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Lab Resource: Single Cell Line

Generation of an erythroid progenitor-derived iPSC line, VRISGi002-A, from a healthy 27-year-old Vietnamese donor under a feeder-free system

Thi-Hong Nhung Nguyen [a], Thi-Tuyet Trinh Tran [a], Thi-Hoa Luong [a,c], Xuan-Hung Nguyen [a,b,*]

[a] Center of Applied Sciences, Regenerative Medicine and Advance Technologies (CARA), Vinmec Healthcare System, Hanoi, Viet Nam
[b] College of Health Sciences, VinUniversity, Hanoi, Viet Nam
[c] VNU, University of Science, Hanoi, Viet Nam

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ABSTRACT

We have established the footprint-free Vietnamese human induced pluripotent stem cell (hiPSC) line, VRISGi002-A, from CD71 + CD235a + erythroid progenitor cells (EPCs) of a 27-year-old healthy donor. The EPCs were enriched from isolated peripheral blood and reprogrammed using Sendai viruses which carried the reprogramming factors c-MYC, SOX2, KLF4, and OCT4 under a feeder-free culture system. The established VRISGi002-A cell line expressed typical pluripotency markers, displayed a normal karyotype, and demonstrated the potential to differentiate into the three germ layers. This hiPSC line could serve as a Vietnamese healthy control model for physiological processes and drug screening.

1. Resource table

Unique stem cell line identifier	VRISGi002-A https://hpscreg.eu/cell-line/VRISGi002-A
Alternative name(s) of stem cell line	ERY01-hiPS-CL2
Institution	Center of Applied sciences, Regenerative medicine and Advance technologies
Contact information of distributor	Dr. Xuan-Hung, Nguyen; v.hungnx@gmail.com
Type of cell line	iPSC
Origin	Human
Additional origin info required for human ESC or iPSC	Age:27 Sex: Female Ethnicity if known: Vietnamese
Cell Source	Erythroid progenitor
Clonality	Clonal cell line
Associated disease	N/A
Gene/locus	N/A
Date archived/stock date	N/A
Cell line repository/bank	https://hpscreg.eu/cell-line/VRISGi002-AStorage system of Vinmec Research Institute of Stem Cell and Gene Technology
Ethical approval	Approval was obtained from the Vinmec International hospital

2. Resource utility

We generated a human iPS cell line VRISGi002-A from the Peripheral blood of a healthy female. This iPS cell line could serve as a healthy control for disease model and drug screening.

3. Resource details

Following our success in generating the first human induced pluripotent stem cell (hiPSC) line of Vietnamese origin from umbilical cord blood-derived hematopoietic stem cells (Tran et al., 2021), we have established and characterized a second footprint-free hiPSC line VRISGi002-A (Table 1) from erythroid progenitor cells (Soares, 2015). Peripheral blood mononuclear cells (PBMCs) isolated from the blood of a 27-year-old healthy female donor with informed consent were cultured to induce the erythropoiesis. Reprogramming of EPCs was induced when the percentage of early-stage erythroid cells (CD71+ CD235a+ cells) reached approximately eighty percent which was identified by Flow cytometry (Supplementary Fig. 1). The detailed procedure and timeline for hiPSC generation are presented in Fig. 1A. Colony morphology after 3 weeks of reprogramming factor transduction is shown in Fig. 1B. Early determination of developing colonies was confirmed with the positive activity of alkaline phosphatase staining (Fig. 1C). The presence of pluripotency protein markers in these colonies

* Corresponding author at: Center of Applied Sciences, Regenerative Medicine and Advance Technologies (CARA), Vinmec Healthcare System, Hanoi, Viet Nam.
E-mail address: v.hungnx@gmail.com (X.-H. Nguyen).

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Table 1
Characterization and validation.

