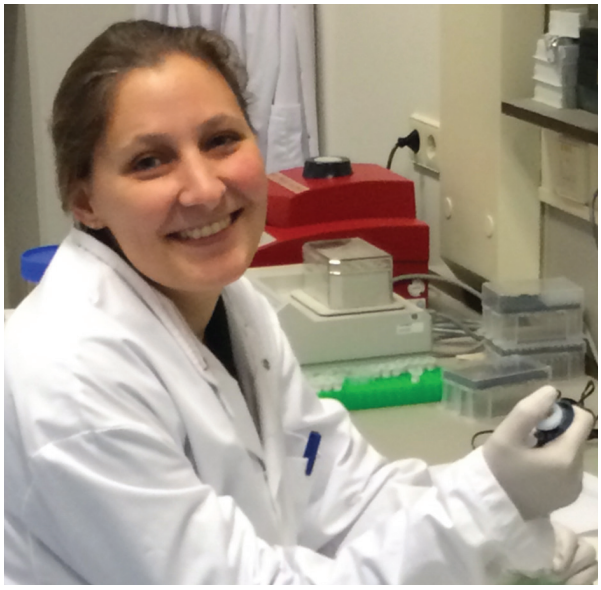
TaqMan Advanced miRNA Assays reliably detect miRNAs from small volumes of nipple aspiration fluid

In this document we demonstrate:

- Successful detection of miRNAs in nipple aspiration fluid (NAF) using Applied Biosystems™ TaqMan™ Advanced miRNA Assays
- Greater sensitivity of TaqMan Advanced miRNA Assays compared to miRCURY™ miRNA assays
- Accurate detection of miRNAs from as low as 2.5 µL of NAF

Customer summary

Dr. Cathy Moelans is a senior researcher in the Department of Pathology at the University Medical Center Utrecht, The Netherlands. Her research focuses on genetic and epigenetic changes during breast cancer initiation, progression, and treatment. One of her projects involves the development of a new breast cancer–screening tool based on biomarker analysis of NAF.



Introduction

MicroRNAs (miRNAs) are small ~22 nt molecules that are defined by structure, regulatory function, and mode of biogenesis. Changes in miRNA abundance have been documented in various diseases including malignancies such as breast cancer [1]. Dysregulated miRNAs have also been detected in blood, plasma, and serum of cancer patients; hence, circulating miRNAs are also being evaluated as potential biomarkers for diseases such as breast and prostate cancer [1,2]. In addition to releasing miRNAs into the blood, mammary epithelia also condition specialized body fluids such as mammary fluid in the resting gland and milk during lactation. Thus, mammary fluids might provide an alternate, local, and noninvasive medium to measure miRNAs implicated in disease [1]. Breast tissue and fluid derived from the ductal system provide a rich source of cells and biomarkers that have the potential to aid in the assessment of short-term risk of breast cancer development, and to assess responses to interventional prevention efforts [1,3]. Release of miRNAs into blood, milk, and ductal fluids is selective and these circulating miRNAs may correlate with malignancy [4,5]. Studying miRNA expression patterns in NAF and ductal lavage has been a challenge due to the small sample volumes (on average 10–20 µL) and the invasiveness of the procedure, respectively.

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Project background

TaqMan Advanced miRNA chemistry

Applied Biosystems™ TaqMan™ Advanced miRNA chemistry enables highly sensitive and specific detection of mature miRNAs using qPCR. The Applied Biosystems™ TaqMan™ Advanced miRNA cDNA Synthesis Kit provides a streamlined workflow that is ideal for analysis of multiple miRNA targets from a single sample, or of low-level RNA samples, such as serum, plasma, or NAF. Universal reverse transcription allows all miRNAs present in the sample to be reverse-transcribed simultaneously followed by detection of individual miRNAs using TaqMan Advanced miRNA Assays.

