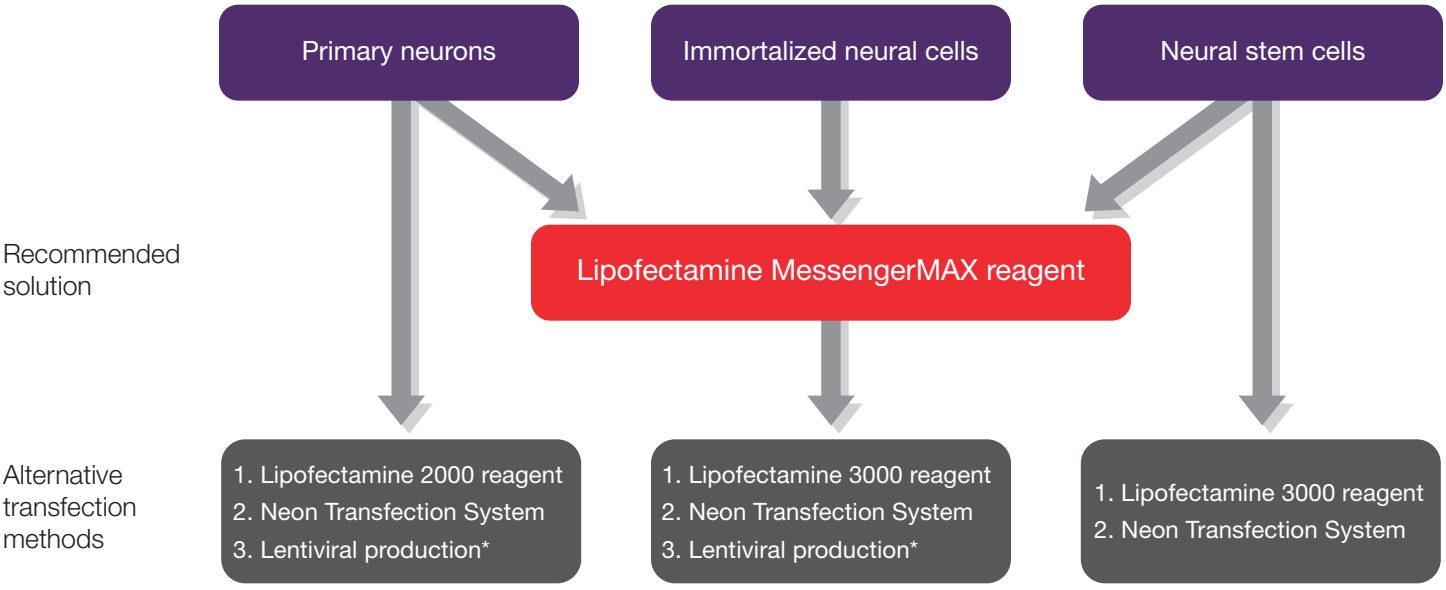
Neurobiology transfection guide

Introduction

The field of neurobiology is rapidly growing, with researchers providing new insights into a variety of areas essential for human health, including memory, mood disorders, aging, and disease. As the field moves toward more physiologically relevant models, such as primary neurons and small animal models, the need for new nucleic acid delivery tools is rapidly increasing. One of the greatest challenges is the ability to manipulate the desired cell models that are traditionally difficult to transfect, including primary neurons and neural stem cells. Methods typically used have not met these needs—reagent-mediated DNA transfection is inefficient for nuclear entry; electroporation requires cell disruption and special instrumentation, and is expensive; and virus-mediated transduction can be very time-consuming [1].

To facilitate the use of more relevant cell models, we have developed Invitrogen™ Lipofectamine™ MessengerMAX™ Transfection Reagent, which we recommend as the primary gene delivery solution (Figure 1). Here we provide an overview for how Lipofectamine MessengerMAX reagent is used for high-efficiency transfection of neuronal cell models, enabling improved results and simplified workflows for applications such as genome editing. We also compare Lipofectamine MessengerMAX reagent to other gene delivery solutions and highlight the advantages and drawbacks of each approach.



*Refer to "Lentiviral production" (page 4) for details on high-efficiency lentiviral production using Lipofectamine 3000 reagent.
Note: The numbers in the alternative transfection methods correspond to our recommended order.

Figure 1. Recommended transfection methods, by cell type. The high transfection efficiency of Lipofectamine MessengerMAX reagent makes it the preferred choice for transfection of neuronal cell models. For researchers interested in other delivery methods, we also offer Lipofectamine™ reagents for DNA transfection and lentiviral production.

