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Supporting Information

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A Genetic Circuit System Based on Quorum Sensing Signaling for Directed Evolution of Quorum-Quenching Enzymes

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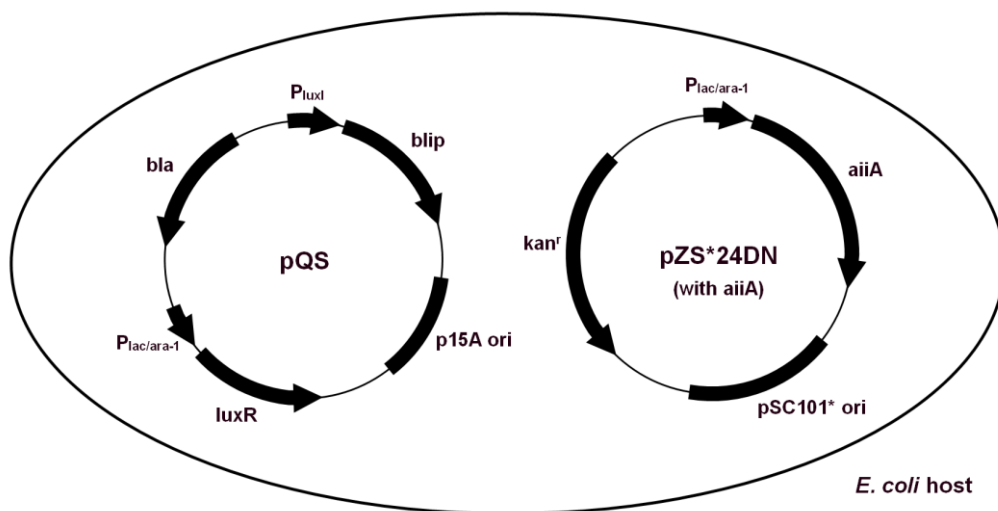


Figure S1. Host cell constructed for the library generation and screening of quorum-quenching enzyme. The AHL-response plasmid pQS and AiiA-expression plasmid pZS*24DN were inserted into the *E. coli* host.

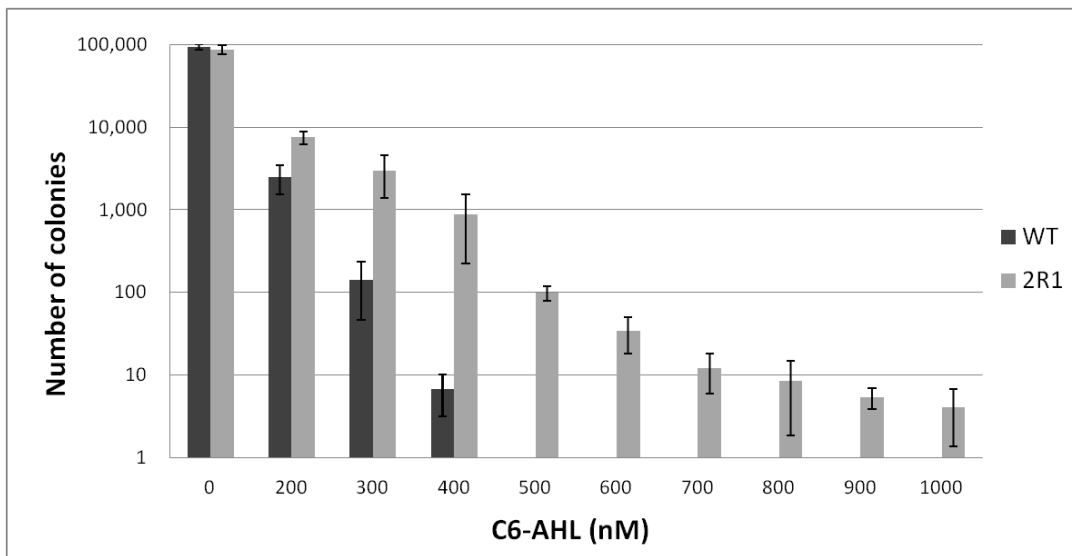


Figure S2. Growth of host cells expressing the wild type (WT) AiiA and the mutant 2R1 (V69L/I190F) on agar plates containing the indicated concentration of C6-AHL. The number of colonies growing on each plate was estimated. Host cells (approximately 100,000 cells per each plate) were cultured at 30°C for 3 days on LB agar plates containing predetermined concentrations of C6-AHL. The concentrations of carbenicillin and kanamycin were 100µg/ml and 30µg/ml, respectively. The number of colonies was uncountable on plates including AHL concentrations below 200nM. Error bars represent the standard deviations in triplicate experiments.

