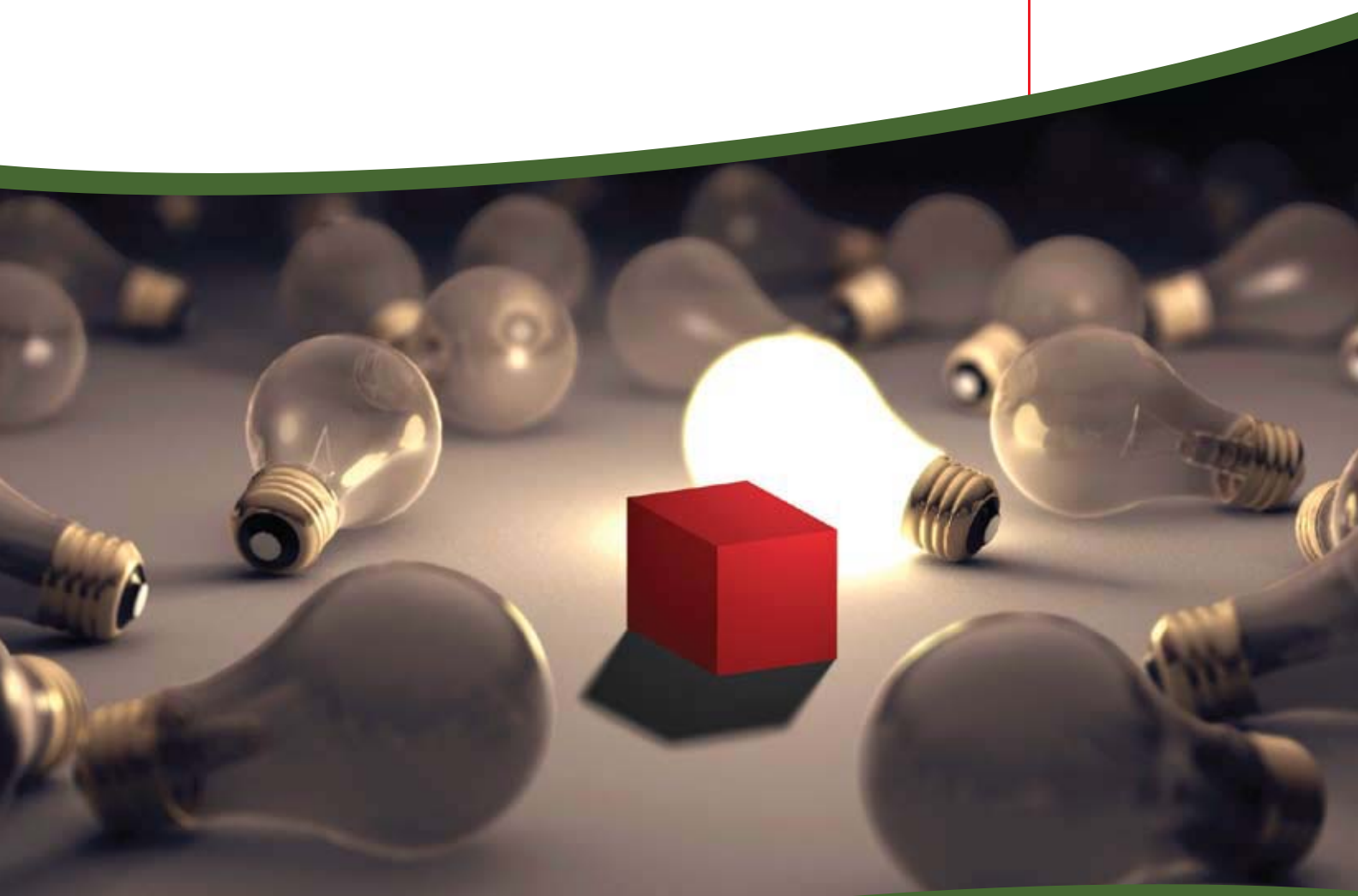


Fluorescent Probes

for Real-Time Quantitative PCR
and other Applications



Assay Design and
Reaction Optimization

Choosing the Right Probe

Dual-Labeled Fluorogenic
Probes

Molecular Beacons

Scorpions™ Probes

LightCycler® Probes

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 our customers receive only 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 training 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. Several sessions are available based on different levels of knowledge and experience. Training materials and webinars can be viewed and downloaded from the website. Check with your local sales representative or our website (sigma.com/qpcrtraining) regarding workshops.



