Alt-R HDR Design Tool & Templates

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

The Alt-R HDR Design Tool enables greatly 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 validation.

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 nematode. The tool also supports custom designs and single or multiple entries.

Industry-leading HDR rates with Alt-R HDR Donor Oligos

Alt-R HDR Donor Oligos are HDR-ready oligos of the highest quality, 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).

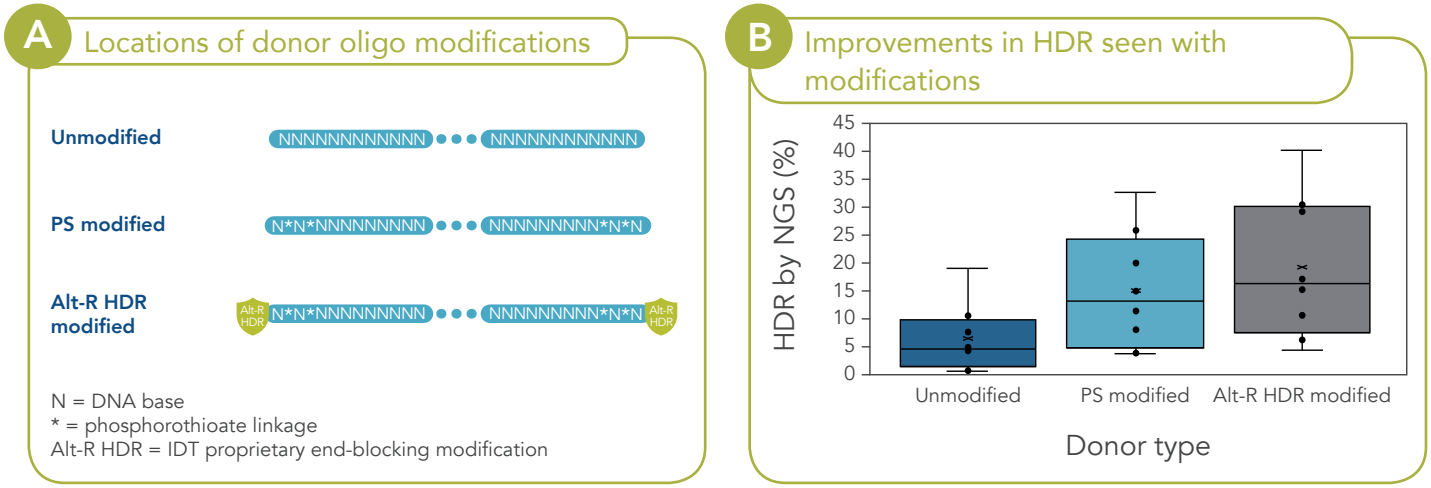

Figure 1. Alt-R HDR modified donors improve HDR efficiency over other donor types. (**A**) Diagram representing donor oligo modification formats. (**B**) Improvement in HDR efficiency. RNP complex (2 µM) targeting 4 genomic loci, along with 0.5 µM single-stranded HDR donor oligo was delivered into Jurkat 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 improves HDR efficiency several fold

We tested several possible chemical compounds for their ability to improve HDR efficiency. Our Alt-R HDR Enhancer more than tripled the rate of HDR, greatly outperforming all other compounds (Figure 2).

