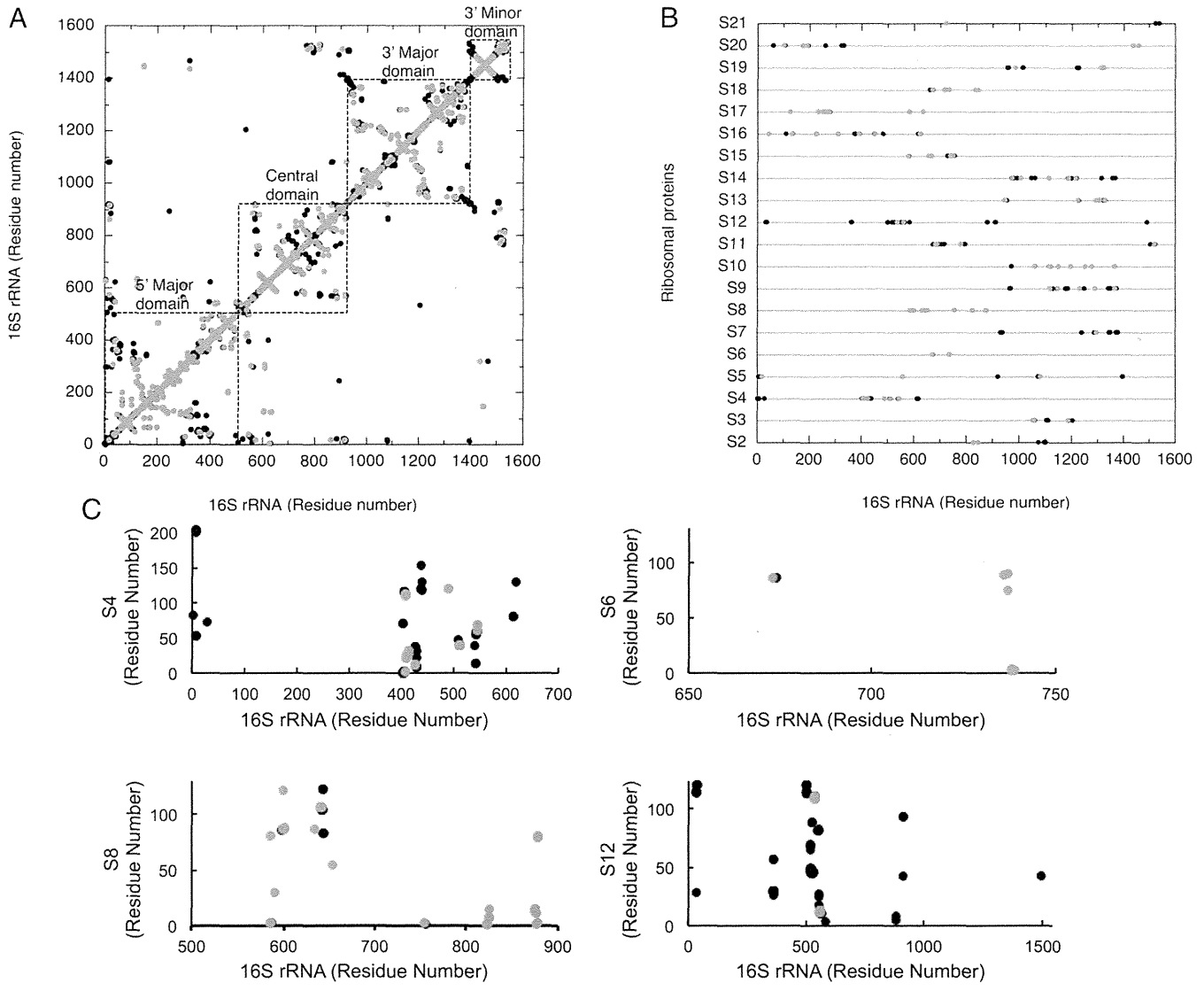

Fig. 3. Variable nucleotides involved in (*A*) intra-16S rRNA and (*B*) 16S rRNA–ribosomal protein interactions. Contact maps were constructed based on the crystal structure of the *E. coli* ribosome (PDB ID code 3R8O) (23). Atomic interactions between oxygen, phosphorus, or nitrogen atoms within 3.4 Å distances were extracted and dotted in black. Contacts that involve variable nucleotides are dotted in red. (*C*) Detailed two-dimensional contact maps of 16S rRNA and some ribosomal proteins. The dots have the same color as those in *A* and *B*. The lengths of the ribosomal proteins S4, S6, S8, and S12 are 206 aa, 131 aa, 130 aa, and 124 aa, respectively.

sequences, we searched for their closest relatives in the database by using the SEQMATCH service supplied by the Ribosomal Database Project at http://rdp.cme.msu.edu/ (29) with the filter setting "isolated type strains, with a length of 1200 bp~". Then, we used the TREE BUILDER service to construct the neighbor-joining tree. The 16S rRNA genes of *E. coli*, *S. ficaria*, and *R. pickettii*, together with some other relevant 16S rRNA genes, such as those from *Salmonella enterica* or *P. vulgaris* (19), were also included in the analysis. The sequence of *Deinococcus aquiradiocola* was used as an outgroup.

