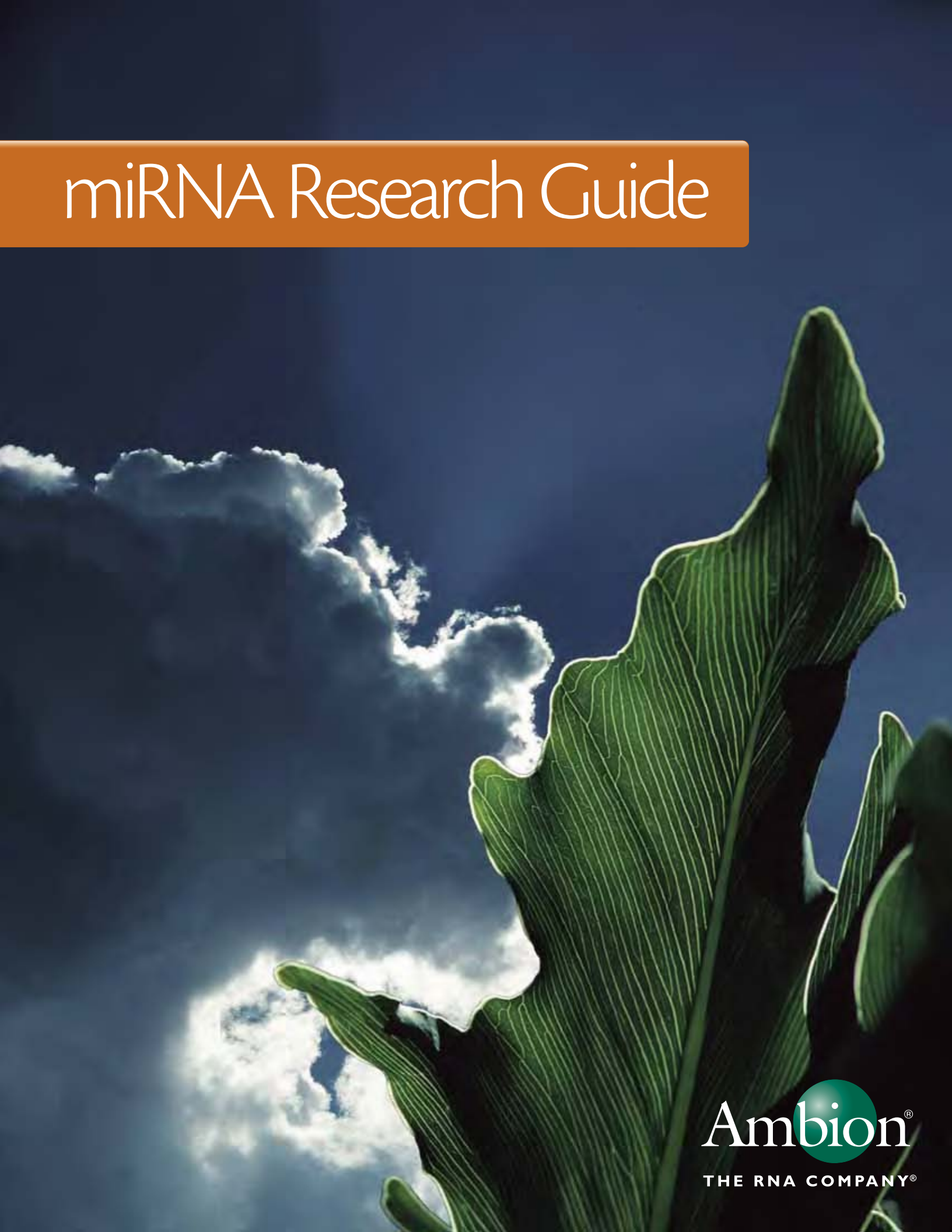
miRNA Research Guide



Ambion®

THE RNA COMPANY®

Introduction to microRNAs

Small regulators with global impact

Description of microRNAs

MicroRNAs (miRNAs) are evolutionarily conserved, small, noncoding RNA molecules that regulate gene expression at the level of translation (Figure 1).

Although the first miRNA was described in 1993, only in the last few years has the breadth and diversity of this gene class been uncovered (Figure 2). Vertebrate genomes are predicted to encode as many as 1000 unique miRNAs [1], which are predicted to regulate expression of at least 30% of genes [2].

miRNAs have been reported to be:

- Critical in the development of organisms [3,4],
- Differentially expressed in tissues [5],
- Involved in viral infection processes [6], and
- Associated with oncogenesis [7,8].

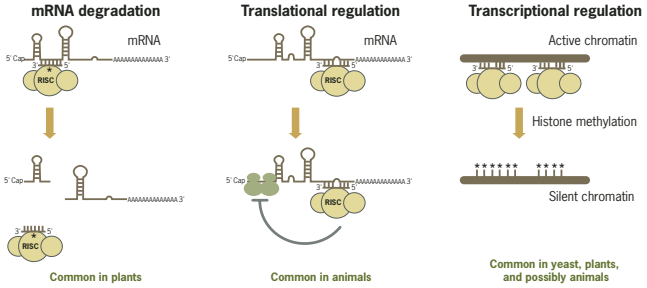


Figure 1. Small RNAs—Key Regulators of Gene Expression.

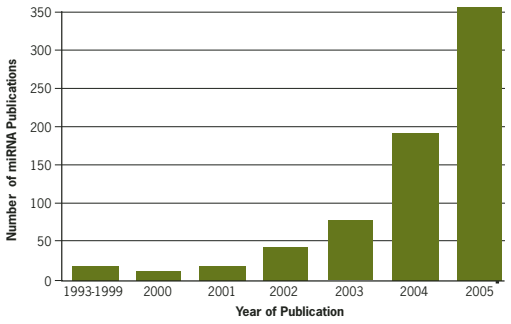


Figure 2. Exponential Growth of miRNA Publications.

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Overview of microRNA processing

miRNAs are transcribed as regions of longer RNA molecules that can be as long as 1000 nt (Figure 3).

The longer RNA molecules are processed in the nucleus into hairpin RNAs of 70–100 nt by the dsRNA-specific ribonuclease Drosha (Figure 4). The hairpin RNAs are transported to the cytoplasm via an exportin-5 dependent mechanism where they are digested by a second dsRNA specific ribonuclease called Dicer. The resulting 19–23 mer miRNA is bound by a complex that is similar to the RNA-Induced Silencing Complex (RISC) that participates in RNA interference (RNAi). In animals, the complex-bound, single-stranded miRNA binds specific mRNAs through sequences that are significantly, though not completely, complementary to the mRNA. By a mechanism that is not fully characterized—but which apparently does not usually involve mRNA degradation as in RNAi—the bound mRNA remains untranslated, resulting in reduced expression of the corresponding gene.

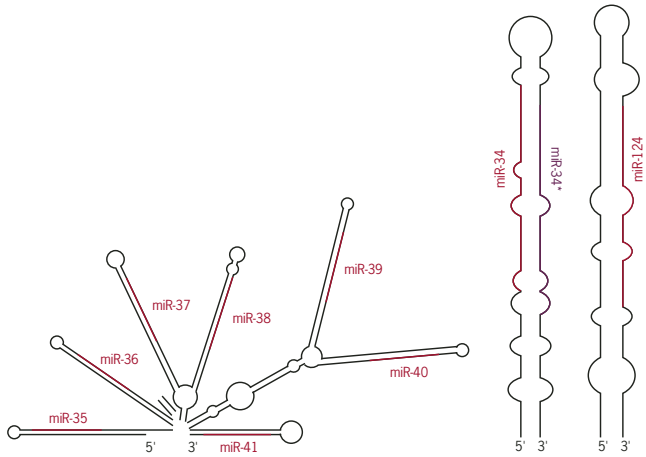

Figure 3. Transcription of miRNAs. Approximately 60% of miRNAs are expressed independently, 15% of miRNAs are expressed in clusters, and 25% are in introns.

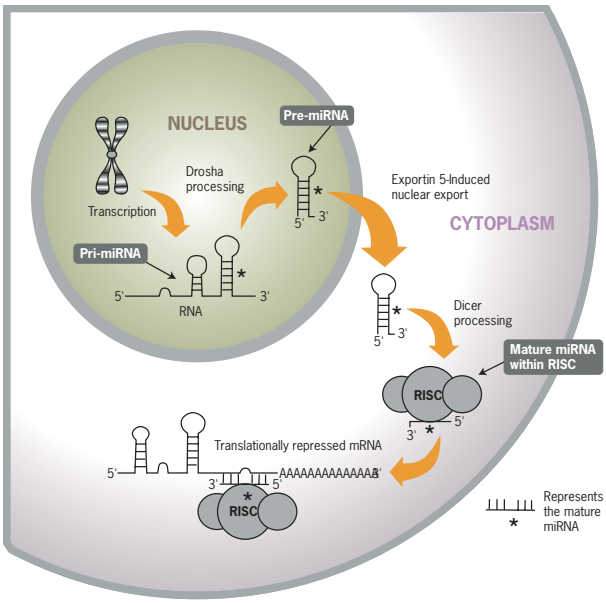

Figure 4. The miRNA Processing Pathway.

miRNA Experimental Overview

As a unique class of small RNA molecules, miRNAs require special tools for accurate and sensitive analysis. Ambion's scientists have developed a portfolio of products that provide a complete solution to accelerate identification and characterization of this gene class. These tools will help you uncover the relationship between mRNA and protein expression, potentially leading to a new segment of targets for diagnostics and therapeutics.

Isolation and Enrichment

Isolation of Small RNAs

The first step in the analysis of miRNAs is purification from a biological sample. Most RNA isolation kits were developed to recover messenger RNA, while ignoring smaller molecules such as miRNAs. The *mir*Vana™ miRNA isolation products are optimized to isolate all RNA species, including small RNA (less than 200 nt). The flashPAGE™ Fractionator can then be used to isolate RNA 10–40 nt in size, including mature miRNA.

PRODUCTS

- *mir*Vana™ miRNA Isolation Kit
- "miRNA Certified" FirstChoice® Total RNA
- *mir*Vana™ PARIS™ Kit
- flashPAGE™ Fractionator
- RecoverAll™ Total Nucleic Acid Isolation Kit for FFPE

Detection & Quantification

Global miRNA Expression*

miRNA Microarray Profiling

The expression levels of miRNAs vary between tissues and developmental stages, and several miRNAs appear to be down-regulated in patients with chronic lymphocytic leukemia, colonic adenocarcinoma, and Burkitt's lymphoma. Evaluation of the global expression patterns of miRNAs provides key opportunities to identify regulation points for many different biological processes.

PRODUCTS

- *mir*Vana™ miRNA Bioarrays
- *mir*Vana™ miRNA Array Probe Set
- *mir*Vana™ miRNA Array Labeling Kit

Specific miRNA Expression

Sensitive Detection of Specific miRNAs

Specialized tools provide sensitive, quantitative detection of miRNAs. These detection products provide highly accurate analysis of specific miRNAs, which is critical for the verification of data from microarray profiling for in-depth analysis of a small number of specific miRNAs.

