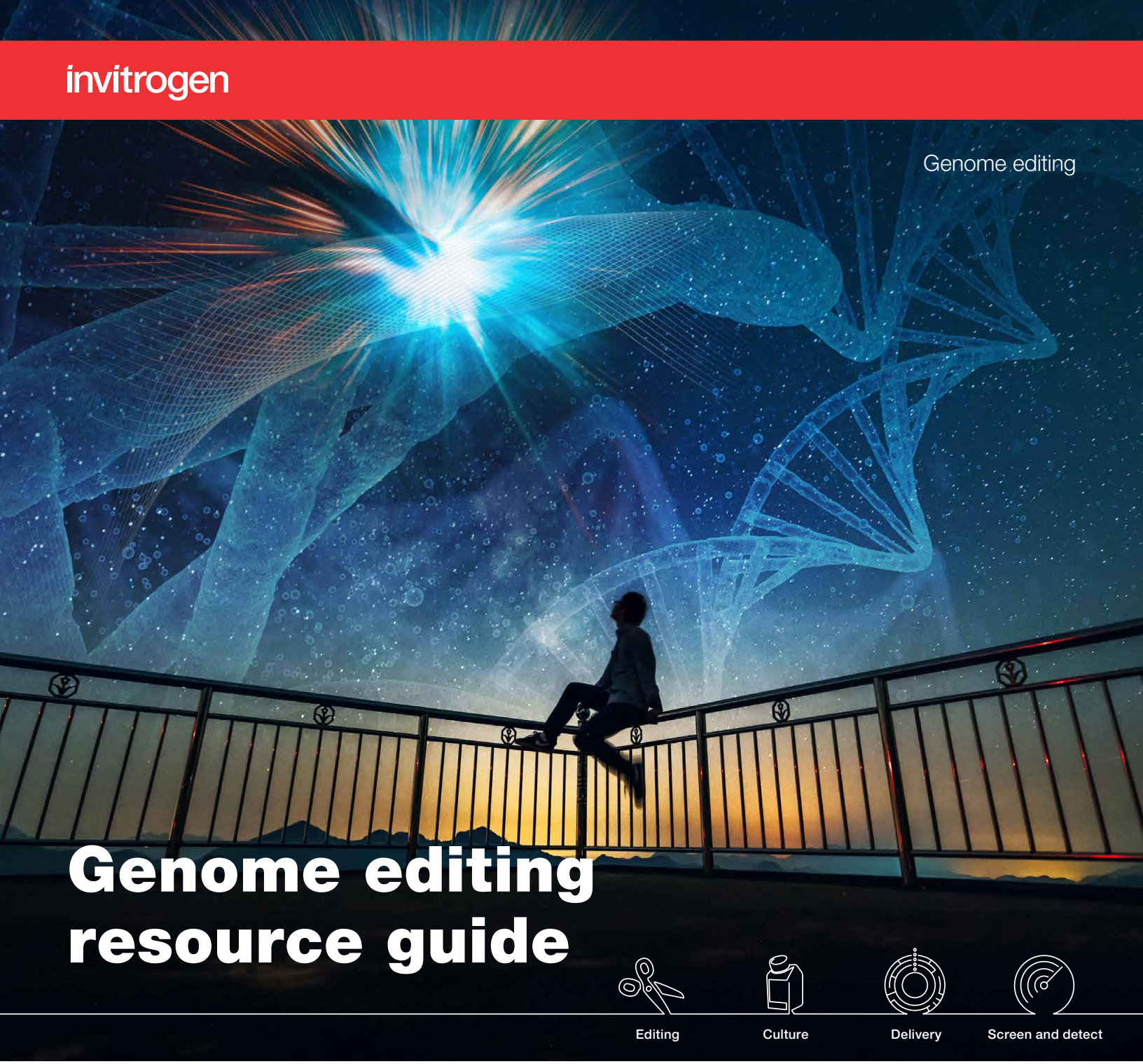
invitrogen

Genome editing

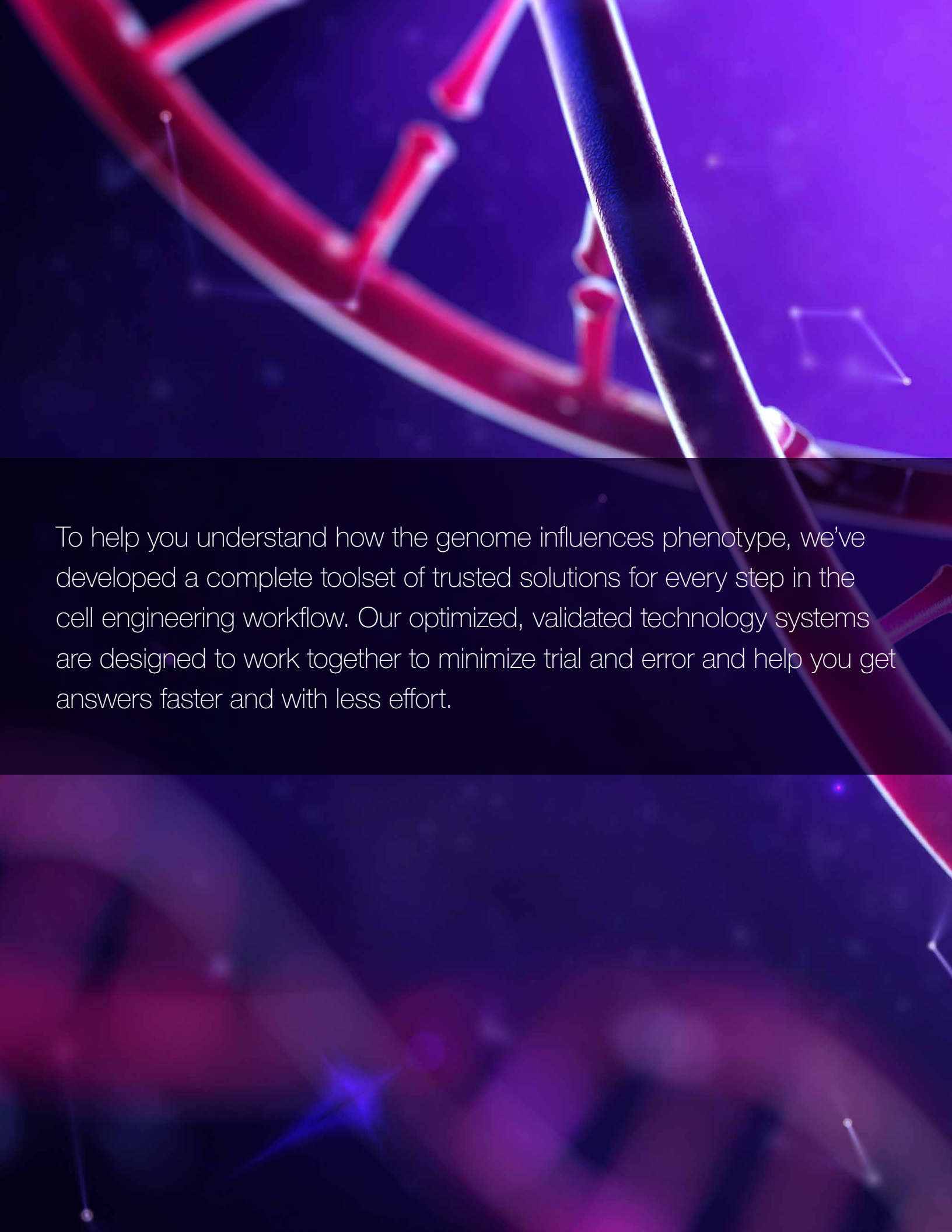


Genome editing resource guide

A complete cell engineering solution from start to discovery

Fourth edition

ThermoFisher SCIENTIFIC



To help you understand how the genome influences phenotype, we've developed a complete toolset of 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.

Contents

As a leader in genome editing, we provide:

1

More choices, better results

Whether you need a solution for CRISPR-Cas9 editing 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.

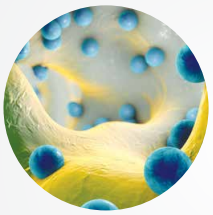
Cell engineering applications and workflow solutions

Key applications

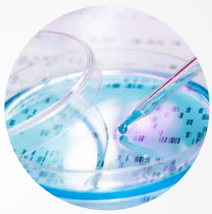
Advances in genome editing have the potential to change the way we create energy, produce food, optimize industrial processing, as well as detect, prevent, and cure diseases. Through innovative design and engineering, this unique science enables researchers like you to study, alter, create, and reconstitute 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.

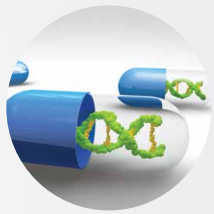
Animal disease models



Tissue disease models



Stem cell engineering



Gene therapy

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	Culture and deliver	Detect and validate
Obtain superior gRNA and donor DNA designs for your specific experiment. Pair these with high-performing enzymes for maximum editing efficiency	**Deliver editing tools to healthy, viable cells with the highest efficiency**	**Confirm editing efficiency, determine percent modification, and validate the edit**

Design and build | Genome editing tools

Genome editing technologies, such as the CRISPR-Cas9 system and transcription activator-like (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).

Find out more about these genome editing products and services at **thermofisher.com/genomeedit**

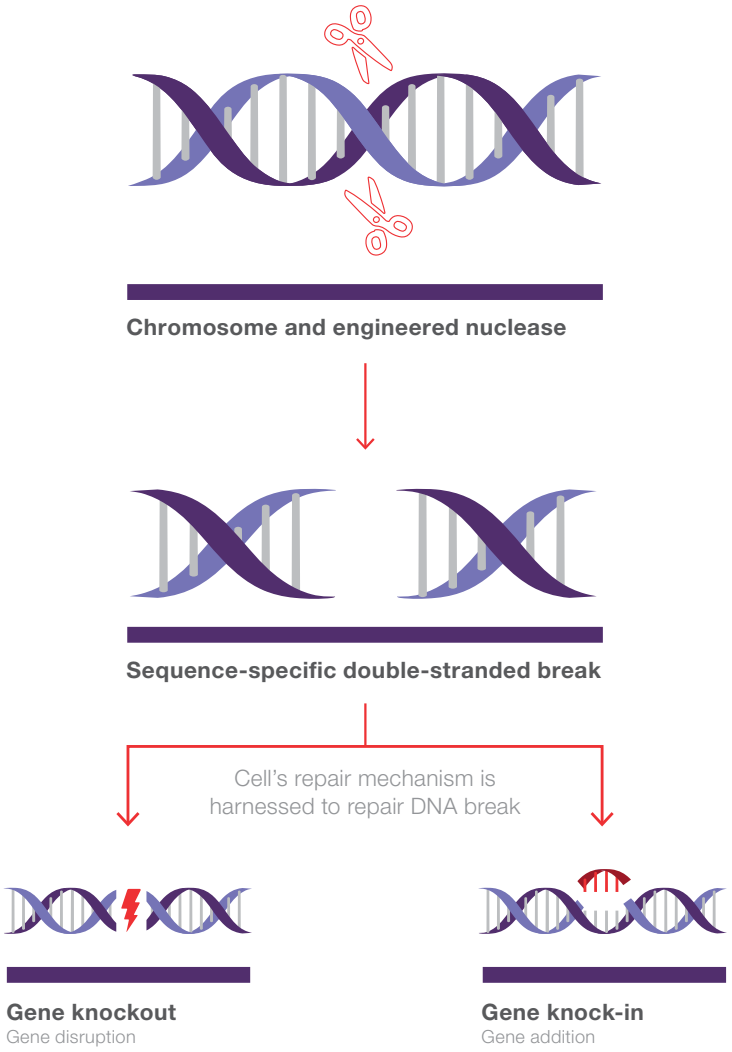


Figure 1. Mechanism of engineered nuclease.

Support resources

- New to genome editing? Access our learning center at **thermofisher.com/genomeedit101**
- Access our collection of validated protocols at **thermofisher.com/crisprprotocols**

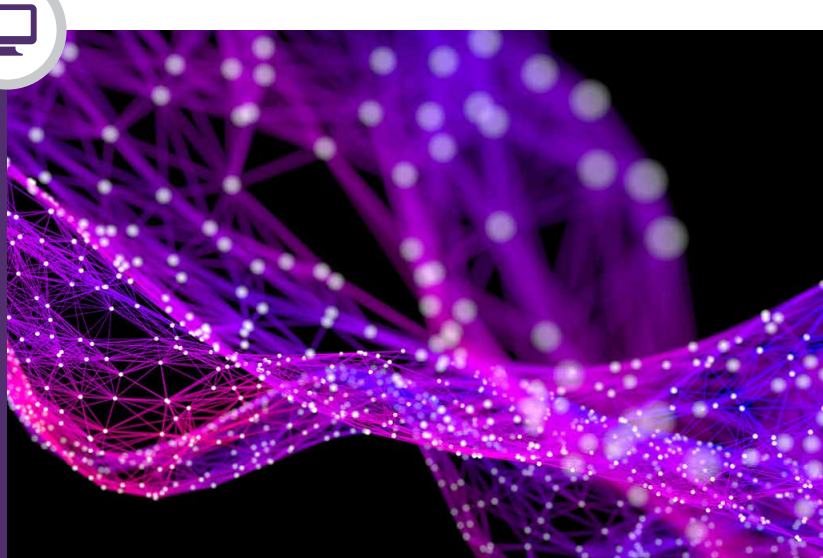
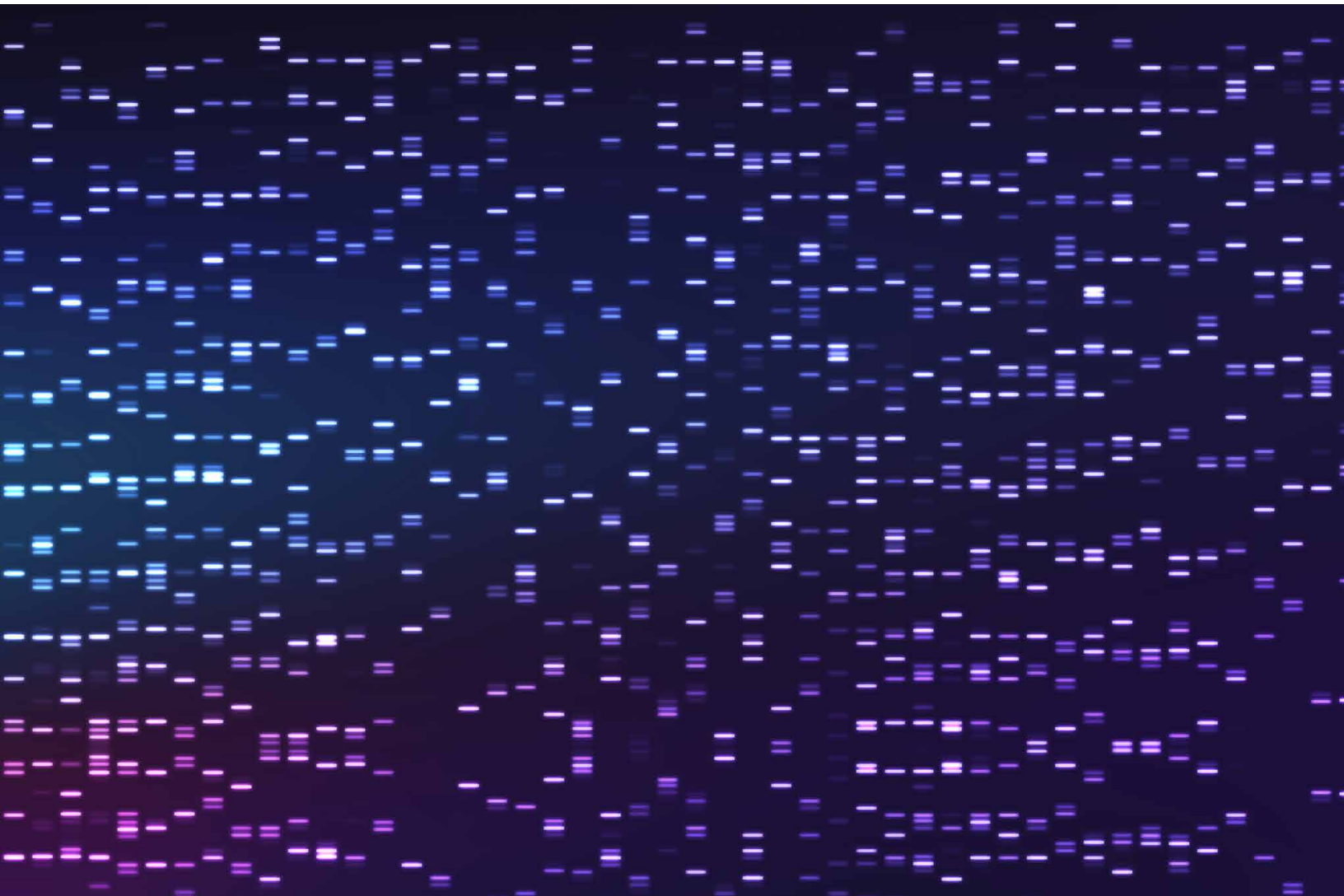


Table 1. Comparison of genome editing and silencing tools.

Technology	CRISPR-Cas9	TALEN	RNAi
End goal	• Permanent gene knockout or knock-in	• Permanent gene knockout or knock-in	• Transient gene knockdown
Benefits	• Superior cleavage efficiency • Simple design and assembly process • Multiplexing capable	• Flexible; no sequence restriction or PAM requirement; ideal for knock-in • Includes rights under foundational TAL intellectual property	• Ultimate flexibility in technology and gene targets • High-potency silencing • Straightforward protocol
Design requirement	• PAM site (NGG)	• Completely flexible, no design restrictions	• Very few design restrictions
Applications	• Single-gene analysis • Arrayed or lentiviral pooled screening	• Single-gene analysis	• Single-gene analysis • Arrayed screening



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 of disease-resistant transgenic plants.

What is CRISPR-Cas9 technology?

The system that comprises clustered regularly interspaced short palindromic repeats (CRISPR) and CRISPR-associated protein 9 (Cas9) 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). In gene editing research, these RNAs are commonly catenated into long structures known as single guide RNA (sgRNA). The gRNA unit guides the Cas9 nuclease to a specific genomic locus, and the Cas9 nuclease 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).

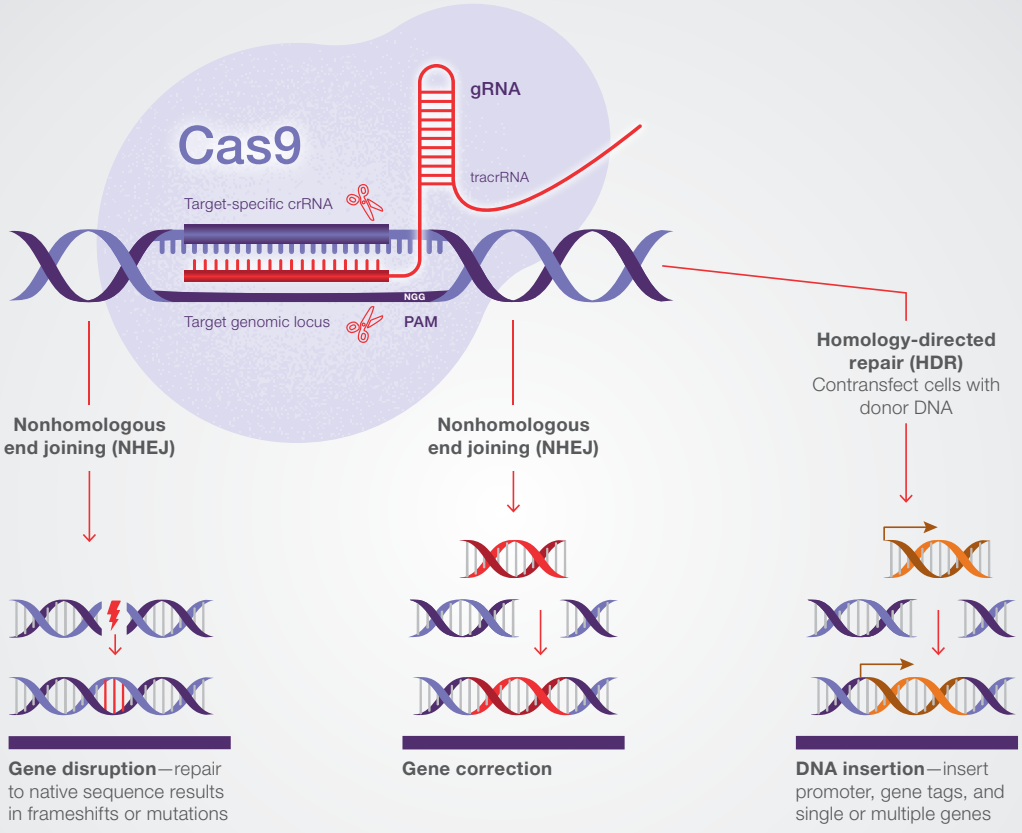


Figure 2. CRISPR-Cas9 double-stranded break and repair pathways. Cleavage occurs on both strands, 3 bp upstream of the NGG in the protospacer adjacent motif (PAM) sequence on the 3′ end of the target sequence.

Tips and tricks for getting started

Set yourself up for CRISPR success

New to CRISPR technology? Before you start, be sure you understand the factors affecting the efficiency, and therefore the outcome, of genome editing.

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, the cleavage site should be as close to the edit site as possible (<10 bp if possible) and the lengths of the donor homology arms should be optimized
- Choose the Cas9 nuclease that is best suited for the experiment and cell type

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-mediated transfection, electroporation, or viral transduction
- When delivering multiple molecules, optimize the ratio of their quantities
- Transfect a validated control gRNA to test transfection efficiency and Cas9 nuclease activity
- For knock-in experiments, deliver the repair template so that it is present when Cas9 is active

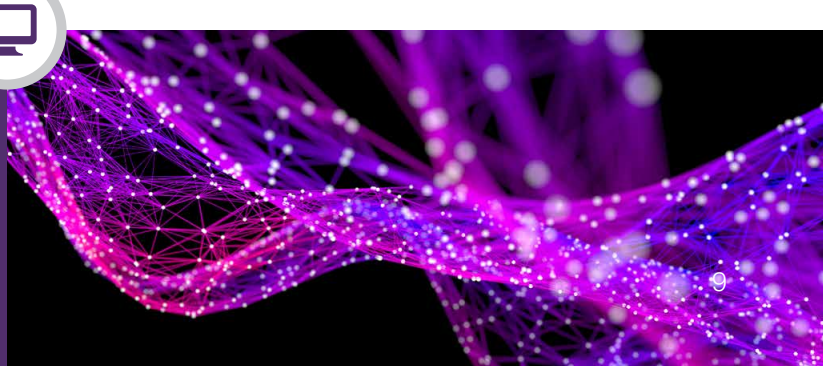
3 Detect

Thing to consider when detecting the edit

- Quickly confirm gRNA cleavage efficiency with a genomic cleavage detection (GCD) assay
- Choose a detection method appropriate for the downstream application
- Isolate clones if a genetically homogeneous 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

Need help with your design?

Utilize our free genome editing design tool.
Find out more at **thermofisher.com/truedesign**



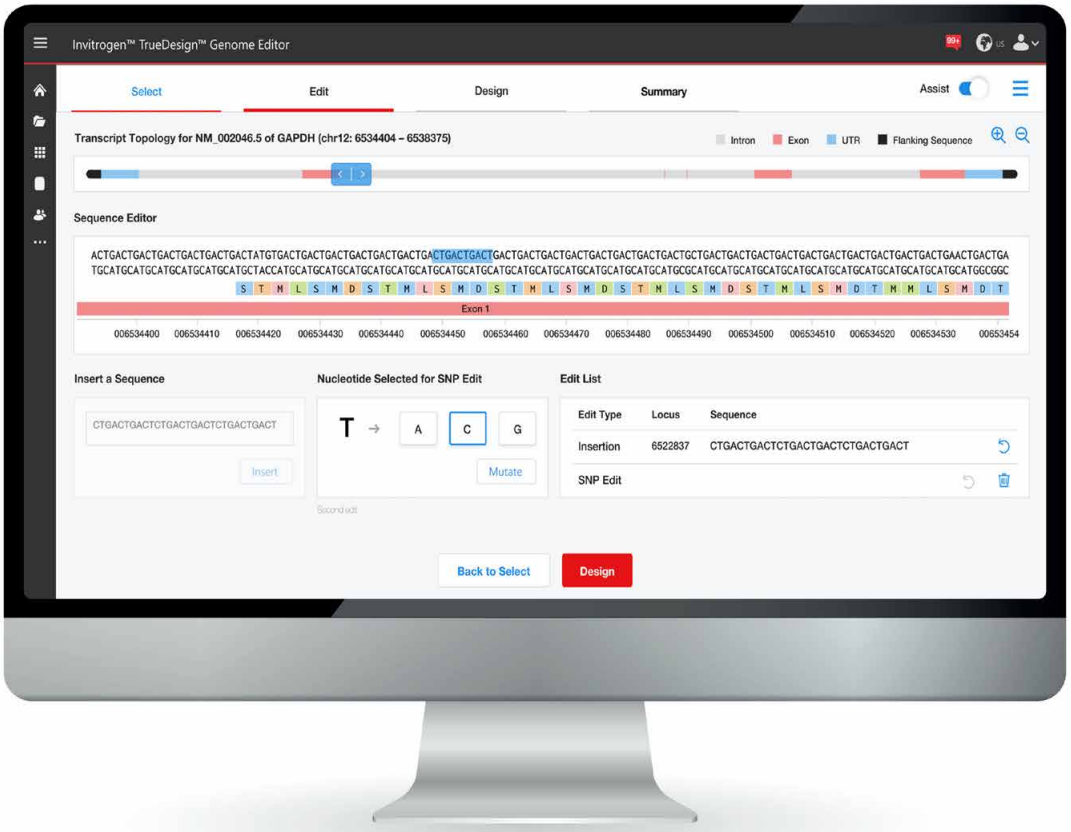
TrueDesign Genome Editor

The Invitrogen™ TrueDesign™ Genome Editor (**thermofisher.com/truedesign**) enables scientists of all experience levels to easily design reagents for accurate and successful knock-in experiments. The tool was built with an intuitive point-and-type interface that allows users to easily find and edit a gene by streamlining the process in three steps: search, edit, and complete.

