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Lentivirus Packaging Handbook

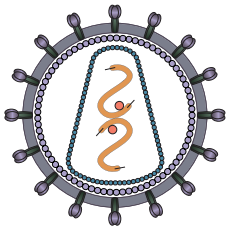
One Stop Service from Gene Synthesis to Lentivirus Packaging

GenScript
Make Research Easy

Content

Introduction of the lentivirus

Lentiviruses have been widely used in gene and cell research, becoming a powerful tool for gene function research. In addition, lentivirus packaging can also play an important role in gene and cell therapy development.



Lentivirus

01 It can infect difficult-to-transfect cells, such as primary cells, stem cells, nerve cells, etc., greatly improving the transduction efficiency of target gene

02 It can infect dividing and non-dividing cells, and introduce larger gene fragments

03 Lentiviruses can efficiently integrate exogenous gene into cellular genome to achieve stable expression

GenScript is using 3rd generation lentivirus packaging system and combining with suspension culture process to achieve higher titer and flexible scalability.Besides, our lentivirus is subjected to multiple centrifugation steps for *in vitro* and *in vivo* propose experiments. Most importantly, GenScript provides one-stop service from gene synthesis to lentivirus packaging as fast as 4 weeks.

Superior Transduction

One Stop Shop

High Titer

Comparison of different virus systems

Compared with adenovirus, adeno-associated virus and retrovirus, lentivirus has the following characteristics:

Table 1. Comparison of characteristics of different virus systems

Characteristics	Lentivirus	Adenovirus	AAV	Retrovirus
Viral genome	ssRNA	dsDNA	ssDNA	ssRNA
Packaging Capacity	9 kb	5.5 kb	4.7 kb	8 kb
Infection	Most dividing and non-dividing cells	Most dividing and non-dividing cells	Most dividing and non-dividing cells	Only infect mitotically active cells
Titer	Up to 10^9 TU/mL	Up to 10^{12} vg/mL	Up to 10^{13} vg/mL	Up to 10^9 TU/mL
Integration	Integrating	Non-integrating	Non-integrating	Integrating
Immunogenicity	Low	High	Very Low	High
Transduction Efficiency	Moderate	Moderate	Moderate	High

Features and advantages of GenScript lentiviral system

Wide range of transfection: Lentivirus has the ability to infect both dividing and non-dividing cells. For some difficult-to-transfect cells, such as primary cells, stem cells, undifferentiated cells, etc., the transduction efficiency and integration probability of the target gene can be significantly improved.

Stable expression: Lentivirus integrates exogenous genes into the genome of host cells, and can express the target gene stably for a long time without losing it with cell division and passage. It is the first choice for functional and assay cell line construction.

Large capacity: Lentiviral vector can accommodate exogenous gene fragments within 9kb.

High biosafety: The 3rd generation lentiviral packaging system, which has not been found to be pathogenic so far, can be used for CAR-T cell therapy development.

Low immunogenicity: In vivo injection would not cause immune response, suitable for animal experiments.

High transfection efficiency: The virus titer can be up to 10^9 IFU/mL, which greatly improves the infection efficiency.

Serum-free system: Reduce pathogenic hazards and contamination of serum impurities.

One-stop service: One-stop service from gene synthesis to lentivirus packaging as fast as 4 weeks.

