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Neural Progenitor Cell (NPC) differentiation

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We use this protocol and it's working

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Disclaimer

This protocol was adapted from the work of Lexi Bounds in the Gersbach lab at Duke University.

Abstract

This protocol describes methods for WTC11 iPSC culture and differentiation into neural progenitor cells.

Materials

WTC11 iPSCs from the Coriell Institute for Biomedical Research (GM25256)
mTeSR1 (StemCell Technologies #85850)
Matrigel (Corning #354230)
Versene (ThermoFisher #15040066)
KnockOut™ Serum Replacement (ThermoFisher #10828028)
Y-27632 (Dihydrochloride) (StemCell Technologies (#72302)
6-well culture plates (Corning #3471)
SB431542 (StemCell Technologies #72234): Resuspend 10 mg in 2.06 mL DMSO.
LDN193189 (StemCell Technologies #72147): Resuspend 10 mg in 2.09 mL of DMSO.
STEMdiff™ Neural Rosette Selection Reagent (StemCell Technologies #05832)
Penicillin-Streptomycin (10,000 units/mL) (ThermoFisher #15140122)
B27 Supplement w/o Vitamin A (50X) (ThermoFisher #12587010)
N-2 Supplement (100X) (ThermoFisher #12587010)
Animal-Free Recombinant Human EGF (Peprotech #AF-100-15): Resuspend 100 ug in 1 mL ultra-pure H20.
Animal-Free Recombinant Human FGF-basic (Peprotech #AF-100-18B): o Resuspend 50 ug in 500 uL ultra-pure H20.

- Neural basal medium (NBM): 500 mL DMEM/F-12 + 5 mL N2 + 10 mL B27 + 5 mL P/S
- Neural induction medium (NIM): 50 mL NBM + 0.5 uL LDN + 50 uL SB431542
- Neural expansion medium (NEM): 50 mL NBM + 10 uL bFGF + 10 uL EGF

iPSC culture

1 All cell lines were cultured at 37C and 5% CO2.

2 WTC11 iPSCs were obtained from the Coriell Institute for Biomedical Research (GM25256)

3 Cells are propagated in mTeSR1 (StemCell Technologies #85850) on Matrigel (Corning #354230)-coated tissue culture plates and passaged as colonies using Versene (ThermoFisher #15040066).

4 Cells are frozen in freezing solution comprised of 60% mTeSR1 media, 10% DMSO, and 30% KnockOut™ Serum Replacement (ThermoFisher #10828028), with 10uM ROCK Inhibitor (RI) Y-27632 (Dihydrochloride) (StemCell Technologies (#72302).

NPC Differentiation

5 WTC11 iPSCs are differentiated into NPCs using embryoid-body (EB) formation and small molecules as previously described with minor modifications (Refs 1, 2)

6 WTC11 iPSCs are seeded in low-adhesion 6-well culture plates (Corning #3471) in NBM containing 10 uM SB431542 (StemCell Technologies #72234) and 0.1 uM LDN193189 (StemCell Technologies #72147) (NIM) with 10 uM Y-27632.

7 24 hours later (Day 1), 2 mL of media is removed and replaced with 2 mL NIM.

8 On Days 2-4, 3 mL of media is removed and replaced with 3 mL NIM.

9 On Day 5, EBs are transferred onto Matrigel-coated 6-well plates containing 1 mL NIM per well.

10 24 hours later (Day 6), all media is removed and replaced with 3 mL NIM.

11 On Days 7-12, 3 mL NIM was changed daily.

12 On Day 13, neural rosettes are harvested using STEMdiff™ Neural Rosette Selection Reagent according to the manufacturer's protocol (StemCell Technologies #05832) and plated onto Matrigel-coated plates in NEM ('Passage 0'):

12.1
1. Aspirate media.
2. Wash w/ 1 mL DMEM/F-12.
3. Aspirate.
4. Add 1 mL neural rosette selection reagent.
5. Incubate 1 hour at 37C. (may need longer incubation, check at 30min, 45min)
6. Aspirate selection reagent.
7. Using P1000, wash each well 2x w/ 1 mL DMEM/F-12 and collect cells.
    a. Try not to break up the rosettes.
8. Centrifuge 200-300g for 5min.
9. Aspirate supernatant.
10. Resuspend cells in 2 mL NEM.
11. Plate to one well of 6-well.

Repeat for all wells.

13 NPCs are propagated for five passages in NEM before characterization as described below. All experiments are performed with NPCs of passage number 7-15.

NPC culture

14 WTC11 NPCs are cultured in NEM. Change media daily with 2 mL NEM.
Passage cells w/ Accutase when 80-90% confluent (include RI in media when passaging, like iPSCs)
Seed cells at 200-500K cells per well (cell line/differentiate dependent).
Collect cells for RNA at P2, P5. Freeze cells when possible in NBM + KSR + DMSO + RI.

Protocol references

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