

## The construction of an engineered bacterium to remove cadmium from wastewater

### DNA CLONING AND CONSTRUCTION OF AEP, ABEP, AND ABET3P

To construct the pAEP plasmid, PGSHA1 and PGSHA2 were used to amplify the gshA gene from the genomic DNA of *Escherichia coli* MG1655. Next, PCYSE1 and PCYSE2 were used to amplify the cysE gene from the genomic DNA of *E. coli* MG1655. In addition, PTCP-CS13 and PTCP-CS12 were used to amplify the TcPCS1 gene from the cDNA of *Thlaspi caerulescens*. The polymerase chain reaction (PCR) products were purified and mixed at a ratio of 1:1:1, and the pET28a plasmid was digested with Bam HI and Hind III. The in-fusion cloning reactions were performed according to the In-Fusion HD Cloning Kit User Manual (Takara, Dalian, China). The recombinant plasmids were identified as described above, and the plasmid containing the identified sequences (GenBank accession numbers: EG10418, EG10187 and AY540104) was named pAEP. The bacteria strain containing the plasmid pTP was named AEP.

To construct the pABEP plasmid, PGSHB1 and PGSHB2 were used to amplify gshB from the genomic DNA of *E. coli* MG1655, and PCYSE3 and PCYSE2 were used to amplify cysE from the genomic DNA of *E. coli* MG1655. The remaining procedure was the same as the procedure for constructing pAEP. The PCR products were purified and mixed at a ratio of 1:1:1:1. The pET28a plasmid was digested with Bam HI and Hind III, and the in-fusion cloning reactions were performed according to the In-Fusion HD Cloning Kit User Manual (Takara, Dalian, China). The recombinant plasmids were identified as described above, and the plasmid containing the identified

sequences (GenBank accession numbers: EG10418, EG10419, EG10187 and AY540104) was named pABEP. The bacteria strain containing the pABEP plasmid was named ABEP.

For constructing pABET3P, PTCHMA31 and PTCHMA32 were used to amplify the TcHMA3 gene from the cDNA of *T. caerulescens*. In addition, PTCP-CS14 and PTCP-CS12 were used to amplify TcPCS1 from the cDNA of *T. caerulescens*. Next, the same procedure described above for constructing pABEP was used. The PCR products were purified and mixed at a 1:1:1:1:1 ratio. The pET28a plasmid was digested with Bam HI and Hind III. The in-fusion cloning reactions were performed according to the In-Fusion HD Cloning Kit User Manual (Takara, Dalian, China). The recombinant plasmids were identified as described above. The plasmid containing the identified sequences (GenBank accession numbers: EG10418, EG10419, EG10187, [Ueno et al. 2011](#); AY540104) was named pABET3P, and the bacterial strain containing the pABET3P plasmid was named ABET3P.

### REFERENCE

- Ueno, D., Milner, M. J., Yamaji, N., Yokosho, K., Koyama, E., Zambrano, M. C., Kaskie, M., Ebbs, S., Kochian, L. V. & Ma, J. F. 2011 [Elevated expression of TcHMA3 plays a key role in the extreme Cd tolerance in a Cd-hyperaccumulating ecotype of \*Thlaspi caerulescens\*](#). *Plant J.* **66**, 852–862.