EXIQON

miRCURY™ LNA research tools for microRNA

As a relatively new but fast-growing area for research, microRNAs (miRNAs) present new challenges for researchers, based primarily on the small size of their target. The intrinsic properties of the nucleotide analog locked nucleic acid (LNA™) are used by Exiqon as the basis for a range of enabling tools for studying miRNA: the miRCURY LNA product line. The miRCURY LNA products include tools for miRNA profiling on arrays, miRNA detection—by *in situ* hybridization and northern blotting, and for studying miRNA function by specific knockdown of miRNAs.

Creating high-affinity probes for microRNA using LNA

miRNAs are a class of short endogenous RNAs that have a role in cancer development and in tissue differentiation. The mature form of miRNAs are found in the cytoplasm of cells as 19–25 nucleotide RNAs, where they act as post-transcriptional regulators of gene expression by base-pairing with their target mRNAs.

Exiqon has enhanced its miRNA technology through the use of LNA, a bicyclic high-affinity RNA mimic in which the sugar ring is locked in the 3′-endo conformation by the introduction of a methylene bridge group connecting the 2′-O atom with the 4′-C atom. It has been regularly demonstrated that the incorporation of LNA into an oligonucleotide probe greatly increases the affinity of that probe for its complementary target. This is expressed as an increase in melting temperature (T_m), or affinity of the oligonucleotide probe against its target. For example, whereas a full-length DNA oligonucleotide probe for an miRNA target would have a T_m of 60 °C, an LNA-enhanced miRNA probe for the same target would have a T_m for target of 74 °C. It is this first characteristic of LNA-enhanced probes that provides the basis for their improved detection of short nucleic acid targets.

A second characteristic of LNA-enhanced oligonucleotides is that the T_m difference between a perfectly matched target and a mismatched target is substantially higher than that observed when a DNA-based oligonucleotide is used. This is particularly important when designing probes for short nucleic acid targets that have sequences that are similar: a T_m difference window of sufficient size can be exploited as a crucial tool to differentiate between closely related targets. Mismatch discrimination can be further improved by decreasing the length of a probe, while maintaining a relatively high T_m.

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miRNAs, like other short noncoding RNAs, present new challenges to researchers. Standard oligonucleotide technologies have insufficient affinity and specificity for these short RNA targets. The introduction of LNA-based tools into the miRNA research field has proven to be an enabling development. The combination of higher T_m with excellent mismatch-discrimination ability means that LNA-based probes are excellent tools for short targets like miRNA, where the length of the target is 18–24 nucleotides and related miRNAs may only differ from each other by a single base. Here we describe the miRCURY LNA research tools for miRNA.

miRCURY LNA Arrays

Exiqon has recently developed an array that uses LNA-based oligonucleotide capture probes for global miRNA profiling. The miRCURY LNA Array uses the intrinsic high-affinity and discriminatory power of LNA to give highly sensitive and specific detection of miRNA in a sample, and to provide an accurate picture of the miRNA profile (**Fig. 1**). By varying

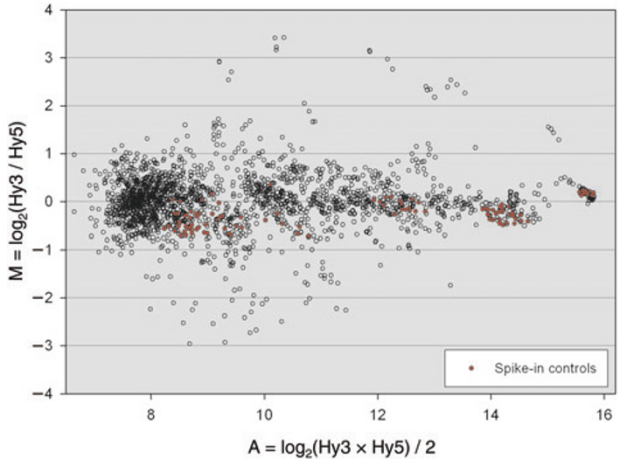

Figure 1 | An MA (ratio intensity) plot of miRNAs from an experimental sample (Hy3™-labeled) against a reference sample (Hy5™-labeled) hybridized to a miRCURY LNA Array. Signals from a set of synthetic miRNA spike-in controls are also shown.

APPLICATION NOTES

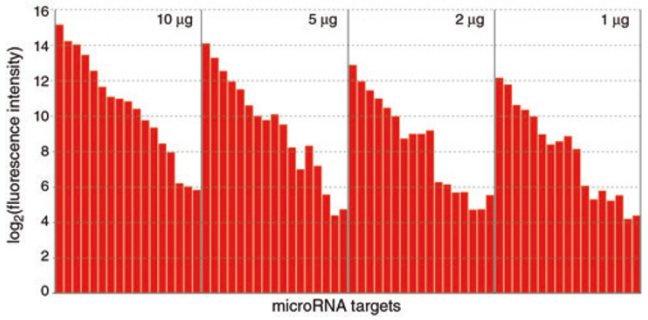

Figure 2 | Identical miRNA profiles were produced from starting amounts of total RNA that span the range from 10 µg to 1 µg, without miRNA enrichment. Represented are 17 different miRNAs detected in human lung total RNA (Ambion). The amount of total RNA used to produce each profile is shown.

Figure 3 | MicroRNA expression patterns in a developing chick embryo. *mir-1* was detected using a miRCURY LNA digoxygenin-labeled *in situ* detection probe. Image contributed by Parker Antin and Diana Darnell, University of Arizona.

the length of the capture probes and their LNA content, the probes on the miRCURY LNA array are T_m-normalized to ensure a uniform affinity for all target miRNAs[1]. Another unique feature of using the miRCURY LNA Arrays is the absence of a requirement for miRNA enrichment from a pool of total RNA. Compared to other methods for studying miRNA on arrays, this provides substantial savings in sample use, because an accurate miRNA profile can be obtained from just 1 µg of a total RNA sample (**Fig. 2**).

miRCURY LNA Detection probes

Virtually all miRNAs have distinct tissue and temporal expression patterns. To determine these patterns—when and where an miRNA is expressed—the *in situ* detection of miRNA has become very important. LNA has proven unique in the case of *in situ* detection of miRNA, where Exiqon has developed miRCURY LNA detection probes, enabling *in situ* detection of miRNAs with a specificity and sensitivity not previously possible (**Fig. 3**). The miRCURY LNA detection probes have been found to work effectively in a wide range of species—vertebrates, insects and plants—and in many sample types, including whole-mount, paraffin-embedded and fresh–frozen tissues as well as suspension cells[2].

miRCURY LNA Knockdown probes

An important aspect of miRNA research is the determination of miRNA function. One method to do this is to simply observe the effect of knocking down, or removing, mature miRNA(s) from the circulating pool of cytoplasmic miRNA.

LNA-based probes have proven to be excellent tools for knockdown of targets; they work excellently as RNA-interference probes, for example. Several recent publications have demonstrated the effectiveness of the miRCURY LNA knockdown probes in miRNA knockdown and inhibition research[3,4]. In this way, LNA technology provides knockdown tools that have high affinity at physiological temperatures, high specificity and low toxicity.

To summarize, Exiqon's miRCURY LNA research tools provide a range of options for studying miRNA, based on the LNA molecule. This nucleic acid analog provides higher affinity and specificity than standard oligonucleotide probes, properties that are particularly advantageous when studying short nucleic acid targets that are often closely related in sequence, like miRNAs.

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