FIRE: THE FIRST-YEAR INNOVATION & RESEARCH EXPERIENCE

MOLECULAR DIAGNOSTICS

One-Pot Ligation LAMP Assay of miRNA for Pancreatic Cancer Screening

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Background

Pancreatic Cancer Incidence and Diagnosis

- 5-year survival rate of 2-9%, ranks last for cancer prognosis[1]
- Asymptomatic during early stages paired with poor screening causes later diagnosis rates[1]
- 30-35% of patients are diagnosed with locally advanced cancer, 50-55% are diagnosed with metastatic (final stage)[1]
- Typical diagnosis methods (combination of endoscopic ultrasound and MRI) are not very accessible nor affordable[2]

miRNA Biomarkers of Pancreatic Cancer

- MicroRNAs (miRNAs) are short-non-coding RNAs that can act as oncogene or tumor suppressor genes[3]
  - Found in blood samples, non-invasive, easy to obtain[3]
  - Primarily detected with qPCR, microarray, and next-generation sequencing[3]
- miR-21, miR-196a, and miR-221 act as biomarkers through their upregulation in patients with pancreatic cancer[4]

Ligation Loop-Mediated Isothermal Amplification (LAMP)

- Combines reverse transcription of target (ligation) with isothermal amplification (LAMP) for nucleic acid detection[5]

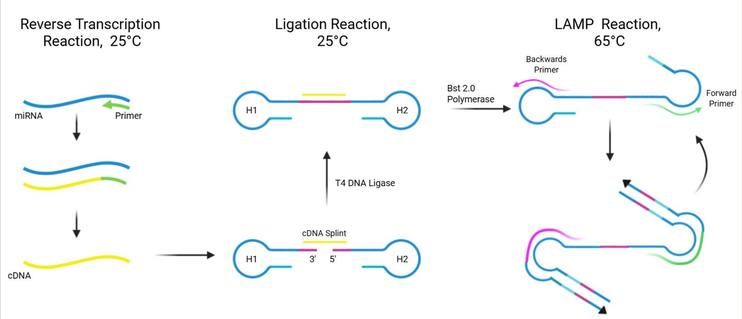

Figure 1: simplified ligation LAMP reaction based on Du et al.[5]

Intent & Motivation

Objective:

To develop a specific and sensitive one-pot colorimetric ligation-LAMP biosensor that targets miRNAs of pancreatic cancer found in whole blood samples in order to screen for cancer.

Motivation:

Make cancer detection easy, affordable, time-efficient, and non-invasive for early detection and improved survival.

References

Scan here for our references!

Methods

Preparation and Testing of LAMP Buffer

- Resuspended 1 nM phenol red and adjusted pH to 8.9
- Prepared 2X buffer using using protocol developed by Martínez and Fernan[6]

Table 1: Lab made 2X buffer synthesized following the table, making 1M stock solutions of $(NH_4)2SO_4$, KCl, and $MgSO_4$, using dNTP aliquots and Tween 20[6]. 1 nM Phenol Red was then added, along with 1M NaOH to adjust pH to ~8-9.

Reagent	Stock Concentration	2x (Buffer Concentration)	1x	Volume for 1 mL
dNTPs	10 mM	2.8 mM	1.4 mM	280 uL
$(NH_4)2SO_4$	1 M	20 mM	10 mM	20 uL
$MgSO_4$	1 M	16 mM	8 mM	16 uL
KCl	1 M	100 mM	50 mM	100 uL
Tween 20	100%	0.2%	0.1%	2 uL
Total Volume				418 uL

- Tested against 10x buffer developed by FIRE's Rapid Diagnostics (RD) lab, as well as the NEB mastermix with different concentrations of HF183 target
- Lab made 2x buffer is more affordable than bought mastermix and individual components are adjustable for increased sensitivity and specificity