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Protocol overview

Lipofectamine MessengerMAX reagent is an mRNA transfection reagent that offers superior transfection efficiency in primary neurons, neural stem cells, and immortalized neural cells. Transfection of mRNA only requires entry into the cell cytoplasm, not the nucleus, and therefore greatly improves transfection efficiency in post-mitotic cells or slowly dividing cells (Figure 2). This mechanism also allows mRNA transfection to be utilized for novel applications such as genome editing where genomic integration is a major concern (see “Genome editing” on page 5 for further discussion).

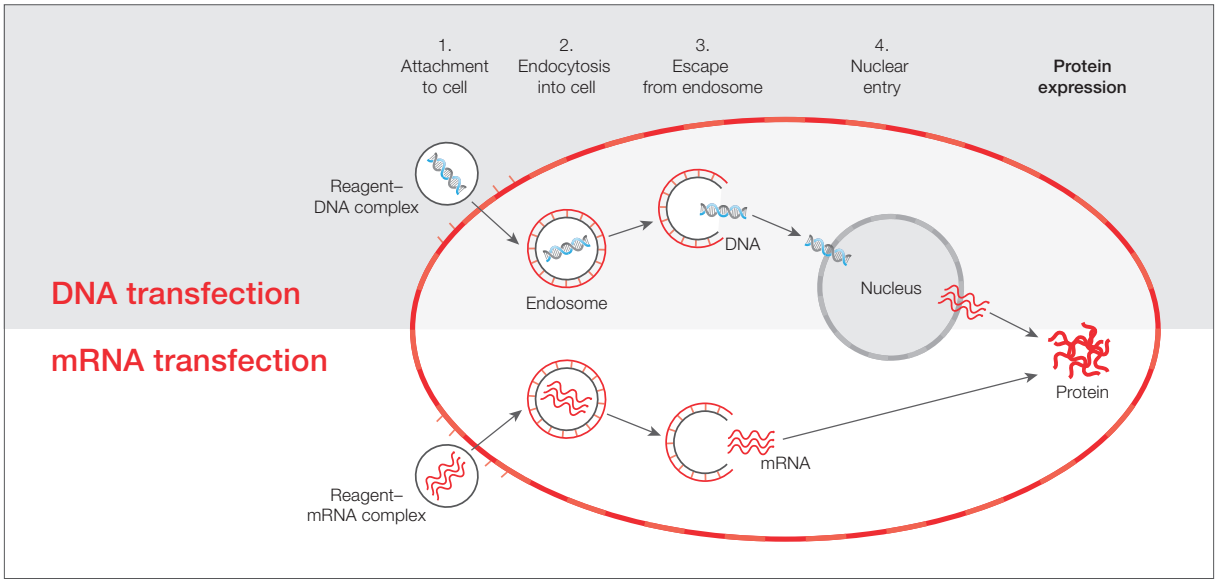


Figure 2. Faster protein expression with no risk of genomic integration. Transfection of mRNA with Lipofectamine MessengerMAX reagent typically results in faster protein expression with greater homogeneity of expression among the transfected cells. Additionally, delivery of mRNA does not require nuclear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

mRNA transfection—it’s as easy as 1-2-3

Any plasmid or PCR product with the gene of interest regulated by a T7 promoter can be used as the template for mRNA preparation (Figure 3). The mMESSAGE mMACHINE™ T7 Ultra Transcription Kit (Cat. No. AM1345) is recommended for high-yield synthesis of mRNA transcripts containing a 5´ cap and poly(A) tail for transfection. Even though mRNA transfection has become a widely used approach for gene delivery in nondividing cells, some misconceptions still remain about the methods involved (Table 1).

