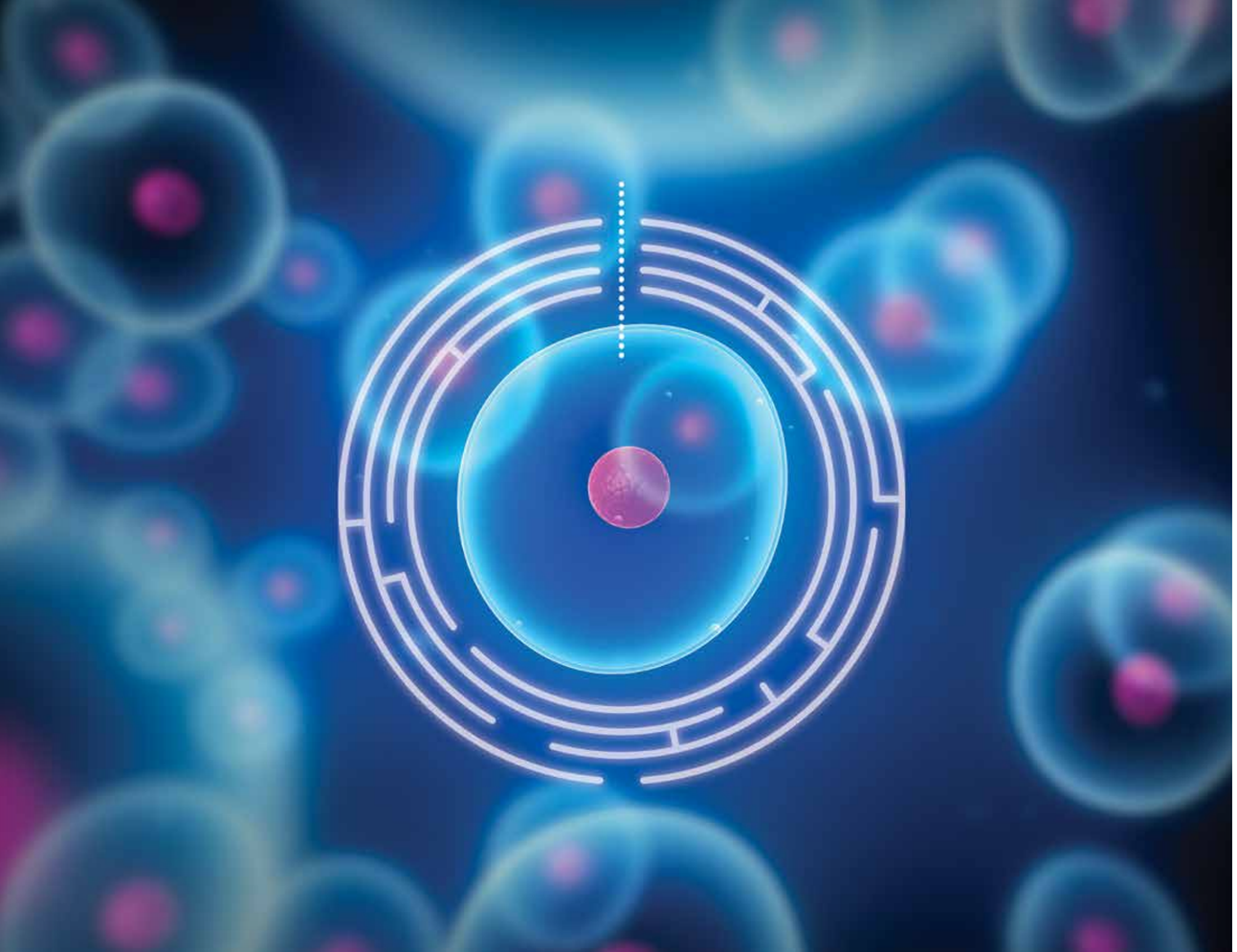
invitrogen by Thermo Fisher Scientific

Transfection and genome engineering

Innovation delivered

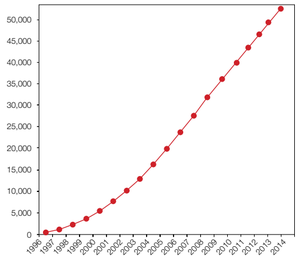
Fisher Scientific



Contents

Transfection selection guide

Transfection is the process by which nucleic acids are introduced into eukaryotic cells. Techniques vary widely and include lipid-based transfection and physical methods such as electroporation. Lipofectamine® transfection reagents are the most trusted and cited in the scientific literature due to their superior transfection performance and broad cell spectrum. An overview of our most effective transfection products is shown in Table 1 to help you choose the solution that's right for you.



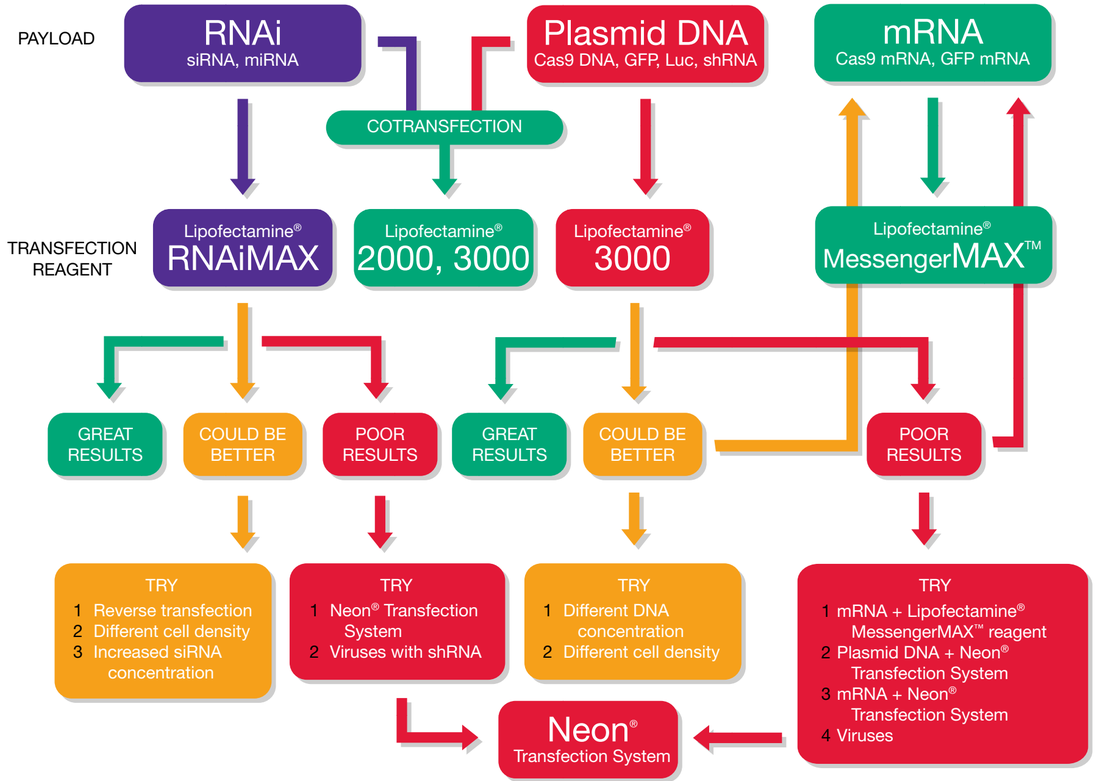
Cumulative number of publications citing the use of Lipofectamine® family of reagents since 1996.

Table 1. Transfection selection guide—more blocks represent higher transfection efficiency into a greater number of cell types.

