

## **Supplementary Material**

Accessibility and conservation in bacterial small RNA-mRNA interactions and implications for genome-wide target predictions

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2

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| sRNA   | Target      | sRNA site | Target site | Interaction                                                                       | Validation                                         | Reference |
|--------|-------------|-----------|-------------|-----------------------------------------------------------------------------------|----------------------------------------------------|-----------|
| OmrB   | <i>cirA</i> | 2 – 24    | -35 – -10   | ((((((((((((...(((((((&)))))))))...)).))))).)))                                   | compensatory mutations                             | [13]      |
| OmrB   | <i>csgD</i> | 2 – 20    | -79 – -61   | (((((((((((((((((((((&)))))))))...))))))                                          | compensatory mutations                             | [14]      |
| OmrB   | <i>ompR</i> | 1 – 19    | -29 – -11   | (((((((((...((...(((&)))...)).))))))                                              | compensatory mutations                             | [13]      |
| OmrB   | <i>ompT</i> | 1 – 32    | -12 – 20    | (((((((((...((...(((((((...(((((&<br>)))))...))))).)).))))))                      | compensatory mutations                             | [13]      |
| OxyS   | <i>fhlA</i> | 22 – 30   | 34 – 42     | (((((((((&)))))))))                                                               | compensatory mutations                             | [1]       |
|        |             | 98 – 104  | -15 – -9    | (((((((&))))))                                                                    |                                                    |           |
| RprA   | <i>rpoS</i> | 33 – 62   | -117 – -94  | (((((...((((((.....(((((((&)))))))))...))))).))))))                               | compensatory mutations                             | [18]      |
| RyhB   | <i>cysE</i> | 34 – 46   | -4 – 9      | (((((((((((((((((&)))))))))                                                       | compensatory mutations                             | [32]      |
| RyhB   | <i>iscS</i> | 40 – 68   | -26 – 3     | (((((((((.....(((((((&)))))))).))))))                                             | <i>in vitro</i> probing                            | [9]       |
| RyhB   | <i>fur</i>  | 38 – 76   | -96 – -47   | (((((((((...((...(((((((...(((((((&<br>)))...))))).)).)...)).)...)).))))))        | compensatory mutations                             | [38]      |
| RyhB   | <i>shiA</i> | 44 – 55   | -59 – -48   | (((((((((((((&)))))))))                                                           | compensatory mutations                             | [28]      |
| RyhB   | <i>sodB</i> | 38 – 46   | -4 – 5      | (((((((((&)))))))))                                                               | <i>in vitro</i> probing                            | [12]      |
| SgrS   | <i>manX</i> | 159 – 172 | 24 – 37     | ((...(((((((((((&)))))))))...))                                                   | compensatory mutations                             | [31]      |
| SgrS   | <i>ptsG</i> | 168 – 187 | -28 – -9    | (((((...(((((((...(((((((&)))))))))...))))                                        | compensatory mutations,<br><i>in vitro</i> probing | [15, 31]  |
| Spot42 | <i>galK</i> | 20 – 61   | -19 – 21    | (((((...(((((((((((...((((....((((...(((((((&<br>)))))))))...))))).))))))         | <i>in vitro</i> probing                            | [21]      |
| Spot42 | <i>gltA</i> | 4 – 13    | -131 – -122 | (((((((((((((&)))))))))                                                           | sRNA mutations                                     | [3]       |
| Spot42 | <i>nanC</i> | 1 – 17    | -33 – -16   | (((((((((((((((((((((&))).)))))))))                                               | compensatory mutations                             | [3]       |
| Spot42 | <i>srlA</i> | 20 – 34   | -15 – -1    | (((((...(((((((((((&)))))))))...))))                                              | compensatory mutations                             | [3]       |
| Spot42 | <i>sthA</i> | 48 – 55   | 15 – 22     | (((((((((&)))))))))                                                               | compensatory mutations                             | [3]       |
| Spot42 | <i>xyIF</i> | 1 – 33    | 2 – 40      | ((...((((...(((((((...(((((((...(((((((&<br>)))))...)))))))).)... . )))))).))))). | sRNA mutations                                     | [3]       |

**Table S2:** Data set of interactions in *Salmonella* used in this study. Target interaction site positions are given relative to the annotated translation start. Interactions are given in bracket notation, where the “&” symbol concatenates the sRNA with its target, matching brackets represent base pairs between the two sequences and dots represent unpaired positions.

