# Supporting Information

## An easy-to-use plasmid toolset for efficient generation and benchmarking of synthetic small RNAs in bacteria

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**Figures S1 to S10**

**Tables S1 and S2**

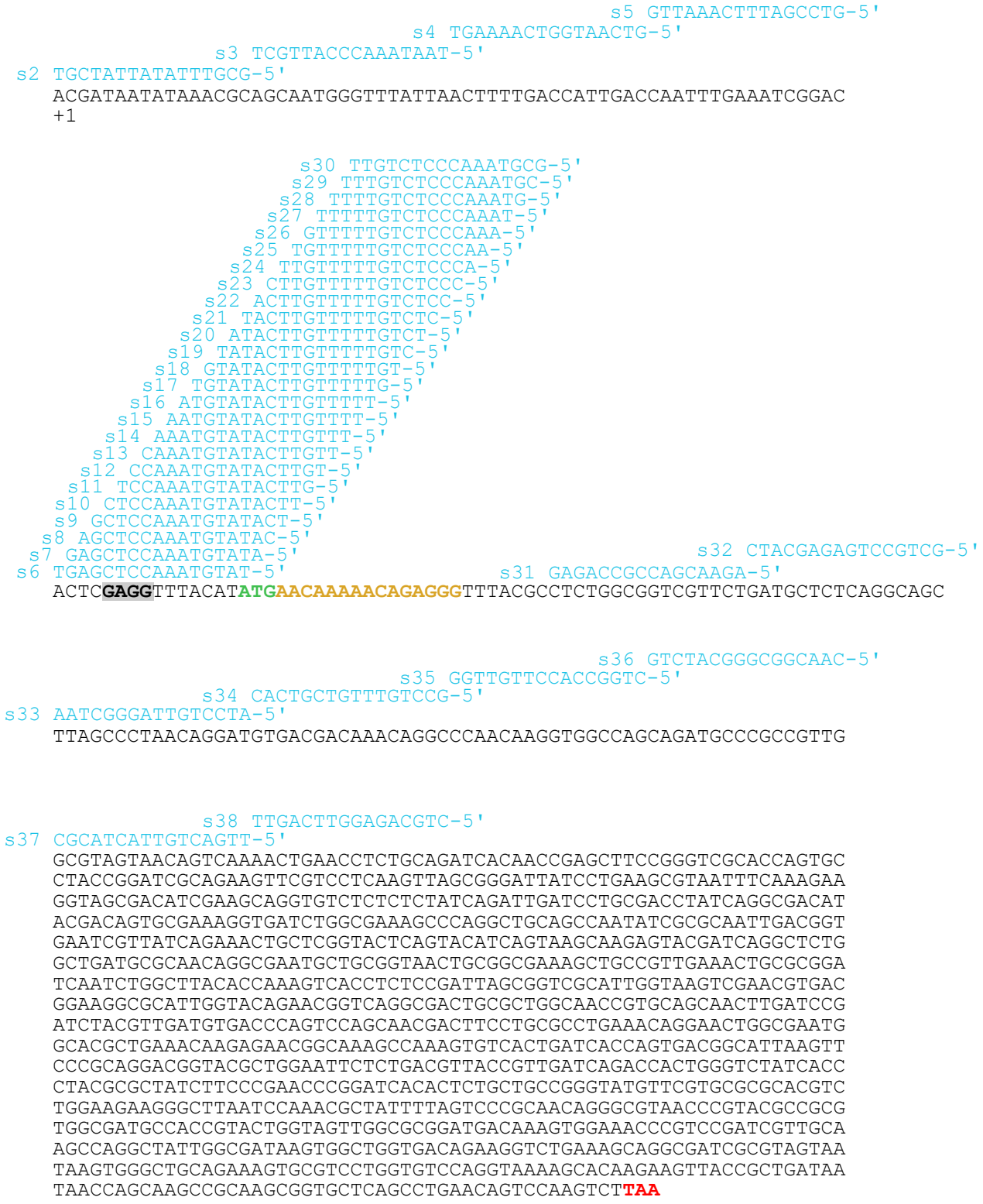
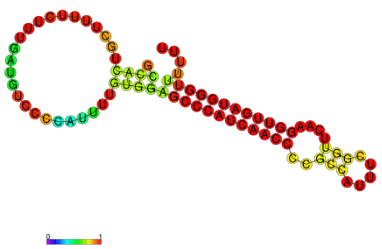



**Figure S1.** Design of seed regions for targeting of *acrA*. The *acrA* sequence is given in black letters. Green and red letters indicate start and stop codons, respectively. Orange letters indicate the 'five codon window'. The Shine-Dalgarno sequence is highlighted in a grey box. +1 indicates the transcriptional start site. Seed regions for *acrA* binding are given in blue letters. To illustrate complementarity to *acrA*, the 5' end of seed regions is on the right-hand side. Seed regions are complementary to the 5' UTR (s2-s5), the TIR (s6-s13), the start codon (s14-s23), the 'five codon window' (s24-s30) and the coding region (s31-s38).

RybB-wt



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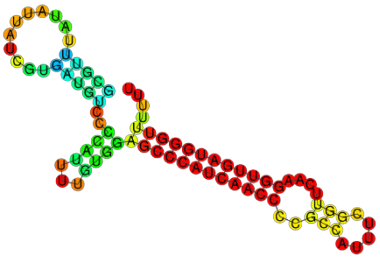

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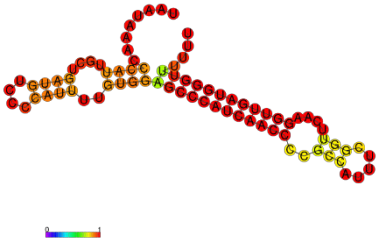

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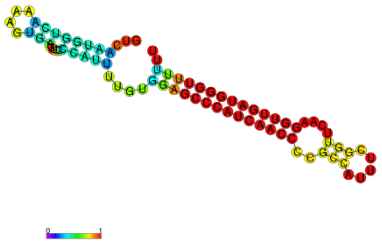

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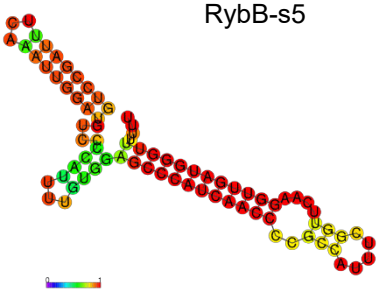

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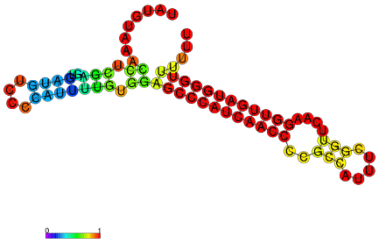

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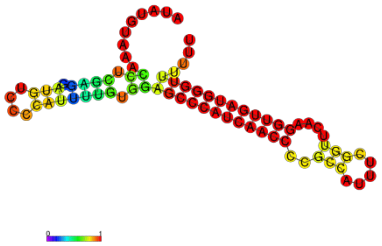

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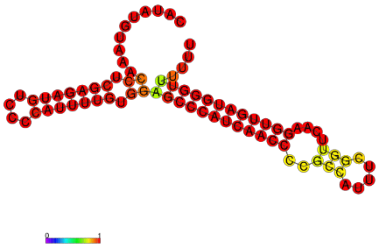

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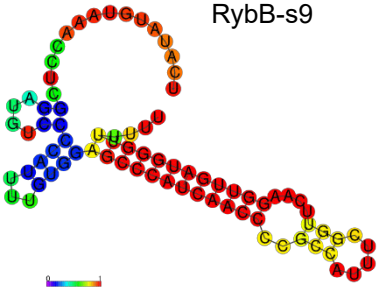

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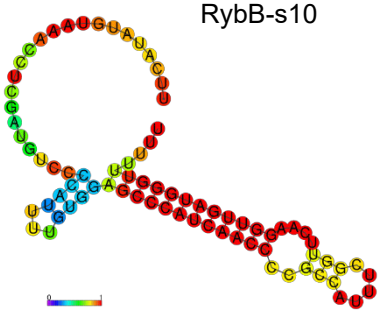

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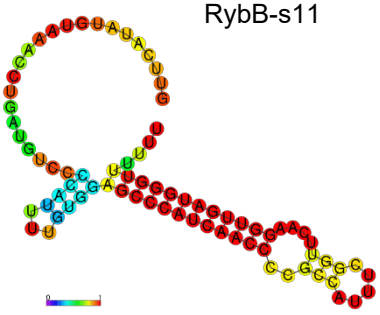

