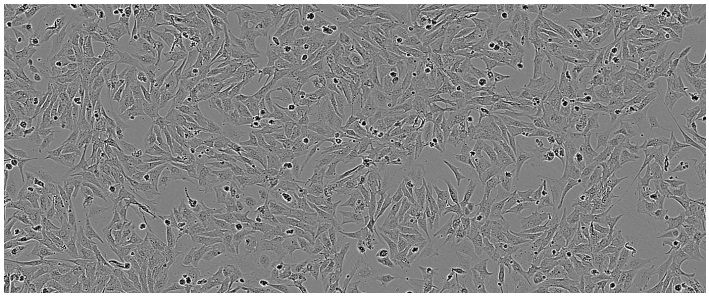
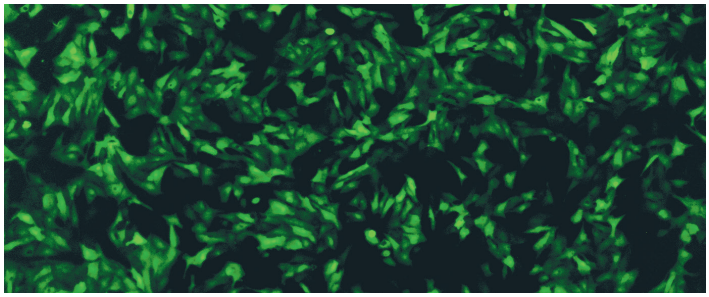
Optimizing cardiomyocyte transfection with Lipofectamine MessengerMAX reagent

Introduction

Cardiovascular disease is the leading cause of mortality in the United States, and understanding the pathophysiology of the disease is a critical research area. In order to study the changes in signaling pathways that give rise to this class of diseases, *in vitro* experimental models are frequently employed. Transfection of cardiac muscle cells, or cardiomyocytes, has posed a particularly challenging problem, and achieving uniform protein expression at a high efficiency has been one of the biggest hurdles for reagent-based systems. Methodologies currently being employed include electroporation and viral overexpression, which come with a range of limitations including cost and time. Reagent-based transfection of DNA in nondividing primary cells is very inefficient due to ineffective nuclear entry and thus is not a viable tool—emphasizing the need for a reagent that can address this limitation. To simplify the process of overexpressing protein in cardiomyocytes, we developed the Invitrogen™ Lipofectamine™ MessengerMAX™ Transfection Reagent. Here we provide a simple protocol and examples of how Lipofectamine MessengerMAX reagent can be used for high-efficiency transfection of cardiomyocytes. The efficacy of this reagent system provides researchers with a superior overexpression system compared to the commonly used methods that include adenovirus and electroporation (Figure 1).



Posttransfection, bright-field



Posttransfection, GFP: 57% transfection efficiency

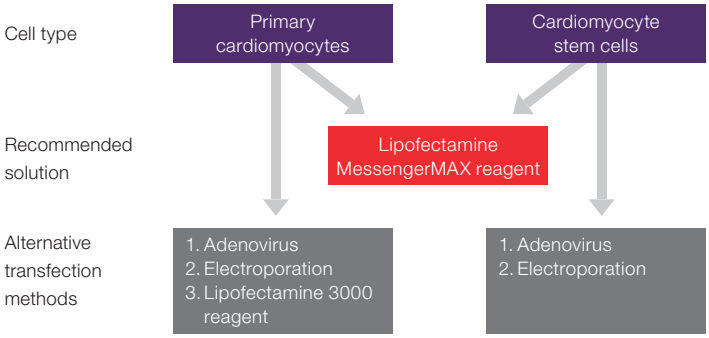


Figure 1. Recommended transfection methods for cardiomyocytes. As can be seen in the bright-field and GFP images above, the high transfection efficiency achieved using mRNA transfection makes Lipofectamine MessengerMAX reagent the recommended choice for use in cardiomyocytes. For researchers interested in other delivery methods, we've listed some additional potential solutions by cell type. The trade-offs of the alternative solutions are provided in Table 1.

