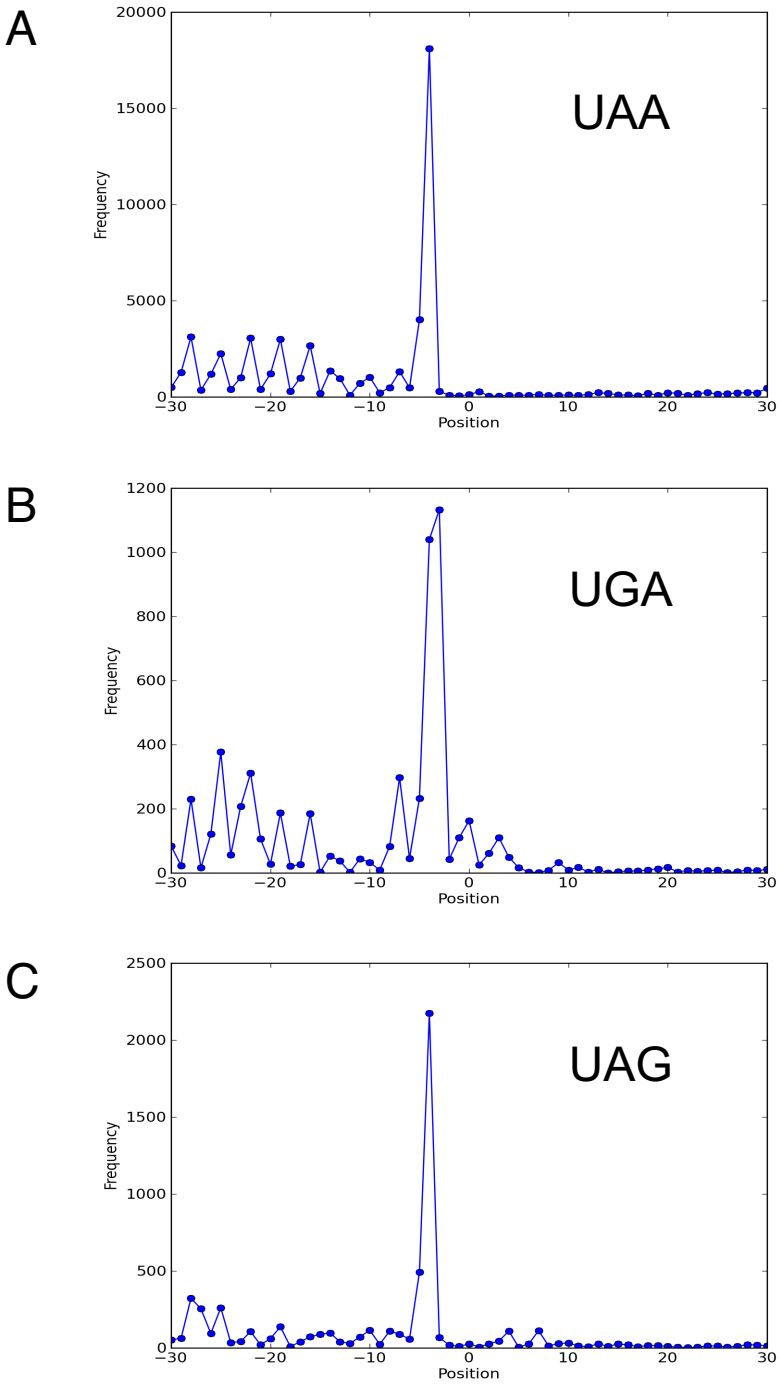


Figure S1. Metagene analysis of termination in *F. johnsoniae*. Ribo-seq read counts are plotted with respect to stop codons UAA (A), UGA (B), and UAG (C). Each read represents one data point and is mapped to the center of the P codon. Position zero corresponds to the last nucleotide of the stop codon.

