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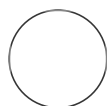
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iNDI PiggyBac-TO-hNGN2 transfection protocol Version 1

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NIH Center for Alzheimer's and Related Dementias



Erika Lara Flores

ABSTRACT

PiggyBac Method for hNGN2 transfection

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

MATERIALS

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 (1000x) 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

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

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Last Modified: Aug 08, 2023

PROTOCOL integer ID:
86174

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

Plasmids

PB-TO-hNGN2 (Addgene #172115)

EF1a-Transposase (sequence):

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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.

Note

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.

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

Note

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 10⁶ cells** into a Matrigel-coated well. Gently swirl plate to evenly 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

Note

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




Note

*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


Note



Vortex after adding every reagent to Opti-MEM

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



45m

Note

Meanwhile, to remove debris and increase transfection efficiency, it 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.

- 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.

Note

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.