Optimization of logic gates for one-step detection of microRNAs via Split loop-mediated isothermal amplification (Split-LAMP)

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Introduction

miRNA biomarkers → To be used in → Development of a diagnostic point-of-care test (POCT)

- ✓ High specificity
- ✓ High stability
- ✓ Easy accessibility from patient samples

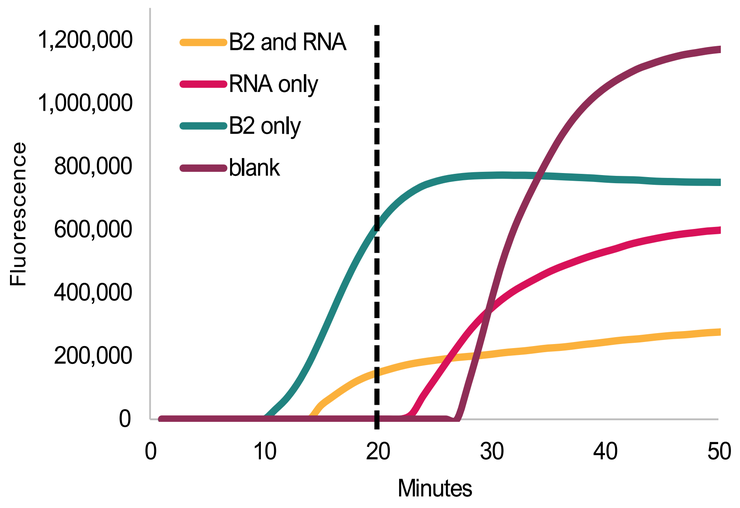
- ✓ Easy-to-use
- ✓ Rapid
- ✓ Customisable to any target disease by detecting miRNA concentration

Most diagnostically valuable in panels

Integrates signals from miRNA panel on a molecular scale into a single diagnostic readout

Aim: Show **proof-of-concept** for an easy-to-use, rapid, customizable novel **miRNA POCT platform** (Split-LAMP)

B Split-LAMP is highly specific



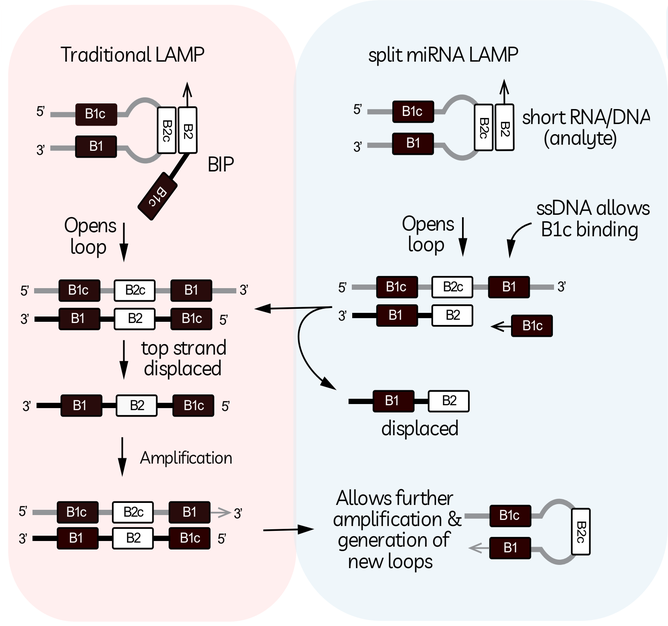
- B2 target is detected even in the presence of background total-cell RNA
- Samples containing background RNA can be clearly distinguished from those containing B2 target

Split-LAMP is **highly specific** with low risk of false positives.

Fig. 2 Real-time fluorescence curves aroused by B2 (target analyte) and HEK-293 total cell RNA (background RNA) in split-LAMP.

Traditional LAMP

- Detects concentrations of long DNA template
- Uses BIP (B1c & B2 concatemer) and FIP (F1c & F2 concatemer) for strand invasion and amplification
- LAMP cannot detect shorter miRNA targets



Novel Split-LAMP

- Detects concentrations of shorter miRNA target
- BIP and FIP are split into component primers: B1c & B2 and F1c & F2 respectively
- B2 and F2 act a miRNAs to be detected

Key Insight:
In Split-LAMP, amplification depends on the presence of **short miRNA analytes** that act as **primers** as opposed to templates.

Key benefits of Split-LAMP:

- ✓ Split-LAMP is a more easily **customizable** platform than Traditional LAMP, which can be designed to detect miRNA biomarkers specific to various target diseases by varying the B2c/F2c sequence in the template.
- ✓ Split-LAMP displays a positive readout only when two miRNA signals are present concurrently. It **integrates multiple signals** from a diagnostically valuable miRNA panel for diagnosis.
- ✓ Adding B1c and B2 as separate oligonucleotides rather than a single BIP primer results in **faster and stronger amplification** due to more strand invasion and amplification.