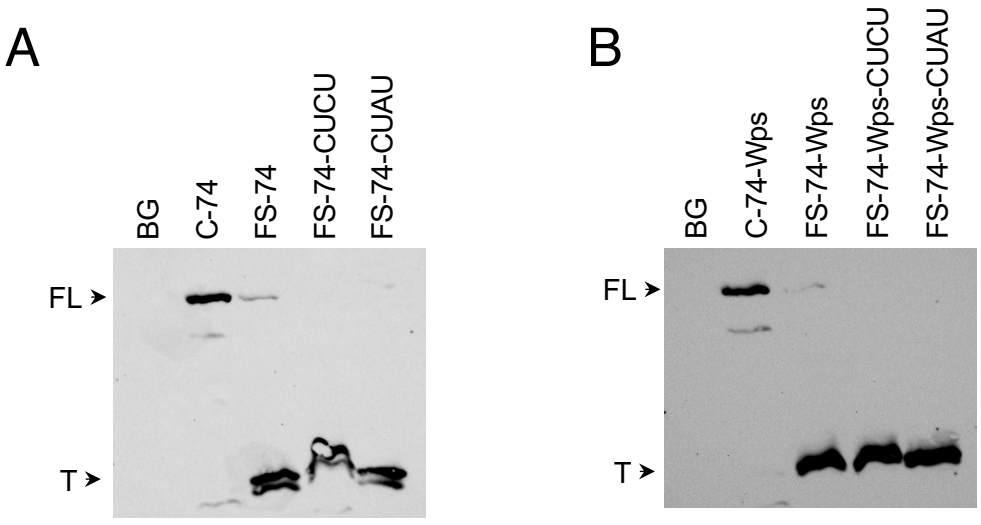


Figure S2. Mutations in the slippery sequence abolish programmed *prfB* frameshifting in *F. johnsoniae*. (A) An example Western blot, comparing products generated from construct FS-74 and its derivatives, which each carry a single nucleotide substitution in the slippery sequence (i.e., CUUU to CUCU or CUAU, as indicated). C-74 corresponds to the no-shift control. Lysate of untransformed cells is loaded in the first lane to assess background (BG). (B) Example of an experiment performed with the analogous *W. psychrotolerans* (Wps) constructs. Three independent experiments like those shown all yielded the same results.

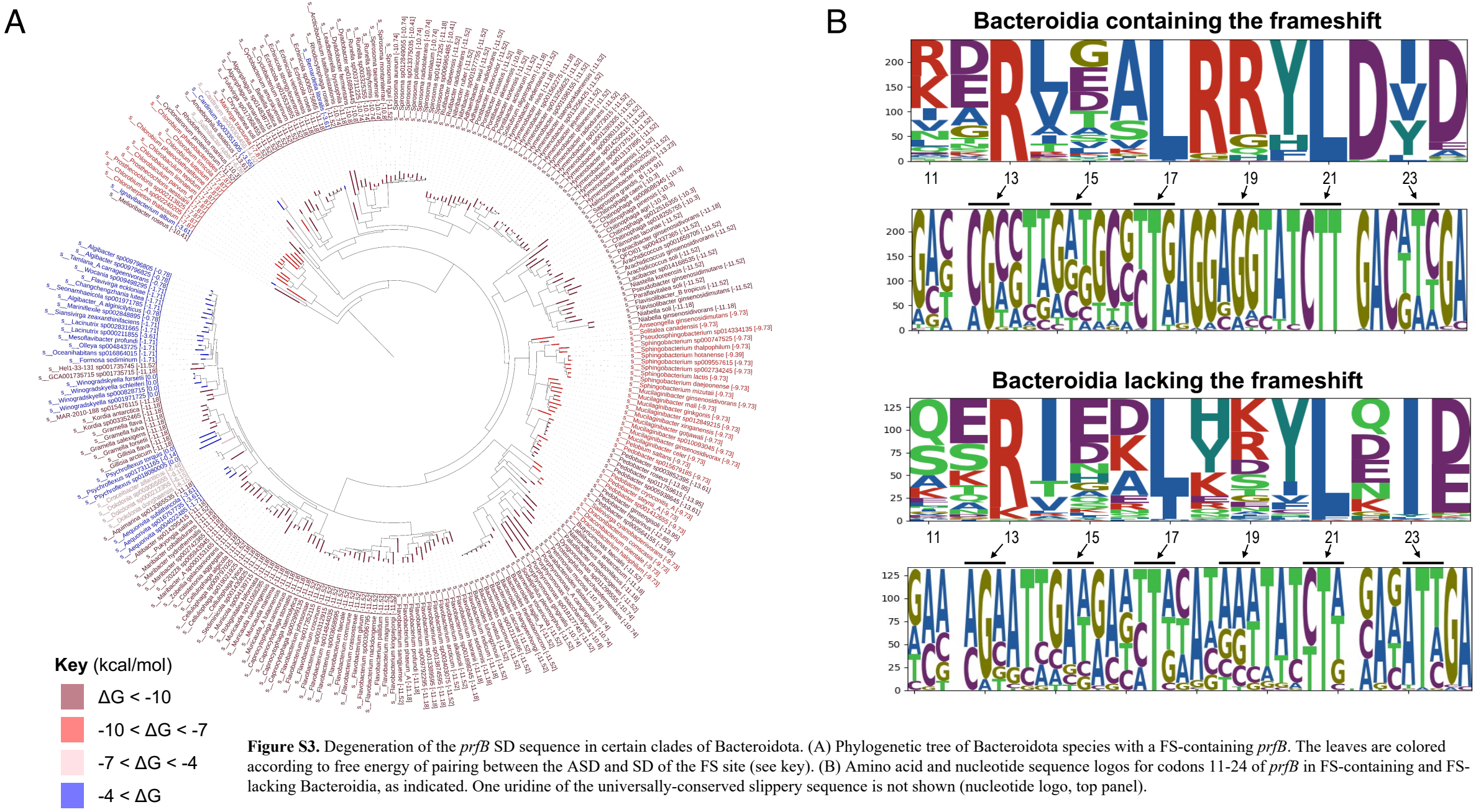
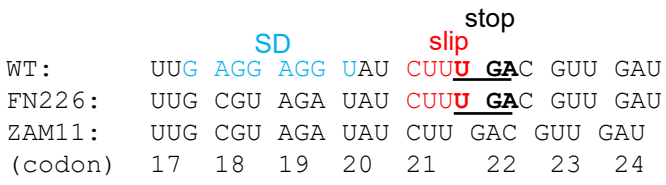


