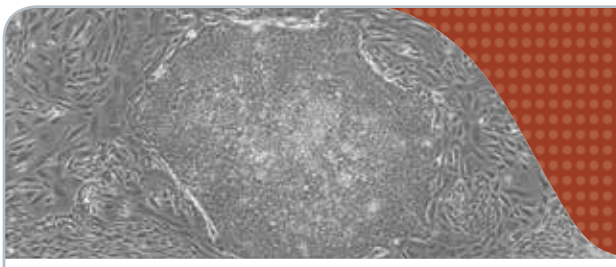

ReproRNA™-OKSGM

Non-Integrating Reprogramming Vector

Generate iPS Cells Using ReproRNA™-OKSGM, a Non-Integrating and Self-Replicating Reprogramming Vector

ReproRNA™-OKSGM is a single-stranded RNA replicon vector that contains five reprogramming factors: *OCT4, KLF-4, SOX2, GLIS1,* and *c-MYC*, as well as a puromycin-resistance gene (Figure 1). This RNA vector reprograms somatic cells, such as fibroblasts, into induced pluripotent stem (iPS) cells with high efficiency and only requires a single transfection step (Figure 2). When used together with ReproTeSR™ reprogramming medium, the generation of iPS cell colonies can be achieved under feeder-free conditions with superior colony morphology and similar reprogramming efficiency to feeder-based systems (Figures 3-4). ReproRNA™-derived iPS cell colonies also express markers of undifferentiated cells and retain a normal karyotype (Figures 5-6). Subsequently, iPS cells generated with ReproRNA™-OKSGM can be maintained in TeSR™ maintenance media (mTeSR™1, TeSR™2, or TeSR™-E8™) and further differentiated into cells of all three germ layers (Figure 7).

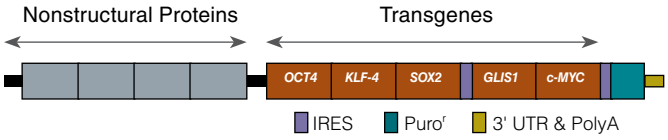

FIGURE 1. Schematic of ReproRNA™-OKSGM, a Single-Stranded RNA Replicon Vector

Advantages:

NON-VIRAL. Non-integrating vector system.

SELF-REPLICATING VECTOR. Only a single transfection is required.

ALL-IN-ONE. Vector contains all reprogramming factors.

HIGHLY EFFICIENT. Comparable fibroblast reprogramming efficiency to Sendai virus.[1]

PRODUCT	SIZE	CATALOG #
ReproRNA™-OKSGM Kit*	1 Kit	05930
ReproRNA™-OKSGM	1 Vial	05931
ReproRNA™ Transfection Reagent Kit**	1 Kit	05934
Recombinant B18R Protein	50 µg	78075

* Kit includes the ReproRNA™-OKSGM vector, ReproRNA™ Transfection Reagent Kit, and Recombinant B18R Protein.
** Kit includes the ReproRNA™ Transfection Reagent (catalog #05932) and ReproRNA™ Transfection Supplement (catalog #05933).

