Use of DNA thermodynamics for low-abundance mutation detection by DNA hybridization

Yannick Stulens, [1] Rebekka Van Hoof,[1,2,3] Michal Szymonik,[2] Karen Hollanders,[2] An Jacobs,[2] Inge Nelissen,[2] Patrick Wagner,[3] and Jef Hooyberghs[1]

yannick.stulens@uhasselt.be

[1] UHasselt, Data Science Institute, Theory Lab, Agoralaan, 3590 Diepenbeek, Belgium
[2] Flemish Institute for Technological Research (VITO), Boeretang 200, 2400 Mol, Belgium
[3] Laboratory for Soft Matter and Biophysics, KU Leuven, Celestijnenlaan 200 D, 3001 Leuven, Belgium

Keywords: nucleic acid thermodynamics, mutation detection, Langmuir adsorption theory

Introduction

Duplex formation via hybridization provides a detection technique where one of the strands is designed to match a mutant sequence and probe its presence. In a majority wild-type background consisting of single-nucleotide variations, the probe is however subject to cross-hybridization, lowering the detection sensitivity of low-abundant mutants. We use thermodynamics-based probe design and Langmuir theory to increase sensitivity and quantify the amount of mutant in a sample.

Results and Discussion

Figure 1 shows a hybridization-based sensor which uses three probe sequences: a wild-type matching probe, a mutant matching probe and a reference probe, the latter designed to have equal affinity to wild-type target as the mutant-matching probe. Single-stranded sample DNA is fluorescently labelled and allowed to bind to the probes. The detection signal is defined as

$$S = \log \frac{I_{Pmut}}{I_{Pref}}$$

A mixture of wild-type and mutant target will result in $I_{Pmut} > I_{Pref}$, signalling the presence of mutant DNA. To limit the amount of cross-hybridization, a large number of wild-type probes are introduced to deplete wild-type target. This enhances the effective mutant to wild-type ratio. The Langmuir adsorption theory provides the theoretical framework and was used in the linear regime to characterize the signal **[1]**. Using synthetic samples at a low mutant ratio, Van Hoof *et al.* showed that depletion of wild-type target achieves an order of magnitude improvement in the limit of detection **[2]**. However, clinical samples suggest a non-linear description is needed at higher mutant ratios, which we have been able to obtain. To characterise the dose-response $S(r)$ in terms of mutant to wild-type ratio r, a calibration experiment needs to be performed.

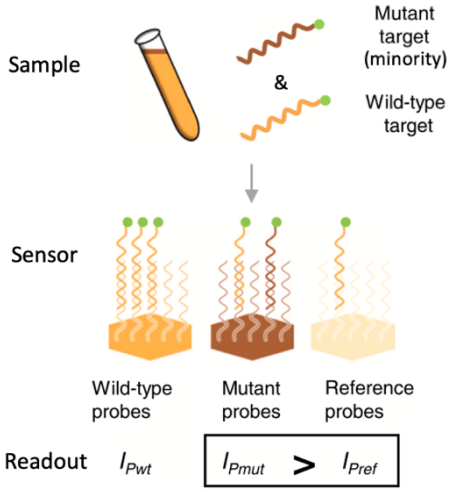

***Figure 1**: Experimental design and readout using reference probes for mutant detection.*

Subsequently, by inverting the theory, a signal measurement allows accurate determination of the amount of mutant in a sample.

Conclusions

We are currently exploring other methods to selectively deplete wild-type targets and extend the model for use with solution-based hybridization sensors **[3]**, which promise a cost-effective biosensing technique usable at room temperature.

References

[1] S.K. Nomidis *et al.*, *Langmuir*, **2019**, doi: 10.1021/acs.langmuir.9b01761

[2] R. Van Hoof *et al.*, *Sensors and Actuators B: Chemical*, **2022**, doi: 10.1016/j.snb.2022.132175.

[3] R.J. Karadeema *et al.*, *Nanoscale*, **2018**, doi: 10.1039/C8NR01107A.

Acknowledgements

This work is supported by Special Research Fund with grant number BOF22OWB11.