

Assay Design and Reaction Optimization  
Choosing the Right Probe  
Dual-Labeled Probes  
Molecular Beacons  
Scorpions™ Probes  
LightCycler® Probes



# Fluorescent Probes

For Quantitative Real-Time PCR  
and other Applications



Sigma Can Provide a Custom Solution  
For Every Project – Guaranteed!

## Sigma's Custom Products

Sigma® is recognized as the world's leading supplier of custom DNA & RNA oligonucleotides and peptide libraries for the global life science research community. Originally founded in 1986 as Genosys Biotechnologies, Genosys was acquired by Sigma-Aldrich in 1998 to form Sigma-Genosys. In 2005 Sigma-Aldrich acquired Prologo and its wide range of specialized DNA and siRNA products. Sigma-Genosys and Sigma-Prologo have harmonized product lines and continue to provide cutting-edge oligonucleotide technology, superior service and competitive prices under the Sigma brand.

### The Sigma Advantage

We continue to invest in our worldwide operations and believe it is not just the quality of our products that sets us apart, but also the quality of our service and technical expertise.

- Our Commitment to Quality
- Outstanding Customer Service & Technical Support
- Global Manufacturing

### Our Commitment to Quality

Quality is an integral part of our manufacturing process. Sigma analyzes all oligonucleotides, including probes, by mass spectrometry, ensuring the highest quality products. Complementary techniques, such as analytical chromatography and electrophoresis are routinely used to verify specifications are met. Our sizeable investment in state-of-the-art analytical equipment provides industry-leading tools to develop and monitor our process.

Our fluorescently labeled probes are manufactured using a rigorous process, including:

- Purification by Ion Exchange and/or Reverse Phase Chromatography
- Electrospray Mass Spectral Analysis
- Quality Control Documentation
- Amber Packaging Ideal for Fluorescent Molecules

### Outstanding Customer Service and Technical Support

Our dedicated staff of highly trained customer service specialists are available via e-mail or telephone to provide timely solutions to every customer inquiry. Providing real-time status for orders, our customer service teams demonstrate total commitment to customer satisfaction. With an extensive staff of Molecular Biologists and Chemists, our technical experts are prepared to assist researchers with experimental design, application support and troubleshooting. Whether contacting us via the web, e-mail, or telephone, Sigma customers are provided with Best-in-Class service and support.

### Global Manufacturing

Sigma has oligonucleotide manufacturing sites around the globe, in 10 countries – Australia, Canada, France, Germany, India, Israel, Japan, Singapore, UK and USA. Our global customers receive consistent high-quality products.



### Expertise of our Scientists

With more than 20 years of experience in oligonucleotide synthesis, we have the expertise to create the most technically challenging custom biomolecules. Our scientists collaborate with researchers around the world and together we develop novel custom products. We meet customer specifications, no matter how complex.

### qPCR Tools

Sigma is pleased to offer a variety of education tools for qPCR users including:

- Webinar Series
- Technical & Troubleshooting Guides
- Workshops

Our webinar series include technical presentations from international speakers discussing the latest advances in qPCR. By offering the webinars in an archived format, researchers are able to access this technical resource at their convenience. Training materials and webinars can be viewed and downloaded from the website. Check with your local sales representative or our website ([sigma.com/qpcrtraining](http://sigma.com/qpcrtraining)) regarding workshops.

# Assay Design and Reaction Optimization

Sigma offers primers, probes, and reagents to support all quantitative real-time PCR (qPCR) assays.

## Detection Options for qPCR

Quantitative real-time PCR relies on real-time detection of amplification products as they are formed in the reaction. This can be accomplished using non-specific DNA binding dyes or sequence specific probes. These techniques and their benefits are described below.

Figure 1. A Theoretical qPCR Assay

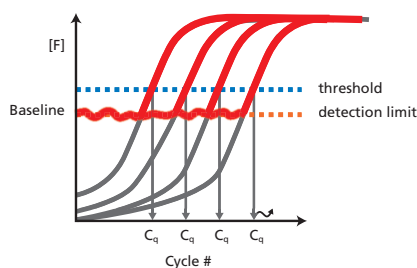


Figure 1. During a qPCR assay, the progress of the reaction is monitored by tracking the increase in fluorescence from an associated reporter molecule. The number of cycles required to reach a threshold level of detection is the  $C_q$ . The lower the  $C_q$  the higher the initial concentration of target and vice versa.

## Non-specific DNA Binding Dye Detection

SYBR® Green I binds non-specifically to double-stranded DNA. Upon binding, the dye undergoes a conformational change resulting in high fluorescent emission, allowing measurement of the total amount of double-stranded product present in the reaction after each amplification cycle.

SYBR Green I detection is a popular option due to its low cost and ease of use. However, multiple double-stranded species that may be present cannot be discriminated when using SYBR. Within any PCR, there is the potential for primer dimer formation and non-specific amplification products. Therefore, melt curves must be used after amplification for estimating the specificity of amplified products. It may be difficult to obtain accurate quantification at low target concentrations.

For this reason, many researchers use SYBR Green I detection for initial screening or proof-of-concept experiments and progress to probe-based detection for greater assay sensitivity and/or for multiplex analysis.

