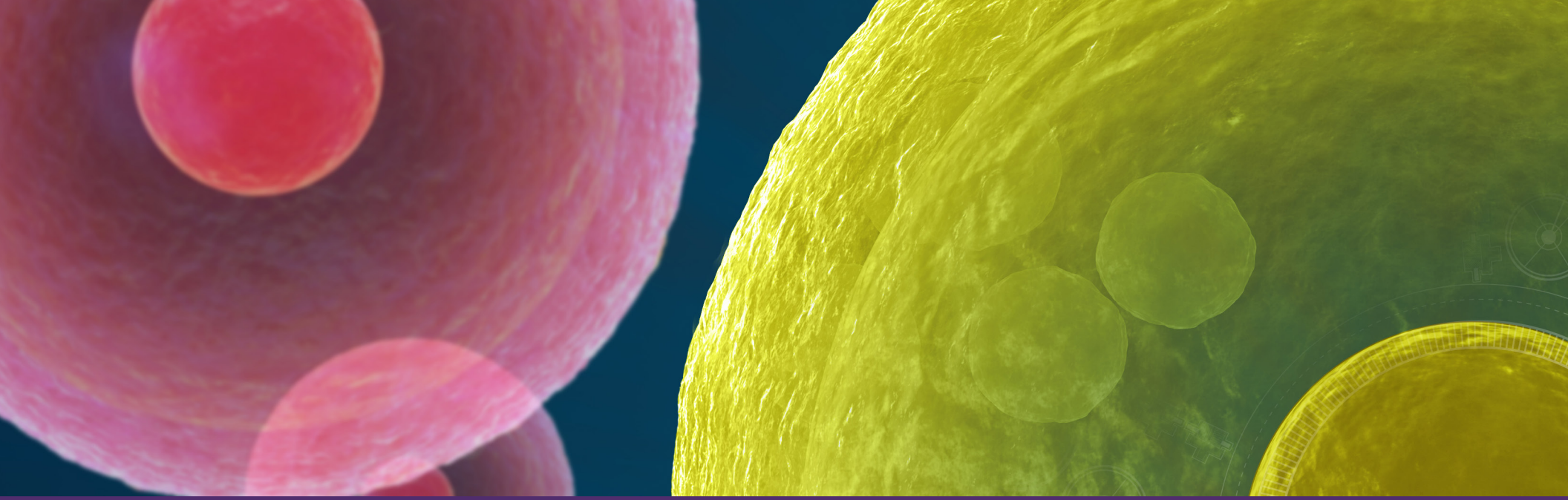
invitrogen

Screening solutions resource guide

New libraries. New capabilities. New discoveries.

ThermoFisher SCIENTIFIC



At Thermo Fisher Scientific, we are dedicated to driving innovative technologies to help accelerate your
we are your trusted partner for products, services, and support throughout your functional genomics
to work better together to deliver great results on your path to discovery.

research. As a leader in life science solutions, screen. Our combined solutions are designed

Contents

Overview of screening solutions

Innovative solutions for every phase of the workflow

With advances in gene editing and RNA interference (RNAi) technologies, it is now possible to efficiently perform genomic screens in a wide variety of model systems. From assay development through target screening and *in vivo* studies, we offer a comprehensive portfolio of products and services for the functional genomics screening workflow (Figure 1). These products empower researchers to expedite discovery of gene targets by performing screens with speed, precision, and accuracy. In addition, our team of experienced scientists can provide custom services to help with your functional genomics screening needs.

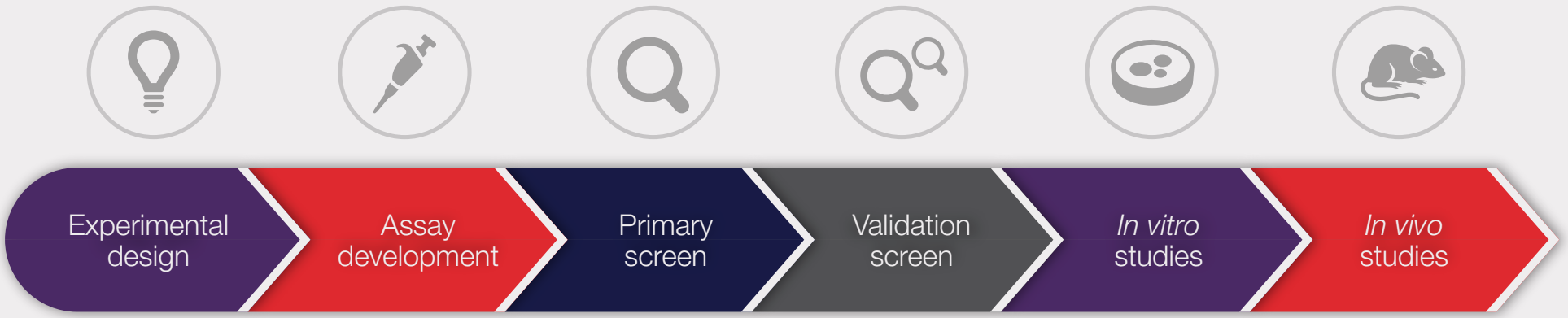


Figure 1. Typical functional genomics screening workflow.

Screening tools available

Accelerate your discovery with the right tool set

Start on the right path to discovery in your gene function studies with advanced tools for experimental design and assay development. Whether you're performing a functional genomics screen in a commonly used cell line or a difficult-to-transfect primary cell type, we offer a large portfolio of workflow tools to enable superior results from your screening studies. Your choice of tool depends on your model system, the length of knockdown time you require, and other experimental parameters. An overview of our most effective technologies is shown in Table 1 to help you choose the solution that's right for you.

Table 1. Library formats available to choose from.

	miRNA mimics and inhibitors	*Silencer* Select siRNA libraries	LentiPool CRISPR libraries	LentiArray CRISPR libraries
Description	Chemically modified, synthetic nucleic acids designed to either mimic mature miRNAs or bind to and inhibit endogenous miRNAs	Ultrapotent siRNAs to knock down gene expression	Pooled libraries of gRNAs in lentiviral expression constructs, providing a potent delivery method for complete gene knockout all in one tube	Arrayed libraries of gRNAs in lentiviral expression constructs, providing a potent delivery method for complete gene knockout
RNA type	miRNA oligonucleotide	siRNA oligonucleotide	Full-length gRNA	Full-length gRNA
Mechanism of action	Endogenous RNAi-induced silencing complex (RISC)	Endogenous RNAi-induced silencing complex (RISC)	Cas9 endonuclease–mediated indel formation	Cas9 endonuclease–mediated indel formation
Effect on gene expression	Transient knockdown	Transient knockdown	Permanent, complete knockout	Permanent, complete knockout
Time to effect	2–3 days	2–3 days	2–4 days	2–4 days
Species	Human, mouse, rat	Human, mouse, rat	Human	Human
Format	Array or pooled	Array or pooled	Pooled, ready-to-use lentivirus	Arrayed, ready-to-use lentivirus or glycerol stock of bacteria containing lentiviral vector

Predefined libraries available

Predefined and custom libraries are available to enable you to focus on defined gene sets or perform more expanded screens (Figure 2). Our libraries are available in miRNA, siRNA, gRNA, and lentiviral formats.

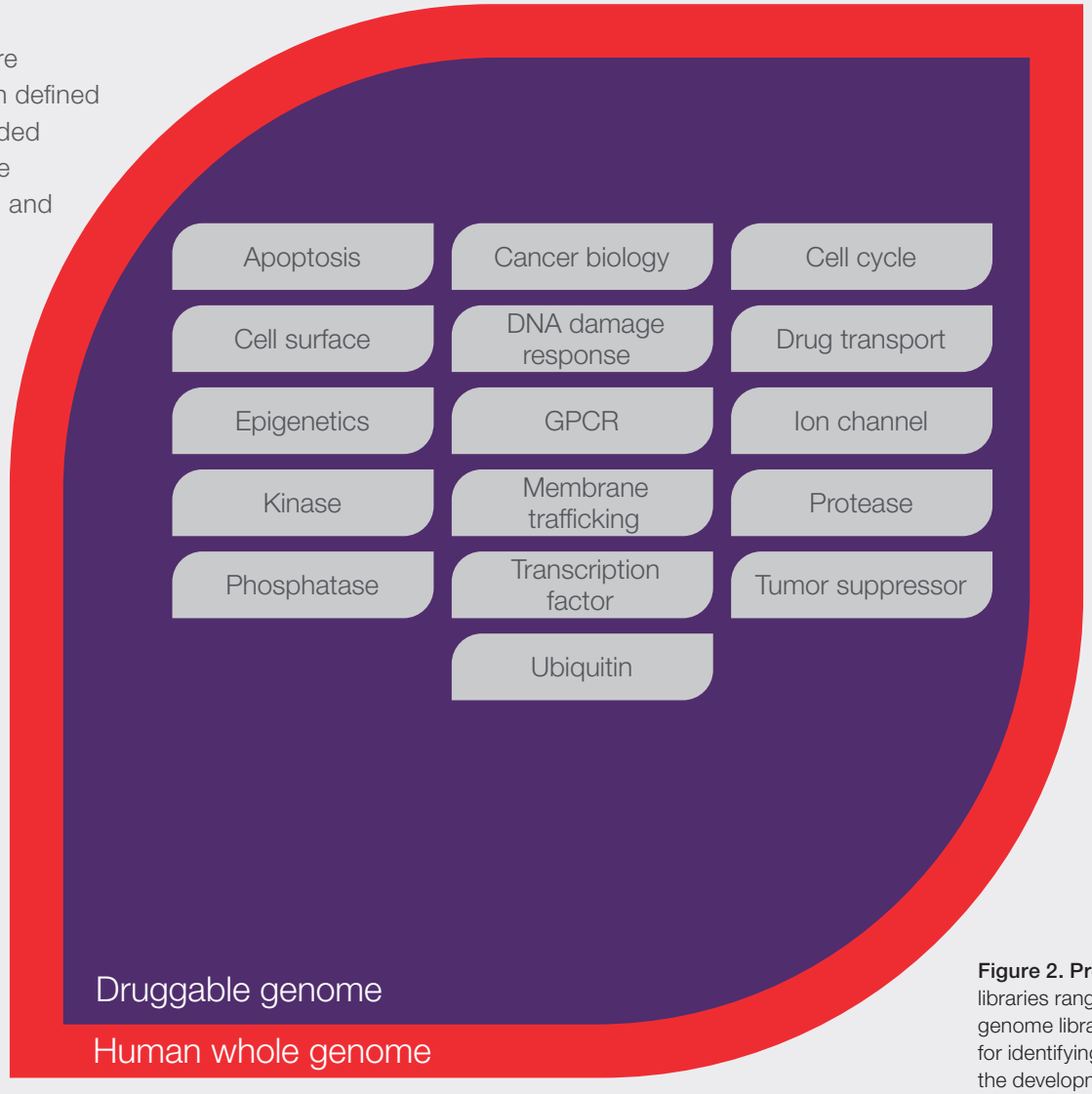


Figure 2. Predefined library options. Available libraries range from focused gene sets to whole-genome libraries. Our druggable genome library is ideal for identifying potential therapeutic targets involved in the development and progression of diseases.

