Real-time qPCR Assay Design Software

www.qpcrdesign.com

REALTIMEDESIGN

Your Blueprint For Success

Informational Guide

BIOSEARCH® TECHNOLOGIES
Advancing Nucleic Acid Technology℠

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REALTIMEDESIGN

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Real-time qPCR Assay Design

Performed at the easy click of a button

RealTimeDesign™ software from Biosearch Technologies, Inc. is a free, easy to use, yet powerful assay design application for real-time quantitative PCR (real-time qPCR). Good probe and primer design is at the heart of any successful real-time qPCR assay, and being equipped with RealTimeDesign software, you're only a few clicks away from:

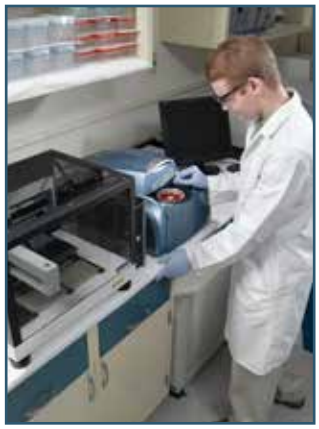
- Designing assays for SNP genotyping (2 probes and 2 primers) and for DNA/RNA quantification (1 probe and 2 primers).
- Designing Dual Labeled BHQ® and BHQ*plus*® probes and matching optimal primer pairs.
- Designing anywhere from single assays to high-throughput batches (design up to 10 different assays simultaneously)
- Saving a list of your custom designs in your account "Design Run History" (up to 100 designs)
- Choosing from Biosearch's wide selection of dyes including our very own BHQ (Black Hole Quencher®) dye
- Ordering your designed probes and primers directly through the Biosearch website: www.biosearchtech.com

"Good probe and primer design is at the heart of any successful real-time qPCR assay..."

REALTIMEDESIGN®

Real-time qPCR Assay Design Software, for both the Novice and Seasoned Expert

Upon designing your probes and primers, RealTimeDesign software performs sophisticated algorithms to select an optimal sequence from your target of interest. What was once considered as an arduous, time-consuming task usually performed through a series of trial and error, qPCR researchers can now efficiently design and order assays during one swift online session. For more advanced users, RealTimeDesign software offers a Custom Mode allowing a fine control over a number of standard and customizable design parameters.



Many qPCR experts put RealTimeDesign software to the test and they agree that this is one of the most useful and easy-to-use online design program available at no cost to the user. Determined to remain at the forefront of qPCR assay design, Biosearch constantly updates RealTimeDesign software with innovative features and increased functionality to provide our customers a step up in assay development.

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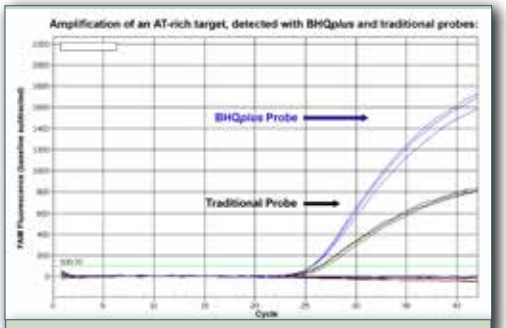
DNA Quantification & Gene Expression Analysis

RealTimeDesign Software Ensures Robust Amplification and Detection

With the "Quantitative PCR" module of RealTimeDesign software, probes can be designed for sensitive measurements of copy number.

When designing probes to quantify DNA for copy number studies (gDNA) or gene expression analysis (cDNA), traditional dual-labeled probes quenched with the BHQ dye are more than sufficient. However, many difficult target sites are AT-rich, and so fortified BHQ*plus* probes are required to maintain an elevated melting temperature.



Replicate PCR Reactions, all amplified using the same primer set, but detected with either a traditional FAM-BHQ1 probe (black) or a BHQ*plus* probe (blue). The amplification product is a region within the AT-rich hemolysin III gene of Bacillus anthracis.

BHQ*plus* probes provide more flexibility in design of assays for DNA quantification: these shorter probes can be accommodated into a greater variety of targets and with a wider range in base composition.

With the "Quantitative PCR" module of RealTimeDesign software, probes can be designed for sensitive measurements of copy number.

