Supplementary Information

Portable environment-signal detection biosensors with cell-free synthetic biosystems

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Materials and methods

Synthetic genetic networks

For the constitutive expression of enzymes GusA, LacZ, and XylE, the genetic circuits were constructed by inserting *gusA*, *lacZ*, and *xylE* downstream of the PT7 promoter in pET-21b.[1–3] For the inducible synthetic genetic circuits, the ArsR-based or LuxR-based GusA, LacZ, and XylE expression circuits were constructed by inserting GusA, LacZ, or XylE downstream of the specifically regulated promoters in pSB3K3. To provide constitutive ArsR or LuxR expression, ArsR or LuxR was cloned downstream of the constitutive J23101 promoter, and these expression cassettes were inserted upstream to the formerly constructed pSB3K3[4,5] (Fig. S5-S8).

Cell extract preparation

For standard cell-free expression reactions, *E. coli* Rosetta (DE3) (as well as BL21 Star (DE3), DH5α) [6,7] were fermented in 4 L of 2xYTP (1.6% tryptone; 1% yeast extraction; 0.5% NaCl; 40 mM K_2HPO_4; 22 mM KH_2PO_4) containing chloramphenicol (34 μg/ml) at 37 °C, 300 rpm. Cells were harvested in the late logarithmic growth phase (~4 h, OD600=2). Cell pellets were washed with ice-cold buffer A (14 mM magnesium glutamate; 60 mM potassium glutamate; 50 mM Tris, pH 7.7) three times. Cells were resuspended in buffer A (1 ml buffer for 1 g of wet cells) for disruption in a high-pressure homogenizer (1000 bar). Then lysate was centrifuged at 12000 x g for 10 min at 4 °C. The supernatant was incubated at 37 °C for 80 min after 3 mM DTT added and centrifuged at 12000 x g for 10 min at 4 °C again. Then it was dialyzed in molecular porous membrane tubing (6-8 KD MWCO) for 3 h at 4 °C with magnetic stirring. The dialysate was then centrifuged at 12000 x g for 10 min at 4 °C, flash frozen, and stored at -80 °C[8].

Cell-free reactions

Cell-free reagents for standard cell-free reaction were assembled on ice as generally described and immediately incubated at 37 °C. The general cell-free reaction mixture consisted of the following components: 30% S12 cell extract (v/v%); 30 ng/μl DNA templates; 175 mM potassium glutamate; 10 mM ammonium glutamate; 2.7 mM potassium oxalate monohydrate; 10 mM magnesium glutamate; 50 mM each of 19 amino acids without glutamic acid; 3 mM phosphenol pyruvate (PEP); 1 mM putrescine; 1.5 mM spermidine; 0.33 mM nicotinamide adenine dinucleotide (NAD); 1.2 mM ATP; 0.86 mM each of CTP, GTP and UTP; 0.27 mM coenzyme A; 170 μg/mL tRNA; 34 μg/mL folinic; 2% PEG8000; T7 RNA polymerase prepared from BL21(DE3) cell extract.

Solution-phase cell-free reaction

For the constitutive PT7 cell-free reactions, the purified genetic circuits were added into the total 20 μL reaction as formally described and incubated at 37 °C overnight. The substrate (2 mg/mL for X-Gluc, 0.6 mg/mL for chlorophenol red-β-D-galactopyranoside and 2 mg/mL for pyrocatechol for the final concentration) was supplied to the 10-fold diluted reactions for the colorimetric analysis. The absorbance of the reactions was measured on the standard ultraviolet spectrophotometer at 660 nm, 470 nm, and 390 nm, respectively, after diluted to 2 mL with ddH_2O.

For inducing cell-free reactions, the *arsR*- and *luxR*-based synthetic genetic circuits were supplied to the assembled reactions along with the inducer. For characterization assays, 0.2 μL arsenic ion solutions in water and AHL stock solutions in DMSO were added as the inducer (within the 20 μL total reaction volume) to give final concentrations of 0.10, 0.20, 0.25, 0.50, and 1.0 μM. The colorimetric reactions were performed in the 10-fold diluted cell-free systems after hours of incubation by adding the substrate mentioned above and measured.

Preparation of reactions and incubation

The 10 μL assembled cell-free reactions (without plasmids) were applied to 4 mm paper disc, which were then frozen at -80 ℃ and freeze-dried in 2 hours. Moreover, 2 mg/ml pyrocatechol as the substrate of XylE could be supplied to the cell-free reaction before the freeze-drying process. Paper discs were cut using a 4-mm puncher. The freeze-dried paper discs were stored at room temperature for days (Fig. S10). The paper reactions were rehydrated with plasmids solution coupled with inducer at the concentrations specified. Rehydrated reactions were incubated at 37 ℃ using the incubator. After hours of incubation, for LacZ-based colorimetric reactions, the chlorophenol red-β-D-galactopyranoside was supplied to the reaction at the final concentration of 0.6 mg/mL. The colorimetric signals of papers were collected by the camera of iPhone and analyzed by Image J software.

Fabrication of matric materials

Although the initial paper-based reactions were successful, there still might be nonspecific interactions between the cell-free components and papers or the activity loss during the freeze-drying processes, which could impede the activity of the reactions. Several commonly used protectants were used for treating papers or the cell-free reactions. For treating paper, the papers were wet with 5 % (w/w) protectants and then cut into paper discs for loading cell-free components and freeze-dried. For treating the cell-free components, the protectants were supplied to the assembled cell-free reactions at the final concentrations of 5 % (w/w) and similarly freeze-dried for the late rehydrated reactions (Fig. S1).

Measurement and analysis

After the conditional expression, images of paper discs were collected by the smartphone camera of iPhone and analyzed by Image J software.

Supplementary Figures

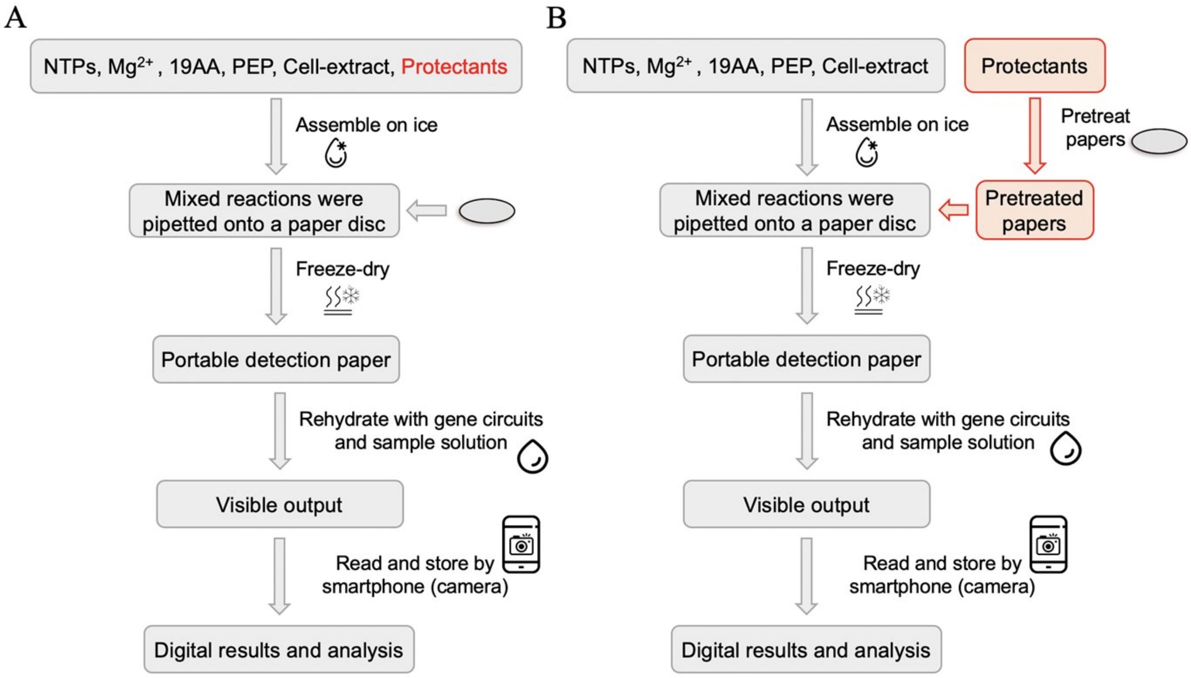

Fig. S1. Cell-free reactions. (A) Cell-free reaction components (including cell extract, NTPs, Mg^{2+}, 19 amino acids, PEP, and others) and protectants were assembled on ice and put onto papers. (B) Cell-free reaction components were assembled on ice and put onto protectant-treated papers. Then the paper could be rehydrated with genetic circuits and sample solutions. The visible output could be distinguished by the naked eye or analyzed by the smartphone after photographed by the camera.

