

Supplemental Material for:

Natural strain variation reveals diverse biofilm regulation in squid-colonizing *Vibrio fischeri*

Ella R. Rotman, Katherine M. Bultman, John F. Brooks II, Mattias C. Gyllborg, Hector L. Burgos, Michael S. Wollenberg, Mark J. Mandel

SUPPLEMENTAL FILES

Table S1 (PDF). Primer pairs for construction of the deletion mutants. Detailed oligonucleotide and construction details for deletions and the MJM1100 *sypE*(ntG33Δ) and MJM1130 *sypE*(nt33::G) strains.

File S1 (PDF). Sequence of the synthetic dsDNA, gBlock_erm. The sequence is provided in FASTA format printed as PDF.

Table S1. Primers pairs for construction of mutants.

Strain	Note	Forward Primer	Reverse Primer	PCR Product Size	Description of PCR product	E. coli with clone
MJM3010 = MJM1100 ΔrscS and MJM3394 = MJM3354 ΔrscS		Gib_ES114_rscS_US_for Gib_ES114_rscS_DS_for ES114_US_ver ES114_US_ver	Gib_ES114_rscS_US_rev Gib_ES114_rscS_DS_rev ES114_DS_ver DAT015F	1.6 kb 1.6 kb 586 bp 520 bp	Gibson assembly cloning of upstream <i>rscS</i> region in MJM1100 into pEVs79 Gibson assembly cloning of downstream <i>rscS</i> region in MJM1100 into pEVs79 Verification of Δ rscS by flanking the upstream/downstream junction Verification of Δ rscS; no product due to one primer binding within <i>rscS</i> .	MJM3008
MJM3017 = MJM1117 ΔrscS		Gib_ES114_rscS_US_for Gib_ES114_rscS_DS_for ES114_US_ver ES114_US_ver	Gib_ES213_rscS_US_rev Gib_ES114_rscS_DS_rev ES114_DS_ver DAT015F	1.6 kb 1.6 kb 586 bp 520 bp	Gibson assembly cloning of upstream <i>rscS</i> region in MJM1117 into pEVs79 Gibson assembly cloning of downstream <i>rscS</i> region in MJM1117 into pEVs79 Verification of Δ rscS by flanking the upstream/downstream junction Verification of Δ rscS; no product due to one primer binding within <i>rscS</i> .	MJM3014
MJM3046 = MJM1130 ΔrscS		Gib_ES114_rscS_US_for Gib_ES114_rscS_DS_for ES114_US_ver ES114_US_ver	Gib_ES114_rscS_US_rev Gib_ES114_rscS_DS_rev ES114_DS_ver DAT015F	1.6 kb 1.6 kb 586 bp 520 bp	Gibson assembly cloning of upstream <i>rscS</i> region in MJM1130 into pEVs79 Gibson assembly cloning of downstream <i>rscS</i> region in MJM1130 into pEVs79 Verification of Δ rscS by flanking the upstream/downstream junction Verification of Δ rscS; no product due to one primer binding within <i>rscS</i>	MJM3043
MJM3042 = MJM2114 ΔrscS		Gib_ES114_rscS_US_for Gib_ES114_rscS_DS_for ES114_US_ver ES114_US_ver	Gib_ES114_rscS_US_rev Gib_ES114_rscS_DS_rev ES114_DS_ver DAT015F	1.6 kb 1.6 kb 586 bp 520 bp	Gibson assembly cloning of upstream <i>rscS</i> region in MJM2114 into pEVs79 Gibson assembly cloning of downstream <i>rscS</i> region in MJM2114 into pEVs79 Verification of Δ rscS by flanking the upstream/downstream junction Verification of Δ rscS; no product due to one primer binding within <i>rscS</i>	MJM3039
MJM3062 = MJM1100 ΔsypA-R		Gib_ES114_syp_US_for Gib_ES114_syp_DS_for Syp_ver_US_for Syp_ver_US_for	Gib_ES114_syp_US_rev Gib_ES114_syp_DS_rev Syp_ver_DS_rev sypA_out	1.6 kb 1.6 kb 503 bp 490 bp	Gibson assembly cloning of upstream <i>sypA</i> region in MJM1100 into pEVs79 Gibson assembly cloning of downstream <i>sypR</i> region in MJM1100 into pEVs79 Verification of Δ sypA-R by flanking the upstream/downstream junction Verification of Δ sypA-R; no product due to one primer binding within <i>sypA</i>	MJM3060
MJM3068 = MJM1117 ΔsypA-R		Gib_ES114_syp_US_for Gib_MB11B1_syp_DS_for Syp_ver_US_for Syp_ver_US_for	Gib_ES114_syp_US_rev Gib_ES114_syp_DS_rev Syp_ver_DS_rev sypA_out	1.6 kb 1.6 kb 503 bp 490 bp	Gibson assembly cloning of upstream <i>sypA</i> region in MJM1117 into pEVs79 Gibson assembly cloning of downstream <i>sypR</i> region in MJM1117 into pEVs79 Verification of Δ sypA-R by flanking the upstream/downstream junction Verification of Δ sypA-R; no product due to one primer binding within <i>sypA</i>	MJM3066
