

# CRISPR genome editing resource guide



Editing



Culture



Delivery

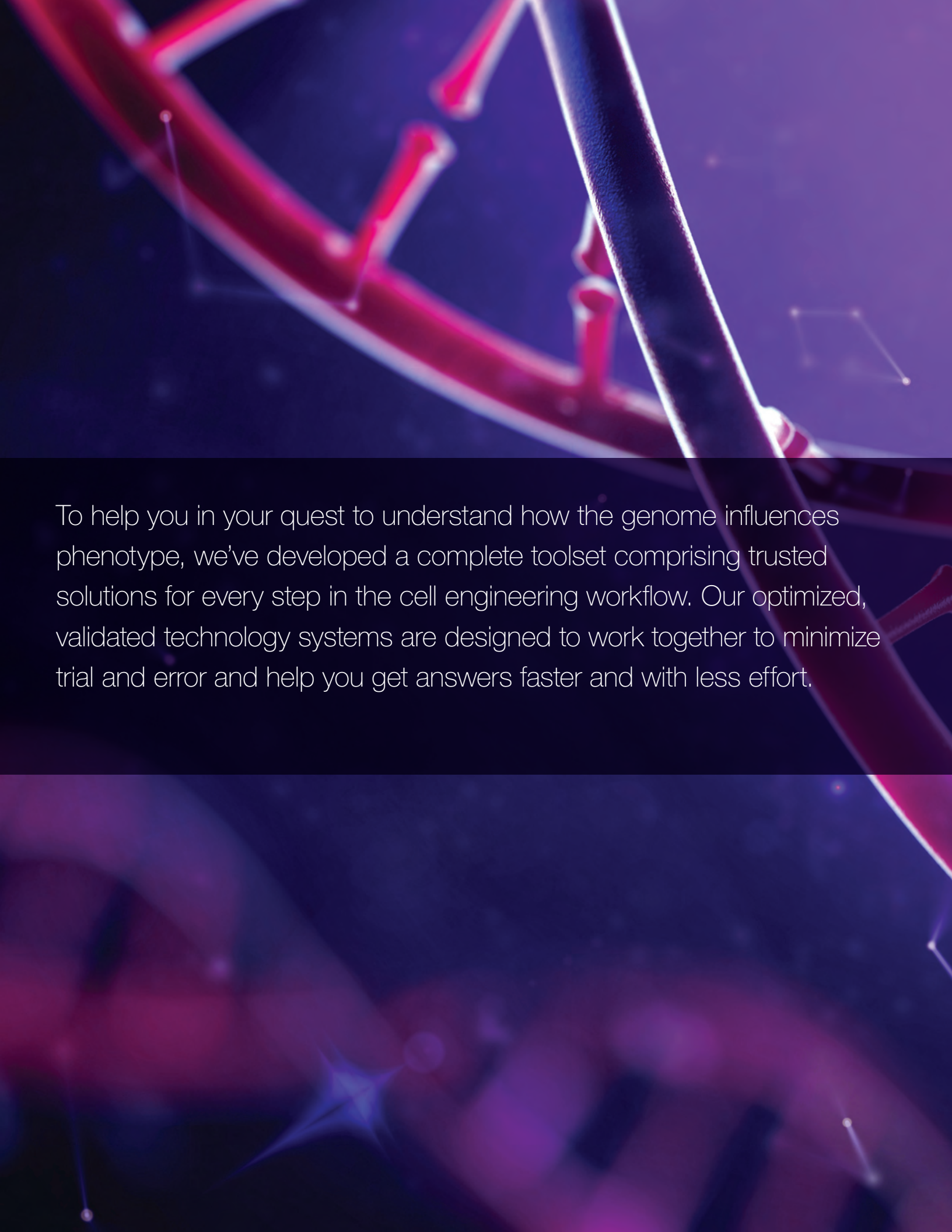


Screen and detect

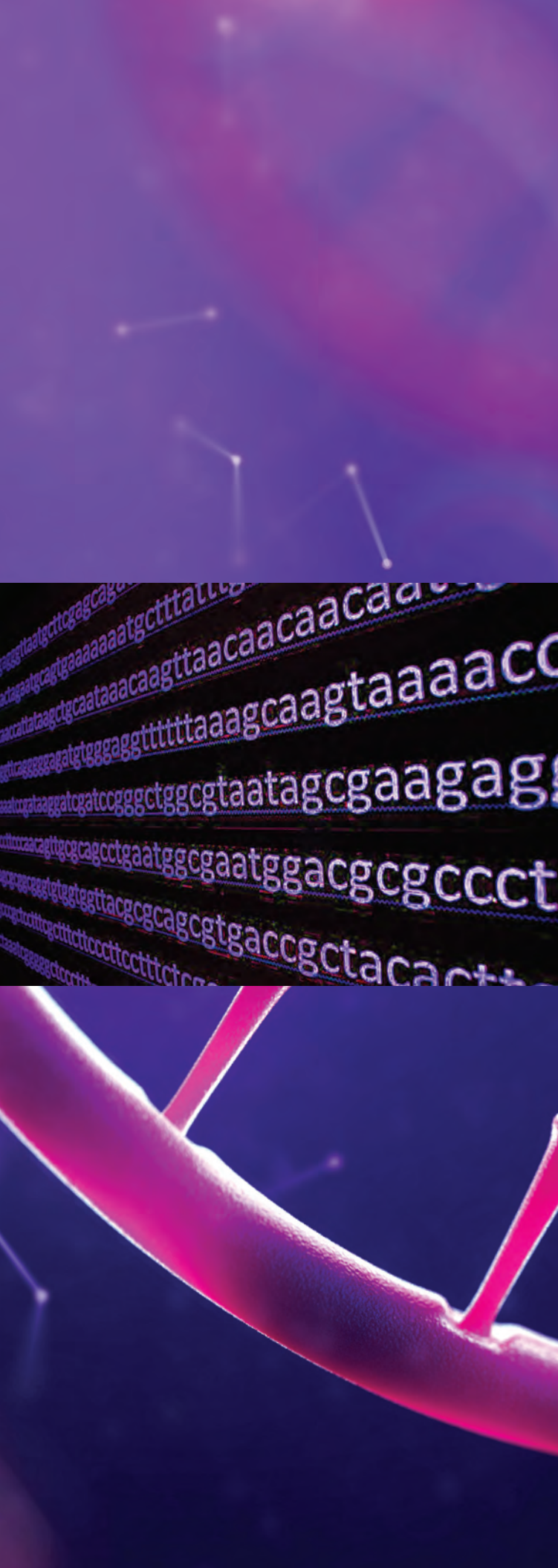
A complete cell engineering solution from start to discovery

Third edition





To help you in your quest to understand how the genome influences phenotype, we've developed a complete toolset comprising trusted solutions for every step in the cell engineering workflow. Our optimized, validated technology systems are designed to work together to minimize trial and error and help you get answers faster and with less effort.



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As a leader in genome editing, we provide:

1



**More choices,  
better results**

Whether you need an economical solution for routine CRISPR-Cas9 editing tasks in standard cell lines, or need to drive maximum editing efficiency in primary cells or induced pluripotent stem cell (iPSC) lines, the Invitrogen™ genome editing suite has a solution to meet your needs.

2



**Superior  
support**

From local technical specialists to our technical support center and dedicated genome editing R&D team—if you have genome editing questions, we've got people who have the answers.

3



**Focused  
innovation**

Our R&D team is dedicated to pushing the boundaries of genome editing and is focused on developing innovative solutions to increase the performance, precision, and ease of use of our editing reagents.

4



**Proven quality**

All of our genome editing reagents are manufactured to meet or exceed the rigorous quality standards that you expect and rely on from Invitrogen products.

5



**Trust**

We leverage 30 years of industry-leading experience in cell and molecular biology to provide effective solutions that help you make new discoveries faster.

6



**Validated  
solutions**

Our extensive line of products includes a complete collection of genome editing tools, each backed by validated protocols for a variety of cell lines and readily available customer service.

# Cell engineering applications and workflow solutions

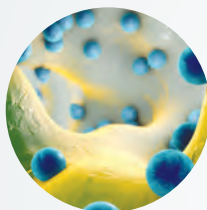
## Key applications

Advances in genome editing have the potential to change the way we create energy, produce food, optimize industrial processing, as well as detect, prevent, and cure diseases. Through innovative design and engineering, this unique science enables researchers like you to study, alter, create, and reconstitute highly complex pathways, DNA sequences, genes, and natural biological systems. With a better understanding of life's most challenging biological questions, we can uncover answers to improve the human condition and the world around us.

We've created this resource to explain the cell engineering technologies and tools available today, and to guide you in choosing the solutions you need to break through to discovery faster. The graphic below highlights just some of the many applications for which genome editing is applicable.



Animal disease models



Tissue disease models



Stem cell engineering



Gene therapy



Disease-resistant transgenic plants

## Workflow solutions

Get everything you need to design, deliver, and detect so you can engineer your cells—all from one place. We're continuing to expand our suite of genome editing products to span the entire cell engineering workflow—from cell culture, delivery, and sample preparation to genome modification, detection, and analysis of known genetic variants.



# Design and build | Genome editing tools

Genome editing technologies, such as the CRISPR-Cas9 system and TAL effectors, provide precise and efficient methods for manipulating genomic DNA sequences. Through innovative design and engineering, these unique tools enable researchers to study, alter, create, and re-create highly complex pathways, DNA sequences, genes, and natural biological systems. Whether you are seeking to knock out a specific gene or introduce (or correct) a specific mutation, the latest genome editing tools allow you to build organ- and disease-specific models to drive understanding of how individual genes and mutations influence disease development and progression (Figure 1).

Our collection of optimized genome editing tools are designed to work together to eliminate the trial-and-error phase and help you develop models faster and with less effort. Every lab is unique, so we offer a range of genome editing solutions to cater to your needs. Whether you want results fast, seek full control over every step in designing your gene edit, or need help with engineering cells to your specific needs, we have solutions that fit (Table 1). Learn more about these genome editing products and services at [thermofisher.com/genomeedit](https://thermofisher.com/genomeedit)

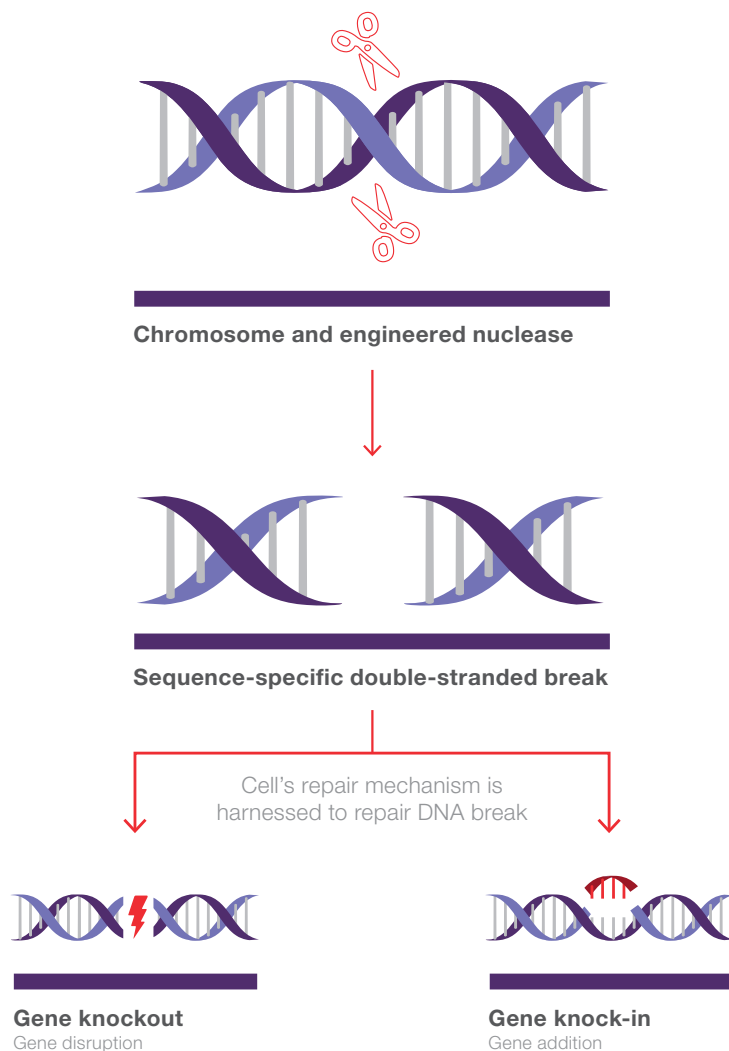


Figure 1. Engineered nuclease mechanism.

## Support resources

- New to genome editing? Access our learning center at [thermofisher.com/genomeedit101](https://thermofisher.com/genomeedit101)
- Join our new, hands-on CRISPR workshop at [thermofisher.com/crisprworkshop](https://thermofisher.com/crisprworkshop)
- Access our collection of validated protocols at [thermofisher.com/crisprprotocols](https://thermofisher.com/crisprprotocols)



**Table 1. Comparison of genome editing and silencing tools.**

	Single-gene analysis		High-throughput screening	
End goal	Permanent gene knockout or knock-in	Permanent gene knockout, knock-in, downregulation, or activation	Transient gene knockdown	Permanent gene knockout
Technology	CRISPR-Cas9	TALEN	RNAi	CRISPR-Cas9
Benefits	<ul style="list-style-type: none"> <li>• Superior cleavage efficiency</li> <li>• Simple design and assembly process</li> <li>• Multiplexing capable</li> </ul>	<ul style="list-style-type: none"> <li>• Flexible; no sequence restriction or PAM requirement; ideal for knock-in</li> <li>• Includes rights under foundational TAL intellectual property</li> </ul>	<ul style="list-style-type: none"> <li>• Ultimate flexibility in technology and gene targets</li> <li>• High potency</li> <li>• Minimal off-target effects</li> </ul>	<ul style="list-style-type: none"> <li>• Superior cleavage efficiency</li> <li>• No cell-specific promoter constraint</li> <li>• No random integration concern</li> </ul>
Design requirement	PAM site (NGG)	Completely flexible, no design restrictions	–	PAM site (NGG)
Ideal products for PSCs	TrueCut Cas9 Protein v2 and TrueGuide Synthetic gRNA	GeneArt Precision TALs	Silencer Select siRNA Libraries	LentiArray CRISPR Libraries or Custom LentiPool CRISPR Libraries
Format	–	–	Arrayed or pooled	Arrayed or pooled

# CRISPR-Cas9 technology

## Revolutionizing the field of genome editing

The transformative CRISPR-Cas9 technology is revolutionizing the field of genome editing. Able to achieve highly flexible and specific targeting, the CRISPR-Cas9 system can be modified and redirected to become a powerful tool for genome editing in broad applications such as stem cell engineering, gene therapy, tissue and animal disease models, and engineering disease-resistant transgenic plants.

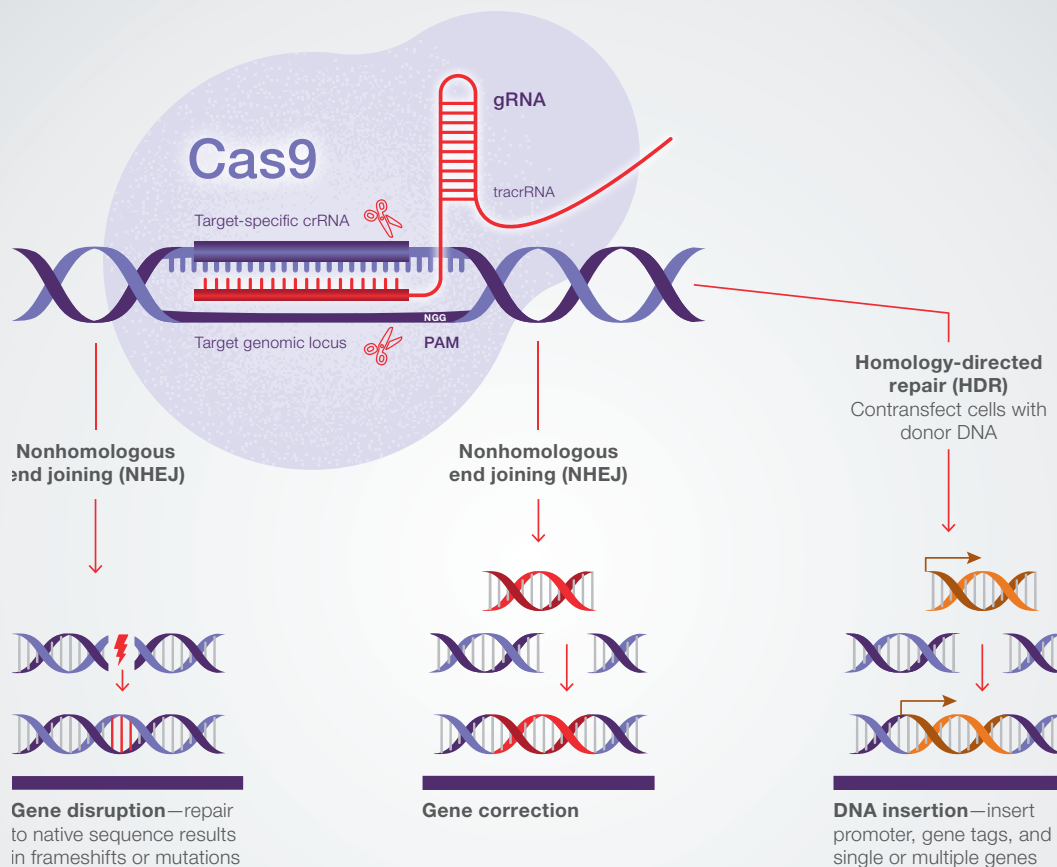
## What is CRISPR-Cas9 technology?

The clustered regularly interspaced short palindromic repeats (CRISPR) and CRISPR-associated protein 9 (Cas9) system is the latest addition to the genome editing toolbox, offering a simple, rapid, and efficient tool. Derived from components of a simple bacterial immune system, the CRISPR-Cas9 system permits targeted gene cleavage and gene editing in a variety of eukaryotic cells. Because

the endonuclease cleavage specificity in the CRISPR-Cas9 system is guided by RNA sequences, editing can be directed to virtually any genomic locus by engineering the guide RNA (gRNA) sequence and delivering it along with the Cas9 endonuclease to your target cell.

## How does CRISPR-Cas9 work?

The CRISPR-Cas9 system is composed of a short noncoding 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 nuclease to a specific genomic locus, and the Cas9 protein induces a double-stranded break at the specific genomic target sequence. Following CRISPR-Cas9–induced DNA cleavage, the double-stranded break can be repaired by the cellular repair machinery using either nonhomologous end joining or a homology-directed repair mechanism (Figure 2).



**Figure 2. CRISPR-Cas9 double-stranded break and repair pathways.** Cleavage occurs on both strands, 3 bp upstream of the NGG in the protospacer adjacent motif (PAM) sequence on the 3' end of the target sequence.



# Tips and tricks for getting started

## Set yourself up for CRISPR success

New to CRISPR technology? Be sure you truly understand the factors affecting the outcome of genome editing efficiency before getting started.

