Development and Optimization of a High Titer Lentivirus System

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Delivery by Mirus

Abstract

Lentivirus is an enveloped single-stranded RNA virus from the *Retroviridae* family. Its ability to infect dividing and non-dividing cells has led to the utilization of recombinant lentivirus as a gene delivery vector. In addition, lentivirus has an efficient integration mechanism which leads to robust and stable transgene expression in target cells. Production of recombinant lentivirus is initiated via transient transfection of the essential lentivirus genes, *gag*, *pol*, and *rev*, along with the vesicular stomatitis virus G protein (VSV-G).

To enable simple and effective high titer lentivirus production, we examined key parameters for the generation of lentivirus vectors including: transfection reagent formulation, transfer vector, and packaging vectors. A targeted compound screening approach was employed to investigate the effect of reagent formulation on functional lentivirus titers. We identified a unique transfection formulation that enables high efficiency transfection of 293T-derived cell types and up to eight-fold higher functional titers compared to other commonly used transfection methodologies. The importance of the transfer vector in recombinant lentivirus generation was analyzed using three commercially available third generation vectors encoding fluorescent reporter genes. Up to a four-fold difference in functional titers was observed among the tested transfer vectors. Packaging vectors are another key variable in lentivirus production. Commercially available packaging premixes are frequently utilized by researchers; therefore, we compared the performance of two commercially available packaging premixes with different transfection reagents. Up to 20-fold difference in functional titers was observed between the lentivirus packaging premixes. As a proof-of-principle, lentivirus stocks generated with different transfection reagents were used to transduce iCell® Motor Neurons. High efficiency transduction was observed via flow cytometry and microscopy. These results illustrate the importance of optimizing transfection processes for high titer recombinant lentivirus production.

Overview of Recombinant Lentivirus Particle Generation and Transduction

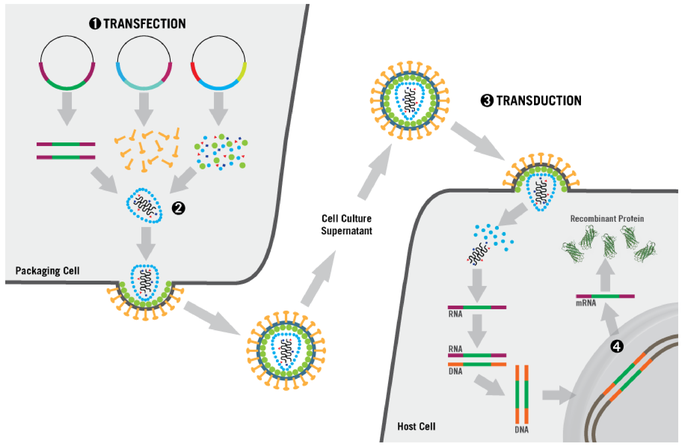


Figure 1. (1) Packaging cells (e.g.293T) are transfected with 3-4 plasmids encoding the gene of interest, vesicular stomatitis G protein (VSV-G) and essential virus proteins (e.g. gag, pol and rev). (2) Virus is assembled and released into the supernatant through budding with the producer cell plasma membrane resulting in an envelope decorated with VSV-G. The medium containing virus is filtered through a 0.45 µm filter to remove any cells. (3) Target cells are frequently transduced with recombinant lentivirus particles in the presence of a polycation to enhance efficency. The virus enters the cell and the capsid is uncoated revealing the RNA genome and viral enzymes. The viral RNA is reverse transcribed into DNA which is then integrated into the host genome. (4) Transcription and translation result in the production of the protein encoded by the gene of interest.

High Efficiency Transfection with the *Trans*IT®-Lenti Transfection Reagent

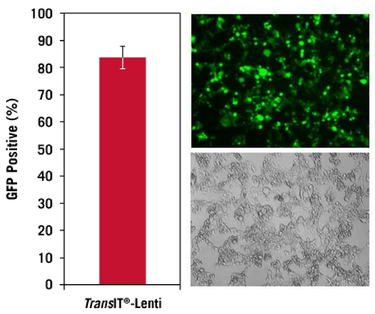


Figure 2. Adherent 293T/17 cells were transfected in a 6-well plate format using the MISSION® vectors (pLKO.1-puro-CMV-TurboGFP™ transfer vector and the Lentivirus Packaging Mix) using the *Trans*IT®-Lenti Transfection Reagent (3:1, vol:wt). GFP efficiency was measured at 48 hours post-transfection using Guava® easyCyte™ 5HT Flow Cytometer. Error bars represent the standard deviation from five transfection complexes. Images were captured at 48 hours post-transfection (10X objective) using a Zeiss Axiovert S100 inverted fluorescence microscope. The observed cell rounding and cell-cell fusion is due to high expression of the vesicular stomatitis virus G protein (VSV-G) for pseudotyping the recombinant lentivirus.

