Notice to the User

It is important that users read the entire manual before commencing work.

Warranty and Liability

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Mouse & Rat miRNA OneArray® User Guide

Getting Started

Please read the introductory information below to help familiarize with miRNA OneArray® before use.

Product Contents

- Mouse & Rat miRNA OneArray® DNA Microarrays
- 2X miRNA OneArray® Hyb Buffer V3
- miRNA OneArray® Hybridization Buffer II
- Spare round cap tube
- Mouse & Rat miRNA OneArray® User Guide
- Spotted Region Guide

Other Necessary Apparatus (Not Supplied)

Apparatus

- Water bath/heating block
- Powder-free gloves
- Clean, blunt forceps
- Micropipettors
- Sterilized and nuclease-free pipet tips
- Sterilized and nuclease-free microcentrifuge tubes
- High-speed microcentrifuge
- Low-speed tabletop microcentrifuge with slide holder attachment
- Vortex mixer
- Hybridization oven
- Hybridization accessories: chamber cover slides, etc.
- Rectangular slide staining dish and slide rack for washing microarrays
- Thermocycler
- Microarray scanner for standard 1" x 3" format (see Table 8 under "miRNA OneArray® Microarray Scanner Specifications" for a list of compatible scanners)
- Hybridization systems (optional)
- Automated hybridization station (optional)

Other Necessary Reagents (Not Supplied)

Reagents

- De-ionized nuclease-free water
- Cyanine 3- or 5-labeled miRNA sample
- 20X SSC stock solution, sterile filtered
- Wash Solutions, sterile filtered (four types, approximately 250 mL of each is required per experiment):
  - Wash I: 2 X SSC, 0.2% SDS
  - Wash II: 2 X SSC
  - Wash III: 0.2 X SSC
  - **NOTE:** SDS must be molecular biology grade.
- 100% Ethanol
- Pre-hybridization Buffer, prepared and sterile filtered immediately prior to pre-hybridization:
  - 5 X SSPE, 0.1% SDS, 1% BSA
  - **NOTE:** BSA must be molecular biology grade.
- Deionized Formamide.

Important Notes on Microarray Handling and Storage

Storage Conditions

- Store unopened miRNA OneArray® product and 2X miRNA OneArray® Hyb Buffer V3 at room temperature **(avoid exposure to light)**.
- Store opened miRNA OneArray® product at 4°C.
- Store miRNA OneArray® Hybridi^{z}ation Buffer II at -20°C temperature.

NOTE: If the product is received with the seal opened or broken, please contact Phalanx Biotech Customer Service for replacement.

Handling Microarrays

Please read this section carefully and follow the instructions!

- Polynucleotide probes are printed on the side of the slide with the barcode, **do not touch** the printing area.
- Whenever possible, handle microarrays with clean blunt forceps to avoid contamination.

Opened arrays should be used within a week.

Product Description and Overview

Mouse & Rat miRNA OneArray® microarrays are made of polydeoxynucleotide probes spotted onto a proprietary chemical layer coated on top of a 1" x 3" (25 mm x 75 mm) standard-format microarray glass slide.

Each probe is spotted onto the array in a highly consistent manner using a proprietary, non-contact spotting technology adapted for microarray manufacturing.

Mouse & Rat miRNA OneArray® v4 Content

Each microarray contains 1,362 unique Mouse & Rat miRNA probes and 144 experimental control probes. Each unique probe has 3 features, and probes contain 100 % of Sanger miRBase v18 miRNA content.

Mouse & Rat miRNA OneArray® v4		
Scientific Name	*Mus musculus*	*Rattus norvegicus*
Common Name	Mouse	Rat
miRBase Code	mmu	rno
Probe No.	1,129	676
miRNA No.	1,157	680
Total probe No.	1362	
Repeat/ Probe	3	
Control Probe No.	144	
Database	Sanger miRBase v18	

T able 1: Mouse & Rat miRNA OneArray® v4 Content

Using Mouse & Rat miRNA OneArray®

This section provides detailed information about how to perform the hybridization process using Mouse & Rat miRNA OneArray®.

