



Technology FAQ's

What does QTL stand for?

QTL stands for Quencher-Tether-Ligand. Please see the description of our technology at our website for more information.

How does QTL technology work?

The QTL technology combines molecular recognition with fluorescent signaling of binding. The key is our proprietary QTL (quencher-tether-ligand) molecule, which turns off or turns on the emission of a fluorescent polymer, upon competition for binding with a ligand. The polymer is a highly fluorescent, water-soluble plastic that may be coated onto a variety of surfaces, including plastic or glass microspheres, micro-titer plate wells, nanoparticles, membranes, micro-fluidic channels, or micro-arrays. Our fluorescence response is tunable over the visible and near infrared, so that our assays can be easily configured to work in any commercial fluorometer. The polymer gives an amplified quenching sensitivity, which leads to increased assay sensitivity over FRET or other homogeneous assay formats.

What is (define) superquenching?

Superquenching is quite simply amplified quench sensitivity. In the context of QTL technology we observe a 10^{6-7} fold greater Stern-Volmer quenching (K_{sv}) constants than have been observed with typical small molecule donor-acceptor pairs. QTL polymer superquenching derives from two separate, but equally important processes: a. A high association constant between the QTL sensor, comprised of polymer co-located with a recognition molecule, and a quencher tagged target species that is captured by the sensor, and b. Rapid excited state energy migration amongst the assembled polymer chains on the sensor surface, which increases the probability of either an energy transfer, or electron transfer event occurring between polymer and quencher.

How do you perform multiplexing with QTL technology?

The simplest approach to multiplexing involves “dressing” different populations of QTL sensors with different capture molecules that recognize different target species. This will also require unique QTL's for each prospective target analyte. In practice one would simply deposit these differently labeled populations of sensors in separate wells of a standard 96, 384 or 1536 well plate, then challenge each well with the samples/QTL's of interest.

Can you tune the wavelength of light emission?

Yes. A wide range of polymers is available to QTL with light emission extending from the UV region to the near IR region. Several polymers throughout this broad window have produced QTL successes with the wavelength of light emission tuned from blue to orange (380nm to 590nm) by selecting different conducting polymers or modifying currently used conducting polymers. For example, the three different conducting polymers listed below emit light at different wavelengths: water-soluble PPE, water-soluble PPV, and water-soluble polythiophene. The conducting polymers used in QTL assays can be categorized into two major types: a) Ionic Conducting Polymers (cationic or anionic) and b) Neutral Conducting Polymers. The latter type could be constructed from two or more types of monomers, amplifying wavelength-tuning effects.

Can a single quencher quench at more than one wavelength?

Yes. Most of our quenchers quench *via* a Förster energy transfer mechanism that requires spectral overlap between the emission band of the quenched material (i.e. the fluorescent polymer) and the absorption band of the quencher (i.e. the QTL). As a result, a single QTL can effectively quench emission over a range of wavelengths, provided they fall within the absorptive spectral envelope of the quencher. Due to the typically broad absorbance spectra of our quenchers, we can both select an optimally matched quencher for any given polymer as well as employ a single quencher for a number of different polymers that have similar emission spectra. Furthermore, some of our quencher molecules have more than a single absorption maximum in the visible light region that overlap with the emission spectrum of the conducting polymer. In such a case, we will observe light quenching from more than one wavelength. For example, dyes from the cyanine or rhodamine families have two absorption peaks; they will be able to quench the light emitted by conducting polymers at two different wavelengths.

What is the sensitivity of the QTL assay?

This question is best answered by providing actual sensitivity data from two representative examples of QTL assay applications. a) DNA detection in the QTL assay is in the nM range; ~500 femtomoles of target DNA/200 μ L total assay volume. b) The detection level for a protease (enterokinase) is in the sub-nanomolar range. We anticipate decreasing these detection limits by one to two orders of magnitude depending upon specific target molecules.

Are QTL microspheres truly “suspension beads”?

