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Piggybac-mediated stable expression of NGN2 in iPSCs for differentiation into excitatory glutamatergic neurons V.2

Cell reports

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Protocols

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We use this protocol and it's working

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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
106KB

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.

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 - Thermo Fisher Scientific** **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 RRID:Addgene_172115
- piggyBac™ transposase vector (Transposagen/Hera BioLabs) #SPB-D10
- KnockOut™ Serum Replacement **Thermo Fisher** **Catalog #**10828010
- DMSO (CATALOG)

Troubleshooting

Safety warnings

Wear proper PPE when transferring cryovials to liquid N2.

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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 Mass Percent 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”).