# Ambion® *mir*Vana™ miRNA Bioarrays Version 9.2

(Cat #4392878)

*Instruction Manual*

**Manual 4396642 Revision A** **Revision Date: December 10, 2007**

**For research use only. Not for use in diagnostic procedures.**



**Literature Citation:** When describing a procedure for publication using this product, we would appreciate that you refer to it as the Ambion *mir*Vana™ miRNA Bioarrays Kit.

If a paper that cites one of Ambion's products is published in a research journal, the author(s) may receive a free Ambion T-shirt by sending in the completed form at the back of this instruction manual, along with a copy of the paper.

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# I. Introduction

## A. Description of Ambion® *mir*Vana™ miRNA Bioarrays

**Probes for a comprehensive list of known miRNAs, spotted using the leading array technology**

Ambion® *mir*Vana™ miRNA Bioarrays Version 9.2 are high-quality microarrays with probes representing a comprehensive panel of human, mouse, and rat microRNAs. Version 9.2 of *mir*Vana miRNA Bioarrays coincides with Version 9.2 of miRBase Sequence Database, which is curated by the Sanger Institute (microrna.sanger.ac.uk/sequences). Version 9.2 also features probes to detect an exclusive set of 153 human miRNAs. Called Ambi-miRs, these novel miRNAs have been identified through a combination of bioinformatic prediction, cloning, and detection in human total RNA samples.

Table 1. Version 9.2 Content for Ambion® *mir*Vana™ miRNA Bioarrays

| Probe Sets | Number |
|---|---|
| human | 471 |
| mouse* | 380 (221) |
| rat† | 238 (41) |
| Ambi-miRs™ | 153 |
| Controls‡ | 22 |
| Total | 908 |

* Unique probes that are not human are in parentheses.

† Unique probes that are not human or mouse are in parentheses.

‡ The positive control provided with the Ambion® *mir*Vana™ miRNA Labeling Kit is complementary to multiple control probe spots on the Ambion *mir*Vana miRNA Bioarrays, which allow for alignment of the array for scanning.

Manufactured for Ambion by Applied Microarrays, Inc., *mir*Vana miRNA Bioarrays are produced using the CodeLink 3-D Gel Matrix slide surface. This provides an aqueous environment that holds the probe away from the surface of the slide, allowing maximum interaction between probe and target.

Ambion *mir*Vana miRNA Bioarrays are supplied ready for use and require no user processing prior to hybridization. They are designed for use with the positive controls, hybridization, and wash components supplied with the Ambion *mir*Vana miRNA Bioarray Essentials Kit (Cat #AM1567) and Ambion *mir*Vana miRNA Bioarray Spike-In Controls (optional; Cat #4382205), and labeled miRNA samples prepared with the Ambion *mir*Vana miRNA Labeling Kit (Cat #AM1562). The optional Ambion *mir*Vana miRNA Reference Panel (Cat #4388891) provides a quality control sample for miRNA labeling and Bioarray processing. The Ambion *mir*Vana miRNA Bioarray system provides an

efficient, user-friendly platform for miRNA expression profiling that results in sensitive, accurate, and highly reproducible data (see *B. Overview of the Ambion® mirVana Array System* on page 2).

