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Generation of hiPSC derived cortical astrocytes

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

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Abstract

This is a modified protocol that describes how to generate cortical region-specific astrocytes based on Gupta et al., 2012, Serio et al., 2013 and Seto-Salvia et al., 2021

Citation

Gupta K, Patani R, Baxter P, Serio A, Story D, Tsujita T, Hayes JD, Pedersen RA, Hardingham GE, Chandran S (2012) . Human embryonic stem cell derived astrocytes mediate non-cell-autonomous neuroprotection through endogenous and drug-induced mechanisms.

https://doi.org/10.1038/cdd.2011.154 LINK

Citation

Serio A, Bilican B, Barmada SJ, Ando DM, Zhao C, Siller R, Burr K, Haghi G, Story D, Nishimura AL, Carrasco MA, Phatnani HP, Shum C, Wilmut I, Maniatis T, Shaw CE, Finkbeiner S, Chandran S (2013) . Astrocyte pathology and the absence of non-cell autonomy in an induced pluripotent stem cell model of TDP-43 proteinopathy.

https://doi.org/10.1073/pnas.1300398110 LINK

Citation

Nuria Seto-Salvia, Noemi Esteras, Rohan de Silva, Eduardo de Pablo-Fernandez, Charles Arber, Christina E Toomey, James M Polke, Huw R Morris, Jonathan D Rohrer, Rickie Patani, Selina Wray, Thomas T Warner (2021). Elevated 4R-tau in Astrocytes From Asymptomatic Carriers of the MAPT 10+16 Mutation. Preprint.

https://doi.org/10.21203/rs.3.rs-117443/v1 LINK

hiPSC culture

1 hiPSCs are maintained on Geltrex in mTeSR (Stem Cell) and passaged using 0.5mM EDTA, as followed by Virdi et al., 2022.

Citation

Virdi GS, Choi ML, Evans JR, Yao Z, Athauda D, Strohbuecker S, Nirujogi RS, Wernick AI, Pelegrina-Hidalgo N, Leighton C, Saleeb RS, Kopach O, Alrashidi H, Melandri D, Perez-Lloret J, Angelova PR, Sylantyev S, Eaton S, Heales S, Rusakov DA, Alessi DR, Kunath T, Horrocks MH, Abramov AY, Patani R, Gandhi S
(2022)
. Protein aggregation and calcium dysregulation are hallmarks of familial Parkinson's disease in midbrain dopaminergic neurons.

https://doi.org/10.1038/s41531-022-00423-7 LINK

Differentiation of hiPSC into Cortical astrocytes 1w 3d

2 Differentiation of cortical region-specific astrocytes is performed using a modified protocol based on Gupta et al., 2012, Serio et al., 2013 and Seto-Salvia et al., 2020.

Citation

Serio A, Bilican B, Barmada SJ, Ando DM, Zhao C, Siller R, Burr K, Haghi G, Story D, Nishimura AL, Carrasco MA, Phatnani HP, Shum C, Wilmut I, Maniatis T, Shaw CE, Finkbeiner S, Chandran S
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(2021)
. Elevated 4R-tau in Astrocytes From Asymptomatic Carriers of the MAPT 10+16 Mutation. Preprint.

https://doi.org/10.21203/rs.3.rs-117443/v1 LINK

3 Neural precursor cells (NPCs) are obtained from hiPSC using the established protocol of

Citation

Shi Y, Kirwan P, Smith J, Robinson HP, Livesey FJ (2012)
. Human cerebral cortex development from pluripotent stem cells to functional excitatory synapses.

https://doi.org/10.1038/nn.3041 LINK

3.1 First, NPCs are induced by dual SMAD inhibition for 10 days, followed by culturing with the N2B27 media for another 15 days.

Note

* how to make 1L of N2B27 media:
100ml DMEM:F12 + glutamax, 5ml N2 supplement, 0.25ml Insulin, 5ml Non Essential Amion Acids, 1ml 2-mercaptoethanol, 2.5ml Pen/Strep, 500ml Neurobasal, 10ml B27, 5ml Glutamax, 2.5ml Pen/Strep

* how to make dual SMAD inhibition media: 10uM SB431542, 1uM Dorsomorphin in N2B27 media

Induction of glial precursor cells (GPCs),

4 NPCs are used to derive glial precursor cells (GPCs) by culturing in an N2B27 medium supplemented with 20 ng/ml of human FGF-2.

4.1 Cells are split twice per week (1:2 or 1:3) using Accutase (Cat No. A1110501, Thermo Fisher Scientific) by vigorously breaking pellets to remove neuronal cells.

Final differentiation into astrocytes

5 Upon the appearance of glial morphology (around day 90 from the neural induction), the GPCs are cultured for 7 days with 10 ng/ml Bone Morphogenetic Protein 4 (BMP4) and 20 ng/ml Leukemia inhibitory factor (LIF), which activates the JAK/STAT signalling pathway, refreshing the medium every other day

5.1 On the 8th day, BMP4 and LIF are withdrawn.

5.2 Cells are further differentiated for the final maturation in the N2B27 media without FGF2.

5.3 3-5 times further passes might be required (1:2) until the complete loss of the precursor property.

Citations

Gupta K, Patani R, Baxter P, Serio A, Story D, Tsujita T, Hayes JD, Pedersen RA, Hardingham GE, Chandran S. Human embryonic stem cell derived astrocytes mediate non-cell-autonomous neuroprotection through endogenous and drug-induced mechanisms.
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Step 1

Virdi GS, Choi ML, Evans JR, Yao Z, Athauda D, Strohbuecker S, Nirujogi RS, Wernick AI, Pelegrina-Hidalgo N, Leighton C, Saleeb RS, Kopach O, Alrashidi H, Melandri D, Perez-Lloret J, Angelova PR, Sylantyev S, Eaton S, Heales S, Rusakov DA, Alessi DR, Kunath T, Horrocks MH, Abramov AY, Patani R, Gandhi S. Protein aggregation and calcium dysregulation are hallmarks of familial Parkinson's disease in midbrain dopaminergic neurons.
https://doi.org/10.1038/s41531-022-00423-7

Step 2

Serio A, Bilican B, Barmada SJ, Ando DM, Zhao C, Siller R, Burr K, Haghi G, Story D, Nishimura AL, Carrasco MA, Phatnani HP, Shum C, Wilmut I, Maniatis T, Shaw CE, Finkbeiner S, Chandran S. Astrocyte pathology and the absence of non-cell autonomy in an induced pluripotent stem cell model of TDP-43 proteinopathy.
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Step 3

Shi Y, Kirwan P, Smith J, Robinson HP, Livesey FJ. Human cerebral cortex development from pluripotent stem cells to functional excitatory synapses.
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