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

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Mutation detection is of great importance in many biological processes and diagnostics. Single-nucleotide variations are common, where a single base-pair is substituted during replication of wild-type DNA. Duplex formation via hybridisation provides an important detection technique where one of the strands is designed to match the mutant sequence and probe its presence. The probe is, however, subject to cross-hybridisation of wild-type DNA, lowering detection sensitivity in case of low-abundant mutants in a majority wild-type background. We use thermodynamics-based probe design and Langmuir theory to increase sensitivity and quantify the amount of mutant in a sample.

Reference probes provide robust signals

Figure 1 shows a hybridisation based sensor on which immobilised probes of a known sequence are grouped and different probe sequences are spatially separated. Sample DNA (which might be a mixture of wild-type and mutant sequences) is fluorescently labeled and will bind to different probe sequences depending on the affinity towards that probe. The amount of bound sample molecules is then determined from fluorescent intensities I_{Pwt}, I_{Pmut} and I_{Pref}. We made use of three probe sequences: a wild-type matching probe, mutant matching probe and a reference probe. The reference probe is designed to have equal affinity to wild-type target as the mutant-matching probe. The signal of a measurement is defined as

$$S = \log \frac{I_{Pmut}}{I_{Pref}}. \tag{1}$$

As illustrated in Figure 1, in case of a pure sample, i.e. containing only wild-type target, the design results in equal binding and fluorescent readout of both reference and mutant probes. More notably, in case of a mixture of wild-type and mutant target $I_{Pmut} > I_{Pref}$, signaling the presence of mutant DNA.

Depletion of wild-type target enhances detection limit

Cross-hybridisation of wild-type target to the mutant and reference probes decreases the signal. To improve the limit of detection, i.e. the lowest detectable mutant ratio in experiment, a large amount of wild-type matching probes are introduced. Wild-type target (but also a proportionally small amount of mutant target) will bind to these probes and enhance the effective mutant to wild-type ratio. We are currently exploring other methods to selectively deplete wild-type targets. Van Hoof *et al.* demonstrated that the technique

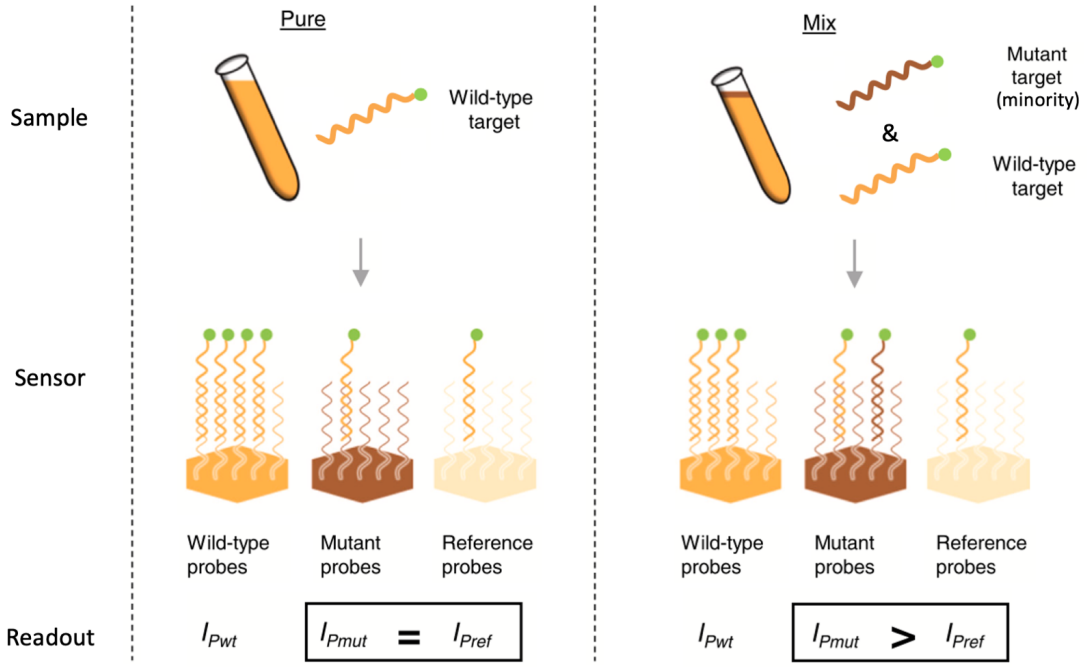

Figure 1: Experimental design and readout using reference probes for mutant detection.

of depletion achieves an order of magnitude improvement in the limit of detection, reaching a comparable sensitivity to digital PCR, which is the gold standard technique for low-abundant mutation detection [1].

Langmuir theory allows accurate determination of mutant ratios

Binding affinity between a target and probe is determined by the free energy difference between bound and unbound state. The Langmuir adsorption theory provides a theoretical framework to describe the system, such that the equilibrium distribution is determined by free energy differences of multiple target-probe pairs and the mutant to wild-type ratio. The theory was previously used in the linear regime to characterise the detection signal [2]. This was in excellent agreement with experiments using synthetic samples at a low mutant ratio [1]. However, clinical samples suggest a non-linear description is needed at higher mutant ratios.

To characterise the dose-response $S(r)$ in function of mutant to wild-type ratio r, free energy differences are determined in a calibration experiment. Subsequently, by inverting the theory, a signal measurement allows accurate determination of the amount of mutant in a sample.

References

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