Gene Assays

Product Guide

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

Custom Fluorescent Molecular Probe Assay Mix

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

Oligo Types & Modifications

Molecular Beacons
TaqMan® Probes
Aptamers
RNA Probes
Fluorophores & Quenchers
Propyne dC and dU labeled Oligos
Phosphorothioate Oligos
2'-5' linked Oligos
Methylated Oligos

Applications

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

20 years
results you can rely on
Gene Link™

Custom Fluorescent Molecular Probe Assay Mix

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 fluorescent molecular probe assay mix.
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 at room temperature.
2. Store at –20^{o}C upon receipt.
3. Store at –20^{o}C after dilution and or reconstitution.
4. Frozen assay should not be exposed to more than 10 freeze-thaw cycles.
5. All fluorescent molecular probe assay mix should be protected from light and stored in the dark.

Fluorescent Molecular Probe Assay Mix

Molecular probe assay for gene expression, SNP analysis or gene detection of any nucleic acid whose sequence is known can be designed, synthesized and custom formulated as a primer/probe mix by Gene Link. Our online GeneAssays™ design page provides a convenient tool for designing and placing orders.

Fluorescent molecular probe assay mix are designed and synthesized by Gene Link with a wide array of fluorophores and quenchers. All TaqMan and Molecular Beacon probes with the primers are supplied in various standard formats or can be custom formulated with differing concentration of primers and probes.

The list below provides the various formats.

Assay Shipment Format: Solution or Lyophilized

Formulation: Standard concentrations of primer and probes or custom formulation.

Probe Type: Molecular Beacons or TaqMan

Assay Type: Gene Expression, genotyping, SNP Analysis or Gene detection.

Concentration Supplied: Various concentration e.g. 10X, 20X, 40X, 60X, 80X or custom concentrations

Quantity: Small (200 µL); Medium (400 µL); Large (800 µL) and custom OEM sizes.

Gene Expression Assays standard 1X Final concentration is two primers each at 500 nM (0.5 micromolar) and one fluorophore labeled probe at 250 nM (0.25 micromolar)

SNP Genotyping Assays standard 1X Final concentration is two primers each at 500 nM (0.5 micromolar) and fluorophore labeled probes each at 200 nM (0.20 micromolar)

The above standard assay conditions are for routine assays. These may need to be optimized for certain specific assays and specially for multiplexing with varying concentrations of primers.

Each assay is provided with detailed sequence information for all probes and primers and their position on the specific DNA segment. Please consult specification information enclosed with each shipment.

Reconstitution, Use & Stability of Lyophilized Fluorescent Probes & Oligos

All Gene Link custom oligo products including, molecular probes, RNA and siRNA includes a datasheet that contains the exact nmols, µg, or A_{260} units (OD Units) and other physical data. This data is important for reconstituting the product.

Reconstitution & Storage

Gene Link fluorescent 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 of time but should preferably be frozen upon receipt. Low TE buffer (10 mM Tris pH 8.0, 0.1 mM EDTA) 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
Alexa dyes	TE Buffer pH 7.5 or 8.0
Cy dyes rapidly degrade in acidic pH	

Further dilution can be made in low 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 Individual Primer & Probe Stock Solution of 100 pmols/µl [100 µM].

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. We recommend preparing a 100 µM stock solution of each individual primer and probes and keep it frozen protecting from light. A 10X assay mix can be prepared using the stock. A general guideline is given below.

Example: 6.6 nmols lyophilized product.
Dissolve the 6.6 nmol probe in 66 µl of low TE pH7.5 (10 mM Tris pH8.0, 0.1 mM EDTA) to get 100 pmols/µL [100 µM] stock solution.

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.
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 several aliquots of the stock solution and stored frozen.

Preparation of 10X Standard Gene Expression Primer & Probe Assay Mix

A general guideline for preparing a 10X standard gene expression assay mix is given below. The worksheet column in the table can be used to prepare other volume.

