**Supplementary materials**

**The small RNA, SdsR, acts as a novel type of toxin in *Escherichia coli***

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|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **No.** | **Gene name** | **Fold change (pHM4T vs pSdsR)a** | **No.** | **Gene name** | **Fold change (pHM4T vs pSdsR)a** | **No.** | **Gene name** | **Fold change (pHM4T vs pSdsR)a** |
| **1** | *aphAb* | -4.39 | **41** | *moaBb* | -2.12 | **81** | *fdnIb* | -1.58 |
| **2** | *flub* | -4.36 | **42** | *ccmFb* | -2.12 | **82** | *yahA* | -1.57 |
| **3** | *narIb* | -3.94 | **43** | *moaEb* | -2.09 | **83** | *rapA* | -1.56 |
| **4** | *isrCb* | -3.80 | **44** | *suhBb* | -2.08 | **84** | *cysMb* | -1.56 |
| **5** | *narGb* | -3.74 | **45** | *moaDb* | -2.08 | **85** | *ydgKb* | -1.55 |
| **6** | *ygiLb* | -3.72 | **46** | *moaCb* | -2.08 | **86** | *purM* | -1.51 |
| **7** | *narHb* | -3.72 | **47** | *xanP* | -2.06 | **87** | *tamA* | -1.50 |
| **8** | *napFb* | -3.62 | **48** | *nikBb* | -2.03 | **88** | *glmZ* | -1.49 |
| **9** | *narJb* | -3.54 | **49** | *sgrS* | -2.03 | **89** | *ybjGb* | -1.49 |
| **10** | *napBb* | -3.47 | **50** | *ydgI* | -2.02 | **90** | *nikD* | -1.48 |
| **11** | *narKb* | -3.43 | **51** | *iadA* | -2.00 | **91** | *purK* | -1.48 |
| **12** | *napAb* | -3.43 | **52** | *ybjEb* | -1.99 | **92** | *yliF* | -1.46 |
| **13** | *napHb* | -3.37 | **53** | *dusBb* | -1.98 | **93** | *yliE* | -1.46 |
| **14** | *nirBb* | -3.33 | **54** | *dsbEb* | -1.96 | **94** | *allB* | -1.45 |
| **15** | *napGb* | -3.23 | **55** | *purE* | -1.94 | **95** | *garP* | -1.44 |
| **16** | *napDb* | -3.18 | **56** | *pyrLb* | -1.92 | **96** | *rnk* | -1.43 |
| **17** | *yeeRb* | -3.18 | **57** | *ccmHb* | -1.92 | **97** | *rsxB* | -1.43 |
| **18** | *yfcC* | -2.94 | **58** | *fdnGb* | -1.91 | **98** | *nrfB* | -1.42 |
| **19** | *napCb* | -2.92 | **59** | *nirCb* | -1.89 | **99** | *gcvB* | -1.42 |
| **20** | *plaPb* | -2.87 | **60** | *rimOb* | -1.89 | **100** | *fimB* | -1.41 |
| **21** | *nirDb* | -2.74 | **61** | *fisb* | -1.89 | **101** | *nrfC* | -1.41 |
| **22** | *ccmAb* | -2.72 | **62** | *garRb* | -1.89 | **102** | *yidD* | -1.40 |
| **23** | *ycgX* | -2.70 | **63** | *infA* | -1.88 | **103** | *potA* | -1.40 |
| **24** | *borDb* | -2.68 | **64** | *mgrR* | -1.85 | **104** | *adkb* | -1.39 |
| **25** | *tolC* | -2.68 | **65** | *nhaBb* | -1.85 | **105** | *yfhH* | -1.39 |
| **26** | *yeeSb* | -2.58 | **66** | *pyrDb* | -1.83 | **106** | *cvpA* | -1.38 |
| **27** | *rzpDb* | -2.51 | **67** | *ygiQ* | -1.82 | **107** | *rpsTb* | -1.37 |
| **28** | *yfaZb* | -2.47 | **68** | *ydiY* | -1.81 | **108** | *ycaM* | -1.37 |
| **29** | *rzoDb* | -2.42 | **69** | *mgtAb* | -1.80 | **109** | *rphb* | -1.37 |
| **30** | *garKb* | -2.40 | **70** | *nikC* | -1.77 | **110** | *nikE* | -1.37 |
| **31** | *ccmCb* | -2.38 | **71** | *yhcB* | -1.71 | **111** | *gptb* | -1.37 |
| **32** | *stpAb* | -2.38 | **72** | *fnrS* | -1.66 | **112** | *treB* | -1.36 |
| **33** | *ccmBb* | -2.32 | **73** | *yegQ* | -1.64 | **113** | *rpsUb* | -1.35 |
| **34** | *dcuAb* | -2.30 | **74** | *nrfA* | -1.64 | **114** | *lysP* | -1.34 |
| **35** | *garLb* | -2.28 | **75** | *artI* | -1.64 | **115** | *yiaF* | -1.33 |
| **36** | *moaAb* | -2.23 | **76** | *malT* | -1.64 | **116** | *aptb* | -1.32 |
| **37** | *ccmDb* | -2.22 | **77** | *flhCb* | -1.63 |  |  |  |
| **38** | *ghxP* | -2.15 | **78** | *fdnHb* | -1.63 |  |  |  |
| **39** | *nikAb* | -2.15 | **79** | *ansPb* | -1.60 |  |  |  |
| **40** | *ccmEb* | -2.13 | **80** | *yhbE* | -1.59 |  |  |  |

