

Defined, feeder-free human induced pluripotent stem cell (hiPSC) generation, selection and expansion from multiple somatic cell types without manual colony picking or scraping

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Introduction

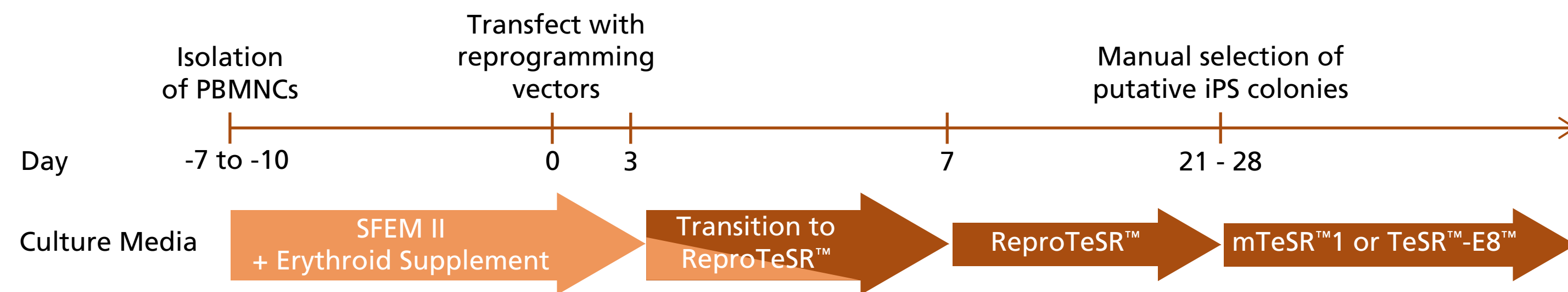
Widespread adoption of human induced pluripotent stem cell (hiPSC) technology has resulted in initiatives to generate large banks of hiPSC lines. The ability to reproducibly generate hiPSCs in a high-throughput manner is hindered by challenges associated with colony identification and requirements for manual isolation of emerging colonies. Here, we show improved identification of emerging hiPSC colonies in feeder-free conditions using defined TeSR™-E7™ Medium for fibroblasts or ReproTeSR™ Medium for peripheral blood (PB)-derived cells. Fibroblasts and blood-derived cells are the two most popular cell types for reprogramming. Further, we use a new reagent, ReLeSR™, for enzyme-free selective detachment of hiPSC aggregates without scraping, removal of differentiated cells or complicated manipulation. Together, these media and dissociation reagents will facilitate automation of large scale reprogramming and banking efforts.

