Agilent microarray hybridization chamber user guide



For use in hybridizing Agilent 60-mer oligo microarrays with Agilent G2534A microarray hybridization chambers

Version 2.0

August 2006

Agilent microarray hybridizations enabled by SureHyb technology

Research use only

Agilent Technologies

Notices

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www.agilent.com/chem/dnasupport

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Introduction

Agilent is enabling researchers to successfully run microarray experiments in their own labs, with enhanced ease-of-use and quality. The Agilent microarray hybridization chamber (G2534A) represents a second generation product advancement in engineering based on Agilent's SureHyb technology–comprising an integrated set of hybridization reagents and other products.

Built to enable "hybridizations anywhere," these new chambers are fabricated from stainless steel for precision fit and finish and long-term durability in most lab environments. To ensure quality hybridizations from run to run, disposable glass gasket slides are used. The silicone gaskets are fabricated in an industrial-scale setting and meet Agilent's stringent quality standards. The gasket slides come in a variety of formats to accommodate most of Agilent's microarray layouts. Because these are disposable, they are not meant for reuse–sparing researchers from potential erroneous results.

When combined with Agilent's 60-mer oligo microarray processing protocol and *In situ* Hybridization kit series of reagents, researchers will benefit from a fully integrated and tested system that will improve productivity while reducing microarray experiment variability.

NOTE

Agilent cannot guarantee microarray performance using non-Agilent protocols in microarray labeling and hybridization and it does not provide support to any non-Agilent protocols.

This user guide may be updated periodically, so we encourage you to register online for product support updates at: www.agilent.com/chem/dnasupport

Updated protocols for all of Agilent's gene expression products are available at: www.agilent.com/chem/dnasupport

Required Equipment

Agilent microarray chamber kit (G2534A) contents

Component	
Microarray hybridization chamber assemblies (Chamber base, cover, clamp assembly)	G2534-60001
Plastic tweezers	8710-0777
Microarray hybridization chamber user guide	G2534-90001

Store protected in a storage box or drawer.
DO NOT stack or drop chamber components as this can cause irreparable harm to the precision stainless steel hybridization surfaces.



Required Equipment

Disposable gasket slides for Agilent microarray hybridization chambers

Component	Part Number
Microarray hybridization chamber, stainless steel	G2534A
Gasket slide, 1 microarray/slide format (5 slides/box)	G2534-60003
Gasket slide, 2 microarrays/slide format (5 slides/box)	G2534-60002
Gasket slide, 4 microarrays/slide format (5 slides/box)	G2534-60011



Practice slides

We strongly recommend that all new users of Agilent's microarray hybridization chambers practice assembling and disassembling these chambers prior to use. Practice slides are available for purchase and provide an excellent means to assess proficiency with handling these microarray hybridization components. Each practice slide surface mimics the surface on an oligo microarray–so wetting and bubble formation will be identical. Use of practice slides will provide users with added confidence that they have mastered the assembly, pipetting and disassembly steps.

Component	Part Number
Practice slide kit (5 slides/kit)	G2534-60515

Required Equipment

Partial listing from the Agilent 60-mer oligo microarray processing protocol

(consult the most up-to-date protocol on our Web site for a complete listing)

- Powder-free gloves
- Micropipettors to pipette a range of 0.1 µL to 1 mL volumes
- Sterile, nuclease-free aerosol barrier pipette tips
- Ice bucket
- Timer
- Three (3) slide staining dishes (Thermo Shandon catalog number 121 or equivalent)
- Slide rack
- Magnetic stir plate (2)
- Magnetic stir bars (2); one large
- 1 L beaker
- Agilent-recommended hybridization oven (e.g., Agilent product number G2545A)
- Oven hybridization rotator for Agilent microarray hybridization chambers (Agilent product number G2530-60029)

Other Required Reagents—Not Included in the Kit

Recommended hybridization kit

Agilent Gene Expression Hybridization Kit (p/n 5188-5242) or Agilent Oligo aCGH Hybridization Kit (p/n 5188-5220) - provides the necessary hybridization reagent, and control targets needed to hybridize cRNA or cDNA targets to an Agilent oligo microarray.