## Probe-based Detection

Probe-based detection methods rely on one or more fluorescently labeled oligonucleotides that are positioned between the two PCR primers. Because the probe is sequence-specific, it will only detect

the presence of a single amplicon within the reaction. There are several types of probe structures that can be used including:

- Dual-labeled Probes
- Molecular Beacons
- Scorpions™ Probes
- LightCycler® Probes

Each probe type enables researchers to measure an increase in fluorescent signal that corresponds to an increase in the copy number of the desired amplicon. In addition to the increase in sensitivity that is gained from using sequence-specific probes for detection, these can also be labeled with different fluorescent dyes, allowing detection of multiple targets within the same PCR reaction.

## Benefits and Challenges of Multiplex Reactions

The use of probes labeled with different reporter dyes allows the simultaneous detection and quantification of multiple target genes in a single (multiplex) reaction.

There are situations in which multiplex reactions are beneficial including:

- **Limited Template Availability:** The number of amplifications can be maximized
- **Large Numbers of Samples:** Reducing the number of reactions leads to cost savings

However, there are a variety of challenges when developing a multiplex assay including:

- **Complex Design:** The degree of difficulty increases with the number of targets to be detected
- **Optimization Reaction Conditions:** All primer/probe sets need similar reaction kinetics and the same buffer, therefore target sensitivity may be reduced compared to similar singleplex reactions

## The MIQE Guidelines

Since the inception of qPCR, researchers have been frustrated by complications caused by variability, and until recently, there has been a lack of consensus about how to deal with these types of obstacles.

An international research team, including Dr. Tania Nolan, Sigma's Global Manager for Applications and Technical Support, published *The MIQE Guidelines* (Clinical Chemistry, 2009) to address the challenges of performing dependable qPCR measurements.

By adhering to The MIQE Guidelines, you will publish uniform, comparable, and reliable data.

Visit our website at [sigma.com/miqe](http://sigma.com/miqe) to learn more and download the paper.

# Reaction Optimization for Increased Sensitivity

Pre-designed probe and primer sets are an attractive option when looking for a fast and simple quantitation solution. However, many of the pre-designed assays that are commercially available have not been properly optimized, leading to reduced efficiency and sensitivity in template detection.

## Primer and Probe Placement

In general, amplicons should be between 50-150 bases in length. Shorter amplicons tend to be more tolerant of less than ideal reaction conditions, improving the consistency of results.

When quantifying RNA targets, select primers spanning exon-exon junction to avoid amplification of contaminating genomic DNA in cDNA samples.

## Primer and Probe Concentration

Optimization of primer and probe concentrations can improve detection level of a particular amplicon by around 10 cycles depending on the sequence. Since a 3C<sub>q</sub> difference in amplicon detection indicates approximately a 10 fold difference in template concentration, this simple step can greatly improve both the accuracy and sensitivity of the reaction.

Figure 1. Primer Optimization Improves Reaction Sensitivity

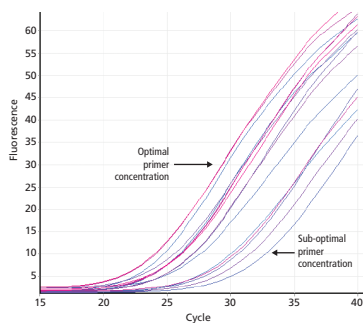


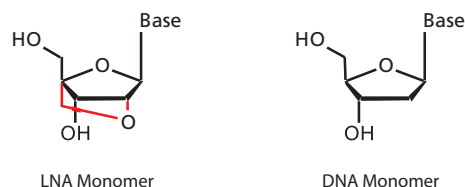
Figure 1. Primer optimization assay using Sigma® SYBR® Green 1 mastermix. Optimization of primers for the human UBC gene. All reactions contain exactly the same template but varying primer concentrations. Assay highlights how variation in primer concentration has an impact on the sensitivity of the assay. Platform: Rotor-Gene™ Corbett Research Ltd. Description of protocol optimization is referenced: Nolan T, Hands RE, Bustin SA Quantification of mRNA using real-time RT-PCR. Nature Protocols 2006; 1:1559-1582

## Buffer Conditions

Assay performance may also benefit from optimization of buffer components (particularly MgCl<sub>2</sub>) and the internal reference dye. Optimizing the concentration of these components is especially important when designing multiplex assays, or singleplex assays in which design of an appropriate probe/primer combination proves to be difficult.

## Improved Assay Sensitivity and Specificity Using Locked Nucleic Acids® (LNA®)

Locked Nucleic Acids (LNA) can be incorporated into any of our qPCR probe types, providing enhanced sensitivity and specificity for your assay. LNA is a novel type of nucleic acid analog containing a 2'-O, 4'-C methylene bridge. A comparison of LNA to DNA is shown below:



When used with any standard bases (A,C,G,T, or U), probes synthesized using LNA have greater thermal stability than conventional DNA or RNA and therefore form a stronger bond with the complementary sequence.

The introduction of LNA chemistry into a qPCR probe may result in an increase in the duplex melting temperature (T<sub>m</sub>) of up to 8 °C per LNA monomer substitution in medium salt conditions compared to a DNA fluorescent probe. It is possible to optimize the T<sub>m</sub> level and the hybridization specificity through specific placement of the LNA base(s) in the probe design as shown below.

