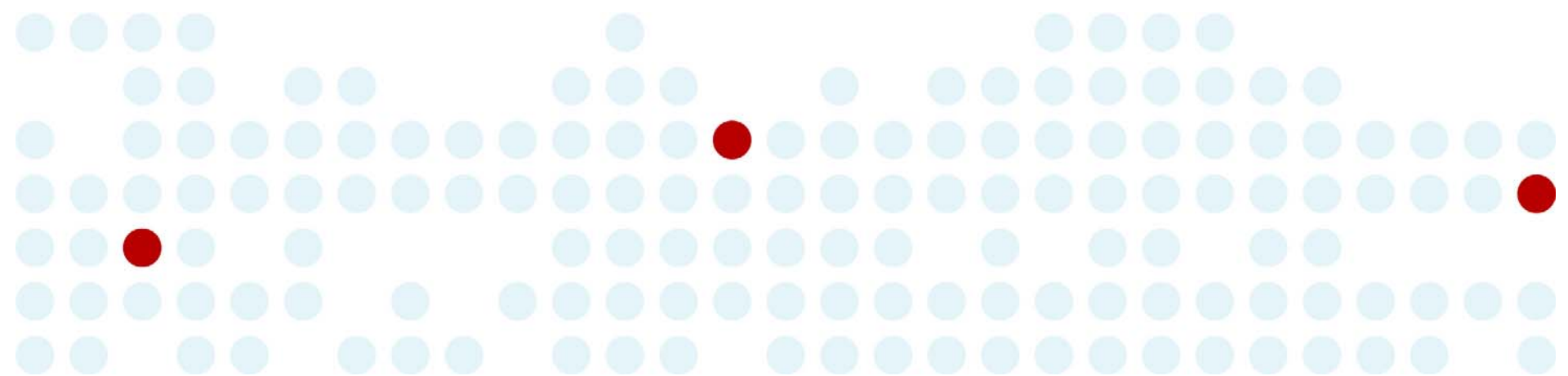


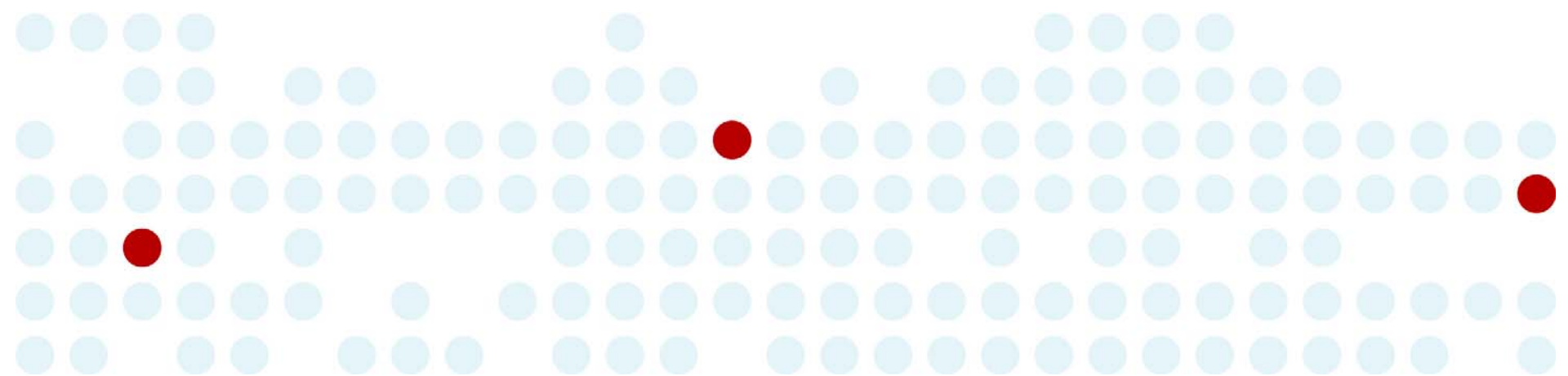
microRNA Array benchmark study  
- January 2008

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## Content

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2. Materials & methods
3. Summary of conclusions
4. Sensitivity benchmark
5. Specificity benchmark
6. Additional information

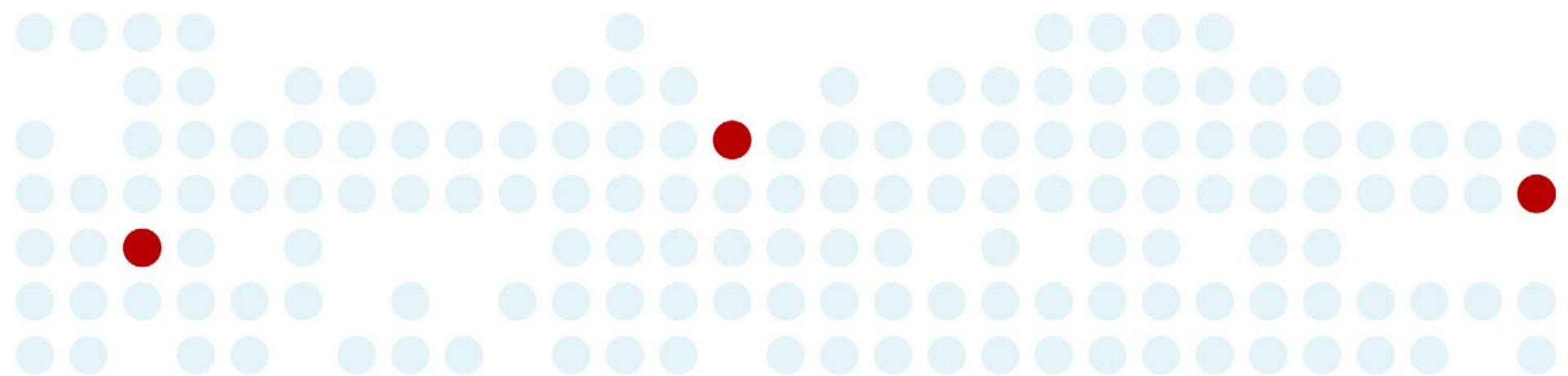


# 1. Purpose



## Purpose

- The purpose of these experiments was to compare the performance of 3 different DNA-based microRNA array platforms with the performance of Exiqon's miRCURY™ LNA Array platform.
- The data presented show how the DNA array platforms perform in our hands. The experiments have been repeated a few times.
- We have evaluated capture probe design, specificity and sensitivity.



## 2. Materials & methods

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## Materials and methods

We have used 557 different synthetic microRNAs (human sequences), divided into 16 pools with 12-54 distantly related microRNAs in each pool. For the miRCURY LNA™ Array and supplier A and C, each pool was spiked into 1 µg of tRNA to create a background RNA level. For supplier B, we did not use tRNA as background because the supplier B labeling kit resulted in very high background signal.

Labeling and hybridization procedures were followed according to each supplier's recommendations.