Transfection product	DNA	mRNA	RNAi	Co-delivery*	Easy-to-transfect cells	Difficult-to-transfect cells	Primary cells	Stem cells	Suspension cells
Superior transfection reagents									
Lipofectamine® 3000 Transfection Reagent	■		■	■	■■■■	■■■■	■■■□	■■■□	■■□□
Lipofectamine® RNAiMAX Transfection Reagent			■		■■■■	■■■■	■■■□	■■■□	■■□□
Lipofectamine® MessengerMAX™ Transfection Reagent		■			■■■■	■■■■	■■■■	■■■□	■■□□
ExpiFectamine™ 293 Transfection Kit	■				Recommended for Expi293F™ cells				
Broad-spectrum transfection reagent									
Lipofectamine® 2000 Transfection Reagent	■		■	■	■■■□	■■□□	■■□□	■■□□	■□□□
Electroporation									
Neon® Transfection System	■	■	■	■	■■■■	■■■■	■■■□	■■■□	■■■□
***In vivo* delivery**									
Invivofectamine 3.0 Reagent		■	■		*In vivo* delivery to liver following tail vein injection				

*Cotransfection of RNAi vector and siRNA.

Transfection decision tree



Superior transfection reagents

Lipofectamine® 3000 Transfection Reagent

Lipofectamine® 3000 reagent leverages our most advanced lipid nanoparticle technology to enable superior transfection performance and reproducible results. It delivers exceptional transfection efficiency into the widest range of difficult-to-transfect and common cell types (Figures 1 and 2) with improved cell viability.

Lipofectamine® 3000 reagent offers:

- **Superior transfection efficiency—**into the broadest spectrum of difficult-to-transfect cell types
- **Improved cell viability—**gentle on your cells, with low toxicity

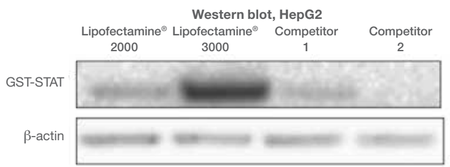


Figure 1. Enhanced protein expression using Lipofectamine® 3000 reagent. HepG2 cells were transfected with a vector expressing GST-tagged STAT protein. A western blot was performed to determine the level of expression using β-actin as a control.

"We were very happy and surprised to see Lipofectamine® 3000 (reagent) provide a more than 10-fold difference in transfection efficiency in our difficult-to-transfect cell line. There was even a reduction in cell death. Awesome results!"
–Rui Eduardo Castro, PhD
University of Lisbon

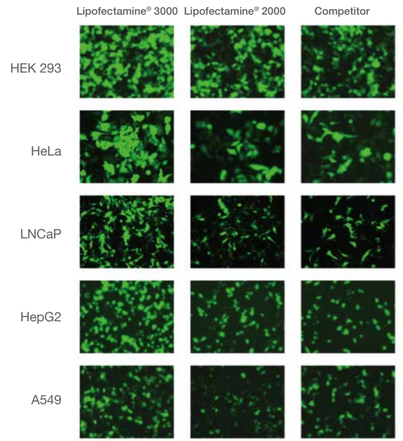


Figure 2. Lipofectamine® 3000 reagent outperforms Lipofectamine® 2000 and competitor reagents. Each reagent was used to transfect HEK 293, HeLa, LNCaP, HepG2, and A549 cell lines in a 96-well format, and green fluorescent protein (GFP) expression was analyzed 48 hours posttransfection. Lipofectamine® 3000 reagent provided higher GFP transfection efficiency than Lipofectamine® 2000 and competitor reagents for all five cell lines.

Gentle with low toxicity

Lipofectamine® 3000 reagent was designed to optimize every step in the transfection process. The superior transfection performance allows you to reduce the reagent dose and improve cell viability when working with your cell line of interest (Figure 3).

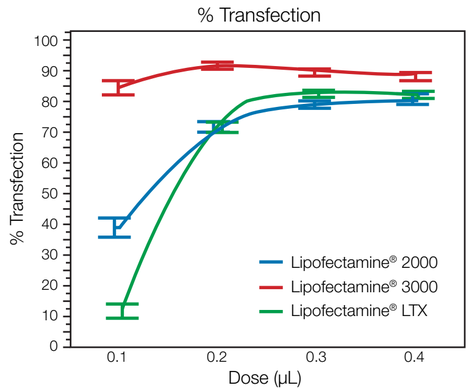


Figure 3. Superior transfection efficiency of Lipofectamine® 3000 reagent at low doses. Each reagent was used to transfect HeLa cells in a 96-well format at the indicated doses with an emerald green fluorescent protein (emGFP)–expressing vector. Analysis was performed 48 hours posttransfection using flow cytometry to determine percent transfection efficiency. Lipofectamine® 3000 reagent delivered higher efficiency than both Lipofectamine® 2000 reagent and Lipofectamine® LTX reagent.

Enhance your cancer research

The superior transfection efficiency and broad cell spectrum of Lipofectamine® 3000 reagent enables excellent flexibility to use your cell line of choice in cancer studies (Figure 4).

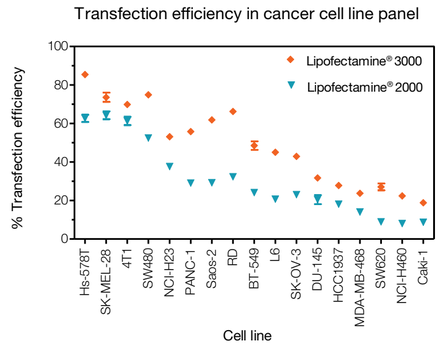


Figure 4. Transfection efficiency in a cancer cell line panel. Lipofectamine® 2000 reagent and Lipofectamine® 3000 reagent were used to transfect 17 cell lines with a GFP-expressing plasmid in a 24-well plate format using 0.5 µg plasmid/well and the recommended protocols for each reagent. GFP expression was analyzed 48 hours posttransfection. Each condition was tested in triplicate, and the data points with error bars show mean transfection efficiency with standard deviation.

Generate induced pluripotent stem cells

Induced pluripotent stem cells (iPSCs) hold immense promise for the future of regenerative medicine and personalized therapeutic treatments for a myriad of diseases and conditions. The superior transfection efficiency of Lipofectamine® 3000 reagent in conjunction with the Epi5™ Episomal iPSC Reprogramming Kit enables highly efficient reprogramming of somatic cells without the need for electroporation (Figure 5).

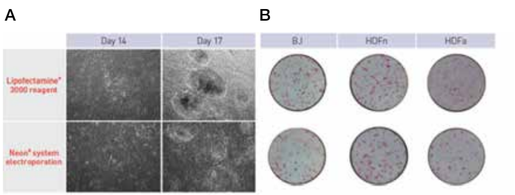


Figure 5. Reprogramming efficiency of Lipofectamine® 3000 reagent compared to electroporation. BJ fibroblasts as well as neonatal (HDFn) and adult (HDFa) human dermal fibroblasts were reprogrammed to iPSCs by transfection of Epi5™ vectors using either Lipofectamine® 3000 reagent or the Neon® Transfection System. Colonies were visualized by **(A)** brightfield microscopy and **(B)** stained for alkaline phosphatase.

Improve gene editing outcomes

Lipofectamine® 3000 reagent was developed to break through the boundaries of traditional delivery methods and facilitate new technologies, such as genome engineering, in more biologically relevant systems. With this reagent, GeneArt® CRISPR vectors targeting the *AAVS1* locus in HepG2 and U2OS cells show improved transfection efficiency (Figure 6), mean fluorescence intensity, and genomic cleavage. High transfection and genome editing efficiency is also observed with GeneArt® Precision TALs. These advancements in delivery help minimize painstaking downstream workflows, enable easier stem cell manipulation, and enhance site-specific insertion of transgenes into the genome.

