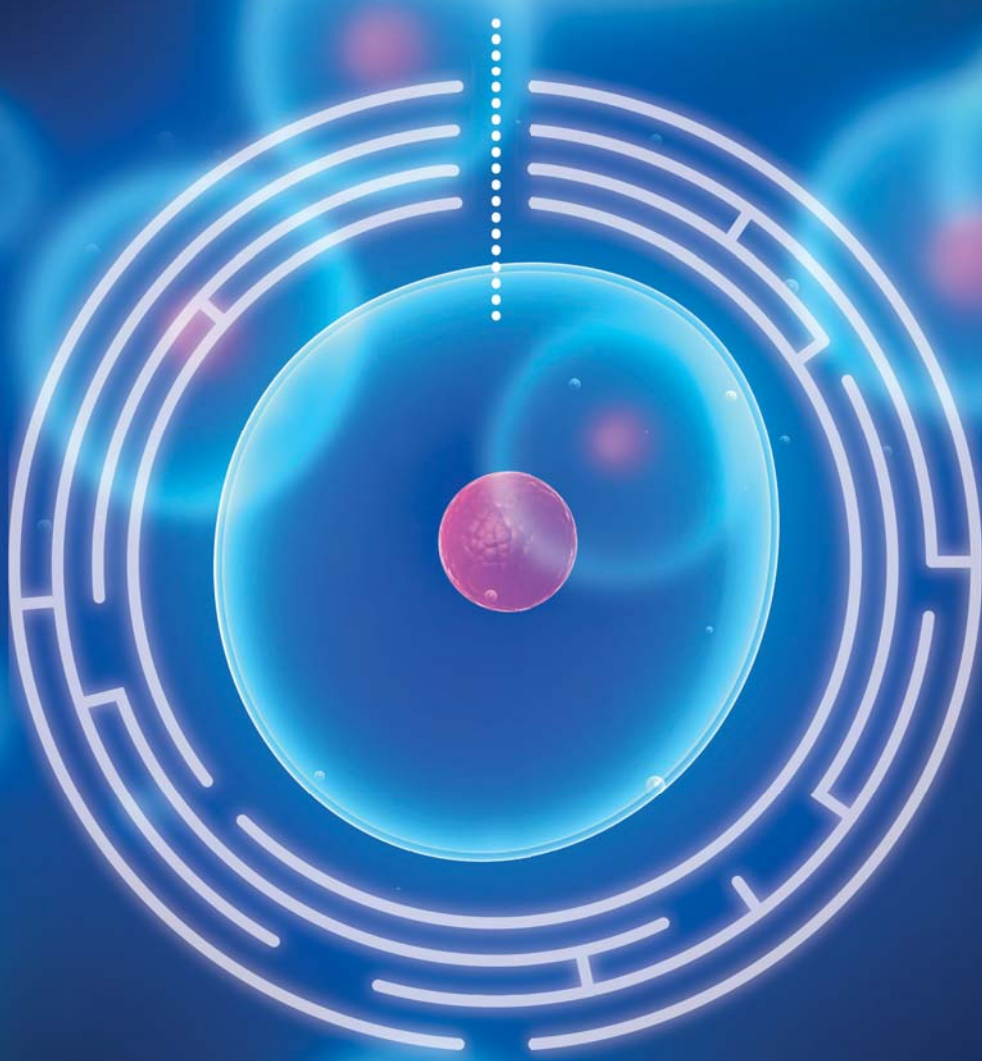


Invitrogen™










# Transfection and genome engineering

Innovation delivered

*life*  
technologies

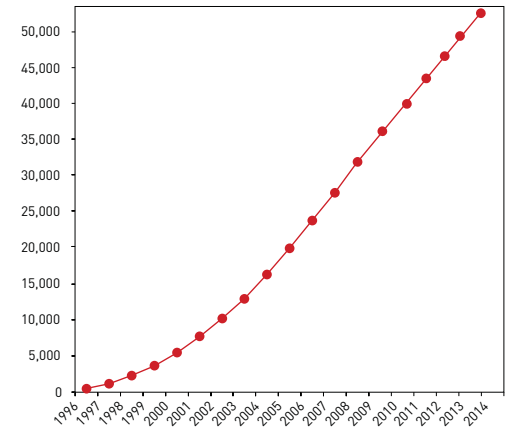


# Contents

<b>Transfection selection guide</b>	<b>4</b>	
<b>Transfection decision tree</b>	<b>5</b>	
<b>Superior transfection reagents</b>	<b>6</b>	
Lipofectamine® 3000 Transfection Reagent	6	
Lipofectamine® RNAiMAX Transfection Reagent	10	
Lipofectamine® MessengerMAX™ Transfection Reagent	11	
ExpiFectamine™ 293 Transfection Kit	14	
<b>Broad-spectrum transfection reagent</b>	<b>15</b>	
Lipofectamine® 2000 Transfection Reagent	15	
<b>Electroporation</b>	<b>16</b>	
Neon® Transfection System	16	
<b><i>In vivo</i> delivery</b>	<b>17</b>	
Invivofectamine® 2.0 Reagent	17	
<b>Transfection-related products</b>	<b>19</b>	
Opti-MEM® I Reduced-Serum Medium	19	
Selection antibiotics	19	
<b>Genome modulation and engineering</b>	<b>20</b>	
Ambion® siRNA	20	
<i>mirVana</i> ™ miRNA Mimics and Inhibitors	22	
GeneArt® CRISPR tools	24	
GeneArt® TAL effector tools	25	
<b>Ordering information</b>	<b>26</b>	

# 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.



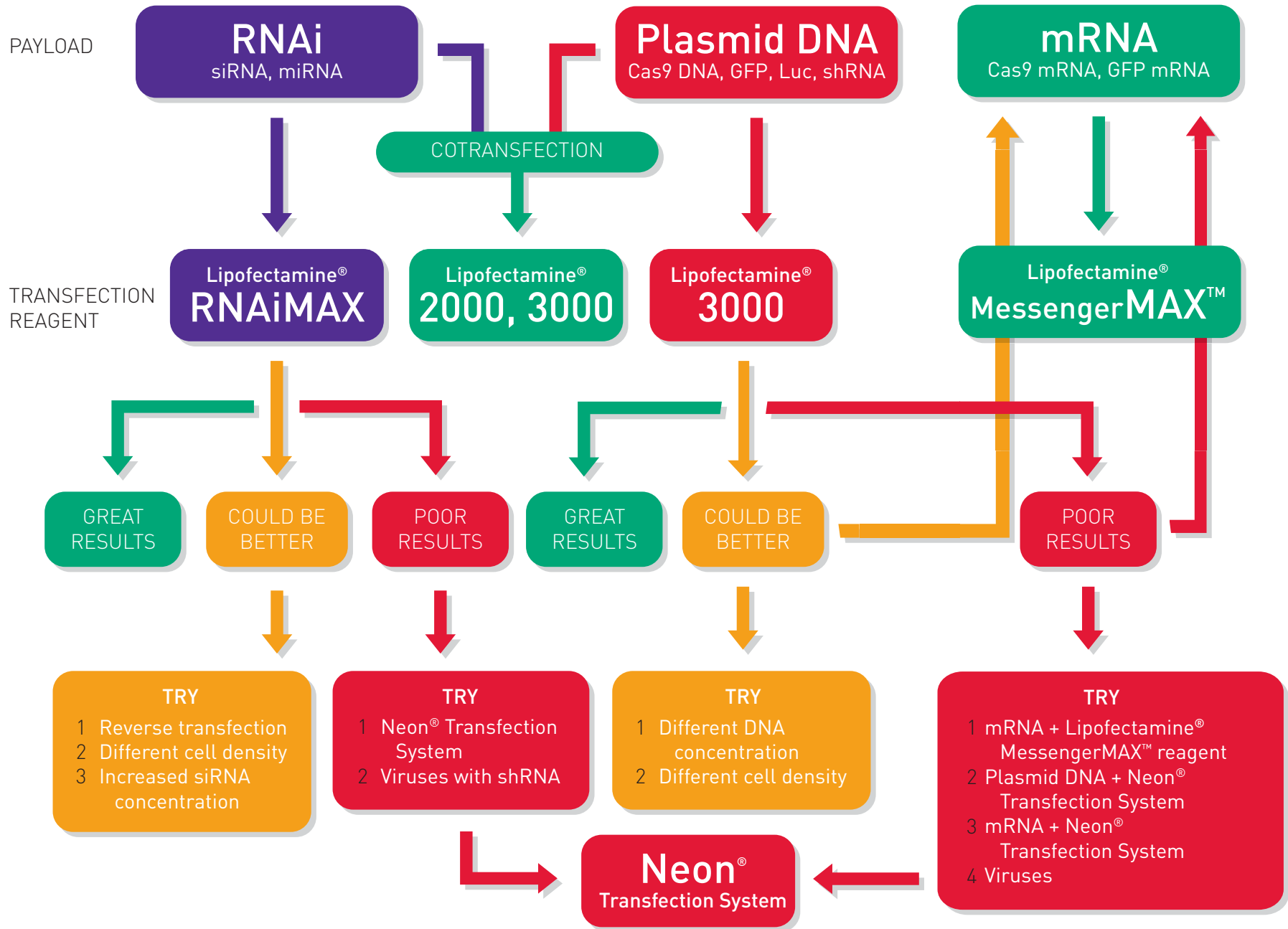
Cumulative number of publications citing the use of Lipofectamine® family of reagents since 1996.