Many commercially available miRNA qPCR assay solutions do not have the sensitivity required to work with low-volume samples. The TaqMan Advanced miRNA chemistry was chosen for this study due to its robust performance and low limit of detection. Notably, the universal reverse transcription minimizes the volume of sample required, with the use of a single reverse transcription step for the 14 different miRNAs investigated. Dr. Moelans evaluated the performance of TaqMan Advanced miRNA Assays in comparison with equivalent miRCURY™ microRNA LNA™ PCR primer sets from Exiqon, within the context of her requirements to reliably detect miRNAs from small-volume NAF samples.

Materials and methods

Sample collection and processing

NAF samples were obtained from 13 healthy volunteers; for 4 of the women, NAF was analyzed from both breasts, resulting in 17 samples. NAF was collected as described in a previous study [6]. Briefly, fluid was obtained by intermittent manual gentle suction after applying local anesthetic cream, heat, and oxytocin nasal spray. RNA was isolated using the Invitrogen™ *mir*Vana™ PARIS™ Kit (Thermo Fisher Scientific, Cat. No. AM1556) with an input amount of 10 µL of NAF. The concentration of total RNA was measured using a Thermo Scientific™ NanoDrop™ 2000 Spectrophotometer (Thermo Fisher Scientific, Cat. No. ND-2000) and RNA quantities were recorded (Table 1).

cDNA synthesis and qPCR

TaqMan Advanced miRNA Assays: cDNA was prepared using the TaqMan Advanced miRNA cDNA Synthesis Kit (Thermo Fisher Scientific, Cat. No. A28007) with 2 µL of RNA followed by qPCR using TaqMan Advanced miRNA Assays, following manufacturer's instructions [7]. miRCURY microRNA assays: cDNA was prepared using the Universal cDNA Synthesis Kit II (Exiqon, Cat. No. 203301) with 4 µL of RNA followed by qPCR with miRCURY microRNA LNA PCR primer sets, following manufacturer's instructions [8]. All qPCR cycling was performed on the Applied Biosystems™ ViiA™ 7 Real-Time PCR System following appropriate manufacturer-recommended protocols for cDNA preparation and subsequent target amplification and detection. Fourteen different miRNA targets (Table 2) were chosen for this study based on their relatively consistent expression in NAF as characterized from a previous profiling study [9].

Table 1. NAF visual appearance and total RNA concentration measured by the NanoDrop 2000 Spectrophotometer.

Sample	NAF appearance	RNA concentration (ng/µL)
NAF_1	Green, thick	4.1
NAF_2_L	White	7.2
NAF_2_R	White	5.1
NAF_3	Green, thick	8.7
NAF_4_L	White	4.5
NAF_4_R	White	5.2
NAF_5	Yellowish, thick	3.6
NAF_6	Clear yellowish	6.1
NAF_7	White	5.8
NAF_8	Clear white	4.0
NAF_9_L	Clear white	4.2
NAF_9_R	Clear white	3.0
NAF_10	Green, thick	5.4
NAF_11	White	8.2
NAF_12	Yellowish, thick	5.2
NAF_13_L	Clear white	5.4
NAF_13_R	Clear brownish	5.9

Table 2. List of 14 miRNAs interrogated in this study.

Name of miRNA	Target sequence	TaqMan Advanced miRNA Assay ID
hsa-miR-145-5p	GUCCAGUUUUCCCAGGAAUCCCU	477916_mir
hsa-miR-146b-5p	UGAGAACUGAAUUCCAUAGGCU	478513_mir
hsa-miR-148a-3p	UCAGUGCACUACAGAACUUUGU	477814_mir
hsa-miR-181a-5p	AACAUUCAACGCUGUCGGUGAGU	477857_mir
hsa-miR-18a-5p	UAAGGUGCAUCUAGUGCAGAUAG	478551_mir
hsa-miR-200c-3p	UAAUACUGCCGGGUAAUGAUGGA	478351_mir
hsa-miR-23a-3p	AUCACAUUGCCAGGGAUUUCC	478532_mir
hsa-miR-335-5p	UCAAGAGCAAUAACGAAAAAUGU	478324_mir
hsa-let-7a-5p	UGAGGUAGUAGGUUGUAUAGUU	478575_mir
hsa-miR-92a-3p	UAUUGCACUUGUCCCGGCCUGU	477827_mir
hsa-miR-125a-5p	UCCCUGAGACCCUUUAACCUGUGA	477884_mir
hsa-miR-877-5p	GUAGAGGAGAUGGCGCAGGG	478206_mir
hsa-miR-652-3p	AAUGGCGCCACUAGGGUUGUG	478189_mir
hsa-miR-21-5p	UAGCUUAUCAGACUGAUGUUGA	477975_mir

