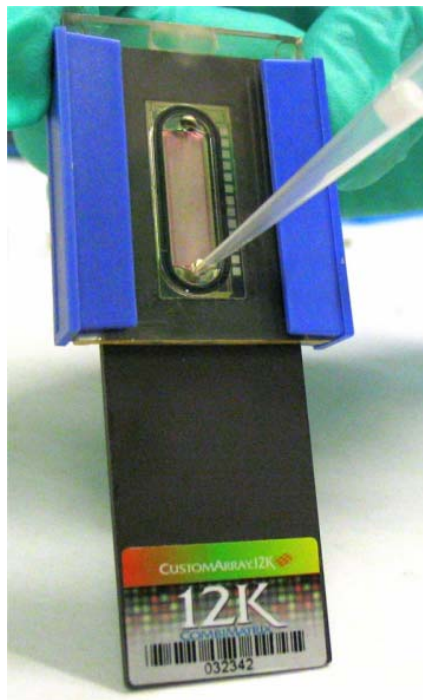
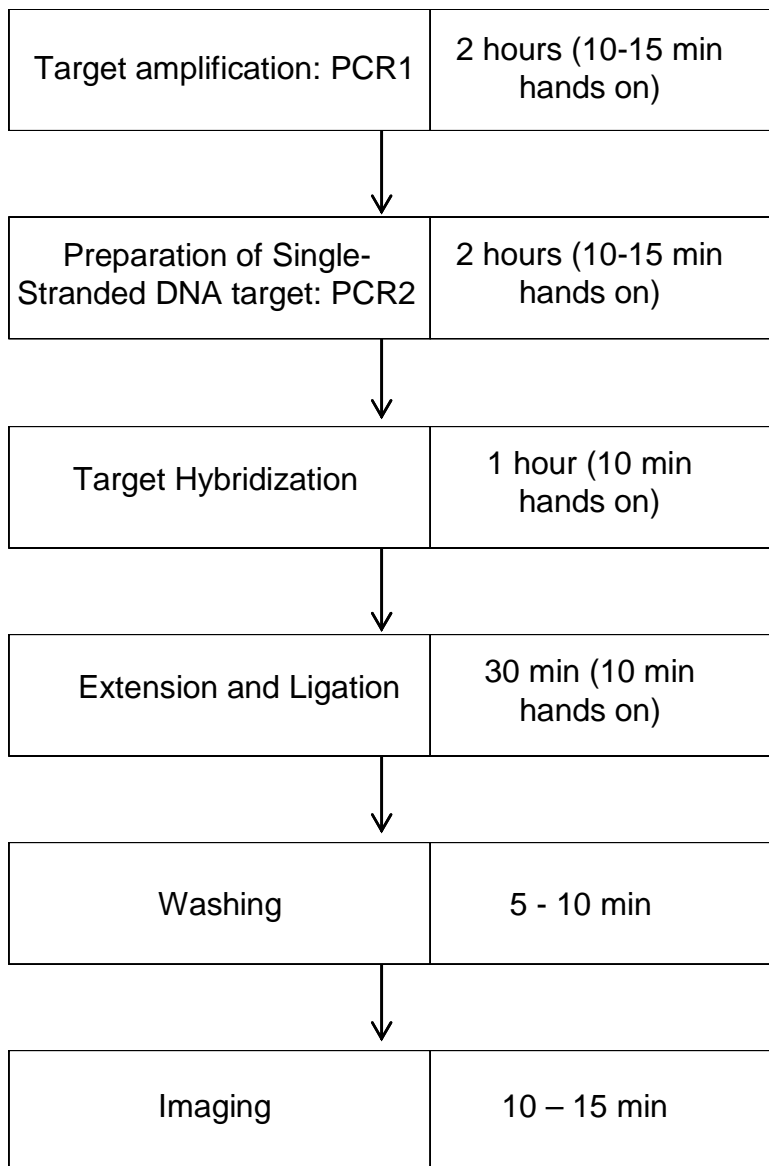


# COMBIMATRIX

Hyb & Seq™ Protocol for SNP Detection and  
Re-sequencing Using CustomArray™  
12K Microarrays (PTL009)



# SNP Detection and Re-sequencing: Hyb & Seq™ Assay Workflow



# Hyb & Seq™ Protocol for SNP Detection and Re-sequencing Using CustomArray™ Microarrays

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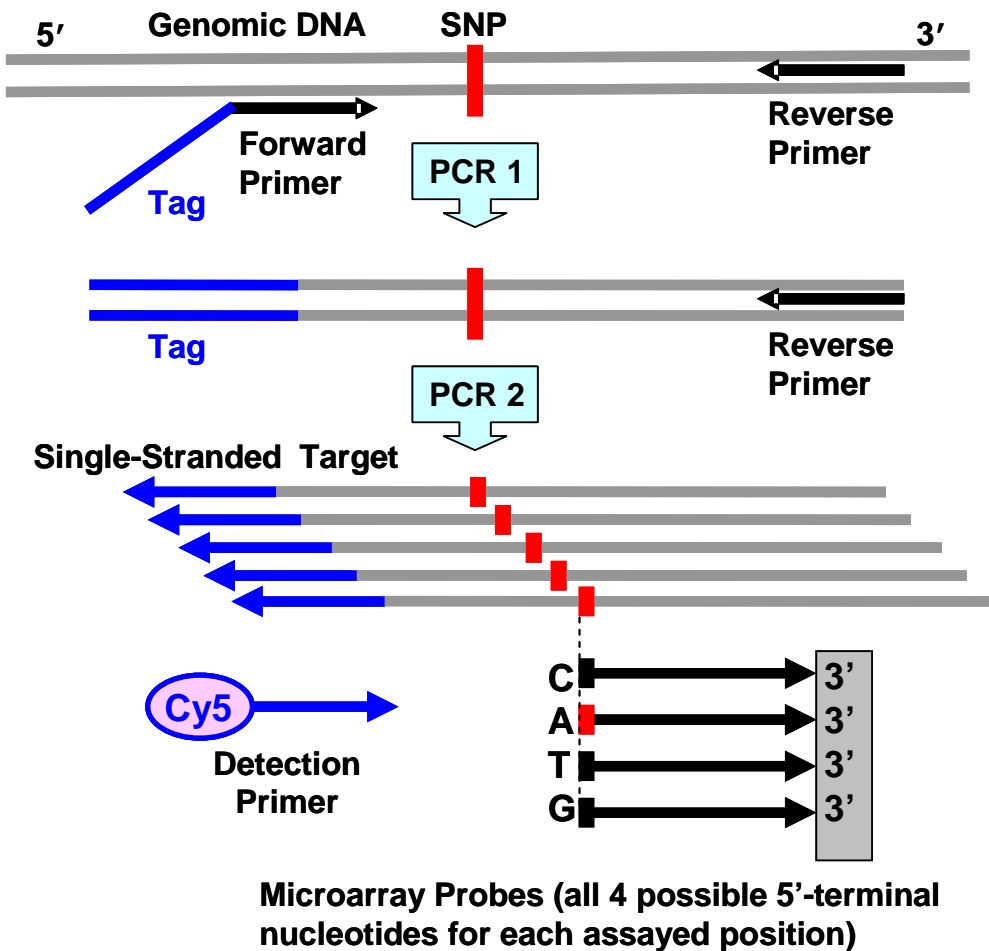
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# Hyb & Seq™ Protocol for SNP Detection and Re-sequencing Using CustomArray™ Microarrays

## Introduction

This manual describes how to perform SNP detection, genotyping, and DNA re-sequencing using the CombiMatrix Hyb & Seq™ method. This method is an enzymatic microarray-based multiplex assay that combines the sensitivity and specificity of ligation with the cost effective strategy of using a common labeled oligonucleotide detection primer that is extended to the site of the match/mismatch. The assay is schematically outlined in Figures 1 and 2.

