

Induced pluripotent stem cells generated from multiple somatic cell types via feeder-free reprogramming in TeSR™-E7™ Medium

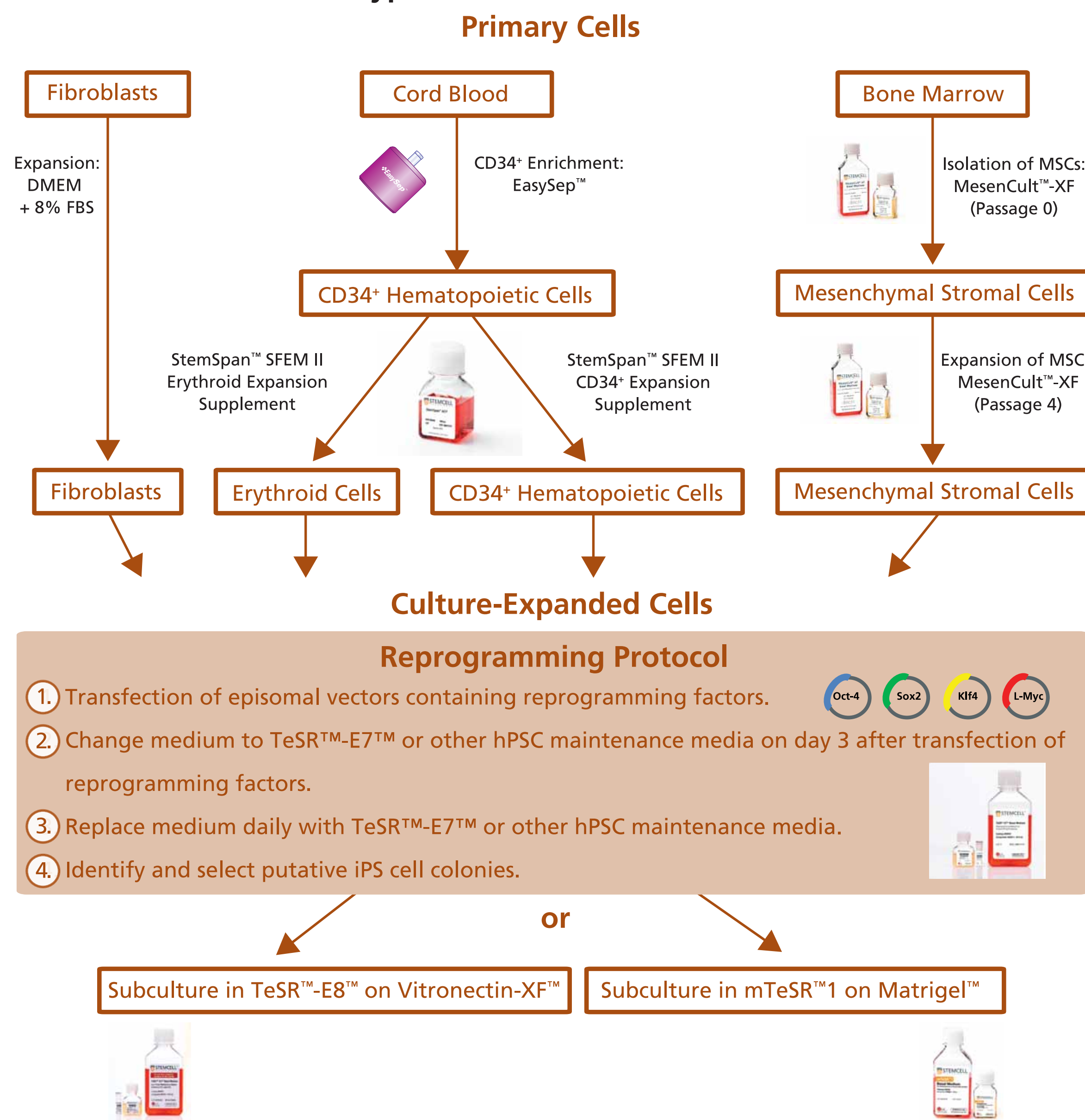
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Introduction

