Supplemental Materials

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Title: Effects of eliminating pyruvate node pathways, and co-expression of heterogeneous carboxylation enzymes, on succinate production by *Enterobacter aerogenes*

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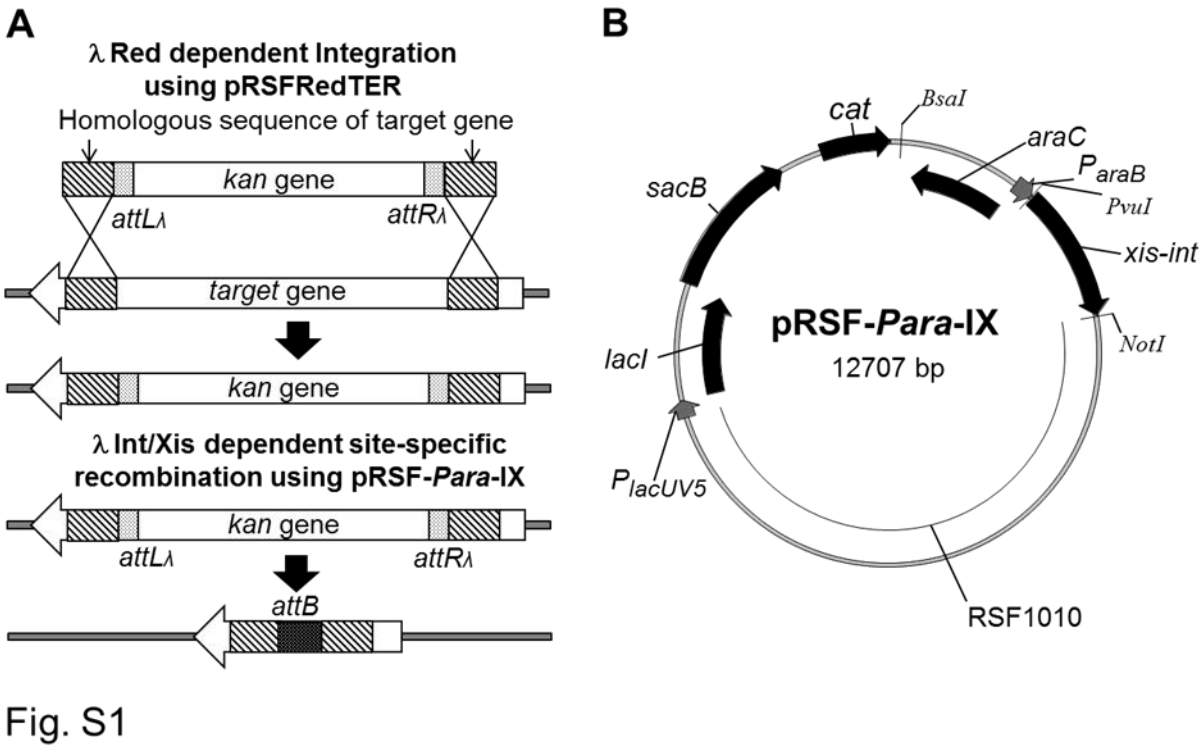

Fig. S1

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A) Schematic of the λ Red recombination system

The kanamycin resistant gene (*kan*) flanked by $attL_{\lambda}$/ $attR_{\lambda}$ was used as the selection marker. A DNA fragment containing 60-nt sequences homologous to the target region was introduced into the target gene on the chromosome via λ Red-dependent homologous recombination. The *kan* gene was then eliminated from the chromosome by λ Int/Xis-dependent site specific recombination leaving the *attB* site on the chromosome.

B) Plasmid map of pRSF-P_{ara}-IX

pRSF-P_{ara}-IX was used for removal of the *kan* gene from the *E. aerogenes* chromosome. The vector length was 12707 bp, and contained the broad-host-range replicon RSF1010 and *sacB* gene under the control of the lacUV5 promoter derived from pRSFRedTER. The

arabinose promoter region containing the *araC* gene and *araB* promoter, derived from pKD46, was integrated into the *Bsa*I and *Pvu*I site. The *xis-int* region derived from pMW-intxis-ts was integrated into the *Pvu*I and *Not*I site.