Universal reporters of mediator probe PCR as target-independent biosensors

S. Wadle*, M. Lehnert*, F. Schuler*, R. Zengerle*,,***, F. von Stetten*,****

*Laboratory for MEMS Applications, IMTEK, University of Freiburg, Georges-Koehler-Allee 103, 79110 Freiburg, Germany
**HSG-IMIT – Institut für Mikro- und Informationstechnik, Georges-Koehler-Allee 103, 79110 Freiburg, Germany
***BIOSS – Centre for Biological Signalling Studies, University of Freiburg, 79110 Freiburg, Germany

Summary

Using the novel mediator probe (RT-)PCR

- **five DNA and RNA target sequences** were detected
- using only **one universal fluorogenic reporter** oligonucleotide (UR)
- with the **same or even better performances as hydrolysis probe** based reactions.

With quenching-efficiency optimized URs lower detection limits can be achieved compared to reference hydrolysis probe based reactions.

Introduction and reaction principle

Hydrolysis probe PCR requires synthesis of a new probe for each sequence to be detected. In comparison, mediator probe PCR (MP PCR) [1,2, fig. 1] replaces fluorescently labeled hydrolysis probes (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 (sequence independent) fluorogenic reporter oligonucleotide (UR). Advantages of MP PCR towards HP PCR in sequence-specific nucleic acid detection are:

- **Design flexibility**: the MP position and length can be adopted without considerations of sequence-based fluorescence quenching or impaired FRET with long probe lengths.
- **Optimized signal generation**: quenching efficiency has to be optimized only once for the UR, which can be used in all assays.
- **Cost savings**: the same UR can be used for all assays and therefore can be ordered in large scale.

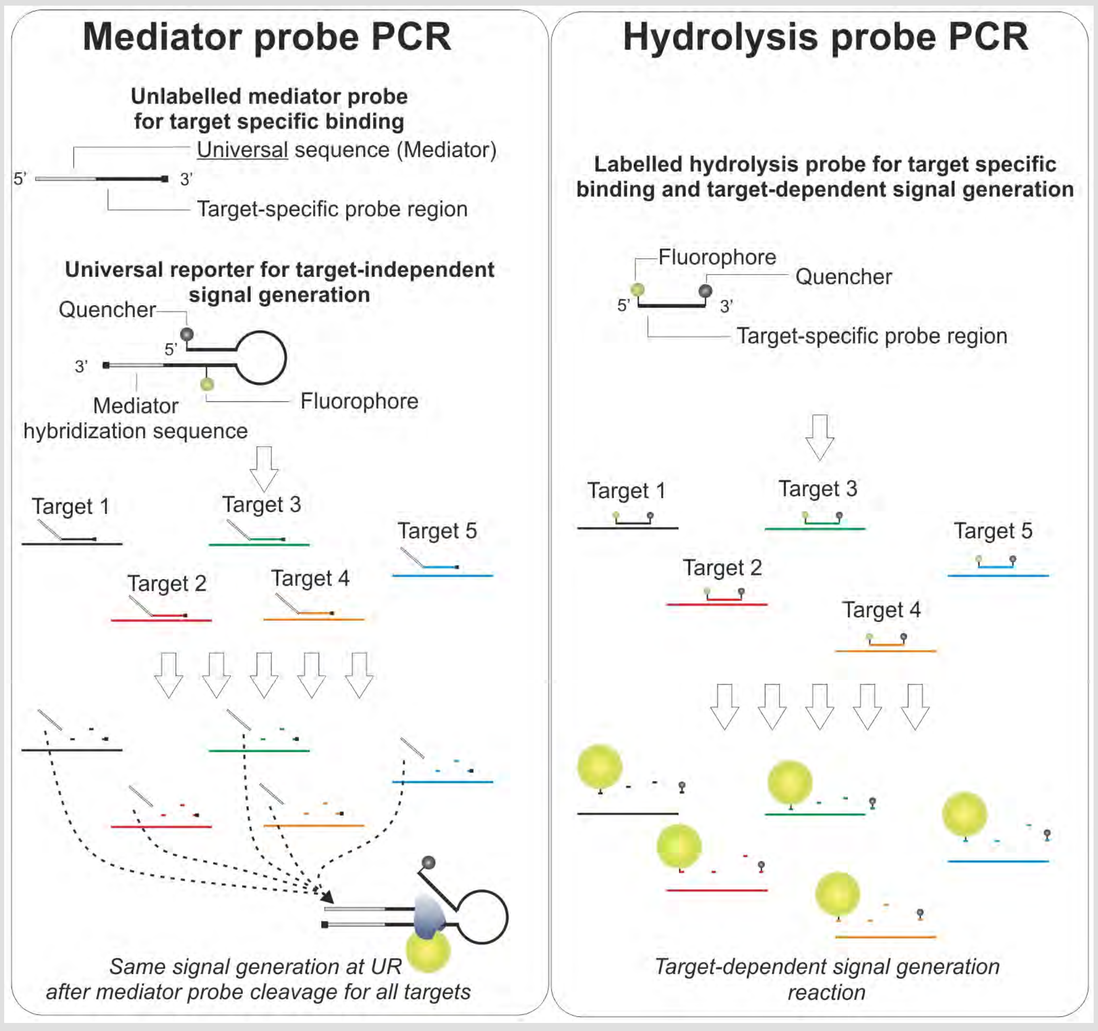

Figure 1: MP and HP PCR as alternative real-time nucleic acid sensing systems.

