Product Information and Certificate of Analysis

Transgenic Human Embryonic Stem Cell Line MEL-1 iFUCCI C1.1

Product Description

MEL-1 iFUCCI C1.1 transgenic human embryonic stem (hES) cell line was generated by StemCore through TALEN-mediated gene targeting at the *AAVS1* locus of parental MEL-1 hES cells. This cell line contains an inducible fluorescence ubiquitination-based cell cycle indicator (iFUCCI) system and enables continuous visualisation of live cells for real-time examination of cell cycle progression in the presence of doxycycline treatment.

Properties

Cell line	MEL-1 iFUCCI C1.1
Cell type	Transgenic human embryonic stem cells
Gender	Male
Bank designation	Working Cell Bank (WCB) #1
Storage conditions	≤ -135°C
Date of cryopreservation	8-Sep-2021
Passage number	P13+28+6
Cell number	5×10^6 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 1:4-1:6 every 5 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	Viable cell count at revival > 65%. Cells reached confluence within 5 days.
Mycoplasma	Bioluminescence (Lonza MycoAlert detection kit)	Negative
Expression of pluripotent stem cell markers	Immunofluorescence	Positive for OCT4 and SSEA4
	Flow cytometry	> 90% positive (SSEA4 and TRA-1-60)
	Gene expression by RT-qPCR	Similar expression levels of NANOG, OCT4, DNMT3B and REX1 compared to pluripotent control
Cytogenetics	G-banding (500 band level)	Normal - 46, XY
Genetic analysis	Copy number variation test qPCR	Abnormal – Chr20q and Chr1q
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) Mesoderm (HAND1, T) Endoderm (FOXA2, SOX17)

1. Expression of Pluripotent Stem Cell Markers

1.1 Immunofluorescence

To assess pluripotency, MEL-1 iFUCCI C1.1 hES cells were stained for nuclear marker OCT4, and a surface marker SSEA4.

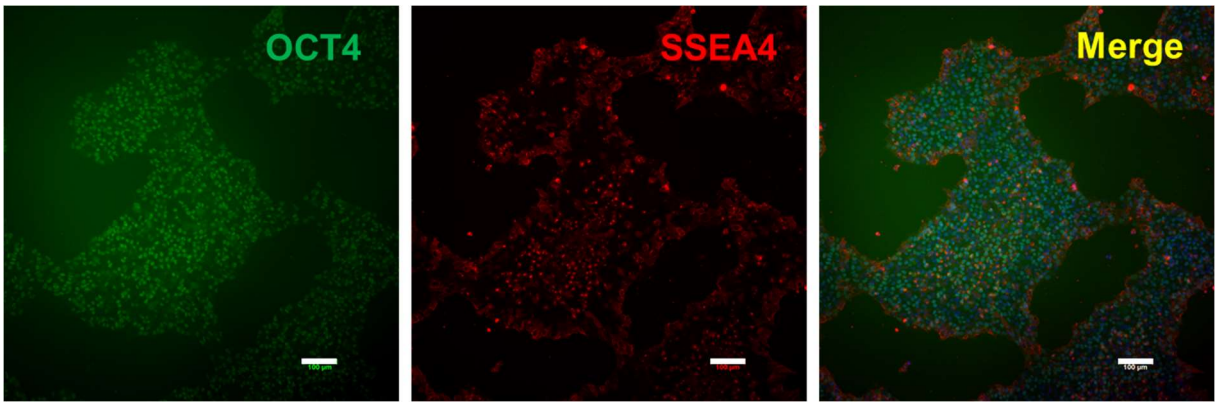

Figure 1. Expression of pluripotency markers OCT4 (green) and SSEA4 (red) in MEL-1 iFUCCI C1.1 cells detected by immunostaining. HB: Hoechst Blue; Scale bar 100 µm.

1.2 Flow Cytometry

In addition to immunofluorescence, flow cytometry analysis confirmed the presence of pluripotent cell surface markers: SSEA4 and TRA-1-60.