Gene Expression Hybridization Kit components

- 25x fragmentation buffer (100 µL)
- 2x hybridization buffer (2.5 mL)
- 2x GEx hybridization buffer HI-RPM (2.5 mL)
- 10x blocking agent (lyophilized)



Oligo aCGH Hybridization Kit components

- 2x oligo aCGH Hybridization solution (7 mL)
- 10x oligo aCGH blocking agent (lyophilized)

Reagents in this kit are sufficient to perform the following number of hybridizations, based on the Agilent microarray format provided:

- Agilent 4 microarrays/slide format–enough reagent to hybridize 10 slides (40 microarrays total)
- Agilent 2 microarrays/slide format–enough reagent to hybridize 10 slides (20 microarrays total)
- Agilent single microarray/slide format–enough reagent to hybridize 10 slides (10 microarrays total)

Large-volume kit availability: For users who desire a larger volume kit and respective hybridization components, Agilent offers the Large Volume *In situ* Hybridization kit-plus, (product number 5185-5977). Please consult the Agilent Web site for more details on ordering this larger volume kit or any of its components separately, www.agilent.com/chem/contactus.

Other required reagents

- Nuclease-free water (Invitrogen catalog number 10977-015)
- Agilent-specified wash solutions listed in the Agilent 60-mer oligo microarray processing protocol
- Deionized water, nuclease-free water (e.g., Milli-Q)

NOTE

Note that Milli-Q water is not guaranteed to be nuclease-free. Improperly maintained Milli-Q systems may harbor nucleases. Milli-Q water systems that are improperly maintained or monitored may introduce nucleases. Please verify the integrity of your own Milli-Q water before use.

Safety Notes

- Observe standard laboratory safety procedures when working in the laboratory.
  - **The edges of the slides may be sharp. Please handle carefully.**
  - **Pipette tips may be sharp. Please handle carefully and dispose of appropriately.**
  - **Wear appropriate protective equipment when working in a laboratory.**
- The recommended Agilent hybridization buffer contains lithium chloride (LiCl) and lithium lauryl sulfate (LLS).
  - **LiCl is toxic.**
  - Its target organ is the central nervous system.
  - It is a potential teratogen and may cause harm to breast-fed babies.
  - It may also impose a risk of impaired fertility.
  - LiCl is harmful by inhalation, by contact with the skin, and if swallowed. Wear suitable protective equipment.
  - **LLS** is harmful by inhalation and is irritating to the eyes, the respiratory system, and the skin. Again, please wear suitable protective equipment.
- The recommended buffer in the Agilent *In situ* Hybridization kit-plus contains Triton X-102:
  - **This compound is harmful if swallowed and may be harmful if inhaled or absorbed through the skin.**
  - **There is a risk of serious damage to your eyes in the event of contact. If either compound contacts your eyes, rinse with copious amounts of water for at least 15 minutes. Seek medical advice.**

For the Material Safety Data Sheets (MSDSs) for these compounds, please visit www.agilent.com/chem/msds.

Specified Use

- Agilent designed these microarray chambers and accessories to be used with its 60-mer oligo microarrays.

NOTE

- Use with non-Agilent microarray formats is up to the sole discretion of the researcher. Agilent cannot support use of these chambers with other microarrays and is not responsible for results generated using non-Agilent microarrays or reagents.
- Gasket slide specifications:

  Gasket dimensions for the 1 pack (one gasket/slide format), 2 pack (two gaskets/slide format), and the 4 pack (four gaskets/slide format) as well as the required printed microarray dimensions on a 1" x 3" (25 mm x 75 mm) glass slide are available in a special Agilent application note (publication number 5988-9939EN). To obtain this guide, please visit the Agilent Web site and register to access this publication number.
  www.agilent.com/chem/dnasupport

  Required microarray glass dimensions (1 mm thick, 25.4 mm wide x 76.2 mm long)

  Fill volumes:

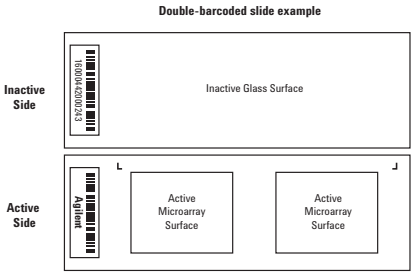
  4 microarrays/slide format = 100 µL/maximum/microarray

  2 microarrays/slide format = 245 µL/maximum/microarray

  1 microarray/slide format = 490 µL/maximum/microarray

Notes and Recommendations on Handling Agilent Oligo Microarrays

- Each slide contains one, two, or four microarrays. They are printed on the side containing the barcode labeled **"AGILENT."** We call this side the **active side.** The numeric barcode alone is on the **inactive** side of the slides.
- Please consult the section "Generating Images of the Hybridized Microarrays" in the Agilent 60-mer oligo microarray processing protocol for the microarray layout, file formats on the microarray kit CD, as well as microarray layout, feature dimensions and spacing information for use with non-Agilent scanners.