PRODUCTS

- *mir*Vana™ qRT-PCR Detection Kit
- *mir*Vana™ miRNA Detection Kit
- *mir*Vana™ Probe and Marker Kit
- *mir*Vana™ miRNA Probe Construction Kit

Functional Analysis

Functional Studies

miRNA functional analysis can be performed with protocols that are similar to standard genes. Up-regulation of the miRNAs can be conducted to identify gain-of-function phenotypes; down-regulation or inhibition can be conducted to identify loss-of-function phenotypes. The combination of up- and down-regulation can be used to identify genes that are regulated by specific miRNAs as well as to identify cellular processes that are affected by specific miRNAs.

PRODUCTS

- Pre-miR™ miRNA Precursor Molecules
- Anti-miR™ miRNA Inhibitors
- siPORT™ *NeoFX*™ Transfection Agent
- pMIR-REPORT™ miRNA Expression Reporter Vector

***REFERENCES**

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miRNA Isolation

Ambion has developed RNA isolation kits for the preparation of miRNA and other small RNAs (Table 1). The *mir*Vana™ RNA isolation products differ significantly from traditional RNA isolation products in that they enable:

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

The first critical step in the analysis of small RNA is purification from a biological sample. Most RNA isolation procedures were developed and optimized to recover mRNA while ignoring smaller molecules. Unfortunately, these procedures can result in the loss of substantial amounts of small RNAs such as miRNAs. The standard glass fiber filter (GFF) or silicate adsorption methods employed by most RNA isolation kits are inefficient at recovering small RNAs (Figure 5). In the GFF lanes in Figures 5A and 5B, even though the 5.8S rRNA is efficiently recovered, other small RNA species, such as U1 snRNA, 5S rRNA, tRNA, and various miRNAs, are partially or completely depleted.

*mir*Vana™ miRNA Isolation Kits

The *mir*Vana™ miRNA Isolation Kit (Figure 6) uses a rapid, enhanced GFF-based procedure to recover all RNA, including small RNA species, from cell and tissue samples. The *mir*Vana™ PARIS™ Kit (Figure 7) goes one step further. With this kit, you can isolate protein and small RNA-containing total RNA from the same sample. Ambion's miRNA isolation products ensure efficient, quantitative recovery of small RNA species for successful downstream applications.

"miRNA Certified" FirstChoice® Total RNA

The method of purification is also an important consideration for RNA prepared commercially from biological samples. If the RNA was prepared using standard glass fiber filter procedures, it probably lacks some or all of the small RNA fraction. Ambion's "miRNA Certified" FirstChoice® Total RNA from human, mouse, and rat tissues has been certified to contain small RNAs including miRNAs.

RecoverAll™ Total Nucleic Acid Isolation Kit

Ambion's new RecoverAll™ Total Nucleic Acid Isolation Kit (patent pending) is designed for quantitative recovery of RNA and/or DNA from FFPE samples. The nucleic acid from the FFPE samples includes the full complement of microRNA (miRNA). These miRNAs can be used for miRNA expression profiling when used in combination with the flashPAGE™ Fractionator System and *mir*Vana™ miRNA Array Technology.

Table 1. Selection Guide for Small RNA Isolation and Enrichment Products.

ISOLATION OF MOLECULES		Small RNA species, including miRNA	Total RNA	Protein
Product	**Major Advantages**			
*mir*Vana™ miRNA Isolation Kit	Fast, easy isolation of small RNA from cultured cells and most tissues (including tissues with high levels of ribonucleases)	●	●	
*mir*Vana™ PARIS™ Kit	Fast, easy isolation of small RNA and protein from cultured cells and many tissues	●	●	●
"miRNA Certified" FirstChoice® Total RNA	Ready-to-use, high quality RNA from a wide selection of human, mouse, and rat tissues & from human cell lines	●	●	
RecoverAll™ Total Nucleic Acid Isolation Kit for FFPE	Optimized for isolation of total nucleic acids, including microRNAs, from FFPE tissue	●	●	

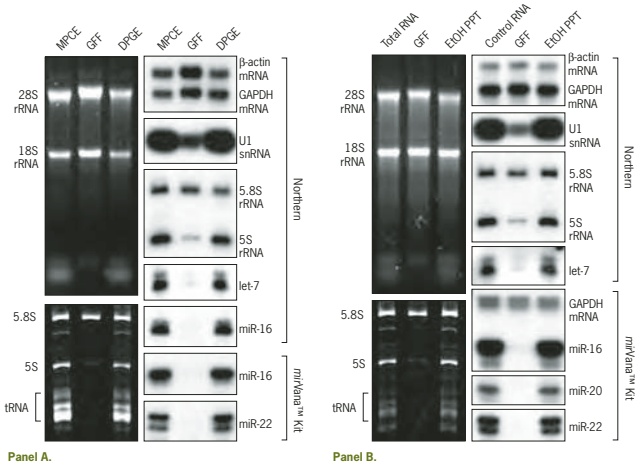


Figure 5. Differential Recovery of Small RNAs. (A) Total RNA was isolated from 1x10^6 HeLa cells using three different techniques: monophasic phenol/chaotropic extraction (MPCE), binding on glass fiber filter in guanidinium solution (GFF) or double phenol/guanidinium extraction (DPGE). Purified RNA (1 µg) was resolved on a 1.2% denaturing agarose gel (top left panel) or 15% denaturing polyacrylamide gel (bottom left panel). The indicated mRNAs or small RNAs were detected by Northern blot or using the *mir*Vana™ miRNA Detection Kit (right panels). **(B)** FirstChoice® Mouse Kidney Total RNA (20 µg) was either bound and eluted from a GFF or precipitated with 0.5 M NH_4OAc and 3 volumes of EtOH (EtOH PPT). The untreated or recovered RNAs (1 µg) were compared by gel analysis, Northern blot, or solution hybridization as described in (A).

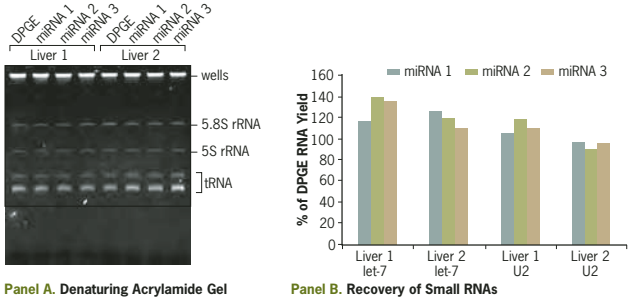


Figure 6. *mir*Vana™ miRNA Isolation Kit for Efficient Recovery of miRNA. (A) Total RNA was isolated from the same mouse liver lysate using a double phenol/guanidinium extraction (DPGE) or the *mir*Vana miRNA Isolation Kit procedure in triplicate (miRNA 1 to 3). The experiment was performed with two different mouse liver lysates. Each sample RNA (1 µg) was analyzed on a denaturing 15% polyacrylamide gel stained with ethidium bromide. **(B)** RNAs from the same gel were transferred to a membrane and probed for U2 snRNA and let-7 miRNA. The relative amount of small RNA in each lane was quantified with a phosphorimager. The graph shows the percentage of recovery with respect to the DPGE prep.

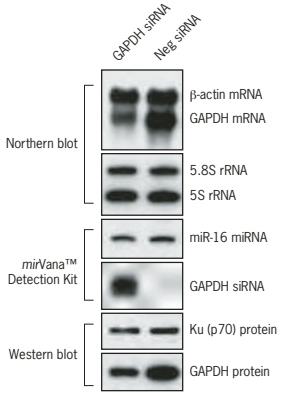


Figure 7. Using the *mir*Vana™ PARIS™ Kit to Analyze RNAi Effects. 1.5x10^6 HUVEC cells were electroporated (800 V, 120 µs, 2 pulses, 0.5 s between pulses) with siRNA (10 µg) targeting GAPDH mRNA (GAPDH) or a negative control sequence (Neg) in 400 µl of siPORT™ siRNA Electroporation Buffer. Total RNA and protein were isolated with the *mir*Vana PARIS Kit 48 hr after electroporation. One µg of total RNA was used to detect the indicated RNA species by Northern blot or with the *mir*Vana miRNA Detection Kit. RNA probes were prepared by in vitro transcription (mRNA probes, MAXIscript® Kit) or by 5' end labeling (rRNA, miRNA, and siRNA probes, *mir*Vana Probe & Marker Kit). Western blots were performed with 15 µg of total protein and antibodies specific for GAPDH or Ku p70 proteins.

miRNA Enrichment

As part of the small population of RNA in cells, miRNAs have evaded detailed analysis partly due to their difficulty to isolate and purify. Mature, functional miRNAs range in size from 19–23 nucleotides, which are the result of processing from longer, primary or "Pri-miRNA" molecules. Accurate microarray profiling as well as advanced study of miRNAs requires polyacrylamide gel electrophoresis (PAGE) separation of mature miRNAs from the longer hairpin pre-miRNAs and primary transcript pri-miRNA molecules.

flashPAGE™ Fractionator System

Fastest and easiest PAGE separation of very small nucleic acids (10–40 nt)

Features:

- Removes >99% of species larger than ~40 nt
- Maintains faithful representation of RNA species
- Handles up to 100 µg total RNA input on each column

Ambion's flashPAGE Fractionator System (Figure 8) is the fastest, easiest, and most reliable method for isolating small nucleic acids (shorter than 40 nt). This system has been designed and optimized for the isolation of mature miRNA (19–23 nt) from longer precursor molecules (Figure 9). The flashPAGE Fractionator System provides an efficient alternative to laborious and time-consuming polyacrylamide gel electrophoresis (PAGE) and subsequent gel elution, which have traditionally been required for isolation of small nucleic acids. With the flashPAGE Fractionator System, you will easily and efficiently enrich mature miRNA populations while excluding longer precursor miRNA molecules—in run times averaging only 12 minutes.

Figure 8. The flashPAGE™ Fractionator Apparatus.

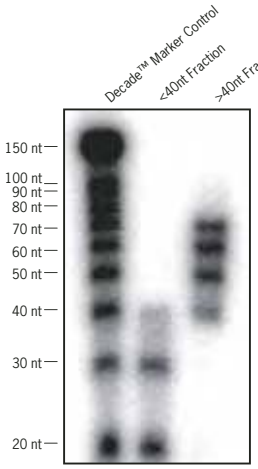


Figure 9. flashPAGE™ Fractionation of Decade Marker. Ambion's Decade Marker, in a background of 10 µg mouse brain total RNA, was loaded onto a pre-cast flashPAGE Gel Cartridge and electrophoresed with the flashPAGE Fractionator. Two successive fractions were collected. For the first fraction, the lower buffer was collected and precipitated when the second dye indicator band had reached the lower end of the gel cartridge (middle lane). After adding fresh lower buffer to the apparatus, the sample was electrophoresed for 10 more minutes. The second lower buffer fraction was then collected and precipitated (right lane).

