

Fig. S1. Characteristics of recombinant ScyA.

A. SDS-PAGE analysis of purified recombinant ScyA by hemestaining. ScyA-His<sub>6</sub> was extracted from the *S. oneidensis* wildtype carrying pHGE-ScyA-His<sub>6</sub>. M represents protein marker. B. Functional analysis of recombinant ScyA and mutant proteins.





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 $\begin{array}{c}
70 \\
55 \\
40 \\
35 \\
25 \\
KDa \\
N^{0}} \\
N^{0} \\
N^{0}$ 

Fig. S2. Development of a new mariner-based transposon vector pHGT01

A. Screening for extremely strong promoters. The activities of the  $P_{SO2503}$ ,  $P_{SO3896}$ ,  $P_{cymA}$ ,  $P_{tn}$ , and  $P_{arcA}$  revealed by *lacZ* reporters. Cells of mid-log phase were sampled for the assay. Both multi-copy (M) and single-copy (S) systems were used. The values are the mean  $\pm$  S.D. (error bars) (n = 5).

B. SO3896 in the outer membrane in the presence or absence of osmotic agents. Wild-type (WT) cells were grown overnight in LB and subcultured 1:200 in fresh medium in the presence or absence of indicated agents. SO3896 protein band is indicated. Experiments were repeated at least three times and similar results were obtained. C. The vector was created by replacing transposon-unrelated DNA sequence with the *R6K* replicon and the *mob* gene and inserting outward  $P_{SO3896}$  inside IS elements.

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