**Table S1. RNA-seq analysis of mRNAs downregulated over two-fold in cells subjected to pulse expression of SdsR**

aFold changes for each mRNA are in log2 scale.

bGenes shared with SdsR(45-103).

**Table S2. RNA-seq analysis of mRNAs upregulated over two-fold in cells subjected to pulse expression of SdsR**

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **No.** | **Gene name** | **Fold change (pHM4T vs pSdsR)a** | **No.** | **Gene name** | **Fold change (pHM4T vs pSdsR)a** | **No.** | **Gene name** | **Fold change (pHM4T vs pSdsR)a** |
| **1** | *bsmA* | 1.30 | **43** | *ybgA* | 1.62 | **85** | *fadB* | 2.66 |
| **2** | *tdcD* | 1.30 | **44** | *prpC* | 1.62 | **86** | *glcC* | 2.79 |
| **3** | *fadE* | 1.30 | **45** | *yfeK* | 1.63 | **87** | *putP* | 2.83 |
| **4** | *srlE* | 1.32 | **46** | *yqeB* | 1.65 | **88** | *astC* | 2.83 |
| **5** | *yjiM* | 1.33 | **47** | *dppC* | 1.66 | **89** | *hmpb* | 2.87 |
| **6** | *srlA* | 1.35 | **48** | *rseA* | 1.66 | **90** | *mhpR* | 2.93 |
| **7** | *ybdD* | 1.35 | **49** | *yigI* | 1.68 | **91** | *acs* | 3.06 |
| **8** | *yabI* | 1.37 | **50** | *asnAb* | 1.69 | **92** | *insI1b* | 3.23 |
| **9** | *yjiL* | 1.37 | **51** | *yjfNb* | 1.71 | **93** | *phoH* | 3.43 |
| **10** | *ydcJ* | 1.39 | **52** | *feaR* | 1.76 |  |  |  |
| **11** | *ytfEb* | 1.39 | **53** | *sstT* | 1.78 |  |  |  |
| **12** | *dtpB* | 1.39 | **54** | *dppB* | 1.78 |  |  |  |
| **13** | *ydhVb* | 1.41 | **55** | *yqeC* | 1.80 |  |  |  |
| **14** | *yibA* | 1.41 | **56** | *csiD* | 1.80 |  |  |  |
| **15** | *dcuBb* | 1.41 | **57** | *cstA* | 1.81 |  |  |  |
| **16** | *dppF* | 1.41 | **58** | *paaJ* | 1.82 |  |  |  |
| **17** | *galS* | 1.42 | **59** | *fadH* | 1.82 |  |  |  |
| **18** | *fucO* | 1.42 | **60** | *lldP* | 1.83 |  |  |  |
| **19** | *puuD* | 1.42 | **61** | *rpoE* | 1.85 |  |  |  |
| **20** | *glgSb* | 1.42 | **62** | *ynfHb* | 1.85 |  |  |  |
| **21** | *lsrC* | 1.43 | **63** | *ynfFb* | 1.86 |  |  |  |
| **22** | *dsdX* | 1.44 | **64** | *aldA* | 1.91 |  |  |  |
| **23** | *ydhT* | 1.44 | **65** | *ydcI* | 1.91 |  |  |  |
| **24** | *ydhWb* | 1.44 | **66** | *ynfGb* | 1.92 |  |  |  |
| **25** | *paaK* | 1.44 | **67** | *dppDb* | 1.92 |  |  |  |
| **26** | *lsrA* | 1.49 | **68** | *ymgE* | 1.94 |  |  |  |
| **27** | *ygeV* | 1.49 | **69** | *yjiHb* | 1.97 |  |  |  |
| **28** | *tdcB* | 1.49 | **70** | *yqaE* | 2.03 |  |  |  |
| **29** | *ytfQ* | 1.50 | **71** | *yghO* | 2.04 |  |  |  |
| **30** | *yciF* | 1.50 | **72** | *paaA* | 2.09 |  |  |  |
| **31** | *betI* | 1.51 | **73** | *hcaR* | 2.09 |  |  |  |
| **32** | *glcD* | 1.52 | **74** | *frvB* | 2.10 |  |  |  |
| **33** | *gadE* | 1.54 | **75** | *argT* | 2.13 |  |  |  |
| **34** | *gltI* | 1.55 | **76** | *putA* | 2.14 |  |  |  |
| **35** | *ybhQ* | 1.55 | **77** | *hcpb* | 2.20 |  |  |  |
| **36** | *srlMb* | 1.55 | **78** | *dppA* | 2.23 |  |  |  |
| **37** | *ydhUb* | 1.57 | **79** | *ugpB* | 2.35 |  |  |  |
| **38** | *aldB* | 1.59 | **80** | *prpR* | 2.37 |  |  |  |
| **39** | *astA* | 1.59 | **81** | *cycA* | 2.40 |  |  |  |
| **40** | *ydhXb* | 1.60 | **82** | *paaB* | 2.44 |  |  |  |
| **41** | *ygiM* | 1.60 | **83** | *hcrb* | 2.48 |  |  |  |
| **42** | *rsd* | 1.61 | **84** | *yahC* | 2.54 |  |  |  |

