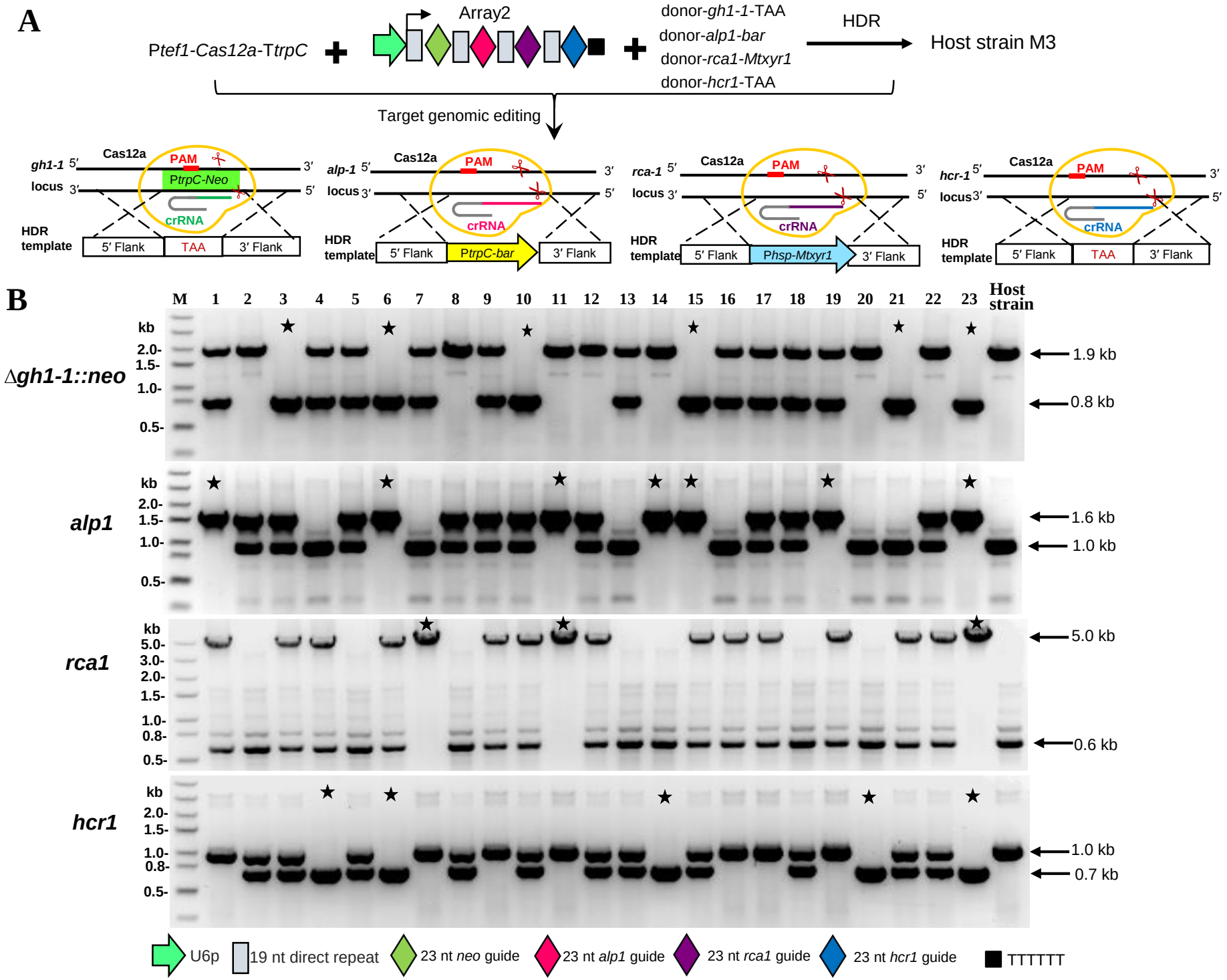

Figure S5. Second round of target genomic editing by CRISPR-Cas12a system. (A) Schematic of homologous recombination (HR) of *neo*, *alp-1*, *rca-1* and *hcr-1* mediated by Cas12a, array2 and donor DNA. (B) PCR analysis of quadruple-gene deletion of *neo*, *alp-1*, *rca-1* and *hcr-1* in selected transformants using one primer (gh1-1-out-F2, alp1/rca1/hcr1-out-F) located upstream of the 5′ flanking region of genomic DNA and the other primer (gh1-1-in-R2, alp1/rca1/hcr1-in-R) located in the 3′ flanking region of genomic DNA. The expected lengths of disrupted transformants of *neo*, *alp1*, *rca-1* and *hcr-1* were 0.8, 1.6, 5.0 and 0.7 kb, respectively, while those of the host strain (rightmost lane) was 1.9, 1.0, 0.6 and 1.0 kb, respectively. Heterokaryotic transformants showed two PCR bands (both of wild-type and knockout). The symbol of star indicated deletion mutant. HDR, homology-directed repair; symbol star indicated deletion mutant.