10X Standard Gene Expression Primer & Probe Assay Mix			
Component	**Volume/500 µL**	**Worksheet***	**10X Final Reaction Concentration**
Primer 1; 100 µM stock	25 µL		5 µM (5 pmol/ µL)
Primer 2; 100 µM stock	25 µL		5 µM (5 pmol/ µL)
Probe; 100 µM stock	12.5 µL		2.5 µM (2.5 pmol/ µL)
Low TE pH 8.0 (10 mM Tris pH 8.0 & 0.1mM EDTA)	437.5 µL		
The final 1X gene expression assay mix contains 500 nM (0.5 pmol/ µL; 0.5 µM) concentration of each primer and 250 nM (0.25 pmol/ µL; 0.25 µM) concentration of probe.			
*Use worksheet space to calculate for other final desired volume			

Preparation of 10X Standard SNP Primer & Probe Assay Mix

A general guideline for preparing a 10X standard SNP assay mix is given below. The worksheet column in the table can be used to prepare other volume.

10X Standard SNP Primer & Probe Assay Mix			
Component	**Volume/500 µL**	**Worksheet***	**10X Final Reaction Concentration**
Primer 1; 100 µM stock	25 µL		5 µM (5 pmol/ µL)
Primer 2; 100 µM stock	25 µL		5 µM (5 pmol/ µL)
Probe 1; 100 µM stock	10 µL		2 µM (2 pmol/ µL)
Probe 2; 100 µM stock	10 µL		2 µM (2 pmol/ µL)
Probe 3; 100 µM stock (if required)	10 µL		2 µM (2 pmol/ µL)
Total volume to 500 µL using Low TE pH 8.0 (10 mM Tris pH 8.0 & 0.1mM EDTA)			
The final 1X SNP assay mix contains 500 nM (0.5 pmol/ µL; 0.5 µM) concentration of each primer and 200 nM (0.2 pmol/ µL; 0.2 µM) concentration of each probe.			
*Use worksheet space to calculate for other final desired volume			

A. Reaction Premix Preparation Worksheet (prepare 10% more than required)

DO NOT ADD TEMPLATE DNA TO THE PREMIX. The template DNA row is only for calculation of the total volume to be added and thus the volume of nuclease free water to be added to arrive at the final volume of the reaction.

Component	/5 µLrxn	Worksheet	/10 µLrxn	worksheet	/20 µLrxn	worksheet
GeneAssays™ Genotyping PCR 2X Master Mix (with or w/o Rox)	2.5 µL		5 µL		10 µL	
10X Primers & Probe(s) assay mix	0.5 µL		1 µL		2 µL	
*Nuclease Free Water						
**Template DNA 10 ng. (max of ~100 ng) Do not add. Only for calculation.						
Final Total Volume	5 µL	5 µL	10 µL	10 µL	20 µL	20 L
*Volume of nuclease free water will be determined by the volume of template DNA or the method of DNA delivery method. Add sufficient nuclease free water to arrive at the final total volume of the reaction.						
**Determine the DNA delivery method, if using wet delivery method then the maximum volume can be used substituting the addition of sterile water. For dry DNA delivery method the volume will be zero.						

B. Plate Setup

1. Dispense appropriate volume of reaction premix to each well.
2. Add template DNA if using the wet DNA delivery method.
3. Seal the plate with optical adhesive film.
4. Gently tap the plate(s) to mix the contents.
5. Centrifuge plate(s) to ensure elimination of bubbles and reaction contents accumulation at the bottom of well.

C. PCR Cycling

1. Place the plate(s) in the real time PCR thermal cycler and start the appropriate programmed file for thermal cycling.
2. Consult instrument manufacturer manual for cycling features and fluorophore optical detection properties.
3. Consult instrument manual for viewing and retrieving result files.
4. Given below is a suggested thermal cycling profile used successfully at Gene Link.