Human induced pluripotent stem (iPS) cells have been derived from a wide range of somatic cell types by the process known as reprogramming. Due to easy accessibility, the two most common tissues used for reprogramming are skin (e.g. dermal fibroblasts) and blood (e.g. mononuclear cell fraction, CD34⁺ cells, or specific blood cell types). In addition to identifying a suitable tissue source, there is a general trend towards reprogramming methods that will generate more clinically relevant iPS cells, such as the use of vectors that do not genetically alter the cell, as well as cell culture systems that are free of xenogeneic material and contain defined components. Towards this goal, we recently released TeSR™-E7™ Medium for reprogramming, which is a low-protein, xeno-free, defined medium for reprogramming without the use of feeder cells. This study demonstrates the efficient generation and detailed characterization of iPS cells derived in TeSR™-E7™ Medium from multiple human fibroblast sources, and further extends the use of TeSR™-E7™ Medium for feeder-free reprogramming of other somatic cell types, including bone marrow-derived mesenchymal stromal cells (MSCs), CD34⁺ hematopoietic cells, and culture-expanded erythroid cells. From these cell sources we demonstrate successful establishment of iPS cell lines which can be further maintained and expanded in human pluripotent stem cell (hPSC) maintenance media such as mTeSR™1 or TeSR™-E8™. iPS cell lines generated by reprogramming of dermal fibroblasts expressed pluripotency markers and differentiated in vitro to cells of the three germ layers. Overall, this study demonstrates the robustness TeSR™-E7™ Medium for reprogramming multiple human somatic cell types, including skin and blood.

Methods

FIGURE 1. Schematic of isolation, expansion, and TeSR™-E7™ reprogramming of different somatic cell types



Prior to reprogramming, cells from skin fibroblasts and hematopoietic tissues were isolated and expanded in their specific expansion medium. Adult normal human dermal fibroblasts (NHDF), neonatal fibroblasts (BJ), and fetal fibroblasts (D551) were cultured in fibroblast medium (DMEM + 8% FBS). MSCs were isolated from bone marrow and expanded in MesenCult™-XF Medium. CD34⁺ hematopoietic cells were isolated from cord blood using EasySep™ Human Cord Blood CD34 Positive Selection Kit and expanded in StemSpan™ SFEM II Medium supplemented with either CD34⁺ Expansion Supplement or Erythroid Expansion Supplement. (1) To initiate reprogramming, each cell type was transfected with episomal vectors containing the reprogramming factors Oct-4, Sox2, Klf4 and L-Myc. (2) Cells were then seeded onto a 6-well plate pre-coated with Matrigel™ and cultured for 3 days in their respective expansion media. (3) After three days, media were replaced with TeSR™-E7™ Medium, which was used for the remainder of the induction phase of reprogramming (day 3 - 28), with daily media changes. (4) Putative iPS cell colonies were typically scored on day 28. iPS cell colonies were manually selected and subcultured either in mTeSR™1 Medium on Matrigel™ or in TeSR™-E8™ Medium on Vitronectin-XF™ for further characterization.

