

# Technical Note

## Locked Nucleic Acid



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### LNA: Fine-tuning of primers and probes.

Locked Nucleic Acid (LNA™) is an RNA derivative in which the ribose ring is constrained by methylene linkage between the 2'-oxygen and 4'-carbon (Figure 1). This conformational restriction increases binding affinity for complementary sequences and provides an exciting new chemical approach to optimize and fine tune primers and probes for sensitive and specific detection of nucleic acids.

LNA can be mixed with DNA, RNA as well as other nucleic acid analogs using standard phosphoramidite DNA synthesis chemistry. Therefore, LNA oligonucleotides can easily be tagged with e.g. amino-linkers, biotin, fluorophores, etc. Thus a very high degree of freedom in the design of primers and probes exists. In order to facilitate efficient design of LNA containing primers and probes software tools have been developed for T<sub>m</sub> prediction which can be accessed at [www.LNA-tm.com](http://www.LNA-tm.com).

LNA may be used to enhance:

- Taqman probes
- In situ hybridization probes
- Primers for PCR
- Primers for multiplex PCR
- Primers for allele specific PCR
- Capture probes for SNP genotyping
- Capture probes for expression analysis
- Probes to monitor exon skipping

LNA should be used in any hybridization assay, which requires high specificity and/or reproducibility. Furthermore, LNA offers the possibility to adjust T<sub>m</sub> values of primers and probes in multiplex assays (Figure 2).

	LNA	RNA	PNA
T <sub>m</sub> increase/monomer against DNA (°C)	2-6	-0.5-0.5	0.5-2
T <sub>m</sub> increase/monomer against RNA (°C)	3-8	1-1.5	0.5-2
ΔT <sub>m</sub> at single mismatch against DNA	LNA>>DNA	RNA>DNA	PNA<DNA*
Compatible with standard DNA synthesis	Yes	Yes	No
Chimera with DNA	Yes	Yes	No
Compatible with standard molecular biology	Yes	Yes	No
Works in PCR primers	Yes	Yes	No
Enhances allele specific priming	Yes	Yes	No
Water solubility	Yes	Yes	Low
Predictive performance in hybridization	Yes	Yes	No

Key parameters of LNA, RNA and PNA. \* May under certain conditions perform equal to DNA.

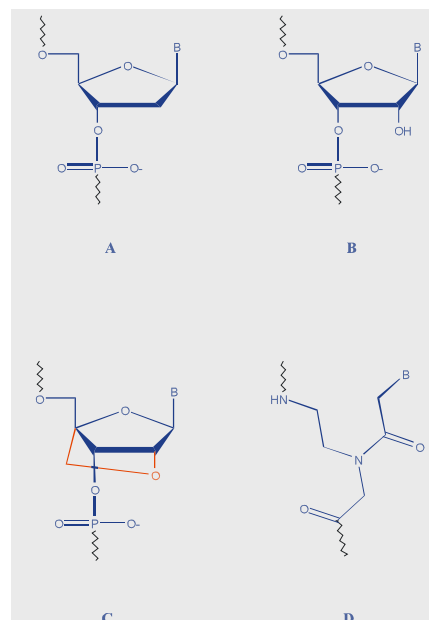


Figure 1. The chemical structures of DNA (A), RNA (B), LNA (C) and PNA (D).

Figure 2 demonstrates how both specificity (ΔT<sub>m</sub>) and T<sub>m</sub> may be enhanced and adjusted for compatibility by including LNA. In the depicted example two capture probes targeting each of the two alleles in a SNP (Single Nucleotide Polymorphism) were enhanced by LNA. Please notice the increased T<sub>m</sub> and ΔT<sub>m</sub>.

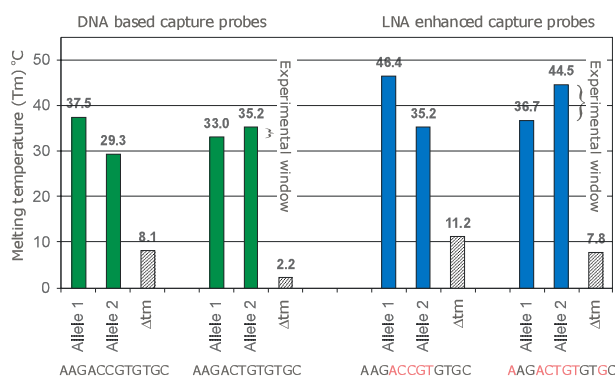


Figure 2. Fine-tuning of specificity (ΔT<sub>m</sub>) and T<sub>m</sub>. The sequences below the bars represent the probes. The underlined positions indicate the site of the SNP and the nucleotides in red represent LNA monomers.

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