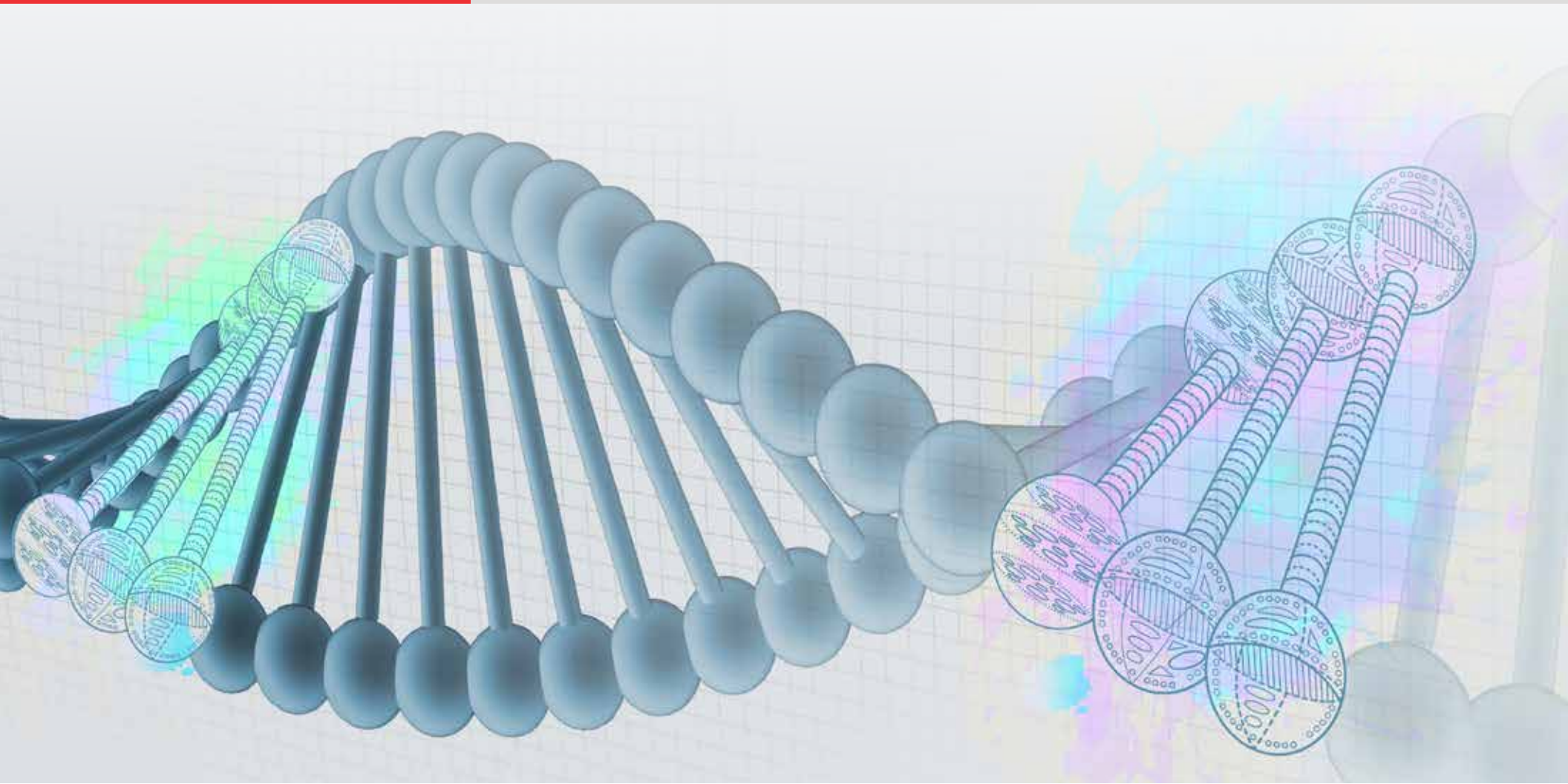


Genome editing resource guide

A complete cell engineering solution from start to discovery

ThermoFisher SCIENTIFIC

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 the trial-and-error phase and help you get answers faster and with less effort.

Contents

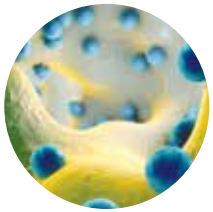
Cell engineering applications and workflow solutions

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

Genome editing for who you are

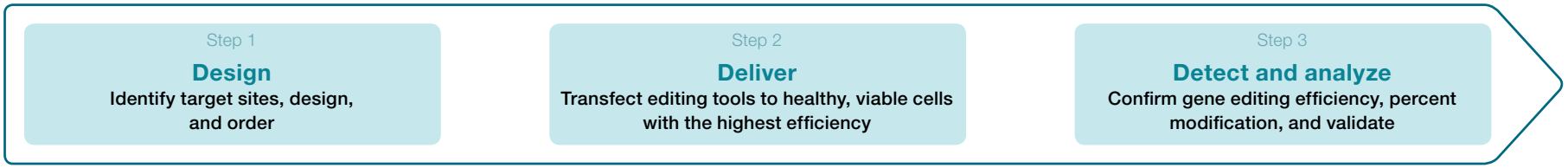
Every lab is unique, so we offer a range of genome editing solutions to cater to your needs. Whether you want results fast, seek full control over every step in designing your gene edit, or need help with engineering cells to your specific needs, we have solutions that fit. Your research. Your way.

Genome modulation and editing workflow

Get everything you need to design, deliver, and detect so you can engineer your cells—all from one place.

thermofisher.com/genomeedit

The cell engineering workflow



Genome editing tools

Minimizes the trial-and-error phase

Complete workflow-optimized and validated Invitrogen™ CRISPR and TAL effector editing toolset

Design

- More than 600,000 predesigned gRNAs available
- Custom gRNA design tool

Delivery

- Multiple delivery formats and matched transfection reagents for optimal efficiency

Detection

- Tools to help you identify and select your edited cells

GeneArt-Engineered Cell Models

Perfect for rapid hypothesis testing

- World's largest collection of ready-to-go, CRISPR-engineered cell lines
- Rapid made-to-order service available for custom genes
- Clonal cell lines available in as little as seven to ten business days

Custom cell line engineering services

Your desired edit in your model system

- Your edit engineered in virtually any mammalian cell line
- Engineered using CRISPR-Cas9 or TAL effector technology
- Stable pools available in as little as five weeks

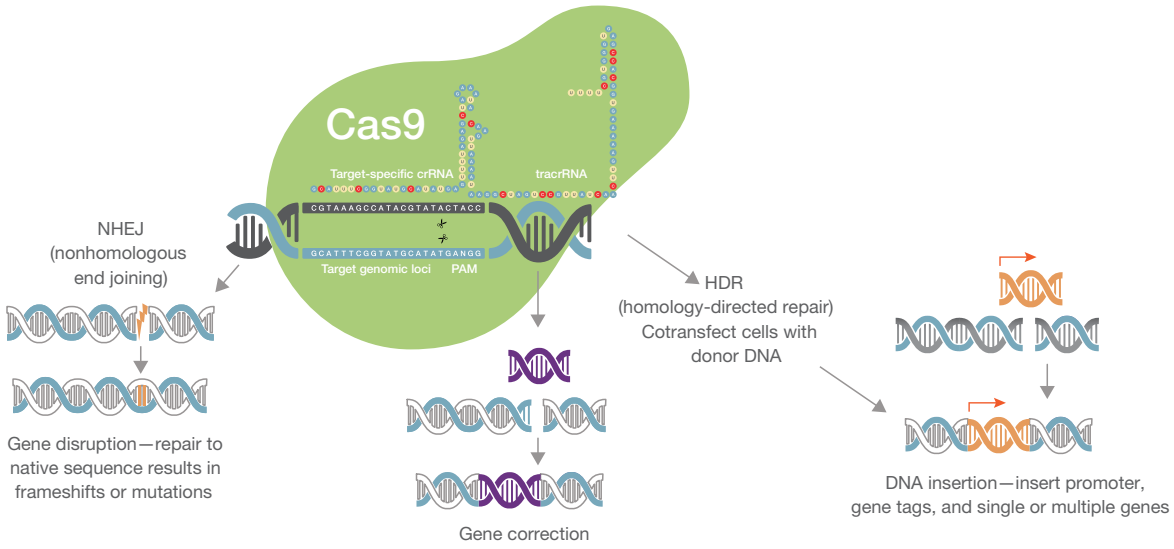
CRISPR-Cas9 technology

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. 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.

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 gRNA has two molecular components: a target complementary 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-strand break at the specific genomic target sequence (Figure 1). Following CRISPR-Cas9–induced DNA cleavage, the double-strand break can be repaired by the cellular repair machinery using either nonhomologous end joining or a homology-directed repair mechanism.

Support resources

New to CRISPR genome editing?
Learn more at
thermofisher.com/genomeedit101

Figure 1. A CRISPR-Cas9 targeted double-strand break. 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.

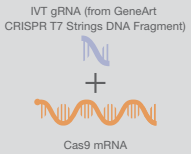
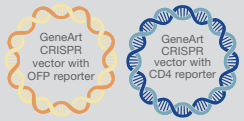
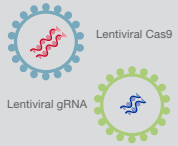
CRISPR-Cas9 products and services

A complete suite of GeneArt CRISPR-Cas9 genome editing tools

We offer a complete suite of genome editing reagents. Our online Invitrogen™ GeneArt™ CRISPR Search and Design Tool, along with GeneArt™ CRISPR-Cas9 editing products, are available in four formats: Cas9 protein, Cas9 mRNA, all-in-one expression vectors, and CRISPR lentiviral library services. These gene editing solutions are paired with optimal cell culture reagents, delivery methods, and analysis tools, based on your application and cell type.

Not sure which CRISPR-Cas9 format to use?

To view our selection guide, go to **thermofisher.com/genomeeditselect**

	CRISPR protein	CRISPR mRNA	CRISPR plasmid	CRISPR libraries
				
	GeneArt Platinum Cas9 Nuclease	**GeneArt CRISPR Nuclease mRNA**	**GeneArt CRISPR Nuclease Vector**	**CRISPR LentiArray Libraries**
gRNA design	Use the GeneArt CRISPR Search and Design Tool for optimal design and minimal off-target effects. Go to **thermofisher.com/crisprdesign**			
gRNA synthesis	GeneArt Precision gRNA Synthesis Kit	GeneArt Precision gRNA Synthesis Kit	DNA oligos cloned into plasmid	
Reporter-based enrichment	GeneArt Genomic Cleavage Selection Kit (sold separately)	GeneArt Genomic Cleavage Selection Kit (sold separately)	■ All-in-one expression plasmid (included)	
No promoter constraint	■	■	Cytomegalovirus (CMV) promoter	
Ready to use	■	■	Required cloning step	Ready-to-use lentiviral particles
No random integration concern	■	■	No, could be a concern	Stable expression of Cas9; lentivirus may randomly integrate
Controlled dosage	■	■		
Fast turnover	■	■		
Microinjection ready	■	■	Larger payload size	
Multiplexing and screening capable	■	■	Larger payload size	High-throughput screening
Ready-to-act, stable ribonucleoprotein (RNP) complex	■			
Modification options	Knockout and knock-in	Knockout and knock-in	Knockout and knock-in	Loss-of-function screening
Delivery method*	Lipofectamine CRISPRMAX reagent	Lipofectamine MessengerMAX reagent	Lipofectamine 3000 reagent	

* For the most efficient transfection of primary cells, stem cells, and difficult-to-transfect cells, use the Neon Transfection System.

