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Neuronal differentiation and MEA plating using an inducible NGN2 hiPSC-line

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Silke Frahm[1]

[1]Stem cell technology platform

Silke Frahm
Stem cell technology platform

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This protocol is an adaptation of the published Star protocol Peitz et al. 2020.

Citation

Peitz M, Krutenko T, Brüstle O (2020)
. Protocol for the Standardized Generation of Forward Programmed Cryopreservable Excitatory and Inhibitory Forebrain Neurons.

https://doi.org/10.1016/j.xpro.2020.100038 LINK

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

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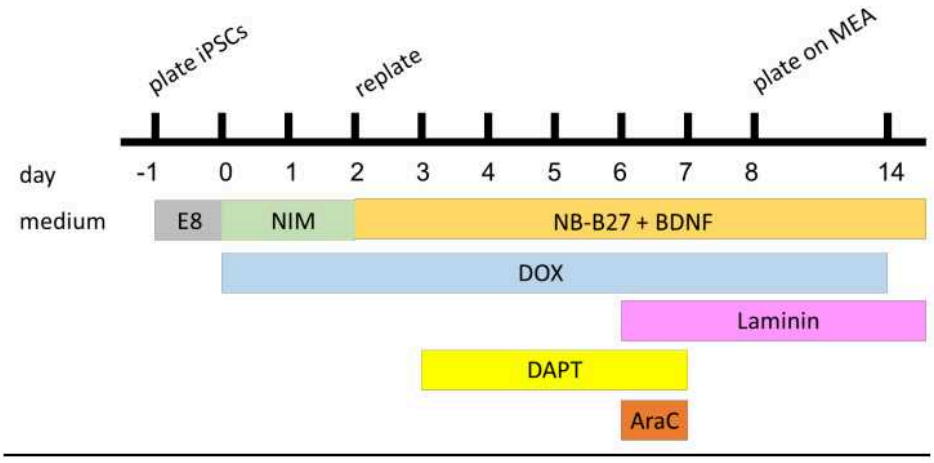
Abstract

This protocol details the neuronal differentiation and MEA plating using an inducible NGN2 hiPSC-line. Neurons generated using this protocol will start showing electrical activity 1-2 weeks after seeding on MEA plate.

Guidelines



- Adapted from Peitz et al. 2020, Star protocol

Well numbers for plating 1 × 48WP MEA:

- **Day -1:** 3 × 6W at 500k/well
- **Day 2:** 4 × 6W at 750k/well

Materials

Neuronal Induction Medium (NIM)

- DMEM/F-12 500 mL
- N2 supplement 5 mL
- Doxycycline 2 µg/mL (final concentration)

Neuronal Medium (NB-B27)

- Neurobasal medium 500 mL
- B-27 supplement (50x) 10 mL stable for one month at 4 °C
- GlutaMAX (100x) 5 mL

+

- BDNF 10 ng/mL (final concentration)
- DAPT 10µM (1:1000)
- Laminin 1:500 dilution
- AraC (stock 4mM, use 1:800)
- Doxycycline 2 µg/mL (final concentration) until day 14

BrainPhys Medium complete

- BrainPhys™ Neuronal Medium **STEMCELL Technologies Inc. Catalog #**05790
- B-27 supplement 1:50
- Penn/Strep 1:100
- BDNF 10ng/ml
- Laminin 1:1000
- GDNF 10ng/ml
- Ascorbic acid 200µM
- cAMP 500µM

Reagents:

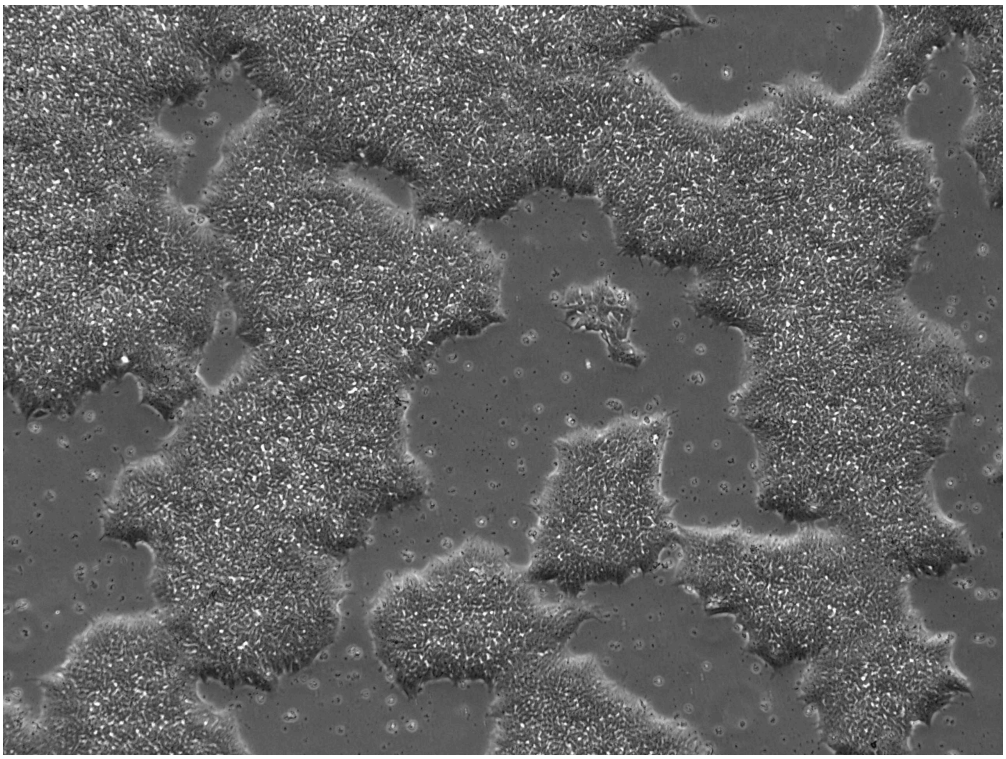
- Pierce™ Concentrated Buffer Stocks (10X and 20X) **Thermo Scientific Catalog #**28341
- Poly(ethyleneimine) solution **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**03880
- mouse Laminin (Sigma-Aldrich) Catalog #L2020
- DMEM/F12 (Gibco) Catalog #11320-033
- Neurobasal (Gibco) Catalog #21103-049

