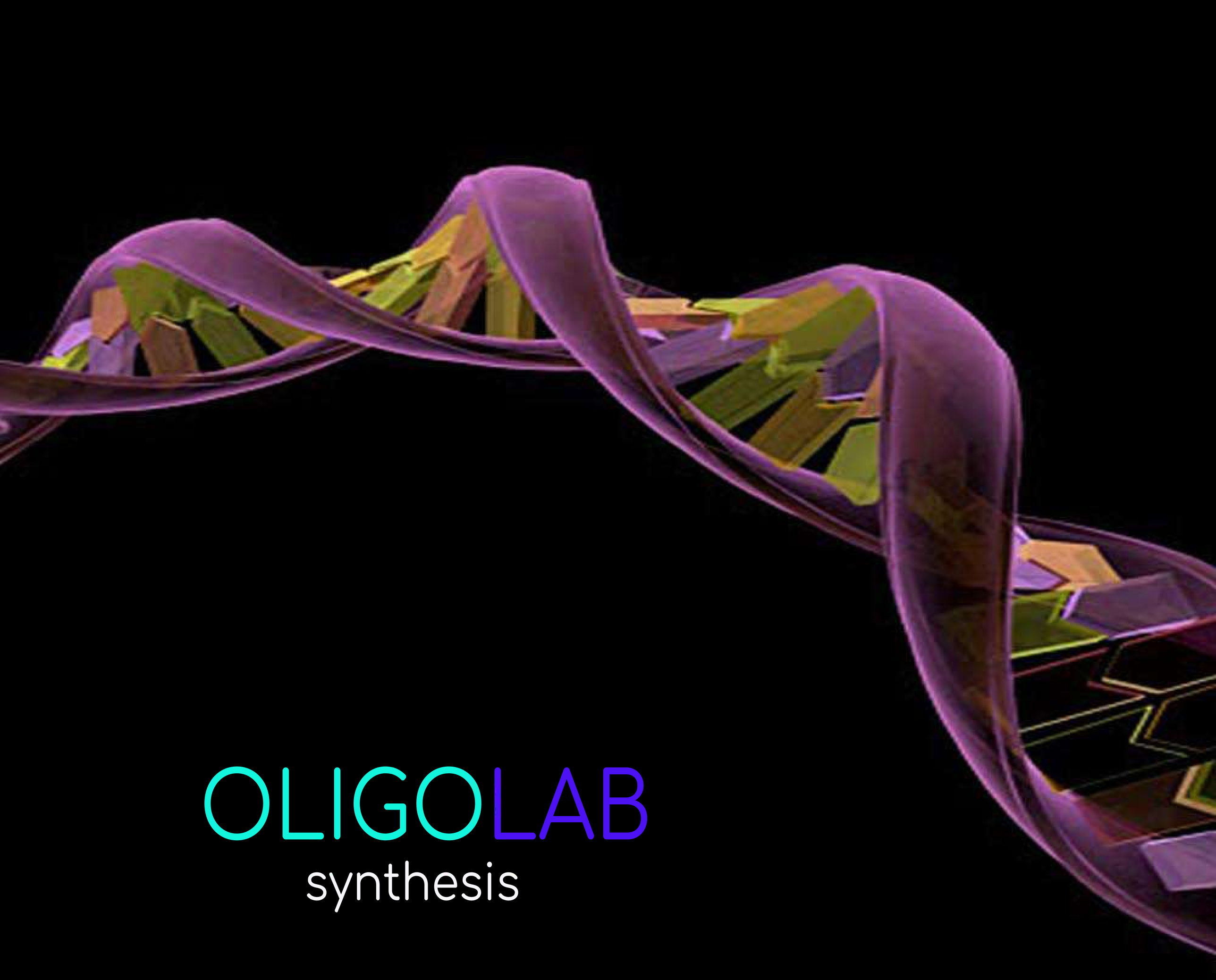


OLIGOLAB

synthesis

ABOUT US

Oligolab is a botechnical company, which specialize in chemical synthesize of oligonucleotites, modified oligonucleotites, fluorescent oligos and TaqMan probes.

By tailoring the synthesis protocol to each specific oligo we give it the best chance to succeeding. We than use our knowledge of purification methods to produce the purest oligo possible and cary out a detailed in depth analysis.If we find anything unexpected we repeat the synthesis underoptimised conditions.

We often work with our customers before ordering, suggesting modifications that will give the best results, both in the synthesis of the oligo and in sunsequent biochemical experiments. Our job isn't done when we dispatch oligos,we welcome customer feedback and if problems arise we make it our mission to help to find out why, suggesting changes to the design thay will improve the results next time

