# Mutation Testing in Colorectal Cancer: Leveraging Blocker Displacement Amplification for Enhanced Treatment Selection

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## 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

**Optimization of EGFR T790 Mutation Assay**

In this phase, we focused on optimizing an assay designed to test for a mutation in EGFR T790. Drawing from prior BDA literature[1], we adjusted our assay to achieve an efficiency close to 90-110% (Fig 1). 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.

**PCR Design**

In this 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

| Trial | Master mix | Cycling Speed | PCR efficiency |
|---|---|---|---|
| Experimenting with various VAFs (Varying Allele Frequency) | ProAmp | Standard | 117.6% |
| Experimenting with very low VAFs (0.01%) | ProAmp | Standard | 116.6% |
| Testing Fast Protocol | SYBR Green | Fast | 154% |
| Testing modified PCR mix | SYBR Green | Fast | 120.4% |
| Significantly increased blocker concentration | SYBR Green | Fast | 136.1% |
| Creating a Standard Curve | ProAmp | Standard | 456.8% |
| Baseline efficiency test (with no blocker) | SYBR Green | Standard | 91.57% |
| Baseline (with blocker) | SYBR Green | Standard | 95.7% |

**Figure 1:** List of all experiments and their resulting efficiencies

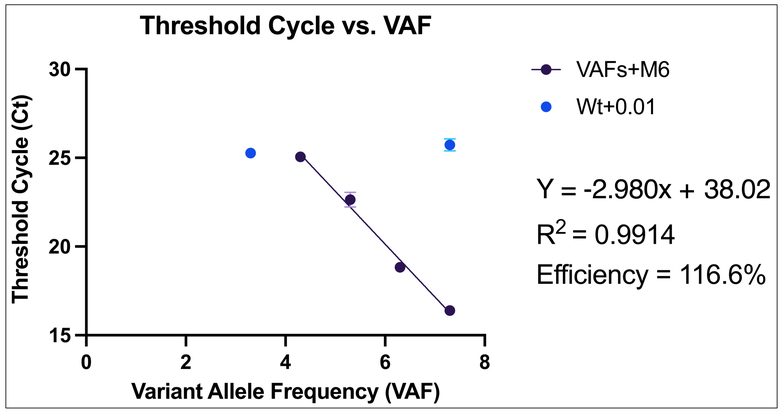


**Figure 2:** Results of most optimized PCR trial, in which SYBR Green was used . An efficiency of 90-110% is optimal (Efficiency = $-1+10^{(-1/\text{slope})}$).

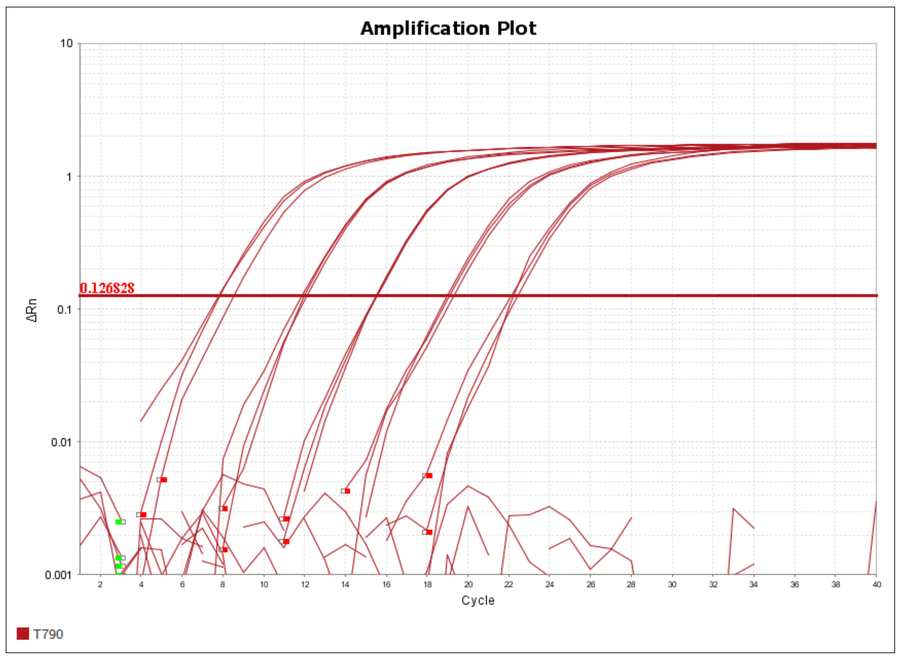


**Figure 3:** Graph depicting the results of of the baseline efficiency PCR (with blocker). As the mutant allele concentration decreases, the cycle threshold (Ct) increases.

## Discussion

- Efficiency peaked at 116.6% throughout all trials (Fig 2)
- Benchmark PCRs:
  - Efficiency with blocker: 95.7%
  - Efficiency without blocker: 91.57%
- SYBR Green results achieved the most favorable efficiency
- Increased wild-type allele concentration increases efficiency
- Wild-type is the main source of noise in assay
- Initial run of new assay had promising amplification of the mutant allele; more tests will be conducted

## Acknowledgments

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## References

1. Wu, L.R., Chen, S.X., Wu, Y. *et al.* Multiplexed enrichment of rare DNA variants via sequence-selective and temperature-robust amplification. *Nat Biomed Eng* **1**, 714–723 (2017). https://doi.org/10.1038/s41551-017-0126-5