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
<b>Superior transfection reagents</b>									
Lipofectamine® 3000 Transfection Reagent									
Lipofectamine® RNAiMAX Transfection Reagent									
Lipofectamine® MessengerMAX™ Transfection Reagent									
ExpiFectamine™ 293 Transfection Kit					Recommended for Expi293F™ cells				
<b>Broad-spectrum transfection reagent</b>									
Lipofectamine® 2000 Transfection Reagent									
<b>Electroporation</b>									
Neon® Transfection System									
<b>In vivo delivery</b>									
InvivoFectamine® 2.0 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
- **Versatility**—single kit for DNA, RNA, and cotransfection

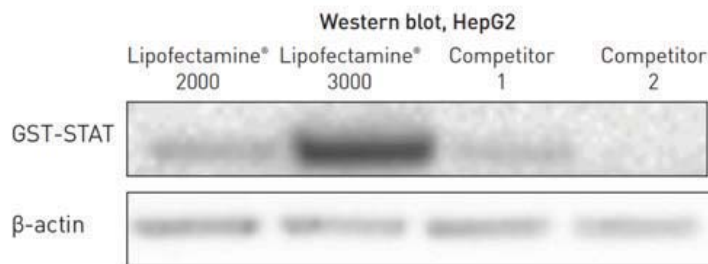


Figure 1. Enhanced protein expression using Lipofectamine® 3000 reagent. HepG2 cells were transfected with a vector expressing GST-tagged STAT protein. A western blot was performed to determine the level of expression using β-actin as a control.

*"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

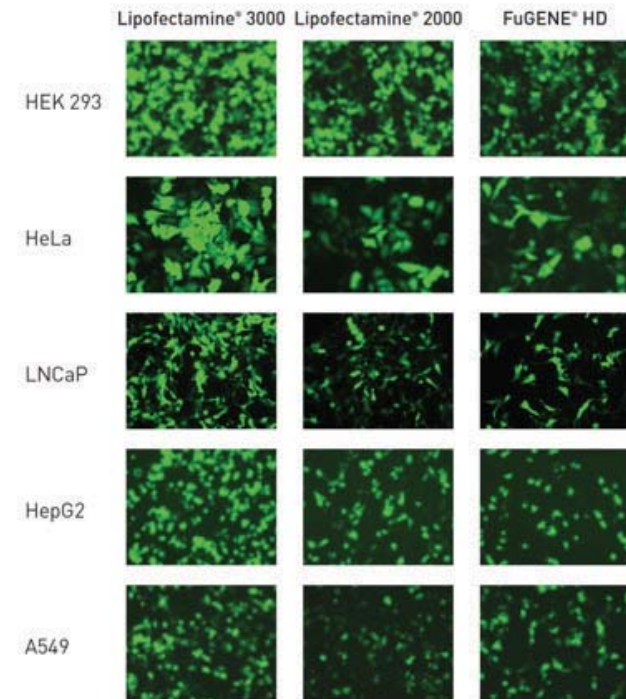
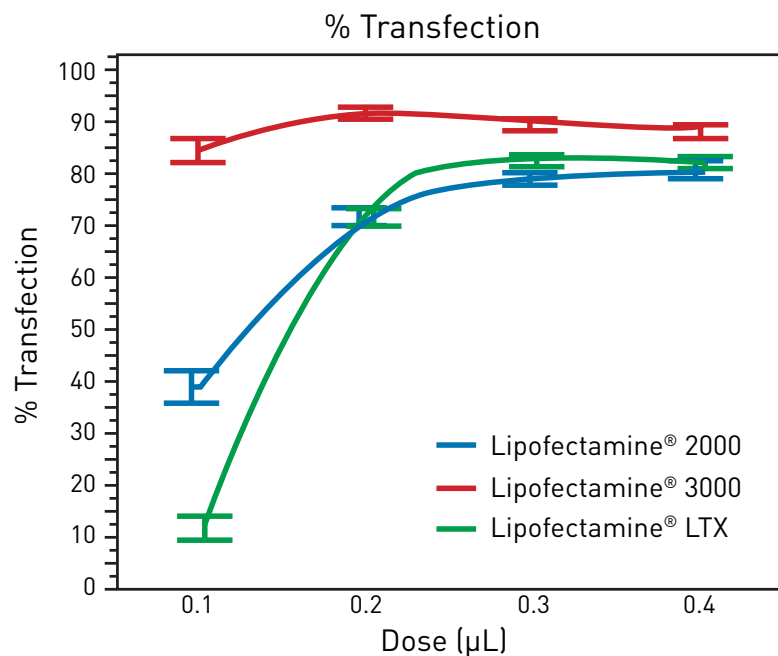


Figure 2. Lipofectamine® 3000 reagent outperforms Lipofectamine® 2000 and FuGENE® HD reagents. Each reagent was used to transfect HEK 293, HeLa, LNCaP, HepG2, and A549 cell lines in a 96-well format, and green fluorescent protein (GFP) expression was analyzed 48 hours posttransfection. Lipofectamine®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

## Gentle with low toxicity

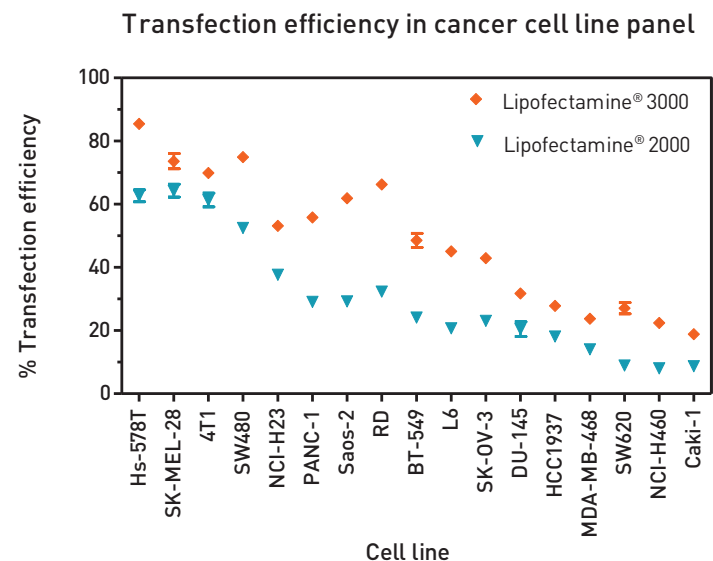
Lipofectamine® 3000 reagent was designed to optimize every step in the transfection process. The superior transfection performance allows you to reduce the reagent dose and improve cell viability when working with your cell line of interest (Figure 3).



**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

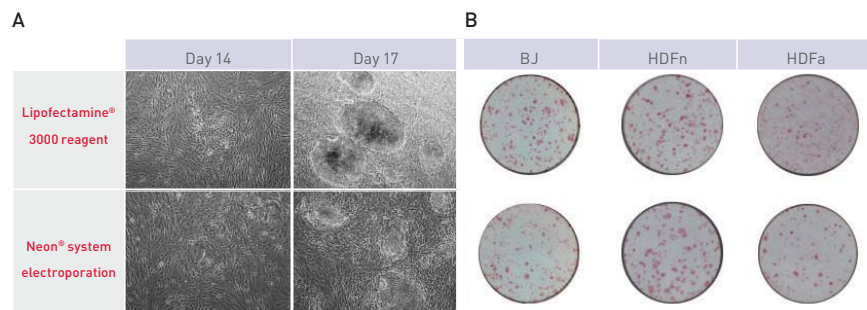
The superior transfection efficiency and broad cell spectrum of Lipofectamine® 3000 reagent enables excellent flexibility to use your cell line of choice in cancer studies (Figure 4).



**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

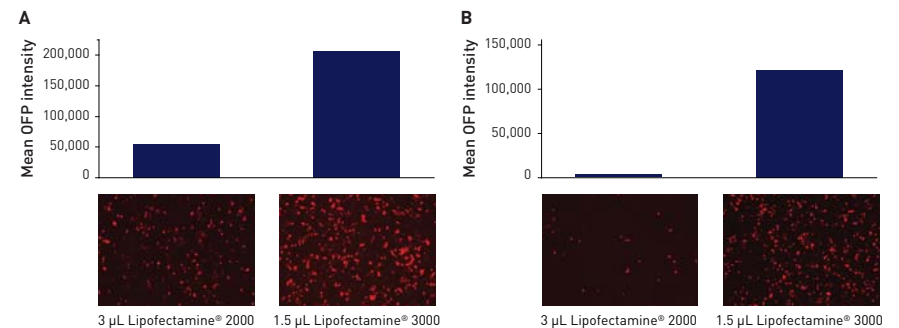
Induced pluripotent stem cells (iPSCs) hold immense promise for the future of regenerative medicine and personalized therapeutic treatments for a myriad of diseases and conditions. The superior transfection efficiency of Lipofectamine® 3000 reagent in conjunction with the Epi5™ Episomal iPSC Reprogramming 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.

