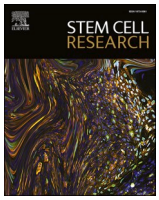
Stem Cell Research 62 (2022) 102815

Contents lists available at ScienceDirect

Stem Cell Research

journal homepage: www.elsevier.com/locate/scr



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)

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A B S T R A C T

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

(*continued on next column*)

(*continued*)

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	*PARK7*
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	*in silico* 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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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

(*continued*)

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://hpscreg.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 FASC 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 GmbBH.

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).

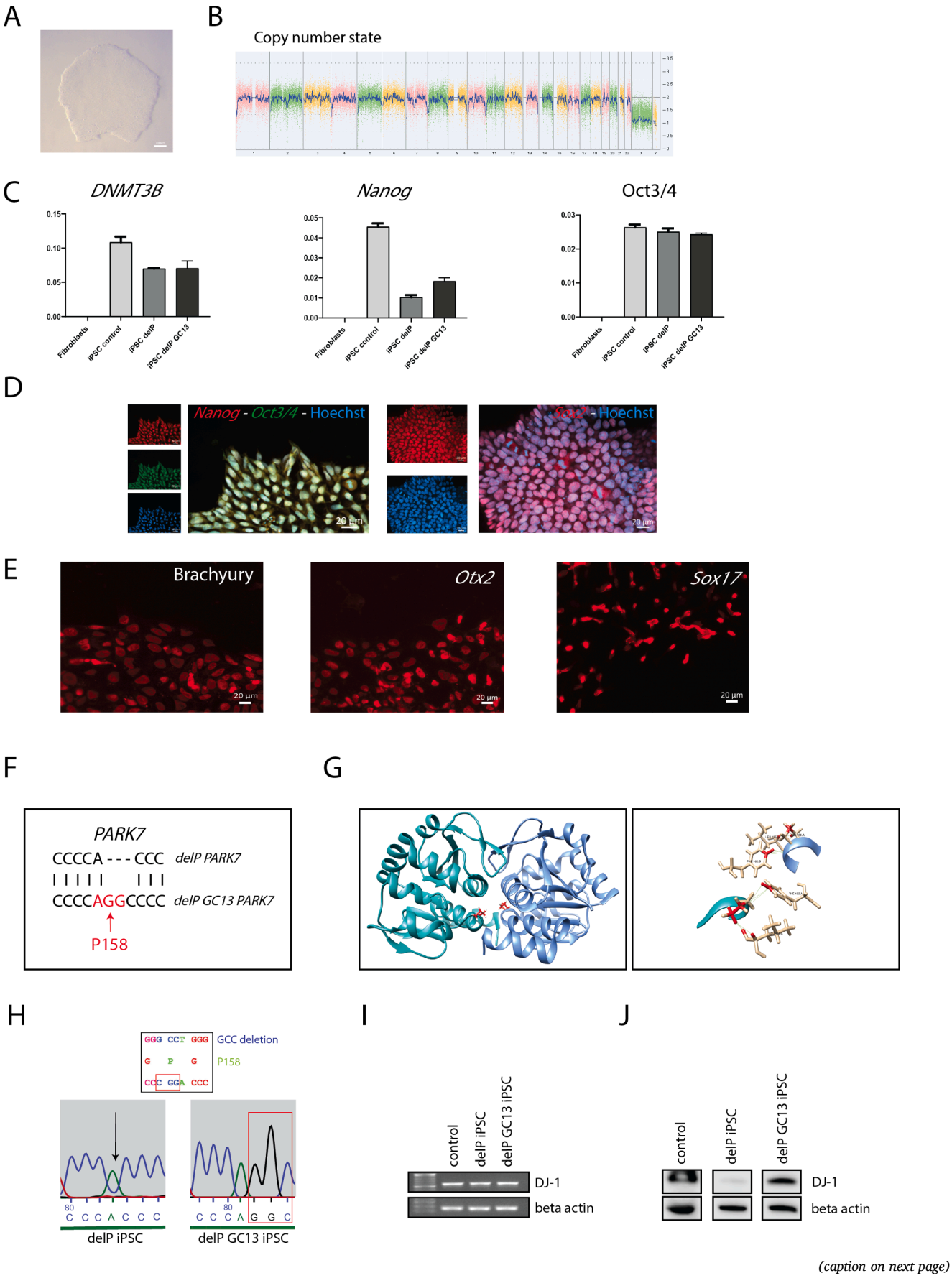

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Fig. 1. A: Brightfield image of DJ-1-delP GC13 iPSC colony. iPSC show the typical iPSC morphology. B: The karyotype of DJ-1-delP GC13 iPSC has no abnormalities. C: Gene expression analysis of pluripotency markers by qPCR shows the expression of *DNMT3B*, *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 *italicized*)	**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 *(Immunocytochemistry)*	staining/expression of pluripotency markers: Oct3/4, *Nanog*, *Sox2*	Fig. 1 panel D
	Quantitative analysis *(RT-qPCR)*	expression of pluripotency markers: *DNMT3B*, *Nanog*, Oct3/4	Fig. 1 panel C
Karyotype	Karyotype	46XY, Resolution 450–500 bhps	Fig. 1 panel B
Genotyping for the desired genomic alteration/allelic status of the gene of interest	PCR across the edited site	PCR + sequencing, Confirmation of the homozygous correction of the mutation	Suppl. Fig. 1
	Transgene-specific PCR	N/A	N/A
Verification of the absence of random plasmid integration events	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
	PCR-based analyses	Detection of correctly-targeted and randomly-integrated selectable targeting construct status	Suppl. Figure 1
	Western blotting	Demonstration of protein rescue in gene corrected line	Fig. 1 panel J
Off-target nuclease analysis-	*in silico* analysis of off-targets	*in silico* tool IDT CRISPR-Cas9 guide RNA design checker was used to identify off-targets, double-stranded breaks that may occur in the genome	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 GmbBH).

