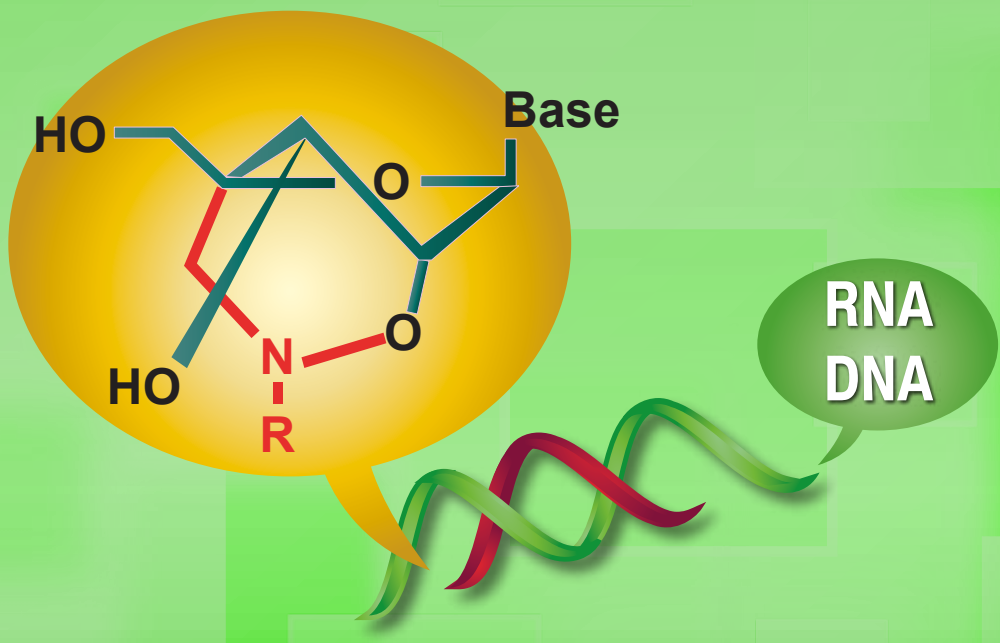
Speed-up Discovery

Bridged Nucleic Acids - BNA3™

Superior hybridization - Enhanced biostability



bioSYNTHESIS
COMMITTED TO BIOMIC RESEARCH

Third Generation Super Functional Bridged Nucleic Acids: BNA3™

BNA3™ (2',4'-BNA^{NC})

A unique chemical tool for developing sensitive detection systems and therapeutic products

Bridged nucleic acids, BNA3™, represent a novel class of nucleic acid analogues 2',4'-BNA^{NC} (2'-O,4'-C-aminomethylene bridged nucleic acid), containing a six-membered bridged structure with an N-O linkage, between the 2' and 4' carbons in the sugar moiety. BNA3™ modified oligonucleotides exhibit an extraordinary level of binding affinity toward target mRNA or target DNA duplex. They also confer significant stability against nuclease degradation when compared to previous generations of bridged nucleic acids, making BNA3™ nucleic acids a superior tool for developing high value detection systems and therapeutic products.

In comparison, natural nucleic acids have a higher degree of freedom in their chemical structure, making them thermodynamically unfavorable for DNA-DNA and RNA-RNA double strand formation which is often subject to degradation by both endo and exonucleases. When incorporating BNA3™ into DNA or RNA oligonucleotide synthesis, the degree of morphological freedom of natural nucleic acids is restricted by its six member methylene bridge structure, causing BNA3™ to be "stacked" in the optimal A type conformation for Watson-Crick binding, thereby enhancing hybridization specificity and duplex stability. In addition, studies have shown that each BNA3™ addition can increase the Tm by 2-9 °C. This feature enables robust detection of all microRNA sequences, regardless of GC content.

BNA3™ Benefits

Increased stability of duplex or triplex formation
- High thermal stability and binding affinity
- Enhanced strand invasion power for the detection of challenging clinical samples

Enables Tm modulation
- Enables robust detection of DNA or RNA targets regardless of GC content
- Enables shorter probe design with high specificity

