Lazzara Lab
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Retrovirus preparation using Phoenix cells

Notes:

- Phoenix cells do not adhere well. So, be really careful when changing media, etc. not to dislodge them.
- Only use cells to passage 5.
- Cells are grown and trypsinized as normal.
- Filter all supernatants used: Phoenix cells grow very fast and will outcompete all others.

Materials:

- 10 cm dishes, filters, FACS tubes, and syringes
- Phoenix (often labeled "ΦNX" in cell bank) cells and target cells
- DMEM +10% FBS +Pen/Strep
- 50 mM chloroquine (1000x) [Sigma C6628]
- 8 mg/mL polybrene/hexadimethrine bromide (1000x) [Sigma H9268]
- 2M $CaCl_2$
- 2x HBS (see recipe at end of protocol)
- DNA (retroviral vector)

Procedure:

Day 1: Phoenix cell preparation

1. Plate cells at 2.5×10^6 cells in 8 mL DMEM in a 10 cm dish (one for each transfection to be done). System works only on dividing cells so plate them at subconfluence for next day.

Day 2: Phoenix cell transfection & target cell preparation

Part 1: Phoenix cell transfection

1. Feed Phoenix cells with DMEM supplemented with chloroquine (diluted from 1000x stock to 1x) 30 min before transfection.
2. In a FACS tube in the hood, add the following:
   15 µg DNA
   40 µL 2M $CaCl_2$
   MilliQ water to final volume of 500 µL
3. Add contents from step #2 to a FACS tube containing sterile filtered 500ul 2x HBS at room temperature.
   a. Add DNA mix slowly starting at tube bottom and moving up while expelling.
   b. Blow some bubbles with the pipette at the bottom of the liquid.
4. Incubate the tube 15 min at room temperature in the hood.
5. Add solution from step #3 drop-wise to a 10 cm dish of Phoenix cells and return to incubator.
6. Feed all transfected plates with 10 mL DMEM after 3-5 hr to remove chloroquine.

Part 2: Target cell preparation

1. Plate 1×10^6 target cells per 10 cm dish per virus to be used for infection. This retroviral system works only on dividing cells. So, plate them at subconfluence for the next day. Also, make a plate (for each target cell type) that will not be infected to use as a comparison ("kill control") when selecting.

Day 3: Target cell infection -- *Be really careful with the virus! Clean things with bleach!*

1. Remove supernatant from Phoenix cells and filter through a 0.45 µm filter syringe.
2. Infect target plates twice: in morning and afternoon at 18 and 24 hr post transfection. Infect cells with undiluted 5 mL supernatant supplemented with polybrene (diluted from 1000x stock to 1x). Remove old supernatants before addition of fresh.
3. Return plates after each infection with viral supernatant to incubator.

Day 4: Last infection

1. Repeat steps from Day 3 once in the morning.

2. Change media after 4-6 hr, either into fresh DMEM or the preferred medium for the target cell.
3. Any confluent plates should be split late in the day. Do not allow target cells to reach confluence before selection added.

Days 5/6: Selection

1. Add selection agent to plates 1 day after each completing infection.
2. Split cells as needed over selection time span to ensure subconfluence is maintained.

Alternatively you can follow these steps for Day 3 and beyond:

Day 3: Target cell infection

Be really careful with the virus! Clean things with bleach!

1. Remove supernatant from Phoenix cells and filter through a 0.45 µm filter syringe.
2. Infect target plates in the afternoon at ~36 hr post transfection. Infect cells with undiluted 5 mL supernatant supplemented with polybrene (diluted from 1000x stock to 1x). Remove old supernatants before addition of fresh.
3. Return plates after infection with viral supernatant to incubator.

Day 4: More infections

1. Repeat Day 3 steps in the morning and in the afternoon.

Day 6 on: Selection

1. Change media in the morning, either into fresh DMEM or the preferred medium for the target cell.
2. Any confluent plates should be split. Do not allow target cells to reach confluence before selection added.
3. Split cells as needed over selection time span to ensure subconfluence.

Notes on selection agents

To decide on the correct concentration of drug to select cells, you can test a range of concentrations on parental cells in a 6-well plate and determine the lowest concentration that kills cells. Typical concentrations: puromycin = 1-3 µg/mL, geneticin = 100-500 µg/mL, hygromycin = 100-500 µg/mL, blasticidin = 2-10 µg/mL.

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.