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Lab Resource: Stem Cell Line

# Generation of an isogenic, gene-corrected iPSC line from a symptomatic 57-year-old female patient with frontotemporal dementia caused by a P301L mutation in the microtubule associated protein tau (MAPT) gene

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ABSTRACT

Frontotemporal dementia with parkinsonism linked to chromosome 17q21.2 (FTDP-17) is an autosomal-dominant neurodegenerative disorder. Mutations in the MAPT (microtubule-associated protein tau)-gene can cause FTDP-17, but the underlying pathomechanisms of the disease are still unknown. Induced pluripotent stem cells (iPSCs) hold great promise to model FTDP-17 as such cells can be differentiated *in vitro* to the required cell type. Furthermore, gene-editing approaches allow generating isogenic gene-corrected controls that can be used as a very specific control. Here, we report the generation of genetically corrected iPSCs from a 57-year-old female FTD-17 patient carrying an P301L mutation in the MAPT-gene.



Resource table:

| | |
|---|---|
| Name of stem cell line | H251 C3 GC |
| Institution | Bioneer A/S |
| Person who created resource | Natakarn Nimsanor, Narisorn Kitiyanant, Benjamin Schmid, Mikkel Aabech Rasmussen |
| Contact person and email | Benjamin Schmid, bsc@bioneer.dk |
| Date archived/stock date | August 2016 |
| Origin | Human induced pluripotent stem cell line H251 C3 |
| Type of resource | Gene-corrected induced pluripotent stem cells; originally derived from skin fibroblasts of patient with frontotemporal dementia |
| Sub-type | iPSC line |

## 1. Resource details

Previously, we have generated an induced pluripotent stem cell (iPSC) line (H251 C3) from a symptomatic, 57-year-old woman carrying a heterozygous P301L mutation in the microtubule-associated protein tau (MAPT) gene (Rasmussen et al., 2016). Reprogramming was performed by electroporation of three episomal plasmids encoding hOCT4, hSOX2, hKLF4, hL-MYC, and hLIN28 (Okita et al., 2011; Takahashi et al., 2007).

Here, we generated a gene-corrected clone of H251 C3 using the CRISPR/Cas9 technology, where the mutated triplet CTG (Leucine) was corrected to the wild-type triplet CCG (Proline) using a single stranded oligodeoxynucleotide (ssODN) as a homologous template (Fig. 1A). Successful gene-correction was validated by sequencing (Fig. 1B). Sequencing analysis of the region around the CRISPR cutting site confirmed that no further cutting occurred and that the DNA sequence remained intact without frameshift or other mutations. We finally ensured that the cells were still pluripotent after gene-correction (Fig. 1C, D and E) and showed a normal karyotype (Fig. 1F).

## 2. Materials and methods

### 2.1. CRISPR design

Isogenic gene-corrected controls were obtained using the CRISPRs/Cas9 system in combination with an ssODN serving as