aFold changes for each mRNA are in log2 scale.

bGenes shared with SdsR(45-103).

**Table S3. RNA-seq analysis of mRNAs downregulated over two-fold in cells subjected to pulse expression of SdsR(45-103)**

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **No.** | **Gene name** | **Fold change [pHM4T vs p(45-103)]a** | **No.** | **Gene name** | **Fold change [pHM4T vs p(45-103)]a** | **No.** | **Gene name** | **Fold change [pHM4T vs p(45-103)]a** |
| **1** | *flub* | -4.55 | **44** | *recR* | -2.28 | **87** | *cysMb* | -1.51 |
| **2** | *cysU* | -4.53 | **45** | *cnu* | -2.24 | **88** | *cysK* | -1.48 |
| **3** | *insA* | -4.52 | **46** | *garKb* | -2.24 | **89** | *plaPb* | -1.47 |
| **4** | *cysW* | -4.28 | **47** | *ccmEb* | -2.22 | **90** | *mgtAb* | -1.46 |
| **5** | *isrCb* | -4.26 | **48** | *flhCb* | -2.19 | **91** | *nikAb* | -1.45 |
| **6** | *yeeE* | -4.24 | **49** | *grxA* | -2.16 | **92** | *intG* | -1.44 |
| **7** | *cysP* | -4.07 | **50** | *nagE* | -2.13 | **93** | *wzzB* | -1.42 |
| **8** | *cysA* | -4.00 | **51** | *nirBb* | -2.12 | **94** | *fdnHb* | -1.42 |
| **9** | *napBb* | -3.83 | **52** | *garLb* | -2.11 | **95** | *gptb* | -1.42 |
| **10** | *cysJ* | -3.77 | **53** | *ygiLb* | -2.10 | **96** | *yajQ* | -1.41 |
| **11** | *cysD* | -3.62 | **54** | *cysH* | -2.09 | **97** | *yicG* | -1.41 |
| **12** | *narHb* | -3.62 | **55** | *ccmDb* | -2.06 | **98** | *nhaBb* | -1.41 |
| **13** | *narIb* | -3.56 | **56** | *suhBb* | -2.04 | **99** | *borDb* | -1.39 |
| **14** | *napFb* | -3.51 | **57** | *rpsTb* | -2.00 | **100** | *yshB* | -1.38 |
| **15** | *iraM* | -3.49 | **58** | *ccmFb* | -1.98 | **101** | *leuE* | -1.38 |
| **16** | *yeeRb* | -3.45 | **59** | *flhD* | -1.97 | **102** | *fliE* | -1.37 |
| **17** | *narGb* | -3.45 | **60** | *acrS* | -1.92 | **103** | *gudX* | -1.37 |
| **18** | *napAb* | -3.44 | **61** | *dsbEb* | -1.92 | **104** | *ves* | -1.35 |
| **19** | *napDb* | -3.38 | **62** | *ccmHb* | -1.89 | **105** | *pyrLb* | -1.34 |
| **20** | *napHb* | -3.37 | **63** | *ansPb* | -1.84 | **106** | *fisb* | -1.34 |
| **21** | *ydjO* | -3.27 | **64** | *moaDb* | -1.84 | **107** | *yeeP* | -1.34 |
| **22** | *narJb* | -3.22 | **65** | *moaBb* | -1.84 | **108** | *yjiA* | -1.33 |
| **23** | *napGb* | -3.17 | **66** | *ybaB* | -1.82 | **109** | *yajG* | -1.33 |
| **24** | *napCb* | -3.04 | **67** | *lamB* | -1.78 | **110** | *gsiC* | -1.32 |
| **25** | *narKb* | -3.02 | **68** | *adkb* | -1.76 | **111** | *gsiA* | -1.32 |
| **26** | *aphAb* | -2.91 | **69** | *gsiB* | -1.75 | **112** | *folA* | -1.31 |
| **27** | *csgF* | -2.90 | **70** | *moaEb* | -1.73 | **113** | *ybjGb* | -1.30 |
| **28** | *cysC* | -2.79 | **71** | *moaCb* | -1.70 | **114** | *fdnIb* | -1.30 |
| **29** | *ryeA* | -2.76 | **72** | *moaAb* | -1.69 | **115** | *rzpDb* | -1.30 |
| **30** | *ccmAb* | -2.75 | **73** | *stpAb* | -1.68 | **116** | *pyrDb* | -1.29 |
| **31** | *cysN* | -2.74 | **74** | *hfq* | -1.67 | **117** | *ndk* | -1.28 |
| **32** | *yciW* | -2.67 | **75** | *ydgKb* | -1.63 | **118** | *fdnGb* | -1.27 |
| **33** | *cysI* | -2.67 | **76** | *nirCb* | -1.63 | **119** | *rsxA* | -1.27 |
| **34** | *yeeD* | -2.62 | **77** | *osmB* | -1.61 | **120** | *yijD* | -1.27 |
| **35** | *yfaZb* | -2.55 | **78** | *cbl* | -1.59 | **121** | *rnk* | -1.27 |
| **36** | *yeeSb* | -2.54 | **79** | *yoaG* | -1.58 | **122** | *queD* | -1.25 |
| **37** | *ydjN* | -2.53 | **80** | *yfbO* | -1.58 | **123** | *tdk* | -1.24 |
| **38** | *dsbA* | -2.42 | **81** | *sbp* | -1.58 | **124** | *aptb* | -1.22 |
| **39** | *icd* | -2.42 | **82** | *emrK* | -1.58 | **125** | *rimP* | -1.22 |
| **40** | *ccmBb* | -2.39 | **83** | *garRb* | -1.53 | **126** | *rpsUb* | -1.21 |
| **41** | *ccmCb* | -2.37 | **84** | *rzoDb* | -1.52 | **127** | *dcuAb* | -1.21 |
| **42** | *nirDb* | -2.36 | **85** | *yncE* | -1.52 | **128** | *yniC* | -1.20 |
| **43** | *sfmH* | -2.32 | **86** | *yedN* | -1.51 | **129** | *narL* | -1.19 |
| **No.** | **Gene name** | **Fold change [pHM4T vs p(45-103)a** |
| **130** | *ybjEb* | -1.18 |
| **131** | *rimOb* | -1.17 |
| **132** | *rarD* | -1.15 |
| **133** | *nikBb* | -1.15 |
| **134** | *rphb* | -1.14 |
| **135** | *ymdA* | -1.13 |
| **136** | *mepS* | -1.12 |
| **137** | *rsxB* | -1.12 |
| **138** | *dusBb* | -1.12 |
| **139** | *slmA* | -1.08 |

