

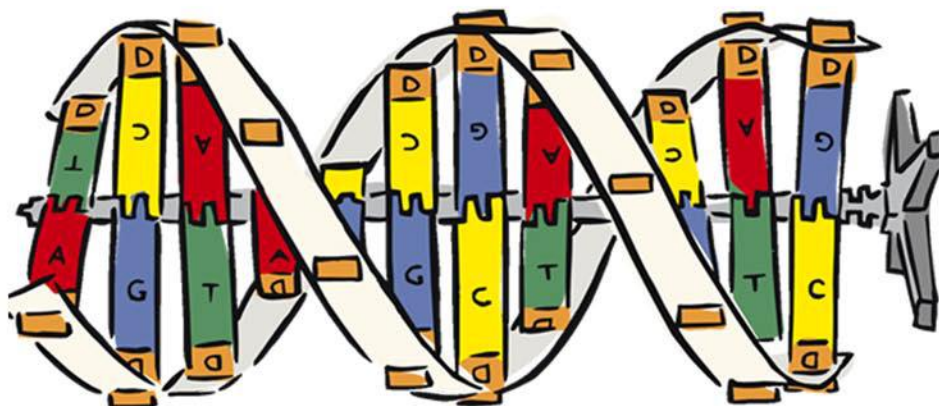
OLIGOLAB  
synthesis

## ABOUT US

Oligolab is a biotechnical company, which specialize in chemical synthesis of oligonucleotides, modified oligonucleotides, fluorescent oligos and TaqMan probes.

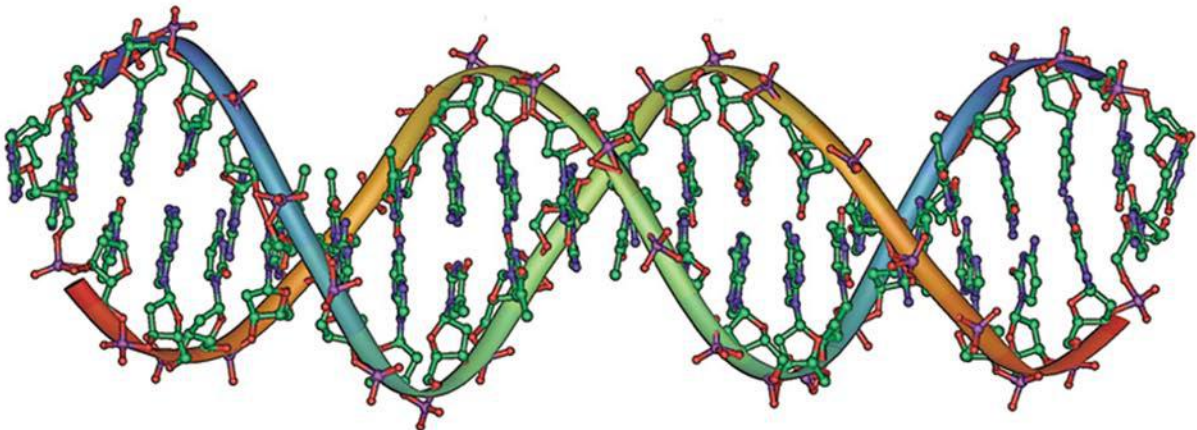
By tailoring the synthesis protocol to each specific oligo we give it the best chance of succeeding. We then use our knowledge of purification methods to produce the purest oligo possible and carry out a detailed in depth analysis. If we find anything unexpected we repeat the synthesis under optimised 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 subsequent 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 that will improve the results next time.



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## APPLICATION OF OLIGONUCLEOTIDES

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

Ologolab provides high quality products to be applied to:

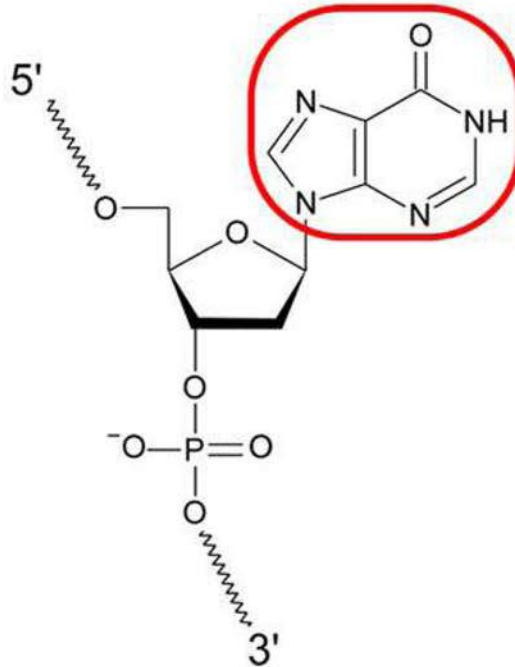
- Polymerase Chain Reactions (PCR),
- Gene expression and gene therapy,
- Molecular diagnostics,
- Epigenetic research,
- Study modern therapeutic strategies,
- Forster Resonance Energy Transfer (FRET), Real-time PCR
- in vitro translations,
- Immobilization on solid support (e.g. microarrays)
- Rolling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