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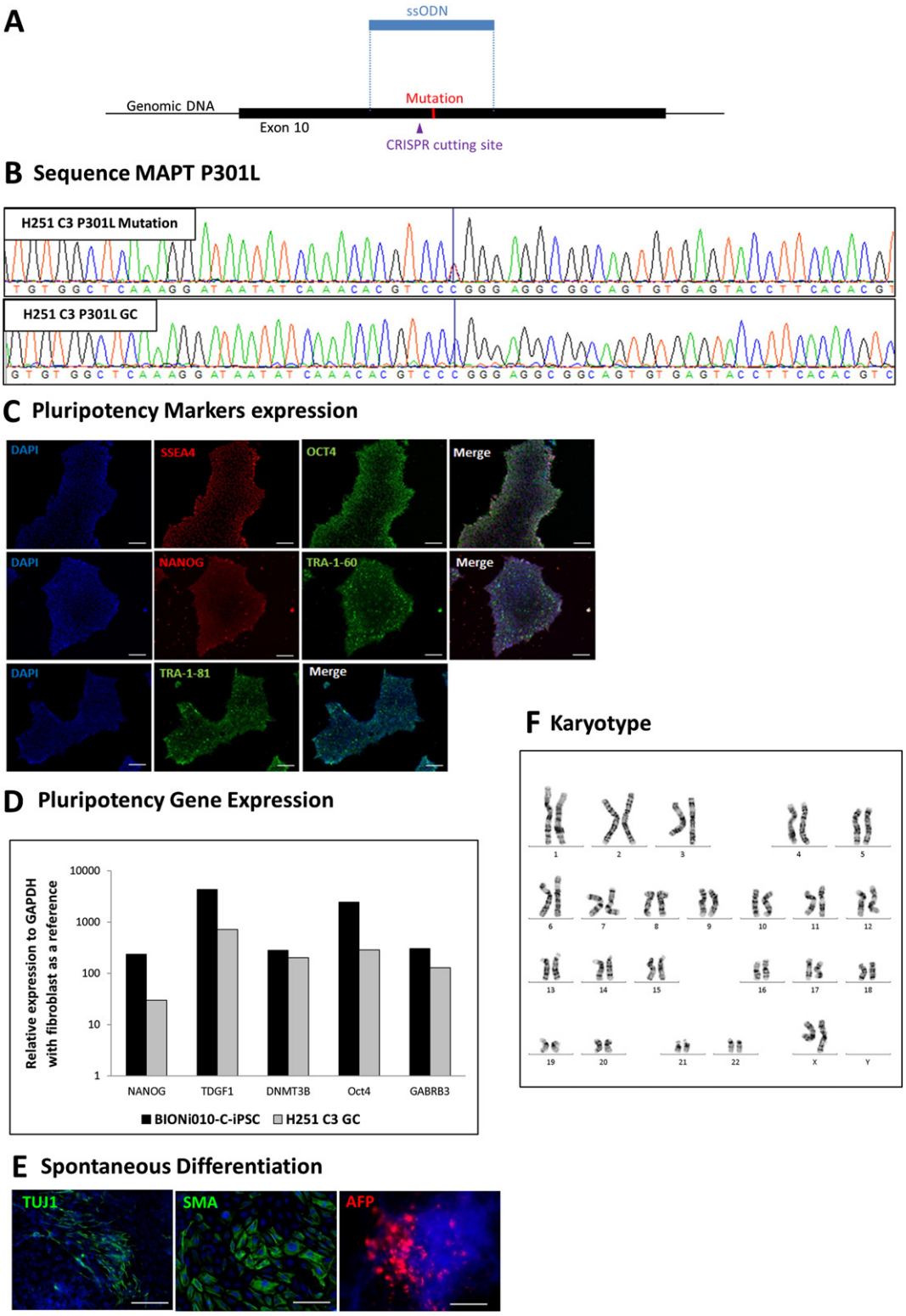


**Fig. 1.** Characterization of iPSCs. (A) Schematic of the gene editing strategy used to correct the MAPT P301L mutation in exon 10 using the CRISPR/Cas9 system in combination with an ssODN as homologous donor construct. (B) Sequencing result of the iPSC line H251 C3 showing a heterozygous C > T mutation (P301L mutation; upper panel). After gene correction (H251 C3 GC), the mutation was no longer present (lower panel). (C) Immunocytochemistry analysis of the pluripotency markers OCT4 (red), TRA1-60 (green) and SSEA4 (green), scale bars = 100 μM. (D) Quantitative PCR analysis of pluripotency genes from the gene-corrected line and a previously established iPSC control line BIONi010-C compared to fibroblasts (set to 1). (E) Immunocytochemistry for proteins representative of the three germ layers, TUJ1 (ectoderm), SMA (mesoderm) and AFP (endoderm), after *in vitro* differentiation by embryoid body formation, scale bars = 200 μM. (F) Representative karyotype of the iPSC line H251 C3 GC (46, XX).

homologous template covering the site of the mutation. A CRISPR targeting exon 10 of the MAPT gene was designed at http://crispr.mit.edu/. The CRISPRs were generated in a single plasmid (a gift from Feng Zhang) containing both sgRNA and the Cas9 (pSpCas9(BB)-2A-Puro (PX459); Addgene plasmid #62988) following the protocol from Ran et al. (2013). The following sequence was

recognized by the CRISPR: GATAATATCAAACACGTCCTGGG (T = mutation site).

### 2.2. Nucleofection

iPSCs were cultured on 100 mm dishes coated with Matrigel (Corning Bioscience) in E8 medium and detached using accutase (Gibco) when they reached a confluency of 70–90%. A total of $1.5 \times 10^6$ cells were co-nucleofected with 10 μg of the CRISPR/Cas9 encoding plasmid and 1 μL of 100 μM ssODN using the P3 Primary Cell Kit (Lonza) using the nucleofection program CA167 following to the manufacturer's instructions (Lonza). iPSCs were subsequently transferred back to a Matrigel-coated 100 mm dish in E8 medium supplemented with 1:200 diluted Revita cell supplement (Gibco). At 4 h post-nucleofection, cells were subjected to selection medium (E8 medium containing 1 μg/mL puromycin) for 20 h and then allowed to recover for a week in normal E8 medium. Finally, colonies were then picked and expanded for genotyping.