aFold changes for each mRNA are in log2 scale.

bGenes shared with SdsR.

**Table S4. RNA-seq analysis of mRNAs upregulated over two-fold in cells subjected to pulse expression of SdsR(45-103)**

|  |  |  |
| --- | --- | --- |
| **No.** | **Gene name** | **Fold change [pHM4T vs p(45-103)]a** |
| **1** | *glgSb* | 1.08 |
| **2** | *dppD* | 1.12 |
| **3** | *ycbJ* | 1.15 |
| **4** | *yjfNb* | 1.15 |
| **5** | *blc* | 1.18 |
| **6** | *fumB* | 1.22 |
| **7** | *yagJ* | 1.22 |
| **8** | *raiA* | 1.23 |
| **9** | *asnAb* | 1.26 |
| **10** | *tdcA* | 1.28 |
| **11** | *ytfEb* | 1.30 |
| **12** | *ydhWb* | 1.30 |
| **13** | *ydhUb* | 1.32 |
| **14** | *ydhY* | 1.35 |
| **15** | *hyi* | 1.35 |
| **16** | *ydhXb* | 1.38 |
| **17** | *srlMb* | 1.41 |
| **18** | *ydhVb* | 1.53 |
| **19** | *ybiY* | 1.54 |
| **20** | *yjiHb* | 1.71 |
| **21** | *tfaR* | 1.74 |
| **22** | *dcuBb* | 1.79 |
| **23** | *ynfE* | 1.93 |
| **24** | *ynfHb* | 2.08 |
| **25** | *ynfGb* | 2.26 |
| **26** | *ynfFb* | 2.29 |
| **27** | *hmpb* | 2.38 |
| **28** | *insI1b* | 2.74 |
| **29** | *hcpb* | 2.88 |
| **30** | *hcrb* | 2.99 |

aFold changes for each mRNA are in log2 scale.

bGenes shared with SdsR.

