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Supplementary Information

A Recombinase-Based Genetic Circuit for Heavy Metal Monitoring

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Table S1. List of oligonucleotides used in this study.

Primer Set	Sequence (5′ - 3′)	Usage
P_{cadA}-FWD	TGCCAAGTGAACACCCTGTAGCCAC-TATAGGGTCAAGCCGCCCG-GATGATCCTGAC	P_{cadA} addition to the output module
P_{cadA}-REV	CTATAGTGGCTACAGGGTGTTCAC-TTGGCAACAGGCGTCGGGGTTT-GTACCGT	
Bxb1-FWD	AGCGGGGTGCACTCATCAAAAGAG-GAGAAAGGTACCATGGGAACGGTG	Overhang primers for Bxb1
Bxb1-REV	TCTTCATGGTAC-CTTTCTCCTCTTTAATACGCGTTTAC-GACATCCCGGTGTG	
CadR-FWD	GAACGGCTACACACCGGGATGTCG-TAAACGCGTATTAAAGAGGA-GAAAGGTACCA	Overhang primers for CadR
CadR-REV	GAGCCTTTCGTTTTATTT-GATGCCCTGCAG-TTAATGCCCGTGGCTTC	

Table S2. List of genetic parts and sequences used in this study.

Part	Type	Sequence (5′ - 3′)
P_{dnaK}	Stress Inducible Promoter	AAAAGCACAAAAAATTTTTGCATCTCCCCCTTGATGACGTGGTTT ACGACCCCATTTAGTAGTCAACCGCAGTGAGTGAGTCTGCAAAA AAATGAAATTGGGCAGTTGAAACCAGACGTTTCGCCCCTATTACA GACTCACAACCACATGATGACCGAATATATAGTGGAGACGTTTA GATG
mProD	Constitutive Promoter	TCTAGATTTACAGCTAGCTCAGTCCTAGGTATAATGCTAGCTACT AGAG
P_{cadA}	Cadmium Inducible Promoter	TTGACTCTGTAGTTGCTACAGGGTGTGCAAT

