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Lab Resource: Single Cell Line

Derivation of YCMi005-A, a human-induced pluripotent stem cell line, from a patient with dilated cardiomyopathy carrying missense variant in *TPM1* (p. Glu192Lys)

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A B S T R A C T

Dilated cardiomyopathy (DCM) is one of the leading causes of heart transplantation. The clinical feature of DCM is characterized by enlarged heart and impaired function of the left or both ventricles, while its etiology is varied. In this study, we generated YCMi005-A, a human-induced pluripotent stem cell (hiPSC) line from a patient with DCM carrying the missense mutation of p.Glu192Lys in the *TPM1* genes. YCMi005-A, an established hiPSC, showed the normal karyotype (46, XX) and high expression of pluripotency markers. In addition, it was confirmed that YCMi005-A has the differentiation potential assessed by staining of three germ layer markers.

1. Resource Table

Unique stem cell line identifier	YCMi005-A
Alternative name(s) of stem cell line	YCMi005-hDCM005-A
Institution	Yonsei University College of Medicine
Contact information of distributor	Seung-Hyun Lee, tiger815@yuhs.ac
Type of cell line	iPSC
Origin	Human
Additional origin info required for human ESC or iPSC	Age: 67 Sex: Female Ethnicity: Korean
Cell Source	Peripheral blood mononuclear cells (PBMCs)
Clonality	Clonal
Method of reprogramming	Episomal plasmid vectors, Transgene-free
Associated disease	Dilated cardiomyopathy
Gene/locus	Heterozygous mutation in TPM1 gene (NM_001018005.1) / c.574G > A, p.Glu192Lys
Date archived/stock date	July 2021
Cell line repository/bank	https://hpscreg.eu/cell-line/YCMi005-A
Ethical approval	Ethical committee: Yonsei University Health System, Severance Hospital, Institutional review board approval number: 4-2020-0112

