

An Adaptable Electrochemical Platform for Detection of Oncogenic Point Mutations Using Rolling Circle Amplification

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Summary:

We report two electrochemical biosensors for rapid detection of oncogenic mutations based on rolling circle amplification and amperometric detection. The platform enables highly selective identification of mutations in *KRAS* and *PIK3CA*, with sensitivity down to ~61 pM and <1% mutant allele fraction. An ultrafast hyperbranched RCA strategy reduces amplification time to 5 minutes while preserving single-nucleotide specificity. The method shows strong agreement with NGS and offers a rapid, cost-effective solution for point-of-care diagnostics.

Keywords: electrochemical biosensor, rolling circle amplification, point mutation detection, *PIK3CA*, *KRAS*

Background

Oncogenes such as *KRAS*, *BRAF* and *PIK3CA* play key roles in oncogenic signaling pathways, where point mutations often drive pathway dysregulation and contribute to cancer development [1]. These biomarkers are commonly associated with poor prognosis, resistance to treatment, or predict response to targeted therapy (e. g. *PIK3CA* E545K is predictive for the response of alpelisib) [2]. Therefore, their reliable detection is crucial.

Conventional detection methods like NGS, or PCR-based techniques are often time consuming, require expensive instrumentation, involve complex data analysis and can be prone to reaction inhibitors. Isothermal amplification techniques, such as rolling circle amplification (RCA), do not require thermal cyclers and are well suited for mutational analysis. When combined with electrochemical (EC) detection, they represent a promising alternative. [3]. However, many RCA-based EC assays remain limited by relatively long amplification times [4].

Description of the New Method or System

We present two EC RCA-based biosensing platforms for the detection of oncogenic point mutations. The first platform is our previously established biosensor for *KRAS* G12V detection, while the second extends this approach to the clinically relevant *PIK3CA* E545K mutation [5]. The biosensors employ variant-specific padlock probe (PP)-mediated RCA with amperometric

readout on screen-printed eight-electrode carbon chips. Additionally, we enriched our *PIK3CA* platform with hyperbranched RCA (HRCA) an exponential amplification variant, to further improve the sensitivity of the biosensor. In both biosensors, target sequences are pre-amplified by recombinase-polymerase amplification and subsequently subjected to (H)RCA, enabling highly selective amplification of either wild-type (wt) or mutant variants. The amplified products are then hybridized to complementary LNA (*KRAS* G12V biosensor) or DNA (*PIK3CA* E545K biosensor) capture probes. Incorporation of biotin-labelled nucleotides during amplification facilitates binding of a streptavidin–horseradish peroxidase conjugate, which catalyzes an electrochemically detectable reaction between hydrogen peroxide and hydroquinone (Fig. 1).

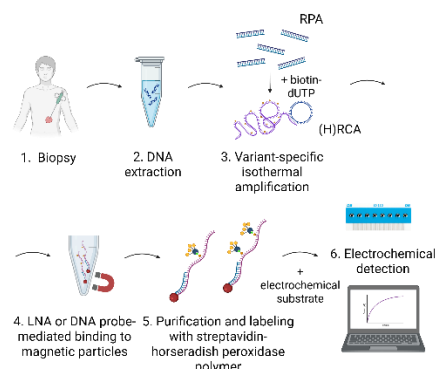


Figure 1: Schematic overview of the experimental workflow for the oncogenic point mutations analysis

In addition, the *KRAS* biosensor incorporates an AI-based logistic regression model that enables automated classification of the G12V mutation presence.

The proposed *PIK3CA* biosensor features an ultrafast HRCA strategy, reducing amplification time from 60 min to 5 min) and total assay time to ~1 h, while maintaining comparable limits of detection (~61 pM) and single-nucleotide specificity. In contrast to our previous designs relying on LNA capture probes, the current approach streamlines the assay architecture by replacing these components with cost-effective DNA probes while maintaining analytical performance.

Results

Both biosensors exhibited a concentration-dependent amperometric response with comparable limits of detection of ~61 pM, whereas the (H)RCA time for the *PIK3CA* biosensor was only 5 minutes, compared to 60-minutes RCA for the *KRAS* biosensor. (Fig. 2).

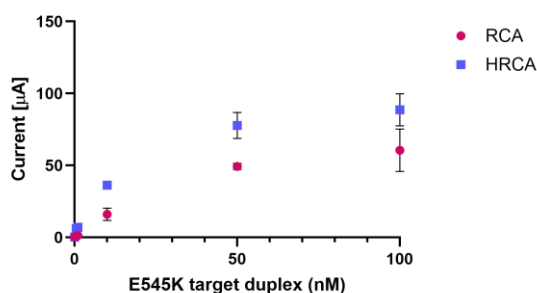


Figure 2: Dependence of electric current values on concentration of *PIK3CA* E545K target (using E545K PP and E545K capture probes). Red dots represent RCA samples, and blue dots represent HRCA samples.

Clear discrimination between mutant and wt sequences, as well as non-complementary controls, confirmed single-nucleotide specificity of both biosensors. This was validated across multiple targets (*KRAS*, *PIK3CA*, *BRAF*, hCMV, HPV), where each padlock probe produced a signal exclusively with its corresponding target.

Furthermore, the system enabled reliable detection of increasing mutant allele fractions in the presence of excess wt DNA, with sensitivity down to <1% mutant allele fraction, demonstrating its applicability for analysis of heterogeneous samples.

The *KRAS* biosensor was validated on 8 cell lines and 11 clinical samples, achieving 100% agreement with NGS in clinical samples (Fig. 3).

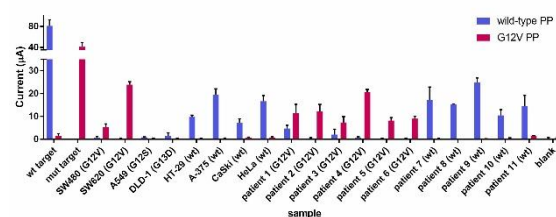


Figure 3: Real sample analysis included 8 cell lines and 11 CRC patient samples with known *KRAS* status (in parenthesis), alongside two synthetic positive controls (wt and G12V mutant targets) and a non-template control.

Ongoing experiments with the *PIK3CA* biosensor focus on validation using cancer cell lines, while future work will address clinical sample validation to assess applicability in real diagnostic settings.

This approach represents a rapid, sensitive, and cost-effective platform for point mutation detection, with strong potential for point-of-care and decentralized diagnostic applications.

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Acknowledgements

The financial support of the Czech Science Foundation (No. 25-15990S), project SALVAGE (OP JAC; reg. no. CZ.02.01.01/00/22_008/0004644) – co-funded by the European Union and by the State Budget of the Czech Republic, BBMRI.cz (No. LM2023033) and MH CZ – DRO (MMCI, 00209805) is greatly acknowledged.