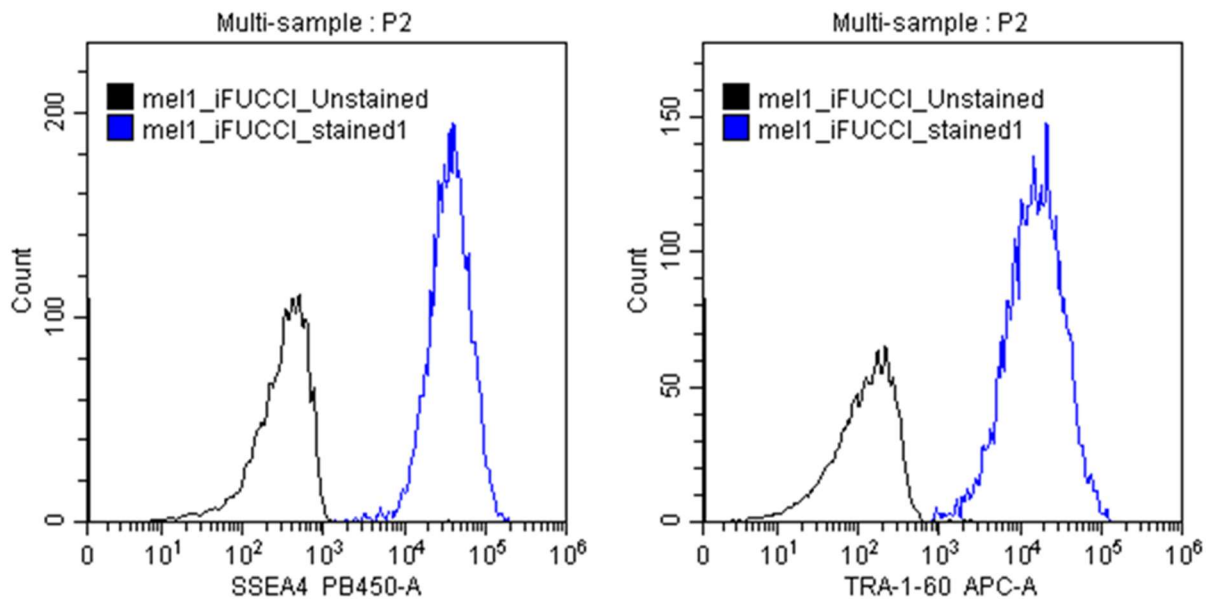

Figure 2. Expression of pluripotency markers SSEA4 (left box) and TRA-1-60 (right box) in the cell line detected by flow cytometry. Black and blue histograms represents unstained and stained cells, respectively.

1.3 Pluripotent 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 is comparable (i.e., difference < 2-fold) between MEL-1 iFUCCI cells and H9 hES cells.

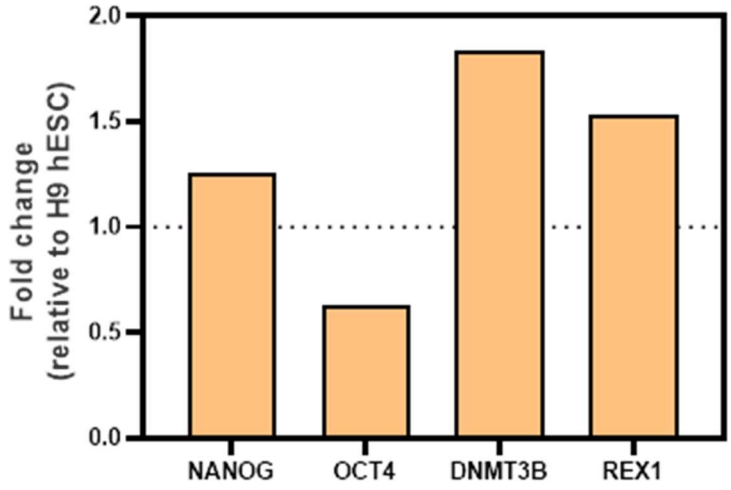

Figure 3. Gene expression analysis of pluripotency markers in MEL-1 iFUCCI C1.1 hES cells demonstrating the expression of NANOG, OCT4, DNMT3B and REX1. The hES cell line H9 (WiCell) is used as positive pluripotent control (dotted line).

2 Genetic Analysis

2.1 Copy Number Variation Test

MEL-1 iFUCCI C1.1 hES cells was screened for recurrent karyotypic abnormalities commonly reported in hES cells using the hPSC Genetic Analysis Kit (STEMCELL Technologies). Genomic DNA was extracted, and qPCR was performed using this kit. Results obtained using the hPSC Genetic Analysis Kit was analysed using the application available at STEMCELL Technologies Genetic Analysis Tool. Amplification of chromosomes 1q and 20q, which are frequently observed sub-karyotypic abnormalities in hPSCs [1], was detected in MEL-1 iFUCCI C1.1 cells.