The simple step-by-step program allows you to:

- Generate a complete knock-in design in minutes
- Edit up to 30 bases in any human gene using CRISPR-Cas9 or TALEN technology
- Design all necessary oligos for precise SNP or amino acid changes
- Design the required reagents to add a GFP or RFP tag to a target gene without the need for cloning
- Download or order all recommended reagents with a single click

Search for your gene **Craft your edit** **Complete your design**

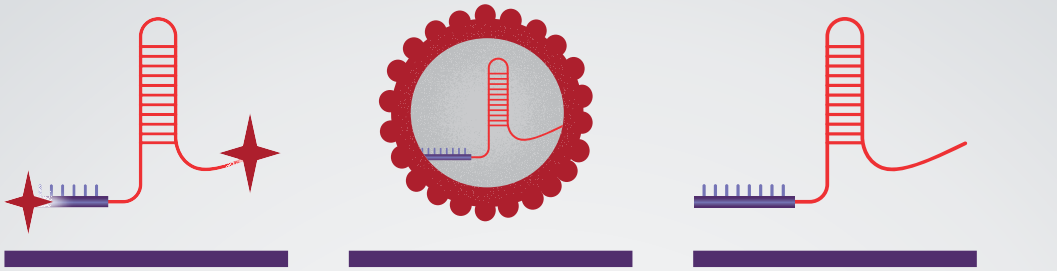


CRISPR-Cas9 editing tools

Maximum flexibility with 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



TrueGuide
Synthetic gRNA

LentiArray
Lentiviral gRNA

Precision gRNA Synthesis Kit

Cas9 nuclease formats

TrueCut
Cas9 Protein v2

GeneArt
CRISPR mRNA

GeneArt
CRISPR all-in-one
plasmid

LentiArray
Cas9 Lentivirus

Figure 3. Available CRISPR-Cas9 formats.

Find out more or place your order at **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 algorithm selects gRNAs for maximum editing efficiency without compromising on specificity. We incorporate the latest in gRNA design research and 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).

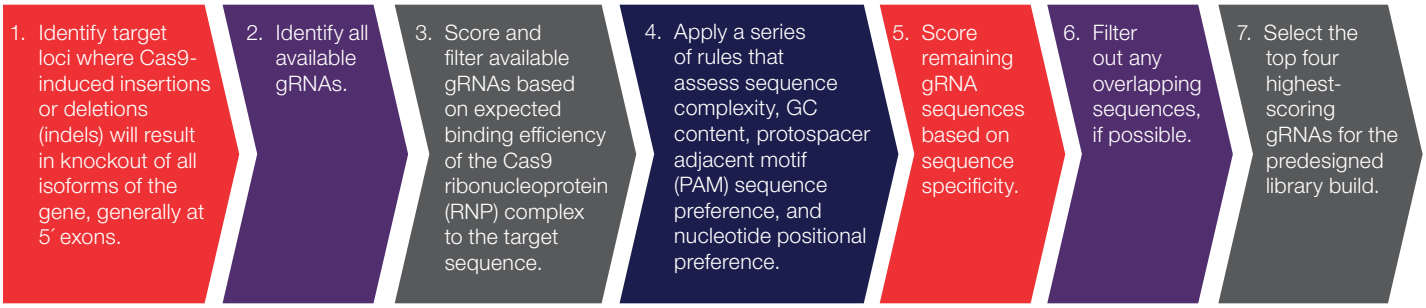
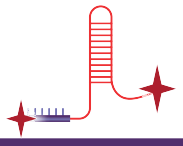
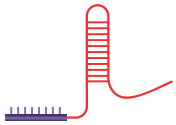


Figure 4. Robust design and selection process for CRISPR gRNAs. These steps are a distilled description of the algorithm used for our predesigned gRNAs.

Table 2. Available CRISPR gRNA formats.

	 TrueGuide Synthetic gRNA	 LentiArray CRISPR gRNA	 Precision gRNA Synthesis Kit
Overview	Choose from our catalog of predesigned gRNAs for gene knockout, order your own gRNA sequence, or order a custom design with our TrueGuide gRNA Ordering Tool	Choose your gRNA design from our catalog of gRNAs packaged as ready-to-use lentivirus	Assembly and synthesis of any gRNA target in as little as 4 hours, including template assembly
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 or Cas9-expressing cells	LentiArray Cas9 Lentivirus	TrueCut Cas9 Protein v2 or Cas9-expressing cells
Controls	Positive and negative controls available	Positive and negative controls available	Available upon request

TrueGuide Synthetic sgRNA

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

- Maximum knockout efficiency without compromising specificity and cell viability
- Easy online ordering—just search for your gene
- Adherence to the rigorous quality standards 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 sgRNAs targeting a number of different genes. 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.

Go to **thermofisher.com/trueguide**

Enter a human or mouse gene identifier

↓

Click "Search"

↓

Select two or more TrueGuide sgRNAs from the Results page

↓

Click "Add to Cart"

↓

View and order related sgRNA controls

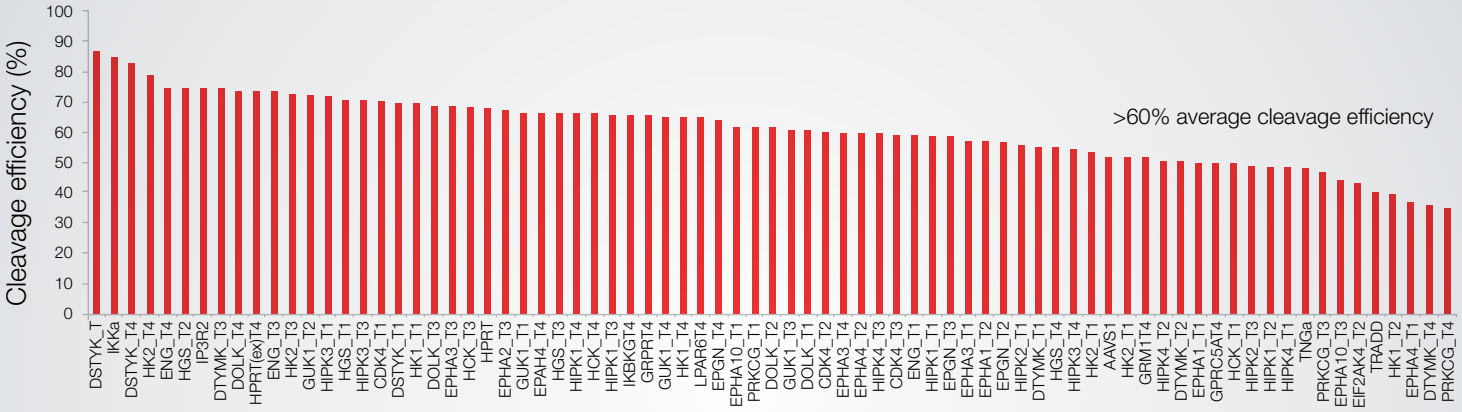


Figure 5. Robust cleavage of kinase genes with TrueGuide Synthetic sgRNAs in Cas9-expressing ME-180 cells.

Find out more at **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

Nuclease-free water

Preparing reagents and resuspending RNA with the appropriate grade of water is a crucial and often ignored 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

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™ RNase*Zap*™ Decontamination Solution and RNase *AWAY*™ Decontamination Reagent.

thermofisher.com/surfacedecontamination

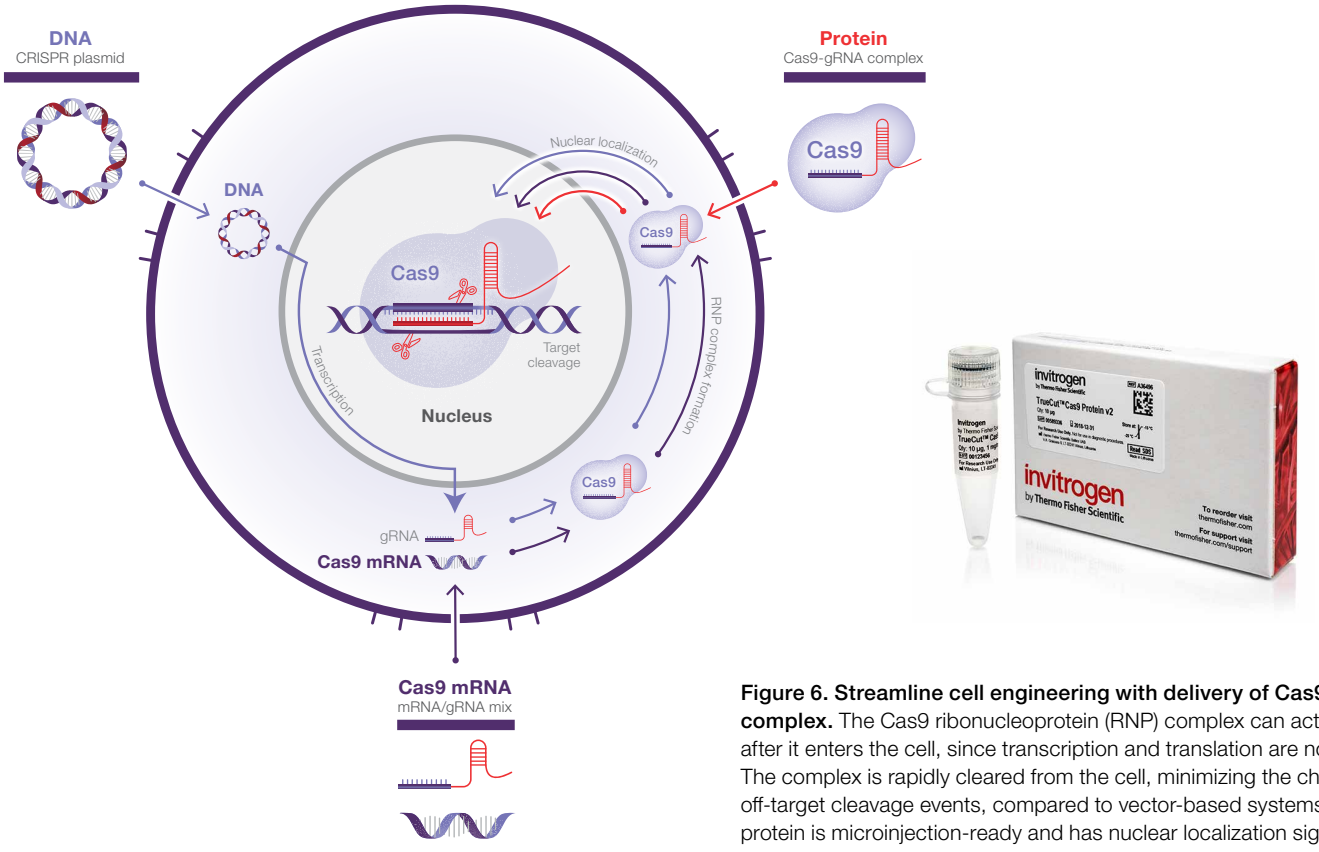


Figure 6. Streamline cell engineering with delivery of Cas9–gRNA complex. The Cas9 ribonucleoprotein (RNP) complex can act immediately after it enters the cell, since transcription and translation are not required. The complex is rapidly cleared from the cell, minimizing the chance for off-target cleavage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

2016 TheScientist TOP 10 INNOVATIONS

Cas9 nuclease

When choosing your Cas9 nuclease format, we recommend Cas9 protein over Cas9-expressing 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)
- Invitrogen™ TrueCut Cas9 Protein v2 is cleared from the cell within 24 hr, minimizing the chance for off-target cleavage events

Award-winning TrueCut Cas9 Protein v2

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

- High editing efficiency achieved consistently in all tested cell lines, including standard, immune, primary, and stem cells
- Up to 2x higher editing efficiency in difficult targets compared to products from other suppliers (Figure 7)
- 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**