Assay Design and Reaction Optimization

In 1993, the first experiments describing real-time PCR detection were published by Higuchi, et al. These experiments described the utility of quantifying DNA. Quantitative PCR (qPCR) is now a standard technique and is used in:

- Gene expression studies
- Validation of RNA-mediated gene suppression
- Pathogen detection
- Genotyping

Since those initial experiments, numerous detection chemistries including novel dyes, quenchers and specialty monomers have been developed to improve sensitivity and multiplexing capabilities. Sigma offers probes and reagents to support all real-time qPCR and end-point assays.

Detection Options for Real-Time Quantitative PCR

Quantitative 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 the benefits of using each are described below.

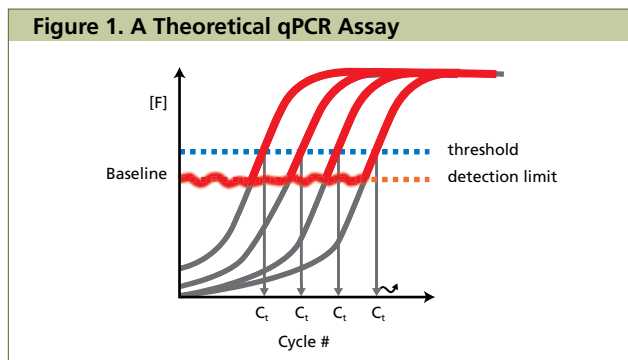


Figure 1. During a qPCR assay, the progress of the reaction is monitored by tracking the increase in fluorescence from an associated reporter molecule. The number of cycles required to reach a threshold level of detection is the C_t . The lower the C_t the higher the initial concentration of target and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

Non-specific DNA Binding Dye Detection

Incorporation of a fluorescent DNA binding dye, such as SYBR® Green I, is the simplest system of 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. Even with the use of melt curves, it is 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 this probe is sequence-specific, it will only detect the presence of a single amplicon within the reaction. There are several different types of probe structures that can be used including:

- Dual-labeled Fluorogenic 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:** Multiplex reactions can maximize the number of amplifications that can be performed when the sample amount is limited
- **Large Numbers of Samples:** Multiplexing can provide a substantial cost savings by reducing the number of reactions required

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

- **Complex Design:** Compatible probe and primer sets can be difficult to design, and the degree of difficulty increases with the number of products to be detected
- **Optimization Reaction Conditions:** All primer/probe sets in the reaction need to have similar reaction kinetics and buffer requirements. This may lead to a reduction in sensitivity for products detected in multiplex reactions versus similar singleplex amplifications

Reaction Optimization for Increased Sensitivity

Pre-designed probe and primer sets are an attractive option for researchers looking for a fast and simple solution for their quantitation needs. 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 & 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 & 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 $3C_t$ 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

