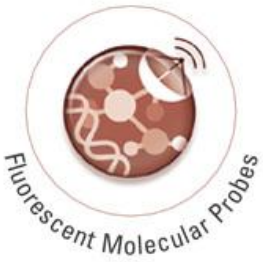


Product Manual

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

Control Fluorescent Molecular 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 Molecular 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^{o}C upon receipt.
3. Store at –20^{o}C after reconstitution.

Endogenous Control Fluorescent Molecular 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, or 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 photo-bleaching. 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 aliquots of the stock solution and frozen.

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. 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
Alexa dyes	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 Probe Stock Solution of 20 pmols/µl [20 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.

Example: 2 nmols lyophilized product.
Dissolve the 2 nmol probe in 100 µl of TE pH7.5 (10 mM Tris pH7.5, 1 mM EDTA) to get 20 pmols/µl stock solution [20 µM].

Working Probe Stock Solution 2 pmols/µl [2 µM]
Dilute 10 fold to prepare a 2 pmols/µl [2 µM]

Preparation of Primer Mix Stock Solution of 30 pmols/µl [30 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. Most Gene Link Genemer™ primer mix are supplied as 10 nmols or 3 nmols lyophilized.

Example: 10 nmols lyophilized product.

Dissolve the 10 nmols of primer mix in 334 µl of TE pH7.5 (10 mM Tris pH7.5, 1 mM EDTA) to get 30 pmols/µl stock solution [30 µM].

Example: 3 nmols lyophilized product.
Dissolve the 3 nmols of primer mix in 100 µl of TE pH7.5 (10 mM Tris pH7.5, 1 mM EDTA) to get 30 pmols/µl stock solution [30 µM].

Working Probe Stock Solution 3 pmols/µl [3 µM]
Dilute 10 fold to prepare a3 pmols/µl [3 µM].

Working Stock solution in TE pH 7.5	
Primer	3 µMolar [3 pmol/µL]
Probe	2 µMolar [2 pmol/µL]

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.

Typical TaqMan Reaction Conditions

Component	Stock Solution	Final Concentration	per 25 µL rxn	5x 25 µL reaction mix
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	~10ng/µL		1 µL	
Taq polymerase	5u/µL	0.025 unit/µL	0.5 µL	
Total Volume			25 µL	

QPCR Reaction

Dispense 25 µL of above QPCR reaction mix to each tube then add 1 µL template and start cycling.

Typical TaqMan Cycling Conditions

	GL TaqMan PCR cycling	Time	Temperature	Cycles
Stage 1	GL Initial denaturation	2 min	95 °C	1
Stage 2	Denature	15 sec	95 °C	40
	Anneal/Extend-OPTICS ON	1 min	60 °C	

Instrument Run & Results

Consult instrument manufacturer protocol and manual

Typical Molecular Beacon Reaction Conditions

Component	Stock Solution	Final Concentration	per 25 µL rxn	5x 25 µL reaction mix
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	~10ng/µL		1 µL	
Taq polymerase	5u/µL	0.025 unit/µL	0.5 µL	
Total Volume			25 µL	

QPCR Reaction

Dispense 25 µL of above QPCR reaction mix to each tube then add 1 µL template and start cycling.

Stage	Molecular Beacon QPCR cycling	Time	Temperature	Cycles
Stage 1	Initial denaturation	2 min	95 °C	1
Stage 2	Denature	15 sec	95 °C	40
	Anneal-OPTICS ON	30 sec	60 °C	
	Extend-OPTICS OFF	30 sec	72 °C	

Instrument Run & Results

Consult instrument manufacturer protocol and manual

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.

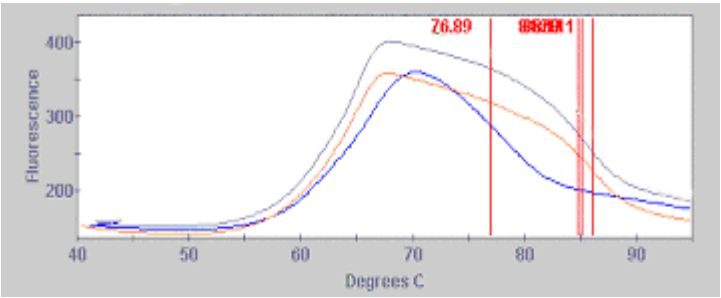
2. Prepare Molecular Beacon working solution at 5 pmols/µl [5 µM (micromolar)] in 1 X PCR Buffer.

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

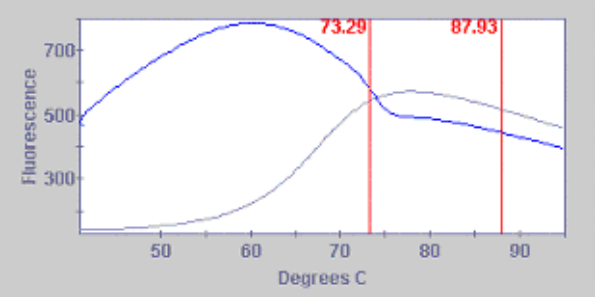
Molecular Beacon Probe Alone	Molecular Beacon Probe plus Target
2.5 µl 10X PCR buffer	2.5 µl 10x PCR buffer
3 µl 25 mM MgCl2	3 µl 25 mM MgCl2
1 µl 5 pmol/ul probe [0.2 pmol/µl final or 200 nM]	1 µl 5 pmol/ul probe [0.2 pmol/µl final or 200 nM]
	3 µl 5 pmol/ul target [0.6 pmol/µl final or 600 nM]
18.5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

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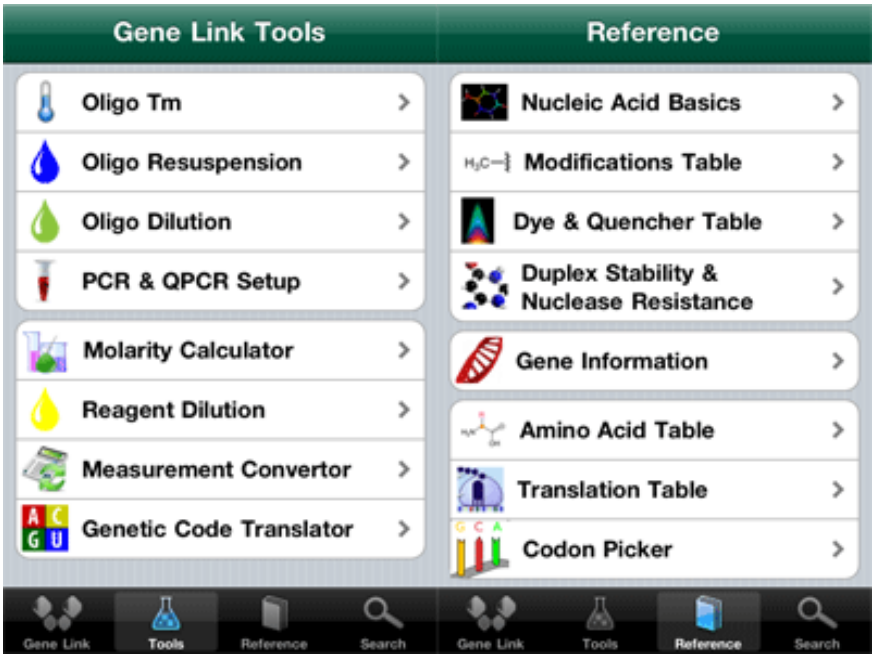
*Alexa Fluor® Dyes are sold under license from Invitrogen Corporation. These may only be used for R&D and may not be used for clinical or diagnostic use.

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.