HMGU12
human iPS cells

GENERAL INFORMATION

Organism	Homo sapiens, human
Tissue	foreskin fibroblast
Source of Cells	BJ (ATCC CRL-2522)
Description	Control Line
Reprogramming Method	Non-modified RNA
Reprogramming Factors	6F (Oct4, Sox2, Klf4, Lin28, c.Myc, Nanog)
Clones	Bulk establishment
Derivation Year	2018
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

HMGU12 hiPSC line was generated from human foreskin fibroblasts by transfection of five mRNA reprogramming factors: Oct4, Sox2, Klf4, Lin28, c.Myc and Nanog (Stemgent StemRNA-NM Reprogramming Kit).

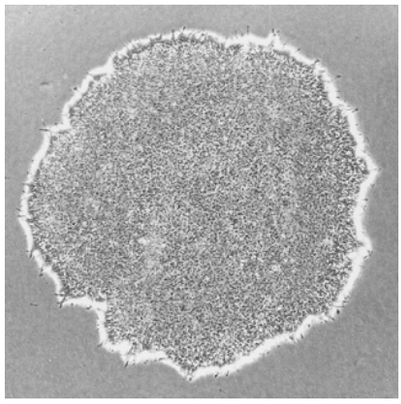
The cells were characterized by immunocytochemical detection of pluripotent factors OCT4, SOX2, NANOG and Lin28 (Figure 2) and in vitro direct differentiation into progenitor progeny of the three germ layers ectoderm, mesoderm and endoderm (Figure 3). The differentiation protocol was adapted from Shi 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 StemMACS Passaging Solution (optionally Collagenase IV) on Matrigel or Geltrex matrix (Figure 1).

HMGU12 showed correct male karyotype at passage 13, using G-Banding staining with 300 resolution banding (Figure 4). Clonal aberration could not be found.

Mycoplasma test: negative based on PCR

Figure 1. Morphology: Established hiPSCs HMGU12 line (p8) cultured in StemMACS iPS-Brew XF on Matrigel display typical colony morphology, Magnification 5x.



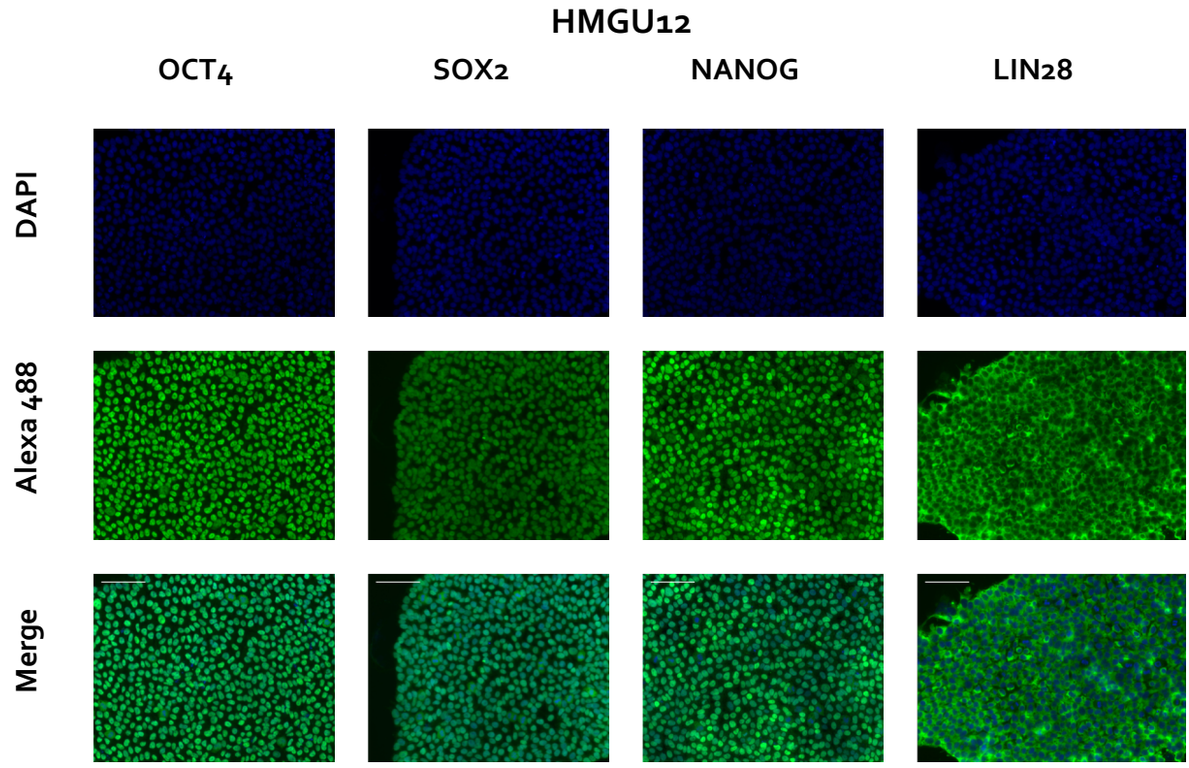


Figure 2. Immunofluorescent staining of pluripotency markers: HMGU12 hiPSC line analysed for OCT4, SOX2, NANOG and LIN28 exhibited expression of the pluripotency markers at passage 15 in StemMACS iPS-Brew XF medium on Matrigel, scale bar 100 uM.

