Product Profile

Custom Oligo Synthesis, antisense oligos, RNA oligos, chimeric oligos,
Fluorescent dye labeled oligos, Molecular Beacons, siRNA, phosphonates
Locked Nucleic Acids (LNA); 2'-5' linked Oligos

Locked Nucleic Acid (LNA)*

For research use only. Not for use in diagnostic procedures for clinical purposes.

Oligo Modifications for Increased Duplex Stability & Nuclease Resistance

Modifications

Locked Nucleic Acids (LNA) Oligos
Propyne dC and dU labeled Oligos
Phosphorothioate Oligos
5-Me-dC & 2-amino dA
2'-5' linked Oligos
Methylated Oligos

Applications

Amplification Primers
Fluorescent Molecular Probes
Molecular Beacons
Taqman Probes
RNA Interference
Allelic Discrimination
Antisense Oligos
SNP Detection

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Gene Link™

Table of Contents

Oligo Modifications for Increased Duplex Stability & Nuclease Resistance

Introduction

Antisense oligonucleotides refer to short, synthetic oligonucleotide that are complementary in sequence and upon specific hybridization to its cognate gene product induces inhibition of gene expression. Oligonucleotides, as short as 15 mer have the required specificity to inhibit gene expression of a particular gene by annealing to the cellular mRNA (1,2). The mechanism of gene expression is based on two properties; the first is the physical blocking of the translation process by the presence of the short double stranded region, secondly the presence of the RNA-DNA duplex is susceptible to cellular RNase H activity. RNase H cleaves the RNA-DNA duplex region of the mRNA thus preventing the faithful translation of the mRNA (3).

The stability of the RNA-DNA duplex in terms of hybridization and half-life is crucial to successful gene inhibition. Vigorous research activity in the area of nucleic acid chemistry has been devoted in developing novel base analogs that are resistant to degradation and that possess strong hybridization properties. This product profile aims at listing some analogs that meet the above criteria and are amenable to be synthesized by currently available standard DNA synthesis chemistry. This includes the classical phosphorothioate linkages (4), propyne analogs (5) and the latest locked nucleic acid (LNA) base analogs (6). We believe from cited reports that LNA substituted oligos with phosphorothioate linkages presents the most stable hybridization and are least susceptible to nuclease degradation (6).

RNA interference studies have shown the effectiveness of short interfering RNA (siRNA) in gene silencing. siRNA technology is now extensively recognized as a powerful tool for the specific suppression of gene expression and is presently being used by researchers in a wide range of disciplines for the assessment of gene function. These are generally 21mer double stranded RNA. Active research to render the siRNA more stable to degradation and to increase the duplex stability has led to the use of modified bases. LNA bases are an attractive substitute together with phosphorothioate linkages to impart greater duplex stability and resistance to nuclease degrdation

At Gene Link offers an extensive array of modifications to accomplish duplex stability and nuclease resistance to synthetic oligos. We have the ability to synthesize complex combinations of modifications, chimeric oligos and fluorescent probes. In addition to the synthesis of these modified oligos, we routinely assist customers in the design of the oligos that are particularly suited to their application.

Modifications

Phosphorothioate

The driving force for the search for novel chemical modification groups compatible with Watson-Crick hybridization of oligonucleotide was based on the observation of the short stability of naturally occurring oligonucleotides with phosphodiester bonds. Oligonucleotides with natural phosphodiester bonds are highly susceptible to rapid degradation by cellular nucleases. Cellular nucleases have endonuclease activity as well such that 3′ and 5′ end caps are not sufficient to prevent from degradation.

Modifications of the phosphodiester bond by replacing one of the non-bridging oxygen by sulfur imparts resistance to nuclease degradation, but in general hybridize to the target sequences with lesser affinity than the phosphodiester counter part. *This can be minimized by the use of LNA and 2′-5′ linked oligos as described in the section below.*

The sulfur-substituted oligonucleotides have a phosphorothioate linkage and are termed as **phosphorothioates** or simply as **S-oligo.** Phosphorothioate oligos are synthesized by Gene Link using the Beaucage (4) sulfurizing reagent. The sulfurization reaction is rapid and is performed on automated DNA synthesizers yielding greater than 96% phosphorothioate linkages; the remainders are phosphodiester linkages. Custom phosphorothioate oligonucleotides synthesized by Gene Link can be specified to have all the diester bonds substituted or only some selected diester linkages depending upon the researcher's experimental requirement. Substitution of all diester linkage is recommended to provide greater nuclease resistance.

