Scientific article

miRNA profiling from blood — challenges and recommendations

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Abstract: The discovery of stable miRNA species circulating in blood has led to increased research focus on disease-related variations in serum and plasma miRNA expression and the possibility that such variations could serve as noninvasive biomarkers for disease. Working with serum and plasma miRNA presents various challenges in purification and characterization. In this paper, we outline QIAGEN® recommendations for robust purification and quantification, as well as reliable data normalization and analysis.

Introduction

In a few short years, microRNA (miRNA) research has advanced from a single publication reporting the discovery of a small, regulatory, noncoding RNA in *Caenorhabditis elegans* to thousands of publications describing both the mechanism behind RNA interference (RNAi) and the many critical roles that miRNAs play in the regulation of gene expression. In humans, the annotated miRNome has rapidly expanded from only a handful of sequences to nearly 2000 in miRBase version 18 (www.mirbase.org).

Large-scale cloning and next-generation sequencing studies have revealed surprising diversity in the mature miRNA population. Heterogeneity is generated by variations in the processing of precursor miRNA (1), as well as by posttranscriptional RNA editing (2). miRNAs have been demonstrated to act synergistically on target mRNAs, further increasing their potential regulatory repertoire. Considering the diversity of mature miRNAs found in the cell and the potential for cooperative regulation of target genes, the potential complexity of miRNA effects on cellular functions is vast.

miRNA research has made a significant impact on all aspects of biomedical research, from elucidating basic functions and mapping pathways in model systems to understanding gene expression changes in cancer and creating new possibilities for molecular diagnostics and nucleic-acid–based drugs. QIAGEN supports this research by providing optimized sample collection, stabilization, and purification solutions, as well as a robust miRNA quantification system that enables efficient profiling of miRNA changes in serum, plasma, and many other sample types. ▶

Contents

Stable, circulating miRNA

The first reports that miRNAs could be found at stable levels in serum and plasma were initially met with skepticism, but were quickly confirmed by others (3, 4, and Figure 1). This discovery generated high interest in the possibility that changes in these miRNA levels could be used as noninvasive biomarkers for a variety of indications. Evidence is accumulating that these circulating miRNAs can indeed be used as biomarkers to identify and monitor a variety of cancers and other diseases (Figures 2 and 3).

Circulating miRNA is presumably not naked miRNA, which would be degraded within seconds due to the high levels of nucleases in blood. Several reports have demonstrated that stability results from the formation of complexes between circulating miRNA and specific proteins (5–7). Other studies have found miRNA contained within circulating exosomes or other microvesicles (8). While there is no longer any doubt that a stable population of miRNAs exist in circulation, nothing is yet known about their purpose and whether they play any role in normal physiology. It is also unknown how and why disease causes changes in the levels of specific circulating miRNAs. It is possible that cell lysis or an increase in the number of exosomes shed from diseased cells contribute to increased levels of certain circulating miRNAs.

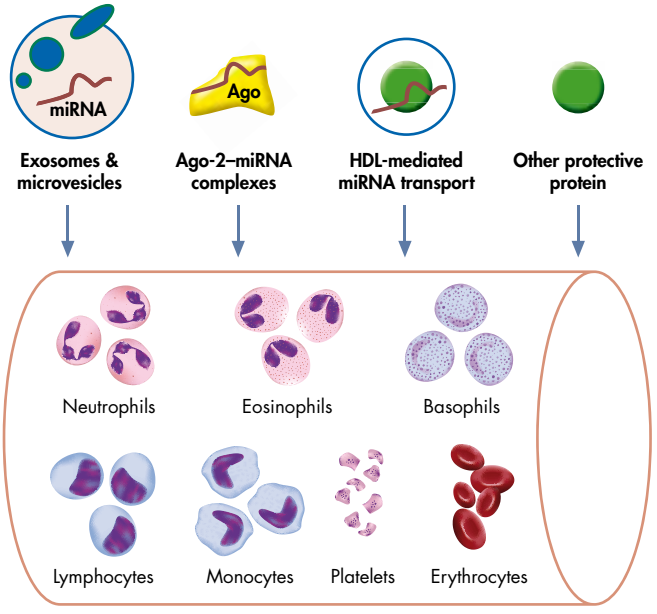


Figure 1. Circulating miRNA in blood. Circulating miRNA may be protected from degradation by a variety of mechanisms, including formation of complexes with proteins or secretion within exosomes. Human blood consists of red blood cells or erythrocytes, platelets, and white blood cells or leukocytes, which include neutrophils, eosinophils, basophils, lymphocytes, and monocytes. These cells are suspended in plasma.