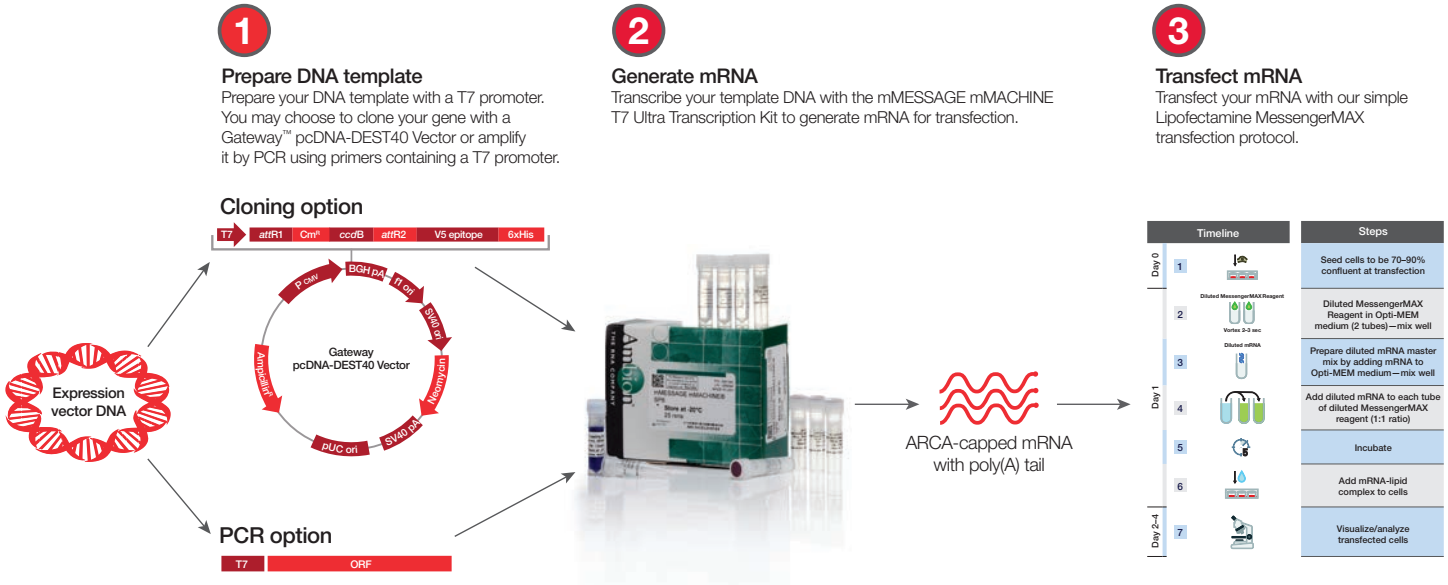


Figure 3. Workflow for mRNA transfection using Lipofectamine MessengerMAX reagent.

Table 1. Common misconceptions about mRNA transfection.

Misconception	Truth
mRNA is unstable and degrades quickly, requiring special handling	• A few simple precautions can help ensure mRNA stability: use RNase-free reagents and tips, aliquot and store mRNA at –80°C, and keep mRNA on ice when in use [2].
mRNA is difficult to prepare	• The mMESSAGE mMACHINE T7 Ultra Transcription Kit provides 20–40 µg of ready-to-use mRNA. • Once prepared, the mRNA can be safely stored for later use in multiple transfection experiments.
Transfection is much easier to perform with DNA than mRNA	• The protocol for Lipofectamine MessengerMAX reagent is straightforward, requiring the same basic steps as protocols for DNA transfection reagents, and we offer a positive control GFP mRNA that can be used to evaluate the system. • For difficult-to-transfect nondividing or primary cells, mRNA transfection obviates the need for nuclear entry and improves protein expression and homogeneity [3].
Exogenous mRNA elicits an immune response	• Chemically modified mRNAs, with 5-methylcytidine and pseudouridine modifications, dramatically reduce innate immune response and improve mRNA translation [4].

High transfection efficiency for neural cell models

Lipofectamine MessengerMAX reagent delivers outstanding transfection efficiency in neural cell models, including primary neurons, human neural stem cells (hNSCs), and immortalized neural cell lines (Figure 4). The high transfection efficiency and homogeneity of expression among transfected cells enables improved application outcomes for a broad range of experiments.

