Lazzara Lab
Evan K. Day
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VSVG-Pseudotype Retrovirus Preparation

Day 1: Plate 293T, 293FT, or LentiX 293T cells (referred to generally in this protocol as 293T)
Plate 2×10^6 rapidly growing 293T cells on a 10 cm dish. [*It's extremely important to make sure your cells are growing quickly. Passaging them twice before using them to make virus may be helpful. Split them when they are 95-100% confluent.*]

Day 2: Transfect 293T cells
Check that cells are approximately 50% confluent. Change media 1-4 hr before transfection.

In a flow tube or DNAse-free microfuge tube, mix in order:

5 µg retroviral vector
4.5 µg pUMVC
0.5 µg pCMV-VSVG
Sterile water to 352 µL
48 µL of 2M $CaCl_2$.

Add the 400 µL total contents above to a tube of 400 µL 2X HBS by putting the pipet tip at the bottom of the HBS tube and slowly ejecting DNA mix while bringing the tip to the top of the fluid. Mix the 800 µL mixture by gently blowing bubbles from the bottom of the tube, repeating 2-3X. Let the tube stand at room temperature for 12 min (fine precipitate forms). Gently pipette up and down, and add dropwise to 293T cells.

Day 3: Change media on 293T cells and plate target cells
In the morning, change the media on the 293T cells. [For 293FT and LentiX 293T cells, make sure to use media without geneticin because this media will go directly on target cells. Geneticin is not used in media for 293T cells.]

NOTE: You may notice a more contracted morphology in the transfected 293T cells than in non-transfected counterparts, as well as the presence of small black DNA precipitate particles in the open spaces of the plate (Fig. 1). While the altered cell morphology may be a sign of successful transfection, in some cases the transfection process may be quite toxic, resulting in death of packaging cells before the end of virus collection. Decreasing the amount of transfected DNA may help improve packaging cell viability.

Plate 1×10^6 target cells in 10 cm dish per transfection. [Alternatively, to use less of the viral supernatant to be generated in subsequent steps, plate 50,000 to 100,000 cells/well in 6-well dishes.]

Day 4: First infection
48 hr post transfection remove media from 293T cells, filter through a 0.45 µm filter. Add to target cells (with polybrene 8 µg/mL). [If working with target cells in 6-wells, add 1-4 mL of filtered virus per well. Extra viral supernatant can be frozen at -80ºC for use later.] Add fresh media to 293T/FT cells. If you notice that target cells respond unfavorably to infection, switch them to fresh media 4 hr after infection. Otherwise, leave viral supernatant on target cells overnight.

Day 5: Second infection
72 hr post transfection remove media from 293T cells, and filter through 0.45 µm filter. Add to cells (with polybrene). Change media after 4 hr if signs of virus-induced toxicity observed. Otherwise, leave overnight.

Day 6 and beyond
Change media on target cells every 24 hr for 2 days to keep them happy. Pass them if they are too crowded, and begin selection. To determine the lowest concentration of selection agent that kills cells, test a range of concentrations on parental cells in a 6-well plate. Typical concentrations: puromycin = 1-3 µg/mL, geneticin = 100-500 µg/mL, hygromycin = 100-500 µg/mL, blasticidin = 2-10 µg/mL.

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293FT medium (500 mL):
425 mL DMEM (high glucose, 4.5 g/L)
50 mL FBS
5 mL L-glutamine (200 mM) [supplements L-gluatamine in Gibco DMEM base]
5 mL Pen/Strep (100X)
5 mL MEM-Non-Essential Amino Acids (MEM-NEAA, 10 mM)
5 mL Na Pyruvate (100 mM)
5 mL geneticin/G418 (50 mg/mL)
** Remember to make media WITHOUT geneticin as well. [We generally just keep the cells in media without geneticin unless we are culturing them to freeze and not just to make virus.]

Lenti-X 293T cells (Clonetech) medium (500 mL):
435 mL DMEM (high glucose, 4.5 g/L)
50 mL FBS
5 mL L-glutamine (200 mM) [supplements L-gluatamine in Gibco DMEM base]
5 mL Pen/Strep (100X)
5 mL Na Pyruvate (100 mM)

2X HEPES Buffered Saline (HBS):
280 mM NaCl
50 mM HEPES
1.5 mM Na_2HPO_4
Adjust the final pH to precisely 7.05; Sterile filter before making 1.5 mL aliquots to keep at -20°C.

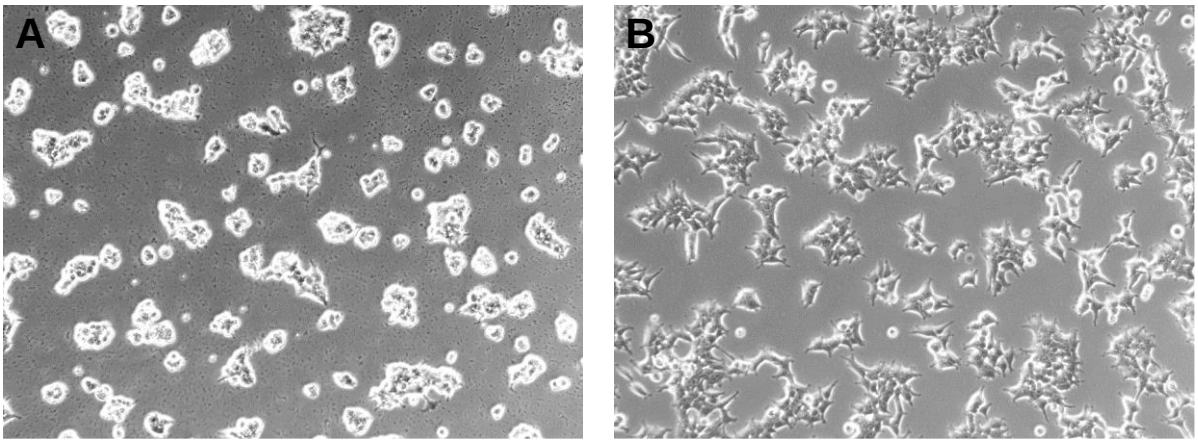

Fig. 1. A. 293T cells one day after transfection with DNA (10X phase contrast image). Cells are more contracted and less well spread than non-transfected cells, and small black particles ($CaHPO_4$/DNA precipitates) can be seen scattered across the plate in the spaces between cell clusters. **B.** Non-transfected 293T cells.