| sRNA | Target      | sRNA site | Target site | Interaction                                                       | Validation              | Reference |
|------|-------------|-----------|-------------|-------------------------------------------------------------------|-------------------------|-----------|
| ArcZ | <i>sdaC</i> | 62 – 71   | -13 – -3    | (((((((((((&)))))).))))))                                         | compensatory mutations  | [25]      |
| ArcZ | STM3216     | 63 – 87   | -25 – -5    | (((((((((.(((....(((((&)))))).))))))))))                          | compensatory mutations  | [25]      |
| ArcZ | <i>tpx</i>  | 66 – 83   | 10 – 26     | (((((((. .... ((((&)))) . .... )))))                              | compensatory mutations  | [25]      |
| ChiX | <i>chbC</i> | 35 – 55   | -66 – -46   | (((((((((.(((((((((((((&)))))))))))).)))))                        | mRNA mutations          | [11]      |
| ChiX | <i>chiP</i> | 42 – 53   | -19 – -8    | (((((((((((((((&)))))))))))))                                     | compensatory mutations  | [11]      |
| CyaR | <i>ompX</i> | 35 – 66   | -30 – -3    | ((..(((((((((((.((((....((((..((((&                               | compensatory mutations  | [24]      |
|      |             |           |             | )))..)))..))..))..))..))..))..))..))                              | compensatory mutations  | [24]      |
| GcvB | <i>argT</i> | 75 – 91   | -57 – -42   | (((((((((.(((((((((((((&)))))))))))))))))                         | <i>in vitro</i> probing | [34]      |
| GcvB | <i>cycA</i> | 72 – 85   | -34 – -19   | (((((((((((((.(&).)).)))))))).))                                  | <i>in vitro</i> probing | [35]      |
|      |             | 138 – 161 | -24 – -8    | (((((((((. .... ((((((((((((&))))))))))))))))))                   |                         |           |
| GcvB | <i>dppA</i> | 65 – 82   | -30 – -14   | (((((((((.(((((((((((((&))))))))))))))))))                        | <i>in vitro</i> probing | [34]      |
| GcvB | <i>gltI</i> | 65 – 76   | -38 – -27   | (((((((((((((((&)))))))))))))                                     | <i>in vitro</i> probing | [34]      |
| GcvB | <i>livJ</i> | 63 – 87   | -51 – -28   | (((((((((.(((((((.(((((((((((((&)))))).)))))))))))).))))))        | <i>in vitro</i> probing | [34]      |
| GcvB | <i>livK</i> | 65 – 77   | -29 – -17   | (((((((((((((((((((&))))))))))))))                                | <i>in vitro</i> probing | [34]      |
| GcvB | <i>oppA</i> | 65 – 89   | -8 – 16     | (((((((((((((..(((((((((((((&)))))))))))).))..))))))              | <i>in vitro</i> probing | [34]      |
| GcvB | STM4351     | 69 – 79   | -54 – -43   | (((((((((((((((&).))))))))))                                      | <i>in vitro</i> probing | [34]      |
| InvR | <i>ompD</i> | 33 – 42   | 56 – 65     | (((((((((((((((&))))))))))                                        | <i>in vitro</i> probing | [27]      |
| MicA | <i>lamB</i> | 8 – 36    | -9 – 18     | (((((((((.((((((((((.(((((((((((((&)))))))))).)))))))).))..)))))) | compensatory mutations  | [4]       |
| MicC | <i>ompD</i> | 1 – 12    | 67 – 78     | (((((((((((((((((((&))))))))))))))                                | compensatory mutations  | [26]      |
| RybB | <i>chiP</i> | 1 – 7     | 12 – 18     | (((((((((((&))))))))                                              | compensatory mutations  | [2]       |
| RybB | <i>fadL</i> | 1 – 8     | 49 – 56     | (((((((((((&))))))))                                              | compensatory mutations  | [23]      |
| RybB | <i>ompA</i> | 1 – 13    | 21 – 32     | (((((((((..(((&).))..))))))                                       | compensatory mutations  | [23]      |
| RybB | <i>ompC</i> | 1 – 10    | -50 – -41   | (((((((((((((((&))))))))))                                        | <i>in vitro</i> probing | [2, 23]   |
| RybB | <i>ompD</i> | 1 – 9     | 18 – 26     | (((((((((((((((&))))))))))                                        | compensatory mutations  | [2, 23]   |
|      |             | 1 – 10    | 10 – 20     | (((((((((((((((&).))..))))))                                      |                         |           |
| RybB | <i>ompF</i> | 1 – 9     | -46 – -38   | (((((((((((((((&))))))))))                                        | compensatory mutations  | [23]      |
| RybB | <i>ompN</i> | 1 – 16    | 5 – 20      | (((((.(((((((((((((((((&)))))))))))).))))                         | compensatory mutations  | [5]       |
| RybB | <i>ompS</i> | 1 – 14    | 7 – 20      | (((((..(((((((((((((((((&)))))))))).))))                          | sRNA deletion mutant    | [23]      |

