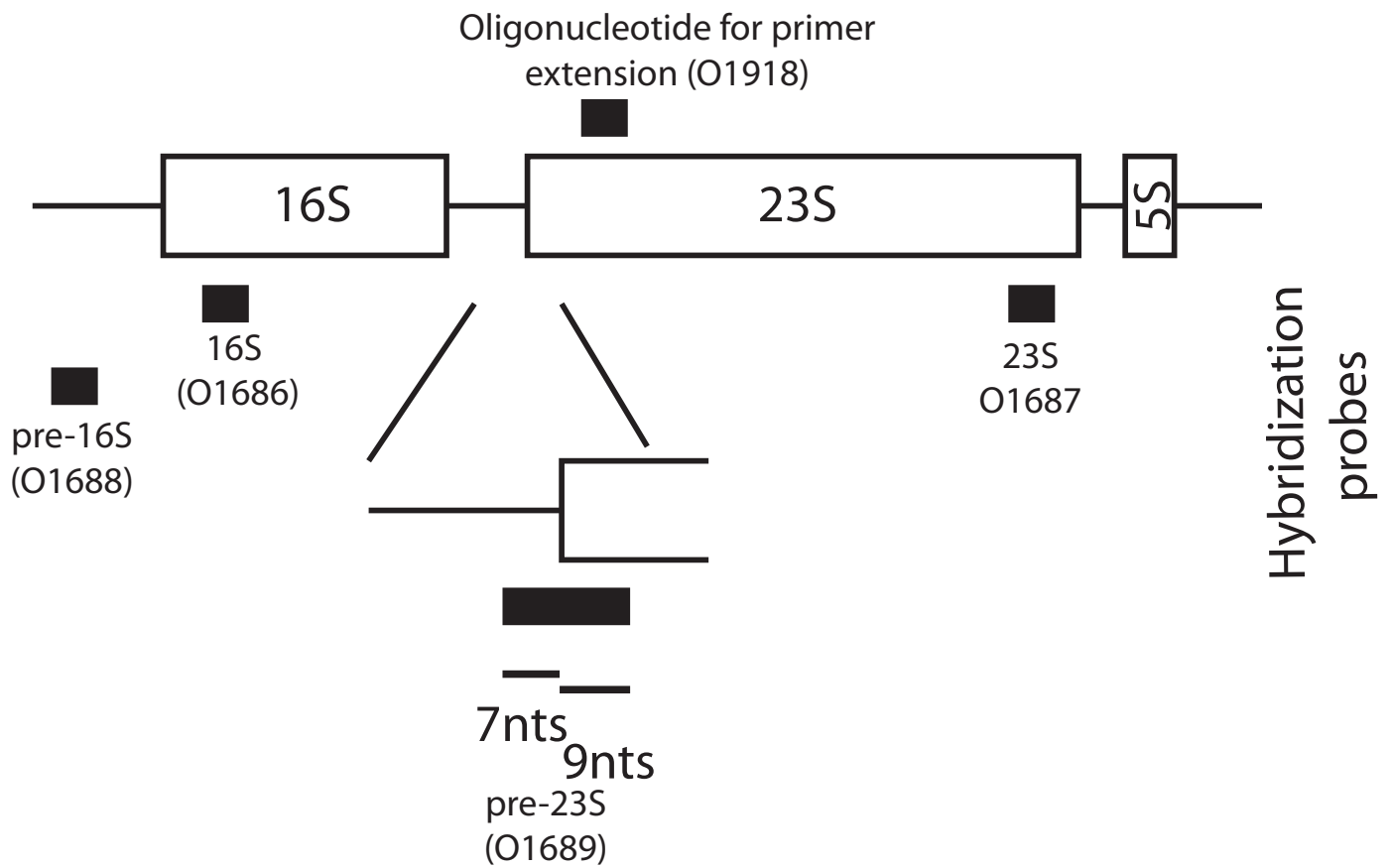


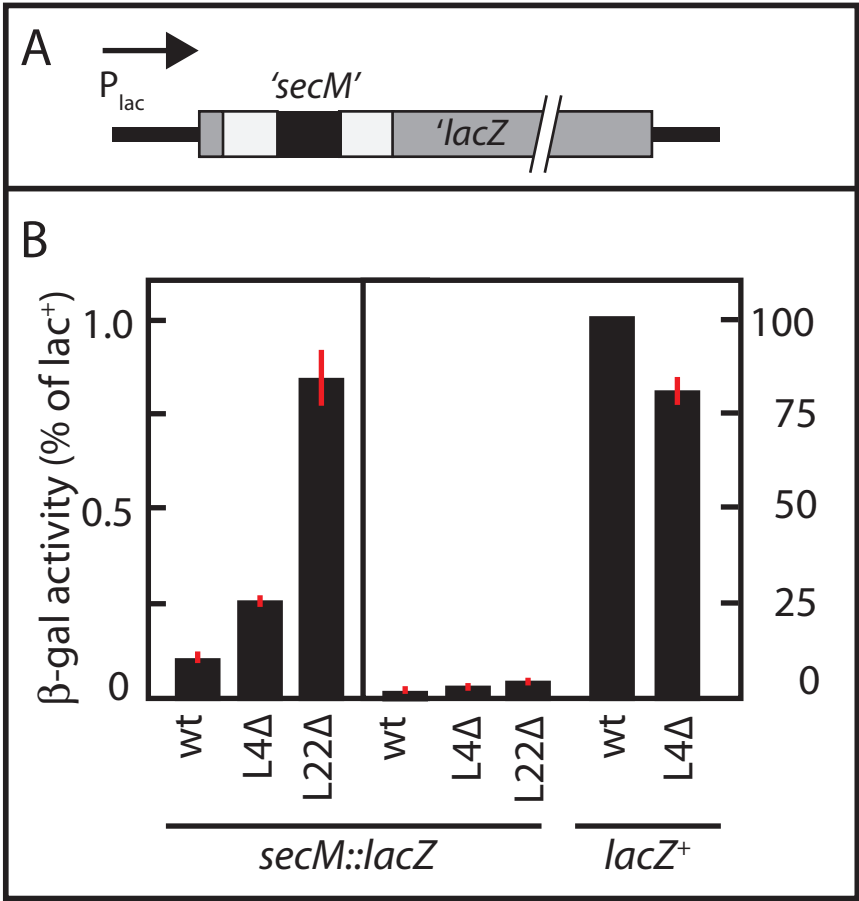
Supplementary Table 1. Oligonucleotides used

Oligo number	Sequence	Complementary to	Used for
O1686	TACTCACCCGTCCGCCACTC	Nucleotide 100-119 of 16S rRNA	Northern probing of 16S rRNA
O1687	AAGGTTAAGCCTCACGGTTC	Nucleotide 2885-2904 of 23S rRNA	Northern probing of 23S rRNA
O1688	CGTATCTTCGAGTGCCACACA	Nucleotide 95-114 upstream of 16S rRNA 5' end	Northern probing of precursor 16S rRNA (p16S)
O1689	CGCTTAACCTCACAAAC	Nucleotides 7 upstream-nucleotide 9 downstream of 23S rRNA 5' end	Northern probing of precursor 23S rRNA (p23S)
O1918	TCACCTTACCGACGCTTA	72-89 of 23S rRNA	Mapping of 5' ends of p23S and 23S rRNA by primer extension

See Supplementary Fig. 1 for details



Supplementary Figure 1



Supplementary Figure 2

## Legends to Supplementary figures

Supplementary Figure 1. Map of position of oligonucleotides with sequences complementary to specific regions of the rRNA transcription unit. The line with open boxes illustrates the *E. coli* rRNA transcription unit. The transcribed spacers (lines between and flanking the boxes) are not to scale. The map does not include tRNA genes in the spacers, because the spacer tRNA genes are not important for this work. The black boxes indicate the position and approximate length of the rRNA regions to which the oligonucleotides hybridize. The oligonucleotides were used for Northern hybridization to identify the indicated rRNA transcripts (Fig. 4B) and mapping the 5' ends of 23S rRNA transcripts by primer extension (fig. 4C-D). See Table 1 for the exact sequences of the oligonucleotides. Note that the p23S hybridization probe spans the end of the 23S rRNA sequence. Under our hybridization conditions, it hybridizes to 23S rRNA with 7-8 nucleotides 5' extensions, but not to mature 23S rRNA or 23S rRNA with shorter 5' extensions. See also text.

Supplementary Figure 2. SecM-mediated translational pausing. A. Map of construct for quantifying pausing. The construct consists of (from left to right) the *lac* promoter, N-terminal end of the *lacZ* gene (gray), a piece of pGEM5 (white box), the sequence for the SecM pausing peptide, another piece of pGEM5, and finally the remaining part of the *lacZ* gene (grey). For more details, see ref. 35. (B)  $\beta$ -galactosidase enzyme activity in the indicated strains. The averages and standard errors of the mean (red lines) of biological triplicates are shown. Three enzyme assays were done for each in duplicate.