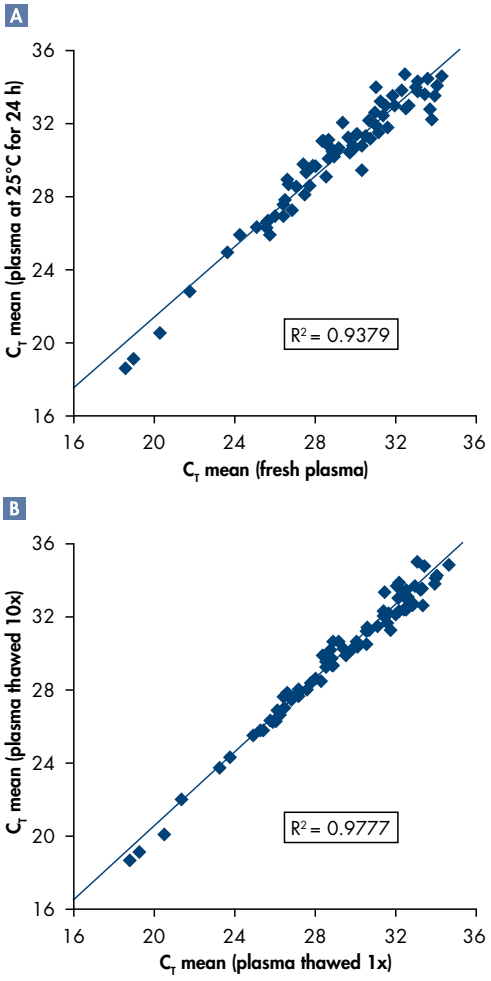


Figure 2. Stability of plasma miRNA. A Fresh plasma samples or plasma samples stored at 25°C for 24 hours were used for RNA purification using the miRNeasy Serum/Plasma Kit. Profiling was performed using the Human Serum & Plasma miScript® miRNA PCR Array. The equivalent of 20 µl plasma was used for each array. Pairwise comparison of the data was performed using a scatter plot of C_T values. Incubation for 24 hours at room temperature had only a minor effect on expression levels. Storage of plasma at room temperature is not recommended. However, these results show that miRNA in these samples is remarkably stable. B Plasma samples were either frozen once or subjected to 10 freeze–thaw cycles prior to RNA purification using the miRNeasy Serum/Plasma Kit. Profiling and data analysis was performed as described above. Multiple freeze–thaw cycles had no effect on reproducibility of miRNA profiling from plasma.

Challenges of miRNA purification from blood

Blood has always presented a unique challenge to RNA isolation technologies. Compared to cells or solid tissue samples, there are relatively few nucleated cells in blood; therefore, larger sample volumes must be processed. Many of the cells in blood are poised to rapidly respond to changes in their environment and, as a result, show changes in gene expression almost immediately after sample collection. The presence of high concentrations of protein, including nucleases and other components, can interfere with downstream enzymatic reactions. For example, heparin, a common anticoagulant, can copurify with RNA and is a potent

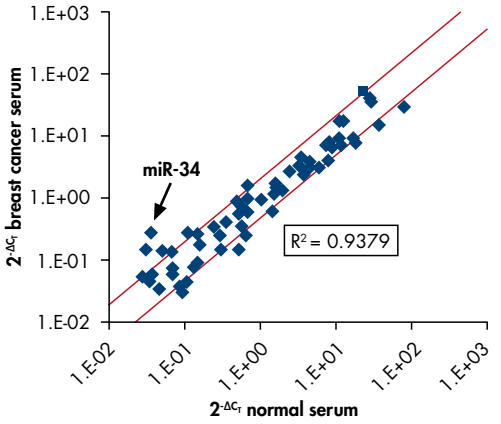


Figure 3. miRNA variation in normal and breast cancer serum. Total RNA was isolated from normal (n = 10) and breast cancer (n = 3) serum samples. Profiling was performed using the Human Serum & Plasma miScript miRNA PCR Array. A scatter plot of $2^{-\Delta C_T}$ values demonstrates several significant differences between mature miRNA expression levels of normal and breast cancer serum pools, including hsa-miR-34a, which has been shown to be a blood-based marker for patients with primary and metastatic breast cancer. Data sets were first calibrated using the *C. elegans* miR-39 miScript Primer Assay which detects miRNeasy Serum/Plasma Spike-In Control, and then normalized using commonly expressed miRNA targets. Analysis of a larger panel of individual samples will be necessary to draw firm conclusions about the expression of specific miRNAs.

inhibitor of enzymatic reactions. Even a high-quality RNA prep from blood can contain contaminants that inhibit real-time RT-PCR if too much sample is used in the reverse-transcription reaction.

QIAGEN sample handling and RNA purification solutions are optimized depending on whether the downstream profiling application requires all miRNA in whole blood, the miRNA component of the nucleated cell fraction, or extracellular circulating miRNA (Table 1).

Table 1. Kits for purification of total RNA including miRNA

Kit	Starting material	Benefit
miRNeasy Serum/Plasma Kit	Serum and plasma	Includes miRNeasy Serum/Plasma Spike-In Control; optimized for circulating, cell-free miRNA
miRNeasy Mini Kit	Animal/human tissues, cells	Optimized for cells and tissues
miRNeasy 96 Kit	Animal/human tissues, cells	High-throughput; optimized for cells and tissues
miRNeasy Micro Kit	Animal/human tissues and cells	Optimized for low RNA amounts and cells and tissues
PAXgene® Blood miRNA Kit	Human blood that has been stabilized in PAXgene Blood RNA Tubes	Optimized for human whole blood miRNA
RNeasy® Protect Animal Blood Kit	Animal blood that has been collected in collected in RNAprotect® Animal Blood Tubes	Optimized for animal whole blood miRNA

