# LRRK2-KO/AIW002-02

☐ Control line ☒ Disease line
Gene edited? Yes

## General information

| | |
|---|---|
| Cell line name | LRRK2-KO/AIW002-02<br>No. 2-2-2 |
| 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 ☒<br>Nanog ☐ SOX2 ☒<br>LIN28 ☐ KLF4 ☒<br>OCT3/4 ☒ |

## Disease status

| | |
|---|---|
| Disease | Parkinson Disease |
| Affected gene | LRRK2 |
| Disease mutation/family history | LRRK2-KO |

## Genetic modification

| | |
|---|---|
| Modification | CRISPR-Cas9 Knock out |
| Gene | LRRK2 |
| Gene ID | ENSG00000188906 |
| Chromosome location | Chromosome 12: 40,196,744-40,369,285 |
| gRNA1 | gRNA1: CAACAGCTGGAGATGGCGGT, |
| gRNA2 | gRNA2: GTCCTAGAGTAAGGCACATT. |
| Delivery method | Lipofectamine TM stem reagent |
| Subclone IDs | No.2-2-2, No.2-5-14 |
| Description | For knocking out the expression of LRRK2 in AIW002-02, guide RNAs (gRNAs) were designed using the “optimized CRISPR design” tool (www.crisp.mit.edu). The locations of gRNA1 and gRNA2 were around exon 1 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: CAACAGCTGGAGATGGCGGT, gRNA2: GTCCTAGAGTAAGGCACATT.<br><br>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: TTCCTCATAAACAGGCGGGC . Reverse primer: |

AAGAGTCCGGGTTTGAGCAG). Wild type PCR product: 427bp. Knock out PCR product: 320bp.

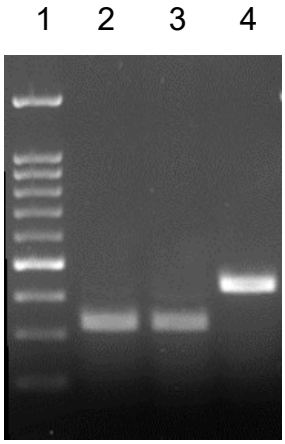
LRRK2 KO/AIW002-02 No. 2-2-2
One chromosome, deletion 106 bases result in reading frame shift

GCCCCCGGGGAGCTGTGGCCGGCGCCCCTGCCGGTTCCCTGAGCA
GCGGACGTTCATGCTGGGAGGGCGGCGGGTTGGAAGCAGGTGCCAC
CATGGCATCCTGGAGGATCTGCTGGTGTTCACGTACTCCGAGCGCGG
TAATCACTTGAAAATAAACTGTGCTTTTATTTTT

The other chromosome, deletion 107 bases result in reading frame shift.

GCCCCTGCCGGTTCCCTGAGCAGCGGACGTTCATGCTGGGAGGGCG
GCGGGTTGGAAGCAGGTGCCACCATGGCTCCTGGAGGATCTGCTGGT
GTTCACGTACTCCGAGCGCGGTAATCACTTGAAAATAAACTGTGCTTTT
ATTTTTG

**1A**




1. 100bp DNA ladder
2. LRRK2-KO/AIW002-02 No. 2-2-2
3. LRRK2-KO/AIW002-02 No. 2-5-14
4. AIW002-02

**1B**

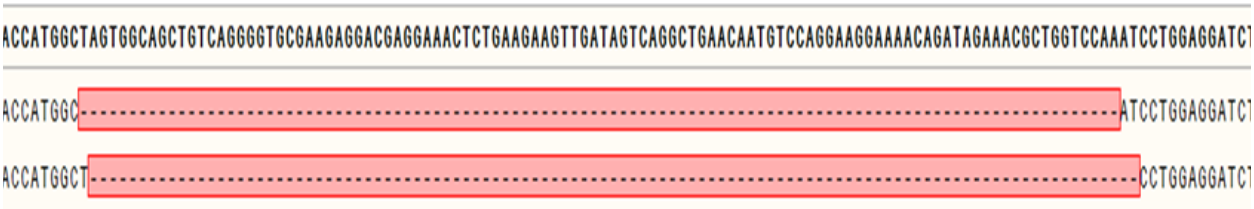



**Figure 1. Sequence verification of LRRK2 knock-out in AIW002-02.**

A. PCR analysis of LRRK2-KO/AIW002-02: 320bp. AIW002-2: 427bp

B. Sequences of LRRK2-KO/AIW002-02 is aligned with AIW002-02 sequence. Top: AIW002-02 genomic DNA sequence. Bottom: two chromosome of LRRK2-KO/AIW002-02 No.2-2-2.

## Characterization:

## Genotyping analysis:

| | |
|---|---|
| Passage number | P12+P6 No.2-2-2 |
| Karyotyping | Normal 46,XY |
| qPCR (Genetic Analysis kit) | Normal |

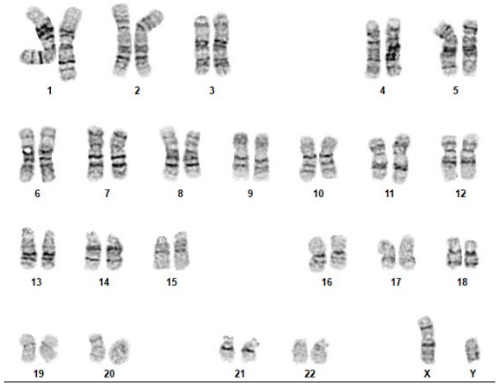


**Figure 2.G-band assay show normal karyotype of LRRK2-KO/AIW002-02 No. 2-2-2, 46, XY**

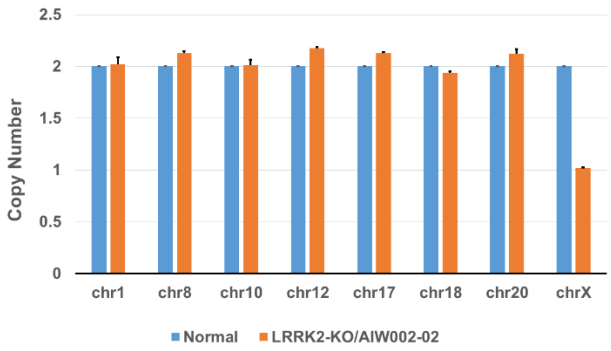



**Figure 3. Genetic stability assay show normal chromosome of LRRK2-KO/AIW002-02 No. 2-2-2**

## Pluripotency analysis:

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

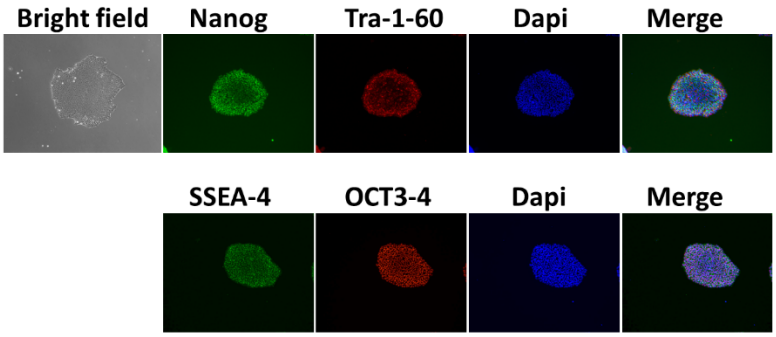



**Figure 4. Bright field image and immunostaining of pluripotency markers on LRRK2-KO/AIW002-02 No.2-2-2.**

## Microbiology/virus screening:

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