Protocol overview

The Lipofectamine MessengerMAX reagent has been optimized for the delivery of mRNA, making it an ideal choice for delivery in isolated primary cardiomyocytes. Utilizing mRNA as a payload bypasses the need for nuclear entry, which greatly improves transfection efficiency and subsequent protein expression, specifically in nondividing primary cells (Figures 2 and 3). A detailed protocol is outlined in Figure 4.

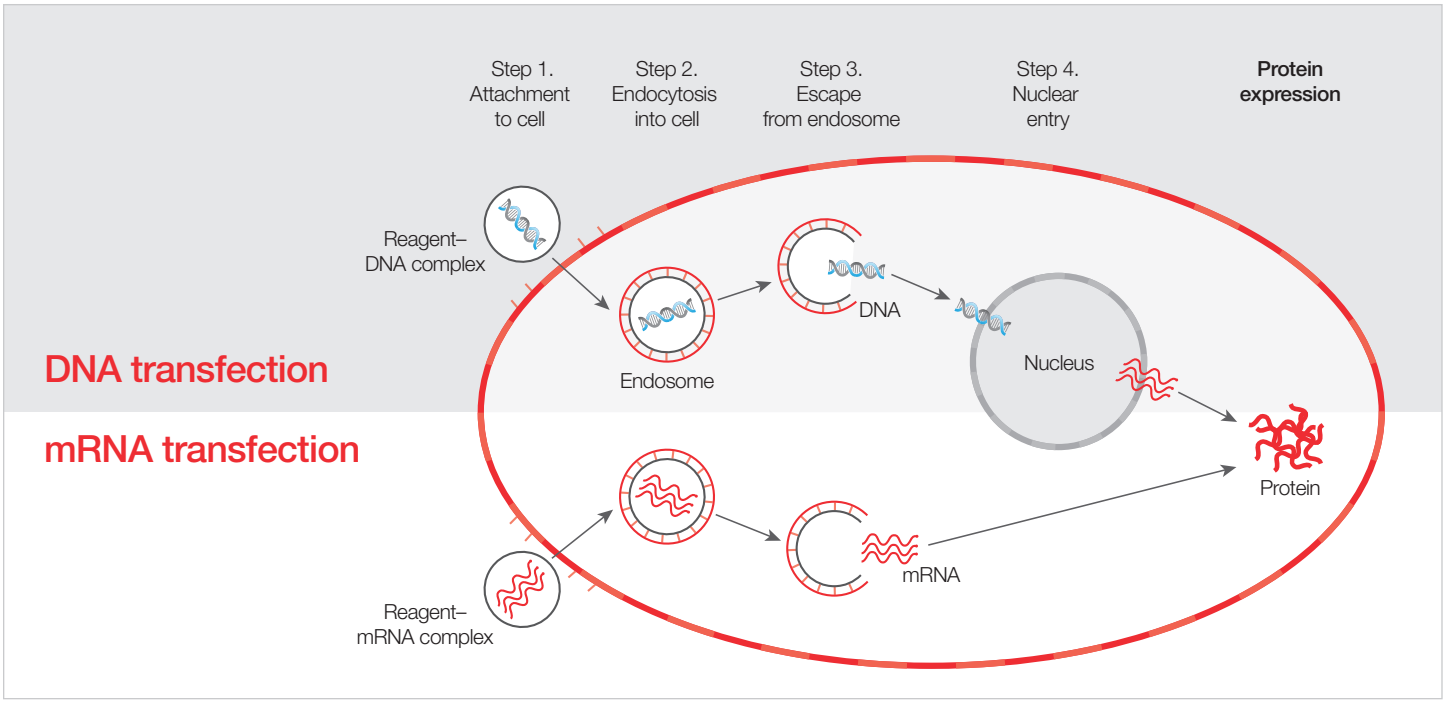


Figure 2. Faster protein expression with no risk of genomic integration. Transfection of mRNA with Lipofectamine MessengerMAX reagent typically results in faster protein expression with greater homogeneity of expression among the transfected cells. Additionally, delivery of mRNA does not require nuclear entry (step 4, DNA transfection), which eliminates the risk of genomic integration and makes transfection effiency cell cycle–independent.

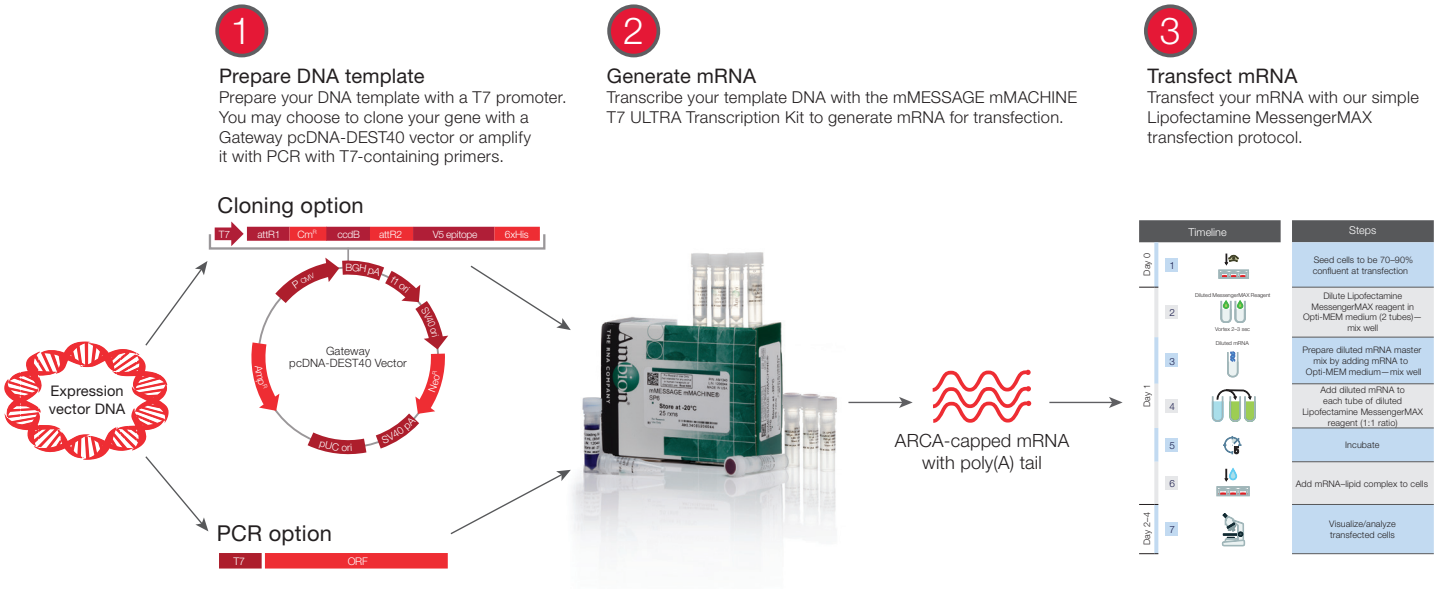


Figure 3. Workflow for mRNA transfection using Lipofectamine MessengerMAX reagent.

High-efficiency transfection of cardiomyocytes

The Lipofectamine MessengerMAX reagent demonstrates high performance in freshly isolated, primary neonatal rat ventricular cardiomyocytes, demonstrating high efficiency and homogeneity of transfection, and significantly outperforming leading DNA transfection reagents. In addition to high transfection efficiency, the reagent has low toxicity to the primary cells, as can be seen by the integrity of the culture system 24 hours posttransfection (Figure 5). Optimization of transfection efficiency, depending on the experimental need of the researcher, can be achieved by modulating plating density, with minimal effect on cell viability (Figure 6).

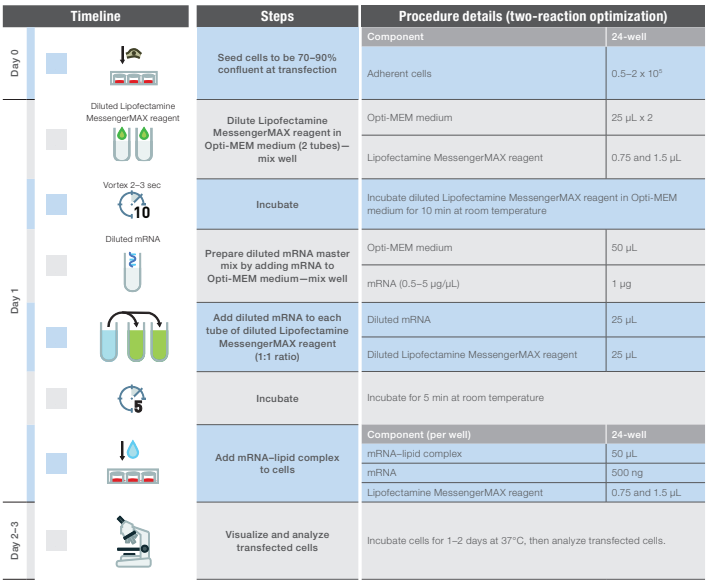


Figure 4. Step-by-step protocol for transfecting cardiomyocytes using Lipofectamine MessengerMAX reagent.

