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Lab Resource: Multiple Cell Lines

Derivation of iPSC lines from two patients with autism spectrum disorder carrying *NRXN1α* deletion (NUIGi041-A, NUIG041-B; NUIGi045-A) and one sibling control (NUIGi042-A, NUIGi042-B)

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A B S T R A C T

NRXN1 encodes thousands of splicing variants categorized into long NRXN1α, short NRXN1β and extremely short NRXN1γ, which exert differential roles in neuronal excitation/inhibition. *NRXN1α* deletions are common in autism spectrum disorder (ASD) and other neurodevelopmental/neuropsychiatric disorders. We derived induced pluripotent stem cells (iPSCs) from one sibling control and two ASD probands carrying *NRXN1α*$^{+/-}$, using non-integrating Sendai viral method. All iPSCs highly expressed pluripotency markers and could be differentiated into ectodermal/mesodermal/endodermal cells. The genotype and karyotype of the iPSCs were validated by whole genome SNP array. The availability of the iPSCs offers an opportunity for understanding *NRXN1α* function in human neurons and in ASD.

1. Resource table

Unique stem cell lines identifier	NUIGi041-A NUIGi041-B NUIGi042-A NUIGi042-B NUIGi045-A
Alternative names of stem cell lines	NUIGi041-A (ND3C2, *NRXN1α*$^{+/-}$) NUIGi041-B (ND3C4, *NRXN1α*$^{+/-}$) NUIGi042-A (CND3C1, *NRXN1α*$^{+/+}$) NUIGi042-B (CND3C3, *NRXN1α*$^{+/+}$) NUIGi045-A (ND2C1, *NRXN1α*$^{+/-}$)
Institution	Regenerative Medicine Institute, National University of Ireland Galway, H91 TK33 Galway, Ireland
Contact information of distributor	*Sanbing Shen* *sanbing.shen@nuigalway.ie*
Type of cell lines	Induced pluripotent stem cells (iPSCs)
Origin	Human
Cell Source	Dermal Fibroblasts
Clonality	Clonal

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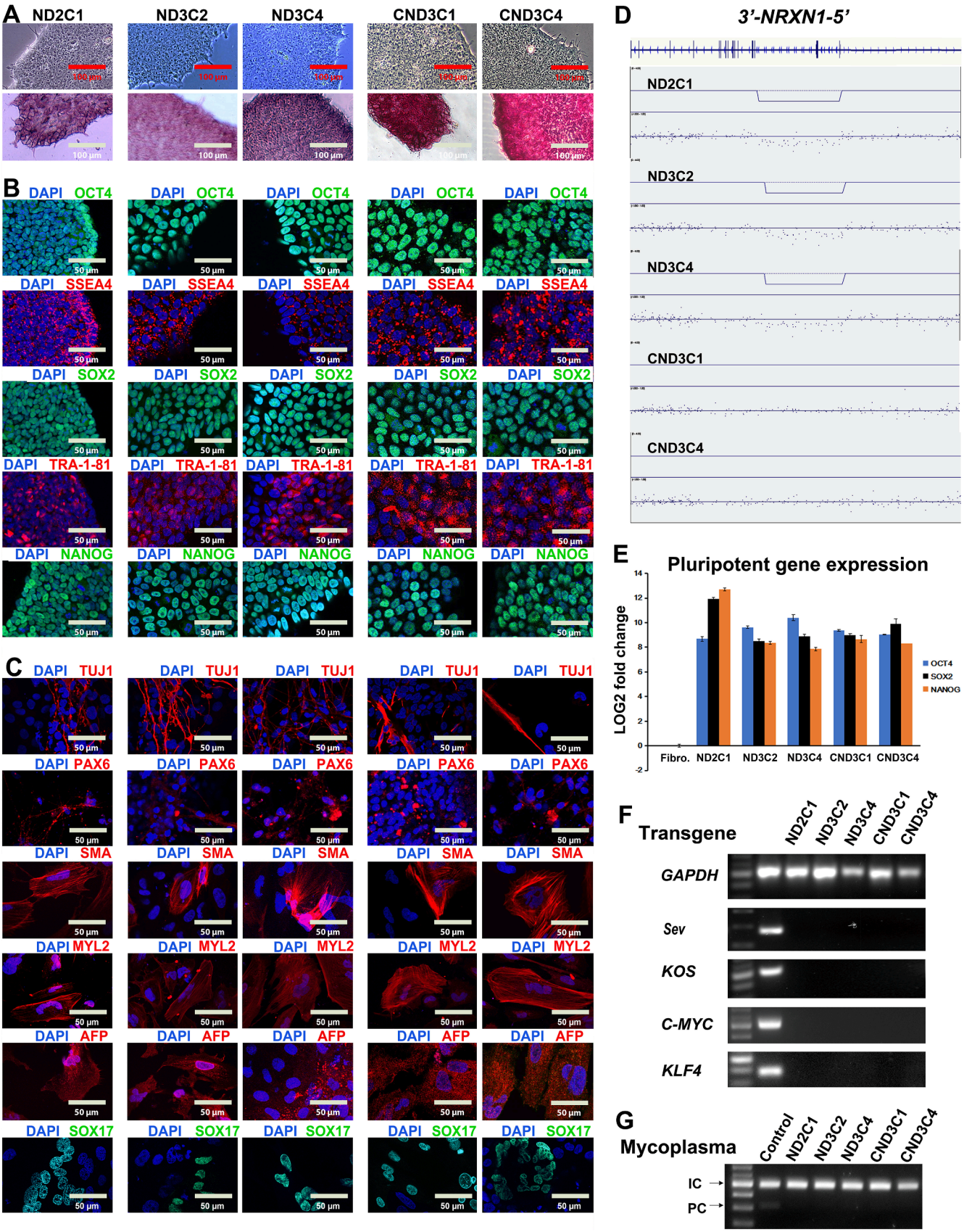

