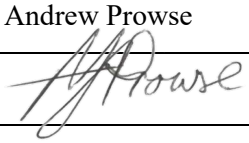
CoA written by:	Mahfuz Chowdhury	CoA approved by:	Andrew Prowse
Signature:		Signature:	
Date:	5th November 2024	Date:	5th November 2024

Product Information and Certificate of Analysis

Human induced Pluripotent Stem Cell (hiPSC) Line SCORe-M-hiPSC

Product Description

SCORe-M-hiPSC line was derived by reprogramming PBMC (Peripheral Blood Mononuclear Cells) collected from a healthy male person (ID: RG4367, Lot: 230771702C, cat: 70025.1, STEMCELL Technologies) in StemCore under ISO9001:2015 quality standards.

Properties

Cell line	SCORe-M-hiPSC (**S**temCore **O**riginal **Re**programming)
Cell type	Human induced Pluripotent Stem Cell
Gender	Male
Somatic origin	PBMC
Reprogramming method	CytoTune iPSC 2.0 Sendai Reprogramming
Bank designation	Working Cell Bank (WCB) #1
Storage conditions	≤ -135°C
Date of cryopreservation	20/09/2024
Passage number	P11
Cell number	3×10^6 cells per vial
Cryopreservation medium	CryoStor® CS10
Culture medium	mTeSR™ Plus
Matrix substrate	Matrigel® - hESC-Qualified Matrix
Recommended passage method and split ratio	ReLeSR™, cells can generally be split 1:8-1:12 every 4 days

Recommended Materials for Use

Reagent	Supplier	Cat. No.
ReLeSR™	STEMCELL Technologies	100-0484
mTeSR™ Plus Kit	STEMCELL Technologies	05825
RevitaCell Supplement (100X)	Thermo Fisher Scientific	A2644501
Corning® Matrigel® hESC-Qualified Matrix	Corning®	354277
Accutase™ Cell Dissociation Reagent	Thermo Fisher Scientific	A1110501

Certificate of Analysis

The following testing specifications have been met for this product:

Test Description	Test Method	Test Results
Post-thaw cell viability and recovery	Cell count	77% viable cells at revival. Cells reached > 80% confluency within 4 days when one stock vial cultured on a Matrigel-coated T75 flask.
Mycoplasma	Bioluminescence (Lonza MycoAlert detection kit)	Negative
Expression of pluripotent stem cell markers	Immunofluorescence	Positive for OCT4 and NANOG
	Flow cytometry	> 90% SSEA4 positive > 72% TRA-1-60 positive
	Undifferentiated state marker expression	Comparable expression of SOX2, NANOG, OCT4, DNMT3B and REX1 to control
Absence of Sendai virus (SeV) and exogenous transcription factor (TF)	PCR followed by gel electrophoresis	No presence of SeV, exogenous TF (c-MYC, KOS, KLF4) at P8
Genetic Analysis	Copy number variations (CNV) test	No CNVs common in pluripotent cells (≤ 1 Mb gain/loss tested in 9 loci) detected
	Karyostat assay	No CNVs (1-2 Mb gain or loss tested in 18,018 loci) and Loss of Heterozygosity (> 5 Mb and ≥ 50 SNPs) were detected.
Differentiation potential	Immunofluorescence	<u>Positive for lineage specific markers:</u> Ectoderm (NESTIN, PAX6) Mesoderm (NCAM1, BRACHYURY) Endoderm (FOXA2, SOX17)
	Gene expression by RT-qPCR	<u>Increased expression of lineage-specific markers:</u> Ectoderm (PAX6, SOX1, NR2F2) Mesoderm (HAND1, T, CDX2) Endoderm (SOX17, FOXA2, GATA6 and EOMES)

1. Expression of Pluripotent Stem Cell Markers

1.1. Immunofluorescence

To assess undifferentiated state, SCOREe-M-hiPSCs were stained for nuclear markers OCT4 and NANOG.

