FIRE: THE FIRST-YEAR INNOVATION & RESEARCH EXPERIENCE

MOLECULAR DIAGNOSTICS & RAPID DIAGNOSTICS

Portable Sensor for One-Pot Ligation LAMP Assay to Detect miRNA Biomarkers

Yedidya Adane, Jason Berdia, Aayush Bhargava, Catarina Fernandes, Roshni Pallavajjala, Mark Vitievsky

Intent and Motivation

Developing a portable sensor for a One-Pot ligation-LAMP assay targeting miRNA, a cancer biomarker, offers a promising method for early cancer detection. Detecting cancer early is crucial for improving survival rates.

Objective:

To develop a sensitive and specific One-Pot ligation-based Loop Mediated Isothermal Amplification (LAMP) assay using Thermally Responsive Alkane Partitions (TRAPs), with integration into a portable sensor to detect miRNA-155 from a patient blood sample for convenient and rapid diagnosis.

Time Efficient | Cost Effective | Non-Invasive

Background

miRNA Overview:

- microRNAs (miRNAs) are short sequences of non-coding RNA that play a vital role in the regulation of gene expression in cells and tumorigenesis[1]
- Specific miRNAs can be used as biomarkers, as patients with cancer exhibited elevated concentrations of miRNA in blood samples[1]

Ligation Loop-Mediated Isothermal Amplification (LAMP) with Thermally Responsive Alkane Partitions (TRAPs):

- Ligation-LAMP detects short nucleic acids sequences.[2]
- LAMP is cost effective, time efficient, easily accessible
  - PCR requires expensive equipment, is time-consuming, and is limited to fluorescent readings.[2]
- Thermally Responsive Alkane Partitions (TRAPs) allow assay steps to be separated in a single tube.[3]
  - Automates reagent mixing through temperature manipulation.[3]
- Changing TRAP geometry allows for magnetic beads to breach without reagents mixing, allowing for a capture step.[3]

Acknowledgements

We would like to express our sincerest gratitude to **Dr. Catherine Spirito** and **Dr. Shannon Hilton** and the **FIRE program** for granting us the opportunity to engage in cutting edge research. Thank you to **Evan Benke** and **Dr. Ian White** for collaborating with us. Lastly, we are tremendously grateful for **Zoya Naseem** for the live animations. Figure 1 was created using BioRender. Figure 5 was created using Canva. Icons are from flaticon.com and poster created in Google Slides.