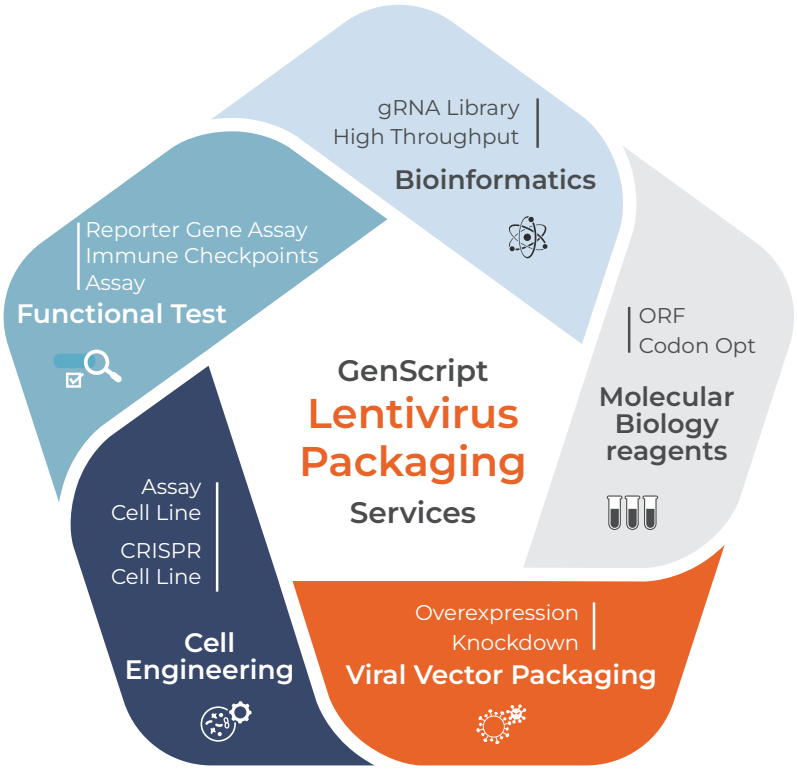


Figure 1. GenScript viral vector services system

The applications of lentivirus system

Based on the characteristics of the lentivirus system, it may be used in the following applications:

Stable Cell Line Construction	Virus Library	Animal Research	Clinical Research	Vaccine Research
• Overexpression • KO/KI/KD	• gRNA virus Library	• Transgenic animal • *In vivo* injection	• Gene therapy • Cell therapy	• Lentiviral vector-based vaccine research

Packaging process of lentivirus in GenScript

Vector preparation: The target sequence is cloned into a transfer vector, then mixed with transfection reagent and incubated;

Transfected cells: The transfer plasmid is co-transfected with helper plasmids into HEK293 cells;

Virus collection: The virus supernatant is collected in 48-72 h after transfection;

Concentration: Virus supernatant is concentrated using ultrafast centrifugation or concentration reagents;

Titration and mycoplasma test: GenScript uses the p24 ELISA to determine lentivirus titers defaultly and reports concentrations in units of IFU/mL.

Workflow

Figure 2. Schematic diagram of lentivirus packaging process

Special note: Although GenScript uses physical titers, which corresponds to the number of lentiviral particles, in order to reflect the functional viral particles more realistically, we measured viral particles through p24 ELISA by dividing 500-1000, then finally get a value close to the functional titer and deliver the viral packaging report.

Calculation method of lentivirus amount

GenScript provides a formula to calculate the amount of lentivirus you need in the experiments:

$$\text{Lentivirus amount (mL)} = \frac{\text{target cell number} * \text{MOI}}{\text{virus titer (IFU/mL)}}$$

Example:

Assuming lentivirus titer= 1×10^8 IFU/mL, and target cell MOI= 10. the number of cells per well at the time of infection is about 5×10^5 (50% confluency).

$$\text{Lentivirus amount (mL)} = \frac{\text{target cell number} * \text{MOI}}{\text{virus titer}} = \frac{5\times10^5\times10}{1\times10^8} = 0.05\ \text{mL} = 50\ \mu\text{L}$$

Table 2. 1×10^8 IFU/mL virus-infected cells required for medium volume and viral load reference

Plate	Single well area	Cell number/well*	Culture volume	Infect volume	Virus amount (MOI=A)
96-well	0.3 cm^2	2.4×10^4	100 μL	100 μL**	0.24×A μL
48-well	0.6 cm^2	4.8×10^4	200 μL	200 μL**	0.48×A μL
24-well	2 cm^2	9.5×10^4	500 μL	500 μL**	0.95 ×A μL
12-well	4 cm^2	1.9×10^5	1000 μL	500 μL	1.9×A μL
6-well	10 cm^2	4.8×10^5	2000 μL	1000 μL	4.8×A μL
T25 falsk	25 cm^2	1.2×10^6	5000 μL	2500 μL	12 ×A μL

*The number of cells per well in the table is estimated based on 293T cells grown to 50% confluency. Due to differences in cell types and experimental conditions, it is recommended to use the cell counting method to determine the number of cells per well. A starting cell seeding confluency of 20-30% is recommended, and it is generally advisable to grow cells to 80%-90% confluency, following 3 days after cell infection.

**Typically, the lentiviral particles in the upper layer of the solution are inaccessible to the cells. Reducing the infection volume of the medium can improve the infection efficiency of the virus. After 1 day of culture, the medium should be replenished to normal volume. However, when the plate wells are small, if the infection volume is halved, the surface tension of the liquid will make the lentiviral particles unevenly distributed, so the infection volume of the small-well culture plate is the same as the normal culture volume.

Guide of optimal antibiotic concentration screening

Taking 293T cells + Puromycin as an example, the minimum lethal concentration of Puromycin on 293T cells was found to be 2 μg/mL from the reference. Set up several more concentration gradients in the 2 μg/mL accessory to obtain a more accurate screening concentration.