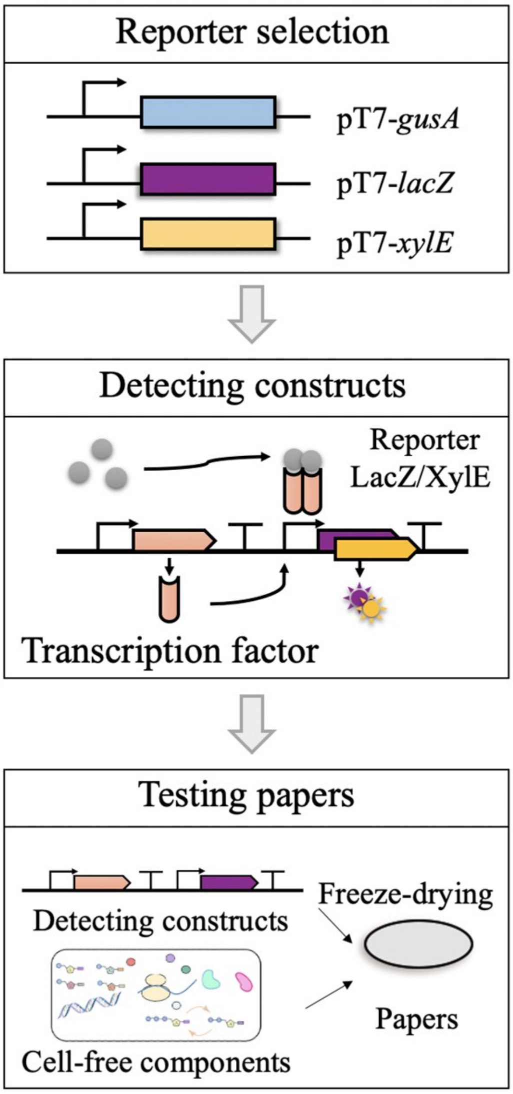

Fig. S2. The construction workflow of paper-based cell-free detecting system. The PT7 system were used for the reporter selection. The selected two reporters LacZ and XylE were constructed into the sensing genetic circuits as the reporter gene. After the construction, the detecting constructs and cell-free components were freeze-dried onto papers for the arsenic ion or 3OC12HSL detection.

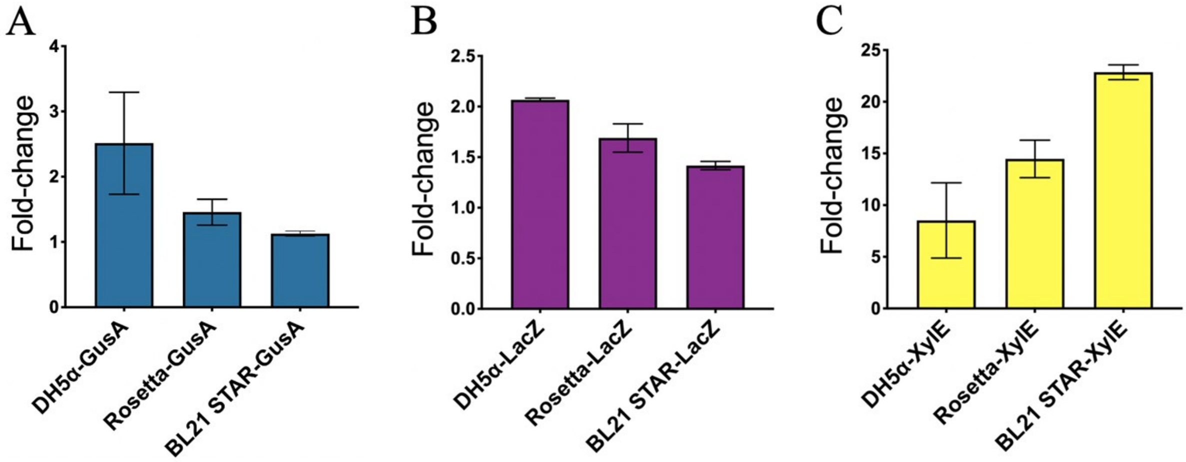

Fig. S3. The visible output change of the constitutive expression of three enzymes (GusA, LacZ, and XylE, respectively) in three cell-free systems compared to the system background control.

A

Construct name	Translation rate of RBS	Construct name	Translation rate of RBS
AL1/LL1	511335.37	AX1/LX1	273544.23
AL2/LL2	51239.36	AX2/LX2	27369.73
AL3/LL3	5140.96	AX3/LX3	2737.27
AL4/LL4	2562.85/930.76	AX4/LX4	3433.74/1144.85
AL5/LL5	50.91	AX5/LX5	27.01

B

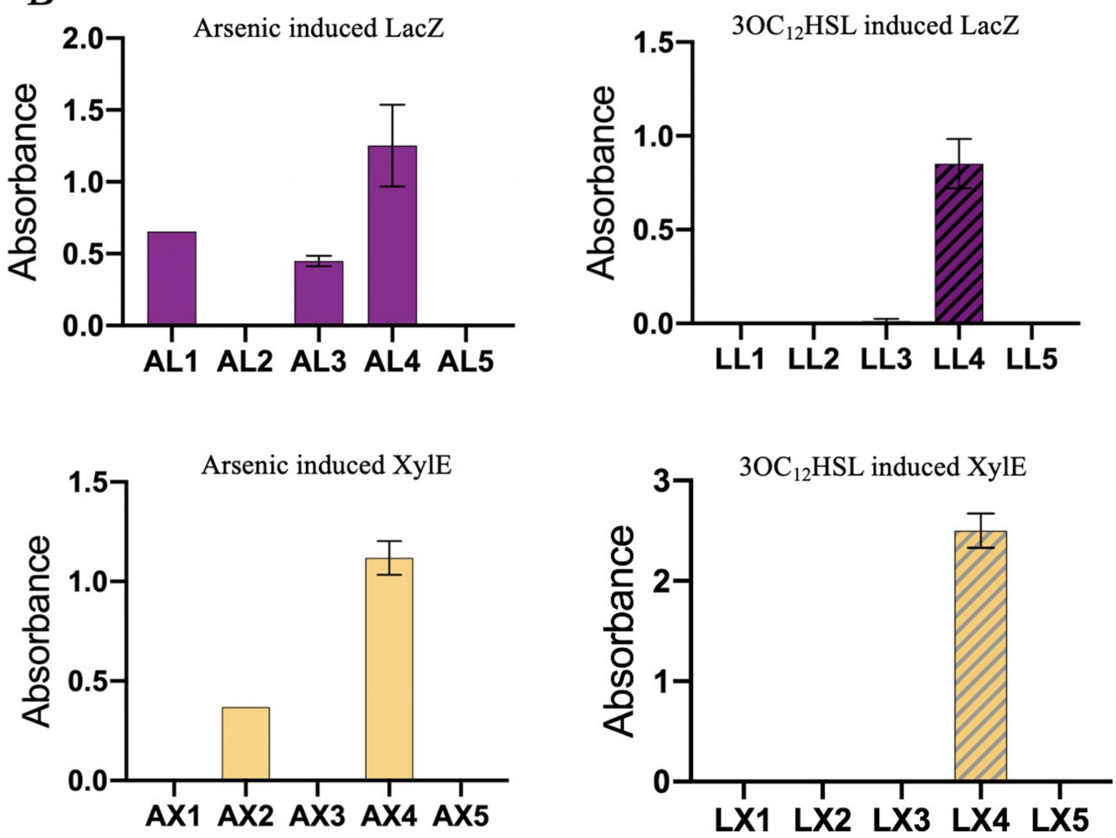

Fig. S4. The selection of RBS for the reporter LacZ and XylE. (A) RBS of LacZ or XylE was predicted and selected from the Salis RBS tools software in a range of predicted expression rate relative to LacZ or XylE[9,10]. (B) The constructs with different RBS were tested in the cell-free reactions with 1 μM inducer. The constructs with AL4, LL4, AX4, and LX4 presented the high signal output.

