Generating Induced Pluripotent Stem Cells from Somatic Cells using a Novel Synthetic Self-Replicating RNA Vector in a Feeder-Free System

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Introduction

Integration of reprogramming factors into the genome of the somatic cells used during induced pluripotent stem cell (iPSC) generation is undesirable because it can potentially affect downstream differentiation capacity or lead to oncogenic transformation. The application of synthetic mRNA encoding reprogramming factors circumvents the risks of integration but typically requires repeated transfections of the reprogramming vector. We have recently developed ReproRNA™-OKSGM, a self-replicating RNA reprogramming vector which expresses reprogramming factors Oct4, Sox2, Klf4, c-Myc, Glis1, and a puromycin selection cassette, for reprogramming of fibroblasts with just a single transfection. When combined with our new transfection reagent and ReproTeSR™ Medium, the complete workflow for generating iPSCs can be achieved with high efficiency and in a feeder-free manner. iPS colonies generated using our optimized workflow are compatible with the TeSR™ family of maintenance medium such as mTeSR™1. In summary, our results described below demonstrate the ease of reprogramming fibroblasts to iPSCs with a single transfection of ReproRNA™-OKSGM non-integrating, self-replicating RNA vector under feeder-free and defined conditions.

Methods

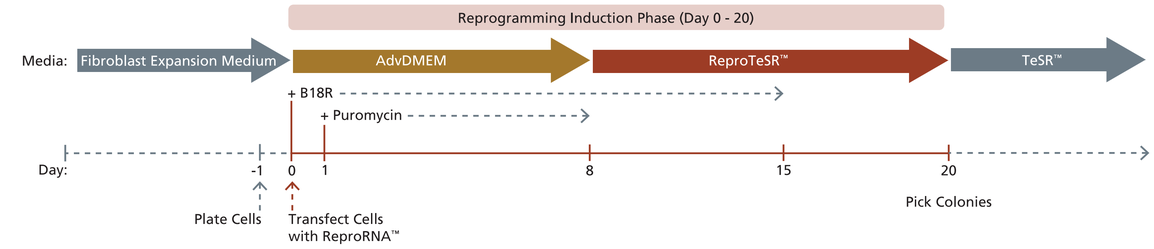

Figure 1: Schematic outlining the application of ReproRNA™-OKSGM for reprogramming fibroblasts to iPSC colonies. Normal adult primary dermal or neonatal fibroblast cells were cultured and expanded in fibroblast medium (DMEM + 10% FBS) prior to reprogramming. One day prior to reprogramming, fibroblasts were harvested with trypsin-EDTA (0.05%, STEMCELL Technologies, Catalog #07910) and seeded at 1 x 10^4 cells/cm^2 in 6-well plates pre-coated with Matrigel® (Corning, Catalog #354277) or Vitronectin XF™ (STEMCELL Technologies, Catalog #07190). On day 0, fibroblasts were transfected with 1 µg of ReproRNA™-OKSGM (STEMCELL Technologies, Catalog #05931) with ReproRNA™ Transfection Reagent (STEMCELL Technologies, Catalog #05932) diluted in OptiMEM® (Life Technologies). The fibroblasts were incubated for 3 hrs with the transfection mixture. The transfection mixture was then replaced with Advanced DMEM containing 10% FBS (Life Technologies). Recombinant B18R protein (e.g. eBiosciences) was added to the media at 200 ng/mL at the time of transfection and during the first 2 weeks of reprogramming to inhibit the interferon response and increase cell viability. In addition, 1 day after transfection, puromycin was added at 0.8 ng/mL until day 8 to select out untransfected cells. On day 8, the media was switched to ReproTeSR™ reprogramming media (STEMCELL Technologies, Catalog #05920) and changed daily for the remainder of the reprogramming induction phase until iPSC colonies emerged. Typically, by day 20, iPSC colonies are large enough to be isolated manually and plated onto Matrigel®-coated wells for propagation in mTeSR™1 medium (STEMCELL Technologies, Catalog #05850).

