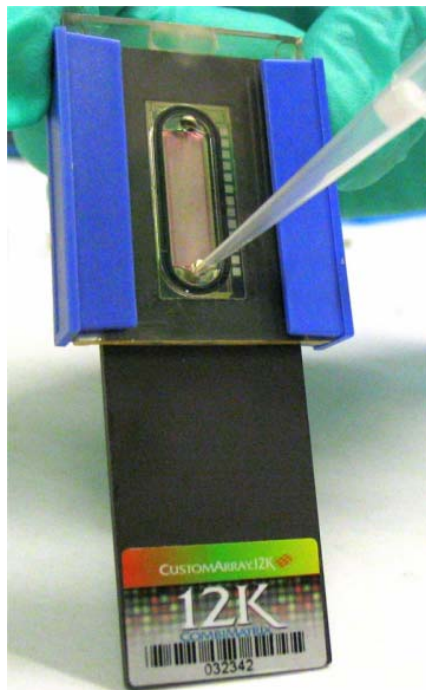
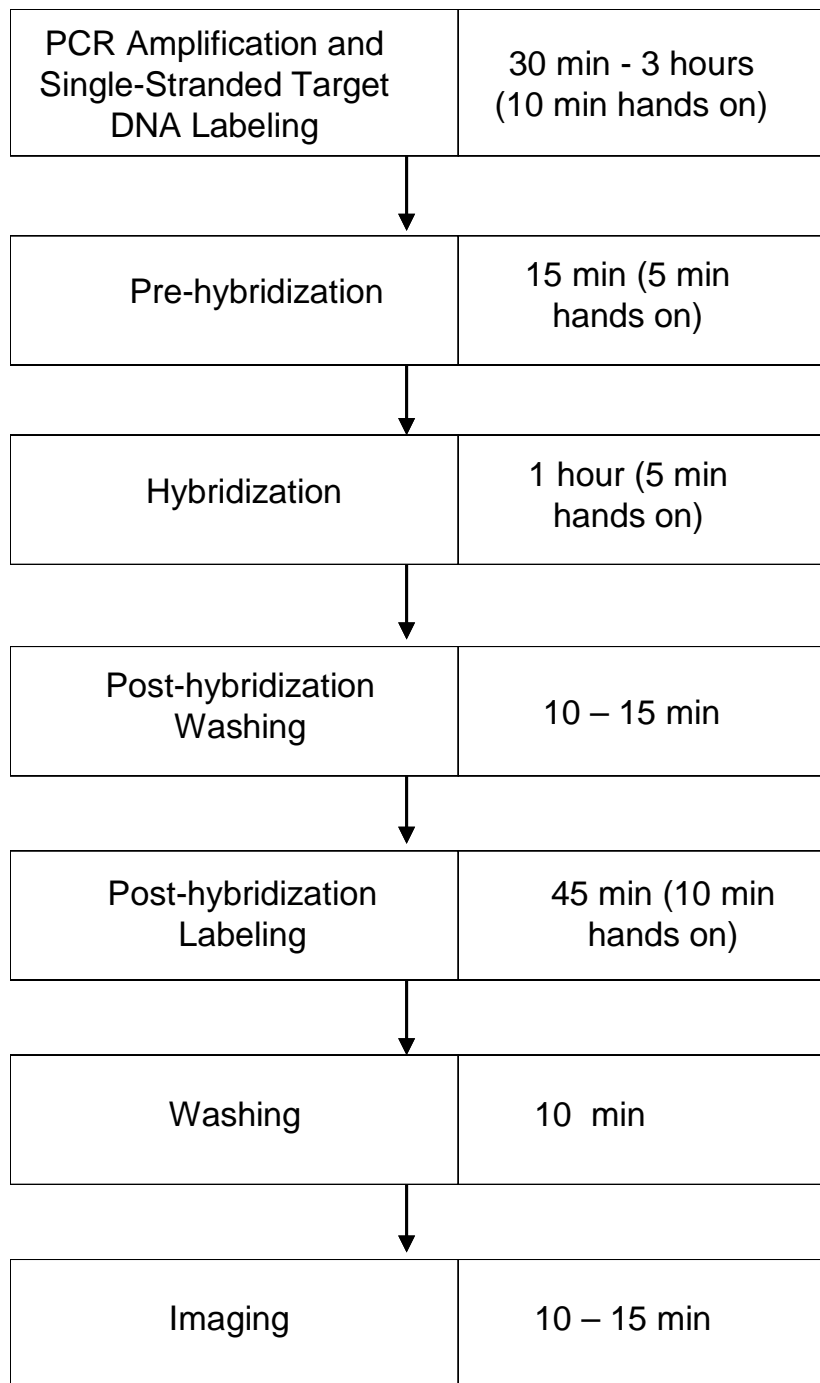


