High Quality Episomal Human iPSC derived from Cord Blood with a Zero Footprint: Applications in Serum-Free and Xeno-Free Culture

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ABSTRACT

Nonviral, non-integrated human induced pluripotent stem cell (hiPSC) lines were generated using cord blood-derived CD34+ progenitors with seven episomal expressed factors (OCT4, SOX2, KLF4, MYC, NANOG, LIN28, SV40 T) expressed with the pCEP4 plasmid system. Zero foot print iPSC lines were derived first on feeders and subsequently adapted to feeder free conditions in serum free and xeno-free culture conditions. In all three culture conditions, the iPSC lines showed robust proliferation with normal karyotype and expressed a panel of pluripotency markers OCT 4 SOX2 and NANOG by RT-PCR and OCT4, SSEA4, TRA-1-60 and TRA-1-81 by ICC. Whole genome expression and epigenetic profiling analyses demonstrated that these lines were of extremely high quality and molecularly indistinguishable from human embryonic stem cell lines. In a directed differentiation and teratoma analysis, the lines retained potential to the three germ layers, ectoderm, endoderm and mesodermal lineages. This report presents an optimized culture system for nonintegrated human iPS cells in a feeder –free, serum – free and Xeno-free conditions, that enable the use of iPSC line as a positive control in reprogramming methods for iPSC generation. In addition, viral free vascular, hematopoietic, neural and cardiac lineages were derived with robust efficiencies that support cell therapy applications.

INTRODUCTION

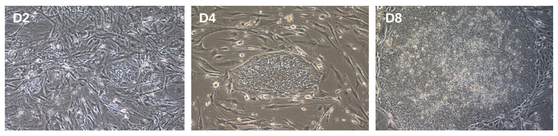
In 2006, Yamanaka and colleagues reported the successful reprogramming of mouse fibroblasts into induced pluripotent stem cells (iPSCs) by transducing them with four genes (OCT4, SOX2, KLF4, c-MYC). Human iPSCs hold great promise for the future of regenerative medicine as they functionally and phenotypically resemble human embryonic stem cells. At an incredibly rapid rate, researchers have been able to reprogram adult human fibroblasts, keratinocytes, neural stem cells, B cells, liver, and stomach epithelial cells into iPSCs that are capable of differentiating into multiple clinically relevant cell types. Initial methods of iPSC generation employing genome-integrating retroviral or lentiviral vectors, displayed caveats such as tumor formation and residual transgene expression that affected their differentiation potential. This field is currently in flux, as iPSCs could provide a system whereby patient-specific pluripotent cells can be readily generated to gain further insights for studying and potentially treating many diseases.

Herein, we describe the generation of nonintegrated, virus-free human iPSC from ex vivo expanded human cord blood $CD34^+$ progenitors with seven episomal expressed factors (OCT4, SOX2, KLF4, c-MYC, NANOG, LIN28, SV40 T).

GIBCO® Episomal hiPSC Line supplied as

- cryopreserved
- ≥1×10^6 cells/mL in iPSC medium with 10% DMSO
- exhibit ≥70% viability
- iPSC line is grown on mouse feeder layer
- characterized for the pluripotency and
- in-vitro and in-vivo differentiation potential

Figure 1. Morphology



Progressive day-wise morphology of the GIBCO® Episomal hiPSC Line on mouse feeder layer

Zero Footprint Gibco® Episomal hiPSC line

Figure 2. Lack of Transgene Expression

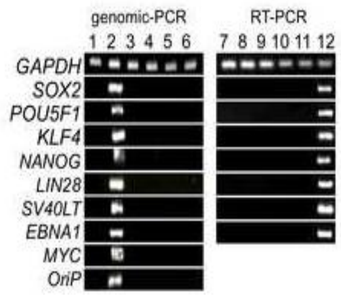
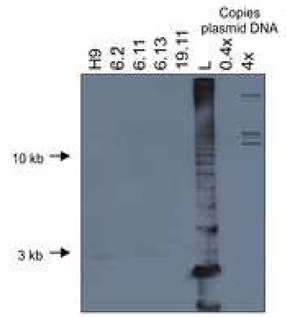


