# iPSC Transduction

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ABSTRACT

This is the iPSC transduction protocol.

ATTACHMENTS

Transduction Protocol .docx

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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 TEXT

**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$^2$)

ATTACHMENTS

Transduction Protocol .docx

## 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, th

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.



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| Well 1 | Well 2 | Well 3 | Control |
|---|---|---|---|
| PLVX mCherry H | PLVX IRES-Puro HI | 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) --> $V_{stock}$ = **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



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