COMBIMATRIX

Re-sequencing and SNP Detection:
Target Preparation, Hybridization, and Imaging of
CustomArray™ 12K Microarrays (PTL014)



Re-sequencing and SNP Detection: Target Preparation, Hybridization, and Imaging of CustomArray™ 12K Microarray Workflow



Re-sequencing and SNP Detection: Target Preparation, Hybridization, and Imaging of CustomArray™ 12K Microarrays

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Re-sequencing and SNP Detection: Target Preparation, Hybridization, and Imaging of CustomArray™ 12K Microarrays

Introduction

This manual describes how to prepare single-strand DNA target samples and to hybridize them to CustomArray™ 12K microarrays for re-sequencing, SNP detection and genotyping. Usage of single-strand targets greatly improves microarray results, because in the case of double-strand targets, self-annealing of complimentary strands is favored over binding to array probes. Even with the denaturing step, the double strand target binding cannot be as robust as the single-strand target binding.

When working with whole genome samples, take approximately 50 ng of DNA per amplification reaction. You can use less material, for example 10 ng, if you work with DNA samples that are already enriched with the sequences of interest. Alternatively, you can amplify the sequence of interest from 10 to 20 ng of cDNA samples obtained from RNA using reverse transcription. The target DNA samples are prepared by using two PCR steps with target-specific primers. First PCR is performed to amplify the double-strand DNA fragment of interest. The resultant product is cleaned using a PCR product purification kit (e.g. from Qiagen). It is then subjected to a second PCR amplification with only one, reverse target-specific primer in the presence of biotin-dCTP to produce the single-strand biotinylated DNA fragment. The target-specific PCR primers should be designed to generate DNA fragments between 100 and 1000 bp (100-200 bp fragments typically generate higher quality results than longer fragments).. Individual DNA targets should be generated in separate PCR reactions. However, multiple different targets can be mixed together for hybridization to the same CustomArray™ 12K microarray using the present protocol.

The method described in this guide can be used for re-sequencing, SNP detection and genotyping based on the standard T_m -based hybridization assay. CustomArray™ 12K microarrays designed for this approach have probes with the average length of 20-mer. Four probes carrying all possible mismatches are designed for each sequence position of interest. Hybridization is performed at 45°C with up to 5-10 µg of biotin-labeled target samples per CustomArray™ 12K microarray.

Imaging of the CustomArray™ 12K 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.

The hybridized CustomArray™ 12K can be stripped of targets using the CombiMatrix Stripping Kit (see Appendix A), and then re-used 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.
Keep in a dry cool place. Store up to 4 months.**

- CustomArray™ 12K microarray
- Hybridization Cap (100 µl Volume)
- Clips for Hybridization Cap
- Gasket (O-ring)
- LifterSlip™ coverslip
- Imaging Solution

Materials and Equipment Required (not provided)

- DNA target samples (10-50 ng)

NOTE: RNA target samples can be reverse-transcribed into cDNA and then PCR-amplified in the same way as genomic DNA targets.

