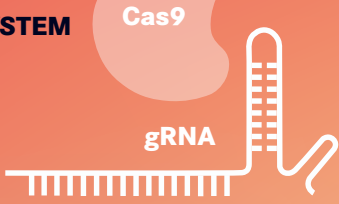


1

SELECT
CRISPR SYSTEM

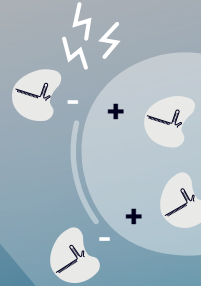


DESIGN
GUIDE
RNAs



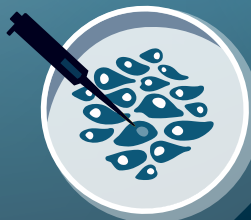
2

CHOOSE
DELIVERY
METHOD



3

PERFORM
EDITS



4

ANALYSE
EDITS



5

CRISPR/CAS GENOME EDITING

A GUIDE

How to Perform a Successful CRISPR Experiment



How does CRISPR work?

Use of CRISPR (clustered regularly interspaced short palindromic repeats) and associated Cas enzymes for genome editing has been a **major technological breakthrough**, making genome modification in cells or organisms faster, more efficient, and more robust than previous genome editing methods.

The most commonly used system combines a guide RNA with the Cas9 enzyme from *Streptococcus pyogenes* to achieve gene disruption or modification via knock-in or knock-out strategies (see below).

Other approaches exist, making use of e.g. modified Cas9 variants (nickases, dead variants, Cas9 fusions to other DNA modifying enzymes). In addition, alternative Cas enzymes are available, such as Cas12a (also termed Cpf1) from *Acidaminococcus* sp. BV3LC, which allow targeting of gene locations inaccessible to the Cas9 system.

In this guide, we focus primarily on the Alt-R[®] CRISPR system from Integrated DNA Technologies (IDT). The Alt-R[®] system employs two different Cas nucleases: CRISPR/Cas9 and CRISPR/Cas12a. We will outline the relative strengths of the two systems and which system to use, depending on your experimental setup.

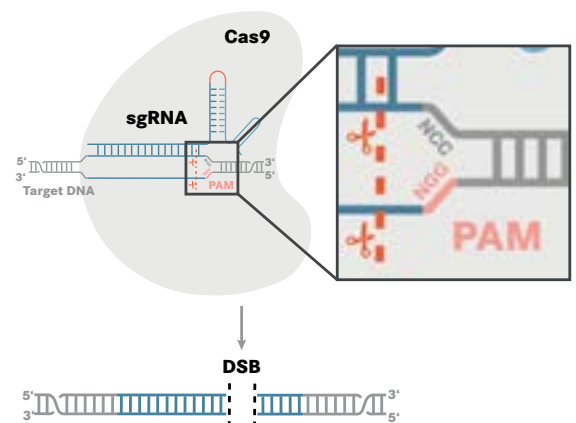
Components and mechanism of action

The Cas enzyme combines with a guide RNA to form an editing complex, the so-called **ribonucleoprotein (RNP)**. The role of the guide RNA is to guide the editing complex to the appropriate location in the genome. Recognition of the genomic region to be targeted is achieved by complementarity between the variable region of the guide RNA and the genomic sequence. The target region must be adjacent to a so-called **PAM (protospacer adjacent) motif**. For Cas9 the PAM motif is NGG, for Cas12a it is TTTV (V = A, C or G). Once bound to the target sequence, the Cas enzyme will effect a double strand cut.

Cas nuclease

The Cas nuclease is the central element of the CRISPR system since it achieves targeting of the RNP complex to the proper genomic region and effects the double strand cuts in the target genome sequence. Cas9 features a bi-lobed architecture with the guide RNA nestled between the alpha-helical lobe and the nuclease lobe. These two lobes are connected through a single bridge helix. There are two nuclease domains located in the nuclease lobe, the RuvC which cleaves the non-target DNA strand, and the HNH nuclease domain which cleaves the target DNA strand.

The most commonly used Cas nuclease is Cas9 but there are also Cas nucleases from other organisms and various Cas mutants which are used in different experimental settings (see «Selecting a CRISPR system» on page 5).



Molecular mechanism of DNA cleavage by the CRISPR-Cas RNP. A Cas nuclease (depicted: Cas9) forms a complex with a guide RNA, targets the genomic region encoded by the guide and effects a double strand cut adjacent to the PAM motif.



Crystal Structure of Cas9. From Nishimasu et al. (2014) Cell 156 (5): 935-49. PMID 24529477.

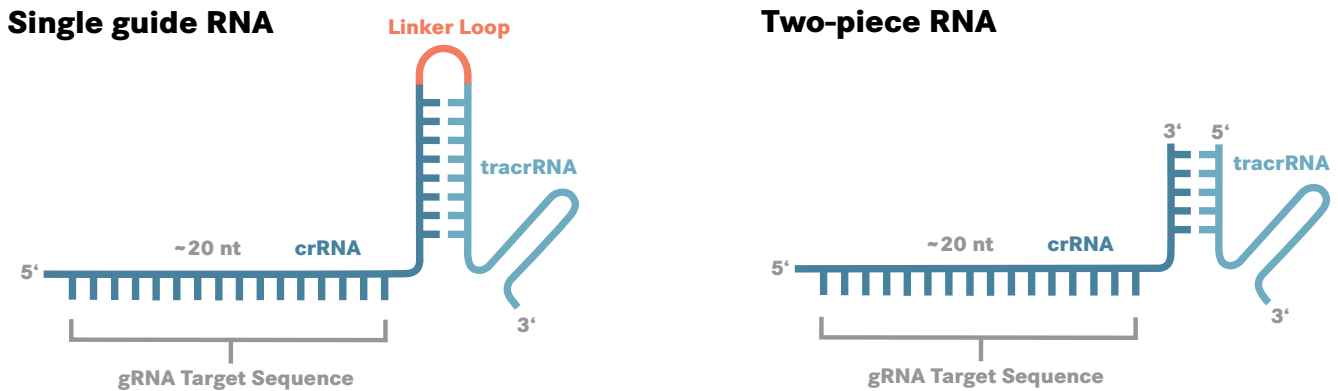


Guide RNAs

A guide RNA consists of **three elements**:

1. The variable sequence targeting the genomic region to be cut
2. The invariable PAM sequence adjacent to the targeting sequence
3. The invariable tracr sequence which binds the guide RNA to the Cas enzyme

Guide RNAs can be either continuous (**single guide RNAs or sgRNAs**) or composed of two separate pieces (**two-piece RNAs**, with a variable crRNA and an invariable tracrRNA).



Guide RNA formats. A single guide RNA consists of the variable crRNA sequence and the invariable tracrRNA sequence linked via a loop structure. Alternatively, a two-piece RNA format can be used, where the crRNA and tracrRNA sequences are encoded by separate RNAs.

RNP delivery

The RNP complex consisting of the Cas enzyme and associated guide RNA has to be delivered to the target cells by an appropriate method. A number of approaches for RNP delivery exist:

- Lipofection
- Electroporation and nucleofection
- Microinjection
- Viral-mediated delivery

The choice of delivery method depends on a number of factors, such as the type of cells to be edited, the type of desired edit (knock-in vs knock-out), the assembly of the RNP complex (in vitro vs in situ) and the availability of equipment. See «Delivery options» on page 11 for a discussion of the advantages and drawbacks of each method.

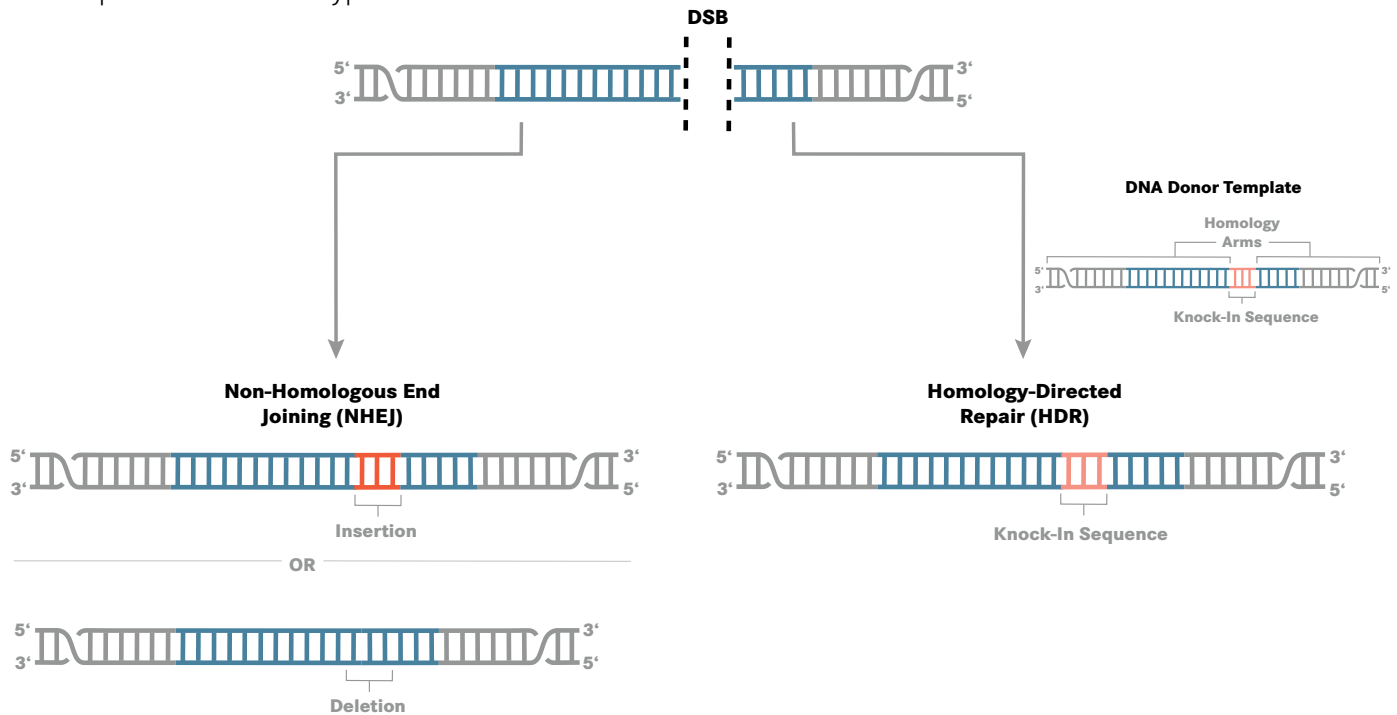
Types of edits

Once the RNP complex has been delivered to the target cells and the target DNA has been cut, two outcomes are possible:

1. Repair via **non-homologous end joining (NHEJ)** occurs when the two strands are «patched together» by enzymes of the NHEJ pathway. This often results in small deletions or insertions (indels) which have the potential to disrupt the open reading frame of the target gene, leading to a knock-out of gene expression. This approach typically yields high editing efficiencies.



