

Description of Additional Supplementary Files

File name: Supplementary Data 1

Description: Multiple sequence alignment of TnsB proteins from type I-F CASTs.

Output from Promals3D¹; Tn7016 (*Pseudoalteromonas* sp. strain S983), WP_029772100.1; Tn6677 (*Vibrio cholerae* strain HE-45), WP_001074608.1; Tn6900 (*Aeromonas salmonicida*), WP_088821968.1; Tn7000 (*Vibrio cholerae* strain 4874), WP_119464978.1; Tn7001 (*Photobacterium iliopiscarium*), WP_107274795.1; Tn7002 (*Vibrio* sp. strain F12), WP_136985358.1; Tn7003 (*Vibrio parahaemolyticus*), WP_015313514.1; Tn7004 (*Vibrio* sp. strain 16), WP_005472985.1; Tn7005 (*Vibrio cholerae* strain M1517), WP_001134621.1; Tn7006 (*Vibrio splendidus*), WP_054541713.1; Tn7008 (*Aliivibrio* sp.), WP_065591601.1; Tn7011 (*Pseudalteromonas* sp. strain P1-25), WP_055018246.1; Tn7012 (*Pseudoalteromonas ruthenica*), WP_138590899.1; Tn7013 (*Vibrio cholerae* strain OYP7G04), WP_114778930.1; Tn7014 (*Vibrio diazotrophicus*), WP_102952857.1; Tn7015 (*Shewanella* sp.), WP_076538387.1; Tn7017 (*Endozoicomonas ascidiicola*), WP_095210281.1². Domain boundaries of PseTnsB are indicated above the alignment; residues described in this study are highlighted in yellow.

File name: Supplementary Data 2

Description: Multiple sequence alignment of TnsA proteins from type I-F CASTs.

Output from Promals3D¹; Tn7016 (*Pseudoalteromonas* sp. strain S983), WP_029772101.1; Tn6677 (*Vibrio cholerae* strain HE-45), WP_000202732.1; Tn6900 (*Aeromonas salmonicida*), WP_088821967.1; Tn7000 (*Vibrio cholerae* strain 4874), WP_119464977.1; Tn7001 (*Photobacterium iliopiscarium*), WP_107274794.1; Tn7002 (*Vibrio* sp. strain F12), WP_136985357.1; Tn7003 (*Vibrio parahaemolyticus*), WP_015313515.1; Tn7004 (*Vibrio* sp. strain 16), WP_005472937.1; Tn7005 (*Vibrio cholerae* strain M1517), WP_000460251.1; Tn7006 (*Vibrio splendidus*), WP_050622132.1; Tn7008 (*Aliivibrio* sp.), WP_065591602.1; Tn7011 (*Pseudalteromonas* sp. strain P1-25), WP_036981971.1; Tn7012 (*Pseudoalteromonas ruthenica*), WP_138590901.1; Tn7013 (*Vibrio cholerae* strain OYP7G04), WP_114778920.1; Tn7014 (*Vibrio diazotrophicus*), WP_102952856.1; Tn7015 (*Shewanella* sp.), WP_076538389.1; Tn7017 (*Endozoicomonas ascidiicola*), WP_157673482.1². Domain boundaries of PseTnsA are indicated above the alignment; residues described in this study are highlighted in yellow.

File name: Supplementary Data 3

Description: Description and sequence of oligonucleotides and plasmids used in this study.

File name: Supplementary Data 4

Description: AlphaFold 3 model of evoTnsAB PEC.