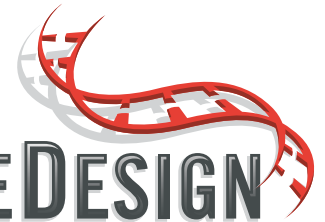




Real-time qPCR Assay Design Software
www.qpcrdesign.com



REALTIMEDESIGN

Your Blueprint For Success

Informational Guide



**BIOSEARCH
TECHNOLOGIES**

Advancing Nucleic Acid TechnologySM

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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, Amplifluor®, and BHQ_{plus}™ probes and primers 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 Black Hole Quencher® (BHQ®) 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...”



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.



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

DNA QUANTIFICATION & GENE EXPRESSION ANALYSIS

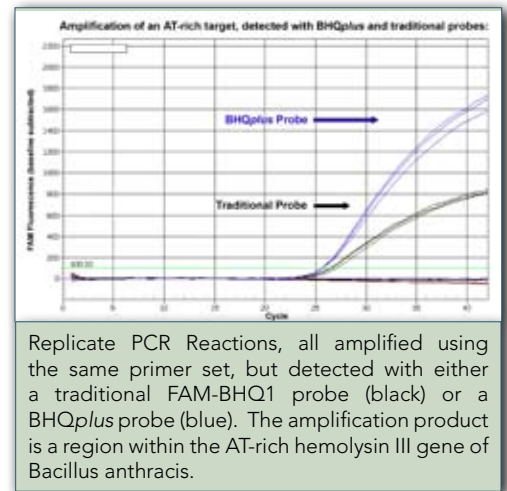
RealTimeDesign Software Ensures Robust Amplification and Detection

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.

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





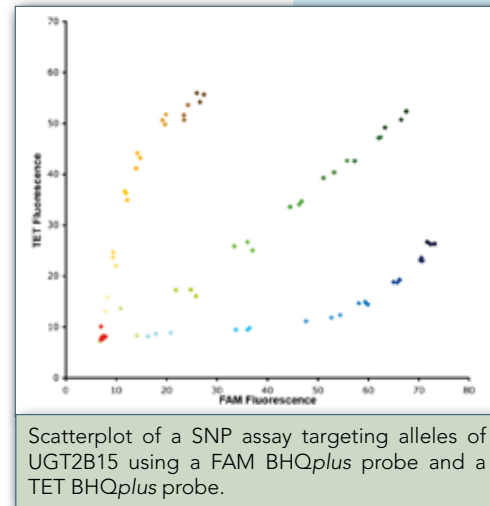
Probe-Based SNP Genotyping

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 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 BHQplus probe and a TET BHQplus probe.

Target	Parameter Set	Assay				
UGT2B15 (U4-R-20)	Most Restrictive	Diagnostic				
Cl Amp: 201	58-65					
Dirn	Tm	GC%	Length	5' Prm	3' Prm	5'-Sequence-3'
Forward	58.2	53	17	216	218	GAGTACTGGGCGTAAC
Reverse	58.2	58	18	149	148	TCTTCGACCACTCTTTC
Allel 1	71.4	41	17	214	198	AACATACACCTCTTCTT
Allel 2	70.8	33	19	218	198	TAACATACACCTCTTCTT

Identified Mismatch Alignments						
Seq Align	Order	3' Align	Margin	Total ΔG°'	Per Align	3' Align ΔG°'
Forward	4	2	3	-10.81	Forward/Reverse	3
Reverse	2	1	0	-10.81	Allel 1/Allel 2	2
Allel 1	3	3	3	-11.44	Allel 1/Reverse	2
Allel 2	4	3	3	-4.36	Allel 1/Forward	2
					Allel 2/Reverse	2
					Allel 2/Forward	2

Amplicon Detail						
Amplicon	Start	End	GC Run	GC%	Length	Variation
Allel 1	86-85	3	0	44	87	C
GAGTACTGGGCTGAACACTTTCAGTACAGCTTTCCTGCTGATTTCTCTCTTCTTCTTCAGTACTTTCAGAGATATGCTGCGAGCA						
Allel 2	84-83	3	0	43	87	T
GAGTACTGGGCTGAACACTTTCAGTACAGCTTTCCTGCTGATTTCTCTCTTCTTCTTCAGTACTTTCAGAGATATGCTGCGAGCA						

ePCR Submission
Reverse/Forward

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

BHQplus™ 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!



