LNA™-based microarray and PCR systems are superior for detection of microRNAs displaying 3'-end heterogeneity

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Carsten Alsbo, Ditte Andreasen, Kim Bundvig Barken, Nana Jacobsen. Exiqon, Skelstedet 16, DK-2950, Denmark

Abstract

It has been demonstrated that a particular microRNA can occur in a cell or tissue as more than one variant. The difference in sequence between the variants is primarily found at the 3'-end of the molecule. The functional significance of this 3'-end heterogeneity is still not fully understood, but it is likely that the regulatory effect of microRNAs is not restricted to one particular variant. When analyzing microRNA expression, it is therefore important that all variants are detected. In this study, we have evaluated the ability of microarray and real-time PCR platforms based on different technologies to detect microRNAs independent of 3'-end heterogeneity. The LNA™-based miRCURY LNA™ microRNA Array and miRCURY LNA™ microRNA PCR System were found to be less vulnerable to common microRNA 3'-end heterogeneity than platforms utilizing loop DNA or full-length DNA probes/primers. The LNA™-based systems, therefore, provide the most accurate data and the most biologically relevant microRNA expression profiles.

Results

Figure 1

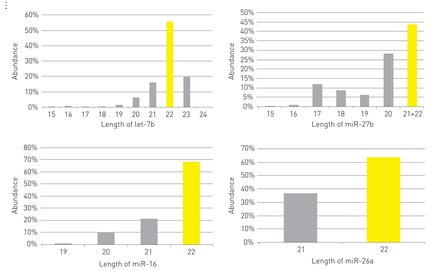

Figure 1. Length variation of microRNAs - sequencing
The figure shows 4 examples of lengths variation of mature microRNAs found by 454 sequencing of breast cancer tissue. Data based on 106 to w1597 sequences of each miR.

Figure 2

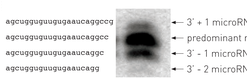

Figure 2. Length variation of microRNA – Northern blot
Northern blot of mmu-miR-138 that shows 3'-end and 5'-end variations of the mature microRNA. From Obernosterer *et al.* RNA, 2006.

Figure 3

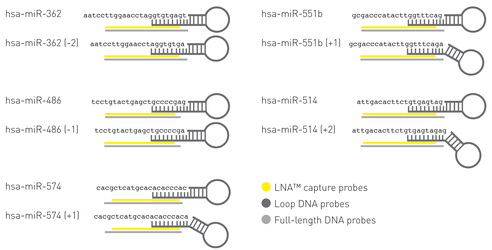

Figure 3. Theoretical binding of LNA™ and DNA based probes to length variations of microRNAs.
The figure illustrates theoretical binding of LNA™ capture probes and full-length and looped DNA capture probes to five different microRNAs annotated in miRBase and to the length variations of these (not annotated in miRBase).

Figure 4

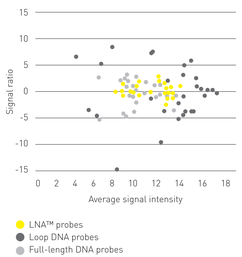

Figure 4. The LNA™-based array is clearly less sensitive to 3'-end variation of microRNA than the full-length DNA- and loop DNA capture probe-based arrays.
Variation in array signal ratios between the miRBase annotated sequences and 3'-end variants. Twenty-nine synthetic microRNA pairs of a miRBase annotated sequence and a selected 3'-end variant were hybridized to three different array platforms, and the annotated/variant signal ratios were calculated. The results are presented in an MA-plot in which the x-axis=½*Log2(signal from annotated sequence*signal from variant) and the y-axis=Log2(signal from annotated sequence/signal from variant). The full-length DNA capture probes (grey) failed to detect both the annotated sequence and the 3'-end variant in two cases.

Figure 5

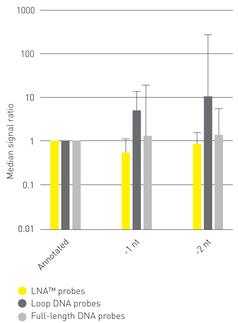

Figure 5. The LNA™-based probes perform equally well on shorter variants of microRNAs.
The figure shows the median array signal ratios of the annotated microRNA to variants with shorter 3'-ends. Each bar represents median ratios for 5-9 different microRNAs. The LNA™-based miRCURY LNA™ Array and the full-length DNA-based array detect all variants with equal sensitivity, while the loop DNA-based array is significantly affected by 3'-end variation with drastically reduced sensitivity towards the shorter variants.

Figure 6

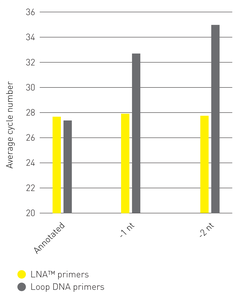

Figure 6. The LNA™-based real-time PCR systems perform equally well on shorter variants of micro-RNAs.
Average real-time PCR cycle number values of annotated microRNAs and variants with shorter 3'-ends. Synthetic microRNAs were used as templates and quantification was done using the miRCURY LNA™ microRNA PCR System (yellow) and a loop primer based real-time PCR system (grey). The values are an average of 3-6 microRNAs in different 3'-end length variants. It is clear, that the LNA™-based system is not affected by 3'-end heterogeneity, while the loop DNA-based system is unable to detect the shorter variants satisfactorily.

Figure 7

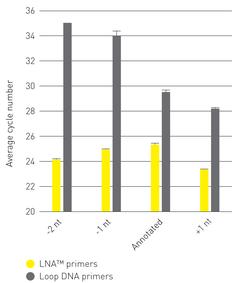

Figure 7. Loop DNA PCR system efficiency varies with microRNA length.
Quantification of human let-7b variants by real-time PCR. Cycle numbers obtained from quantification by the miRCURY LNA™ microRNA PCR System (yellow) and a loop DNA primer based real-time PCR system (grey) are shown. Synthetic 5'-phosphorylated microRNAs were used as target molecules. The loop DNA real-time PCR system was very sensitive to a reduction of target length, whereas the miRCURY LNA™ microRNA PCR System was unaffected by 3'-end variation.

Conclusion

The LNA™-based systems (miRCURY LNA™ microRNA Arrays and miRCURY LNA™ microRNA PCR System) are less vulnerable to 3'-end heterogeneity than competitor platforms utilizing pure DNA or stemloop probes/primers.

The LNA™-based systems are therefore likely to provide researchers with the most biologically relevant microRNA expression profiles. LNA™ makes it possible to design capture probes or primers that have the optimal T_m while at the same time avoiding that the probe/primer efficiency is dependent on the most 3'-end positions of the microRNA. This makes the use of loop probes/primers or full-length probes superfluous, and provides the LNA™-based systems with a superior advantage.

References on 3' heterogeneity of microRNAs:

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