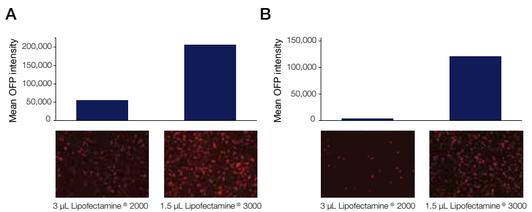
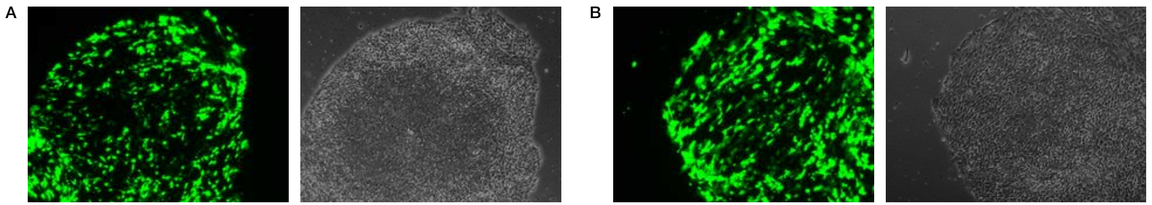


Figure 6. Transfection efficiency and protein expression using GeneArt® CRISPR Nuclease Vector. The vector contained an OFP reporter gene and was transfected with Lipofectamine® 2000 or Lipofectamine® 3000 reagent into **(A)** U2OS and **(B)** HepG2 cell lines. Bar graphs show reporter gene expression; images show fluorescence of corresponding cells expressing OFP.

Transfect stem cells

Lipofectamine® 3000 reagent was developed to unleash the power of stem cells by providing a highly efficient, cost-effective nucleic acid delivery alternative to electroporation (Figure 7). This advanced lipid nanoparticle technology minimizes the stress on cells caused by electroporation, simplifies the reprogramming workflow, and enables advanced gene-editing technologies.



DNA: 1.0 µg
Lipofectamine® 3000: 1.5 µL
SSEA4+/GFP+: 42%
GFP MFI 247344

DNA: 1.3 µg
Lipofectamine® 3000: 1.5 µL
SSEA4+/GFP+: 69%
GFP MFI 456741

Figure 7. Transfection of stem cells. (A) H9 embryonic stem cells (ESCs) or **(B)** iPSCs were transfected using Lipofectamine® 3000 reagent. Cells were visualized by fluorescence microscopy and processed using flow cytometry to determine transfection efficiency.

Lipofectamine® RNAiMAX Transfection Reagent

Lipofectamine® RNAiMAX reagent offers an advanced, efficient solution for siRNA or miRNA delivery. No other transfection reagent for RNA interference (RNAi) experiments provides such easy and efficient delivery into a broad spectrum of cell types including primary cells, stem cells, and other hard-to-transfect cell types (Figure 8).

Superior transfection efficiency at low siRNA concentrations

When it comes to achieving effective gene knockdown, Lipofectamine® RNAiMAX reagent easily outperforms other siRNA transfection reagents. High knockdown levels of target genes can be achieved with as little as 1 nM siRNA.

Low cytotoxicity profile for easy optimization

Lipofectamine® RNAiMAX reagent enables maximal knockdown and excellent cell viability across a 10-fold concentration range of the reagent. This makes Lipofectamine® RNAiMAX reagent easy to optimize for the lowest siRNA concentration that can be used while reducing cytotoxicity in your experimental system. Transfection-mediated cytotoxicity can mask the true phenotype of a target gene being studied, so minimizing the amount of reagent during transfection is a critical factor for successful RNAi experiments.

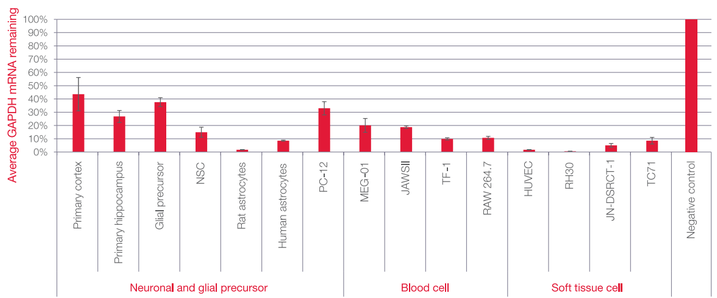


Figure 8. *Silencer*® Select siRNA transfection with Lipofectamine® RNAiMAX. Cells were transfected with Lipofectamine® RNAiMAX complexed with *Silencer*® Select siRNA at 30 nM/well. Knockdown of GAPDH mRNA was assessed by qPCR. The cell density per well and amount of Lipofectamine® RNAiMAX used per well were as follows: rat primary cortex cells: 10^4 cells/well, 0.6 µL; rat primary hippocampus cells: 10^4 cells/well, 0.6 µL; rat glial precursor cells: 8 x 10^3 cells/well, 0.4 µL; neuroblastoma-spinal cord (NSC) cells: 1.2 x 10^4 cells/well, 0.3 µL; rat astrocyte cells: 1.2 x 10^4 cells/well, 0.3 µL; human astrocyte cells: 8 x 10^3 cells/well, 0.3 µL; PC-12 cells (derived from a pheochromocytoma of the rat adrenal medulla): 8 x 10^3 cells/well, 0.3 µL; megakaryoblastic leukemia cells (MEG-01): 2 x 10^4 cells/well, 0.3 µL; murine dendritic cells (JAWSII): 1.2 x 10^4 cells/well, 0.3 µL; human erythroleukemia cells (TF-1): 10^4 cells/well, 0.3 µL; RAW 264.7 macrophages: 10^4 cells/well, 0.3 µL; human umbilical vein endothelial cells (HUVEC): 8 x 10^3 cells/well, 0.3 µL; rhabdomyosarcoma cells (RH30): 8 x 10^3 cells/well, 0.3 µL; human desmoplastic small round cell tumor cells (JN-DSRCT-1): 8 x 10^3 cells/well, 0.3 µL; human Ewing's sarcoma cells (TC71): 1.6 x 10^4 cells/well, 0.3 µL.

To learn more, go to **lifetechnologies.com/rnaimax**

Lipofectamine® MessengerMAX™ Transfection Reagent

Lipofectamine® MessengerMAX™ mRNA Transfection Reagent delivers amazing transfection efficiency in neurons and a broad spectrum of primary cells, enabling improved application outcomes and more biologically relevant research (Figure 9). That's because our novel lipid nanoparticle technology is optimized to deliver the highest amount of mRNA possible without the nuclear entry step that is required with DNA.