Your Blueprint for Success

ADVANCED FEATURES OF REALTImeDESIGN

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

Status

Processing 4 of 10 sequence(s)...

Using 'Most Restrictive v2.0' parameter set

Target: AW342287 #Bases: 806

Primers Design

Primersets Design

Probes Design

Assays Design

Successful Sequences: 3

High-Throughput

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

Current Design Runs

Select	Name/Description	Design Date	#Seq	#w/ Assays	
<input type="checkbox"/>	BG536849, CX698698, ...	02/02/08 03:24 PM	10	10	Details
<input type="checkbox"/>	AY859741, NM_0010035...	02/02/08 03:19 PM	10	9	Details
<input type="checkbox"/>	NM_000740	01/24/08 09:33 AM	1	1	Details
<input type="checkbox"/>	AY289206	01/16/08 11:22 AM	1	1	Details
<input type="checkbox"/>	UGT2B15 A/G, UGT2B15...	12/17/07 01:22 PM	10	8	Details
<input type="checkbox"/>	TBXAS1 A/G, PTCIS G/...	12/17/07 01:17 PM	10	9	Details
<input type="checkbox"/>	CYP3A5 A/-	12/14/07 10:00 AM	1	1	Details
<input type="checkbox"/>	DPYD - /ATGA	12/14/07 09:56 AM	1	1	Details
<input type="checkbox"/>	NAT1 GG/CC	12/14/07 09:55 AM	1	1	Details

1 2 3 4 5 6

Archive

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.

Parameters for BHQ® Probe-Quantitative PCR-Custom

primerset assay Forward Reverse TaqMan Probe

Parameter	Settings	Weight	Use
Amplicon Length	Min: 60, Ideal: 80, Max: 100	3	<input type="checkbox"/>
Amplicon GC Percent	Min: 40.0, Ideal: 50.0, Max: 60.0	5	<input checked="" type="checkbox"/>
Amplicon ACT Mono Run Length	Ideal: 1, Max: 4	0	<input checked="" type="checkbox"/>
Amplicon G Mono Run Length	Ideal: 1, Max: 4	0	<input checked="" type="checkbox"/>
Forward/Reverse Pair - Duplex	Ideal: 0, Max: 4	2	<input checked="" type="checkbox"/>
Forward/Reverse Pair - Match Count	Ideal: 0, Max: 8	2	<input checked="" type="checkbox"/>
Forward/Reverse	Ideal: Max, Analysis Len		<input checked="" type="checkbox"/>

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.



CUSTOM DNA SYNTHESIS PRODUCTS & PRICING

Black Hole Scorpions® Probes and Primer					
5' Label	5' Ex/Em (nm)	3' Label	Catalog #	Synthesis Scale	Price
FAM	495 / 520	Internal BHQ-1	SCP-FB1-2	200 nmol	\$450
			SCP-FB1-1	1 µmol	\$650
CAL Fluor Gold 540	522 / 544	Internal BHQ-1	SCP-CGB1-2	200 nmol	\$500
			SCP-CGB1-1	1 µmol	\$700
CAL Fluor Orange 560	538 / 559	Internal BHQ-1	SCP-COB1-2	200 nmol	\$500
			SCP-COB1-1	1 µmol	\$700
TAMRA	557 / 583	Internal BHQ-2	SCP-TB2-2	200 nmol	\$550
			SCP-TB2-1	1 µmol	\$700
CAL Fluor Red 610	590 / 610	Internal BHQ-2	SCP-CAB2-2	200 nmol	\$500
			SCP-CAB2-1	1 µmol	\$700
Quasar 670	647 / 667	Internal BHQ-2	SCP-Q6B2-2	200 nmol	\$600
			SCP-Q6B2-1	1 µmol	\$750

