AFFINITY PLUS™ qPCR PROBES

Compared to unmodified probes, these probes have increased SNP target specificity and can be made with shorter sequences

Improve SNP discrimination over traditional probe use [1]

Cost-effective approach

Adjust T_m with greater flexibility for increasing hybridization specificity compared to MGB™ Probes [2]

USE qPCR PROBES WITH ENHANCED STRUCTURAL STABILITY

When performing qPCR-based SNP analysis, your experiments will always benefit from use of probes with increased hybridization specificity and enhanced discrimination. Affinity Plus qPCR Probes are custom probes you design. They can include up to 6 locked nucleic acid nucleotides (Figure 1), which impart heightened structural duplex stability to the probes.

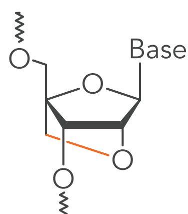

Figure 1. A locked nucleic acid monomer. These modified nucleotides contain a methylene bridge (orange) between the 2′ oxygen and the 4′ carbon of the pentose ring. The bridge fixes the pentose ring in the 3′-endo conformation.

OBTAIN ENHANCED PROBE STABILITY, PLUS ABILITY TO MODULATE MELTING TEMPERATURE

In comparison to unmodified probe sequences, the higher melting temperature (T_m) of the locked nucleic acid–containing Affinity Plus qPCR Probes provides better stability in qPCR assays, especially with target regions of low GC content. Affinity Plus qPCR Probes have identical annealing properties leading to the same increased sensitivity as other manufacturers locked nucleic acid qPCR probes (Figure 2). Furthermore, you can modulate probe T_m by the number of Affinity Plus nucleotides you include. Modulating T_m helps you adjust and shorten probe length. The shorter the probe, the higher the impact of the mismatch on probe duplex stability and mismatch discrimination.

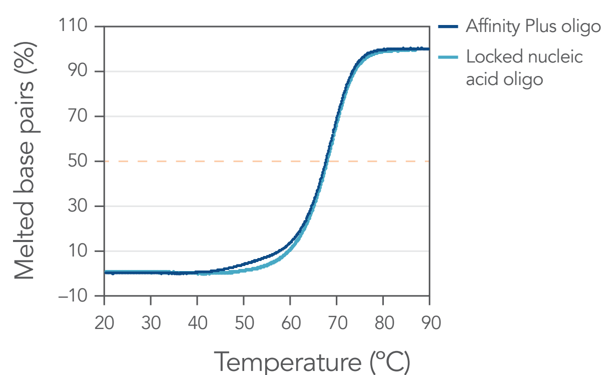

Figure 2. Sequences containing Affinity Plus locked nucleic acids show identical annealing properties to those from another manufacturer. The 15-mer sequence, GGTCCT+T+A+CTTGGTG, was synthesized with either Affinity Plus or locked nucleic acid modifications incorporated at the +T, +A, and +C sites. These oligos were mixed with the complementary DNA strand (1 µM each strand) in 1 M Na+ buffer (pH 7). Melt curves were performed as described in Owczarzy, et al. [3]. The percentages of duplex melted base pairs are plotted as a function of temperature. The plots of duplex melted base pairs were averaged from at least 7 heating and cooling melting curves. Melting temperature was measured to be 67.7 ± 0.3°C for the Affinity Plus Oligo and 67.9 ± 0.3°C for the locked nucleic acid oligo. Free energy of duplex hybridization at 37°C was determined to be –18.1 ± 0.9 kcal/mol for the Affinity Plus Oligo and –18.7 ± 0.9 kcal/mol for the locked nucleic acid oligo. These results demonstrate the identical annealing properties of both manufacturers locked nucleic acid sequences.

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IMPROVE SNP ANALYSIS

Figure 3 compares cluster plots resulting from assay sequences with Affinity Plus or locked nucleic acid probes.

