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Packaging Lentiviral Particles

Some cell lines are resistant to transfection. Lentiviral particles offer an alternative delivery method. Transomic lentiviral vectors for CRISPR, shRNA, and LentiORF select-vectors™ can be packaged into lentiviral particles for efficient delivery into target cell lines. Constitutive vectors may be packaged with 2nd, 3rd, or 4th generation packaging plasmids. Inducible vectors must be packaged with 2nd or 4th generation packaging plasmids as they are Tat-dependent.

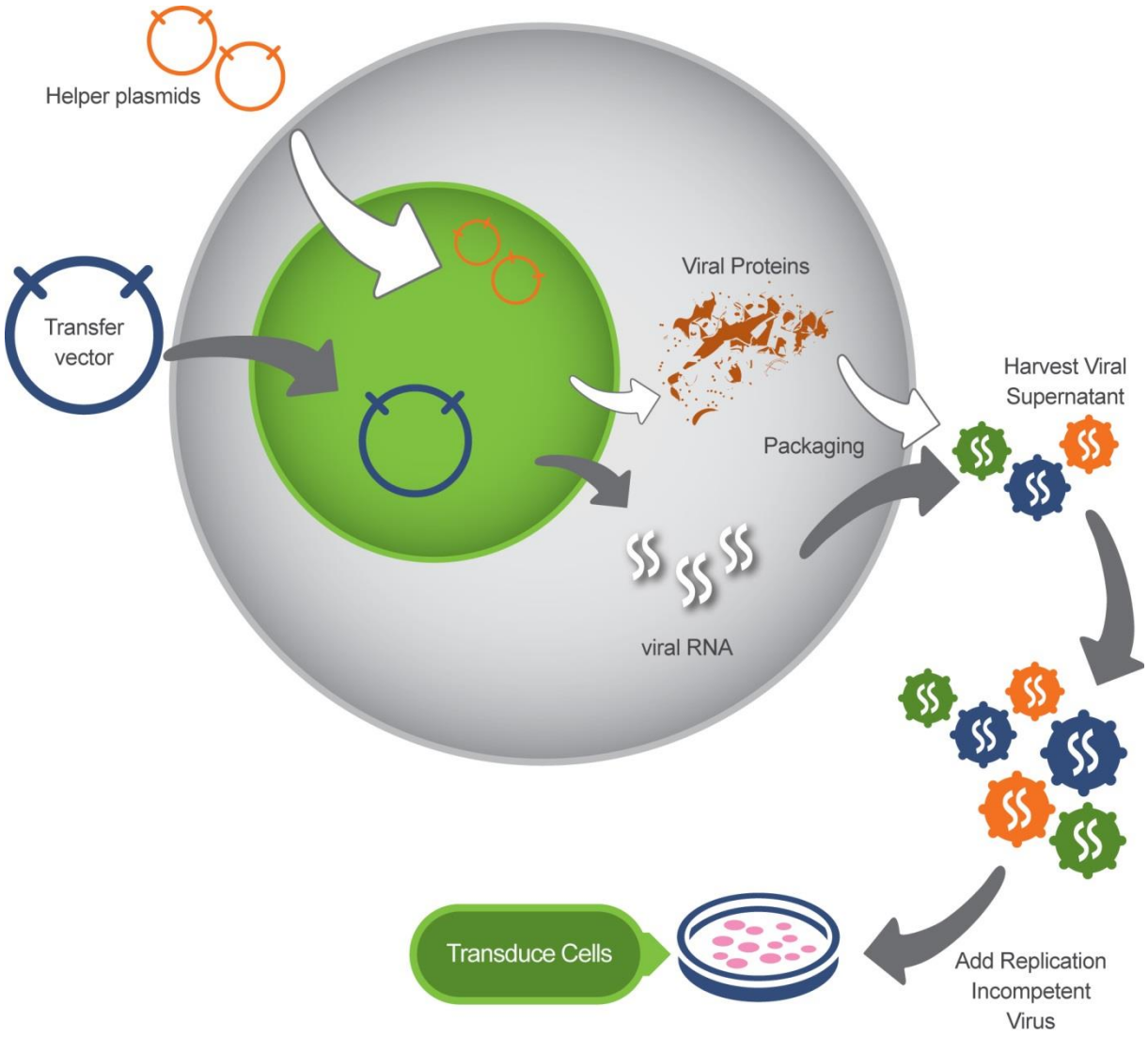

Figure 1: Schematic depicting lentiviral packaging of lentiviral vectors

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When packaging lentivirus, the genetic elements required for assembly of replication incompetent viral particles are transfected into the cell in trans. The lentiviral transfer vector is co-transfected with the desired packaging vectors (helper plasmids) encoding the *env, gag* and *pol* protein into a packaging cell line. *gag*, *pol* and *env* provide the proteins necessary for viral assembly and maturation. The transfer vector contains sequences that will be packaged as the viral genome and code for the ORF and selection cassette that will integrate into the targeted cell's genome. Viral particles are released from the packaging cell and can be harvested from the supernatant of the packaging cell. The resulting viral supernatant can be concentrated or applied directly to the targeted cell line. A filter can be used to remove cellular debris from the generated virus, but the filter should not be nitrocellulose. Nitrocellulose binds proteins present in the membrane of lentivirus and destroys the viral particles.

