A FEEDER-INDEPENDENT CULTURE SYSTEM TO CONVERT AND MAINTAIN HUMAN PLURIPOTENT STEM CELLS IN A NAÏVE-LIKE STATE

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Introduction

The cells that make up the developing embryo transition through a number of pluripotent states as they become increasingly lineage-restricted. Naïve human pluripotent stem cells (hPSCs) are representative of the inner cell mass of the pre-implantation embryo, whereas primed hPSCs are representative of the inner cell mass of the early post-implantation embryo and are classically dependent on basic fibroblast growth factor (bFGF). The ability to capture these states in vitro requires specialized culture media and protocols.

A simplified and reproducible culture protocol for naïve-like hPSCs in vitro would provide a platform to study the regulatory pathways of naïve and primed pluripotency. To date, conditions that maintain hPSCs in naïve-like states have depended on the use of feeder cells for long-term expansion and results vary depending on the quality of the feeder cells. RSeT™ Feeder-Free (RSeT™-FF) Medium supports the reversion of primed hPSCs to a naïve-like state and supports their long-term feeder-independent maintenance in a bFGF-free system.

Methods

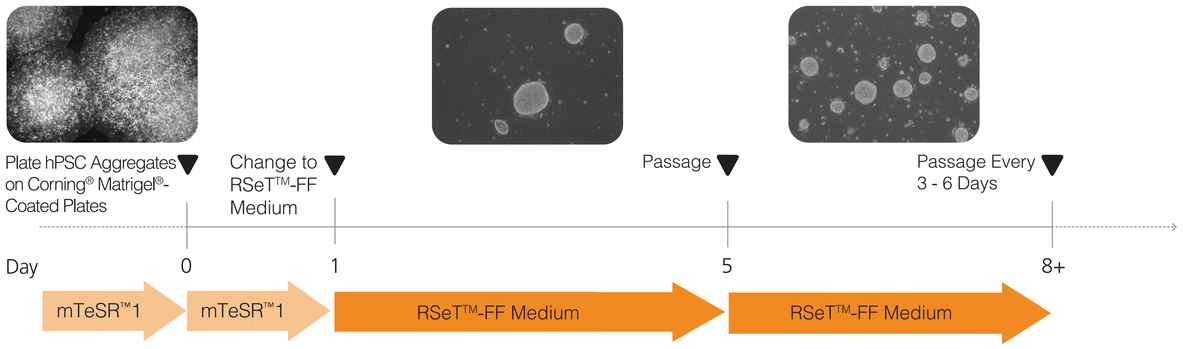

FIGURE 1. RSeT™ Feeder-Free Workflow
Human embryonic stem (ES) and induced pluripotent stem (iPS) cells are reverted to a naïve-like state within one passage in RSeT™-FF Medium. On day 0, seed 500 - 1000 hPSC aggregates per well of a Corning® Matrigel®-coated tissue culture (TC)-treated 12-well plate in mTeSR™1 or TeSR™-E8™. Change medium to RSeT™-FF Medium 24 - 36 hours after seeding. Culture for 4 days in RSeT™-FF Medium and perform full medium changes every other day. Passage cells by incubating cultures with TrypLE™ at 37°C for 8 minutes. Count and plate 20,000 cells/cm^2 into a non-coated TC-treated plate prepared with RSeT™-FF Medium + 5 µM Y-27632. Perform a full medium change 24 hours after passaging and every other day between passages. Passage cells grown in RSeT™-FF Medium every 3 - 6 days.