miRNA purification from fresh whole blood

The optimal approach for miRNA purification from whole blood is to collect fresh blood and process the sample as quickly as possible. We recommend the PAXgene Blood miRNA Kit for human blood (with blood collected and stabilized in PAXgene Blood RNA Tubes) or the RNeasy Protect Animal Blood Kit for mouse, rat, and other small animal blood (with blood collected and stabilized in RNAprotect Animal Blood Tubes). A single PAXgene Blood RNA Tube enables collection of 2.5 ml blood, while RNAprotect Animal Blood Tubes are available in 100 µl or 500 µl capacities. Use of either of these tube types results in disruption of all cellular content and stabilization of RNA prior to sample preparation. Since cells are lysed, these methods do not allow later separation of blood into cellular and fluid components for miRNA analysis in these fractions. Ideally, fresh blood should be used, since freezing and thawing blood prior to processing can result in cell lysis and RNA degradation. RNA preps from blood contain large quantities of globin mRNA; however, this does not affect miRNA quantification by real-time RT-PCR.

miRNA purification from nucleated cells

The simplest way to isolate miRNA from the cellular, non-red blood cell (RBC) fraction of fresh whole blood is to first lyse RBCs by addition of 1.5 volumes cold 1 mM EDTA and then pellet the intact cells by centrifugation. Carefully remove the entire supernatant, then follow the protocol provided in the *miRNeasy Micro Handbook*, *miRNeasy Mini Handbook*, or *miRNeasy 96 Handbook* for cells, starting with cell pellets. If the blood was previously frozen, it will no longer be possible to separate white blood cells (WBCs) from other blood components because freezing and thawing causes cells to burst. In this case, the only option is to purify whole blood.

RNA purified from anticoagulated blood stored for any length of time may not provide a meaningful miRNA profile from the cellular component due to changes in gene expression in the cells. If subfractionation of immune cells in blood by cell sorting is performed during research, these purified cells can also be treated as a cell pellet and processed using the miRNeasy Micro, Mini, or 96 Kit.

miRNA purification from serum and plasma

Plasma is a clear, yellow fluid that makes up about 55% of the total blood volume. It contains fibrinogen and other clotting factors. Serum is blood plasma without fibrinogen or other clotting factors. Plasma is prepared by centrifugation of whole blood to remove RBCs and WBCs. Preparation of serum is more complicated and involves allowing blood to clot, followed by centrifugation to remove the clot. We recommend using EDTA or citrate anticoagulated blood, and processing plasma rather than serum. This minimizes procedural variation caused by differences in clotting and subsequent collection of serum.

Since the mechanisms by which miRNAs enter circulation and are protected from nucleases are still unknown, we currently recommend profiling miRNA expression from total serum or plasma rather than performing any enrichment of RNA–protein complexes or exosomes. Purification of circulating miRNA from serum or plasma can be performed using the miRNeasy Serum/Plasma Kit. We recommend a starting volume of 100–200 µl serum or plasma for RNA purification with the kit. Use of more than 200 µl serum or plasma per RNA prep is not recommended, as the amount of contaminants present in larger volumes reduce the binding capacity of the RNeasy MinElute® spin column and increase the risk of copurification of inhibitors.

Purification and downstream real-time RT-PCR profiling work equally well whether serum or plasma miRNA preps are used as starting material. In our experience, profiling data is similar from serum and plasma samples from the same donor. However comparing a serum sample to a plasma sample from the same donor shows more variability than comparison of 2 samples of the same type (either serum or plasma). For this reason, we recommend comparing the same sample types where possible to minimize variability.

miRNA profiling by real-time RT-PCR

Real-time RT-PCR is a reliable, easily applicable technique for miRNA quantification. Arrays of miRNA assays can be used to profile hundreds of miRNAs to gain a snapshot of the expressed miRNAs in the cell at a certain timepoint, in a diseased or healthy state, or after stress or drug treatment. Alternatively, individual miRNA assays can be used for experiments studying the expression of a single miRNA or a small group of miRNAs. Individual miScript Primer Assays and miScript miRNA PCR Arrays enable high-quality expression analysis from single miRNAs to miRNome-wide profiling, using a straightforward, optimized real-time RT-PCR protocol (Figure 4). Patent-pending miRNA technology ingeniously integrates a universal tailing and reverse-transcription reaction with accurate expression analysis of distinct miRNA sequences that may only differ by a single nucleotide base. This technology allows the researcher to easily perform a comprehensive survey of relative miRNA expression in samples of interest. All miScript Primer Assays are designed to work under a common set of cycling conditions and are thoroughly wet-lab verified to ensure high specificity and high sensitivity.

Reverse transcription from serum and plasma samples

Upstream of real-time PCR, the miScript PCR System includes an integrated kit for reverse transcription: the miScript II RT Kit. The kit comes with 2 different buffer chemistries, each optimized for specific advantages. miScript HiSpec Buffer is a patent-pending breakthrough chemistry that severely inhibits the activity of the tailing reverse-transcription reaction on templates other than miRNA-sized templates. This provides an exceptionally specific cDNA synthesis reaction, virtually eliminating background from longer RNA species. This advantage is not provided by any other currently available chemistry, and is ideal for profiling experiments, as melt curve and gel analysis become virtually unnecessary.