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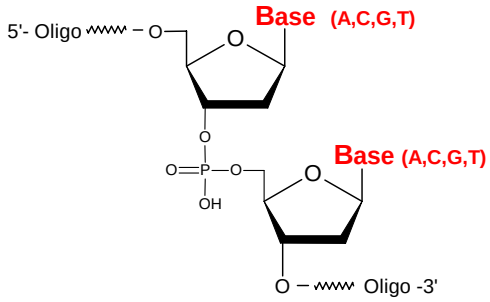
<td> 

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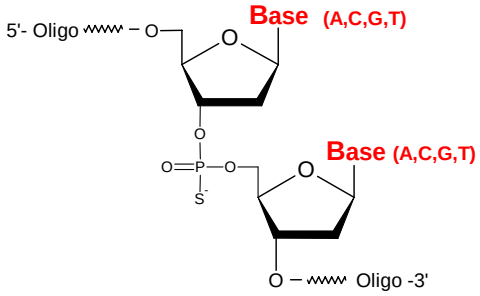
<td> 

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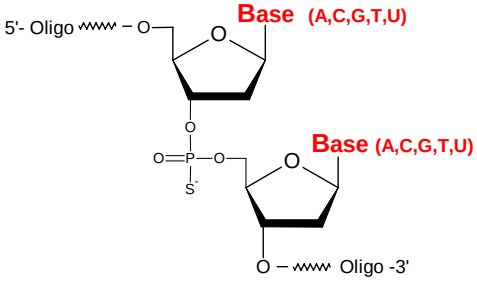
<td> 

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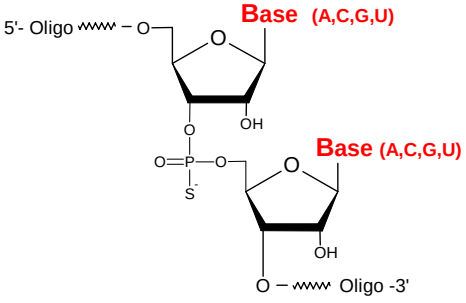
<td> 

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<td>Chimeric Phosphorothioate Linkages
(DNA,RNA, LNA, 2'O Me and other bases)</td>
<td>RNA phosphorothioate Linkage</td>
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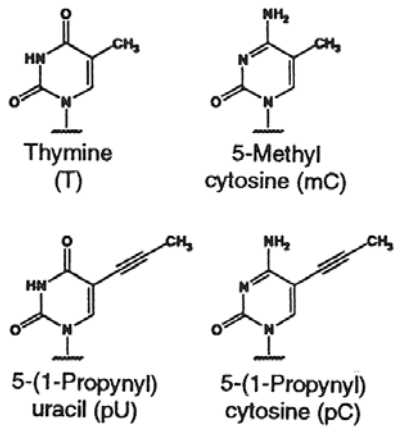
Propyne* Analogs

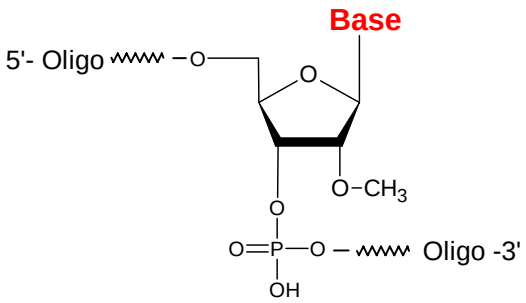
It has been shown that C-5 propyne analogs of dC and dT when substituted in phosphorothioate oligonucleotide imparts greater inhibition of gene expression due to increased binding affinity to the target mRNA and increased stability (5). Based on the above information antisense oligonucleotide could either be Phosphorothioated at all diester linkages or combined with substitutions of dC and dT by C-5 propyne analogs pdC and pdU.

The use of propyne analogs is covered by patents and licensing agreements. The sale of propyne-modified oligos is for research use only. See license agreement*



2'-O-methyl RNA oligonucleotides

RNA oligos are susceptible to degradation to the same extent as native RNA extracted from various sources. An attractive alternate to prevent degradation from nucleases is the use of 2'-O- methyl RNA bases, when specific 2'OH is not required. The 2'-O- methyl oligonucleotides confer considerable nuclease resistance and are similar in hydrogen bonding properties to RNA/RNA than the lower RNA/DNA binding property (7). The coupling efficiency of 2'-O- methyl phosphoramidite is also higher than the RNA monomers resulting in higher yield of full-length oligos.