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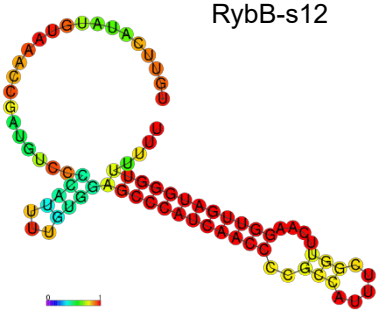

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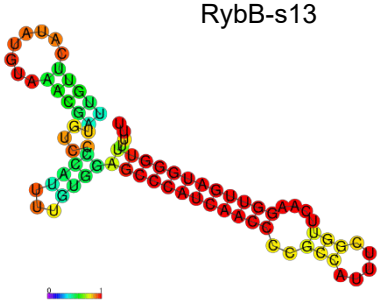

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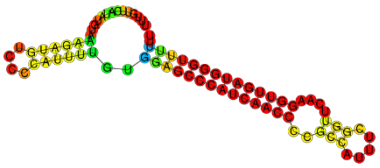

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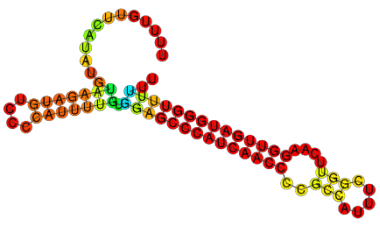

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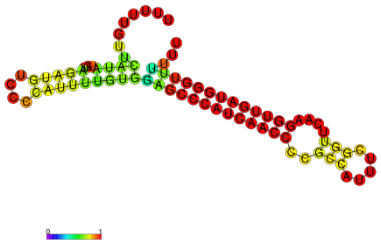

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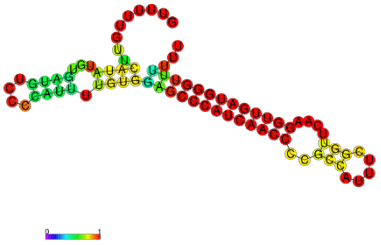

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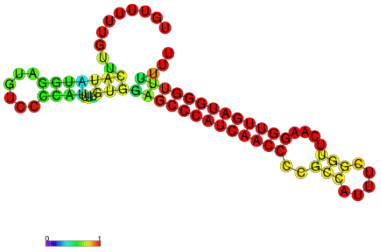

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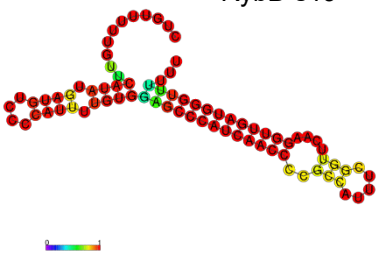

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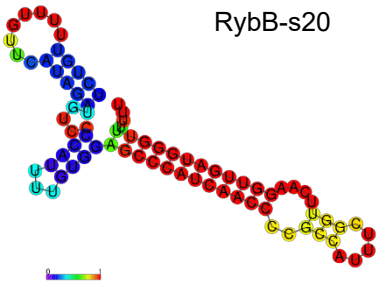

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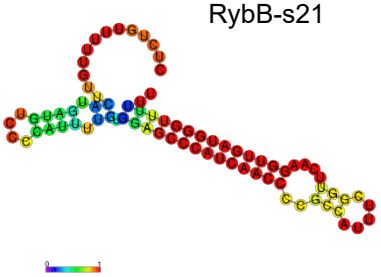

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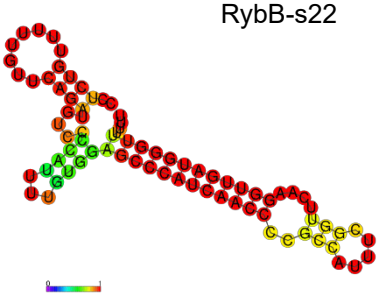

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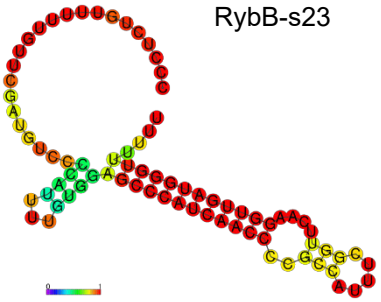

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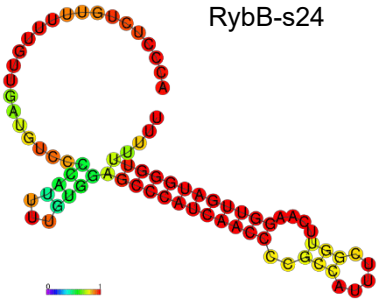

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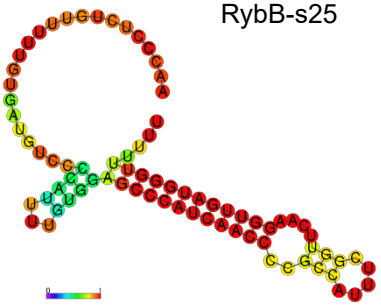

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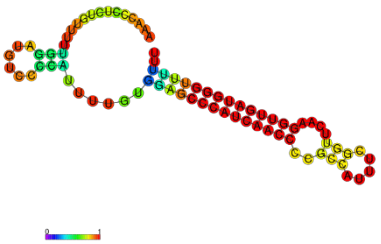

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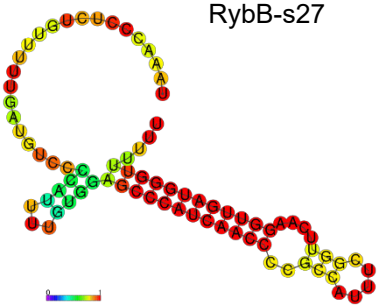

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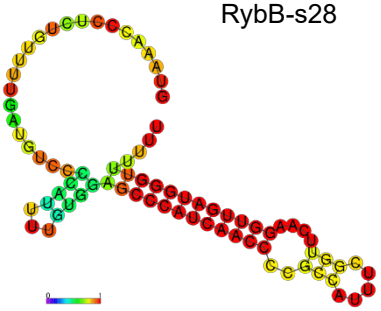

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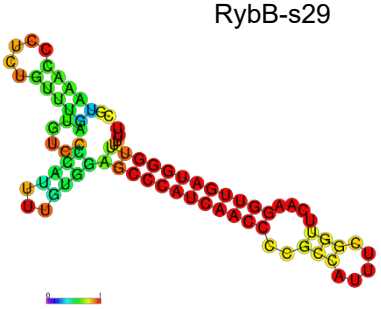

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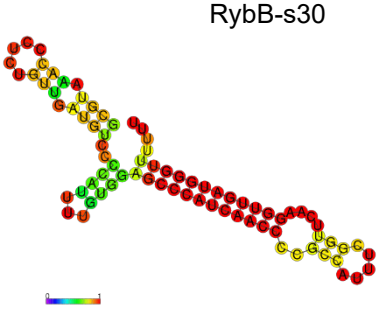

