Supporting Information

Evolved bacterial biosensor for arsenite detection in environmental water

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Contents: five figures (Figure S1-S5); two tables (Table S1-S2)

Annotation I: Construction of the plasmid pUC18-ars-gfp and pUC18-AID-gfp is shown in Figure S1. The plasmid of pUC18-ars-gfp was used for directed evolution, including the arsenite sensing elements and *GFP* reporter gene. The arsenite sensing elements comprised by ArsR DNA binding site (abs), ArsR repressor, *ars* promoter, etc were amplified and introduced with restriction site of *Bgl*II and *Xho*I with primer pair PP1 and PP2 from the plasmid pPR-arsR-ABS.[1] The *GFP* gene was amplified and introduced with restriction site of *Xho*I and *Cla*I with primer pair of PP3 and PP4. *Bgl*II-*Xho*I digested arsenite sensing elements were cloned into the vector of pUC18 to substitute lac promoter, then *Xho*I-*Cla*I digested *GFP* gene was inserted down stream of arsenite sensing elements to yield pUC18-ars-gfp (Figure S1(A)). The null variant plasmid of pUC18-AID-gfp was used as a negative control to set the gate of background fluorescence for FACS screening. A non-functional *AID* gene fragment was amplified and introduced with restriction site of *Hin*dIII and *Xho*I from the plasmid of pCI-AID.[2] The *AID* gene was digested with *Hin*dIII and *Xho*I and ligated into *Hin*dIII-*Xho*I digested pUC18-ars-gfp to substitute arsenite sensing elements to yield pUC18-AID-gfp (Figure S1(B)).

Annotation II: In this study, several concepts of "relative fluorescence intensity", "folds of relative fluorescence" and "relative induction activity" were used to exhibit the performance of arsenite-responsive biosensors.

"Relative fluorescence intensity" is the value of fluorescence directly read by flow cytometer. "Folds of relative fluorescence" is calculated by dividing "relative fluorescence intensity" value of arsenite treated bacteria with untreated bacteria. "Induced fluorescence activity (IFA)" is calculated with the formula:

$$IFA = \frac{TP * TF}{CP * CF}.$$

Here, TP is the percentage of bacteria in positive region of arsenite treated bacteria, TF is the value of "relative fluorescence intensity" in positive region of arsenite treated bacteria. CP is the percentage of positive bacteria in positive region of arsenite untreated bacteria, and CF is the value of "relative fluorescence intensity" in positive region of arsenite untreated bacteria.

"Relative induction activity" equals the IFA of mutants divided by the IFA of WT.

For example, *E. coli* with wild-type pUC18-ars-gfp (WT) and the evolved mutant pUC18-ep3ars-gfp (EP3) were induced with and without arsenite (Figure S2). As shown in Figure S2, the population of P1 represented all normal bacteria detected by flow cytometer. The bacteria untreated with arsenite was used to set the negative region, a gate of P2 was drawn to separate the background fluorescence from the induced fluorescence according to negative bacteria. So, the bacteria in P2 region were considered as positive population. The value of "relative fluorescence intensity" of arsenite treated WT was 9545 and that of EP3 was 10643. The value of "folds of relative fluorescence" of WT was 9545/778, and EP3 was 10643/311. The "Induction fluorescence activity (IFA)" of EP3 was 258.8, and that of WT was 21.0. In detail, they were calculated respectively as follow:

$$IFA_{EP3} = \frac{95\% * 11258}{3.1\% * 1333},$$

$$IFA_{WT} = \frac{96.2\% * 9947}{24.7\% * 1849}.$$

The "relative induction activity" of EP3 over WT was 258.8/21.0.

Annotation III: A multiple sequence alignment of the wild-type *arsR* gene and three variants was performed using Clustal 2.1 (Figure S3).[3] The accession number is listed on the left of the sequence alignment with each of the sequence name. The mutation site of the sequence is given a yellow background.

Annotation IV: The response of evolved ep3 biosensor to arsenate was monitored. The biosensor plasmid was transformed into *E.coli* DH5α. There existed *ArsC* in *E.coli* strain, which could convert intracellular arsenate to arsenite.[4] Theoretically, the biosensor could response to both arsenite an arsenate. The *E. coli* with pUC18-ep3ars-gfp were treated with a serial of concentration of both arsenite and arsenate. Figure S4 showed the evolved EP3 arsenite biosensor could response to both arsenite and arsenate in a very similar dose-dependent manner. Since arsenite is around 60 times more toxic than arsenate, this study focused mainly on the detection of arsenite.[5]

Annotation V: For the specifity test of evolved ep3 biosensor, all the selected elements were tested with three different concentrations (Table S1). The middle one of the three different concentrations was chosen according to the regulated contamination levels in drinking water (Table S2). Except for arsenite, the evolved ep3 biosensor showed no significant fluorescence response to all the other elements (Figure S5).