Probe Sequence	LNA Bases	T <sub>m</sub> *	ΔT <sub>m</sub>	ΔT <sub>m</sub> /LNA
GTGATATGC	0	29 °C	--	--
<b>GT</b> <u>GAT</u> ATGC	3	44 °C	15 °C	+5 °C
<b>GT</b> <u>GATATGC</u>	9	64 °C	35 °C	+3.9 °C

\* T<sub>m</sub> of duplex between probe and its complementary sequence

Note: The **bolded** and underlined bases denote LNA base

Incorporation of LNA into your qPCR probe can improve performance of many assays, including:

- **SNP Discrimination:** The presence of a single base mismatch has a greater destabilizing effect on the duplex formation between a LNA fluorescent probe and its target nucleic acid than with a conventional DNA fluorescent probe
- **Multiplex Assays:** Incorporation of LNA bases allows simpler T<sub>m</sub> optimization, providing more flexibility in probe placement
- **Problematic Target Sequences:** Shorter probes can be designed to address traditionally problematic target sequences, such as AT- or GC-rich regions, highly repetitive sequences or regions with difficult secondary structure. Short regions of homology in aligned sequences can also be targeted.

Contact your local technical service professional at [oligotechserv@sial.com](mailto:oligotechserv@sial.com) for support with your specific application.

# Primer and Probe Design Services

## OligoArchitect™ Primer and Probe Design Solutions

Sigma® is pleased to offer OligoArchitect for all of your primer and probe design requirements. OligoArchitect includes both our complimentary online design tool and our unique consultative service.

### OligoArchitect Online

For routine needs, improve your assay with our OligoArchitect online design tool powered by the industry standard Beacon Designer™ (Premier Biosoft). The user-friendly interface utilizes the latest algorithms, provides results in real time, supports templates up to 10,000 base pairs, and allows for the adjustment of input parameters such as homopolymer run/repeat maximum length, G/C clamp length, and maximum primer pair Tm mismatch.

Designs can be completed for traditional PCR or quantitative real-time PCR (qPCR) using the following detection chemistries:

- SYBR® Green I
- Dual-Labeled Probes

Our online design tool can be used for the following applications:

- Traditional PCR
- Allele discrimination
- Endpoint genotyping
- SNP detection
- Gene expression analysis
- Haplotyping
- Genomic copy number determination

All reported sequences, associated properties, and assay parameters are available for export to Excel and convenient email ordering. Visit [wherebiobegins.com/probedesignonline](http://wherebiobegins.com/probedesignonline) to try OligoArchitect Online.

### OligoArchitect Consultative

For more complex and demanding applications, utilize our consultative service to ensure the success of your assay. With personal consultation from our expert, technical support scientists, your request, including all sequences and data analysis, will be MIQE compliant and returned to you within 24 hours. If required, you will also receive follow-up assay optimization, data analysis assistance, and troubleshooting support.

Designs can be completed for traditional PCR or qPCR using the following detection chemistries (Locked Nucleic Acid® (LNA®) can be included in probe and beacon designs):

- SYBR® Green I
- Scorpions™ Probes
- Dual-Labeled Probes
- Molecular Beacons
- LightCycler® Probes

Designs can typically be completed for the following PCR and qPCR applications:

- Traditional PCR
- Endpoint genotyping
- Methylation specific PCR
- Gene expression analysis

- Genomic copy number determination
- Transcript detection across exon junctions
- High resolution melting analysis
- Northern blotting
- Southern blotting
- Allele discrimination
- SNP detection
- Haplotyping
- Multiplexing for up to four assays

### Other applications:

- Fluorescence *in situ* hybridization

Sigma's consultative service uses Beacon Designer from Premier Biosoft International. Blastn and mFold are used to determine assay specificity.

## Bioinformatic Services

With recent growth in the volume and complexity of genomics and proteomics research, it is necessary for scientists to be able to capture and use this information. Sigma recognized this need and developed state-of-the-art bioinformatics capabilities, including:

- A dedicated team of bioinformatics professionals with extensive expertise in genomics and proteomics computing applications.
- An in-house system of hardware, software, tools, and databases. Both proprietary and commercial software packages allow greater flexibility in addressing researcher needs.
- Microarray oligonucleotide design, AQUA™ Peptide design, PEPscreen®: peptide library design, gene / transcript / proteomic annotation, gene / protein function classification, microarray data analysis, and more.
- Rapid and secure completion of projects. Entire genome oligonucleotide microarray designs are completed in less than 2 weeks. All sequence information is retained in a secure environment using internal Blast databases for searches.

## Biostatistics Service

Sigma offers this confidential service for your qPCR research. Before beginning your experiment, our biostatisticians will evaluate and offer recommendations for developing protocols. After completing your experiment, they will process your raw data into a comprehensive results report, including all statistical analyses as well as publication-ready tables and graphs.