ORDERING INFORMATION

Product	CAT #	SIZE
*mir*Vana™ miRNA Isolation Kit	1560	40 purifications
*mir*Vana™ PARIS Kit	1556	40 purifications
RecoverAll™ Total Nucleic Acid Isolation Kit for FFPE	1975	40 purifications

"miRNA Certified" FirstChoice® Total RNA

Human Total RNA-Normal Tissue	CAT #	SIZE
Adrenal	7994	100 µg
Aorta	6844	100 µg
Left Atrium	6854	100 µg
Right Atrium	6858	100 µg
Bladder	7990	100 µg
Brain	7962	100 µg
Breast	7952	100 µg
Cervix	7992	100 µg
Colon	7986	100 µg
Distal Colon	6836	100 µg
Proximal Colon	6834	100 µg
Duodenum	6832	100 µg
Esophagus	6842	100 µg
Fallopian Tube	6862	50 µg
Heart	7966	100 µg
Ileum	6828	100 µg
Jujenum	6830	100 µg
Kidney	7976	100 µg
Liver	7960	100 µg
Lung	7968	100 µg
Lymph Node	7894	50 µg
Ovary	7974	100 µg
Pancreas	7954	100 µg
Pericardium	6852	100 µg
Placenta	7950	100 µg
Prostate	7988	100 µg
Skeletal Muscle	7982	100 µg
Small Intestine	7984	100 µg
Spleen	7970	100 µg
Stomach	7996	100 µg
Testes	7972	100 µg
Thymus	7964	100 µg
Thyroid	6872	50 µg
Trachea	6846	100 µg
Uterus	7892	100 µg
Vena Cava	6848	50 µg
Left Ventricle	6856	100 µg
Right Ventricle	6860	100 µg

Mouse Total RNA-Normal Tissue	CAT #	SIZE
Liver	7810	200 µg
Brain	7812	200 µg
Thymus	7814	200 µg
Heart	7816	200 µg
Lung	7818	200 µg
Spleen	7820	200 µg
Testicle	7822	200 µg
Ovary	7824	200 µg
Kidney	7826	200 µg
Embryo (10–12 days)	7828	200 µg
Assorted	7800	10x25 µg

Rat Total RNA-Normal Tissue	CAT #	SIZE
Liver	7910	200 µg
Brain	7912	200 µg
Thymus	7914	200 µg
Heart	7916	200 µg
Lung	7918	200 µg
Spleen	7920	200 µg
Testicle	7922	200 µg
Ovary	7924	200 µg
Kidney	7926	200 µg
Embryo (10–12 days)	7928	200 µg
Assorted	7900	10x25 µg

NEW!

Product	CAT #	SIZE
flashPAGE™ Fractionator	13100	1 apparatus
flashPAGE™ Pre-cast Gels (Type A)	10010	10 gels
flashPAGE™ Buffer Kit (Type A)	9015	1 kit
flashPAGE™ Reaction Clean-up Kit	12200	20 reactions

miRNA Expression Profiling

Ultrasensitive miRNA microarray profiling of all known human, mouse, and rat miRNAs

Ambion's *mir*Vana™ miRNA array platform provides:

- Ultrasensitive miRNA expression profiling between samples
- Comprehensive expression analysis corresponding to all known human, mouse, and rat miRNAs
- Optimized, extensively validated probes and reagents

The *mir*Vana miRNA array platform has been extensively validated with human, rodent, and synthetic miRNA samples, yielding greater than 98% correlation between similar samples. The *mir*Vana miRNA products provide highly sensitive, accurate, and reproducible miRNA array data—enabling evaluation of miRNA expression under any condition, including during development, differentiation, viral infection, and oncogenesis (Figures 10 to 13).

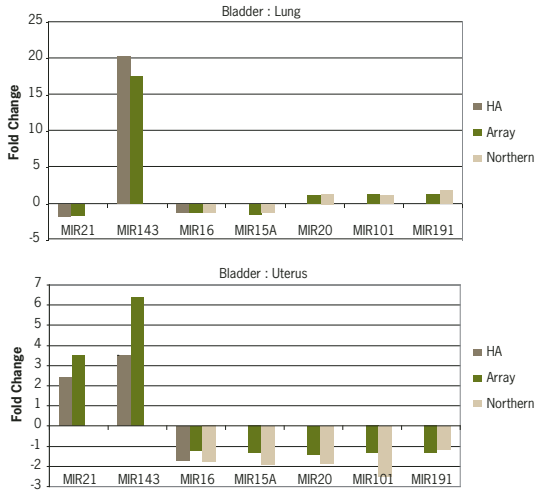


Figure 10. The *mir*Vana™ miRNA Array Procedure Yields Quantitative Data Consistent With Other miRNA Analysis Methods. The miRNA in human bladder, lung, and uterus was analyzed by Northern, *mir*Vana array, and hybridization assay (HA). Total RNA was used for both the Northern and hybridization assay analysis. For array analysis, the miRNAs were purified, labeled, and analyzed using the *mir*Vana miRNA array procedure. Northern signal from probes for five different miRNAs was quantified by phosphorimaging. The hybridization assay, which features chemiluminescent detection, was used to measure the expression of three different miRNAs in the three total RNA samples. The miRNA array compared the expression of 167 different miRNAs using an Axon scanner. The expression of each of the seven miRNAs that were evaluated by at least two methods was compared between each of the three samples. As seen in the bar graphs, all three methods of miRNA analysis showed very similar expression profiles for the various miRNAs tested.

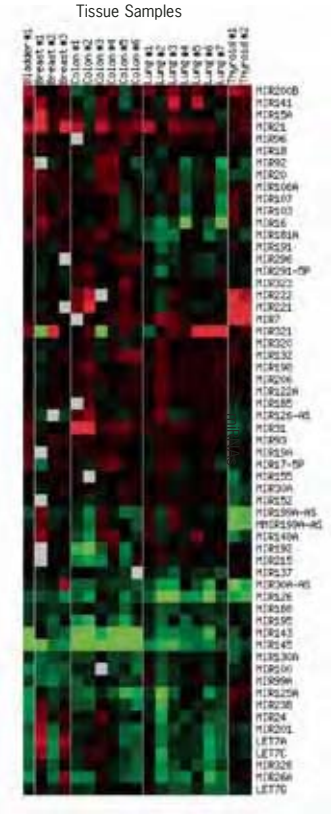


Figure 12. miRNA Expression Profiles of Tumor vs. Normal Adjacent Tissue from Cancer Patients. The miRNA expression profiles (y axis) in tumor vs normal adjacent human tissues (x axis) were compared for 19 cancer patients. Green in this heat map shows miRNAs that are down-regulated in the tumor sample relative to the normal adjacent tissue sample, and red shows miRNAs that are up-regulated in the tumor sample relative to the normal adjacent tissue sample.

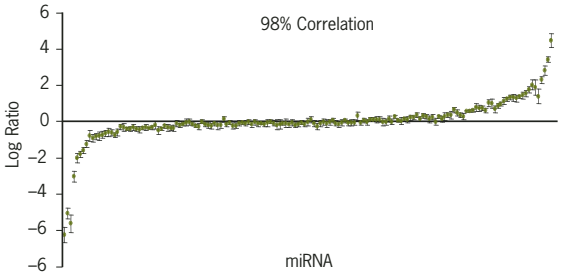


Figure 13. The miRNA Array Procedure is Highly Reproducible. The reproducibility of the miRNA array procedure was tested by repeatedly comparing the miRNA profiles of human prostate and colon samples. Signal ratios (colon:prostate) at each element are expressed as a log ratio. Standard deviation of Log Ratio between the 6 replicates was determined for each miRNA. Average correlation was 98%. Distribution of Log Ratio for the 6 replicates was centered and normal-like (data not shown).

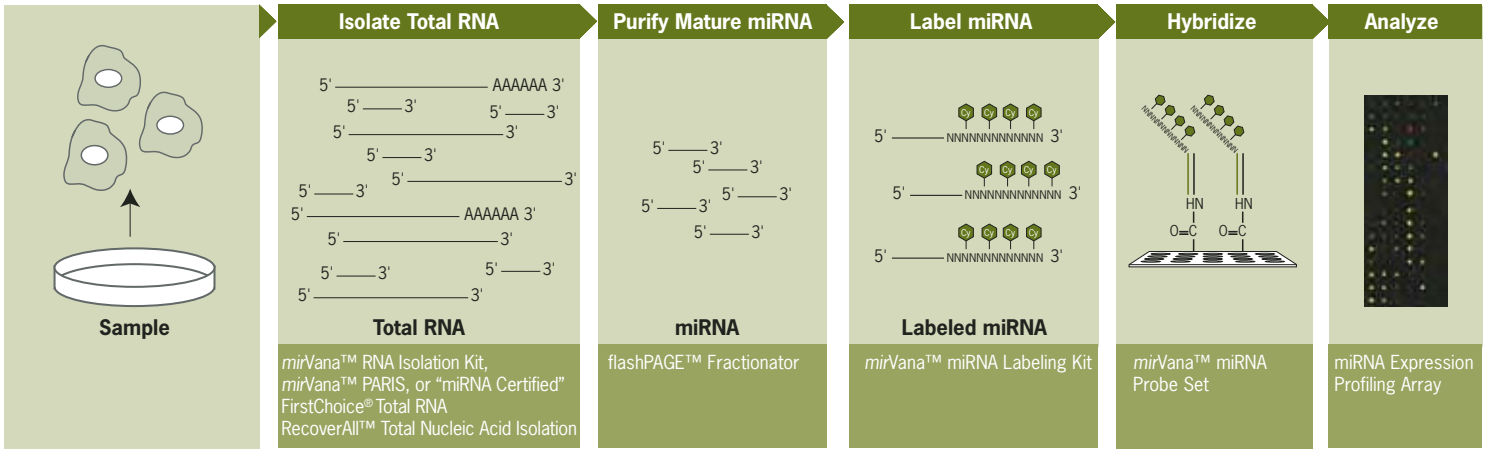
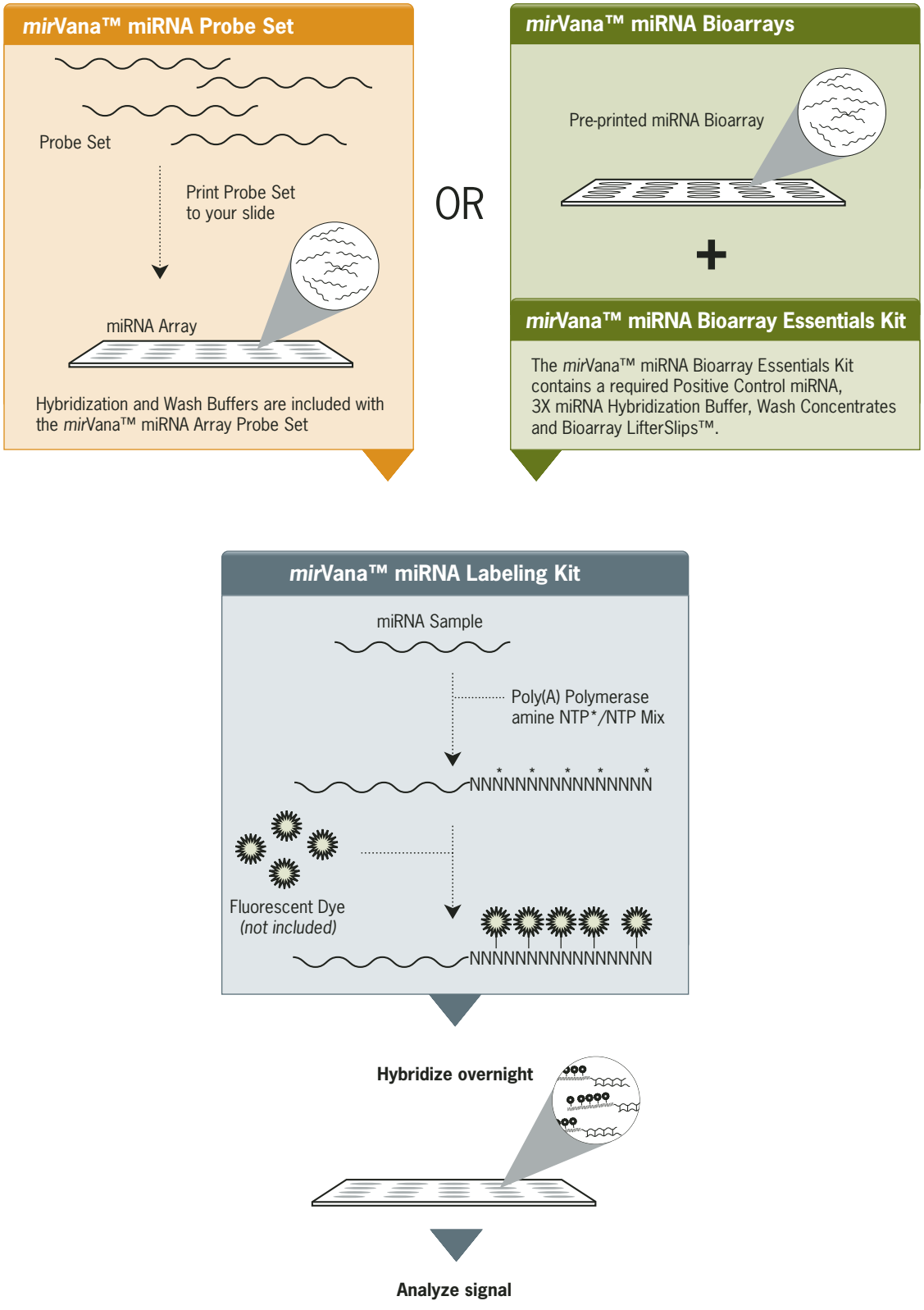