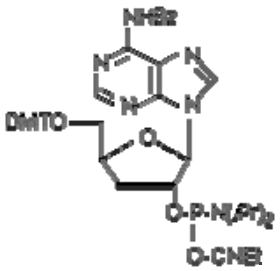
2' O-Methyl Base

[27-6410-XX]

Gene Link also offers custom synthesis of RNA and DNA chimeric oligos with investigator specified ribo or deoxy bases or 2'-O-methyl bases.
The chimeric oligos can also be synthesized with the regular phosphodiester bonds or substituted with phosphorothioate linkages. The combination of 2'-O- methyl RNA bases with phosphorothioate internucleotide linkages imparts these oligos greater nuclease resistance, which is particularly useful for antisense studies. Custom phosphorothioate oligonucleotides synthesized by Gene Link can be specified to have all the diester bonds substituted or only some selected diester linkages depending upon the researcher's experimental requirement. Substitution of all diester linkage is recommended to provide greater nuclease resistance.

2'-5' Linked Oligonucleotides

Cellular DNA and RNA are made up of ribo- and 2'-deoxyribonucleic acids linked together via 3'-5' phosphodiester linkages and by far comprise the bulk of polynucleic acids found in cells. Much less common are oligonucleotides which have 2'-5' linkages. However, a unique feature of 2'-5' linked oligonucleotides is their ability to bind selectively to complementary RNA (12-13). These features suggest a number of interesting uses for 2'-5' linked oligos such as their use as RNA specific probes or in antisense oligos.



3'-dA-CE Phosphoramidite

2-Amino-dA & 5-Me-dC

The underlying principle of genetic molecular interaction is Watson and Crick base pairing. Consistent efforts have been expended to introduce different modifications to the bases to increase duplex stability in turn making the hybridization stronger. Two such modifications are discussed below that can be easily substituted in almost all primer, oligo, probe and antisense oligonucleotide design.

As shown in Figure below, A-T base pairs have two hydrogen bonds whereas G-C base pairs have three hydrogen bonds. The simplest approach to improving primers would be to substitute A sites with 2-amino-A which forms three hydrogen bonds with T on hybridization. 2-Amino-A also destabilizes A-G wobble mismatches, thus increasing specificity.

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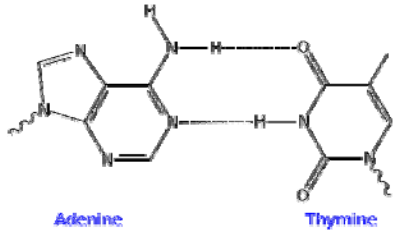
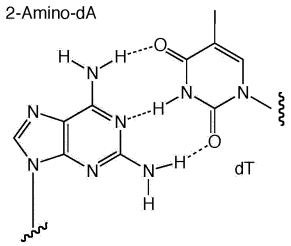
<tr><td> 
</td><td> 
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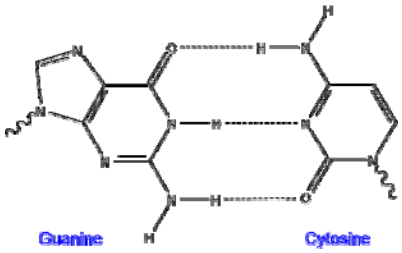
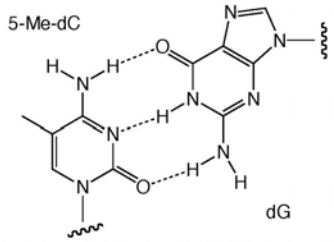
<tr><td>A-T base pairing</td><td>2-Amino dA-dT base pairing</td></tr>
<tr><td> 
</td><td> 
</td></tr>
<tr><td>G-C base pairing</td><td>5-Me-dC-dG base pairing</td></tr>
</table>

Pyrimidine Analogues

C-5 methyl pyrimidine nucleosides are known to stabilize duplexes relative to the non-methylated bases. Therefore, enhanced binding can be achieved using 5-methyl-dC in place of dC, duplex melting temperature being increased by 1.3°. Improved stacking in this case is believed to be brought about by elimination of water molecules from the duplex. 2,6-Diaminopurine 2'-deoxyriboside (2-amino-dA) forms an additional hydrogen bond with Thymidine, thereby leading to duplex stabilization with a melting temperature increase of 3°.