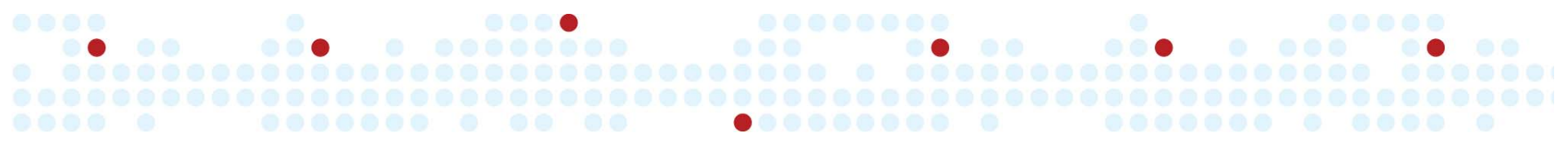
### **The sensitivity experiment**

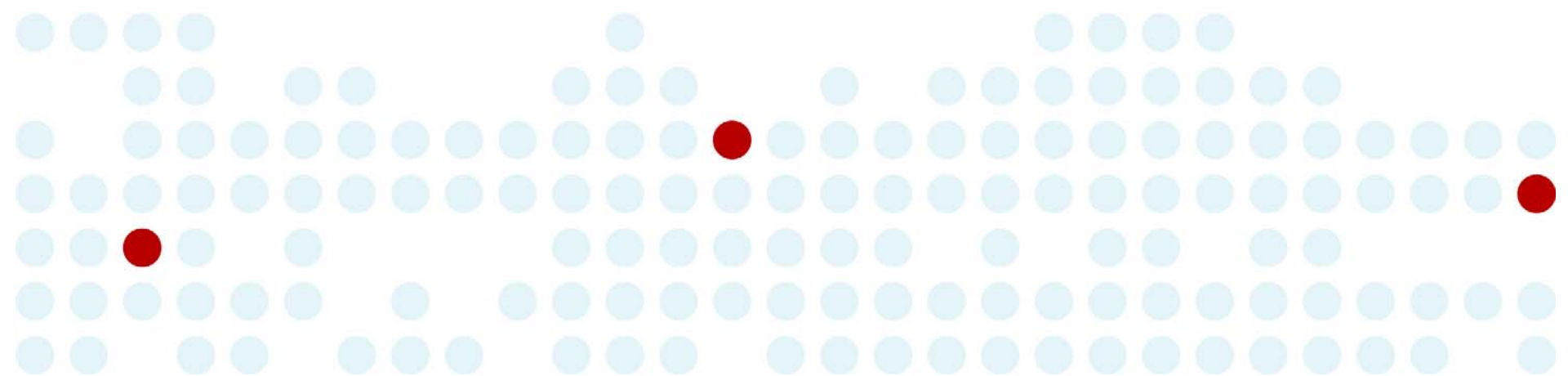
The 16 pools were combined and dilution series from 0.4 to 1000 amol were made.

Signals from all the relevant 557 capture probes above background were measured. Detection limits were defined as 5x standard deviation above background level, unless a software for calculation of the microRNAs had been supplied with the particular platform (Supplier A).

### **The specificity experiment**

We used 1000 amol of each of the 16 pools and performed individual hybridizations of each pool. Unspecific signal was defined as signal from a capture probe, targeting a microRNA that was NOT present in the pool. The cross hybridization was calculated as the percentage of signal compared to the signal for the matching pool which includes the target miRNA.

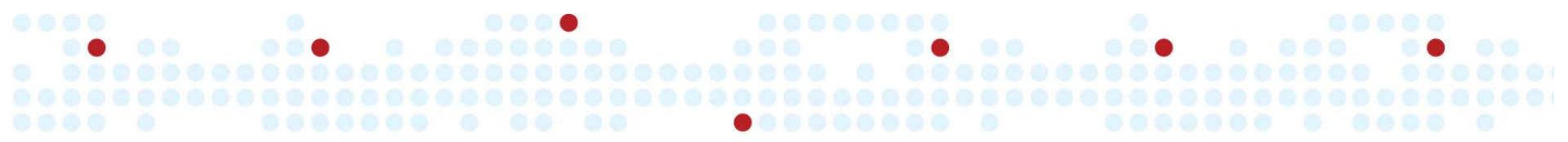


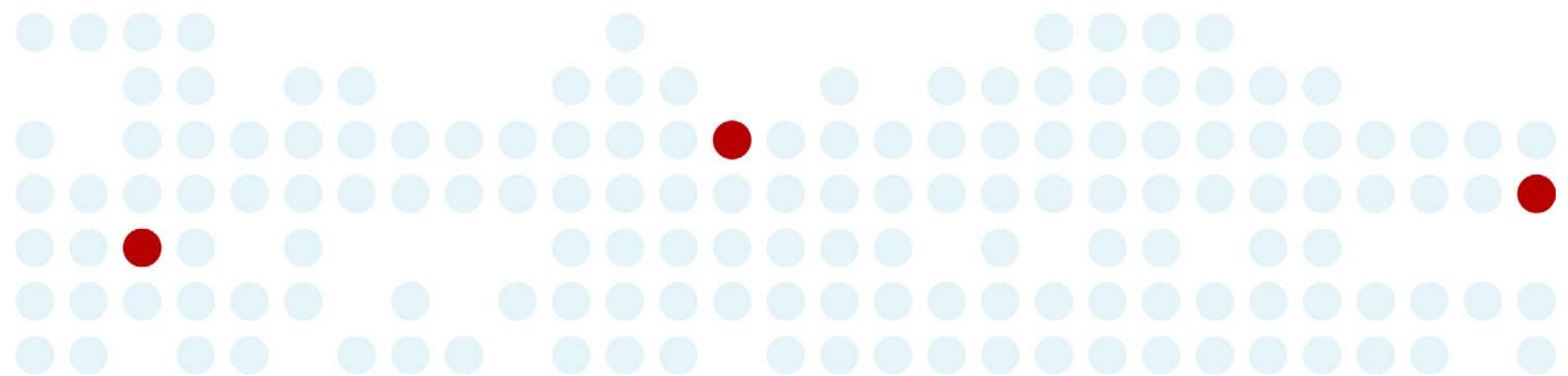


### 3. Summary of conclusions

## Summary of conclusions

- The data from Supplier C was excluded from further data analysis, because of very low signal to noise ratios. Therefore, only data from The miRCURY LNA™ Array and supplier A and B are shown.
- The miRCURY LNA™ Array platform is significantly more sensitive and specific compared to the DNA based array platforms.
- Average sensitivity for the miRCURY LNA™ Array was found to be 50-fold and 100-fold better compared to supplier A and B, respectively.
- In the few cases where the specificity of the miRCURY LNA™ Array did not perform optimally, the platforms from supplier A & B also performed suboptimally.
- This study shows, that DNA-based probes (supplier A + B) do not perform satisfactory across the full GC-span of the microRNA sequences. In contrast, the miRCURY LNA™ Array offers optimal performance of all probes by utilizing Tm-normalization of probes using LNA™.
- The unmatched sensitivity and specificity of miRCURY LNA™ Array is partly explained by the optimal design of the LNA™ capture probes. Another part of the explanation is that LNA™ simply improves discriminative power with respect to their nucleic acid targets, compared to DNA (read more on [www.exiqon.com/LNA](http://www.exiqon.com/LNA)).