Growth Phenotype Analysis. Phenotypes of the KT103 derivatives were quantitatively analyzed from the DT as described previously (8, 14, 17). Briefly, 0.4 μL (0.2%, vol/vol) of overnight preculture was inoculated into 200 μL of 2× YT supplemented with 100 μg/mL Zeocin and 5% (wt/vol) sucrose in a flat-bottom 96-well microplate. The microplate was incubated at 37 °C with vigorous agitation in a VersaMax plate reader (Molecular Devices), and the absorbance at 600 nm (A_{600}) was monitored every 15 min. The average DT was calculated from at least five independent cultures.

Calculation of Atomic Contacts in the 30S Ribosome. Interatomic contacts within 16S rRNA and between 16S rRNA and ribosomal proteins were determined with the CONTACT software (30) by using the atomic coordinates of the *E. coli* 30S ribosome (PDB ID code, 3R8O) (23). The contacts were defined with an interatomic distance of <3.4 Å. To sort out specific interactions (i.e., electrostatic and hydrogen-bonding interactions), carbon atoms were excluded from the calculation.

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Supporting Information

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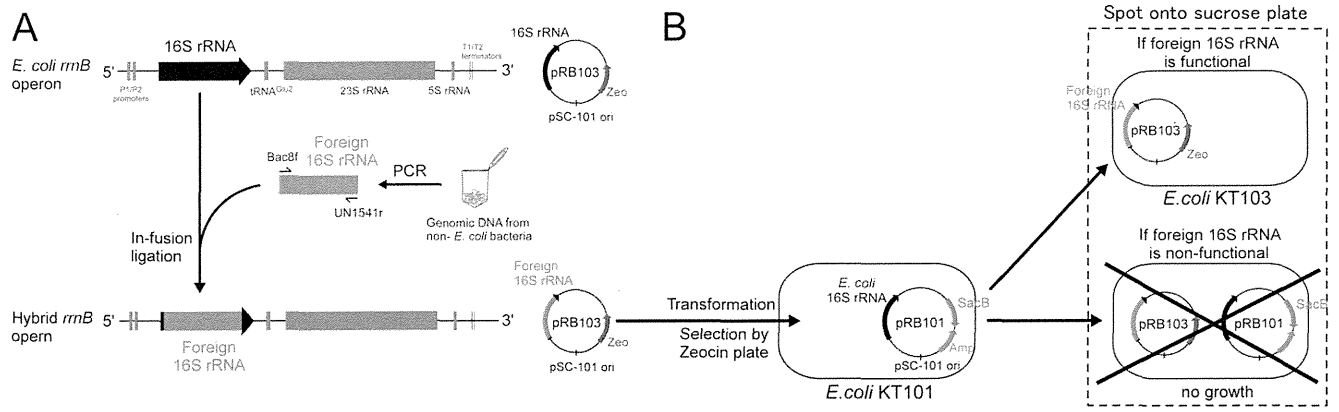

