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

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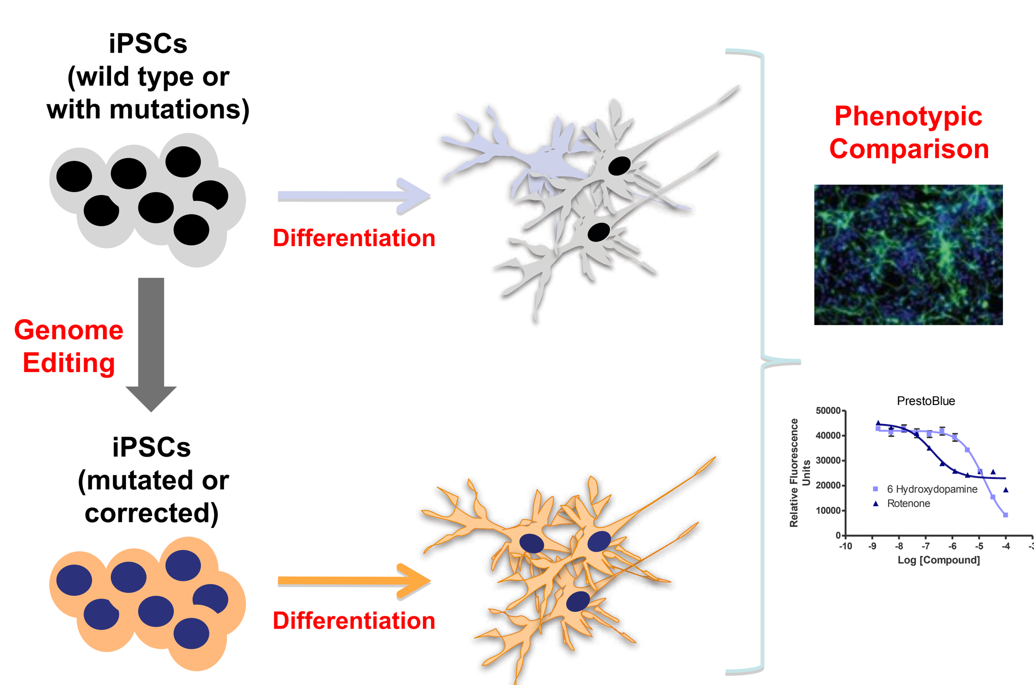
ABSTRACT

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 technologies has been hindering the current pace of research and discovery. One of the greatest challenges lies in the manipulation of stem cells. Lipofectamine® 3000, a new transfection reagent, has been developed for nucleic acid delivery to enable the use of new technologies for stem cell applications. In the present study, we first demonstrated that Lipofectamine® 3000 can be used to efficiently deliver the EPi5™ episomal reprogramming vectors to BJ skin fibroblasts for the generation of iPSCs. This method allows researchers to perform efficient in-situ reprogramming at lower cost, providing a great alternative over electroporation techniques typically used for iPSC generation. Furthermore, it was discovered that Lipofectamine® 3000 can achieve optimal transfection efficiency of various sizes of plasmid DNA and low toxicity in both embryonic stem cells (ESCs) and iPSCs which have been traditionally proven to be hard to transfect. More importantly, manipulation of stem cells can be achieved utilizing TALs and CRISPRs for genome engineering purposes. Transcription activator-like effector nucleases (TALENs) and clustered regularly interspaced short palindromic repeats (CRISPRs) allow for editing and engineering of DNA at specific loci. However, the effectiveness of these tools depends on efficient delivery and the intrinsic properties of the locus of interest. Lipofectamine® 3000 demonstrated improved delivery of CRISPRs into iPSCs for targeted genome engineering. Taken together these advancements in delivery greatly improve downstream workflows, enable easier stem cell manipulation, and enhance site-specific insertion or deletion of transgenes for the generation of knock-in or knock-out cell models and transgenic small animal models.

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 iPS 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 (Figure 1.). 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 iPS cells.