Results

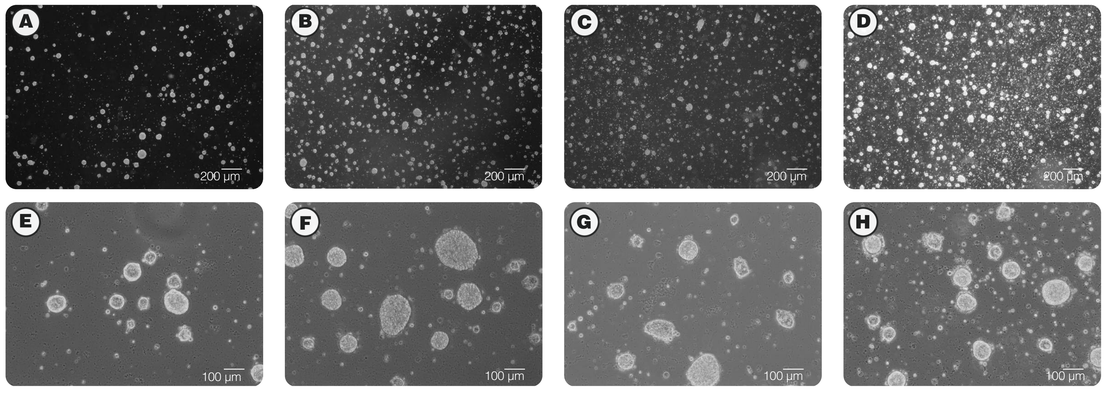

FIGURE 2. hPSCs Cultured in RSeT™ Feeder-Free Medium have a Highly Domed Morphology
Consistent domed morphology of hPSCs cultured in RSeT™-FF Medium in multiple cell lines including: **(A, E)** H7 hES cells, p10; **(B, F)** H9 hES cells, p5; **(C, G)** WLS-1C hiPS cells, p5; and **(D, H)** STiPS-F016 hiPS cells, p10. These cultures also display very low levels of spontaneous differentiation. Additionally, Corning® Matrigel® supplemented in RSeT™-FF Medium eliminates the need to pre-coat plates.

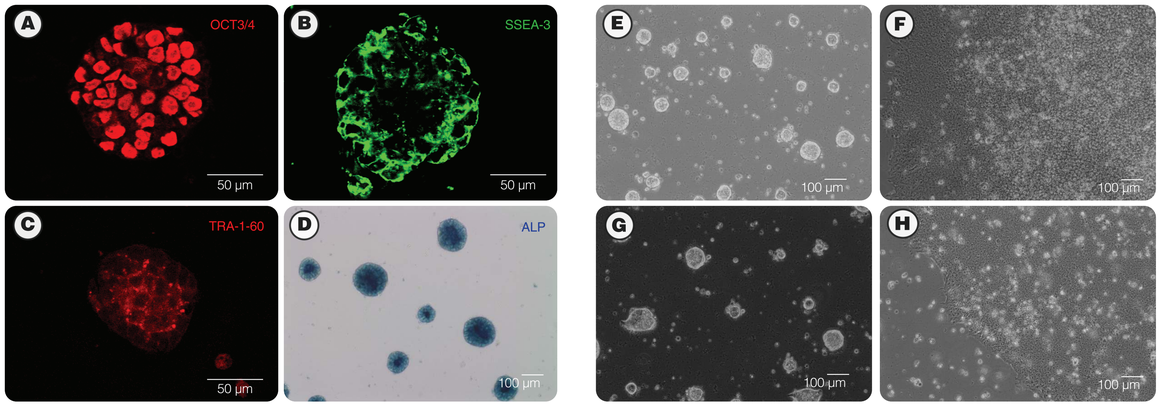

FIGURE 3. hPSCs Cultured in RSeT™-FF Medium Express hPSC Markers and Can Efficiently Transition to a Primed State
Naïve-like hPSCs cultured in RSeT™-FF Medium express **(A)** OCT3/4 (WLS-1C hiPS cells, p7), **(B)** SSEA-3 (WLS-1C hiPS cells, p7) **(C)** TRA-160 (WLS-1C hiPS cells, p7) and **(D)** stain positive for alkaline phosphatase (ALP) (STiPS-F016 hiPS cell line, p2). Additionally, RSeT™-FF Medium cultures (**E:** H9 hES cells, **G:** WLS-1C hiPS cells) can easily transition to a primed state by passaging into either mTeSR™1 (**F:** H9 hES cells, p1 in mTeSR™1) or TeSR™-E8™ (**H:** WLS-1C hiPS cells, p2 in TeSR-E8™).

