Cost-effective real-time analysis by mediator probe (RT-)PCR

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Summary

Using the novel mediator probe (RT-)PCR

- **four different** DNA and RNA target sequences were detected
- using only **one universal fluorogenic reporter** oligonucleotide
- with the **same performances as hydrolysis probe** based reactions.

The mediator probe PCR combines label-free mediator probes with universal fluorogenic reporters. Thus it gives the opportunity of oligonucleotide cost-savings in comparison to the usage of dual-labeled probes in real-time PCR by more than 60 %.

Introduction and reaction principle

In real-time PCR high synthesis costs for sequence-specific dual-labeled detection probes are still one reason why researchers are reluctant when larger numbers of probes need to be ordered. In order to reduce costs universal sequence specific PCR variants have been developed [1, 2]. As one kind, the mediator probe PCR [3] replaces fluorescently labeled hydrolysis probes 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 (UR) oligonucleotide. The key to cost savings is that the same UR can be used for all assays and therefore can be ordered in large scale.

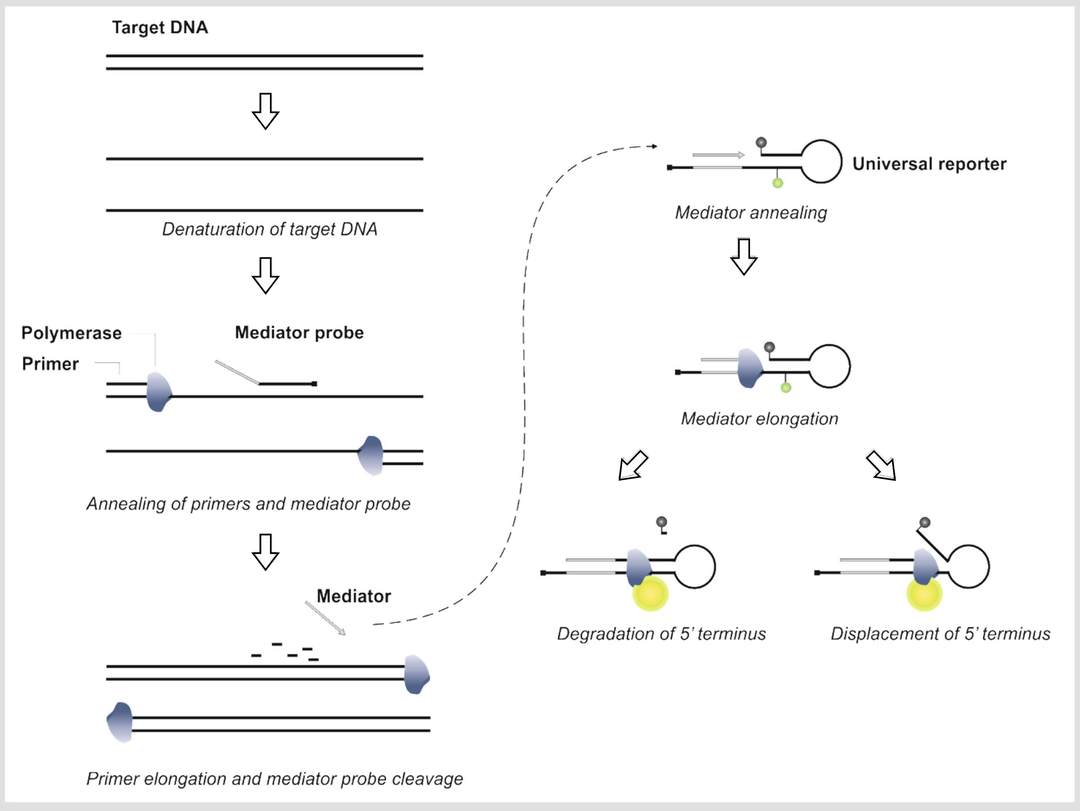

Figure 1: Reaction steps of mediator probe PCR.

Experimental setup

In this work, performance characteristics of mediator probe PCR (MP PCR) were compared to hydrolysis probe PCR (HP PCR). Probe sequences of the MPs were based on the corresponding HP sequences.