Scan for References and Our Assay in Action

Methodology

One-Pot Assay

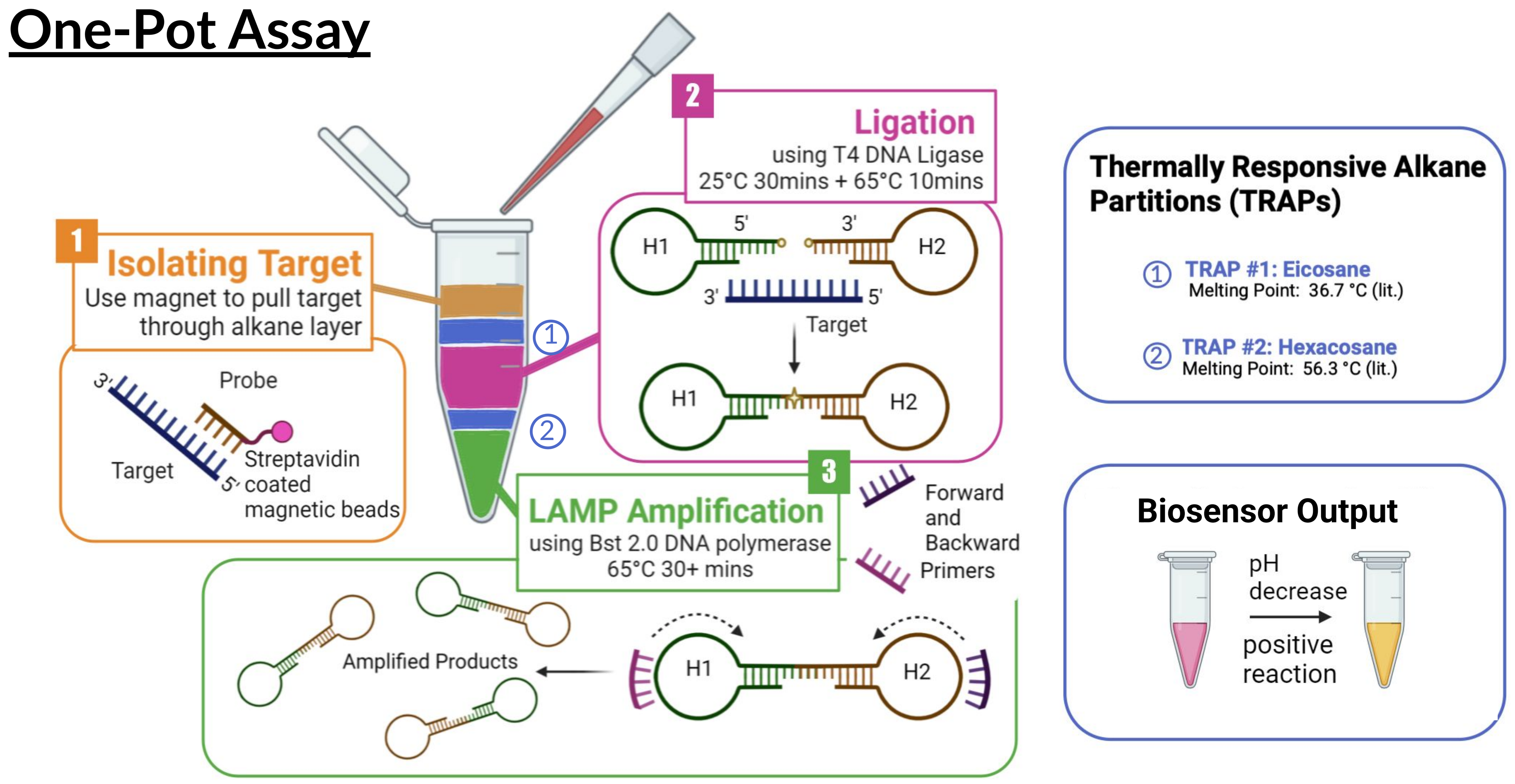

Figure 1: The three main steps to the One-Pot Ligation-LAMP Assay. Ligation and LAMP steps follow the process outlined in the Du et al.[2] TRAP system utilizes system similar to that of the White Lab.[3] Our assay is specific to the detection of miRNA-155. LAMP reaction decreases pH, causing phenol red color change to occur.