### Ambion miRNA Array Web Resource

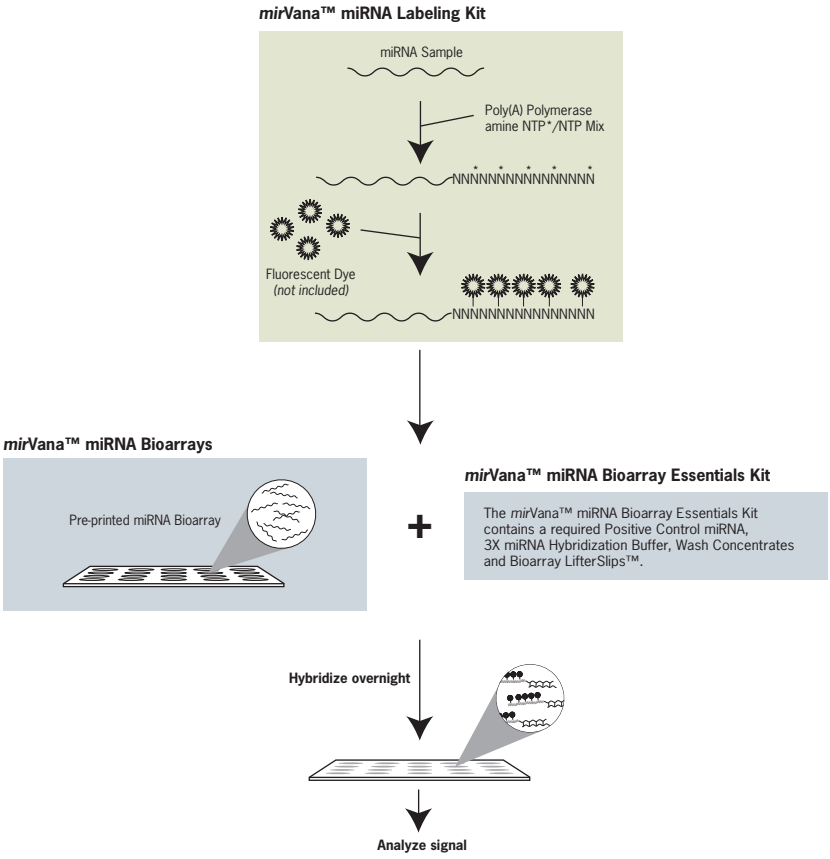
Both the miRNA and microarray fields are rapidly evolving. To provide you with the most up-to-date information on important developments in the miRNA field, and the latest recommendations for analyzing array data, see the dynamic and comprehensive Ambion miRNA Array Resource on the web at: www.ambion.com/miRNA/array

The miRNA Array Resource includes frequently updated miRNA annotation files, miRNA array tips, troubleshooting, support for data analysis, and current versions of all the data files that are included with Ambion *mir*Vana miRNA Bioarrays and *mir*Vana miRNA Probe Set. Visit this resource frequently to ensure you have the most up-to-date miRNA and miRNA analysis information.

## B. Overview of the Ambion® *mir*Vana Array System

The Ambion *mir*Vana Array System includes: the *mir*Vana miRNA Labeling Kit, *mir*Vana miRNA Bioarrays, the *mir*Vana miRNA Bioarray Essentials Kit, the *mir*Vana miRNA Bioarray Spike-In Controls, the *mir*Vana miRNA Reference Panel v9.1, and the *mir*Vana miRNA Probe Set. The system is designed to facilitate analysis of miRNA expression profiles in human, mouse, and rat RNA samples. Figure 1 shows how the *mir*Vana miRNA Bioarrays procedure simplifies sample labeling and purification, and miRNA array hybridization and washing, providing a robust, reproducible platform for miRNA expression profile analysis. Bioarray results for individual targets can be validated using real-time RT-PCR with TaqMan® MicroRNA Assays.

Figure 1. Overview of the Ambion® *mir*Vana™ miRNA Bioarray Procedure



## C. Kit Components and Storage Conditions

| Amount | Component |
|---|---|
| 3 | *mir*Vana miRNA Bioarray V9.2 Slides (2 Bioarrays per slide) |
| 1 | Compact Disc (CD) |

Store *mir*Vana miRNA Bioarrays in their foil packaging at room temperature until use.

Properly stored *mir*Vana miRNA Bioarrays are guaranteed until the expiration date printed on the package.

The supplied CD contains the following data files:

- miRNA Annotations from the miRNA Registry: microrna.sanger.ac.uk/sequences/index.shtml
- Genepix® Array List (GAL) File
- Genepix Settings files for each array on the slide
- Files necessary for CodeLink Software users (Note that CodeLink software is not required for analysis of *mir*Vana miRNA Bioarrays.)