Methods

A) Fibroblast Reprogramming



B) Erythroid Cell Reprogramming



C) CD34+ Cell Reprogramming

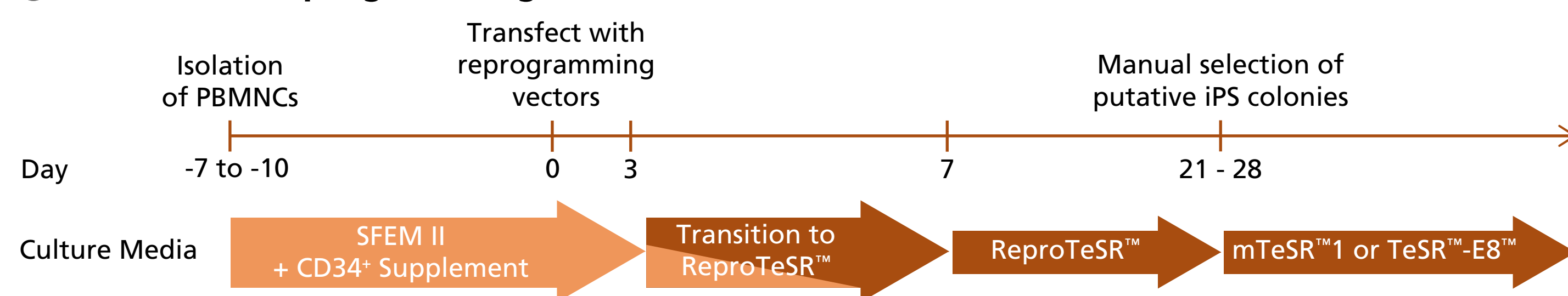


Figure 1. Schematic of reprogramming systems with TeSR™-E7™ and ReproTeSR™ **A)** Neonatal or adult fibroblasts, expanded in fibroblast medium (DMEM + 8% FBS), were transfected with episomal vectors containing OCT4, SOX2, KLF4, L-MYC and Lin28. Post-transfection (day 0), cells were seeded on Matrigel®-coated plates at 5,000 cells/cm² (for later manual isolation), or 135 - 270 cells/cm² (density for clonal reprogramming and later ReLeSR™ isolation). On day 3 post-transfection, media was changed to TeSR™-E7™ Medium and changed daily until day 21 - 28, when iPSC colonies were identified by microscopic examination of morphology and counted. Selected colonies were transferred manually to Matrigel®-coated plates and cultured in mTeSR™1 or TeSR™-E8™ Media for further propagation of hiPSC lines. Alternatively, hiPSC colonies generated from fibroblasts seeded at a density for clonal reprogramming resulted in single colonies per well were isolated without manual selection using ReLeSR™ and expanded in TeSR™-E8™ Medium. **B, C)** Mononuclear cells (MNCs) were isolated from PB by fractionation over a Ficoll® density gradient in SepMate™-50 tubes. Erythroid precursor cells were expanded from PB MNCs for 7 - 10 days in StemSpan™ SFEM II Medium with Erythroid Expansion Supplement. Alternatively, CD34+ cells were enriched from PB MNCs by immunomagnetic separation using EasySep™ CD34+ Positive Selection Kit, and expanded for 7 days in StemSpan™ SFEM II Medium with CD34+ Expansion Supplement. Culture expanded erythroid precursor or CD34+ cells were transfected with episomal vectors and immediately transferred to Matrigel®-coated plates at 30,000 cells/cm². Cells were transitioned from StemSpan™ media to ReproTeSR™ Medium by addition of 1 mL of ReproTeSR™ on days 3 and 5. From day 7 onwards, ReproTeSR™ reprogramming medium was replaced daily, until day 21 - 28. As above, iPSC colonies were identified by microscopic examination and counted. Selected colonies were transferred manually to Matrigel®-coated plates and cultured in mTeSR™1 or TeSR™-E8™ Media for further propagation of hiPSC lines.

Results

TABLE 1: Enrichment and expansion of CD34+ cells from human peripheral blood

	% CD34+ Cells After Enrichment	Total Nucleated Cell Fold Expansion	CD34+ Cell Fold Expansion	% CD34+ Cells After Expansion	Number of CD34+ Cells After Expansion per Initial 100 mL PB
Mean (n = 6)	56	18	19	60	1.3 x 10 ⁶
95% CL	39 - 74	12 - 25	15 - 22	47 - 63	0.1 - 2.5 x 10 ⁶

TABLE 2: Expansion of erythroid precursor cells from human peripheral blood

	% CD71+/GlyA+ Cells in PB MNCs	Total Number of Cells After Expansion	% CD71+/GlyA+ Cells After Expansion	Number of CD71+/GlyA+ Cells After Expansion per Initial 10 mL PB
Mean (n = 7)	0.7%	3.3 x 10 ⁶	82	2.0 x 10 ⁶
95% CL	0.23 - 1.17	2.0 - 4.6 x 10 ⁶	72 - 92	1.5 - 4.0 x 10 ⁶

TABLE 3: Reprogramming efficiencies of fibroblasts in TeSR™-E7™ and CD34+ and erythroid precursor cells in ReproTeSR™

Somatic Cell Type	# of Experiments	Reprogramming Efficiency (%) (mean ± SEM)	Avg. # iPSC Cell Colonies
TeSR™-E7™ (per 50,000 input cells)			
Neonatal Fibroblasts (BJ line)	6	0.026 ± 0.007	13
Adult Normal Human Dermal Fibroblasts	6	0.076 ± 0.004	38
ReproTeSR™ (per 10 mL blood)			
PB-derived CD34+ Cells	3	0.012 ± 0.002	38
PB-derived Erythroid Cells	2	0.014 ± 0.003	520

