Product Guide

Fluorescent Molecular Probes, Molecular Beacons, TaqMan Probes
FRET Probes, Chimeric Probes, BHQ quenchers,
Thermal denaturation profiles, Real time monitoring of PCR
Procedure, Troubleshooting, FAQ's, Pricing, Ordering Information

Fluorescent Molecular Probes

Gene Link™

Fluorescent Molecular Probes

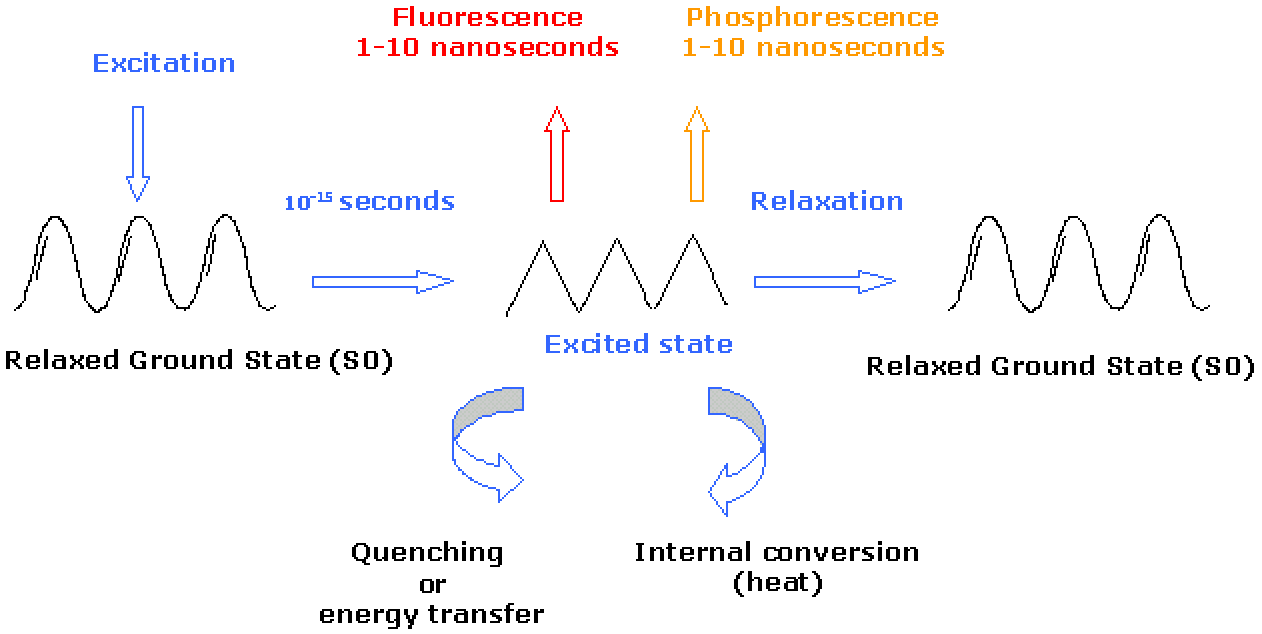
The use of fluorescent dyes in molecular biology has rapidly transformed from just single dye labeled primers for fragment analysis to the use of double labeled dyes and quenchers as probes for quantitative analysis. Fluorescence based detection offers a safe and sensitive method for quantitative detection. This also means that Molecular Biologist have to understand new terms like donors, acceptors, quenchers, FRET, Stokes shift etc. Molecular basis of some of the probe design are simply elegant and thus has led to an exponential use of molecular probes and consequently furthering new developments. It is essential to understand the basic concepts of fluorescence.

Gene Link offers synthesis of all different forms of molecular probes. We provide technical service in the design of novel probes and have synthesized numerous combinations of dyes, quenchers, RNA, phosphorothioate, 2'O methyl and chimeric probes.

Luminescence, Phosphorescence & Fluorescence Defined

Molecules absorb energy as photons of light are shined on them. The property to absorb photons of light depends on the atomic configuration of the molecules, leading to an excited state of energy level. The energy that is absorbed can be translated into rotational, vibrational or electronic modes. The exact fate of the energy depends on the wavelength of the incoming light. The longer the wavelength, the lower the energy. The vibrational and rotational energy levels are closer together than the electronic levels. Thus changes in these levels are often associated with the absorption of infrared radiation and release of energy as heat. However, light in the visible and UV regions of the spectrum has enough energy to cause changes in the electronic states of a molecule without a relative elevation of temperature.

● Luminescence	The release of energy as light at low temperature is termed as luminescence. The molecules emitting light are relatively cool. It is in contrast to light bulbs, fire etc. Luminescence is a general term not limited to the duration of the emitted light.
● Phosphorescence	Delayed luminescence after absorption of energy. The wavelength of the released light energy is at a different wavelength and it continues for an extended period of time, even after excitation has ceased.
● Fluorescence	Instantaneous luminescence after absorption of energy. The fluorescence is usually at a different wavelength and it ceases almost at once when excitation has ceased.

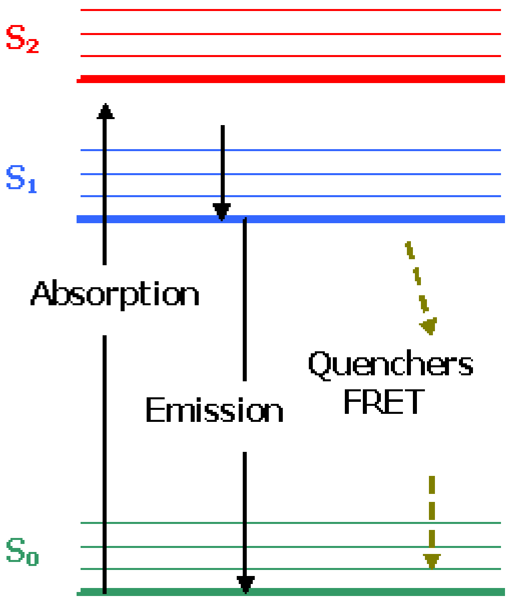


Excitation and Emission

The excitation level of molecules varies at different wavelength. Molecules exposed to a beam of light absorb more at a particular wavelength. This specific wavelength is termed as the Excitation Maxima. The emission maxima 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. The lifetime is measured in nanoseconds, example the lifetime of Cy3 is <0.3ns and Cy5 is 1.0ns. Excitation and Emission is a cyclic process and consequently can be repeated to an extent before it starts to fade, termed as photobleaching.

We will now focus our discussion referring to fluorescent dyes used for molecular probes and primer design. The popular ones in use are Fluorescein, 6-FAM (6-fluorescein amidite), HEX (hexachloro fluorescein), TET (tetrachloro fluorescein), all fluorescein derivatives, Cy series dyes, the Alexa series dyes and rhodamine derivatives. The dyes are selected based on the excitation and emission wavelengths, bleaching, quenching and various other biophysical factors. All dyes when excited undergo a conformational change based on the interaction with other molecules, bases and dye in close proximity. The emission is thus bound by these variables of the microenvironment interaction. The amount of emission will vary and not all the energy consumed and absorbed during excitation is released as fluorescence.

The excitation time is usually is in picoseconds to nanoseconds; Similarly the emission time is in nanoseconds time frame. The energy used for excitation elevates the dye from a stable and relaxed state, termed as S0 to S1-S2. The transition from S2 to S1 is without any release of light energy. The photons emitted from the excited S1 state on its return to the relaxed S0 state are not equal to the difference between the excitation and emission maxima, this is termed as the Stokes shift. The Stokes shift represents the energy lost while the molecule was in the excited state.



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. Generally, it would be an impediment if the emission were reduced. 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.

Natural quenching occurs due to 'fading' after repeated cycles of excitation and relaxation. The decrease in the ability of further excitation of a proportion of molecules is termed as photobleaching. Some dyes are much more sensitive than other to photobleaching, for example fluorescein photobleaches very easily. Often the rate of decomposition is proportional to the intensity of illumination. So a simple practical way to overcome this is to reduce the incident radiation. It is sometimes possible to introduce antioxidants such as phenylalanine or azide to reduce bleaching. Quenching is also observed when the concentration of the dye is too high and the overall brightness decreases. This is 'self quenching'. It is observed that multiple labeling of an oligo with the same dye does not always lead to an increase in fluorescence.

Placing a molecule that absorbs light in close proximity to the fluorophore can induce quenching. Quenching is distance dependent quite similar to FRET, and can be assumed that the energy transfer typically occur over a distance of 1-10 nm. The quenching effect is exhibited by fluorescent as well non-fluorescent molecules. A non-fluorescent quencher is the basis of the design of Molecular Beacons. This molecule could be non-fluorescent and acts as energy sink, and termed as a quencher. In other instances this molecule could itself be a dye with overlapping spectral absorption and emission spectra, in such cases energy is transferred from one to another without any emission of light energy. This is termed as resonance energy transfer. Generally, the term 'quencher' is used for non-fluorescent molecules in probe design and 'double dye' or 'dual dye' used for probes with two dyes with spectral overlap.

Fluorescence Resonance Energy Transfer (FRET)

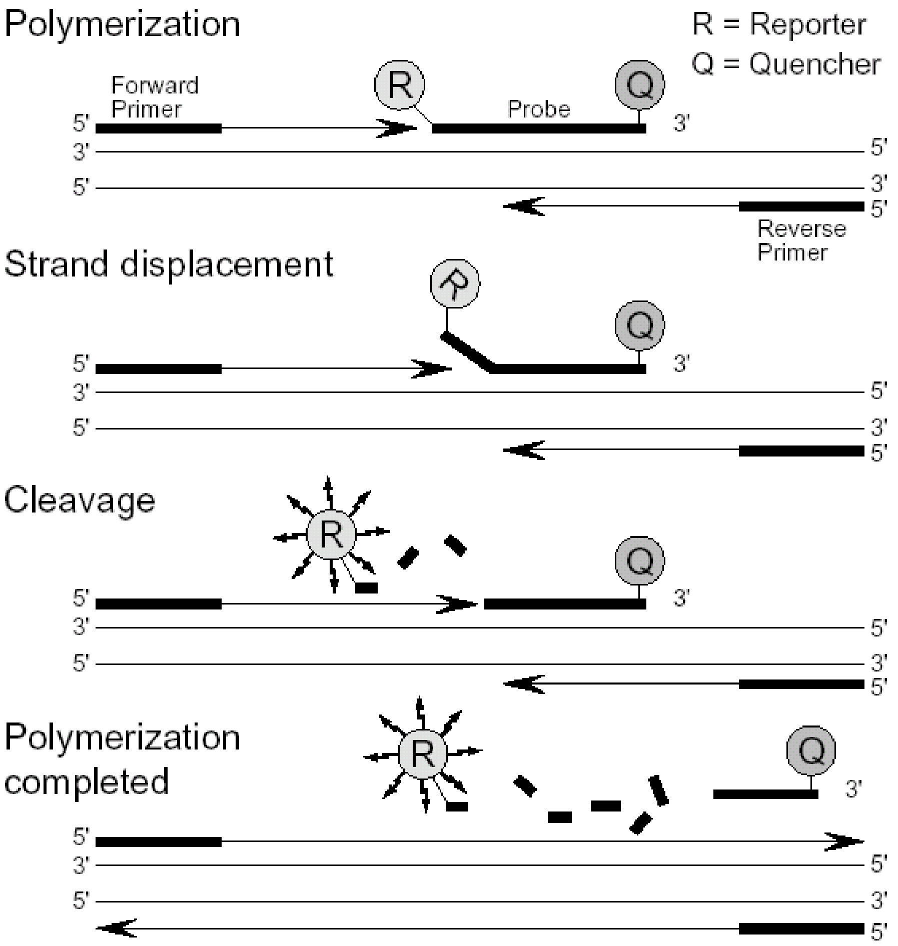
Resonance energy transfer, often known as fluorescence resonance energy transfer (FRET) or Förster energy transfer. It is the radiationless transfer of excitation energy from a donor to an acceptor. An important consequence of this transfer is that there is no emission of light by the donor. The acceptor may or may not be fluorescent. FRET is a distance-dependent interaction where the energy transfer occurs typically over a distance of 1-10 nm. The distance dependent nature of FRET is highlighted by the fact that it is proportional to the inverse sixth power of the intermolecular separation. The fact that FRET typically occurs in the 1-10 nm region means that these separation distances are comparable with the dimensions of biological macromolecules. This means that FRET can be a valuable tool in studying proximity events in biological systems. One helical turn is ~3.4nm and comprises of 10 nucleotides. A nucleotide is thus ~0.34 nm or 3.4Å (1 Å= 0.1nm). Thus, to observe FRET and quenching the donor and acceptor should not be placed more than 30 nucleotides away.