Yes and no. Microspheres by definition are suspensions when dispersed in liquid. The larger the particle diameter of the microspheres, the more likely they are to sediment. QTL microspheres routinely range in diameter from 50nm to 1000nm. Latex or silica microspheres in this size range remain in suspension for several minutes. The QTL microspheres are highly charged due to the polymers coated on them; therefore they do not readily aggregate or sediment. Due to the true “mix-and-measure” scope of QTL

assays, even larger diameter (1000nm-10000nm) microspheres do not settle during the QTL assay duration.

[What are some of the advantages of the QTL technology over FRET assays?](#)

The principal advantages of the QTL technology over FRET are: a) Light harvesting capability of our polymers and b) Superquenching! It is important to point out that an analogous sensor formed by co-assembling small organic dyes, like those used in FRET, with a capture species do not work for two reasons; 1) Upon concentration, small organic dyes undergo extensive electronic interactions resulting in non-emissive excited state complexes, i.e. the dye quantum yields drop dramatically, ii) The unique electronic structure of the QTL polymer affords facile inter/intra-molecular energy migration absent in small molecules. This latter point is particularly important because it increases the effective quench radius of the QTL polymer-quencher interaction, compared to highly localized FRET events. And a greater effective quench radius translates directly to greater sensitivity.

[What is the distance dependence of quench response? Is it similar to FRET? Will quench response be attenuated or disappear entirely at 100 Å between polymer and quencher?](#)

The actual distance dependence of quenching has not been accurately measured and remains unknown. However, we do know from employing spatially large QTL reagents such as antibody-quencher ensembles, that there is a reduction in quench response due in part to the greater average distance between a potential quencher, and the QTL polymer at the QTL sensor surface. The functional form of this roll-off in quench sensitivity with distance is likely to be much weaker than the inverse r^6 characteristic of FRET. In solution studies of sensing polymers similar to those employed in QTL sensing, quench radii as high as 300 Å have been measured. Also, in organized films of QTL polymers exciton diffusion lengths of ~100 Å were observed.

[How many reactions can be performed using a single QTL Assay test kit?](#)

QTL Biosystems will customize QTL Assay kit for the number of reactions that a customer wishes to perform. QTL Biosystems customers will initially utilize unique QTL Assay test kits for each target or application using single colored polymers. QTL Biosystems will develop QTL assay kits that permit multiplexing within a well either by using a spatial array or different color polymers.

[Will QTL assay work with any type of fluorometer?](#)

Yes. QTL assay will work with any make, model, and brand of fluorometer from any manufacturer, in any format, tube-based and microplate-based. However, some of the cheaper filter based instruments might require proper selection of excitation/emission filters. The main concern in performing QTL assays in fluorometers is the intensity of the light source. QTL sensors rapidly photo bleach under the incident flux densities

common to most fluorometers employing high output Xe arc sources. However, employing either neutral density or spatial filters can circumvent photobleaching.

Can QTL assays be adapted for HTS applications?

Yes. The deposition of QTL polymer-coated microspheres into 96 – 1536 wells and subsequent homogeneous quench assay could be scaled to a HTS format. Alternately, biotinylated QTL polymers could be deposited onto streptavidin-coated microplate wells to facilitate HTS homogeneous and/or heterogeneous assay formats.

What is coated on the microsphere – QTL polymer or the quencher?

The QTL polymer is coated onto the microspheres. The Quencher is also “coated onto the microsphere” *via* any specific interactions that occur with the capture ligand that is also coated onto the surface of the microspheres.

How complete or uniform is the coating of the polymer on the microspheres?

Statistically, the QTL polymer coatings should be uniform. Sufficient care is taken to relate the surface area of the specific microsphere being coated to the necessary parking area of the QTL polymer repeat units in order to effectively coat a monolayer. Analysis of the number of repeat units of the QTL polymer coated onto the parking area per polymer repeat unit do indicate a monolayer in the bulk of the coated microspheres.