- PCR primers:
 - target-specific forward primer, 10 µM solution in nuclease-free water
 - target-specific reverse primer, 10 µM solution in nuclease-free water
- Thermal cycler (PCR machine)
- Taq DNA Polymerase with 10X ThermoPol PCR Buffer (New England BioLabs Cat. #M0267L)
- 10 mM dNTP mix (New England BioLabs Cat. #N0447S)
- 10 mM solutions of dATP, dGTP, dTTP, and dCTP
- 0.4 mM Biotin-14-dCTP (Invitrogen, Cat. #19518-018)
- DMSO (Sigma Cat. #D8418)
- QIAquick PCR Purification kit, (Qiagen Cat. #28106)
- 95°C Heating Block
- Micropipettors and tips
- Sterile plastic ware
- Gloves (powder-free)
- Nuclease-free water
- Rotisserie oven for array hybridization
- CombiMatrix Holders for rotisserie oven
- 95°C Heating Block
- 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)
- 50x Denhardt's solution
- 0.5 M EDTA (pH 8.0)
- 10% Tween-20
- 20x SSPE Buffer
- 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)
- 5X PBS-Casein Blocking Buffer, BioFX Laboratories, Cat.# PBSC-0100-01
- Biotin detection reagent: Fluorolink™ Cy5®-labeled streptavidin, GE Healthcare/Amersham Biosciences Cat. # PA45001
- Standard, high-resolution fluorescent microarray scanner (CombiMatrix recommends the Axon Instruments Genepix® 4000B and 4200A, and the Perkin Elmer ScanArray® 4000, 5000, Lite and Express microarray scanners).

Preparation of Single-Stranded DNA Targets

Two-way PCR Amplification

When working with whole genome samples, take approximately 50 ng of DNA per reaction. You can use less material (10 ng), if you work with DNA samples that are enriched with the sequences of interest. Alternatively, you can use 10 to 20 ng of cDNA samples that have been generated from RNA using reverse transcription.

1. Prepare PCR mixtures in PCR tubes on ice, one for each DNA fragment of interest, use the corresponding target-specific primers:

Reagent	Volume for one PCR (100 μl)
Nuclease-free water	67 μl
DNA template (10-50 ng)	5 μl
10X ThermoPol PCR Buffer	10 μl
DMSO	10 μl
10 mM dNTP	3 μl
10 μM Forward primer	2 μl
10 μM Reverse primer	2 μl
Total Volume	99 μl

2. Mix well by pipetting, and spin the tubes 5 seconds in a microcentrifuge. Place into the thermal cycler preheated to 94°C, and incubate for 2 minutes. Pause the thermal cycler and add 1 μ l (5 units) of Taq DNA Polymerase to each tube. Proceed with the thermal cycler program.

NOTE: CombiMatrix recommends the use of a "hot start" approach to reduce background caused by nonspecific priming. Commercially available "hot start" DNA polymerase preparations can also be used, however check these preparations for compatibility with DMSO.

3. Incubate the tubes at 94°C for 5 minutes to denature the template.
4. Perform 40 cycles of PCR amplification as follows:
 - Denature at 94°C for 30 seconds,
 - Anneal at 55°C for 30 seconds,
 - Extend at 72°C for 30 seconds.

NOTE: The annealing temperature may be optimized depending on the design (T_m) of target-specific primers.

5. Perform a final incubation at 72°C for 5 minutes.
6. Maintain the reactions at 4°C after cycling.
7. Spin the tubes briefly in a microcentrifuge to collect condensate.
8. Purify the PCR products by using a QIAquick PCR purification Kit according to the manufacturer's protocol. Elute with 60 μ l of nuclease-free water pre-warmed to 60°C. The purified reactions can be stored at -20°C until use.

