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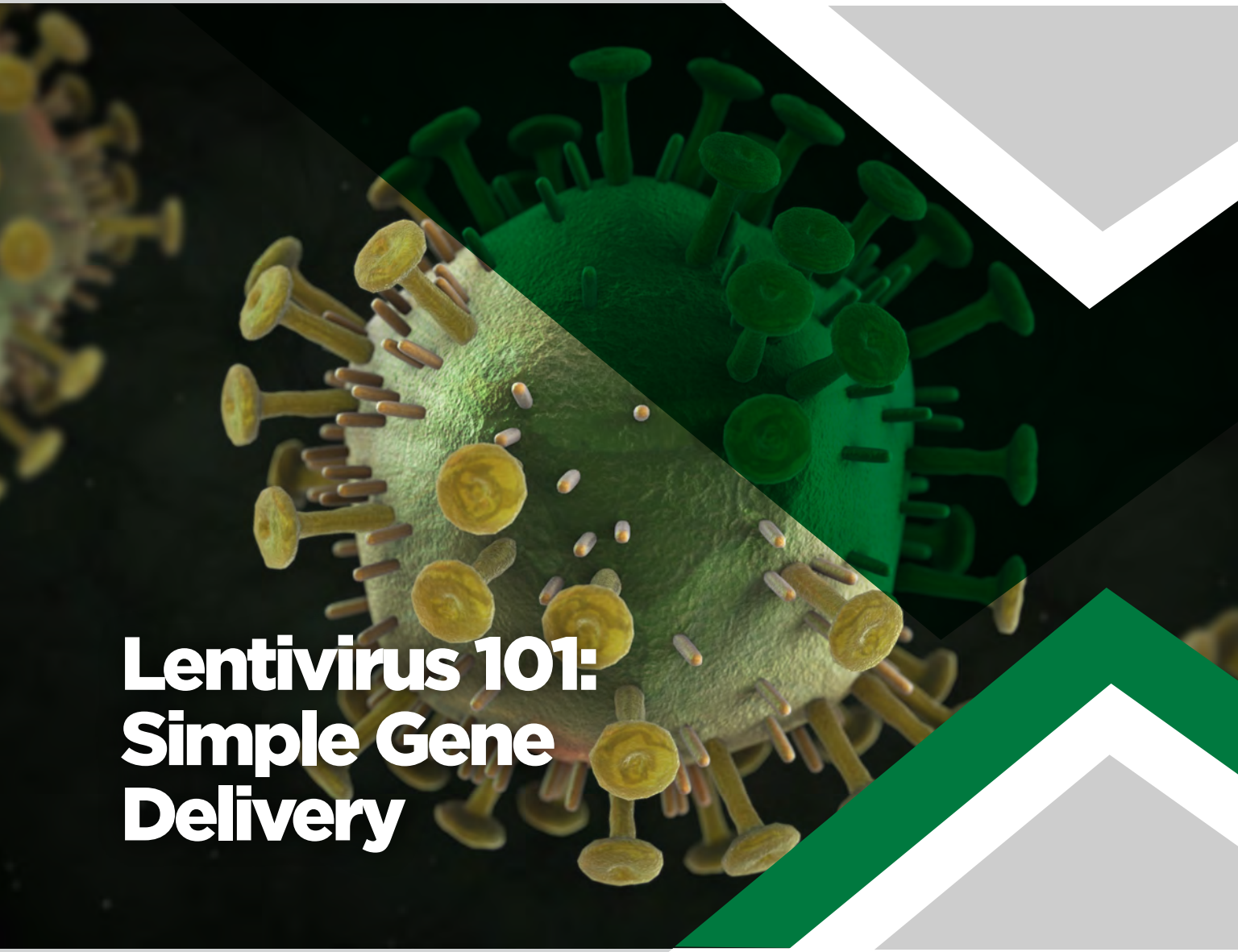

