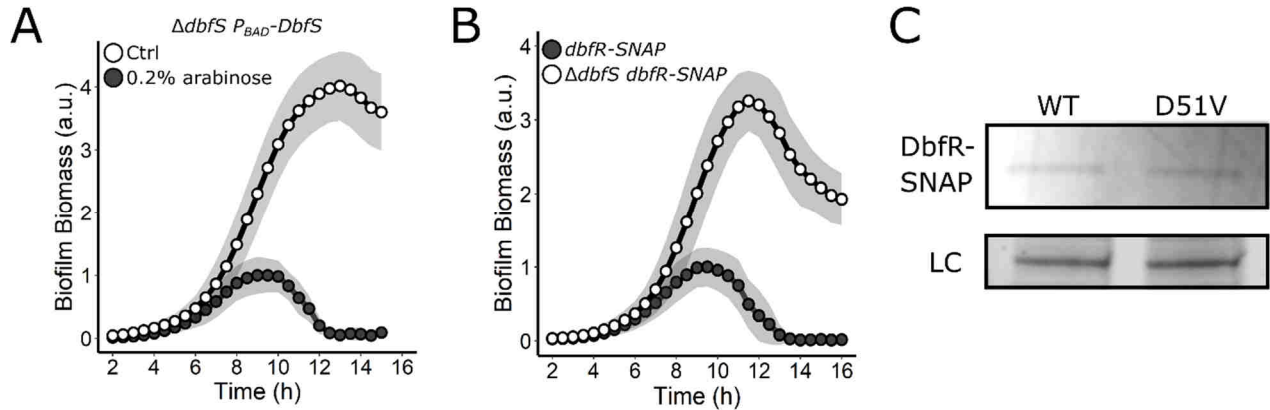


Extended Data Fig. 1



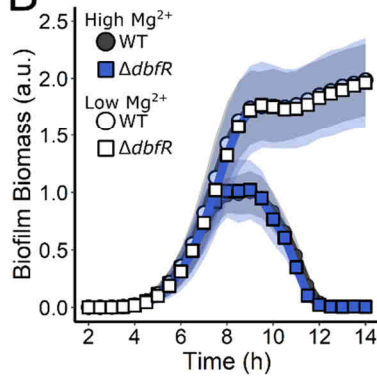
Extended Data Fig. 1. **Complementation, functional tagging, and mutagenesis of the DbfS-DbfR two-component system.** (A) Quantitation of biofilm biomass over time measured by time-lapse microscopy for the  $\Delta dbfS P_{BAD}-dbfS$  strain following addition of water (Ctrl) or 0.2% arabinose. (B) As in A for SNAP-tagged DbfR in the WT and  $\Delta dbfS$  strains. (C) Top panel: representative in-gel SDS-PAGE fluorescence following electrophoresis of *V. cholerae* cell lysates containing WT DbfS-SNAP or DbfS<sup>D51V</sup>-SNAP that had been incubated with SNAP-Cell TMR Star. Bottom panel: Coomassie stained loading control (LC). For all biofilm measurements,  $N = 3$  biological and  $N = 3$  technical replicates,  $\pm$  SD (shaded). a.u., arbitrary unit.

