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Lentiviral Infection of Endothelial Cells (HDMEC)

Overall Schedule for Transfection and Infection:

Day 1: Seed 293T cells in 100 mm dish (400,000 cells/dish)

Day 2 (am): $CaPO_4$ Transfection of 293T cells with packaging constructs + lentiviral construct
Day 2 (pm): Change media to remove chloroquine

Day 3: Seed endothelial cells for infection

Day 4 (am): 1st infection of cells with virus + Replace DMEM of 293T cells to produce new virus for 2nd infection round

Day 4 (noon): 2nd infection of cells with virus ~ 4-5 hours later
Discard 293T cells after 2nd infection (or save it for Western Blot analysis)

Day 4 (pm): change media of infected cells to remove sequabrene ~
4-5 hours later

Day 5: Start to monitor GFP expression and split cells with necessary

- Transfection of 293T cells for lentiviral packaging:

293T cells do NOT attach well to the plate. Thus, make sure you add new media to the plates to the side of the plate, carefully!!!

All solutions must be at 37°C for an efficient transfection

293T cells should be at 50-60% confluent at the day of transfection

Just prior to transfection (some authors recommend 3-4 hours before) change media of the 293T cells. The fresh media should contain 25 µM Chloroquine (Sigma). Return the plate to the incubator as soon as possible (to keep everything at 37°C). Chloroquine should not be frozen-thawed. Prepare stock solution 100 mM (2.5 (µl in 10 ml).

Solutions for CaP04 transfection

Solution A: DNA $CaCl_2$ solution

4 µg DNA construct (and GFP as your positive control)
4 µg each viral packaging vector (p59, p60, p61)
62.5 µl of 2M $CaCl_2$ (stock)
Mq Water up to 500 µl total volume

Solution B: 2X HBS solution - 500 µl for each plate

Mix A and B dropwise with mixing. Use a 1 ml pipette to blow bubbles through solution B. Incubate the mixed solution (A+B) for 15 min RT. A fine white precipitate should form. The

process of mixing solutions must be very slow, 1-2 minutes. Add the 1 ml solution to the related the 293T plate.

Incubate ~ 8-12 hours at 37°C. Change media (10 ml DMEM/10% FBS) to remove chloroquine

- Infection of endothelial cells with lentivirus:

1) Plate HDMEC the day prior to infection

2) Infection: Collect the supernant from transfected 293T cells. Replace 293T cell dishes with fresh DMEM.

3) Centrifuge the viral supernant for 10 minutes at 3,000 rpm at 4°C, and collect supernatant into a new Falcon tube..

4) Add 8 µg/ml sequabrene (Sigma) to the viral supernatant.

5) Add the centrifuged viral supernant containing sequabrene dropwise to the cells to be infected. Dilute the supernant so each plate is infected with approximately:

6 well plate ~ 1 ml/well
6 cm^2 plate ~ 2 ml each
10 cm^2 plate ~ 3 ml each

6) 4-5 hours later repeat the infection procedure above

7) 4-5 hours later after the second infection replace with fresh media to remove sequabrene

8) Start monitoring GFP expression 24-48 hours later