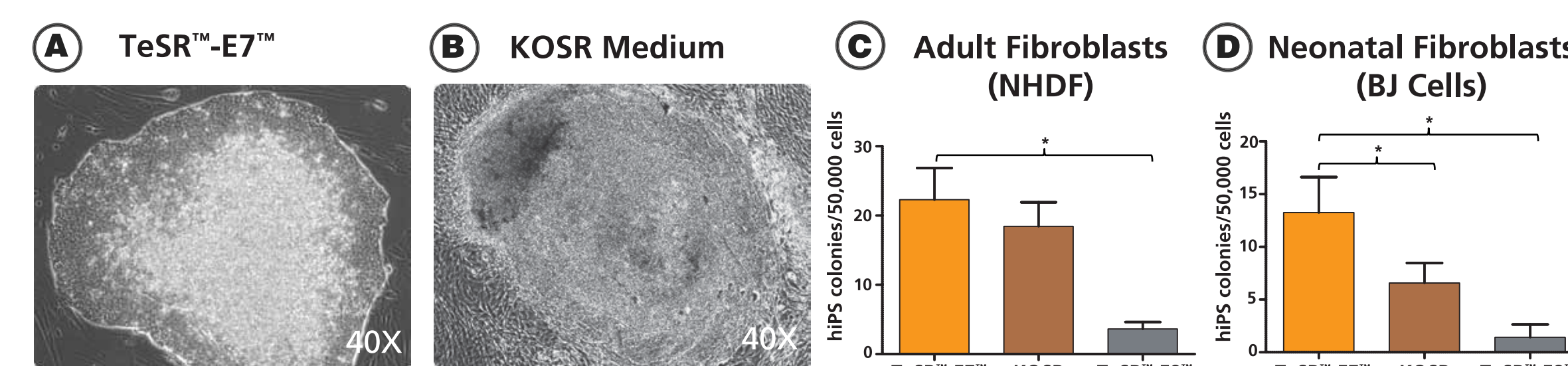
Results

TABLE 1. Fibroblast reprogramming efficiencies in TeSR™-E7™ Medium

Fibroblast Cell Type	# of Experiments	# of Input Cells	Avg. # of iPS Cell Colonies	*Reprogramming Efficiency (%)
Fetal Fibroblasts (D551)	1	5 x 10 ⁴	7	0.015
Neonatal Fibroblasts (BJ)	6	5 x 10 ⁴	13	0.026 ± 0.007
Adult Normal Human Dermal Fibroblasts (NHDF)	6	5 x 10 ⁴	38	0.076 ± 0.004