Data Collection and Portable Sensor

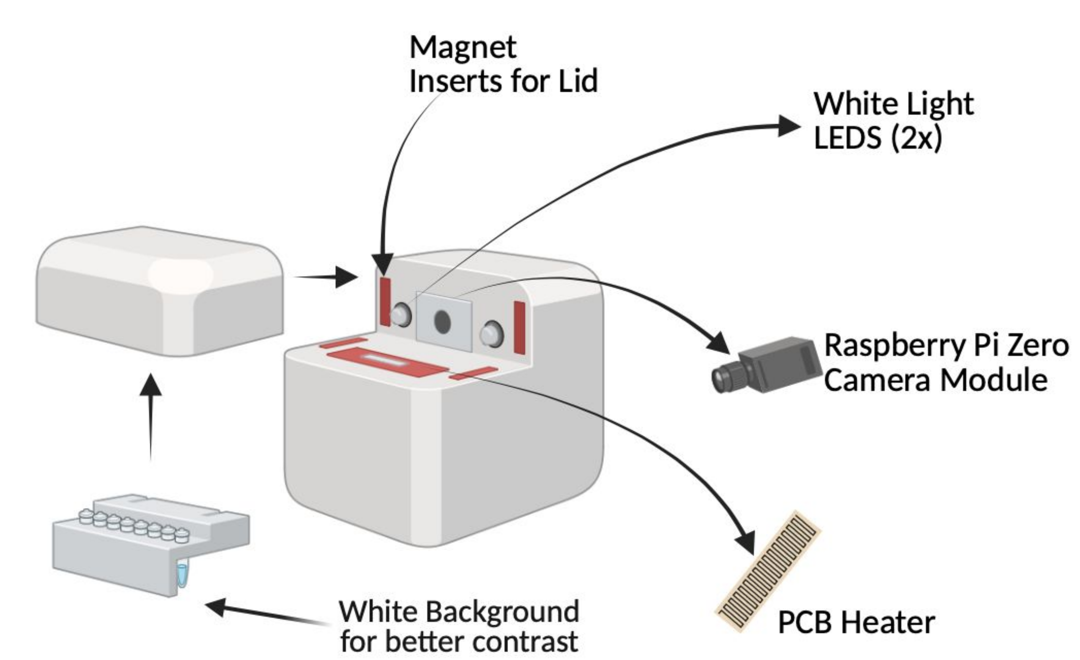

Figure 2: Portable sensor design adapted from Papadakis et al.[4] for colorimetric LAMP. Features include LEDs for lighting, camera module for automated pictures, PCB heater for LAMP, and magnet inserts for lid.

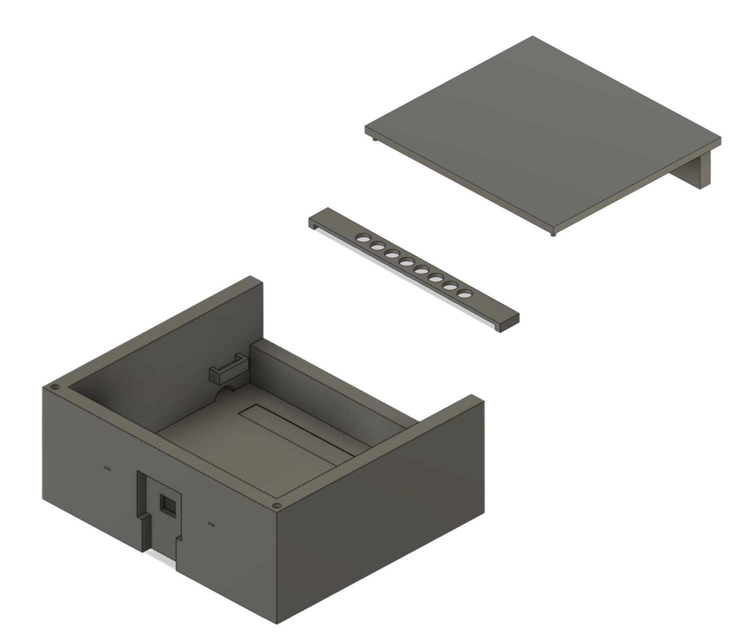

Figure 3: Our Computer-Aided Design (CAD) for portable sensor, which includes tube holder, lid notches, and insert for camera.