Extended Data Fig. 2

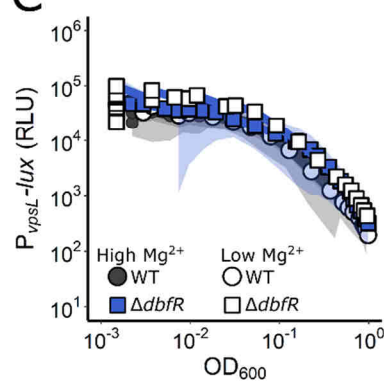
A



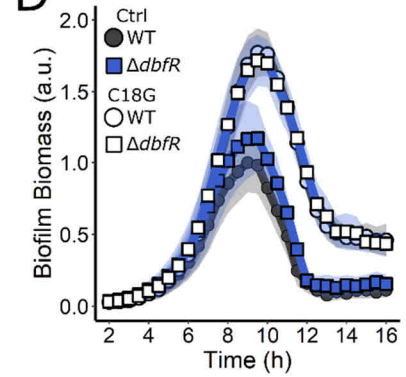
B



C

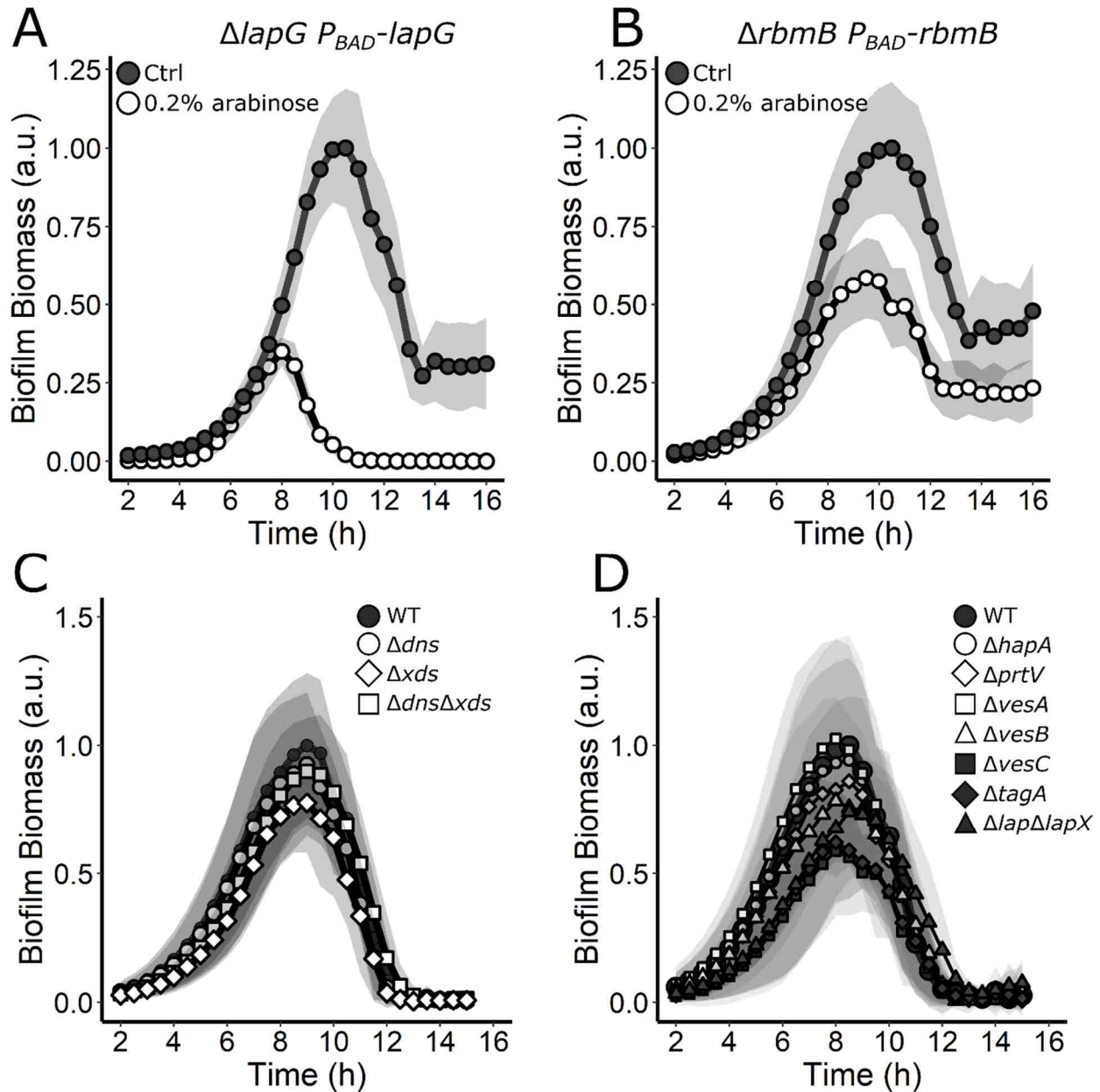


D



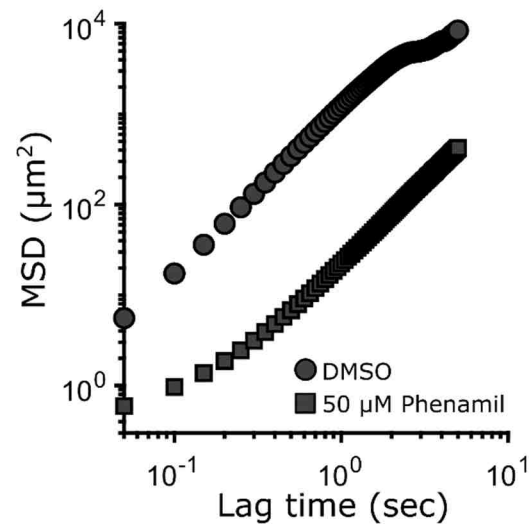
Extended Data Fig. 2. **DbfS is not functionally equivalent to PhoQ.** (A) Alignment of the sensory domains of PhoQ from *E. coli*, *S. enterica*, and *P. aeruginosa* against that of *V. cholerae* DbfS. Black boxes indicate residues involved in  $Mg^{2+}$  binding in PhoQ. (B) Quantitation of biofilm biomass over time measured by time-lapse microscopy in high magnesium (10 mM) and limiting magnesium (10  $\mu$ M) conditions for WT *V. cholerae* and the  $\Delta dbfR$  strain. (C) The corresponding  $P_{vpsL-lux}$  outputs for strains and growth conditions in B over the growth curve. (D) As in B except following the addition of water or 5  $\mu$ g/mL C18G. In all cases,  $N = 3$  biological and  $N = 3$  technical replicates,  $\pm$  SD (shaded). a.u., arbitrary unit. For  $vpsL-lux$  measurements,  $N = 3$  biological replicates,  $\pm$  SD (shaded). RLU, relative light units.

Extended Data Fig. 3



Extended Data Fig. 3. **Introduction of *lapG* and *rbmB* complements the  $\Delta lapG$  and  $\Delta rbmB$  biofilm defects, respectively, and assessment of the roles of extracellular DNases and secreted proteases in *V. cholerae* biofilm dispersal.** (A) Quantitation of biofilm biomass over time measured by time-lapse microscopy for the  $\Delta lapG P_{BAD-lapG}$  strain following addition of water (Ctrl) or 0.2% arabinose. (B) As in A, but for the  $\Delta rbmB P_{BAD-rbmB}$  strain. (C) Quantitation of biofilm biomass over time measured by time-lapse microscopy for WT *V. cholerae* and mutants lacking the designated DNases. (D) Quantitation of biofilm biomass over time measured by time-lapse microscopy for WT *V. cholerae* and mutants lacking the designated proteases. In all cases,  $N = 3$  biological and  $N = 3$  technical replicates,  $\pm$  SD (shaded). a.u., arbitrary unit.

Extended Data Fig. 4



Extended Data Fig. 4. **Phenamil inhibits *V. cholerae* motility.** Mean squared displacement (MSD) of cell trajectories versus lag time for WT *V. cholerae* treated with DMSO solvent or 50  $\mu\text{M}$  phenamil.

