Alt-R™ CRISPR Systems

A complete workflow solution, from design to analysis

Use of the CRISPR (clustered regularly interspaced short palindromic repeats) and associated enzymes for genome editing has been a major technological breakthrough, making genome modification in cells or organisms faster, more efficient, and more accurate than previous genome editing methods. The Alt-R CRISPR Systems are optimized genome editing solutions for producing on-target, double-stranded DNA breaks.

The Alt-R CRISPR Systems were developed through comprehensive research on each component of the CRISPR-driven, double-stranded break generation critical for gene disruption (knock-out) and DNA insertion by homologous recombination (knock-in, aka HDR).

Applications and IDT product solutions

A complete workflow solution

Design	Guides & protein	Knock-in (HDR) reagents	Analysis
Alt-R Cas9 gRNA Design Tool • Predesigned guides • Custom designs • Design checking **Alt-R Cas9 HDR Design Tool** • Friendly UI • Empirically defined design rules • Integration with Cas9 gRNA designs	**Alt-R gRNAs** • Cas9 crRNA:tracrRNA • Fluorescently labeled tracrRNAs • Cas9 sgRNA • Cas12a crRNA • Custom ordering for any gRNA (e.g., pegRNA, Cas13) **Alt-R CRISPR proteins** • WT Cas9 • HiFi Cas9 • Cas9 nickases, dCas9 • *A.s.* Cas12a *Ultra* • *L.b.* Cas12a *Ultra* • Fluorescently labeled Cas9 • Glycerol-free Cas9 **Alt-R Electroporation Enhancers**	**Alt-R HDR Donor Oligos** • Up to 200 nt • Modified ssODNs **Alt-R HDR Donor Blocks** • Modified to reduce blunt integration • Up to 3000 nt • Sequence-verified by NGS **Alt-R HDR Enhancers** • v2 small molecule **Megamer Fragments** • ssDNA 200–2000 bases • Sequence-verified via NGS	**Genome Editing Detection Kit** • T7EI assay **rhAmpSeq CRISPR Analysis System** • Multiplexed amplicon NGS sequencing (singleplex on-target, multiplex off-target) • rhAmpSeq CRISPR Analysis Tool

CRISPR gene knock-out

Designing guide RNAs

The **IDT guide RNA design tool** can be used to help you design gRNA sequences for use with Cas9 with predicted high on-target and low off-target activity in human, mouse, rat, zebrafish, and *C. elegans* for your specific research application.

You can choose from a library of **predesigned guide RNAs** for the five species, or target a custom sequence from any species using the **custom guide RNA design tool**. The IDT **Cas9 gRNA design checker tool** allows you to assess on- and off-target potential of guide RNA sequences you have designed yourself, or have obtained from publications, before ordering.

We offer three guide RNA formats: crRNA:tracrRNA duplex, crRNA XT:tracrRNA duplex, and single guide RNA (sgRNA). For crRNAs use with *A.s.* Cas12a, use the IDT **CRISPR-Cas12a (Cpf1) crRNA Oligo ordering tool**.

In addition to Cas9 and Cas12a systems, we also offer chemically synthesized and modified custom guide RNAs for specialized research applications. **Alt-R Custom Guide RNAs** are ideal for prime editing (pegRNA) projects, CRISPR-Cas13 applications, and most alternative CRISPR-Cas systems.

All our CRISPR guide RNAs are available with chemical modifications that increase stability, potency, and/or nuclease resistance. Some modifications can decrease unwanted immune responses in your experiment

- sgRNAs in days, not weeks, with fast synthesis time (3–5 business days*)
- Guaranteed function with predesigned sgRNAs
- Custom features to meet your needs, such as a variety of deliverable sizes, chemical modifications, and purification
- Trusted quality and manufacturing, delivering optimized synthesis and purification to mitigate oligo cross contamination risk

* 3–5 business days for most standard requests. Custom requests may require additional manufacturing time.

CRISPR nucleases

Our Alt-R Cas enzymes are designed to improve on-target CRISPR editing and are available in a variety of formats to fit your project needs. From the widely adopted *S. pyogenes* Cas9 to our proprietary engineered mutants, we're sure to have the perfect option for your next gene editing venture.

