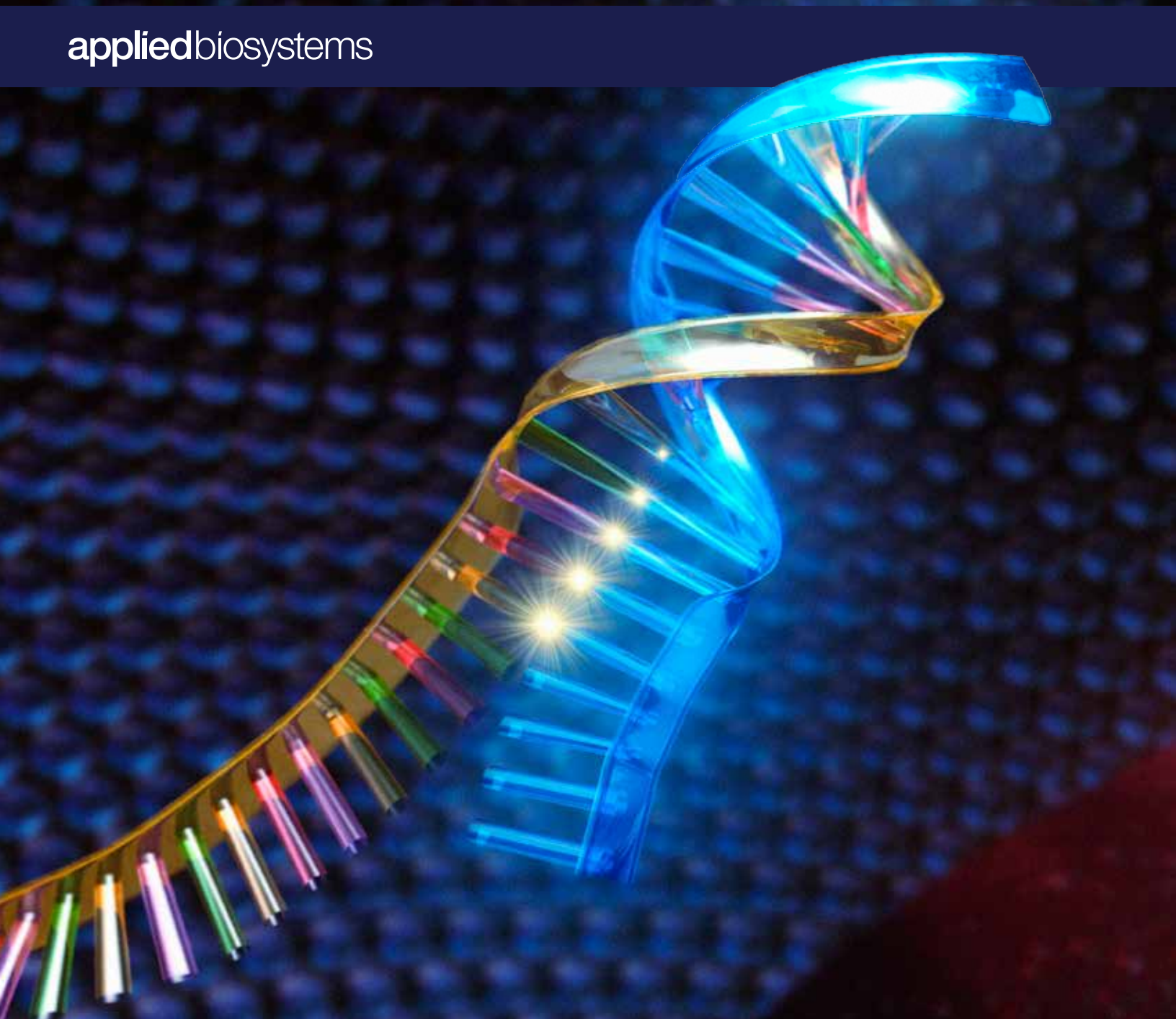
applied biosystems



MicroRNA: having a big impact on biology

A collection of innovative tools for microRNA research

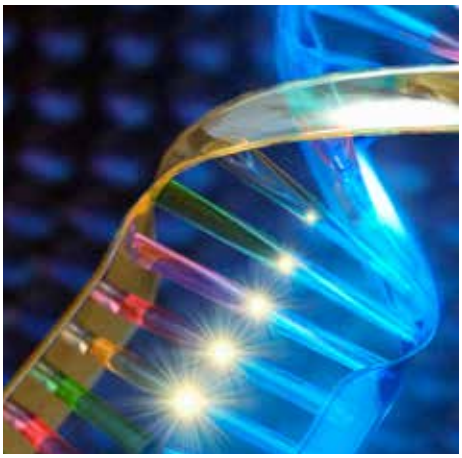
ThermoFisher SCIENTIFIC

MicroRNA: a new frontier in biology

MicroRNAs (miRNAs) are small, noncoding RNA molecules that direct posttranscriptional suppression of gene expression. First referred to as the “biological equivalent of dark matter” [1], miRNAs have been shown to act as key regulators in many basic biological processes such as development, cell proliferation, differentiation, and the cell cycle. Emerging evidence also implicates miRNAs in the pathogenesis of human diseases such as cancers, metabolic diseases, neurological disorders, infectious diseases, and other illnesses [2].

Active, mature miRNAs are typically 17- to 24- nucleotide, single-stranded RNA molecules that are excised from larger precursors. Because of their small size, the study of mature miRNA and other classes of small RNA requires specialized techniques. We provide innovative tools developed specifically for miRNA research, from isolation through discovery, profiling, quantitation, validation, and functional analysis. These tools enable scientists to not only address the fundamental questions about miRNA, but to use these advances to realize the full potential of this new and exciting chapter in biological science.

Contents



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Genome-wide small-RNA discovery

Discover the sequence and expression level of every small RNA in your sample

The discovery of new small RNAs is ongoing; nearly 10,000 new sequences have been added to the miRBase Registry in the last two years [3]. Our RNA sequencing analysis solutions for the Ion PGM™ System include a suite of products that enable rapid discovery of previously unreported small RNAs and the sensitive quantitation of expression levels.

Unlike other detection methods that only assay known or predicted small-RNA sequences, the Ion Total RNA-Seq Kit v2 provides a digital readout of the small-RNA molecules in a particular sample without prior sequence information. This capability enables both the discovery of novel small-RNA molecules, and the detection of different isoforms generated by alternative processing.

Features of the workflow

- Ability to discover novel small-RNA molecules and isoforms
- Sensitivity to detect small RNAs present at less than 1 copy per cell
- Orientation information is preserved for strand-specific expression analysis

How it works

Small RNAs are isolated from the sample and reverse-transcribed to make a cDNA library of the small-RNA population. The cDNA in the library is then amplified and sequenced, generating sequence fragments, commonly called “tags”, corresponding to the cDNA of each small RNA. The tags are then mapped back to reference sequence databases to identify both new and known small-RNA species. Relative expression levels can be calculated based on the number of tags obtained for each small RNA. Follow-up validation can be performed using Applied Biosystems™ TaqMan™ 5′ nuclease chemistry (Figure 1).

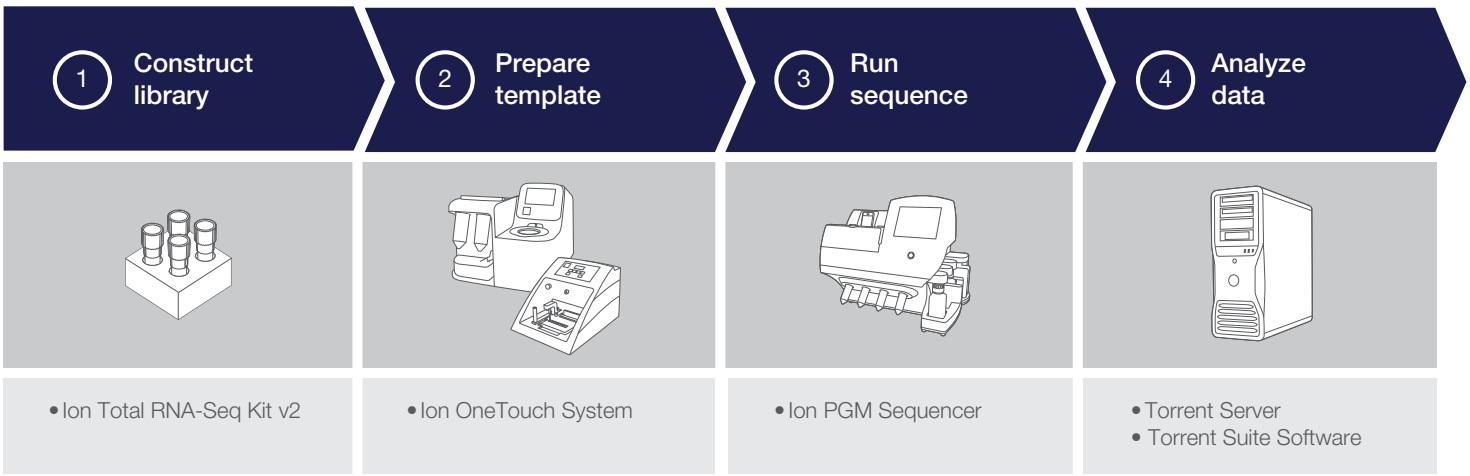


Figure 1. Small-RNA discovery and validation workflow.