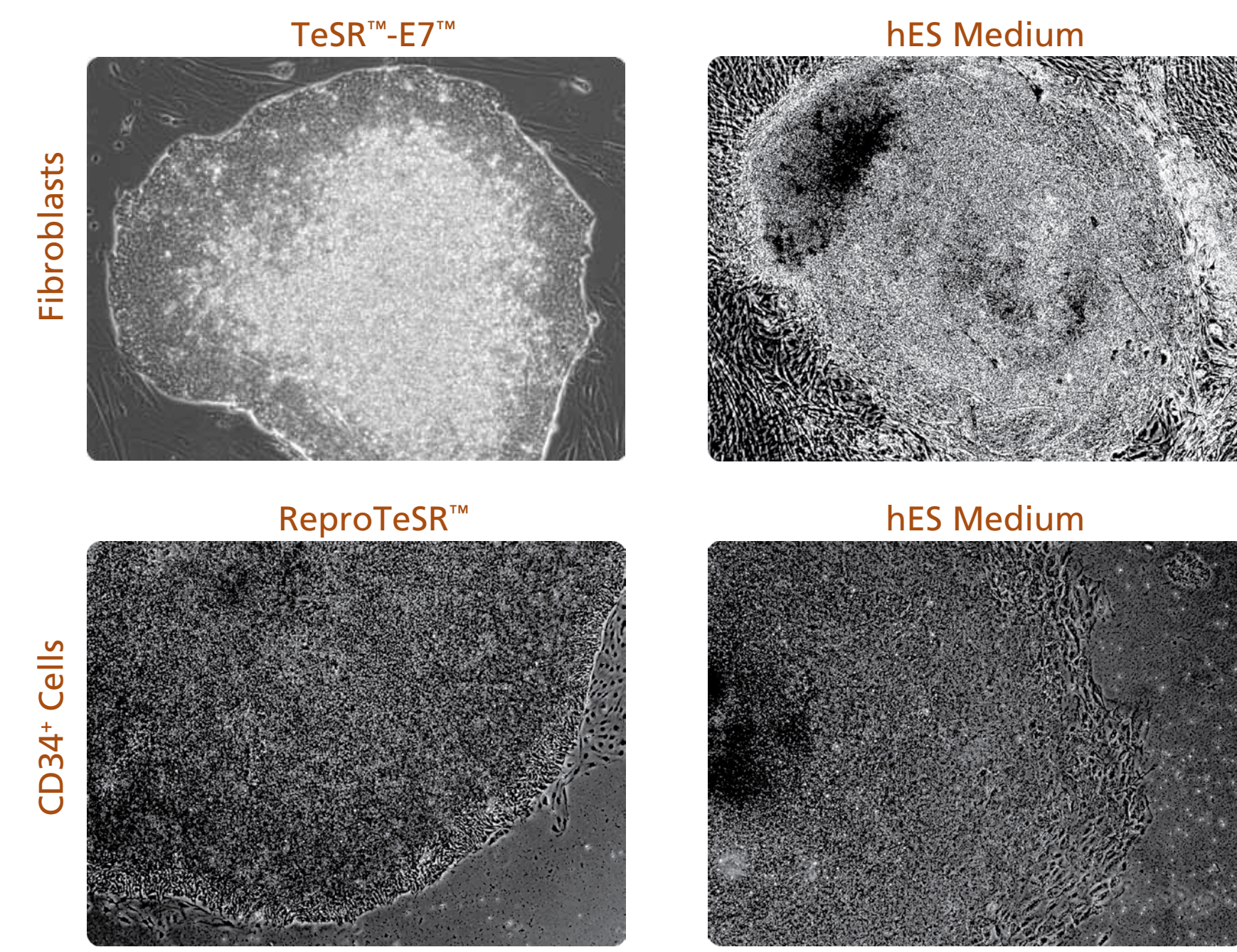


Figure 2. Superior iPSC colony morphology derived in TeSR™-E7™ or ReproTeSR™ under feeder-free conditions. iPSC colonies were derived from fibroblasts or PB-derived CD34+ cells in defined reprogramming media TeSR™-E7™ or ReproTeSR™, respectively, and compared microscopically to iPSC colonies derived in hES Medium (DMEM/F12, 20% KSR, 10 ng/mL bFGF, L-glutamine, NEAA, 2-mercaptoethanol). Colonies derived in TeSR™-E7™ or ReproTeSR™ defined media exhibited more defined borders, compact morphology, and reduced differentiation compared to those in hES Medium.

