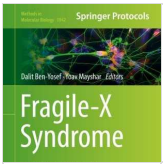

Sep 02, 2021

3.1 Human iPSC Culture

Book Chapter

In 1 collection

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ABSTRACT

This is part 3.1 of the "Induced Neurons for the Study of Neurodegenerative and Neurodevelopmental Disorders" collection of protocols.

Collection Abstract: Patient-derived or genomically modified human induced pluripotent stem cells (iPSCs) offer the opportunity to study neurodevelopmental and neurodegenerative disorders. Overexpression of certain neurogenic transcription factors (TFs) in iPSCs can induce efficient differentiation into homogeneous populations of the disease-relevant neuronal cell types. Here we provide protocols for genomic manipulations of iPSCs by CRISPR/Cas9. We also introduce two methods, based on lentiviral delivery and the piggyBac transposon system, to stably integrate neurogenic TFs into human iPSCs. Furthermore, we describe the TF-mediated neuronal differentiation and maturation in combination with astrocyte cocultures.

ATTACHMENTS

Sauter2019_Protocol_InducedNeuronsForTheStudyOfNeu.pdf

DOI

dx.doi.org/10.17504/protocols.io.bqhbmt2n

EXTERNAL LINK

https://link.springer.com/protocol/10.1007/978-1-4939-9080-1_9

PROTOCOL CITATION

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COLLECTIONS

Induced Neurons for the Study of Neurodegenerative and Neurodevelopmental Disorders

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KEYWORDS

Human induced pluripotent stem cells, Nucleofection, PiggyBac transposon, Lentiviral transduction, CRISPR/Cas9, Transcription factor-mediated neuronal differentiation, Astrocyte coculture

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PARENT PROTOCOLS

Part of collection

Induced Neurons for the Study of Neurodegenerative and Neurodevelopmental Disorders

MATERIALS TEXT

2. Materials

2.1 Human iPSC Culture

1. Human iPSC line with or without genomic modifications (e.g., PGP1 cells (Personal Genome Project iPS cell line, derived from Participant #1 (PGP1, hu43860C)), can be obtained from Coriell #GM23338, the matching primary fibroblast line is #GM23248) or iNGN cells (modified from PGP1, contains the neurogenic TFs Neurogenin-1 and Neurogenin-2 under the control of the doxycycline-inducible promoter. This cell line is part of the ENCODE catalogue (https://www.encodeproject.org) #ENCBS369AAA [4]).
2. Coating medium: DMEM medium, [M]**1 % penicillin–streptomycin** . Store at **4 °C** .
3. Matrigel hESC-qualified matrix (Corning). Store aliquots at **-20 °C** (*see* **Note 1**).
4. mTeSR™1 medium: **400 mL mTeSR™1 Basal medium (Stemcell Technologies)** ,

   100 mL mTeSR™1 5× Supplement (Stemcell Technologies) ,

   5 mL penicillin–streptomycin , sterile-filter (0.45 µm). Store at **4 °C** for a maximum of 2 weeks. Do not prewarm before usage.
5. mTeSR™1 medium with ROCK inhibitor (ROCKi): add ROCK inhibitor to mTeSR™1 medium ([M]**3.3 µg/mL** final concentration). Store at **4 °C** for a maximum of 2 weeks.
6. Dissociation reagent: TrypLE Express (Thermo Fisher Scientific). Store at **Room temperature** .
7. Freezing medium: mFreSR™ (Stemcell Technologies). Store aliquots at **-20 °C** .
8. [M]**1 x PBS pH 7.2** without calcium and magnesium. Store at **Room temperature** .

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SAFETY WARNINGS

For hazard information and safety warnings, please refer to the SDS (Safety Data Sheet).

BEFORE STARTING

NB Introduction, Notes, and References are in the Collection Guidelines tab

3.1 Human iPSC Culture 2d 2h 50m

1 For the coating of cell culture plates, resuspend one aliquot of Matrigel in the appropriate amount of cold coating medium (*see* **Note 1**). Add the Matrigel solution to the cell culture plates and distribute equally so that the entire well is covered (*see* **Note 6**).

