Getting the most out of your samples – microRNA enrichment by depleting abundant undesirable small RNAs in biofluids

RNA sequencing has revolutionized our understanding of the transcriptome and led to a paradigm shift in biology from seeing RNA as a mere transit molecule between the genetic information stored in DNA and protein synthesis, to identifying discrete, regulatory functions of RNAs. Depending on these functions, RNAs can be classified into different categories, with microRNAs (miRNAs) as the most prominent class with an integral role in gene regulation. Due to this particular function and the dynamic nature of RNAs in general, miRNAs emerged as potential biomarkers. Sequencing of miRNAs, as part of the small RNA fraction, is a well established approach that works reliably and efficiently with high quality samples or miRNA-rich sample types, e.g. fresh or fixed tumor tissue. In the frame of personalized medicine, there is an increasing interest in detection of miRNAs in sample types obtained through minimally invasive procedures, such as serum or plasma. However, sequencing of these sample types is challenging as the derived RNA is of poor quality, of low concentration and contains a large fraction of RNAs that are not of primary interest, e.g. tRNA fragments.

This tech note describes the technical assessment of an improved small RNA sequencing approach with the goal to enrich the miRNA fraction in the sample without affecting the miRNA composition itself.

Undesirable RNAs in your samples

Using TruSeq small RNA library prep, Pippin Prep size selection, NextSeq 500 single end 75 nucleotide sequencing and (proprietary) Cobra RNA sequencing data-analysis of various challenging sample types, we observed that many of these samples can contain a large fraction of undesirable RNAs, when the main interest lays in miRNAs. These observations confirm previous findings (Kumar et al., trends in biochemical sciences, 2016). The undesirable RNAs are often characteristic to the sample type. Sequencing the small RNA fraction from serum of different mouse strains, for instance, revealed that a large fraction of sequencing reads aligned to various tRNA fragments (Figure 1).

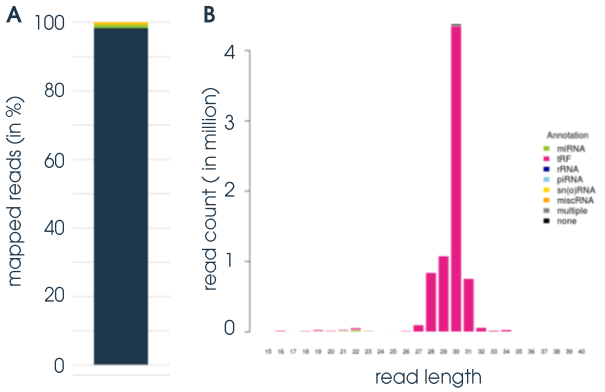

Figure 1: small RNA sequencing results of a typical mouse serum sample. A, Distribution of mapped reads with around 1% of miRNA reads. green=miRNA, yellow=not annotated, blue=other small RNAs. B, Read histogram of mapped reads illustrating that the majority of reads aligns to tRNA fragments.

Depending on the strain, as many as 98% of the mapped reads in this sample type are aligned to tRNA fragments, and only around 1% can be assigned to miRNAs. This severely limits the pool of detectable miRNAs to an extent that the data from such a sample is seemingly unusable.

A similar observation was made with samples derived from human plasma (Figure 2). Of the mapped reads, on average, only around 20% align to miRNAs and 70% to other small RNAs with the vast majority aligning to Y RNA genes.

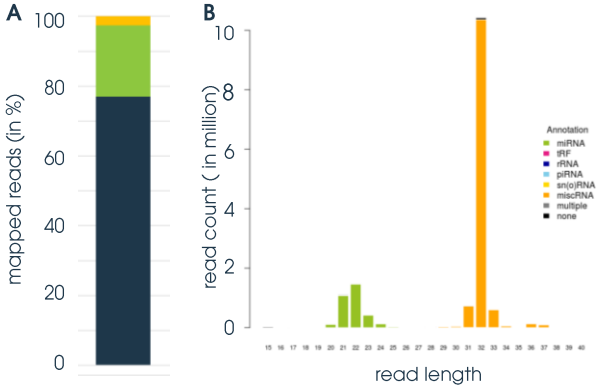

Figure 2: small RNA sequencing results of a typical human plasma sample. A, Distribution of mapped reads with around 20% of miRNA reads. green=miRNA, yellow=not annotated, blue=other small RNAs. B, Read histogram of mapped reads illustrating that the majority of reads aligns to Y RNA genes (in the miscellaneous RNA category).