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 of choice for high-throughput screening. The CRISPR-Cas9 system provides an efficient method for specific, complete, and permanent gene knockout. We are applying the power of the CRISPR-Cas9 system to high-throughput screening applications with our award-winning Invitrogen™ LentiArray™ libraries. These arrayed CRISPR libraries are designed to provide you with flexible systems that can be adapted to your needs and help drive new discoveries.

2016 TheScientist TOP 10 INNOVATIONS

Features of LentiArray libraries

- Arrayed libraries in flexible formats to allow for a wide variety of assays
- 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
- Complete set of controls to streamline assay development
- 19 predefined libraries and custom options available, allowing you to focus on defined gene sets or perform unbiased whole-genome surveys
- Individual gRNAs and custom arrays available to support rapid assay development and validation of hits

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 4 high-quality gRNAs to help ensure that the library will provide high-efficiency knockout of target genes across a wide array of cell types.

LentiArray CRISPR libraries

LentiArray CRISPR libraries are arrayed libraries of gRNAs that are constructed in lentiviral expression vectors, providing a potent delivery method that allows you to perform high-throughput screens in a wide variety of cell types. The libraries are available as either high-titer, ready-to-use lentivirus or as glycerol stocks of bacteria containing lentiviral vector (Figure 3). A full selection of controls and accessory products provides everything you need to successfully develop and perform your screens.

Figure 3. Lentiviral map. All constructs contain sequence-verified gRNA with expression driven by a U6 promoter, as well as a puromycin resistance gene driven by an EF-1α promoter. Puromycin resistance allows you to select for cells that were transduced with gRNA and therefore enrich the population for cells with edits to the target gene, which will ultimately produce stronger phenotypes in your screen.

Case study

Functional characterization studies of the human genome have delivered a tremendous amount of information using genome-based loss-of-function screening in diverse models. RNAi has been the predominant method for loss-of-function screening, but is limited by variable efficiency, frequent incompleteness of protein depletion, and confounding off-target effects. In addition, certain types of mammalian cells are difficult to transfect using lipid reagents or electroporation.

To circumvent these difficulties, lentiviral vectors are commonly used as another delivery method, as they can be easily titrated to manipulate transgene copy number and are stably maintained by integration into genomic DNA during subsequent cell replication. Figure 4 shows how a human kinase loss-of-function screen using our Invitrogen™ CellSensor™ NF-κB-*bla* ME-180 cell line easily enables identification of genomic targets associated with the NF-κB pathway.

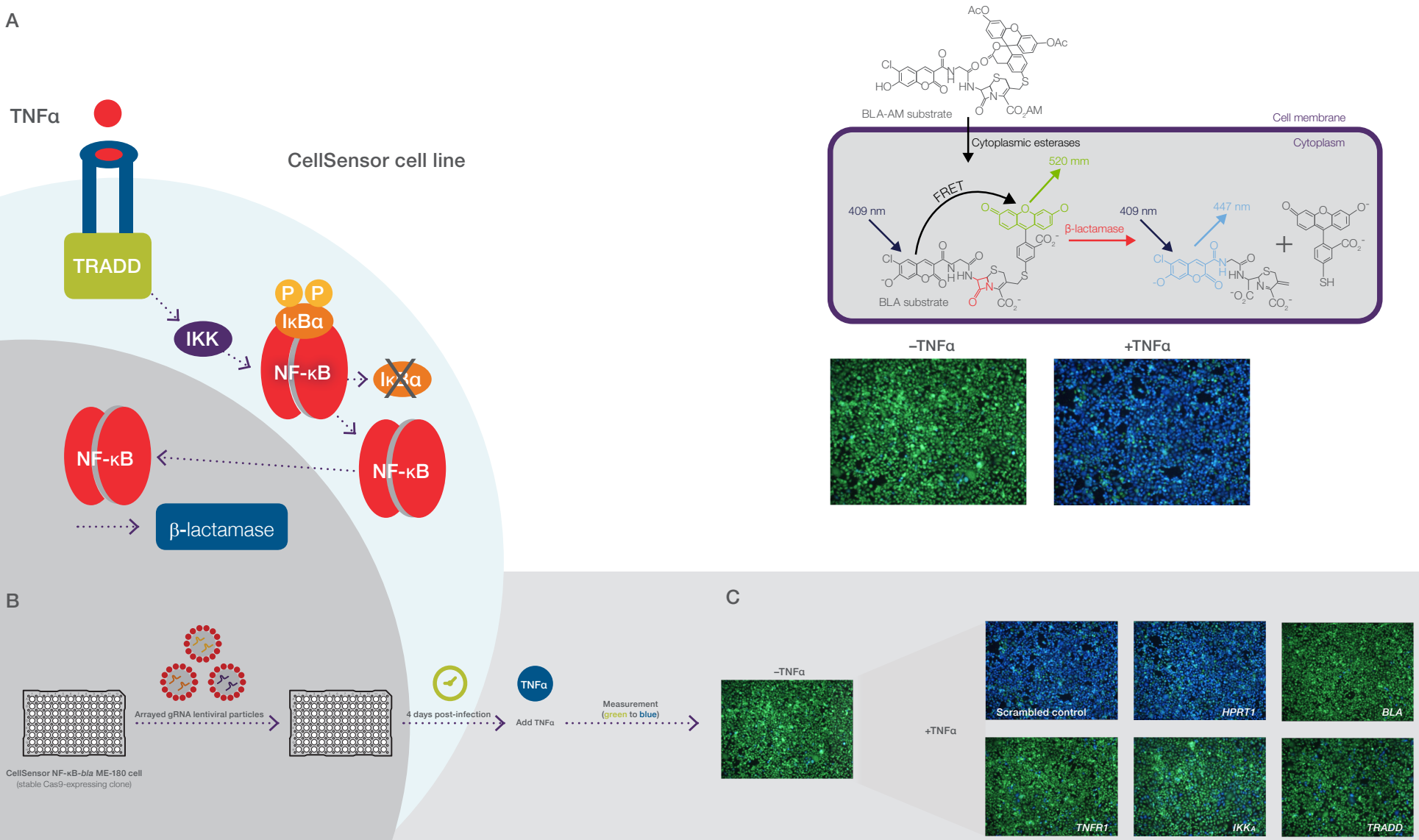


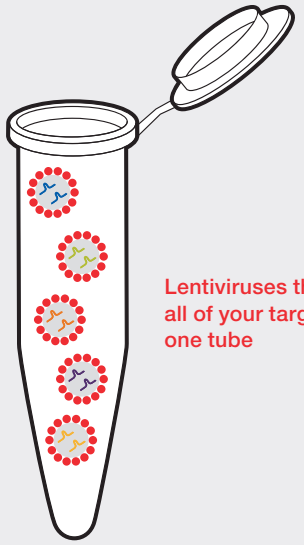
Figure 4. A subset of kinases screened in CellSensor NF-κB-*bla* ME-180 cells with a ratiometric two-color reporter assay. (A) CellSensor cell lines use β-lactamase reporter technology to provide a rapid and sensitive method of analyzing activation of signal transduction pathways. **(B)** Overview of 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.

Learn more at **thermofisher.com/lentiarraycrispr**

LentiPool CRISPR libraries

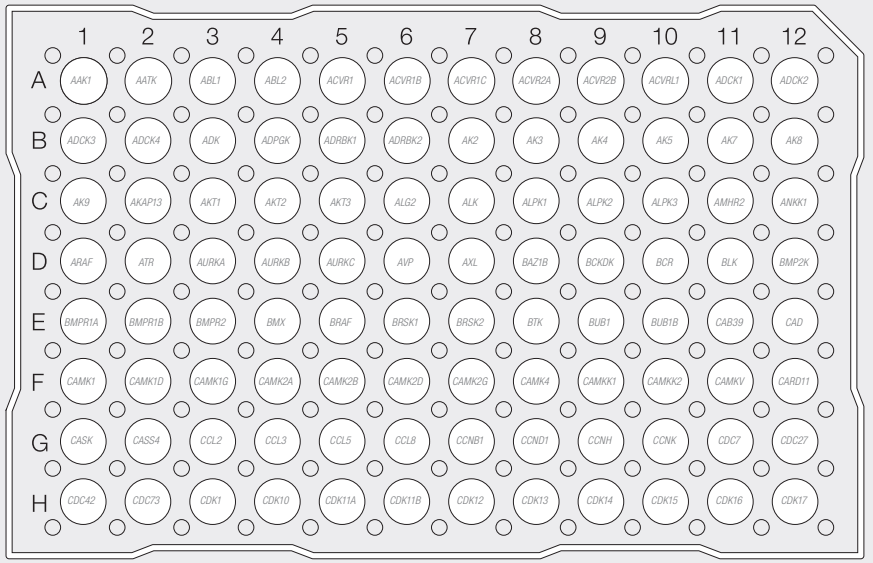
Our Invitrogen™ LentiPool™ CRISPR libraries are an affordable method to screen a large number of genes, as there is no high-throughput instrumentation required. The key difference between LentiPool and LentiArray CRISPR libraries is the format you receive them in. The pooled CRISPR library includes lentiviruses that encode gRNA to all your target genes, all in one tube, versus an array plate format where you receive one gene per well (Figure 5). All analysis can be performed using a next-generation sequencing (NGS) system. Our LentiPool CRISPR libraries are quality controlled by NGS to confirm gRNA and gene representation.

