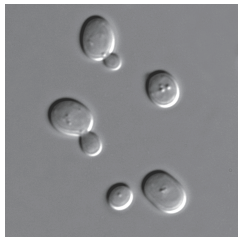
OneArray™



Yeast OneArray™ User Guide

www.OneArray.com

Phalanx Biotech Group

Notice to the User

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

Warranty and Liability

Phalanx Biotech Group's products are intended for research use only, and not intended for any other uses. OneArray® microarray products are designed and manufactured for research use only. Buyers and users agree and understand that they are not granted the right to use OneArray® products for clinical diagnostic purposes unless they obtain written approval from the appropriate government authority. Phalanx Biotech Group (Phalanx Biotech) will not be liable for any damages arising from the use of its products in any manner other than their intended use or for the use of its products for clinical diagnostic purposes without written approval from the appropriate government authority. The manufacture, sale, or importation of products from Phalanx Biotech is not permitted without the prior written consent from Phalanx Biotech. Buyers and users agree and acknowledge that Phalanx Biotech is the owner and has the copyrights to the probe sequence information of all OneArray® products.

Phalanx Biotech is founded on the mission to offer researchers high-quality and user-friendly solutions at an affordable price. Your satisfaction in using our products is very important to us. Therefore, if any of our products is not performing to the standard we promised, we are willing to replace the product, or credit the product purchase price. Phalanx Biotech accepts liability of ONLY the purchase price of its products, and has no other liabilities.

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✧ User Guide and Technical Support

Electronic version of this manual is available on the enclosed Product Support CD, and online at:
www.onearray.com

To reach technical support by telephone, call
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Outside the US: 886.3.5781168

✧ Feedback

We welcome your feedback regarding our products and this manual. Please contact us at:
twsales@phalanxbiotech.com

All comments are welcome.

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Last updated February 2010

Thank You

Phalanx Biotech Group would like to extend special thanks to our customers who have provided feedback that enabled us to improve the Yeast OneArray® User Guide.

User Guide

Getting Started

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

Product Contents

- Yeast OneArray® DNA Microarray
- OneArray® 1.5X Hybridization Buffer

- Each tube contains buffers sufficient for 3-10 microarray hybridization procedures (depending on the hybridization system)
  - Spare round cap tube
  - Yeast OneArray® User Guide
  - Spotted Region Guide
  - Product Support CD, which contains the following:

- Sample Images
- miRNA Yeast OneArray® gal file(s)
- Yeast OneArray® annotation and probe sequence file
- Yeast OneArray® microarray layout
- Yeast OneArray® Control Probe list
- Yeast OneArray® User Guide (electronic version)
- SimpleMeasure experimental control analysis program

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/PCR (polymerase chain reaction) machine
- Microarray scanner for standard 1" x 3" format (see Table 8 under "Yeast 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 yeast sample

- 20X SSPE stock solution, sterile filtered:
  - 3.6 M Sodium chloride
  - 0.2 M Sodium phosphate (pH 7.7)
  - 20 mM EDTA

- **Wash Solutions**, sterile filtered (four types, approximately 250 mL of each is required per experiment):
  - 2 X SSPE, 0.1% SDS
  - 2 X SSPE
  - 0.1X SSPE, 0.1% SDS
  - 0.1X SSPE

  NOTE: ***SDS must be molecular biology grade.***

- 100% **Ethanol**

- Pre-hybridization Buffer, prepared and sterile filtered immediately prior to pre-hybridization:
  - 5X SSPE, 0.1% SDS, 1% BSA

  NOTE: ***BSA must be molecular biology grade.***

- Deionized formamide to be added to the OneArray® Hybridization Buffer prior to use (see Step 4).

- DNA Blocking Mixture:
  - Ambion® sheared Salmon Sperm DNA (10 µg/µl), or Invitrogen™ Cot-1 DNA® (2.5 10 µg/µl), or Invitrogen™ Poly-A (2.5 10 µg/µl)

-

Important Notes on Microarray Handling and Storage

Storage Conditions

- Store unopened Yeast OneArray® product at room temperature.
- Store opened Yeast OneArray® product at 4°C.
- Store OneArray® 1.5X Hybridization Buffer at room temperature.
- NOTE: If the product is received with an open bag, please contact Phalanx Biotech Customer Service for an immediate 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.
- To avoid irreparable damage of the printing area, **do not touch** the surface with bare hands, or with any other objects.
- Whenever possible, handle microarrays with clean blunt forceps to avoid contamination.

Open arrays should be used within a week.

Product Description and Overview

Yeast OneArray® DNA 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.

