Abstract

Loop-mediated isothermal amplification (LAMP) is an isothermal nucleic acid quantification concept compatible with point-of-care testing for various cancers. As miRNA panels are more diagnostically valuable than single miRNAs, we developed a novel miRNA LAMP approach that produces a positive output only if two miRNA signals are present by splitting the forward and backward internal primers of traditional LAMP into their component primers, F1c & F2 and B1c & B2 respectively. In this work, we demonstrated a proof-of-concept for diagnostic miRNA quantification via Split-LAMP. The optimal primer concentrations to produce a signal (measured in amplification time) responsive to a 100-fold change in concentrations of the two miRNAs were empirically determined based on the ability to discriminate analyte concentrations most clearly across the amplification time (slope) and the log linearity of the analyte concentration with amplification time (correlation coefficient R^2). From these concentration parameters, we approximated the effective dynamic range of Split-LAMP and identified a practical cutoff value such that a positive output is observed only if both [B2] and [F2] are above a threshold concentration. We found that split-LAMP is highly specific with low risk of false positives. Additionally, we developed a Bayesian Optimization model to introduce new points in the solution space to optimize the F2 dynamic range. One primer concentration ratio suggested by the model outperformed expectations. In the future, the optimized split-LAMP technique may be significant in point-of-care tests to diagnose cancers.

Keywords: Loop mediated isothermal amplification, miRNA detection, ANDgate

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