Supplemental materials

Control of competence in *Vibrio fischeri*

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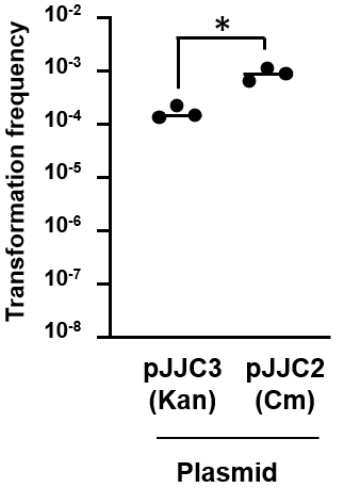
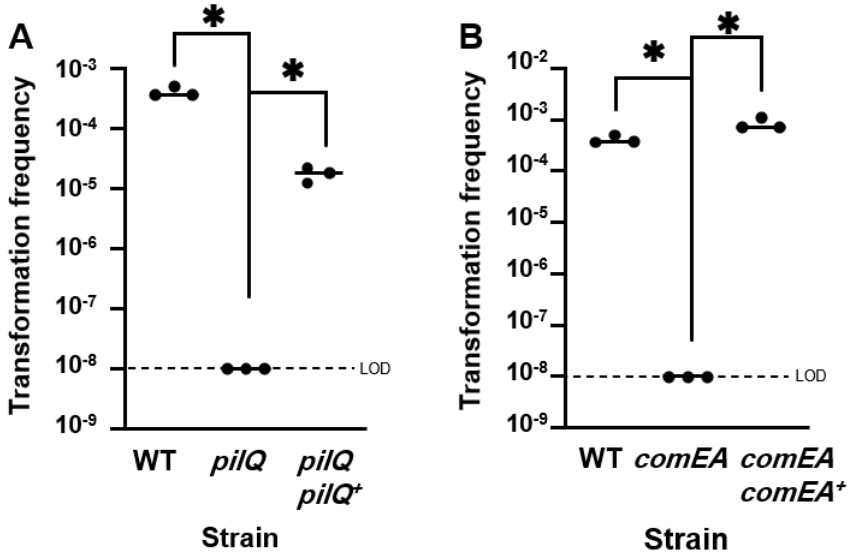
Running title: *V. fischeri* competence

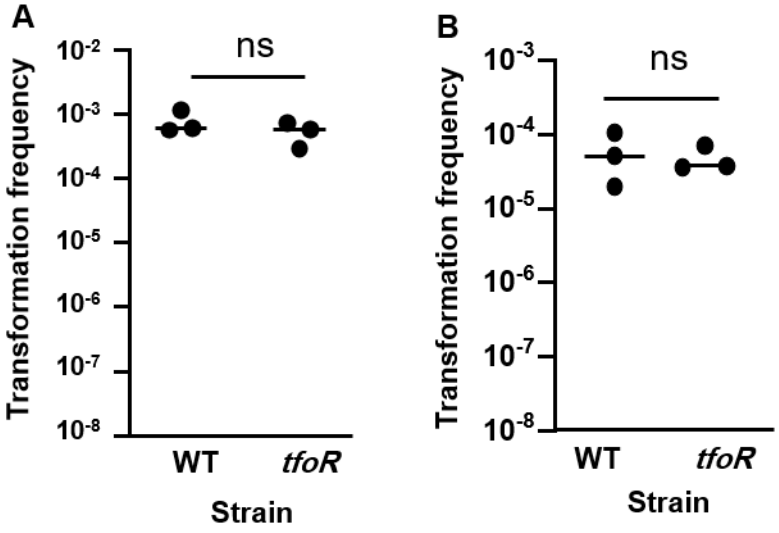
Key words: Competence; *Vibrio fischeri*; *litR*; *hapR*; quorum sensing; luminescence