Yeast OneArray® Genome Content

Each microarray contains 7,642 oligonucleotides: *6,958 yeast genome probes*, and *684 experimental control probes*. Yeast OneArray™ content was selected from Operon Yeast Genome Array-Ready Oligo Set (AROS) v1.1 and Yeast Brown Lab Oligo Extension (YBOX) v1.0.
Yeast AROS is a set of 70-mer probes specially designed within 750 bases from the 3' end of the open reading frames obtained from the *Saccharomyces* Genome Database (Jane 2004).
YBOX is a set of 70-mer probes designed by P. Brown lab at Stanford based on *Saccharomyces* Genome Database (July 2005).
For more information about AROS and YBOX, access the following Web sites:

http://omad.operon.com/download/storage/s_cerevisiae_V1.1.2_datasheet.pdf

http://omad.operon.com/download/storage/s_cerevisiae_core_V1.0.2_datasheet.pdf

Table 1, below, provides an example of the contents of a yeast genome that can be studied using the Yeast OneArray®

Table 1: Yeast Genome Content

Probe type	**Number of Probes**
Yeast genome	**6,958**
***Saccharomyces cerevisiae* open reading frames**	**6,787**
***Saccharomyces cerevisiae* non-coding RNAs including tRNAs, snoRNAs, rRNAs,etc)**	**171**

Yeast OneArray® Control Features

There are 684 control probes including alignment, extrinsic target quality, positive and negative controls built into the Yeast OneArray® DNA microarray that monitor the sample quality and hybridization process. These control probes provide valuable information to ensure experiments are done correctly to ensure higher quality results for analysis.

SimpleMeasure™ is a small, free Java-based applet designed to analyze control probe data and generate easy-to-interpret graphs. The program can be downloaded from http://www.onearray.com

NOTE: ***Detailed control information, gene lists, gene annotations, and probe sequences can be found on the Product Support CD that accompanied this product, or at: http://www.onearray.com***

Table 2: Alignment Controls		
Phalanx Probe Index	**Gene Name**	**Description**
PH_c_0000001	GAM Control	Grid alignment marks, Cy3 labeled
PH_c_0000002	Cy3 Control	Cy3 intensity ladder no. 1, 0.39 µM
PH_c_0000003	Cy3 Control	Cy3 intensity ladder no. 2, 0.78 µM
PH_c_0000004	Cy3 Control	Cy3 intensity ladder no. 3, 1.56 µM
PH_c_0000005	Cy3 Control	Cy3 intensity ladder no. 4, 3.13 µM
PH_c_0000006	Cy3 Control	Cy3 intensity ladder no. 5, 6.25 µM
PH_c_0000007	Cy3 Control	Cy3 intensity ladder no. 6, 12.5 µM
PH_c_0000008	Cy3 Control	Cy3 intensity ladder no. 7, 25 µM
PH_c_0000009	Cy3 Control	Cy3 intensity ladder no. 8, 50 µM
PH_c_0000010	Cy5 Control	Cy5 intensity ladder no. 1, 0.39 µM
PH_c_0000011	Cy5 Control	Cy5 intensity ladder no. 2, 0.78 µM
PH_c_0000012	Cy5 Control	Cy5 intensity ladder no. 3, 1.56 µM
PH_c_0000013	Cy5 Control	Cy5 intensity ladder no. 4, 3.13 µM
PH_c_0000014	Cy5 Control	Cy5 intensity ladder no. 5, 6.25 µM
PH_c_0000015	Cy5 Control	Cy5 intensity ladder no. 6, 12.5 µM
PH_c_0000016	Cy5 Control	Cy5 intensity ladder no. 7, 25 µM
PH_c_0000017	Cy5 Control	Cy5 intensity ladder no. 8, 50 µM
PH_c_0000072	CGAM Control	Corner Grid alignment marks, Cy3 and Cy5 labeled

Table 3: Extrinsic Target Quality Controls		
Phalanx Probe Index	**Gene Name**	**Description**
AM_c_0000015	Ambion Spike Control_1	Ambion ArrayControl RNA SPIKE 1
AM_c_0000016	Ambion Spike Control_2	Ambion ArrayControl RNA SPIKE 2
AM_c_0000017	Ambion Spike Control_3	Ambion ArrayControl RNA SPIKE 3
AM_c_0000018	Ambion Spike Control_4	Ambion ArrayControl RNA SPIKE 4
AM_c_0000019	Ambion Spike Control_5	Ambion ArrayControl RNA SPIKE 5
AM_c_0000020	Ambion Spike Control_6	Ambion ArrayControl RNA SPIKE 6
AM_c_0000021	Ambion Spike Control_7	Ambion ArrayControl RNA SPIKE 7
AM_c_0000022	Ambion Spike Control_8	Ambion ArrayControl RNA SPIKE 8
ST_c_0000001	Stratagene Alien Control_1	Stratagene Alien 1
ST_c_0000002	Stratagene Alien Control_2	Stratagene Alien 2
ST_c_0000003	Stratagene Alien Control_3	Stratagene Alien 3
ST_c_0000004	Stratagene Alien Control_4	Stratagene Alien 4
ST_c_0000005	Stratagene Alien Control_5	Stratagene Alien 5
ST_c_0000006	Stratagene Alien Control_6	Stratagene Alien 6
ST_c_0000007	Stratagene Alien Control_7	Stratagene Alien 7
ST_c_0000008	Stratagene Alien Control_8	Stratagene Alien 8
ST_c_0000009	Stratagene Alien Control_9	Stratagene Alien 9
ST_c_0000010	Stratagene Alien Control_10	Stratagene Alien 10
PH_c_0000066	Phalanx ETQ Control_1	Probe for Extrinsic Target Quality Control No.1
PH_c_0000067	Phalanx ETQ Control_2	Probe for Extrinsic Target Quality Control No.2
PH_c_0000068	Phalanx ETQ Control_3	Probe for Extrinsic Target Quality Control No.3
PH_c_0000069	Phalanx ETQ Control_4	Probe for Extrinsic Target Quality Control No.4
PH_c_0000070	Phalanx ETQ Control_5	Probe for Extrinsic Target Quality Control No.5
PH_c_0000071	Phalanx ETQ Control_6	Probe for Extrinsic Target Quality Control No.6

