The possible influence of growth stages and viable counts on transformation frequency

In our experiments, the samples used in the transformation tests might have exhibited different growth rates and viable counts. To avoid influencing transformation frequency as a result of different growth rates in the samples, cultures were always grown to stationary phase in shaking culture stage before being transferred to a beaker for static cultivation. Shaking cultivation usually lasted for 14 h to ensure that all samples reached stationary phase. All growth curves of BW25113, Δ*rpoS*, and BW25113 with glucose added reached stationary phase after incubation for 14 h (Fig. S1); however, Δ*crp* and Δ*cyaA* exhibited a 12-h delay in reaching stationary phase (Fig. S2A). To avoid the effect of different growth phases on transformation, the shaking cultivation of Δ*crp* and Δ*cyaA* was prolonged to 26 h before transformation experiments were performed. Similar to our previous results, the transformation frequencies of Δ*crp* and Δ*cyaA* were nearly an order of magnitude higher than for the wild type (Fig. S2B). The relationship between cell density and transformants has been examined in previous studies. A linear relationship was observed between transformation frequency and cell density which ranges between 2×10^7 and 1.28×10^8 CFU/plate with strain ZK126 (1). In this work, we investigated the effect of cell density on transformation with strain BW25113. A transformation experiment in which the total CFUs ranged from ~10^8 to ~10^{10} CFU/ml was performed. A linear relationship was observed between cell density and the number of transformants (Fig. S3). Thus, the transformation frequency precisely reflects the transformability of samples with viable counts in the range of ~10^8 to ~10^{10} CFU/ml. Another experiment also confirmed this. The viable count of the wild-type strain supplemented with glucose exhibited no significant difference compared to the control. The viable counts of Δ*rpoS*, Δ*crp*, and Δ*cyaA* were 95%, 76%, and 74% lower than that of the wild-type, respectively. We concentrated the Δ*rpoS*, Δ*crp* and Δ*cyaA* cultures by the appropriate

multiples to obtain similar viable counts to the wild-type. As expected, the numbers of transformants increased accordingly and the transformation frequencies remained unchanged (Fig. S4AB).

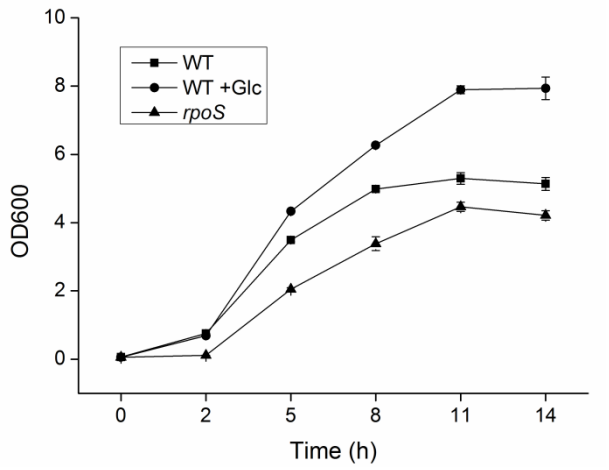

FIG. S1. Growth curves of BW25113, BW25113 with additional glucose and Δ*rpoS*.

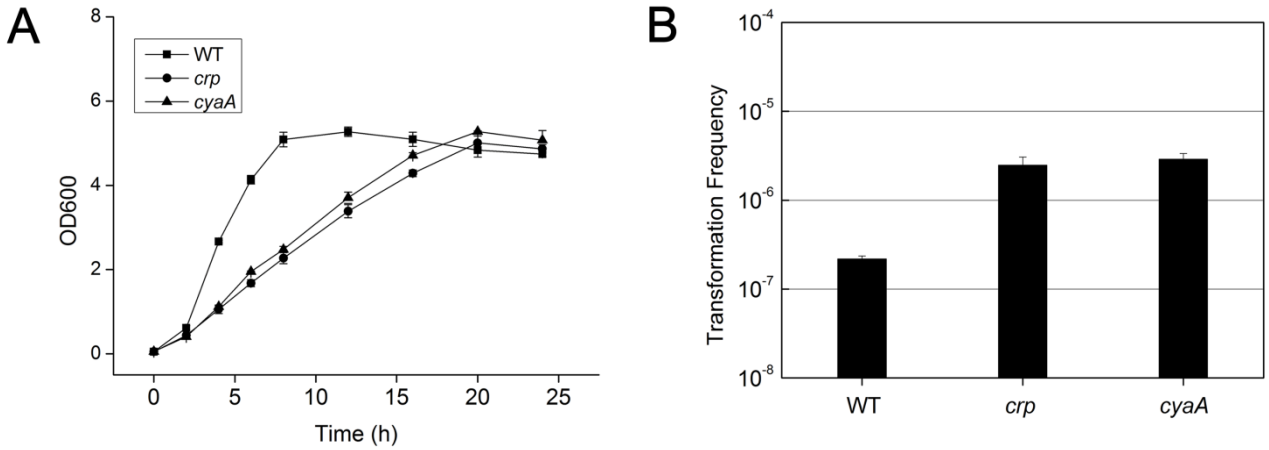

FIG. S2. (A) Growth curves of BW25113, Δ*crp* and Δ*cyaA*. (B) The transformation frequencies of Δ*crp* and Δ*cyaA* with a prolonged shaking cultivation.