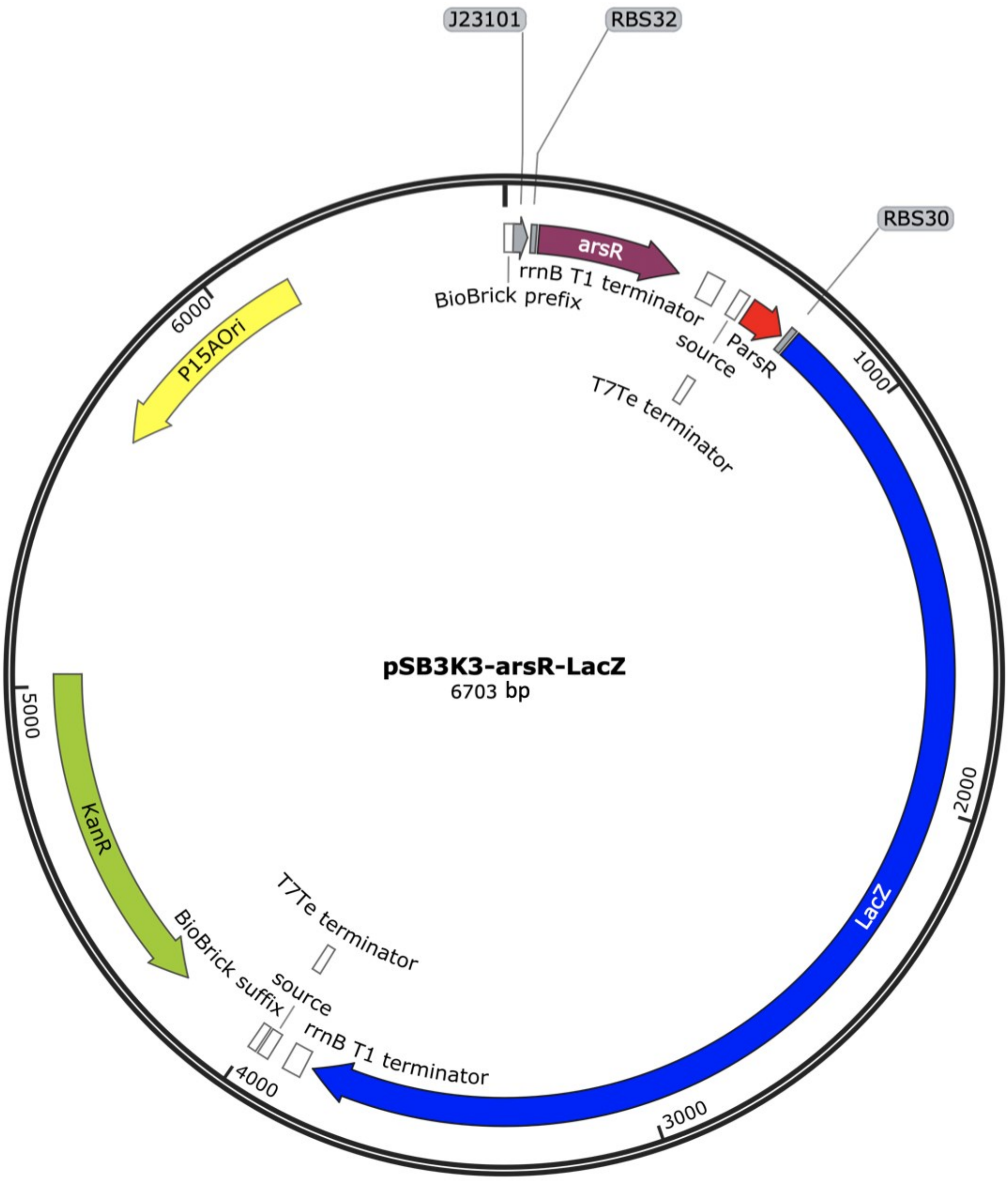

Fig. S5. The plasmid map of LacZ-based arsenic ion sensing operon. Detailed DNA or amino acid sequences were shown in supplementary tables.

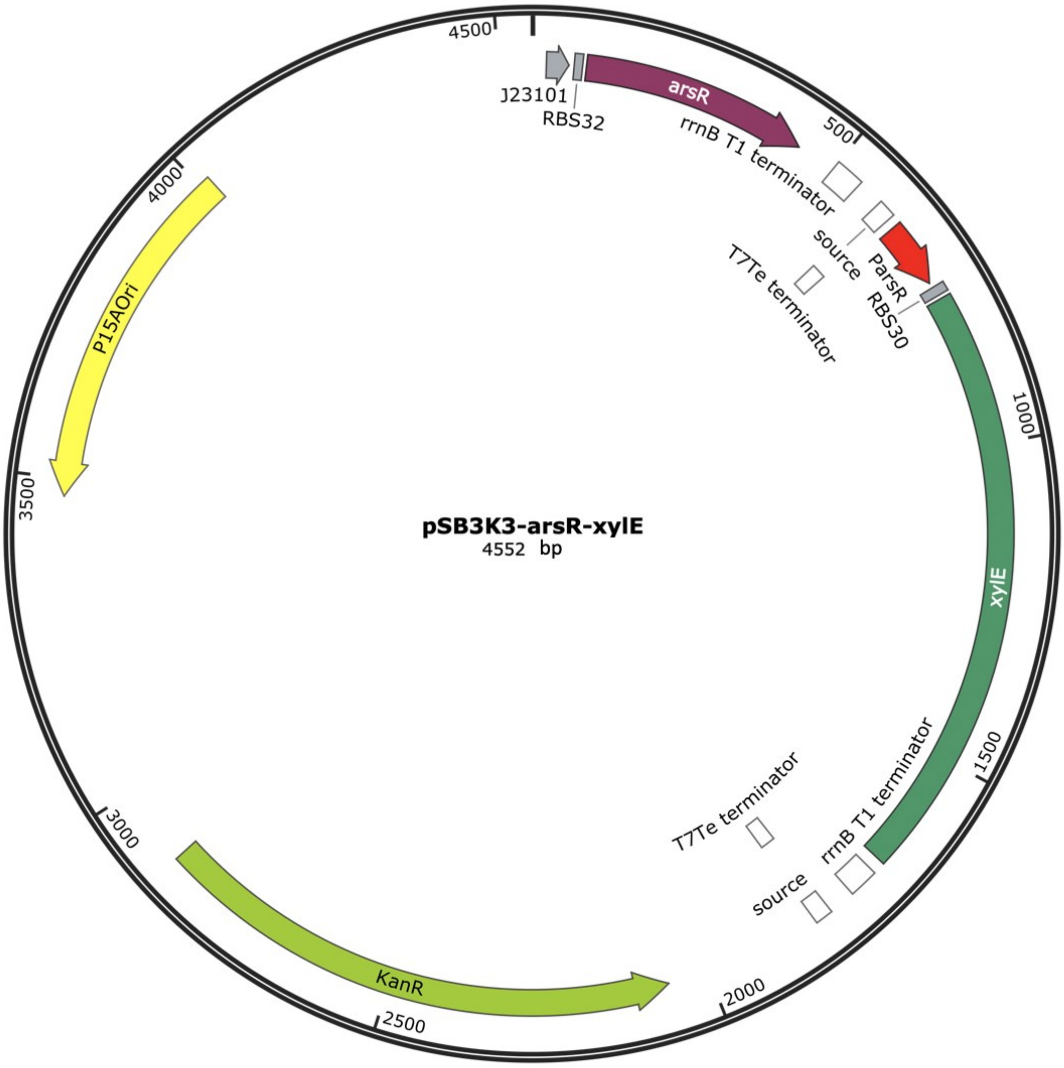

Fig. S6. The plasmid map of XylE-based arsenic ion sensing operon. Detailed DNA or amino acid sequences were shown in supplementary tables.