- Choose BHQ or BHQ*plus* probes partnered with the ideal fluorophore for the instrument optics in use
- Use NCBI gene symbols, RefSeq IDs, or GenBank accession numbers, or use your own optimized sequence
- Pick the optimal assays designed by RealTimeDesign software or choose alternate primers and probes using Custom Mode
- Order directly through the Biosearch website

REALTIMEDESIGN

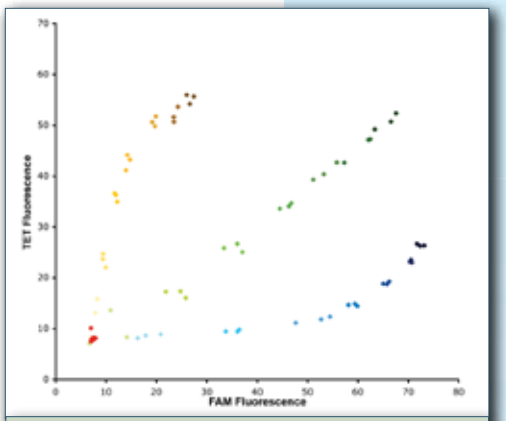
Probe-Based SNP Genotyping

RealTimeDesign for Allelic Discrimination

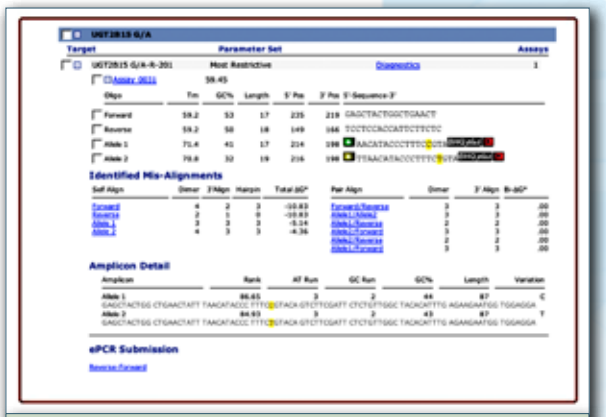
Because the BHQplus technology stabilizes probe hybridization to the template, it allows for the design of shorter probes that offer increased resistance against hybridizing to a mismatch. This makes BHQplus probes ideal for genotyping applications such as screening, linkage associations, or fine-mapping studies.

Designing optimal probes is simple with RealTimeDesign's "SNP Genotyping" module (found under qPCR - BHQ Probe > Application > SNP Genotyping).

- » Use NCBI SNP Reference ID (rs#), gene symbols or RefSeq IDs derived from a SNP database such as dbSNP as a starting point for design
- » Choose the fluorophore that matches your instrument optics.
- » Review the optimal assay proposed by RealTimeDesign software
- » Order directly through the Biosearch website



Scatterplot of a SNP assay targeting alleles of UGT2B15 using a FAM BHQ*plus* probe and a TET BHQ*plus* probe.



Screenshot from RealTimeDesign software, displaying the oligo sequences of the genotyping assay targeting UGT2B15, a gene involved in the metabolism of certain drugs such as tamoxifon.

BHQ*plus* Probes - Fortified Probes Achieve Powerful Discrimination

- *A duplex stabilizing technology is used to elevate the oligo Tm over traditional fluorescence-quenched probes*
- *Permits a shorter probe sequence allowing flexible and discriminating assay design.*
- *Relies on the proven performance of the Black Hole Quencher (BHQ) dyes, the industry standard in dark quenchers.*

Save Time and Money, Let RealTimeDesign Software do the Prep Work!

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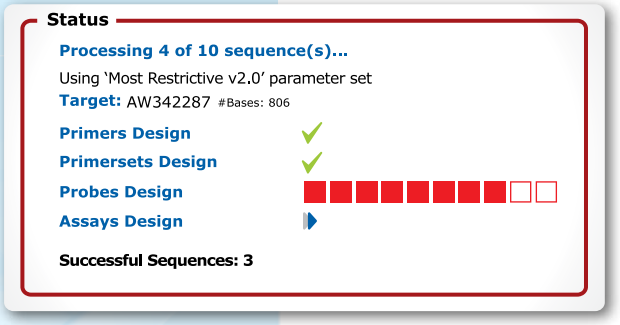
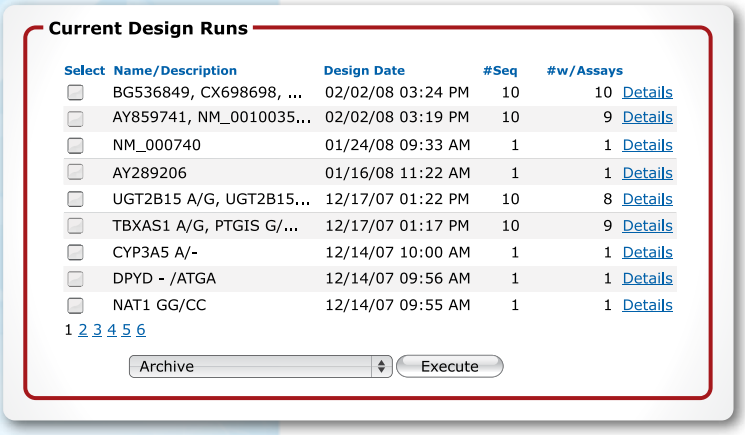
Advanced Features of RealTimeDesign Software

RTD™ software offers sophisticated features for the qPCR expert. By providing powerful control over oligo sequences, RTD software is your genome-grade software engine.



