FIRE: THE FIRST-YEAR INNOVATION & RESEARCH EXPERIENCE

MOLECULAR DIAGNOSTICS

Ligation Loop-Mediated Isothermal Amplification for the Detection of Prostate Cancer

Betiel Amanuel, Yanar Hanieh, Alexander Negussie

BACKGROUND

Prostate cancer causes over 360,000 U.S. deaths annually due to late diagnoses and limitations of current invasive methods like biopsies, which can yield false positives[1]. Our project aims to non-invasively detect the biomarker miR-101-3p for early diagnosis using rapid and efficient loop-mediated isothermal amplification technology.

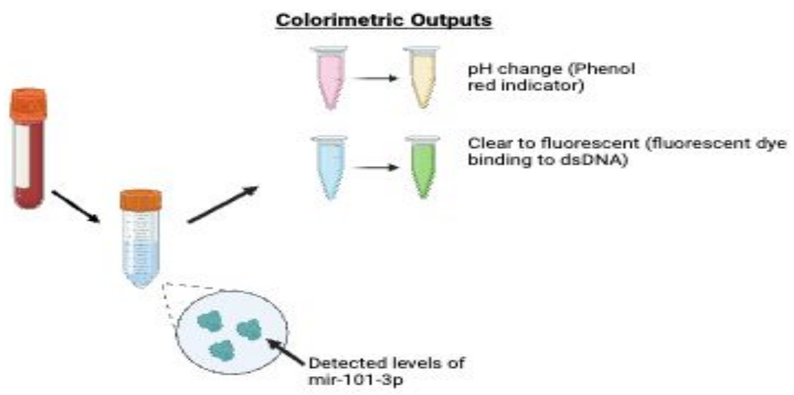


Figure 1: A blood sample is tested for our biomarker, miR-101-3p, using ligation loop-mediated amplification.

INTENT & MOTIVATION

Develop a Ligation LAMP-based biosensor to detect Hsa-miR-101-3p, a prostate cancer biomarker.
Optimize biosensor performance for accurate diagnosis by increasing sensitivity and specificity.
Evaluate effectiveness for point-of-care diagnostics, focusing on cost-effectiveness, speed, and ease of use.
Point-of-care integration of this biosensor could encourage earlier screening and more accurate diagnoses.

METHODS

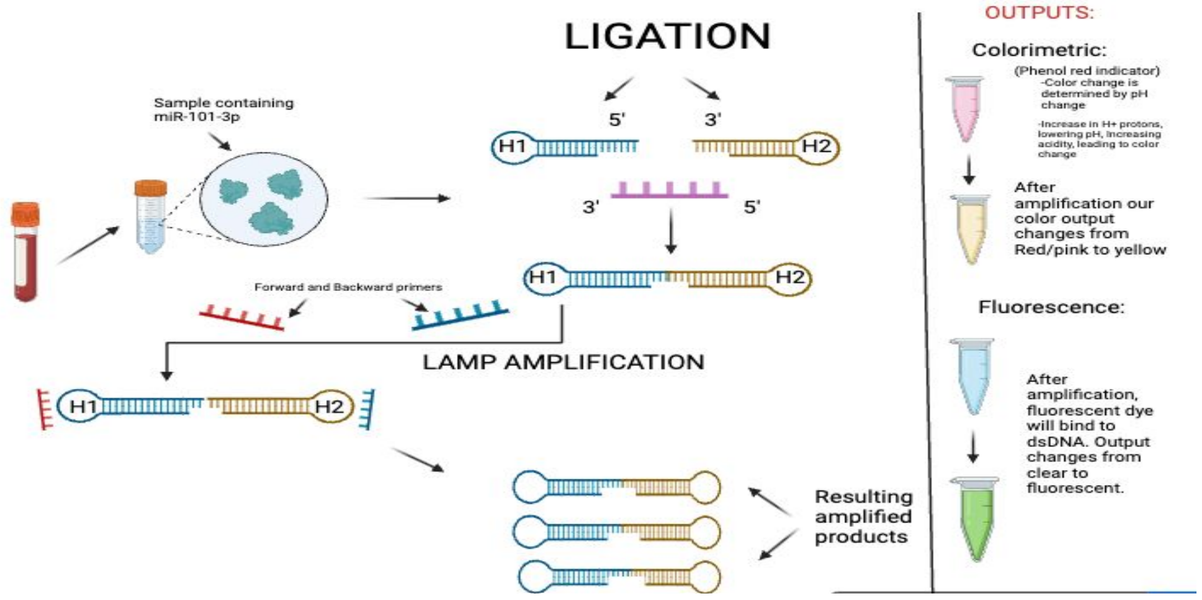


Figure 2: Target acts as a splint for the ligation of H1 and H2 to form a dumbbell. LAMP amplification results in a colorimetric output change from red to yellow or from clear to fluorescent.

