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Lab resource: Multiple stem cell lines

Generation of two isogenic iPSC lines with either a heterozygous or a homozygous E280A mutation in the PSEN1 gene

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\textbf{ABSTRACT}
Alzheimer's disease (AD) is the most common form of dementia. Mutations in the gene \textit{PSEN1} encoding Presenilin1 are known to cause familial forms of AD with early age of onset. The most common mutation in the \textit{PSEN1} gene is the E280A mutation. iPSCs are an optimal choice for modeling AD, as they can be differentiated \textit{in vitro} into neural cells. Here, we report the generation of two isogenic iPSC lines with either a homozygous or a heterozygous E280A mutation in the \textit{PSEN1} gene. The mutation was introduced into an iPSC line from a healthy individual using the CRISPR-Cas9 technology.

Resource table

| Unique stem cell lines identifier | 1. BIONi010-C + homozygous E280A = BIONi010-C-29  
| Alternative names of stem cell lines | 2. BIONi010-C + heterozygous E280A = BIONi010-C-30  
| Institution | Bioneer A/S Hørsholm Denmark and University of Copenhagen (UCPH) Copenhagen Denmark
| Contact information of distributor | Contact at Bioneer: Benjamin Schmid,bsc@bioneer.dk– Contact at UCPH: Kristine Freude,kkf@sund.ku.dk
| Type of cell lines | iPSCs
| Origin | Human
| Cell Source | Fibroblasts
| Clonality | Clonal
| Method of reprogramming | Episomal plasmids (Okita et al., 2011)
| Multiline rationale | Mutated isogenic clones
| Gene modification | YES
| Type of modification | Induced point mutation
| Associated disease | Alzheimer's disease (AD)
| Gene/locus | PSEN1/Chr14:73664808
| Method of modification | CRISPR-Cas9
| Name of transgene or resistance | N/A
| Inducible/constitutive system | N/A
| Date archived/stock date | September 2017
| Cell line repository/bank | 1. BIONi010-C-29:https://hpscreg.eu/cell-line/BIONi010-C-29  
| Ethical approval | 2. BIONi010-C-30:https://hpscreg.eu/cell-line/BIONi010-C-30

The study was approved by the Ethics Committee of the Capital Region of Denmark (H-4-2011-157), and written informed consent was obtained from the participant before enrolment.

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1873-5061/ © 2019 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/BY-NC-ND/4.0/).
The exact mechanisms leading to Alzheimer’s disease (AD) remain still unknown. In order to better understand disease development and underlying cellular pathological mechanisms, we have established two isogenic iPSC lines containing either a heterozygous or a homozygous E280A mutation in the PSEN1 gene, which are known to result in AD.

The mutation E280A in the PSEN1 is one of the most common mutations related to familial forms of Alzheimer’s disease (AD). Ample patient based information is available based on studies from a large kindred from the Colombian state of Antioquia. (Sepulveda-Falla et al., 2012). This specific mutation is therefore highly relevant for establishment of an iPSC based disease model, as the cell model is substantially supported by patient phenotype information, which will facilitate validation of the in vitro model.

For this study, the E280A mutation was inserted into the human iPSC line BIONi010-C, which had earlier been established from a skin biopsy obtained from a healthy male individual aged 18 (Rasmussen et al., 2014).

The point mutation resulting in an amino acid change from glutamic acid (E) to alanine (A) was knocked into the PSEN1 gene by using the CRISPR-Cas9 system (Jinek et al., 2012). The nucleotide substitution was confirmed by restriction digest (data not shown) followed by DNA sequencing. Sequencing analysis confirmed a successful A > C transition causing the E280A mutation on protein level in both a heterozygous and homozygous manner. The two lines are further referred to as BIONi010-C-E280A +/− and BIONi010-C-E280A +/+, respectively (Fig. 1A, Table 1).

Pluripotency of the gene-edited lines was confirmed by immunocytochemistry (ICC) and quantified by flow analysis. Both cell lines show clear expression of OCT4, NANOG, SSEA4, SSEA3, TRA-1-60 and TRA-1-81 (Fig. 1B). The differentiation potential of the iPSCs was confirmed via spontaneous differentiation into endo- and mesodermal cell types (Fig. 1D). Cells were stained to neural progenitor cells and stained for PAX6 and Nestin (Fig. 1D) the cells hereby showed capability of ectodermal differentiation. A karyotype analysis was carried out to confirm chromosomal integrity. The results showed a normal 46, XY karyotype without detectable abnormalities (Supplementary Fig. 1A). Investigation of cells by light microscopy confirmed a normal iPSC morphology throughout the gene editing process (Fig. 1C). Finally, an STR analysis was performed to confirm cell line identity (Available with author) and cells were tested negative for mycoplasma (Supplementary Fig. 1B).

Materials and methods

Gene editing

Cells were grown on Matrigel coated plates in E8 media and incubated at 37°C and 5% CO2. At 80% confluency, cells were detached by treating cells with Accutase for 5 min after which 0.8 × 10⁶ cells were nucleofected. Nucleofection was made by using the Lonza nucleofector, P3 solution and the CA167 pulse setting. Nucleofected cells were transferred to Matrigel coated plates and single colonies were picked manually for analysis after 7 days.