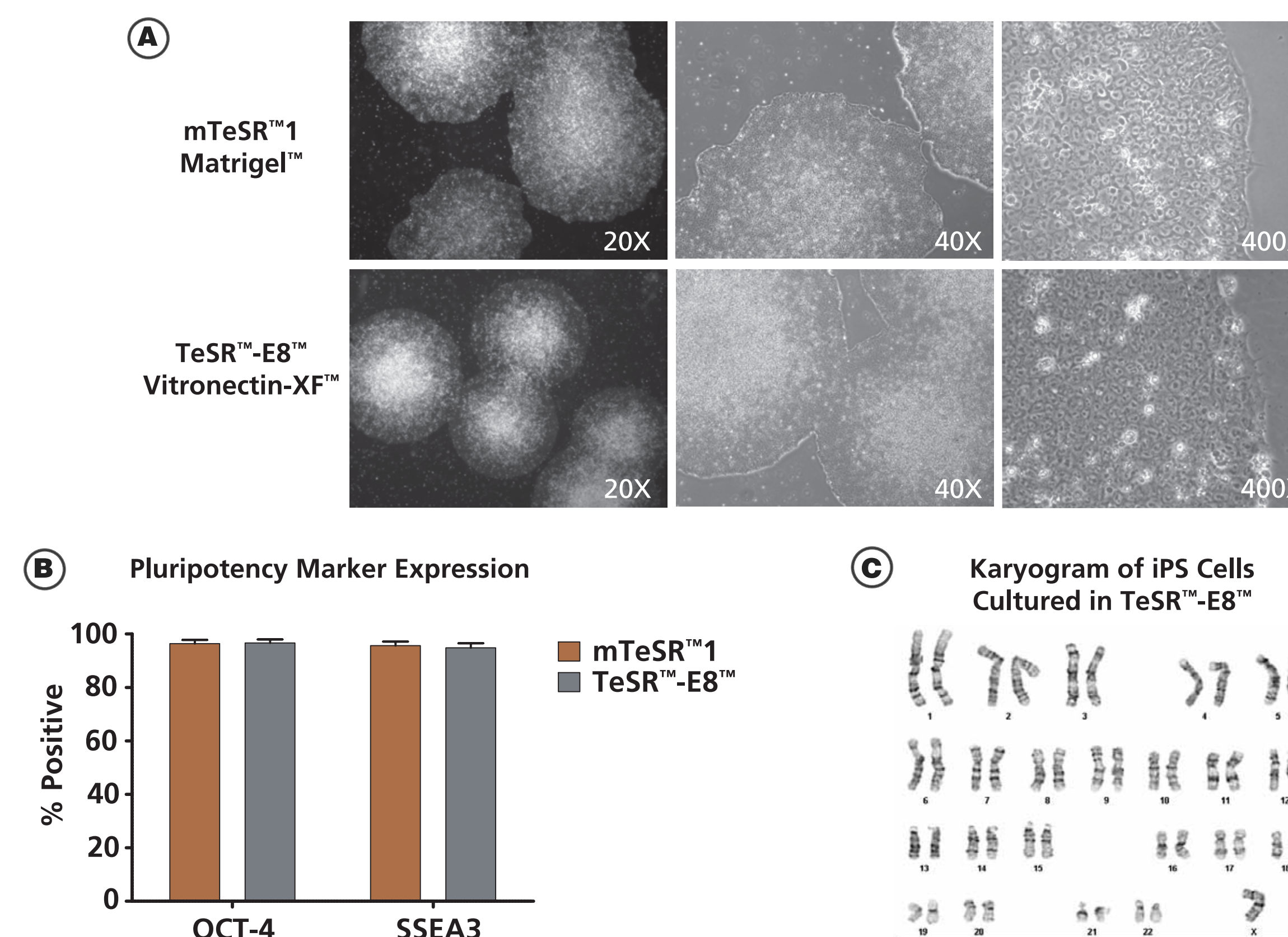
*mean ± SEM

FIGURE 2. TeSR™-E7™ yields higher quality iPS cell colonies and improved reprogramming efficiencies compared to standard pluripotent maintenance media



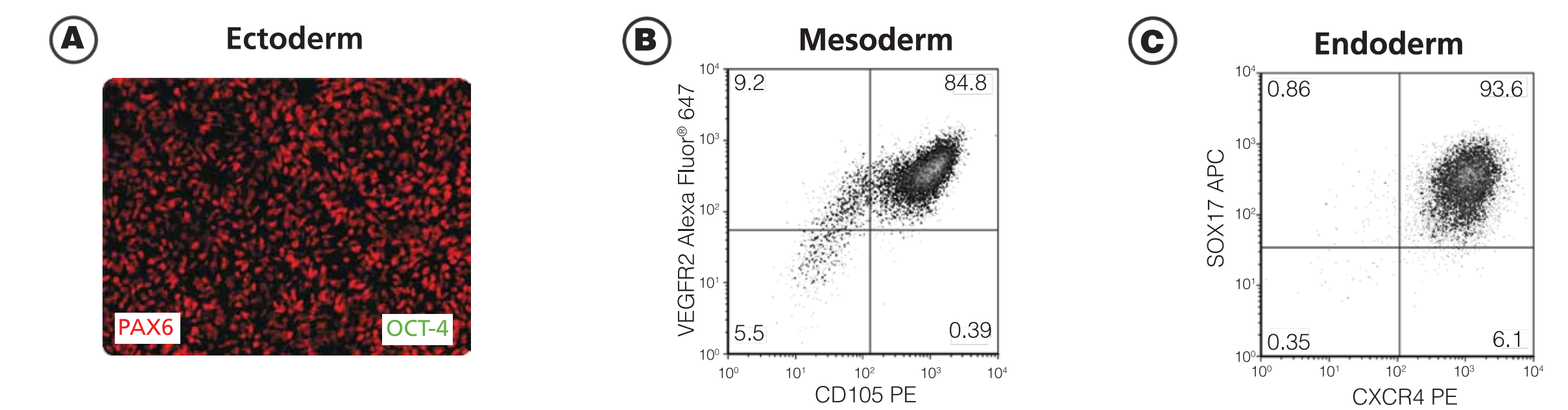
(A - B) Reprogramming of human fibroblasts in TeSR™-E7™ (A) typically yields a greater proportion of high quality iPS cell colonies with defined borders and results in less overgrowth of background fibroblasts compared to KOSR medium (B; DMEM/F12, 20% KnockOut Serum Replacement, bFGF). (C) Reprogramming efficiency for adult fibroblasts in TeSR™-E7™ Medium was comparable to that in KOSR medium, and 6.1-fold higher than in TeSR™-E8™ Medium. (D) For neonatal fibroblasts, reprogramming efficiency in TeSR™-E7™ Medium was 2-fold and 9.4-fold higher than in KOSR medium and TeSR™-E8™ Medium, respectively. Data represents average ± SEM of 6 independent experiments *p<0.05.

