FIRE: THE FIRST-YEAR INNOVATION & RESEARCH EXPERIENCE

MOLECULAR DIAGNOSTICS

One-Pot Ligation LAMP Assay to Detect miRNA-222: A Glioma Biomarker

Yedidya Adane, Catarina Fernandes, Jillian Kaiser, Roshni Pallavajjala, Khushi Patel

BACKGROUND

Glioma: Brain Cancer Diagnosis

- Conventional diagnostic methods include MRI scans, CAT scans, and tissue biopsies, which are invasive and expensive.[1]

Ligation Loop Mediated Isothermal Amplification (LAMP)

- Amplifies short nucleic acids sequences through amplification.[2]
- Quicker/easier alternative to PCR; less complex lab equipment → cheaper/faster point of care diagnostic tool.[2]

Thermally Responsive Alkane Partitions (TRAPs)

- Allow assay steps to be separated in a single tube.[3]
- Changing TRAP geometry allows for magnetic beads to breach without reagents mixing, allowing for a capture step.[3]

INTENT & MOTIVATION

To develop a **sensitive** and **specific** one-pot ligation-based Loop Mediated Isothermal Amplification (LAMP) assay that detects **miRNA-222**, a glioma biomarker, from a patient blood sample using Thermally Responsive Alkane Partitions (TRAP).

Time Efficient **Cost Effective** **Less-Invasive**

METHODS

Our target: Upregulation of miRNA-222 is correlated to increased glioma cell proliferation and migration, while down regulation inhibits angiogenesis of glioma cells [4].

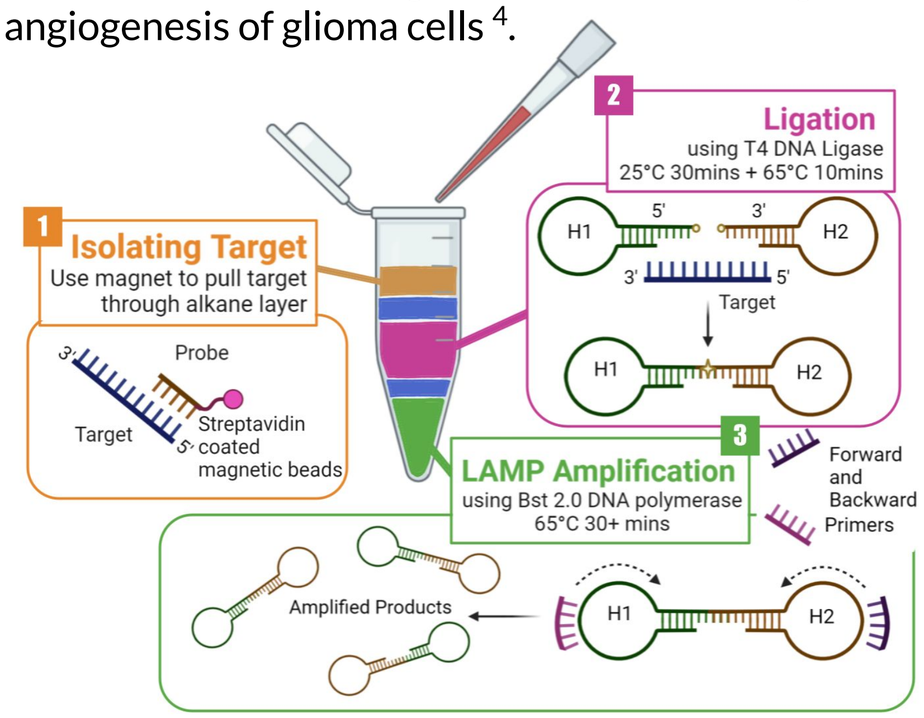

Figure 1: The three main steps to the One-Pot Ligation-LAMP Assay. Ligation and LAMP steps follow the process outlined in the Du et al.[2] TRAP system utilizes system similar to that of the White Lab.[5] Our assay is specific to the detection of miRNA-222.