GeneArt CRISPR Search and Design Tool

Instant access to over 600,000 predesigned CRISPR gRNAs

The GeneArt CRISPR Search and Design Tool allows you to search our database of more than 600,000 predesigned CRISPR gRNAs targeting human and mouse genes, or analyze your sequence of interest for *de novo* gRNA designs using our proprietary algorithms. Up to 25 gRNA sequences per gene are provided with recommendations based on potential off-target effects for each CRISPR sequence (Figure 2). Optimize your CRISPR design—quickly and easily.

thermofisher.com/crisprdesign

Figure 2. *BRCA2* gene search results. The results from a predesigned gRNA search are shown here for the *BRCA2* gene. Displayed are the top six CRISPR sequences with the top four recommendations based on predicted off-target binding sites that were calculated based on the number and position of mismatches to target.

Support resources

Download your copy of our Quick Reference Guide at **thermofisher.com/crisprdesign**

GeneArt Precision gRNA Synthesis Kit

A complete system for rapid *in vitro* synthesis of gRNA

The Invitrogen™ GeneArt™ Precision gRNA Synthesis Kit is a complete system for rapid synthesis of gRNA. Starting with two short, single-stranded oligos that code for the target sequence, the gRNA template is assembled with a T7 promoter in a single-tube PCR reaction. The assembled product is then used as a template in an *in vitro* transcription (IVT) reaction, followed by a rapid purification step yielding transfection-ready gRNA. The resulting gRNA can be cotransfected with Invitrogen™ GeneArt™ CRISPR Nuclease mRNA or GeneArt™ Platinum™ Cas9 Nuclease for high-efficiency cleavage of target (Figure 3). The GeneArt Precision gRNA Synthesis Kit offers:

- Fast synthesis of any gRNA in as little as four hours, including template assembly
- High yield (>10 µg) and concentration (>200 ng/µL) of gRNA

thermofisher.com/crispr

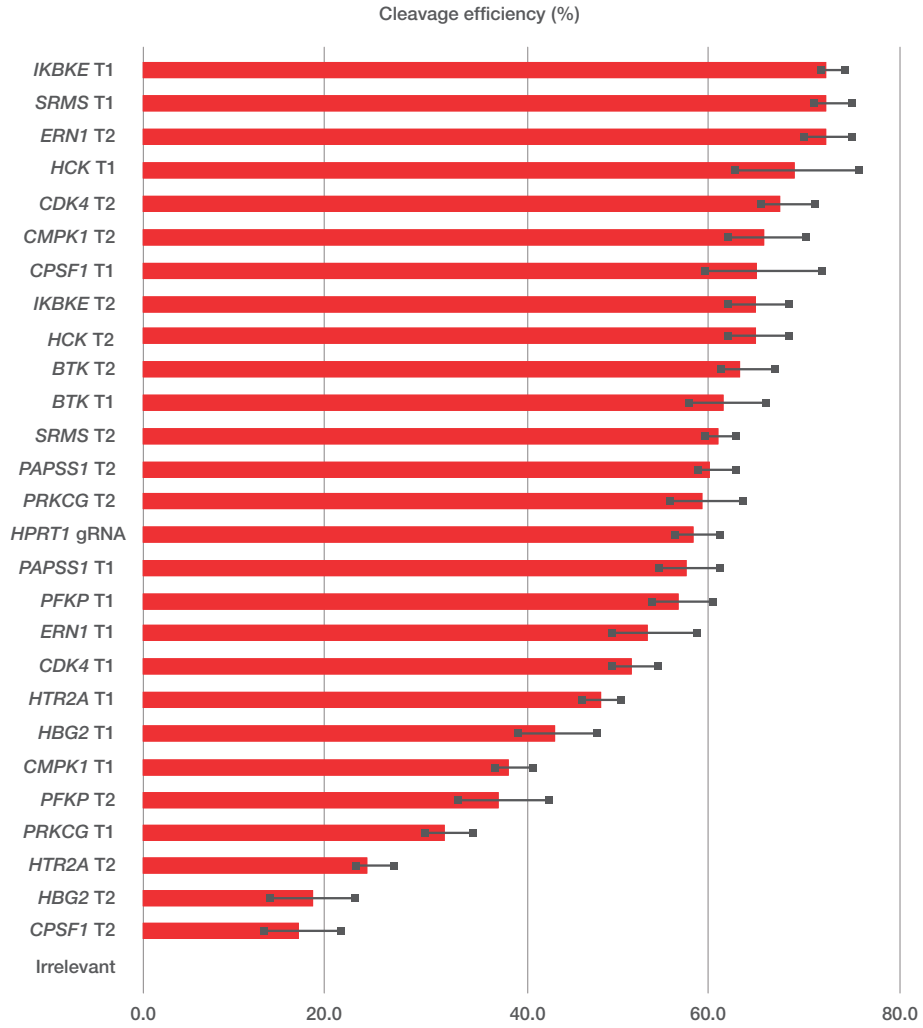


Figure 3. High-efficiency editing with the GeneArt Precision gRNA Synthesis Kit. Cleavage-efficiency data were generated from 13 different genes from the human kinome (two targets tested in each case), *HPRT1* gene target control (synthesized from the control oligo in the gRNA synthesis kit), and one irrelevant target (negative control). *In vitro*–synthesized gRNA was generated using a gRNA template and the GeneArt Precision gRNA Synthesis Kit. Following synthesis and purification, the gRNA was transfected into stable Cas9-expressing U2OS cell lines using Invitrogen™ Lipofectamine™ RNAiMAX™ Transfection Reagent. The Invitrogen™ GeneArt™ Genomic Cleavage Detection Kit was used to assay for double-strand breaks at 48 hours posttransfection. More than 70% of the targets from this list show greater than 50% cleavage efficiency. We designed two different targets using the GeneArt CRISPR Search and Design Tool (T1 and T2), and in each case we had successful target locus cleavage.

CRISPR protein

Maximum efficiency, minimal off-target cleavage

GeneArt Platinum Cas9 Nuclease is purified, wild-type Cas9 protein that is ready for cotransfection with gRNA. Cas9 protein and gRNA form a very stable ribonucleoprotein protein (RNP) complex that provides the next level of cleavage efficiency over CRISPR-Cas9 vector and mRNA systems.

Streamline cell engineering with preformed Cas9 protein–gRNA RNPs

The Cas9 RNP complex can act immediately after it enters the cell since transcription and translation are not required, and it is rapidly cleared from the cell, minimizing the chance for off-target cleavage events compared to vector-based systems (Figure 4). The Cas9 protein is microinjection-ready and has nuclear localization signals.

thermofisher.com/crisprprotein

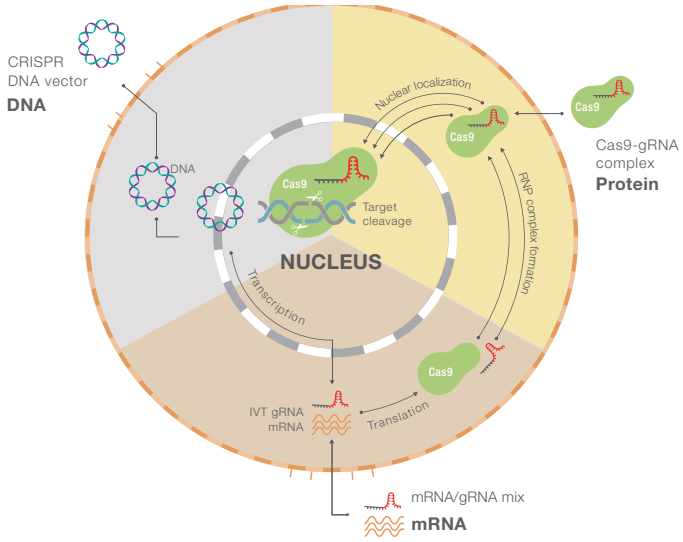


Figure 4. CRISPR protein delivery. Cas9 protein transfection that streamlines cell engineering by eliminating transcription and translation in the cell.

Design to analysis in less than 4 days

Step 1	Step 2	Step 3	Step 4
Prepare	**Make gRNA**	**Deliver gRNA and Cas9 to cells**	**Analyze editing efficiency**
• Design CRISPR targets • Order primers (next-day delivery) • Seed cells for transfection	• Assemble gRNA template • Perform IVT reaction • Purify gRNA	• Complex gRNA and Cas9 protein • Perform lipid transfection or electroporation • Incubate 48 hours	

Step 1: Prepare
Order oligos:
thermofisher.com/crisprdesign

Step 2: Make gRNA
- GeneArt Precision gRNA Synthesis Kit

Step 3: Deliver gRNA
- Neon Transfection System
- Lipofectamine CRISPRMAX Cas9 Transfection Reagent
- GeneArt Platinum Cas9 Nuclease

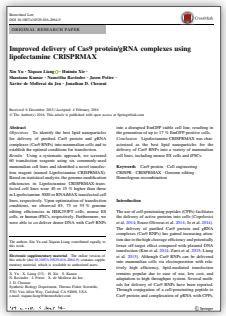
Step 4: Analyze
- GeneArt Genomic Cleavage Detection Kit

High editing efficiency demonstrated in over 20 cell lines

CRISPR-Cas9 RNP complexes can be introduced into cell lines by lipid-mediated transfection or electroporation, resulting in high cleavage efficiency of gene targets. Table 1 summarizes cleavage efficiency as measured by the percentage of insertions and deletions (indels) occurring in targets after either lipid-mediated delivery with Invitrogen™ Lipofectamine™ CRISPRMAX™ Cas9 Transfection Reagent or via electroporation with the Invitrogen™ Neon™ Transfection System. Both approaches led to successful genome editing in standard and difficult-to-transfect cell lines. Cleavage efficiency has been demonstrated in over 20 cell types, including iPSCs, mESCs, N2A, CHO, A549, HCT116, HeLa, HEK 293, and several others.

Table 1. Comparison of lipid-mediated transfection and electroporation of CRISPR-Cas9 RNP complexes and the resulting cleavage efficiencies (% indels) for 20 cell lines.

