## FISH Probes Introduction

Probes for fluorescent in situ hybridization (FISH) are derived from repetitive chromosomal regions to paint a chromosome or are directed towards unique gene(s) for identification. These probes are usually developed from clones (BAC and cosmids) and more recently synthesized chemically as oligonucleotides appropriately labeled with fluorescent dyes. The oligo probes can be synthesized as tiles or longer sequences up to 250mer in length by Gene Link. Gene Link presents various design options for synthesizing 'Smart' FISH probes. Smart FISH probes can be synthesized in a predetermined way to exhibit the features that is desired; for instance to increase nuclease resistance we can substitute standard bases with 2' Fluoro bases. Standard DNA probes are like natural molecules that are prone to degradation under normal conditions, ubiquitous nucleases as well as chemical instability lead to fast degradation with a finite half life. The premise of this product guide is to discuss various modifications that are offered by Gene Link that may be used for design and synthesis of Smart FISH Probes. There are four common features that are desirable and in particular can be improved by using a combination of available nucleic acid modifications that modify the phosphodiester linkages, nucleic acid bases, the sugar moieties and addition of various other functional groups.

# FISH Probes Design Protocols

**Design Guidelines for FISH Probes**

1. Design multiple 24 to 30mer probes. Avoid stretches of more than 3 G or C bases.
2. To impart exonuclease resistance substitute 3-4 bases at the 5' and 3' end with 2'F bases. The 2' F bases imparts resistance to exonuclease degradation and increases duplex stability by 4-6 degrees.
3. Several internal bases can be substituted with 5me dC and 2 Amino dA to further increase duplex stability.
4. Affinity ligands such as Digoxigenin or Biotin or fluorescent dye e.g Cy3, Cy5 or any other can be labeled at the 3' and 5' end. Multiple internal sites can also be labeled with affinity ligands or fluorescent dyes to increase sensitivity.
5. Multiple dye sites should be spaced apart by 10 or more bases.
6. The above guidelines are for all initial FISH probe design. Design rules may have to be established empirically for very specific or novel assay settings, but following the above recommendations will provide a good start.

## FISH Probes Applications

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## References

1. Milligan, J.F., Matteucci, M.D. and Martin, J.C. (1993) Current concepts in antisense drug design. J. Medicinal Chem. 36:1923-1937.
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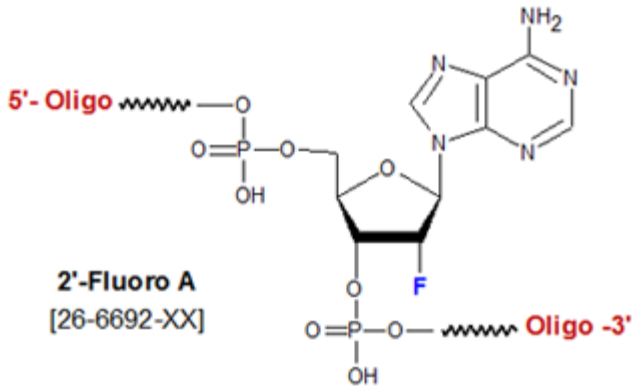
# Modificaton Code List

| Modification | Code | Catalog Number |
|---|---|---|
| 2'-Fluoro deoxyadenosine (2'-F-A) | [fA] | 26-6692 |
| 2'-Fluoro deoxycytosine (2'-F-C) | [fC] | 26-6463 |
| 2'-Fluoro deoxyguanosine (2'-F-G) | [fG] | 26-6693 |
| 2'-Fluoro deoxyuridine (2'-F-U) | [fU] | 26-6462 |
| 2'-O Me-5-Me-C | [m5mC] | 27-6508 |
| 2'-O methyl adenosine A | [mA] | 27-6410A |
| 2'-O methyl cytosine C | [mC] | 27-6410C |
| 2'-O methyl guanosine G | [mG] | 27-6410G |
| 2'-O methyl uridine U | [mU] | 27-6410U |
| 2-Amino dA | [Am-dA] | 26-6525 |
| Cy3-5' (Cyanine 3) | [5-Cy3] | 26-6437 |
| Cy5 (5'-Cyanine 5) | [5-Cy5] | 26-6436 |
| 5-methyl deoxycytosine [5mdC] | [5mdC] | 26-6413 |
| Amino C6 U (RNA) | [AmC6U] | 27-6422 |
| Amino deoxyadenosine dA C6 | [Am-dA-C6] | 26-6666 |
| Amino deoxycytosine dC C6 | [Am-dC-C6] | 26-6670 |
| Amino deoxyguanosine dG C6 | [Am-dG-C6] | 26-6669 |
| Amino deoxythymidine dT C6 | [Am-dT-C6] | 26-6438 |
| Cy3.5 (Cyanine 3.5) | [Cy3.5] | 26-6461 |
| Cy5.5 (Cyanine 5.5) | [Cy5.5] | 26-6460 |

| | | |
|---|---|---|
| Cy7 (Cyanine 7) | [Cy7-N] | 26-6474 |
| Fluorescein dT (Fam dT) | [Fam-dT] | 26-6422 |
| Fluorescein-5' | [Fl-5] | 26-6426 |
| Hex-5' (Hexachloro-Fluorescein) | [5-Hex] | 26-6432 |
| Tet-5' (Tetrachloro-Fluorescein) | [Tet-5] | 26-6433 |

# 2'-F-A

| | |
|---|---|
| Category | Nuclease Resistance |
| Modification Code | fA |
| Reference Catalog Number | 26-6692 |
| 5 Prime | Y |
| 3 Prime | Y |
| Internal | Y |
| Molecular Weight(mw) | 331.2 |

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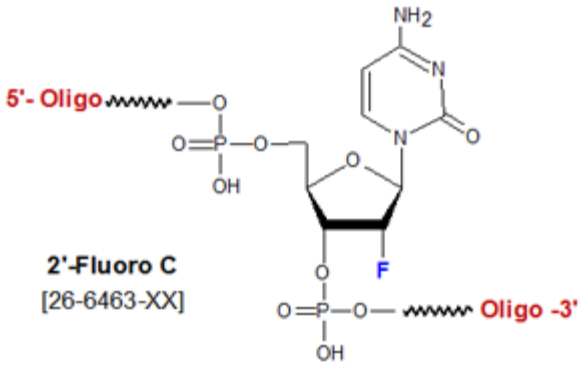
## 2'-F-C

| | |
|---|---|
| Category | Nuclease Resistance |
| Modification Code | fC |
| Reference Catalog Number | 26-6463 |
| 5 Prime | Y |
| 3 Prime | Y |
| Internal | Y |
| Molecular Weight(mw) | 307.17 |

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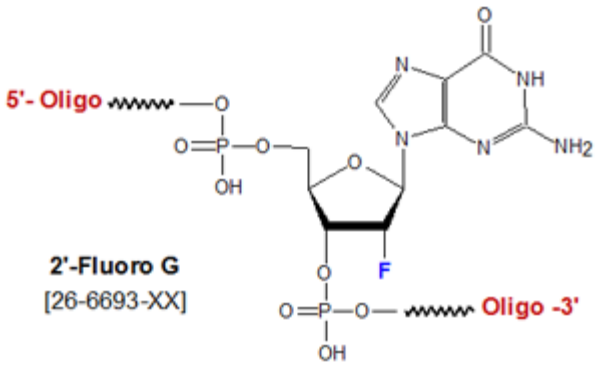
# 2'-F-G

| | |
|---|---|
| Category | Nuclease Resistance |
| Modification Code | fG |
| Reference Catalog Number | 26-6693 |
| 5 Prime | Y |
| 3 Prime | Y |
| Internal | Y |
| Molecular Weight(mw) | 347.16 |

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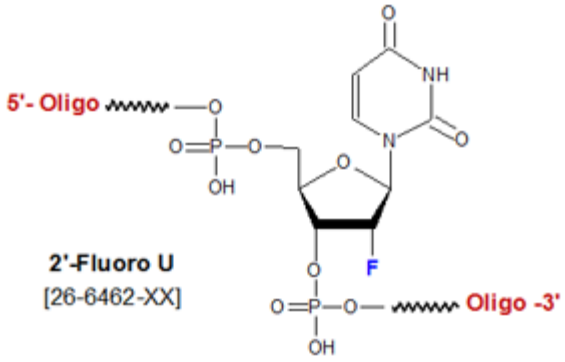
# 2'-F-U

| | |
|---|---|
| Category | Nuclease Resistance |
| Modification Code | fU |
| Reference Catalog Number | 26-6462 |
| 5 Prime | Y |
| 3 Prime | Y |
| Internal | Y |
| Molecular Weight(mw) | 308.16 |