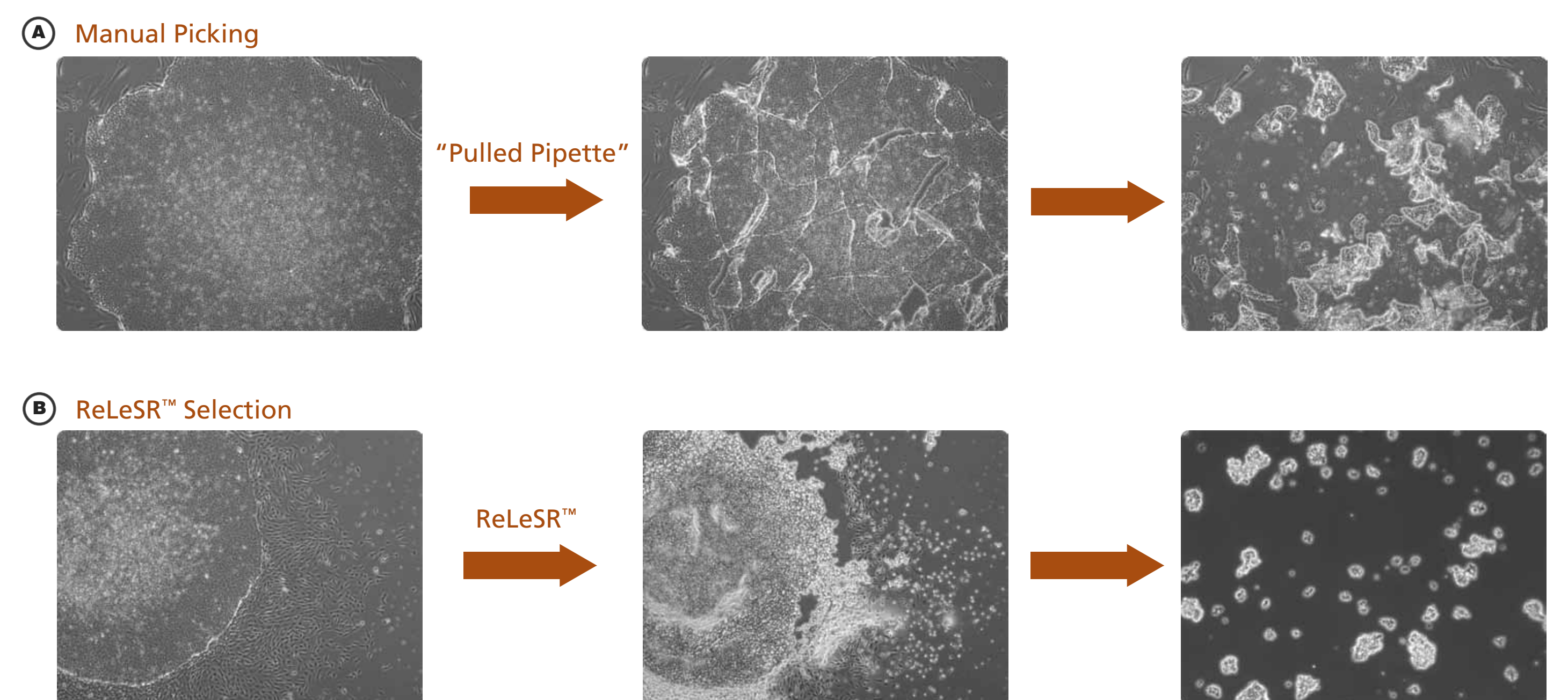


Figure 3. iPSC colonies can be selected using manual picking or ReLeSR™ selection. **A)** Using a pulled Pasteur pipette, selected undifferentiated areas of primary fibroblast-derived iPSC colonies were manually divided into sections of 50 - 200 µm size, and then transferred to a fresh plate for sub-cloning. **B)** Using ReLeSR™, primary iPSC colonies derived from fibroblasts seeded at a density for clonal reprogramming (135 or 270 cells/cm²) were selectively detached, leaving non-reprogrammed cells attached. Aggregates of the ideal 50 - 200 µm size for sub-cloning were generated by tapping or shaking the plate after ReLeSR™ treatment.