		% indels	
Cell type	**Cell type source**	**Lipofectamine CRISPRMAX Transfection Reagent**	**Neon Transfection System** (–) not tested
HCT116	Human colon carcinoma	85 ±5	–
293FT	Human kidney	85 ±5	88 ±3
mESC	Mouse embryonic stem cell	75 ±3	74 ±4
HEK 293	Human kidney	75 ±3	–
N2A	Mouse neuroblastoma	70 ±5	81 ±2
NIH/3T3	Mouse embryonic fibroblast	57 ±4	50 ±2
CHO-K1	Chinese hamster ovary	57 ±1	–
iPSC	Human induced pluripotent stem cell	55 ±3	85 ±2
U2OS	Human osteosarcoma	55 ±4	70 ±3
HeLa	Human cervical cancer	50 ±7	–
A549	Human lung carcinoma	48 ±3	66 ±3
COS-7	Monkey kidney	44 ±3	–
MDA-MB-231	Human breast cancer	39 ±5	–
HepG2	Human liver cancer	30 ±3	52 ±3
K562	Human lymphoblastoid	20 ±2	91 ±1
Jurkat	Human T cell leukemia	19 ±3	94 ±2
HEKa	Human primary epidermal keratinocytes	14 ±2	32 ±2
THP-1	Human monocytes	12 ±3	31 ±3
HUVEC	Human umbilical vein endothelium	9 ±3	26 ±2
MCF-7	Human mammary gland	8 ±4	22 ±5



Improved delivery of Cas9 protein/gRNA complexes using lipofectamine CRISPRMAX

For a complete set of data that illustrates cutting efficiencies by cell line and delivery method, download the publication “Rapid and highly efficient mammalian cell engineering via Cas9 protein transfection” at **thermofisher.com/crisprprotein**

CRISPR mRNA

High-efficiency CRISPR genome editing tools for multiplex editing

GeneArt CRISPR Nuclease mRNA is a wild-type Cas9 mRNA that has been capped and polyadenylated. This ready-to-transfect Cas9 mRNA circumvents the need for the time-consuming cloning steps needed when using CRISPR vector systems. Simply cotransfect Cas9 mRNA with IVT gRNA or a synthetic gRNA expression cassette (Figure 6). Following transfection, the Cas9 protein is directed by the gRNA to target a specific genomic locus. Genome editing results depend on setting the proper conditions for cells and transfection, but an example of results obtained by this approach in iPSCs is shown in Figure 7.

This system can also be used for multiplex editing. When multiple gRNAs are added, it allows multiplex genome editing of the corresponding target gene sequences simultaneously in a single transfection reaction. The system is versatile and simple to use, and changing target specificity only requires a change in the gRNA design.

thermofisher.com/crisprmRNA

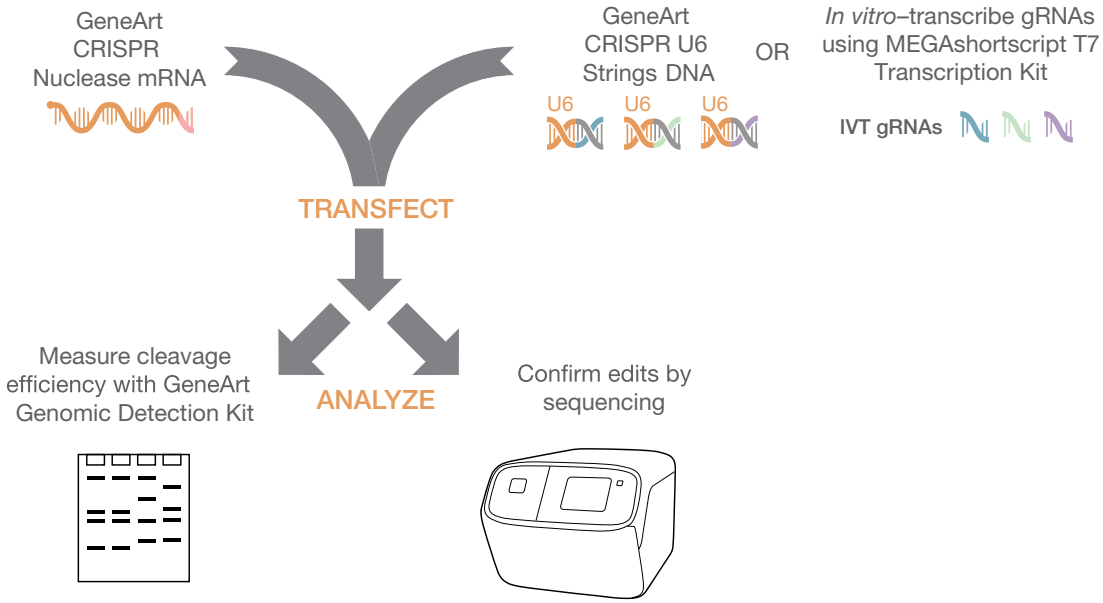


Figure 6. The CRISPR mRNA workflow.

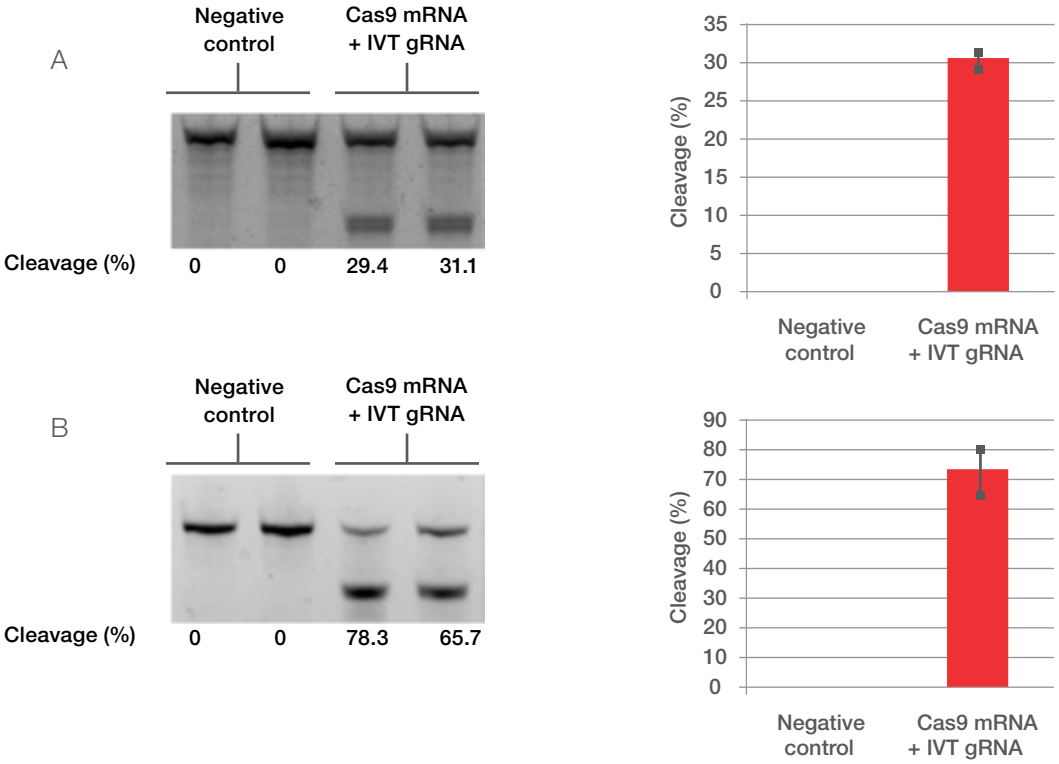


Figure 7. Cas9 nuclease mRNA functions robustly in iPSCs. GeneArt CRISPR Nuclease mRNA was transfected along with IVT gRNA using Invitrogen™ Lipofectamine™ MessengerMAX™ Transfection Reagent. The editing efficiency at the *HPRT1* locus was determined by a cleavage assay using the GeneArt Genomic Cleavage Detection Kit 72 hours posttransfection. **(A)** iPSCs grown in feeder-free conditions. **(B)** iPSCs grown on feeder cells and made feeder free prior to transfection. Transfection was performed in a 24-well format with 0.5 × 10^5 cells per well, 150 ng *HPRT1*-specific IVT gRNA and 500 ng Cas9 mRNA per well, and 1.5 µL Lipofectamine MessengerMAX reagent per well.

CRISPR plasmid

All-in-one expression vector system

The Invitrogen™ GeneArt™ CRISPR Nuclease Vector Kit offers an all-in-one expression vector consisting of both a Cas9 nuclease expression cassette and gRNA cloning cassette (Figure 8) for simple and efficient cloning of a double-stranded DNA oligo encoding a target-specific crRNA. This system allows you to edit and engineer a genomic locus of choice in a sequence-specific manner from a single plasmid in mammalian cells.

The system has a straightforward workflow for cloning and analysis (Figure 9), and offers a choice of orange fluorescent protein (OFP) and CD4 gene reporters for downstream enrichment of transfected cells expressing the Cas9 construct (Figure 10).

thermofisher.com/crisprvectors

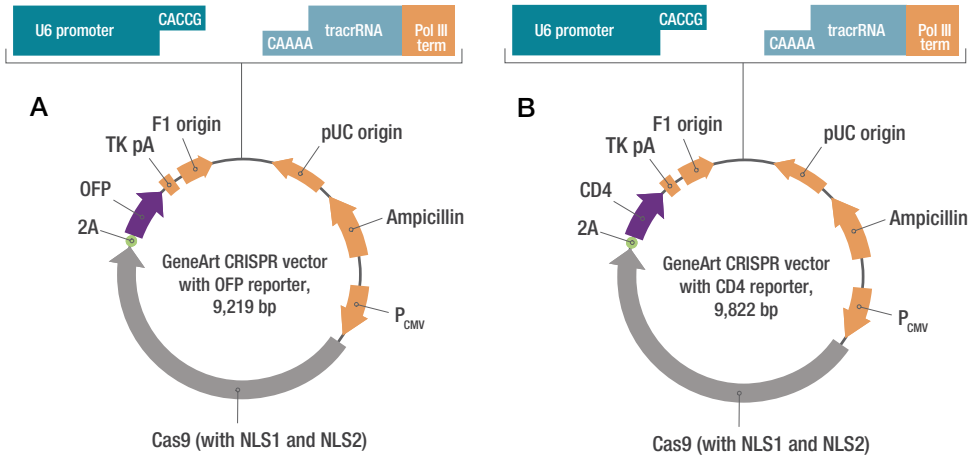


Figure 8. GeneArt CRISPR Nuclease Vector maps. The vector comes linearized with 5 bp overhangs for easy cloning of your double-stranded DNA oligo that encodes a target-specific crRNA. Maps are shown of the vectors with **(A)** OFP reporter and **(B)** CD4 reporter. OFP allows for fluorescence-based tracking of transfection efficiency as well as fluorescence-activated cell sorting and enrichment of edited cells. CD4 provides an option for magnetic bead–based sorting (FACS) and enrichment of edited cells. Expression of Cas9 and the reporter gene (either OFP or CD4) is driven by the CMV promoter. Cas9 is directed to the nucleus by nuclear localization signals (NLS1 and NLS2).