## D. Required Materials Not Provided with the Kit

### Reagents

- 70% ethanol, to clean coverslips
- The *mir*Vana miRNA Bioarray Essentials Kit (Cat #AM1567) is required for *mir*Vana miRNA Bioarray hybridization and washing; it contains the following materials:
  - Positive Control miRNA (provides a saturated signal at Bioarray ID BA10001 to enable alignment of the array for scanning)
  - 3X miRNA Hybridization Buffer
  - Bioarray LifterSlips™
  - Salt Concentrate
  - Detergent Concentrate
- (Optional) Ambion *mir*Vana™ miRNA Bioarray Spike-In Controls, for positive controls that span the signal dynamic range of the *mir*Vana miRNA Bioarray
- (Optional) Ambion *mir*Vana™ miRNA Reference Panel (Cat #4388891), for processing and quality control of the *mir*Vana miRNA Bioarray procedure (requires a *mir*Vana miRNA Bioarray in addition to that used for experimental samples)

### Equipment and supplies

#### Incubators

- Incubator at 95°C
- Incubator at 65°C (hybridization oven recommended)
- Waterbath incubator at 42°C

### General laboratory equipment and supplies

- Microcentrifuge for microcentrifuge tubes
- Nuclease-free microcentrifuge tubes
- Adjustable pipettors, and nuclease-free tips

### Array handling and scanning equipment

- Lint-free or low-lint laboratory wipes: Laboratory wipes are used to clean and dry coverslips for hybridization of *mir*Vana miRNA Bioarrays. Using lint-free wipes, such as those used in cleanrooms, is recommended because it considerably speeds up the process of removing dust particles from coverslips. However, ordinary low-lint laboratory wipes are adequate.
- Array hybridization chamber: we recommend Corning Hybridization Chamber (Product #2551).
- Slide holders and slide wash containers
- Centrifuge or microcentrifuge with a slide carrier or a microtiter plate holder capable of drying slides
- Microarray scanner and image processing software

## E. Related Products Available from Ambion

| | |
|---|---|
| *mir*Vana™ miRNA Labeling Kit<br>Cat #AM1562 | The *mir*Vana miRNA Labeling Kit employs a simple and highly efficient labeling strategy for universal fluorescence labeling of microRNA (miRNA) samples in preparation for microarray analysis. The kit was developed using CyDye™ fluorescent dyes (GE Healthcare), and is fully compatible with other amine reactive dyes. Reagents for purification of labeled miRNA from unincorporated nucleotides and other labeling reaction components are included, resulting in low background signal on miRNA arrays. |
| *mir*Vana™ miRNA Bioarray Essentials Kit<br>Cat #AM1567 | The *mir*Vana miRNA Bioarray Essentials Kit provides all the necessary materials and reagents for hybridization and post-hybridization washing of the *mir*Vana miRNA Bioarrays when using a miRNA preparation that has been labeled using the *mir*Vana miRNA Labeling Kit. The kit also includes a positive control miRNA to spike into your miRNA preparation prior to labeling and hybridization. |
| flashPAGE™ Fractionator<br>Cat #AM13100 | The flashPAGE Fractionator is a specialized electrophoresis instrument for rapid PAGE-purification of small nucleic acids. Designed for use with flashPAGE Pre-Cast Gels and the optimized running buffers supplied in the flashPAGE Buffer Kit, the flashPAGE Fractionator purifies small nucleic acid molecules more quickly, easily, and efficiently than traditional PAGE purification. |
| flashPAGE™ Reaction Clean-Up Kit<br>Cat #AM12200 | The flashPAGE Reaction Clean-Up Kit is a fast and convenient filter-based purification/concentration system for small nucleic acids obtained using the flashPAGE Fractionator. It is a rapid and simple alternative to overnight precipitation. |