Fig. 1. Charaterization of iPSCs.

Table 1
Summary of lines.

iPSC line names	Abbreviation in figures	Gender	Age	Ethnicity	Genotype of locus	Disease
NUIGi045-A	ND2C1	Male	20	Caucasian	NRXN1$^{+/-}$ Hg38 chr2:50887567-51218714	ASD
NUIGi041-A	ND3C2	Male	20	Caucasian	NRXN1$^{+/-}$ Hg38 chr2:50910347-51140703	ASD
NUIG041-B	ND3C4	Male	20	Caucasian	NRXN1$^{+/-}$ Hg38 chr2:50910347-51140703	ASD
NUIGi042-A	CND3C1	Male	21	Caucasian	NRXN1$^{+/+}$	Sibling control
NUIGi042-B	CND3C4	Male	21	Caucasian	NRXN1$^{+/+}$	Sibling control

(*continued*)

Method of reprogramming	Integration-free Sendai Virus expressing OCT4, SOX2, c-MYC, KLF4
Multiline rationale	Two clones NUIGi042-A (CND3C1, *NRXN1α*$^{+/+}$) and NUIGi042-B (CND3C3, *NRXN1α*$^{+/+}$) from a sibling control and three clones from two ASD probands carrying *NRXN1α* $^{+/-}$ deletion on exons 1–5 [NUIGi041-A, ND3C2, *NRXN1α*$^{+/-}$; NUIGi041-B, ND3C4, *NRXN1α*$^{+/-}$; and NUIGi045-A, ND2C1, *NRXN1α*$^{+/-}$) are essential for overcoming the heterogeneity of iPSCs and for creating isogenic lines to minimize the genetic background effects.
Gene modification	Yes
Type of modification	*de novo*
Associated disease	Autism spectrum disorder (ASD)
Gene/locus	*NRXN1α*$^{+/-}$ for NUIGi041-A (ND3C2) and NUIGi041-B (ND3C4) carrying 230,357 bp heterozygous *NRXN1α* deletion on exons 1–5 (chr2:50910347-51140703, Hg38). *NRXN1α*$^{+/-}$ for NUIGi045-A (ND2C1) carrying 331,148 bp heterozygous deletion on exons 1–5 of *NRXN1α* (chr2:50887567–51218714, Hg38). *NRXN1α*$^{+/+}$ for NUIGi042-A (CND3C1) and NUIGi042-B (CND3C3) carrying no NRXN1 deletion or no other specific and consistent CNV.
Method of modification	N/A
Name of transgene or resistance	N/A
Inducible/constitutive system	N/A
Date archived/stock date	10/2018, 11/2018, 3/2019
Cell line repository/bank	Regenerative Medicine Institute, National University of Ireland, Galway
Ethical approval	This study has been approved Galway University Hospitals Clinical Ethics Committee (C.A.750). Patient gave their written informed consent for skin biopsy donation.

2. Resource utility

The ND2/ND3 ASD iPSCs with heterozygous deletion of exons 1–5 of the *NRXN1* gene (*NRXN1α*$^{+/-}$) offer a great opportunity for coupling NRXN1α isoform function in human neurons with clinical symptoms. The CND3 offers sibling iPSCs to create closely related isogenic lines for phenotypic comparison with minimal genetic background effects.

