# Rolling Circle Amplification-Coupled Nanopore for Quantification of miRNAs

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## ABSTRACT

Mild traumatic brain injury (mTBI) is the most common type of traumatic brain injury. However, the mTBI is underdiagnosed and underreported due to delayed onset of symptoms and the conventional subjective assessment. Recent findings suggest that salivary miRNA could be biomarkers for mTBI diagnosis. However, detecting and differentiating miRNAs are challenging due to the short length and high homogeneity. We developed a nanopore coupled rolling circle amplification (RCA) method to identify and quantify mTBI-related miRNAs. We believe this platform could be further developed for the clinical diagnosis of mTBI.

## KEYWORDS:

mTBI, miRNA, RCA, Nanopore

## INTRODUCTION

The mTBI diagnosis relies on patient-reported symptoms and subjective clinical assessment. Therefore, objective assessment tools are needed to help identify mTBI. Previous findings showed that perturbation in salivary miRNA levels after traumatic brain injury could persist for weeks, and can be used to predict the occurrence of post-concussive symptoms [1]. Therefore, salivary miRNAs are potential biomarkers for mTBI diagnosis. Besides, simultaneously monitoring levels of multiple miRNAs could help diagnose mTBI with higher accuracy [2]. The traditional techniques for miRNAs detection include northern blotting, RT-PCR, and microarrays [3]. However, these methods have limitations like possible contamination, lengthy experiment time, and costly instruments. Rapid identification and quantification of miRNAs thus remain a critical unmet need. The RCA-based miRNA detection via nanopore provides the possibility of parallel quantification of multiple miRNAs at the same time, which could be further applied to diagnose mTBI.

## THEORY

The working scheme of the RCA-based miRNA detection by nanopore is illustrated in **Figure 1 A**. First, miRNA is hybridized to the complementary region of the specific probe. Next, the probes will be ligated by T4 RNA ligase 2 and become circularized. After that, the miRNA can act as the primer to initiate RCA reaction via phi29 DNA polymerase. Finally, the elongated products can be verified by agarose gel electrophoresis, and quantified by glass nanopore through blockage event rate (**Figure 1B**). Therefore, we can further quantify the amount of miRNA by comparing the event rate.

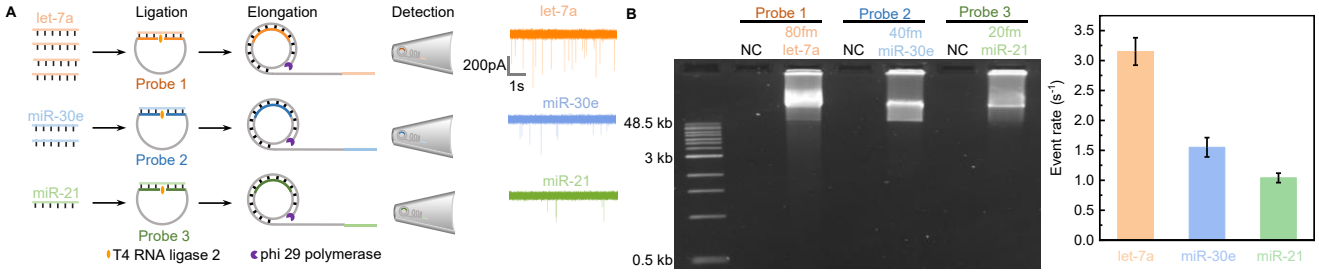


***Figure 1.*** *RCA-based miRNA assay validation. **(A)** The schematics of rolling circle amplification and the current trace of nanopore detection. **(B)** Gel image (1% agarose) of RCA amplicons and event rate for different miRNAs' amplicons (NC means negative control, i.e., no miRNA added).*

## RESULTS AND DISCUSSION

Prior to the quantification of miRNAs, we first validate the RCA assay and specificity of designed probes. **Figure 1 B** shows the gel image of RCA amplicons. All negative controls show no band, and bands longer than 48.5 kb are clearly observed for all three types of miRNAs. The miRNAs are intestinally added with different amounts: 80 fm let-7a, 40 fm miR-30e and 20 fm miR-21. The gel image shows decreasing intensity, and the event rate shows decreasing values for decreasing amount of miRNA. This trend indicates the possible miRNA quantification ability of the RCA-based platform. The amount ratio of three miRNAs is 4:2:1. However, the event

rate ratio between miR-30e and miR-21 is not close to the 2:1. This is probably due to the different binding efficiency between miRNAs and probes.

