Product Guide

Custom Oligo Synthesis, antisense oligos, RNA oligos, chimeric oligos, Fluorescent dye labeled oligos, Molecular Beacons, TaqMan Probes siRNA, Aptamers

Control Fluorescent Probes

For research use only. Not for use in diagnostic procedures for clinical purposes.

Applications

Real Time Quantitative PCR Analysis (QPCR) Probes
Fluorescent Genotyping
siRNA Gene Knockout Validation
Allelic Discrimination
Antisense Targeting
SNP Detection
Aptamers Detection Probes

Gene Link™

Endogenous Control Fluorescent Probes

IMPORTANT NOTE

1. **Consult product specification sheet and material supplied for specifications of product received.**
2. **This product guide is not specific to any particular endogenous control fluorescent probe.**
3. **This product guide should be used in conjunction with the particular instrument manual and specifications and is not intended to replace those specifications.**

Storage Instructions:
1. Shipped lyophilized at room temperature.
2. Store at –20°C upon receipt.
3. Store at –20°C after reconstitution.

Endogenous Control Fluorescent Probes

Reconstitution, Use & Stability of Fluorescent Probes

All Gene Link custom oligo products including, molecular probes, RNA and siRNA includes a datasheet that contains the exact nmols, µg, A_{260} units(OD Units) and other physical data. This data is important for reconstituting the product. All fluorescent probes are shipped in amber tubes to prevent exposure to light and minimize photobleaching. Gene Link guarantees the stability of oligos for 1 year and fluorescent molecular probes for 6 months if reconstituted and stored appropriately as detailed below.

In our experience unmodified oligos are stable for numerous years if reconstituted and stored properly. Avoid multiple freeze thaws; do not exceed 6-10 freeze thaw cycles. If the same oligo is intended to be used repeatedly then it is prudent to make a numerous aliquot of the stock solution and stored frozen.

Reconstitution & Storage
Gene Link probes are supplied lyophilized in amber tubes to protect from light and to reduce photo bleaching. These are stable at room temperature for an extended period but should preferably be frozen upon receipt. TE buffer is recommended for dissolving the probes and oligonucleotides; EDTA inhibits the activity of the nucleases.

Preferred TE Buffer Reconstitution & Storage pH for Fluorescent Probes	
6-FAM, HEX, TET, ROX, and TAMRA	TE Buffer pH 7.5 or 8.0
Cy3, Cy3.5, Cy5, and Cy5.5	TE Buffer pH 7.0 or 7.5
AFDyes	TE Buffer pH 7.5 or 8.0
Cy dyes rapidly degrade in acidic pH	

Further dilution can be made in TE buffer. After reconstitution store the stock solution at -80°C or -20°C. Fluorescently labeled oligos should be stored protected from light.

Preparation of Stock Solution of 100 pmols/µl [100µM]
Gene Link provides the exact amount of nmols of each probe supplied on the tube and on the Product Specifications Sheet included with the product.

Multiply the 'nmol' amount by 10 to arrive at the volume of TE to be added.
Example: 2 nmols x 10 = 20 µL
Dissolve the oligo in 20 µL to get 100 pmols/µl stock solution [100 µM].

Working Stock Solution.
Dilute 10 fold to prepare a 10pmols/µL [10µM]. Dilute further as required.

Storage
For optimal long-term storage, it is recommended that the oligonucleotides should be stored dry at -20°C in the dark. If numerous experiments are planned using the same oligonucleotide, prepare aliquots, dry them and store the aliquots at -20°C.

Stability
Gene Link guarantees the stability of oligos for 1 year and fluorescent molecular probes for 6 months if reconstituted and stored appropriately as recommended by Gene Link. The stability can be increased several fold by instituting proper handling conditions, avoiding exposure to light and multiple freeze thaws.

Fluorescent Probes PAGE Purified Yield*			
Dye/Quencher	**50 nmol scale Yield**	**200 nmol scale Yield**	**1 µmol scale Yield**
	PAGE Purified*	PAGE Purified*	PAGE Purified*
5' 6-Fam/3'-Tamra, BHQ1 or Dabcyl	10 nmol	25 nmol	60 nmol
5'-Hex, Tet, Cy3 or Cy5/BHQ2	5 nmol	15 nmol	40 nmol
5' -Cy3.5, Cy5.5,/quencher	5 nmol	12 nmol	25 nmol
AFDyes and other NHS Dyes/quencher	2 nmol	5 nmol	16 nmol
*Approximate yield for dye and quencher listed. Lower yield with additional modifications. ** Purified yield is for gel purified (PAGE, polyacrylamide gel electrophoresis). Inquire for higher scale of synthesis			