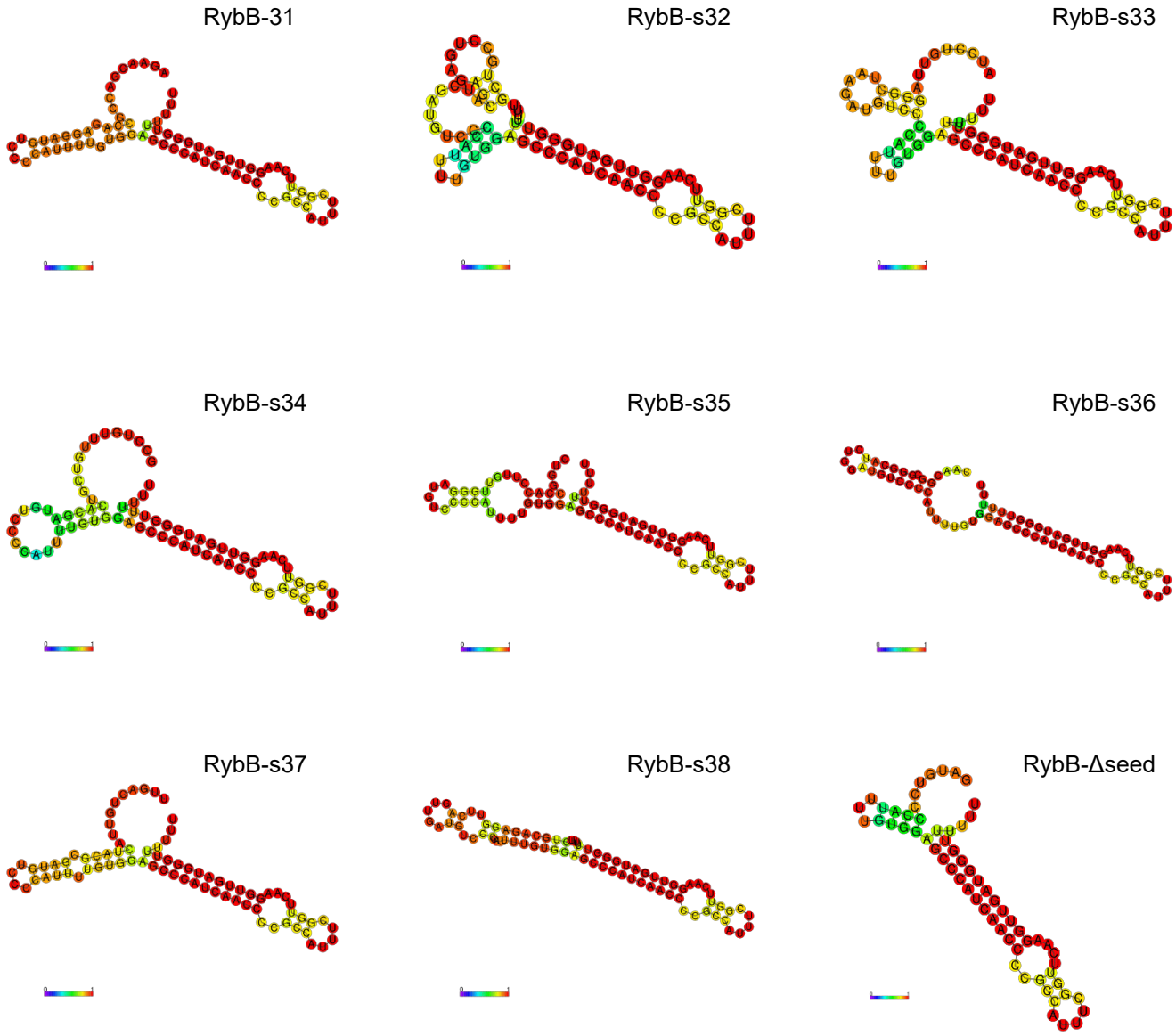


**Figure S2.** Secondary structure predictions for synthetic RybB sRNAs. Secondary structures were predicted using the RNAfold web server (http://rna.tbi.univie.ac.at/cgi-bin/RNAWebSuite/RNAfold.cgi). The colored bar indicates the base-pair probabilities. Wild-type (wt) RybB and the RybB scaffold (RybB-Δseed) are shown for comparison.

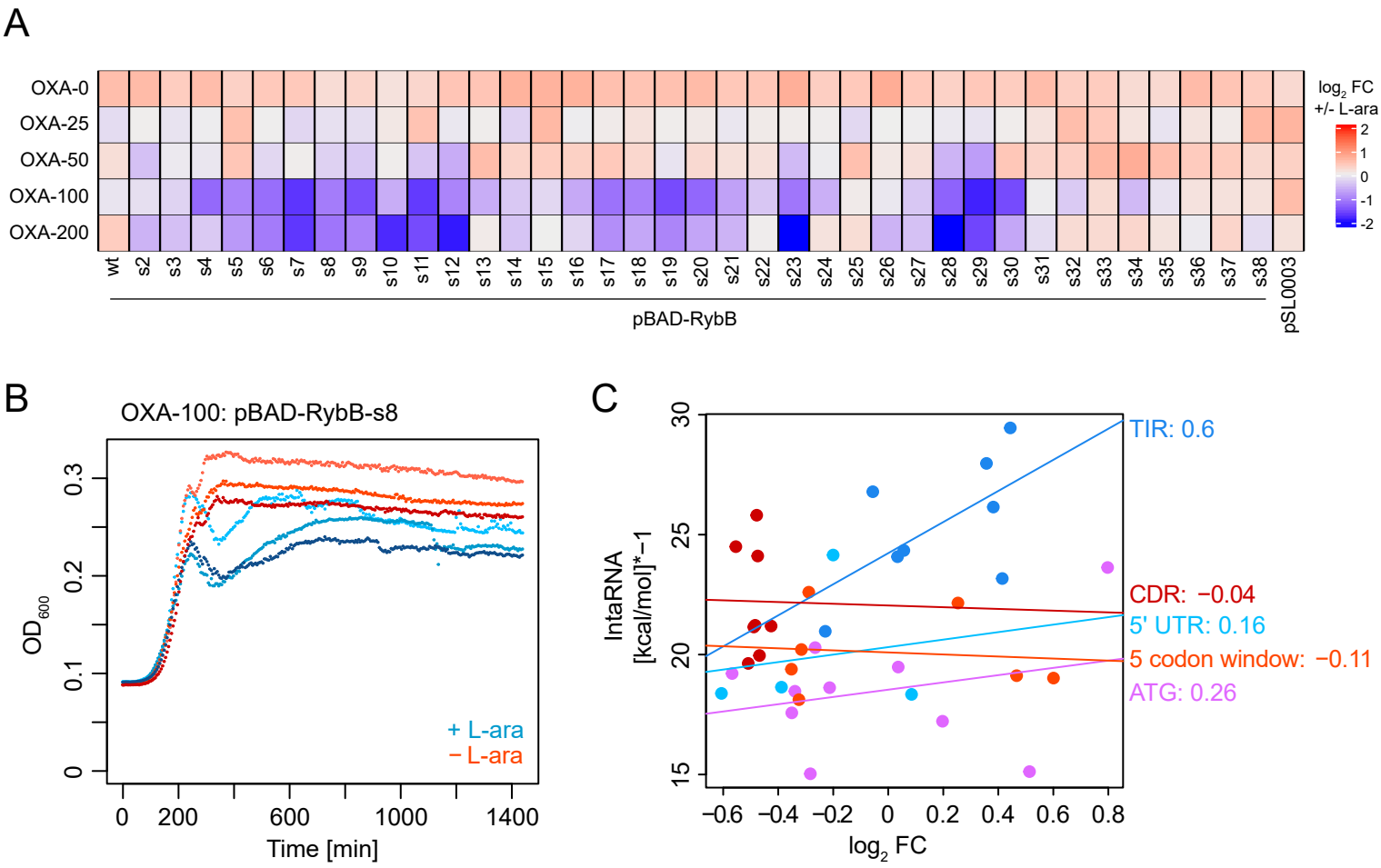


**Figure S3.** Phenotypic screening of synthetic RybB sRNAs with seed regions s2-s38. Seed regions were cloned into the pBAD derivative pSL0004 for inducible expression of synthetic RybB sRNAs. Stationary-phase cultures were inoculated in 96-well plates to monitor growth ($OD_{600}$) in a plate reader. **(A)** LB medium contained oxacillin (OXA) at the indicated concentrations (0-200 µg/ml). Strains were treated with L-arabinose (+ L-ara) to induce sRNA expression or left untreated (- L-ara). The areas under the curves (AUC) were calculated and AUC values were subsequently used to calculate $log_2$ fold-changes (FC). $Log_2$ FC are illustrated in a heatmap. Wild-type (wt) RybB and the empty plasmid pSL0003 served as controls. **(B)** Growth curves for pBAD-RybB-s8 at 100 µg/ml oxacillin (OXA-100) with (blue) and without (red) addition of L-arabinose (L-ara). The dots show the measured optical density (OD) at 600 nm. The presence of oxacillin causes irregular growth curves with peaks at ~300 min due to cellular filamentation. **(C)** Correlation analysis of $log_2$ FC (-/+ L-ara) and IntaRNA energy predictions for sRNA-*acrA* pairs (multiplied by -1). Numbers at the right-hand side represent Pearson's *r* for individual subsets (5' UTR: 5' untranslated region; TIR: translation initiation region; ATG: start codon; CDR: coding region).