**Table S5. Validation of putative SdsR-targeted mRNAs predicted from RNA-seq data by qRT-PCR**

|  |  |  |  |
| --- | --- | --- | --- |
| **Gene** | **RNA-seq fold regulationa** | **qRT-PCRa** | **Description** |
| *adk* | -1.39 | -0.69 | Adenylate kinase |
| *aphA* | -4.39 | -1.89 | Acid phosphatase/phosphotransferase |
| *crp* | -1.15 | -1.32 | DNA-binding transcriptional dual regulator CRP |
| *mutS* | -1.03 | -0.92 | DNA mismatch repair protein MutS |
| *nhaB* | -1.85 | -1.16 | Sodium: H+ antiporter |
| *stpA* | -2.38 | -0.61 | H-NS-like DNA-binding transcriptional repressor with RNA chaperone activity |
| *tolC* | -2.68 | -1.56 | Outer membrane channel TolC |
| *yhcB* | -1.71 | -0.95 | Inner membrane protein YhcB |

aFold changes for each mRNA are in log2 scale.

**Table S6. Strains and plasmids used in this study**

|  |  |  |
| --- | --- | --- |
| **Name** | **Description** | **Source** |
| *Strains* |  |  |
| MG1655 | *E. coli* MG1655 wild type | Laboratory stock |
| BW25113 | *E. coli* BW25113 wild type | Laboratory stock |
| ∆*hfq* | BW25113 *Δhfq::kanR* | Keio collection 1 |
| MG1655*ΔlacZ* | MG1655 *ΔlacZ::kanR* | This study |
| ∆*yhcB* | MG1655 *ΔyhcB::kanR* | This study |
| MG1655Δ*ryeA/sdsR* | MG1655Δ*ryeA/sdsR::kanR* | This study |
| SDF204(*rnc*+*)* | W3110 *rnc+, TD1-17::*Tn*10* | 2 |
| SDF205(*rnc*-*)* | W3110 *rnc-, TD1-17::*Tn*10* | 2 |
| BL21-Gold(DE3) | Chemically competent *E. coli* BL21 cells to obtain high-level of Hfq protein | Invitrogen |
| PM1205 | MG1655 *mal::lacIq, DaraBAD araC+, lacI'::PBAD-cat-sacB-lacZ, mini λ tetR* | 3 |
| JS201 | PM1205 lacI’∷PBAD-yhcB-lacZ | This study |
| JS202 | JS201 with a +19 C to G mutation in the *yhcB* coding sequence | This study |
|  |  |  |
|  |  |  |
|  |  |  |
|  |  |  |
| *Plasmids* |  |  |
| pHM4T | A derivative of pHM1, AmpR, IPTG-inducible RNA expression vector | 4 |
| pSdsR | A pHM4T derivative carrying the full *sdsR* sequence | This study |
| pRyeA | A pHM4T derivative carrying the full *ryeA* sequence but a -10 promoter mutation (TATGGT🡪CTCGAG) on *sdsR* for inhibiting *sdsR* expression | This study |
| p(29-103) | A pHM4T derivative carrying the 29-103 sequence of *sdsR* | This study |
| p(37-103) | A pHM4T derivative carrying the 37-103 sequence of *sdsR* | This study |
| p(45-103) | A pHM4T derivative carrying the 45-103 sequence of *sdsR* | This study |
| p(53-103) | A pHM4T derivative carrying the 53-103 sequence of sdsR | This study |
| pΔ(59-68) | A pHM4T derivative carrying the 28-58::69-103 sequence of *sdsR* | This study |
| pΔ(49-58) | A pHM4T derivative carrying the 28-48::59-103 sequence of *sdsR* | This study |
| pΔ(39-48) | A pHM4T derivative carrying the 28-38::49-103 sequence of *sdsR* | This study |
| p(1-68T) | A pHM4T derivative carrying the 1-68 sequence of *sdsR* and the terminator sequence of *sibC* | This study |
| p(29-68T) | A pHM4T derivative carrying the 29-68 sequence of *sdsR* and the terminator sequence of *sibC* | This study |
| pM(29-33) | A p29-68T derivative carrying a 5-nt complementary mutation in the 29-33 sequence (AACTT🡪TTGAA) | This study |
| pM(34-38) | A p29-68T derivative carrying a 5-nt complementary mutation in the 34-38 sequence (TTAGC🡪AATCG) | This study |
| pM(39-43) | A p29-68T derivative carrying a 5-nt complementary mutation in the 39-43 sequence (CGACG🡪GCTGC) | This study |
| pM(44-48) | A p29-68T derivative carrying a 5-nt complementary mutation in the 44-48 sequence (GCTCT🡪CGAGA) | This study |
| pM(49-53) | A p29-68T derivative carrying a 5-nt complementary mutation in the 49-53 sequence (CTCCC🡪GAGGG) | This study |
| pM(54-58) | A p29-68T derivative carrying a 5-nt complementary mutation in the 54-58 sequence (AAGAG🡪TTCTC) | This study |
| pM(59-63) | A p29-68T derivative carrying a 5-nt complementary mutation in the 59-63 sequence (CCATT🡪GGTAA) | This study |
| pM(64-68) | A p29-68T derivative carrying a 5-nt complementary mutation in the 64-68 sequence (TCCCT🡪AGGGA) | This study |
| pSdsRm | A pSdsR derivative carrying a G to C mutation at position 37 | This study |
| pARtolC | A pHM4T derivative expressing an afsRNA 5 that can repress *tolC* expression | This study |
| pARgpt | A pHM4T derivative expressing an afsRNA 5 that can repress *gpt* expression | This study |
| pARyhcB | A pHM4T derivative expressing an afsRNA 5 that can repress *yhcB* expression | This study |
| pARnhaB | A pHM4T derivative expressing an afsRNA 5 that can repress *nhaB* expression | This study |
| pARdcuA | A pHM4T derivative expressing an afsRNA 5 that can repress *ducA* expression | This study |
| pARtamA | A pHM4T derivative expressing an afsRNA 5 that can repress *tamA* expression | This study |
| pARinfA | A pHM4T derivative expressing an afsRNA 5 that can repress *infA* expression | This study |
| pAKA | A derivative of pACYC184, TcR, IPTG-inducible RNA expression vector | 4 |
| pASdsR | A pAKA derivative carrying the full *sdsR* sequence | This study |
| pET21b-Hfq | A pET21b derivative expressing Hfq | 6 |
|  |  |  |