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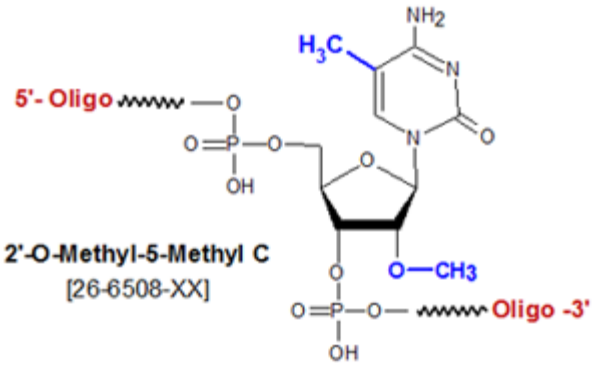
# 2'-O Me-5-Me-C

| | |
|---|---|
| Category | Structural Studies |
| Modification Code | m5mC |
| Reference Catalog Number | 27-6508 |
| 5 Prime | Y |
| 3 Prime | Y |
| Internal | Y |
| Molecular Weight(mw) | 333.24 |




Antisense Oligos (ODN) & siRNA Oligo Modifications
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2'-OMethyl-5-methyl cytosine (2'-OMe-5-Me-C) is an RNA monomer that pairs with G, and when substituted for C in an oligonucleotide, both increases the stability of the resulting duplex relative to the comparable unmodified form, and confers nuclease resistance at that position(1). This "double-methylated"-modified cytosine thus is an excellent choice for incorporation into anti-sense oligos, where both properties are particularly desirable. Furthermore, because anti-sense oligonucleotides containing a CpG motif are known to induce pro-inflammatory responses after *in vivo* administration to animals, including human, via activation of Toll-like receptor 9 (TLR9), substitution of 2'-OMe-5-Me-dC for C in these motifs can prevent or sharply reduce these undesirable immune responses (2,3).
Modifications Increasing Duplex Stability and Nuclease Resistance

Modification

Duplex Stability [Tm Increase]

Nuclease Resistance Locked Analog Bases Increased [2- 4C per substitution] Increased 2-Amino-dA Increased [3.0C per substitution] Similar to DNA C-5 propynyl-C Increased [2.8C per substitution] Increased C-5 propynyl-U Increased [1.7C per substitution] Increased 2'-Fluoro Increased [1.8C per substitution] Increased 5-Methyl-dC Increased [1.3C per substitution] Similar to DNA 2'-O Methyl Increased Increased Phosphorothioate Slightly decreased Increased

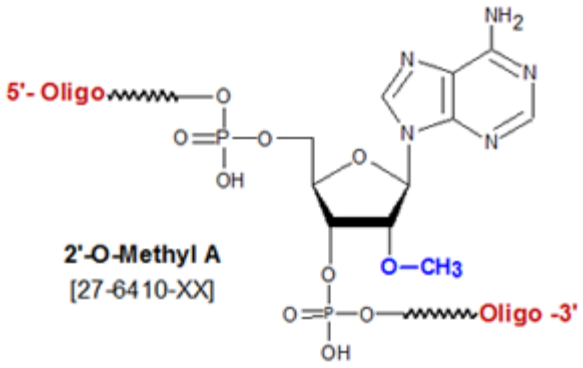
asp?mod_sp_cat_id=19 >Click here for complete list of duplex stability modifications **References**

1. Bundock, P.; de Both, M.T.J.; Hogers, R.C.J. 2006. Alternative nucleotides for improved targeted nucleotide exchange. Patent No. 2007073149, filed Dec 22. 2005, issued June 28, 2007. 2. Henry, S.P.; Stecker, K.; Brooks, D.; Monteith, D.; Conklin, B.; Bennett, C.F. Chemically modified oligonucleotides exhibit decreased immune stimulation in mice. *J. Pharmacol. Exp. Ther.* (2000), **292**: 468-479.

3. Yu, D.; Wang, D.; Zhu, F.-G.; Bhagat, L.; Dai, M.; Kandimalia, E.R.; Agrawal, S. Modifications Incorporated in CpG Motifs of Oligodeoxynucleotides Lead to Antagonist Activity of Toll-like Receptors 7 and 9. *J. Med. Chem.* (2009), **52**: 5108-5114.

# 2'-O methyl A

| | |
|---|---|
| Category | Nuclease Resistance |
| Modification Code | mA |
| Reference Catalog Number | 27-6410A |
| 5 Prime | Y |
| 3 Prime | Y |
| Internal | Y |
| Molecular Weight(mw) | 343.24 |

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genelink.com/oligo_modifications_reference/OMR_mod_category_intro.asp?mod_sp_cat_id=19 >Click here for complete list of duplex stability modifications

**ASO's and siRNA Modifications.**
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**References**

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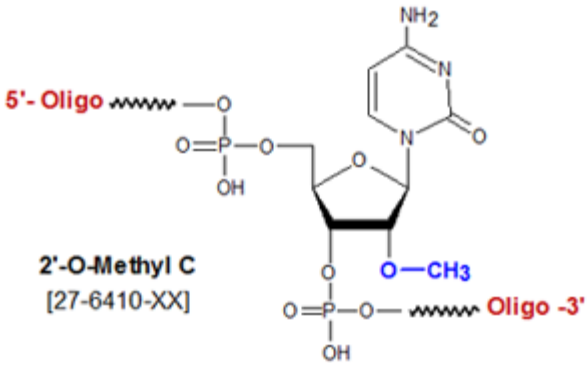
# 2'-O methyl C

| | |
|---|---|
| Category | Nuclease Resistance |
| Modification Code | mC |
| Reference Catalog Number | 27-6410C |
| 5 Prime | Y |
| 3 Prime | Y |
| Internal | Y |
| Molecular Weight(mw) | 319.21 |

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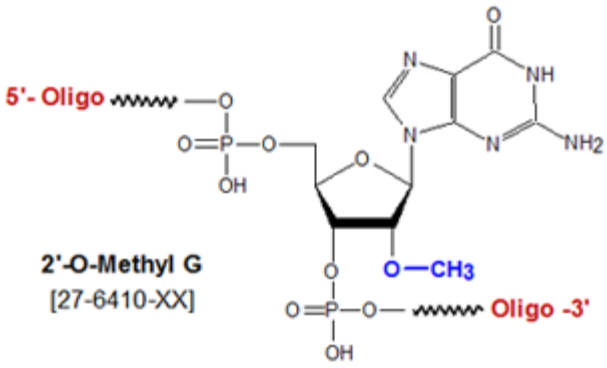
# 2'-O methyl G

| | |
|---|---|
| Category | Nuclease Resistance |
| Modification Code | mG |
| Reference Catalog Number | 27-6410G |
| 5 Prime | Y |
| 3 Prime | Y |
| Internal | Y |
| Molecular Weight(mw) | 359.24 |

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genelink.com/oligo_modifications_reference/OMR_mod_category_intro.asp?mod_sp_cat_id=19 >Click here for complete list of duplex stability modifications

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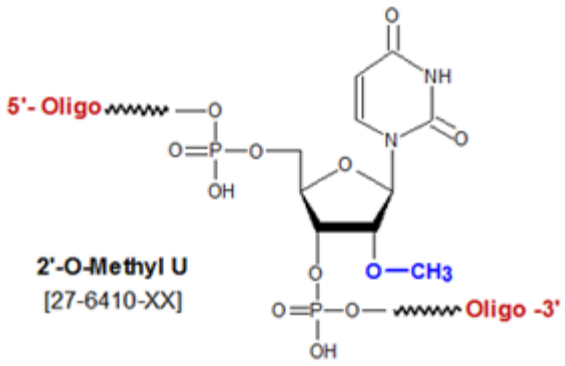
# 2'-O methyl U

| | |
|---|---|
| Category | Nuclease Resistance |
| Modification Code | mU |
| Reference Catalog Number | 27-6410U |
| 5 Prime | Y |
| 3 Prime | Y |
| Internal | Y |
| Molecular Weight(mw) | 320.2 |

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genelink.com/oligo_modifications_reference/OMR_mod_category_intro.asp?mod_sp_cat_id=19 >Click here for complete list of duplex stability modifications