Editing was performed using the CRISPR-Cas9 system in combination with an ssODN (single stranded oligo deoxynucleotide) as homologous template containing the E280A mutation. Two silent mutations (changing the DNA sequence but not the protein sequence) were inserted to avoid repeated cutting by the CRISPR-Cas9 and to add a restriction enzyme recognition site for Bsa1. The 20nt crRNA was designed with the software http://crispr.mit.edu/. crRNA, tracrRNA, ssODN, and Cas9 protein were all ordered from Integrated DNA Technologies (IDT).

Genotyping and restriction enzyme

To screen for positive clones, DNA was extracted using the prepGEM kit (ZyGEM) followed by PCR using the AmpliTaq GOLD DNA polymerase (Thermo Fisher) according to the manufacturer’s instructions. 40 cycles consisting of 30 s of denaturation at 94°C, 30 s of annealing at 60°C and 60 s of elongation at 72°C was performed in a T100 thermocycler from Bio-Rad. The primers SURV PSEN1 FW/RV (IDT) used...
for PCR, were designed to cover the mutation at locus Chr14:73664808 and produces a 400 bp product (Table 3). The screening was carried out by restriction digest using BsaI for 1 h at 37 °C (New England Biolabs).

Sequencing

Positive clones were sequenced by Sanger sequencing using primer SURV PSEN1 seq (Table 3).

Karyotyping and quality control

Quality control was performed after the lines had been banked and are listed in Table 2. The general morphology of the cells was investigated daily by light microscopy. When cells were 70–85% confluent, karyotyping was initiated by treating cells with Colcemid (Gibco) for 1.5 h after which they were detached with Accutase for 5–10 min and 0.2×105 cells were pressed by the cell lines using the Staining Buffer Set from Invitrogen. Flow cytometry was used to quantify pluripotency markers expressed by the cell lines using the Staining Buffer Set from Invitrogen. Cells were detached with Accutase for 5–10 min and 0.2×105 cells were fixed in 0.5 mL of the fixation/permeabilization buffer according to standard ICC procedures by permeabilizing fixed cells with 2% Triton X-100 in PBS for 20 min at RT followed by 30 min of blocking with 3% BSA. All antibodies were diluted in 3% BSA (OCT4, NANOG, SSEA4, SSEA3, Tra-1-60 and Tra-1-81) and incubated overnight at 4 °C. The primary antibodies (Table 3) were visualized with Alexa488 or Alexa647 as secondary antibodies diluted 1:200 (Life Technologies). Secondary antibodies were incubated in darkness for one hour at RT and all samples were stained with Hoechst bisbenzimide 33,258.

Differentiation potential

For analysis of differentiation potential, embryoid bodies were formed for 7 days in E8 media after which, cells were spontaneously differentiated in fibroblast media for 14 days on Matrigel coated plates. Cells were afterwards immunocytochemically stained for smooth muscle actin (mesoderm), alpha fetoprotein (endoderm). Fluorescent images showing the expression of the two markers were acquired on a Leica DMRGB-fluorescence microscope. iPSCs were also differentiated into neural progenitor cells (ectoderm), by forming embryoid bodies for 7 days in neural induction media and plating the embryoid bodies on Matrigel coated plates for another 7 days in neural induction media. Neural induction media consisted of Neurobasal media (Thermo Fisher), DMEM-F12 (Gibco), B-27 (Thermo Fisher), N-2 (Thermo Fisher), SB431542 (SMS), LDN193189 (Sigma), Glutamax (Thermo Fisher), Pen/Strep (Sigma). Cells were immunocytochemically stained for PAX6 and Nestin and images acquired on a Leica DMRGB-fluorescence microscope.

Flow cytometry

Flow cytometry was used to quantify pluripotency markers expressed by the cell lines using the Staining Buffer Set from Invitrogen. Cells were detached with Accutase for 5–10 min and 0.2 × 105 cells were fixed in 0.5 mL of the fixation/permeabilization buffer according to the guidelines. After 30 min incubation at RT, the cells were washed three times with permeabilization buffer and then incubated for 45 min with 100 μL of the diluted antibody. Cells were washed additionally three times and afterwards run at a calibrated BD Accuri C6 flow cytometer (analyze 50,000 cells at high speed in 150 μL buffer). The analysis was done using the FlowJo software.
### Table 3

Reagents details.

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<th>Antibody</th>
<th>Dilution</th>
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<tr>
<td><strong>Pluripotency Marker</strong></td>
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<tr>
<td>Goat anti-OCT4</td>
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<td>Santa Cruz Cat: sc-8628 RRID:AB_653551</td>
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<td>Peprotech Cat: 500-P236 RRID:AB_1268805</td>
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<td>SOX2 AF647</td>
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### Acknowledgments

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### Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scr.2019.101403.

### References


