Microsynth

Probes for qPCR and dPCR Applications

Broad Portfolio of Dyes and Modifications (MGB, LNA, etc.)
Combinations of Different Tm Enhancing Solutions Possible
Exceptionally Fast Delivery Times

Introduction

Probe-based qPCR or digital PCR relies on the sequence–specific detection of a desired PCR product. Unlike dye-based methods that detect all double–stranded DNA, probe-based approaches utilize a fluorescent–labeled target-specific probe resulting in increased specificity and sensitivity. Therefore, use high-quality probes from Microsynth to improve the sensitivity and specificity of your qPCR or dPCR assay. Furthermore, benefit from various Tm enhancers (MGB, LNA etc.) or even combine them to tailor your assay to your individual needs.

Dual-Labeled Probes

Dual-Labeled probes are the most common probe type for qPCR and are often referred to as TaqMan probes. Within the intact probe no overall fluorescence occurs as the emitted light is absorbed by an adjacent quencher. During PCR the target-bound probe gets hydrolyzed by the exonuclease activity of the polymerase thereby releasing the fluorophore from the quencher.

***Figure 1**. Principle design of dual-labeled probes. The primer is elongated by the polymerase, and the probe binds to the specific DNA template. Hydrolysis releases the reporter from the probe/target hybrid, causing an increase in fluorescence. The measured fluorescence is directly proportional to the amount of target DNA.*

Double-Quenched Probes

While traditional probes have 20-30 nucleotides between the dye and quencher, this probe has an additional internal quencher 8-10 bases from the 5′ fluorophore. This shortened distance, particularly when combined with the standard 3′ quencher, decreases background fluorescence and increases sensitivity.

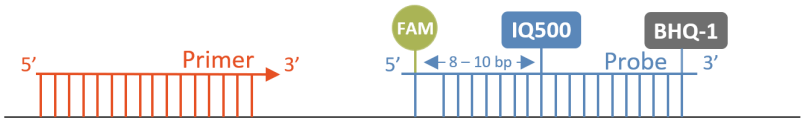

***Figure 2.** Principle design of double-quenched probes. As both quenchers absorb the emitted light of the fluorophore, the overall quenching effciency is higher compared to single-quenched qPCR probes. Consistently low backgrounds for probe sequences of even 40 base pairs or longer are possible.*

Molecular Beacons

Molecular Beacons are hairpin-shaped hybridization probes that are highly sensitive, sequence specific, and are used for sequence detection in qPCR and in vitro studies. The 5′ and 3′ ends of the probe contain a reporter and a quencher molecule, respectively. The loop is a single-stranded DNA sequence complementary to the target sequence.

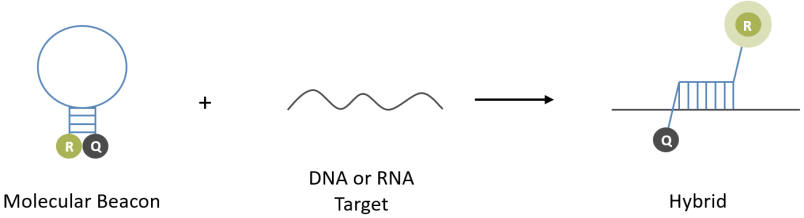

***Figure 3**. Principle design of molecular beacons. Molecular Beacons hybridize to their specific target sequence causing the hairpin-loop structure to open and separate the 5′ end reporter from the 3′ end quencher. As the quencher is no longer in proximity to the reporter, fluorescence emission takes place.*

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

Abs (nm)	Em (nm)	5' Dye	3' Quencher	Synthesis Scale		
				0.04	0.2	1
495	520	FAM	TAM, BHQ1,	x	x	x
495	520	FAM	iQ500-TAM, iQ500-BHQ1		x	x
521	536	TET	BHQ1	x	x	x
522	548	JOE	BHQ1		x	x
530	549	Yakima Yellow	BHQ1		x	x
535	556	HEX	BHQ1	x	x	x
546	563	Cy3	BHQ2	x	x	x
564	579	TAMRA	BHQ2		x	x
576	601	ROX	BHQ2		x	x
586	610	Texas Red	BHQ2		x	x
646	662	Cy 5	BHQ2	x	x	x
683	705	Cy 5.5	BHQ2	x	x	x
750	773	Cy7	BHQ2	x	x	x