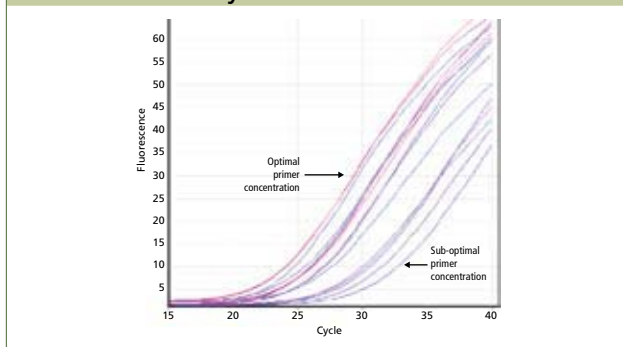


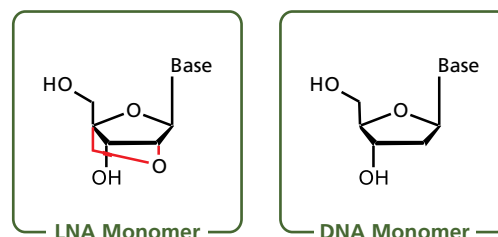
Figure 1. Primer optimization assay using Sigma® SYBR® Green 1 mastermix. Optimization of primers for the human UBC gene. All reactions contain exactly the same template but varying primer concentrations. Assay highlights how variation in primer concentration has an impact on the sensitivity of the assay. 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 $MgCl_2$) and the internal reference dye. Optimizing 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 probes to provide enhanced sensitivity and specificity for your assay. LNA is a novel type of nucleic acid analog that contains 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_m) 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_m 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_m^*	ΔT_m	$\Delta T_m/LNA$
GTGATATGC	0	29 °C	—	—
<u>G</u>TGATATGC	3	44 °C	15 °C	+5 °C
<u>GTGATATGC</u>	9	64 °C	35 °C	+3.9 °C

* T_m 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_m 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 techsrv@sial.com for support with your specific application.

Design Services & Related Products

Sigma® offers a complementary world-class design service specifically for real-time qPCR needs. Highlights of our comprehensive design service include:

- Personal consultation with our design experts
- All sequences and analysis data are provided
- Rapid design – The majority of design requests are completed within 24 hours
- Singleplex and multiplex assays for dual-labeled probes and probes containing LNA®, Molecular Beacons and Scorpions™
- Optimization and troubleshooting consultation
- Unique probes and primers crafted using the latest design algorithms
- Applications support

Highly Efficient Assays: Primers and probes on multiple sequences are designed in a single search run and screened for secondary structures and cross homology. Melting temperature (T_m) is calculated using nearest neighbor thermodynamic theory and Santa Lucia values. We can also evaluate efficiency of new probes in conjunction with pre-designed assays.

We will BLAST your sequence and design highly specific primers that avoid regions of cross-homology.

Multiplex Assays: The additional constraints of a multiplex assay are that none of the participating oligos must interact, showing significant cross-homology. During the design process, up to four compatible assays are chosen in regions specifically selected to avoid such interactions.

Allelic Discrimination Probes: Probes for detecting both wild type and mutant alleles of your target of interest can be designed. Effective designs are achieved through access to thousands of SNPs in available databases.

Template Secondary Structure: Oligonucleotide sequences are designed to avoid secondary structure in the template when designing primer and probe sets.

For assistance in the design of your probes and assays, submit your request at sigma.com/designmyprobe.

Bioinformatic Services

With the recent growth in volume and complexity of genomic and proteomic information, it is necessary for scientists to be able to capture and use this information for their own research needs. 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

- Capabilities include, but are not limited to, siRNA design, microarray oligonucleotide design, qPCR primer design, methylation specific primer design, AQUA Peptide™ design, PEPscreen®: peptide library design, gene/transcript/proteomics annotation, gene/protein function classification and microarray data analysis
- Rapid and secure completion of projects. Entire genome oligonucleotide microarray design is completed in less than 2 weeks. All sequence information is retained in a secure environment using internal BLAST databases for searches.

Contact your local technical service (techsrv@sial.com) or sales professional for bioinformatics services specific to your research.

qPCR and qRT-PCR Kits

Sigma offers a broad array of qPCR kits for all detection chemistries and all instrument platforms. Researchers using our custom qPCR probes may find value in the kits below:

Standard qPCR

Compatible Platforms: ABI instruments; Roche LightCycler® 480; Bio-Rad / MJ instruments; Stratagene instruments; Corbett Rotor-Gene™ 6000 and Rotor-Gene 3000; Eppendorf Mastercycler® ep realplex instruments

