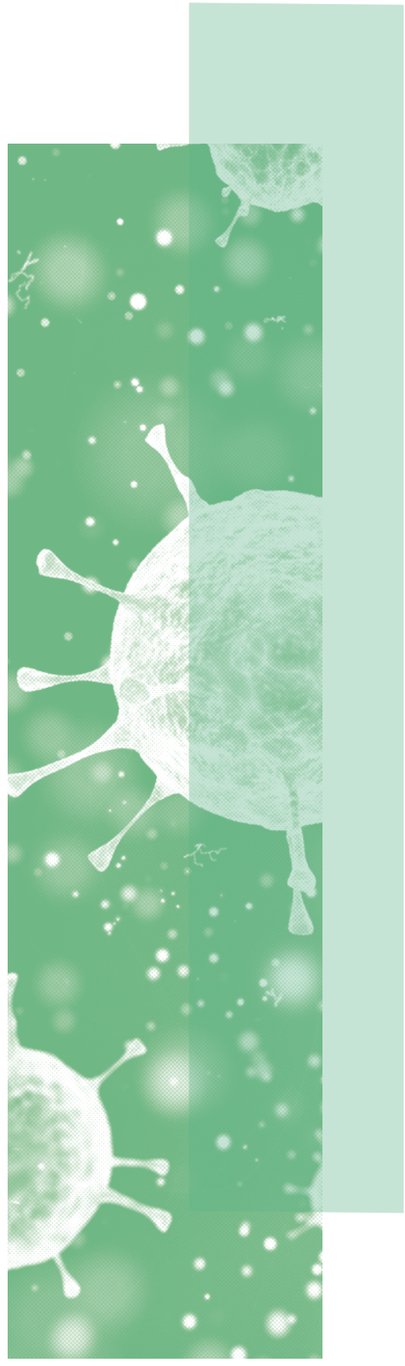


Figure 5 Comparison of titer, yield and cost of adherent and suspension processes. Left: crude titer improved 10 times from adherent to suspension process, and the batch yield of 10L and 50L improved 4~5 times from adherent to suspension process. **Right:** comparing the total yield and total cost of adherent and suspension process in 50L, the unit cost of suspension process reduced 40%~60% from adherent process.



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

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

- 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.

LVV Product Quality Control

In the process of manufacturing LVV, 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 LVV 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 Lentiviral Vector.
- 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.

GenScript ProBio Lentiviral Vector Services

GenScript ProBio Lentiviral Vector Services

GenScript ProBio is dedicated to providing the best solutions for overcoming industrial bottlenecks in lentiviral vector 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 lentiviral vector services that help clients advance gene and cell therapy products smoothly from research to commercialization.

State-of-the-art Facility

GMP Viral Vector Manufacturing Center

GenScript ProBio currently operates a 1200 m^2 GMP facility for viral vector 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 in 2022.

Dedicated Process Development and Analytical Laboratories

A dedicated process development 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.

Well-established Quality Management Systems

GenScript ProBio has employed Phase Appropriate Compliance method by referring to PDA TR NO.56 (Parenteral Drug Association Technical Report) Application of Phase-Appropriate Quality System and cGMP to Development of Therapeutic Protein Drug Substance (API or Biological Active Substance). An integral Quality Management System is established by adopting international guidelines such as FDA, PDA, ICH, EMA, WHO, etc.
General Procedures include Quality manual, Site Master File, Deviation Management, Change Control, Out of Specification, Training, Supplier Management, Documentation Management, Complaint, Risk Assessment Management, Validation, and etc.

- 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.
- Document control: All the documents are managed based on classifications, unique serial No. and specific written procedures.
- Validation management: Validation is performed for equipment, instrument, facility and auxiliary system, computerized system, analytical methods, manufacturing processes and cleaning processes.

Lentiviral Vector One-stop Solution

Cell Banking

Ready-to-use Cell Banks

For adherent culture, GenScript ProBio has screened several 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.
For suspension culture process, GenScript ProBio in-house developed the **proprietary suspension cell line, PowerS™-293T**, which is adapted from HEK 293T. PowerS™-293T is proved to produce lentiviral vector that is comparable with other commercially available cell lines. Master Cell Bank (MCB) and Working Cell Banks (WCB) are available to start GMP manufacturing of lentiviral vector.

Cell Banking Services

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
- Full characterization delivering with cell banks
- Appropriate for regulatory submissions

Process Development

GenScript ProBio has developed **adherent culture system,** as well as **suspension culture system** in a dedicated PD lab, and is experienced in developing and optimizing process of customer's projects. Successful deliveries over the past year have enabled us to reduce development cycle and streamline processes.

