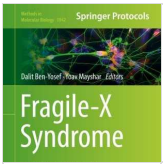

Sep 02, 2021

3.2 Nucleofection of iPSCs

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.bqhcmt2w

Springer Nature Books

satyavati Kharde

ABSTRACT

This is part 3.2 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.bqhcmt2w

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. 3.2 Nucleofection of iPSCs. **protocols.io** https://dx.doi.org/10.17504/protocols.io.bqhcmt2w

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). 3.2 Nucleofection of iPSCs. https://dx.doi.org/10.17504/protocols.io.bqhcmt2w

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

45316

PARENT PROTOCOLS

Part of collection

Induced Neurons for the Study of Neurodegenerative and Neurodevelopmental Disorders

MATERIALS TEXT

2. Materials

2.2 Nucleofection of PiggyBac Plasmids

1. 4D-Nucleofector™ System: 4D-Nucleofector™ Core Unit and 4D-Nucleofector™ X-Unit (Lonza).
2. P3 Primary Cell 4D-Nucleofector™ X Kit: Containing the Nucleofector™ Solution, Supplement and 100 µl Cuvettes (Lonza). Store the Nucleofector™ Solution and the Supplement at **4 °C**.
3. PiggyBac vector containing the gene of interest under the control of a doxycycline-inducible promoter, such as Addgene plasmid #104454 (*see* **Note 2** and Fig. 2a). Store at **-20 °C**.
4. Transposase vector, such as System Biosciences #PB210PA-1. Store at **-20 °C**.
5. Antibiotic: If you would like to select the cells for the integrated piggyBac construct, use the appropriate antibiotic (e.g., blasticidin or puromycin). Store aliquots at **-20 °C**. After thawing store at **4 °C**, protected from light.
6. PiggyBac copy number kit (System Biosciences #PBC100A-1) including UCR1 primer mix, PBcopy primer mix, and cell lysis buffer. Store at **4 °C**.
7. Real time PCR master mix, such as Power SYBR® Green PCR Master Mix (Thermo Fisher Scientific). Store at **4 °C**.
8. Real time PCR system, such as StepOnePlus™Real-Time PCR System (Thermo Fisher Scientific).

SAFETY WARNINGS

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

BEFORE STARTING

Citation: Evelyn J. Sauter, Lisa K. Kutsche, Simon D. Klapper, Volker Busskamp (09/02/2021). 3.2 Nucleofection of iPSCs. https://dx.doi.org/10.17504/protocols.io.bqhcmt2w

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

Nucleofection of iPSCs 2d 0h 16m

1 In order to electroporate piggyBac and transposase vectors into iPSCs in suspension, use the X-Unit of the 4D-Nucleofector™ System in combination with the P3 Primary Cell 4D-Nucleofector™ X Kit according to the manufacturer's guidelines.

2

First of all, prepare the DNA, the Nucleofector™ solution and the cell culture plates. For a nucleofection reaction in 100 µl cuvettes, mix **10 µg piggyBac vector** and **2.5 µg transposase vector** in less than **10 µl** volume (maximum **10 %** of the final sample volume) in a 1.5 ml tube.

3

In a separate tube, mix **82 µl Nucleofector™ solution** with **18 µl supplement** per nucleofection reaction and bring to **Room temperature** . Prepare Matrigel-coated cell culture plates with the desired volume of mTeSR™1 medium with ROCKi and prewarm in the incubator (*see* **Note 11**).

4 Switch on the X-Unit of the 4D-Nucleofector™ System and choose the cell-type specific program for the human embryonic stem cell line H9, the cuvette size, P3 primary solution and the pulse CB-156 or CB-150 (*see* **Note 12**).

5

Dissociate the cells to be nucleofected using TrypLE, centrifuge (**400 x g, 00:04:00**) and resuspend in mTeSR™1 with ROCKi.

6

Determine the cell number, transfer 800,000 cells for each nucleofection into a 1.5 ml tube and centrifuge (**400 x g, 00:04:00**).

7

Aspirate the supernatant and resuspend the cells in **100 µl room temperature Nucleofector™solution with supplement** , mix with the DNA and transfer into an electroporation cuvette and close the lid. Avoid air bubbles while pipetting. Gently tap the cuvette to make sure that the sample covers the bottom.

8 Quickly put the cuvette(s) into the Nucleofector™ and press the start button to apply the pulse CB-156 or CB-150.

9

Immediately after, carefully remove the samples, add mTeSR™1 with ROCKi into the cuvette, mix by gently pipetting up

Citation: Evelyn J. Sauter, Lisa K. Kutsche, Simon D. Klapper, Volker Busskamp (09/02/2021). 3.2 Nucleofection of iPSCs. https://dx.doi.org/10.17504/protocols.io.bqhcmt2w

and down two to three times and transfer the complete solution onto a Matrigel-coated plate with prewarmed medium and place in the incubator (*see* **Note 13**).