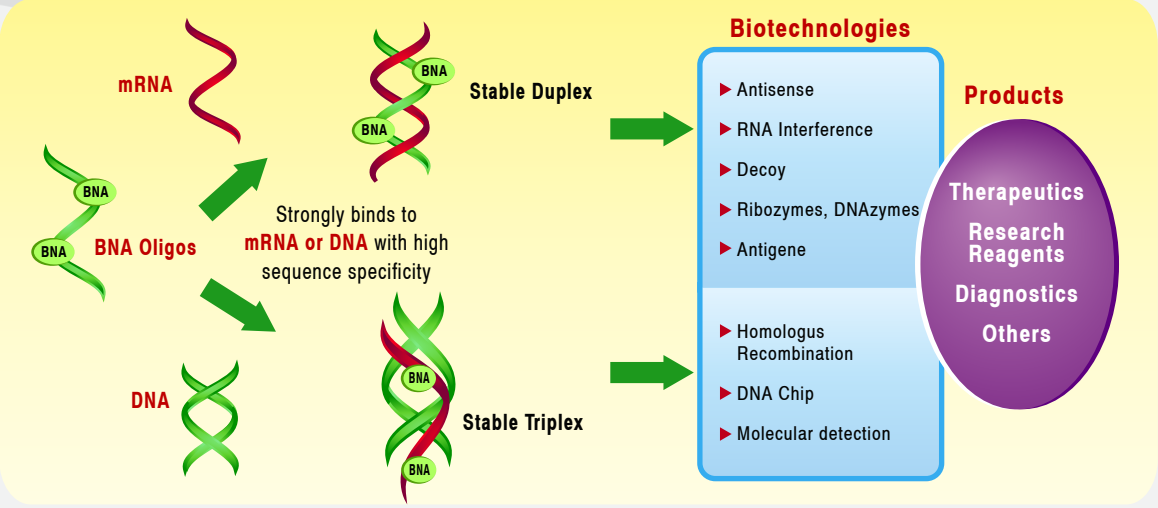
Superior discriminatory power
- Enables detection of single nucleotide mismatches
- Superior discrimination of microRNA families

Increased target specificity
- Faster target-specific binding compared to DNA, RNA and earlier generation of BNA

Enhanced biostability
- Resistant to exo- and endonucleases resulting in high *in vivo* and *in vitro* stability
- Enables high potency and binding affinity to RNA and DNA
- Superior antisense inhibition of small RNA targets

Enzyme compatibility
- Serves as a substrate to standard molecular biology enzymes

Offers flexibility and ease of use
- Easily adaptable to many DNA or RNA detection systems



Enhance Your Applications with BNA3™

BNA3™ has been proven to be a powerful tool in many molecular biology applications due to its unique structural feature (N-O bond) in the sugar moiety which lowers electrostatic repulsion between the negatively charged phosphate backbone. It allows a higher affinity to complementary nucleic acids and stronger biological, thermal and chemical stability.

Aptamer Capping

Aptamers are used as research tools in both diagnostic and therapeutic applications. With this technique, nucleic acid aptamers can be selected from a library of single-stranded oligonucleotides usually 40 to 60 bases in length. An initial pool of completely random sequences may be narrowed down through successive rounds of selection to yield a number of defined sequences which possess the desired properties. Selected oligonucleotides used for this technique must be chemically modified to impart nuclease stability, which is important for both *in vitro* and *in vivo* applications. They must also be correctly read during the reverse transcription and PCR amplification steps to make the next refined pool of modified aptamers, or the final aptamer itself.

The number of enzymes available that can efficiently incorporate modified nucleotides is currently very limited. In 2010, Kasahara et. al. showed that the capping of the 3'-ends of thrombin binding aptamers (TBAs) with bridged nucleotides BNA3™ increased the nuclease resistances and the stabilities in human serum. Furthermore, they have shown that the binding abilities of the aptamers were not affected by the capping. The capping BNA3™ monomer was added to the aptamer with a one step enzymatic process using 2',4'-bridged nucleoside 5'-triphosphate and terminal deoxynucleotidyl transferase.

RNA Targeting and Gene Silencing Application

The use of oligonucleotides as therapeutic tools to modulate gene expression has been investigated in a large number of biological studies. Many of these studies have been focused on gene silencing experiments using modified oligonucleotides with the ability to form RNA duplex in antisense strategy or triplex-forming oligonucleotide in antigene technology. In antisense technology, a single stranded oligonucleotide may be added externally to bind with a target mRNA. The formation of oligonucleotide RNA duplexes block the translation apparatus or cleaves the target mRNA by recruitment of RNase H using small interfering RNA (siRNA) to reduce mRNA expression. Likewise, in antigene strategy, a single-stranded homopyrimidine triplex-forming oligonucleotide (TFO) added from outside the cell may bind with a homopurine-pyrimidine stretch in target duplex DNA by Hoogsteen hydrogen bonding to form pyrimidine motif triplex, where T • A:T and C •G:C base triplets are formed. The formed triplex inhibits RNA polymerase and transcription of the target gene due to its steric hindrance, which may result in downregulation of target gene expression.