MJM3501 = MJM1125 ΔsypA-R		Gib_SR5_syp_US_for Gib_SR5_syp_DS_for SR5_syp_ver_for Gib_SR5_syp_US_for	Gib_SR5_syp_US_rev Gib_SR5_syp_DS_rev SR5_syp_ver_rev sypA_out	1.6 kb 1.6 kb 506 bp 1.9 kb	Gibson assembly cloning of upstream <i>sypA</i> region in MJM1125 into pEVs79 Gibson assembly cloning of downstream <i>sypR</i> region in MJM1125 into pEVs79 Verification of Δ sypA-R by flanking the upstream/downstream junction Verification of Δ sypA-R; no product due to one primer binding within <i>sypA</i>	MJM3500
MJM3065 = MJM1130 ΔsypA-R		Gib_MB11B1_syp_US_for Gib_MB11B1_syp_DS_for Syp_ver_US_for Syp_ver_US_for	Gib_MB11B1_syp_US_rev Gib_ES114_syp_DS_rev Syp_ver_DS_rev sypA_out	1.6 kb 1.6 kb 503 bp 490 bp	Gibson assembly cloning of upstream <i>sypA</i> region in MJM1130 into pEVs79 Gibson assembly cloning of downstream <i>sypR</i> region in MJM1130 into pEVs79 Verification of Δ sypA-R by flanking the upstream/downstream junction Verification of Δ sypA-R; no product due to one primer binding within <i>sypA</i>	MJM3063
MJM3071 = MJM2114 ΔsypA-R		Gib_ES114_syp_US_for Gib_ES114_syp_DS_for Syp_ver_US_for Syp_ver_US_for	Gib_ES114_syp_US_rev Gib_ES114_syp_DS_rev Syp_ver_DS_rev sypA_out	1.6 kb 1.6 kb 503 bp 490 bp	Gibson assembly cloning of upstream <i>sypA</i> region in MJM2114 into pEVs79 Gibson assembly cloning of downstream <i>sypR</i> region in MJM2114 into pEVs79 Verification of Δ sypA-R by flanking the upstream/downstream junction Verification of Δ sypA-R; no product due to one primer binding within <i>sypA</i>	MJM3069
MJM3084 = MJM1130 ΔbinK		Gib_ES114_binK_US_for Gib_ES114_binK_DS_for JFB_287_MB11B1 JFB_287_MB11B1	Gib_ES114_binK_US_rev Gib_ES114_binK_DS_rev JFB_288 JFB_365	1.6 kb 1.6 kb 767 bp 624 bp	Gibson assembly cloning of upstream <i>binK</i> region in MJM1130 into pEVs79 Gibson assembly cloning of downstream <i>binK</i> region in MJM1130 into pEVs79 Verification of Δ binK by flanking the upstream/downstream junction Verification of Δ binK; no product due to one primer binding within <i>binK</i>	MJM3082
MJM3417 = MJM1100 ΔsypE and MJM3423 = MJM3010 ΔsypE		Gib_ES114_sypE_US_for Gib_ES114_sypE_DS_for syp4F syp5F	Gib_ES114_sypE_US_rev Gib_ES114_sypE_DS_rev syp4R syp4R	1.6 kb 1.6 kb 780 bp 772 bp	Gibson assembly cloning of upstream <i>sypE</i> region in MJM1100 into pEVs79 Gibson assembly cloning of downstream <i>sypE</i> region in MJM1100 into pEVs79 Verification of Δ sypE by flanking the upstream/downstream junction Verification of Δ sypE; no product due to one primer binding within <i>sypE</i>	MJM3416
MJM3410 = MJM1130 ΔsypE		Gib_MB11B1_sypE_US_for Gib_MB11B1_sypE_DS_for for_ver_sypE MB11B1_indel_for	Gib_MB11B1_sypE_US_rev Gib_MB11B1_sypE_DS_rev rev_ver_sypE MB11B1_indel_rev	1.6 kb 1.6 kb 732 bp 779 bp	Gibson assembly cloning of upstream <i>sypE</i> region in MJM1130 into pEVs79 Gibson assembly cloning of downstream <i>sypE</i> region in MJM1130 into pEVs79 Verification of Δ sypE by flanking the upstream/downstream junction Verification of Δ sypE; no product due to both primers binding within <i>sypE</i>	MJM3409
MJM3354 = MJM1100 <i>sypE</i>(ntG33A)		Gib_ES114_sypE_N_for Gib_ES114_sypE_C_for Gib_pEVs79_ES_sypE_for ES114_indel_for	Gib_ES114_sypE_N_rev Gib_ES114_sypE_C_rev Gib_pEVs79_ES_sypE_rev ES114_indel_rev	332 bp 1786 bp 2118 bp 802 bp	Gibson assembly cloning of N-terminal <i>sypE</i> in MJM1100 into pEVs107 Gibson assembly cloning of C-terminal <i>sypE</i> in MJM1100 into pEVs107 Gibson assembly cloning of <i>sypE</i> (ntG33A) into pEVs79 Verification of <i>sypE</i> (ntG33A); stronger band with original allele.	MJM3340 and MJM3352
MJM3397 = MJM1130 <i>sypE</i>(nt33::G)		Gib_MB11B1_sypE_N_for Gib_MB11B1_sypE_C_for Gib_pEVs79_MB_sypE_for MB11B1_indel_for	Gib_MB11B1_sypE_N_rev Gib_MB11B1_sypE_C_rev Gib_pEVs79_MB_sypE_rev MB11B1_indel_rev	332 bp 1786 bp 2118 bp 779 bp	Gibson assembly cloning of N-terminal <i>sypE</i> in MJM1130 into pEVs107 Gibson assembly cloning of C-terminal <i>sypE</i> in MJM1130 into pEVs107 Gibson assembly cloning of <i>sypE</i> (nt33::G) into pEVs79 Verification of <i>sypE</i> (nt33::G); stronger band with corrected allele.	MJM3338 and MJM3351

