qPCR probes and custom oligonucleotides

Discover the future

fisherbiotec AUSTRALIA

BIOSEARCH™ TECHNOLOGIES

GENOMIC ANALYSIS BY LGC

Integrated tools. Accelerated science.

Black Hole Quencher probes and assays

Black Hole Quencher (BHQ)-labeled probes are ideal for your qualitative and quantitative PCR (qPCR) experiments such as single – and multiplex gene expression, copy number variation (CNV), SNP genotyping, and presence/absence due to their superior quenching, enhanced specificity, and optimal signal-to-noise ratios. These attributes yield highly accurate and reproducible data across all your experiments.

BHQ probes are designed using our highly efficient Black Hole Quencher dyes with broad absorption spectra that are then optimally paired with a single fluorescent dye from our wide range of proprietary or commonly available fluorophores.

Dual labeled BHQ

5’ Fluorophore 3’ BHQ

20 - 30 complementary bases to the target sequence

STANDARDISE YOUR ASSAY Dual-labeled BHQ probes are traditional probes that contain a 5’ fluorophore and a 3’ Black Hole Quencher dye covalently bound to an oligo. Choose from a broad spectrum of proprietary and common market fluorophores and their corresponding Black Hole Quencher dye to obtain high quality qPCR data.

BHQplus®

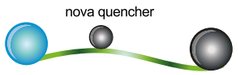
5’ Fluorophore 3’ BHQ

15 - 25 complementary bases to the target sequence

STRENGTHEN YOUR ASSAY with shorter probes specifically designed to enhance specificity and improve mismatch discrimination through the use of modified C and T nucleotides. Reduce assay cost and improve data quality by redesigning MGB probe assays with BHQplus technology.

BHQnova™

5’ Fluorophore 3’ BHQ



>25 complementary bases to the target sequence

POWER YOUR ASSAY when longer probes are needed by substantially reducing background interference and increasing the signal-to-noise ratio with double-quenched BHQ probes utilising the power of both an internal nova quencher and a 3’ BHQ quencher.

ValuMix Assays

SIMPLIFY YOUR ASSAY We custom build your qPCR assay at a desired primer:probe ratio to enable the greatest experimental flexibility all the while saving you valuable time and effort. Available for Dual-labeled BHQ and BHQplus probe formats.

BHQ Probe format selection guide

This table is intended to guide you through the BHQ probe selection process for your experiment.

Specifications	BHQ Probes	BHQplus Probes	BHQnova Probes
Differentiator	Dual-labeled probes using Black Hole Quencher dyes for probes that are 20-30 bases	Enhanced specificity for mismatch discrimination for probes that are 15-25 bases	Dual fortified quenching and optimal S:N ratio for probes that are >25 bases
Applications			
SNP genotyping Associations, validation, screening, InDels	—	●●	—
Gene expression Relative quantification	●●	●●	●●
Absolute quantification Viral load, RNA copy number	●●	●●	●●
Copy number variation (CNV) Zygosity	●	●●	●●
Presence \ Absence Mutation detection, pathogen detection	●	●●	●
Plex (assays per reaction well)			
Singleplex	✓	✓	✓
Duplex	✓	✓	✓
Multiplex	✓	✓	✓
Fluorophore choices	15	6	5
Target sequence content			
35-65% GC	✓	✓	✓
<35% (AT-rich)	—	✓	✓
>65% (GC-rich)	✓	✓	✓
Probe length in bases	20-30	15-25	>25

● suitable ●● recommended

Fluorophore and BHQ dye selection chart

This chart is intended to guide you through the dye selection process for your oligonucleotide. A fluorophore and quencher combination may be selected for applications such as probe-based qPCR and SNP genotyping.

Fluorophore	Alternate dyes	5' Dye Excitation	5' Dye Emission	Recommended quencher
● **Biosearch Blue™**		352	447	BHQ-1
FAM		495	520	BHQ-1
TET		521	536	BHQ-1
● **CAL Fluor® Gold 540**	*VIC/TET/JOE*	522	544	BHQ-1
JOE		529	555	BHQ-1
HEX		535	556	BHQ-1
● **CAL Fluor Orange 560**	*VIC/HEX/JOE*	538	559	BHQ-1
● **Quasar® 570**	*CY3*	548	566	BHQ-2
TAMRA		557	583	BHQ-2
● **CAL Fluor Red 590**	*TAMRA*	569	591	BHQ-2
ROX		586	610	BHQ-2
● **CAL Fluor Red 610**	*TEXAS RED/ROX/ ALEXA FLUOR® 594*	590	610	BHQ-2
● **CAL Fluor Red 635**	*LC RED® 640*	618	637	BHQ-2
● **Pulsar® 650**		460	650	BHQ-2
● **Quasar 670**	*CY5*	647	670	BHQ-2*, BHQ-3
● **Quasar 705**	*CY5.5*	690	705	BHQ-2*, BHQ-3