Results

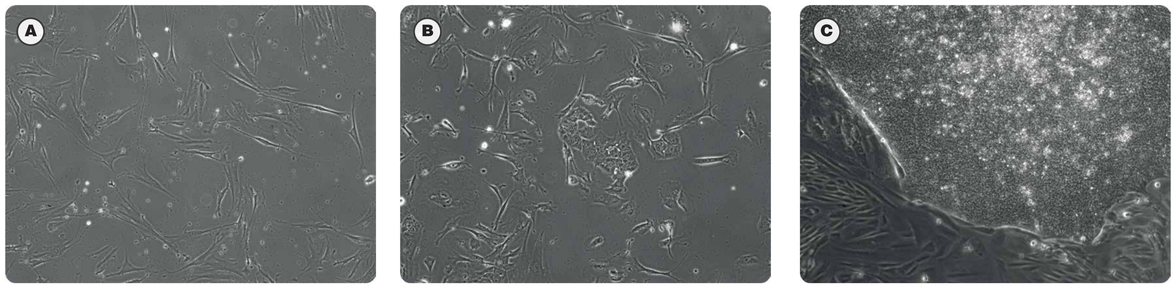

Figure 2: Morphology of emerging iPSC colonies during the induction phase of reprogramming of fibroblasts with ReproRNA™-OKSGM. Emerging iPSC colonies were identified by observing morphological changes and imaged at several time points of the induction phase of reprogramming. **(A)** Morphology of human dermal fibroblasts plated at 1 x 10^5 cells per well onto Matrigel®-coated 6-well plates one day prior to transfection with ReproRNA™-OKSGM. **(B)** Small clusters of epithelial-like cells were observed within the first week after transfection as the fibroblasts undergo a mesenchymal-to-epithelial transition. **(C)** iPSC colonies with hES-like cell morphology exhibiting high nuclear-to-cytoplasmic ratio and prominent nucleoli are ready to be isolated and subcultured by day 20. Images taken at 100X.

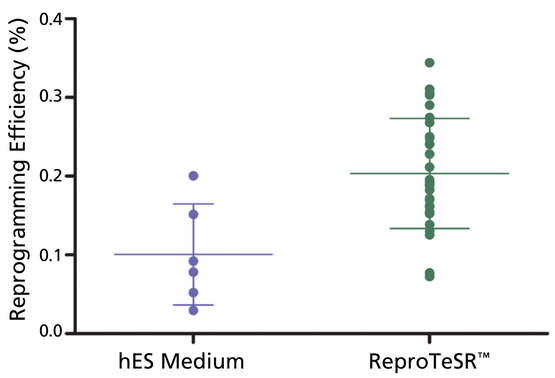

Figure 3: ReproRNA™-OKSGM generates iPSCs with high efficiency and is compatible with both ReproTeSR™ and feeder-free conditions. Reprogramming efficiencies [(# iPSC colonies / # input fibroblasts) x 100%] using ReproRNA™-OKSGM were compared between a feeder-based system on iMEFs and a feeder-free system on Matrigel®. Reprogramming adult human dermal fibroblasts under feeder-free conditions with ReproTeSR™ exhibited higher reprogramming efficiency than a feeder-based system with standard hESC medium with efficiencies of 0.203 ± 0.012% and 0.101 ± 0.026% (n ≥ 6, mean ± SEM), respectively.

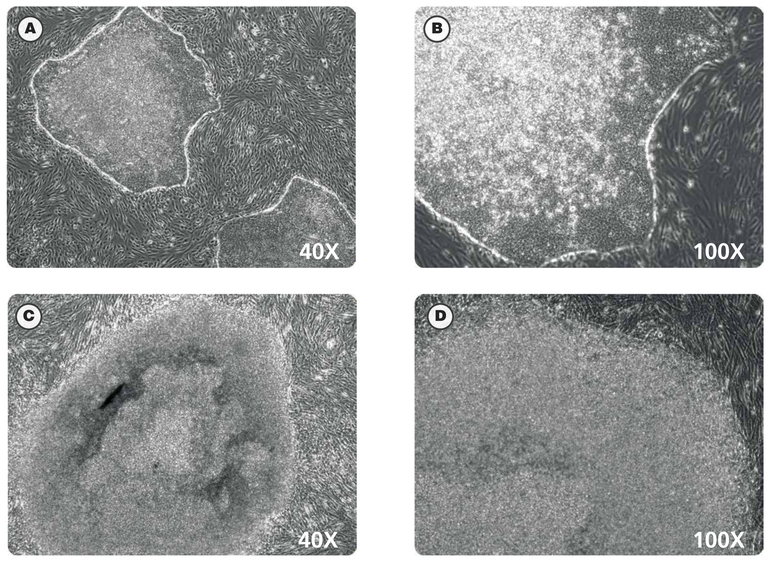

Figure 4: Generating iPSCs with ReproRNA™-OKSGM in ReproTeSR™ under feeder-free conditions results in superior colony morphology and are easier to identify. ReproRNA™-OKSGM reprogrammed iPSC colonies derived in ReproTeSR™ (**A** and **B**) exhibited more defined borders, compact morphology, and reduced differentiation, compared to iPSC colonies derived in standard hES medium (**C** and **D**), allowing simpler and unmistakable identification of iPSC colonies.