LentiPool CRISPR library



Lentiviruses that encode gRNA to all of your target genes are all in one tube

LentiArray CRISPR library



Lentivirus is organized to target one gene per well

Figure 5. Comparison between LentiPool and LentiArray CRISPR libraries.

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

CRISPR library controls

Optimize your assay design with quality controls

High-quality controls are a cornerstone of successful screens. Our libraries provide a suite of controls to help you quickly develop your assay and define your hit criteria with confidence.

All of the content within the libraries is available as individual, ready-to-transfect gRNA to help you with rapid assay development and hit validation.

Negative controls

Negative controls are nontargeting gRNAs that don't recognize any sequence in the human genome. The negative controls provided are available in multiple package sizes for use in assay development and as on-plate controls when running your screens.

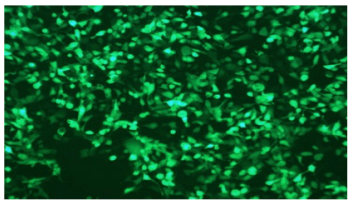
Positive controls

Positive controls are validated gRNA sequences that have demonstrated high-level editing efficiencies of up to 90% in several cell types. These controls are recommended for use in assay development to determine the conditions that provide maximum editing efficiency in your cell models.

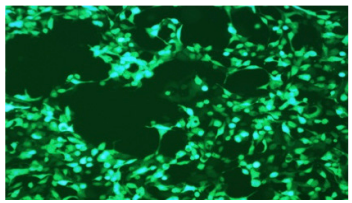
Individual gRNAs against specific genes are available to serve as biological controls for use in assay development and as on-plate positive controls when running your screens.

Delivery controls

Specific to LentiArray CRISPR libraries, we offer a set of delivery controls. These control lentiviruses are available as either negative or positive controls and also express GFP. The inclusion of the GFP marker provides a visual readout to aid in the rapid optimization of viral delivery conditions (Figure 6).



Negative control, scrambled gRNA sequence



Positive control, *HPRT1* gene

Figure 6. Delivery controls. HT1080 cells were infected with a negative control lentivirus expressing a scrambled gRNA sequence, or a positive control lentivirus expressing a gRNA specific to the *HPRT1* gene. GFP is expressed from both lentivirus controls, which provides a visual readout of transduction efficiency and helps to determine multiplicity of infection (MOI) for the cell line being used in the screen.

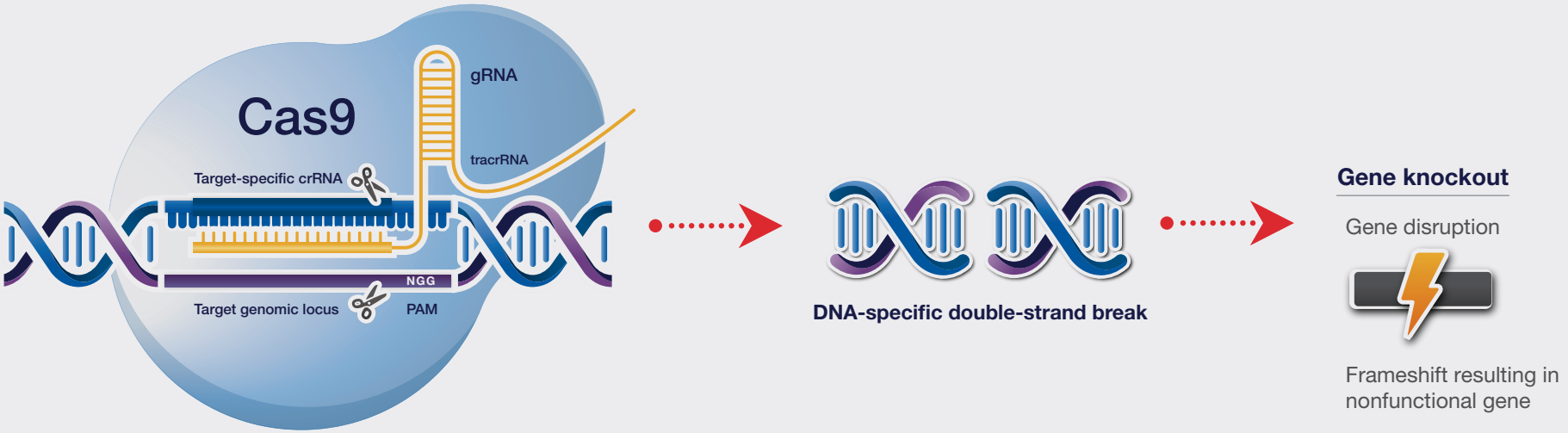


Figure 7. A CRISPR-Cas9 targeted double-strand break. Cleavage occurs on both strands, 3 bp upstream of the NGG in the protospacer adjacent motif (PAM) sequence on the 3′ end of the target sequence.

Support resources

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

High-efficiency gene knockout with the CRISPR-Cas9 system

The CRISPR-Cas9 system offers specific, complete, and permanent gene knockout, making it a potent tool for new discoveries about gene function. Derived from components of a simple bacterial immune system, this tool enables targeted gene cleavage and gene editing in a variety of eukaryotic cells. Since Cas9 nuclease specificity is determined by a gRNA sequence, editing can be directed to virtually any genomic locus by engineering the gRNA sequence and delivering it along with the Cas9 nuclease to your target cell.

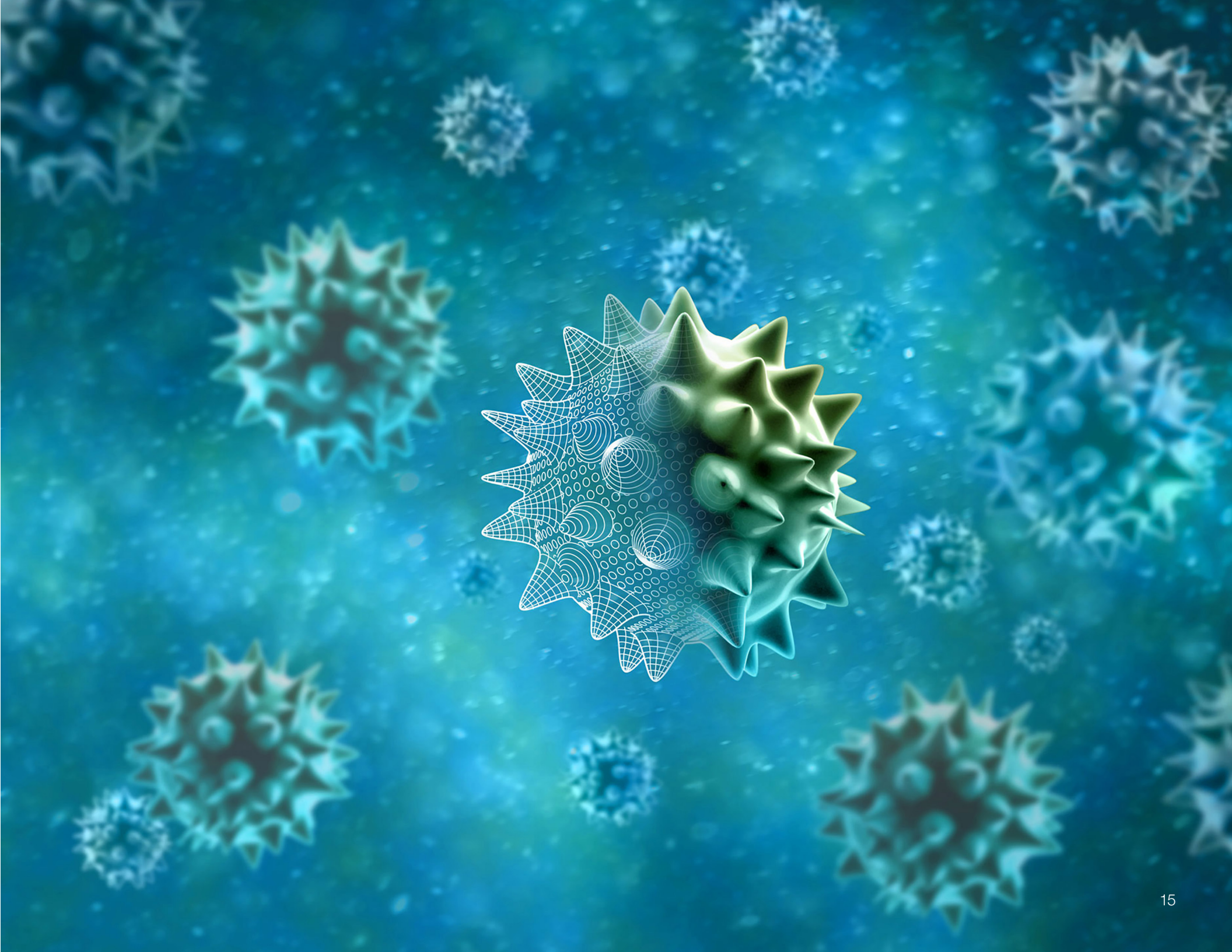
Knockouts are created by the CRISPR-Cas9 system by introducing a double-strand break at a site within the target gene. This double-strand break is then repaired by nonhomologous end joining (NHEJ). The error-prone nature of NHEJ leads to the introduction of insertion or deletion (indel) mutations, resulting in a frameshift and ultimately a nonfunctional gene (Figure 7).

