

**Manuscript title: Coupling the recombineering to Cre-lox system
enables simplified large-scale genome deletion in *Lactobacillus casei***

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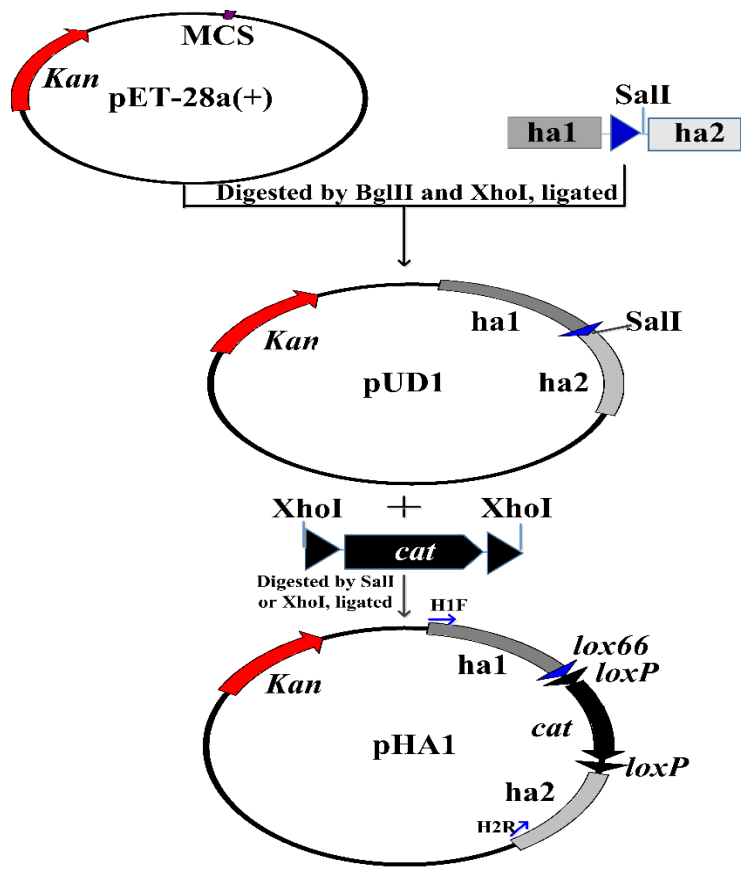


Figure S1. Strategies for obtaining linear donor disruption cassette H1-lox66-loxP-cat-loxP-H2.

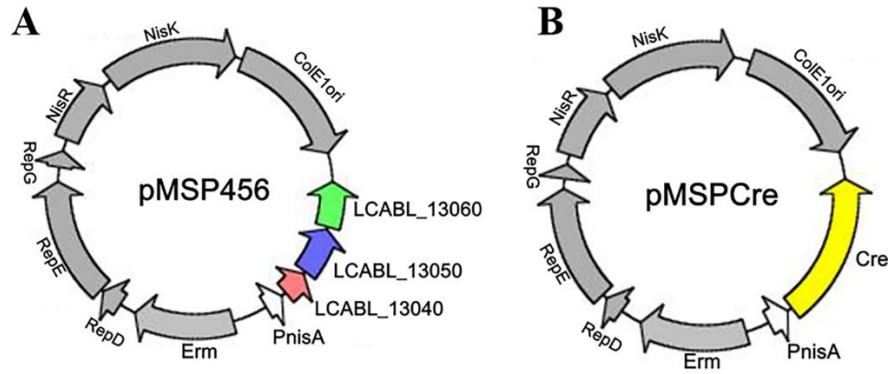


Figure S2. Structure of plasmids used in this study. Arrow heads show genes and their direction. (A) LCABL_13040-50-60-expression plasmid pMSP456. (B) Cre-expression plasmid pMSPCre. LCABL_13040-50-60 and Cre were transcribed from PnisA that is controlled by the product of nisin.