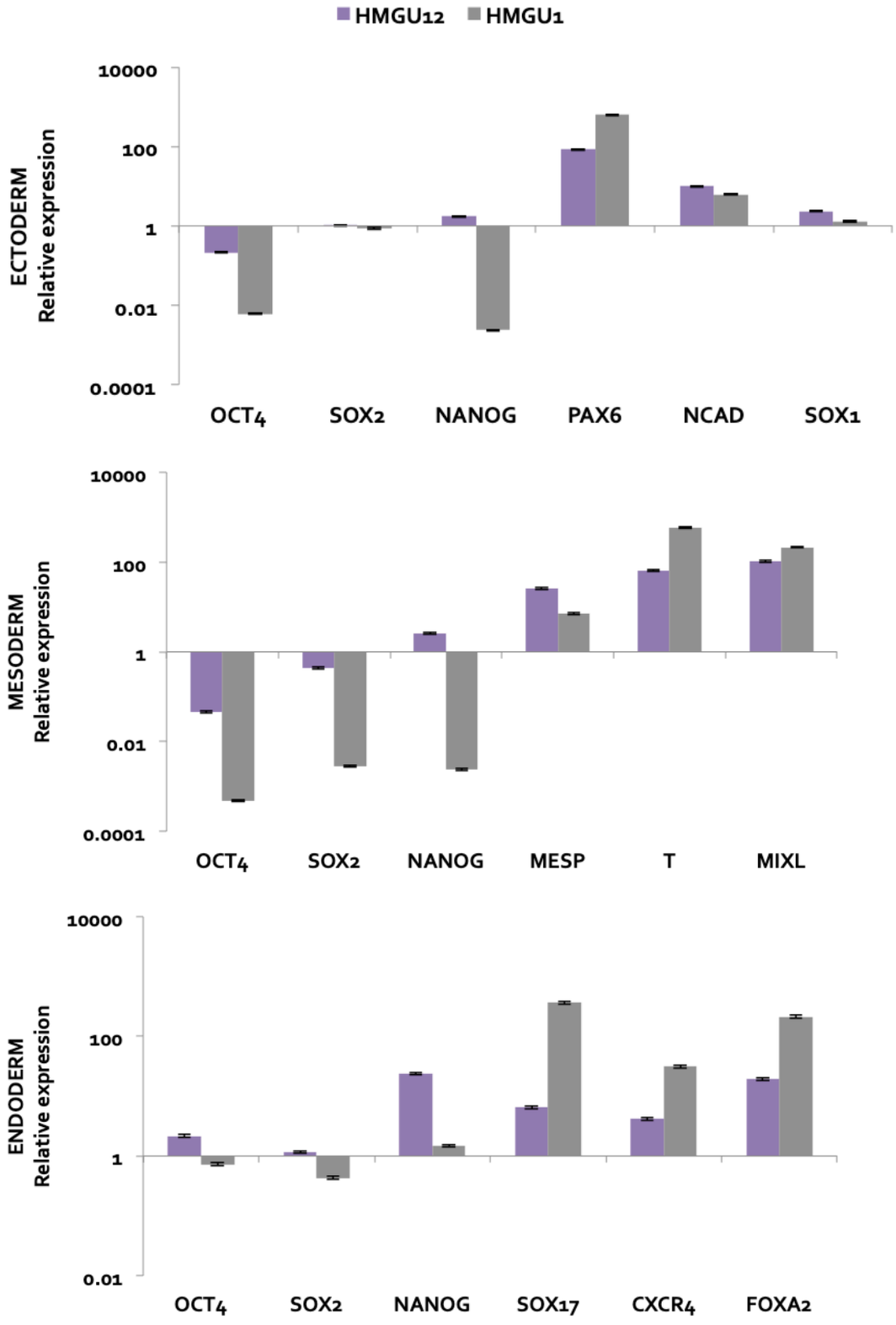


Figure 3. Comparison of the relative expression (log100) changes during differentiation of hiPSC HMGU1 (grey bars) and HMGU12 (StemMACS iPS-Brew XF conditions, violet bars), down-regulation of pluripotency genes and up-regulation of respective lineage markers is consistent for the lines.