IMPORTANT! CombiMatrix recommends to quality control the amplified DNA targets after the first amplification step by gel electrophoresis. Run a 2 μ l aliquot of each purified reaction on an acrylamide gel, or a 5 μ l aliquot on an agarose gel. Since the applied PCR conditions may not be stringent, expect to see multiple bands of PCR products. The PCR step is successful if you observe one or several bands in the size range expected for your target of interest. The presence of additional bands of various sizes does not adversely affect the results of microarray hybridization.

One-way PCR Amplification and Labeling of Single-Strand DNA Targets

Preparation and biotin labeling of the single-stranded DNA targets is done by a one-way PCR. Prepare PCR mixtures, one per each fragment of interest, using the corresponding reverse primers. For each reaction use 5 μ l of the PCR-amplified double-strand DNA template from the previous step (purified using QIAquick Kit).

1. Prepare one-way PCR mixtures in PCR tubes on ice:

<u>Reagent</u>	<u>Volume for one PCR (50 μl)</u>
Nuclease-free water	26.4 μ l
Double-strand DNA template from the previous step	5 μ l
10X PCR buffer	5 μ l
DMSO	5 μ l
10 mM dATP	0.7 μ l
10 mM dGTP	0.7 μ l
10 mM dTTP	0.7 μ l
10 mM dCTP	0.5 μ l
0.4 mM Biotin-14-dCTP	4 μ l
10 μ M Target-specific reverse	1.5 μ l
Total Volume	49.5 μl

2. Mix well by pipetting, and spin the tubes 5 seconds in a microcentrifuge. Place into the thermal cycler preheated to 94°C, and incubate for 2 minutes. Pause the thermal cycler and add 0.5 μ l of Taq DNA Polymerase to each tube. Proceed with the thermal cycler program.

NOTE: CombiMatrix recommends the "hot start" approach to reduce background caused by nonspecific priming.

3. Denature at 94°C for 5 min.
4. Perform 50 cycles (can be reduced to 35-40 cycles to decrease the assay time) of PCR amplification as follows:
 - Denature at 94°C for 30 seconds,
 - Anneal at 55°C for 30 seconds,
 - Extend at 72°C for 30 seconds.
5. Perform a final incubation at 72°C for 5 minutes.
6. Maintain the reactions at 4°C after cycling.
7. Spin the tubes briefly in a microcentrifuge to collect condensate.
8. Purify the PCR products by using a QIAquick PCR purification Kit. Elute with 60 μ l of nuclease-free water. The purified reactions can be stored at -20°C until use.

CustomArray™ 12K Assembly

IMPORTANT! The Hybridization Cap, Clips and O-ring are intended for single use only.

1. Insert the provided Gasket (O-ring) in the groove of the Hybridization Cap.
2. Align the Hybridization Cap over the slide so that the top edge of the slide is flush against the stop on the Hybridization Cap, and the Cap is centered over the semiconductor area.
3. Secure the Hybridization Cap in place with the Clips provided (see Fig. 1 and 2). The Clips can be attached only if the Hybridization Cap is positioned with the top edge of the slide at the stop of the Cap.

