

Previous studies have shown that a DNA double strand break (DSB) at or near a chromosomal target site enhances homologous recombination (HR) mediated genome editing. As such, designer endonucleases that recognize specific DNA sequences and generate a DSB at a site of interest have rose in popularity, however, concerns exist for in vivo therapeutic applications including the immune response to foreign epitopes and off-target mutagenic activity. A reported alternative to site-specific endonucleases are proximal inverted repeats (IRs), structured DNA elements processed to DSBs via endogenous DNA repair. To date, the influence of IRs engrafted on the DNA repair molecule have not been well characterized in a gene editing context. In this work, a truncated adeno-associated virus (AAV) IR enhanced for gene editing was randomly mutated in a manner that conserved sequences necessary for vector production thereby generating a mutant IR library of  $>10^6$  diversity. Screening of individual mutant IRs in a gene editing selection identified AAV IR sequences decreased and enhanced for gene editing compared to the library parent and the wild-type AAV IR sequence. Currently, bioinformatic analyses correlating sequence/structure to chromosomal repair efficiencies and AAV vector production are in progress. Together, these results demonstrate that mutated viral IR sequences on the repair molecule effect gene editing frequency and offer mechanistic insights into the generation of AAV viral vectors that mediate high efficiency genetic engineering in the absence of a site-specific endonuclease.