FIRE: THE FIRST-YEAR INNOVATION & RESEARCH EXPERIENCE

MOLECULAR DIAGNOSTICS

# Ligation LAMP Assay Targeting miRNA-155 Biomarker for Diagnosing Acute Myeloid Leukemia

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## BACKGROUND

**ACUTE MYELOID LEUKEMIA (AML)**

**Standard Diagnostic Methods**

- Standard methods of diagnosis for AML are expensive, invasive, time-consuming, painful, complex, and pose a high risk of generating false positives/negatives.[1]
- Includes: Complete Blood Count (CBC) & White Blood Cell Count (WBC) Blood Tests, Bone Marrow Biopsy, Peripheral Blood Smear[1]

**miRNA-155 Biomarker**

- Promotes tumor growth by targeting tumor suppressor genes[2]
- Suppresses immune response targeting malignant cells[2]
- Overexpression in AML patients leads to tumor cell proliferation[2]

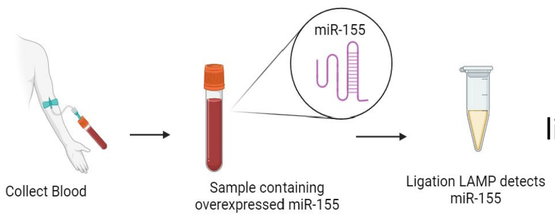


**Figure 1.** Proposal for ligation LAMP diagnostic method.

[1]USA Annual Death Rate: 11,220 deaths/year

## INTENT & MOTIVATION

- Create a rapid, minimally invasive, and cost-effective diagnostic tool for Acute Myeloid Leukemia with high sensitivity and specificity.
- Measure miRNA-155 levels, significantly elevated in AML patients, using a ligation Loop-mediated Isothermal Amplification (LAMP) assay.
- Facilitate earlier AML diagnosis to improve patient outcomes with timely treatment.

## METHODOLOGY

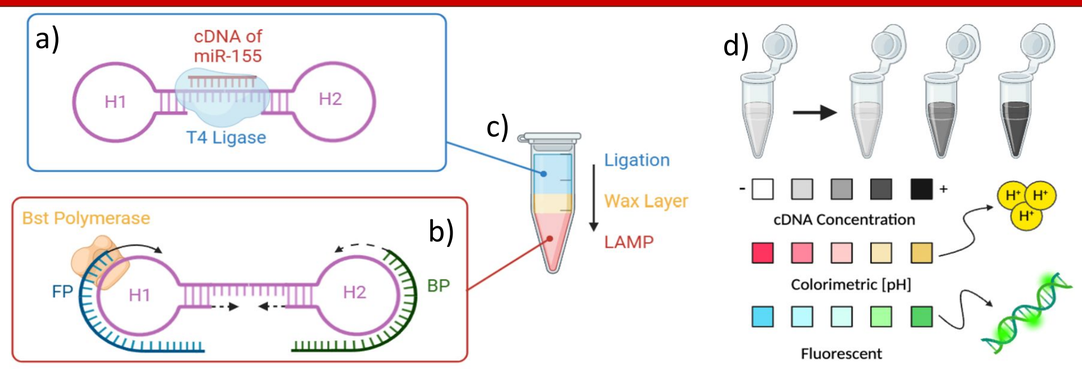


**Figure 2.** Ligation LAMP One-Pot Assay. a) Ligation. cDNA serves as splint to bind H1 and H2 probes together. Allows T4 Ligase to form dumbbell structure. b) LAMP reaction. Exponential amplification of dumbbell structure due to Bst Polymerase strand displacement. c) One-pot assay. Wax layer melts at 65°C with initiation of LAMP. d) Biochemical mechanisms of colorimetric and fluorescent LAMP.

**Ligation LAMP Assay**

- A DNA amplification method that uses primers and DNA polymerase to detect short nucleotide sequences (microRNAs) with high sensitivity and specificity. [3]
- It is faster and cheaper and compared to traditional methods like PCR.[3]

**Fluorescent LAMP**

- Luminescence generated through binding of fluorescent dye to double stranded DNA

**Colorimetric LAMP**

- Red → Yellow phenol red indicator color change generated by H+ ions during LAMP reaction
- Red → Yellow color change quantified through ImageJ software

## RESULTS

**Figure 3.** Real-time fluorescent ligation-LAMP data

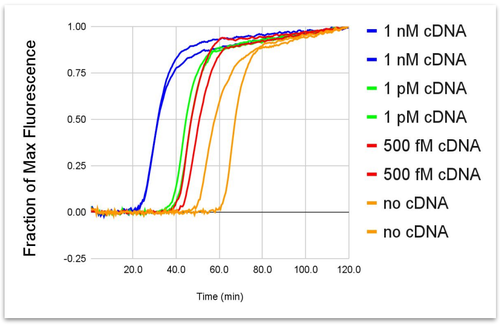


**Figure 3.1**. Various cDNA concentrations with replicates.

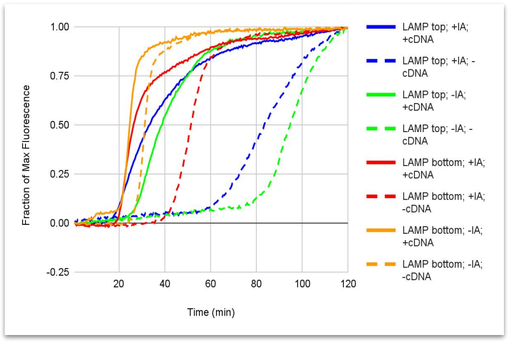


**Figure 3.2** One-pot assay. Effect of adding isothermal amplification(IA) buffer to ligation.

Colorimetric LAMP (Phenol Red) at 0.75 uM Primer Concentration; H1 & H2 at 1nM Concentration; Testing Different cDNA Concentrations