FRET varies based on the degree of spectral overlap of the donor and acceptor. That is the degree to which the emission band of the donor and the absorption band of the acceptor overlap. 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.

There are many other applications and consequences arising from these equations. For example if the donor and acceptor are the same molecular species it is still possible to observe FRET. This is called homotransfer and could be thought of as energy migration. The obvious conclusion from this is that the observed fluorescence would not be changed. However, at high concentration of dye it is possible to observe concentration quenching. In this case the transfer of the energy does not result in emission, the explanation for this is that the transfer is occurring at less than a critical distance and some of the dyes are acting as energy sinks (Source: AmershamBiosciences Website).

TaqMan

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. Black Hole Quencher. 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. If the target of interest is present, these probes specifically anneal between the forward and reverse primer sites. During the reaction, the 5′ to 3′ nucleolytic activity of Taq polymerase cleaves the probe between the reporter and the quencher only if the probe hybridizes to the target. The probe fragments are displaced from the target, separating the reporter dye from the quencher dye and thus resulting in increased fluorescence of the reporter. Accumulation of PCR products is detected directly by monitoring the increase in fluorescence of the reporter dye. Because increase in fluorescence signal is detected only if the target sequence is complementary to the probe, nonspecific amplification is not detected.



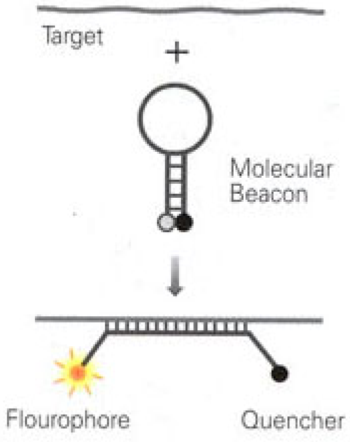
Design of TaqMan Primers and Probes

Some guidelines for TaqMan probes and primers selection are as follows:

- G-C content between 20% and 80%
- Avoid runs of an identical nucleotide, especially guanine
- Avoid G to be on the 5′ end
- Probes and primers should contain more C than G
- Melting temperature (T_m) should be 68-70°C for probes and 58-60°C for primers
- The five nucleotides at the 3′ end of each primer should have no more than two Gs and/or Cs
- Give precedence to better probes over primers
- Probe should be as close to 5′ primer as possible without overlapping