1. Prepare a 24-well culture plate, inoculate 293T cells according to the number of 1-5×10^4 cells per well (approximately equal to 20%-35% confluence), and seed in 6-well plate;

2. Generally, the concentration of Puromycin stock solution is 10 mg/mL. Dilute Puromycin stock solution 1000 times with cell culture medium to obtain Puromycin dilution with a final concentration of 10 μg/mL;

3. As shown in Table, add the corresponding cell culture medium and Puromycin to each well;

4. The 24-well plate was placed in a 37°C, 5% CO_2 incubator for overnight incubation;

5. Periodically observe the cell state under a fluorescence microscope.

6. The concentration of antibiotics can be used as the optimal screening concentration when the 293T cells are all died.

Table 3. Purimycin concentration gradients reference

Group	Group's specs	Puromycin final Conc.
1	250 μL Puromycin Diluent* + 250 μL Cell Culture Medium	5 μg/mL
2	100 μL Puromycin Diluent* + 400 μL Cell Culture Medium	2 μg/mL
3	75 μL Puromycin Diluent* + 425 μL Cell Culture Medium	1.5 μg/mL
4	50 μL Puromycin Diluent* + 450 μL Cell Culture Medium	1 μg/mL
5	25 μL Puromycin Diluent* + 475 μL Cell Culture Medium	0.5 μg/mL
6	500 μL Cell Culture Medium	0 μg/mL

*The Puromycin concentration used in the table is 10 μg/mL, obtained by 1000-fold dilution of 10 mg/mL Puromycin stock solution in cell culture medium.
The settings in the above table are for reference only.

Method of lentivirus titer detection

Table 4. Comparison of different lentivirus titer detection methods

Method	Principle	Advantage	Disadvantage
p24 ELISA	Detection of viral capsid p24 protein level	Simple, fast, and stable operation	Overestimates virus titer
FACS	Detection of reporter gene level	Detection of functional virus	Complex operation, high instrument requirements, and non-fluorescent proteins require specific antibodies
qPCR	Infect target cells with virus, extract genomic DNA, and compare with standard	Detection of functional virus	Complex operation, high quality requirements for instruments and kits
Antibiotic Selection	After the virus is serially diluted, the standard the titer is calculated according to the number of live clones.	Detection of functional virus	Time consuming, and prone to experimental error

Lentivirus Shipping and Storage Conditions

1. The lentivirus is shipped on dry ice

2. After receiving the virus, if it is used within a week, it can be temporarily stored in 4°C

3. For long-term storage, it needs to be stored in a -80°C refrigerator. In order to avoid repeated freezing and thawing (every freezing and thawing will reduce the virus titer by 10%), GenScript has aliquot the virus (100 µL/tube) and please stored it directly at -80°C after receiving.

4. If the virus is stored for more than six months, the virus titer needs to be re-measured before use.

5. Dilution of lentivirus: When the user needs to dilute the virus, please place it in an ice bath to dissolve, and mix well with PBS or serum-free medium (serum or antibiotic does not affect virus infection) and store at 4°C (please finish using within a week).

Methods of infecting target cells with lentivirus

Cell preparation

Inoculate the target cells into a 6-well plate, so that the cell concentration is 1×10^5 cells/mL. The number of inoculated cells varies slightly depending on the growth rate of the cells. Generally, the cell fusion rate is guaranteed between 50% to 70% when the virus is infected in next day.

Virus infection

1. Polybrene selection:

Polybrene is a positively charged small molecule that binds to anions on the cell surface to improve the infection efficiency of lentivirus. Usually, adding polybrene can increase the infection efficiency by 2-10 times. Polybrene has certain cytotoxicity, and some cells have obvious toxicological reactions to polybrene, so it is necessary to explore whether polybrene is suitable for your cell infection; different cells have different sensitivities to polybrene, and the range of 1-10 μg/mL can be used to screen the appropriate concentration , it is better to have no obvious toxic reaction in cells within 24h, you can refer to the literature and conduct preliminary experiments to explore, the most commonly used working concentration of polybrene is 6-8 μg/mL.

2. Determination of optimal MOI:

MOI (Multiplicity of Infection) refers to the number of viruses infected per cell. Generally, the higher the MOI, the higher the number of viruses integrated into chromosomes and the higher the expression level of the target gene. For actively dividing cells, such as Hela and HEK293 cells, when MOI=1-3, more than 80% of the cells express the target gene. For non-dividing cells, such as primary cells, the infection efficiency is lower. It is necessary to conduct a MOI gradient experiment, and select a suitable MOI for the experiment.