Procedure

2h 40m

1 **Day -1:**

Dissociate 80% confluent hPSCs with Accutase or TryplE and seed them on Geltrex-coated 6-well plates at a density of 450,000 cells/well in E8 supplemented with [M] 10 micromolar (µM) RI (2 mL /well).



Example of a 80% confluent iPSC culture.

2 **Day 0:**

Change medium to neuronal induction medium (NIM) supplemented with 2 µg/ml doxycycline (dox) to induce transgene expression. Dox is added freshly to the medium from now on. Start PO/laminin coating for neural progenitor split on day 2.

3 **Day 1:**

Change medium with NIM supplemented with 2 µg/ml doxycycline (dox).

4 **Day 2:**

10m

Dissociate cells with Accutase for 00:10:00 at 37 °C. Plate cells on PO/laminin-coated wells at a density 7.5 × 10^4 cells/cm for the generation of glutamatergic neurons. At this point, change to neuronal medium supplemented with 2 µg/ml dox and [M] 10 micromolar (µM) RI.

5 **Day 3:**

Medium change (dox-supplemented neuronal medium containing [M] 10 micromolar (µM) DAPT; DAPT remains in the medium from day 3 until day 7).

6 **Day 5:**

Medium change (dox-supplemented neuronal medium containing [M] 10 micromolar (µM) DAPT). From this day on, carefully remove the medium with a serological pipet instead of an aspirator to avoid cell detachment.

7 **Day 6:**

Medium change (dox-supplemented neuronal medium containing [M] 10 micromolar (µM) DAPT, [M] 5 micromolar (µM) Ara-C and 1:500 laminin). Ara-C will induce a growth arrest of remaining proliferative cells.

8 **Day 7:**

Medium change (dox-supplemented neuronal medium containing 1:500 laminin). Pre-treat MEA plate and dry o/n.

9 **Day 8: MEA plating:**

9.1 Remove complete medium and directly add (no PBS wash) pre-warmed Accutase (1 mL /6W) supplemented with [M] 10 micromolar (µM) RI, incubate for 01:00:00 at 37 °C . 1h

9.2 Wash off the cells with same amount of medium using a 10ml pipette, gently pipet up and down 5-10times, collect in 50ml Falcon tube.

9.3 Use same amount of medium to wash once more the wells and add to tube (at the end should be double volume medium:accutase).

9.4 Count cell number in suspension before spinning or after resuspending in max. 500 µL medium.

9.5 Spin down, resuspend in NB-B27 medium containing Laminin, BDNF, dox and RI at 50k cells per 8 µL (= 6,25Mio/ml).

9.6 Seed 8µl as a drop with the pipet tip in the center of the MEA well covering the electrodes (48WP pretreated with PEI) without touching them and incubate for 00:15:00 - 00:30:00 . 45m

9.7 After 30min, add Astrocytes in 10 µL medium at a ratio of 1:5 (astrocyte:neuron) and incubate for another 00:15:00 - 00:30:00 (10.000 cells in 10 µL per well = 1M/ml). 30m

9.8 Add 300 µL medium after 00:30:00 . 30m

9.9 Change medium next day (without RI) and half medium every other day thereafter.

9.10 After day 14, change gradually to BrainPhys medium complete (half medium changes 3x per week).

PO/Laminin coating 2d

10 Apply 15 µg/ml polyornithine (diluted in ddH_2O and then sterile-filtered) and incubate for 16:00:00 – 24:00:00 at 37 °C . 1d

11 Remove polyornithine and wash three times with PBS.

12 Apply laminin (diluted in DPBS++ 1:100) and incubate Overnight for 16:00:00 – 24:00:00 at 4 °C . 1d

Note

Use 1.5 mL /6W

MEA coating 1h

13 Dot 8 µL 0.07% PEI solution over the recording electrodes.

14 Incubate 01:00:00 at 37 °C , 5% CO_2. 1h

15 Wash 3-4x with ddH_2O (PEI is toxic).

16 Air dry Overnight in culture hood (no UV).

Preparing 0.07 % PEI solution

17 Dilute the 20x borate buffer to 1x in water (Thermo Fisher #28341).

18 Prepare a 0.07 % PEI solution in borate buffer using a 50 % PEI stock solution (Sigma: #03880), e.g. by diluting 28 µL of the 50%PEI stock solution in 20 mL 1x

Borate Buffer.

19 Filter the solution through a 0.22 µm filter, store 1 mL aliquots at -20 °C .

Protocol references

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Citations

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