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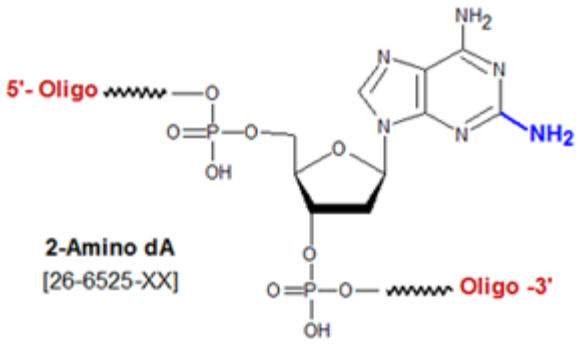
# 2-Amino dA

| | |
|---|---|
| Category | Duplex Stability |
| Modification Code | Am-dA |
| Reference Catalog Number | 26-6525 |
| 5 Prime | Y |
| 3 Prime | Y |
| Internal | Y |
| Molecular Weight(mw) | 328.22 |




2-Amino-deoxyadenosine dA can be used to **improve the ability of an oligo to hybridize to its target**. The 2-Amino-A nucleotide base forms three hydrogen bonds with thymine (T), compared with only two H-bonds between unmodified A and T. 2-Amino A:T base pairs thus have the same number of H-bonds as G:C base pairs do. Consequently, when a 2-Amino-dA oligo binds to its unmodified target, the Tm of the duplex **is raised by 3oC per 2-Amino-dA residue added**, compared with the unmodified case (1). In addition, 2-Amino-A also destabilizes A-G wobble mismatches, presumably due to a steric clash between the 2-amino on A and the 2-amino on G. Thus 2-Amino-dA modified oligos show better specificity for a target than their unmodified counterparts.
Modifications Increasing Duplex Stability and Nuclease Resistance

Modification

Duplex Stability [Tm Increase]

Nuclease Resistance Locked Analog Bases Increased [2- 4C per substitution] Increased 2-Amino-dA Increased [3.0C per substitution] Similar to DNA C-5 propynyl-C Increased [2.8C per substitution] Increased C-5 propynyl-U Increased [1.7C per substitution] Increased 2'-Fluoro Increased [1.8C per substitution] Increased 5-Methyl-dC Increased [1.3C per substitution] Similar to DNA 2'-O Methyl Increased Increased Phosphorothioate Slightly decreased Increased Click here for complete list of duplex stability modifications

2-Amino dA has been shown to be particularly useful in the following oligo-based applications:

(a)Strong-binding PCR primers: 2-Amino-dA-modified PCR primers have been shown to prime far better than their unmodified counterparts in PCR reactions, consistently yielding more product per cycle, permitting amplification at very high annealing temperatures (as high as 72oC), and interestingly, allowing excellent priming from within palindromic sequences (1).

The improvement in priming efficiency could significantly reduce the number of amplification-related mutations in PCR products. 2-Amino-dA primers also could be useful in several PCR applications, *e.g.*, when short, specific primers are required, when only a limited quantity of template is available (*e.g.*ancient DNA), when DNA secondary structure in the primer binding site prevents binding of an unmodified primer, or when primer extension is blocked by downstream DNA secondary structure in the template.

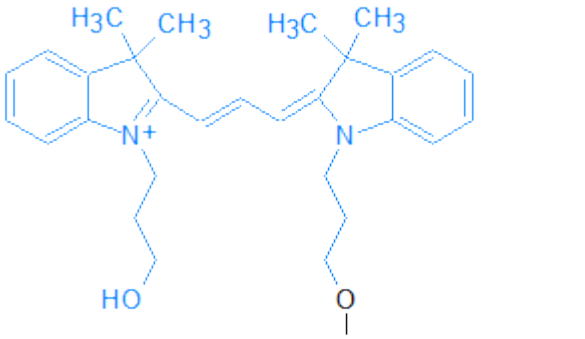
(b)Selective Binding Complementary (SBC) Oligos: SBC oligos are complementary pairs of oligos that contain one or more modified base pairs (that is, each member of the pair is modified). Each individual modified base does not form a stable base pair with its modified partner, but does form a particularly stable base pair with its natural (unmodified) counterpart. Thus, two complementary SBC oligos do not form a stable duplex with each other, but each individual SBC oligo does form a very stable duplex with an unmodified complementary target. This property enables an SBC duplex to effectively bind with both the sense and anti-sense strands of a DNA or RNA duplex target. SBC oligos. SBC oligos are useful as probes for investigation of branching secondary structures, and as anti-sense reagents against mRNA that has significant secondary structure.

An excellent pair of SBC oligos can be made by substituting 2-Amino-dA for A, and 2-Thio-dT for T. Because 2-Amino-dA only forms one hydrogen bond with 2-Thio-dT, these modified base pairs are very weak, and the corresponding duplex is unstable. However, both 2-Amino-dA and 2-Thio-dT bind effectively with T and A bases, respectively. In a classic study, SBC 20mers annealed against a DNA 20mer target exhibited Tm values 10° higher than the corresponding DNA-DNA hybrid, whereas the SBC-SBC hybrid yielded Tm values 30° lower (2). **References**

Lebedev, Y., *et al.* Oligonucleotides containing 2-aminoadenine and 5-methylcytosine are more effective as primers for PCR amplification than their unmodified counterparts. *Genetic Analysis: Biomolecular Engineering* (1996), **13**: 15-21.
Kutyavin, I.V.; Rhinehart, R.L.; Lukhtanov, E.A.; Gorn, V.V.; Meyer, R.B.; Gamper, H.B. *Oligonucleotides Containing 2-Aminoadenine and 2-Thiothymine Act as Selectively Binding Complementary Agents. Biochemistry* (1996), **35**: 11170-11176.

# 5'-Cy3

| | |
|---|---|
| Category | Fluorescent Dyes |
| Modification Code | 5-Cy3 |
| Reference Catalog Number | 26-6437 |
| 5 Prime | Y |
| 3 Prime | N |
| Internal | N |
| Molecular Weight(mw) | 630.78 |




**5'-Cy3 Label**
**[26-6437-XX]**

**Click here for a list of fluorophores.**

Prices listed above are for 5' modification. Internal and 3' incurs additional charges and are post synthesis conjugation to a primary amino group thus an additional modification with an amino group is required. A C6 or C12 amino group can be placed at the 5' or for the 3' end a C3 or C7 amino and for internal positions an amino modified base is used, e.g Amino dT C6.

Cyanine 3 (Cy3) is a fluorescent dye that belongs to the Cyanine family of synthetic polymethine dyes. Cy3 is reactive, water-soluble, and has an absorbance maximum of 550 nm and an emission maximum of 570 nm. It is available as both a phosphoramidite and an NHS ester, and is used to fluorescently label oligonucleotides at either the 5' or 3' end, or internally. Cy3 plays a particularly important role in real-time PCR applications, being used as the reporter moiety in TaqMan probes (1), Scorpion primers (2) and Molecular Beacons (3). For such probes, Cy3 is most commonly paired with the dark quencher BHQ-2, as the two have excellent spectral overlap.

Cy3 can also be used to label DNA oligos for use as hybridization probes in other applications, such as Fluorescent In-Situ Hybridization (FISH). In 2010, Stoeckler and co-workers (4) reported that Cy3 double-labeling of FISH probes (at both ends) that were specific to ribsosomal RNA targets in microorganisms at least doubles FISH signal intensity without affecting specificity. This Double Labeling of Oligonucleotide Probes for Fluorescence In Situ Hybridization (DOPE-FISH 0 strategy may provide an effective solution to the problem of low signal intensity, which is commonly observed when using corresponding singly-labeled FISH probes for microbe identification. As an added benefit, Cy3-doubly labeled probes were shown to increase the in situ accessibility of rRNA targets sites in microbes, which allows for greater probe design flexibility.

Applied Biosystems Proprietary Dyes & Possible Substitutions

Fluorophore

Color

Absorbance max (nm)

Emission max (nm) VIC Pink Red 538 554 Cal Orange 560 Pink Red 537 558 HEX Pink Red 535 556 NED Red Orange 546 575 Cy3 Red Orange 550 570 PET Red Orange 558 595 Cy3.