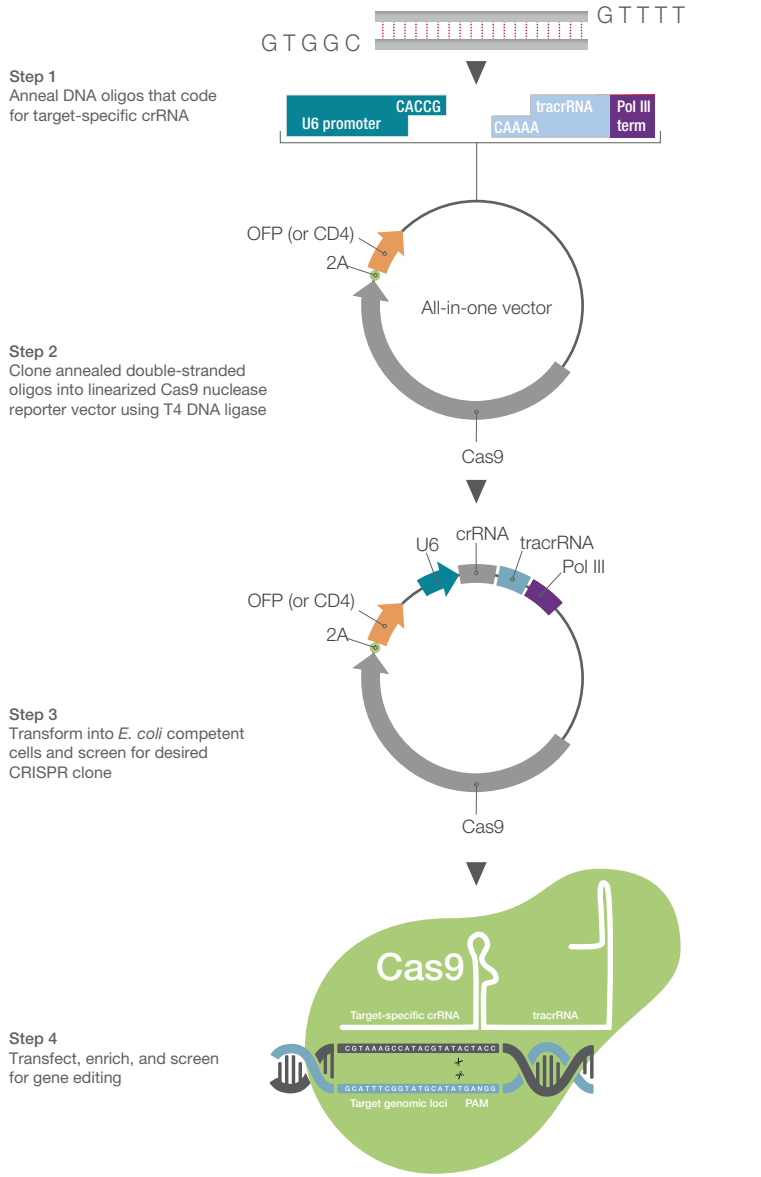


Figure 9. The cloning and analysis workflow for the GeneArt CRISPR Nuclease Vector Kit. After transfection, the samples can be analyzed for transfection efficiency based on the choice of reporter used or for cleavage efficiency using our GeneArt Genomic Cleavage Detection Kit (Cat. No. A24372), or can be enriched for the Cas9-expressing cell population using the OFP and CD4 reporters.

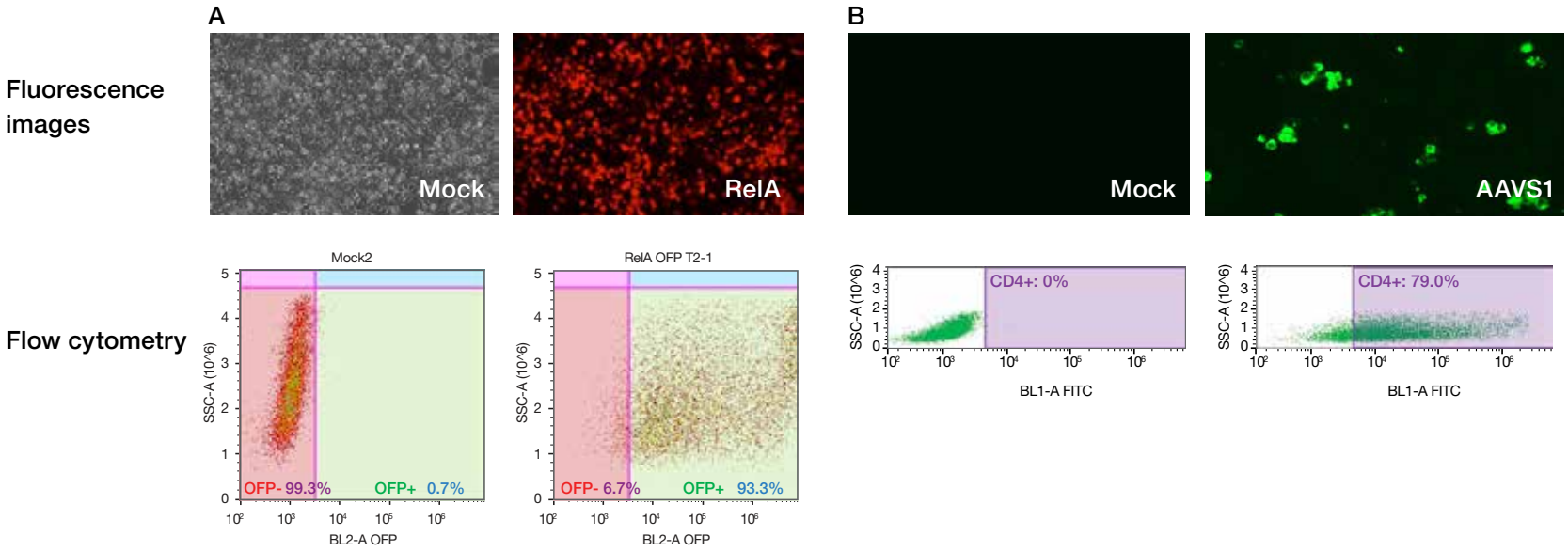


Figure 10. Enrichment of the Cas9-expressing cell population. (A) Transfection efficiency in 293T cells using the GeneArt CRISPR Nuclease OFP Vector encoding crRNA specific for the RelA locus. Data shows >90% OFP-positive cells in transfected samples. **(B)** CD4 functionality for the GeneArt CRISPR Nuclease CD4 Vector. 293FT cells were transfected with AAVS1-specific GeneArt CRISPR Nuclease Vector with CD4 reporter. Cells were harvested and stained with anti-CD4 FITC antibody and analyzed by flow cytometry FACS to measure transfection efficiency. A portion of the stained cells was also seeded on a plate for analysis by fluorescence microscopy.

Support resources

Check out our
Learning Support Center at
thermofisher.com/crispr101

CRISPR libraries

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.

LentiArray CRISPR libraries

New libraries. New capabilities. New discoveries.

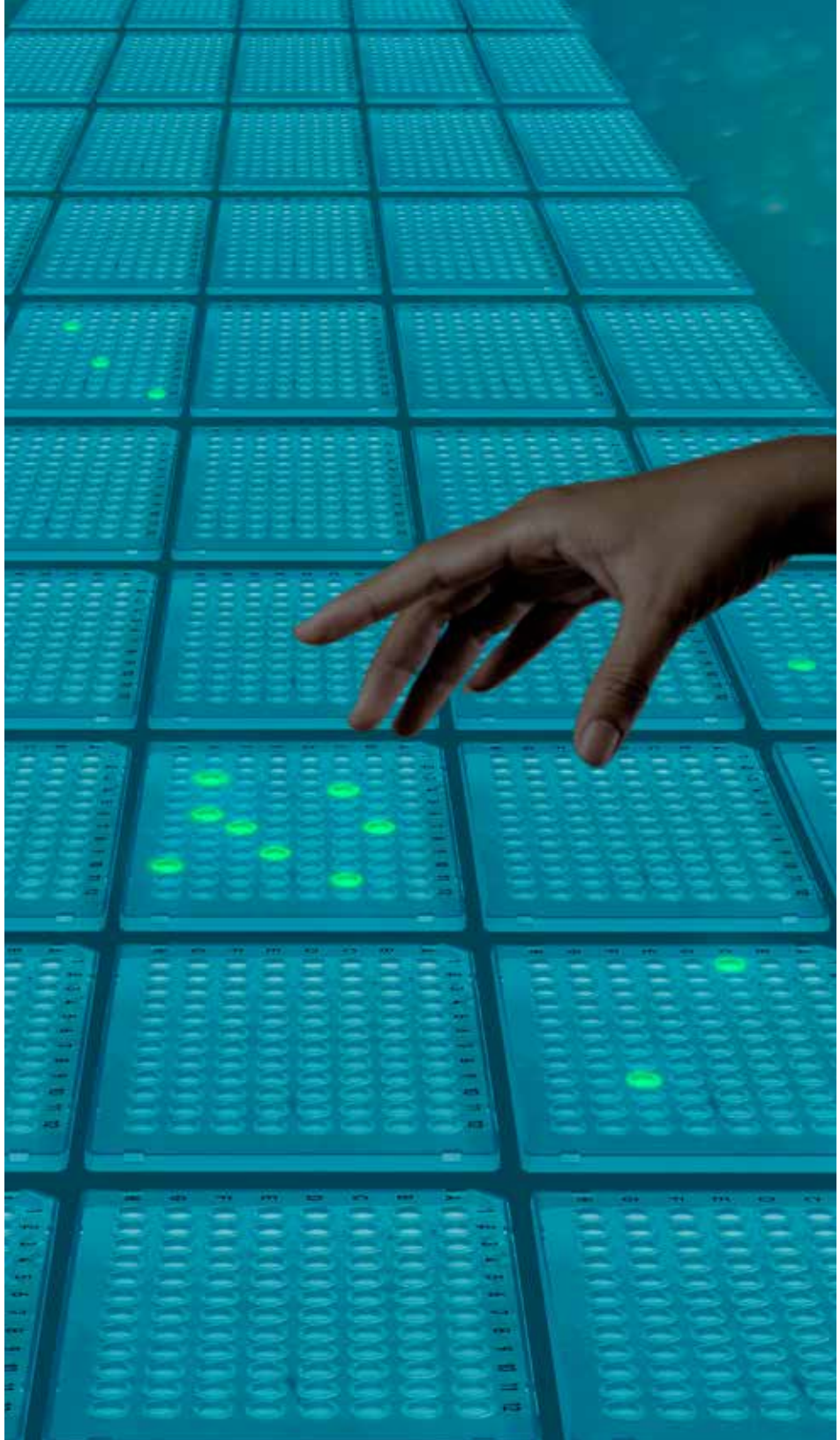
Introducing the Invitrogen™ LentiArray™ CRISPR library product line: a suite of tools that apply the power of CRISPR-Cas9 technology to high-throughput functional genomics screening. CRISPR-Cas9 provides an efficient method for specific, complete, and permanent gene knockout, making it a potent tool for new discoveries about gene function. LentiArray libraries help enable you to utilize breakthrough CRISPR-Cas9 technology to rapidly interrogate thousands of genes, and to 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 compatible with your existing high-throughput screening infrastructure and have been designed and constructed to provide a flexible system that doesn't impose any limitations on your assay design and research goals. The LentiArray library product line provides all the tools you need to expand your screening capabilities with CRISPR-Cas9 technology and help you make your next big discovery.

