FIRE: THE FIRST-YEAR
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MOLECULAR DIAGNOSTICS

Colorimetric Detection of miRNA with DNA-Functionalized Gold Nanoparticles

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Intent & Motivation

Intent: Develop a miRNA detection assay with a colorimetric output that can be quantified using a **portable sensor**.

Motivation: Increase the **accessibility** of initial cancer screenings by providing a **portable** and **cost-effective** method for miRNA detection.

Background

miRNAs and Early Detection

- Early detection of cancer is important for improving patient prognosis.[1]
- miRNAs are short, non-coding RNA sequences that regulate genes involved in oncogenesis.[1]
- miRNAs circulate in peripheral blood and can serve as cancer biomarkers.[1]
- A portable sensor eliminates the need for expensive equipment involved in current miRNA detection methods like RT-PCR.[1]

Cas12a in Detection Assays

- The Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) complex cleaves DNA.[2]
- Cas12a, a CRISPR enzyme, has trans cleavage activity, allowing for cleavage of surrounding DNA.[2]
- Cas12a triggers an output when a target sequence is present, allowing for diagnostic applications.[2]

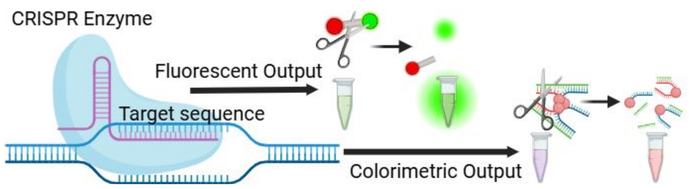

Figure 1: Schematic of CRISPR mediated outputs. Trans cleavage activity activates when crRNA in the Cas12a complex binds to a target sequence.[2]

Gold Nanoparticles for Colorimetric Output

- Gold nanoparticles (AuNPs) serve as colorimetric reporters because nanoparticle proximity changes visual output.[3]
- Conjugation of thiolated single-stranded DNA onto AuNPs enables manipulation of particle aggregation.[3]

Methods

Ligation-Initiated Transcription & CRISPR Activation (LTC) Assay

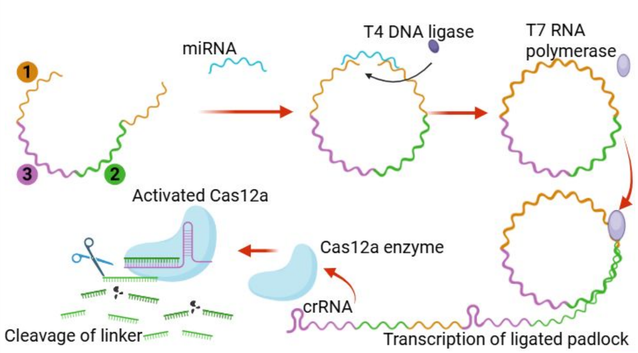

Figure 2. Presence of miRNA target initiates LTC assay, resulting in Cas12a activation. Cas12a cleaves linker sequence. Absence of linker sequence prevents aggregation

Testing Colorimetric Output with Gold Nanoparticles

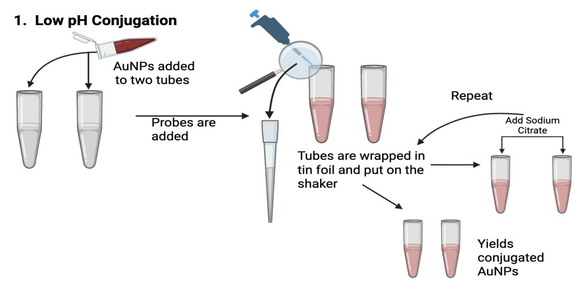

Figure 3a. (1) Gold nanoparticles conjugated with ssDNA probes using the low-pH method with Sodium Citrate.[4,5]