Ion semiconductor sequencing: fast, simple workflow solutions

The Ion PGM System offers a complete solution for performing small RNA-Seq studies, from fast library preparation and simple, rapid sequencing workflows to intuitive data analysis tools.

The Ion Total RNA-Seq Kit v2 provides simple workflows to generate small-RNA libraries from human and nonhuman samples, starting with as little as 1 ng of small RNA. Unlike methods that ligate adapters to double-stranded cDNA, the Ion Total RNA-Seq Kit v2 utilizes proprietary technology to attach the adapters in a directional manner that preserves strand information in the resulting libraries (patent pending). In addition, both the 3´ and 5´ adapters are attached simultaneously, reducing ligation and clean-up steps.

The Ion OneTouch™ System enables preparation of templates in about 3 hours with just minutes of hands-on time, and the sequencing run on the Ion PGM™ Sequencer takes less than 1 hour. Sequence reads are processed via Torrent Suite™ Software typically in less than 1 hour, and a simple file is exported to common genomics data analysis software tools.

Detection of all miRNA isoforms with the Ion PGM Sequencer

The Ion PGM Sequencer also has the ability to detect both the -3p and -5p forms (formerly known as leading and star(*) forms) for annotated miRNAs (Table 1). A representative example is illustrated with the analysis of Ion PGM sequence data for mature miRNA forms derived from the stem loop hsa-mir-126. These reads from placenta libraries also show detection of both 5´ and 3´ isomirs (data not shown). Accurate isomir detection is very difficult using microarrays.

Table 1

Platform	hsa-miR-126	hsa-miR-126*
TaqMan chemistry	21.4	26.5
Microarray	15.1	Not detected
Ion PGM Sequencer	**13.2**	**10.9**

Ability of the Ion PGM Sequencer to distinguish -3p vs. -5p of mature miRNA forms. Shown are the C_t values for TaqMan™ MicroRNA Assays, the $\log_2$ values of the hybridization signals for microarrays, and the normalized $\log_2$ values for mapped Ion PGM Sequencer reads.

Hypothesis-free, digital data generation for data better than from microarrays

Ion Torrent™ small RNA-Seq is a hypothesis-free method for discovery, profiling, and quantitation of small RNAs. Using Ion RNA-Seq reagents, miRNAs from human lung and placenta samples were sequenced on the Ion 314™ and Ion 316™ Chips (3 replicates of lung for both chips, 3 replicates of placenta for the Ion 314 Chip, and 2 for the Ion 316 Chip). Sequencing data was compared to the results of expression profiling experiments conducted on Affymetrix™ GeneChip™ miRNA microarrays (3 replicates per tissue type) and Applied Biosystems™ TaqMan™ Array MicroRNA Cards (2 replicates per tissue type). The number of different miRNA molecules detected with the Ion PGM Sequencer exceeds those detected with microarrays (Table 2), demonstrating that the sensitivity for detecting miRNA species with Ion semiconductor sequencing surpasses that of microarrays.

Moreover, the digital nature of Ion RNA-Seq allows for wider dynamic range of LogRatio values compared with that of microarrays (Figure 2), which are limited by the signal intensity thresholds of the detection devices.

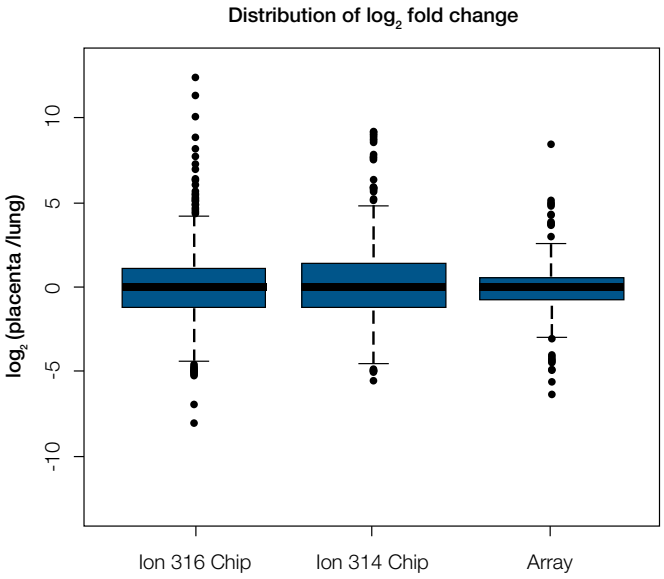


Figure 2. Ion small RNA-Seq dynamic range is greater than that of arrays. Dynamic ranges of LogRatio values obtained with the Ion 316 and Ion 314 Chips, and with GeneChip miRNA arrays, are shown as a box-and-whisker plot.

Table 2

	Total assays	Intersection assays	Intersection detected
TaqMan chemistry	748	708	308
GeneChip Array	847	708	216
Ion 314 Chip	**904**	**708**	**230**
Ion 316 Chip	**904**	**708**	**358**

Superior sensitivity with Ion small RNA-Seq data. The first column shows the total number of miRNAs that can be detected by each platform based on their respective content. The Ion PGM Sequencer detection threshold is not content-dependent, and the number represents the total number of miRNAs included in the up-to-date miRBase. Detection by TaqMan chemistry is calculated at $C_t < 40$. The number of miRNAs that all 3 platforms can theoretically detect is shown in the second column, and the actual number of miRNAs detected by each platform is indicated on the last column.

Genome-wide small-RNA discovery

Product	No. of reactions	Cat. No.
Ion PGM Sequencer	1 sequencer	4462921
Ion OneTouch System	1 system	4470001
Torrent Server	1 server	4462918
Ion Total RNA-Seq Kit v2	12 rxns	4475486
Ion Xpress RNA-Seq BC 01-16 Kit	10 rxns (each barcode)	4475485

MicroRNA expression and profiling

Gold standard TaqMan technology: profile microRNA expression in a day

Profiling the differences in global miRNA expression among samples is a useful first step in identifying specific miRNAs that influence a biological process. For example, a researcher might compare the miRNA profiles in diseased vs. healthy tissue, compound-treated vs. untreated samples, or different organs from a single subject, with the intention of identifying those miRNAs that are expressed at different levels between the sample types.

Since miRNAs can influence cellular function even at very low concentrations and can be expressed over an extremely wide range, miRNA quantitation requires tools with high sensitivity and a broad dynamic range. Applied Biosystems™ TaqMan™ OpenArray™ MicroRNA Panels, and TaqMan Array MicroRNA Cards, in conjunction with Applied Biosystems™ Megaplex™ Primer Pools, are ideal for such experiments.

Using these tools, researchers can generate as many as 48 miRNA expression profiles from human, mouse, or rat samples, in a single working day, starting with as little as 100 ng of input total RNA. Taking full advantage of the gold-standard sensitivity, specificity, and dynamic range afforded by TaqMan Assay chemistry, and incorporating our innovative stem-loop RT primer design for PCR of small targets, TaqMan OpenArray MicroRNA Panels and TaqMan Array MicroRNA Cards provide significant benefits over microarrays, which require several days and hundreds of nanograms of input RNA to generate data (Figure 3).

Features of the workflow

- Results the same day—complete an experiment profiling hundreds of miRNAs in as little as five hours
- Ideal for human, mouse, and rat profiling
- Extensive coverage of known miRNAs consistent with Sanger miRBase

How it works

Using medium-throughput TaqMan Array MicroRNA Cards, up to two sets of 377 miRNAs are reverse-transcribed in a single reaction. Or, using high-throughput TaqMan OpenArray MicroRNA Panels, 754 miRNAs are reverse-transcribed in two reactions using Applied Biosystems™ Megaplex™ RT primers, a mixture of miRNA-specific stem-loop primers, and an Applied Biosystems™ real-time PCR instrument capable of running either format. Next, a preamplification step is performed using Applied Biosystems™ Megaplex™ PreAmp Primers. This unbiased amplification step significantly increases the concentration of miRNAs in the sample for maximum sensitivity and detection using real-time PCR. For the final quantitation step, Applied Biosystems™ TaqMan™ Universal PCR Master Mix II is added to each sample, and the mixtures are easily loaded with the Applied Biosystems™ OpenArray™ AccuFill™ System into Applied Biosystems™ TaqMan™ OpenArray™ MicroRNA Panels—preconfigured nanofluidic plates containing 3 sets of up to 754 matching TaqMan MicroRNA Assays. Alternatively, the mixtures can be pipetted into the sample-loading ports of two TaqMan Array MicroRNA Cards—preconfigured microfluidic cards that contain up to 377 miRNA assays each. The real-time PCR step is run and the data are then analyzed.