General Procedural Notes

- Follow Biosafety Level 1 (BL1) safety rules.
- To prevent contamination of reagents by ribonucleases, always wear powder-free laboratory gloves and use dedicated solutions and pipettors with nuclease-free aerosol-resistant tips.
- Maintain a clean work area.
- When preparing hybridization or wash reagents and handling them, **NEVER** use an apparatus that has been used to handle RNAse solutions.
- Remember to set your hybridization oven to 65°C before you need to put your assembled microarray hybridization chambers into it.
- Microarray handling precaution: When handling slides by their edges, always wear gloves, as the edges may be sharp. **Never touch the surfaces of the slides.**

Before You Begin

Intended use of this guide

This user guide is a supplement to the Agilent 60-mer oligo microarray processing protocol (available online at: www.agilent.com/chem/dnasupport) and is intended to demonstrate the assembly and disassembly procedures of the Agilent microarray chambers (G2534A).

Recommendations

1. **Handling recommendations:**

   Treat these hybridization chambers and accessories with care. The stainless steel surfaces, if not stored or handled properly, may be prone to damage that could adversely affect the performance of the microarray chamber sealing capability.

   The GASKET SLIDE should remain in its packaging prior to placement into the Microarray CHAMBER BASE. Care should be taken to handle the GASKET SLIDE by its edges only.



2. **Storage recommendations:**

   Microarray chamber assemblies

   We strongly recommend that the chambers be stored in a protected environment, free from any potential damaging forces that include "stacking, dropping, or nuclease contamination." When storing, dry each part and then assemble the chambers and line up neatly in a drawer or store in protective plastic containers.



   Microarray gasket slides

   These gasket slides come packaged in a protective sleeve and assembled in a shipping box. We recommend storing these gasket slides in their native shipping boxes or, alternatively, storing them in a protected container to avoid breakage and potential contamination by nucleases.



NOTE

3. **Labeling recommendations:**

Microarray chamber assemblies

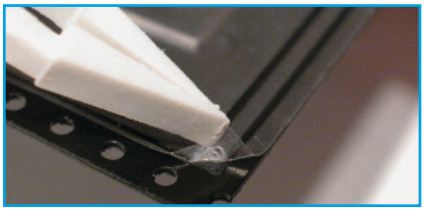
To better track samples during processing, we suggest that you pre-label your Chamber Bases. One way to do this is to scribe the numbers onto the outer, top edges of the pieces. Alternatively, you can mark them with a BLACK waterproof marking pen.



4. **Assembly recommendations:**

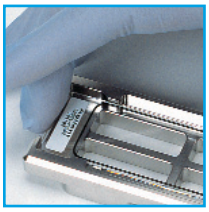
Removing gasket slide from protective sleeve

Take care not to slice or cut open the GASKET SLIDE protective packaging. With tweezers, carefully lift up the corner of the clear plastic covering and slowly pull back the protective sheath. With clean powder-free gloved fingers, remove the GASKET SLIDE from its package being sure to handle only the edges of the slide. Immediately place in the CHAMBER BASE to avoid any potential contamination.



Gasket slide placement

Always place the GASKET SLIDE into the CHAMBER BASE with the "Agilent" label facing up and aligned in the rectangular end section of the base.

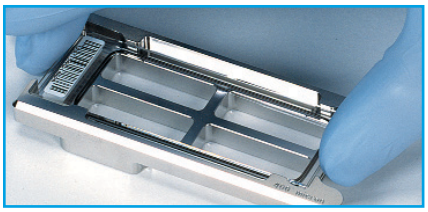




Rectangular Section

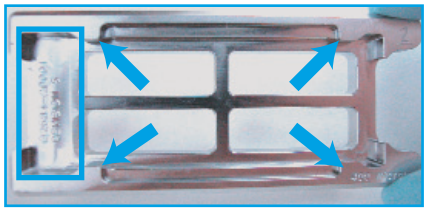
Oligo microarray slide placement

Always place the oligo microarray slide into the CHAMBER BASE with the NUMERIC BARCODE FACING UP and aligned in the rectangular end section of the base.



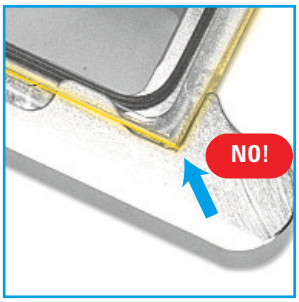
Gasket slide alignment in chamber base

In addition to the rectangular alignment area, there are 4 guideposts in the CHAMBER BASE. These facilitate alignment of both the GASKET SLIDE and oligo microarray slide into the CHAMBER BASE.



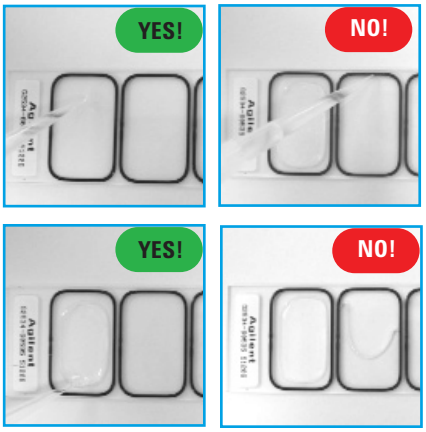
When placing either of these slides into the base, inspect to make sure that the slide does not rest on the edge of the CHAMBER BASE.

To familiarize new users with these placement techniques, we recommend that they practice placing two ordinary 1″ x 3″ (25 mm x 75 mm) glass slides into the CHAMBER BASE prior to running an experiment.



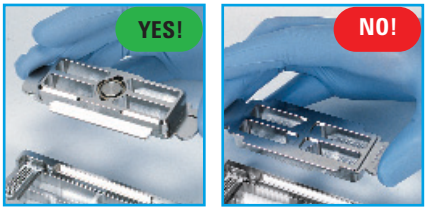
Dispensing Hybridization Solution

To avoid spillage/leakage of the hybridization solution, dispense the hybridization solution to the center of the gasket well without touching the gasket with the pipette tip.



Cover placement

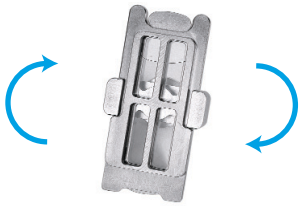
To ensure a proper seal, the CHAMBER COVER must be placed correctly on the CHAMBER BASE containing the slides. See the figures at right that detail the correct and incorrect placement and orientation of the CHAMBER COVER component.



5. **Wet the gasket slide seal after assembling the chamber:**

   Rotate assembled chamber clockwise

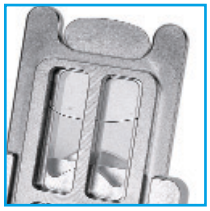
   Wetting the GASKET SLIDE seal will help ensure that the mixing bubble will rotate freely during the hybridization step. Once the entire chamber has been assembled, rotate it vertically 2 to 3 times to help wet the gasket lining(s). While you are rotating it, observe the bubble formation as described in the next step.

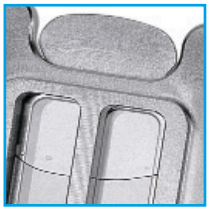


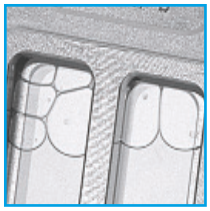
6. **Inspect for proper bubble formation:**

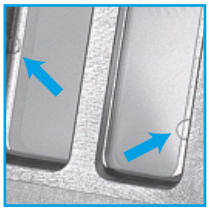
Good bubble

Hybridization is best facilitated by a single, large mixing bubble (top 2 figures to the right) which freely moves around the chamber when rotated. After assembly, verify that there are NO STRAY/STATIONARY BUBBLES that stay in one place when rotating the assembly clockwise in your hands. These can be removed by gently tapping each corner of the chamber assembly on a firm surface as you rotate it.









Multiple bubbles are acceptable so long as they move when you rotate the chamber.

DO NOT Load into the hybridization oven/rotator rack until these stray or stationary bubbles move freely!

7. **Cleaning chamber assemblies:**

Avoid stacking, piling, throwing or other mishandling of the assemblies

As described in the Storage Recommendations, these chambers are precision engineered and prone to damage by mishandling resulting from throwing chamber pieces into a sink or stacking them haphazardly in a wash basin. Take care to line up each chamber piece side by side in a wash basin for cleaning and rinsing to avoid potential damage to the stainless steel surfaces.



RNAse cleaning solutions or like cleaners should be thoroughly rinsed off each part as any remaining solution can potentially damage the stainless steel surface. Rinse with copious amounts of water. Dry each part completely and then assemble and store appropriately.