Cat. No.	Product Description	Quantity
D7440	JumpStart™ Taq ReadyMix™ for Quantitative PCR	100 reactions
		400 reactions

High-Throughput qPCR

Compatible Platforms: ABI 7300, 7700, 7900, and StepOne

Cat. No.	Product Description	Quantity
D6442	JumpStart Taq ReadyMix for High-Throughput Quantitative PCR	400 reactions
		2000 reactions

One-Step qRT-PCR

Compatible Platforms: ABI instruments; Roche LightCycler 480; Roche LightCycler 2.0 & 1.0; Bio-Rad / MJ instruments; Stratagene instruments; Corbett Rotor-Gene 6000 and Rotor-Gene 3000; Eppendorf Mastercycler ep realplex instruments

Cat. No.	Product Description	Quantity
QR0200	Quantitative RT-PCR ReadyMix	1000 reactions

We continually add to our portfolio of products and services. Please check our website at sigma.com/probes for updated information.

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

	SYBR® Green 1	Dual-Labeled Probes	Dual-Labeled LNA® Probes	Molecular Beacons*	LightCycler® Probes*	Scorpions™ Probes*
APPLICATION						
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				••		••
<i>In vitro</i> 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 5) 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 non-fluorescent 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 5.

Choosing the Right Probe

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 ¹	522	541	BHQ-1
HEX™ ²	535	555	BHQ-1, TAMRA
Cal Fluor Orange 560 ²	540	561	BHQ-1
TAMRA™	555	576	BHQ-2

¹JOE/TET alternative
²VIC® alternative

³Cy3 alternative
⁴TAMRA alternative

⁵Cy5 alternative

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

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	•	•	•	•					•			
Bio-Rad iCycler iQ	•	•	•	•	•	•	•	•	•	•	•	•
Bio-Rad Opticon® 2	•	•	•			•			•			
Bio-Rad Chromo4™	•	•	•	•	•	•	•	•	•	•		
Stratagene Mx4000®	•	•	•	•	•	•	•	•	•	•		
Stratagene Mx3000P®	•	•	•	•	•	•	•	•	•	•		
Stratagene Mx3005P®	•	•	•	•	•	•	•	•	•	•		
Roche LightCycler®	•	•									•	•
Roche LightCycler 2	•	•	•								•	•
Roche LightCycler 480	•	•	•					•			•	•
Cepheid SmartCycler®	•	•				•	•			•		
Cepheid SmartCycler II	•	•				•	•	•		•		
Corbett Rotor-Gene™ 6000	•	•		•	•	•	•	•	•	•		
Eppendorf Mastercycler® <i>realplex</i>	•	•	•	•	•				•	•		

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

Dual-Labeled Fluorogenic Probes

Dual-labeled probes are the most common probe type for real-time quantitative PCR and are often referred to as hydrolysis probes.

Choose Dual-Labeled Fluorogenic 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

Dual-labeled probes are highly sensitive, bi-labeled fluorescent probes and are designed to be sequence specific. They can be used with most real-time quantitative PCR instruments and multiplex analysis systems due to their straightforward design and the extensive range of fluorophores.

Benefits of Using Dual-Labeled Fluorogenic Probes Include:

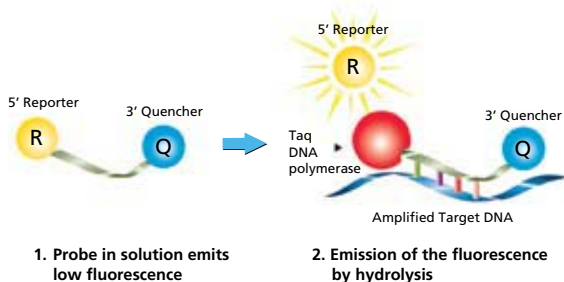
- Design simplicity for sequence specificity
- Increased sensitivity
- Extensive availability of fluorophore combinations