Find out more at **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

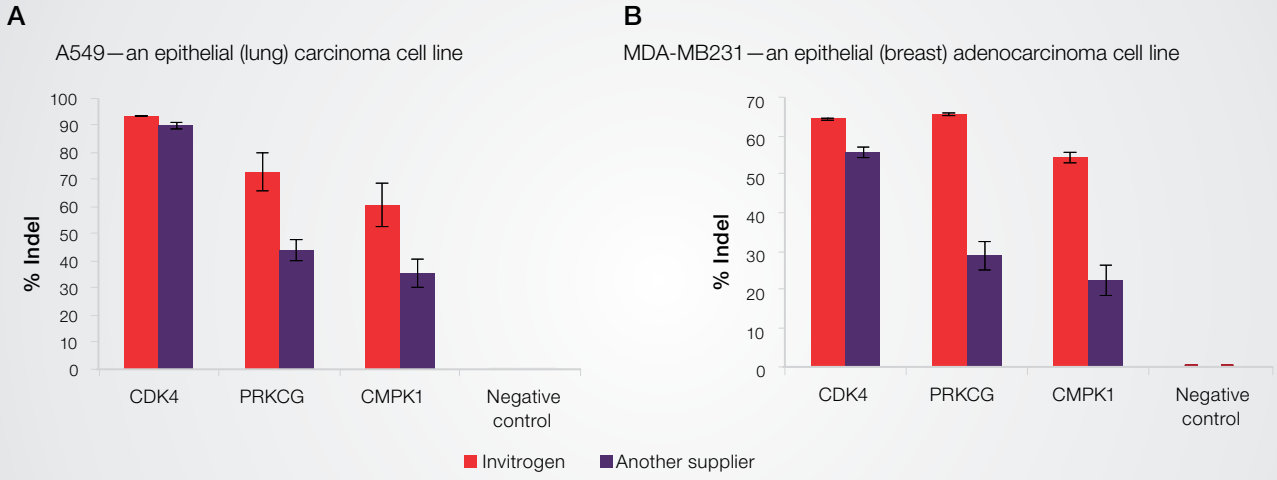


Figure 7. Invitrogen CRISPR tools consistently outperform products from other suppliers. Genome editing of multiple gene targets was performed with TrueCut Cas9 Protein v2 and corresponding TrueGuide Synthetic gRNAs. Delivery was achieved using optimized transfection protocols and Invitrogen™ Lipofectamine™ 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.

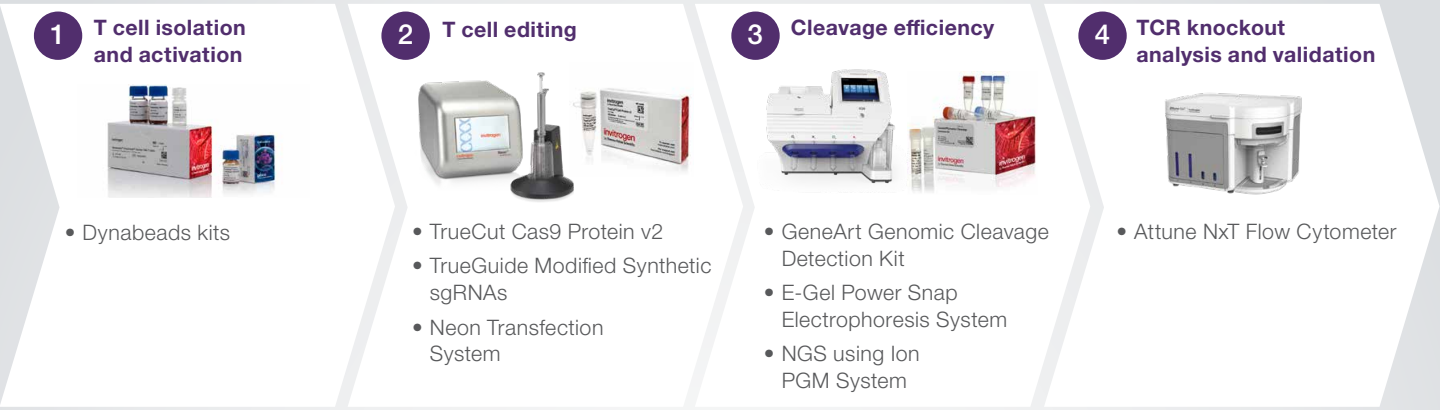


Figure 8. An end-to-end workflow solution for primary T cell editing.

Up to 90% functional knockout in human primary T cells

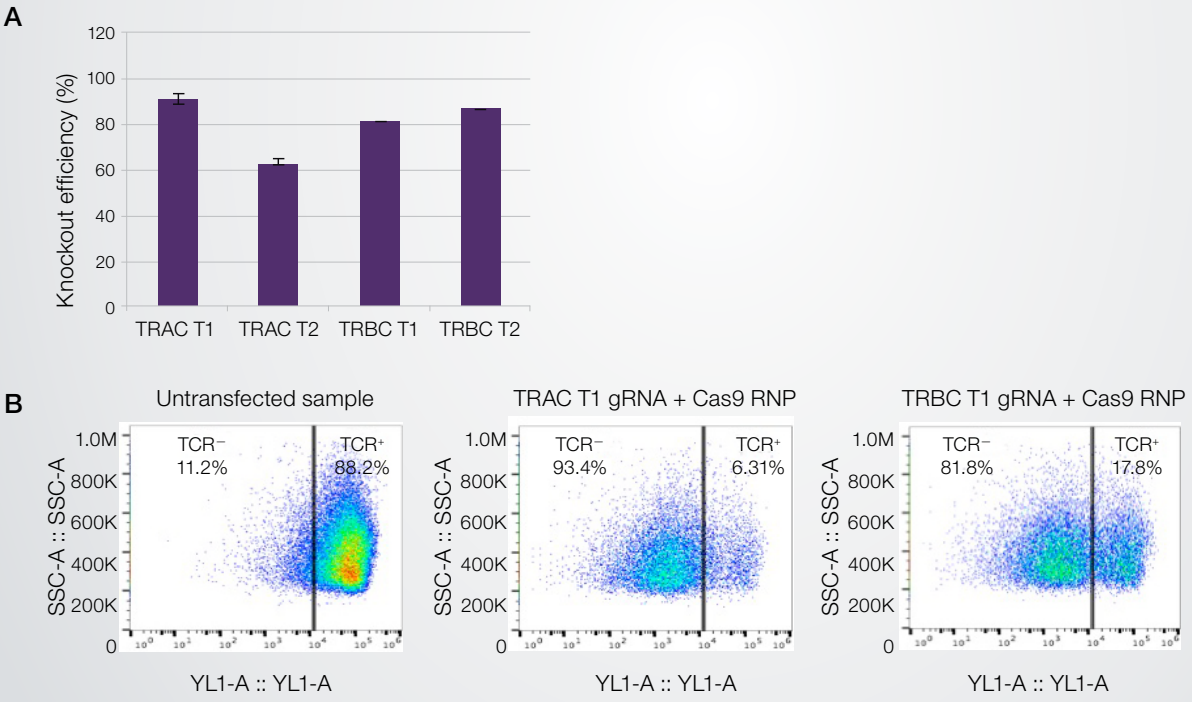


Figure 9. High-efficiency functional knockout in T cells. T cells were isolated from PBMCs (from a healthy donor) using Dynabeads magnetic beads, and then transfected with TrueCut Cas9 Protein v2 and TrueGuide Modified Synthetic sgRNAs targeting T cell receptor alpha (TRAC) or beta (TRBC) regions using the Neon Transfection System. **(A)** Analysis by flow cytometry following binding with antibody specific to the T cell receptor (TCR) shows >90% functional knockdown of the receptor. For both TRAC and TRBC, gRNAs specific for two different genomic DNA targets (T1 and T2) were tested, and results are shown only for the T1 target in each case. **(B)** Summary of NGS-based analysis of cleavage efficiency at two different genomic DNA targets (T1 and T2) for both TRAC and TRBC loci.

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 better signal-to-noise ratios and give you more confidence in the hits that emerge from your screens.

Type of control	Description	Recommended use
Negative control	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 for nonspecific cellular effects when running your screens.
Positive control	Positive controls are validated gRNA sequences that have demonstrated high 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 control	Specific to LentiArray Lentiviral CRISPR gRNA libraries, these control constructs are available as either negative or positive controls that also express GFP.	The GFP marker provides a visual readout to aid in the rapid optimization of viral transduction conditions.

CRISPR workshops

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 of scientists has designed a series of course options to help you learn the tools and processes needed to design more efficient genome edits.

Find out more or register today at **thermofisher.com/educationresources**

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 loss-of-function screening. The system provides complete, permanent knockout of the target gene, resulting in strong phenotypes and providing confidence in your screening results.

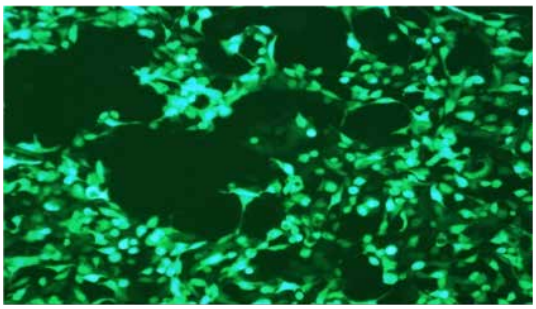
Award-winning LentiArray CRISPR gRNA libraries

The Invitrogen™ LentiArray™ CRISPR library product line provides 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 functional genomics discoveries. LentiArray lentiviral gRNA 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.

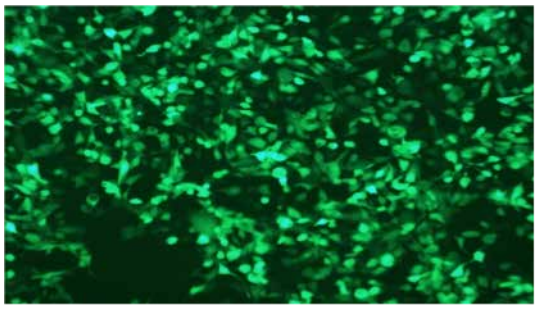
LentiArray lentiviral CRISPR gRNA libraries are provided in an arrayed format that is designed to be compatible with your existing high-throughput screening infrastructure. The LentiArray library product line provides the flexibility you need to expand your screening capabilities with CRISPR-Cas9 technology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

Optimize your assay design with LentiArray GFP-expressing control gRNAs

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 transduction and assay design.



LentiArray CRISPR Positive Control Lentivirus, human, with GFP



LentiArray CRISPR Negative Control Lentivirus, human, nontargeting, with GFP

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

LentiArray CRISPR libraries feature:

- Advanced gRNA designs for maximum knockout efficiency without sacrificing specificity
- Up to four high-quality gRNAs per gene target combined in a single well for efficient knockout in a wide variety of cell types
- Delivered as high-titer, ready-to-use lentivirus or glycerol stocks in 96-well plates
- Complete set of controls and lentiviruses against single-gene targets to support pre-screen assay development and rapid post-screen hit validation
- 19 pre-defined libraries and custom collections are available, enabling you to focus on defined gene sets or perform unbiased whole-genome surveys (Figure 11)