Classification	Test	Result	Data
Morphology	Photography	Normal	Fig. 1 panel B
Phenotype	Alkaline phosphatase staining	Positive	Fig. 1 panel C
	Qualitative analysis RT-qPCR	Positive for Oct4, Sox2, c-Myc, Klf4, Rex1f transcription factors	Fig. 1 panel E
	Quantitative analysis Immunocytochemistry	Positive for Oct4, Sox2, Nanog, TRA1-60 and SSEA-4 markers	Fig. 1 panel D
Genotype	Karyotype (G-banding)	46, XX	Fig. 1 panel H
Identity	STR analysis	24 loci tested, completely matched	submitted in archive with journal
Mutation analysis	Sequencing	N/A	N/A
Microbiology and virology	Colorimetric test	Mycoplasma testing by luminescence, negative	Supplementary, Table 1
Differentiation potential	In vitro trilineage differentiation	Proof of three germ-layers formation by positive immunostaining for TUBB3, OTX2 (Ectoderm), α-ACTIN, Brachyury (Mesoderm), SOX17, GATA-4 (Endoderm)	Fig. 1 panel F And supplementary, figrure 1
Donor screening	HIV, Hepatitis B, Hepatitis C	Negative	Not shown but available with author
Genotype additional information	Blood group genotyping	N/A	N/A
	HLA tissue typing	N/A	N/A

was also verified by immunocytochemistry at 20X magnification (Fig. 1D, scale bar: 50 μm). In addition, quantitative real-time PCR data proved the expression of pluripotency genes in the developed iPSC line, in comparison with the first established iPSC line derived from hematopoietic stem cells (Fig. 1E). These iPS cells also illustrated the ability to differentiate into different cell types of all three germ layers, as shown by the positive staining with specific markers TUBB3, α-ACTIN and SOX17 (Fig. 1F, 20X magnification, scale bar: 50 μm). Subsequently loss of Sendai virus vector expression in the established cells after continuous passaging was confirmed by RT-PCR at passage 18 (Fig. 1G) in which GapDH was used as an internal control. The reprogrammed VRISGi002-A cell line retained a normal chromosome pattern after 20 passages in maintaining culture (Fig. 1H) and had an identical short tandem repeat (STR) profile with the original PBMCs isolated from the same donor (submitted along with the paper).

The study was approved by the Hospital Board of Vinmec International Hospital in Hanoi, Vietnam (No. ISC 18.16).

4. Materials and methods

4.1. Reprogramming of erythroid cells using Sendai virus system

PBMCs were isolated from the donated blood by Ficoll gradient centrifugation and expanded in the Erythroid expansion medium (StemCELL) for 9 days. The purity of the EPCs was assessed by population proportion of transferrin receptor (CD71) and Glycophorin A (CD235a, GLyA) positive erythroblasts. Approximately $2x10^6$ of EPCs were reprogrammed by a non-integrative reprogramming method (Sendai Cytotune^{TM} 2.0, Invitrogen) and cultured in StemMACS medium (Miltenyi). After 24 h, the cells were passed into a 0.1 mg/ml Matrigel-coated plate, fed daily with mTESR1 (StemCELL) and incubated at 37 °C, 5% CO_2. When the iPSC colonies reach 80% of confluence, cells are dissociated using Versene (Gibco) and passed at 1:20 split ratios onto plate coated with 10 μg/ml Vitronectin in mTESR1 medium.

4.2. Alkaline phosphatase (ALP) staining

The alkaline phosphatase staining (Abcam) was performed at developing iPSC passage 3 according to the manufacturer's protocol.

4.3. Sendai virus genome detection and pluripotency gene expression

To detect the retention of the SeV genome and transgenes in the cell line, we isolated 50 ng of total RNA at different passages with RNeasy kit (Qiagen), then converted into cDNA using qScript cDNA synthesis kit (Quantabio), all following the manufacturer's instructions. The PCR reactions were performed on a thermocycler (Techne, TC-312). Next, 50 ng cDNA was used to evaluate the pluripotency gene expression by Real-time PCR with the SYBR PerfeCTa SuperMix (Qiagen) using the ABI-7500 (Applied Biosystem). Relative gene expression of the targets was calculated according to the ΔΔCt method with GapDH as endogenous control and PBMC as the baseline.