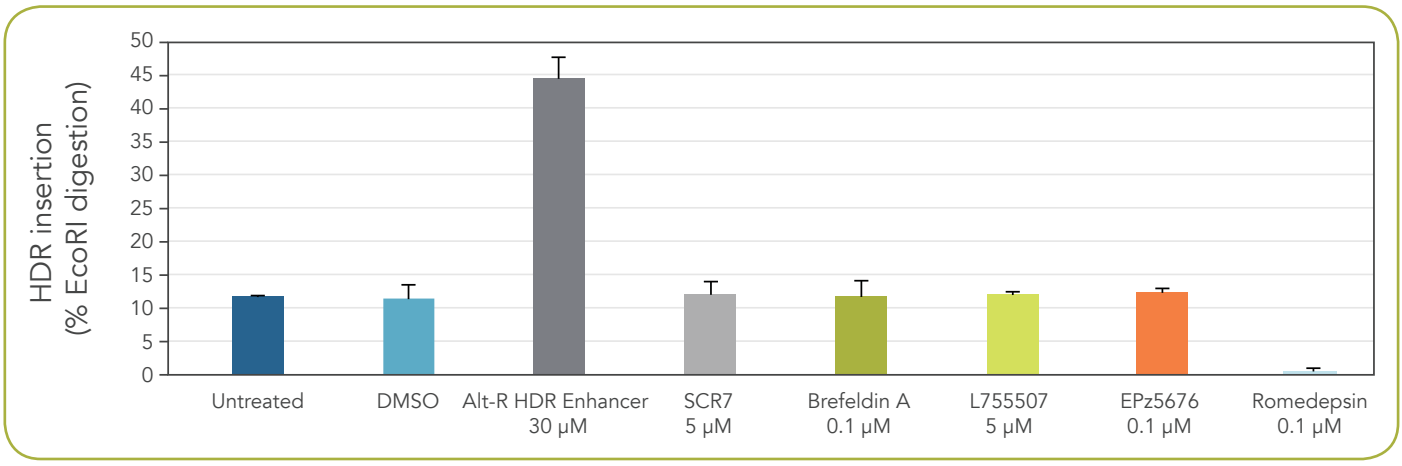

Figure 2. Alt-R HDR Enhancer improves HDR efficiency by more than 3-fold compared to other tested compounds. RNP complex (4 µM) targeting human *HPRT1* and 3 µM PS-modified HDR donor oligos designed to insert an EcoRI restriction site were delivered together into Jurkat cells by electroporation using 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 either 30 µM Alt-R HDR Enhancer or a recommended dose of small molecules (SCR7, Brefeldin A, L 755507, EPz5676, and Romedepsin) proposed to improve HDR rates. Similar volumes of 1X PBS or DMSO were used as the untreated and negative controls, respectively. Genomic DNA was isolated 72 hours after electroporation, and HDR efficiency was measured by EcoRI cleavage of PCR amplicons from the target region in the *HPRT1* gene.

Alt-R HDR modified donors and Alt-R HDR Enhancer 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. Our data confirmed that this works well, leading to the highest HDR rates (Figure 3).

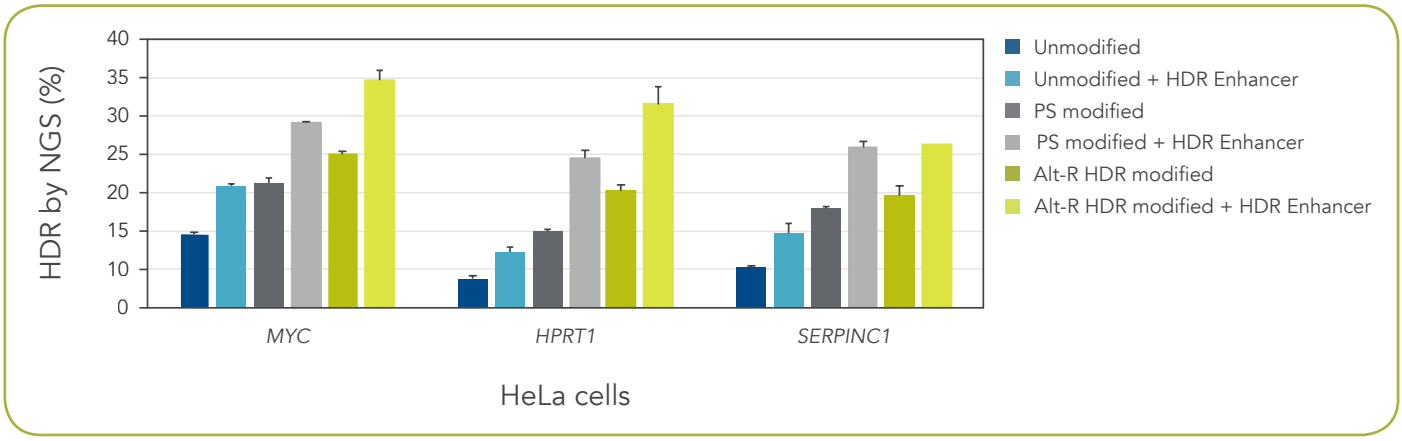

Figure 3. Combined use of Alt-R HDR modified donors and Alt-R HDR Enhancer have an additive effect on HDR improvement. RNP complex (2 µM) targeting 3 genomic loci along with 0.5 µM single-stranded HDR donor oligo were delivered to HeLa cells by electroporation using the 4D-Nucleofector™ System (Lonza). The RNP complex comprised Alt-R S.p. HiFi Cas9 Nuclease V3 complexed with Alt-R CRISPR-Cas9 crRNA and tracrRNA. Unmodified, PS-modified, or Alt-R HDR modified donor templates were used. Immediately after electroporation, cells were plated in media with or without 30 µM Alt-R HDR Enhancer. 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).

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, order them at **www.idtdna.com/HDRdonoroligos** and take advantage of our HDR donor oligos specifically built for successful homology-directed repair.

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