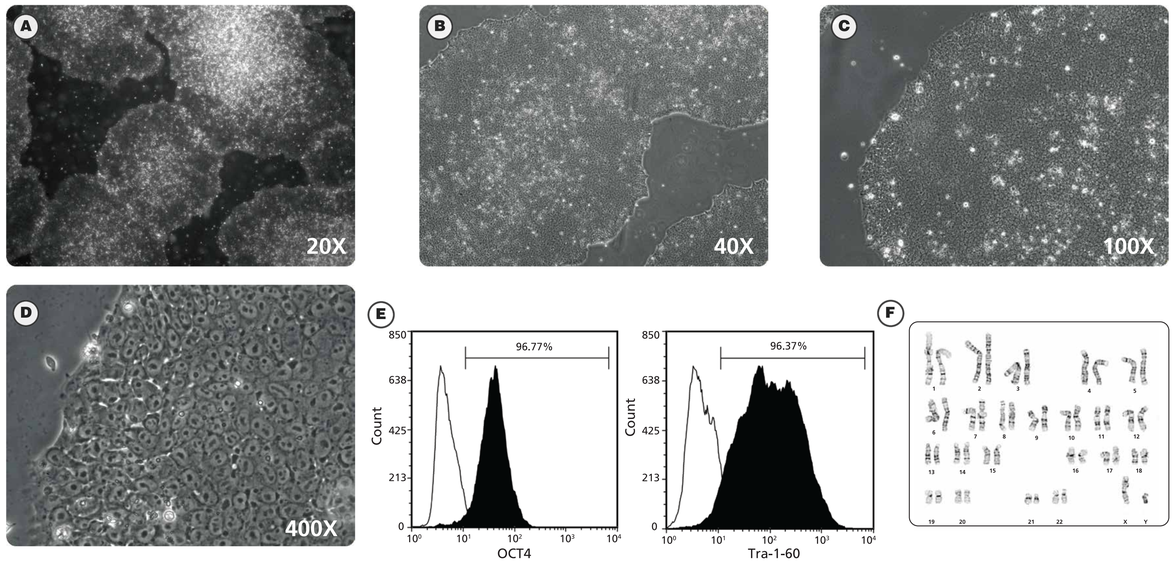

Figure 5: iPSCs generated with ReproRNA™-OKSGM can be readily subcultured in mTeSR™1 Medium, express pluripotent markers, and retain normal karyotype. (A - D) iPSC generated with ReproRNA™-OKSGM and subcultured in mTeSR™1 Medium on Matrigel® displayed typical iPSC-like morphology with defined colony borders and high nuclear-to-cytoplasmic ratio. **(E)** Flow cytometry analysis of an iPSC line generated with ReproRNA™-OKSGM and cultured in mTeSR™1 Medium for 12 passages show high percentage of cells expressing OCT4 and Tra-1-60, markers associated with pluripotency. **(F)** A representative iPSC line generated with ReproRNA™-OKSGM retain normal karyotype. Karyotype analysis was performed at passage 8.

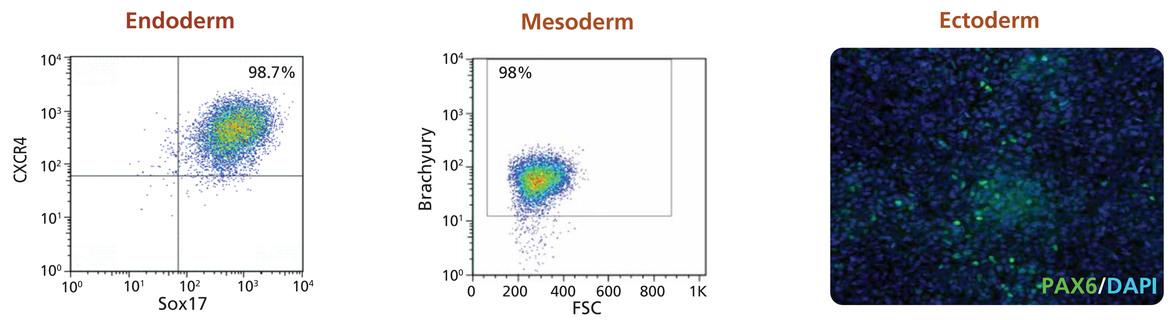

Figure 6: iPSCs generated with ReproRNA™-OKSGM demonstrated the capacity to differentiate *in vitro* to cells of the three germ layers using the STEMdiff™ systems. (A) iPSCs differentiated using STEMdiff™ Definitive Endoderm Kit exhibited 98.7% SOX17 and CXCR4 double positive cells. **(B)** Differentiation to mesoderm lineage using STEMdiff™ Mesoderm Induction Medium generated 98% Brachyury positive cells. **(C)** Neural progenitors expressing PAX6 were generated with STEMdiff™ Neural Induction Medium. Differentiation assays were performed at passage 7 or higher.

Summary

- ReproRNA™-OKSGM is a self-replicating reprogramming RNA vector for generating iPSCs with just one transfection
- Rapid induction of iPSC colonies is observed using ReproRNA™-OKSGM with colonies arising within 2 weeks post-transfection
- ReproRNA™-OKSGM is performs with ReproTeSR™ in a feeder-free culture system
- iPSC colonies derived with ReproRNA™ can be easily subcultured in TeSR™ media, express markers of pluripotency, differentiate to cells of the three germ layers using the optimized STEMdiff™ kits