

SNCA-KO /AIW002-02

Control line Disease line
Gene edited? Yes

General information

Cell line name	SNCA KO/ AIW002-02 No. 2-13-9
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	SNCA
Disease mutation/family history	SNCA-KO

Genetic modification

Modification	CRISPR-Cas9 Knock out
Gene	SNCA (alpha-synuclein)
Gene ID	ENSG00000145335
Chromosome location	Chromosome 4: 89,724,099-89,838,315
gRNA1	GCCATGGATGTATTCATGAAAGG
gRNA2	AAGCACCAAAGTACATTTGGGG
Delivery method	Lipofectamine TM stem reagent
Subclone IDs	No. 1-7-7; No. 2-7-2 and No. 2-13-9.

Description	<p>For knocking out the expression of SNCA 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 around exon1 with ATG start codon. 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: GCCATGGATGTATTCATGAAAGG, gRNA2: AAGCACCAAAGTACATTTGGGG.</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: TCCGTGGTTAGGTGGCTAGA . Reverse primer:</p>
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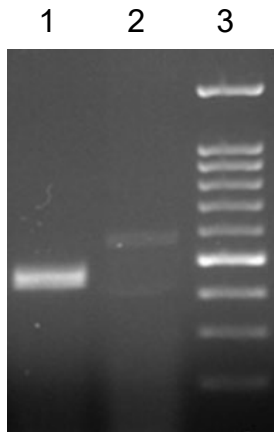
CTGGAAAAGCAAACAGTCGCA). Wild type PCR product: 572bp. Knock out PCR product: 450bp. SNCA KO cell lines were identified using agarose gel electrophoresis and confirmed by Sanger sequencing.

Sanger DNA sequence: **No. 2-13-9**

One chromosome, deletion 122 bases result in reading frame shift.

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GGAGGGTTTCTCATGATTTTTTCAGTGTTTTTTGTGTTATTTTTCCCCGA
AAGTTCTCATTCAAAGTGTATTTTATGTTTTCCAGTGTGGTGTAAGG
AATTCATTAGCCATGGATGTATTCATATGTCAGTTTGGTGCTTGTTCA
TGAGTGATGGGTTAGGATAATCAATACTCTAAATGCTGGTAGTTCTC
TCTCTTGATTCATTTTTGCATCATTGCTTGTCAAAAAGGTGGACTGAG
TCAGAGGTATGTGTAGGTAGGTGAATGTGAACGTGTGATTTGAGCT
AATAGTAA
```

1A



1. SNCA-KO/AIW002-02 No. 2-13-9
2. AIW002-02
3. 100bp DNA ladder

1B

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ATGTATTCATGAAAAGGACTTTCAAAGCCAAAGGAGGGAGTTGTGGCTGCTGCTGAGAAAACCAACAGGGTGTGGCAGAAGCAGCAGGAAAGACAAAAGAGGGTGTCTCTATGTAGGTAGGTAACCCCAATGTCAGTTTGGTGC
atgtattcat-----atgtcagtttggtgc
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Figure 1. Sequence verification of SNCA knock-out in AIW002-02.

A. PCR analysis of No. 2-13-9. AIW002-2: 572bp. SNCA-KO/AIW002-02: 450bp.

B. Sequences of No. 2-13-9 is aligned with AIW002-02 sequence. Top: AIW002-02 genomic DNA sequence. Bottom: SNCA-KO/AIW002-02 No.2-13-9.

Characterization:

Genotyping analysis:

Passage number	P12+6 No. 2-13-9
Karyotyping	Normal 46, XY
qPCR (Genetic Analysis kit)	Normal

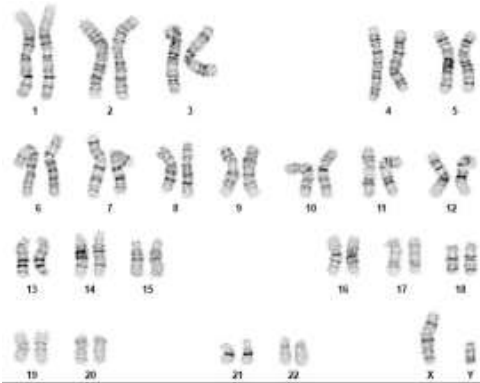


Figure 2. G-band assay show normal karyotype of SNCA-KO/AIW002-02 No.2-13-9

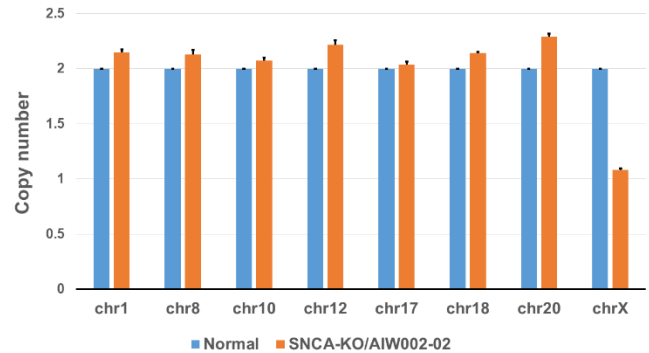


Figure 3. Genetic stability assay show normal chromosome of SNCA-KO/AIW002-02 No.2-13-9.

Pluripotency analysis:

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

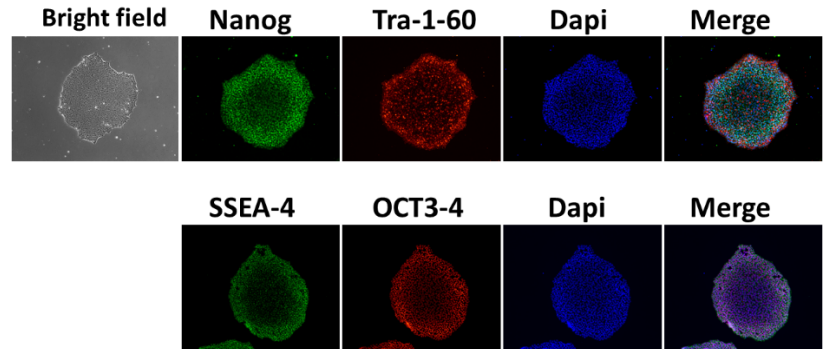


Figure 4. Bright field image and immunostaining of pluripotency markers on SNCA-KO/AIW002-02 No.2-13-9.

Microbiology/virus screening:

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