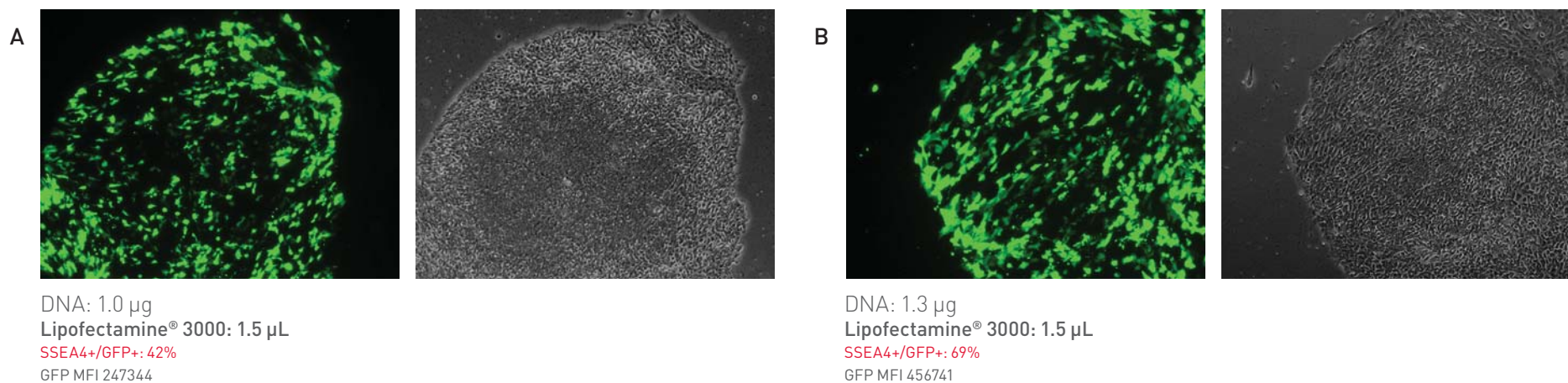


**Figure 6. Transfection efficiency and protein expression using GeneArt® CRISPR Nuclease Vector.** The vector contained an GFP reporter gene and was transfected with Lipofectamine® 2000 or Lipofectamine® 3000 reagent into (A) U2OS and (B) HepG2 cell lines. Bar graphs show reporter gene expression; images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



## 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.



**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.

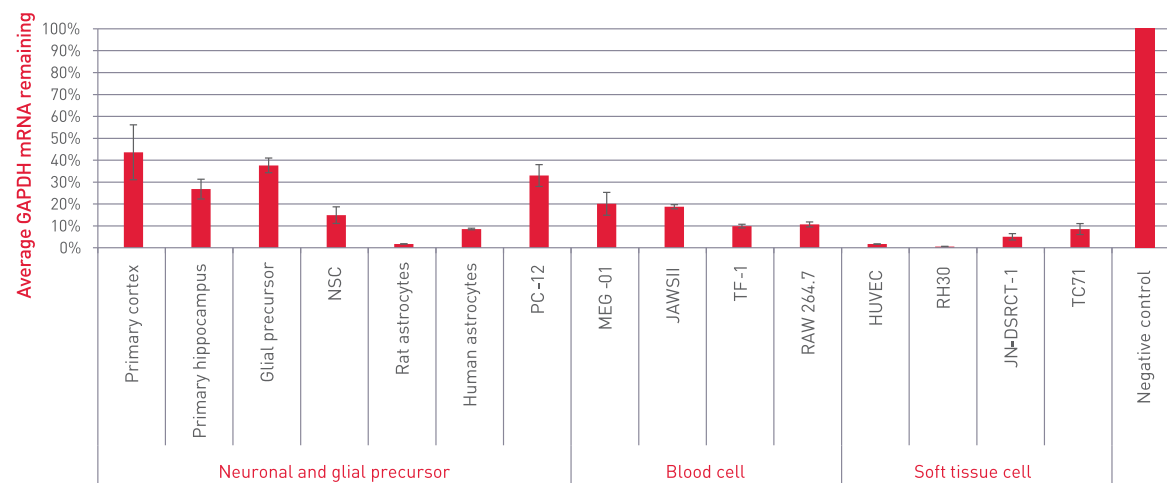
To learn more or place an order, go to [lifetechnologies.com/3000](https://www.lifetechnologies.com/3000)

## 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.



**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  $\mu$ L; rat primary hippocampus cells:  $10^4$  cells/well, 0.6  $\mu$ L; rat glial precursor cells:  $8 \times 10^3$  cells/well, 0.4  $\mu$ L; neuroblastoma-spinal cord (NSC) cells:  $1.2 \times 10^4$  cells/well, 0.3  $\mu$ L; rat astrocyte cells:  $1.2 \times 10^4$  cells/well, 0.3  $\mu$ L; human astrocyte cells:  $8 \times 10^3$  cells/well, 0.3  $\mu$ L; PC-12 cells (derived from a pheochromocytoma of the rat adrenal medulla):  $8 \times 10^3$  cells/well, 0.3  $\mu$ L; megakaryoblastic leukemia cells (MEG-01):  $2 \times 10^4$  cells/well, 0.3  $\mu$ L; murine dendritic cells (JAWSII):  $1.2 \times 10^4$  cells/well, 0.3  $\mu$ L; human erythroleukemia cells (TF-1):  $10^4$  cells/well, 0.3  $\mu$ L; RAW 264.7 macrophages:  $10^4$  cells/well, 0.3  $\mu$ L; human umbilical vein endothelial cells (HUVEC):  $8 \times 10^3$  cells/well, 0.3  $\mu$ L; rhabdomyosarcoma cells (RH30):  $8 \times 10^3$  cells/well, 0.3  $\mu$ L; human desmoplastic small round cell tumor cells (JN-DSRCT-1):  $8 \times 10^3$  cells/well, 0.3  $\mu$ L; human Ewing's sarcoma cells (TC71):  $1.6 \times 10^4$  cells/well, 0.3  $\mu$ L.

## 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.

To learn more or place an order, go to [lifetechnologies.com/rnaimax](http://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.

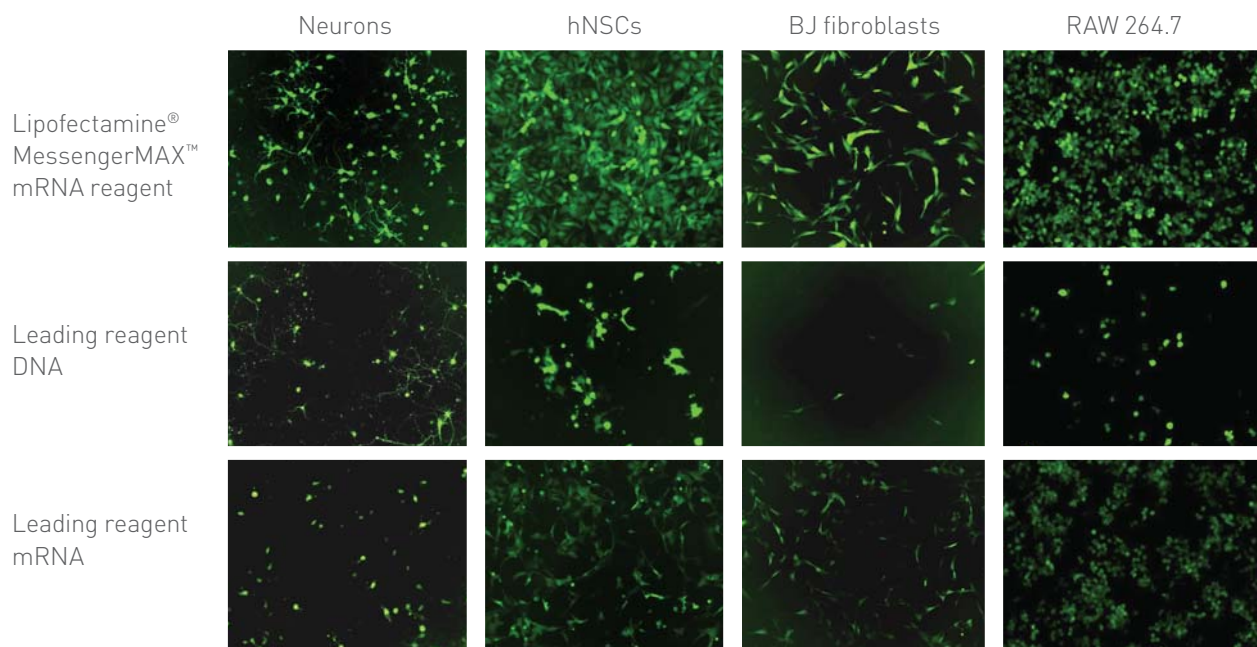
Successfully transfect more predictive cell models with a reagent that provides:

- 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.