Lentiviral vector process development services include:

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

GenScript PROBIO GENE AND CELL THERAPY

GMP Lentiviral Vector Manufacturing

The lentiviral vector 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 lentiviral vectors adopting both adherent production system and suspension production 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
- Robust **suspension culture system,** over 50% cost reduction
- In-process control for whole monitoring

Analytics

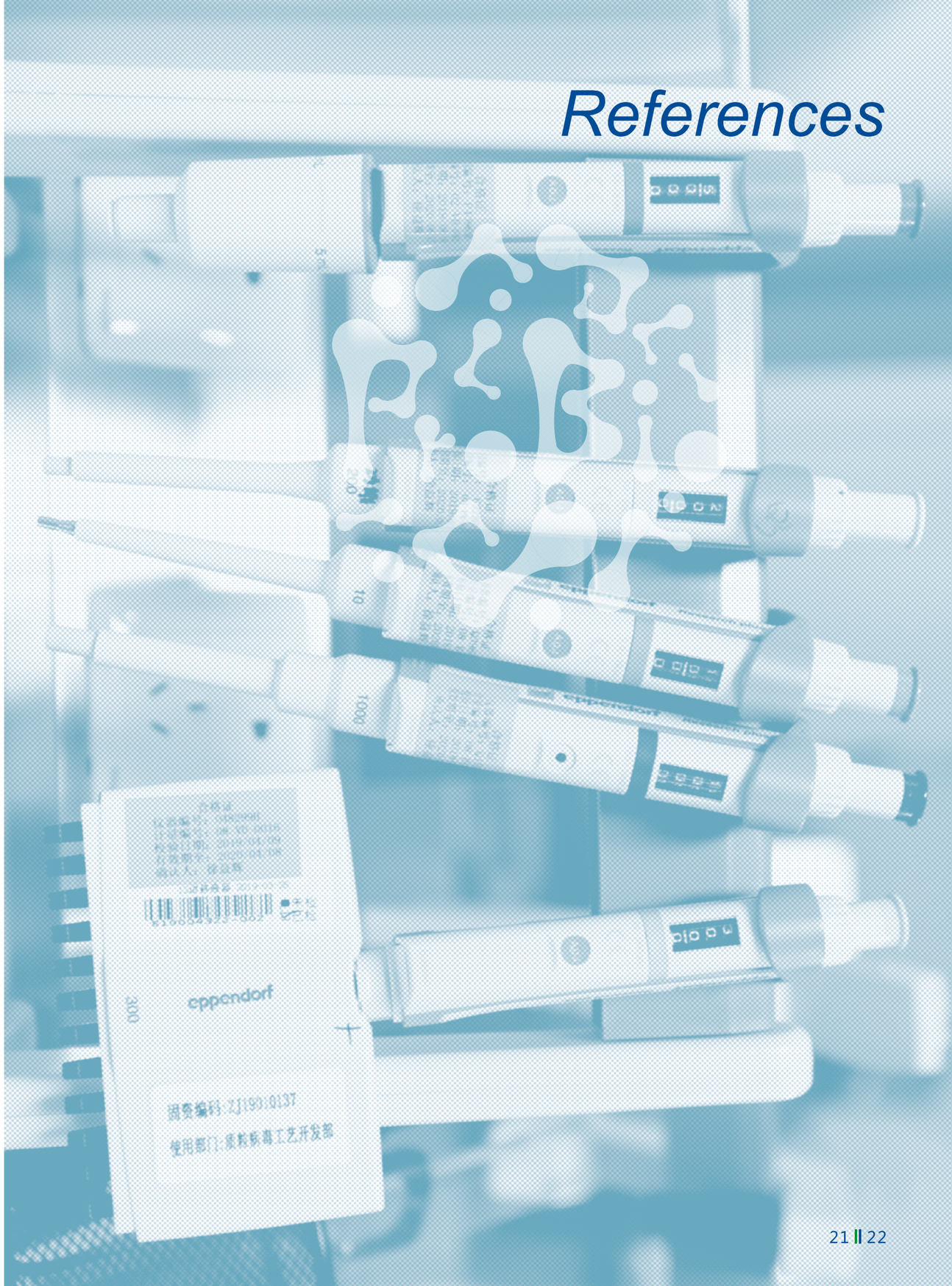
Analytical development, qualification and validation services, and stability test are available to serve clients' varied requirements, including preparing for regulatory filings.
Various testing capabilities applying highly sensitive methods are accessible for characterization and quality controls of 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 with regulatory support. Our highly experienced teams in process, quality and regulatory can help with technical transfer, document filing, regulatory consulting and etc. to guarantee clients' projects go smoothly from early stage development to clinical trials and commercialization.