Table 4: Positive & Negative Controls		
Phalanx Probe Index	**Gene Name**	**Description**
YLL039C_01	Operon positive control_UBI4	Ubiquitin, becomes conjugated to proteins, marking them for selective degradation via the ubiquitin-26S proteasome system; essential for the cellular stress response
YBL092W_01	Operon positive control_RPL32	Protein component of the large (60S) ribosomal subunit, has similarity to rat L32 ribosomal protein; over expression disrupts telomeric silencing
YBR181C_01	Operon positive control_RPS6A	Protein component of the small (40S) ribosomal subunit; identical to Rps6Ap and has similarity to rat S6 ribosomal protein
YER103W_01	Operon positive control_SSA4	Heat shock protein that is highly induced upon stress; plays a role in SRP-dependent cotranslational protein-membrane targeting and translocation; member of the HSP70 family; cytoplasmic protein that concentrates in nuclei upon starvation
YFL039C_01	Operon positive control_ACT1	Actin, structural protein involved in cell polarization, endocytosis, and other cytoskeletal functions
YIL115C_01	Operon positive control_NUP159	Subunit of the nuclear pore complex that is found exclusively on the cytoplasmic side, forms a subcomplex with Nup82p and Nsp1p, required for mRNA export
YJL052W_01	Operon positive control_TDH1	Glyceraldehyde-3-phosphate dehydrogenase, isozyme 1, involved in glycolysis and gluconeogenesis; tetramer that catalyzes the reaction of glyceraldehyde-3-phosphate to 1,3 bis-phosphoglycerate; detected in the cytoplasm and cell-wall
YJL061W_01	Operon positive control_NUP82	Subunit of the nuclear pore complex (NPC), forms a subcomplex with Nup159p and Nsp1p, interacts with Nup116p and is required for proper localization of Nup116p in the NPC
YJR009C_01	Operon positive control_TDH2	Glyceraldehyde-3-phosphate dehydrogenase, isozyme 2, involved in glycolysis and gluconeogenesis; tetramer that catalyzes the reaction of glyceraldehyde-3-phosphate to 1,3 bis-phosphoglycerate; detected in the cytoplasm and cell-wall
YLL026W_01	Operon positive control_HSP104	Heat shock protein that cooperates with Ydj1p (Hsp40) and Ssa1p (Hsp70) to refold and reactivate previously denatured, aggregated proteins; responsive to stresses including: heat, ethanol, and sodium arsenite; involved in [PSI+] propagation
YLR212C_01	Operon positive control_TUB4	Gamma-tubulin, involved in nucleating microtubules from both the cytoplasmic and nuclear faces of the spindle pole body
YPL016W_01	Operon positive	Subunit of the SWI/SNF chromatin

	control_SWI1	remodeling complex, which regulates transcription by remodeling chromosomes; required for transcription of many genes, including ADH1, ADH2, GAL1, HO, INO1 and SUC2
YCONTROL02	Operon Negative control_1	Randomly_generated_negative_control
YCONTROL04	Operon Negative control_2	Randomly_generated_negative_control
YCONTROL19	Operon Negative control_3	Randomly_generated_negative_control
YCONTROL57	Operon Negative control_4	Randomly_generated_negative_control
YCONTROL60	Operon Negative control_5	Randomly_generated_negative_control
YCONTROL63	Operon Negative control_6	Randomly_generated_negative_control
YCONTROL77	Operon Negative control_7	Randomly_generated_negative_control
YCONTROL91	Operon Negative control_8	Randomly_generated_negative_control
YCONTROL97	Operon Negative control_9	Randomly_generated_negative_control
YCONTROL98	Operon Negative control_10	Randomly_generated_negative_control
YCONTROL99	Operon Negative control_11	Randomly_generated_negative_control
YCONTROLAF	Operon Negative control_12	Randomly_generated_negative_control

NOTE: Detailed control information, gene lists, gene annotations, and probe sequences can be found on the Product Support CD that accompanied this product, or at:

http://www.phalanxbiotech.com

Using OneArray®

This section provides you with detailed information about how to perform the steps necessary to complete the hybridization process to study gene expressions using the OneArray® microarray.

