

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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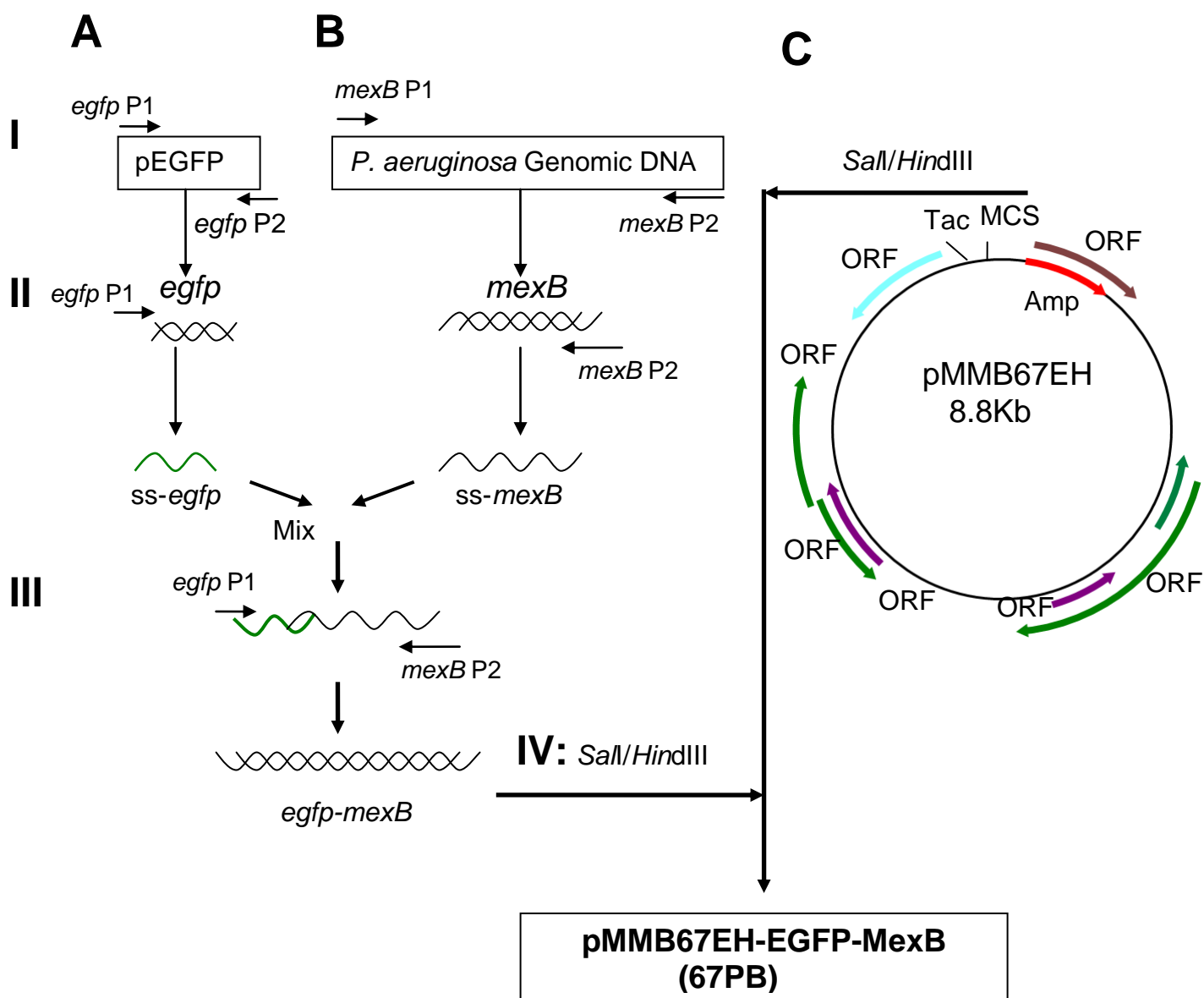


Fig. S1: The schematic outlines construction of fusion *egfp-mexB* gene. **(A): (I)** The ds-*egfp* gene is amplified using EGFP plasmid as a template, and both *egfp* P1 (5'-CTTGTGCGACAAGGGGATCCACCATGGTGAGCAAGG-3') and *egfp* P2 (5'-CAATGAAAACTTCGACATCTTGTACAGCTCGTCCATGC-3') as primers. **(II)** The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

CTTGTCGACAAGGGGATCCACC**atg**gtgagcaagggcgaggagctgttaccggggtggtgccatcctggtcgagctggac
 ggcgacgtaaacggccacaagttcagcgtgtccggcgagggcgagggcgatgccacctcggcaagctgacctgaagttcatctgcacc
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 gcgtgatcggcggcatggtcactgcgaccgtcctggcgtatcttctgggtaccgctgttctacgtggcggctcagcagcgtgttcaaggacgaggg
 gtccaagcagcagggcgtccgtcgaaaaggggcaa**TGATAAGCTT**ATC

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.