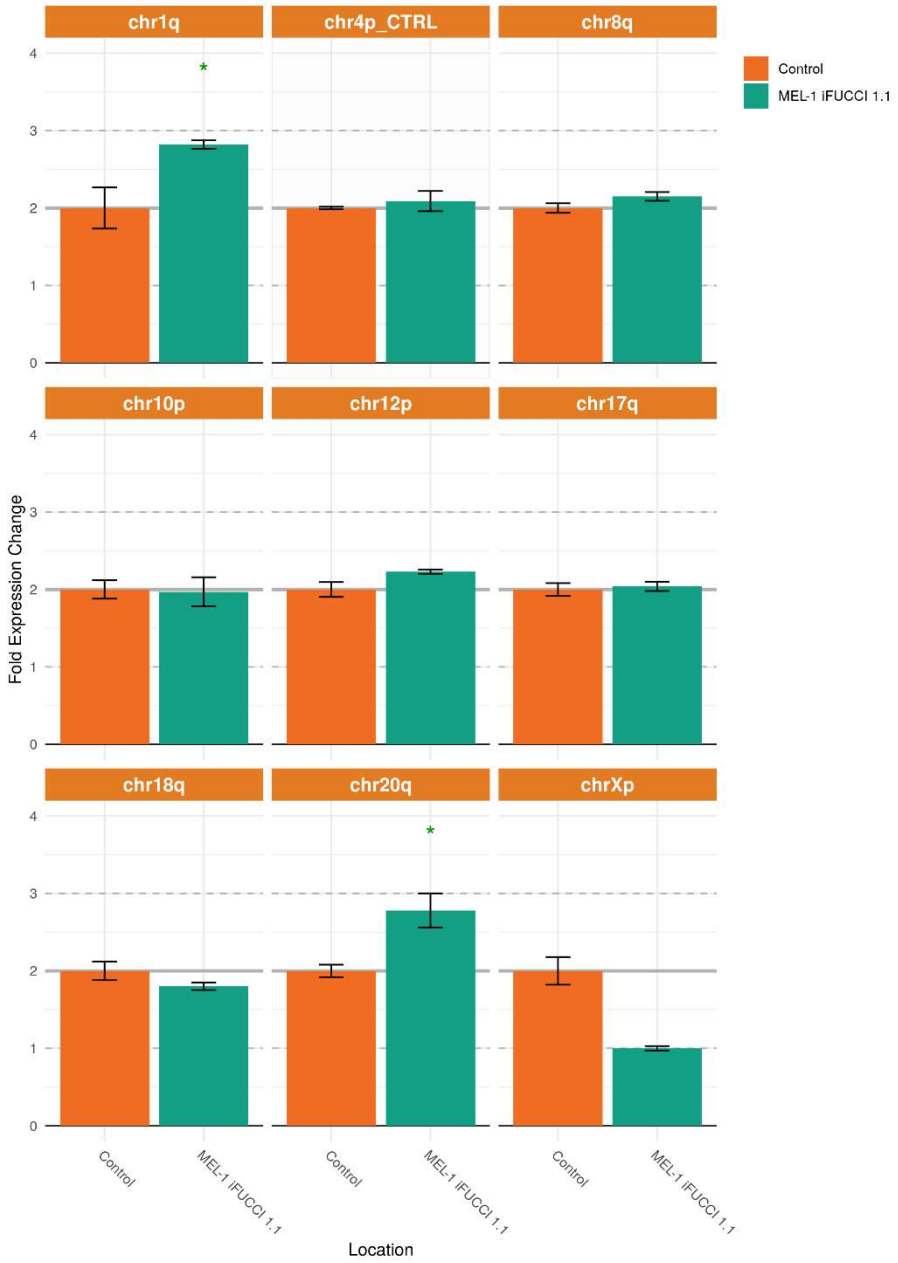

Figure 4. Genetic abnormalities screening showing amplification of chromosome 1q and 20q in MEL-1 iFUCCI C1.1 hES cells. **NOTE:** Only one copy of chrXp detected as expected for sex difference between male sample and female control. Data is summarised from three technical replicate and error bar represent mean ± SD.