Follow these steps for optimal experimental results.

- **Step 1:** Prepare the RNA Sample
- **Step 2:** miRNA isolation
- **Step 3:** Preparation of the Labeled Targets
- **Step 4:** Pre-Hybridize the Microarray
- **Step 5:** Hybridization Protocol
- **Step 6:** Wash the Hybridized Microarray
- **Step 7:** Scan and Extract Gene Expression Results
- **Step 8:** Check Control Probe Data

Step 1: Prepare the RNA Sample

High-quality, intact RNA is essential for all expression microarray experiments.

Enriched small RNA is recommended for expression profiling on Phalanx's miRNA OneArray. There are many different small RNA isolation protocols and commercially available total RNA isolation kits (containing small RNA). You can isolate small RNA during RNA isolation from tissue or cell samples, or enrich small RNA from total RNA.

We recommend starting from 0.5 - 2.5 μg total RNA or 100-500 ng small RNA is suggested for single Phalanx's miRNA microarray.

RNA Isolation Protocols Recommend:	
Ambion®	miRVana™ miRNA Isolation Kit
Life	Trizol®

Step 2: miRNA isolation

General Guidelines for miRNA isolation

There are many commercially available miRNA isolation kits for microarray analysis. Select an isolation kit or isolation method that is most suitable for your specific needs.

Follow the instructions provided by the reagent supplier. Table 3, below, contains a list of products that have been tested for use with Mouse & Rat miRNA OneArray®. The following protocol is as Pall Nanosep 100K as example.

Table 3: Mouse & Rat miRNA Preparation Products

Manufacturer	Product Name and Description
Ambion®	miRVana™ miRNA Isolation Kit
Pall	Nanosep 100K (miRNA isolation)
Millpore	YM-100

1) Add 50 ul of nuclease-free water to the membrane of the sample reservoir of Nanosep 100K.
2) Spin at 14,000 x g for 10 min, and transfer the sample reservoir of Nanosep 100K to 1.5 ml collect tube
3) Add the ULS labeling sample to sample reservoir of Nanosep 100K. Spin at 5,000 x g for 10 min; collect the flow-through sample.
4) Proceed OD reading and calculate the small RNA amount.

Step 3: Preparation of the Labeled Target

Kreatech ULS miRNA labeling kit is highly recommended.

Table 4 : Compatible miRNA Labeling Kits:	
Kreatech	ULS miRNA labeling kit
Mirus	*Label* IT® miRNA Labeling Kit

Step 3A: ULS Labeling Procedure

1) Mix all kit components thoroughly by vortexing and briefly spin down.
2) Use the same input amount of labeled small RNA and adjust the sample volume to final 15.5μl.

Small RNA (100-500 ng)	10 x labeling solution	Cy5 ULS	Total volume
15.5 μl	2 μl	2.5 μl	20μl

3) Label sample by incubation for 15 minutes at 85 ℃.
4) Add 10 μl of Nuclease-free Water and spin the sample and place on ice till purification using the KREApure columns.

Step 3B: Dye removal using KREApure columns

1) Resuspend the column by vortex.
2) Loosen the cap and snap off the bottom closure.
3) Place the column into a 2ml collection tube and pre-spinning the column for 1 min at 13000rpm. Discard flow-through.
4) Wash the column with 300 μl RNase-free water for spinning column for 1 minute at 13000 rpm.
5) Discard collection tube and flow-through and put column in a new (RNase free) 1.5 ml tube.
6) Add ULS labeled sample on the middle of the column bed without touching it and centrifuge for 1 min at 13000rpm and

collect the flow-through. Keep the labeled samples on ice, in dark and proceed the hybridization.