GeneAssays™ Real Time Gene Expression TaqMan Thermal Cycling Profile			
Description	**Temperature**	**Time**	**Cycle(s)**
Taq Hot start Activation	95°C	10 minutes	1 cycle
Denaturation	95°C	15 sec	40 Cycles
Annealing & Extension	60°C	1 minute	
If required a hold cycle at 12°C for infinity can be inserted at the end to retrieve amplified product.			

GeneAssays™ Real Time qPCR Molecular Beacon® Thermal Cycling Profile			
Description	**Temperature**	**Time**	**Cycle(s)**
Taq Hot start Activation	95°C	10 minutes	1 cycle
Denaturation	95°C	15 seconds	40 Cycles
Annealing-OPTICS ON	60°C	30 seconds	
Extension-OPTICS OFF	72°C	30 seconds	
If required a hold cycle at 12°C for infinity can be inserted at the end to retrieve amplified product.			

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.

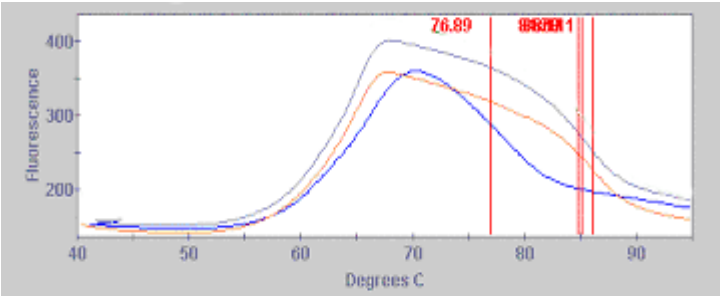
2. Prepare Molecular Beacon working solution at 5 pmols/µl [5 µM (micromolar)] in 1 X PCR Buffer.

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

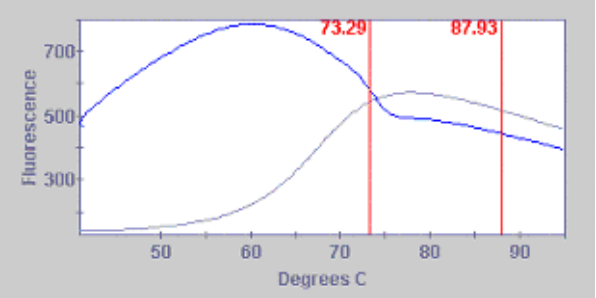
Molecular Beacon Probe Alone	Molecular Beacon Probe plus Target
2.5 µl 10X PCR buffer	2.5 µl 10x PCR buffer
3 µl 25 mM MgCl2	3 µl 25 mM MgCl2
1 µl 5 pmol/ul probe [0.2 pmol/µl final or 200 nM]	1 µl 5 pmol/ul probe [0.2 pmol/µl final or 200 nM]
	3 µl 5 pmol/ul target [0.6 pmol/µl final or 600 nM]
18.5 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 fluorescence), and the probe+target is completely open (shows maximal fluorescence). 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

Fluorophore Absorbance and Emission Data

Dye	Color	Absorbance max (nm)	Emission max (nm)	Extinction Coefficient
6-FAM (Fluorescein)	Green	494	525	74850
TET	Orange	521	536	85553
HEX	Pink	535	556	95698
Cy 5	Violet	646	667	250000
Cy 5.5	Blue	683	707	190000
Cy 3	Red	552	570	150000
Cy 3.5	Purple	588	604	150000
Cy 7	Near IR	743	767	200000
Tamra	Rose	565	580	87000
ROX	Purple	587	607	105000
JOE	Mustard	528	554	105000
Alexa Dye Series	Varies	Varies	Varies	Varies