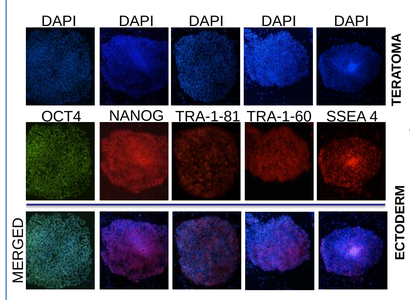
Figure 3. Southern Blot



Lack of genomic episomal vector or transgene integration was demonstrated by PCR and Southern Blot

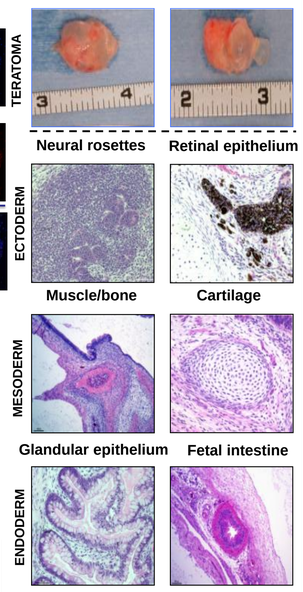
Pluripotent Analyses of Gibco® Episomal hiPSC line

Figure 4. Immunocytochemistry



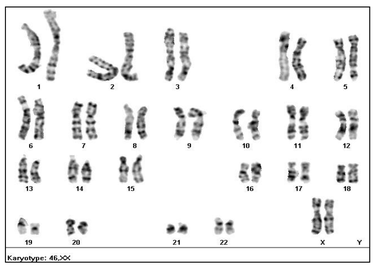
Immunocytochemistry (ICC) analysis for pluripotency markers shows nuclear OCT4 and NANOG proteins, as well as surface Tra-1-81, Tra-1-60 and SSEA4 antigens, with DAPI nuclear counter stains

Figure 5. Teratoma Results



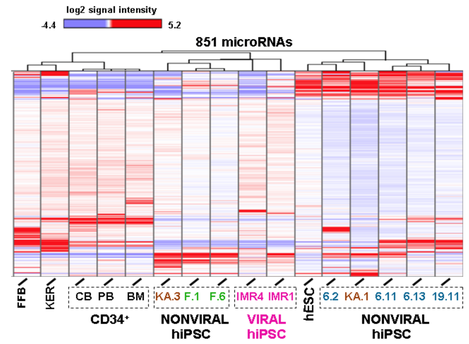
Teratoma Formation results show the gross morphology of cystic teratomas which were formed on injection of CD34 iPSC into immunodeficient mice (upper panel). Analysis of H&E stains of tumor sections showed robust differentiation to a diverse array of well-differentiated ectoderm, mesoderm, endoderm germ layers (lower panels)

Figure 6. Karyotype Analysis



G banding chromosome analysis of GIBCO® Episomal hiPSC Line shows a normal karyotype

Figure 7. Microarray Analysis

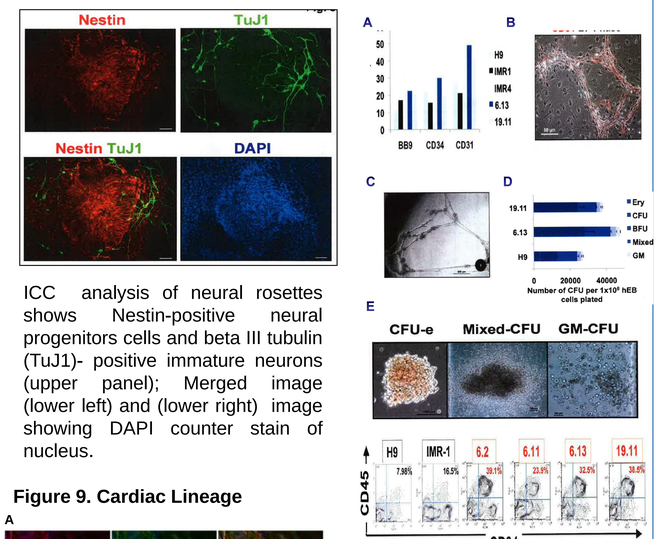


Micro RNAs Expression Profile in Viral and Nonviral iPSCs:

- Viral iPSCs have expression profile close to starter cells.
- Nonviral iPSCs have less MiRNA Expression profile
- Micro RNA expression resembles core hESC like in episomal derived iPSCs.
- High quality human episomal iPSC line