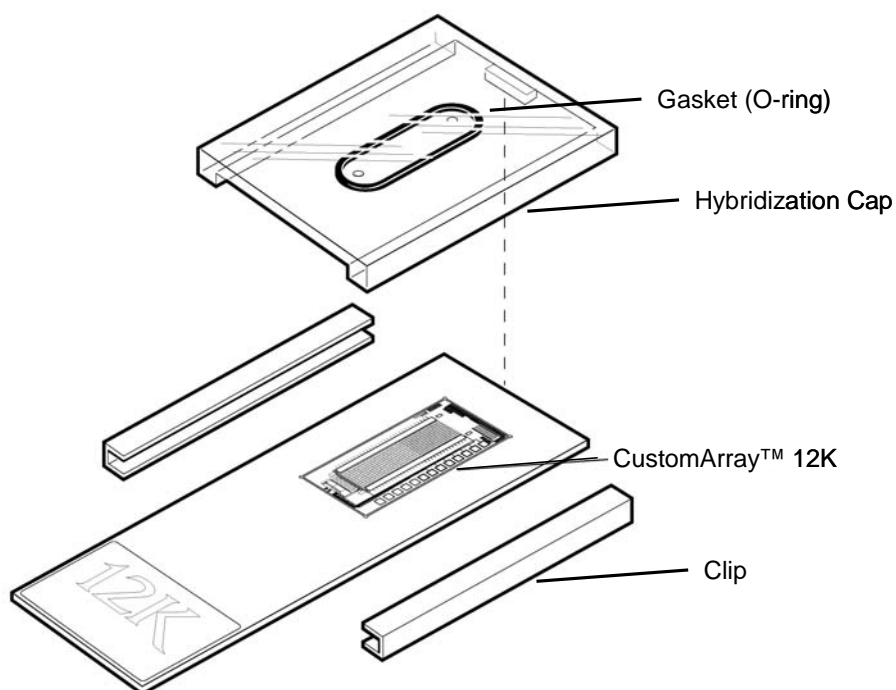
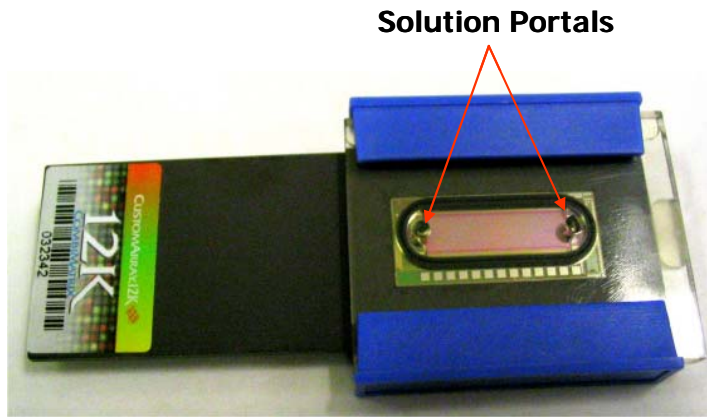


Figure 1. CustomArray™ 12K, Hybridization Cap and Clips.

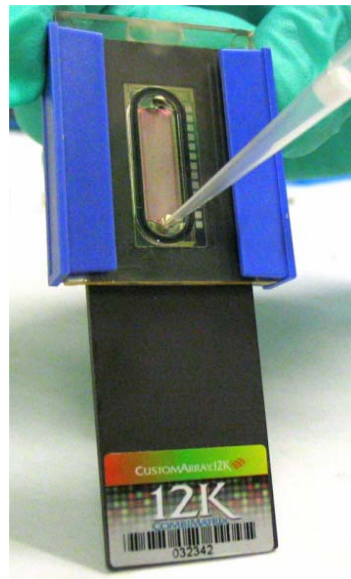
Figure 2. Assembled CustomArray™ 12K microarray with Hybridization Cap and Clips.



Handling of Assembled Microarrays

1. Wear gloves at all times when handling microarrays and reagents.
2. After a CustomArray™ 12K microarray is assembled and re-hydrated, keep the Hybridization Cap in place during all hybridization and wash steps. Do NOT allow the semiconductor microarray surface to become dry at any step in the protocol.
3. To add or remove solutions, hold the microarray with the assembled Hybridization Cap at a 45 degree angle. Use a 200 µl micropipettor with nuclease-free pipet tips to add or remove solutions through the lower solution portal of the Hybridization Cap (see Fig. 3).

Figure 3. Pipetting of solutions into the hybridization chamber of the assembled CustomArray™ 12K microarray.



