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 biotnylated 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'-TACTCACCCGTCCGCCACTC-3'); 17S- 5' probe (5'-CGTATCTTCGAGTGCCCACA-3'); 17S- 3' probe (5'- ACGCTTCTTTAAGGTAAGG-3').

References:

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