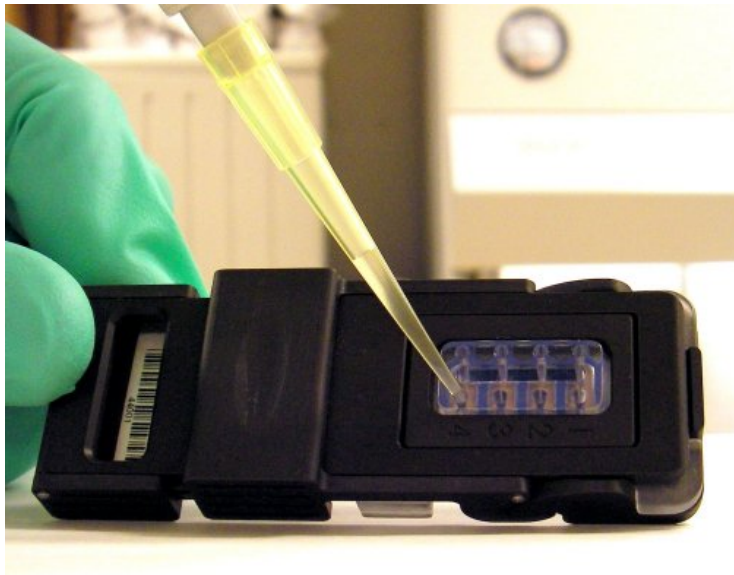
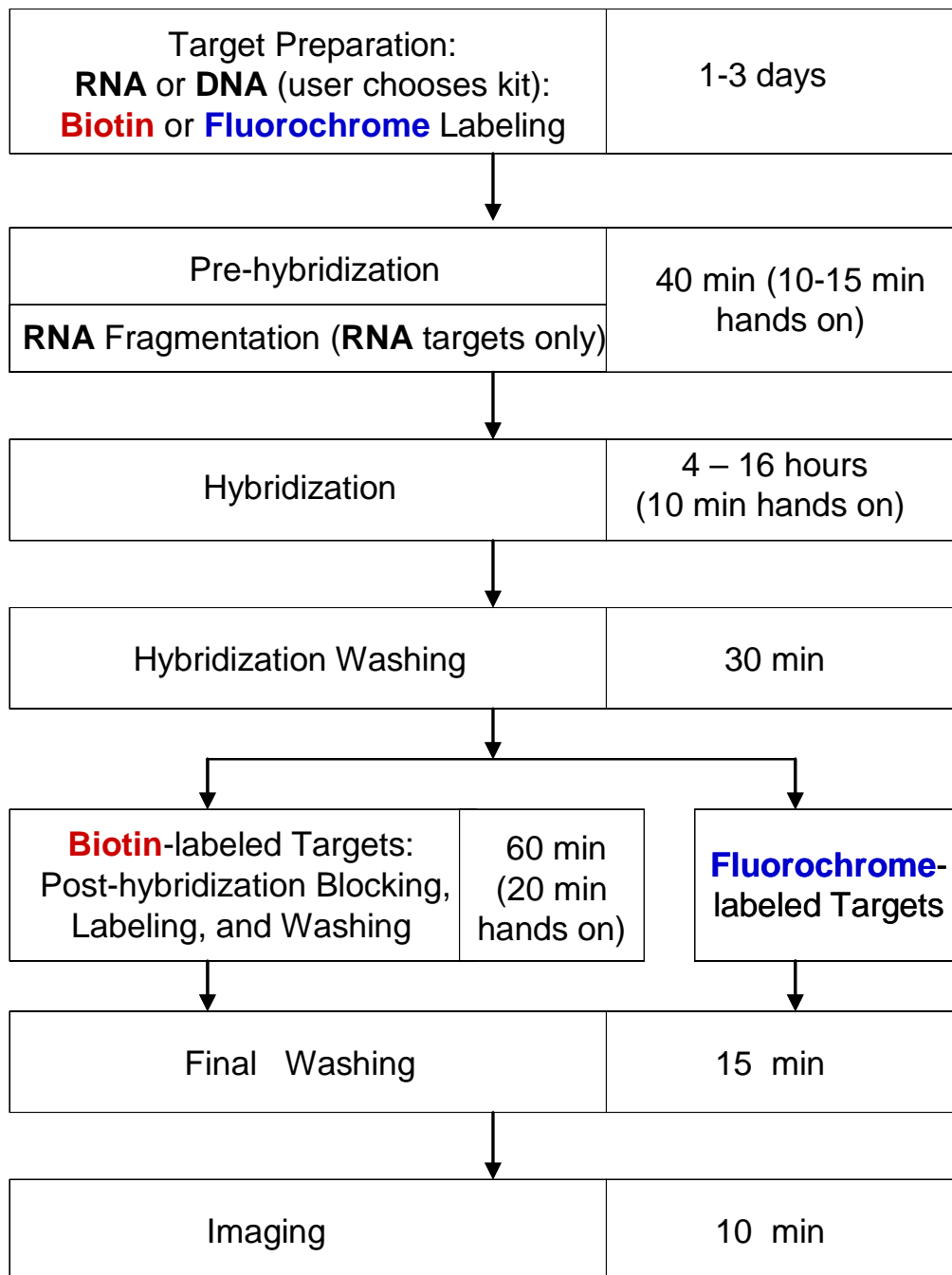