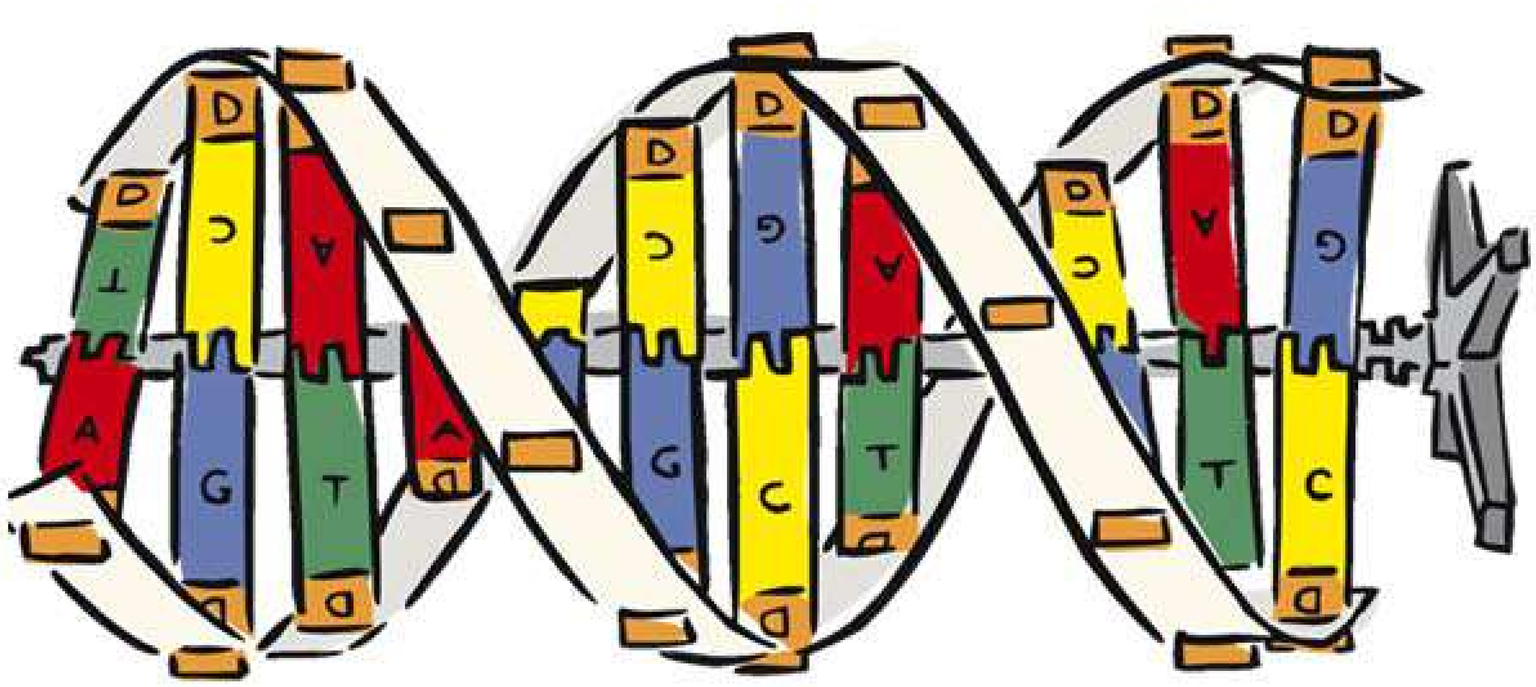
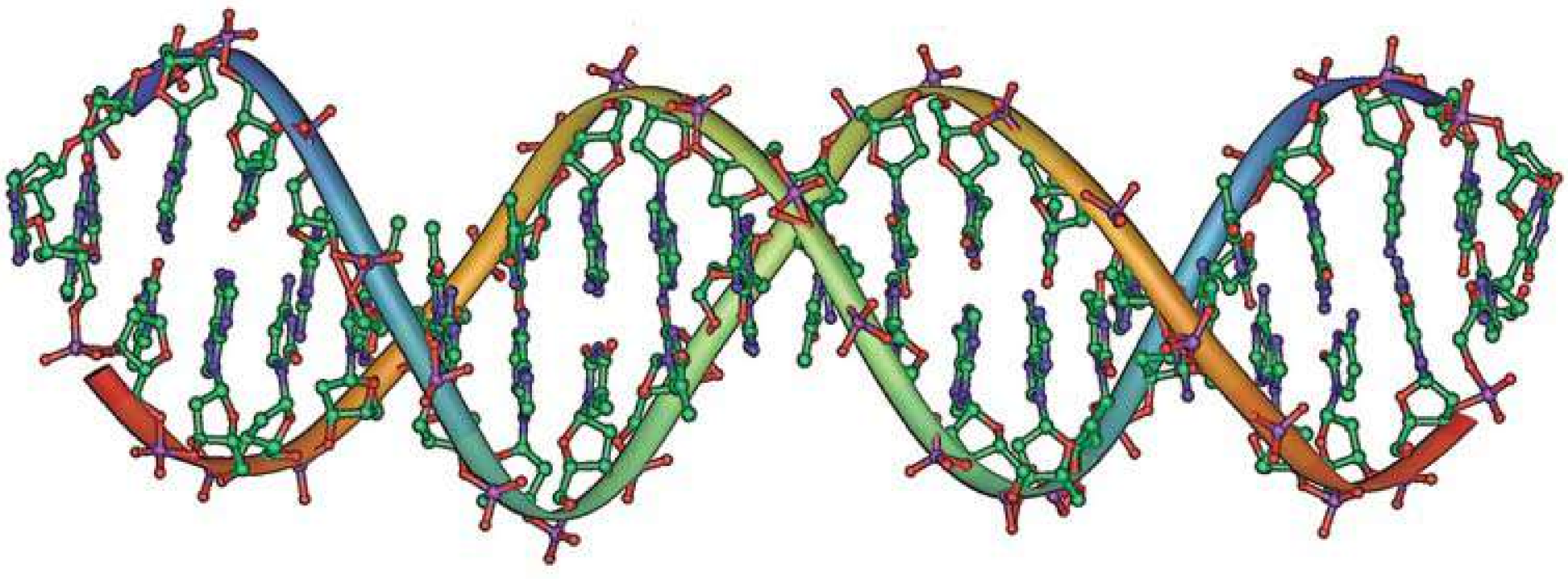


Table of content



APPLICATION OF OLIGONUCLEOTIDES

Oligonucleotides are now in extensive use in various scientific fields, including bio-technology, molecular biology, genetic engineering, proteomics, immunology and pharmacy.