2. Resource utility

Because of genetic heterogeneity, the exact pathomechanism of DCM

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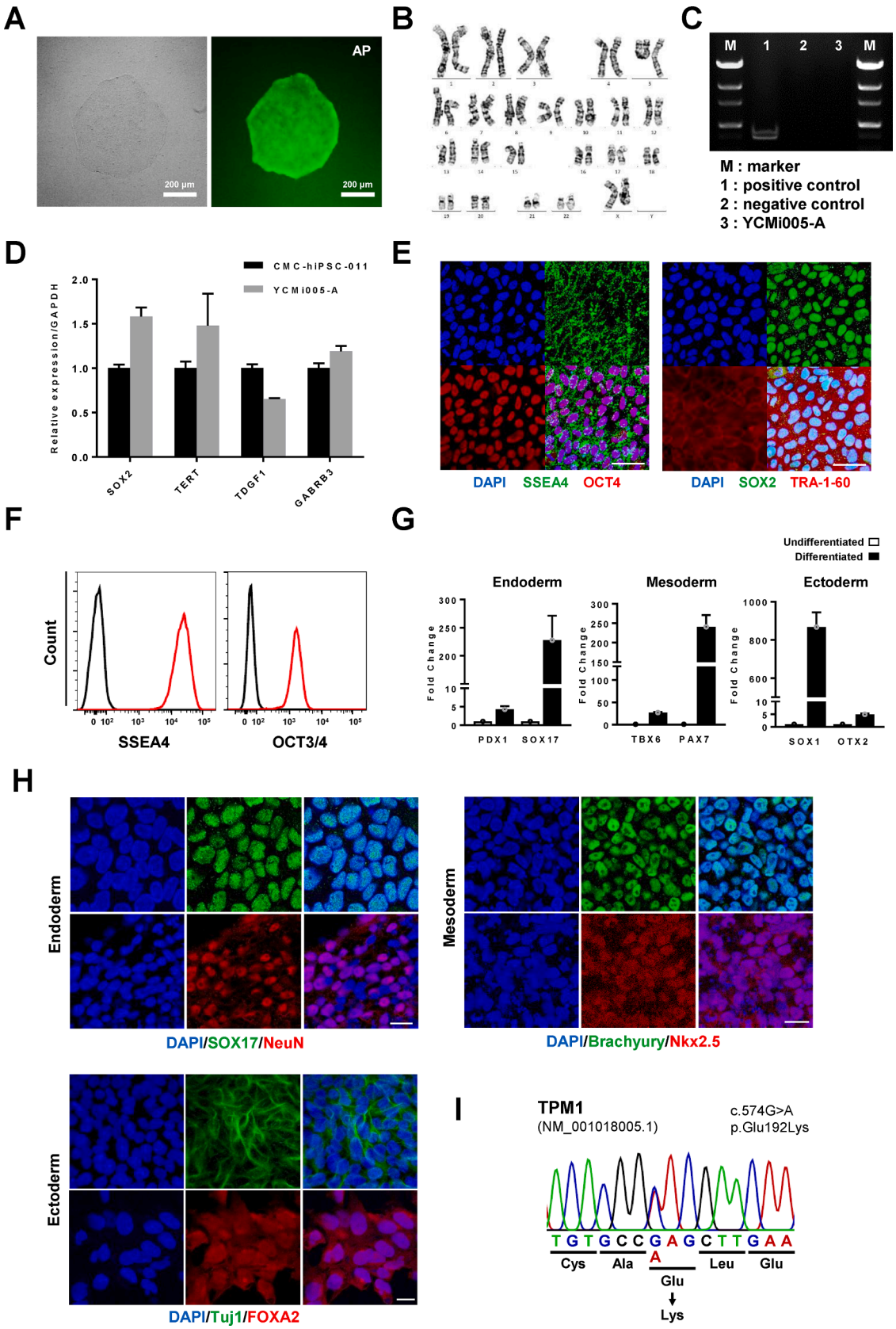
A
AP
200 μm
200 μm
B
C
M 1 2 3 M
M : marker
1 : positive control
2 : negative control
3 : YCMi005-A
D
CMC-hiPSC-011
YCMi005-A
Relative expression/GAPDH
2.0
1.5
1.0
0.5
0.0
SOX2
TERT
TDGF1
GABRB3
E
DAPI SSEA4 OCT4
DAPI SOX2 TRA-1-60
F
Count
SSEA4
OCT3/4
G
Undifferentiated
Differentiated
Endoderm
Mesoderm
Ectoderm
Fold Change
PDX1 SOX17
TBX6 PAX7
SOX1 OTX2
H
Endoderm
DAPI/SOX17/NeuN
Mesoderm
DAPI/Brachyury/Nkx2.5
Ectoderm
DAPI/Tuj1/FOXA2
I
TPM1
(NM_001018005.1)
c.574G>A
p.Glu192Lys
T G T G C C G A G C T T G A A
Cys Ala Glu Leu Glu
Glu
Lys

Fig. 1.

Table 1
Characterization and validation.

Classification	Test	Result	Data
Morphology	Photography Bright field	Normal	Fig. 1 panel A
Phenotype	Alkaline Phosphatase staining	Positive	Fig. 1 panel A
	Quantitative analysis RT-qPCR	Positive for SOX2, TERT, TDGF1, GABRB3	Fig. 1 panel D
	Quantitative analysis Immunocytochemistry	Positive for pluripotency markers including OCT4, SOX2, SSEA4, and TRA-1-60	Fig. 1 panel E
	Quantitative analysis Flow cytometry	SSEA4 positive: 100% OCT3/4 positive: 99.1%	Fig. 1 panel F
Genotype	Karyotype (G-banding) and resolution	46XX, Resolution 450-500	Fig. 1 panel B
Identity	Microsatellite PCR (mPCR)	Not performed	NA
	STR analysis	16 loci tested, all matched	Submitted in archive with journal
Mutation analysis	Sequencing	Heterozygous mutation	Fig. 1 panel I
	Southern Blot OR WGS	NA	NA
Microbiology and virology	Mycoplasma	Mycoplasma testing by RT-PCR, negative	Fig. 1 panel C
Differentiation potential	Directed differentiation *In vitro* trilineage differentiation Immunocytochemistry	Endoderm: αSOX17, αNeuN Mesoderm: αBrachyury, αNkx2.5 Ectoderm: αTuj1, αFOXA2	Fig. 1 panel H
	Directed differentiation *In vitro* trilineage differentiation RT-qPCR	Endoderm: PDX1, SOX17 Mesoderm: TBX6, PAX7 Ectoderm: SOX1, OTX2	Fig. 1 panel G
Donor screening	HIV 1 + 2 Hepatitis B, Hepatitis C	Negative	Not shown but available with author
Genotype additional info	Blood group genotyping	Not performed	NA
	HLA tissue typing	Not performed	NA

