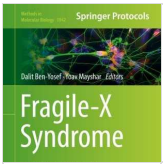

Sep 02, 2021

3.5 Doxycycline-Induced Differentiation

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.bqhgmt3w

Springer Nature Books

satyavati Kharde

ABSTRACT

This is part 3.3 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.bqhgmt3w

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. 3.5 Doxycycline-Induced Differentiation. **protocols.io** https://dx.doi.org/10.17504/protocols.io.bqhgmt3w

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

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

45320

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). 3.5 Doxycycline-Induced Differentiation. https://dx.doi.org/10.17504/protocols.io.bqhgmt3w

MATERIALS TEXT

2. Materials

2.5 Doxycycline-Induced Differentiation

1. Poly-L-lysine (PLL) solution: Dilute PLL hydrobromide in ddH_2O to a stock concentration of **[M]1 mg/mL** . Store at **4 °C** .
2. Laminin solution: **[M]1 mg/mL stock** . Store aliquots at **-20 °C** .
3. **[M]1 x PBS** with calcium and magnesium. Store at **4 °C** .
4. Doxycycline solution: dissolve **10 mg doxycycline hyclate powder** in **20 mL PBS** (**[M]0.5 mg/mL** = **[M]1000 x**), sterile-filter (0.22 µm). Store aliquots at **-20 °C** ; after thawing store at **4 °C** , protected from light.
5. Differentiation medium: mTeSR™1 medium supplemented with **[M]0.5 µg/mL doxycycline** . Store at **4 °C** for a maximum of 2 weeks.
6. Maturation medium: **10 mL BrainPhys™ Neuronal Medium** (Stemcell Technologies) supplemented with **200 µl Neuro- Cult™ SM1 Neuronal Supplement** (Stemcell Technologies), **100 µl N2 Supplement-A** (Stemcell Technologies), **20 µl 10 µg/mL recombinant Human BDNF** to a final concentration of **[M]20 ng/mL** (Peprotech), **20 µl 10 µg/mL recombinant Human GDNF** to a final concentration of **[M]20 ng/mL** (Peprotech), **98 µl 50 mg/ml dibutyryl cAMP** to a final concentration of **[M]1 Milimolar (mM)** (Sigma), **50 µl 40 mM ascorbic acid** 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

3.5 Doxycycline-Induced Differentiation 4h

1

Neurons can be grown on Matrigel-coated cell culture dishes, however, especially for long-term neuronal differentiation, it is recommended to grow the neurons on cell culture plates coated with poly-L-lysine (PLL) and laminin. Dilute the PLL in ddH_2O to a final concentration of **[M]40 µg/mL** , add the diluted PLL solution to the cell culture plates and distribute equally so that the entire well is covered.

2

Incubate at **37 °C** **Overnight** .

3

Wash *three times* with ddH_2O on the next day.

4 4h

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Dilute the laminin in [M]**1 x PBS with Ca2+ and Mg2+** to a final concentration of [M]**20 µg/mL** and add to the PLL-coated cell culture plates. Incubate at **37 °C** for approximately **04:00:00** . Prior to use, simply aspirate the coating solution and seed the cells without washing the plates.

5 Seed the iPSCs at a density of 30,000–50,000 cells per cm^2 in mTeSR™1 medium with ROCKi supplemented with [M]**0.5 µg/mL doxycycline** .

6

On the next day, wash the cells with [M]**1 x PBS w/o Ca2+ and Mg2+** and change the medium to mTeSR™1 w/o ROCKi supplemented with [M]**0.5 µg/mL doxycycline** . Change the medium daily until day 4 (Fig. 4).

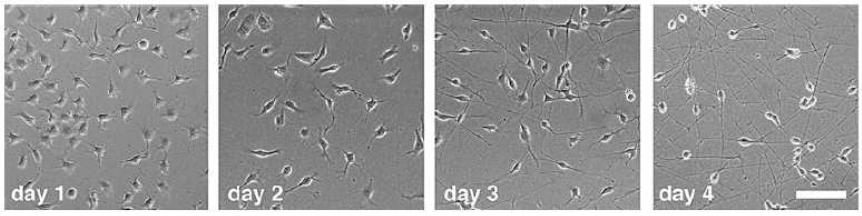

Fig. 4 Representative images of neuronal differentiation of human iPSCs expressing the neurogenic TFs Neurogenin-1 and Neurogenin-2 (iNGN cells) under the control of a doxycycline-inducible promoter [4]. Scale bar represents 100 µm

7 When culturing the neurons for longer time periods, it is recommended to change the stem cell medium (mTeSR™1) to maturation medium (BrainPhys™ with supplements).

7.1 Change half of the medium on day 5 of differentiation to BrainPhys™ medium with supplements. Repeat changing half of the medium 2 days later.

7.2 After those two adaptation medium changes, it is sufficient to change half of the medium once per week. Volume loss due to evaporation should be compensated with ddH_2O.

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