

TABLE S1 Primers for RACE and cloning

| primer name | primer sequence | use in this study | reference |
|----------------------|---|--|------------|
| sinl-5'-RACE1 | ATCGGTGACCGTGACGATATGG | 5'-RACE of SMc00168 | 50 |
| sinl-5'-RACE2 | ATGGTGACCTGGTTCGATGC | 5'-RACE of SMc00168 | 50 |
| Psinl-H-fwd | CCTAAAGCTTCAACGATTCTCCGGCATATCC | Construction of pLK64 | 50 |
| Psinl-X-rev | TCCCTCTAGAACCGTTCCGTTCACTATCCT | Construction of pLK64 | 50 |
| Psinl-X-revII | GGCTCTAGACATTTTCGCTCCATGCGT | Construction of pLK60 | This study |
| sinl-noTGA+ATG-X-rev | GATTCTAGACATGGCGGCGCGTGCCTCAAGC | Construction of pLK61 | This study |
| PsinR-H-fwd | TGCCAAGCTTCGCAATTCTGTCGCCGT | Construction of pLK65 | 50 |
| PsinR-X-rev | CAAATCTAGACATGCCGTAACACCGAAAC | Construction of pLK65 | 50 |
| PcspA3-H-fwd | TGCCAAGCTTGAAGACGCAATACCTCGTCA | Construction of pLK002 | 24 |
| PcspA3-X-rev | CAAATCTAGACTCGGCCATCGTCAGGT | Construction of pLK002 | 24 |
| PcspA3-219-rev | ACAGTAAGAGTGATGGTTT | Fusion primer for construction of pLKpLKrec01 | This study |
| PcspA3_msinl-fwd | AAACCCATCACTCTTACTGTATCGCGTAATCACGCATGG | Fusion primer for construction of pLKpLKrec01 | This study |
| Psinl-29-rev | GTAGCGATGCTGTCAGGCT | Fusion primer for construction of pLKpLK rec02 | This study |
| Psinl_mcspA3-fwd | AGCCTGACAGCATCGCTACGTTGGGCAATCTCGA | Fusion primer for construction of pLKpLKrec02 | This study |
| rne-Ndel-fwd | CGCACATATGGCAGAGAAAATGCTTATC | Construction of pBSrne and pWBrne | This study |
| rne-XbaI-rev | TGCTCTAGATTAGAAGAACCGCGCG | Construction of pBSrne and pWBrne | This study |
| rne-T5stop-H-rev | CATAAGCTTCAATCTCGTGGACTCCT | Construction of pWBrne675 and pK1123-2024 | This study |
| rne-B-1123-fwd | ATCGGATCCCTCTACAACAAGGACATCAGCGA | Construction of pK1123-2024 | This study |
| rne-E-79-fwd | GAAGAATTGATTCGAATCGG | Construction of pK79-926 | This study |
| rne-B-926-rev | ATCGGATCCCGCATCTCCTCAGACGCTT | Construction of pK79-926 | This study |

TABLE S2 Primer pairs for qRT-PCR analysis

| target gene | forward strand | reverse strand | primer pair efficiency or reference |
|---|-----------------------|----------------------|-------------------------------------|
| SMc00170; <i>sinR</i> | CTGGATATCGTGGAAATATGG | GGTCGATCCTGTAAAGC | 1.951 |
| SMc00168, <i>sinl</i> , amplification of bp 4-251 | TCAGGATAGTGAACGGAAA | GTATCGTCCAGCATATTG | 1.933 |
| SMc00168, <i>sinl</i> , amplification of bp 232-434 | AATATGCTGGACGATACGTT | GTGACGATATGGCTGATG | 1.856 |
| SMc01336, <i>rne</i> , upstream of mini-Tn5 | ACATCATCATCAACCAGACC | AGGCAATCCTTAGCTTCTT | 1.924 |
| SMc01336, <i>rne</i> , downstream of mini-Tn5 | CGAGAGTGTGATGAAGAAG | CCCTTTGGGTTTGT | 1.908 |
| SMc01317, <i>rpoB</i> | ATCCTCGACACCTTCTACAC | GATAGTTGCCGTAGAGATCG | 1.987 |
| SMc03224, 16S | TCTACGGAATAACGCAGG | GTGTCAGTCCCAGT | ref. 55 |