Differentiation Potential of Gibco® Episomal hiPSC line

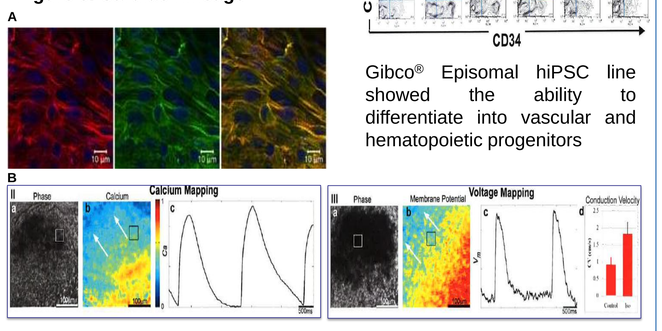
Figure 8. Neural and Blood Lineages



ICC analysis of neural rosettes shows Nestin-positive neural progenitors cells and beta III tubulin (TuJ1)- positive immature neurons (upper panel); Merged image (lower left) and (lower right) image showing DAPI counter stain of nucleus.

Gibco® Episomal hiPSC line showed the ability to differentiate into vascular and hematopoietic progenitors

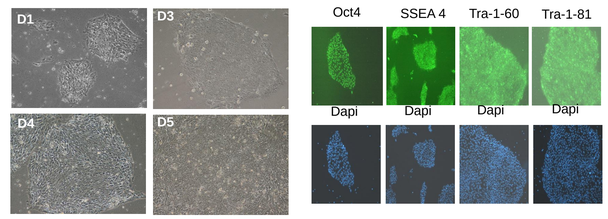
Figure 9. Cardiac Lineage



Differentiation into Cardiac Lineage. A. ICC of cardiac markers in hEB differentiated from the iPSC line B. Calcium Micromapping of the beating hEB (left panel), Voltage micromapping of the beating hEB (right panel)

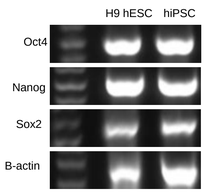
Feeder Free Adaptation of Gibco® Episomal hiPSC line

Figure 10. Morphology and Immunocytochemistry



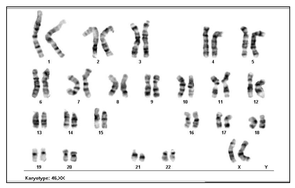
Progressive day-wise morphology (left panel) and ICC for pluripotent markers of the Gibco® Episomal hiPSC Line on Geltrex in StemPro® hESC SFM medium (right panel)

Figure 12. RT-PCR



RT-PCR shows expression of pluripotent markers

Figure 13. Karyotype Analysis



Karyotyping of hiPSC Line cultured in StemPro® hESC SFM medium

CONCLUSIONS

- GIBCO® Episomal hiPSC line is a **footprint- free** iPSC line derived from $CD34^+$ progenitors using episomal vectors to deliver reprogramming genes Oct4, Sox2, Klf4, c-Myc, Nanog, Lin28 and SV40T.
- GIBCO® Episomal hiPSC line shows robust proliferation with a normal karyotype and expresses a panel of **pluripotency markers** as determined by **RT-PCR** (Oct4, Sox2, and Nanog) and by **immunocytochemistry** (Oct4, SSEA4, TRA-1-60, and TRA-1-81) in both feeder-based and feeder-free culture conditions
- GIBCO® Episomal hiPSC line is **molecularly indistinguishable** from hESCs in terms of the whole genome expression and epigenetic profiling analyses. The line shows **directed differentiation** (***vascular, hematopoietic, neural and cardiac lineages***) and **teratoma** analyses, into all the three lineages with robust efficiency

REFERENCES

1. Burridge P.W. et al. (2011) A universal system for highly efficient cardiac differentiation of human induced pluripotent stem cells that eliminates interline variability. PLoS ONE 6(4): e18293.
2. Yu J. et al. (2009) Human Induced Pluripotent Stem Cells Free of Vector and Transgene. Science 324: 797–801.

ACKNOWLEDGEMENTS

We thank Dr. Purushotham Reddy for help with characterization.

TRADEMARKS/LICENSING