Successfully transfect more predictive cell models with a reagent that provides:

- Amazing transfection efficiency in neurons and primary cell types
- Faster protein expression with no risk of genomic integration
- Up to 10x higher cleavage efficiency using CRISPR mRNA

"With Lipofectamine® MessengerMAX™ (reagent), I can transfect Cas9 and CRE recombinase mRNA into preadipocytes and bone marrow–derived mesenchymal stem cells at high efficiency and without the toxic effects of viral vectors."
–Daniel Cohen, PhD
University of Pennsylvania

Amazing transfection efficiency in neurons and primary cell types

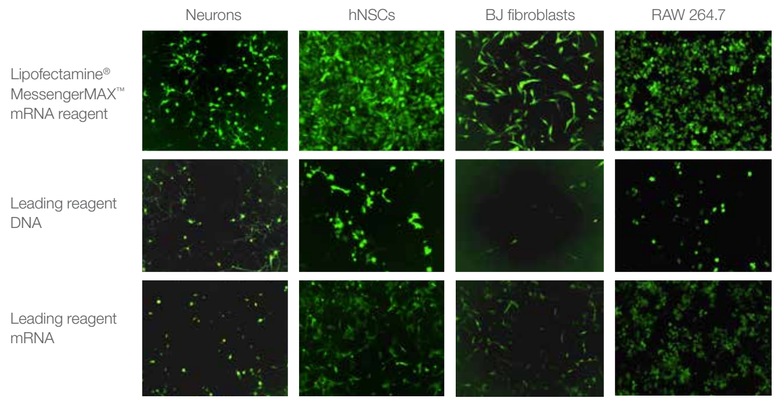


Figure 9. Lipofectamine® MessengerMAX™ mRNA reagent outperforms a leading DNA delivery reagent and a leading mRNA delivery reagent in fresh isolated mouse cortical neurons, hNSCs, BJ fibroblasts, and RAW 264.7 cells. For all cells except hNSCs, Lipofectamine® MessengerMAX™ reagent and the leading mRNA delivery reagent were used to deliver GFP mRNA (500 ng/well) in a 24-well format. For all cells except hNSCs, the leading DNA delivery reagent was used to deliver GFP DNA (500 ng/well), and GFP expression was analyzed 24 hours posttransfection. For hNSCs, Lipofectamine® MessengerMAX™ reagent and the leading mRNA delivery reagent were used to deliver GFP mRNA (250 ng/well) in a 48-well format. The leading DNA delivery reagent was used to deliver GFP DNA (250 ng/well), and GFP expression was analyzed 24 hours posttransfection.

Faster protein expression with no risk of genomic integration

Transfection of mRNA with Lipofectamine® MessengerMAX™ reagent results in faster protein expression because translation of mRNA occurs in the cytoplasm. Additionally, delivery of mRNA does not require nuclear entry (Figure 10, step 4), which eliminates the risk of genomic integration, and transfection efficiency becomes cell cycle–independent.

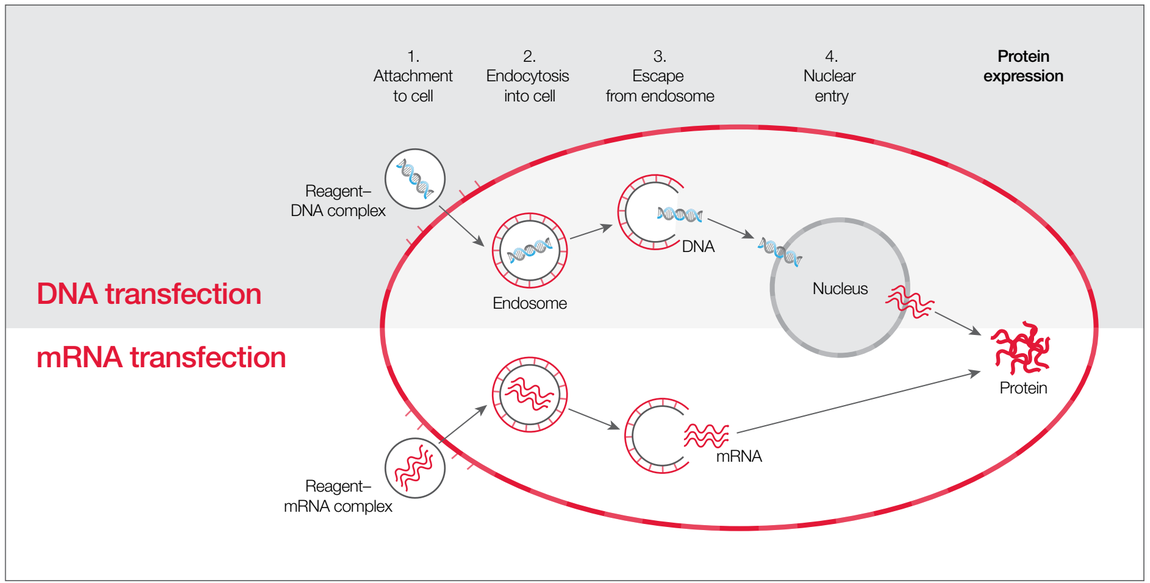


Figure 10. DNA vs. mRNA transfection.

Getting started is as easy as 1-2-3!

1 Prepare DNA template
Prepare your DNA template with a T7 promoter. You may choose to clone your gene with a Gateway® pcDNA™-DEST40 Vector or amplify it with PCR and T7-containing primers.

2 Generate mRNA
Transcribe your template DNA with the mMESSAGE mMACHINE® T7 ULTRA Transcription Kit to generate mRNA for transfection.

3 Transfect mRNA
Transfect your mRNA with our simple Lipofectamine® MessengerMAX™ transfection protocol.

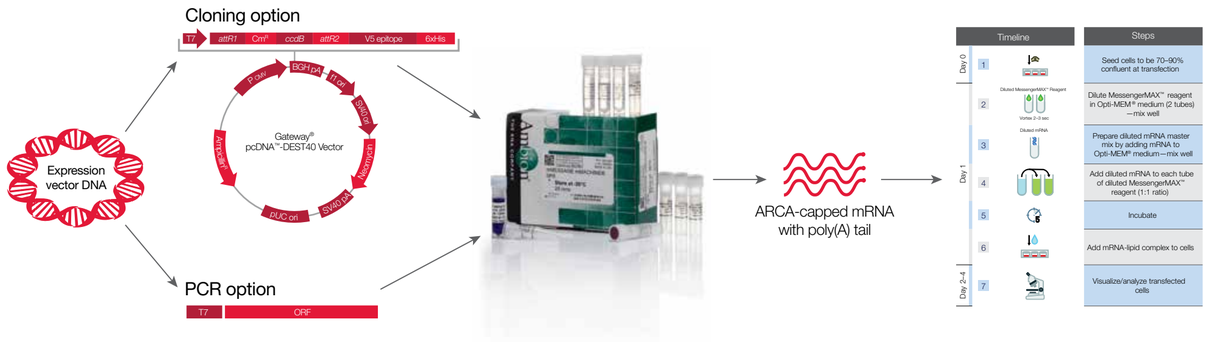


Figure 11. mRNA transfection workflow.

To learn more, go to **lifetechnologies.com/messengermax**

ExpiFectamine™ 293 Transfection Kit

The ExpiFectamine™ 293 Transfection Kit is a core component of the Expi293™ Expression System. It is designed for transient transfection of high-density cultures of human embryonic kidney (HEK) 293 cells. The high-efficiency, cationic, lipid-based transfection reagent and transfection enhancers are designed to power the highest possible level of protein expression from Expi293F™ Cells cultured in Expi293™ Expression Medium.

The ExpiFectamine™ 293 Transfection Kit:

- Is designed specifically for transfection of high-density suspension cells in culture, with enhancers that boost transfection performance and protein expression
- Achieves protein yields 2- to 10-fold higher than other transfection reagents that are used with high-density HEK 293 cell cultures (Figure 12)
- Employs the same transient expression protocols typically used in current low-density HEK 293 suspension culture systems, allowing you to to easily switch from low-density systems to the Expi293™ Expression System
- Provides robust and reproducible transfection results, giving you greater confidence in results
- Allows you to scale transfections for culture volumes less than 1 mL to greater than 10 L while maintaining equivalent volumetric protein yields

"In all my years working with transient expression systems, the Expi293™ expression system is the first one to achieve 2.3 grams/L beating every other HEK293 transient expression system."
–Jelte-Jan Reitsma
Research associate

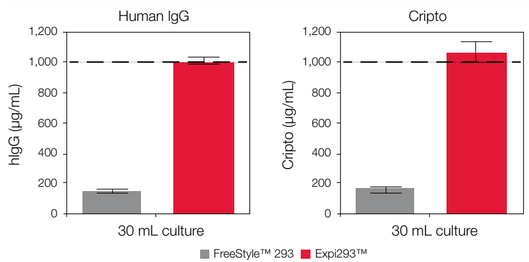


Figure 12. Expression of human IgG and Fc-tagged Cripto protein achieve expression levels of over 1 g/L in the Expi293™ Expression System.

