A high-throughput platform to select nucleic acid-based bio-recognition elements for electrochemical biosensors

Thijs Van der Snickt [a], Simone Bassini [b,c], Yannick Stulens [d], Laura Claudino [a], Scott Ailliet [a], Jef Hooyberghs [d], Karolien De Wael [a,b], and Pieter Mestdagh [a]

[a] OncoRNALab, Department of Biomolecular Medicine, Ghent University, 9000 Ghent, Belgium.

[b] A-Sense Lab, University of Antwerp, 2020 Antwerp, Belgium.

[c] NANOlab Centre of Excellence, University of Antwerp, 2020 Antwerp, Belgium.

[d] Theory Lab, Department of Science, University of Hasselt, 3500 Hasselt, Belgium.

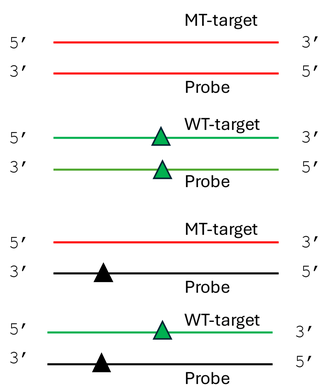
thijs.vandersnickt@ugent.be

INTRODUCTION

- **Challenge:** Detecting rare mutations in a high-abundance wild-type (WT) background is key for precision cancer diagnostics, but standard methods (qPCR, MPS) are costly, complex, and time-consuming.
- **Alternative approach:** Electrochemical biosensors are simpler and cost-effective but need better selectivity for low-abundance mutation detection.
- **Presented solution:** We developed a high-throughput platform to systematically evaluate and optimize the hybridization affinity of nucleic acid-based bio-recognition elements for biosensor applications.
- **Results:** Screening 884 probes across 12 hybridization conditions in varying WT backgrounds (0%, 50%, 75%) identified highly selective probes for KRAS G12C, validated using a photoelectrochemical (PEC) assay.

METHODOLOGY

Tailored probe design for enhanced target discrimination



RED = Perfect match to the MT-target
GREEN = Perfect match to the WT-target
BLACK = Alternative probe with 1 additional MM to the WT-target

Fig.1: *Introducing **an artificial mismatch** between the capture probe and target enhances discrimination by creating **a double mismatch with the WT sequence**, reducing hybridization stability [1]. Using a 17-nt probe, 884 variants were designed by shifting the mutation across 17 positions and testing four base substitutions (A, C, T, G) at each site.*

Screening of bio-recognition elements

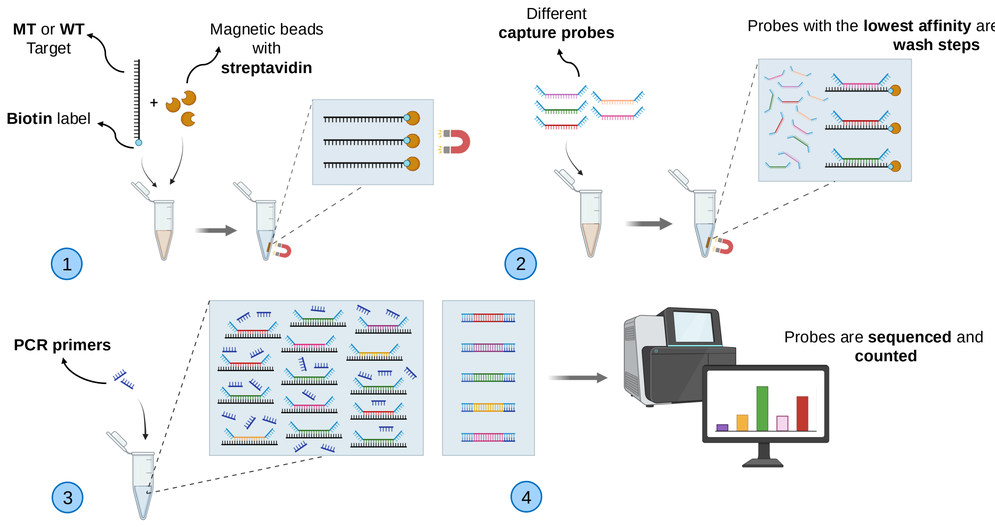


Fig.2: *1) Hundreds of candidate bio-recognition elements (**capture probes**) that are flanked by PCR handles are **hybridized** to a biotinylated MT or WT target. 2) Following **streptavidin pull-down**, 3) probes are converted into a **probe library** 4) which is quantified by next generation sequencing (**NGS**).*

PEC biosensor for KRAS G12C biomarker detection

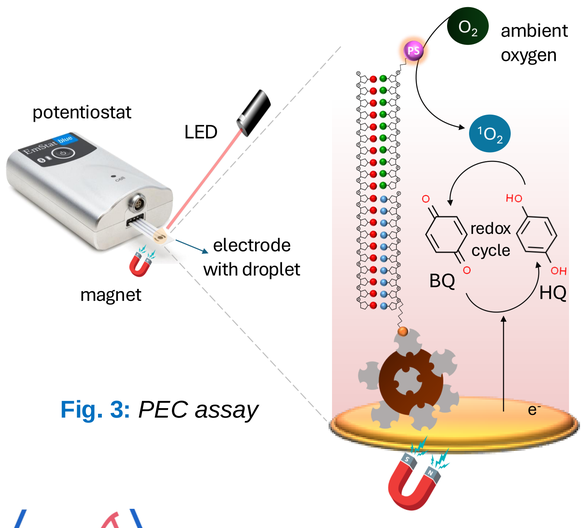


Fig. 3: *PEC assay*

- Sandwich assay with a capture probe (**blue**) on magnetic beads and **a photosensitiser-labelled** detection probe (**green**) to detect the target of interest (**red**).
- Upon target recognition and illumination, **singlet oxygen ($^1O_2$)** oxidizes hydroquinone (HQ) to benzoquinone (BQ).
- Electrochemical regeneration of the redox reporter (HQ) at -0.2 V enables an electrocatalytic cycle. Amperometric responses are recorded with light-chopped illumination [2].

RESULTS

Experimental and theoretical data of probe hybridization to KRAS G12C and WT sequences