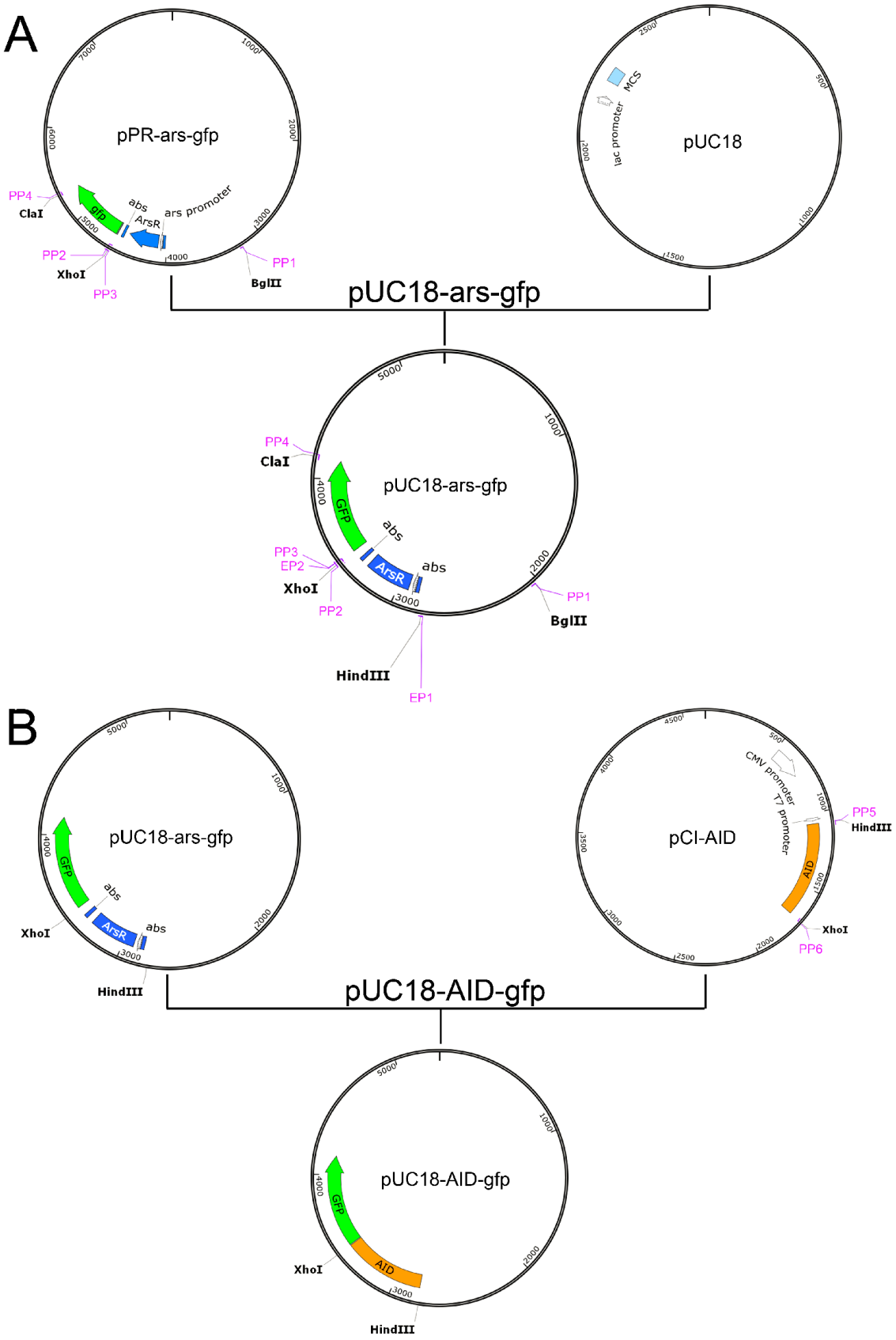

Figure S1. A schematic representation of the plasmid construction pUC18-ars-gfp (A) and pUC18-AID-gfp (B).