LentiArray CRISPR libraries feature:

- Advanced gRNA designs for maximum knockout efficiency without sacrificing specificity
- Up to 4 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 prescreen assay development and rapid postscreen hit validation
- 19 defined libraries and custom options available, enabling you to focus on defined gene sets or perform unbiased whole genome surveys

For more information, go to **thermofisher.com/lentiarraylibraries**



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 11), 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).

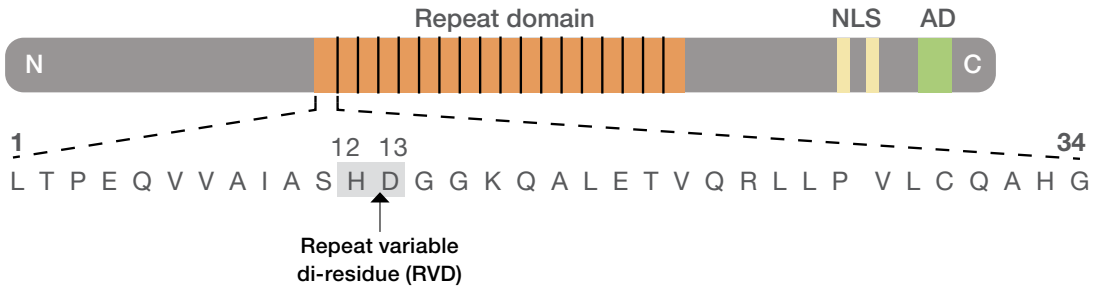


Figure 11. TAL effector DNA-binding domain. The structure of the DNA-binding domain can be manipulated to produce a protein domain that binds specifically to any DNA sequence in the genome. TAL effector repeats can be assembled modularly, varying the RVDs to create a TAL protein that recognizes a specific 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**

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 delivery of effectors with different functionalities, including nucleases, activators, repressors, chromatin modifiers, genomic labels, and crosslinking molecules (Table 2).

Based on your research needs, you can select from two formats of TAL effector tools: Invitrogen™ GeneArt™ Precision TALs or GeneArt™ PerfectMatch TALs (Figure 12). 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 13.

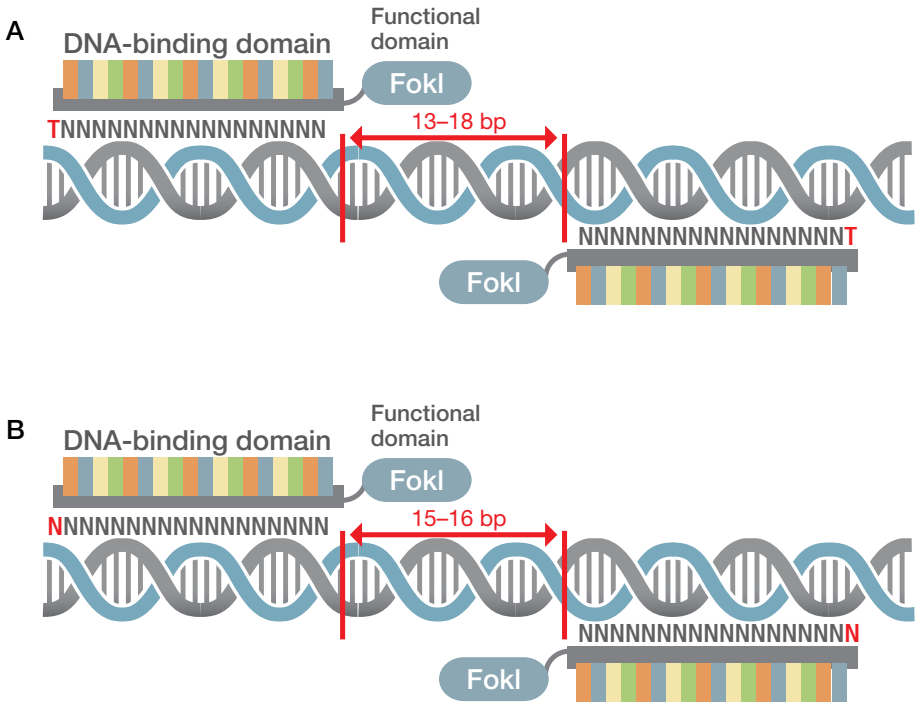


Figure 12. Designing target sites for maximal binding of customized TAL effectors.
(A) GeneArt Precision TALs encode a DNA-binding protein specific to a customer-submitted sequence, fused to a FokI nuclease domain for genome editing. The sequence targeted by our first-generation TAL effectors must have 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-strand 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.

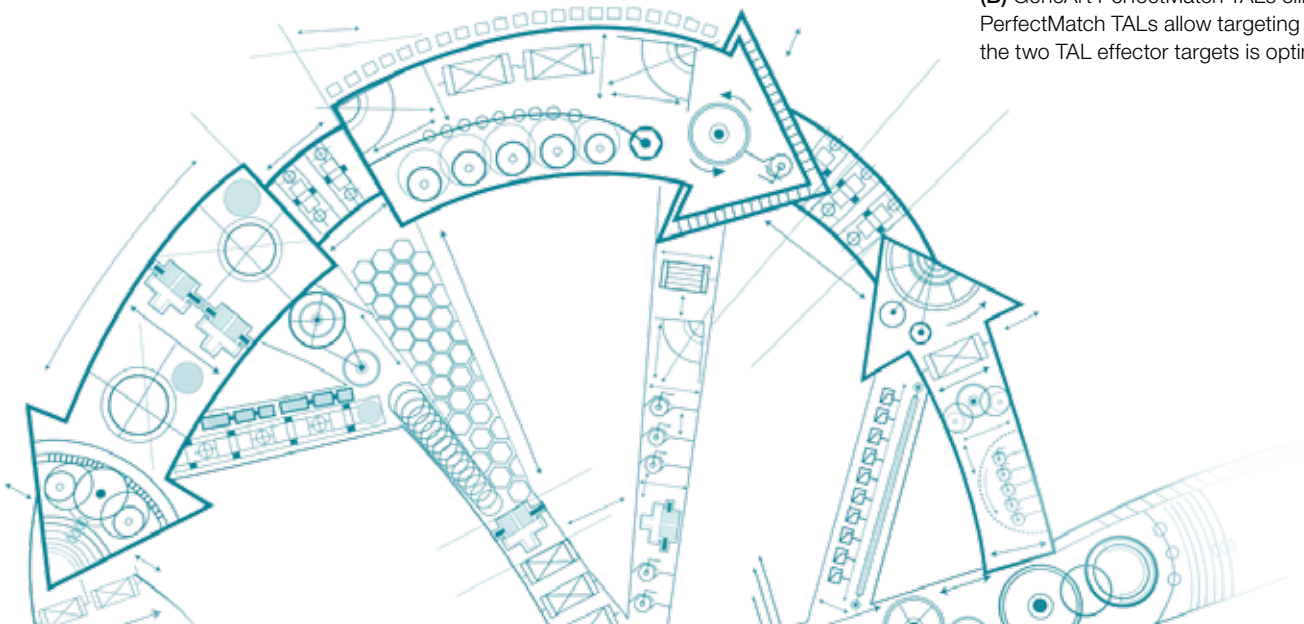


Table 2. TAL effector domains and their applications.

Product	Effector domain	Functionalities	Applications
GeneArt PerfectMatch TAL	FokI endonuclease	Gene targeting (truncated TAL)	Silencing Gene editing (e.g., introduction of SNPs, incorporation of exogenous DNA)
	FokI endonuclease with CMV promoter	Gene targeting in mammalian systems (truncated TAL)	Silencing Gene editing (e.g., introduction of SNPs, incorporation of exogenous DNA)
	FokI endonuclease	Gene targeting in plant systems (native TAL)	Silencing Gene editing (e.g., introduction of SNPs, incorporation of exogenous DNA)
GeneArt Precision TAL	VP16 activator	Activation of transcription (native TAL VP16)	Increasing expression of endogenous gene isoforms
	VP64 activator	Activation of transcription (native TAL VP64)	Increasing expression of endogenous gene isoforms
	KRAB repressor	Epigenetic repression of transcription (TAL repressor)	Heritable knockdown of gene expression
	Multiple cloning site (MCS)	Steric repression and custom design (modified TAL MCS)	Transient knockdown of gene expression Targeting any locus in the genome with the effector domain of your choice
	Prevalidated FokI endonuclease	*LRRK2* gene targeting (truncated TAL)	Correction of *LRRK2* mutation, linked to Parkinson's disease, to wild type

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**. For GeneArt PerfectMatch TALs, you can download and complete the TAL order form at **thermofisher.com/tals** and email it to **geneartsupport@lifetech.com**

To order, follow these three simple steps:

1. Select the functionality (effector domain) of your TAL from Table 2.
2. Select the product name from Table 2.
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 **geneartsupport@lifetech.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**

Superior performance of GeneArt PerfectMatch TALs

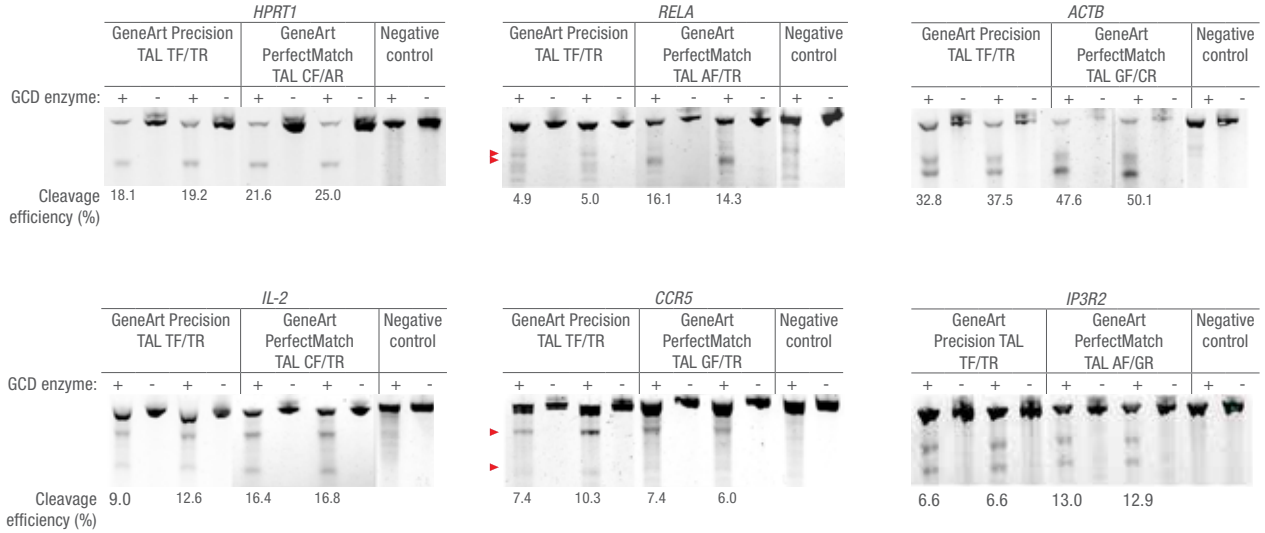


Figure 13. Cleavage efficiencies of GeneArt PerfectMatch TALs compared to GeneArt Precision TALs. To determine the functionality of GeneArt PerfectMatch TALs, we compared the genome cleavage efficiencies of GeneArt PerfectMatch TALs to those of GeneArt Precision TALs at specific loci using the GeneArt 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.

