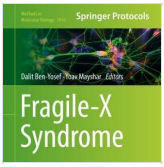

Sep 02, 2021

3.6 Coculturing with Astrocytes

Book Chapter

In 1 collection

Evelyn J. Sauter[1], Lisa K. Kutsche[1], Simon D. Klapper[1], Volker Busskamp[1]

[1]Center for Regenerative Therapies, Technische Universität Dresden, Dresden, Germany

dx.doi.org/10.17504/protocols.io.bqhhmt36

Springer Nature Books

satyavati Kharde

ABSTRACT

This is part 3.4 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 cell s (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.bqhhmt36

EXTERNAL LINK

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

PROTOCOL CITATION

Evelyn J. Sauter, Lisa K. Kutsche, Simon D. Klapper, Volker Busskamp 2021. 3.6 Coculturing with Astrocytes. **protocols.io** https://dx.doi.org/10.17504/protocols.io.bqhhmt36

MANUSCRIPT CITATION please remember to cite the following publication along with this protocol

Sauter E.J., Kutsche L.K., Klapper S.D., Busskamp V. (2019) Induced Neurons for the Study of Neurodegenerative and Neurodevelopmental Disorders. In: Ben-Yosef D., Mayshar Y. (eds) Fragile-X Syndrome. Methods in Molecular Biology, vol 1942. Humana Press, New York, NY. https://doi.org/10.1007/978-1-4939-9080-1_9

COLLECTIONS

Induced Neurons for the Study of Neurodegenerative and Neurodevelopmental Disorders

Citation: Evelyn J. Sauter, Lisa K. Kutsche, Simon D. Klapper, Volker Busskamp (09/02/2021). 3.6 Coculturing with Astrocytes. https://dx.doi.org/10.17504/protocols.io.bqhhmt36

KEYWORDS

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

CREATED

Dec 07, 2020

LAST MODIFIED

Sep 02, 2021

OWNERSHIP HISTORY

Dec 07, 2020	Lenny Teytelman	protocols.io
Jul 05, 2021	Emma Ganley	protocols.io
Aug 24, 2021	Satyavati Kharde	
Aug 26, 2021	satyavati Kharde	

PROTOCOL INTEGER ID

45321

PARENT PROTOCOLS

Part of collection

Induced Neurons for the Study of Neurodegenerative and Neurodevelopmental Disorders

Citation: Evelyn J. Sauter, Lisa K. Kutsche, Simon D. Klapper, Volker Busskamp (09/02/2021). 3.6 Coculturing with Astrocytes. https://dx.doi.org/10.17504/protocols.io.bqhhmt36

MATERIALS TEXT

2. Materials

2.6 Coculturing with Astrocytes

1. Rat primary cortical astrocytes (Thermo Fisher Scientific).
2. Astrocyte medium: DMEM + [M]**4.5 g/L D-Glucose** + [M]**1 Milimolar (mM) Pyruvate supplemented with N2 Supplement** (Thermo Fisher Scientific), [M]**10 % OneShot fetal bovine serum** (Thermo Fisher Scientific) and [M]**1 % penicillin–streptomycin** . Store aliquots at **-20 °C** . After thawing, working aliquots can be stored at **4 °C** for a maximum of 2 weeks.
3. Accutase. Store aliquots at **-20 °C** .
4. [M]**1 Molarity (M) HCl** . Store at **Room temperature** .
5. [M]**100 % ethanol** . Store at **Room temperature** .
6. Low-melting paraffin. Store at **Room temperature** .
7. [M]**1 x PBS with calcium and magnesium** . Store at **4 °C** .
8. Ara-C: dissolve **11 mg cytosine β-D-arabinofuranoside hydrochloride powder** in **15 mL ddH2O** ([M]**2.5 Milimolar (mM)** = [M]**500 x**), sterile-filter (0.22 µm). Store aliquots at **-20 °C** , after thawing store at **4 °C** for several weeks, protected from light.
9. [M]**1 % BSA** : **8.7 mL BrainPhys™ Neuronal Medium** (Stemcell Technologies) supplemented with **128 µl 1 M HEPES** and **1.33 µl 7.5% BSA** . Prewarm and use immediately.
10. [M]**0.2 % BSA** : **4 mL BrainPhys™ Neuronal Medium** (Stemcell Technologies) supplemented with **59 µl 1 M HEPES** and **1 mL 1% BSA** from previous step. Prewarm and use immediately.
11. Minimal maturation medium (*see* **Note 5**): **10 mL BrainPhys™ Neuronal Medium** (Stemcell Technologies) supplemented with **200 µl NeuroCult™ SM1 Neuronal Supplement** (Stemcell Technologies), **100 µl N2 Supplement-A** (Stemcell Technologies), **50 µl 40 mM ascorbic acid (dissolved in ddH2O)** to a final concentration of [M]**200 Nanomolar (nM)** (Sigma) and **100 µl 100 × penicillin–streptomycin** (*see* **Note 4**). Mix thoroughly. Store at **4 °C** for a maximum of 2 weeks.

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

3.6 Coculturing with Astrocytes 1d 6h 16m

1 In order to increase the maturation of neurons for electrophysiological measurements, coculturing with astrocytes is highly recommended [4, 15]. We adapted the protocol from Kaech and Banker [16] to our cell culture.