### The following design and analysis options are available:

- Evaluation or development of assay design
- Quantification of unknown samples
- Determination of statistical significance
- Hierarchical clustering of expression profiles
- Identification of optimal reference genes
- Identification of marker gene combinations

Contact your local technical service professional at [oligotechserv@sial.com](mailto:oligotechserv@sial.com) for support with our design, bioinformatics, and biostatistics products and services.

# Choosing the Right Probe

There are a selection of possible probes that can be used for qPCR, and each has advantages for different applications. The summary below highlights the applications of commonly used probes.

## Application Reference Guide

APPLICATION	SYBR® Green 1	Dual-Labeled Probes	Dual-Labeled LNA® Probes	Molecular Beacons*	LightCycler® Probes*	Scorpions™ Probes*
Mass screening	••					
Microarray validation	••	•				
Multiple target genes / few samples	•	•				
SNP detection			••	•	•	••
Allelic discrimination			••	•	•	••
Pathogen detection	•	•	••	•	•	••
Multiplex		••	••	••	•	••
Viral load quantification		•	••	•	•	••
Gene expression	•	••	••	••	••	••
Gene copy determination		•	••	•	•	••
End point genotyping				••		••
In vitro quantification or detection			•	••		

\*LNA can be incorporated into the probes for improved specificity.

## Fluorophores, Quenchers and Instrument Compatibility

Modern qPCR platforms typically have multiple detection channels enabling flexibility in the choice of probe labels. It is important to select the fluorescent labels which are compatible with the detection channels for the qPCR instrument and to ensure the correct filter settings or detection calibration for the instrument. The Fluorophores and Instrument Compatibility table (see page 7) lists a selection of some of the most widely used qPCR platforms and indicates which fluorescent labels may be used. Please note that not all labels are listed and many alternative fluorophores are available. For information on the use of non-standard labels with these platforms, please contact your local technical service professional.

## Dye Substitutes

Several qPCR instruments utilize proprietary dyes which are not generally available commercially, such as VIC™ and NED™. When seeking dye alternatives, the following criteria are important:

- The excitation and detection wavelength are compatible with the instrument light source and detection system
- For probes, the quencher effectively absorbs light at the emission wavelength of the fluorophore
- The higher the extinction coefficient the brighter the dye, which contributes to sensitive detection
- When using multiple dyes (multiplex) the excitation and emission wavelengths of each dye must be independent to avoid cross talk

## Quenchers

Quenching molecules are typically placed at the 3' end of single molecule probes such as Dual-labeled probes, Molecular Beacons and Scorpions. Quenchers may be fluorescent (TAMRA™) or nonfluorescent molecules (DABCYL, Black Hole Quenchers (BHQ™)). For optimal performance, the quencher's absorbance spectrum should match the fluorophore's emission spectrum as closely as possible. Recommended fluorophore/quencher combinations can be found in the Spectral Properties table on page 7.

Spectral Properties Table

Dye	Max. EX (nm)	Max. EM (nm)	Compatible Quencher
6-FAM™	494	515	BHQ-1, TAMRA
JOE™	520	548	BHQ-1, TAMRA
TET™	521	536	BHQ-1, TAMRA
Cal Fluor® Gold 540 <sup>1</sup>	522	541	BHQ-1
HEX™ <sup>2</sup>	535	555	BHQ-1, TAMRA
Cal Fluor Orange 560 <sup>2</sup>	540	561	BHQ-1
TAMRA™	555	576	BHQ-2

<sup>1</sup>JOE/TET alternative  
<sup>2</sup>VIC alternative

<sup>3</sup>Cy3 alternative  
<sup>4</sup>TAMRA alternative

<sup>5</sup>Cy5 alternative

Dye	Max. EX (nm)	Max. EM (nm)	Compatible Quencher
Cy3®	550	570	BHQ-2
Quasar® 570 <sup>3</sup>	548	566	BHQ-2
Cal Fluor Red 590 <sup>4</sup>	565	588	BHQ-2
ROX™	573	602	BHQ-2
Texas Red®	583	603	BHQ-2
Cy5®	651	674	BHQ-3
Quasar 670 <sup>5</sup>	647	667	BHQ-3
Cy5.5®	675	694	BHQ-3

Sigma® is a licensed supplier of a variety of dyes and quenchers and continually adds to its portfolio of new chemistries. For assistance in the design of your probe and/or assays, submit your request at [sigma.com/designmyprobe](http://sigma.com/designmyprobe).