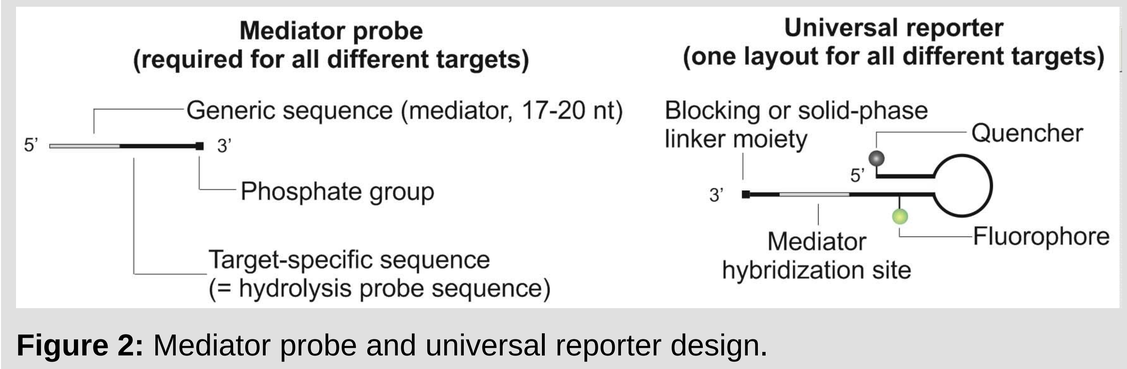

Figure 2: Mediator probe and universal reporter design.

Experimental results

Analysis of serial dilutions of three respitory pathogen sequences HAdV B7, FluB and RSV as well as HPV 18 revealed good agreement between MP and HP (RT-)PCR in terms of linearity, accuracy, precision and detection limits.

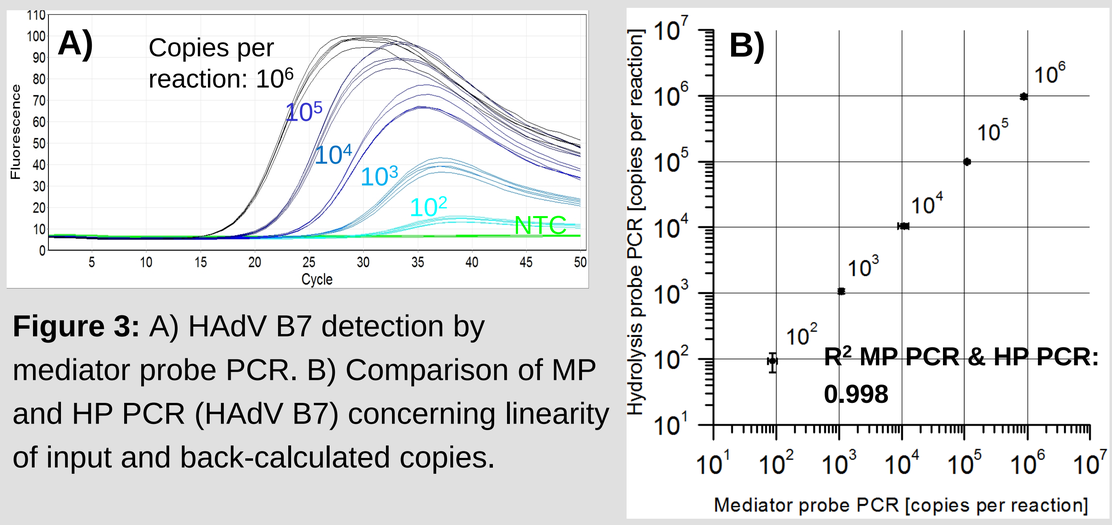

Figure 3: A) HAdV B7 detection by mediator probe PCR. B) Comparison of MP and HP PCR (HAdV B7) concerning linearity of input and back-calculated copies.

Due to the usage of one fluorogenic reporter, fluorescence background variation can be reduced (4 assays): HP PCR: 35.3 % and MP PCR: 7.9 %. Different analytes can thus be detected in one run without any reference dye such as ROX.

Conclusions and outlook

MP PCR can reduce oligonucleotide synthesis costs and is recommended where a multitude of probes with low batch size is required. This can be in research laboratories, during assay development or in low resource settings. Our current research aims at (1) extending the design rules and (2) increasing the degree of multiplexing.

Acknowledgements

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References

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[3] B. Faltin *et al.*, Clin Chem, vol. 58 (2012), pp. 1546-1556