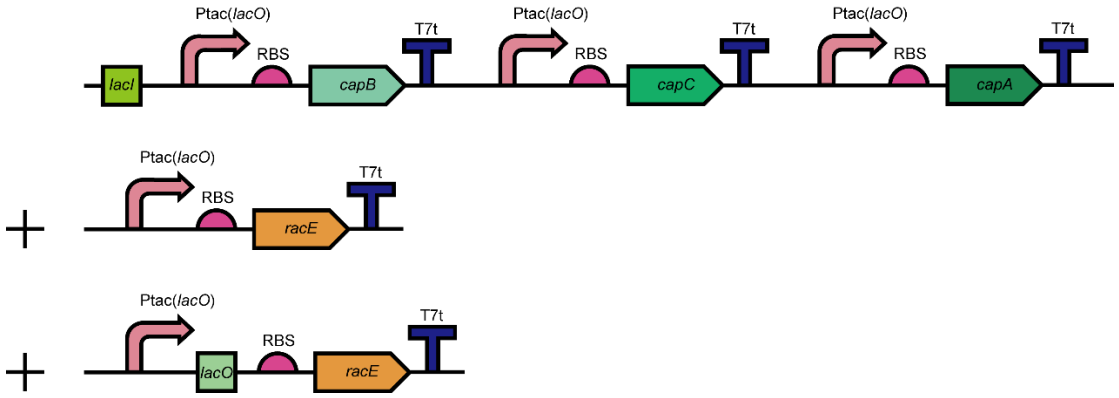
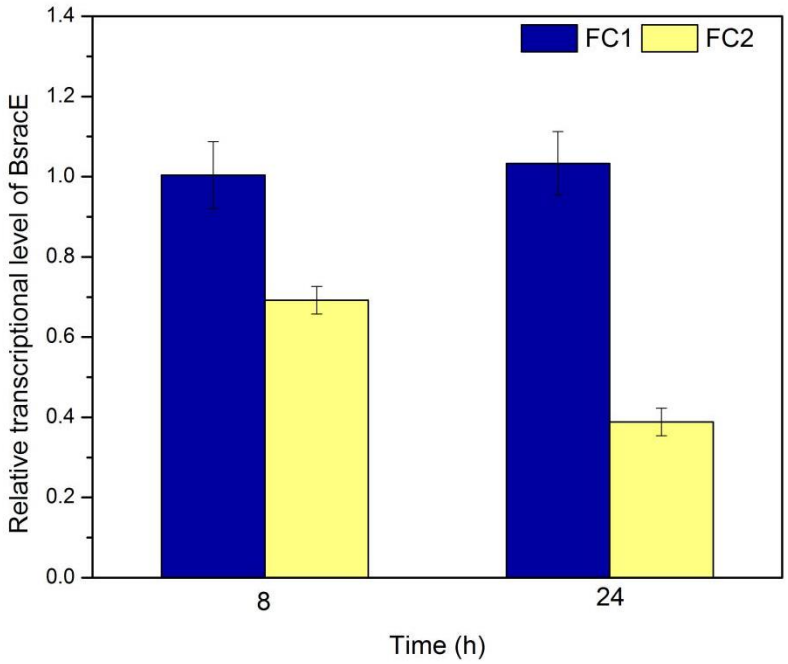
Part II Biosynthesis of γ-PGA with tailored L/D glutamate ratio

In γ-PGA produced by the engineered *C. glutamicum* F343, the major monomer unit was L-glutamate as demonstrated by the observation that 97.1% of the glutamate units were L-glutamate. This is possibly due to the low intrinsic activity of glutamate racemase, which can convert L-glutamic acid to D-glutamic acid. To change the ratio of L-glutamate units in the product γ-PGA and investigate the effect of the L-glutamate supply with different ratios of L-glutamate and D-glutamate on γ-PGA production, the glutamate racemase gene *racE* derived from *B. subtilis* Ia1a was expressed with different transcriptional levels in the engineered strain *C. glutamicum* F343 pZM1-Ptac-*capB*CA* (strain FC0) (see Fig.4A).