Image Analysis

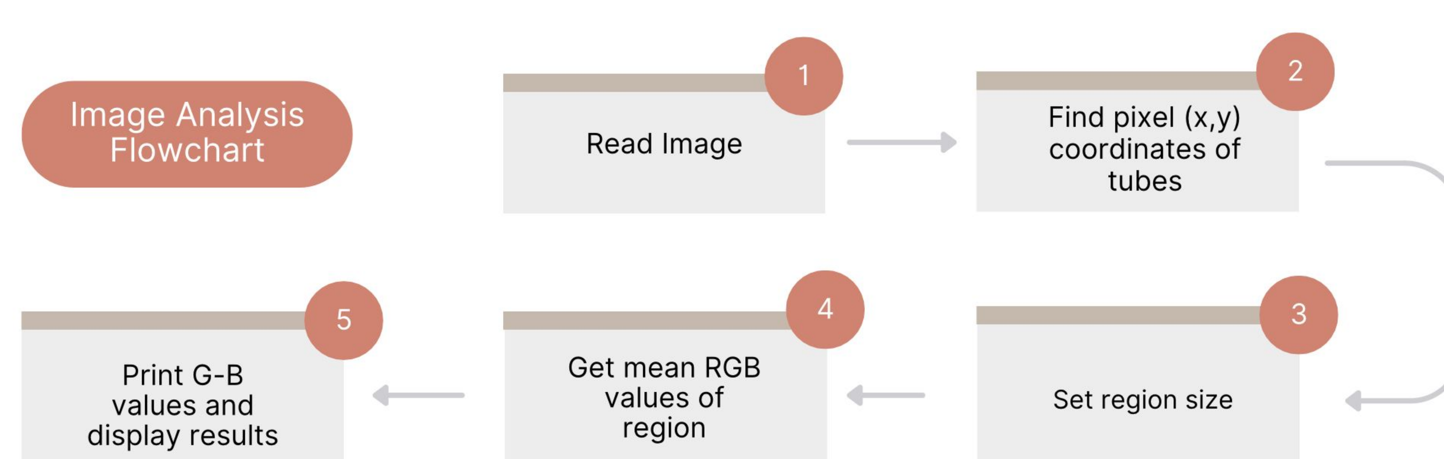

Figure 4: Flowchart of image analysis algorithm in Python. G-B formula used for the quantification of colorimetric LAMP.[4]

Results

One-Pot Colorimetric LAMP Image Results

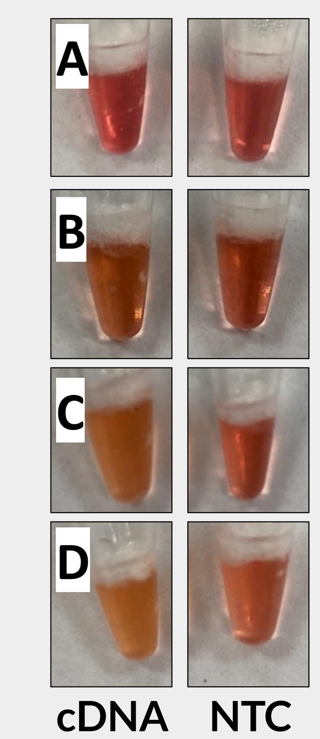

Figure 5: One-Pot Colorimetric LAMP with phenol red at **A.** 10 min, **B.** 25 min, **C.** 45 min, and **D.** 60 min with target (miRNA 222 cDNA) vs. water as negative control (NTC).

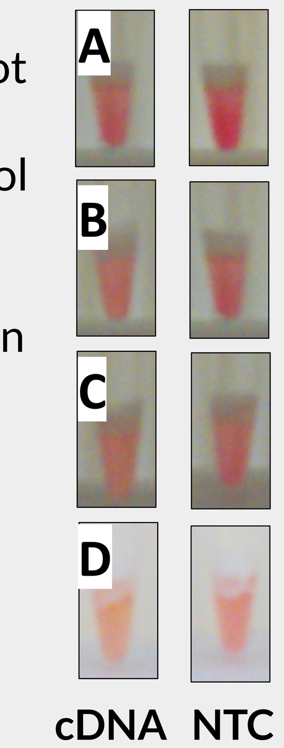

Figure 6: One-Pot Colorimetric LAMP with phenol red at **A.** 10 min, **B.** 20 min, **C.** 30 min, and **D.** 40 min with target (miRNA 155 cDNA) vs. water as negative control (NTC) using our portable sensor camera.