Materials

- pCMV-dR8.2 Packaging Plasmid (Addgene, Plasmid 8455) (2nd generation)
- pCMV-VSVG Envelope Plasmid (Addgene, Plasmid 8454)
- 6-well tissue culture plate
- HEK293T cells
- Complete cell culture medium – (DMEM supplemented with 10% fetal calf serum, 1X L-Glutamine, and 1X Pen-Strep)
- Antibiotic-free complete medium – (DMEM supplemented with 10% fetal calf serum, 1X L-Glutamine)
- Transfection Reagent (examples: Lipofectamine®, Fugene®)
- OPTI-MEM® I + GlutaMAX Reduced Serum Media (Gibco, Catalog # 51985-034)
- Sterile 1.5 ml microfuge tubes

Equipment

- Automatic pipette/Pipette-aid (for tissue culture)
- Disposable or autoclaved tissue culture pipettes
- CO_2 cell culture incubator at 37°C

Protocol for 6-well tissue culture plate

1. Plate the target cells and HEK293T cells 18-24 hours prior to transduction in a 6-well tissue culture plate. Plate at a density of 800,000 to 1,000,000 cells per well in 2 ml complete cell culture medium. It is important to seed enough cells so that the cell confluency ranges between 70 and 80% at the time of transfection.
2. Incubate overnight with 5% CO_2 at 37°C.
3. Two hours prior to transfection, remove the culture media and replace with 2 ml fresh, antibiotic-free culture medium.
4. Preparation of plasmids and lentiviral vector packaging mix for transfection (note, all plasmids are re-suspended in dH_2O):
    a. Transfer vector – dilute plasmid to 0.2 μg/μl
    b. Lentiviral packaging mix (0.5 μg/μl):

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   i. 100 µl pCMV-dR8.2 (0.5 µg/µl)
   ii. 50 µl pCMV-VSVG (0.5 µg/µl)
5. Just prior to transfection, allow transfection reagent and OPTI-MEM® I to come to room temperature.
6. Plasmid DNA preparation:
   a. Add 5 µl of transfer vector (1.0 µg) and 3 µl lentiviral packaging mix (1.5 µg) in a sterile microfuge tube containing OPTI-MEM© I Reduced Serum Media to a total volume of 100 µl.
7. Transfection reagent preparation: In a separate microfuge tube, add 5 µl of transfection reagent into 95.0 µl OPTI-MEM© I Reduced Serum Media for a total volume of 100 µl.
8. Final transfection complex: Transfer the diluted DNA solution to the diluted transfection reagent (total volume = 200 µl. Mix gently and incubate at room temperature for 10 minutes.
9. Add the 200 µl of transfection complex to each well containing HEK293T cells and medium.
10. Incubate cells at 37°C in a CO_2 incubator.
11. Collect viral particles (supernatant) 48-60 hours post-transfection.
12. Clarify supernatant by low-speed centrifugation (800xg) for 10 minutes using a tabletop centrifuge.

Aliquot supernatant into sterile cryovials and store at -80°C. *Note: 50 µl aliquots will be used in the functional titering protocol. They should be stored at -80°C overnight prior to titering to reflect any loss of function due to freeze/thaw cycle that will occur for the transduction aliquots. Freshly harvested viral particles from well-transfected cells should have a titer of approximately 1-5 x 10^6 TU/ml when measured on NIH-3T3 or HEK293T cells.*

Protocol for 10 cm tissue culture plate (recommended for All-in-One CRISPR vectors)

1. Plate the target cells and HEK293T cells 18-24 hours prior to transduction in a 10 cm tissue culture plate. Plate at a density of 5-6 x 10^6 cells in a total volume of 12 ml complete culture medium. It is important to seed enough cells so that the cell confluency ranges between 70 and 80% at the time of transfection.
2. Incubate overnight with 5% CO_2 at 37°C.
3. Two hours prior to transfection, remove the culture media and replace with 10 ml fresh, antibiotic-free culture medium.
4. Preparation of plasmids and lentiviral vector packaging mix for transfection (note, all plasmids are re-suspended in dH_2O):
   a. Transfer vector – dilute plasmid to 0.2 µg/µl
   b. Lentiviral packaging mix (0.5 µg/µl):
      i. 100 µl pCMV-dR8.2 (0.5 µg/µl)
      ii. 50 µl pCMV-VSVG (0.5 µg/µl)
5. Just prior to transfection, allow transfection reagent and OPTI-MEM® I to come to room temperature.
6. Plasmid DNA preparation:

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a. Add 30 µl of transfer vector (6.0 µg) and 18 µl lentiviral packaging mix (9 µg) in a sterile microfuge tube containing OPTI-MEM© I Reduced Serum Media to a total volume of 600 µl. Mix immediately and incubate an additional 15 min at room temperature.

7. Transfection reagent preparation: In a separate microfuge tube, add 30 µl of transfection reagent into 570 µl OPTI-MEM© I Reduced Serum Media for a total volume of 600 µl.
8. Final transfection complex: Transfer the diluted DNA solution to the diluted transfection reagent (total volume = 1200 µl. Mix gently and incubate at room temperature for 10 minutes.
9. Add the 1200 µl of transfection complex to each well containing HEK293T cells and medium.
10. Incubate cells at 37°C in a CO_2 incubator.
11. Collect viral particles (supernatant) 48-60 hours post-transfection.
12. Clarify supernatant by low-speed centrifugation (800xg) for 10 minutes using a tabletop centrifuge.
13. Aliquot supernatant into sterile cryovials and store at -80°C.

Aliquot supernatant into sterile cryovials and store at -80°C. *Note: 50 µl aliquots will be used in the functional titering protocol. They should be stored at -80 ℃ overnight prior to titering to reflect any loss of function due to freeze/thaw cycle that will occur for the transduction aliquots. Freshly harvested viral particles from well-transfected cells should have a titer of approximately 1-5 x 10^6 TU/ml when measured on NIH-3T3 or HEK293T cells.*