Results

The overall effectiveness of each assay system was evaluated by plotting the full range of C_t values obtained from the 13 samples (10 µL extractions) across each of the 14 targets (Figure 1). TaqMan Advanced miRNA Assays recorded lower average C_t values for all but one target, with 4.1 being the median C_t difference between TaqMan Advanced miRNA Assays and miRCURY microRNA LNA PCR primer sets. Endogenous control assay expression was found to be consistent between the two platforms (data not shown).

The suitability of TaqMan Advanced miRNA Assays for low-volume samples was determined by comparing average C_t values of duplicate qPCR reactions across all 14 miRNA targets, obtained from 2.5, 5, or 10 µL of NAF from a single specimen (Figure 2). C_t values were detected for every target regardless of input volume, with overall expression patterns maintained. Comparison with the Universal cDNA Synthesis Kit II from Exiqon was not possible, as consistent results could not be obtained for samples under 10 µL (data not shown).

Assay-centric expression patterns were plotted by Pearson average linkage cluster analysis using Applied Biosystems™ DataAssist™ Software (Figure 3). The cluster patterns show similarities for individuals where NAF samples were obtained from the left and right breast.

Discussion

Routine miRNA analysis using qPCR can be challenging, particularly for a low-volume sample such as NAF. Dr. Moelans was looking for a reliable method to assist her research and said, “the TaqMan Advanced miRNA Assays are the perfect match for our limited NAF samples. They offer high sensitivity and accuracy, and allow us to save more sample for parallel biomarker analysis and technical duplicates.”

TaqMan Advanced miRNA Assays proved to be robust and sensitive across all 14 targets chosen for evaluation, with consistently lower C_t values compared with miRCURY microRNA LNA PCR primer sets. Further, the TaqMan Advanced miRNA cDNA Synthesis Kit was the only means tested to successfully prepare samples for effective detection across all 14 targets from as little as 2.5 µL of NAF. The similarities seen in the cluster analysis support the reproducibility of TaqMan Advanced miRNA Assays and allow for further investigation into the suggested role of the endocrine environment in regulating NAF miRNA expression levels.

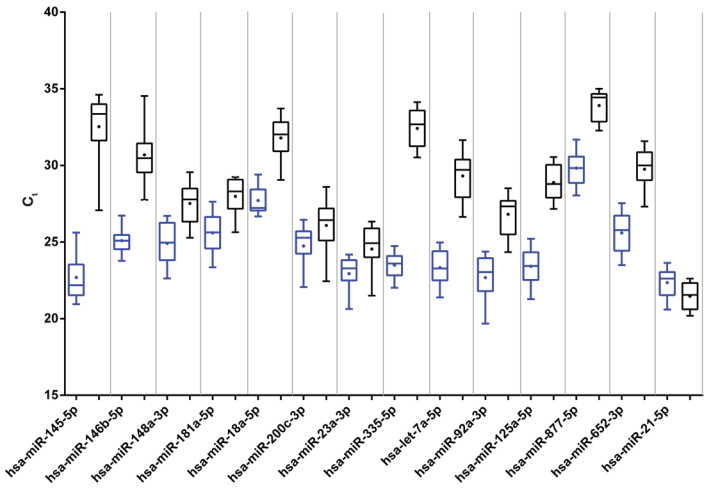

Figure 1. Box plot showing expression of 14 different miRNA targets in 13 NAF samples as detected by Taqman Advanced miRNA Assays (blue) and miRCURY microRNA LNA primers (black). The line in the middle of each box is plotted at the median and the dot is plotted at the mean. The box extends from the 25th to 75th percentiles. The whiskers are drawn down to the 10th percentile and up to the 90th percentile. The median C_t difference between the two platforms was found to be 4.1.