(A)



(B)



(C)

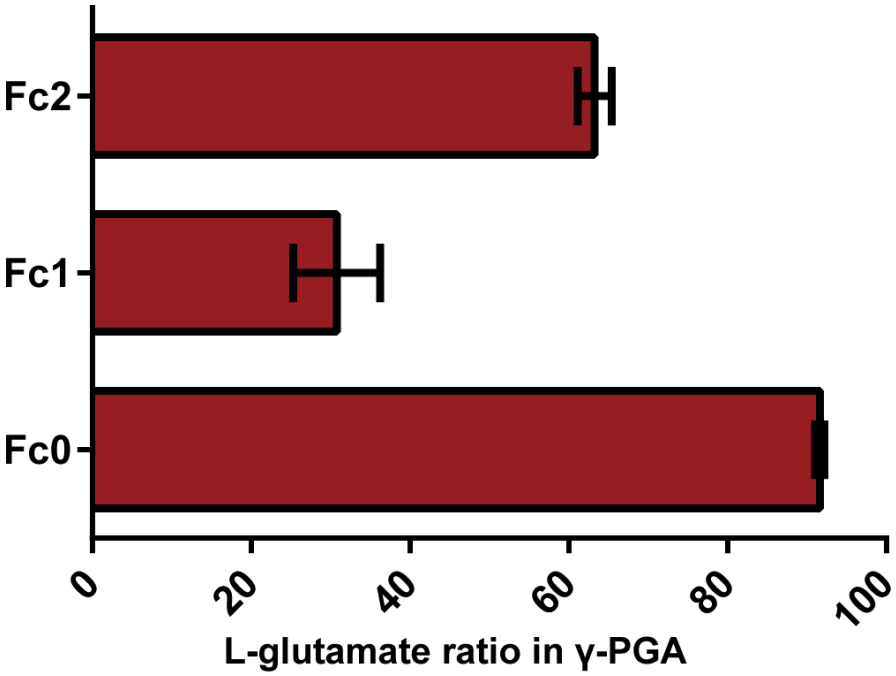


Fig.4A. Construction of FC1 and FC2

B. Relative transcriptional level of *racE* in FC1 and FC2.

C. L-glutamate ratio of γ-PGA produced by FC0, FC1 and FC2.

FC0, *C. glutamicum* F343 pZM1-Ptac-*capB*CA*;

FC1, *C. glutamicum* F343 pZM1-*capB*CA*-(1lacO)*BsracE*;

FC2, *C. glutamicum* F343 pZM1-*capB*CA*-(2lacO)*BsracE*.

In γ-PGA produced by the engineered *C. glutamicum* F343, the major monomer unit was L-glutamate as demonstrated by the observation that 97.1% of the glutamate units were L-glutamate(see Fig.4C). Real-time quantitative PCR was employed to measure the expression levels of *BsracE*. The transcriptional levels of *BsracE* in *C. glutamicum* F343 pZM1-*capB*CA*-(2lacO)*BsracE* (strain FC2), where two lacO operators were incorporated, were approximately 70.0% and 37.6% ($P<0.05$) of that in strain *C. glutamicum* F343 pZM1-*capB*CA*-(1lacO)*BsracE* (strain FC1) at 8 h and 24 h, respectively(see Fig.4B). Then the effect of different expression of *BsracE* on the ratio of L-glutamate in γ-PGA was monitored. As expected, 97.1% of the glutamate units is L-glutamate in the engineered strain FC0, and the percentage of L-glutamic acid in PGA decreased to 36.9% in the engineered strain FC1, while in engineered strain FC2, the ratio of L-glutamate monomer was decreased to almost half the PGA moieties (62.2%)(see Fig.4C). Thus, a large and even range of

L-glutamate (36.9-97.1%) was achieved in the γ-PGA-producing *C. glutamicum* strains.