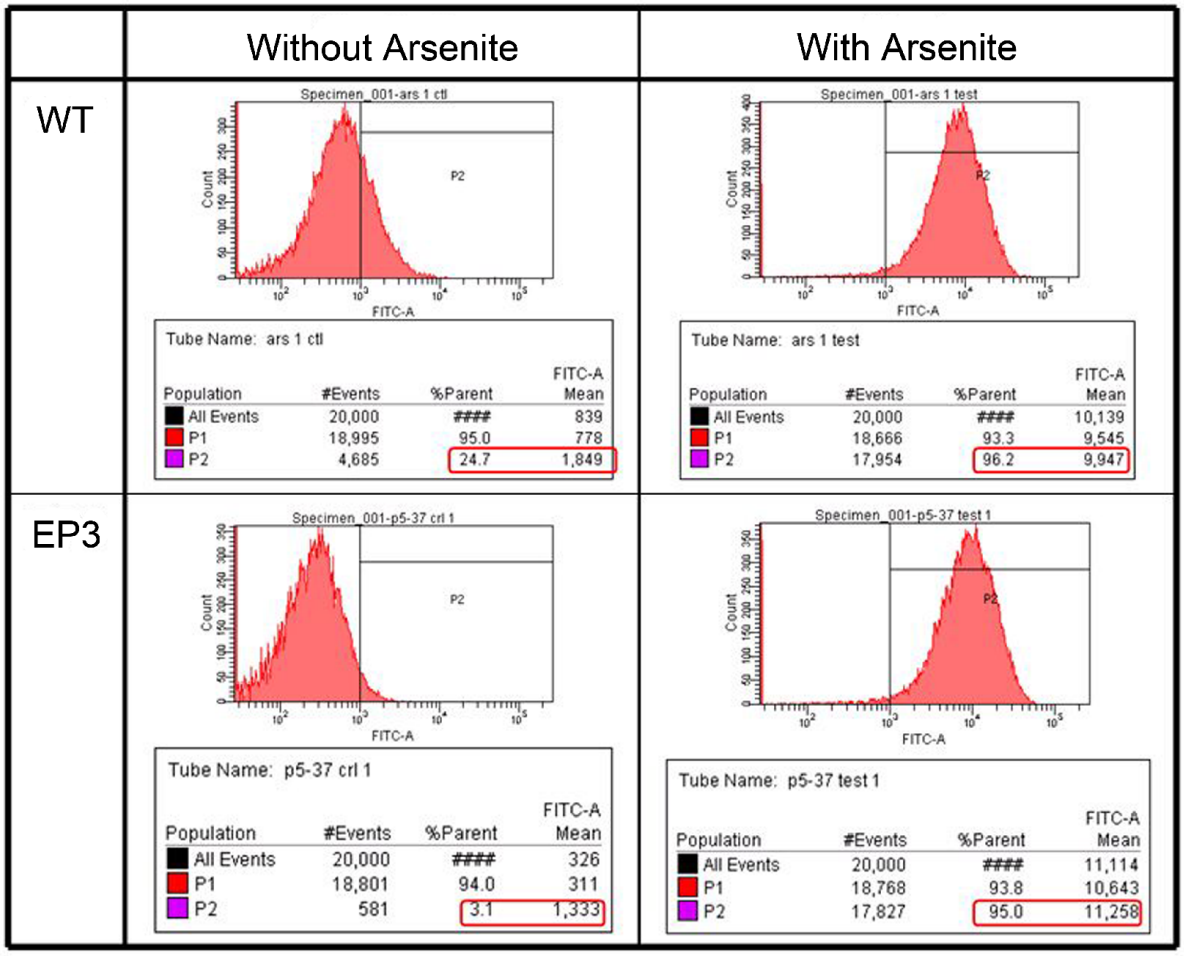

Figure S2. Fluorescence detection of arsenite treated and untreated bacteria by flow cytometer.

