Recommendations for Bacterial Ribosome Profiling Experiments Based on Bioinformatic Evaluation of Published Data

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Abstract

Ribosome profiling (RIBO-Seq) has improved our understanding of bacterial translation, including finding many unannotated genes. However, protocols for RIBO-Seq and corresponding data analysis are not yet standardized. Here, we analyzed 48 RIBO-Seq samples from nine studies of Escherichia coli K12 grown in lysogeny broth medium and particularly focused on the size-selection step. We show that for conventional expression analysis, a size range between 22 and 30 nucleotides is sufficient to obtain protein-coding fragments, which has the advantage of removing many unwanted rRNA and tRNA reads. More specific analyses may require longer reads and a corresponding improvement in rRNA/tRNA depletion. There is no consensus about the appropriate sequencing depth for RIBO-Seq experiments in prokaryotes, and studies vary significantly in total read number. Our analysis suggests that 20 million reads that are not mapping to rRNA/tRNA are required for global detection of translated annotated genes. We also highlight the influence of drug-induced ribosome stalling, which causes bias at translation start sites. The resulting accumulation of reads at the start site may be especially useful for detecting weakly expressed genes. As different methods suit different questions, it may not be possible to produce a "one-size-fits-all" ribosome profiling data set. Therefore, experiments should be carefully designed in light of the scientific questions of interest. We propose some basic characteristics that should be reported with any new RIBO-Seq data sets. Careful attention to the factors discussed should improve prokaryotic gene detection and the comparability of ribosome profiling data sets.

Keywords: bacterial genetics; bacterial transcription; computational biology; gene mapping; mRNA; polyribosome; ribosome profiling (RIBO-Seq); size selection; transcriptomics; translation.

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