Follow these detailed steps *exactly* to achieve the best experimentation results.

- **Step 1:** Prepare the RNA Sample
- **Step 2:** Label the Target
- **Step 3:** Pre-Hybridize the Microarray
- **Step 4:** Perform the Hybridization Protocol
- **Step 5:** Wash the Hybridized Microarray
- **Step 6:** Scan and Extract Gene Expression Results
- **Step 7:** Check Control Probe Data

Step 1: Prepare the RNA Sample

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

There are many different RNA isolation protocols and commercially available RNA isolation kits. You should choose a solution that meets your specific needs. Qiagen, Ambion, Invitrogen, and other reagent companies offer many different RNA isolation products. For more information, you can visit each company's Web site.

Once the RNA samples are isolated, you must confirm the quantity and quality of the samples. Similarly, many different protocols are available and you should choose a solution that is suitable for your needs.

For faster and more automated RNA analysis, you may want to consider the "No Cuvettes" Spectrophotometer from NanoDrop®, or the 2100 Bioanalyzer from Agilent Technologies. For more information, visit each company's Web site.

Step 2: Label the Target

For best results, it is recommended that you use one of the commercially available labeling kits that has been tested for use with the OneArray® microarray—please refer to Tables 3 and 4 below.

General Guidelines for Target Labeling

There are many commercially available labeling kits for microarray analysis. Select a labeling kit or labeling method that is most suitable for your specific needs. If you use a labeling kit that is not listed in Tables 5, it is recommended that you validate the method to test and determine its compatibility with the OneArray®

You may want to confirm the quality of the labeled target with the "No Cuvettes" Spectrophotometer from NanoDrop®.

RNA Sample Amounts

Generally, the amount needed of quality RNA is ~20 μg for each labeling reaction.

If you have an *ample* supply of RNA samples, you have the *choice* of using a protocol that either amplifies or does not amplify the RNA sample.

If you have a *limited* amount of RNA samples, it is recommended that you use a protocol that includes a linear amplification of the RNA samples.

Dye Incorporation Efficiency

Good dye incorporation rates are important for yielding the best data from microarray hybridization. Incorporation rates of 30-60 dye molecules per 1000 bases (17-33 bases/dye molecule) yield the most usable data. Rates below 15 dyes per 1000 bases (50 bases/dye) are very low and may lead to a loss of signal of many

targets. It is not recommended to perform hybridization with samples of low dye incorporation efficiency.

For aRNA Hybridization

Follow the instructions provided by the reagent supplier. Indirect labeling with NHS ester dye is recommended. Table 5, below, contains a list of products that have been tested for use with OneArray®.

Table 5: aRNA Preparation Products

Manufacturer	Product Name and Description
Ambion®	Amino Allyl MessageAmp II™ aRNA Kit
Ambion®	aRNA Fragmentation Reagent
Epicentre® Biotechnologies	TargetAmp™ 1-Round Aminoallyl -aRNA Amplification Kit

For aRNA labeling, >20 µg of quality aRNA is recommended. Smaller volumes can lead to significant loss of sample and may increase the concentration of contaminants in the labeled aRNA sample, leading to higher background signal.

It is best to use aRNA as soon as possible after labeling, as exposure to air and light can reduce the signal of some dyes. If it must be left overnight, it is best to aliquot your labeled aRNA and store in the dark at -80°C. Avoid thawing and refreezing aRNA if possible, as freeze-thaw cycles can damage the aRNA.

Finally, aRNA fragmentation is best performed immediately prior to hybridization (Step 4).

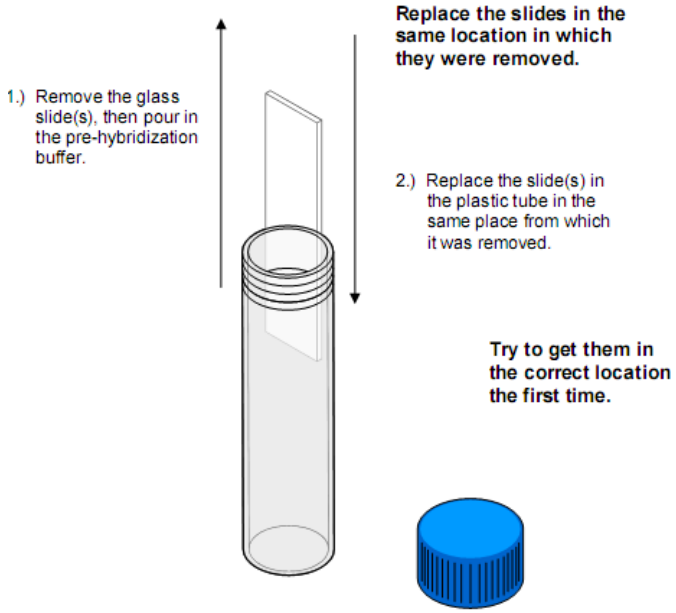
Step 3: Pre-Hybridize the Microarray

General Instructions

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 prehybridization step by carefully following the instructions below.