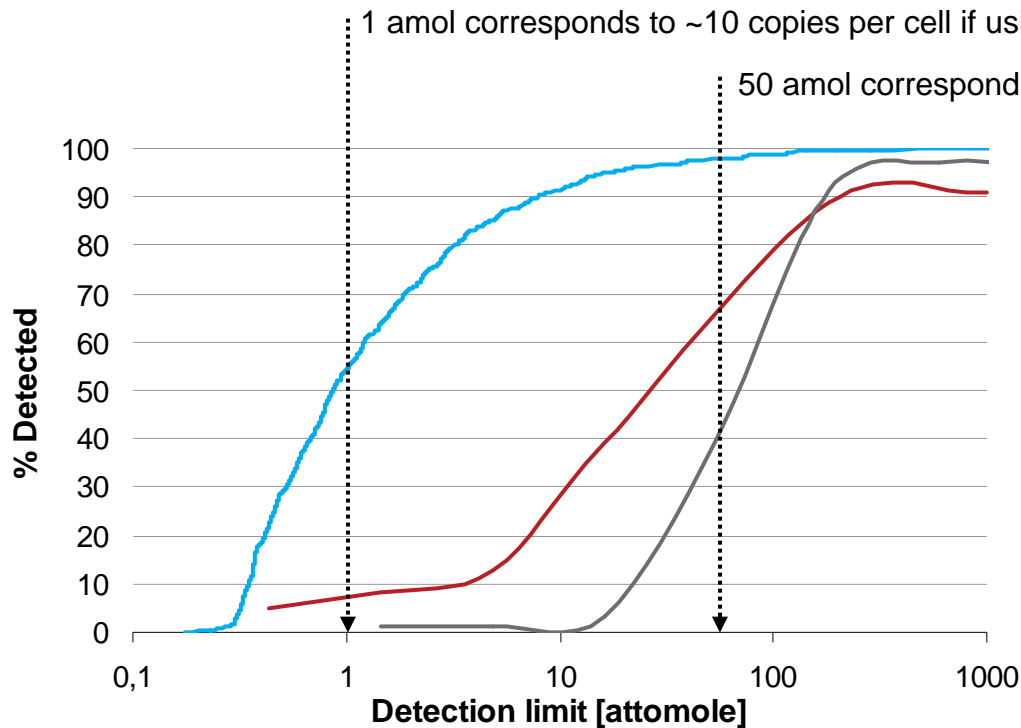




## 4. Sensitivity benchmark

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## Exiqon's miRCURY™ LNA Arrays have unmatched sensitivity



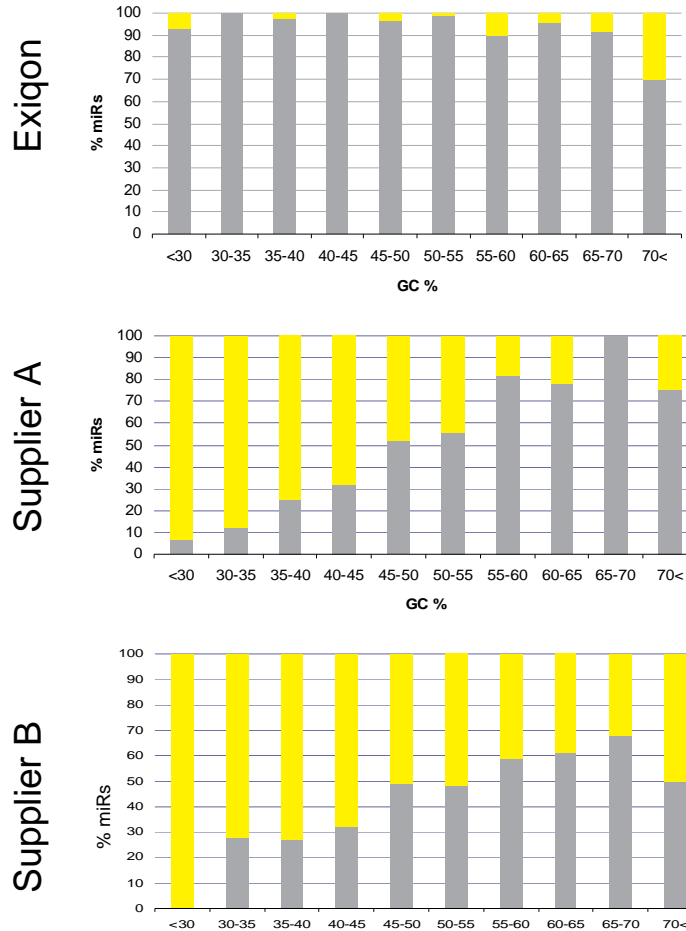
### Experimental set-up:

A dilution series of 557 synthetic microRNAs was hybridized, and for each array the percentage of capture probes detecting microRNAs was plotted.

At 50 amol only 40% of the probe of Supplier B and 66% of Supplier A detect their targets. Exiqon capture probes detect 96% of their targets.

All arrays were processed according to the manufacturers' protocols.

## Exiqon's miRCURY™ LNA Arrays have unmatched sensitivity for all microRNAs



■ Working capture probes  
■ Capture probes not working

It is clear that Supplier A + B have difficulties in designing optimal probes spanning the entire region of GC%.

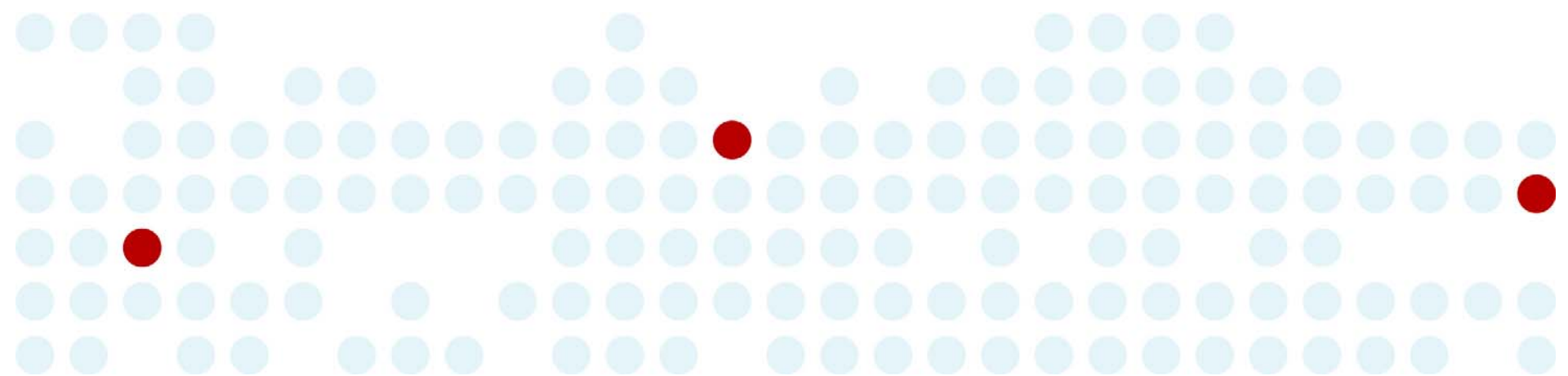
In contrast, the miRCURY LNA™ Array offers optimal performance of all probes by utilizing Tm-normalization of probes using LNA™.