Figure 1. Disease Model Development

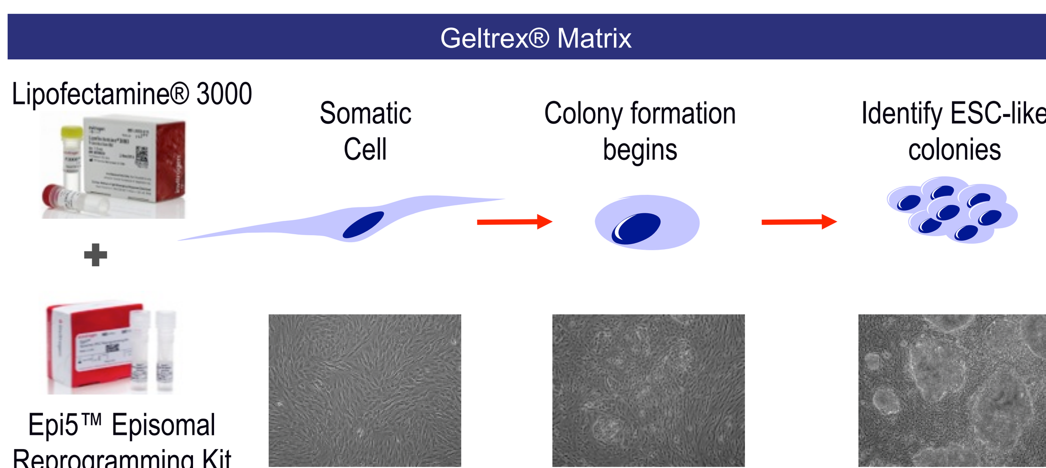


PART I – Reprogramming MATERIALS & METHODS

Figure 2. Protocol Outline for generating iPSCs

▪ Day (-1): Seed cells on Geltrex® Matrix coated dishes	▪ Day 0: Transfect in Fibrobast Media for 24 hour ▪ Day 1-14: Culture in N2B27 Medium with 100ng/mL bFGF (change daily)	▪ Day 15-20: Culture in E8 Medium (change daily) ▪ Day 21+: Manually pick and expand colonies
Day (-1)	Day 0	Day 15 — Day 21
Fibroblast Medium	N2B27 Medium with bFGF (100ng/mL)	Essential™ 8 Medium

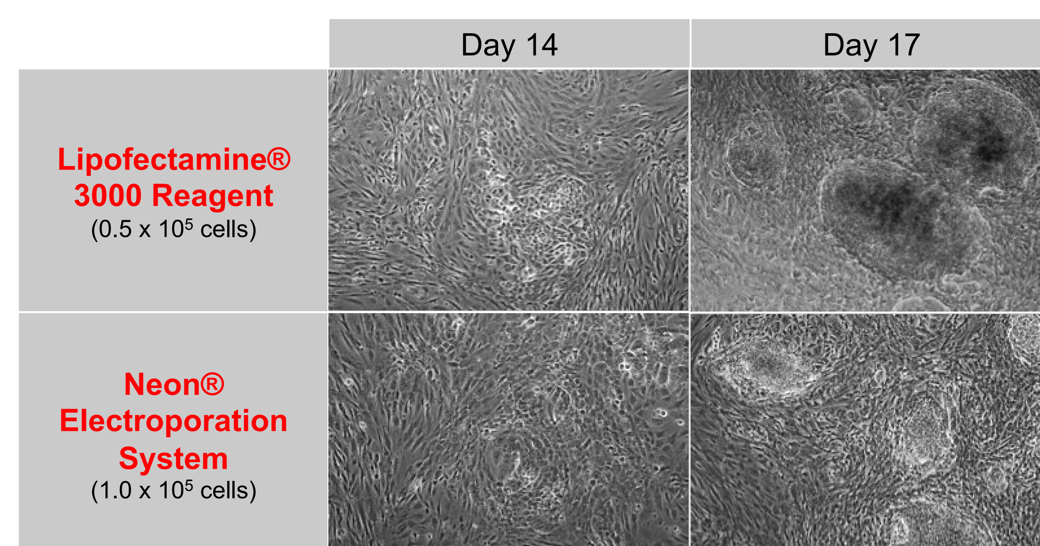
Geltrex® Matrix



Overview of protocol outline for generating iPS cells with Epi5™ Episomal reprogramming kit and Lipofectamine® 3000 reagent. For a complete protocol detailing required materials and step-by-step instructions, please visit: *www.lifetechnologies.com/3000*

