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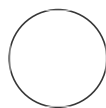
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dopaminergic neurons from iPSC

Rachel Bates¹

¹UCL



rachel.bates

ABSTRACT

This protocol describes our method for the differentiation of human floor plate neural progenitor cells into human midbrain-dopaminergic neurons. This protocol has been developed using a combination of several published protocols.

Adapted from

CITATION

Jo J, Xiao Y, Sun AX, Cukuroglu E, Tran HD, Göke J, Tan ZY, Saw TY, Tan CP, Lokman H, Lee Y, Kim D, Ko HS, Kim SO, Park JH, Cho NJ, Hyde TM, Kleinman JE, Shin JH, Weinberger DR, Tan EK, Je HS, Ng HH (2016). Midbrain-like Organoids from Human Pluripotent Stem Cells Contain Functional Dopaminergic and Neuromelanin-Producing Neurons.. Cell stem cell.

LINK

<https://doi.org/10.1016/j.stem.2016.07.005>

CITATION

Mohamed NV, Sirois J, Ramamurthy J, Mathur M, Lépine P, Deneault E, Maussion G, Nicouveau M, Chen CX, Abdian N, Soubannier V, Cai E, Nami H, Thomas RA, Wen D, Tabatabaei M, Beitel LK, Singh Dolt K, Karamchandani J, Stratton JA, Kunath T, Fon EA, Durcan TM (2021). Midbrain organoids with an SNCA gene triplication model key features of synucleinopathy.. Brain communications.

LINK

<https://doi.org/10.1093/braincomms/fcab223>



















Keywords: ASAPCRN

PROTOCOL MATERIALS

Funders

Acknowledgement:

Asap

-  CHIR99021 R&D Systems Catalog #4423 In [3 steps](#)
 -  MEM, NEAA, no glutamine Thermo Fisher Catalog #10370021 In [5 steps](#)
 -  N-2 max supplement R&D Systems Catalog #AR009 In [5 steps](#)
 -  Glutamax (100x) Gibco - Thermo Fischer Catalog #35050-061 In [5 steps](#)
 -  FGF8 Novus Biologicals Catalog #423-F8-025 In [4 steps](#)
 -  Stemolecule LDN-193189 Stemgent - Bio-connect Catalog #04-0074 Step 1.1
 -  N21-MAX Media Supplement (50X) R&D Systems Catalog #AR008 In [5 steps](#)
 -  SB431542 Cell Signaling Technology Catalog #14775 Step 1.1
 -  Neurobasal Plus Medium Gibco, ThermoFisher Catalog #A3582901 In [5 steps](#)
 -  Laminin from Engelbreth-Holm-Swarm murine sarcoma basement membrane Merck MilliporeSigma (Sigma-Aldrich) Catalog #L2020
- Step 7.1
-  DMEM/F-12 Thermo Fisher Catalog #11320033 In [3 steps](#)
 -  recombinant human SHH protein Qkine In [4 steps](#)
 -  Insulin Merck MilliporeSigma (Sigma-Aldrich) Catalog #I2643-25MG Step 7.1
 -  GDNF Qkine Catalog #QK051 Step 10.1
 -  L-Ascorbic acid Merck MilliporeSigma (Sigma-Aldrich) Catalog #A4403 In [5 steps](#)
 -  SAG 1 mg STEMCELL Technologies Inc. Catalog #73412 In [2 steps](#)
 -  BDNF Qkine Catalog #QK050 Step 10.1
 -  Db-cAMP (dibutyryl-cyclic AMP) Merck MilliporeSigma (Sigma-Aldrich) Catalog #D0627
- Step 10.1

Day 0

- 1 Human floor plate neuronal progenitor cells (mfNPC) were derived using Smits 2019 protocol. They were

maintained for a minimum of 5 passages before being used to generate dopaminergic neurons.

CITATION

Fedele S, Collo G, Behr K, Bischofberger J, Müller S, Kunath T, Christensen K, Gündner AL, Graf M, Jagasia R, Taylor V (2017). Expansion of human midbrain floor plate progenitors from induced pluripotent stem cells increases dopaminergic neuron differentiation potential.. Scientific reports.

LINK

<https://doi.org/10.1038/s41598-017-05633-1>

CITATION

Smits LM, Reinhardt L, Reinhardt P, Glatza M, Monzel AS, Stanslowsky N, Rosato-Siri MD, Zanon A, Antony PM, Bellmann J, Nicklas SM, Hemmer K, Qing X, Berger E, Kalmbach N, Ehrlich M, Bolognin S, Hicks AA, Wegner F, Sternecker JL, Schwamborn JC (2019). Modeling Parkinson's disease in midbrain-like organoids.. NPJ Parkinson's disease.

LINK

<https://doi.org/10.1038/s41531-019-0078-4>

1.1 mfNPC maintenance medium

[M] 50 % volume



DMEM/F-12 Sigma
Aldrich Catalog #11320033

[M] 50 % volume



Neurobasal Plus Medium Sigma
Aldrich Catalog #A3582901

1:50



N21-MAX Media Supplement (50X) Sigma
Aldrich Catalog #AR008

1:100



N-2 max supplement Sigma
Aldrich Catalog #AR009

[M] 1 % volume



Glutamax (100x) Sigma
Aldrich Catalog #35050-061

[M] 1 % volume



MEM, NEAA, no glutamine Sigma
Aldrich Catalog #10370021

[M] 10 micromolar (μ M)



SB431542 Sigma
Aldrich Catalog #14775

[M] 0.5 micromolar (μM)

⊗ SAG 1 mg Sigma
Aldrich Catalog #73412

[M] 250 millimolar (mM)



⊗ Stemolecule LDN-193189 Sigma
Aldrich Catalog #04-0074

[M] 200 micromolar (μM)

⊗ L-Ascorbic acid Sigma
Aldrich Catalog #A4403

[M] 3 micromolar (μM)

⊗ CHIR99021 Sigma
Aldrich Catalog #4423

2 mfNPCs were detached using accutase at  37 °C for  00:03:00 .

