

**Supplemental Figure S1.** Read length distribution after trimming procedure across all datasets [58–64].

**Supplemental Figure S2.** Part of the reads passed the trimming procedure across all datasets [58–64] using all upper bounds.

**Supplemental Figure S3.** Kallisto alignment rates by kmer length across all datasets [58-64].

**Supplemental Figure S4.** Salmon alignment rates by kmer length across all datasets [58-64].

**Supplemental Figure S5.** (A) Distribution of sRNA types expression values by datasets and pipelines (B) Distribution of sRNA types transcripts by datasets and pipelines.

**Supplemental Figure S6.** Number of transcripts filtered using 6 thresholds (A) by dataset [58-64] and (B) by pipeline.

**Supplemental Figure S7.** Number of significant differentially expressed transcripts using three DE packages (DESeq2 - top; edgeR - middle; limma - bottom) and two thresholds of significance (adjusted p-value <0.05 - left; p-value<0.05 - right) by two filtering (*mean*>5 and *median*>5) and by pipeline.

**Supplemental Figure S8.** H-score of significant differentially expressed transcripts using three DE packages (DESeq2 - top; edgeR - middle; limma - bottom) and two thresholds of significance (adjusted p-value <0.05 - left; p-value<0.05 - right) by two filtering (*mean*>5 and *median*>5) and by pipeline.

**Supplemental Figure S9.** H-score of significant differentially expressed transcripts using two thresholds of significance (adjusted p-value <0.05 - upper; p-value<0.05 - low) and two filtering (*mean*>5 - left, *median*>5 - right) by DE package and by pipeline.

**Supplemental Figure S10.** Distribution of H-scores using three DE packages (DESeq2 - top; edgeR - middle; limma - bottom) and two filtering (*mean*>5 and *median*>5) by pipeline groups for all data [58-64].

**Supplemental Figure S11.** Kallisto alignment rates by dataset [58-64], kmer length and by aligner for tsRNA.

**Supplemental Figure S12.** Number of reads by dataset [58-64], kmer length and by aligner for tsRNA.

**Supplemental Table S1.** Alignment rates for alignment-based pipelines across datasets [58-64].

**Supplemental Table S2.** Assignment rates for alignment-based pipelines across datasets [58-64].

**Supplemental Table S3.** Assignment rates for pseudoalignment-based methods across datasets [58-64].

**Supplemental Table S4.** Number of differentially expressed transcripts with adjusted p-value < 0.05 by three DE packages (DESeq2; edgeR; limma) and two filtering (mean>5, median>5) across contrasts of datasets [58-64] and pipelines.

**Supplemental Table S5.** Hobotnica scores for sRNAs by three DE packages (DESeq2; edgeR; limma), two filtering (mean>5, median>5), two thresholds of significance (adjusted p-value <0.05; p-value<0.05) across contrasts of datasets [58-64] and pipelines.

**Supplemental Table S6.** Assignment rates for tsRNA (assigned reads and reads assigned to tRNA ratio) by aligner and kmer length across datasets [58-64].

**Supplemental Table S7.** Assignment rates for tsRNA (assigned reads and processed reads ratio) by aligner and kmer length across datasets [58-64].

**Supplemental Table S8.** Number of differentially expressed tsRNA with adjusted p-value < 0.05 by three DE packages (DESeq2; edgeR; limma) and two filtering (mean>5, median>5) across contrasts of datasets [58-64] and pipelines.

**Supplemental Table S9.** Hobotnica scores for tsRNAs by three DE packages (DESeq2; edgeR; limma), two filtering (mean>5, median>5), two thresholds of significance (adjusted p-value <0.05; p-value<0.05) across contrasts of datasets [58-64] and pipelines.

**Supplemental Table S10.** Assignment rates for sRNA-based pipelines across datasets [58-64].