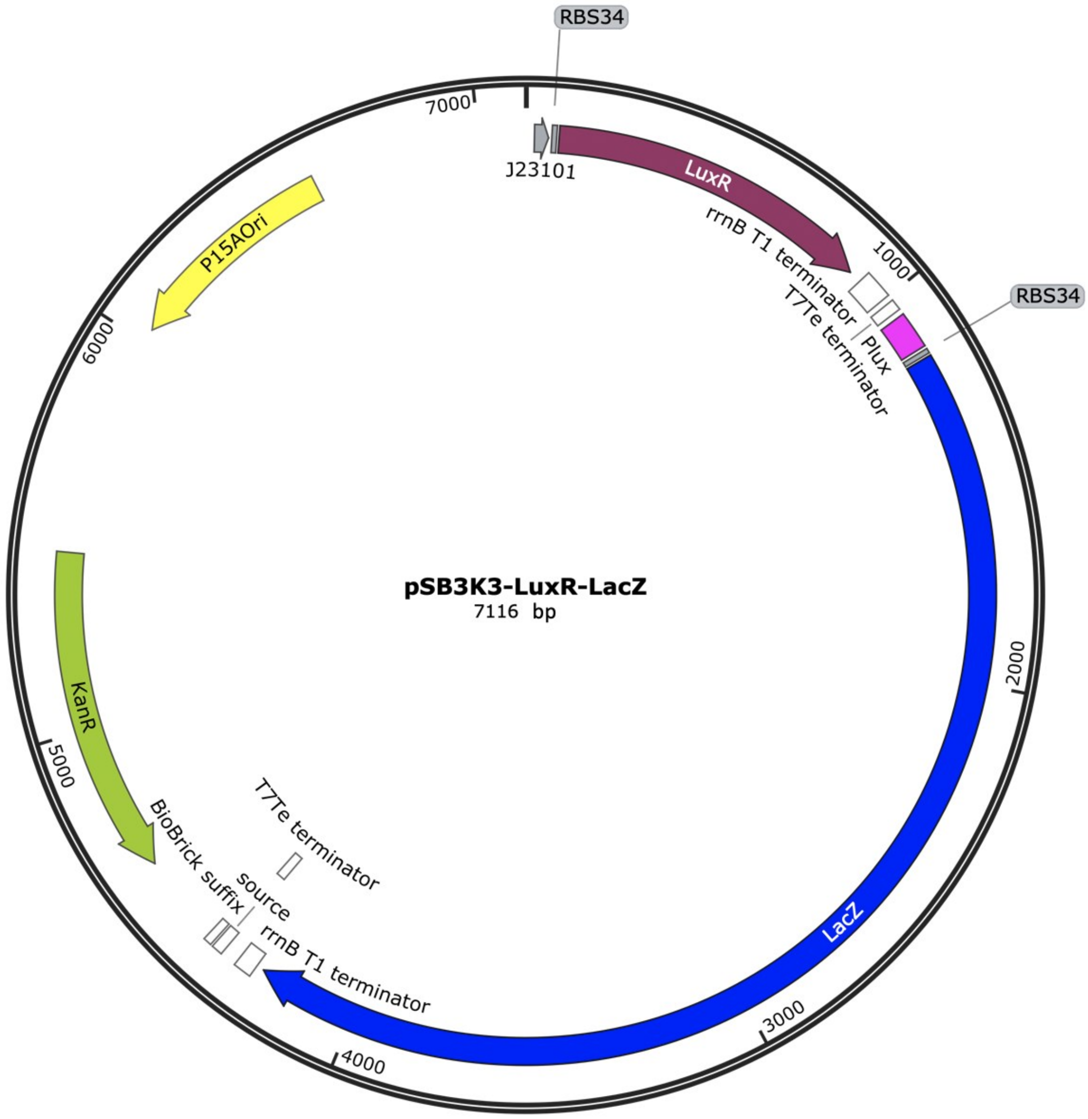

Fig. S7. The plasmid map of LacZ-based AHL sensing operon. Detailed DNA or amino acid sequences were shown in supplementary tables.

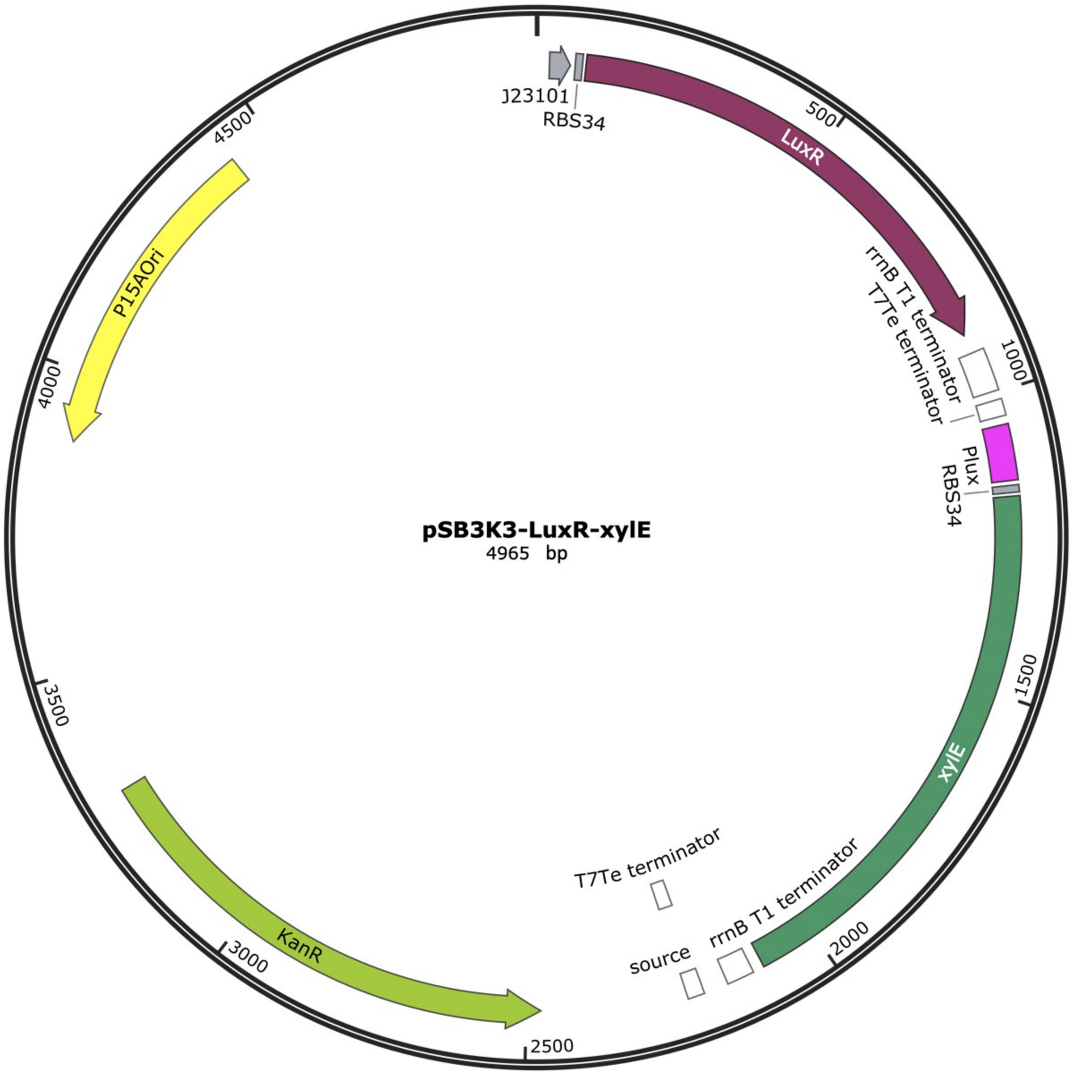

Fig. S8. The plasmid map of XylE-based AHL sensing operon. Detailed DNA or amino acid sequences were shown in supplementary tables.

