NutriStem® hPSC XF Medium Supports Long-term Culture of Human Pluripotent Stem Cells as Clumps on Corning® Matrigel® Matrix and Single Cells on Corning PureCoat™ rLaminin-521 Cultureware

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Introduction

Human pluripotent stem cells (hPSCs) including human embryonic stem cells (hESCs) and human induced pluripotent stem cells (hiPSCs), are typically cultured using complex media containing animal-derived components. In addition, adherent culture of hPSCs requires either a layer of feeder cells (*e.g.,* mouse or human fibroblasts), a complex mixture of naturally derived extracellular matrices (*e.g.,* Corning® Matrigel® matrix) or defined surfaces (*e.g.*, recombinant Laminin). In this study, we demonstrated long-term culture of hiPSCs in NutriStem® hPSC XF medium – a serum-free, xeno-free medium – widely used for long-term growth and expansion of hPSCs. Human iPSCs were cultured as clumps on Matrigel matrix or as single cells on Corning PureCoat™ rLaminin-521 cultureware in NutriStem medium for at least ten passages.
Cells exhibited typical hPSC morphology and remained undifferentiated as demonstrated by the expression of Oct-3/4 (>94%), SSEA-4 (>95%) and the absence of SSEA-1. After ten passages, pluripotency was shown by differentiation into the three germ layers and cells retained a normal karyotype.

Materials and Methods

Matrigel coated plates were prepared using Corning Matrigel hESC-qualified matrix (Cat. No. 354277). Corning PureCoat rLaminin-521 cultureware (Cat. No. 356290) was used according to manufacturer's guidelines. Human episomal iPSCs (Gibco®) were routinely maintained on Matrigel coated 6-well plates in mTeSR®1 medium (STEMCELL Technologies™).

On Matrigel coated 6-well plates, cells were clump passaged every three to five days using Cell Dissociation Buffer (Life Technologies) and seeded at a split ratio of 1:14 - 1:21 in NutriStem XF/FF medium (manufactured by Biological Industries).

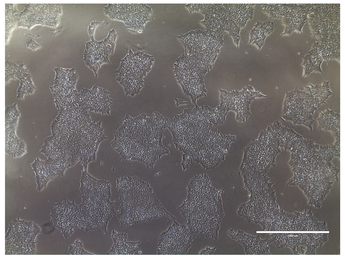
Cells were seeded as single cells onto rLaminin-521 cultureware (6-well format) in NutriStem medium. Cells were passaged every four to five days using Accutase® and seeded at a density of 50,000 cells/cm^2.

At each passage, the morphology of the cells and the cell yields (PureCoat only) were monitored. After ten passages, cells were karyotyped at Cell Line Genetics® (WI), expression of Oct-3/4, SSEA-4, and SSEA-1 was evaluated using flow cytometry (BD FACSCalibur™) and directed differentiation into three germ layers was performed using human pluripotent stem cell functional identification kit (R&D Systems); nuclei were counterstained with Hoechst 33342 (blue).

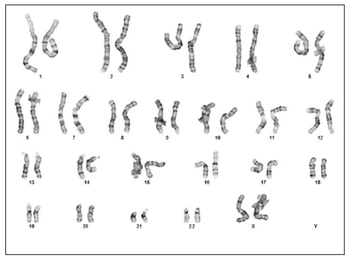
Results

Cell morphology: Using NutriStem, cells displayed typical hPSC morphology on both surfaces.
Undifferentiated marker expression: hiPSCs expressed undifferentiation markers (Oct-3/4 and SSEA-4); differentiation marker (SSEA-1) was not detected.
Maintenance of pluripotency: hiPSCs were able to differentiate into three germ layers.
Karyotype: hiPSCs retained a normal karyotype.

