ALT-R™ HDR DESIGN TOOL & TEMPLATES

Streamlining CRISPR-Cas9 homology-directed repair (HDR) research with an intelligent and easy-to-use design tool

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.

FLEXIBLE INPUT AND DESIGN PARAMETERS

The Alt-R HDR Design Tool provides exceptional flexibility. It allows entry by gene name, accession number, genomic coordinates, or sequence in FASTA format for multiple species including human, mouse, rat, zebrafish, and *C. elegans*. The tool also supports custom designs and single or multiple entries.

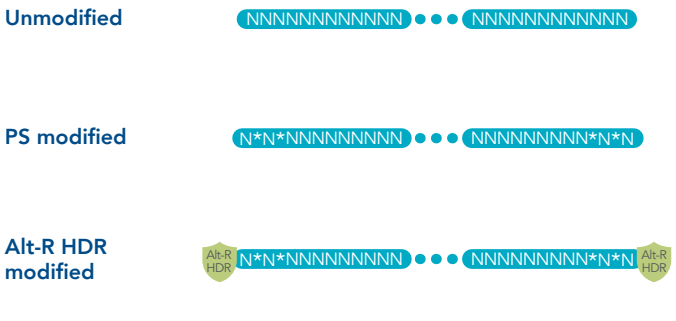
REMARKABLE HDR RATES WITH Alt-R HDR DONOR OLIGOS

Alt-R HDR Donor Oligos are HDR-ready oligos of up to 200 bases. They include 2 phosphorothioate (PS) linkages and an IDT proprietary end-blocking modification at each end to provide increased stability (Figure 1A). Ideal for introducing point mutations or short insertions, Alt-R HDR Donor Oligos are offered in tube or plate formats. Donor oligos are also available unmodified or PS modified for your HDR experiments.

Alt-R HDR MODIFICATIONS IMPROVE HDR EFFICIENCY

IDT offers donor ssDNA strands in 3 formats: unmodified, PS modified, and Alt-R HDR modified. Our data show that use of the Alt-R HDR modification results in the highest HDR efficiency (Figure 1B).

A. Locations of donor oligo modifications



N = DNA base
* = phosphorothioate linkage
Alt-R HDR = IDT proprietary end-blocking modification

B. Improvements in HDR seen with modifications

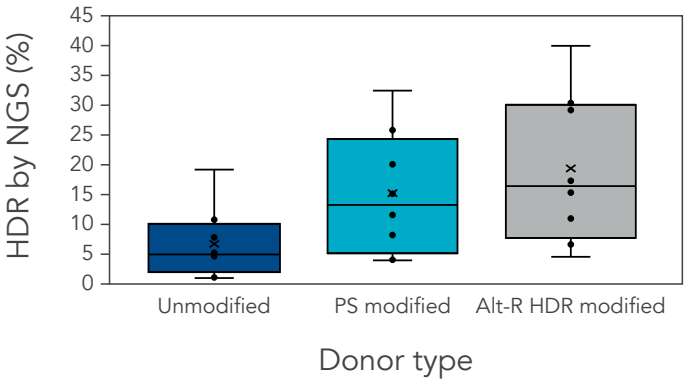


Figure 1. Alt-R HDR modified donors improve HDR efficiency over other donor types. (**A**) Diagram representing donor oligo modification formats. (**B**) Improvement in HDR efficiency. RNP complexes (2 µM) targeting 4 genomic loci, along with 0.5 µM single-stranded HDR donor oligo, were delivered into Jurkat and HeLa cells by electroporation using the 4D-Nucleofector™ System (Lonza). The RNP complex comprised Alt-R S.p. HiFi Cas9 Nuclease V3 complexed with Alt-R CRISPR-Cas9 crRNA and tracrRNA. Donor templates contained no modifications (Unmodified), phosphorothioate linkages (PS modified), or the Alt-R HDR modification (Alt-R HDR modified). Genomic DNA was isolated 48 hours (HeLa) or 72 hours (Jurkat) after electroporation, and HDR efficiency was measured by amplicon sequencing on an Illumina™ MiSeq™ system (v2 chemistry, 150 bp paired-end reads).

