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

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Key words

iPSC, differentiation, iNeuron, Piggybac, NGN2

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."

Materials

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

Reagents

- Growth Factor Reduced Matrigel (Corning, Cat# 354230)
- Essential 8 media (ThermoFisher, Cat# A1517001)
- Accutase (Sigma, Cat# A6964-100ML)
- Y-27632 ROCK inhibitor (Selleck Chemicals, Cat# S1049)
- OptiMEM media (Thermo Fisher, Cat# 31985-070)
- Lipofectamine Stem (Thermo Fisher, Cat# STEM00008)
- PB-TO-hNGN2-puro-BFP vector (Addgene plasmid #172115)
- piggyBac™ transposase vector (Transposagen)
- Knockout serum replacement (Thermo Fisher, Cat# 10828010)
- DMSO (CATALOG)

Safety warnings

Wear proper PPE when transferring cryovials to liquid N2.

Steps

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 10 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: 200 μL OptiMEM, 0.75 μg PB-TO-hNGN2-puro-BFP plasmid, 0.37 μg EF1α-transposase plasmid, 4 μL Lipofectamine Stem.
4. Check for transfection efficiency (BFP-labeled cells) on the next day using fluorescence microscopy. Passage iPSCs with Accutase to a 10 cm dish when cells are confluent enough for splitting. 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 hours after transfection, select for transfected iPSCs with 0.5 μg/mL puromycin. Confirm purity of surviving transfected cells with fluorescence microscopy. When population is pure, withdraw puromycin.
6. Cryopreserve selected iPSCs with 70% Essential 8 media, 20% knockout serum replacement, 10% DMSO, supplemented with 10 μM ROCK inhibitor. Proceed to culture and induction to neuronal fate using doxycycline (see “Protocol: iNeuron differentiation from human iPSCs”).

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.