Despite the large number of chemically modified nucleic acids developed in the past, regulation of gene expression using oligonucleotides is often limited by their poor binding ability to target mRNA or duplex DNA. It is also limited by stability of oligonucleotides against nuclease degradation, and often limits antisense and antigene applications *in vivo*.

These drawbacks can be overcome by BNA3™ (BNA^{NC}) nucleobase having a nitrogen atom in the sugar moiety which improves duplex and triplex stability by lowering repulsion between the negatively charged backbone phosphates. This conformational restriction results in unprecedented thermal stabilities when hybridized with their RNA target molecules. Thus, an increase in melting temperature (Tm value) of +2 to +10 °C per BNA3™ monomer against complementary RNA compared to unmodified duplexes has been reported. (Rahman et al. 2010).

Incorporating of BNA3™ into oligonucleotides further increases resistance to endo- and exonucleases which leads to high *in vitro* and *in vivo* stability. Rahman et al. in 2010 evaluated the thermal stability, nuclease resistance and gene silencing properties against cultured mammalian cells of BNAs and compared their characteristics with those of natural siRNAs. The thermally stable siBNA3s composed of slightly modified sense and antisense strands were capable of suppressing gene expression and were remarkably stable in serums with promising RNAi properties superior to those exhibited by natural siRNAs or previous generations of bridged nucleic acids and thus, makes BNA uniquely suited for mimicking RNA structure and sequence specific targeting of RNA *in vitro* or *in vivo*.

Third Generation Super Functional Bridged Nucleic Acids: BNA3™

BNAClamp™ PCR Technology

Mutation detection using real-time PCR clamping has been widely used as an accurate diagnostic method for the detection of pathogen, quantitation of changes in gene expression, validation of results obtained from array analyses and other gene expression evaluation techniques.

Bio-Synthesis' BNAClamp™ Technology is a BNA-based PCR clamping technique that selectively amplifies only the mutated target DNA sequence as a minor portion in the mixture with the major wild type. Pitfalls encountered during primer and probe design can be easily overcome by using BNA3™ enhanced oligonucleotides to allow for a stable duplex formation. BNAClamp™ oligonucleotides have a much larger ΔCt than comparable clamp oligos, which permits highly selective amplification of the target gene (as little as 0.01% mutated DNA in the sample analyzed). BNA3™ based probes are ideal for detecting sequences with low complexity or low integrity, which can often lead to off-target effects in assays caused by poor binding, non-binding or less specific binding primers and probes. Compared to other probe technologies, BNA3™ probes have proven to be a sensitive, accurate and robust detection system. (Rahman et al. (2007)

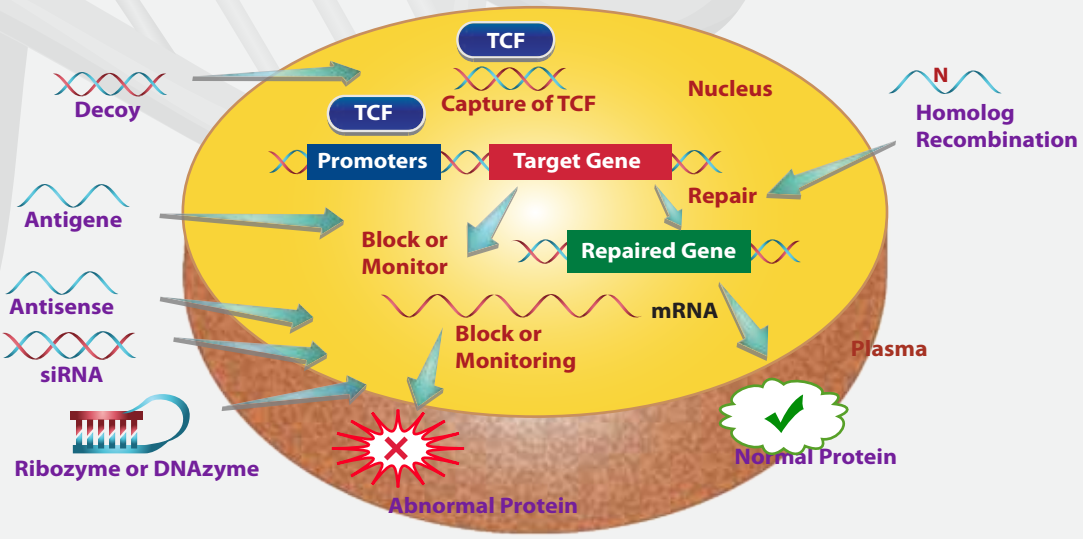
In situ Hybridization Probes

In situ hybridization is one of the most frequently used technique for studying gene expression. While the use of small oligonucleotide probes allows for easy penetration into the cells or tissue of interest, shortening of the probes is often hampered by the inability to modulate the melting temperature (Tm) of oligonucleotide/microRNA duplex with varying GC content. This results in high uncertainty and low robustness, limiting the use of such oligonucleotides for *in situ* hybridization reactions to detect short transcripts.