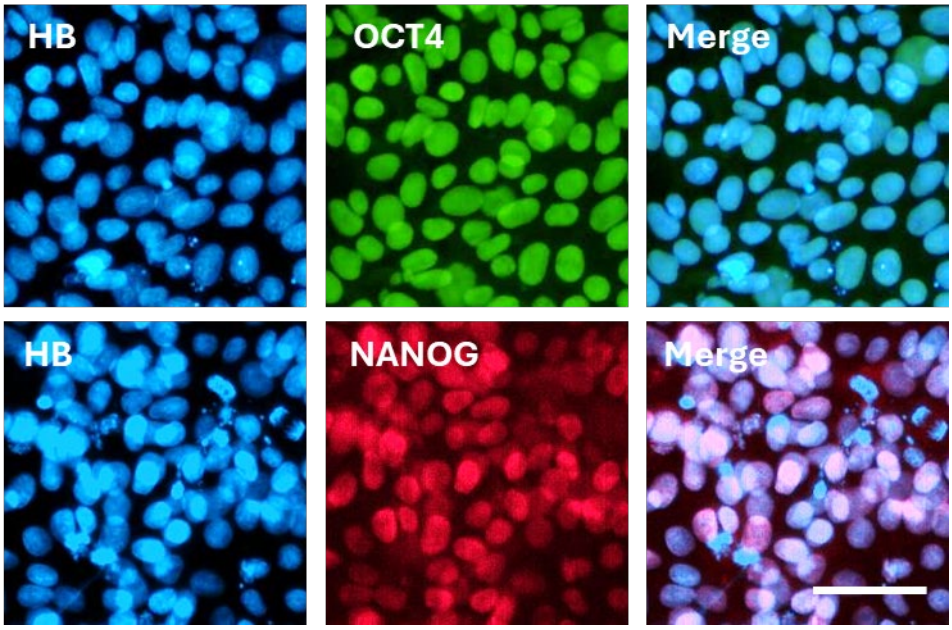


Figure 1. Expression of markers for the undifferentiated state, OCT4 and NANOG in SCOREe-M-hiPSCs detected by immunostaining. HB: Hoechst Blue; Scale bar represents 50 µm.

1.2. Flow Cytometry

In addition to immunofluorescence, flow cytometry analysis confirmed that SCORe-M-hiPSCs expressed undifferentiated state markers SSEA4 and TRA-1-60.

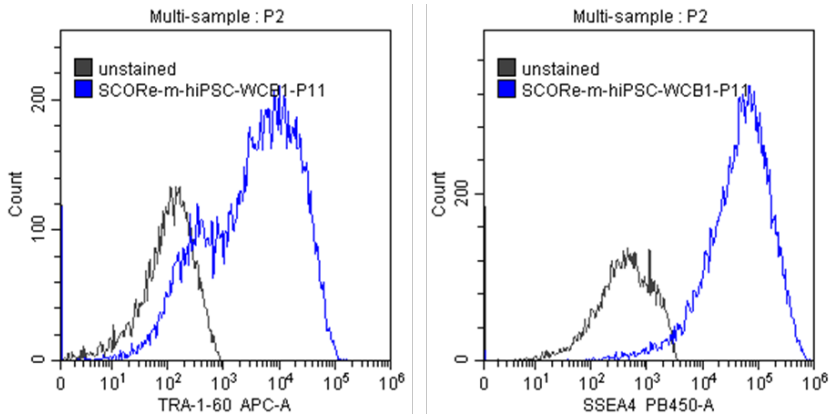


Figure 2. Expression of undifferentiated cell markers SSEA4 (left box) and TRA-1-60 (right box in SCORe-M-hiPSCs at P11 detected by flow cytometry. Black and blue histograms represent unstained and stained cells, respectively.

1.3. Gene Expression Analysis

Relative gene expression was measured by Reverse Transcription Quantitative PCR (RT-qPCR) using mRNA extracted from the cells. GAPDH was used as the endogenous housekeeping gene. Relative gene expression demonstrated that pluripotent gene expression was comparable between SCORe-M-hiPSCs and MEL-2 (used as the control) hES cells.