Fluorophores and Instrument Compatibility Table

Platform	SYBR® Green I	FAM	HEX	JOE	ROX	TET	Cy3	Cy5	TAMRA	Texas Red	LC Red 640	LC Red 705
ABI 7900HT	•	•	•	•	•	•			•			
ABI 7300	•	•	•	•	•				•			
ABI 7500	•	•	•	•	•		•	•	•	•		
ABI 7700	•	•	•	•		•			•			
ABI 7000	•	•	•	•					•			
ABI StepOne™	•	•	•	•	•		•		•			
ABI StepOnePlus™	•	•	•	•	•		•		•			
Bio-Rad iQ™ 5	•	•	•	•	•	•	•		•	•		
Bio-Rad Opticon™ 2	•	•	•			•			•			
Bio-Rad Chromo4™	•	•	•	•	•	•	•	•	•	•		
Bio-Rad MyiQ™	•	•										
Bio-Rad MiniOpticon™	•	•										
Bio-Rad CFX96™	•	•	•	•	•	•	•	•	•	•	•	•
Bio-Rad SFX384™	•	•	•	•	•	•	•	•	•	•	•	•
Agilent Mx4000®	•	•	•	•	•	•	•	•	•	•		
Agilent Mx3000P®	•	•	•	•	•	•	•	•	•	•		
Agilent Mx3005P®	•	•	•	•	•	•	•	•	•	•		
Roche LightCycler®	•	•									•	•
Roche LightCycler 2	•	•	•								•	•
Roche LightCycler 480	•	•	•					•			•	•
Cepheid SmartCycler®	•	•				•	•			•		
Cepheid SmartCycler II	•	•				•	•	•		•		
Qiagen Rotor-Gene® 6000	•	•		•	•	•	•	•	•	•		
Eppendorf Mastercycler® ep realplex	•	•	•	•	•				•	•		

**Note:** Not all qPCR instruments or fluorophores are listed. Contact the instrument manufacturer for details on compatible fluorophores

# Dual-Labeled Probes

Dual-labeled probes are the most common probe type for qPCR and are often referred to as hydrolysis probes.

## Choose Dual-Labeled Probes for:

- Gene expression
- Multiplex assay development
- Pathogen detection
- Viral load quantitation
- Microarray validation
- si/shRNA knockdown
- Gene copy determination
- Mutation detection
- Allelic discrimination
- SNP detection

## Benefits of Using Dual-Labeled Probes Include:

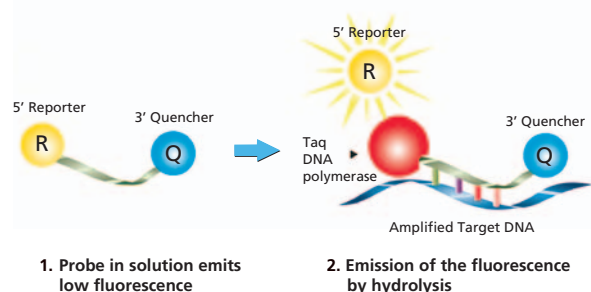
- Design simplicity for sequence specificity
- Increased sensitivity
- Extensive availability of fluorophore combinations

## Add LNA® to Your Probe for:

- Increased thermal stability and hybridization specificity
- Greater accuracy in gene quantitation and allelic discrimination
- Easier and more sensitive probe designs for problematic target sequences

### How Dual-Labeled Probes Work

A dual-labeled probe is a single-stranded oligonucleotide labeled with two different dyes. A reporter dye is located at the 5' end and a quencher molecule located at the 3' end. The quencher molecule inhibits the natural fluorescence emission of the reporter dye by Forster-type energy transfer. The illustration below depicts the mechanism.



The primer is elongated by the polymerase and the probe binds to the specific DNA template, hydrolysis releases the reporter dye from the probe/target hybrid, causing an increase of fluorescence. The measured fluorescence signal is directly proportional to the amount of target DNA.

## Product Features Include:

- Available in lengths of 15–40 bases
- Simple pricing – HPLC purification and base charges are included
- Stringent quality control including electrospray mass spectroscopy
- Shipped within 3–5 working days for 6-FAM™, HEX™, or TET™ labeled
- Shipped in an amber tube to protect dye integrity
- Custom formats available (normalization, special plates, etc.)

Sigma's probes are provided in a format to simplify your experimental planning.

## Guaranteed Yields

Guaranteed OD Yield	Appx. No. of nmoles	Appx. No. of µg	Appx. No. of Reactions*
1	4	32	800
3	12	96	2,400
5	20	160	4,000
10	40	320	8,000

\* Estimate is based on 4 nmoles or 32 µg for 1 OD and 200 nM in 25 µl reaction (5.0 pmol/reaction). Estimate is based on an average sequence length of 25 bases.

The most common fluorophore and quencher combinations are listed below:

## Spectral Properties Table

Dye	Max. EX (nm)	Max. EM (nm)	Compatible Quencher
6-FAM™	494	515	BHQ-1, TAMRA
JOE™	520	548	BHQ-1, TAMRA
TET	521	536	BHQ-1, TAMRA
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<sup>3</sup>Cy3 alternative  
<sup>4</sup>TAMRA alternative

<sup>5</sup>Cy5 alternative

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# Molecular Beacons

Molecular Beacons are structured probes that are highly sensitive, sequence-specific and used for sequence detection in real-time qPCR and *in vitro* studies.