Lentivirus 101: Simple Gene Delivery

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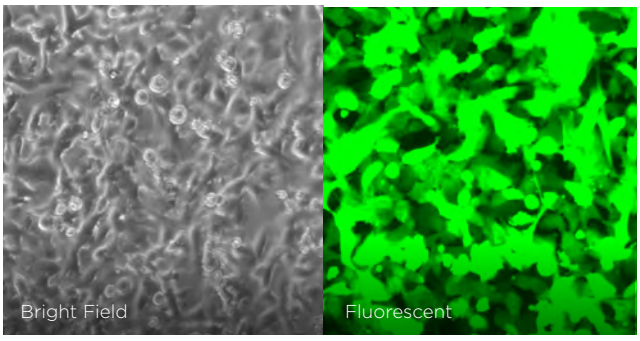
How is Lentivirus Prepared? An Introduction to Lentivirus Production Methods

"[Lentiviruses] can transduce both dividing and non-dividing cells, meaning they have wide application potential."



GFP Lentiviral Particles

Lentiviruses (LV) are single-stranded RNA viruses and the second major subgroup of the retroviruses. They have long incubation periods and are capable of delivering relatively large loads of genetic information into the DNA of a broad range of host cells.

Why Lentiviruses?

LVs are powerful tools in molecular biology. They have many features that make them excellent gene-delivery tools:

- Lentiviruses contain the enzyme reverse transcriptase, which produces cDNA from an RNA template. When a LV infects a host cell, viral RNA is released and cDNA is produced, which then migrates to the nucleus and integrates into the host genome. This leads to long-term expression while avoiding activation of the immune system.[1]
- They can transduce both dividing and non-dividing cells, meaning they have wide application potential.
- They have a 9 kb carrying capacity.

Packed to Perfection: The Lentivirus Delivery System

LV vectors are most commonly based on the human immunodeficiency virus-1 (HIV-1) with many of the viral genes removed. The components necessary for virus production are divided across multiple plasmids.

The newest LV vectors are *third-generation systems* that consist of four plasmids (*see infographic, page 4*):

- A transfer plasmid containing the insert of interest
- Two plasmids containing packaging genes
- An envelope plasmid (also involved in packaging)

The transfer plasmid, containing the insert of interest, comprises long terminal repeats (LTRs) flanking the transgene sequence, and Psi (Ψ) the packaging signal. Transfer plasmids are incapable of replication, usually containing a deletion in the 3' LTR and a deactivated 5' LTR. This renders the plasmid self-inactivating after host-genome integration.

The first packaging plasmid encodes the lentiviral structural genes *gag* and *pol*, and the second encodes *rev*. The fourth and final plasmid, also involved in packaging, encodes the LV envelope. The VSV-G envelope protein is commonly used, which confers broad tropism over a range of species and cell types.

New and Improved: Second Generation vs. Third Generation

The four-plasmid format of third-generation systems is safer compared to three-plasmid second-generation systems, which have packaging genes *gag*, *pol*, *rev*, and *tat* encoded on a single packaging plasmid. Splitting the packaging genes across two plasmids reduces the likelihood of replication-competent lentiviruses (RCLs).

Producing Lentiviruses in the Laboratory

LVs are classified as Biosafety Level 2 (BSL-2) due to their ability to infect primary human cells. Laboratories may wish to perform an RCL assessment before working with them.

The lenti transfer plasmid DNA preparation (that contains your gene of interest) requires a recombination deficient *E. coli* due to the LTR regions. LV production involves a simple co-transfection of the lenti transfer plasmid with the lenti packaging plasmids *(see infographic, page 4*). Here is the summary:

- Day 1: Plate HEK293T cells
- Day 2: Co-transfect lenti transfer plasmid along with the lenti packaging plasmids
- Day 3: Change media, incubate for an additional 48 hours
- Day 5: Harvest cell culture supernatants containing viral particles

LV particles can then be stored for later use: short-term at +4_° C, or long-term at -80_° C (*see page 6*), or be titered and used immediately for transduction of target cells (*see page 5*).