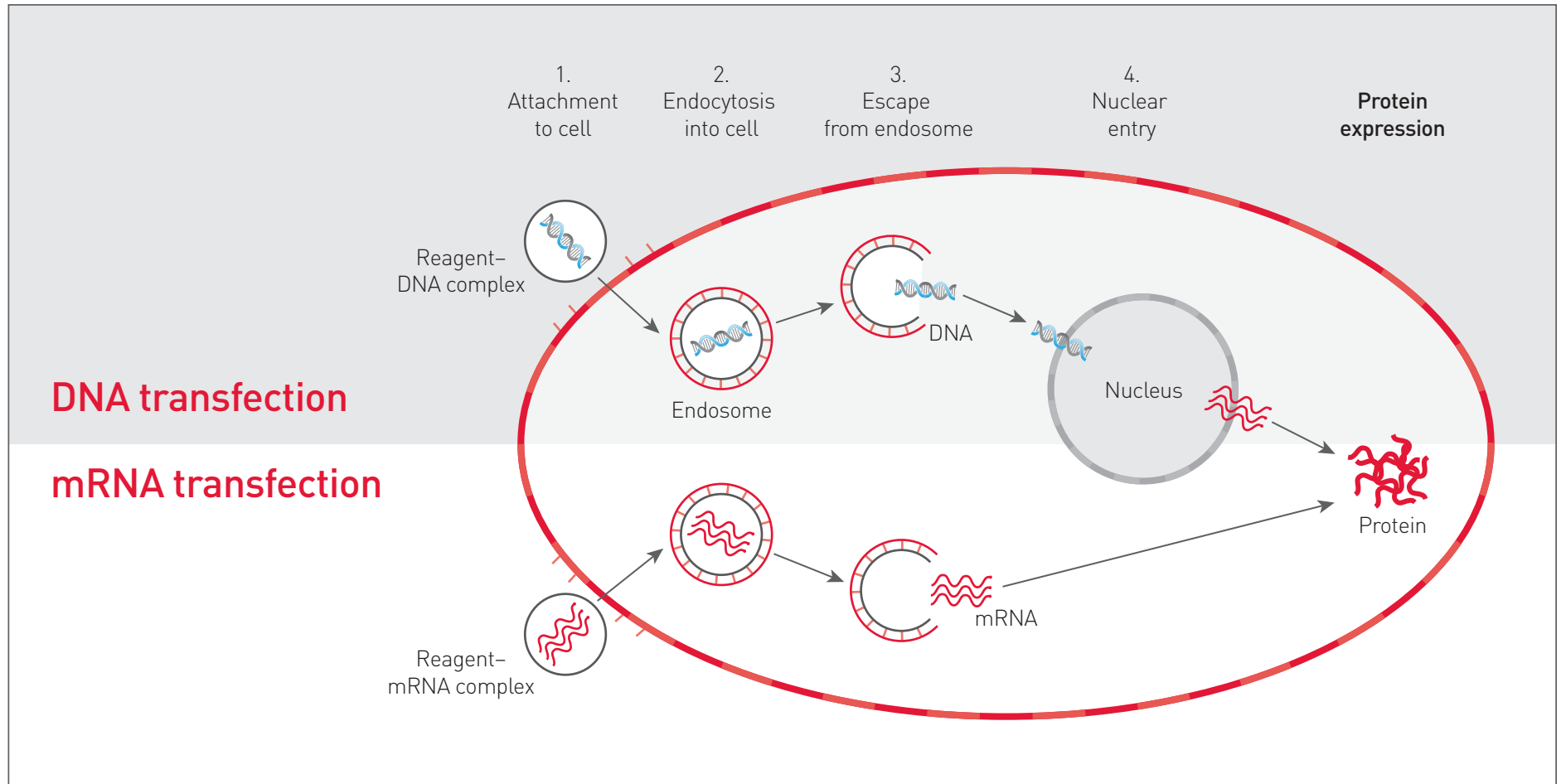


Figure 10. DNA vs. mRNA transfection.

# 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 or place an order, go to [lifetechnologies.com/messengermax](http://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 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

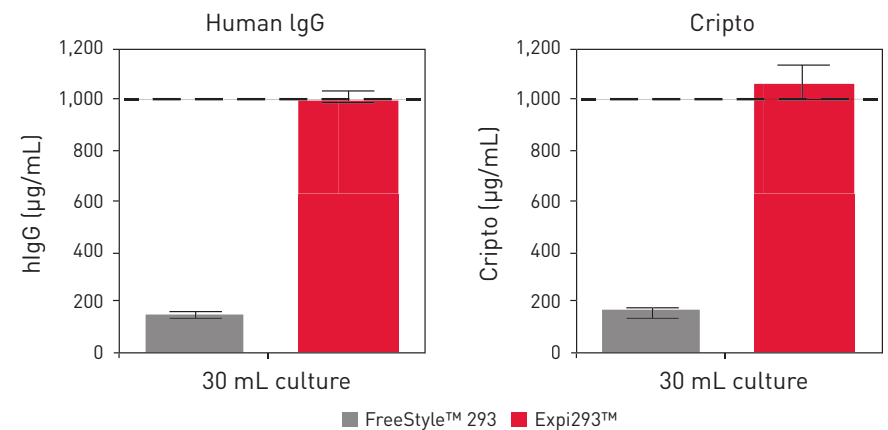


Figure 12. Expression of human IgG and Fc-tagged Cripto protein achieve expression levels of over 1 g/L in the Expi293™ Expression System.

To learn more or place an order, go to [lifetechnologies.com/exp293](https://lifetechnologies.com/exp293)

# 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

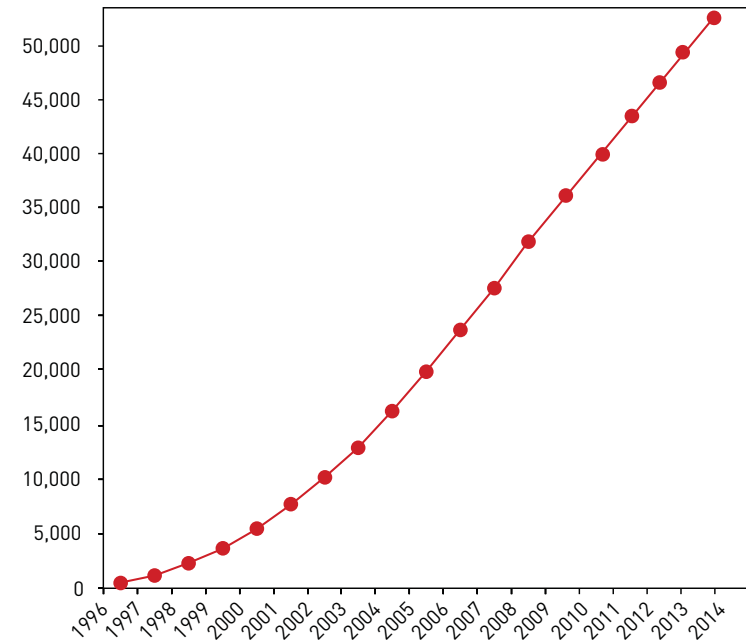


Figure 13. Cumulative number of publications citing the use of Lipofectamine® family of reagents since 1996.



To learn more or place an order, go to [lifetechnologies.com/2000](http://lifetechnologies.com/2000)

# Electroporation

## Neon<sup>®</sup> Transfection System

The Neon<sup>®</sup> 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  $2 \times 10^4$  cells per reaction. A novel reaction chamber provides a dramatic increase in transfection efficiency and cell viability.

The Neon<sup>®</sup> 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  $2 \times 10^4$  cells to  $6 \times 10^6$  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

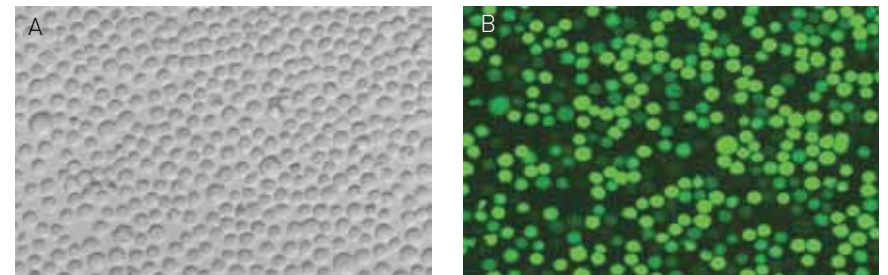


Figure 14. High transfection efficiency of Jurkat cells with the Neon<sup>®</sup> Transfection System. Cells were analyzed by (A) brightfield and (B) fluorescence microscopy for intracellular uptake of enhanced GFP (EGFP) reporter vector 24 hours following transfection.

