Supplementary materials

Combining 26s rDNA and Cre-loxP system for iterative gene integration and efficient marker curation in *Yarrowia lipolytica*

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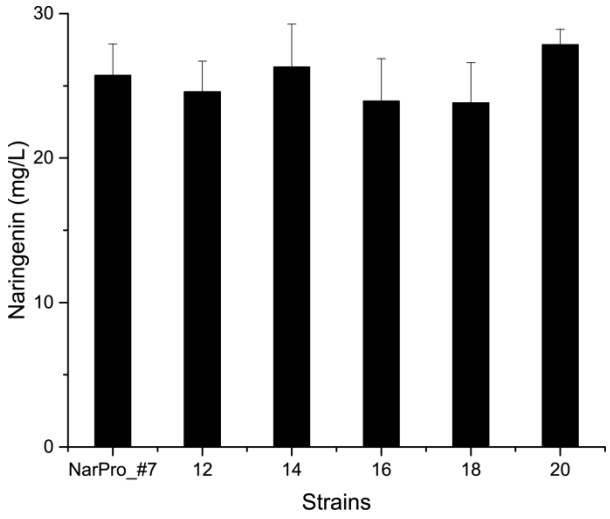
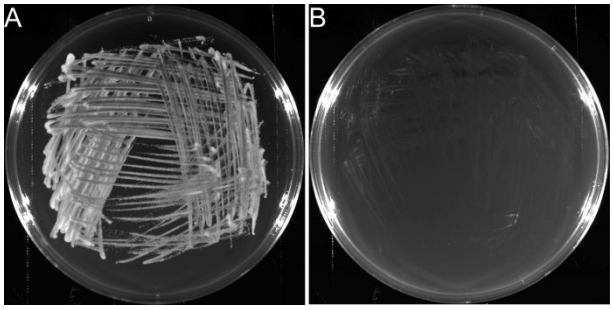


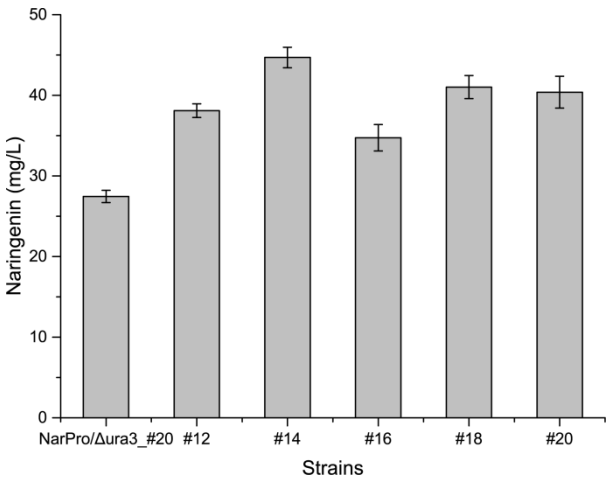
Supplementary Figure 1. Analysis of naringenin pathway stability after *URA3* marker removing.

Strain NarPro_#7 was used as control. Strains 12, 14, 16, 18, and 20 were from the colonies of Figure 2E, respectively. These strains produced similar naringenin, indicating that the naringenin pathway is stable after *URA3* marker removing.

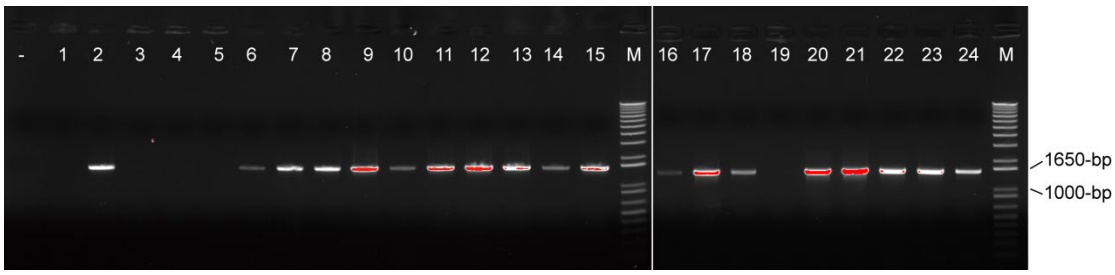


Supplementary Figure 2. Confirmation of pYLXP'-Cre removing by growing on YPD and CSM-Leu plates.

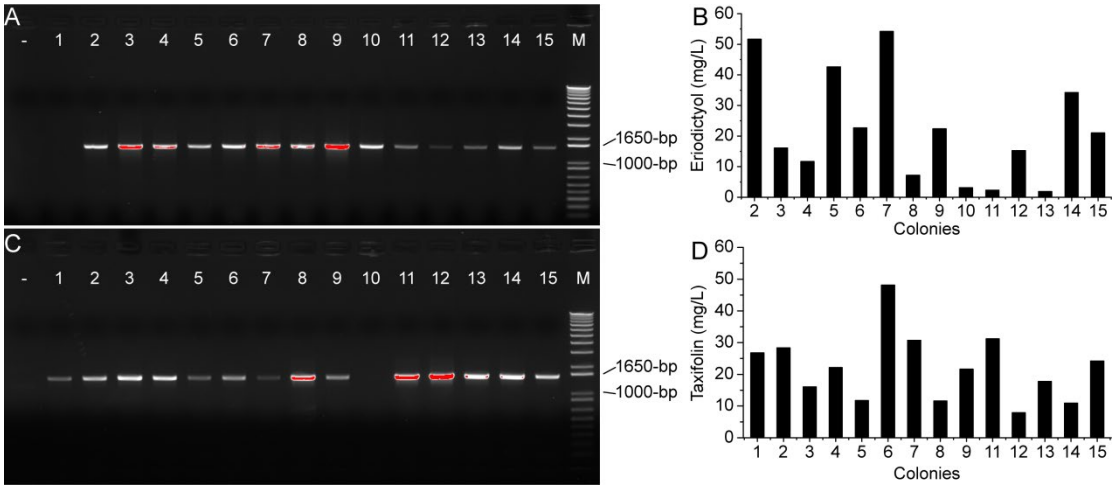
A. Cell growth on YPD plate. **B.** Cell growth on CSM-Leu plate. After growing in YPD medium at 30^{o}C for 48 h, the cells were innoculated to YPD and CSM-Leu plates. The photoes were taken after incubating at 30^{o}C for another 24 h.



Supplementary Figure 3. Screening of ARO1 cassette transformants in YPD medium. Five colonies were picked into YPD medium randomly. Fermentation was carried out in 250-mL shaking flasks for 144 h.



Supplementary Figure 4. Colony PCR screening of YlACS2-YlACC1 transformants. Primer pair TEF_F2/YlACS2$_{veri.}$ R was used in the colony PCR. The positive colony will produce a 1515-bp specific band, while the negative colony will not. "-" refers to the negative control, which used a NarPro/ASC colony as template.



Supplementary Figure 5. Colony PCR and HPLC screening of HR_{x2} and HRH_{x2} transformants. **A.** Colony PCR screening of HR_{x2} transformants. **B.** HPLC screening of eriodictyol producing colonies. **C.** Colony PCR screening of $HR_{x2}H$ transformants. **D.** HPLC screening of taxifolin producing colonies. For the colony PCR screening of HR_{x2} and $HR_{x2}H$ transformants, primer pair GhF3'H F/GhF3'H R was used for screening HR_{x2} and $HR_{x2}H$ trandformants. The positive colony will produce a 1536-bp specific band. "-" refers to the negative control, which used a NarPro/ASC colony as template.