Introduction: Colorectal cancer ranks as the third most common diagnosed cancer and is the third leading cause of cancer-related deaths in the United States. Traditional core tumor biopsies have limitations, such as not accounting for tumor heterogeneity and being difficult to perform. Liquid biopsy offers a minimally invasive alternative, capturing information despite tumor heterogeneity and allowing for repeated monitoring.

In this study, we utilized blocker displacement amplification (BDA) PCR to selectively amplify specific mutations, such as T790, with high sensitivity, thereby guiding the selection of EGFR-targeted therapies in colorectal cancer. Although our initial focus was on the T790 mutation in the EGFR gene due to its association with non-small cell lung cancer, we have since shifted our attention to colorectal cancer. BDA PCR allows for the amplification of single nucleotide polymorphisms (SNPs) from the wild-type, facilitating the detection of low variant allele frequencies (VAFs) in liquid biopsies. By identifying resistance to treatment options in a timely manner, this test aims to improve treatment selection and patient outcomes. My research primarily involved assay development and optimization of its sensitivity and specificity.

Methods:

In the first phase, we focused on optimizing an assay designed to test for a mutation in EGFR T790. Drawing from prior BDA literature, we adjusted our assay to achieve an efficiency close to 90-110%. These adjustments included experimenting with two types of master mix (SYBR Green and TaqPath ProAmp) and altering blocker/primer concentrations. We also conducted a PCR with only varying concentrations of the mutant allele in order to have a baseline efficiency when running future trials. In one of the baseline trials, blockers were removed, while the other, they were retained.

In the second phase, a PCR assay was designed to detect two new mutations. This was accomplished using different genome viewing tools including NCBI BLAST and NCBI Genome Viewer, as well as the IDT PrimerQuest Tool.

Results: The efficiency of the assay was evaluated through a series of trials, with the highest efficiency observed at 116.6% (Fig 1). Benchmark PCRs were performed to compare the efficiency with and without the blocker. The efficiency with the blocker was found to be 95.7%, while the efficiency without the blocker was slightly lower at 91.57%. Among the different methods tested, SYBR Green yielded the most favorable efficiency.

![Figure 1. Results of most optimized PCR trial, in which SYBR Green was used.](image)

Conclusion: Our findings also revealed that increasing the concentration of the wild-type allele led to an increase in efficiency. However, the wild-type allele was identified as the main source of noise in the assay. The initial run of the newly developed assay demonstrated promising amplification of the mutant allele. Further tests will be conducted to validate and optimize the assay performance.

References:


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