Figure 8. LentiArray Cas9 Lentivirus map. An EF-1α promoter drives expression of a Cas9 gene linked to the blasticidin resistance gene by a self-cleaving P2A peptide. Blasticidin resistance allows for the isolation of stable Cas9-expressing cells.

Invitrogen™ LentiArray™ Cas9 Lentivirus provides an efficient method to drive high-level Cas9 nuclease expression in a wide variety of cell types. The LentiArray Cas9 Lentivirus construct includes a human codon–optimized version of Cas9 with 2 nuclear localization signals (NLS, one on the C-terminus and one on the N-terminus) to facilitate efficient delivery into the nucleus (Figure 8). LentiArray Cas9 Lentivirus gives you the ability to create a stable pool of Cas9-expressing cells or isolate stable Cas9-expressing clones. LentiArray Cas9 Lentivirus can be used in screening applications with LentiArray CRISPR libraries. LentiArray Cas9 Lentivirus is provided as high-titer, ready-to-use lentiviral particles.

Cas9 formats available

We offer Cas9 nuclease in multiple formats. For screening applications, we offer Cas9 protein, Cas9 mRNA, and Cas9 lentiviral particles. These gene editing solutions are paired with optimal cell culture reagents, delivery methods, and analysis tools based on your application and cell type.



RNAi libraries

Transient knockdown of multiple gene targets

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

siRNA libraries

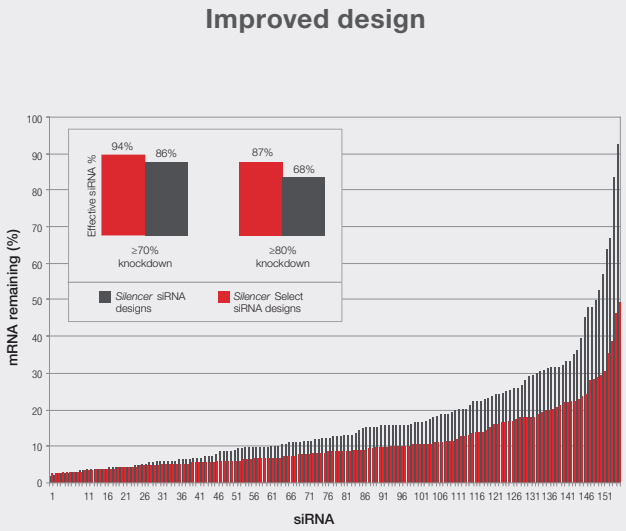
Ultrapotent siRNA for *in vitro* RNAi applications is the best way to effectively knock down gene expression to study protein function in a wide range of cell types. Traditional RNAi methods for gene knockdown in mammalian cells involve the use of synthetic RNA duplexes consisting of two unmodified 21-mer oligonucleotides annealed together to form siRNAs. Invitrogen™ *Silencer*™ Select siRNA products (Table 2) incorporate the latest improvements in siRNA design, off-target effect prediction algorithms, and chemical modifications to enhance strand bias.

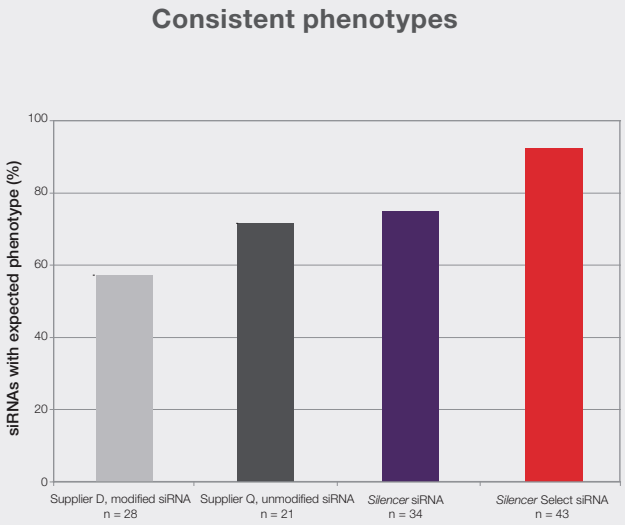
* Guarantee details at **thermofisher.com/sirnaguarantee**

With our expanded line of Invitrogen™ *Silencer*™ and *Silencer*™ Select siRNA libraries, you are empowered to expedite your functional genomics screen with precision and accuracy using an array of gene classes. Our premium siRNAs are designed to provide superior results for your loss-of-function studies, helping you to achieve your goals and confidently move to the next phase of your work (Figures 9–11).

We synthesize all siRNAs in-house to exacting quality standards. All *Silencer* and *Silencer* Select siRNA libraries, whether predefined or custom-made, are supplied with full siRNA sequence information and, when available, with siRNA validation data. *Silencer* Select siRNA consistently offers:

- **Efficacy**—guaranteed that with at least two of the siRNAs you will achieve >70% reduction in target mRNA levels, to help prevent additional siRNA testing, false negatives, and missed discoveries*
- **Specificity**—precise mRNA target recognition helps eliminate false positives, helping to reduce time-consuming and costly off-target validations
- **Potency**—100-fold more potent than other siRNAs, resulting in lower cost per experiment and low off-target effects





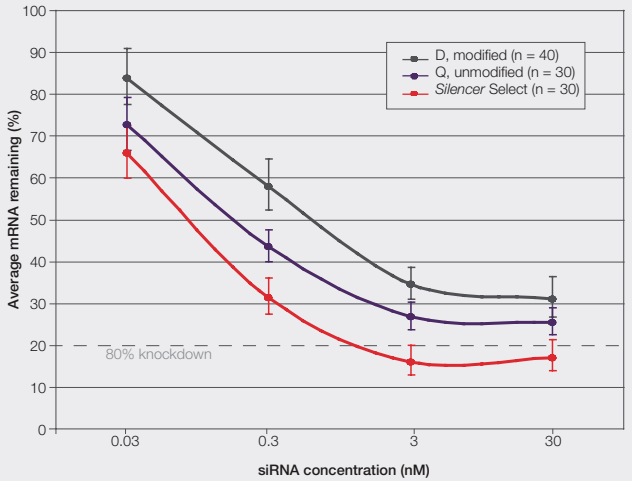


Figure 9. *Silencer* Select siRNA design algorithm significantly improves effective siRNA prediction accuracy. The *Silencer* Select siRNA algorithm was used to design 155 siRNAs to 40 different targets. These siRNAs were tested side by side with siRNAs designed using the previous algorithm, at 5 nM in HeLa cells. mRNA knockdown was measured 48 hours posttransfection via qRT-PCR using Applied Biosystems™ TaqMan® Gene Expression Assays. Results are expressed as percentage of mRNA remaining compared to cells treated with Invitrogen™ *Silencer*™ Select Negative Control No. 1 siRNA. The inset shows the percentages of siRNAs that elicited ≥70% and ≥80% mRNA knockdown.

Figure 10. *Silencer* Select siRNAs elicit the expected phenotype at a higher rate than other siRNAs. siRNAs to 7 gene targets with well-understood RNAi-induced phenotypes were individually transfected at 3 nM and phenotypes measured 48 hours later. Each bar represents the percentage of siRNAs that gave the expected, silenced phenotype. siRNAs to *BUB1B*, *AURKB*, *WEE1*, and *PLK1* were assessed using a multiparametric cell growth/apoptosis assay in U2OS human osteosarcoma cells. siRNAs to *HMGCR*, *LDLR*, and *FDFT1* were assessed using an LDL uptake assay in HUH7 human hepatoma cells.

Figure 11. *Silencer* Select siRNAs provide up to 100x higher potency compared to other siRNAs. *Silencer* Select siRNAs and siRNAs from two other suppliers to the same 10 gene targets were individually transfected into HeLa cells in triplicate at the indicated siRNA concentrations. mRNA knockdown levels were tested 48 hours later by qRT-PCR. Average percentage of mRNA remaining is shown for each set of siRNAs.

Table 2. siRNA product selection guide.

	Silencer siRNA	Stealth RNAi™ siRNA	*Silencer* Select siRNA
	Good knockdown, cost-effective siRNA	Excellent knockdown, low off-target effects	Best knockdown, lowest off-target effects
Potency	20 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
Specificity	Moderate	High	Highest
Coverage	Coding RNA	Coding RNA	Coding and noncoding RNA
Target species	Human, mouse, rat (use custom tool for other species)	Human, mouse, rat (use custom tool for other species)	Human, mouse, rat (use custom tool for other species)
Recommended product	*Silencer* Pre-designed siRNA, 5 nmol (Cat. No. AM16708)	Stealth RNAi siRNA, tube (Cat. No. 1299001)	*Silencer* Select Pre-designed siRNA, 5 nmol (Cat. No. 4390771)

