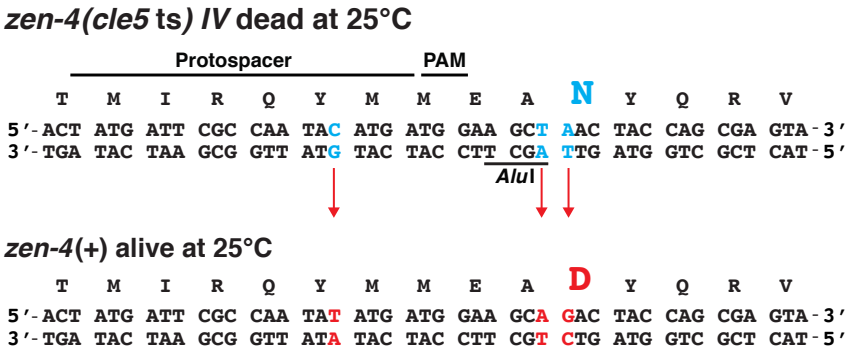
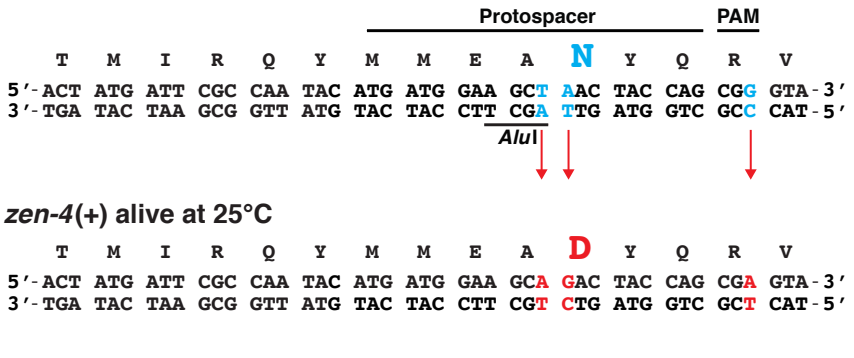


0 rescued *zen-4* (+) F1 hermaphrodites were recovered from 40 *zen-4(cle5* ts*)* P0s injected with Cas9 RNPs and a single-stranded protospacer repair template.

In control experiments conducted at 15° in which 62 *zen-4(cle5* ts*)* P0 animals were injected with Cas9 RNPs containing *zen-4* and *dpy-10* guides plus a single-stranded protospacer *zen-4* (+) repair template and a *dpy-10* (gf) repair template, 41% of 110 *dpy-10* (gf) animals had imprecise repair at *zen-4*, but 0% had templated repair to *zen-4* (+), indicating that Cas9 made DSBs at the expected location, but HDR repair did not occur with the *zen-4* protospacer template.



112 rescued *zen-4* (+) F1 hermaphrodites were recovered from 40 *zen-4(cle10* ts*)* P0s injected with Cas9 RNPs and a single-stranded protospacer repair template.

Figure S3 Strategy to develop a co-conversion marker that enabled selection of viable, edited animals

(A) To develop *zen-4*(+) as a co-conversion marker we used Cas9 editing to create a temperature-sensitive lethal allele of *zen-4* that had the death-inducing GAC to AAC transition at codon 520 [*zen-4(cle5* ts)]. The strain also included an *Alu*I site that was made by converting codon 519 from a GCA alanine codon to a GCT alanine codon to distinguish edited genes from true revertants. These DNA changes were located 3' of the PAM. In initial attempts to convert the *zen-4(cle5* ts) allele to a wild-type allele and thereby rescue the lethal phenotype, we used a protospacer stand repair template in which codon 515 had been converted to TAT from TAC to prevent the repaired *zen-4*(+) gene from being cleaved by Cas9. HDR failed, but imprecise repair succeeded, indicating that Cas9 RNPs cleaved the DNA at the expected location. This failure contributed to the evidence that protospacer strand HDR templates are not successful for repair of mutations 3' of the PAM (see Figures 2 and 3). (B) We then created a second *zen-4* mutant strain (allele *cle10* ts) to change the location of Cas9 RNP binding. The strain carried the GAC to ACC transition at codon 520 to create the temperature-sensitive lethal mutation and also included the CGA to CGG transition at codon 523 to create a PAM near the target sequence for the guide RNA. In addition, an *Alu*I site was introduced by converting codon 519 from a GCA alanine codon to a GCT alanine codon to distinguish edited animals from true revertants. All these changes were 5' of the PAM. Fortuitously, we chose a single-stranded HDR repair template that corresponded to the protospacer strand and found that the combination of guide and repair template was successful for HDR. This result reinforced the evidence in Figures 2 and 3 that a protospacer strand repair template is efficient for repair of sequences 5' of the PAM.