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Non-guided neural organoids differentiation

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We use this protocol and it's working

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Abstract

This protocol describes how to perform non-guided neural organoid differentiation

hiPSCs lines (LINC01876 KD)

- 1 To generate the human cerebral-like organoids we followed the protocol detailed in [Johansson et al., 2022](#), based on the protocol published by [Lancaster et al., 2013](#).

We used three hiPSC6-derived lines obtained by transduction and FACS sorting as described in detail in the protocol [CRISPR inhibition of LINC01876 in hiPSCs and fbNPCs](#).

The cell lines included one control line (with a gRNA against LacZ) and two LINC01876 CRISPRi KD lines (guide 2 and guide 3).

Embroid body formation

- 2 8000 cells/well were plated in a 96-wells plate (Costar, Ultra Low Attachment, round bottom; REF 7007) with 250 μ L of mTeSR1 (StemCell Technologies, Inc.) and RY27632 10 μ M. This is considered day -5 of the differentiation of the iPSCs-derived non-guided neural organoids.
- 3 On days -3 and -1 the medium was changed (150 μ L and 200 μ L of mTeSR1, respectively).

Neural induction

- 4 At day 0 the cells are fed with Neural Induction Medium (NIM; DMEM/F12 media, N2 Supplement (1:100), L-Glutamine (2mM), Penicillin/Streptomycin (1:500), Non-Essential Amino acids (1:100) and Heparin (2ug/ml).) enriched with 3% KSR.
- 5 At day 2, 4, and 6, the organoids were fed with NIM with no added KSR.

Embedding

- 6 On day 8 the organoids were embedded in 30-50 μ L of Matrigel (Corning) and incubated at 37°C for 25 minutes to allow the Matrigel to solidify.

Differentiation

- 7 The organoids were then transferred in Corning REF 3471 6-wells plates with flat bottom containing 4ml/well of Cortical Differentiation Medium (CDM; F12 Media (-Glut) (48.5%), Neurobasal (48.5%), N2 Supplement (1:200), B27 Supplement (-Vit.A, 1:100), L-



- Glutamine (2mM), Penicillin/Streptomycin (1:500), Non-Essential Amino acids (1:200), Beta MercaptoEtOH (50uM) and Insulin (2.5 ug/mL)).
- 8 On day 10 and 12 of the differentiation, the medium was changed exchanging 3 ml/well for 3 mL of fresh CDM.
- 9 On day 15, 17, 19, 21 and 23, ~4 mL the medium was replaced with 4 mL of Improved Differentiation Medium + A (IDM, F12 Media (-Glut) (48.5%), Neurobasal (48.5%), N2 Supplement (1:200), B27 Supplement (+Vit.A, 1:50), L-Glutamine (2mM), Penicillin/Streptomycin (1:500), Non-Essential Amino acids (1:200), Beta MercaptoEtOH (50uM), Insulin (2.5 ug/mL) and Ascorbic Acid (400uM)).
- 10 From day 25, the medium was changed every 3 days with 3-4 mL of Cortical Terminal Differentiation Medium (CTDM, F12 Media (-Glut) (48.5%), Neurobasal (48.5%), N2 Supplement (1:200), B27 Supplement (+Vit.A) – (1:50) 800uL, L-Glutamine (2mM), Penicillin/Streptomycin (1:500), Non-Essential Amino acids (1:200), Beta MercaptoEtOH (50uM), Insulin (2.5 ug/mL) and Ascorbic Acid (400uM), BDNF (10ng/uL), cAMP (200uM), GDNF (10ng/uL)).

Organoids' size measurements

- 11 All the diameter measurements of the organoids were taken with the Measure tool from the Image J software. The chosen measuring unit was mm.