Comparison of DQAMmiR with qRT-PCR for microRNA quantification under various matrices

Liang Hu, David W. Wegman, Alexander S. Stasheuski, and Sergey N. Krylov

Centre of Research on Biomolecular Interaction, York University, Toronto, ON, Canada M3J 1P3

Our Group

On-line poster version

Introduction

MicroRNAs (miRNAs) are shown to have great potential as biomarkers for practical diagnostics, causing urgent need for reliable and effective miRNA analysis tools. Direct quantitative analysis of multiple miRNAs (DQAMmiR) is a recently developed technique that utilizes capillary-electrophoresis (CE) and laser-induced fluorescence (LIF) detectors to separate and detect multiple miRNAs with remarkable accuracy, specificity and sensitivity.[1] In order to validate the capability of DQAMmiR for practical analysis, here we conduct a comparative study of DQAMmiR with quantitative reverse-transcription PCR (qRT-PCR), which is usually considered as a "gold standard" strategy for miRNAs profiling.[2]

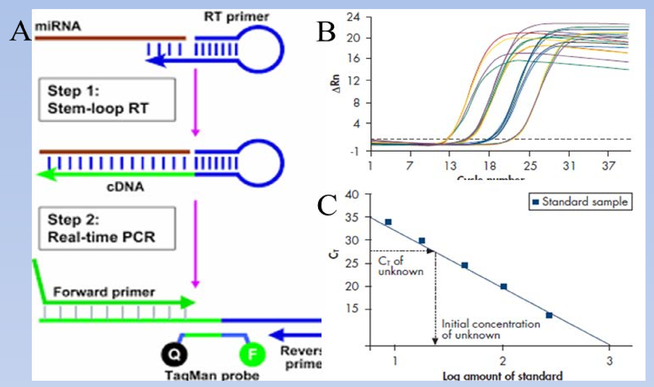


Figure 1: qRT-PCR (TaqMan assay) for absolute quantitative miRNA analysis. . (A) Mechanism of miRNA TaqMan assay. (B) Amplification plot curves. The dashline is set as signal threshold line. (C) Standard curve built by standard samples.

Since accurate quantitative analysis of biological samples is an essential requirement for miRNA practical analysis, it is necessary to investigate the ability of DQAMmiR with samples under different biological matrices. In this study, five different matrices, including buffer solution, tRNA library, total RNA extract, cell lysate and bio-fluid, are introduced to investigate the ability of DQAMmiR under different biological matrices in comparison with qRT-PCR.

As it requires standard curve, qRT-PCR is usually subject to interferences from the biological matrices. Therefore, accurate quantification of bio-samples is a challenging task for qRT-PCR. In contrast, the preliminary results indicate that DQAMmiR has excellent ability of multiple miRNA quantification under either buffer solution or complex cell lysate. As a result, DQAMmiR is inherently capable of working with complex biological matrices, while qRT-PCR can be significantly affected by complex bio-matrices (especially cell lysate and biofluid). Therefore, DQAMmiR is anticipated to become a standard procedure complementary to qRT-PCR for practical miRNAs analysis.

DQAMmiR

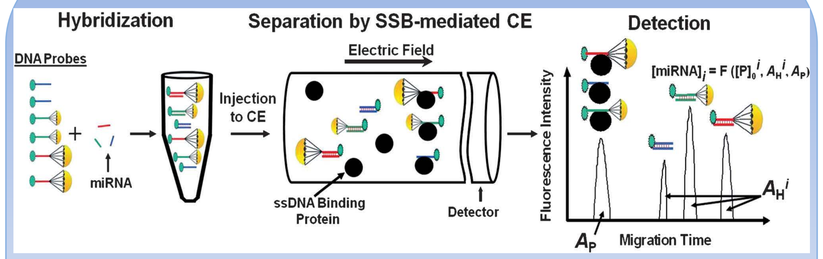


Figure 2: Schematic representation of DQAMmiR. (1) Hybridization. MiRNAs bind to excess specific ssDNA probes that are conjugated with specific drag tags to generate hybridizations mixture. (2) Capillary-electrophoresis (CE) separation mediated by Single-strand DNA binding protein (SSB) and drag tags. SSB in running buffer is used to separate excess un-bound ssDNA probes by binding to excess ssDNA probes. Various miRNA-DNA probes hybrids are separated by different drag tags. (3) Detection. Laser-induced fluorescence (LIF) detector is applied.