| Tube | Area | Mean | Min | Max |
|---|---|---|---|---|
| **0 Minutes** | | | | |
| 1 | 190 | **129.63** | 106 | 167 |
| 2 | 190 | **129.63** | 106 | 167 |
| 3 | 190 | **129.63** | 106 | 167 |
| 4 | 190 | **129.63** | 106 | 167 |
| **35 Minutes** | | | | |
| 1 | 136 | **101.294** | 91 | 126 |
| 2 | 136 | **128.934** | 107 | 171 |
| 3 | 136 | **152.397** | 135 | 159 |
| 4 | 136 | **155.794** | 134 | 172 |
| **70 Minutes** | | | | |
| 1 | 190 | **105.184** | 93 | 125 |
| 2 | 190 | **138.089** | 121 | 158 |
| 3 | 190 | **141.158** | 128 | 158 |
| 4 | 190 | **149.111** | 128 | 183 |

**Figure 4**: ImageJ Analysis for Colorimetric LAMP. Average Quantified Red-Yellow Color Change. cDNA concentrations of 0M, 1fM, 1pM, 1nM in tubes 1, 2, 3, 4, respectively. Data correspond to Figure 5.

**Figure 5.** Colorimetric LAMP utilizing phenol red 0.75uM primer concentration. Tube 1 = no cDNA. Tube 2 = 1fM cDNA. Tube 3 = 1pM cDNA. Tube 4 = 1nM

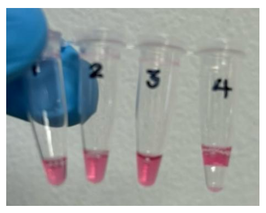

**Figure 5.1.** 0 Minutes

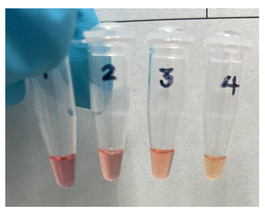

**Figure 5.2.** 35 Minutes

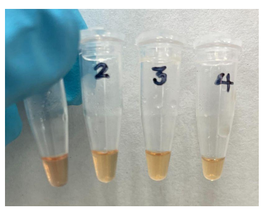

**Figure 5.3.** 70 Minutes

**Figure 6.** Colorimetric One-Pot with different ratios of Ligation:LAMP volumes. Experimental samples in top row contain isothermal amplification buffer.

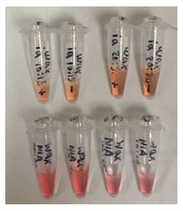

**Figure 6.1.** 10 Minutes

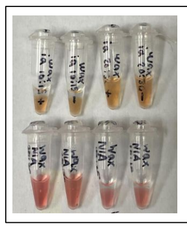

**Figure 6.2.** 65 Minutes

SUMMARY

**Figures 3 and 6** tested the sensitivity of our assay, with higher concentrations of cDNA signalling earlier than lower ones. **Figure 3** also tested replicates of each cDNA concentration, where the same concentration fluorescing at similar times indicates a more reliable assay. **Figure 4** tests the addition of a surfactant(IA) to the Ligation section of One-Pot, as well as whether LAMP is on top or bottom. Better results are indicated by a larger difference in time to fluorescence between + and -cDNA, and LAMP on bottom without IA performed the worst. **Figure 5** similarly tested the use of IA, as well as increasing the volumes of the Ligation and LAMP reagents, but the results were inconclusive.

## FUTURE WORK

- Threshold optimization using machine learning models like RandomForest and XGBoost to determine the concentration of cDNA at which a test produces a binary result for whether a patient should be diagnosed with acute myeloid leukemia
- Testing patient blood samples to quantify miRNA-155 concentration to diagnose acute myeloid leukemia, with addition of dextran additives to remove red blood cells from sample to avoid interference of red blood cells with colorimetric LAMP results
- Further optimization of the One-Pot assay, as inconclusive and deviating results were found.

## REFERENCES

1. *MicroRNAs and acute myeloid leukemia: Therapeutic implications and emerging concepts | blood | american Society of Hematology* Available at: https://ashpublications.org/blood/article/130/11/1290/36448/MicroRNAs-and-acute-myeloid-leukemia-therapeutic. (Accessed: 12th October 2024)
2. Narayan, N. *et al.* Functionally distinct roles for different mir-155 expression levels through contrasting effects on gene expression, in acute myeloid leukaemia. *Leukemia* 31, 808–820 (2016).
3. Du, W., Lv, M., Li, J., Yu, R., & Jiang, J. (2016). A ligation-based loop-mediated isothermal amplification (ligation-LAMP) strategy for highly selective microRNA detection. Chemical Communications, 52(86), 12721-12724.

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Figures 1 and 2 were created using BioRender.