Oligo Modifications for Increased Duplex Stability & Nuclease Resistance

Increased duplex stability and nuclease resistance are underlying requirements for most oligonucleotide-based applications.

Gene Link offers several modifications that can render the oligo less susceptible to nuclease degradation as well as increase hybridization stability. In addition to the synthesis of these modified oligos, we routinely assist customers in the design of oligos that are particularly suited to their application.

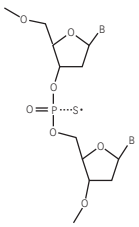
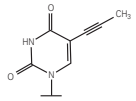
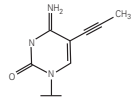
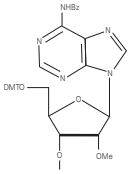
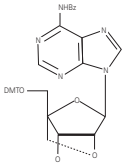
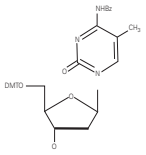
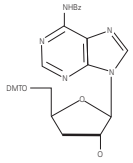
Antisense research requires short oligonucleotides that are complementary in sequence, and upon specific hybridization to its cognate gene product, induce inhibition of gene expression.

Increased stability of the RNA-DNA duplex in terms of hybridization and half-life is crucial to successful gene inhibition. These modifications can also be used for molecular probes and primers. Listed are some of the common modifications that impart these properties.

Antisense ODN Design Options

Traditionally phosphorothioate ODN's are used for antisense research. Consider inclusion of modified bases imparting increased duplex stability.

Oligo Modifications

Modification	Phosphorothioate	Propyne analogs	2'-O-methyl RNA	Locked Nucleic Acids	5-Me-dC	2'-5' Linked Oligonucleotides	Chimeric Linkages
Molecular Structure	 2'-Deoxy thioate	 5-(1-Propynyl) uracil (pU)  5-(1-Propynyl) cytosine (pC)	 2'-O-methyl A	 dA LNA	 5-Me-dC	 3' dA (2'-5' linked)	Structure varies
Chemical Characteristics	Modification of the phosphodiester bond by replacing one of the non-bridging oxygens by sulfur	C-5 propyne analogs of dC and dT	2'-O-methyl at the 2' hydroxyl position	Bicyclic nucleic acid where a ribonucleoside is linked between the 2'-oxygen and the 4'-carbon atoms with a methylene unit	C-5 methylated dC	2'-5' linked phosphodiester linkage, 3' deoxy bases	Mixed phosphorothioate and phosphodiester linkages and modifications
Duplex Stability	Hybridizes to the target sequences with lesser affinity than oligos with phosphodiester backbone	Increased binding affinity to the target mRNA and increased stability	Binding similar to DNA	Highest thermal stability of all available modifications	Increased	Increased binding efficiency to RNA	Increased
Nuclease Resistance	Imparts resistance to nuclease degradation	Increased nuclease resistance	Increased	Increased	Similar to DNA	Increased	Increased

APPLICATION	RECOMMENDED MODIFICATIONS
Antisense Gene Target	• 2'-O-Me-nucleotides (2'-O-Me-RNA) form more stable hybrids with complementary RNA strands than equivalent DNA and RNA sequences. • Phosphorothioate linkages confer resistance to nuclease degradation. • Locked Nucleic Acids (LNA) demonstrate unsur–passed duplex stability. Use phosphorothioate linkages to impart nuclease resistance and LNA bases to achieve the most stable hybridization. • Propyne modified with phosphorothioate linkages are 50x more effective than the corresponding phosphodiester oligo.
Real-Time PCR probes, QPCR	• 5-Me-dC enhances duplex stability, thus shorter probes can be synthesized. • LNA bases render the probe greater duplex stability than the use of single MGB (minor groove binders) at the 3' end. It is an excellent substitute for TaqMan MGB modifications. • All combinations of modifications, fluorescent dyes, and backbone modifications can be performed.
SNP Genotyping, Allelic Discrimination	• LNA substituted bases impart greater specificity with higher T_m. • All types of fluorescent dyes and backbone modifications can be performed. • 5-Me-dC behaves similar to LNA bases in imparting duplex stability.
Hybridization Probes and PCR Amplification Primers	• LNA substituted bases impart greater specificity with higher T_m. Substitute 4-6 DNA bases with LNA bases. • 5-Me-dC behaves similar to LNA bases in imparting duplex stability.

Modifications Increasing Nuclease Resistance and Duplex Stability

	Price ($)/site	
Product	**200 nmol scale**	**1 µmol scale**
Phosphorothioates	4.25	6.50
5-Propyne pdC, pdU	130.00	200.00
5-Me-dC	75.00	125.00
2'-O-methyl bases	14.00	20.00
2'-5' linked bases	275.00	300.00
LNA bases	190.00	250.00
Chimeric linkage	75.00	75.00

*minimum charge for 15-20 mer applies depending upon modification.
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The Gene Link Advantage

- Stringent Quality Control Measures
- Specializing in Challenging Combinations of Modifications
- Antisense ODN combinations
- SNP Genotyping & Allelic Discrimination
- Real Time PCR Probes
- All Oligo Types and Modifications Available
- Easy Online Ordering System
- Online Design and Analysis Tools
- Rapid Turn Around Time
- Knowledgeable Technical Support
- Personalized, Friendly Customer Service

GOLD STANDARD

Unique Modifications

Gene Link specializes in the design and synthesis of challenging combinations of modifications.

You are invited to compare.