

Mediator Probe PCR:

Improved real-time PCR detection using mediator probes and universal reporters

Mediator Probe PCR enables highly sensitive nucleic acid detection and precise quantification, using label free mediator probes in combination with optimized fluorogenic universal reporter molecules.

In Mediator Probe PCR label-free, target-specific mediator probes are used. During PCR amplification, these probes are cleaved, and the mediator sequences are released, which subsequently activate the fluorogenic universal reporters.

Since universal reporters are target sequence independent, once optimized, they will improve the fluorescence signal generation of all assays they are used in.

By the separation of target DNA detection and fluorescence signal generation, several advantages arise:

Advantages

- High fluorescence signal-to-noise ratios using universal reporter molecules
- Sensitive, selective and precise PCR-multiplexing
- Robust design guidelines
- Compatible with all PCR mastermixes

Application

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

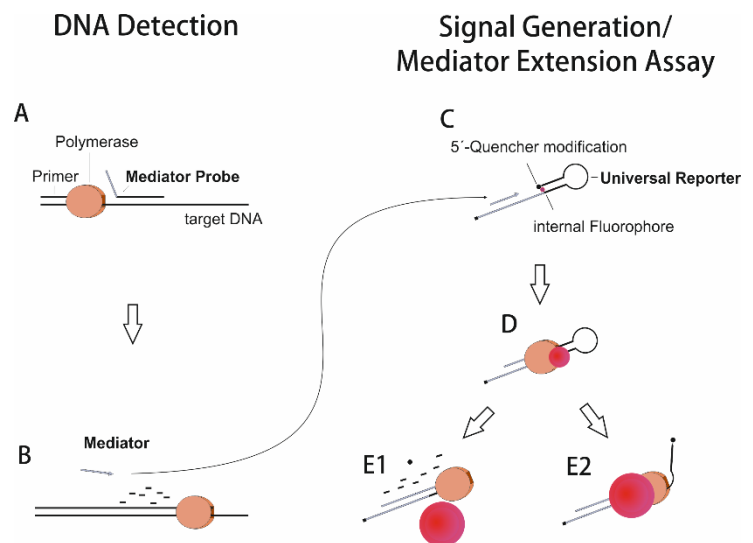


Fig. 1: Reaction mechanism of Mediator Probe PCR: Target sequence detection (left) and fluorescence signal generation (right) are two independent processes. This leads to several advantages.¹

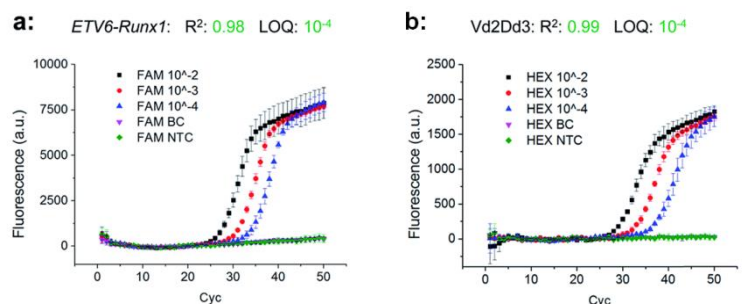


Fig. 2: Biplex Mediator Probe PCR, simultaneously quantifying the gene-fusion *ETV6-Runx1* (a) and the gene-rearrangement *Vd2Dd3* (b). In each reaction 1 000 – 10 copies of target DNA were detected in a background of 100 000 copies of buffy coat (BC) background DNA.¹

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-