SUPPLEMENTAL MATERIALS

Poly(A) polymerase is required for RyhB sRNA stability and function in *Escherichia coli*

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Running title: Poly(A) polymerase promotes sRNA stability

Key words: Poly(A) polymerase, *pcnB*, Hfq, small RNAs, RNase E
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SUPPLEMENTAL MATERIALS AND METHODS
### TABLE S1. Strains and Plasmid

| Strains or plasmids | Relevant features | Reference or Source |
|---------------------|-------------------|---------------------|
| **Strains**         |                   |                     |
| MG1655 rph-1        |                   | (Blattner et al. 1997) |
| KR10000 MG1655 rph’|                   | D. Court, NCI       |
| NM600T rph-1 ΔlacZ mini tet lambda | | N. Majdalani, NCI |
| EM1238 rph-1 ΔlacX74 ΔryhB::cat | | (Masse et al. 2003) |
| EM1377 rph-1 ΔlacX74 me-131 zce-726::Tn10 | | (Masse et al. 2003) |
| JW3617 rph-1 Δ(araD-araB)567 Δ(rhaD-rhaB)568 ΔlacZ4787 (:rrnB-3) hsdR514 ΔpyrE::kan | | (Baba et al. 2006) |
| JW4130 rph-1 Δ(araD-araB)567 Δ(rhaD-rhaB)568 ΔlacZ4787 (:rrnB-3) hsdR514 Δhfq::kan | | (Baba et al. 2006) |
| JW5808 rph-1 Δ(araD-araB)567 Δ(rhaD-rhaB)568 ΔlacZ4787 (:rrnB-3) hsdR514 ΔpcnB::kan | | (Baba et al. 2006) |
| DJS2812 rph-1 ΔlacX74 mal::lacI ΔaraBAD Δhfq::cat-sacB ΔpurA::kan | | (Schu et al. 2015) |
| DJS2817 rph-1 ΔlacX74 mal::lacI ΔaraBAD hfqQ8A | | (Schu et al. 2015) |
| DJS2820 rph-1 ΔlacX74 mal::lacI ΔaraBAD hfqR17A | | (Schu et al. 2015) |
| DJS2864 rph-1 ΔlacX74 mal::lacI ΔaraBAD hfqY25D | | (Schu et al. 2015) |
| TC279 rph-1 ΔcyaR ΔmicA::zeo ΔryhB::micA | | (Cameron and De Lay 2016) |
| NM600T rph-1 ΔlacZ mini-λ tet | | N. Majdalani, NCI |
| NM1200 rph-1 mini-Δcm (cat) | | N. Majdalani, NCI |
| NRD463 rph-1 Δpnp::cat | | (De Lay and Gottesman 2011) |
| NRD473 rph-1 ΔlacX74 mal::lacI Δpnp::kan | | (De Lay and Gottesman 2011) |
| NRD576 rph-1 ΔlacX74 mal::lacI Δpnp::kan me-131 zce-726::Tn10 | | NRD473 + P1 (EM1377) |
| NRD698 rph-1 ΔlacZ ΔpcnB::cat | | This study |
| NRD999 rph-1 Δpnp::cat | | KR10000 + P1 (NRD463) |
| NRD1038 λ rph-1 ΔryhB::kan-pBAD-ccdB mini-λ tet | | This study |
| NRD1138 rph-1 | | (Cameron and De Lay 2016) |
| NRD1198 rph-1 ΔpcnB::cat | | NRD1138 + P1 (NRD698) |
| NRD1243 rph-1 Δpnp-3XFLAG | | (Bandrya et al. 2016) |
| NRD1362 rph-1 Δfur::zeo | | This study |
| NRD1410 rph-1 hfqY25D | | DS057 + P1 (DJS2864) |
| NRD1455 rph-1 ΔmalQ::nptII | | NRD1138 + P1 (JW3379) |
| NRD1530 rph-1 ΔasnB::tet | | This study |