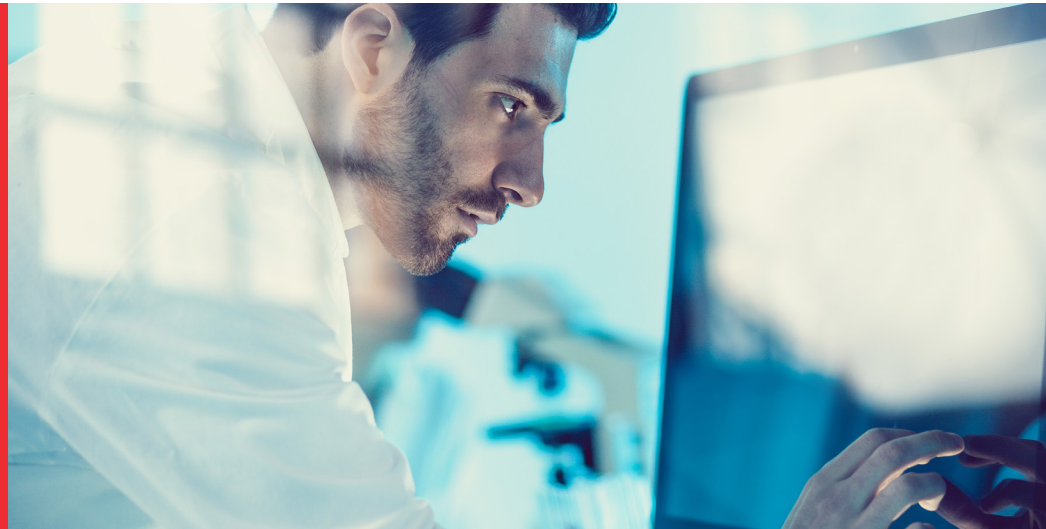
* Guarantee details at **thermofisher.com/sirnaguarantee**

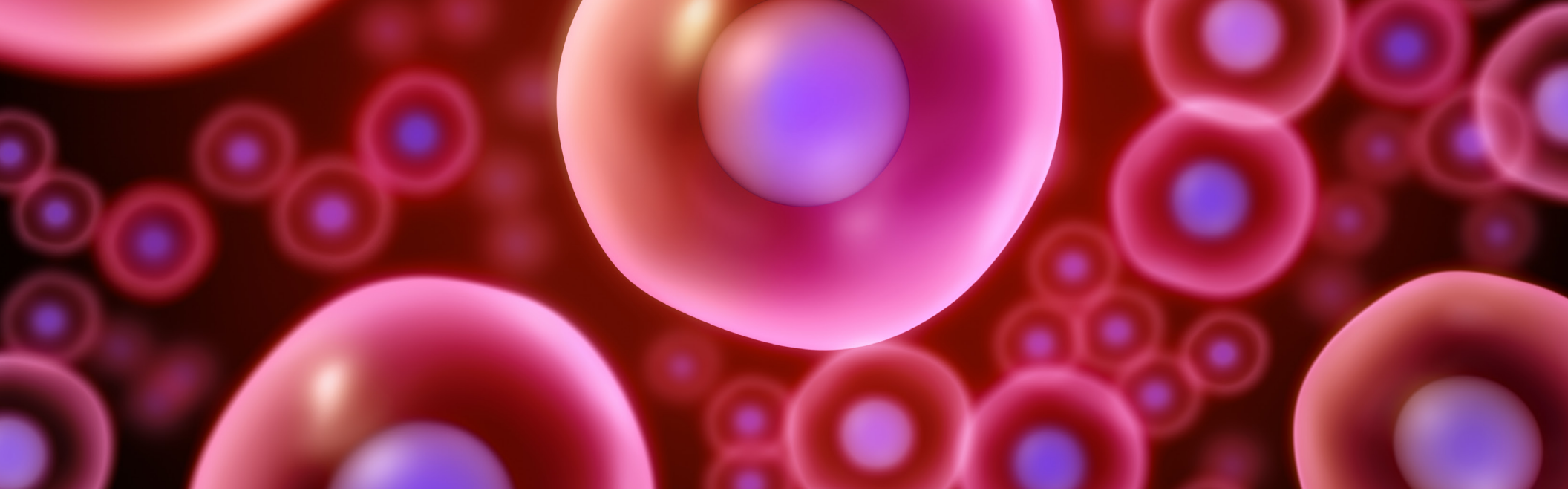
Request more information at **thermofisher.com/sirnaquote**
To learn more or place an order, go to **thermofisher.com/sirna**

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. We offer positive and negative controls as well as Invitrogen™ GeneArt™ gene synthesis for siRNA-resistant genes that can be used in RNAi rescue experiments.

To learn more, go to **thermofisher.com/sirnacontrols**





miRNA mimics and inhibitors

For artificial regulation of target mRNA translation, Invitrogen™ *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 are:

- **Versatile**—ready-to-use *in vitro* and *in vivo*, allowing consistency and specificity throughout your entire study (Figures 13 and 14)
- **Highly potent**—lack toxicity and immunostimulation essential to *in vivo* studies
- **Available as high-throughput screening libraries**—most up-to-date miRBase sequence content
- **Current**—updated frequently using miRBase database content

Resources

- **RNAi Handbook**—go to **thermofisher.com/rnai**
- **Genome Editing Selection Guide**—go to **thermofisher.com/geneedit** and select "Genome Editing Resource Center"
- **Transfection Protocol Calculator**—go to **thermofisher.com/transfection**
- **Publications**—go to **thermofisher.com/rnaipublications**

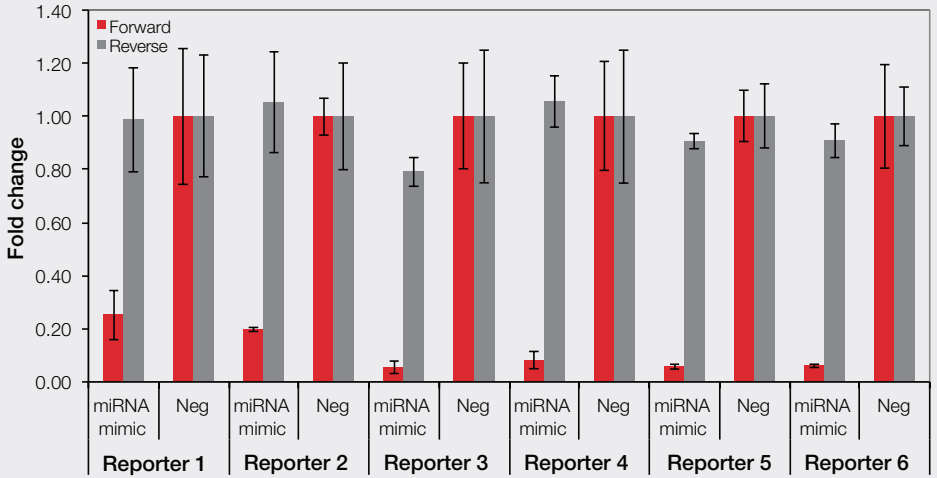


Figure 12. Superior miRNA specificity. The guide strand of *mir*Vana miRNA mimics is highly potent and the star strand is inactivated. The key advantage of *mir*Vana miRNA mimics is inactivation of the star strand. miRNA mimics, like natural microRNAs, have 2 strands. The guide strand is functional and used by argonaute protein to target miRNAs. The star, or passenger strand, is nonfunctional and is normally cleaved and expelled from the silencing complex. Most scientists want to analyze one strand of miRNA at a time while the other strand remains totally inactive. *mir*Vana miRNA mimics achieve this specificity. For this assay we measured activity from both strands of miRNA mimics. One reporter had a target in the forward orientation to measure activity of the guide miRNA strand. Another reporter had the target cloned in reverse orientation to test activity of the star strand of the miRNA mimic. For all 6 sequences, activity of the guide strand is high (resulting in 75–90% lower expression of the reporter), and activity of the star strand is minimal (similar to negative control).

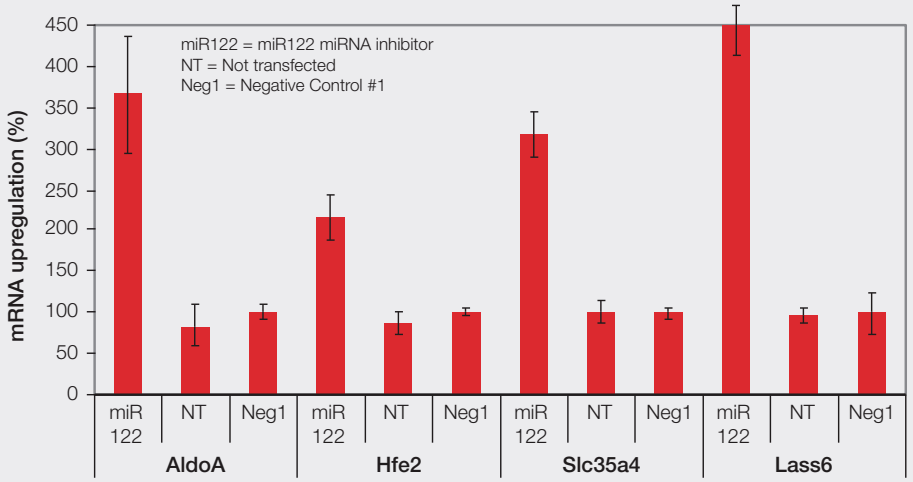


Figure 13. *mir*Vana miRNA inhibitors effectively suppress miRNA *in vivo*. Invitrogen™ *mir*Vana™ miR122 inhibitor or Negative Control #1 (Neg1) were complexed with Invitrogen™ Invivofectamine™ 2.0 Reagent and delivered to BALB/c mouse liver via tail vein injection on three consecutive days at a dose of 7 mg/kg body weight. Expression of four mRNA targets (AldoA, Hfe2, Slc35a4, and Lass6), natural targets of miR122, were measured using Applied Biosystems™ TaqMan® MicroRNA Assays in transfected livers of mice injected with miR122 inhibitor or Negative Control #1 and livers of mice that were not transfected (NT). The results indicate that the inhibitor is efficiently delivered to the liver with Invivofectamine 2.0 Reagent and inactivate miR122, leading to upregulation of genes naturally suppressed by miR122.

miRNA libraries

Complete Invitrogen™ *mir*Vana™ libraries containing mimics and inhibitors for human, mouse, and rat miRNA are available.