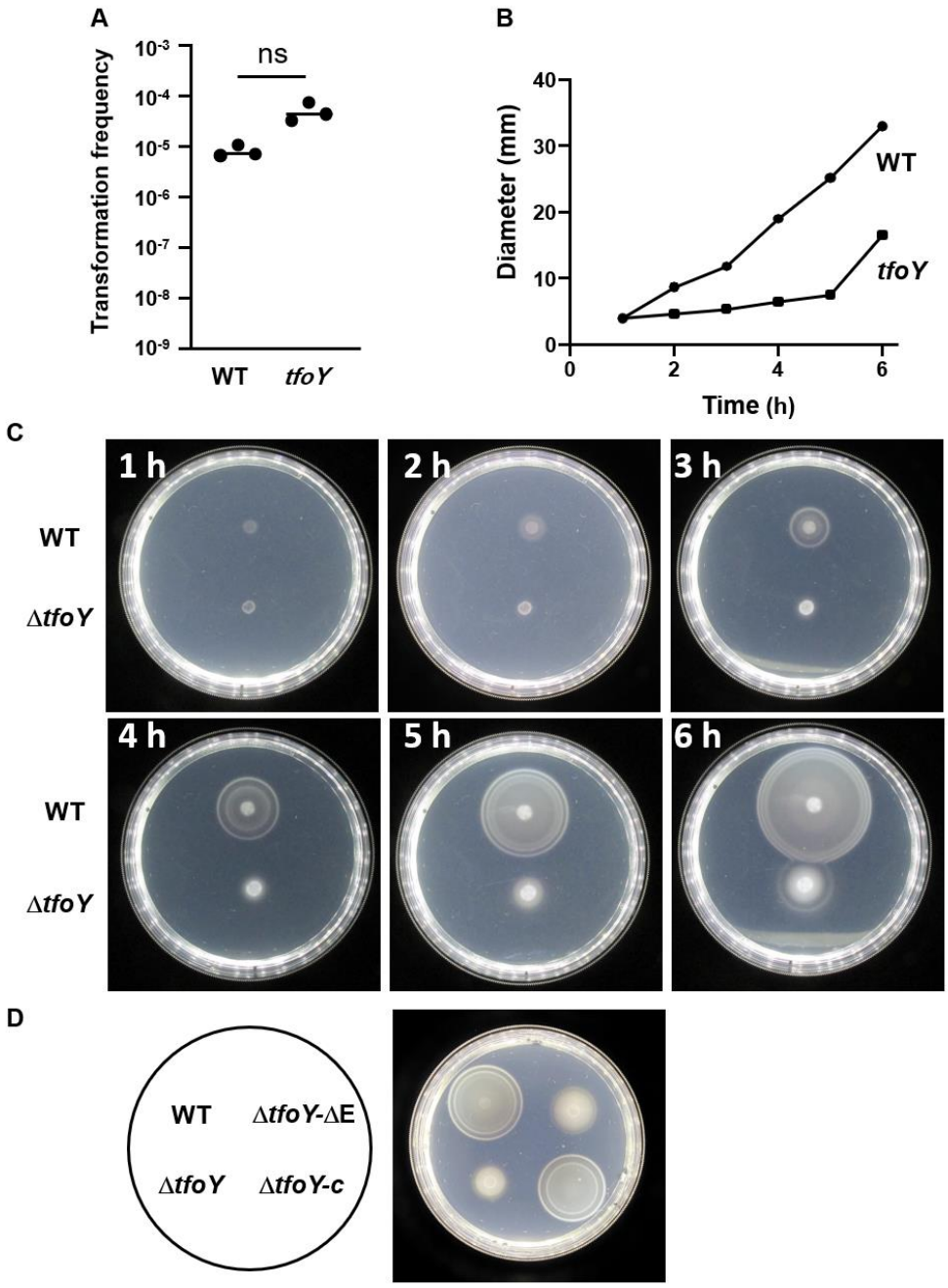
Supplemental Figure 1. Cm^R –encoding *tfoX* overexpression plasmids confer greater transformation frequencies relative to the Kan^R derivatives. Transformation frequencies (Trim^R CFU/Viable CFU) of wild-type strain ES114 carrying the following plasmids: plostfoX-Cm, pJJC2 (Cm^R), plostfoX-Kan, and pJJC3 (Kan^R). For pJJC2 and pJJC3-containing strains, cells were grown in the presence of 0.2% arabinose to induce *tfoX* transcription from the arabinose-inducible promoter. All strains were exposed to gDNA from KV8300 (*fliQ*::Trim). * $p<0.05$



