Mediator Probe PCR

A cost-effective Approach for Real-Time PCR

Technology

The Mediator Probe PCR enables cost-effective real-time nucleic acid detection by combination of labelfree primary probes standardized fluorogenic reporter oligonucleotides. Specific probe-based real-time PCR commonly requires fluorescently-labeled detection oligonucleotides, which cause high costs in synthesis. In contrast, the Mediator Probe PCR replaces fluorogenic target-specific probes by unlabeled target-specific Mediator Probes. During amplification a Mediator is released by nucleolytic activity of the amplification enzyme, a polymerase. The Mediator is subsequently detected using standardized fluorescently labeled reporter oligonucleotides, which can be fabricated in large batches. Synthesis costs for specific nucleic acid detection oligonucleotides can be reduced by more than 60 % compared to typically used TaqMan PCRs giving a significant cost reduction in nucleic acid assay development.

Innovation

- Replacement of target-specific fluorogenic probes (e.g. TaqMan probes) by target-specific unlabeled Mediator Probes and standardized fluorogenic reporter oligonucleotides
- Reduction of assay-sepcific oligonucleotide synthesis costs by more than 60 %
- Reduction of background signal variation using standardized fluorogenic reporter oligonucleotides

Application

- PCR based nucleic acid quantification
- Real-Time PCR
- Multiplexed Real-Time PCR
- Reverse Transcription Real-Time PCR
- Digital PCR
- Medical Diagnostics
- Gene expression analysis
- Pharmacologic research
- Food analysis (Pathogens, GMO)

Market Potential

- The standardized detection format can give significant cost reduction in assay development and performance. It is of interest for all industrial and academic facilities working in real-time nucleic acid analysis.

.

Responsible Scientist

Dr. Felix von Stetten

Dept. of Microsystems Engineering

Branch

Biotech/Research

Patent Status

Patent Applications pending in
DE, EP, US, CA, CN, JP
WO 2013 079307 A1

Filed (PRD) Nov. 10th 2011

Reference Number

ZEE20110901

Status: Sept-16

CTF – The R & D Company of the
Freiburg University and the Freiburg
University Medical Center

Contact
Dr. Claudia Skamel
Campus Technologies Freiburg GmbH
Stefan-Meier-Str. 8 | D-79104 Freiburg
Email: Claudia.Skamel@campus-technologies.de
Tel: +49 (0)761 203-4987
Fax:+49 (0)761 203-5021

Reaction Principle Mediator Probe PCR

Target amplification and detection take place simultaneously in a concerted reaction, resulting in a detectable signal increase (figure 1). For several different DNA- or RNA-targets a sensitivity and precision concordant to state-of-the-art hydrolysis probe assays could be shown (figure 2) (B. Faltin *et al.*, Clin Chem, vol. 58, pp. 1546-1556, 2012).

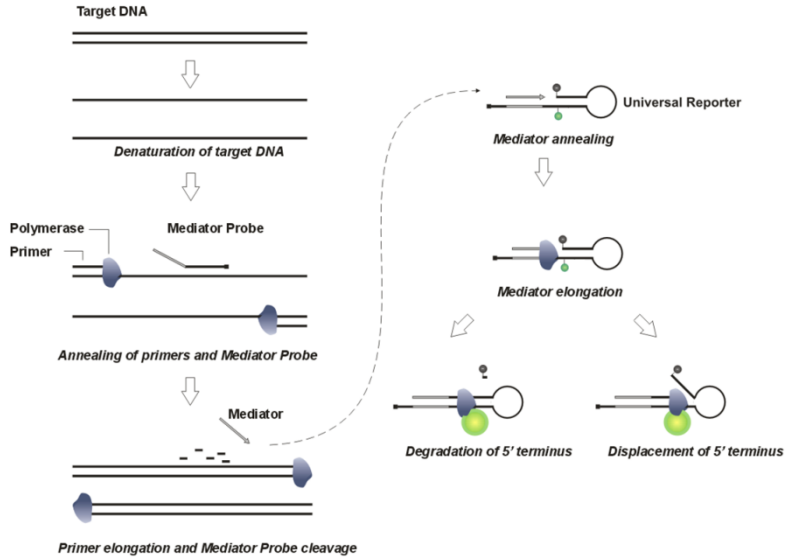

Figure 1: The nucleic acid target (A) is denaturated at increased temperatures (B). (C), Annealing of MP and primer molecules. The 5' portion of the MP does not anneal to the target. (D), Primer elongation and cleavage of MP. With each target duplication 1 mediator is released to the bulk solution. Subsequently, the mediator anneals to the UR (E). Mediator elongation (F) leads to dequenching of the fluorophore (G & H). All reaction steps take place within 1 thermocycle.

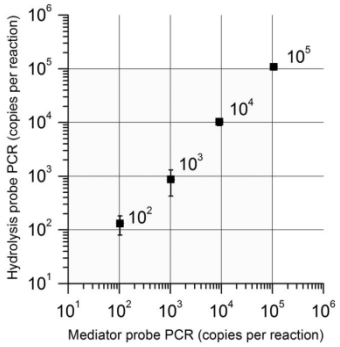

Figure 2: A DNA dilution series of HPV18 was amplified with the MP PCR and the state-of-the-art hydrolysis probe PCR. The backcalculated copy values for the MP PCR (abscissa) were plotted against values for the hydrolysis probe PCR (ordinate).

Cost Reduction in Comparison to Hydrolysis Probe PCR

The Mediator Probe PCR enables replacement of typically dual-labeled hydrolysis probes by unlabeled Mediator Probes, which cost less than 40 % in synthesis. Dual-labeled standardized Universal Reporter oligonucleotides can be used for different detection assays, and thus fabricated in larger batches. Therefore, oligonucleotide synthesis costs for multiple different analyses approach solely the costs of the Mediator Probes.

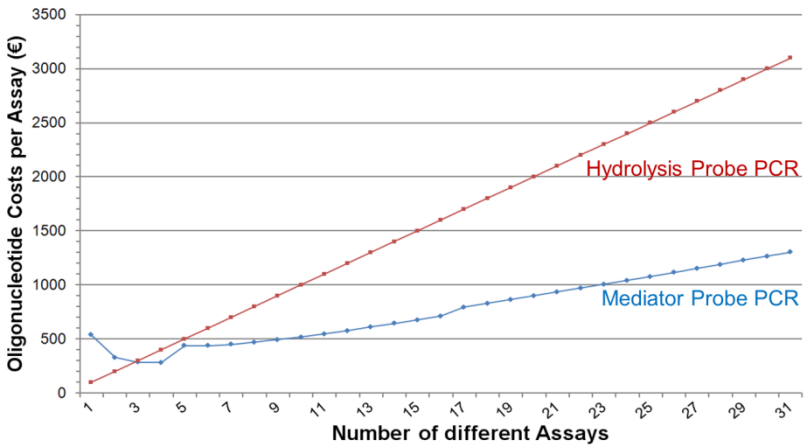

Figure 3: With increased number of target specific assays, total synthesis costs approach the costs solely of the Mediator Probes due to the repeated usage of the same standardized Reporter oligonucleotide. The graph accounts for larger synthesis batches of Universal Reporter oligonucleotides with increased assay numbers.
(Values are based on costs for oligonucleotide synthesis from the company Biomers, Germany).