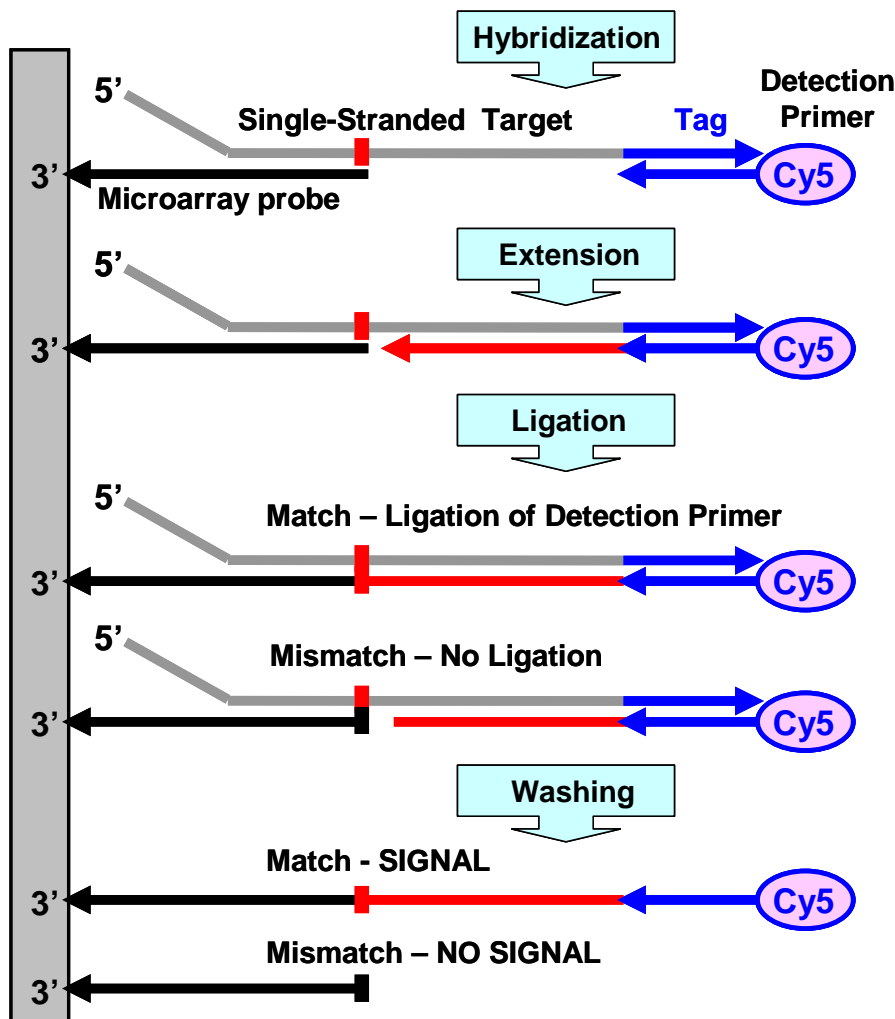
A DNA target sequence is first amplified from the genome of interest, and a sequence tag is added during amplification that is anti-sense (complementary) to the common labeled detection primer (Fig. 1). The length of amplified DNA targets should be between 100 and 1000 bp (100-200 bp fragments typically generate higher quality results than longer fragments). The second round of target amplification is performed to prepare single-stranded DNA (Fig. 1). Multiple different single-stranded DNA targets can be tested in the same assay.



**Figure 1.** Preparation of the single-stranded DNA targets and the design of microarray probes for the Hyb & Seq™ assay.

A CustomArray™ 12K microarray is synthesized with oligonucleotide probes that are complimentary to the target sequence and carry 5'-terminal mismatches corresponding to the assayed positions of the target. For each assayed position, four probes are generated carrying all possible substitutions. Thus, in total one CustomArray™ 12K microarray is sufficient to analyzed 3000 positions.

The amplified single-stranded targets are hybridized to the microarray in the presence of the common fluorochrome-labeled detection primer (Fig. 2). Microarray oligonucleotide probes and the detection primer anneal to the target. DNA polymerase and ligase enzymes are added (Fig. 2). The detection primer is extended by DNA polymerase that uses the target sequences as a template (Fig. 2). If the probe terminal nucleotide is the exact match to the target sequence, the extended detection primer gets ligated to the probe. In the case of a mismatch between the probe terminal nucleotide and the target sequence the ligation event does not happen. After the enzymatic reactions are completed, DNA:DNA hybrids are denatured by washing at high stringency (Fig. 2). If the hybridized detection primer is not ligated to the probe, it is washed away. Therefore, the hybridization signals remain only at the probes that are exact 5'-terminal matches to the target sequence (Fig. 2). Since the targets are covalently bound to the microarray probes, the hybridized microarrays can not be stripped and re-used after completion of the Hyb & Seq™ assay.



**Figure 2.** The CombiMatrix Hyb & Seq™ assay performed on the surface of CustomArray™ microarrays.

Imaging of the hybridized CustomArray™ 12K is then performed using a high-resolution fluorescent scanner. The scanner must be compatible with a 1"x3" slide format, with a minimum resolution of 5µm and an adjustable focus.

## Materials and Equipment Provided

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**IMPORTANT! Do not touch the semiconductor microarray surface. Wear gloves when handling. CustomArray 12K microarrays can be stored in a cool dry place for up to 4 months.**

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- ❑ 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)

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

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- ❑ Cy5®-Labeled oligonucleotide detection primer, CombiMatrix recommends to use 5'/Cy5/GCATCCTAATACGACTCACTATAGG, 100 µM solution in nuclease-free water
- ❑ PCR primers, 10 µM solutions in nuclease-free water:
  - a target-specific forward primer, containing the tag for the detection primer at the 5' end (5'/TAATACGACTCACTATAGGG/specific sequence),
  - a target-specific reverse primer
- ❑ 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)
- ❑ DMSO (Sigma Cat. #D8418)
- ❑ QIAquick PCR Purification kit, (Qiagen Cat. #28106)
- ❑ Rotisserie oven
- ❑ CombiMatrix CustomArray™ Holder for rotisserie oven (optional, see Appendix A)
- ❑ 37°C Incubator
- ❑ 95°C Heating Block
- ❑ Pipettors and tips, sterile plastic ware
- ❑ Gloves (powder-free)
- ❑ Adhesive tape: Scotch® Brand Magic® Transparent Tape, or a PCR sealing tape such as Nunc Brand PN 232702 (clear polyolefin liner) or PN 276014 (aluminum liner)
- ❑ Nuclease-free water
- ❑ T4 polynucleotide kinase (PNK) with 10X PNK Buffer (New England BioLabs Cat. #M0201S)
- ❑ 100 mM rATP (Promega Cat. #E601B)
- ❑ DNA ligase (*E. coli*; NAD) with 10X Ligase Buffer (New England BioLabs Cat. #M0205L)
- ❑ AmpliTaq® DNA polymerase, Stoffel Fragment (Applied Biosystems Cat. #N8080038)
- ❑ 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)
- ❑ 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:

<b>Reagent</b>	<b>Volume for one PCR (100 <math>\mu</math>l)</b>
<b>Nuclease-free water</b>	<b>67 <math>\mu</math>l</b>
<b>DNA template (10-50 ng)</b>	<b>5 <math>\mu</math>l</b>
<b>10X ThermoPol PCR Buffer</b>	<b>10 <math>\mu</math>l</b>
<b>DMSO</b>	<b>10 <math>\mu</math>l</b>
<b>10 mM dNTP</b>	<b>3 <math>\mu</math>l</b>
<b>10 <math>\mu</math>M Forward primer</b>	<b>2 <math>\mu</math>l</b>
<b>10 <math>\mu</math>M Reverse primer</b>	<b>2 <math>\mu</math>l</b>
<b>Total Volume</b>	<b>99 <math>\mu</math>l</b>

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  $\mu$ l (5 units) of Taq DNA Polymerase to each tube. Proceed with the thermal cycler program.

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NOTE: We recommend 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.

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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.

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

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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  $\mu$ l of nuclease-free water pre-warmed to 60°C. The purified reactions can be stored at -20°C until use.

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**IMPORTANT!** CombiMatrix recommends to examine the amplified DNA targets after the first amplification step by gel electrophoresis. Run a 2  $\mu$ l aliquot of each purified reaction on an acrylamide gel, or a 5  $\mu$ l aliquot on an agarose gel. Since the applied PCR conditions may not 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.

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

Preparation 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  $\mu$ 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:

<b>Reagent</b>	<b>Volume for one PCR (100 <math>\mu</math>l)</b>
<b>Nuclease-free water</b>	<b>67 <math>\mu</math>l</b>
<b>Purified double-strand DNA template from the previous step</b>	<b>7 <math>\mu</math>l</b>
<b>10X ThermoPol PCR Buffer</b>	<b>10 <math>\mu</math>l</b>
<b>DMSO</b>	<b>10 <math>\mu</math>l</b>
<b>10 mM dNTP</b>	<b>3 <math>\mu</math>l</b>
<b>10 <math>\mu</math>M Reverse primer</b>	<b>2 <math>\mu</math>l</b>
<b>Total Volume</b>	<b>99 <math>\mu</math>l</b>

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  $\mu$ l (5 Units) of Taq DNA Polymerase to each tube. Proceed with the thermal cycler program.

