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iPSC Transduction

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Protocol status: Working
We use this protocol and it's working

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Abstract

This is the iPSC transduction protocol.

Attachments

DOCX

Transduction Protoco...

38KB

Guidelines

Note:
Cells will need to be maintained in StemFlex medium to reform healthy iPSCs colony-like morphology (4-5 days)
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

Materials

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/cm^2)

Troubleshooting

Begin transduction with cell line 7026

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Day 1:

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

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

Day 2: Calculations are for 24-well plates. (If using a 6 well plate, then seed at 1×105 cells per well, 850µL of medium the next day with 150µL)

4 Virus infection:

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

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

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

7 Add Polybrene into the infected well to make the final concentration to be 1ug/ml.

7.1 Stock Polybrene (10mg/mL)

7.2 Make a 100x dilution to make a working concentration of 100µg/mL

$(10mg/mL)\ (V_{stock}) = (100µg/mL)(0.5mL)$
V_{stock} = 5µL of stock into 495µL of DPBS (-)(-)

$(100µg/mL)\ (V_{stock}) = (1µg/mL)(0.3mL) \rightarrow V_{stock}$ = **3µL**of working concentration per 300µL of medium added

Day 3:

8 Monitor the cells for the next 48 hours

Day 4 (or 48 hours later):

9 Check IRES-GFP or HLA-A2's expression at least **48 hours** after the transduction.

10 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