Fluorescent Dyes Introduction

Fluorescent dyes are routinely used in oligonucleotide-based research as detection labels for primers and probes. Single-dye-labeled oligos are effective as primers for sequencing, AFLP and microsatellite fragment analysis, and single-dye-labeled probes for fluorescent in situ hybridization (FISH) and oligonucleotide ligation assay (OLA) applications. Dual-labeled probes incorporating various matched dye and quencher combinations are often indispensable for quantitative experiments. Fluorescence-based detection offers a safe and sensitive method for both qualitative and quantitative detection of target sequences in vitro and in vivo. The elegant design of the newer probes has led to an exponential increase in the use of molecular probes, furthering new developments. Gene Link offers synthesis of all different forms of molecular probes and knowledgeable technical service in the design of novel probes, including chimerics.

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Fluorescent Dyes Design Protocols

Use of Controls in Fluorescence-based Real-Time Quantitative PCR (RT-qPCR) reactions—Design Considerations

A variety of fluorescence-based systems can be used to analyze samples using RT-qPCR, including sequence-specific nucleic acid probes (ex: 5'-nuclease probes, molecular beacons, FRET hybridization probes, Scorpions) and non-sequence-specific fluorescent intercalating dyes (ex: SYBR Green, Cyto dyes). No matter which system you choose, however, it is critically important that the proper negative and positive controls be run to ensure that your experimental results can be correctly interpreted. The following controls should be part of every RT-qPCR experiment. I. Negative Controls (each run in a separate tube)

a) a no-reverse-transcriptase control: For this control, the reverse transcriptase enzyme is not included in the reverse transcription reaction. Performing this control allows you to determine the amount of genomic DNA contamination in your RNA preparation.

b) a no-template control: For this control, the cDNA template is not included in the PCR reaction. Performing this control allows you to determine the amount of nucleic acid contamination and/or primer-dimer formation. It is particularly important to do this control if you are using a fluorescent intercalating dye for detection.

c) a no-amplification control: For this control, the DNA polymerase is not included in the PCR reaction. Performing this control allows you to determine background fluorescence in the PCR reaction. II. Positive Controls

a) exogenous positive control (run in a separate tube): For this control, external RNA or DNA, containing the target of interest (but NOT one of your experimental samples), is added to the reverse transcription or PCR reaction in place of an experimental sample. This control allows you to verify that the reaction is working properly, that the reaction is not being inhibited by something in the reaction mix, and that the fluorescence signal is being generated and detected. Although PCR-generated and purified amplicons can be use as exogenous positive controls, using a synthetic ultra-long oligo (Synthetic Positive Control –SPCT) is more robust because it provides you with a known copy number.

b) endogenous positive control (can be run in a separate tube or in multiplex with the experimental sample): For this control, a second target, native to the species of interest and known to be in the experimental sample, is used to normalize the various fluorescent signals obtained from those samples. Most commonly, reference genes are selected for this. Such genes are ubiquitously expressed in all tissues. For example, a list of such reference genes for mouse is found here, and for human, here.
Fluorescent Dyes Applications

Fluorescent dyes are particularly advantageous in biological research because they combine very high sensitivity and selectivity in target detection with low toxicity. As such, they have now become the detection method of choice for tracing the presence of specific biomolecules in cells, cell culture whole organisms, and in vitro assays.

Single-dye labeled oligonucleotides are routinely used as cytogenetic probes in Fluorescence In Situ Hybridization (FISH) to detect and localize specific DNA sequences on chromosomes. FISH is also used to detect and localize specific mRNAs within tissues, thereby revealing spatial-temporal patterns of gene expression within both cells and tissues (1,2). Besides FISH, other common applications of single-dye oligos are as fluorescence-based sequencing and genotyping primers (3), and as probes for oligonucleotide ligation assay (OLA) systems (4), or for SNP detection on microarrays (5).

Dual-dye labeled oligos are particularly useful in fluorescence resonance energy transfer (FRET) experiments for determination of intra- and intermolecular distances at very high resolution (1-10 nm) (6). In addition, dual-labeled oligos containing fluorescent dye/dark quencher pairs are now routinely used in quantitative and qualitative real-time PCR experiments and assays (5′-nuclease assay, Molecular Beacon, Scorpions, etc.) Details of how such dual-labeled probes work for detection of minute amounts of target are found in the Quenchers modifications category.

Fluorescent modifications can also be combined with non-fluorescent modifications in a wide variety of combinations for use in highly specializing applications or research projects.

Dye & Quencher Selection Table

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References