Alt-R HDR ENHANCER V2 IMPROVES HDR EFFICIENCY SEVERAL FOLD

We tested several possible chemical compounds for their ability to improve HDR efficiency. Our Alt-R HDR Enhancer V2 has shown increased rate of HDR (Figure 2).

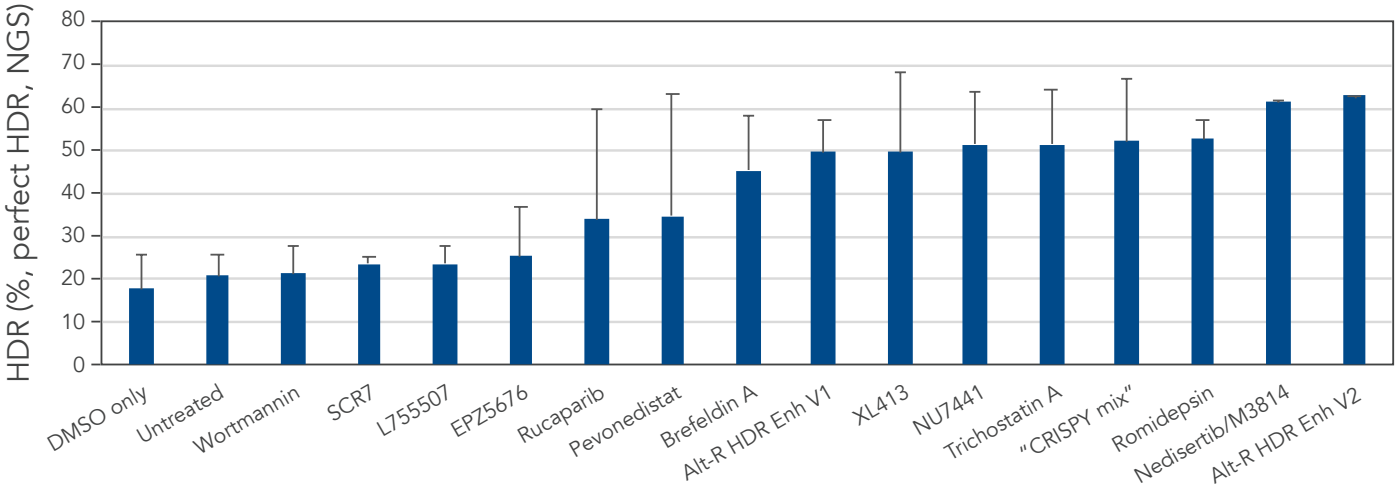


Figure 2. Alt-R HDR Enhancer V2 outperforms other small molecules from the literature. RNP complex (2 µM) targeting human HPRT1 and 3 µM Alt-R HDR Donor designed to insert an EcoRI restriction site were delivered together into Jurkat cells by electroporation using the 4D-Nucleofector™ System (Lonza). The RNP complex comprised Alt-R *S.p.* Cas9 Nuclease V3 complexed with Alt-R CRISPR-Cas9 crRNA and tracrRNA. Immediately after electroporation, cells were cultured in media containing no treatment, DMSO (vehicle control), or a recommended dose of small molecule (0.02 µM wortmannin, 5 µM SCR7, 5 µM L755507, 0.1 µM EPZ5676, 5 µM rucaparib, 1 µM pevonedistat, 0.1 µM brefeldin A, 30 µM Alt-R HDR Enhancer V1, 10 µM XL413, 2.5 µM NU7441, 10 µM trichostatin A, "CRISPY mix" [30 µM NU7026, 10 µM trichostatin A, 1 µM MLN4924], 0.1 µM romidepsin, 20 µM nedisertib/M3814, or 1 µM Alt-R HDR Enhancer V2). Genomic DNA was isolated 48 hours after lipofection, and target regions were amplified by PCR and sequenced on an Illumina™ MiSeq™ instrument. HDR efficiency was quantified using the IDT in-house NGS CRISPAltRations™ pipeline. The highest HDR rate was achieved when using the Alt-R HDR Enhancer V2 at 1 µM.