1



### Design

#### Things to consider when designing your experiment

- Design and test 3 gRNAs for every target
- Run bioinformatic analysis to choose gRNAs with minimal predicted off-target effects
- For knockout experiments, target early constitutive exons to disrupt the reading frame
- For knock-in experiments, cleavage site should be as close to the edit site as possible
- Choose the Cas9 nuclease format best suited for the experiment

2



### Deliver

#### Things to consider when transfecting cells

- Start with optimal cell density and cell health
- Know the best method for transfecting your cells: lipid, electroporation, or viral
- When delivering multiple molecules, optimize the ratio of their quantities
- Transfect a validated control gRNA to test transfection efficiency and Cas9 activity
- For knock-in experiments, deliver the repair template so that it is present when Cas9 is active

3



### Detect

#### Thing to consider when detecting the edit

- Confirm gRNA cleavage efficiency quickly with a genomic cleavage detection (GCD) assay
- Choose a detection method appropriate for the downstream application
- Isolate clones if a genetically homogenous population is desired
- Understand that the most efficient gRNA for indel-based knockout is typically the most efficient gRNA for knock-in
- Verify lack of off-target editing using targeted sequencing

## Support resources

New to CRISPR genome editing?  
Learn more at [thermofisher.com/crispr101](https://thermofisher.com/crispr101)



# CRISPR-Cas9 editing tools

## Maximum flexibility of high-quality gRNA and Cas9 nuclease

The CRISPR-Cas9 system is a two-component system, consisting of the target-specific CRISPR gRNA and Cas9 nuclease. We offer both of these components in multiple formats to give you flexibility in experimental design (Figure 3).

### gRNA formats



### Cas9 nuclease formats

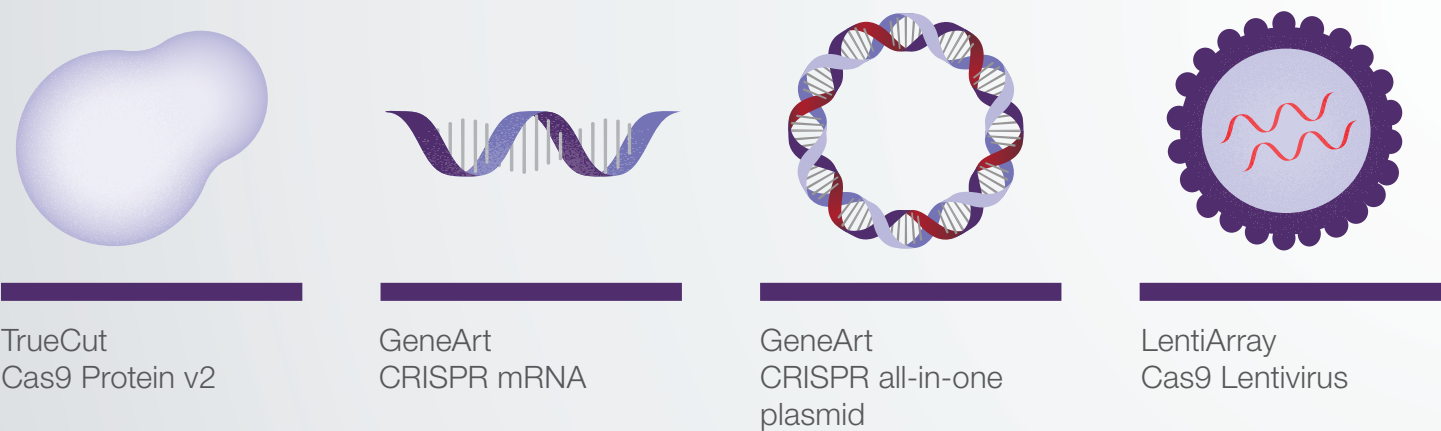


Figure 3. Available CRISPR-Cas9 formats.

Find out more or place your order at [thermofisher.com/crispr](https://thermofisher.com/crispr)

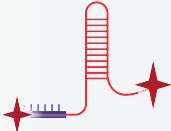

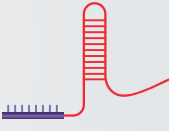
CRISPR gRNA design and formats

Critical to the editing efficiency of the CRISPR-Cas9 system is the design of the gRNA. Our proprietary design algorithms select gRNAs for maximum editing efficiency without compromising specificity. Our tool incorporates the latest in gRNA design research with our extensive in-house experience to bring you the most efficient gRNA sequences (Figure 4). The gRNAs are available in multiple formats for greater flexibility in your applications (Table 2).



Figure 4. Robust design and selection process for CRISPR gRNAs. These steps are a distilled description of the algorithm rules used for our predesigned gRNAs.

Table 2. Available CRISPR gRNA formats.

			
	TrueGuide Synthetic gRNA	LentiArray CRISPR gRNA	IVT gRNA Custom Service
Overview	Choose from our catalog of predesigned gRNAs, or upload your sequence to our TrueGuide gRNA Ordering Tool	Choose your gRNA design from our catalog of gRNAs packaged as ready-to-use lentivirus	Utilize our in-house expertise and have our custom services team design and synthesize ready-to-transfect gRNAs for you, or design them yourselves using our GeneArt CRISPR Search and Design Tool
Format	Synthetic gRNA oligo	Lentiviral gRNA	In vitro-transcribed (IVT) gRNA
Application	Knockout or knock-in	Knockout or library screening	Knockout or knock-in
Species	All species	Human	All species
Delivery method	Lipid-mediated transfection or electroporation	Lentiviral delivery	Lipid-mediated transfection or electroporation
Recommended Cas9 format	TrueCut Cas9 Protein v2	LentiArray Cas9 Lentivirus	TrueCut Cas9 Protein v2
Controls	Positive and negative controls available	Positive and negative controls available	Available upon request



## TrueGuide Synthetic gRNA

Invitrogen™ TrueGuide™ Synthetic gRNAs are ready-to-transfect synthetic gRNAs designed and validated to work with the Invitrogen™ suite of genome editing tools. These predesigned gRNAs offer:

- Maximum knockout efficiency without compromising specificity and cell viability
- Simple custom ordering
- Adherence to the rigorous quality standards that you expect and rely on from Invitrogen products

## Performance of predesigned synthetic gRNAs

ME-180 cells that stably express Cas9 were edited with TrueGuide Synthetic gRNAs in 2-piece format, which consist of separate crRNA and tracrRNA that were annealed together prior to delivery into cells. The cells were harvested and assayed for gene editing efficiency using the Invitrogen™ GeneArt™ Genomic Cleavage Detection Kit to assess the performance of gRNA designs (Figure 5). An average of >60% cleavage efficiency was achieved.

## CRISPR gRNA design basics

Identify target sequence:

- 18–20 nucleotides
- Adjacent to the PAM (NGG) site
  - PAM site is 3' of target sequence
  - Target sequence can be on the sense or antisense strand
  - Align sequence against host genome for off-target effects:

- gRNA–Cas9 nuclease complexes tolerate mismatches
- Published articles offer insights into choosing target sequence (Fu et al., 2013; Mali et al., 2013; Yang et al., 2014)

Confirm there is no NGG in double-stranded HDR template:

- Including the NGG will make it a target for cleavage
- It is not necessary to confirm that there is no NGG site in the gRNA sequence

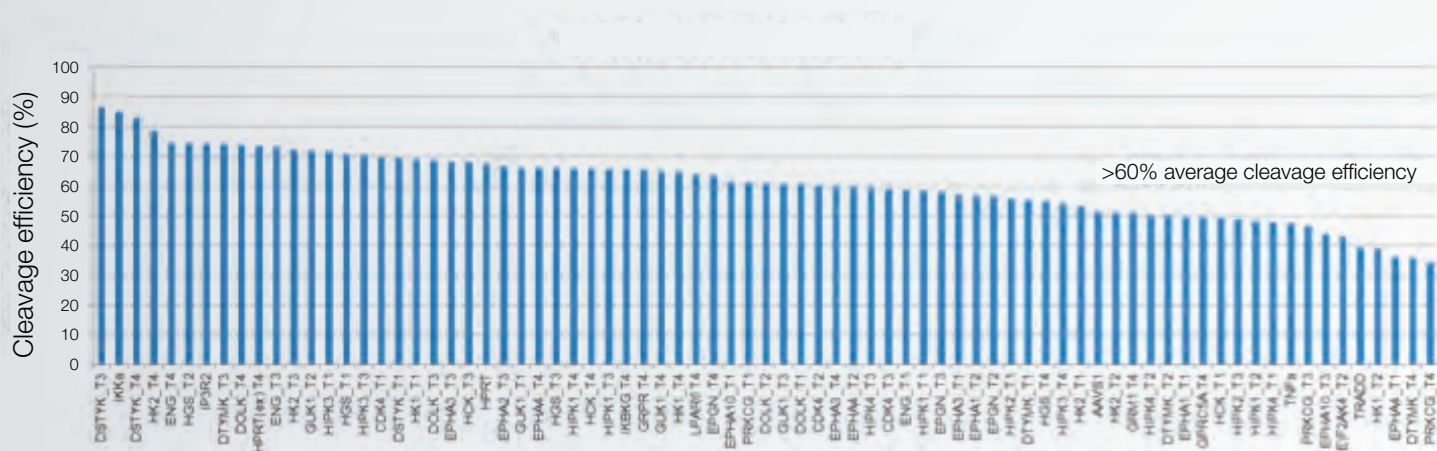


Figure 5. Robust cleavage of kinase genes with TrueGuide Synthetic gRNAs in Cas9-expressing ME-180 cells.

Learn more at [thermofisher.com/trueguide](https://thermofisher.com/trueguide)

## Essentials for working with your CRISPR gRNAs

### Nuclease-free tips and tubes

Pipette tips and tubes are an easily overlooked source of RNase contamination. We offer a range of RNase-free plastic pipette tips, PCR tubes, microcentrifuge tubes, and conical tubes. Each lot of tips and tubes undergoes rigorous testing and is certified to be nuclease-free.

[thermofisher.com/nucleasefreeplastics](https://thermofisher.com/nucleasefreeplastics)

### Nuclease-free water

Preparing reagents and resuspending precipitated RNA with the appropriate grade of water is a crucial and often ignored first step for ensuring consistent experimental results. We offer several grades of nuclease-free water—diethyl pyrocarbonate (DEPC)-treated water, nuclease-free water (not DEPC-treated), and RT-PCR-grade water—all rigorously tested for contaminating nonspecific endonuclease, exonuclease, and RNase activity.

[thermofisher.com/nucleasefreewater](https://thermofisher.com/nucleasefreewater)

## Surface decontamination

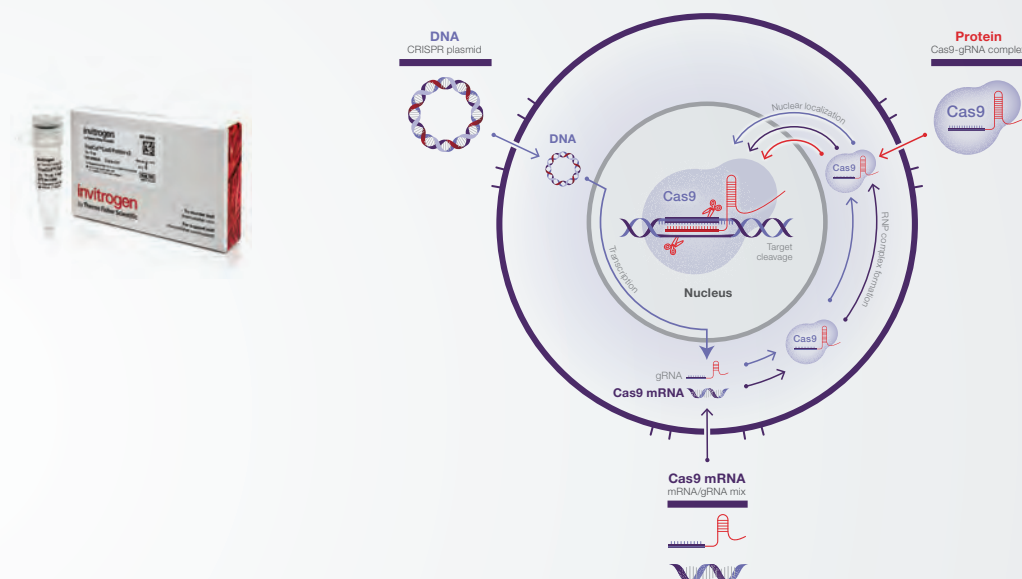
Most laboratory surfaces are likely to be contaminated with RNases, and even trace quantities of these enzymes can lead to RNA degradation. A suite of trusted products that are proven effective at removing RNase contamination from lab surfaces is available, including Invitrogen™ RNaseZap™ Decontamination Solution and RNase AWAY™ Decontamination Reagent.

[thermofisher.com/surfacedecontamination](https://thermofisher.com/surfacedecontamination)

## Cas9 nuclease

When choosing your Cas9 nuclease format, we recommend Invitrogen™ TrueCut™ Cas9 Protein v2 over CRISPR plasmids for the following reasons:

- CRISPR plasmids remain in the cell for more than 72 hr, contributing to potential off-target events
- Transfection of Cas9 protein and gRNA bypasses transcription and translation, helping to greatly increase editing efficiencies (Figure 6)
- Award-winning TrueCut Cas9 Protein v2 is cleared from the cell within 24 hr, minimizing the chance for off-target cleavage events



**Figure 6. Streamline cell engineering with preassembled Cas9–gRNA complex.** The Cas9 ribonucleoprotein (RNP) complex can act immediately after it enters the cell since transcription and translation are not required. The complex is rapidly cleared from the cell, minimizing the chance for off-target cleavage events compared to vector-based systems. The Cas9 protein is microinjection-ready and has nuclear localization signals.



### Award-winning TrueCut Cas9 Protein v2

TrueCut Cas9 Protein v2 is a next-generation Cas9 protein engineered to deliver maximum editing efficiency.

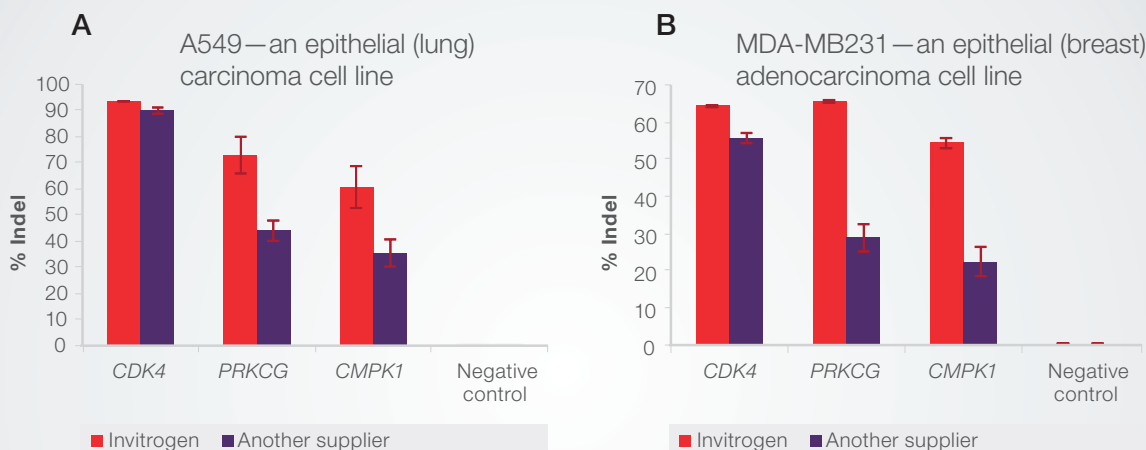
- Consistently high editing efficiency in all tested cell lines, including standard, immune, primary, and stem cells, with up to 2x higher editing efficiency in difficult targets compared to products from other suppliers
- Manufactured under strict ISO 13485 quality standards
- Validated protocols for a large number of cell types help you achieve success faster—access these protocols at [thermofisher.com/crisprprotocols](https://thermofisher.com/crisprprotocols)

Learn more at [thermofisher.com/crisprprotein](https://thermofisher.com/crisprprotein)

### Truly stunning performance, reliable results, and more choices

TrueCut Cas9 Protein v2 and TrueGuide Synthetic gRNA offer consistently high editing efficiency in a broad range of cell types and applications (Figures 7–9).

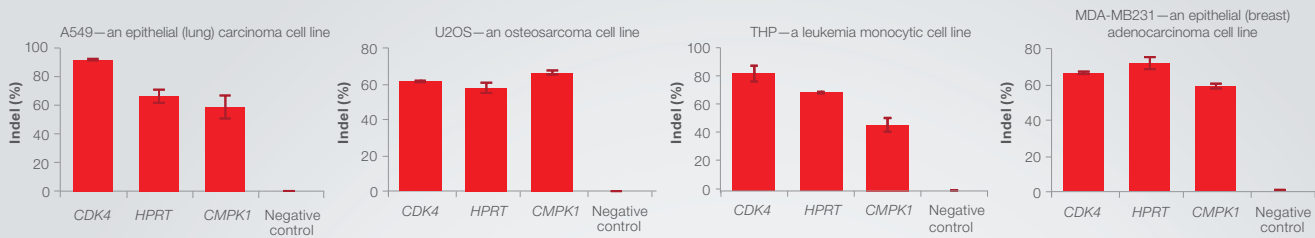
### Consistently outperforms products from other suppliers



**Figure 7. Invitrogen CRISPR tools consistently outperform products from other suppliers.** Genome editing of multiple gene targets was performed with TrueCut Cas9 Protein v2 and corresponding TrueGuide Synthetic gRNAs. Delivery was achieved using optimized transfection protocols and Invitrogen™ Lipofectamine™ CRISPRMAX Transfection Reagent in two cell lines: **(A)** A549, a human lung carcinoma cell line, and **(B)** MDA-MB231, a human breast cancer cell line. The graphs also compare the same experiments using products and recommended protocols from another supplier. With the Invitrogen system, cleavage efficiency is improved for low-efficiency loci (*PRKCG* and *CMPK1*) and shows consistently superior efficiency, up to a 2-fold increase, when compared to products and protocols from other suppliers.

