We will begin transduction with cell line 7026

**Material needed:**

1. Packaged virus
2. Polybrene (diluted 100µg/mL)
3. Stem flex (+) warmed to room temperature ~ 10 to 15min
4. Vitronectin coated plates (coating to final concentration of 1.0µg/cm2)

**Day 1:**

1. Split iPS cells into single cells in complete medium with RVC. (*500µl per well)*

2. Seed cells at 5.0X104 cells in glass-bottom 24-well plates

**7/12/2022**

Will try a higher density of 5.0x104 cells/well

*The density on next day should be around* ***60%.***

**Day 2:** Calculations are for 24-well plates. (If using a 6 well plate, then seed at 1x105 cells per well**, 850µL** of medium the next day with **150µL**)

Virus infection:

1. Remove the culture medium from cells, add (**300µL)** fresh medium without RVC into each well

2. Add **45µl** virus and 1µg/ml polybrene into cells and mix it. Incubate in 37 degree, 5% CO2.

|  |  |  |  |
| --- | --- | --- | --- |
| well 1  PLVX mCherry HLA-A2 | Well 2  PLVX IRES-Puro HLA-A2 | Well 3  pLoc Mlana | Control |

1. Add Polybrene into the infected well to make the final concentration to be 1ug/ml.
   1. Stock Polybrene (10mg/mL)
   2. Make a 100x dilution to make a working concentration of 100µg/mL
      1. (10mg/mL) (Vstock) = (100µg/mL)(0.5mL)

Vstock = 5µL of stock into 495µL of DPBS (-)(-)

(100µg/mL) (Vstock) = (1µg/mL)(0.3mL) 🡪 Vstock = **3µL** of working concentration per 300µL of medium added

**Day 3:**

1. Monitor the cells for the next 48 hours

**Day 4: or 48 hours later!**

1. Check IRES-GFP or HLA-A2’s expression at least **48 hours** after the transduction.
2. For antibiotic-based selection, the cells will be maintained to reform colonies and subsequently grown in medium with **1µg/mL** of Puromycin to initiate selection

Note:

1. Cells will need to be maintained in StemFlex medium to reform healthy iPSCs colony-like morphology (4-5 days)
2. Given that we don’t know what viral titer will be toxic to the cells during transduction we will have to optimize the transduction as we progress