For information on all our predefined and custom miRNAs libraries, contact us at **RNAiLibraries@lifetech.com**

To learn more or place an order, go to **thermofisher.com/mirna**

Custom library services

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

You're not on this journey alone. Our experienced scientists including technical specialists, project support specialists, and other professionals who appreciate your challenges 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 RNAi and gene editing team can work with you to design and implement the solutions that fit. We offer custom pooled libraries, custom libraries with genes selected from our pooled libraries, and more. 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 **GEMServices@thermofisher.com**

Transfection technologies

Transfection selection guide

Transfection is the process by which nucleic acids are introduced into eukaryotic cells. Transfection techniques vary widely, including 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 3.

Table 3. Transfection product selection guide.

	siRNA, miRNA	mRNA	Protein	Lentivirus production
Lipofectamine CRISPRMAX Cas9 Transfection Reagent			✔	
Lipofectamine 3000 Transfection Reagent*				✔
Lipofectamine RNAiMAX Transfection Reagent	✔			
Neon Transfection System	✔	✔	✔	
Lipofectamine MessengerMAX Transfection Reagent		✔		

* Invitrogen™ Lipofectamine™ 3000 Transfection Reagent can be used to produce lentivirus.

Choose the solution that's right for you at **thermofisher.com/transfection**

Lipofectamine CRISPRMAX Cas9 Transfection Reagent

First transfection reagent optimized for CRISPR-Cas9 protein delivery

With Invitrogen™ Lipofectamine™ CRISPRMAX™ Cas9 Transfection Reagent, it is now possible to use a lipid-based reagent to deliver CRISPR-Cas9 protein complexes (Table 4). Lipofectamine CRISPRMAX reagent is the first lipid-based transfection reagent for optimized delivery of CRISPR-Cas9 protein complexes, providing up to 85% cleavage efficiency when combined with Invitrogen™ GeneArt™ Platinum™ Cas9 Nuclease. It is also cost-effective and gentle on cells.

Deliver our superior GeneArt Platinum Cas9 Nuclease with a reagent that provides:

- **Demonstrated cleavage efficiency**—tested in over 20 cell types, including iPSCs, mESCs, N2a, CHO, A549, HCT116, HeLa, HEKa, 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

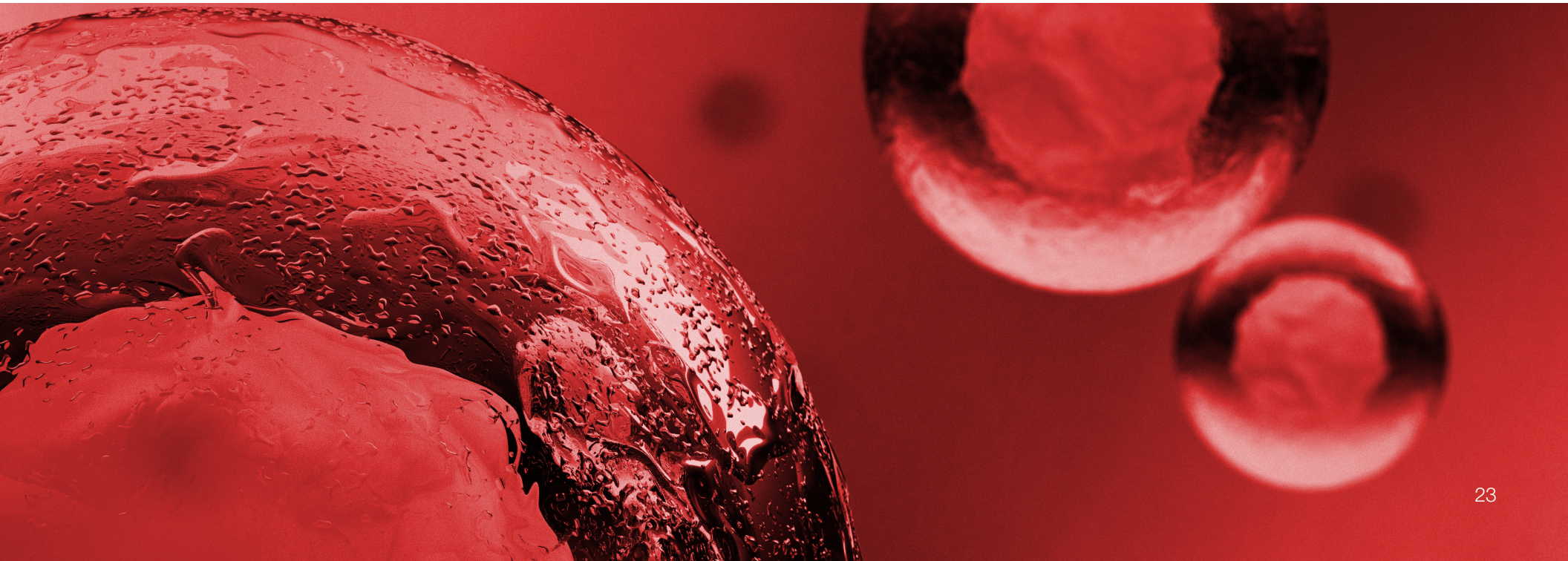
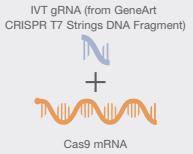
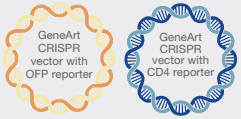
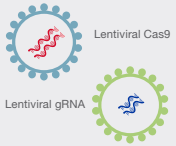


Table 4. Summary of CRISPR-Cas9 delivery methods.

	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	
Multiplexing and screening capable	✔	✔	Larger payload	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.

Learn more or see what cell types we've tested at **thermofisher.com/crisprtransfection**

Lipofectamine RNAiMAX Transfection Reagent

Invitrogen™ Lipofectamine™ RNAiMAX™ Transfection Reagent offers an advanced, efficient solution for siRNA or miRNA delivery. It can also be used for cotransfection of gRNA and Cas9 mRNA. No other transfection reagent for RNAi experiments provides such easy and efficient delivery into a broad spectrum of cell types including primary cells, stem cells, and other hard-to-transfect cell types (Figure 14).

Superior transfection efficiency at low siRNA concentrations

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

Low cytotoxicity profile for easy optimization

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

Support resources

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

Download your copy of our transfection and genome engineering handbook at **thermofisher.com/transfectionhandbook**

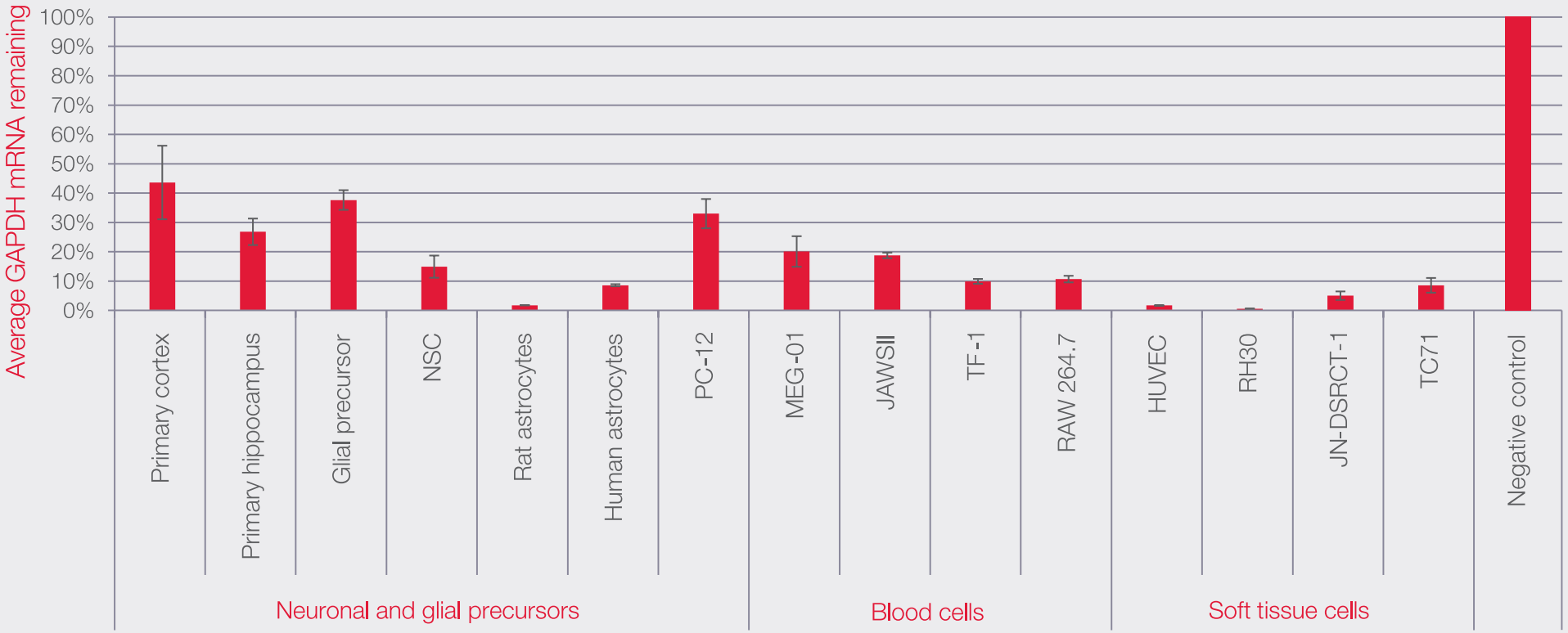


Figure 14. Invitrogen™ *Silencer*™ Select siRNA transfection with Lipofectamine RNAiMAX reagent. Cells were transfected with Lipofectamine RNAiMAX reagent complexed with *Silencer* Select siRNA at 30 nM/well. Knockdown of GAPDH mRNA was assessed by qPCR. The cell density per well and amount of Lipofectamine RNAiMAX reagent used per well were as follows: rat primary cortex cells: 10^4 cells/well, 0.6 µL; rat primary hippocampus cells: 10^4 cells/well, 0.6 µL; rat glial precursor cells: 8 x 10^3 cells/well, 0.4 µL; neuroblastoma-spinal cord (NSC) cells: 1.2 x 10^4 cells/well, 0.3 µL; rat astrocyte cells: 1.2 x 10^4 cells/well, 0.3 µL; human astrocyte cells: 8 x 10^3 cells/well, 0.3 µL; PC-12 cells (derived from a pheochromocytoma of the rat adrenal medulla): 8 x 10^3 cells/well, 0.3 µL; megakaryoblastic leukemia cells (MEG-01): 2 x 10^4 cells/well, 0.3 µL; murine dendritic cells (JAWSII): 1.2 x 10^4 cells/well, 0.3 µL; human erythroleukemia cells (TF-1): 10^4 cells/well, 0.3 µL; RAW 264.7 macrophages: 10^4 cells/well, 0.3 µL; human umbilical vein endothelial cells (HUVEC): 8 x 10^3 cells/well, 0.3 µL; rhabdomyosarcoma cells (RH30): 8 x 10^3 cells/well, 0.3 µL; human desmoplastic small round cell tumor cells (JN-DSRCT-1): 8 x 10^3 cells/well, 0.3 µL; human Ewing's sarcoma cells (TC71): 1.6 x 10^4 cells/well, 0.3 µL.

