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Alt-R™ CRISPR-Cas9 System

For increased efficiency of genome editing

The Alt-R CRISPR-Cas9 System includes all of the reagents needed for successful genome editing. Based on the natural *S. pyogenes* CRISPR-Cas9 system, the Alt-R CRISPR-Cas9 System offers numerous advantages over alternative methods:

- Higher on-target editing efficiency than other CRISPR systems
- Increased precision and control by delivering Cas9 ribonucleoprotein (RNP)
- Efficient delivery of the RNP using lipofection or electroporation
- No toxicity or innate immune response activation as observed with *in vitro* transcribed *Cas9* mRNA and sgRNAs

benefits

Focus on results with higher potency CRISPR reagents.

Save time and money with an efficient workflow and ready-to-use reagents.

Increase precision and reduce off-target editing using CRISPR-Cas9 ribonucleoprotein.

Adapt to your experimental needs with compatibility for lipofection or electroporation.

Discover more at
www.idtdna.com/CRISPR-Cas9

Potent editing with Alt-R S.p. Cas9 Nuclease 3NLS

The Alt-R CRISPR-Cas9 System includes the potent Alt-R S.p. Cas9 Nuclease 3NLS. The system outperforms other editing methods by combining the Alt-R S.p. Cas9 Nuclease 3NLS with the optimized, chemically modified Alt-R CRISPR-Cas9 crRNA and tracrRNA into an RNP (Figure 1). RNP transfections allow optimal dose control of editing complexes, and the non-renewable Cas9 RNP is cleared after a short duration by endogenous mechanisms, limiting off-target editing.

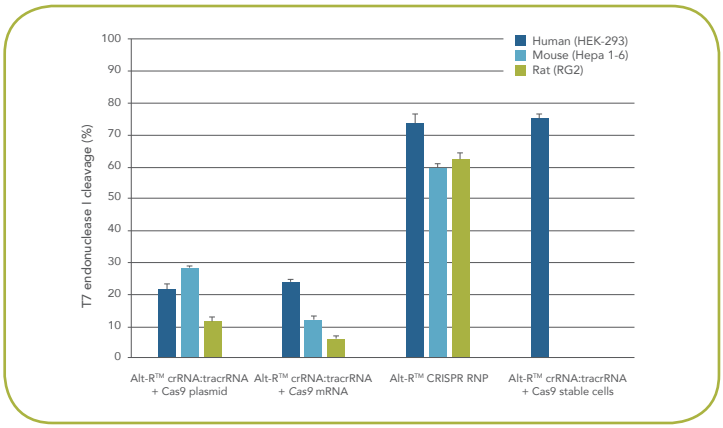

Figure 1. Alt-RTM CRISPR-Cas9 System ribonucleoprotein outperforms other transient CRISPR-Cas9 editing methods. Alt-R CRISPR HPRT Control crRNAs for human, mouse, or rat were complexed with Alt-R CRISPR-Cas9 tracrRNA. Resulting complexes were transfected with Cas9 expression plasmid, Cas9 mRNA, or as a Cas9 RNP (containing Alt-R S.p. Cas9 Nuclease 3NLS precomplexed with the crRNA and tracrRNA) into human (HEK-293), mouse (Hepa1-6), or rat (RG2) cell lines. Mutation detection using T7EI assays showed that the Alt-R CRISPR-Cas9 RNP outperformed the other transient Cas9 expression methods, and performed comparably to reference HEK-293–Cas9 cells that stably express S. pyogenes Cas9.

Potent performance simplifies design

The optimized crRNA and tracrRNA of the Alt-R CRISPR-Cas9 System outperform other CRISPR guide formats (Figure 2). In fact, the system is so potent that minimal design effort is needed for the crRNA. The only requirement is a site-specific, 19- or 20-base sequence immediately adjacent to the PAM (protospacer adjacent motif; NGG) sequence in your gene. Using Alt-R CRISPR-Cas9 crRNAs, on average, >80% of target sequences will show potent editing under standard conditions (Figure 3).