| NRD1533 rph-1 ΔasnB::tet Δfur::zeo | | NRD1530 + P1 (NRD1362) |
| Strain | Description | Notes |
|--------|-------------|-------|
| NRD1546 | rph-1 ΔryhB::cat Δfur::zeo | DS132 + P1(NRD1533) |
| NRD1547 | rph-1 ΔpcnB::kan ΔryhB::cat Δfur::zeo | DS135 + P1(NRD1533) |
| NRD1550 | rph-1 me -131 zce-726::Tn10 ΔryhB::cat Δfur::zeo ΔpcnB::kan | DS137 + P1(NRD1362) |
| NRD1551 | rph-1 me -131 zce-726::Tn10 ΔryhB::cat | DS137 + P1(JW5808) |
| NRD1576 | rph-1 Δfur::leuZ3'ETS-kan | This study |
| NRD1577 | rph-1 Δfur::leuZ3'ETS G113C C114G G120C C121G-kan | This study |
| NRD1579 | rph-1 ΔpcnB::cat leuZ3'ETS-kan | NRD1138 + P1(NRD1576) |
| NRD1580 | rph-1 ΔpcnB::cat leuZ3'ETS G113C C114G G120C C121G-kan | NRD1138 + P1(NRD1578) |
| DS021 | rph-1 ΔhfpQ::kan | NRD1138 + P1(JW4130) |
| DS024 | rph-1 Δfur::zeo | NRD1138 + P1(NRD1362) |
| DS025 | rph-1 ΔpcnB::cat Δfur::zeo | NRD1198 + P1(NRD1362) |
| DS027 | rph-1 ΔhfpQ::kan Δfur::zeo | DS021 + P1(NRD1362) |
| DS057 | rph-1 ΔhfpQ::cat-sacB ΔpurA::kan | NRD1138 + P1(DJS2812) |
| DS058 | rph-1 hfpQ8A | DS057 + P1(DJS2817) |
| DS060 | rph-1 hfpR17A | DS057 + P1(DJS2820) |
| DS071 | rph-1 ΔpcnB::kan | NRD1138 + P1(JW5808) |
| DS072 | rph-1 hfpQ8A ΔpcnB::cat | DS058 + P1(NRD1198) |
| DS073 | rph-1 hfpR17A ΔpcnB::cat | DS060 + P1(NRD1198) |
| DS090 | rph-1 ΔhfpQ::kan me-131 zce-726::Tn10 | DS024 + P1(EM1377) |
| DS082 | rph-1 ΔhfpQ::kan me-131 zce-726::Tn10 | DS025 + X P1(EM1377) |
| DS089 | rph-1 ΔpurA Δmca::zeo ΔryhB::micA ΔpcnB::kan | NRD1243 + P1(JW3617) |
| DS090 | rph-1 ΔpurA Δmca::zeo ΔryhB::micA ΔpcnB::kan | DS089 + P1(MG1655) |
| DS102 | rph-1 ΔpurA Δmca::zeo ΔryhB::micA ΔpcnB::kan | DS090 + P1(NRD1198) |
| DS106 | rph-1 ΔpurA Δmca::zeo ΔryhB::micA ΔpcnB::kan | NRD1138 + P1(EM1377) |
| DS120 | rph-1 ΔpurA Δmca::zeo ΔryhB::micA ΔpcnB::kan | NRD1138 + P1(EM1377) |
| DS130 | rph-1 ΔpurA Δmca::zeo ΔryhB::micA ΔpcnB::kan | NRD1138 + P1(EM1377) |
| DS132 | rph-1 ΔpurA Δmca::zeo ΔryhB::micA ΔpcnB::kan | NRD1138 + P1(EM1377) |
| DS135 | rph-1 ΔpurA Δmca::zeo ΔryhB::micA ΔpcnB::kan | DS071 + P1(EM1328) |
| DS137 | rph-1 ΔpurA Δmca::zeo ΔryhB::micA ΔpcnB::kan | DS102 + P1(EM1238) |
| DS153 | rph-1 ΔpurA Δmca::zeo ΔryhB::micA ΔpcnB::kan | DS130 + P1(NRD1362) |
| DS185 | rph-1 hfpY25D ΔpcnB::cat | NRD1410 + P1(NRD1198) |
| LM06 | rph-1 ryhB G44C C45G | This study |
| LM10 | rph-1 ΔmalQ ΔlacX74 ΔryhB::cat | NRD1455 + P1(EM1238) |
| LM11 | rph-1 ryhB G44C C45G | LM10 + P1(LM06) |
| LM13 | rph-1 ryhB G44C C45G ΔpcnB::cat | LM11 + P1(NRD1198) |

