HMGU1

human iPS cells

GENERAL INFORMATION

Organism	Homo sapiens, human
Tissue	foreskin fibroblast
Source of Cells	BJ (ATCC CRL-2522)
Description	Control Line
Reprogramming Method	Modified mRNA
Reprogramming Factors	5F (Oct4, Sox2, Klf4, Lin28, c.Myc)
Clones	Bulk establishment
Derivation Year	2013
Biosafety Level	1 (HBV, HCV, HIV-1 negative)
Disease	Normal
Age	Newborn
Gender	Male
Ethnicity	Unknown
Storage	Liquid Nitrogen

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LINE SPECIFICATION

HMGU1 hiPSC line was generated from human foreskin fibroblasts by transfection of five mRNA reprogramming factors: Oct4, Sox2, Klf4, Lin28 and c.Myc (Kunze et al., 2017).

The cells were characterized by immunocytochemical detection of pluripotent factors OCT4, SOX2 and NANOG (Figure 2) and in vitro direct differentiation into progenitor progeny of the three germ layers ectoderm, mesoderm and endoderm (Figure 3). The differentiation protocol was adapted from Shi et al., 2012, Borchin et al., 2013, D'Amour et al., 2005, respectively.

The established lines showed round compact shape colonies with defined borders when propagated in mTeSR medium or StemMACS iPS-Brew XF and passaged using Collagenase IV or StemMACS Passaging Solution on Matrigel or Geltrex matrix (Figure 1).

HMGU1 showed correct male karyotype at passage 15 and 34, using G-Banding staining with 300 and 500 resolution banding, respectively (Figure 4).

Mycoplasma test: negative based on PCR

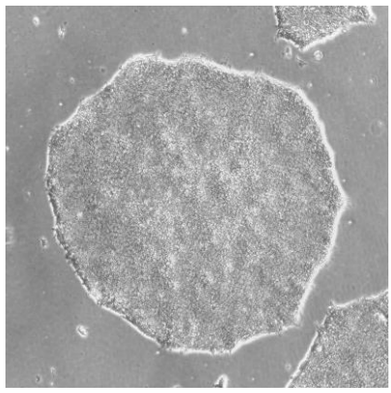


Figure 1. Morphology: Established hiPSCs HMGU1 line cultured in mTeSR on Matrigel display typical colony morphology, Magnification 5x.