4. Proceed rapidly when changing solutions during the pre-hybridization, hybridization, and washing steps. Do not leave the hybridization chamber empty for any significant length of time.
5. 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 for hybridization temperatures of 50°C or less; for higher temperatures use 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.
6. If you plan to strip and re-use a hybridized CustomArray™ 12K microarray (see Appendix A), do not allow the semiconductor surface to dry, as this will prevent any further use. Avoid prolonged storage of hybridized microarrays prior to stripping; instead, strip the microarrays, then 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.

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		
Reagent	Volume for 10 ml	Final Concentration
20X SSPE	6 ml	12X
10% Tween-20	100 µl	0.1%
0.5M EDTA	560 µl	40mM^a
Nuclease-free water	3.34 ml	
Total Volume	10 ml	

^aFinal concentration includes the EDTA from the SSPE.

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		
Step	Solution	For 10 ml
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

3. 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.

Pre-Hybridization

NOTE: The CustomArray™ 12K must be re-hydrated prior to hybridization. A pre-hybridization step is recommended to block non-specific binding of targets.

1. Pre-heat an incubator to 65°C.
2. Set a hybridization rotisserie oven to 45°C.
3. Prepare the fresh **Pre-hybridization Solution** (see Table 3).

Table 3. Pre-hybridization Solution		
Reagent	Volume for 100 µl	Final Concentration
2X Hyb Solution Stock	50 µl	6X SSPE, 0.05% Tween-20, 20mM EDTA
Nuclease-free water	35 µl	
50X Denhardt's solution	10 µl	5X
1% SDS	5 µl	0.05%
Total Volume	100 µl	

4. Fill the hybridization chamber with nuclease-free water. Avoid introducing air bubbles into the chamber. Cover the solution portals with adhesive tape to prevent evaporation. Incubate at 65°C for 10 min. Remove the microarray from the incubator and bring to room temperature. Remove the adhesive tape and aspirate the water out of the hybridization chamber.
5. Fill the hybridization chamber with the 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.
6. Load the microarray onto the rotisserie in the hybridization oven and incubate at 45°C for 30 minutes with gentle rotation.

NOTE 1: To improve microarray performance, use of a rotisserie oven or a rotating incubator is recommended to ensure mixing during hybridization. The CustomArray™ microarray can be attached to standard rotisseries using holders available from CombiMatrix (see Appendix A).

NOTE 2: Some CustomArray™ designs may require optimization of the hybridization temperature.

Hybridization

1. Mix together the purified biotinylated single-strand DNA target samples. Keep the volume of the resultant mixture below 45 μ l.
2. Denature the single-strand DNA target mixture by incubating at 95°C for 10 minutes.
3. Place on ice.
4. When you are ready to hybridize microarrays, prepare the **Hybridization Solution** as shown in Table 4.

NOTE: The Hybridization Solution should be prepared fresh each time you hybridize microarrays.

Table 4. Hybridization Solution		
Reagent	Volume for 100 μl	Final Concentration
Denatured single-strand DNA target mixture	Varies (up to 45 μl)	
2X Hyb Solution Stock	50 μl	6X SSPE, 0.05% Tween-20, 20mM EDTA
1% SDS	5 μl	0.05%
Nuclease-free water	to 100 μl	
Total Volume	100 μl	

5. Denature the Hybridization Solution at 95°C for 3 minutes, and then cool for 1 minute on ice.
6. Spin down the solution in a microcentrifuge for 5 seconds at maximum speed to collect condensate.
7. Remove the adhesive tape from the microarray and pipet the Pre-hybridization Solution out of the hybridization chamber.
8. Fill the hybridization chamber with the Hybridization Solution and mix gently with repeated pipetting. A small air bubble can be introduced to improve the mixing process if the arrays are rotated during hybridization.

NOTE: Usually the volume of Hybridization Cap is slightly less than 100 μ l. You can adjust the final volume of Hybridization Solution to 95 instead of 100 μ l, if you want the whole amount to fit into the chamber.

