Small RNA-sequencing for Analysis of Circulating miRNAs: Benchmark Study

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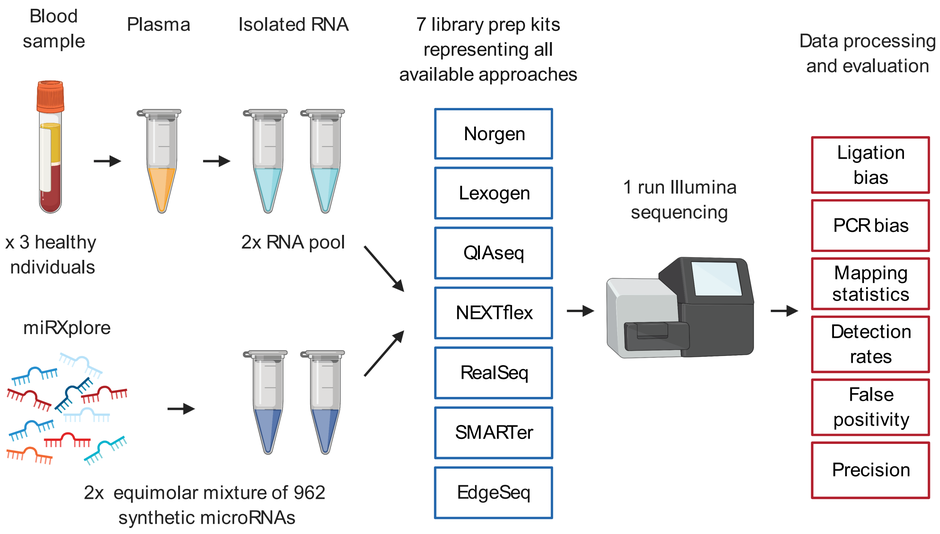
Background

The application of **small RNA sequencing** (small RNA-seq) for profiling of **circulating microRNAs** (miRNAs), novel **promising biomarkers**, is increasing. However, the accuracy of the method is **limited by bias** introduced by ligation, PCR or polyadenylation. Therefore recently, **new approaches** were developed to prevent the biases. Here, we present **comparison of all small RNA-Seq library preparation approaches** that are commercially available for quantification of miRNAs in biofluids.

Experimental design



Conclusions

- There is **no single protocol outperforming others** across all metrics.
- **Protocol-specific biases** give rise to limited overlap of measured miRNAs profiles between methods and cause their low correlation.
- The correlation can be improved by **normalization** approach utilizing bias ratios **learnt from miRXplore** sample.
- The application of UMIs has only marginal effect on overall quantification bias.
- Traditional two-steps ligation methods introduce large bias.
- Protocols performing well in majority of metrics use capture probes (EdgeSeq) or randomized adaptors (NEXTflex).

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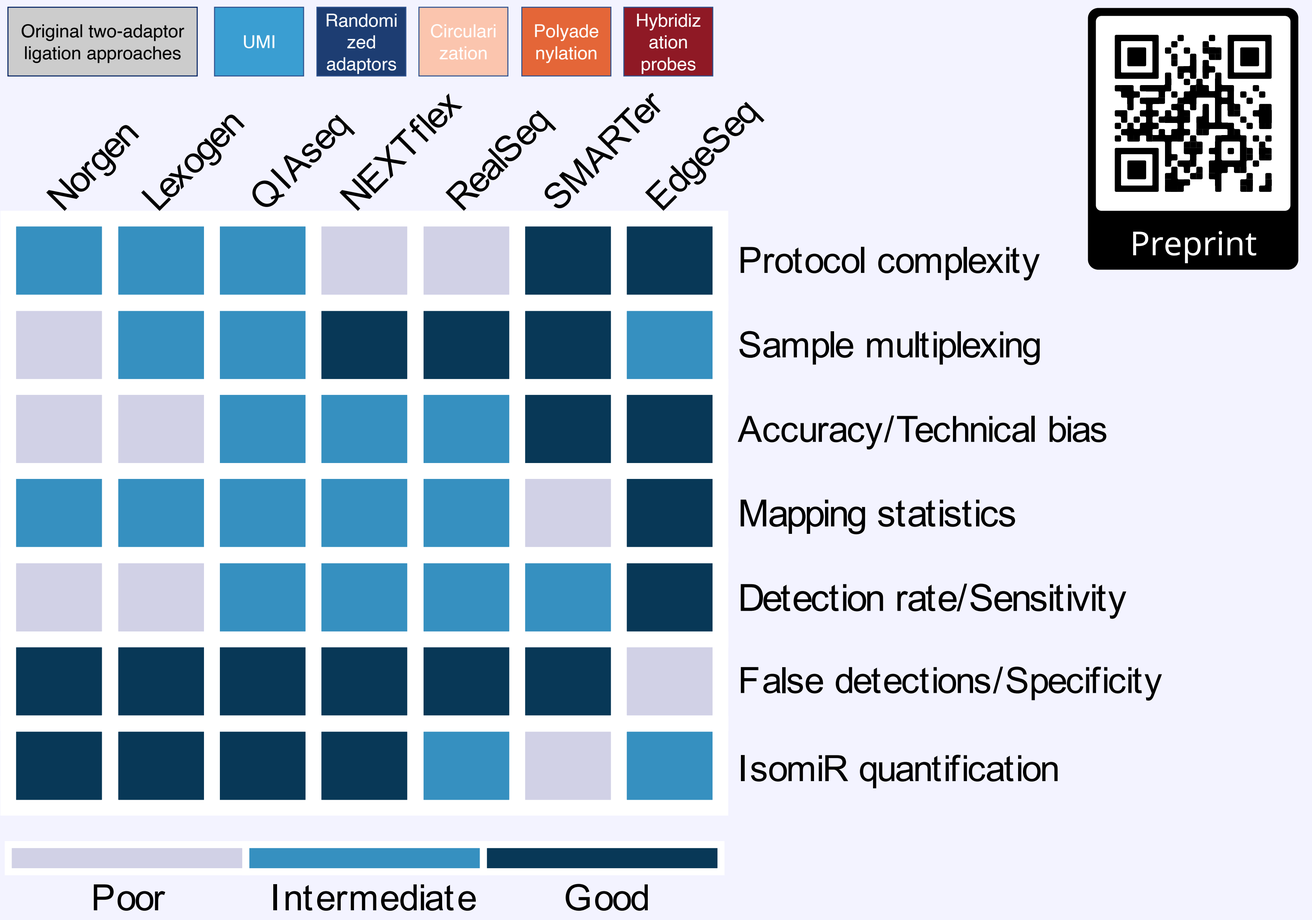
Selection of suitable protocol for small RNA-seq



