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Generation of SLC45A1 Knockout iPSC Line (WTC11-Ngn2 Line)

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We use this protocol and it's working

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Abstract

This protocol describes the generation of an **SLC45A1** knockout (or other any gene) in WTC11-Ngn2 induced pluripotent stem cells (iPSCs) by targeting the coding sequence (CDS) using the CRISPR-spCas9 system.

Materials

Reagents:

- Thiazovivin (ROCK1 inhibitor), 10 µM
- ReLeSR™ (Stemcell Technologies, for cell dissociation)
- Cas9 enzyme (IDT Alt-R S.p. Cas9 Nuclease V3), 10 µg/µL
- sgRNA (Synthego, 2'-O-methyl modified), 3.2 µg/µL
- P3 Primary Cell Nucleofector™ Solution (Lonza, V4XP-3032)
- 16-well Nucleocuvette™ Strips
- 4D-Nucleofector™ System (Lonza)
- iPSC growth medium (e.g., StemFlex, Stemcell Technologies)
- Sterile PBS
- 24-well tissue culture plates

Designing the Single Guide RNA (sgRNA)

1 • Clone 1 sgRNA: **AGATGCCCTCTTCCCCAGCG**
• Clone 2 sgRNA: **ACTTCAAACGACACCCCAAG**

Pre-treatment of iPSCs (Day -1)

2 **Objective:** Enhance iPSC viability prior to nucleofection.
1. Plate iPSCs at ~50% confluency in iPSC growth medium.
2. Add 10 µM Thiazovivin.
3. Incubate overnight at 37 °C with 5% CO_2.

Preparation of Cas9-sgRNA RNP Complex

3 Assemble the Cas9-sgRNA ribonucleoprotein (RNP) complex.

3.1 In a PCR tube, mix 6 µg Cas9 protein with 3.2 µg sgRNA.

3.2 Incubate at 37 °C for 15 minutes.

3.3 Keep the RNP complex on ice until use.

Dissociation of iPSCs

4 Prepare a single-cell suspension for nucleofection.

4.1 Aspirate the medium and add ReLeSR™ to the iPSCs.

4.2 Incubate at 37 °C for 3–5 minutes.

4.3 Gently tap the plate to detach cells and resuspend them in iPSC growth medium.

4.4 Count viable cells and adjust to 0.3 × 10^6 cells per nucleofection.

4.5 Centrifuge at **300 × g for 3 minutes**.

4.6 Wash the pellet with sterile PBS and centrifuge again at **300 × g for 3 minutes**.

4.7 Resuspend the cell pellet in 20 µL P3 Nucleofector™ Solution per nucleofection (16.4 µl of P3 solution was mixed with 3.6 µl of Supplement).

Post-Transfection Treatment (Day 1–3)

5 Facilitate iPSC recovery and expansion.

5.1 After 24 hours, replace the medium with fresh iPSC growth medium.

5.2 Continue treatment with 10 µM Thiazovivin.

5.3 Culture cells for an additional 72 hours.

Validation of SLC45A1 Knockout

6 Amplify the targeted genomic region via PCR.

6.1 Perform Sanger sequencing of the PCR amplicons.

6.2 Analyze chromatograms using ICE software (Synthego) to determine indel frequency and knockout efficiency by comparing edited cells to wild-type or mock-treated controls.