- More editing options from multiple Cas systems
- Flexible sizing and formulations
- Complete CRISPR workflow solutions

Alt-R CRISPR enzymes are available in a variety of formats, with stock sizing available up to 50 mg. Larger formats and lot matching are also available for all products upon request.

Cas protein	Available versions	Key features
S.p. Cas9 Nuclease V3	Wild-type, 50% Glycerol	Targeting GC-rich regions
	Wild-type, Glycerol-free	Low viscosity for high-throughput applications
	High Fidelity (HiFi)	Reduced off-target activity*
	Wild-type Fluorescent-fusion	Fluorescent label for enrichment (GFP or RFP)
A.s. Cas12a (Cpf1) V3	Wild-type	Targeting AT-rich regions
	Ultra	Increased on-target activity*
L.b. Cas12a (Cpf1)	*Ultra*	Increased on-target activity and low-temperature tolerance*

* when compared to corresponding Wild-type controls

Homology-directed repair (HDR, a.k.a. knock-in) tool and reagents

Alt-R HDR Design Tool

Streamline your homology-directed repair (HDR) research by using IDT's **HDR Design Tool**. Simply provide basic information about your target site, then use the HDR tool to design and visualize your desired edit within the sequence The Alt-R HDR Design Tool enables increased HDR rates by providing optimized donor template design and Cas9 guide RNA selection. The higher HDR rates result from clear design rules based on extensive wet bench testing and customer experimentation.

Alt-R HDR Donor Oligos are enhanced Ultramer™ DNA oligos specifically built for homology-directed repair (HDR) in research applications. Our proprietary synthesis process delivers HDR-ready oligos of high quality up to 200 bases manufactured under ISO 9001 standards. Alt-R HDR Donor Oligos are ideal for introducing point mutations or short insertions.

Alt-R HDR Donor Blocks are developed for researchers creating large changes or insertion (>120 bases) in the genome. Utilizing the same high-fidelity process as IDT gBlocks™ HiFi Gene Fragments, the HDR Donor Blocks incorporate advanced chemical modifications at each end of the sequence to boost HDR rates and aid in inhibiting the occurrence of blunt-end integration of the donor sequence. Alt-R HDR Donor Blocks are available from 201 to 3000 bases in length.

Improved large knock-ins

Reduced off-target integration

NGS-verified

Alt-R DHR Enhancer V2 is a small molecule compound that increases homology-directed repair. Alt-R HDR Enhancer V2 exhibits its activity in multiple cell lines, including both adherent and suspension cell lines. Its activity is independent of the enzyme employed; for example, it can be used either with Alt-R *S.p.* Cas9 nucleases or A.s. Cas12a (Cpf1) nucleases. This versatile reagent is also compatible with **electroporation and lipofection methods**.

CRISPR Screening

Alt-R Custom CRISPR gRNA libraries are available for all CRISPR nucleases, including Cas9, Cas12a, Cas13, prime editing enzymes, and others. These libraries were developed to address the need for better CRISPR screening solutions. They are chemically modified guide RNAs (gRNAs) synthesized on the IDT proprietary high-fidelity RNA manufacturing platform to provide high quality, reliable gRNA libraries with fast delivery.

Fast turnaround time, flexible formuation

Adaptable for different CRISPR systems

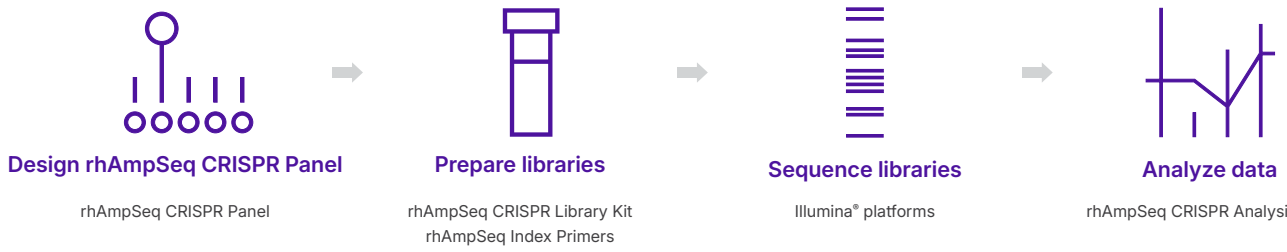
Complete CRISPR workflow solution from custom design to analysis