Figure S3. Degeneration of the *prfB* SD sequence in certain clades of Bacteroidota. (A) Phylogenetic tree of Bacteroidota species with a FS-containing *prfB*. The leaves are colored according to free energy of pairing between the ASD and SD of the FS site (see key). (B) Amino acid and nucleotide sequence logos for codons 11-24 of *prfB* in FS-containing and FS-lacking Bacteroidia, as indicated. One uridine of the universally-conserved slippery sequence is not shown (nucleotide logo, top panel).

A



B

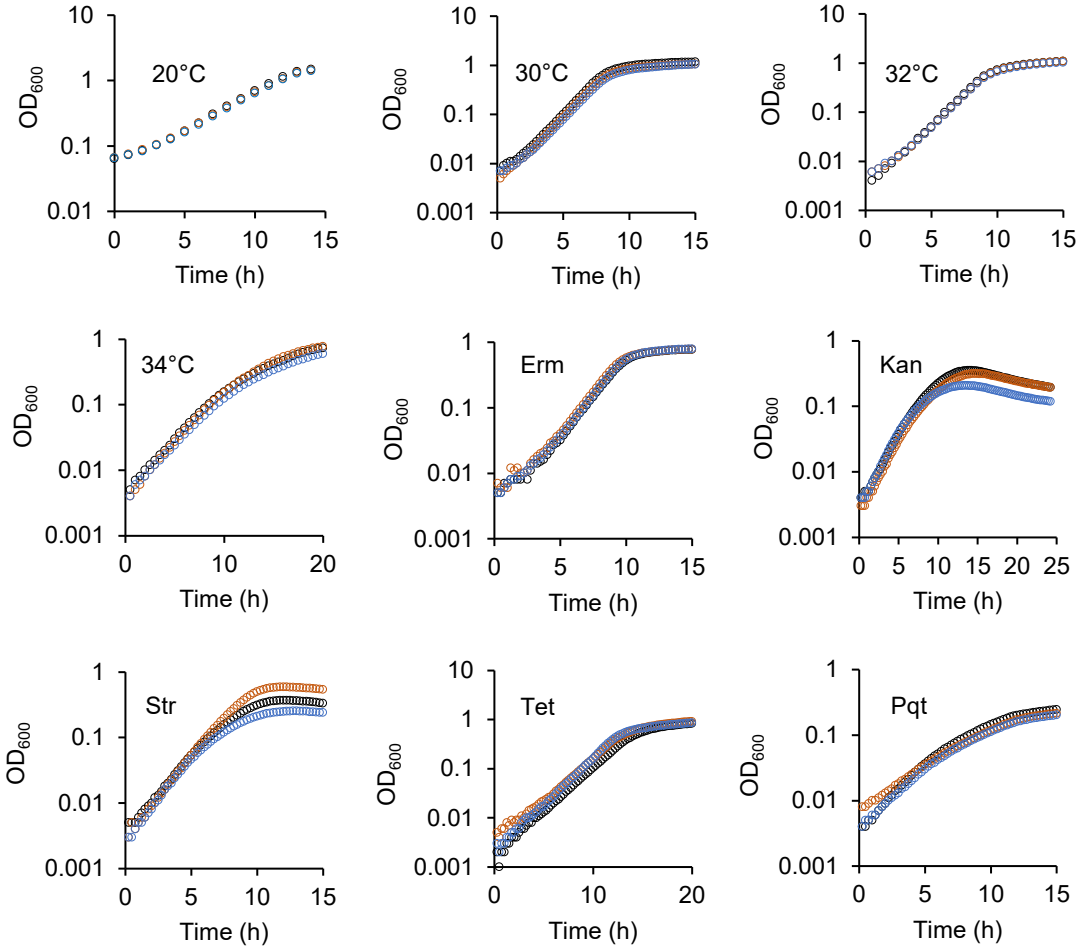


Figure S4. Mutations that remove the *prfB* SD or frameshift confer subtle growth defects in *F. johnsoniae*. (A) Sequence of codons 17-24 of *prfB* in control (WT) and mutant (FN266, ZAM11) strains, as indicated. (B) Examples of growth curves of WT (black), FN266 (blue), and ZAM11 (orange) strains under various conditions, as indicated. Growth experiments at 20°C were performed in Erlenmeyer flasks; all other experiments were done using a microplate reader. Erm, erythromycin; Kan, kanamycin; Str, streptomycin; Tet, tetracycline; Pqt, paraquat.