To learn more, go to **lifetechnologies.com/expi293**

Broad-spectrum transfection reagent

Lipofectamine® 2000 Transfection Reagent

Lipofectamine® 2000 reagent is the most referenced transfection reagent in the scientific literature (Figure 13). Lipofectamine® 2000 reagent's success can be attributed to its reliable performance, delivering a high level of transfection efficiency for DNA, RNA, or cotransfection in a broad range of common cell types. The reagent offers:

- **Reliable performance**—high transfection efficiency in a broad range of common cell types
- **Versatility**—single reagent for DNA, RNA, and cotransfection
- **Proven efficacy in the presence of serum**—eliminates the need to change media following transfection

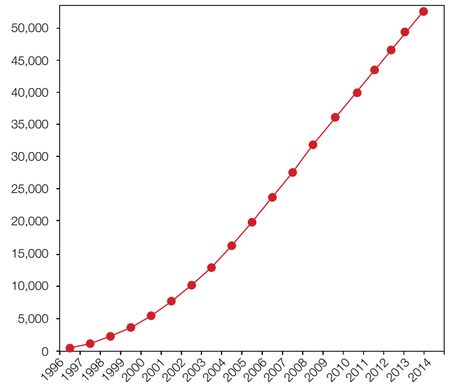


Figure 13. Cumulative number of publications citing the use of Lipofectamine® family of reagents since 1996.

To learn more, go to **lifetechnologies.com/2000**

Electroporation

Neon® Transfection System

The Neon® Transfection System is a next-generation electroporation device for highly efficient transfection of primary cells, stem cells, and other difficult-to-transfect cells (Figure 14). The flexible and open system allows you to perform high-quality transfections using optimized or user-defined protocols in three simple steps with as few as 2 × 10^4 cells per reaction. A novel reaction chamber provides a dramatic increase in transfection efficiency and cell viability.

The Neon® Transfection System is:

- **Efficient**—up to 90% efficiency in many cell types, including difficult-to-transfect cells, primary cells, and stem cells
- **Flexible**—easily transfect from 2 × 104 cells to 6 × 106 cells per reaction
- **Simple**—easy to use, with a single kit that includes reagents for all cell types
- **Versatile**—open system allows electroporation parameters to be optimized freely

"I would recommend it simply because it was very easy to use, it was actually a little bit cheaper than the system we've used in the past and…I really like the features such as the database."
—Lydia Wunderley, PhD
University of Manchester

To learn more, go to **lifetechnologies.com/neon**

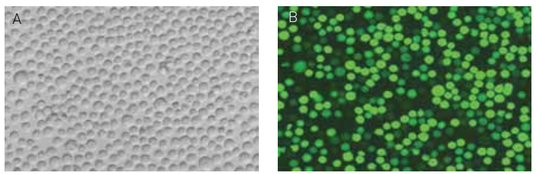


Figure 14. High transfection efficiency of Jurkat cells with the Neon® Transfection System. Cells were analyzed by **(A)** brightfield and **(B)** fluorescence microscopy for intracellular uptake of enhanced GFP (EGFP) reporter vector 24 hours following transfection.

In vivo delivery

Invivofectamine 3.0 Reagent

Invivofectamine® 3.0 Reagent is a breakthrough reagent for *in vivo* RNAi delivery, with greatly improved performance and up to 85% knockdown achieved using microgram levels of siRNA.

- **Easy to use**—siRNA complexes are ready to deliver in just a few steps
- **Effective, targeted knockdown**—up to 85% knockdown observed with targets tested
- **Use less siRNA sample**—up to 90% less siRNA than required with previous reagent for effective knockdown
- **Sustained knockdown**—prolonged knockdown observed following a single injection
- **Low toxicity**—complexes exhibit extremely low *in vivo* toxicity

Easy to use

Creating complexes of Invivofectamine® 3.0 Reagent and RNAi duplexes for delivery is easy: simply mix, incubate, dilute, and inject (Figure 1).

Effective, targeted knockdown

Complexes of Invivofectamine® 3.0 Reagent and Ambion® *in vivo* siRNA targeting Factor VII or Stealth™ RNAi PPIB have been successfully delivered by mouse tail vein injection to liver tissue (Figure 2). We have demonstrated effective knockdown of Factor VII and PPIB at the mRNA level.

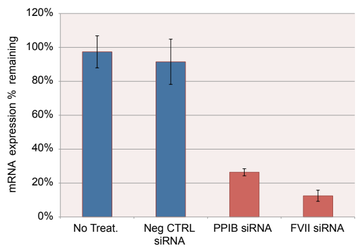


Figure 2. Use of Invivofectamine® 3.0 Reagent enables targeted knockdown in the liver after a single intravenous injection. Invivofectamine® 3.0 Reagent complexed with siRNA targeting mRNA for Factor VII (FVII) or PPIB, injected at doses of 1 mg per kilogram mouse body weight (mg/kg), achieved as much as 85% knockdown of target mRNA levels (knockdown assessed via TaqMan® Assay).

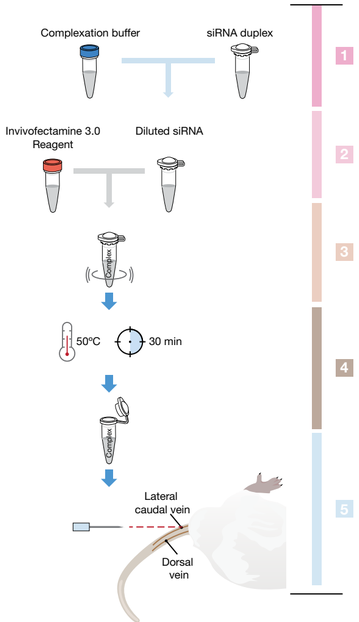


Figure 1. An injection-ready RNAi/Invivofectamine® 3.0 Reagent complex can be prepared quickly and in just a few steps.

Less siRNA required to achieve effective knockdown

Complexes of Invivofectamine® 3.0 Reagent and siRNA in a range of amounts were introduced via tail vein injection. FVII protein levels in the serum were measured using a chromogenic assay 24 hours after injection (Figure 3). The amount of knockdown is correlated with the amount of siRNA in the complex. The ED50 of Invivofectamine® 3.0 Reagent is 0.1 mg/kg, compared to previous levels of 1.0 mg/kg.

Low *in vivo* toxicity

To evaluate *in vivo* toxicity of Invivofectamine® 3.0 Reagent, several detailed studies focusing on analysis of a blood chemistry panel, hematology, and cytokine levels at multiple time points post-injection were performed (Figure 4). The results show that the levels of various biomarkers in mice transfected using Invivofectamine® 3.0 Reagent were not significantly different from those of untreated mice.

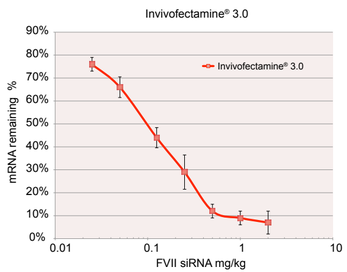


Figure 3. Invivofectamine® 3.0 Reagent and siRNA targeting FVII produce dose-response knockdown in liver after a single intravenous injection. Invivofectamine® 3.0 Reagent complexed with Ambion® *in vivo* siRNA targeting FVII was injected at doses ranging from 0.02 to 2 mg/kg. Blood serum was isolated and assayed for FVII protein levels (Biophen® chromogenic assay).