4.4. Immunofluorescence staining

Cells were fixed with IC Fixation (Invitrogen) for 15 min at 4 °C, permeabilized with permeabilization (Invitrogen) for 30 min and blocked with 5% BSA (Sigma) for 1 h at RT. Then, the cells were stained with the appropriate antibodies at 4 °C, overnight. The excess antibodies were removed by washing with PBS and nuclei were then stained with DAPI for 5 min at RT. A stained cell image was captured under the Confocal Imaging System.

4.5. Differentiation into three germ layers

At passages 6, the establishing hiPSCs were differentiated into the three germ layers using the StemMACS™ Trilineage Differentiation Kit (Miltenyi). In short, cells were dissociated into single cells and seeding with desired density in Vitronectin-coated plate, cultured by mTeSR1 supplemented with Y-27632 (Sigma). One day later, the medium was replaced by differentiation inducing media and incubated at 37 °C. The differentiation media was refreshed daily until day 5 (mesoderm and endoderm) or day 7 (ectoderm). Fixed differentiated cells were then labelled with specific antibodies (Table 2).

4.6. Karyotyping

Karyotype analysis by KaryoMAX kit (Gibco) was achieved for iPSCs at passage 20 at Vinmec Hospital, Vietnam. At least 20 metaphase

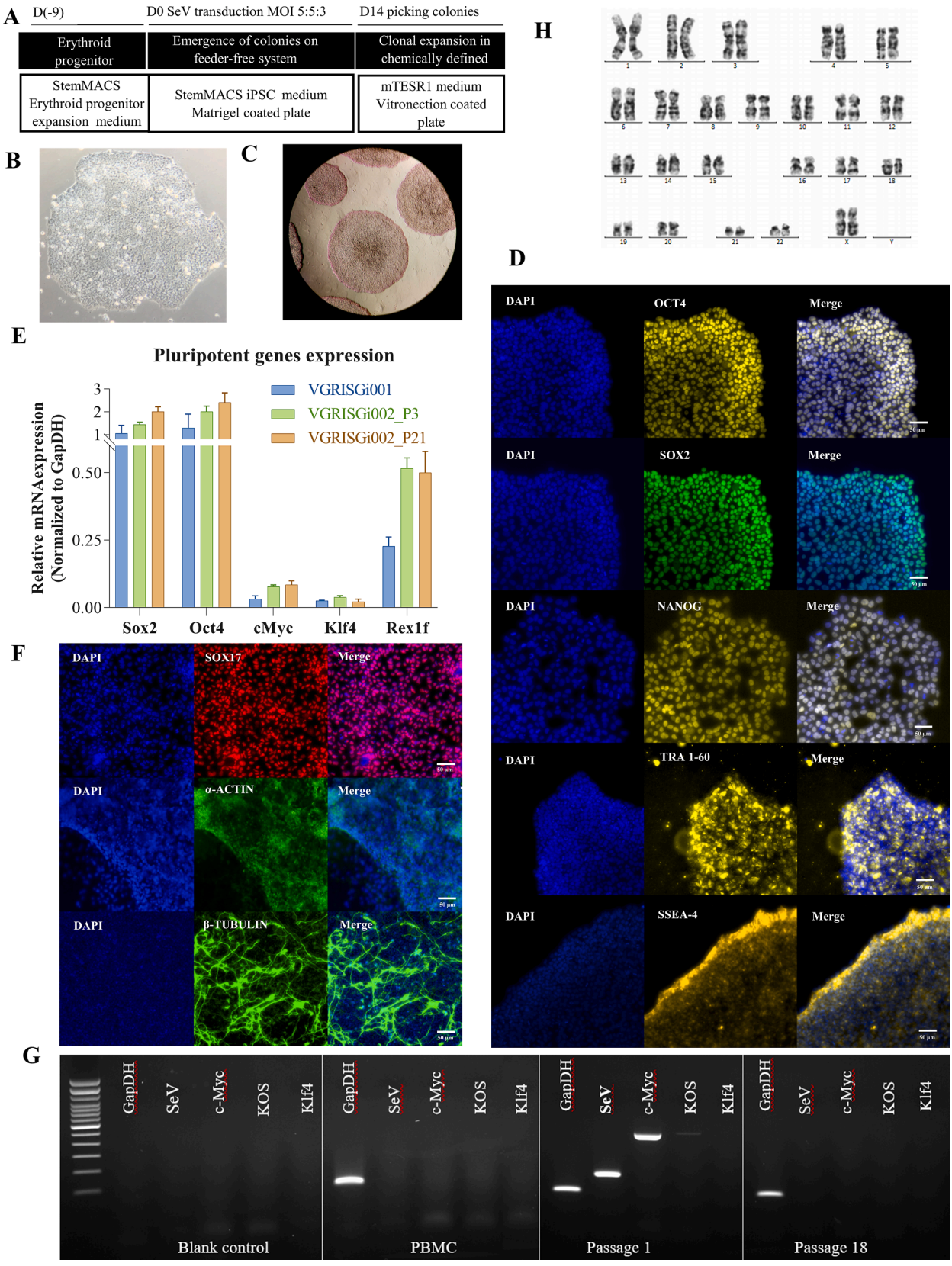

Fig. 1. hiPSC characterization.

Table 2
Reagents details.