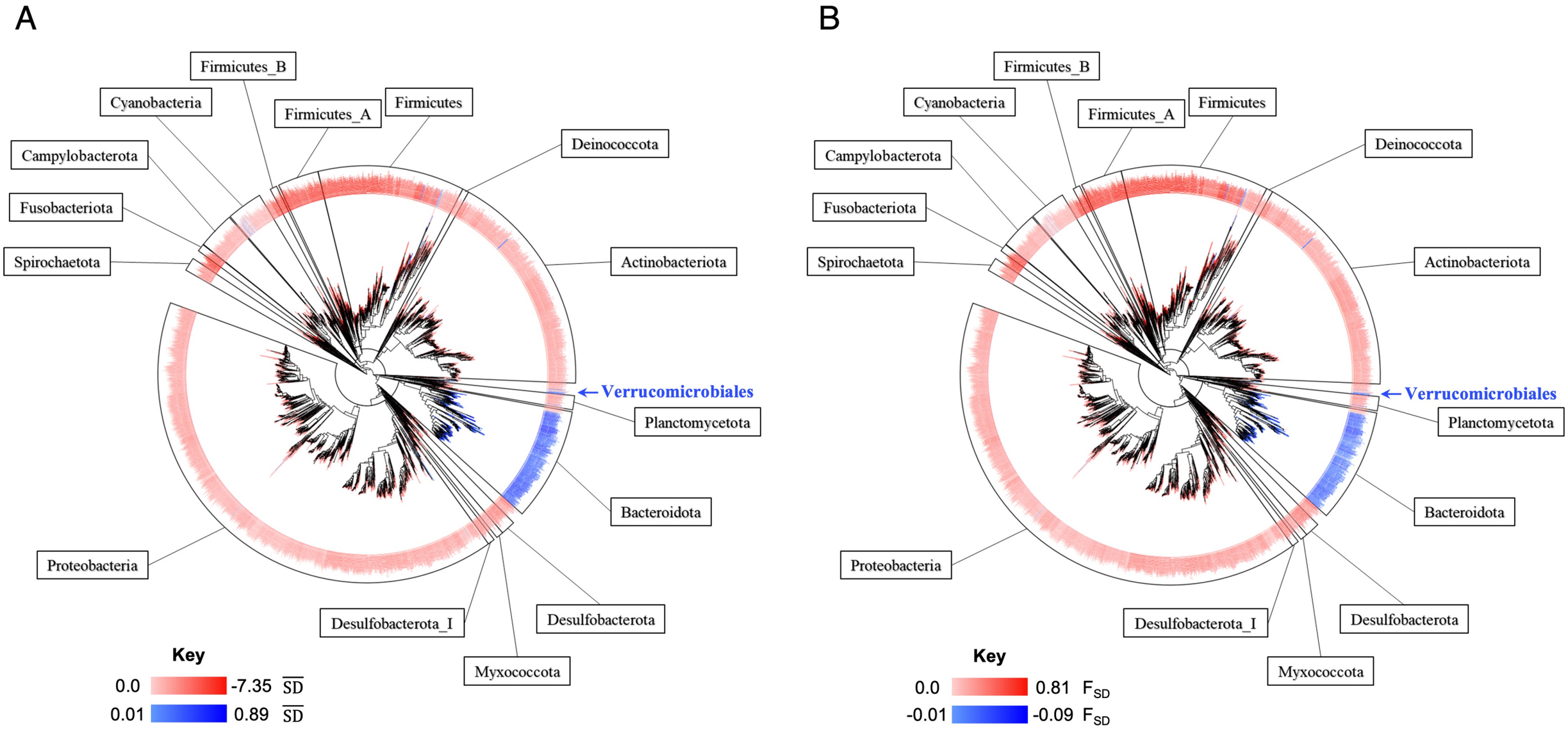


Figure S5. SD usage across bacteria. Genome-wide SD usage was assessed for 4362 organisms by calculating the average SD strength, $\overline{SD}$, and the fraction of genes with a SD, F_{SD}. Shown is the phylogenetic tree with leaves color-coded based on either $\overline{SD}$ (A) or F_{SD} (B). See keys for ranges of parameter values. All values are listed in Table S1, and an expanded rectangular version of the tree of panel B is provided as Fig. S6 (separate pdf file). Phyla with ≥ 25 representative organisms are indicated, as are the Verrucomicrobiales (blue text).