| Culture Nr. | Strain | IPTG | Position \ time (min) | +1 | +20 | +24 | +114 | +117 | +133 | +219 | +239 | +243 | +262 | +268 | +284 | +295 | +297 | +306 | +313 | +330 | +326 | +332 | +337 | +338 | +357 | +359 | | |
|-------------|-------------------|------|-----------------------|----|-----|-----|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|---|--|
| 1 | 2011 | - | - | 9 | 6 | | | | | | | | | | | 1 | | | | | | | | | 8 | | | |
| 2 | 2011 | + | 60 | | 12 | 5 | | | | | | | | | | | | | | | | | | 1 | 6 | | | |
| 3 | 2011 (pWBrne) | - | - | 4 | 2 | 2 | | | 2 | | | | | 2 | | | | | | | | | 2 | | | | | |
| 4 | 2011 (pWBrne) | + | 20 | | | 1 | | | | 1 | | | | | | | | | | | | | | | 1 | 1 | | |
| | 2011 (pWBrne) | + | 40 | | | 2 | | 2 | | | | | | | | | | | | | | | | | | 1 | | |
| | 2011 (pWBrne) | + | 60 | | | 1 | | | | | | | 1 | | | 1 | | | | | | | | | | 1 | | |
| 5 | 2011 (pWBrne) | - | - | 6 | 2 | | | | | | | | | 1 | | | | | | | | | | | | | | |
| 6 | 2011 (pWBrne) | + | 20 | | | 3 | | | | | | | | | | | | | | 2 | | | | | 1 | 2 | 1 | |
| | 2011 (pWBrne) | + | 40 | | | | | | | | | | | | | | | | | | | | | | | | | |
| | 2011 (pWBrne) | + | 60 | | | | | | | | | | | | | | | | | | | | | | | | | |
| 7 | rne::Tn5 (pWBrne) | - | - | 15 | | | | | | | | | | | | | | | | | | | | | | | | |
| 8 | rne::Tn5 (pWBrne) | + | 20 | 3 | | | | | | | | | | | | | | | | | | | | | | | | |
| | rne::Tn5 (pWBrne) | + | 40 | 3 | 2 | | | | | | | | | 1 | | | | | | | | | | | | | | |
| | rne::Tn5 (pWBrne) | + | 60 | 3 | 1 | | | | | | | | | | | | | | | | | | | | | | | |
| 9 | rne::Tn5 (pWBrne) | - | - | 9 | | | | | | | | | | | | | | | | | | | | | | | | |
| 10 | rne::Tn5 (pWBrne) | + | 20 | 3 | | | | | | | | | | | | | | 2 | | | | | | | | | | |
| | rne::Tn5 (pWBrne) | + | 40 | 3 | 3 | | | | | | | | | | | | | | | | | | | | | | | |
| | rne::Tn5 (pWBrne) | + | 60 | | | | | | | | | | | | 2 | | | | | | | | | | | | | |

Table S3. The results of all 5'-RACE experiments in detail. For the determination of 5'-ends of RNA by 5'-RACE *S. meliloti* cells were grown in TY medium to an OD₆₀₀ of 1.0. Ectopic expression of *rne* was induced by addition of 1 mM IPTG. Cells were harvested 20 min, 40 min, and 60 min after induction. No IPTG was added to the control cultures. RNA was isolated and 5'-RACE analysis of *sinl* was performed. Given is the number of the clones corresponding to a certain position in the *sinl* transcript (+1 is the transcriptional start site, +24 is the putative RNase E cleavage site between the Shine-Dalgarno sequence and the start codon). Results are listed separately for each independent culture. For cultures with IPTG, the results are shown in a time resolution manner.