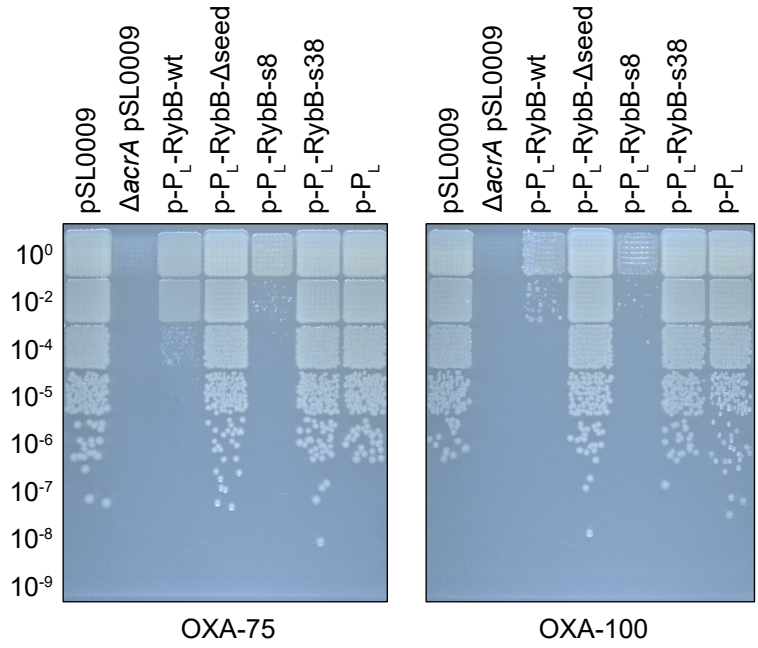


**Figure S4.** Oxacillin susceptibility assay. Stationary-phase cultures were serially diluted as indicated and spotted onto LB agar plates containing varying concentrations of oxacillin (OXA, 75 and 100 µg/ml; *cf*. Fig. 3E for 0-50 µg/ml). Plates were incubated overnight at 37 °C. The empty plasmid pSL0009, wild-type RybB (RybB-wt), RybB lacking a seed region (RybB-Δseed) and a plasmid containing the $P_L$lacO-1 promoter (p-$P_L$) were used as controls.

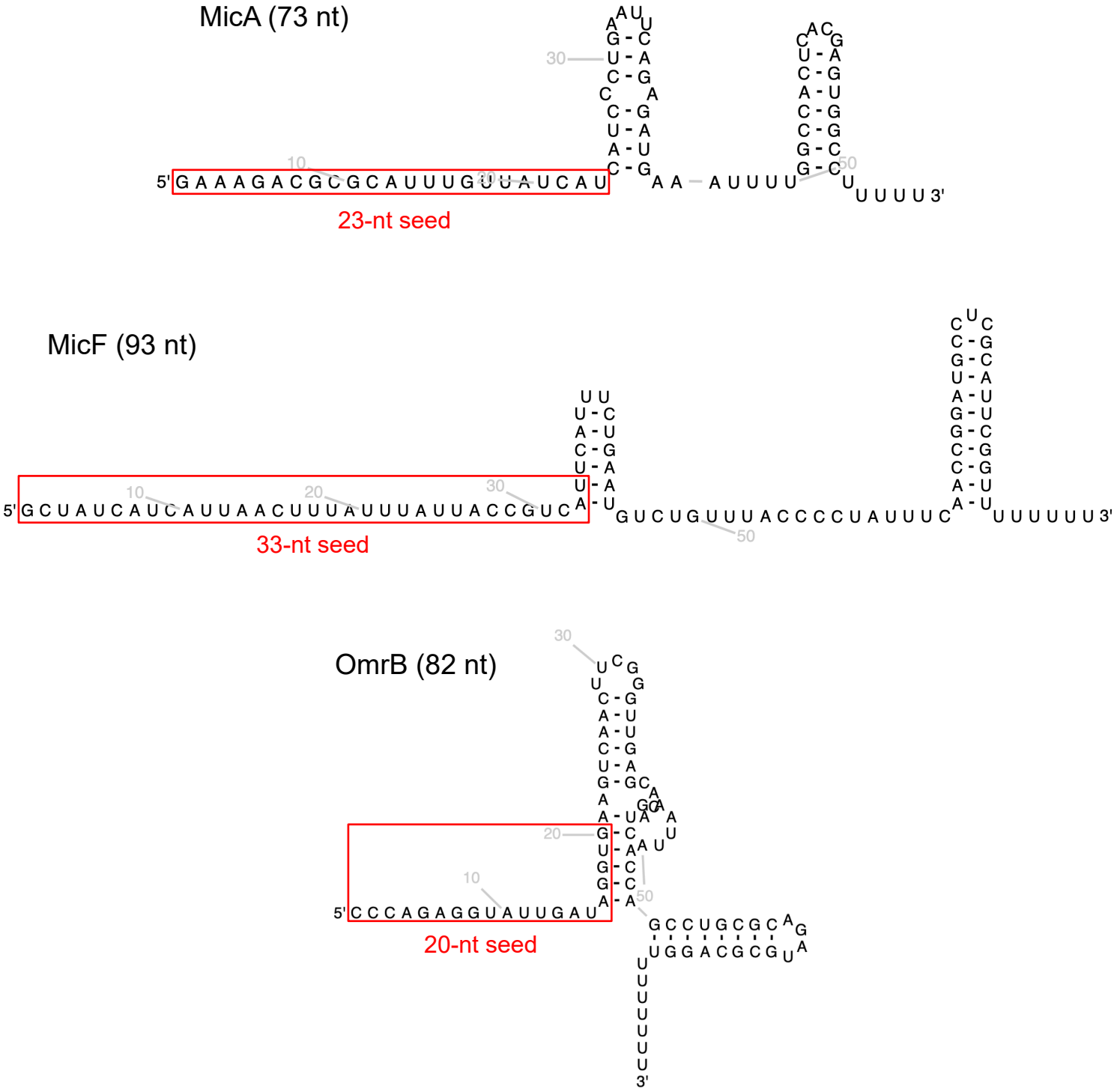


**Figure S5.** Secondary structures and seed regions for MicA, MicF and OmrB. Secondary structures were retrieved from RNAcentral (https://rnacentral.org/). Corresponding seed regions are marked in red.

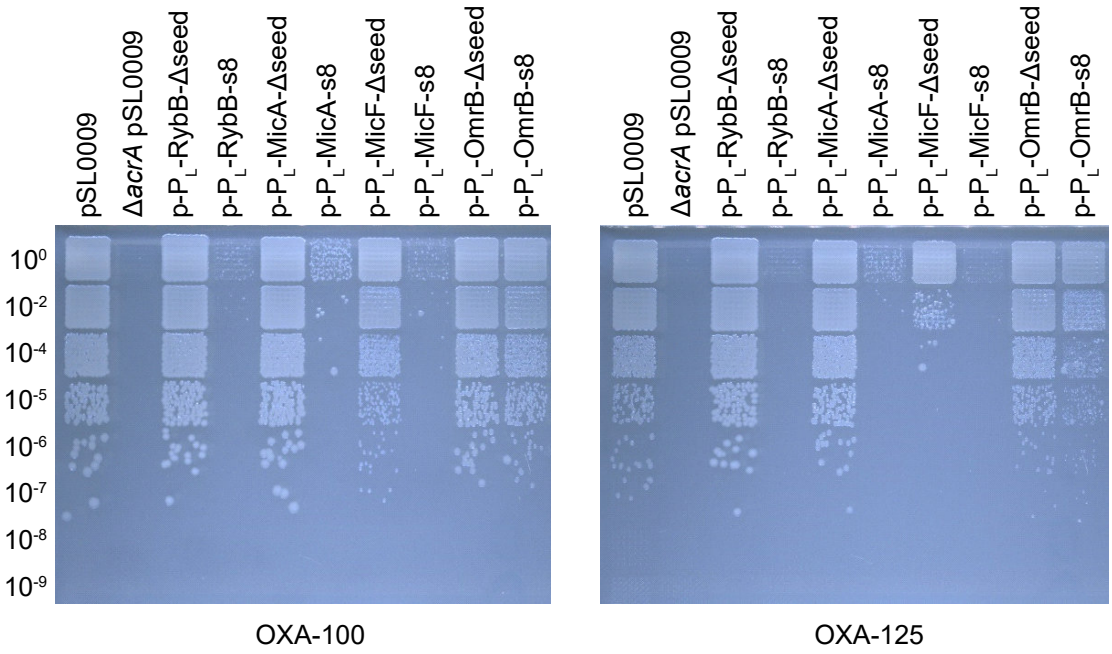


**Figure S6.** Oxacillin susceptibility assay. Stationary-phase cultures were serially diluted as indicated and spotted onto LB agar plates containing varying concentrations of oxacillin (OXA, 100 and 125 µg/ml; *cf*. Fig. 5 for 0-75 µg/ml). Plates were incubated overnight at 37 °C. Synthetic sRNAs containing the s8 seed region were compared to variants lacking a seed region (Δseed). The empty plasmid pSL0009 was used as a control.