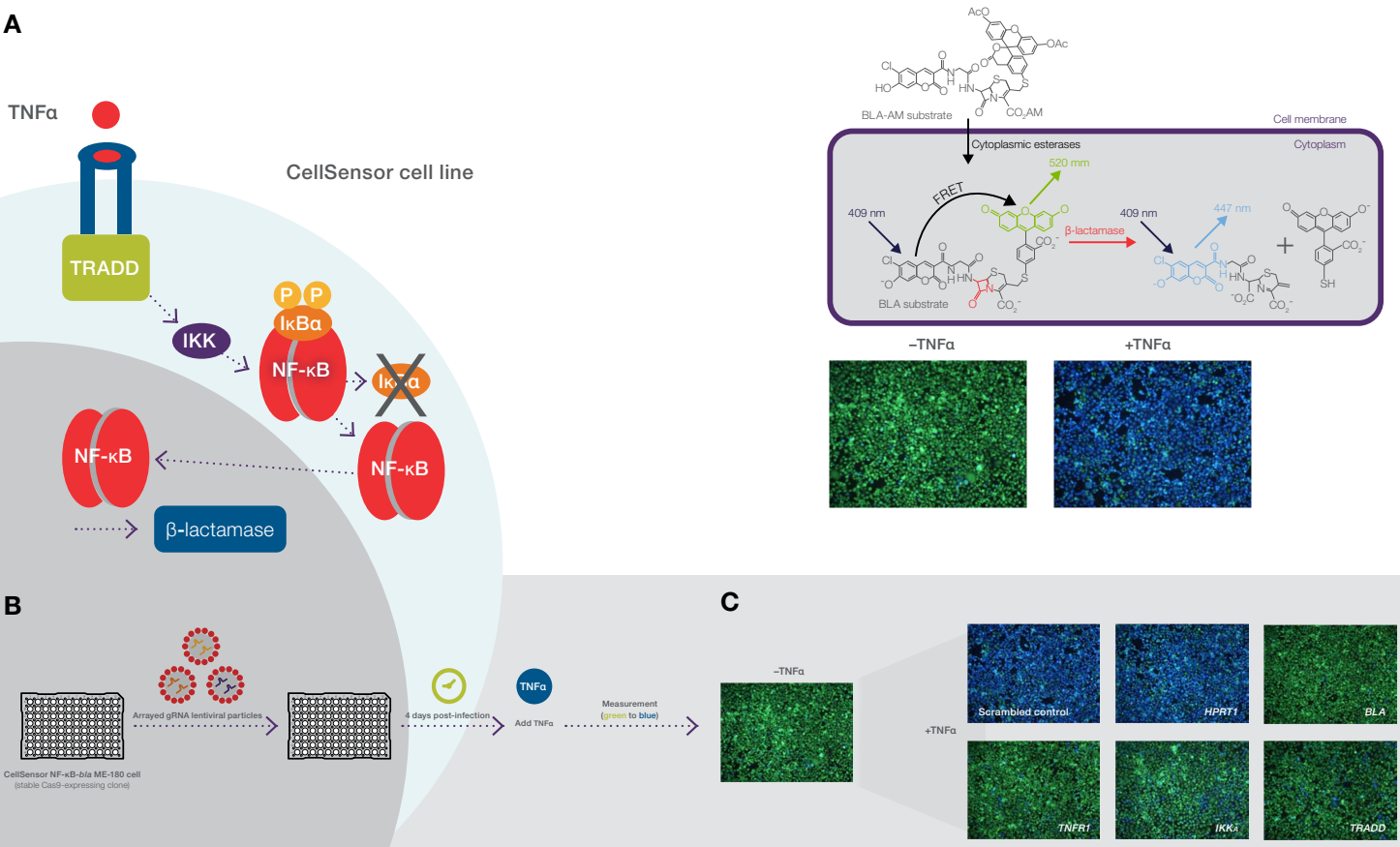


Figure 11. A subset of kinases screened in CellSensor NF-κB-*bla* ME-180 cells with a ratiometric two-color reporter assay. (A) CellSensor cell lines use β-lactamase reporter technology to provide a rapid and sensitive method of analyzing activation of signal transduction pathways. **(B)** Overview of the screening workflow. **(C)** After stimulation with TNFα, the ratio of green to blue fluorescence decreased in unedited cells. Cells infected with lentiviral particles carrying gRNA that effectively disrupted the NF-κB pathway remained green, with a high ratio of green to blue fluorescence. Complete knockout of *TNFR1* was observed in nearly 100% of the cells.

For more information, go to **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 ensure that the library will provide high-efficiency knockout of target genes across a wide array of cell types.

Pooled lentiviral CRISPR libraries

In addition to our LentiArray libraries we offer pooled lentiviral libraries. Invitrogen™ LentiPool™ CRISPR libraries provide an affordable method to screen a large number of genes, as there is no high-throughput instrumentation required.

- Four high-quality gRNAs per gene target in pools for screening without complex automation
- Delivered at high titer: >1 x 10^8 TU/mL
- Rigorous QC process includes sequencing to confirm gRNA representation
- Many libraries are on the shelf and ready to ship

For more information, contact us at
GEMServices@thermofisher.com

Table 3. Gene family collections available as LentiPool or LentiArray lentiviral gRNA libraries.

Predefined CRISPR gRNA libraries	Number of genes
Kinase CRISPR Library	822
Phosphatase CRISPR Library	288
Cancer Biology CRISPR Library	510
Epigenetics CRISPR Library	396
Ubiquitin CRISPR Library	943
Cell Cycle CRISPR Library	1,444
Membrane Trafficking CRISPR Library	141
Transcription Factor CRISPR Library	1,817
Nuclear Hormone Receptor CRISPR Library	47
Apoptosis CRISPR Library	904
Drug Transporter CRISPR Library	98
Ion Channel CRISPR Library	328
Cell Surface CRISPR Library	778
Protease CRISPR Library	475
Tumor Suppressor CRISPR Library	716
DNA Damage Response CRISPR Library	561
GPCR CRISPR Library	446
Druggable CRISPR Library	10,128
Whole-Genome CRISPR Library	18,453

Predesigned donor solutions

The Invitrogen™ TrueTag™ Donor DNA Kit is the fastest and easiest way to generate transfection-ready donor DNA for gene tagging and knock-in experiments. TrueTag kits come with all the necessary reagents to prep the donor DNA, and the easy-to-follow protocols enable that even novice users can perform high-efficiency knock-in edits in their own lab.

- **Tag your gene**—use your choice of GFP or RFP tags (Figure 12)
- **No cloning is required**—create donor templates in hours, not days (Figure 13)
- **Get superior efficiency**—obtain up to 100% edited cells (Figure 14)

The TrueTag kit provides a simple and fast workflow that delivers up to 100% knock-in efficiency

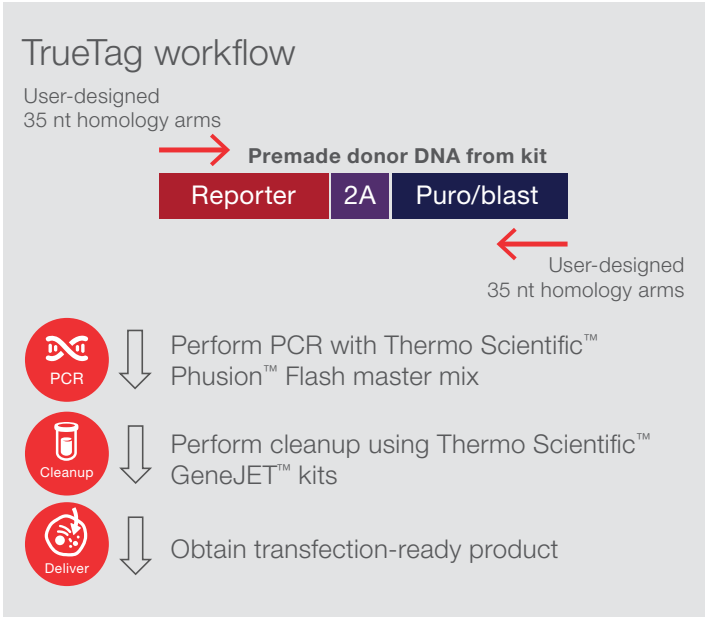


Figure 13. The TrueTag Donor DNA Kit workflow can be done in one afternoon. Using the materials included with the kit, just complete a single PCR run, column-purify the double-stranded DNA product, and then transfect the donor DNA (with appropriate CRISPR-Cas9 or TAL effector nucleases) to knock in the tag into your gene of interest.

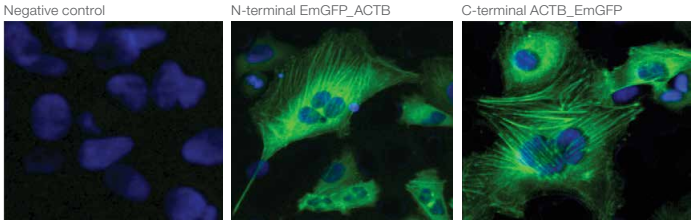


Figure 12. U2OS cells were transfected with Invitrogen™ TrueCut™ Cas9 Protein v2, a TrueTag dsDNA donor to insert GFP, and an Invitrogen™ TrueGuide™ gRNA for either the N-terminal or C-terminal end of the *ACTB* locus. Transfection was performed with Invitrogen™ Lipofectamine™ CRISPRMAX™ reagent following donor DNA preparation using the TrueTag Donor DNA Kit, GFP (Cat. No. A42992). Seven days after transfection, the cells were counterstained with Invitrogen™ NucBlue™ Live ReadyProbes™ Reagent and images were captured on an Invitrogen™ EVOS™ Imaging System.

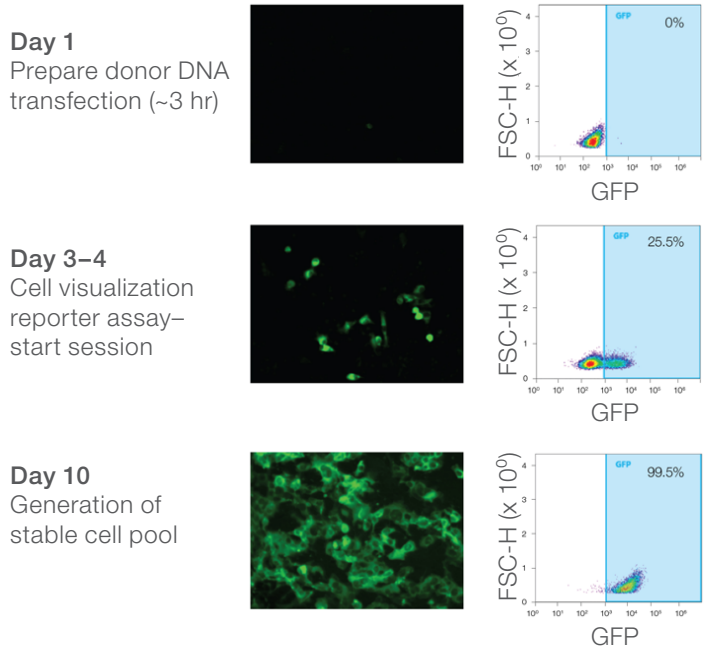


Figure 14. Generation of a stable pool of edited cells in 10 days. On day 1, 293FT cells are transfected with TrueCut Cas9 v2, a TrueTag dsDNA donor for homology-directed repair at the C-terminal end of the *ACTB* locus to insert GFP-puromycin, and a TrueGuide gRNA for the C-terminal end of *ACTB*, using the Lipofectamine CRISPRMAX reagent. TrueTag donor generation is a quick process that takes 3 hours or less to complete. On day 3 or 4, homology-directed repair is complete, and the fusion tag is expressed from the native locus. Approximately 25% of the cells in this example were GFP-positive. Selection can begin 72 to 96 hours after transfection. By day 10, the population of cells stably selected with puromycin is >99% positive for GFP. Data were collected on an Invitrogen™ Attune™ NxT Flow Cytometer.

Find out more at
thermofisher.com/truetag

Designer TAL effector proteins

Precise and flexible editing with the freedom to innovate

TAL effector proteins are produced by bacteria of 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 15), 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).

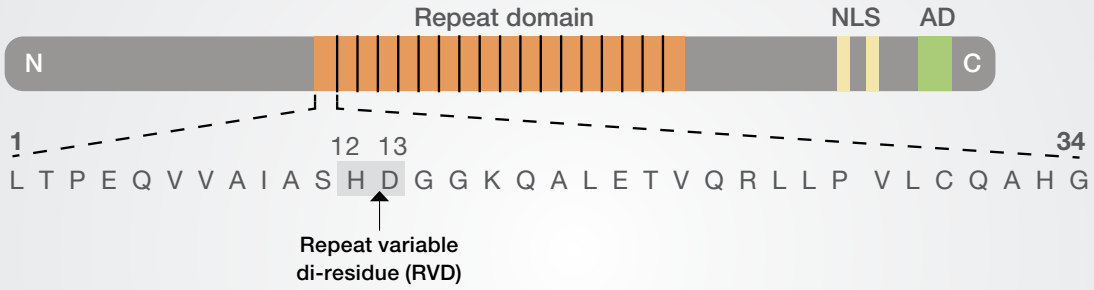
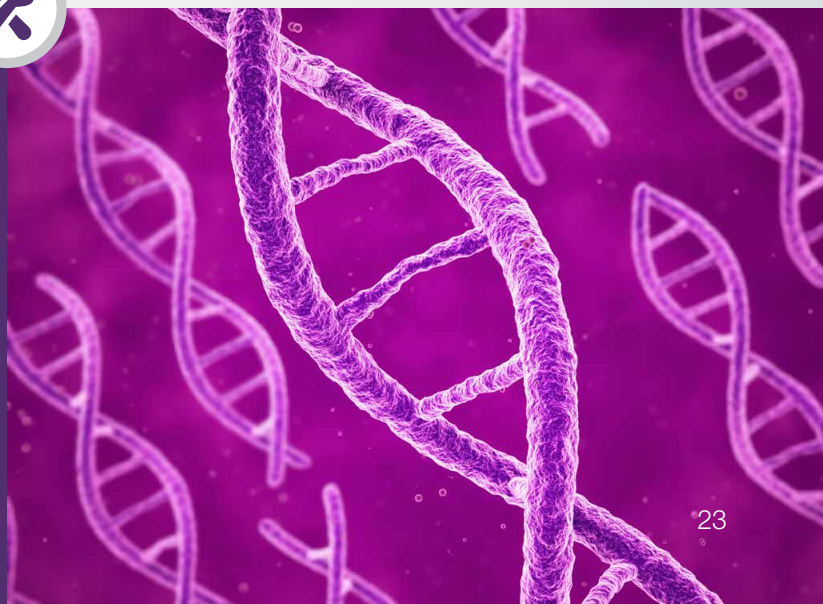


Figure 15. TAL effector DNA-binding domain. The structure of the DNA-binding domain can be manipulated to produce a protein domain that binds specifically to any DNA sequence in the genome.