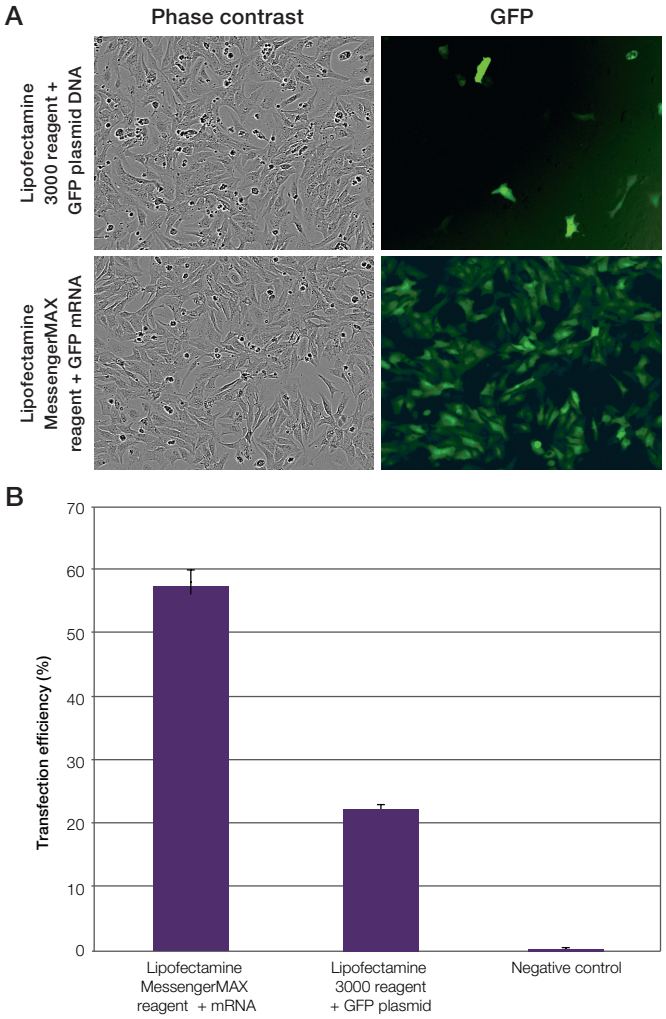


Figure 5. Superior transfection achieved in cardiomyocytes using mRNA transfection vs. DNA transfection. (A) Phase-contrast and fluorescence images are shown for comparison. Fluorescence imaging shows GFP-positive primary cardiomyocytes 24 hours following transfection using either Invitrogen™ Lipofectamine™ 3000 reagent + GFP plasmid DNA, or Lipofectamine MessengerMAX reagent + GFP mRNA. **(B)** Summary of flow cytometry analysis of transfection efficiency demonstrates the high efficiency of transfection achieved using mRNA delivery.

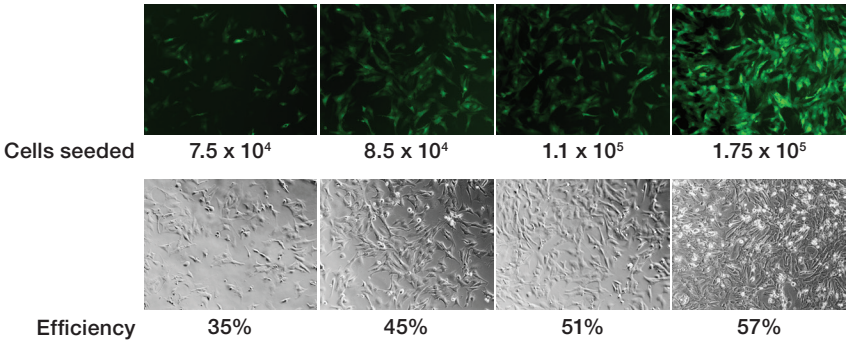


Figure 6. Optimization of mRNA expression. Fluorescence images of primary cardiomyocytes 24 hours after transfection with mRNA + Lipofectamine MessengerMAX reagent, demonstrating increasing transfection efficiency with increasing plating density of the cardiomyocytes. This system overcomes a variety of experimental design constraints, as researchers can still achieve high-efficiency transfection with minimal toxicity at lower plating densities.