Table 5. Lentivirus MOI infection reference

Cell Line Name	Description	MOI Range	Polybrene or not
K562	Human Leukemia Cells	~30	Yes
Jurkat	Human T Lymphocyte Cells	~80	No
Kasumi	Human acute myeloid leukemia cells	10~30	No
NB4	Human acute promyelocytic leukemia cell line	50~80	No
U937	Human myelomonocytic cells	20~40	Yes
THP-1	Human monocytic cell line	50~80	Yes
GBC-SD	Human gallbladder carcinoma cell line	30~50	No
H929	Human multiple myeloma cell line	100~150	No
H1299	Human non-small cell lung cancer cells	1~3	Yes
95D	Human lung giant cell carcinoma	2~4	Yes
A549	Human alveolar basal epithelial cell line	20~40	Yes
SPC-A-1	Human lung cancer cell line	100~150	Yes
7402	Human hepatoma cell line	10~15	Yes
Hep 3B	Human hepatocellular carcinoma cell line	10~30	Yes
Hep G2	Human hepatocellular carcinoma cell line	10~30	Yes
SMMC-7721	Human hepatocellular carcinoma cell line	10~30	Yes
Huh-7	Human hepatocellular carcinoma cell line	10~30	Yes
Hela	Human cervical cancer cell line	10~30	Yes
HOS	Human osteosarcoma cell line	20~40	Yes

Table 5. Lentivirus MOI infection reference

Cell Line Name	Description	MOI Range	Polybrene or not
Hep-2	Human laryngeal carcinoma cell line	10~30	Yes
HL-60	Human leukemia cell line	>100	Yes
HT-29	Human colon cancer cell line	10~30	Yes
PKO	Human colon cancer cell line	2~4	Yes
SW480	Human colon cancer cell line	10~30	Yes
DLD-1	Human colorectal adenocarcinoma cell line	10~30	Yes
SK-OV-3	Human ovarian adenocarcinoma cell line	2~4	Yes
SHG-44	Human malignant glioma cell line	10~30	Yes
U251	Human malignant glioblastoma cell line	1~3	Yes
U87	Human glioblastoma astrocytoma cell line	1~3	Yes
293T	Human embryonic kidney cell line	1~3	Yes
HUVEC-2C	Human umbilical vein endothelial cell line	10~30	Yes
PC-3	Human prostate cancer cell line	20~40	Yes
MDA-MB-231	Human breast cancer cell line	10~30	Yes
MCF-7	Human breast cancer cell line	20~40	No
Tca8113	Human tongue squamous cell carcinoma	10~30	Yes
RPE	Human Retinal pigment epithelial cell line	10~30	Yes
AGS	Human gastric adenocarcinoma cell line	100~150	Yes
BGC-823	Human gastric cancer cell line	100~150	Yes

Table 5. Lentivirus MOI infection reference

Cell Line Name	Description	MOI Range	Polybrene or not
SGC-7901	Human gastric cancer cell line	10~30	Yes
MKN-28	Human well-differentiated stomach adenocarcinoma cells	20~40	Yes
MKN-45	Human gastric adenocarcinoma cell line	20~40	Yes
BxPc-3	Human pancreatic cancer cell line	20~40	Yes
CFPAC-1	Human pancreatic adenocarcinoma cell line	50~80	Yes
Panc-1	Human pancreatic cancer cell line	2~4	Yes
HEC-1-B	Human endometrial cancer cell line	2~4	Yes
NIH-3T3	Mouse fibroblast cell line	20~40 (Differentiation after infection)	Yes
Raw264.7	Mouse monocyte/macrophage-like cell line	10~30	No
CHO	Chinese hamster ovary cell line	20~40	Yes
HSC-T6	Rat hepatic stellate cell line	10~30	No
C6	Rat glioma cell line	>100	Yes
NRK	Normal rat kidney epithelial cell line	10~30	Yes

Note: Due to the influence of factors such as cell origin, generation, and cell status, the MOI value may slightly vary. The data above were obtained for cells in good condition when infection efficiency was 85-100% and for reference only.

3. Infection steps

- **For adherent cells**

  Plate cells as needed for the experiment (e.g 12-well plate). The number of cells should be about 50% of confluence in next day. Incubate at 37°C overnight. After infection for 24 hours, the virus-containing medium was aspirated, replaced with fresh medium, continued culturing at 37°C. 48 hours after infection, for viruses with GFP reporter gene, the GFP expression efficiency can be observed by fluorescence microscope. For viruses with Puromycin resistance gene, the culture medium is changed to fresh medium with concentration of Puromycin, and screen stably transduced cells (see Table 6 for details).