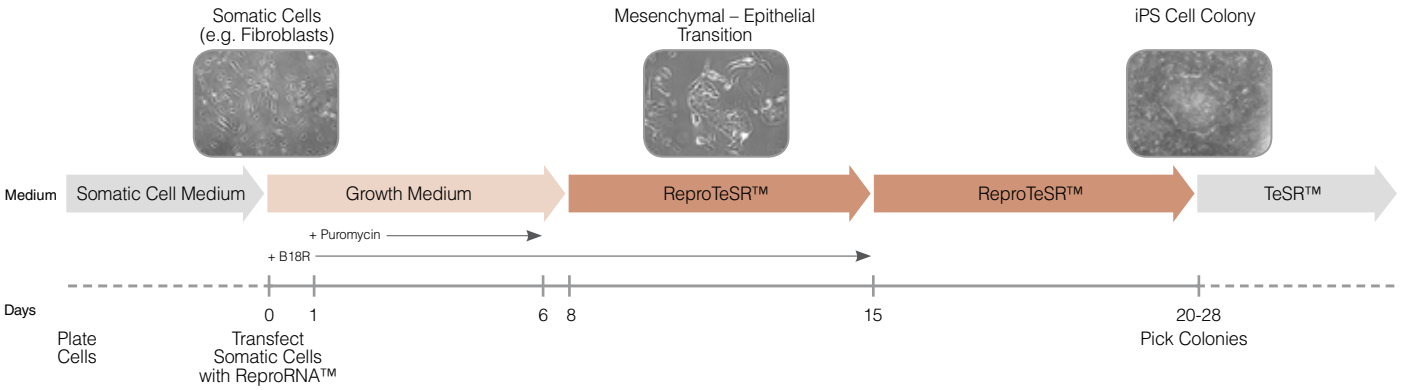

FIGURE 2. Timeline for Reprogramming with ReproRNA™-OKSGM

Somatic cells are transfected with ReproRNA™-OKSGM at day 0, and cultured in Growth Medium (containing puromycin). After 5 days of puromycin selection post-transfection, cells are cultured in ReproTeSR™ for the remainder of the reprogramming induction phase until iPS cell colonies emerge. Recombinant B18R Protein is also added during the first 2 weeks after transfection to inhibit the interferon response and increase cell viability. Typically, by day 20, iPS cell colonies are large enough to be isolated and propagated in *TeSR™ media. *TeSR™ = TeSR™ family media (mTeSR™1, TeSR™2, and TeSR™-E8™)

ReproRNA™-OKSGM

Non-Integrating Reprogramming Vector

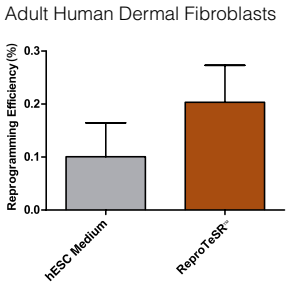

FIGURE 3. ReproRNA™-OKSGM Vector Efficiently Reprograms Fibroblasts

Dermal fibroblasts were transfected with the ReproRNA™-OKSGM vector and reprogrammed under feeder-dependent (standard KOSR-containing hES cell medium on irradiated mouse embryonic fibroblasts (iMEFs)) or feeder-independent conditions (ReproTeSR™ on Corning® Matrigel®). Fibroblasts (passage 4) were reprogrammed with average efficiencies of 0.10 ± 0.06% (hES cell medium) and 0.20 ± 0.07% (ReproTeSR™). Reprogramming efficiency of fibroblasts with ReproRNA™ and ReproTeSR™ is comparable to that reported with Sendai virus.[1] (n ≥ 6; Data shown are mean ± SD).

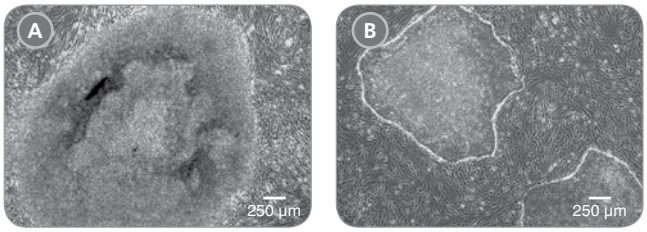

FIGURE 4. Feeder-Free Reprogramming with ReproRNA™-OKSGM Vector and ReproTeSR™ Generates iPS Cell Colonies with Superior Colony Morphology

Representative images of iPS cell colonies were generated using ReproRNA™-OKSGM and cultured in **(A)** standard hES cell medium on irradiated mouse embryonic fibroblasts (iMEFs) or **(B)** ReproTeSR™ on Corning® Matrigel®. iPS cell colonies derived using ReproTeSR™ exhibit more defined borders, compact morphology, and reduced differentiation as compared to the ES cell medium.