5 Red 588 604 ROX Red 575 602 Texas Red Red 583 603

**Click here for a list of fluorophores.**

**References**

1. Livak, K.J., Flood, S.J.A., Marmaro, J., Giusti, W., Deetz, K. Oligonucleotides with fluorescent dyes at opposite ends provide a quenched probe system useful for detecting PCR product and nucleic acid hybridization.*PCR Methods Appl.* (1995), **4**: 1-6.

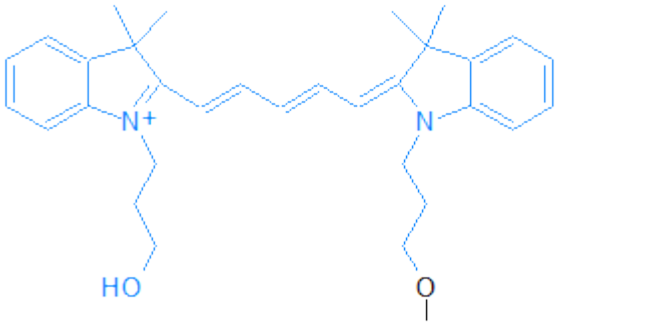
2. Thelwell, N., Millington, S., Solinas, A., Booth, J., Brown, T. Mode of action and application of Scorpion primers to mutation detection. *Nucleic Acids Res.* (2000), **28**: 3752-3761.

3. Tyagi, S., Kramer, F.R. Molecular beacons: probes that fluoresce upon hybridization. *Nat. Biotechnol.* (1996), **14**: 303-308.

4. Stoecker, K., Dorninger, C., Daims, H., Wagner, M. Double Labeling of Oligonucleotide Probes for Fluorescence In Situ Hybridization (DOPE-FISH) Improves Signal Intensity and Increases rRNA Accessibility. *Appl. Environ. Microb..* (2010), **76**: 922-926.

# 5'-Cy5

| | |
|---|---|
| Category | Fluorescent Dyes |
| Modification Code | 5-Cy5 |
| Reference Catalog Number | 26-6436 |
| 5 Prime | Y |
| 3 Prime | N |
| Internal | N |
| Molecular Weight(mw) | 533.63 |

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**[26-6436-XX]**

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**Click here for list of quenchers.**

**Click here for a list of fluorophores.**

Near Infrared Fluorophore Spectral Data & Quencher Selection Guide

| Fluorophore Name | Excitation Max, nm +/-10 | Emission Max, nm +/-10 | Extinction Coefficient* | Color** | Quencher |
|---|---|---|---|---|---|
| **Cy5** | **650** | **665** | **250,000** | | |
| **IRDye 650 NHS** | **650** | **665** | **230,000** | | |
| **AZ647 NHS** | **655** | **680** | **191,800** | | |
| **Cy5.5** | **684** | **710** | **198,000** | | |
| **IRDye 700 NHS** | **684** | **710** | **288,000** | | |
| **Cy7 NHS** | **740** | **773** | **199,000** | | |

**IRDye 750 NHS 756 776 260,000**

**cy7.5 NHS 788 808 223,000**

**IRDye 800 NHS 795 819 240,000**

*** Extinction coefficient at λ (max) in cm-1M-1. ** Typical emission color seen through the eyepiece of a conventional fluorescence microscope with appropriate filters. Near-IR region. Human vision is insensitive to light beyond ~650 nm; it is not possible to view near-IR fluorescent dyes.**

**Click here for a list of fluorophores.**

**Click here for list of quenchers.**

**References**

**1. Livak, K.J., Flood, S.J.A., Marmaro, J., Giusti, W., Deetz, K. Oligonucleotides with fluorescent dyes at opposite ends provide a quenched probe system useful for detecting PCR product and nucleic acid hybridization.*PCR Methods Appl.* (1995), 4: 1-6.**

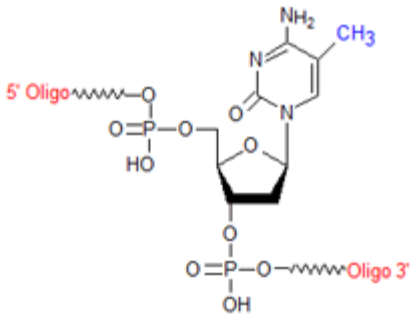
**2. Thelwell, N., Millington, S., Solinas, A., Booth, J., Brown, T. Mode of action and application of Scorpion primers to mutation detection. *Nucleic Acids Res.* (2000), 28: 3752-3761.**

**3. Tyagi, S., Kramer, F.R. Molecular beacons: probes that fluoresce upon hybridization. *Nat. Biotechnol.* (1996), 14: 303-308.**

**4. Stoecker, K., Dorninger, C., Daims, H., Wagner, M. Double Labeling of Oligonucleotide Probes for Fluorescence In Situ Hybridization (DOPE-FISH) Improves Signal Intensity and Increases rRNA Accessibility. *Appl. Environ. Microb..* (2010), 76: 922-926.**

# 5-Me dC

| | |
|---|---|
| Category | Epigenetics |
| Modification Code | 5mdC |
| Reference Catalog Number | 26-6413 |
| 5 Prime | Y |
| 3 Prime | Y |
| Internal | Y |
| Molecular Weight(mw) | 303.21 |




**5-methyl deoxycytosine (Me-dC)**
**[26-6413-XX]**

5-methyl deoxycytosine (5-Me-dC) pairs with dG, and when substituted for dC in an oligonucleotide, increases the stability of the resulting duplex relative to the comparable unmodified form, raising the Tm by 1.3degC per 5-Me-dC residue added (1,2). 5-Me-dC thus can be used to **improve the ability of an oligo to hybridize to its target**. The presence of the hydrophobic 5-methyl group presumably acts to exclude water molecules from the duplex.
Modifications Increasing Duplex Stability and Nuclease Resistance

Modification

Duplex Stability [Tm Increase]

Nuclease Resistance Locked Analog Bases Increased [2- 4C per substitution] Increased 2-Amino-dA Increased [3.0C per substitution] Similar to DNA C-5 propynyl-C Increased [2.8C per substitution] Increased C-5 propynyl-U Increased [1.7C per substitution] Increased 2'-Fluoro Increased [1.8C per substitution] Increased 5-Methyl-dC Increased [1.3C per substitution] Similar to DNA 2'-O Methyl Increased Increased Phosphorothioate Slightly decreased Increased Click here for complete list of duplex stability modifications

5-Me-dC is particularly useful in the following applications:

(a)Strong-binding PCR primers: 5-Me-dC-modified PCR primers have been shown to prime far better than their unmodified counterparts in PCR reactions, consistently yielding more product per cycle, permitting amplification at very high annealing temperatures (as high as 72degC), and interestingly, allowing excellent priming from within palindromic sequences (1). The improvement in priming efficiency could significantly reduce the number of amplification-related mutations in PCR products.

5-Me-dC primers also could be useful in several PCR applications, *e.g.*, when short, specific primers are required, when only a limited quantity of template is available (*e.g.*ancient DNA), when DNA secondary structure in the primer binding site prevents binding of an unmodified primer, or when primer extension is blocked by downstream DNA secondary structure in the template.

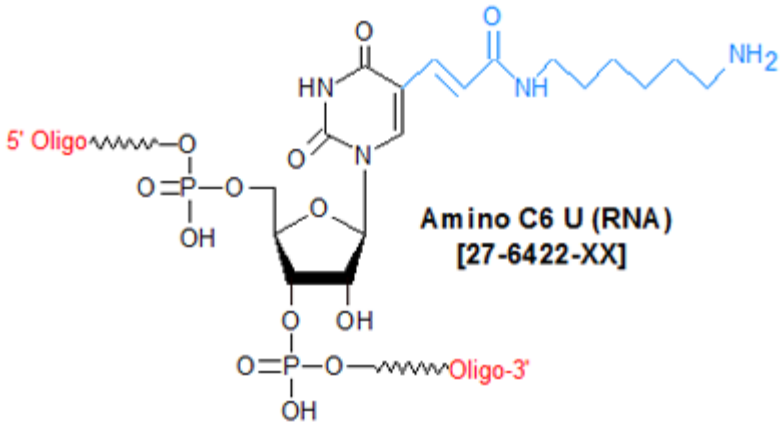
(b) Anti-sense: Anti-sense oligonucleotides containing a CpG motif induce pro-inflammatory responses after *in vivo* administration to animals, including human, via activation of Toll-like receptor 9 (TLR9). Substitution of 5-Me-dC for dC in these motifs can prevent or sharply reduce these undesirable immune responses (3).