3. Resource details

Human *NRXN1* coding region spans 1.2 Mb of genomic sequence with thousands of Neurexin splicing variants falling into three major classes of long NRXN1α, short NRXN1β and extremely short NRXN1γ, which play differential roles in neuronal excitation and inhibition. Additionally, *NRXN1* gene comprises of 3.3 Mb intergenic DNA which are highly conserved throughout evolution and anticipated to closely regulate *NRXN1* expression. The NRXN1 and family members are therefore proposed to encode language of neuronal communication (Südhof, 2017).

The excitation/inhibition imbalance is considered as a major mechanism for ASD and other neurodevelopmental and neuropsychiatric disorders. Deletions of *NRXN1* gene are known to be a shared risk factor among hundreds of rare factors for ASD (Pinto et al., 2014; Al Shehhi et al., 2019) schizophrenia, intellectual disability, epilepsy and developmental delay (Grayton et al., 2012). The availability of the iPSCs from different disease cohorts with different *NRXN1* deletion regions will offer an opportunity to create 3-D organoid disease models (Lancaster et al., 2013), which may unveil NRXN1 functions in normal neuronal physiology and in disease pathology. The resources may also assist identification of a second hit of differential risk factors among different individuals, which may hold a key for expressing diverse clinical symptoms together with *NRXN1* lesions.

In this study, we report the iPSCs derived from three Caucasian donors using Sendai virus vectors to express OCT4, SOX2, KLF4 and C-MYC in fibroblasts. The 20-year-old ND2 male was diagnosed with ASD, language delay, IQ of 78 at age 11, but attended mainstream education. He carries 331,148 bp *NRXN1α*$^{+/-}$ deletion from upstream to intron 5 (chr2:50887567-51218714, Hg38, Fig. 1E). One of his parents had language delay, one grandfather and one cousin had ASD. The 20-year-old ND3 was diagnosed of ASD, speech and language delay, moderate intellectual disability, Beckwith-Wiedemann syndrome, carrying 230,357 bp *NRXN1α*$^{+/-}$ deletion (chr2:50910347-51140703, Hg38, Fig. 1E) from upstream to intron 5. The 21-year-old CND3 is a healthy male sibling of ND3.

Fibroblasts at passage 6 were used to generate iPSCs. The derived cells showed typical embryonic stem cell-like morphology with small cell body and a large nucleus/cytoplasm ratio, which formed packed colony structure (Fig. 1A). They contained high alkaline phosphatase activity (Fig. 1A), expressed high level of mRNA from the endogenous *OCT4*, *SOX2* and *NANOG* genes (Fig. 1E), and were positively immunostained for pluripotency markers of OCT4, SSEA4, SOX2, NANOG and TRA-1–81 (Fig. 1B). They could spontaneously differentiate into cells of three embryonic germ layers from embryoid bodies with positive immunoreactivity for endodermal markers of alpha-fetoprotein (AFP) and SRY-Box Transcription Factor 17 (SOX17), mesodermal markers of alpha smooth muscle actin (α-SMA) and Myosin Light Chain 2 (MYL2), and ectodermal markers of beta-III tubulin (TUJ1) and Paired box protein Pax-6 (PAX6) (Fig. 1C).

We carried out whole genome SNP array and confirmed the *NRXN1α*$^{+/-}$ deletions in two ASD probands (Fig. 1D, Supplementary Fig. 1) with no other consistent/specific CNVs among the cell lines (Table 1, Supplementary Fig. 1), with a limitation to detect balanced chromosomal translocations. The iPSCs were free of transgene integration (Fig. 1F), or mycoplasma contamination (Fig. 1G), which were validated by RT-PCR and PCR. The full characterization were summarized in Table 2 and Fig. 1. The iPSCs from two ASD *NRXN1α*$^{+/-}$ donors and a sibling control will become valuable resources for investigating molecular mechanisms of ASD and contribution of NRXN1α lesion to neuronal dysfunction.

Table 2
Characterization and validation.