## Supplemental Discussion

### *DbfS is not equivalent to PhoQ*

In *E. coli*, low  $Mg^{2+}$  and cationic peptides activate PhoQ kinase activity.<sup>40</sup> Sequence alignment of the DbfS sensory domain with that from PhoQ of *E. coli*, *Salmonella enterica*, and *Pseudomonas aeruginosa* revealed that DbfS lacks all of the key residues involved in  $Mg^{2+}$  binding (Extended Data Fig. 2A).<sup>41</sup> To test if  $Mg^{2+}$  alters DfbS activity, we measured the *V. cholerae* biofilm lifecycle in response to low  $Mg^{2+}$  conditions in WT *V. cholerae* and in the  $\Delta dbfR$  mutant. If, analogous to PhoQ, DfbS kinase activity is activated by low  $Mg^{2+}$ , when  $Mg^{2+}$  is limiting, WT *V. cholerae* should exhibit an altered biofilm dispersal phenotype while the  $\Delta dbfR$  mutant would be impervious to  $Mg^{2+}$  changes.<sup>40</sup> Extended Data Fig. 2B shows that  $Mg^{2+}$  limitation does indeed inhibit *V. cholerae* biofilm dispersal, however, inhibition occurs in *both* the WT and the  $\Delta dbfR$  strains.  $Mg^{2+}$  limitation did not alter *vpsL-lux* expression in either strain (Extended Data Fig. 2C). Thus,  $Mg^{2+}$  does not control DfbS activity. We obtained the same results following exogenous addition of the cationic peptide C18G (Extended Data Fig. 2D). Together, these results demonstrate that DfbS does not respond to the ligands that control PhoQ activity.

## Supplementary Table 1

Strains used in this study.

| Strain Number | Genotype  | Plasmid                           | Antibiotic Resistance | Parent     |
|---------------|---|-----------------------------------|-----------------------|------------|
| BB_Vc_0090    | WT O1 EI Tor biotype C6706str2                                      | -                                 | Sm                    | -          |
| AB_Vc_761     | $\Delta vlc1807::Cm^R$ (Referred to as WT)                          | -                                 | Sm, Cm                | BB_Vc_0090 |
| AB_Vc_705     | $\Delta cheY \Delta vlc1807::Cm^R$                                  | -                                 | Sm, Cm                | BB_Vc_0090 |
| AB_Vc_708     | $\Delta bjpA \Delta vlc1807::Cm^R$                                  | -                                 | Sm, Cm                | BB_Vc_0090 |
| AB_Vc_839     | $\Delta mbaA \Delta vlc1807::Cm^R$                                  | -                                 | Sm, Cm                | BB_Vc_0090 |
| AB_Vc_711     | $\Delta potD1 \Delta vlc1807::Cm^R$                                 | -                                 | Sm, Cm                | BB_Vc_0090 |
| AB_Vc_757     | $\Delta lapG \Delta vlc1807::Cm^R$                                  | -                                 | Sm, Cm                | BB_Vc_0090 |
| AB_Vc_758     | $\Delta rocS \Delta vlc1807::Cm^R$                                  | -                                 | Sm, Cm                | BB_Vc_0090 |
| AB_Vc_775     | $\Delta dbfS \Delta vlc1807::Cm^R$                                  | -                                 | Sm, Cm                | BB_Vc_0090 |
| AB_Vc_777     | $\Delta cdgI \Delta vlc1807::Cm^R$                                  | -                                 | Sm, Cm                | BB_Vc_0090 |
| AB_Vc_778     | $\Delta cdgG \Delta vlc1807::Cm^R$                                  | -                                 | Sm, Cm                | BB_Vc_0090 |
| AB_Vc_485     | $\Delta rbmB \Delta vlc1807::Kan^R$                                 | -                                 | Sm, Kan               | BB_Vc_0090 |
| AB_Vc_801     | $\Delta vlc1807::Kan^R$   | pEVS143- $P_{vpsL}$ - $lux::Cm^R$ | Sm, Cm, Kan           | AB_Vc_479  |
| AB_Vc_825     | $\Delta cheY \Delta vlc1807::Kan^R$                                 | pEVS143- $P_{vpsL}$ - $lux::Cm^R$ | Sm, Cm, Kan           | AB_Vc_705  |
| AB_Vc_829     | $\Delta lapG \Delta vlc1807::Kan^R$                                 | pEVS143- $P_{vpsL}$ - $lux::Cm^R$ | Sm, Cm, Kan           | AB_Vc_757  |
| AB_Vc_802     | $\Delta rbmB \Delta vlc1807::Kan^R$                                 | pEVS143- $P_{vpsL}$ - $lux::Cm^R$ | Sm, Cm, Kan           | AB_Vc_485  |
| AB_Vc_815     | $\Delta dbfS \Delta vlc1807::Kan^R$                                 | pEVS143- $P_{vpsL}$ - $lux::Cm^R$ | Sm, Cm, Kan           | AB_Vc_775  |
| AB_Vc_773     | $\Delta dbfR \Delta vlc1807::Cm^R$                                  | -                                 | Sm, Cm                | BB_Vc_0090 |
| AB_Vc_701     | $\Delta dbfRS \Delta vlc1807::Cm^R$                                 | -                                 | Sm, Cm                | BB_Vc_0090 |
| AB_Vc_788     | $dbfR^{D51V} \Delta vlc1807::Cm^R$                                  | -                                 | Sm, Cm                | BB_Vc_0090 |
| AB_Vc_891     | $dbfR^{D51V} \Delta dbfS \Delta vlc1807::Kan^R$                     | -                                 | Sm, Kan               | BB_Vc_0090 |
| AB_Vc_863     | $dbfR$ -SNAP $\Delta dbfS \Delta vlc1807::P_{BAD}$ - $dbfS::Spec^R$ | -                                 | Sm, Spec              | BB_Vc_0090 |
| AB_Vc_865     | $\Delta dbfS \Delta vlc1807::P_{BAD}$ - $dbfS::Spec^R$              | -                                 | Sm, Spec              | BB_Vc_0090 |
| AB_Vc_879     | $dbfR$ -SNAP $\Delta vlc1807::Kan^R$                                | -                                 | Sm, Kan               | BB_Vc_0090 |
| AB_Vc_881     | $dbfR^{D51V}$ -SNAP $\Delta vlc1807::Kan^R$                         | -                                 | Sm, Kan               | BB_Vc_0090 |
| AB_Vc_859     | $\Delta lapG \Delta vlc1807::P_{BAD}$ - $lapG::Spec^R$              | -                                 | Sm, Spec              | AB_Vc_757  |
| AB_Vc_898     | $\Delta lapD \Delta vlc1807::Kan^R$                                 | -                                 | Sm, Kan               | BB_Vc_0090 |
| AB_Vc_900     | $\Delta lapD \Delta lapG \Delta vlc1807::Kan^R$                     | -                                 | Sm, Kan               | BB_Vc_0090 |
| AB_Vc_862     | $\Delta rbmB \Delta vlc1807::P_{BAD}$ - $rbmB::Spec^R$              | -                                 | Sm, Spec              | AB_Vc_485  |
| BB_Vc_0252    | $\Delta dns$  | -                                 | Sm                    | BB_Vc_0090 |
| BB_Vc_0253    | $\Delta xds$  | -                                 | Sm                    | BB_Vc_0090 |
| BB_Vc_0254    | $\Delta dns \Delta xds$   | -                                 | Sm                    | BB_Vc_0090 |
| MJ_552        | $\Delta hapA \Delta vlc1807::Kan^R$                                 | -                                 | Sm, Kan               | BB_Vc_0090 |
| MJ_553        | $\Delta prtV \Delta vlc1807::Kan^R$                                 | -                                 | Sm, Kan               | BB_Vc_0090 |
| MJ_554        | $\Delta vesA \Delta vlc1807::Kan^R$                                 | -                                 | Sm, Kan               | BB_Vc_0090 |
| MJ_555        | $\Delta vesB \Delta vlc1807::Kan^R$                                 | -                                 | Sm, Kan               | BB_Vc_0090 |
| MJ_562        | $\Delta vesC \Delta vlc1807::Kan^R$                                 | -                                 | Sm, Kan               | BB_Vc_0090 |

