Fluorescein Amidites and Supports

Fluorescent Dye Labeled Oligonucleotides:

- Conjugation of fluorophores to an oligonucleotide can be achieved in several ways. However, it is more advantageous to use fluorophore phosphoramidites to incorporate into an oligonucleotide using automated oligonucleotide synthesizer. This method obviates more laborious steps of solution phase labeling and purification.
- Changes in fluorescence emission intensity can reflect changes in the environment and molecular motion of a fluorophore. Researchers can capitalize on these characteristics to assess the affinity and specificity of oligonucleotides with target such as receptors, proteins or nucleic acids.
- Fluorescent dye labeled oligonucleotides have been used extensively in wide variety of applications such as genetic analysis, DNA sequencing, amplification-based diagnostic assays, forensic identity tests and other methods for detecting and quantitating target DNA.
- The fluorescent detection assays are simple to read, sensitive and very fast in kinetics. These assays do not require laborious washing and separation steps. These assays can be automatized for several molecular biology applications.
- Since majority of oligonucleotides are designed for sequencing analysis and amplification experiments. Probes for these applications require the 3'-end to be available for extension. So, 5'-labelling is required for these applications.
- High yield of final fluorophore coupled oligonucleotides requires an efficient coupling of fluorescent dye, which can be achieved with a high purity of the fluorophore amidite or supports.

Fluorescein (FAM) labeling:

Fluorescein (abbreviated as FAM) is the most commonly used fluorescent dye for labeling of oligonucleotides. ChemGenes offer isomerically pure 5 or 6-FAM amidites and supports with different linker as shown in Figure 1. FAM amidites (CLP-9777, CLP-4282, CLP-9780 and CLP-9789) incorporates a fluorescent fluorescein moiety at the 5' terminus of an oligonucleotide. On the other hand, it can be conjugated at 3'-end of the oligonucleotide using FAM supports (N-9985-05 and N-9969-05).

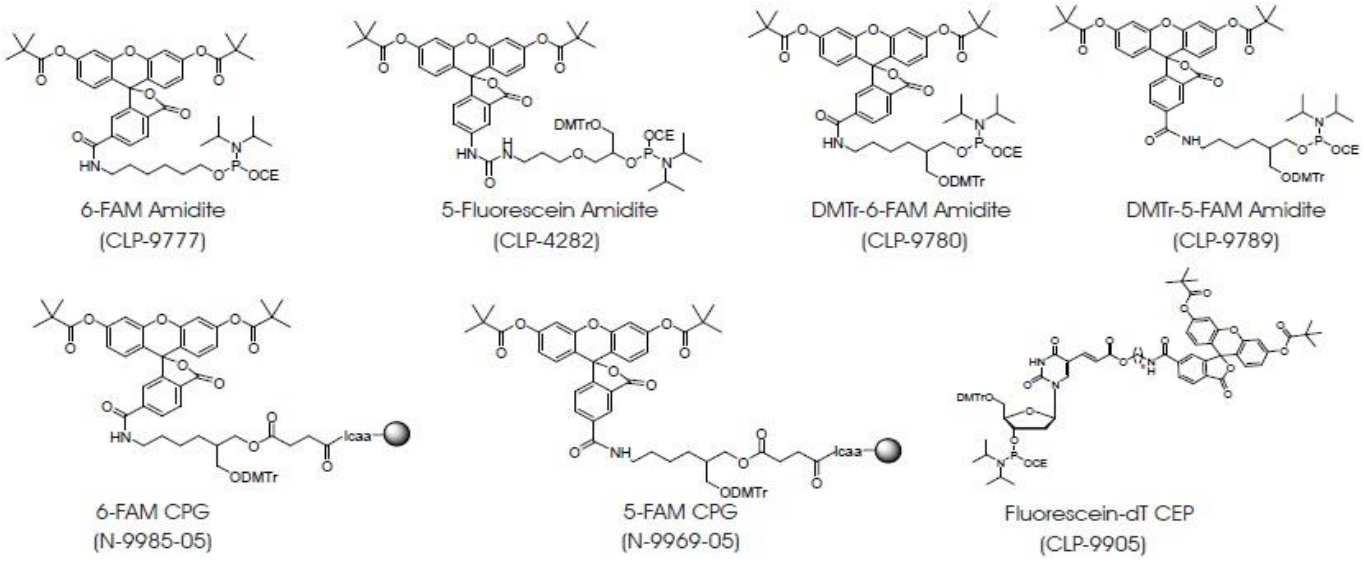

Figure 1: Chemical structures of FAM supports and Fluorescein dT amidite.

Applications:

- FAM has maximum absorbance at λ 494 nm and emission at λ 521 nm.

- 5'-FAM modified oligonucleotides can be used in a wide array of applications, including dual-labeled fluorogenic probes for real-time PCR.[1]
- FAM fluoresces in the green region of the visible spectrum and its fluorescence can be effectively quenched by BHQ-1 dye.
- Fluorescein can be used to label DNA oligos for use as hybridization probes in a variety of *in vivo* and *in vitro* research or diagnostic applications, as well as for structure-function studies of DNA, RNA and protein-oligonucleotide complexes.
- Oligos labeled with fluorescein at the 5'-end can be used as PCR and DNA sequencing primers to generate fluorescently-labeled PCR, sequencing or genetic analysis products.
- 6-FAM-labeled primers have also been used for bacterial SNP genotyping by allele-specific real-time PCR.[2]

Fluorescein-dT Amidite:

- Fluorescein-dT has maximum absorbance at λ 494 nm and emission at λ 521 nm.
- Fluorescein-dT is a deoxythymidine nucleoside derivatized with 6-FAM (6-carboxyfluorescein) through a spacer arm (Figure 1, CLP-9905).
- Fluorescein-dT is used to internally label an oligonucleotide at a dT position.
- Fluorescein-dT can be useful for Fluorescence Resonance Energy Transfer (FRET) DNA oligonucleotide probe with a fluorophore.
- Such a labeling strategy is pertinent in cases where the distance between the quencher and fluorophore needs optimization for efficient quenching. For such applications, fluorescein is most commonly paired with the dark quencher BHQ-1.
- Oligos internally labeled with fluorescein-dT also can be used as PCR and DNA sequencing primers to generate fluorescently-labeled PCR, sequencing or genetic analysis products.

References:

1. Nazarenko, I. et. al. *Nucl. Acids Res.* **2002**, *30*, e37.

2. Huygens, F. et. al. *J. Clin. Microbiol.* **2006**, *44,* 3712-3718.