



Lab Resource: Genetically-Modified Single Cell Line



Generation of isogenic control DJ-1-delP GC13 for the genetic Parkinson's disease-patient derived iPSC line DJ-1-delP (LCSBi008-A-1)

Pauline Mencke^a, Zoé Hanss^a, Javier Jarazo^b, François Massart^a, Arkadiusz Rybicki^a,
 Elizabet Petkovski^c, Enrico Glaab^d, Ibrahim Boussaad^a, Vincenzo Bonifati^e,
 Jens Christian Schwamborn^b, Wim Mandemakers^e, Rejko Krüger^{a, f, g, *}

^a Translational Neuroscience, Luxembourg Centre for Systems Biomedicine, University of Luxembourg, Luxembourg

^b Developmental & Cellular Biology Group, Luxembourg Centre for Systems Biomedicine, University of Luxembourg, Luxembourg

^c Forensic Genetics, Dept. Legal Medicine, Laboratoire national de santé, Luxembourg

^d Biomedical Data Science Group, Luxembourg Centre for Systems Biomedicine, University of Luxembourg, Luxembourg

^e Erasmus MC, University Medical Center Rotterdam, Dept. Clinical Genetics, Rotterdam The Netherlands

^f Parkinson Research Clinic, Centre Hospitalier de Luxembourg (CHL), Luxembourg

^g Transversal Translational Medicine, Luxembourg Institute of Health (LIH), Luxembourg

ABSTRACT

We describe the generation of an isogenic control cell line DJ-1-delP GC13 from an induced pluripotent stem cell (iPSC) line DJ-1-delP LCSBi008-A that was derived from fibroblasts obtained from a Parkinson's disease (PD) patient. Using CRISPR/Cas9 technology, we corrected the disease causing c.471_473delGCC homozygous mutation in the *PARK7* gene leading to p.158P deletion in the encoded protein DJ-1. The generated isogenic pair will be used for phenotypic analysis of PD-patient derived neurons and astrocytes.

1. Resource table

| | |
|-----------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Unique stem cell line identifier | LCSBi001-A-1 |
| Alternative name(s) of stem cell line | DJ-1-delP GC13 delP GC13 |
| Institution | Luxembourg Centre for Systems Biomedicine (LCSB) |
| Contact information of the reported cell line distributor | Dr. Wim Mandemakers, w.mandemakers@erasmusmc.nl |
| Type of cell line | Induced pluripotent stem cell line (iPSC) |
| Origin | Human |
| Additional origin info (applicable for human ESC or iPSC) | Age at biopsy, 66 years Sex: male Ethnicity: Caucasian |
| Cell Source | fibroblasts |
| Method of reprogramming | electroporation using three episomal plasmids Oct3/4 (pCXLE-hOCT3/4 (Addgene #27076)), Sox2 and Klf4 (pCXLE-hSK ((Addgene #27078)), Lin28, L-Myc combined with a short hairpin RNA for p53 |
| Clonality | Clonal |

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| | |
|-------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------|
| Unique stem cell line identifier | LCSBi001-A-1 |
| Evidence of the reprogramming transgene loss (including genomic copy if applicable) | RT-/q-PCR, ICC, western blotting, etc. If piggyBac: evidence of excision/lack of insertion, PCR |
| Cell culture system used | Cells were grown on Geltrex or Matrigel |
| Type of Genetic Modification | Correction of mutation |
| Associated disease | Parkinson's disease |
| Gene/locus | <i>PARK7</i> |
| Method of modification/site-specific nuclease used | CRISPR/Cas9 |
| Site-specific nuclease (SSN) delivery method | Plasmid transfection |
| All genetic material introduced into the cells | HDR donor vector |
| Analysis of the nuclease-targeted allele status | Sequencing of the targeted allele |
| Method of the off-target nuclease activity surveillance | <i>in silico</i> tool IDT CRISPR-Cas9 guide RNA design checker was used to identify off-targets, double-stranded breaks that may occur in the genome |
| Name of transgene | N/A puromycin |

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* Corresponding author.

E-mail address: rejko.krueger@uni.lu (R. Krüger).