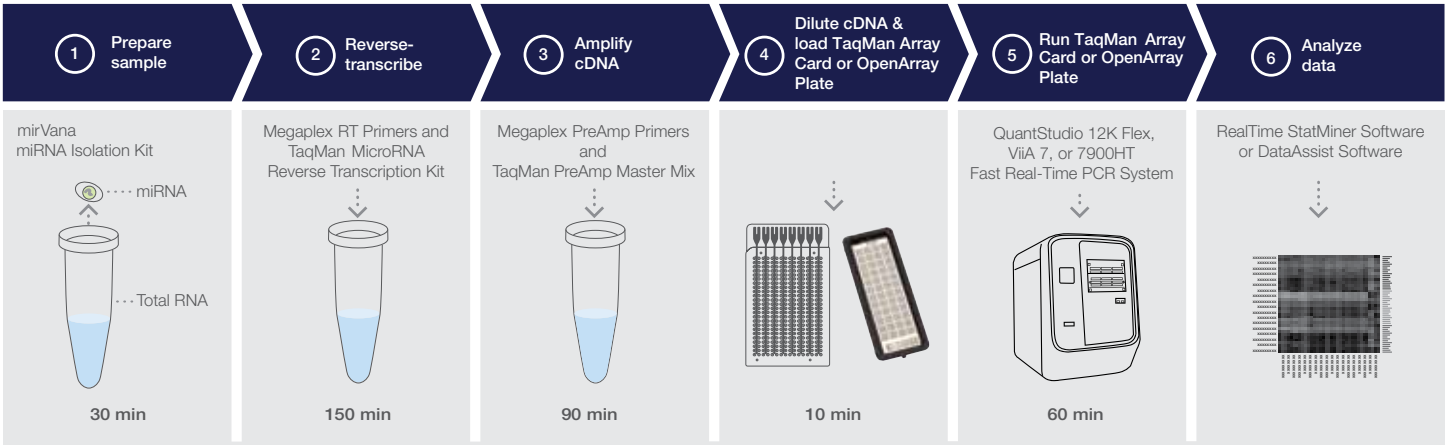


Figure 3. TaqMan OpenArray MicroRNA Panel workflow. Get an extensive human or rodent miRNA profile from as many as 12 samples in only 5.5 hours with the optional Megaplex PreAmp Primers using TaqMan OpenArray MicroRNA Panels; if TaqMan Array MicroRNA Cards are used, get results in as little as 4.25 hours, or 5.75 hours with the Megaplex PreAmp Primers per sample.

Megaplex Primer Pools: cards or panels

Whether your profiling experiment requires ultimate sensitivity, broad coverage, or both, Megaplex Primer Pools offer the flexibility to accomplish your research goals. When used with TaqMan OpenArray MicroRNA Panels or TaqMan Array MicroRNA Cards, Megaplex Primer Pools provide extensive coverage of the Sanger miRBase registry and deliver the ideal miRNA profiling solution for both human and rodent species.

- Megaplex RT Primers—designed to streamline miRNA profiling, Megaplex RT Primers are available in pools ideal for global miRNA expression profiling. They can also be used to prepare cDNA for individual TaqMan MicroRNA Assays.
- Megaplex PreAmp Primers—when using TaqMan Array MicroRNA Cards and samples are limiting or assay sensitivity is of utmost importance, Megaplex PreAmp Primers significantly help increase detection of human and rodent miRNAs with low expression levels or from limited amounts of sample. Megaplex PreAmp Primers enable the generation of an extensive miRNA expression profile, using as little as 1 ng of input total RNA (Figure 4).

TaqMan MicroRNA Arrays and Panels

Ideal for human or rodent profiling, TaqMan MicroRNA Arrays provide all the advantages of TaqMan Assays in a convenient, preconfigured microfluidic card sets or OpenArray panels. Their content is matched to the Megaplex Primers, reducing setup time and experimental variability. For higher throughput, TaqMan OpenArray MicroRNA Panels contain three replicates of the entire profiling set, enabling three samples to be run simultaneously. In both cases, reverse transcription of miRNA targets using Megaplex RT Primers, and preamplification with Megaplex PreAmp Primers, TaqMan Universal PCR Master Mix II is simply combined with each reaction. This greatly helps simplify sample handling and increases sample throughput.

	MicroRNA microarrays	TaqMan™ Array MicroRNA Card and TaqMan™ OpenArray™ panel
Time-to-results	At least two days: one full day labeling samples and setting up the hybridization, and a second day washing and scanning the arrays.	One day and two steps (three if preamplification is included) to go from total RNA to real-time PCR data.
Dynamic range	Typically 2–3 logs (some platforms report as much as 4 logs for some probes).	Up to 7 logs, enabling the detection of both high- and low-expressed miRNAs in a single experiment.
Specificity	Cross-hybridization is a concern given that microarray measurements rely on a single hybridization event. Hybridization to capture probe does not differentiate between miRNA precursors and biologically active mature miRNA.	High specificity is achieved with TaqMan™ chemistry—hybridization of four unique oligonucleotides is necessary for amplification signals. Use of innovative stem-loop RT primer provides specificity for the biologically active mature miRNA.
Input RNA amount	Typically 1 µg or more (some platforms support down to 100 ng).	Anywhere within 1–1,000 ng.
Coverage of known miRNAs	Microarray design typically relies solely on in silico selection and design of capture probes without any wet bench validation. This results in reduced coverage due to nonresponsive probes. In addition, due to the limited sensitivity afforded by a hybridization-based technology, low-expressed miRNAs are unlikely to be detected.	All assays designed through the highly successful and validated *in silico* design pipeline are wet bench–validated prior to inclusion in the Megaplex™ Primer Pools and TaqMan™ Array Cards. Performance of the assay with the Megaplex™ Primer Pools is revalidated at the bench. As a result, all assays included are deemed informative.

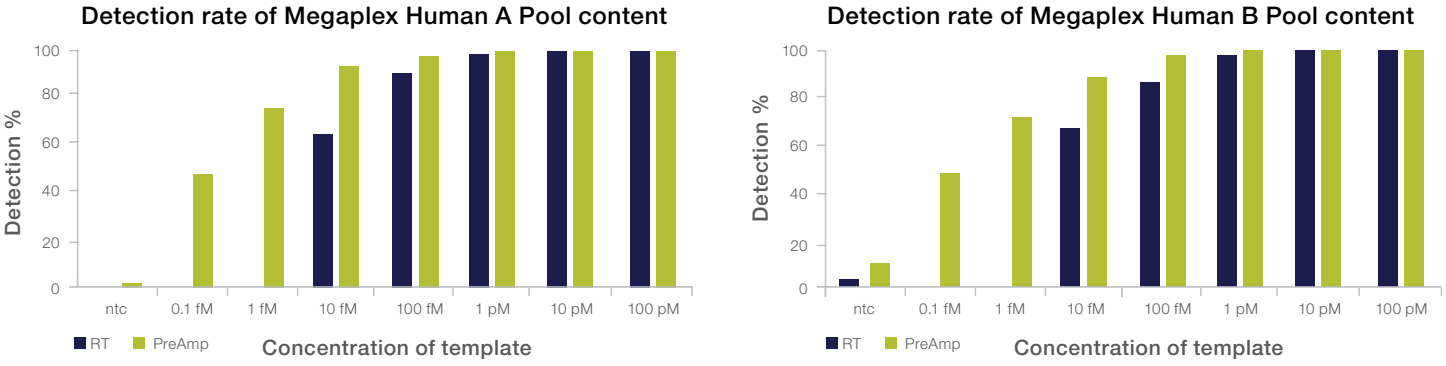


Figure 4. MicroRNA detection using Megaplex™ Primer Pools and TaqMan® Array MicroRNA Cards. Synthetic artificial targets (10 pM) for each assay represented on the A and B TaqMan® MicroRNA Arrays were spiked into a complex total RNA background (10 ng/µL). The mixture was then serially diluted across a range of 6 logs or 4 logs and detection of the artificial targets was tested using the Megaplex™ workflow with and without the preamplification step. In addition, no-template control (NTC) reactions were performed to confirm assay specificity.