**Plasmid**

- **pKD3**
  - Amp', Cm'; oriR6K; cat cassette flanked by FRT sites
  - (Datsenko and Wanner 2000)

- **pKD4**
  - Amp', Kan'; oriR6K; kan cassette
  - (Datsenko and Wanner 2000)
### TABLE S2. Primers and Probes

| Probes          | Sequence (5’ - 3’)                                                                 |
|-----------------|-----------------------------------------------------------------------------------|
| RyhB            | AAGTAATACTGGAAGCAATGTGAGCAATGTCGTGCTTTGAGTTCTC                                   |
| RyhB-LM         | GTCGTGCTTTTCAGGTTTCTCCGCGAGGGTCTTTCC                                             |
| MicA            | CCAAAATTTTCATCTCTGATCCAGGGATGATGATGATAAACAAATG                                   |
| GcvB            | CCAGAACACGATTCCCAGTAAAAACTTTGCTTTCCGGCTCAGG                                      |
| CyaR            | TGGTTCTCGGTACAGCTAGCATTTTATGCGTTGATG                                          |
| ChiX            | CATTTTTTTTATTATTATCGCGTCATTAAAGCGACCGTG                                          |
| MgrR            | CGAACTCAAAAACACTAGCGATTAAAGCGACCGTTCAGG                                         |
| SsrA            | GCGCCACTAAACAAACTAGCGATTAAAGCGACCGTTCAGG                                         |
| 5S              | ATGGGGTCTAGGGAGGACCACCGCGCTACTGC                                                 |
| sodB            | TGCAGTAGTCGATTCAAATAAGCGTGTTCCACGACATCAAAC                                       |
| sodB 5' - UTR   | GGTAGTGCGAGTTAATGCGACATTGCATCTACTCTCC                                            |
| uof             | GCTCTTGGAGATAATGCGTATCATTATAAGCGACCGC                                            |
| ompA            | CCATTTTGTGTTATGAAAAACAGTTATCGATCTAGGAGCCACCAGC                                   |
| ompX            | TACGGAACTCTCGGTGAAAAACGACCGTGGCA                                                  |
| 3'ETS<sub>LeuZ</sub> | CCACCCGAAGGGTTGTTTACGAGCAGCAGTCTCAGTTAAGGGTGTTT                                    |
| LeuZ term       | AAAAACACCCGAAGGTTGTTTACGAGCAGCAGCAGTCTCAGTTAAGGGTGTTTAAAAAACCCCGCAGGTTGTTTACGAGCAGC |
| 16S             | CCTGTTACGCTGACTG                                                              |

#### qRT-PCR primers

| qsdhC For       | CGATAGCGTCCATTCTCCATC                                                          |
| qsdhC Rev       | GACGAAGAAGCTGCCCAAA                                                             |
| qsdhD For       | TATCGTCCTACGGCTCTACA                                                          |
| qsdhD Rev       | CCAGGCGATGATCAAGATGGCC                                                        |
| qssrA For       | TTTGTAGTGCCGCTGTCCT                                                          |
| qssrA Rev       | GAACCCGGCGTCCGAAAT                                                          |