Molecular Beacons					
5' Label	5' Ex/Em (nm)	3' Label	Catalog #	Synthesis Scale	Price
FAM	495 / 520	BHQ-1	MBO-FB1-5	50 nmol	\$375
			MBO-FB1-1	1 µmol	\$590
FAM	495 / 520	DABCYL	MBO-FD-5	50 nmol	\$375
			MBO-FD-1	1 µmol	\$590
TET	521 / 536	BHQ-1	MBO-TEB1-5	50 nmol	\$400
			MBO-TEB1-1	1 µmol	\$630
CAL Fluor Gold 540	522 / 544	BHQ-1	MBO-CGB1-5	50 nmol	\$390
			MBO-CGB1-1	1 µmol	\$615
HEX	535 / 556	BHQ-1	MBO-HB1-5	50 nmol	\$410
			MBO-HB1-1	1 µmol	\$645
CAL Fluor Orange 560	538 / 559	BHQ-1	MBO-COB1-5	50 nmol	\$390
			MBO-COB1-1	1 µmol	\$615
CAL Fluor Red 610	590 / 610	BHQ-2	MBO-CAB2-5	50 nmol	\$415
			MBO-CAB2-1	1 µmol	\$650
Quasar 670	647 / 667	BHQ-2	MBO-Q6B2-5	50 nmol	\$415
			MBO-Q6B2-1	1 µmol	\$650

BHQplus™ Probes					
5' Label	5' Ex/Em (nm)	3' Label	Catalog #	Synthesis Scale	Price
FAM	495 / 520	BHQ-1 plus	DLO-FBP-5	50 nmol	\$255
			DLO-FBP-1	1 µmol	\$600
TET	521 / 536	BHQ-1 plus	DLO-TBP-5	50 nmol	\$255
			DLO-TBP-1	1 µmol	\$600
CAL Fluor Orange 560	538 / 559	BHQ-1 plus	DLO-CBP-5	50 nmol	\$255
			DLO-CBP-1	1 µmol	\$600
CAL Fluor Red 610	590 / 610	BHQ-1 plus	DLO-RBP-5	50 nmol	\$255
			DLO-RBP-1	1 µmol	\$600
Quasar 670	647 / 667	BHQ-1 plus	DLO-QBP-5	50 nmol	\$255
			DLO-QBP-1	1 µmol	\$600

Dual Labeled BHQ® Probes					
5' Label	5' Ex/Em (nm)	3' Label	Catalog #	Synthesis Scale	Price
FAM ValuProbe™ (single RP-HPLC)	495 / 520	BHQ-1	DLO-RFB-5	50 nmol	\$95
FAM (dual HPLC)	495 / 520	BHQ-1	DLO-FB1-5	100 nmol	\$150
			DLO-FB1-1	1 µmol	\$365
FAM	495 / 520	TAMRA	DLO-FT-5	100 nmol	\$150
			DLO-FT-1	1 µmol	\$365
TET	521 / 536	BHQ-1	DLO-TEB1-5	50 nmol	\$200
			DLO-TEB1-1	1 µmol	\$420
TET	521 / 536	TAMRA	DLO-TET-5	50 nmol	\$200
			DLO-TET-1	1 µmol	\$420
CAL Fluor Gold 540 (replaces TET)	522 / 544	BHQ-1	DLO-CGB1-5	50 nmol	\$195
			DLO-CGB1-1	1 µmol	\$400
HEX	535 / 556	BHQ-1	DLO-HB1-5	50 nmol	\$205
			DLO-HB1-1	1 µmol	\$450
CAL Fluor Orange 560 (replaces VIC/HEX/JOE)	538 / 559	BHQ-1	DLO-COB1-5	50 nmol	\$195
			DLO-COB1-1	1 µmol	\$400
TAMRA	557 / 583	BHQ-2	DLO-TB2-5	50 nmol	\$200
			DLO-TB2-1	1 µmol	\$400
CAL Fluor Red 610 (replaces Texas Red® dye)	590 / 610	BHQ-2	DLO-CAB2-5	50 nmol	\$215
			DLO-CAB2-1	1 µmol	\$475
Quasar 670 (replaces Cy5™ dye)	647 / 667	BHQ-2	DLO-Q6B2-5	50 nmol	\$230
			DLO-Q6B2-1	1 µmol	\$475
Quasar 705	690 / 705	BHQ-2	DLO-Q7B2-5	50 nmol	\$230
			DLO-Q7B2-1	1 µmol	\$475

This is a sample list of our real-time qPCR oligonucleotides. Other probe & primer formats, 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**



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