Molecular Beacons

Molecular beacons are oligonucleotide probes that can report the presence of specific nucleic acids in homogenous solutions (Tyagi S, Kramer FR. Molecular beacons: probes that fluoresce upon hybridization, Nature Biotechnology 1996; 14: 303-308.) They are useful in situations where it is either not possible or desirable to isolate the probe-target hybrids from an excess of the hybridization probes, such as in real time monitoring of polymerase chain reactions in sealed tubes or in detection of RNAs within living cells. Molecular beacons are hairpin shaped molecules with an internally quenched fluorophore whose fluorescence is restored when they bind to a target nucleic acid (Figure 1). They are designed in such a way that the loop portion of the molecule is a probe sequence complementary to a target nucleic acid molecule. The stem is formed by the annealing of complementary arm sequences on the ends of the probe sequence. A fluorescent moiety is attached to the end of one arm and a quenching moiety is attached to the end of the other arm. The stem keeps these two moieties in close proximity to each other, causing the fluorescence of the fluorophore to be quenched by energy transfer. Since the quencher moiety is a non-fluorescent chromophore and emits the energy that it receives from the fluorophore as heat, the probe is unable to fluoresce. When the probe encounters a target molecule, it forms a hybrid that is longer and more stable than the stem and its rigidity and length preclude the simultaneous existence of the stem hybrid. Thus, the molecular beacon undergoes a spontaneous conformational reorganization that forces the stem apart, and causes the fluorophore and the quencher to move away from each other, leading to the restoration of fluorescence.



Operation of molecular beacons: On their own, these molecules are non-fluorescent, because the stem hybrid keeps the fluorophore close to the quencher. When the probe sequence in the loop hybridizes to its target, forming a rigid double helix, a conformational reorganization occurs that separates the quencher from the fluorophore, restoring fluorescence.

In order to detect multiple targets in the same solution, molecular beacons can be made in many different colors utilizing a broad range of fluorophores (Tyagi S, Bratu DP, Kramer FR. Multicolor molecular beacons for allele discrimination, Nature Biotechnology 1998; 16: 49-53.) DABCYL, a non-fluorescent chromophore, serves as the universal quencher for any fluorophore in molecular beacons. Owing to their stem, the recognition of targets by molecular beacons is so specific that single-nucleotide differences can be readily detected.