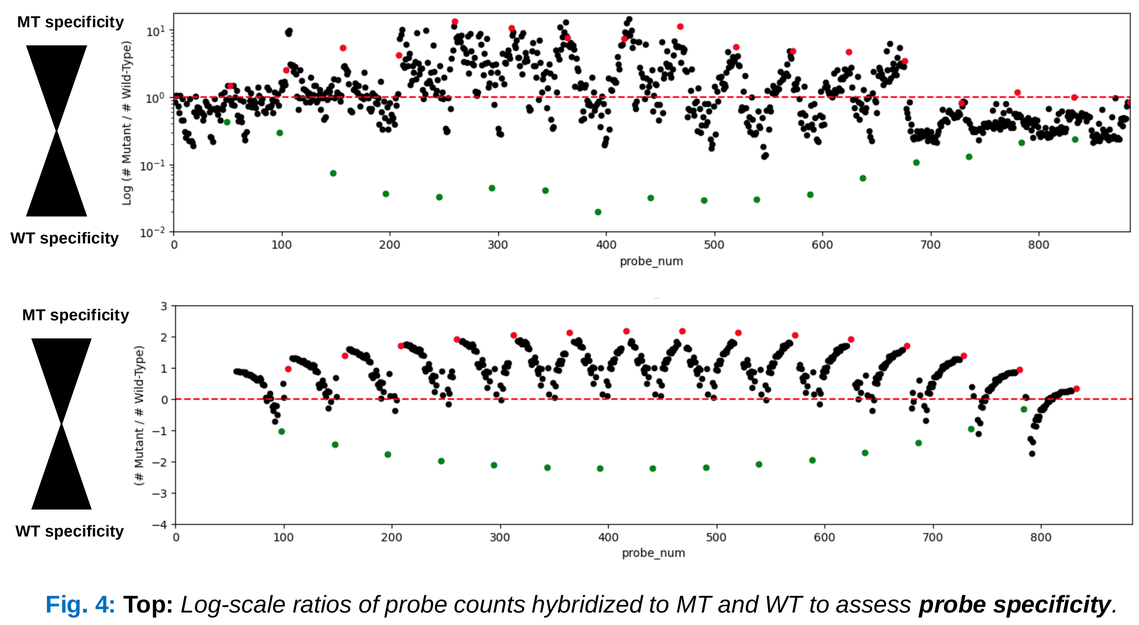


Fig. 4: Top: *Log-scale ratios of probe counts hybridized to MT and WT to assess **probe specificity**.* **Bottom:** *Computational predictions based on **the nearest-neighbor model** and microarray data [3]. **Red**: MT-perfect match; **Green**: WT-perfect match; **Black**: an extra single-mismatch to the WT.*

Probe discrimination between KRAS G12C and WT targets

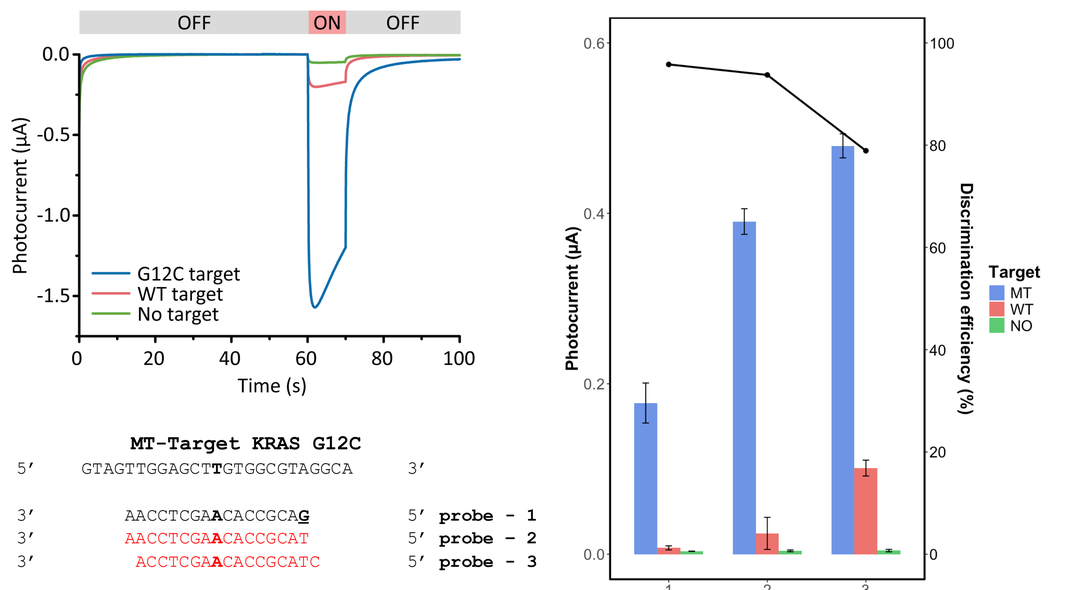


Fig. 5: Top left: *PEC results of the KRAS G12C biomarker, including WT and No target controls.* **Bottom left:** *The design of 3 selected probes; probe 1 carries an additional mismatch to the WT-target, and probe 2 and 3 are perfect matches to the MT target with different binding position.* **Right:** *PEC measurements of MT, WT or No target.*

CONCLUSIONS

- **High-Performing Probes Identified** – among 884 probes tested, top candidates showed strong selectivity for the MT-target.
- **Advancing Precision Diagnostics** – this method accelerates biosensor development for improved cancer detection.

[1] Lefever, S. et al. Cost-effective and robust genotyping using double-mismatch allele-specific quantitative PCR. Scientific Reports Vol. 9, (2019).
[2] Daems, E., Singlet oxygen-based photoelectrochemical detection of single-point mutations in the KRAS oncogene. Biosensors and Bioelectronics Vol 249, (2024)
[3] Hadiwikarta, W.W et al. Probing hybridization parameters from microarray experiments: nearest-neighbor model and beyond. Nucleic Acid Research Vol. 40, (2012).