

**Table S1.** Related to Figure 1. DNA templates used in this study.

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| <p>Pre-16S<br/>DNA</p>                      | <p><b>GATCCTAATACGACTCACTATA</b><b>GGGTGAGTGAGAGATGGATGGGTAGAGAGTTAGTAGTA</b><br/> <b>AGG</b>CCGCTGAGAAAAAGCGAAGCGGCACTGCTCTTTAACAAATTTATCAGACAATCTGTGT<br/> GGGCACTCGAAGATACGGATTCTTAACGTCGCAAGACGAAAAATGAATACCAAGTCTCAA<br/> GAGTGAACACGTAATTCATTACGAAGTTTAATTCTTTGAGCGTCAAACCTTTTAAATTGAA<br/> GAGTTTGATCATGGCTCAGATTGAACGCTGGCGGCAGGCCTAACACATGCAAGTCGAACG<br/> GTAACAGGAAGAAGCTTGCTTCTTTGCTGACGAGTGGCGGACGGGTGAGTAATGTCTGGG<br/> AAACTGCCTGATGGAGGGGGATAACTACTGGAACCGGTAGCTAATACCGCATAACGTCGC<br/> AAGACCAAAGAGGGGGACCTTCGGGCCTCTTGCCATCGGATGTGCCCAGATGGGATTAGC<br/> TAGTAGGTGGGGTAACGGCTCACCTAGGCGACGATCCCTAGCTGGTCTGAGAGGATGACC<br/> AGCCACACTGGAAGTGAACACGGTCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATT<br/> GCACAATGGGCGCAAGCCTGATGCAGCCATGCCCGGTGTATGAAGAAGGCCTTCGGGTTG<br/> TAAAGTACTTTTACGCGGGGAGGAAGGGAGTAAAGTTAATACCTTTGCTCATTGACGTTAC<br/> CCGCAGAAGAAGCACCGGCTAACTCCGTGCCAGCAGCCGCGGTAATACGGAGGGTGCAAG<br/> CGTTAATCGGAATTACTGGGCGTAAAGCGCACGCAGGCGGTTTGTAAAGTCAGATGTGAA<br/> ATCCCCGGGCTCAACCTGGGAAGTGCATCTGATACTGGCAAGCTTGAGTCTCGTAGAGGG<br/> GGGTAGAATTCAGGTGTAGCGGTGAAATGCGTAGAGATCTGGAGGAATACCGGTGGCGA<br/> AGGCGGCCCCCTGGACGAAGACTGACGCTCAGGTGCGAAAGCGTGGGGAGCAAACAGGAT<br/> TAGATACCCTGGTAGTCCACGCCGTAACGATGTCGACTTGGAGGTTGTGCCCTTGAGGC<br/> GTGGCTTCCGGAGCTAACCGTAAAGTCGACCGCCTGGGGAGTACGGCCGCAAGGTTAAA<br/> ACTCAAATGAATTGACGGGGGCCGCACAAGCGGTGGAGCATGTGGTTTAATTTCGATGCA<br/> ACGCGAAGAACCTTACCTGGTCTTGACATCCACGGAAGTTTTTACAGAGATGAGAATGTGCC<br/> TTCGGGAACCGTGAGACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTGTGAAATGTTGG<br/> GTTAAGTCCCAGCAACGAGCGCAACCCTTATCCTTTGTTGCCAGCGGTCCGGCCGGGAACT<br/> CAAAGGAGACTGCCAGTGATAAACTGGAGGAAGGTGGGGATGACGTCAAGTCATCATGGC<br/> CCTTACGACCAGGGCTACACACGTGCTACAATGGCGCATACAAAGAGAAGCGACCTCGCG<br/> AGAGCAAGCGGACCTCATAAAGTGCCTGATGTCAGTCCGGATTGGAGTCTGCAACTCGACTCC<br/> ATGAAGTCGGAATCGTAGTAATCGTGGATCAGAATGCCACGGTGAATACGTTCCCGGGC<br/> CTTGATACACACCGCCCGTACACCATGGGAGTGGGTTGCAAAGAAGTAGGTAGCTTAAC<br/> CTTCGGGAGGGCGCTTACCCTTTGTGATTGATGACTGGGGTGAAGTCGTAACAAGGTAA<br/> CCGTAGGGGAACCTGCGGTTGGATCACCTCCTTACCTTAAAGAAGCGTACTTTGTAGTGC<br/> TCACACAGATTGTCTGATAGAAAGTAAAAGCAAGGCGTTTACGCGTTGGGAGTGAAGGCT<br/> GAAGAGAATAAGGCGTTCGCTTTCTATTAATGAAAGCTCACCTACACGAAAATATCAC<br/> GCAACGCGTGATAAGCAATTTTCGT<b>GGACGACACACTTTGGACAGGACACACAGGACACA</b><br/> <b>GGCTAGCATAACCCCTTGGGCCTCTAACGGGTCTTGAGGGTTTTTTG</b></p> |
| <p>16S<br/>1-100 DNA</p>                    | <p><b>GATCCTAATACGACTCACTATA</b><b>GGGTGAGTGAGAGATGGATGGGTAGAGAGTTAGTAGTA</b><br/> <b>AGAAATTGAAGAGTTTGAT</b>CATGGCTCAGATTGAACGCTGGCGGCAGGCCTAACACATGC<br/> AAGTCGAACGGTAACAGGAAGAAGCTTGCTTCTTTGCTG<b>GGACGACACACTTTGGACAGG</b><br/> <b>ACACACAGGACACAGGCTAGCATAACCCCTTGGGCCTCTAACGGGTCTTGAGGGTTTT</b><br/> <b>TTTG</b></p>  |
| <p>5'<br/>truncated<br/>pre-16S<br/>DNA</p> | <p><b>GATCCTAATACGACTCACTATA</b><b>GGGTGAGTGAGAGATGGATGGGTAGAGAGTTAGTAGTA</b><br/> <b>AGA</b>CTGGGCGTAAAGCGCACGCAGGCGGTTTTGTTAAGTCAGATGTGAAATCCCCGGGCTC<br/> AACCTGGGAAGTGCATCTGATACTGGCAAGCTTGAGTCTCGTAGAGGGGGGTAGAATTCC<br/> AGGTGTAGCGGTGAAATGCGTAGAGATCTGGAGGAATACCGGTGGCGAAGGCGGCCCCCT<br/> GGACGAAGACTGACGCTCAGGTGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGG<br/> TAGTCCACGCCGTAAACGATGTCGACTTGGAGGTTGTGCCCTTGAGGCGTGGCTTCCGGA<br/> GCTAACGCGTTAAGTCGACCGCCTGGGGAGTACGGCCGCAAGGTTAAAACCTCAAATGAAT<br/> TGACGGGGGCCCGCACAAAGCGGTGGAGCATGTGGTTTTAATTCGATGCAACGCGAAGAACC<br/> TTACCTGGTCTTGACATCCACGGAAGTTTTTACAGAGATGAGAATGTGCCTTCGGGAACCGT<br/> GAGACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTGTGAAATGTTGGGTTAAGTCCCGC<br/> AACGAGCGCAACCCTTATCCTTTGTTGCCAGCGGTCCGGCCGGGAACCTCAAAGGAGACTG</p>   |