<https://doi.org/10.1016/j.scr.2022.102815>

Received 6 May 2022; Received in revised form 12 May 2022; Accepted 16 May 2022

Available online 17 May 2022

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| Unique stem cell line identifier | LCSBi001-A-1 |
|-------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Eukaryotic selective agent resistance (including inducible/gene expressing cell-specific) | |
| Inducible/constitutive system details | N/A |
| Date archived/stock date | 22.09.2020 |
| Cell line repository/bank | IBBL Luxembourg https://hpscereg.eu/user/cellline/edit/LCSBi008-A-1 |
| Ethical/GMO work approvals | Medical Ethical Committee, Erasmus MC Rotterdam, The Netherlands; MEC-2012-001/NL38860.078.11 |
| Addgene/public access repository recombinant DNA sources' disclaimers (if applicable) | HDR donor plasmid was synthesized by GeneArt®, sequence is available as suppl. File pX330 plasmid sequence available as suppl. file |

2. Resource utility

The p.158Pdel mutation in the *PARK7* gene encoding the protein DJ-1 leads to autosomal recessive early onset PD. By generating an isogenic control for the PD-patient derived iPSC line DJ-1-delP (Mencke et al., 2022), the obtained isogenic pair of DJ-1-delP and DJ-1-delP GC13 will be used to investigate underlying pathological mechanisms of genetic PD that are caused by the loss of DJ-1 protein due to the p.158Pdel mutation (Mencke et al., 2022).

3. Resource details

To generate the presented iPSC line, iPSC of the parental patient line DJ-1-delP (Mencke et al., 2022) were used. Using an established protocol for CRISPR/Cas9 fluorescent guided biallelic HDR targeting selection with PiggyBac system removal for gene editing (Jarazo et al., 2019), the mutation was homozygously corrected.

The generated isogenic control line shows the typical morphology of iPSC colonies (Fig. 1A). The cell line has a structurally and numerically normal karyotype (46, XY) after correction of the mutation (Fig. 1B). qPCR results for fibroblasts, control iPSC GM23338 (Larsen et al., 2020), DJ-1-delP and the newly generated DJ-1-delP GC13 iPSC in triplicates confirmed that the isogenic line express *Nanog*, Oct3/4 (*POU5F1*) and *DMNT3B* mRNA in the same range as the parental line (Fig. 1C). The DJ-1-delP GC13 iPSC express the stemness marker *Sox2*, Oct3/4 and *Nanog*, as validated by immunocytochemistry (Fig. 1D). DJ-1-delP GC13 iPSC are capable of differentiating into the 3 germ layers mesoderm, endoderm and ectoderm (Fig. 1E), as shown by immunofluorescence staining for the germ layer marker Brachyury (mesoderm), *Otx2* (endoderm) and *Sox17* (ectoderm) (Fig. 1E).

The c.471_473delGCC mutation in the exon 7 of the *PARK7* gene leading to the deletion of proline 158 of the encoded DJ-1 protein (Fig. 1F) is expected to impair homodimerization of the DJ-1 monomers as the proline residues lie in the contact site of the two monomers (Fig. 1G).

The mutation does not affect the mRNA level of DJ-1 (Fig. 1I), but leads to loss of DJ-1 protein due to protein instability followed by degradation (Fig. 1J) (Ramsey and Giasson, 2010). The successful gene correction was confirmed by Sanger sequencing (Fig. 1H) and rescues the levels of DJ-1 protein (Fig. 1J) (Table 1).

4. Materials and methods

iPSC were cultured in freshly prepared E8 medium (DMEM F-12 + HEPES, Life Technologies; 1% Pen/Strep, Life Technologies; 1% Insulin-Transferrin-Selenium, Life Technologies; 2 µg/L TGFβ1, Peprotech; 10 µg/L FGF2, Peprotech; 64 mg/L ascorbic acid 2 PM, Sigma-Aldrich; 100 ng/mL Heparin, Sigma-Aldrich; 10% mTesR, StemCell Technologies). The medium was changed each day. The iPSC were passaged using EDTA (Life Technologies) once a week at a 1:5 ratio. iPSC were cultured at 37 °C under 5% CO₂.

5. Gene editing

To correct the mutation, we followed the steps described in the protocol for CRISPR/Cas9 fluorescent guided biallelic HDR targeting selection with PiggyBac system removal for gene editing (Jarazo et al., 2019). The donor plasmid (homology arms spanning the EGFP and the puromycin resistance) was synthesized by GeneArt®. The gRNA was designed using the broadinstitute gRNA design tool (sequence see Table 2, predicted off-targets see [suppl. file 1](#)). Nucleofection of the donor plasmid and the Cas9 plasmid was performed in DJ-1-delP iPSC at passage 18 using the Amaxa™ P3 Primary Cell 4D-Nucleofector™ X Kit L (24 RCT) and the Lonza Nucleofector™ (H9 program). Screening of the EGFP + colonies was performed using a Yokogawa CellVoyager CV7000 microscope. Green colonies were picked and analysed for random events and successful integration of EGFP (suppl. Fig. 1A and B, primers in Table 2). Non random EGFP integrated colonies were expanded and sorted to obtain 100% EGFP + cells with BD FACS Aria II, and sent for sequencing using primers spanning the region of the mutation (suppl. Fig. 1C, primers in Table 2). DJ-1-delP clone 13 showed the correction of the mutation (suppl. Fig. 1C, primers in Table 2). After the excision of the EGFP, cells were analysed for absence of integration of the donor plasmid and pX330 and sent for sequencing again (suppl. Fig. 1D + E, primers in Table 2).

