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

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Preparation of human iPSC-derived cortical neuronal progenitors for transplantation into the rodent brain

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ABSTRACT

This protocol describes how we prepare human iPSC-derived cells, that have been differentiated into cortical neuronal
progenitors, for transplantation into the brain of immunocompromised athymic mice. Neuronal progenitors mature within the mouse brain and are used to study the pathogenesis of Parkinson's disease.

MATERIALS

Material input

- Adult (8-10 week) athymic mice (BALBc/Nu)
- IPSCs differentiated into cortical progenitors (D25-30 of differentiation depending on desired experimental outcomes), typically grown in 48 well plates. Full details of the cortical differentiation protocol: dx.doi.org/10.17504/protocols.io.bu6znzf6.

Equipment

- Microscope
- Microscope (brightfield with Phase contrast)
- Heamocytometer
- Pipettes (P20, P200, P1000)

Consumables

- Falcon tubes
- PCR tubes

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Keywords: ASAPCRN, human iPSC, transplantation, cortical progenitors, neurons, human-to-mouse xenograft

- pipette tips

Key reagents

- 1X PBS (Phosphate-buffered saline)
- ACCUTASE™ 100 mL **STEMCELL Technologies Inc. Catalog #7920**
- Rock inhibitor Y-27632 dihydrochloride **Tocris Catalog #125410**
- Trypan Blue 100 mL **STEMCELL Technologies Inc. Catalog #7050**

Solutions

- Cortical Base Media (recipe dx.doi.org/10.17504/protocols.io.bu6znzf6): DMEM/F12, Neurobasal media, B27 supplement, N2 supplement, ITS-A, Glutamax, Pen/Strep, β-mercaptoethanol

SAFETY WARNINGS

For hazard information and safety warnings, please refer to the SDS (Safety Data Sheet).

Preparation of cell suspension

1 23m

1. Prepare a Falcon tube with 2 mL base cortical media plus Rock inhibitor (Ri, 1:1000 dilution)
2. Wash cells with 300 µL PBS -/- (gently run PBS down the side of each well)
3. Incubate cells in 300 µL Accutase (per well) at 37 °C to lift cells off wells in small clumps

- Monitor the Accutase incubation after 00:10:00; tap the plate to dislodge cells and look for cells lifting in a sheet before proceeding
- Triturate 5 times to break the cells into clumps
- Incubate clumps for a further 00:05:00 at 37 °C
- Triturate 10 times to break the cells into single cells and small clumps

4. Transfer cells from the wells into the 15ml Falcon tube containing media + Ri (Step 1)
5. Rinse with plate with a further 300 µL of Accutase to ensure all cells are collected
6. Spin cells at 4 °C, 1300 rpm for 00:04:00
7. Discard supernatant
8. Resuspend cell pellet in 1-2ml base cortical media and add Ri (1:1000)
9. Count cells
    - Take 2 x 10 µL aliquot of cells in two separate tubes

Dilute cells 1:1 using 10 µL of trypan blue

    - Count cells in a 10 µL aliquot from each tube using a haemocytometer
    - Calculate total number of cells (Total cells = Average count of quadrants x Dilution factor x Volume (ml) x 10^4)

10. Calculate the total volume needed to resuspend cells to a final density of 100 000/uL
11. Spin cells at 4 °C, 1300 rpm for 00:04:00
12. Whilst cells are spinning, make up 1 mL media + Ri which will be used to resuspend the cells for implantation surgery
13. At end of the spin, collect tube and discard supernatant
14. Using a P20, add 5 µL and resuspend pellet in base cortical media + Ri
15. Transfer resuspended pellet to PCR tube and precisely measure the volume
16. Add the remaining volume needed for cells to reach a density of 100 000 cells/ul as calculated in step 10.
17. Label the PCR tube containing the cells with details of the cell line and concentration
18. Store cells on ice and transport to animal surgical room.

Transplantation

2 1. Athymic mice undergo stereotactic surgery to receive a unilateral graft of 100,000 cortical progenitors in 1 µL volume. The surgical coordinates from Bregma are AP: +1, ML: +/-1.5, DV: -1.5

For full details of procedures for implantation of cell suspension can be found at https://www.protocols.io/view/transplantation-of-fetal-midbrain-dopamine-progeni-261ge4jq7v47/v1