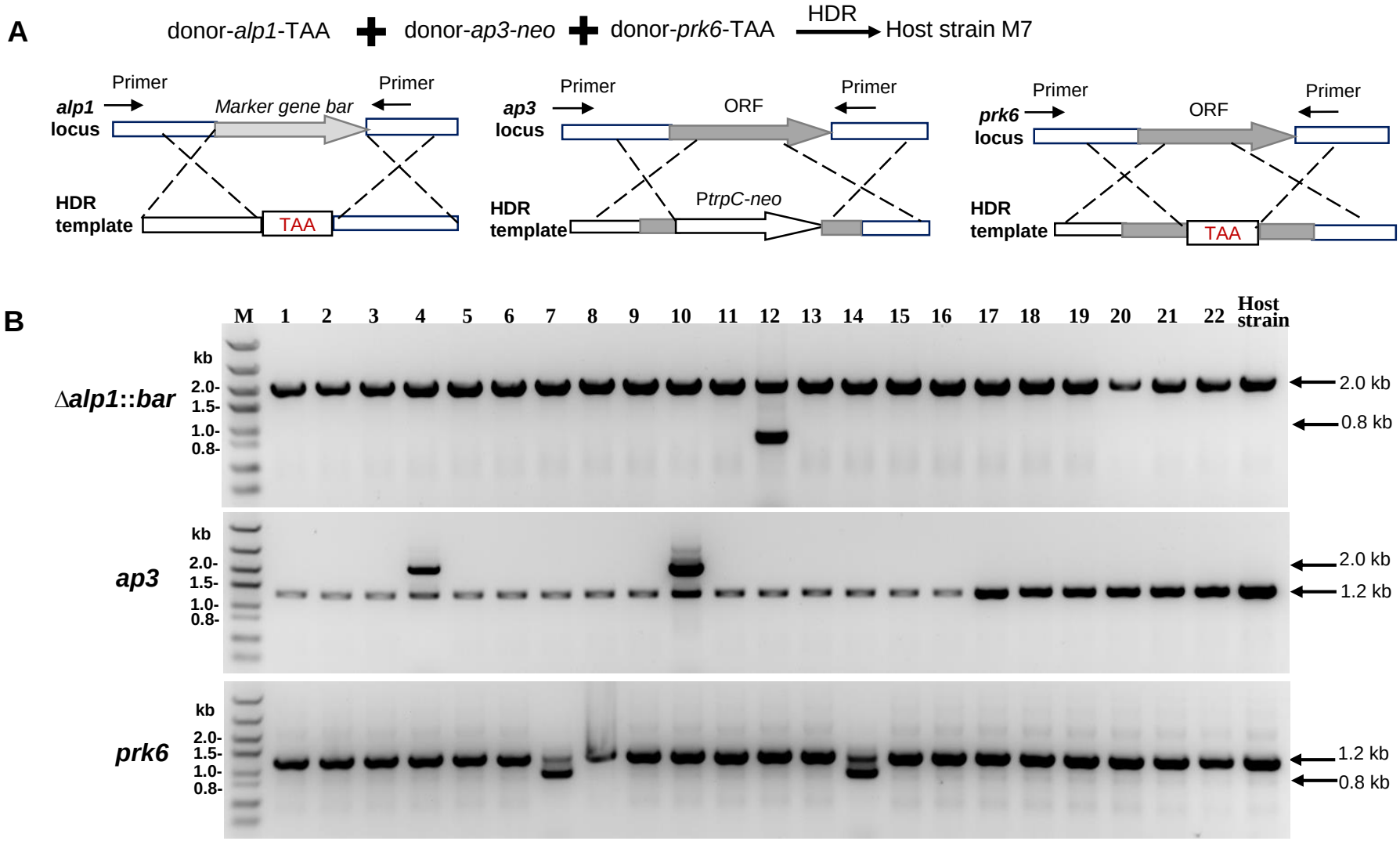

Figure S8 Verification of triple-gene deletions of *bar*, *ap-3* and *prk-6* in selected 22 transformants with co-transformation of three donor-DNAs without CRISPR expressing cassettes. (A) Schematic of homologous recombination (HR) of target genes mediated by donor DNA. (B) PCR analysis of triple-gene deletion of *bar*, *ap-3* and *prk-6* in selected 22 transformants using one primer (alp1-out-F2, ap3/prk6-out-F) located upstream of the 5′ flanking region of genomic DNA and the other primer (alp1-in-R2, gh1-1/res1-in-R) located in the 3′ flanking region of genomic DNA. The expected lengths of disrupted transformants of *bar*, *ap-3* and *prk-6* were 0.8, 2.0 and 0.8 kb, respectively, while those of the host strain (rightmost lane) was 2.0, 1.2 and 1.2 kb, respectively. Heterokaryotic transformants showed two PCR bands (both of wild-type and knockout). HDR, homology-directed repair.