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iPSC editing with TALENs

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1 Works for me dx.doi.org/10.17504/protocols.io.8dahs2e

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THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION

Tian et al (2019). CRISPR Interference-Based Platform for Multimodal Genetic Screens in Human iPSC-Derived Neurons. Neuron pii: S0896-6273(19)30640-3. [Epub ahead of print] PubMed PMID: 31422865.

Editing_iPSCs_with_TALENs.pdf

MATERIALS

NAME	CATALOG #	VENDOR
pZT-C13-L1	62196	addgene
pZT-C13-R1	62197	addgene
DPBS (no Ca, no Mg)	14190144	Thermofisher
Essential 8™ Medium	A1517001	Gibco, ThermoFisher
StemPro™ Accutase™ Cell Dissociation Reagent	A1110501	Thermo Fisher Scientific
Lipofectamine™ Stem Transfection Reagent	STEM00008	Thermo Fisher Scientific
StemFlex™ Medium	A3349401	Thermo Fisher Scientific
Matrigel	356231	Corning
KnockOut™ DMEM	10829018	Thermo Fisher Scientific
Opti-MEM™ I Reduced Serum Medium	31985070	Thermo Fisher Scientific
Y-27632 dihydrochloride (Rock Inhibitor)	1254/10	R&D Systems

MATERIALS TEXT

- BCL-XL plasmid (pEF1-BCL-XL-wpre-polyA P1102): Gift from Xiaobing Zhang, described in PMID: 30239926

SAFETY WARNINGS

Please see SDS (Safety Data Sheet) for hazards and safety warnings.

Pre-coating 6 well plate

1 

Pre-coat 6 well plate with Matrigel (**diluted 1:50** with Knockout DMEM) by adding **1 ml** to each well.

2 

Let it sit in incubator for at least **00:20:00** .

Passaging and Plating iPSC Cells

3 Grow iPSCs until they are **~ 85 %** confluent in one well of a 6 well plate or larger format.

4 

Remove old medium and wash iPSCs with DPBS 1x.

5 Add Accutase for **00:03:00** – **00:05:00** to lift the cells.

6 

Singularize the cells by gently pipetting them up and down several times.

7 In a 15 ml conical tube, add DPBS and then add the lifted cells in Accutase.

8 

Spin down at **200 x g** for **00:05:00** .

9 Aspirate supernatant and resuspend cells in Stemflex.

10 Perform cell count.

11 Remove the Matrigel in the pre-coated plate and add appropriate amount of Stemflex medium with Rock inhibitor (1000x).

12 Re-seed 0.5 M cells into a 6 well plate so that it can reach **~ 60 %** confluency the next day.

13 

Put the plate in incubator and culture overnight.

Transfection

14 The following day, change the media with new E8 media.

15 Prepare the following mixes:

Tube 1:

- **100 µl Opti-Mem**
- **10 µl Lipofectamine Stem Reagent:**

Tube 2:

- **100 µl Opti-Mem**
- DNA mix (**0.5 µg** – **5 µg** total)

  Optimized TALENS ratio
  - **1.5 µg Your Plasmid of choice**
  - **0.75 µg TALENS L**
  - **0.75 µg TALENS R**
  - **0.3 µg BCL-XL**

16 

Add tube 2 to tube 1 and mix well.

17 

Incubate the mixture for **00:10:00** .

18 Add the whole mixture to your cells in the 6 well plate.

19 

Incubate overnight and change the media to Stemflex the next day.

20 If confluent, passage and expand them into 10 cm plate.

21 When confluent in 10 cm plate, continue to selection protocol.