## Choose Molecular Beacons for:

- End-point genotyping
- *In vitro* quantification or detection studies
- Multiplexing
- SNP detection
- Allelic discrimination
- Pathogen detection

## Benefits for Using Molecular Beacons Include:

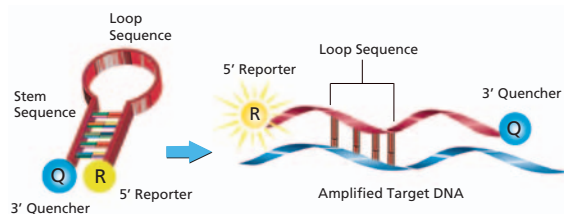
- Increased specificity
- Probe preserved during the reaction

## Add LNA to Your Probe for:

- Increased thermal stability and hybridization specificity
- Greater accuracy in gene quantitation and allelic discrimination
- Easier and more sensitive probe designs for problematic target sequences

### How Molecular Beacons Work

A Molecular Beacon is a single-stranded bi-labeled fluorescent probe held in a hairpin-loop conformation (around 20 to 25 nt) by complementary stem sequences (around 4 to 6 nt) at both ends of the probe. The 5' and 3' ends of the probe contain a reporter dye and a quencher dye, respectively. The loop is a single-stranded DNA sequence complementary to the target sequence. The close proximity of the reporter and quencher dyes causes the quenching of the natural fluorescence emission of the reporter dye. The structure and mechanism of a Molecular Beacon is shown below.



1. Unbound beacon with quenched fluorescence

2. Bound beacon with unquenched fluorescence

Molecular Beacons hybridize to their specific target sequence causing the hairpin-loop structure to open and separate the 5' end reporter dye from the 3' end quencher dye. As the quencher dye is no longer in proximity to the reporter dye, fluorescence emission takes place. The measured fluorescence signal is directly proportional to the amount of target DNA.

## Product Features Include:

- Available in lengths of 15–40 bases
- Simple pricing – HPLC purification and base charges are included
- Stringent quality control including electrospray mass spectroscopy
- Shipped within 5–6 business days for 6-FAM™, HEX™, or TET™ labeled
- Shipped in an amber tube to protect dye integrity
- Custom formats available (normalization, special plates, etc.)

Sigma's probes are provided in a format to simplify your experimental planning.

## Guaranteed Yields

Guaranteed OD Yield	Appx. No. of nmoles	Appx. No. of µg	Appx. No. of Reactions*
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5	15	160	3,000
10	30	320	6,000

\* Estimate is based on 3 nmoles or 32 µg for 1 OD and 200 nM in 25 µl reaction (5.0 pmol/reaction). Estimate is based on an average sequence length of 30 bases.

The most common fluorophore and quencher combinations are listed below:

Dye	Max. EX (nm)	Max. EM (nm)	Compatible Quencher
6-FAM™	494	515	BHQ-1, DABCYL
Fluorescein	495	520	BHQ-1, DABCYL
JOE™	520	548	BHQ-1, DABCYL
TET	521	536	BHQ-1, DABCYL
HEX <sup>2</sup>	535	555	BHQ-1, DABCYL
Cy3 <sup>®</sup>	550	570	BHQ-2, DABCYL
ROX™	573	602	BHQ-2, DABCYL
Texas Red <sup>®</sup>	583	603	BHQ-2, DABCYL
Cy5 <sup>®</sup>	651	674	BHQ-3, DABCYL
Cy5.5 <sup>®</sup>	675	694	BHQ-3, DABCYL

Sigma<sup>®</sup> is a licensed supplier of a variety of dyes and quenchers and continually adds to its portfolio of new chemistries. For assistance in the design of your Molecular Beacons and assays, submit your request at [sigma.com/designmyprobe](http://sigma.com/designmyprobe).

# Scorpions™ Probes

Scorpions probes are highly sensitive, sequence-specific, bi-labeled fluorescent probe/primer hybrids designed for qPCR.

## Choose Scorpions for:

- SNP detection
- Allelic discrimination
- Pathogen detection
- Viral load quantification
- End-point genotyping
- Multiplex assay development

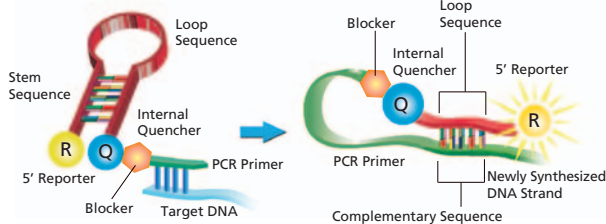
Because the probe and primer are incorporated into a single molecule, the reaction kinetics of this probe are extremely fast. The reaction leading to generation of a fluorescent signal is essentially instantaneous and occurs prior to any competing side reactions. This enables Scorpions probes to provide stronger signals, shorter reaction times, and better discrimination than other conventional bi-molecular mechanisms. It also allows for more reliable probe design.

## Benefits of Using Scorpions Probes Include:

- Increased specificity
- Fast amplicon detection
- Exceptional signal-to-noise (bi-probes typically yield stronger signal when compared to uni-probes)

### How Uni-Probe Scorpions Probes Work

The Scorpions uni-probe consists of a single-stranded bi-labeled fluorescent probe sequence held in a hairpin-loop conformation with a 5' end reporter dye and an internal quencher dye directly linked to the 5' end of a PCR primer via a blocker. The blocker prevents the polymerase from extending the PCR primer.



**1. Quenching of the fluorescence**      **2. Emission of the fluorescence**

At the beginning of the real-time qPCR reaction, the polymerase extends the PCR primer and synthesizes the complementary strand of the specific target sequence. During the next cycle, the hairpin-loop unfolds and the loop-region of the probe hybridizes intramolecularly to the newly synthesized target sequence. Now that the reporter dye is no longer in close proximity to the quencher dye, fluorescence emission may take place. The fluorescent signal is detected by the qPCR instrument and is directly proportional to the amount of target DNA.

