mRNA Reprogramming, Frequently Asked Questions

December 2011

What is mRNA reprogramming and how is it different from other reprogramming methods?
Messenger RNA reprogramming is a non-viral and non-integrating method of generating iPS cells. mRNA-based reprogramming completely eliminates the risk of genomic integration and mutagenesis inherent to DNA and viral-based technologies. The mRNA reprogramming method is the safest and most efficient (>3%) method currently available, eliminating safety and biocontainment concerns associated with virus as well as the need for the screening of cells to confirm viral remnants no longer remain.

What is Stemgent's mRNA reprogramming system? The Stemgent® mRNA Reprogramming System includes five mRNA reprogramming factors (Oct4, Sox2, Klf4, c-Myc, and Lin28), GFP marker, the Pluriton™ Reprogramming Medium, B18R Recombinant Protein Carrier-Free, and a validated protocol. The entire system can be purchased as a kit, where all components within the kit have been validated together for successful mRNA reprogramming. Some components of the system are also available individually. However, the B18R protein is currently manufactured through eBiosicence and is distributed through Stemgent as part of the mRNA Reprogramming Kit only.

Product Description	Cat. No.
Stemgent mRNA Reprogramming Factors Set: hOKSML **Contains:** 2 vials of human Oct4 mRNA, 1 vial of human Klf4, Sox2, c-Myc, Lin-28 mRNA, and 1 vial nGFP mRNA	00-0067
Stemgent mRNA Reprogramming Kit **Includes:** Pluriton Medium, B18R Recombinant Protein Carrier-Free*	00-0071
Pluriton Reprogramming Medium	00-0070
Stemgent Oct4 mRNA, Human	05-0014
Stemgent Klf4 mRNA, Human	05-0015
Stemgent Sox2 mRNA, Human	05-0016
Stemgent Lin-28 mRNA, Human	05-0017
Stemgent c-Myc mRNA, Human	05-0018
Stemgent nGFP mRNA	05-0019
Stemgent eGFP mRNA Transfection Control	05-0020

*B18R is manufactured by eBioscience.

How much mRNA is in each vial of the set? Each vial contains 20 µg of mRNA in a 200 µL volume. The concentration of mRNA is 100 ng/µL, diluted in RNase-free TE buffer, pH 7.0. The contents of one mRNA Reprogramming Factors Set: hOKSML is sufficient to reprogram 4 wells of target cells in a 6-well plate format.

What is the recommended medium to use for mRNA reprogramming? Pluriton Reprogramming Medium was specifically developed to support iPS cell generation by mRNA and has been found to be essential for successful reprogramming. Pluriton Reprogramming Medium (Cat. No. 00-0070) contains two components: one 500 mL bottle of Pluriton Medium and one 200 µL vial of Pluriton Supplement 2500X. Pluriton Reprogramming Medium is sufficient to support reprogramming of two full mRNA experiments (8 wells total in a 6-well plate format).

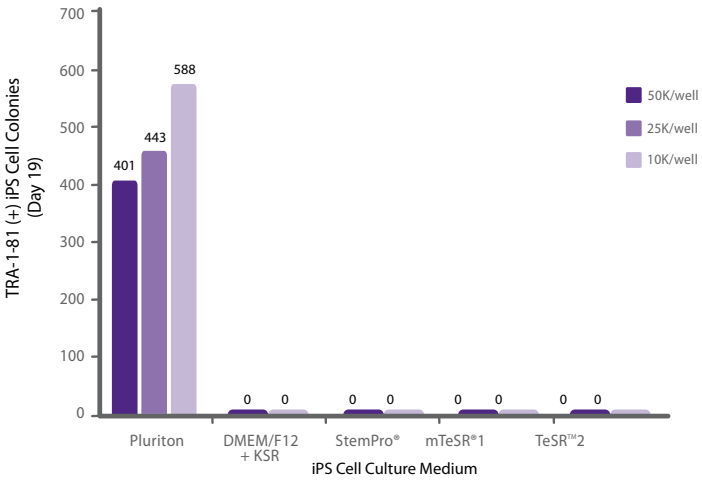

FIGURE: mRNA iPS cell derivation media comparison of Stemgent Pluriton Reprogramming Medium and other common human ES culture media. Different target cell densities (50K, 25K, and 10K for Pluriton medium; 50K and 10K for all others) of BJ fibroblasts were plated on human fibroblast feeders (250K/well) in a single well of a 6-well plate. Each bar in the graph is individually labeled with the number of iPS cell colonies generated.

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How should the Pluriton Reprogramming Medium and Supplement be stored? Store the Pluriton Medium at -20°C and the Pluriton Supplement at less than -70°C. Upon receipt, we recommend initially thawing both components and refreezing multiple aliquots in single-use volumes. Refreeze the amount of Pluriton Medium that will not be used within the first two weeks of the mRNA reprogramming experiment in 40 mL aliquots and store at -20°C until use. The Pluriton Supplement should be aliquoted into small, single-use volumes and refrozen at -70°C until use. These aliquots are intended to be thawed again only once, just before use.

Is B18R protein necessary for mRNA reprogramming? Yes. B18R protein is required to suppress the interferon-induced apoptosis that results from repeated transfection with exogenous mRNA. We recommend pretreating the target cells with B18R prior to the first transfection. The Stemgent mRNA reprogramming protocol requires that the target cells are continually cultured in the presence of B18R while transfecting with mRNA. B18R is not required in the culture system once the transfections have been completed.