Fig. S1. Functionality test of metagenomically retrieved foreign 16S rRNA. (*A*) In-fusion cloning scheme of foreign 16S rRNA genes into an expression vector pRB103 containing the entire *rrnB* operon. The *Escherichia coli* 16S rRNA gene in pRB103 was substituted with foreign 16S rRNA genes amplified from the environmental DNA (metagenome). (*B*) A genetic method to test the functionality of foreign 16S rRNA genes. *E. coli* Δ7 strain KT101was used to screen for foreign 16S rRNA genes compatible with an *E. coli* genetic background.

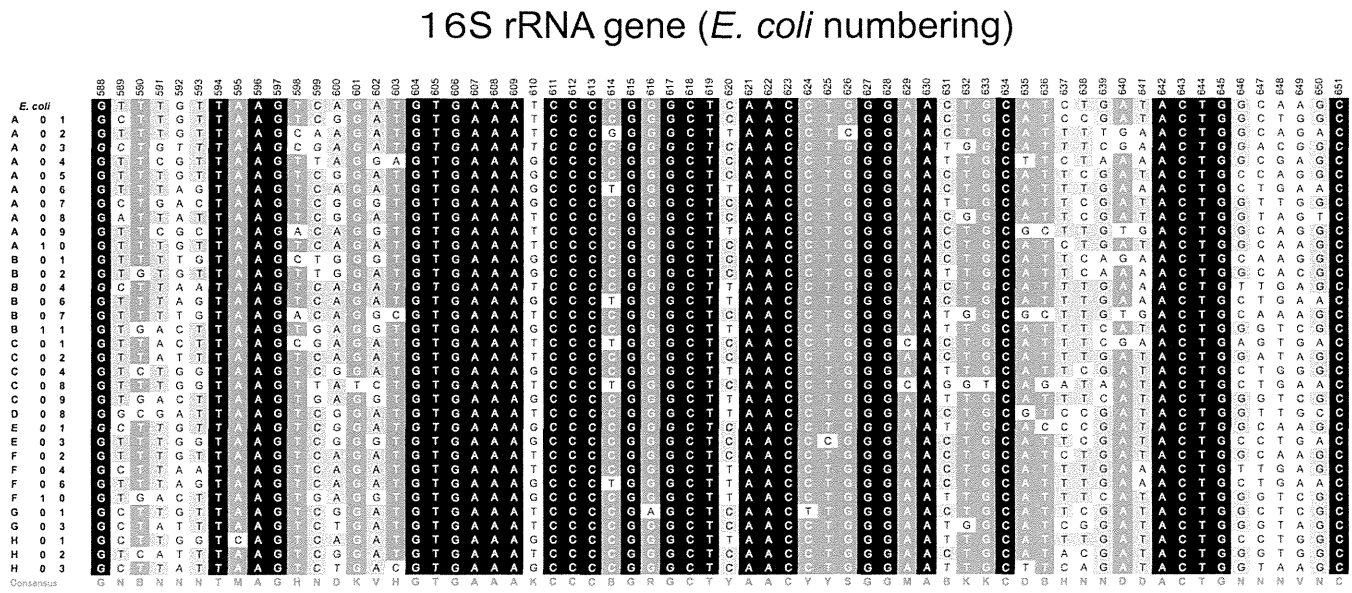

Fig. S2. Multiple alignment of the h21 region (G588–C651) of the 16S rRNA genes obtained from the environmental metagenome. The residues conserved in all of the sequences are shaded in black, those conserved in 80% of the sequences are in dark gray, and those conserved in 60% are in light gray. The consensus sequence is shown in red at the bottom of the alignment.

