



Figure S1. Analysis of an *sgrS* allele with a premature *sgrT* stop codon on regulation of *ptsG*. A) The *sgrS14* allele was created by deleting the nucleotide highlighted in grey to create a premature *sgrT* stop codon. Thus, the distance between the new stop codon and the beginning of the *sgrS* base pairing region was increased to 121 nt. B) Cultures of JH111 harboring the plasmid carrying P_{lac} -*ptsG'-'gfp* (pZEDB03) and a compatible plasmid with the P_{tet} -*sgrS* (pZADB01) and the P_{tet} -*sgrS14* (pZADB05) alleles were growth to mid-logarithmic phase and expression of *ptsG'-'gfp* and SgrS induced with 0.75mM IPTG and 30ng/ml aTc, respectively. Black bars represent cultures having only the translational fusion induced (-SgrS). Gray bars represent cultures having both the translational fusion and the *sgrS* allele induced (+SgrS). Kinetic fluorescence assays were performed, and the Relative Fluorescence Units (RFU) were measured and normalized to culture density (OD₆₀₀). The RFU/OD₆₀₀ value of cultures that only induced for the translational fusion was normalized to 1.



Figure S2. Levels of SgrS RNA from wild-type and translation-impaired sgrS alleles. Wild-type (JH111) cells containing the P_{tet} -sgrS alleles described in Fig. 2A and Fig. 3A were grown to mid-logarithmic phase, induced with 30ng/ml aTc and total RNA extracted 20 min. after induction. The samples were subjected to Northern blot using a biotinylated SgrS probe.



Figure S3. Translational regulation of *manX* by wild-type and translationimpaired *sgrS* alleles. Cultures of JH111 harboring the plasmid carrying P_{lac} *manX'-'gfp* (pZEMB11) and a compatible plasmid with the P_{tet} -sgrS alleles indicated in Fig. 3A were growth to mid-logarithmic phase and expression of *manX'-'gfp* and SgrS induced with 0.75mM IPTG and 30ng/ml aTc, respectively. Black bars represent cultures having only the translational fusion induced (-SgrS). Gray bars represent cultures having both the translational fusion and the *sgrS* allele induced (+SgrS). Kinetic fluorescence assays were performed, and the Relative Fluorescence Units (RFU) were measured and normalized to culture density (OD₆₀₀). The RFU/OD₆₀₀ value of cultures that only induced for the translational fusion was normalized to 1.







Figure S5. SgrT produced by wild-type and base pairing-deficient *sgrS* alleles. Cultures of JH111 containing the P_{lac} -plasmid vector (pBRCS12), *S*. Typhimurium wild-type *sgrS* plasmid (pBRCS22) or *sgrS1* plasmid (pBRCS27) were grown to mid-logarithmic phase. Transcription of P_{lac} -sgrS alleles was induced, total protein extracted and subject to Western blot as described in Fig. 4B.



Figure S6. Analysis of SgrS stability A) A culture of JH111 containing the *S*. Typhimurium wild-type *sgrS* plasmid (pBRCS22) was grown to mid-logarithmic phase, induced with 0.1mM IPTG for 10 min. and total RNA extracted (0 min. time point). Rifampicin was added to inhibit both SgrS and target synthesis, and total RNA extracted at the time points indicated. RNA samples were subjected to Northern blot and probed for SgrS. B) A culture of JH111 harboring pBRCS22 was grown to mid-logarithmic phase, induced with 0.1mM IPTG for 10 min. and total RNA extracted (0 min. time point). The inducer IPTG was then washed out by filtration and re-suspended in same volume of media to stop SgrS synthesis, but allow for continued target mRNA transcription. Total RNA was extracted at the time points indicated. RNA samples were subjected to Northern blot and probed for SgrS.