## Custom Oligo Specifications

Gene Link custom oligonucleotides are supplied desalted and lyophilized. They are ready to use after appropriate reconstitution. Dry oligonucleotides are stable at room temperature for an extended period of time.

**Storage & Reconstitution**
The oligonucleotide should preferably be frozen upon receipt. TE buffer (10 mM Tris, 1 mM EDTA, pH 7.5) is recommended for dissolving the oligonucleotides. After reconstitution store the stock solution at -80°C or -20°C.

**Gel Photo Documentation**
An actual gel picture of the synthesized custom oligonucleotide is supplied. A major single band represents high purity of the crude oligonucleotide.

**Purity & Usage**
The crude, desalted oligonucleotide supplied is suitable for all amplification and sequencing protocols. Gel purification is advised for all oligos used for cloning applications and for oligos longer than 50 mer.

**Biophysical Data**
Each oligo after desalting is quantified by recording $A_{260}$. Exact nmols and µg is determined by the extinction coefficient and molecular weight of the oligo.

### Oligo Scale of Synthesis and Typical Yield of Unmodified Oligos*

| | Crude Desalted | | RPC Purified*** | | Gel Purified | |
|---|---|---|---|---|---|---|
| | 20 mer oligo** | | 30 mer oligo** | | 50 mer oligo** | |
| **Scale** | **$A_{260}$ Units** | **nmols** | **$A_{260}$ Units** | **nmols** | **$A_{260}$ Units** | **nmols** |
| **50 nmol** | 8-10 | 30+ | 4-5 | 12+ | NR* [1-2] | NR* [2-4] |
| **200 nmol** | 20-25 | 80+ | 8-12 | 24+ | 4-6 | 8+ |
| **1 µmol** | 100-120 | 400+ | 40-50 | 30+ | 20-25 | 40+ |
| **Purity & Yield** | Purity is more than 80% depending on oligo sequence and structure. | | Purity 85% to 95% depending on oligo sequence and structure. Not recommended for oligos longer than 35 mer. | | Purity 98% to ~100% depending on oligo sequence and structure. Yield will gradually decrease as length of oligo increases. | |

*The yield of modified oligos varies based on modification.
**Yield of 30 µg/$A_{260}$ unit for oligos is calculated for an ~equimolar base composition. Long stretches of a single base or homopolymers will have variable yields. Example for homopolymeric 50mer: A(50) = ~20/$A_{260}$ Unit; G(50) = ~28/$A_{260}$ Unit; T(50) = ~35/$A_{260}$ Unit and C(50) = ~39/$A_{260}$ Unit.
***RPC is reverse phase purification using a cartridge; a substitute for HPLC.
NR* Not Recommended.

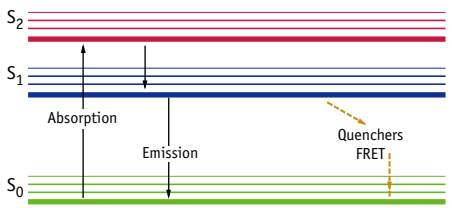
## Fluorescent Molecular Primers & Probes

The use of fluorescent dyes in molecular biology has rapidly transformed from just single dye labeled primers for fragment analysis to the use of multiple labeled dyes and quenchers as probes for real time quantitative PCR analysis. Fluorescence based detection offers a safe and sensitive method for quantitative detection. Gene Link offers synthesis of all different forms of molecular primers and probes. We provide technical service in the design of novel probes and synthesize numerous combinations of dyes, quenchers, RNA, phosphorothioate, 2'O methyl and chimeric probes.

**Excitation and Emission**
The excitation level of molecules varies at different wavelengths. Molecules exposed to a beam of light absorb more at a particular wavelength. This specific wavelength is termed as the Excitation Maxima. The emission maximum is the wavelength at which the maximum amount of light is released. The molecule stays in the excited state for a finite time, usually <1-10 nanoseconds and returns to the relaxed state upon emission of energy. Excitation and Emission is a cyclic process and consequently can be repeated to an extent before it starts to fade, termed as photo-bleaching.

Different fluorescent dyes are used for molecular probes and primer design. The dyes are selected based on the excitation and emission wavelengths, bleaching, quenching and various other biophysical factors.

### Detection Methods




Absorption of a photon and excitation to $S_1$ or $S_2$. Radiationless energy loss and return to $S_1$. Return to $S_0$ from $S_1$ with emission of fluorescence or by energy transfer to quenchers or other acceptor dye (FRET).

**Quenching**
Reduction in the expected fluorescence emission is termed as quenching. The phenomenon of quenching forms the basis of the mode of action of molecular probes; the designed and controlled fluorescence based on hybridization to the target sequence.

### Quenchers

| Dye | Absorbance max (nm) |
|---|---|
| Dabcyl | 453 |
| *BHQ-1 | 534 |
| *BHQ-2 | 579 |
| *BHQ-3 | 672 |

Placing a molecule that absorbs light in close proximity to the fluorophore can induce quenching. The quenching effect is exhibited by fluorescent as well as non-fluorescent molecules. A non-fluorescent quencher is the basis of the design of Molecular Beacons.

**Fluorescence Resonance Energy Transfer (FRET)**
Resonance energy transfer, often known as fluorescence resonance energy transfer (FRET) or Förster energy transfer. It is the radiation-less transfer of excitation energy from a donor to an acceptor. An important consequence of this transfer is that there is no emission of light by the donor. The acceptor may or may not be fluorescent.

FRET varies based on the degree of spectral overlap of the donor and acceptor. This is called the "spectral overlap" or sometimes the "Förster overlap integral". This describes the amount of overlap where resonance can occur, i.e. where the donor and acceptor have the same frequencies.

**TaqMan Probes**
TaqMan (also known as Fluorogenic 5' nuclease assay) probes contain two dyes, a reporter dye (e.g. 6-FAM) at the 5' end and a 3' acceptor dye, usually TAMRA. Recent designs substitute the 3' TAMRA fluorescent acceptor quencher dye with non-fluorescent quencher, e.g. BHQ-1. The proximity of the quencher to the reporter in an intact probe allows the quencher to suppress, or "quench" the fluorescence signal of the reporter dye through FRET.

**Molecular Beacons**
Molecular beacons are hairpin shaped oligos with a fluorophore and a quencher at either ends. The loop serves as the specific target sequence. The stem is formed by the annealing of complementary arm sequences on the ends of the probe sequence. The stem keeps the fluorophore and the quencher in close proximity to each other, causing the fluorescence of the fluorophore to be quenched by energy transfer. When the probe encounters a target molecule, it forms a hybrid that is longer and more stable than the stem leading to the restoration of fluorescence.

### 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 |

*Color and fluorescence data vary with pH. Consult appropriate dye manufacturer for details.

*BHQ is a registered trademark of Biosearch Technologies. Complete disclaimer of license statement for Molecular Beacons products, PHRI Molecular Beacon and BHQ license agreement can be viewed at the following link: www.genelink.com/newsite/products/MBPriceList.asp

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