The limitation to this extraordinary chemistry is that miScript HiSpec Buffer can only be used for mature miRNA quantification. To measure pre-miRNA (with miScript Precursor Assays) or mRNA (with QuantiTect® Primer Assays) from the same reverse-transcription reaction, it is necessary to use miScript HiFlex Buffer (also included in the miScript II RT Kit). Reverse transcription with miScript HiFlex Buffer is an extremely efficient but nonbiased reaction. With cDNAs prepared using miScript HiFlex Buffer, a small percentage of miScript Primer Assays will show increased background signal from cross reactivity with sequences from a total RNA prep. These are easily distinguished by performing a melt curve analysis, as miScript Primer Assays melt in a specific and very narrow temperature range.

We recommend a starting volume of 100–200 µl serum or plasma for RNA purification using the miRNeasy Serum/Plasma Kit. After elution, 1.5 µl of a 14 µl eluate should be used as a template for reverse transcription with the miScript II RT Kit. This provides enough cDNA for either one 384-well plate or four 96-well plates. Up to 10 µl RNA eluate has routinely been used as template without inhibiting the reverse-transcription reaction,

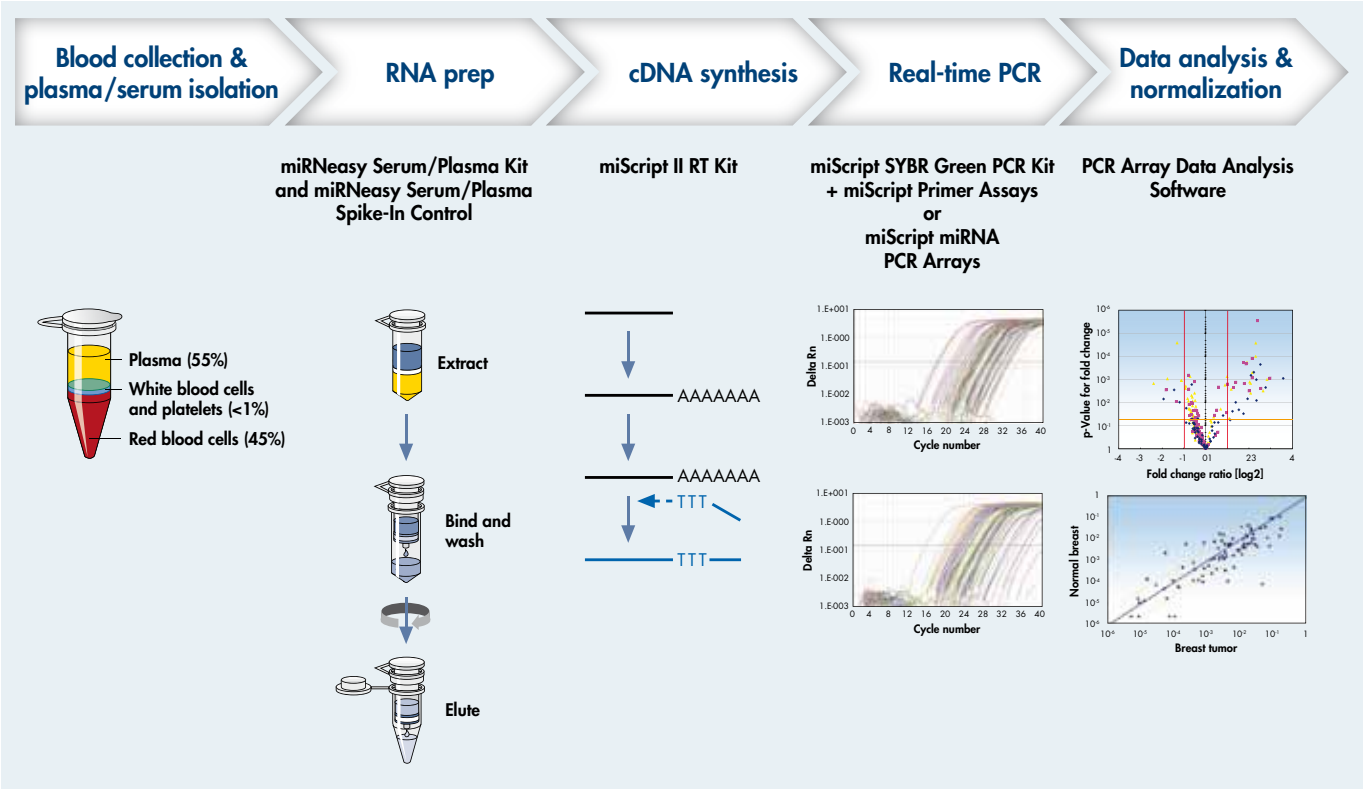


Figure 4. miScript PCR System workflow.

which would provide enough cDNA for more than six 384-well plates or two copies of the Human miRNome miScript miRNA PCR Array.