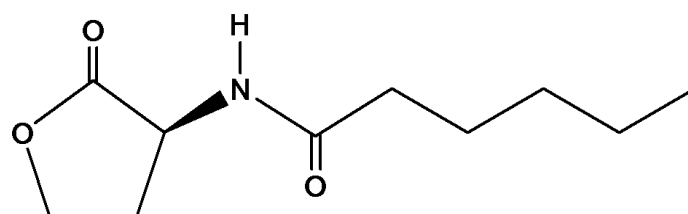


Figure S3. Molecular structure of N-hexanoyl-L-homoserine lactone (C6-AHL).

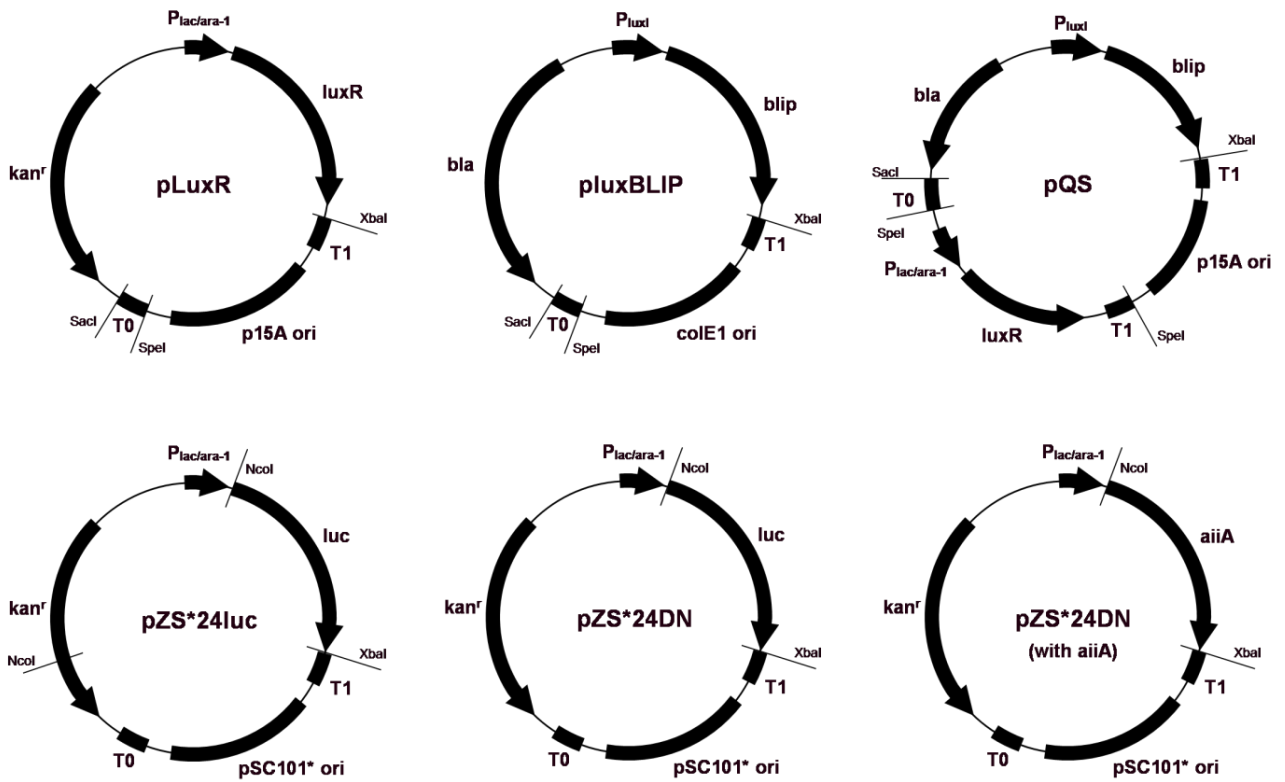


Figure S4. Plasmids used in this study. The AHL-response plasmid, pQS, was constructed from fragments of pLuxR and pluxBLIP. The plasmid pZS*24DN for the expression of quorum quenching enzymes was constructed by substitution of the luciferase gene in the plasmid pZS*24luc.

Table S1. Kinetic parameters of the mutants toward C6-AHL.				
Enzyme	k_{cat} (s ⁻¹) ^[a]	K_{M} (mM) ^[a]	$k_{\text{cat}}/K_{\text{M}}$ (M ⁻¹ s ⁻¹)	Fold
Wild type AiiA	85 ± 17	8.1 ± 1.4	1.0 × 10 ⁴	
I190F	55 ± 10	5.6 ± 0.60	9.8 × 10 ³	0.98
G207V	110 ± 8.5	9.2 ± 1.1	1.2 × 10 ⁴	1.2
[a] Each value represents mean and standard deviation in triplicate experiments.				