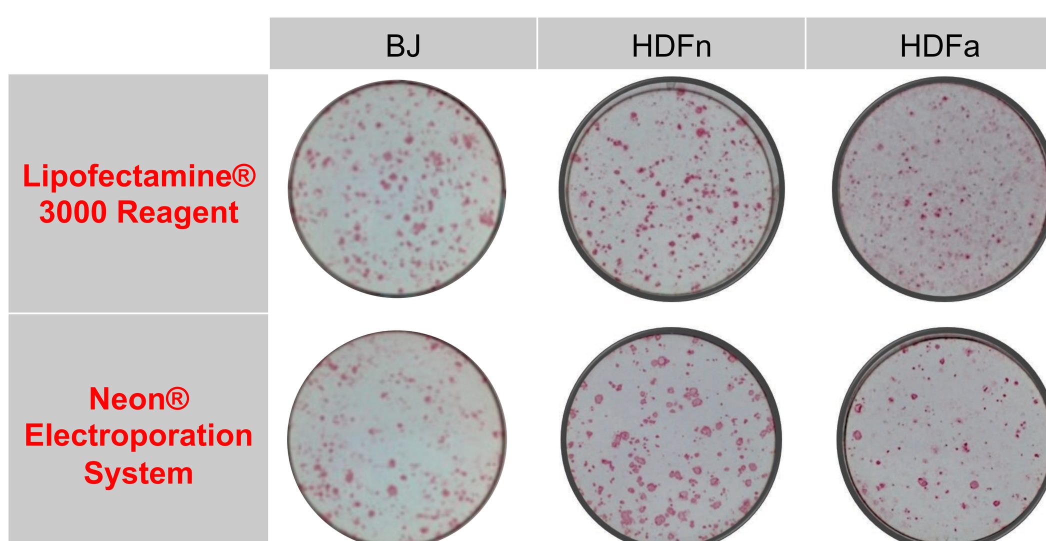
RESULTS

Figure 3. Morphological changes in BJ Fibroblasts with Epi5™ Episomal vectors delivered with Lipofectamine® 3000 or Neon® Electroporation system



Transfection performed in BJ fibroblast using Neon® Transfection System at recommended conditions and Lipofectamine® 3000, 3.6ul per well for a 6-well culture plate and the Epi5™ Episomal Reprogramming vectors. Media changes performed daily according to Life Technologies™ protocol: *Generation of Human Induced Pluripotent Stem Cells (hiPSCs) from Fibroblasts using Episomal Vectors.* Results obtained via bright field microscopy for Neon® and Lipofectamine® 3000 indicate that reprogramming was successful in generating iPSC colonies.

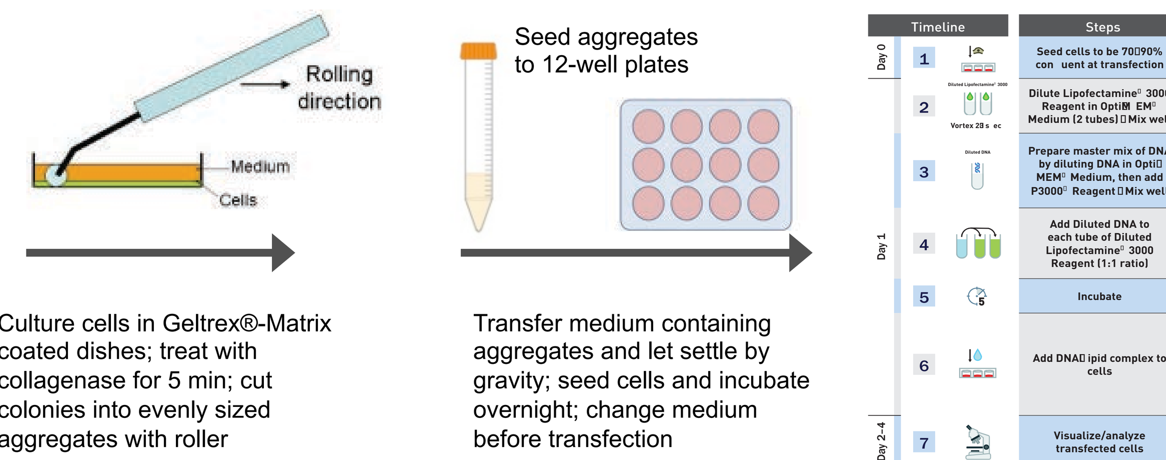
Figure 4. Alkaline phosphatase stain for iPSC colony visualization of cells transfected with Epi5™ Episomal reprogramming vectors with Lipofectamine® 3000 or Neon® Electroporation System



Transfection performed in BJ fibroblast, neonatal human dermal fibroblast (HDFn) and adult human dermal fibroblast cells using Neon® Transfection System at recommended conditions and Lipofectamine® 3000, 3.6ul per well in a 6-well culture plate. Epi5™ Episomal Reprogramming vector was used. Media changes performed daily according to Life Technologies™ protocol: *Generation of Human Induced Pluripotent Stem Cells (hiPSCs) from Fibroblasts using Episomal Vectors.* A terminal stain was performed with red alkaline phosphatase at 18 days post-transfection.