To learn more or place an order, go to **thermofisher.com/rnaimax**

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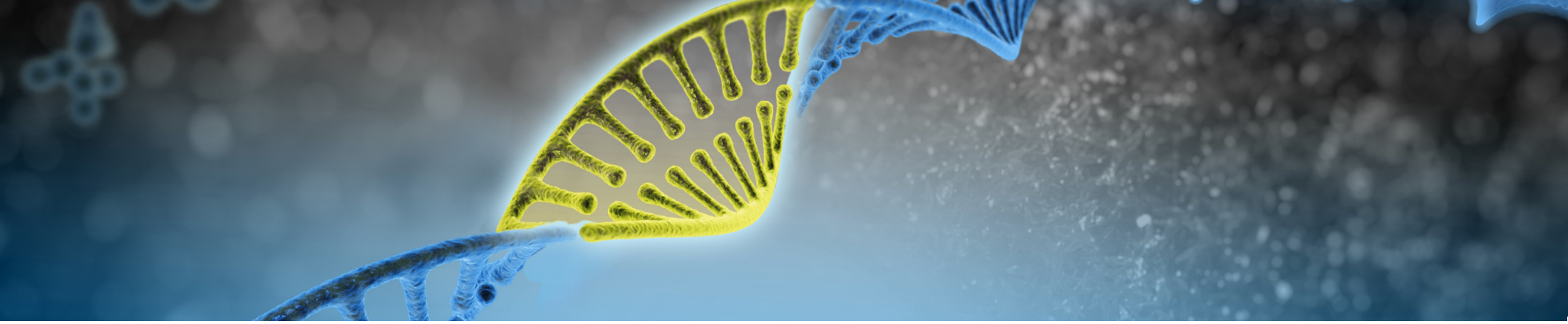
Find trusted cell culture products at **thermofisher.com/custommedia**



High-content analysis

CRISPR analysis steps using HCA

- Process development
- Rapid phenotyping
- Off-target screening

When screening a CRISPR library involves large cell numbers and complex phenotypic responses, HCA is an obvious choice. HCA provides automation throughout the process, and the imaging format allows for quantitative morphological analysis.

Process optimization

Cell analysis using fluorescent protein reporters of transfection or dsDNA cleavage is routine in automated workflows generally run at high throughput in HCA. Cell health assays prior to editing can be a stringent QC step to avoid wasting time and reagents, and after editing they are critical metrics for process optimization.

Thermo Scientific™ CellInsight™ CX7 High Content Analysis Platform

Transfection efficiency assay

Cell viability assay

General intensity measurement tool

Cell health profiling assay

Cell phenotyping

The CRISPR-Cas9 system is routinely used for knockout, knock-in, and modulation of gene expression. HCA provides automated analysis with quantitative rigor to directly analyze changes in protein expression levels, compartmentalization, or cell morphology (Figure 15).

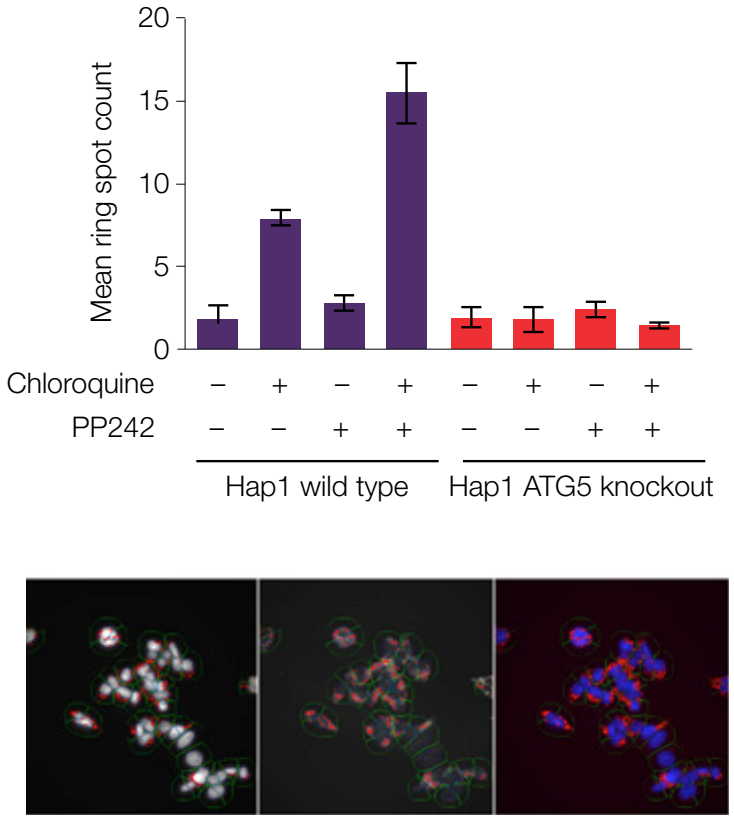


Figure 15. Automated quantitation of autophagy using HCS. The CellInsight CX7 High Content Analysis (HCA) Platform was used to identify and count LC3B granules in CRISPR-edited cells. Nuclei (blue overlay), cell perimeter (green outline), and LC3B granules (red overlay) are shown.

Off-target analysis

HCA allows you to track your targeted protein and also monitor its impact on cell health and behavior. Invitrogen™ reagents and the CellInsight CX5 High Content Analysis (HCA) Platform makes analysis of multiple cell health parameters routine (Figure 16).

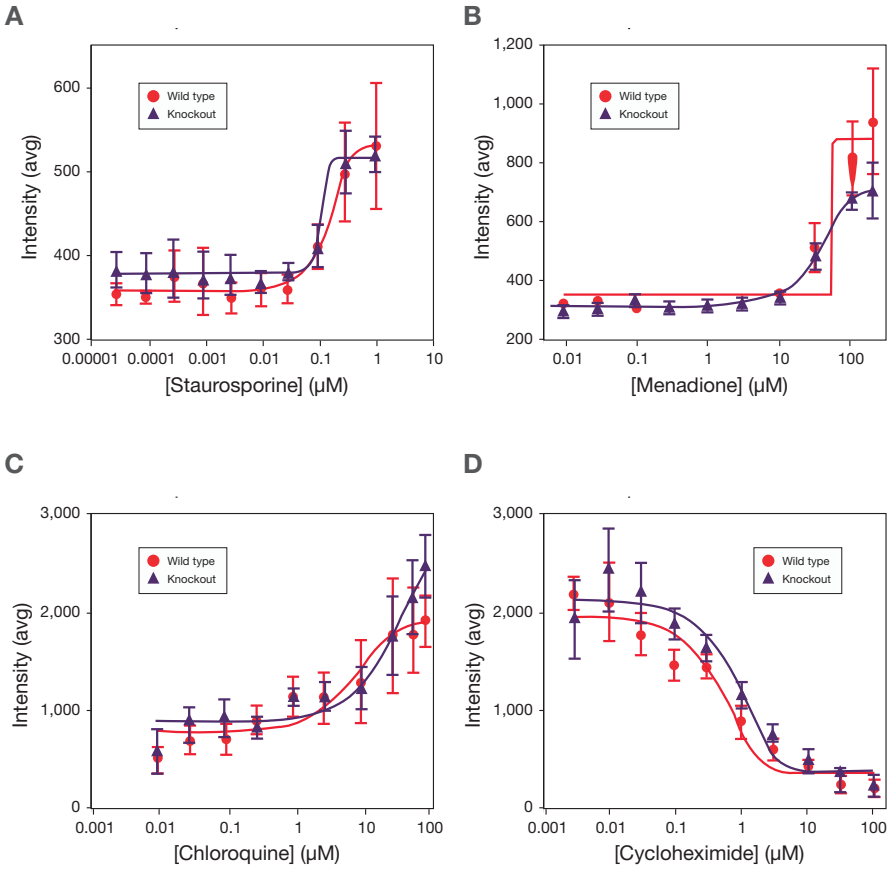


Figure 16. Rapid analysis using the CellInsight CX5 High Content Analysis Platform and Invitrogen™ reagents. Wild type and CRISPR-edited cells were compared for **(A)** apoptosis, using CellEvent™ Caspase-3/7 Green Detection Reagent, **(B)** oxidative stress, using CellROX™ reagents, **(C)** protein degradation, using LysoTracker™ reagents, and **(D)** protein synthesis, using the Click-iT™ Plus OPP kit.