- **For suspension cells**

  For suspended or semi-suspended cells, centrifugation is required. After adding an appropriate amount of virus liquid to the cell culture dish, seal and, put it into a flat angle centrifuge, centrifuge it at 500-1000 g/min for 30-180min. Then put it in the incubator for overnight culture. If there is no flat angle centrifuge due to limited experimental conditions, a centrifuge tube can be used instead. The cells are pipetted into the centrifuge tube, and centrifuged at low speed. Most of the supernatant is removed, and then an appropriate amount of virus solution is added, placed at room temperature for 15min, then transfer the cells and virus solution into petri dish for overnight culture.

For difficult-to-infect cells, one can choose to halve the medium before infection and then change to fresh medium 24-48 hours after infection. Either perform two infections or increase the infection efficiency by centrifugation and adjusting the amount of Polybrene.

4. Puromycin resistance screening

Puromycin is commonly used at a final concentration range between 1～10 μg/mL. However, Puromycin's exact working concentration varies among different cell types. Please refer to relevant literature, (see Table 6 for recommended concentration for some cell types). Remember to set up a negative control (uninfected wild-type cells), and culture with the same amount of Puromycin.

Table 6. Puromycin concentration reference

Cell Line	Species	Puromycin (μg/mL)
293	Human	3
HeLa	Human	3
B16	Mouse	1-3
PC1.0	Hamster	10
MC3T3-E1	Mouse	10
H9C2	Rat	1
MCF-7	Human	1-3
MDA-MB-231	Human	1-3
HepG2	Human	2
HT1080	Human	1
A549	Human	1.5
H1299	Human	2
Human embroyonic stem cells (Human ESCs)	Human	1

- **The medium containing puromycin is changed every 2-3 days until the cells in the control group are completely killed by Pruomycin. The following two-step operations can be performed (selected according to specific experimental needs):**

a) No single clones were picked: the infected and screened cells were passaged and continued to apply Puromycin for maintenance screening culture. Cryopreserved stable cell pools.

b) Single clones were picked: Pick at least 10 clones of the infected and screened cells for cell expansion, and continue to apply puromycin for maintenance screening culture. After amplification, the expression of the target protein was detected (FACS/WB/qPCR). Picked the single clone with stable expression and cryopreserve stable cell clones.

Precautions of using lentivirus

The lentivirus provided by GenScript is a replication-deficient virus, it means that after the virus infects the target cell, it will not infect other cells, nor will it use the host cell to generate new virus particles. The virulence gene in lentivirus has been deleted and replaced by exogenous target gene, which belongs to pseudotype virus. However, the virus still has possible potential biohazard. We recommend against the use of pseudotype viruses encoding genes that are known or likely to be oncogenic. Pseudotype virus is not recommended unless it is fully recognized that a gene is definitely not carcinogenic.

Please refer to the following content for experiments when using lentivirus:

1) Please use a biological safety cabinet when handling viruses.

2) For lentivirus-related experiments, please wear a lab coat, mask and gloves.

3) Please be careful when performing lentivirus-related experiments. If the ultra-clean bench is contaminated with viruses, wipe it with alcohol.

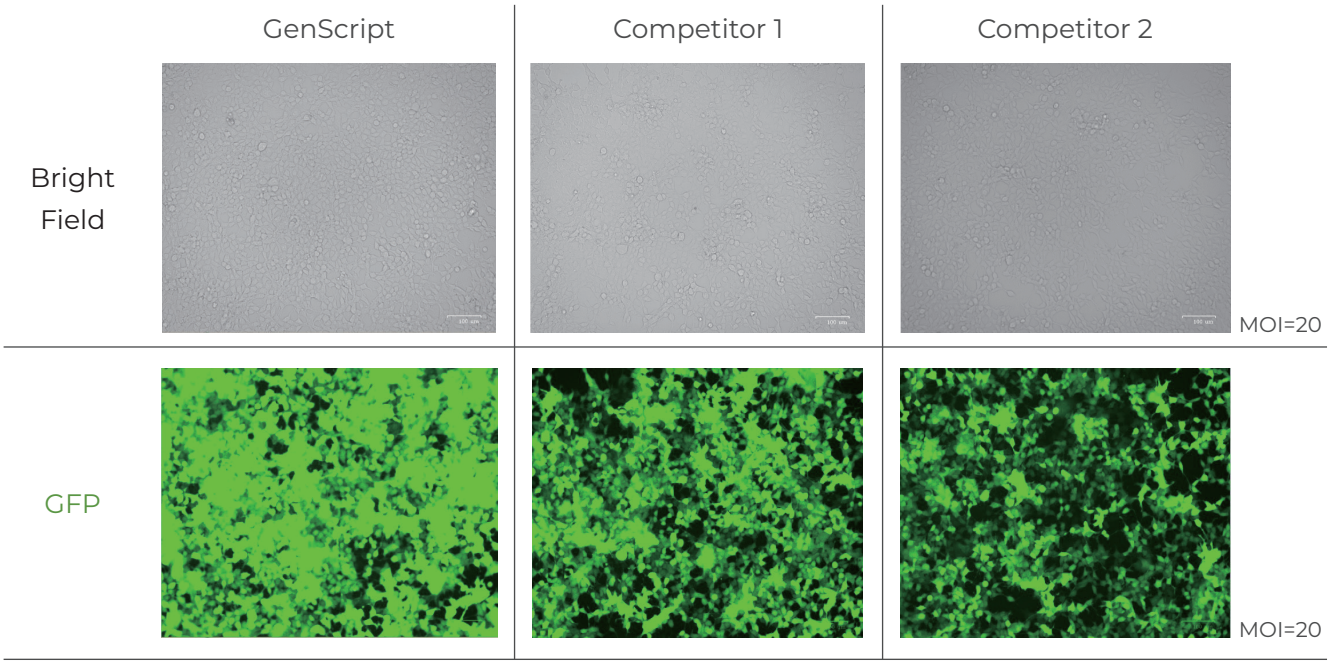
4) For centrifugation, a sealed centrifuge tube should be used, or the centrifuge should be sealed with parafilm. If possible, try to use only centrifuges in cell culture laboratories.