MicroRNA expression and profiling

Product	Quantity	Cat. No.
QuantStudio 12K Flex TaqMan OpenArray MicroRNA Panels		
Human Panel	1 panel	4470187
Rodent Panel	1 panel	4470188
TaqMan OpenArray MicroRNA Panels		
Human Panel	1 panel	4461104
Rodent Panel	1 panel	4461105
Associated products for use with microRNA panels		
Megaplex Primer Pools, Human Pools Set v3.0		4444750
Megaplex Primer Pools, Rodent Pools Set v3.0		4444766
TaqMan MicroRNA Reverse Transcription Kit	200 rxns 1,000 rxns	4366596 4366597
TaqMan PreAmp Master Mix	40 rxns	4391128
TaqMan OpenArray Real-Time PCR Master Mix	1.5 mL 5 mL	4462159 4462164
TaqMan Array Human MicroRNA Cards		
A+B Cards Set v3.0	8-pack	4444913
B Cards v3.0	4-pack	4444910
A Cards v2.0	4-pack	4398965

Megaplex Primer Pools, Human Pools					
Human primer pools		**Human RT primers**		**Human PreAmp primers**	
Pool Set A+B v3.0	4444750	Pool Set A+B v3.0	4444745	Pool Set A+B v3.0	4444748
Pool B v3.0	4444749	Pool B v3.0	4444281	Pool B v3.0	4444303
Pool A v2.1	4401009	Pool A v2.1	4399966	Pool A v2.1	4399233

TaqMan Array Rodent MicroRNA Cards		
A+B Cards Set v3.0	8-pack	4444909
B Cards v3.0	4-pack	4444899
A Cards v2.0	4-pack	4398967

Megaplex Primer Pools, Rodent Pools					
Rodent primer pools		**Rodent RT primers**		**Rodent PreAmp primers**	
Pool Set A+B v3.0	4444766	Pool Set A+B v3.0	4444746	Pool Set A+B v3.0	4444747
Pool B v3.0	4444752	Pool B v3.0	4444292	Pool B v3.0	4444308
Pool A v2.0	4401090	Pool A v2.0	4399970	Pool A v2.0	4399203

Targeted microRNA quantitation

Detect and quantify specific microRNAs

Detailed studies on specific miRNAs are often conducted to validate previous results or to learn more about these miRNAs. Through the use of novel adaptations in assay design, we bring the benefits of TaqMan Assays and quantitative real-time PCR—unparalleled sensitivity, specificity, and dynamic range—to miRNA detection and quantitation.

TaqMan MicroRNA Assays incorporate a target-specific stem-loop reverse transcription primer. This innovative design addresses a fundamental problem in miRNA quantitation: the short length of mature miRNAs (~22 nt). The stem-loop structure provides specificity for only the mature miRNA target and forms an RT primer/mature miRNA chimera that extends the 3′ end of the miRNA.

The resulting longer RT product presents a template amenable to standard TaqMan real-time PCR.

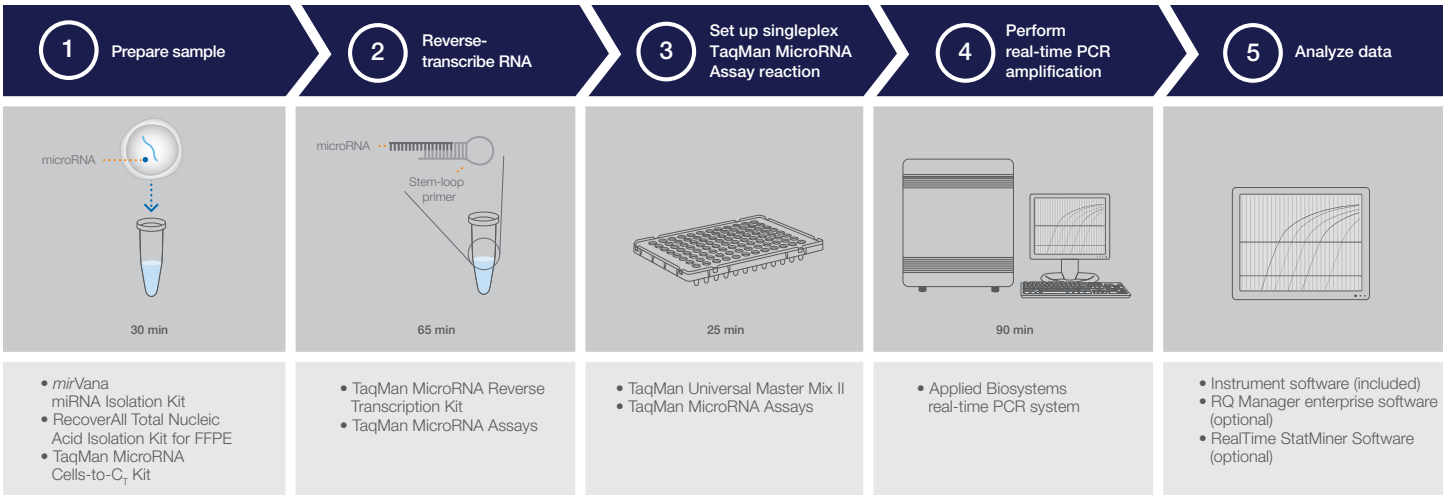
We offer a comprehensive collection of TaqMan MicroRNA Assays covering all the species listed in the Sanger database, including human, mouse, rat, *Arabidopsis*, *C. elegans*, and *Drosophila*. On a regular basis, new assays are promptly added in accordance with any update to the Sanger miRBase registry.

Features of the workflow

- Ideal for quantitating individual miRNAs from any species in the Sanger miRBase, including human, mouse, rat, *Drosophila*, *C. elegans*, and *Arabidopsis*
- Each kit includes the TaqMan Assay and the reverse transcription primer specific for the mature miRNA target of interest
- Assays are continually added to help ensure comprehensive coverage and alignment with the Sanger miRBase Registry

How it works

TaqMan MicroRNA Assays employ an innovative target-specific stem-loop reverse transcription primer to address the challenge of the short length of mature miRNA. The primer extends the 3′ end of the target to produce a template that can be used in standard TaqMan real-time PCR. Also, the stem-loop structure in the tail of the primer confers a key advantage to these assays: specific detection of the mature, biologically active miRNA (Figure 5).



Individual TaqMan MicroRNA Assays

Two-step TaqMan MicroRNA Assays, containing the TaqMan Assay and the reverse transcription primer specific for the target of interest, are designed for any miRNA in the Sanger miRBase database. We continually increase the number of assays for these species to remain aligned with the Sanger miRBase Registry.

TaqMan MicroRNA Assay Endogenous Controls

This selection of candidate endogenous control assays for human, mouse, rat, *Arabidopsis*, *C. elegans*, and *Drosophila* simplifies data normalization. Designed against carefully selected, small, noncoding RNAs that are unrelated to miRNAs, these controls are expressed at consistent levels across a wide variety of cell types, tissues, and experimental conditions. For more information, refer to the application note, “Endogenous controls for real-time quantitation of miRNA using TaqMan MicroRNA Assays”.

Custom TaqMan Small RNA Assays

The novel adaptations in TaqMan Assay design developed for the study of miRNAs using TaqMan MicroRNA Assays are ideal for analysis of any small nucleic acid less than 200 bases long. With Applied Biosystems™ Custom TaqMan™ Small RNA Assays, the benefits of these assays are available for any small RNA, from any species. This includes newly discovered miRNAs that are not yet in the registry and other classes of small RNAs, such as piwi-interacting RNA (piRNA), small nuclear RNA (snRNA), and small nucleolar RNA (snoRNA).