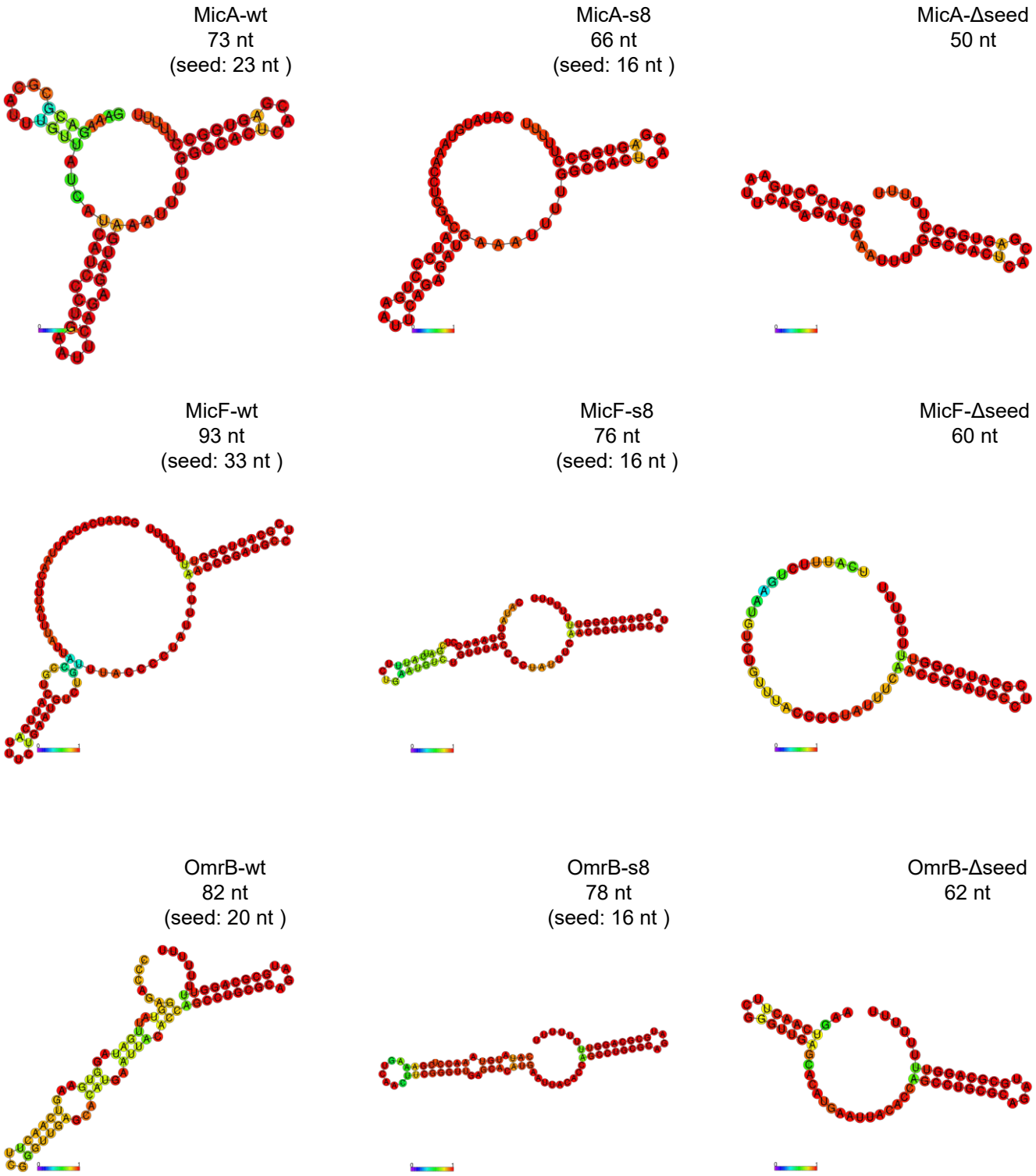


**Figure S7.** Secondary structure predictions for synthetic sRNAs containing seed region s8. Secondary structures were predicted using the RNAfold web server (http://rna.tbi.univie.ac.at/cgi-bin/RNAWebSuite/RNAfold.cgi). The colored bar indicates the base-pair probabilities. Wild-type (wt) MicA, MicF and OmrB sRNAs and variants lacking a seed region (Δseed) are shown for comparison.

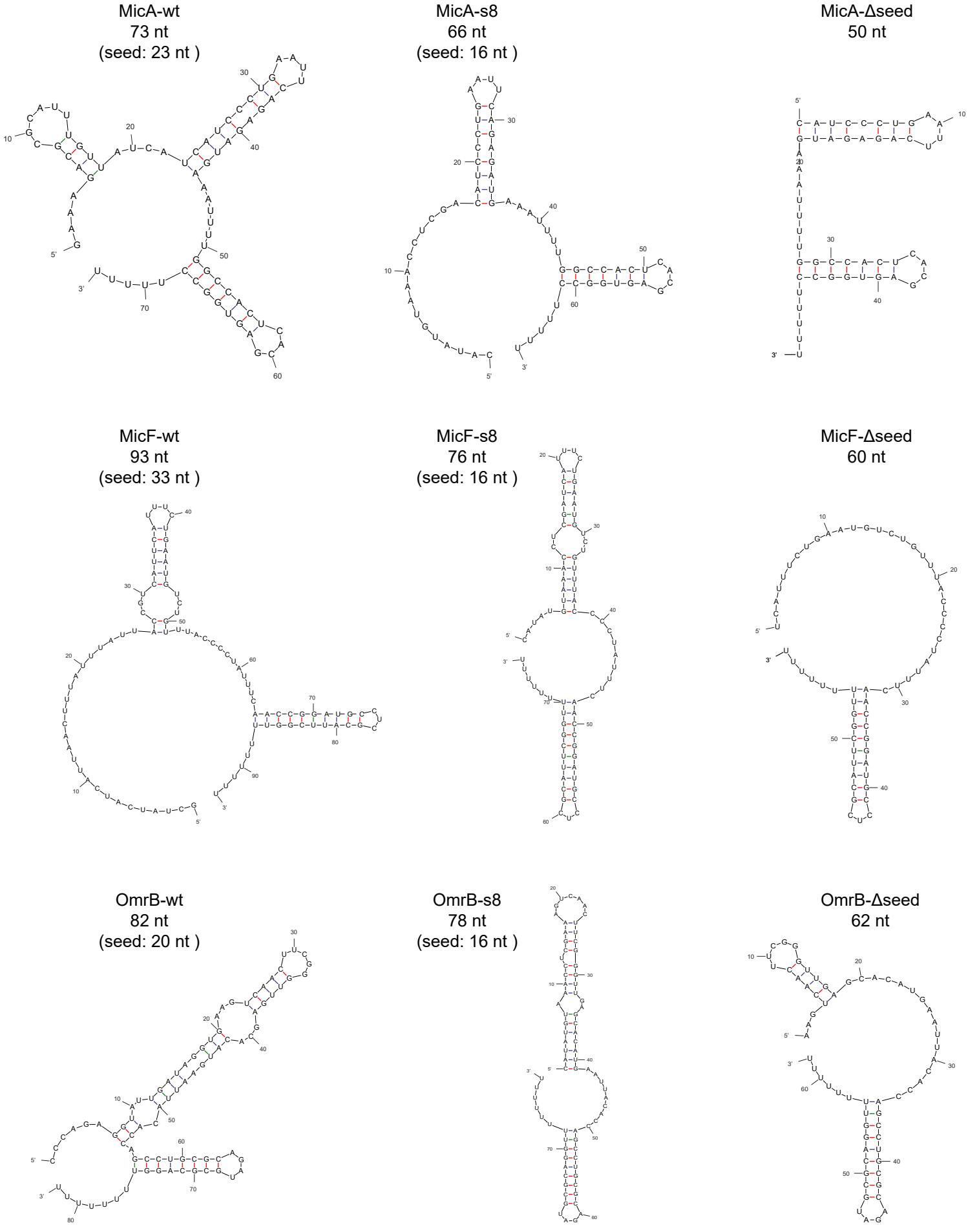


**Figure S8.** Secondary structure predictions for synthetic sRNAs containing seed region s8. Secondary structures were predicted using the Mfold web server (http://www.unafold.org/mfold/applications/rna-folding-form.php). Wild-type (wt) MicA, MicF and OmrB sRNAs and variants lacking a seed region (Δseed) are shown for comparison.