2.2 Cytogenetics

Test Method	G-Banding
G-band level	500
Passage at analysis	P13+28+11
Metaphase cells counted	10
Metaphase cells analysed	5
Short ISCN	46, XY

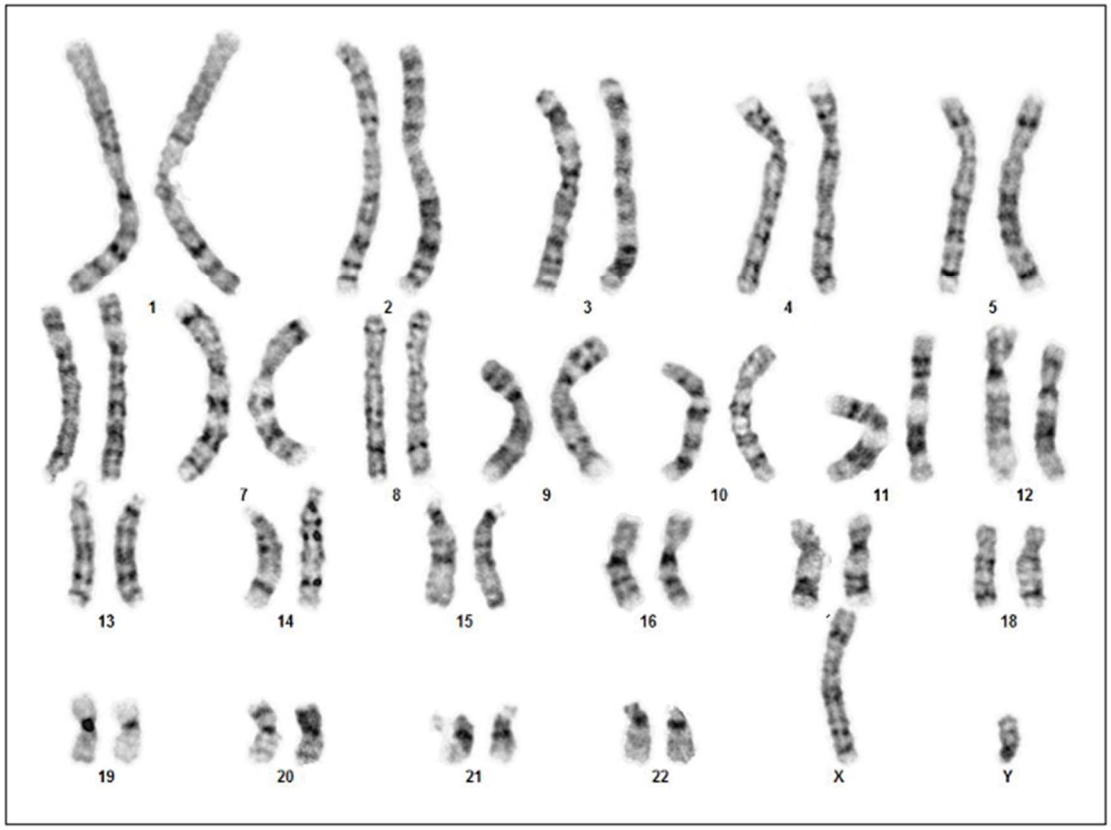

Figure 5. G-banding karyogram of MEL-1 iFUCCI C1.1 hES cells at passage P13+28+11 showing a normal male karyotype – 46, XY (testing performed by Virtus Diagnostics).

3 Differentiation Potential Analysis

3.1 Immunofluorescence

MEL-1 iFUCCI C1.1 hES cells were differentiated using the STEMdiff™ Trilineage Differentiation Kit (STEMCELL Technologies) and stained for two markers of each germ layer.

