IDT Alt-R System: Enhancing genome editing and homology-directed repair (HDR) with improved CRISPR reagents and novel design tools

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Overview

The Alt-R CRISPR-Cas9 design tool has improved editing performance by using novel Alt-R gRNAs, which are optimized for HDR efficiency. These gRNAs are designed to improve HDR efficiency by using a combination of computational and experimental approaches. The Alt-R CRISPR-Cas9 design tool has also been used to develop a new CRISPR-Cas12a system, which has been shown to be highly efficient and versatile.

Al-R Protein Development

The Alt-R CRISPR-Cas9 system can be used to improve the efficiency of CRISPR genome editing by using a combination of computational and experimental approaches. These approaches include using Alt-R gRNAs, which are optimized for HDR efficiency, and using a novel CRISPR-Cas12a system, which has been shown to be highly efficient and versatile.

Figure 4. Off-target editing by WT and HiFi Cas9, comparing specificity between RNP delivery vs. constitutive Cas9-expressing cells. On-target (●) bars were measured in the different settings indicated, using rhAmpSeq™ amplicon-based NGS enrichment. Off-target % editing is indicated directly above the orange off-target bar. All amplicons are rank-ordered (highest to lowest) by INDEL formation percentage as determined for the WT Cas9 stable cell line. Pie charts indicate the fractional percentage of total on-target (●) and off-target (♦) editing. The HiFi Cas9 greatly reduces off-target editing, while maintaining on-target activity.

Figure 5. Broadly enhanced genome editing using A. Cas12a Ultra in human cells. A) Jurkat & HEK293 cells were electroporated with either WT or A. Cas12a Ultra RNP targeting 120 or 96 genomic loci, respectively. On-target editing as determined by NGS is presented, segregated by unique PAM sequence. B) 96 gRNAs in the same genomic region were electroporated as RNPs with A or Alt-R Cas9. A. Cas12a Ultra editing was assessed by NGS amplicon sequencing using the same assay for each Cas9/Cas12a pairing.

Figure 6. Insertion placement and selection of the appropriate gRNA for HDR. The location of an Alt-R guide insertion on or adjacent to the Cas9 cleavage site (A, with data shown from 4 µM RNP electroporations in HEK293 cells targeting the ZFPM1C locus) and selection of a potent gRNA to mediate the desired insertion at the targeted site (B, with data shown from 3 µM RNP electroporations at the GAPDH locus in K562 cells) are both strong drivers of HDR efficiency. These and other predictive rules based on functional data sets provide the foundation of the Alt-R HDR Design Tool (C). Further enhancements in HDR frequency can be made by using Alt-R HDR modified ssODNs in conjunction with the Alt-R HDR Enhancer (D).

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