FIRE: THE FIRST-YEAR
INNOVATION & RESEARCH
EXPERIENCE

**MOLECULAR DIAGNOSTICS & RAPID 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

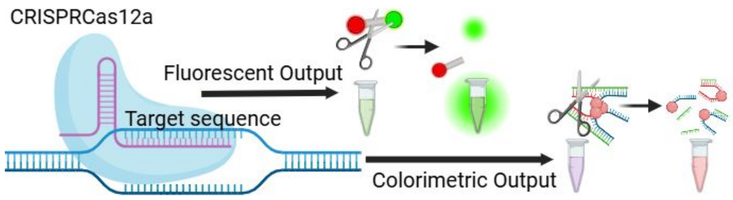


**Figure 1.** Schematic of CRISPR mediated outputs. Nonspecific ssDNA trans-cleavage activity of CRISPR/Cas12a can trigger fluorescent output using a fluorophore-quencher reporter, or a colorimetric output using cross-linked AuNPs, allowing for diagnostic applications.[2,3]

## 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 for their contributions to our research. This project was a part of the First-Year Innovation and Research Experience Program at the University of Maryland. Figures were created in BioRender.

## Methods

### Synthesis, Conjugation, and Cross-Linking of Gold Nanoparticles

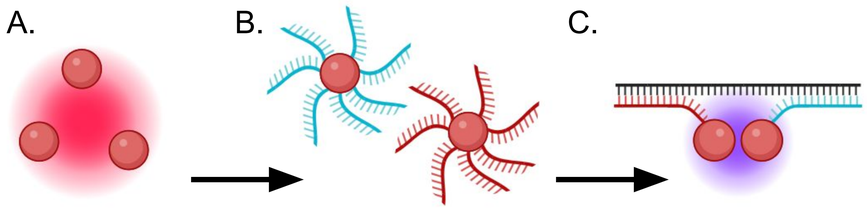


**Figure 2.** AuNPs were synthesized using the citrate reduction method **(A)**[4]. Thiolated ssDNA probes were conjugated onto AuNP surface using the low pH method **(B)**[5]. Cross linking of AuNPs occurs in presence of sequence complementary to probes**(C)**[6].

### Ligation-Initiated Transcription & CRISPR Activation (LTC) Assay

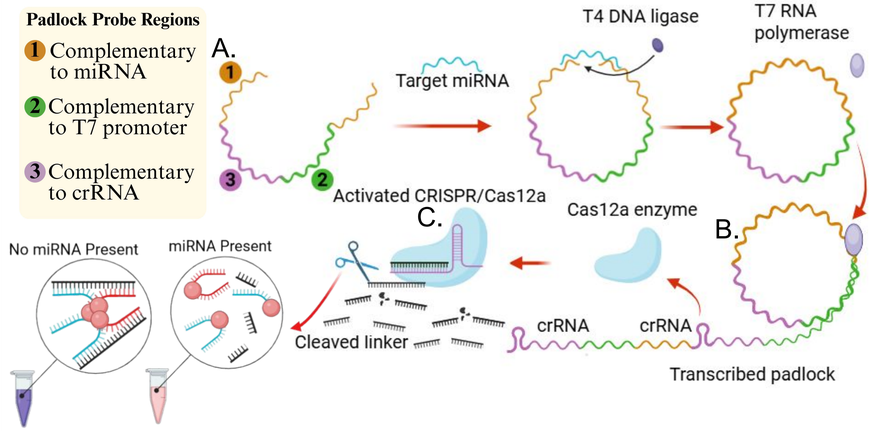


**Figure 3. (A)** Target miRNA acts as splint for padlock DNA ligation. Reaction occurs at 25 C for 1 hour. **(B)** Rolling Circle Transcription(RCT) of circular padlock at 37 C for 1 hour generates crRNA repeats. **(C)** crRNA-Cas12a complex cleaves linker DNA. Cleaves linker cannot cross-link conjugated AuNPs. LTC Assay has been adapted from Cheng et al. 2025 and Zhang et al. 2021.