| | |
|---|---|
| *mir*Vana™ miRNA Bioarray Spike-In Controls<br>Cat #4382205 | The *mir*Vana™ miRNA Bioarray Spike-In Controls are a set of 10 synthetic 21-mer RNA oligonucleotides designed for use as positive controls with *mir*Vana miRNA Bioarrays. Add *mir*Vana miRNA Bioarray Spike-In Controls, which are premixed at concentrations that span the signal dynamic range of the *mir*Vana miRNA Bioarrays, to miRNA-containing RNA samples before labeling and hybridization to Bioarrays. Use *mir*Vana miRNA Bioarray Spike-In Controls to monitor sample labeling and bioarray processing and to indicate the high and low signal limits on each bioarray, thus providing an indicator of confidence in *mir*Vana miRNA Bioarray data. |
| *mir*Vana™ miRNA Reference Panel v9.1<br>Cat #4388891 | The *mir*Vana™ miRNA Reference Panel v9.1 is a mixture of synthetic RNA oligonucleotides representing an extensive collection of known human, mouse, and rat microRNAs (miRNAs), as annotated in miRBase v9.1 (microrna.sanger.ac.uk/sequences/). The *mir*Vana miRNA Reference Panel is designed for use as a positive control and a quality control sample, validated extensively for microarray analysis and real-time PCR using *mir*Vana miRNA Bioarrays and available TaqMan® MicroRNA Assays, respectively. |
| *mir*Vana™ miRNA Isolation Kit<br>Cat #AM1560 | The *mir*Vana miRNA Isolation Kit (patent pending) is designed especially for the isolation of small RNAs, such as microRNA (miRNA), small interfering RNA (siRNA), and small nuclear RNA (snRNA), from tissues and cells. The kit uses a fast and efficient glass fiber filter (GFF) based procedure to isolate total RNA ranging in size from kilobases down to 10-mers. It also includes a procedure to enrich the population of RNAs that are 200 bases and smaller, which enhances the sensitivity of small RNA detection by solution hybridization and Northern blot analysis. |
| *mir*Vana™ PARIS™ Kit<br>Cat #AM1556 | The *mir*Vana PARIS Kit employs a unique and versatile procedure for quantitative recovery of native protein and all RNA species, including small RNAs such as microRNA (miRNA), small interfering RNA (siRNA), small nuclear RNA (snRNA), and small nucleolar RNA (snoRNA), from the same sample. The kit also includes a procedure to enrich the population of RNAs <200 nt, which can dramatically enhance sensitivity in downstream applications. |

# II. miRNA Bioarray Hybridization and Washing

## A. Preparation of Labeled miRNA Sample

### Obtaining miRNA

Detailed information on obtaining miRNA samples for analysis on *mir*Vana miRNA Bioarrays is provided in the Instruction Manual sent with the *mir*Vana miRNA Labeling Kit and is available for download from the Ambion website by searching for Cat #AM1562, and clicking the *Instruction Manual* link.

Briefly, we recommend the following:

1. Start with total RNA that contains the miRNA fraction. Ambion offers "miRNA certified" FirstChoice® Total RNA as well as kits for isolating RNA that includes small RNA: the *mir*Vana miRNA Isolation Kit (Cat #AM1560) and the *mir*Vana PARIS™ Kit (Cat #AM1556).
2. If the experiment requires small RNA enrichment, e.g., for samples devoid of precursor miRNAs, isolate the miRNA fraction using the Ambion flashPAGE™ rapid electrophoresis system (Cat #AM13100, AM9015, AM10010, AM12200). Alternatively, miRNA can be obtained using traditional PAGE; a protocol, *Gel Purification of miRNA from Total RNA* is available from the Ambion *mir*Vana miRNA Labeling Kit web catalog page or from the Technical Resources/Appendix/Supplemental Protocols section of the Ambion website.

### Labeling miRNA with fluorescent dye(s)

We strongly recommend using the *mir*Vana miRNA Labeling Kit to prepare labeled miRNA samples for analysis on *mir*Vana miRNA Bioarrays.

*mir*Vana miRNA Bioarrays were developed and extensively tested with Cy™5-labeled samples, but samples labeled with any of the following fluorescent dyes are compatible with analysis using *mir*Vana miRNA Bioarrays:

- CyDye™ Post-Labelling Reactive Dye Pack, GE Healthcare (Amersham Biosciences) Product Codes: RPN 5661, 25-8010-80, 25-8010-79
- Invitrogen (Molecular Probes) AlexaFluor® Reactive Dye Decapacks for Microarray Applications
  – AlexaFluor 555, Cat #A32756
  – AlexaFluor 594, Cat #A32751
  – AlexaFluor 647, Cat #A32757

## B. *mir*Vana miRNA Bioarray Hybridization

NOTE

*Since each Bioarray Slide contains 2 Bioarrays, plan to hybridize both Bioarrays at the same time.*

**Incubators needed:**

65°C incubator (for microcentrifuge tubes)

95°C incubator (for microcentrifuge tubes)

42°C waterbath incubator for array hybridization

**1. Preheat 3X miRNA Hybridization Buffer to 65°C for >5 min**

Heat the 3X miRNA Hybridization Buffer to 65°C for at least 5 min immediately before use. Vortex the tube a few times at maximum speed for ~15 sec during this preheating incubation. Even after heating and vortexing, you may see precipitate in the bottom of the tube; it will not affect hybridization.