|           |   |   |          |            |
|-----------|---|---|----------|------------|
| MJ_561    | <i>Δlap ΔlapX lacZ::Ptac-mKO Δvc1807::Kan<sup>R</sup></i>                         | - | Sm, Kan  | BB_Vc_0090 |
| AB_Vc_792 | <i>ΔtagA Δvc1807::Cm<sup>R</sup></i>  | - | Sm, Cm   | BB_Vc_0090 |
| AB_Vc_715 | <i>cheY<sup>D16K, Y109W</sup> Δvc1807::Kan<sup>R</sup></i>                        | - | Sm, Kan  | BB_Vc_0090 |
| AB_Vc_732 | <i>ΔvpsL Δvc1807::Ptac-mScarlett::Spec<sup>R</sup></i>                            | - | Sm, Spec | BB_Vc_0090 |
| AB_Vc_735 | <i>ΔcheY ΔvpsL Δvc1807::Ptac-mScarlett::Spec<sup>R</sup></i>                      | - | Sm, Spec | AB_Vc_705  |
| AB_Vc_745 | <i>cheY<sup>D16K, Y109W</sup> ΔvpsL Δvc1807::Ptac-mScarlett::Spec<sup>R</sup></i> | - | Sm, Spec | AB_Vc_715  |

## Supplementary Table 2

DNA oligonucleotides and gene fragments used in this study.