COMBIMATRIX

CustomArray™ 4X2K Microarray: Hybridization and Imaging Protocol (PTL005)



Hybridization and Imaging of CustomArray™ 4X2K Workflow



Hybridization and Imaging of CustomArray™ 4X2K

Table of Contents

Table of Contents	ii
Introduction	1
Materials and Equipment Provided.....	2
Materials and Equipment Required (not provided).....	3
CustomArray™ 4X2K Assembly.....	4
Handling of Assembled Microarrays	5
Preparation for Hybridization.....	6
Solutions Required for All Targets.....	6
Solutions Required Only for Biotin-labeled Targets	7
Pre-Hybridization	8
RNA Fragmentation (RNA Target Samples Only)	9
Hybridization	10
Hybridization Washing.....	11
Post-hybridization Labeling for Biotin-labeled Samples.....	12
Final Washing.....	12
Imaging of the Hybridized CustomArray™ 4X2K.....	13
Appendix A. Related Products Available from CombiMatrix.....	15
CombiMatrix Rotisserie Holders for 4X2K Microarrays.....	15
CombiMatrix CustomArray™ Stripping Kit and Clamp	15
Appendix B. CombiMatrix CustomArray™ Synthesizer	16

Hybridization and Imaging Protocol

Introduction

This manual describes how to hybridize labeled target samples to the CombiMatrix CustomArray™ 4X2K microarray, and how to prepare the microarray for imaging. Each 4X2K microarray has four identical array sectors that can be hybridized with different target samples using the sectorized Hybridization Cap provided. All four sectors must be hybridized simultaneously. Hybridization is performed with 0.5 to 2 µg of a labeled target sample per array sector. If two labeled samples are to be analyzed on the same array (for a dual-color experiment), use 0.5 to 2 µg of each sample (for a total of 1-4 µg). DNA or RNA target samples can be labeled by incorporation of either **biotin** or a **fluorochrome** (Cy3®, Cy5®, or AlexaFluor® 555 and 647 fluorescent dyes). Preparation of labeled targets is the user's responsibility. The hybridization conditions recommended in this protocol are designed for gene expression analysis. For other microarray applications, please refer to the Combimatrix Website (www.combimatrix.com), or contact customer support (support@combimatrix.com).

Imaging of the CustomArray™ 4X2K must be performed using a high-resolution fluorescent scanner that is compatible with a 1"x3" slide format, with a minimum resolution of 5µm and an adjustable focus. All four sectors are scanned simultaneously.

The hybridized CustomArray™ 4X2K can be stripped of targets using the CombiMatrix Stripping Kit (see Appendix A), and then re-used up to three times according to the hybridization protocol described in this manual.

Materials and Equipment Provided

IMPORTANT! Do not touch the semiconductor microarray surface. Wear gloves when handling. CustomArray™ 4X2K microarrays can be stored in a cool dry place for up to 4 months.

- ❑ CustomArray™ 4X2K microarray
- ❑ Sectored Hybridization Cap
- ❑ LifterSlip™ coverslip for imaging
- ❑ Imaging Solution

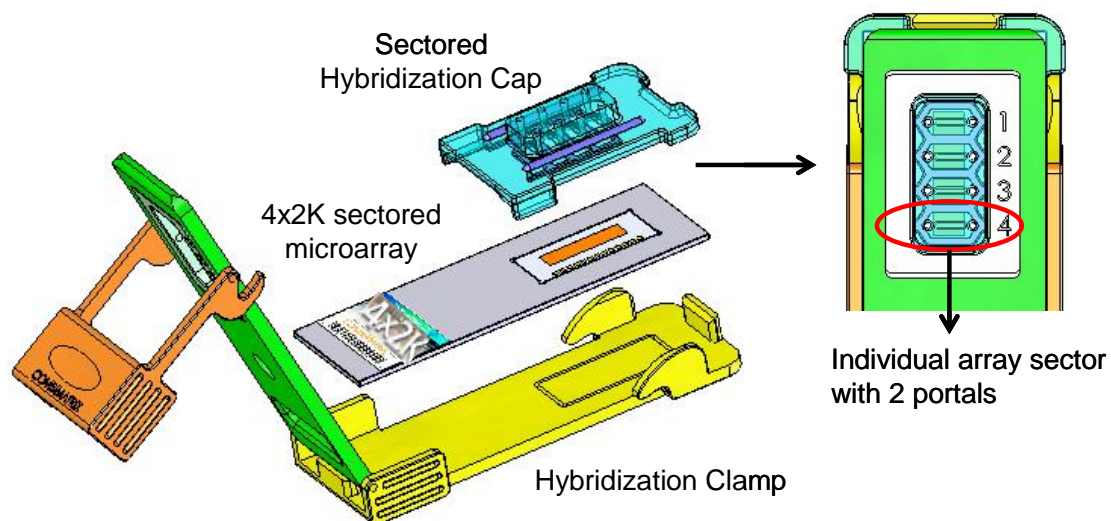


Figure 1. CustomArray™ 4X2K and accessory components. The colorization of the Hybridization Clamp is for illustrative purposes and does not reflect the real color (see Fig. 2).