Figure 11. *mir*Vana™ miRNA Expression Profiling.

Overview of the *mir*Vana™ Array System



*mir*Vana™ miRNA Labeling Kit

Efficient and robust system for universal labeling of miRNA

The *mir*Vana miRNA Labeling Kit (Figure 14) is designed to prepare miRNA samples for microarray analysis using a simple and highly efficient labeling strategy. When used with Ambion's *mir*Vana miRNA Isolation Kit and *mir*Vana miRNA Probe Set, the system:

- Provides subfemtomole sensitivity, allowing detection of most miRNAs with as little as 10 µg of input total RNA
- Enables detection of less than 2 fold differential expression
- Provides reproducible results (CV <20%, both between experiments and within experiments, for one color detection; CV <10% for two color detection)
- Provides greater than 2.5 logs dynamic range
- Fully compatible with Amersham, Axon, and Agilent scanners

The *mir*Vana miRNA Labeling Kit is ideal for analyzing and comparing miRNA expression levels across tissues and among different disease states. The kit is fully compatible with any amine reactive dye, including Cy™3 and Cy5. The kit also provides reagents for purification of labeled miRNA—resulting in minimal contaminating unincorporated nucleotides, and low background signals.

The *mir*Vana miRNA Labeling Kit provides a non-isotopic miRNA labeling procedure using poly(A) polymerase. Multiple amine-modified nucleotides are appended to the 3' ends of purified miRNAs in a sample—providing significantly more sensitivity than the current labeling methods for miRNAs. The *mir*Vana miRNA Labeling procedure incorporates optimized, proprietary solutions to enhance the labeling efficiency of the very limiting quantities of nucleic acid in a typical miRNA sample.

ORDERING INFORMATION		
Product	Cat. #	SIZE
*mir*Vana™ miRNA Labeling Kit	1562	20 reactions

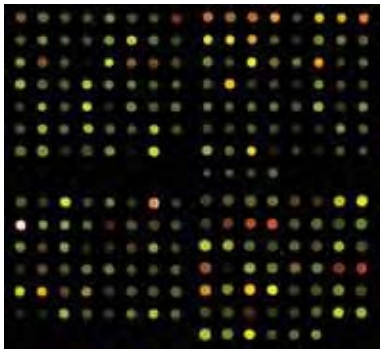


Figure 14. *mir*Vana™ miRNA Probe Set Array Hybridized with Samples Labeled Using the *mir*Vana miRNA Labeling Kit. The mature miRNA fraction of FirstChoice® Total RNA (20 µg) from human bladder and lung was purified using the flashPAGE™ Fractionator Apparatus. The miRNA samples were then nonisotopically labeled using the *mir*Vana miRNA Labeling Kit and hybridized to a glass slide arrayed with the *mir*Vana miRNA Probe Set. Analysis of the array is shown here.

*mir*Vana™ miRNA Probe Set

Highly specific microRNA probes for accurate microarray profiling

The *mir*Vana miRNA Probe Set provides specific, functionally validated miRNA microarray probes against a complete set of registered human, rat, and mouse miRNAs. These probes have been extensively validated to produce highly accurate and reproducible data.

The kit also includes optimized hybridization and wash buffers for precise miRNA array analysis. With the *mir*Vana miRNA Probe Set, you can immediately and cost-effectively print microarray slides and perform sensitive miRNA profile analysis—without the need for extensive, time-consuming probe design and evaluation.

The *mir*Vana miRNA Probe Set is a collection of amine-modified oligonucleotides. The oligonucleotide probes are 42–46 nt in length with an amine-modification that make the probe set ideally suited for epoxy and aldehyde slide surface chemistries. Ambion uses and recommends Nexterion Schott Slide E for spotting miRNA arrays with our probe set. Multiple labels are efficiently incorporated into the oligonucleotides using the *mir*Vana™ miRNA Labeling Kit. Ambion's miRNA array system was used in two recently published peer-reviewed journals:

REFERENCES

1. Johnson SM, Grosshans H, Shingara J, Byrom M, Jarvis R, Cheng A, Labourier E, Reinert KL, Brown D, Slack FJ. (2005) RAS is regulated by the let-7 microRNA family. *Cell* **120:**635-647.
2. Shingara J, Keiger K, Shelton J, Laosinchai-Wolf W, Powers P, Conrad R, Brown D, Labourier E. (2005) An optimized isolation and labeling platform for accurate microRNA expression profiling. *RNA* (in press).

*Verified miRNAs are reported by the miRNA Registry and published at www.sanger.ac.uk/Software/Rfam/mirna/index.shtml. The *mir*Vana Probe Set contains a complete profile of probes against the published miRNA sequences at each time of production.

ORDERING INFORMATION		
Product	Cat. #	SIZE
*mir*Vana™ miRNA Probe Set (including all buffers) *complete set of current miRNA probes; optimized miRNA buffers*	1564V1	
The following buffers are also available as stand-alone products:		
*mir*Vana™ miRNA Probe Set Detergent Concentrate	9823G5	200 ml
*mir*Vana™ miRNA Probe Set Salt Concentrate	9763G6	1 L
*mir*Vana™ miRNA Probe Set 3X miRNA Hybridization Buffer	8860G	2 ml

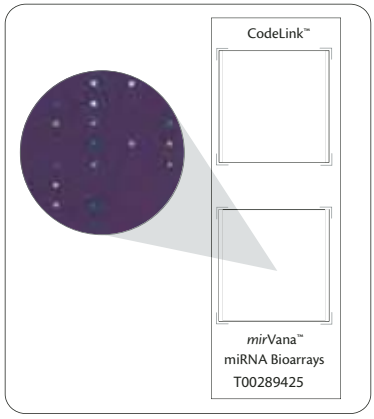
NEW!

*mir*Vana™ miRNA Bioarrays—
high-quality and highly consistent pre-spotted miRNA arrays (Cat. #1566V1)

miRNA Array Services —
have Ambion perform your miRNA microarray experiments Contact Ambion for additional information.

*mir*Vana™ miRNA Bioarrays

*mir*Vana miRNA Bioarrays, ready-to-use, high quality microarrays that contain probes for detection of a comprehensive panel of the human, mouse and rat miRNAs catalogued in the miRNA Registry (microrna.sanger.ac.uk/sequences/index.shtml). In addition, probes targeting an exclusive set of newly identified human miRNAs, called Ambi-miRs™, are included. Ambi-miRs are novel miRNAs that have been identified through a combination of bioinformatic prediction, cloning and sequencing, and detection in human total RNA samples.



Manufactured by GE Healthcare, *mir*Vana miRNA Bioarrays are produced using the CodeLink 3-D Gel Matrix slide surface, providing an aqueous environment that holds the probe away from the surface of the slide, allowing maximum interaction between probe and target. These enhanced hybridization conditions result in superior probe specificity, higher signal-to-noise ratio, and increased sensitivity, making it easier to accurately detect low abundance miRNAs. As shown in Figure 15, spiking different amounts of synthetic miRNA oligonucleotides in human total RNA showed linear detection down to 2.8x10-16 moles. In addition, the superior quality of the Bioarrays enables accurate one-color labeling and detection, providing robust and highly reproducible results. Analysis of the same human total RNA sample on 42 different Bioarrays on different days showed excellent technical and day-to-day reproducibility with average CVs lower than 10% (Figure 16).

Each package of *mir*Vana miRNA Bioarrays contains 3 slides for a total of 6 arrays. A CD containing miRNA Annotations from the Sanger miRNA Registry, a GenePix® Array (GAL) file, GenePix Settings files for each array, and files necessary for CodeLink software users is also included.

Be Confident in Your Profiling Data

The *mir*Vana miRNA Bioarray Essentials Kit (Cat #1567) provides a positive control, an optimized hybridization buffer, Bioarray Lifterslips™, and all wash solutions required for successful analysis of *mir*Vana miRNA Bioarrays.

NEW!		
Product	Cat. #	SIZE
*mir*Vana™ miRNA Bioarrays	1566V1	3 slides (6 arrays)
*mir*Vana™ miRNA Bioarrays Essentials	1567	25 washes

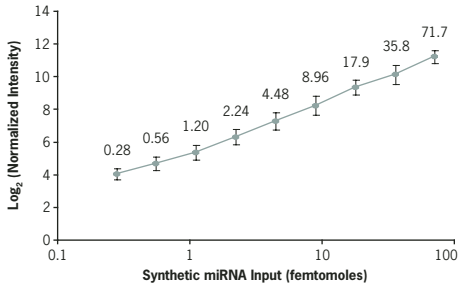


Figure 15. Linearity and Sensitivity of the *mir*Vana Bioarray System. Chemically synthesized oligonucleotides corresponding to ten mature miRNA sequences were spiked into FirstChoice® Total RNA samples. The synthetic spikes represented a series of miRNAs not expressed in the chosen tissue, and were spiked in at known amounts (0.28–71.68 femtomoles) and arrayed in a Latin Square format (i.e., each sample contained synthetic miRNAs representing the entire range of amounts). Samples were subjected to the whole *mir*Vana Bioarray process (fractionation, labeling, and hybridization on independent Bioarrays). The graph shows the average of the normalized signal intensities of the ten spiked synthetic miRNAs for each input amount.

