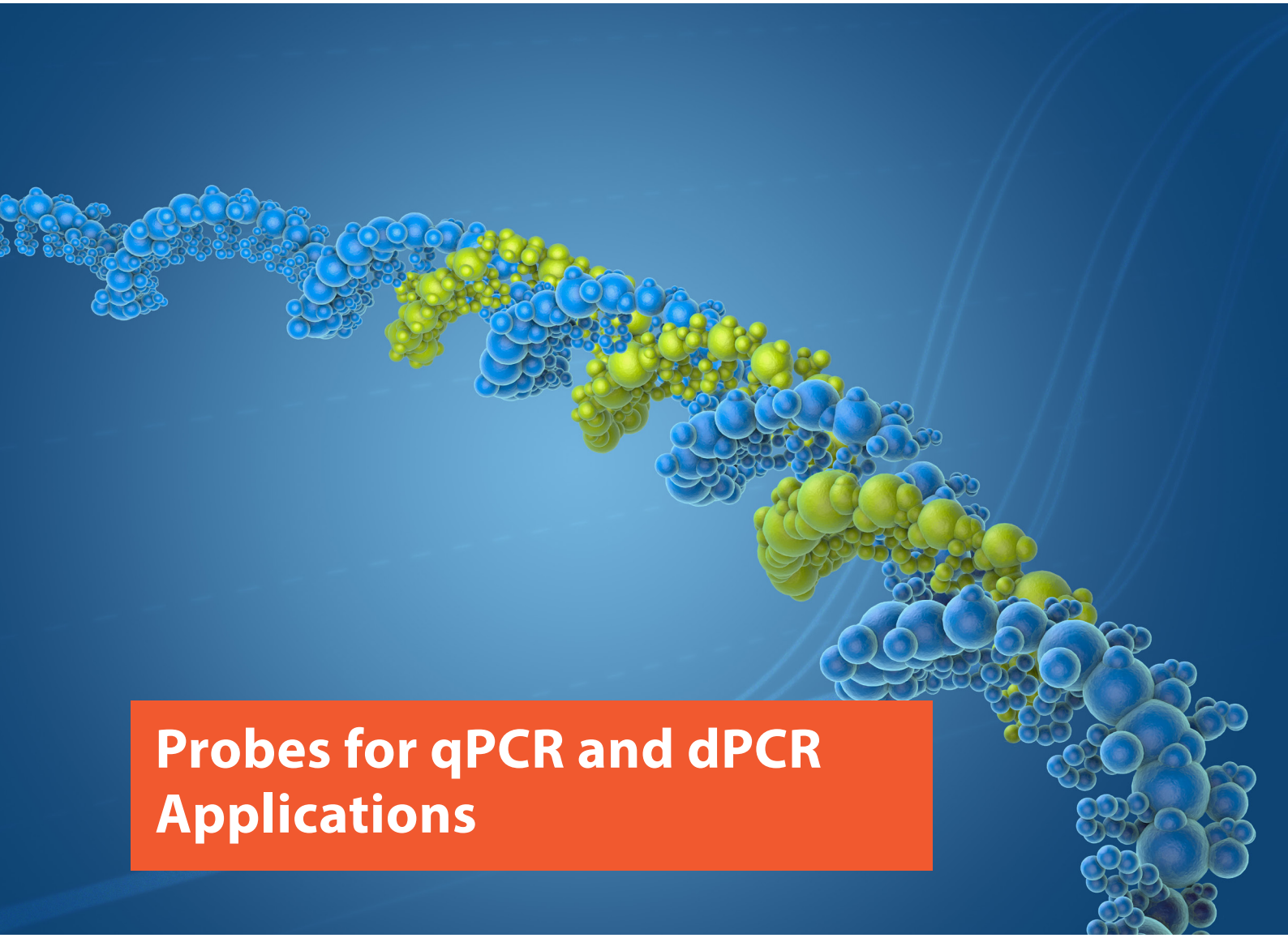
Microsynth



Probes for qPCR and dPCR Applications

Exceptionally Fast Delivery Times

Get your probes delivered in just 3–5 business days, ensuring your projects stay on schedule and reducing downtime in your workflows.

Broad Portfolio

Access a diverse selection of fluorophore-quencher combinations and probe designs, giving you the flexibility to create assays perfectly suited to your specific requirements.

Precise Tm Enhancing

Customize your assay with advanced Tm-enhancing options such as MGB, LNA, and other modifications. Combine multiple enhancers to achieve optimal binding affinity and unmatched assay performance.

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

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 reporter from the quencher.