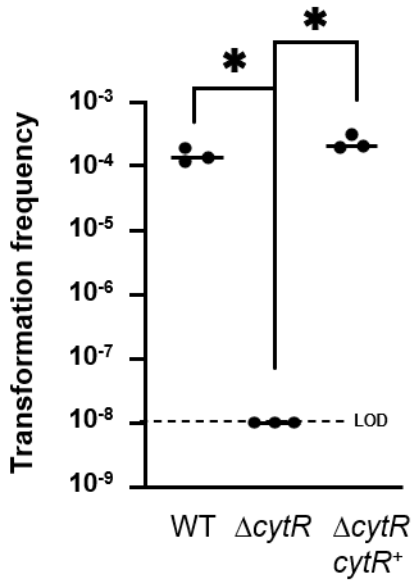
Supplemental Figure 2. Competence defects of the *pilQ* and *comEA* mutants can be complemented. Transformation frequencies ($Trim^R$ CFU/Viable CFU) of plostfoX-Cm-containing wild-type, mutant, and complemented *V. fischeri* cells exposed to gDNA from KV8300 (*fliQ*::Trim). (A) Transformation frequencies of plostfoX-Cm-containing ES114 (WT), *pilQ*::mTn (*pilQ*; KV8879), and $pilQ^+$-expressing *pilQ*::mTn (KV9633) strains. (B) Transformation frequencies of plostfoX-Cm-containing ES114 and Δ*comEA* (KV9219), and $comEA^+$-expressing Δ*comEA* (KV9632) strains. The Δ*pilQ* and Δ*comEA* strains exhibited transformation frequencies below the limit of detection (LOD). * $p<0.05$



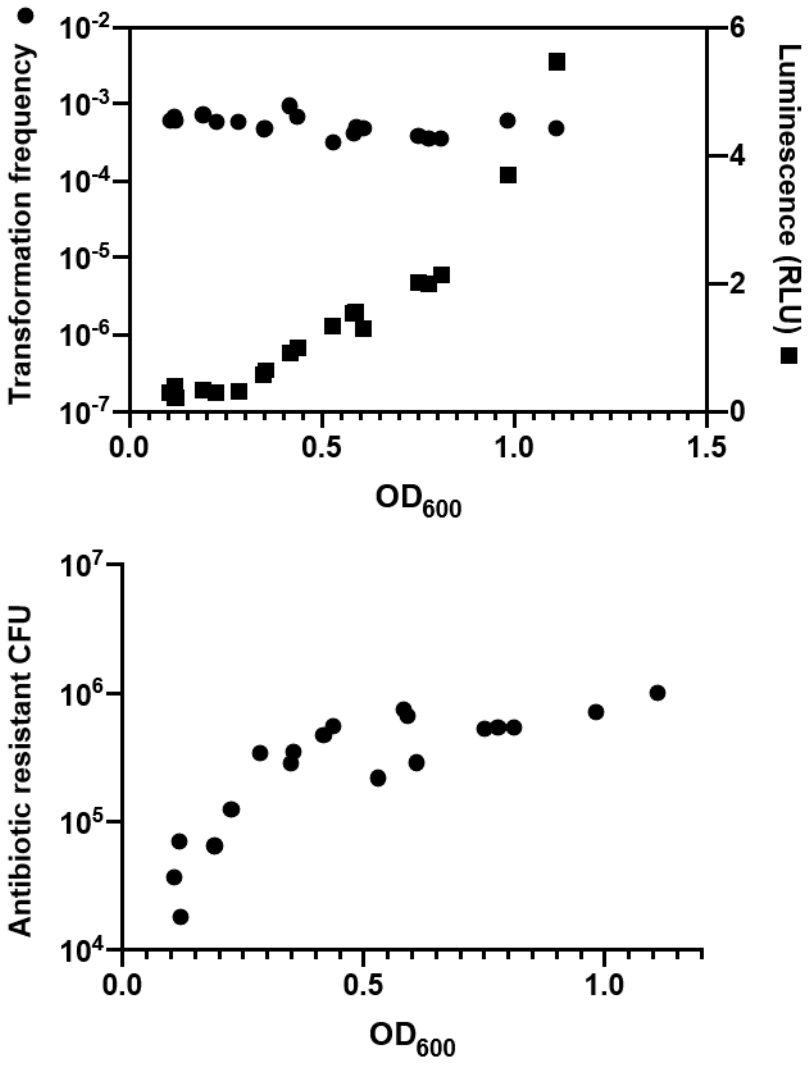
Supplemental Figure 3. Loss of TfoR does not impact *V. fischeri* transformation under *tfoX*-overexpression conditions. Transformation frequencies (total Trim^R CFU/Viable CFU) of plostfoX-Cm- (A) or plostfoX-Kan- (B) containing wild-type (ES114) (WT) and the *tfoR* mutant (KV8162) (A) or KV9605 (B) strains exposed to gDNA from KV8300 (*fliQ*::Trim). ns, not significant