Materials and Equipment Required (not provided)

- ❑ Labeled target DNA or RNA samples (labeled with **biotin** or **fluorochrome**)
- ❑ CombiMatrix Hybridization Clamp for the CustomArray™ 4X2K (see Fig. 1 and 2), Product Number 610009
- ❑ Rotisserie oven for microarray hybridization
- ❑ CombiMatrix rotisserie holder for CustomArray™ 4X2K microarrays (see Appendix A)
- ❑ Standard, high-resolution fluorescent microarray scanner (CombiMatrix recommends the Axon Instruments Genepix® 4000B and 4200A microarray scanners, and the Perkin Elmer ScanArray® 4000, 5000, Lite and Express microarray scanners).
- ❑ 95°C Heating Block
- ❑ Micropipettors and tips
- ❑ Sterile plastic ware
- ❑ Gloves (powder-free)
- ❑ Adhesive tape: Scotch® Brand Magic® Transparent Tape is suitable for hybridization temperatures of 50°C or less; for extended incubations at higher temperatures use a PCR sealing tape such as Nunc Brand PN 232702 (clear polyolefin liner) or PN 276014 (aluminum liner)
- ❑ Nuclease-free water
- ❑ 50X Denhardt's solution
- ❑ 0.5 M EDTA (pH 8.0)
- ❑ 10% Tween-20
- ❑ 20x SSPE Buffer
- ❑ 10 mg/ml Salmon Sperm DNA (Sheared), Ambion, Cat.#9680
- ❑ 1% SDS
- ❑ 10X Phosphate-buffered Saline (PBS: 1.37M Sodium Chloride, 0.027M Potassium Chloride, 0.08M Sodium Phosphate dibasic, 0.02M Sodium Phosphate monobasic, pH 7.4; Ambion, Cat.# 9625)

For RNA targets:

- ❑ Deionized (DI) Formamide
- ❑ RNA Fragmentation Reagents (see the section "RNA fragmentation")

For Biotin-labeled targets:

- ❑ Acetylated Bovine Serum Albumin (BSA, 20 mg/ml solution), Ambion Cat.#2614, or 5X PBS-Casein Blocking Buffer, BioFX Laboratories, Cat.# PBSC-0100-01
- ❑ Biotin detection reagent: Fluorolink™ Cy5®-labeled streptavidin, GE Healthcare/Amersham Biosciences Cat. # PA45001

CustomArray™ 4X2K Assembly

IMPORTANT! The Hybridization Cap is intended for single use only. Dispose of the cap upon completion of this protocol.

1. Open the Hybridization Clamp and place the CustomArray™ 4X2K with the semiconductor array side up, as shown in Figures 1 and 2.



Figure 2. Placement of the CustomArray™ 4X2K in the Hybridization Clamp.

2. Align the Hybridization Cap over the slide so that the top edge of the slide is positioned against the stop on the Hybridization Cap, and the Cap is centered over the semiconductor array (see Figures 1 and 3). There is only one way to position the Hybridization Cap inside the clamp.

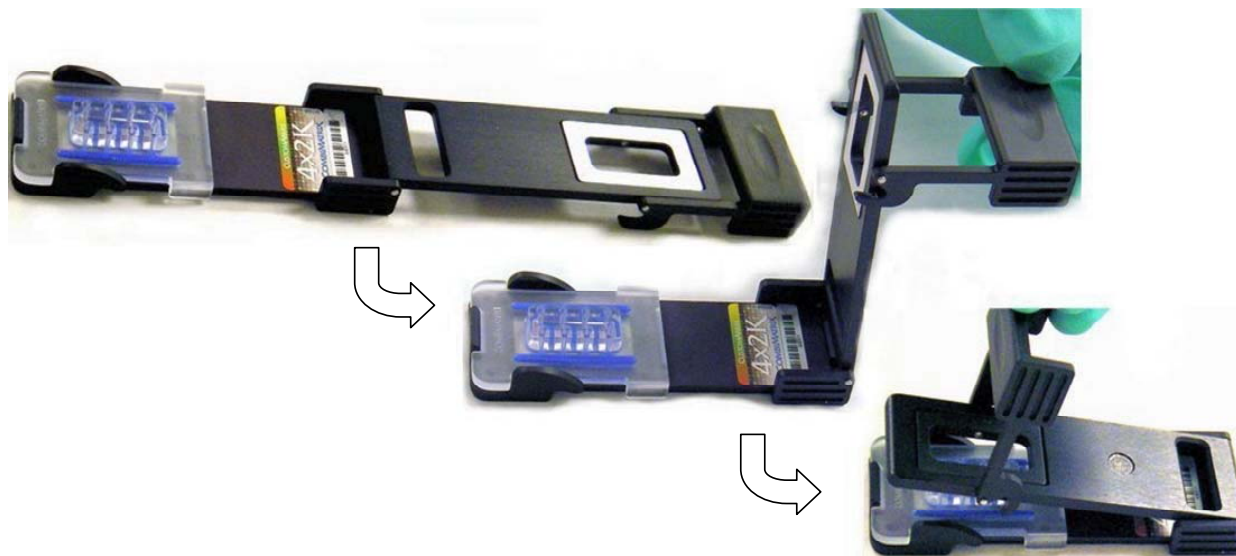


Figure 3. Placement of the Hybridization Cap on the top of the CustomArray™ 4X2K, and closing of the Hybridization Clamp.