Molecular Beacon Example Sequence

Fluorophore at 5' end; 5'-GCGAGCTAGGAAACACCAAAGATGATATTTGCTCGC -3'-DABCYL,

```
5' GCGAGCTAGGAAACACCA
   ||||||           ]
3' CGCTCGTTTATAGTAGAA
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STEM AT 1 IS 6 BP LONG. LOOP = 24.

The vertical lines at the 5' and 3' ends identify the arm sequences that are complementary.

The length of the probe sequence (10-40 nt) is chosen in such a way that the probe target hybrid is stable in the conditions of the assay. The stem sequence (5-7 nt) is chosen to ensure that the two arms hybridize to each other but not to the probe sequence. Folding of the designed sequence with the help of a computer program can indicate whether the intended stem-and-loop conformation will occur. The computer program can also predict the melting temperature of the stem.

Signal to background ratio

1. Determine the fluorescence (F_{buffer}) of 200 µl of molecular beacon buffer solution using 491 nm as the excitation wavelength and 515 as the emission wavelength. If the fluorophore is not fluorescein, chose wavelengths that are optimal for the fluorophore in the molecular beacon.
2. Add 10 µl of 1 µM molecular beacon to this solutio. Record the new level of fluorescence (F_{close})
3. Add a two-fold molar excess of the oligonucleotide target and monitor the rise in fluorescence until it reaches a stable level (F_{open}).
4. Calculate the signal to background ratio as $(F_{open}-F_{buffer})/(F_{close}-F_{buffer})$.

Thermal denaturation profiles

1. Prepare two tubes containing 50 µl of 200 nM molecular beacon dissolved in 3.5 mM $MgCl_2$ and 10 mM Tris-HCl, pH 8.0 and add the oligo target to one of the tubes at a final concentration of 400 nM.
2. Determine the fluorescence of each solution as a function of temperature using a thermal cycler with the capacity to monitor fluorescence. Decrease the temperature of these tubes from 80 °C to 10 °C in 1 °C steps, with each hold lasting one min, while monitoring the fluorescence during each hold.

Real time monitoring of polymerase chain reactions

Utilize molecular beacons that are complementary to a sequence in the middle of the expected amplified target fragment. The length of their arm sequences should be chosen so that a stem is formed at the annealing temperature of the polymerase chain reaction. The length of the loop sequence should be chosen so that the probe-target hybrid is stable at the annealing temperature. Whether a molecular beacon actually exhibits these designed features is determined by obtaining thermal denaturation profiles. The molecular beacons with appropriate thermal denaturation characteristics are included in each reaction at a concentration similar to the concentration of the primers. During the denaturation step, the molecular beacons assume a random coil configuration and fluoresce. As the temperature is lowered to allow annealing of the primers, stem hybrids form rapidly, preventing fluorescence. However, at the annealing temperature, molecular beacons also bind to the amplified target fragment and generate fluorescence. When the temperature is raised to allow primer extension, the molecular beacons dissociate from their targets and do not interfere with polymerization. A new hybridization takes place in the annealing step of every cycle, and the intensity of the resulting fluorescence indicates the amount of accumulated amplified target fragment.

Procedure

1. Set up six 50 µl reactions so that each contains a different number of targets, 0.34 µM molecular beacon, 1 µM of each primer, 2.5 units of Taq polymerase, 0.25 mM of each deoxyribonucleotide, 3.5 mM $MgCl_2$, 50 mM KCl, and 10 mM Tris-HCl, pH 8.0.
2. Program the thermal cycler to incubate the tubes at 95 °C for 10 min to activate Amplitaq Gold DNA polymerase, followed by 40 cycles of 30 sec at 95 °C, 60 sec at 50 °C and 30 sec at 72 °C. Monitor fluorescence during the 50 °C annealing steps.

Troubleshooting

- The assay medium may contain insufficient salt. There should be at least 1 mM $MgCl_2$ in the solution, in order to ensure that the stem hybrid forms.
- The molecular beacon may fold into an alternate conformation that results in a sub-population that is not quenched well. Change the stem sequence (and probe sequence, if necessary) to eliminate that possibility. Incomplete restoration of fluorescence at low temperatures.