**Table S7. Oligonucleotides used in this study**

|  |  |  |
| --- | --- | --- |
| **Name** | **Sequence (5’ to 3’)** | **Used for** |
| SdsRFw | CGG AAT TCG GCA AGG CAA CTA AGC | Cloning of pSdsR |
| SdsRRv | GCT CTA GAA AAA AGA GAC CGA AC | Cloning of pSdsR |
| 29-103Fw | CGG AAT TCA ACT TTT AGC GCA CG | Cloning of p(29-103) |
| 37-103Fw | CGG AAT TCG CGC ACG GCT CTC TC | Cloning of p(37-103) |
| 45-103Fw | CGG AAT TCC TCT CTC CCA AGA GC | Cloning of p(45-103) |
| 53-103Fw | CGG AAT TCC AAG AGC CAT TTC CCT | Cloning of p(53-103) |
| Δp1 | CGG TCA GTT TTA CCT GAT GT | Cloning of pΔ(59-68), pΔ(49-58), and pΔ(39-48) |
| Δp2 | CGC TAC TGC CGC CAG GCA AA | Cloning of pΔ(59-68), pΔ(49-58), and pΔ(39-48) |
| Δ(59-68)Fw | ACG ATT CCT GTA TTC GGT CCC TCT TGG GAG AGA GCC GTG C | Cloning of pΔ(59-68) |
| Δ(59-68)Rv | GGA CCG AAT ACA GGA ATC GT | Cloning of pΔ(59-68) |
| Δ(49-58)Fw | TAT TCG GTC CAG GGA AAT GGA GAG CCG TGC GCT AAA AGT T | Cloning of pΔ(49-58) |
| Δ(49-58)Rv | CCA TTT CCC TGG ACC GAA TA | Cloning of pΔ(49-58) |
| Δ(39-48)Fw | AGG GAA ATG GCT CTT GGG AGG CTA AAA GTT GAA TTC ATT A | Cloning of pΔ(39-48) |
| Δ(39-48)Rv | CTC CCA AGA GCC ATT TCC CT | Cloning of pΔ(39-48) |
| T(sibC)Fw | GCT CTA GAG GTA AAG CCC TCA CCG AAG CGA GGG CAG GGA AAT GGC TCT TGG GAG | Cloning of p(1-68T) and p(23-69T) |
| M(29-33)Fw | GAT AAC AAT ATA ATG AAT TCT TGA ATT AGC  GCA CGG CTC TCT CC | Cloning of pM(29-33) |
| M(29-33)Rv | GGA GAG AGC CGT GCG CTA ATT CAA GAA TTC  ATT ATA TTG TTA TC | Cloning of pM(29-33) |
| M(34-38)Fw | CAA TAT AAT GAA TTC AAC TTA ATC GGC ACG  GCT CTC TCC CAA GAG | Cloning of pM(34-38) |
| M(34-38)Rv | CTC TTG GGA GAG AGC CGT GCC GAT TAA GTT GAA TTC ATT ATA TTG | Cloning of pM(34-38) |
| M(39-43)Fw | TAA TGA ATT CAA CTT TTA GCC GTG CGC TCT  CTC CCA AGA GCC | Cloning of pM(39-43) |
| M(39-43)Rv | GGC TCT TGG GAG AGA GCG CAC GGC TAA AAG TTG AAT TCA TTA | Cloning of pM(39-43) |
| M(44-48)Fw | AAT TCA ACT TTT AGC GCA CGC GAG ACT CCC  AAG AGC CAT TTC CC | Cloning of pM(44-48) |
| M(44-48)Rv | GGG AAA TGG CTC TTG GGA GTC TCG CGT GCG CTA AAA GTT GAA TT | Cloning of pM(44-48) |
| M(49-53)Fw | CTT TTA GCG CAC GGC TCT GAG GGA AGA GCC ATT TCC CTG | Cloning of pM(49-53) |
| M(49-53)Rv | CAG GGA AAT GGC TCT TCC CTC AGA GCC GTG CGC TAA AAG | Cloning of pM(49-53) |
| M(54-58)Fw | CAC GGC TCT CTC CCT TCT CCC ATT TCC CTG  CCC | Cloning of pM(54-58) |
| M(54-58)Rv | GGG CAG GGA AAT GGG AGA AGG GAG AGA GCC GTG | Cloning of pM(54-58) |
| M(59-63)Fw | CTC TCT CCC AAG AGG GTA ATC CCT GCC CTC  GC | Cloning of pM(59-63) |
| M(59-63)Rv | GCG AGG GCA GGG ATT ACC CTC TTG GGA GAG AG | Cloning of pM(59-63) |
| M(64-68)Fw | CTC CCA AGA GCC ATT AGG GAG CCC TCG CTT  CGG TG | Cloning of pM(64-68) |
| M(64-68)Rv | CAC CGA AGC GAG GGC TCC CTA ATG GCT CTT  GGG AG | Cloning of pM(64-68) |
| RyeAFw | CGG AAT TCA AAG TCA GCG AAG GAA ATG C | Cloning of pRyeA |
| RyeARv | GCT CTA GAG CGA AGA GGT GAT GCT G | Cloning of pRyeA |
| SdsR\_pAKAFw | CCG TGA CGT CGG CAA GGC AAC TAA | Cloning of pASdsR |
| SdsR\_pAKARv | CCG TAA GC TTC AGC GAA GGA AAT GC | Cloning of pASdsR |
| IVT\_SdsRFw | TAA TAC GAC TCA CTA TAG GGC AAG GCA ACT AAG CCT GCA T | *In vitro* transcription of SdsR |
| IVT\_SdsRRv | AAA AAG AGA CCG AAC ACG ATT CCT G | *In vitro* transcription of SdsR |