Alt-R HDR MODIFIED DONORS AND Alt-R HDR ENHANCER V2 LEAD TO THE HIGHEST HDR RATES

We investigated whether combining Alt-R HDR Enhancer with Alt-R modifications of HDR donor oligos would improve HDR rates further. Based upon internal experiments, our data has shown that this works well and leads to higher HDR rates.

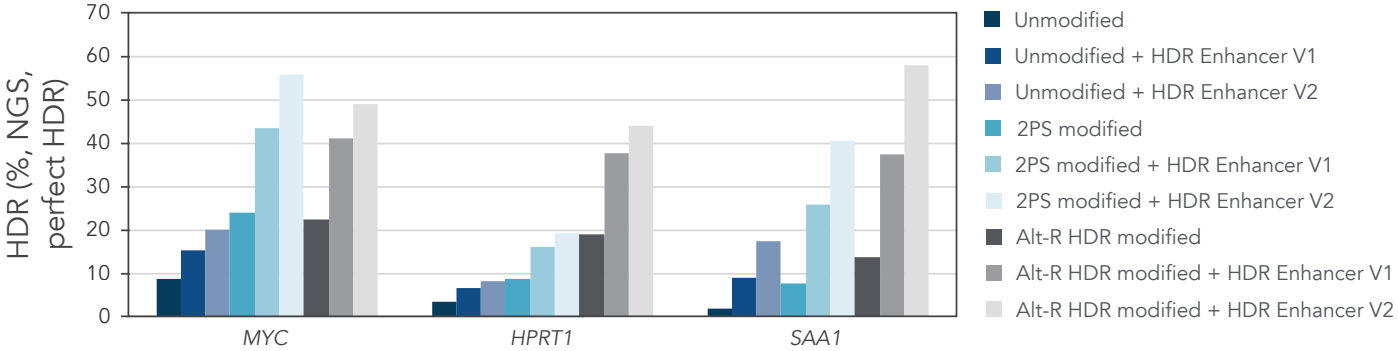


Figure 3. Alt-R HDR modified donors and Alt-R HDR Enhancer have an additive effect on HDR improvement. RNP complexes (2 µM) targeting three genomic loci (MYC, HPRT, and SAA1) along with 0.5 µM single-stranded Alt-R HDR donor oligo were delivered to HeLa cells by electroporation with 3 µM Alt-R Cas9 Electroporation Enhancer using the 4D-Nucleofector™ System (Lonza). The RNP complex comprised Alt-R *S.p.* HiFi Cas9 Nuclease V3 complexed with Alt-R CRISPR-Cas9 crRNA and tracrRNA. Unmodified, PS modified, or Alt-R HDR modified donor templates were used. Immediately after electroporation, cells were plated in media with either no treatment, 30 µM Alt-R HDR Enhancer (V1), or 1 µM HDR Enhancer V2. Genomic DNA was isolated 48 hours after electroporation, and HDR efficiency was measured by amplicon sequencing on an Illumina™ MiSeq™ system (v2 chemistry, 150 bp paired-end reads). The highest HDR rate was achieved with the combination of the Alt-R HDR Donor and HDR Enhancer V2.

Design and order your HDR donor templates and associated Cas9 guide RNAs for genome editing of human, mouse, rat, zebrafish, or *C. elegans* targets with the Alt-R HDR Design Tool at **www.idtdna.com/HDRdesigntool**.

If you already have your HDR donor design(s), order them at **www.idtdna.com/HDRdonoroligos** and take advantage of our HDR donor oligos specifically built for successful homology-directed repair in your research applications.