- If the stem of a molecular beacon is too strong, at low temperatures it may remain closed while the probe is bound to the target. This may happen inadvertently if the probe sequence can participate in the formation of a hairpin that results in a stem longer and stronger than originally designed. Change the sequence at the edges of the probe and the stem sequence to avoid this problem.

Molecular Beacon Design

The PCR primers themselves should have been optimized in a regular PCR to see that it performs well. Assuming the melting temperature of the primers are ~55 degrees.

There are two independent features to control in the design of the MB probe. The stem and the target loop sequence. Design the probe sequence and see that there is minimal secondary structures, loop formation and dimers and the TM is ~5 degree higher than the PCR primer annealing temp. For a good guideline keep it at ~60 degrees. Add the stem sequence of 5-7 bp. The TM of the stem itself will be ~ 60-70 degrees. You are done!.

General guidelines for MB design at Gene Link are as follows:

1. Design regular 18-24mer PCR primers for amplification with a TM around 55^0C. The optimal amplified fragment should be between 100-300 bp. Perform PCR, optimize conditions. Should get good clean amplification product visible on ethidium bromide stained gels.
2. Design target probe sequence with a TM ~8-10 degrees higher than the PCR primers annealing temp. Example 60-65 degrees. The probe should be designed near the center of the amplified fragment avoiding stretches of strong secondary structure. [Taqman probes are designed ~ 5-10 bases near the primer of the same strand]
3. Add the stem 5- 7 bp stem sequence with a GC content of 70-80%. Avoid a G at the 5′ end next to the fluorophore. G's seem to quench. Hairpin Stem TM should be 7-10 degrees higher than the PCR annealing temperature. Example 65-70 degrees.
Caution: See that by adding the stem you have not created secondary structures with the loop sequence. Try variation of stem sequence to avoid secondary structure with the loop sequence.

The Hairpin stem TM is based on free energy stabilization and folding, the following is a good guideline.

GC rich stem
5 bp = 55^0C- 60^0C
6 bp = 60^0C-65^0C
7 bp = 65^0C-70^0C

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.

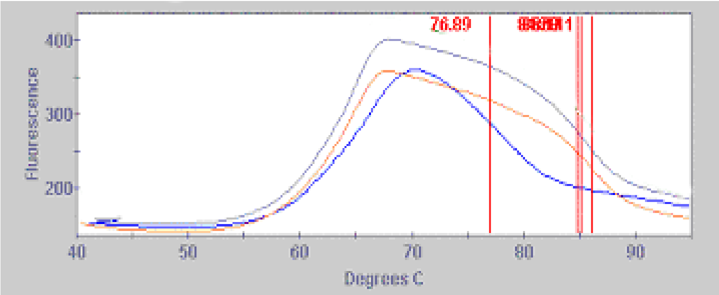
2. Prepare Molecular Beacon working solution at 5 pmols/μl [5 μM (micromolar)] in 1 X PCR Buffer.

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

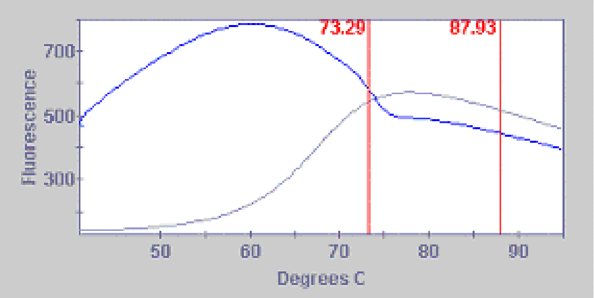
Molecular Beacon Probe Alone	**Molecular Beacon Probe plus Target**
2.5 μl 10X PCR buffer	2.5 μl 10x PCR buffer
3 μl 25 mM MgCl2	3 μl 25 mM MgCl2
1 μl 5 pmol/ul probe [0.2 pmol/μl final or 200 nM]	1 μl 5 pmol/ul probe [0.2 pmol/μl final or 200 nM]
	3 μl 5 pmol/ul target [0.6 pmol/μl final or 600 nM]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

Fluorescent Probes / Molecular Beacons Frequently Asked Questions

In a small-scale synthesis (eg. ~5-10 nmole; 2000-5000 assays), what is the amount of fluorophore-conjugated oligonucleotide typically obtained after purification?

The smallest scale of synthesis offered by Gene Link for Molecular Beacons is at the 200 nmol scale. Synthesis at the lower scale does NOT appreciably reduce cost, as cost of synthesis is NOT directly proportional to the scale of synthesis. Whereas, the higher scale affords Gene Link the ability to comfortably purify the product by polyacrylamide gel, and thus provide our customers with a product of the highest quality. **Gene Link provides between 7-15 nmols of purified product at the 200 nmols scale synthesis that is sufficient for 2000-5000 assays.**

How are these purified (eg. PAGE or HPLC)?

Answered partly above. Gene Link performs and strongly recommends gel purification and believes it to be the "gold standard" of purification. All other forms of purification depend on either hydrophobic (Reverse Phase HPLC) or ionic affinity (Ion-exchange HPLC); both of these techniques are sequence dependent and do not yield the purity achieved by gel purification. Gene Link provides a before-and-after gel picture, i.e of the crude and the gel purified oligo.

Is there mass spectroscopic confirmation of the purified product?