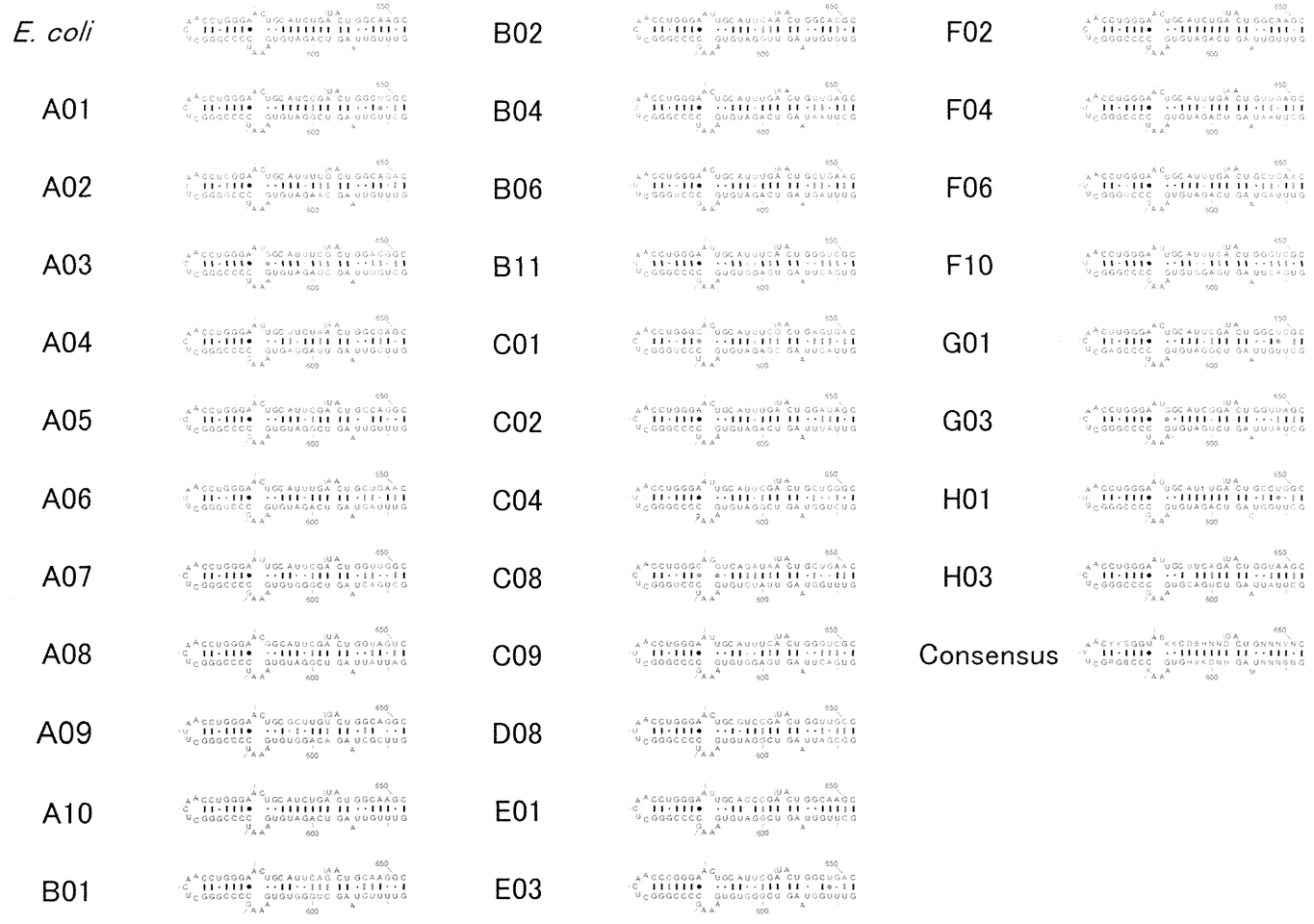

Fig. S3. Conserved nature of the secondary structure of h21 of the 16S rRNA obtained from the environmental metagenome.