Portable Sensor Image Analysis

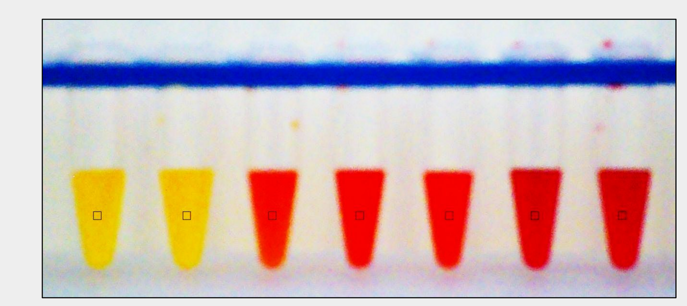

Figure 7: The average RGB values of a square region at the center of every tube was taken.

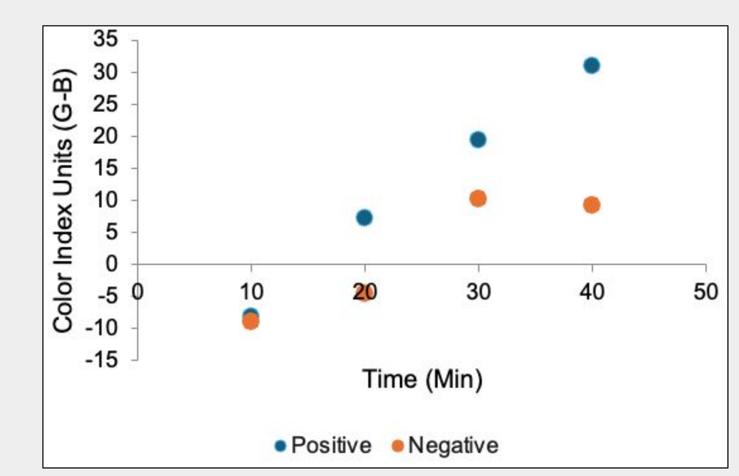

Figure 8: One-Pot Colorimetric LAMP results from Figure 7 compared using the G - B formula that subtracts the blue channel value from the green channel value in an image's average RGB values. Used to quantify the reaction rate.

Key Findings

- Using Raspberry Pi camera module, pictures in Figure 6 were comparable to manual picture-taking in Figure 5. We saw color change at similar time points, with Figure 5 at 25 mins and Figure 6 at 20-30 mins.
- Figure 7 demonstrates the ability to conduct real-time quantification analysis through Python and RGB values.
- Figure 8 demonstrates the ability to analyze rate of color change for sample

Next Steps

- Regulate temperature with a PCB heater controlled by a solid-state relay and temperature sensor to maintain temperature @ 65°C, test Ligation-LAMP for optimal heating.
- Test the fully automated picture-taking program along with heating for a positive and negative control sample with attached LEDs.
- Test different image analysis formulas.
- Test One-Pot system with surfactant (Tween 0.1%) for reducing interactions with wax, and test One-Pot system sensitivity and specificity and optimize Ligation on top

Abstract

microRNAs (miRNAs) are short sequences of non-coding RNA that play a vital role in the regulation of gene expression in cells and tumorigenesis. Previous research has identified specific miRNAs can be used as biomarkers, as patients with cancer exhibited elevated concentrations of miRNA in blood samples. Patients can have poor prognosis rates without early detection; however, current detection methods are invasive, time-consuming, and expensive. In this study, we have developed a point-of-care screening tool to detect miRNA using ligation-loop-mediated isothermal amplification (ligation-LAMP) and a 3D-printed sensor. Specifically, we designed a colorimetric ligation LAMP assay that has high sensitivity and specificity to miRNA-155, a cancer biomarker, and created a One-Pot system using Thermally Responsive Alkane Partitions (TRAPs). Furthermore, we have developed a portable sensor for the assay, with controlled heating and real-time quantification, to make the assay more accessible at the point of care. Combining the One-Pot assay with the portable sensor allows for rapid and inexpensive detection of miRNA biomarkers.