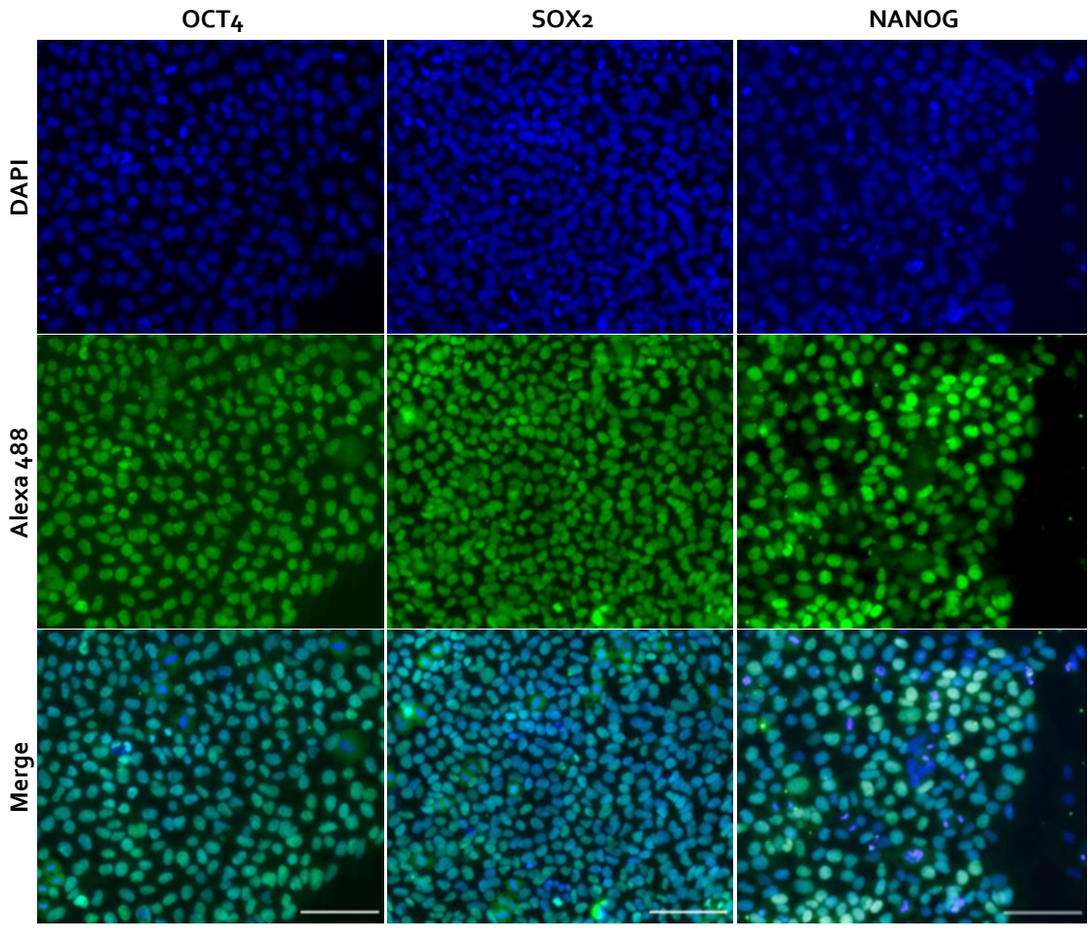


Figure 2. Immunofluorescent staining of pluripotency markers: HMGU1 hiPSC line analysed for OCT4, SOX2 and NANOG exhibited expression of the pluripotency markers at passage 35 in mTeSR medium on Matrigel, scale bar 100 uM.

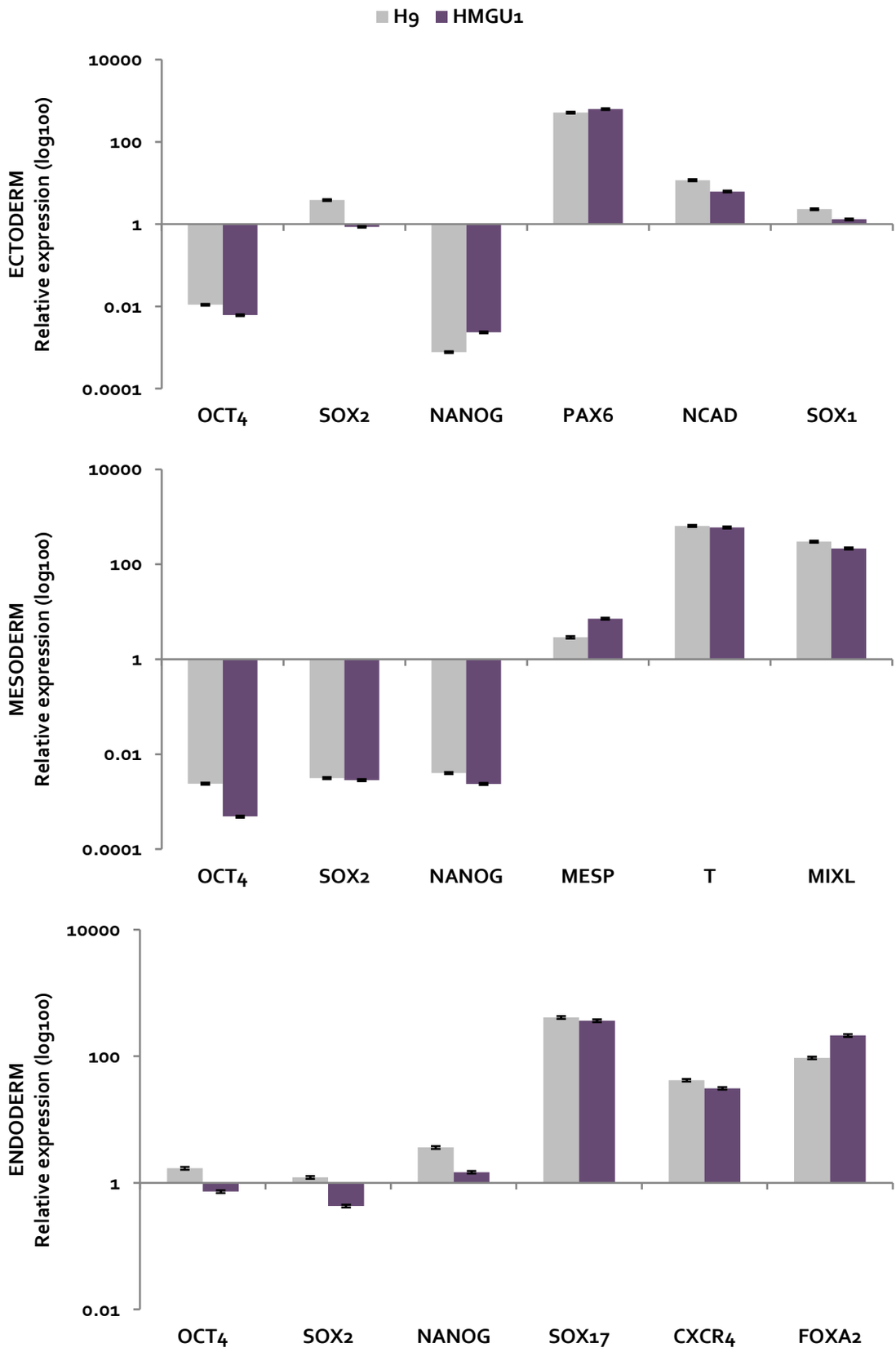
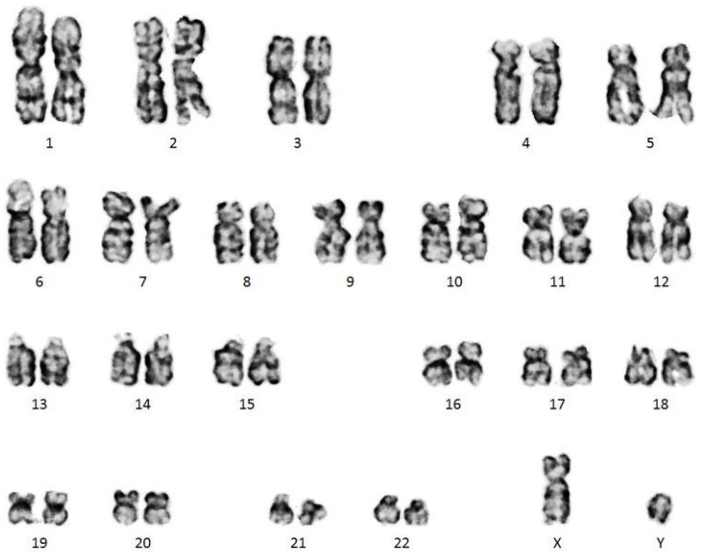