High Functional Titers with the *Trans*IT®-Lenti Transfection Reagent

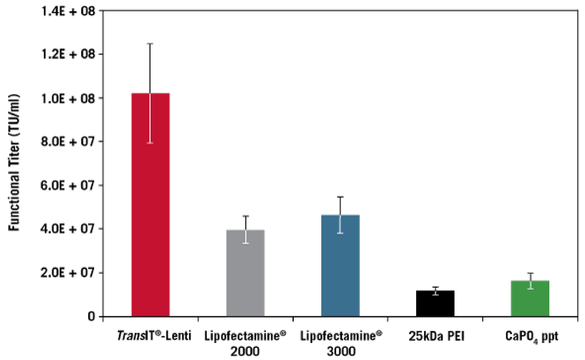
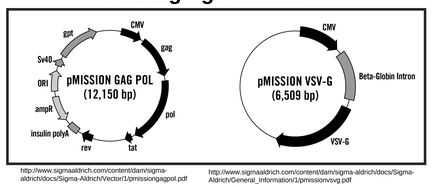


Figure 3. Adherent 293T/17 cells were transfected in a 6-well plate with pLKO.1-puro-CMV-TurboGFP™ transfer vector and the Lentivirus Packaging Mix powered by MISSION® (1:1 ratio, 2 µg/well) with the following reagents: *Trans*IT®-Lenti (3:1, vol:wt), Lipofectamine® 2000 (3:1), Lipofectamine® 3000 (3:1:1), 25 kDa PEI (6:1), or $CaPO_4$ precipitation (4 µg pDNA/well). The supernatant was harvested, filtered (0.45 µm), and titered using 293T/17 cells. Lentivirus transductions were performed in the presence of 8 µg/ml *Transduce*IT™ and GFP expression was measured 72 hours post-transduction using Guava® easyCyte™ 5HT Flow Cytometer. Error bars represent the standard deviation of triplicate transfection complexes titered individually. Functional titers were calculated using virus dilutions with less than 20% GFP positive cells.

Transfer Vector Selection Greatly Affects Functional Lentivirus Titers

Packaging Premix:

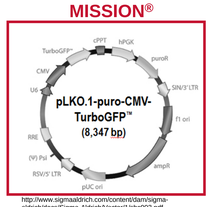


http://www.sigmaaldrich.com/content/dam/sigma-aldrich/docs/Sigma-Aldrich/Vector/1/pmissiongagpol.pdf

http://www.sigmaaldrich.com/content/dam/sigma-aldrich/docs/Sigma-Aldrich/General_Information/1/pmissionvsvg.pdf

Transfer Vector:

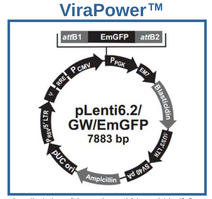
MISSION®



http://www.sigmaaldrich.com/content/dam/sigma-aldrich/docs/Sigma-Aldrich/Vector/1/shc003.pdf

OR

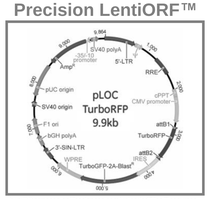
ViraPower™



https://tools.thermofisher.com/content/sfs/manuals/plenti6_2_gw_emgfp_man.pdf

OR

Precision LentiORF™



http://dharmacon.gelifesciences.com/uploadedFiles/Resources/precision-lentiorf-manual.pdf

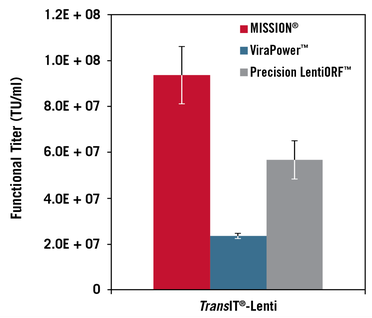


Figure 4. Adherent 293T/17 cells were transfected in a 6-well plate with one of three transfer vectors: MISSION® pLKO.1-puro-CMV-TurboGFP™ (Millipore Sigma), ViraPower™ pLenti6.2-GW/EmGFP (Thermo Fisher Scientific), or Precision Lenti-ORF™ RFP (GE Life Sciences), and the MISSION® Lentivirus Packaging Mix (2 µg/well) using the *Trans*IT®-Lenti Transfection Reagent (3:1, vol:wt). The supernatant was harvested, filtered (0.45 µm), and titered using 293T/17 cells. Lentivirus transductions were performed in the presence of 8 µg/ml *Transduce*IT™ and GFP was measured 72 hours post-transduction using Guava® easyCyte™ 5HT Flow Cytometer. Error bars represent the standard deviation of triplicate transfection complexes titered individually. Functional titers were calculated using virus dilutions with less than 20% GFP positive cells.