TaqMan technology goes small with big benefits for miRNA research

- Highly specific—quantitate only the biologically active mature miRNAs, not precursors—with single-base discrimination of homologous family members (Figure 6)
- Sensitive—requires only 1–10 ng of total RNA or equivalent to conserve limited samples
- Wide dynamic range—up to 7 logs—detect high and low expressors in a single experiment (Figure 7)
- Custom assays available—you specify the sequence, and we will design an assay
- Fast, simple, and scalable—two-step real-time RT-PCR assay quickly provides high-quality results

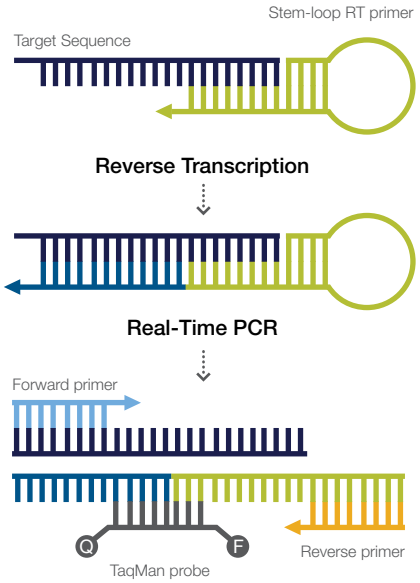


Figure 5. TaqMan MicroRNA Assay approach. A simple two-step process brings the advantages of real-time PCR to miRNA research.

miRNA Assays		Mature miRNAs			Precursors	
		Perfectly matched C_t	Mismatched C_t	ΔC_t (mismatch vs. match)	C_t	ΔC_t (Precursor vs. mature)
let-7a	Looped	16.5	33.1	16.6	29.5	13.0
	Linear	23.6	38.3	14.7	30.4	6.8

Figure 6. The stem-loop primer strategy for reverse transcription in TaqMan MicroRNA Assays confers specificity for biologically active mature microRNA. An off-the-shelf TaqMan MicroRNA Assay for let-7a, containing a stem-loop RT primer, was compared with a comparable-sequence linear RT primer/PCR primer/TaqMan probe set. Next, 1.5 x 10^8 copies of synthetic miRNA mimicking mature let-7a, mature let-7e (a closely related miRNA differing at only two base positions), and the stem-loop let-7a precursor were added to RT reactions primed with either the stem-loop TaqMan MicroRNA Assay RT primer or linear RT primer of comparable sequence. The cDNA was then amplified using real-time PCR. The data indicate that the stem-loop RT primer confers better discrimination between mature versus precursor miRNAs and closely related targets.

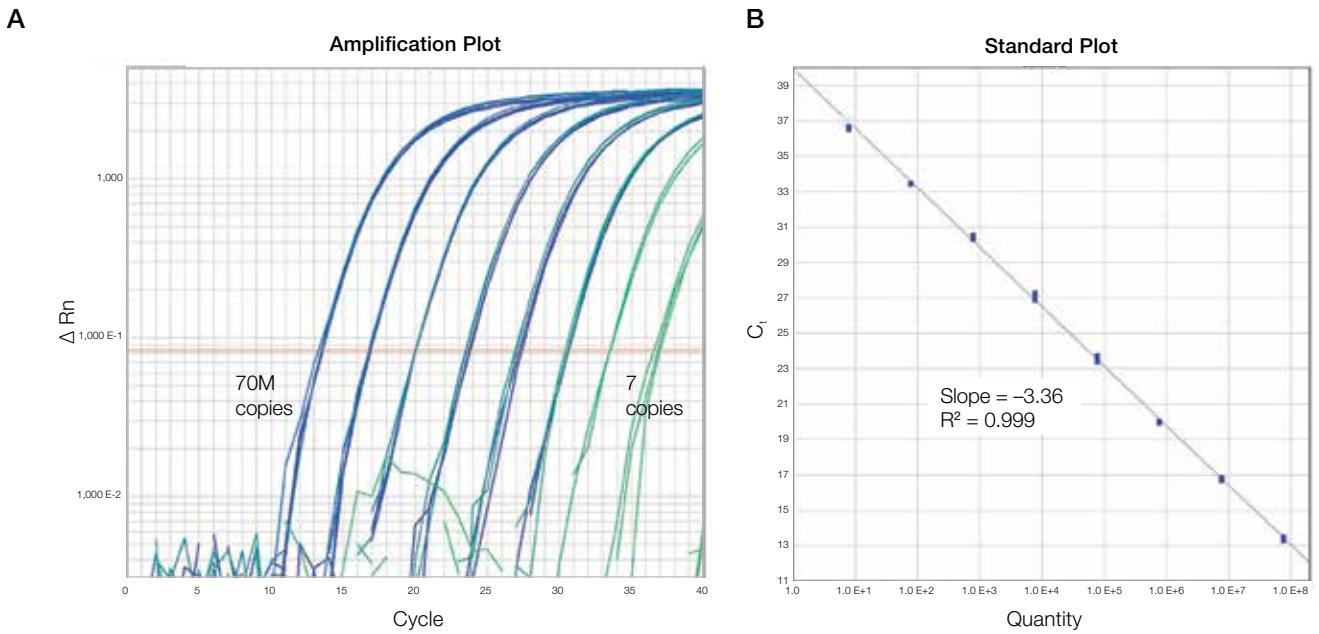


Figure 7. TaqMan MicroRNA Assays provide up to 7 logs of dynamic range. This wide dynamic range enables miRNA targets that vary in abundance from a few copies to millions of copies to be accurately quantitated in the same experiment—an important factor given the wide range of miRNA concentrations within and across different cells, tissue types, and disease states. To illustrate the dynamic range and sensitivity of TaqMan MicroRNA Assays, a synthetic lin-4 miRNA was serially diluted and amplified using the lin-4 TaqMan MicroRNA Assay. **(A)** Amplification plot of synthetic lin-4 miRNA over 7 orders of magnitude. Synthetic RNA input ranged from 1.3 x 10^{-3} fM (equivalent to 7 copies per reaction) to 1.3 x 10^{4} fM (equivalent to 7 x 10^{7} copies per reaction) in PCR. **(B)** Standard curve of synthetic lin-4 miRNA amplification.

Targeted microRNA quantitation

Product	No. of reactions (RT/PCR)	Assay mix formulation (RT/PCR)	Quantity	Cat. No.
Inventoried Predesigned TaqMan MicroRNA Assays				
Small-Scale	50/150	5X/20X		4427975
Made-to-order Predesigned TaqMan MicroRNA Assays				
Extra Small-Scale	25/75	5X/20X		4440885
Small-Scale	50/150	5X/20X		4440886
Medium-Scale	750/750	20X20X		4440887
Large-Scale	2,900/290	60X/60X		4440888
Custom TaqMan MicroRNA Assays				
Extra Small-Scale	25/75	5X/20X		4440418
Small-Scale	50/150	5X/20X		4398987
Medium-Scale	750/750	20X20X		4398988
Large-Scale	2,900/290	60X/60X		4398989
Associated products for use with microRNA panels				
*mir*Vana miRNA Isolation Kit			40 purifications	AM1560
TaqMan MicroRNA Cells-to-C_T Kit			100 rxns	4391848
TaqMan MicroRNA Reverse Transcription Kit			200 rxns	4366596
TaqMan Universal Master Mix II, no UNG			200 rxns	4440040

MicroRNA functional analysis

Analyze microRNA function

Analyses of miRNA function are performed using strategies that are similar to those used for protein-coding genes. Transfecting cultured cells with miRNA mimics can help identify gain-of-function phenotypes; down-regulation or inhibition experiments using miRNA inhibitors can be conducted to identify loss-of-function phenotypes.

The combination of up-regulation and down-regulation can be used to identify genes and cellular processes that are regulated by specific miRNAs.

Our microRNA mimics and inhibitors were the first commercially available tools for influencing miRNA function in cells. Invitrogen™ *mir*Vana™ miRNA Mimics and Invitrogen™ *mir*Vana™ miRNA Inhibitors are designed to mimic or inhibit specific miRNAs for gain-of-function or loss-of-function studies, respectively. Both can be introduced into cells using transfection or electroporation parameters similar to those used for siRNAs (Figure 8).

*mir*Vana miRNA Mimics are chemically modified small double-stranded RNA molecules that are similar to, but not identical to, siRNAs and designed to mimic endogenous mature miRNAs. The chemical modifications ensure that the correct strand, representing the desired mature miRNA, is taken up into the RNA-induced silencing complex (RISC) (Figure 9). Thanks to their small size, they are easier to transfect than vectors, and can be delivered using conditions similar to those used for siRNAs by transfection or electroporation. In contrast to miRNA expression vectors, these synthetic molecules can be used in dose response studies.