Figure 3. Comparison of the relative expression changes during differentiation of hESC (H9, grey bars) & hiPSC (HMGU#1, mTeSR conditions, violet bars), down-regulation of pluripotency genes and up-regulation of respective lineage markers is consistent for the lines.

HMGU1, Passage 15



HMGU1, Passage 34

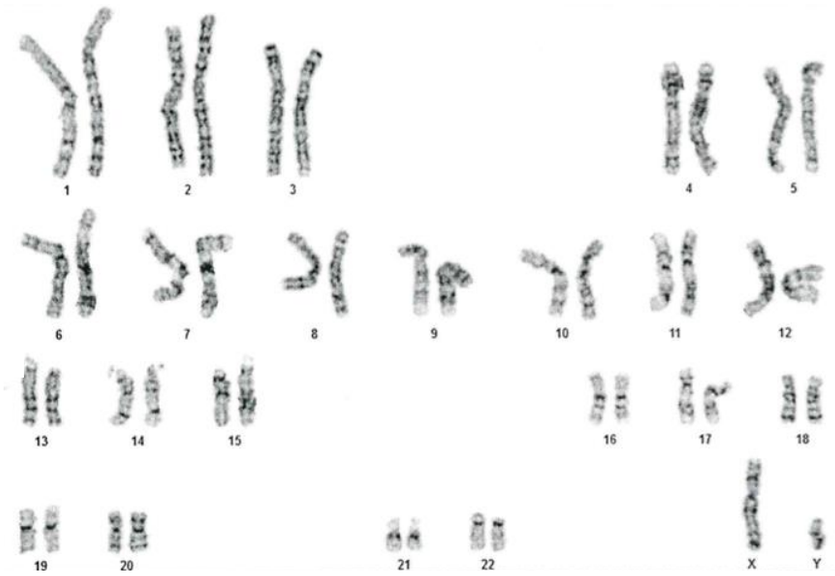


Figure 4. Karyogram of HMGU1 at passage 15 and passage 34 stained with G-banding.

CULTURE METHOD

General Information

The HMGU iPSCs are adapted to feeder- and serum-free culture conditions. The base medium for this cell line is mTeSR (Stemcell technologies) / StemMACS iPS-Brew XF (Miltenyi Biotec), which is a ready to use medium for serum-free and feeder-free iPSC culture.