| Oligo # | Name                   | Purpose  | Direction | 5' to 3' Sequence   |
|---------|------------------------|--|-----------|---|
| 551     | <i>cheY_3000up</i>     | Cloning at <i>cheY3</i> locus                          | F         | CAAGCGTTACAACCTCGCAGCCTAG   |
| 552     | <i>cheY_3000down</i>   | Cloning at <i>cheY3</i> locus                          | R         | CACAACCAGACCTACGCGCTGAC   |
| 553     | <i>cheY_100up</i>      | Cloning at <i>cheY3</i> locus                          | F         | GGTGAGGTACTTGGAGTTAGTGAATCTC  |
| 554     | <i>cheY_100down</i>    | Cloning at <i>cheY3</i> locus                          | R         | CACTGAAGCGCTCATCAATCTGAAAG  |
| 555     | <i>cheY_B</i>          | <i>cheY3</i> deletion                                  | R         | GAGCACCTTTTGCCGACGAAAAGCCTGAGTTTGAGATCAG<br>TGATATTTAGTCATTCC   |
| 556     | <i>cheY_C</i>          | <i>cheY3</i> deletion                                  | F         | GGAATGACTAAATATCACTGATCTCAAACCTCAGGCTTTTGCT<br>GCGGCAAAGGTGCTC  |
| 561     | <i>cheY_2700up</i>     | Cloning at <i>cheY3</i> locus                          | F         | GATGACCGTGTGAGTTTGAATCGAG   |
| 562     | <i>cheY_2700down</i>   | Cloning at <i>cheY3</i> locus                          | R         | CTTCGGTGCTAACCAGTTTTGTAAGTAGAAC   |
| 563     | <i>cheY_up_R</i>       | Cloning at <i>cheY3</i> locus                          | R         | GAGTTTGAGATCAGTGATATTTAGTCATTCCGAGTCC   |
| 564     | <i>cheY_down_R</i>     | Cloning at <i>cheY3</i> locus                          | R         | GGCTTTTGCTGCGGCAAAGGTGCTCTATTC  |
| 566     | <i>cheY_D16K_Y109W</i> | Gblock for introduction of <i>cheY3</i> point mutation | F         | GTAAAGTTCTTGACTCGGAATGACTAAATATCACTGATCTC<br>AAACTCAGTGGAGGCAATTTTGAATAAAAAACATGAAGATCCT<br>TATTGTTGATAAGTTTTCAACAATGCGCCGAATCGTTAAAAA<br>CCTACTTCGAGATCTGGGGTTCAATAACACGCAGGAAGCGG<br>ACGATGGCCTAACGGCATTGCCTATGCTCAAGAAAGGTGAT<br>TTTGACTTTGTAGTCACAGACTGGAATATGCCCGGTATGCAA<br>GGTATTGACTTGCTTAAAAATATCCGTGCCGACGAAGAAGCTG<br>AAGCACCTGCCTGTAATAATGATCACAGCAGAAGCCAAACG<br>TGAGCAAATCATCGAAGCCGCTCAAGCAGGCGTGAATGGTT<br>GGATCGTAAAACCATTTACCGCTGCTACGCTTAAAGAAAAAT<br>TAGACAAAATTTTGAGCGTTTATAAGGCTTTTGCTGCGGCA<br>AAAGGTGCTCTATTACACGCGCAAAAG |
| 545     | <i>bipA_3000up</i>     | Cloning at <i>bipA</i> locus                           | F         | GCTGCGTGAGCAGTTGTAATCGAG  |
| 546     | <i>bipA_3000down</i>   | Cloning at <i>bipA</i> locus                           | R         | CAACGCTTTGTAGTTCGGGATTAGCATATA  |
| 547     | <i>bipA_100up</i>      | Cloning at <i>bipA</i> locus                           | F         | GTCGACGATTTACGCGCAGACATC  |
| 548     | <i>bipA_100down</i>    | Cloning at <i>bipA</i> locus                           | R         | GAGGTATTTCTGGATAGGTGGCATAGC   |
| 549     | <i>bipA_B</i>          | <i>bipA</i> deletion                                   | R         | GATGACTTATCTTACCAAACGAAAGTCAGTGACGGGGTTTG<br>CTTCACTTTTTTCATTGAGGCTG  |
| 550     | <i>bipA_C</i>          | <i>bipA</i> deletion                                   | F         | CAGCCTCAATGAAAAAGTGAAGCAAACCCGTCAGTACTT<br>TCGTTTGTAAGATAAGTCATC  |
| 567     | <i>bipA_2700up</i>     | Cloning at <i>bipA</i> locus                           | R         | CAGTGACTCGTCCAAAATGAGCACTG  |
| 568     | <i>bipA_2700down</i>   | Cloning at <i>bipA</i> locus                           | R         | GATCTAAATCGCCACTGATCCCATCAAG  |
| 571     | <i>mbaA_3000up</i>     | Cloning at <i>mbaA</i> locus                           | F         | GCGCGCTAATCTGAACTCAACCCATAAG  |
| 572     | <i>mbaA_2700up</i>     | Cloning at <i>mbaA</i> locus                           | F         | CGTTAGCATTCCACGCGGTGAGTTAG  |
| 711     | <i>mbaA_KO2_B</i>      | <i>mbaA</i> deletion                                   | R         | GGAGGCATGAAGCCATGGGGAGATCTCGCTATGGTTAGCT<br>TCATATTGGTAAGTCACACTG   |
| 712     | <i>mbaA_KO2_C</i>      | <i>mbaA</i> deletion                                   | F         | CAGTGTGACTTACCAATATGAAGCTAAACCATAGCGAGATCT<br>CCCCATGGCTTCATGCCTCC  |
| 575     | <i>mbaA_2700down</i>   | Cloning at <i>mbaA</i> locus                           | R         | GATCTCATGACGCGCCTGACGGTATTTAAG  |
| 576     | <i>mbaA_3000down</i>   | Cloning at <i>mbaA</i> locus                           | R         | CATCGTTGCGGATAGTGGGAAATTCAATAAAATG  |