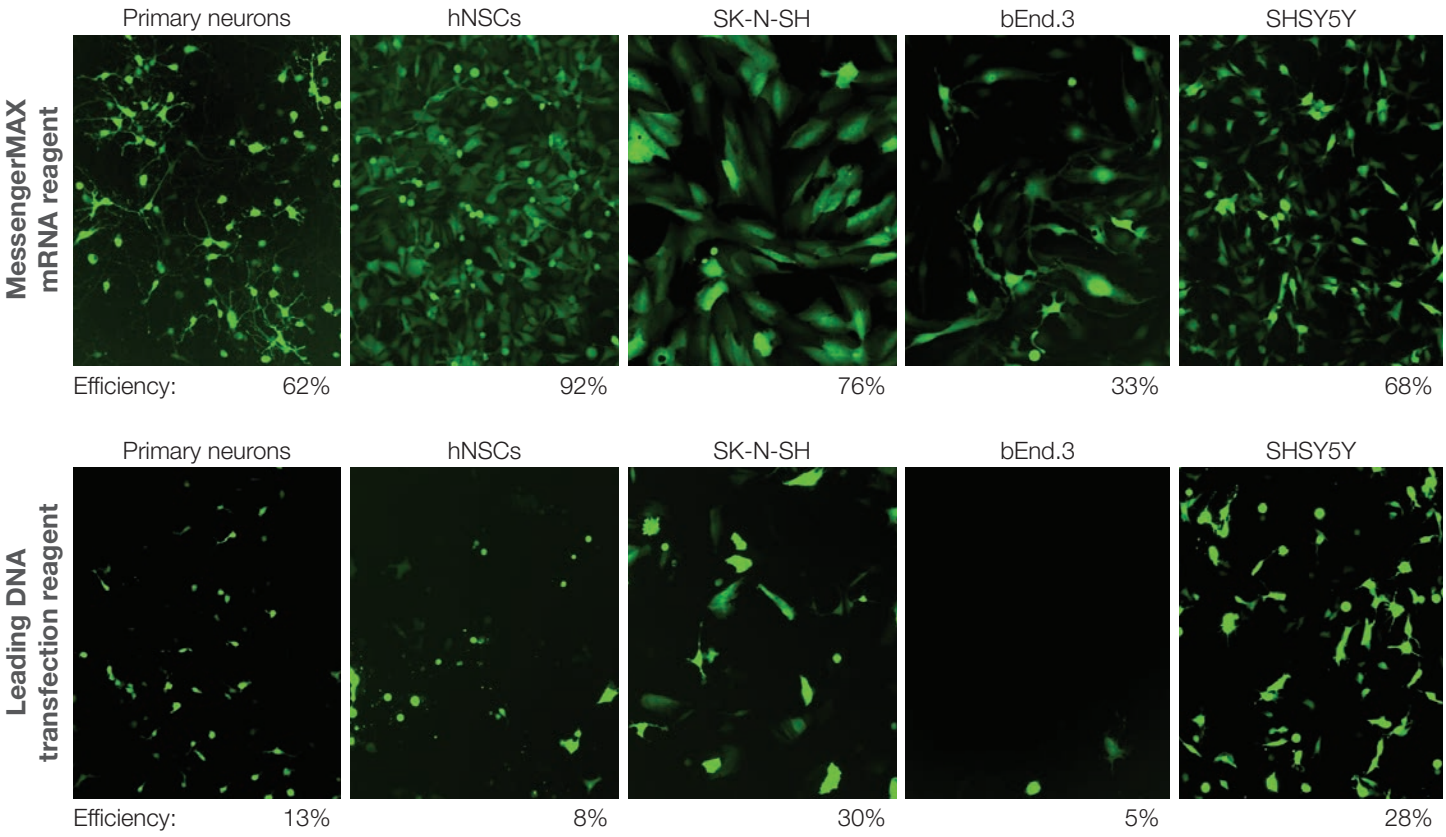


Figure 4. Transfection of neural cell models. Lipofectamine MessengerMAX reagent was used to deliver mRNA encoding GFP (500 ng/well) in primary cortical neurons (freshly isolated from E16 mouse; 5 days *in vitro*), SK-N-SH cells (neuroblastoma), bEnd.3 cells (endothelial polyoma from cerebral cortex), and SHSY5Y cells (neuroblastoma) in a 24-well format. For Gibco™ Human Neural Stem Cells (hNSCs), 250 ng/well of mRNA was used in a 48-well format. Results are compared to plasmid DNA delivered with the leading DNA transfection reagent. GFP expression was analyzed 24 hours posttransfection.

Product selection guide

We offer a variety of other gene delivery options based on your research needs, including lentiviral delivery and physical methods such as electroporation. However, there are advantages and limitations for each method based on the cell models being used and the end goal of the experiment: gene expression or gene knockdown. An overview of our most effective delivery solutions for neurobiology research is shown in Table 2.

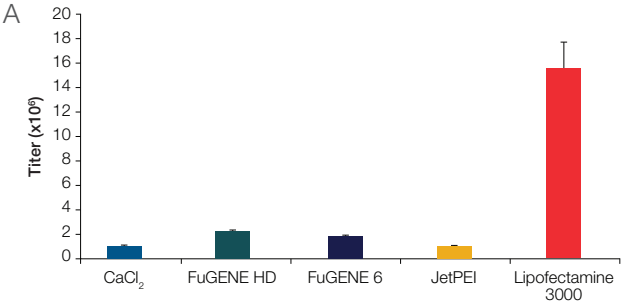
Table 2. Gene delivery products for neurobiology research by nucleic acid type.

