



Figure S1 Schematic representation of deletion/insertion experiments on *K08F4.2* gene

Black lines represent genomic DNA. Red lines represent inserted sequence and blue lines are homology arms on template DNA. Scissors denote the sgRNA recognition sequence (pairing site + PAM). HDR – homology-direct repair. NHEJ – non-homologous end-joining. RE: restriction enzyme site.

(A) and (B) Experiments 2 and 3 (also see Table 1). Simultaneous small deletion (24bp) and restriction enzyme site (RE) insertion.

(C) Experiment 22. The homology arms in the donor template do not extend to the cuts and as a result, NHEJ is preferred over HDR and the donor template is not used. This experiment demonstrates that separation of the homology arms from the cut sites by as little as 10-31 bp favors NHEJ. (Note that we know that at least sgRNAs APs1, 4 and 5 are functional based on Exp. 2, 3 and 6).

(D) Experiment 25. The homology arms in the donor template extend to the cuts and as a result, HDR is preferred over NHEJ, and the donor template is integrated as evidenced by insertion of the restriction enzyme site (RE).

(E) Experiment 26. Same as in Exp. 25 except that only one sgRNA (APs5) was used. No large deletions were obtained.