Features	Options
Design	Predesigned, custom, user-provided
CRISPR systems	Cas9, Cas12a, Cas13, prime editing, and other alternative systems
Guaranteed yield	0.5 nmol, 2 nmol, 5 nmol, and custom normalized deliverables
Cas9 gRNA formats	2-part crRNA:tracrRNA complex and sgRNA
Custom lengths supported	30–150 nt
Chemical modifications	2′-O-methyl RNA, PS linkages, end-blocking Alt-R modifications
Plate types	96- & 384-well PCR, Deep-well, V-bottom, ECHO, custom options available
Formulation options	Multi-guide per well; pooled by gene Arrayed (single gRNA/well) Custom formulations upon request
QC	Individual ESI/MS
Supporting reagents & functional analysis pipeline (optional)	WT Cas9, HiFi Cas9, Cas12a and Cas12a Ultra Glycerol-free options available in tubes or plates (ideal for robotics) Electroporation Enhancers rhAmpSeq™ CRISPR Analysis System (NGS-based on-/off-target editing analysis)

Genome Editing Analysis

rhAmpSeq™ CRISPR Analysis System allows quick and accurate quantification of CRISPR-Cas edits. Our proprietary RNAse H2-dependent PCR technology generates amplicon libraries for targeted sequencing on Illumina® NGS platforms. The system also includes an advanced but accessible cloud-based data analysis pipeline for quantification of on- and off-target edits.

The rhAmpSeq CRISPR Analysis System offers a convenient workflow that takes you from sample to results in under a week.



- **rhAmpSeq CRISPR Panels** build your custom panels online for CRISPR on- and off-target editing analysis in a variety of species.
- **rhAmpSeq CRISPR Library Kit** is a rapid, cost-effective library preparation for rhAmpSeq CRISPR targeted sequencing. The rhAmpSeq CRISPR Library Kit also comes with analysis credits to enable data processing and quantification of editing events via the rhAmpSeq CRISPR Analysis Tool. Each analysis credit allows for the analysis of up to 500 targets for one, indexed sample.
- **rhAmpSeq Index Primers** enables combining rhAmpSeq amplicon libraries in a single sequencing run for maximum efficiency.
- **rhAmpSeq CRISPR Analysis Tool** is a flexible, cloud-based tool for interrogation of CRISPR-mediated, double-strand breaks. Obtain publication-ready data using our advanced cloud-based data analysis pipeline without the need for coding or advanced bioinformatics experience.

Alt-R Genome Editing Detection Kit

Alt-R Genome Editing Detection Kit was developed for detection of on-target or known off-target CRISPR events in cultured cells. It is a T7EI mismatch endonuclease cleavage assay (gel-based) and provides a good estimate of genome editing efficiency. However, because T7EI endonuclease does not recognize single-base insertions or deletions, this method underestimates editing efficiency when compared to next generation sequencing (NGS).

* We guarantee that predesigned Alt-R CRISPR-Cas9 guide RNAs will provide successful editing at the target site, when delivered as a ribonucleoprotein complex as described in the Alt-R User Guides, using Alt-R CRISPR-Cas9 guide RNAs (crRNA:tracrRNA duplex or sgRNA) and either Alt-R S.p. Cas9 nuclease or Alt-R S.p. HiFi Cas9 nuclease. Analysis of editing must be at the DNA level, such as with the Alt-R Genome Editing Detection Kit or DNA sequencing. If successful editing is not observed for a predesigned guide RNA while an appropriate positive control is successful, a one-time "no-cost" replacement of the predesigned Alt-R CRISPR-Cas9 guide RNA will be approved. **Contact us** for more information. This guarantee does not extend to any replacement product, or to any other incurred or incidental costs or expenses.

For more information, visit **idtdna.com/CRISPR**