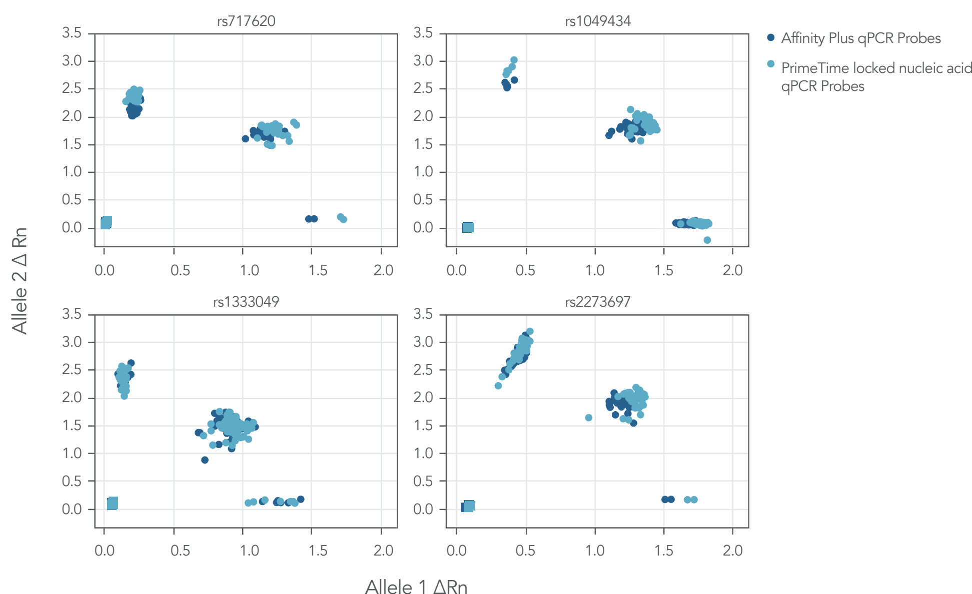

Figure 3. Affinity Plus qPCR Probes generate identical genotyping calls as PrimeTime locked nucleic acid Probes. Genotyping assays (n = 4) were run using 46 unique Coriell gDNA samples and the same probe sequences made as either Affinity Plus qPCR Probes or PrimeTime locked nucleic acid qPCR Probes. There were no differences in calls observed between Affinity Plus and locked nucleic acid probes. All genotype calls matched previous results (data not shown). The sequences of the primers and probes are listed in Table 1 of the Product data tab on the Affinity Plus qPCR Probes webpage. All but rs1333049 are ADME (absorption, distribution, metabolism and excretion) assays

COST-EFFECTIVE LOCKED NUCLEIC ACID PROBES

Affinity Plus qPCR Probes give you a cost-effective alternative to other locked nucleic acid probes, while providing a wide selection of formats, dyes & quenchers, and customization.

ORDERING INFORMATION

Affinity Plus qPCR Probes are available for screening small sample sets or for performing just a few reactions when optimizing probe designs. The mini size delivers 0.5 nmol of probe with a selection of 3 reporter dyes and a single quencher. See the table below.

Affinity Plus qPCR Probes are also available in 250 nmol and 1 µmol synthesis scales, best suited for large-scale or high-throughput applications. They come with a wider selection of dyes and quenchers.

Table 1. Selection of Mini Affinity Plus qPCR Probes and Affinity Plus qPCR Probes.

Product	Delivery	5′ Reporter dye	3′ Quencher
Mini Affinity Plus qPCR Probes	0.5 nmol	Cy® 5, 6-FAM, HEX, SUN™	Iowa Black™ FQ
Affinity Plus qPCR Probes	250 nmol, 1 µmol	6-FAM, HEX	Iowa Black FQ or Black Hole Quencher® 1
		SUN	Iowa Black FQ
		Cy 3 and Cy 5	Iowa Black RQ-Sp or Black Hole Quencher 2

Black Hole Quencher is a registered trademark of and licensed from Biosearch Technologies, Inc. Cy™ is a trademark of Cytiva.

For questions about Affinity Plus qPCR Probes, including additional dye options, visit www.idtdna.com/ContactUs.

REFERENCES

1. You Y, Moreira BG, Behlke MA, et al. Design of LNA probes that improve mismatch discrimination. Nucleic Acids Res. 2006;34(8):e60.
2. Kutyavin IV, Afonina IA, Mills A, et al. 3′-minor groove binder-DNA probes increase sequence specificity at PCR extension temperatures. Nucleic Acids Res. 2000;28(2):655-661.
3. Owczarzy R, You Y, Groth CL, et al. Stability and mismatch discrimination of locked nucleic acid-DNA duplexes. Biochemistry. 2011;50(43):9352-9367.

> FOR MORE INFORMATION, VISIT **WWW.IDTDNA.COM/AFFINITYPROBES**

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Doc ID: RUO22-1165_001 09/22