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Generation of induced pluripotent stem cells and gene correction

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Protocol status: Working

We use this protocol and it's working



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Abstract

iPSC generation and gene correction (CRISPR-CAS9) protocol

Generation of induced pluripotent stem cells and gene correction

- 1 Skin fibroblasts were reprogrammed by nucleofection with pCXLE- hOct3/4 (RRID:Addgene_27076), pCXLE-hSK (RRID:Addgene_27078), using the Amaxa nucleofection kit for human dermal fibroblasts (Lonza, VPD-100) and program P-022 of the Nucleofector 2b (Lonza).
- 2 Nucleofected fibroblasts were plated in six-well plates coated with Matrigel (Corning) in DMEM supplemented with 10% FBS (Gibco) and 1% GlutaMAX Supplement (Gibco).
- 3 The following day, the medium was changed to DMEM+/+ (DMEM with 10% FBS and 1% GlutaMAX Supplement and 1% Pen/Strep (Millipore)) supplemented with 2 ng/ml recombinant basic human fibroblast growth factor (FGF2, Peprotech).
- 4 On day 3 or 4 post nucleofection, the medium was changed to E8 medium composed of DMEM F12 with HEPES (Gibco), 128 ng/ml ascorbic acid (Sigma –Aldrich), 1x insulin-transferrin-selenium (Thermo Fisher Scientific), 10 ng/mL FGF2 (Peprotech), 500 ng/ml heparin (Sigma-Aldrich), and 2 ng/ml TGFβ1 (Peprotech). E8 medium was supplemented with 100 μm sodium butyrate and 0.1% Pen/Strep.
- 5 Colonies started to appear from day 14 onward.
- 6 Induced pluripotent stem cells (iPSCs) were cultured on Vitronectin XF (StemCell Technologies) in E8 medium.
- 7 Gene correction for the L444P mutation was performed as previously described in (Schöndorf, D. C. et al., 2014)
- 8 One hour before nucleofection, 10 μM Rockinhibitor was added to the iPSC medium. About 240 nM crRNA (IDT): Atto550-labeled tracrRNA (IDT) duplex was complexed with 124 μM Cas9 to form the ribonucleoprotein complex (RNP complex).
- 9 iPSCs (1.6×10^6) were nucleofected with the RNP complex and 16 μg of ssODN using 100 μl of Ingenio nucleofection solution (Mirus) with program B-016 of Nucleofector 2b.
- 10 Following nucleofection, the cells were FACSsorted for Atto550-positive cells using a FACS Aria II with a 100-μm nozzle. After sorting, 1×10^4 cells were plated per 10-cm dish.
- 11 Colonies were picked and sequenced by Sanger sequencing.

