Protocol S11. Ribosome profiles, translation fidelity, and cellular RNA analyses

The crude S30 extracts were loaded on linear sucrose density gradients as described [1], and 40% sucrose was used as cushion and ribosomes or subunits were isolated by high-speed ultracentrifugation at 4°C for 16 hrs essentially as previously described [2]. Translational fidelity assay on the deletion mutants were performed with the reported expression plasmids as described earlier [3-5].

The cellular rRNA was analyzed by growing the overnight cultures of the parental WT (BW25113) and the Keio ΔyaiF and ΔrsgA single gene deletion mutant strains in fresh LB media essentially as previously described [6,7]. The rRNA from the indicated cells was purified using the RNeasy kit (QIAGEN) following manufacturer's protocols. RNA samples were resolved on a 0.7% agarose and 0.9% Synergel for 4h (Diversified Biotech) and processed by Northern hybridization essentially as described [7], except that the primer extension of 16S or 17S rRNAs was performed using a biotynlated oligonucleotide probes. Immunoblot analysis was carried out using HRP-conjugated streptavidin. Single-stranded DNA probe complementary to the internal fragment of 16S rRNA was used as control. The sequences for the probes used are as follows: 16S internal probe (5’-TACTCACCGTCCGCCCTC-3’); 17S- 5’ probe (5’-CGTATCTTCTCGAGTGCCCACA-3’); 17S- 3’ probe (5’-ACGCTTCTTTAAGGTAGG-3’).

References:

