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

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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


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