Alt-R™ HDR DONOR BLOCKS

gBlocks™ HiFi Gene Fragments specifically built for homology-directed repair (HDR) research

Improved large knock-ins

Reduced off-target integration

NGS-verified

HIGH-QUALITY DONOR FRAGMENTS FOR LARGE GENOME EDITING PROJECTS

Alt-R HDR Donor Blocks† were developed to address the need for better HDR solutions when creating larger changes or inserts in the genome. Utilizing the same high-fidelity process as IDT's gBlocks HiFi Gene Fragments, Alt-R HDR Donor Blocks incorporate advanced chemical modifications within universal terminal sequences to boost HDR rates and inhibit the occurrence of non-homologous (blunt) integration of the donor sequence.

BENEFITS

- Ideal for making large genomic changes and insertions
- Modified to increase successful HDR events
- Lower unintended blunt integrations at on- and off-target sites
- Sequence-verified by next generation sequencing
- Provides the highest HDR rates when combined with Alt-R HDR Enhancer V2

PRODUCT SPECIFICATIONS

Product type	Chemically end-modified dsDNA fragment
Fragment size	201–3,000 bp
Error rate	1: 12,000
Verification method	Next generation sequencing (NGS)
Shipping time	201–2,000 bp • starting from 12 business days* 2.001–3,000 bp • starting from 18 business days*

* Since manufacturing Alt-R HDR Donor Blocks depends on many factors, the actual delivery times may vary.

IMPROVED SOLUTION FOR EFFICIENT GENERATION OF LARGE KNOCK-INS

HDR efficiency of inserting a green florescent protein (GFP) tag using either long, single-stranded DNA (ssDNA) or double-stranded DNA (dsDNA) templates, was investigated. Use of Alt-R HDR Donor Blocks resulted in the highest HDR rates compared to unmodified dsDNA, or long ssDNA (Figure 1A). When combined with the Alt-R HDR Enhancer V2, modified Alt-R HDR Donor Blocks exhibited the highest HDR rates at multiple genomic loci and in multiple cell lines tested (Figure 1B).