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 fluorogenic 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	Approx. No. of nmoles	Approx. No. of µg	Approx. 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
Cal Fluor® Gold 540 ¹	522	541	BHQ-1
HEX ²	535	555	BHQ-1, TAMRA
Cal Fluor Orange 560 ²	540	561	BHQ-1
TAMRA™	555	576	BHQ-2
Cy3®	550	570	BHQ-2
Quasar® 570 ³	548	566	BHQ-2
Cal Fluor Red 590 ⁴	565	588	BHQ-2
ROX™	573	602	BHQ-2
Texas Red®	583	603	BHQ-2
Cy5®	651	674	BHQ-3
Quasar 670 ⁵	647	667	BHQ-3
Cy5.5®	675	694	BHQ-3

¹JOE/TET alternative

³Cy3 alternative

⁵Cy5 alternative

²VIC® alternative

⁴TAMRA alternative

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 dual-labeled probes and assays, submit your request at sigma.com/designmyprobe.

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 of Using Molecular Beacons Include:

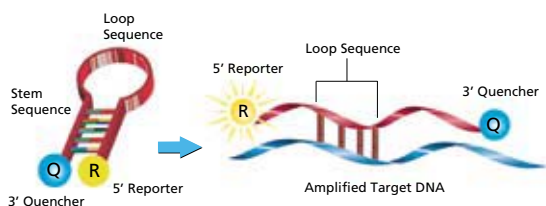
- Increased specificity
- Probe preserved during the reaction

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	Approx. No. of nmoles	Approx. No. of µg	Approx. No. of Reactions*
1	3	32	600
3	9	96	1,800
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	535	555	BHQ-1, DABCYL
Cy3®	550	570	BHQ-2, DABCYL
ROX™	573	602	BHQ-2, DABCYL
Texas Red®	583	603	BHQ-2, DABCYL
Cy5®	651	674	BHQ-3, DABCYL
Cy5.5®	675	694	BHQ-3, DABCYL

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 Molecular Beacons and assays, submit your request at sigma.com/designmyprobe.

Scorpions™ Probes

Scorpions probes are highly sensitive, sequence-specific, bi-labeled fluorescent probe/primer hybrids designed for real-time quantitative PCR.

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:

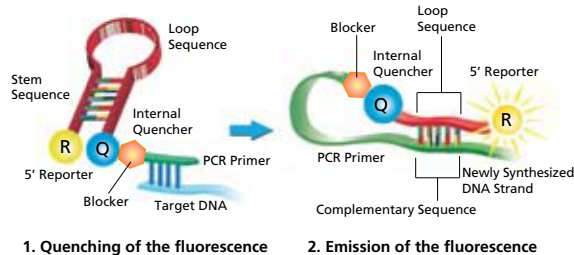
- Increased specificity
- Fast amplicon detection
- Exceptional signal-to-noise (bi-probes typically yield stronger signal when compared to uni-probes)

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 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.



At the beginning of the real-time quantitative PCR 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 real-time PCR instrument and is directly proportional to the amount of target DNA.

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	Approx. No. of nmoles	Approx. No. of µg	Approx. 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, DABYCL, BHQ-1, BHQ-2, BHQ-3

The Bi-Probe Scorpions Probe mechanism is not shown, but can be viewed at 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.

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.

Benefits of Using LightCycler Probes Include:

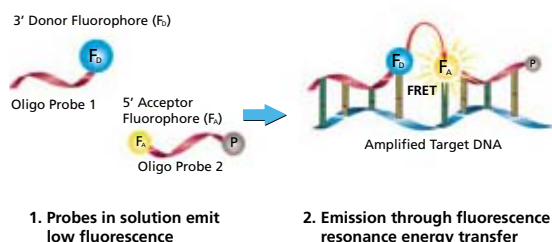
- Increased specificity
- Probe preserved during the reaction
- Increased thermal stability and hybridization specificity
- Greater accuracy in gene quantitation and allelic discrimination
- Easier and more sensitive probe designs for problematic target sequences

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 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.



During the annealing step of real-time quantitative PCR, 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.

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	Approx. No. of nmoles	Approx. No. of µg	Approx. 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.

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