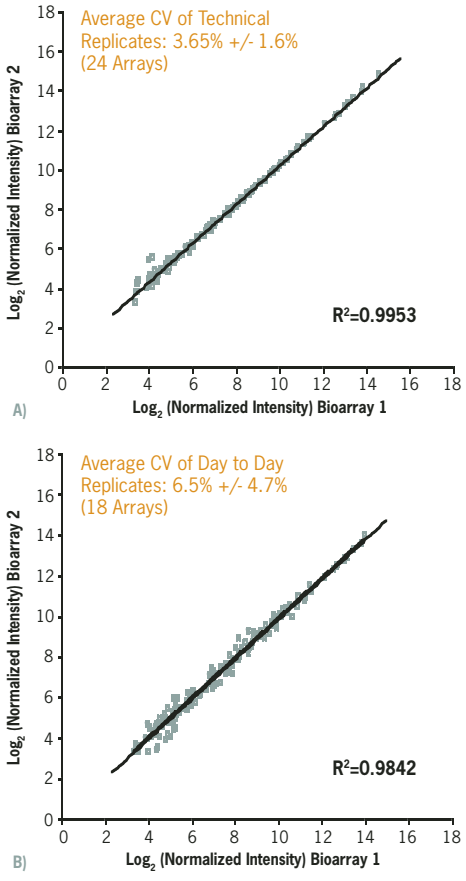


Figure 16. Bioarray Reproducibility. Comparison of normalized signal intensities from independent arrays demonstrated that the *mir*Vana™ miRNA Bioarray System exhibits excellent array-to-array (technical), and day-to-day (process) reproducibility. **(A)** The same labeled miRNA sample was hybridized on two independent bioarrays on one day. **(B)** The same total RNA sample was processed independently (flashPAGE™ Purification, miRNA labeling, and hybridization on Bioarrays) on two different days.

*mir*Vana™ miRNA Detection Kit

Ultrasensitive detection of small RNAs

- Sensitive—detect miRNA or siRNA in as little as 50 µg total RNA
- Multiple target detection—detect multiple small RNAs and mRNAs in the same sample
- Specific—extremely low background
- Simple and fast—single tube procedure followed by PAGE

The patented *mir*Vana miRNA Detection Kit provides a fast and sensitive method for detecting small RNAs. The assay is 100–500 times more sensitive than Northern analysis and is able to detect as little as 10 attomoles (10–17 mol) of target RNA. In addition, the kit can be used to simultaneously detect several small RNAs of the same size, or both small RNA and longer RNA species in the same sample.

The *mir*Vana miRNA Detection Kit is based on a simple solution hybridization principle (Figure 17). The RNA sample containing the target RNA(s) of interest is simply mixed with one or more radiolabeled RNA probes and the included Hybridization Buffer. After heat denaturation, the mixture is incubated at 42°C to hybridize the probe to its complement. Unhybridized RNA and excess probe are then removed by a ribonuclease digestion step. The hybridized, protected RNA fragments are recovered using Ambion's patented single-step technology for simultaneous ribonuclease inactivation and nucleic acid precipitation. RNA samples are then resuspended and analyzed on a denaturing polyacrylamide gel. The entire procedure was specifically developed to provide optimal sensitivity and specificity with short antisense probes, and is ideal for detecting small RNA molecules such as miRNA or siRNA.

ORDERING INFORMATION		
Product	Cat. #	SIZE
*mir*Vana™ miRNA Detection Kit	1552	100 rxns

*mir*Vana™ miRNA Probe and Marker Kit

End labeling and purifying small RNA probes and markers

- 5' end label and clean up small RNA and DNA probes
- Compatible with chemically synthesized oligonucleotides—no need to gel purify after labeling
- Includes 10–150 nt Decade™ Markers—ideal for gel analysis of small RNAs
- Simple and rapid post-labeling probe clean-up procedure
- Compatible with the *mir*Vana miRNA Detection Kit and Northern analysis

The *mir*Vana Probe & Marker Kit is designed for rapid 5' end labeling and clean-up of small RNA or DNA probes. For maximal utility, the kit also contains reagents to prepare small radiolabeled RNA size markers (Decade Markers; 150, 100, 90, 80, 70, 60, 50, 40, 30, 20, and 10 nt) and single-nucleotide RNA ladders (Figure 18).

One advantage of end labeling chemically synthesized oligonucleotides is that there is typically no need for gel purification of probes for use in the *mir*Vana miRNA Detection Kit or RPA procedures, as is often required for probes prepared by in vitro transcription. However, unincorporated label must typically be removed for the cleanest results. The purification columns included in the *mir*Vana Probe & Marker Kit allow rapid and efficient clean-up of 5' end labeled RNA probes.

Radiolabeled RNA probes prepared with the *mir*Vana Probe & Marker Kit have been successfully used for detection of miRNA by Northern blot, and by solution hybridization using the *mir*Vana miRNA Detection Kit. DNA probes can also be efficiently labeled with the Probe & Marker Kit for use in many downstream applications.

ORDERING INFORMATION		
Product	Cat. #	SIZE
*mir*Vana™ miRNA Probe and Marker Kit	1554	30 rxns; 10 marker rxns

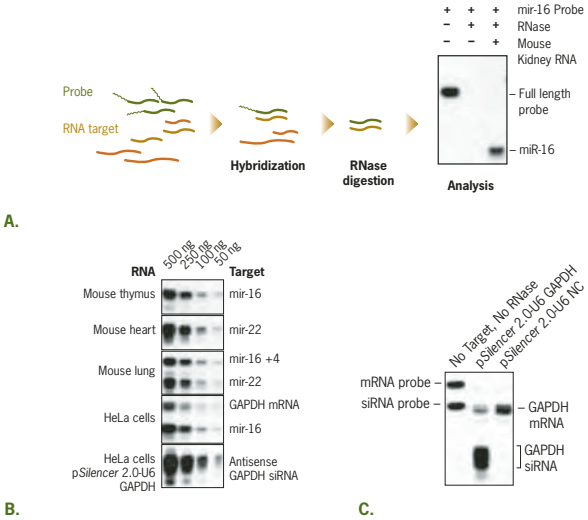


Figure 17. Single and Multiple Target Detection with the *mir*Vana™ miRNA Detection Kit. **(A)** The *mir*Vana™ miRNA Detection Kit procedure. **(B)** The indicated target RNAs were detected in 500, 250, 100 and 50 µg of total RNA from mouse tissues (3.5 hours exposure) or HeLa cells (6 hours exposure). Probes were prepared using the *mir*Vana™ miRNA Probe Construction Kit. The miR-16 +4 probe (36 nt) carries four A residues adjacent to the leader sequence, providing a 26 nt protected fragment after hybridization and RNase digestion. The probe specific for GAPDH mRNA (39 nt) produces a 29 nt long protected fragment with the same specific activity as the mir-16 protected fragment. **(C)** HeLa cells were transfected with p*Silencer*™ 2.0-U6 engineered to express either an siRNA targeting GAPDH or a negative control siRNA (NC). Three days after transfection, total RNA was isolated and 1 µg was assessed using the *mir*Vana™ miRNA Detection Kit as described for Panel (B).

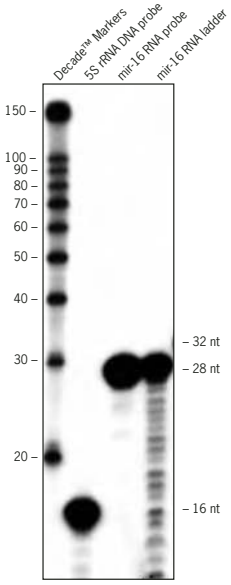


Figure 18. Probes and Markers Prepared with the *mir*Vana™ Probe & Marker Kit. Radiolabeled probes were prepared following the *mir*Vana Probe & Marker Kit Instruction Manual. The Purification Cartridges and reagents were used to remove unincorporated nucleotides from all the labeled nucleic acids except the Control DNA. 1.25% of the purified Control RNA probe was subjected to alkaline hydrolysis as described in the Kit Instruction Manual to produce the RNA Ladder. The indicated percentage of each reaction was resolved on a 15% denaturing polyacrylamide gel. The gel was exposed for 15 min.

*mir*Vana™ miRNA Probe Construction Kit

Rapid Preparation of Small RNA Probes by IVT

- Rapid procedure—prepare transcription templates and synthesize RNA probes in less than 1 hour
- More economical than labeling chemically synthesized RNA
- Produces probes with higher specific activity than 5' end labeling, resulting in greater sensitivity
- Ideal for preparing radiolabeled and nonisotopically labeled small RNA probes
- Compatible with the *mir*Vana miRNA Detection Kit, Northern analysis, and in situ hybridization

With the *mir*Vana miRNA Probe Construction Kit, short (<100 nt) radiolabeled or nonisotopically labeled RNA probes can be generated by in vitro transcription (IVT) in less than 1 hour (Figure 19). Depending on the probe sequence and the radiolabeled nucleotide used, in vitro transcribed probes typically result in ~2-fold greater sensitivity than 5' end labeled probes due to their higher specific activity (Figure 20). In addition, because they are synthesized enzymatically, RNA oligonucleotide probes prepared by IVT are less expensive than probes prepared by chemical synthesis followed by 5' end labeling.

Radiolabeled probes prepared with the *mir*Vana miRNA Probe Construction Kit have been successfully used for the detection of microRNA (miRNA), small nuclear RNA (snRNA), and small interfering RNA (siRNA), as well as mRNA. Nonisotopically labeled probes generated with the kit have been used to study the distribution of miRNA and mRNA in tissues by in situ hybridization.

Rapid and Easy RNA Probe Synthesis

The kit uses a Klenow fragment fill-in reaction followed by in vitro transcription to generate the RNA probe. All you need to supply is a short, inexpensive DNA oligonucleotide specific for the target of interest that also includes an 8 base sequence complementary to the 3' end of the T7 promoter primer included in the kit. This target-specific oligonucleotide is annealed to the provided T7 promoter primer, and a 30 minute fill-in reaction generates a double-stranded transcription template. The resulting template is simply mixed with the provided T7 RNA Polymerase, rNTPs, and Transcription Buffer along with your choice of labeled rNTP. The reaction is then incubated for as little as 10 minutes at 37°C to generate labeled probe.

ORDERING INFORMATION		
Product	Cat. #	SIZE
*mir*Vana™ miRNA Probe Construction Kit	1550	30 rxns

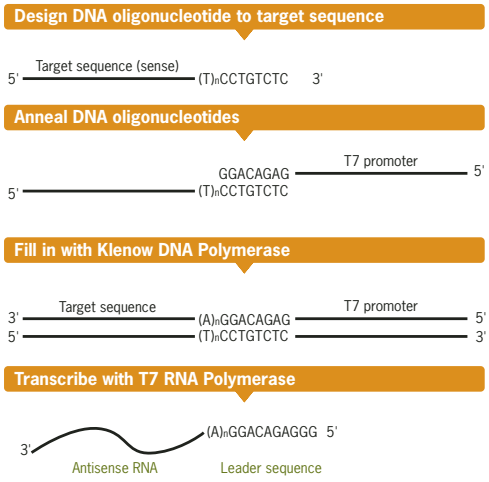


Figure 19. *mir*Vana™ miRNA Probe Construction Kit Procedure.