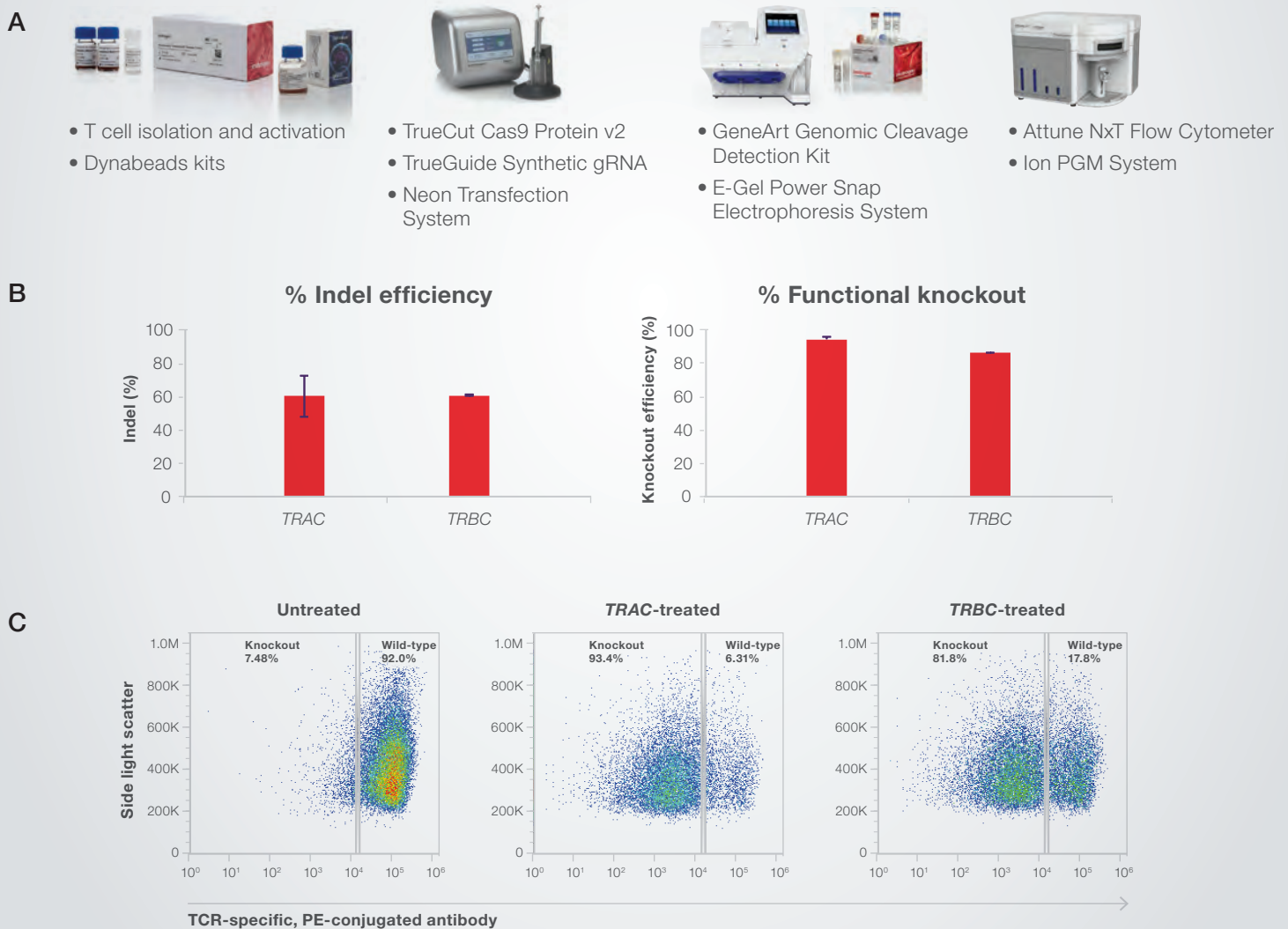


## Robust editing efficiencies in broad cell types and gene targets



**Figure 8. High-efficiency gene editing across a broad range of cell types and targets.** A wide range of adherent and suspension cell lines were tested to determine editing efficiencies using TrueGuide Synthetic gRNA complexed with TrueCut Cas9 Protein v2. Here, the Cas9 RNP complex was delivered into cell lines using Lipofectamine CRISPRMAX Cas9 Transfection Reagent. At 72 hours posttransfection, cells were harvested and tested for efficiency.

## Up to 90% functional knockout in human primary T cells



**Figure 9. End-to-end solution for T cell editing.** (A) Human T cells were isolated and activated using Invitrogen™ Dynabeads™ magnetic beads. Cells were then transfected with TrueCut Cas9 Protein v2 and TrueGuide Synthetic gRNA for the T cell receptor alpha (TRAC) or beta (TRBC) regions using the Invitrogen™ Neon™ Transfection System. Following transfection, editing efficiency was measured by (B) next-generation sequencing using the Ion PGM™ System or (C) determining the percentage of T cell receptor–negative cells using the Invitrogen™ Attune™ NxT Flow Cytometer. Cells analyzed by flow cytometry were stained with a T cell receptor–specific antibody conjugated to PE.

# Hands-on CRISPR training course

The powerful gene editing technology known as CRISPR has the potential to transform science at an astonishingly rapid rate. At Thermo Fisher Scientific, we are committed to helping you stay ahead and advance your science through education. Our experienced team has designed a comprehensive four-day CRISPR workshop composed of both lectures and hands-on laboratory work at our state-of-the-art training facility.



Learn more or register today at [thermofisher.com/crisprworkshop](https://thermofisher.com/crisprworkshop)

## CRISPR controls

High-quality controls play an integral role in the successful development and performance of high-throughput screens or the optimization of gene editing conditions in your cell type of choice. We provide a complete collection of validated positive and negative controls to help you

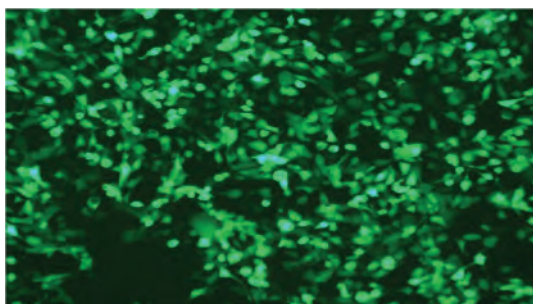
optimize delivery conditions, maximize editing efficiency, and establish hit selection criteria. These controls can help you build assays with a better signal-to-noise ratio and give you more confidence in the hits that emerge from your screens.

Type of control	Description	Recommended use
Negative controls	Negative controls are nontargeting gRNA sequences that don't recognize any sequence in the human genome. The negative controls are available in multiple package sizes.	Negative controls are used during assay development and as on-plate controls when running your screens.
Positive controls	Positive controls are validated gRNA sequences that have demonstrated high-level editing efficiencies across different cell types with up to 90% editing efficiency in some cell types. Individual LentiArray and TrueGuide gRNAs against specific genes are available.	Positive controls are used during assay development to determine the conditions that provide maximum editing efficiency in cell models. They can also serve as on-plate positive controls when performing your screens.
Delivery optimization controls	Specific to LentiArray CRISPR libraries, we offer a set of delivery controls. These control lentiviruses are available as either negative or positive controls and also express GFP.	The GFP marker provides a visual readout to aid in the rapid optimization of viral delivery conditions.

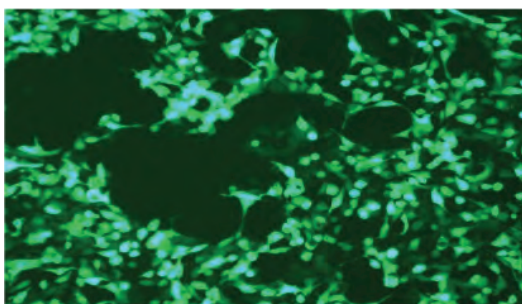
## Optimize your assay design with GFP control particles

HT1080 cells infected with control lentiviral particles expressing GFP provide a visual readout of transduction efficiency and help to determine multiplicity of infection

(MOI) for the cell line being used in the screen (Figure 10). This example illustrates how GFP control particles can be used to optimize assay design.



Negative-control lentivirus (nontargeting) with GFP



Positive-control lentivirus (*HPRT* target) with GFP

Figure 10. HT1080 cells infected with control particles expressing GFP.

## CRISPR libraries

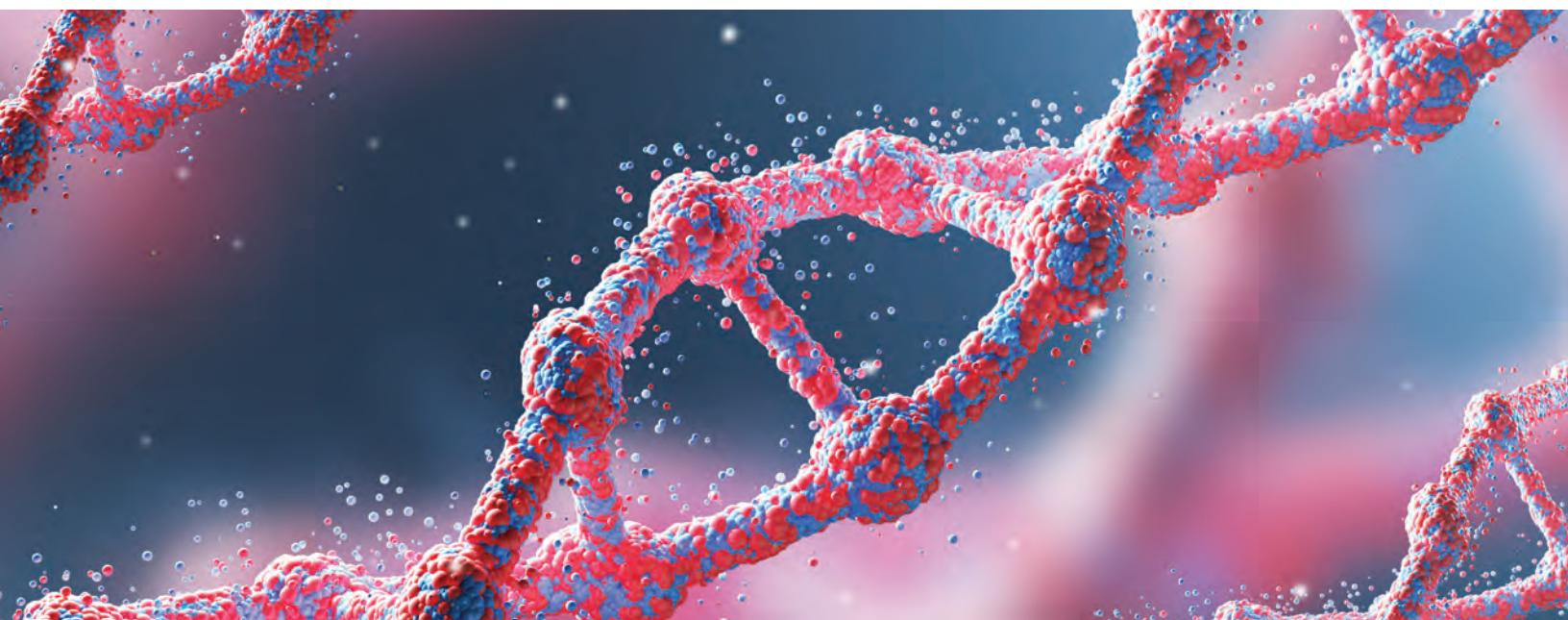
Bring the power of CRISPR-Cas9 technology to high-throughput screening. The CRISPR-Cas9 system is the premier technology for knocking out gene expression and is emerging as the next-generation tool for screening. The system provides complete, permanent knockout of the target gene, resulting in strong phenotypes and providing confidence in your screening results.

### Award-winning LentiArray CRISPR libraries

Introducing the Invitrogen™ LentiArray™ CRISPR library product line: a suite of tools that apply the power of CRISPR-Cas9 technology to high-throughput functional genomics screening. CRISPR-Cas9 provides an efficient method for specific, complete, and permanent gene

knockout, making it a potent tool for new discoveries about gene function. LentiArray libraries enable you to utilize breakthrough CRISPR-Cas9 technology to rapidly interrogate thousands of genes and determine which are key members of specific biological pathways and whether they are involved in disease development and progression.

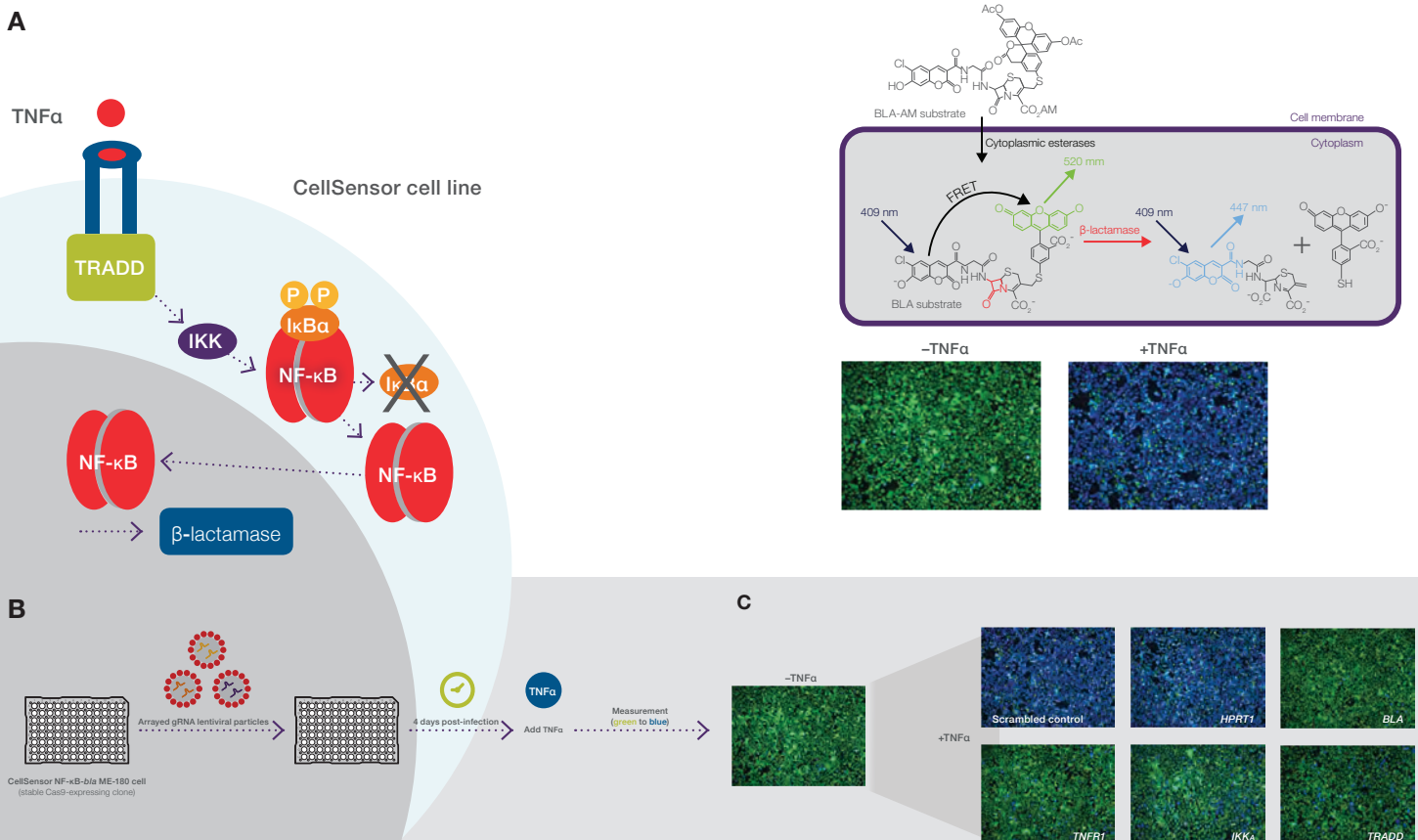
LentiArray libraries are provided in an arrayed format that is designed to be compatible with your existing high-throughput screening infrastructure. The LentiArray library product line provides the flexibility you need to expand your screening capabilities with CRISPR-Cas9 technology and can help you make your next big discovery.





## LentiArray CRISPR libraries feature:

- Advanced gRNA designs for maximum knockout efficiency without sacrificing specificity
- Up to four high-quality gRNAs per gene target for efficient knockout in a wide variety of cell types
- Delivered as high-titer, ready-to-use lentivirus or glycerol stocks
- Complete set of controls and lentiviruses against single-gene targets to support pre-screen assay development and rapid post-screen hit validation
- 19 defined libraries and custom options available, enabling you to focus on defined gene sets or perform unbiased whole-genome surveys (Figure 11)



For more information, go to [thermofisher.com/lentiarraylibraries](https://thermofisher.com/lentiarraylibraries)

## Designed to enable your success

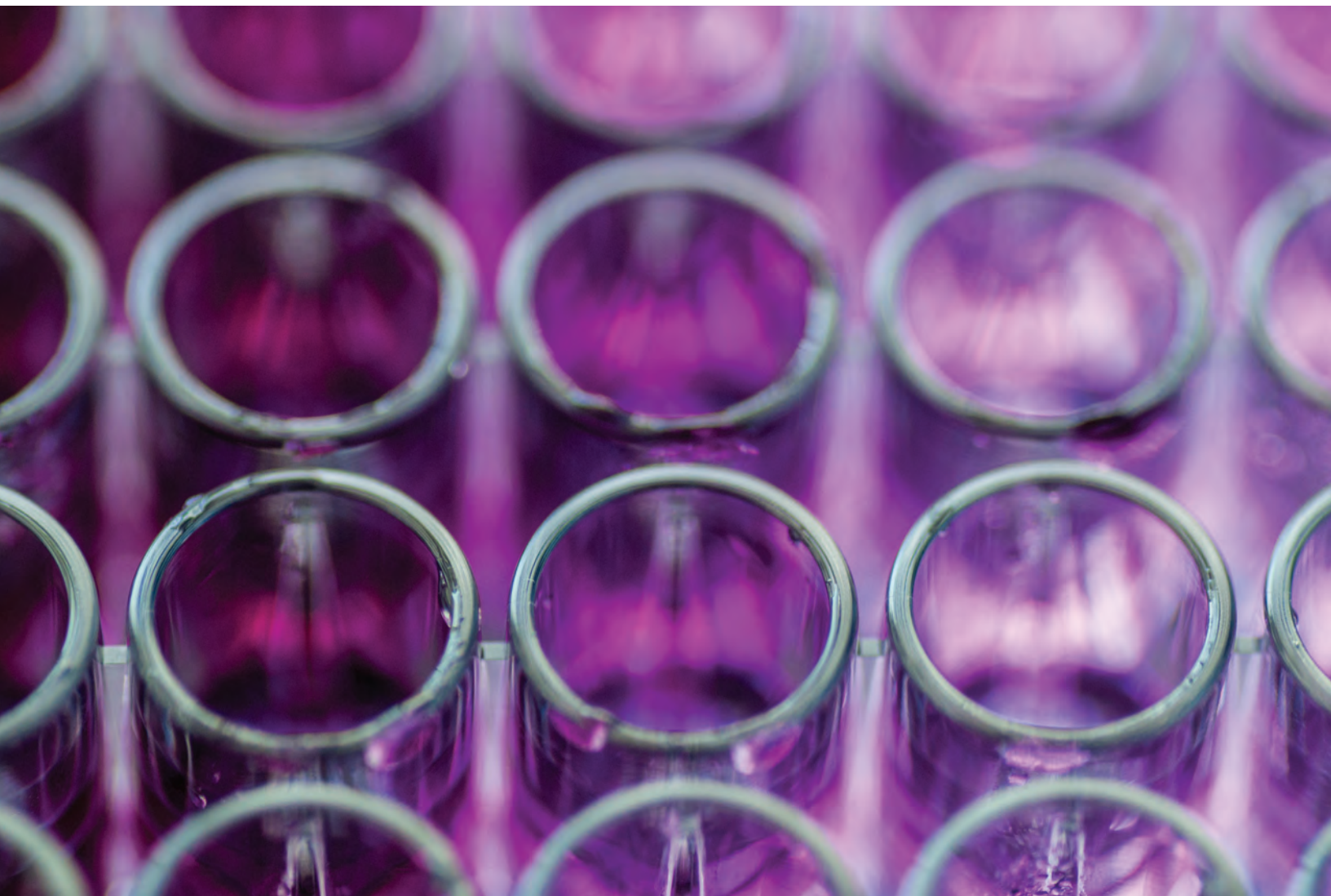
CRISPR libraries are constructed using our proprietary gRNA design algorithm, which incorporates the latest research and our extensive in-house experience. The gRNA designs are selected for maximum knockout efficiency without compromising specificity. For each gene target, we include up to four high-quality gRNAs to help ensure that the library will provide high-efficiency knockout of target genes across a wide array of cell types.