#### Primers for strain construction

| fur::zeo For    | TCTAAATGAGTGAAACCGGTTAGTAACAGGACAGATTCCGCTGTTGACAATTAACATCGGCC |
| fur::zeo Rev    | AAAGCACAACCCGAGGTTGCTTTTTCGAGGCTGCGGTTGAGTCTGTTATCTG          |
| leuZKochk For   | CGGATGTCTGCTGGAAGG                                                  |
| leuZKochk Rev   | CCAGGACCTCTATATCCGATG                                              |
| leuZwtKoin For  | CCGGTTACGATGAAAAGATAAAGATAAATCAAGACAAATAGGAAGCGAGGCTGCTGTTATCCG |
| leuZwtKoin Rev  | GGTGATTTAAAAATTAAAGGAGGTGTGAACGACAGAGTTCCGACCATATGAATATCCTCCTTAG |
| primer name | sequence |
|-------------|----------|
| IeuZdblinvKOin For | CCGGGTACCATGGGAAGATAAGATAATAAAAAATCAACGAATAACGA GTGTCGTGAAACCACCTCCGGGTGTGTTTTTTGTTGAGCTGGAG CTCCTTC |
| pcnB KO For | TTTTTGTCTGAATGATGGTTTGACACTACGGAGGTGTACTGTGTAG GCTGGAGCTGCTTC |
| pcnB KO Rev | CCTATGGCAATATACGCTATGCAGGTACCCTACGCATAT GAATATCCTCCTTAG |
| pcnB For | GTGGCTTTTCAAGGATATCG |
| pcnB Rev | TTTTCAAGCAGAATCATGC |
| furKOchk For | GTGGCAATTTCTAATGATACGC |
| furKOchk Rev | GAGAGCTGTAACCTTCGC |
| RyhBKOchk For | CAAATCGCAGTCAAATGC |
| RyhBKOchk Rev | GTGTTTCCTACTGAGTGTC |
| RyhBmut gBlock | GTTGGGACAAGTGCGAATGAGATGATTATTATTGTCACGCATC AGGAAGACCCCTCCGGAGGAACTCAGACAGCAGACTTCCGTACAT TGCTTCCAGTATTACCTAGCCAGCGGTGCTGCTTTTTTTTGTGC TCTTCCGTCTCAATTTATCCACGGGACTGCTTGTGTT |

**3' RACE primers**

| primer name | sequence |
|-------------|----------|
| RyhB3'RACE | GCGATCAGGAAGACCCTC |
| E1 DNA adapter | TTCACCTGTCTTACGCGGCGCATGCTC |
| M13 For | GTAAAACGACGGCCAG |
| M13 Rev | CAGGAAACAGCTATGAC |
SUPPLEMENTAL FIGURES