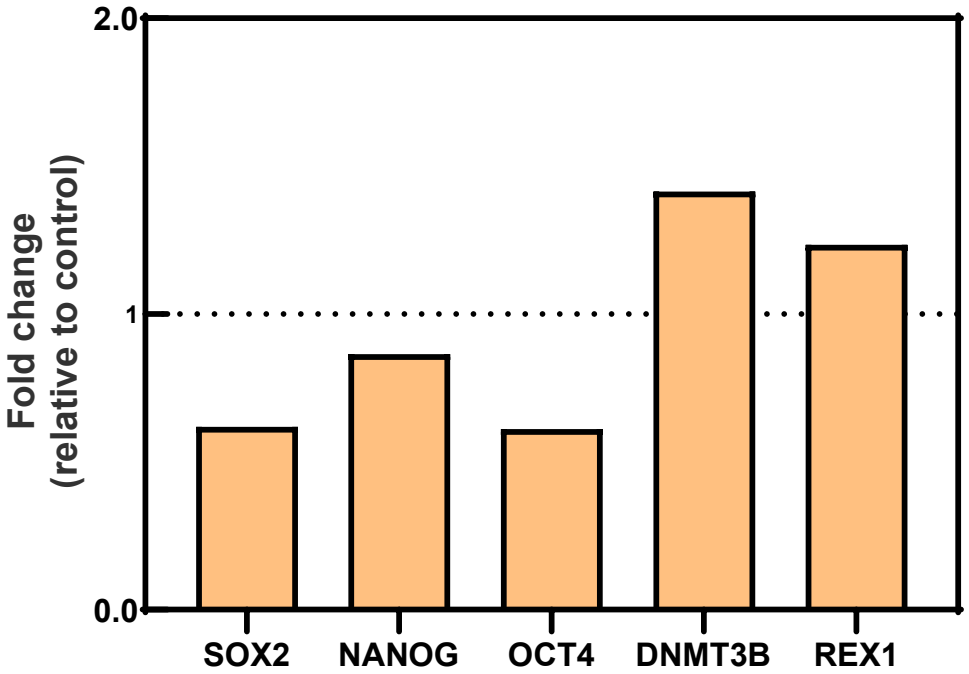


Figure 3. Gene expression analysis of pluripotency markers in SCORe-M-hiPSCs demonstrating the expression of SOX2, NANOG, OCT4, DNMT3B and REX1. Expression (dotted line) in MEL-2 hES line represents positive pluripotent control. Data represents mean for three technical replicates from one biological replicate.

2. Absence of Sendai Virus (SeV) and exogenous transcription factors (exo-TFs)

Using SeV-marker and associated reprogramming factors (i.e., exo-TFs: c-MYC, KOS and KLF4) specific primers, product was amplified using PCR and size (i.e., presence) of the product was determined using gel electrophoresis. In conjunction with relevant controls, gel electrophoresis images confirmed absence of SeV and exo-TFs in SCORe-M-hiPSCs.

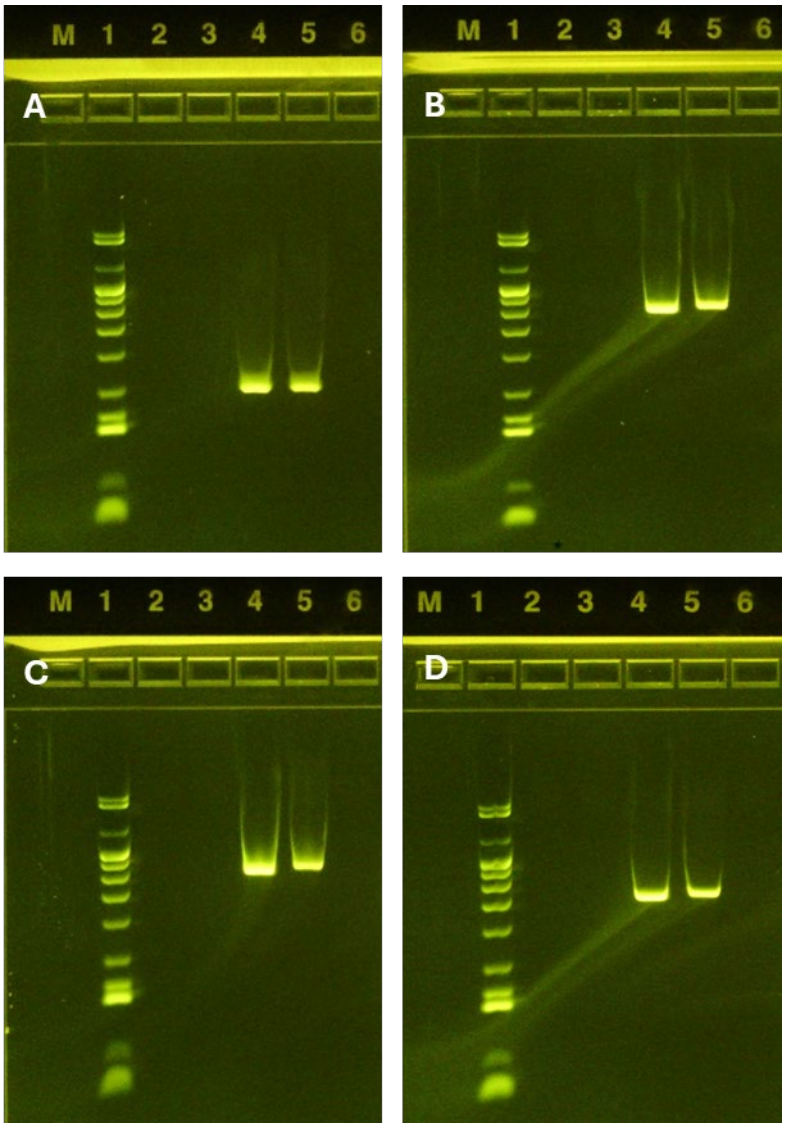


Figure 4. Gel electrophoresis of PCR-amplified products (A: SeV, B: c-MYC, C: KOS and D: KLF4) using specific primers for amplifying Sendai Virus (SeV)-specific gene and exogenous transcription factors (exo-TFs) used for reprogramming. Lane 1: 25bp DNA Ladder; Lane 2: SCORe-M-hiPSC P8; Lane 3: MEL-1 hESC (as a SeV or exo-TFs-negative control); Lane 4 and 5: two hiPSC lines as SeV or exo-TFs positive controls; Lane 6: NTC (no template control)