The unmatched sensitivity and specificity is partly explained by the optimal design of the LNA™ capture probes.

Method: 557 synthetic microRNAs have been used.

Each at a concentration of 50 amol (~500 copies per cell if using 1 µg total RNA).

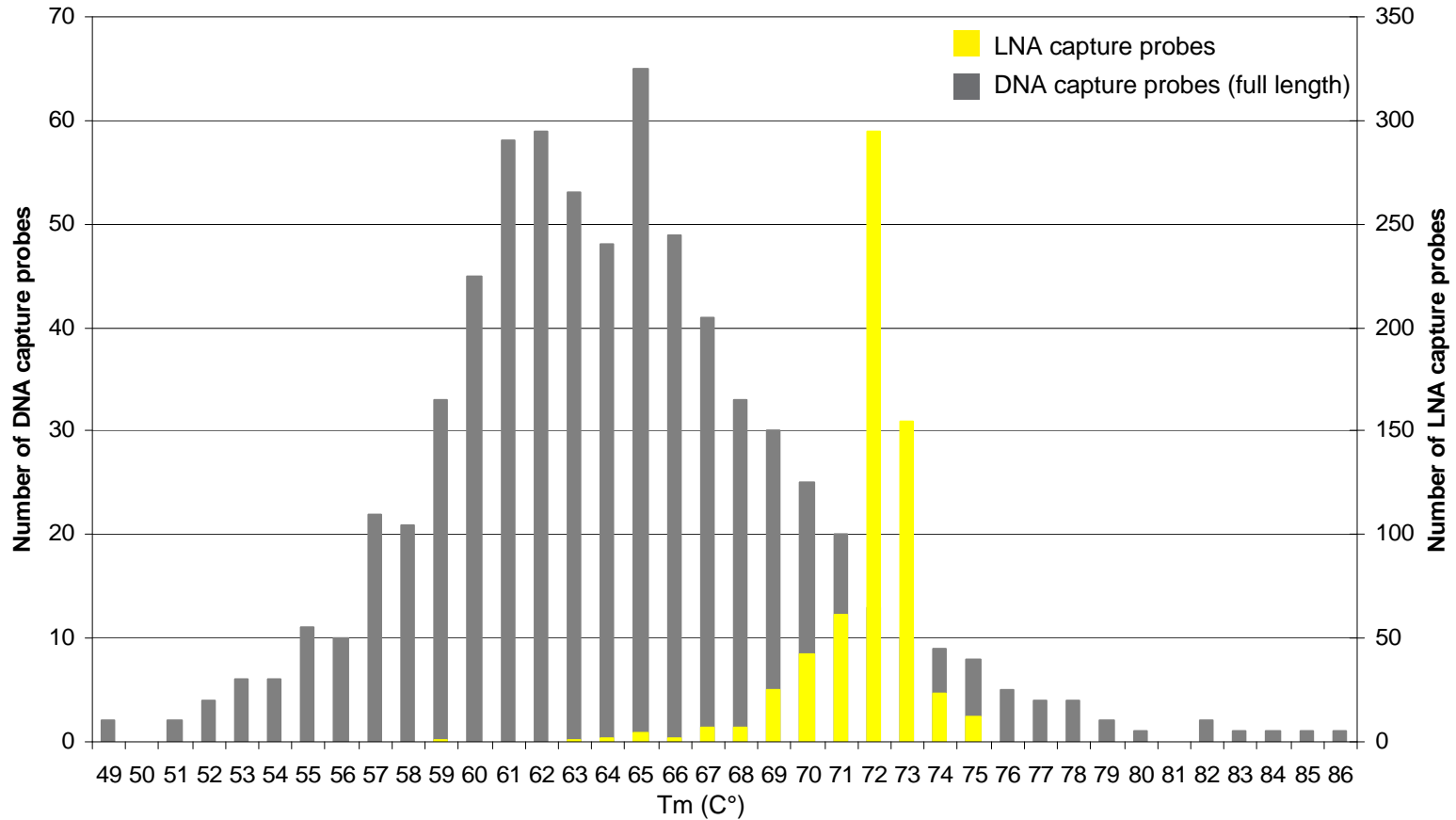
GC%	<30	30-35	35-40	40-45	45-50	50-55	55-60	60-65	65-70	70<
# of miRs	15	40	48	91	92	89	39	28	16	12



## 5. Specificity benchmark

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## Optimally designed LNA™ capture probes result in unmatched specificity



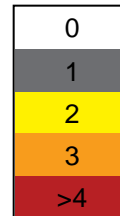
Using LNA™, the T<sub>m</sub> is increased significantly and the T<sub>m</sub> range is narrowed significantly, compared to DNA probes. This results in increased stringency and optimal hybridization conditions for the LNA™ capture probes.

## How to interpret the specificity data

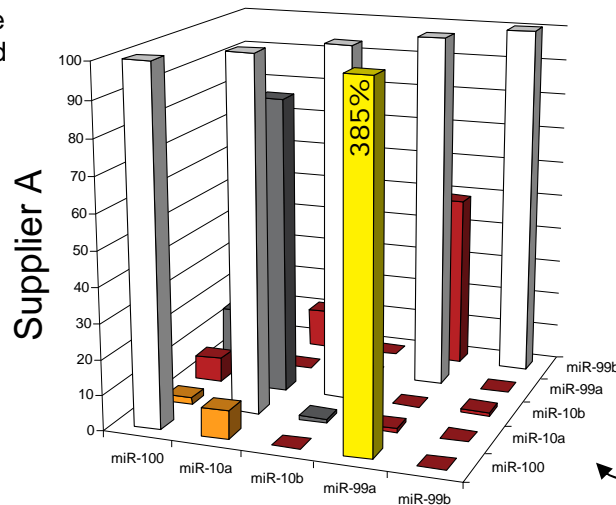
Alignment of microRNA sequences. Letters with underscore show mismatches compared to the sequence mentioned first

mi croRNA	Sequence (5' -3' )
mi R-100	aaccg-gtagatccgaacttgtg
mi R-10a	<u>t</u> acc <u>c</u> tgtagatccgaatttgtg
mi R-10b	<u>t</u> acc <u>c</u> tgtagaaccgaatttgtg
mi R-99a	aaccg-gtagatccga <u>t</u> cttgtg
mi R-99b	<u>c</u> accg-gtaga <u>a</u> ccgac <u>c</u> ttgtg
mi R-100	aaccg-gtagatccgaacttgtg

The color code shows the number of sequence mismatches compared to the sequence of the microRNA spiked in.



The Y-axis shows the relative signal intensity (%) compared to the corresponding fully matching capture probe.



The X-axis-1 shows the names of the synthetic microRNAs that are spiked in.

**Example**

### Example:

miR-99a is spiked in.

Signal from the miR-99a capture probe is set to 100% and the bar is white (0 mismatches).