| IVT\_RyeAFw | TAA TAC GAC TCA CTA TAG GGA AAG TCA GCG AAG GAA ATG C | *In vitro* transcription of RyeA |
| IVT\_RyeARv | AAA AAC CGC CTC AGT TCT TTC AC | *In vitro* transcription of RyeA |
| IVT\_29-103Fw | TAA TAC GAC TCA CTA TAG GAA CTT TTA GCG CAC GGC TCT | *In vitro* transcription of 29-103 |
| IVT\_37-103Fw | TAA TAC GAC TCA CTA TAG GGC GCA CGG CTC TCT CCC AAG | *In vitro* transcription of 37-103 |
| IVT\_45-103Fw | TAA TAC GAC TCA CTA TAG GCT CTC TCC CAA GAG CCA TTT | *In vitro* transcription of 45-103 |
| SdsR\_np | GAG ACC GAA CAC GAT TCC TGC G | Northern probe for SdsR |
| SdsR\_np2 | GGC TCT TGG GAG AGA GCC GTC G | Northern probe for SdsR |
| RyeA\_np | CAG CTG ATG ACC ACC A | Northern probe for RyeA 7 |
| RyeA\_np2 | CGT GTT CGG TCT CTT TTT ATC TG | Northern probe for RyeA |
| T(sibC)\_np | AGG GAA ATG GCT CTT GGG AG | Northern probe for *sibC* terminator |
| ARgptFw | TGT GAA GTG TCC CAG CCT GT | ARgpt cloning into pHM4T derivative 8 |
| ARgptRv | ACA GGC TGG GAC ACT TCA CA | ARgpt cloning into pHM4T derivative8 |
| ARyhcBFw | TGA ACA TCT CCC GTT GTC TT | ARyhcB cloning into pHM4T derivative8 |
| ARyhcBRv | AAG ACA ACG GGA GAT GTT CA | ARyhcB cloning into pHM4T derivative8 |
| ARnhaBFw | TGA ACA TCT CCC GTT GTC TT | ARnhaB cloning into pHM4T derivative8 |
| ARnhaBRv | AAG ACA ACG GGA GAT GTT CA | ARnhaB cloning into pHM4T derivative8 |
| ARdcuAFw | TAT TAG CCT TCC TTG TTT TT | ARdcuA cloning into pHM4T derivative8 |
| ARdcuARv | AAA AAC AAG GAA GGC TAA TA | ARdcuA cloning into pHM4T derivative8 |
| ARtamAFw | CAT TTT CTC CTG AAT ATC CT | ARtamA cloning into pHM4T derivative8 |
| ARtamARv | AGG ATA TTC AGG AGA AAA TG | ARtamA cloning into pHM4T derivative8 |
| gapAFw | GCA CCA CCA ACT GCC TGG CT | qPCR primer for *gapA* 9 |
| gapARv | CGC CGC GCC AGT CTT TGT GA | qPCR primer for *gapA* 9 |
| tolCFw | TAG TAA CCC GGA ATT GCG TAA G | qPCR primer for *tolC* |
| tolCRv | AGC CGT TGC TAT AGG TGT AAT C | qPCR primer for *tolC* |
| yhcBFw | GGT TAG TCG TCG GCA TCA TTA | qPCR primer for *yhcB* |
| yhcBRv | AAG TGG CTA ACC AGC TCT TC | qPCR primer for *yhcB* |
| adkFw | GCC GTC TGG TCG TGT TTA T | qPCR primer for *adk* |
| adkRv | GGT GCT GTC ATC TGA TGG TAT T | qPCR primer for *adk* |
| aphAFw | GCA AGA TCA CAC AGG CAA TC | qPCR primer for *aphA* |
| aphARv | CGA CCG AAA CCC AAT GAA TG | qPCR primer for *aphA* |
| crpFw | CCA TCC AAG AGC ACG CTT AT | qPCR primer for *crp* |
| crpRv | CCC TGA TTC AGA TAG GAG AGG A | qPCR primer for *crp* |
| mutSFw | AAG CAC TGG CAC TGG AAA | qPCR primer for *mutS* |
| mutSRv | GCA GGT GTA GTT CAG GGT ATA G | qPCR primer for *mutS* |
| nhaBFw | TGG CTA TCG AAG CGG TAT TC | qPCR primer for *nhaB* |
| nhaBRv | GCT TAA CAG CAA ACG GGT AAA T | qPCR primer for *nhaB* |
| stpAFw | CGA ATT CTC CAT TGA CGT TCT TG | qPCR primer for *stpA* |
| stpARv | GTG CAG CAG CAG AGC TAT TA | qPCR primer for *stpA* |
| ΔRyeABFw | ATA GCC TTT TGA TTT CCA ATA AAA AAA CCG CCT CAG TTC TTG TAG GCT GGA GCT GCT TCG | Constructing *ΔryeA/sdsR* |
| ΔRyeABRv | ATA AAC GGG CGC AGG CTG TTT CAT CAA TAT TTT CCT CCC GAT TCC GGG GAT CCG TCG ACC | Constructing *ΔryeA/sdsR* |
| ΔyhcBFw | GCC AAC ACC CTA CGG AAA CAA AAG ACA ACG  GGA GAT GTT CTG TAG GCT GGA GCT GCT TCG | Constructing *ΔyhcB* |
| ΔyhcBRv | AGA GAA GAG GAG GGC GCA ATG GCT GCG CCC  GAA AAA TAA AAT TCC GGG GAT CCG TCG ACC | Constructing *ΔyhcB* |

