Classical transduction and antibiotic selection

Note 1.
Polybrene is a small, positively charged molecule that binds to cell surfaces, neutralizes surface charge, increases binding between pseudoviral capsid and the cellular membrane. Polybrene has been proved to greatly enhance transduction efficiency. Some cells, like primary neurons, are sensitive to polybrene. Do not add polybrene to these types of cells. If working with a cell type for the first time, a non-transduced control well with polybrene should be used to determine cell sensitivity.

Note 2.
Before beginning, determine the optimal dose of selective reagent for your target cell line. To do this, treat target cells with a range of doses of antibiotic and determine the lowest dose that kills all of the cells.

Note 3.
Prepare a range of dilutions of the lentivirus in complete medium + 8 µg/ml polybrene. Please see the MOI determination protocol for a sample of possible dilutions in most common cell lines. You may want to try higher/lower dilutions, depending on your downstream applications.

Day 1:

The protocol describes volumes in a 6-well plate, scale volume up or down according to culture dish format.

Seed cells to the plates. As a gauge, we seed 150.000 mouse embyonic fibroblasts (MEFs) on a single well of a 6-well plate in 2 ml complete medium.

Day 2:

Prepare a range of dilutions of the lentivirus in complete medium + 8 µg/ml polybrene.

Drain the media from the wells and replenish with 0.5 ml virusparticles + 1.5 ml media per well and 8 µg/ml polybrene (use stock 8 mg/ml at 1:1000).

Incubate for approximately 7 h (after 10 h polybrene is cytotoxic for some cells). Remove the virus-containing medium and feed cells with 2 ml fresh complete medium to allow cells to recover overnight.

Day 3:

In the morning, split cells and seed e.g. 1:3, 1:30 and 1:300 in growth medium. EGFP expression usually peaks around 24 - 48 h after transduction. If you aim to perform antibiotic selection, add antibiotics around 24 h post-infection (in the evening of day 3). Include a dish of uninfected cells as a control for antibiotic killing.

Change medium every 2 or 3 days until the selection is complete.

Note 1. For amphotropic infections, test the target cell culture supernatants for virus production (they should score negative) by reverse transcriptase (RT) assay. Contact us at fugu-support@helsinki.fi for information on RT assay services.
A service for exclusion of replication competent virus (RCV) to confirm the biosafety of a cell line is available through BVC Core Unit (contact: fugu-support@helsinki.fi).

Note 2. You aim would be to use minimal antibiotic concentration that kills all uninfected cells. If no data available for your cells, establish a kill curve.