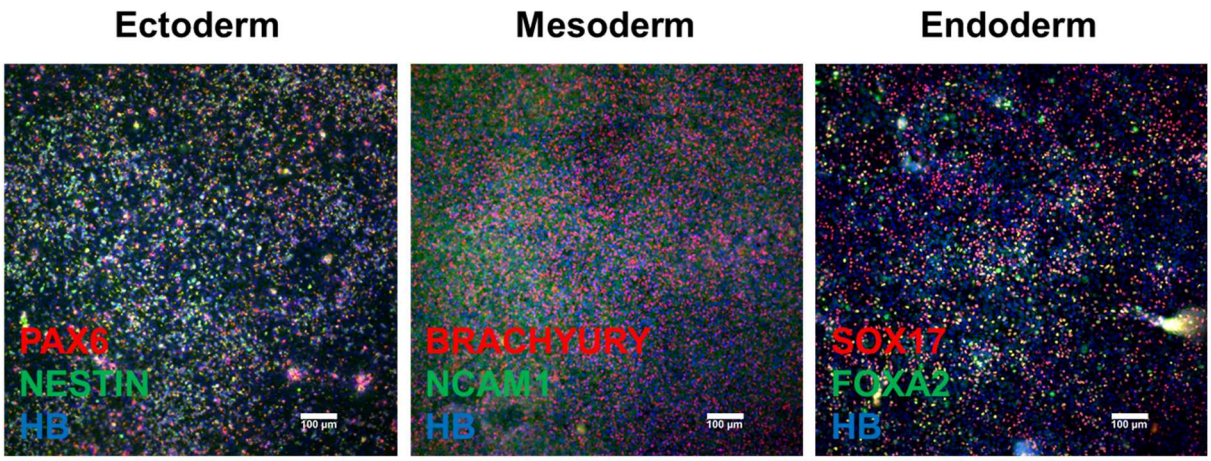

Figure 6. Trilineage differentiation of MEL-1 iFUCCI C1.1 cells demonstrating the presence of markers from the three germ layers: Ectoderm (PAX6 and NESTIN), Mesoderm (Brachyury and NCAM1) and Endoderm (SOX17 and FOXA2). HB: Hoechst Blue; Scale bar 100 µm.

3.2 Gene Expression Analysis

Using the STEMdiff™ Trilineage Differentiation Kit, cells were differentiated towards three lineages (ecto-, meso- and endo-derm) and characterised using gene expression analysis. Relative gene expression was measured from extracted mRNA by using RT-qPCR. GAPDH was used as the endogenous housekeeping gene.

Germ Layer Gene Expression During Lineage Differentiation

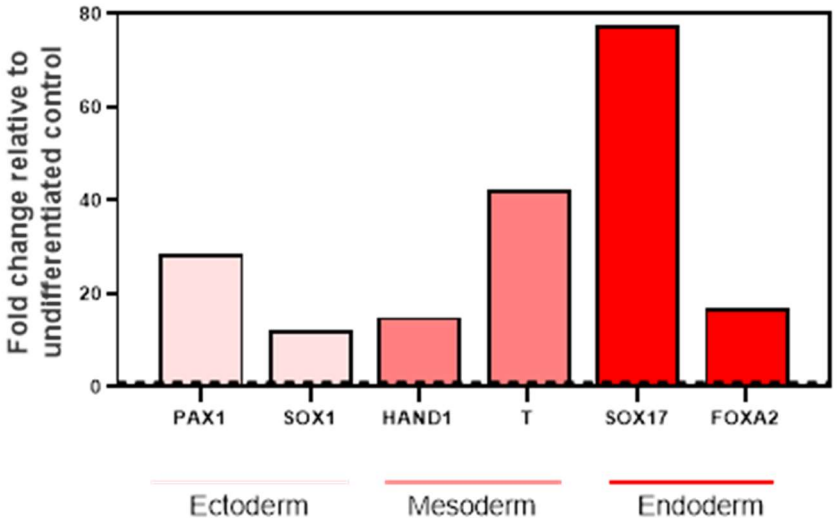

Figure 7. Gene expression analysis of differentiated MEL-1 iFUCCI C1.1 cells, demonstrating the expression of genes from the three germ layers - Ectoderm (PAX6 and SOX1), Mesoderm (HAND1 and T) and Endoderm (SOX17 and FOXA2) relative to undifferentiated control (dotted line).