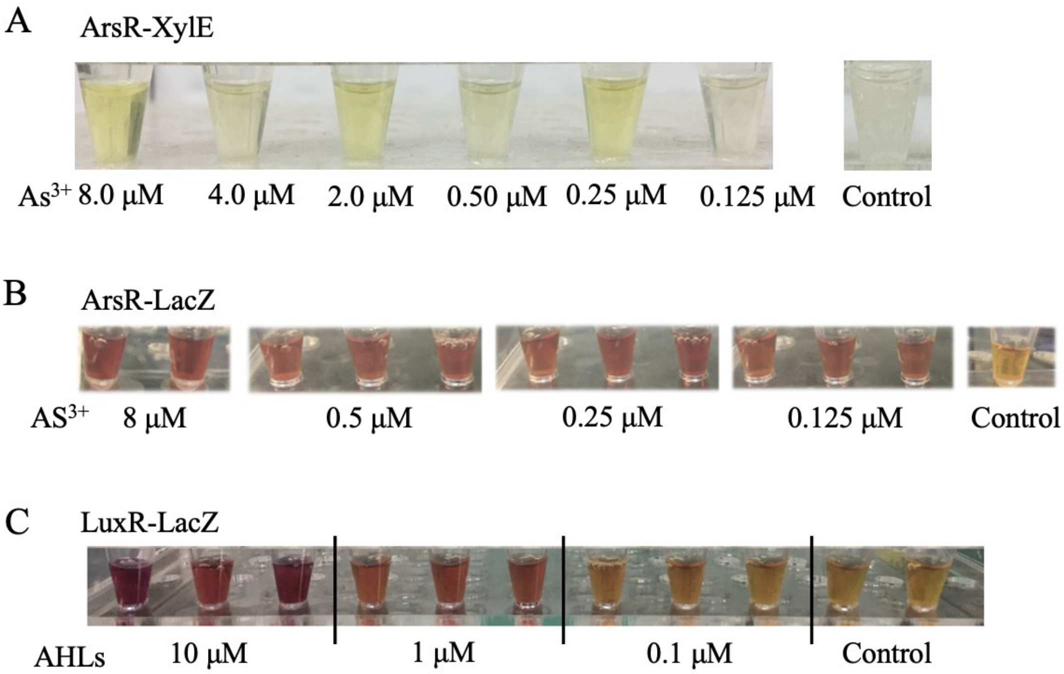

Fig. S9. Pictures of solution-phase *in vitro* sensing systems response to arsenic ion and AHL. (a) Pictures of the ArsR-XylE-based sensing system response to arsenic ion. (b) Pictures of ArsR-LacZ-based sensing system response to arsenic ion. (c) Pictures of LuxR-LacZ-based sensing system response to AHL.

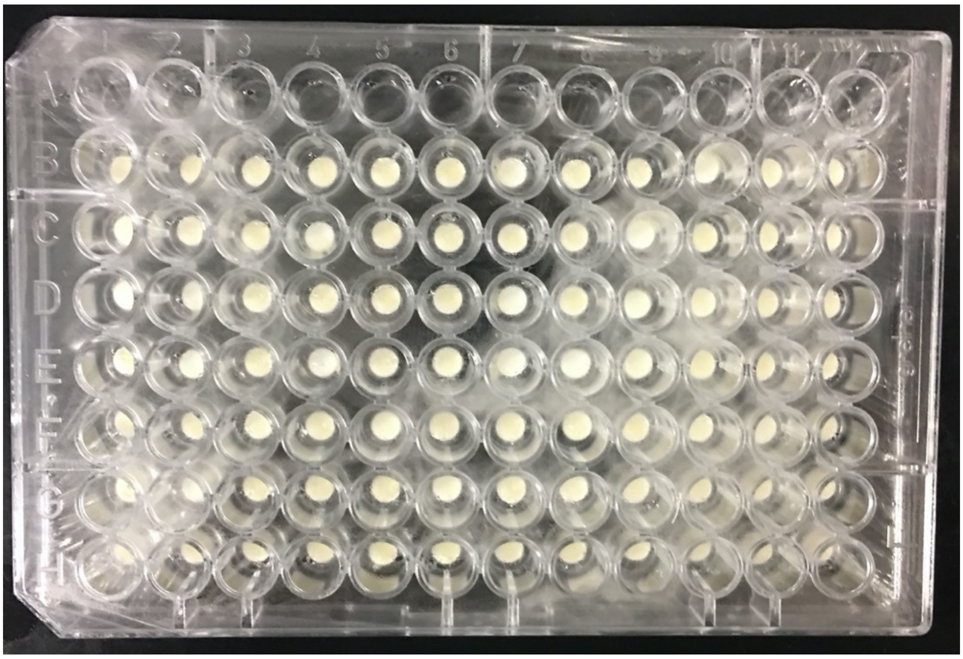

Fig. S10. Picture of freeze-dried *in vitro* sensing paper discs that were stored in the 96-well plate at room temperature. The 4 mm paper discs were put into the wells and embedded with 10 μL assembled cell-free reactions with free-drying.

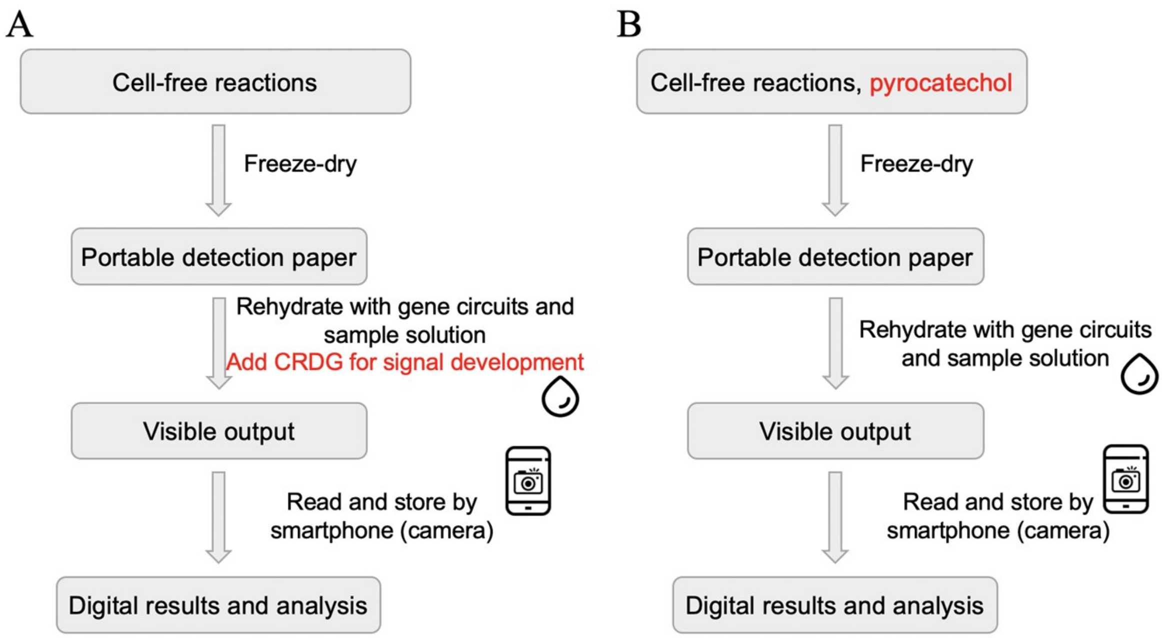

Fig. S11. The procedure of adding the two analytes of LacZ and XylE. (A) The analyte of LacZ was added after the detecting reaction for the signal development. (B) The analyte of XylE were preadded into the cell-free systems before freeze-dried onto the papers.

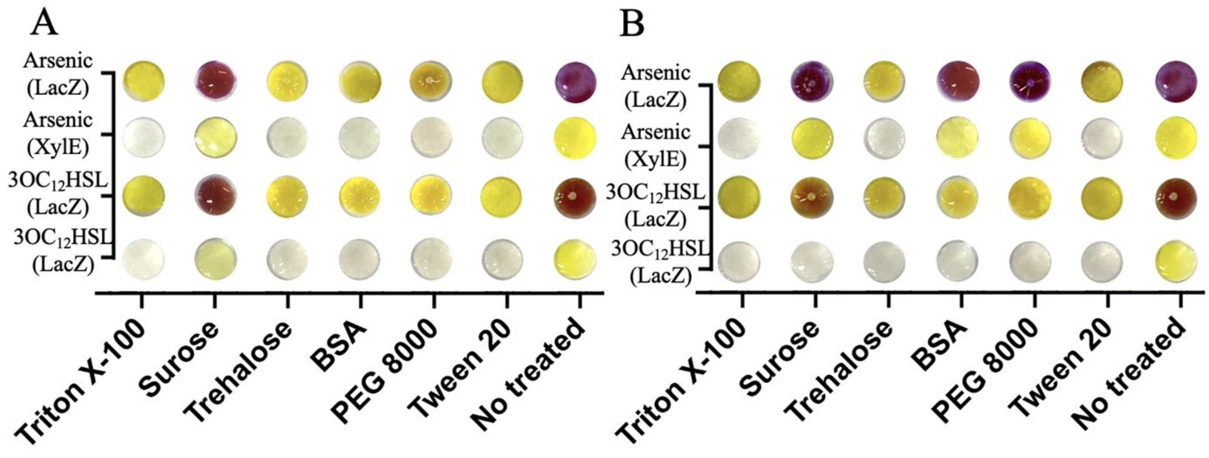

Fig. S12. Pictures of paper discs after inducible incubation. (A) Testing paper discs that were pretreated with cell-free reactions before freeze-dried. (B) Testing paper discs that were treated with different protectants.

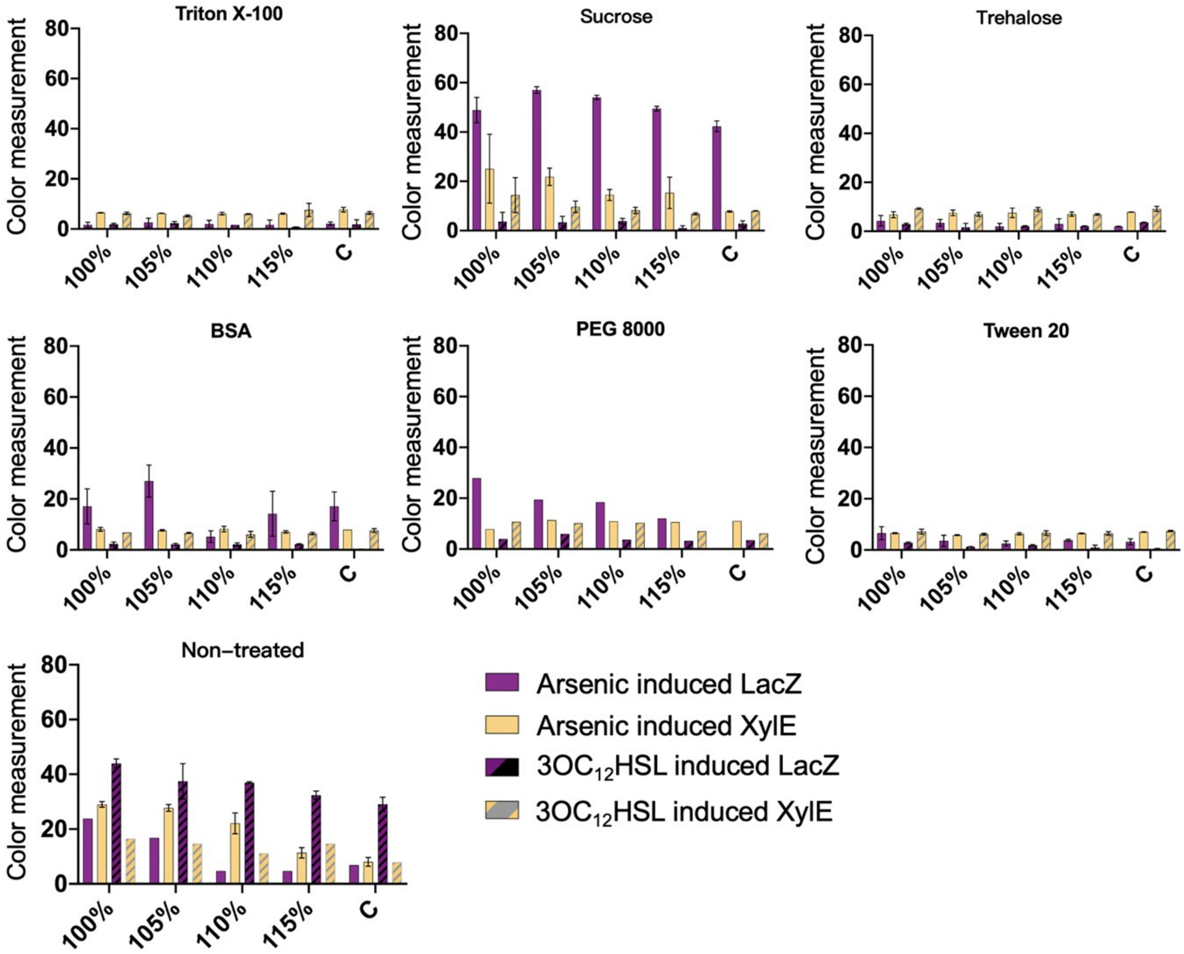

Fig. S13. Cell-free reactions on protectant-treated papers were rehydrated with water of 100%, 105%, 110%, and 115% volume of original cell-free reaction. Cell-free reactions on the sucrose or PEG 8000-treated papers developed the signal.

Supplementary Tables

Table S1 DNA sequences of expression elements involved in two sensing systems

Gene name	**Sequence**
P_{ars}	CCAACTCAAAATTCACACCTATTACCTTCCTCTGCACTTACACAT TCGTTAAGTCATATATGTTTTTGACTTATCCGCTTCGAAGAGAGA CACTACCTGCAA
arsR	ATGTCATTTCTGTTACCCATCCAATTGTTCAAAATTCTTGCTGAT GAAACCCGTCTGGGCATCGTTTTACTGCTCAGCGAACTGGGAGA GTTATGCGTCTGCGATCTCTGCACTGCTCTCGACCAGTCGCAGCC CAAGATCTCCCGCCACCTGGCATTGCTGCGTGAAAGCGGGCTAT TGCTGGACCGCAAGCAAGGTAAGTGGGTTCATTACCGCTTATCA CCGCATATTCCAGCATGGGCGGCGAAAATTATTGATGAGGCCTG GCGATGTGAACAGGAAAAGGTTCAGGCGATTGTCCGCAACCTGG CTCGACAAAACTGTTCCGGGGACAGTAAGAACATTTGCAGTTAA
P_{lux}	ACTATTGTATCGCTGGGAATACAATTACTTAACATAAGCACCTG TAGGATCGTACAGGTTTACGCAAGAAAATGGTTTGTTATAGTCG AATAT
luxR	ATGATATATAACACGCAAAACTTGCGACAAACAATAGGTAAGG ATAAAGAGATGGGTATGAAAAACATAAATGCCGACGACACATA CAGAATAATTAATAAAATTAAAGCTTGTAGAAGCAATAATGATA TTAATCAATGCTTATCTGATATGACTAAAATGGTACATTGTGAAT ATTATTTACTCGCGATCATTTATCCTCATTCTATGGTTAAATCTG ATATTTCAATTCTAGATAATTACCCTAAAAAATGGAGGCAATAT TATGATGACGCTAATTTAATAAAATATGATCCTATAGTAGATTAT TCTAACTCCAATCATTCACCAATTAATTGGAATATATTTGAAAAC AATGCTGTAAATAAAAAATCTCCAAATGTAATTAAAGAAGCGAA AACATCAGGTCTTATCACTGGGTTTAGTTTCCCTATTCATACGGC TAACAATGGCTTCGGAATGCTTAGTTTTGCACATTCAGAAAAAG ACAACTATATAGATAGTTTATTTTTACATGCGTGTATGAACATAC CATTAATTGTTCCTTCTCTAGTTGATAATTATCGAAAAATAAATA TAGCAAATAATAAATCAAACAACGATTTAACCAAAAGAGAAAA AGAATGTTTAGCGTGGGCATGCGAAGGAAAAAGCTCTTGGGATA TTTCAAAAATATTAGGCTGCAGTGAGCGTACTGTCACTTTCCATT TAACCAATGCGCAAATGAAACTCAATACAACAAACCGCTGCCAA AGTATTTCTAAAGCAATTTTAACAGGAGCAATTGATTGCCCATA CTTTAAAAATTAA
gusA	ATGTTACGTCCTGTAGAAACCCCAACCCGTGAAATCAAAAAACT CGACGGCCTGTGGGCATTCAGTCTGGATCGCGAAAACTGTGGAA TTGATCAGCGTTGGTGGGAAAGCGCGTTACAAGAAAGCCGGGC