|     |                        |                               |   |   |
|-----|------------------------|-------------------------------|---|---|
| 577 | <i>mbaA</i> _100up     | Cloning at <i>mbaA</i> locus  | F | GAAACCTGACATTGCCGCAATCAATGC   |
| 578 | <i>mbaA</i> _100down   | Cloning at <i>mbaA</i> locus  | R | CCTGCTTCCAATCCGACATAATACTCTGC   |
| 539 | <i>potD1</i> _3000up   | Cloning at <i>potD1</i> locus | F | CTGGAATCCGGTATGTGTGTGATGGTTAG   |
| 540 | <i>potD1</i> _3000down | Cloning at <i>potD1</i> locus | R | AGAGCGACTAGGTGTTATTGAACTTGGG  |
| 541 | <i>potD1</i> _100up    | Cloning at <i>potD1</i> locus | F | CTAAGAAAAGCATCAAATAGGCAGCCATTG  |
| 542 | <i>potD1</i> _100down  | Cloning at <i>potD1</i> locus | R | GATCTGGAAGAGATTAAGGCGCTCTC  |
| 543 | <i>potD1</i> _B        | <i>potD1</i> deletion         | R | GGTGGCTTTTTAATGGGAGATAAAAGGCTACGTTCCCATAGT<br>GTATAGAAAGAACC          |
| 544 | <i>potD1</i> _C        | <i>potD1</i> deletion         | F | GGTTCTTTCTATACACTATGGGAACGTAGCCTTTTATCTCCC<br>ATTA AAAAGCCACC         |
| 569 | <i>potD1</i> _2700up   | Cloning at <i>potD1</i> locus | F | CTGATGATTATTGGTACGAGTTTTCTGACTCGTG                                    |
| 570 | <i>potD1</i> _2700down | Cloning at <i>potD1</i> locus | R | CGATAATCCAAATCAAATCGAGGTGCAGG   |
| 602 | <i>lapG</i> _3000up    | Cloning at <i>lapG</i> locus  | F | CAAACAATTACCCGGTTATTGGGGATG   |
| 603 | <i>lapG</i> _2700up    | Cloning at <i>lapG</i> locus  | F | GCATTCCGTCAAAGTGCTCGATATTCATC   |
| 604 | <i>lapG</i> _100up     | Cloning at <i>lapG</i> locus  | F | GATCATTCCGGGAATGACCGCTTC  |
| 605 | <i>lapG</i> _B         | Cloning at <i>lapG</i> locus  | R | CGACTAGTTGTTTGTATAGCGTCATAGTGCAGGGCGGGCTA<br>TTCCCTCAGCGCATTGCTTTG    |
| 606 | <i>lapG</i> _C         | <i>lapG</i> deletion          | F | CAAAGCAATGCGCTGAGGGAATAGCCCGCCCTGCACTATGA<br>CGCTATACAAACA ACTAGTCG   |
| 607 | <i>lapG</i> _100down   | <i>lapG</i> deletion          | R | GTGTTGTTGACTTCAGAGCGTTGTTG  |
| 608 | <i>lapG</i> _2700down  | Cloning at <i>lapG</i> locus  | R | GTCCAGCCATTAACCAGATCAACAC   |
| 609 | <i>lapG</i> _3000down  | Cloning at <i>lapG</i> locus  | R | CAGCGGTACTGGAATTGTCCTTGC  |
| 774 | <i>lapD</i> _3000up    | Cloning at <i>lapD</i> locus  | F | CGCGAATACAAGAAGCGATCATGCAG  |
| 775 | <i>lapD</i> _2700up    | Cloning at <i>lapD</i> locus  | F | GCAAACCTTGCTTAAGCTCAAGATACTTGC  |
| 776 | <i>lapD</i> _100up     | Cloning at <i>lapD</i> locus  | F | CAATTGGCTGGGGACTCTTCGAGAC   |
| 777 | <i>lapD</i> _B         | <i>lapD</i> deletion          | R | GTATCTTGCATGCCTCTGACCTTGGAGTGCCTACTCATCATA<br>GCTAAC                  |
| 778 | <i>lapD</i> _C         | <i>lapD</i> deletion          | F | GTTAGCTATGATGAGTAGGCACTCCAAGGTCAGAGGCATGC<br>AAGATAC                  |
| 779 | <i>lapD</i> _100down   | Cloning at <i>lapD</i> locus  | R | GTAAGCCGTTGATCAGTGCTTCAGGAG   |
| 780 | <i>lapD</i> _2700down  | Cloning at <i>lapD</i> locus  | R | CTAACTACGCGCAGTATGTTGAGTTACAAGCG                                      |
| 781 | <i>lapD</i> _3000down  | Cloning at <i>lapD</i> locus  | R | CGTTCAAGCACAAGGCGATA TAGACG   |
| 784 | <i>lapDG</i> _B        | <i>lapDG</i> deletion         | R | GTATCTTGCATGCCTCTGACCTTGGAGGGCGGGCTATTCCC<br>TCAGCGCATTG              |
| 785 | <i>lapDG</i> _C        | <i>lapDG</i> deletion         | F | CAATGCGCTGAGGGAATAGCCCGCCCTCCAAGGTCAGAGG<br>CATGCAAGATAC              |
| 610 | <i>rocS</i> _3000up    | Cloning at <i>rocS</i> locus  | F | CAACTCGAGCTTTTCTACCAACCTCAG   |
| 611 | <i>rocS</i> _2700up    | Cloning at <i>rocS</i> locus  | F | GCATTTTACCGCCCCATTTTCGC   |
| 612 | <i>rocS</i> _100up     | Cloning at <i>rocS</i> locus  | F | CTTCAGGCCAAGATCCTTTTCTACTGTG  |
| 613 | <i>rocS</i> _B         | <i>rocS</i> deletion          | R | GGTTTCCACCAATCAGAGTAAAATTAACCCCTTAAAATACTA<br>CCAACTGTCCGTGCGCGACGACG |
| 614 | <i>rocS</i> _C         | <i>rocS</i> deletion          | F | CGTCGTCGCGCACGGACAGTTGGTAGTATTTAAGGGGTTA<br>ATTTACTCTGATTGGTGGAAACC   |