5) Put the discarded virus-containing culture medium solution and consumables into the disinfectant for sterilization, soak for 24 hours and then process. Solid waste can also be autoclaved.

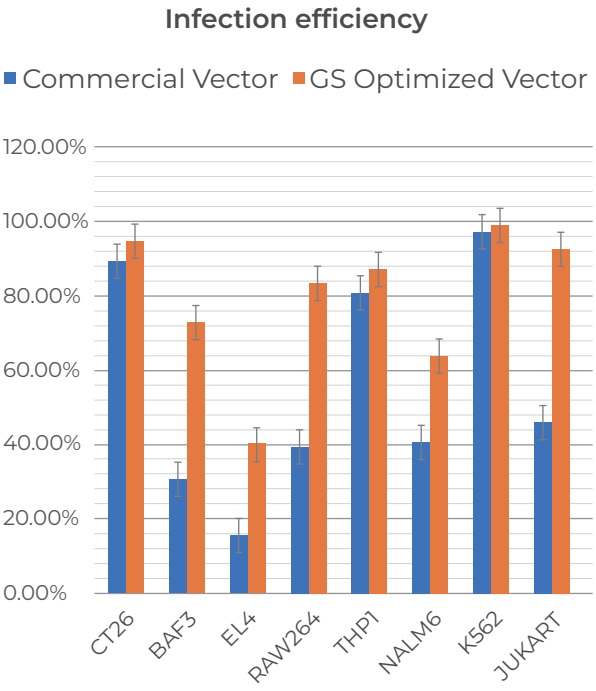
6) After the experiment, take off your gloves and wash your hands with hand sanitizer and water; the lab coat you should be properly placed or cleaned and disinfected.

Case study

Case 1. Superior transduction performance test



Case 2. Optimized vector performance test



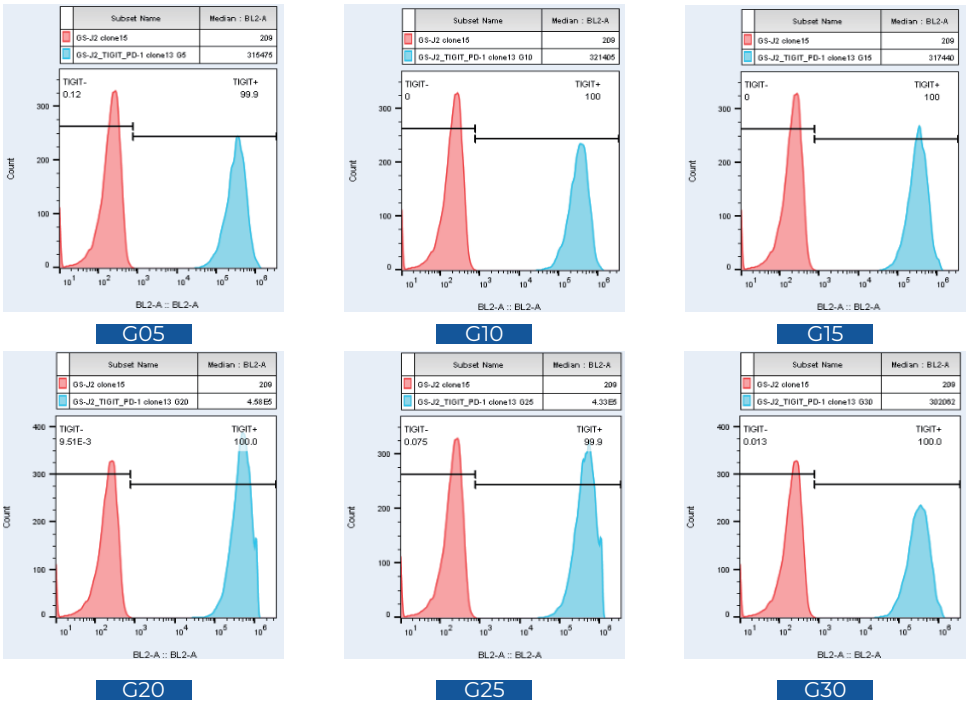
Cell Line	Description	MOI
CT26	Mouse colony	100
BAF3	Mouse B cell	
EL4	Mouse T lymphoma	
RAW 264	Monocyte/macrophage-like cells	
Jurkat	Human acute T cell Leukemia	
K562	Meylogenous leukemia cell line	
NALM6	B cell precursor leukemia cell	
THP-1	Acute monocytic leukemia	