In addition to our LentiArray libraries we now offer pooled libraries. Invitrogen™ LentiPool™ CRISPR libraries are an affordable method to screen a large number of genes, as there is no high-throughput instrumentation required. For more information contact us at [GEMServices@thermofisher.com](mailto:GEMServices@thermofisher.com)

### **Donor DNA design and synthesis**

Invitrogen™ GeneArt™ Gene Synthesis services offer chemical synthesis and sequence verification of virtually any desired genetic sequence, making it ideal for the production of donor DNA. To learn more, visit [thermofisher.com/genesynthesis](http://thermofisher.com/genesynthesis)

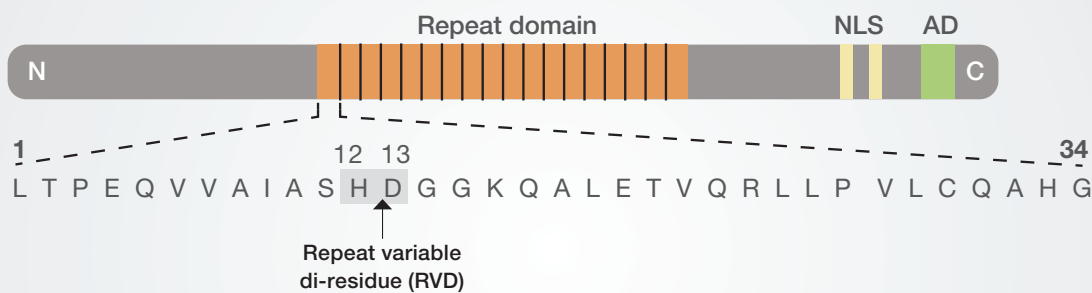


# Designer TAL effector proteins

## Precise and flexible editing with the freedom to innovate

Transcription activator–like (TAL) effector proteins are produced by bacteria in the genus *Xanthomonas*, which are widely distributed plant pathogens. Natural TAL effectors bind to specific sequences of host DNA, altering the infected plant's gene expression in ways that further the disease process. The natural TAL effector proteins have two distinct domains: an effector domain and an

extraordinarily specific DNA-binding domain. The DNA-binding domain consists of a variable number of amino acid repeats (Figure 12), each containing 33 to 35 amino acids and recognizing a single DNA base pair. The DNA recognition occurs via two hypervariable amino acid residues at positions 12 and 13 within each repeat, called repeat variable di-residues (RVDs).



**Figure 12. TAL effector DNA-binding domain.** The structure of the DNA-binding domain can be manipulated to produce a protein domain that binds specifically to any DNA sequence in the genome. TAL effector repeats can be assembled modularly, varying the RVDs to create a TAL protein that recognizes a specific target DNA sequence. Linking the repeats is straightforward, and long TAL effectors can be designed to specifically target any locus in the genome.

## Licensing TALEN technology

We are currently the only provider of TAL effector nuclease (TALEN) technology, which includes rights under foundational TAL intellectual property invented at Martin-Luther-Universität Halle-Wittenberg, the University of Minnesota, and Iowa State University. For more information on licensing TALEN technology, please contact us at [outlicensing@lifetech.com](mailto:outlicensing@lifetech.com)



Gene editing with TAL effectors

TAL effectors are a widely used technology for precise and efficient gene editing in live cells. This genome editing technology functions in a variety of host systems, including bacteria, yeast, plants, insects, fish, and mammals.

The deciphering of the TAL effector “code” led to the engineering of designer TAL effector proteins. Invitrogen™ GeneArt™ TALs provide custom DNA-binding proteins for accurate DNA targeting and precise genome editing. GeneArt TALs offer site-specific delivery of a wide variety of effectors with different functionalities, including nucleases, activators, repressors, chromatin modifiers, genomic labels, and crosslinking molecules (Table 3).

Based on your research needs, you can select from two formats of TAL effector tools: Invitrogen™ GeneArt™ Precision TALs or GeneArt™ PerfectMatch TALs (Figure 13). Choose GeneArt Precision TALs when working with plants, or if you have no design constraints. Choose GeneArt PerfectMatch TALs when you need complete flexibility in target design, as it has no 5′ T constraint for targets. GeneArt PerfectMatch TALs are derived from GeneArt Precision TALs and contain a truncated TAL effector fused to a FokI nuclease domain that converts the 5′ T binding motif at its terminus to a universal binding motif, so it will bind to any base: A, G, C, or T. Cleavage efficiencies at several genomic loci are shown in Figure 14.

Table 3. TAL effector domains and their applications.

Product	Effector domain	Functionalities	Applications
GeneArt PerfectMatch TAL	FokI endonuclease	Gene targeting (truncated TAL)	<ul style="list-style-type: none"><li>• Silencing</li><li>• Gene editing (e.g., introduction of SNPs, incorporation of exogenous DNA)</li></ul>
	FokI endonuclease with CMV promoter	Gene targeting in mammalian systems (truncated TAL)	<ul style="list-style-type: none"><li>• Silencing</li><li>• Gene editing (e.g., introduction of SNPs, incorporation of exogenous DNA)</li></ul>
	FokI endonuclease	Gene targeting in plant systems (native TAL)	<ul style="list-style-type: none"><li>• Silencing</li><li>• Gene editing (e.g., introduction of SNPs, incorporation of exogenous DNA)</li></ul>
GeneArt Precision TAL	VP16 activator	Activation of transcription (native TAL VP16)	<ul style="list-style-type: none"><li>• Increasing expression of endogenous gene isoforms</li></ul>
	VP16 activator	Activation of transcription (native TAL VP16)	<ul style="list-style-type: none"><li>• Increasing expression of endogenous gene isoforms</li></ul>
	KRAB repressor	Epigenetic repression of transcription (TAL repressor)	<ul style="list-style-type: none"><li>• Heritable knockdown of gene expression</li></ul>
	Multiple cloning site (MCS)	Steric repression and custom design (modified TAL MCS)	<ul style="list-style-type: none"><li>• Transient knockdown of gene expression</li><li>• Targeting any locus in the genome with the effector domain of your choice</li></ul>
	Prevalidated FokI endonuclease	LRRK2 gene targeting (truncated TAL)	<ul style="list-style-type: none"><li>• Correction of LRRK2 mutation, linked to Parkinson's disease, to wild type</li></ul>

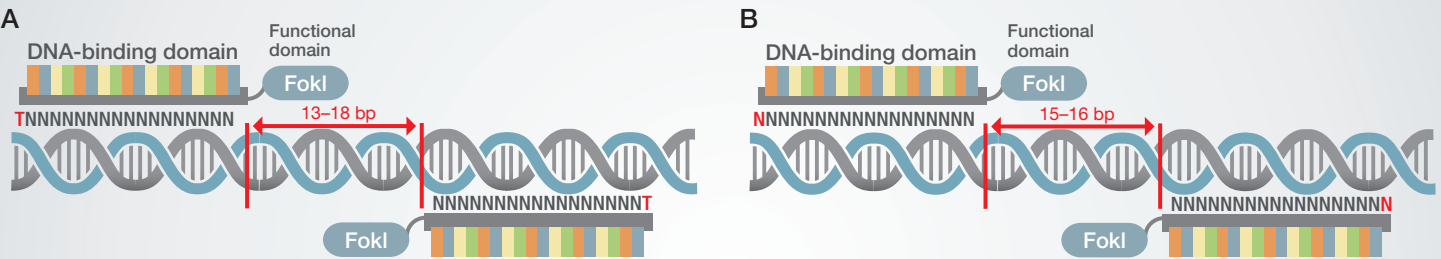
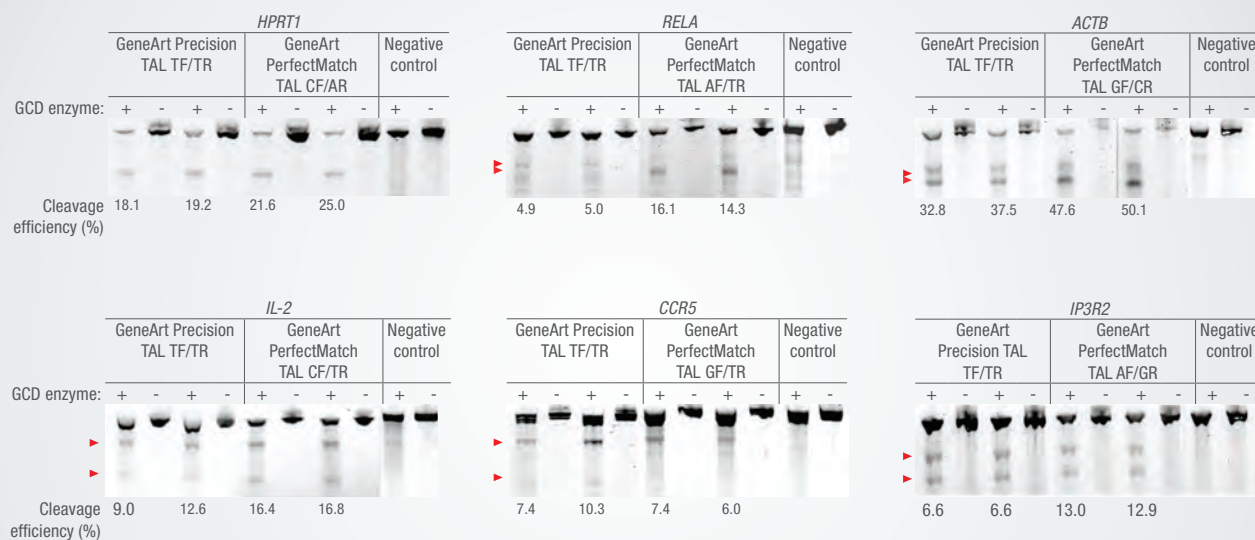


Figure 13. Designing target sites for maximal binding of customized TAL effectors. (A) GeneArt Precision TALs encode a DNA-binding protein specific to a customer-submitted sequence, fused to a FokI nuclease domain for genome editing. The sequence targeted by our first-generation TAL effectors must have a T at its 5′ end, and spacing between forward and reverse TALs must be 13–18 bp for proper pairing of the FokI nucleases and creation of a double-stranded break. (B) GeneArt PerfectMatch TALs eliminate the 5′ T constraint of GeneArt Precision TALs. GeneArt PerfectMatch TALs allow targeting of any sequence across the genome; 15–16 bp spacing between the two TAL effector targets is optimal for GeneArt PerfectMatch TALs.

## Superior performance of GeneArt PerfectMatch TALs



**Figure 14. Cleavage efficiencies of GeneArt PerfectMatch TALs compared to GeneArt Precision TALs.** To determine the functionality of GeneArt PerfectMatch TALs, we compared the genome cleavage efficiencies of GeneArt PerfectMatch TALs to those of GeneArt Precision TALs at specific loci using the GeneArt Genomic Cleavage Detection Kit (Cat. No. A24372). The function of GeneArt PerfectMatch TALs is equal to or better than GeneArt Precision TALs in 293FT cells when the targeting sequences of forward and reverse TAL effectors are preceded by different (nonidentical) bases. The red arrowheads point to the cleavage products of the genomic cleavage detection (GCD) enzyme if multiple bands were observed in a GCD assay. TF: TAL effector target site with 5' T on forward strand; TR: TAL effector target site with 5' T on reverse strand. CF: TAL effector target site with 5' C on forward strand; CR: TAL effector target site with 5' C on reverse strand. GF: TAL effector target site with 5' G on forward strand; GR: TAL effector target site with 5' G on reverse strand. AF: TAL effector target site with 5' A on forward strand; AR: TAL effector target site with 5' A on reverse strand.

## Ordering GeneArt TALs

The fastest and easiest way to design, edit, optimize, and order GeneArt Precision TALs is through the GeneArt portal at [thermofisher.com/geneartportal](https://thermofisher.com/geneartportal). For GeneArt PerfectMatch TALs, you can download and complete the TAL order form at [thermofisher.com/tals](https://thermofisher.com/tals) and email it to [GEMservices@thermofisher.com](mailto:GEMservices@thermofisher.com)

To order, follow these simple steps:

1. Select the functionality (effector domain) of your TAL from Table 3.
2. Select the product name from Table 3.
3. Place your order through our online design tool within the GeneArt portal, or download and complete the TAL order form and email it to [GEMservices@thermofisher.com](mailto:GEMservices@thermofisher.com)

If you have a question or need free design consultation, contact us and we'll be happy to assist you. We'll ship you a clone with a verified, optimized sequence approximately two weeks after confirming your order.

To find out more, or to place an order, go to [thermofisher.com/tals](https://thermofisher.com/tals)

## Support resources

Visit our TALEN learning center at [thermofisher.com/talen101](https://thermofisher.com/talen101)

# Gene silencing with RNAi tools

## Transient knockdown of multiple transcripts

RNA interference (RNAi) is a specific, potent, and highly successful approach for loss-of-function studies in virtually all eukaryotic organisms. We have developed products that include two types of small RNAs that function in RNAi: short interfering RNA (siRNA) and microRNA (miRNA). These products are designed for RNAi analysis *in vitro* and *in vivo*, and include libraries for high-throughput applications. Your choice of tool depends on your model system, the length of time you require knockdown, and other experimental parameters.

## siRNA libraries

Superior siRNAs for *in vitro* RNAi applications is the best way to effectively knock down gene expression to study protein function in a wide range of cell types. Traditional RNAi methods for gene knockdown in mammalian cells involve the use of synthetic RNA duplexes consisting

of two unmodified 21-mer oligonucleotides annealed together to form siRNAs. Invitrogen™ *Silencer*™ Select siRNA products (Table 4) incorporate the latest improvements in siRNA design, off target effect prediction algorithms, and chemistry, to offer:

- **High potency**—improved siRNA prediction accuracy compared to Invitrogen™ *Silencer*™ siRNA
- **Minimal off-target effects**—locked nucleic acid (LNA) chemical modifications reduce off-target effects by up to 90%
- **Open access**—65,000 siRNA sequences and associated data on PubChem from our *Silencer* Select siRNA library

[thermofisher.com/rnai](http://thermofisher.com/rnai)

Table 4. Selection guide for siRNA products.

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

## siRNA controls

Proper controls are essential to help ensure success in every RNAi experiment. The number and types of controls chosen depend on the ultimate research goal, and we offer positive and negative controls, as well as optimized GeneArt Gene Synthesis for siRNA-resistant genes that can be used in RNAi rescue experiments.

[thermofisher.com/sirnacontrols](http://thermofisher.com/sirnacontrols)

## Silencer Select siRNA libraries

We also offer predefined collections of Silencer Select siRNAs against popular human gene classes—kinase, phosphatase, GPCR, ion channel, nuclear hormone receptor, and protease—as well as the genome and druggable genome. Custom libraries are also available for all human, mouse, and rat genes. For more information, please contact [GEMservices@thermofisher.com](mailto:GEMservices@thermofisher.com) or go to:

[thermofisher.com/sirnalibraries](http://thermofisher.com/sirnalibraries)



## mirVana miRNA libraries

Complete Invitrogen™ *mirVana*™ libraries containing mimics and inhibitors for every human, mouse, and rat miRNA are available. For information on all of our predefined and custom miRNA libraries, contact us at [GEMservices@thermofisher.com](mailto:GEMservices@thermofisher.com) or go to:

[thermofisher.com/mirna](http://thermofisher.com/mirna)

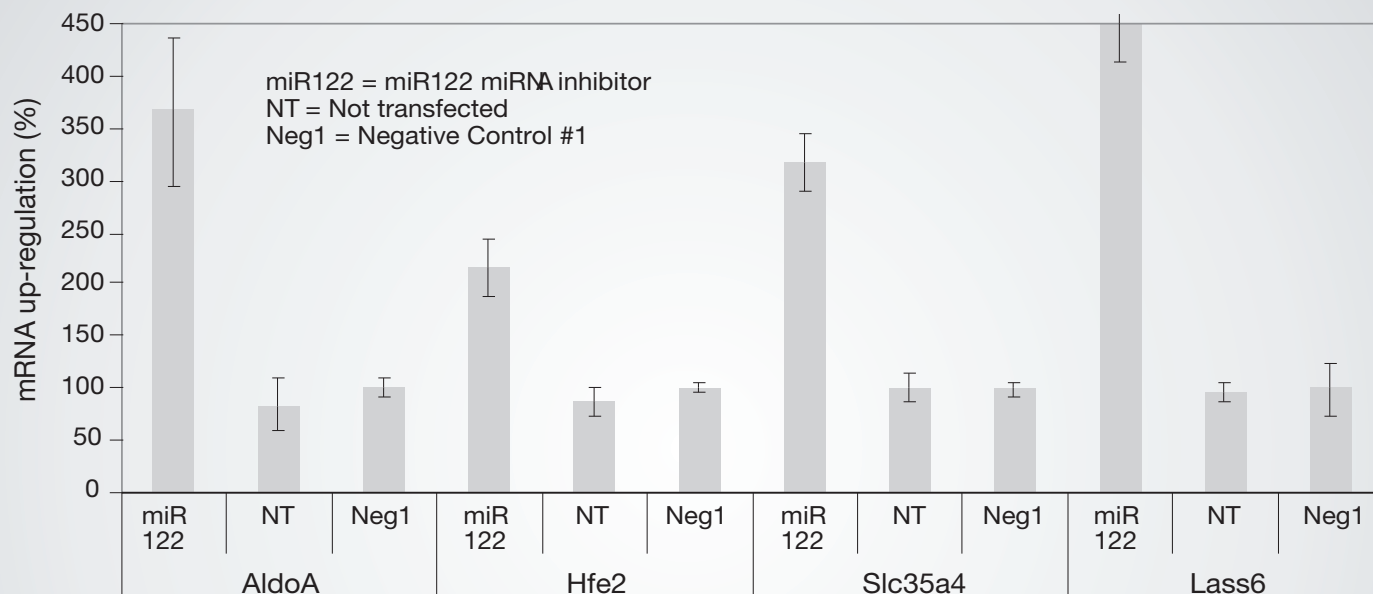
## mirVana miRNA mimics and inhibitors

For artificial regulation of target mRNA translation, Invitrogen™ *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 Invitrogen™ Lipofectamine™ RNAiMAX™ Transfection Reagent for use in cell-based systems, and with Invitrogen™ 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 15)
- **High-throughput compatible**—generate libraries for effective simultaneous screening of multiple miRNAs
- **Current**—content is regularly updated based on miRBase content

[thermofisher.com/mirna](http://thermofisher.com/mirna)



**Figure 15. *mirVana* miRNA inhibitors effectively suppress miRNA *in vivo*.** miR122 or negative control *mirVana* miRNA inhibitor was complexed with InvivoFectamine 2.0 Reagent and delivered to BALB/c mouse liver via tail vein injection on 3 consecutive days at a dose of 7 mg per kg of body weight. Expression of four mRNA targets (AldoA, Hfe2, Slc35a4, and Lass6), natural targets of miR122, were measured in transfected livers of mice injected with miR122 miRNA inhibitor or Negative Control #1 (Neg 1) and livers of mice that were not transfected (NT), using Applied Biosystems™ TaqMan® MicroRNA Assays. The results indicate that *mirVana* miRNA inhibitors are efficiently delivered to the liver with InvivoFectamine 2.0 Reagent, leading to upregulation of genes naturally suppressed by miR122.

# Culture and deliver | Cell culture and transfection technologies

## Cell culture

Gibco™ media, supplements, and cell culture reagents are designed to deliver reproducibility and performance for results you can count on every day.

### Cell culture reagents—media

Time-tested and trusted, our Gibco cell culture media include products designed to support the growth and maintenance of a variety of mammalian cells and cell lines.

[thermofisher.com/media](https://thermofisher.com/media)

### Cell culture reagents—sera

Gibco sera have earned the trust of researchers around the world because the products deliver consistent quality and superior confidence.

[thermofisher.com/fbs](https://thermofisher.com/fbs)

### Cell culture reagents—growth factors

Select pure, high-quality growth factors to help you achieve consistent cell culture.

[thermofisher.com/growthfactors](https://thermofisher.com/growthfactors)

### Cell culture—custom media

Not all projects are alike—each experiment can present unique needs and challenges. We offer cell culture products that are customized to your individual requirements.