Real-time PCR of serum and plasma samples

Once completed, a reverse-transcription reaction as described above provides sufficient cDNA for real-time PCR profiling using miScript miRNA PCR Arrays. Various array options are available for profiling miRNA expression in serum or plasma samples (Table 2). Every array includes replicates of a miRNA reverse transcription control assay (miRTC) and a positive PCR control (PPC). These are quality control assays that can be used to determine the presence of reverse transcription and real-time PCR inhibitors.

For quantification of smaller numbers of miRNAs, each reverse-transcription reaction can be diluted to 220 µl with RNase-free water and 1 µl can be used as template for single miRNA assays, regardless of the final PCR reaction volume.

Table 2. miScript miRNA PCR Array solutions for serum and plasma samples

miScript miRNA PCR Array	Species
Complete miRNome	Human, mouse, rat, dog, rhesus macaque
Serum & Plasma	Human, mouse, rat
Serum & Plasma 384 HC	Human

As an overview, profiling the miRNome with the miScript PCR System involves setting up a reverse-transcription reaction, performing reverse transcription for 1 hour, diluting the cDNA, adding master mix, and aliquoting into the miScript miRNA PCR Array. After cycling under standardized conditions on any real-time PCR cycler, resultant data can be exported and uploaded to the QIAGEN Website for analysis (Figure 5). ▶

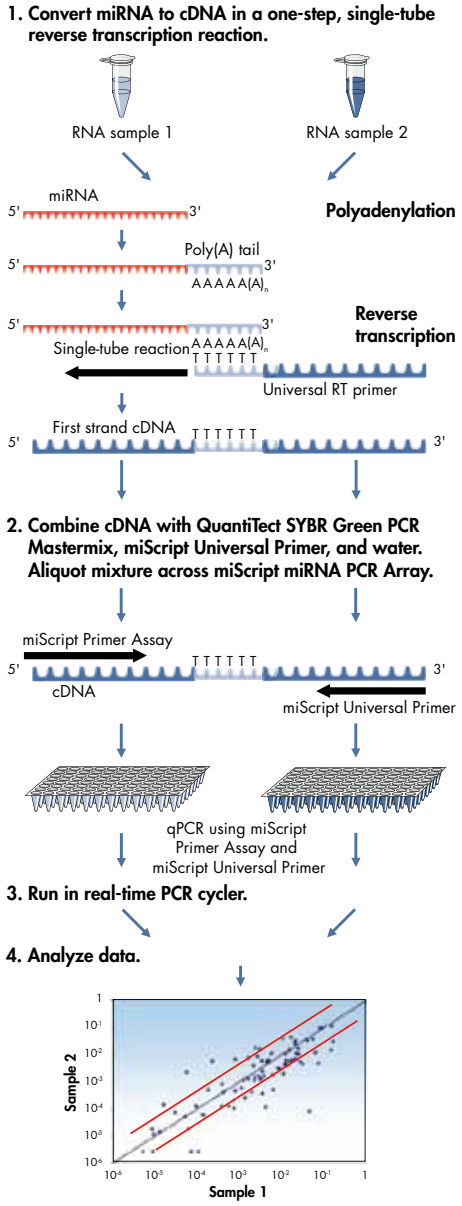


Figure 5. miRNA profiling workflow.

Quality control of serum and plasma miRNA

The amount of RNA recovered from serum or plasma is typically too small to be quantified or qualified by traditional methods such as spectrophotometry readings with a Nanodrop or assessments with a Bioanalyzer. In addition, long RNA will likely be degraded, making it impossible to specifically measure the miRNA contribution. However, several miRNA assays can serve as controls, such as those listed in Table 3. These should be readily detectable in human serum or plasma samples, thereby serving as a check that the sample contains miRNA and performs normally in real-time RT-PCR.

Table 3. miRNAs commonly expressed in serum and plasma samples*

hsa-let-7a	hsa-miR-92a
hsa-let-7c	hsa-miR-93
hsa-miR-21	hsa-miR-103a
hsa-miR-22	hsa-miR-126
hsa-miR-23a	hsa-miR-145
hsa-miR-24	hsa-miR-146a
hsa-miR-25	hsa-miR-191
hsa-miR-26a	hsa-miR-222
hsa-miR-26b	hsa-miR-423-5p

* Visit the GeneGlobe® Web portal (www.qiagen.com/GeneGlobe) to order miScript Primer Assays for detection of these miRNAs.

Exogenous controls for circulating miRNA profiling

One of the challenges of miRNA profiling from serum or plasma is the lack of established housekeeping genes for data normalization when using the $\Delta\Delta C_T$ method of quantification. Normalization strategies will be discussed in greater detail in the next section, but it is worth mentioning here that one frequently used strategy for helping to identify and minimize variations in circulating RNA recovery is to spike a synthetic RNA sequence into the sample. In conjunction with the miRNeasy Serum/Plasma Kit, QIAGEN offers the miRNeasy Serum/Plasma Spike-In Control that can be added into serum and plasma preparations during the purification process after homogenization with QIAzol® Lysis Reagent. This enables normalization for any nonspecific losses incurred during miRNA purification. Even in the absence of specific miRNA signals, the spike-in control should give a positive signal with a reasonably constant C_T value. The miScript Primer Assay for this *C. elegans* miRNA target is included in the miRNeasy Serum/Plasma Kit, and is also included on every miScript miRNA PCR Array. This control does not replace normalization with invariant internal controls, but helps control for variations in recovery and amplification efficiency between RNA preps.

