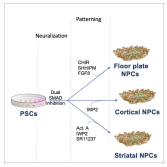

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Modular generation of cortical, striatal and ventral midbrain progenitor cells

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ABSTRACT

The present protocol describes the modular generation of cortical, striatal, and ventral midbrain human neural progenitor cells from human-induced pluripotent stem cells (hiPSC). The different elements of the differentiation protocols have been optimized so that they only differ in the patterning factors (protein ligands or small molecules) that are required for each lineage.

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KEYWORDS

A9 dopamine neurons, cortical neurons, striatal neurons, medium spiny neurons, hiPSC, differentiation, lateral ganglionic eminence

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MATERIALS TEXT

N2B27:
Neurobasal:DMEM/F12 (1:1)
0.5x B27 without Vit. A
0.5x N2
1x PenStrep
1x GlutaMAX
1x NEAA

KSR:
KO-DMEM
15% KSR
1x PenStrep
1x GlutaMAX
1x NEAA
0.1mM b-Mercaptoethanol

Freezing media:
N2B27 + 10% DMSO

Vendors:
SB431542 (Tocris; 1614 - 50mg)
LDN193189 hydrochloride (SIGMA; SML0559-5MG)
IWP2 (Preprotech; 6866167)
Activin A (R&D Systems; 338-AC-050)
SR11237 (Tocris; 3411/10)
Shh (C25II) N-Terminus (R&D Systems; 464-SH-200/CF)
Purmorphamine (SIGMA; 540223-5MG)
FGF8b (R&D Systems; 423-F8-025/CF)
CHIR99021 (StemCell Technologies; 72054
BDNF (R&D Systems; 248-BDB-050/CF)
GDNF (R&D Systems; 212-GD-050/CF)
Ascorbic Acid (SIGMA; A4544-25G)
DAPT (Tocris; 2634/10)
dbcAMP (SIGMA; D0627-100MG)
Rock Kinase Inhibitor (Miltenyi Biotech; 130-104-169)
StemPro Accutase Cell Dissociation Reagent (Life Technologies; A1110501)

Preparation of the hiPSC for starting the neuralization 1w

1 **Notes before starting:**

- We maintain hPSC in mTESR-Plus medium on Matrigel-coated plates and split the cells as clumps using RELESR or 0.5 mM EDTA. **Accutase** is used when cells need to be counted such as in the initial step of the protocol. Or for the first passages of the NPCs (breaking the monolayer). Accutase needs to be 1) fresh and 2) washed by dilution and centrifugation. It is advisable to prolong accutase treatment (7 to 10 minutes) rather than singularize cells by repeated pipetting.
- It is important to coat the plates with **fresh Matrigel (1:50)** for the neuralization step (days 0 to 13). Otherwise, the borders of the monolayer may detach during the course of the neuralization
- In order to have enough cells for the initial seeding, we generally grow the cells in 60mm or 100mm plates. A confluent 100mm contains enough cells to seed an entire 6 well plate.

2 **Day -1:**

- Pretreat the PSC with RI for 2h to O/N.
- Remove the medium (this medium can be saved for diluting the accutase).
- Add 3 mL of pre-warmed accutase to a 100mm plate (alternatively 1.5 mL to a 6mm). Incubate the cells with the accutase for 5-10' in the incubator at 37^{o}C. Before cells start to detach, remove the accutase. Pipette medium (fresh or the medium saved before) on top of the cells vigorously in order to detach them from the plate. It is very important not to pipette medium with cells suspended in it as this would harm the cells.
- Count the cell suspension, and Seed 300k cells per cm^2 in the presence of a 1:1 mixture of mTESR-Plus and KSR

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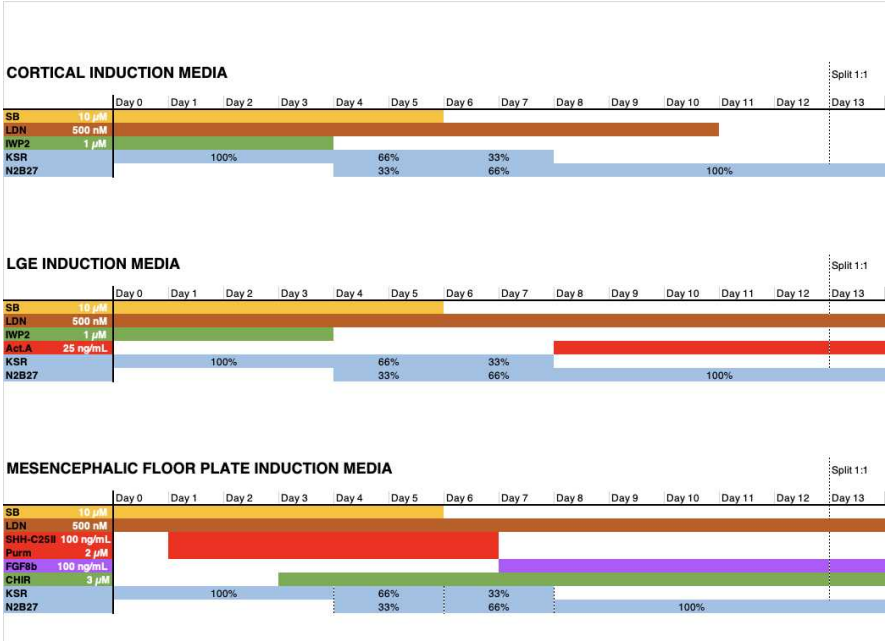
supplemented with RI.

