Supplementary Materials

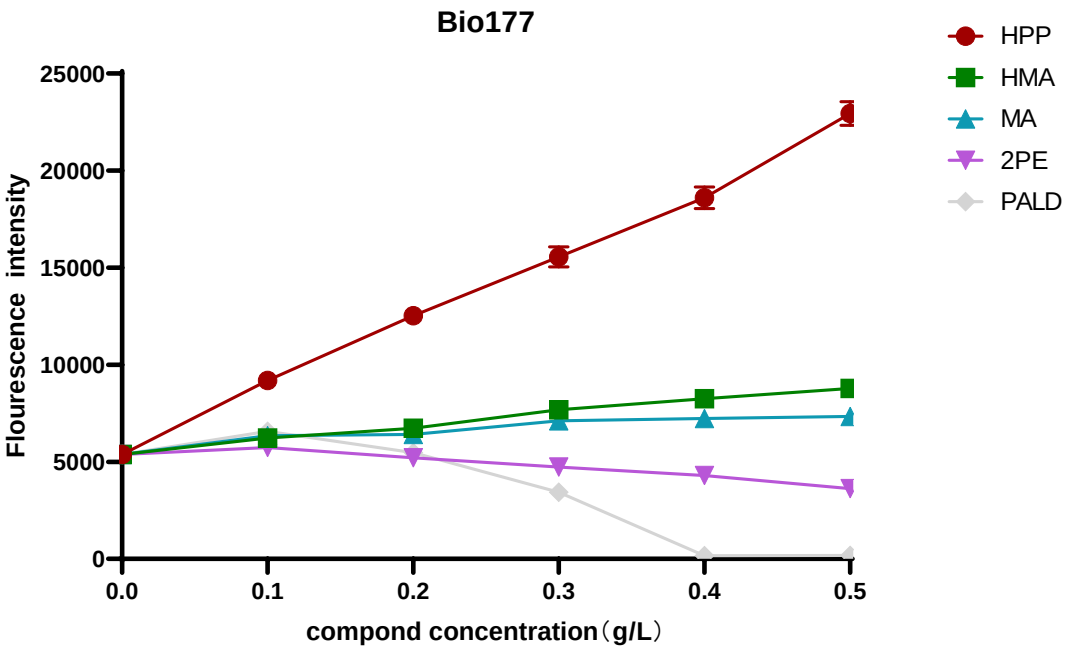
Supplementary Table 1. Strains and plasmids used in this study.

Strains and plasmids	Description	Sources
Strains		
E. coli DH5α	F⁻*ΔlacU169(Φ80 lacZ ΔM15) hsdR17 recA1 endA1 supE44 gyrA96 thi-1 relA1*	Invitrogen
BW25113	*F-, λ-, E. coli K-12 strain BD792 (CGSC6159) lacZ*	Invitrogen
BWΔCD	*BW25113 strain knocked out codA*	Invitrogen
Plasmids		
gY9s-dual T7-Trrnb-HmaS(Scpa1)-Bio 177	*Str*^{R}, CPA1 promoter, Trrnb, T7 promoter, HmaS Am, CD enzyme, mCherry, pobA promoter, PobR	This study
pHyo094	*Cmr*^{R}, f1 ori,p15A ori, P_{BAD}-*UGI*	Hyojin, et al. (2020)
PSC101-dCas9(sg-eGFP-1)	*Kan*^{R}, pSC101 ori, Rep 101, *lacI*, *sgRNA1-eGFP*, *gRNA scaffold*, *dCas9*, *tracrRNA*	This study
R6K-dCas9(sg-eGFP-1)	*Kan*^{R}, R6K ori, *pir*, *lacI*, *sgRNA1-eGFP*, *gRNA scaffold*, *dCas9*, *tracrRNA*	This study
R6K-dCas9(sg-vec)	*Kan*^{R}, R6K ori, *pir*, *lacI*, *trc promoter*, *sgRNA1-eGFP*, *gRNA scaffold*, *dCas9*, *tracrRNA*	This study
R6K-dCas9(sg-pykF-1)	*Kan*^{R}, R6K ori, *pir*, *lacI*, trc promoter, *spacer(pykF)*, *gRNA scaffold*, *dCas9*, *tracrRNA*	This study
R6K-dCas9(sg-tyrB-2)	*Kan*^{R}, R6K ori, *pir*, *lacI*, trc promoter, *spacer(tyrB)*, *gRNA scaffold*, *dCas9*, *tracrRNA*	This study
R6K-dCas9(sg-tyrR-3)	*Kan*^{R}, R6K ori, *pir*, *lacI*, trc promoter, *spacer(tyrR)*, *gRNA scaffold*, *dCas9*, *tracrRNA*	This study
R6K-dCas9(sg-pheA-3)	*Kan*^{R}, R6K ori, *pir*, *lacI*, trc promoter, *spacer(pheA)*, *gRNA scaffold*, *dCas9*, *tracrRNA*	This study
R6K-dCas9-gRNA-pykF-tyrB-tyrR-pheA	*Kan*^{R}, R6K ori, *pir*, *lacI*, trc promoter, *spacer(pheA)*, trc promoter, *spacer(tyrR)*, *trc promoter*, *spacer(tyrB)*, trc promoter, *spacer(pykF)*, *gRNA scaffold*, *dCas9*, *tracrRNA*	This study