## INTERNAL MODIFICATIONS OF NUCLEIC ACIDS

### Desoxy inosine (Di)

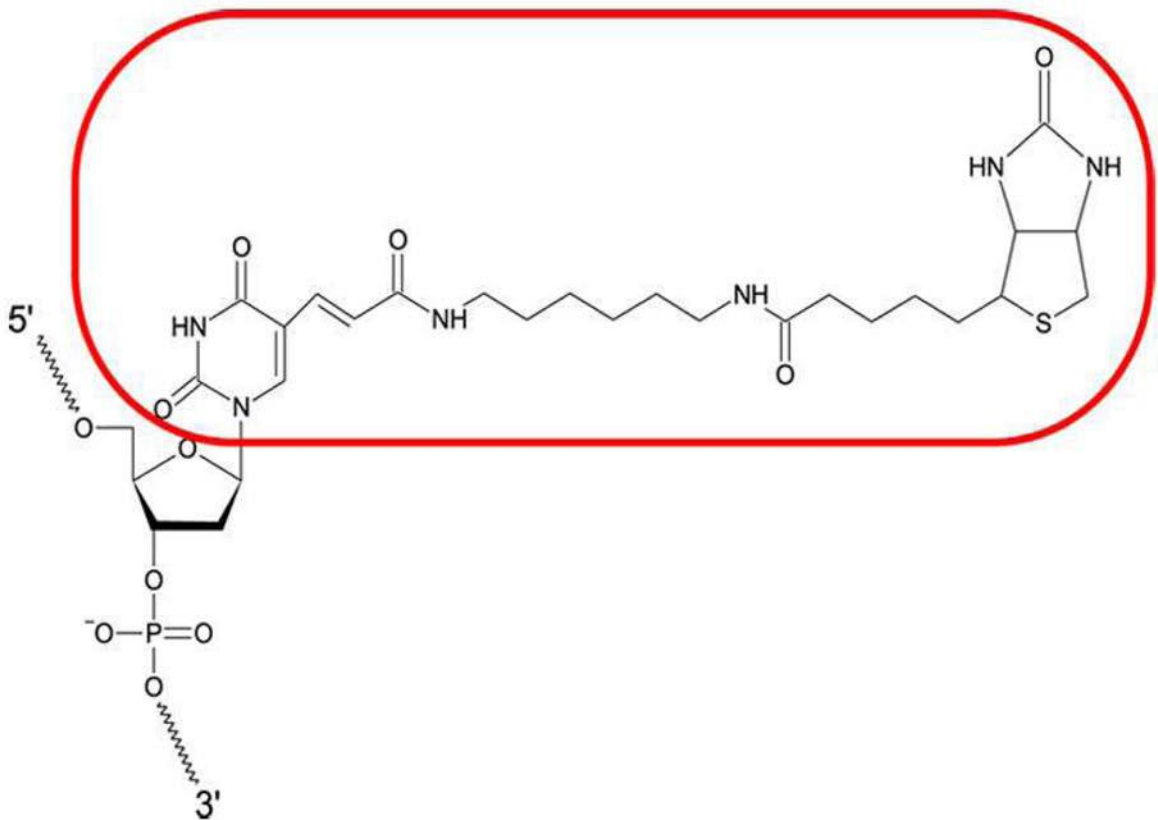
Inosine is a purine nucleoside containing hypoxanthine as heterocyclic base. The inosine nucleotide is used as a universal base because it may form stable base pairs with any of the canonic heterocyclic bases. Therefore it is commonly used in designing starters for PCR



Inosine displays the following 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.



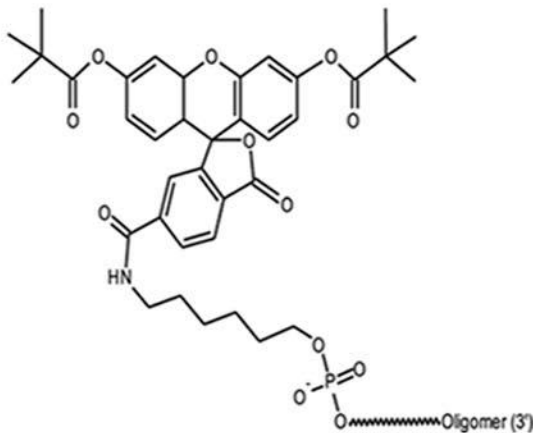
Biotin strongly interacts with streptavidin, forming a very stable complex. The property is extensively used in molecular biology, e.g. in fluorescent labelling or oligonucleotide immobilization on solid support.

