MAY 08, 2023

OPEN ACCESS

DOI:
dx.doi.org/10.17504/protocols.io.e6nvwj54dlmk/v1

Protocol Citation: Dan Dou, C. Alexander Boecker, Erika L.F. Holzbaur 2023. Piggybac-mediated stable expression of NGN2 in iPSCs for differentiation into excitatory glutamatergic neurons. **protocols.io** https://dx.doi.org/10.17504/protocols.io.e6nvwj54dlmk/v1

MANUSCRIPT CITATION: 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.

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

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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.

Created: Oct 12, 2022

Last Modified: May 08, 2023

PROTOCOL integer ID: 71238

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 (µM) 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 µL
	PB-TO-hNGN2-puro-BFP plasmid	0.75 µg
	EF1α-transposase plasmid	0.37 µg
	Lipofectamine Stem	4 µL

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 µg/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 µM

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