Data normalization for serum and plasma samples

Small, noncoding RNAs, such as snoRNAs and snRNAs, are typically used for data normalization in miRNA profiling experiments in the same way that housekeeping genes are used for normalization of mRNA expression data. Many of these small

RNAs are widely expressed and usually show little variation in different cell types. In reverse-transcription reactions using the miScript II RT Kit, these small RNAs are polyadenlyated and reverse transcribed in the same way as miRNAs. As a result, they serve as controls for variability in sample loading and real-time RT-PCR efficiency. However, these widely used controls are not well expressed in human serum and plasma samples (Table 4). In fact, if the mean C_T of the snRNA/snoRNA miScript PCR Controls is less than 32, this indicates that the samples have >0.1% cellular contamination. For this reason, it is necessary to use alternative methods of normalization for serum and plasma samples. As a first step, if the miScript Serum/Plasma Spike-In Control (a synthetic *C. elegans* miR-39) was added to the homogenized serum or plasma samples prior to RNA purification, C_T values obtained using the *C. elegans* miR-39 miScript Primer Assay can be used to calibrate the data sets being analyzed. This calibration can resolve differences in recovery that may occur during the purification procedure and in amplification efficiency. Using the data presented in Figure 3, the mean C_T values for the *C. elegans* miR-39 miScript Primer Assay were 18.90 and 19.55 for normal serum and breast cancer serum samples, respectively. For calibration, 0.65 (the difference between the mean C_T values) was added to every primer assay in the normal serum sample. Depending on the type of experiment and the number of 'present calls', several methods of normalization are then available that yield acceptable results (see box). Normalization by either plate mean or commonly expressed miRNA targets are ideal methods for profiling experiments using either miRNome or Pathway-Focused miScript miRNA PCR Arrays. Normalization using a panel of invariant miRNAs is ideal for Custom miScript miRNA PCR Arrays or experiments where single miScript Primer Assays are used.

Table 4. miScript PCR Control expression in human serum samples

miScript PCR Control	C_T mean: normal serum	C_T mean: cancer serum
SNORD61	35.47	33.87
SNORD68	34.38	32.28
SNORD72	34.89	34.78
SNORD95	32.92	31.33
SNORD96A	34.20	33.68
RNU6-2	36.08	33.31

Methods for normalization of serum and plasma miRNA expression data

- **Normalization by plate mean:** The plate mean is the mean C_T value of all the miRNA targets on the plate (Figure 7A).
- **Normalization using commonly expressed miRNA targets:** When profiling the miRNome for example, many targets will not be expressed in the samples. In this simple strategy, only the targets that are expressed in all samples are used to calculate the mean value (9). The greater the percentage of negatives on the plate, the more accurate this approach is compared to the plate mean approach (Figure 7B).
- **Normalization using a panel of invariant miRNA:** When comparing 2 sample types, it is common that many miRNAs are relatively invariant (Figure 6 and Figure 7C). These are easy to recognize if the samples are of equal miRNA content and there was no significant difference in real-time RT-PCR efficiency caused by poor technique or contaminants. When sample input amount is less well controlled, a software package such as NormFinder (www.mdl.dk/publicationsnormfinder.htm) can be used to analyze the data set to determine which assays are the least variable and thus, the best candidates for use as normalizers. NormFinder can run as a template within Microsoft® Excel®.

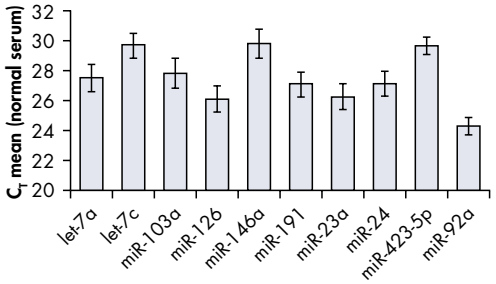


Figure 6. Relatively invariant circulating miRNAs in healthy volunteers. Raw C_T values are shown for 10 miRNAs whose expression levels vary less than 2-fold in plasma between 9 normal serum samples, each from a different donor. Expression profiling was performed using the miScript PCR System and the Human Serum & Plasma miScript miRNA PCR Array. These data suggest that, for a given cohort of samples, suitable miRNAs can be found that can serve as endogenous normalizers.

Once normalization values for the dataset have been obtained, a classic $\Delta\Delta C_T$ calculation and a log2 transformation provide normalized fold-difference values for the miRNA targets. If technical replicates have been included, the variance for each sample/assay combination can be calculated. The miScript PCR System and miScript miRNA PCR Arrays are extremely robust, and array-to-array variability is negligible. For this reason, rather than simple technical replicates of the PCR array, we recommend biological replicates, for example, increasing the number of each sample type to be analyzed.