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**



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™ FlexCut™ TALEN mRNA pairs provide custom DNA-binding proteins for accurate DNA targeting and precise genome editing.

Based on your research needs, our FlexCut TALENs can provide you with complete flexibility in target design for difficult loci. FlexCut TALENs are available now in an mRNA format, and are designed to have 5′ T constraint (which were required in earlier-generation TAL effectors) and are fused to FokI nuclease domain to support genome editing experiments (Figure 16).

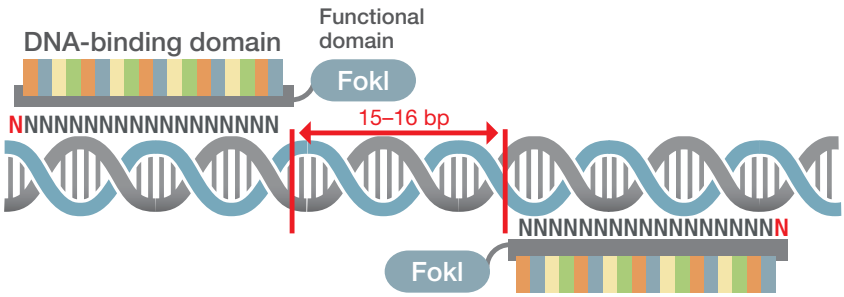
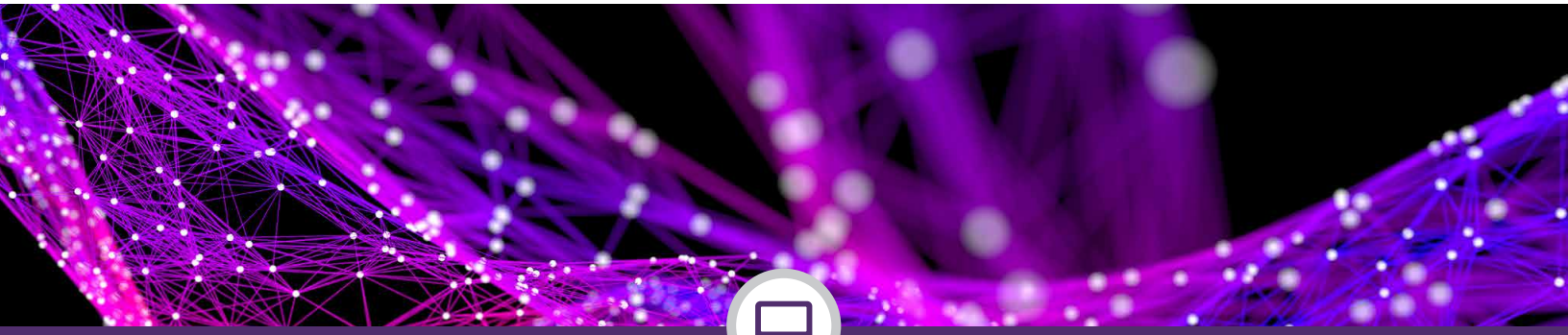


Figure 16. Designing target sites for maximal binding of customized TAL effectors. Invitrogen TALs eliminate the 5′ T constraint found in earlier generations of the technology. Invitrogen TALs allow targeting of any sequence across the genome; 15–16 bp spacing between the two TAL effector targets is optimal.

Find out more or place an order at **thermofisher.com/tals**



Support resources

Go to our TALEN learning center at **thermofisher.com/talen101**

Ordering Invitrogen TALs

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

RNAi tools for gene silencing and microRNA analysis

A mechanism for regulation of gene expression

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 short interfering RNA (siRNA) for targeted gene knockdown and microRNA (miRNA) reagents to mimic or inhibit endogenous gene regulators. 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 for gene silencing

Superior siRNAs for *in vitro* RNAi applications are 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, and strategies to reduce or eliminate off-target effects, 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 from our *Silencer* Select siRNA library are available in the PubChem database at NCBI.

thermofisher.com/sirna

Table 4. Selection guide for siRNA products.

	Silencer Select siRNA (Recommended) Highest knockdown, fewest off-targets	**Stealth RNAi siRNA** Good knockdown, reduced off-targets	**Silencer siRNA** Cost-effective, guaranteed siRNA
Potency (recommended working conc.)	5 nM	20 nM	50 nM
Silencing guarantee	2 of 2 siRNAs	2 of 3 siRNAs	2 of 3 siRNAs
Target specificity	Highest	High	Moderate
Chemical modifications, length	LNA-modified 19-bp duplex 2 nt overhangs	Modified 25-bp duplex no overhangs	Unmodified 19-bp duplex 2 nt overhangs
Gene types targeted	Protein-coding genes Long noncoding RNA (lncRNA)	Protein-coding genes	Protein-coding genes
siRNA libraries	Custom gene lists Predefined collections, including the whole human genome	Not available	Custom gene lists Predefined collections
Target species	Human, mouse, rat (Other species: order custom designs)		

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, but all experiments should be optimized with a validated positive control (targeting a ubiquitously expressed gene) and a nontargeting control to identify nonspecific effects.

thermofisher.com/sirnacontrols

Silencer Select siRNA libraries

We offer predefined collections of Silencer Select siRNAs against popular human gene classes, as well as the whole human genome and druggable genome. Custom libraries are also available for your particular genes of interest.

For more information, please contact **RNAiSupport@thermofisher.com** or go to: **thermofisher.com/sirnalibraries**

*mir*Vana miRNA mimics and inhibitors

For artificial regulation of target mRNA translation, Invitrogen™ *mir*Vana™ miRNA mimics and inhibitors are chemically modified, synthetic RNA reagents designed to either mimic mature miRNAs or bind 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. *mir*Vana miRNA mimics and inhibitors have been validated with Invitrogen™ Lipofectamine™ RNAiMAX™ Transfection Reagent for use in cell-based systems, and with Invitrogen™ Invivofectamine™ 3.0 Reagent for *in vivo* delivery. *In vivo*–ready *mir*Vana miRNA mimics and inhibitors have been purified by HPLC and dialysis, making them ready for immediate use in animal models. *mir*Vana miRNA mimics and inhibitors are:

- **Versatile**—do functional studies of specific miRNAs using *in vitro* or *in vivo* systems
- **Potent**—validate miRNA regulation of gene expression with minimal off-target effects (Figure 17)
- **High throughput–compatible**—generate libraries for effective simultaneous screening of multiple miRNAs
- **Current**—content is regularly updated based on miRBase database entries

thermofisher.com/mirna

*mir*Vana miRNA libraries

Complete Invitrogen™ *mir*Vana™ 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 **RNAiSupport@thermofisher.com** or go to:

thermofisher.com/mirna

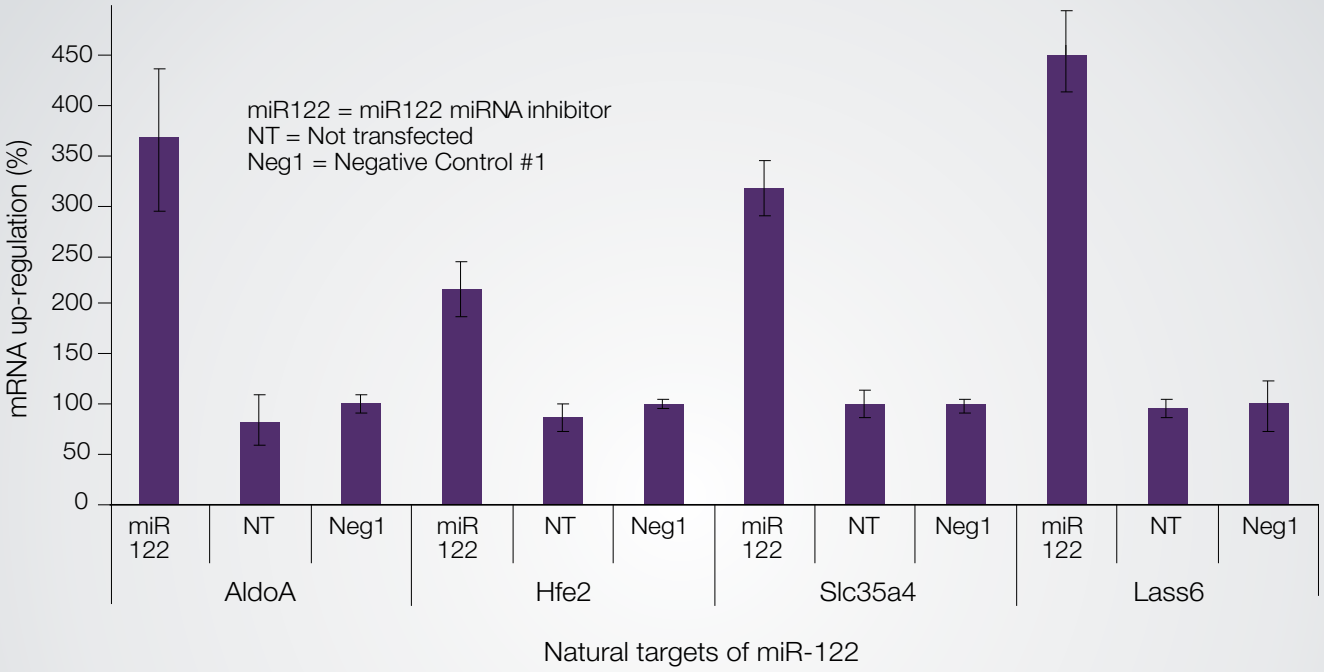


Figure 17. *mir*Vana miRNA inhibitors effectively suppress miRNA *in vivo*. miR122 or negative control *mir*Vana miRNA inhibitor was complexed with Invivofectamine 2.0 Reagent and delivered to BALB/c mouse liver via tail vein injection on 3 consecutive days at a dose of 7 mg per kg of body weight. Expression 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 *mir*Vana 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

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

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

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

Cell culture—plastics
Thermo Scientific™ Nunc™ cell culture treated plastics with the 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

Support resources

Explore virtual training labs at
thermofisher.com/gibcoeducation

Download your copy of our cell culture handbook at
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 Invitrogen™ 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

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

	Plasmid DNA	mRNA, TALs	Synthetic RNA reagents (gRNA, siRNA, miRNA)	Protein	Lentivirus
Lipofectamine CRISPRMAX reagent				•	
Lipofectamine 3000 reagent	•				
Neon Transfection System	•	•	•	•	
Lipofectamine MessengerMAX reagent		•			
Viral delivery*					•
Lipofectamine RNAiMAX reagent			•		

* Lipofectamine 3000 reagent can be used to produce lentivirus.

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 synthetic gRNA into stable Cas9-expressing cells
  - **Lipofectamine MessengerMAX reagent:** to co-transfect Cas9 mRNA with synthetic sgRNA or *in vitro*–transcribed gRNA
  - **Neon transfection system:** to maximize your efficiency with Cas9 RNP in difficult cell types
- **Knock-in applications:** we recommend delivering TrueCut Cas9 Protein v2 with synthetic sgRNA and relevant donor template using the Neon Transfection System; for more information, refer to Liang et al. (2016) *Biotech Letters* 38(6)
- **Knockout applications:** LentiArray lentiviral gRNA for difficult-to-transfect cells or TrueGuide synthetic sgRNA with TrueCut Cas9 v2 protein delivery with Lipofectamine CRISPRMAX reagent
- **Gene silencing and miRNA modulation:** RNAiMAX reagent is the superior-performing and most widely used siRNA/miRNA transfection reagent available.

Support resources

View transfection protocols at **thermofisher.com/transfectionprotocols**

Download the Transfection and Genome Engineering Handbook at **thermofisher.com/transfectionhandbook**

Lipofectamine CRISPRMAX Cas9 Transfection Reagent

The first optimized transfection reagent for CRISPR-Cas9 protein delivery

Lipofectamine CRISPRMAX reagent is the first optimized lipid nanoparticle transfection reagent for delivery of the CRISPR-Cas9 protein–gRNA complex (RNP), providing up to 85% cleavage efficiency when combined with TrueCut Cas9 Protein v2 (Figure 18).