	AATTGCTGTGCCAGGCAGTTTTAACGATCAGTTCGCCGATGCAG ATATTCGTAATTATGCGGGCAACGTCTGGTATCAGCGCGAAGTC TTTATACCGAAAGGTTGGGCAGGCCAGCGTATCGTGCTGCGTTT CGATGCGGTCACTCATTACGGCAAAGTGTGGGTCAATAATCAGG AAGTGATGGAGCATCAGGGCGGCTATACGCCATTTGAAGCCGAT GTCACGCCGTATGTTATTGCCGGGAAAAGTGTACGTATCACCGT TTGTGTGAACAACGAACTGAACTGGCAGACTATCCCGCCGGGAA TGGTGATTACCGACGAAAACGGCAAGAAAAAGCAGTCTTACTTC CATGATTTCTTTAACTATGCCGGGATCCATCGCAGCGTAATGCTC TACACCACGCCGAACACCTGGGTGGACGATATCACCGTGGTGAC GCATGTCGCGCAAGACTGTAACCACGCGTCTGTTGACTGGCAGG TGGTGGCCAATGGTGATGTCAGCGTTGAACTGCGTGATGCGGAT CAACAGGTGGTTGCAACTGGACAAGGCACTAGCGGGACTTTGCA AGTGGTGAATCCGCACCTCTGGCAACCGGGTGAAGGTTATCTCT ATGAACTGTGCGTCACAGCCAAAAGCCAGACAGAGTGTGATATC TACCCGCTTCGCGTCGGCATCCGGTCAGTGGCAGTGAAGGGCGA ACAGTTCCTGATTAACCACAAACCGTTCTACTTTACTGGCTTTGG TCGTCATGAAGATGCGGACTTGCGTGGCAAAGGATTCGATAACG TGCTGATGGTGCACGACCACGCATTAATGGACTGGATTGGGGCC AACTCCTACCGTACCTCGCATTACCCTTACGCTGAAGAGATGCTC GACTGGGCAGATGAACATGGCATCGTGGTGATTGATGAAACTGC TGCTGTCGGCTTTAACCTCTCTTTAGGCATTGGTTTCGAAGCGGG CAACAAGCCGAAAGAACTGTACAGCGAAGAGGCAGTCAACGGG GAAACTCAGCAAGCGCACTTACAGGCGATTAAAGAGCTGATAG CGCGTGACAAAAACCACCCAAGCGTGGTGATGTGGAGTATTGCC AACGAACCGGATACCCGTCCGCAAGGTGCACGGGAATATTTCGC GCCACTGGCGGAAGCAACGCGTAAACTCGACCCGACGCGTCCG ATCACCTGCGTCAATGTAATGTTCTGCGACGCTCACACCGATAC CATCAGCGATCTCTTTGATGTGCTGTGCCTGAACCGTTATTACGG ATGGTATGTCCAAAGCGGCGATTTGGAAACGGCAGAGAAGGTA CTGGAAAAAGAACTTCTGGCCTGGCAGGAGAAACTGCATCAGCC GATTATCATCACCGAATACGGCGTGGATACGTTAGCCGGGCTGC ACTCAATGTACACCGACATGTGGAGTGAAGAGTATCAGTGTGCA TGGCTGGATATGTATCACCGCGTCTTTGATCGCGTCAGCGCCGTC GTCGGTGAACAGGTATGGAATTTCGCCGATTTTGCGACCTCGCA AGGCATATTGCGCGTTGGCGGTAACAAGAAAGGGATCTTCACTC GCGACCGCAAACCGAAGTCGGCGGCTTTTCTGCTGCAAAAACGC TGGACTGGCATGAACTTCGGTGAAAAACCGCAGCAGGGAGGCA AACAATGA
lacZ	ATGACCATGATTACGGATTCACTGGCCGTCGTTTTACAACGTCGT GACTGGGAAAACCCTGGCGTTACCCAACTTAATCGCCTTGCAGC ACATCCCCCTTTCGCCAGCTGGCGTAATAGCGAAGAGGCCCGCA

	CCGATCGCCCTTCCCAACAGTTGCGCAGCCTGAATGGCGAATGG CGCTTTGCCTGGTTTCCGGCACCAGAAGCGGTGCCGGAAAGCTG GCTGGAGTGCGATCTTCCTGAGGCCGATACTGTCGTCGTCCCCTC AAACTGGCAGATGCACGGTTACGATGCGCCCATCTACACCAACG TGACCTATCCCATTACGGTCAATCCGCCGTTTGTTCCCACGGAGA ATCCGACGGGTTGTTACTCGCTCACATTTAATGTTGATGAAAGCT GGCTACAGGAAGGCCAGACGCGAATTATTTTTGATGGCGTTAAC TCGGCGTTTCATCTGTGGTGCAACGGGCGCTGGGTCGGTTACGG CCAGGACAGTCGTTTGCCGTCTGAATTTGACCTGAGCGCATTTTT ACGCGCCGGAGAAAACCGCCTCGCGGTGATGGTGCTGCGCTGGA GTGACGGCAGTTATCTGGAAGATCAGGATATGTGGCGGATGAGC GGCATTTTCCGTGACGTCTCGTTGCTGCATAAACCGACTACACA AATCAGCGATTTCCATGTTGCCACTCGCTTTAATGATGATTTCAG CCGCGCTGTACTGGAGGCTGAAGTTCAGATGTGCGGCGAGTTGC GTGACTACCTACGGGTAACAGTTTCTTTATGGCAGGGTGAAACG CAGGTCGCCAGCGGCACCGCGCCTTTCGGCGGTGAAATTATCGA TGAGCGTGGTGGTTATGCCGATCGCGTCACACTACGTCTGAACG TCGAAAACCCGAAACTGTGGAGCGCCGAAATCCCGAATCTCTAT CGTGCGGTGGTTGAACTGCACACCGCCGACGGCACGCTGATTGA AGCAGAAGCCTGCGATGTCGGTTTCCGCGAGGTGCGGATTGAAA ATGGTCTGCTGCTGCTGAACGGCAAGCCGTTGCTGATTCGAGGC GTTAACCGTCACGAGCATCATCCTCTGCATGGTCAGGTCATGGA TGAGCAGACGATGGTGCAGGATATCCTGCTGATGAAGCAGAAC AACTTTAACGCCGTGCGCTGTTCGCATTATCCGAACCATCCGCTG TGGTACACGCTGTGCGACCGCTACGGCCTGTATGTGGTGGATGA AGCCAATATTGAAACCCACGGCATGGTGCCAATGAATCGTCTGA CCGATGATCCGCGCTGGCTACCGGCGATGAGCGAACGCGTAACG CGAATGGTGCAGCGCGATCGTAATCACCCGAGTGTGATCATCTG GTCGCTGGGGAATGAATCAGGCCACGGCGCTAATCACGACGCGC TGTATCGCTGGATCAAATCTGTCGATCCTTCCCGCCCGGTGCAGT ATGAAGGCGGCGGAGCCGACACCACGGCCACCGATATTATTTGC CCGATGTACGCGCGCGTGGATGAAGACCAGCCCTTCCCGGCTGT GCCGAAATGGTCCATCAAAAAATGGCTTTCGCTACCTGGAGAGA CGCGCCCGCTGATCCTTTGCGAATACGCCCACGCGATGGGTAAC AGTCTTGGCGGTTTCGCTAAATACTGGCAGGCGTTTCGTCAGTAT CCCCGTTTACAGGGCGGCTTCGTCTGGGACTGGGTGGATCAGTC GCTGATTAAATATGATGAAAACGGCAACCCGTGGTCGGCTTACG GCGGTGATTTTGGCGATACGCCGAACGATCGCCAGTTCTGTATG AACGGTCTGGTCTTTGCCGACCGCACGCCGCATCCAGCGCTGAC GGAAGCAAAACACCAGCAGCAGTTTTTCCAGTTCCGTTTATCCG GGCAAACCATCGAAGTGACCAGCGAATACCTGTTCCGTCATAGC GATAACGAGCTCCTGCACTGGATGGTGGCGCTGGATGGTAAGCC