2

Rat primary cortical astrocytes are cultured in astrocyte medium at **37 °C** and [M]**5 % CO2** according to the manufacturer's instructions.

Citation: Evelyn J. Sauter, Lisa K. Kutsche, Simon D. Klapper, Volker Busskamp (09/02/2021). 3.6 Coculturing with Astrocytes. https://dx.doi.org/10.17504/protocols.io.bqhhmt36

3

For passaging, aspirate the culture medium and store it in a Falcon tube as a washing solution (*see* **Note 26**). Rinse the cells once with [M] **1 x PBS w/o Ca2+ and Mg2+** .

4

5m

Add prewarmed Accutase and incubate the cells at **37 °C** until all of them are detached (usually **00:05:00** are sufficient).

5

Stepwise add the cell culture medium stored in step 3 to flush cells and collect all cells to a prerinsed 15 ml Falcon tube.

6

Centrifuge at **400 x g, 00:05:00** . Aspirate the supernatant and resuspend the pellet in prewarmed astrocyte growth medium.

7

Count the cells using Trypan Blue and seed the appropriate amount in uncoated tissue-culture treated dishes at a seeding density of approximately 5000 cells per cm^2. Change the growth medium every 3–4 days.

8

For the coculture with neurons, prepare astrocytes to be ~80% confluent at day 4 of neuronal differentiation. One day before the reseeding of neurons, wash the astrocytes *three times* with [M] **1 x PBS w/o Ca2+ and Mg2+** and add BrainPhys™ medium with minimal supplements.

9

4h

Thoroughly clean the coverslips in a big glass petri dish. First, rinse the coverslips in ddH_2O for **02:00:00** and then shake in **50 mL 1 M HCl** **Overnight** .

9.1

2h 6m

Rinse *three times* with ddH_2O by shaking for **00:02:00** , and rinse *another two times* with ddH_2O by shaking for **02:00:00** . Shake *three times* in [M] **100 % ethanol** for **00:02:00** and one time **Overnight** .

9.2

22h

Sterilize the coverslips at **225 °C** for **06:00:00** – **16:00:00** (can be done overnight) (*see* **Note 27**) [16].

Citation: Evelyn J. Sauter, Lisa K. Kutsche, Simon D. Klapper, Volker Busskamp (09/02/2021). 3.6 Coculturing with Astrocytes. https://dx.doi.org/10.17504/protocols.io.bqhhmt36

10 

Autoclave ~ **100 mL low melting paraffin** in a 500 ml bottle. Melt in a boiling water bath (1 l beaker with **400 mL H2O** on a heat plate with **350 °C**). Use a 2 ml aspiration pipette with a 200 µl pipette tip attached and soak it in melted paraffin. Shake off extra drops and place small drops on coverslips to create paraffin feet as spacer (*see* **Note 28**). Coat the coverslips with PLL and laminin (*see* protocol 3.5) on the side with the paraffin dots.

11 Differentiate the iPSCs on Matrigel-coated cell culture dishes using **0.5 µg/mL doxycycline in mTeSR™1 medium** for 4 days (*see* protocol 3.5). Add **5 Micromolar (µM) Ara-C** to the culture for 1 day to remove occasionally undifferentiated cells.

12 

At day 5, reseed predifferentiated neurons on PLL and laminin coated coverslips with paraffin feet. Collect the old medium from the culture well and wash the cells very carefully with prewarmed **1 x PBS w/o Ca2+, Mg2+** .

13 

5m

Dissociate the cells by adding Accutase and place in the incubator for approximately **00:05:00** until the neuronal network detaches. Add the cell culture medium stored in step 3 and transfer the cell solution to a 15 ml Falcon tube.

14 

Rinse the well *1–2 times* with **1 % BSA** to collect all cells and centrifuge at **400 x g, 00:05:00** .

15 Aspirate the supernatant and resuspend the cell pellet in **200 µl 0.2% BSA** slowly and carefully (this step is crucial for the survival of single cells).

16 

Add **800 µl 0.2% BSA** to a total volume of **1 mL** .

17 

Centrifuge at **20 x g, 00:01:00** and collect **800 µl** supernatant in a fresh tube (this is the single cell suspension).

18 Repeat the dissociation of the pellet for a maximum of five times until no pellet is visible any more. **go to step #13**

19 

Centrifuge the single cell suspension at **400 x g, 00:05:00** and resuspend the pellet in **0.5 mL BrainPhys™ medium** . Count the cells if necessary and seed on coated coverslips on the side with the

Citation: Evelyn J. Sauter, Lisa K. Kutsche, Simon D. Klapper, Volker Busskamp (09/02/2021). 3.6 Coculturing with Astrocytes. https://dx.doi.org/10.17504/protocols.io.bqhhmt36

paraffin feet (*see* **Note 29**).

20 After 02:00:00 , place the coverslips with the differentiated iPSCs upside down into culture wells containing 80% confluent rat astrocytes. Every 7 days, exchange 50% of the BrainPhys™ medium and compensate the volume loss due to evaporation with ddH_2O (*see* **Note 30**). 2h

Citation: Evelyn J. Sauter, Lisa K. Kutsche, Simon D. Klapper, Volker Busskamp (09/02/2021). 3.6 Coculturing with Astrocytes. https://dx.doi.org/10.17504/protocols.io.bqhhmt36