3. Genetic Analysis

3.1. Copy Number Variation (CNV) Test

SCORe-M-hiPSCs were screened for recurrent CNVs commonly reported in hES cells using hPSC Genetic Analysis Kit (STEMCELL Technologies). Genomic DNA was extracted, and qPCR was performed using the kit. qPCR data was analysed using the applicate available at STEMCELL Technologies Genetic Analysis Tool. SCORe-M-hiPSC WCB#1 cells (as well as parental PBMC) showed no abnormalities (≤ 1 Mb gains) in all tested loci. Presence of one copy of chrXp confirmed the male sex identity of SCORe-M-hiPSCs.

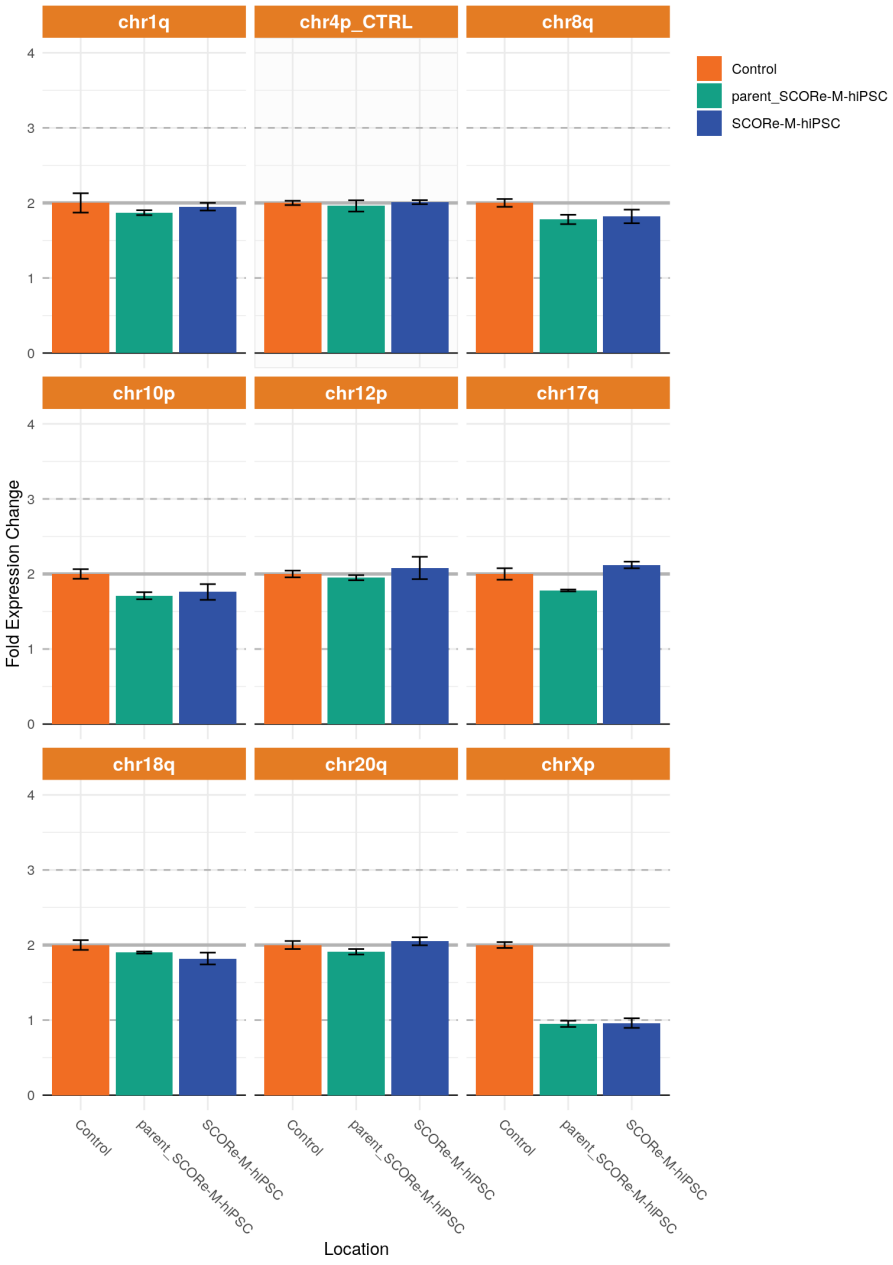


Figure 5. Copy Number Variation test result summary for SCORe-M-hiPSC WCB#1 and its parental PBMC (i.e., parent_SCORe-M-hiPSC). Data represents mean ± SD for three technical replicates of each sample.