### Portable miRNA Detector

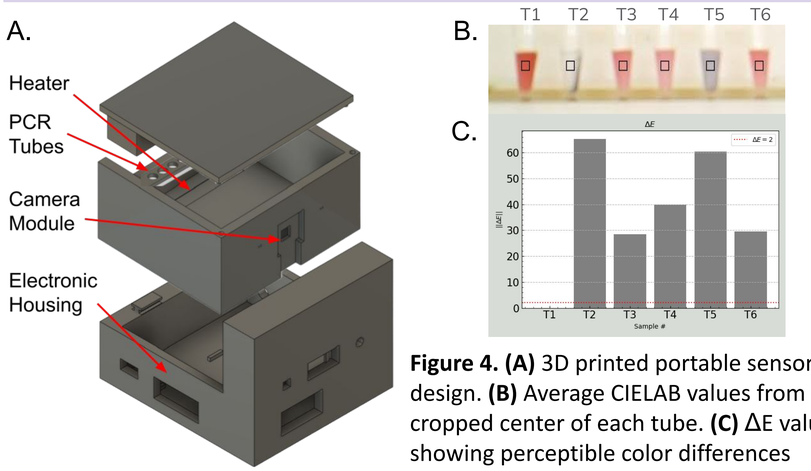


**Figure 4. (A)** 3D printed portable sensor design. **(B)** Average CIELAB values from the cropped center of each tube. **(C)** ΔE values showing perceptible color differences between the tubes in (**B**).

## Results & Discussion

### Successful RCT Triggers CRISPR/Cas12a Activation

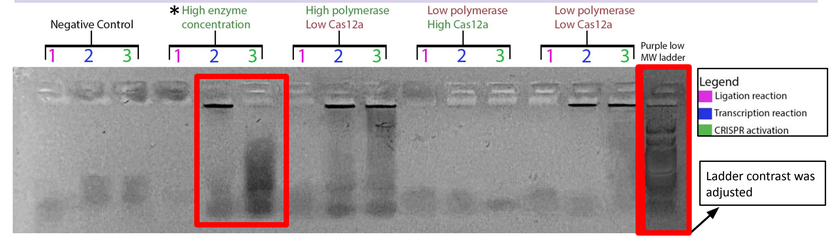


**Figure 5.** 3% Agarose gel stained with GelRed displaying successful ligation of padlock probe in the presence of DNA analog of miRNA target **(1)**, followed by rolling circle transcription (RCT) of the padlock probe **(2)**, and subsequent activation of Cas12a **(3)**, which results in cleavage of transcription product. Negative control contains padlock probe, but no miRNA target.
*High enzyme = high T7 RNA polymerase & high Cas12a.

### Resistance to Salt Indicates Successful Conjugation of AuNPs

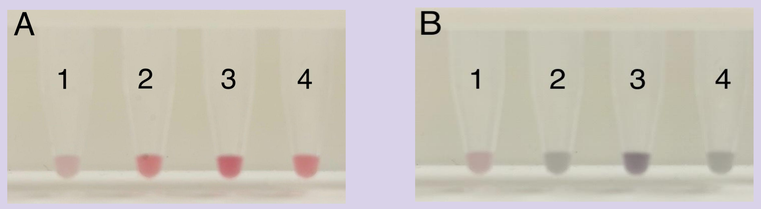


**Figure 6A and 6B** represent before (A) and after (B) adding 1 µL of saturated NaCl. Red color indicates successful DNA conjugation and no aggregation.

1. 10 µL AuNPs (12 nm diameter) with probes 1 + 2
2. 10 µL of unconjugated AuNPs (12 nm diameter)
3. 10 µL AuNPs (20 nm diameter) with probes 1 + 2
4. 10 µL of unconjugated AuNPs (20 nm diameter)

### Discussion

- Activation of CRISPR/Cas12a trans-cleavage activity in the presence of DNA analog of miRNA target (**Fig. 5**).
- Successful conjugation of ssDNA probes onto synthesized AuNPs (**Fig. 6**).
- Larger AuNP sizes were more sensitive to salt induced aggregation regardless of conjugation (**Fig. 6**).

## Future Work

- Optimize assay conditions to troubleshoot cross-linking of AuNPs.
- Combine LTC assay with cross-linked AuNPs 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.