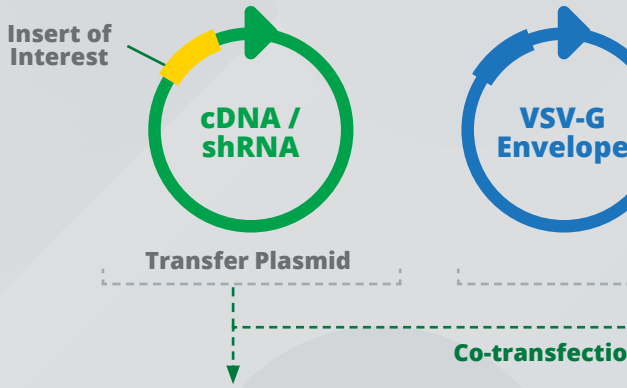
What Factors Affect the Packaging Efficiency of Lentivirus?

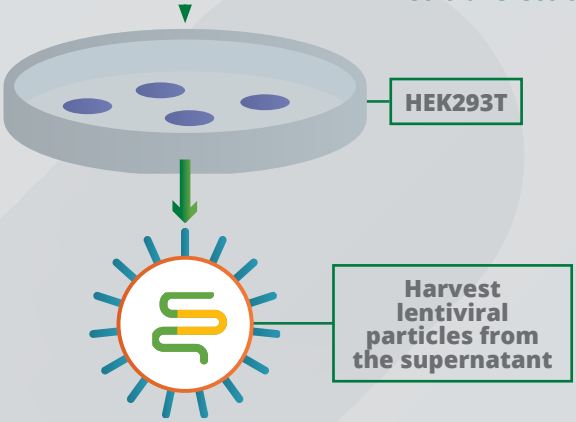
- **DNA quality of the lenti transfer plasmid:** Since lenti transfer plasmids contain LTRs, potential recombination events must be tested for. Plasmid recombination can be tested using a restriction-enzyme digestion to check plasmid DNA size, and also by sequencing.
- **DNA quality and optimal ratio of the packaging plasmids:** The use of ion-exchange plasmid purification and an endo-free kit is highly recommended for preparation of plasmid DNA.
- **Fragment length between LTRs:** Since the packaging limit for lentivirus is around 9 kb, larger inserts may lead to lower packaged viral titer.
- **Health of HEK293T cells:** Firstly, HEK293T cells usually lose packaging efficiency after many passages. Cells should not be used after culturing for 1-2 months. Secondly, cells are happier when seeded the day before transfection, which results in higher transfection efficiency
- **Transfection efficiency:** Since all required plasmids need to be transfected into HEK293T cells to produce viral particles, transfection efficiency is critical for high titer production. A transfection reagent that results in high transfection efficiency in HEK293T cells is required.

References:

1. M. Carter and J. Shieh, "Gene Delivery Strategies," in *Guide to Research Techniques in Neuroscience (second edition)*, Elsevier, 2015, pp. 239-252.

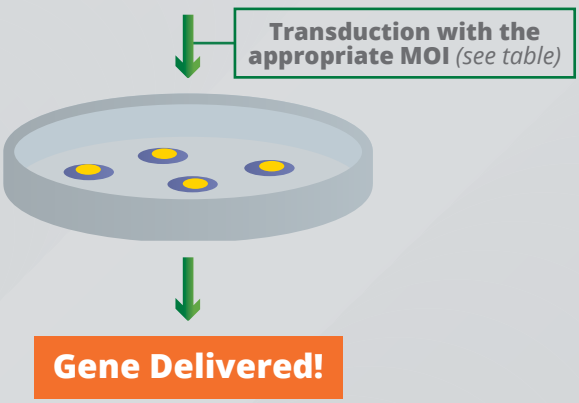
Lentivirus Workflow
Production and Transduction





Optional Procedures before Transduction

• PCR • p24 ELISA	• Increase Titer • Increase stability	• -80 °C • Longer-term storage • Buffer is critical
Titration	Concentration	Storage