PART II – Transfection of Stem Cells MATERIALS & METHODS

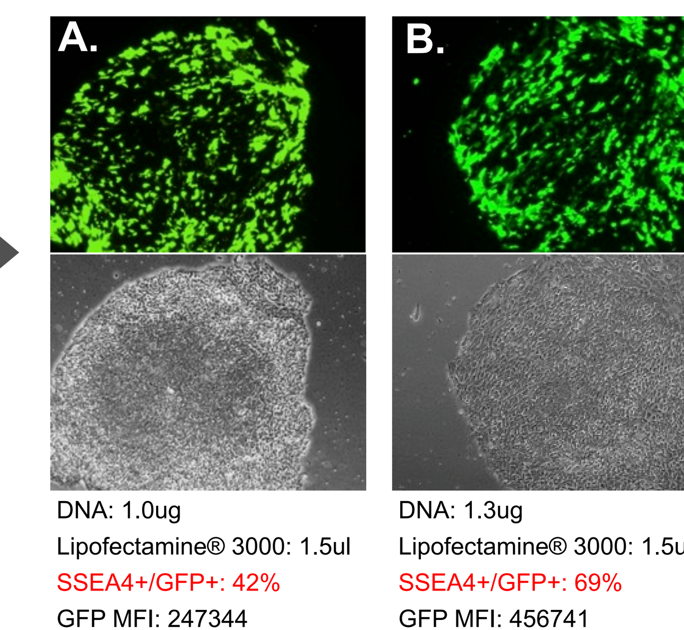
Figure 5. Protocol overview for transfection of stem cells



Transfection was performed in H9 embryonic stem cells (Figure 6. A) and iPSC (Figure 6. B) with a GFP expressing plasmid and the indicated 1.5ul of Lipofectamine® 3000 in a 12-well format. Cells were prepared one day prior to transfection using the protocol in Figure 5. Transfection efficiency was determined 24 hours post-transfection using flow cytometry and fluorescence imaging. SEEA4 staining was performed with an AlexaFluor® 647 antibody to ensure pluripotency of stem cell colonies post-transfection via flow cytometry.

RESULTS

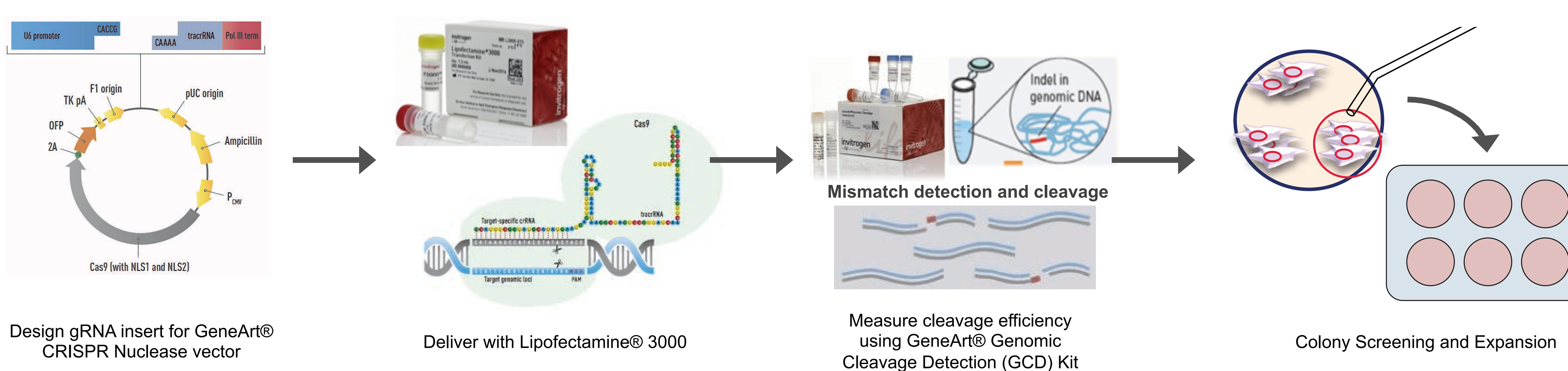
Figure 6. Transfection of Stem Cells in H9 ESC (A) or iPSC (B) using Lipofectamine® 3000



A.	B.
DNA: 1.0ug	DNA: 1.3ug
Lipofectamine® 3000: 1.5ul	Lipofectamine® 3000: 1.5ul
SSEA4+/GFP+: 42%	SSEA4+/GFP+: 69%
GFP MFI: 247344	GFP MFI: 456741

