[Inv2]

Universal reporters of mediator probe PCR as target-independent biosensors for detection of five different RNA and DNA sequences

S. Wadle[1], M. Lehnert[1], R. Zengerle[1,2], F. von Stetten*[1,2]

[1]University of Freiburg, Germany, [2]HSG-IMIT - Institut für Mikro- und Informationstechnik, Germany, [3]BIOSS – Centre for Biological Signalling Studies, University of Freiburg, Germany

Molecular diagnostics often uses hydrolysis probes (HP) for real-time nucleic acid sensing. However, each target sequence requires synthesis of specific dual-labelled HPs, which are expensive, especially when used at low batch sizes. Also, HPs must be individually optimized for signal generation efficiencies for each target sequence to be detected. We have published a novel approach, the mediator probe PCR (MP PCR) [1, Fig.1] which overcomes these issues by using a labelled but **universal reporter** oligonucleotide (UR) as a **biosensor for target-independent signal** generation. It is triggered by unlabelled and thus **cost-effective sequence-specific mediator probes**. Compared to [1] we improved UR quenching efficiencies and reaction setup of MP PCRs to detect **5 different DNA and also RNA target sequences** of viruses causing respiratory tract infections. HP based assays, which required 5 different dual-labelled probes were run as references.

MPs and the UR designs were adapted from [1] with the sequence-specific MP section equal to corresponding HP sequences. Nucleic acid standards from human adenovirus (hAdV), influenza virus A&B (InfA & B), human metapneumovirus (hMPV), and respiratory syncytial virus (RSV) were serially diluted enabling efficiency calculation and detection limit determination.

Figure 2 presents reaction efficiencies and the correlation of input- with back-calculated output concentrations for both, MP and HP RT-PCRs. 95 % detection limits were: hAdV 7 / 7 copies per reaction (MP / HP (RT-)PCR), InfA 4 / 18, InfB 10 / 14, hMPV 11 / 29, RSV 14 / 22. These **correspond well to commercially available assays** [2].

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 as with 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**.

References

[1] B. Faltin *et al.*: Clin Chem, vol. 58, pp. 1546-1556, 2012

[2] L. Van Wesenbeeck *et al.*: J. Clin Microbiol., vol. 51, pp. 2977-2985, 2013

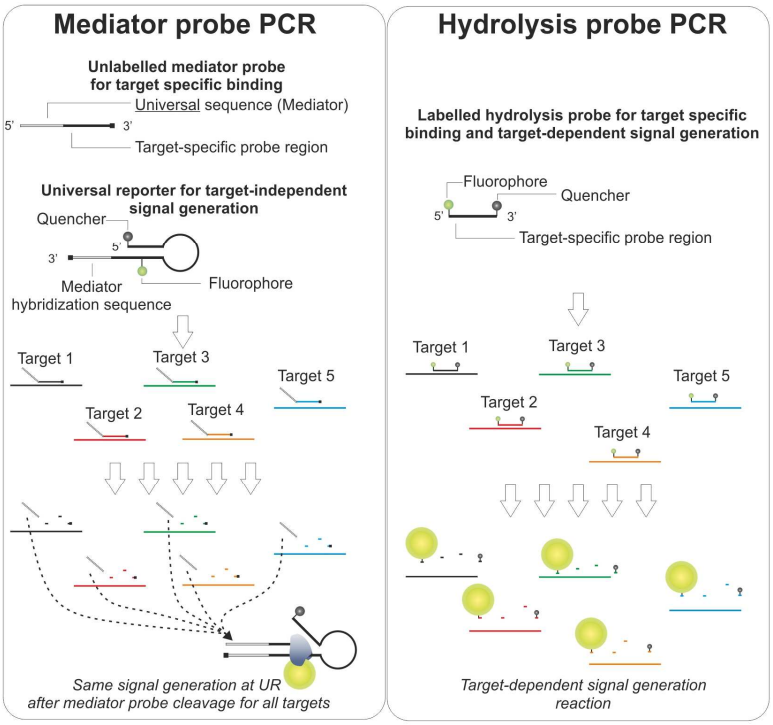

Fig. 1: MP and HP PCR as alternative real-time nucleic acid sensing systems. In MP PCRs the signal is generated after cleavage of the target-specific MP by extension of the released mediator at the UR. The latter thus functions as a target-sequence independent biosensor and can be used for signal generation in several detection reactions. In comparison, HP PCR requires a specific dual-labelled probe for each target sequence to be detected.

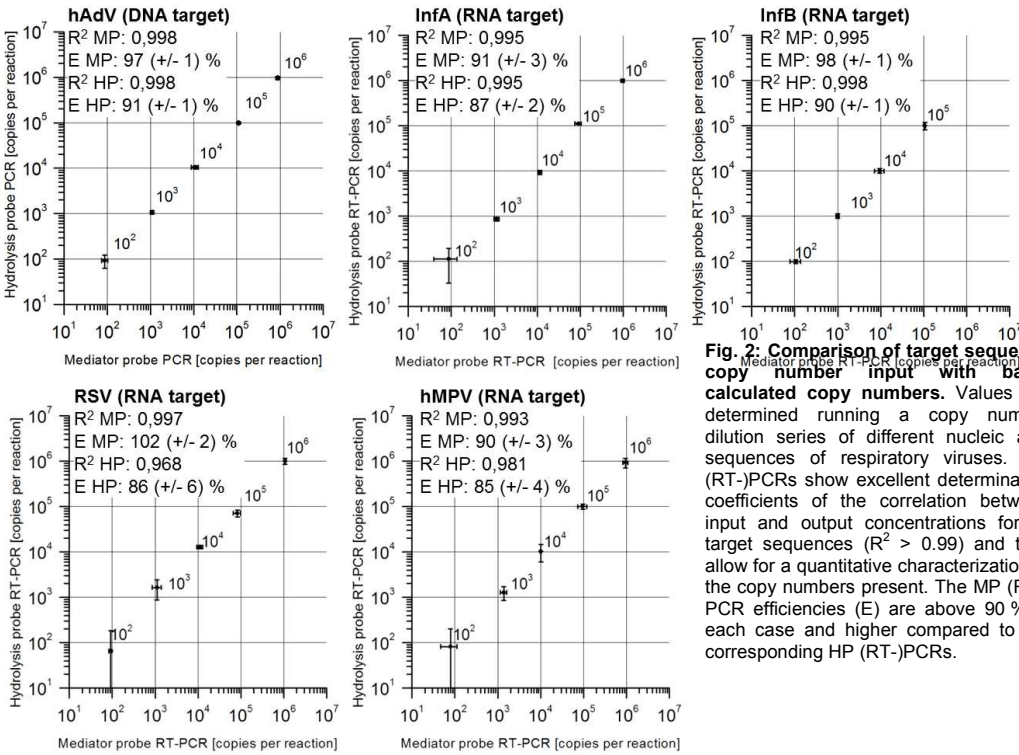

Fig. 2: Comparison of target sequence copy number input with back-calculated copy numbers. Values are determined running a copy number dilution series of different nucleic acid sequences of respiratory viruses. MP (RT-)PCRs show excellent determination coefficients of the correlation between input and output concentrations for all target sequences (R^2 > 0.99) and thus allow for a quantitative characterization of the copy numbers present. The MP (RT-)PCR efficiencies (E) are above 90 % in each case and higher compared to the corresponding HP (RT-)PCRs.

Keywords: Mediator probe PCR, Molecular diagnostics, Universal detection, Microarray