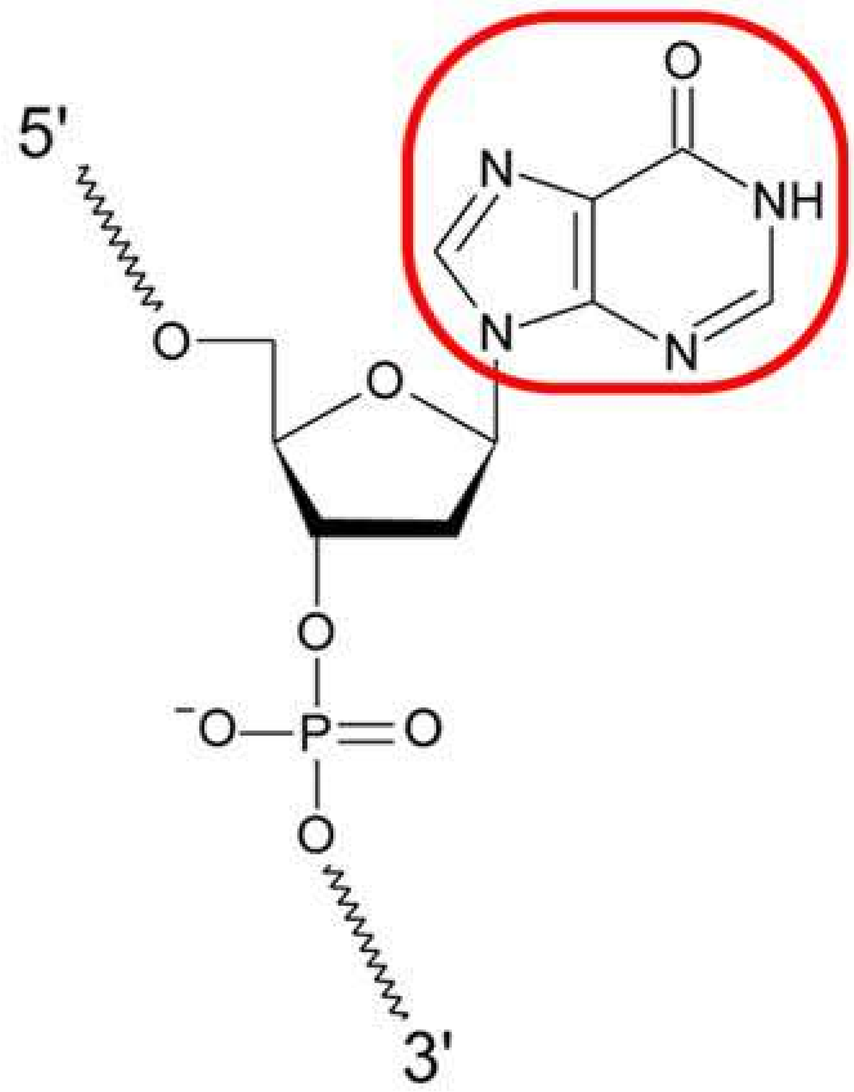
Oligolab provides high quality products which can be applied for:

- Polymerase Chain Reactions (PCR),
- Gene expression and gene therapy,
- Molecular diagnostics,
- Epigenetic research,
- Study modern therapeutic strategies,
- Forster Resonance Energy Transfer (FRET), Real-time PCR
- in vitro translations,
- Immobilization on solid support (e.g. microarrays)
- Rolling circle amplification.

INTERNAL NODIFICATION OF NUCLEIC ACIDS

Desoxy inosine (Di)