To learn more or place an order, go to [lifetechnologies.com/neon](https://www.lifetechnologies.com/neon)



# In vivo delivery

## Invivolectamine® 2.0 Reagent

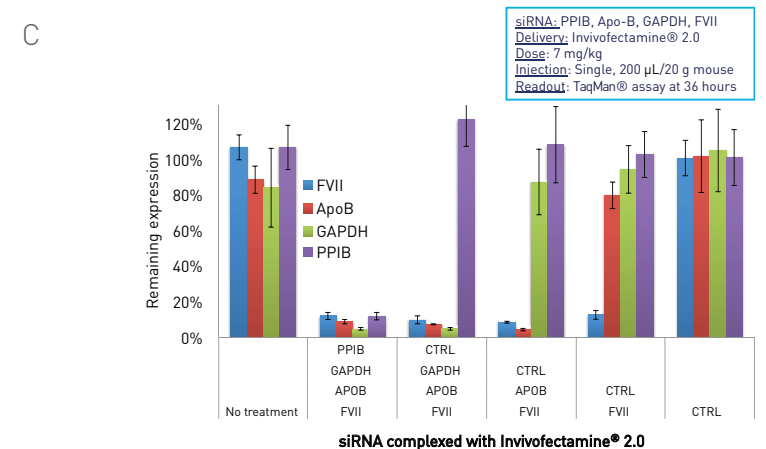
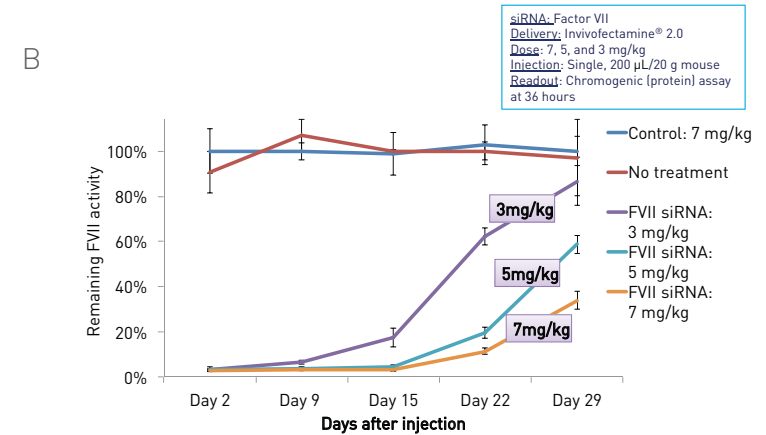
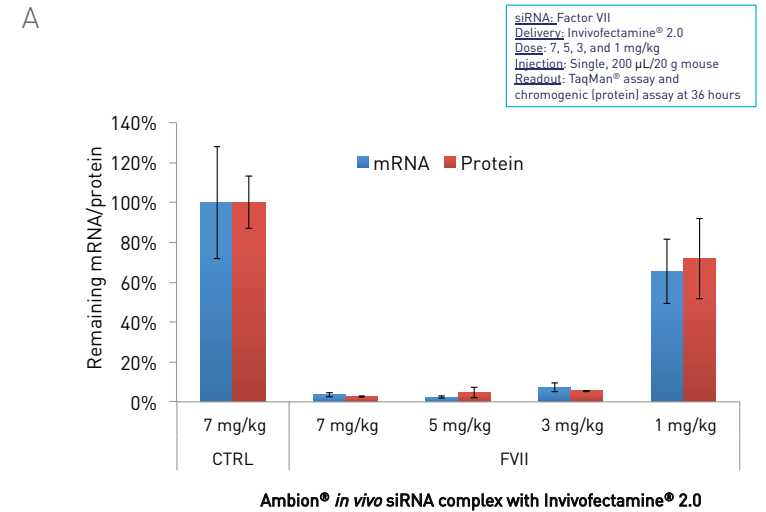
Invivolectamine® 2.0 Reagent is our latest innovation for *in vivo* siRNA delivery. This reagent is designed for systemic siRNA delivery with high transfection efficiency in liver following tail vein injection, producing highly effective mRNA and protein knockdown. The reagent demonstrates a low toxicity profile and is exceptionally easy to use.

## Performance

Complexes composed of Invivolectamine® 2.0 Reagent and Ambion® *In Vivo* siRNA targeting factor VII were successfully delivered by tail vein injection at 7, 5, 3, and 1 mg/kg (Figure 15A). Effective knockdown of factor VII mRNA and protein was demonstrated (Figure 15A) and loss of protein activity in serum was effective over several days after injection (Figure 15B).

Invivolectamine® 2.0 Reagent was complexed with siRNA for factor VII, ApoB, GAPDH, and PPIB. Effective gene knockdown of multiple target genes was demonstrated in liver after a single intravenous injection (Figure 15C).

**Figure 15. Invivolectamine® 2.0 Reagent and Ambion® *In Vivo* siRNA demonstrate knockdown in liver after a single intravenous injection.** (A) Invivolectamine® 2.0 Reagent complexed with factor VII siRNA achieves effective knockdown of mRNA and protein. Invivolectamine® 2.0 Reagent complexed with factor VII siRNA was injected at doses of 7, 5, 3, and 1 mg/kg. Liver tissue and plasma were collected 36 hours postinjection and analyzed for factor VII mRNA via TaqMan® qRT-PCR assay and factor VII protein levels via chromogenic assay. (B) Knockdown of factor VII protein is effective over several days. Factor VII siRNA molecules were complexed with Invivolectamine® 2.0 Reagent and injected at doses of 3, 5, and 7 mg/kg. Plasma samples were collected on days 2, 9, 15, 22, and 29 postinjection and analyzed for enzymatic factor VII activity with a chromogenic substrate. (C) Invivolectamine® 2.0 Reagent achieves effective knockdown of multiple target genes after a single intravenous injection. Invivolectamine® 2.0 Reagent was complexed with a combination of the following siRNA: ApoB (25%), factor VII (15%), GAPDH (35%), and PPIB (25%); ApoB (25%), factor VII (15%), GAPDH (35%), and negative control (25%); ApoB (25%), factor VII (15%), and negative control (85%); factor VII (15%) and negative control (85%); negative control only. Complexes were injected at 7.0 mg/kg into BALB/c mice on a normal diet via intravenous route. Liver tissue was collected 36 hours postinjection and analyzed for mRNA via TaqMan® qRT-PCR assay.

