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Generation of induced pluripotent stem cells and gene correction

In 2 collections

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ABSTRACT

iPSC generation and gene correction (CRISPR-CAS9) protocol

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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 or4 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, 10µM Rockinhibitor was added to the iPSC medium. About 240 nM crRNA (IDT): Atto550-labeled tracrRNA (IDT) duplex was complexed with 124µM Cas9 to form the ribonucleoprotein complex (RNP complex).

9 iPSCs(1.6 × 10^6) were nucleofected with the RNP complex and 16µg of ssODN using 100µl 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-µmnozzle. After sorting, 1 × 10^4 cells were plated per 10-cm dish.

11 Colonies were picked and sequenced by Sanger sequencing.