Product	Nucleic acid type	Cell model	Performance	Cell viability	Ease of use
Lipofectamine MessengerMAX reagent	**mRNA**	**Primary cells, stem cells, and difficult-to-transfect cells**	**+++**	**++++**	**++++**
Lipofectamine 3000 reagent	DNA	Easy- and difficult-to-transfect immortalized cell lines	++	++++	++++
Lipofectamine 2000 reagent	DNA	Easy-to-transfect cells, primary neurons	+	++++	++++
Neon Transfection System	DNA, mRNA, siRNA	All neuronal cell models	++++	+++	+++
Lipofectamine 3000 reagent for lentiviral production	DNA	Difficult-to-transfect cells (viral delivery)	++++	+++	++
Invivofectamine 3.0	siRNA, mRNA	*In vivo* direct brain injection	++	++	++
Lipofectamine RNAiMAX reagent	siRNA	All neuronal cell models (gene knockdown)	++++	++++	++++

Lentiviral production

If viral delivery is the preferred method for transfecting neurons, Invitrogen™ Lipofectamine™ 3000 Transfection Reagent can be used as a highly efficient tool for lentiviral production. This versatile reagent enables high viral titers even with genes that are large or difficult to package. Production of high-quality, high-titer lentivirus can be achieved while minimizing time and reagent usage. Higher viral titers are obtained with Lipofectamine 3000 reagent than with other commonly used transfection reagents, even with larger gene sizes (Figure 5). However, viruses present challenges in production and handling, making mRNA transfection an attractive alternative to lentiviral delivery.

A



B

Production cell line	Insert size	Comparative increase in viral titer			
		Lipofectamine 3000 vs. FuGENE™ HD	Lipofectamine 3000 vs. $CaCl_2$	Lipofectamine 3000 vs. FuGENE™ 6	Lipofectamine 3000 vs. JetPEI™ reagent
293FT	0.7 kb	3x	26x	9x	4x
	3 kb	8x	16x	9x	17x
293T	0.7 kb	4x	10x	5x	1x
	3 kb	4x	10x	4x	4x

Figure 5. Lipofectamine 3000 reagent performs significantly better than leading competitor reagents. (A) Titers obtained using Lipofectamine 3000 reagent compared to commonly used transfection reagents in 293FT cells using a 3 kb insert gene. **(B)** Summary of increases in titer levels using two different cell lines and insert sizes.

Emerging transfection segments

In vivo delivery

There is considerable interest in using *in vivo* delivery of siRNA and mRNA to better understand diseases and for research as therapeutic molecules [5,6]. Current technologies such as viral or hydrodynamic delivery are powerful but can be time-consuming and expensive. Invitrogen™ Invivofectamine™ 3.0 Reagent is an animal origin–free transfection reagent designed for *in vivo* delivery of siRNA and miRNA to hepatocytes following tail-vein injection. Complexes of Invivofectamine 3.0 Reagent and siRNA are prepared and delivered using a simple procedure with no measured toxicity or stress response in the animal. Recently, we have also demonstrated that Invivofectamine 3.0 Reagent can successfully deliver mRNA to the liver, spleen, muscle, and xenograft tumors, making this reagent a promising tool for gene delivery to other areas of the body, including direct injection to the brain (Figure 6) [7].