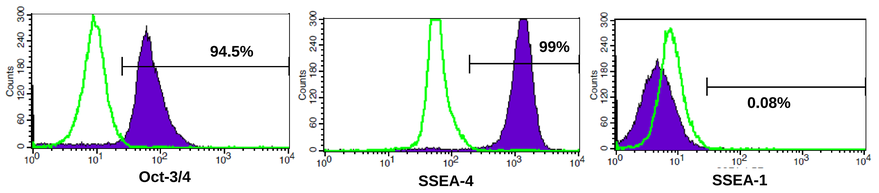
Clump Passaging on Corning Matrigel Matrix (10 passages)

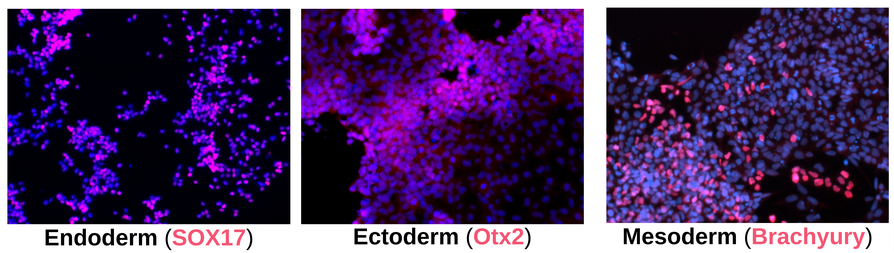


Morphology



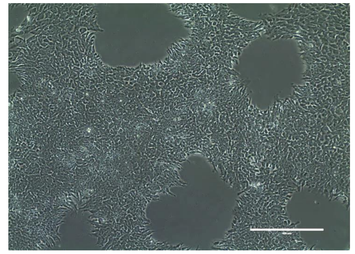
Karyotype



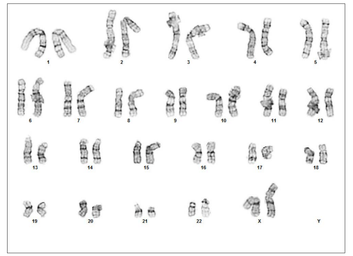


Endoderm (SOX17) | Ectoderm (Otx2) | Mesoderm (Brachyury)

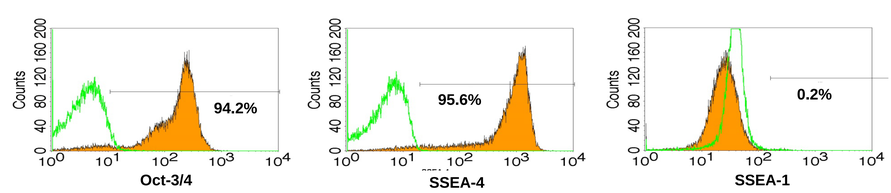
Single Cell Passaging on Corning PureCoat rLaminin-521 (10 passages)

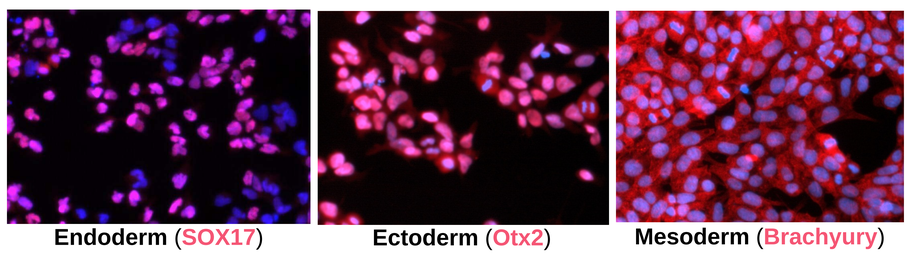


Morphology



Karyotype





Endoderm (SOX17) | Ectoderm (Otx2) | Mesoderm (Brachyury)

Conclusions

The xeno-free medium NutriStem allows usage of multiple surfaces and passaging methodologies for long-term culture and expansion of hPSCs.

- Clump passaging on Matrigel matrix
- Single cell passaging on PureCoat rLaminin-521 cultureware

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