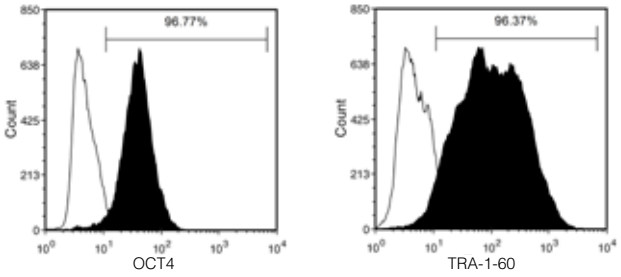

FIGURE 5. Human iPS Cells Generated with ReproRNA™-OKSGM Express Undifferentiated Cell Markers

Human iPS cells generated with ReproRNA™-OKSGM display high expression of undifferentiated cell markers (OCT4 and TRA-1-60) as shown by flow cytometry analysis after 12 passages in mTeSR™1. (Filled histogram = sample, hollow histogram = secondary antibody only).

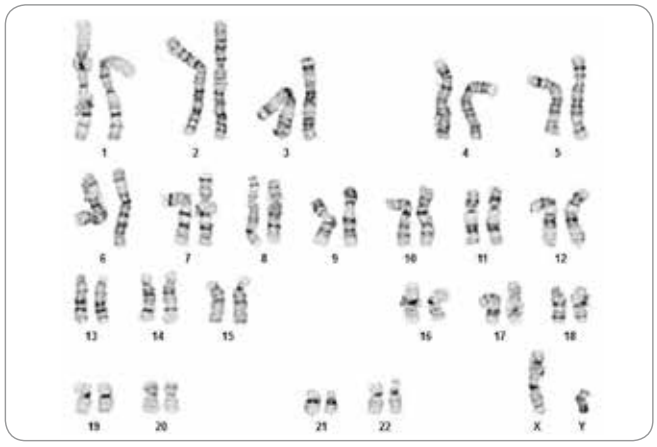

FIGURE 6. iPS Cells Derived Using ReproRNA™-OKSGM Display a Normal Karyotype.

Karyogram of iPS cells derived with ReproRNA™-OKSGM and cultured in mTeSR™1 for 8 passages shows that a normal karyotype is retained.

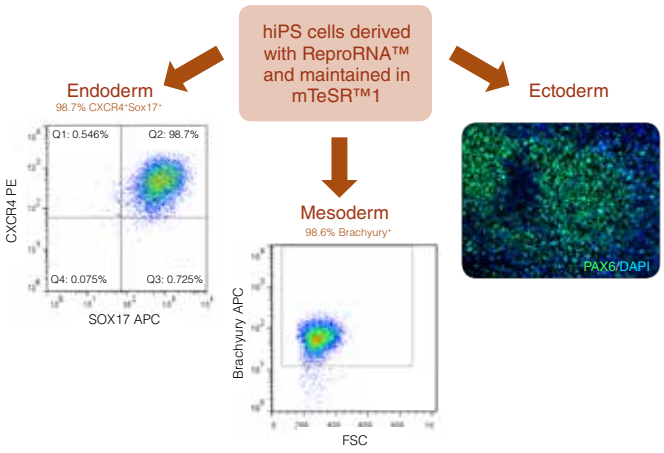

FIGURE 7. ReproRNA™-OKSGM Derived iPS Cells Have the Capacity to Differentiate into Cells of the Three Germ Layers

Human iPS cells derived with ReproRNA™-OKSGM and maintained in mTeSR™1 for 7 passages were differentiated into cells of the three germ layers. Endoderm specification was achieved using the STEMdiff™ Definitive Endoderm Kit, and flow cytometry analysis shows a high percentage of cells (98.7%) positive for endoderm markers (CXCR4+SOX17+). Mesoderm induction was achieved with STEMdiff™ Mesoderm Induction Medium as shown by the high percentage of cells (98.6%) expressing Brachyury (T). Ectoderm specification was demonstrated using STEMdiff™ Neural Induction Medium. CNS-enriched NPC cultures expressing PAX6 (green) and stained with DAPI (blue) are shown.

For a complete list of related products, including specialized cell culture and storage media, matrices, antibodies, cytokines and small molecules, visit **www.stemcell.com/hPSCworkflow** or contact us at **techsupport@stemcell.com**.

Reference:

1. Schlaeger TM, et al. (2015) Nat Biotechnol 33(1): 58-63.