|     |                                 |  |   |   |
|-----|---------------------------------|--|---|---|
| 615 | <i>rocS_100down</i>             | Cloning at <i>rocS</i> locus   | R | GAAACCGATATAAACCGCATCGGCA   |
| 616 | <i>rocS_2700down</i>            | Cloning at <i>rocS</i> locus   | R | GTCACGTTATTAGGCTTGGCGTATTTTC  |
| 617 | <i>rocS_3000down</i>            | Cloning at <i>rocS</i> locus   | R | GCTGTTTGTTCACCTTAGGCTCG   |
| 533 | <i>vc1639_3000up</i>            | Cloning at <i>dbfS</i> locus   | F | GCTTAGTGATCGCAGAGCTTGC  |
| 534 | <i>vc1639_3000down</i>          | Cloning at <i>dbfS</i> locus   | R | GTGCACTGCATTATTGACTCGCTTAGC   |
| 535 | <i>vc1639_100up</i>             | Cloning at <i>dbfS</i> locus   | F | CAAGATTTTGACCGCGATTCCAATAC  |
| 536 | <i>vc1639_100down</i>           | Cloning at <i>dbfS</i> locus   | R | G TAGAGTTTCCAAACCTATAGGAG   |
| 626 | <i>vc1639_Real_B</i>            | <i>dbfS</i> deletion   | R | CAACTGAAAATCCGTTTTTGCACCGCATTTAATTGGCATGCA<br>ACTGATACCCAAG   |
| 627 | <i>vc1639_Real_C</i>            | <i>dbfS</i> deletion   | F | CTTGGGTATCAGTTGCATGCCAATTAATGCGGTGCAAAAAC<br>GGATTTTCAGTTG  |
| 559 | <i>vc1639_2700up</i>            | Cloning at <i>dbfS</i> locus   | F | CAATCGGTGGTGCACAACCTATCTGAG   |
| 560 | <i>vc1639_2700down</i>          | Cloning at <i>dbfS</i> locus   | R | GTTAATGACTTGGAGCAGAATTAAGTTAGCCGC   |
| 527 | <i>vc1638_3000up</i>            | Cloning at <i>dbfR</i> locus   | F | GTAGGTCTTCTCGCACTTGTGTTTTG  |
| 528 | <i>vc1638_3000down</i>          | Cloning at <i>dbfR</i> locus   | R | GTCCATAACCTTAGCGGAACTCATG   |
| 529 | <i>vc1638_100up</i>             | Cloning at <i>dbfR</i> locus   | F | GACAATCAAGTCTTTCGTGTCGAATACAAC  |
| 530 | <i>vc1638_100down</i>           | Cloning at <i>dbfR</i> locus   | R | CTTCCAGCAAATATTGATGGATGAGATTTGGG  |
| 628 | <i>vc1638_Real_B</i>            | <i>dbfR</i> deletion   | R | GAGATTTAATTGGCATGCAACTGATACCCAAGGTCTGCTCG<br>ATTATTTTTTGTATGGCAGC   |
| 629 | <i>vc1638_Real_C</i>            | <i>dbfR</i> deletion   | F | CGTGCCATCAAAAATAATCGAGCAGACCTTGGGTATCAGTT<br>GCATGCCAATTAATCTC  |
| 557 | <i>vc1638_2700up</i>            | Cloning at <i>dbfR</i> locus   | F | CACCATCCGGTTTGTGCATCATGATG  |
| 558 | <i>vc1638_2700down</i>          | Cloning at <i>dbfR</i> locus   | R | GTGGCGTCAGATCCCAAACTTGTTTC  |
| 650 | <i>dbfR_D51V_B</i>              | Generating <i>dbfR<sup>D51V</sup></i>  | R | CAATTCGGTAGGCCGAGTACGAGTACGATGACGTCC  |
| 651 | <i>dbfR_D51V_C</i>              | Generating <i>dbfR<sup>D51V</sup></i>  | F | GGACGTCATCGTACTCGTACTCGGCCTACCGAAATTG   |
| 736 | <i>dbfR_SNAP_delta_S_Gblock</i> | Gblock for generating <i>dbfR-SNAP</i> and simultaneously deleting <i>dbfS</i> | F | CGCGGTCTTGGGTATCAGTTGCATGCCAATTCAGGAAGCGG<br>CTCAGGCAGCGGATCAGGAATGGATAAGGATTGTGAAATGA<br>AGAGAACAACCTTAGATTCCCCACTAGGTAAT TAGAATTAT<br>CCGTTTGCGAACAGGATTACATCGTATTATATTTTTAGGAA<br>AAGGAACCAAGTGCAGCAGACGCGG TAGAAGTACCAGCCCC<br>CGCCGAGTTTTAGGAGGACCAGAACCCTAATGCAAGCCA<br>CCGCTTGGTTAAACGCATATTTTCATCAACCAGAAGCCA TAG<br>AAGAATCCCAGTACCAGCCCTACACCACCCAGTATTTCAAC<br>AAGAATCATTTACGAGACAAGTATTATGGAAATTAATAAAGT<br>CGTCAAATTCGGAGAAGTTATCAGCTATAGTCACCTAGCCG<br>CTCTTGCCGGTAATCCAGCAGCCACTGCCGAGTTAAAACC<br>GCATTATCAGGTAACCCAGTTCCCATATTAATTCCATGCCA T<br>AGAGTAGTACAAGGAGATT TAGACGTCGGCGGATATGAAGG<br>AGGTTTAGCAGTTAAGAATGGTTACTAGCACATGAAGGACA<br>TAGATTAGGTAACCAGGATTAGGTTAATGCGGTGCAAAAAC<br>CGGATTTTCAGTTGC |
| 734 | <i>dbfR_R</i>                   | Generating <i>dbfR-SNAP</i> and deleting <i>dbfS</i>                           | R | TTGGCATGCAACTGATACCCAAGACCGCG   |
| 735 | <i>dbfS_down_F</i>              | Generating <i>dbfR-SNAP</i> and deleting <i>dbfS</i>                           | F | ATGCGGTGCAAAAACGGATTTTCAGTTGC   |
| 672 | SNAP_UnivR                      | Generating <i>dbfR-SNAP</i>  | R | TTAACCTAATCCTGGTTTACCTAATCTATGTCTTCATGTGCT<br>AGTAACC   |