Duplex Stabilization

Using these base substitutions, duplex stability and therefore melting temperatures are raised by the approximate amounts shown below:

2-Amino-A	3.0° per substitution
5-Methyl-C	1.3° per substitution
C-5 propynyl-C	2.8° per substitution
C-5 propynyl-U	1.7° per substitution

While these modifications would also have a desirable effect on antisense oligonucleotides, the increased costs associated with most of them may limit their use. However, primers are less cost-sensitive because of the smaller scale, so the effects of the modified bases may be more generally useable. Potential improvements would include: the ability to use shorter oligos when sequence information is incomplete; higher melting temperatures, which should minimize the frequency of mutations; and enhanced binding, which should break any secondary structure in the target.

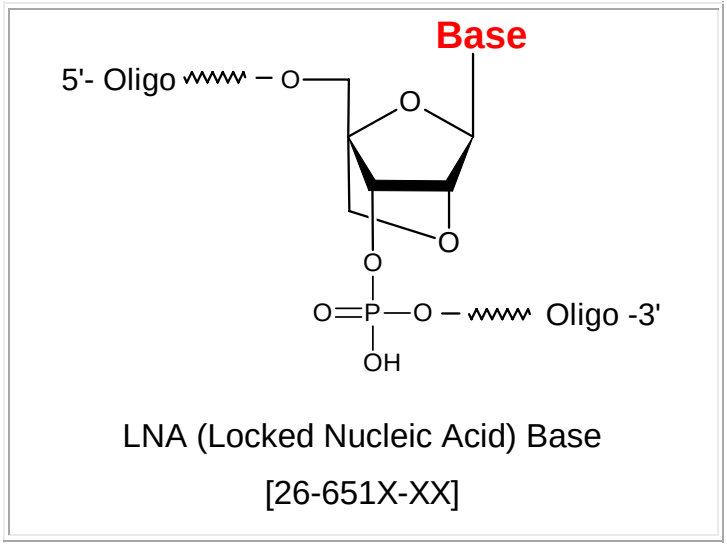
Locked Nucleic Acids (LNA)

Features

- LNA Oligonucleotides exhibit unprecedented thermal stability towards complementary DNA and RNA.
- Excellent mismatch discrimination.
- The high binding affinity of LNA oligos allows for the use of short probes & hybridization assays.
- LNA offers the possibility to adjust Tm values of primers and probes in multiplex assays.
- Use in antisense protocols, hybridization assay, in situ hybridization probes, dual labeled probes, molecular beacons and PCR primers.

Introduction

LNA is a bicyclic nucleic acid where a ribonucleoside is linked between the 2'-oxygen and the 4'-carbon atoms with a methylene unit. Locked Nucleic Acid (LNA) was first described by Wengel and co-workers in 1998 (8-10) as a novel class of conformationally restricted oligonucleotide analogues.



LNA (Locked Nucleic Acid) Base

[26-651X-XX]

The design and ability of oligos containing locked nucleic acids (LNAs) to bind supercoiled, double-stranded plasmid DNA in a sequence-specific manner has been described by Hertoghs et al (6). The main mechanism for LNA oligos binding plasmid DNA is demonstrated to be by strand displacement. LNA oligos are more stably bound to plasmid DNA than similar peptide nucleic acid (PNA) `clamps' for procedures such as particle mediated DNA delivery (gene gun). It is shown that LNA oligos remain associated with plasmid DNA after cationic lipid-mediated transfection into mammalian cells. LNA oligos can bind to DNA in a sequence-specific manner so that binding does not interfere with plasmid conformation or gene expression (6).

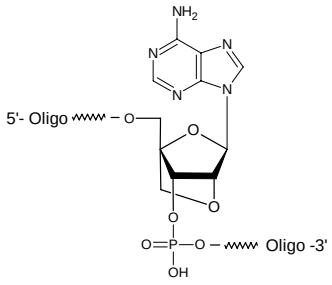
LNA Oligonucleotides exhibit unprecedented thermal stabilities towards complementary DNA and RNA, which allow excellent mismatch discrimination (8). The high binding affinity of LNA oligos allows for the use of short probes in antisense protocols and LNA is recommended for use in any hybridization assay that requires high specificity and/or reproducibility, e.g., dual labeled probes, in situ hybridization probes, molecular beacons and PCR primers. Furthermore, LNA offers the possibility to adjust Tm values of primers and probes in multiplex assays. Each LNA base addition in an oligo increases the Tm by approximately 2-4^{o}C. As a result of these significant characteristics, the use of

LNA-modified oligos in antisense drug development is now coming under investigation, and recently the therapeutic potential of LNA has been reviewed (11).