MOI: Multiplicity of infection

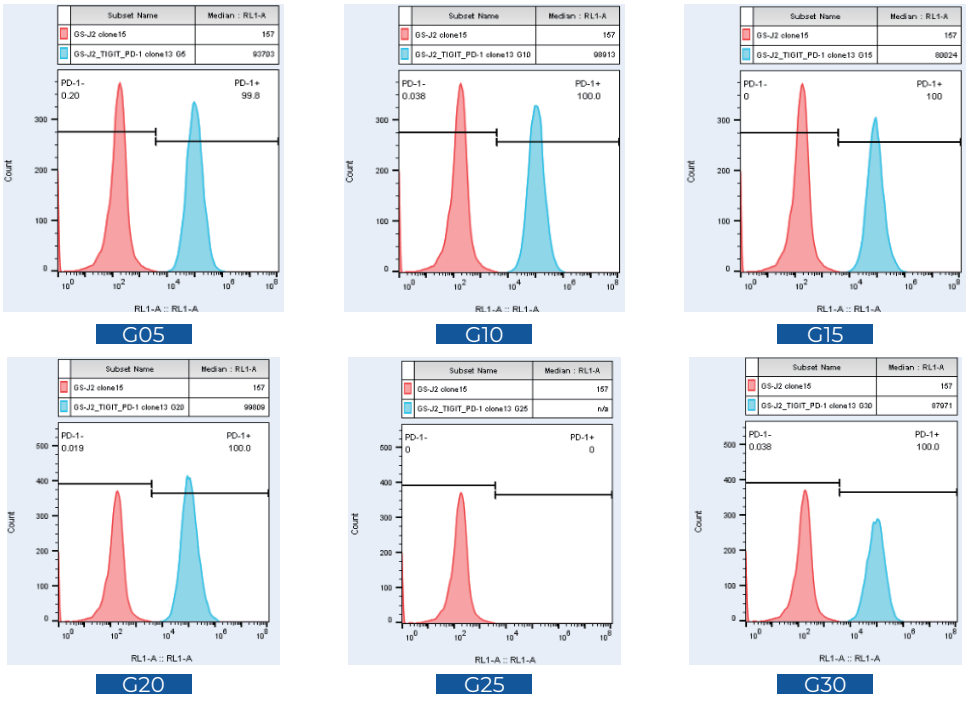
Case 3. Gene expression stability test

To evaluate Gene A/PD-1 expression stability in 30 generations

Jurkat /Gene A Stability



Jurkat /PD-1 Stability



Common Questions

1. What is the maximum size of the target gene sequence that can be inserted into the transfer vector?

Based on the HIV gene backbone, the target gene sequence inserted is usually less than 4kb, otherwise it will affect the virus titer and even gene expression

2. The infection efficiency of lentivirus to target cells is very low, how to improve the infection efficiency of the virus?

Generally, we improve the transfection efficiency of the virus by increasing the MOI value, and if necessary, add Polybrene (4~8 μg/mL) to the medium to improve the infection efficiency of the virus. Meanwhile, the the health condition of target cells and the lower generations (usually within 20 generations) are the guarantees for obtaining normal infection efficiency.

3. Why the GFP fluorescence intensity is very weak after lentivirus transduction?

GFP fluorescence intensity depends on the particle amount of virus that infected cells, the proliferation state of the cells, cell types, and observation time. Generally speaking, the more virus particles the target cell is infected with, the faster the cell itself proliferates, and the stronger the GFP fluorescence will be. Lentivirus is a member from retrovirus family. so, the expression of GFP gene reaches its peak only after 72-96 hours of virus infection in fast-proliferating cells, for slower proliferating cells, GFP gene expression peak was appeared later.