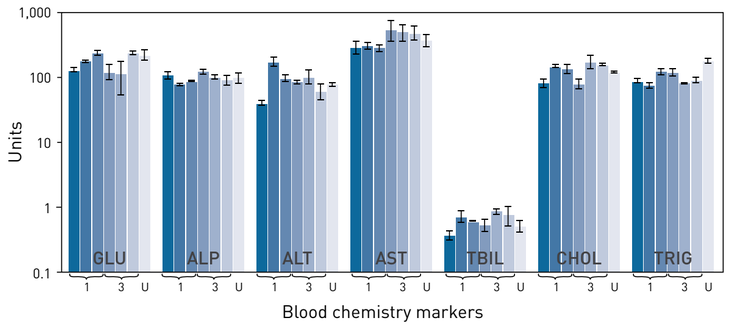


Figure 4. Evaluating *in vivo* toxicity markers over time and with varying reagent doses. Invivofectamine® 3.0 Reagent complexed with Ambion® *in vivo* siRNA targeting FVII was injected into mice at doses of 1 and 3 mg/kg. Blood samples were collected at 2, 24, and 48 hr and were evaluated using clinical chemistry assays for several biomarkers (Antech).

To learn more, go to **lifetechnologies.com/invivofectamine**

Transfection-related products

Opti-MEM® I Reduced-Serum Medium

Opti-MEM® I Reduced-Serum Medium is a modification of Eagle's Minimal Essential Medium, buffered with HEPES and sodium bicarbonate, and supplemented with hypoxanthine, thymidine, sodium pyruvate, L-glutamine, trace elements, and growth factors. Most cells grown in serum-supplemented medium can be transferred to Opti-MEM® medium with a minimum of 50% reduction in serum. We recommend Opti-MEM® medium for dilution of transfection reagent and nucleic acids prior to complex formation.

To learn more, go to **lifetechnologies.com/optimem**

Selection antibiotics

Gibco® high-quality selection agents provide unique solutions for your research needs, such as dual selection and rapid generation of stable cell lines (Table 2).

Table 2. Eukaryotic selection antibodies.

Selection antibiotic	Most common selection usage	Common working concentration	Available powder sizes	Available liquid sizes
Blasticidin	Eukaryotic and bacterial cells	1–20 µg/mL	50 mg	10 x 1 mL, 20 mL
Geneticin (G418)	Eukaryotic cells	100–200 µg/mL (bacteria) 200–500 µg/mL (mammalian cells)	1, 5, 10, or 25 g	20 mL, 100 mL
Hygromycin B	Eukaryotic cells and dual-selection experiments	200–500 µg/mL	–	20 mL
Mycophenolic acid	Mammalian and bacterial cells	25 µg/mL	500 mg	–
Puromycin	Eukaryotic and bacterial cells	0.2–5 µg/mL	–	10 x 1 mL, 20 mL
Zeocin	Mammalian, insect, yeast, bacterial, and plant cells	50–400 µg/mL	–	8 x 1.25 mL, 50 mL

To learn more, go to **lifetechnologies.com/selectionantibiotics**

Genome modulation and engineering*

Ambion® siRNA

RNA interference (RNAi) is the best way to effectively knock down gene expression to study protein function in a wide range of cell types. Traditional methods for gene knockdown in mammalian cells involved the use of synthetic RNA duplexes consisting of two unmodified 21-mer oligonucleotides annealed together to form small interfering RNAs (siRNAs). *Silencer*® Select siRNA products have been validated with Lipofectamine® RNAiMAX Transfection Reagent and incorporate the latest improvements in siRNA design, off-target effect prediction algorithms, and chemistry to offer:

- **High potency**—improved siRNA prediction accuracy compared to Silencer® siRNA (Figure 17, Table 3)
- **Minimal off-target effects**—locked nucleic acid (LNA) chemical modifications reduce off-target effects by up to 90%
- **Guaranteed results**—100% guaranteed† to silence for increased confidence in your reagents
- **Open access**—65,000 siRNA sequences and associated data on PubChem from our Silencer® Select siRNA library

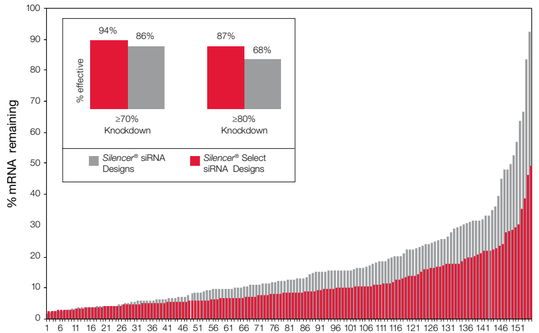


Figure 17. *Silencer*® Select siRNA design algorithm significantly improves effective siRNA prediction accuracy. The *Silencer*® Select siRNA design algorithm was used to design 155 siRNAs to 40 different targets. These siRNAs were tested side by side with siRNAs designed using the previous algorithm at 5 nM in HeLa cells. mRNA knockdown was measured 48 hours posttransfection by real-time PCR using TaqMan® Gene Expression Assays. Results are expressed as percent of mRNA remaining compared to *Silencer*® Select Negative Control No. 1 siRNA–treated cells. The inset shows the percentage of siRNAs that elicited ≥70% and ≥80% mRNA knockdown.

*Gene modulation and engineering products are currently not available from Fisher Scientific. For more information, go to lifetechnologies.com

†We guarantee that when you purchase two *Silencer*® Select Predesigned siRNAs to the same target, those two siRNAs will silence the target mRNA by 70% or more. To qualify for the guarantee, siRNAs must have been transfected at ≥5 nM and mRNA levels detected 48 hours posttransfection. Real-time PCR is recommended but not required for this application. Customers must also show >80% knockdown with a positive control siRNA to demonstrate transfection efficiency. If the guaranteed level of knockdown is not observed and an appropriate positive control is successful, a one-time replacement of up to two new *Silencer*® Select siRNA designs will be provided free of charge. This guarantee does not extend to any replacement product.

Table 3. Ambion® siRNA selection guide.

	Cost-effective siRNA	Good knockdown, low off-target effects	Highest knockdown, lowest off-target effects
	Silencer® siRNA	Stealth RNAi™ siRNA	*Silencer*® Select siRNA
Potency	100 nM recommended conc.	20 nM recommended conc.	5 nM recommended conc.
Efficacy (>70% knockdown)	2 of 3 siRNA guaranteed	2 of 3 siRNA guaranteed	2 of 2 siRNA guaranteed
Target specificity	Moderate	High	Highest
Coverage	Coding RNA	Coding RNA	Coding and noncoding RNA
Target species	Human, mouse, rat (use custom tool for other species)	Human, mouse, rat (use custom tool for other species)	Human, mouse, rat (use custom tool for other species)

A solution for successful *in vivo* RNAi

In vivo RNAi experiments are more challenging than their *in vitro* counterparts due to the high levels of ribonucleases present in all body fluids. These added challenges necessitate the use of RNase-resitant Ambion® *In Vivo* siRNAs to obtain reproducible results. Ambion® *In Vivo* siRNAs have been validated with Invivofectamine 3.0 Reagent and retain all the characteristics of *Silencer*® Select siRNAs including potency, specificity, and efficacy, as well as an enhanced stability and safety profile for animal studies.