Classification	Test	Result	Data
Morphology	Photography	Normal morphology	Fig. 1A
Phenotype	Alkaline phosphatase staining	Positive staining	Fig. 1A
	Immunocytochemistry	Positive for OCT4, SSEA4, SOX2, TRA-1–81 and NANOG.	Fig. 1B
	qRT-PCR	Positive for SOX2, OCT4 and NANOG	Fig. 1E
	RT-PCR	Negative for Sendai vectors	Fig. 1F
Genotype	Single Nucleotide Polymorphism	No gross chromosomal alteration by reprogramming detected	Suppl. Fig. 1
Identity	Fingerprinting (STR analysis)	Tested 16 sites (CSF1PO, D2S1338, D3S1358, D5S818, D7S820, D8S1179, D13S317, D16S539, D18S51, D19S433, D21S11, FGA, TH01, TPOX, vWA, and the gender marker Amelogenin), all matched	The data will be archived with the journal as per the instructions.
Mutation analysis	Single Nucleotide Polymorphism	NRXN1α+/− for NUIGi041-A (ND3C2) and NUIGi041-B (ND3C4) harbors 230357 bp deletion spanning from upstream to intron 5 (chr2:50910347-51140703, Hg38) NRXN1α+/− for NUIGi045-A (ND2C1) carrying deletion from upstream to intron 5 (chr2:50887567-51218714, Hg38)	Fig. 1D
Microbiology and virology	Mycoplasma	Detection by PCR, negative	Fig. 1G
Differentiation potential	Embryonic body formation	Alpha-fetoprotein (AFP) and SRY-Box Transcription Factor 17 (SOX17) for endoderm, alpha smooth muscle actin (α-SMA) Myosin Light Chain 2 (MYL2) for mesoderm, and beta-III tubulin (TUJ1) and Paired box protein Pax-6 (PAX6) for ectoderm	Fig. 1C

4. Materials and methods

4.1. Cell reprogramming

Fibroblasts derived from skin biopsy were cultured at 37 °C, 5% CO_2, in high glucose DMEM (Gibco), supplemented with 1% NEAA solution (Gibco), 10% FBS (Sigma-Aldrich) and 1% penicillin-streptomycin. Fibroblasts at P6 were transduced with Cytotune-iPS 2.0 Sendai Reprogramming kit (ThermoFisher Scientific, Cat. A16518) under manufacturer's instructions. Isolated iPSCs were cultured on Geltrex-coated 6-well plates in Essential 8 medium (Gibco). All the iPSC lines were passaged to P14 or higher by Gentle Cell Dissociation Reagent (STEMCELL) for full characterization (Table 1).

4.2. Pluripotency validation

Alkaline Phosphatase Staining Kit II (Stemgent) was used to detect alkaline phosphatase activity. For immunofluorescence staining iPSCs were fixed in 4% PFA for 20 min, permeabilized with 0.1% Triton X-100 (Sigma) for 15 min, blocked for 1 h in 1% BSA-DPBS, and incubated overnight with primary antibodies against OCT4, SSEA4, SOX2 or TRA-1–81 (Table 2) at 4 °C. Alexa Fluor 488- or 555-conjugated secondary antibodies (Cell Signaling Technology) were used to visualize the immunoreactivity, and Hoechst 33342 for cell nuclei (Life Technologies). Cells were imaged under the Olympus FluoView 1000 system. qRT-PCR reaction was performed on the StepOne Plus Real Time PCR System. Fast SYBR Green Master Mix (Applied Biosystems) and specific primers (Table 3) were used to quantify the expression of endogenous *OCT4*, *SOX2* and *NANOG*, which were adjusted with GAPDH as an internal control, and then converted to log2 fold of expression over fibroblast mRNA as a negative control.

4.3. Three germ layer differentiation

iPSCs were scraped and transferred to uncoated 6-well plates and shaken at 50 rpm on an orbital shaker inside a 37 °C incubator. EBs were formed in suspension culture with DMEM/F12 medium supplemented with 20% FBS, 1% L-Glutamine (200 mM), 1% non-essential amino acids solution, 1% penicillin-streptomycin and 0.2% β-mercaptoethanol. Five days later EBs were plated on Geltrex-coated 8-well chambers (iBiDi) for 3–4 weeks of spontaneous differentiation and stained with antibodies against AFP, SOX17, α-SMA, MYL2, PAX6 and TUJ1 (Table 3).

4.4. DNA fingerprinting analysis

STR analysis was perfomred commercially with 16 independent markers including CSF1PO, D2S1338, D3S1358, D5S818, D7S820, D8S1179, D13S317, D16S539, D18S51, D19S433, D21S11, FGA, TH01, TPOX, vWA, and the gender marker AMEL (Amelogenin) by Eurofins Genomics Europe Applied Genomics GmbH, with the Applied Biosystems AmpFLSTR Identifier Plus PCR Amplification Kit. Data were analyzed by Applied Biosystems GeneMapper Software 6 according to ANSI/ATCC standard ASN-0002.

4.5. Karyotyping

The molecular karyotype was analyzed by Beijing Hyslar Biotech Limited Corporation (Beijing, China) with 990k SNP array. The SNP data was analyzed by Axiom Analysis software (ThermoFisher, USA) which generated LogR ratio and B allele plots, using 83 samples to create an internal control. IGV software was used then to examine the molecular karyotyping of fibroblasts and derived iPSC lines aligned to Hg38 genome.

