

**Figure S1** Verification of *cre-1* gene deletions in selected transformants with co-transformation of only Cas12a and donor DNA, only crRNA and donor DNA, or only donor DNA. (A) Schematic of homologous recombination (HR) of target gene *cre-1*. (B–D) PCR analysis of *cre-1* deletion (B–D) with one primer (cre1-out-F) located upstream of the 5' flanking region of genomic DNA and the other (cre1-in-R) located in the 3' flanking region of genomic DNA. The expected length of disrupted transformants was 1.9 kb, while that of the WT host strain, used as a negative control, was 1.0 kb (rightmost lane). Heterokaryotic transformants showed two PCR bands (both of wild-type and knockout). HDR, homology-directed repair; WT, wild type.