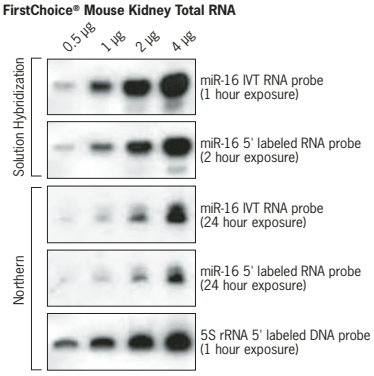


Figure 20. Comparison Between In Vitro Transcribed (IVT) and 5' End Labeled Probes. The expression of miR-16 miRNA and 5S rRNA was analyzed in 0.5, 1, 2, or 4 µg of FirstChoice® Total RNA from mouse kidney included in the *mir*Vana™ miRNA Detection Kit using the indicated DNA or RNA probes included in the *mir*Vana™ miRNA Probe Construction Kit (IVT probes) or *mir*Vana Probe & Marker Kit (5' end labeled probes).

*mir*Vana™ qRT-PCR miRNA Detection Kit

The *mir*Vana qRT-PCR miRNA Detection Kit has been optimized to amplify specific small RNAs enabling accurate quantitation of miRNA expression levels (Figure 21). This novel detection system can be used to characterize miRNA expression via traditional end-point RT-PCR or real-time RT-PCR using SYBR® Green 1.

Since miRNAs represent only a minute fraction of total RNA, detection of these molecules can be difficult. However, the *mir*Vana qRT-PCR miRNA Detection Kit is extremely sensitive and can preferentially detect mature miRNA from as little as 25 pg of total RNA input. The *mir*Vana qRT-PCR miRNA Detection Kit exhibits linearity over 5 logs.

*mir*Vana qRT-PCR Primer Sets are available for nearly all of the annotated human, mouse, and rat miRNAs. In addition, Primer Sets specific for the small RNA species U6 snRNA and 5S rRNA can be used to control input variability and sample normalization. For a list of our most currently available primers, please visit our website at: www.ambion.com/miRNA/primers.

For analysis of individual miRNAs, the *mir*Vana qRT-PCR miRNA Detection Kit is unparalleled in sensitivity and speed.

Each kit contains sufficient reagents for 200 reactions, human heart total RNA, and a control primer set to amplify ubiquitously expressed miR-24.

ORDERING INFORMATION

Product	Cat. #	SIZE
*mir*Vana™ qRT-PCR miRNA Detection Kit	1558	200 rxns
SuperTaq™ Polymerase (Cloned) 5 U/µl	2052	250 U
*mir*Vana™ qRT-PCR Primer Set for Normalization (5S)	30302	200 rxns
*mir*Vana™ qRT-PCR Primer Set for Normalization (U6)	30303	200 rxns
*mir*Vana™ qRT-PCR Primer Sets	30xxx	200 rxns

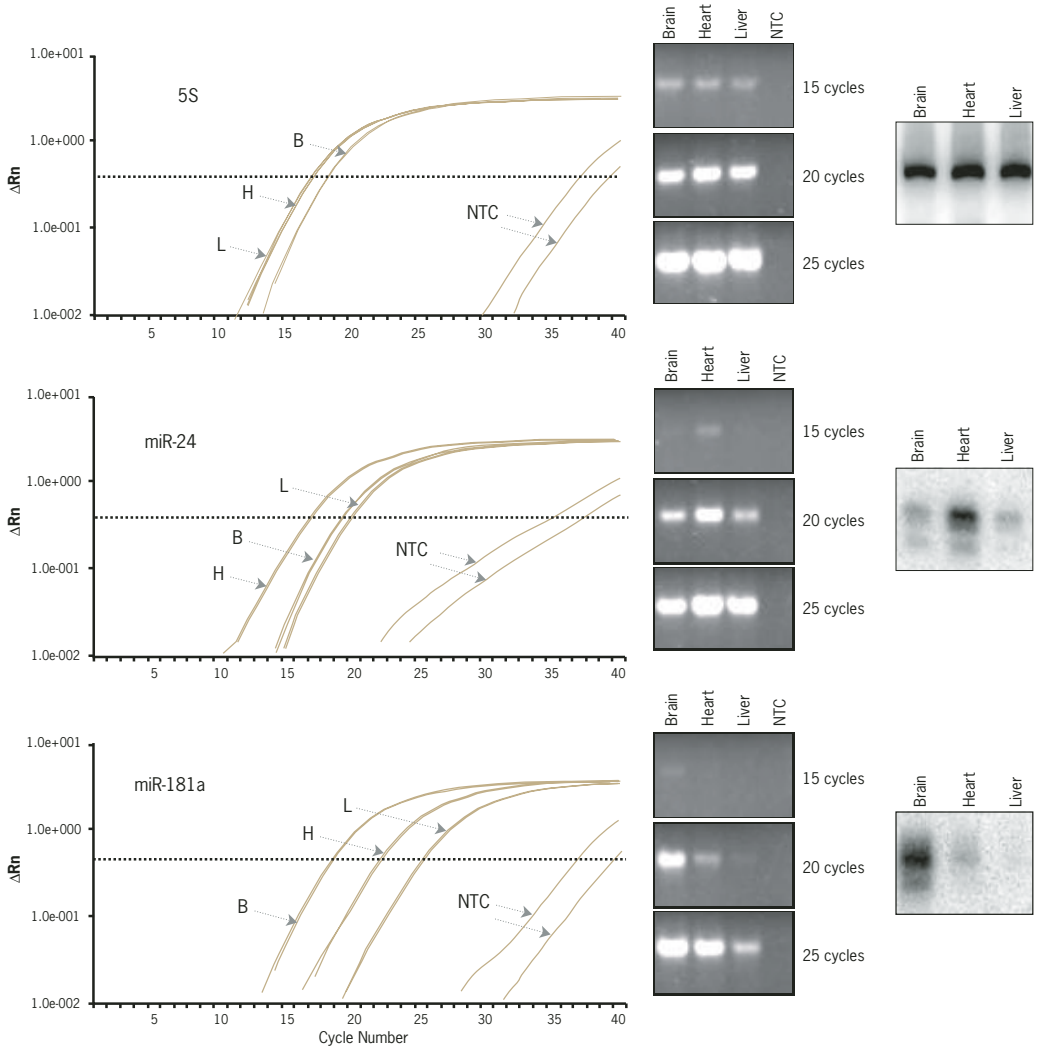


Figure 21. Real-Time RT-PCR, End-Point RT-PCR, and Northern Blot Analysis of miRNA Expression. FirstChoice® Total RNA (25 ng) from human brain (B), heart (H), and liver (L) was evaluated for expression of the indicated small RNA species using the *mir*Vana™ qRT-PCR miRNA Detection Kit, the corresponding *mir*Vana qRT-PCR Primer Sets, and 1 U SuperTaq™ per reaction. Experiments were conducted using both real-time and end-point RT-PCR. Northern analysis was performed on 10 µg of RNA (right). NTC=No template control.

miRNA Functional Analysis

miRNA functional analysis can be performed with protocols that are similar to standard genes. Up-regulation of the miRNAs can be conducted to identify gain-of-function phenotypes; down-regulation or inhibition can be conducted to identify loss-of-function phenotypes (Table 2 and Figure 22). The combination of up- and down-regulation can be used to identify genes that are regulated by specific miRNAs as well as to identify cellular processes that are affected by specific miRNAs (Figure 23).

Key applications include:

- miRNA target site identification and validation
- Screening for miRNAs that regulate the expression of a gene
- Screening for miRNAs that affect a cellular process

Table 2. Products for miRNA Functional Analysis.

Product	Function
Pre-miR™ miRNA Precursor Molecules	Synthetic miRNA molecules for up-regulation of miRNA activity
Anti-miR™ miRNA Inhibitors	Synthetic miRNA inhibitors that reduce miRNA activity
pMIR-REPORT™ miRNA Expression Reporter Vector	Accurate, quantitative miRNA reporter vector to monitor miRNA activity

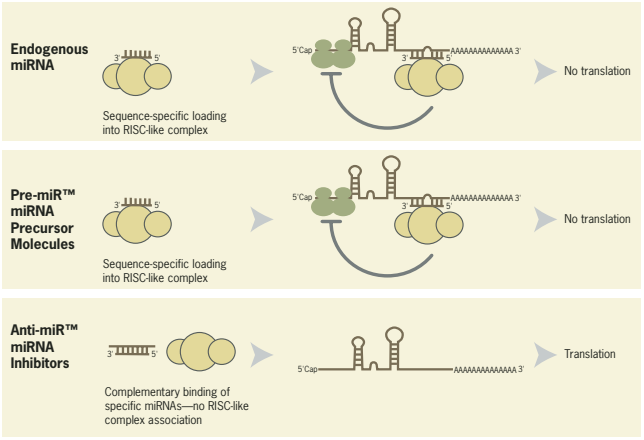


Figure 22. Activity of Pre-miR™ miRNA Precursor Molecules and Anti-miR™ miRNA Inhibitors.

Up-regulation of miRNAs in cells can be accomplished either by transfecting cells with synthetic miRNAs [1, 2] or plasmids that express miRNAs [3]. The use of synthetic molecules has several advantages (Table 3):

- The transfection efficiency of small RNAs can approach 100% for immortalized cells. The high transfection efficiencies of synthetic miRNAs are particularly beneficial for high-throughput applications like miRNA functional screening.
- Small RNAs like miRNAs can be readily electroporated into primary cells without inducing significant cell death.
- Synthetic miRNAs can be transfected at several different concentrations, facilitating dose response studies.
- Unlike plasmid-induced miRNAs, the sequence of the synthetic miRNA that is altering translation is defined. The rules for miRNA excision and activation are not fully understood, making it difficult to ensure uptake and activation of the intended miRNA molecule expressed from a plasmid.

Table 3. Comparison of Synthetic miRNAs Versus Other Technologies.

	Pre-miR™ Synthetic miRNA Design	Vector Expression Design	siRNA Design
High transfection efficiency	●		●
Correct strand uptake	●	●	
Tight regulation of miRNA concentration	●		●
Defined functional sequence	●		

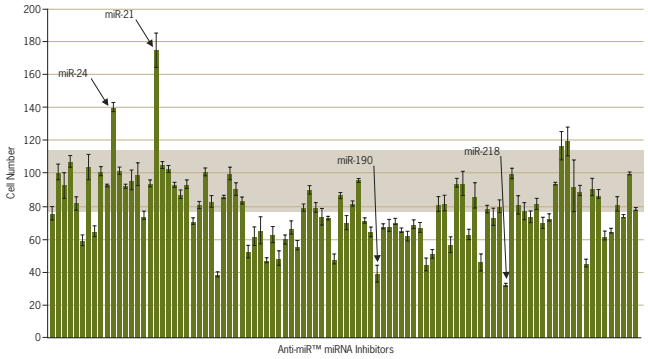


Figure 23. Identification of miRNAs that Alter Cell Proliferation. HeLa cells (5 x 10^3) were transfected with individual Anti-miR™ miRNA Inhibitors in triplicate using siPORT™ *NeoFX*™ Transfection Agent (Ambion). 72 hr post-transfection, cells were fixed and stained with propidium iodide to look at total cell number. Cells were then stained for β-actin using immunofluorescence. The shaded horizontal area represents the normal range of cell number for this cell type, as exemplified by cells transfected with a GAPDH antisense transcript that does not affect cell proliferation (second bar from the right).