● Fluorophores proprietary to LGC, Biosearch Technologies

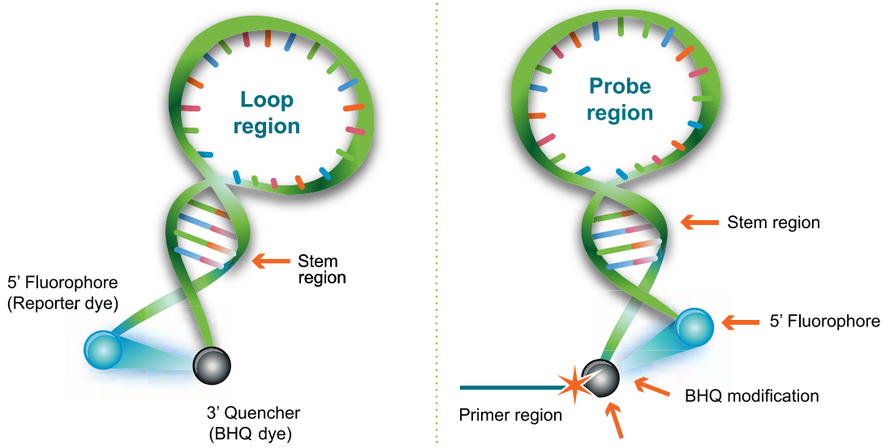
** BHQ-2 dye is recommended for Quasar 670 and Quasar 705 fluorophores due to static quenching*

Molecular Beacons and Scorpions Primers

These qPCR primers and probes are available as alternatives to MGB probes when greater discrimination and faster reaction times are desired.

Can be ordered with a variety of fluorophores. Choose from up to 15 different fluorophores and corresponding Black Hole Quencher dye.

Contain BHQ dyes for improved signal-to-noise ratio compared to other commercially available versions.



Molecular Beacons
A dual-labeled probe that relies on a hairpin conformation to heighten assay specificity. This property allows Beacons to discriminate mismatches as specific as a single nucleotide polymorphism (SNP). Beacons generate fluorescence through hybridisation and under non-hydrolytic conditions allowing post application PCR melt curve analyses.

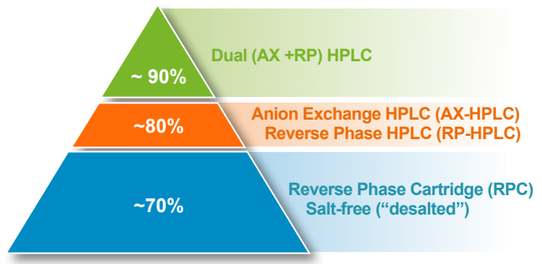
Scorpions® Primers
A dual-labeled probe that combines a hairpin structure and a PCR primer element in a single oligo, allowing for target detection through a unimolecular mechanism. Mismatched hybrids are less stable than the reformed stem region. Therefore, Scorpions Primers only produce signal when the probe region hybridises to a perfect-match target sequence within the primer extension product.

Oligo purification

We can also accommodate a minimum purity (e.g. 95%) that is elevated above our typical purity range for custom projects.

We make a number of purification options available depending on your needs. Choosing the appropriate purification option will ensure that your oligos are suitable for their application. Choosing the right purification option will also ensure you have the best value in your oligos because increasing stringency of purification will by necessity diminish the final yield.

- Salt-free ("desalted") oligos are suitable for applications such as microarrays, sequencing or qPCR primers.
- Reverse Phase Cartridge (RPC) purification is suitable to enrich the full-length product for oligos which contain 50 bases or less.
- For oligonucleotides containing modifications, such as fluorescent probes, we recommend more stringent purification such as single HPLC or dual HPLC.
- Final QC specifications for custom-synthesised oligos are such that the observed mass must be within ±0.1% difference from the theoretical mass, as determined by electrospray mass spectrometry.



RealTimeDesign qPCR assay design software

RealTimeDesign™ is our web-based qPCR assay design software provided FREE of charge for all users. This design software is accessible from any internet browser and contains express and custom features to suit both novice and expert users in qPCR. Take the guess work out of probe and primer design - let RealTimeDesign select the optimal sequences for you! Visit our qPCR resources website at www.biosearchtech.com/support/tools to learn more.

qPCR design collaborations

If you have a large project that requires great attention to detail by experienced assay designers, please contact our customer support group to discuss feasibility of assay design. We will work with your team to understand the requirements for your application.

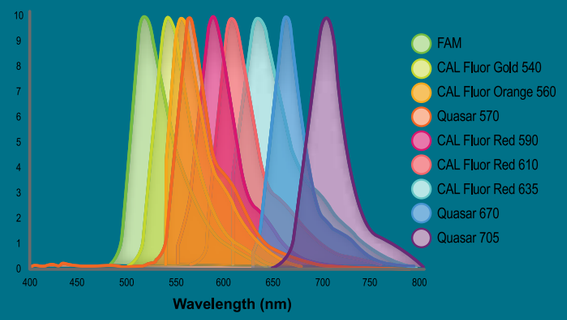
We have a team of highly trained molecular biologists and oligonucleotide chemists to assist with your industrial projects. Our team can provide guidance for the selection of the optimal modified oligos for your particular instrumentation and applications.