File S1

>gBlock_erm

```
GGTCAGCCTCTAATGGCTCGTAAGATAGTGTAGGAAGCTTTATCGAACTGCGCGAAAGATCCCGAAGTTCCTATTCTCT
AGAAAGTATAGGAACTTCCTTAGAAGCAAACCTAAGAGTGTGTTGATAGTGCAGTATCTTAAAATTTTGTATAATAGGA
ATTGAAGTTAAATTAGATGCTAAAAATTTGTAATTAAGAAGGAGTGATTACATGAACAAAAATATAAAATATTCTCAA
ACTTTTTAACGAGTGAAAAAGTACTCAACCAAATAATAAAACAATTGAATTTAAAAGAAACCGATACCGTTTACGAAAT
TGGAACAGGTAAAGGGCATTAAACGACGAAACTGGCTAAAATAAGTAAACAGGTAACGTCTATTGAATTAGACAGTCAT
CTATTCAACTTATCGTCAGAAAAATTA AAACTGAATACTCGTGTCACTTTAATTCACCAAGATATTCTACAGTTTCAAT
TCCCTAACAAACAGAGGTATAAAATTTGTTGGGAGTATTCCCTTACCATTTAAGCACACAAATTATTA AAAAGTGGTTTT
TGAAAGCCATGCGTCTGACATCTATCTGATTGTTGAAGAAGGATTCTACAAGCGTACCTTGGATATTCACCGAACACTA
GGTTTGCTCTTGACACTCAAGTCTCGATTTCAGCAATTGCTTAAGCTGCCAGCGGAATGCTTTCATCCTAAACAAAAG
TAAACAGTGTCTTAATAAAAACCTACCCGCCATACCACAGATGTTCCAGATAAATATTGGAAGCTATATACGTACTTTGT
TTCAAATGGGTCAATCGAGAATATCGTCAACTGTTTACTAAAAATCAGTTTCATCAAGCAATGAAACACGCCAAAGTA
AACAATTTAAGTACCGTTACTTATGAGCAAGTATTGTCTATTTTTAATAGTTATCTATTATTTAACGGGAGGAAATAAT
CTAGAATGCGAGAGTAGGGAAGTCCGAAGTTCCTATTCTCTAGAAAGTATAGGAACTTCAGCGCTCATGCACTTGATT
CCGGATCCCTAATTAGCGAACGCAGCTGGCTCTCCAA
```