Microarray Hybridization Chamber Instructions

CAUTION

1. Preheat the hybridization oven to 65 °C

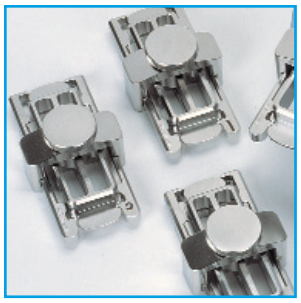
Adhere to the hybridization temperature and time settings specified in the Agilent 65-mer oligo microarray processing protocol. Agilent cannot support its microarrays where these hybridization conditions are not followed.

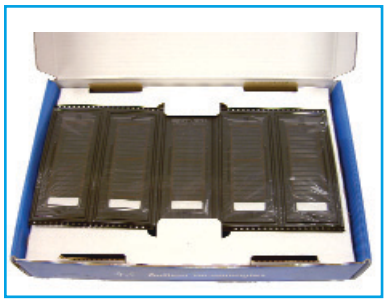


2. Prepare materials prior to loading samples

a. Ensure that the work area is clean and free from potential contaminants.

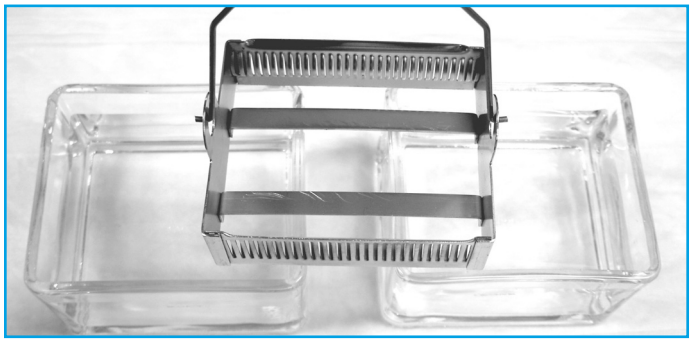
b. Gather the necessary quantity of labeled chamber assemblies and packaged gasket slides to process samples. Disassemble chambers, if needed, and line up bases, corresponding covers and clamp components.





c. Prepare pipettes and tips for sample addition steps.

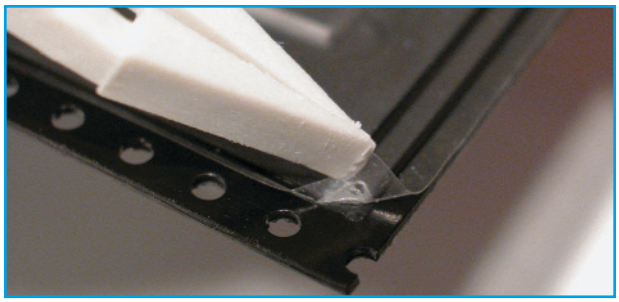
d. **For the wash steps:** Organize wash staining dishes, wash accessories and any needed wash solutions.



Microarray Hybridization Chamber Instructions

3. Remove gasket slide from its packaging

With tweezers, carefully lift up the corner of the clear plastic covering and slowly pull back the protective sheath. With clean, powder-free gloved fingers, remove the GASKET SLIDE from its package–being sure to handle the slide only from its edges. Immediately place in the CHAMBER BASE to avoid any potential contamination from surrounding surface materials.

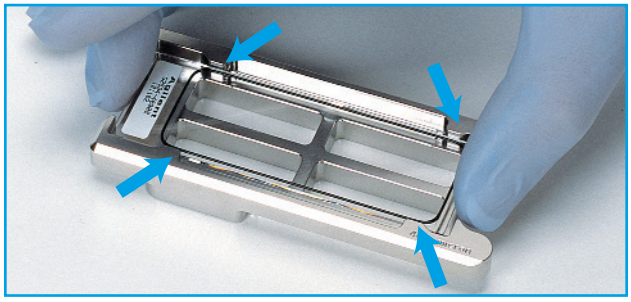


4. Insert hybridization gasket slide into chamber base

a. Prior to adding the GASKET SLIDE to the CHAMBER BASE, ensure that you are holding the slide with the label reading "Agilent" facing up–denoting the "gasket-side" of the slide.