We can also assist with your pre-existing probe designs, to evaluate *in silico* for compatibility with our chemistries. Let us accelerate the development of your assays from inception to commercialisation. We have decades of experience worked with a broad range of customers and fields including molecular diagnostics, pharmaceuticals, government agencies, and agricultural sciences.

Calibration and reference dyes

Instrument calibration dyes

Improve signal deconvolution in real-time qPCR thermal cyclers that require spectral calibration. They enable the instrument to store the fluorescent profile of each dye and control for crosstalk.



Passive reference dye

SuperROX® is a specially formulated version of the ROX (carboxy-X-rhodamine) fluorophore that provides a more uniform passive reference signal compared to standard ROX dyes. This dye is commonly used as passive reference to normalise signal variations not related to the PCR amplification. Irregularities controlled with a ROX passive reference include inconsistency in pipetting, evaporation of solution, instability of baseline fluorescence, and laser or light source anomalies, which all produce well-to-well variation.

Custom oligonucleotides

As the original custom oligo manufacturing company, our automated, vertically-integrated high-throughput facility synthesises superior performing oligos in certified environments depending on your project requirements. Whether you need research-grade, product design verification and validation, or GMP-compliant oligos, we manufacture to the highest standards in a cost-effective manner and with rapid turnaround times to enable your timely project completion.

	Research-grade oligos	PilotDx® oligos	GMP oligos
Key differentiator	Standard quality synthesis provides reliable and cost-effective oligonucleotides for routine studies	Specifically synthesised for product design verification and validation (test methods, PQ, stability studies)	Specifically synthesised for IVD and molecular diagnostic applications
Delivery amounts	10s to 100s nmol	100s to 1000s nmol	>10 µmol
Purification methods*	Salt-free (desalted), Reverse Phase Cartridge (RPC), Reverse Phase HPLC (RP-HPLC), Anion Exchange HPLC (AX-HPLC), Dual (AX + RP) HPLC		
Compound modifications** (5' internal, 3')	1000s	1000s	100s
Delivery formats	Lyophilised and in-solution; single tube and strips, 96- and 384-well plates, custom labeling/kitting		
Manufacturing facility	ISO 9001:2015 certified facility	ISO 13485:2016 certified facility	ISO 13485:2016 and GMP compliant facility, per 21 CFR Part 820
Quality control	Standard mass spectrometry	Standard mass spectrometry and analytical HPLC depending on requirements	

* *We make a number of purification options available depending on the needs of you particular molecular method. Choosing the appropriate purification option will ensure that your oligos are suitable for your application. Choosing just the right purification option will also ensure you have the best value in your oligos because increasing stringency of purification will by necessity diminish the final yield. Final purity is determined by sequence, oligo length, selected modifications, and oligo type.*

** *Chemical synthesis capabilities to make unique linkers, fluorophores, and other compounds as well as the ability to couple commercially available compounds. Typical offerings include, but not limited to: amines, biotin, Black Hole Quencher dyes, fluorophores, methylene blue, non-standard bases and linkages, phosphate, spacers, thiol.*

Pre-clinical and therapeutics

Larger scale oligonucleotide synthesis has been growing rapidly in demand for therapeutic and pre-clinical applications. The surge in the need for the development of oligos as aptamers, antisense, and RNAi therapeutics, and the requirement for highly modified oligos falls into perfect harmony with our manufacturing expertise.

Our ‘Quality by Design’ approach offers the ability to optimise production from milligram to hundreds of grams according to your custom project requirements. We are committed to delivering solutions for difficult problems in a timely fashion without compromising quality, price, reliability, and reproducibility.

OEM and kit manufacturing

We serve a number of assay kit companies globally. We can design and assemble part, or most of your kits that are tailored to your specifications, and provided to you with your private label.

Equipped with state of the art instruments and ample production capacity, we are the preferred OEM manufacturer for many reputable institutions in biotech, pharmaceutical, public health, and AgBio sectors.

Our proprietary DNA synthesis instruments produce oligos more rapidly and cost-effectively than any commercially available system. The automated methodologies that we’ve developed give us the ability to synthesise large volumes of high value custom oligos and assay components as well as mixing different oligos into an exacting concentration specification. We also maintain excellent cost control by manufacturing our own amidites, dyes, and synthesis columns and pass these efficiencies onto our customers and OEM partners.

Products and services

Services

GMP and Commercial Services, pre-clinical and therapeutics, OEM and kit manufacturing

- IVD and Molecular Dx oligos
- PilotDx oligos
- GMP-compliant oligos
- Analyte Specific Reagents
- RealTimeDesign qPCR Assay Design Software (online)

Products

Black Hole Quencher Probes

- Dual-Labeled BHQ Probes
- BHQplus Probes
- BHQnova Probes

ValuMix Assays

- ValuMix for Gene Expression and qPCR
- ValuMix for SNP Genotyping

Molecular Beacons

Scorpions primers

Custom oligonucleotides

- Research-grade
- PilotDx
- GMP

Calibration dyes

SuperROX

Integrated tools.
Accelerated science.

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