Ligation Process:

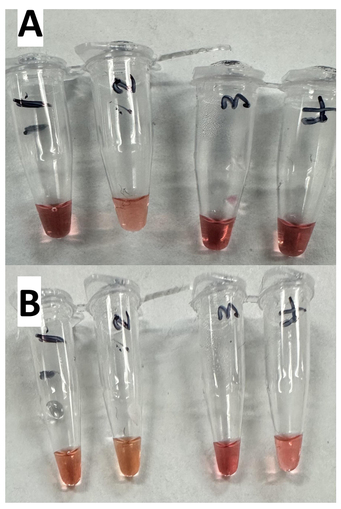
- H1 and H2 oligonucleotides.
- T4 DNA ligase.
- cDNA binds the overhangs of H1 and H2, forming dumbbell-shaped DNA structures

Colorimetric LAMP: Phenol red works as a pH indicator, changing color from pink to yellow

Fluorescent LAMP: Fluorescent dye binds to dsDNA, changing from clear to fluorescent.

RESULTS

Colorimetric LAMP w/ and without Betaine



- Betaine appears to be slowing down the reaction; The 2 right tubes (with betaine) change color at a slower rate than the left 2 (**3A**)
- Reactions with cDNA experience a color change quicker than without- tubes 2&4 (**3B**)
- Tubes 1&3 both don't have cDNA but tube 3 (with betaine) does not undergo solid color change (**3B**)

Figure 3. PCR tubes 1 and 2 have no betaine while tubes 3 and 4 have betaine added. Tubes 1 and 3 have no cDNA while tubes 2 and 4 have cDNA. At minute 20 our first color change occurred(**3A**), followed by our 2nd and 3rd color change(**3B**) at minute 40.

Colorimetric LAMP with Different Concentrations of cDNA

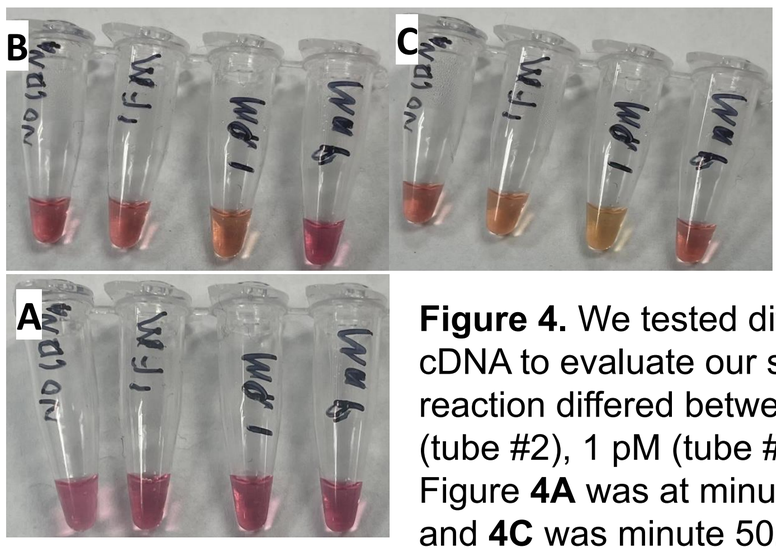


Figure 4. We tested different concentrations of cDNA to evaluate our sensitivity. We saw how the reaction differed between a no cDNA (tube #1), 1 fM (tube #2), 1 pM (tube #3), and 1 nM (tube #4). Figure **4A** was at minute 0, **4B** was at minute 40, and **4C** was minute 50.

Fluorescent LAMP w/ Betaine

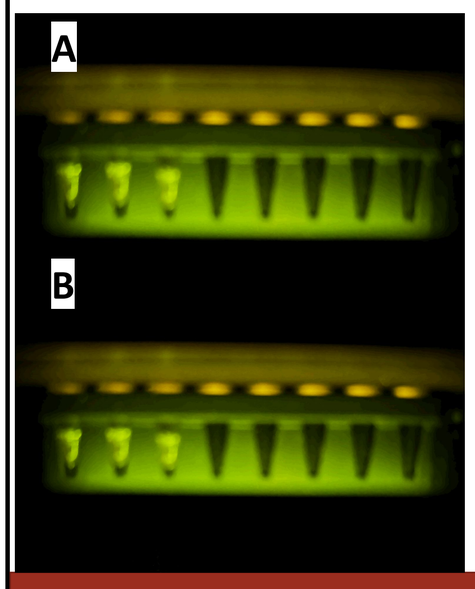


Figure 5. Fluorescent LAMP with betaine; 1 nM H1, H2 & cDNA with portable fluorescent viewer. Positive was control (miR-101-3p) and negative controls were incubated at 65°C. **5A** shows the initial reaction setup, while **5B** displays samples after 35 minutes, still lacking fluorescence.

- Neither positive nor negative controls exhibited fluorescence, indicating unsuccessful amplification.

Gel to Confirm Ligation

Figure 6. Gel Analysis to confirm ligation with 100 nM concentrations of H1, H2, and cDNA (positive control) or no cDNA (negative control).

FUTURE WORK

- Optimize sensitivity and specificity of our miR-101-3P assay
- Develop a one-pot assay method that combines all reactions: reverse transcription, ligation, LAMP amplification in a single tube
- Explore the adaptability of our LAMP-based assay for detecting other cancer biomarkers, such as miR-21 or miR-155

REFERENCES ACKNOWLEDGEMENTS

References

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