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/crisprmax

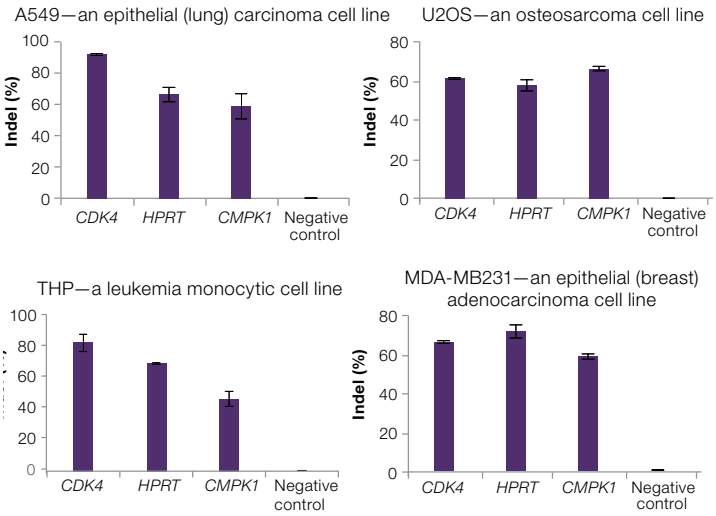


Figure 18. High-efficiency gene editing across a broad range of cell types and targets. A wide range of adherent and suspension cell lines were tested to determine editing efficiencies using TrueGuide Synthetic sgRNA complexed with TrueCut Cas9 Protein v2. Here, the Cas9 RNP complex was delivered into cell lines using Lipofectamine CRISPRMAX Cas9 Transfection Reagent. At 72 hours posttransfection, cells were harvested and tested for efficiency.

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 19).

thermofisher.com/messengermax

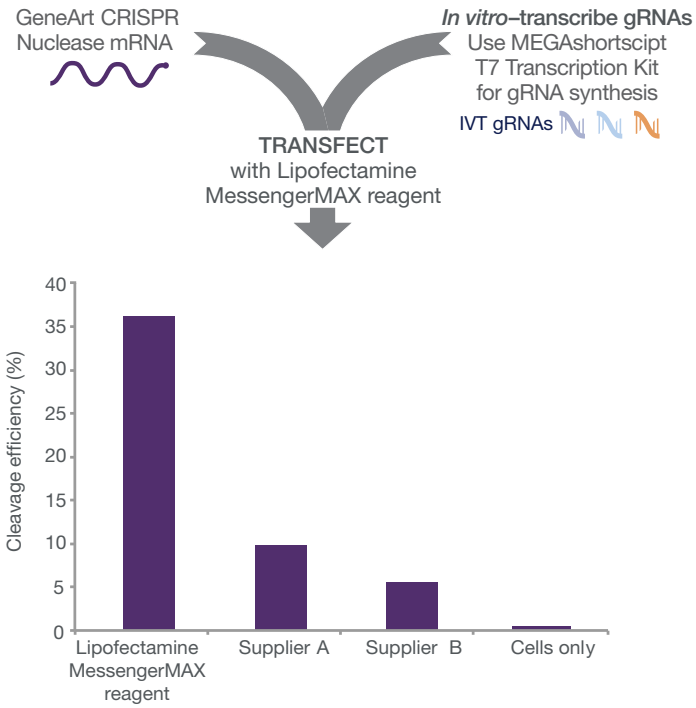


Figure 19. Up to 10x higher cleavage efficiency using GeneArt CRISPR mRNA and Lipofectamine MessengerMAX reagent. Lipofectamine MessengerMAX reagent and two leading mRNA delivery reagents were used to deliver the complete CRISPR format (Cas9 mRNA + IVT gRNA) targeting the *HPRT1* locus in Gibco™ iPSCs. 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.

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, Invitrogen™ GeneArt™ CRISPR vectors targeting the *AAVS1* locus in HepG2 and U2OS cells show improved transfection efficiency, mean fluorescence intensity, and genomic cleavage (Figure 20). High transfection and genome editing efficiency is also observed with Invitrogen™ 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

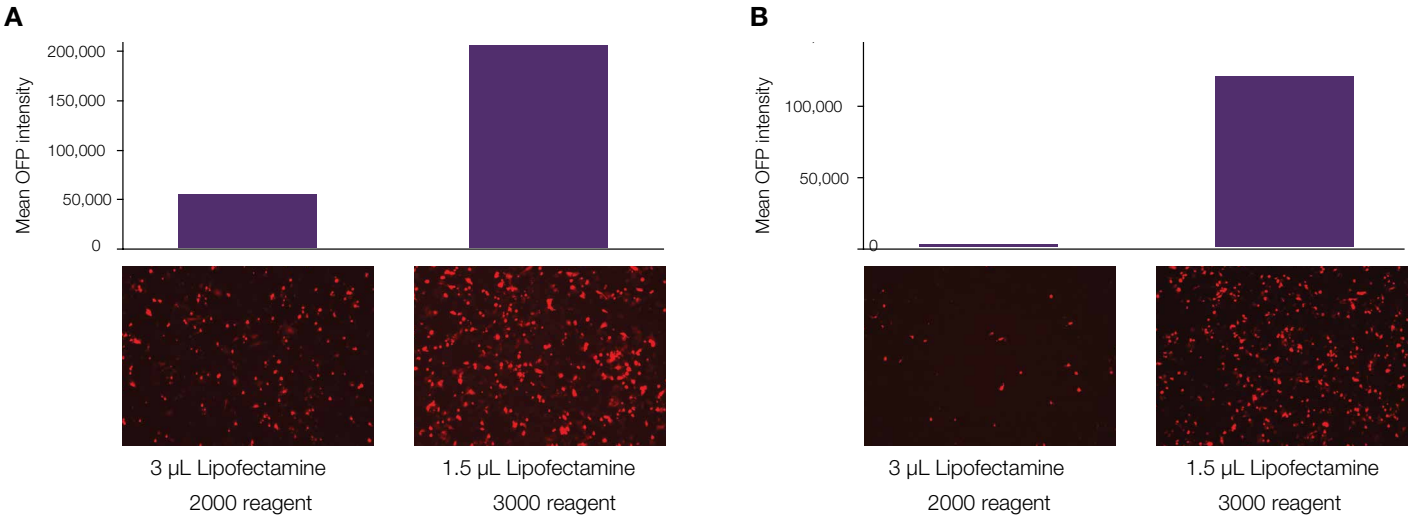


Figure 20. Transfection efficiency and protein expression using GeneArt CRISPR Nuclease Vector. The vector contained an Orange Fluorescent Protein (OFP) reporter gene and was transfected with Invitrogen™ Lipofectamine™ 2000 or Lipofectamine™ 3000 Reagent into **(A)** U2OS and **(B)** HepG2 cell lines. Bar graphs show reporter gene expression; images show fluorescence of corresponding cells expressing OFP.

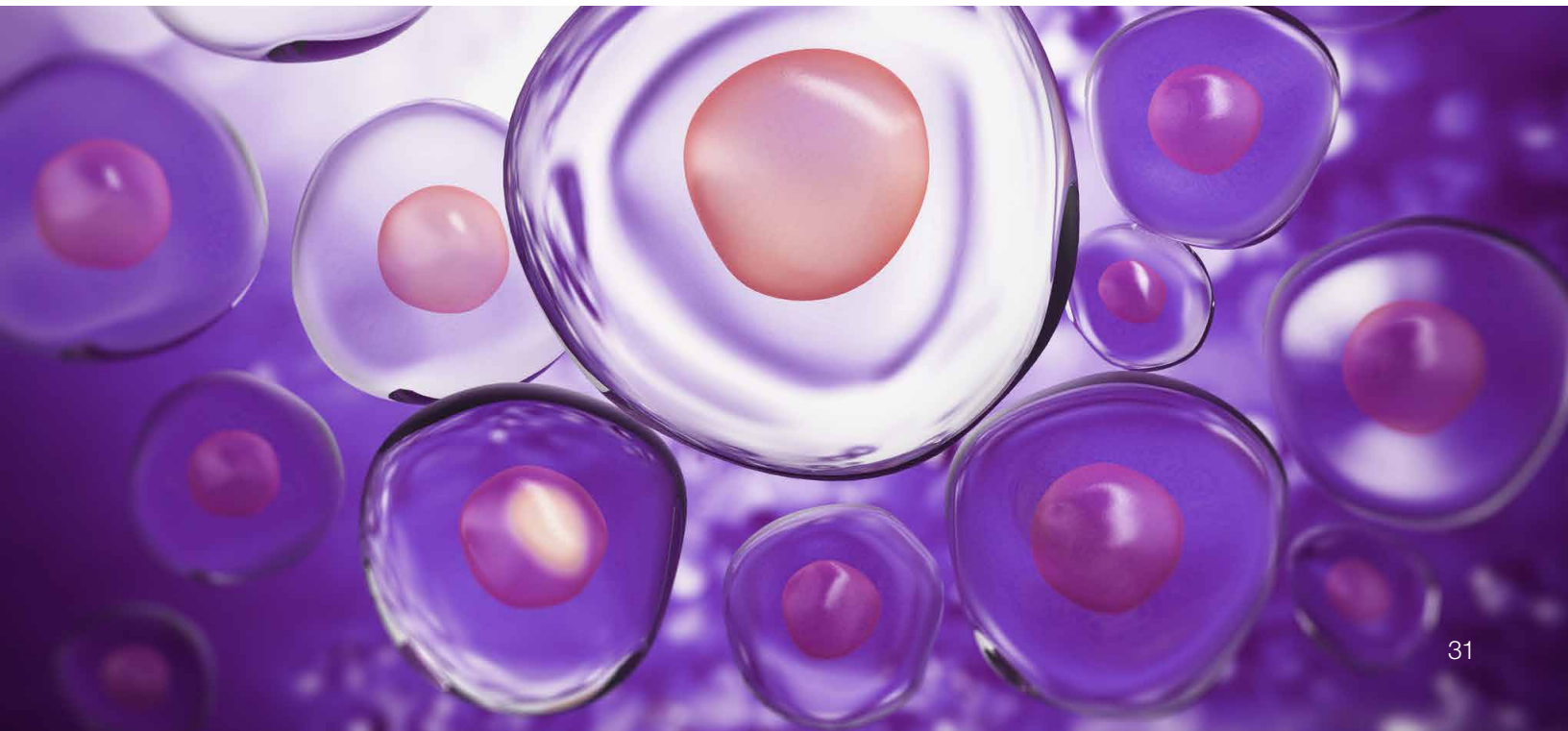
Neon transfection system

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 x 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 x 10^4 cells to 6 x 10^6 cells per reaction
- **Simple**—easy to use with no cell-specific buffers; uses a 10 µL or 100 µL transfection kit with reagents for all cell types
- **Versatile**—includes preprogrammed and user-configurable electroporation parameters that can be optimized freely

thermofisher.com/neon



Visualization and confirmation of your gene edit

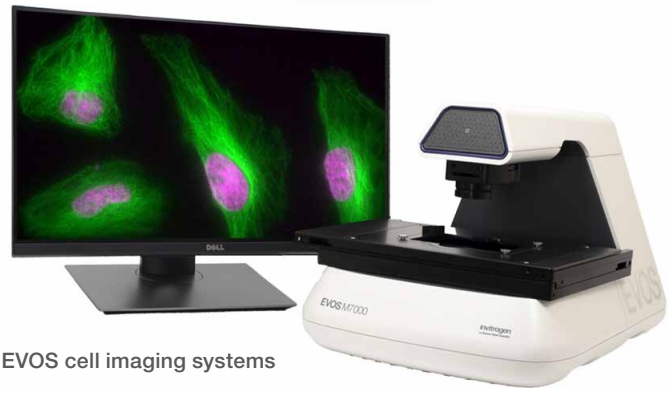
Whichever gene editing strategy you use, careful monitoring of the process will help you generate robust and reliable results. Start with accurate cell counts and viability determinations before you commit more expensive resources. Select monitoring tools to optimize the editing steps based on your biological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

Countess 3 FL Automated Cell Counter

Cell counting

The Invitrogen™ Countess™ 3 FL Automated Cell Counter is a benchtop assay platform equipped with state-of-the-art optics, autolighting, autofocus, and onboard image analysis functionality for rapid assessment of cells. With three-channel flexibility—brightfield and two user-selected fluorescence channels—you can count cells, monitor fluorescent protein expression, and measure cell viability to optimize your gene editing experiments.

thermofisher.com/countess



EVOS cell imaging systems

Cell imaging systems

Designed to eliminate the complexities of the microscopy, generate publication quality images and empower you with the most advanced features and cutting-edge technology without compromising performance and user safety, 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 cells right in your cell culture room and in the biosafety cabinets.

thermofisher.com/evos

CellInsight CX7-LZR

High-content screening/analysis (HCS/HCA)