is diverse and remains to be elucidated. To increase understanding of the development of this disease, a novel iPSC line containing the missense mutation in *TPM1* was established. This iPSC can be differentiated into cardiomyocytes for DCM-related disease modeling, which will serve as a valuable resource for DCM-related research.

3. Resource details

DCM is a disease of the heart muscle, and the causes of DCM are heterogeneous (Schultheiss et al., 2019). More than 50 genes have been reported to be associated with DCM and these genes encode various protein including cytoskeletal, mitochondrial, and calcium binding proteins (McNally et al., 2013). Tropomyosin1, encoded by *TPM1*, is an essential component of sarcomeres, which are the basic units of contractile muscle fibers. *TPM1* mutations have been mainly found and studied in hypertrophic cardiomyopathy. But several mutations of *TPM1* were identified in the DCM and pathogenicity of *TPM1* mutations has also been reported in DCM (Lakdawala et al., 2010; van de Meerakker et al., 2013). Although reports on *TPM1* mutation linked to DCM are increasing, their pathophysiologic roles in DCM are unclear. Therefore, we generated a novel hiPSC line from a patient with DCM carrying the missense mutation p.Glu192Lys in *TPM1* gene. Peripheral blood mononuclear cells (PBMCs) were isolated from a 67-year-old female patient with DCM. The PBMCs were reprogrammed using an Epi5 Episomal iPSC Reprogramming Kit (Thermo Fisher Scientific) containing five reprogramming factors (Oct4, Sox2, Lin28, Klf4, and L-Myc). The YCMi005-A line showed a typical hESC-like morphology. Alkaline phosphatase expression was detected using an Alkaline Phosphatase Live Stain kit (Thermo Fisher Scientific) (Fig. 1A). This cell line has an XX karyotype by G-banding analysis (Fig. 1B). YCMi005-A cells were free from contamination with mycoplasma assessed by polymerase chain reaction (PCR) (Fig. 1C). Expression of endogenous pluripotency markers, including SOX2, TERT, TDGF1, and GABRB3, were confirmed to be comparable to that of CMC-hiPSC-011 by quantitative real-time PCR (qRT-PCR) (Fig. 1D). Immunocytochemistry showed that YCMi005-A cells express pluripotency markers, such as SSEA4, OCT4, SOX2, and TRA-1-60. Scale bars, 50 μm (Fig. 1E). In addition, the surface expression of SSEA4 and intracellular expression of OCT3/4 were confirmed using flow cytometry. Lines in black were used as isotype controls (Fig. 1F). Differentiation YCMi005-A into three germ layers *in vitro* was examined using a STEMdiff™ Trilineage Differentiation Kit (Stemcell Technologies, 05230). Fig. 1G shows that YCMi005-A could differentiate into endoderm (PDX1, SOX17), mesoderm (TBX6, PAX7), and ectoderm (SOX1, OTX2) based on qRT-PCR. Expression of differentiation markers for endoderm (SOX17, NeuN), mesoderm (Brachyury, Nkx2.5), and ectoderm (Tuj1, FOXA2) of YCMi005-A cells were confirmed using immunofluorescence staining. Scale bars, 20 μm (Fig. 1H). DNA sequence determination confirmed the missense mutation of c.574G > A change in exon 6 of the *TPM1* gene (Fig. 1I). The identity of this iPSC line was also confirmed using short tandem repeat analysis.