Anti-miR™ miRNA Inhibitors

The easiest way to knockdown specific miRNA activity

Product Description

The Anti-miR miRNA Inhibitors are sequence-specific and chemically modified to specifically target and knockdown individual miRNA molecules.

REFERENCES

1. Lewis BP, Shih IH, Jones-Rhoades MW, Bartel DP, Burge CB. (2004) Prediction of mammalian microRNA targets. *Cell* **115(7):**787–798.
2. Cheng, A. M., Byrom, M. W., Shelton, J., Ford, L. P. (2005) Antisense inhibition of human miRNAs and indications for an involvement of miRNA in cell growth and apoptosis. *Nucleic Acids Res* **33:**1290–1297.
3. Zeng Y, Wagner EJ, Cullen BR. (2002) Both natural and designed micro RNAs can inhibit the expression of cognate mRNAs when expressed in human cells. *Mol Cell* **9**:1327–1333.

ORDERING INFORMATION

Product	Cat. #	SIZE
*mir*Vana™ miRNA Probe and Marker Kit	1554	30 rxns; 10 marker rxns
Anti-miR™ miRNA Inhibitors	17000	5 nmol
	17001	20 nmol (4x5 nmol)
order online at www.ambion.com/catalog/mirna_search.php		
Anti-miR™ miRNA Inhibitors – custom defined sequence	17003	20 nmol
order online at www.ambion.com/catalog/mirna_search.php		
Anti-miR™ Negative Control #1	17010	5 nmol

Pre-miR™ miRNA Precursor Molecules

Synthetic miRNA molecules for up-regulation of specific miRNA levels in cells

Pre-miR miRNA Precursor Molecules (patent pending) are chemically modified and optimized nucleic acids designed to closely mimic the miRNA molecules in cells. The Pre-miR miRNA Precursor Molecules are designed to directly enter into the miRNA processing pathway, and are treated identically to endogenous miRNA within the cell. With the Pre-miR miRNA Precursor Molecules, you can:

- Control specific miRNA activity
- Tightly regulate miRNA cellular levels
- Achieve optimal delivery efficiency with minimal cytotoxicity

For a synthetic miRNA to be useful, it must be active, robust, and most importantly, stranded. As pointed at by Schwarz et al.*, small double-stranded RNAs like siRNAs and miRNAs exhibit strandedness wherein one of the two complementary RNAs in the dsRNA molecule is preferentially incorporated into the miRNA (or siRNA) pathway. The sequence compositions and duplex stabilities of the small RNA molecules dictate which strand will be active in the cell. Transfected miRNAs should be similarly stranded to ensure efficient uptake of the active miRNA strand and occlusion of the incorrect, complementary strand.

Since the Pre-miR miRNA Precursor Molecules directly enter the miRNA pathway, they eliminate any non-miRNA cellular response that is seen with siRNA-like designs. Unlike plasmid-based miRNA expression, the Pre-miR miRNA Precursor Molecules are easy to transfect with minimal cellular stress, and are able to be tightly regulated for specific dose response analysis.

ORDERING INFORMATION

Product	Cat. #	SIZE
Pre-miR™ miRNA Precursor Molecules	17100	5 nmol
order online at www.ambion.com/catalog/mirna_search.php	17101	20 nmol (4x5 nmol)
Pre-miR™ miRNA Precursor Molecules custom defined sequence	17103	20 nmol
order online at www.ambion.com/catalog/mirna_search.php		
Pre-miR™ Negative Control #1	17110	5 nmol
Pre-miR™ Negative Control #2	17111	5 nmol

siPORT™ *NeoFX*™ Transfection Agent

Transfection of small RNAs and co-transfection of miRNAs/pMIR-REPORT™

siPORT *NeoFX*'s lipid-based formulation can be used to efficiently transfect many cell types—especially adherent cells as they are subcultured—without increased cytotoxicity. From start to finish, successful gene silencing experiments can be completed in as early as 24 hours. This streamlined "neofection" protocol can be adapted to a wide range of cells and experimental designs, including high-throughput applications. *NeoFX* Transfection Agent efficiently transfects low concentrations of miRNA and siRNA. For example, high levels of siPORT *NeoFX*-mediated gene silencing (>80%) were achieved by transfection of as little as 0.1–10 nM siRNA (targeting GAPDH) into HeLa cells.

ORDERING INFORMATION

Product	Cat. #	SIZE
siPORT™ *NeoFX*™ Transfection Agent	4510	0.4 ml
	4511	1 ml

*Schwarz DS, Hutvagner G, Du T, Xu Z, Aronin N, Zamore PD. (2003) Asymmetry in the assembly of the RNAi enzyme complex. *Cell.* **115(2)**:199-208.

pMIR-REPORT™ miRNA Expression Reporter Vector

Sensitive, quantitative monitoring of miRNA expression

The pMIR-REPORT miRNA Expression Reporter Vector provides accurate, quantitative, in-cell measurement of miRNA expression. This validated reporter system contains two mammalian expression vectors (Figure 24). pMIR-REPORT contains Luciferase under the control of a mammalian promoter/terminator system, with a miRNA-target cloning region downstream of the luciferase translation sequence. This vector is optimized for cloning of miRNA targets in order to evaluate miRNA regulation. A second vector, pMIR-REPORT Beta-gal Control Vector, is provided to normalize for transfection efficiency.

The pMIR-REPORT miRNA Expression Reporter Vector is designed for the cloning and testing of putative miRNA binding sites. pMIR-REPORT can be transfected into mammalian cells to evaluate endogenous miRNA expression, or used to evaluate up- and down-regulation from Pre-miR™ miRNA Molecules and Anti-miR™ miRNA Inhibitor Molecules, respectively. The pMIR-REPORT miRNA Expression Reporter Vector can also be used as a sequence screening tool to identify miRNA targets or screen libraries of Pre-miR miRNA Molecules to identify genes that regulate expression.

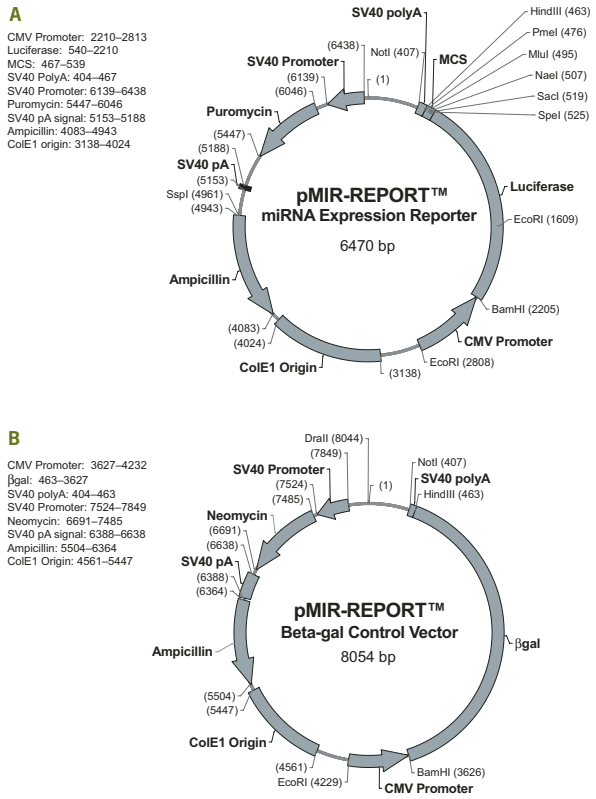


Figure 24. (A) pMIR-REPORT™ miRNA Expression Reporter Vector. (B) pMIR-REPORT™ β-Galactosidase Control Vector.

ORDERING INFORMATION

Product	Cat. #	SIZE
pMIR-REPORT™ miRNA Expression Reporter Vector	5795	1 kit (supercoiled plasmids in E. coli)

microRNA Information Resources

Ambion is committed to providing the research community with products and information necessary to advance microRNA (miRNA) research. We develop innovative products specifically for miRNA experimental needs. Ambion's Research and Development scientists:

- Develop novel, market-leading miRNA products that we use in our own grant-funded miRNA research
- Have decades of RNA experience, with greater than three years of specific experience in the miRNA field
- Establish critical research collaborations with thought-leaders in the miRNA field

As part of the miRNA research community, Ambion provides extensive access to our miRNA expertise, by providing free, in-depth experimental design consultation and support. We provide the following information resources:

miRNA Resource

www.ambion.com/miRNA

A gateway to all types of information about microRNAs. This resource includes links to articles, tools, news, products, other websites, and more.

miRNA Array Resource

www.ambion.com/techlib/resources/miRNA_array/index.html

A resource for those conducting microRNA array experiments that includes tips, troubleshooting advice, frequently updated miRNA annotation files, and data analysis support.

miRNA Database

www.ambion.com/catalog/mirna_search.php

A comprehensive database of known microRNAs. Search by stem loop name, mature name, accession number, and/or species. Search results include information about the miRNA, as well as a list of associated Ambion products--simply click to order!

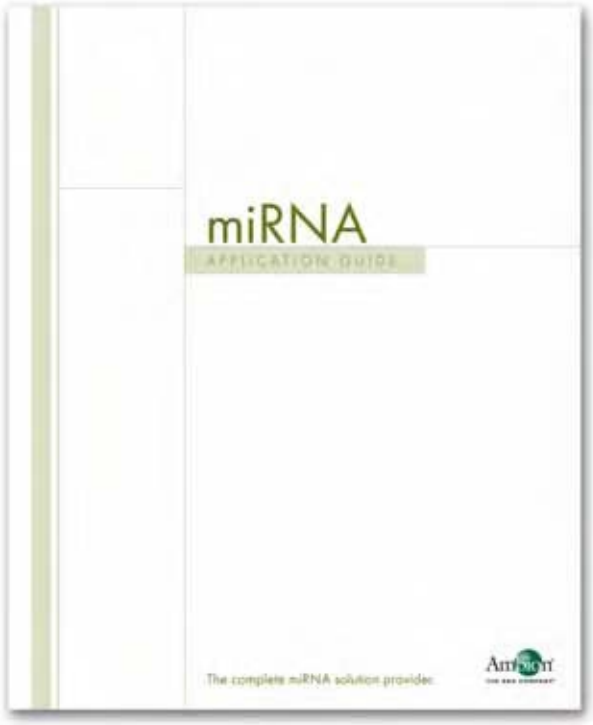
Introduction to microRNAs

www.ambion.com/main/explorations/mirna.html

This interactive web feature answers the questions "What are miRNAs?", "How do they work?", "How do I study them?", and "Where can I learn more about them?".

miRNA Application Guide

The new miRNA Application Guide is the first reference publication devoted exclusively to the field of miRNA. The miRNA Application Guide includes an extensive miRNA literature review, experimental design workflows, miRNA-specific research protocols, and research reprints.



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Ambion's scientists travel worldwide to provide technical miRNA seminars at conferences, academic institutions, and biotech companies. Our seminars are tailored to your interests and provide a vehicle to open direct lines of communication between our scientists and other members of the miRNA research community. If you're interested in hosting a seminar in your area, please contact us at **seminars@ambion.com**.