Thermo Scientific™ HCS/HCA platforms are designed for exceptional resolution with subcellular, automated detection, and phenotyping with intact, fixed, or live cells. These platforms provide superior images and high content imaging data in no time by performing simultaneous imaging with cell-level quantification and advanced statistics. This real-time analysis approach assures immediate data availability post-scan and aids researchers in making informed decisions more quickly. Moreover, our platforms are empowered with advanced 3D analysis tools that guarantees accurate and efficient investigation as well as superior and intelligent object identification accuracy with incredible speed and efficiency for complex 3D scientific and experimental models of HCS.

thermofisher.com/hcs

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

Detect and validate | Essential analysis tools

Confirm gene editing efficiency and validate the edit

Access/verify → **Characterize** → **Perform**

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 Invitrogen™ 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 21). 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 predesigned positive gRNA controls to 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**

Rapid method for cleavage detection

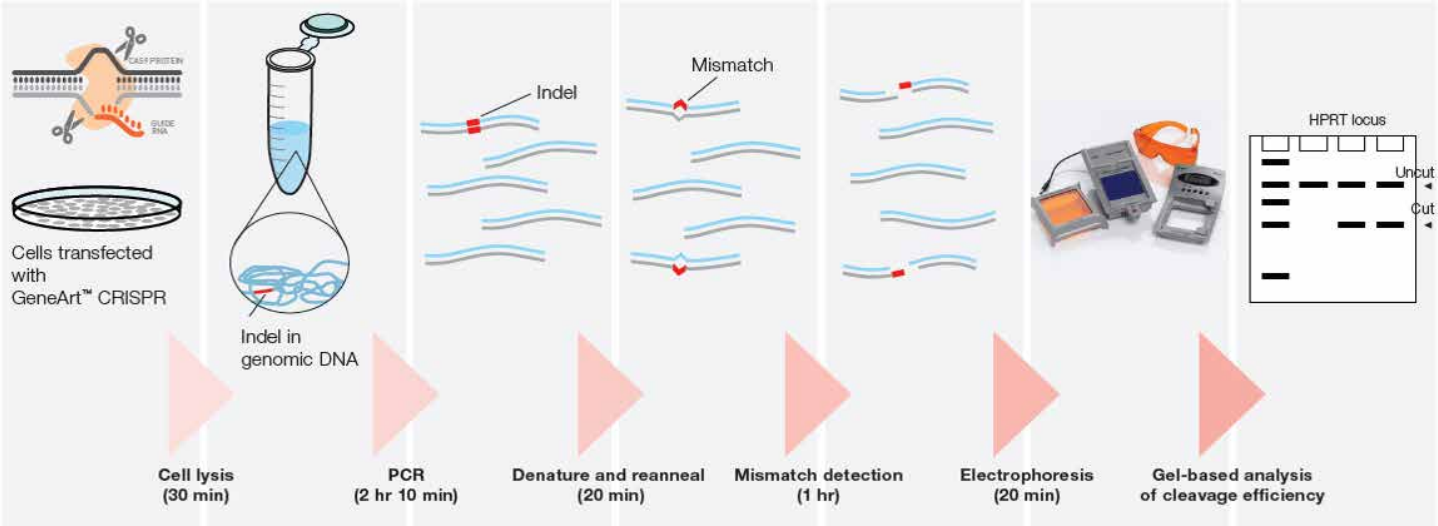
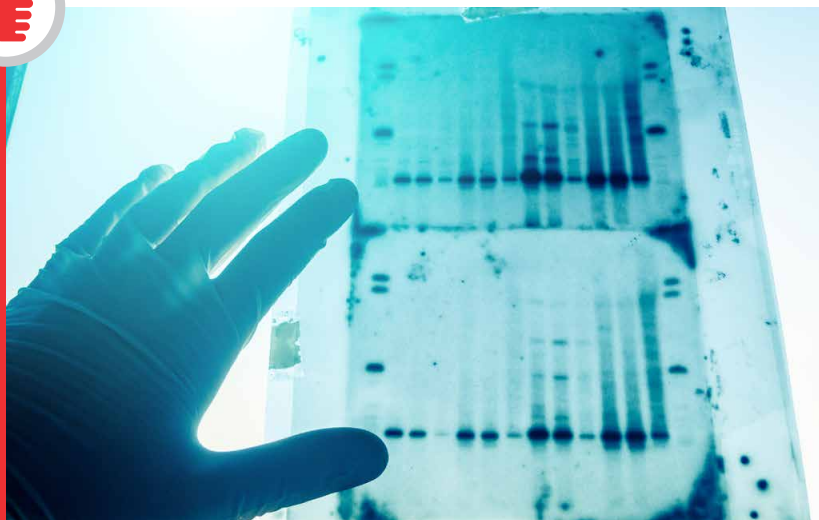


Figure 21. Simple, reliable, and rapid method for the detection of locus-specific cleavage of gDNA using the GeneArt Genomic Cleavage Detection Kit. Loci where the gene-specific double-strand breaks occur are amplified by PCR. The PCR product is denatured and reannealed so that mismatches are generated as strands with an indel re-annealed to strands with no indel or a different indel. The mismatches are subsequently detected, cleaved by detection enzyme, and then the resultant bands are analyzed by gel electrophoresis and band densitometry.

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 Torrent™ 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**

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.

Find out more at **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 Applied Biosystems™ 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.

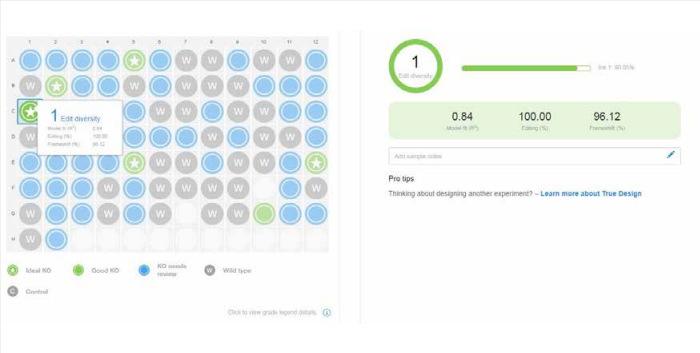


Figure 22. Easy-to-use software for quick identification of successful gene edits.

Figure 23. Robust algorithm calculates and grades the editing outcomes in a matter of seconds.

Screen your gene editing outcomes with the Applied Biosystems™ SeqScreener™ Gene Edit Confirmation Application

The SeqScreener Gene Edit Confirmation Application is an app available through Thermo Fisher™ Connect platform used to determine the spectrum and frequency of targeted mutations generated in a pool of cells by genome editing tools such as CRISPR-Cas9. This app will screen and validate gene editing results obtained using capillary electrophoresis Sanger sequencing technology. The SeqScreener application enables you to:

- Apply an algorithm that accurately reconstructs the spectrum of insertions and deletions (indels) from the sequence traces
- Screen populations of edited cells to understand and quickly identify ideal edited clones (Figure 22)
- Identify the detected indels and their frequencies (Figure 23)
- Detect designed mutations generated by homologous recombination using a donor template
- Characterize results from both knock-in and knockout experiments
- Analyze results from a pool or a single clone

Isolate clones

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.

For more information, please visit
thermofisher.com/clariomassays

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-upgradable design of up to four lasers and 14 colors to meet some of your most challenging research needs.

thermofisher.com/attune

Characterize edited clones

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

Invitrogen detection 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

Western workflow devices and reagents

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.

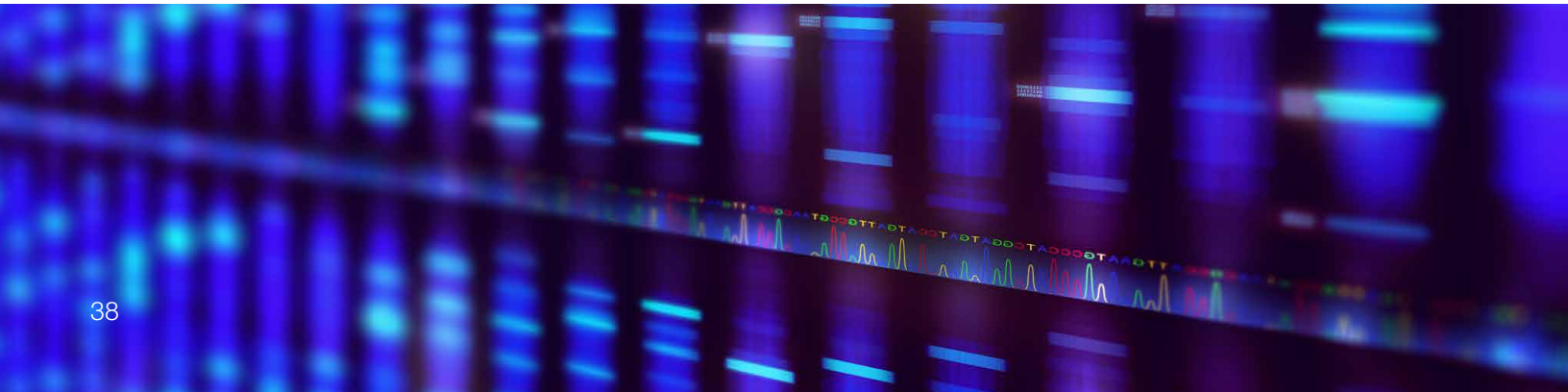
thermofisher.com/antibodies

Advanced Verification

Thermo Fisher Scientific is committed to adopting validation* standards for our Invitrogen antibody portfolio. The Advanced Verification badge is applied to products that have passed application and specificity testing. This badge can be found in the search results and at the top of the product specific web pages. Data supporting the Advanced Verification badges can be found in product specific data galleries.

thermofisher.com/antibodyvalidation

*The use or any variation of the word "validation" refers only to research use antibodies that were subject to functional testing to confirm that the antibody can be used with the research techniques indicated. It does not ensure that the product(s) was validated for clinical or diagnostic uses.



Perform 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

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.

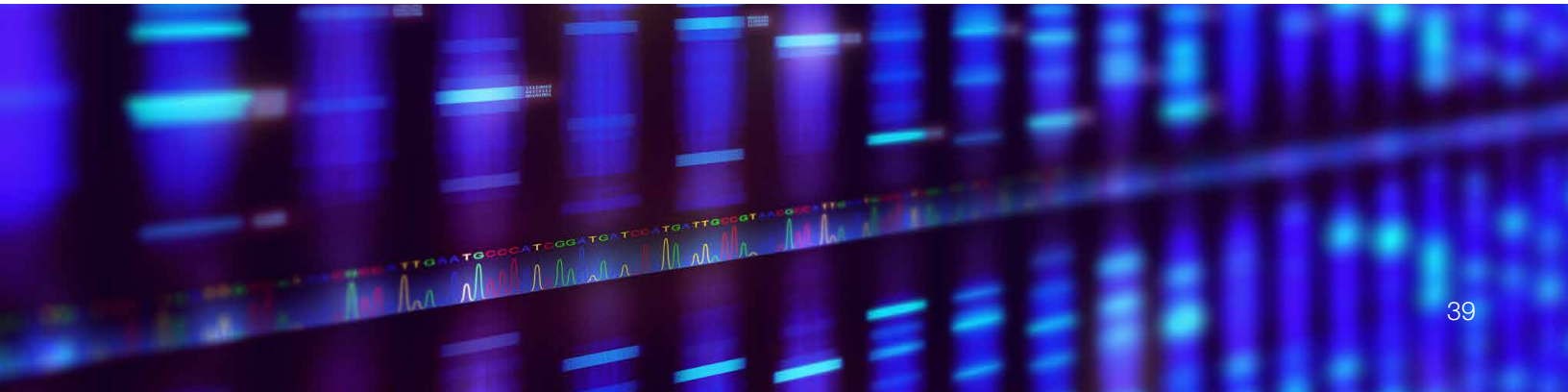
Gene expression analysis

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.

thermofisher.com/taqman



Custom engineering tools and designer cell lines

Collaborating as partners to accelerate your discovery

As the trusted, experienced developers of 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

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 TAL or CRISPR tools and your customer-supplied cell line. See our service packages in Table 6.

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			•

Timeline

Stable cell line engineering services can be completed in as little as 10 weeks, but will depend on the individual cell line growth characteristics and culturing requirements.

Figure 24. Cell line design and engineering process.

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 24).

Additional services available:

- Design and production of *in vitro*–transcribed (IVT) gRNA
- CRISPR-Cas9 nuclease vector with OFP or CD4 reporter
- LentiArray lentiviral gRNA with customized vector backbone
- Single-stranded oligo and double-stranded donor DNA template
- Cas9 stable cell lines
- Cas9-expressing iPSCs

invitrogen

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 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** or **800.955.6288**, option 4.

Learn more about our comprehensive resources for your genome editing needs at **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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Get true results at **thermofisher.com/genomeedit**

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