Preprint

Normalization of data with factors learnt from equimolar pool leads to higher between protocol correlations

Between-protocol reproducibility for plasma samples before and after data correction using bias ratios learnt on miRXplore samples. P-value from two-tailed paired t-test.

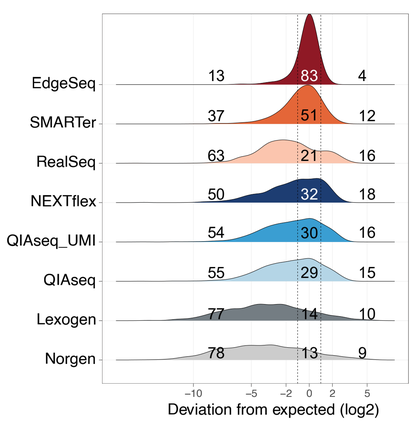
Ligation is the main contributor of the overall bias in the small RNA-seq

Percentage of variance in QIAseq data (miRXplore sample) explained by ligation bias, PCR bias and replicates.

First two bases of miRNA affects ligation of adaptor in RealSeq

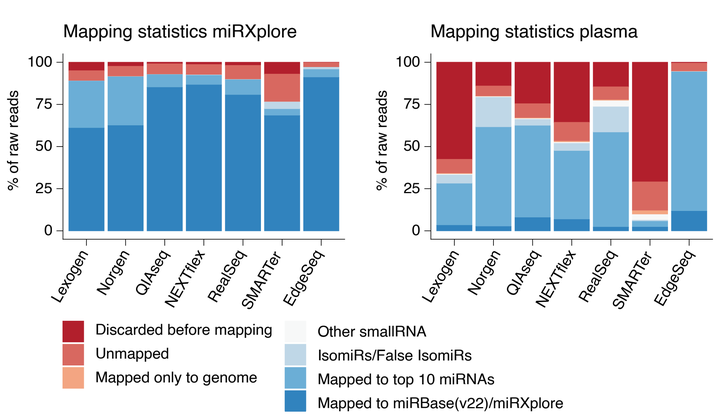
Percentage variance in QIAseq data (miRXplore sample) explained by miRNA sequence characteristics.

Accuracy/Technical bias



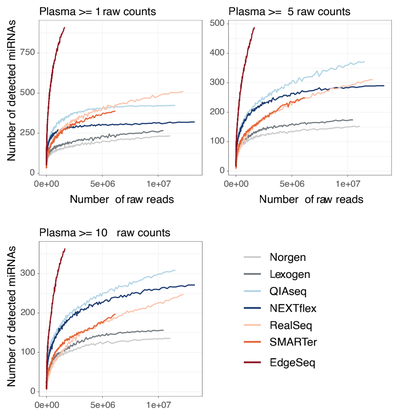
Density plots show distribution of log_2-fold change between measured and expected value in miRXplore. Dashed lines show two-fold deviation from expected value; numbers indicate percentage of miRNAs within and outside of the two-fold range.

Mapping statistics



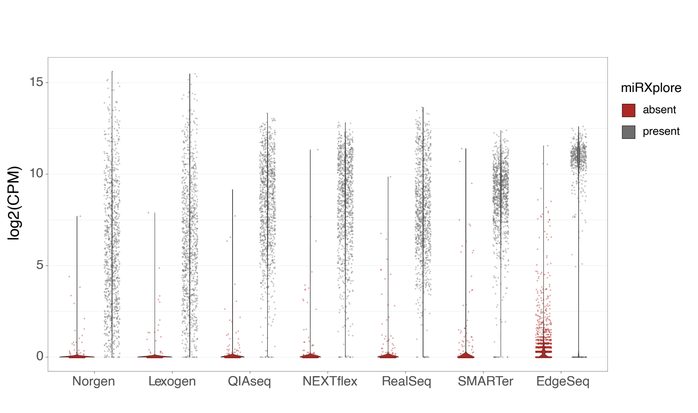
Mapping statistics for miRXplore and plasma samples. Top 10 = 10 most expressed miRNAs; discarded before mapping = sequence length out of 16-29 bp or only adaptors.

Detection rate/Sensitivity



Dependency of number of detected miRNAs in plasma on sequencing depth and various detection thresholds (1, 5, 10 raw reads).

False detections/Specificity



Violin plots showing measured level of true and false positive miRNAs measured in miRXplore samples.