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

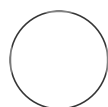
🌐 Piggybac-mediated stable expression of NGN2 in iPSCs for differentiation into excitatory glutamatergic neurons

Dan Dou^{1,2}, C. Alexander Boecker³, Erika L.F. Holzbaur^{1,2}

¹Department of Physiology, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA 19104, USA;

²Aligning Science Across Parkinson's (ASAP) Collaborative Research Network, Chevy Chase, MD, USA;

³Department of Neurology, University Medical Center Goettingen, 37077 Goettingen, Germany



Dan Dou

ABSTRACT

We adapted a previously-described method (Pantazis et al., 2022) for employing Piggybac transfection to stably express doxycycline-inducible NGN2 in human iPSCs. After stable integration of NGN2, proceed to differentiate iPSCs using protocol "iNeuron differentiation from human iPSCs."

ATTACHMENTS

[549-1145.pdf](#)

GUIDELINES

Citations:

- Pantazis, C.B., Yang, A., Lara, E., McDonough, J.A., Blauwendraat, C., Peng, L., Oguro, H., Zou, J., Sebesta, D., Pratt, G., et al. (2022). A reference induced pluripotent stem cell line for large-scale collaborative studies. *BioRxiv* 2021.12.15.472643.

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Keywords: iPSC, Differentiation, iNeuron, Piggybac, NGN2

MATERIALS

Materials

- 10 cm cell culture dish
- 6-well cell culture dish
- Cryovials

Reagents

-  Growth Factor Reduced (GFR) Matrigel® Corning Catalog #354230
-  Essential 8™ Medium Gibco, ThermoFisher Catalog #A1517001
-  Accutase® solution Merck MilliporeSigma (Sigma-Aldrich) Catalog #A6964
-  Y-27632 2HCl Selleckchem Catalog #S1049
-  Opti-MEM™ I Reduced Serum Medium Thermo Fisher Catalog #31985070
-  Lipofectamine™ Stem Transfection Reagent Thermo Fisher Scientific Catalog #STEM00008
-  PB-TO-hNGN2 addgene Catalog #172115
- piggyBac™ transposase vector (Transposagen)
-  KnockOut™ Serum Replacement Thermo Fisher Catalog #10828010
- DMSO (CATALOG)

SAFETY WARNINGS



Wear proper PPE when transferring cryovials to liquid N₂.

Piggybac-mediated stable expression of NGN2 in iPSCs for ...

3d 6h

- 1 Culture iPSCs in a 10 cm dish coated with Growth Factor Reduced Matrigel (Corning) and feed daily with Essential 8 media (ThermoFisher).
- 2 Passage iPSCs with warm Accutase into Essential 8 media with **[M] 10 micromolar (μ M)** ROCK inhibitor. Plate 800,000 iPSCs into one Matrigel-coated well of a 6-well plate.



- 3 3 - 6 hours after plating, cells should be healthy and attached. Perform transfection using Lipofectamine Stem and a 2:1 ratio of donor plasmid to transposase:



A	B
OptiMEM	200 μ L
PB-TO-hNGN2-puro-BFP plasmid	0.75 μ g
EF1 α -transposase plasmid	0.37 μ g
Lipofectamine Stem	4 μ L

- 4 Check for transfection efficiency (BFP-labeled cells) on the next day using fluorescence microscopy.



- 4.1 Passage iPSCs with Accutase to a 10 cm dish when cells are confluent enough for splitting.



Note

Continue to feed iPSCs daily with Essential 8 media without ROCK inhibitor, and confirm division of stably-expressing transfected cells (should observe local clusters of BFP-fluorescent cells).

- 5 **🕒 72:00:00** after transfection, select for transfected iPSCs with **[M] 0.5 μ g/ml** puromycin.

3d

- 5.1** Confirm purity of surviving transfected cells with fluorescence microscopy. When population is pure, withdraw puromycin.



6 Cryopreserve selected iPSCs with



A	B
Essential 8 media	70%
Knockout serum replacement	20%
DMSO	10%
ROCK inhibitor (Supplement)	10 μ M

- 6.1** Proceed to culture and induction to neuronal fate using doxycycline (see “Protocol: iNeuron differentiation from human iPSCs”).