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Trade-offs

A comparison of the main transfection techniques that are commonly used for cardiomyocytes highlights the benefits and drawbacks of each system. Electroporation requires physical disruption of the cell, which results in high cell mortality. It also requires specialized instrumentation and corresponding consumables, which can be expensive. Adenoviral overexpression is a highly efficient technique and frequently used for cardiomyocytes; however, there are several drawbacks associated with the use of this methodology. In addition to requiring adherence to specific laboratory guidelines and considerations for handling and use, generating adenoviral constructs is a time-consuming and expensive process. While high expression efficiency is achievable using adenovirus, the ease of use of this method in experimental design is limited to making a separate virus for each individual protein, which can be very time-consuming and high in cost. The practicality of making a library of constructs for mRNA or DNA transfection is more feasible than generating individual virus constructs for each experimental target. Table 1 compares mRNA transfection using Lipofectamine MessengerMAX reagent to the more commonly used transfection techniques that are used for cardiomyocytes.

Table 1. Comparison of different transfection techniques.

Transfection technique	Expression efficiency	Cost effectiveness	Cell viability	Experimental ease of use
Electroporation	++	+	+	++
Adenoviral overexpression	+++	++	+++	+
Leading DNA transfection reagent	+	++	++	+++
Lipofectamine MessengerMAX reagent	+++	+++	+++	+++

Additional applications for cardiomyocyte models

Transfection of human induced pluripotent stem cell (iPSC)-derived cardiomyocytes is an important experimental platform for studying cardiovascular disease. Differentiated iPSCs comprise a very sensitive cell culture system and offer a physiologically relevant model that can be used for translational research. Using mRNA with Lipofectamine MessengerMAX reagent, transfection efficiency greater than >30% was achieved in these cells as seen from the fluorescence images taken 18 hours following transfection with GFP mRNA (Figure 7).

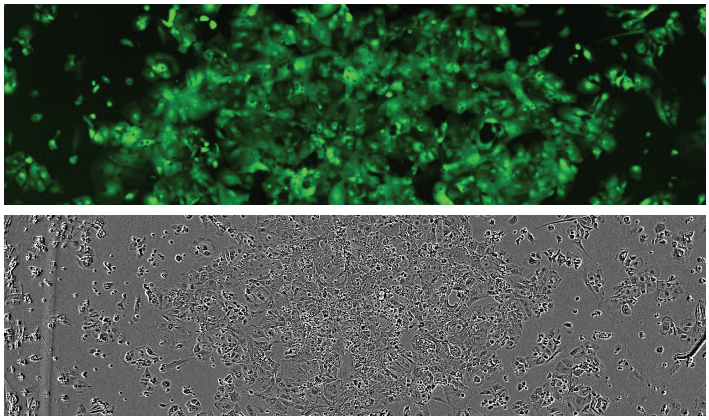

Figure 7. High-efficiency mRNA transfection in iPSC-derived cardiomyocytes using Lipofectamine MessengerMAX reagent.

Conclusions

As demonstrated here, Lipofectamine MessengerMAX reagent offers a highly effective solution for protein overexpression in difficult-to-transfect primary cell models, providing several benefits including ease of use and high efficiency. This reagent is very gentle on isolated primary cardiomyocytes and differentiated cardiomyocytes, compared to other commonly used transfection methods. While several methodologies exist for transfecting cardiomyocytes, we recommend Lipofectamine MessengerMAX reagent as the primary gene delivery solution.

See all of our application notes at
thermofisher.com/transfectionbasics

Find out more about Lipofectamine MessengerMAX reagent at **thermofisher.com/messengermax**

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