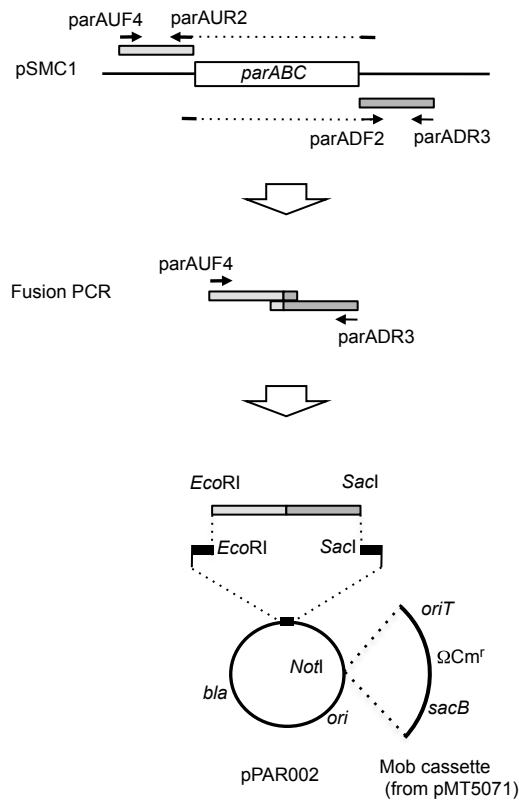


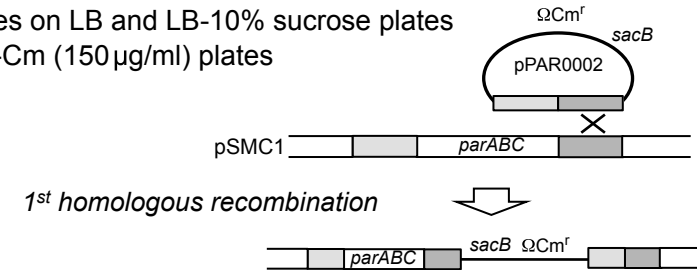
Figure S1

## A. Construction of pPAR002



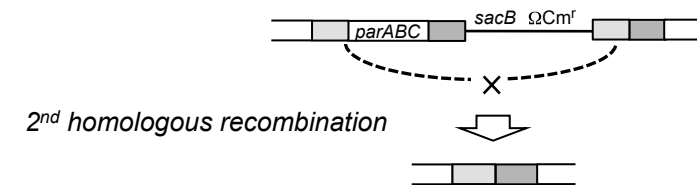
## B. Curing of pSMC1 from SM39

1. Transformation of pPAR002 into *E. coli* S17-1
2. Conjugal transfer of pPAR002 into SM39 on LB plates
3. Select trans-conjugants (SM39 carrying pPAR002) on BM2-Cm (150  $\mu$ g/ml) plates\*
4. 1<sup>st</sup> recombination event:  
Screening of sucrose<sup>s</sup> colonies on LB and LB-10% sucrose plates  
Confirmation of Cm<sup>r</sup> on BM2-Cm (150  $\mu$ g/ml) plates



Confirmation of the 1<sup>st</sup> recombination by PCR

5. 2<sup>nd</sup> recombination event:  
Screening sucrose<sup>r</sup> colonies on LB-10% sucrose plates



Confirmation of the 2<sup>nd</sup> recombination by PCR  
and lack of growth on LB-Cm (150  $\mu$ g/ml) plates

pSMC1-cured derivatives were confirmed by the loss of HgCl<sub>2</sub>-resistance on LB-HgCl<sub>2</sub> (16  $\mu$ g/ml) plates

\*BM2 medium: 0.7 % K<sub>2</sub>HPO<sub>4</sub>, 0.3% KH<sub>2</sub>PO<sub>4</sub>, 0.1 % (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, 0.01 % MgSO<sub>4</sub>·7H<sub>2</sub>O, 1.08 % sodium succinate, 1.5 % agarose