|                      |  |
|----------------------|--|
|                      | <p>CCAGTGATAAACTGGAGGAAGGTGGGGATGACGTCAAGTCATCATGGCCCTTACGACCAG<br/> GGCTACACACGTGCTACAATGGCGCATACAAAGAGAAGCGACCTCGCGAGAGCAAGCGGA<br/> CCTCATAAAGTGCCTCGTAGTCCGGATTGGAGTCTGCAACTCGACTCCATGAAGTCGGAA<br/> TCGCTAGTAATCGTGGATCAGAATGCCACGGTGAATACGTTCCCGGGCCTTGTACACACC<br/> GCCCCGCACACCATGGGAGTGGGTTGCAAAGAAGTAGGTAGCTTAACCTTCGGGAGGGC<br/> GCTTACCACCTTTGTGATTCATGACTGGGGTGAAGTCGTAACAAGGTAACCGTAGGGGAAC<br/> CTGCGGTTGGATCACCTCCTTACCTTAAAGAAGCGTACTTTGTAGTGCTCACACAGATTG<br/> TCTGATAGAAAGTGAAAAGCAAGGCGTTTACGCGTTGGGAGTGAGGCTGAAGAGAATAAG<br/> GCCGTTTCGCTTTCTATTAATGAAAGCTCACCTACACGAAAATATCACGCAACGCGTGAT<br/> AAGCAATTTTCGT<b>GGACGACACACTTTGGACAGGACACACAGGACACAGGCTAGCATAAC</b><br/> <b>CCCTTGGGGCCTCTAACGGGTCTTGAGGGGTTTTTTG</b></p>   |
| 16S 5WJ<br>DNA       | <p><b>GATCCTAATACGACTCACTATA</b><b>GGGTGAGTGAGAGATGGATGGGTAGAGAGTTAGTAGTA</b><br/> <b>AGGG</b><u>C</u>TAGATTGAACGCTGGCGGCAGGctcaaCCATGCCGCGTGTATGAAGAAGGCCTT<br/> CGGGTTGTAAAGTACTTTTCAGCGGGGAGGAAGGGAGTAAAGTTAATACCTTTGCTCATTG<br/> ACGTTACCCGCAGAAGAAGCACCGGCTAACTCCGTGCCAGCAGCCGCGGTAATACGGAGG<br/> GTGCAAGCGTTAATCGGAATT<b>GGACGACACACTTTGGACAGGACACACAGGACACAGGCT</b><br/> <b>AGCATAACCCCTTGGGGCCTCTAACGGGTCTTGAGGGGTTTTTTG</b></p>   |
| 16S 5WJ +<br>5'L DNA | <p><b>GATCCTAATACGACTCACTATA</b><b>GGGTGAGTGAGAGATGGATGGGTAGAGAGTTAGTAGTA</b><br/> <b>AGG</b><u>C</u>CGCTGAGAAAAAGCGAAGCGGCCTGCTCTTTAACAATTTATCAGACAATCTGTGT<br/> GGGCACTCGAAGATACGGATTCTTAACGTCGCAAGACGAAAAATGAATACCAAGTCTCAA<br/> GAGTGAACACGTAATTCATTACGAAGTTTAATTCCTTTGAGCGTCAAACCTTTTGGCTCAGA<br/> TTGAACGCTGGCGGCAGGctcaaCCATGCCGCGTGTATGAAGAAGGCCTTCGGGTTGTAA<br/> AGTACTTTTCAGCGGGGAGGAAGGGAGTAAAGTTAATACCTTTGCTCATTGACGTTACCCG<br/> CAGAAGAAGCACCGGCTAACTCCGTGCCAGCAGCCGCGGTAATACGGAGGGTGAAGCGT<br/> TAATCGGAATT<b>GGACGACACACTTTGGACAGGACACACAGGACACAGGCTAGCATAACCC</b><br/> <b>CTTGGGGCCTCTAACGGGTCTTGAGGGGTTTTTTG</b></p>  |
| 16S 5'<br>domain     | <p><b>GATCCTAATACGACTCACTATA</b><b>GGGTGAGTGAGAGATGGATGGGTAGAGAGTTAGTAGTA</b><br/> <b>AGAAATTGAAGAGTTTGAT</b><u>C</u>ATGGCTCAGATTGAACGCTGGCGGCAGGCCTAACACATGC<br/> AAGTCGAACGGTAACAGGAAGAAGCTTGCTTCTTTGCTGACGAGTGGCGGACGGGTGAGT<br/> AATGTCTGGGAAACTGCCTGATGGAGGGGGATAACTACTGGAAACGGTAGCTAATACCGC<br/> ATAACGTCGCAAGACCAAAGAGGGGGACCTTCGGGCCTCTTGCCATCGGATGTGCCCAGA<br/> TGGGATTAGCTAGTAGGTGGGGTAACGGCTCACCTAGGCGACGATCCCTAGCTGGTCTGA<br/> GAGGATGACCAGCCACACTGGAAGTGGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGT<br/> GGGGAATATTGCACAATGGGCGCAAGCCTGATGCAGCCATGCCGCGTGTATGAAGAAGGC<br/> CTTCGGGTTGTAAAGTACTTTTCAGCGGGGAGGAAGGGAGTAAAGTTAATACCTTTGCTCA<br/> TTGACGTTACCCGCAGAAGAAGCACCGGCTAACTCCGTGCCAGCAGCCGCGGTAATACGG<br/> AGGGTGAAGCGTTAATC<b>GGACGACACACTTTGGACAGGACACACAGGACACAGGCTAGC</b><br/> <b>ATAACCCCTTGGGGCCTCTAACGGGTCTTGAGGGGTTTTTTG</b></p> |