Examples of data normalization

In the following example (Figure 7), we take the same data set derived from profiling 3 normal serum and 3 breast cancer serum samples using the Human Serum & Plasma miScript miRNA PCR Array and apply each of the normalization methods described above.

In general, if technical variations in sample handling, sample prep, and real-time RT-PCR set up are kept to a minimum, only small differences should be expected from these different data normalization methodologies. Targets that differ by less than 2-fold would be expected to show the most impact from changes in normalization methodology, while targets with large differences in expression level would be least affected. To be valuable as a biomarker, any correlation between circulating miRNA level and disease would need to be robust enough to withstand the normal variations of collection and sample prep and hold true in a statistically significant population.

Biomarker discovery recommendations

Serum and plasma samples provide a unique opportunity for biomarker discovery. As the number of annotated mature miRNAs continues to grow, it becomes increasingly important to optimize miRNA biomarker screening methods, as statistical power requirements can push experimental sample requirements into the hundreds or even thousands. We have successfully used the procedure outlined below to identify key serum or plasma miRNAs associated with disease (see box). With clearly stratified populations (for example, serum from normal donors and serum from donors exclusively with breast cancer), we first screen 3 pools of 10 samples of each clearly defined population using either the Human miRNome miScript miRNA PCR Array or Human Serum & Plasma 384HC miScript miRNA PCR Array. This method averages out individual variances and finds robust differences between the populations. Next, the miRNAs of interest are organized in a Custom miScript miRNA PCR Array and screened with every individual sample in each population.

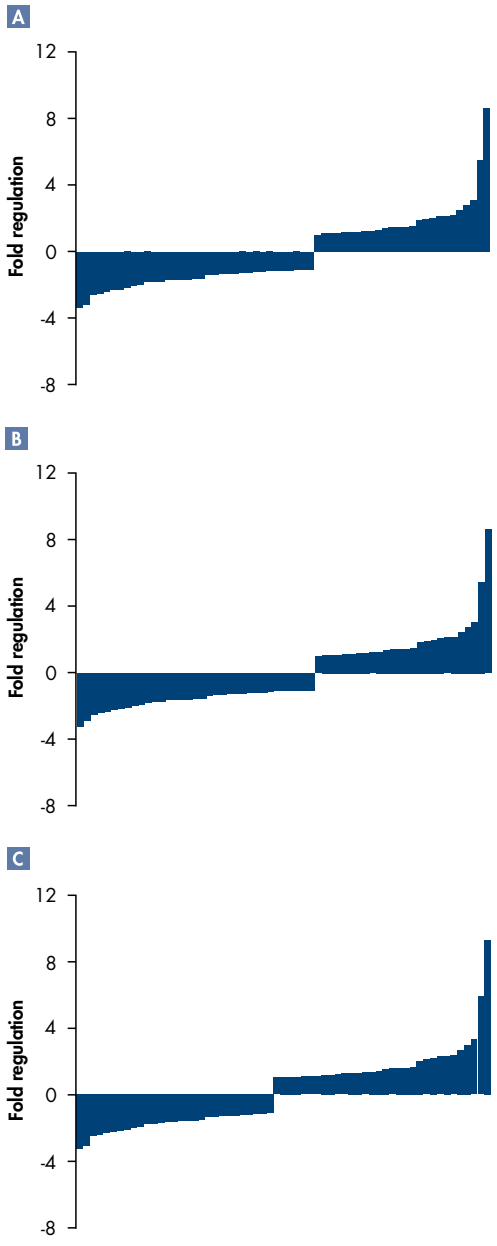
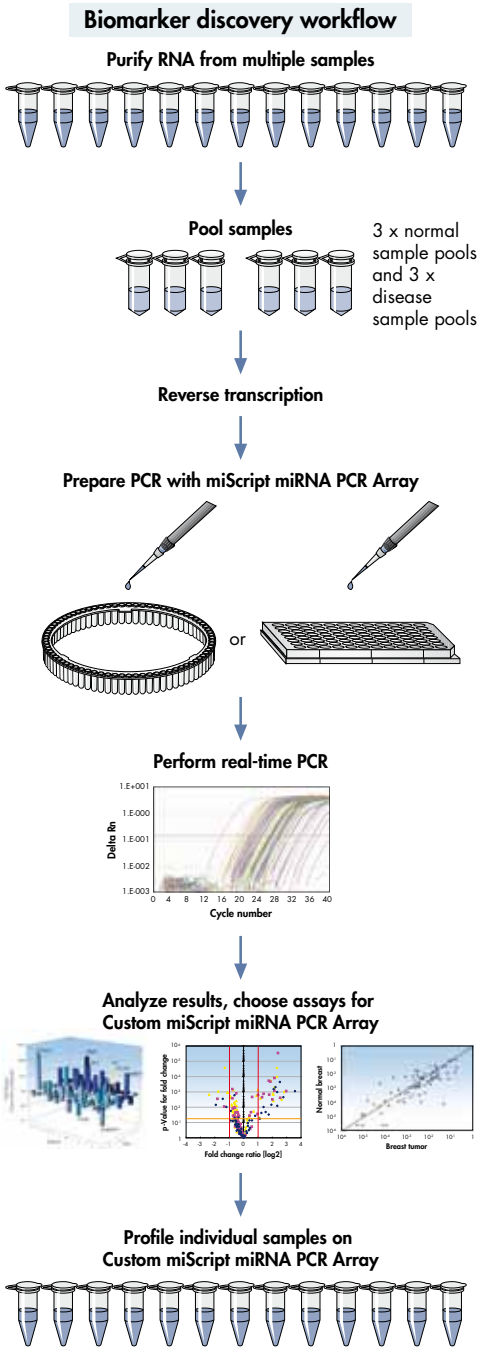