	Antibodies used for immunocytochemistry/flow-cytometry			
	Antibody	**Dilution**	**Company Cat #**	**RRID**
Cell viability and purity	7AAD staining solution	1:100	130-111-568,Miltenyi	
	Anti-human CD71 APC	1:50	17-0719-42, Invitrogen	RRID: AB_10671393
	Anti-human CD235a FITC	1:50	11-9987-82, Invitrogen	RRID: AB_465477
Pluripotency markers	Anti-human Oct3/4_PE	1:100	130-105-606, Miltenyi	RRID: AB_2653084
	Anti-human Sox2_FITC	1:100	130-104-993, Miltenyi	RRID: AB_2653499
	Anti-human Nanog_PE	1:100	130-120-774, Miltenyi	RRID: AB_2784440
	Anti-human TRA-1-60_PE	1:100	130-100-635, Miltenyi	RRID: AB_2654226
Differentiation markers	Anti-human TUBB3	1:20	Human Three Germ layer 3-color Immunocytochemistry kit (R&D, SC022)	
	Anti-human α-ACTIN	1:20		
	Anti-human SOX17	1:20		
	Primers			
	Target	**Size of band**	**Forward/Reverse primer (5′-3′)**	
Pluripotency markers (qPCR)	Oct4	164 bp	Forward: CCTCACTTCACTGCACTGTA	
			Reverse: CAGGTTTTCTTTCCCTAGCT	
	Sox2	140 bp	Forward: ATGTCCCAGCACTACCAGAG	
			Reverse: GCACCCTCCCATTTCCC	
	Myc	190 bp	Forward: CTGAAGAGGACTTGTTGCGGAAAC	
			Reverse: TCTCAAGACTCAGCCAAGGTTGTG	
	Endo-Klf4	172 bp	Forward: GGTCGGACCACCTCGCCTTACAC	
			Reverse: CTCAGTTGGGAACTTGACCA	
	Rex1f	125 bp	Forward: CCGAGACCACGTCTGTGCGG	
			Reverse: AGCGCTTTCCGCACCCTTCA	
House-Keeping Genes (qPCR)	GapDH	87 bp	Forward: GGCATGGACTGTGGTCATGAG	
			Reverse: TGCACCACCAACTGCTTAGC	
Detection of Sendai virus vectors (RT-PCR)	SeV	181 bp	Forward: GGATCACTAGGTGATATCGAGC	
			Reverse: ACCAGACAAGAGTTTAAGAGATATGTATC	
	KOS	528 bp	Forward: ATGCACCGCTACGACGTGAGCGC	
			Reverse: ACCTTGAGAATGGTGATGTGG	
	Klf4	410 bp	Forward: TTCCTGCATGCCAGAGGAGCCC	
			Reverse: AATGTATCGAAGGTGCTCAA	
	cMyc	532 bp	Forward: TAACTGACTAGCAGGCTTGTCG	
			Reverse: TCCACATACAGTCCTGGATGATGATG	
	Antibodies used for immunocytochemistry/flow-cytometry			
	Antibody	**Dilution**	**Company Cat #**	**RRID**
Cell viability and purity	7AAD staining solution	1:100	130-111-568,Miltenyi	
	Anti-human CD71 APC	1:50	17-0719-42, Invitrogen	RRID: AB_10671393