The synthesis and incorporation of LNA bases can be achieved by using standard DNA synthesis chemistry. Detailed research results have not yet concluded as to the amount of LNA bases and regular DNA base combination in successful antisense and gene delivery experiments. The investigator can elect to substitute individual bases in the oligo to LNA bases or use a combination. Due to the high affinity and thermal stability of the LNA: DNA duplex it is not advised to have more than 15 LNA bases in an oligo; this induces strong self-hybridization

The use of LNA C base requires special synthesis and post synthesis protocols. LNA-containing oligonucleotides can be purified and analyzed using the same methods employed for standard DNA. LNA can be mixed with DNA and RNA, as well as other nucleic acid analogues, modifiers and labels. LNA oligonucleotides are water soluble, and can be separated by gel electrophoresis and precipitated by ethanol.

LNA MONOMER STRUCTURES



LNA-A

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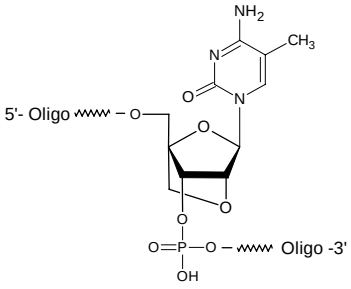

LNA-C

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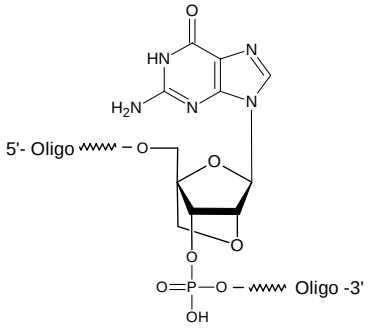

LNA-G

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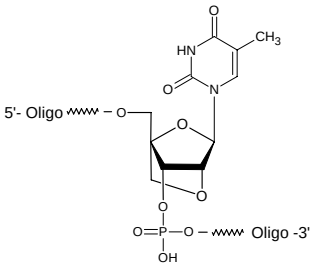

LNA-T

[26-6520-XX]

Applications & Recommended Modifications

LNA bases incorporated in oligonucleotides show remarkable hybridization specificity to both RNA and DNA. The higher Tm from 2-4°C of each modified LNA base offers excellent duplex stability. LNA-C and 5-methyl-C are equivalent in terms of duplex stability.

LNA containing oligonucleotides are substrates for most enzymes commonly used in molecular biology. A selection is listed below:

- 3' LNA is substrate for polymerases
- LNA primers and as template are read by different polymerases (Klenow, Taq polymerase) and reverse transcriptase.
- LNA can be cut by restriction enzymes
- 32P labeling with T4 polynucleotide kinase
- 3' LNA enhances resistance to exonuclease I

The following guideline represents the wide application of LNA use for almost all oligo design and use. Gene Link technical service offers advice on oligo design and use of modifications based on application.

Application	Recommended Modifications
Antisense Gene Target	• Oligonucleotides containing 2'-OMe-nucleotides (2'-OMe-RNA) forms more stable hybrids with complementary RNA strands than equivalent DNA and RNA sequences. • Phosphorothioate linkages confer oligonucleotides resistance to nuclease degradation. • Locked Nucleic Acids (LNA) has demonstrated an unsurpassed duplex stability. Use phosphorothioate linkages to impart nuclease resistance and LNA bases to achieve most stable hybridization and thus duplex stability.
RNA Interference (siRNA)	• LNA substituted bases at the 3' and 5' end of siRNA enhances duplex stability and increases exonuclease resistance. It has been shown that siRNA with end modified LNA bases have ~10 fold longer half life. [Olaf et al. Mol Cancer Ther 2007; 6(3):833–43]. • 3-4 LNA base substitution in the sense strand increases duplex stability. • Phosphorothioate linkages confer oligonucleotides resistance to nuclease degradation. • 2'F U and C substituted siRNA are more resistant to RNAse degradation. • 3' Cholesterol modification helps in cellular