[thermofisher.com/custommedia](https://thermofisher.com/custommedia)



### Cell culture—plastics

Thermo Scientific™ Nunc™ cell culture treated plastics with Nunclon™ Delta surface endure rigorous testing with Gibco media to help ensure consistent cell growth across multiple cell lines. It's a proven combination for happy cells—and even happier scientists.

[thermofisher.com/cellcultureplastics](https://thermofisher.com/cellcultureplastics)

## Support resources

Explore virtual training labs at  
[thermofisher.com/gibcoeducation](https://thermofisher.com/gibcoeducation)

Download your copy of our cell culture handbook at  
[thermofisher.com/cellculturebasics](https://thermofisher.com/cellculturebasics)



# Transfection technologies

Transfection is the process by which nucleic acids are introduced into eukaryotic cells. Techniques vary widely and include lipid nanoparticle-mediated transfection and physical methods such as electroporation. Our Invitrogen™ Lipofectamine™ family of reagents paired with the Neon Transfection System provide complete delivery solutions for your genome editing needs. We have optimized protocols to achieve high cleavage efficiency and ease of delivery.

An overview of our most effective transfection products is shown in Table 5 to help you choose the solution that's right for you.

[thermofisher.com/transfection](https://thermofisher.com/transfection)

Table 5. Selection guide for delivery of genome editing tools.

	Plasmid DNA	mRNA	Protein	Lentivirus
Lipofectamine CRISPRMAX reagent			●	
Lipofectamine 3000 reagent	●			
Neon Transfection System	●	●	●	
Lipofectamine MessengerMAX reagent		●		
Viral delivery*				●

\* Lipofectamine 3000 reagent can be used to produce lentivirus.

## Lipofectamine CRISPRMAX Cas9 Transfection Reagent

### The first optimized transfection reagent for CRISPR-Cas9 protein delivery

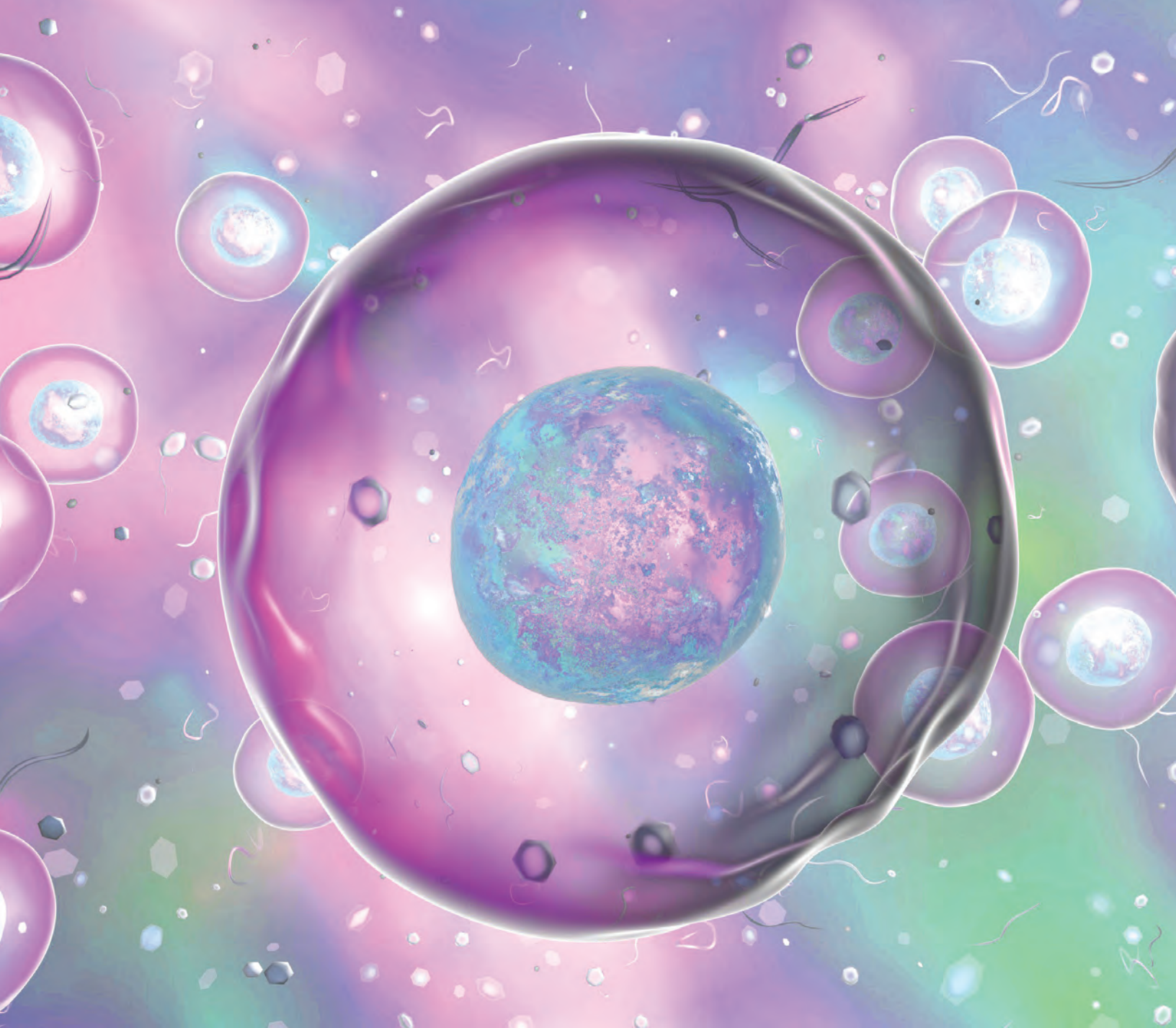
With Lipofectamine CRISPRMAX reagent, it's now possible to use a lipid-based reagent to deliver CRISPR-Cas9 protein complexes. Lipofectamine CRISPRMAX reagent is the first optimized lipid nanoparticle transfection reagent for CRISPR-Cas9 protein delivery, providing up to 85% cleavage efficiency when combined with TrueCut Cas9 Protein v2 (Figure 8). It's also gentle on cells and cost-effective.

Deliver our superior TrueCut Cas9 Protein v2 as well as other CRISPR-Cas9 proteins with a reagent that provides:

- **Demonstrated cleavage efficiency**—tested in over 20 cell types including iPSCs, mESCs, N2A, CHO, A549, HCT116, HeLa, HEK 293, and several others
- **Low cell toxicity**—fewer cells needed to initiate your experiment
- **Cost savings**—whether cost per reaction or initial investment
- **Easy scalability**—an ideal delivery solution for high-throughput experiments

[thermofisher.com/crisprprotein](https://thermofisher.com/crisprprotein)





To see what cell types we've tested, go to [thermofisher.com/crisprtransfection](https://thermofisher.com/crisprtransfection)



## Support resources

View transfection protocols at  
[thermofisher.com/transfectionprotocols](https://thermofisher.com/transfectionprotocols)

Download the Transfection and Genome Engineering  
Handbook at [thermofisher.com/transfectionhandbook](https://thermofisher.com/transfectionhandbook)





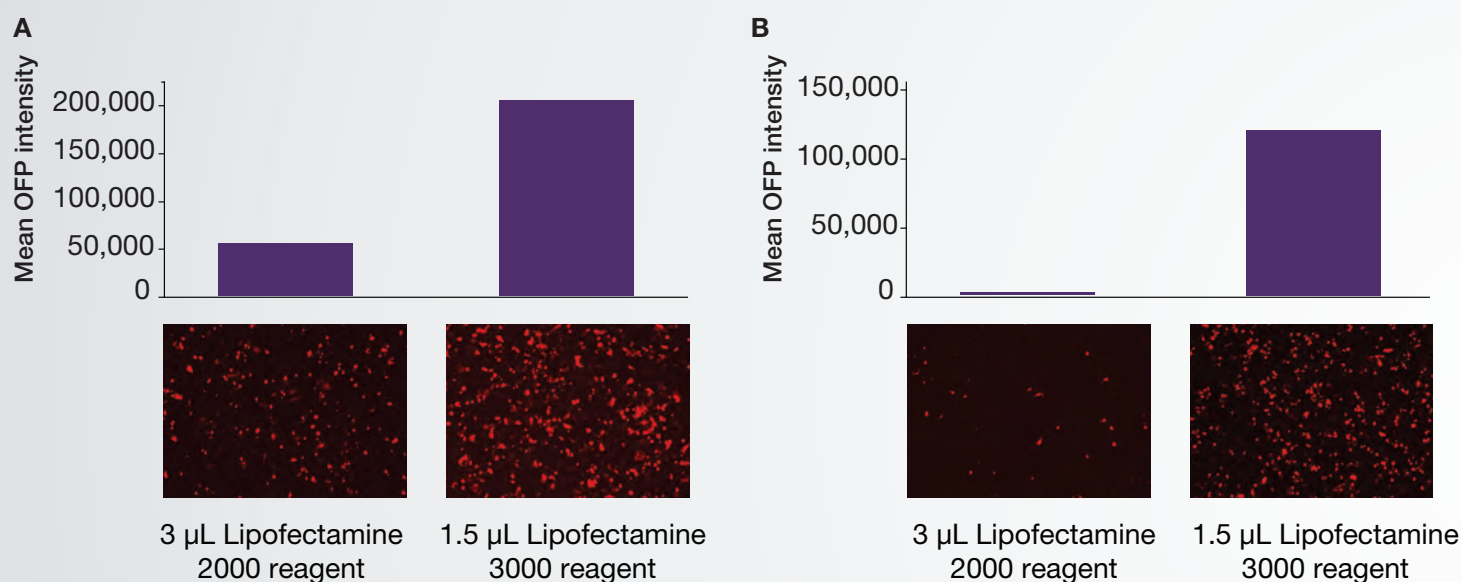
## Lipofectamine 3000 Transfection Reagent

### Improve gene editing outcomes

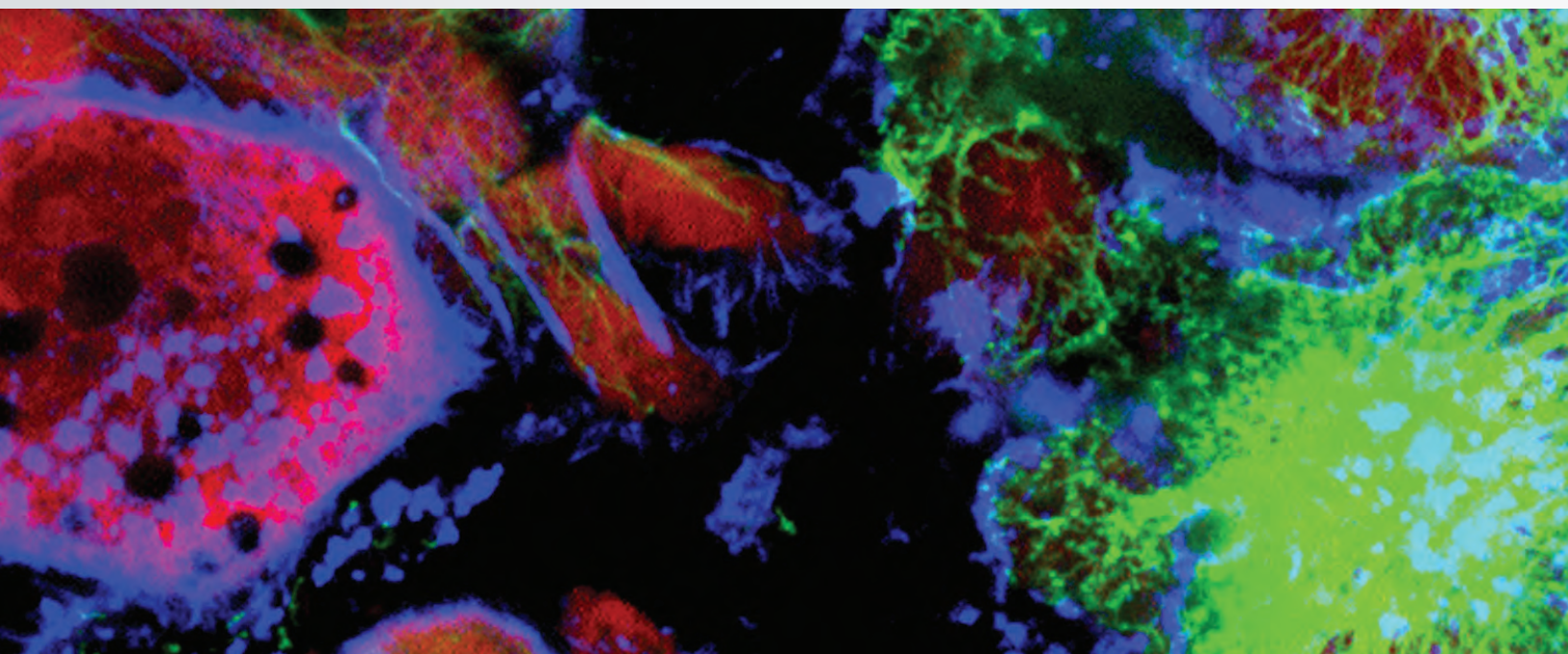
Invitrogen™ Lipofectamine™ 3000 Transfection Reagent was developed to break through the boundaries of traditional delivery methods to specifically deliver DNA and facilitate new technologies, such as genome engineering, in more biologically relevant systems. With this reagent, GeneArt CRISPR vectors targeting the AAVS1 locus in HepG2 and U2OS cells show improved transfection efficiency, mean fluorescence intensity, and genomic

cleavage (Figure 16). High transfection and genome editing efficiency is also observed with GeneArt Precision TAL plasmids. These advancements in delivery help minimize painstaking downstream workflows, enable easier stem cell manipulation, and enhance site-specific insertion of transgenes into the genome.

[thermofisher.com/3000](https://thermofisher.com/3000)



**Figure 16. Transfection efficiency and protein expression using GeneArt CRISPR Nuclease Vector.** The vector contained an Orange Fluorescent Protein (OFP) reporter gene and was transfected with Invitrogen™ 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.



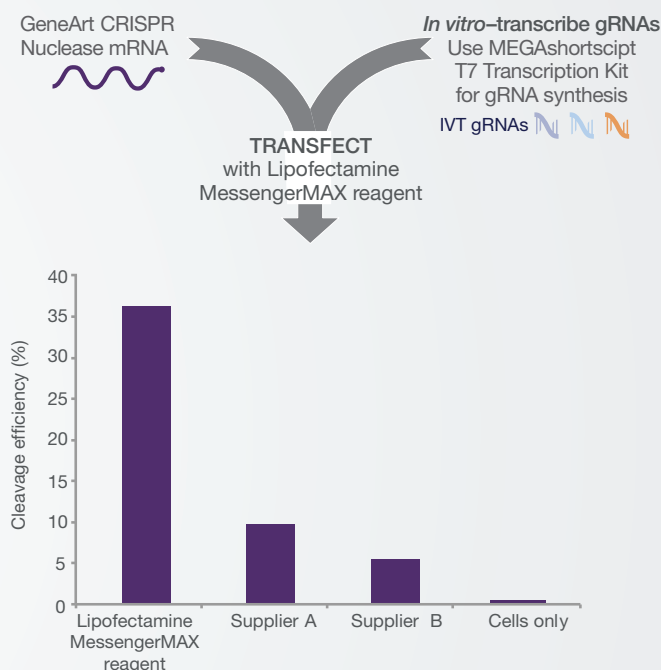
## Lipofectamine MessengerMAX Transfection Reagent

### Up to 10x higher cleavage efficiency with Cas9 mRNA

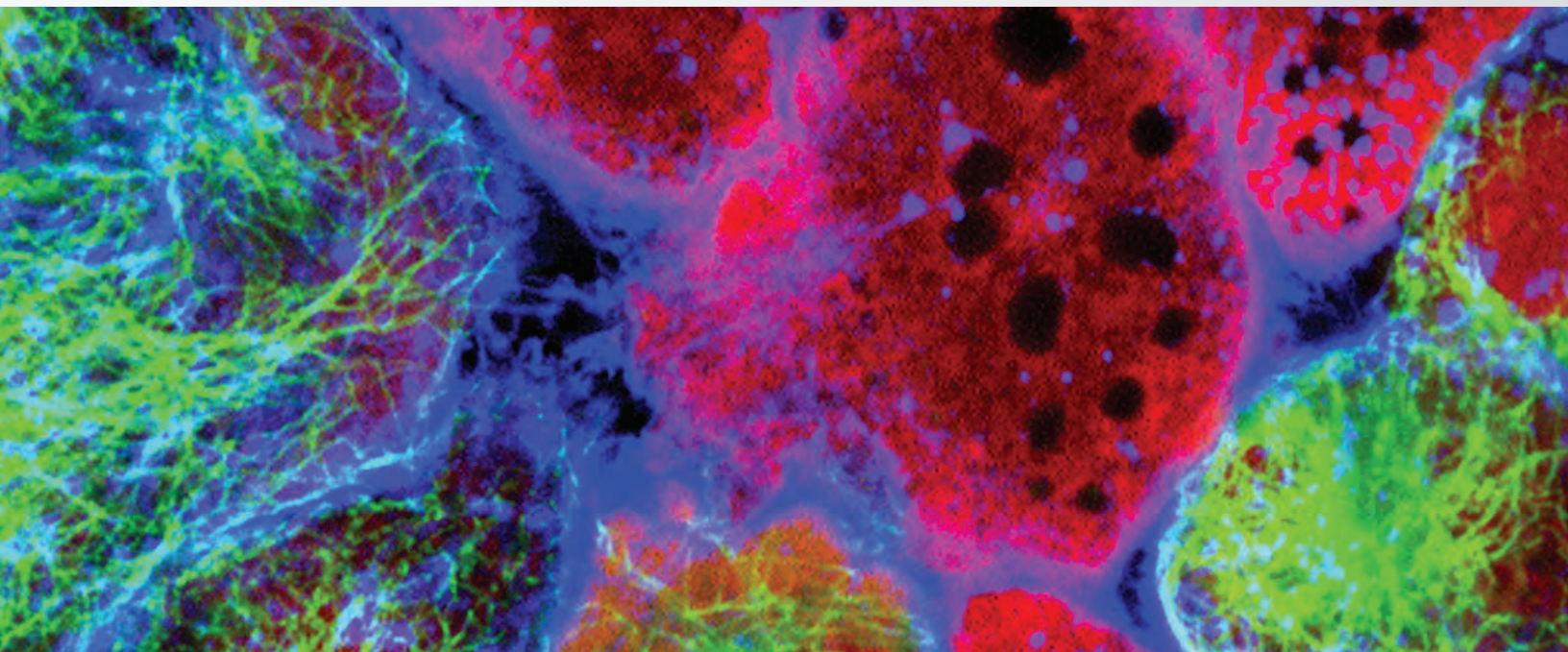
Invitrogen™ Lipofectamine™ MessengerMAX™ Transfection Reagent helps increase the likelihood of cleavage and recombination with Invitrogen™ GeneArt™ CRISPR Nuclease

mRNA through highly efficient transfection, maximizing the efficiency of genetic modifications and simplifying the downstream processes (Figure 17).