A B2 and F2 can be detected concurrently for diagnosis

- Preliminary experiments were conducted with DNA rather than RNA primers.
- 2 miRNAs modelled by B2 and F2.

1 **Optimal primer concentrations** to produce a signal most responsive to a 100-fold change in concentrations of B2 and F2 were **empirically identified**.

Nucleic acid component	Template	B1C	F1C	LB	LF
Empirically optimized concentration	53pg/ml	100nM	100nM	10nM 100nM	0nM

2 The effective dynamic range of Split -LAMP for B2 and F2 is **~1-200nM.**

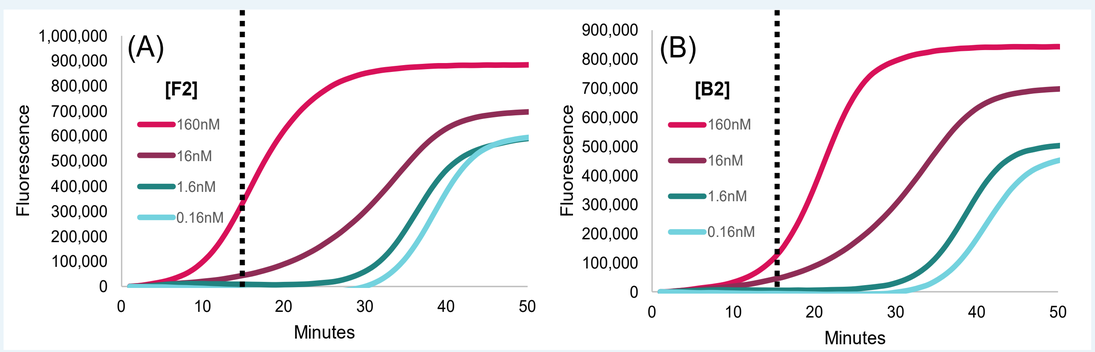
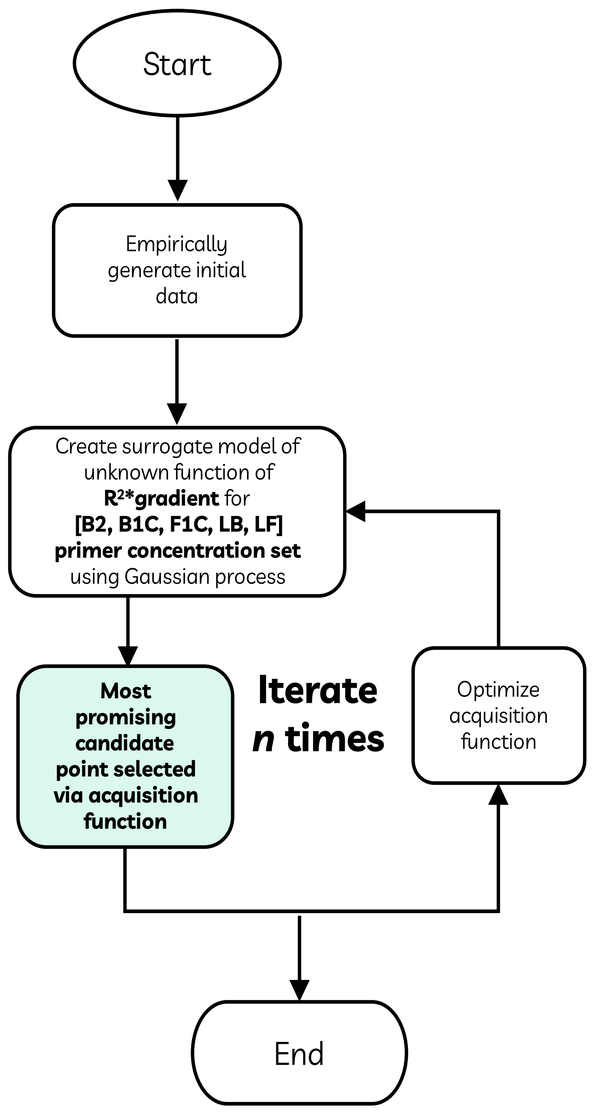


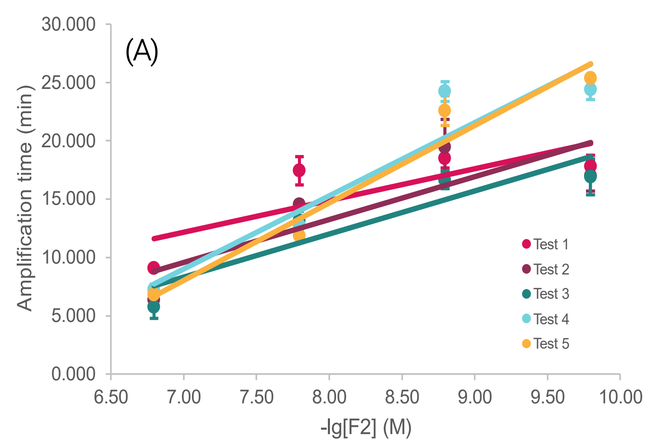
Fig. 1 (A) represents real-time fluorescence curves aroused by varying [F2] in a serial dilution, where [B2] = 16nM. (B) represents similar curves aroused by varying [B2] in a serial dilution, where [F2] = 16nM. Suitable cutoff amplification time (dotted line) can be applied to determine is readout is positive or negative.