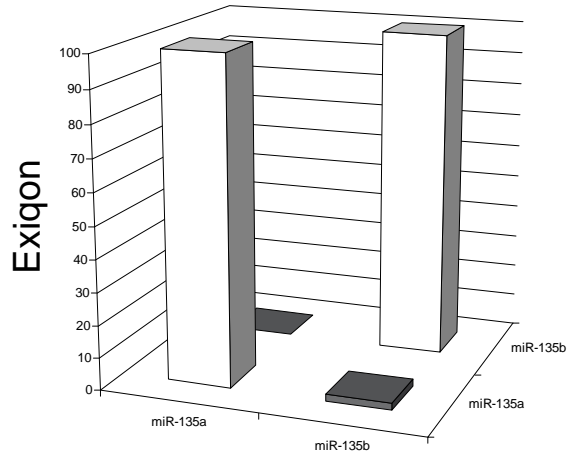
Signal from miR-100 capture probe is 385%, the bar is yellow (2 mismatches, compared to the sequence of miR-99a).

Signal from miR-10a and 10b capture probes are 0%, the bars are red (4 mismatches).

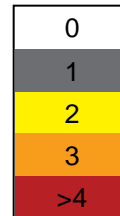
Signal from miR-99b capture probe is 58%, the bar is red (4 mismatches).

The X-axis-2 shows the name of the capture probes for the microRNA's.

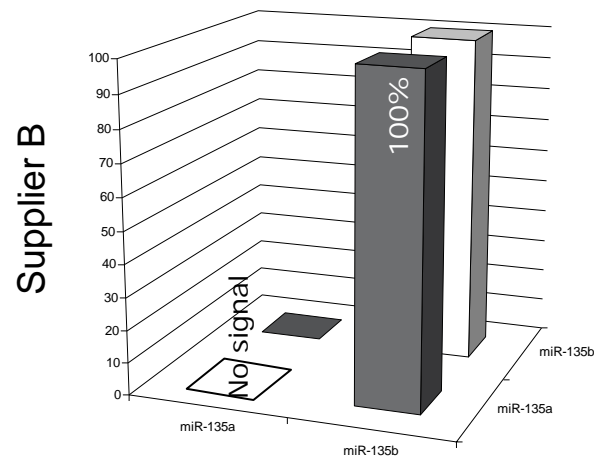
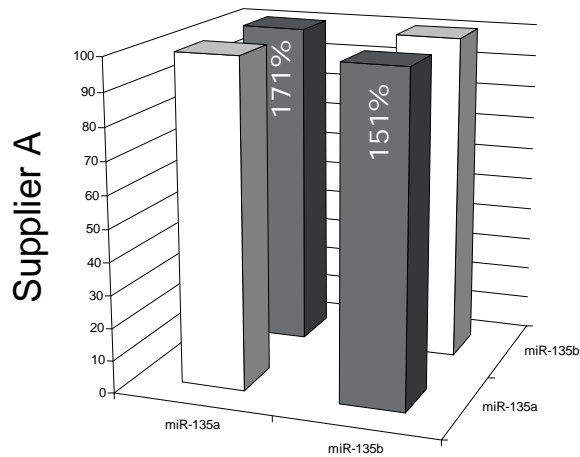
## Exiqon's miRCURY LNA™ Array is the most specific – example 1 of 6



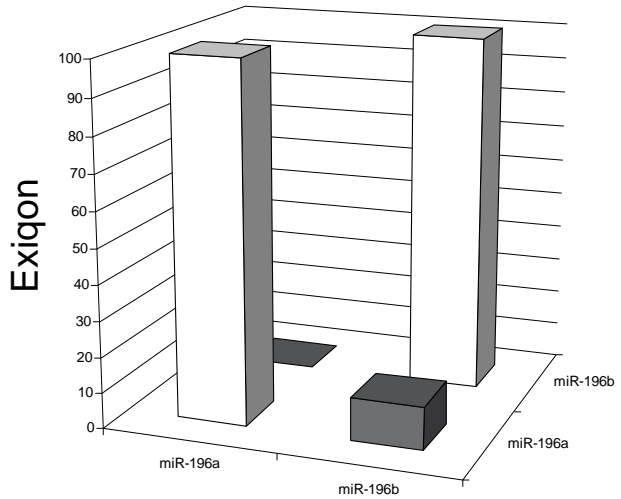
Number of mismatches



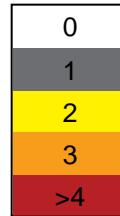
mi croRNA	Sequence (5' -3' )
mi R-135a	tatggctttttattcctatgtga
mi R-135b	tatggcttttt <u>a</u> ttcctatgtga



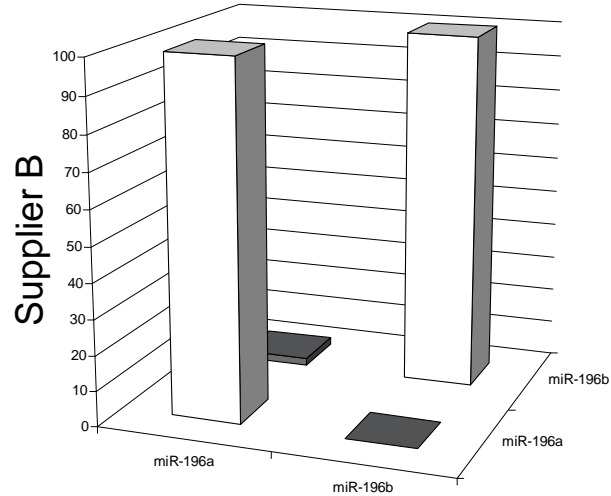
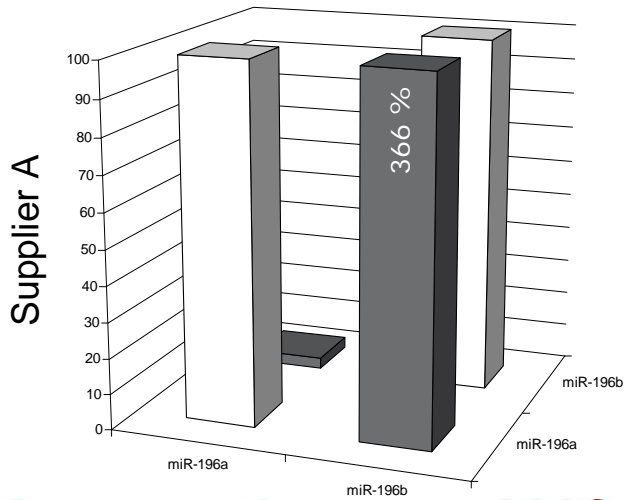
## Exiqon's miRCURY LNA™ Array is the most specific – example 2 of 6



