

# High-Sensitivity Electrochemical Detection of Point Mutations: Optimization of *BRAF* V600E Analysis from Liquid Biopsy

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## Summary:

This study presents electrochemical approaches for detecting the *BRAF* V600E mutation in circulating tumor DNA (ctDNA) from liquid biopsies. The V600E mutation was successfully identified in clinical samples using SNP-specific primers in Loop-mediated isothermal amplification (LAMP). Additionally, a novel strategy employing CRISPR/Cas12a cleavage to selectively degrade wild-type (WT) sequence was evaluated to enhance mutation-specific detection.

**Keywords:** *BRAF*, V600E point mutation, ctDNA, liquid biopsy, electrochemistry

## Background

The *BRAF* proto-oncogene encodes serine/threonine protein kinase, a key component of the MAPK/ERK signaling pathway. *BRAF* mutations drive tumorigenesis; specifically, the V600E mutation is present in up to 50% of melanoma and 10% of metastatic colorectal carcinomas and is associated with poorer prognosis and treatment response. Accurate detection is therefore essential for optimizing personalized treatment.

Since ctDNA concentrations are typically low, highly sensitive detection methods are required. This challenge can be addressed by combining isothermal amplification techniques (IATs) with electrochemical (EC) detection. This approach offers a low-cost, simple, and highly selective method for point mutation detection [1].

IATs enable nucleic acid amplification at constant temperatures using specialized polymerases with strand-displacement activity, providing simplicity and rapidity for point-of-care (POC) diagnostics. Furthermore, EC detection enhances the method's potential for integration into fast, simple and cost-effective instrument in clinical diagnostics [2].

## Methods

The first approach for point mutation detection utilizes single nucleotide polymorphism (SNP)-specific primers in Loop-mediated isothermal amplification (LAMP). These primers were designed to selectively recognize the V600E

variant of the *BRAF* gene while excluding the wild type (WT) sequence (Fig. 1). This ensures that only mutant sequences will be amplified for subsequent endpoint detection.

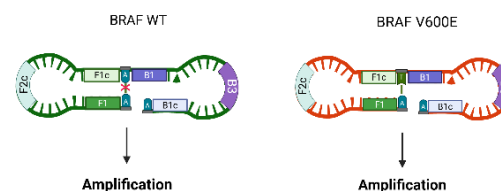


Fig. 1. Principle of SNP-specific recognition of WT and V600E mutation of *BRAF* gene

Second approach relies on CRISPR/Cas12a cleavage of the WT sequence. The guide RNA (gRNA) was designed to specifically recognize the WT *BRAF* sequence, leading to its degradation while keeping the mutated sequence intact and allowing further recombinase-polymerase (RPA) amplification and detection (Fig. 2).

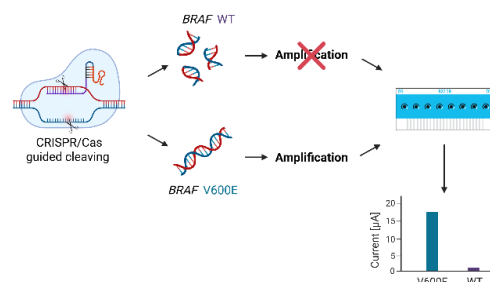


Fig. 2. Principle of CRISPR/Cas12a guided detection of *BRAF* V600E mutation

In both approaches, biotin-labeled dUTPs alongside classical dTTP are randomly incorporated into newly synthesized strands. Additional specificity is achieved by mutation-specific locked nucleic acid (LNA) capture probes attached to magnetic beads (MBs), enabling sample washing and preconcentration. The incorporated biotin-dUTPs are subsequently conjugated with a streptavidin peroxidase polymer (SPP) for EC detection.

The EC measurement is performed on screen printed carbon electrodes (SPCE), where the prepared magnetic beads are loaded and covered with hydroquinone (HQ) and  $H_2O_2$  substrate. During measurement,  $H_2O_2$  is reduced while HQ is oxidized to benzoquinone; the subsequent reduction of benzoquinone back to HQ generates changes in electric current measured via amperometry (Fig. 3).

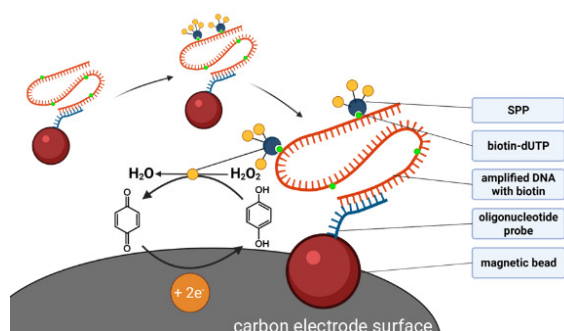


Fig. 3. Electrochemical detection of nucleic acids on screen printed carbon electrode

## Results and Discussion

SNP-specific LAMP combined with EC detection was successfully applied to detect the *BRAF* V600E mutation in clinical liquid biopsy samples. Fig. 4 shows the analysis of 27 blood serum samples, which successfully distinguished mutated from WT samples. These results correspond perfectly with prior validation via sequencing.

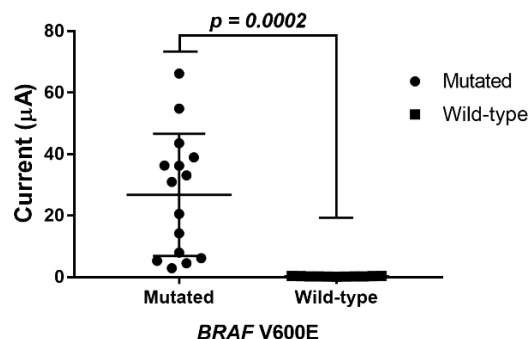


Fig. 4. EC detection of 27 blood samples successfully distinguishing WT and *BRAF* V600E mutated samples

The CRISPR-based approach was tested on cell lines under several conditions. Fig. 5 demonstrates performance across different Cas12a:gRNA ratios (excess Cas12a, equimolar, and excess gRNA). The results indicate that an excess of gRNA over Cas12a enzyme significantly improves the ability to distinguish between mutated and WT DNA samples.

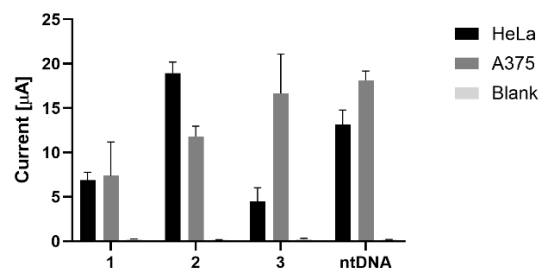


Fig. 5. Electrochemical detection of *BRAF* V600E mutation in cell lines (HeLa – homozygote WT; A375 – homozygote mutant) using CRISPR approach in various ratios of Cas12a and gRNA – 1: excess of Cas12a; 2: equimolar ratio; 3: excess of gRNA

## Conclusion

This study validates two electrochemical platforms for sensitive *BRAF* V600E detection. The LAMP-based approach demonstrates high clinical accuracy, while the CRISPR system offers a promising, selective alternative. Together, these methods provide a rapid, cost-effective framework for point-of-care diagnostics and personalized oncology monitoring.

## References

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