A **single cut-off amplification time** can be applied such that positive readout is observed only if **both B2 and F2 are above threshold concentrations**. This ensures that B2 and F2 can be detected **concurrently for diagnosis.**

C Bayesian Optimization (BO) identifies new points in the solution space

Need for further finetuning of difference between F2 amplification curves from (A) → Implemented BO to introduce **novel** points in the space of **primer concentration sets** which we did not investigate from empirical data from (A)





(B)

Test #	[B2, B1C, F1C, LB, LF]
1	[15, 165, 175, 180, 30]
2	[75, 120, 155, 165, 35]
3	[90, 195, 65, 195, 10]
4	[90, 80, 120, 40, 60]
5	[75, 100, 25, 70, 125]

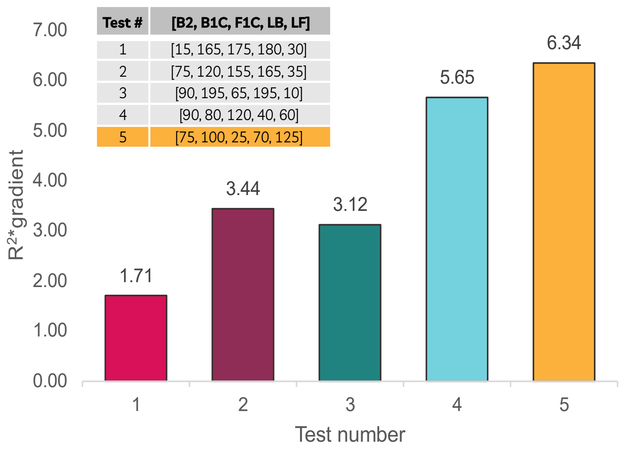


Fig. 3 (A) Standard plots for F2 serial dilutions at 5 different [B2, B1C, F1C, LB, LF] primer concentration sets generated by algorithm. (B) Bar graphs of R^2*gradient values derived from standard plots in (A) with [B2, B1C, F1C, LB, LF] primer concentration set values (nM) in each test shown in table.

Although the points introduced by the preliminary BO model did not outperform the empirical data from (A), the model outputs **competitive points** in the space of primer concentration sets. The model **shows promise** in outperforming empirical data if fine tuned with more complexity and iterations.

Conclusions and Future Work

Showed proof-of-concept for Split-LAMP → To identify a potential disease diagnostic application → To develop a POCT device for a target disease

- ✓ Integrates multiple miRNA signals into a single readout
- ✓ Customizable to detect any target disease
- ✓ Highly specific with low rate of false positives

- ➢ Development of OR/NOT logic gates

- ✓ Easy-to-use
- ✓ Rapid
- ✓ Low cost

All media objects are self-created.

[1] Abdullah AL-maskri, A. A., Ye, J., Talap, J., Hu, H., Sun, L., Yu, L., Cai, S., & Zeng, S. (2020). Reverse transcription-based loop-mediated isothermal amplification strategy for real-time miRNA detection with phosphorothioated probes. *Analytica Chimica Acta, 1126*, 1–6. https://doi.org/10.1016/j.aca.2020.06.007
[2] Biolabs, N. E. (n.d.). *Loop-mediated isothermal amplification*. NEB. Retrieved November 17, 2022, from https://www.neb.sg/applications/dna-amplification-pcr-and-qpcr/isothermal-amplification/loop-mediated-isothermal-amplification-lamp
[3] Deng, R., Zhang, K., & Li, J. (2017). Isothermal amplification for microRNA detection: From the test tube to the cell. *Accounts of Chemical Research*, *50*(4), 1059–1068. https://doi.org/10.1021/acs.accounts.7b00040
[4] Li, C., Li, Z., Jia, H., & Yan, J. (2011). One-step ultrasensitive detection of microRNAs with loop-mediated isothermal amplification (LAMP). *Chem. Commun.*, *47*(9), 2595–2597. https://doi.org/10.1039/c0cc03957h
[5] Sun, Y., Tian, H., Liu, C., Sun, Y., & Li, Z. (2017). One-step detection of microRNA with high sensitivity and specificity via target-triggered loop-mediated isothermal amplification (TT-Lamp). *Chemical Communications*, *53*(80), 11040–11043. https://doi.org/10.1039/c7cc06140d
[6] Rasmussen, C. E. Gaussian processes for machine learning. MIT Press, 2006.