Not applicable. Gene Link performs gel purification and supplies a real gel picture. Molecular Beacons provided by Gene Link should have better than 50:1 signal to background ratio, usually in the range of 200. The purity is greater than 98%.

Do beacons undergo reverse complement (ie. target) verification and is a close to open ratio analysis performed?

Gene Link currently does not perform these analyses as they consider "after the fact". These analyses do NOT constitute quality of oligo synthesis but rather of design of oligonucleotide. Customers designing their probes should keep the parameters in mind. Gene Link can guide customers to the design of the stem and the loop and the Tm. A good initial reading of Fred Kramer's articles suffices the need, just as designing oligos for PCR, sequencing etc.

What fluorophores are available for conjugation?

The most popular ones are FAM, Fluorescein, HEX and TET, Cy5 and Cy3 in that order; others available as post synthesis conjugation are Alexa Series Dyes, Tamra,Texas Red, Coumarin, etc.

What quenchers are available for conjugation?

In additional to Dabcyl, BHQ-1, BHQ-2 and BHQ-3 are also available.

How quickly and in what form are fluorescent probes shipped?

Gel purified fluorescent probes are routinely shipped in 4-5 business days. These are shipped lyophilized ready to use.

What type of guarantees could be provided in the event of an inferior or incorrect fluorescent probes synthesis (eg. free replacement)?

All Gene Link products are replaced at no charge for not meeting specifications. This rarely happens. This means free replacement for the same sequence. We usually suggest designing a new probe/sequence or to avoid stretches of G" and C's etc.

Would a free fluorescent probes sample be available for testing by our laboratory?

Yes. New customers are offered, "Buy One, Get One Free" of the same fluorophore.

Would there be on-going technical support in the form of updating us on new developments in beacon synthesis?

Yes. We get to know our customers and talk frequently. The latest development is the wavelength shift, incorporating a different fluorophore in the stem in addition to the 5' end. This is similar to FRET.

Gene Link is not an "oligo factory." We believe as scientist and researchers that each oligo/probe/gene construction/genotyping etc. is an experiment to be well performed. We believe in quality, consistency and gaining confidence ourselves in our products, and ultimately gaining our customer's total confidence in Gene Link products. We hope we have answered most of the frequently asked questions. Please feel free to call (1-800-436-3546) or email (support@genelink.com) if you require more information.

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

Alexa Dye Series

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. Source: Molecular Probes www.probes.com

Ordering Information

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 quantitative 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.

The table below lists the common dyes for use in TaqMan probes with Tamra or BHQ at 3' end and Molecular Beacons with universal fluorescence quencher dabcyl or BHQ at 3' end. Price includes gel purification. Gene Link considers gel purification to be the best method of purification and essential for optimum performance of fluorescent dye labeled oligonucleotides. Customers may request fluorescent probes without gel purification for reduced pricing (not recommended).

Fluorophore*	Color	Absorbance max (nm)	Emission max (nm)	$, 200 nmol scale	$, 1 µmol scale
Dabcyl (Quencher)		453		$290	$350
BHQ-1** (Quencher)		534		$290	$350
BHQ-2** (Quencher)		579		$290	$350
BHQ-3** (Quencher)		672		$290	$350
6-FAM (Fluorescein)	Green	494	525	$390	$450
TET	Orange	521	536	$390	$450
HEX	Pink	535	556	$390	$450
Cy 3	Red	552	570	$390	$450
Cy 3.5	Purple	588	604	$390	$450
Cy 5	Violet	646	667	$390	$450
Cy 5.5	Blue	683	707	$390	$450
Cy 7	Near IR	743	767	$390	$450
Tamra	Rose	565	580	$540	$600
ROX	Purple	587	607	$540	$600
JOE	Mustard	528	554	$540	$600
Texas Red-X	Red	583	603	$540	$600
Cascade Blue	Blue	396	410	$540	$600
Marina Blue	Blue	362	459	$540	$600
Alexa Dye Series	Varies	Varies	Varies	$540	$600
Please see our complete list at www.genelink.com or call at 1800-436-3546					

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