7) 1.5 µl of labeled miRNA sample, proceed OD reading (260, 280, 550, 649 nm) and calculate labeled small RNA amount and the labeling efficiency.(If you start labeling with 100-500 ng small RNA , you should get 80-400 ng labeled small RNA. Be sure to use the same input amount of small RNA for hybridization)

Labeling Efficiency: # dye molecules/per 1000 nucleotides
Calculation:
Cy5:Cy5 (pmole/ul)/[Conc. (ng/ul)*1000 (pg/ng)/330(pg/NTP)]*1000
Cy3:Cy3 (pmole/ul)/[Conc. (ng/ul)*1000 (pg/ng)/330(pg/NTP)]*1000

Step 4: Pre-Hybridize the Microarray

Mouse & Rat miRNA OneArray® requires a pre-hybridization step prior to hybridization of the labeled target. The pre-hybridization step reduces background signals and increases the performance of the microarray. Complete the pre-hybridization step by the following the instructions.

1) Warm the pre-hybridization solution (5X SSPE, 0.1% SDS, and 1% BSA) to 42°C.
2) Pour 25 ml room temperature 100% ethanol into the spare array tube.
3) Preheat the Mouse & Rat miRNA OneArray® in the round cap tube at 60°C for 10 min (hybridization oven recommended).
4) Remove the Mouse & Rat miRNA OneArray® from the round cap tube, place in the two outermost slots inside the tube containing 100% ethanol, close the cap, and let sit for approximately 15 sec.
5) Shake the round cap tube for 20 sec.
6) Remove and thoroughly rinse each array with deionized water to remove any residual ethanol.

7) Carefully and slowly, fully submerge the Mouse & Rat miRNA OneArray® in an abundant amount of pre-hybridization solution for 2 hr at 42°C (35 ml is sufficient if using a round cap tube).
8) After 2 hr, transfer the slide to room temperature, distilled water and wash gently for 2 min
9) Spin dry the slide for 2 min. Store in a dry, dark place until hybridization. It is recommended to use the slides in the hybridization protocol within 1 hr after completing the pre-hybridization process.

Try to insert the slides into the correct position the first time. Avoid inserting and removing the slides more than once in the pre-hybridization buffer.

Step 5: Hybridization Protocol

There are many different hybridization protocols, apparatus, and instruments available that may be compatible for use with the Mouse & Rat miRNA OneArray® microarray. Detailed instructions for using the glass cover slide method are described below.

It is recommended to use the miRNA OneArray® Hybridization Buffers included with this product to complete the hybridization process.

Step 5A: Complete the Hybridization Using the Glass Cover Slide Method

To complete this step, you will need to select a type of chamber or glass cover slide. Table 5, below, contains a list of products that have been confirmed compatible for use with the miRNA OneArray®.

Table 5: Compatible Glass Cover Slide Products	
Manufacturer	**Product Name**
Corning®	**Cover Glass (22 X 22 mm)**
Erie Scientific Company®	**mSeries LifterSlip™ 22X25I-M5226**

1) Ensure your work and experimentation area, as well as the Mouse & Rat miRNA OneArray®, are clean before adding the Hybridization Buffers solution to the target array.
2) Prepare the labeled miRNA sample to a final volume of 7.3 µL in a 0.2 ml microtube.
3) Add hybridization buffers to each sample according to the Table 6, and briefly vortex and spin-down:

Table 6: Hybridization Components	
For each array: 55 µl	
Component	***Volume***
2 X miRNA OneArray® Hyb Buffer V3	**27.5 µl**
miRNA OneArray® Hybridization Buffer II	**1 µl**
100% deionized Formamide	**5.5 µl**
KREAblock	**13.7 µl**
Labeled miRNA	**7.3 µl**

4) Heat the mixture to 95°C for 2 minutes and soaking at 60°C on a thermocycler[3], and proceed the hybridization in 30 min.
5) Place the Mouse & Rat miRNA OneArray® slide, bar code up, atop the "Probe Printed Region Guide" (included, see Figure 1).