Support resources

Visit our designer TALEN learning center at **thermofisher.com/TALEN101**

Pre-engineered cell lines and engineering services

Designer cell lines to accelerate your research

Even with advanced genome editing tools it can take time to isolate and validate edited clones. To help ensure you have what you need to get your results faster, we now offer both off-the-shelf engineered cell lines and custom design and cell engineering services. With our broad, integrated cell engineering workflow solution, we bring together the power of cutting-edge science from multiple disciplines to create a single source for genome engineering tools and engineered cell lines.

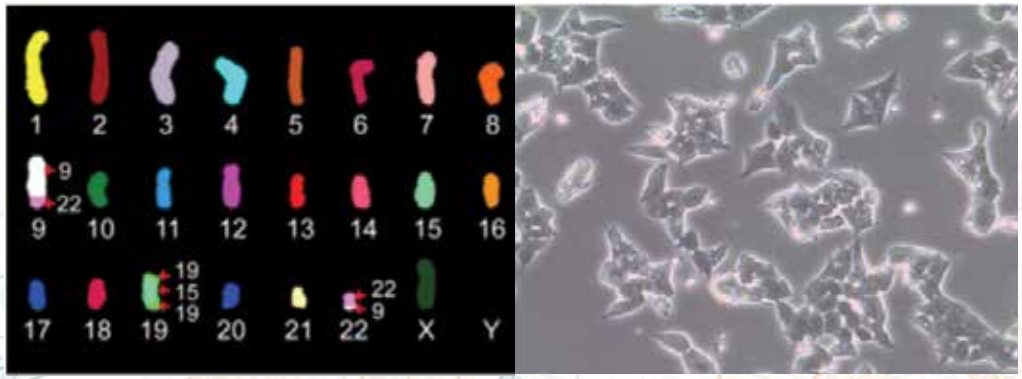


Figure 14. Spectral karyotyping and bright-field imaging of HAP1 cells. HAP1 cells have defined copy number and unambiguous genotyping.

GeneArt Engineered Cell Models

Contemporary workhorse cell lines for genome editing

Get fast access to the world's largest collection of ready-to-go cell lines engineered using CRISPR-Cas9 technology. The Invitrogen™ GeneArt™ Engineered Cell Models collection offers a superior resource for functional characterization of genes and their role in cellular processes, signaling pathways, disease development and progression, and drug response. With more than 2,000 cell lines on the shelf and ready to ship, along with a rapid made-to-order service, the GeneArt Engineered Cell Models collection can provide the cell lines you need to move your research forward.

Traditional cell-based assays compare patient cells with cells from healthy individuals, which can differ genetically in thousands of ways. GeneArt Engineered Cell Models are provided as an isogenic pair, where the unedited control cells and the edited cell lines differ only by the mutation you wish to test, enabling greater confidence in your results. Now you can transition from genomic information to functional characterization faster than ever before—accelerate your research with GeneArt Engineered Cell Models.

thermofisher.com/engineeredcells

Cell line engineering service

Collaborating as partners to accelerate your discovery

As the trusted, experienced developers of GeneArt TAL and GeneArt 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

Stable pool generation

Service packages	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

Service packages	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 FACS 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 the table at left.

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.

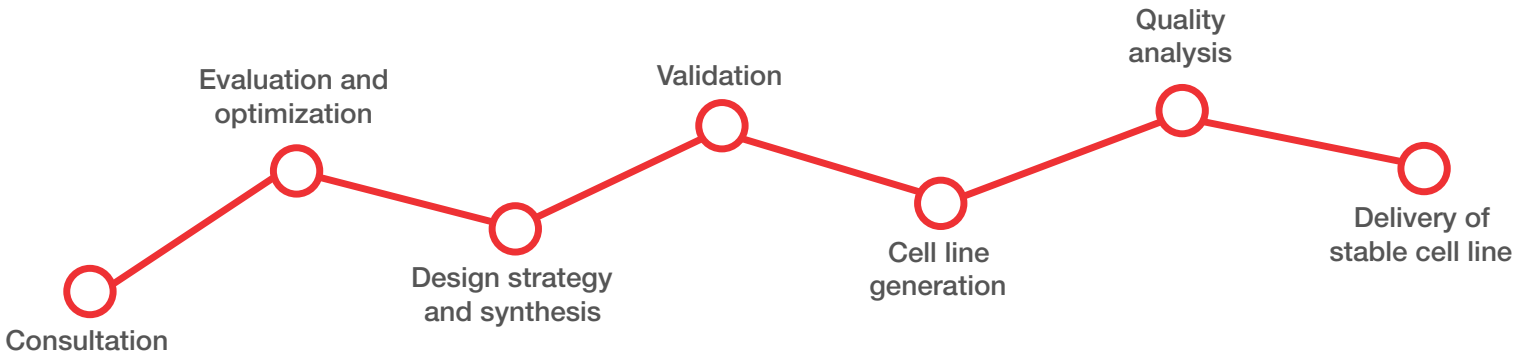


Figure 15. Cell line design and engineering process.

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.

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

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 two types of small RNAs that function in RNAi: short interfering RNA (siRNA) and microRNA (miRNA). We offer products for RNAi analysis *in vitro* and *in vivo*, including 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 small interfering RNAs (siRNAs). Invitrogen™ *Silencer*™ Select siRNA products (Table 3) 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

Table 3. 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 and noncoding RNA
Target species	Human, mouse, rat (use custom tool for 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 GeneArt™ optimized gene synthesis for siRNA-resistant genes that can be used in RNAi rescue experiments.

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, 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 **RNAiLibraries@lifetech.com** or go to:

thermofisher.com/sirnalibraries

*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 our predefined and custom miRNAs libraries, contact us at **RNAiLibraries@lifetech.com** or go to: **thermofisher.com/mirna**

*mir*Vana miRNA mimics and inhibitors

For artificial regulation of target mRNA translation, *mir*Vana 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. *mir*Vana miRNA mimics and inhibitors have been validated with Lipofectamine RNAiMAX Transfection Reagent for use in cell-based systems, and with Invitrogen™ Invivofectamine™ 2.0 Reagent for *in vivo* delivery. *In vivo*–ready *mir*Vana miRNA mimics and inhibitors have been purified by HPLC and dialysis, making them ready for immediate use. *mir*Vana miRNA mimics and inhibitors are:

- **Versatile**—functionally study specific miRNAs using *in vitro* or *in vivo* systems
- **Potent**—validate miRNA regulation of gene expression with minimal off-target effects (Figure 16)
- **High throughput–compatible**—generate libraries for effective screening of multiple miRNAs simultaneously
- **Current**—content is regularly updated based on the miRBase—an miRNA database

thermofisher.com/mirna

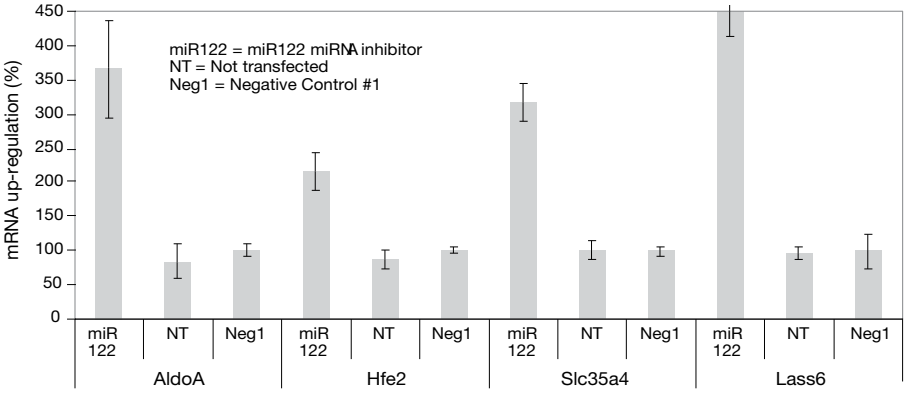


Figure 16. *mir*Vana miRNA inhibitors effectively suppress miRNA *in vivo*. miR122 or negative control *mir*Vana miRNA inhibitor was complexed with Invivofectamine 2.0 Reagent and delivered to BALB/c mouse liver via tail vein injection on 3 consecutive days at a dose of 7 mg per kg of body weight. Expression 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. This indicates 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.

Delivery | 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 includes 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 they 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

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 Lipofectamine™ family of reagents paired with the Neon Transfection System provides the 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 below to help you choose the solution that's right for you.

thermofisher.com/transfection

	Plasmid DNA	mRNA	Protein	Lentivirus
Lipofectamine CRISPRMAX reagent			■	
Lipofectamine 3000 reagent	■			
Neon transfection system	■	■	■	
Lipofectamine MessengerMAX reagent		■		
Viral delivery*				■

*Lipofectamine 3000 can be used to produce lentivirus.

To see what cell types we've tested, go to
thermofisher.com/crisprtransfection

Lipofectamine CRISPRMAX Cas9 Transfection Reagent

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 GeneArt Platinum Cas9 Nuclease. It's also gentle on cells (Figure 17) and cost-effective.