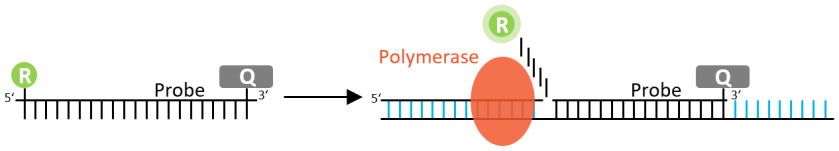


***Figure 1**. Principle design of dual-labeled probes. The primer is elongated by the polymerase, and the probe binds to the specific DNA template. Hydrolysis releases the reporter from the probe/target hybrid, causing an increase in fluorescence. The measured fluorescence is directly proportional to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

Double-Quenched Probes

Compared to dual-labeled probes, this probe features an additional quencher positioned 8–10 bp from the 5′ reporter. This reduces the distance between the reporter and quencher, enhancing energy transfer efficiency. The result is lower background fluorescence, an improved signal-to-noise ratio, and the ability to design longer probes of up to 40 nucleotides.

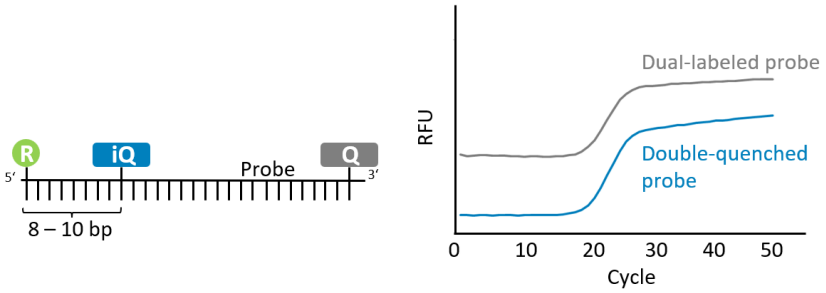


***Figure 2.** Design and qPCR performance of double-quenched probes. The additional quencher placed 8–10 bp from the 5′ reporter (left) enhances energy transfer, making these probes ideal for longer designs. This results in reduced background fluorescence compared to dual-labeled probes, as demonstrated in qPCR using a 30 nt probe (right, no baseline subtraction applied).*

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.

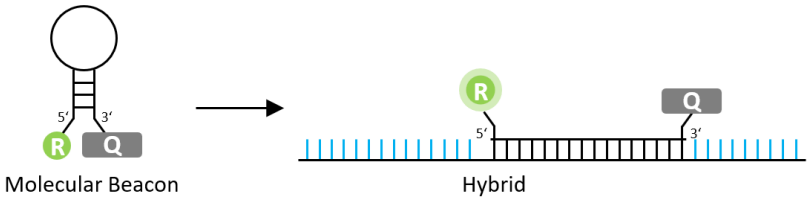


***Figure 3**. Principle design of molecular beacons. Molecular beacons hybridize to their specific target sequence causing the hairpin-loop structure to open and separate the 5′ end reporter from the 3′ end quencher. As the quencher is no longer in proximity to the reporter, fluorescence emission takes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

***Table 1**. Possible standard dyes and quenchers for probes. Various synthesis scales available. For a full list visit our website or contact us directly.*

Abs [nm]	Em [nm]	5′ Dye	3′ Quencher	Internal
495	520	FAM	BHQ1, TAMRA	iQ500/ iQ530
521	536	TET	BHQ1	-
522	548	JOE	BHQ1	-
530	549	Yakima Yellow	BHQ1	iQ530
535	556	HEX	BHQ1, BHQ2	iQ530
546	563	Cy3	BHQ2	-
564	579	TAMRA	BHQ2	-
576	601	ROX	BHQ2	iQ530
586	610	Texas Red	BHQ2	-
595	615	CalRed	BHQ2	iQ530
646	662	Cy 5	BHQ2	iQ530
646	664	A647N	BHQ2	iQ530
683	705	Cy 5.5	BHQ2	-
750	773	Cy7	BHQ2	-

MGB Probes

MGB probes are dual-labeled probes conjugated with a minor groove binder (MGB) moiety, which enhances the probe's melting temperature (Tm) through its ability to bind the minor groove of DNA. This allows MGB probes to form highly stable duplexes with their targets, enabling the design of shorter probes (as short as 13 bases) without compromising efficiency. As a result, MGB probes offer superior specificity, efficiency, and sensitivity compared to conventional single- or double-quenched probes.

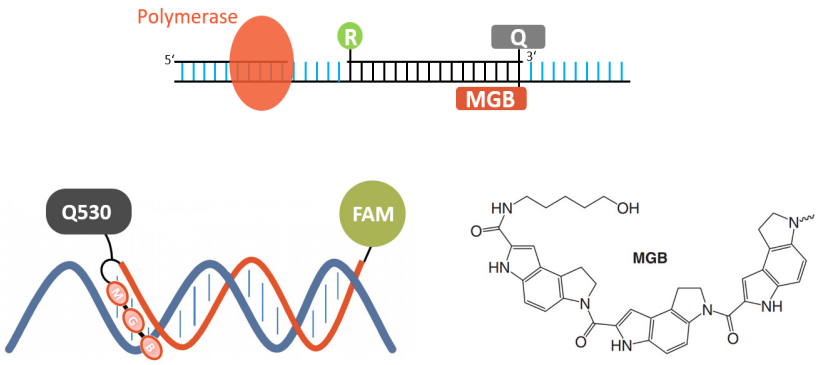


***Figure 4**. Principle design of MGB probes. Incorporating a minor groove binder (MGB) stabilizes the probe-target duplex and increases the melting temperature (Tm), enabling the use of shorter probes. The chemical structure is shown (bottom right), along with a schematic representation of probe hybridization with the target (bottom left)..*