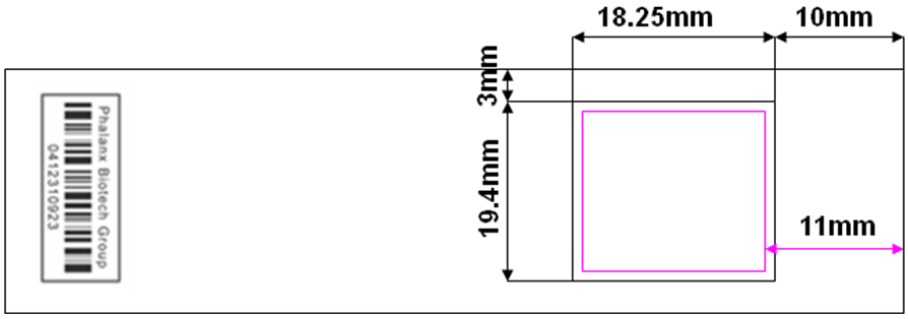


Figure 1: Mouse & Rat miRNA OneArray® Microarray Glass Slide with "Probe Printed Region Guide" Plastic Underlay. (One array per slide)

6) Pipette the hybridization mixture onto the spotted region of Mouse & Rat miRNA OneArray® Microarray. Avoid creating any bubbles.
7) Carefully place the glass cover slide over the spotted area in an even manner.
8) Place the entire labeled target plus the microarray set-up into a closable, chambered box* that is humidified by 2 X SSC buffer in the 37°C oven for 14 to 16 hours. A sealed chamber ensures that the appropriate humidity level is maintained during incubation. (See Figure 2).

Figure 2, below, provides an illustration of Step 5A, where the hybridization protocol is completed using the glass cover slide method, and specifically, the Mouse & Rat miRNA OneArray® Microarray is placed into the chambered box.

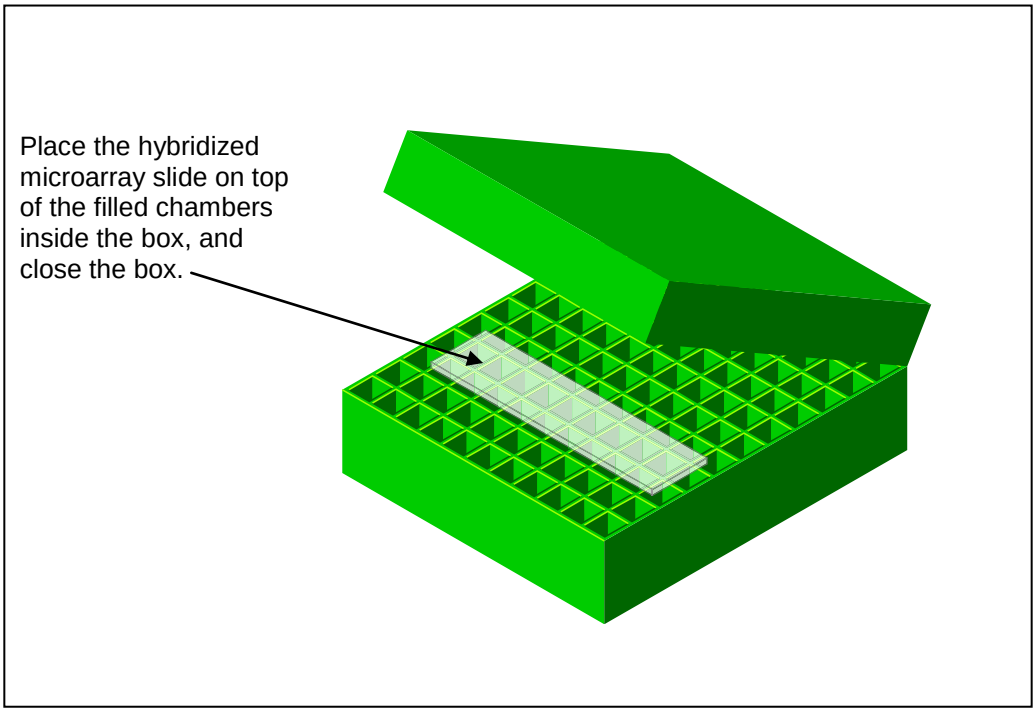


Figure 2: Step 5A → Glass Slide inside Chamber Box[2]

[2] The Hinged 100-Place Storage & Freezer Polypropylene Box from USA Scientific has been used to complete this step with frequent success. The small (approximately ½ inch x ½ inch) chambers within the box are filled about ¾ full of buffer, then the microarrays are laid on top of the chambers. The box is then closed and placed inside the oven. For information about this product or other USA Scientific products, access their Web site at: www.usascientific.com