4. Materials and methods

4.1. Ethical statementss

This study and the experimental procedures conducted were approved by the Institutional Review Board (IRB) and ethics committee of the Yonsei University Health System, and informed consent was obtained from the patient (NO. 4-2020-0112).

4.2. Reprogramming of human peripheral blood mononuclear cells (PBMC)

A whole blood sample was obtained from a 67-year-old Korean female patient who had a missense mutation (E192K) in exon 6 of the *TPM1* gene. PBMCs were isolated using SepMate™ (StemCell Technologies, 15410), according to the manufacturer's recommendations. To induce cell reprogramming, isolated PBMCs were electroporated with Epi5™ Episomal iPSC Reprogramming plasmids Kit (Thermo Fisher Scientific, A15960) using a Neon Electroporation system, according to the manufacturer's instructions. After electroporation, the cells were plated on Matrigel (hESC-qualified, Corning) coated six-well plate in ReproTeSR™ medium (Stemcell Technologies, 05920). The cells were cultured over 20 days with daily change of medium until the appearance of iPS cell colonies. The obtained clones were cultured onto vitronectin coated plates (Truncated VTN-N recombinant human protein, Gibco, A31804) in TeSR™-E8™ medium (Stemcell Technologies, 05990). All cells were cultured at 37 °C in humidified atmosphere containing 5% CO_2, and cells were changed with fresh medium every 24 hours until the cells reached 80–90% confluency. Cells were dissociated using ReLeSR™ (Stemcell Technologies, 05872) and passaged at 1:10–1:20 ratio with 10 μM Y-27632 (Tocris, 1254).

Table 2
Reagents details.