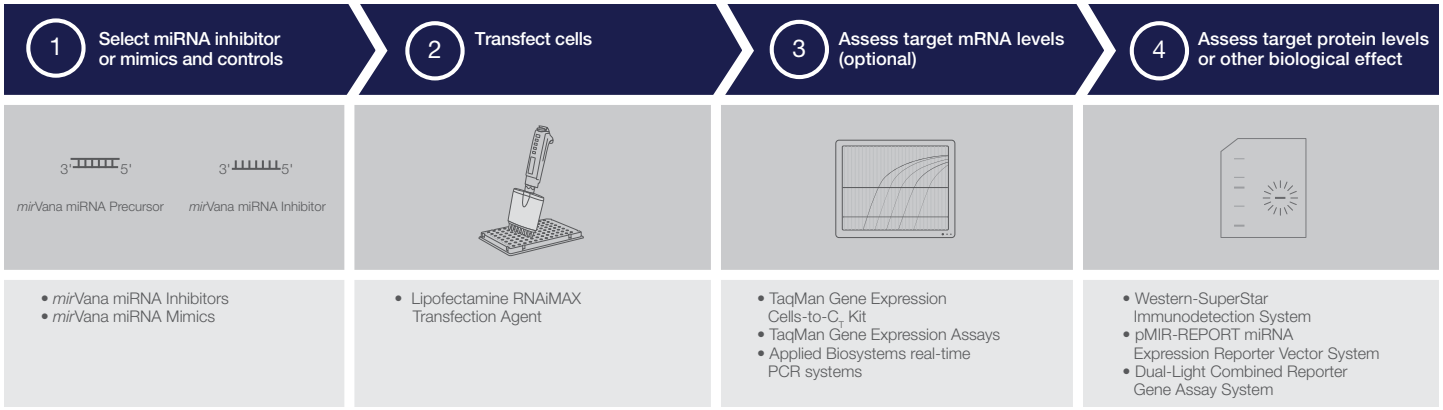
*mir*Vana miRNA Inhibitors are chemically modified, single-stranded nucleic acids designed to specifically bind to and inhibit endogenous miRNAs. When tested using individual reporter constructs containing the appropriate miRNA-binding site, these inhibitors induced, on average, an approximately 4-fold increase in the expression of the reporter relative to cells co-transfected with a negative control *mir*Vana™ miRNA Inhibitor, indicating their strong inhibitory properties.

Features of the workflow

- Gain- and loss-of-function phenotypes identify genes and processes regulated by miRNAs
- Western blotting and/or real-time RT-PCR are used to validate miRNA targets
- Target site interaction and impact on protein expression can be evaluated using reporter gene and immunodetection systems

How it works

- *mir*Vana miRNA Mimics and *mir*Vana miRNA Inhibitors are designed to mimic or inhibit specific miRNAs for artificial up-regulation and down-regulation of target mRNA translation, respectively
- miRNA targets are validated by quantitating target protein and/or messenger RNA levels in reponse to miRNA up-regulation or down-regulation
- Western analysis is used to investigate the impact on protein expression
- Reporter gene assays are used to study the interaction between miRNAs and their target sites



MicroRNA mimics and inhibitors and the means to deliver them to cells

All *mir*Vana miRNA Mimics and Inhibitors:

- Are nontoxic
- Target the most updated miRBase content
- Exhibit maximum and consistent effect *in vitro* at low concentrations
- Can be used *in vitro* and *in vivo*, offering consistency throughout your entire research project

In vivo–ready *mir*Vana miRNA Inhibitors and Mimics

The *in vivo* research for miRNA analysis is rapidly growing. More and more scientists use TaqMan chemistry to obtain accurate quantitative information, which is why *mir*Vana mRNA Inhibitors are fully compatible with TaqMan Assays (Figure 10).

One of the key requirements for *in vivo* use of oligonucleotides such as mimics and inhibitors is lack of toxicity and immunostimulation. During their development, neither mimics nor inhibitors induced any toxic effects.

*mir*Vana miRNA Inhibitor and Mimic libraries and controls

Libraries of *mir*Vana miRNA Inhibitors and Mimics are also available. Each *mir*Vana miRNA Inhibitor or Mimic included in the library is provided in a quantity of 0.25 nmol, dried in individual wells of a 96-well plate. This is a sufficient quantity for 50 transfections when used at 50 nM each. Positive and negative controls are also available for use with *mir*Vana miRNA inhibitors and mimics in miRNA.

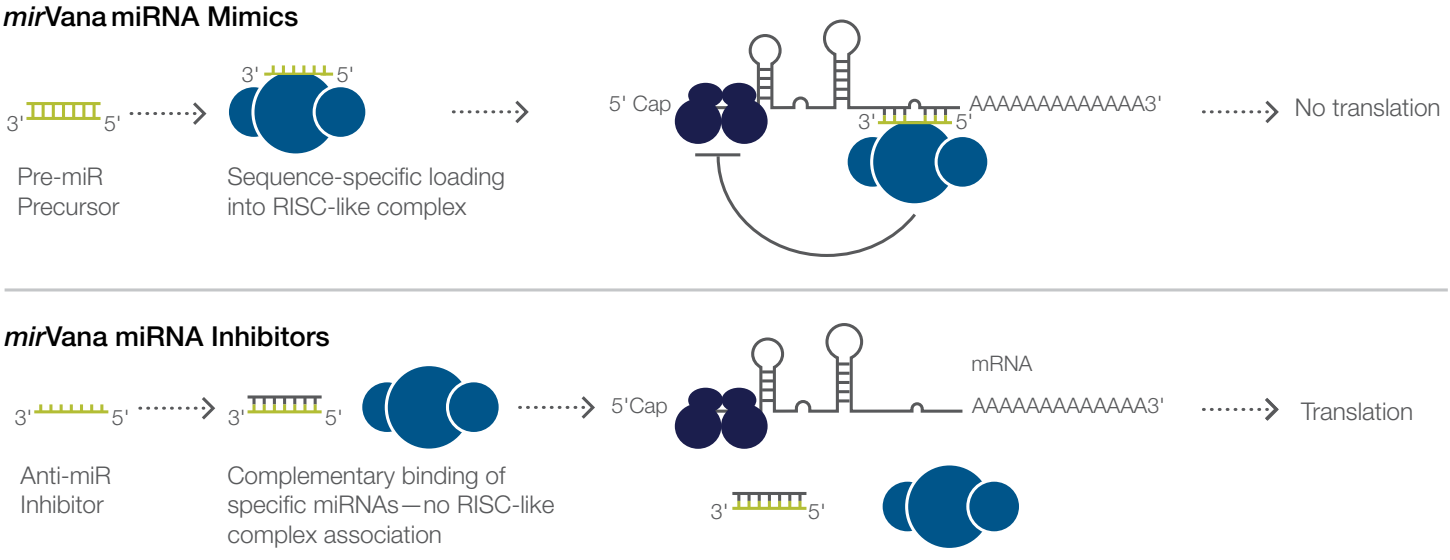


Figure 8. Activity of *mir*Vana miRNA Mimics and *mir*Vana miRNA Inhibitors.

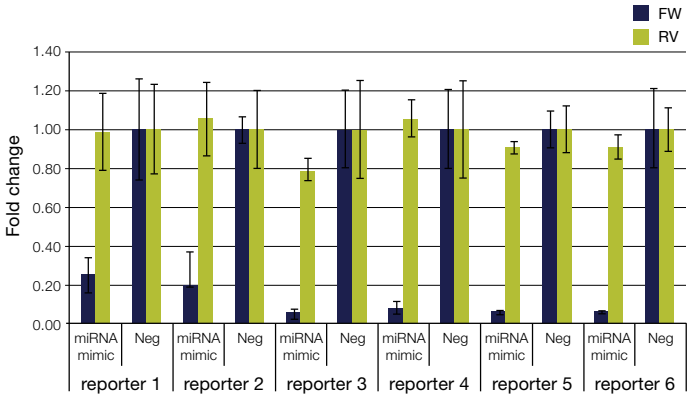


Figure 9. *mir*Vana miRNA Mimic mature strand is highly potent while star strand is inactivated. Reporter constructs were used to measure target and reverse (star) strand activity on their respective targets. For all six sequences, activity of the mature strand is high (signal reduced 5- to 10-fold compared to negative control), and activity of the star strand is low (similar to negative control).

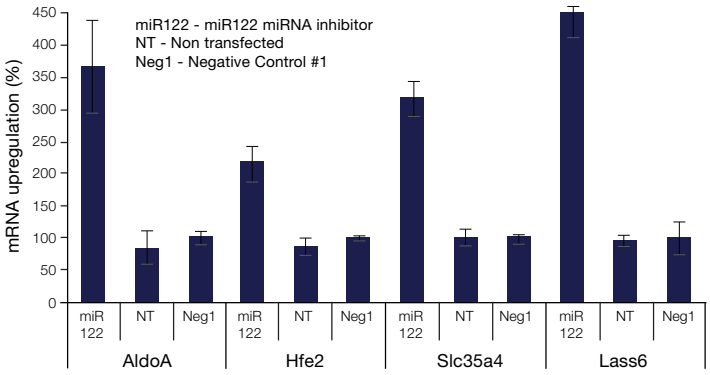


Figure 10. *mir*Vana miRNA Inhibitors effectively suppress miRNA *in vivo*. miR122 or Negative Control #1 *mir*Vana miRNA Inhibitors were complexed with Invitrogen™ Invivofectamine™ 2.0 Reagent and injected in the tail veins of Balb-C mice on 3 consecutive days at 7 mg/kg dose. Twenty-four hours after the last injection, expression levels of the four natural targets of miR122 were measured in the livers of mice using TaqMan MicroRNA Assays. Significant up-regulation of all four mRNA was detected in livers of mice treated with miR122, compared to untreated and negative control–treated mice.