We deliver our superior GeneArt Platinum Cas9 Nuclease 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
- **Easily scalable**—an ideal delivery solution for high-throughput experiments

thermofisher.com/crisprprotein

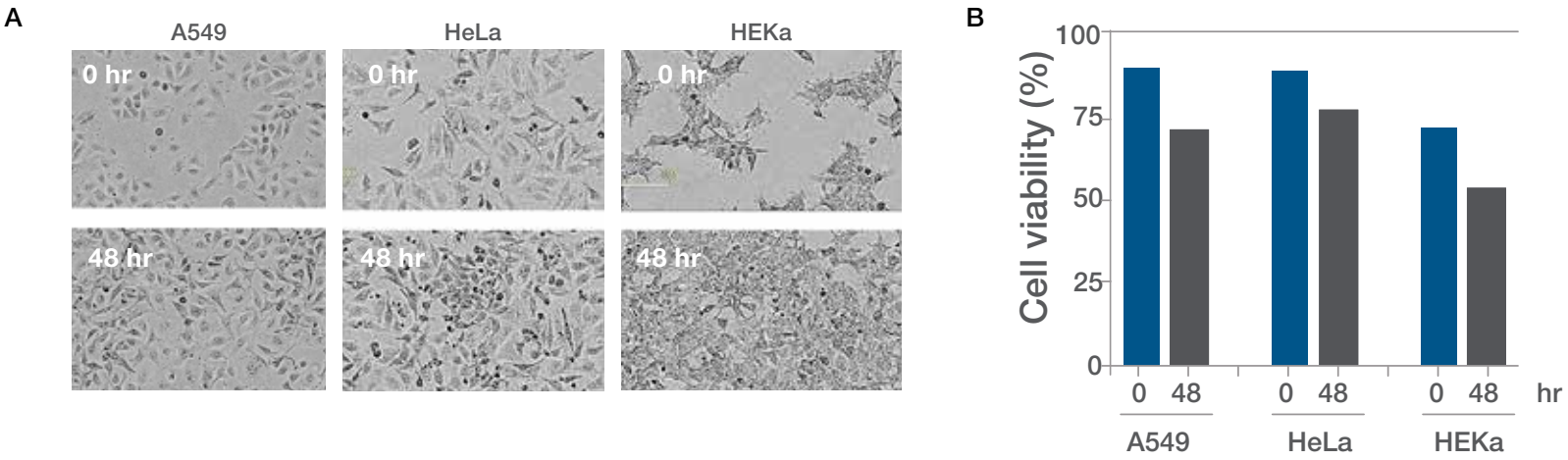


Figure 17. Low toxicity of Lipofectamine CRISPRMAX reagent on cultured cell lines. (A) Cell morphology captured from an Invitrogen™ EVOS™ XL Imaging System. **(B)** Viability remains high even after 48 hours of incubation.

Support resources

View transfection protocols at
thermofisher.com/transfectionprotocols

Download the Transfection and Genome Engineering Handbook at
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 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 18). High transfection and genome editing efficiency is also observed with GeneArt Precision TALs. These advancements in delivery help minimize painstaking downstream workflows, enable easier stem cell manipulation, and enhance site-specific insertion of transgenes into the genome.

thermofisher.com/3000

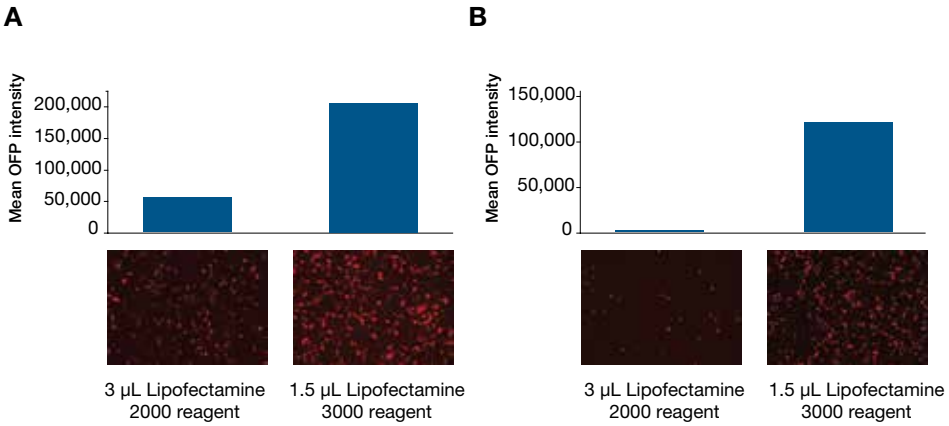


Figure 18. Transfection efficiency and protein expression using GeneArt CRISPR Nuclease Vector. The vector contained an orange fluorescent protein (OFP) reporter gene and was transfected with Invitrogen™ Lipofectamine™ 2000 or Lipofectamine™ 3000 Reagent into **(A)** U2OS and **(B)** HepG2 cell lines. 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

Lipofectamine MessengerMAX reagent helps increase the likelihood of cleavage and recombination with GeneArt CRISPR Nuclease mRNA through highly efficient transfection, maximizing the efficiency of genetic modifications and simplifying the downstream processes (Figure 19).

thermofisher.com/messengermax

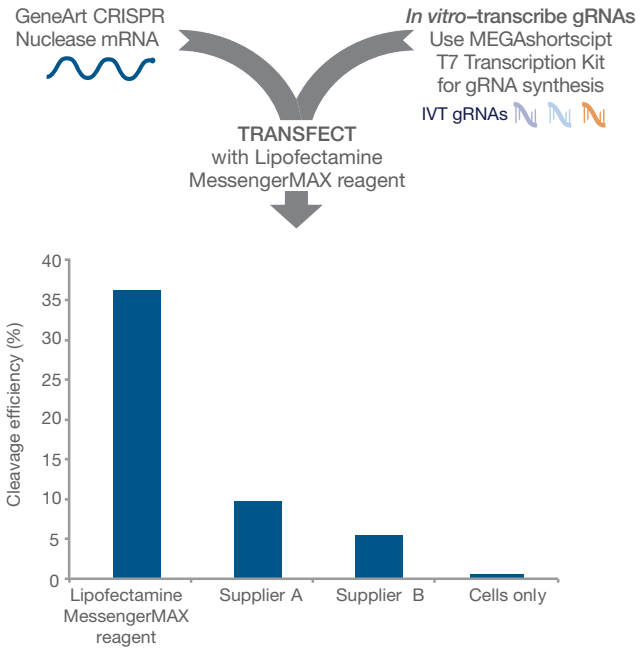


Figure 19. Up to 10x higher cleavage efficiency using GeneArt CRISPR mRNA and Lipofectamine MessengerMAX reagent. Lipofectamine MessengerMAX reagent and two leading mRNA delivery reagents were used to deliver the complete CRISPR format (Cas9 mRNA + IVT gRNA) targeting the *HPRT1* locus in Gibco™ iPSCs. 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 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, the flexible and open system allows you to perform high-quality transfections using optimized or user-defined protocols in three simple steps with as few as 2 × 10^4 cells per reaction. A 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 from 2 × 10^4 cells to 6 × 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 to be optimized freely

thermofisher.com/neon

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

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

EVOS cell imaging systems

Flow cytometry

The Invitrogen™ Attune™ NxT Flow Cytometer is a benchtop cytometer designed for fast, efficient multiparametric detection at the single-cell level. This revolutionary acoustic focusing technology aligns individual cells prior to laser interrogation. In combination with its clog-resistant design, 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. This allows for true walk-away screening of 96- and 384-well plates for the interrogation of tens of thousands of cells per second. The Attune NxT Flow Cytometer accommodates a variety of experimental protocols and labs with any budget with its customizable and field-upgradeable design, with up to four lasers and 14 colors for detection to meet some of your most challenging research needs.

thermofisher.com/attune

Attune NxT Flow Cytometer

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

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 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

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

Antibodies

Western workflow devices and reagents

Screening and validation

Essential tools for monitoring the efficiency of your genome editing experiments

When using genome editing tools such as the CRISPR-Cas9 system, TAL effectors, or zinc finger nucleases to obtain targeted mutations, you need to determine the efficiency with which these nucleases cleave the target sequence prior to continuing with labor-intensive and expensive experiments. We've developed a set of tools that will enable you to quickly determine which cells have been edited.

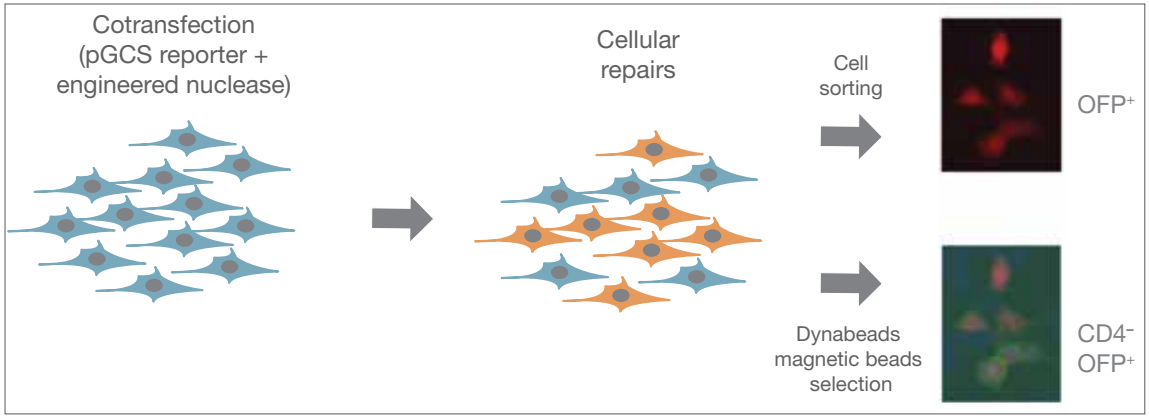


Figure 20. GeneArt Genomic Cleavage Selection Kit workflow.

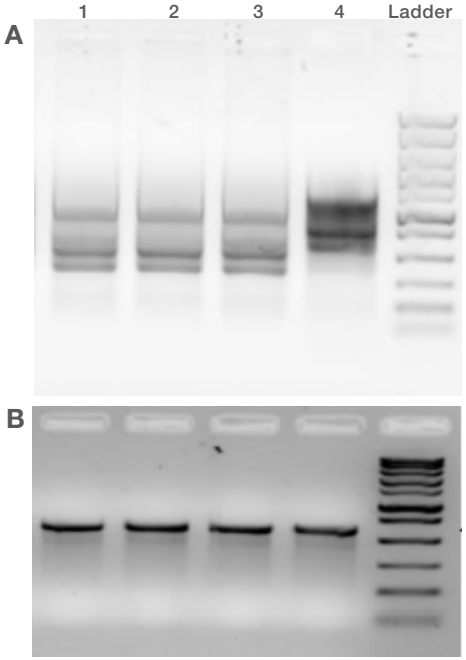


Figure 21. Comparison of the GeneArt Genomic Cleavage Detection Kit with another commercial kit. (A) With the other commercial kit, the expected 620 bp product is not observed on the gel; 10 µL of the 50 µL PCR was loaded on the gel. **(B)** The GeneArt Genomic Cleavage Detection Kit results show a visible and specific product at the expected 620 bp mark; 3 µL of the 50 µL PCR was loaded on the gel. Data courtesy of BioMarin Pharmaceuticals Inc.

GeneArt Genomic Cleavage Detection Kit

A quick, simple, and reliable method for detecting and quantifying locus-specific double-strand breaks

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-strand breaks have occured and resulted in indel introduction. The mismatches are recognized and cleaved by the detection enzyme. This cleavage is easily detectable and quantifiable by gel analysis (Figure 20). This approach is:

- **Easy**—with direct PCR amplification, there's no need for genomic DNA isolation
- **Rapid**—five-hour 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

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-strand 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 OFP gene for a quick visual check of the functionality of the engineered nuclease. In addition, the reporter genes OFP and CD4 can be used to enrich for edited cells (Figure 21). 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

Genome sequencing

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.