Keep the solution at 65°C until use.

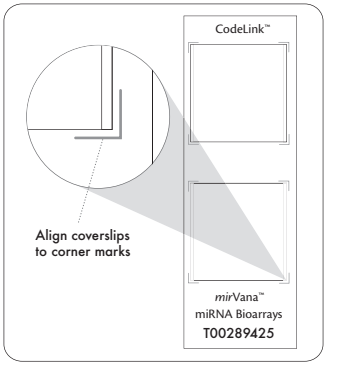
**2. Clean a Bioarray LifterSlip™ and place it over the Bioarray**

a. Clean a Bioarray LifterSlip coverslip with 70% ethanol, and wipe it dry with a lint-free laboratory wipe. (Ordinary low-lint laboratory wipes can be used, but they will deposit some dust particles that must be removed in the next step.)

b. Using the same wipe, remove all dust particles from the coverslip.

c. Examine the Bioarray LifterSlip to determine on which side the raised edges are located. We do this by carefully examining the reflection of overhead lights on the glass; the white strips that "lift" the coverslip away from the slide appear dull because they do not reflect the light. Using Figure 2 as a guide, gently place the Bioarray LifterSlip (raised-edge down) over both miRNA Bioarrays on the slide so that the raised edges align with the long edges of the slide, and with the alignment marks on the slide.

Figure 2. Placement of Bioarray LifterSlips on miRNA Bioarrays



**3. Assemble hybridization mixture with labeled miRNA and 1X final concentration miRNA Hybridization Buffer**

a. Add Nuclease-free Water to bring the volume of the labeled sample to 26 µL. (Use sample prepared with the *mir*Vana miRNA Labeling Kit.)

b. Add 13 µL preheated 3X miRNA Hybridization Buffer and mix thoroughly.
Using the Bioarray LifterSlips provided in the *mir*Vana miRNA Bioarray Essentials Kit, ~36 µL of miRNA hybridization mixture will completely fill the space under the coverslip.

**IMPORTANT**
*It is important to prepare enough miRNA hybridization mixture to completely fill the area under the coverslip; the required volume can vary from 24–36 µL. Also, the final concentration of miRNA Hybridization Buffer in the mixture must be 1X for optimal hybridization.*

**4. Heat to 95°C for 2 min, then let the mixture cool to room temp**

a. Heat the miRNA hybridization mixture to 95°C for 2 min.

b. Briefly centrifuge to bring the sample to the bottom of the tube, and vortex to mix the contents.

c. Centrifuge at 13,000 x g for 1 min to pellet any particulates that might be present.

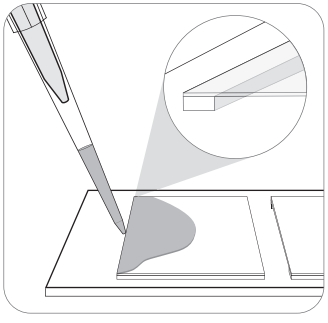
**5. Hybridize for 12–16 hr at 42°C**

a. Place the array slide(s) in a hybridization chamber (e.g., Corning® Product #2551).

b. Carefully remove 36 µL of miRNA hybridization mixture without disturbing any pelleted material, if present.

c. Position the pipette tip at an open side of the coverslip and slowly pipette the miRNA hybridization mixture under the Bioarray LifterSlip onto the *mir*Vana miRNA Bioarray (see Figure 3). Do not reposition the pipette tip while you are dispensing the miRNA hybridization mixture, as this may result in a trapped bubble.