(Continued)

| sRNA | Target      | sRNA site | Target site | Interaction                       | Validation             | Reference |
|------|-------------|-----------|-------------|-----------------------------------|------------------------|-----------|
| RybB | <i>ompW</i> | 1 – 13    | 3 – 20      | ((((((((.&)))))).....))))))       | compensatory mutations | [23]      |
| RybB | <i>tsx</i>  | 1 – 16    | -26 – -7    | ((((((((...(((.&)))))).....)))))) | compensatory mutations | [23]      |

**Table S3:** Organisms used in this study and the respective NCBI RefSeq database genome accession numbers.

| Organism                                 | RefSeq genome accession number |
|------------------------------------------|--------------------------------|
| <i>Citrobacter koseri</i>                | NC_009792                      |
| <i>Citrobacter rodentium</i>             | NC_013716                      |
| <i>Cronobacter sakazakii</i>             | NC_009778                      |
| <i>Enterobacter</i> sp. 638              | NC_009436                      |
| <i>Escherichia coli</i>                  | NC_000913                      |
| <i>Escherichia fergusonii</i>            | NC_011740                      |
| <i>Klebsiella pneumoniae</i>             | NC_009648                      |
| <i>Pectobacterium carotovorum</i>        | NC_012917                      |
| <i>Photorhabdus luminescens</i>          | NC_005126                      |
| <i>Proteus mirabilis</i>                 | NC_010554                      |
| <i>Salmonella typhimurium</i>            | NC_003197                      |
| <i>Salmonella enterica</i> serovar Typhi | NC_003198                      |
| <i>Serratia proteamaculans</i>           | NC_009832                      |
| <i>Shigella boydii</i>                   | NC_007613                      |
| <i>Shigella dysenteriae</i>              | NC_007606                      |
| <i>Shigella flexneri</i>                 | NC_004337                      |
| <i>Shigella sonnei</i>                   | NC_007384                      |
| <i>Sodalis glossinidius</i>              | NC_007712                      |
| <i>Yersinia enterocolitica</i>           | NC_008800                      |
| <i>Yersinia pestis</i>                   | NC_003143                      |
| <i>Yersinia pseudotuberculosis</i>       | NC_006155                      |