Figure 7. Data normalization strategies. Mature miRNA expression was profiled in 3 normal serum and 3 breast cancer serum samples using the Human Serum & Plasma miScript miRNA PCR Array. Fold-changes for each miRNA (breast cancer versus normal serum) were calculated with C_T values normalized as follows: A C_T values normalized to whole plate C_T mean, B C_T values normalized to the plate C_T mean of commonly expressed miRNAs (those miRNAs with C_T values <35 in both sample sets), C C_T values normalized to the C_T mean of at least 4 miRNAs with little C_T variation. In these experiments, hsa-miR-23a, hsa-miR-24, hsa-miR-92a, and hsa-miR-126 exhibited a C_T variation less than 1 between the samples being compared.

To demonstrate the robustness of this approach: instead of profiling the human miRNome (three 384-well plates) with 100 normal samples and 100 breast cancer samples, which would take over 2 months of constant real-time PCR profiling, the method outlined above can trim the miRNome to just 24 miRNAs of interest, allowing 16 samples to be processed on one 384-well Custom miScript miRNA PCR Array. This condensed panel would, in contrast, take only take 1.5 days to assess, providing substantial time and cost savings.



Sample-to-results workflow for miRNA biomarker discovery

- **Step 1:** Prepare total RNA using the miRNeasy Serum/Plasma Kit.
- **Step 2:** Randomly choose 30 normal samples and 30 disease samples.
- **Step 3:** Combine the RNA to make 3 normal pools and 3 disease pools. Each pool contains RNA from 10 samples.
- **Step 4:** Perform reverse transcription (using the miScript II RT Kit and miScript HiSpec Buffer), followed by real-time PCR quantification (using the miScript SYBR® Green PCR Kit) on each of the pools. Just 5 µl total RNA from the miRNeasy Serum/Plasma Kit provides enough cDNA to profile the entire Human miRNome miScript miRNA PCR Array.
- **Step 5:** Analyze the data and choose the miRNA assays for a Custom miScript miRNA PCR Array.
- **Step 6:** Profile all samples in the cohort using the Custom miScript miRNA PCR Array.

Conclusion

The study of circulating miRNAs in blood has the potential to reveal insights into the function of miRNAs, their transport in the cell, and their role in gene regulation in disease and nondisease states. In addition, the use of circulating miRNAs as biomarkers may be a useful, practical application of this knowledge. The nature of circulating miRNAs results in several challenges in experimental research not faced when examining miRNA from sources of abundant RNA, such as cells and tissues. Special considerations must be taken with purification, quality control, experimental controls, normalization, and experimental design. In this paper, we have outlined experimentally verified recommendations and QIAGEN kit solutions that enable the researcher to get projects up and running as rapidly as possible and ensure reliable, easy-to-interpret data.

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Ordering Information

Product	Contents	Cat. no.
miRNeasy Serum/Plasma Kit (50)	Columns, plasticware, and reagents for 50 preps	217184
miRNeasy Serum/ Plasma Spike-In Control	10 pmol *C. elegans* miR-39 miRNA mimic spike-in control for serum/ plasma samples	219610
miRNeasy Micro Kit (50)	Columns, plasticware, and reagents for 50 preps	217084
miRNeasy Mini Kit (50)	Columns, plasticware, and reagents for 50 preps	217004
miRNeasy 96 Kit (4)	Columns, plasticware, and reagents for 4 x 96 preps	217061
RNeasy Protect Animal Blood Kit (50)	Columns, plasticware, and reagents for 50 preps	73224
PAXgene Blood miRNA Kit (50)	Columns, plasticware, and reagents for 50 preps	763134
miScript II RT Kit (12)	Reagents for 12 x 20 µl cDNA synthesis reactions	218160
miScript II RT Kit (50)	Reagents for 50 x 20 µl cDNA synthesis reactions	218161
miScript SYBR Green PCR Kit (200)	Reagents for 200 x 50 µl PCRs	218073
miScript SYBR Green PCR Kit (1000)	Reagents for 1000 x 50 µl PCRs	218075
miScript PCR Starter Kit (80)	Reagents for 10 x 20 µl cDNA synthesis reactions and 80 x 25 µl PCRs	218193
miScript Primer Assay (100)	miRNA-specific primer for 100 x 50 µl PCRs	Varies*
miRNome miScript miRNA PCR Array	miRNome panels of miRNA assays	331222
miScript miRNA PCR Array	Pathway or disease panels of miRNA assays	331221
Custom miScript miRNA PCR Array	Custom panels of miRNA assays	331231

* Visit GeneGlobe to search for and order these products (www.qiagen.com/GeneGlobe).

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