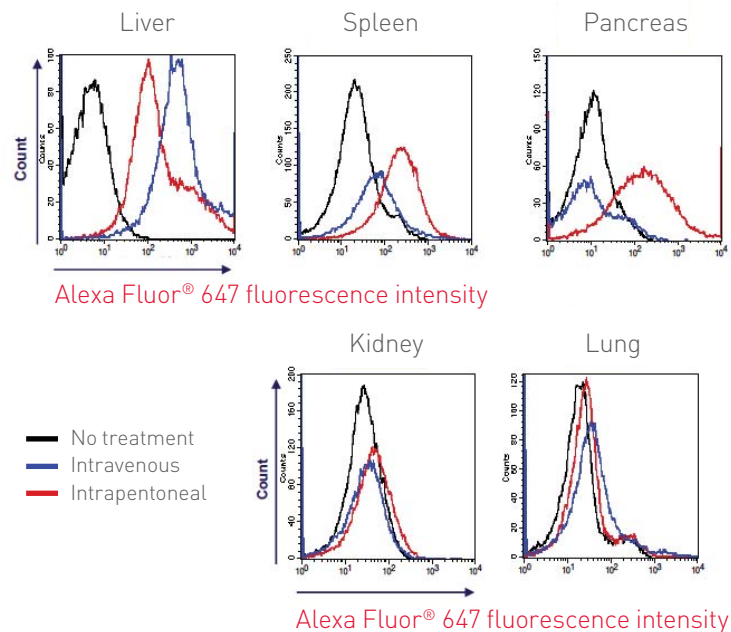


In vivo delivery

## Biodistribution

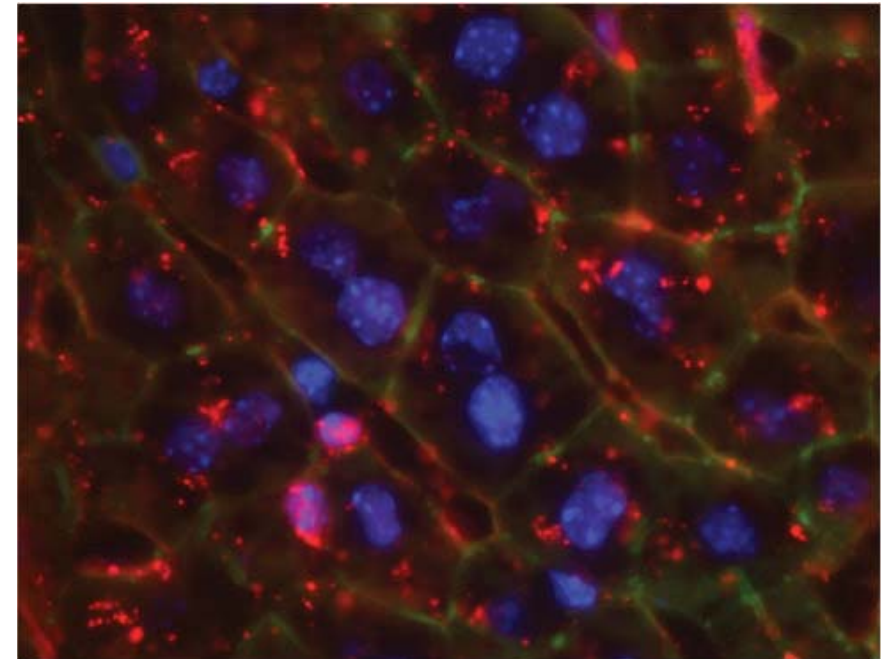
The biodistribution of InvivoFectamine® 2.0 with Alexa Fluor® 647-labeled siRNA is shown in Figure 16. Majority of the siRNA was delivered to hepatocytes after intravenous injection; however, there was also some delivery to the spleen and kidney. Cryosectioning of the kidney revealed that the labeled siRNA was specifically delivered to the glomeruli (data not shown). For intraperitoneal injection, a different biodistribution pattern was observed. Delivery of labeled siRNA occurred mostly to the liver, although to a lesser degree than intravenous injection, as well as to the spleen and pancreas (Figure 16A).

A



**Figure 16. Biodistribution of Alexa Fluor® 647-labeled Stealth RNAi™ siRNA after injection.** (A) Tissues were harvested 4 hours after intravenous or intraperitoneal injection of Alexa Fluor® 647-labeled Stealth RNAi™ siRNA complexed with InvivoFectamine® 2.0 Reagent (7 mg/kg). Single cells from each tissue were analyzed by flow cytometry. (B) Liver tissue was harvested and prepared for immunofluorescence 24 hours after intravenous injection of Alexa Fluor® 647-labeled Stealth RNAi™ siRNA complexed with InvivoFectamine® 2.0 Reagent (7 mg/kg). Tissue was fixed and stained with Alexa Fluor® 488 phalloidin to outline the cell membrane and Hoechst DNA stain to identify nuclei.

B



We also offer InvivoFectamine® Rx, an *in vivo* delivery option that can be used for potential therapeutic applications. More information and product detail can be obtained by contacting [outlicensing@lifetech.com](mailto:outlicensing@lifetech.com)

To learn more or place an order, go to [lifetechnologies.com/invivoFectamine](https://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.

## 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).

To learn more or place an order, go to [lifetechnologies.com/optimem](https://lifetechnologies.com/optimem)



Table 2. Eukaryotic selection antibiotics.

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 (bacterial) 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 or place an order, go to [lifetechnologies.com/selectionantibiotics](https://lifetechnologies.com/selectionantibiotics)

# Genome modulation and engineering

## Ambion<sup>®</sup> 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*<sup>®</sup> Select siRNA products have been validated with Lipofectamine<sup>®</sup> 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*<sup>®</sup> 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*<sup>®</sup> Select siRNA library

\*We guarantee that when you purchase two *Silencer*<sup>®</sup> Select Predesigned siRNAs to the same target, then those two siRNAs will silence the target mRNA by 70% or more. To qualify for the guarantee, siRNAs must have been transfected at  $\geq 5$  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*<sup>®</sup> Select siRNA designs will be provided free of charge. This guarantee does not extend to any replacement product.

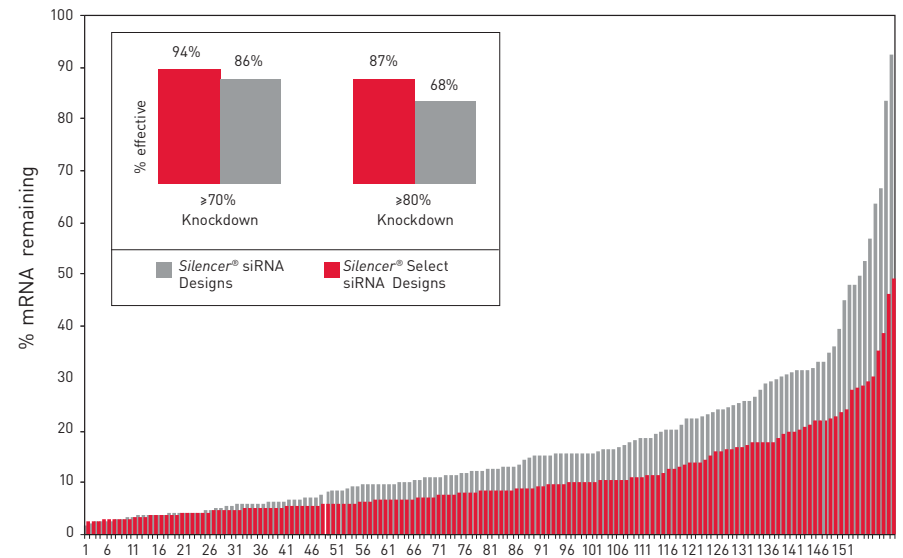


Figure 17. *Silencer*<sup>®</sup> Select siRNA design algorithm significantly improves effective siRNA prediction accuracy. The *Silencer*<sup>®</sup> Select siRNA design algorithm was used to design 155 siRNAs to 40 different targets. These siRNAs were tested side by side with siRNAs designed using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

Table 3. Ambion® siRNA selection guide.

	Cost-effective siRNA	Good knockdown, low off-target effects	Highest knockdown, lowest off-target effects
	<i>Silencer</i> ® siRNA	Stealth RNAi™ siRNA	<i>Silencer</i> ® 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-resistant Ambion® *In Vivo* siRNAs to obtain reproducible results. Ambion® *In Vivo* siRNAs have been validated with InvivoFectamine® 2.0 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](mailto:RNAiLibraries@lifetech.com)

To learn more or place an order, go to [lifetechnologies.com/sirna](https://www.lifetechnologies.com/sirna)

## mirVana™ miRNA Mimics and Inhibitors

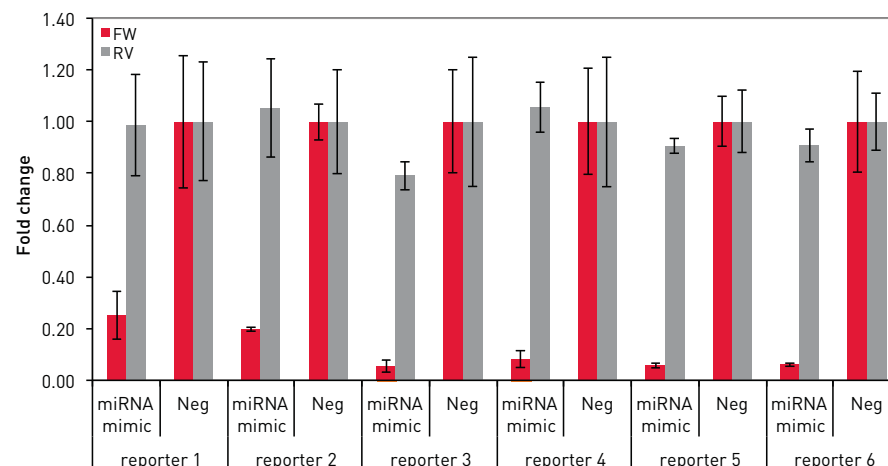
mirVana™ miRNA Mimics and Inhibitors are chemically modified, synthetic nucleic acids designed to either mimic mature miRNAs, or to bind to and inhibit endogenous miRNAs. These products provide a means to functionally study the role of specific miRNAs within cellular systems, or to validate the role of miRNAs in regulating target genes. mirVana™ miRNA Mimics and Inhibitors have been validated with Lipofectamine® RNAiMAX Transfection Reagent for use in cell-based systems, and with InvivoFectamine® 2.0 Reagent for *in vivo* delivery. *In vivo*-ready mirVana™ miRNA Mimics and Inhibitors have been purified by HPLC and dialysis, making them ready for immediate use.

mirVana™ 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

## mirVana™ miRNA Libraries

Complete mirVana™ 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@lifetechnologies.com](mailto:RNAiLibraries@lifetechnologies.com)



