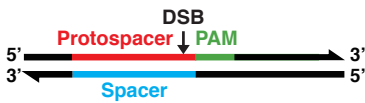


Endogenous target and HDR repair template



3' exonucleolytic degradation of protospacer by RuvC and 5' to 3' resection of spacer and protospacer

annealing to template, primer extension

repair outcome

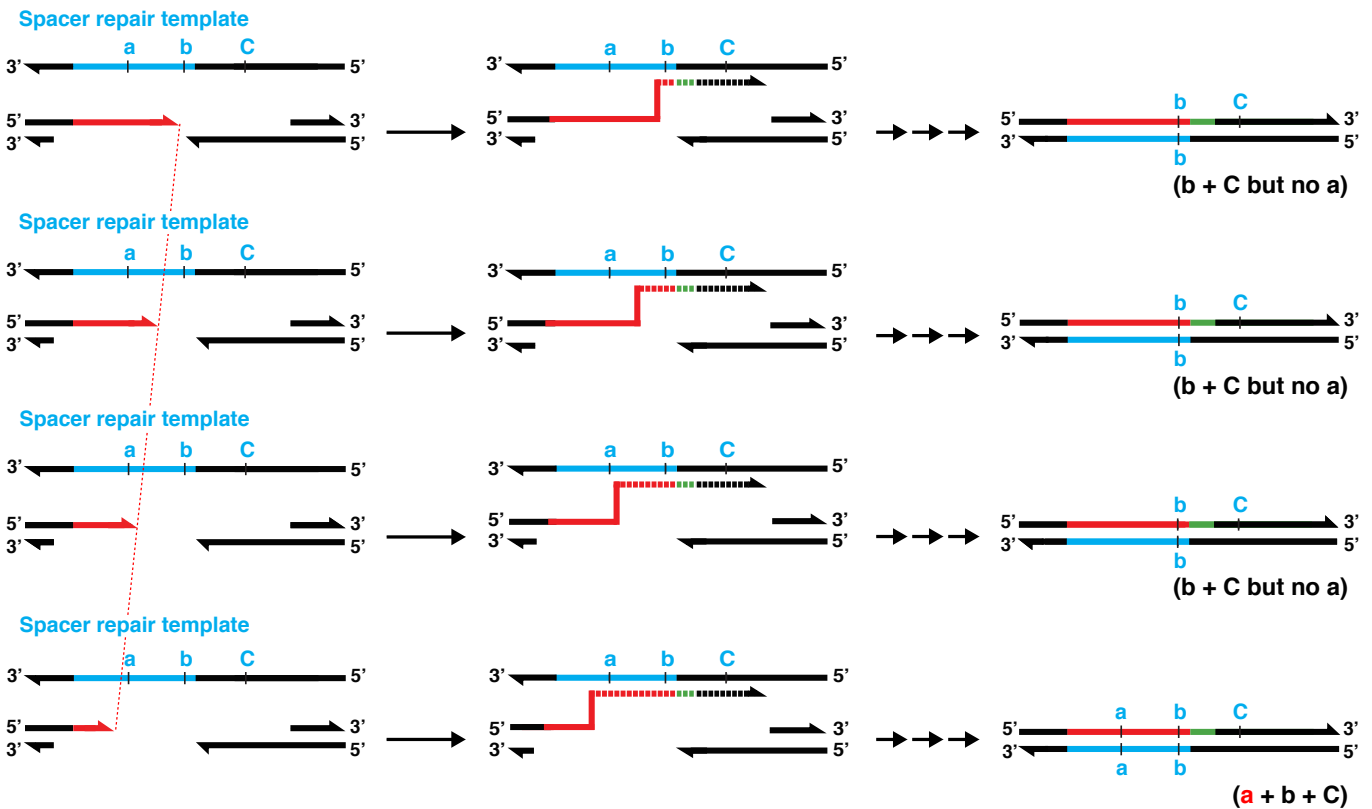


Figure S1 RuvC single-stranded 3' exonucleolytic activity provides a plausible mechanism for insertion of polymorphisms 5' of the PAM from a spacer strand repair template

This model for the infrequent insertion of SNPs 5' of the PAM from a spacer strand repair template requires the 3' exonucleolytic activity of the single-stranded Cas9 nickase RuvC. This nickase resects single-stranded DNA *in vitro* up to 10 nucleotides in the 3' - 5' direction. Since polymorphism a is only 10 bp from the DSB, the RuvC nickase should occasionally resect the protospacer strand of the endogenous DNA past the location of the polymorphism and allow incorporation of this polymorphism from primer extension off the spacer repair template. The model shows different degrees of 3' resection by RuvC on the protospacer strand (dashed red line) and the subsequent difference in repair outcomes for the inclusion of polymorphism a.