These challenges can be overcome by using BNA3™ enhanced oligonucleotides by simply varying the BNA3™ content with DNA or RNA. Oligonucleotides with a narrow range of melting temperatures can be designed with similar affinity towards all types of sequences regardless of the GC-content of the target gene. This permits a significant increase in hybridization temperature, thereby allowing for a shorter probe design as required in miRNA detection. It's strong resistance to nucleases, makes BNA3™ an ideal method for detecting specific mRNA sequences in preserved tissue sections or cell preparations.

Single Nucleotide Polymorphisms Discrimination

With the increasing demand for single nucleotide polymorphisms (SNP) in disease association studies, the development of appropriate genotype validation systems to elucidate ambiguous genotypes has become critical for more specific SNP assays. Detection of single base substitution using high affinity DNA analogues known as Bridged Nucleic Acids for use in allelic discrimination assays has been achieved by Real-Time PCR. Comparable in sensitivity and specificity, BNA hybridization probes have been proven to be an effective alternative assay to MGB TaqMan systems.



Comparison of BNA3™ with Other Nucleic Acid Analogs

Criteria	BNA	LNA	PNA	DNA
Hybridization affinity with DNA	1-3 °C higher Tm per base	2-3 °C higher Tm per base	At least 1 °C higher Tm per base	
Salt concentration for hybridization	Dependent	Dependent	Independent	Dependent
Tm for each single mismatch	Ca. 4 °C	Ca. 3-4 °C	Lowering 1 - 5 °C	Lowering 1 °C
Chemical stability	Stable to moderately stable	Stable to moderately stable	Stable	Unstable or moderately stable
Biological stability	Very stable	Stable	Stable to nuclease and protease	Degradation by nuclease
Thermal stability	Excellent	Good	Good	Moderate
Water solubility	Excellent	Good to Excellent	Soluble	Soluble
Probe length for diagnostic use	10-25 bases	10-25 bases	13-18 bases	20-30 bases
PCR compatible	Yes	Yes	No	Yes
Ability to introduce other nucleic acids in oligonucleotide	Yes	Yes	No	Yes
Body clearance ability	Yes	Yes	No	Yes
Inhibition of RNAse H	Yes	Less	No	Yes
Triplex formation	Yes	Yes	Yes	Yes
Hepatotoxicity	No	Moderate	Moderate	No
Nephrotoxicity	No	Very low	High	No
Innate immunity stimulation	No	No	Yes	Yes
Gene Silencing	Yes	Yes	Yes	na
Triplex Formation Ability	Excellent	Moderate	Good	Yes
RNA/DNA binding selectivity	Excellent	Good	No preference	na
Hybridization affinity with RNA	5-6 °C higher Tm per base	5-6 °C higher Tm per base	At least 1 °C higher Tm per base	na

Third Generation Super Functional Bridged Nucleic Acids: BNA3™

BNA3™ Oligonucleotide Synthesis

BNA3™ can be spiked with DNA, RNA, and other nucleic acid analogues using standard phosphoramidite nucleic acid chemistry. Therefore, oligos containing BNA can be modified with a wide range of chemical modifications by using our nucleic acid synthesis services.

Oligos containing BNA3™ are deprotected, desalted to remove small molecule impurities, quantitated by UV spectrophotometry to provide an accurate measure of yield and undergo final quality control by MALDI-TOF, ESI mass spectrometry and analytical HPLC.

Length: up to 200
Scale: µg to multi-gram sizes
Bases and Linkages: BNA3 or BNA3/ DNA, RNA, analogs and all linkages (PO/PS)
Modifications: More than 100 modifications!
Purifications: RP-HPLC , PAGE, IE-HPLC, dual HPLC/PAGE or RNase free HPLC/PAGE
Quality Control: MALDI-TOF MS or LC-MS
Format: Dried, in solution, annealed, mixed
Packaging: 2 ml tube, 96-well or 384-well plates
Documentation: Technical Data Sheet
Additional Services: Custom preparative service available upon request

Purifications

HPLC purification is highly recommended for many modified oligonucleotides or oligonucleotide with high amounts of BNA3™ substitutions.