[3] It may be helpful to set a Denature program in the thermocycler as follows:
95°C – 2 minutes
60°C – Hold

Step 5B: Complete the Hybridization Using the miRNA OneArray® Double Chamber Method

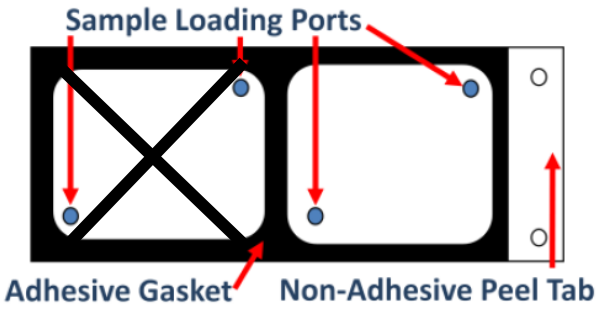


Figure 3: miRNA OneArray® Double Chamber. (Mouse & Rat miRNA OneArray® v3 is one array per slide only)

1) Ensure your work and experimentation area, as well as the Mouse & Rat miRNA OneArray®, are clean before adding the Hybridization Buffers solution to the target array.
2) Remove the clear liner on the back of the hybridization chamber.
3) Align the tab-end of the chamber to the edge of the microarray opposite to the barcode.
4) Slowly guide the chamber onto the microarray. If necessary, pivot the chamber at the tab- end to align the chamber to the microarray.
5) After the chamber has adhered, flip the microarray upside-down with the barcode facing down. On a hard and flat surface, use the applicator stick provided to press along the edges on the glass slide to ensure a secure seal.
6) Allow the adhesive to set for 5 minutes at 42 ℃ before filling samples.
7) Prepare the labeled miRNA sample to a final volume of 12.5 µL in a 0.2ml microtube.
8) Prepare the hybridization mix to each sample according to the Table 7, below.

Table 7: Hybridization Components	
For each array: 90 µl	
Component	***Volume***
2 X miRNA OneArray® Hyb Buffer V3	**45 µl**
miRNA OneArray® Hybridization Buffer II	**1 µl**
100% deionized Formamide	**9 µl**
KREAblock	**22.5 µl**
Labeled miRNA	**12.5 µl**

9) Heat the mixture to 95°C for 2 minutes and soaking at 60°C on a thermocycler[3], and proceed the hybridization in 30 min.

10) Place the Mouse & Rat miRNA OneArray® slide, bar code up, atop the "Probe Printed Region Guide" (included, see Figure 4).

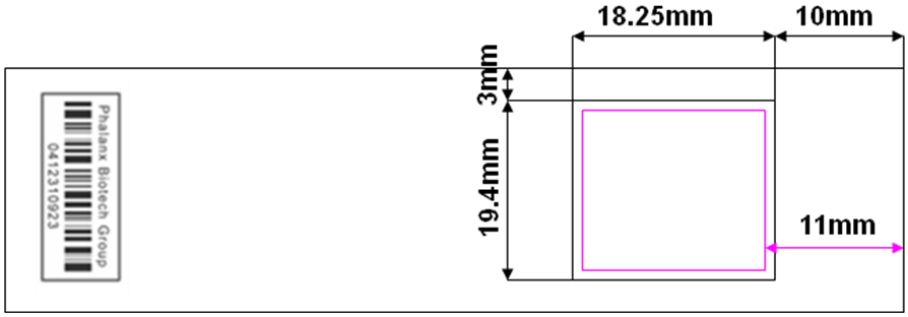


Figure 4: Mouse & Rat miRNA OneArray® Microarray Glass Slide with "Probe Printed Region Guide" Plastic Underlay. (One array per slide)

9) Pipette 75 - 80 µl of labeled RNA solution through one port of the chamber of the array printing area while allowing air to escape through the other port. Avoid creating any bubbles.