Preliminary Results

$$[miRNA]_i = \frac{A_H^i \sum_i^N [P]_0^i q_P^i}{q_H^i \left[\left(\sum_i^N \frac{A_H^i q_P^i}{q_H^i}\right) + A_P\right]}$$

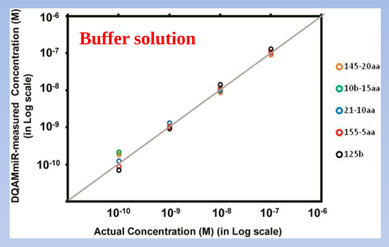
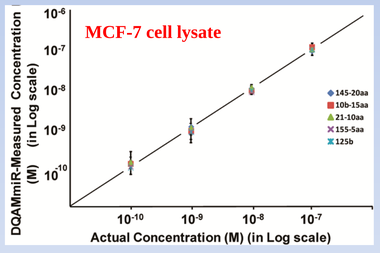


Figure 3: Quantitative analysis of five miRNA under buffer solution was processed simultaneously using the equation above. Excess (500 nM) of the five DNA probes was incubated with all five respective miRNA, ranging in concentration from 100pM to 100nM.

Figure 4: Quantitative analysis of five miRNA under MCF-7 cell lysates. 100 pM to 100 nM of five miRNAs were incubated with 500nM of their respective DNA-peptide probes in the presence of MCF-7 cell lysate for 10 min. Concentrations of miRNA were validated by light absorbance at 260 nM.



The results indicate that DQAMmiR can accurate quantitative analysis of multiple miRNAs. Moreover, this method can be applied to samples under either simple or complex biological matrices with excellent performance.

Prospective Results

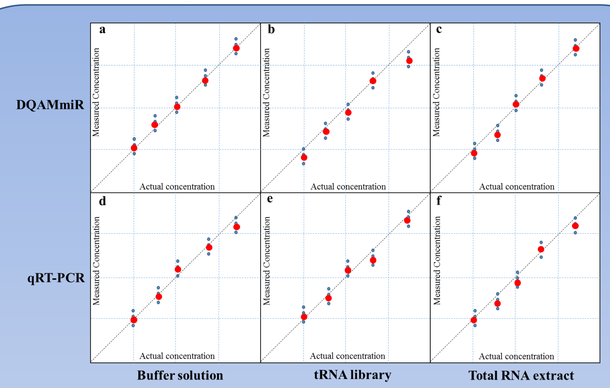


Figure 5: "Anticipated" results of DQAMmiR (a-c) and qRT-PCR (d-f) under buffer solution, tRNA library and total RNA extract. In these three matrices, both DQAMmiR and qRT-PCR will exhibit great performance in miRNA absolute quantification. The concentration is plot in log scale. Each concentration (red points) comes from three measurements (blue points).

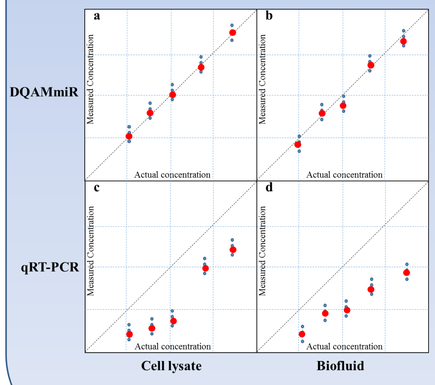


Figure 6: "Anticipated" results of DQAMmiR (a-b) and qRT-PCR (c-d) under complex bio-matrices, cell lysate and biofluid. In this case, qRT-PCR will be subject to significant interferences from complex matrices, resulting in unexpected errors. In contrast, DQAMmiR will shows excellent results which indicate its ability for miRNA quantification of complex biological samples. The concentration is plot in log scale. Each concentration (red points) comes from three measurements (blue points).

Conclusion

- DQAMmiR has shown direct, quantitative analysis of multiple analysis with remarkable accuracy, specificity and sensitivity.
- In comparison with qRT-PCR, DQAMmiR is anticipated to be inherently capable of working with biological samples without calibration curve and complex sample extraction.
- Therefore, DQAMmiR is anticipated to become a standard procedure complementary to qRT-PCR for practical miRNAs analysis.

References

1. Wegman, David W., and Sergey N. Krylov. *Angewandte Chemie International Edition* 50.44 (**2011**): 10335-10339.
2. Chen, Caifu, et al. *Nucleic acids research* 33.20 (**2005**): e179-e179.