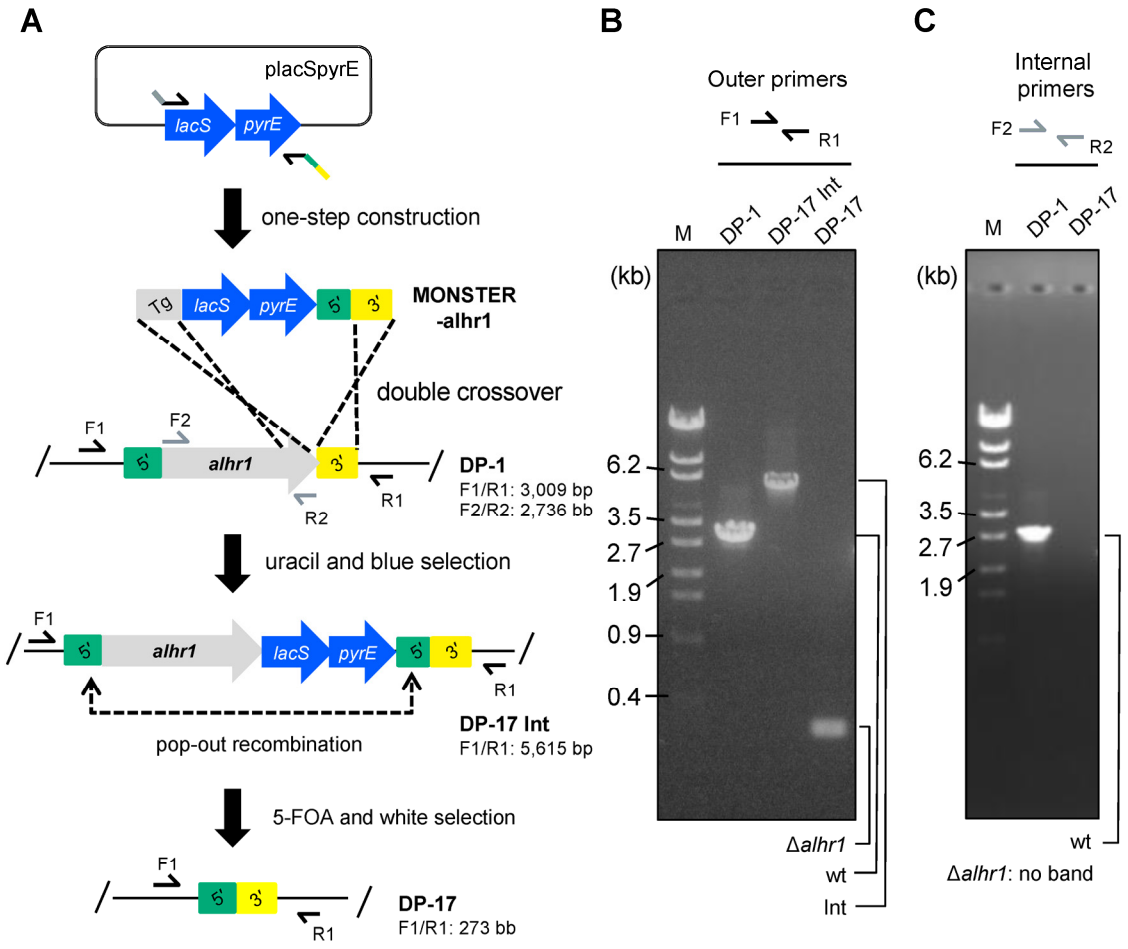
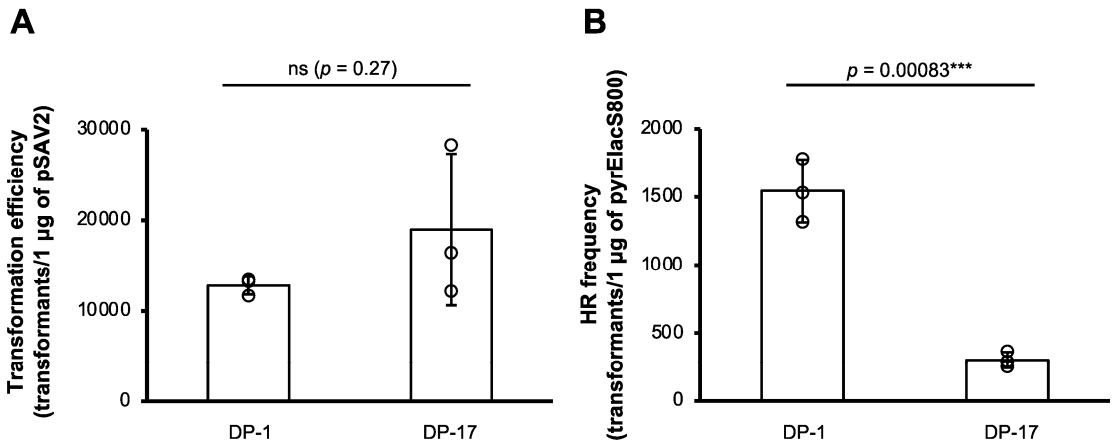


Supplemental Figure S1. Construction of *alhr1*-deleted strain using MONSTER. (A) Schematic drawing of the construction of an *alhr1* gene-deletion mutant. The MONSTER-alhr1 cassette was amplified and electroporated into the DP-1 strain. A double crossover between the MONSTER-alhr1 and the chromosome at the Tg and 3′-flanking regions resulted in the insertion of the *lacS-pyrE* marker and the 5′-flanking region at the *alhr1* locus. Transformants forming blue colonies were selected on uracil-free plates. The excision of *alhr1* and counterselectable marker genes was achieved through pop-out recombination. PCR analyses were performed on genomic DNA using the outer primer set F1/R1 (B) and the internal primer set F2/R2 (C). Expected sizes of the products were 3,009 bp (wt), 5,615 bp (Int), and 273 bp (Δ*alhr1*) in B, in addition to 2,736 bp (wt) and no band (Δ*alhr1*) in C. A λ EcoT14 ladder was loaded into lane M.



Supplemental Figure S2. Transformation efficiency and HR frequency. Transformation of DP-1 and DP-17 with pSAV2 (A) and pyrElacS800 (B) was carried out. Data are presented as mean ± SD, calculated from the values of three independent experiments, which are labeled as dots on each bar. Statistical significance was determined using two-tailed Student's *t*-test analysis.