**Supplementary Figure Legends**

**Supplementary Fig. 1.** Correlation analysis of miRNA quantifications across the six tested sRNA-seq methods.

Scatter plots show log2 transformed and DESeq2 normalized data for miRNAs with greater than 1 normalized count in all methods. Correlation values shown are Pearson correlation coefficients. Distribution curves on the diagonal demonstrate that the distributions were fairly similar and normal for each method. The y-axis for these plots indicates the proportion of the miRNAs with the quantifications given on the x-axis.

**Supplementary Fig. 2.** Concordance plots of the rank of synthetic miRNA quantification error for the six tested sRNA-seq methods.

The concordance of the rank of “accuracy error” between two given methods, is depicted by each line. The x-axis shows the rank of the difference of each synthetic sequence quantification from the mean quantification of the 962 synthetic sequences for each method. The gray line indicates what two methods with zero concordance would look like, while the doted red line indicates what perfect concordance would look like across the 962 synthetic sequences. The dotted green line shows what concordance for accuracy error of only the top miRNAs would look like between two methods. The Illumina and Fivepercent methods show the highest concordance for the top ranking most miRNAs with the most dissimilar quantifications from the average for each method. Overall the concordance between the methods was weak.

**Supplementary Fig. 3.** Boxplot and heatmap of the influence of estimated secondary structure on accuracy error.

**(A)** The percent expression relative to the mean of all the synthetic sequences is plotted for each individual synthetic sequence. Sequences are grouped by the Gibb’s free secondary structure estimate. Overall most methods show a positive quantification relationship with higher or less negative Gibb’s free energy estimates. **(B)** Theheatmap also depicts the percent expression relative to the mean of all the synthetic sequences for sequences with various rounded Gibb’s free energy estimates.

**Supplementary Fig. 4.** Boxplot and heatmap of the influence of the identity of the last 2 bases of the synthetic sequences on accuracy error.

**(A)** The percent expression relative to the mean of all the synthetic sequences is plotted for each individual synthetic sequence. Sequences are grouped by the identity of the last 2 bases of the individual synthetic sequences. Overall most methods show consistent quantification despite the identity of the last two bases, however NEB and Illumina show more inconsistent quantification. **(B)** Theheatmap also depicts the percent expression relative to the mean of all the synthetic sequences for sequences grouped by the identity of the last two bases.

**Supplementary Fig. 5.** Boxplot and heatmap of the influence of the identity of the first 2 bases of the synthetic sequences on accuracy error.

**(A)** The percent expression relative to the mean of all the synthetic sequences is plotted for each individual synthetic sequence. Sequences are grouped by the identity of the first 2 bases of the individual synthetic sequences. Overall all of the methods show fairly consistent quantification despite the identity of the first two bases, however the Deduped method showed more consistent quantification. **(B)** The heatmap also depicts the percent expression relative to the mean of all the synthetic sequences for sequences grouped by the identity of the first bases.

**Supplementary Fig. 6.** Boxplot and heatmap of the influence of the number of Cs in the synthetic sequences on accuracy error.

**(A)** The percent expression relative to the mean of all the synthetic sequences is plotted for each individual synthetic sequence. Sequences are grouped by the number of Cs in the individual synthetic sequences. Overall, most of the methods show quantification with increasing numbers of Cs, however the Clontech method showed the opposite relationship, and the Deduped method was quite consistent. **(B)** The heatmap also depicts the percent expression relative to the mean of all the synthetics sequences for sequences grouped by number of Cs in the synthetic sequences.

**Supplementary Fig. 7.** miRNA detection across various starting amounts.

The number of miRNAs detected above 10 normalized counts in all triplicates of batch 1 for each method is plotted on the y-axis. The starting total RNA amount is indicated on the x-axis in nanograms. Only starting amounts in the range of suggested inputs were tested for each method.

**Supplementary Fig. 8.** Inconsistency of miRNA detection across triplicates.

The percentage of miRNAs detected above the 10 normalized counts threshold by a single triplicate that were not detected the threshold by the other two triplicates is plotted on the y-axis. The starting total RNA amount is indicated on the x-axis in nanograms. Only starting amounts in the range of suggested inputs were tested for each method. The relationship between the percentage of inconsistently detected miRNAs and starting amount is plotted as a line using a locally estimated scatterplot smoothing regression (LOESS).

**Supplementary Fig.9.** Overlap of isomiR detection.

The overlap of isomiRs detected above the 100 normalized count threshold for all methods except for the Fivepercent control are plotted. Only 24 isomiRs were detected by all of the 5 of the methods shown.

**Supplementary Fig.10.** isomiR detection across various starting amounts.

The number of non-canonical miRNA sequences detected above 100 normalized counts in all triplicates of batch 1 for each method is plotted on the y-axis. The starting total RNA amount is indicated on the x-axis in nanograms. Only starting amounts in the range of suggested inputs were tested for each method.

**Supplementary Fig.11.** Concordance plots of the rank of batch error for individual miRNAs.

The concordance of the rank of “batch error” is depicted by each line for two given methods. This x-axis is the rank of the difference of each miRNA measurement from the mean of all three triplicates in batch 1 to the measurements in batch 2. The gray line indicates what two methods with zero concordance would look like, while the dotted red line indicates what perfect concordance would look like across all detected miRNAs. Overall the concordance between the methods was moderate.

**Supplementary Fig.12.** Concordance plots of the rank of triplicate error for individual miRNAs.

The concordance of the rank of “triplicate error” is depicted by each line for two given methods. This x-axis is the rank of the difference of each miRNA measurement from the mean of all three triplicates. The gray line indicates what two methods with zero concordance would look like, while the dotted red line indicates what perfect concordance would look like across all detected miRNAs. Overall the concordance between the methods was moderate.

**Supplementary Fig.13.** Concordance plots of the rank of miRNA quantification estimates using different starting amounts.

The concordance of the rank of “triplicate error” is depicted by each line for two given starting amounts. This x-axis is the rank of the difference of each miRNA measurement from the mean of all three triplicates. Overall the concordance between the starting amounts was moderate.