Ambion® siRNA libraries

We provide collections of premium quality siRNAs for performing RNAi experiments in human, mouse, and rat cell systems. siRNA libraries can be custom made or predefined to major sets of gene targets, and are backed by a 100% guarantee. For more information, please contact **RNAiLibraries@lifetech.com**

To learn more, go to **lifetechnologies.com/sirna**

*mir*Vana™ miRNA Mimics and Inhibitors

*mir*Vana™ miRNA Mimics and Inhibitors are chemically modified, synthetic nucleic acids designed to either mimic mature miRNAs, or to bind to and inhibit endogeneous miRNAs. These products provide a means to functionally study the role of specific miRNAs within cellular systems, or to validate the role of miRNAs in regulating target genes. *mir*Vana™ miRNA Mimics and Inhibitors have been validated with Lipofectamine® RNAiMAX Transfection Reagent for use in cell-based systems, and with Invivofectamine 3.0 Reagent for *in vivo* delivery. *In vivo*–ready *mir*Vana™ miRNA Mimics and Inhibitors have been purified by HPLC and dialysis, making them ready for immediate use.

*mir*Vana™ miRNA Mimics and Inhibitors are:

- **Versatile**—functionally study specific miRNAs using in vitro or *in vivo* systems
- **Potent**—validate miRNA regulation of gene expression with minimal off-target effects (Figure 18)
- **High-throughput compatible**—generate libraries for effective screening of multiple miRNAs simultaneously
- **Current**—content is regularly updated based on the miRBase miRNA database

*mir*Vana™ miRNA Libraries

Complete *mir*Vana™ libraries containing mimics and inhibitors for every human, mouse, and rat miRNA are available. For information on all our predefined and custom miRNAs libraries, contact us at **RNAiLibraries@lifetech.com**

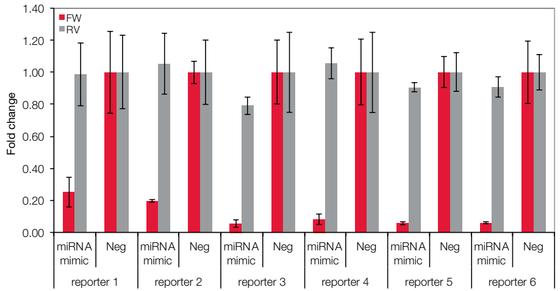


Figure 18. The mature strand of *mir*Vana™ miRNA Mimics are highly potent. miRNA mimics, like natural miRNAs, have 2 strands—the mature strand (guide strand) that is functional and used by Ago protein to target mRNAs, and the star or passenger strand that is nonfunctional and is normally cleaved and expelled from the complex. Most scientists want to analyze one strand of miRNA at a time, and want the other strand to be totally inactive. A key advantage of *mir*Vana™ miRNA Mimics is inactivation of the star strand. For this assay we measured activity from both strands of miRNA mimics. One reporter has a target in forward orientation to measure activity of the mature miRNA strand, and the other reporter has the target cloned in reverse/complement orientation to test activity of the star strand of the miRNA mimic. For all 6 sequences, activity of the mature strand is high (5-10–fold knockdown compared to negative control), and activity of the star strand is low (similar to negative control).

To learn more, go to **lifetechnologies.com/mirna**

Genome editing

Engineering made easy

With the wealth of data available through advanced sequencing technologies, the next challenge for the research community is to apply this information for more valuable, clinically relevant output. The ability to edit the genome in a precise and targeted manner can be used to provide a more comprehensive understanding of biology and disease mechanisms. Advances in molecular biology tools have enabled scientists to modulate or edit genes with simple yet powerful genome editing techniques (Figure 19). Genome editing has a variety of applications such as creating disease-resistant transgenic plants, stem cell engineering and gene therapy, and is also widely used in creating tissue and animal disease models.

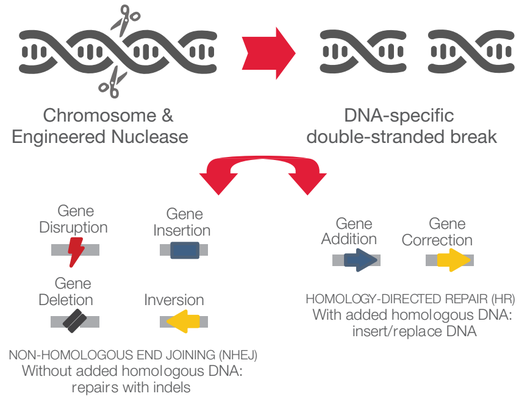
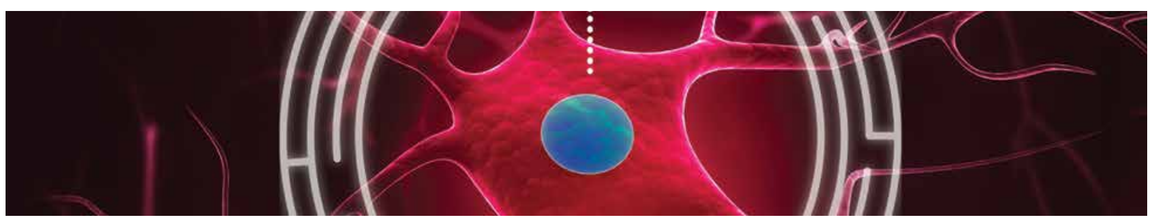


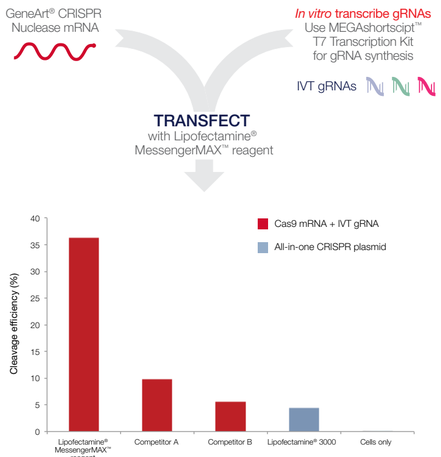
Figure 19. Engineered nuclease-mediated genome editing. The CRISPR-Cas9 system or TAL effector nucleases can be designed to target specific sites in the genome, creating double-strand breaks (DSBs) at desired locations. The natural repair mechanisms of the cell repair the break by either homologous recombination (HR) or non-homologous end joining (NHEJ). HR is more precise, since it requires a template, allowing the introduction of foreign DNA into the target gene. Homologous DNA "donor sequences" can be used with homology-directed repair (HDR) to introduce a defined new DNA sequence. DSB repair by NHEJ is likely to introduce errors such as insertions or deletions (indels), leading to a non-functional gene.



GeneArt® CRISPR tools

Rapid and efficient editing with multiplexing capabilities

The CRISPR-Cas9 system is a new approach to genome editing that provides rapid, efficient editing with multiplexing capabilities. With their highly flexible but specific targeting, CRISPR-Cas9 systems can be manipulated and redirected to become powerful tools for genome editing. The endonuclease cleavage specificity in CRISPR-Cas9 systems is guided by RNA sequences, so editing can be directed to virtually any genomic locus by engineering the guide RNA sequence and delivering it along with the Cas9 endonuclease to your target cell. Based on your research needs, you can choose from our two different formats of CRISPR tools: GeneArt® CRISPR Nuclease all-in-one expression plasmids when working with mammalian systems, or the GeneArt® CRISPR Nuclease mRNA System for improved editing efficiencies and multiplexing capabilities (Figures 20 and 21).