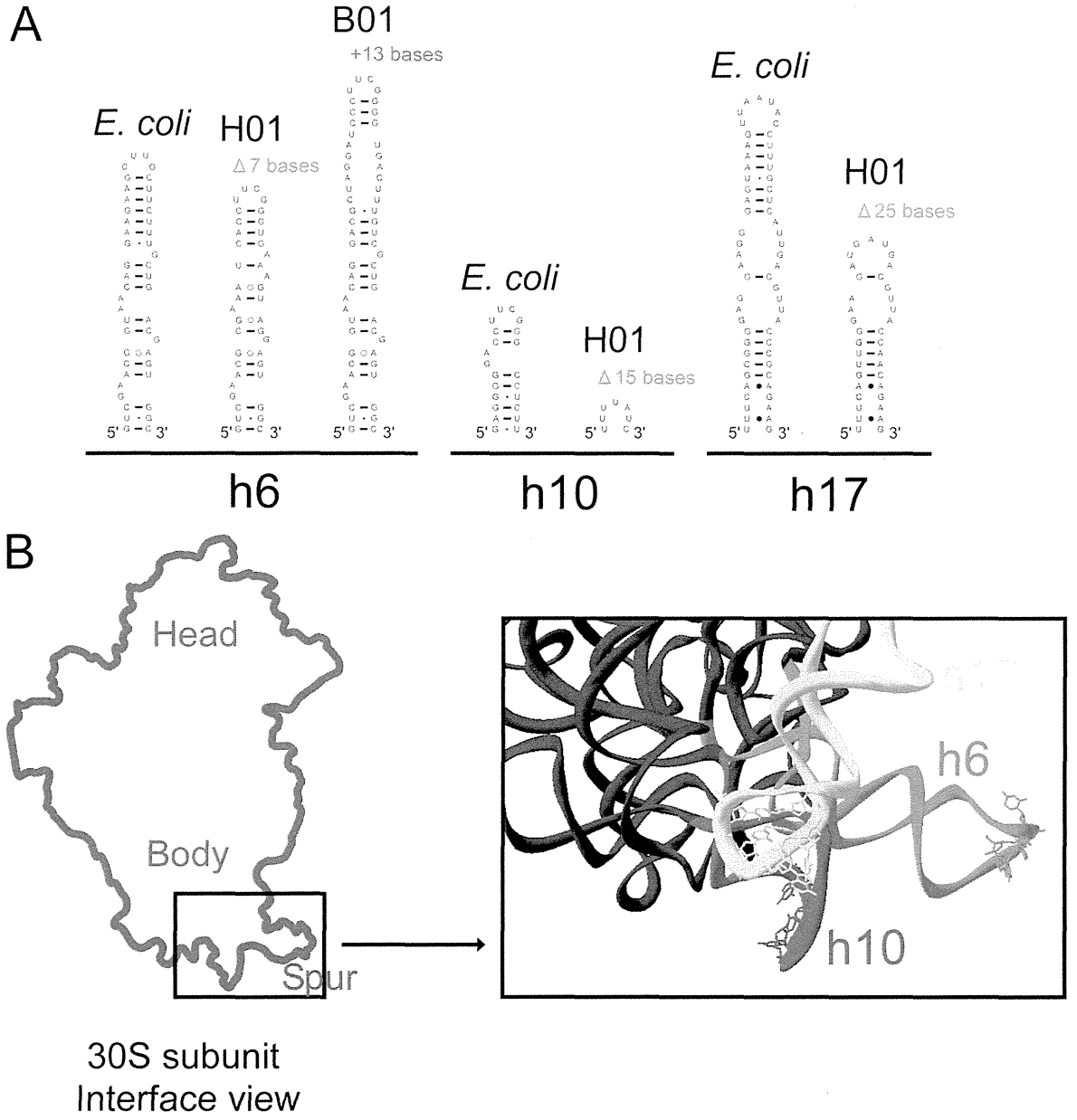

Fig. S4. Length polymorphism of RNA helices. (*A*) Comparison of secondary structures. The RNA h6, h10, and h17 of H01 are shorter than those of *E. coli* by 7, 15, and 25 bases, respectively. h6 of B01 is elongated by 13 bases. (*B*) Positions of h6, h10, and h17 in the crystal structure of the *E. coli* ribosome.