# CHARACTERIZATION OF THERMINAL MODIFICATION

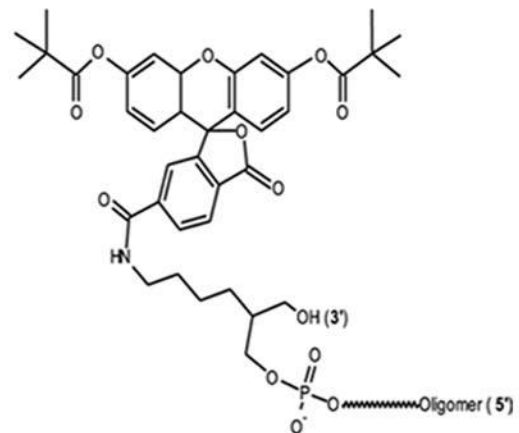
## Fluorescein

Fluorescein 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 wavelength 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

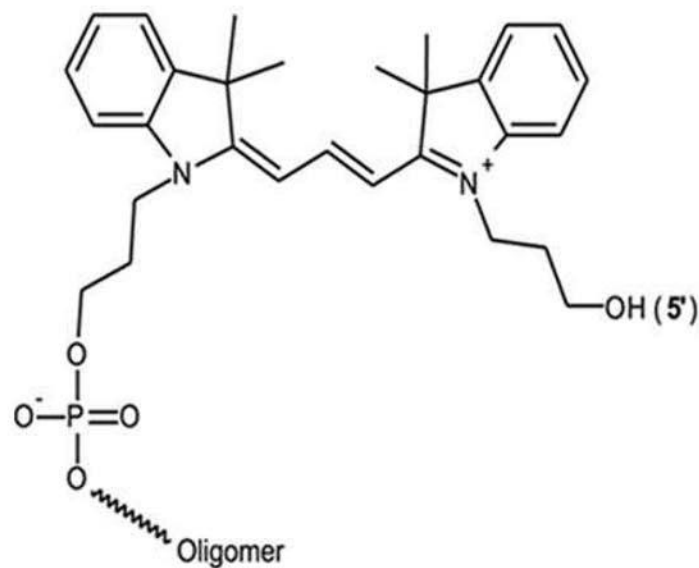


3' - fluoresceine



## Cyanine 3 (Cy3)

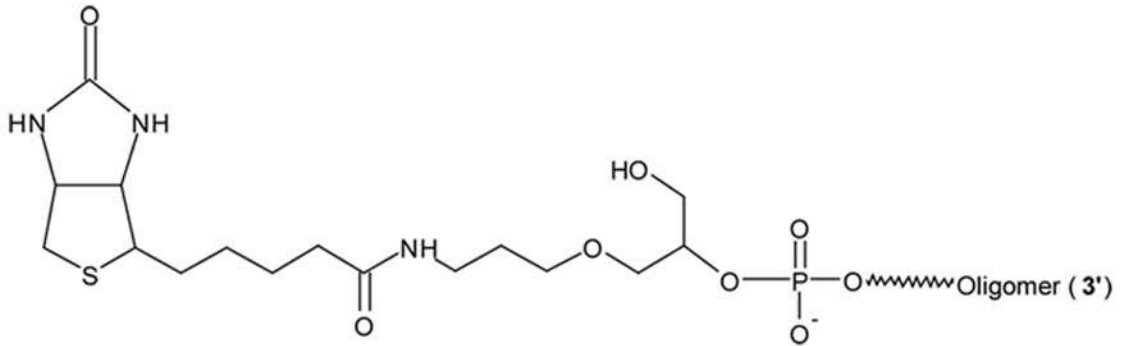
Cyanine 3 (Cy3) is a fluorescent dye that belongs to the Cyanine family of synthetic polymethine dyes. Cy3 is reactive, water-soluble, and has an absorbance maximum of 550 nm and an emission maximum of 570 nm. It is available as both a phosphoramidite and an NHS ester, and is used to fluorescently label 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

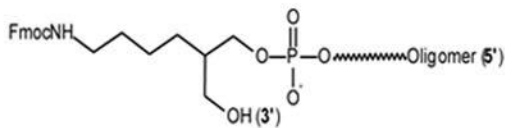
Biotin is a heterocyclic organic compound that occurs naturally in living organisms (vitamin H) as coenzyme of numerous enzymes. It has been widely applied in biological research due to its high affinity with avidin and streptavidin. As an oligonucleotide label it is used to immobilize DNA on slides in PCR - ELISA reactions.