Figure 3. Pipetting miRNA Hybridization Mixture Under the Bioarray LifterSlip



d. Add 1X Hybridization Buffer (dilute the 3X Hybridization Buffer to a 1X concentration with nuclease-free water) to the designated places in the hybridization chamber to maintain humidity during hybridization. This is critical for avoiding problems caused by drying out of the miRNA hybridization mixture.

e. Check the position of the Bioarray LifterSlips, and gently push them back into the alignment marks if they have drifted. Then seal the hybridization chamber completely.

**IMPORTANT**
*Keep the hybridization chamber in the dark during hybridization to avoid photobleaching.*

f. Place the sealed hybridization chamber in a 42°C waterbath (resting on the floor of the waterbath) and incubate for 12–16 hr.
If the waterbath has a clear cover, or a hole for a thermometer, cover the lid to prevent light from entering the waterbath during hybridization.

## C. Washing *mir*Vana miRNA Bioarrays

**IMPORTANT**
*During the wash procedure, carefully avoid exposing the slides to air for more than 1–2 sec. Longer exposures typically result in drying, which causes the formation of precipitates on the array. It is also very important to complete the wash procedure quickly (i.e., in 15 min or less), and in as little light as possible (to avoid photobleaching of the labeled sample).*

### 1. Prepare array wash solutions

- Assemble 2 slide holders and 4 slide wash containers, and determine the volume of wash solution needed to fill the containers so that the slides in a slide holder will be completely immersed in solution.
- You will need 2 containers of Low Stringency Wash, and 2 containers of High Stringency Wash.
- Starting with the nuclease-free water, assemble the wash solutions and mix thoroughly.

Table 2. Wash Solutions for 400 mL Wash Containers

| Low Stringency Wash | High Stringency Wash | |
|---|---|---|
| 752 mL | 780 mL | Nuclease-free Water |
| 8 mL | – – | Detergent Concentrate |
| 40 mL | 20 mL | Salt Concentrate |

### 2. Submerge miRNA Bioarray in Low Stringency Wash and wash for ~30 sec

a. Remove the hybridized miRNA Bioarray slide from the hybridization chamber and submerge it in Low Stringency Wash at room temperature. The coverslip will disengage from the slide and fall to the bottom of container. Quickly place the slide in a slide rack in the second wash container of Low Stringency Wash.

b. Leave the slide in the Low Stringency Wash, and wash at room temperature for ~30 sec by dipping the slide rack up and down in the container of wash solution or with gentle stirring (150–200 rpm).

**3. Change slide rack and wash in High Stringency Wash for 1 min with gentle stirring**

a. Transfer the miRNA Bioarray to a clean slide rack that is already submerged in the High Stringency Wash, being careful not to expose the array to the air for more than 1–2 sec.

b. Wash in High Stringency Wash at room temperature for 1 min by dipping the slide rack up and down in the container of wash solution or with gentle stirring (150–200 rpm).

**4. Wash in fresh High Stringency Wash for 1 min**

Transfer the slide rack with the miRNA Bioarray to a clean container of fresh High Stringency Wash, and wash for 1 min by dipping the slide holder up and down in the container of wash solution or with gentle stirring (150–200 rpm).

**5. Centrifuge the slide to dry it**

a. Rapidly transfer the miRNA Bioarray slide to a centrifuge equipped with a slide holder. Alternatively, the entire slide rack can be centrifuged in a microtiter plate rotor adapter.

b. Start the centrifugation immediately after transferring the slides to the centrifuge. At Ambion, we typically cover the top of the centrifuge to keep out the light.
- In a picofuge, centrifuge for 1–2 min (speed is not adjustable).
- In a tabletop centrifuge, spin at 600 x g for 3 min.

## D. Image Acquisition

Digital images are made up of many small square pixels, each with its own intensity value. The resolution of an image is the total number of pixels and is expressed as the number of rows and columns of pixels in the image (e.g., 1600 x 1200). The number of possible values for an individual pixel is determined by the color depth; 16 bits/pixel is typical for array analysis systems. This means that individual pixels can have intensity values ranging from 0 to 65,535. Pixels exhibiting the maximum intensity value or higher are "saturated" and are assigned the intensity value 65,535. In other words, all signals at the maximum intensity value and higher are assigned the same value.