Color indicates common features of the DNA template design: T7 promoter (green), C-less cassette (blue), Stall site (red highlighted nucleotide), SA5 oligo binding site (orange), Terminator (purple).

**Table S2.** Related to Figures 3 – 5. Kinetic parameters for maximum likelihood fitting of the S4 lifetime data.

| Experiment                                    | $\tau_1$         | $\tau_2$        | $\tau_3$       | $a_1$           | $a_2$            | $a_3$ |
|---|------------------|-----------------|----------------|-----------------|------------------|-------|
| pre-16S<br>post-txn                           | $1.2 \pm 0.1$ s  | $6.5 \pm 0.7$ s | $60 \pm 21$ s  | $0.66 \pm 0.04$ | $0.31 \pm 0.04$  | 0.03  |
| pre-16S<br>co-txn                             | $0.5 \pm 0.1$ s  | $5 \pm 1.5$ s   | $29 \pm 12$ s  | $0.96 \pm 0.01$ | $0.03 \pm 0.001$ | 0.01  |
| 16S <sub>100bp</sub>                          | $0.5 \pm 0.01$ s | —               | —              | —               | —                | —     |
| 5' truncated<br>pre-16S                       | $0.6 \pm 0.1$ s  | $8 \pm 2.1$ s   | —              | $0.98 \pm 0.01$ | 0.02             | —     |
| 16S <sub>5WJ</sub>                            | $1 \pm 0.03$ s   | $8 \pm 1.5$ s   | $95 \pm 31$ s  | $0.89 \pm 0.01$ | $0.08 \pm 0.01$  | 0.03  |
| 16S <sub>5WJ+5'L</sub>                        | $0.6 \pm 0.2$ s  | $9 \pm 1$ s     | $114 \pm 57$ s | $0.90 \pm 0.01$ | $0.07 \pm 0.01$  | 0.03  |
| 16S <sub>5' domain</sub>                      | $0.75 \pm 0.1$ s | $9 \pm 2.2$ s   | $236 \pm 28$ s | $0.90 \pm 0.02$ | $0.07 \pm 0.01$  | 0.03  |
| pre-16S<br>+ S16<br>S17<br>S20                | $0.9 \pm 0.04$ s | $4.5 \pm 0.7$ s | $68 \pm 37$ s  | $0.90 \pm 0.01$ | $0.09 \pm 0.01$  | 0.01  |
| pre-16S<br>+ S16, S17,<br>S20, S5,<br>S8, S12 | $0.7 \pm 0.2$ s  | $6 \pm 1.5$ s   | $98 \pm 20$ s  | $0.89 \pm 0.01$ | $0.09 \pm 0.01$  | 0.02  |
| pre-16S<br>+ S9                               | $0.6 \pm 0.1$ s  | $3 \pm 0.9$ s   | $27 \pm 12$ s  | $0.97 \pm 0.02$ | $0.02 \pm 0.002$ | 0.01  |
| pre-16S<br>+ MS2-MBP                          | $0.7 \pm 0.1$ s  | $15 \pm 5.9$ s  | —              | $0.99 \pm 0.01$ | 0.01             | —     |

Experiments were fit to Equations 1 and 2 outlined (see Methods). Errors in the fitting parameters were calculated by bootstrapping;  $A_3 = 1 - a_1 - a_2$ .