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**NOTE:** We recommend 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.

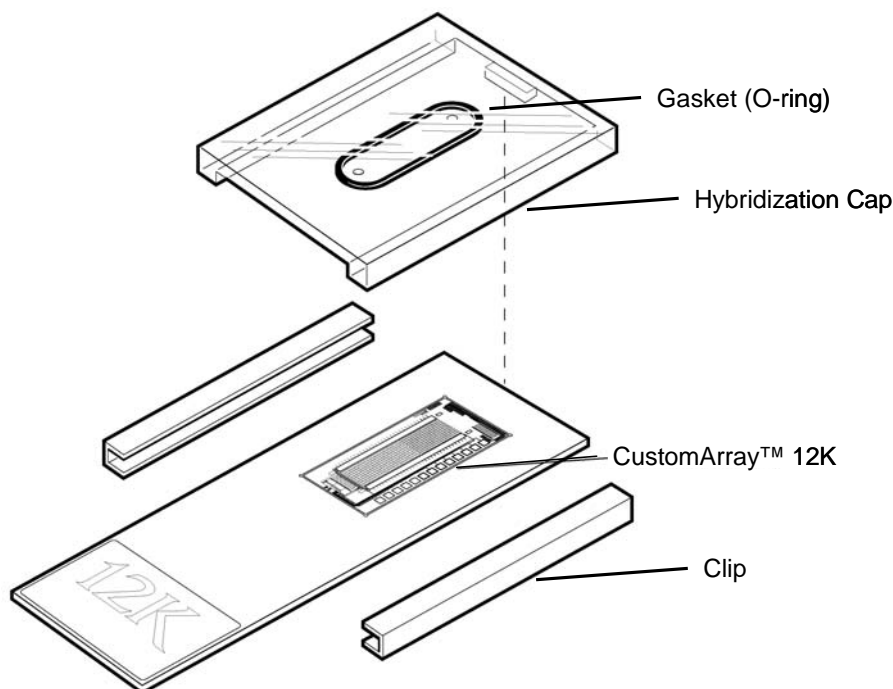
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3. Denature at 94°C for 5 min.
4. Perform 50 cycles (can be reduced to 35-40 cycles to decrease the assay time) of PCR amplification as follows:
  - Denature at 94°C for 30 seconds,
  - Anneal at 55°C for 30 seconds,
  - Extend at 72°C for 30 seconds.
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 according to the manufacturer's protocol. Elute with 60  $\mu$ l of nuclease-free water. The purified reactions can be stored at -20°C until use.

## CustomArray™ 12K Assembly

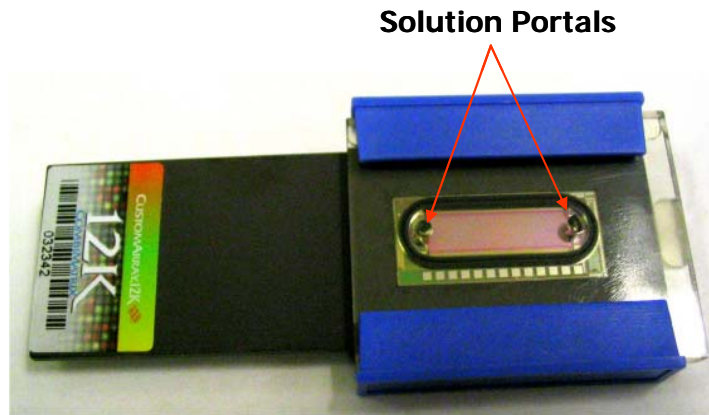
**IMPORTANT! The Hybridization Cap, Clips and O-ring are intended for single use only.**

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. 3 and 4). 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 3.** CustomArray™ 12K microarray, Hybridization Cap and Clips.