Cell culture dishes are coated with Matrigel Matrix (BD or Corning Life Sciences) or Geltrex Matrix (Thermo Fisher Scientific, Waltham, MA USA) to provide a surface for the attachment of iPSCs. The coating is done according to the manufacturer's instructions.

Cells are passaged by 2 mg/ml collagenase IV (Thermo Fisher Scientific, Order No. 17104019) or by using StemMACS Passaging solution (Myltenyi Biotec, Order No. 130-104-688).

Cell culture initiation protocol

This protocol is designed to begin cell culture in a 6-well dish.

1. Coat 6-well plates with an appropriate attachment substrate according to the manufacturer's instructions.
2. Thaw the cells by gentle agitation in a 37°C water bath. Thawing should be rapid (approximately 1 to 2 minutes).
3. Remove the vial from the water bath as soon as the contents are thawed, and decontaminate by dipping in or spraying with 70% ethanol.
    - ❖ **Note:** All operations from this point onward should be carried out under strict aseptic conditions.
4. Add the appropriate volume of complete growth medium [volume = (1 mL x number of wells to be seeded) - 1 mL] into a sterile conical tube. Using a sterile pipette, transfer the cells from the cryovial to the conical tube. Gently pipette the cells to homogenize the suspension. Do not centrifuge.
5. Transfer 1.0 mL of the cell suspension to each of the pre-equilibrated culture dish. Gently rock the plate to distribute the solution evenly.
6. Place the seeded culture flasks in the incubator at 37°C, 5% CO_2 atmosphere. Incubate for at least 24 hours before processing the cells further.

Subculturing protocol
(passaging in cell clusters wit Collagenase IV)

This protocol is designed to passage stem cell colonies cultured in a 6-well dish.

1. Coat 6-well plates with an appropriate attachment substrate according to the manufacturer's instructions.
2. Aspirate the cell culture supernatant.
3. Wash the cell layer with 1 mL of DPBS without Ca^{2+} and Mg^{2+} per well.
4. Add 0.5 mL of 2 mg/ml collagenase IV per well. Gently rock the plate to distribute the solution evenly.
5. Incubate at 37°C for 40 minutes. Monitor the detachment process under the microscope.
    - ❖ **Note:** Colonies must not detach completely. Only wait until the colony edges lift off
6. Add 1 mL of pre-warmed cell culture medium.
7. Gently detach the colonies by rinsing the well with a 1 mL tip.
8. Transfer the cell suspension into a 15 mL conical tube.
9. Carefully pipette up and down 2–3 times to break up the colonies into smaller cell clusters.
    - ❖ **Note:** Take care to minimize break-up of colonies. Do not create single cells!
10. Centrifuge for 3 minutes at 200xg.
11. Aspirate the cell culture supernatant leaving behind the cells pellet.
12. Transfer the cell clusters into a fresh, appropriately coated 6-well cell culture plate. Use 2 mL cell culture medium (optionally supplemented with ROCK inhibitor Y27632 at 10 uM concentration) per well and a splitting ratio 1:10.
    - ❖ **Note:** The optimal splitting ratio will depend on the cell line and must be determined empirically.
13. After 48 hours, replace media with fresh pre-warmed cell culture medium (without ROCK inhibitor) and continue with daily media changes.
    - ❖ **Note:** Many ES and iPS cell lines will also tolerate every-other-day media changes.

Cryopreservation

For optimal results, cryopreserve stem cell colonies when the cell cultures are 80% confluent. This protocol is designed to cryopreserve stem cell colonies cultured in a 6 cm dish.

1. Detach stem cell colonies from the dish as described in the subculturing protocol (steps 1-11). Gently tap the bottom of the tube to loosen the cell pellet.
2. Take the stem cell freezing media from storage and swirl to mix. Keep cold. Decontaminate by dipping in or spraying with 70% alcohol.
3. Add 2 mL of cold freezing media (recommended CryoStor CS10, Sigma, Order No. C2874-100ML) to the tube. Gently resuspend the pellet by pipetting up and down 2 to 3 times with a 1 mL tip, maintaining the cell aggregates.
4. Immediately transfer 1 mL each of the cell suspension into two labeled cryovials.
5. Freeze the cells gradually at a rate of -1°C/min until the temperature reaches -70°C to -80°C.
    - ❖ **Note:** A cryopreservation container (e.g. CoolCell® freezing container) may also be used.
6. The cells should not be left at -80°C for more than 24 to 48 hours. Once at -80°C, frozen cryovials 6should be transferred to the vapor phase of liquid nitrogen for long-term storage.

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