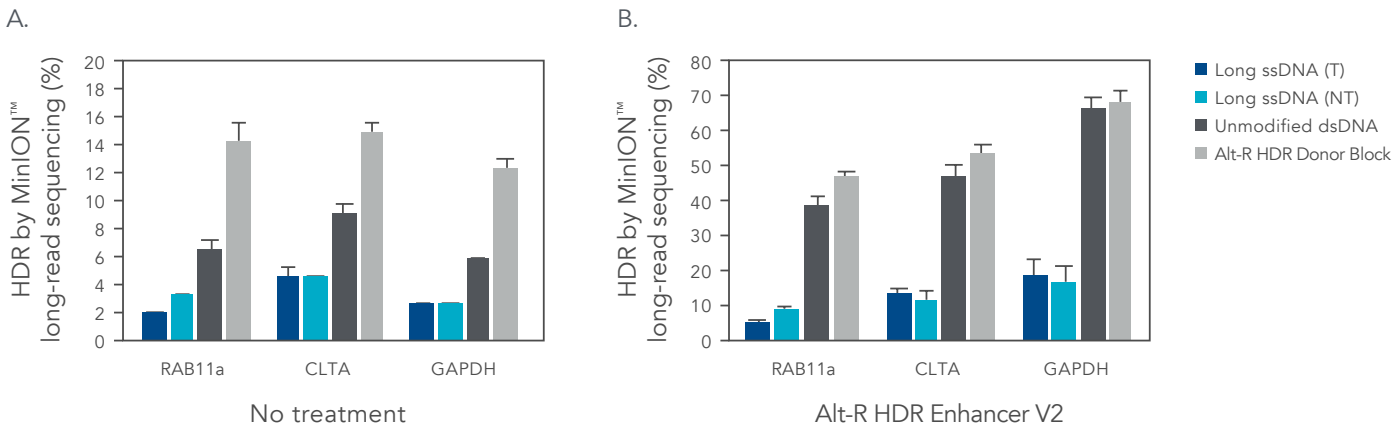

Figure 1. Alt-R HDR Donor Blocks improve large knock-in rates. Use of Alt-R HDR Donor Blocks increases large knock-in rates relative to long ssDNA HDR templates (**A**). When combined with Alt-R HDR Enhancer V2, Alt-R HDR Donor Blocks demonstrated the highest HDR rates (**B**). HEK 293 and K562 cells were electroporated with 2 µM Cas9 RNP complexes and 50 nM dsDNA or ssDNA donor templates using the Nucleofector™ system (Lonza). HDR templates were designed to mediate a GFP-tagging event (700 bp insert, 200 bp homology arms) at three genomic loci. The dsDNA templates contained either no modifications (unmodified), or the Alt-R HDR Donor Block modification. Both the targeting (T) and non-targeting (NT) strands were tested for the long ssDNA templates. After electroporation, cells were plated in media with or without 1 µM Alt-R HDR Enhancer V2 with a media change after 24 hours. Genomic DNA was isolated 48 hours after electroporation. Editing was assessed by long-read amplicon sequencing on the MinION™ system (Oxford Nanopore Technologies) and processed using an internal analysis pipeline.

COMBINED USE OF Alt-R HDR DONOR BLOCKS AND Alt-R HDR ENHANCER V2 MITIGATES THE RISK FOR OFF-TARGET INTEGRATION EVENTS

Since use of dsDNA templates may pose a risk for unwanted off-target integrations, the ability of Alt-R HDR Donor Blocks to reduce the occurrence of these unwanted events was examined. As shown in Figure 2, use of Alt-R HDR Donor Blocks reduced the blunt insertions at a mock off-target double strand break by 65% compared to unmodified dsDNA. Addition of Alt-R HDR Enhancer V2 further reduced the off-target integration, lowering blunt insertion levels to 1% (Figure 2).

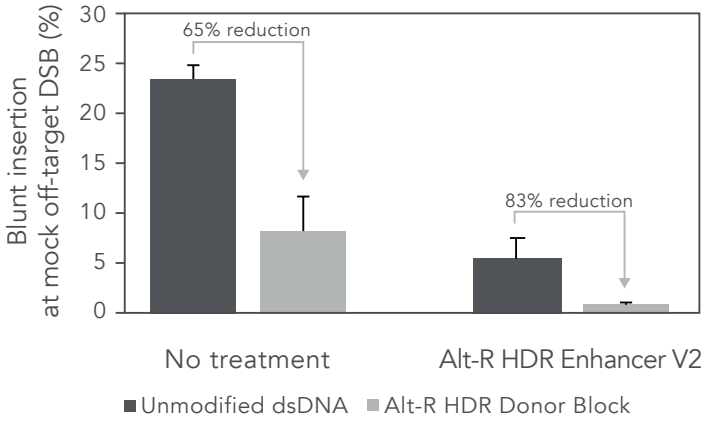

Figure 2. Combined use of Alt-R HDR Donor Blocks and Alt-R HDR Enhancer V2 reduces the rate of non-homologous (blunt) integrations at off-target DSBs. A mock off-target DSB was generated by delivering 2 µM Cas9 RNP targeting the SERPINC1 locus into HEK-293 cells using the Nucleofector™ system (Lonza). Next, 50 nM dsDNA donor templates mediating GFP insertions at alternative genomic loci (n=4 sequences) were codelivered with the mock off-target RNP. After electroporation, cells were plated in media with or without 1 µM Alt-R HDR Enhancer V2, followed 24 hrs later by a media change. Genomic DNA was isolated 48 hours after electroporation. The SERPINC1 locus was PCR-amplified, and blunt insertion events were measured by size discrimination on a Fragment Analyzer (Agilent).

ORDERING INFORMATION

Product	Length	Size	Ordering
Alt-R™ HDR Donor Block	201–500 bp, 501–2000 bp, 2001–3000 bp	3 or 10 µg	www.idtdna.com/HDRDonorBlocks
Alt-R™ HDR Enhancer V2		30 or 150 µL	10007910, 10007921

If you do not have a template design of your own, use our **Alt-R HDR Design Tool** to design your template. Simply provide basic information about your target site, then use the tool to design and visualize your desired edits within the sequence. The Alt-R HDR Design Tool will provide the recommended HDR donor template along with gRNA(s) for your specifications.

> FOR MORE INFORMATION, VISIT **WWW.IDTDNA.COM/HDRDONORBLOCKS**.