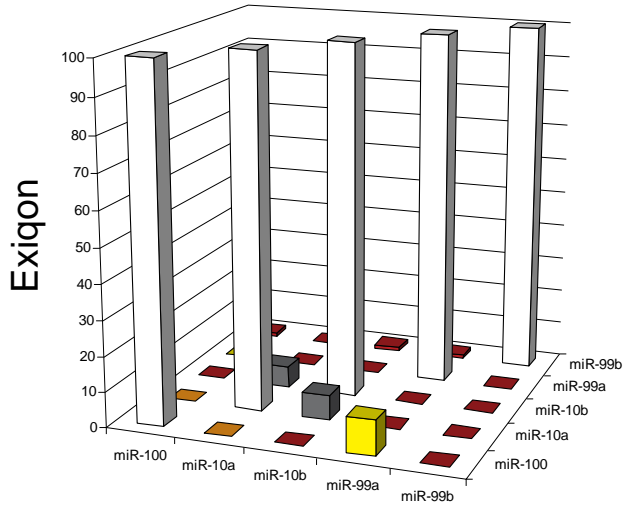
Number of mismatches



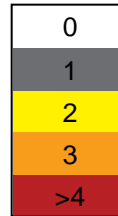
mi croRNA	Sequence (5' -3' )
mi R-196a	taggtagtttcatgttgttggg
mi R-196b	taggtagtttcc_tgttgttggg



## Exiqon's miRCURY LNA™ Array is the most specific – example 3 of 6



Number of mismatches



**mi croRNA**    **Sequence (5' -3')**

mi R-100    aacc-gtagatccgaacttgtg

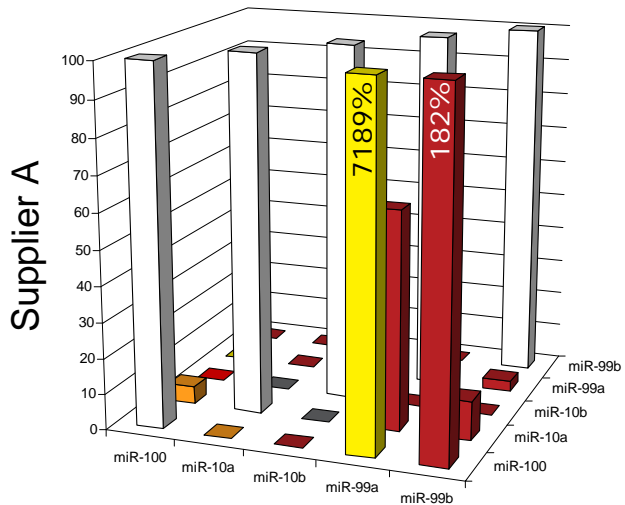
mi R-10a    taccctgtagatccgaatttgtg

mi R-10b    taccctgtagaaccgaatttgtg

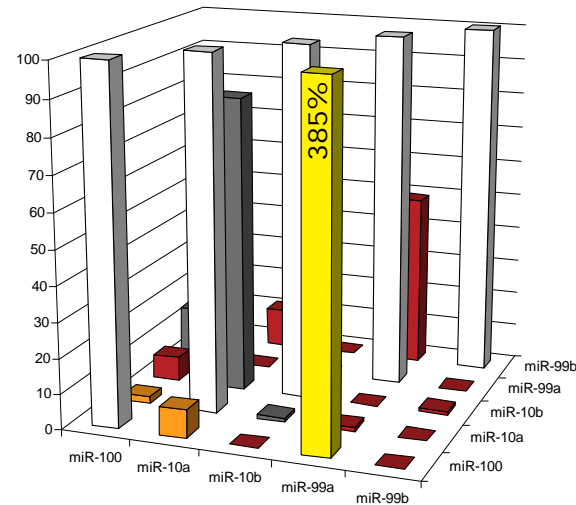
mi R-99a    aacc-gtagatccgatccttgtg

mi R-99b    cacc-gtagaaccgaccttgcg

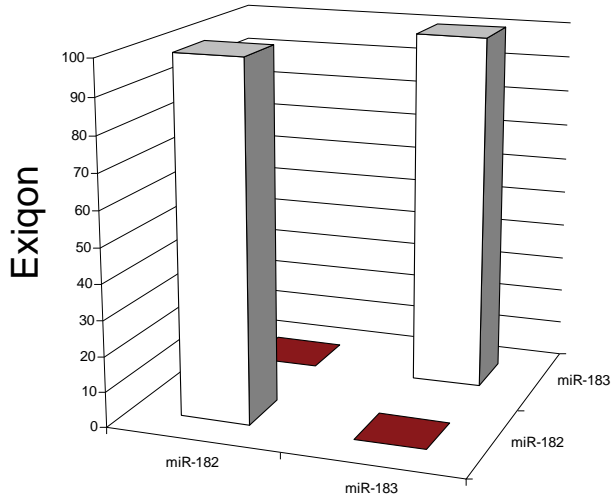
mi R-100    aacc-gtagatccgaacttgtg



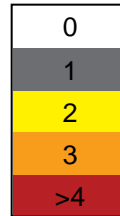
Supplier B



## Exiqon's miRCURY LNA™ Array is the most specific – example 4 of 6



Number of mismatches



mi croRNA

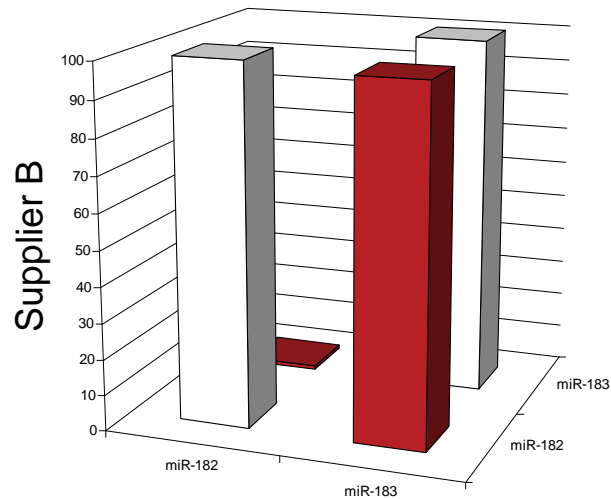
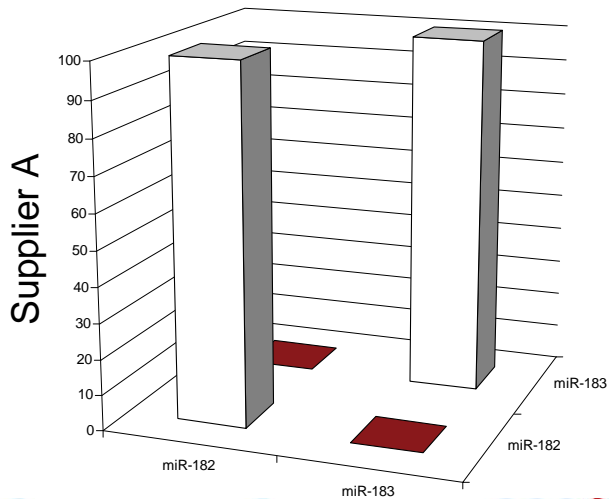
Sequence (5' -3' )

mi R-182

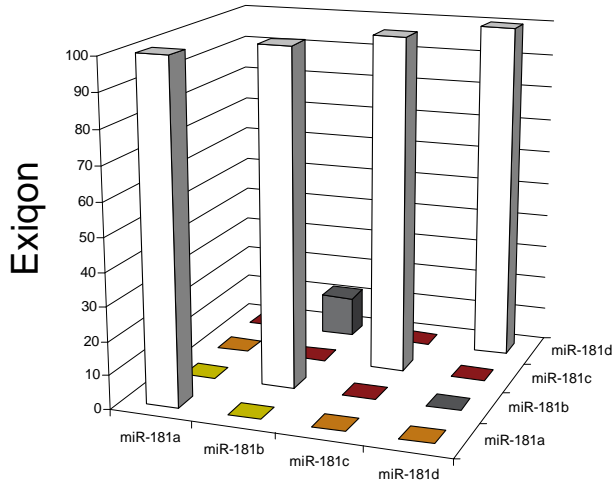
t t t g g c a a t g g t a g a a c t c a c a c t

mi R-183

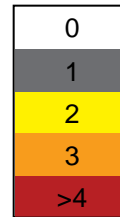
t a t g g c a c t g g t a g a a - t - t c a c t