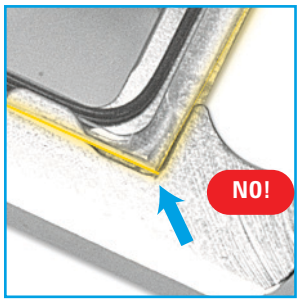


b. Locate the 4 guideposts and rectangular barcode guide in the CHAMBER BASE. Position the GASKET SLIDE between the 4 guideposts (see illustration) with the "Agilent Label" resting over the base's "rectangular barcode shaped" guide area. Gently place the GASKET SLIDE into the CHAMBER BASE.



NOTE

c. Check for any potential misalignment where the GASKET SLIDE may not be resting flush against the CHAMBER BASE. Re-adjust to a flush position against the CHAMBER BASE if necessary.





5. Pipette the sample

a. Select the correct volume of hybridization mixture to pipette onto the GASKET SLIDE surface. Refer to the Agilent 60-mer oligo microarray processing protocol (available online at: www.agilent.com/chem/dnasupport) for target and hybridization solution preparation steps.

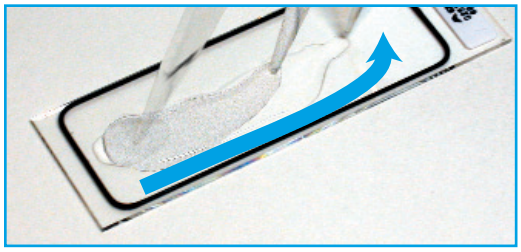
4 microarrays per slide = 100 uL maximum/microarray

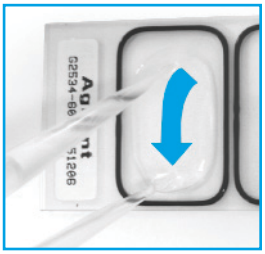
2 microarrays per slide = 245 µL maximum/microarray

1 microarray per slide = 490 µL maximum/microarray

b. Aspirate the required amount of sample and apply to the microarray surface in the following manner:

Starting close to one end of the inner gasketed well area, slowly dispense your sample while moving your pipette to the opposite end of the well without touching the slide–we call this "drag and dispense" pipetting method. This facilitates even distribution of sample across the surface and avoids any potential of mis-pipetting the sample over the gasket edge.





CAUTION

The images above are for demonstration purposes only. The backing slide is always placed in the chamber base before dispensing the hybridization solution.

6. Add the oligo microarray slide

With gloved fingers, retrieve a 60-mer oligo microarray from its slide holder. Grab the slide between your thumb and index finger, NUMERIC BARCODE SIDE FACING UP, and carefully lower it on top of the GASKET SLIDE.

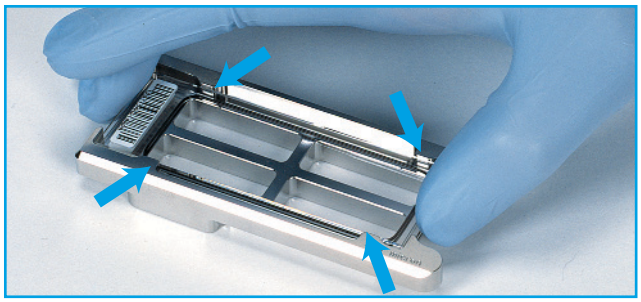




NOTE

Barcode ends of both slides must line up at rectangular end of the CHAMBER BASE.

Use the 4 CHAMBER BASE guideposts and rectangular end of the base to position the oligo microarray slide as you lower it to within 3 mm (1/8") above the GASKET SLIDE. Once positioned, gently rest it on the lower GASKET SLIDE.



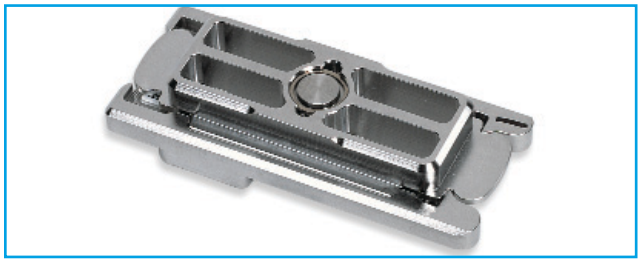
NOTE

Be sure not to drop the array slide onto the gasket slide as this will increase the chances of sample mixing between gasket wells. Ensure that the oligo microarray is not ajar. Quickly reposition the slide if needed. Once correctly placed–**do not attempt to move the chamber and sandwiched slides** as this could cause leakage of the hybridization solution.