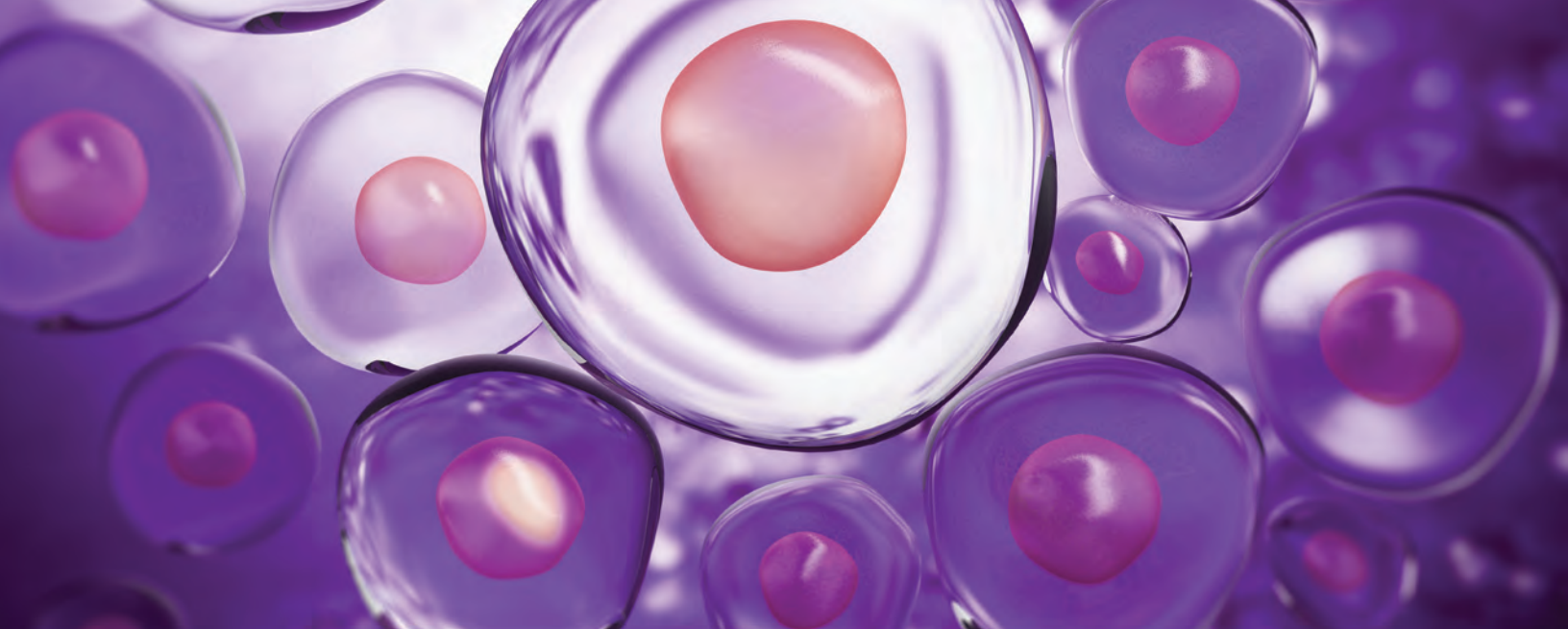
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**Figure 17. Up to 10x higher cleavage efficiency using GeneArt CRISPR mRNA and Lipofectamine MessengerMAX reagent.** Lipofectamine MessengerMAX reagent and two leading mRNA delivery reagents were used to deliver the complete CRISPR format (Cas9 mRNA + IVT gRNA) targeting the *HPRT1* locus in Gibco™ iPSCs. Invitrogen™ GeneArt™ CRISPR Strings™ DNA fragments with a T7 promoter were *in vitro*-transcribed into gRNA using our Invitrogen™ MEGAshortscript™ T7 Transcription Kit prior to transfection. Cleavage efficiency was determined using the GeneArt Genomic Cleavage Detection Kit at 72 hours posttransfection.







## Neon transfection system

### Shockingly simple electroporation

The Neon Transfection System enables superior cleavage efficiency in CRISPR gene editing applications, delivering Cas9 protein or Cas9 plasmid DNA into mammalian cell types, including primary, stem, and difficult-to-transfect cells. Unlike other electroporation instruments, this 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 unique reaction chamber provides a dramatic increase in transfection efficiency and cell viability.

The Neon Transfection System is:

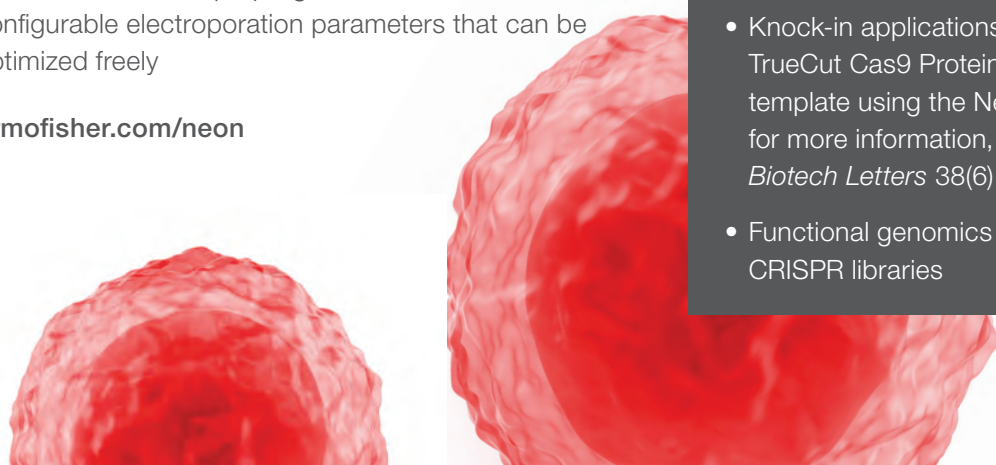
- **Efficient**—up to 94% cleavage efficiency in difficult-to-transfect cells, primary cells, and stem cells
- **Flexible**—easily transfect  $2 \times 10^4$  cells to  $6 \times 10^6$  cells per reaction
- **Simple**—easy to use with no cell-specific buffers; uses a 10  $\mu$ L or 100  $\mu$ L transfection kit with reagents for all cell types
- **Versatile**—includes preprogrammed and user-configurable electroporation parameters that can be optimized freely

[thermofisher.com/neon](https://thermofisher.com/neon)



### Choose the delivery method that best fits your downstream application and workflow

- Gene knockout:
  - Lipofectamine CRISPRMAX reagent: for Cas9 RNP complex
  - Lipofectamine RNAiMAX reagent: to deliver gRNA into stable Cas9-expressing cells
  - Neon transfection system: to maximize your efficiency with Cas9 RNP in difficult cell types
- Knock-in applications: we recommend delivering TrueCut Cas9 Protein v2 and relevant donor template using the Neon Transfection System; for more information, refer to Liang et al. (2016) *Biotech Letters* 38(6)
- Functional genomics screening: LentiArray CRISPR libraries





# Cell analysis instrumentation

## Cell counting

The Invitrogen™ Countess™ II FL Automated Cell Counter is a benchtop assay platform equipped with state-of-the-art optics, full autofocus, and image analysis software for rapid assessment of cells. With three-channel flexibility—bright-field and two optional fluorescence channels—you can count cells, monitor fluorescent protein expression, and measure cell viability to optimize your gene editing experiments.

[thermofisher.com/countess](https://thermofisher.com/countess)



Countess II FL Automated Cell Counter

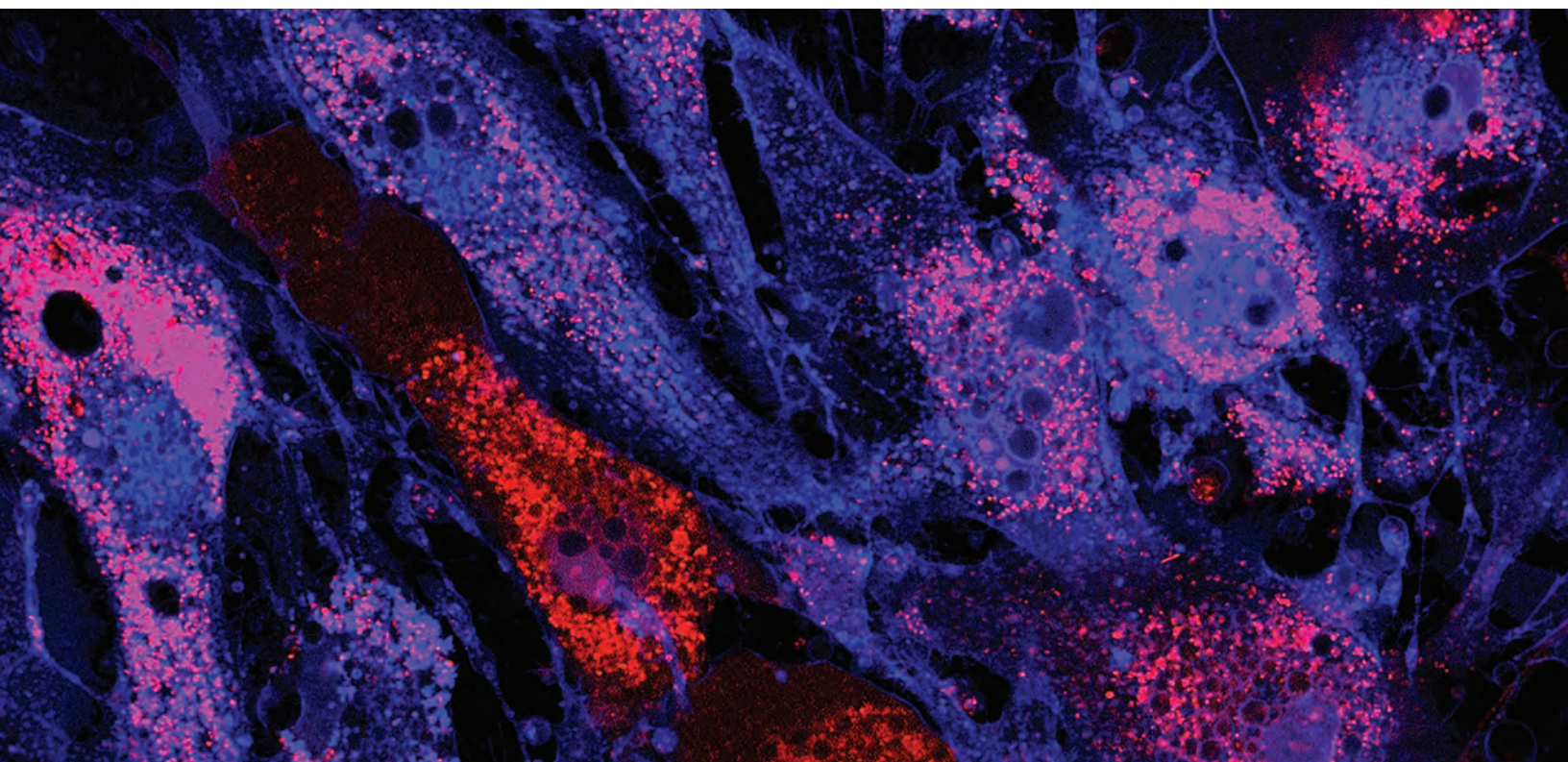
## Cell imaging systems

Designed to eliminate the complexities of microscopy without compromising performance, Invitrogen™ EVOS™ cell imaging systems make cell imaging accessible to almost every lab and budget. From cell culture to complex protein analysis to multichannel fluorescence imaging, EVOS cell imaging systems allow you to visualize your CRISPR-edited cells right in your cell culture room.

[thermofisher.com/evos](https://thermofisher.com/evos)



EVOS cell imaging systems



# Detect and validate | Essential analysis tools

## Confirm gene editing efficiency and validate the edit

Whichever gene editing strategy you use, careful monitoring of the process will help you generate robust and reliable results. Start with accurate cell counts and viability determinations before you commit more expensive resources. Select monitoring tools to optimize the editing steps based on your biological models. Then analyze your new cell phenotypes for targeted and off-target effects. This section walks you through the various tools and strategies available.

### Verify gene editing efficiency

Once your cells have been transfected with the CRISPR-Cas9 system you will want to verify the gene editing efficiency of the control target and select the condition that shows the highest level of editing efficiency in future screening experiments. To estimate the CRISPR-Cas9 editing efficiency in a pooled cell population, use the GeneArt Genomic Cleavage Detection Kit or perform sequencing using either Ion Torrent™ next-generation sequencing or Sanger sequencing.

#### GeneArt Genomic Cleavage Detection Kit

The GeneArt Genomic Cleavage Detection Kit provides a relatively quick, simple, and reliable assay that allows the assessment of the cleavage efficiency of genome editing tools at a given locus. A sample of the edited cell population is used as a direct PCR template for amplification with primers specific to the targeted region. The PCR product is then denatured and reannealed to produce heteroduplex mismatches where double-stranded breaks have occurred and introduced indels. The mismatches are recognized and cleaved by the detection enzyme.

This cleavage is easily detectable and quantifiable by gel analysis (Figure 18). This approach is:

- **Easy**—with direct PCR amplification, there's no need for genomic DNA isolation
- **Rapid**—5 hr total processing time
- **Quantitative**—gel band density is directly correlated to target indel introduction
- **Convenient**—a quick method for screening the functionality of nuclease cleavage and enrichment of edited cell populations

#### Recommendation

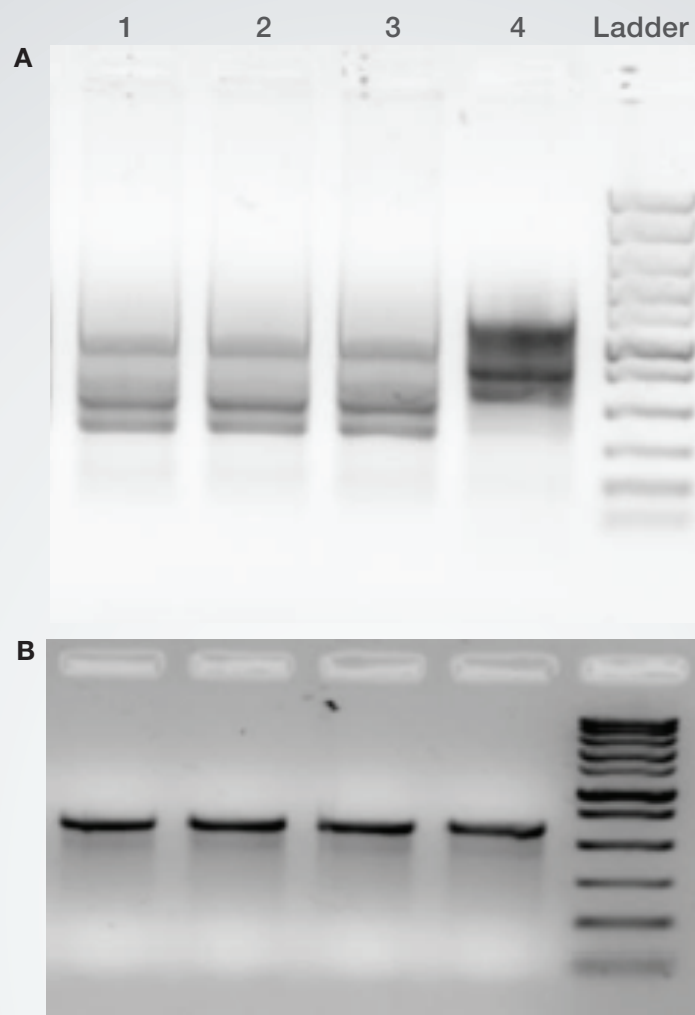
We recommend using our control targets to help determine optimal conditions based on the highest level of editing efficiency.

#### Custom DNA primers

You can design and order target-specific primer sets for the cleavage assay or sequencing through our OligoPerfect™ Designer Tool, available at [thermofisher.com/oligoperfect-designer](http://thermofisher.com/oligoperfect-designer)



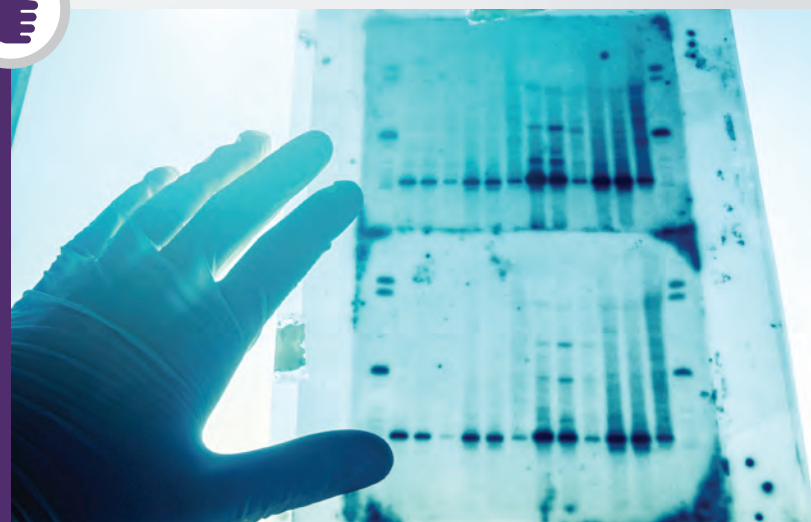




**Figure 18. Comparison of the GeneArt Genomic Cleavage Detection Kit with another commercial kit. (A)** With the other commercial kit, the expected 620 bp product was not observed on the gel; 10  $\mu$ L of the 50  $\mu$ L PCR was loaded on the gel. **(B)** The GeneArt Genomic Cleavage Detection Kit gave a specific product of the expected 620 bp size; 3  $\mu$ L of the 50  $\mu$ L PCR was loaded on the gel. Data courtesy of BioMarin Pharmaceuticals Inc.

## Quick tip

While the genomic cleavage detection (GCD) assay provides a rapid method for evaluating the efficiency of indel formation following an editing experiment, next-generation sequencing (NGS) of the amplicons from the edited population or Sanger sequencing of amplicons cloned into plasmids give more accurate estimates of the percent editing efficiency and indel types.



## Sequencing solutions

Genome sequencing allows you to uncover the genetic makeup of cells. Sequencing edited and unedited genomes is becoming easier and more cost-effective—this is true even for *de novo* sequencing projects aimed at obtaining the primary genetic sequence of your species of interest. We have an extensive portfolio of sequencing instruments, reagents, and analysis software to help get you there faster, and with greater accuracy and reliability.

### Next-generation sequencing

The Ion S5™ and Ion S5™ XL Systems provide the simplest DNA-to-data workflow for targeted sequencing with industry-leading speed and affordability. That means you can spend less time doing repetitive lab work and more time answering the critical questions in your research. These improvements in sequencing technology are changing the way genome engineers look at genomics, and are paving the way for the next wave of remarkable discoveries. Next-generation sequencing is an ideal high-throughput solution for analyzing editing efficiency and indel types or validating the edited sequence in multiple samples. It can also be leveraged for off-target analysis. Learn more at [thermofisher.com/ions5](https://thermofisher.com/ions5)

### Sanger sequencing

Sanger sequencing by capillary electrophoresis (CE) is a simple and well-established method for analyzing the results of genome editing workflows. From determining the efficiency of edits in a primary transfected culture to verifying an edit at a locus in a purified secondary culture, the Sanger method remains the gold standard for analyzing sequences at a single locus.

### BigDye reagents for your sequencing needs

The Applied Biosystems™ BigDye™ family of reagents provides quality results, long read lengths, and optimal base calling for a multitude of Sanger sequencing applications.

