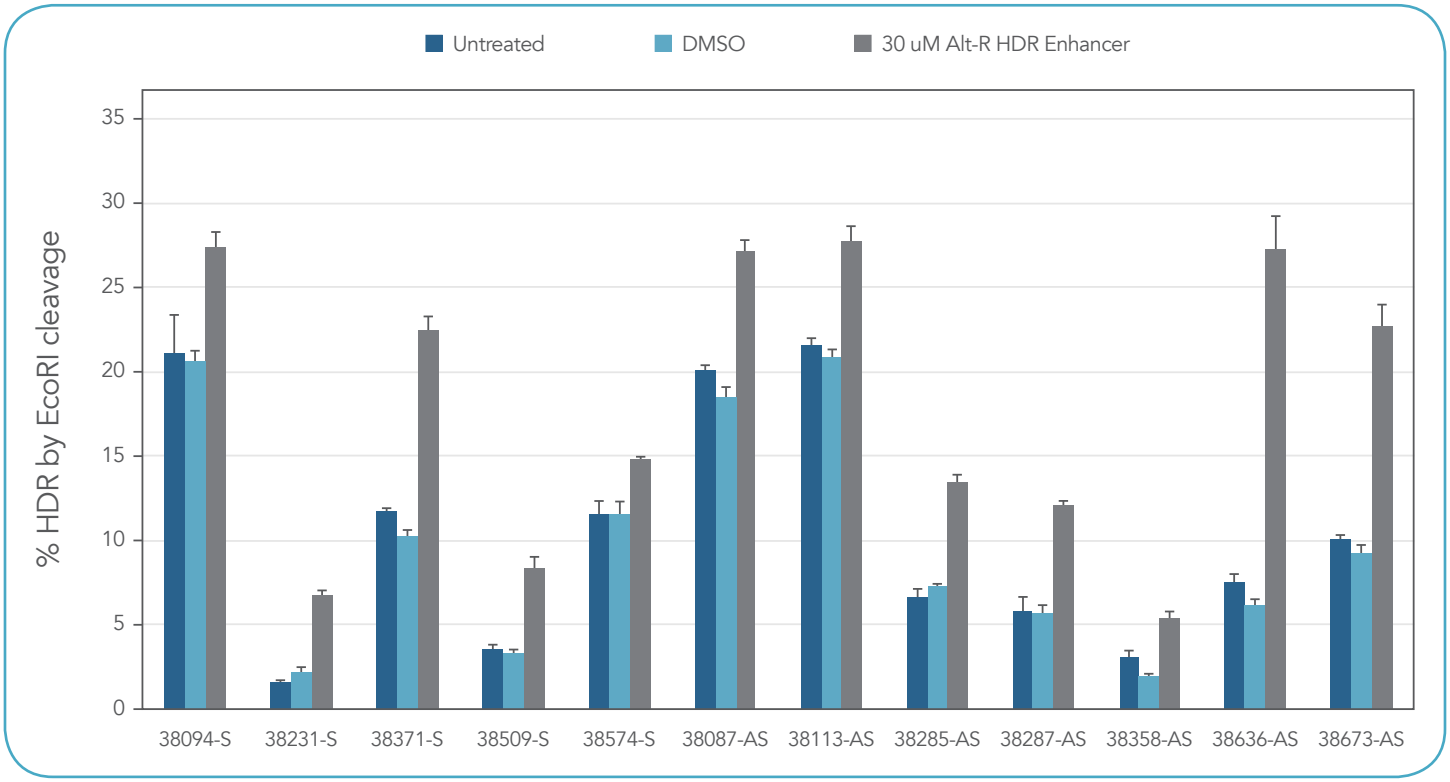
Alt-R HDR Enhancer improves efficiency of homology-directed repair (HDR) in cells transfected via lipofection

CRISPR-mediated HDR efficiency varies by cell line, editing site, and the desired insert. Our results (shown here) demonstrate the versatility of the Alt-R HDR Enhancer to increase HDR efficiency in multiple cell lines when using electroporation to deliver different Cas enzymes with their guide RNAs. The following data shows that the Alt-R HDR Enhancer also improves HDR efficiency significantly when cells are transfected via lipofection.



Alt-R HDR Enhancer improves HDR efficiency in cells transfected via lipofection. HEK-293 cells were reverse transfected with 10 nM of RNP complex (Alt-R S.p. Cas9 Nuclease V3 complexed with Alt-R CRISPR-Cas9 crRNA and tracrRNA) targeting multiple sites in the human *HPRT1* gene using 1.2 µL of Lipofectamine® RNAiMAX reagent (Thermo Fisher Scientific) along with 3 nM of IDT Ultramer oligonucleotides as HDR template. The HDR template contains a 6-base Eco RI recognition site that enables assessment of HDR efficiency by restriction fragment length polymorphism (RFLP). Immediately after lipofection, cells were cultured in media containing either 30 µM Alt-R HDR Enhancer (gray bar), or the same volume of DMSO as HDR enhancer (negative control; light blue bar). A "no treatment" control group with no HDR enhancer (dark blue bar) was also included in the experiment. Twenty-four hours after lipofection, the cell culture media was changed to fresh media containing no HDR Enhancer or DMSO. Genomic DNA was isolated 48 hr after lipofection, and target regions were amplified by PCR. HDR efficiency was assessed by EcoRI cleavage of PCR products amplified from the target regions within the *HPRT1* gene.

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