FIGURE 3. iPS cells derived in TeSR™-E7™ Medium are readily subcultured in mTeSR™1 or TeSR™-E8™ Media, express pluripotent markers, and exhibit normal karyotype



(A) iPS cell lines derived from dermal fibroblasts in TeSR™-E7™ and subcultured in mTeSR™1 on Matrigel™ or TeSR™-E8™ on Vitronectin-XF™ displayed typical iPS-like morphology with defined borders and high nuclear to cytoplasmic ratio. (B) iPS cell lines derived in TeSR™-E7™ and cultured in either mTeSR™1 or TeSR™-E8™ showed high expression of pluripotency markers SSEA-3 and Oct-4 at passage 2. (C) Karyotype analyses of TeSR™-E7™-derived iPS cells at passage 6 showed no chromosomal anomalies.

FIGURE 4. iPS cells derived in TeSR™-E7™ Medium demonstrated the capacity to differentiate in vitro to cells of ectoderm, mesoderm and endoderm lineages

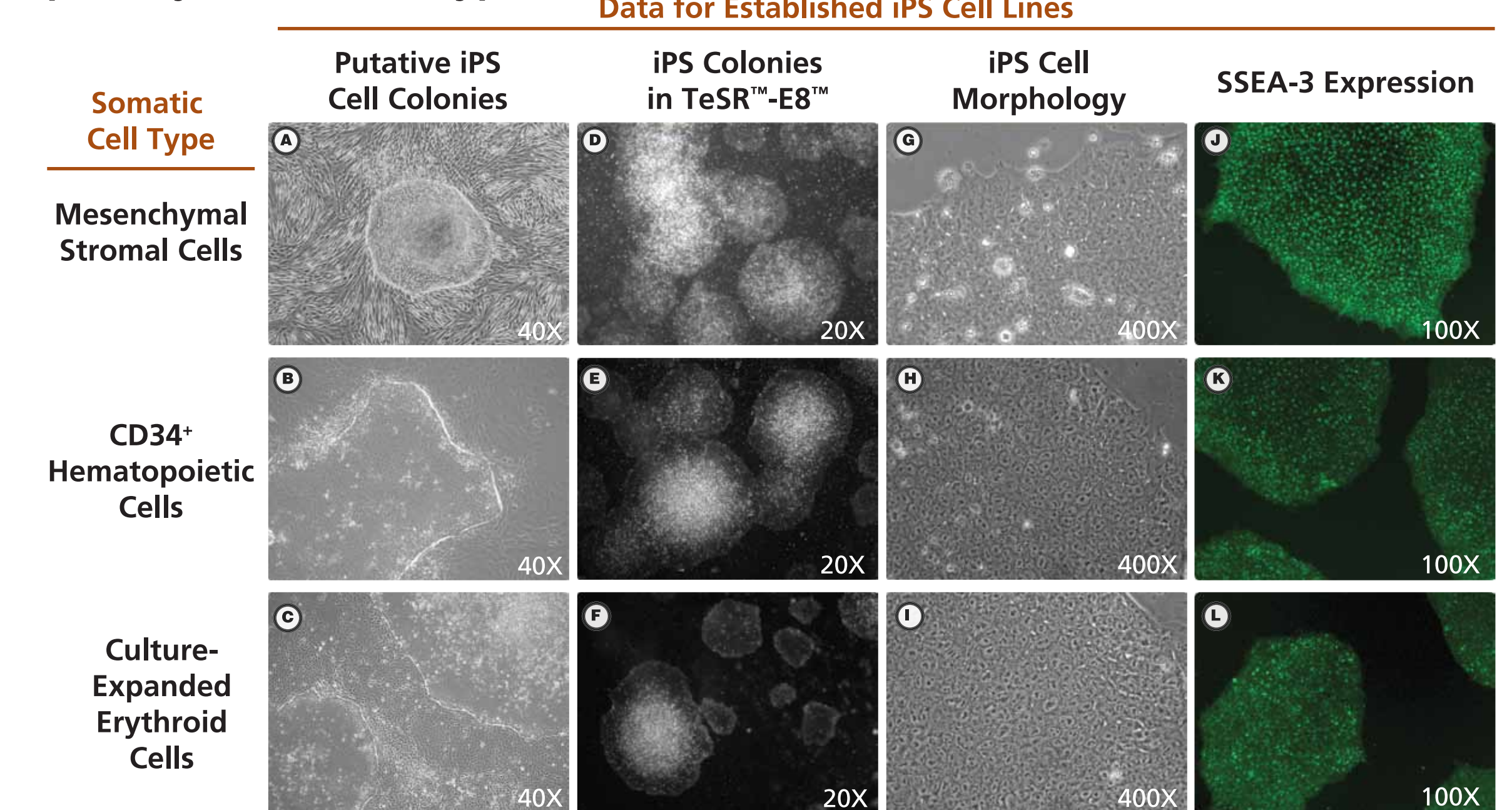


To demonstrate pluripotency, iPS cell lines derived from fibroblasts in TeSR™-E7™ were differentiated to cells of the three germ layers using the separate STEMdiff™ systems. (A) iPS cells differentiated with STEMdiff™ Neural Induction Medium generated greater than 90% neural progenitor cells expressing PAX6. (B) Mesoderm potential was demonstrated by differentiation of iPS cells to endothelial cells using STEMdiff™ AP-EL™ Medium supplemented with cytokines. This generated a population of cells with greater than 80% CD105 and VEGFR2 expression. (C) iPS cells differentiated using STEMdiff™ Definitive Endoderm kit exhibited greater than 90% SOX17 and CXCR4-positive cells.

TABLE 2. Reprogramming efficiencies with other somatic cell types in TeSR™-E7™ Medium

