Lipofectamine® 3000: A new transfection reagent for iPSC generation and stem cell genomic engineering

Nektaria Andronikou, Yue Geng, Natasha Roark, Xin Yu, Mahalakshmi Sridharan, Uma Lakshmipathy, Namritha Ravinder, Xavier de Mollerat du Jeu – Thermo Fisher Scientific, Life Technologies, 5791 Van Allen Way, Carlsbad, CA 92008

ABSTRACT

Stem cells, specifically induced pluripotent stem cells (iPSCs), hold immense promise for the future of regenerative medicine and personalized therapeutics. However, the lack of advanced advancements in delivery greatly improve downstream editing and engineering of DNA at specific loci. However, the lack of advanced reagents for iPSC generation and stem cell engineering techniques. We would also like to extend a special thanks to the transfection team at Life Technologies™, Thermo Fisher Scientific.

INTRODUCTION

Patient-derived iPSCs offer exciting potential in both cell therapy and in vitro disease modeling by enabling access to cell populations that are otherwise unavailable from living donors. With the recent discovery of site-specific gene editing, this true power is fast approaching. For example, an iPSC cell can be genetically altered at a specific locus, using genome engineering tools such as CRISPR or TAL, differentiated to a neuronal cell type and be used for more targeted drug screening and design. Having the ability to develop two cell models with identical genetic background, except for the site specific edit, gives researchers the potential to study the true effects of a single mutation in a pathway or syndrome and in turn develop advanced therapies and treatments.

Lipofectamine® 3000 is a new transfection reagent optimized for stem cell manipulation from reprogramming to genome editing in iPSC cells.

RESULTS

Figure 1. Disease Model Development

Figure 2. Protocol Outline for generating iPSCs

Figure 3. Morphological changes in BJ fibroblasts with Epis™ Episomal vectors delivered with Lipofectamine® 3000 or Neon® Electroporation system

Figure 4. Alkaline phosphatase stain for iPSC colony visualization of cells transfected with Epis™ Episomal reprogramming vectors with Lipofectamine® 3000 or Neon® Electroporation System

Figure 5. Protocol overview for transfection of stem cells

Figure 6. Transfection of Stem Cells in H9 ESC (A) or iPSC (B) using Lipofectamine® 3000

Figure 7. Workflow overview and design of CRISPR vector, delivery with Lipofectamine® 3000, cleavage detection and colony expansion

CONCLUSIONS

Lipofectamine® 3000 is a transfection reagent that was developed to improve transfection efficiency in many difficult to transfect cell types. In this study, we demonstrated that using an improved transfection reagent such as Lipofectamine® 3000, helps to eliminate many of the pain points associated with current protocols where stem cell generation and manipulation is required. We showed, that the ability to utilize a transfection reagent for reprogramming instead of electroporation eliminates the need for large numbers of cells, minimizes cell perturbation, and is far more cost effective. We also demonstrated that having a transfection reagent that will enhance delivery, will improve the cleavage efficiency of a CRISPR based gene editing system, ultimately maximizing genetic modifications, and simplifying the downstream processes, such as clonal selection.

Lipofectamine® messagemAX™ is a new reagent that has been developed to improve delivery of mRNA. Alternatively to DNA, transcription of mRNA requires that the cargo enter only the cell cytoplasm, not the nucleus. We briefly demonstrated that using an mRNA based from of Cas9 with a guide-RNA for gene editing and Lipofectamine® messagemAX™ for transfection resulted in far more targeted cleavage of the host cell genome when compared to standard DNA based electroporation approaches.

mRNA delivery has become the “go-to” method for a variety of applications that have been difficult to execute in the past, and will help to propel many new and exciting applications and technologies forward.

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