10 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. Change the medium every day until next passaging (*see* Fig. 2b). Starting **48:00:00** after nucleofection, select the cells with an integrated construct with the appropriate antibiotic (*see* **Note 14**). 2d

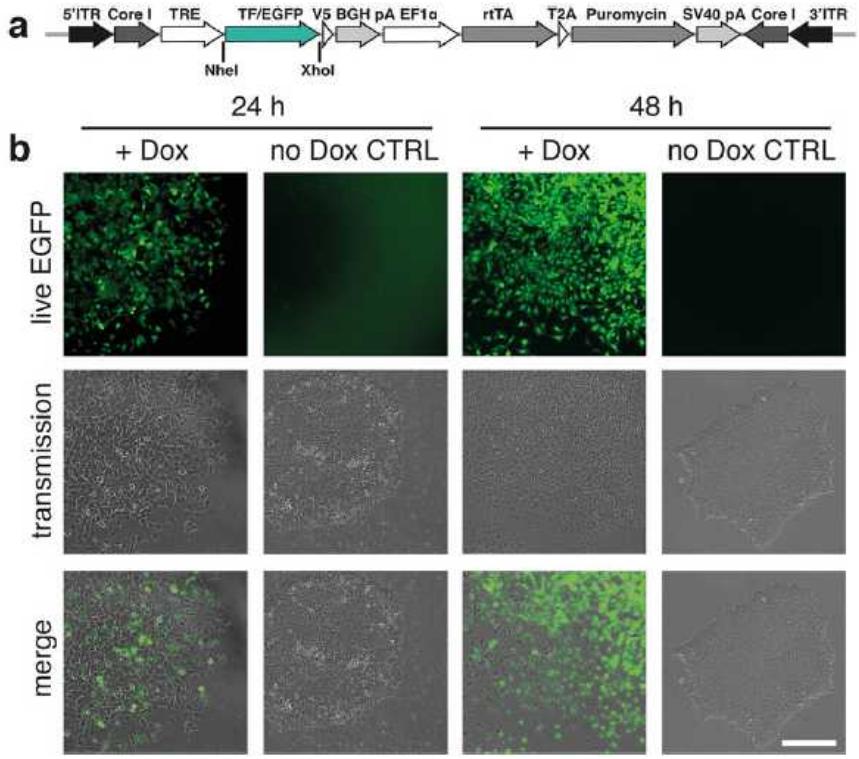

Fig. 2 Nucleofection of iPSCs with the piggyBac plasmid. (**a**) Schematic representation of the piggyBac plasmid containing the 50 and 30 inverted terminal repeats (ITR), core insulator (Core I), the doxycyclineinducible TRE promoter driving the expression of a transcription factor (TF) or in our example of EGFP which can be excised be the restriction enzymes NheI and XhoI and replaced by a TF of interest, followed by a V5 tag and a bGH poly A signal. Furthermore, the plasmid contains an EF1α promoter driving the expression of the doxycycline-sensitive transactivator rtTA followed by a T2A signal and a puromycin resistance gene. (**b**) Representative images of iPSCs nucleofected with the plasmid depicted in (**a**) 24 and 48 h after doxycycline (Dox) induction and respective controls without doxycycline (no Dox CTRL). Scale bar represents 200 µm

11

In order to determine the number of the integrated piggyBac constructs, use the piggyBac copy number kit from System Biosciences (*see* **Note 15**). To prepare genomic DNA, seed the cells in a 12-well plate (*see* **Note 16**). When confluent, wash once with [M] **1 x PBS w/o Ca2+ and Mg2+** and add **250 µl lysis buffer** to each well.

12 Freeze the cells at **-80 °C** and thaw the plate at **Room temperature** to ensure complete cellular lysis.

13 2m

Detach the cells by pipetting up and down, transfer the lysates to 1.5 ml tubes and heat them at **95 °C** for

Citation: Evelyn J. Sauter, Lisa K. Kutsche, Simon D. Klapper, Volker Busskamp (09/02/2021). 3.2 Nucleofection of iPSCs.
https://dx.doi.org/10.17504/protocols.io.bqhcmt2w

00:02:00 .

14

Centrifuge at **17000 x g, 00:02:00** and transfer the supernatant to a new 1.5 ml tube. The lysates should be placed **On ice** if used immediately or stored at **-20 °C** .

15

Prepare two master mixes of **4.75 µl ddH2O** , **6.25 µl SYBR® Green** , and **0.5 µl primers** per sample (one master mix with UCR1 primers for genomic DNA detection and one with PBcopy primers for piggyBac detection).

16

Aliquot **12 µl master mix** per well of a 96-well plate and add **0.5 µl lysate** (≤ **500 ng DNA**). Seal the plate, carefully, mix by vortexing and briefly spin down.

14m

17

Run the qPCR with the following program:
00:02:00 at **50 °C** ,
00:10:00 at **95 °C** ,
40 cycles of **95 °C** for **00:00:15** and **60 °C** for **00:01:00** ,
followed by **00:00:15** at **95 °C** ,
00:00:15 at **60 °C** , and **00:00:15** at **95 °C** (*see***Note 17**).

18

Calculate the copy number as follows [11]:
$\Delta\Delta C_t = 2^{(\text{average PBcopy Ct} - \text{average UCR1 Ct})}$, divide the ΔΔCt by 2 as there are two copies of the UCR1 sequence per genome.

Citation: Evelyn J. Sauter, Lisa K. Kutsche, Simon D. Klapper, Volker Busskamp (09/02/2021). 3.2 Nucleofection of iPSCs. https://dx.doi.org/10.17504/protocols.io.bqhcmt2w