6. Mutation analysis

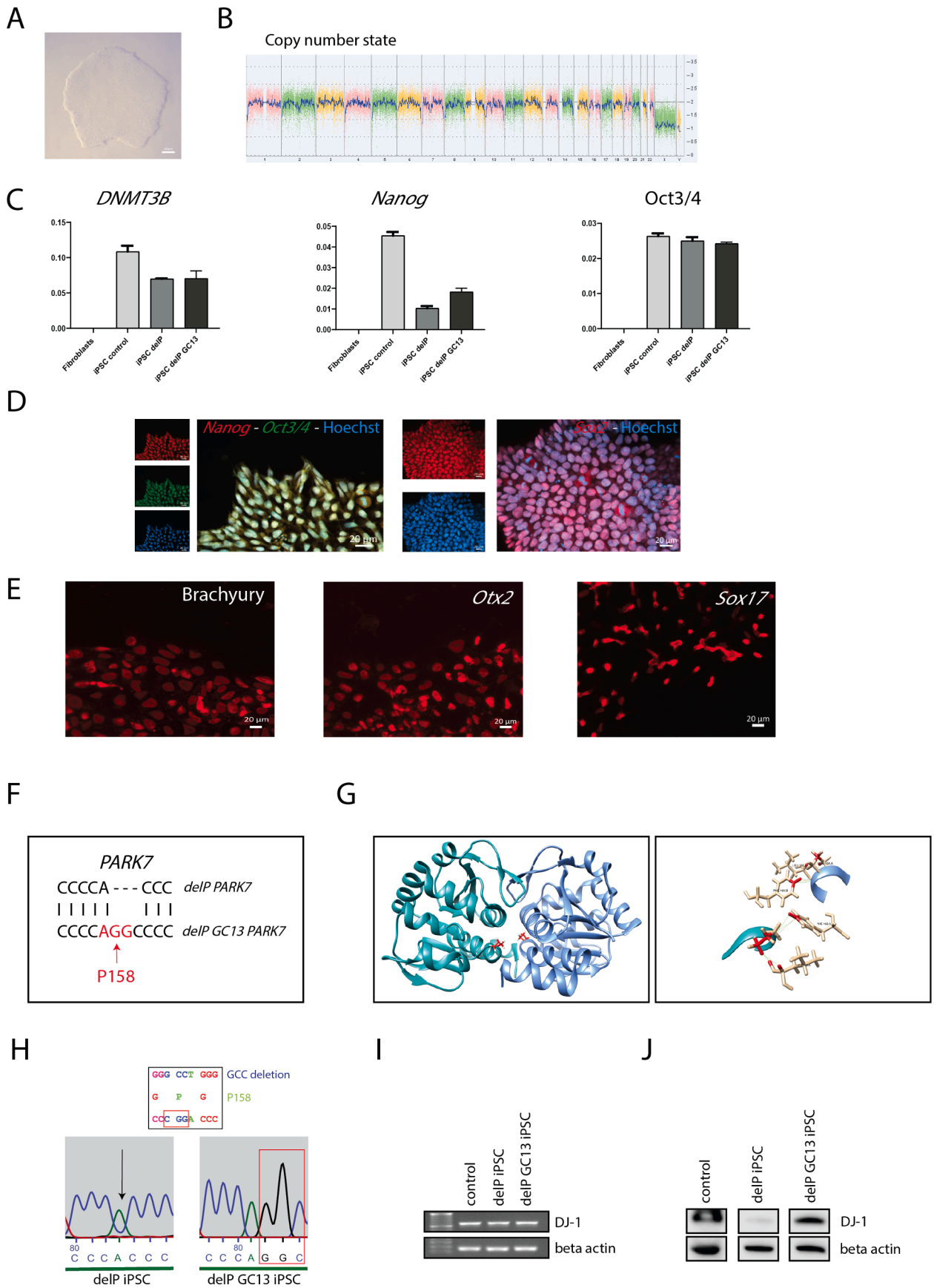
Genomic DNA was purified from fibroblasts and iPSC using the QIA Blood and Tissue kit (Qiagen). Using the primers listed in Table 2, the exon 7 of the *PARK7* gene was amplified by PCR and Sanger sequenced at Eurofins Genomics Germany GmbH.