One-pot Ligation LAMP to Detect miRNA

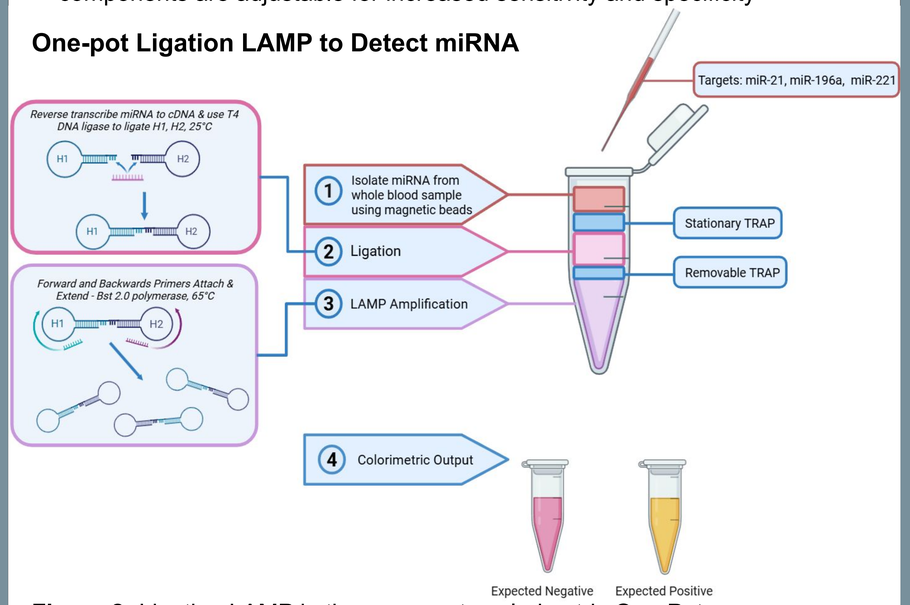

Figure 2: Ligation LAMP in three separate colorimetric One-Pot assays, detecting miR-21, miR-196a, and miR-221.

1. Magnetic beads isolate and pull target miRNA through the stationary thermally responsive alkane partition (TRAP)[7]
2. Ligation, at 25°C, reverse transcribes miRNA to cDNA using T4 DNA ligase
3. At 65°C, removable TRAP is melted and LAMP amplification occurs as Bst 2.0 polymerase attaches the forward and backwards primers, extending and amplifying H1 and H2 dumbbells
4. Increased release of H+ ions during amplification causes pH to decrease; color change indicates presence of miRNA

Results & Discussion

Comparing Lab Made vs Commercial LAMP buffers

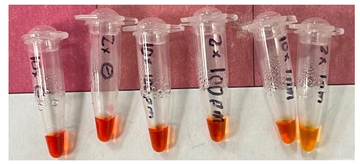

Figure 3: Individual colorimetric LAMP of lab-made 2x buffer and RD's 10x buffer, different HF183 target concentrations. 60 minutes - from left to right: 10x - control, 2x - control, 10x 100pM, 2x 100 pM, 10x 1nM, 2x 1nM.

Gel of Buffer

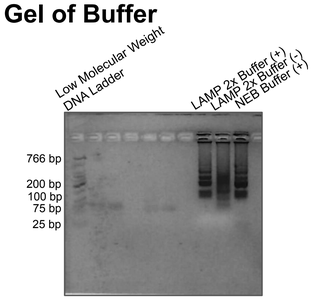

Figure 4: + and - controls for LAMP 2X buffer (individual colorimetric LAMP) and NEB mastermix.

One Pot Colorimetric Ligation LAMP

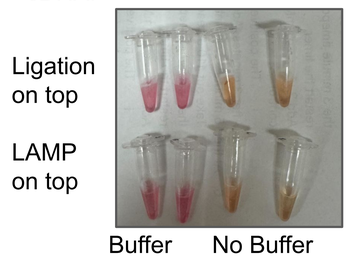

Figure 5: One-pot colorimetric LAMP with/without 10 nM miR-222 cDNA, different orientations, with/without 40 U/uL 1x T4 DNA ligase buffer (NEB) after 60 minutes.

Key Findings

- 2x buffer successfully detected the amplified DNA target (figure 4) at 1nm, however color needs to be more distinguishable (figure 5)
- No color change was observed in the one-pot after 60 minutes, meaning something in the 2x buffer is interfering with the color change

Future Work

- First, we'd like to get one-pot ligation LAMP working with ligation on top
- Continue optimizing buffer to get more of a color change
- Utilize a heatable portable sensor to run and collect continuous photos of one-pot Colorimetric LAMP assays
- Expand testing on real clinical samples (i.e. blood samples) in order to help with accuracy
- Broaden the selection of miRNA biomarkers to increase specificity for detecting pancreatic cancer exclusively

Acknowledgements

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