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**Figure S1. Cell death by SdsR can occur without lysis.** Ectopic SdsR expression was induced with 1 mM IPTG at a growth time point of 1 h. Cells grown for 8 h were diluted 1:10 with fresh LB medium or spent LB medium. (A) Lysis of pSdsR-containing cells was tested by reading OD600 after 10 min from the dilution. (B) Cell viability was assessed by measuring cfu per OD600 of the diluted cells. Mean ± SD; n = 3, \**P* ≤ 0.01, \*\**P* ≤ 0.001 by Student’s t-test.

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**Figure S2. Effects of the deletion of *sdsR/ryeA* gene on SdsR-driven growth defects.**

Lysis (A) and cell viability (B) were assessed using the *sdsR/ryeA* deletion mutant (*ΔsdsR/ryeB*) cells as in Fig. S1 (mean ± SD; n = 3, \**P* ≤ 0.05, \*\**P* ≤ 0.001 by Student’s t-test).

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**Figure S3. Half-lives of SdsR and RyeA.** (A) Total cellular RNA was prepared from cells at the indicated times after rifampicin treatment. Rifampicin was added to 4-h grown cultures (E) for RyeA and 8 h grown cultures (S) for SdsR. Cellular levels of SdsR and RyeA were measured using Northern blot analysis. 5S rRNA was detected as a loading control. The membrane was probed with an anti-RyeA oligonucleotide and analyzed for RyeA signals. Then the membrane was briefly washed and reprobed with an anti-SdsR oligonucleotide. The remaining RyeA signals in the anti-SdsR probed membrane were indicated by asterisks. (B) RNA levels are presented relative to that in cells right after rifampicin treatment (0 min).

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**Figure S4. Secondary structure models of SdsR(37-103) and SdsR(45-103).** The 45-60 sequences in SdsR are boxed in blue.

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