Lenti-X™ Packaging Single Shots (96-well, VSV-G) Protocol-At-A-Glance

I. Introduction

This protocol is provided for high-titer lentivirus production with **Lenti-X Packaging Single Shots (96-well, VSV-G)**, a kit consisting of 4 x 96-well plates pre-aliquoted with lyophilized Xfect™ Transfection Reagent premixed with an optimized formulation of VSV-G-pseudotyped Lenti-X lentiviral packaging plasmids. Lenti-X Packaging Single Shots (96-well, VSV-G) simplify the process of high-throughput lentiviral vector production for applications such as arrayed screening or vector optimization.

The amount of reagent and packaging vectors in each well of the Lenti-X Packaging Single Shots 96-well Plate (VSV-G) has been carefully optimized such that high-titer lentivirus can be obtained by adding a fixed volume of transfer vector plasmid to each well, mixing together the respective components, and then applying the mixtures to packaging cells in a separate plate. Transfection of packaging cells can be carried out entirely in the presence of serum.

NOTE: Use of tetracycline-free FBS is critical for achieving high titers with this technology.

II. General Considerations

A. Storage and Handling

- Store each plate in its supplied foil pouch with desiccant sachet at –20°C.
- Return any unused plate to its supplied foil pouch with desiccant sachet, and store at –20°C.

B. Mock Transfections

Use a plasmid that does not contain your gene of interest. You should include a source of nucleic acids to assemble with the Xfect polymer.

III. Transfection and Virus Production Protocol

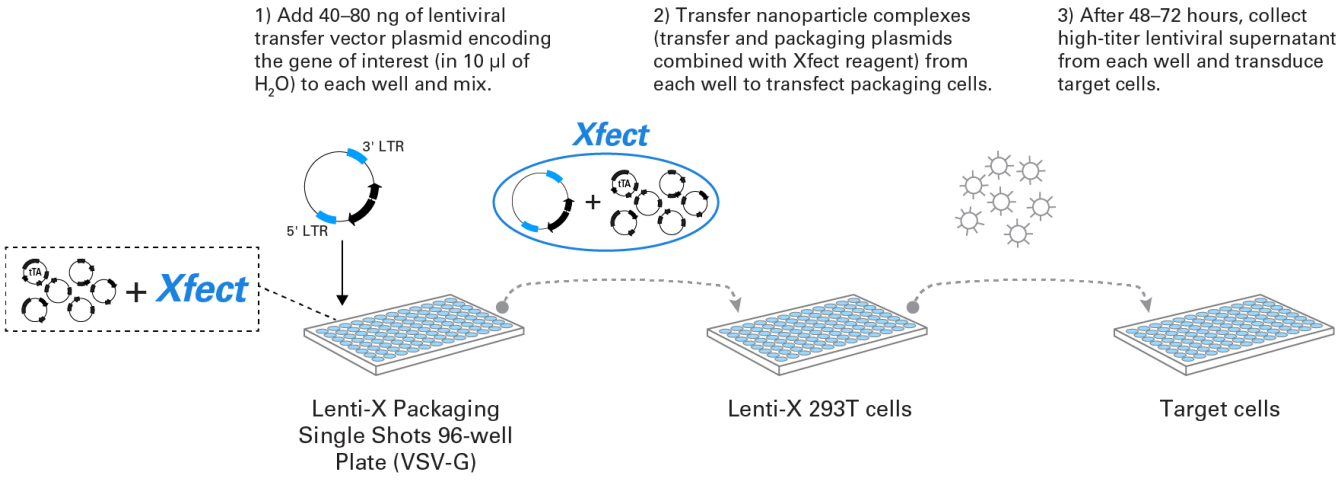

Figure 1. The Lenti-X Packaging Single Shots 96-well Plate (VSV-G) protocol.

IMPORTANT: All of the following steps should be performed in a sterile tissue culture hood. Lentivirus requires the use of a Biosafety Level 2 facility. Pseudotyped lentiviruses packaged from HIV-1-based vectors are capable of infecting human cells. Know and use appropriate safety precautions.

- Transfections are performed using **collagen-coated 96-well tissue culture plates**. Tetracycline-free FBS should be used in both the transfection medium and the medium used to collect the virus.
- One day prior to transfection, plate cells in complete growth medium to allow for ~60% confluence at the time of transfection.

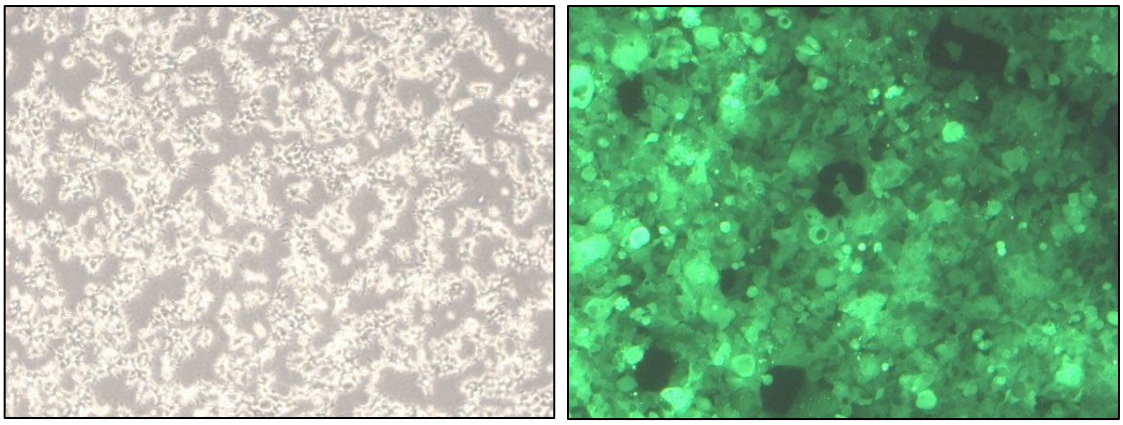

Figure 2. Optimal density of Lenti-X 293T cells at the point of transfection (left panel) and harvest (right panel), shown here using a transfer vector encoding ZsGreen1.