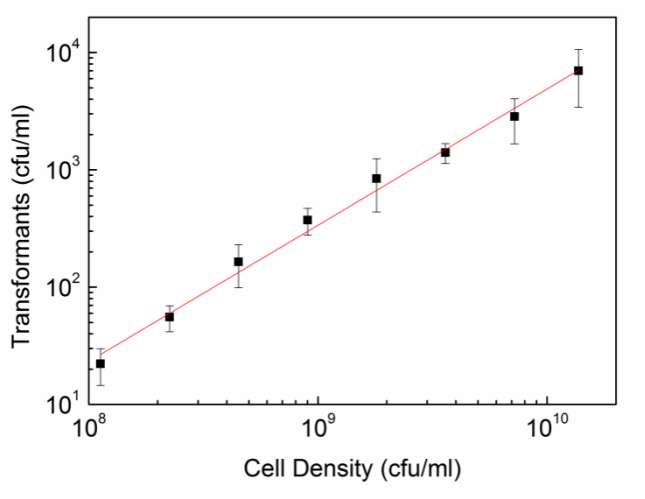

FIG. S3. The relationship between cell density and transformants. Twofold serial concentrations and dilutions from the BW25113 culture were subject to transformation experiment.

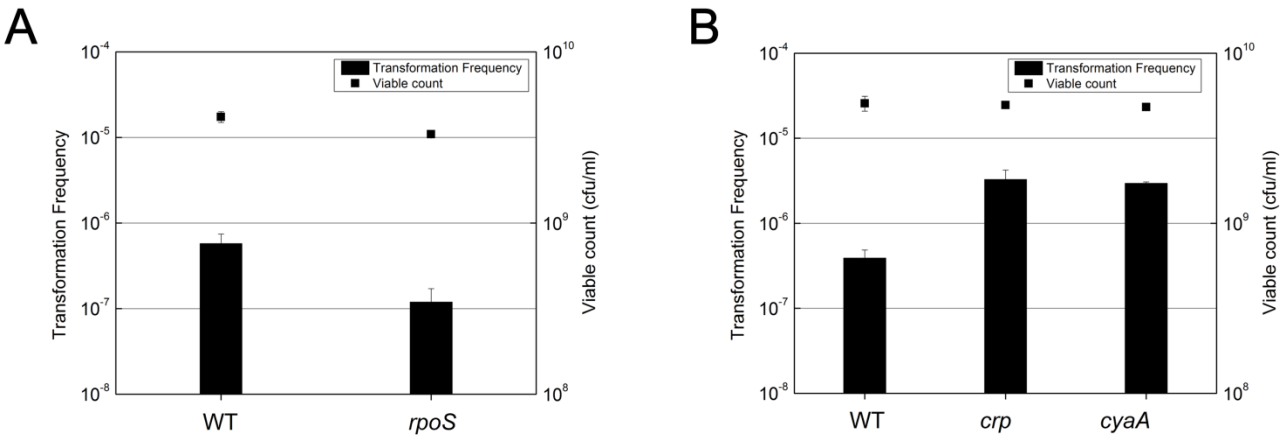

FIG. S4. (A) The transformation frequency and viable count of Δ*rpoS* after concentrating the culture by 20-fold. (B) The transformation frequencies and viable counts of Δ*crp* and Δ*cyaA* after concentrating the cultures by 4-fold.

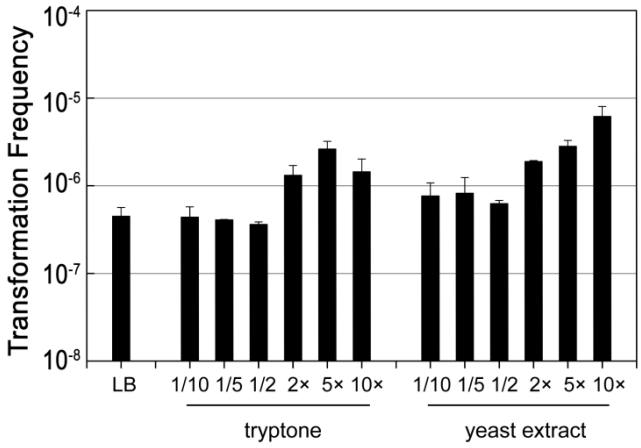

FIG. S5. Transformation frequencies of *E. coli* incubated in modified LB medium. Strains were inoculated in modified LB medium with various concentrations of tryptone or yeast extract. The transformation procedure was performed with standard protocol. Sample grown in normal LB broth served as controls.