6.1. RT-qPCR

Total RNA was extracted from native fibroblasts passage 16, control iPSC passage 24, DJ-1-delP iPSC passage 9 and DJ-1-delP GC13 iPSC passage 40 using the RNeasy Mini Kit (Qiagen). Transcriptor High Fidelity cDNA Synthesis Kit (Roche) was used to synthesize cDNA. Quantification of pluripotency markers by multiplex qPCR was performed using the LightCycler® 480 Probes Master kit (Roche) and hydrolysis probes (Table 2). cDNA from DJ-1-delP fibroblasts passage 16 was used as a negative control.

7. RNA and protein status analysis by PCR and Western blotting

RNA and protein levels of *PARK7*/DJ-1 were evaluated by PCR and Western blotting using the primers and antibodies listed in Table 2 following our standard protocols (Boussaad et al., 2020).



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Fig. 1. A: Brightfield image of DJ-1-delP GC13 iPSC colony. iPSC show the typical iPSC morphology. B: The karyotype of DJ-1-delP GC13 iPSC has no abnormalities. C: Gene expression analysis of pluripotency markers by qPCR shows the expression of *DNMT3B*, *Nanog* and *Oct3/4* in control and DJ-1-delP GC13 iPSC and no expression of the markers in control fibroblasts. D: Expression of stemness markers by immunocytochemistry. DJ-1-delP GC13 iPSC express the stemness marker *Sox2*, *Oct3/4* and *Nanog*. E: DJ-1-delP GC13 iPSC are able to differentiate into the three germ layers, as assessed by three germ layer differentiation and subsequent immunocytochemistry analysis of the markers for Brachyury (mesoderm), *Otx2* (endoderm) and *Sox17* (ectoderm). F: Graphical scheme of the location of the c.471_473delGCC mutation in the *PARK7* gene. The mutation leads to the deletion of proline 158. G: 3D protein structure model of the DJ-1 protein dimer (PDB: 1UCF). One subunit of the dimer is highlighted in blue and one in green, the proline 158 residues in these subunits are marked in red (left). The residues are located in the contact site of the two DJ-1 monomers (left) and contribute to dimer interactions via van-der-Waals contacts (right, visualized using the software UCSF Chimera). The loss of proline 158 is therefore expected to impair homodimerization of the DJ-1 protein leading to subsequent proteasomal degradation. H: The mutation in the DJ-1-delP line and the correction of the mutation in the isogenic counterpart DJ-1-delP GC13 was confirmed by Sanger sequencing. I: DJ-1-delP and DJ-1-delP GC13 iPSC have normal DJ-1 mRNA levels, as assessed by RT-PCR. J: The mutation in the DJ-1-delP iPSC leads to loss of DJ-1 protein, as seen by Western blotting. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 1
Characterization and validation.

