On-Line Supplementary Information

Design and Study of Efflux Function of EGFP Fused MexAB-OprM Membrane Transporter in *Pseudomonas aeruginosa* Using Fluorescence Spectroscopy

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The on-line supplementary information includes:

Two figures:

**Figure S1:** Construction of fusion *egfp-mexB*

**Figure S2:** DNA sequence of *egfp-mexB*

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Fig. S1: The schematic outlines construction of fusion \textit{egfp-mexB} gene. (A): (I) The ds-\textit{egfp} gene is amplified using EGFP plasmid as a template, and both \textit{egfp} P1 (5'-CTTGTCGACAAGGGGATCCACCATTGGTGAGCAAGG-3') and \textit{egfp} P2 (5'-CAATGAAAAACTTCGACATCTTGTACGCTCGTCCATGC-3') as primers. (II) The coding strand of \textit{egfp} gene (ss-\textit{egfp}) is generated using the amplified ds-\textit{egfp} as the template and \textit{egfp} P1 as the primer. (B): (I) The ds-\textit{mexB} gene is produced using genomic DNA of \textit{P. aeruginosa} as a template, and both \textit{mexB} P1 (5'-GCAATGGACGAGCTGTAAGATGTCGAAAGTTTTTCA-3') and \textit{mexB} P2 (5'-GATAAGCTTATCATTGCCCCCTTTTCGAC-3') as primers. (II) The template strand of \textit{mexB} gene (ss-\textit{mexB}) is created using the ds-\textit{mexB} as a template, and \textit{mexB} P2 as a primer. (III) By mixing the ss-\textit{egfp} with ss-\textit{mexB}, it creates 20 base-pair (bp) ds-DNA that links the ss-\textit{egfp} with ss-\textit{mexB}, which is used as a template to generate and amplify \textit{egfp-mexB} fusion gene using both \textit{egfp} P1 and \textit{mexB} P2 as primers. (IV) The purified \textit{egfp-mexB} gene and (C) vector (pMMB67EH) are digested by a pair of restriction enzymes (SalI/HindIII). The digested and purified \textit{egfp-mexB} gene is inserted into the digested vector (pMMB67EH) to produce pMMB67EH-EGFP-MexB vector via ligation.
CTTGTCGACAAGGGGATCCACC

**Fig. S2**: DNA sequence of *egfp-mexB* constructed and purified as described in the main text and sequenced using DNA sequencer (Applied Biosystem, 3730xl DNA Analyzer). Start codon of ATG and stop codon of TGA are marked in red and green, respectively. The underlined GTCGAC and AAGCTT are SalI and HindIII sites, respectively.