## Supplementary References

- [1] L. Argaman and S. Altuvia. *fhlA* repression by OxyS RNA: kissing complex formation at two sites results in a stable antisense-target RNA complex. *Journal of Molecular Biology*, 300(5):1101–12, 2000.
- [2] R. Balbontín, F. Fiorini, N. Figueroa-Bossi, J. Casadesús, and L. Bossi. Recognition of heptameric seed sequence underlies multi-target regulation by RybB small RNA in *Salmonella enterica*. *Mol Microbiol*, 78(2):380–94, 2010.
- [3] C. L. Beisel and G. Storz. The base-pairing RNA Spot 42 participates in a multioutput feedforward loop to help enact catabolite repression in *Escherichia coli*. *Mol Cell*, 41(3):286–97, 2011.
- [4] L. Bossi and N. Figueroa-Bossi. A small RNA downregulates LamB maltoporin in *Salmonella*. *Mol Microbiol*, 65(3):799–810, 2007.
- [5] M. Bouvier, C. M. Sharma, F. Mika, K. H. Nierhaus, and J. Vogel. Small RNA binding to 5' mRNA coding region inhibits translational initiation. *Mol Cell*, 32(6):827–37, 2008.
- [6] S. Chen, A. Zhang, L. B. Blyn, and G. Storz. MicC, a second small-RNA regulator of Omp protein expression in *Escherichia coli*. *J Bacteriol*, 186(20):6689–97, 2004.
- [7] A. Coornaert, A. Lu, P. Mandin, M. Springer, S. Gottesman, and M. Guillier. MicA sRNA links the PhoP regulon to cell envelope stress. *Mol Microbiol*, 76(2):467–79, 2010.
- [8] N. De Lay and S. Gottesman. The Crp-activated small noncoding regulatory RNA CyaR (RyeE) links nutritional status to group behavior. *J Bacteriol*, 191(2):461–76, 2009.
- [9] G. Desnoyers, A. Morissette, K. Prévost, and E. Massé. Small RNA-induced differential degradation of the polycistronic mRNA *iscRSUA*. *EMBO J*, 28(11):1551–61, 2009.
- [10] S. Durand and G. Storz. Reprogramming of anaerobic metabolism by the FnrS small RNA. *Mol Microbiol*, 75(5):1215–31, 2010.
- [11] N. Figueroa-Bossi, M. Valentini, L. Malleret, F. Fiorini, and L. Bossi. Caught at its own game: regulatory small RNA inactivated by an inducible transcript mimicking its target. *Genes Dev*, 23(17):2004–15, 2009.
- [12] T. A. Geissmann and D. Touati. Hfq, a new chaperoning role: binding to messenger RNA determines access for small RNA regulator. *EMBO J*, 23(2):396–405, 2004.
- [13] M. Guillier and S. Gottesman. The 5' end of two redundant sRNAs is involved in the regulation of multiple targets, including their own regulator. *Nucleic Acids Research*, 36(21):6781–94, 2008.
- [14] E. Holmqvist, J. Reimegård, M. Sterk, N. Grantcharova, U. Römling, and E. G. H. Wagner. Two antisense RNAs target the transcriptional regulator CsgD to inhibit curli synthesis. *EMBO J*, 29(11):1840–50, 2010.
- [15] H. Kawamoto, Y. Koide, T. Morita, and H. Aiba. Base-pairing requirement for RNA silencing by a bacterial small RNA and acceleration of duplex formation by Hfq. *Mol Microbiol*, 61(4):1013–22, 2006.
- [16] R. A. Lease, M. E. Cusick, and M. Belfort. Riboregulation in *Escherichia coli*: DsrA RNA acts by RNA:RNA interactions at multiple loci. *Proc. Natl. Acad. Sci. USA*, 95(21):12456–61, 1998.
- [17] N. Majdalani, C. Cunniff, D. Sledjeski, T. Elliott, and S. Gottesman. DsrA RNA regulates translation of RpoS message by an anti-antisense mechanism, independent of its action as an antisilencer of transcription. *Proc. Natl. Acad. Sci. USA*, 95(21):12462–7, 1998.
- [18] N. Majdalani, D. Hernandez, and S. Gottesman. Regulation and mode of action of the second small RNA activator of RpoS translation, RprA. *Mol Microbiol*, 46(3):813–26, 2002.
- [19] P. Mandin and S. Gottesman. A genetic approach for finding small RNAs regulators of genes of interest identifies RybC as regulating the DpiA/DpiB two-component system. *Mol Microbiol*, 72(3):551–65, 2009.
- [20] P. Mandin and S. Gottesman. Integrating anaerobic/aerobic sensing and the general stress response through the ArcZ small RNA. *EMBO J*, 29(18):3094–107, 2010.
- [21] T. Møller, T. Franch, C. Udesen, K. Gerdes, and P. Valentin-Hansen. Spot 42 RNA mediates discoordinate expression of the *E. coli* galactose operon. *Genes Dev*, 16(13):1696–706, 2002.
- [22] M. Overgaard, J. Johansen, J. Møller-Jensen, and P. Valentin-Hansen. Switching off small RNA regulation with trap-mRNA. *Mol Microbiol*, 73(5):790–800, 2009.
- [23] K. Papenfort, M. Bouvier, F. Mika, C. M. Sharma, and J. Vogel. Evidence for an autonomous 5' target recognition domain in an Hfq-associated small RNA. *Proc. Natl. Acad. Sci. USA*, 107(47):20435–40, 2010.