List of Other Molecular Probes Fluorescent Dyes

Dye	Color	Absorbance max (nm)	Emission max (nm)	Extinction Coefficient
Cascade Blue	Blue	396	410	29,000
Marina Blue	Blue	362	459	19,000
Oregon Green 500	Green	499	519	78,000
Oregon Green 514	Green	506	526	85,000
Oregon Green 488	Green	495	521	76,000
Oregon Green 488-X	Green	494	517	84,000
Pacific Blue	Blue	416	451	36,000
Rhodamine Green	Green	504	532	78,000
Rhodol Green	Green	496	523	63,000
Rhodamine Green-X	Green	503	528	74,000
Rhodamine Red-X	Red	560	580	129,000
Texas Red-X	Red	583	603	136,000

Applied Biosystems Proprietary Dyes & Possible Substitutions		
Dye	**Absorbance max (nm)**	**Emission max (nm)**
VIC	538	554
HEX	535	556
NED	546	575
Cy3	550	570
PET	558	595
Cy3.5	588	604
ROX	575	602
Texas Red	583	603
LIZ	630	652
Cy5	646	667

Alexa Fluor® Dyes

Dye	Color	Absorbance max (nm)	Emission max (nm)	Extinction Coefficient
Alexa Fluor 350	Blue	346	442	19,000
Alexa Fluor 405	Green	401	421	34,000
Alexa Fluor 430	Green	433	541	16,000
Alexa Fluor 488	Green	495	519	71,000
Alexa Fluor 532	Yellow	532	553	81,000
Alexa Fluor 546	Red	556	573	104,000
Alexa Fluor 555	Red	555	565	150,000
Alexa Fluor 568	Red	578	603	91,000
Alexa Fluor 594	Red	590	617	73,000
Alexa Fluor 633	Violet	632	647*	100,000
Alexa Fluor 647	Violet	650	665*	239,000
Alexa Fluor 660	Purple	663	690*	123,000
Alexa Fluor 680	Blue	679	702*	184,000
Alexa Fluor 700	Near IR	702	723*	192,000
Alexa Fluor 750	Near IR	749	775*	240,000

Spectral characteristics of the Alexa Fluor dyes. Extinction coefficient at l max in cm -1m-1. *Human vision is insensitive to light beyond~650nm, and therefore it is not possible to view the far -red- fluorescent dyes by looking through the eyepiece of a conventional fluorescent microscope. Alexa Fluor® Dyes is sold under license from Invitrogen Corporation. These may only be used for R&D and may not be used for clinical or diagnostic use.

*Disclaimer of License Statement for Molecular Beacons Products -

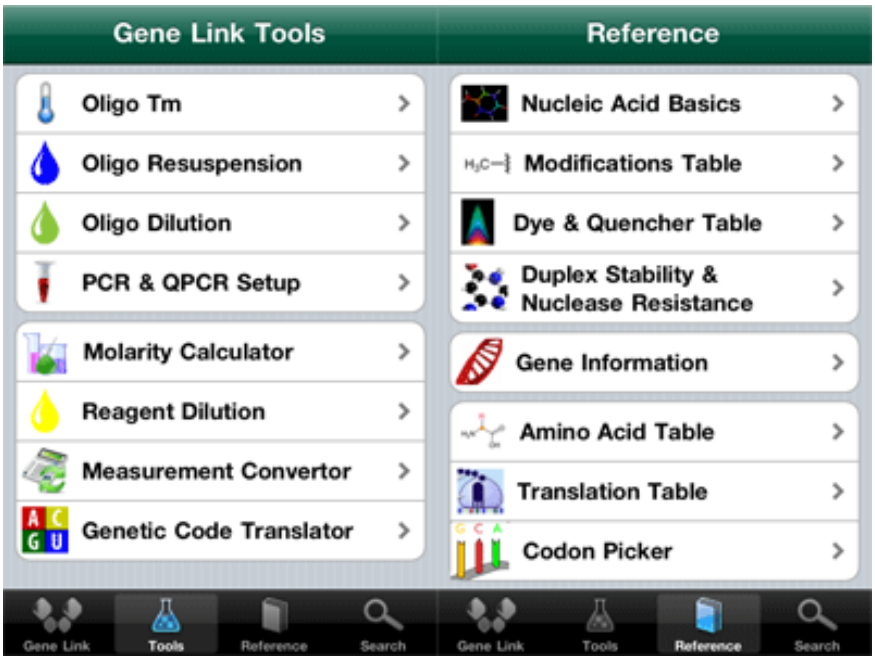
*PHRI Molecular Beacon License Agreement -"This product is sold under license from the Public Health Research Institute. It may be used under PHRI Patent Rights only for the purchaser's research and development activities". **Black Hole Quencher License Agreement