IR3	Regulatory	GCAAGCTTGAGCGGGGTGCACTCATCA
Bxb1 attP	Regulatory	GTCGTGGTTTGTCTGGTCAACCACCGCGGTCTCAGTGGTGTACGG TACAAACCCCGAC
Bxb1 attB	Regulatory	GCCCGGATGATCCTGACGACGGAGACCGCCGTCGTCGACAAGCC GGCCGA
RBS	Regulatory	AAAGAGGAGAAA
rrnBT1	Terminator	CAAATAAAACGAAAGGCTCAGTCGAAAGACTGGGCCTTTCGTTT TATCTGTTGTTTGTCGGTGAACGCTCTCCTGAGTAGGACAAAT
GFP	CDS	ATGCGTAAAGGAGAAGAACTTTTCACTGGAGTTGTCCCAATTCTT GTTGAATTAGATGGTGATGTTAATGGGCACAAATTTTCTGTCAGT GGAGAGGGTGAAGGTGATGCAACATACGGAAAACTTACCCTTAA ATTTATTTGCACTACTGGAAAACTACCTGTTCCATGGCCAACACTT GTCACTACTTTCGGTTATGGTGTTCAATGCTTTGCGAGATACCCAG ATCATATGAAACAGCATGACTTTTTCAAGAGTGCCATGCCCGAAG GTTATGTACAGGAAAGAACTATATTTTTCAAAGATGACGGGAACT ACAAGACACGTGCTGAAGTCAAGTTTGAAGGTGATACCCTTGTTA ATAGAATCGAGTTAAAAGGTATTGATTTTAAAGAAGATGGAAAC ATTCTTGGACACAAATTGGAATACAACTATAACTCACACAATGTA TACATCATGGCAGACAAACAAAAGAATGGAATCAAAGTTAACTT CAAAATTAGACACAACATTGAAGATGGAAGCGTTCAACTAGCAG ACCATTATCAACAAAATACTCCAATTGGCGATGGCCCTGTCCTTT TACCAGACAACCATTACCTGTCCACACAATCTGCCCTTTCGAAAG ATCCCAACGAAAAGAGAGACCACATGGTCCTTCTTGAGTTTGTAA CAGCTGCTGGGATTACACATGGCATGGATGAACTATACAAATAA
HspR	CDS	ATGGCCAAAAATCCGAAAGATGGCGAAAGCCGCACCTTCCTGAT TAGCGTGGCCGCCGAACTGGCCGGTATGCATGCCCAGACCCTGC GCACCTATGATCGTCTGGGTCTGGTGAGCCCGCGTCGTACCAGTG GTGGTGGTCGTCGTTATAGCCTGCATGATGTGGAGCTGCTGCGCC AGGTTCAGCATCTGAGCCAGGATGAAGGCGTGAATCTGGCCGGC ATCAAACGCATCATTGAACTGACCAGCCAGGTGGAAGCACTGCA GAGCCGCCTGCAGGAAATGGCCGAAGAACTGGCCGTGCTGCGCG CCAATCAGCGTCGTGAAGTGGCCGTGGTGCCGAAAAGCACCGCC CTGGTGGTGTGGAAACCGCGTCGTTAA
CadR	CDS	ATGAAGATCGGAGAACTGGCCAAAGCCACCGACTGCGCCGTGGA AACCATCCGCTACTACGAGCGTGAACAGCTGCTGCCGGAGCCGG CACGCAGCGACGGCAACTACCGGCTGTACACCCAGGCCCACGTC GAGCGGCTTACCTTCATCCGCAACTGCCGCACCCTGGACATGACC CTGGATGAAATCCGCAGCCTGCTACGCCTGCGCGACAGCCCCGA TGATTCGTGCGGCAGCGTCAATGCGCTGATCGACGAGCATATCGA GCATGTGCAGGCACGGATCGATGGTCTGGTGGCGTTGCAGGAAC AGCTGGTGGAGCTGCGGCGGCGCTGCAATGCACAAGGGGCGGA GTGTGCGATCTTGCAGCAACTGGAGACGAACGGGGCGGTATCGG TGCCGGAAACCGAGCATTCGCATGTAGGGCGAAGCCACGGGCAT TAA
Bxb1	CDS	ATGGGAACGGTGGCGCAGATGGAATTAGAAGCGATCAAAGAGC GGAACCGTTCGGCTGCGCATTTCAATATCCGCGCCGGGAAATACC GAGGATCCCTGCCGCCGTGGGGATACCTGCCTACGCGCGTGGAC GGGGAGTGGCGGCTGGTGCCGGACCCTGTGCAGCGAGAGCGCAT CCTCGAGGTGTATCACCGCGTCGTCGACAACCACGAGCCGCTGC ATCTGGTGGCCCACGACCTGAACCGGCGTGGTGTCCTGTCGCCGA AGGACTACTTCGCGCAGCTGCAAGGCCGCGAGCCGCAGGGCCGG

GAGTGGTCGGCTACCGCGCTGAAGCGATCGATGATCTCCGAGGC
GATGCTCGGGTACGCGACTCTGAACGGTAAGACCGTCCGAGACG
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GAGCAGCTGGAGGCGCTGCGCGCCGAGCTCGTGAAGACCTCCCG
GGCGAAGCCCGCGGTGTCTACCCCGTCGCTGCTGCTGCGGGTGTT
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GAGGACGTAAGCACCCGCGCTACCGCTGCCGCTCGATGGGGTTC
CCGAAGCACTGCGGGAACGGCACGGTGGCGATGGCCGAGTGGG
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GAGCGTCTGGAGAAAGTCTGGGTAGCGGGCTCGGACTCCGCGGT
CGAACTCGCGGAGGTGAACGCGGAGCTGGTGGACCTGACGTCGC
TGATCGGCTCCCCGGCCTACCGGGCGGGCTCTCCGCAGCGAGAA
GCACTGGATGCCCGTATTGCGGCGCTGGCCGCGCGGCAAGAGGA
GCTGGAGGGCCTGGAGGCTCGCCCGTCTGGCTGGGAGTGGCGCG
AGACCGGGCAGCGGTTCGGGGACTGGTGGCGGGAGCAGGACAC
CGCGGCAAAGAACACCTGGCTTCGGTCGATGAACGTTCGGCTGA
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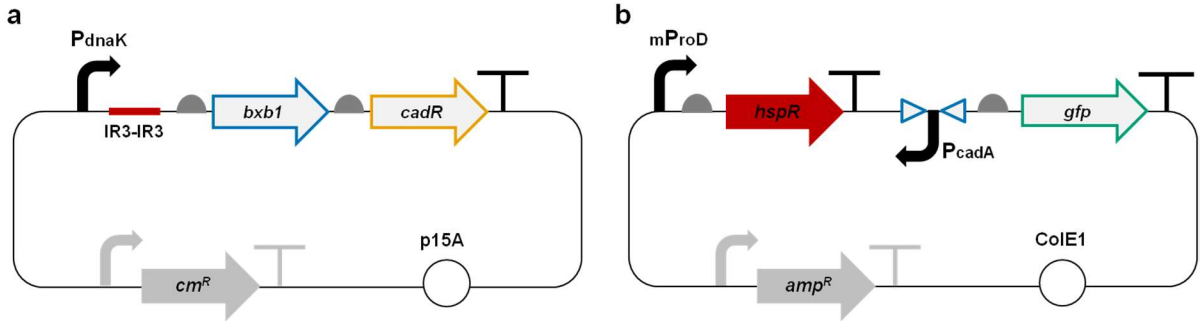