HMGU12, Passage 13

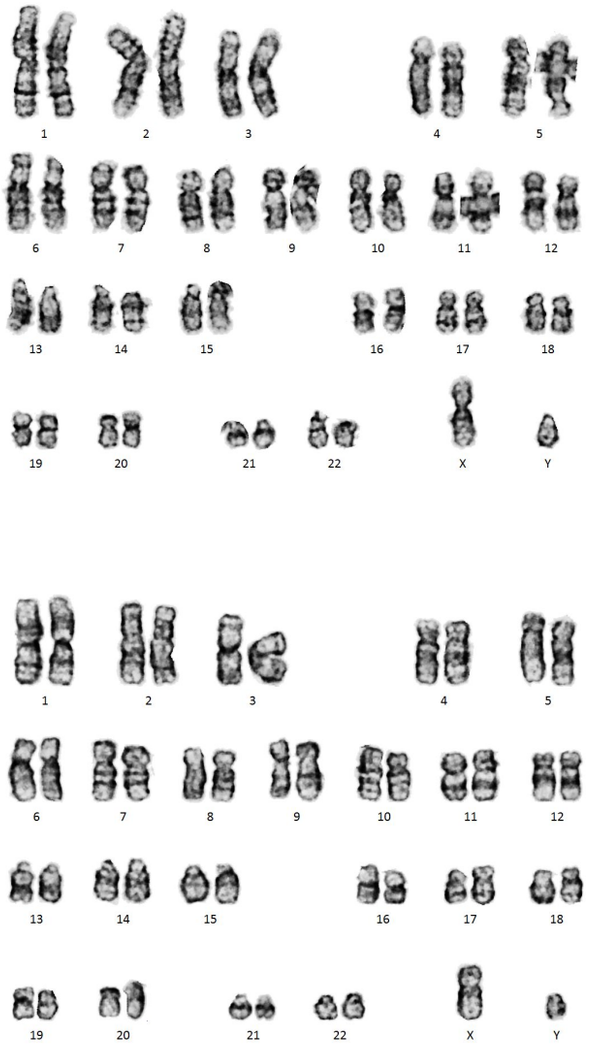


Figure 4. Karyogram of HMGU12 at passage 13 stained with G-banding.

CULTURE METHOD

General Information

The HMGU iPSCs are adapted to feeder- and serum-free culture conditions. The base medium for this cell line is StemMACS iPS-Brew XF (Miltenyi Biotec) / mTeSR (Stemcell technologies), 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 using StemMACS Passaging Solution XF (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 80% ethanol.
    - ❖ **Note:** All operations from this point onward should be carried out under strict aseptic conditions.
4. Transfer cells into a 15 ml conical tube with 2-3 ml of fresh medium.
5. Centrifuge for 3 minutes at 300xg.
6. Aspirate the cell culture supernatant leaving behind the cells pellet.
7. Gently resuspend the cell pellet in the appropriated volume of medium supplemented with ROCK inhibitor Y27632 at 10 uM concentration and plate the cells on the coated 6-well dish.
    - ❖ **Note:** The optimal plating ratio will depend on the cell line. Advisable after thawing plate your cells in maximal 3 wells of 6-well dish.
8. Gently rock the plate to distribute the solution evenly.
9. 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

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 1 mL of StemMACS Passaging Solution XF per well. Gently rock the plate to distribute the solution evenly.
5. Incubate at RT°C for 4-6 minutes. Monitor the detachment process under the microscope.
6. Carefully remove the StemMACS Passaging Solution XF.
7. Per well, add 1 ml of medium (optionally supplemented with 10 uM Rock inhibitor Y27632)
8. Gently detach the colonies by rinsing with a 1 ml pipette to break up the colonies into smaller cell clusters.
9. Transfer the cell clusters into a fresh, appropriately coated 6-well cell culture plate. Use 2 ml StemMACS iPS-Brew XF (optionally supplemented with ROCK inhibitor) per well and splitting ratio between 1:6 and 1:20.
    - ❖ **Note:** The optimal splitting ratio will depend on the cell line and must be determined empirically.
10. After 24h replace the media with a fresh StemMACS iPS Brew XF 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 1 well of the 6 well plate into 2 freezing tubes.

1. Detach stem cell colonies from the dish as described in the subculturing protocol (steps 2-8).
2. Collect cells in the 15 ml conical tube and centrifuge for 3 min at 300 xg.
3. Aspirate the supernatant/medium leaving behind the cell pellet.
4. Take the stem cell freezing media from storage and swirl to mix. Keep cold. Decontaminate by dipping in or spraying with 80% alcohol.
5. 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.
6. Immediately transfer 1 mL each of the cell suspension into to labelled cryovials.
7. 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.
8. The cells should not be left at -80°C for more than 24 to 48 hours. Once at -80°C, frozen cryovials should be transferred to the vapor phase of liquid nitrogen for long-term storage.

REFERENCES

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