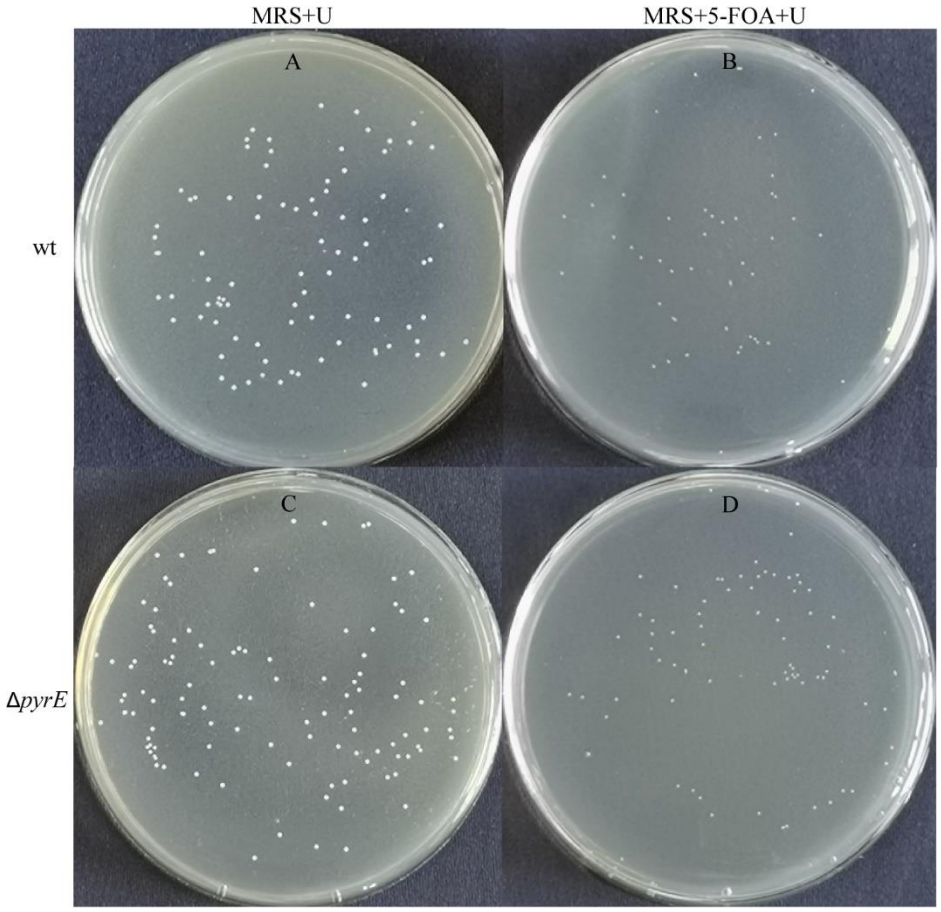

FIG S1 Verification of counter-selection function of *pyrE* gene marker. (A and B) Colonies of the wildtype strain *P. acidilactici* LA412 (wt) formed in modified MRS medium containing 20 μg/mL U alone or both 2 mg/mL 5-FOA and 20 μg/mL U. (C and D) Colonies of the Δ*pyrE* strain formed in modified MRS medium containing 20 μg/mL U alone or both 2 mg/mL 5-FOA and 20 μg/mL U. U, Uracil; 5-FOA, 5-fluoroorotic acid.

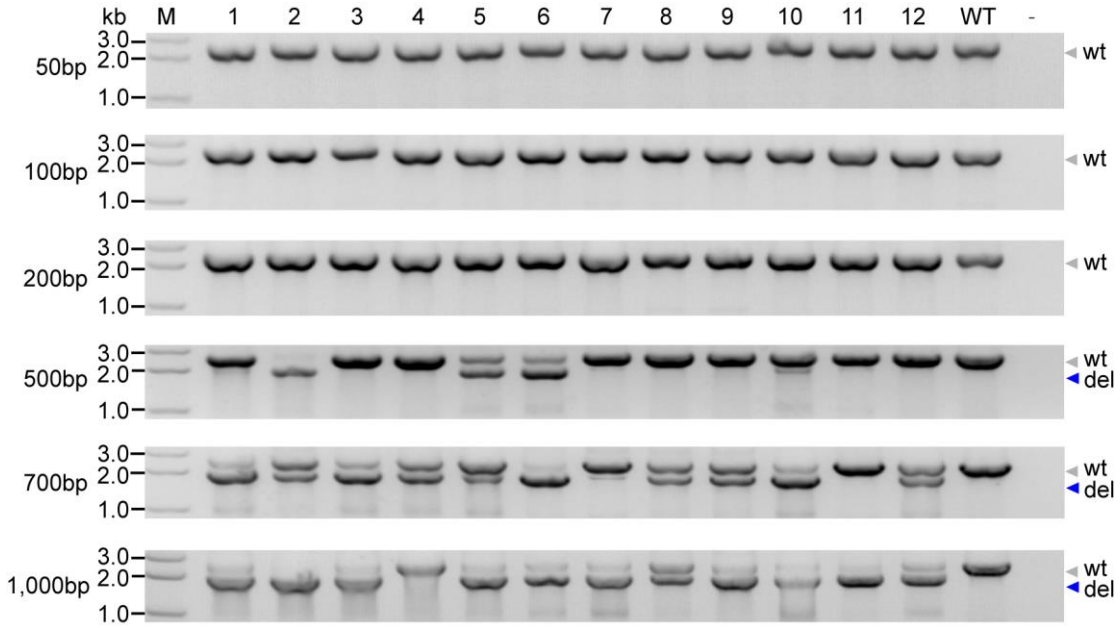

FIG S2 PCR validation of *pyrE* gene deletion in 12 randomly selected transformants (lane 1 to 12) containing editing plasmids with donor DNAs whose length ranged from 50 bp to 1,000 bp (50, 100, 200, 500, 700, and 1,000 bp, respectively). M, DNA ladder; WT, control group with *P. acidilactici* LA412 total DNA as template; and "-", control group with ddH_2O as the template. wt, wildtype band; and del, *pyrE*-deleted band. The sizes for upstream/downstream donors are indicated on the left.

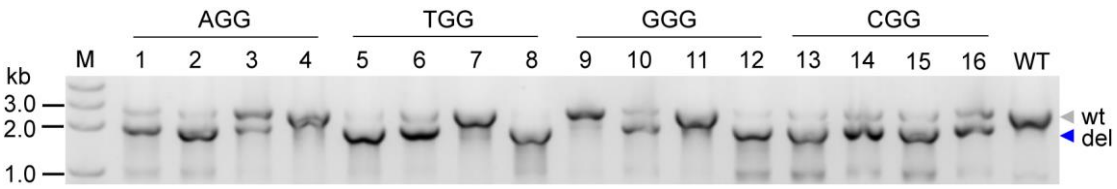

FIG S3 Agarose gel analysis of PCR products of *pyrE* gene locus amplified from the single colonies carrying the *pyrE* gene-deleting plasmids with 700 bp donor in the *cas9*-corrected strain (*P. acidilactici* LA412*cas9*). Four protospacers with different adjacent PAMs (AGG, TGG, GGG, or CGG) were targeted. M, DNA ladder; WT, control group with *P. acidilactici* LA412 total DNA as the template. “wt” and “del” indicate the bands of wildtype *pyrE* gene and the deleted gene, respectively.