Pluripotent Gene Expression During Lineage Differentiation

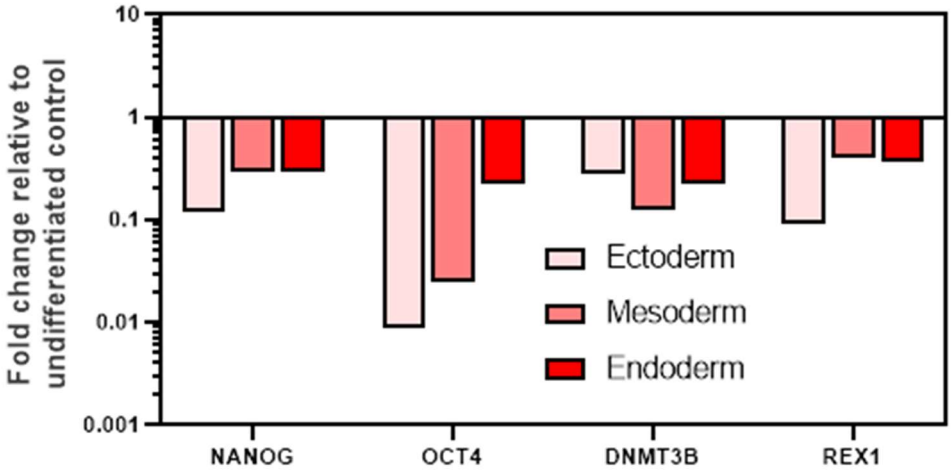

Figure 8. Gene expression analysis of differentiated MEL-1 iFUCCI C1.1 cells, demonstrating downregulation of pluripotency genes in the three germ layers relative to undifferentiated control.

4. Directions for Use

The following instructions are for preparing one cryovial of MEL-1 iFUCCI C1.1 hES cells for plating in 1 × 75cm^2 flask. Use aseptic technique throughout the protocol.

4.1 Thawing and plating MEL-1 iFUCCI C1.1 hES cells

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 pre-warmed to 37°C and the mTeSR™ Plus medium warmed to room temperature.
2) Gently warm the cryovial in a water bath until a small ice pellet remains and transfer into a biosafety cabinet.
3) Add 1 mL of mTeSR™ Plus media to cryovial and transfer content into a 15 mL centrifuge tube containing 2 mL of mTeSR™ Plus media.
4) Centrifuge at 200 × g for 3 min.
5) Add RevitaCell to mTeSR™ Plus medium according to manufacturer's specifications.
6) Aspirate the media avoiding disruption to the cell pellet and add 1 mL of mTeSR™ Plus with RevitaCell.
7) Remove the substrate coating prior to transferring the cells and add mTeSR™ Plus medium with RevitaCell to flask (11 mL/T75 flask).
8) Gently re-suspend the cell pellet and transfer to the Matrigel-coated flask containing mTeSR™ Plus and RevitaCell.
9) Transfer flask into an incubator at 37°C and do not disturb for 24 hrs.
10) Perform a full media exchange 24 hrs later (without RevitaCell) and then replace daily or every second day (depending on cell confluence).

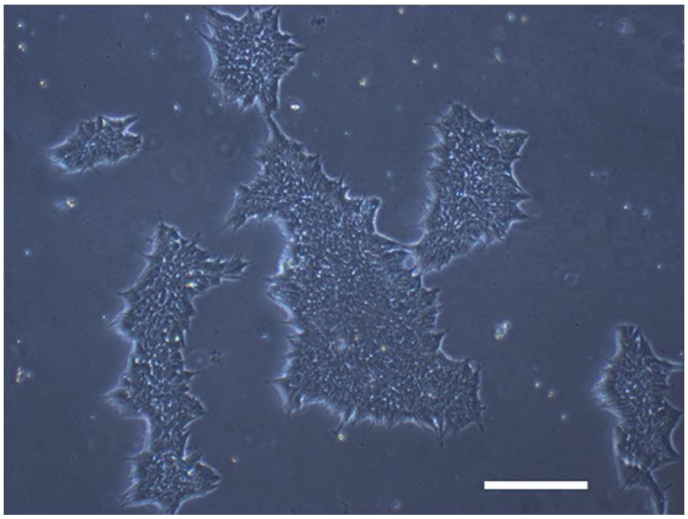

Figure 9. MEL-1 iFUCCI C1.1 hESC colonies at Day 4 post-recovery from cryostorage. Scale bar 200 µm.

4.2 Passaging MEL-1 iFUCCI C1.1 hES cells

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) 15 mL mTeSR™ Plus (pre-warm to 37°C) containing 1x RevitaCell at recommended dilution (or 10 µM ROCK inhibitor).
6) 5 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.
15) Wash the surface with the 5 mL DMEM/cell suspension and transfer to a centrifuge tube.
16) Pellet the cells at 300 × g for 3 min.
17) Aspirate supernatant and add 1 mL of mTeSR™ Plus with RevitaCell. Mix well to resuspend pellet.
18) Aspirate ECM from T75 and immediately add 12 mL of mTeSR™ Plus with RevitaCell down the side of the flask to avoid removing ECM from surface.
19) Take 1:6 to 1:8 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) Change media 24 hrs later to remove RevitaCell. 12-20 mL per T75 flask is recommended based on confluence at the time.
22) Cells will reach confluence within 4-5 days.