(b) DNA methylation studies: Methylation of dC to 5-methyl-dC, when it occurs in CpG sites near promoters is associated with gene silencing, and is an important epigenetic mechanism in living organisms. Oligonucleotides incorporating 5-Me-dC have been used by a number of research groups as research tools to study the epigenetic effects of DNA methylation in such areas as tumorigenesis and the effects of cocaine on fetal heart development (4-6). **References**

1. Lebedev, Y.; Akopyants, N.; Azhikina, T.; Shevchenko, Y.; Potapov, V.; Stecenko, D.; Berg, D.; Sverdlov, E.. Oligonucleotides containing 2-aminoadenine and 5-methylcytosine are more effective as primers for PCR amplification than their nonmodified counterparts. *Genet Anal.* (1996), **13**: 15-21.
2. Xodo, L.E.; Manzini, G.; Quadrifoglio, F.; van der Marel, G.A.; van Boom, J.H. Effect of 5-methylcytosine on the stability of triple-stranded DNA—a thermodynamic study*Nucleic Acids Res.* (1991), **19**: 5625-5631.
3. Henry, S.P.; Stecker, K.; Brooks, D.; Monteith, D.; Conklin, B.; Bennett, C.F. Chemically modified oligonucleotides exhibit decreased immune stimulation in mice. *J. Pharmacol. Exp. Ther.* (2000), **292**: 468-479.
4. Mancini, D.N.; Singh, S.M.; Archer, T.K.; Rodenhiser, D.I. Site-specific DNA methylation in the neurofibromatosis (NF1) promoter interferes with binding of CREB and SP1 transcription factors. *Oncogene.* (1999), **18**: 4108-4119.
5. Zhang, H.; Darwanto, A.; Linkhart, T.A.; Sowers, L.C.; Zhang, L. Maternal Cocaine Administration Causes an Epigenetic Modification of Protein Kinase Ce Gene Expression in Fetal Rat Heart, *Mol. Pharmacol.* (2007), **71**: 1319-1328.
6. Ishii, T.; Fujishiro, M.; Masuda, M.; Teramoto, S.; Matsuse, T. A methylated oligonucleotide induced methylation of GSTP1 promoter and suppressed its expression in A549 lung adenocarcinoma cells. *Cancer Letters* (2004), **212**: 211-223.

# Amino C6 U

| | |
|---|---|
| Category | Minor Bases |
| Modification Code | AmC6U |
| Reference Catalog Number | 27-6422 |
| 5 Prime | Y |
| 3 Prime | Y |
| Internal | Y |
| Molecular Weight(mw) | 474.4 |



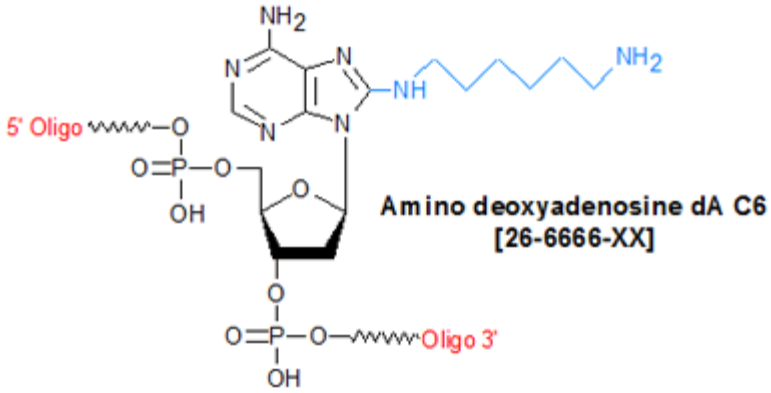

Amino C6-U can be used to internally incorporate an active primary amino group into either an RNA oligonucleotide or a chimeric oligo. The presence of the primary amino group allows the user to label the oligo with a variety of different ligands for affinity, reporter or protein moieties (as NHS esters or isothiocyanates), depending on the application. Examples include biotin, digoxigenin, and fluorescent dyes or quenchers, magnetic beads and enzymes (for example, alkaline phosphatase).

-

The primary amine labelled oligos can also be conjugated to carboxyl functional groups usually for solid supports applications using EDC mediated reaction as shown in the figure below.

# Amino dA C6

| | |
|---|---|
| Category | Minor Bases |
| Modification Code | Am-dA-C6 |
| Reference Catalog Number | 26-6666 |
| 5 Prime | Y |
| 3 Prime | Y |
| Internal | Y |
| Molecular Weight(mw) | 427.4 |



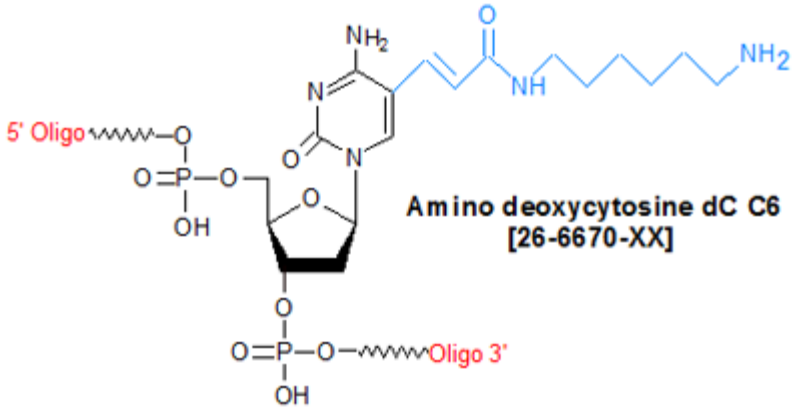

Amino dA C6 can be used to internally incorporate an active primary amino group into either a DNA oligonucleotide or a chimeric oligo. The presence of the primary amino group allows the user to label the oligo with a variety of different ligands for affinity, reporter or protein moieties (as NHS esters or isothiocyanates), depending on the application. Examples include biotin, digoxigenin, and fluorescent dyes or quenchers, magnetic beads and enzymes (for example, alkaline phosphatase).

-

The primary amine labelled oligos can also be conjugated to carboxyl functional groups usually for solid supports applications using EDC mediated reaction as shown in the figure below.

# Amino dC C6

| | |
|---|---|
| Category | Minor Bases |
| Modification Code | Am-dC-C6 |
| Reference Catalog Number | 26-6670 |
| 5 Prime | Y |
| 3 Prime | Y |
| Internal | Y |
| Molecular Weight(mw) | 457.42 |



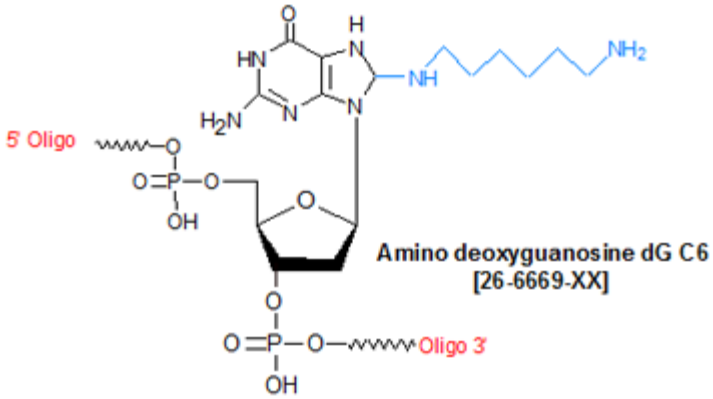

Amino dC C6 can be used to internally incorporate an active primary amino group into either a DNA oligonucleotide or a chimeric oligo. The presence of the primary amino group allows the user to label the oligo with a variety of different ligands for affinity, reporter or protein moieties (as NHS esters or isothiocyanates), depending on the application. Examples include biotin, digoxigenin, and fluorescent dyes or quenchers, magnetic beads and enzymes (for example, alkaline phosphatase).

-

The primary amine labelled oligos can also be conjugated to carboxyl functional groups usually for solid supports applications using EDC mediated reaction as shown in the figure below.