3. Secure the CustomArray™ 4X2K and Hybridization Cap by closing the Clamp (Fig. 3).

Handling of Assembled Microarrays

1. Wear gloves at all times when handling microarrays and reagents.
2. After a CustomArray™ 4X2K is assembled and re-hydrated, keep the Hybridization Cap in place during all hybridization and wash steps, and do not open it. Do **NOT** allow the semiconductor microarray surface to become dry at any step in the protocol. Proceed rapidly when changing solutions, and do not leave the hybridization chambers empty for any significant length of time.
3. Use a 200 µl micropipettor with nuclease-free pipet tips to add or remove solutions through the solution portals of the Hybridization Cap. Hold the microarray with the assembled Hybridization Cap at a 45 degree angle, and add/remove solutions through the lower portals (Fig. 4).

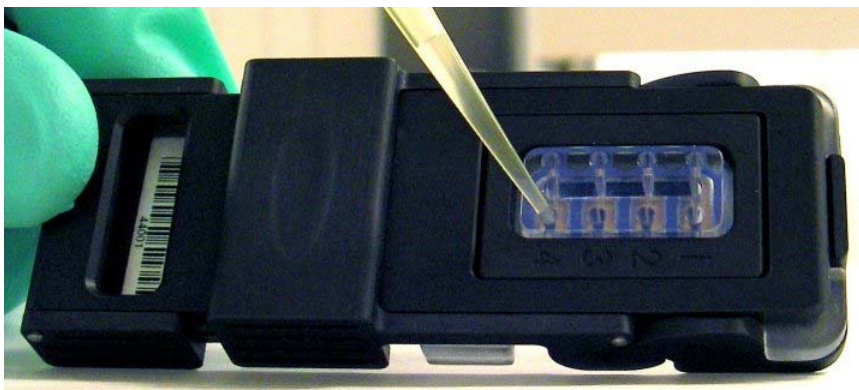


Figure 4. Ready to use CustomArray™ 4X2K assembled with the Hybridization Cap in the Hybridization Clamp. To add/remove solutions, hold the assembled microarray at a 45 degree angle and pipet through the lower portals of hybridization chambers.

4. The volume of each chamber of the Hybridization Cap is 35 µl. However, when filling a chamber with hybridization buffer containing labeled targets, add only 30 µl per chamber to prevent overfilling and spilling of the labeled target solution to a neighboring chamber. The air bubbles that form in the chambers will improve mixing when arrays are rotated during hybridization incubation.

IMPORTANT! Do **NOT** overfill the chambers with hybridization buffer. Carefully wipe all spilled solution from the upper surface of the hybridization cap. This is critical to prevent cross contamination between array sectors that may be caused by solution leaking from one portal and being absorbed by capillary action into a neighboring portal.

5. Use of a rotisserie oven or a rotating incubator is recommended to ensure mixing during hybridization, and it is critical to improve performance of 4X2K microarrays. Special holders are available from CombiMatrix for attachment of CustomArray™ 4X2K to standard rotisseries (see Appendix A).
6. For all incubations longer than 10 minutes, seal the solution portals of Hybridization Cap with non-permeable adhesive tape to prevent evaporation. Use Scotch® Brand Magic® Transparent Tape or a PCR sealing tape such as Nunc Brand PN 232702 (clear polyolefin liner) or PN 276014 (aluminum liner). Wipe the surface clean with a lint-free tissue before sealing.
7. If you plan to strip and re-use a hybridized CustomArray™ 4X2K with the CombiMatrix CustomArray™ Stripping Kit (see Appendix A), do not allow the semiconductor microarray surface to dry, as this will prevent any further use. Avoid prolonged storage of hybridized microarrays prior to stripping; instead, strip the microarrays first, and store them wet in Imaging Solution or 1X PBS at 4°C for a maximum of 2 weeks.

Preparation for Hybridization

The following solutions can be prepared beforehand. All reagents used should be RNase and DNase-free.

Solutions Required for All Targets

1. Prepare the **2X Hyb Solution Stock** (see Table 1). This stock will be used for preparation of the Pre-hybridization and Hybridization Solutions. The 2X Hyb Solution Stock should be filter-sterilized and can be stored at room temperature for up to 6 months.

Table 1. 2X Hyb Solution Stock		
<u>Reagent</u>	<u>Volume for 10 ml</u>	<u>Final Concentration</u>
20X SSPE	6 ml	12X
10% Tween-20	100 µl	0.1%
0.5M EDTA	560 µl	40mM
Nuclease-free water	3.34 ml	
Total Volume	10 ml	

^aFinal concentration includes the EDTA from the SSPE.