3.2. KaryoStat Assay

The KaryoStat Assay provides much better resolution than G-banding karyotyping offering detection of multiple types of chromosomal abnormalities in the whole-genome. The KaryoStat assay on SCORe-M-hiPSC sample was performed by using CytoScan Optima Suite that can detect copy number variations (CNVs) in 18,018 regions, single-nucleotide polymorphism (SNP) in 148,450 regions as well as allelic imbalance, loss of heterozygosity (LOH).

The analysis of the assay data demonstrated that there was no detectable CNVs (1-2 Mb gain or loss) and LOH (> 5 Mb and ≥ 50 SNPs) in any chromosomes of SCORe-M-hiPSC as well as parental PBMCs (data not shown).

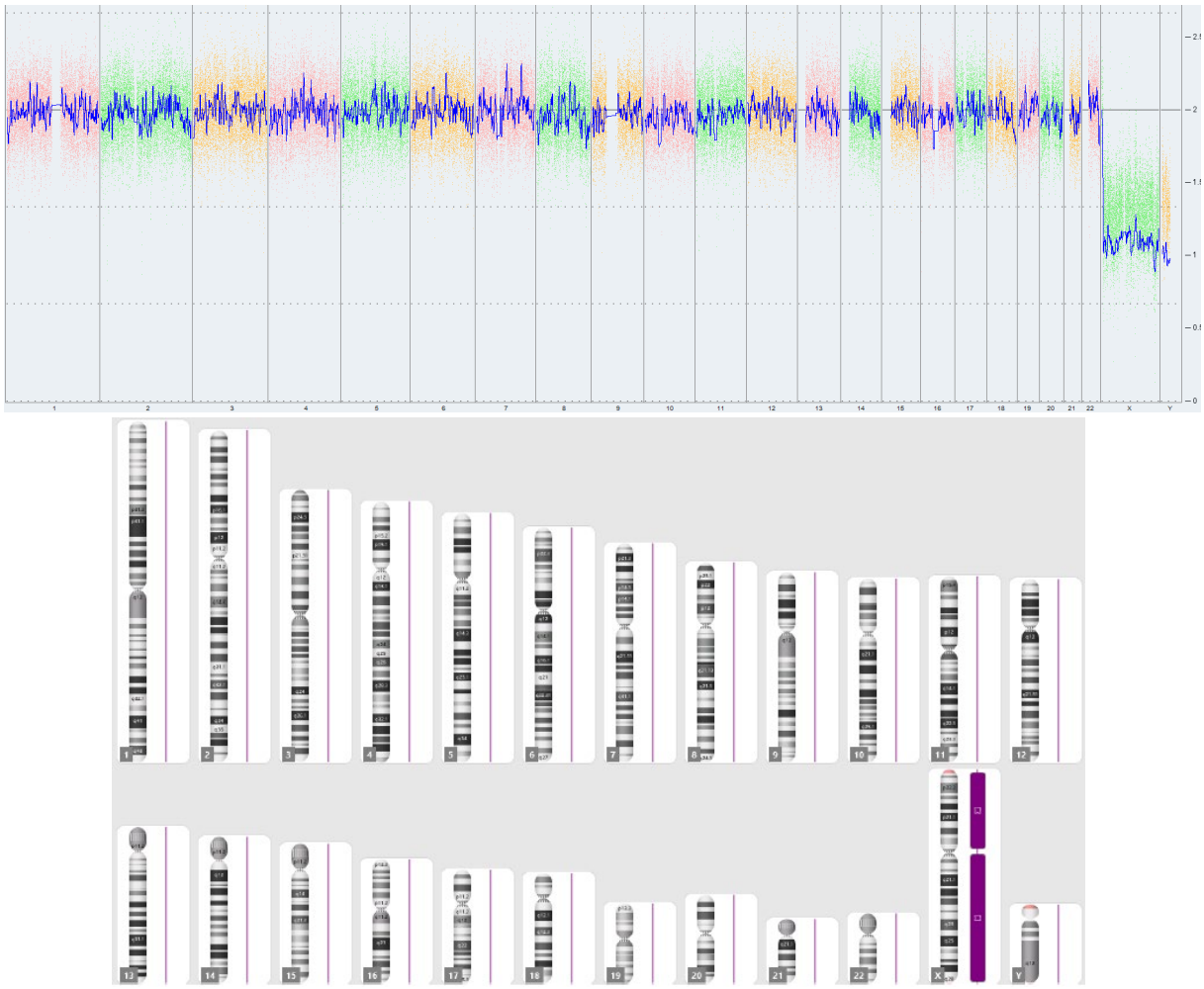


Figure 6. Summary of the KaryoStat assay data analysed with ChAS 4.4. Top panel shows fold change (right y-axis, blue line indicates mean of the signal) of the regions analysed for CNVs. Region bounded by consecutive x-axis major gridlines represent data for a single chromosome and chromosome number increases from left (starting at chromosome 1) to right (ending at chromosome X). Mean value hovering around value of 2 or 1 (for X and Y chromosomes, because of male identity of the cells) indicates absence of CNVs in SCORE-M-hiPSC cells. Karyoview (bottom panel) confirm the absence of LOH. LOH marked by bar on the side of X-chromosome is due to male origin of cells containing only one X-chromosome.

