Biogazelle Tech Note 201502A

Small RNA sequencing of RNA isolated from body fluids

microRNA (miRNA) expression analysis of clinically relevant body fluids offers a promising approach for the identification of candidate miRNA biomarkers for disease. Today, Biogazelle offers a workflow for small RNA sequencing of RNA isolated from various body fluids, including serum and plasma. Our workflow, starting from total RNA extracted from 200 µl of body fluid, is optimized to tackle the particular challenges associated with analysis of degraded RNA samples. It enables researchers to start applying the power of next-generation sequencing technology to miRNA expression studies on RNA isolated from various body fluids.

Introduction

Twenty years after their first discovery, microRNAs (miRNAs), a class a small non-coding RNAs, are emerging as excellent candidate biomarkers for disease and therapy response[1]. In this setting, miRNAs hold a major advantage over mRNA biomarkers as they are characterized by superior stability in degraded RNA samples[2]. This enables miRNAs to be accurately quantified in challenging samples such as clinically relevant body fluids[3].

During the last decade, next generation sequencing of cDNA has emerged as a valuable technology for transcript profiling. In particular, the quantitative nature, depth and dynamic range of RNA sequencing has made it a useful method for gene expression compared with RT-qPCR or microarray. However, small RNA sequencing is not routinely performed on samples of low RNA quality as sequencing reads are polluted by fragmented RNA, making small RNA sequencing of challenging samples inefficient and expensive.

To overcome these challenges, Biogazelle has developed a workflow for small RNA sequencing of RNA extracted from various body fluids.

This tech note describes the technical assessment of our optimized workflow on RNA extracted from serum, plasma and cerebrospinal fluid (CSF) and zooms in to differential miRNA expression in serum samples from patients diagnosed with colorectal cancer and from health individuals.

Methods summary

To assess the performance of our workflow, we performed small RNA sequencing on RNA extracted from a plasma sample, 2 cerebrospinal fluid samples and 8 serum samples, taken from 4 healthy individuals and 4 patients diagnosed with colorectal cancer (CRC).

In brief, total RNA was extracted from 200 µl of body fluid. Subsequently, half of the extracted RNA was used to prepare libraries for small RNA sequencing. Resulting cDNA libraries were purified to recover the fractions containing mature miRNAs and small RNA libraries were sequenced on a NextSeq 500 (Illumina). Sequencing reads were processed using Ant, our custom small RNA sequencing analysis pipeline, part of Cobra, our RNA sequencing data analysis platform.

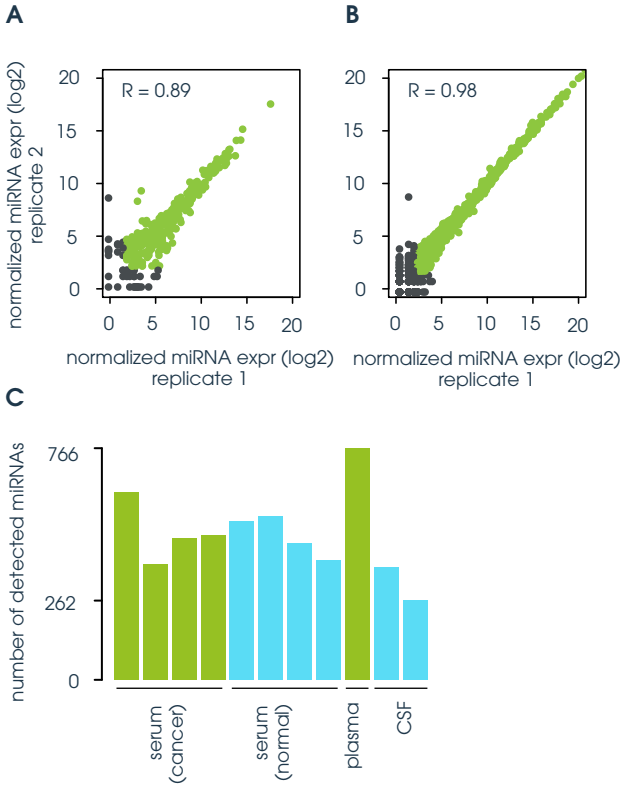

Figure 1: Highly reproducible and sensitive small RNA sequencing workflow. High correlation between normalized miRNA expression of replicates of (A) serum and (B) plasma. Gray dots: miRNAs below the detection threshold (≥ 4 reads) in both sample replicates. (C) Number of detected miRNAs (≥ 4 reads) in RNA extracted from distinct body fluids.