***Table 1**. Standard dyes and quenchers for dual-labeled probes (TaqMan, molecular beacons, LNA probes). Double-quenched probes are currently available with FAM as reporter dye, only. For a full list visit our website or contact us directly.*

MGB Probes

Minor Groove Binder (MGB) Probes are dual-labeled probes conjugated with MGB. The MGB moiety increases the Tm of a probe because of its minor groove binding ability. MGB probes form highly stable duplexes with their targets, allowing shorter probes compared to standard dual-labeled probes, (down to 13 bases) to be highly efficient. Therefore, MGB probes are more specific, more efficient and more sensitive than conventional single or double-quenched probes.

Microsynth offers customized high-quality MGB probes for R&D purposes at affordable prices in two different versions. Whereas the cost-effective 3'MGB-Q500 quencher moiety is available with FAM as reporter dye only, 3'MBG-Q530 quencher moiety can be combined with FAM, JOE Yakima Yellow and HEX.

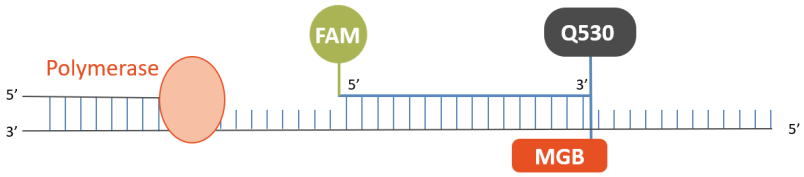

***Figure 4**. Principle design of MGB probes. The incorporation of a minor groove binder (MGB) stabilizes probe-target hybridization and increases melting temperature, allowing the use of shorter probes which are better suited for allelic discrimination and targeting AT-rich regions in qPCR assays..*

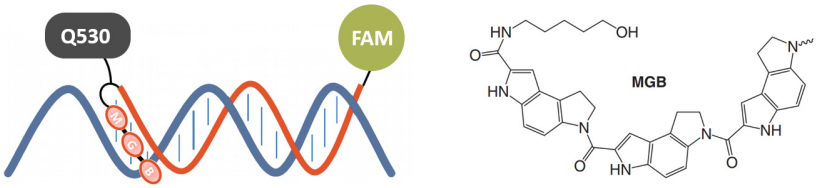

***Figure 5**. Depiction of an MGB probe (red) hybridized to the target DNA (blue); and chemical structure of MGB.*

Abs (nm)	Em (nm)	5' Dye	3' Quencher	Synthesis Scale		
				0.04	0.2	1.0
495	520	FAM	MGB-Q500	x	x	x
495	520	FAM	MGB-Q530	x	x	x
520	548	JOE	MGB-Q530	x	x	x
526	548	YYE	MGB-Q530	x	x	x
535	556	HEX	MGB-Q530	x	x	x

***Table 2**. Standard dyes available for MGB probes at Microsynth.*

LNA Probes

Locked nucleic acid (LNA) is a synthetic nucleic acid analogue containing a bridged, bicyclic sugar moiety. The superior hybridization characteristics of LNA allow a fine-tuning of the Tm in the design of qPCR probes. This significantly broadens the scope of assay conditions and permits more successful qPCR. When probes consist of LNA nucleotides, affinity and specificity of the hybridization to the target sequence are improved. This in turn reduces background fluorescence from spurious binding and leads to a better signal-to-noise ratio.