Dual-SMAD inhibition and regional patterning 2w 2d

3 **Day 0:** follow the following feeding schemes (see below).
Days 1-6: change medium every other day (3 mL per 6wp well).
Days 6-13: change medium every day (3 mL per 6wp well).



4 **Expansion and subsequent cryopreservation of neural progenitor cells.**

Day 13: reseed cells 1:1 in a matrigel-coated plate using accutase. This cell passaging step facilitates the homogenization of the differentiating cells and the elimination of dead cells trapped in the monolayer. Pre- and post-incubate cells with RI.
Day 15: feed cells. Add RI to the medium.
Day 16: split cells 1:2 using accutase. Reseed in medium supplemented with RI.
Day 18: feed cells. Add RI to the medium.
Day 20: freeze cells in freezing medium. Reserve 1:6 of the cells for seeding in 2x 24wp wells and for RNA analysis.

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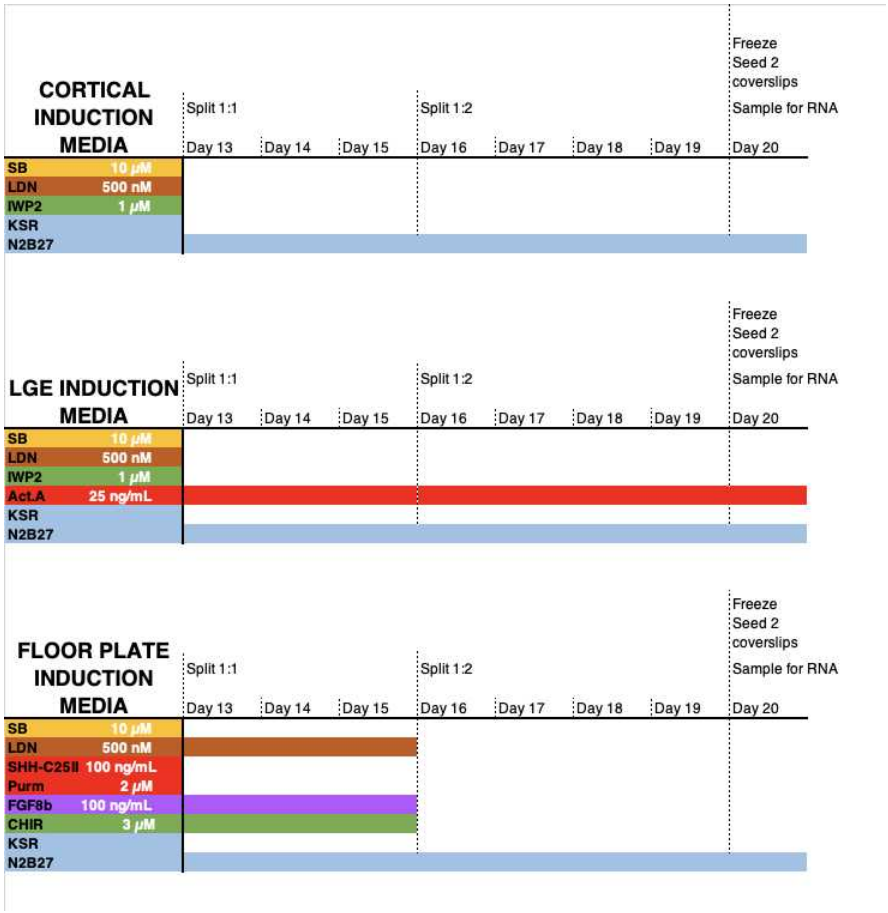

Quality control 2d

5 Immunofluorescence analysis

The different progenitors should express markers specific to the ventricular and subventricular zones of the corresponding brain regions:

- Cortical progenitors: FOXG1/OTX2/PAX6/TBR2
- Striatal progenitors: FOXG1/OTX2/GSX2/DLX2
- Mesencephalic floor plate progenitors: FOXA2/LMX1A/OTX2/EN1

RT-qPCR

It is advisable to test a battery of markers that are specific to the developing brain regions of interest but also to include markers specific to the pluripotent state as well as markers specific to neighboring regions to rule out contaminations. With respect to the latter, we regularly include markers specific to the lateral (GSX2 and DLX2) and medial (NKX2.1 and DLX2) ganglionic eminences when checking cortical progenitors. We include markers specific to the developing cortex and to the medial ganglionic eminence (NKX2.1) when checking lateral ganglionic eminence (striatal) progenitors. And finally, we include markers specific to regions anterior (BARHL1), posterior (HOXA2), or dorsal (NKX6-1) to the mesencephalic floor plate when checking mesencephalic floor plate progenitors.

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