2. If a DNA donor template is supplied along with the CRISPR components, knock-in of a desired sequence can be achieved via the **homologous directed repair (HDR)** pathway. The donor template contains the sequence of interest (knock-in sequence) flanked by sequences that are homologous to the target genomic sequence (homology arms). Because a defined template is used, HDR is typically accurate and the sequence of interest is inserted into the genome seamlessly. However, HDR only occurs in the S and G2 phases of the cell cycle and is less efficient than NHEJ. Efficiencies around 20% may be achieved but the outcome strongly depends on the cell type used.



KNOCK-OUTS

- Genomic location repaired by the NHEJ pathway
- Imprecise repair, indels occur
- Indels may or may not lead to loss of gene function
- Typically high editing efficiencies

KNOCK-INS

- Genomic location repaired by the HDR pathway
- Donor template for repair must be provided
- Enables precise modification of a target sequence via insertion of a donor template
- Typically less efficient than knock-outs, some cells may have indels from repair by NHEJ

Analysing your edits and performing downstream analysis

Once your target cells have been edited, you are faced with a heterogeneous cell population consisting of unedited and edited cells. Edited cells may either be heterozygous (one allele edited) or homozygous (both alleles edited). The fraction of edited vs unedited cells depends on several factors, including the cell type, genomic region and the delivery method used.

The first step of downstream analysis is usually to determine your editing efficiency. There are a number of approaches for doing this, which are described in more detail in the section «Analysis» on page 12. Once you have determined the editing efficiency of your experiment and edited cells have been selected/expanded, the next step is usually one or several functional assays to determine the effect(s) of the gene edit on the target cells.



Planning your CRISPR experiment in five easy steps

Planning your first CRISPR experiment can be challenging, as there are many different factors to consider – choice of CRISPR system, what type of guide RNA to use and how to deliver the RNP complex into your target cells. Our guide will help you in making the right choices and divides the CRISPR workflow into five easy steps:

- Selecting the right CRISPR system for your purpose
- Designing your guide RNAs
- Selecting the Cas nuclease and enhancers
- Choosing a delivery option
- Analysing your edited cells

In the following pages, URL links can be recognized by the icon seen down below.

The respective URL paths are preceded by www.lubio.ch as in this example:

www.lubio.ch/idt-cas9





Selecting a CRISPR system

Your first step is to decide which CRISPR/Cas system to use, as there are many different homebrew and commercial variants available. We recommend using the Alt-R[®] system from Integrated DNA Technologies, as it is heavily optimized, is being widely used, and has a strong publication record.

The Alt-R[®] system uses either a single guide RNA or a two-part guide RNA, giving you maximum flexibility. Several Cas nuclease variants are also available, based on your experimental needs. Finally, the Alt-R[®] system delivers a finished RNP complex into your target cells, ensuring a high editing efficiency and low off-target activity.

There are two Alt-R[®] systems available which differ in the type of Cas nuclease used. In most cases, the CRISPR/Cas9 system is the best starting point and will yield good results. If editing efficiencies are low with the CRISPR/Cas9 system, consider switching to the CRISPR/Cas12a system to improve your chances of obtaining correctly edited cells.

CRISPR/Cas9 system

This is the **current gold standard** – start with this system if this is your first attempt at editing your gene of interest.

- Required PAM site: NGG
- Benefit from optimized on- and off-target design and improved chemical stability
- Get optimal editing with high on-target potency and reduced off-target activity
- Precisely control editing with efficient delivery of the RNP by lipofection or electroporation
- Protect your cells from toxicity or innate immune response activation

 /idt-cas9

CRISPR/Cas12a system

The Alt-R CRISPR-Cas12a system allows **targeting of alternative sites** that are not available to the CRISPR/Cas9 system and produces a staggered cut with a 5' overhang. May work better than CRISPR/Cas9 for **AT-rich genomes**.

- Required PAM site: TTTV (V = A, C or G)
- Enables genome editing in organisms with AT-rich genomes
- Allows interrogation of additional genomic regions compared to Cas9
- Requires simple complexing of crRNA with the Cas12a protein – no tracrRNA needed

 /idt-cas12a



Choosing a guide RNA format

When using the CRISPR/Cas9 system, two approaches are possible. You may either use a sgRNA, which combines the variable crRNA and constant tracrRNA sequences into one long RNA, or you may use a two-part system with separate crRNA and tracrRNA.

One-part RNA (sgRNA)

Single RNA molecule comprised of both crRNA and tracrRNA sequences. Suitable for **challenging experimental conditions** (e.g. high nuclease environments or with Cas9 mRNA). Contains chemical modifications for the highest level of stability.

Alt-R® CRISPR-Cas9 sgRNA (sizes: 2, 10, 50, 100 nmol)

Two-part RNA (crRNA & tracrRNA)

CRISPR-Cas9 crRNA

To be used together with a tracrRNA to form a functional guide RNA duplex. Suitable for most applications. Contains chemical modifications to **protect from degradation by cellular RNases**.

Alt-R® CRISPR-Cas9 crRNA (sizes: 2, 10 nmol)

Alt-R® CRISPR-Cas9 tracrRNA (sizes: 5, 20, 100 nmol)

CRISPR-Cas9 crRNA XT

To be used together with a tracrRNA to form a functional guide RNA duplex. Suitable for challenging experimental conditions (e.g. high nuclease environments or with Cas9 mRNA). Contains additional chemical modifications compared to standard crRNA to provide a **cost-effective option for increased stability**.

