Microsynth

Locked Nucleic Acid (LNA)

Increased thermal stability and hybridization specificity
Improved signal-to-noise ratio in qPCR assays
Enhanced single nucleotide discrimination

Background Information

Locked Nucleic Acid (LNA) is a synthetic nucleic acid analogue containing a bridged, bicyclic sugar moiety. The extra methylene group attached between the 2'-O- and the 4'-positions "locks" the ribofuranosyl-ring in its 3'-endo conformation (see ***Figure 2***). This conformation leads to the characteristic structure of A-form RNA. As a consequence of the constraint bicyclic sugar skeleton, LNA exclusively forms A-type duplexes. Furthermore, LNA fully complies with Watson-Crick base pairing rules. LNA:DNA hybrid duplexes are formed spontaneously from complementary DNA- and LNA-sequences, and it was found that LNA:DNA hybrids show strongly improved annealing temperatures compared to their DNA:DNA counterparts [1]. Since the synthesis of LNA is compatible with standard oligonucleotide synthesis, site-selective incorporation of single or multiple LNA nucleotides into DNA sequences can be achieved straightaway. These LNA-containing oligonucleotides anneal with their DNA complements to form chimeric LNA:DNA hybrids. Any such duplex adopt A-form conformation, and again Tm are substantially increased compared to analogous DNA:DNA double-strands. As a rough guess, the incorporation of LNA-nucleotides into short DNA primers (< 30 nt) increases the Tm by 3-8 °C for each substituted nucleotide [2].

All in all, the major advantage of LNA lies in the design options for primers and probes as the Tm can be fine-tuned according to the needs of a desired oligonucleotide. Due to the enhanced binding affinity, shorter probes can be realized and as a result, binding specificity to the target DNA is increased. Therefore, LNA is recommended for use in any hybridization assay that requires high specificity and/or reproducibility, e.g. PCR primers, dual-labeled probes, in-situ hybridization probes, and molecular beacons. Furthermore, but for the same reasons, LNA modified oligonucleotides are equally interesting as candidates in antisense drug development [3].

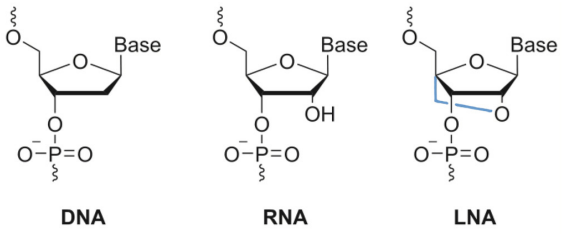

***Figure 1**: Structural drawings of DNA, RNA and LNA nucleotides*

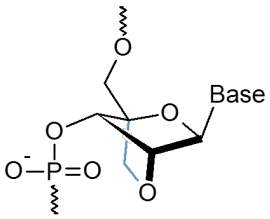

***Figure 2**: LNA 3'-endo conformation*

Increased Thermal Stability of qPCR Probes

The **superior hybridization characteristics** of LNA allow a **fine-tuning of the Tm** in the design of qPCR probes [4]. This significantly broadens the scope of assay conditions and permits more successful qPCR. When probes consist of LNA nucleotides, affinity and specificity of the hybridization to the target sequence are improved. This in turn **reduces background fluorescence** from spurious binding and leads to a **better signal-to-noise ratio**.

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Multiplex qPCR Systems

As the Tm of qPCR probes can be adjusted using LNA, **normalization of the Tm** across several short sequences with varying GC-content becomes accessible. For example, AT-rich native-state DNA qPCR probes often need to be over 30 bases long (sometimes over 40 nt) to satisfy amplicon design guidelines but may still perform poorly. With LNA qPCR probes, the selective positioning of LNA nucleotides **facilitates the optimal design** of highly-specific, shorter probes. A narrow Tm range is **in particular beneficial for microarray and multiplex PCR** applications, where simultaneous binding of probes to many different targets must occur under the same conditions.

Enhanced Single Nucleotide Discrimination

The ability of qPCR probes to **discriminate between alleles via single nucleotide polymorphism (SNP)** is greatly enhanced by the incorporation of LNA nucleotides since there is strong preference of LNA to form Watson-Crick base pairs over mismatches [5]. This is reflected by thermal denaturation studies that show a remarkable difference in Tm between a perfect match and a mismatch when LNA is incorporated at the particular position of the oligonucleotide (see Figure 3). Therefore, in SNP assays, LNA qPCR probes have an **enhanced destabilizing effect** on target hybridization and permit **better mismatch discrimination** compared to native-state DNA probes.

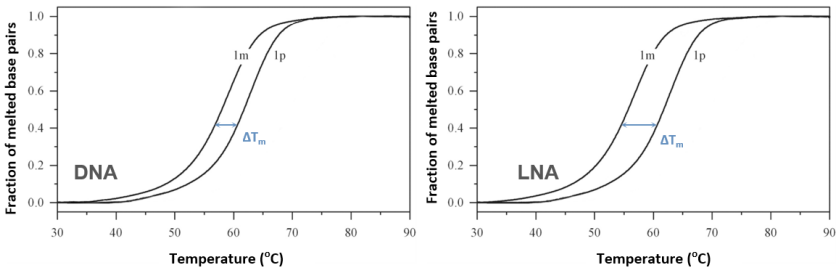

***Figure 3**: Influence of LNA on the melting temperature (Tm) and the resulting larger difference between specific (1p) and non-specific (1m) signals*

Antisense Technology

Due to the enhanced binding affinity to complementary nucleic acids, there is **great potential for LNA to be used in antisense technology**. The concomitant **high nuclease resistance** of LNAs is an important benefit for *in vivo* and *in vitro* applications. Numerous studies confirm the superior properties of LNA as antisense agents. LNA oligonucleotides are transfected by the conventional techniques. For knockdown of microRNA or other small RNAs, LNA antisense oligonucleotides are designed to hybridize to their target sequence by base pairing. A further increase in nuclease resistance is obtained by the introduction of a phosphorothioate backbone.

Literature

1. *Vester, B.; Wengel, J. LNA (Locked Nucleic Acid): High-Affinity Targeting of Complementary RNA and DNA. Biochemistry 2004, 43 (42), 13233-13241.*
2. *Singh, S.; Nielsen, P.; Koshkin, A.; Wengel, J. LNA (locked nucleic acids): synthesis and high-affinity nucleic acid recognition. Chemical Communications 1998, No. 4, 455-456.*
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4. *Letertre, C.; Perelle, S.; Dilasser, F.; Arar, K.; Fach, P. Evaluation of the performance of LNA and MGB probes in 5'-nuclease PCR assays. Molecular and cellular probes 2003, 17 (6), 307-11.*
5. *Johnson, M. P.; Haupt, L. M.; Griffiths, L. R. Locked nucleic acid (LNA) single nucleotide polymorphism (SNP) genotype analysis and validation using real-time PCR. Nucleic acids research 2004, 32 (6), e55.*

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