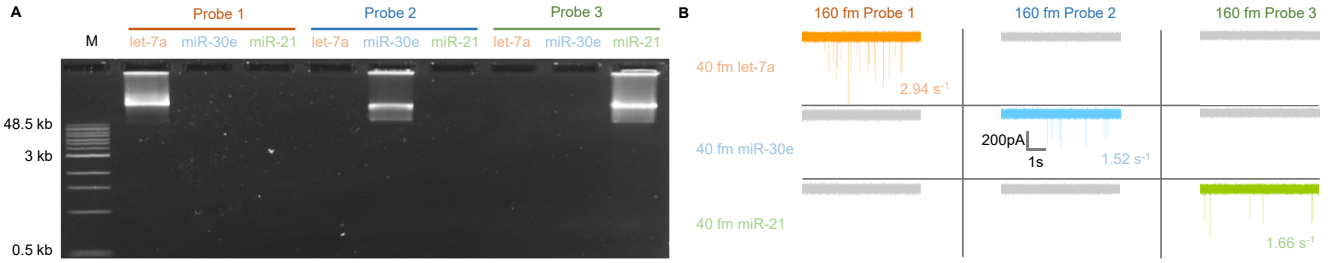


***Figure 2.*** *Validation of probe specificity. (A) Gel image of combinations of probes with different miRNAs. (B) The corresponding current traces for each combination.*

To evaluate the specificity of designed probes, we performed RCA reactions with combinations of different miRNAs and probes. Probe 1 is specific to let-7a, probe 2 is specific to miR-30e, and probe 3 is specific to miR-21. The RCA products are confirmed by gel electrophoresis (**Figure 2 A)**. According to the gel image, no band is observed for non-specific combinations, and only combinations of miRNA and its specific probe show bands for the RCA products. All samples are further tested with glass nanopore to get the blockage event rate. Only combinations of miRNA and corresponding probe have events, and no event is captured for non-specific combinations **(Figure 2 B)**. Therefore, we can conclude that the designed probes have good specificity. However, the binding efficiency between different miRNAs and probes varies since the gel band intensity and event rate is different for the same amount of miRNAs.

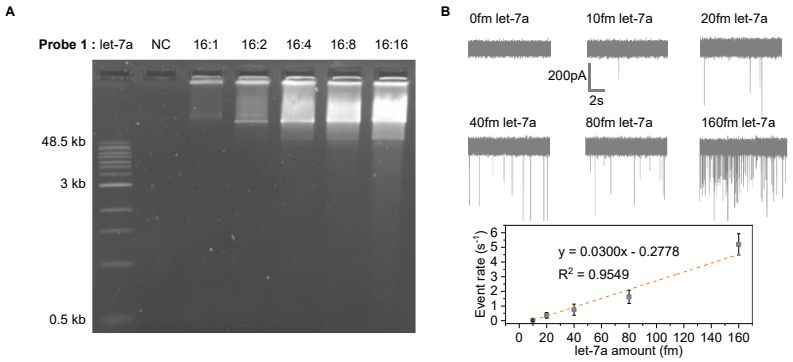


***Figure 3.*** *Quantification of miRNA. (A) Gel image of different amount let-7a's RCA amplicon. (B) The corresponding event rate for different amount let-7a.*

Next, the quantification ability of this platform is evaluated. RCA reactions are performed with different amounts of let-7a. As shown in **Figure 3 A**, the intensity of bands is increasing with increasing let-7a amount. The following glass nanopore counting result (**Figure 3 B**) shows a linear relationship between the let-7a amount and event rate. Therefore, with validated specificity, we can identify and quantify certain miRNA by measuring the event rate of the RCA assay solution.

## CONCLUSION

In summary, we developed a RCA-based miRNA detection platform. With verified good specificity, multiple mTBI-related miRNAs could be identified and quantified at the same time. We believe this nanopore coupled platform could be further developed for the clinical diagnosis of mTBI.


## ACKNOWLEDGEMENTS

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## REFERENCES


**[1]** Atif, H., et al., Journal of experimental neuroscience, 2019, 13, 1179069519832286.

**[2]** Hicks, S.D., et al., Clinical and translational medicine, 2020, 10(6), e197.

**[3]** Ye, J., et al., Journal of pharmaceutical analysis, 2019, 9(4), 217-226.


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