Transfection Reagent and Packaging Vector Comparison

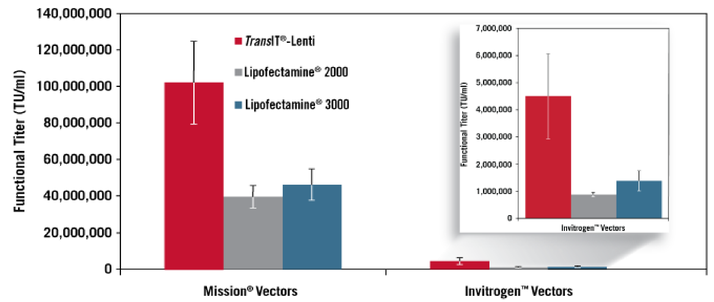


Figure 5. Adherent 293T/17 cells were transfected in a 6-well plate with MISSION® vectors (pLKO.1-puro-CMV-TurboGFP™ transfer vector and the Lentivirus Packaging Mix) or Invitrogen™ vectors (pLenti6.2-GW/EmGFP transfer vector and the ViraPower™ Lentiviral Packaging Mix) (2 µg/well) with the following reagents: *Trans*IT®-Lenti (3:1, vol:wt), Lipofectamine® 2000 (3:1), or Lipofectamine® 3000 (3:1:1). The supernatant was harvested, filtered (0.45 µm), and titered using 293T/17 cells. Lentivirus transductions were performed in the presence of 8 µg/ml *Transduce*IT™ and GFP was measured 72 hours post-transduction using Guava® easyCyte™ 5HT Flow Cytometer. Error bars represent the standard deviation from triplicate transfection complexes titered individually. Functional titers were calculated using virus dilutions with less than 20% GFP positive cells.

High Efficiency Transduction of iPSC-derived Motor Neurons

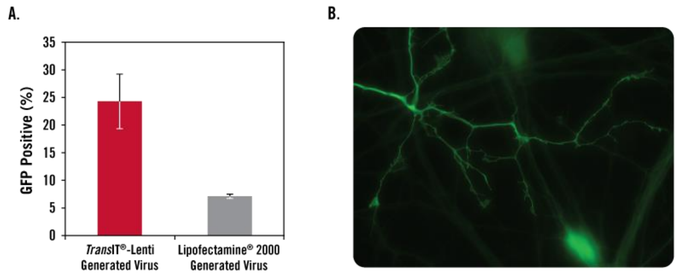


Figure 6. Lentivirus was produced using *Trans*IT®-Lenti Transfection Reagent (3:1, vol:wt) or Lipofectamine® 2000 using the MISSION® vectors (pLKO.1-puro-CMV-TurboGFP™ transfer vector and the Lentivirus Packaging Mix powered by MISSION®) in 293T/17 cells. The supernatant was harvested, filtered (0.45 µm), and frozen. Lentivirus transductions were performed 5 days post-plating with iCell® Motor Neurons (Cellular Dynamics International). For both *Trans*IT®-Lenti and Lipofectamine® 2000, one microliter of unconcentrated supernatant was added per well of a 96-well plate. GFP efficiency was measured 72 hours post-transduction using Guava® easyCyte™ 5HT Flow Cytometer. Error bars represent the SEM of duplicate wells. (B) iCell® Motor Neurons were plated in 35 mm dishes (Ibidi) and transduced with lentivirus produced using the *Trans*IT®-Lenti Transfection Reagent and MISSION® vectors. Images were captured at 72 hours post-transduction with a Zeiss Axiovert S100 inverted fluorescence microscope using a 63X objective under oil.

Conclusions

- Greater than two-fold differences in functional titers can be obtained based on transfection reagent formulation during recombinant lentivirus production
- Transfer vector selection can affect titers by up to four-fold
- Recombinant lentivirus system-to-system comparisons (transfection reagent and packaging/transfer vectors) reveal up to 100-fold differences in functional titers
- Higher titer lentivirus results in higher transduction efficiencies with unconcentrated virus in iPSC-derived motor neurons