	uptake. • LNA-DNA-LNA gapmers shown to activate RNase H activity.
Real-Time PCR probes, QPCR	• LNA substituted bases in the probe enhances the duplex stability and thus shorter probes can be synthesized. • The use of LNA bases renders the probe greater duplex stability than the use of single MGB (minor grove binders) at the 3’ end. It is an excellent substitute for TaqMan MGB probes. • All types of fluorescent dyes and backbone modifications can be performed.
SNP Genotyping, Allelic Discrimination.	•LNA substituted bases imparts greater specificity with higher Tm. • All types of fluorescent dyes and backbone modifications can be performed. • C-5 methylated pyrimidine deoxy-nucleosides behave similar to LNA bases in imparting duplex stability.
Hybridization Probes and PCR Amplification Primers.	•LNA substituted bases imparts greater specificity with higher Tm. Substitute 4-6 bases with LNA .

LNA Oligonucleotide Melting Temperature

LNA is a unique base modification due to its higher Tm. This extreme duplex stability restricts the use of to many LNA bases especially in short oligos. Introduction of more than 4 LNA bases consecutively induces irreversible intra oligo LNA base binding and should be avoided. A maximum of 8 LNA bases is recommended in a 18mer oligo.

Prediction of Melting Temperature (Tm) for DNA and LNA Modified Oligonucleotides

The Tm prediction given below was calculated by the LNA Tm predictor available at http://lnatools.com/

Melting temperature
In order to design optimal probes and primers it is important to predict the melting temperature (Tm) of the LNA modified oligonucleotides. Tm is a necessary, but not sufficient, factor for determining the efficiency of capture probes and primers. Other important factors are the secondary structure, the discrimination ability and the steepness of the melting curve. LNA gives a higher degree of freedom with regard to optimising the melting temperature range of the oligonucleotides.

Parameter estimation in the Tm model
LNA modified oligonucleotides have eight possible monomers as opposed to the four DNA nucleotides, therefore the number of possible nearest neighbour pairs is 64 instead of 16. Since both entropy (ΔS) and enthalpy (ΔH) are estimated using statistical learning, up to 128 parameters needs to be estimated from the experimental data. Because of experimental error, more than one sample per parameter is necessary, thus in the order of 300 measurements of hybridisations are used, in order to get good parameter estimates. To get even better parameter estimates several parameter reduction techniques have been used. The model parameters were trained by optimising a sum-of-squared-residual error function using gradient descent (16).

Tm Comparison of Standard DNA bases and LNA substituted bases

Oligo Type	18 mer Oligo Sequence Lower case DNA and Upper case are LNA bases	# of LNA bases	Tm at 115 mM salt and 2µM Traget+Probe	Tm at 200 mM salt and 2µM Traget+Probe
All DNA	5′-caagttcctcctgtttag-3′	Zero	55°C	58 °C
All LNA	5′-CAAGTTCCTCCTGTTTAG-3′	18	98 °C	102 °C
Mixed	5′-caagTtcctcctgtttag-3′	1 T LNA base	56 °C	59 °C
Mixed	5′-caAgttcctcctgtttag-3′	1 A LNA base	57 °C	60 °C
Mixed	5′-caaGttcctcctgtttag-3′	1 G LNA base	57 °C	60 °C
Mixed	5′-caagttCctcctgtttag-3′	1 C LNA base	57 °C	60 °C
Mixed	5′-caagTtcctcctgtTtag-3′	2 T LNA base	57 °C	59 °C
Mixed	5′-caagTtcctcctGtttag-3′	1 T & 1G LNA	58 °C	61 °C
Mixed	5′-caAgttCctcctgtTtag-3′	3 LNA (T,C & A)	60 °C	62 °C
Mixed	5′-caAGttCctcctgtTtag-3′	4 LNA (G,A,T& C)	61 °C	64 °C
Mixed	5′-caAGttCctCctgtTtag-3′	5 LNA	64 °C	66 °C
Mixed	5′-cAAgttCctCctGtTtag-3′	6 LNA	66 °C	69 °C
Mixed	5′-cAAgttCcTCctGtTtag-3′	7 LNA	69 °C	72 °C
Mixed	5′-cAAgttCcTCctGtTtAg-3′	8 LNA	72 °C	75 °C

