Specific SNP detection by mediator probe digital droplet PCR

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Summary

Mediator probe digital droplet PCR (MP ddPCR) was used to detect single nucleotide polymorphisms (SNP) at the cKit V559D gene locus, a gastrointestinal cancer biomarker [1]. In comparison to conventional PCR with dual-labelled hydrolysis probes (HP) the MP PCR combines label-free mediator probes with universal fluorogenic reporters [2].

In the presented experiment, specificity of the MP-based SNP detection was as high as SNP detection using HPs with locked-nucleic acid (LNA) modified nucleotides.

Introduction and reaction principle

In order to reach the analytical specificity required for SNP detection by digital PCR costly MGB- or LNA-modified HPs are currently used [3, 4]. This requires synthesis of a new probe for each sequence to be detected. In comparison, MP PCR [2, fig. 1] replaces fluorescently labeled HPs by sequence-specific label free mediator probes (MP). Cleavage of the MP during amplification results in release of a mediator which is detected by a universal fluorogenic reporter oligonucleotide (UR). Advantages of MP PCR towards HP PCR in sequence-specific nucleic acid detection are:

- **Cost savings**: the same UR can be used for all assays and therefore can be ordered in large scale.
- **Design flexibility**: the MP position and length can be adopted without considerations of sequence-based fluorescence quenching or impaired FRET with long probe lengths.
- **Selectivity of signal generation**: the 2-step process leading to signal generations adds additional sequence detection selectivity to the assay (fig. 2).

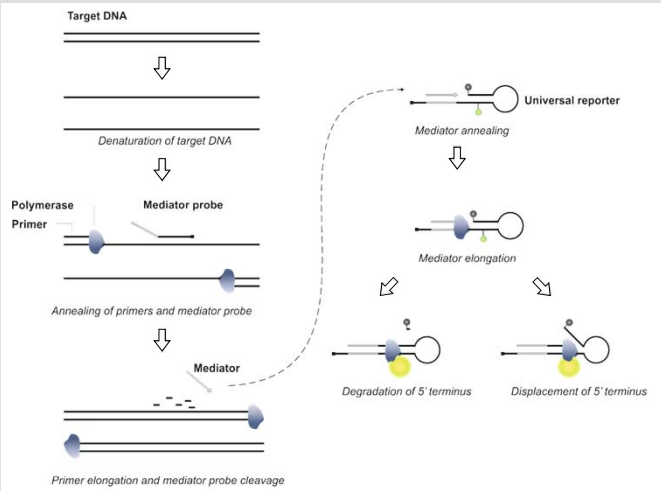

Figure 1: Reaction steps of mediator probe PCR.

Experimental setup

Specificity of the novel MP ddPCR was compared to ddPCR with HPs bearing LNA-modified nucleotides (fig. 2).

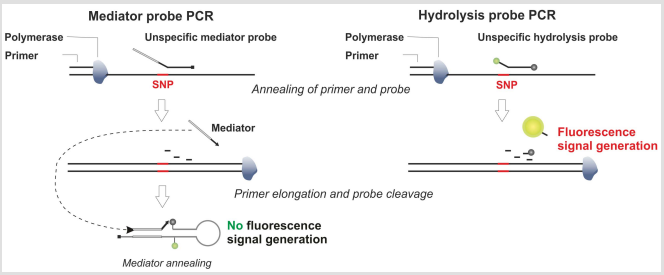

Figure 2: Principle of SNP detection by MP PCR compared to HP PCR.

Experimental results

Amplification detection of ~$6{\cdot}10^3$ wildtype (wt) sequence copies by mutant (mut)-specific MPs gave false positive signals in 8 (HP: 4) out of $3.4{\cdot}10^4$ droplets. Detection of ~$7{\cdot}10^4$ mut-sequence copies by wt-specific MPs gave false positive signals in 9 (HP: 44) out of $3.3{\cdot}10^4$ droplets. Back-calculated concentrations of wt- and mut-sequences with the corresponding specific target-probe combinations were essentially the same for both methods.

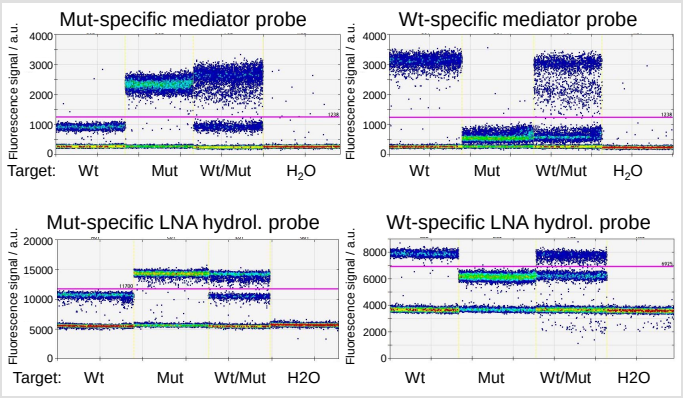

Figure 3: Fluorescence dot blots (Bio-Rad QX100) of SNP detection by MP and HP ddPCR. The pink line is the discrimination threshold between positive and negative signals of the droplets.

Conclusions and outlook

Analytical specificity of ddPCR for SNP detection using MPs was as good as using LNA-modified HPs, whilst the MP synthesis costs are ~10 % of the LNA-modified HP costs. Next, parallel detection of wt and mut sequences by MP ddPCR shall be enabled using a second fluorogenic UR.

Acknowledgements

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References

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