How should the B18R protein be stored? After thawing the stock vial of B18R protein, aliquot the contents into single-use tubes and refreeze at -70°C until use. Do not subject the B18R protein to more than this one additional freeze/thaw cycle.

Which transfection reagent should I use for mRNA reprogramming? Our current protocol for reprogramming human fibroblasts with mRNA has been optimized and validated using the RNAiMAX transfection reagent, as was published in the original protocol by Warren et al. However, Stemgent's Stemfect™ RNA Transfection Kit (Cat. No. 00-0069) provides excellent transfection efficiency of mRNA into a range of cell types with greater than 95% viability.

Can I prepare any materials prior to beginning my experiment? Yes, material preparation can begin several days prior to beginning your experiment. To begin, aliquot the Plurition™ Supplement, B18R protein, and prepare the mRNA cocktail up to one week prior to Day -2 . The bottle of Pluriton™ Medium can be aliquoted a few days prior to beginning your experiment. We also recommended that you prepare your conditioned media beginning on Day -2 of the experiment. For the complete protocol, visit www.stemgent.com/mrna

Do I need to make the mRNA cocktail fresh every day? No. Prior to beginning your experiment, combine the mRNA transcription factors according to the protocol to make a "master mix" of the mRNA cocktail. Aliquot the mRNA cocktail into single-use volumes and freeze the tubes at -70°C. The mRNA aliquots should not be re-frozen after they are thawed.

What type of cells should I use? The Stemgent mRNA Reprogramming System has been validated on inactivated human newborn foreskin fibroblasts (NuFFs) from GlobalStem (GlobalStem Cat. No.'s GSC-3001M and GSC-3001G) as a feeder layer for mRNA reprogramming.

Can I switch to a MEF feeder layer for my hiPS cells after reprogramming? Yes. Once you pick the new hiPS cell colonies from the reprogramming wells, the new colonies can be re-plated directly onto a new, freshly-inactivated MEF feeder layer. New hiPS cell cultures can be continually maintained on MEFs beginning with the first passage.

Why do I need to plate 3 wells of my target cells? While it is best to utilize healthy, proliferative cells, cell cultures growing to confluence within the first week of the reprogramming protocol can inhibit the overall reprogramming process. When working with a new cell type, titrating the starting cell densities is important. Transfecting multiple wells of target cells in a single experiment can streamline optimization by minimizing the influence of outside variables on the efficiency of reprogramming.

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Do I have to change the medium every day? Yes. It is important to change the cell culture medium daily to maintain a healthy culture environment for hiPS cell generation. mRNA transfection with the RNAiMAX transfection reagent can be toxic to the cells if left in the culture medium longer than the 4 hours needed for transfection.

Can I leave the transfection reagent in my culture overnight? No. When transfecting cells using the RNAiMAX transfection reagent, the transfection time must be limited to 4 hours, as described in the mRNA reprogramming protocol. RNAiMAX can cause severe cellular toxicity if left in the reprogramming culture longer than 4 hours or overnight.

What if my cells become too confluent within the first week of the reprogramming experiment? Based on the degree of confluence of your target cells by Day 6 of the mRNA reprogramming protocol, one or more wells of target cells can be passaged and replated at a lower density before Day 8.

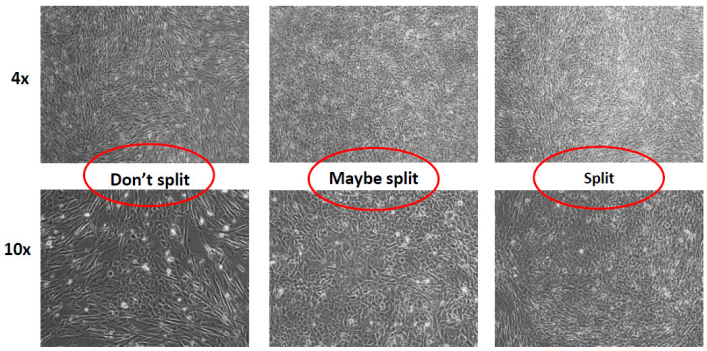

FIGURE: Time course images of the growth of iPS cells using the Stemgent mRNA Reprogramming System shown at Day 6. The guide above shows density of cells and when to split and replate.

Can I use a Rho kinase inhibitor when plating my target cells? Yes. We have found that the use of the Rho kinase inhibitor Y27632 (Cat. No. 04-0012) can promote cell survival and increase attachment when replating transitioning fibroblasts onto new NuFF feeder plates when passaged during the first week of the reprogramming process. For the complete protocol, visit www.stemgent.com/mrna

Can I stain my cells for pluripotency markers while keeping them in culture? Yes, Stemgent's StainAlive™ antibodies have been designed to effectively stain live iPS cells without the need to fix or otherwise disturb the cultures. StainAlive™ DyLight™ 488 Tra-1-60 (Cat. No. 09-0068) and StainAlive™ DyLight™ 488 Tra-1-81 (Cat. No. 09-0069) can be a very useful tool for monitoring emerging hiPS cell colonies in an mRNA reprogramming experiment.

Where can I find more information on mRNA reprogramming? Visit our website at **www.stemgent.com/mrna** or call Technical Support at **877.783.6911** for more product information, data, protocols, and troubleshooting tips.

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