1.

2. Prepare the **Wash Solutions** (see Table 2). The prepared solutions should be filter-sterilized and can be stored at room temperature for up to 6 months.

Table 2. Wash Solutions		
<u>Step</u>	<u>Solution</u>	<u>For 10 ml</u>
6X SSPET Wash	6X SSPE, 0.05% Tween-20	3 ml 20X SSPE 50 µl 10% Tween-20 6.95 ml Nuclease-free water
3X SSPET Wash	3X SSPE, 0.05% Tween-20	1.5 ml 20X SSPE 50 µl 10% Tween-20, 8.45 ml Nuclease-free water
0.5X SSPET Wash	0.5X SSPE, 0.05% Tween-20	250 µl 20X SSPE 50 µl 10% Tween-20 9.7 ml Nuclease-free water
PBST Wash	2X PBS, 0.1% Tween-20	2 ml 10X PBS, 100 µl 10% Tween-20, 7.9 ml Nuclease-free water
PBS Wash	2X PBS	2 ml 10X PBS, 8 ml Nuclease-free water

Solutions Required Only for **Biotin**-labeled Targets

NOTE: If working with **fluorochrome**-labeled targets, skip this step.

1. Prepare the 1 mg/ml stock solution of the Fluorolink™ Cy5®-labeled streptavidin. This reagent is supplied as a lyophilized powder. Dissolve in 1.0 ml of nuclease-free water, dispense into 10-20 µl aliquots, freeze and store at -20°C. Avoid repeated freeze-thaw cycles.
2. Prepare the **Biotin Blocking Solution** (see Table 3). All reagents should be nuclease-free. The prepared Biotin Blocking Solution should be filter-sterilized and can be stored at 4°C for up to 1 month.

Table 3. Biotin Blocking Solution		
<u>Reagent</u>	<u>Volume for 1 ml</u>	<u>Final Concentration</u>
10X PBS	200 µl	2X
10% Tween-20	10 µl	0.1%
BSA (20 mg/ml)	500 µl	1%
Nuclease-free water	290 µl	
Total Volume	1 ml	

NOTE: You can replace the Biotin Blocking Solution with the 5X PBS-Casein Blocking Buffer for both blocking and labeling steps.

Pre-Hybridization

NOTE: The CustomArray™ 4X2K microarray must be re-hydrated prior to hybridization. A pre-hybridization step is then recommended to block non-specific binding of targets.

1. Pre-heat an incubator to 65°C.
2. Set a hybridization rotisserie oven to the desired hybridization temperature. See Table 4 for recommended hybridization temperatures.

Table 4. Recommended Hybridization Temperatures		
<u>Sample</u>	<u>Hybridization Solution</u>	<u>Hybridization Temperature (°C)</u>
RNA	25% Formamide	45
DNA	No Formamide	50

IMPORTANT! The hybridization protocol has been optimized for standard oligonucleotide lengths (35-40mers), with a balanced melting temperature (T_m) of 72°C. If non-standard oligonucleotide lengths are used, conditions such as hybridization temperature and hybridization buffer composition must be optimized empirically.

3. Prepare fresh **Pre-hybridization Solution**, 150 µl per one CustomArray™ 4X2K microarray (Table 5).

Table 5. Pre-hybridization Solution		
<u>Reagent</u>	<u>Volume for 150 µl</u>	<u>Final Concentration</u>
2X Hyb Solution Stock	75 µl	6X SSPE, 0.05% Tween-20, 20mM EDTA
Nuclease-free water	51 µl	
50X Denhardt's solution	15 µl	5X
Denatured Salmon sperm DNA (10mg/ml) ^a	1.5 µl	100 ng/µl
1% SDS	7.5 µl	0.05%
Total Volume	150 µl	

^a Heat the Salmon sperm DNA solution to 95°C for at least 5 minutes and then place on ice for at least 1 minute before use.

3. Fill the hybridization chambers with nuclease-free water. Avoid introducing air bubbles into the chambers. Cover the solution portals with adhesive tape to prevent evaporation. Incubate at 65°C for 10 minutes. Remove the microarray from the incubator and bring to room temperature. Remove the adhesive tape and aspirate the water out of the hybridization chambers.
4. Fill the hybridization chambers with the fresh Pre-hybridization Solution. Mix gently by pipetting. A small air bubble can be introduced to improve the mixing process if the arrays are rotated during incubation. Cover the solution portals with adhesive tape.
5. Load the microarray onto the rotisserie in the hybridization oven and incubate at the desired hybridization temperature for 30 minutes with gentle rotation.

RNA Fragmentation (RNA Target Samples Only)

IMPORTANT! CombiMatrix recommends that RNA target samples be fragmented prior to hybridization. In general, 50 to 200-base fragments work best to maximize binding specificity and detection sensitivity. This step can be completed during the Pre-hybridization incubation.

NOTE: Other established fragmentation protocols may be used in place of this protocol. Alternatively, the RNA Fragmentation Reagents from Ambion (Cat#8740) can be used according to the manufacturer's protocol.

