iPSC editing with TALENs

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Protocol status: Working
 We use this protocol and it's working

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iPSC editing with TALENs

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Neurodegeneration Method Development Community

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ATTACHMENTS

[Editing_iPSCs_with_TALENs.pdf](#)

MATERIALS

MATERIALS

pZT-C13-L1 **addgene Catalog #62196**

pZT-C13-R1 **addgene Catalog #62197**

DPBS (no Ca, no Mg) **ThermoFisher Catalog #14190144**

Essential 8™ Medium **Gibco, ThermoFisher Catalog #A1517001**

StemPro™ Accutase™ Cell Dissociation Reagent **Thermo Fisher Scientific Catalog #A1110501**

Lipofectamine™ Stem Transfection Reagent **Thermo Fisher Scientific Catalog #STEM00008**

StemFlex™ Medium **Thermo Fisher Scientific Catalog #A3349401**

Matrigel **Corning Catalog #356231**

KnockOut™ DMEM **Thermo Fisher Scientific Catalog #10829018**

Opti-MEM™ I Reduced Serum Medium **Thermo Fisher Scientific Catalog #31985070**

Y-27632 dihydrochloride (Rock Inhibitor) **R&D Systems Catalog #1254/10**

- 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.

Keywords: iPSC, TALENs, CRISPR interference, CRISPRi, CROP-seq, Perturb-Seq, essential genes, functional genomics, high-content microscopy, neuron, single-cell RNA sequencing, stem cell

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 – 5.0 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.