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IDT® INTEGRATED DNA TECHNOLOGIES

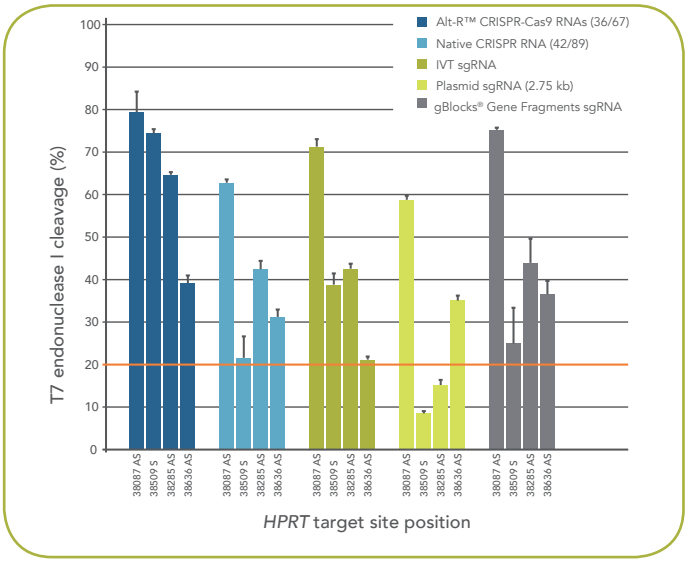

Figure 2. Optimized crRNA:tracrRNA outperforms other guide RNA types. Alt-R™ CRISPR-Cas9 RNAs, native *S. pyogenes* CRISPR RNAs, *in vitro* transcribed (IVT) single-guide RNAs (sgRNA), and sgRNAs expressed from an expression plasmid or gBlocks® Gene Fragments targeted 4 human *HPRT* gene sites (38087 AS, 38509 S, 38285 AS, and 38636 AS). The guide RNAs were transfected into HEK-293–Cas9 cells, that stably express Cas9. Mutation detection using a T7EI assay showed that the Alt-R CRISPR-Cas9 RNAs outperformed other guide RNA types at most sites.

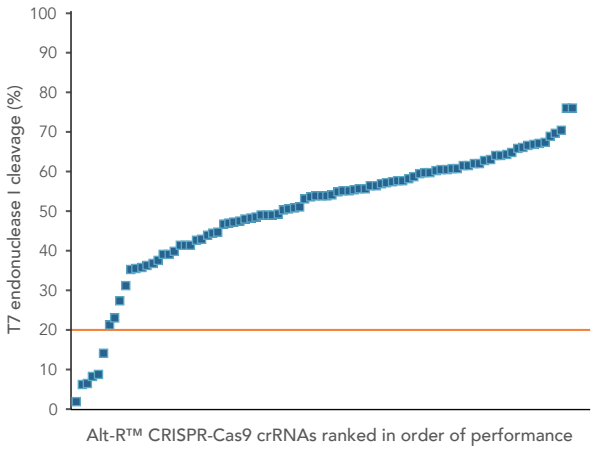

Figure 3. Alt-R™ CRISPR-Cas9 crRNA designs for 92 PAM sites in the STAT3 gene deliver potent editing efficiency. Alt-R CRISPR-Cas9 crRNAs (n = 92) were generated, targeting every PAM site in the *STAT3* locus. Mutation detection using T7EI assays showed that 93% of the crRNAs provided good to excellent performance in HEK-293–Cas9 cells, with editing efficiency >20%. Note: T7EI does not detect single-base deletions or insertions, and underestimates editing efficiency.

Ordering information

CRISPR guide RNAs

Product	Size	Catalog #
Alt-R™ CRISPR-Cas9 crRNA	2, 10 nmol tubes or plates	Order at www.idtdna.com/CRISPR-Cas9
Alt-R™ CRISPR-Cas9 tracrRNA	5 nmol	1072532
	20 nmol	1072533
	100 nmol	1072534

Cas9 endonuclease

Product	Size	Catalog #
Alt-R™ S.p. Cas9 Nuclease 3NLS	100 µg	1074181
	500 µg	1074182

Control kits*

Product	Catalog #
Alt-R™ CRISPR-Cas9 Control Kit, Human (2 nmol)	1072554
Alt-R™ CRISPR-Cas9 Control Kit, Mouse (2 nmol)	1072555
Alt-R™ CRISPR-Cas9 Control Kit, Rat (2 nmol)	1072556

* Control kit components are also available individually.

Control kit contents

- Alt-R™ CRISPR HPRT Positive Control crRNA
- Alt-R™ CRISPR Negative Control crRNA #1
- Alt-R™ CRISPR-Cas9 tracrRNA
- Alt-R™ HPRT PCR Primer Mix
- Nuclease-Free Duplexing Buffer

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For Research Use Only. Not for use in diagnostic procedures.

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