1. Prepare the **5X RNA Fragmentation Solution** (see Table 6). The prepared solution should be filter-sterilized and can be stored at room temperature for up to 6 months.

Table 6. 5X RNA Fragmentation Solution (RNA samples only)		
<u>Reagent</u>	<u>Volume for 10 ml</u>	<u>Final Concentration</u>
1 M Tris Acetate pH 8.1 (adjust pH with glacial acetic acid)	2 ml	200 mM
KOAc	0.49 g	500 mM
MgOAc	0.32 g	150 mM
Water	To 10 ml	
Total volume	10 ml	

2. Set up the RNA fragmentation reaction (see Table 7). Use 0.5 to 2 μg (1 to 4 μg if using two samples for the dual color experiment) of the labeled RNA for each sector of CustomArray™ 4X2K microarray.

Table 7. RNA Fragmentation Reaction	
<u>Reagent</u>	<u>Volume for 6 μl (for 30 μl hybridization chamber)</u>
Labeled RNA	0.5 to 2 μg per array for single color; 1 to 4 μg per array for dual color
Nuclease-free water	to 4.8 μl
5X RNA Fragmentation Solution	1.2 μl
Total Volume	6 μl

3. Incubate at 95°C for 20 minutes. Place on ice.
4. Add the entire Fragmentation Reaction volume to the Hybridization Solution.

Hybridization

1. Prepare the **Hybridization Solution** (see Table 8)

IMPORTANT! The hybridization buffer composition depends on the type of nucleic acids used as targets. For RNA (but not DNA) targets, it should include 25% formamide.

Table 8. Hybridization Solution		
<u>Reagent</u>	<u>Volume for 30 μl</u>	<u>Final Concentration</u>
2X Hyb Solution Stock	15 μ l	6X SSPE, 0.05% Tween-20, 20mM EDTA
DI Formamide (for RNA targets) ^a	7.5 μ l	25%
Labeled targets: DNA or fragmented RNA	Varies (up to 6 μ l)	15-67 ng/ μ l recommended
Salmon sperm DNA (10mg/ml) ^b	0.3 μ l	100 ng/ μ l
1% SDS	1.2 μ l	0.04%
Nuclease-free water	to 30 μ l	
Total Volume	30 μl	

^a Replace formamide with water when working with the labeled DNA targets.

^b Salmon sperm DNA solution should be heat-denatured (as for preparation of the pre-hybridization solution).

2. Denature the Hybridization Solution at 95°C for 3 minutes, and then cool for 1 minute on ice. Spin down the solution in a microcentrifuge for 5 seconds at maximum speed to collect condensate.
3. Remove the adhesive tape from the microarray and pipet the Pre-hybridization Solution out of the hybridization chambers.
4. Fill the hybridization chambers with the Hybridization Solution (30 μ l per chamber) and mix gently with repeated pipetting. A small air bubble will form, and it will improve the mixing process if the arrays are rotated in the hybridization rotisserie oven.

IMPORTANT! Do NOT overfill the chambers with hybridization buffer. This is critical to prevent cross contamination between array sectors that may be caused by solution leaking from one portal and being absorbed by capillary action into a neighboring portal.

5. Carefully wipe excess solution from the surface of the Hybridization Cap with a lint-free tissue, and cover the solution portals with adhesive tape.
6. Load the microarray onto the rotisserie in the hybridization oven and incubate at the desired hybridization temperature for 4-16 hours with gentle rotation.

NOTE: To improve microarray performance, use of a rotisserie oven or a rotating incubator is recommended to ensure mixing during hybridization. The CustomArray™ 4X2K can be attached to standard rotisseries using holders available from CombiMatrix (see Appendix A).

Hybridization Washing

IMPORTANT! Do not allow the microarray to become dry at any time. Proceed rapidly when changing solutions. Do not leave the hybridization chamber empty for any significant length of time.

NOTE 1: For every wash step, CombiMatrix recommends rinsing the hybridization chamber with the corresponding Wash Solution prior to the wash incubation. Add the Wash Solution to the chamber, gently mix by pipetting, remove it, and fill the chamber again with the same solution.

NOTE 2: If the microarray has been hybridized with **fluorochrome**-labeled targets, protect it from light by covering with aluminum foil during all incubations exceeding 5 minutes.

NOTE 3: With the exception of the first wash step, microarrays should be incubated in the Hybridization Wash solutions for a minimum of 1 minute. However, if processing multiple microarrays, you can extend the wash incubation time until you rinse and fill all hybridization chambers.

