Lentiviral infection of iAEC2s (human induced pluripotent stem cell-derived alveolar epithelial type 2 cells; e.g. alveolospheres) in single cell suspension

Kotton Laboratory

From Kotton Lab alveolosphere protocol (see alveolosphere protocol for all reagents and recipe details):

Alveolosphere dissociation and passaging method (Single cell)
1) Aspirate media from matrigel drop.
2) Add 1 ml dispase (2mg/ml), leave at 37C for 1 hour, pipetting up and down once after ~30 mins.
3) Transfer 1ml dissociated organoids in dispase into a 15ml conical, add 10ml DMEM to wash.
4) Centrifuge 200g x 4min, aspirate supernatant, repeat once more
   a) If a clear haze is seen above the pellet, the dispase has not totally dissolved and more dispase can be added to the pellet for another 20 minutes at 37C.
5) Aspirate supernatant. Further Aspirate any leftover supernatant with a P200 tip, leaving as little supernatant as possible.
   a) It is important to remove all dispase since it will dissolve matrigel in culture as well.
6) Resuspend cells in 1ml trypsin/ well, incubate at 37C for 10-12 minutes, until pipetting only 3-5 times results in single cell suspension.
   a) Dissociate cells in a tissue culture plate so that you can visualize them in a microscope. Cells are sensitive to over-pipetting at this stage, so leave them in trypsin long enough for most of the dissociation to be enzymatic. If alveolospheres have not dissociated into single cells by 12 minutes, spin down, add fresh trypsin, and leave another 5 minutes.
7) While spinning, prepare infection media:
   a) CK+DCI + 10 µM Y-27632 + 5mcg/mL polybrene
8) Stop with FBS-containing media, spin at 300g x 5min, wash once with 10ml DMEM.
   a) Resuspend cells in FACS buffer (PBS + 1% FBS + 10 µM Y-27632)
   b) Measure cell number and transfer appropriate volume to 1.5 mL Eppendorf tube
   c) Spin down at 300g for 5 minutes
   d) Resuspend cells in 100 – 200 ul of infection media (above).
9) Add appropriate volume of virus to resuspended cells and flick side of tube to mix.
   a) MOI of 10 – 20 yields efficient viral transduction.
10) Place Eppendorf in 37C incubator for 4 hours. Mix up cells by tapping the side of the tube at 2 hours after incubation has begun.
11) After incubation period add ~500 ul of empty media and spin down at 300g for 5 minutes.
12) Resuspend cells in 3D matrigel at desired concentration (250 – 500 cells/ml is reasonable).
   a) Place in 37C incubator for 15-20 minutes without adding media to allow the matrigel to solidify.
   b) Add 1ml CK+DCI+ 10 µM Y-27632 media over matrigel drop.

Example:
Aliquot 100,000 cells into 1.5 mL Eppendorf tube. Spin down at 300 g x 5 min. Resuspend in 150 ul infection media.
Calculate volume of virus needed to get MOI 20 (2,000,000 virions for 100,000 cells).
Add virus to resuspended cells. Flick tube to mix and place in incubator.

Virus: pHAGE-EF1αL-eGFP-W
Titer: 6.71x10^8 virions/mL

Volume of virus for MOI 20:

\[ 2x10^8 \text{ virions} \times \frac{1 \text{ mL}}{6.71x10^8 \text{ virions}} \times \frac{1000 \text{ ul}}{1 \text{ mL}} = 2.98 \text{ ul virus} \]
## VERSION HISTORY

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<tr>
<td>2018-12-17</td>
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