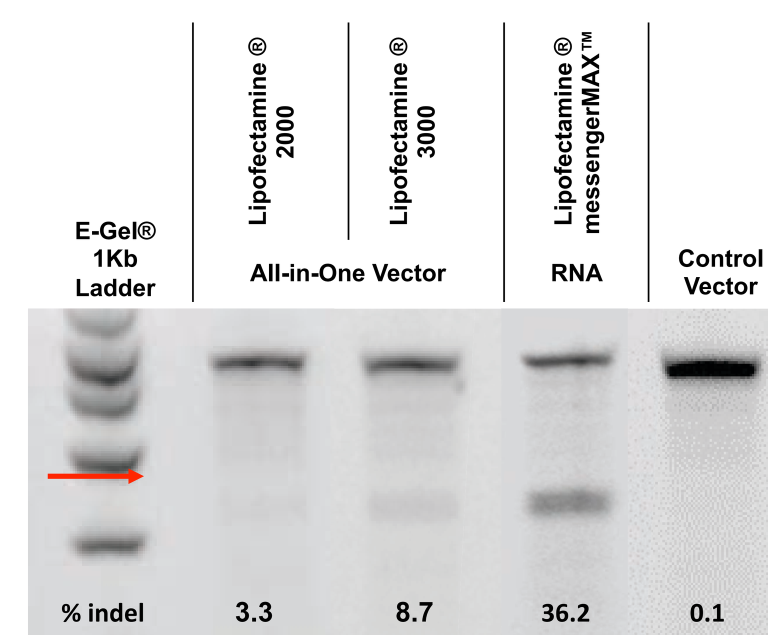
PART III – Genome Editing of Stem Cells MATERIALS & METHODS

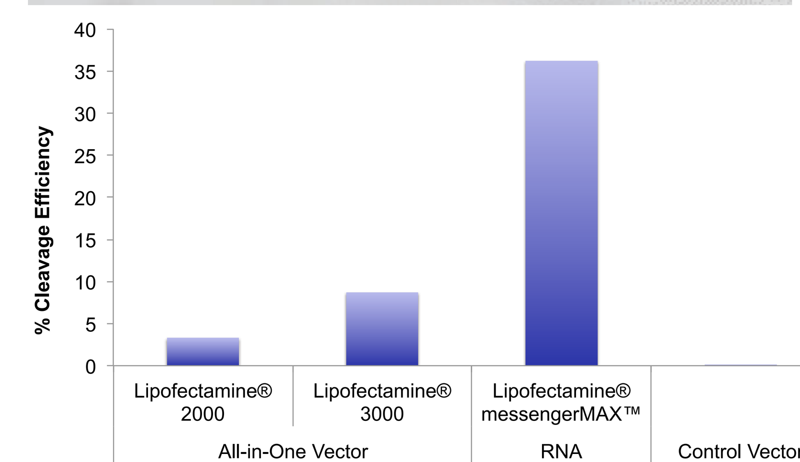
Figure 7. Workflow overview and design of CRISPR vector, delivery with Lipofectamine® 3000, cleavage detection and colony expansion



RESULTS

Figure 8. Lipofectamine® 3000 Improves cleavage efficiency of GeneArt® CRISPR Nucleases in iPSCs





iPS cells were prepared according to protocol listed above (Figure 5.) and seeded in a Geltrex®-Matrix coated 12-well culture dish. Transfection was performed in iPSCs with 3ul of Lipofectamine® 2000 and 3ul of Lipofectamine® 3000 to deliver a GeneArt® CRISPR Nuclease vector targeting the HPRT locus. Transfection was also performed with GeneArt® CRISPR Nuclease RNA editing system targeting the HPRT locus and 3ul of the newly developed Lipofectamine® messengerMAX reagent. RNA editing system utilizes a Cas9 mRNA, which was prepared via in vitro transcription with the Ambion® mMessage mMachine® Kit, and a gRNA which was transcribed using the Ambion® MEGAshortscript™ Kit. Cells were harvested 72-hours post-transfection and cleavage efficiency was determined using the GeneArt® Genomic Cleavage Detection Kit.

*** For more detailed information regarding the GeneArt® CRISPR Nuclease editing system, please visit poster F-2125*

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 eliminated 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® messengerMAX™ is a new reagent that has been developed to improve delivery of mRNA. Alternatively to DNA, transfection 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® messengerMAX™ for transfection resulted in far more targeted cleavage of the host cell genome when compared to standard DNA based editing 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.

ACKNOWLEDGMENTS

The transfection team at Life Technologies™, Thermo Fisher Scientific would like to thank the synthetic biology team for their support in providing the required CRISPR editing tools and expertise in design and genome engineering techniques. We would also like to extend a special thanks to the stem cell team for their support in providing all the necessary cell models for many of the above referenced experiments.