[3] It may be helpful to set a Denature program in the thermocycler as follows:
95°C – 2 minutes
60°C – Hold

10) Wipe excess solution from the ports. Covert ports with supplied circular seals.
11) Apply pressure to both seals simultaneously to ensure a secure adhesion.
12) Keep the chamber/microarray assembly at 37 ℃ for 14-16 hrs with vertically rotation overnight at 2 rpm.
13) Pre-warm the wash solution of 2 X SSC, 0.2 % SDS at 37°C in oven overnight.

Step 6: Wash the Hybridized Microarray

Do not allow the microarray(s) to be exposed to air to dry during wash steps or the background signal could be increased.

1) Submerge the over-night hybridized microarray set-up into a large container filled with 37°C 2X SSC, 0.2% SDS.
2) Carefully remove the cover slide either from the glass by gently shaking or tearing the OneArray Chamber apart while the array remains submerged in the buffer.
3) Wash the slide(s) in a fresh container with the excess fresh amount of pre-warmed 2X SSC, 0.2% SDS solution for 5 min at 37°C, 80rpm.
4) Discard the wash buffer and quickly fill with 2X SSC and wash for 5 min at 37°C, 80rpm.
5) Discard the wash buffer and quickly fill with 2X SSC and wash for 5 min at room temperature, 80rpm
6) Rinse each array with 0.2X SSC and spin dry.
7) Store arrays in dry and dark, and scan within 1 day.

Microarrays should be scanned within a couple of hours.

Step 7:

Step 7 Scan and Extract Gene Expression Results

Mouse & Rat miRNA OneArray® Microarray Scanner Specifications

Mouse & Rat miRNA OneArray® is compatible for many microarray scanners with list in Table 9. Select and use a microarray scanner that meets the specifications below.

Microarray Scanner Specifications

Format capabilities:	**1" x 3" (one inch by three inch) glass slide**
Molecular capabilities:	**Able to accurately detect, activate and read Cy3 and Cy5 fluorescent molecules with minimum resolution of 10 μ M**

Table 9: Compatible Microarray Scanners

Manufacturer	Product Name and Description
Molecular Devices	Axon GenePix® 4000B and 4200 series
Genomic Solutions®, Inc.	GeneTAC™ 2000
TECAN®	LS 200/300/400
Agilent Technology	DNA Microarray Scanner G2565B

Use the .gal file and Gene List provided with this product, or refer to our Web site at:

www.onearray.com

Step 8:

Step 8 Check the Control Probe Data

Mouse & Rat miRNA OneArray® Microarrays contains built-in control probes for performance monitoring of the hybridization process. Please visit our website for more information.

http://www.phalanx.com.tw/Support/HmiOA_CP.php

Additional information about the control probes is included on the Product Support CD, and on our Web site at:

www.onearray.com

OneArray® Product Family

- **Human OneArray® v5**

  - 29,187 human genome probes
  - 1,088 experimental control probes
  - Composition: RefSeq release 38 and Ensembl release 56

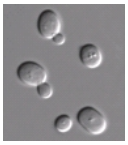
- **Mouse OneArray® v2**

  - 26,423 mouse genome probes
  - 872 experimental control probes
  - Composition: RefSeq release 42 and Ensembl release 59

- **Rat OneArray® v1**

  - 24,358 rat genome probes
  - 980 experimental control probes
  - Composition: RefSeq release 42 and Ensembl release 59

- **Yeast OneArray® v1**



  - 6,958 yeast genome probes
  - 684 experimental control probes
  - Composition: AROS v1.1 and YBOX v1.0

- **Rice OneArray® v1**

  - 22,003 rice genome probes
  - 824 experimental control probes
  - Composition: RGAP v6.1 and BGI 2008

- **Human miRNA OneArray® v4**

  - 1,884 unique miRNA probes
  - 144 experimental control probes
  - 3 features per probe
  - 100 % of Sanger miRBase v18 Human miRNAs

- **Mouse & Rat miRNA OneArray® v4**

  - 1,362 unique miRNA probes
  - 144 experimental control probes
  - 3 features per probe
  - 100% of Sanger miRBase v18 miRNAs