Figure S6. SD usage across bacteria. Genome-wide SD usage was assessed for 4362 organisms by calculating F_{SD}, the fraction of genes with a SD. This large pdf file shows an expanded rectangular version of the tree shown in Fig. S5B. All values are additionally listed in Table S1.

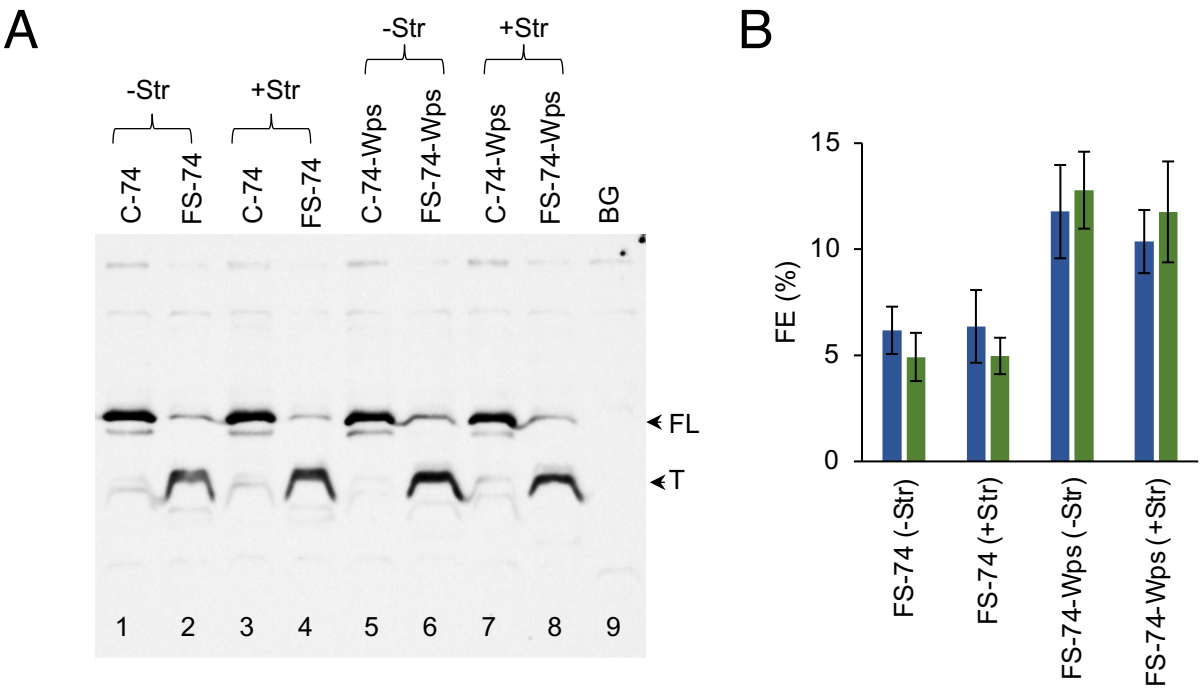


Figure S7. Streptomycin has no apparent effect on *prfB* frameshifting in *F. johnsoniae*. (A) An example Western blot, comparing products generated from various constructs in the absence or presence of streptomycin (Str), as indicated. (B) Frameshifting efficiency (FE) was quantified in two ways (method A, blue bars; method B, green bars). Data represent mean ± SEM values (n = 3), and raw data are given in Table S1.