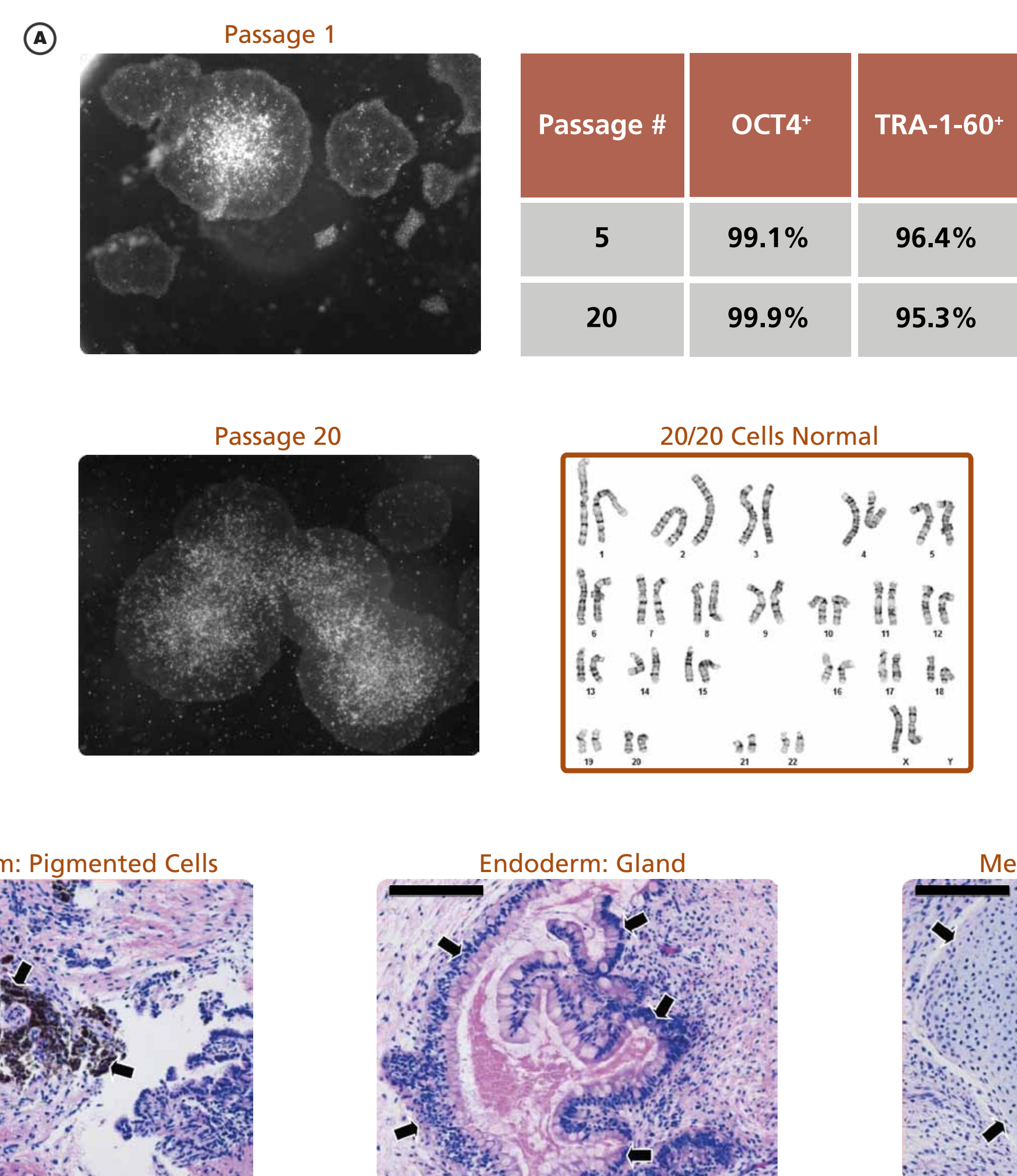


Figure 4. Derivation and characterization of iPSC lines generated without manual selection using ReLeSR™. An iPSC colony generated at a density for clonal reprogramming was selected using ReLeSR™ and expanded in TeSR™-E8™ Maintenance Medium. **A)** Colonies exhibited ideal morphology from passage 1 through passage 20; maintained high expression of markers of the undifferentiated state (OCT4 and TRA-1-60) and maintained a normal karyotype (20/20 normal cells at passage 20). **B)** Histological assessment of day 78 teratomas formed from passage 12 cells in the kidney or testis of SCID mice demonstrated tissues of all three germ layers. Representative tissues included: endodermal gland; mesodermal cartilage, muscle (not shown), and bone (not shown); and ectodermal pigmented cells and primitive neural cells (not shown). Scale bars = 100 µm.

Conclusions

- Peripheral blood-derived CD34+ or erythroid cells can be efficiently expanded using StemSpan™ SFEM II Medium with lineage-specific expansion supplements.
- Reprogramming in feeder-free conditions with TeSR™-E7™ (fibroblasts) and ReproTeSR™ results in well-defined iPSC colonies which can be easily identified for selection.
- ReLeSR™ can be used to select iPSC colonies for further expansion without the need for manual picking.
- iPSCs derived in TeSR™-E7™ or ReproTeSR™ and selected with ReLeSR™ are easily subcultured in mTeSR™1 or TeSR™-E8™ Media to maintain a pluripotent state.