***Table 2**. Standard dyes for MGB probes at Microsynth. Various synthesis scales are available.*

Abs [nm]	Em [nm]	5′ Dye	3′ Quencher
495	520	FAM	MGB-Q500
495	520	FAM	MGB-Q530
520	548	JOE	MGB-Q530
526	548	YYE	MGB-Q530
535	556	HEX	MGB-Q530

LNA Probes

Locked nucleic acid (LNA) is a synthetic nucleic acid analogue featuring a bridged, bicyclic sugar moiety. The exceptional hybridization properties of LNA enable precise tuning of the melting temperature (Tm) during qPCR probe design, expanding the range of assay conditions and increasing the likelihood of successful qPCR.

By incorporating LNA nucleotides, probes achieve enhanced affinity and specificity for their target sequences. This reduces background fluorescence from nonspecific binding and results in an improved signal-to-noise ratio.

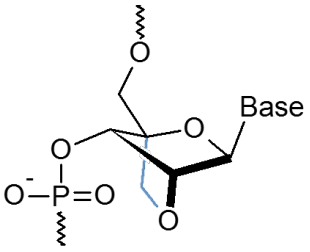


***Figure 6**. LNA 3'-endo conformation*

Tm Enhancing Nucleobase Modifications

Substituting propynyl-dC (pdC) for dC and C-5 propynyl-dU (pdU) for dT is a proven strategy for enhancing base pairing. These modifications significantly increase duplex stability and melting temperature (Tm), with each substitution raising the Tm by approximately 2.8°C for propynyl-dC and 1.7°C for propynyl-dU.

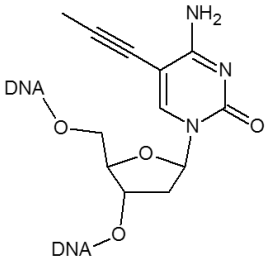


***Figure 7**. Propynyl-dC*

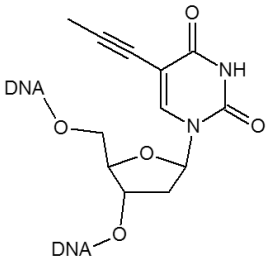


***Figure 8**. Propynyl-dU*

Specifications

***Table 3**. Minimum manufacturing times and yields for different probe types (13–40 nt). The yields, indicated in nmol, are calculated for a 20mer using the following rule of thumb: nmol of oligo = OD × 100 ÷ length of oligo. This calculation assumes a nearly homogeneous distribution of the four DNA bases. Actual yields may vary for sequences with high GC content (>70%) or other compositional biases.*

Probe Type	Manufacturing Times [wd]	Yield [nmol]		
		0.04 µmol	0.2 µmol	1.0 µmol
Dual-Labeled Probes/ Molecular Beacons	3	3.75	7.5	40
Double-Quenched Probes	3	5	10	40
MGB Probes	4	5	10	50
LNA Probes	5	2.5	5	30

For larger scales, please inquire.

Advantages of Choosing Microsynth

- **Fast Turnaround Times:** Receive your probes within 3–5 business days.
- **Customizable Binding Affinity:** Tailor your assay by adjusting binding affinity with MGB, LNA, and other Tm enhancers, or combine multiple enhancers for optimal results.
- **Extensive Fluorophore-Quencher Options:** Choose from a wide range of combinations to fit your assay needs.
- **Expert Design Support:** Benefit from our professional probe design service.
- **Certified Quality:** Enjoy peace of mind with our EN ISO 13485:2016-certified production process.
- **Comprehensive Services:** Outsource your qPCR and digital PCR assay development, validation, manufacturing, and testing to Microsynth for a seamless experience.

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Overview

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

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 dual-labeled probes.	More expensive than conventional dual-labeled 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.

How to Order Probes with Internal Modifications

To order probes with internal modifications such as LNA, propynyl-dC, or internal quenchers, please follow these steps:

1. **Include "5" in your sequence**
   Add the number "5" in your sequence where the modification is required (e.g., catattgaa5actgggttaacggaatt).

2. **Select the modification type**
   Under the InnerModification section, choose the appropriate modification (e.g., Internal Quencher 530) and assign it to "5".

3. **Handle multiple internal modifications**
   For sequences with different internal modifications (e.g., LNA-A, LNA-C, etc.), use additional numbers. Always use:
   "5" for nucleotide base A
   "6" for nucleotide base C
   "7" for nucleotide base G
   "8" for nucleotide base T

4. **Choose 5' and 3' modifications**
   Specify your desired 5' and 3' modifications, as well as the purification method.

Remark

If your sequence includes an internal quencher, it will be automatically recognized as a double-quenched probe and processed accordingly.

Need More Information?

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

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