## Add LNA to Your Probe for:

- Increased thermal stability and hybridization specificity
- Greater accuracy in gene quantitation and allelic discrimination
- Easier and more sensitive probe designs for problematic target sequences

## Product Features Include:

- Available in lengths of 30 to 60 mers (uni-probe) and 15 to 45 mers (bi-probe)
- Simple pricing – HPLC purification and base charges are included
- Stringent quality control including electrospray mass spectroscopy
- Shipped within 7–10 business days
- Shipped in an amber tube to protect dye integrity
- Custom formats available (normalization, special plates, etc.)

Sigma's probes are provided in a format to simplify your experimental planning.

## Guaranteed Yields

Guaranteed OD Yield	Appx. No. of nmoles	Appx. No. of µg	Appx. No. of Reactions*
1	2	32	400
5	10	160	2,000
10	20	320	4,000

\* Estimate is based on 2 nmoles or 32 µg for 1 OD and 200 nM in 25 µl reaction (5.0 pmol/reaction). Estimate is based on an average sequence length of 50 bases (uni-probe).

The available fluorophore and quencher combinations are listed below. Scorpions Probes include a HEG (hexathylene glycol) blocker.

## Uni-Probe

5' Fluorophore	Internal Quencher
FLC, 6-FAM™, HEX™, TET™, TAMRA™, JOE™, ROX™, Cy3®, Cy5®, Cy5.5®, Texas Red®, Rhodamine, Rhodamine Green™, Rhodamine Red™, Oregon Green® 488, Oregon Green 500, Oregon Green 514	DABCYL dT, BHQ-1, BHQ-2, BHQ-3

## Bi-Probe

5' Fluorophore	Internal Quencher
FLC, 6-FAM, HEX, TET, TAMRA, JOE, ROX, Cy3, Cy5, Cy5.5, Texas Red, Rhodamine, Rhodamine Green, Rhodamine Red, Oregon Green 488, Oregon Green 500, Oregon Green 514	TAMRA, DABCYL, BHQ-1, BHQ-2, BHQ-3

The Bi-Probe Scorpions Probe mechanism is not shown, but can be viewed at [sigma.com/probes](http://sigma.com/probes).

Sigma® is a licensed supplier of a variety of dyes and quenchers and continually adds to its portfolio of new chemistries. For assistance in the design of your Scorpions probes and assays, submit your request at [sigma.com/designmyprobe](http://sigma.com/designmyprobe).

# LightCycler® Probes

LightCycler probes are highly sensitive and sequence-specific fluorescent probes designed for use with the Roche LightCycler instruments.

## Choose LightCycler Probes for:

- SNP detection
- Allelic discrimination
- End point detection

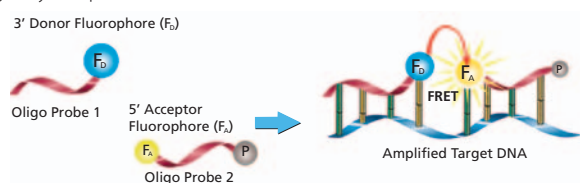
Several fluorophores are available and are suitable for multiplex analysis.

## Benefit of Using LightCycler Probes include:

- Increased specificity
- Probe preserved during the reaction
- Increased thermal stability and hybridization specificity
- Greater accuracy in gene quantitation and allelic discrimination
- Easier and more sensitive probe designs for problematic target sequences

### How LightCycler Probes Work

A LightCycler probe system consists of a pair of single-stranded fluorescent-labeled oligonucleotides. Oligo Probe 1 is labeled at the 3' end with a donor fluorophore dye and Oligo Probe 2 is labeled at its 5' end with one of two available acceptor fluorophore dyes. The free 3' hydroxyl group of Probe 2 must be blocked with a phosphate group (P) to prevent DNA polymerase extension. There should be a spacer of 1 to 5 nt to separate the two probes from each other. The structures and mechanism of a LightCycler probe are shown below.



**1. Probes in solution emit low fluorescence**

**2. Emission through fluorescence resonance energy transfer**

During the annealing step of real-time qPCR, the PCR primers and the LightCycler probes hybridize to their specific target regions bringing the donor dye into close proximity to the acceptor dye. When the donor dye is excited by light from the LightCycler instrument, energy is transferred by Fluorescence Resonance Energy Transfer (FRET) from the donor to the acceptor dye. The acceptor fluorophore's emission wavelength is detected. The increase in fluorescence signal is directly proportional to the amount of target DNA.

## Add LNA to Your Probe for:

- Increased thermal stability and hybridization specificity
- Greater accuracy in gene quantitation and allelic discrimination
- Easier and more sensitive probe designs for problematic target sequences

## Product Features Include:

- Available in lengths of 15 to 40 mers (optimal length: 20 to 30 mers)
- Simple pricing – HPLC purification and base charges are included
- Stringent quality control including electrospray mass spectroscopy
- Shipped within 7–10 business days
- Shipped in an amber tube to protect dye integrity
- Custom formats available (normalization, special plates, etc.)

