A Validated mRNA Reprogramming Protocol for the Reproducible Generation of Integration-free Human iPS Cells

Chenmei Luo, Kerry Mahon, Kevin Yi, Shuya Zhai, Amelia Cianci, and Brad Hamilton*
Stemgent, One Kendall Square, Cambridge, MA 02139
*Corresponding Author: brad.hamilton@stemgent.com

ABSTRACT

To date, the broad implementation of induced pluripotent stem (iPS) cells in regenerative medicine and drug screening applications has been limited by the inability to efficiently derive iPS cell lines that are free from genomic integration. Past publications have highlighted the use of DNA-based reprogramming technologies that 1) utilize retroviral or lentiviral vectors to integrate into the target cell genome, 2) directly integrate into the target cell genome, but can be removed, or 3) minimally integrate into the target cell genome, but still require screening to ensure no genomic alteration. More recent works have utilized recombinant protein or mRNA to derive iPS cell lines that are inherently free from any target integration. However, these methods have been plagued by low efficiency and/or the lack of reproducibility. Here, we demonstrate a validated protocol utilizing the Stemgent® mRNA Reprogramming Factor Set for the efficient and reproducible generation of iPS cells from both BJ foreskin fibroblasts and patient-derived dermal fibroblasts. This reproducibility is dependent on the use of Stemgent® Plurisphere™ Reprogramming Medium that is essential for complete conversion to iPSC. In addition, the highly efficient StemCell® RNA Transfection Reagent was developed to ensure that mRNA can be delivered to a range of cell types, including fibroblasts and lymphocytes, with titratable control over expression. This methodology provides a reproducible, non-integrating method for generating iPS cells which has the potential to be expanded to multiple cell types.

INTRODUCTION

Several recent publications have demonstrated the potential of mRNA to reprogram somatic cells to induced pluripotent stem (iPS) cells, albeit with varying levels of efficiency, reproducibility, and colony stability.1-3 Messenger RNA reprogramming is a desirable method for iPS cell generation for a number of reasons. First, it eliminates the need for DNA-based reprogramming factor delivery, thereby eliminating any risk for insertional mutagenesis of the patient cell genome. Second, published methods have demonstrated an increased yield in the number of iPSC colonies generated per defined starting cell population, thereby potentially reducing the number of target cells required to initiate the reprogramming process. Third, this method has generated iPSC colonies in as little as over two weeks and in doing so has minimized the primary cell culturing time during the reprogramming process when compared to other DNA-based delivery methods. In an effort to develop a more robust and reproducible method for iPS cell generation using this integration-free technology, our laboratory evaluated both the mRNA reprogramming protocol as whole as well as the individual materials and reagents employed.

Early experiments using the Stemgent® mRNA Reprogramming Factor Set and the mRNA reprogramming protocol as published4, revealed that while we were able to detect properly localized protein expression from each mRNA delivered to our target fibroblasts (Figure 6), overall the culture was unable to sustain the morphological changes recognized very early in the reprogramming process. As a result, we incorporated five significant changes to generate a more streamlined and reproducible protocol (Figure 1). Most significantly, we developed Plurisphere™ mRNA Reprogramming Medium which allows for better mRNA reprogramming factor delivery and protein expression (data not shown) as well as exclusive support of iPS cell colony generation when compared to other commonly used human ES cell culture/reprogramming media (Figure 3). Second, we converted the protocol to a no-split format to minimize the inherent variability associated with enzymatic passage of the reprogramming culture and thereby allow for an accurate reprogramming efficiency determination. Third, we included three different target cell plating densities to ensure that there is a cell culture condition that is amenable to iPS cell colony generation regardless of the target cells’ propensity for expansion. Thereby allowing for healthy, expanding cultures to enter the reprogramming protocol as little as 10K starting cells and still generate significant numbers of iPSC colonies. Fourth, we incorporated conditioned medium into the protocol to ensure that the culture environment remains healthy as the feeder layer benefit typically deteriorates after 6-7 days in culture. Lastly, we reduced the plating density of the human feeders from 300K/well to 250K/well to allow for more culture expansion and iPS cell colony growth. Incorporation of the above modifications has ensured that reprogramming with mRNA is not only reproducible, but also extremely efficient and rapid as demonstrated by a reprogramming efficiency as high as 5.88% (588 colonies from 10K starting BJ fibroblasts) after only sixteen transfections and nineteen days in culture. Importantly, the optimized protocol has also been validated for the derivation of iPSC lines from patient derived fibroblast lines (Figure 10).

In concert, to enhance this process, we have developed a new RNA delivery method, based on leading edge lipid-based delivery systems, that allows for fine-tuned control over the amount of mRNA delivered and expressed. This reagent, StemCell™ RNA transfection kit, has been shown to deliver mRNA at 95% efficiency in a broad range of cell types (Figure 4) and could enable the reprogramming of cell lines that are normally refractory to transfection.

SUMMARY

1. Developed functionally validated mRNA reprogramming protocol
   - Integration-free
   - Increased reprogramming efficiency
   - Faster iPS cell colony generation – colony isolation within 3 weeks
   - Demonstrated on diseased patient fibroblasts
   - Validated at 5% O2 and 21% O2
2. Developed novel, xenotrauma-free method effective for mRNA reprogramming success
3. Developed highly efficient RNA transfection reagent for broadened cell application

REFERENCES