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iNDI PiggyBac-TO-hNGN2 transfection protocol Version 1

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Neurodegeneration Method Development Community | iNDI Protocol Development

Erika Lara Flores

ABSTRACT

PiggyBac Method for hNGN2 transfection

- Transfection protocol
- Use of CEPT: *Nature Methods 18, 528-541, 2021*

DOI

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EXTERNAL LINK

https://www.jax.org/jax-mice-and-services/ipsc

PROTOCOL CITATION

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KEYWORDS

iNeurons, NGN2, Piggybac, transfection, iNDI, Jackson Laboratory, CARD, NIH

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57655

MATERIALS TEXT

Reagents

Opti-MEM™ I Reduced Serum Medium **Gibco - Thermo Fischer Catalog #31985062**

Lipofectamine™ Stem Transfection Reagent **Thermo Fisher Scientific Catalog #STEM00008**

StemPro™ Accutase™ Cell Dissociation Reagent **Gibco - Thermo Fisher Catalog #A1110501**

PBS 1x **Lonza Catalog #BE17-516F**

• Chroman I **MedChemExpress Catalog #HY-15392**

Emricasan (IDN-6556) **Selleckchem Catalog #S7775**

Polyamine Supplement (1000×) **Sigma Aldrich Catalog #P8483**

Trans-ISRIB **Tocris Catalog #5284**

Essential 8™ Medium **Gibco, ThermoFisher Catalog #A1517001**

Matrigel hESC-Qualified Matrix, LDEV-free **Corning Catalog #354277**

Plasmids

PB-TO-hNGN2 (Addgene #172115)

EF1a-Transposase (sequence):
GACGGATCGGGAGATCTCCCGATCCCCTATGGTGCACTCTCAGTACAATCTGCTCTGATGCCGCATAGT
TAAGCCAGTATCTGCTCCCTGCTTGTGTGTTGGAGGTCGCTGAGTAGTGCGCGAGCAAAATTTAAGCTA
CAACAAGGCAAGGCTTGACCGACAATTGCATGAAGAATCTGCTTAGGGTTAGGCGTTTTGCGCTGCTTC

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CAGGGTTATTGTCTCATGAGCGGATACATATTTGAATGTATTTAGAAAAATAAACAAATAGGGGTTCCG

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CGCACATTTCCCCGAAAAGTGCCACCTGACGTC

Transfection protocol 1h 30m

1 Observe **KOLF2.1** iPSCs under a phase contrast microscope to assess confluency and presence of cells debris. Dish should be dissociated at ~70% to 90% confluency. 30m

Coat **a well of 6 well plate** to be used for transfection with **1 mL** of Matrigel solution, tilting to ensure coverage of entire surface area. Place in **37 °C** incubator for **00:30:00** .

2 Prepare E8 medium supplemented with CEPT and place in at **Room temperature** to warm during dissociation.

C.E.P.T is a cocktail that has been shown to improve the survival while transfection, however it can be used any other rock inhibitor as Y-27632 knowing that survival/transfection efficiency is not going to be as good as with CEPT.

Components of C.E.P.T cocktail

A	B	C	D	E
Reagent	**Company**	**Cat. #**	**Final concentration**	**Target**
Chroman 1	MedChem Express	HY-15392	50 nM	ROCK 2 inhibitor
Emricasan	Selleckchem	S7775	5 uM	Activated Caspase inhibitors
Polyamine supplement (1000X)	Sigma	P8483	1x (1:1000)	Cell growth booster
Trans-ISRIB	Tocris	5284	0.7 uM	Integrated stress response inhibitor

All the component were prepared as desired concentration following the manufacture instructions of each one.

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3 Aspirate culture medium from well/plate that should be dissociated and wash with PBS 1X.

4 Aspirate PBS and add half of culture volume of Accutase.

5 Transfer to **37 °C** incubator for **00:10:00** . 10m

The time can vary by cell line and density (the optimal density is 70-90%) and the goal to use accutase is singularize as single cells.

6 Meanwhile aspirate Matrigel from well and add **2 mL** culture medium E8 supplemented with CEPT.

7 When Incubation is ready, tilt the plate and pipet the accutase solution two to three times up and down the culture surface to break the colonies.

8 Quench the Accutase adding half of the culture volume of PBS.

9 Transfer to a new conical tube and rinse with more PBS the culture surface, combine with the cell solution in the tube.

10 Centrifuge **00:05:00** at 200 - 300 x g at **Room temperature** . 5m

11 Aspirate supernatant and resuspend the cell pellet in culture medium E8 supplemented with CEPT.

12 Count cells and **plate 1 - 1.5 x 10^6 cells** into a Matrigel-coated well. Gently swirl plate to evenly

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distribute cells.

13 Return plate to **37 °C** incubator.

14 **After ~** **01:00:00** to **02:00:00** of plating cells , warm **OptiMEM** at **Room temperature** 3h .

Do not bring Lipofectamine Stem out of refrigerator until ready to be used!

15 Prepare one tube with:

- **200 µL** of OptiMEM
- Total of **3 µg** of DNA mix in a 1:3 (Transposase:DNA) ratio

EF1a-transposase sequence can be found in the materials section.
Alternatively investigators can try the commercial superpiggybac transposase from SBI, but activity may be lower in iPSCs due to the use of a CMV promoter (which is rapidly silenced) rather than an EF1a promoter

- **5 µL** to **7.5 µL** of Lipofectamine Stem

Vortex after adding every reagent to Opti-MEM

16 Incubate transfection mix for **00:15:00** to **00:30:00** at **Room temperature** . 45m

Meanwhile, to remove debris and increase transfection efficiency, is recommended to wash the wells with PBS 1X and add **2 mL** *of fresh E8 supplemented with CEPT.*
This step can be skipped when using CEPT but not when using rock inhibitor.

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17 Add transfection mix from step 15 to cells drop wise and immediately swirl the plate to evenly distribute the mix to cells.

18 Return plate to **37 °C** incubator.

19 Next day, check for nuclear BFP positive signal (for PB-TO-hNGN2-BFP plasmid). If a positive transfection occurred and the well is confluent, re-plate the cells and puromycin select 24-48 h after transfection (it all depends on the health and transfection efficiency of the cells) with **8 µg/mL** for 2 to 14 days.

> *One can start puromycin selection with low dose as* **1 µg/mL** *and see how the cells react, then increase as convenient to reach* **8 µg/mL** *.*

20 Change media accordingly and expand cells when they reach 70-90% confluency.

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