2  45m

Incubate at **Room temperature** for at least **00:45:00** . Use immediately or store at **4 °C** for a maximum of 2 weeks. Prior to use, simply aspirate the coating medium and add the cell suspension. No washing step is required.

3 To thaw iPSCs, get the frozen vials from the liquid nitrogen tank and keep on dry ice. Thaw carefully in a **37 °C** water bath or alternatively with the ThawSTAR™ Automated Cell Thawing System (BioCision™) until only a small ice cube remains.

4 

Transfer the cell solution to a 15 ml Falcon tube and add dropwise **2 mL** – **3 mL** **1× PBS w/o Ca2+ and Mg2+** .

5 

Spin down at **400 x g, 00:04:00** and aspirate the supernatant.

6 

Resuspend the cell pellet in mTeSR™1 with ROCKi and transfer to a cell culture plate coated with Matrigel and place in the incubator (**37 °C** , **5 %** **CO2**).

7  1d

After **24:00:00** , wash the cells once with **1 x PBS w/o Ca2+ and Mg2+** and change the medium to mTeSR™1 w/o ROCKi. Change the medium every day until the next passaging (*see* **Note 7**).

8  5m

In order to passage iPSCs, aspirate the culture medium and wash the cells once with **1 x PBS w/o Ca2+ and Mg2+** . Dissociate the cells by adding TrypLE and place in the incubator for approximately **00:02:00** – **00:03:00** .

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9

Add [M]**1 x PBS w/o Ca2+ and Mg2+** and pipet up and down to collect all cells.

10

Transfer the cell solution to a 15 ml Falcon tube and spin down at **400 x g, 00:04:00** . Aspirate the supernatant and resuspend the cell pellet in mTeSR™1 with ROCKi.

11

Count the cells using Trypan Blue (e.g., with the Countess™ II FL Automated Cell Counter or hemocytometer) and seed the appropriate number of cells in Matrigel- or poly-L-lysine + laminin-coated cell culture plates (*see* **Note 8**). Mix well and place in the incubator (**37 °C** , [M]**5 % CO2**).

1d

12

After **24:00:00** , wash the cells once with [M]**1 x PBS w/o Ca2+ and Mg2+** and change the medium to mTeSR™1 w/o ROCKi. Change the medium every day until next passaging (*see* **Note 7** and Fig. 1). It is recommended to check the iPSCs regularly for mycoplasma contamination (*see* **Note 9**).

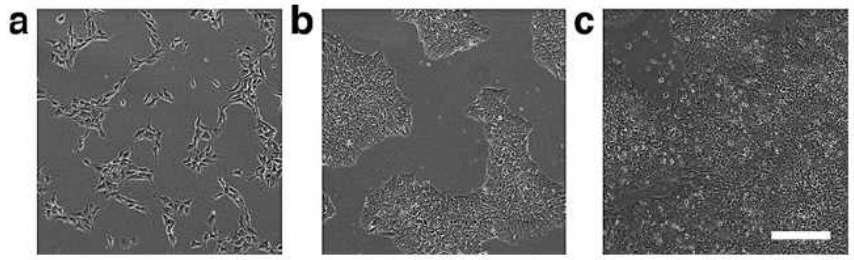

Fig. 1 Representative images of human iPSCs [4] grown on Matrigel-coated cell culture plates. (**a**) iPSCs 1 day after passaging in mTeSR™1 with ROCKi. (**b**) iPSC colonies in mTeSR™1 w/o ROCKi. (**c**) Confluent iPSC colonies. Scale bar represents 200 µm

13

To freeze iPSCs, dissociate the cells with TrypLE and collect in [M]**1 x PBS w/o Ca2+ and Mg2+** , spin down and resuspend the pellet in mFreSR™ medium. If necessary, count the cells and aliquot the appropriate amount into cryotubes (*see* **Note 10**).

14 Put the tubes in a freezing container and store at **-80 °C** for at least **02:00:00** . Subsequently, store in liquid nitrogen.

2h

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