Microsynth

Double-Quenched qPCR Probes

Reduced Background and Increased End-Point Fluorescence
Optimal Primer/Probe Design
Proficient Customer Support

Background Information

Quantitative PCR (qPCR or real-time PCR) is an important tool to detect specific DNA sequences in a sample, even in trace amounts. Due to the simplicity of the experimental set-up and the relatively short time needed, the use of dual-labelled hybridization probes in qPCR has become the most favoured method among all different approaches.

In order to obtain reliable results in qPCR experiments, background fluorescence should be reduced to a minimum level. This in turn is achieved by maximizing self-quenching of the initial probe or to completely transfer the emitted light of the 5'-fluoresence donor to the 3'- acceptor, respectively. As FRET (or the quenching effect) depends on the distance between donor and acceptor fluorophore, short hybridization probes, where the donor is in close proximity to the acceptor, would be desirable. Usually probes with a length of 15 nt show high quenching efficiencies. When probes are 25 nt or longer, the resonance energy transfer is considerably reduced and background fluorescence increases.

Smart Probe Design

An elegant way to increase self-quenching of qPCR probes is to equip the probe internally with an additional quencher in optimal distance from the fluorescence donor (Figure 1). These kinds of probes are called double-quenched probes. Microsynth has evaluated a considerable number of probes that consists of various internal quenchers and that differ in their positions relative to the 5'-FAM label. Among all tested probes the one with the internal quencher iQ500 showed lowest background fluorescence. Optimal behavior of the probe was obtained, when the quencher was positioned 9 nt from the 5'-FAM label. However marginal variations in the positioning are well tolerated.

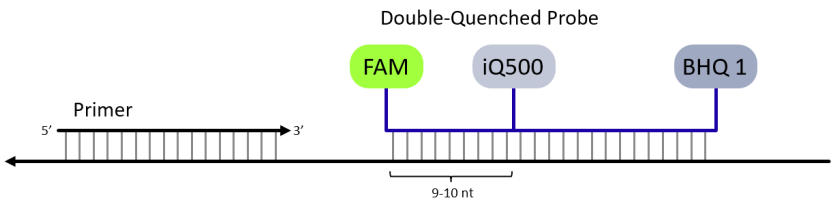


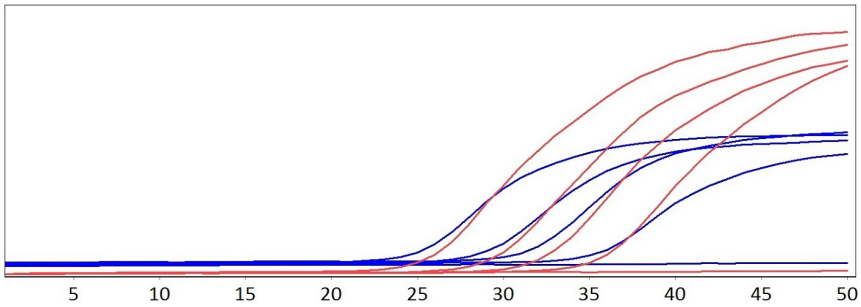
***Figure 1**. Principle design of double-quenched probes. As both quenchers absorb the emitted light of the fluorophore, the overall quenching efficiency is higher compared to conventional single-quenched qPCR probes.*

Microsynth AG, Switzerland
Schützenstrasse 15 · P.O. Box · CH-9436 Balgach · Phone +41 71 722 83 33 · Fax +41 71 722 87 58 · info@microsynth.ch · www.microsynth.com

Standard Probe vs. Double-Quenched Probe

The effect of double-quenched probes compared to standard probes is easily visible from the raw data and the normalized curves below (Figure 2). Double-quenched probes show reduced background fluorescence and increased end-point fluorescence.
As a consequence the accuracy and the robustness of qPCR experiments are increased.

a)



b)

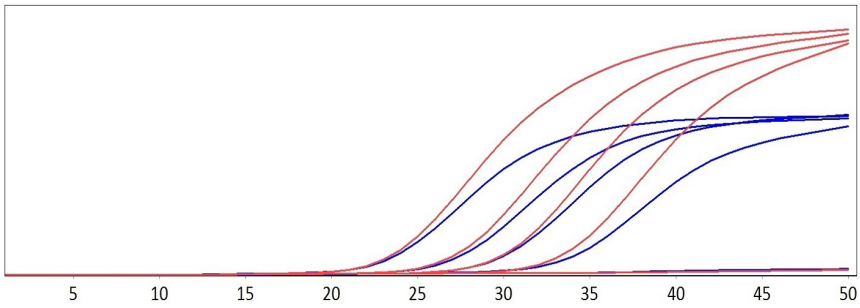


Figure 2. *qPCR amplification plots of a double-quenched probe vs. a standard probe.*
a) Raw data. b) Normalized curves. .
red: FAM-5'-ACT CCG GTG - iQ500 - AG ACT CTC TCT ACA GGG G-3'-BHQ 1
blue: FAM-5'-ACT CCG GTG AG ACT CTC TCT ACA GGG G-3'-BHQ 1

Closing Remark

The effect of double-quenched probes is unquestioned and the advantages are apparent from the qPCR plots. The chemical structure of the internal quencher is based on a planar aromatic system that potentially intercalates into DNA. Therefore, also higher hybridization temperatures can be expected from this type of probes as a positive side effect.
The internal quencher iQ500 should be used in combination with FAM/BHQ1 only. In some cases iQ500 can also be used in combination with either JOE/BHQ1 or HEX/BHQ1, however the effect is less distinctive than with FAM.

How to Order Double-Quenched Probes

Include ordering symbol "5" into your sequence (e.g. catattgaa5actgggttaacggaatt) and choose "Internal Quencher 500" under Inner Modification ("5=…"). Select your desired 5', 3' modification as well as purification, and your oligo is recognized and processed as a double-quenched probe.

Need More Information?
Call us at +41 71 722 83 33 or
Email us at oligo.support@microsynth.ch

Microsynth AG, Switzerland
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