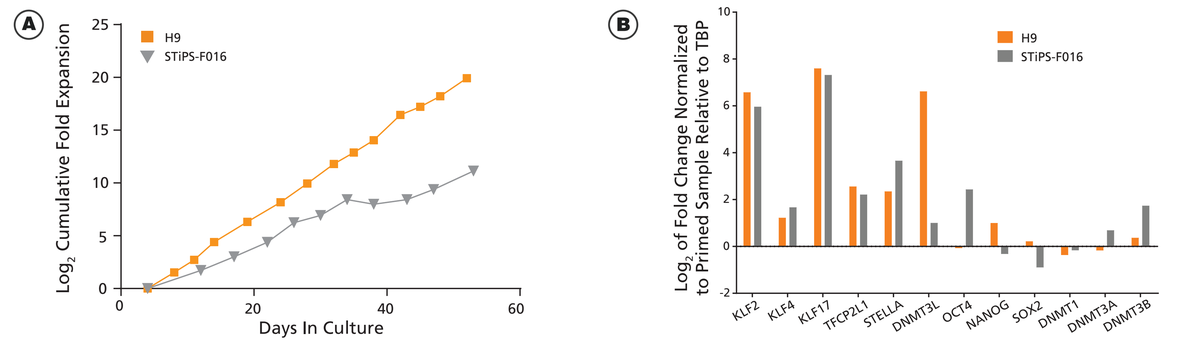

FIGURE 4. hPSCs Cultured in RSeT™-FF Medium Express Genes Associated with the Naïve-Like State
hPSCs maintained in RSeT™-FF Medium **(A)** display continued expansion and **(B)** express higher levels of KLF2, KLF4, KLF17, TFCP2L1, STELLA, and DNMT3L compared to primed hPSC controls. Genes associated with pluripotency (OCT4, NANOG, and SOX2) are expressed by naïve-like hPSCs at similar levels compared to primed hPSC controls.

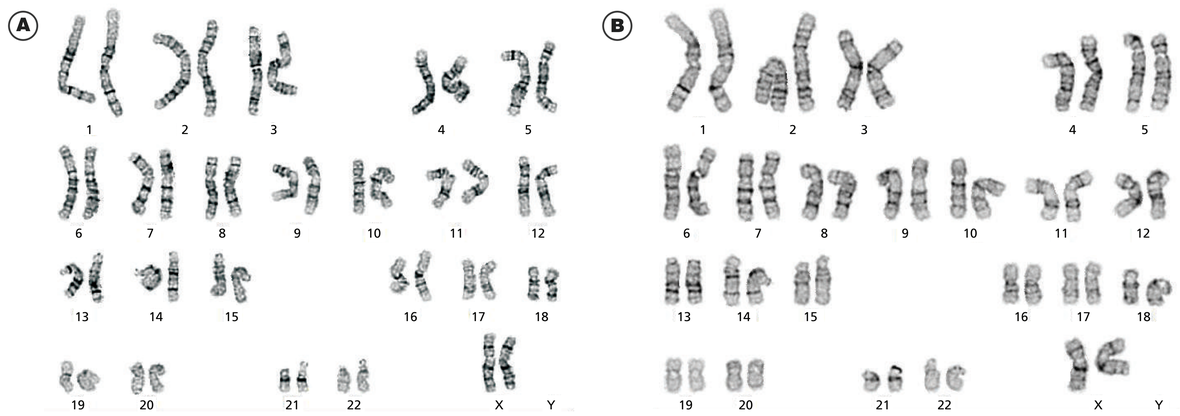

FIGURE 5. hPSCs Maintained in RSeT™-FF Medium Retain a Normal Karyotype After 10 Passages
Representative karyogram results for a **(A)** H9 hES cells and **(B)** STiPS-F016 hiPS cells cultured in RSeT™-FF Medium for 10 passages.