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 OneArray® (s) in the round cap tube at 60°C for 10 min (hybridization oven recommended).
4) Remove the OneArray® (s) 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 1-2 minutes.
6) Remove and thoroughly rinse each array with deionized water to remove any residual ethanol.
7) Carefully and slowly, fully submerge the OneArray® in an abundant amount of pre-hybridization solution for 2 hrs at 42°C (35 ml is sufficient if using a round cap tube).

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.



8) After 2 hrs, transfer the slide(s) to room temperature, distilled water and wash by reversing the cap tube for 2 min.
9) Spin dry the slide(s) for 2 min. Store in a dry, dark place until hybridization. It is recommended that you use the slides in the hybridization protocol within 1 hr of completing the pre-hybridization process.

Step 4: Complete the Hybridization Protocol

Once you have completed the pre-hybridization step using one of the methods outlined in the Step 3: Pre-Hybridize the Microarray section, you are ready to complete the hybridization protocol.

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

For best performance and consistent hybridization results, it is recommended that you use the OneArray® Hybridization Buffer, included with this product to complete the hybridization process.

A: Hybridization Using the Glass Cover Slide Method

Step 4A(i): →Prepare Hybridization Solution Using the OneArray® Hybridization Buffer (Included)

For correct use of this buffer, you must add a specific amount of formamide and labeled target. Please follow the instructions below carefully.

1) Spin down the stock OneArray® Hybridization Buffer (~ 410 µl in each tube).
2) Add 90 µl of deionized formamide.
3) Warm the mixture to 42°C to completely dissolve the solution. Mix thoroughly.
   Yield: 500 µl of 1.5X Hybridization Buffer solution.
4) Make up 1 X Hybridization Buffer by adding nuclease-free H_2O.
5) Aliquot the solution into individual tubes according to usage and store in darkness at -20°C.

Step 4A(ii): →Prepare Target for Hybridization

- Hybridization Using Labeled Targets from aRNA Labeling Approaches

1) Mix 2 μg of your aRNA sample with nuclease-free H_2O to yield a final volume of 9 μL.

NOTE: ***It is essential to use at least 2 μg of labeled target for each hybridization. If you are performing a dual-dye experiment, use at least 2 μg of each labeled aRNA sample.***

2) Add 1 μl 10x Fragmentation Reagent, and incubate at 70°C for 15 minutes.

3) Add 1 μl Stop Solution, and mix well.

4) Mix with nuclease-free H_2O to yield a final volume of 17 μL.

5) Keep on ice and in darkness until hybridization (Step 4A(iii)).

Step 4A(iii): →Complete the Hybridization

NOTE: ***If you perform hybridization using methods other than the basic glass cover slide method, it is recommended that you validate the protocol experimentally. For example, the phalanx hybridization system, the MAUI System from BioMicro Systems, or HS Series of Hybridization Stations from TECAN offer a higher throughput and more automated hybridization methods.***

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

Table 5: Compatible Glass Cover Slide Products	
Manufacturer	**Product Name**
BioRad® Laboratories	**SLS 6001 (24x60 mm)**
Erie Scientific Company®	**mSeries LifterSlip™ 25x601-M-5439**
Corning®	**Cover Glass (24 X 60 mm)**

1) Ensure your work and experimentation area, as well as the OneArray®, are clean before adding the Hybridization Buffer solution to the target array.

2) Pre-warm the Hybridization Buffer with formamide at 42°C for 10 minutes.

3) Prepare the hybridization mix in a 1.5 ml Eppendorf tube according to the Table 6, below.

Table 6: Hybridization Mix Measurements	
For each slide: 55 µl	
Component	***Final Volume***
1.5X OneArray® Hybridization Buffer	**37 µl**
Sheared Salmon Sperm DNA (10 µg/µl)*	**1 µl**
Target preparation plus nuclease-free ddH_2O	**17 µl**

* Alternatives to Salmon Sperm DNA Blocking Mixtures:
  Ambion® sheared Salmon Sperm DNA (10 µg/µl), or
  Invitrogen™ Cot-1 DNA® (2.5 10 µg/µl), or
  Invitrogen™ Poly-A (2.5 10 µg/µl)

4) Spin down the mixture for 5 minutes to eliminate potential debris.