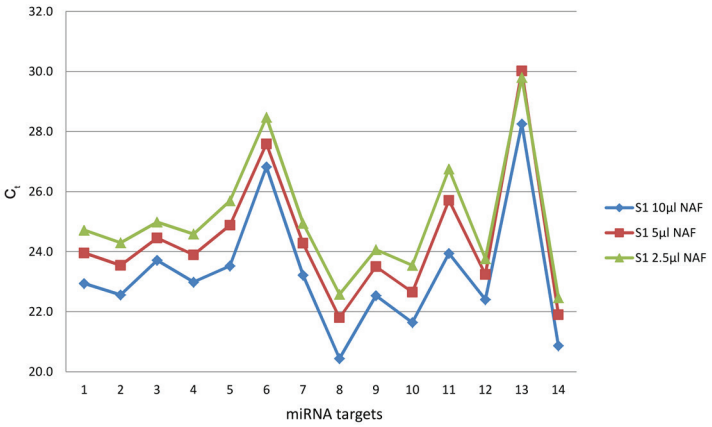

Figure 2. Average C_t values of 14 miRNAs from varying amounts of NAF from a single specimen (No. 13). Total RNA was isolated from 2.5, 5, or 10 µL of NAF using the *mir*Vana PARIS Kit. 2 µL of total RNA input was then used with the TaqMan Advanced miRNA cDNA Synthesis Kit followed by detection with TaqMan Advanced miRNA Assays.

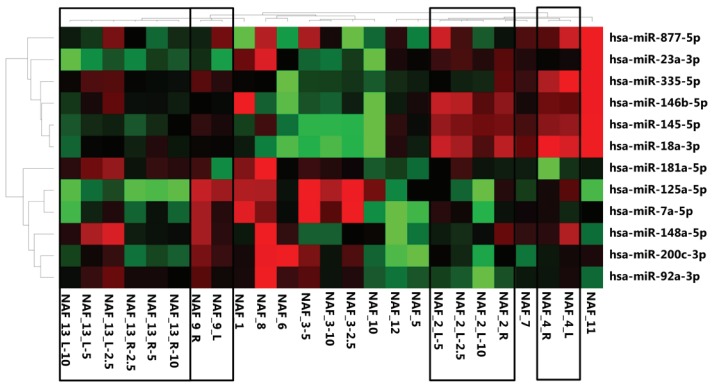

Figure 3. Assay-centric Pearson average linkage cluster analysis generated with C_t values from TaqMan Advanced miRNA Assays using DataAssist Software. The expression pattern of miRNAs was similar when comparing NAF samples obtained from left (L) and right (R) breast.

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Conclusion

TaqMan Advanced miRNA Assays can be used to reliably detect miRNAs from low-volume NAF samples. The TaqMan Advanced miRNA Assay workflow demonstrated superior sensitivity, with lower average C_t values when compared to miRCURY microRNA assays, and the ability to detect miRNAs from as low as 2.5 µL of NAF, where miRCURY microRNA assays required at least 10 µL. When sample volume is limited and sensitivity is paramount, TaqMan Advanced miRNA Assays are a superior tool, supporting the important research of Dr. Moelans and the University Medical Center Utrecht as they work towards the development of a new breast cancer–screening method.

References

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7. TaqMan Advanced miRNA Assays User Guide. Pub. No. 100027897, Revision B.
8. miRCURY LNA Universal RT microRNA PCR Instruction Manual v6.1, April 2015.
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Ordering information

Product	Quantity	Cat. No.
TaqMan Advanced miRNA cDNA Synthesis Kit	50 reactions	A28007
TaqMan Advanced miRNA Assays	250 qPCR reactions (20 µL)	A25576

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