Figure S1. Representative plasmid maps of the cadmium sensor. Plasmid map containing actuating module **(a)** and sensing and output modules **(b)**. "T" represents terminator.

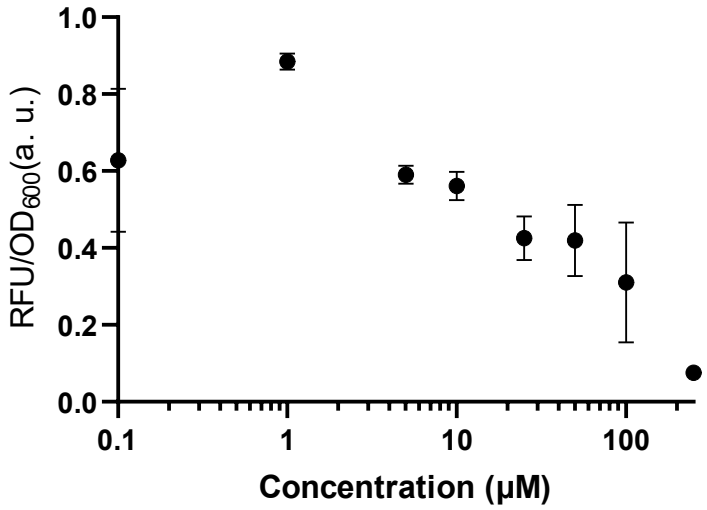

Figure S2. Cadmium-related toxicity for Escherichia coli DH5α strain. The initial number of cells was 1:50 of culture volume. The cells were cultured in MOPS minimal media with a pH of 7.5 at 37°C for 14h. Measurements were done on 96-well plates. The fluorescence intensity of each group

was compared with each other and normalized according to the formula stated in the Materials and Methods section. Data were visualized with mean ± standard error mean (SEM) in each graph. At least three biological replicates were used for each analysis.

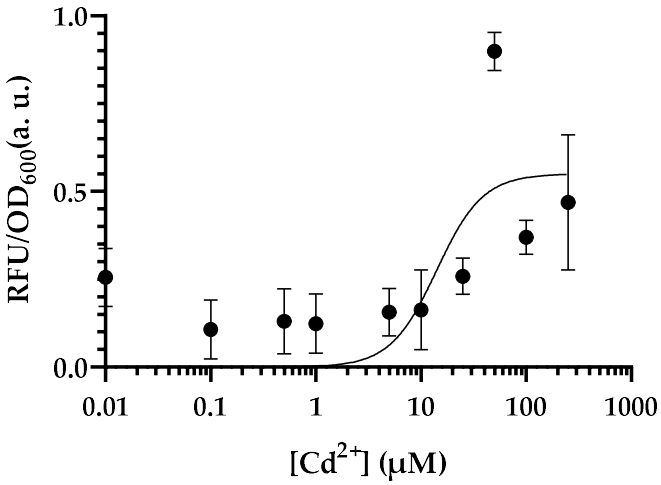

Figure S3. The system response with 100 μM and 250 μM. The initial number of cells was 1:50 of culture volume. The cells were cultured in MOPS minimal media with a pH of 7.5 at 37°C for 10h. Measurements were done on 96-well plates. The fluorescence intensity of each group was compared with each other and normalized according to the formula stated in the Materials and Methods section. Data were visualized with mean ± standard error mean (SEM) in each graph. At least three biological replicates were used for each analysis.