9. Carefully wipe excess solution from the surface of the Hybridization Cap with a lint-free tissue, and cover the solution portals with adhesive tape.
10. Load the microarray onto the rotisserie in the hybridization oven and incubate at 45°C for 1 hour 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™ microarray 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: Keep the Hybridization Cap in place during all wash steps. Hold the microarray with the assembled Hybridization Cap at a 45° angle (as shown in Figure 3), and add/remove solutions through the lower solution portal by using a 200 µl micropipette with nuclease-free pipet tip.

NOTE 2: 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 3: With the exception of the first wash step, microarrays should be incubated in the Wash solutions for a minimum of 1 minute. However, if processing multiple arrays, 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 45°C.
2. Remove the microarray from the hybridization oven. Remove the adhesive tape, and pipet the Hybridization Solution out of the chamber.
3. Using the pre-heated **6X SSPET Wash** solution, rinse the hybridization chamber, fill the chamber, 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 chamber.
4. Using the **3X SSPET Wash** solution, rinse the hybridization chamber, fill the chamber, and incubate the microarray at room temperature for 1 minute (see NOTE 3). Remove the 3X SSPET Wash solution from the hybridization chamber.
5. Using the **0.5X SSPET Wash** solution, rinse the hybridization chamber, fill the chamber, and incubate the microarray at room temperature for 1 minute. Remove the 0.5X SSPET Wash solution from the hybridization chamber.
6. Using the **PBST Wash** solution, rinse the hybridization chamber, fill the chamber, and incubate the microarray at room temperature for 1 minute. Remove the PBST Wash Solution from the hybridization chamber.
7. Using the **PBS Wash** solution, rinse the hybridization chamber, fill it, and incubate the microarray at room temperature for 1 minute. Retain the PBS Wash Solution in the hybridization chamber until you are ready to proceed to the Post-hybridization Blocking step.

Post-hybridization Blocking, Labeling, and Washing

1. Bring the 5X PBS-Casein Blocking Buffer to room temperature.
2. Remove the PBS Wash Solution from the hybridization chamber.
3. Add the 5X PBS-Casein Blocking Buffer to the hybridization chamber. Cover the solution portals with adhesive tape, and incubate the microarray at room temperature for 15 minutes.
4. Prepare the **Dye Labeling Solution** using the 1 mg/ml stock solution of the Fluorolink™ Cy5®-labeled streptavidin. Thaw an aliquot, and make a 1:1000 dilution (v/v, 1 µl per 1 ml) in the 5X PBS-Casein Blocking Buffer. Discard the used aliquot.

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

5. Remove the 5X PBS-Casein Blocking Buffer, and fill the hybridization chamber with the **Dye Labeling Solution**. Cover the solution portals with adhesive tape, and incubate the microarray at room temperature for 30 minutes. Protect the microarray from light by covering with aluminum foil.
6. Using the PBST Wash solution, rinse the hybridization chamber, fill it, and incubate the microarray at room temperature for 1 minute. Repeat the PBST Wash one more time.
7. Using the PBS Wash solution, rinse the hybridization chamber, fill it, and incubate the microarray at room temperature for 1 minute. Repeat the PBS Wash one more time.
8. Retain the PBS Wash Solution in the hybridization chamber until you are ready to proceed to the Imaging step.

Imaging of CustomArray™ 12K Microarray

IMPORTANT! The CustomArray™ 12K microarray must be scanned wet using the Imaging Solution supplied. The LifterSlip™ coverslip provided with the microarray has been specifically designed to retain the Imaging Solution without contacting the microarray surface. Do not use a standard coverslip.

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 chamber.
2. Carefully remove the hybridization chamber from the microarray by removing the clips and lifting the Hybridization Cap off the slide surface.
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. 4). 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.