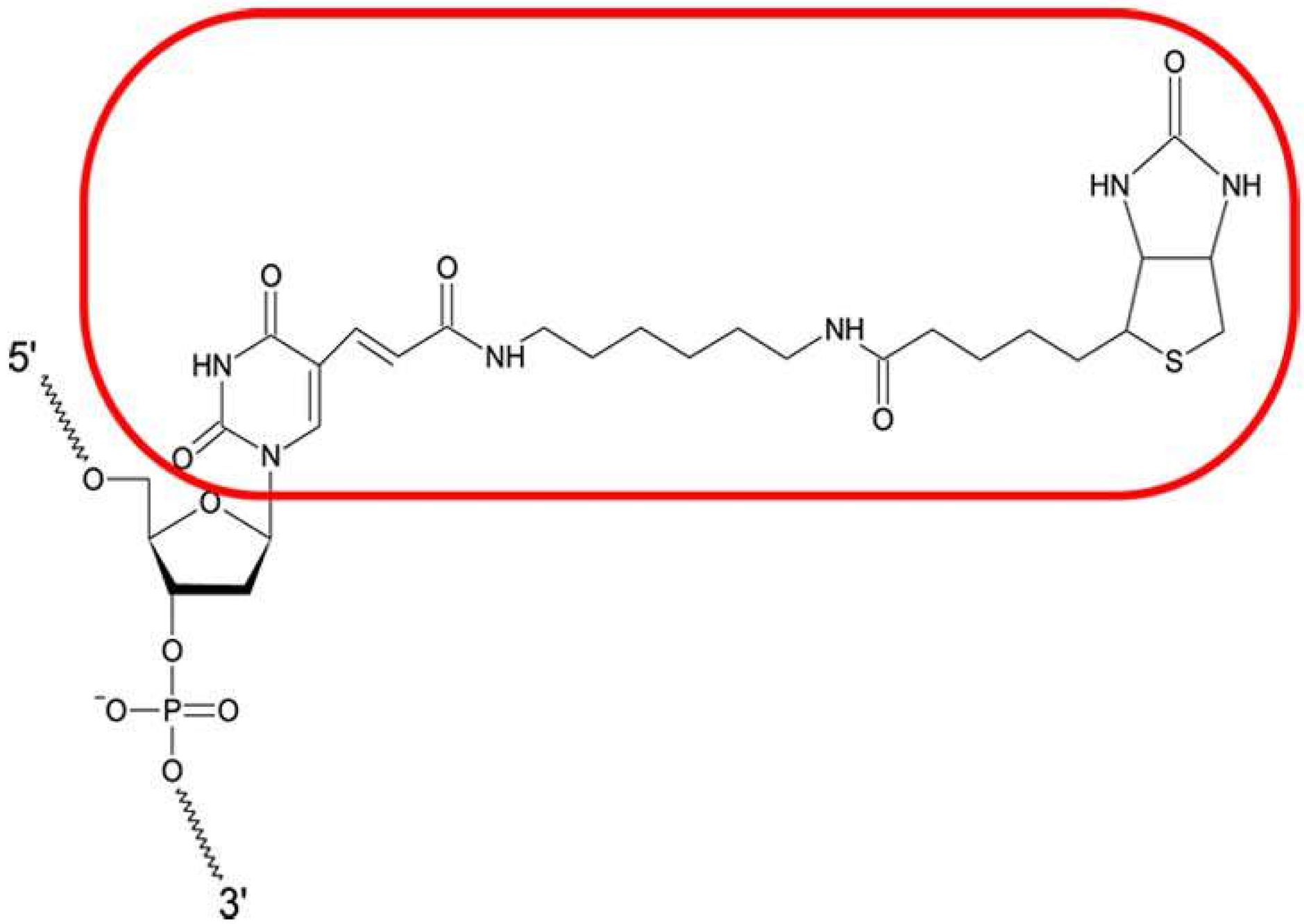
Inosine is a purine nucleoside containing hypoxantine as heterocyclic base. The inosine nucleotide is used as a universal base because it may form stable base pairs with any of the canonic heterocyclic bases. Therefore it is commonly used for designing starters in PCR.



Inosine displays the folowing preferences in forming Watson-Crick base pairs: I-C> I-A> I-G = I-T (With significant preference of the I-C pair).

Biotin-dT

Biotinylated deoxythymidine is a deoxynucleotide connected with biotin by a flexible alkyl linker.