| Classification (optional <i>italicized</i>) | Test | Result | Data |
|---------------------------------------------------------------------------------------------|--------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------|
| Morphology | Photography | typical primed pluripotent human stem cell morphology | Fig. 1 panel A |
| Pluripotency status evidence for the described cell line | Qualitative analysis (<i>Immunocytochemistry</i>) | staining/expression of pluripotency markers: <i>Oct3/4</i> , <i>Nanog</i> , <i>Sox2</i> | Fig. 1 panel D |
| Karyotype | Quantitative analysis (<i>RT-qPCR</i>) | expression of pluripotency markers: <i>DNMT3B</i> , <i>Nanog</i> , <i>Oct3/4</i> | Fig. 1 panel C |
| | Karyotype | 46XY, | Fig. 1 panel B |
| Genotyping for the desired genomic alteration/allelic status of the gene of interest | PCR across the edited site | Resolution 450–500 bbps PCR + sequencing, Confirmation of the homozygous correction of the mutation | Suppl. Fig. 1 |
| Verification of the absence of random plasmid integration events | Transgene-specific PCR | N/A | N/A |
| | PCR | Random integration of donor plasmid in edited cell line not detected Integration of CRISPR/Cas9 (modified pX330) plasmid in edited cell line not detected | Suppl. Fig. 1 |
| Parental and modified cell line genetic identity evidence | STR analysis | DNA Profiling | Submitted in the archive with journal |
| Mutagenesis / genetic modification outcome analysis | Sequencing (genomic DNA PCR) | Confirmation of the homozygous correction of the mutation | Fig. 1 panel H |
| Off-target nuclease analysis- | PCR-based analyses | Detection of correctly-targeted and randomly-integrated selectable targeting construct status | Suppl. Figure 1 |
| | Western blotting <i>in silico</i> analysis of off-targets | Demonstration of protein rescue in gene corrected line <i>in silico</i> tool IDT CRISPR-Cas9 guide RNA design checker was used to identify off-targets, double-stranded breaks that may occur in the genome | Fig. 1 panel J Suppl. File 1 |
| Specific pathogen-free status | Mycoplasma | Mycoplasma testing by luminescence. Negative | Suppl. Fig. 1 |
| Multilineage differentiation potential | Directed differentiation | Demonstration of ability to differentiate into derivatives of all 3 germ layers | Fig. 1 panel E |
| Donor screening (OPTIONAL) | HIV 1 + 2 Hepatitis B, Hepatitis C | N/A | |
| Genotype - additional histocompatibility info (OPTIONAL) | Blood group genotyping HLA tissue typing | N/A N/A | |

7.1. Immunofluorescence staining

Expression of stemness markers was analysed via immunocytochemistry. iPSC were fixed at passage 36 with 4% paraformaldehyde in PBS for 15 min and stained according to a standard immunofluorescence protocol using antibodies against *Sox2*, *Oct3/4* and *Nanog* ([Table 2](#)). Nuclei were stained with Hoechst. Images were acquired using a Zeiss spinning disk confocal microscope (Carl Zeiss Microimaging GmbH).

7.2. In vitro differentiation

The ability of the iPSC passage 36 to differentiate into the three germ layers was tested using the manufacturer's differentiation protocol of the Human Pluripotent Stem Cell Functional Identification Kit (R&D

Systems). Expression of the germ layer marker was confirmed after differentiation by immunocytochemistry (antibodies in [Table 2](#)). Images were acquired using a Zeiss spinning disk confocal microscope (Carl Zeiss Microimaging GmbH).

7.3. Karyotyping and identity analysis

Molecular karyotyping of iPSC passage 35 was performed at Thermo Fisher using the KaryoStat™ Assay. STR analysis of iPSC DJ-1-delP passage 26 and iPSC DJ-1-delP GC13 passage 40 was performed at the Laboratoire national de santé (LNS) Luxembourg.

Table 2
Reagents details.