### 2.3. Genotyping

DNA for genotyping was extracted using the prepGem kit from ZyGEM. PCR genotyping was performed using TEMPase Hot Start DNA Polymerase (Ampliqon) according to the manufacturer's instructions at an annealing temperature of 62 °C. The following screening primers were designed covering the P301L mutation in the MAPT gene: Exon 10 forward 5′-TCACTCATCGAAAGTGGAGG-3′ and Exon 10 reverse 5′-CAGTGTCTCGCAAGTGTACG-3′. The PCR products were digested using XmaI for 1 h to detect gene-corrected clones (correction of the mutation produces an XmaI restriction enzyme cutting site). Positive clones were then sequenced using the forward primer to confirm correction of the mutation.

### 2.4. qRT-PCR analysis of stem cell markers

Total RNA was purified from H251 C3 GC iPSCs, fibroblasts and the iPSC line BIONi010-C (Rasmussen et al., 2014) as a positive control using RNeasy mini kit (Qiagen). Conversion to cDNA was performed with RevertAid First Strand cDNA synthesis kit (Thermo Scientific). qPCR analysis was carried out using the TaqMan primers GAPDH Hs03929097_g1, NANOG Hs02387400_g1, OCT4 Hs00999632_g1, TDGF1 Hs02339497_g1, DMNT3B Hs00171876_m1 and GABRB3 Hs00241459_m1 (Thermo Scientific). Data was analyzed using the $2^{-\Delta\Delta Ct}$ method relative to GAPDH with fibroblasts as a reference (set to 1).

### 2.5. Immunostaining of pluripotency markers

Cells were seeded on matrigel-coated coverslips. After 2 days, the cells were fixed at room temperature with 4% paraformaldehyde in PBS for 10 min, permeabilized with 0.1% Triton X-100 and blocked for unspecific binding with 2% BSA in PBS for 1 h. Immunostaining was performed with primary antibodies against OCT4, NANOG, TRA-1-60, TRA-1-81, and SSEA4 (Table 1). After incubation with the primary antibody, the cells were washed 3 times with PBS and then incubated with fluorescence-conjugated secondary antibodies Alexa fluor 488 (goat anti-mouse, 1:1000) or Cyanine 3 (goat anti-rabbit, 1:1000) for 1 h (both Life technologies). The cells were mounted on glass slides with mounting solution-containing DAPI (Life Technologies).

### 2.6. In vitro differentiation

iPSCs were dissociated with EDTA (Gibco) and allowed to form aggregates in non-coated cell culture dishes with E8 medium. On day 2, the E8 medium was changed to E6 medium (E6 medium = E8 medium without FGF2 and TGF-β1). On day 7, the aggregates were transferred to matrigel-coated dishes and medium was switched to differentiation medium. For mesodermal differentiation: DMEM/F12 containing 10% FBS, 1% L-glutamine, and 1% non-essential amino acids (all Gibco). For endodermal differentiation: MCDB131-1 containing 0.5% BSA, 0.1% pen/strep, 3 μM CHIR99021 (Selleckchem), 100 ng/mL Activin A (Cell Guidance). For ectodermal differentiation: DMEM/F12 mixed with neurobasal medium in a ratio of 1:1, 1X B27, 1X N2, and 1% L-glutamine (all Gibco), 10 μM SB 431542 and 0.1 μM LDN 193189 (both Selleckchem). Cells were fixed for immunocytochemistry on day 21. ICC analysis was performed with the antibodies against TUJ1, SMA and AFP (Table 1).

**Table 1**
Antibodies used for immunochemistry.

| | Antibodies and host species | Dilution | Company and catalog number |
|---|---|---|---|
| Pluripotency | Goat anti-OCT4 | 1:500 | Santa Cruz, sc-8628 |
| | Rabbit anti-NANOG | 1:100 | Millipore, AB5731 |
| | Mouse anti-SSEA4 | 1:500 | BioLegend, 330402 |
| | Mouse anti TRA-1-60 | 1:500 | BioLegend, 330602 |
| | Mouse anti TRA-1-81 | 1:500 | BioLegend, 330702 |
| *In vitro* differentiation | Mouse anti-Smooth muscle actin (SMA) | 1:500 | Dako, M0851 |
| | Rabbit anti-Alpha-1-fetoprotein (AFP) | 1:500 | Dako, A0008 |
| | Mouse anti-Beta-III-tubulin (TUJ1) | 1:500 | Sigma-Aldrich, T8660 |

### 2.7. Karyotyping

iPSCs and fibroblasts were treated for 1 h with KaryoMAX colcemid (Life Technologies) followed by hypotonic treatment and then harvested in fresh fixative containing 25% acetic acid and 75% methanol. Karyotyping was performed on G-banded metaphase chromosomes using standard cytogenetic procedures.

### 2.8. Verification and authentication

An intact genome was demonstrated by karyotyping using G-banding of 15 mitoses. Analysis was performed at the Institute of Medical Genetics and Applied Genomics, University of Tübingen, Germany. The results showed a normal 46, XX karyotype, free of any discernible abnormalities iPSC line identity (Fig. 1F).

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