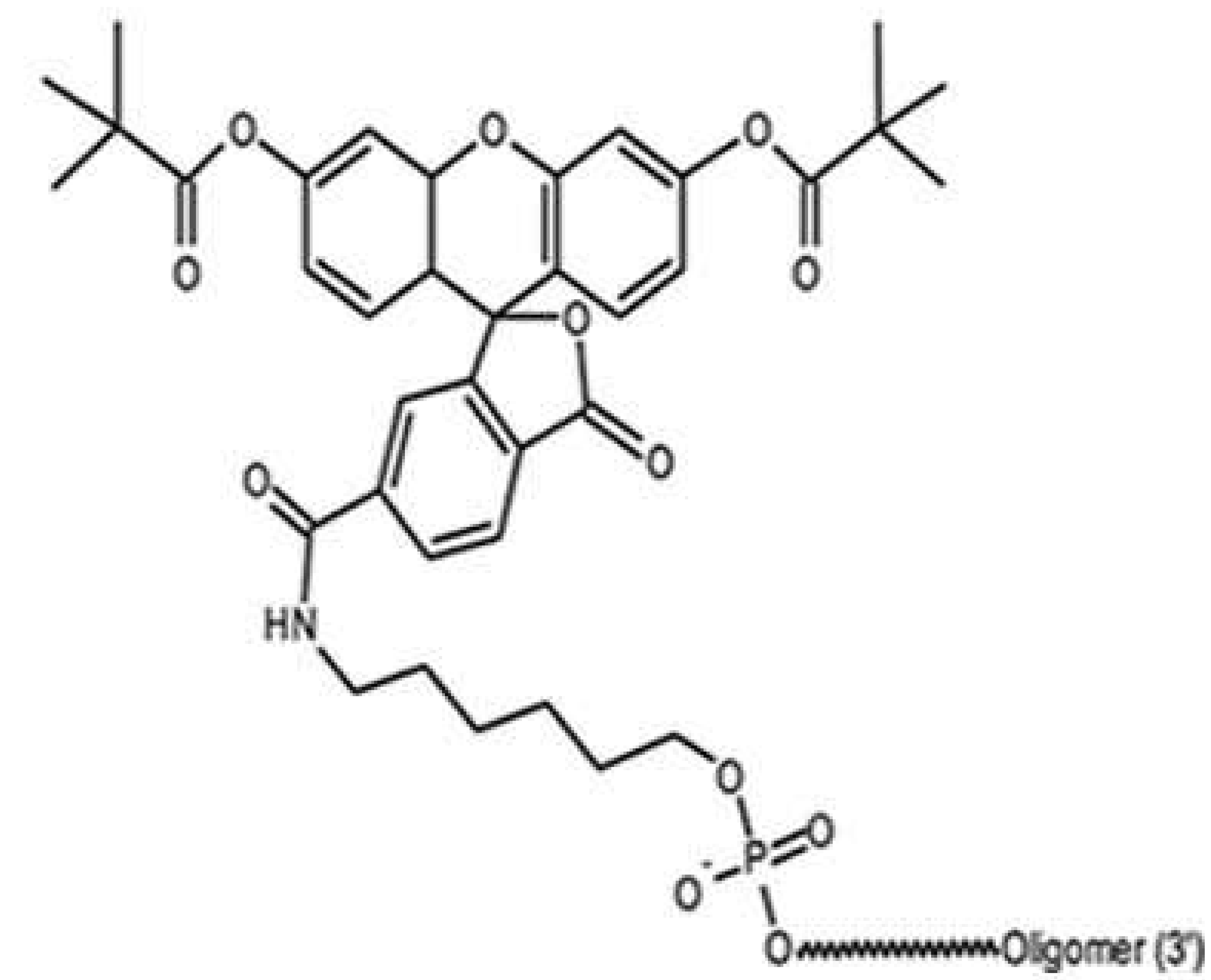


Biotin strongly interacts with streptavidin, forming a very stable complex. The property is extensively used in molecular biology, e. g. in fluorecent labeling or oligonucleotide immobilization on solid support.

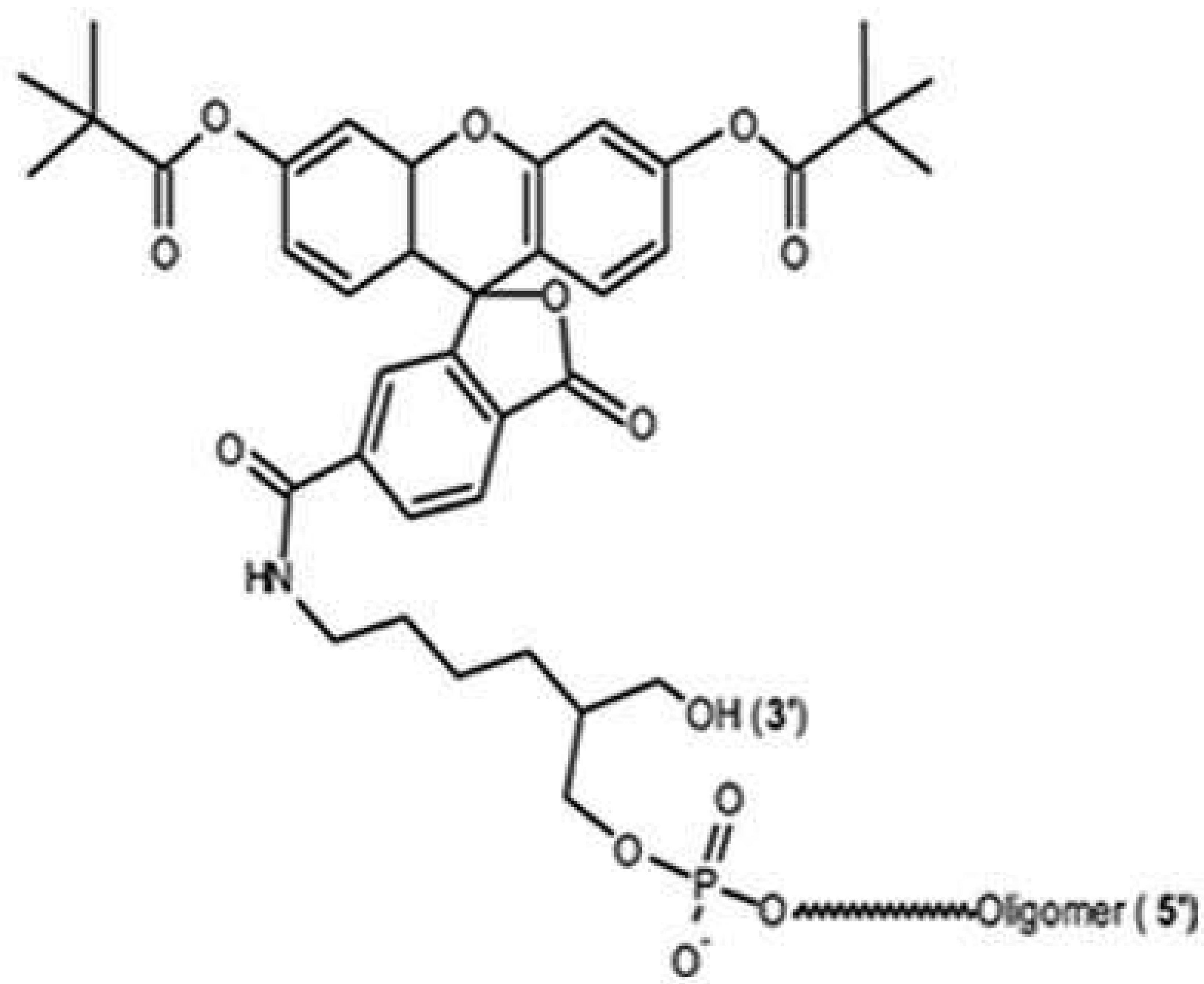
Fluorescein

Fluoreiscein is an organic dye which displays fluorescence in yellow and green in basic environment. Absorbtion maximum for fluorescein is 495 nm, but after excitation it emits light whose wavelenght is of 520nm (in aqueous solution).It is comonly used to label cells and antibodies. Coupled with oligonucleotide, it may be used in hybridization, as labelled starters or molecular probes.