|     |  |   |   |  |
|-----|--|---|---|--|
| 718 | <i>dbfR</i> _SNAP_E                          | Generating <i>dbfR</i> -SNAP                    | F | GACA TAGATTAGGTAACCAGGATTAGGTTAAGATGTGATCA<br>AAACTGTGCGCGGTC            |
| 634 | <i>cdgl</i> _3000up                          | Cloning at <i>cdgl</i> locus                    | F | CGATGCAAGTAGCTGAACAAGCAC   |
| 635 | <i>cdgl</i> _2700up                          | Cloning at <i>cdgl</i> locus                    | F | GAATACATTGACGCCGAGCGCTTTG  |
| 636 | <i>cdgl</i> _100up                           | Cloning at <i>cdgl</i> locus                    | F | GGGAGCAACTTCACTGTATTCAATGAGTG  |
| 637 | <i>cdgl</i> _B                               | <i>cdgl</i> deletion                            | R | GATGCGATCATCATGAGCTACCTATTTTTGTAAAGGCCCGAC<br>TTCATTTTTTTCTACTCTC        |
| 638 | <i>cdgl</i> _C                               | <i>cdgl</i> deletion                            | F | GAGAGTAGAAAAAATGAAGTCGGGCCTTTACAAAAATAGG<br>TAGCTCATGATGATCGCATC         |
| 639 | <i>cdgl</i> _100down                         | Cloning at <i>cdgl</i> locus                    | R | GGTCAGCAGCTTTTGCAGCACTTTATTG   |
| 640 | <i>cdgl</i> _2700down                        | Cloning at <i>cdgl</i> locus                    | R | GAGGTGCAACCTGCGTGTAACTGGATTTTC   |
| 641 | <i>cdgl</i> _3000down                        | Cloning at <i>cdgl</i> locus                    | R | CCAGTGAGGCTATCAATATGCGCATC   |
| 642 | <i>cdgG</i> _3000up                          | Cloning at <i>cdgG</i> locus                    | F | GTGTCGATTCCAGCGACAAGTGCCAATTTG   |
| 643 | <i>cdgG</i> _2700up                          | Cloning at <i>cdgG</i> locus                    | F | GAATACACCGCAGAGCCGATAGTGAC   |
| 644 | <i>cdgG</i> _100up                           | Cloning at <i>cdgG</i> locus                    | F | GATAAATGCTGCCAGTCGGCATAAACACTGAG   |
| 645 | <i>cdgG</i> _B                               | <i>cdgG</i> deletion                            | R | GCACAAATTAATAGTTAATTAGCTTAAATATTAATCAGACTGG<br>ATAGTTGAGGATCAATCCTGATCC  |
| 646 | <i>cdgG</i> _C                               | <i>cdgG</i> deletion                            | F | GGATCAGGATTGATCCTCAACTATCCAGTCTGATTAATATTT<br>AAGCTAATTAATAATTTGTGC      |
| 647 | <i>cdgG</i> _100down                         | Cloning at <i>cdgG</i> locus                    | R | TTGAGGCCATGC TAGAGCATGATGTTGAGC  |
| 648 | <i>cdgG</i> _2700down                        | Cloning at <i>cdgG</i> locus                    | R | CCAGTAAATTCGGTTATGAGGTAAAGGATG   |
| 649 | <i>cdgG</i> _3000down                        | Cloning at <i>cdgG</i> locus                    | R | GATCGCCACTTTCCGCGATTGGATG  |
| 105 | BBC1881                                      | Cloning at <i>vc1807</i> locus                  | F | TTTAAAGGGGATCAGTGACCG  |
| 106 | BBC1882                                      | Cloning at <i>vc1807</i> locus                  | R | CAATTTTGCTTTTGACCATCCC   |
| 270 | <i>1807</i> _2700up                          | Cloning at <i>vc1807</i> locus                  | F | GGCCGGCACTTTGATTACAATC   |
| 271 | <i>1807</i> _2700down                        | Cloning at <i>vc1807</i> locus                  | R | GTCTATATCAGAGCGCTTAAAGAGCG   |
| 721 | <i>P<sub>BAD</sub></i> _1807_Univ_B          | Generating <i>P<sub>BAD</sub></i> - <i>dbfS</i> | R | CATTTACACCTCCTGCAGGTAC   |
| 722 | <i>P<sub>BAD</sub></i> - <i>dbfS</i> -1807_C | Generating <i>P<sub>BAD</sub></i> - <i>dbfS</i> | F | GTACCTGCAGGAGGTGTGAAATGGGTATCAGTTGCATGCCA<br>ATTAATCTCG                  |
| 723 | <i>P<sub>BAD</sub></i> - <i>dbfS</i> -1807_D | Generating <i>P<sub>BAD</sub></i> - <i>dbfS</i> | R | GTCGACGGATCCCCGGAATTTAATGGATTTGACGGCTTTG<br>GCTG                         |
| 232 | ABD123                                       | Generating <i>P<sub>BAD</sub></i> - <i>dbfS</i> | F | ATTCCGGGGATCCGTCGAC  |
| 729 | <i>P<sub>BAD</sub></i> - <i>lapG</i> -1807_C | Generating <i>P<sub>BAD</sub></i> - <i>lapG</i> | R | GTACCTGCAGGAGGTGTGAAATGAAACGTTGGATTGTGCTG<br>TCTCTGG                     |
| 730 | <i>P<sub>BAD</sub></i> - <i>lapG</i> -1807_D | Generating <i>P<sub>BAD</sub></i> - <i>lapG</i> | F | GTCGACGGATCCCCGGAATCTACTCATCATAGCTAAC TAGA<br>GG                         |
| 731 | <i>P<sub>BAD</sub></i> - <i>rbmB</i> -1807_C | Generating <i>P<sub>BAD</sub></i> - <i>rbmB</i> | R | GTACCTGCAGGAGGTGTGAAATGCTGTTATACTTAAATCAAT<br>TCAATAAAGAGGGTGG           |
| 732 | <i>P<sub>BAD</sub></i> - <i>rbmB</i> -1807_D | Generating <i>P<sub>BAD</sub></i> - <i>rbmB</i> | F | GTCGACGGATCCCCGGAATTCATCTTTAATAAAGTGCTGTA<br>TATAAATGGTCCG               |
| 587 | <i>tagA</i> _3000up                          | Cloning at <i>tagA</i> locus                    | F | GGGCTGCAAGAAGCTGGATCTGCTAC   |
| 588 | <i>tagA</i> _2700up                          | Cloning at <i>tagA</i> locus                    | F | GAGCAAATTACAAGCTCGATCTCAGCTAAG   |
| 662 | <i>tagA</i> _103bpD_B                        | Removes first 103 codons of                     | R | GTCAAATACTGGTCGTTACTGGATGTTGCATTCTTTAACAAA<br>AAAATAAAGACAAGGGAAACGTATTG |

|            |                       | <i>tagA</i> including start                             |   |  |
|------------|-----------------------|---|---|--|
| <b>663</b> | <i>tagA</i> _103bpD_C | Removes first 103 codons of <i>tagA</i> including start | F | CAATACGTTTCCCTTGTCTTTATTTTTTTGTTAAAGAATGCAA<br>CATCCAGTAACGACCAGTATTTGAC |
| <b>591</b> | <i>tagA</i> _2700down | Cloning at <i>tagA</i> locus                            | R | CCACCGAGGATACCATCCATCTTGATAAATATG  |
| <b>592</b> | <i>tagA</i> _3000down | Cloning at <i>tagA</i> locus                            | R | CTCTTGCCATCCATATGACATGATGTCTTTTG   |
| <b>593</b> | <i>tagA</i> _100up    | Cloning at <i>tagA</i> locus                            | F | GTGTGGCTTCATCCATTGACCTCCAATG   |
| <b>594</b> | <i>tagA</i> _100down  | Cloning at <i>tagA</i> locus                            | R | CCACTGCGAAATTAATTTTAGGATCAGCTTTAGC                                       |
| <b>664</b> | <i>tagA</i> _150down  | Cloning at <i>tagA</i> locus                            | R | GCAACCATACATCTTCCATTACTACCATAAGAG  |