Somatic Cell Type	# of Experiments	# of Input Cells	Avg. # of iPS Cell Colonies	Reprogramming Efficiency (%)
Mesenchymal Stromal Cells	1	5 x 10 ⁵	5	0.001
CD34 ⁺ Hematopoietic Cells	3	5 x 10 ⁵	115	0.023
Culture-expanded Erythroid Cells	1	5 x 10 ⁵	35	0.007

FIGURE 5. TeSR™-E7™ Medium supports the reprogramming of multiple primary somatic cell types



Mesenchymal stromal cells (top row), CD34⁺ hematopoietic cells (middle row) and culture-expanded erythroid cells (bottom row) were reprogrammed in TeSR™-E7™ Medium with episomal reprogramming vectors as described in Figure 1. (A - C) Three to four weeks post-transfection, putative iPS cell colonies began to emerge and were identified microscopically. (D - L) iPS cells generated from all three somatic cell sources subcultured in either TeSR™-E8™ or mTeSR™1 (data not shown), exhibited iPS-like colony morphology (D - F), high nuclear to cytoplasmic ratio (G - I), and expressed the pluripotency marker SSEA-3 detected by immunocytochemistry (J - L).

Conclusions

- Improved fibroblast reprogramming efficiency and iPS cell colony morphology was achieved with TeSR™-E7™ Medium compared to common hPSC maintenance media.
- iPS cells can be generated from multiple somatic cell types in TeSR™-E7™ Medium, including mesenchymal stromal cells, CD34⁺ hematopoietic cells, and culture-expanded erythroid cells.
- TeSR™-E7™ derived iPS cell colonies can be easily subcultured in mTeSR™1 or TeSR™-E8™ Media.
- iPS cells derived in TeSR™-E7™ Medium expressed pluripotency markers and had the capacity to differentiate to cells of the three germ layers.