Alt-R® CRISPR-Cas9 crRNA XT (sizes: 2, 10 nmol)

Alt-R® CRISPR-Cas9 tracrRNA (sizes: 5, 20, 100 nmol)

GLOSSARY

- sgRNA: single guide RNA
- crRNA: CRISPR RNA
- tracrRNA: trans-activating RNA

ORDERING OPTIONS

Predesigned guide RNAs

- Human
- Mouse
- Rat
- Zebrafish
- *C. elegans*

Custom designed guide RNAs

Submit your own design for analysis

FORMATS

- Tubes
- 96 well plates
- 384 well plates

DESIGN TOOLS

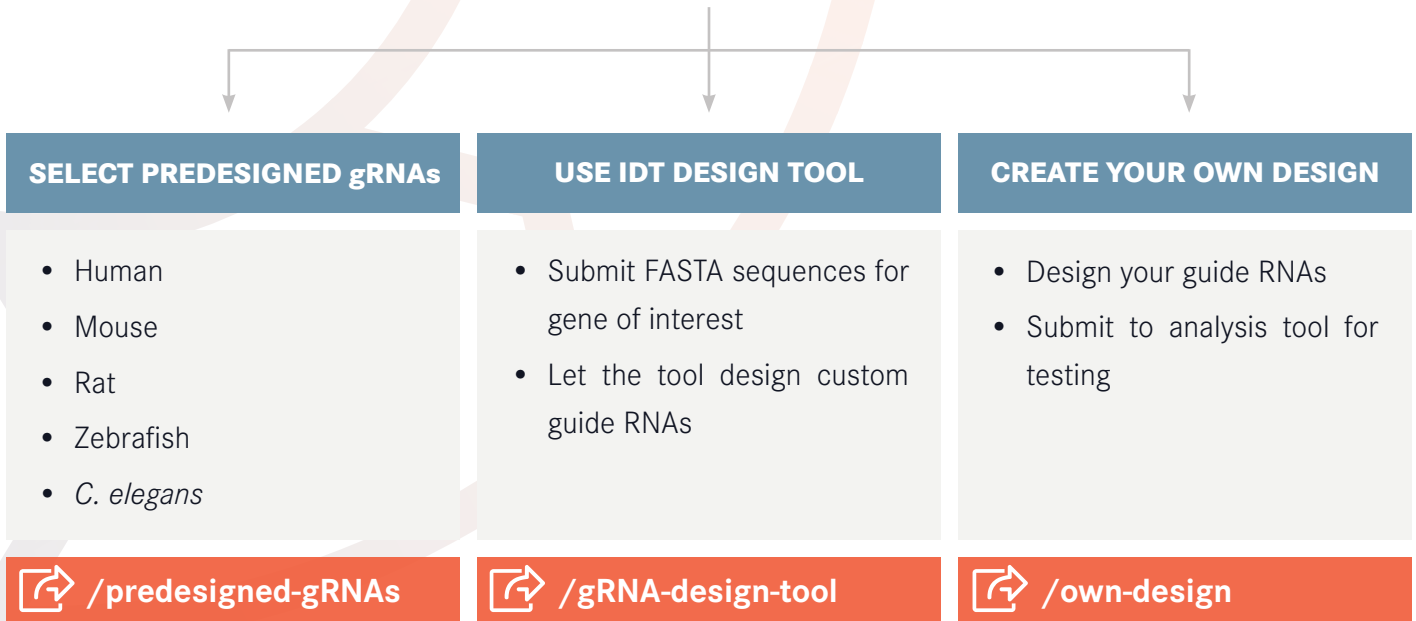
- > IDT Design Tools
- > CHOPCHOP
- > Design tips
- > FAQs



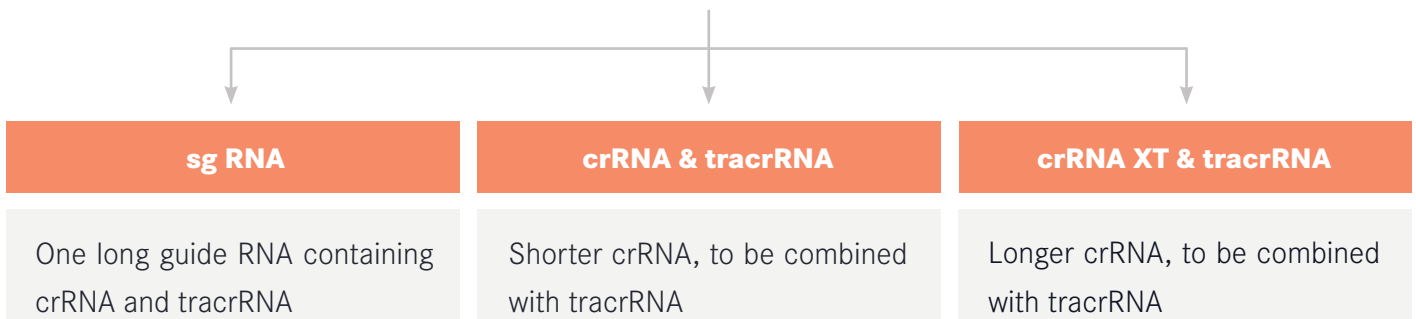


Using the IDT Design & Ordering Tool

Choose gene(s) to be modified and decide on design approach



Select from available guide RNAs



Optional: design HDR donor templates for knock-ins

- A complete solution for industry-leading HDR rates based on extensive wet bench testing and customer validation
- Design and order HDR donor templates and associated Cas9 guide RNAs for genome editing of human, mouse, rat, zebrafish, or *C. elegans* targets
- Optimized donor template design and Cas9 guide RNA selection
- Flexible input and design parameters
- Outstanding HDR rates with Alt-R HDR Donor Oligos

 /crispr-hdr



Choosing a Cas nuclease

IDT offers a range of Cas9 and Cas12a nucleases to fit your editing needs. Choose the Cas nuclease which is optimal for your desired edit and experimental design. Target sequences must contain an adjacent «NGG» (Cas9) or «TTTV» (Cas12a) PAM site.

