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

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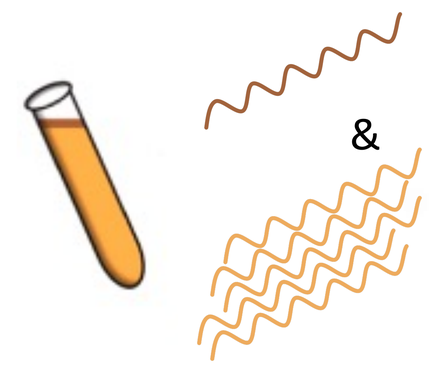
Problem Statement

In cancer diagnostics, hybridization-based sensors detect mutant DNA by matching probes. The presence of a large amount of wild-type DNA lowers the detection sensitivity, as wild-type also binds to the probe.

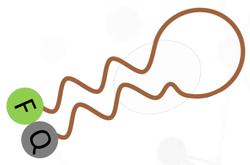


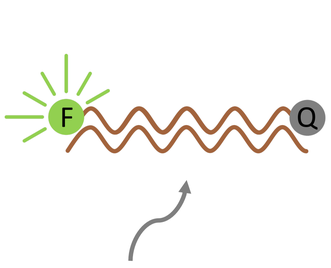
Mutant (minority)

&

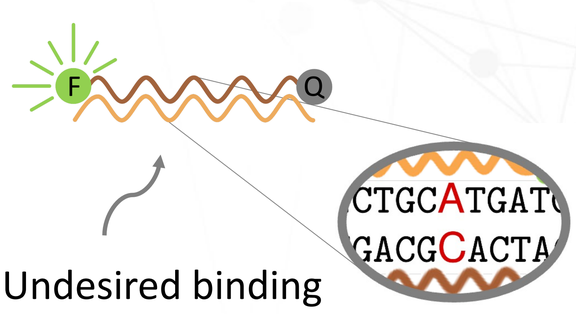
Wild-type (majority)

Mutant matching probe:





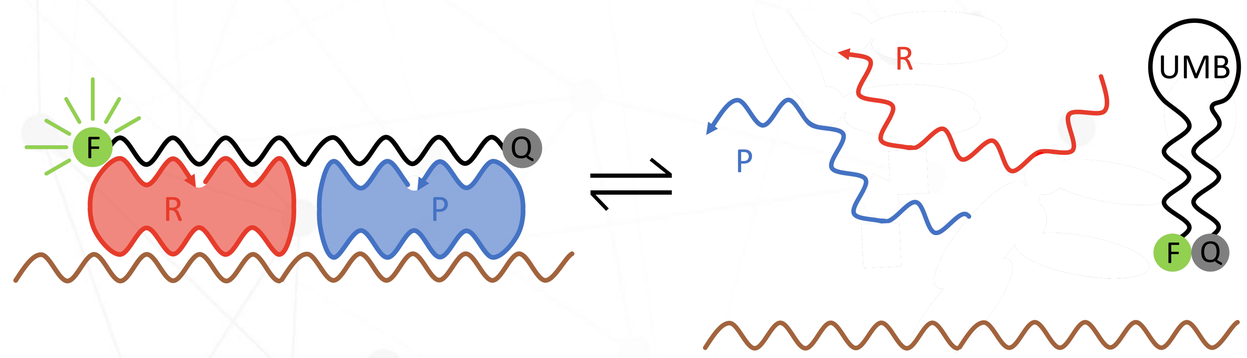
Desired binding



Undesired binding with mismatch

Sensor Design & Depletion

The OWL sensor consists of two DNA adaptor strands (R & P) and a universal molecular beacon (UMB). The 'fragile' structural rigidity of the P-strand provides **highly selective** binding to mutant DNA [1].

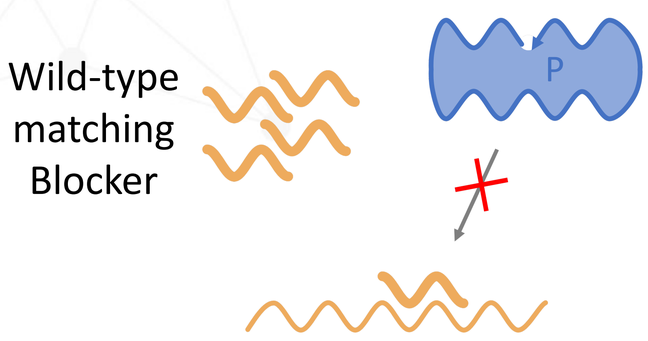


The sensor differentiates mutant DNA at room temperature in a wide temperature range. It has recently been equiped with additional strands, capable of unfolding secondary structures of sample DNA [2].

Interactions are described by Langmuir theory [3]:

$$I \sim e^{-\frac{\Delta G}{RT}}$$

Wild-type binding and experimental noise determines limit of detection. This limit is improved by the **technique of depletion**:



Blocker strands selectively reduce DNA concentrations:

$$\frac{c^*_{\mathrm{mut}}}{c^*_{\mathrm{tot}}} > \frac{c_{\mathrm{mut}}}{c_{\mathrm{tot}}}$$

Enhanced mutant ratio improves limit of detection

Results

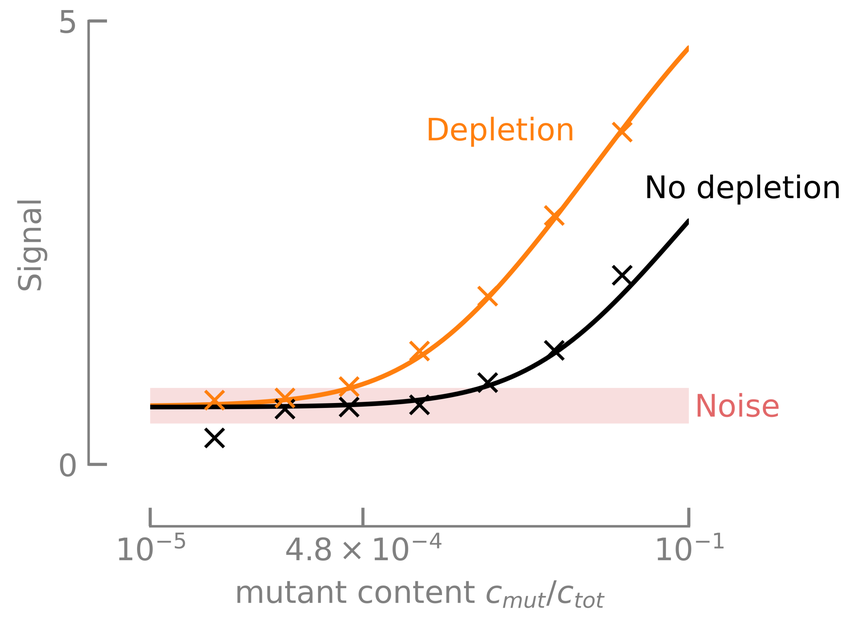


Figure: Surface-based sensors show depletion results in a ~10-fold improvement in limit of detection [4].

To test the OWL sensor and the effect of depletion, experiments were conducted on lung cancer biomarker EGFR. Results show that depletion greatly improves the limit of detection. As yet, the sensor allows discrimitation of mixtures containing more than 0.07% mutant DNA, reaching the accuracy of clinical methods. We are currently exploring the kinetics of the OWL sensor, which could provide a new detection method for mutant DNA.

References & Acknowledgement

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