7. Assemble the chamber

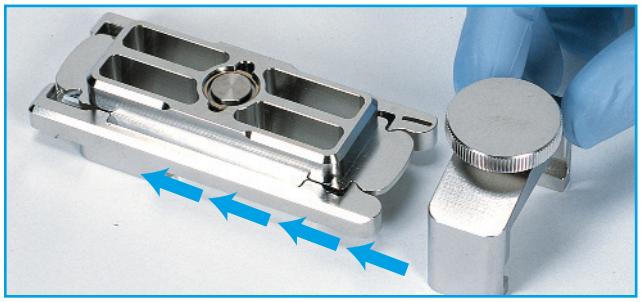
a. CHAMBER COVER placement:

Place the CHAMBER COVER, correct side facing up, onto the CHAMBER BASE containing both "sandwiched" slides.



b. Attach CLAMP ASSEMBLY:

From the rounded corner of the CHAMBER BASE, slip the CLAMP ASSEMBLY onto the CHAMBER BASE and COVER until it stops firmly in place–resting at the center of the two pieces.



c. Tighten the completed CHAMBER ASSEMBLY:

Before you start to tighten the THUMBSCREW, be sure to leave the CHAMBER ASSEMBLY flat on the lab bench. This will help avoid spillage of the hybridization solution.

Next, firmly tighten the THUMBSCREW **fully handtight.**

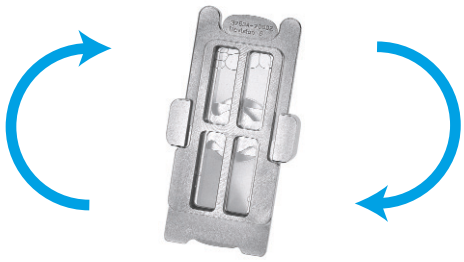
NOTE

The slides will not be harmed by hand-tightening. DO NOT use tools to tighten the THUMBSCREW. The use of pliers or other tools can damage the parts and will void the warranty.



8. Wet the gasket

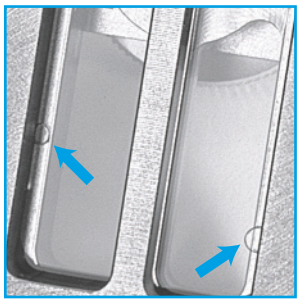
Rotate the final assembled chamber in a VERTICAL ORIENTATION, 2 to 3 times to wet the gaskets. This helps ensure that the hybridization solution will coat the entire surface of the microarray during the incubation process.

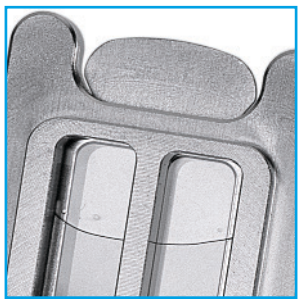


Microarray Hybridization Chamber Instructions

9. Inspect for good bubble formation

Once you've completed the "wetting" procedure (step 8), hold the chamber vertically and inspect for stray or small bubbles that don't move as you rotate the chamber. You should have a "large mixing bubble" that should dislodge these small stray or stationary bubbles.





NOTE

If the small stray or stationary bubbles persist, gently tap the assembled chamber on a firm surface, rotating the chamber on its sides as you tap. This is the preferred means to dislodge these types of bubbles. Inspect again and repeat if necessary until the small stray or stationary bubbles dissipate.

10. Continue the process of loading samples and assembling chambers

Repeat steps 2 through 9 until you've loaded all of your samples and assembled your chambers.

11. Place assembled chambers into the hybridization oven

Load each assembled chamber into the oven rotator rack. Once loaded, close the door and adjust the rotator speed to match what is specified in the Agilent 60-mer oligo microarray processing protocol.





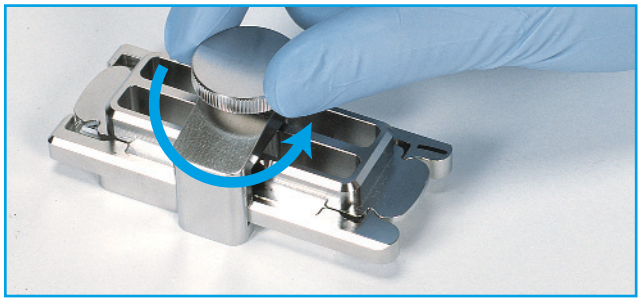
12. Disassemble the hybridization chamber

NOTE

a. Prepare the wash staining dishes as described in the Agilent 60-mer oligo microarray processing protocol. Fill with Wash Solutions. Have the slide racks, stir bars and magnetic stirrer ready to go. Locate a pair of blunt end tweezers and place them next to the FIRST wash staining dish for quick disassembly of slides.

b. Remove 1 microarray hybridization CHAMBER ASSEMBLY from the oven at a time. This prevents any unnecessary cooling of the chambers during the disassembly process.

