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iPSC-derived organoid generation

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Irene Fernández-Carasa[1,2], Veronica Testa[1,2], Antonella Consiglio[1,2], Miquel Vila[3]

[1]Department of Pathology and Experimental Therapeutics, Bellvitge University Hospital- IDIBELL, 08908 Hospitalet de Llobregat, Spain;
[2]Institute of Biomedicine of the University of Barcelona (IBUB), Carrer Baldiri Reixac 15-21, Barcelona 08028, Spain.;
[3]VHIR-CIBERNED-ASAP

Vilalab Public

Miquel Vila
VHIR-CIBERNED-ASAP

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We use this protocol and it's working

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Abstract

iPSC-derived organoid generation

1 iPSCs are grown until 90% of confluence and 10 µM of RI is added to culture plate 4/6 hours before starting the protocol. Then, cells are dissociated in 1:1 accutase/DPBS, detached and centrifuged at 800x*rpm* for 5 minutes.

2 Cell pellet is resuspended in 1 mL of fresh mTeSR™1, passed through a 40 µm cell strainer (Clear Line®) and counted. Dissociated iPSCs are plated in ultra-low attachment 6-well plastic plate (Corning®) at a density of 4×10^6 cells per well, with mTeSR™1 medium supplemented with 10 µM of RI.

3 From then on, cells are kept under rotation on CO_2 resistant orbital shaker (Leica) at 120x*rpm* and maintained under normal incubation conditions.

4 After 2 days, medium is replaced with SRM supplemented with LDN and SB, a dual SMAD inhibition to promote neuronal commitment.

5 On day 4, and every 3 days, half of SRM medium is replaced with fresh factors and the addition of SAG, Purmorphamine, CHIR and AA.

6 On day 12, SRM medium is replaced by NBN2B27-vitA, which consisted of Neurobasal™ supplemented with 1x N2, 2x B27™-vitA, 1% of P/S and 1% of Glut. N2B27-vitA is changed every other day by the addition of 100 nM of LDN, 1 µM of SAG, 2 µM of Purmorphamine, CHIR and AA, 50 ng/mL of FGF-2, and 50 ng/mL of Endothelial Growth Factor (EGF; R&D Systems), to promote conversion towards a DAn fate.

7 From day 22 onwards, spheres are matured to vmO in B27-vitA supplemented with BDNF, GDNF, TGF-β3, cAMP and AA. Half of the medium is changed every 2-3 days and vmO are cultured until day 45, before being used for experiments.