Cas9 nucleases

The most widely used nuclease. Use Cas9 if this is your first attempt at editing your gene of interest and if your target organism has a standard GC content.

CAT-NO.	NUCLEASE	SIZE	COMMENTS
1081058	Alt-R® S.p. Cas9 Nuclease V3	100 µg	Wild-type version with strong nuclear localization signal (NLS). High on-target efficiency due to effective delivery to the nucleus.
1081060	Alt-R® S.p. HiFi Cas9 Nuclease V3	100 µg	Improved version with increased specificity. High on-target efficiency paired with strongly reduced off-target activity. Use when off-target modifications are of concern.
1081062	Alt-R® S.p. Cas9 D10A Nickase V3	100 µg	This Cas9 D10A Nickase V3 creates a single-stranded cut in the targeted strand of DNA.
1081064	Alt-R® S.p. Cas9 H840A Nickase V3	100 µg	This Cas9 H840A Nickase V3 creates a single-stranded cut in the non-targeted strand of DNA.
1081066	Alt-R® S.p. dCas9 Protein V3	100 µg	Enzymatically «dead» Cas9 mutant, lacks cleavage activity on both DNA strands.

[/idt-cas9](#)

Cas12a nucleases

The Alt-R CRISPR-Cas12a system allows targeting of alternative sites that are not available to the CRISPR-Cas9 system and produces a staggered cut with a 5' overhang. Enables genome editing in organisms with AT-rich genomes. Target sequences must contain an adjacent «TTTV» PAM site (V = A, C or G).

CAT-NO.	NUCLEASE	SIZE	COMMENTS
1081068	Alt-R® A.s. Cas12a V3	100 µg	Wild-type version with strong NLS. High on-target efficiency due to effective delivery to the nucleus.
10001272	Alt-R® A.s. Cas12a Ultra	100 µg	Improved version with increased specificity. High on-target efficiency paired with strongly reduced off-target activity. Use when off-target modifications are of concern.

[/idt-cas 12a](#)



Choosing enhancers and controls

Improve delivery of the RNP complex and HDR donor template by using enhancers. Do not forget to include positive and negative controls to monitor correct delivery of the RNP complex into cells.

Cas9 Electroporation Enhancer

Purified carrier DNA that improves delivery of Cas9 ribonucleoprotein (RNP) by electroporation. Specifically designed to avoid homology to human, mouse, and rat genomes.

CAT-NO.	ENHANCER	SIZE	COMMENTS
1075915	Alt-R® Cas9 Electroporation Enhancer	2 nmol	Purified carrier DNA.

HDR Enhancer

A small molecule compound that has demonstrated an ability to increase the rate of homology-directed repair (HDR).

CAT-NO.	ENHANCER	SIZE	COMMENTS
1081072	Alt-R® HDR Enhancer	100 µl	HDR enhancer, provided as 3 mM stock in DMSO.

CRISPR-Cas9 Control Kits

Separate kits for human, mouse, or rat. Includes tracrRNA, HPRT positive control crRNA, negative control crRNA#1, HPRT Primer Mix, and Nuclease-Free Duplex Buffer. Purchase the Cas9 enzyme (or expression plasmid) and PCR reagents separately.

CAT-NO.	KIT	SIZE	COMMENTS
1072554	Alt-R® CRISPR-Cas9 Control Kit, human	2 nmol	Contains control sequences specific to human.
1072555	Alt-R® CRISPR-Cas9 Control Kit, mouse	2 nmol	Contains control sequences specific to mouse.
1072556	Alt-R® CRISPR-Cas9 Control Kit, rat	2 nmol	Contains control sequences specific to rat.



CRISPR Starter Kit

Our CRISPR/Cas9 Starter Kit supplies all reagents necessary for a complete genome editing experiment. It is an ideal starting point for performing your first editing experiments and offers a complete solution, including crRNAs, tracrRNA, Cas9 enzyme, enhancers, controls, and detection kit.

KIT ITEM	ORDER #	AMOUNT	DESCRIPTION
2 crRNAs	Custom	2 nmol each	Two custom crRNAs targeting two different loci adjacent to a PAM site in your gene of interest.
tracrRNA	1072532	5 nmol	The tracrRNA assembles together with a custom crRNA and the Cas9 protein into the RNP complex.
Cas9 nuclease	1074181 1078727	100 µg	Standard or HiFi Cas9 enzyme, part of the RNP complex.
Electroporation enhancer	1075915	2 nmol	Enhances efficiency when introducing the RNA complex into target cells via electroporation.
CRISPR-Cas9 control kit	1072554 1072555 1072556	1 kit	Separate kits for human, mouse, or rat. Includes tracrRNA, HPRT positive control crRNA, negative control crRNA#1, HPRT Primer Mix, and Nuclease-Free Duplex Buffer.
Genome editing detection kit	1075931	1 kit (25 rxn)	For detection of on-target or known off-target CRISPR events in cultured cells. The T7 endonuclease I (T7EI) mismatch cleavage assay detects on-target genome editing and estimates genome editing efficiency in CRISPR-treated cells.
IDTE buffer, pH 7.5	11-01-02-02	10 x 2 ml	1x TE buffer pH 7.5, nuclease-free, sterile, for resuspension of RNP complexes.