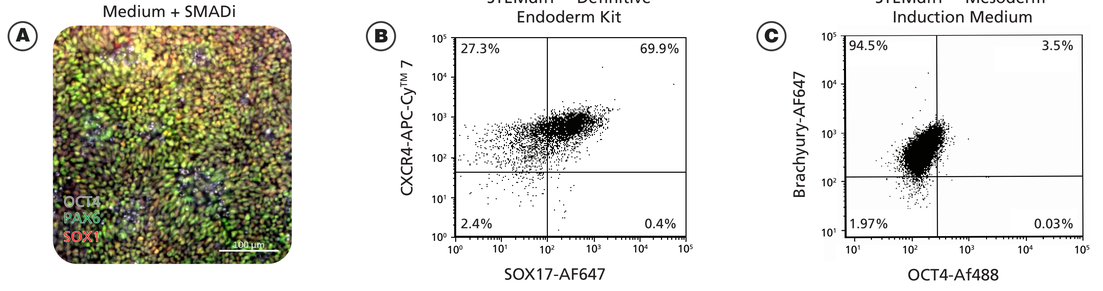

FIGURE 6. hPSCs Maintained in RSeT™-FF Medium are Capable of Direct Trilineage Differentiation Without Re-Priming
(A) Representative image of WLS-1C hiPS cells previously maintained in RSeT™-FF Medium for 11 passages and subjected to neural induction using STEMdiff™ Neural Induction Medium + SMADi monolayer culture. Cultures were processed for immunocytochemistry on day 6 where the majority of cells were positive for neural progenitor markers PAX6 (green) and SOX1 (red), and negative for OCT4 (grey). **(B)** Representative flow cytometry plot for H9 human ES cells previously maintained in RSeT™-FF Medium and subjected to differentiation using the STEMdiff™ Definitive Endoderm Kit. Cultures were processed for flow cytometry on day 5 where 69.9% of cells were SOX17$^+$/CXCR4$^+$, indicating differentiation to endodermal lineage. **(C)** Representative flow cytometry plot for STiPS-F019 hiPS cells previously maintained in RSeT™-FF Medium for 23 passages and subjected to differentiation using STEMdiff™ Mesoderm Induction Medium. Cultures were processed for flow cytometry on day 5 where 94.5% of cells were T$^+$/OCT4$^-$, indicating efficient differentiation to mesoderm lineage.

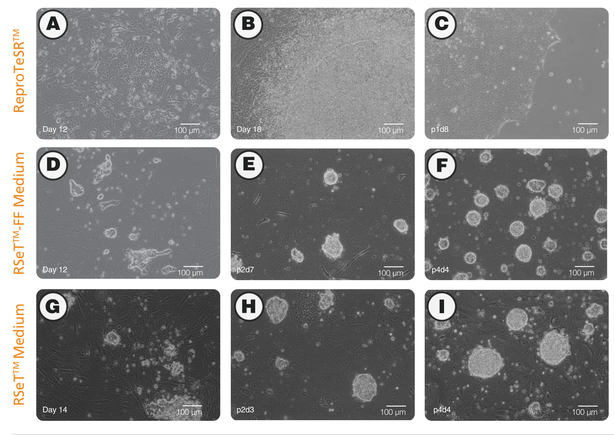

FIGURE 7. Normal Human Dermal Fibroblasts (NHDFs) can be Efficiently Reprogrammed Directly to the Naïve-Like State in Both RSeT™-FF Medium and RSeT™ Medium
Colonies that arise from NHDFs reprogrammed using ReproRNA™-OKSGM + B18R and either ReproTeSR™ **(A, B)**, RSeT™-FF Medium **(D)**, or RSeT™ Medium **(G)**. Cultures were passaged on day 12 (RSeT™-FF Medium) or day 14 (RSeT™ Medium) and could be propagated long-term with domed morphology and low levels of differentiation (**E,F** and **H, I**; respectively). On day 18, the ReproTeSR™ control **(B)** contained colonies large enough to be isolated and propagated in mTeSR™1 **(C)**.

Summary

- RSeT™-FF Medium efficiently reverts primed hPSCs to a naïve-like state with stable domed morphology, naïve gene expression profiles, and low levels of spontaneous differentiation as early as passage 1
- RSeT™-FF Medium maintains hPSCs in a naïve-like state without bFGF or feeder cells in long-term cultures (>p 25)
- Somatic fibroblasts can be reprogrammed directly to the naïve-like state in RSeT™ Medium and RSeT™-FF Medium