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Genetic Modification of Human Induced Pluripotent Stem Cells (hiPSCs) by Lentiviral Transduction Protocol

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Roshni Jaffery[1,2], Ningbo Zheng[1,2], Jiakai Hou[1,2], Ashley Guerrero[1,2], Si Chen[1,2], Chunyu Xu[1,2], Nicholas A. Egan[1,2], Ritu Bohat[1,2], Weiyi Peng[1,2]

[1]Department of Biology and Biochemistry, University of Houston, Houston, TX, USA;
[2]Aligning Science Across Parkinson's Collaborative Research Network

Sarfraz Ahmed
Mass General Hospital

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We use this protocol and it's working

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Abstract

This is the protocol for the genetic modification of human induced pluripotent stem cells (hiPSCs) using lentiviral transduction method.

Materials

1. Cell Lines: 293T and hiPSCs of interest
2. Plasmid of interest
3. Packaging plasmids: VSVG and psPAX2
4. JetPRIME buffer
5. jetPRIME transfection reagent
6. DMEM media + 10% FBS complete mixture
7. 0.45 um PDVF membrane filter
8. 5 mL syringe
9. MilliporeSigma™ Amicon™ Ultra-15 Centrifugal Filter Units
10. StemFlex media and supplement (ThermoFisher Scientific Cat.No.A3349401)
11. Vitronectin (VTN)
12. Polybrene

Day 1

1 Grow and culture 293T cell line in 10 cm plates.

2 Grow and culture hiPSCs in pre-coated VTN 6 well plates.

3 When there are enough 293T cells, seed 5 million 293T cells a 10cm plates in 10mL DMEM
complete media per plasmid of interest.

4 Grow overnight at 37 deg C and 5% CO2 until 80%-90% confluence.

Day 2: Transfection

5 Change media of wells. Make a master mix in 1.5 mL Eppendorf tubes for each plate as follows:

	A	B	C	D	E
	JetPRIME Buffer (Polyplus Transfection, Ref #: 114-15)	Plasmid interest	Packaging plasmid VSVG	Packaging plasmid psPAX2	jetPRIME transfection reagent (Polyplus Transfection, Ref #: 114-15)
	500 uL	500 uL	2 ug	3 ug	1:2 ratio of total DNA (ug): transfection reagent (uL) =20 uL

6 Vortex 10s.

7 Incubate 10 min RT *NEVER OVER 30 MIN*.

8 Add all of mix to wells dropwise and rock plate and incubate overnight 37 deg C and 5% CO2.

Day 3

9 Check cells 24 hours post-transfection.

10 First thing in the morning, exchange the media – add 15 mL pre-warmed DMEM complete media to transfected wells. (This removes the jetPRIME transfection reagent and residue plasmid.).

11 Split hiPSCs into clumps in StemFlex complete medium. Seed 20% confluency or ~0.1M per well in 2 mL of 6 well plate. Note: Cell number depends on cell type.

Day 4: Virus Collection

12 Check cells 48 hours post-transfection. Collect virus in 50 mL tube.

13 Centrifuge 1500 rpm for 5 minutes.

14 Using a 0.45 um PDVF membrane filter and 5 mL syringe, collect virus into new 50 mL tubes. Avoid bottom of tube with cell debris

15 Concentrate 1:40 [~42-45mL of diluted virus makes approximately 0.8-1mL of concentrated virus]

15.1 Collect and label MilliporeSigma™ Amicon™ Ultra-15 Centrifugal Filter Units.

15.2 Rinse column with PBS and spin 2,000 × g for 5 min.

15.3 Add up to 10 mL of sample to the Amicon® Ultra filter device.

15.4 Centrifuge at 2,000 × g maximum for 10 minutes.

15.5 o
Repeat adding rest of virus to Amicon® Ultra filter device and centrifuge again at 2,000 × g maximum for 10 minutes until all virus is concentrated.

15.6 Collect concentrated virus and aliquot 0.2mL per Eppendorf tube. Use virus, other tubes store -80degC for future use if necessary.

Day 4 (continued): Virus infection

16 Remove the culture medium from cells, add 850uL fresh Stemflex complete medium into each well of 6 well plate.

17 Add 150ul virus and 1ug/ml polybrene into cells. Rock plate.

18 Centrifuge at 1000rcf for 90min at room temperature.

19 Incubate at 37 C^0, 5% CO2 overnight.

Day 5: Change medium

20 Replace the medium with 2ml fresh Stemflex complete medium.

Day 6: Check cells

21 Check the expression of plasmid via microscopy (if applicable).

22 Begin selection (if applicable).

Day 7 and onward

23 Check the cell status.

24 Split cells if necessary.

25 Freeze cells to store.