1. Prior to starting the wash procedure, preheat the **6X SSPET Wash** Solution to the hybridization temperature (45 or 50°C depending on the sample hybridized).
2. Remove the microarray from the hybridization oven. Remove the adhesive tape, and pipet the Hybridization Solution out of the chambers.
3. Using the pre-heated **6X SSPET Wash** solution, rinse the hybridization chambers, fill the chambers, cover the portals with adhesive tape, and return the microarray to the hybridization oven for 5 minutes (with gentle rotation). Remove the 6X SSPET Wash Solution from the hybridization chambers.
4. Using the **3X SSPET Wash** solution, rinse the hybridization chambers, fill the chambers, and incubate the microarray at room temperature for 1 minute (see NOTE 3). Remove the 3X SSPET Wash Solution from the hybridization chambers.
5. Using the **0.5X SSPET Wash** solution, rinse the hybridization chambers, fill the chambers, and incubate the microarray at room temperature for 1 minute. Remove the 0.5X SSPET Wash Solution from the hybridization chambers.
6. Using the **PBST Wash** solution, rinse the hybridization chambers, fill the chambers, and incubate the microarray at room temperature for 1 minute. Remove the PBST Wash Solution from the hybridization chambers.
7. If the microarray has been hybridized with **fluorochrome**-labeled target samples, proceed to the final wash step.
8. If the microarray has been hybridized with **biotin**-labeled target samples, proceed to the Post-hybridization Labeling Step. Retain the PBST Wash Solution in the hybridization chambers until you are ready to proceed to the Post-hybridization Blocking step.

Post-hybridization Labeling for **Biotin**-labeled Samples

NOTE: If working with **fluorochrome**-labeled targets, skip this step.

1. Prepare the **Dye Labeling** Solution using the 1 mg/ml stock solution of Fluorolink™ Cy5®-labeled streptavidin. Thaw an aliquot, and make a 1:1000 dilution (v/v, 1 µl per 1 ml) in the Biotin Blocking Solution (or 5X PBS-Casein Blocking Buffer). We recommend that you discard any unused Cy5®-labeled streptavidin.

NOTE: Prepare the Dye Labeling Solution fresh each time you hybridize microarrays.

2. Remove the PBST Wash Solution from the hybridization chambers.
3. Add the **Biotin Blocking** Solution (or 5X PBS-Casein Blocking Buffer) to the hybridization chambers, mix gently by pipetting, and incubate the microarray at room temperature for 15 minutes. Remove the solution from the hybridization chambers.
4. Add the **Dye Labeling** Solution to the hybridization chambers, mix gently by pipetting, and cover the solution portals with adhesive tape. Incubate the microarray at room temperature for 30 minutes. Protect from light by covering with aluminum foil. Remove the Dye Labeling Solution from the hybridization chambers.
5. Using the **PBST** Wash solution, rinse the hybridization chambers, fill it, and incubate the microarray at room temperature for 1 minute. Remove the PBST Wash Solution from the hybridization chambers.
6. Repeat the PBST washing one more time.

Final Washing

1. Remove the PBST Wash Solution from the hybridization chambers.
2. Using the **PBS Wash** solution, rinse the hybridization chambers, fill the chambers, and incubate the microarray at room temperature for 1 minute. Remove the PBS Wash Solution from the hybridization chambers. Repeat a second time.
3. Retain the PBS Wash Solution in the hybridization chambers until you are ready to proceed to the Imaging step.

Imaging of the Hybridized CustomArray™ 4X2K

IMPORTANT! The CustomArray™ 4X2K must be scanned wet using the Imaging Solution supplied. The LifterSlip™ coverslip provided with the CustomArray™ 4X2K has been specifically designed to retain the Imaging Solution without contacting the array surface.

NOTE: The Imaging Solution contains phosphate buffer, which may precipitate during shipping. If a precipitate is visible, heat the Imaging Solution at 60-70°C for about 5 minutes until it dissolves. Allow the solution to cool to room temperature before applying it to the microarray.

1. Remove the PBS Wash Solution from the hybridization chambers.
2. Open the Hybridization Clamp and carefully lift the Hybridization Cap off the slide surface. Remove the microarray from the Clamp and place it horizontally.
3. Immediately cover the semiconductor microarray surface with the Imaging Solution.
4. Using thin-tipped forceps, pick up a fresh LifterSlip™ and hold it so that the raised edges face the microarray. The raised edges can be detected by gently rubbing an edge with the tip of the forceps – the raised edge will feel rougher than the glass surface.
5. Lay the LifterSlip™ at an angle onto the microarray so that it is centered over the semiconductor area (see Fig. 5). First touch the Imaging Solution with one side of the LifterSlip™, then slowly lower the slip down, taking care not to introduce air bubbles. If bubbles still form, lift one side of the LifterSlip™ with forceps (or a razor blade) to let the bubbles out, and lower it down again.

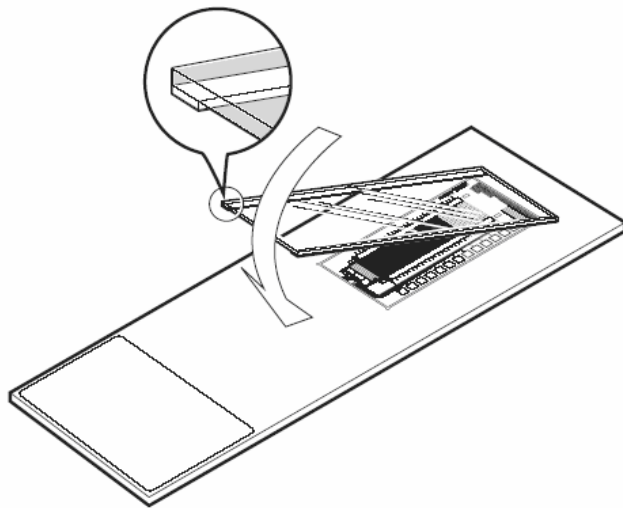


Figure 5. A CombiMatrix microarray with LifterSlip™ coverslip

6. Carefully remove any excess Imaging Solution from the edges of the LifterSlip™ using a lint-free tissue, until it is resting evenly over the microarray.
7. Load the 4X2K microarray into the scanner, taking care not to disturb the LifterSlip™ coverslip. Follow the manufacturer's recommendations for loading the slide into the scanner.