FIGURE S1. Representative northern blots corresponding to sRNA stability curves shown in Figure 2. Northern blot analysis was used to determine the expression of the following sRNAs: GcvB, MicA, CyaR, ChiX, and MgrR, and their corresponding loading controls (SsrA or 5S) at the indicated time points following rifampicin addition in strains TC279 (A and B), NRD1138 (C and D), or the derived ΔpcnB mutant strains DS120 and NRD1198, respectively, under exponential growth conditions as described in the legend of Figure 2 and in Materials and Methods.
FIGURE S2. Northern Blot analysis of *sodB* mRNA turnover in *pcnB* mutant. RyhB expression was induced in exponentially growing cultures of the wild-type (NRD1138) and ∆pcnB (NRD1198) strain by addition of dipyridyl, and samples were collected at different time points for RNA extraction. *sodB* and RyhB levels were detected by northern blotting, and both the sRNA and mRNA signals were normalized to the SsrA loading control. Representative blots are shown in (A). *sodB* mRNA decay curves (B) were generated by fitting the normalized signal intensities for each time point in GraphPad Prism. Results represent the mean of at least two independent experiments and bars indicate SEM.
FIGURE S3. Analysis of the effects of deletions in pcnB, hfq and rne on sdhD transcript steady-state levels in an E. coli Δfur strain. qRT-PCR analysis was used to determine sdhD relative transcript levels as described in Materials and Methods. Strains and growth conditions used are described in Figure 3. sdhD transcript levels were normalized to that of the internal control (reference gene) SsrA. Results represent the mean of at least three independent experiments and bars indicate SEM.
FIGURE S4. Determination of the steady-state levels of MicA targets *ompA* and *ompX* in Δ*pcnB* mutant. Northern blot analysis was performed to determine *ompA* and *ompX* transcript steady-state levels in exponentially growing cultures of a parent (TC279) and an isogenic Δ*pcnB* mutant strain (DS120) under MicA non-inducing (-Dipyridyl) and inducing (+Dipyridyl) conditions; in these strains *micA* is under the control of the *ryhB* promoter. *ompX* and MicA signals were normalized to SsrA loading control while *ompA* signals were normalized to 16S rRNA. Representative northern blots are shown in (A). (B) and (C) show graphs representing the normalized *ompA* and *ompX* mRNA levels, respectively. Results represent the mean of at least three independent experiments and bars indicate SEM. Data analysis was performed as described in the legend of Figure 3B. Probes used are listed in Table S2.
**FIGURE S5.** Northern blot analysis to determine RyhB stability in the wild-type strain and its derived isogenic *hfq* and *pcnB* mutants. (A) Representative northern blots corresponding to RyhB stability curves shown in Figure 4 F. Northern blot analysis was used to determine RyhB and SsrA (loading control) expression at indicated time points following rifampicin addition in the indicated strain backgrounds under exponential growth conditions. (B) RyhB decay curves corresponding illustrating RyhB stability in the wild-type strain (NRD1138; WT (fur⁺)) and its derived mutants (NRD1198; ΔpcnB, DS060; *hfqR17A*, and DS073; *hfqR17A ΔpcnB*). sRNA decay curves were generated as described in Figure 4 legend and corresponding half-life measurements are listed in Table 1. Points and error bars in the curves represent the means and the standard errors (SEM) of at least three independent experiments.
FIGURE S6. Determination of the relative abundance of sodB transcript in wild-type (fur+; WT) and its derived isogenic fur and pcnB mutants. Northern blot analysis performed to determine the transcript steady state levels of RyhB target sodB under RyhB inducing and non-inducing conditions as described in the legend of Figure 5B in a wild-type (WT (fur+); NRD1138) and its derived isogenic mutants (ΔpcnB, NRD1198; ryhBmut, LM11; ryhBmut ΔpcnB; Δfur, DS024; Δfur ΔpcnB, DS025). Samples for RNA extraction from DS024 and DS025 were collected as described in the legend of Figure 3A. Representative blots showing relative steady-state levels of sodB, RyhB, and SsrA in the indicated strain backgrounds are presented. SsrA is used as the loading control.
FIGURE S7. The defect in regulation of the *sdhCDAB* mRNA by RyhB in the absence of poly(A) polymerase consequently impacts the ability of *E. coli* to utilize succinate as the sole carbon source. (A, B) Overnight cultures of strain NRD1138 (WT; *fur*⁺), an isogenic Δfur strain (DS024), or derivatives of this Δfur strain harboring additional mutations in *pcnB* (Δfur ΔpcnB; DS025), *hfq* (Δfur Δhfq; DS027) *rne* (Δfur *rne*-131; DS069), *rne* and *pcnB* (Δfur *rne*-131 ΔpcnB; DS082) and, *rne* and *hfq* (Δfur *rne*-131Δhfq) were grown in M9-glucose media and subcultured in 5 mL of M9-glucose or M9-succinate media to a starting OD₆₀₀ of 0.01. Cultures were subsequently incubated at 37°C with constant shaking. After 24 h and 48 h, growth of each culture was determined by measuring absorbance at 600 nm (OD₆₀₀). Results represent the mean of the ratio of growth in M9-succinate to M9-glucose of at least three independent experiments for each dataset. Error bars indicate SEM.
FIGURE S8. Northern blot analysis to determine transcript steady-state levels of LeuZ, 3'ETS$^{LeuZ}$ and RyhB in wild-type and derived isogenic $pcnB$ and $leuZ$ mutants under RyhB inducing and non-inducing conditions. Representative northern blots corresponding to Figure 6C. Strains and growth conditions used are described in the legend of Figure 6C. Experiment was performed in triplicate and northern blots representing transcript steady-state levels of LeuZ, 3'ETS$^{LeuZ}$ and RyhB are shown. SsrA was used as the loading control. LeuZ term probe (Table S2) was used to determine LeuZ and 3'ETS$^{LeuZ}$ levels.
FIGURE S9. Analysis of 3′-end polyadenylation state of RyhB. 3′ RACE was used to determine the sequence at the 3’ ends of RyhB from exponential cultures of the ∆fur mutant and the wild type (WT; control) strain. Full-length RyhB (90 nt.) sequence was detected in 7 out of 47 clones sequenced from the ∆fur mutant and 5 out of 7 clones consisted of a template independent terminal adenine at the 3′ end (A). RyhB sequence as annotated in E. coli MG1655 is highlighted in red (A). The remaining 40 clones from the ∆fur mutant and all clones sequenced from the WT control yielded RyhB degradation products of varying lengths (C) and most RyhB degradation products terminated at nucleotide positions 40, 61 and 64 (B).
SUPPLEMENTAL MATERIALS AND METHODS

