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iPSC editing with TALENs

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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.

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

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

Attachments

PDF

Editing_iPSCs_with_T...

58KB

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 - Thermo Fisher Scientific** **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.

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.