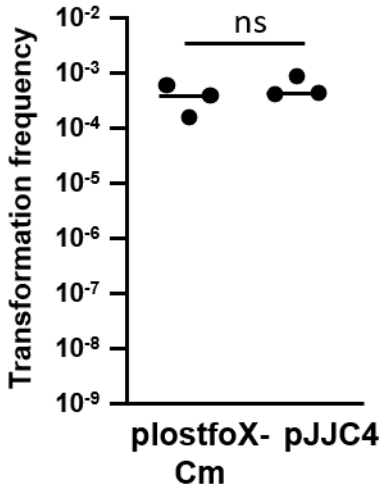
Supplemental Figure 4. TfoY required for motility but not competence. (A) Transformation frequencies (Total Trim^R CFU/viable CFU) of plostfoX-Kan-containing wild-type (ES114) (WT) and *tfoY* mutant (KV9353) strains exposed to gDNA from KV8300 (*fliQ*::Trim). ns, not significant (B and C) Motility of ES114 and *tfoY* mutant (KV9353) strains in TBS + Mg soft agar plates. To observe and quantify migration patterns, photographs (B) and measurements (C) were taken hourly. (D) Cartoon of strain placement on motility agar (left) and r**epresentative picture of the migration** (right) of ES114 (WT) and Δ*tfoY* (Erm^R) (KV9353) (Δ*tfoY*) strains along with Erm^S Δ*tfoY* derivative (KV9607) (Δ*tfoY*-ΔE) and complemented *tfoY* mutant (KV9627) (Δ*tfoY*-c).



Supplemental Figure 5. The competence defect of the *cytR* mutant can be complemented. Transformation frequencies (Total Trim^R CFU/viable CFU) of plostfoX-Cm-containing wild-type (WT) (ES114), Δ*cytR* (KV8840), and *cytR*$^+$ expressing Δ*cytR* mutant (KV9650) cells exposed to gDNA from KV8300 (*fliQ*::Trim). The Δ*cytR* mutant exhibited a transformation frequency below the limit of detection (LOD). * $p<0.05$



Supplemental Figure 6. Transformation frequencies during *V. fischeri* growth. The data from Figure 7 are combined here with a second experiment performed the same way. (A) Transformation frequencies ($Trim^R$ CFU/Viable CFU) (left Y-axis) (circles) and relative light units (RLU) (right Y-axis) (squares) of plostfoX-Cm-containing ES114 assessed at the indicated optical densities (OD_{600}) during growth of *V. fischeri* in minimal medium. (B) The same transformation experiments as shown in panel A, plotted instead as numbers of transformants at each optical density (not normalized to total cell number) (circles). Cells were exposed to gDNA from KV8300 (*fliQ*::Trim). The data shown are from two combined experiments each performed by sampling from two flasks.



Supplemental Figure 7. Transformation of ES114 containing pLostfoX-Cm or pJJC4. Transformation frequencies ($Trim^R$ CFU/Viable CFU) of wild-type strain ES114 carrying plostfoX-Cm or pJJC4 (Cm^R) were assessed following exposure to gDNA of KV8300 (*fliQ*::Trim). ns, not significant

Supplemental Materials and Methods

Strain construction. Strains and plasmids used for work shown in the Supplemental data section are listed in Supplemental Tables 1 and 2, respectively. Primers used for their construction are listed in Supplemental Table 3. As needed, the Erm^R cassette was removed using the FLP recombinase protein expressed from plasmid pKV496 [1] by conjugation using *E. coli* strains carrying that plasmid and another carrying conjugal plasmid pEVS104 [2].

Strains for complementation experiments were generated as described in Visick et al., 2018 [1] with primers as indicated in Supplemental Table 3. Because this method uses Erm^R as a selectable marker, the Erm^R resistance marker was removed from the *tfoY* mutant before the Erm^R-marked complement was introduced. The *pilQ*, *comEA*, and *cytR* mutants were not able to be transformed. Therefore, the Erm^R-marked mutations were instead introduced into strains that carried the corresponding gene complement lacking the associated Erm^R marker. For example, the *pilQ*::FRT-Erm mutation was introduced into a strain that carried a wild-type copy of *pilQ* at the *yeiR-glmS* region [1].

Plasmid construction. For plasmids pJJC2 and pJJC3, a PCR product was first cloned into pJET1.2 using the Thermo Scientific™ CloneJET PCR cloning kit. pJJC2 was derived from plostfoX-Cm digested with KpnI and SacI, which removes the *tfoX* gene, and ligated with a KpnI and SacI-digested DNA fragment containing *araC* and P*ara-tfoX*. This DNA fragment was derived from a PCR SOE reaction using EMD Millipore KOD DNA polymerase using primer sets 2764 & 2765 (with template pSW7848 [3] and 2766 & 2767 (with template ES114), that was cloned into pJET1.2. pJJC3 was made in the same way, except that plostfoX-Kan was used as the vector.