4. After being infected with the virus, the target cells die more, why?

Lentivirus may have certain toxicity to your target cells, please adjust and reduce the MOI value of infection, and change the medium after 4h, 8h or 12h, and continue to culture and observe with fresh medium. In addition, reducing the amount of polybrene is also an important strategy. If the desired result cannot be obtained after optimization, it may be necessary to consider whether the virus itself contains a lot of impurities, and the virus needs to be further purified.

5. What is the process of lentivirus packaging in GenScript?

After the transfer plasmid was constructed, the transfer plasmid and the helper plasmids were co-transfected into HEK293 cells, the supernatant was collected, the virus was purified and concentrated, the titer, and the mycoplasma was detected. In addition, GenScript also has a unique suspension lentivirus production system to obtain higher quality viruses.

6. What method does GenScript use to detect the titer of lentivirus?

GenScript uses the p24 ELISA to measure lentivirus titers by default. The p24 protein is the most abundant marker protein in the lentiviral coat. There are about 2000 p24 protein molecules in a lentiviral particle (LP). The content of p24 protein molecules is detected by ELISA to determine the titer of the virus. If you need other titer detection methods such as FACS or qPCR, please inform in advance, and additional costs and timeline will be required.

7. How to charge for lentiviral packaging services for genes of different lengths?

Gene Length<1500bp, GenScript guarantees at least 10^7 IFU/mL. If it is less than 10^7 IFU/mL, it will be repackaged once free of charge, and will be shipped at the highest titer. The cost will be charged according to the price corresponding to the actual titer. If both times are lower than 10^7 IFU/mL, no fee will be charged for lentivirus packaging.

1500≤Gene Length<3000bp, GenScript guarantees at least 10^7 IFU/mL. If it is less than 10^7 IFU/mL, it will be repackaged once free of charge, and will be shipped at the highest titer. The cost will be charged according to the price corresponding to the actual titer. If both times are lower than 10^7 IFU/mL, only 50% of the order price will be charged for lentivirus packaging.

3000≤Gene Length<4000bp, GenScript guarantees at least 10^6 IFU/mL. If it is less than 10^6 IFU/mL, it will be repackaged once free of charge, and will be shipped at the highest titer. The cost will be charged according to the price corresponding to the actual titer. If both times are lower than 10^6 IFU/mL, only 50% of the order price will be charged for lentivirus packaging.

8. What is Polybrene? In lentiviral particle infection, is more Polybrene the better?

Polybrene is a commonly used infection additive, usually at a concentration of 4-10 μg/mL. Polybrene can significantly improve the infection efficiency of lentivirus, usually 2-10 times, and even increase the infection efficiency for some cells by 10-20 times. When the target cell MOI is higher than 20, we recommend adding ploybrene (about 4-10 μg/mL) to the culture medium to appropriately increase the infection efficiency.

However, polybrene has certain cytotoxicity. Different cells have different sensitivities and tolerances to Polybrene, and some cells react significantly to polybrene, which can easily lead to poor cell state, morphological changes, and even death. Therefore, not every cell is suitable for polybrene.

Before using the Polybrene, it is recommended to set a gradient concentration in the range of 1-10 μg/mL, and observe whether the cells still maintain healthy growth after 24 hours at this concentration, so as to determine the optimal polybrene concentration for the cells.

9. What is MOI?

MOI means the multiplicity of infection, which means the ratio of virus particle number to infecting cells number. We defined the ratio as the MOI value when 80% cells were infected during the experiment.

10. Lentiviral particles are accidentally contaminated during storage, can they continue to be used?

If the lentivirus particles are accidentally infected with bacteria and the experimental materials are limited, a 0.45 μm filter can be used to filter the lentivirus. Of course, this procedure will result in a certain loss of titer and total loss of lentiviral particles. If necessary, we recommend repurchasing this lentivirus.

11. What are the advantages of using lentivirus to generate stable cell line?

Lentivirus has a much higher positive clone rate with the selectable marker. Using lentivirus to generate stable cell line can save much more time, cost and labor than non-viral based stable cell line generation.

12. What are the advantages of lentivirus over retrovirus and adenovirus?

Lentiviruses comprise a subtype of retroviruses. Lentiviruses can stably integrate into the host genome in dividing, non-dividing and post-mitotic mammalian cells, while retroviruses are less active in this scenario. Adenoviruses can also transduce non-dividing cells, but can not stably integrate into the host cells genome. Adenoviruses also take much more time to design and prepare. In addition, lentiviruses are much less immunogenic than the retroviruses and adenoviruses, making lentivirus more suitable for application in most types of cells and animal models.

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