

**Parkin-KO/AIW002-02**

Control line  Disease line  
Gene edited? Yes

**General information**

Cell line name	Parkin-KO/AIW002-02 No. 2-5-7
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 <input type="checkbox"/> Myc <input checked="" type="checkbox"/> Nanog <input type="checkbox"/> SOX2 <input checked="" type="checkbox"/> LIN28 <input type="checkbox"/> KLF4 <input checked="" type="checkbox"/> OCT3/4 <input checked="" type="checkbox"/>

**Disease status**

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

**Genetic modification**

Modification	CRISPR-Cas9 Knock out
Gene	Parkin
Gene ID	ENSG00000185345
Chromosome location	Chromosome 6: 161,347,420-162,727,771
gRNA1	gRNA1: CTCCAGCCATGGTTTCCCAG
gRNA2	gRNA2: CTGCGAAAATCACACGCAAC.
Delivery method	Lipofectamine TM stem reagent
Subclone IDs	No.1-5-5, No. 2-5-7 and No. 2-6-4

Description	<p>For knocking out the expression of Parkin in AIW002-02 iPSCs, Guide RNAs (gRNAs) were designed using the “optimized CRISPR design” tool (<a href="http://www.crisp.mit.edu">www.crisp.mit.edu</a>). 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.</p> <p>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:</p>
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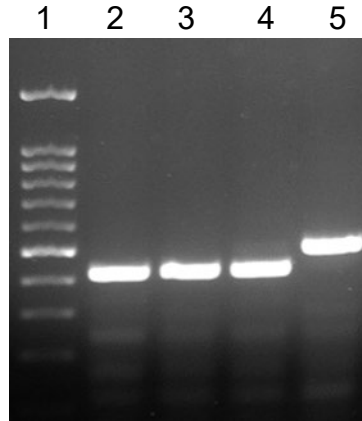
CCTTGCTGCTCCTGTAGTCA). Wild type PCR product: 505bp. Knock out PCR product: 428bp.

AIW002-02 Parkin KO NO2-5-7

Both chromosome, deletion 77 bases result in reading frame shift.

AATTTCTTGGTCAGTGTTCAGGTTCAACTCCAGCCATGGTTTCCC  
AGTGGCGTGTGATTTTCGCAGGGAAGGAGCTGAGGAATGACTGGACT  
GTGCAGGTGAGTCTCCCTTGGCGGCCGTTCTTGGGA

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

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CCCA GTGGAGGTCGATTCTGACACCAGCATCTTCCAGCTCAAGGAGGTGGTTGCTAAGCGACAGGGGGTTCCGGCTGACCAGTTGCGTGTGATTT
CCCA GTGG-----CGTGTGATTT

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**Figure 1. Sequence verification of Parkin knock-out in AIW002-02.**

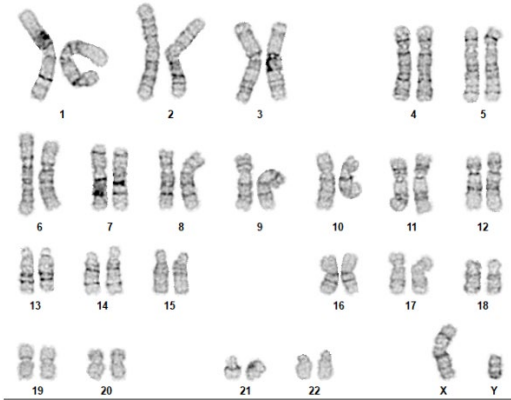
A. PCR analysis of Parkin-KO/AIW002-02 No. 2-5-7; 428bp. AIW002-2: 505bp

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

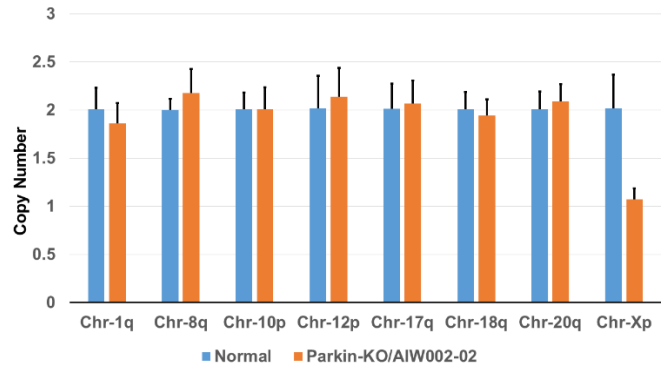
**Characterization:**

**Genotyping analysis:**

Passage number	P11 No. 2-5-7
Karyotyping	Normal 46, XY
qPCR (Genetic Analysis kit)	Normal



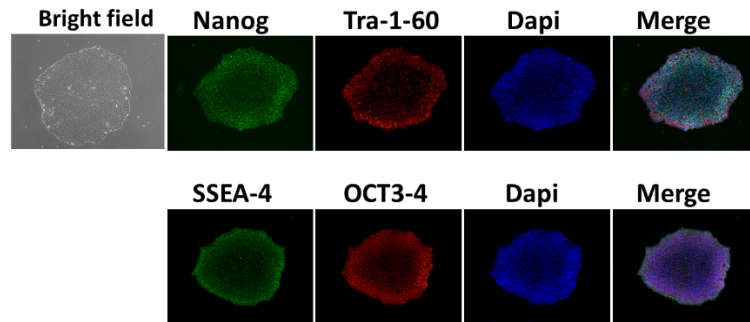
**Figure 2.**G-band assay show normal karyotype of Parkin-KO/AIW002-02 No.2-5-7



**Figure 3.** Genetic stability assay show normal chromosome of Parkin-KO/AIW002-02 No. 2-5-7.

**Pluripotency analysis:**

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



**Figure 4.** Bright field image and immunostaining of pluripotency markers on Parkin-KO/AIW002-02 No.2-5-7.

**Microbiology/virus screening:**

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