4 Differentiation Potential Analysis

Pluripotent state of SCORe-M-hiPSCs was confirmed by differentiating them in three germ layers (Ecto, Meso and Endoderm) by using the STEMdiff Trilineage Differentiation Kit (STEMCELL Technologies) and characterising the differentiated cells with immunofluorescence and qPCR.

4.1. Immunofluorescence

Differentiated cells were stained for two markers of each germ layer.

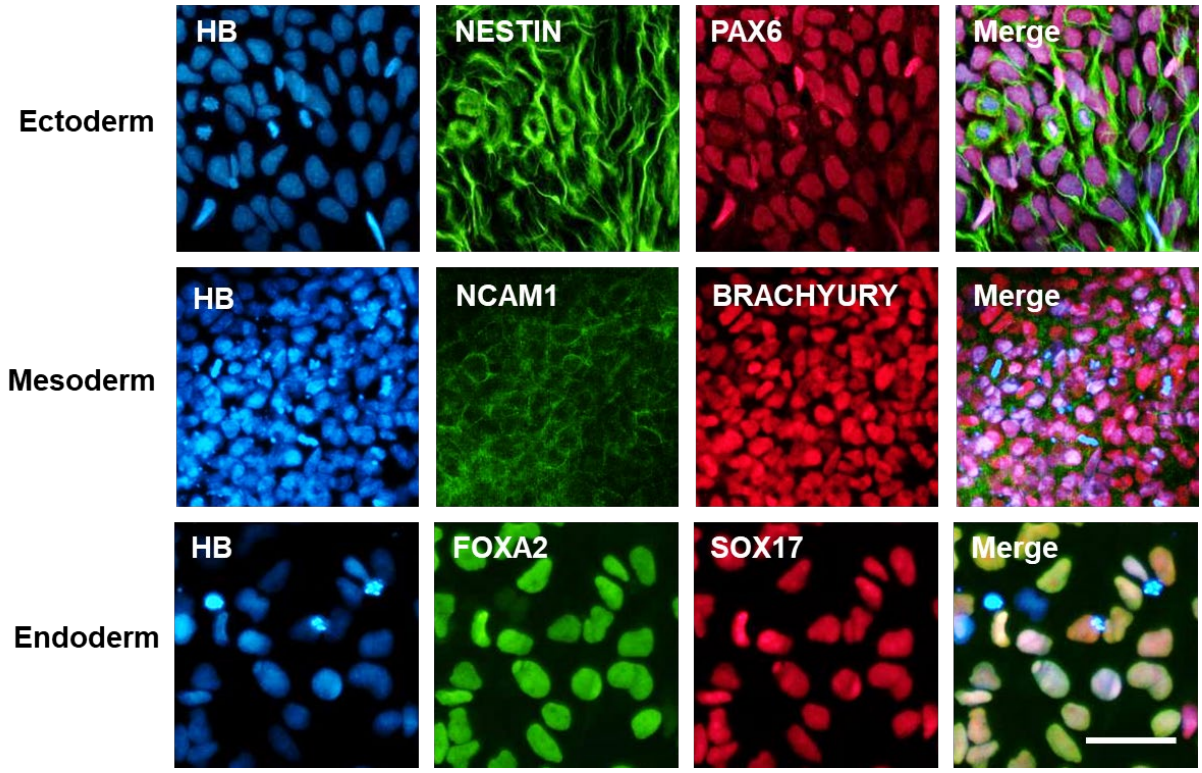


Figure 7. Trilineage differentiation of SCORe-M-hiPSCs demonstrating the presence of markers from the three germ layers – Ectoderm (NESTIN and PAX6), Mesoderm (NCAM1 and BRACHYURY) and Endoderm (FOXA2 and SOX17) and. HB: Hoechst blue; Scale bar represents 50 µm.

4.2. Gene Expression Analysis

Relative gene expression was measured from extracted mRNA by using RT-qPCR. GAPDH was used as the endogenous housekeeping gene.