Supplementary Table 2. Primers used in this study.

Primers	Sequences
gY9s-dual T7-Trrnb-HmaS(Scpa1)-Bio 177 construction	
(1) Bio 177 cloning	
Bio-F=0811	GTGCTGGGTCTCGCCTTTTACCGTTTGTAATCGATGG
Bio-R=0811	AGCGTGGGTCTCTTATATCTCCTTCTTAAAAGATCTTTTGAA
(2) dual T7-HmaS cloning	
AM-gg-F=0709	GTGCTGGGTCTCGGCAATAATACGACTCACTATAGGATGGCGGCGCAGGCGGG
AM-R=0811	AGCGTGGGTCTCTTAATACGACTCACTATAGGTTAACG
(3) RE-TrrnB cloning	
RET-F=0811	GTGCTGGGTCTCGTATAAAGGCCCAGTCTTTCGACTG
RET-R=0811	AGCGTGGGTCTCTTTGCTGCCTGGCGGCAGTAGCG
(4) TrrnB cloning	
T-F=0811	GTGCTGGGTCTCGATTATGCCTGGCGGCAGTAGCG
T-R=0811	AGCGTGGGTCTCTAAGGCCCAGTCTTTCGACTG
(5) Bio 177-Str construction	
CD-F==0617	GTGCTGGGTCTCGATTCTTACCGTTTGTAATCGATGGC
Str-R=0617	AGCGTGGGTCTCTTGTGTTATTTGCCGACTACCTTG
(6) HmaS-scpa1 construction	
cpa1-F=0725	GTGCTGGGTCTCGTAATTATCAAAAAGAGTATTGACATAAAGTC
Hmas-R=0617	AGCGTGGGTCTCTGAATTCCGACATACAGCAGG

Supplementary Figure 1



Supplementary Figure 1. Evaluating the specificity of biosensor Bio177. HPP: 4-hydroxyphenylpyruvate, HMA: 4-hydroxymandelate, MA: Mandelic acid, 2PE: 2-Phenylethanol, PALD: phenylacetaldehyde.

Supplementary Figure 2

0.01 mg/L 5-FC **0.05 mg/L 5-FC** **0.1 mg/L 5-FC**

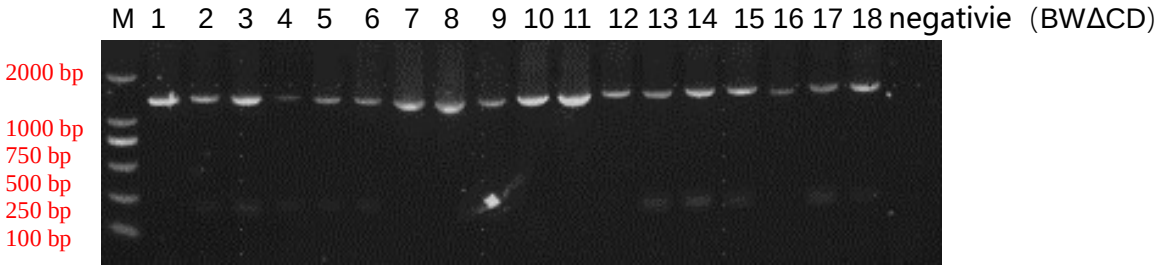
Supplementary Figure 2. Comparison of the functionality between the Bio177 plasmid (low activity) and the gY9s-HmaS-Bio177 plasmid (high activity) in bacteria under stress conditions. Both strains were plated on media with 0.01, 0.05 and 0.1 mg/L of 5-FC and clones containing the hmaS gene were selected. A 100% selection efficiency was achieved on the plate with 0.01 mg/L 5-FC medium. The wild-type strain was impaired in 0.05 mg/L 5-FC medium and eliminated in 0.1 mg/L 5-FC medium.