Antibodies used for immunocytochemistry/flow-cytometry				
	Antibody	Dilution	Company Cat #	RRID
Pluripotency Markers (Immunocytochemistry)	Rabbit anti-OCT4	1:300	Cell Signaling Technology Cat# 9656	AB_1658242
	SOX2	1:100	Thermo Fisher Scientific Cat# 53-9811-82	AB_2574479
	TRA-1-60	1:100	Thermo Fisher Scientific Cat# 13-8863-82	AB_891594
	SSEA-4	1:100	Thermo Fisher Scientific Cat# 46-8843-42	AB_2573850
Pluripotency Markers (Flow cytometry)	SSEA4	0.03 μg/test	Thermo Fisher Scientific Cat# 46-8843-42	AB_2573850
	OCT3/4	0.5 μg/test	Thermo Fisher Scientific Cat# 50-5841-80	AB_11218890
Trilineage Differentiation Markers	Goat IgG anti-hSOX17	1:100	R&D Systems Cat# AF1924	AB_355060
	Rabbit IgG anti-h/m/rNeuN	1:100	Thermo Fisher Scientific Cat # PA5-78639	AB_2736207
	Goat IgG anti-h/mBrachyury	1:100	R&D Systems Cat# AF2085	AB_2200235
	Rabbit IgG anti-h/mNkx2.5	1:100	Thermo Fisher Scientific Cat # PA5-49431	AB_2634885
	Mouse anti-Neuron-specific beta-III Tubulin (Clone TuJ-1)	1:100	R&D Systems Cat# MAB1195	AB_357520
	Rabbit IgG anti-h/m/FOXA2	1:100	Thermo Fisher Scientific Cat # PA5-35097	AB_2552407
Secondary antibodies	Alexa® Fluor 488 chicken anti-rabbit IgG	1:500	Thermo Fisher Scientific Cat# A-21441	AB_2535859
	Alexa® Flour 546 goat anti-mouse IgG	1:500	Thermo Fisher Scientific Cat# A-11030	AB_2534089
Primers				
	Target	Size of band	Forward/Reverse primer (5′-3′)	
Pluripotency Markers (qPCR)	SOX2	215 bp	5′-TGG ACA GTT ACGC GC ACA T-3′ 5′-ACC TAC AGC ATG TCC TAC TCG-3′	
	TERT	96 bp	5′-TCA CGG AGA CCA CGT TTC AAA-3′ 5′-CGT CCG TAG AAG GAG GGA GG-3′	
	TDGF1	96 bp	5′-ACA GCA CAG TAA GGA GCT AAA C-3′ 5′-CGT CCG TAG AAG GAG GGA GG-3′	
	GABRB3	153 bp	5′-GAA AAA CCG CAT GAT CCG TCT-3′ 5′-TCC GTG GTG TAG CCA TAG CTT-3′	
Trilineage Differentiation Markers (qPCR)	PDX1	193 bp	5′-ATC TCC CCA TAC GAA GTG CC-3′ 5′-CGT GAG CTT TGG TGG ATT TCA T-3′	
	SOX17	94 bp	5′-GTG GAC CGC ACG GAA TTT G-3′ 5′-GGA GAT TCA CAC CGG AGT CA-3′	
	TBX6	128 bp	5′-AGG CCC GCT ACT TGT TTC TTC-3′ 5′-GGG GTG AAT GTA GAC ACG GT-3′	
	PAX7	121 bp	5′-ACC CCT GCC TAA CCA CAT C-3′ 5′-GCG GCA AAG AAT CTT GGA GAC-3′	
	SOX1	287 bp	5′-CAG TAC AGC CCC ATC TCC AAC-3′ 5′-GCG GGC AAG TAC ATG CTG A-3′	
	OTX2	179 bp	5′-CAA AGT GAG ACC TGC CAA AAA GA-3′ 5′-TGG ACA AGG GAT CTG ACA GTG-3′	
House-Keeping Genes (qPCR)	GAPDH	197 bp	5′-GGA GCG AGA TCC CTC CAA AAT-3′ 5′-GGC TGT TGT CAT ACT TCT CAT GG-3′	
Mutation sequencing primer	TPM1	510 bp	5′-GGA TTT GGT CAC CCT-3′ 5′-ATC CAC TTG GCA CTT-3′	

4.3. Alkaline phosphatase staining

YCMi005-A cells were seeded on Matrigel™ coated six-well plates. On day 40 after reprogramming, cells were stained using the Alkaline Phosphatase Live stain kit (Thermo Fisher Scientific, A14353). Briefly, the cultured iPSCs were washed with DMEM/F-12 prior to staining, and appropriate amount of the stain solution was applied directly on to the iPSCs, incubated for 20 min. Plates were analyzed with a fluorescence microscope (OLYMPUS, IX71).

4.4. Quantitative RT-PCR

Total RNA was isolated using a Ribospin™ total RNA purification kit (GeneAll Biotechnology, 314-150) from iPSC at passage 15. Complementary DNA (cDNA) was synthesized using PrimeScript™ RT reagent Kit (Takara, 2680A) according to the manufacturer's recommendations. The qPCR was conducted on QuantStudio™ 3 Real-Time PCR system (Applied Biosystems™, A28567) with FastStart Universal SYBR® Green Master Mix (Roche Applied Science). They were then standardized according to endogenous control gene *GAPDH*. Validated human iPSC (CMC-hiPSC-011) was used as a positive control.

