

Supplementary Figure for Harshaw, et al. 2021

From Antibiotics used in empiric treatment of ocular infections trigger the bacterial Rcs stress response system independent of antibiotic susceptibility

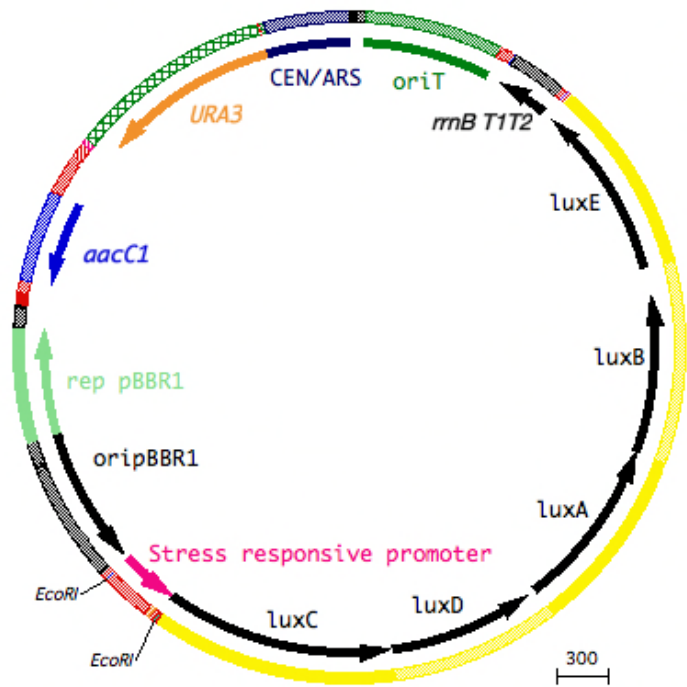


Figure S1. Diagram of pMQ747 used in this study. Starting from the origin, the origin of conjugal transfer, *oriT*, is from RP4; the *rmB* T1T2 are transcriptional terminators from *Escherichia coli*. The *luxCDABE* operon provides luminescence. The stress responsive promoter was altered in each of the plasmids used in this study. The *oriPBBR1*-*rep pBBR1* is a broad host-range medium copy replicon that is sufficient for replication in a large number of Gram-negative bacterial species. The *aacC-1* gene provides gentamicin resistance, and the *URA3* and *CEN/ARS* portions allow replication in *Saccharomyces cerevisiae* for recombineering purposes. The size bar represents 300 base pairs.

Supplemental tables for Antibiotics used in empiric treatment of ocular infections trigger the bacterial Rcs stress response system independent of antibiotic susceptibility

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Table S1. *S. marcescens* strains and plasmids used in this study.

Strain	Description	Source
K904	Wild-type <i>S. marcescens</i> keratitis isolate	[1]
$\Delta gumB$	K904 with deletion of <i>gumB</i> gene	[2]
$\Delta rcsB$	K904 with <i>rscB</i> ORF replaced by <i>mClover</i>	[3]
$\Delta gumB$ $\Delta rcsB$	K904 with deletion mutations of <i>gumB</i> and <i>rscB</i>	[3]
S17-1 λ - <i>pir</i>	<i>Escherichia coli</i> conjugal plasmid donor strain	[4]
pMQ414	pBBR1-replicon plasmid with <i>nptII</i> promoter driving <i>tdtomato</i> expression	[5]
pMQ589	RSF1010-IncQ plasmid with <i>nptII</i> promoter driving <i>luxCDABE</i> expression	This study
pMQ670	pBBR1-replicon plasmid with <i>xut</i> promoter driving <i>luxCDABE</i> expression	[6]
pMQ713	pBBR1-replicon plasmid with <i>pigA</i> promoter - <i>luxCDABE</i> expression	[7]
pMQ747	pMQ713 with SMDB11_1637 promoter - <i>luxCDABE</i>	This study
pMQ748	pMQ713 with SMDB11_2817 promoter - <i>luxCDABE</i>	This study
pMQ749	pMQ713 with SMDB11_1194 promoter - <i>luxCDABE</i>	This study

Table S2. Oligonucleotides used in this study.