Scanning is the process of illuminating a microarray with laser light and collecting the light emitted by bound fluorescent dye. The laser is set to emit the wavelength that excites the fluorescent dye used to label the

sample, and the resulting fluorescence is then measured. Any of a number of commercially available scanners can be used to collect and analyze microarray data. Ambion uses the Axon® GenePix 4000B scanner and associated GenePix software.

**Orientation of the Ambion *mir*Vana miRNA Bioarrays on the slide**

In a low resolution scan, *mir*Vana miRNA Bioarrays will be visible as a small square in the middle of the area covered by the coverslip. By zooming in on the array, the positive control spots should be visible in the first and last row. There are two additional control spots on the bottom left of the image.

**Scan settings for *mir*Vana miRNA Bioarrays**

Scan *mir*Vana miRNA Bioarrays at a photomultiplier tube (PMT) setting of 600 with 100% power and 5 µm resolution. Do not adjust these setting for individual arrays.

## E. Evaluating *mir*Vana miRNA Bioarray Data

Currently accepted best practice for evaluating array data is constantly being refined. To access the most timely information, visit the Ambion miRNA Array Web Resource at: www.ambion.com/miRNA/array

The high and low detection limits of each bioarray can be determined by mixing the *mir*Vana™ miRNA Bioarray Spike-In Controls (Cat #4382205) with your miRNA-containing samples prior to labeling. The *mir*Vana miRNA Bioarray Spike-In Controls hybridize to individual probes on *mir*Vana miRNA Bioarrays and are premixed at concentrations that span the signal dynamic range of the bioarrays. However, they should not be used for signal normalization across multiple bioarrays.

# III. Troubleshooting

**Check the Ambion miRNA Array Web Resource**

As new information on troubleshooting *mir*Vana miRNA Bioarrays becomes available, we will post it on the miRNA Array Resource on the web at: www.ambion.com/miRNA/array

## A. Low Overall Fluorescent Signal Intensity

Consider these suggestions if the average signal intensity from both your sample RNA and the Positive Control miRNA are very low (<500 RFU).

**1. Troubleshoot miRNA sample labeling**

The most common cause of low overall fluorescence signal is poor labeling of the miRNA sample. Troubleshooting suggestions for the *mir*Vana miRNA Labeling Kit are provided in the Instruction Manual supplied with the product, and available on our website at www.ambion.com/catalog/CatNum.php?1562

**2. Excessive exposure of the labeled sample to light**

Fluorescent dyes are subject to photobleaching. Exposing them to light, either before or after coupling, will reduce signal intensity. Limit the exposure of fluorescent dyes to light by conducting coupling reactions, labeled miRNA clean-up, array hybridization, and array washing in the dark when possible.

**3. The array slide was placed in the scanner incorrectly**

Improper placement of slides in the scanner, for example, upside down or backwards, can result in no signal from the scanned region. miRNA Bioarrays have writing on them that is legible when they are array-side-up.

**4. Hybridization problems**

**a. Incorrect miRNA Hybridization Buffer concentration**

The dilution of 3X miRNA Hybridization Buffer with labeled miRNA sample must be precise so that the final concentration is 1X miRNA Hybridization Buffer. This ensures the proper formamide concentration for optimal hybridization stringency.

**b. Hybridization and/or wash temperatures were higher than recommended**

The recommended hybridization temperature is 42°C. If in doubt, verify the incubator temperature with a calibrated thermometer.

Conduct posthybridization washes at room temperature. If the ambient room temperature in your lab is over 25°C, washing may be too stringent and could result in loss of legitimate signal.

**5. Nuclease-contaminated tubes, tips, or equipment**

Using pipette tips, tubes, or other plasticware that is contaminated with nucleases during purification and/or handling of miRNA samples can degrade miRNA, reducing yield and the size of miRNA. Both RNases and DNases can be removed from surfaces using Ambion RNase*Zap*® Solution (Cat #AM9780).

## B. Array Signal Not Associated with Probe

If your array image shows blotchy spots, signal that is not associated with probe spots, and/or high background, consider the following troubleshooting suggestions.