3m

3 Re-suspend cells in d0 induction medium and plate 0.1×10^5 cells per well 12 well plate coated with Ultimatrix for 1 hour beforehand.

Day 2


4 Change the medium to mfNPC medium supplemented with [M] 0.0001 mg/mL

⊗ FGF8 Sigma
Aldrich Catalog #423-F8-025

[M] 0.0001 mg/mL

⊗ recombinant human SHH protein Sigma
Aldrich

Day 4

5 Change the medium to patterning I medium adding  1 mL per well.

5.1 patterning I medium

[M] 50 % volume	DMEM/F-12 Sigma Aldrich Catalog #11320033
[M] 50 % volume	Neurobasal Plus Medium Sigma Aldrich Catalog #A3582901
1:50	N21-MAX Media Supplement (50X) Sigma Aldrich Catalog #AR008
1:100	N-2 max supplement Sigma Aldrich Catalog #AR009
[M] 1 % volume	Glutamax (100x) Sigma Aldrich Catalog #35050-061
[M] 1 % volume	MEM, NEAA, no glutamine Sigma Aldrich Catalog #10370021
[M] 0.5 micromolar (μ M)	SAG 1 mg Sigma Aldrich Catalog #73412
[M] 200 micromolar (μ M)	L-Ascorbic acid Sigma Aldrich Catalog #A4403
[M] 3 micromolar (μ M)	CHIR99021 Sigma Aldrich Catalog #4423
[M] 0.0001 mg/mL	FGF8 Sigma Aldrich Catalog #423-F8-025
[M] 0.0001 mg/mL	recombinant human SHH protein Sigma Aldrich

Day 6

6 Change medium to patterning II medium with reduced CHIR adding 1 mL per well.

6.1 Patterning II medium

[M] 50 % volume	DMEM/F-12 Sigma Aldrich Catalog #11320033
[M] 50 % volume	Neurobasal Plus Medium Sigma Aldrich Catalog #A3582901
1:50	N21-MAX Media Supplement (50X) Sigma Aldrich Catalog #AR008
1:100	N-2 max supplement Sigma Aldrich Catalog #AR009

[M] 1 % volume

✕ Glutamax (100x) Sigma
Aldrich Catalog #35050-061

[M] 1 % volume

✕ MEM, NEAA, no glutamine Sigma
Aldrich Catalog #10370021

[M] 200 micromolar (μ M)

✕ L-Ascorbic acid Sigma
Aldrich Catalog #A4403

[M] 0.7 micromolar (μ M)

✕ CHIR99021 Sigma
Aldrich Catalog #4423

[M] 0.0001 mg/mL

✕ FGF8 Sigma
Aldrich Catalog #423-F8-025

[M] 0.0001 mg/mL

✕ recombinant human SHH protein Sigma
Aldrich

Day 8

30m

7 Carefully add 1 mL of tissue induction medium to each well.

7.1 Tissue induction medium

[M] 100 % volume

✕ Neurobasal Plus Medium Sigma
Aldrich Catalog #A3582901

1:50

✕ N21-MAX Media Supplement (50X) Sigma
Aldrich Catalog #AR008

1:100

✕ N-2 max supplement Sigma
Aldrich Catalog #AR009

[M] 1 % volume

✕ Glutamax (100x) Sigma
Aldrich Catalog #35050-061

[M] 1 % volume

✕ MEM, NEAA, no glutamine Sigma
Aldrich Catalog #10370021

[M] 200 micromolar (μ M)

✕ L-Ascorbic acid Sigma
Aldrich Catalog #A4403

[M] 0.0001 mg/mL

✕ FGF8 Sigma
Aldrich Catalog #423-F8-025

[M] 0.0001 mg/mL

✕ recombinant human SHH protein Sigma
Aldrich

[M] 0.00025 mg/mL



Laminin from Engelbreth-Holm-Swarm murine sarcoma basement membrane Sigma
Aldrich Catalog #L2020


[M] 0.025 mg/mL



Insulin Sigma
Aldrich Catalog #I2643-25MG

Day 9

30m

8 Add  2 mL of Tissue induction medium to 12 well plate.

9 Incubate plates at  37 °C .

Day 10

10 Change medium to Differentiation medium.

10.1 Differentiation medium

[M] 100 % volume



Neurobasal Plus Medium Sigma
Aldrich Catalog #A3582901

1:50



N21-MAX Media Supplement (50X) Sigma
Aldrich Catalog #AR008

1:100



N-2 max supplement Sigma
Aldrich Catalog #AR009

[M] 1 % volume



Glutamax (100x) Sigma
Aldrich Catalog #35050-061

[M] 1 % volume



MEM, NEAA, no glutamine Sigma
Aldrich Catalog #10370021

[M] 200 micromolar (μM)



L-Ascorbic acid Sigma
Aldrich Catalog #A4403

125 micromolar (μM)

Db-cAMP (dibutyl-cyclic AMP) Sigma
Aldrich Catalog #D0627

10ng/mL BDNF Sigma
Aldrich Catalog #QK050

10ng/mL GDNF Sigma
Aldrich Catalog #QK051

11 After day 10 perform a 75% medium change every 2-3 days.

12 Use neurons after a further 45 days differentiation.