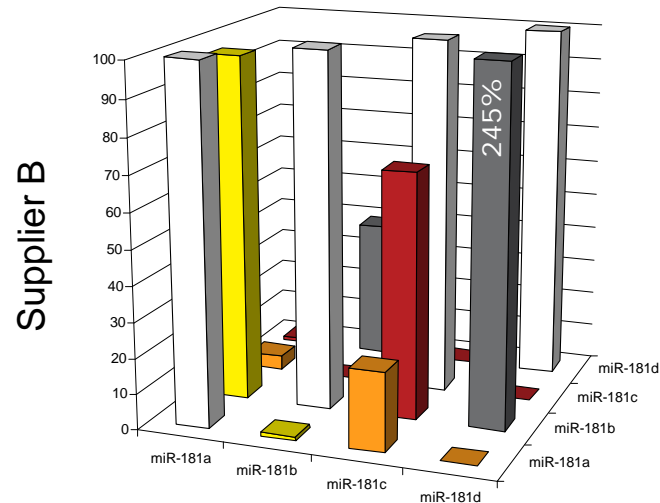
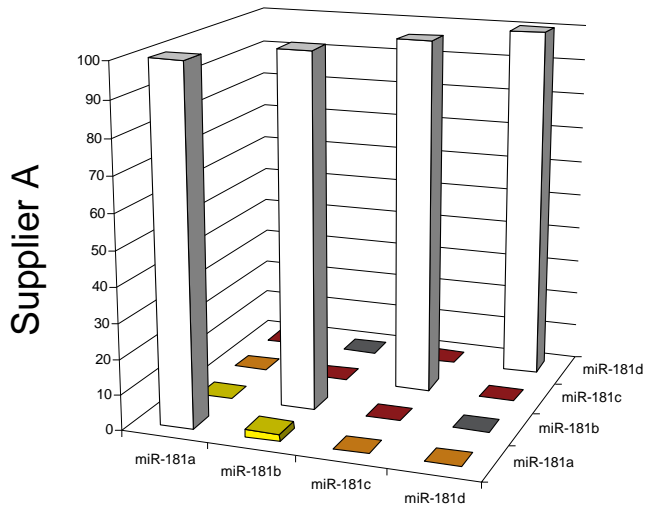
## Exiqon's miRCURY LNA™ Array is the most specific – example 5 of 6



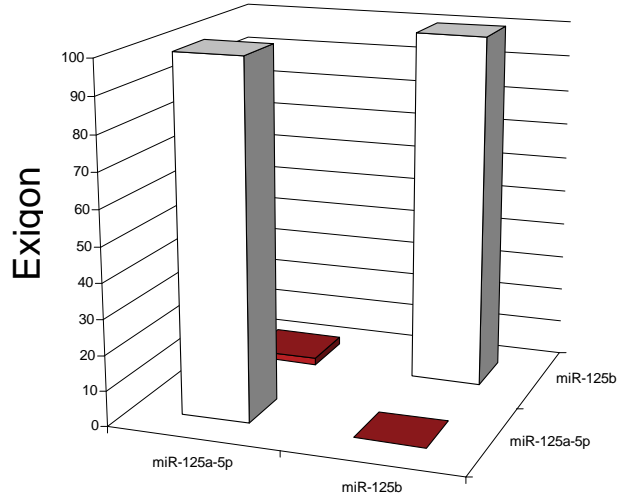
Number of mismatches



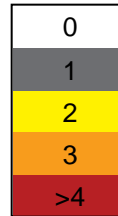
mi croRNA	Sequence (5' -3')
mi R-181a	aacattcaacgctgtcggtgagt
mi R-181b	aacattcattgctgtcggtgagg
mi R-181c	aacattcaac-ctgtcggtgagt
mi R-181d	aacattcattgtgtcggtgagg



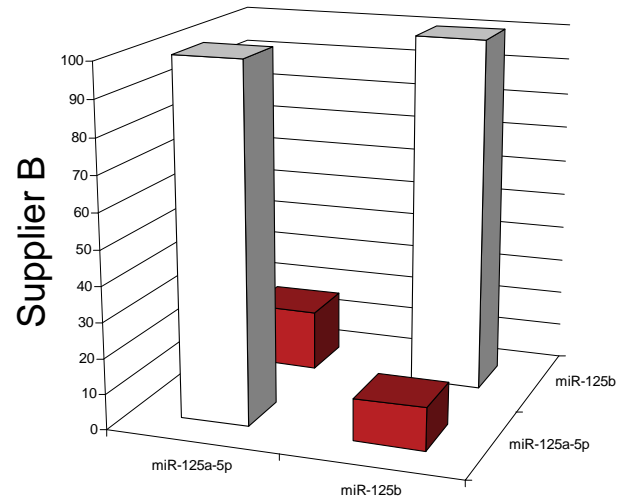
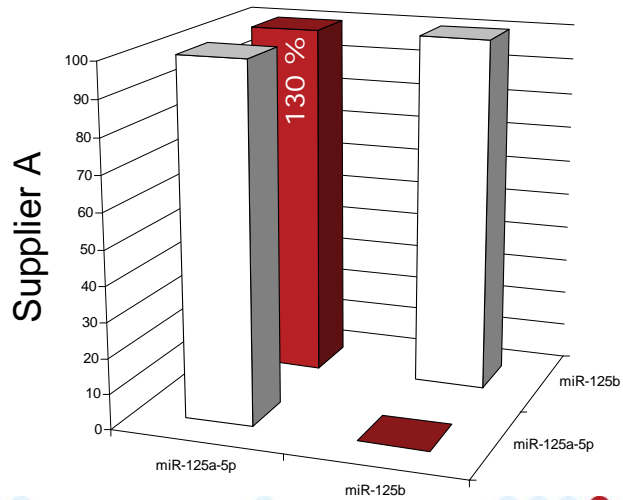
## Exiqon's miRCURY LNA™ Array is the most specific – example 6 of 6