Arabinose-inducible plasmid transformations. *V. fischeri* strain ES114 carrying pJJC2 (Cm^R) or pJJC3 (Kan^R), which contain *tfoX* under the control of an arabinose-inducible promoter, were grown overnight in TMM containing 5 µg/ml Cm or 100 µg/ml Kan, respectively. The strains were subcultured in the same medium, but supplemented with arabinose at a final concentration of 0.5%. As with the other experimental transformations reported in the main text, the strains were grown until an OD_{600} between 0.5 and 0.7 was achieved and transformed with 500 ng of genomic DNA from KV8300 (Δ*fliQ*::FRT-$Trim^R$).

Motility experiments. For motility experiments, *V. fischeri* was grown in TBS broth, which contains 1% Difco tryptone and 2% sodium chloride, and inoculated onto TBS containing 35 mM $MgSO_4$, solidified with 0.3% agar (final concentration) [4, 5]. Wild-type and *tfoY* mutant (KV9353) cells were grown overnight in TBS at 28C, then subcultured in the same medium for 1-2 h at 28 degrees. An aliquot (10 μl) of cells normalized to a similar OD_{600} (equal to about 0.2) were spotted onto TBS soft agar plates (solidified with 0.3% agar (final concentration)) that contained 35 mM $MgSO_4$ [5]. Migration was examined over time and photos were taken at a representative point.

Supplemental Table 1. Strains used for work shown in the Supplemental data section

Strain	Genotype[1]	Derivation or description[2, 3]	Source or Reference
ES114	Wild Type	N/A	[6]
KV8162	Δ*tfoR*::FRT-Erm	TT ES114 with PCR SOE generated with primer sets 2270 & 2271 (ES114), 2089 & 2090 (pKV494), and 2272 & 2273 (ES114)	This study
KV8232	IG (*yeiR-glmS*)::Erm' $Trim^R$	Parent strain for inserting complementing genes into the *V. fischeri* genome	[1]
KV8300	Δ*fliQ*::FRT-Trim	N/A	[1]
KV8399	Δ*fliQ*::FRT IG (*yeiR*-FRT-Erm/*glmS*)::P_{nrdR}-*fliQ*	Template for PCR for inserting complements at the *yeiR-glmS* intergenic (IG) region, used in lieu of pKV506 and/or pKV503	[1]
KV8840	Δ*cytR*::FRT-Erm	See Table 1, main document	This study
KV8879	*pilQ*::mTn5	See Table 1, main document	This study
KV9219	Δ*comEA*::FRT-Erm	See Table 1, main document	This study
KV9353	Δ*tfoY*::FRT-Erm	TT ES114 with PCR SOE with primers 2777 & 2778 (ES114), 2089 & 2090 (pKV494), and 2779 & 2780 (ES114)	This study
KV9605	Δ*tfoR*::FRT	Introduction of pKV496 into KV8162 to remove Erm^R cassette	This study

KV9607	Δ*tfoY*::FRT	Introduction of pKV496 into KV9353 to remove Erm^R cassette	This study
KV9620	IG (*yeiR-glmS*)::P_{nrdR}-*tfoY*$^+$ [Erm^R]	TT KV8232 with PCR SOE with primers 2290 & 2090 (pKV506), 2951 & 2952 (ES114), and 2196 & 1487 (pKV503)	This study
KV9627	Δ*tfoY*::FRT P_{nrdR}-*tfoY*$^+$	TT KV9607 with gKV9620	This study
KV9628	IG (*yeiR-glmS*)::P_{nrdR}-*comEA*$^+$	TT KV8232 with PCR SOE with primers 2290 & 2090 (KV8399), 2949 & 2950 (ES114), and 2196 & 1487 (KV8399), then introduction of pKV496 to remove Erm^R cassette	This study
KV9629	IG (*yeiR-glmS*)::P_{nrdR}-*pilQ*$^+$	TT KV8232 with PCR SOE with primers 2290 & 2090 (KV8399), 2963 & 2964 (ES114), and 2196 & 1487 (KV8399), then introduction of pKV496 to remove Erm^R cassette	This study
KV9632	P_{nrdR}-*comEA*$^+$ Δ*comEA*::FRT-Erm^R	TT KV9628 with gKV9219	This study
KV9633	P_{nrdR}-*pilQ*$^+$ Δ*pilQ*::mTn5	TT KV9629 with gKV8879	This study
KV9638	IG (*yeiR-glmS*)::P_{nrdR}-*cytR*$^+$	TT KV8232 with PCR SOE with primers 2290 & 2090 (KV8399), 2947 & 2948 (ES114), and 2196 & 1487 (KV8399), then	This study

		introduction of pKV496 to remove Erm^R cassette	
KV9650	P_{nrdR}-*cytR*$^+$ Δ*cytR*::FRT-Erm^R	TT KV9638 with gKV8840	This study