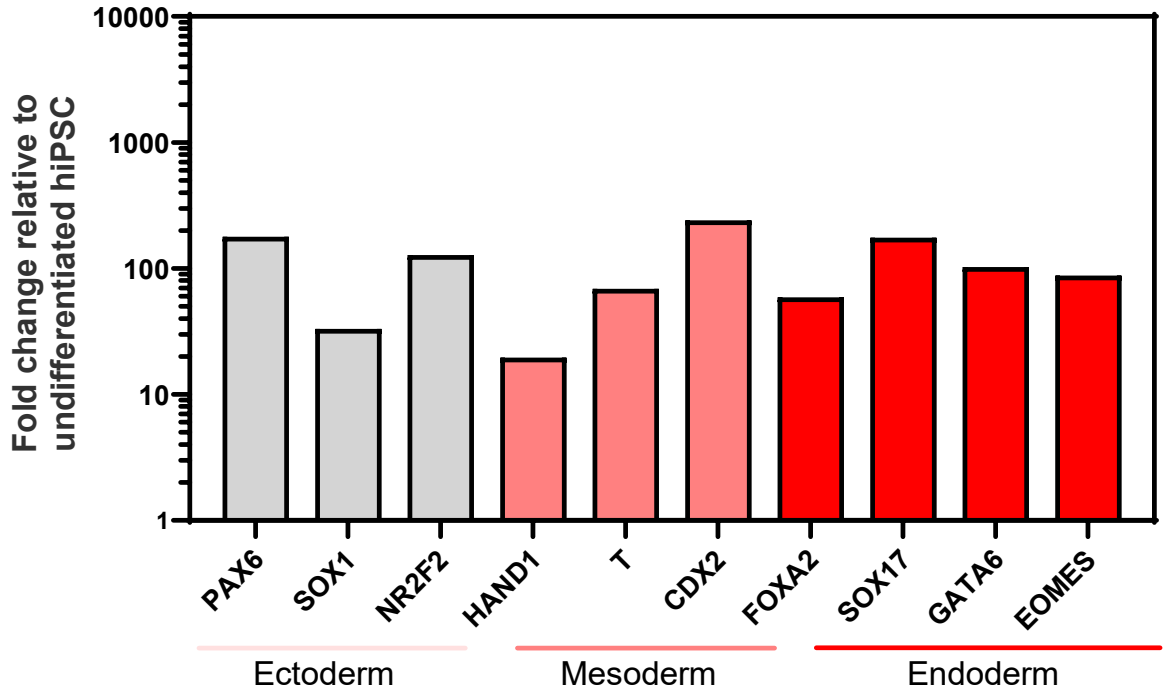


Figure 8. Gene expression analysis of differentiated SCORe-M-hiPSCs demonstrating the expression of genes from the three germ layers - Ectoderm (PAX6, SOX1 and NR2F2), Mesoderm (HAND1, T and CDX2) and Endoderm (FOXA2, SOX17, GATA6 and EOMES) relative to undifferentiated control (expression level = 1). Data represents mean for three technical replicates of each sample.

5. Directions for Use

The following instructions are for preparing one cryovial of SCORe-M-hiPSC for plating in 1 × 75cm^2 flask. Use aseptic technique throughout the protocol.

5.1. Thawing and plating SCORe-M-hiPSCs

1) Coat culture-ware flask with Matrigel® (made to manufacturer's specifications) and incubate for at least 30 min at 37°C or overnight at 4°C. Prior to cell seeding, the flask should be brought to room temperature.
2) Aliquot required volume of mTeSR plus media (~ 15 mL to 20 mL for seeding in one T75) in a 50 mL tube, add Y-27632 (i.e., rock inhibitor) for 10 µM final concentration and pre-warm to 37°C. Use this media in steps 3 to 9.
3) Gently warm the cryovial in a water bath until a small ice pellet remains and transfer into a biosafety cabinet.
4) Add 1 mL of mTeSR media to cryovial and transfer content into a 15 mL centrifuge tube containing 2 mL of mTeSR media.
5) Centrifuge at 300 × g for 3 min.
6) Aspirate the media avoiding disruption to the cell pellet and add 1 mL of mTeSR media.
7) Remove the substrate coating prior to transferring the cells and add mTeSR to flask (11 mL/T75 flask).
8) Gently re-suspend the cell pellet and transfer to the flask.
9) Transfer flask into an incubator at 37°C and do not disturb it for 24 hrs.
10) Perform a full media exchange after 24 hrs (without Y-27632) and then every other day.