 [/crispr-kit](#)



Delivery options

There are a number of methods for expressing a Cas nuclease and its associated gRNA in cells, which can be classified into physical, chemical and viral-mediated methods. The most common approaches are:

- Lipofection
- Electroporation and nucleofection
- Microinjection
- Viral-mediated delivery

Please keep in mind that for RNP complexes generated with IDT's AltR® CRISPR systems, only lipofection, electroporation/nucleofection and microinjection are viable options.

	LIPOFECTION	ELECTROPORATION/ NUCLEOFECTION	MICROINJECTION	VIRUS
Principle	Lipid complexed with CRISPR components fuses with the cell membrane.	Electric pulse forms pores in the cell membrane for entry of CRISPR components.	Microneedle injects CRISPR components inside cells, oocytes, or zygotes.	DNA/RNA is packaged into infectious particles and introduced into cells.
Advantages	Cost-effective High throughput	Easy, fast High efficiency	High efficiency	High efficiency
Limitations	Less efficient	Requires optimization Requires specialized equipment	Time-consuming Technically demanding Low throughput	Time-consuming Safety requirements Expensive

Discussion: transient vs stable transfection

In principle, Cas9 nuclease and its guide RNA can either be transiently expressed (i.e. a limited amount of Cas9 and guide RNA are expressed in cells and decay over time) or they can be stably integrated into the cell's genome, ensuring long-term expression.

	TRANSIENT TRANSFECTION	STABLE TRANSFECTION
Principle	Lipofection, electroporation/nucleofection or microinjection of RNP complexes or expression plasmids	Lipofection, electroporation/nucleofection or microinjection of expression plasmids, viral transduction
Advantages	Easy to achieve No screening for Cas9 and/or guide RNA expressing cells needed Lower risk of off-target edits due to transient expression of Cas9 nuclease	Ideal if long-term expression of CRISPR components is needed
Limitations	Switching guide RNAs requires repeating the entire transfection procedure	Requires selection via e.g. antibiotic selection or FACS sorting, followed by expansion



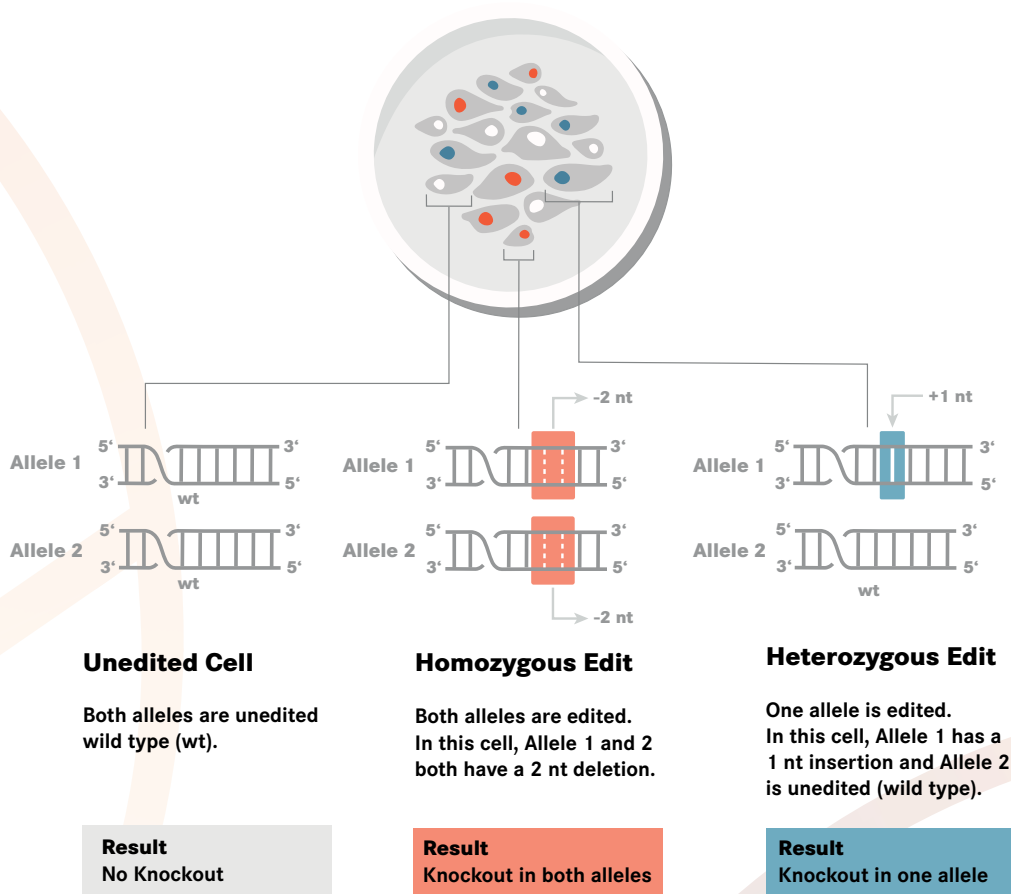
Analysis

After delivery of the editing complex, you normally have a heterogeneous cell population consisting of unedited and edited cells. Edited cells may either be heterozygous (one allele edited) or homozygous (both alleles edited).

Depending on the goal of your experiment and your downstream analysis, you may either continue with a heterogeneous cell population or you may have to pick, analyse and expand individual edited cells.

You will find a number of assays and kits below which are useful for analysis of your edited cell population.

Knockout Cell Pool





DNA isolation from edited cells

QuickExtract™ DNA Extraction Solution

A simple, rapid extraction of PCR-ready DNA for screening your edited cells.