Protein expression analysis

miRNA functional studies may require simultaneous analyses of RNA and protein expression. The Invitrogen™ Western-SuperStar™ Immunodetection System is a highly sensitive chemiluminescent immunodetection system, providing an ideal solution for measuring the effect of miRNA function on the expression of specific proteins.

TaqMan Gene Expression Assays

Applied Biosystems™ TaqMan™ Gene Expression Assays can be used with miRNA gain-of-function and loss-of-function experiments to quantitate effects on target mRNA expression.

We offer more than 1.3 million TaqMan Gene Expression Assays for 23 species, the most comprehensive set of predesigned real-time PCR assays available.

MicroRNA functional analysis

Product	Contents/No. of reactions	Cat. No.
***mir*Vana miRNA Inhibitors**		
Small-Scale	5 nmol	4464084
Large-Scale	250 nmol	4464088
Negative Control, Small-Scale	5 nmol	4464076
Negative Control, Large-Scale	250 nmol	4464079
***mir*Vana miRNA Mimics**		
Small-Scale	5 nmol	4464066
Large-Scale	250 nmol	4464070
Negative Control, Small-Scale	5 nmol	4464058
Negative Control, Large-Scale	250 nmol	4464061
*mir*Vana miRNA Isolation Kit	40 purifications	AM1560
Lipofectamine RNAiMAX Transfection Agent	1.5 mL	13778-150
TaqMan Gene Expression Cells-to-C_T Kit	100 rxns	AM1728
TaqMan Gene Expression Assays	250 20 µL rxns	4331182

MicroRNA sample preparation: kits and selection guide

We offer a variety of kits for the isolation and analysis of miRNA and other small RNAs. Each kit is ideal for use in miRNA analysis because they are optimized to enable:

- Quantitative recovery of small RNA (<200 nt)
- Maintenance of representative amounts of small RNA (eliminating experimental bias)

TaqMan miRNA ABC Purification Kit

The Applied Biosystems™ TaqMan™ miRNA ABC (anti-miRNA Bead Capture) Purification Kit is specially designed for use with our TaqMan human miRNA assays and array cards. Invitrogen™ Dynabeads™ Magnetic Beads, conjugated with anti-miRNA oligos, are used to purify miRNAs from difficult sample types such as whole blood, serum, plasma, and FFPE tissue sections with minimal sample input. Typical input volumes range from 10 µL for whole blood, to 50 µL for serum and plasma samples. Because miRNAs are captured by the magnetic beads, RT-PCR inhibitors commonly found in blood-related samples are easily washed away. The process uses no hazardous chemicals such as phenol, chloroform, Invitrogen™ TRIzol™ reagent, or guanidine isothiocyanate, and the entire procedure is completed in 75 minutes. In addition, unlike other sample prep methods that produce a mixed population of RNAs, the miRNA isolated with the TaqMan miRNA ABC Purification Kit is a pure and specific miRNA population.

The kits are offered in two configurations, Human Panel A and Human Panel B. Each kit is used to purify specific miRNAs corresponding to the miRNA assays contained in the Applied Biosystems™ TaqMan™ Array Human MicroRNA A and B cards (a total of 754 human miRNAs).

*mir*Vana miRNA Isolation Kit

Samples are lysed in a denaturing lysis solution that both stabilizes RNA and inactivates RNases. The lysate is then extracted with acid-phenol:chloroform, yielding a semi-pure RNA sample. The RNA is further purified over a glass-fiber filter to yield either total RNA or a size fraction enriched in miRNAs. The kit reagents are specifically formulated for miRNA retention to avoid the loss of small RNAs that is typically seen with standard glass-fiber filter methods. This quick and easy procedure is compatible with virtually all cell and tissue types, and can be used for efficient isolation of small RNA–containing total RNA, or for enrichment of the small-RNA fraction (<200 nt), to increase sensitivity in downstream analyses.

RecoverAll Total Nucleic Acid Isolation Kit for FFPE tissues

The Invitrogen™ RecoverAll™ Kit procedure requires about 45 minutes of hands-on time and can easily be completed in less than 1.5 hours when isolating RNA. Formalin- or paraformalin-fixed, paraffin-embedded (FFPE) samples are deparaffinized using a series of xylene and ethanol washes and are then subjected to a rigorous protease digestion with an incubation time tailored for recovery of either RNA or DNA. Nucleic acids are purified using a rapid glass-filter method that includes an on-filter nuclease treatment, and are finally eluted into either water or the low-salt buffer provided. This RNA can be readily analyzed by real-time RT-PCR and generates profiles equivalent to those seen in RNA isolated from fresh or flash-frozen samples.

TaqMan MicroRNA Cells-to-C_T Kit

Start with 10–100,000 cultured cells/sample, either in multiwell plates or individual tubes, and be ready for real-time RT-PCR in 10 minutes. Cells are washed in PBS and lysed for 8 minutes at room temperature; DNase treatment can be performed concurrently. Lysis is terminated by adding Invitrogen™ Stop Solution and incubating for two additional minutes at room temperature. Because samples can be processed directly in culture plates (96- or 384-well), sample handling is reduced, and the risk of sample loss or transfer error is minimized. No heating, washing, or centrifugation is required; the Applied Biosystems™ TaqMan™ MicroRNA Cells-to-C_T™ Kit greatly reduces a traditionally time-consuming, labor-intensive process to just 10 minutes. Also included in the kit are Applied Biosystems™ TaqMan™ MicroRNA Reverse Transcription Reagents and TaqMan Universal PCR Master Mix II to complete the gold standard TaqMan miRNA profiling workflow.

Quantitative recovery of small RNAs from a variety of sample types

	TaqMan miRNA ABC Purification Kit	TaqMan MicroRNA Cells-to-C_T Kit	*mir*Vana miRNA Isolation Kit	*mir*Vana PARIS Kit	RecoverAll Total Nucleic Acid Isolation Kit for FFPE Tissues	MagMAX-96 for Microarrays Total RNA Isolation Kit
Technology	Magnetic beads, conjugated with anti-miRNA oligos, are used to capture and purify human miRNAs from small sample inputs	Cells-to-C_T technology, with optional DNase treatment for preparation of cultured cell lysates that can be used in real-time RT-PCR without RNA isolation	Acid-phenol plus rapid, enhanced glass-fiber filter purification	Cell Disruption Buffer combined with acid-phenol:chloroform extraction and glass-fiber filter purification	Deparaffinization, protease digestion and glass-fiber filter purification	Cell lysis using TRI Reagent solution and magnetic bead purification
Sample input amounts	10–50 µL blood-related samples, 10 to 10^6 cultured cells, 1–10 mg tissue, 5–10 µm FFPE sections, or 50 µL saliva	10 to 10^5 cultured cells	10^3–10^7 cultured cells or 0.5–250 mg tissue	100–10^7 cultured cells or up to 100 mg tissue	Up to four 20 µm FFPE sections	Up to 5 x 10^6 cultured cells or up to 100 mg tissue
Features	• No hazardous chemicals such as phenol, chloroform, TRIzol reagent, or guanidine isothiocyanate used • Simple miRNA purification using magnetic beads from complex samples in under 75 minutes • Superior RT-qPCR results with respect to sensitivity, specificity and reproducibility • Specially developed for use with our TaqMan Human MicroRNA Assays and Array Cards	• Go from cells in culture to real-time RT-PCR in 10 min at room temperature • Simple procedure with no sample transfers, no centrifugation, and no vacuum manifold needed • For real-time RT-PCR • Superior results when used with TaqMan MicroRNA Assays Cards and Panels	• Fast, easy isolation of small RNA from cultured cells and most tissues (including tissues with high levels of ribonucleases) • Ideal for miRNA profiling experiments and other gene expression applications	• Simple, 30 minute procedure • Protein can also be recovered • Optional small-RNA enrichment procedure • Ideal for correlating mRNA, miRNA, and/or siRNA with protein levels	• Isolate total nucleic acids, including DNA and microRNAs, from FFPE samples • No overnight Proteinase K digestion required—deparaffinize in the morning and perform real-time RT-PCR in the afternoon • Routinely obtain yields of >50% that of unfixed tissue from the same sample source • For real-time RT-PCR and PCR, mutation screening, and microarray analyses	• Highly consistent results from experiment to experiment • Streamlined RNA purification • Requires less hands-on time than competitor kits • Walk away—integrate with established robotic platforms • Modified protocol to recover small RNAs