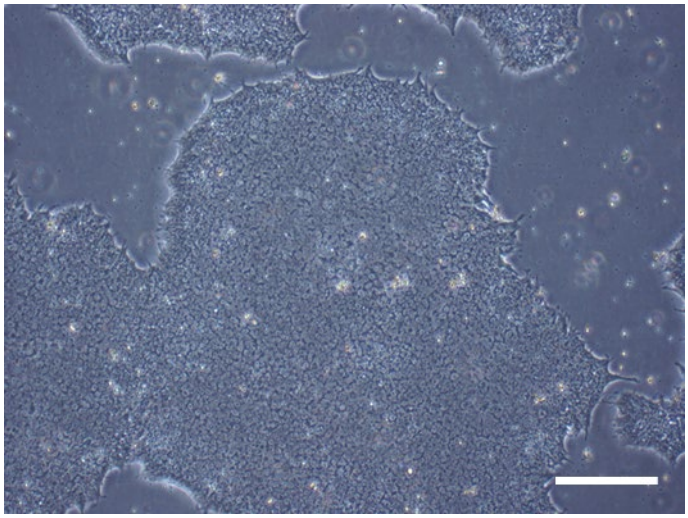


Figure 9. SCORe-M-hiPSC colonies at Day 4 post-recovery from cryostorage. Scale bar 200 µm.

5.2. Passaging SCORe-M-hiPSCs

1) When cells reach 80-90% confluence prepare the following reagents and volumes per T75 flask (adjust volumes as required for specific culture flask):
2) 5 mL ReLeSR™ (pre-warm to 37°C).
3) 10 mL of PBS (calcium and magnesium free (CMF), pre-warm to 37°C).
4) T75 flasks pre-coated with extracellular matrix (ECM) of choice according to manufacturer's instructions.
5) 18 mL mTeSR™ Plus (pre-warm to 37°C) containing 10 µM Y-27632.
6) 10 mL of DMEM (pre-warm to 37°C, specific formulation not strictly important).
7) Remove flask from incubator and transfer to biosafety cabinet.
8) Aspirate media and wash cells with 10 mL PBS CMF. Aspirate PBS.
9) Add 5 mL of ReLeSR™. Lay down flask and ensure entire surface is covered by ReLeSR™.
10) Incubate in biosafety cabinet for no more than 30 sec.
11) Aspirate ReLeSR™ and immediately transfer flask to 37°C incubator.
12) Incubate for 2 min. Check cells under microscope for rounding of cells. Incubate for 1 min longer if required.
13) In the biosafety cabinet, add 5 mL of DMEM down the side of the flask.
14) Tilt flask so surface is covered with DMEM and tap the side of the flask 2 or 3 times. If incubation time in ReLeSR™ is sufficient, cells will lift off with tapping. Collect the cell suspension in a 15 mL centrifuge tube.
15) Wash the surface with another 5 mL DMEM and transfer to the centrifuge tube.
16) Pellet the cells at 300 × g for 3 min.
17) Aspirate supernatant and add 5 mL of mTeSR™ Plus with Y-27632. Mix well to resuspend pellet.
18) Aspirate ECM from T75 and immediately add 12 mL of mTeSR™ Plus with Y-27632 down the side of the flask to avoid removing ECM from surface.
19) Take 1:8 to 1:12 (or 0.75×10^6 to 1×10^6 cells) of the cell suspension and transfer to one T75 with media.
20) Transfer to CO_2 incubator. Gently agitate flask back and forth to ensure even spread of cells in the flask.
21) Exchange media after 24 hrs with 20 mL mTeSR plus without Y-27632 and then every other day.
22) Cells will reach 80-90% confluence within 4 days.

5.3. Cryopreservation of SCORe-M-hiPSCs

1) Follow the passaging protocol up to step 17 and take 0.5 or 1 mL of the cell suspension into a 15 mL tube for cell counting.
2) Centrifuge the 15 mL tube at 300 × g for 3 min.
3) Resuspend cell count aliquot in 0.5 mL of pre-warmed Accutase™ (include a PBS CMF wash before this if desired. Cells are currently in small clusters of 5 - 10 cells and don't require much effort for single cell dissociation).
4) Incubate at 37°C for 3 min with regular gentle agitation. After incubation, add 0.5 mL mTeSR+ media and pipette 5-10 times using a 200 µL pipette.
5) Perform cell count using desired method.
6) Back calculate to determine cell number of original cell suspension. Depending on confluence, each T75 flask may contain 15 - 25 × 10^6 cells.
7) Centrifuge the original cell suspension at 300 × g for 3 min and aspirate media.
8) Gently flick tube to dislodge cell pellet.
9) Add cold, CryoStor® CS10 to a final cell density of 2 - 5 × 10^6 cells/mL. We don't recommend cryopreserving densities lower than 2 × 10^6 cells/mL. Higher densities improve cell recovery at thaw.
10) Aliquot ≥ 500 µL of cell suspension in CS10 per cryovial.
11) Transfer the cryovials to Mr. Frosty Cryo freezing containers, store the containers at -80°C for overnight and then transfer the vials to LN2 cryostorage.