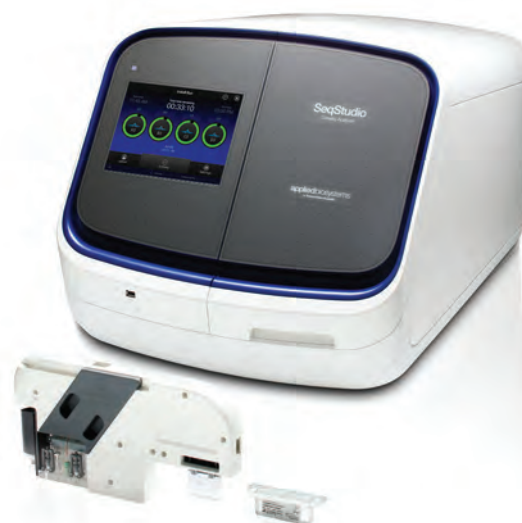


Learn more at [thermofisher.com/sequencing](https://thermofisher.com/sequencing)

## Sanger sequencing instruments

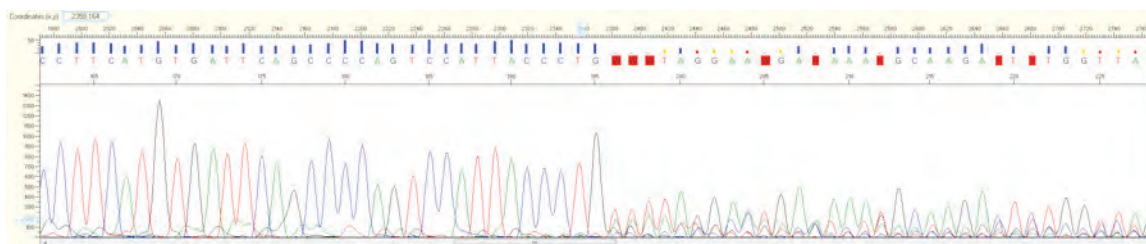
Applied Biosystems™ genetic analyzers for DNA sequencing applications feature proven CE technology and utilize our high-quality capillaries, polymers, and BigDye™ terminator chemistry. Three different instruments offer maximal flexibility for throughput needs:

- The Applied Biosystems™ SeqStudio™ Genetic Analyzer is the newest offering in our portfolio, designed to maximize ease of use and connectivity. A removable plug-and-play cartridge contains four capillaries, running buffers, and a new polymer matrix that can run both sequencing and fragment experiments without modification. The user interface was redesigned so that run setup, run monitoring, and real-time data analysis is simple and intuitive. Finally, the SeqStudio Genetic Analyzer was designed to work seamlessly with Thermo Fisher Cloud, facilitating remote monitoring, data access, and cloud-enabled applications.
- The Applied Biosystems™ 3500 series of CE instruments couples medium-throughput options with maximal configuration flexibility. The 3500 instruments are available with either 8-capillary or 24-capillary capabilities and capillary lengths of 36 cm or 50 cm. These instruments can run Applied Biosystems™ POP-6™ or POP-7™ polymers, depending on the needs of the run. Finally, consumables are monitored on-instrument using RFID tags, making the instruments ideal for validated and process-controlled environments.
- The Applied Biosystems™ 3730 family of instruments gives maximal throughput and scalability. These instruments, available in either 48- or 96-capillary formats, can be configured with 36 cm or 50 cm capillaries and can run POP-7 polymers. Up to sixteen 96-well sample plates can be loaded on the instrument and run completely unattended, providing the opportunity to collect very large amounts of sequencing data.



## Data analysis tools

Analysis of sequencing data is facilitated using Applied Biosystems™ software (Figure 19). To learn more about these packages, visit [thermofisher.com/sangersequencing](https://thermofisher.com/sangersequencing)



**Figure 19. Sanger sequencing trace of *HPRT* locus modified to generate random deletions.** Note the degradation of sequence, visible as mixed-nucleotide peaks, downstream of the target edit.



## Microarray analysis

Transcriptome-wide analysis can be complex. Matching your experimental requirements to the most appropriate tool can streamline your study, thereby reducing time-to-results and simplifying analysis. Expression microarrays simultaneously measure expression levels of thousands of RNA transcripts, so you can screen for unintended neighboring and large-scale off-target effects of your genome editing event, beyond the site of DNA insertion or deletion. They're ideal for scientists who want to quickly and easily find expression differences between biological groups. With a history of over 20 years, Applied Biosystems™ array technology has proven to be extremely reproducible, reliable, sensitive, and accurate. Combined with our novel reagents for challenging, precious samples and intuitive analysis software, our solutions allow you to go from sample to insights in just three days.

Applied Biosystems™ Clariom™ D and Clariom™ S assays (for human, mouse, and rat) are designed for whole-transcriptome expression profiling and biomarker discovery. Use as little as 100 pg total RNA input from a wide variety of sample types, including cells, fresh/fresh-frozen and formalin-fixed, paraffin-embedded (FFPE) tissues, and whole blood. There's no need to remove globin mRNA or rRNA—which helps preserve sample integrity and reduces data variability. They are available in single-sample array cartridge and multisample array plate formats for different throughput needs. They all include our fast, flexible analysis software at no additional cost.

## Clonal isolation

### Flow cytometry

The Invitrogen™ Attune™ NxT Flow Cytometer is a benchtop cytometer designed for fast, efficient multiparametric detection at the single-cell level.

The revolutionary acoustic-assisted hydrodynamic focusing technology precisely aligns cells into the center of the sample stream resulting in uniform laser illumination, regardless of the sample input rate. Designed as clog-resistant, the Attune NxT Flow Cytometer enables you to run a variety of cell types, including large and clumpy cells previously not compatible with flow cytometry. For true walk-away screening of 96- and 384-well plates, the Attune NxT Autosampler allows for the interrogation of tens of thousands of cells per second. The Attune NxT Flow Cytometer accommodates a variety of experimental protocols and fits most laboratory budgets with its field-upgradeable design of up to four lasers and 14 colors to meet some of your most challenging research needs.

[thermofisher.com/attune](http://thermofisher.com/attune)



For more information, please visit [thermofisher.com/clariomresource](http://thermofisher.com/clariomresource)

## GeneArt Genomic Cleavage Selection Kit

The Invitrogen™ GeneArt™ Genomic Cleavage Selection Kit is a rapid and reliable tool for detecting functionality of engineered nucleases in transfected cells as well as enriching for modified cells (Figure 20). When using engineered nucleases to create double-stranded breaks in genomic DNA, you need to know whether the engineered nucleases are functional. Furthermore, to efficiently screen for modified cells, you also need a way to enrich for the edited cells, particularly if the engineered nuclease has low efficiency or the cell line used is difficult to transfect. The GeneArt Genomic Cleavage Selection Kit contains a vector with the GFP gene for a quick visual check of the functionality of the engineered nuclease. In addition, the reporter genes GFP and CD4 can be used to enrich for edited cells (Figure 20). The kit can be used in conjunction with genome editing tools such as zinc finger nucleases, TALENs, and the CRISPR-Cas9 system to:

- **Screen**—for functionality of engineered nucleases as early as 24 hours posttransfection using standard fluorescence microscopy
- **Enrich**—for modified cells using fluorescence-activated cell sorting or Invitrogen™ Dynabeads™ CD4 magnetic beads

[thermofisher.com/genomeeditdetect](http://thermofisher.com/genomeeditdetect)

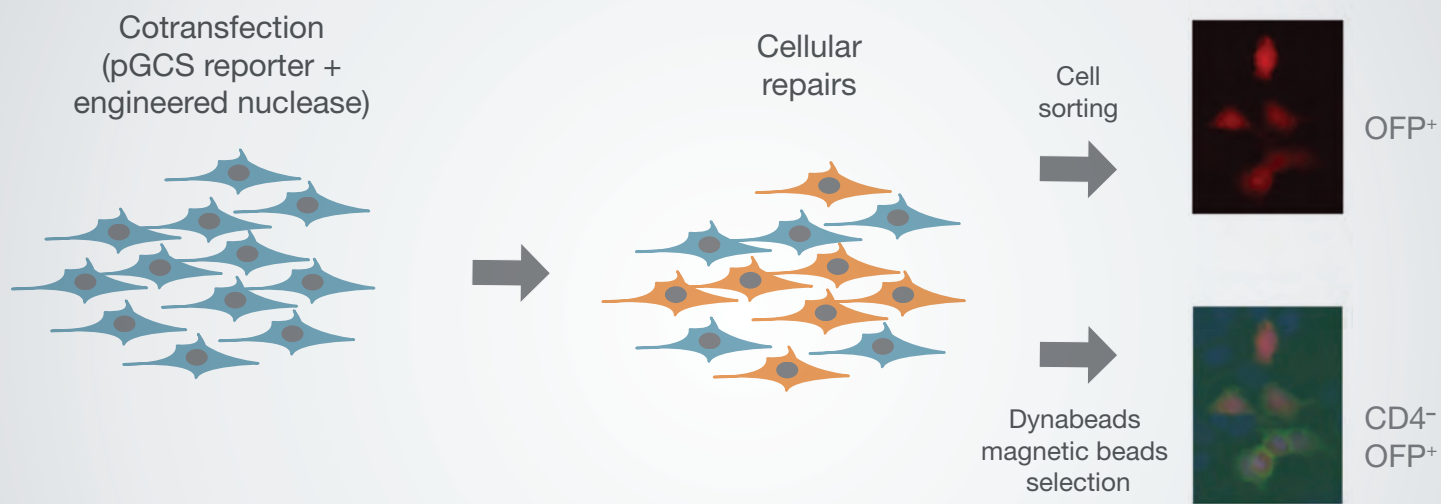


Figure 20. GeneArt Genomic Cleavage Selection Kit workflow.

# Characterize edited clones

## Thermo Scientific high-content instruments

For cell phenotyping by high-content analysis, you want to extract the maximum information from your sample in a robust and reproducible manner to make reliable decisions. We offer a choice of Thermo Scientific™ platforms with all the applications tools you need to phenotype your CRISPR-edited cells.

[thermoscientific.com/hcs](https://thermoscientific.com/hcs)

## Complete western workflow solutions

Improve the quality of your western data while simultaneously reducing your time and effort. For each of the three steps of the western workflow—separate, transfer, and detect—we offer high-performance tools and technologies to make the process quick, easy, and efficient. Explore our innovative products for western blotting, from gel electrophoresis to digital imaging, to obtain reliable results faster and with greater sensitivity.

[thermofisher.com/western](https://thermofisher.com/western)

## Invitrogen reagents

For beautiful results, Invitrogen™ reagents support a broad range of detection platforms and cell phenotyping applications. Invitrogen reagents allow you to label proteins and monitor a diverse array of physiological and morphological dynamics including apoptosis, cell health, the cell cycle, cell proliferation, and more. Together, these reagents enable superior results from your targeting and staining protocols.

[thermofisher.com/molecularprobes](https://thermofisher.com/molecularprobes)

## Antibodies

Make use of our extensive portfolio of more than 40,000 high-quality antibodies, supported by an extensive range of antibody-related products and custom services. Our antibody assay results are validated by thousands of citations worldwide and backed by a performance guarantee. Track your edited gene products and monitor off-target effects with confidence.

[thermofisher.com/antibodies](https://thermofisher.com/antibodies)



Antibodies



Western workflow devices and reagents



# Genotyping

## Genetic analysis tools for validating genome editing experiments

To validate genome editing experiments, tools to analyze how well they have succeeded are essential. For genotyping applications, PCR-based techniques offer quick reliability combined with ease of use.

## Detecting changes in single genes with the QuantStudio 3D Digital PCR System

The Applied Biosystems™ QuantStudio™ 3D Digital PCR System is a simple, affordable, and easy-to-implement platform, making digital PCR accessible for any lab. Digital PCR expands the application boundaries of traditional real-time PCR by enabling absolute quantification without the use of a standard curve. With digital PCR, you can go beyond measuring threshold cycle ( $C_t$ ) to detecting individual DNA molecules—gaining additional sensitivity and precision for a variety of experiments, including but not limited to:

- Copy number variation analysis
- Pathogen detection and load determination
- Absolute quantification of standards
- Library quantification for next-generation sequencing
- Characterization of low-fold changes in mRNA and miRNA expression
- Genetically modified organism (GMO) detection and contamination assessment

[thermofisher.com/digitalpcr](https://thermofisher.com/digitalpcr)

## Detecting changes in one or multiple genes with TaqMan Assays and reagents

Applied Biosystems™ TaqMan® Assays are the most comprehensive products available for analysis of gene expression, miRNA, copy number variation, SNP genotyping, and protein expression. TaqMan Assays include a range of solutions, from off-the-shelf, gene-specific probe and primer sets to custom probes and primers manufactured with your desired sequences. All assay products use TaqMan probe-based chemistry—the gold standard in allelic discrimination and quantitative gene expression—offering high sensitivity, specificity, reproducibility, and broad dynamic range. To get from sample to result, a wide range of reagents tailored for quantitative PCR provides unrivaled performance for both routine and challenging applications.

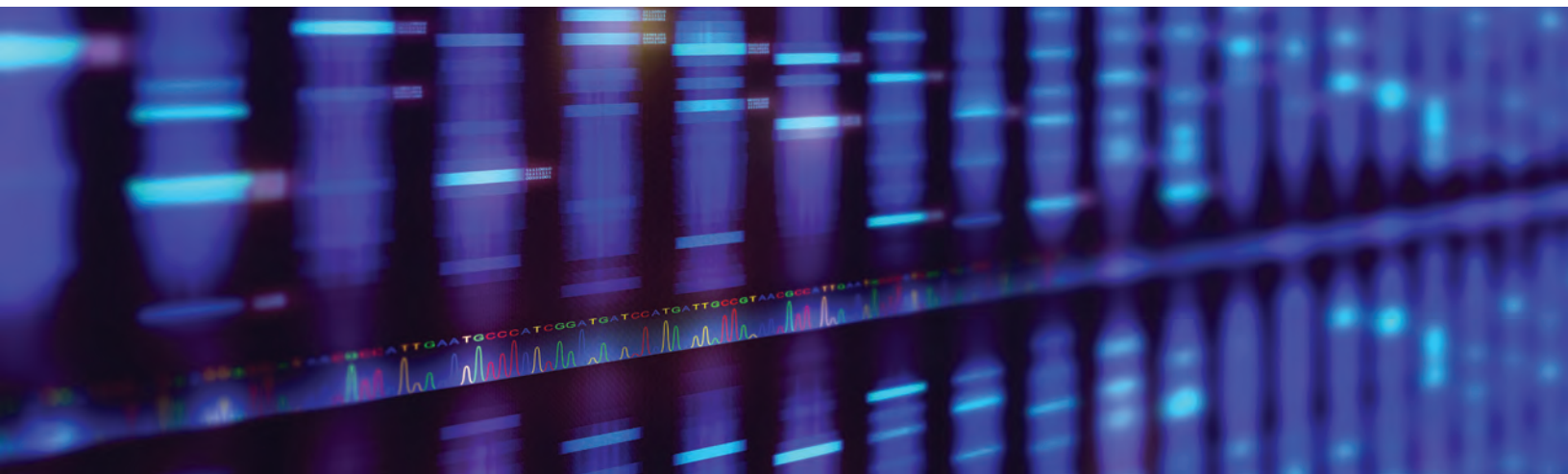
## Gene expression analysis

Applied Biosystems™ TaqMan® Gene Expression Assays provide more than 1.4 million primer/probe sets for 29 species, in four sizes, including your choice of FAM™ or VIC™ dye labels. It's the most comprehensive set of quantitative gene expression assays available. Custom assays enable you to study the expression of any gene or splice variant in any organism.

## SNP genotyping

The precision of TaqMan probe-based chemistry makes SNP genotyping studies easy. Choose from 4.5 million predesigned human and mouse Applied Biosystems™ TaqMan® SNP Genotyping Assays, or custom genotyping assays, in various sizes.

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# Custom engineering tools and designer cell lines

## Collaborating as partners to accelerate your discovery

As the trusted, experienced developers of GeneArt TAL and Invitrogen CRISPR tools, we offer you custom-designed, stable cell lines generated using one of the most robust and reliable technologies on the market. Deploying quality products throughout the process—everything from Gibco™ cell culture media, reagents, and cell health assays to next-generation sequencing (NGS) using

Ion Torrent™ sequencers—our scientists will work with you to design your stable cell line, and to develop and perform quality-control testing to help ensure the cell line meets your requirements.

We collaborate with you as partners, from start to finish, to accelerate your discovery.

[thermofisher.com/celllineservice](https://thermofisher.com/celllineservice)

Table 6. Stable cell line service packages.

Stable pool generation		Standard	Premium	
Design and synthesis of genome editing tool		●	●	
Stable pool generation (stable transfection plus enrichment or selection)		●	●	
Editing efficiency analysis (GCD assay; TaqMan Gene Expression Assay)		●	●	
On- and off-target NGS analysis (CRISPR only)			●	
Stable cell line generation		Standard	Premium	Elite
Design and synthesis of genome editing tool	●	●	●	●
Stable pool generation (stable transfection plus enrichment or selection)	●	●	●	●
Editing efficiency analysis (GCD assay; TaqMan Gene Expression Assay)	●	●	●	●
Limiting dilution cloning or cell sorting	●	●	●	●
Clonal identification and consolidation	●	●	●	●
Sanger sequencing	●	●	●	●
On- and off-target NGS analysis (CRISPR only)		●		●
Clonality analysis: on- and off-target NGS analysis (CRISPR only)		●		●
Custom-made package available upon request				●

### Mammalian cell line service

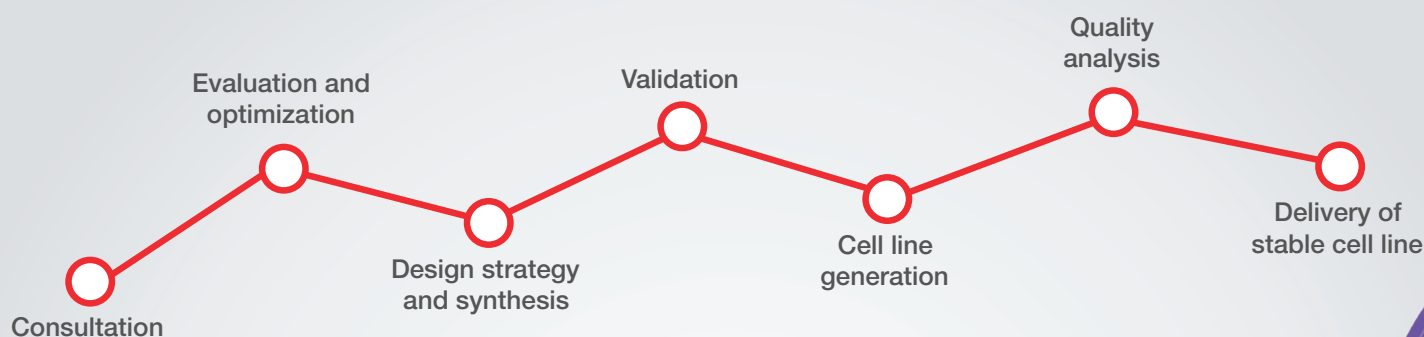
We'll apply our expertise and work with you to design, develop, and validate a custom stable cell line using validated GeneArt TAL or CRISPR tools and your customer-supplied cell line. See our service packages in Table 6.



## Timeline

How rapidly we can deliver a custom stable cell line depends on the individual cell line growth characteristics and culturing requirements. Stable cell line engineering services can be completed in as little as 10 weeks.