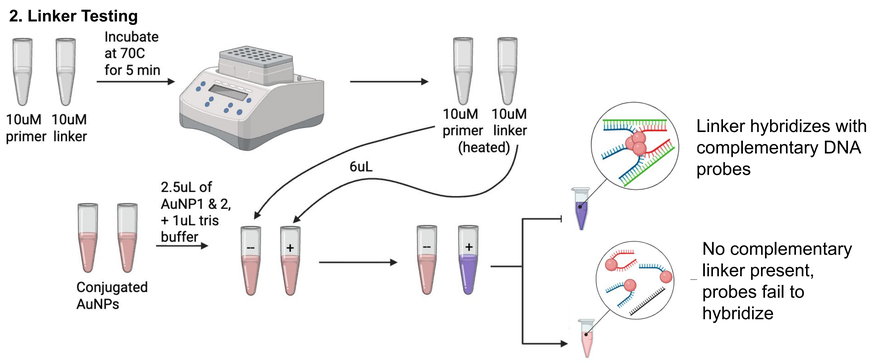

Figure 3b. (2) Linker testing to form AuNP-linker complex; successful binding of linker to probes forms complex, producing a purple output; unsuccessful binding prevents formation of complex, maintaining a pink output. The linker test negative control simulates assay output when linker sequence is cleaved.[6]

References and Acknowledgements

QR code leads to a document containing references. We would like to express our gratitude towards Dr. Catherine Spirito, Dr. Shannon Hilton, Dr. Ian White, and the Rapid Diagnostics lab for their contributions to our research. This project was a part of the First-Year Innovation and Research Experience Program. Figures 1-3 were created in BioRender.

Results & Discussion

Visualizing Successful CRISPR Activation

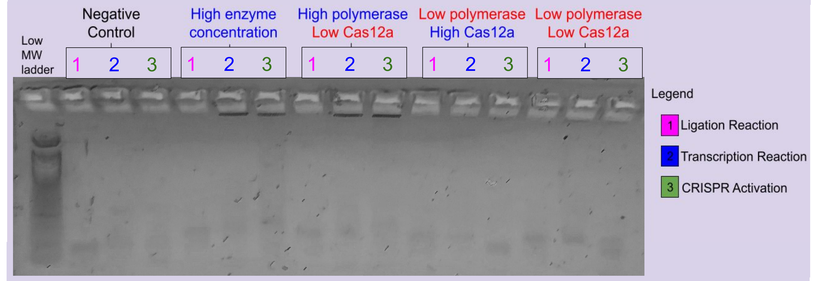

Figure 4. 3% Agarose gel displaying successful ligation of padlock probe (1), followed by rolling circle transcription (RCT) of the padlock probe(2), and subsequent activation of Cas12a (3), which results in cleavage of transcription product. Higher concentration of T7 RNA polymerase resulted in successful RCT.

Linker Testing

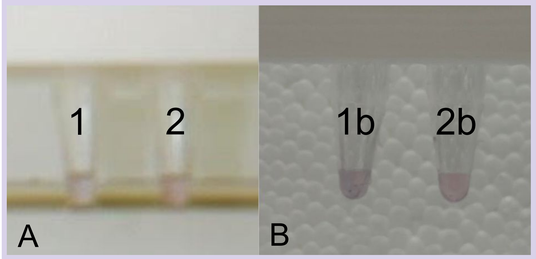

Figure 5. Linker test results captured on portable sensor camera (A) and phone camera (B). All tubes contain conjugated AuNPs. Tubes 1 and 1b contain 10 uM of linker DNA . Tubes 2 and 2b contain 10 uM of primer, roughly the same length as the linker DNA.

Discussion

Figure 4. Demonstrated the effectiveness of the LTC assay, showing that Cas12a is activated successfully in the presence of the miRNA target. When implemented with the AuNP output, Cas12a should go on to cleave the linker sequence, preventing AuNP aggregation.

Figure 5. Presence of linker sequence results in AuNP aggregation. The linker is complementary to the AuNP probes, so no aggregation occurs when a primer which is not complementary to the probes is used in place of linker.

Future Work

- Combine LTC assay with AuNP output to detect miRNA.
- Heat and quantify full assay using portable sensor
- Determine the specificity and sensitivity of the assay with differing miRNAs and concentrations.
- Compare the performance of the assay to other methods of miRNA detection, like RT-PCR and ligation-LAMP.
- Assess if the LTC-AuNP assay can be utilized in a one-pot system.