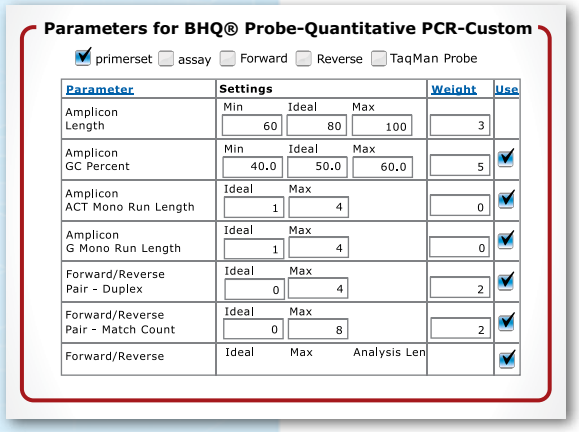
High-Throughput

10 different assays can be designed simultaneously. Simply enter all of your targets in FASTA format, or as comma-separated accession numbers.



Design Archives

Rely upon the Design Run History to store your newly proposed sequences. Up to 100 different assays can be recorded for later review, in a unique database for every user.



Customizing Parameters

Adjustable parameters provide complete control over design. Values can be fine-tuned and then made default for your future designs.

Method

- Click "Manage Parameter Sets" from the Main Menu;
- Clone one of the current sets and rename it;
- Click on its name to adjust the values within;
- Once done, click "Save" and return to Manage Parameters;
- Click the "Use" checkbox to make your custom set the default.

REALTIMEDESIGN®

CUSTOM DNA SYNTHESIS PRODUCTS & PRICING

This is a sample list of our real-time qPCR oligonucleotides. Other synthesis scales and dye selections are also available. For Biosearch's complete panel of dye-quencher combinations, please visit:
www.biosearchtech.com or call **1.800.GENOME.1**

Dual Labeled BHQ® Probes

5′ Label	5′ Ex/Em (nm)	3′ Label	Catalog #	Synthesis Scale
FAM ValuProbe™ (single RP-HPLC)	495 / 520	BHQ-1	DLO-RFB-5	50 nmol
FAM (dual HPLC)	495 / 520	BHQ-1	DLO-FB1-5 DLO-FB1-1	100 nmol 1 µmol
FAM	495 / 520	TAMRA	DLO-FT-5 DLO-FT-1	100 nmol 1 µmol
TET	521 / 536	BHQ-1	DLO-TEB1-5 DLO-TEB1-1	50 nmol 1 µmol
TET	521 / 536	TAMRA	DLO-TET-5 DLO-TET-1	50 nmol 1 µmol
CAL Fluor® Gold 540 (replaces TET)	522 / 544	BHQ-1	DLO-CGB1-5 DLO-CGB1-1	50 nmol 1 µmol
HEX	535 / 556	BHQ-1	DLO-HB1-5 DLO-HB1-1	50 nmol 1 µmol
CAL Fluor Orange 560 (replaces VIC/HEX/JOE)	538 / 559	BHQ-1	DLO-COB1-5 DLO-COB1-1	50 nmol 1 µmol
TAMRA	557 / 583	BHQ-2	DLO-TB2-5 DLO-TB2-1	50 nmol 1 µmol
CAL Fluor Red 610 (replaces Texas Red® dye)	590 / 610	BHQ-2	DLO-CAB2-5 DLO-CAB2-1	50 nmol 1 µmol
Quasar® 670 (replaces Cy5™ dye)	647 / 667	BHQ-2	DLO-Q6B2-5 DLO-Q6B2-1	50 nmol 1 µmol
Quasar 705	690 / 705	BHQ-2	DLO-Q7B2-5 DLO-Q7B2-1	50 nmol 1 µmol

BHQ*plus*® Probes

5′ Label	5′ Ex/Em (nm)	3′ Label	Catalog #	Synthesis Scale
FAM	495 / 520	BHQ-1 *plus*	DLO-FBP-5 DLO-FBP-1	50 nmol 1 µmol
TET	521 / 536	BHQ-1 *plus*	DLO-TBP-5 DLO-TBP-1	50 nmol 1 µmol
CAL Fluor Gold 540	522 / 544	BHQ-1 *plus*	DLO-GBP-5 DLO-GBP-1	50 nmol 1 µmol
CAL Fluor Orange 560	538 / 559	BHQ-1 *plus*	DLO-CBP-5 DLO-CBP-1	50 nmol 1 µmol
CAL Fluor Red 610	590 / 610	BHQ-2 *plus*	DLO-RBP-5 DLO-RBP-1	50 nmol 1 µmol
Quasar 670	647 / 667	BHQ-2 *plus*	DLO-QBP-5 DLO-QBP-1	50 nmol 1 µmol

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