References



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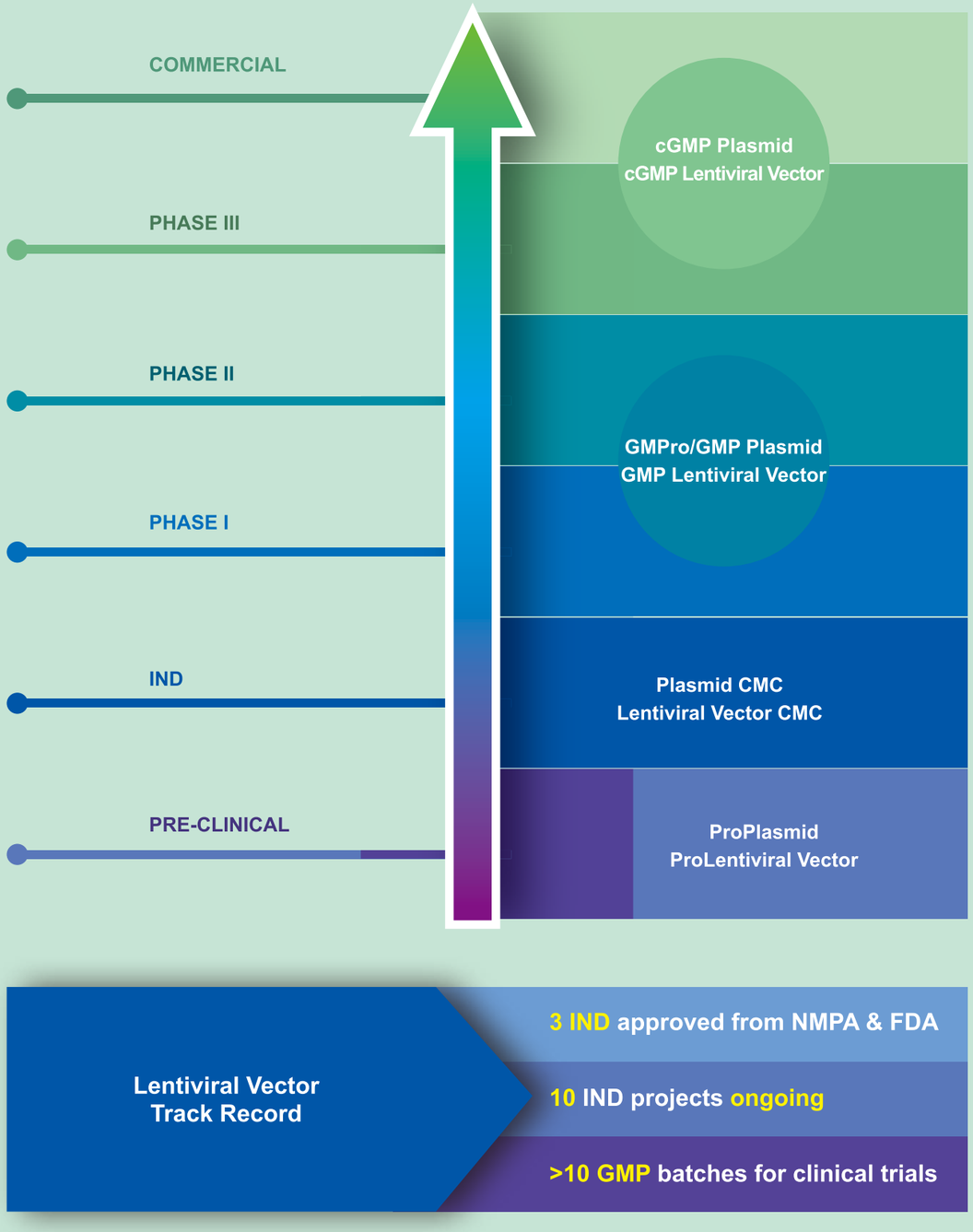

Lentiviral Vector Track Record

- **3 IND** approved from NMPA & FDA
- **10** IND projects **ongoing**
- **>10 GMP** batches for clinical trials

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ENGINEERED PROBIOTICS AND THEIR THERAPEUTIC APPLICATIONS