# GBA-KO /AIW002-02

☐ Control line ☒ Disease line
Gene edited? Yes

## General information

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|---|---|
| Cell line name | GBA KO/ AIW002-02 No. 18 |
| 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

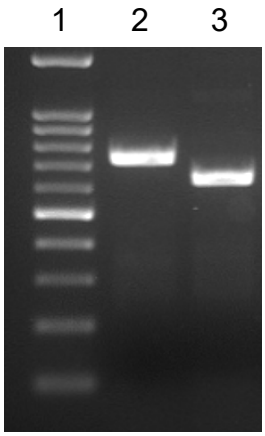
| | |
|---|---|
| Disease | Parkinson Disease |
| Affected gene | GBA |
| Disease mutation/family history | GBA-KO |

## Genetic modification

| | |
|---|---|
| Modification | CRISPR-Cas9 Knock out |
| Gene | GBA |
| Gene ID | ENSG00000177628 |
| Chromosome location | Chromosome 1: 155,234,452-155,244,699 |
| gRNA1 | TAAAAGCTTCGGCTACAGCT |
| gRNA2 | GCTATGAGAGTACACGCAGT |
| Delivery method | Neon electroporation |
| Subclone IDs | NO18 |
| Description | For knocking out the expression of GBA 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 4. 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: TAAAAGCTTCGGCTACAGCT, gRNA2: GCTATGAGAGTACACGCAGT.<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: TTTTGGCTCATTCCAACCTC. Reverse primer: |

| | |
|---|---|
| | TTGAGAGCAGCAGCATCTGT). Wild type PCR product: 739bp. Knock out PCR product: 641bp.<br><br>Sanger DNA sequence:<br><br>AIW002-02 GBA KO NO18<br><br>Both chromosome, deletion 98 bases result in reading frame shift.<br><br>AGGTAGCTGGGATTACAGGCGGCCACCACTACGCCCAGCTAATTTT<br>TGTATTTTTAGTAGAGACGGGGTTTCACCATGCTGGCAAGGCAGGT<br>CTCAAACTCCTCACCTCAGGTGATCCGCCCACCTCGGCCTCCTAAA<br>GTGCTAGGATTACAGGTGTGAGCCCCTGCGCCCGGCCAAGGGGTG<br>AGGAATTTTGAAACCGTGTTCAGTCTCTCCTAGCAGATGTGTCCATT<br>CTCCATGTCTTCATCAGACCTCACTCTGCTTGTACTCCCTCCCTCCC<br>AGGTGCCCGCCCCTGCATCCCTAAAAGCTTCGGCTACAAGTGGGCG<br>ACGGATGGAGCTGAGTATGGGGCCCATCCAGGCTAATCACACG |

**1A**




1. 100bp DNA ladder
2. AIW002-02
3. GBA-KO/AIW002-02 No.18

**1B**

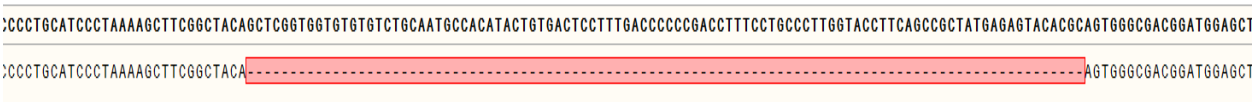



**Figure 1. Sequence verification of GBA knock-out in AIW002-02.**

A. PCR analysis of GBA-KO/AIW002-02 No.18: 641bp. AIW002-2: 739bp

B. Sequences of GBA-KO/AIW002-02 is aligned with AIW002-02 sequence. Top: AIW002-02 genomic DNA sequence. Bottom: GBA-KO/AIW002-02 No.18.

## Characterization:

### Genotyping analysis:

| | |
|---|---|
| Passage number | P10 No.18 |
| Karyotyping | Normal 46, XY |
| qPCR (Genetic Analysis kit) | Normal |

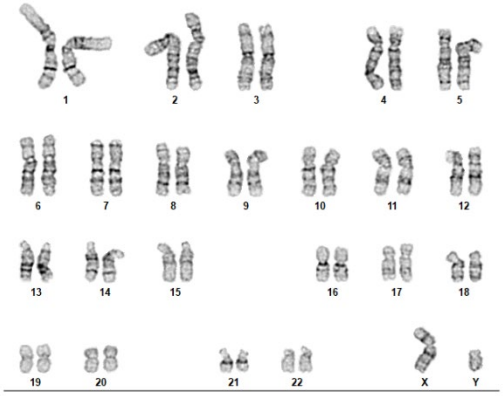


**Figure 2.G-band assay show normal karyotype of GBA-KO/AIW002-02 No.18**

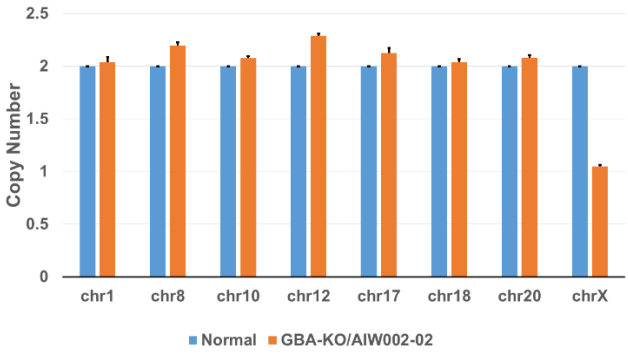



**Figure 3. Genetic stability assay show normal chromosome of GBA-KO/AIW002-02 No.18.**

### Pluripotency analysis:

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

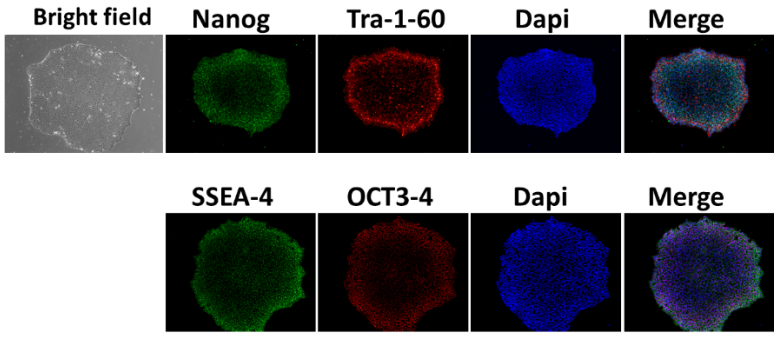



**Figure 4. Bright field image and immunostaining of pluripotency markers on GBA-KO/AIW002-02 No.18.**

### Microbiology/virus screening:

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