Biogazelle Tech Note 201501A

Small RNA sequencing of RNA isolated from FFPE tissues

microRNA (miRNA) expression analysis of archived formalin-fixed paraffin-embedded (FFPE) tissue blocks 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 FFPE samples. The workflow, starting from 100 ng of total RNA, 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 FFPE samples.

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 archived formalin-fixed paraffin embedded (FFPE) tissue blocks[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 generally not 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 FFPE tissue.

This tech note describes the technical assessment of our optimized workflow and zooms in to differential miRNA expression in colon cancer and normal colon FFPE tissue.

Methods summary

To assess the performance of our workflow, we performed small RNA sequencing on RNA isolated from 4 colorectal cancer (CRC) FFPE samples and 4 normal control FFPE samples.

In brief, total RNA was extracted from 4 scrolls per sample, yielding an average of 2 µg total RNA per sample. Isolated RNA was of low quality with DV200 values between 8% and 26%. Libraries for small RNA sequencing were prepared starting from 100 ng of total RNA. Resulting libraries were purified to recover the fractions containing mature miRNAs and purified libraries were sequenced on a NextSeq 500 (Illumina). Sequencing reads were analysed using Ant, our custom small RNA-sequencing analysis pipeline, part of Cobra, our RNA-sequencing data analysis platform.

To assess the reproducibility of our workflow, a technical replicate of one FFPE sample was included in the entire workflow.

Highly reproducible and sensitive workflow

Our workflow is characterized by high reproducibility (R = 0. 97) over a wide dynamic range of miRNA expression (Fig. 1A).

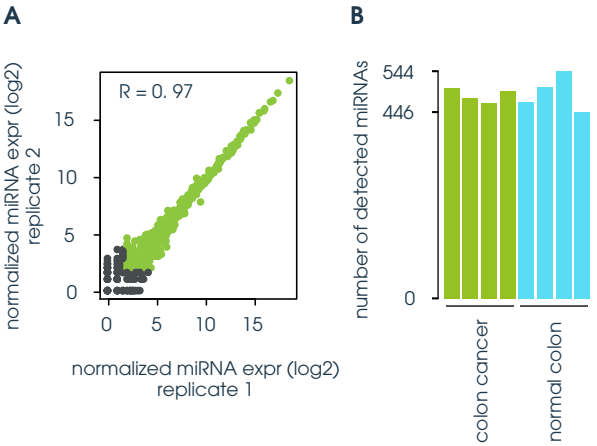

Figure 1: Highly reproducible and sensitive small RNA sequencing workflow. (A) High correlation between normalized miRNA expression of replicate samples (Pearson's correlation: 0.97). Gray dots: miRNAs below detection threshold (≥ 4 reads) in both replicates. (B) Number of detected miRNAs (≥ 4 reads) in RNA extracted from colorectal cancer and normal colon tissue.

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). In total, 709 unique miRNAs were detected (miRNA read count ≥ 4) across all eight samples, with on average 489 miRNAs detected per sample (Fig. 1B).

Detection of differentially expressed miRNAs

Given the high number of detected miRNAs, we next tested for differential expression of miRNAs between FFPE samples originating from CRC tissue (n = 4) and from normal colon tissue (n = 4).

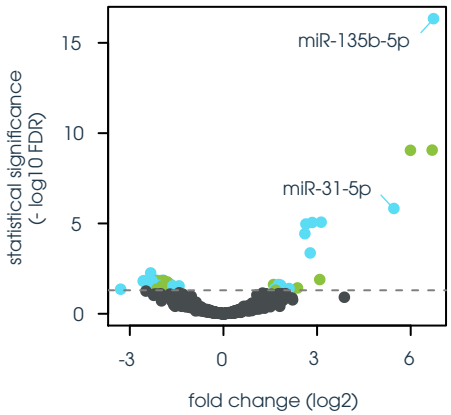

Figure 2. Detection of differentially expressed miRNAs in colon cancer versus control samples. Each dot represents one miRNA that lacks (gray) or shows (green/blue) differential expression; blue: miRNAs reported in literature; gray line: significance cut-off off FDR = 0.05. Two well-established colon cancer specific miRNAs are highlighted.

Using a generalized linear model in edgeR software to fit the pair-wise data, 39 miRNAs were differentially expressed between CRC and normal colon at a false discovery rate (FDR) < 0.05 (Fig. 2). Of the 39 differentially expressed miRNAs, 22 were downregulated and 17 were upregulated in the CRC samples. Notably, 14/22 downregulated and 11/17 upregulated miRNAs have been reported in literature to be respectively lower and higher in CRC compared to normal colon, thus confirming the validity of our workflow and results.

Conclusion

To enable miRNA biomarker discovery in FFPE tissue, 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 colon cancer based on the study of FFPE tissue. This optimized workflow enables researchers to apply the power of next-generation sequencing technology to miRNA expression studies on RNA isolated from FFPE samples.

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

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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