Improved CRISPR HDR using modified dsDNA donors for large insertions

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Optimized HDR reagents result in robust HDR rates with large insertions

Figure 1. Alt-R modified dsDNA or Megamer donors mediating a 200bp insertion at the AAVS1 locus, where both strands of the ssDNA donor were tested (C). Use of Alt-R HDR Enhancer V2 for 24 hrs post-transfection, followed by a media change. Genomic DNA was isolated from cells after 48 hrs. Rates of HDR and blunt integration were assessed by long-read sequencing using targeted amplification with the Oxford Nanopore Technologies MinION™ system (R9.4). Data were analyzed via the CRISPAltRations data analysis pipeline.

Identification of Alt-R modification for dsDNA donors

Increased perfect HDR and decreased blunt integration drives desired repair outcomes at on- and off-target double-stranded breaks

Comparison between long ssDNA and dsDNA repair

Discontinuous 5’ and 3’ integration events detected with ssDNA but not dsDNA

Figure 2. Alt-R modified dsDNA or Megamer donors mediating 300, 500, or 1000 bp insertions (with symmetrical 100-bp homology arms) at 2 genomic loci were nucleofected into HEK293 cells (100 nM donor) with 2 µM Cas9 RNP using a Lonza 4D nucleofector™. Cells were grown in media with (A,B) or without (B) 1 µM Alt-R HDR Enhancer V2 for 24 hrs post-transfection, followed by a media change. Genomic DNA was isolated from cells after 48 hrs. Rates of HDR and blunt integration were assessed by long-read sequencing using targeted amplification with the Oxford Nanopore Technologies MinION™ system (R9.4). Data were analyzed via the CRISPAltRations data analysis pipeline.

Figure 3. Repair events mediated by Alt-R modified dsDNA or Megamer donors targeting the SERPINC1 locus (reported in Fig. 2) were visualized using IGV to assess integration rates across the full HDR insert (A). While primarily complete HDR insertion events were observed for the dsDNA donors, use of long ssDNA donors resulted in partial HDR insertion events in which lower integration rates were observed at the 5’ junction of the donor, particularly for larger insertions. These observations were further confirmed using ddPCR assessment of the individual 5’ and 3’ HDR junctions (B). A similar finding was observed using Alt-R modified dsDNA and Megamer donors mediating a 200bp insertion at the 7MP03 locus, where both strands of the ssDNA donor were tested (C).