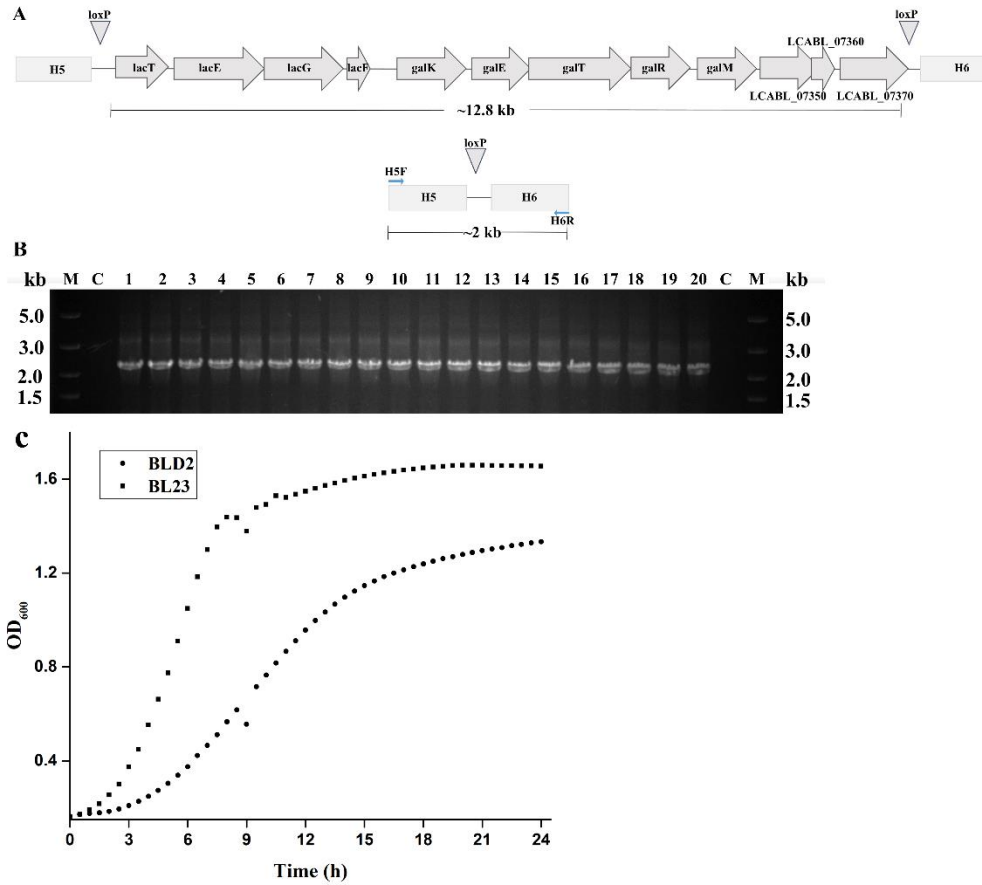
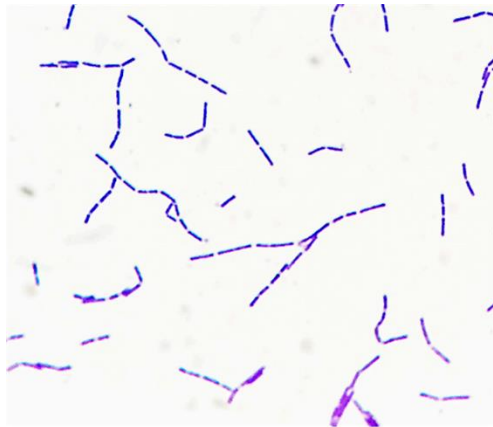
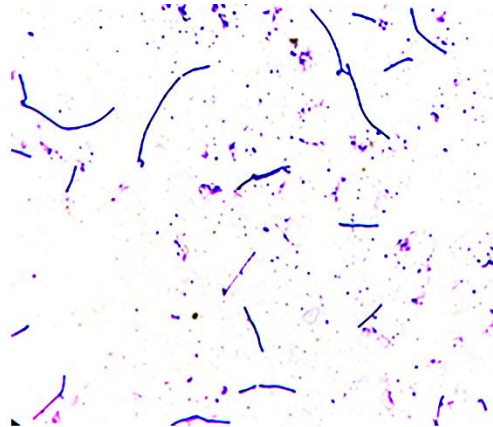


Figure S3. Deletion of large-scale genome region containing important gene(s) in *L. casei* BL23. (A) Layout and genetic context of the ~12.8 kb genome region and the size of PCR products amplified with primers H1F and H2R from BLD2 strains. (B) Colony PCR results. M: DNA marker; C: wild-type strain; 1–20: chloramphenicol resistance colonies. (C) Growth of *L. casei* BL23 and the deletion strain *L. casei* BLD2 on MRS.



Lactobacillus casei BL23



Lactobacillus casei BLD2

Figure S4. Cellular morphologies of *L. casei* strains BL23 and BLD2.

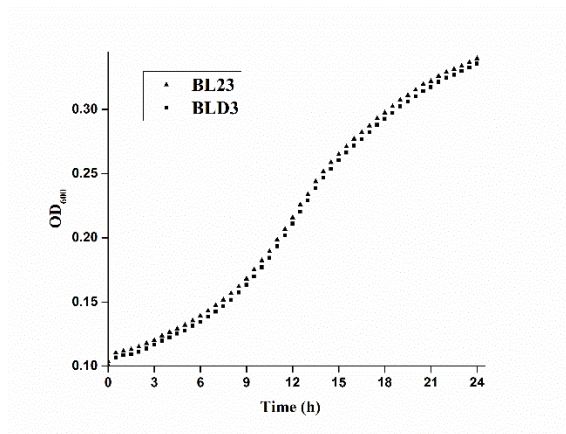


Figure S5. Growth of *L. casei* BL23 and the deletion strain *L. casei* BLD3 on CDM medium containing galactose.