5' - fluoresceine



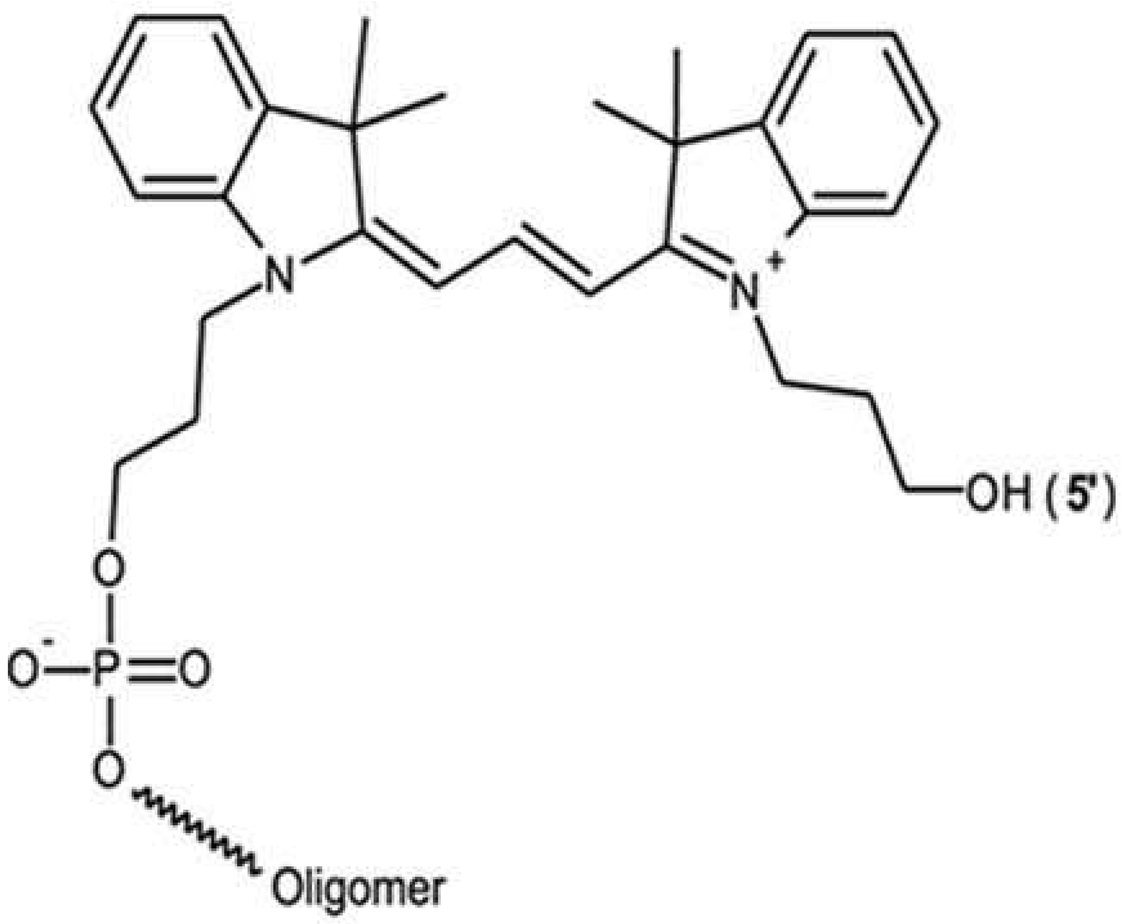
3' - fluoresceine



Fluorescein is the most commonly used fluorescent dye for labeling oligo-nucleotides. Fluorescein plays a particularly important role in real-time PCR applications, being used as a reporter moiety in TaqMan probes, Scorpion primers and Molecular Beacons. For such probes, fluorescein is most commonly paired with the dark quencher BHQ-1,as the two have excellent spectral overlap.

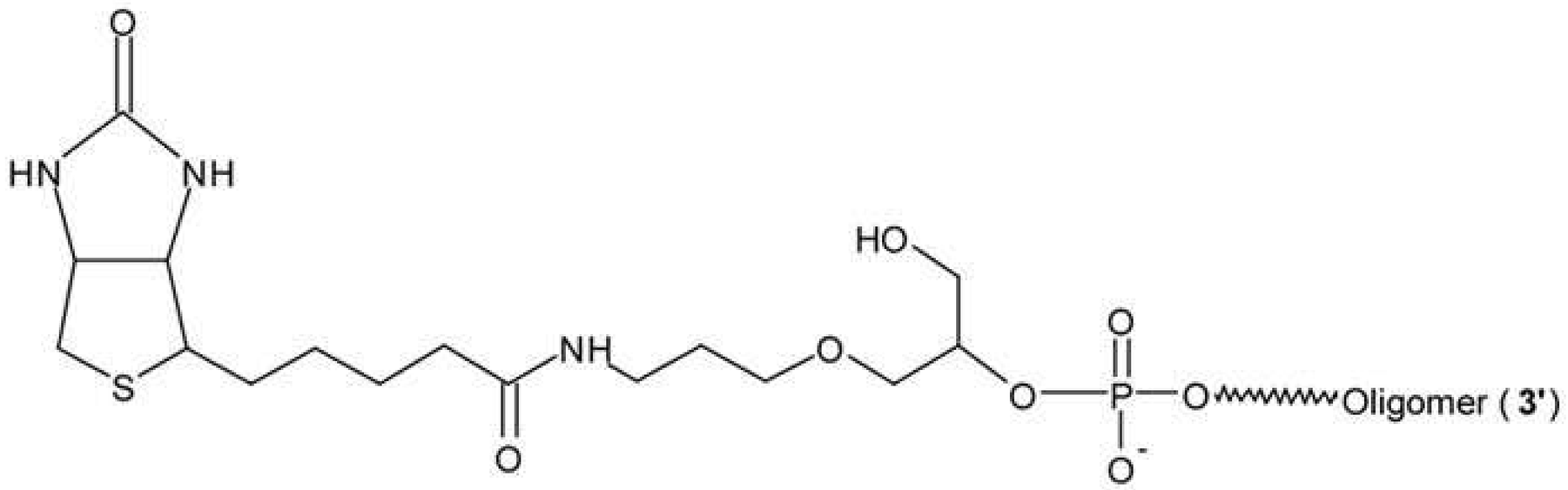
Cyanine 3 (Cy3)