Removal of undesirable RNAs in your samples

In order to enrich the miRNA fraction, the undesirable RNA fraction (tRNA and Y RNA fragments, etc.) is specifically removed using biotinylated DNA probes and streptavidin coated magnetic beads (Van Goethem et al., in preparation). This allows an unbiased enrichment of the miRNA fraction as miRNAs are not directly targeted, avoiding the risk that e.g. unknown miRNAs / isomiRs are not efficiently selected.

In the example of mouse serum, the removal of tRNA fragments resulted in an average enrichment of miRNAs of around 50 X (~1% mapped miRNA and ~97% mapped other small RNAs (with the majority (>99%) being tRNA fragments) before removal compared to ~49% mapped miRNA and ~9% mapped other small RNAs (with the majority (~70%) being tRNA fragments) after removal) (Figure 3).

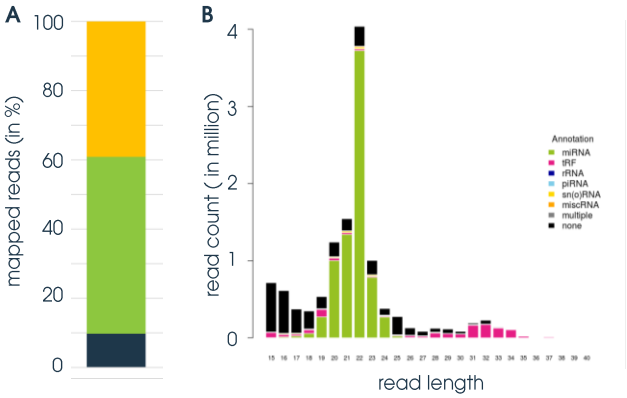

Figure 3: small RNA sequencing results of a typical mouse serum sample after tRNA fragment removal. A, Distribution of mapped reads with around 50% of miRNA reads and only around 10% undesirable other RNAs. green=miRNA, yellow=not annotated, blue=other small RNAs. B, Read histogram of mapped reads illustrating that the majority of reads aligns to miRNAs.

Removal of undesirable small RNAs did not negatively impact the miRNA composition of samples as indicated by comparison of tRNA-fragment-depleted and untreated samples (Figure 4A). In fact, as additional miRNAs can be detected at a similar sequencing depth in samples with removed undesirable RNAs, these samples provide a more accurate and richer representation of the miRNA spectrum of a given sample. On average more than twice as many miRNAs could be detected in mouse serum samples with tRNA fragments removed (Figure 4B).

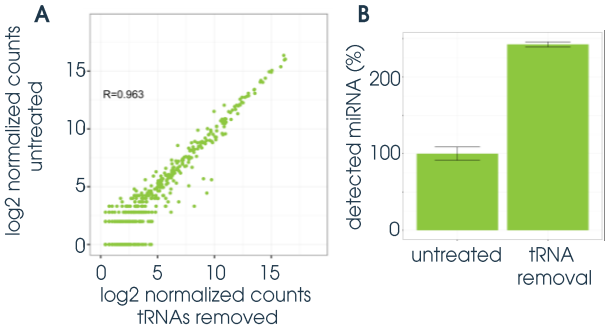

Figure 4: Impact of tRNA fragment removal on miRNA composition. A, Example of a typical mouse serum sample (25 µl equivalent) containing large amounts of tRNA fragments was sequenced with and without removal and log2 transformed normalized read counts plotted. R = Pearson product-moment correlation coefficient. B, Average detection rate with and without tRNA fragment removal indicating a more than twofold increase of detected miRNAs. Error bars = SEM.

Conclusion

Small RNA sequencing of samples derived from body fluids is challenging and often inefficient as the RNA concentration is low and contains a large fraction of RNAs that are not of primary interest, e.g. tRNA fragments. A modified sequencing approach was developed in which the miRNA fraction could be enriched by removal of the undesired RNA fraction. With this approach, Y RNAs in human plasma and tRNA fragments in mouse serum could be efficiently removed. The very customizable nature of this approach also allows to easily extent this to other challenging sample types and species to unveil the true microRNA potential of your samples.

Your small RNA body fluid sequencing project @ Biogazelle – 8 steps to success

1. upfront discussion with our PhD-level project managers
2. RNA extraction (optional)
3. library preparation
4. library QC
5. sequencing
6. data processing
7. reporting
8. discussion of results with our PhD-level project managers

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