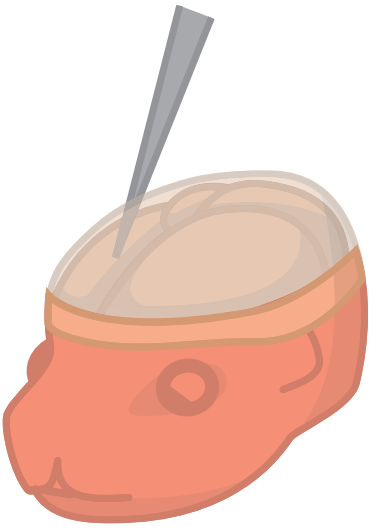
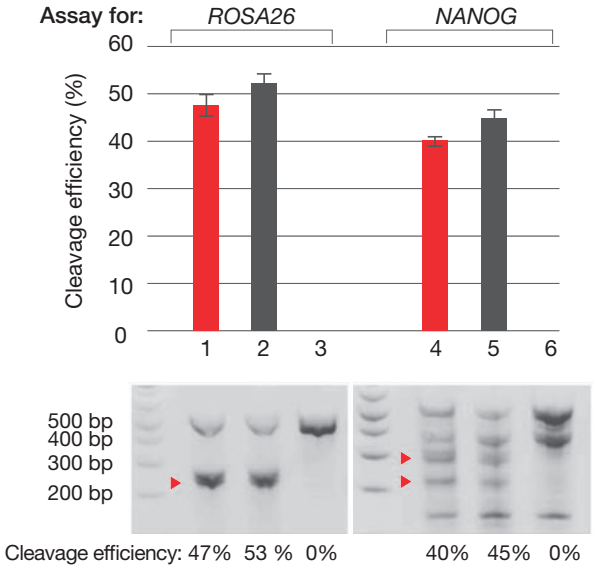


Figure 6. Invivofectamine 3.0 Reagent for gene targeting and knockdown in brain. Invivofectamine 3.0 complexes, prepared according to the reagent protocol, can be safely injected directly into the brain for gene targeting and gene knockdown experiments according to institutional guidelines for the care and use of animals in research.

Genome editing

mRNA transfection is widely used for genome editing using the CRISPR-Cas9 system (Figure 7). The effectiveness of the CRISPR-Cas9 system is contingent upon maximum expression of the Cas9 nuclease and guide RNA (gRNA) that targets the genomic locus of interest. Precise cleavage of DNA at a specific locus allows for the knockout of a specific gene or the insertion of a new sequence using endogenous cellular repair mechanisms.

A



CRISPR-Cas9 format	Lane	
	ROSA26	*NANOG*
All-in-one plasmid	1	4
Cas9 mRNA + IVT gRNA	2	5
Control (Cas9 mRNA only)	3	6

B

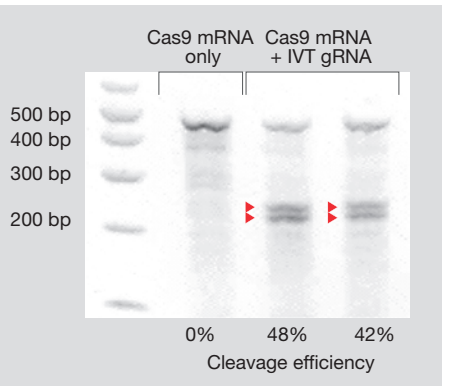


Figure 7. Lipofectamine MessengerMAX reagent enables efficient genome editing using the CRISPR-Cas9 system. (A) Mouse Neuro 2A cells were transfected with Invitrogen™ GeneArt™ CRISPR Nuclease mRNA encoding Cas9 and *in vitro*–transcribed (IVT) gRNA targeting the *ROSA26* or *NANOG* locus in a 24-well plate format. Results are compared to an all-in-one plasmid expressing both Cas9 and gRNA that was transfected into cells using Lipofectamine 3000 reagent. **(B)** Induced pluripotent stem cells were transfected with GeneArt CRISPR Nuclease mRNA and IVT gRNA. Cells were harvested 72 hours posttransfection and genome editing efficiency was assessed using the Invitrogen™ GeneArt™ Genomic Cleavage Detection Kit. The cleavage products are indicated with arrowheads.

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Conclusions

As we have shown, mRNA transfection with Lipofectamine MessengerMAX reagent provides numerous benefits when working with difficult-to-transfect cell models, including ease of use and superior efficiency. This reagent is also gentle on cells and enables high cleavage efficiency when used with genome editing tools such as the CRISPR-Cas9 system. Therefore, while there are many options for transfecting neurons, we recommend Lipofectamine MessengerMAX reagent as the primary gene delivery solution.

Additional resources

Detailed Lipofectamine MessengerMAX reagent protocol and product information: **thermofisher.com/messengermax**

Lentiviral production protocol using Lipofectamine 3000 reagent: **thermofisher.com/3000**

Invivofectamine 3.0 Reagent for *in vivo* delivery: **thermofisher.com/invivofectamine**

Protocol for isolation and transfection of mouse primary neurons: **https://www.thermofisher.com/us/en/home/references/protocols/neurobiology/neurobiology-protocols/isolation-of-mouse-primary-neurons.html**

References

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Find out more at **thermofisher.com/transfection**

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