4.3 Cryopreservation MEL-1 iFUCCI C1.1 hES cells

1) Follow the passaging protocol up to step 10.
2) Take a small aliquot of the cell suspension (volume of your choice) and transfer to a second centrifuge tube to perform a cell count.
3) Centrifuge both tubes at 300 × g for 3 min.
4) Aspirate media and resuspend cell suspension for cryopreservation in 5 mL of mTeSR™ Plus.
5) Resuspend cell count aliquot in 1 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).
6) Incubate at room temperature for 3 min with regular gentle agitation using a 200 µL pipette.
7) Perform cell count using desired method.
8) Back calculate to determine cell number of original cell suspension.
9) Centrifuge cells at 300 × g for 3 min.
10) Aspirate mTeSR™ Plus
11) Gently flick tube to dislodge cell pellet.
12) Add cold, CryoStor® CS10 to a final cell density of 1 - 5 × 10^6 cells/mL. We recommend not cryopreserving densities lower than 1 × 10^6 cells/mL. Higher densities improve cell recovery at thaw.
13) Aliquot ≥ 500 µL of cells in CryoStor® CS10 per cryovial.
14) Transfer to isopropanol tanks and then to -80°C for 2 hrs before transfer to cryostorage.
15) Depending on confluence, each T75 flask contains 18 - 25 × 10^6 cells.

4.4. Activating iFUCCI in MEL-1 iFUCCI C1.1 hES cells for visualising cell cycle progression

Add doxycycline at 1µg/mL for 24 hrs in the cell culture to activate the expression. Cells will start expressing GFP, RFP or both depending on their cell cycle status [2]. Doxycycline concentration or treatment duration may need to be adjusted to improve the expression intensity.

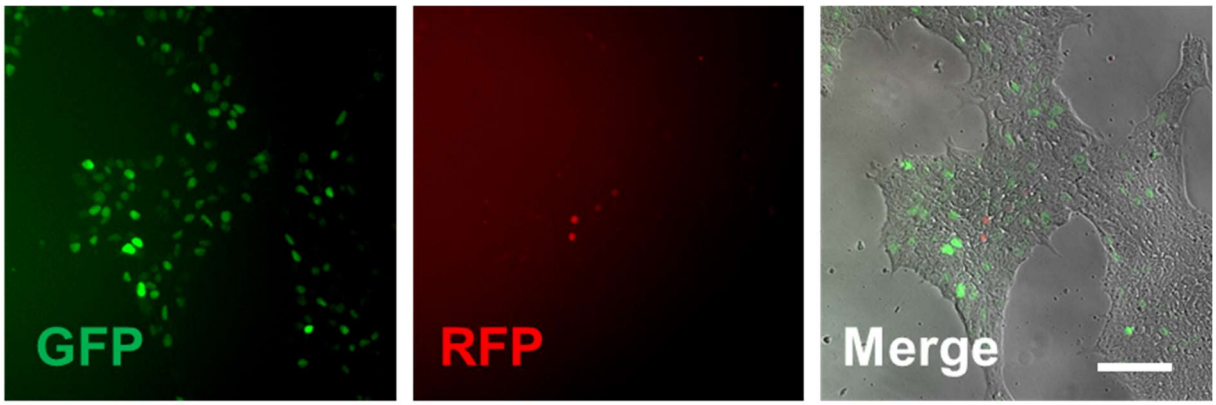

Figure 10. MEL-1 iFUCCI C1.1 cell showing GFP, RFP or expression of both. Scale bar 100 µm.

References

[1] Molina-Ruiz, F. J., Introna, C., Bombau, G., Galofre, M., & Canals, J. M. (2022). Standardization of Cell Culture Conditions and Routine Genomic Screening under a Quality Management System Leads to Reduced Genomic Instability in hPSCs. *Cells*, *11*(13), 1984.
[2] Koh, S. B., Mascalchi, P., Rodriguez, E., Lin, Y., Jodrell, D. I., Richards, F. M., & Lyons, S. K. (2017). A quantitative FastFUCCI assay defines cell cycle dynamics at a single-cell level. Journal of cell science, 130(2), 512-520.