RESULTS

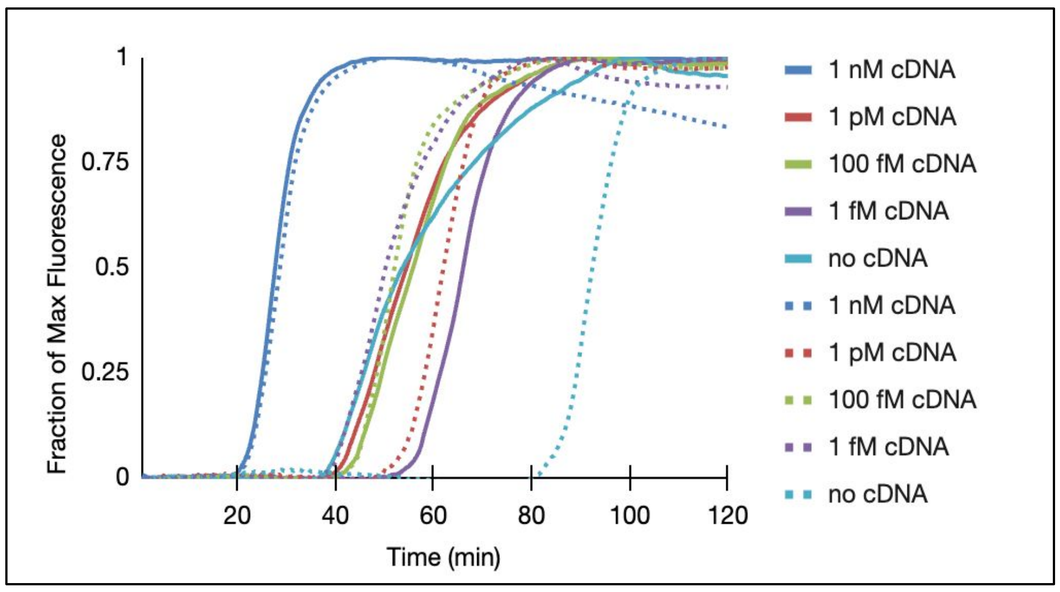

Figure 2: Real-Time One-Pot Fluorescent LAMP with different concentrations of cDNA plotted as fraction of max fluorescence against time. Reactions were run in duplicate.

Key Findings

Figure 2 shows that performing LAMP with 1 nM cDNA consistently allows for fastest reaction; fluorescence begins at 20 min. and peaks at 40 min. Generally, higher cDNA concentration yields quicker fluorescence

Figure 3 shows visible color change of LAMP with 1nM of miRNA-222 occurs at around 25 min but no color change with negative control

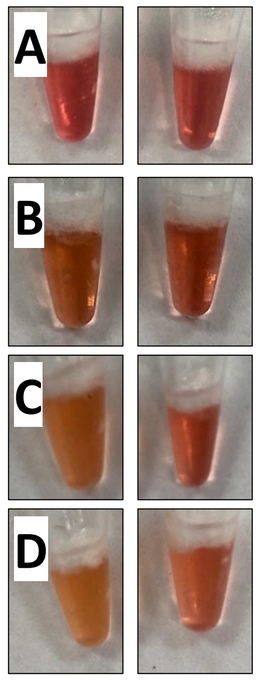

Figure 3: One-Pot Colorimetric LAMP with phenol red at **A.** 10 min, **B.** 25 min, **C.** 45 min, and **D.** 60 min with target (miRNA 222 cDNA) vs. water as negative control (NTC).

FUTURE WORK

One-Pot Assay Optimization

- Optimizing colorimetric LAMP for one-pot ligation LAMP system
- Testing strand displacement with biotinylated probe and cDNA using streptavidin-coated magnetic beads

Accessibility

- Optimizing ligation LAMP using portable heater instead of thermocycler

Scan to Watch Assay in Action and References

REFERENCES & ACKNOWLEDGEMENTS

We would like to express our sincerest gratitude to **Dr. Catherine Spirito** and the **FIRE program** for granting us the opportunity to engage in cutting-edge research. Thank you to **Dr. Shannon Hilton, Evan Benke** and **Dr. Ian White** for collaborating with us and allowing us to use their equipment. We would like to acknowledge **Azkah Anjum, Alejandra Bogusch, Zoya Naseem, and Nitya Vempaty** for their contribution towards this poster; we thank **Zoya Naseem** for creating figure 1 in the methods section. Figures 1, 2, and 3 were created using **BioRender**. Icons are from **flaticon.com** and poster created in **Google Slides.**