[1]IG, intergenic region between the genes in parentheses

[2]TfoX-induced Transformation (TT) was performed with *tfoX* overexpressing versions of the indicated strains using either PCR SOE DNA or genomic (g) DNA from the indicated strain

[3]pKV496 was introduced into strains by conjugation as described in the Supplemental Methods section

Supplemental Table 2. Plasmids used for work shown in the Supplemental data section

Plasmid	Description	Resistance marker	Source or Reference
pEVS104	Conjugal plasmid	Kan	[2]
pJJC2	plostfoX-Cm[1] with *tfoX* replaced with P*ara-tfoX*	Cm	This study
pJJC3	plostfoX-Kan with *tfoX* replaced with P*ara-tfoX*	Kan	This study
pKV496	pJET + FLP recombinase gene	Kan	[1]
pKV503	pJET + *glmS* sequences + linker	Ap	[1]
pKV506	pJET + *yeiR*, erm^R, and P_{nrdR} sequences + linker	Ap, Erm	[1]
pSW7848	Suicide vector; used as source of *araC* P*ara*	Cm	[3]

Supplemental Table 3: Primers used for the construction of strains and plasmids used for work shown exclusively in the Supplemental data section

Primer Number	Purpose	Sequence[1]
1487	Amplify *glmS*	GGTCGTGGGGAGTTTTATCC
2090	Amplify Erm^R	CCATGGCCTTCTAGGCCTATCC
2196	Amplify *glmS*	TCCATACTTAGTGCGGCCGCCTA
2270	Delete *tfoR*	CCAAGAATGGTTATTAAAAGTCTACC
2271	Delete *tfoR*	taggcggccgcactaagtatggCCCATCCGATTAAATAACTCATATTGAACCCC
2272	Delete *tfoR*	ggataggcctagaaggccatggCGATGTAATTAACTATAACTGC
2273	Delete *tfoR*	CCATTAACTTCTTTCTTGTGTCGGTTGG
2290	Amplify Erm^R	AAGAAACCGATACCGTTTACG
2764	Clone *araC* and P*ara*	aaagagctcTCGTTACCAATTATGACAACTTGACG
2765	Clone *araC* and P*ara*	aaaattgacctcctAACGGGTATGGAGAAACAGTAGAG
2766	O/E *tfoX* with strong RBS	cccgttaggaggTCAATTTTATGACAGGGACGGG
2767	O/E *tfoX* with strong RBS	aaaggtaccATTTGTTTCGGTATAACGAGG
2777	Delete *tfoY*	TGAAATGCAACCAGAGGTTGTTACAC
2778	Delete *tfoY*	taggcggccgcactaagtatggAGACGAAGTGAGTCTTTTAATACTGG
2779	Delete *tfoY*	ggataggcctagaaggccatggGTAATTTCTGCCGAAACACGTAATGAA
2780	Delete *tfoY*	CTGTTGTTATTGCGATGCAAGCTG
2947	Complement *cytR*	ggataggcctagaaggccatggATGGACTTTAGATATTGAGCCAC
2948	Complement *cytR*	taggcggccgcactaagtatggaCGTGGCTTAAAGAATGAATCGAG
2949	Complement *comEA*	ggataggcctagaaggccatggGCGTTCTCAAACTCAGCAAG
2950	Complement *comEA*	taggcggccgcactaagtatggaTTGTACCAATGAGCTGATTAGC
2951	Complement *tfoY*	ggataggcctagaaggccatggGTAGTAGTGTTTCAAACCGCC
2952	Complement *tfoY*	taggcggccgcactaagtatggaGAGCCTGTAGATTAAGGCTG
2963	Complement *pilQ*	ggataggcctagaaggccatggAGGATTAGGAATCTCTCATGTCG
2964	Complement *pilQ*	taggcggccgcactaagtatggaCATGCTGAATTTGAATACTATCTC

[1]Lowercase letters indicate non-native sequences

References

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