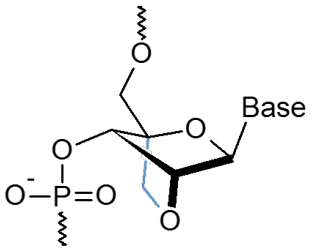

***Figure 6**. LNA 3'-endo conformation*

Tm Enhancing Nucleobase Modifications

Substitution of propynyl-dC (pdC) for dC and C-5 propynyl-dU (pdU) for dT are effective strategies to enhance base pairing. Using these base substitutions, duplex stability and melting temperatures are raised by the following amounts: propynyl-dC 2.8°C per substitution; propynyl-dU 1.7°C per substitution.

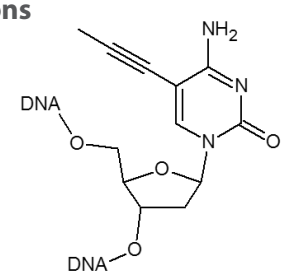

***Figure 7**. Propynyl-dC*

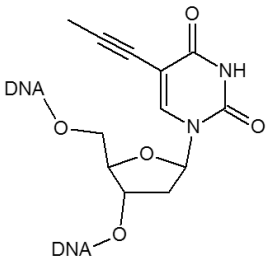

***Figure 8**. Propynyl-dU*

Manufacturing Times

Probe Type	Manufacturing Time [wd]
Dual-Labeled Probes	3
Double-Quenched Probes	3
Molecular Beacons	4
MGB Probes	4
LNA Probes	5

***Table 3**. Minimal manufacturing times for different types of probes. Add one additional working day for synthesis scales ≥ 1.0 µmol.*

Advantages of Using Microsynth

- Possibility to adjust binding affinity via MGB, LNA and other Tm enhancers or even combine different Tm enhancers to tailor your assay
- Fast turnaround times: 3-5 business days
- Wide variety of fluorophores - quencher combinations
- Professional design service
- EN ISO 13485:2016 certified production process
- qPCR & digital PCR assay development, validation, manufacturing and testing can be outsourced to Microsynth

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

Overview

Probe Type	Advantages	Disadvantages	Applications
Dual-Labeled Probes	Most popular PCR chemistry relying on the activity of Taq polymerase. Major benefits are the increased sensitivity & specificity compared to dye-based qPCR/dPCR.	Well suited for simple experiments, but might not work for more complex targets.	Chemistry of choice for most quantification as well as for multiplexing applications. Widely used in academic, food, environmental and medical research.
Double-Quenched Probes	Marked decrease in background fluorescence compared to identical single-quenched probes.	More expensive than conventional single-quenched probes.	Especially suitable for demanding qPCR applications that require greater flexibility in sequence selection without sacrificing the sensitivity of the highest performing probe designs.
Molecular Beacons	The stem probe structure of a molecular beacon makes it better able to discriminate single base-pair mismatches because the hairpin makes mismatched hybrids thermally less stable than hybrids.	The main disadvantage associated with molecular beacons is the accurate design of the hybridization probe. Optimal design of the molecular beacon stem annealing strength is crucial.	Molecular beacons have become popular for standard analyses such as quantification of DNA and RNA. Molecular beacons can also be used in non-PCR amplification assays.
MGB Probes	Higher target binding selectivity Less background fluorescence Higher quality of the probe due to shorter length (between 13 and 18 nucleotides). Very robust technology.	Freedom to operate in diagnostic procedures is limited due to various patents in the field.	Incorporation of minor groove binders results in higher accuracy and confidence for difficult targets.Typical applications are multiplex PCR systems and low copy assays.
LNA Probes	Enhanced binding affinity of LNA. Tm can be fine-tuned according to the needs of a desired oligonucleotide.	Freedom to operate in diagnostic procedures is limited due to various patents in the field.	Essentially same uses as MGB probes. Especially useful for single nucleotide polymorphism (SNP) testing since LNA can be incorporated at exact position.
Propynyl -dC, -dU Modified Probes	Enhance Tm selectively with the substitution of C and T.	Smaller increase of Tm than LNA or MGB. Freedom to operate in diagnostic procedures is limited due to various patents in the field.	Multiplex PCR Systems.

Table 4. *Applications for the different probe types available at Microsynth*

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

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