Accurate MicroRNA Analysis in Crude Cell Lysate by Capillary Electrophoresis-Based Hybridization Assay in Comparison with Quantitative Reverse Transcription-Polymerase Chain Reaction

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Abstract

Here we report on accurate miRNA quantitation in crude cell lysate by a capillary electrophoresis-based hybridization assay termed direct quantitative analysis of multiple miRNAs (*DQAMmiR*). Accuracy and precision of miRNA quantitation were determined for miRNA samples in a crude cell lysate, RNA extract from the lysate, and a pure buffer. The results showed that the measurements were matrix-independent with inaccuracies of below 13% from true values and relative standard deviations of below 11% from the mean values in a miRNA concentration range of 2 orders of magnitude. We compared DQAMmiR-derived results with those obtained by a benchmark miRNA-quantitation method–quantitative reverse transcription-polymerase chain reaction (*qRT-PCR*). *qRT-PCR*-based measurements revealed multifold inaccuracies and relative standard deviations of up to 70% in crude cell lysate. Robustness of *DQAMmiR* to changes in sample matrix makes it a perfect candidate for validation and clinical use of miRNA-based disease biomarkers.

Introduction

The deregulation of specific subsets of miRNA in cancer shows that they have potential to be used as disease biomarkers. Accurate quantitation of microRNA (miRNA) in tissue samples is required for validation and clinical use of miRNA-based disease biomarkers. Since sample processing, such as RNA extraction, introduces undesirable biases, it is advantageous to measure miRNA in a crude cell lysate. Recently a capillary electrophoresis-based hybridization assay was designed and developed in our lab to be capable of Direct, Quantitative Analysis of Multiple miRNAs, termed as *DQAMmiR*, in a sensitive and specific manner. Meanwhile, we also demonstrated that sampling the cell lysate did not greatly affect the CE separation pattern. These results led us to a hypothesis that *DQAMmiR* may be robust to changes in the sample matrix and potentially allow highly accurate miRNA analysis in crude cell lysates. Here we test this hypothesis by investigating the influence of the sample matrix on accuracy and precision of the miRNA analysis. To put our results into a context of the benchmark method, a comparative study with *qRT-PCR* was also conducted in the present work.

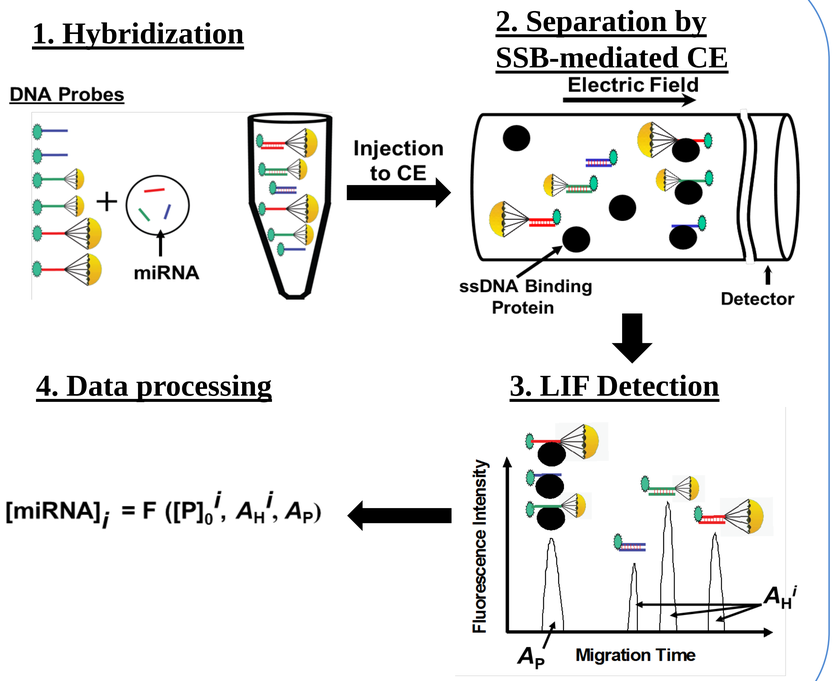

Figure 1: Schematic of *DQAMmiR*

Results and Discussion

Test the robustness of *DQAMmiR* for measuring miRNA in crude cell lysate.