References

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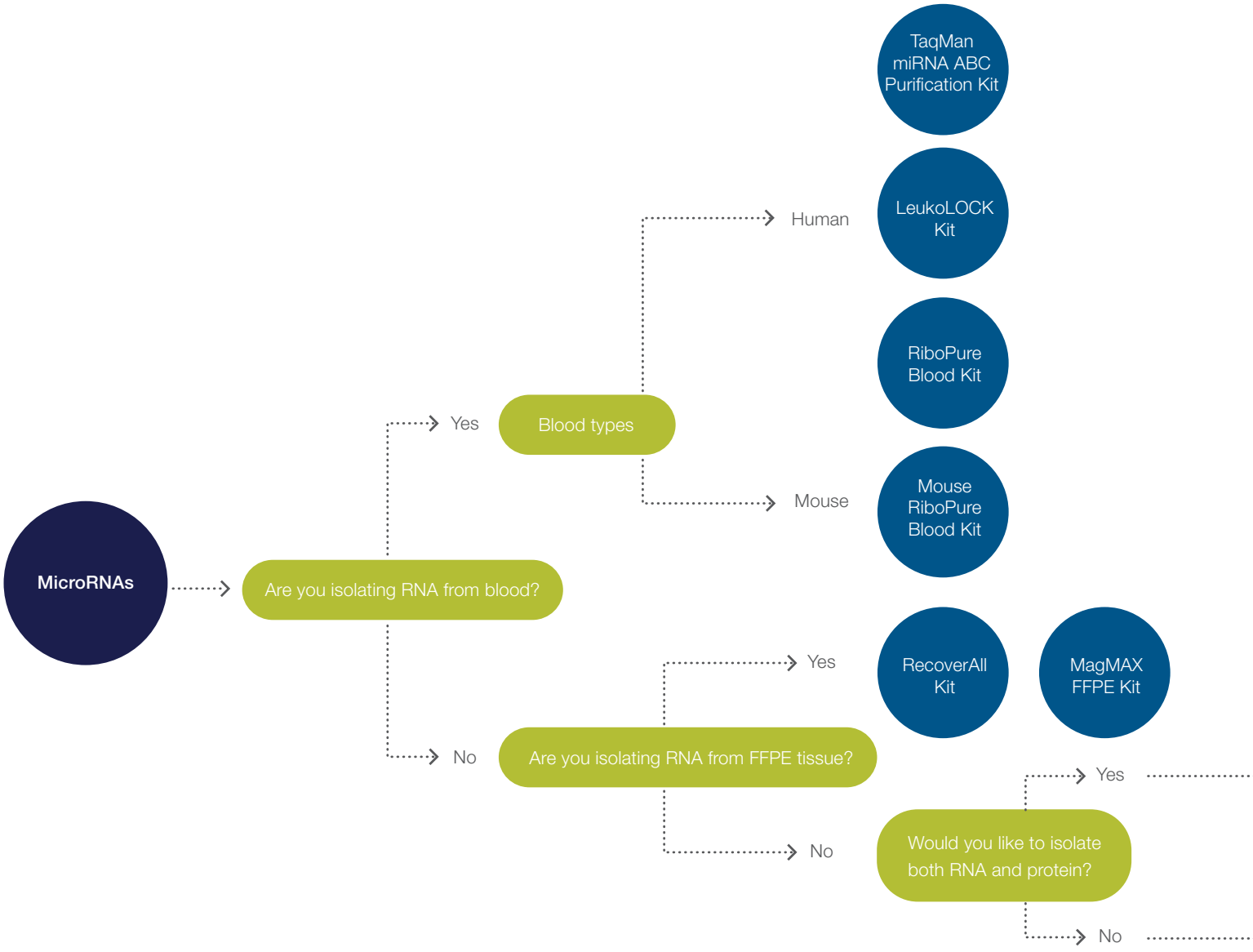
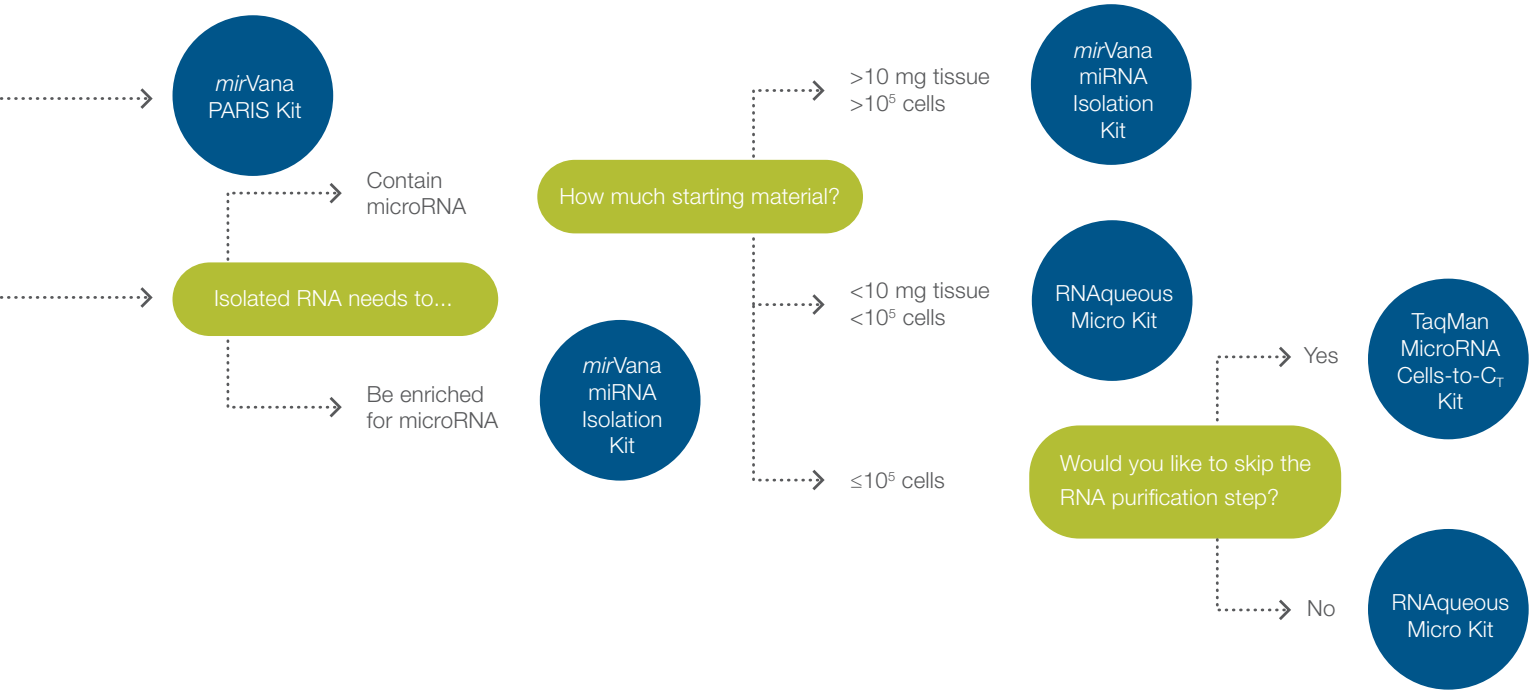
2. Soifer HS, Rossi JJ, Saetrom P (2007) MicroRNAs in disease and potential therapeutic applications. *Mol Ther* 15(12): 2070–2079.
3. Griffiths-Jones S, Saini HK, van Dongen S, Enright AJ (2008) miRBase: tools for microRNA genomics. *Nucleic Acids Res* 36: D154–D158.

Sample preparation selection guide




*mir*Vana PARIS Kit
Contain microRNA
Isolated RNA needs to...
Be enriched for microRNA
*mir*Vana miRNA Isolation Kit
How much starting material?
>10 mg tissue
>10^5 cells
<10 mg tissue
<10^5 cells
≤10^5 cells
*mir*Vana miRNA Isolation Kit
RNAqueous Micro Kit
Would you like to skip the RNA purification step?
Yes
No
TaqMan MicroRNA Cells-to-C_T Kit
RNAqueous Micro Kit

applied
biosystems

Find out more at **thermofisher.com/microrna**

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