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



Biotin

Biotin is an affinity label that can be incorporated at either the 5'- or 3'-end of an oligonucleotide, or at an internal position. Biotin has a high affinity for the bacterial protein, streptavidin, which can be conjugated to a solid support (such as magnetic beads) for use as a capture and immobilization medium for a biotinylated oligo. In the biotin phosphoramidite, the biotin is attached to a long spacer arm, which acts to minimize steric hindrance between the biotin moiety and the oligo, thereby providing streptavidin easy access to the biotin. Biotinylated oligos are most commonly used as probes or primers in a variety of in vitro and in vivo applications

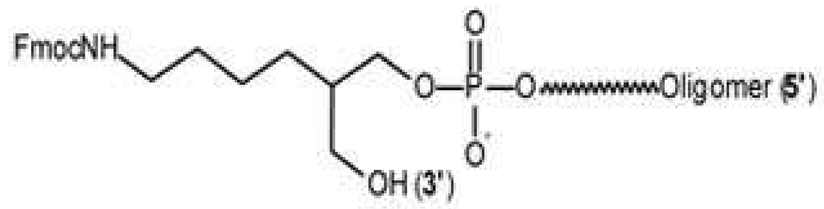


Besides their importance as nucleic acid probes, biotinylated oligonucleotides are also useful for the purification of DNA binding proteins. In this context, the biotinylated oligonucleotide can be bound to a streptavidin matrix and used for either column or spin chromatography. For isolation of DNA binding proteins, the streptavidin-biotin-oligonucleotide complex is incubated with a crude cell extract containing nuclear proteins. Following appropriate washes, the proteins that bind selectively to the oligonucleotide sequence can be eluted under conditions that disrupt the protein: DNA complex. Because the binding of biotin to streptavidin is essentially irreversible and is resistant to chaotropic agents and extremes of pH and ionic strength, the elution conditions can be relatively stringent

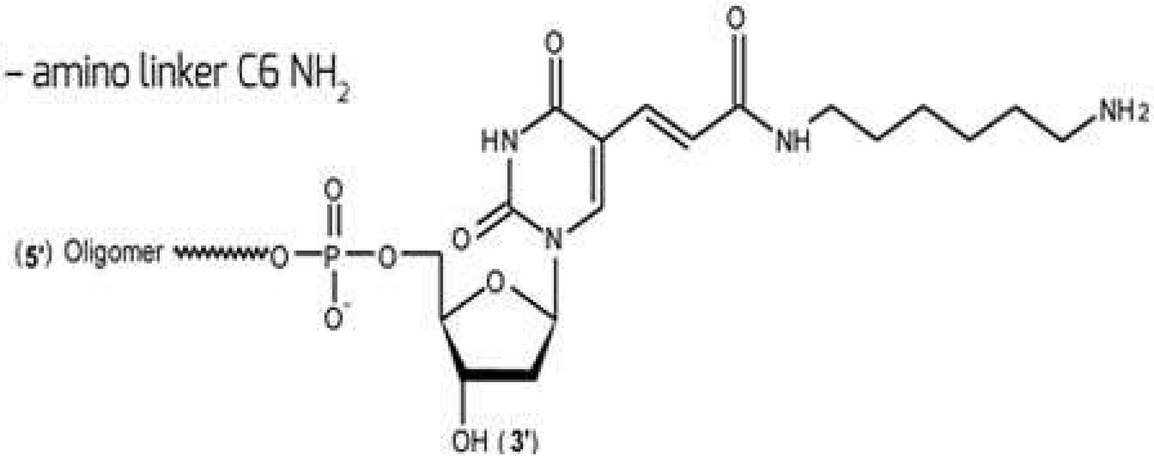
AMINE LINKER C6 NH2

It is applied to functionalize an oligonucletide with an amine group, which may be used to form covalent bonds with numerous other molecules. A long alkyl linker it posible to attach a fluorescenct label whose interaction with oligonucleotide might decrease their functionality. It is often used to immibilize oligonucleotides on solid suport, e.g. microarray slides.