NOTE: To achieve the highest titers, it is critical to pay close attention to the transfection. You may want to perform a cotransfection with a lentiviral vector that contains a fluorescent protein. You should be able to achieve transfection efficiencies of greater than 90%.

1. Approximately 24 hr before transfection, seed 3 x 10^4 Lenti-X 293T cells/well in 120 µl of growth medium. Make sure that the cells are plated evenly. Incubate at 37°C, 5% CO_2 overnight. Continue to incubate the cells until you are ready to add the transfection mixture in Step 8. The cells should be ~60% confluent at the time of transfection.
2. For each vector, dilute 40–80 ng of your lentiviral vector plasmid DNA with sterile water to a final volume of 10 µl. Mix thoroughly.
3. The Lenti-X Packaging Single Shots 96-well Plate (VSV-G) has a pierceable/peelable foil. For peeling, gently and slowly peel the seal. For piercing, disinfect the foil with 70% ethanol, allowing it to dry before piercing.
4. Add 10 µl of diluted DNA to each well of the plate.
5. Tap the plate gently to collect the diluted DNA at the bottom of each well. The liquid will dissolve the cake on contact. Do not mix or vortex.

NOTE: Always dilute your DNA in water prior to adding it to a Lenti-X Packaging Single Shots 96-well Plate. (Undiluted DNA should not be mixed with the transfection reagent).

6. Incubate the samples for 10 min at room temperature to allow nanoparticle complexes to form.
7. After the 10-min incubation, ensure that each cake has been completely dissolved.

8. Mix the solution by gently pipetting up and down, then transfer all 10 µl of each transfection complex solution dropwise to the cultured Lenti-X 293T cells.

   NOTE: It is normal for the medium to change color slightly upon addition of the nanoparticle complex solution.

9. Incubate the cells at 37°C, 5% CO_2.

10. After 16 hr, add an additional 80 µl of fresh complete growth medium to each well and incubate at 37°C, 5% CO_2 for an additional 24–48 hr. Virus titers will generally be highest 48 hr after the start of transfection.

11. Harvest the lentiviral supernatants and pool similar stocks, if desired (a 48-hr sample may be stored at 4°C until a 72-hr sample is harvested).

    CAUTION: Supernatants contain infectious lentivirus.

12. Verify virus production using Lenti-X GoStix™ Plus (for details, see the Lenti-X GoStix Plus Protocol-At-A-Glance).

13. Use the virus to transduce target cells, or store at –80°C.

    NOTE: Titers can drop as much as 2- to 4-fold with each freeze-thaw cycle.

14. For protocols describing how to transduce your target cells or create frozen stocks, see the Lenti-X Lentiviral Expression Systems User Manual.

IV. Expected Results

Typical results are shown below in Figure 3.

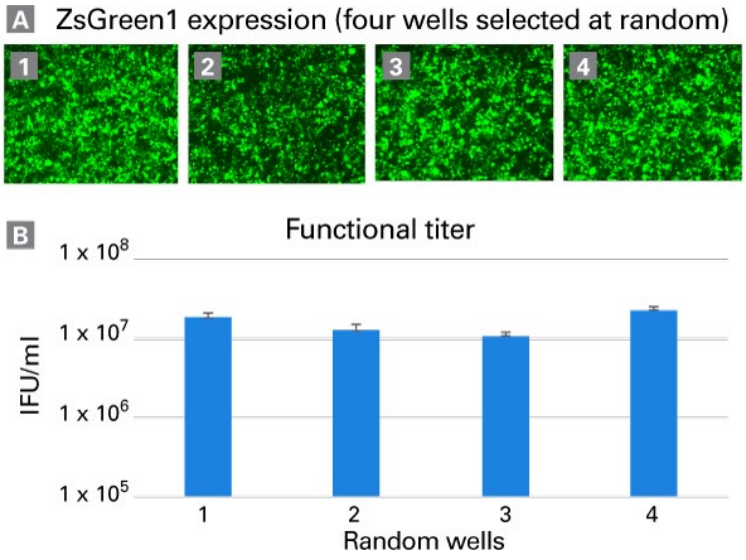

Figure 3. Transfection efficiency and infectious titer using the Lenti-X Packaging Single Shots 96-well Plate (VSV-G). Random wells of the Lenti-X Packaging Single Shots 96-well Plate (VSV-G) were used to produce lentivirus encoding the fluorescent protein ZsGreen1. **Panel A.** High transfection efficiencies were observed for each of the selected wells via fluorescence microscopy**.** Clarified supernatant was harvested at 48 hr, then serially diluted and titrated using Lenti-X 293T cells in the presence of 6 µg/ml polybrene. Transduced cells were analyzed by flow cytometry 72 hr post-transduction. **Panel B.** Titers were calculated using culture volumes that resulted in cell populations transduced at <10% efficiency to ensure one copy per cell. The resulting titers for each well were ~1 x 10^7 IFU/ml.

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