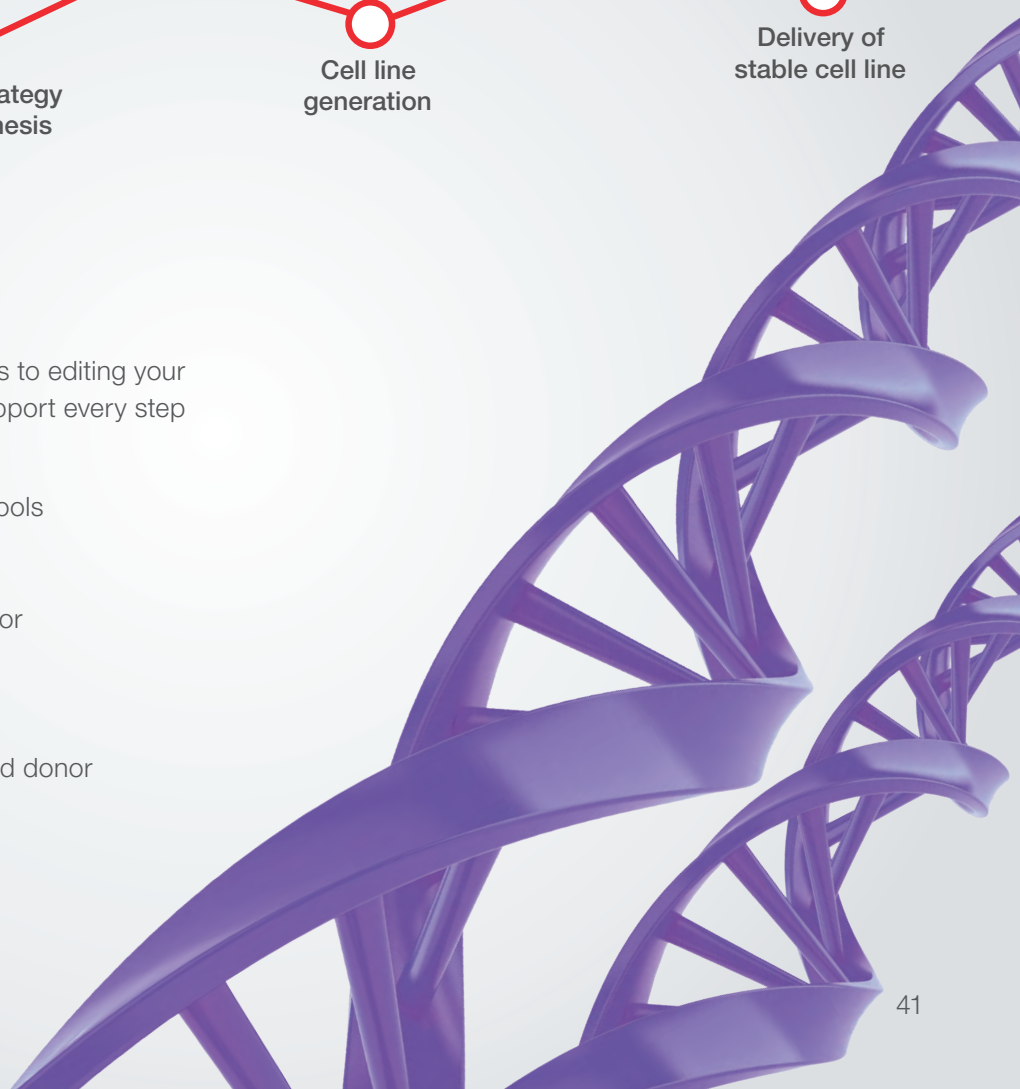
Figure 21. Cell line design and engineering process.



## Additional services available

From synthesizing your genome editing tools to editing your cell line, we offer end-to-end services to support every step of the genome editing workflow (Figure 21).

- Design and synthesis of genome editing tools
- *In vitro*-transcribed (IVT) gRNA
- CRISPR-Cas9 nuclease vector (with GFP or CD4 reporter)
- CRISPR-Cas9 lentiviral particles
- Single-stranded oligo and double-stranded donor DNA template
- Cas9 stable cell lines
- Cas9 iPSCs





# Experienced support at every stage of discovery

You're not on this journey alone. Our technical and project support specialists are experienced scientists and other professionals who appreciate your challenges and can help you find answers efficiently and accurately. Whether you're validating an assay, setting up your experiment, purchasing supplies, or verifying compatibility, our team is here to assist you.

Especially at a time when you're constantly challenged to do more with less, a problem with an assay is the last thing your lab needs. Through a consultative approach to all services offered, our genome modulation and engineering team can work with you to design and implement the solutions that fit.

From smaller validation projects and consulting engagements to complete turnkey solutions on a regional or nationwide scale, we can help you achieve your goals.



## Questions? Ready to get started?

Contact our dedicated technical support team today at [custom.services@thermofisher.com](mailto:custom.services@thermofisher.com) or **800.955.6288**, option 4.

Learn more about our comprehensive resources for your genome editing needs at [thermofisher.com/genomeedit](https://thermofisher.com/genomeedit)

## Genome Editing Support Center

Explore our genome editing support center to find answers, information, and resources to help you with your research. Read through frequently asked questions, view on-demand webinars, download the latest application notes, or check out tips and tricks. Access it at any time, day or night, and let us help you break through to discovery.

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# Ordering information

Product	Quantity	Cat. No.
<b>CRISPR nuclease</b>		
TrueCut Cas9 Protein v2 (1 mg/mL)	10 µg	A36496
	25 µg	A36497
TrueCut Cas9 Protein v2 (5 mg/mL)	100 µg	A36498
	500 µg	A36499
LentiArray Cas9 Lentivirus, 1 x 10 <sup>7</sup> TU/mL	100 µL	A32064
	1 mL	A32069
GeneArt CRISPR Nuclease mRNA	15 µg	A29378
GeneArt CRISPR Nuclease Vector with OFP Reporter Kit	10 rxn	A21174
GeneArt CRISPR Nuclease Vector with OFP Reporter Kit (with competent cells)	10 rxn	A21178
GeneArt CRISPR Nuclease Vector with CD4 Enrichment Kit	10 rxn	A21175
GeneArt CRISPR Nuclease Vector with CD4 Enrichment Kit (with competent cells)	10 rxn	A21177
<b>CRISPR gRNA</b>		
TrueGuide sgRNA, modified predefined	3 nmol	A35511
TrueGuide sgRNA, modified custom	3 nmol	A35514
TrueGuide sgRNA, predefined	3 nmol	A35510
TrueGuide sgRNA, custom	3 nmol	A35513
TrueGuide crRNA, modified predefined	2 nmol	A35509
TrueGuide crRNA, modified custom	2 nmol	A35512
	5 nmol	A35506
	20 nmol	A35507
TrueGuide tracrRNA	100 nmol	A35508
	200 µL	A32042
LentiArray Lentiviral sgRNA, 1 x 10 <sup>6</sup> TU/mL	200 µL	A32042
GeneArt Precision gRNA Synthesis Kit	25 rxn	A29377
<b>CRISPR controls</b>		
TrueGuide crRNA Positive Control, AAVS1 (Human)	2 nmol	A35515
TrueGuide crRNA Positive Control, CDK4 (Human)	2 nmol	A35516
TrueGuide crRNA Positive Control, HPRT1 (Human)	2 nmol	A35517
TrueGuide crRNA Positive Control, Rosa26 (Mouse)	2 nmol	A35518
TrueGuide crRNA Negative Control, Nontargeting	2 nmol	A35519
TrueGuide sgRNA Positive Control, AAVS1 (Human)	3 nmol	A35522
TrueGuide sgRNA Positive Control, CDK4 (Human)	3 nmol	A35523
TrueGuide sgRNA Positive Control, HPRT1 (Human)	3 nmol	A35524
TrueGuide sgRNA Positive Control, Rosa26 (Mouse)	3 nmol	A35525
TrueGuide sgRNA Negative Control, Nontargeting	3 nmol	A35526
LentiArray CRISPR Positive Control Lentivirus, human HPRT, 1 x 10 <sup>7</sup> TU/mL	100 µL	A32056
LentiArray CRISPR Positive Control Lentivirus, human HPRT, 1 x 10 <sup>7</sup> TU/mL, with GFP	100 µL	A32060
LentiArray CRISPR Negative Control Lentivirus, human nontargeting, 1 x 10 <sup>7</sup> TU/mL	100 µL	A32062
	1 mL	A32327
LentiArray CRISPR Negative Control Lentivirus, human nontargeting, 1 x 10 <sup>7</sup> TU/mL, with GFP	100 µL	A32063

# Ordering information continued

Product	Quantity	Cat. No.
<b>CRISPR delivery</b>		
Lipofectamine CRISPRMAX Cas9 Transfection Reagent	10 rxn	CMAX00001
	25 rxn	CMAX00003
	75 rxn	CMAX00008
	150 rxn	CMAX00015
Lipofectamine Stem Transfection Reagent	0.1 mL	STEM00001
	0.3 mL	STEM00003
	0.75 mL	STEM00008
	1.5 mL	STEM00015
Neon Transfection System Starter Pack	1 pack	MPK5000S
Neon Transfection System Kit, 100 µL	25 x 2 rxn	MPK10025
	96 x 2 rxn	MPK10096
Neon Transfection System Kit, 10 µL	25 x 2 rxn	MPK1025
	96 x 2 rxn	MPK1096
Neon Transfection System	1 each	MPK5000
Neon Transfection System Pipette	1 each	MPP100
Neon Transfection System Pipette Station	1 each	MPS100
Neon Transfection Tubes	1 pack	MPT100
Lipofectamine MessengerMAX Transfection Reagent	0.1 mL	LMRNA001
	0.3 mL	LMRNA003
	0.75 mL	LMRNA008
	1.5 mL	LMRNA015
	15 mL	LMRNA150
Lipofectamine RNAiMAX Transfection Reagent	0.1 mL	13778100
	0.3 mL	13778030
	0.75 mL	13778075
	1.5 mL	13778150
	15 mL	13778500
<b>CRISPR detection</b>		
GeneArt Genomic Cleavage Detection Kit	20 rxn	A24372
PrestoBlue Cell Viability Reagent	25 mL	A13261
	100 mL	A13262
Propidium Iodide, 1.0 mg/mL solution in water	10 mL	P3566
ProFlex 3 x 32-Well PCR System	1 each	4484073
SimpliAmp Thermal Cycler	1 each	A24811

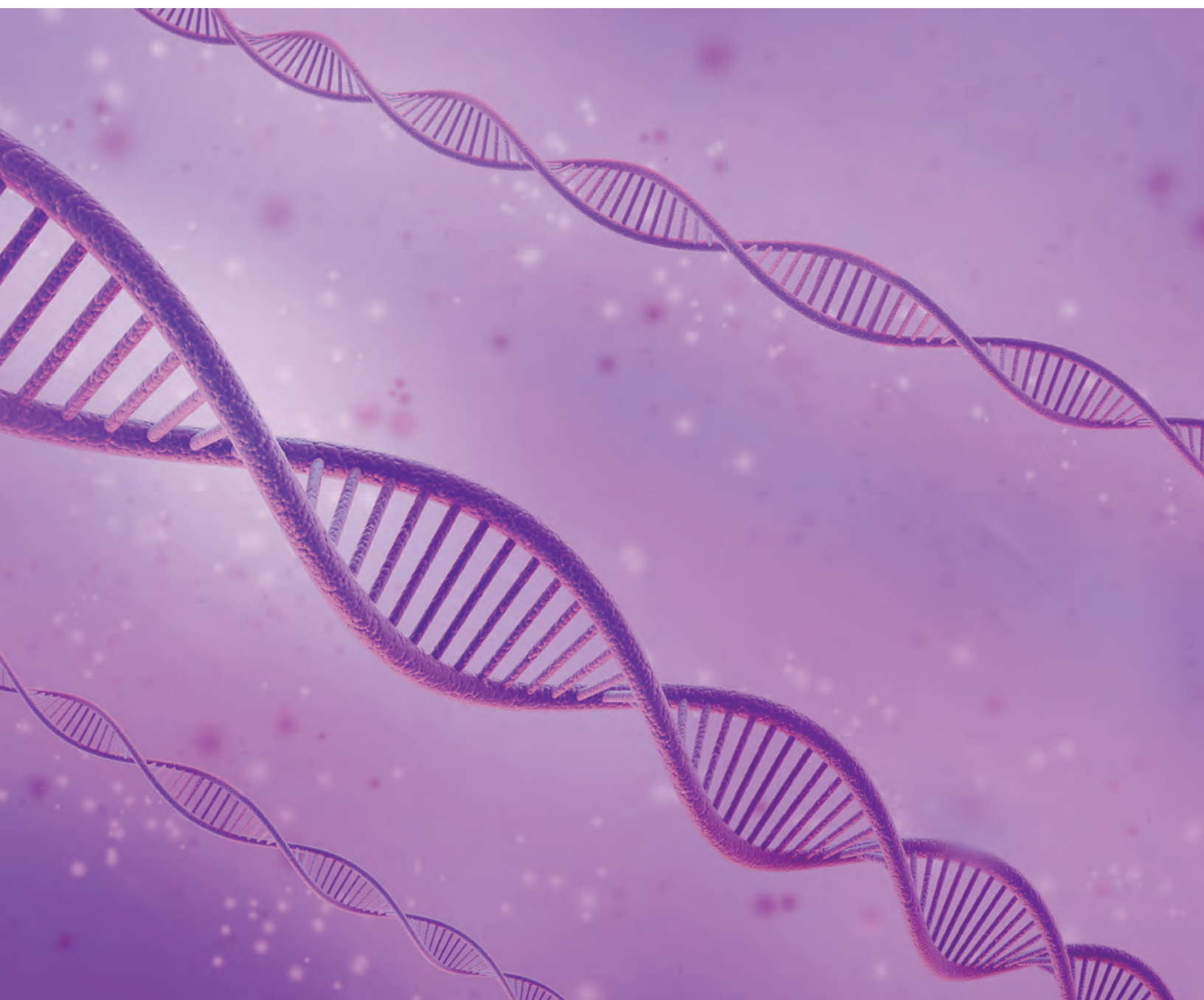


Product	Quantity	Cat. No.
<b>Flow cytometry and microscopy</b>		
FluoroBrite DMEM	500 mL	A1896701
	10 x 500 mL	A1896702
GlutaMAX Supplement	100 mL	35050061
	20 x 100 mL	35050079
Attune NxT Acoustic Focusing Cytometer, blue/red/violet/yellow	1 each	A24858
Attune Focusing Fluid (1X)	1 L	4488621
	10 L	424904
	6 x 1 L	4449791
Attune Wash Solution	250 mL	A24974
Attune Performance Tracking Beads	3 mL	4449754
Attune Shutdown Solution (1X)	250 mL	A24975
EVOS FL Imaging System	1 each	AMF4300
EVOS FL Auto Imaging System	1 each	AMAFD1000
EVOS FL Color Imaging System	1 each	AMEFC4300
FLoid Cell Imaging Station	1 each	4471136
Countess II FL Automated Cell Counter	1 each	AMQAF1000
<b>Cell culture</b>		
StemFlex Medium	500 mL	A3349401
Essential 8 Medium	500 mL	A1517001
293FT Cell Line	3 x 10 <sup>6</sup> cells	R70007
DMEM, high glucose, GlutaMAX Supplement, pyruvate	500 mL	10569010
	10 x 500 mL	10569044
	5 L	10569069
	10 L	10569077
Sodium Pyruvate (100 mM)	100 mL	11360-070
DMEM, high glucose, GlutaMAX Supplement	500 mL	10566016
	10 x 500 mL	10566024
	5 L	10566032
Fetal Bovine Serum, certified, US origin	100 mL	16000036
	500 mL	16000044
	1,000 mL	16000069
	50 mL	A3160401
	10 x 50 mL	A3160402
	100 mL	11140050
MEM Non-Essential Amino Acids Solution (100X)	20 x 100 mL	11140076
TrypLE Express Enzyme (1X), phenol red	100 mL	12604013
1X DPBS, no calcium, no magnesium	500 mL	14190094
1X PBS, pH 7.4	500 mL	10010023
Nunc T-Flasks (as needed for preculture: T-75)	1 case (100)	156499
Nunc T-Flasks (as needed for preculture and Neon system: T-25)	1 case (200)	156367
Nunc Cell Culture Treated Multidishes, 24-well	1 case (75)	142475
Antibiotic-Antimycotic (100X)	100 mL	15240062

# Ordering information continued

Product	Quantity	Cat. No.
<b>Cell culture, cont.</b>		
Countess Cell Counting Chamber Slides with Trypan Blue	50 slides	C10228
Opti-MEM I Reduced Serum Medium	100 mL	31985062
Nunc EZFlip Conical Centrifuge Tubes, 15 mL	Case of 500	362694
Nunc EZFlip Conical Centrifuge Tubes, 50 mL	Case of 500	362696
<b>Gel electrophoresis</b>		
E-Gel Simple Runner Device	1 device	G8000
E-Gel Power Snap Electrophoresis Device	1 device	G8100
E-Gel Power Snap Camera	1 camera	G8200
E-Gel Power Snap Electrophoresis System	1 system	G8300
E-Gel Power Snap Electrophoresis Device Starter Kit, EX 1%	1 kit	G8141ST
E-Gel Power Snap Electrophoresis Device Starter Kit, EX 2%	1 kit	G8142ST
E-Gel Power Snap Electrophoresis Device Starter Kit, SYBR Safe 1.2%	1 kit	G8151ST
E-Gel Power Snap Electrophoresis Device Starter Kit, SYBR Safe 2%	1 kit	G8152ST
E-Gel Power Snap Electrophoresis Device Starter Kit, CloneWell	1 kit	G8168ST
E-Gel Power Snap Electrophoresis Device Starter Kit, SizeSelect	1 kit	G8162ST
E-Gel Power Snap Electrophoresis System Starter Kit, EX 1%	1 kit	G8341ST
E-Gel Power Snap Electrophoresis System Starter Kit, EX 2%	1 kit	G8342ST
E-Gel Power Snap Electrophoresis System Starter Kit, SYBR Safe 1.2%	1 kit	G8351ST
E-Gel Power Snap Electrophoresis System Starter Kit, SYBR Safe 2%	1 kit	G8352ST
TrackIt Ultra Low Range DNA Ladder	50 µg	10488023
Ultra Low Range DNA Ladder	50 µg	10597012
E-Gel 1 Kb Plus Express DNA Ladder	2 x 1.25 mL	10488091
E-Gel Sizing DNA Ladder	2 x 1.25 mL	10488100
E-Gel Ultra Low Range DNA Ladder	2 x 1 mL	10488096
E-Gel CloneWell II Agarose Gels, 0.8%	10 gels	G661818
E-Gel SizeSelect II Agarose Gels, 2%	10 gels	G661012
RNA Gel Loading Dye (2X)	1 mL	R0641
Gel Loading Buffer II (for denaturing PAGE)	1 mL	AM8546G
E-Gel EX Agarose Gels, 2%	20 gels	G402002
	10 gels	G401002
Century-Plus RNA Markers	50 µL	AM7145
TrackIt 100 bp DNA Ladder	0.5 mL	10488058
E-Gel iBase and E-Gel Safe Imager Combo Kit	1 each	G6465
E-Gel Imager System with UV Light Base	1 each	4466611

Product	Quantity	Cat. No.
Other related products		
Qubit RNA BR Assay Kit	100 assays	Q10210
Water, nuclease-free	4 x 1.25 mL	R0581
	30 mL	R0582
	250 mL	AM9780
RNaseZAP RNase Decontamination Solution	6 x 250 mL	AM9782
	4 L	AM9784
MicroAmp Reaction Tube with Cap, 0.2 mL, autoclaved	1,000	N8010612
Thin-walled, frosted lid, RNase-free PCR tubes (0.2 mL)	1,000	AM12225
RNase-Free Microfuge Tubes (1.5 mL)	500	AM12400
RNase-Free Microfuge Tubes (2.0 mL)	500	AM12425





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