Technical Note

Custom Oligo Synthesis, antisense oligos, RNA oligos, chimeric oligos,
Fluorescent dye labeled oligos, Molecular Beacons, TaqMan Probes
Locked Nucleic Acids (LNA), siRNA, Aptamers

Fluorescent Molecular Probes Reconstitution & Stability

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) Probes
Fluorescent Genotyping
siRNA Gene Knockout Validation
Allelic Discrimination
Antisense Targeting
SNP Detection
Aptamers Detection Probes

Gene Link™

Reconstitution & 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 numerous aliquots of the stock solution and frozen.

Reconstitution & Storage
Gene Link oligos are supplied lyophilized. These are stable at room temperature for an extended period of time. The oligonucleotide should preferably be frozen upon receipt. TE buffer (10mM Tris, 1mM EDTA, pH 7.5) is recommended for dissolving the 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
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 in light-free conditions.

Sterile water with a pH of 7.5 can also be used if desired to avoid EDTA and Tris. Note that standard water mostly has an acidic pH.

Preparation of Stock Solution of 100 pmols/µl [100µM]
Gene Link provides the exact amount of nmols of each oligo supplied on the tube and on the Oligo Report. Multiply the 'nmol' amount by 10 to arrive at the volume of TE to be added.
Example: 45.10nmols x 10 = 451µl
Dissolve the oligo in 451µl to get 100pmols/µl stock solution.
Use as required.
Dilute 10 fold to prepare a 10pmols/µl [10µM]. Use as required.

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 to use the same oligonucleotide, prepare aliquots, dry them and store the aliquots at -20°C.

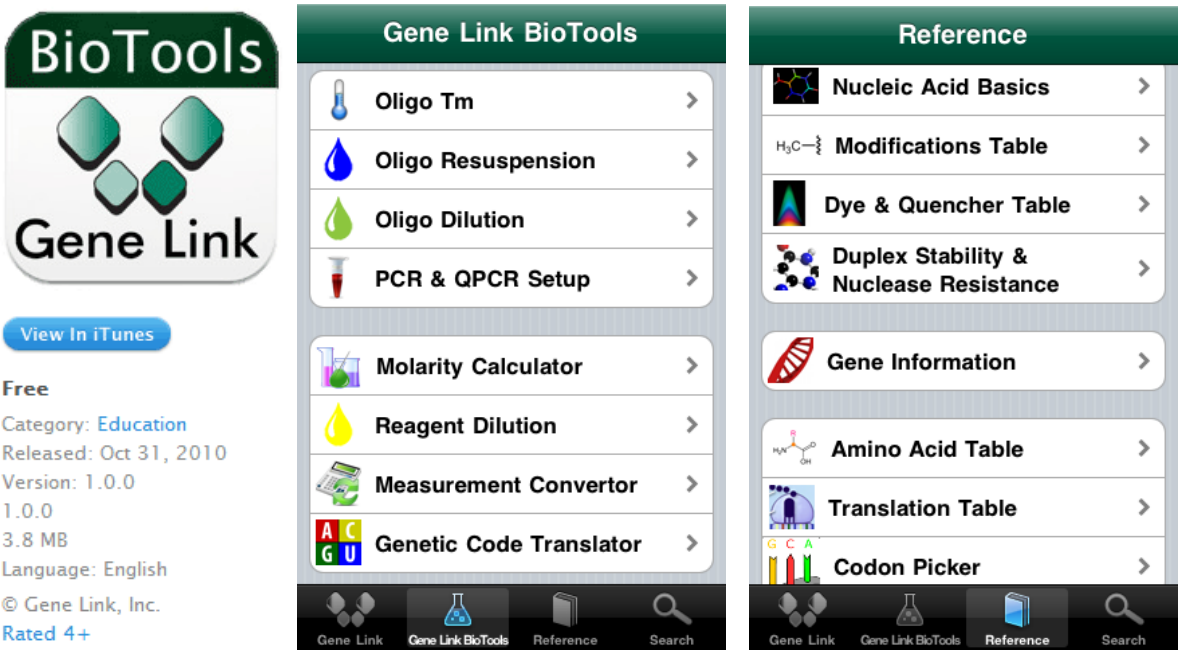
Stability
Gene Link guarantees the stability of oligos for 1 year and fluorescent molecular probes for 6 months if reconstituted and stored appropriately as recommended by Gene Link. The stability can be increased several fold by instituting proper handling conditions, avoiding exposure to light and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

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	Yellow-Green	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		
Cal Fluor Gold 540	552	543	81,100	Yellow	
JOE	**520**	**548**	**75,000**		
Yakima Yellow	**531**	**549**	**83,800**		
AFDye-532 NHS	530	555	81,000		
HEX	**535**	**556**	**98,000**		
Cal Orange 560	**537**	**558**	**81,000**		
Cy3	**550**	**570**	**150,000**	Yellow-Orange	BHQ-2 λ (max) = 579 nm Range = 550-650 nm
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**		
ROX NHS	575	602	82,000	Orange	
AFDye-568 NHS	578	602	88,000		
Cal Red 610	**590**	**610**	**108,000**		
TXRed-616 NHS	589	616	69,000		
AFDye-594 NHS	590	617	92,000	Orange-Red	
CAL Fluor Red 635	616	637	112,000	Red	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**		
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	223000		

* 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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