| Antibodies and stains used for immunocytochemistry/flow-cytometry | | | |
|-------------------------------------------------------------------|-----------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| | Antibody | Dilution | Company Cat # and RRID |
| Pluripotency Markers | Goat anti <i>Sox2</i> (Y-17) | 1:250 | Santa Cruz, Cat #: sc-17320; RRID: AB_2286684 |
| Pluripotency Markers | Mouse anti Oct3/4 | 1:1000 | Santa Cruz, Cat #: sc-5279; RRID: AB_628051 |
| Pluripotency Markers | Rabbit anti <i>Nanog</i> | 1:1000 | Abcam, Cat #: ab21624; RRID: AB_446437 |
| DJ-1 | Rabbit anti DJ-1 (D29E5)XP | 1:1500 | cell signaling, Cat #: 5933; RRID: AB_11179085 |
| β -Actin | mouse anti β -Actin (8H10D10) | 1:20.000 | cell signaling, Cat #: 3700S; RRID: AB_2242334 |
| Secondary antibody | Alexa Fluor 488 Goat anti Mouse IgG (H + L) | 1:1000 | Invitrogen, Cat #: A11029; RRID: AB_138404 |
| Secondary antibody | Alexa Fluor 568 Goat anti Rabbit IgG (H + L) | 1:1000 | Invitrogen, Cat #: A11036; RRID: AB_143011 |
| Secondary antibody | Alexa Fluor 647 Donkey anti Goat IgG (H + L) | 1:1000 | Invitrogen, Cat #: A-21447; RRID: AB_2535864 |
| Secondary antibody | Goat anti Rabbit IgG (H + L) Secondary Antibody, HRP, 0.5 mg GTXRB IgG F AB'2 HRP X ADS | 1:5000 | Invitrogen, Cat #: A24537; RRID: AB_2536005 |
| Secondary antibody | Goat anti Mouse IgG (H + L) Secondary Antibody, HRP 0.5 mg GTXMU IgG F AB'2 HRP X ADS | 1:10.000 | Invitrogen, Cat #: A24524; RRID: AB_2535993 |
| Site-specific nuclease | | | |
| Cas9 | Cas9 | | |
| Delivery method | | Nucleofection | |
| Selection/enrichment strategy | | Puromycin | |
| Primers and Oligonucleotides used in this study | | | |
| Pluripotency Markers (qPCR) | Target <i>NANOG</i> -FAM <i>OCT4</i> -FAM <i>DNMT3B</i> <i>ACTB</i> | Forward/Reverse primer (5'-3') <i>NANOG</i> -FAM (Hs02387400_g1, Thermo Fisher Scientific) <i>OCT4</i> -FAM (Hs00999632_g1, Thermo Fisher Scientific) <i>DNMT3B</i> (Hs00171876_m1, Thermo Fisher Scientific) <i>ACTB</i> (Hs03023880_g1, Thermo Fisher Scientific) was used as a housekeeping gene | Representative PCR gel (+/-) Fig. 1 I <i>PARK7</i> fw 5'-ACGAATTCGAATGGCTTCCAAAAGAGCTCTGGT-3' <i>PARK7</i> rev 5'-AGCGGCCCGCTAGTCTTTAAGAACAAGTGGAGCC-3' Beta actin fw 5'-AAACTGGAACGGTGAAGGTG-3' Beta actin rev 5'-AGAGAAGTGGGTGGCTTTT-3' |
| House-Keeping Genes (qPCR) | | | |
| Genotyping (desired allele/transgene presence detection) | PCR specific for the targeted allele | | |
| Targeted mutation analysis/sequencing | Sequencing data from both alleles | Sanger sequencing chromatograms Fig. 1 H No integration PCR primer (for detection of homozygous/heterozygous gene editing and for sequencing after removal of the cassette) 5'-CAATGCTGCGAGGGCAGTAA-3' 5'-CTCTTTCCCTTCCCCAGGTA-3' Sequencing primer 5'-GCCCATTAGGATGTCACCTT-3' 5'-GCAGTTCGCTGCTAGTCTT-3' | |
| Potential random integration-detecting PCRs | plasmid backbone, vector/homology arm end PCRs | Suppl. Figure 1 Left homology arm (LHA) primer #246 5'-CAATGCTGCGAGGGCAGTAA-3' #861 5'-AGATGTCCTAAATGCACAGCG-3' Right homology arm (RHA) primer #43 5'-CGATATACAGACCGATAAAACACATGC-3' #247 5'-CTCTTTCCCTTCCCCAGGTA-3' Cas9 primer 5'-AGGAAATCGGCAAGGCTACC-3' 5'-TTCGCCGTTGTCTCGATCA-3' Random left primer #1321 5'-AGATGTCCTAAATGCACAGCG-3' #861 5'-GCTGCCTATCAGAAGGTGGTG-3' Random right primer #43 5'-CGATATACAGACCGATAAAACACATGC-3' | |

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Table 2 (continued)

| Site-specific nuclease | |
|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Cas9 | Cas9 |
| gRNA sequence | <i>PARK7</i> |
| Genomic target sequence(s) | <i>PARK7</i> |
| e.g. Top off-target mutagenesis predicted site sequencing (for CRISPR/Cas9 and TALENs) primers | #1752 5'-GCAGCCACTGGTAACAGGAT-3' 5'-CTGATTCTTACAAGCCGGGG-3' gRNA context sequence 5'-CGGCCTGATTCTTACAAGCCGGGGTGGGAC-3' PAM: TGG Gene: <i>PARK7</i> Location: 1.0.878 Length: 878 nt [Positional Info] XM_008975660.3 position: 527 <i>in silico</i> tool IDT CRISPR-Cas9 guide RNA design checker was used to identify off-targets, double-stranded breaks Suppl. File 1 |
| ODNs/plasmids/RNA templates used as templates for HDR-mediated site-directed mutagenesis. Backbone modifications in utilized ODNs have to be noted using standard nomenclature. | N/A |

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements and funding

We would like to thank the patient for providing fibroblasts for the generation of the described cell line.

The current work was supported by the Fonds National de Recherche (FNR) within the PEARL Excellence Programme [FNR/P13/6682797] to RK, and the MiRisk project [C17/BM/11676395], and by the Stichting ParkinsonFonds, The Netherlands [grant SPF-1870].

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.scr.2022.102815>.

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