**Supplemental Figure 1.** Read length distribution after trimming procedure across all datasets.

**Supplemental Figure 2.** Part of the reads passed the trimming procedure across all datasets using all upper bounds.

**Supplemental Figure 3.** Kallisto alignment rates by kmer length across all datasets.

**Supplemental Figure 4.** Salmon alignment rates by kmer length across all datasets.

**Supplemental Figure 5.** (A) Distribution of sRNA types expression values by datasets and pipelines (B) Distribution of sRNA types transcripts by datasets and pipelines.

**Supplemental Figure 6.** Number of transcripts filtered using 6 thresholds (A) by dataset and (B) by pipeline.

**Supplemental Figure 7.** 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 8.** 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 9.** 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 10.** 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.

**Supplemental Figure 11.** Kallisto alignment rates by dataset, kmer length and by aligner for tsRNA.

**Supplemental Figure 12.** Number of reads by dataset, kmer length and by aligner for tsRNA.

**Supplemental Table 1.** Alignment rates for alignment-based pipelines across datasets.

**Supplemental Table 2.** Assignment rates for alignment-based pipelines across datasets.

**Supplemental Table 3.** Assignment rates for pseudoalignment-based methods across datasets.

**Supplemental Table 4.** 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 and pipelines.

**Supplemental Table 5.** 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 and pipelines.

**Supplemental Table 6.** Assignment rates for tsRNA (assigned reads and reads assigned to tRNA ratio) by aligner and kmer length across datasets.

**Supplemental Table 7.** Assignment rates for tsRNA (assigned reads and processed reads ratio) by aligner and kmer length across datasets.

**Supplemental Table 8.** 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 and pipelines.

**Supplemental Table 9.** 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 and pipelines.

**Supplemental Table 10.** Assignment rates for sRNA-based pipelines across datasets.