Typical Real Time PCR Component Mix				
Component	Stock Solution	Final Concentration	per 25 µL rxn	5x 25 µL reaction
Water	Water		10 µL	50 µL
10X PCR buffer	10X	1X	2.5 µL	12.5 µL
dNTP	2 mM	200 µM	2.5 µL	12.5 µL
MgCl2	25 mM	3 mM	3 µL	15 µL
Primer Mix	3 µM	300 nM (0.3 pmol/ul)	3 µL	15 µL
Probe	2 µM	200 nM (0.2 pmol/ul)	3 µL	15 µL
Template			1 µL	
Taq polymerase	5u/µL	0.025 unit/µL	0.5 µL	
Total Volume			25 µL	

Gene Link Molecular Beacon Melt Curve Protocol

1. Prepare Molecular Beacon stock solution at 100 pmols/µl [100 µM (micromolar)] in 1 X PCR Buffer. Gene Link provides the exact amount of nmoles of each oligo supplied on the tube and on the Oligo Report. Multiply the 'nmol' amount by 10 to arrive at the volume of solvent to be added.

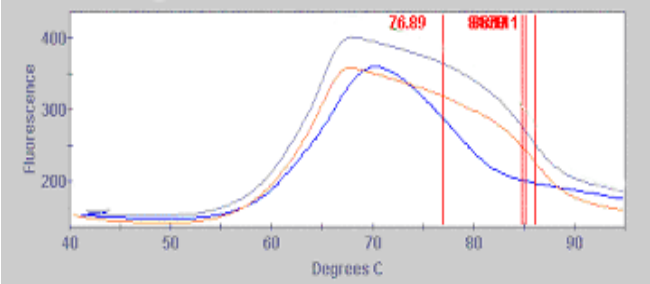
2. Prepare Molecular Beacon working solution at 5 pmols/µl [5 µM (micromolar)] in 1 X PCR Buffer.

3. Set up two 25 µl reactions, one with probe alone, one with target + probe as follows.

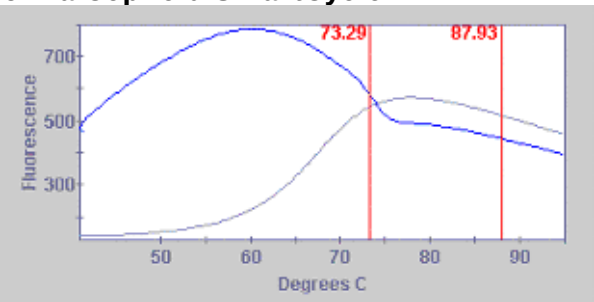
Molecular Beacon Probe Alone	Molecular Beacon Probe plus Target
2.5 µl 10X PCR buffer	2.5 µl 10x PCR buffer
3 µl 25 mM MgCl2	3 µl 25 mM MgCl2
1 µl 5 pmol/ul probe [0.2 pmol/µl final or 200 nM]	1 µl 5 pmol/ul probe [0.2 pmol/µl final or 200 nM]
	3 µl 5 pmol/ul target [0.6 pmol/µl final or 600 nM]
18.5 µl H_20	15.5 µl H_2O

The protocol for Molecular Beacon Melt Curve ramps from 40 to 95 degrees C at 0.2 degrees/second

Screen capture of graphs from a Cepheid SmartCycler



Multiple Molecular Beacon Probe Alone Melt Curve



Molecular Beacon Probe plus Target and Probe Alone Melt Curve

Notes:
-$MgCl_2$ needs to be higher for MB reactions than for regular PCR as it helps to stabilize the stem structure of the probe during the high ramp rate. Final concentration of $MgCl_2$ should be between 2.5 and 4 mM. Here we use 3 mM final. This is the same range of concentration used in an actual amplification reaction.
-Final concentration of probe should be 200-600 nM. Here we use 200 nM. This is also the same concentration range used for the real time reaction.
-For a melt curve it is important to saturate the probe with target. Use 2-3 X the *Molar* amount of target. Here we use 3X target for a final concentration of 600 nM. For real time monitoring, 500 ng genomic DNA, diluted 10X to various concentrations can be used as a starting point.

QPCR
Once you have your melt curve you want to select an annealing temperature for your real time PCR where the probe alone is completely closed (shows no flourescence), and the probe+target is completely open (shows maximal fluoresence). This temperature should be about 5-8 degrees below the Tm of the probe/target hybrid (red vertical line on melt curve of probe+target). It is important to test your primers at the annealing T to ensure that you will have strong, clean amplification at this temperature.