Highly reproducible and sensitive workflow

Our workflow is characterized by high reproducibility over a wide dynamic range of miRNA expression (Fig. 1A-B). We also observe high concordance in detected targets between replicates: 82% of miRNAs are detected in both replicates, with failing miRNAs in the lower range of detection (miRNA read count < 10). On average 482 and 317 miRNAs were detected in serum and CSF samples (Fig. 1C). Finally, a large number of miRNAs was detected in the plasma sample (n = 766; Fig. 1C).

Detection of differentially expressed miRNAs

We next tested for differential expression of miRNAs between samples originating from patients diagnosed with CRC (n = 4) and from healthy individuals (n = 4). Using a generalized linear model in edgeR software to fit the data, 4 miRNAs were differentially expressed between serum from CRC patients and normal individuals at a false discovery rate < 0.05 (Fig. 2); all 4 differentially expressed miRNAs were upregulated in the cancerous serum samples. Notably, for the top differentially expressed miRNA, miR-27a-5p (previously named miR-27a*), overexpression in natural killer cells was shown to be associated with reduced cytotoxic effects on CRC xenografts, resulting in sustained tumor growth[4]. In addition, the second most strongly regulated miRNA, miR-1246, was found overexpressed in serum of patients diagnosed with CRC[5]. These findings support the relevancy of our optimized workflow for studying miRNA biomarkers in serum samples.

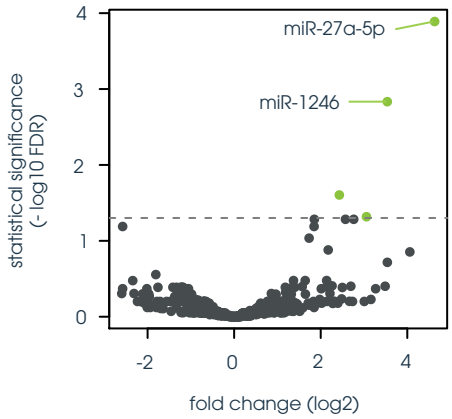

Figure 2. Detection of differentially expressed miRNAs in colon cancer versus control samples. Each dot represents one miRNA that lacks (gray) or shows (green) differential expression; gray line: significance cut-off off FDR = 0.05.

Conclusion

To enable miRNA biomarker discovery in various body fluids, Biogazelle offers an optimized workflow for small RNA sequencing. Using a highly reproducible and sensitive method, we have detected known and potential new miRNA biomarkers for CRC in serum samples. This optimized workflow enables researchers to apply the power of next-generation sequencing technology to miRNA expression studies on RNA isolated from various body fluids.

References

(1) Lu J *et al.*, Nature, 2005
(2) Hall JS *et al.*, British journal of cancer, 2012
(3) Jung M *et al.*, Clinical chemistry, 2010
(4) Kim T *et al.*, Blood, 2011
(5) Ogata-Kawata H *et al.*, PloS one, 2014

Learn more

To learn more about miRNAs as biomarkers or the Cobra RNA-sequencing analysis pipeline, visit Biogazelle's Knowledge Center:

https://www.biogazelle.com/biogazelle-shares-its-knowledge

Your small RNA sequencing project @ Biogazelle – eight steps to success

1. upfront discussion with our PhD-level project managers
2. RNA extraction (optional)
3. small RNA library prep
4. library quality control
5. sequencing
6. data processing and analysis
7. reporting
8. discussion of results with our PhD-level project managers

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