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Pluripotency markers	Anti-human Oct3/4_PE	1:100	130-105-606, Miltenyi	RRID: AB_2653084
	Anti-human Sox2_FITC	1:100	130-104-993, Miltenyi	RRID: AB_2653499
	Anti-human Nanog_PE	1:100	130-120-774, Miltenyi	RRID: AB_2784440
	Anti-human TRA-1-60_PE	1:100	130-100-635, Miltenyi	RRID: AB_2654226
Differentiation markers	Anti-human TUBB3	1:20	Human Three Germ layer 3-color Immunocytochemistry kit (R&D, SC022)	
	Anti-human BRACHYURY	1:20		
	Anti-human SOX17	1:20		
	Primers			
	Target	**Size of band**	**Forward/Reverse primer (5′-3′)**	
Pluripotency markers (qPCR)	Oct4	164 bp	Forward: CCTCACTTCACTGCACTGTA	
			Reverse: CAGGTTTTCTTTCCCTAGCT	
	Sox2	140 bp	Forward: ATGTCCCAGCACTACCAGAG	
			Reverse: GCACCCTCCCATTTCCC	
	Myc	190 bp	Forward: CTGAAGAGGACTTGTTGCGGAAAC	
			Reverse: TCTCAAGACTCAGCCAAGGTTGTG	
	Endo-Klf4	172 bp	Forward: GGTCGGACCACCTCGCCTTACAC	
			Reverse: CTCAGTTGGGAACTTGACCA	
	Rex1f	125 bp	Forward: CCGAGACCACGTCTGTGCGG	
			Reverse: AGCGCTTTCCGCACCCTTCA	
House-Keeping Genes (qPCR)	GapDH	87 bp	Forward: GGCATGGACTGTGGTCATGAG	
			Reverse: TGCACCACCAACTGCTTAGC	
Detection of Sendai virus vectors (RT-PCR)	SeV	181 bp	Forward: GGATCACTAGGTGATATCGAGC	
			Reverse: ACCAGACAAGAGTTTAAGAGATATGTATC	
	KOS	528 bp	Forward: ATGCACCGCTACGACGTGAGCGC	
			Reverse: ACCTTGAGAATGGTGATGTGG	
	Klf4	410 bp	Forward: TTCCTGCATGCCAGAGGAGCCC	
			Reverse: AATGTATCGAAGGTGCTCAA	
	cMyc	532 bp	Forward: TAACTGACTAGCAGGCTTGTCG	
			Reverse: TCCACATACAGTCCTGGATGATGATG	

spreads were counted.

4.7. Short tandem repeat (STR) analysis

STR analysis of VRISGi002 passage 22 was carried out at Medlatec Hospital, Vietnam in comparison with PBMC.

4.8. Mycoplasma test

The absence of mycoplasma in the culture medium was analysed at passage 20 using MycoAlert™ PLUS Mycoplasma Detection Kit (Lonza).

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.

Data availability

Data will be made available on request.

Acknowledgments

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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.2022.102824.

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