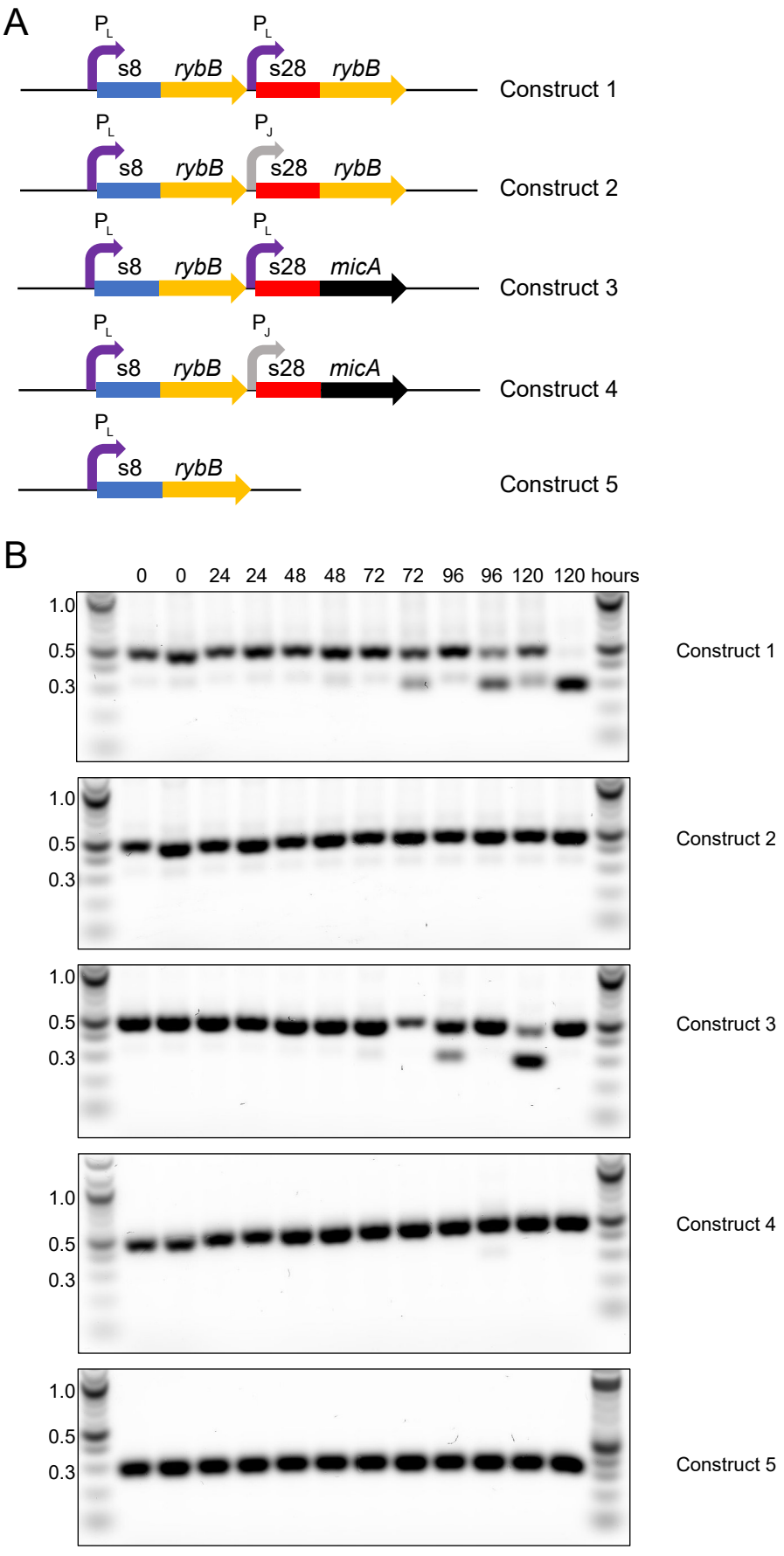


**Figure S9.** Stability assay for assembled constructs. **(A)** Constructs with dual sRNA TUs were assembled using high-complexity Golden Gate cloning with plasmid pSL0011 (Constructs 1-4; ranging from high identity to no identity). A construct with a single sRNA TU was used for comparison (Construct 5). Colors indicate identity of DNA parts. **(B)** Clones containing constructs were incubated separately (as biological duplicates) in a 96-well flat-bottom plate with 150 µl of LB and 50 µg/ml kanamycin per well. The plate was incubated at 180 rpm and 37 °C. After a 24-hours period, 1 µl of the cell suspension was collected in 50 µl $dH_2O$ and frozen for a screening PCR. At the same time, 1 µl of the cell suspension was inoculated in a new plate containing 150 µl LB and 50 µg/ml kanamycin for a new incubation period. This was repeated for five days (120 hours). The screening PCR was performed in a total volume of 10 µl, consisting of 5 µl OneTaq Quick-Load 2x Master Mix (NEB), 1 µl of frozen cell samples, 2 µl $dH_2O$, and 1 µl of each 10 µM colony PCR primer (Table S2). The resulting PCR fragments were separated in a 2% agarose gel.

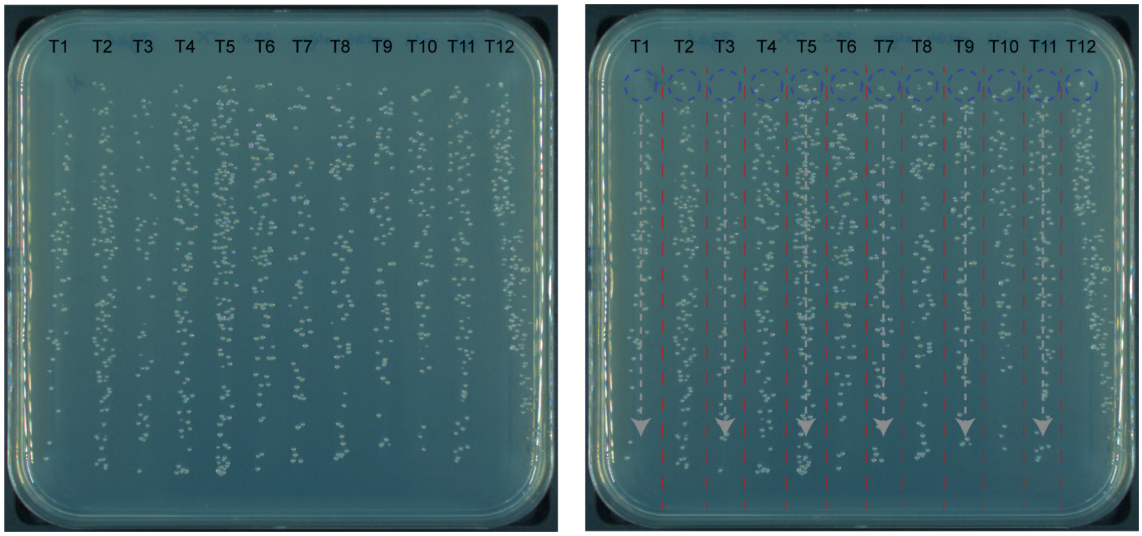


**Figure S10.** Exemplary transformation plate of the high-throughput plating procedure. Twelve transformation mixtures were plated in parallel by gravity flow of 20 µl drops using a multi-channel pipette. Both panels show the same representative plate. The left panel shows the transformation plate without explanatory features, just the indication of the individual transformation (T1 to T12). The right panel indicates the positions where the drops were set (blue dotted circles). Grey arrows indicate the gravity flow direction. Red dotted lines separate the individual transformations (lanes) for clarification.

## Oligodeoxynucleotides

All oligodeoxynucleotides were ordered from Integrated DNA Technologies (IDT, Coralville, USA) or Microsynth Seqlab (Göttingen, Germany) with standard desalting purification.