- 8-minute extraction protocol for most sample types
- No centrifugation steps or spin columns
- Automation-friendly: simple protocol integrates easily into automated workflows
- Safe: uses only non-toxic reagents
- Recommended for rapid, easy sample prep for CRISPR mutation detection assays

SUPPLIER	CAT-NO.	ITEM	SIZE
Lucigen	QE09050	QuickExtract™ DNA Extraction Solution	50 ml (100 extractions)

Alternative options

DNA extraction kits from Norgen Biotek offer a rapid and convenient spin column procedure. Purified DNA is of the highest quality and integrity for sensitive downstream applications. The Cells and Tissue DNA Isolation Micro Kit is optimized for small inputs of cells and tissues, such as laser-captured microdissection (LCM).

SUPPLIER	CAT-NO.	ITEM	SIZE
Norgen	24700	Genomic DNA Isolation Kit	50 preps
Norgen	57300	Cells and Tissue DNA Isolation Micro Kit	50 preps



Alt-R® Genome Editing Detection Kit

For detection of on-target or known off-target CRISPR events in cultured cells. This T7 endonuclease I (T7EI) mismatch cleavage assay detects on-target genome editing and estimates genome editing efficiency in CRISPR-treated cells.

- Fast – does not require purification of PCR products before analysis
- Consistent – analyse easy-to-read electrophoresis results
- Quantifiable – gel band intensities relate to genome editing efficiency
- Versatile – convenient for a single sample or high-throughput analysis in plate format

SUPPLIER	CAT-NO.	ITEM	SIZE
IDT	1075931	Alt-R® Genome Editing Detection Kit	25 rxn (larger sizes available)

rhAmpSeq for evaluation of edits

The rhAmpSeq system enables highly accurate amplicon sequencing on Illumina™ next-generation sequencing (NGS) platforms. The fast and easy rhAmpSeq workflow, based on IDT's proprietary RNase H2-dependent PCR technology, generates NGS-ready amplicon libraries for deep, targeted resequencing.

- Achieve cost-effective library preparation using custom or pre-designed panels
- Reduce the formation of primer dimers or misprimed PCR products
- Perform only two PCR amplification steps in a fast, easy workflow

SUPPLIER	CAT-NO.	ITEM	SIZE
IDT	N/A	Custom rhAmpSeq Panels	Custom



Storage of edited cell pools and clonal lines

Store your valuable cell pools and clonal cell lines using Bambanker™ in order to ensure maximum viability during long-term storage. Its innovative formulation simplifies the freezing process and greatly increases cell viability. With Bambanker™, the viability of cells during freeze/thaw cycles is greatly enhanced, even for fragile cells such as embryonic stem cells.

SUPPLIER	CAT-NO.	ITEM	SIZE
Nippon Genetics	BB01	Bambanker™ Standard	120 ml
Nippon Genetics	BBD01	Bambanker™ Direct	20 ml
Nippon Genetics	BBH01	Bambanker™ HRM	20 ml
Nippon Genetics	BBF01	Bambanker™ DMSO-free	20 ml