Sigma's probes are provided in a format to simplify your experimental planning.

## Guaranteed Yields of LightCycler Probes

Guaranteed OD Yield	Appx. No. of nmoles	Appx. No. of µg	Appx. No. of Reactions*
0.1	0.4	3.2	80
0.25	1	8	200
1.5	6	48	1,200
15	60	480	12,000

\* Estimate is based on 4 nmoles or 32 µg for 1 OD and 200 nM in 25 µl reaction (5.0 pmol/reaction). Estimate is based on an average sequence length of 25 bases.

Please inquire for alternative quantities.

The recommended constructs for LightCycler Probe 1 and LightCycler Probe 2 are listed in the tables below:

## Labels and Modifications for LightCycler Probes

Probe 1	
3' donor fluorophore	Fluorescein
Probe 2*	
3' end	Phosphate
5' end	LightCycler Red 610, 640, 670 and 705

\*For enhanced discrimination, LNA can be incorporated into Probe 2

Sigma® is a licensed supplier of a variety of dyes and quenchers and continually adds to its portfolio of new chemistries. For assistance in the design of LightCycler probes and assays, submit your request at [sigma.com/designmyprobe](http://sigma.com/designmyprobe).

## Sigma-Aldrich® Worldwide Offices

### Argentina

Free Tel: 0810 888 7446  
Tel: (+54) 11 4556 1472  
Fax: (+54) 11 4552 1698

### Australia

Free Tel: 1800 800 097  
Free Fax: 1800 800 096  
Tel: (+61) 2 9841 0555  
Fax: (+61) 2 9841 0500

### Austria

Tel: (+43) 1 605 81 10  
Fax: (+43) 1 605 81 20

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Free Tel: 0800 14747  
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Fax: (+32) 3 899 13 11

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Free Tel: 0800 701 7425  
Tel: (+55) 11 3732 3100  
Fax: (+55) 11 5522 9895

### Canada

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Free Fax: 1800 265 3858  
Tel: (+1) 905 829 9500  
Fax: (+1) 905 829 9292

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Tel: (+56) 2 495 7395  
Fax: (+56) 2 495 7396

### China

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Tel: (+86) 21 6141 5566  
Fax: (+86) 21 6141 5567

### Czech Republic

Tel: (+420) 246 003 200  
Fax: (+420) 246 003 291

### Denmark

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Tel: (+358) 9 350 9250  
Fax: (+358) 9 350 9255

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Free Fax: 0800 031 052  
Tel: (+33) 474 82 28 88  
Fax: (+33) 474 95 68 08

### Germany

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Free Fax: 0800 64 90 000  
Tel: (+49) 89 6513 0  
Fax: (+49) 89 6513 1160

### Hungary

Ingyenes telefonszám: 06 80 355 355  
Ingyenes fax szám: 06 80 344 344  
Tel: (+36) 1 235 9063  
Fax: (+36) 1 269 6470

### India

#### Telephone

Bangalore: (+91) 80 6621 9400  
New Delhi: (+91) 11 4358 8000  
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Hyderabad: (+91) 40 4015 5488  
Kolkata: (+91) 33 4013 8003

#### Fax

Bangalore: (+91) 80 6621 9550  
New Delhi: (+91) 11 4358 8001  
Mumbai: (+91) 22 4087 2364  
Hyderabad: (+91) 40 4015 5488  
Kolkata: (+91) 33 4013 8000

### Ireland

Free Tel: 1800 200 888  
Free Fax: 1800 600 222  
Tel: (+353) 402 20370  
Fax: (+353) 402 20375

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Free Tel: 1 800 70 2222  
Tel: (+972) 8 948 4100  
Fax: (+972) 8 948 4200

### Italy

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Free Tel: 01 800 007 5300  
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Tel: (+31) 78 620 5411  
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Free Fax: 0800 937 777  
Tel: (+61) 2 9841 0555  
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### Norway

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Fax: (+47) 23 17 60 10

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Tel: (+48) 61 829 01 00  
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Free Tel: 800 202 180  
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Tel: (+351) 21 924 2555  
Fax: (+351) 21 924 2610

### Russia

Tel: (+7) 495 621 5828  
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### Singapore

Tel: (+65) 6779 1200  
Fax: (+65) 6779 1822

### Slovakia

Tel: (+421) 255 571 562  
Fax: (+421) 255 571 564

### South Africa

Free Tel: 0800 1100 75  
Free Fax: 0800 1100 79  
Tel: (+27) 11 979 1188  
Fax: (+27) 11 979 1119

### Spain

Free Tel: 900 101 376  
Free Fax: 900 102 028  
Tel: (+34) 91 661 99 77  
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### Sweden

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Free Fax: 0800 80 00 81  
Tel: (+41) 81 755 2828  
Fax: (+41) 81 755 2815

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Free Tel: 0800 717 181  
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Toll-Free Fax: 800 325 5052  
Tel: (+1) 314 771 5765  
Fax: (+1) 314 771 5757

### Vietnam

Tel: (+84) 3516 2810  
Fax: (+84) 6258 4238

### Internet

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**[sigma-aldrich.com](http://sigma-aldrich.com)**

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