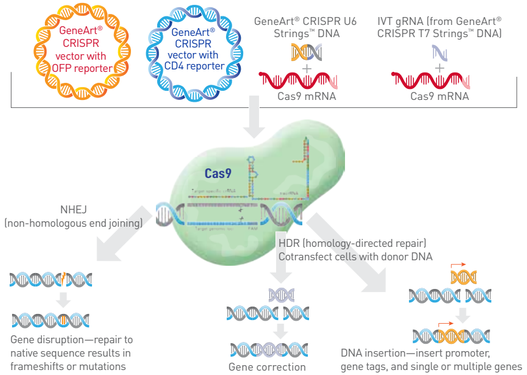


Figure 20. Available GeneArt® CRISPR-Cas9 genome editing tools. The CRISPR-Cas9 system is composed of a short noncoding guide RNA (gRNA) that has two molecular components, a target-specific CRISPR RNA (crRNA), and an auxiliary trans-activating crRNA (tracrRNA). The gRNA unit guides the Cas9 protein to a specific genomic locus via base pairing between the crRNA sequence and the target sequence. Upon binding to the target sequence, the Cas9 protein induces a double-strand break at the specific target sequence. Following CRISPR-Cas9–induced DNA cleavage, the break can be repaired by the cellular repair machinery using either non-homologous end joining (NHEJ) or a homology-directed repair (HDR) mechanism.

Figure 21. Cleavage efficiency of various GeneArt® CRISPR formats targeting the HPRT locus in Gibco® iPS cells in a 12-well format. Lipofectamine® 3000 reagent was used to deliver CRISPR nuclease all-in-one plasmid DNA; Lipofectamine® MessengerMAX™ and two leading mRNA delivery reagents were used to deliver an all-RNA CRISPR format (Cas9 mRNA + IVT gRNA). Cleavage efficiency was determined using the GeneArt® Genomic Cleavage Detection Kit 72 hours posttransfection.

To learn more, go to **lifetechnologies.com/CRISPR**

GeneArt® TAL effector tools

Precise and flexible editing with freedom to innovate

TAL effectors are a widely used technology for precise and efficient gene editing. The deciphering of the TAL effector “code” led to the engineering of designer TAL effector proteins. GeneArt® TALs provide custom DNA binding proteins engineered and designed for accurate DNA targeting and precise genome editing. GeneArt® TALs have been validated with Lipofectamine® 3000 (Figure 22) and offer site-specific delivery of nucleases, activators, repressors, chromatin modifiers, genomic labels, and crosslinking molecules (Figure 23). Based on your research needs you can choose from our two different formats of TAL effector tools: GeneArt® Precision TALs when you are working with plants or have no design constraints, or GeneArt® PerfectMatch TALs that do not require a 5’ T for complete design flexibility.

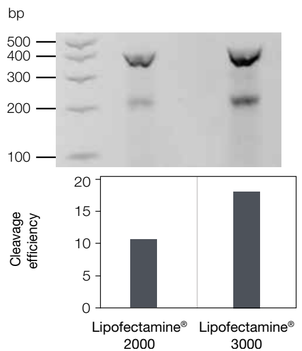
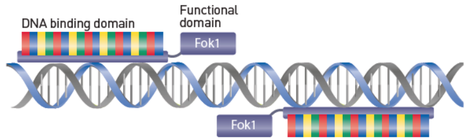
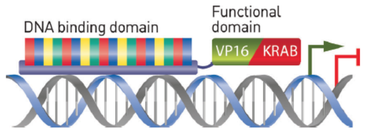


Figure 22. Cleavage efficiency for GeneArt® TALs. TALs targeting the *AAVS1* locus were transfected by Lipofectamine® 2000 or Lipofectamine® 3000 reagent in the U2OS cell line. Cleavage was assayed using the GeneArt® Genomic Cleavage Detection Kit.

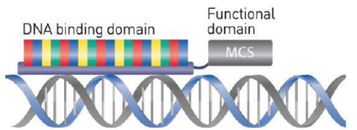
To learn more, go to **lifetechnologies.com/tals**



Precision TALs fused to a Fok1 nuclease allow sequence-specific, double-stranded DNA breaks to be introduced.



Precision TALs fused to a vp16/KRAB functional domain allow specific activation/repression of gene expression.



An MCS version of the GeneArt® Precision TAL vector allows you to customize the resulting TAL fusion protein with an effector domain of your choice.

Figure 23. Available TAL effector domains.

Ordering information

Superior transfection reagents	Quantity	Cat. No.
Lipofectamine® 3000 Transfection Reagent	0.1 mL	L3000001
Lipofectamine® 3000 Transfection Reagent	0.75 mL	L3000008
Lipofectamine® 3000 Transfection Reagent	1.5 mL	L3000015
Lipofectamine® 3000 Transfection Reagent	5 x 1.5 mL	L3000075
Lipofectamine® 3000 Transfection Reagent	15 mL	L3000150
Lipofectamine® RNAiMAX Transfection Reagent	0.1 mL	13778100
Lipofectamine® RNAiMAX Transfection Reagent	0.3 mL	13778030
Lipofectamine® RNAiMAX Transfection Reagent	0.75 mL	13778075
Lipofectamine® RNAiMAX Transfection Reagent	1.5 mL	13778150
Lipofectamine® RNAiMAX Transfection Reagent	15 mL	13778500
Lipofectamine® MessengerMAX™ Transfection Reagent	0.1 mL	LMRNA001
Lipofectamine® MessengerMAX™ Transfection Reagent	0.3 mL	LMRNA003
Lipofectamine® MessengerMAX™ Transfection Reagent	0.75 mL	LMRNA008
Lipofectamine® MessengerMAX™ Transfection Reagent	1.5 mL	LMRNA015
Lipofectamine® MessengerMAX™ Transfection Reagent	15 mL	LMRNA150
ExpiFectamine™ 293 Transfection Kit	1 x 1 L culture	A14524
ExpiFectamine™ 293 Transfection Kit	1 x 10 L culture	A14525
ExpiFectamine™ 293 Transfection Kit	5 x 10 L culture	A14526
Broad-spectrum transfection reagents	**Quantity**	**Cat. No.**
Lipofectamine® 2000 Transfection Reagent	0.3 mL	11668030
Lipofectamine® 2000 Transfection Reagent	0.75 mL	11668027
Lipofectamine® 2000 CD Transfection Reagent	1 mL	12566014
Lipofectamine® 2000 Transfection Reagent	1.5 mL	11668019
Lipofectamine® 2000 Transfection Reagent	15 mL	11668500
Electroporation	**Quantity**	**Cat. No.**
Neon® Transfection System	1 each	MPK5000
Neon® Transfection System Starter Pack	1 pack	MPK5000S
Neon® Transfection System Pipette	1 each	MPP100
Neon® Transfection System Pipette Station	1 each	MPS100
Neon® Transfection Tubes	1 pack	MPT100
Neon® Transfection System 100 µL Kit	25 x 2 reactions	MPK10025

Electroporation *continued*	Quantity	Cat. No.
Neon® Transfection System 100 µL Kit	96 x 2 reactions	MPK10096
Neon® Transfection System 10 µL Kit	25 x 2 reactions	MPK1025
Neon® Transfection System 10 µL Kit	96 x 2 reactions	MPK1096
***In vivo* delivery**	**Quantity**	**Cat. No.**
Invivofectamine 3.0 Reagent	1 mL	IVF3001
Invivofectamine 3.0 Reagent	5 x 1 mL	IVF3005
Transfection-related products	**Quantity**	**Cat. No.**
Opti-MEM® I Reduced-Serum Medium	100 mL	31985062
Blasticidin S HCl (10 mg/mL)	10 x 1 mL	A1113903
Geneticin® Selective Antibiotic (G418 Sulfate) (50 mg/mL)	100 mL	10131027
Hygromycin B (50 mg/mL)	20 mL	10687010
Mycophenolic Acid	500 mg	11814019
Puromycin Dihydrochloride	10 x 1 mL	A1113803
Zeocin™ Selection Reagent	8 x 1.25 mL	R25001

invitrogen
by Thermo Fisher Scientific

Find out more at **lifetechnologies.com**

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For customer service, call 1-800-766-7000
To fax an order, use 1-800-926-1166
To order online: www.fishersci.com

In Canada:
For customer service, call 1-800-234-7437
To fax an order, use 1-800-463-2996
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