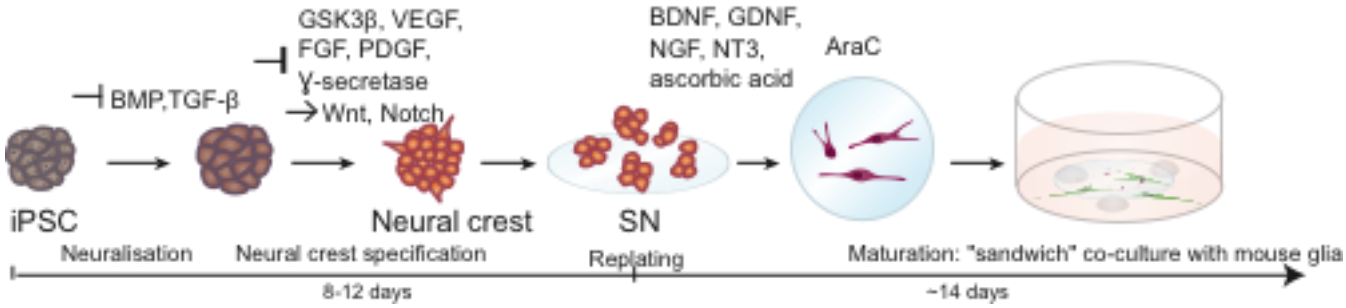
Sensory neuron differentiation from FF iPSC



Materials
ascorbic acid (Sigma)
B-Mercaptoethanol (B-ME, 14.3 M, Sigma, cat. no. M7522)
BDNF (R&D, cat. no. 2837)
CHIR99021 (Sigma)
Cytarabine (Ara-C)
DAPT (Sigma, D5942)
Dispase 1U/ml (Stemcell Technologies?)
DMSO (cat. no. D8418)
Dulbecco's modified eagle medium/Nutrient mixture F-12 (DMEM/F12) (1:1) (Gibco-BRL, cat. no. 11330)
Dulbecco's phosphate-buffered saline (DPBS) (Gibco-BRL, cat. no. 14190)
Fetal bovine serum (FBS) (Gibco-BRL, cat. no. 10437)
GDNF (R&D, cat. no. 212-GD)
Geltrex
KOSR
Laminin
LDN193189 (Stemgent, cat. no. 130-096-226)
MEM non-essential amino acids solution (NEAA) (Gibco-BRL, cat. no. 11140)
mTesR
Recombinant Human β-NGF (cat#450-01, PeproTech)
Recombinant Human NT-3 (cat# 450-03, PeptroTech)
PDL
SB431542 (Axon Medchem, cat. no. Axon 1661)
SU5402 (Sigma, SML0443)

Media:

hES medium w/o FGF (hES0):
up to 500 ml DMEM/F12
100 ml KOSR
5 ml Glutamax
5 ml NEAA
0.01 uM B-ME
2,5 ml P/S

hMN medium (name stems from initial application for human Motor Neurons)
500 ml Neurobasal
5 ml Glutamax
5 ml NEAA
5 ml N2
10 ml B27-vit A
2,5 ml P/S

d0
Replate 10^6 FF iPSCs in mTeSR + Y27 (1:1000) in a geltrex-coated 6cm dish using accutase:

A full 35mm dish, e.g. a backup the day after splitting, contains enough cells.
Wash 2x in warm PBS
0.4 ml Accutase (for 35mm dish), 4 min in incubator
Quench in 4ml medium (mTeSR, or hES if available which is cheaper)
Count
Centrifuge: 200 rcf 4 min
Resuspend in a small amount (1ml or less) of mTeSR, and seed a small volume (about 50-100 ul) of cells in dish with 3ml mTeSR + Y27. Shake to disperse the cells immediately.

d1
Normal FF medium change (3ml mTesR)

d2
hES0 5ml/dish
1uM LDN193189 (50 000x)
10 uM SB431542 (10 000x)

d4-5
75% hES0 / 25%hMN 5ml/dish
1µM LDN193189 (50 000x)
10µM SB431542 (10 000x)
10µM CHIR99021 (8000x)
10µM DAPT (4000x)
10µM SU5402 (3000x)

d6-7
50% hES0 / 50%hMN (5ml/dish)
1µM LDN193189 (50 000x)
10µM SB431542 (10 000x)
10µM CHIR99021 (8000x)
10µM DAPT (4000x)
10µM SU5402 (3000x)

d8-9
25% hES0 / 75%hMN (5ml/dish)
10µM CHIR99021 (8000x)
10µM DAPT (4000x)
10µM SU5402 (3000x)

Around day 8-10: Plating.

SN maintenance Medium:
hMN
10 ng/ml BDNF (10 000x)
10 ng/mlGDNF (10 000x)
10 ng/ml NT3 (10 000x)
10 ng/ml NGF (10 000x)
ascorbic acid (1000x)

dx/0
After a few days with these inhibitors, cells start forming 3d layer and look brownish, and detach easily, and a while after this, neurite outgrowth starts to appear. At this point, replate the cells (Both the adhering ones and detached spheres can be used) on PDL-laminin coated coverslips using Dispase:

Aspirate medium
Add 1 ml 1U/ml dispase
37°C 4 min
Prepare 10 ml medium and an empty falcon.
Collect cells with P1000 and spray with medium to get them loose 1 ml at the time. Cells that don't come off with this method have a different morphology and can stay behind as they don't form neurons.
(Optional: take some out for counting – as cells are still in clumps, reliability of counting is variable – cell numbers are typically underestimated)
Centrifuge at 160rcf for 5 min.
Wash pellet once with medium as dispase is not inhibited by serum, then resuspend for plating.

Seed 0.5ml in 24w plate or 1ml in 12w plate

d1 AraC selection: the day after plating

Add 100% of volume (0.5 ml for 24w, 1ml fo12w):
hMN
AraC (1:10 000)
10 ng/ml BDNF (10 000x)
10 ng/mlGDNF (10 000x)
10 ng/ml NT3 (10 000x)
10 ng/ml NGF (10 000x)
ascorbic acid (1000x)

Prime glia as for MNs

d2 The day after AraC selection:
Change medium of glia to 1.5 ml of SN maintenance medium with freshly added factors.
Transfer coverslips with SNs to glia.

Half medium change every 3rd day or monday, (wednesday), friday.