Signal-to-noise (S:N) ratios is calculated by dividing the fluorescence signal of a 25-mer in the presence of a two to five-fold excess of an exactly complementary target sequence by the fluorescence intensity of the probe alone.

Fluorophore Spectral Data & Quencher Selection Guide

Fluorophore Name	Excitation Max, nm	Emission Max, nm	Extinction coefficient*	Color**	Quencher
AFDye-350 NHS	346	445	19,000	Blue	Dabcyl λ (max) = 453 nm Range = 380-530 nm
AFDye-405 NHS	402	424	33,000		
PBlue-455 NHS	410	455	46,000		
MBlue-460 NHS	362	459	20,000	Blue-Green	
AFDye-488 NHS	494	517	73,000		BHQ-1 λ **(max)** = 534 nm Range = 480-580 nm
FAM	**495**	**520**	**75,850**		
TET	**521**	**536**	**99,000**		
AFDye-430 NHS	430	539	15,000	Yellow-Green	
Cal Fluor Gold 540	552	543	81,100		
JOE	**520**	**548**	**75,000**		
Yakima Yellow	**531**	**549**	**83,800**		
AFDye-532 NHS	530	555	81,000		
HEX	**535**	**556**	**98,000**	Yellow	
Cal Orange 560	**537**	**558**	**81,000**		BHQ-2 λ (max) = 579 nm Range = 550-650 nm
Cy3	**550**	**570**	**150,000**		
AFDye-555 NHS	555	572	155,000		
TAMRA	**555**	**576**	**65,000**		
CAL Fluor Red 590	**569**	**591**	**79,000**		
Redmond Red	579	595	52,300		
Cy3.5	**581**	**596**	**150,000**	Yellow-Orange	
ROX NHS	575	602	82,000		
AFDye-568 NHS	578	602	88,000		
Cal Red 610	**590**	**610**	**108,000**	Orange	
TXRed-616 NHS	589	616	69,000		
AFDye-594 NHS	590	617	92,000	Orange-Red	
CAL Fluor Red 635	616	637	112,000		BBQ-650 λ (max) = 650nm Range = 550-750 nm
LC Red 640 NHS	625	640	110,000		
AFDye-647 NHS	649	671	270,000		
Cy5	**649**	**670**	**250,000**	Red	
Cy5.5	**675**	**694**	**190,000**		
AFDye-680 NHS	678	701	185,000	Near-IR region. Human vision is insensitive to light beyond ~650 nm; it is not possible to view near-IR fluorescent dyes.	
Cy7 NHS	750	773	199,000		
IR 750 NHS	756	776	260,000		
Cy7.5 NHS	788	808	223,000		

* Extinction coefficient at λ (max) in cm–1M–1. ** Typical emission color seen through the eyepiece of a conventional fluorescence microscope with appropriate filters.

BioTools application from Gene Link for iPhone / iPod / iPad
BioTools: An Array of Genetic Tools

The BioTools app also has advanced modules for setup of Polymerase Chain Reaction (PCR) and Quantitative Real Time PCR (Q-PCR).

The main focus of this app is to have a handy source of calculation modules and quick reference sections for designing and executing experiments involving PCR and Q-PCR.

BioTools	Reference
1. Oligo Tm: A robust oligo melting temperature calculation module using three methods; it also calculates other physical attributes. 2. Oligo Resuspension 5. Oligo Dilution 6. PCR & QPCR: Convenient calculator for multiple reaction setup for PCR, TaqMan QPCR and Molecular Beacon QPCR setup. Includes stock solution information and cycling profiles 7. Molarity Calculator 8. Reagent Dilution 9. Measurement Convertor: A convenient selection of calculators to convert length, area, mass, temperature and volume units. 10. Genetic Code Translator: Enter DNA sequence to see coding pattern.	A selection of topics, relevant to life scientists for quick access to basic information. This section includes the following sections and sub sections. 1. Nucleic Acid Basics 2. Modifications Table: A list of common modifications with molecular structure and basic properties. 3. Dye & Quencher Table: A convenient list of fluorophores and quencher matching the emission max. 4. Duplex Stability & Nuclease Resistance 5. Gene Information: Simply enter the accession number and retrieve detailed gene information from NCBI database, 6. Amino Acid Table: Molecular structure and detailed physical properties of all amino acids. 7. Translation Table: Genetic code for all amino acids. 8. Codon Picker: Select codon sequence and see the corresponding amino acid and detailed information.

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