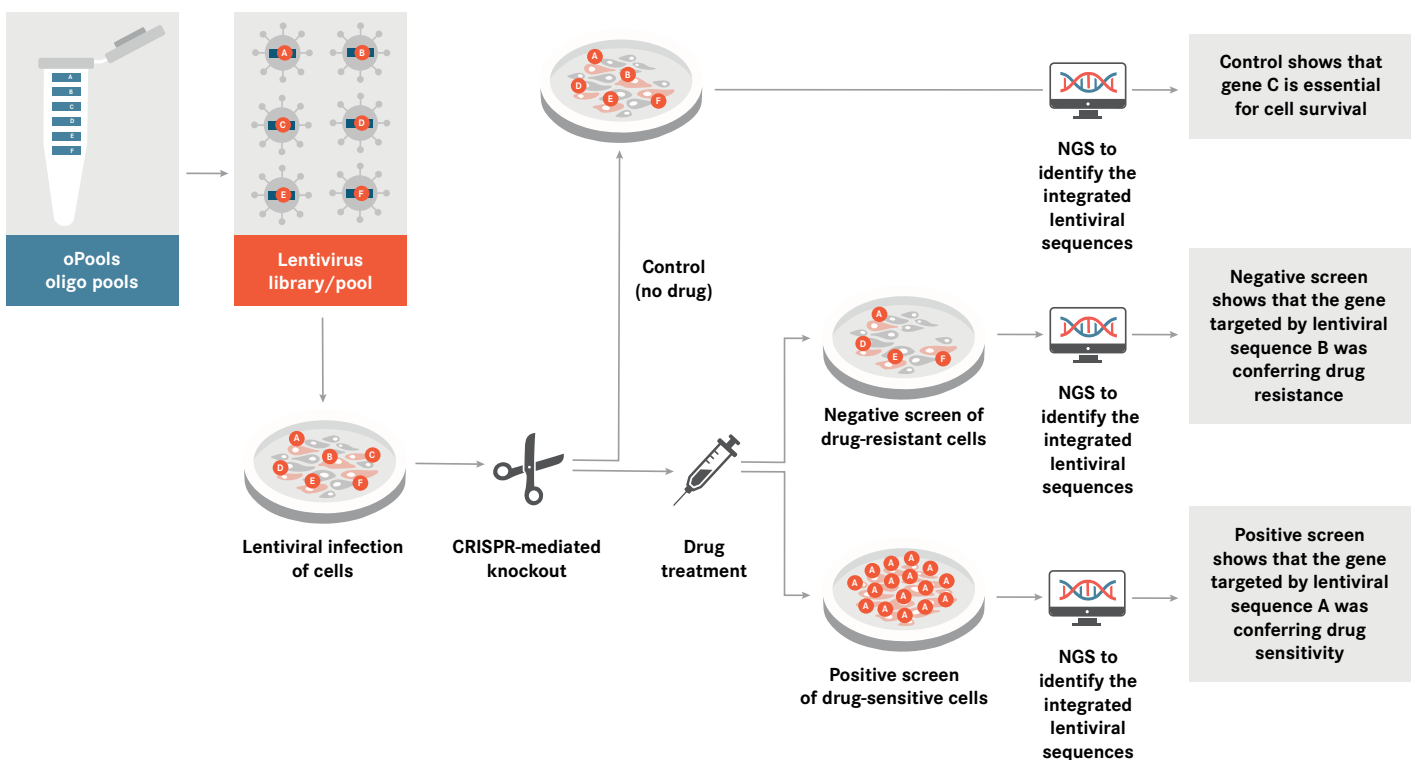
CRISPR screening

CRISPR is also used in genome-wide functional screening and is rapidly replacing older technologies, such as RNAi. Using CRISPR in combination with gRNA libraries, hundreds of genes can be efficiently knocked out in a single experiment.

A pool of oligos is designed to target a massive number of genes. A library of lentiviruses is produced from the oligos and used to infect cells. CRISPR genome editing knocks out different genes in different cells. Next-generation sequencing is used to determine which genes are present and which are absent. Genes for drug resistance or for drug sensitivity can be identified; negative screens determine genes conferring resistance, and positive screens determine genes conferring sensitivity.

CRISPR SCREENING

If you want to understand the possibilities of CRISPR screening, read IDT's Screening Primer at www.lubio.ch/crispr-screens



CRISPR array screening workflow. CRISPR-mediated knock-out screens are a powerful tool to investigate gene function directly in mammalian cells. Genes of interest are targeted via a library of oligo pools, resulting in a collection of knock-out cell clones. These can be challenged e.g. with a drug to identify genes which confer drug sensitivity or resistance, respectively.



oPools Oligo Pools

Use oPools Oligo Pools for accurate, reliable, and affordable CRISPR libraries. These pools of custom single-stranded DNA sequences offer high fidelity, uniformity, low error rates, and low dropout rates. This means you can avoid amplification bias, varying concentrations, or high error rates that are often encountered when using pooled oligos from other suppliers.

- Get started immediately: fast delivery and no amplification required
- Reduce experimental variability with more complete coverage

SUPPLIER	CAT-NO.	ITEM	SIZE
IDT	N/A	oPools custom oligo pools for CRISPR screening	Custom

 </crispr-screening>

Endura competent cells for CRISPR library construction

Clone repetitive sequences and lentiviral CRISPR libraries with high efficiency.

- Stabilize direct repeats and generate lentiviral guide RNA libraries
- Choose electrocompetent or chemically competent cells
- Highest efficiency commercially available cells for lentiviral cloning: over 1×10^7 cfu/ μg (chem) or 1×10^{10} cfu/ μg (electro)

SUPPLIER	CAT-NO.	ITEM	SIZE
Lucigen	60240-1	Endura chemically competent cells (DUO)	12 rxn
Lucigen	60241-1	Endura chemically competent cells (SOLO)	12 rxn
Lucigen	60242-1	Endura electrocompetent cells (DUO)	12 rxn



Further reading

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Zhang F, Wen Y, Guo X (2014). “CRISPR/Cas9 for genome editing: progress, implications and challenges”. *Human Molecular Genetics*. **23**(R1): R40–6. doi:10.1093/hmg/ddu125. PMID 24651067.

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Lander ES (January 2016). “The Heroes of CRISPR”. *Cell*. **164**(1–2): 18–28. doi:10.1016/j.cell.2015.12.041. PMID 26771483.

Quadros RM, Miura H, Harms DW et al. (2017). “Easi-CRISPR: a robust method for one-step generation of mice carrying conditional and insertion alleles using long ssDNA donors and CRISPR ribonucleoproteins”. *Genome Biology*. **18**: 92.

Jacobi AM, Rettig GR, Turk R, Collingwood MA, Zeiner SA, Quadros RM, Harms DW, Bonthuis PJ, Gregg C, Ohtsuka M, Gurumurthy CB, Behlke MA (2017). “Simplified CRISPR tools for efficient genome editing and streamlined protocols for their delivery into mammalian cells and mouse zygotes”. *Methods*. **121–122**: 16–28.



Links

Online design tools

<https://eu.idtdna.com/pages/tools> (overview of all IDT web tools)

https://eu.idtdna.com/site/order/designtool/index/CRISPR_PREDESIGN (predesigned gRNA search)

https://eu.idtdna.com/site/order/designtool/index/CRISPR_CUSTOM (design custom gRNA)

https://eu.idtdna.com/site/order/designtool/index/CRISPR_SEQUENCE (CRISPR-Cas9 gRNA checker)

<https://eu.idtdna.com/pages/tools/alt-r-crispr-hdr-design-tool> (Alt-R CRISPR HDR Design Tool)

<https://chopchop.cbu.uib.no/> (web selection tool for CRISPR target site)

Information

<https://eu.idtdna.com/pages/support/guides-and-protocols> (overview of IDT user guides and protocols)

<https://eu.idtdna.com/pages/education/videos/genome-editing> (online videos on genome editing)

<https://eu.idtdna.com/pages/support/faqs> (IDT FAQs)

<https://eu.idtdna.com/pages/education/publications> (IDT publications)

<https://www.lubio.ch/crispr>

Product order

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