- [24] K. Papenfort, V. Pfeiffer, S. Lucchini, A. Sonawane, J. C. D. Hinton, and J. Vogel. Systematic deletion of *Salmonella* small RNA genes identifies CyaR, a conserved CRP-dependent riboregulator of OmpX synthesis. *Mol Microbiol*, 68(4):890–906, 2008.
- [25] K. Papenfort, N. Said, T. Welsink, S. Lucchini, J. C. D. Hinton, and J. Vogel. Specific and pleiotropic patterns of mRNA regulation by ArcZ, a conserved, Hfq-dependent small RNA. *Mol Microbiol*, 74(1):139–58, 2009.
- [26] V. Pfeiffer, K. Papenfort, S. Lucchini, J. C. D. Hinton, and J. Vogel. Coding sequence targeting by MicC RNA reveals bacterial mRNA silencing downstream of translational initiation. *Nat Struct Mol Biol*, 16(8):840–6, 2009.
- [27] V. Pfeiffer, A. Sittka, R. Tomer, K. Tedin, V. Brinkmann, and J. Vogel. A small non-coding RNA of the invasion gene island (SPI-1) represses outer membrane protein synthesis from the *Salmonella* core genome. *Mol Microbiol*, 66(5):1174–91, 2007.
- [28] K. Prévost, H. Salvail, G. Desnoyers, J.-F. Jacques, É. Phaneuf, and E. Massé. The small RNA RyhB activates the translation of *shiA* mRNA encoding a permease of shikimate, a compound involved in siderophore synthesis. *Mol Microbiol*, 64(5):1260–73, 2007.
- [29] S. C. Pulvermacher, L. T. Stauffer, and G. V. Stauffer. The small RNA GcvB regulates *sstT* mRNA expression in *Escherichia coli*. *J Bacteriol*, 191(1):238–48, 2009.
- [30] A. A. Rasmussen, J. Johansen, J. S. Nielsen, M. Overgaard, B. Kallipolitis, and P. Valentin-Hansen. A conserved small RNA promotes silencing of the outer membrane protein YbfM. *Mol Microbiol*, 72(3):566–77, 2009.
- [31] J. B. Rice and C. K. Vanderpool. The small RNA SgrS controls sugar-phosphate accumulation by regulating multiple PTS genes. *Nucleic Acids Research*, 39(9):3806–19, 2011.
- [32] H. Salvail, P. Lanthier-Bourbonnais, J. M. Sobota, M. Caza, J.-A. M. Benjamin, M. E. S. Mendieta, F. Lépine, C. M. Dozois, J. Imlay, and E. Massé. A small RNA promotes siderophore production through transcriptional and metabolic remodeling. *Proc. Natl. Acad. Sci. USA*, 107(34):15223–8, 2010.
- [33] M. Schmidt, P. Zheng, and N. Delihias. Secondary structures of *Escherichia coli* antisense *micF* RNA, the 5'-end of the target *ompF* mRNA, and the RNA/RNA duplex. *Biochemistry*, 34(11):3621–31, 1995.
- [34] C. M. Sharma, F. Darfeuille, T. H. Plantinga, and J. Vogel. A small RNA regulates multiple ABC transporter mRNAs by targeting C/A-rich elements inside and upstream of ribosome-binding sites. *Genes Dev*, 21(21):2804–17, 2007.
- [35] C. M. Sharma, K. Papenfort, S. R. Pernitzsch, H.-J. Mollenkopf, J. C. D. Hinton, and J. Vogel. Pervasive post-transcriptional control of genes involved in amino acid metabolism by the Hfq-dependent GcvB small RNA. *Mol Microbiol*, 2011. doi:10.1111/j.1365-2958.2011.07751.x.
- [36] K. I. Udekwi, F. Darfeuille, J. Vogel, J. Reimegård, E. Holmqvist, and E. G. H. Wagner. Hfq-dependent regulation of OmpA synthesis is mediated by an antisense RNA. *Genes Dev*, 19(19):2355–66, 2005.
- [37] J. H. Urban and J. Vogel. Two seemingly homologous noncoding RNAs act hierarchically to activate *glmS* mRNA translation. *PLoS Biol*, 6(3):e64, 2008.
- [38] B. Večerek, I. Moll, and U. Bläsi. Control of Fur synthesis by the non-coding RNA RyhB and iron-responsive decoding. *EMBO J*, 26(4):965–75, 2007.