4.6. Transgene-free confirmation

Total RNA from cells were extracted from iPSCs with RNeasy Mini Kit (Qiagen), and reversely transcribed to single strand cDNA with sensiFAST cDNA Synthesis Kit (Sigma-Aldrich). The cDNA was diluted 1:10 and amplified by PCR with TopTaq® Master Mix (Qiagen) under standard conditions using a set of commercially supplied transgene-specific primers (Table 3).

Table 3
Reagents details.

Antibodies used for immunocytochemistry/flow-cytometry			
	Antibody	Dilution	Company Cat # and RRID
Pluripotency Markers	Rabbit anti-OCT4	1:1000	Cell Signaling Technology Cat# 2840, RRID:AB_2167691
Pluripotency Markers	Mouse anti-SSEA4	1:500	Cell Signaling Technology Cat# 4755, RRID:AB_1264259
Pluripotency Markers	Rabbit anti-SOX2	1:1000	Cell Signaling Technology Cat# 3579, RRID:AB_2195767
Pluripotency Markers	Mouse anti-TRA-1–81	1:500	Cell Signaling Technology Cat# 2840, RRID:AB_2119060
Pluripotency Markers	Rabbit anti-NANOG	1:1000	Cell Signaling Technology Cat# 3580, RRID: AB_2150399
Differentiation Markers	Mouse anti-AFP	1:200	Sigma-Aldrich Cat# A8452, RRID:AB_258392
Differentiation Markers	Mouse anti-TUJ1	1:500	Abcam Cat# ab78078, RRID:AB_2256751
Differentiation Markers	Mouse anti-SMA	1:500	Cell Marque Corp Cat# 202M-96, RRID:AB_1157940
Differentiation Markers	Rabbit anti-PAX6	1:200	Abcam Cat# ab195045 RRID:AB_2750924
Differentiation Markers	Rabbit anti-MYL2	1:200	Proteintech Cat# 10906-1-AP, RRID:AB_2147453
Differentiation Markers	Goat anti-SOX17	1:20	R and D Systems Cat# AF1924, RRID:AB_355060
Secondary antibodies	AF488 Goat Anti-Rabbit IgG	1:1000	Cell Signaling Technology Cat# 4412, RRID:AB_1904025
Secondary antibodies	AF555 Goat Anti-Mouse IgG	1:1000	Cell Signaling Technology Cat# 4412, RRID:AB_1904022
Secondary antibodies	AF488 Goat Anti-Rabbit IgG	1:1000	Cell Signaling Technology Cat# 4412, RRID:AB_1904025
Secondary antibodies	AF555 Goat Anti-Mouse IgG	1:1000	Cell Signaling Technology Cat# 4412, RRID:AB_1904022

Primers		
	Target	Forward/Reverse primer (5′–3′)
Sendai Reprogramming Vector (RT-PCR)	SeV/181 bp	For: GGATCACTAGGTGATATCGAGC Rev: ACCAGACAAGAGTTTAAGAGATATGTATC
Sendai Reprogramming Vector (RT-PCR)	KOS (KLF4, OCT3/4, SOX2)/528 bp	For: ATGCACCGCTACGACGTGAGCGC Rev: ACCTTGACAATCCTGATGTGG
Sendai Reprogramming Vector (RT-PCR)	KLF4/410 bp	For: TTCCTGCATGCCAGAGGAGCCC Rev: AATGTATCGAAGGTGCTCAA
Sendai Reprogramming Vector (RT-PCR)	C-MYC/532 bp	For: TAACTGACTAGCAGGCTTGTCG Rev: TCCACATACAGTCCTGGATGATGATG
Pluripotency Markers (qPCR)	NANOG/149 bp	For: ATAACCTTGGCTGCCGTCTC Rev: AGCCTCCCAATCCCAAACAA
Pluripotency Markers (qPCR)	OCT4/229 bp	For: AACTTCACTGCACTGTACTCCTC Rev: CACCCTTTGTGTTCCCAATTCC
Pluripotency Markers (qPCR)	SOX2/187 bp	For: AGACTTCACATGTCCCAGCACT Rev: CGGGTTTTCTCCATGCTGTTTC
House-Keeping Genes (qPCR)	GAPDH/206 bp	For: AGGGCTGCTTTTAACTCTGGT Rev: CCCCACTTGATTTTGGAGGGA

4.7. Mycoplasma detection

MycoSensor PCR Assay Kit (Agilent) were used to detect the contamination of mycoplasma.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scr.2021.102222.

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