Viral transduction of cells using 'spinfection'

1. Background and purpose of the procedure

Lentiviral transduction is an effective method for creating a stable cell line with a DNA cassette of interest integrated into its genomic DNA. Transducing cells while centrifuging ('spinfection' or 'spinoculation'), is a method to achieve efficient transduction of a large number of cells. Cells are typically infected in the presence of polybrene, a polycationic chain that neutralizes the charge repulsion between the virus and cell target surface and helps viral integration into the cell.

2. Materials and equipment

- Single cell suspension
- Complete cell culture medium with FBS and antibiotics
- D-PBS, without Ca^{++} and Mg^{++} (Gibco, 14190144)
- Lentivirus for the expression of gene of interest
- Polybrene transfection reagent (Millipore-Sigma, TR-1003-G)

- 6-well plate
- Cell counter/Hemacytometer
- Swinging bucket centrifuge pre-warmed at 33˚C

3. Method

a. Pre-warm the centrifuge at 33˚C by spinning the empty rotor at 1000 g for 30-60 min
b. Plate cells at a density of 300,000-500,000 cells per well in a 6-well plate, it is important to have them as a single cell suspension *[1 to 1.5 mL maximum per well]*
c. Thaw virus at room temperature or use fresh virus conserved at 4˚C
d. Add 3.33 µg/mL of polybrene *[Do a 1:1 dilution of stock polybrene (10 mg/mL) in culture media and add 1 µL to a final volume of 1.5 mL or 1.33 µL to a final volume of 2 mL]*
e. Parafilm the edges of plate and spin at 800 g for 90 min at 33˚C
f. Change the media by washing cells three times with 1 mL of D-PBS
g. Add fresh complete cell culture media
h. Incubate for 24 hrs in TC incubator at 37˚C / 5% CO_2
i. If transduction is difficult, repeat steps c through g
j. Assess transduction efficacy 72 hrs after the last infection cycle using flow cytometry or by adding selection antibiotics

4. References

Berggren WT, Lutz M, Modesto V. General Spinfection Protocol. 2012 Dec 10. In: StemBook [Internet]. Cambridge (MA): Harvard Stem Cell Institute; 2008-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK133260/ doi: 10.3824/stembook.1.85.1

Optimization of Lentiviral Transduction Using Spinfection, Broad Institute

Transduction using spinfection protocol from Nathanson Lab, UCLA David Geffen School of Medicine

5. Revision history

Revision #	Date	Prepared by
1.0	**2021-10-04**	**Elie Besserer-Offroy**
Summary of modifications		
Initial version of the protocol		