Experimental setup

Performance characteristics of MP PCR and HP PCR were compared. MP sequences are based on the corresponding HP sequences.

Experimental results

Analysis of serial dilutions of five respiratory pathogen sequences revealed good agreement between MP and HP (RT-)PCR in terms of linearity, efficiency, and precision.

95 % detection limits were: human Adenovirus 7B (hAdV) 7 / 7 copies per reaction (MP / HP (RT-)PCR), Influenza virus A 4 / 18, Influenza virus B 10 / 14, human Metapneumovirus 11 / 29, Respiratory syncytial virus 14 / 22. These correspond well to commercially available assays [3].

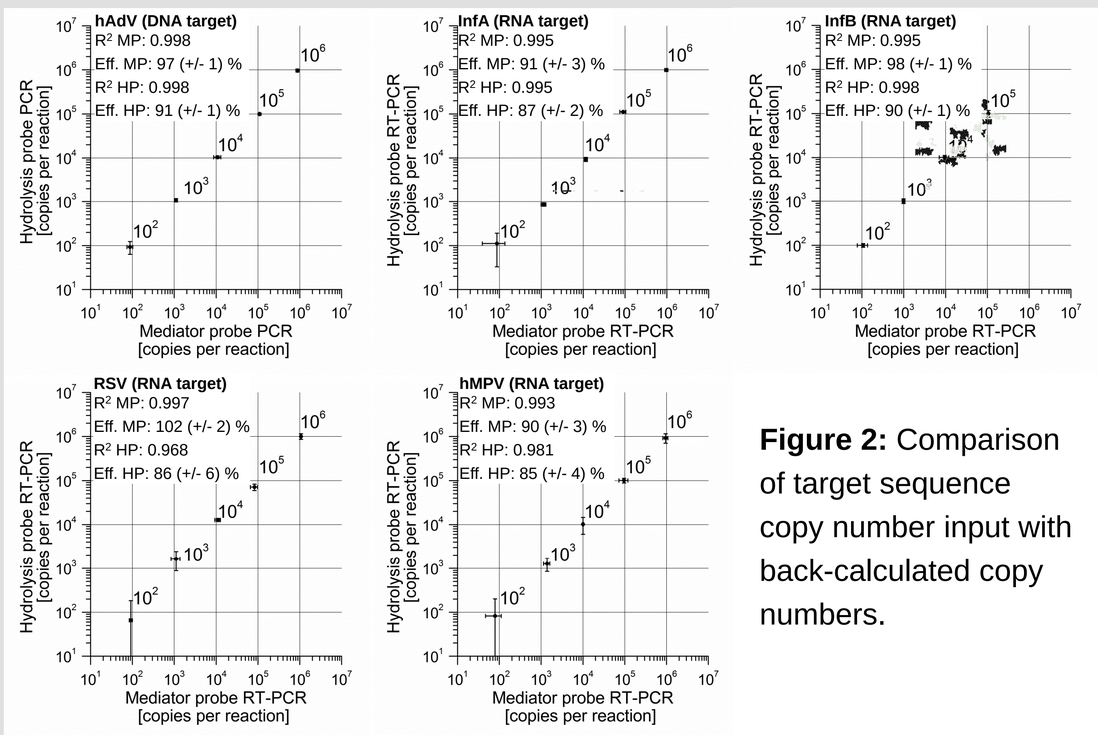

Figure 2: Comparison of target sequence copy number input with back-calculated copy numbers.

Conclusions and outlook

As conclusion, one UR was used for sensing 5 different DNA and RNA targets by MP (RT-) PCR. Even higher reaction efficiencies and lower detection limits compared to the more expensive HP (RT-) PCRs could be reached. The method is especially recommended if many different target-specific probes are required at low batch sizes. In future, multiplexing degrees shall be increased using UR-microarrays or differently labelled URs.

Acknowledgements

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References

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[2] B. Faltin *et al.*, Clin Chem, vol. 58 (2012), pp. 1546-56
[3] L. Van Wesenbeeck *et al.*: J. Clin Microbiol., vol. 51 (2013), pp. 2977-85

Contact: simon.wadle@imtek.de; +49 761 20373228