# Amino dG C6

| | |
|---|---|
| Category | Minor Bases |
| Modification Code | Am-dG-C6 |
| Reference Catalog Number | 26-6669 |
| 5 Prime | Y |
| 3 Prime | Y |
| Internal | Y |
| Molecular Weight(mw) | 428.38 |



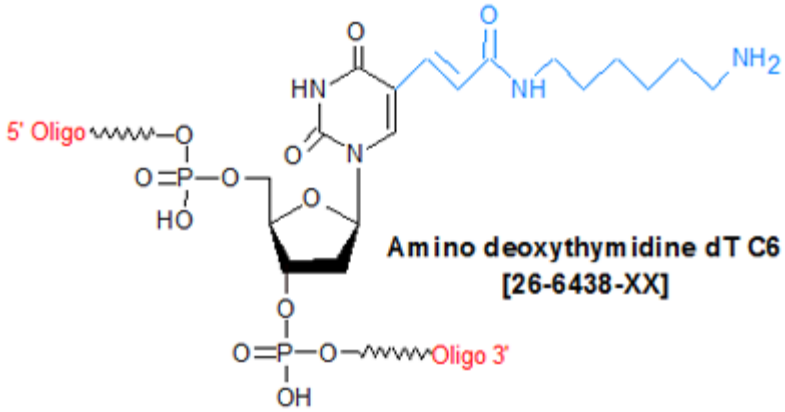

Amino dG C6 can be used to internally incorporate an active primary amino group into either a DNA oligonucleotide or a chimeric oligo. The presence of the primary amino group allows the user to label the oligo with a variety of different ligands for affinity, reporter or protein moieties (as NHS esters or isothiocyanates), depending on the application. Examples include biotin, digoxigenin, and fluorescent dyes or quenchers, magnetic beads and enzymes (for example, alkaline phosphatase).

-

The primary amine labelled oligos can also be conjugated to carboxyl functional groups usually for solid supports applications using EDC mediated reaction as shown in the figure below.

# Amino dT C6

| | |
|---|---|
| Category | Minor Bases |
| Modification Code | Am-dT-C6 |
| Reference Catalog Number | 26-6438 |
| 5 Prime | Y |
| 3 Prime | Y |
| Internal | Y |
| Molecular Weight(mw) | 458.41 |



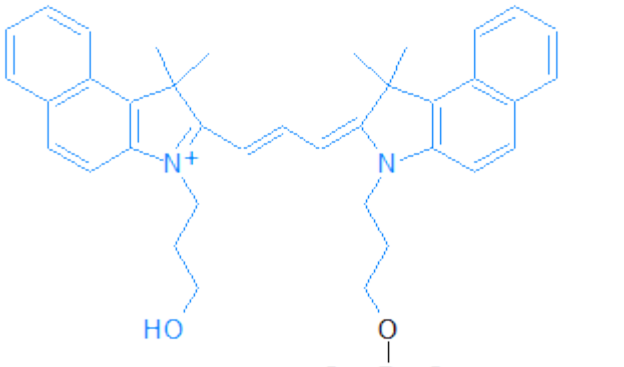

Amino dT C6 can be used to internally incorporate an active primary amino group into either a DNA oligonucleotide or a chimeric oligo. The presence of the primary amino group allows the user to label the oligo with a variety of different ligands for affinity, reporter or protein moieties (as NHS esters or isothiocyanates), depending on the application. Examples include biotin, digoxigenin, and fluorescent dyes or quenchers, magnetic beads and enzymes (for example, alkaline phosphatase). NHS ester-activated ligands react with primary amines to yield stable amide bonds. The reaction releases N-hydroxysuccinimide (NHS). NHS ester reaction scheme for chemical conjugation to a primary amine in an oligo is given below.

-

The primary amine labelled oligos can also be conjugated to carboxyl functional groups usually for solid supports applications using EDC mediated reaction as shown in the figure below.

# Cy3.5

| | |
|---|---|
| Category | Fluorescent Dyes |
| Modification Code | Cy3.5 |
| Reference Catalog Number | 26-6461 |
| 5 Prime | Y |
| 3 Prime | N |
| Internal | N |
| Molecular Weight(mw) | 607.7 |




**5'-Cy3.5 Label**
**[26-6461-XX]**

Cyanine 3.5 (Cy3.5) is a fluorescent dye that belongs to the Cyanine family of synthetic polymethine dyes. Cy3.5 is reactive, water-soluble, and has an absorbance maximum of 581 nm and an emission maximum of 596 nm. It is available as a phosphoramidite, and is used to fluorescently label oligonucleotides at either the 5' or 3' end, or internally. Cy3.5 plays a particularly important role in real-time PCR applications, being used as a reporter moiety in TaqMan probes (1), Scorpion primers (2) and Molecular Beacons (3). For such probes, Cy3.5 is most commonly paired with the dark quencher BHQ-2, as the two have excellent spectral overlap.

Cy3 can also be used to label DNA oligos for use as hybridization probes in other applications, such as Fluorescent In-Situ Hybridization (FISH).
Applied Biosystems Proprietary Dyes & Possible Substitutions

Dye

Color

Absorbance max (nm)

Emission max (nm) VIC Pink Red 538 554 Cal Orange 560 Pink Red 537 558 HEX Pink Red 535 556 NED Red Orange 546 575 Cy3 Red Orange 550 570 PET Red Orange 558 595 Cy3.

5 Red 588 604 ROX Red 575 602 CAL Fluor Red 590 Red 569 591 Texas Red Red 583 603

**Click here for a list of fluorophores.**

**References**

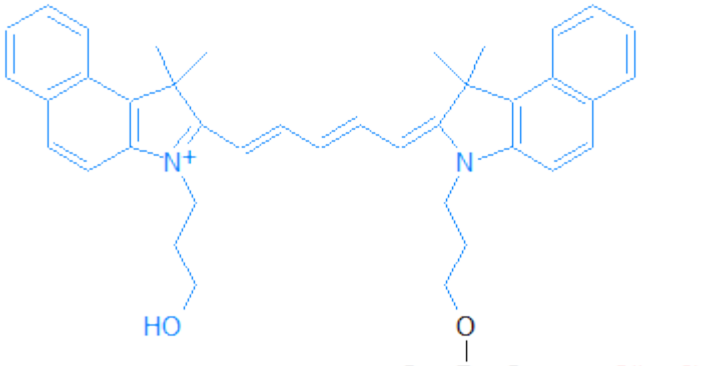
1. Livak, K.J., Flood, S.J.A., Marmaro, J., Giusti, W., Deetz, K. Oligonucleotides with fluorescent dyes at opposite ends provide a quenched probe system useful for detecting PCR product and nucleic acid hybridization.*PCR Methods Appl.* (1995), **4**: 1-6.

2. Thelwell, N., Millington, S., Solinas, A., Booth, J., Brown, T. Mode of action and application of Scorpion primers to mutation detection. *Nucleic Acids Res.* (2000), **28**: 3752-3761.

3. Tyagi, S., Kramer, F.R. Molecular beacons: probes that fluoresce upon hybridization. *Nat. Biotechnol.* (1996), **14**: 303-308.

# Cy5.5

| | |
|---|---|
| Category | Fluorescent Dyes |
| Modification Code | Cy5.5 |
| Reference Catalog Number | 26-6460 |
| 5 Prime | N |
| 3 Prime | Y |
| Internal | N |
| Molecular Weight(mw) | 633.74 |




**5'-Cy5.5 Label**
**[26-6460-XX]**

Cyanine 5.5 (Cy5.5) is a far-red/near infra red (NIR) fluorescent dye that belongs to the Cyanine family of synthetic polymethine dyes. Cy5.5 is reactive, water-soluble, and has an absorbance maximum of 675 nm and an emission maximum of 694 nm. It is available as a phosphoramidite, and is used to fluorescently label oligonucleotides at either the 5' or 3' end, or internally. Cy5.5 plays a particularly important role in real-time PCR applications, being used as a reporter moiety in TaqMan probes (1), Scorpion primers (2) and Molecular Beacons (3). For such probes, Cy5.5 is most commonly paired with the dark quencher BHQ-3, as the two have excellent spectral overlap.

Cy5.5 can also be used to label DNA oligos for use as hybridization probes in other applications, such as Fluorescent In-Situ Hybridization (FISH).