Highly Trained miRNA Technical Support Scientists

Ambion employs scientists with advanced training and extensive experience to provide personalized support to researchers. Our miRNA technical specialists are closely tied to Ambion R&D scientists and can provide you with answers to most of your miRNA questions. The miRNA specialists provide:

- Experimental design development
- Product selection assistance
- Technical troubleshooting
- A primary contact for any communication with Ambion

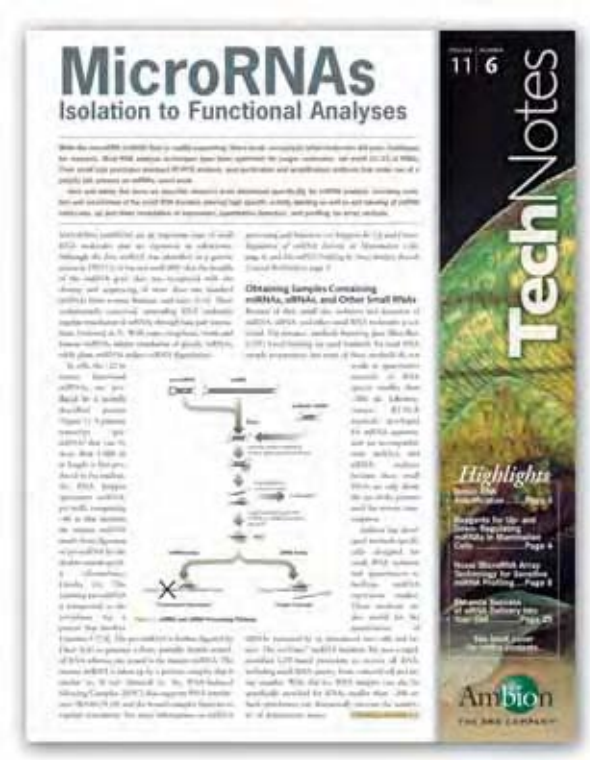
Contact us in the US by telephone at 800-888-8804 option #2, or worldwide via email at **techserv@ambion.com**.

miRNA e-Updates

Be the first to learn about new products and services for miRNA research. Visit us online and sign-up for *miRNA Messenger* e-Updates at **www.ambion.com/contact/litreq/index.html** Individuals on this list will be sent e-mail alerts when new miRNA products or services are available—enabling you to be among the first to have access to these innovative technologies. This list is for miRNA-specific e-mails only, and individuals will receive e-mails that directly relate to miRNA research and products.

TechNotes Newsletter

Ambion's *TechNotes* newsletter is published several times a year and has informative articles on new RNA applications, tips for improving your results, and descriptions of Ambion's latest products. Previous editions of *TechNotes* are available online at **www.ambion.com/techlib/tn/index.html**. The print version of *TechNotes* contains additional articles. If you would like to receive a print copy of *TechNotes* by mail, please sign up online at **www.ambion.com/contact/litreq/index.html**



Relative miRNA Expression Among Common Cell Types

Relative miRNA Expression Among Cell Types. The miRNA expression profiles of seven common cell types were assessed using the miRNA array procedure (see pages 7–8). miRNAs that generate very high signal on the microarray are denoted by ++++, high-signal miRNAs are denoted by +++, medium-signal miRNAs are denoted by ++, and low-signal miRNAs are denoted by +. An "0" indicates a miRNA with signal that does not exceed that of negative control elements on the microarray.

miRNAs	293	MCF7	HeLa	HeLa S3	A549	HepG2	K562
let-7A	+	++	+	+	++	+	+
let-7C	+	+	++	++	++	+	+
let-7D-AS	0	0	+	0	0	0	0
let-7G	+	++	0	+	+	+	+
miR-1	+	++	+	+	++	++	+
miR-7	+	++	+	0	+	++	+
miR-9	0	0	0	0	0	0	0
miR-10A	0	0	0	0	0	0	+
miR-15A	0	+	+	0	+	+	0
miR-16	++	++	+++	+++	+++	++++	+++
miR-17-3P	+	++++	+++	++++	+	++	++++
miR-17-5P	+	0	++	++	0	0	+++
miR-18	+	+++	++	++	0	+	++++
miR-19A	+	++	0	0	+	+	++
miR-20	+	++++	++	+	+	++	+++
miR-21	++	++++	+++	++	++++	++	++
miR-22	+	++	++	0	0	+	+
miR-23B	+	++	++++	+++	+	0	+++
miR-24	++	++	++++	++++	+	+	+++
miR-25	+	++	++	+++	++	++	+++
miR-26A	++	+++	++	++	++	++	+++
miR-27A	+	++	+	++	++	+	++
miR-28	0	+	0	+	+	+	++
miR-29B	0	0	0	0	0	0	0
miR-30A	+	++	+++	+++	++	+	+
miR-31	+++	+	++++	++++	+	+	+
miR-32	0	0	0	0	0	0	0
miR-33	0	+	0	0	+	0	+
miR-34A	0	++	+	+	+	+	+
miR-92	0	+++	++	+++	+	0	++++
miR-93	+	++	+++	++++	++	+	++++
miR-95	0	0	+	0	0	0	0
miR-96	+	+	+	+	+	0	0
miR-98	0	0	0	0	+	0	+
miR-99A	+	++	+	++	++	+	+
miR-100	+	+	++	++	+	+	+
miR-101	0	0	0	0	0	0	0
miR-103	0	++	0	+	+	+	+
miR-105	0	0	+	0	0	0	+

miRNAs	293	MCF7	HeLa	HeLa S3	A549	HepG2	K562
miR-106A	0	++++	++	+++	+	+	++++
miR-107	0	++	++	+++	+	+	+++
miR-122A	+	++	0	+	++	+	+
miR-124A	0	0	0	0	0	0	0
miR-125A	0	0	+	+	+	+	+
miR-126	++	0	0	0	0	+	+++
miR-127	0	0	0	0	+	+	0
miR-128A	0	0	0	0	0	0	0
miR-129	0	0	0	0	0	0	0
miR-130A	+	++	+	+	+	+	+
miR-132	+	+	+	+	+	+	+
miR-133A	0	0	0	0	0	0	0
miR-134	+	0	+	0	0	+	0
miR-135A	0	0	+	0	0	0	0
miR-136	0	0	+	0	0	0	0
miR-137	0	0	+	0	0	0	+
miR-138	0	0	+	0	0	0	+
miR-139	0	0	0	+	0	0	0
miR-140	+++	+++	++	++	++	++	+++
miR-141	0	0	+	0	0	0	0
miR-142-5P	+	+	+	0	+	+	+
miR-143	+	+	+	0	+	+	0
miR-144	0	0	+	0	0	0	0
miR-145	+++	+++	+++	++	++	++	+++
miR-146	+	+	+	0	+	+	0
miR-147	+	+	+	+	+	++	+
miR-148A	+	+	0	0	+	+	0
miR-149	+	+	0	0	+	0	0
miR-150	0	0	0	0	0	0	0
miR-151	0	0	0	0	0	0	0
miR-152	0	0	0	0	0	0	0
miR-153	0	+	0	+	+	+	+
miR-154	0	0	0	0	+	0	0
miR-155	+	++	+	+	+	+	+
miR-181A	0	0	++	+	0	0	+
miR-182	++	++	+	+	++	++	++
miR-183	0	+	0	+	+	+	+
miR-184	0	0	0	0	0	0	0
miR-185	0	0	+	++	0	0	+++

miRNAs	293	MCF7	HeLa	HeLa S3	A549	HepG2	K562
miR-186	0	0	+	0	0	0	0
miR-187	0	0	0	0	0	0	+
miR-188	+	+	++	0	+	0	0
miR-189	+	+	0	0	+	+	+
miR-190	0	0	0	0	0	0	0
miR-191	+	+++	+++	+++	++	+	++++
miR-192	+	+++	0	+	+	0	+
miR-193	0	0	0	0	0	0	0
miR-194	+	++++	0	+	+	+	+
miR-195	0	0	0	0	+	0	0
miR-196-2	0	0	0	++	0	0	0
miR-197	0	0	0	0	0	0	+
miR-198	+	++	+	+	+	+	+
miR-199	0	0	+	0	0	0	0
miR-199A-AS	+	++	+	+	+	+	+
miR-200B	+	++	0	0	++	+	0
miR-201	0	0	0	0	0	0	0
miR-202	+	+	0	0	0	0	+
miR-203	0	0	0	0	0	0	0
miR-204	0	0	+	0	0	0	0
miR-205	0	0	0	0	0	0	0
miR-206	+	+	+	0	+	+	0
miR-207	0	0	0	0	0	0	0
miR-208	0	0	0	0	0	+	0
miR-210	0	+	++	+++	0	0	++
miR-211	0	0	+	0	0	0	0
miR-212	0	0	0	0	0	0	0
miR-214	0	0	0	0	0	0	0
miR-215	0	0	+	0	0	0	0
miR-216	+	+	+	0	+	+	+
miR-217	+	+	0	0	+	+	+
miR-218	+	+	+	0	+	+	0
miR-219	0	0	+	0	0	0	0
miR-220	0	0	0	0	0	0	0
miR-221	++	++	+++	+++	+	++	+++
miR-222	+	+	+++	+++	+	+	+
miR-223	+	+	+	0	+	+	+
miR-224	+	+	++	0	0	+	+
miR-290	0	0	+	0	0	0	0

miRNAs	293	MCF7	HeLa	HeLa S3	A549	HepG2	K562
miR-291-5P	+++	+	+	++	+	+	++
miR-292-5P	0	+	0	0	+	0	+
miR-293	0	0	0	0	0	0	0
miR-294	0	0	0	0	0	0	0
miR-295	0	0	0	0	0	0	0
miR-296	++	++	++	+	++	++	++
miR-297	0	0	+	0	0	0	0
miR-298	0	+	0	0	+	0	+
miR-299	0	0	0	0	0	0	0
miR-300	0	0	+	0	0	0	0
miR-301	0	+	0	0	+	0	0
miR-302	+	+	0	0	+	+	0
miR-320	+	++	++++	++++	+	0	++++
miR-321	++++	++++	+++	+++	++++	+++	++++
miR-322	0	0	+	0	0	0	0
miR-323	+	+	+	0	+	+	0
miR-324-5P	0	0	0	0	0	0	0
miR-325	+	0	0	0	+	+	0
miR-326	0	0	0	0	0	0	0
miR-328	0	0	0	0	0	0	0
miR-329	0	0	+	0	0	0	0
miR-330	+	0	0	0	0	0	0
miR-331	0	0	+	0	0	0	0
miR-337	++	+	+	+	++	++	+
miR-338	+	+	+	0	+	+	+
miR-339	0	0	0	0	0	0	0
miR-340	0	0	+	0	0	0	0
miR-341	+	0	0	0	0	0	+
miR-342	+	++	++	++	+	0	+++
miR-344	0	0	+	0	0	0	0
miR-345	0	0	+	0	0	0	+
miR-346	+	0	+	0	0	0	+
miR-350	0	0	0	0	0	0	0
miR-351	++	+	+	0	+	+	+

This table is provided as a resource for selecting cells to use for target-site validation with Pre-miR™ miRNA Precursor Molecules and Anti-miR™ miRNA Inhibitors. The number of +'s indicate the likelihood that detectable levels of a specific miRNA are expressed in a cell type. Note that the arrays that were used to create the table are not designed to provide absolute quantification of specific miRNAs in samples since the hybridization efficiencies of the probes vary for the different miRNAs.

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