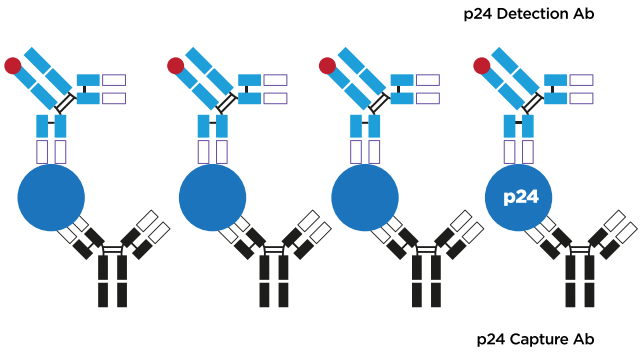
What is MOI?

MOI stands for Multiplicity Of Infection, the number of lentiviral particles per cell. The optimal MOI for each cell line varies.

For each cell line, a range of MOI from 5-100 needs to be tested the first time it is used. This can be done using positive control particles containing fluorescent protein markers.

Cell Line	Recommended MOI
A549	40
C2C12	200
Caco2	100
HCT116	20
HEK293T	20
HeLa	80
Hep3B	50
HuH7	20
Jurkat	40
MCF-7	60
NK92	50
PC3	40
SH-SY5Y	5
SKNMC	20
THP1	80
U2OS	20

How Can Lentivirus Particles be Counted? Quantification and Analysis of Virus Particles



"This vital step allows control of multiplicity of infection (MOI), the number of lentiviral particles per cell."

Congratulations, you've packaged, produced, and harvested lentivirus! But what's next? Before beginning transduction into a stably producing cell line, it's important to determine the amount of virus you have in your prep. This vital step allows control of multiplicity of infection (MOI), the number of lentiviral particles per cell.

Taking the Time to Titer

Titering your lentivirus prep will save you time in the long term. Titering generally comes in two forms: physical or functional.

Physical Titers: ELISAs and qPCR

Commonly used physical titer methods are p24 measurements using ELISA and viral RNA measurements using qPCR. P24 is an LV capsid protein, encoded by *gag*.

Commercially available p24 ELISA kits are rapid, easy ways to titer LV. They take less than a day to complete and are based on a straightforward sandwich ELISA, which usually involves five steps: Collecting LV supernatant, lysing, binding, washing, and detecting.

p24 ELISA is the most common LV titration method. Although p24 ELISA assays cannot distinguish between assembled particles versus free p24, studies show that approximately 1 out of 100 viral particles is functional, and that 40% of p24 protein detected in a p24 ELISA is of the free form. Therefore, it's possible to calculate the number of functional viral particles using a p24 ELISA.

An alternative to p24 assays is to use qPCR to measure lentiviral RNA. Viral RNA is converted to cDNA, which can then be quantified using qPCR primers targeted to various LV sequences including universal features such as LTRs or LV structural genes, or to the transgene itself. The drawback is that qPCR also targets defective LV particles, which can lead to titer overestimation. This issue can be overcome by using a reverse transcriptase mutant in a control reaction.[1]

Functional Titers

Infectious or functional titers are considerably more accurate at determining titer because they quantify only functional viral particles. Two popular methods are fluorescence titering using flow cytometry using fluorescence-activated cell sorting; FACS or microscopy, and transfer-plasmid antibiotic-resistance gene titering by counting colony-forming units (CFU).

The fluorescence method is ideal for LV constructs that contain a fluorescence marker such as GFP. Transductions with serial dilutions of LV preparation are carried out, and then target cells are counted via flow cytometry or using a microscope. The serial dilutions are necessary to obtain an ideal dilution for flow analysis. Ideally, cell populations should be somewhere between 2-20% fluorophore-positive. This assay provides the number of transducing units per mL (TU/mL). However, the method is prone to underestimation of titer, as it cannot differentiate between cells with single or multiple integration events.