**1. Partial or complete drying of the hybridization reaction on the array slide**

- Exposure to air during washing is a common problem encountered by people attempting array analysis for the first time. When miRNA Bioarrays are exposed to air for more than 1–2 sec, it can allow partial drying of the array and problems with blotches, streaks, and dots of signal that are not associated with probe.
- Make sure that the repositories in the hybridization chamber are filled with 1X miRNA Hybridization Buffer to maintain humidity.
- Make sure that the area under the coverslip is completely filled with miRNA hybridization mixture before starting the hybridization.
- Be sure to check the position of the coverslip over each miRNA Bioarray just before sealing the hybridization chamber. If a coverslip gets too close to the edge of the slide, the miRNA hybridization mixture can wick under the slide, leaving the miRNA Bioarray with insufficient hybridization mixture.

**2. Incomplete washing**

If there was a problem with washing the array, then repeating the entire wash procedure may help remove the excess signal from the array. Following are some precautions to observe in subsequent experiments to avoid having to re-wash your array:

- Follow the washing procedure exactly, and be sure to completely submerge the miRNA Bioarray in wash buffer at each step.
- Be sure to put the array slides into a clean slide rack when switching them from the Low Stringency Wash to the High Stringency Wash. Otherwise detergent may be carried over, causing detergent residue on the slides.

## C. High Signal from More Probes Than Expected

### 1. Incomplete reactive dye quenching and removal

If the labeling moiety is not removed by column purification, it can interact nonspecifically with elements on the array. Check the color of the solution of the labeled, purified miRNA; it should be clear or lightly tinted. If the solution is more is more than just lightly tinted, it is an indication that uncoupled dye is present, and it cannot be used for miRNA array hybridization. This could occur if too much fluorescent dye is used in the labeling reaction, or if the glass fiber filter inside the miRNA Labeling Column is not properly positioned and sample bypasses the filter during the purification.

### 2. Hybridization stringency was too low

The recommended hybridization temperature is 42°C. Lower temperatures can lead to nonspecific interactions between miRNAs in the sample and probes on the array. If in doubt, verify the incubator temperature with a calibrated thermometer.

### 3. Nonspecific binding of the labeled RNA

We have found that expression profiles on *mir*Vana miRNA Bioarrays obtained with Ambion FirstChoice Total RNA (which is certified to contain miRNA) are similar to those obtained with RNA enriched for miRNA using the Ambion flashPAGE™ Fractionator. In some cases, however, labeling total RNA may lead to nonspecific binding to the relatively short probes used for miRNAs. If highly purified RNA samples, or samples devoid of precursor miRNA are desired, we recommend purifying miRNAs using the flashPAGE Fractionator before labeling for analysis with the *mir*Vana miRNA Bioarrays. Alternatively, miRNA can be isolated using traditional gel electrophoresis; a detailed protocol is available at the following address:

www.ambion.com/catalog/CatNum.php?1562

# IV. Appendix

## A. *mir*Vana miRNA Bioarrays Specifications

### Kit contents and storage conditions

| Amount | Components |
|---|---|
| 3 | *mir*Vana miRNA Bioarray V2 Slides (2 Bioarrays per slide) |
| 1 | Compact Disc (CD) |

Store *mir*Vana miRNA Bioarrays in their foil packaging at room temperature until use.

Properly stored *mir*Vana miRNA Bioarrays are guaranteed until the expiration date printed on the package.

### To obtain Material Safety Data Sheets

- Material Safety Data Sheets (MSDSs) can be printed or downloaded from product-specific links on our website at the following address:
  www.ambion.com/techlib/msds
- Alternatively, e-mail your request to MSDS_Inquiry_CCRM@appliedbiosystems.com. Specify the catalog or part number(s) of the product(s), and we will e-mail the associated MSDSs unless you specify a preference for fax delivery.
- For customers without access to the internet or fax, our technical service department can fulfill MSDS requests placed by telephone or postal mail. (Requests for postal delivery require 1–2 weeks for processing.)

## B. Quality Control

### Functional testing

Total RNA, including small RNA, from a reference tissue source is labeled and hybridized to a sample set of *mir*Vana miRNA Bioarrays from each production lot. Array signal intensities are correlated with expected results.