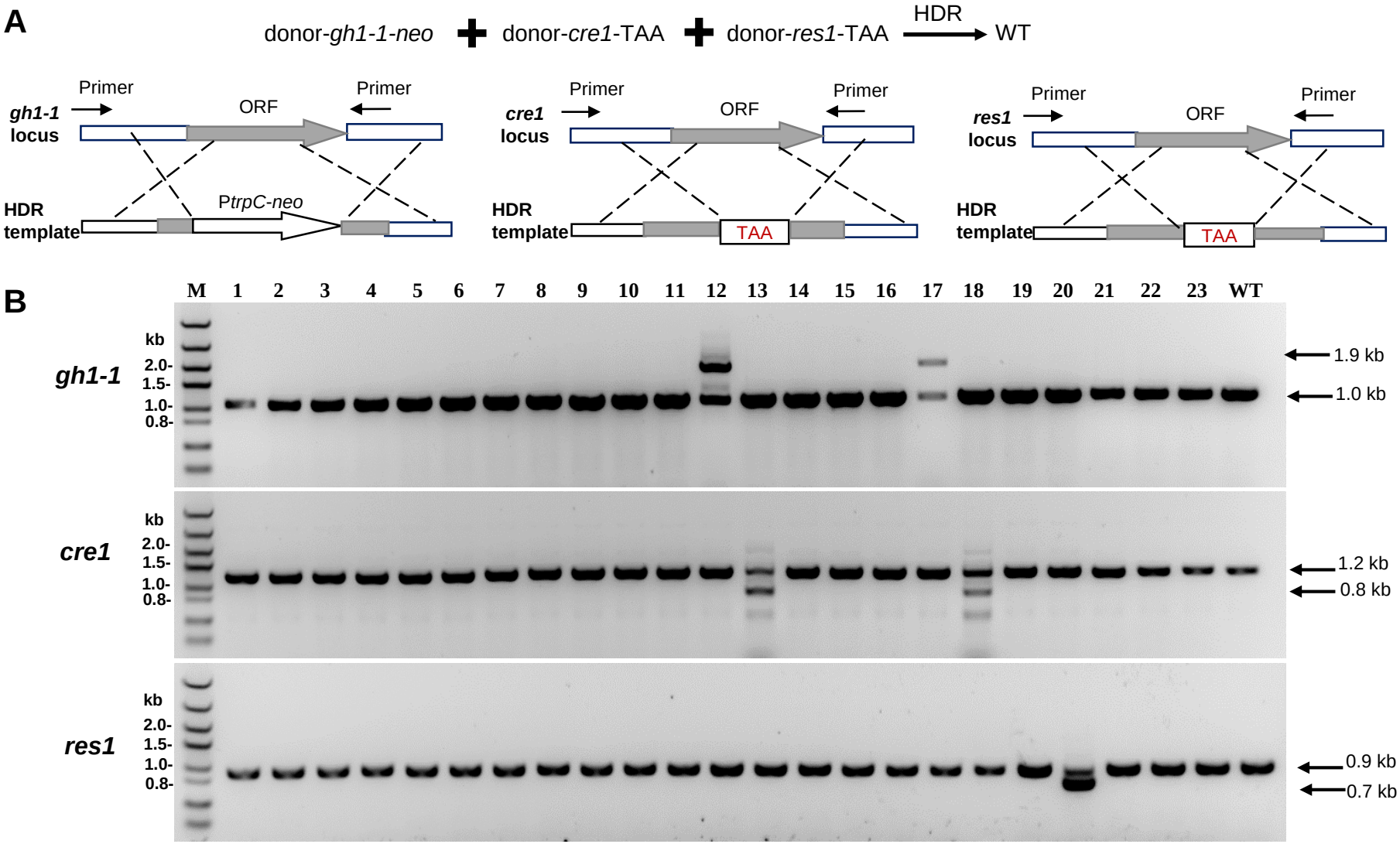

Figure S3 Verification of triple-gene deletions of *gh1-1*, *cre-1* and *res-1* in selected transformants with co-transformation of only three donor-DNAs without CRISPR expressing cassettes. (A) Schematic of homologous recombination (HR) of target genes mediated by each donor DNA. (B) PCR analysis of triple-gene deletion of *gh1-1*, *cre-1* and *res-1* in selected 23 transformants using one primer (cre1/gh1-1/res1-out-F) located upstream of the 5′ flanking region of genomic DNA and the other primer (cre1/gh1-1/res1-in-R) located in the 3′ flanking region of genomic DNA. The expected lengths of disrupted transformants of *gh1-1*, *cre-1* and *res-1* were 1.9, 0.8 and 0.7 kb, respectively, while those of the host strain (rightmost lane) was 1.0, 1.2 and 0.9 kb, respectively.