General LNA Oligonucleotide Design Guidelines

1. LNA's should be introduced at the positions where specificity and discrimination is needed (e.g. 3'end in allele specific PCR and in the SNP position in allele specific hybridization probes).
2. Avoid stretches of more than 4 LNA bases. LNA hybridizes very tightly when several consecutive residues are substituted with LNA bases.
3. Avoid LNA self-complimentarity and complementarity to other LNA containing oligonucleotides in the assay. LNA binds very tightly to other LNA residues.
4. Typical primer length of 18mer should not contain more than 8 LNA bases.
5. Each LNA bases increases the Tm by approximately 2-4ºC.
6. Do not use blocks of LNA near the 3' end.
7. Keep the GC-content between 30-60 %.
8. Avoid stretches of more than 3 G DNA or LNA bases.
9. Tm of the primer pairs should be nearly equal.

For very specific or novel assay settings, design rules may have to be established empirically, but following the above recommendations will provide a good start.

Design Guidelines for SNP Microarray Genotyping

Guidelines for designing LNA containing oligonucleotides for genotyping using SNP chip microarrays are listed below. Please note that all the general design guidelines also apply. The guidelines should be considered as a rule of thumb.

1. Capture probes should be approximately 12 bp in length.
2. 2-3 LNA bases should be positioned directly at the SNP site.
3. The position of the mismatch in the capture probe is flexible – however, positioning the SNP at the very 3' or 5' end or 1 position from the ends may compromise discrimination.
4. A Tm of approximately 65ºC is recommended.
5. No LNA bases should be positioned in palindrome sequences (GC base pairs are not allowed, while AT base pairs are less critical).

Design Guidelines for PCR primers

1. LNA's should be introduced at the positions where specificity and discrimination is needed (e.g. 3'end in allele specific PCR and in the SNP position in allele specific hybridization probes).
2. Avoid stretches of more than 4 LNA bases. LNA hybridizes very tightly when several consecutive residues are substituted with LNA bases.
3. Start by spiking LNA in the 5' end of the primer (allow the 5' end of the primer to anneal at high Tm avoiding random priming by unspecific annealing of the 3' end).
4. Avoid LNA self-complementarity and complementarity to other LNA containing oligonucleotides in the assay. LNA binds very tightly to other LNA residues.
5. Typical primer length of 18mer should not contain more than 8 LNA bases.
6. Each LNA bases increases the Tm by approximately 2-4^{o}C.
7. Do not use blocks of LNA near the 3' end.
8. Keep the GC-content between 30-60 %.
9. Avoid stretches of more than 3 G DNA or LNA bases.
10. Tm of the primer pairs should be nearly equal.

Design Guidelines for Allele specific PCR

For improvement of allele specific PCR a single LNA nucleotide should be placed in the terminal 3' or the 3'-1 position. In both cases the LNA base should correspond to the position of the polymorphism. Follow general design guidelines given above.

Design guidelines for Real Time QPCR Probes

1. The 3'-end of the dual labeled probe should be blocked with PO4, NH2 or a blocked nucleotide to prevent extension unless a quencher or dye is placed at the 3' end.
2. Tm of the dual labeled probe should optimally be 10 °C higher that Tm of the forward primer. For single mutation detection the Tm-difference should be 7 °C.
3. Typical Tm of PCR primers for dual labeled assays: 58-60 °C.
4. Typical Tm of dual labeled probes: 65-70 °C (i.e. slightly lower than the extension temperature).
5. Optimal length of LNA substituted dual labeled probes: 15-18 nucleotides (Please note that these are 5-8 bases shorter than the corresponding DNA probes).
6. Maintain Tm with LNA substitutions to match the Tm of the corresponding longer DNA probe.
7. Substitute every third base with LNA in the central segment of the probe. Usually 4-6 LNA substitutions are required to obtain a useful Tm.
8. Avoid stretches of more than 3 G DNA or LNA bases.
9. When detecting single nucleotide mutations, select the probe sequence so that the mutation is located centrally in the probe. Make a single LNA substitution at the position of the single nucleotide mutation.
10. Avoid LNA substitutions participating in formation of secondary structures.
11. Position the dual labeled probe as close as possible to the forward primer.
12. Avoid Guanine (G) in the 5'-position next to the fluorophore.
13. Select the strand giving the lowest concentration of G's in the probe.
14. Avoid longer stretches of identical nucleotides and especially G's.
15. Keep the GC-content between 30-60 %.
16. All fluorescent dyes offered by Gene Link can be conjugated to LNA containing probes. (FAM, TET, HEX, TAMRA, ROX, CY3, CY3.5, Texas Red, CY5, CY5.5, CY7 and Alexa series dyes).