De novo sequencing solutions

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.

Genome sequencing for all

Fast and affordable genome sequencing is accessible with the semiconductor sequencing technology in the Ion S5 systems, empowering researchers to sequence economically important species, large or small. The system offers:

- Chips with 60–80 million reads—ideal for exomes, transcriptomes, and genotyping by sequencing
- High accuracy
- 2–4 hour sequencing run time

thermofisher.com/ions5

Genotyping

Genetic analysis tools for validating genome editing experiments

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

Detecting known and unknown changes in single genes by digital PCR

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 detection and contamination assessment

thermofisher.com/digitalpcr

Detecting changes or variants in one or multiple genes

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—and everything in between. 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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Nucleic acid isolation

Gene editing requires careful sample preparation and isolation of RNA, plasmid DNA, and other materials that will be used at various steps in the selected workflow.

RNA isolation

RNA isolation is a crucial step in your CRISPR editing journey. Be confident that you're getting started on the right foot with solutions that enable you to:

- Isolate from any sample type
- Obtain high-purity, intact RNA
- Achieve high yields, even from small sample quantities

Starting with high-quality, pure, and intact total RNA is critical to successful genome editing. Table 4 will help you choose the right product to purify total RNA from your specific sample type and sample size. For your convenience, we also offer an online kit selection guide.

thermofisher.com/rnaselection

Essentials for working with RNA

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

Table 4. Selection guide for RNA isolation and analysis products.

	TRIzol reagents Process a large amount of tissue	**PureLink kits** Fast isolation of RNA from a variety of samples	**MagMAX kits** High-throughput purification of RNA and DNA	**Cells-to-C_T kits** Process cells for gene expression analysis
Prep time	60 min	<20 min	45 min	10 min
Sample types	Most samples, particularly more difficult to lyse	Bacteria, liquid, blood, cells, yeast, plants, tissue	Cells, blood, plants	Cultured cells
Starting material	100 mg of tissue or 10^7 cells	10^8 cells, 200 mg of tissue, 250 mg of plant, 0.2 mL of blood, 5 x 10^8 yeast, 10^9 bacteria	100 mg of tissue or 5 x10^6 cells	1–100,000 cells
Yield	1 x 10^6 epithelial cells: 8–15 µg; tobacco leaf: 73 µg	Up to 350 µg	Variable, depending on sample	NA
High-throughput compatible	—	Yes	Yes	Yes
Technology	Organic extraction	Silica membrane spin column or filter plate	Magnetic beads	Crude lysate

Nuclease-free water

Preparing reagents and resuspending precipitated RNA with the appropriate grade water is a crucial and often ignored first step for ensuring consistent experimental results. Even purified water can have a high pH and minerals that can interfere with certain types of reactions. We offer several grades of nuclease-free water—diethylpyrocarbonate (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

It's safe to assume that most laboratory surfaces are contaminated with RNases, since they're exposed to the bacteria, fungi, flaked skin, and hair present in the environment. Unfortunately, even trace quantities of RNases can lead to lower yields from IVT reactions, degradation during RNA purification protocols, and variable results with qRT-PCR. Fortunately, a suite of trusted products proven effective at removing RNase contamination from lab surfaces is available, including Invitrogen™ RNaseZap™ Decontamination Solution and Invitrogen™ RNase *AWAY*™ Decontamination Reagent.

thermofisher.com/surfacedecontamination

Sample stabilization

In order to isolate high-quality RNA, the tissue has to be either processed immediately after harvest, snap-frozen, or stabilized in an intermediary solution to preserve RNA integrity and allow for storage. We offer several Invitrogen™ RNA*later*™ products designed specifically to stabilize and preserve the quality of RNA either at the point of collection or even post-collection.

thermofisher.com/stabilizeRNA

Plasmid DNA isolation

Sufficient yields of plasmid DNA free of impurities such as endotoxin, protein, RNA, or genomic DNA contamination is key in genome editing workflows. Plasmid purification kits are available in the full range of purity grades to support your CRISPR workflow at every step from cloning to transformation to transfection. Table 5 will help you choose the right products to purify plasmid DNA for your application.

thermofisher.com/plasmidprep

Did you know you could automate nucleic acid isolation?

Learn how at **thermofisher.com/kingfisher**

Table 5. Selection guide for plasmid isolation products.

	GeneJET Plasmid DNA Purification Kits Rapid isolation of high-quality DNA for molecular biology applications	**PureLink HiPure Plasmid Purification Kits** Simple, low-endotoxin, transfection-grade plasmid DNA	**PureLink Expi Endotoxin-Free Plasmid Purification Kits** Endotoxin-free plasmid DNA in about half the time
Purity grade	Molecular	Transfection	Advanced transfection
Prep size	Mini–Maxi	Mini–Giga	Mega–Giga
Protocol time	15–60 min	90–120 min	120 min
Yield	20 µg–1,000 µg	20 µg–15 mg	5 mg–15 mg
Throughput	Medium	Low	Medium
Technology	Silica membrane	Anion exchange membrane	Anion exchange membrane

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.

gemservices@thermofisher.com

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.

thermofisher.com/genomeeditsupport

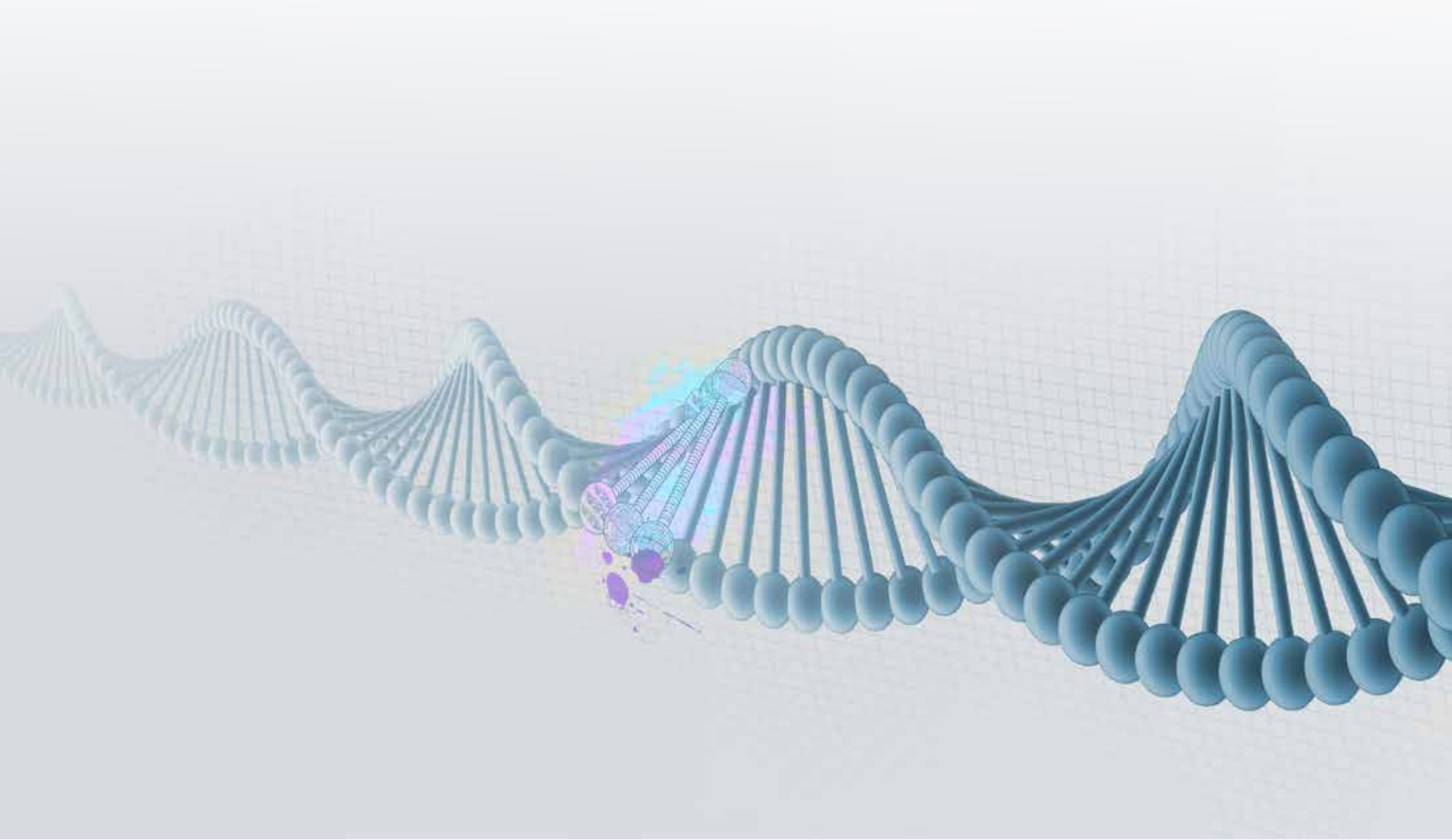
Questions? Ready to get started?

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**

Ordering information

Product	Quantity	Cat. No.
CRISPR protein		
GeneArt Platinum Cas9 Nuclease (1 µg/µL)	10 µg	B25642
GeneArt Platinum Cas9 Nuclease (1 µg/µL)	25 µg	B25640
GeneArt Platinum Cas9 Nuclease (3 µg/µL)	75 µg	B25641
CRISPR mRNA		
GeneArt CRISPR Nuclease mRNA	15 µg	A29378
GeneArt Strings U6 DNA	>200 ng	Contact: **geneartsupport@lifetech.com**
GeneArt Strings T7 DNA	>200 ng	
Custom *in vitro*–transcribed gRNA	250 nmol	
CRISPR plasmid		
GeneArt CRISPR Nuclease Vector with OFP Reporter Kit	10 reactions	A21174
GeneArt CRISPR Nuclease Vector with OFP Reporter Kit (with competent cells)	10 reactions	A21178
GeneArt CRISPR Nuclease Vector with CD4 Enrichment Kit	10 reactions	A21175
GeneArt CRISPR Nuclease Vector with CD4 Enrichment Kit (with competent cells)	10 reactions	A21177
CRISPR gRNA synthesis		
GeneArt Precision gRNA Synthesis Kit	25 reactions	A29377
Detection and analysis reagents		
GeneArt Genomic Cleavage Detection Kit	20 reactions	A24372
GeneArt Genomic Cleavage Selection Kit	10 reactions	A27663
Transfection reagents and instruments		
Lipofectamine CRISPRMAX Cas9 Transfection Reagent	75 reactions	CMAX00008
Lipofectamine 3000 Transfection Reagent	1.5 mL	L3000015
Lipofectamine 2000 Transfection Reagent	1.5 mL	11668019
Lipofectamine RNAiMAX Transfection Reagent	1.5 mL	13778150
Lipofectamine MessengerMAX Transfection Reagent	1.5 mL	LMRNA015
Neon Transfection System Starter Pack	1 pack	MPK5000S



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

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