Supplementary Figure 3

gY9s-HmaS^{mut}-Bio177 mutant library plates 1, 2, 3

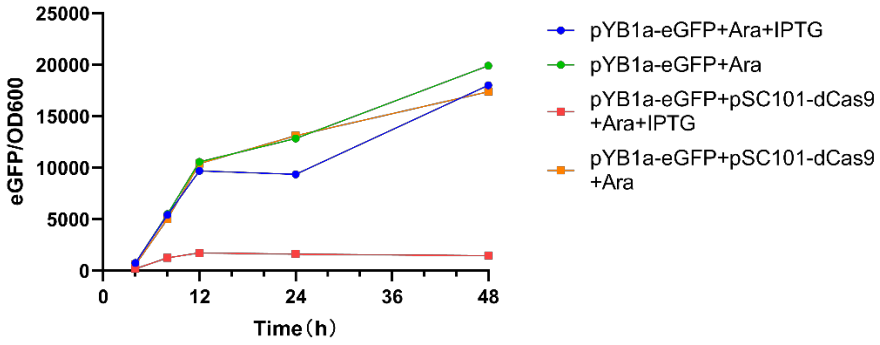
Supplementary Figure 3. By using the error-prone PCR with a low-fidelity DNA polymerase and adjusting Mn^{2+} and Mg^{2+} concentrations and their ratio, the mutation rate was manipulated. The Golden Gate assembly procedure was optimized to increase the insertion probability of mutated fragments into the vector. A mutant library with 10^5 clones was constructed.

Supplementary Figure 4.



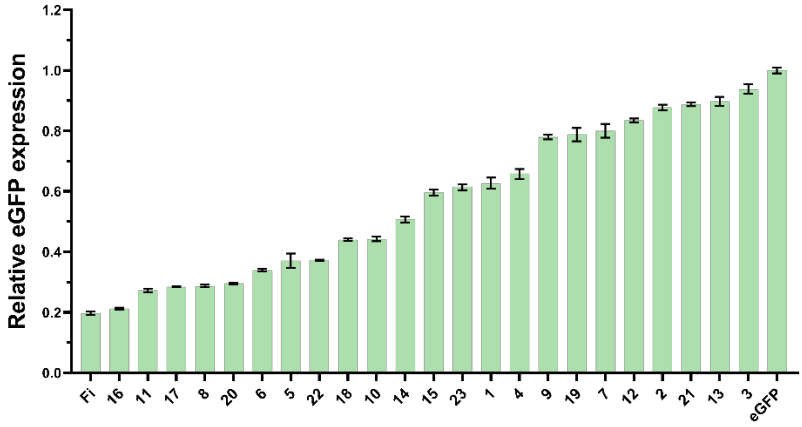
Supplementary Figure 4. Colony PCR to screen for plasmids containing HmaS mutants.

Supplementary Figure 5



Supplementary Figure 5. The inhibitory effect of pSC101-dCas9-eGFP plasmid on CRISPRi. IPTG was used to induce sgRNA expression and Ara was used to induce eGFP expression.

Supplementary Figure 6



Supplementary Figure 6. Evaluation of eGFP inhibition by a sgRNA library using the CRISPRi technology. After co-transformation of R6K-dCas9-eGFP mutant library plasmid and pYB1a-eGFP plasmid, 24 colonies were selected for fluorescence characterization and DNA sequencing of the sgRNA 7-8bp spacer. Fi means Fully Interference, transferred to the strain that containing the non-mutated R6K-dCas9-eGFP plasmid.