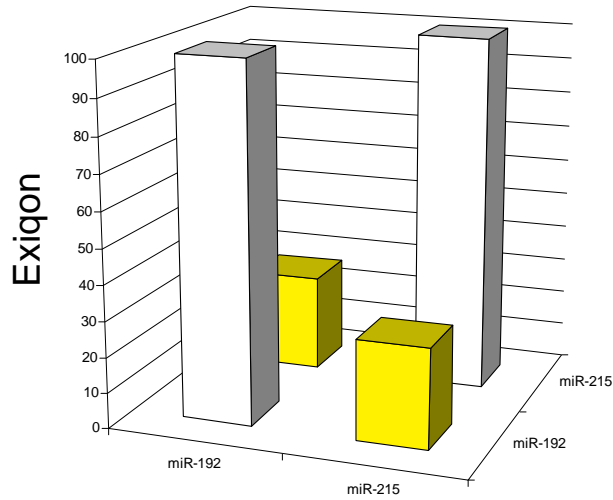
Number of mismatches



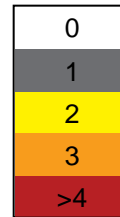
mi croRNA	Sequence (5' -3' )
mi R-125a-5p	tccctgagaccctttaacctgtga
mi R-125b	tccctgagaccct <u>act</u> --tgtga



## None of the tested arrays show optimal specificity for miR-192 and miR-215



Number of mismatches



mi croRNA

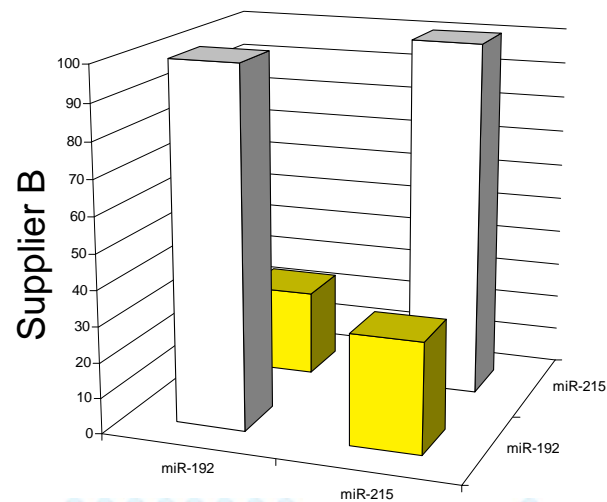
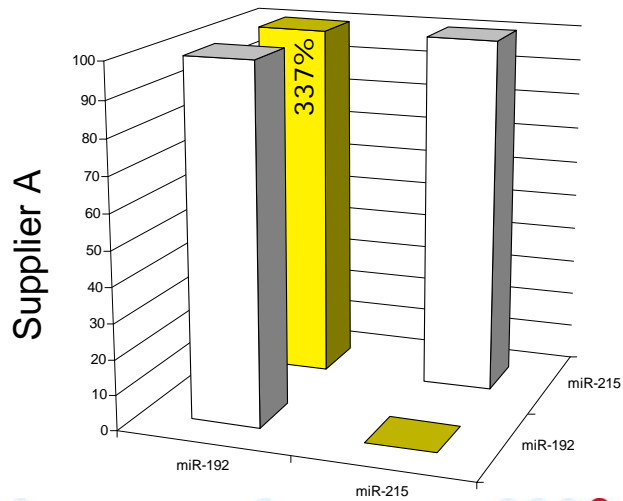
Sequence (5' -3')

mi R-192

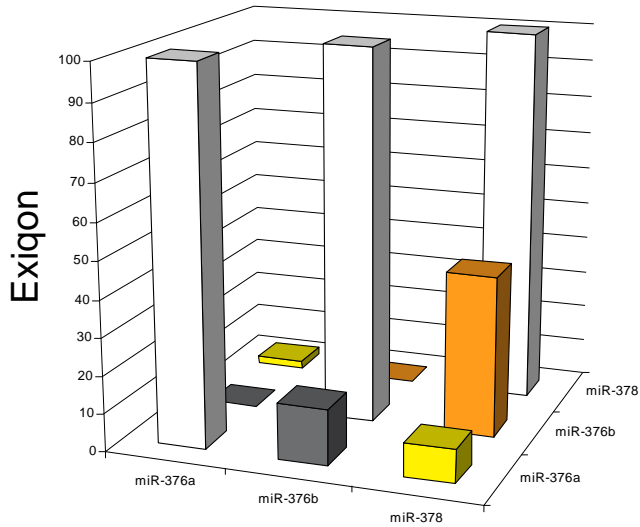
ctgacctatgaattgacagcc

mi R-215

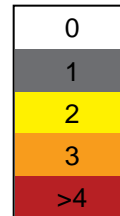
atgacctatgaattgacagac



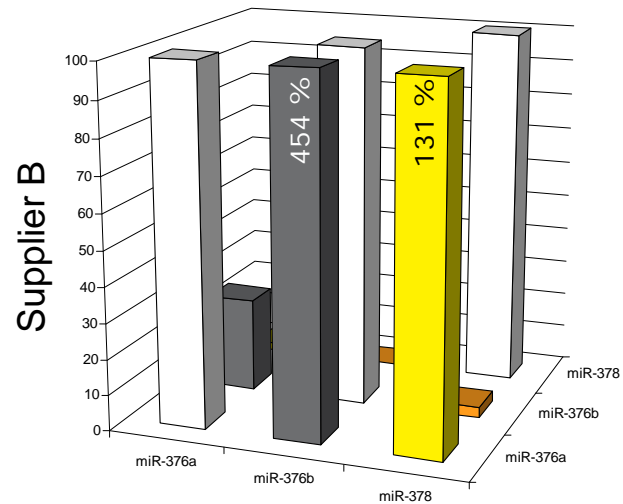
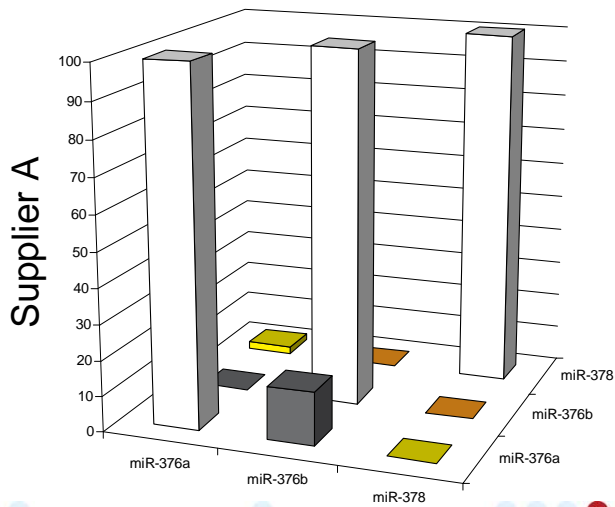
## Supplier A is more specific than the miRCURY LNA™ Array for the mir-367 family



Number of mismatches



mi croRNA	Sequence (5' -3' )
mi R-376a	atcatagaggaaaatccacgt
mi R-376b	atcatagaggaaaatccatggt
mi R-378	aacatagaggaaaatccacgt



Thank you for your attention

Please contact [support@exiqon.com](mailto:support@exiqon.com) to get more information

or

Go to [www.exiqon.com/array](http://www.exiqon.com/array) to learn more about the miRCURY LNA™ Array