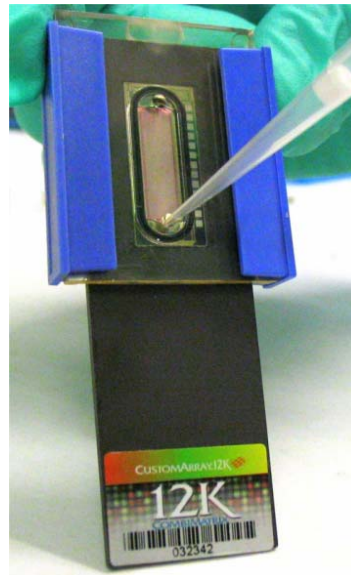
**Figure 4.** Assembled CustomArray™ 12K microarray with Hybridization Cap and Clips.



## 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. 5).

**Figure 5.** 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. After completion of the Hyb & Seq™ assay, the hybridized microarrays can not be stripped and re-used since the targets are covalently bound to the microarray probes.

## 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.

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

<sup>a</sup>Final concentration includes the EDTA from the SSPE.

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

<b>Table 2. Wash Solutions</b>		
<u>Step</u>	<u>Solution</u>	<u>For 10 ml</u>
<b>PBST Wash</b>	<b>2X PBS, 0.1% Tween-20</b>	<b>2 ml 10X PBS, 100 µl 10% Tween-20, 7.9 ml Nuclease-free water</b>
<b>PBS Wash</b>	<b>2X PBS</b>	<b>2 ml 10X PBS, 8 ml Nuclease-free water</b>

3. Prepare 100 µl of the 1X Ligase Buffer Wash per one microarray. Take 10 µl of the 10X Ligase buffer (supplied with E. coli DNA ligase) and add 90 µl of nuclease-free water. The 1X Ligase Buffer Wash solution can be stored at -20°C.
4. Prepare the 0.2 N NaOH solution using nuclease-free water. The prepared solution can be stored at room temperature for up to 6 months.

## Phosphorylation of Microarray Oligonucleotide Probes

The oligonucleotides synthesized on the microarray do not have a 5' phosphate, and they need to be phosphorylated with T4 polynucleotide kinase (PNK). This step also results in re-hydration of the CustomArray™ 12K prior to hybridization.

1. Prepare the Phosphorylation Solution using the reagents supplied with PNK (see Table 3). Pre-heat an incubator to 37°C.

<b>Table 3. Phosphorylation Solution</b>	
<b>Reagent</b>	<b>Volume 100 µl</b>
<b>Nuclease-free water</b>	<b>87 µl</b>
<b>10X PNK Buffer</b>	<b>10 µl</b>
<b>100 mM rATP stock solution</b>	<b>1 µl</b>
<b>T4 polynucleotide kinase (PNK)</b>	<b>2 µl</b>
<b>Total Volume</b>	<b>100 µl</b>

2. Fill the hybridization chamber with the Phosphorylation Solution, and cover the solution portals with adhesive tape to prevent evaporation.
3. Incubate the CustomArray™ microarray at 37°C for 30 min.
4. Wash the microarray twice with nuclease-free water at room temperature. For each wash step, add water to the chamber, gently mix by pipetting, and remove water from the chamber.
5. Leave the chamber filled with water until you are ready to proceed to the next step.

---

**IMPORTANT! Do not allow the microarray to become dry at any step in the protocol. Proceed rapidly when changing solutions during the washing steps. Do not leave the hybridization chamber empty for any significant time.**

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# Hybridization

1. Mix together the purified single-strand DNA target samples. Keep the volume of the resultant mixture below 46  $\mu$ 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.

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

---

<b>Table 4. Hybridization Solution</b>	
<b>Reagent</b>	<b>Volume 100 <math>\mu</math>l</b>
<b>2X Hyb Solution Stock</b>	<b>50 <math>\mu</math>l</b>
<b>1% SDS</b>	<b>2 <math>\mu</math>l</b>
<b>100 <math>\mu</math>M Oligonucleotide detection primer, 5'/Cy5/GCATCCTAATACGACTCACTATAGG</b>	<b>2 <math>\mu</math>l</b>
<b>Denatured single-strand DNA target solution</b>	<b>up to 46</b>
<b>Nuclease-free water</b>	<b>to 100 <math>\mu</math>l</b>
<b>Total Volume</b>	<b>100 <math>\mu</math>l</b>

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 water 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.

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

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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 45-60 minutes with gentle rotation.

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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).

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# Hybridization Washing

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**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.