	GCTGGCAAGCGGTGAAGTGCCTCTGGATGTCGCTCCACAAGGTA AACAGTTGATTGAACTGCCTGAACTACCGCAGCCGGAGAGCGCC GGGCAACTCTGGCTCACAGTACGCGTAGTGCAACCGAACGCGAC CGCATGGTCAGAAGCCGGGCACATCAGCGCCTGGCAGCAGTGG CGTCTGGCGGAAAACCTCAGTGTGACGCTCCCCGCCGCGTCCCA CGCCATCCCGCATCTGACCACCAGCGAAATGGATTTTTGCATCG AGCTGGGTAATAAGCGTTGGCAATTTAACCGCCAGTCAGGCTTT CTTTCACAGATGTGGATTGGCGATAAAAAACAACTGCTGACGCC GCTGCGCGATCAGTTCACCCGTGCACCGCTGGATAACGACATTG GCGTAAGTGAAGCGACCCGCATTGACCCTAACGCCTGGGTCGAA CGCTGGAAGGCGGCGGGCCATTACCAGGCCGAAGCAGCGTTGTT GCAGTGCACGGCAGATACACTTGCTGATGCGGTGCTGATTACGA CCGCTCACGCGTGGCAGCATCAGGGGAAAACCTTATTTATCAGC CGGAAAACCTACCGGATTGATGGTAGTGGTCAAATGGCGATTAC CGTTGATGTTGAAGTGGCGAGCGATACACCGCATCCGGCGCGGA TTGGCCTGAACTGCCAGCTGGCGCAGGTAGCAGAGCGGGTAAAC TGGCTCGGATTAGGGCCGCAAGAAAACTATCCCGACCGCCTTAC TGCCGCCTGTTTTGACCGCTGGGATCTGCCATTGTCAGACATGTA TACCCCGTACGTCTTCCCGAGCGAAAACGGTCTGCGCTGCGGGA CGCGCGAATTGAATTATGGCCCACACCAGTGGCGCGGCGACTTC CAGTTCAACATCAGCCGCTACAGTCAACAGCAACTGATGGAAAC CAGCCATCGCCATCTGCTGCACGCGGAAGAAGGCACATGGCTGA ATATCGACGGTTTCCATATGGGGATTGGTGGCGACGACTCCTGG AGCCCGTCAGTATCGGCGGAATTCCAGCTGAGCGCCGGTCGCTA CCATTACCAGTTGGTCTGGTGTCAAAAATAA
xylE	ATGAACAAAGGCGTTATGCGCCCGGGTCATGTGCAACTGCGCGT GCTGGATATGAGCAAAGCGCTGGAACATTATGTGGAACTGCTGG GCCTGATTGAAATGGATCGCGATGATCAGGGCCGCGTGTATTTA AAAGCGTGGACCGAGGTGGATAAATTTAGCCTGGTGCTGCGCGA AGCAGATGAACCGGGCATGGATTTTATGGGCTTTAAGGTGGTGG ATGAAGATGCGCTGCGCCAGTTAGAACGTGATCTGATGGCGTAT GGCTGCGCGGTGGAACAATTACCTGCGGGCGAACTGAATAGCTG TGGTCGTCGCGTTCGTTTTCAAGCGCCTAGCGGCCATCATTTTGA ACTGTACGCGGACAAAGAATATACCGGCAAATGGGGCCTGAAC GATGTGAACCCTGAAGCATGGCCGCGCGATTTAAAAGGTATGGC GGCGGTGCGTTTTGATCATGCGCTGATGTATGGCGATGAACTGC CGGCGACCTATGATCTGTTTACCAAAGTGCTGGGCTTTTATCTGG CGGAACAGGTGCTGGATGAAAACGGCACTCGCGTGGCGCAATTT TTAAGCCTGAGCACCAAAGCGCATGATGTGGCGTTTATTCATCA TCCGGAAAAAGGCCGCCTGCATCATGTGAGCTTTCATCTGGAAA CCTGGGAAGATCTGTTACGCGCGGCGGATCTGATTAGCATGACC GATACCAGCATTGATATTGGCCCGACCCGTCATGGCTTAACCCA

	TGGCAAAACCATCTACTTCTTTGATCCGAGCGGCAACCGCAACG AAGTTTTTTGCGGCGGCGATTATAACTATCCGGATCATAAACCG GTGACCTGGACCACTGATCAACTGGGCAAAGCGATTTTTTACCA CGATCGCATTCTGAACGAACGCTTTATGACCGTGCTGACCTAA

Table S2 Amino acid sequences of proteins involved in two sensing systems

Protein name	Sequence
ArsR	MSFLLPIQLFKILADETRLGIVLLLSELGELCVCDLCTALDQSQPKI SRHLALLRESGLLLDRKQGKWVHYRLSPHIPAWAAKIIDEAWRCE QEKVQAIVRNLARQNCSGDSKNICS*
LuxR	MIYNTQNLRQTIGKDKEMGMKNINADDTYRIINKIKACRSNNDIN QCLSDMTKMVHCEYYLLAIIYPHSMVKSDISILDNYPKKWRQYY DDANLIKYDPIVDYSNSNHSPINWNIFENNAVNKKSPNVIKEAKTS GLITGFSFPIHTANNGFGMLSFAHSEKDNYIDSLFLHACMNIPLIVP SLVDNYRKINIANNKSNNDLTKREKECLAWACEGKSSWDISKILG CSERTVTFHLTNAQMKLNTTNRCQSISKAILTGAIDCPYFKN*
GusA	MLRPVETPTREIKKLDGLWAFSLDRENCGIDQRWWESALQESRAI AVPGSFNDQFADADIRNYAGNVWYQREVFIPKGWAGQRIVLRFD AVTHYGKVWVNNQEVMEHQGGYTPFEADVTPYVIAGKSVRITV CVNNELNWQTIPPGMVITDENGKKKQSYFHDFFNYAGIHRSVML YTTPNTWVDDITVVTHVAQDCNHASVDWQVVANGDVSVELRDA DQQVVATGQGTSGTLQVVNPHLWQPGEGYLYELCVTAKSQTEC DIYPLRVGIRSVAVKGEQFLINHKPFYFTGFGRHEDADLRGKGFD NVLMVHDHALMDWIGANSYRTSHYPYAEEMLDWADEHGIVVID ETAAVGFNLSLGIGFEAGNKPKELYSEEAVNGETQQAHLQAIKELI ARDKNHPSVVMWSIANEPDTRPQGAREYFAPLAEATRKLDPTRPI TCVNVMFCDAHTDTISDLFDVLCLNRYYGWYVQSGDLETAEKVL EKELLAWQEKLHQPIIITEYGVDTLAGLHSMYTDMWSEEYQCAW LDMYHRVFDRVSAVVGEQVWNFADFATSQGILRVGGNKKGIFTR DRKPKSAAFLLQKRWTGMNFGEKPQQGGKQ*
LacZ	MTMITDSLAVVLQRRDWENPGVTQLNRLAAHPPFASWRNSEEAR TDRPSQQLRSLNGEWRFAWFPAPEAVPESWLECDLPEADTVVVPS NWQMHGYDAPIYTNVTYPITVNPPFVPTENPTGCYSLTFNVDESW LQEGQTRIIFDGVNSAFHLWCNGRWVGYGQDSRLPSEFDLSAFLR AGENRLAVMVLRWSDGSYLEDQDMWRMSGIFRDVSLLHKPTTQI SDFHVATRFNDDFSRAVLEAEVQMCGELRDYLRVTVSLWQGETQ VASGTAPFGGEIIDERGGYADRVTLRLNVENPKLWSAEIPNLYRA VVELHTADGTLIEAEACDVGFREVRIENGLLLLNGKPLLIRGVNRH EHHPLHGQVMDEQTMVQDILLMKQNNFNAVRCSHYPNHPLWYT LCDRYGLYVVDEANIETHGMVPMNRLTDDPRWLPAMSERVTRM VQRDRNHPSVIIWSLGNESGHGANHDALYRWIKSVDPSRPVQYEG GGADTTATDIICPMYARVDEDQPFPAVPKWSIKKWLSLPGETRPLI LCEYAHAMGNSLGGFAKYWQAFRQYPRLQGGFVWDWVDQSLIK YDENGNPWSAYGGDFGDTPNDRQFCMNGLVFADRTPHPALTEA KHQQQFFQFRLSGQTIEVTSEYLFRHSDNELLHWMVALDGKPLAS

	GEVPLDVAPQGKQLIELPELPQPESAGQLWLTVRVVQPNATAWSE AGHISAWQQWRLAENLSVTLPAASHAIPHLTTSEMDFCIELGNKR WQFNRQSGFLSQMWIGDKKQLLTPLRDQFTRAPLDNDIGVSEATR IDPNAWVERWKAAGHYQAEAALLQCTADTLADAVLITTAHAWQ HQGKTLFISRKTYRIDGSGQMAITVDVEVASDTPHPARIGLNCQLA QVAERVNWLGLGPQENYPDRLTAACFDRWDLPLSDMYTPYVFPS ENGLRCGTRELNYGPHQWRGDFQFNISRYSQQQLMETSHRHLLH AEEGTWLNIDGFHMGIGGDDSWSPSVSAEFQLSAGRYHYQLVWC QK*
XylE	MNKGVMRPGHVQLRVLDMSKALEHYVELLGLIEMDRDDQGRVY LKAWTEVDKFSLVLREADEPGMDFMGFKVVDEDALRQLERDLM AYGCAVEQLPAGELNSCGRRVRFQAPSGHHFELYADKEYTGKW GLNDVNPEAWPRDLKGMAAVRFDHALMYGDELPATYDLFTKVL GFYLAEQVLDENGTRVAQFLSLSTKAHDVAFIHHPEKGRLHHVSF HLETWEDLLRAADLISMTDTSIDIGPTRHGLTHGKTIYFFDPSGNR NEVFCGGDYNYPDHKPVTWTTDQLGKAIFYHDRILNERFMTVLT*

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