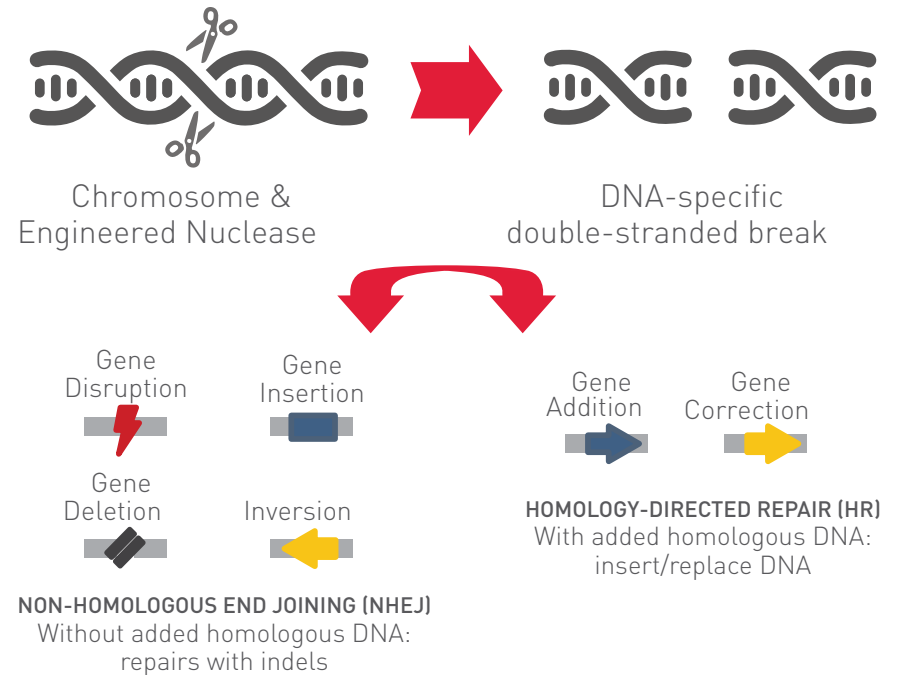
**Figure 18. The mature strand of mirVana™ miRNA Mimics are highly potent.** miRNA mimics, like natural miRNAs, have 2 strands—the mature strand (guide strand) that is functional and used by Ago protein to target mRNAs, and the star or passenger strand that is nonfunctional and is normally cleaved and 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 mirVana™ 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 or place an order, go to [lifetechnologies.com/mirna](http://lifetechnologies.com/mirna)

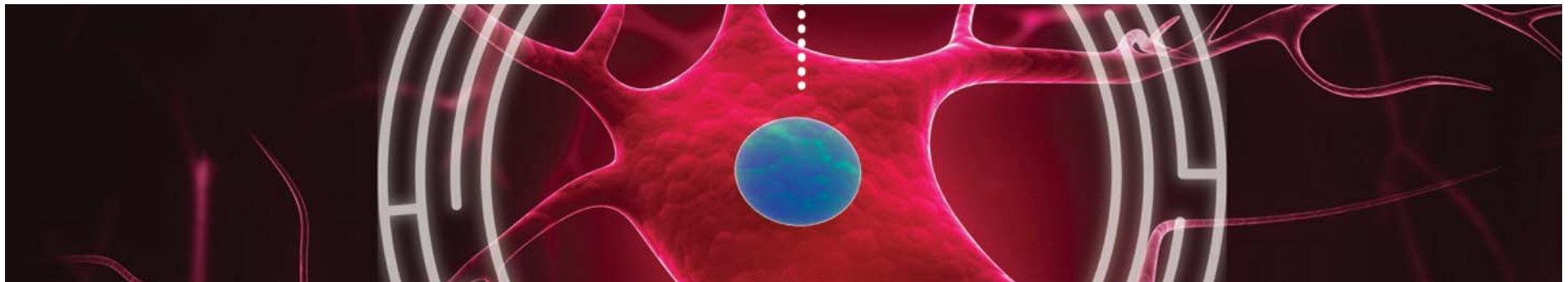
# Genome editing

## 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.



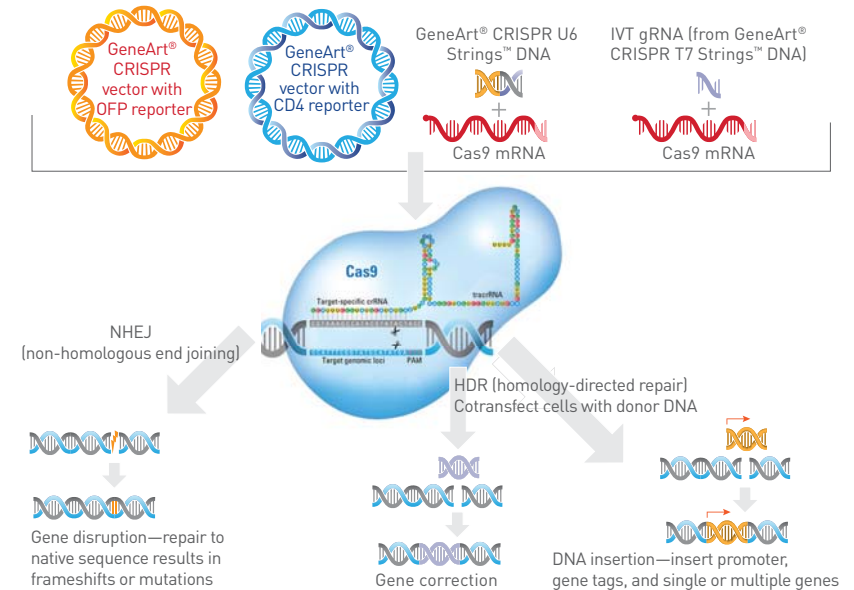
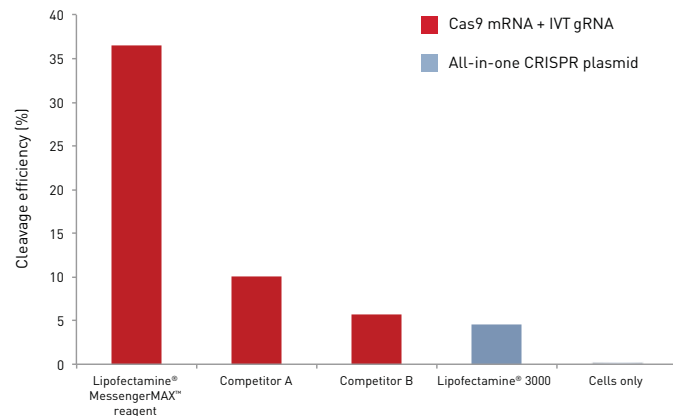
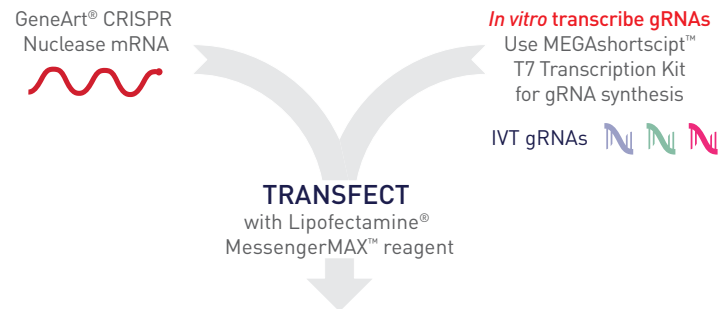
**Figure 19. Engineered nuclease-mediated genome editing.** The CRISPR-Cas9 system or TAL effector nucleases can be designed to target specific sites in the genome, creating double-strand breaks (DSBs) at desired locations. The natural repair mechanisms of the cell repair the break by either homologous 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 or place an order, go to [lifetechnologies.com/CRISPR](https://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.

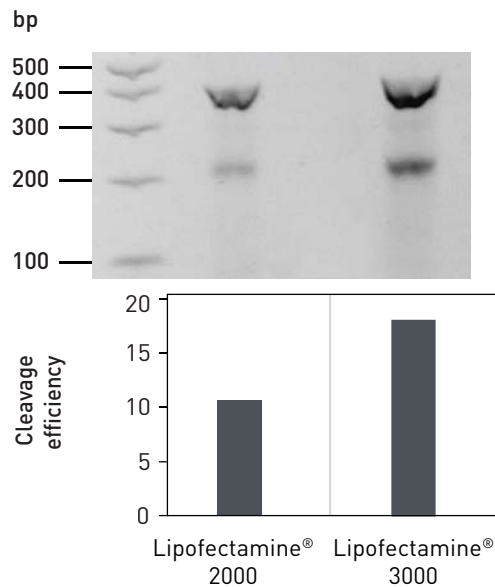
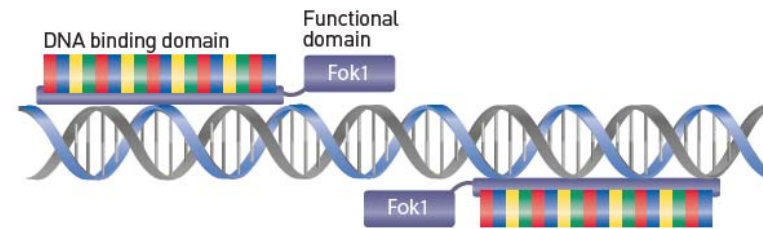
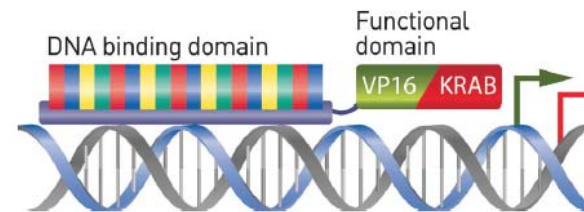


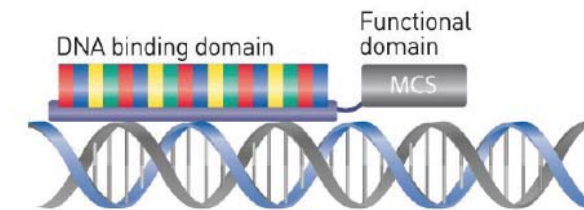
Figure 22. Cleavage efficiency for GeneArt® TALs. TALs targeting the *AAVS1* locus were transfected by Lipofectamine® 2000 or Lipofectamine® 3000 reagent in the U2OS cell line. Cleavage was assayed using the GeneArt® Genomic Cleavage Detection Kit.