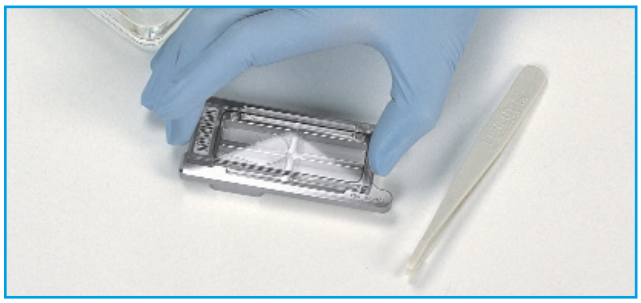
c. Place the hybridization CHAMBER ASSEMBLY on a flat surface and loosen the THUMBSCREW, turning COUNTERCLOCKWISE .



d. Slide off the CLAMP ASSEMBLY and remove the CHAMBER COVER

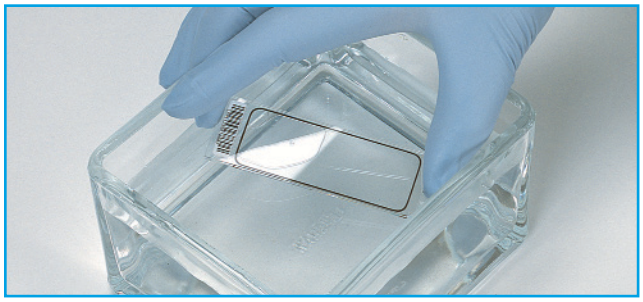
NOTE

e. With gloved fingers, remove the "sandwiched slides" from the CHAMBER BASE by grabbing the slides from their ends. Keep the oligo microarray slide NUMERIC BARCODE FACING UP as you quickly transfer the sandwiched slides to the **FIRST** wash staining dish (step 13).



13. Remove the oligo microarray slide

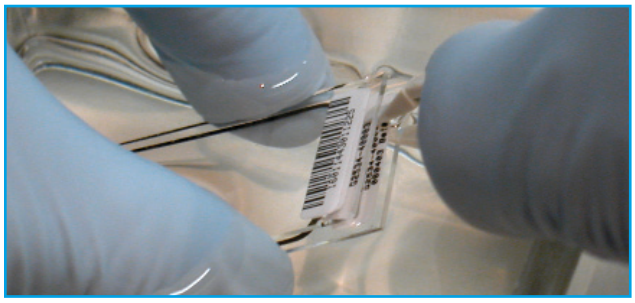
a. Without letting go of the "sandwiched slides," submerge the slides into the **FIRST** wash staining dish containing **Wash Solution 1**.



NOTE

b. With the "sandwiched slides" **completely submerged** in the wash solution, pry the two slides apart from the **barcode end only**.

Do this by slipping one of the blunt ends of the tweezers between the slides and then GENTLY turn the tweezers upward or downward to separate the slides.



Let the GASKET SLIDE drop to the bottom of the wash staining dish.



NOTE

c. Remove the oligo microarray slide quickly and place into the slide rack contained in the **SECOND** wash staining dish–containing **Wash Solution 1**. Minimize exposure of the slide to air.



14. Repeat disassembly procedure

Remove the next CHAMBER ASSEMBLY from the oven and load the slides into the rack following steps 12b to 13c. Place up to 8 slides in each rack.

NOTE

Microarray Hybridization Chamber Instructions

15. Follow slide washing, drying and storage procedures

Agilent provides detailed washing and drying procedures for its 60-mer oligo microarrays. It is important that you follow the appropriate protocol chosen for your particular experimental design.

Agilent 60-mer oligo microarray processing protocols are available on the Agilent Web site at: www.agilent.com/chem/dnasupport

16. Dispose of gasket slides in a safe manner

Once all gasket slides have been removed, dispose of them in a "sharp objects" disposal vessel.



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Global Web site: www.agilent.com/chem/dna

Information, descriptions and specifications are subject to change without notice. Please register online with Agilent to receive new product updates at:
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Part Number: G2534-90001, August 2006, Version 2.0





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