**Table S1.** Oligodeoxynucleotides used for Golden Gate cloning.

| Forward sequence [5'-3'] | Reverse sequence [5'-3'] | Assembly piece | Resulting plasmid |
|---|---|---|---|
| **CCAT**GCCACTGCTTTTCTTT | **CATC**AAAGAAAAGCAGTGGC | 16 nt native RybB seed region, variant 1 | pSLcol_01.s01<br>p-$P_L$-RybB-wt |
| **CCAT**GCGTTTATATTATCGT | **CATC**ACGATAATATAAACGC | 16 nt seed region, variant 2, target: *acrA* | pSLcol_01.s02 |
| **CCAT**TAATAAACCCATTGCT | **CATC**AGCAATGGGTTTATTA | 16 nt seed region, variant 3, target: *acrA* | pSLcol_01.s03 |
| **CCAT**GTCAATGGTCAAAAGT | **CATC**ACTTTTGACCATTGAC | 16 nt seed region, variant 4, target: *acrA* | pSLcol_01.s04 |
| **CCAT**GTCCGATTTCAAATTG | **CATC**CAATTTGAAATCGGAC | 16 nt seed region, variant 5, target: *acrA* | pSLcol_01.s05 |
| **CCAT**TATGTAAACCTCGAGT | **CATC**ACTCGAGGTTTACATA | 16 nt seed region, variant 6, target: *acrA* | pSLcol_01.s06 |
| **CCAT**ATATGTAAACCTCGAG | **CATC**CTCGAGGTTTACATAT | 16 nt seed region, variant 7, target: *acrA* | pSLcol_01.s07 |
| **CCAT**CATATGTAAACCTCGA | **CATC**TCGAGGTTTACATATG | 16 nt seed region, variant 8, target: *acrA* | pSLcol_01.s08<br>p-$P_L$-RybB-s8 |
| | **GATG**TCGAGGTTTACATATG | 16 nt seed region, variant 8, target: *acrA* | p-$P_L$-MicA-s8 |
| | **ATGA**TCGAGGTTTACATATG | 16 nt seed region, variant 8, target: *acrA* | p-$P_L$-MicF-s8 |
| | **ACTT**TCGAGGTTTACATATG | 16 nt seed region, variant 8, target: *acrA* | p-$P_L$-OmrB-s8 |
| **CCAT**TCATATGTAAACCTCG | **CATC**CGAGGTTTACATATGA | 16 nt seed region, variant 9, target: *acrA* | pSLcol_01.s09 |
| **CCAT**TTCATATGTAAACCTC | **CATC**GAGGTTTACATATGAA | 16 nt seed region, variant 10, target: *acrA* | pSLcol_01.s10 |
| **CCAT**GTTCATATGTAAACCT | **CATC**AGGTTTACATATGAAC | 16 nt seed region, variant 11, target: *acrA* | pSLcol_01.s11 |
| **CCAT**TGTTCATATGTAAACC | **CATC**GGTTTACATATGAACA | 16 nt seed region, variant 12, target: *acrA* | pSLcol_01.s12 |
| **CCAT**TTGTTCATATGTAAAC | **CATC**GTTTACATATGAACAA | 16 nt seed region, variant 13, target: *acrA* | pSLcol_01.s13 |
| **CCAT**TTTGTTCATATGTAAA | **CATC**TTTACATATGAACAAA | 16 nt seed region, variant 14, target: *acrA* | pSLcol_01.s14 |
| **CCAT**TTTTGTTCATATGTAA | **CATC**TTACATATGAACAAAA | 16 nt seed region, variant 15, target: *acrA* | pSLcol_01.s15 |
| **CCAT**TTTTTGTTCATATGTA | **CATC**TACATATGAACAAAAA | 16 nt seed region, variant 16, target: *acrA* | pSLcol_01.s16 |
| **CCAT**GTTTTTGTTCATATGT | **CATC**ACATATGAACAAAAAC | 16 nt seed region, variant 17, target: *acrA* | pSLcol_01.s17 |
| **CCAT**TGTTTTTGTTCATATG | **CATC**CATATGAACAAAAACA | 16 nt seed region, variant 18, target: *acrA* | pSLcol_01.s18 |
| **CCAT**CTGTTTTTGTTCATAT | **CATC**ATATGAACAAAAACAG | 16 nt seed region, variant 19, target: *acrA* | pSLcol_01.s19 |
| **CCAT**TCTGTTTTTGTTCATA | **CATC**TATGAACAAAAACAGA | 16 nt seed region, variant 20, target: *acrA* | pSLcol_01.s20 |
| **CCAT**CTCTGTTTTTGTTCAT | **CATC**ATGAACAAAAACAGAG | 16 nt seed region, variant 21, target: *acrA* | pSLcol_01.s21 |
| **CCAT**CCTCTGTTTTTGTTCA | **CATC**TGAACAAAAACAGAGG | 16 nt seed region, variant 22, target: *acrA* | pSLcol_01.s22 |
| **CCAT**CCCTCTGTTTTTGTTC | **CATC**GAACAAAAACAGAGGG | 16 nt seed region, variant 23, target: *acrA* | pSLcol_01.s23 |
| **CCAT**ACCCTCTGTTTTTGTT | **CATC**AACAAAAACAGAGGGT | 16 nt seed region, variant 24, target: *acrA* | pSLcol_01.s24 |
| **CCAT**AACCCTCTGTTTTTGT | **CATC**ACAAAAACAGAGGGTT | 16 nt seed region, variant 25, target: *acrA* | pSLcol_01.s25 |
| **CCAT**AAACCCTCTGTTTTTG | **CATC**CAAAAACAGAGGGTTT | 16 nt seed region, variant 26, target: *acrA* | pSLcol_01.s26 |
| **CCAT**TAAACCCTCTGTTTTT | **CATC**AAAAACAGAGGGTTTA | 16 nt seed region, variant 27, target: *acrA* | pSLcol_01.s27 |
| **CCAT**GTAAACCCTCTGTTTT | **CATC**AAAACAGAGGGTTTAC | 16 nt seed region, variant 28, target: *acrA* | pSLcol_01.s28 |
| **CCAT**CGTAAACCCTCTGTTT | **CATC**AAACAGAGGGTTTACG | 16 nt seed region, variant 29, target: *acrA* | pSLcol_01.s29 |
| **CCAT**GCGTAAACCCTCTGTT | **CATC**AACAGAGGGTTTACGC | 16 nt seed region, variant 30, target: *acrA* | pSLcol_01.s30 |
| **CCAT**AGAACGACCGCCAGAG | **CATC**CTCTGGCGGTCGTTCT | 16 nt seed region, variant 31, target: *acrA* | pSLcol_01.s31 |
| **CCAT**GCTGCCTGAGAGCATC | **CATC**GATGCTCTCAGGCAGC | 16 nt seed region, variant 32, target: *acrA* | pSLcol_01.s32 |
| **CCAT**ATCCTGTTAGGGCTAA | **CATC**TTAGCCCTAACAGGAT | 16 nt seed region, variant 33, target: *acrA* | pSLcol_01.s33 |
| **CCAT**GCCTGTTTGTCGTCAC | **CATC**GTGACGACAAACAGGC | 16 nt seed region, variant 34, target: *acrA* | pSLcol_01.s34 |
| **CCAT**CTGGCCACCTTGTTGG | **CATC**CCAACAAGGTGGCCAG | 16 nt seed region, variant 35, target: *acrA* | pSLcol_01.s35 |
| **CCAT**CAACGGCGGGCATCTG | **CATC**CAGATGCCCGCCGTTG | 16 nt seed region, variant 36, target: *acrA* | pSLcol_01.s36 |
| **CCAT**TTGACTGTTACTACGC | **CATC**GCGTAGTAACAGTCAA | 16 nt seed region, variant 37, target: *acrA* | pSLcol_01.s37 |
| **CCAT**CTGCAGAGGTTCAGTT | **CATC**AACTGAACCTCTGCAG | 16 nt seed region, variant 38 for *acrA* | pSLcol_01.s38<br>p-$P_L$-RybB-s38 |
| **ACCA**TTGTGAGCGGATAACAA TTGACATTGTGAGCGGATAAC AAGATACTGAGCAC | **ATGG**GTGCTCAGTATCTTGTT ATCCGCTCACAATGTCAATTG TTATCCGCTCACAA | $P_L$lacO-1 promoter | p-$P_L$-RybB-wt<br>p-$P_L$-RybB-s8<br>p-$P_L$-RybB-s28<br>p-$P_L$-RybB-s38<br>p-$P_L$-MicA-s8<br>p-$P_L$-MicF-s8<br>p-$P_L$-OmrB-s8<br>p-$P_L$-s8-RybB-$P_L$-s28-RybB<br>p-$P_L$-s8-RybB-$P_J$-s28-RybB<br>p-$P_L$-s8-RybB-$P_L$-s28-MicA<br>p-$P_L$-s8-RybB-$P_J$-s28-MicA |
| | **CATC**GTGCTCAGTATCTTGTT ATCCGCTCACAATGTCAATTG TTATCCGCTCACAA | $P_L$lacO-1 promoter | p-$P_L$-RybB-Δseed |