Strain construction

Strains generated by P1 vir transduction are indicated in Supplementary Table S1 with the donor strain indicated in brackets, using the protocol described by Miller (Miller 1992) and appropriate antibiotic selection.

Strain NRD698: Electrocompetent lambda Red induced NM600T cells were transformed with DNA generated from PCR amplification of the chloramphenicol resistance cassette from pKD3 using pcnB KO For and Rev primers. Successful recombinants were isolated on LB plates containing chloramphenicol and were validated by colony PCR using pcnB for and rev primers.

Strain NRD1038: Lambda Red induced NM600T cells were transformed via electroporation with a PCR product generated from amplification of the kan-pBAD-ccdB cassette from NM570 with Taq DNA polymerase using RyhBccdBkan For and Rev primers. Recombinants were selected for on LB plates containing kanamycin, then screened for arabinose sensitivity and tetracycline resistance (maintenance of mini-λ). Successful recombinants that were kanamycin and tetracycline resistant, but arabinose sensitive were validated by colony PCR using RyhBKOCchk For and Rev primers.

Strain NRD1362: Electrocompetent lambda red induced KR10000 harboring a mini-lambda phage were transformed with DNA generated from PCR amplification of the zeocin resistance cassette was amplified from NRD676 genomic DNA using fur::zeo For and Rev primers. Successful recombinants were isolated on LB plates containing zeocin and validated by colony PCR using furKOCchk For and Rev primers.

NRD1530: Lambda Red induced NM1200 cells were transformed via electroporation with a PCR product generated by amplification of a tetracycline resistance cassette from NRD654 with Taq DNA polymerase using asnBKOtet For and Rev primers. Cells were subsequently isolated
on LB plates containing tetracycline and validated by colony PCR using asnBKOrchck For and Rev primers.

**NRD1576**: A kanamycin resistance cassette was inserted immediately after the Rho-independent terminator encoded downstream of *leuZ* by transforming electrocompetent Lambda Red recombinase induced KR10000 cells with a PCR product created by amplification of the kanamycin resistance cassette from pKD4 with primers *leuZwtKOin* For and *leuZKOin* Rev using Taq DNA polymerase. Successful recombinants were selected on LB plates containing kanamycin and validated by colony PCR using *leuZKOchk* For and Rev primers.