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.

To learn more or place an order, go to [lifetechnologies.com/tals](https://lifetechnologies.com/tals)

# 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

<b>Electroporation <i>continued</i></b>	<b>Quantity</b>	<b>Cat. No.</b>
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
<b><i>In vivo</i> delivery</b>	<b>Quantity</b>	<b>Cat. No.</b>
InvivoFectamine® 2.0 Reagent	1 mL	1377501
InvivoFectamine® 2.0 Reagent	5 x 1 mL	1377505
<b>Transfection-related products</b>	<b>Quantity</b>	<b>Cat. No.</b>
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
<b>Genome modulation and engineering</b>	<b>Quantity</b>	<b>Cat. No.</b>
<b><i>Silencer</i>® Select siRNA—highest knockdown, lowest off-target effects</b>		
<i>Silencer</i> ® Select Predesigned siRNA	5 nmol	4390771
<i>Silencer</i> ® Select Validated siRNA	5 nmol	4390824
<i>Silencer</i> ® Select Custom-Designed siRNA	5 nmol	4399665
<i>Silencer</i> ® Select Custom-Sequence siRNA	5 nmol	4390827
<i>Silencer</i> ® Select Negative Control siRNA # 1	5 nmol	4390843
<i>Silencer</i> ® Select Negative Control siRNA # 2	5 nmol	4390846
<i>Silencer</i> ® Select GAPDH Positive Control siRNA	5 nmol	4390849
<i>Silencer</i> ® Select MALAT1 Positive Control siRNA	5 nmol	4455877
<b><i>Stealth</i> siRNA—good knockdown, low off-target effects</b>		
Stealth RNAi™ Predesigned siRNA, Human	20 nmol	1299001
Stealth RNAi™ Predesigned siRNA, Human Set of 3	20 nmol x 3	1299003
Stealth RNAi™ Predesigned siRNA, Mouse	20 nmol	1320001
Stealth RNAi™ Predesigned siRNA, Mouse Set of 3	20 nmol x 3	1320003
Stealth RNAi™ Predesigned siRNA, Rat	20 nmol	1330001
Stealth RNAi™ Predesigned siRNA, Rat Set of 3	20 nmol x 3	1330003
Stealth RNAi™ siRNA Negative Control, low GC	250 µL	12935200

Genome modulation and engineering <i>continued</i>	Quantity	Cat. No.
Stealth RNAi™ siRNA Negative Control, medium GC	250 µL	12935300
Stealth RNAi™ siRNA Negative Control, high GC	250 µL	12935400
BLOCK-IT™ Alexa Fluor® Red Fluorescent Control	2 x 125 µL	14750100
<b>Silencer® siRNA—cost-effective siRNA</b>		
<i>Silencer®</i> Validated siRNA	5 nmol	AM51331
<i>Silencer®</i> Predesigned siRNA	5 nmol	AM16708
<i>Silencer®</i> Custom-designed siRNA	5 nmol	4399670
<i>Silencer®</i> GAPDH Positive Control siRNA (including 2 nmol negative control siRNA)	5 nmol	AM4624
<i>Silencer®</i> Negative Control # 1 siRNA	5 nmol	AM4611
<b>mirVana™ miRNA Mimics and Inhibitors*—next-generation miRNA chemistries for lowest off-target effects and highest potency</b>		
<i>mirVana™</i> Predesigned miRNA Mimic	5 nmol	4464066
<i>mirVana™</i> Predesigned miRNA Mimic, <i>in vivo</i> use	250 nmol	4464070
<i>mirVana™</i> Predesigned miRNA Inhibitor	5 nmol	4464084
<i>mirVana™</i> Predesigned miRNA Inhibitor, <i>in vivo</i> use	250 nmol	4464088
<i>mirVana™</i> miRNA Mimic, Negative Control #1	5 nmol	4464058
<i>mirVana™</i> miRNA Inhibitor, Negative Control #1	5 nmol	4464076
<i>mirVana™</i> miRNA Mimic, miR-1 Positive Control	5 nmol	4464062
<i>mirVana™</i> miRNA Inhibitor, let-7c Positive Control	5 nmol	4464080
<i>mirVana™</i> Custom miRNA Mimic	5 nmol	4464068
<i>mirVana™</i> Custom miRNA Mimic, <i>in vivo</i> use	250 nmol	4464071
<i>mirVana™</i> Custom miRNA Inhibitor	5 nmol	4464086
<i>mirVana™</i> Custom miRNA Inhibitor, <i>in vivo</i> use	250 nmol	4464089
<b>Pre-miR™ Precursors and Anti-miR™ Inhibitors**—trusted, affordable miRNA</b>		
<b>Predesigned</b>		
Anti-miR™ miRNA Inhibitor	5 nmol	AM17000
Pre-miR™ miRNA Precursor	5 nmol	AM17100
<b>Controls</b>		
Pre-miR™ hsa-miR-1 miRNA Precursor Positive Control	5 nmol	AM17150
Pre-miR™ miRNA Precursor Negative Control #1	5 nmol	AM17110
Cy3® Dye-Labeled Pre-miR™ Negative Control #1	5 nmol	AM17120
Anti-miR™ hsa-let-7c miRNA Inhibitor Positive Control	5 nmol	4392431

Genome modulation and engineering <i>continued</i>	Quantity	Cat. No.
Anti-miR™ miRNA Inhibitor Negative Control #1	5 nmol	AM17010
Cy3® Dye-Labeled Anti-miR™ Negative Control #1	5 nmol	AM17011
<b>Custom</b>		
Anti-miR™ Inhibitor	Custom	AM17004
Pre-miR™ miRNA Precursor	Custom	AM17104
<b>Genome editing</b>		
GeneArt® CRISPR Nuclease mRNA	15 µg	A25640
GeneArt® Strings™ U6 DNA	200 ng	Contact <a href="mailto:geneartsupport@lifetech.com">geneartsupport@lifetech.com</a>
GeneArt® Strings™ T7 DNA	200 ng	Contact <a href="mailto:geneartsupport@lifetech.com">geneartsupport@lifetech.com</a>
GeneArt® CRISPR mRNA with custom <i>in vitro</i> transcribed gRNA	Custom	Contact <a href="mailto:geneartsupport@lifetech.com">geneartsupport@lifetech.com</a>
GeneArt® CRISPR Nuclease Vector with OFP Reporter Kit	10 reactions	A21174
GeneArt® CRISPR Nuclease Vector with CD4 Enrichment Kit	10 reactions	A21175
GeneArt® CRISPR Nuclease Vector with CD4 Enrichment Kit (with competent cells)	10 reactions	A21177
GeneArt® CRISPR Nuclease Vector with OFP Reporter Kit (with competent cells)	10 reactions	A21178
Custom CRISPR for every gene—we'll design your target and clone it for you; ready to transfect	100 µg	Contact <a href="mailto:geneartsupport@lifetech.com">geneartsupport@lifetech.com</a>
GeneArt® Precision TALs	Custom	Contact <a href="mailto:geneartsupport@lifetech.com">geneartsupport@lifetech.com</a>
GeneArt® PerfectMatch TALs	Custom	Contact <a href="mailto:geneartsupport@lifetech.com">geneartsupport@lifetech.com</a>
GeneArt® Genomic Cleavage Detection Kit	20 reactions	A24372

\*Covers human, mouse, rat, and other species.

\*\*Covers human.





Find out more at [lifetechnologies.com/transfection](http://lifetechnologies.com/transfection)

For Research Use Only. Not for use in diagnostic procedures. © 2014 Thermo Fisher Scientific Inc. All rights reserved. All trademarks are the property of Thermo Fisher Scientific and its subsidiaries unless otherwise specified. Cy is a trademark of GE Healthcare UK Limited. C035627 1114



A Thermo Fisher Scientific Brand

---