Design guidelines for siRNA containing LNA bases

1. At the 5′ and 3′-end 2-3 bases can be substituted with LNA bases to impart resistance to exonuclease degradation.
2. Substitute every third base with LNA in the central segment of the sense strand. Avoid stretches of more than 3 G RNA or LNA bases.
3. Avoid LNA substitutions participating in formation of secondary structures.
4. Substitute with 2′F U and 2′F C to impart resistance to RNase.
5. Place cholesterol at 3′ end for ease of uptake.

Single strand antisense oligos

1. LNA-DNA-LNA gapmers. Substitue 4 LNA bases at the ends with 8-10 DNA bases in the middle. This activates RNase H activity.
2. ~10 times more stable than siRNA but ~6 fold less knockout
3. Place cholesterol at 3′ end for ease of uptake.
4. The IC values of different modified antisense oligos are given below. A combination of various modifications may lead to higher stability and longer half life.

Antisense Oligos	IC values
siRNA	0.06 nM
LNA-DNA-LNA	0.4 nM
Phosphorothioate (PS)	70 nM
2′O me	220 nM

Design Guidelines for FISH Probes

1. LNA substituted probes are excellent improvements in the design of FISH probes.
2. Design 24 to 30mer probes.
3. 5′ Biotin, Cy3, Cy5 or any other fluorescent dye can be used.
4. Substitute with LNA bases at every second or third base.
5. Avoid stretches of more than 3 G or C LNA bases.
6. Avoid LNA self-complimentarity and complementarity to other LNA containing oligonucleotides in the assay. LNA binds very tightly to other LNA residues.

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.

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Ordering Information

Locked Nucleic Acid Oligo Synthesis						
Product	**Catalog No.**	**50 nmol scale (XX=05)**	**200 nmol scale (XX=02)**	**1 µmol scale (XX=01)**	**10 µmol scale (XX=10)**	**15 µmol scale (XX=15)**
Locked Nucleic Acid (LNA)*	26-6517-XX	190.00	190.00	250.00	2000.00	2500.00

Please inquire about volume discounts

Related Products Ordering Information

Modification Oligo Synthesis						
Product	**Catalog No.**	**50 nmol scale (XX=05)**	**200 nmol scale (XX=02)**	**1 µmol scale (XX=01)**	**10 µmol scale (XX=10)**	**15 µmol scale (XX=15)**
Phosphorothioates	26-6401-XX	3.50	4.25	6.50	50.00	65.00
Propyne dC or propyne dU*	26-6408-XX	130.00	130.00	200.00	1600.00	2000.00
Propyne dC or propyne dC	26-6501-XX	130.00	130.00	200.00	1600.00	2000.00
2'O methyl bases	27-6410-XX	14.00	14.00	20.00	170.00	250.00
2'-5' linked bases	26-6414-XX	275.00	275.00	300.00	2400.00	3000.00
5-Me-dC	26-6413-XX	75.00	75.00	125.00	825.00	1250.00
Spacer 9	26-6440-XX	100.00	100.00	140.00	840.00	1400.00
2'O-Me-5-Me-C	26-6508-XX	250.00	250.00	310.00	2500.00	2900.00
Chimeric Linkage	26-6420-XX	75.00	75.00	75.00	675.00	675.00
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Purification						
Product	**Catalog No.**	**50 nmol scale (XX=05)**	**200 nmol scale (XX=02)**	**1 µmol scale (XX=01)**	**10 µmol scale (XX=10)**	**15 µmol scale (XX=15)**
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Fluorophore*	**Color**	**Absorbance max (nm)**	**Emission max (nm)**	**$, 200 nmol scale**	**$, 1 µmol scale**
Dabcyl	(Quencher)	453		290.00	350.00
BHQ-1, BHQ-2, BHQ-3	(Quencher)	534, 579, 672		290.00	350.00
6-FAM (Fluorescein)	Green	494	525	390.00	450.00
TET	Orange	521	536	390.00	450.00
HEX	Pink	535	556	390.00	450.00
Cy 3	Red	552	570	490.00	550.00
Cy 5	Violet	643	667	490.00	550.00
Tetramethylrhodamine	Rose	565	580	540.00	600.00
Alexa Dye Series	Varies	Varies	Varies	540.00	600.00
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