8. After you complete the scan, save the image as a .tiff image file. The data can be extracted from the image using the CombiMatrix Microarray Imager Software. Please refer to the Quick Start Guide or the Microarray Imager User's Manual on our web site (<https://webapps.combimatrix.com>).
9. After imaging is completed, you can proceed to stripping of the CustomArray™ 4X2 for subsequent re-hybridization using the CombiMatrix CustomArray™ Stripping Kit (see Appendix A). Do not allow the semiconductor microarray surface to dry; keep the microarray wet, either in a tube or slide-holder containing 1X PBS, or in the Imaging Solution with the LifterSlip™ attached. Avoid prolonged storage of hybridized microarrays prior to stripping; instead, first strip the microarray, then store wet in Imaging Solution or 1X PBS at 4°C for a maximum of 2 weeks.

Appendix A. Related Products Available from CombiMatrix

CombiMatrix Rotisserie Holders for 4X2K Microarrays

To improve microarray performance, use of a rotisserie oven or a rotating incubator is recommended to ensure mixing during hybridization. Microarrays can be attached to standard rotisseries using holders available from CombiMatrix. Please note that the rotisserie holders for CombiMatrix 4X2K microarrays are different from the 12K ones, and they are not interchangeable. The appropriate holder is necessary because the rotation axis is different for these two types of microarrays.

- **CombiMatrix 8 CustomArray™ Rotisserie for 4X2K:**
 - Product Number for 3/8" diameter shaft is 610020,
 - Product Number for 3/4" diameter shaft is 610014,
 - Product Number for 1/2" diameter shaft is 610021,
 - Product Number for 10 mm diameter shaft is 610022,
 - Product Number for 13 mm diameter shaft is 610023.

The 8 CustomArray™ Rotisserie for 4X2K allows the hybridization of 8 microarray slides (32 microarray sectors) at one time. This custom engineered product will mount on a shaft of an incubation oven with simple spring clasps. CombiMatrix have several models adapted for different oven shaft diameters. Please indicate the shaft diameter of your rotisserie oven when ordering.

CombiMatrix CustomArray™ Stripping Kit and Clamp

- **CombiMatrix CustomArray™ Stripping Clamp**, Product Number 610010.

The CombiMatrix CustomArray™ Stripping Clamp enables the microarrays to be stripped and re-hybridized up to three times. The Stripping Clamp is specially designed to withstand chemicals used for stripping and to provide appropriate pressure to prevent leakage from the stripping cap. The CustomArray™ Stripping Clamp is recommended for up to 100 stripping procedures.

- **CombiMatrix CustomArray™ Stripping Kit**, Product Number 610024.

The CustomArray™ Stripping Kit enables re-hybridization of a microarray three times, which makes the use of a single CombiMatrix microarray four times. Each kit contains reagents for 25 microarray stripping reactions, and accessories for microarray re-hybridizations.

Appendix B. CombiMatrix CustomArray™ Synthesizer

The CustomArray Synthesizer enables researchers to make custom microarrays to their exact specifications. While fulfilling their existing microarray requirements researchers can take microarrays to new frontiers by exploring unique and novel applications.

The platform consists of the CustomArray™ DNA Synthesizer instrument and freely programmable CustomArray™ microarrays. The CustomArray™ technology utilizes a modified semiconductor adapted for biological applications. The integrated circuits built into CustomArray™ contain arrays of microelectrodes that are individually addressable using embedded logic circuitry on the chip. Placed in a specially-designed fluidic chamber, the chip (under direction from software) digitally directs the molecular assembly of oligonucleotides. During this process a chip can rapidly synthesize several thousand different oligonucleotide probes in parallel, each above a distinct electrode. Additionally, the platform utilizes standard phosphoramidite chemistry and the resultant DNA microarray slides can be read on common scanners.

CustomArray™ Synthesizer, Product Number 610002

- ❑ **Production Efficiency:** Manufacture eight of 12K or 4X2K custom arrays in less than 24 hours.
- ❑ **Versatility:** Use the same design or different designs in a single run.

CustomArray™ Synthesizer Specifications:

- ❑ **Min/Max Oligo Length:** Up to 50 'mer
- ❑ **Min/Max Feature Capacity for Array:** 2,240, 12,544
- ❑ **Microarray Format:** 1" x 3", CustomArray™ slide
- ❑ **Production Capacity:** One to eight slides per run