When using antibiotic-resistance genes to titer LV preparations, like the fluorescence method, target cells are first transduced with serial dilutions of the LV preparation. Target cells are then treated with an antibiotic and CFU are counted. This method also carries the risk of titer underestimation, for the same reasons as noted for the fluorescence method above.[2]

Each titer prediction method has its drawbacks and benefits, and the method chosen may depend on many factors including reagents available, time, experience, and equipment available in the laboratory. However, performing titration is an essential step in an LV vector experiment and is critical for success.

References:

1. R.H. Kutner et al., "Production, concentration and titration of pseudotyped HIV-1-based lentiviral vectors," *Nat Protoc*, 4(4):495-505, 2009.
2. M. Geraerts et al., "Comparison of lentiviral vector titration methods," *BMC Biotechnol*, 6:34, 2006.

What's the Best Way to Store Lentivirus? Smart and Safe Storage for Lentivirus Particles

"Treat your lentivirus right, and it will repay you with a tremendous transduction."

Using freshly prepared lentivirus (LV) is optimal, but not always feasible. However, storing LV without impacting infective qualities is no small task. The way in which LV is stored should be carefully considered. Treat your lentivirus right, and it will repay you with a tremendous transduction.

No Extremes: Optimal Temperature

While LVs are happiest being aliquoted and stored for long periods (more than one week) at -80 °C, they do not do well with freeze-thawing, which can cause titers to drop 2-3 fold with each freeze-thaw cycle.[1] To avoid freeze-thawing, aliquot LV preps before freezing and thaw LV aliquots on ice just before use, or store sample preps at +4 °C for up to one week. Snap freezing (flash freezing) LV particles, rapidly freezing using dry ice or liquid nitrogen to -80 °C, may may help to maintain sample integrity.

A Comfy Environment: Buffers

LVs must always be stored in a suitable liquid and must never be dried, which results in membrane disruption and subsequent virus inactivation. However, since the VSV-G protein is particularly pH sensitive, and serum can affect transduction efficiency, a serum-free media buffered to pH 7.2 should be used. The addition of 10 mM HEPES to an LV preparation will ensure the viral particles remain at a comfortable pH.[2] Many such buffers are now available commercially. Furthermore, commercially available LV stabilizing solutions may also be used to decrease loss of LV infectivity that occurs when storing LV long term.

Potential Contaminants

Mycoplasma is a common laboratory contaminant and something to be aware of when storing LV preps. Signs of mycoplasma contamination include clumping, increased confluence, and decreased growth rates. However, filtering an LV preparation is not recommended as viral particles can stick to the filter. If filtering is absolutely necessary, a 0.45 µM or larger pore-size filter is recommended. Mycoplasma contamination can also be prevented by following correct biosafety guidelines.[3]

Air bubbles are another potential form of contamination because they can denature the envelope protein. Therefore, laboratory techniques that may introduce bubbles such as vortexing are not recommended.[2]

Five Star Storage: Containing Lentivirus Particles

Lentiviruses are surrounded by a predominantly hydrophobic membrane, which causes them to be "sticky" in certain environments. For this reason, LVs should not be stored in containers composed of hydrophobic plastics such as polystyrene. LVs are safest when stored in low-protein binding tubes made from polypropylene or silicone. The same considerations should be applied for pipette tips.[2]

Remember: happy LV particles = successful transductions!

References:

1. W. Jiang et al., "An optimized method for high-titer lentivirus preparations without ultracentrifugation," *Sci Rep*, 5: 13875, 2015.
2. "Care and Handing of VSV-G Pseudotyped Lentiviruses," med.stanford.edu/gvvc/lentiviruses, Stanford Medicine, 2018. Accessed on August 15, 2018.
3. C. Delenda et al., "Biosafety issues in lentivector production," in *Lentiviral Vectors*, D. Trono, ed., Berlin Heidelberg: Springer-Verlag, 2002, pp. 123-141.

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