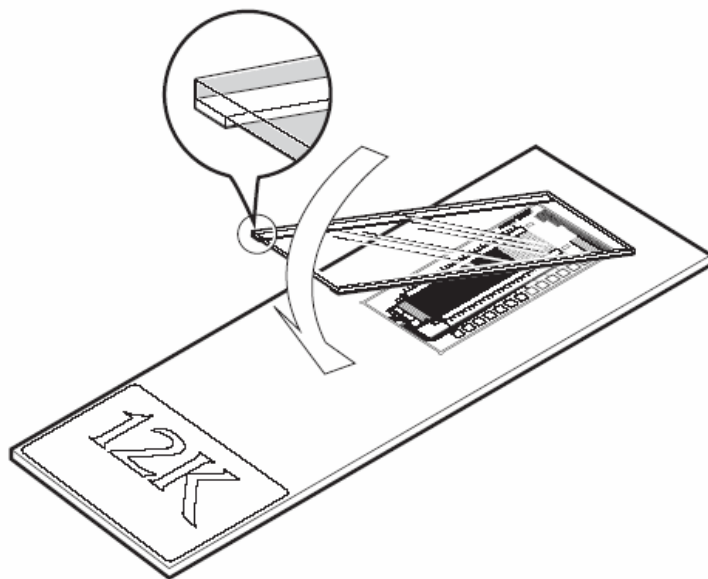


Figure 4. CustomArray™ 12K with LifterSlip™ coverslip

6. Carefully remove any excess Imaging Solution from the edge of the LifterSlip™ using a lint-free tissue, until it is resting evenly over the microarray.
7. Load the 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 microarray 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.
10. CombiMatrix has created an Excel worksheet for analysis of re-sequencing and SNP detection data. This tool can be obtained by contacting support@combimatrix.com or calling CombiMatrix Customer Support at 800-985-2269.

Appendix A. Related Products Available from CombiMatrix

CombiMatrix Rotisserie Holders for CustomArray™ 12K Microarrays

To improve microarray performance, use of a rotisserie oven or a rotating incubator is recommended to ensure mixing during hybridization. CustomArray™ 12K microarrays can be attached to standard rotisseries using holders available from CombiMatrix.

- **CombiMatrix 32 CustomArray™ Rotisserie for 12K**, Product Number 610004.

The 32 CustomArray™ Rotisserie for 12K allows the hybridization of 32 microarrays at a time. The unit has four plates, each with eight positions, using ball detent clamps to secure the microarray. The 32 CustomArray™ Rotisserie is supplied with a shaft that fits most Fisher Scientific Isotemp Hybridization Incubators. The Rotisserie can also be adapted to fit other incubation ovens.

- **CombiMatrix 8 CustomArray™ Rotisserie for 12K:**
 - Product Number for 3/8" diameter shaft is 610012,
 - Product Number for 3/4" diameter shaft is 610013.

The 8 CustomArray™ Rotisserie for 12K allows the hybridization of 8 microarrays at a time. The Rotisserie will mount on a 3/8" or 3/4" diameter shaft of an incubation oven with simple spring clasps. The model can also be adapted to fit other incubation ovens. Please indicate the oven shaft diameter when ordering.

- **CombiMatrix 3-Tray CustomArray™ for 12K**, Product Number 610008.

CustomArray™ 3-Tray for 12K is a three-position tray that fits in place of the standard Affymetrix tray/bucket in an Affymetrix oven. As many as eight trays can be loaded in one oven, allowing for 24 of CustomArray™ 12K microarrays to be hybridized at a time.

CombiMatrix CustomArray™ Stripping Kit and Clamp

- **CombiMatrix CustomArray™ Stripping Kit**, Product Number 610011.

The Stripping Kit enables re-hybridization of a CombiMatrix microarray three times, thus allowing the use of a single microarray up to four times. Each kit contains reagents for 25 stripping reactions, plus accessories for subsequent re-hybridizations.

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

The CombiMatrix Stripping Clamp is designed to be used with the **CombiMatrix CustomArray™ Stripping Kit**. The CustomArray™ Stripping Clamp is recommended for up to 100 stripping procedures.

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 12K 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