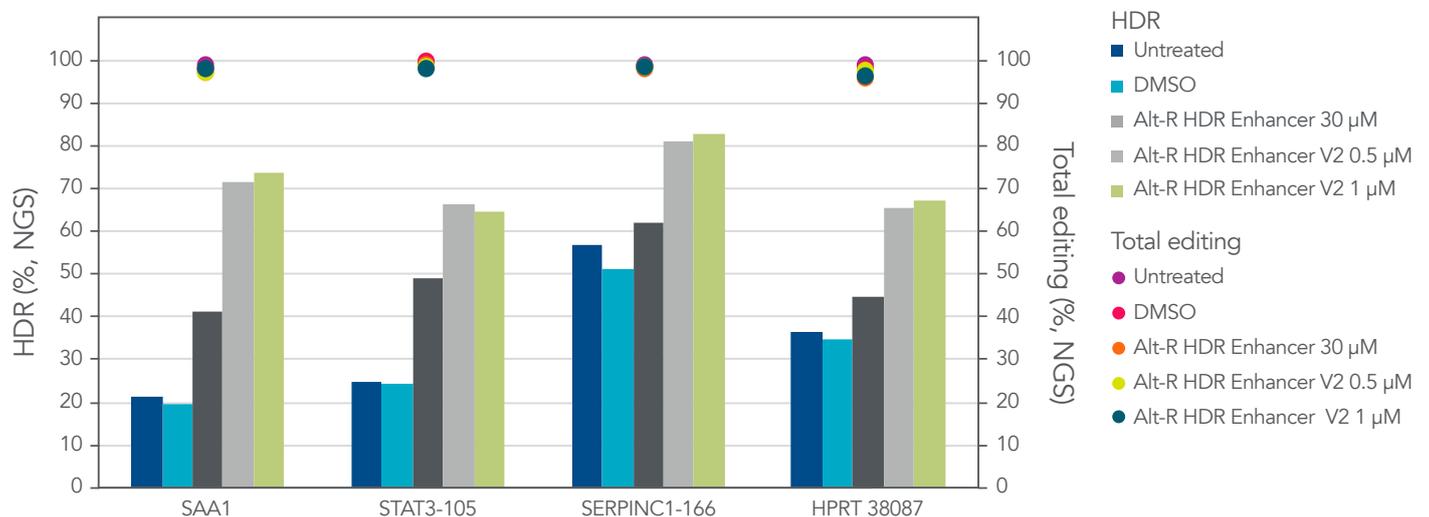


Alt-R™ HDR ENHANCER V2 IMPROVES EFFICIENCY OF HOMOLOGY-DIRECTED REPAIR (HDR) IN CELLS TRANSFECTED BY LIPOFECTION

CRISPR-mediated HDR efficiency varies by cell line, editing site, and the desired insert. IDT's internal experimental results demonstrated the versatility of the Alt-R HDR Enhancer V2* to increase HDR efficiency in multiple cell lines when using electroporation to deliver different Cas enzymes with their guide RNAs. The following data shows how Alt-R HDR Enhancer V2 also showed improved HDR efficiency when cells are transfected by lipofection.



Alt-R HDR Enhancer V2 improves HDR efficiency in cells transfected by lipofection. HEK-293 cells stably expressing Cas9 were reverse transfected with gRNA complex (Alt-R CRISPR-Cas9 crRNA complexed with tracrRNA) targeting SAA1, STAT3, SERPINC1, and HPRT 38087 in the human genome (final concentration = 10 nM) and Alt-R HDR Donor Blocks designed to insert six bases at the Cas9 cleavage site (final concentration = 3 nM) using 0.75 µL of Lipofectamine® RNAiMAX® reagent (Thermo Fisher Scientific). Immediately after lipofection, cells were cultured in media containing either no treatment (dark blue), DMSO (vehicle control, light blue), 30 µM Alt-R HDR Enhancer (dark gray), 0.5 µM Alt-R HDR Enhancer V2 (light gray), or 1 µM Alt-R HDR Enhancer V2 (green). After 24 hours, the old culture media was removed and replaced with fresh cell culture media without DMSO, Alt-R HDR Enhancer, or Alt-R HDR Enhancer V2. Genomic DNA was isolated from each sample type 48 hours after lipofection, and the targeted editing sites were amplified by PCR. The efficiency was quantified using the rhAmpSeq™ CRISPR Analysis System that uses NGS analysis to assess the percentage of HDR for on-target locations. NGS reads were analyzed using the rhAmpSeq CRISPR Analysis Tool. The highest HDR was achieved using the Alt-R HDR Enhancer V2 with a final concentration of 1 µM.

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