| | | | |
|---|---|---|---|
| | **GATG**GTGCTCAGTATCTTGTTATCCGCTCACAATGTCAATTGTTATCCGCTCACAA | $P_L$lacO-1 promoter | p-$P_L$-MicA-Δseed |
| | **ATGA**GTGCTCAGTATCTTGTTATCCGCTCACAATGTCAATTGTTATCCGCTCACAA | $P_L$lacO-1 promoter | p-$P_L$-MicF-Δseed |
| | **ACTT**GTGCTCAGTATCTTGTTATCCGCTCACAATGTCAATTGTTATCCGCTCACAA | $P_L$lacO-1 promoter | p-$P_L$-OmrB-Δseed |
| **TTC**TTGTGAGCGGATAACAATTGACATTGTGAGCGGATAACAAGATACTGAGCACCCATG | **CCG**CATGGGTGCTCAGTATCTTGTTATCCGCTCACAATGTCAATTGTTATCCGCTCACAA | $P_L$lacO-1 promoter | p-$P_L$ |
| GCGAAGACAA**CATC**CCTGAATTCAGAGATG | GCGAAGACAA**ATCC**GATACCGAACCGTTTGCG | MicA scaffold | p-$P_L$-MicA-s8<br>p-$P_L$-MicA-Δseed |
| GCGAAGACAA**TCAT**TTCTGAATGTCTGTTTAC | GCGAAGACAA**ATCC**CTGTGGTAGCACAGAATAATG | MicF scaffold | p-$P_L$-MicF-s8<br>p-$P_L$-MicF-Δseed |
| GCGAAGACAA**AAGT**CAACTTCGGGTTGAG | GCGAAGACAA**ATCC**GTCGGTTACTGTTACAGATTG | OmrB scaffold | p-$P_L$-OmrB-s8<br>p-$P_L$-OmrB-Δseed |
| **CCAT**CATATGTAAACCTCGA | **GTAC**TCGAGGTTTACATATG | 16 nt seed region, variant 8, target: *acrA* | p-$P_L$-s8-RybB-$P_L$-s28-RybB<br>p-$P_L$-s8-RybB-$P_J$-s28-RybB<br>p-$P_L$-s8-RybB-$P_L$-s28-MicA<br>p-$P_L$-s8-RybB-$P_J$-s28-MicA |
| **CTTG**GTAAACCCTCTGTTTT | **TGCA**AAAACAGAGGGTTTAC | 16 nt seed region, variant 28, target: *acrA* | p-$P_L$-s8-RybB-$P_L$-s28-RybB<br>p-$P_L$-s8-RybB-$P_J$-s28-RybB<br>p-$P_L$-s8-RybB-$P_L$-s28-MicA<br>p-$P_L$-s8-RybB-$P_J$-s28-MicA |
| **GCAA**TTGTGAGCGGATAACAATTGACATTGTGAGCGGATAACAAGATACTGAGCAC | **CAAG**GTGCTCAGTATCTTGTTATCCGCTCACAATGTCAATTGTTATCCGCTCACAA | $P_L$lacO-1 promoter | p-$P_L$-s8-RybB-$P_L$-s28-RybB<br>p-$P_L$-s8-RybB-$P_L$-s28-MicA |
| **GCAA**TTGACAGCTAGCTCAGTCCTAGGTATAATGCTAGC | **CAAG**GCTAGCATTATACCTAGGACTGAGCTAGCTGTCAA | $P_{BBa\_J23119}$ promoter | p-$P_L$-s8-RybB-$P_J$-s28-RybB<br>p-$P_L$-s8-RybB-$P_J$-s28-MicA |
| GCGAAGACAA**GTAC**GATGTCCCCATTTTGTGGAG | GCGAAGACAA**TTGC**GAGGGTTGCAGGGTAGTAG | RybB scaffold | p-$P_L$-s8-RybB-$P_L$-s28-RybB<br>p-$P_L$-s8-RybB-$P_J$-s28-RybB<br>p-$P_L$-s8-RybB-$P_L$-s28-MicA<br>p-$P_L$-s8-RybB-$P_J$-s28-MicA |
| GCGAAGACAA**TGCA**GATGTCCCCATTTTGTGGAG | GCGAAGACAA**ATCC**GAGGGTTGCAGGGTAGTAG | RybB scaffold | p-$P_L$-s8-RybB-$P_L$-s28-RybB<br>p-$P_L$-s8-RybB-$P_J$-s28-RybB |
| GCGAAGACAA**TGCA**CATCCCTGAATTCAGAGATG | GCGAAGACAA**ATCC**GATACCGAACCGTTTGCG | MicA scaffold | p-$P_L$-s8-RybB-$P_L$-s28-MicA<br>p-$P_L$-s8-RybB-$P_J$-s28-MicA |

(bold: overhangs for Golden Gate cloning; red: BbsI recognition sites)

**Table S2.** Oligodeoxynucleotides used for λ red recombineering, screening and Northern blot analysis.

| Name | Sequence [5'-3'] | Purpose |
|---|---|---|
| acrAB-KO-1 | **ACCATTGACCAATTTGAAATCGGACACTCGAGGTTTACAT**GCTCATATGAATATCCTCCTTAG | Forward primer for deletion of *acrA* and *acrAB* |
| acrAB-KO-2 | **AAAAAGGCCGCTTACGCGGCCTTAGTGATTACACGTTGTA**GCCTTTGAGTGAGCTGATAC | Reverse primer for deletion of *acrAB* |
| acrA-KO-2 | **GATAAAGAAATTAGGCATGTCTTAACGGCTCCTGTTTAAG**CTAGAGCTAACTAACTTGTAGGCTG | Reverse primer for deletion of *acrA* |
| acrA-yfp-1 | **AGCCGCAAGCGGTGCTCAGCCTGAACAGTCCAAGTCTTAA**CCGAATTCAGAGAAAGAGGAG | Forward primer for transcriptional fusion of *acrA* to *syfp2* |
| acrA-yfp-2 | **GATAAAGAAATTAGGCATGTCTTAACGGCTCCTGTTTAAG**CTAGAGCTAACTAACTTGTAGGCTG | Reverse primer for transcriptional fusion of *acrA* to *syfp2* |
| acrA-yfp-5 | **TCGAGGTTTACATATGAACAAAAACAGAGGGTTTACGCCT**GTTAGCAAGGGCGAAGAACTTTTTAC | Forward primer for translational fusion of *acrA* first 9 codons to *syfp2* |
| acrA-yfp-6 | **GATAAAGAAATTAGGCATGTCTTAACGGCTCCTGTTTAAG**CTAGAGCTAACTAACTTGTAGGCTG | Reverse primer for translational fusion of *acrA* first 9 codons to *syfp2* |
| acrAB-scr-1 | GTATGTACCATAGCACGACG | Screening of *acrA* and *acrAB* manipulations |
| acrAB-scr-2 | GAGATCCTGAGTTGGTGG | Screening of *acrA* and *acrAB* manipulations |
| sYFP2_out | CGCGTCTTGTAGTTACCG | Screening of *acrA-syfp2* fusions |
| rybB-KO-1 | **AACCGCAGAACTTTTCCGCAGGGCATCAGTCTTAATTAGT**GCTCATATGAATATCCTCCTTAG | Forward primer for deletion of *rybB* |
| rybB-KO-2 | **GTTGAGAGGGTTGCAGGGTAGTAGATAAGTTTTAGATAAC**GCCTTTGAGTGAGCTGATAC | Reverse primer for deletion of *rybB* |
| rybB-scr-1 | GGTATGGCCAGGATTAGG | Screening of *rybB* deletion |
| rybB-scr-2 | GAGGATGGTTGAGAGGG | Screening of *rybB* deletion |
| RybB-probe-2 | GAAATGGCGGGGTTGATGGGCTCCACAAAATGGGGACATC | Detection of RybB |
| 5S probe-2 | CCTGGCAGTTCCCTACTCTCGCATGAGGAG | Detection of 5S rRNA |
| Mult-Targ-Scr-Fw | CTGTCAAATGGACGAAGCAG | Forward primer for colony PCR of Golden Gate constructs |
| Mult-Targ-Scr-Rev | CAGGCAAATTCTGTTTTATCAGACC | Reverse primer for colony PCR of Golden Gate constructs |

(bold: overhangs for homologous recombination; underlined: sequences for PCR amplification)