5) Transfer the mixture to a new tube.

6) Heat the mixture to 95°C for 5 minutes (thermocycler recommended).

7) Maintain the mixture at a temperature of 60°C until pipetting onto the array (thermocycler recommended[1]).

8) Place the OneArray® slide, bar code up, atop the "Probe Printed Region Guide" (included, see Figure 1).

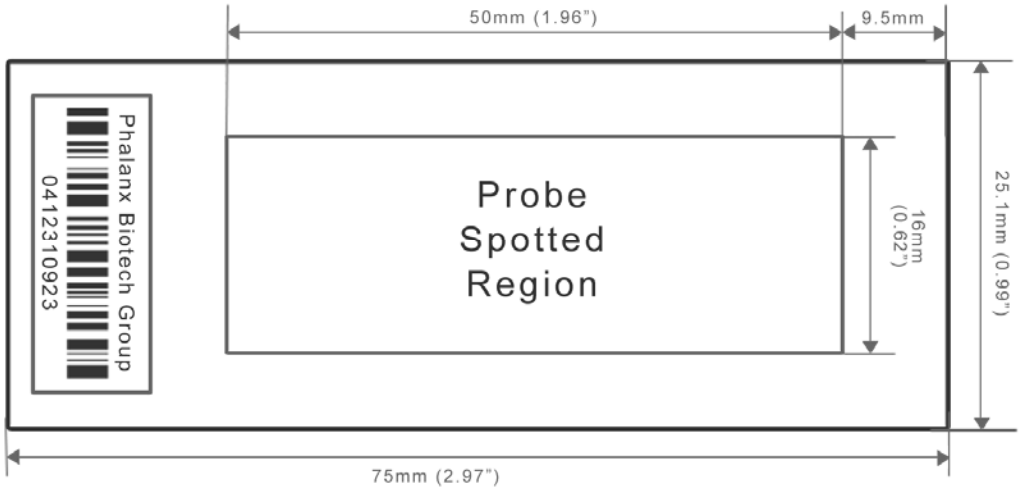


Figure 1: OneArray® Microarray Glass Slide with "Probe Printed Region Guide" Plastic Underlay.

[1] It may be helpful to set a Denature program in the thermocycler as follows:

95°C – 5 minutes

60°C – Hold

9) Pipette the hybridization mixture onto the spotted region of OneArray® DNA Microarray. Avoid creating any bubbles.

10) Carefully place the glass cover slide over the spotted area in an even manner.

11) Place the entire labeled target plus the microarray set-up into a closable, chambered box* that is humidified by 2X SSPE buffer in the 50°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 4A(iii), where the hybridization protocol is completed using the glass cover slide method, and specifically, the OneArray® DNA Microarray is placed into the chambered box.

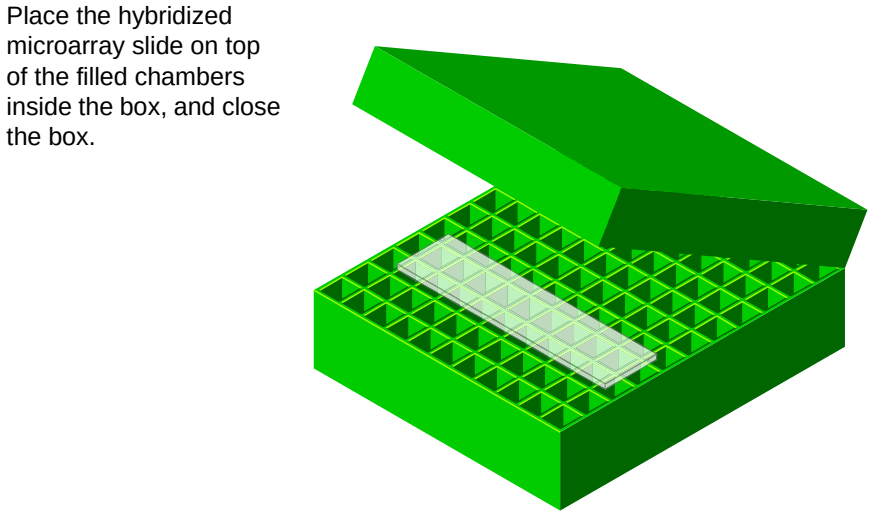


Figure 2: Step 4A(iii) → aRNA Hybridization—Glass Slide Inside Chamber Box[2]

[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

B. Using OneArray® Full Length Chamber

Step 4B(i): →Prepare Hybridization Solution Using the OneArray® Hybridization Buffer (Included)

For correct use of this buffer, you must add a specific amount of formamide and labeled target. Please follow the instructions below carefully.

1) Spin down the stock OneArray® Hybridization Buffer (~410µl in each tube).

2) Add 90 µl of deionized formamide.