Near Infrared Fluorophore Spectral Data & Quencher Selection Guide

Fluorophore Name

Excitation Max, nm +/-10

Emission Max, nm +/-10

Extinction Coefficient*

Color**

Quencher

**Cy5 650 665 250,000**

**IRDye 650 NHS 650 665 230,000**

asp?modid=516">AZ647 NHS 655 680 191,800

**Cy5.5 684 710 198,000**

**IRDye 700 NHS 684 710 288,000**

**Cy7 NHS 740 773 199,000**

**IRDye 750 NHS 756 776 260,000**

**cy7.5 NHS 788 808 223,000**

**IRDye 800 NHS 795 819 240,000**

*** Extinction coefficient at λ (max) in cm-1M-1. ** Typical emission color seen through the eyepiece of a conventional fluorescence microscope with appropriate filters. Near-IR region. Human vision is insensitive to light beyond ~650 nm; it is not possible to view near-IR fluorescent dyes.**

**Click here for a list of fluorophores.**

**Click here for list of quenchers.**

**References**

**1. Livak, K.J., Flood, S.J.A., Marmaro, J., Giusti, W., Deetz, K. Oligonucleotides with fluorescent dyes at opposite ends provide a quenched probe system useful for detecting PCR product and nucleic acid hybridization.*PCR Methods Appl.* (1995), 4: 1-6.**

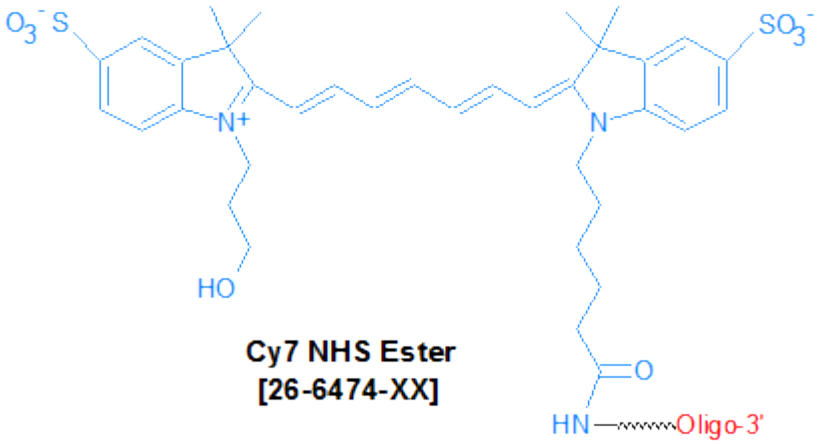
**2. Thelwell, N., Millington, S., Solinas, A., Booth, J., Brown, T. Mode of action and application of Scorpion primers to mutation detection. *Nucleic Acids Res.* (2000), 28: 3752-3761.**

**3. Tyagi, S., Kramer, F.R. Molecular beacons: probes that fluoresce upon hybridization. *Nat. Biotechnol.* (1996), 14: 303-308.**

**4. Stoecker, K., Dorninger, C., Daims, H., Wagner, M. Double Labeling of Oligonucleotide Probes for Fluorescence In Situ Hybridization (DOPE-FISH) Improves Signal Intensity and Increases rRNA Accessibility. *Appl. Environ. Microb..* (2010), 76: 922-926.**

# Cy7 NHS

| | |
|---|---|
| Category | Fluorescent Dyes |
| Modification Code | Cy7-N |
| Reference Catalog Number | 26-6474 |
| 5 Prime | Y |
| 3 Prime | Y |
| Internal | Y |
| Molecular Weight(mw) | 682 |




**Cy7 NHS Ester**
**[26-6474-XX]**

Cy7 modification is a post synthesis conjugation to a primary amino group thus an additional modification with an amino group is required. A C6 or C12 amino group can be placed at the 5' or for the 3' end a C3 or C7 amino and for internal positions an amino modified base is used, e.g Amino dT C6. **YIELD** NHS based modifications are post synthesis conjugation performed using a primary amino group. The yield is lower as compared to direct automated coupling of modifications that are available as amidites. Approximate yield for various scales are given below.
~2 nmol final yield for 50 nmol scale synthesis.
~5 nmol final yield for 200 nmol scale synthesis.
~16 nmol final yield for 1 umol scale synthesis

Cyanine 7(Cy7) NHS ester is a fluorescent dye that belongs to the Cyanine family of synthetic polymethine dyes. Cy7 is reactive, water-soluble, and has an absorbance maximum of 747 nm and an emission maximum of 776 nm, which is in the near IR. It is available as an NHS ester, and is used to fluorescently label oligonucleotides at either the 5' or 3' end, or internally. Because it is a near IR dye, Cy7 has very little background fluorescence associated with it (1). It is thus an excellent choice for labeling oligo probes slated for in vivo applications, because the minimal scattering and absorption of near-IR photons by cellular tissue ensures higher S/N ratio, and better sensitivity. For example, Fluorescent Resonance Energy Transfer (FRET) oligonucleotide duplexes using Cy5.5 as the donor on one strand and Cy7 as the acceptor on the complementary strand have been used to detect and characterize transcription factor NF-kappaB p50 protein binding to DNA (2)

Caution: Cy7 is intensely colored and very reactive. Care should be exercised when handling the vial containg the C7-labeled oligo to avoid staining clothing, skin, and other items. Also, because Cy7 is in the form of an NHS ester, the oligo first must be synthesized with an Amino C6 Linker (for the ends) or the Amino C6 version of the base phosphoramidite (for internal labeling). The Cy7-NHS ester is then manually attached to the oligo through the amino group in a separate reaction post-synthesis.

Near Infrared Fluorophore Spectral Data & Quencher Selection Guide

Fluorophore Name

Excitation Max, nm +/-10

Emission Max, nm +/-10

Extinction Coefficient*

Color**

Quencher

genelink.com/newsite/products/mod_detail.asp?modid=27">Cy5 650 665 250,000

**IRDye 650 NHS 650 665 230,000**

**AZ647 NHS 655 680 191,800**

**Cy5.5 684 710 198,000**

**IRDye 700 NHS 684 710 288,000**

**Cy7 NHS 740 773 199,000**

**IRDye 750 NHS 756 776 260,000**

**cy7.5 NHS 788 808 223,000**

**IRDye 800 NHS 795 819 240,000**

*** Extinction coefficient at λ (max) in cm-1M-1. ** Typical emission color seen through the eyepiece of a conventional fluorescence microscope with appropriate filters. Near-IR region. Human vision is insensitive to light beyond ~650 nm; it is not possible to view near-IR fluorescent dyes.**

**Click here for a list of fluorophores.**

**Click here for list of quenchers.**

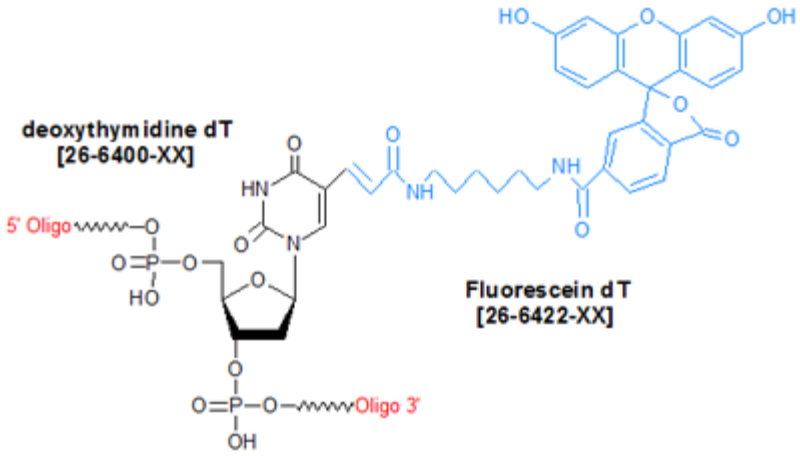
**References**

**1. Benson, R.C., Kues, H.A. Absorption and Fluorescence Properties of Cyanine Dyes.*J. Chem. Eng. Data* (1977), 22: 379-383.**

**2. Zhang, S., Metelev, V., Tabatadze, D., Zamecnik, P.C., Bogdanov, A. Fluorescence resonance energy transfer in near-infrared fluorescent oligonucleotide probes for detecting protein-DNA interactions. *Proc. Nat. Acad. Sci. USA.* (2008), 105: 4156-4161.**

# Fam dT

| | |
|---|---|
| Category | Fluorescent Dyes |
| Modification Code | Fam-dT |
| Reference Catalog Number | 26-6422 |
| 5 Prime | Y |
| 3 Prime | Y |
| Internal | Y |
| Molecular Weight(mw) | 815.71 |