Figure S2

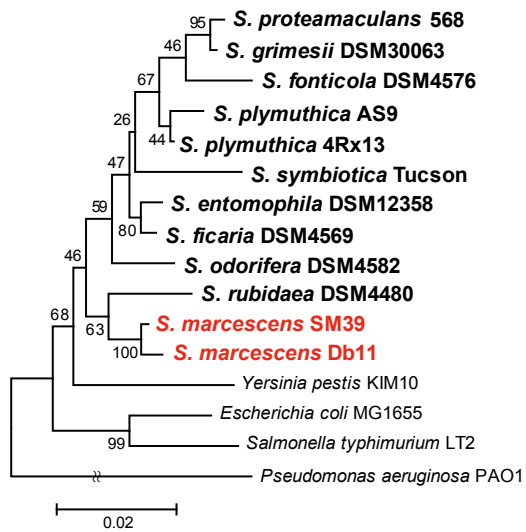


Figure S3

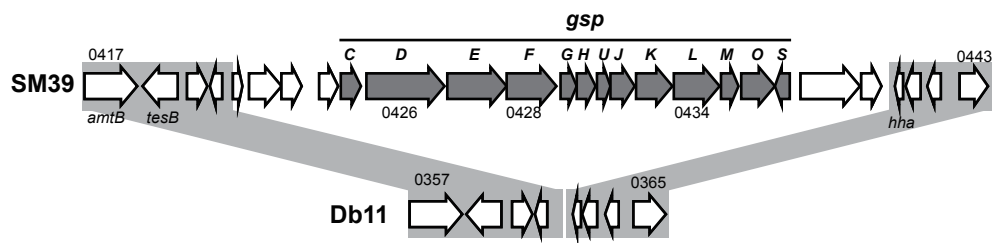


Figure S4

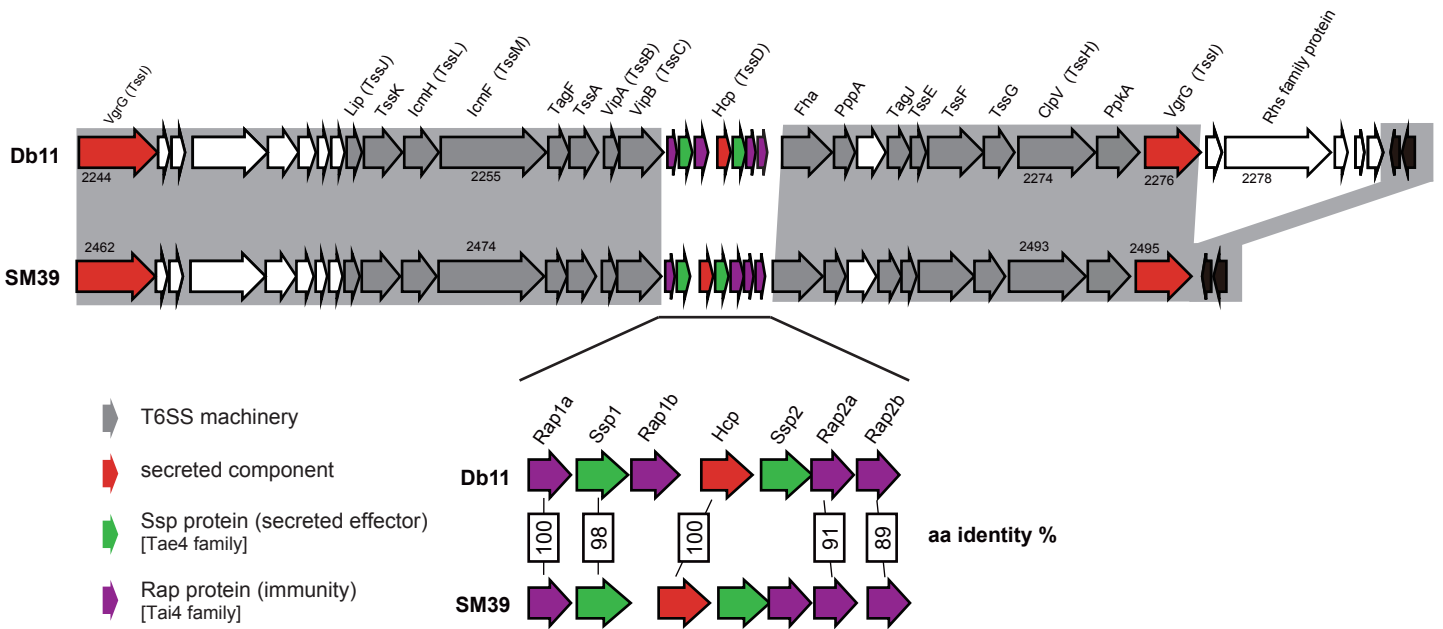


Figure S5