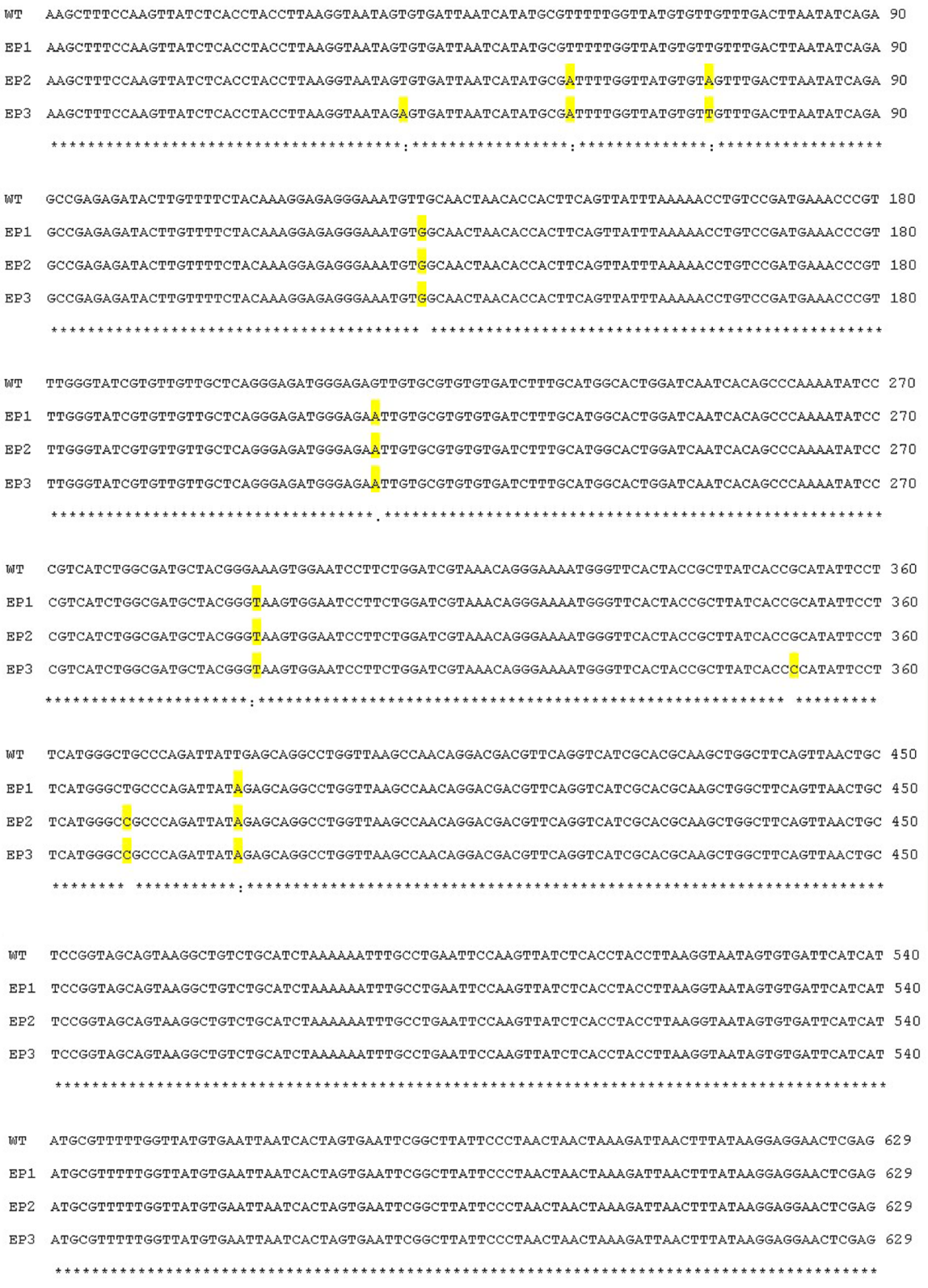

Figure S3. Sequence alignment of the wild-type *arsR* gene and three variants. Wt refers to the wild-type promoter. EP1, EP2 and EP3 are the first, second and third round evolution mutants. Nucleotides that are different from the other sequences are highlighted in yellow.

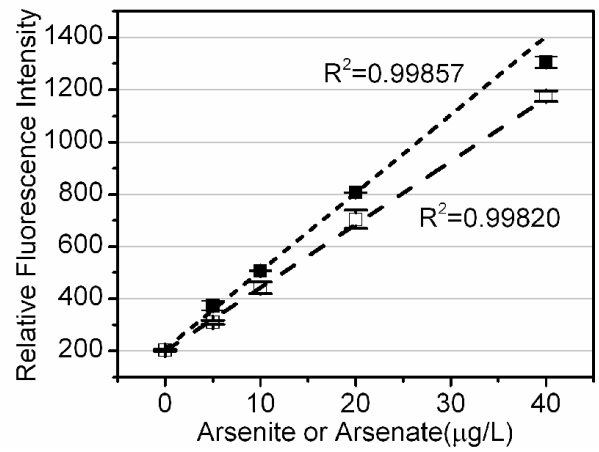

Figure S4. The does-effect relationship of evolved EP3 arsenite biosensor in response to the same concentrations of arsenite (black square) and arsenate (white square).