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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 5), 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: 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.

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1. Remove the microarray from the hybridization oven. Remove the adhesive tape, and pipet the Hybridization Solution out of the chamber.
2. 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.
3. Repeat the PBST Wash step (step 2) one more time for a total of 2 washes.
4. Using the **PBS Wash** solution, rinse the hybridization chamber, fill the chamber, and incubate the microarray at room temperature for 1 minute. Remove the PBS Wash Solution from the hybridization chamber.
5. Repeat the PBS Wash step (step 4) one more time for a total of 2 washes.
6. Using the **1X Ligase Buffer Wash** solution, rinse the hybridization chamber, fill the chamber, and incubate the microarray at room temperature for 1 minute. Retain the 1X Ligase Buffer Wash solution in the chamber until you are ready to proceed to the next step.

## Enzymatic Reaction on Microarray

1. Prepare the Enzyme Solution using the 10X Ligase buffer supplied with *E. coli* DNA Ligase (see Table 5).

<b>Table 5. Enzyme Solution</b>	
<b>Reagent</b>	<b>Volume 100 <math>\mu</math>l</b>
<b>Nuclease-free water</b>	<b>84 <math>\mu</math>l</b>
<b>10X DNA ligase buffer</b>	<b>10 <math>\mu</math>l</b>
<b>10mM dNTP</b>	<b>2 <math>\mu</math>l</b>
<b>AmpliTaq® DNA polymerase, Stoffel Fragment</b>	<b>2 <math>\mu</math>l</b>
<b>DNA ligase (<i>E. coli</i>)</b>	<b>2 <math>\mu</math>l</b>
<b>Total Volume</b>	<b>100 <math>\mu</math>l</b>

2. Fill the hybridization chamber with the Enzyme Solution. Avoid introducing air bubbles into the chamber. Cover the solution portals with adhesive tape to prevent evaporation.
3. Incubate the microarray at 37°C for 30 minutes.
4. Remove the microarray from the incubator. Remove the adhesive tape, and pipet the Enzyme Solution out of the chamber.
5. Using the **0.2 N NaOH** solution, rinse the hybridization chamber, fill the chamber, and incubate the microarray at room temperature for 5 minutes. Remove the NaOH Solution from the hybridization chamber.
6. Repeat the NaOH washing step.
7. Rinse and fill the hybridization chamber with the PBS Wash solution. Proceed to the imaging step.

## Imaging of CustomArray™ 12K

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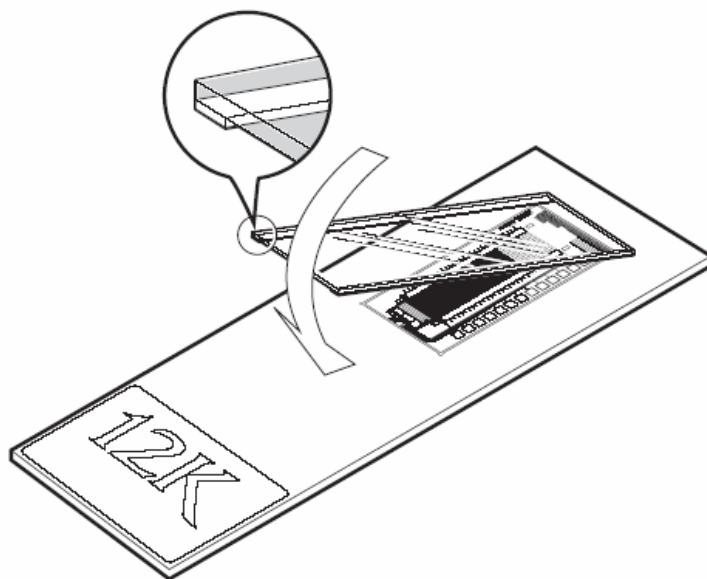
**IMPORTANT!** The CustomArray™ 12K must be scanned wet using the Imaging Solution supplied. The LifterSlip™ coverslip provided with the CustomArray™ has been specifically designed to retain the Imaging Solution without contacting the microarray surface. Do not use a standard coverslip with CustomArray™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

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**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.

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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. 6). 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 6.** 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 CustomArray™ 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. 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](mailto: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™ 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 CustomArray™ 12K microarrays to be hybridized at a time.