**NRD1578**: Four point mutations were introduced into the sequence encoding the 3'ETS\(^{LeuZ}\) and a *kan* cassette was simultaneously inserted downstream of its encoded Rho-independent terminator by Lambda Red-mediated recombineering. Electrocompetent Lambda Red induced KR10000 cells were transformed with a PCR product generated from the template plasmid pKD4 with Taq DNA polymerase using primers *leuZdblinvKOin* For and *leuZKOin* Rev. Successful recombinants were selected on LB plates containing kanamycin and validated by sequencing of the DNA produced from colony PCR using *leuZKOchk* For and Rev primers.

**LM06**: Strain NRD1038\(\lambda\) harboring mini-lambda phage and the Kn-pBAD-ccdB cassette was transformed with the RyhBmut gBlock (IDT, Table S2) containing the mutated RyhB sequence with 40 bp of homology on either side of RyhB. Successful recombinants (Ara\(^R\)Kan\(^S\)) were sequence verified for the RyhB mutation after colony PCR amplification using RyhBKOchk forward and reverse primers.

**Succinate growth assay**

Strains were initially grown on M9-glucose agar plates and single colonies for each strain was inoculated in 2 mL of M9-glucose broth and grown overnight at 37°C aerobically.
Each overnight culture was subcultured separately into 5 mL of fresh M9-glucose broth and M9-succinate broth to a starting OD$_{600}$ of 0.01. Cultures were grown aerobically at 37°C and growth was determined by measuring OD$_{600}$ after 24 h and 48 h. Final growth yield in succinate at the end of each time point was expressed as a ratio of the OD$_{600}$ obtained for growth in M9-succinate to that in M9-glucose corresponding to each strain culture.

**Northern blot analysis of ompA**

Northern blot analysis of ompA was carried out as described previously (De Lay and Gottesman 2009). Briefly, 8 µg of each RNA sample was loaded on a 1.2% agarose gel that was pre-run at 12V/cm for at least 5 min and subsequently run at 5V/cm for 2 h in 1X MOPS (morpholinepropanesulfonic acid) buffer. Next, the RNA samples were transferred to a Zeta-Probe GT membrane (Bio-Rad) via capillary transfer. Transferred RNA was UV crosslinked and hybridized overnight with 100 ng/mL of 5’ biotinylated OmpA probe (Supplemental Table S2) as described in the Materials and Methods. Signal intensity corresponding to ompA was normalized to that of 16S rRNA, which served as internal loading control.

**3’ RACE**

3’-RACE assays were carried out as described previously (Argaman et al. 2001) with minor modifications. Total RNA was isolated from exponential phase cultures growing in MOPS EZ rich defined media as described in Materials and Methods section and was subjected to DNase treatment (DNase Turbo; Ambion) according to manufacturer’s guidelines. 15 µg of DNase-treated RNA was dephosphorylated using calf intestine alkaline phosphatase (CIP; NEB). 3’ RNA adapter (E1, 5’-phosphate UUCACUGUUCUUAGCGGCCGCAUGCUC-idT-3’) ligation was performed overnight (16 h) at 37°C with T4 Ligase (NEB). Reverse transcription of
the ligated RNA samples was carried out as described in Materials and Methods section, but with 100 pmol of E1 DNA adapter (primer complementary to E1 RNA adapter (Table S2)). The products of RT-reactions (2 μL) were PCR amplified with Taq DNA polymerase (NEB) using the following primer pairs - RyhB 3′RACE and E1 DNA adapter (Table S2). PCR products were size checked and cloned into pCR 2.1 TOPO-vector (Invitrogen) following manufacturer’s protocol. Bacterial clones obtained after transformation were assayed for the presence of inserts of expected size by colony PCR using primer pairs M13 For and M13 Rev (Table S2). Plasmids from clones containing appropriate sized inserts were purified using QIAprep Spin miniprep kit (Qiagen) and sequenced with M13 For primer.

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