For demanding applications, such as single nucleotide discrimination, dual label probes, antisense or siRNA application, purification may improve the performance of oligonucleotides. For such situations, we may apply single or dual RP-HPLC, ion-exchange HPLC.

For oligo >60 bases in applications requiring high purity, we recommend PAGE, IE-HPLC (ion-exchange), or RNase free HPLC for applications that are sensitive to ribonucleases.

When used in cells or live animals, we recommend Na^+ salt exchange for oligonucleotides used in applications where the presence of even minute amounts of toxic salt can cause unwanted results after HPLC purification.

Custom BNA3™ Oligonucleotides - Labels and Modifications

Modifications

- Mixed base and linkage modification
- Spacers and linkers
- 5' -terminal cap
- Fluorescent and non-fluorescent labels
- Quenchers
- Azide
- Digoxigenin
- Cholesteryl
- Adenylation
- Amino modifier
- Alkynes
- Biotinylation
- Thiol Modifications
- Non-fluorescent conjugates
- Many others

Real-Time qPCR

A wide range of fluorophores and quenchers in various combination are available for your dual labeled BNA oligonucleotides.

In situ hybridization

BNA custom probes for mRNAs, small RNAs, snRNAs and chromosome FISH applications

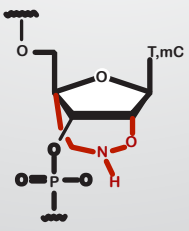
BNAClamp™

In combination with real-time PCR technology, BNAClamp™ can be used to detect mutated DNA sequences as low as 0.01% of patient sample. Custom BNA3™ based PCR clamping primer/probes can be designed using a mixture of DNA and BNA3™ with 3' phosphate or a C3 spacer.

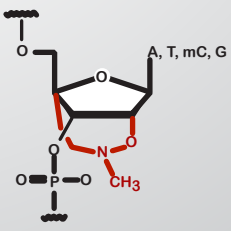
BNA3™ Bioconjugation Services

We offer many types of oligo bioconjugation services including but not limited to:

- Peptide conjugates
- Nanoparticle conjugates
- Lipid conjugates
- Small drug conjugates
- Bead conjugation
- siRNA modified BNA conjugates and more



BNA-NC(N-H)



BNA-NC(N-Me)

Design Recommendations

BNA3™ oligonucleotide can be synthesized as either 100% BNA3™ modified bases, or a mixture of BNA/DNA, BNA/RNA bases or other chimeric designs. However, only oligonucleotides that are between 7 and 15 mers in length can be made up of 100% BNA3™. For longer oligonucleotides, the 100% modified BNA3™ oligo may bind tightly to itself (self complement) rather than to the target sequence. Therefore, for oligonucleotides longer than 15 mers, we recommend decreasing the BNA content accordingly as the oligonucleotide increases in length. We continuously update the design guidelines to reflect the latest scientific developments as well as to integrate custom requirements. For novel applications, design guidelines may have to be established empirically. Visit **biosyn.com** for details.

Bio-Synthesis will be happy to assist you with the design of your BNA3™ oligonucleotides. Please provide us with the details of your application and preferred experimental conditions.

How to Order?

Enter the oligonucleotide sequence from 5' to 3' direction.

- DNA bases: Enter as A, T, C, G
- BNA™ bases: Enter as +A, +T, +C, +G
- RNA bases: Enter as rA, rC, rU, rG
- Phosphorothioated linkage: A*, C*, T*, G*
- 2'O-methyl RNA bases: Enter as mA, mC, mG, mU)

Contact Information

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Patents and Trademarks

"Bridged Nucleic Acids" (BNA^{NC}) and their use are covered by patents and patent applications owned by BNA Inc. BNA3™ is a trademark owned by Bio-Synthesis. Bio-Synthesis has the sole right to sell BNA3™ (BNA^{NC}) oligonucleotides worldwide, excluding Japan. The BNA^{NC} technology is protected by patents JP: 4731324(2011.4.28), USP: 7427672(2008.9.23), EP: 1551905(2012.4.25).

Disclaimers and Licensing

BNA3™ (BNA^{NC}) products are sold for research use only and in their original or any modified form may be used only for the buyer's internal research purposes and not for commercial, diagnostic, clinical, therapeutic or other uses, including use in humans. Buyer may not provide products to third parties in their original or any modified form. The purchase of products does not include or carry an implied right or license for the buyer to use such products in their original or any modified from for commercial use, including contract research or in the provision of services to third parties. Commercial applications including but not limited to diagnostic, clinical, therapeutic or other uses require a separate license agreement; please contact Bio-Synthesis Inc. at 800-227-0627 for further information.

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