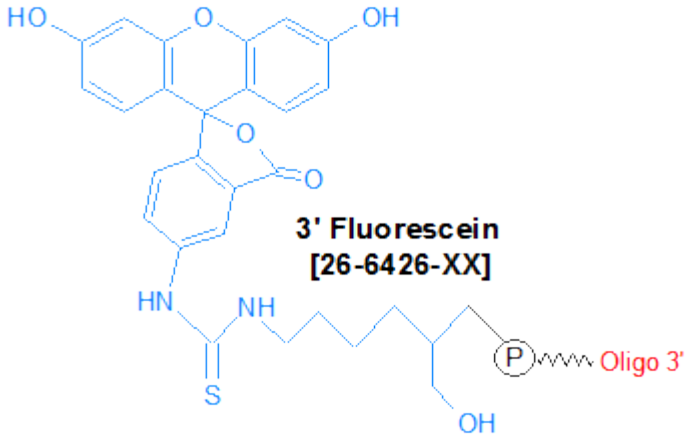



Fluorescein-dT is a deoxythymidine nucleoside derivitized with 6-FAM (6-carboxyfluorescein) through a spacer arm. 6-FAM is the most commonly used fluorescent dye for labeling oligonucleotides; Fluorescein-dT is used to internally label an oligonucleotide at a dT position. Fluorescein-dT has an absorbance maximum of 492 nm and an emission maximum of 517 nm. Fluorescein-dT can be used to internally label a 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 probes, fluorescein is most commonly paired with the dark quencher BHQ-1, as the two have excellent spectral overlap.

Fluorescein-dT also 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 internally labeled with fluorescein-dT also can be used as PCR and DNA sequencing primers to generate fluorescently-labeled PCR, sequencing or genetic analysis (AFLP or microsatellite) products.

# Fluorescein-5'

| | |
|---|---|
| Category | Fluorescent Dyes |
| Modification Code | Fl-5 |
| Reference Catalog Number | 26-6426 |
| 5 Prime | Y |
| 3 Prime | N |
| Internal | N |
| Molecular Weight(mw) | 537.46 |



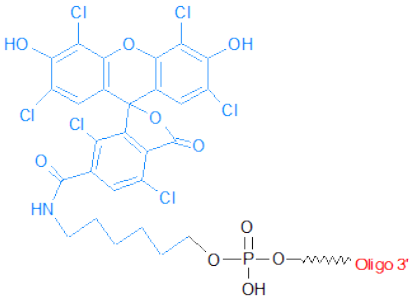

Fluorescein is the most commonly used fluorescent dye for labeling oligonucleotides. Fluorescein has an absorbance maximum of 494 nm and an emission maximum of 521 nm. The difference between the fluorescein and 6-FAM modifications is that the fluorescein dye is attached to the 6-carbon spacer via an N-hydroxysuccinimide (NHS) group instead of a carboxy group. Fluorescein plays a particularly important role in real-time PCR applications, being used as a reporter moiety in TaqMan probes (1), Scorpion primers (2) and Molecular Beacons (3). For such probes, fluorescein is most commonly paired with the dark quencher BHQ-1, as the two have excellent spectral overlap.

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 (AFLP or microsatellite) products. **References**

1. Livak, K.J., Flood, S.J.A., Marmaro, J., Giusti, W., Deetz, K. Oligonucleotides with fluorescent dyes at opposite ends provide a quenched probe system useful for detecting PCR product and nucleic acid hybridization.*PCR Methods Appl.* (1995), **4**: 1-6.
2. Thelwell, N., Millington, S., Solinas, A., Booth, J., Brown, T. Mode of action and application of Scorpion primers to mutation detection. *Nucleic Acids Res.* (2000), **28**: 3752-3761.
3. Tyagi, S., Kramer, F.R. Molecular beacons: probes that fluoresce upon hybridization. *Nat. Biotechnol.* (1996), **14**: 303-308.

# Hex-5'

| | |
|---|---|
| Category | Fluorescent Dyes |
| Modification Code | 5-Hex |
| Reference Catalog Number | 26-6432 |
| 5 Prime | Y |
| 3 Prime | N |
| Internal | N |
| Molecular Weight(mw) | 744.13 |




**5' Hexachloro-Fluorescein (HEX)**
**[26-6432-XX]**

**Click here for a list of fluorophores.**

Hexachloro-fluorescein (HEX) is hexachlorinated version of the fluorescent dye fluorescein, and is used for labeling oligonucleotides at either the 5' or 3' end. HEX has an absorbance maximum of 535 nm and an emission maximum of 556 nm. HEX can be used in real-time PCR applications as a reporter moiety in TaqMan probes (1), Scorpion primers (2) and Molecular Beacons (3). For such probes, HEX is most commonly paired with the dark quencher BHQ-1, as the two have good spectral overlap.

HEX also 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 HEX at the 5' end can be used as PCR and DNA sequencing primers to generate fluorescently-labeled PCR, sequencing or genetic analysis (AFLP or microsatellite) products. NOTE: If HEX is on the 3' end of the oligo, it cannot be used as a primer in PCR-based applications.

Applied Biosystems Proprietary Dyes & Possible Substitutions

Dye

Color

Absorbance max (nm)

Emission max (nm) VIC Pink Red 538 554 Cal Orange 560 Pink Red 537 558 HEX Pink Red 535 556 NED Red Orange 546 575 Cy3 Red Orange 550 570 PET Red Orange 558 595 Cy3.

5 Red 588 604 ROX Red 575 602 Texas Red Red 583 603

**Click here for a list of fluorophores.**

**References**

1. Livak, K.J., Flood, S.J.A., Marmaro, J., Giusti, W., Deetz, K. Oligonucleotides with fluorescent dyes at opposite ends provide a quenched probe system useful for detecting PCR product and nucleic acid hybridization.*PCR Methods Appl.* (1995), **4**: 1-6.

2. Thelwell, N., Millington, S., Solinas, A., Booth, J., Brown, T. Mode of action and application of Scorpion primers to mutation detection. *Nucleic Acids Res.* (2000), **28**: 3752-3761.

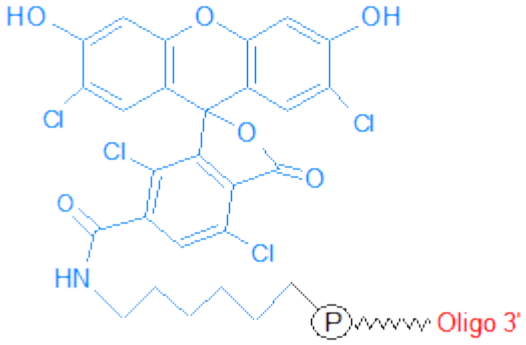
3. Tyagi, S., Kramer, F.R. Molecular beacons: probes that fluoresce upon hybridization. *Nat. Biotechnol.* (1996), **14**: 303-308.

# Oligo Modifications



## Tet-5'

| | |
|---|---|
| Category | Fluorescent Dyes |
| Modification Code | Tet-5 |
| Reference Catalog Number | 26-6433 |
| 5 Prime | Y |
| 3 Prime | N |
| Internal | N |
| Molecular Weight(mw) | 675.24 |




**5'-Tetrachloro-Fluorescein (TET)**
**[26-6433-XX]**

**Click here for a list of fluorophores.**

Tetrachloro fluorescein (TET) is tetra-chloro derivative of fluorescein that is used to fluorescently label oligonucleotides. TET has an absorbance maximum of 522 nm and an emission maximum of 538 nm. TET plays a role in real-time PCR applications, being used as a reporter moiety in TaqMan probes (1), Scorpion primers (2) and Molecular Beacons (3). For such probes, TET is most commonly paired with the dark quencher BHQ-1, as the two have excellent spectral overlap.

TET 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 TET at the 5' end can be used as PCR and DNA sequencing primers to generate fluorescently-labeled PCR, sequencing or genetic analysis (AFLP or microsatellite) products. **References**

1. Livak, K.J., Flood, S.J.A., Marmaro, J., Giusti, W., Deetz, K. Oligonucleotides with fluorescent dyes at opposite ends provide a quenched probe system useful for detecting PCR product and nucleic acid hybridization.*PCR Methods Appl.* (1995), **4**: 1-6.
2. Thelwell, N., Millington, S., Solinas, A., Booth, J., Brown, T. Mode of action and application of Scorpion primers to mutation detection. *Nucleic Acids Res.* (2000), **28**: 3752-3761.
3. Tyagi, S., Kramer, F.R. Molecular beacons: probes that fluoresce upon hybridization. *Nat. Biotechnol.* (1996), **14**: 303-308.