

MAPT-KO/AIW002-02

Control line Disease line
Gene edited? Yes

General information

Cell line name	MAPT-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 <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	Alzheimer's Disease
Affected gene	MAPT
Disease mutation/family history	MAPT-KO

Genetic modification

Modification	CRISPR-Cas9 Knock out
Gene	MAPT
Gene ID	ENSG00000276155
Chromosome location	Chromosome CHR_HSCHR17_1_CTG5: 46,069,784-46,203,150
gRNA1	gRNA1: GGATAAGTTCTGAGGAGTGT
gRNA2	gRNA2: TGATCTGGGCCTGCTGTGCA
Delivery method	Lipofectamine TM stem reagent
Subclone IDs	No. 24-6. No. 31-4

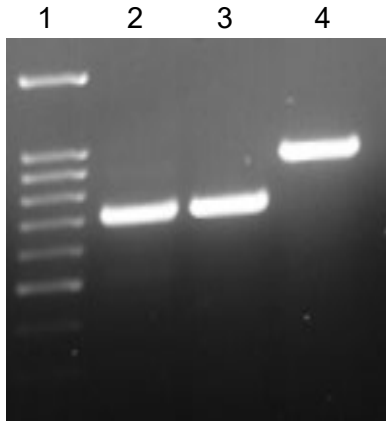
Description

For knocking out the expression of MAPT 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 exon 2 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: GGATAAGTTCTGAGGAGTGT, gRNA2: TGATCTGGGCCTGCTGTGCA.

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: AGCTGGGAATAACAAGTCGGG . Reverse primer: GCTTGAGTTATCTTGGCCAC). Wild type PCR product: 918bp. Knock

out PCR product: 697-714bp. MAPT-KO cell lines confirmed by Sanger sequencing.

1A



1. 100bp DNA ladder
2. MAPT-KO/AIW002-02 No. 24-6
3. MAPT-KO/AIW002-02 No. 31-4
4. AIW002-02

1B



Fig1. Sequence verification of TAU knock-out in AIW002-02 cell.

A. PCR analysis of MAPT-KO/AIW002-02. MAPT-KO/AIW002-02: 697-714bp. AIW002-2: 918bp

B. Sequences of MAPT-KO/AIW002-02 is aligned with AIW002-02 sequence. 1. AIW002-02 genomic DNA sequence. 2. MAPT-KO/ AIW002-02 No. 31-4 chromosome DNA sequence. 3. MAPT-KO/ AIW002-02 No. 24-6 one chromosome DNA sequence. 4. MAPT-KO/ AIW002-02 No. 24-6 the other genomic DNA sequence.

Characterization:

Genotyping analysis:

Passage number	«Genome_stability_passage_number»
Karyotyping	«Karyotyping»
qPCR (Genetic Analysis kit)	«qPCR»

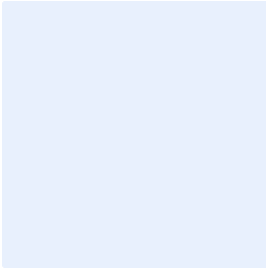


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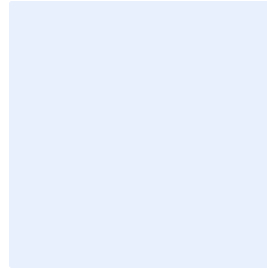


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Pluripotency analysis:

Marker	Expressed?
Nanog	«Nanog»
Tra-1-60	«Tra160»
SSEA-4	«SSEA4»
OCT3/4	«Oct_34»

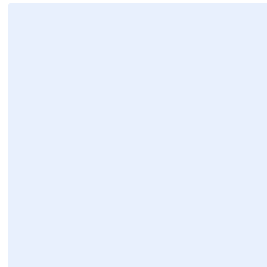


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Microbiology/virus screening:

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