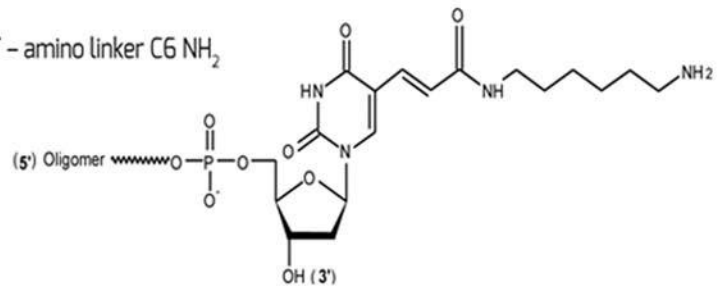
## AMINE LINKER C6 NH2

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

3' - amine linker C6 NH<sub>2</sub>

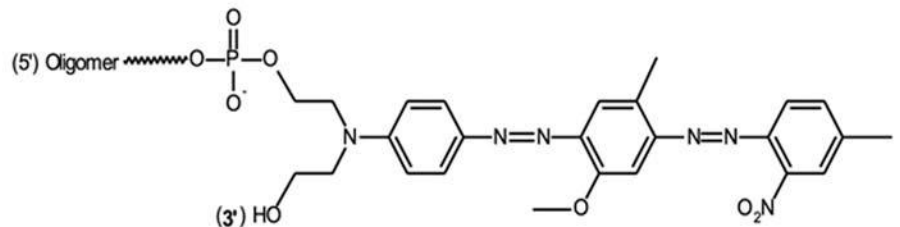


dT - amino linker C6 NH<sub>2</sub>

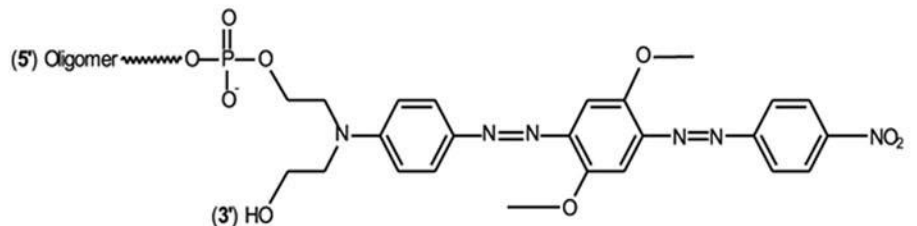


## BHQ-1, BHQ-2

BHQ-1<sup>®</sup>



BHQ-2<sup>®</sup>



# Supplement

## COMPABILITY OF FLUORESCENT LABELS WITH QUENCHERS

FLUOROPHORE	ALTERNATE DYES	DYE-5'-T		RECOMMENDED QUENCHER	BHQ Dye*
		EX	EM		
🌀 Biosearch Blue™		352	447	BHQ-1	BHQ-0 $\lambda_{max}$ 495 nm QR = 430-520 nm
FAM		495	520	BHQ-1	
TET		521	536	BHQ-1	
🌀 CAL Fluor® Gold 540	VIC/TET/JOE	522	544	BHQ-1	BHQ-1 $\lambda_{max}$ 534 nm QR = 480-580 nm
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		BHQ-2 $\lambda_{max}$ 579 nm QR = 559-670 nm
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	* BHQ-2 dye is recommended for Pulsar 650, Quasar 670, and Quasar 705 dyes due to static quenching
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		BHQ-3 $\lambda_{max}$ 672 nm QR = 620-730 nm
🌀 Quasar 705	CY5.5	690	705	BHQ-2*, BHQ-3	

## CALCULATING THE AMOUNT OF MATERIAL

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

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

$m[\text{nmol}]$  - number of nmols

$n[\text{OD}]$  - amount of OD

A, G, C, T - number of proper bases in oligonucleotide

Calculating the amount of material in  $\mu\text{g}$  depending on the number of nmols and molar mass of oligonucleotide.

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

$n [\mu\text{g}]$  - amount in  $\mu\text{g}$

$m[\text{nmol}]$  - 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

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

$v [\mu\text{l}]$  - volume of sample  $\mu\text{l}$

$n[\text{nmol}]$  - amount of nmols

$c [\text{pmol}/\mu\text{l}]$  - concentration  $\text{pmol}/\mu\text{l}$

# OLIGOLAB

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