Name of sequence	Sequence (5' to 3')
3805	agaccgcttctgcgttctgatttaatctgtatca <u>GGATCCT</u> CAACTATCAAACGCTTCGG
3806	gatcaagatctgatcaagagacaggatgagga <u>GAATTC</u> ATGACTAAAAAAATTCATTCA
4858 SMDB11_1637	ttgcactaaatcatcactttcgggaagatttcaacctggccggttaatgatgaatgaaatcttttagtcat <u>GAATTC</u> TTCCTGGTAAATTGGCTGATTAATGGATTAAAAATAATCCGATTAAACC CACCGGTAGAAGTTGGGATGAATGTATTCTCCGAAAGAAAATCGAAC AGGTAAAAATTCTTAATTTTTCGGCGGTGAAAAGCGATTGCTCGCCGA ATGTGCGGCCACCGACAAAACCGTGGGTCTATACTGGGAAGACGCCTC GCGGGGTGCGGGGCACACACTCTGGAGGCCGCCATGTCCGATCTCGTT TCCGCGTCCGGCAAACCGGTGAAAATCCCCGGCCCGGACCACCAATC ACCTTGACCCGCCATCCGGCGCGGGTTGTCGTGCTGCTGCCGGTCAG ACCCTGGCCGACAGTCGCAGAATTCgtcgactccagtcgggaacctgtcgtgccagctgca ttaatgcgccctacgggcttgct
4860 SMDB11_2817	ttgcactaaatcatcactttcgggaagatttcaacctggccggttaatgatgaatgaaatcttttagtcat <u>GAATTC</u> CTTCTCCTTCCCGCTTGCGCGGCCTGGCCGTTTGTCACTTTATGAAGCG CGTTCATCAGTTTAAAAAGCGGCCCTATCGTTATTTGCTGCAATATC GGCATCATAGCGCCGCGCATAGGGTTGAATCTGAGAGCCACTCTGAA AATGATGCAGGAGGTTAAGATTTGAGATTTTCTGGAGTATCGCCGCT CGATTGAACGGCGGAATTTGTAAGCGATTACAATAAGATGTTCTCGAT CTGCGCGAGCTCTTCTTCACTGAAGTGACGATTGGCCAGCATGCCCAC CGCGTCTTCTATCTGCGCATTCTTGCTGGCGCCGATCAGCACTGAGGGA <u>ATTC</u> gtcgactccagtcgggaacctgtcgtgccagctgcattaatgcgccctacgggcttgctctccgggcttcg ccctg
4862 SMDB11_1194	gattgcactaaatcatcactttcgggaagatttcaacctggccggttaatgatgaatgaaatcttttagtcat <u>GAATT</u> <u>C</u> AGGAAACCTCCTGAAAGTGTTACCACGCATTTTTTCCGGGGTCTGTTA CCGGCATGGCACCGCGCTTATTATTTTGCATGGGCGGCTTGCGGTAT CCCGGTTTACGGCGTGCGCGACGCGCCGTCTGTAAACGAGGTAAAGTC TGCACAATGATTTGAGGGCTCGCAATAAGACAAGGGGACCAAAAATG CGGAAAACGGCCGTTTTGCCGTTGTTTAGGAGGAATCTTAAGAATTT TACCCATGTCGGCGACAGCTTATGCTGAAGAATCAAGCAACCGCCGCC GTTAGCCTCGCCCCGCCATAAAAAAACCCGCGCAGGCGAACCGGCGC GGGGAATTCgtcgactccagtcgggaacctgtcgtgccagctgcattaatgcgccctacgggcttgctctcc gggcttcgcc

Lower case sequence represents DNA for targeting recombination, upper case is for priming or promoter sequence. Underlined sequence indicates an introduced restriction enzyme cleavage site.

References

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