3' – amine linker C6 NH_2

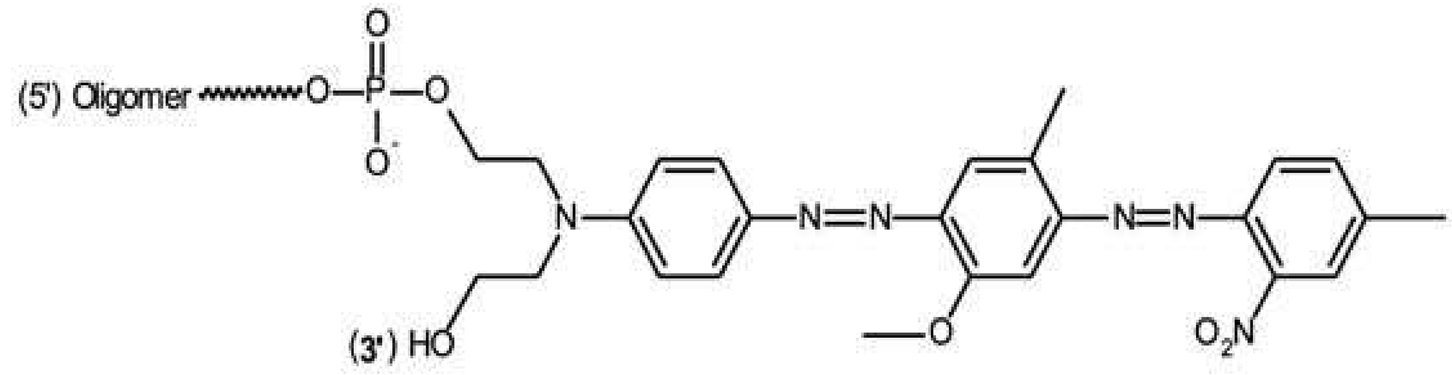


dT – amino linker C6 NH_2

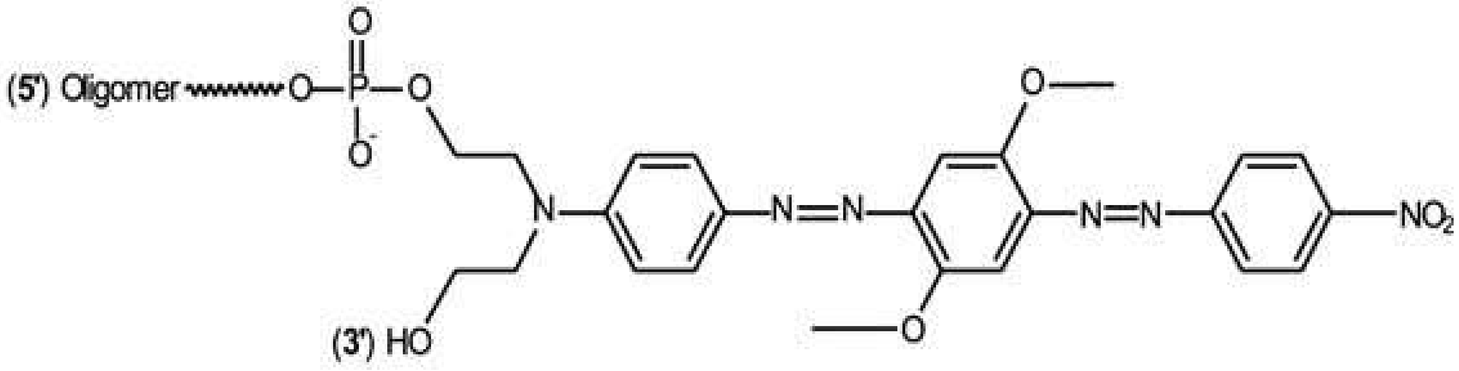


BHQ-1, BHQ-2

BHQ-1®



BHQ-2®



Supplement

COMPABILITY OF FLUORESCENT LABELS WITH QUENCHERS

FLUOROPHORE	ALTERNATE DYES	DYE-5'-T EX	DYE-5'-T EM	RECOMMENDED QUENCHER
Biosearch Blue™		352	447	BHQ-1
FAM		495	520	BHQ-1
TET		521	536	BHQ-1
CAL Fluor® Gold 540	VIC/TET/JOE	522	544	BHQ-1
JOE		529	555	BHQ-1
VIC		538	554	
HEX		535	556	BHQ-1
CAL Fluor Orange 560	VIC/HEX/JOE	538	559	BHQ-1
Quasar® 570	CY3	548	566	BHQ-2
Cy™ 3		549	566	
NED		546	575	
TAMRA		557	583	BHQ-2
CAL Fluor Red 590	TAMRA	569	591	BHQ-2
Cy 3.5		581	596	
ROX		586	610	BHQ-2
CAL Fluor Red 610	TEXAS RED/ROX/ ALEXA FLUOR® 594	590	610	BHQ-2
Texas Red®		597	616	
CAL Fluor Red 635	LC RED® 640	618	637	BHQ-2
Pulsar® 650		460	650	BHQ-2
Cy 5		646	669	
Quasar 670	CY5	647	670	BHQ-2*, BHQ-3
Cy 5.5		675	694	
Quasar 705	CY5.5	690	705	BHQ-2*, BHQ-3

BHQ-0 λ_{max} 495 nm
QR = 430-520 nm

BHQ-1 λ_{max} 534 nm
QR = 480-580 nm

BHQ-2 λ_{max} 579 nm
QR = 559-670 nm

** BHQ-2 dye is recommended for Pulsar 650, Quasar 670, and Quasar 705 dyes due to static quenching*

BHQ-3 λ_{max} 672 nm
QR = 620-730 nm

CALCULATING THE AMOUNT OF MATERIAL

Calculating the amount of material in nmols depending on the amount of OD and oligonucleotide sequence.

$$m\,[nmol] = \frac{100 \times n\,[OD]}{1{,}54 \times A + 1{,}17 \times G + 0{,}75 \times C + 0{,}92 \times T}$$

m[nmol] - number of nmols
n[OD] - amount of OD
A, G, C, T - number of proper bases in oligonucleotide

Calculating the amount of material in µg depending on the number of nmols and molar mass of oligonucleotide.

$$n\,[\mu g] = \frac{m\,[nmol] \times MW\ [g/mol]}{1000}$$

n [µg] - amount in µg
m[nmol] - amount in nmols
MW - molar mass

Calculating the volume of a sample in order to obtain oligonucleotide solution of defined concentration depending on the number of nmols

$$v\,[\mu l] = \frac{n\,[nmol] \times 1000}{c\,[pmol/\mu l]}$$

v [µl] - volume of sample µl
n[nmol] - amount of nmols
c [pmol/µl] - concentration pmol/µl

OLIGOLAB

www.oligolab.com

oligsynthesis@gmail.com

Kyiv, Ukraine