3) Warm the mixture to 42 °C to completely dissolve the solution. Mix thoroughly.
   Yield: 500 µl of 1.5X Hybridization Buffer solution.

4) Make up 1 X Hybridization Buffer by adding nuclease-free H_2O.

5) Aliquot the solution into individual tubes according to usage and store in darkness at -20 °C.

Step 4B(ii): →Prepare Target for Hybridization

- Hybridization Using Labeled Targets from aRNA Labeling Approaches

6) Mix 10 µg of your aRNA sample with nuclease-free H_2O to yield a final volume of 27 µL.

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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.usasciesntific.com

NOTE: ***It is essential to use at least 10 μg of labeled target for each hybridization. If you are performing a dual-dye experiment, use at least 10 μg of each labeled aRNA sample.***

7) Add 3 μl 10x Fragmentation Reagent, and incubate at 70 °C for 15 minutes.

8) Add 3 μl Stop Solution, and mix well.

9) Mix with nuclease-free H_2O to yield a final volume of 67 μL.

10) Keep on ice and in darkness until hybridization (Step 4B(iii)).

Step 4B(iii): →Complete the Hybridization

11) Thaw and re-suspend the 1.5X and 1X Working Hybridization buffer at 42°C for 10 minutes.

12) Prepare Target Hybridization Mix:

Final Total Volume of Target Hybridization Mix	200 μl
Labeled target mix	67 μl
1.5X Working Hybridization Buffer Add RNAase free ddH_2O to reach the final volume	133 μl

NOTE: ***Different volumes of labeled target mix may be obtained due to different labeling protocols. If the final volume of the labeling target mix is less, use distilled water to make up the volume.***

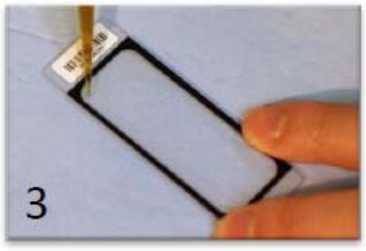
13) Denature the Target Hybridization Mix from the previous step in a PCR machine at 95 °C for 5 minutes and hold at 60 °C.

14) Assembling Process:

i. Remove the clear liner on the back of the hybridization chamber. Align the tab-end of the chamber to the edge of the microarray opposite to the barcode. It is easier to hold the long edges of the chamber in one hand and press down the tab with the other hand.(Figure 1)

ii. Use the applicator stick provided to press along the adhesive areas to ensure a secure seal. Visually inspect the seal from underneath the microarray; inconsistent patterns in the black adhesive may indicate an insecure seal. Re-use the applicator stick if needed (Figure 2)

iii. Allow the adhesive to set for 30 minutes



iv. Pipette 200 µL of the labeled RNA solution through one port of the chamber while allowing air to escape through the other port. Make sure there are no bubbles in the pipette tip. If air bubbles form within the chamber, light pressure may be applied to the surface to dislodge them. (Figure 3)

v. Wipe excess solution from the ports. Be careful not to draw solution from the chamber.

vi. Cover ports with supplied circular seals. Seals should be removed from the liner and applied using forceps. The seals will adhere to most wet surfaces. Apply pressure to both seals simultaneously to ensure a secure adhesion. (Figure 4)

vii. Keep the chamber/microarray assembly at 50°C for 14-16 hrs. Rotation of the assembly during hybridization has been shown to increase the signal intensity.

Step 5: Wash the Hybridized Microarray

Washed and dried microarrays should be scanned within a couple of hours.

NOTE: ***Do not allow the microarray(s) to be exposed to air for a significant amount of time; otherwise, an increased fluorescent background signal could appear.***

1) Submerge the entire labeled target and microarray set-up with the cover slide still intact into a large container filled with 42°C 2X SSPE, 0.1% SDS solution.
2) If cover slide was used for hybridization, carefully remove the cover slide from the glass by gently shaking the glass slide so that the cover slide is freed while the slide is submerged. If OneArray® Full Length Chamber was used for hybridization, use forceps to slowly lift and remove the chamber starting from the

tab-end. Use the holes in the tab for a better grip. Be sure to keep the microarray under the wash solution during removal.

NOTE: ***At this stage, the microarray has the highest concentration of unhybridized target and dye. Transfer the array quickly to the slide rack to minimize exposure to air.***

3) Wash the slide(s) in the "rectangular, slide staining dish and slide rack" with the excess amount of pre-warmed 2X SSPE, 0.1% SDS solution for 5 min at 42°C under slightly rocking (around 80 rpm) condition.
4) Transfer the slide rack to a second slide staining dish that contains 0.1X SSPE, 0.1% SDS solution and wash for 5 min at 42°C under slightly rocking (around 80 rpm) condition.
5) Transfer the slide rack to a third slide staining dish that contains 0.1X SSPE and wash for 5 min at room temperature under slightly rocking (around 80 rpm) condition.
6) Rinse each array carefully with 0.1X SSPE using a squeeze bottle.
7) Spin dry with a centrifuge for at least 1 min.
8) Keep the microarray dry and in the dark until ready to scan.

