NEW CLINICAL DIAGNOSTIC APPROACH FOR miRNA QUANTIFICATION USING THE CHLORELLA VIRUS DNA LIGASE.

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Introduction

miRNAs (miRNAs) are small non-coding RNA molecules playing an important regulatory role in gene regulation through base-pairing and/or degradation of target mRNAs. There has been tremendous interest in using miRNAs as diagnostic, prognostic and prediction biomarkers in the whole field of oncology. Their utilization in liquid biopsy as a non-invasive diagnostic tool is very promising as well [1]. miRNA expression can be measured by many techniques, the three most common being microarray, next generation sequencing and reverse transcription quantitative PCR (RT-qPCR), which is considered to be a gold standard in miRNA detection [2]. To our knowledge, these methods are not suitable for clinical use - they are too in reproducibility and sensitivity, high technology demanding or very time consuming. As a principal-based methods are struggling to overcome these problems, for example immunoassay-based method [3], called miREIA (BioVendor – Laboratorní medicína a.s.).

Another promising technology is utilizing the enzyme Chlorella virus DNA ligase (SplintR® ligase, New England Biolabs) which has recently been found to efficiently ligate two DNA oligonucleotides splinted by RNA [4]. This enzyme has been used for detection of multiple miRNAs by ligation and to potentially complex regulatory proteins and following qPCR [5]. Our aim was to optimize this methodology for potential clinical use.

Methods

1. SplintR® ligation PCR

A) Hybridization/ligation

B) Real-time PCR with Syber Green or probe visualization

2. TaqMan ADVANCED (ThermoFisher Scientific)

A) Reverse transcription

B) Visualization

3. miREIA (BioVendor – Laboratorní medicína a.s.)

A) Hybridization

B) Visualization

Results

Figure 1: Amplification plot and standard curve of SplintR® ligation qPCR for detection of miR-142-5p. Synthetic miRNA 142-5p was serially diluted in water. (Blue – standard, Red – no template controls). Dynamic range was determined to 7 logs and sensitivity to 1 amol/µl in original clinical sample.

Figure 2: Comparison of Syber Green and Q5 labeledprobe detection mechanisms for miR-142-5p SplintR® ligation PCR. Samples: RNA isolates from whole blood (N = 25), peripheral blood mononuclear cells (N = 13) and plasma (N = 11) of healthy donors.

Figure 3: Comparison of miR-142-5p expression measured with SplintR® ligation qPCR and TaqMan ADVANCED method. Samples: RNA isolates from peripheral blood mononuclear cells (N = 13) of healthy donors.

Figure 4: Comparison of miR-142-5p expression measured with SplintR® ligation qPCR and miREIA immunoassay method. Samples: RNA isolates from whole blood (N = 25), peripheral blood mononuclear cells (N = 13) and plasma (N = 11) of healthy donors.

Figure 5: Comparison of parameters of three tested methods for miR-142-5p quantification.

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Conclusion

We have optimized SplintR® ligation PCR for clinical use and developed an assay for detection of miR-142-5p, potential marker of colorectal cancer. Optimized SplintR® ligation PCR showed excellent correlation with TaqMan qPCR and miREIA.

References