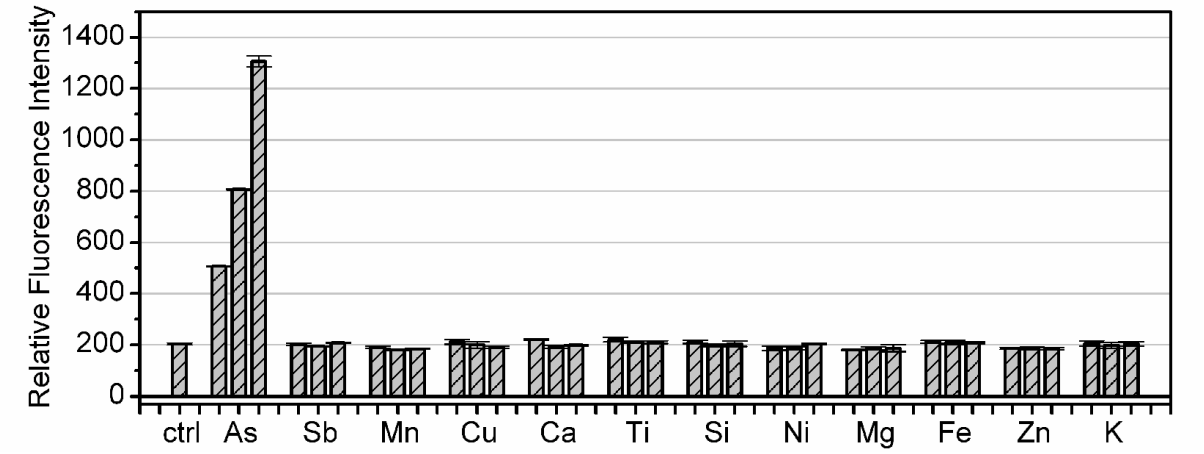

Figure S5. Specificity test of arsenite induced bacterial biosensors. The concentrations of individual element of each bar were listed in Table S1.

Table S1. List of chemical concentrations in the specificity test.

	Left column (mg/L)	Middle column(mg/L)	Right column(mg/L)
As(III)	0.01	0.02	0.04
Sb(III)	0.01	0.02	0.04
Mn	0.02	0.5	5
Cu	0.02	1	5
Ca	0.02	0.5	5
Ti	0.02	0.5	5
Si	0.02	0.5	5
Ni	0.02	1	5
Mg	0.02	0.5	5
Fe^{2+}	0.02	0.3	5
Zn	0.02	1	5
K	0.02	0.5	5

Table S2. List of chemicals regulated contamination levels in drinking water.

	China	WHO (1993,1998)	EPA (~2001)
As	0.05mg/L	0.01mg/L	0.05mg/L
Sb	0.005mg/L	0.005mg/L	0.006mg/L
Fe	0.3mg/L	0.3mg/L	0.3mg/L
Mn	0.1mg/L	0.1mg/L	0.05mg/L
Cu	1.0mg/L	1.0mg/L	1.0mg/L
Zn	1.0mg/L	3.0mg/L	5.0mg/L
Ni	0.02mg/L	0.02mg/L	N.A.

References

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(2) Wu, X., Geraldes, P., Platt, J.L., and Cascalho, M. The double-edged sword of activation-induced cytidine deaminase. *J Im-munol.* **2005**, *174*(2), 934–941.

(3) ClustalW2 Website; http://www.ebi.ac.uk/Tools/msa/clustalw2/.

(4) Ji, G.; Silver, S. Reduction of arsenate to arsenite by the ArsC protein of the arsenic resistance operon of Staphylococcus aureus plasmid pI258. *Proc Natl Acad Sci U S A*. **1992**, *89*(20), 9474-9478.

(5) Chatterjee, A, et al. Arsenic in ground water in six districts of West Bengal, India: the biggest arsenic calamity in the world. Part I. Arsenic species in drinking water and urine of the affected people. *Analyst.* **1995**, *120*(3), 643–650.