What is the size of the QTL quencher and what is it made of?

QTL Biosystems quenchers are typically energy transfer; water-soluble, organic dye molecules with extended conjugated π -systems. They can be neutral, cationic or anionic. Suitable functional groups on these quenchers allow easy coupling of DNA, proteins, peptides, enzymes or antibodies. Various base structures and chemical modifications are employed to tune the spectral properties of the quenchers. Overall, the typical size of the quencher portion of a QTL is similar to that of fluorescein. The size of the quenchers also varies somewhat from one group to another. In general, quenchers range in size from a few Å to ~ 30 Å.

Are fluorescent polymers susceptible to photobleaching?

Fluorescent polymers are susceptible to photobleaching and QTL polymers are no exception especially when exposed to intense UV or Visible light. However, the care required for handling QTL reagents is not extreme. If the exposure of QTL polymers to normal light is kept short and by following good laboratory practices such as capping the QTL polymer vial immediately after use and storing the QTL polymer in the supplied vial and in the refrigerator at all times except during use, then the QTL polymer will remain stable for three months.

What is the photostability of the QTL reagents? Could assays be performed under fluorescent lighting conditions?

The photostability of QTL reagents depends upon the type of specific application being developed. In general, QTL polymers and quenchers are light sensitive and should be stored protected from light. However, QTL assays being rapid and true “mix-and-measure” could be performed under normal laboratory lighting conditions of 30-40 foot candles, 320-430 lux. When not in use, QTL Biosystems recommends that its reagents are kept refrigerated and protected from light. Occasionally, certain QTL polymers could be especially photosensitive and are not intended for multiple measurements. With such reagents, a 30 second scan on the fluorometer over the 450-700 nm range could reduce the fluorescence intensity by ~ 20%. Such QTL reagents will be delivered with appropriate assay conditions described in detail and the precautions to be adopted during fluorescence measurements.

Why are the QTL polymer and receptor placed together on QTL microspheres?

The QTL fluorescent assay takes place upon QTL microspheres surface. In order for the fluorescent QTL polymer to be susceptible to the externally delivered target analyte, the receptor to the analyte is also co-localized with the QTL polymer upon the microspheres surface. In this way, when the QTL reagent interacts with the receptor, the quencher is able to turn off the fluorescence. Fluorescence is regained in the presence of competing target analyte present in the customer’s sample.

In the scenario of non-specific quench of polymer by quencher, what drives the complexation?

In buffered aqueous solutions the principal thermodynamic driving force for polymer-QTL associations is the same as that responsible for the folded conformation of proteins, i.e. hydrophobic interactions. Charge based interactions are also likely to play a role since it is the surface potential of the QTL sensor that ultimately dictates the nature of its immediate chemical environment, which in turn influences the diffusion of solutes from the bulk solution to the sensor surface.

What is the quench response susceptibility to time? Will quench response be the same whether measured immediately or after several hours?

The stability of quench response is affected by factors such as stability of QTL polymer to assay conditions, the competition to binding the receptor by the QTL reagent and target, and the target assay under investigation. If the QTL polymer is not exposed to bright direct visible or UV light, it is stable for several hours. If the competition between the QTL and target analyte for the receptor is balanced completely in favor of the target, the signal is likely to remain stable over time. If the competition is not complete when the first measurement is made, then it is likely that subsequent measurements will produce different results.

This is best illustrated with examples of actual QTL assays. In the case of a protease assay for example, the use of a stop reagent that inactivates the target enzyme will prevent continuous cleavage of substrate by enzyme. The quench response of a step-wise DNA QTL assay does not change by more than 10% between $t = 15$ minutes and $t = 3$ hours. The small change that does occur actually improves the dynamic range of quenching. In the case of a competitive Ovalbumin QTL assay, the quench response, quench reversal and dynamic range was unaffected by storing the reaction mixtures at 4°C for 24 hours.