Step 6:

Scan and Extract Gene Expression Results

There are many scanners available to extract signals from miRNA OneArray®. Data extraction using GenePix™ 4100 from Molecular Devices is described below. Please refer to the respective company product instructions for appropriate use.

Table 8, below, lists the setting for using the GenePix 4100. For a list of scanners that are compatible with the OneArray®, please refer to Table 8, below.

NOTE: *The performance of each scanner may differ. Therefore, to ensure best results, it is recommended that the scanner be adjusted based on standard microarray calibration parameters. Turn on and warm up the scanner for the duration according to manufacture instructions for the scanner.*

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

www.phalanxbiotech.com

Table 8: Scanner Settings Using GenePix™4100 from Molecular Devices

Wavelength	**635 nm**	**532 nm**
PMT	**630V**	**590V**
Minimum diameter (%)	**50**	
Maximum diameter (%)	**200**	
CPI Threshold	**100**	

NOTE: *For lower versions of GenePix software, adjust the property parameter to 142.8 μm manually to obtain best results.*

Figure 3, below provides a visual example of the OneArray® glass slide with spotted probe region.

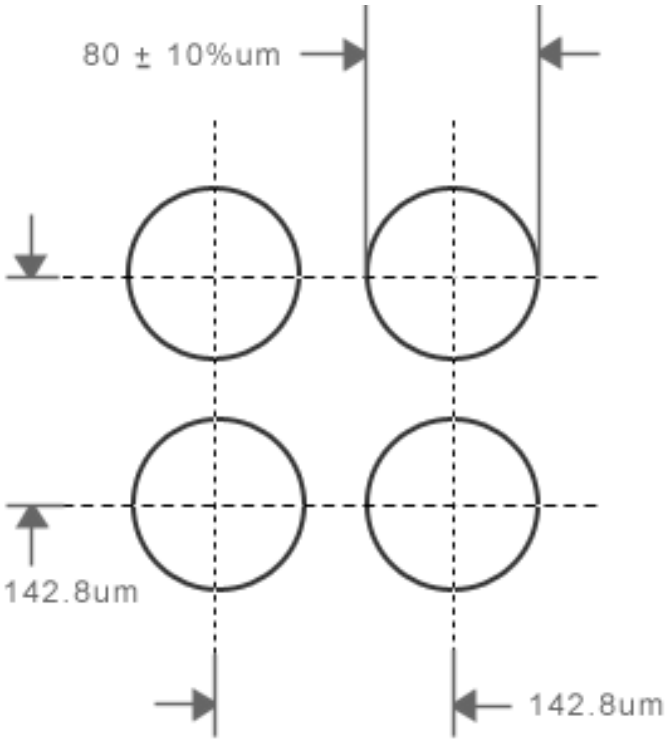


Figure 3: OneArray® Glass Slide with Spotted Probe Region.

OneArray® Microarray Scanner Specifications

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**

Table 9, below, contains a partial list of microarray scanner products that are compatible for use with the OneArray® microarray. Please refer to the respective company website for more information about the products listed below.

Table 9: Compatible Microarray Scanners

Manufacturer	Product Name and Description
Molecular Devices	**Axon GenePix® 4000, 4100, and 4200 series**
Genomic Solutions,® Inc.	**GeneTAC™ 2000**
Perkin Elmer,® Inc.	**ScanArray™ 5000**
TECAN®	**LS 200/300/400**
Agilent Technology	**DNA Microarray Scanner G2565B**

Step 7: Check the Control Probe Data

OneArray® Microarrays contains built-in control probes for performance monitoring of the hybridization process. They are used to confirm or deny whether the experiment was completed successfully. Please visit http://www.phalanxbiotech.com/Support/Support.html

for more detailed information about the experimental controls on your OneArray® product.

Alternatively, SimpleMeasure™ is a small, free Java-based applet designed to analyze control probe data and generate easy-to-interpret graphs. The program is included on your product CD or can be downloaded from

http://www.phalanx.com.tw/tech_support/support_tab.html

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

- **Yeast OneArray®**

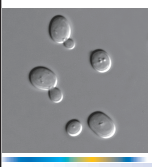
  - 6,958 yeast genome probes
  - 684 experimental control probes
  - Composition: AROS v1.1 and YBOX v1.0.

- **Human miRNA OneArray® v2**

  - 1,087 unique miRNA probes
  - 105 experimental control probes
  - 3 features per probe
  - 100% of Sanger miRBase v15 Human miRNAs

- **Mouse & Rat miRNA OneArray® v2**

  - 785 unique miRNA probes
  - 105 experimental control probes
  - 3 features per probe
  - 100% of Sanger miRBase v15 miRNAs



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