4.5. Immunocytochemistry

The expression of pluripotency markers was detected by Immunocytochemistry. YCMi005-A cells were seeded onto a Matrigel-coated slide chamber and cultivated for 5 days. Passage 15 iPSCs were washed in PBS and fixed with 4% paraformaldehyde for 20 min, then blocked with 3% bovine serum albumin (LPS solution, 9048-46-8) with 0.3% Triton-X (USB®, 9002-93-1) in PBS. After blocking, cells were stained with primary antibodies (OCT4, SOX2, SSEA4, TRA-1-60) and incubated at 4 °C overnight. On the next day, after washing in PBS, the solution was replaced by diluted secondary antibodies (Alexa® Fluor 488 chicken anti-rabbit IgG [(1:500, Thermo Fisher Scientific, A21441)

or Alexa® Flour 546 goat anti-mouse IgG (1:500, Thermo Fisher Scientific, A11030)] (Table 2) and cells were incubated for 3 hours at RT. After washing, cell nuclei were stained with Hoechst 33342 (Thermo Fisher Scientific, 62249) for 10 min at RT. The sample slides were imaged with a confocal microscope (Zeiss, LSM710) and analysed using the ZEN software. All antibody information is listed in Table 2.

4.6. Flow cytometry

YCMi005-A cells were dissociated using Gentle Cell Dissociation Reagent (Stemcell Technologies, 07174). For intracellular staining, the single cell suspension was fixed and permeabilized with Fixation/Permeabilization solution kit (BD Bioscience, 554714) for 15 min. YCMi005-A cells were incubated with pluripotency-associated markers, SSEA4 (PerCP-eFluor710, 0.03 μg/test) and OCT3/4 (eFluor 660, 0.5 μg/test). All antibodies were obtained from ThermoFisher Scientific. An irrelevant isotype-match antibody was used as a negative control. Stained cells were analysed using LSR II flow cytometer (BD Bioscience) and FLOWJO v10.0.7 software (Tree Star, Inc.).

4.7. Karyotyping

YCMi005-A cells at passage 15 were cultured in a feeder-free culture system with TeSR™-E8™ medium and treated with KaryoMAX Colcemid (Thermo Fisher Scientific) for 76 min at 37 °C. They were then dissociated into single cell using Gentle Cell Dissociation Reagent (Stemcell Technologies, 07174). Single-dissociated iPSCs were incubated in 0.1 M KCl solution for 30 min at 37 °C. Cells were then fixed with 4% paraformaldehyde for 5 min. The karyotype of iPSC was analysed using Ikaros (MetaSystems, Neon 1.2.7) software at a band resolution of 450–500 nm.

4.8. In vitro trilineage differentiation

Directed differentiation into all trilineage differentiation was achieved using the STEMdiff™ Trilineage Differentiation Kit (Stemcell Technologies, 05230), according to manufacturer's instructions. Briefly, dissociated iPSCs were seeded onto a Matrigel coated coverslips in 12 wells. Cells were cultured in lineage-specific medium with daily replacement until day five for meso- and endodermal, and until day seven for ectodermal differentiation, respectively. To assess trilineage differentiation, qRT-PCR for lineage-specific markers and immunocytochemistry were performed as described (Table 1).

4.9. DNA sequence analysis of the mutation site

The genomic DNA from YCMi005-A cells were isolated using the G-spin™ Genomic DNA Extraction Kit (iNtRON Biotechnology, 17121), according to the manufacturer's instructions. Polymerase chain reaction was performed using Q5® High-Fidelity DNA Polymerase (New England Biolabs, M0491) and primers (Table 2) flanking the mutation. The heterozygous mutation c.574G > A, p.Glu192Lys in *TPM1* was confirmed via Sanger sequencing (Bionics, Seoul, Korea).

4.10. STR analysis

An STR analysis was performed on the iPSCs and the parental PBMCs, with detection of 16 loci (D8S1179, D21S11, D7S820, CSF1PO, D3S1358, TH01, D13S317, D16S539, D2S1338, D19S433, vWA, TPOX, D18S51, AMEL, D5S818). The results were analysed by ANALYZER software.

4.11. Mycoplasma screening

Mycoplasma contamination was detected using TaKaRa PCR Mycoplasma Detection Set (Takara, 6601), according to the manufacturer's recommendations. The correct size band indicates the presence of mycoplasma species in the cell culture. YCMi005-A cells at passage 15 were used and the result was negative.

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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