

Mediator Probe PCR:

Advanced PCR detection using label free mediator probes and optimized universal reporters

In Mediator Probe PCR, DNA detection and fluorescence signal generation are two separate processes. This enables highly sensitive and precise nucleic acid detection and quantification.

Mediator Probes are target-specific DNA-probes without fluorescence label. These probes are cleaved base specifically during PCR amplification. After cleavage, a mediator sequence is released, which subsequently activates a specific dual labeled universal reporter.

Since universal reporters are target sequence independent, once optimized, a set of universal reporters with high fluorescence signals can be used in all assays. From this separation of DNA detection and fluorescence signal generation, several advantages arise:

Advantages

- Improved quality management by standardization of assay components
- High fluorescence signal-to-noise ratios using universal reporter molecules
- Sensitive, selective and precise PCR-multiplexing
- Precise design guidelines
- Compatible with all PCR mastermixes

Application

- Quantitative multiplex real-time PCR
- Reverse transcription real-time PCR
- Digital PCR
- SNP detection

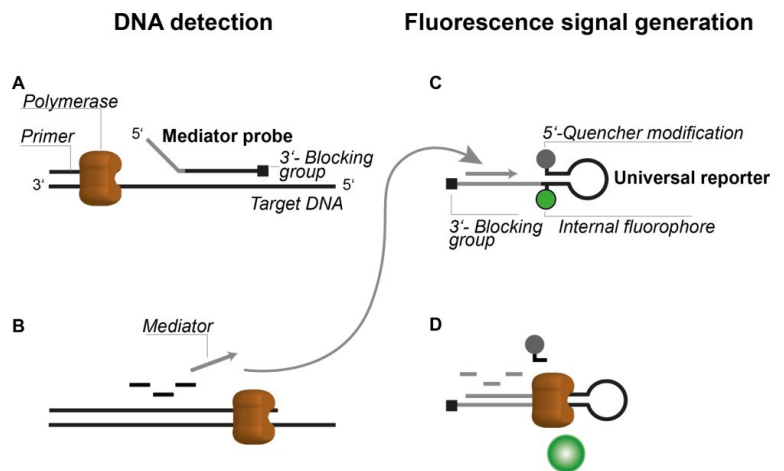


Fig. 1: Reaction mechanism of Mediator Probe PCR: Target sequence detection (left) and fluorescence signal generation (right) are two independent processes. This leads to several advantages (see left).

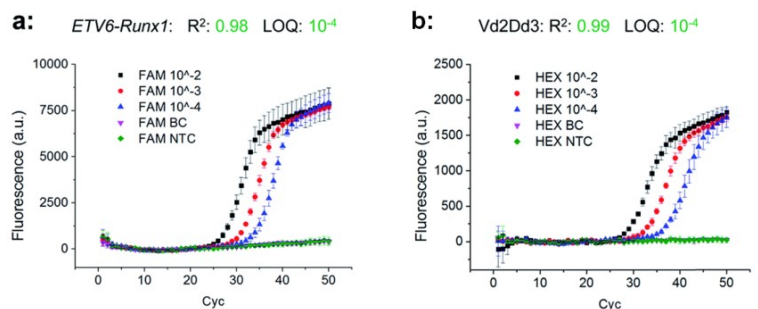


Fig. 2: Biplex Mediator Probe PCR, simultaneously quantifying the gene-fusion *ETV6-Runx1* (a) and the gene-rearrangement *Vd2Dd3* (b). In each reaction 1 000 – 10 copies of target DNA were detected in buffy coat (BC) background DNA in 100 000 DNA copies total. From Lehnert et al.¹ under CC BY-NC license (<https://creativecommons.org/licenses/by-nc/3.0/>).

References

- ¹ Lehnert et al.: Fluorescence signal-to-noise optimisation for real-time PCR using universal reporter oligonucleotides. *Anal. Methods*, (2018), DOI: 10.1039/c8ay00812d. –open access-
- ² Wadle et al.: Simplified development of multiplex real-time PCR through master mix augmented by universal fluorogenic reporters. *BioTechniques*, (2016), DOI: 10.2144/000114443 –open access-