"Black Hole Quencher", "BHQ-1", "BHQ-2" and "BHQ-3" are registered trademarks of Biosearch Technologies, Inc., Novato, CA. The BHQ technology is licensed and sold under agreement with Biosearch and these products are sold exclusively for R&D use by the purchaser. They may not be used for clinical or diagnostic purposes and they may not be resold, distributed or re-packaged.

*Alexa Fluor® Dyes are sold under license from Invitrogen Corporation. These may only be used for R&D and may not be used for clinical or diagnostic use.

Tools: An Array of Genetic Tools

Tools application from Gene Link for iPhone/iPod/iPad



The Gene Link Tools 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.

Tools Modules

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.

Reference Modules

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.

Document Warranty and Liability

Information in this document is subject to change without notice. This document and all information presented in this document are written as a guide. Gene Link, Inc. does not warrant this document to be free of errors and assumes no responsibility for any errors that may appear in this document.
Gene Link disclaims all warranties with respect to this document, expressed or implied, including but not limited to those of merchantability or fitness for a particular purpose. In no event shall Gene Link be liable, whether in contract, tort, warranty, or under any statute or on any other basis for special, incidental, indirect, punitive, multiple or consequential damages in connection with or arising from this document, including but not limited to the use thereof.

Website

As the receipt of information on the Internet is highly dependent upon factors, including without limitations to, the user's computer, browser, operation system, etc., information may be perceived incorrectly. Therefore, Gene Link does not warrant or guarantee that the information contained on its website www.genelink.com is error free.

Product Warranty and Liability

Warranty: Gene Link makes no warranty of any kind, specifically disclaims and excludes all other warranties of any kind or nature, directly or indirectly, express or implied, including, without limitation, as to the suitability, productivity, durability, fitness for a particular purpose or use, merchantability, condition, or any other matter with respect to Gene Link products. Gene Link products are for research purposes only including custom products. There is no warranty or claim of its performance for any specific research application. All Gene Link products are guaranteed to meet or exceed the specifications stated. Each Gene Link product is shipped with documentation stating specifications and other technical information. If the product fails to meet the stated specifications the sole remedy is prompt replacement by Gene Link or within 30 days of purchase a refund of the purchased price.

Liability: Under no circumstances shall Gene Link be liable for any damages directly or indirectly related to Gene Link's products and services. Whether direct, incidental, foreseeable, consequential, or special (including but not limited to loss of use, revenue or profit), whether based upon warranty, contract, tort (including negligence) or strict liability arising in connection with the sale or the failure of Gene Link products to perform in accordance with the stated specifications.

Research Use Only. Not for use in diagnostic or clinical procedures.

Notice to Purchaser: The purchase of this product conveys to the purchaser the limited, non-transferable right to use the purchased amount of the product only to perform internal research for the sole benefit of the purchaser. No right to resell this product or any of its components is conveyed expressly, by implication, or by estoppel. This product is for internal research purposes only and is not for use in commercial applications of any kind, including, without limitation, quality control and commercial services such as reporting the results of purchaser's activities for a fee or other form of consideration. For information on obtaining additional rights, please contact support@genelink.com

Gene Link, Inc.
190 Saw Mill River Road
Hawthorne, NY 10532
USA

Tel: (914) 769-1192
Email: support@genelink.com
www.genelink.com