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 perfomed 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 *Sox2* (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 *Nanog*	1:1000	Abcam, Cat #: ab21624; RRID: AB_446437
DJ-1	Rabbit anti DJ-1 (D29E5)XP	1:1500	cell signaling, Cat #: 5933; PRRID: 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		
	Target	**Forward/Reverse primer (5′-3′)**
Pluripotency Markers (qPCR)	*NANOG*-FAM	*NANOG*-FAM (Hs02387400_g1, Thermo Fisher Scientific)
	OCT4-FAM	OCT4-FAM (Hs00999632_g1, Thermo Fisher Scientific)
	DNMT3B	*DNMT3B* (Hs00171876_m1, Thermo Fisher Scientific)
House-Keeping Genes (qPCR)	*ACTB*	*ACTB* (Hs03023880_g1, Thermo Fisher Scientific) was used as a housekeeping gene
Genotyping (desired allele/transgene presence detection)	PCR specific for the targeted allele	Representative PCR gel (+/-) Fig. 1 I *PARK7* fw 5́-ACGAATTCGAATGGCTTCCAAAAGAGCTCTGGT-3́ *PARK7* rev 5́-AGCGGCCGCCTAGTCTTTAAGAACAAGTGGAGCC-3́ Beta actin fw 5́-AAACTGGAACGGTGAAGGTG-3́ Beta actin rev 5́-AGAGAAGTGGGGTGGCTTTT-3́
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) 5́-CAATGCTGCGAGGGCAGTAA-3́ 5́-CTCTTTTCCCTTCCCCAGGTA-3́ Sequencing primer 5́-GCCCATTAGGATGTCACCTTT-3́ 5́-GCAGTTCGCTGCTCTAGTCTT-3́
Potential random integration-detecting PCRs	plasmid backbone, vector/ homology arm end PCRs	Suppl. Figure 1 Left homology arm (LHA) primer #246 5́-CAATGCTGCGAGGGCAGTAA-3́ #861 5́-AGATGTCCTAAATGCACAGCG-3́ Right homology arm (RHA) primer #43 5́-CGATATACAGACCGATAAAACACATGC-3́ #247 5́-CTCTTTTCCCTTCCCCAGGTA-3́ Cas9 primer 5́-AGGAAATCGGCAAGGCTACC-3́ 5́-TTCGCCGTTTGTCTCGATCA-3́ Random left primer #1321 5́-AGATGTCCTAAATGCACAGCG-3́ #861 5́-GCTGCCTATCAGAAGGTGGTG-3́ Random right primer #43 5́-CGATATACAGACCGATAAAACACATGC-3́

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Table 2 (*continued*)

Site-specific nuclease		
Cas9	Cas9	
		#1752 5́-GCAGCCACTGGTAACAGGAT-3́
gRNA sequence	*PARK7*	5́-CTGATTCTTACAAGCCGGGG-3́
Genomic target sequence(s)	*PARK7*	gRNA context sequence 5́-CGGCCTGATTCTTACAAGCCGGGGTGGGAC-3́ PAM: TGG Gene: *PARK7* Location: 1.0.878 Length:878 nt [Positional Info] XM_008975660.3 position: 527
e.g. Top off-target mutagenesis predicted site sequencing (for CRISPR/Cas9 and TALENs) primers		*in silico* 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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