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

Ordering information

Product	Product and Cat. No.			
	Silencer siRNA (unmodified)	*Silencer* Select siRNA (chemically modified)	LentiArray CRISPR (ready to use)	LentiArray CRISPR (glycerol stock)
Screening libraries				
Human Whole Genome Library	NA	NA	A31949	A32185
Human Druggable Genome Library	A30077	NA	A31948	A32184
Human Apoptosis Library	A30095	A30095	A31940	A32176
Human Cancer Biology Library	A30143	A30142	A31933	A32169
Human Cell Cycle Regulation Library	A30137	A30136	A31936	A32172
Human Cell Surface Library	A30145	A30144	A31943	A32179
Human DNA Damage Response Library	A30090	A30089	A31946	A32182
Human Drug Transporter Library	A30135	A30133	A31941	A32177
Human Epigenetics Library	A30086	A30085	A31934	A32170
Human GPCR Library	A30082	NA	A31947	A32183
Human Ion Channel Library	A30084	NA	A31942	A32178
Human Kinase Library	A30079	NA	A31931	A32167
Human Membrane Trafficking Library	A30139	A30138	A31937	A32173
Human Nuclear Hormone Receptor Library	A30083	NA	A31939	A32175
Human Phosphatase Library	A30080	NA	A31932	A32168
Human Protease Library	A30081	NA	A31944	A32180
Human Transcription Factor Library	NA	A30087	A31938	A32174
Human Tumor Suppressor Library	A30147	A30146	A31945	A32181
Human Ubiquitin Library	A30141	A30140	A31935	A32171

Product	Quantity	Cat. No.
CRISPR library controls		
LentiArray CRISPR Positive Control Lentivirus, human HPRT	100 µL	A32056
LentiArray CRISPR Positive Control Lentivirus, human HPRT	1 mL	A32829
LentiArray CRISPR Positive Control Lentivirus, human HPRT with GFP	100 µL	A32060
LentiArray CRISPR Positive Control Lentivirus, human HPRT with GFP	1 mL	A32830
LentiArray CRISPR Negative Control Lentivirus, human non-targeting	100 µL	A32062
LentiArray CRISPR Negative Control Lentivirus, human non-targeting	1 mL	A32327
LentiArray gRNA	Custom	A32042
LentiArray Custom CRISPR Plate	Custom	A32045

Ordering information (continued)

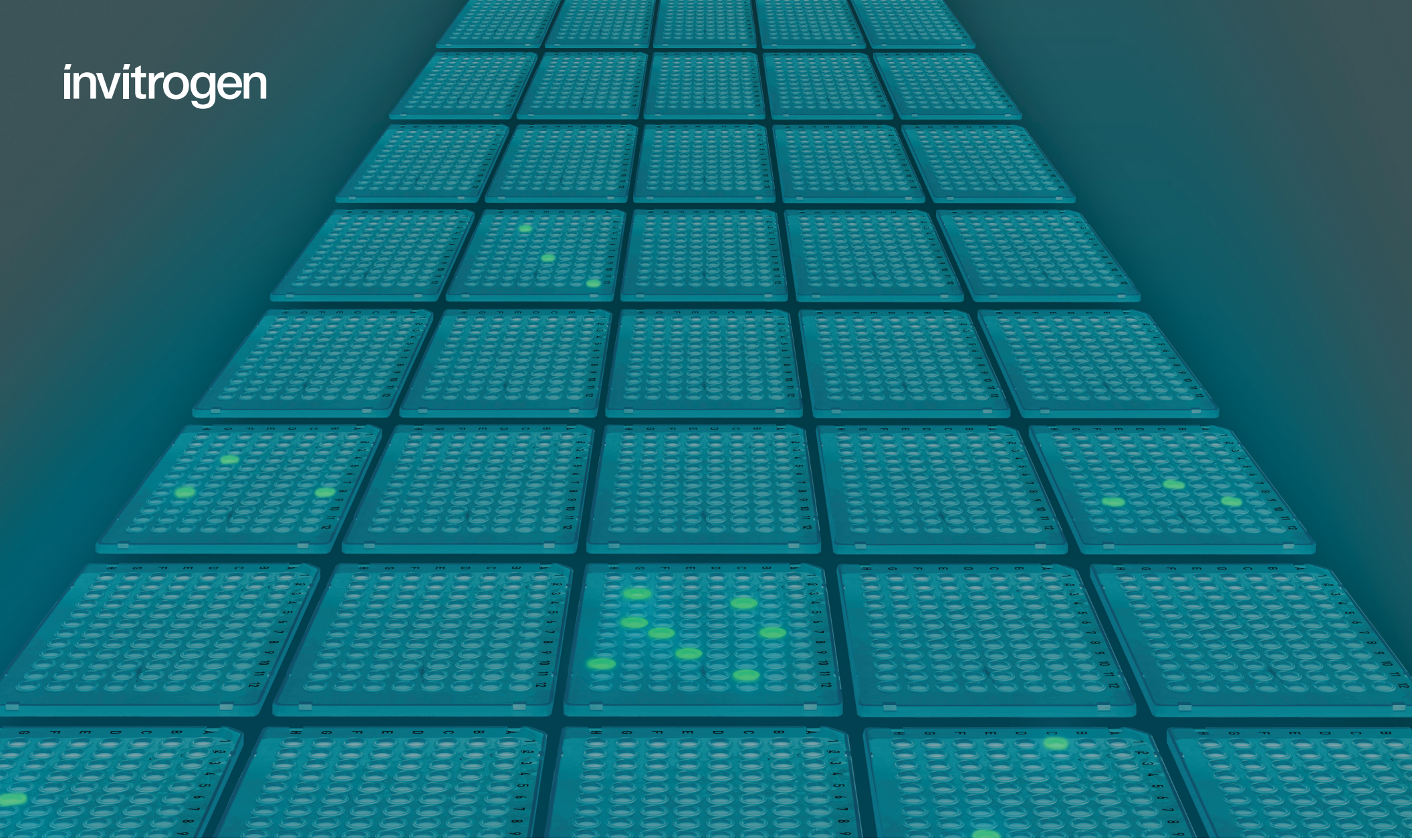
Product	Quantity	Cat. No.
Cas9 formats		
LentiArray Cas9 Lentivirus	100 µL	A32064
LentiArray Cas9 Lentivirus	1 mL	A32069
GeneArt Platinum Cas9 Nuclease (1 µg/µL)	10 µg	B25642
GeneArt Platinum Cas9 Nuclease (1 µg/µL)	25 µg	B25640
GeneArt Platinum Cas9 Nuclease (3 µg/µL)	75 µg	B25641
GeneArt CRISPR Nuclease mRNA	15 µg	A29378
Related products		
GeneArt Genomic Cleavage Detection Kit	20 rxns	A24372
THE RNA Storage Solution	50 mL	AM7001
Lipofectamine CRISPRMAX Cas9 Transfection Reagent	10 rxns	CMAX00001
Lipofectamine CRISPRMAX Cas9 Transfection Reagent	25 rxns	CMAX00003
Lipofectamine CRISPRMAX Cas9 Transfection Reagent	75 rxns	CMAX00008
Lipofectamine CRISPRMAX Cas9 Transfection Reagent	150 rxns	CMAX00015
Lipofectamine RNAiMAX Transfection Reagent	1.5 mL	13778150
Lipofectamine MessengerMAX Transfection Reagent	1.5 mL	LMRNA015
Neon Transfection System Starter Pack	1 pack	MPK5000S

Product	Quantity	Cat. No.
***mir*Vana miRNA mimics and inhibitors*—next-generation miRNA chemistries for lowest off-target effects and highest potency**		
*mir*Vana Predesigned miRNA Mimic	5 nmol	4464066
*mir*Vana Predesigned miRNA Mimic, *in vivo* use	250 nmol	4464070
*mir*Vana Predesigned miRNA Inhibitor	5 nmol	4464084
*mir*Vana Predesigned miRNA Inhibitor, *in vivo* use	250 nmol	4464088
*mir*Vana miRNA Mimic, Negative Control #1	5 nmol	4464058
*mir*Vana miRNA Inhibitor, Negative Control #1	5 nmol	4464076
*mir*Vana miRNA Mimic, miR-1 Positive Control	5 nmol	4464062
*mir*Vana miRNA Inhibitor, let-7c Positive Control	5 nmol	4464080
*mir*Vana Custom miRNA Mimic	5 nmol	4464068
*mir*Vana Custom miRNA Mimic, *in vivo* use	250 nmol	4464071
*mir*Vana Custom miRNA Inhibitor	5 nmol	4464086
*mir*Vana Custom miRNA Inhibitor, *in vivo* use	250 nmol	4464089
Pre-miR precursors and anti-miR inhibitors—trusted, affordable miRNA		
Predesigned		
Anti-miR miRNA Inhibitor	5 nmol	AM17000
Pre-miR miRNA Precursor	5 nmol	AM17100

Ordering information (continued)

Product	Cat. No.
Positive controls	
Silencer Select GAPDH Positive Control siRNA	4390850
Silencer GAPDH siRNA (human, mouse, rat)	AM4624
Silencer GAPDH siRNA (human)	AM4633
Silencer Select MALAT1 Positive Control siRNA	4455877
Silencer Cy®3-labeled GAPDH siRNA (human, mouse, rat)	AM4649
Silencer GFP (eGFP) siRNA	AM4626
Silencer Firefly Luciferase (GL2 + GL3) siRNA	AM4629
*mir*Vana miRNA Mimic, miR-1 Positive Control	4464062
*mir*Vana miRNA Inhibitor, let-7c Positive Control	4464080
Negative controls	
Silencer Select Negative Control No. 1 siRNA	4390843
Silencer Select Negative Control No. 2 siRNA	4390846
Silencer Negative Control No. 1 siRNA	AM4611
Silencer Negative Control No. 2 siRNA	AM4613
*mir*Vana miRNA Inhibitor, Negative Control #1	4464077
*mir*Vana miRNA Mimic, Negative Control #1	4464058

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



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