

Retroviral/Lentiviral Transduction Protocol – Adapted from the Stewart Lab

Day 1

1. Plate 1.5×10^6 293T cells in a p60 containing 5ml media

Day 2

Retroviral Transduction

In polypropylene tubes (polystyrene tubes don't work!) add the following:

- 1) 1 μ g retroviral plasmid containing your gene of interest
- 2) The packaging plasmid (pUMVC3) at an 8:1 ratio with the envelope plasmid (pCMV-VSV-G) for a total of 1 μ g
- 3) DME without serum to 94 μ l total
- 4) 6 μ l fugene transfection reagent

Notes:

-Add DME first, followed by the plasmids.

-Add fugene last, directly to the solution (not the sides of the tube).

- 5) Mix by pipetting up and down, and incubate for 20-30 minutes at room temperature.
- 6) Add directly to the 293T cells media. Be careful not to touch the sides of the dish.
- 7) Leave overnight.

Day 2

Lentiviral Transduction

Also in polypropylene tubes. Protocol is the same as with retroviral transduction except the packaging plasmids differ.

- a) 1 μ g lentiviral plasmid containing your gene of interest
- b) The packaging plasmid (pHR'8.2 Δ R) at an 8:1 ratio with the envelope plasmid (pCMV-VSV-G) for a total of 1 μ g
- c) DME without serum to 94 μ l total
- d) 6 μ l fugene transfection reagent

Notes:

-Add DME first, followed by the plasmids.

-Add fugene last, directly to the solution (not the sides of the tube).

- e) Mix by pipetting up and down, and incubate for 20-30 minutes at room temperature.
- f) Add directly to the 293T cells media. Be careful not to touch the sides of the dish.

Leave overnight.

Day 3

- 1) Change the media on the 239T cells to 5-6ml of whatever media you want to use when infecting the target cells. Be careful to not cause detachment of the 293T cells when adding the media – run it down the side of the dish.
- 2) Plate target cells at 0.8×10^6 per plate, in p60 plates. For each condition make an extra plate of target cells to be used as a “canary” during drug selection.

Day 4 – collect the virus.

It is useful to place a beaker containing bleach in the hood to collect the used needles, and an autoclavable bag by the side of the hood to collect anything else (pipets etc.) that comes in contact with the virus infected medium.

- 1) Collect medium from the 293T cells using a 10ml syringe.
- 2) After collecting the medium in the syringe, remove the needle and replace with a 0.45 μ m filter.
- 3) Filter the 293T medium into a 15 ml tube
- 4) Aspirate the media from your target cells (plated the day before), and add 3ml infecting media to the cells. (As little as 1ml has been effectively used by other labs to infect the target cells, but I have never tried this).
- 5) Add polybrene (hexadimethrine bromide) or protamine sulfate directly to the media on the target cells, for a final concentration of 8-10 μ g/ml.
- 6) Infect for 4 hours in the incubator.
- 7) Replace the virus-containing media with fresh media.

Day 5

Repeat the infection.

Day 6+

-Allow cells to recover and start expressing the virus (24-48 hrs). Check GFP expression in the target cells to see infection efficiency.

-Add selection drug. When the canaries have died then it's safe to assume that selection is complete. With BJ cells and MEFs I have used the following concentrations:

- Puromycin: 1-2 $\mu\text{g}/\text{ml}$ (takes 1-3 days)
- Neomycin: 1mg/ml (takes 4-5 days)