Pink1-KO/Parkin-KO/AIW002-02

☐ Control line ☒ Disease line
Gene edited? Yes

General information

Cell line name	Pink1-KO/ Parkin-KO /AIW002-02
Biosample ID	2889
Lines from same donor	

Donor information

Sex	MALE
Age	37 YEARS
Race	CAUCASIAN

Culture conditions

Coating/ medium	MATRIGEL/mTeSR1
Passage method	Gentle cell dissociation reagent

Derivation

Primary cell line	PBMC
Reprogramming method	RETROVIRUS
Reprogramming factors	Bcl-XI ☐ Myc ☒ Nanog ☐ SOX2 ☒ LIN28 ☐ KLF4 ☒ OCT3/4 ☒

Disease status

Disease	Parkinson Disease
Affected gene	Pink1 and Parkin
Disease mutation/family history	Pink1-KO/Parkin-KO

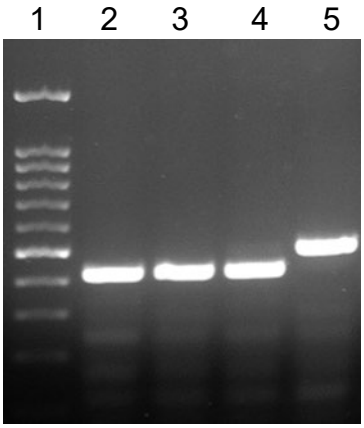
Genetic modification

Modification	CRISPR-Cas9 Knock out
Gene	Parkin-Pink1
Gene ID	Parkin; ENSG00000185345 Pink: ENSG00000158828
Chromosome location	Parkin: Chromosome 6: 161,347,420-162,727,771 Pink: Chromosome 1: 20,633,455-20,651,511 forward strand
gRNAs	Parkin: gRNA1: CTCCAGCCATGGTTTCCCAG, gRNA2: CTGCGAAAATCACACGCAAC Pink gRNA: ACTGGCGGAAGAAGCGGAGA
Delivery method	For Parkin KO: Lipofectamine TM stem reagent For Pink KO: Lonza electroporation
Subclone IDs	PINK1 and Parkin double KO
Description	For knocking out the expression of Parkin in AIW002-02 iPSCs, Guide RNAs (gRNAs) were designed using the “optimized CRISPR design” tool (www.crisp.mit.edu). The locations of gRNA1 and gRNA2 were in exon 2. Oligonucleotides with Bsb1 cleavage overhang were ordered from Life Technologies, annealed and cloned into Cas9/puromycin expressing vector (PX459 from Addgene #48139). The following gRNA sequences were used to create the KO line. gRNA1: CTCCAGCCATGGTTTCCCAG, gRNA2: CTGCGAAAATCACACGCAAC.

Genotyping: genomic DNA was extracted with QuickExtract (Lucigen) and PCR was performed using Q5® High-Fidelity DNA Polymerase according to the manufacturer's protocol (Forward primer: AAGGGCTTCGAGTGATGCTC. Reverse primer: CCTTGCTGCTCCTGTAGTCA). Wild type PCR product: 505bp. Knock out PCR product: 428bp.

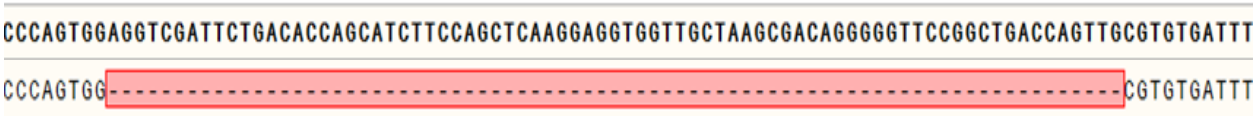
For knocking out the expression of Pink in AIW002-02 iPSCs, Guide RNAs (gRNAs) were designed using the "optimized CRISPR design" tool (www.crisp.mit.edu). The locations of gRNA1 were around exon 1 with ATG start codon. Indel cell line is identified by ddPCR, and then finally verified by DNA sequence. There are 5 base deletions which result in reading frameshift.

1A



1. 100bp DNA ladder
2. Parkin-KO/AIW002-02 No.1-5-5
3. Parkin-KO/AIW002-02 No. 2-5-7
4. Parkin-KO/AIW002-02 No. 2-6-4
5. AIW002-02

1B



1C

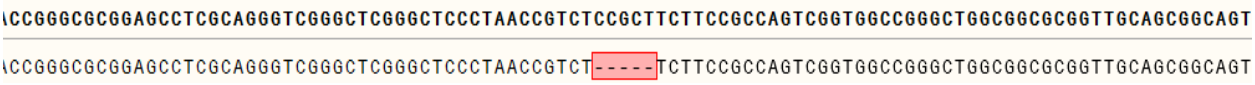


Figure 1. Sequence verification of Pink1 and Parkin double knock-out in AIW002-02.

A. PCR analysis of Parkin-KO/AIW002-02 No. 2-5-7; 428bp. AIW002-2: 505bp

B. Sequences of Parkin-KO/AIW002-02 is aligned with AIW002-02 sequence. Top: AIW002-02 genomic DNA sequence. Bottom: Parkin-KO/AIW002-02 No.2-5-7

C. Sequences of Pink1-KO/AIW002-02 is aligned with AIW002-02 sequence. Top: AIW002-02 genomic DNA sequence. Bottom: Pink1-KO/AIW002-02

Characterization:

Genotyping analysis:

Passage number	
Karyotyping	
qPCR (Genetic Analysis kit)	

Image description: Click or tap here to enter text.

Image description: Click or tap here to enter text.

Pluripotency analysis:

Marker	Expressed?
Nanog	
Tra-1-60	
SSEA-4	
OCT3/4	

Image description: Click or tap here to enter text.

Microbiology/virus screening:

Mycoplasma test	Negative
Hepatitis B	Negative
Hepatitis C	Negative
HIV1 or 2	Negative