- Samples: Spiking-in single miRNA target into different matrices. (cel-miR-39-3p, it is foreign for human; *concentration range:* 0.1nM – 10 nM)
- Matrices: 1) pure TAE buffer; 2) cell lysate from MCF-7 cells; 3) total RNA extract from MCF-7 cells.

Considerations of measuring in biological matrices:

- ➢ Degradation of the target miRNAs and DNA probes by nucleases.
- ➢ Slower or incomplete hybridization because of the influences of matrix components, pH, salts, *etc*.

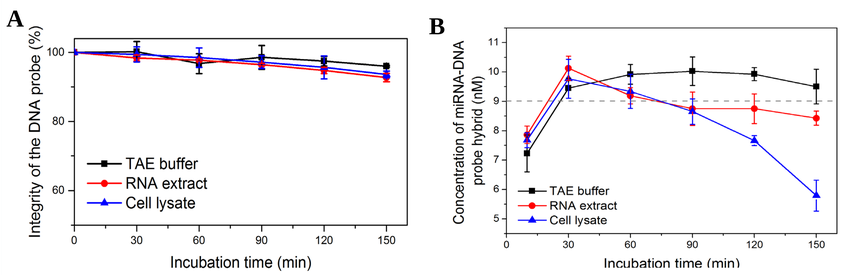

Figure 2: A) Probe integrity in different matrices. Samples: 100nM DNA probe and Internal standard. B) Effects of sample matrix on hybridization. Samples: 100nM DNA probe and 10nM miRNA.

Measurements in different matrices

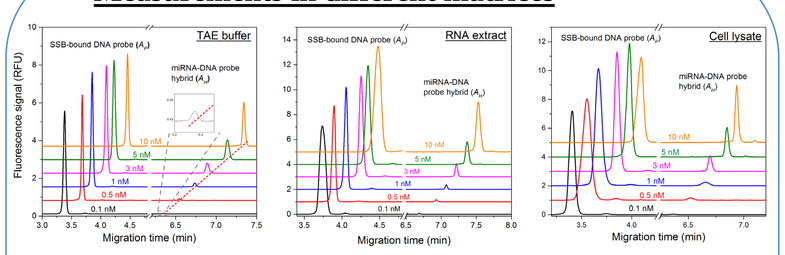

Figure 3: Electropherograms of *DQAMmiR* measurements in all matrices. There was no significant matrix-associated effect on CE-separation, and quantifiable peaks of the miRNA-probe hybrid of all concentrations (0.1–10 nM) were sucessfully produced in all matrices.

Measured results in comparison with *qRT-PCR*

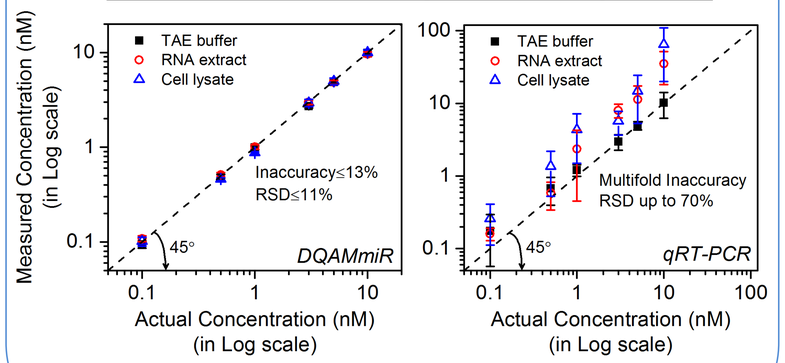

Figure 4: Comparison with *qRT-PCR*. The measured results by two methods were compared. The dashlines refers to 100% of recovery.

Conclusions

- *DQAMmiR* is able to quantify miRNA equally well in pure buffer and biological matrices such as cell lysates, which suggests it is robust to the changes of sample matrix.
- The robustness of *DQAMmiR* indicates that it may directly quantitate miRNAs in crude clinical samples without the need of bias-prone RNA extractions, making *DQAMmiR* a very promising candidate for validation and clinical use of miRNA-based biomarkers.

References

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2. Wegman, D.W.; Krylov, S.N. *Angew. Chem., Int. Ed.* **2011**, *50*, 10335-10339.

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