



Agilent G2534A Microarray Hybridization Chamber User Guide

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Agilent Technologies

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

Agilent lets you successfully run microarray experiments in your own lab, 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 chambers are made 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 made 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 gasket slides are disposable, they are not meant for reuse, which reduces potential erroneous results.

When combined with Agilent's microarray processing protocol and hybridization reagents, you benefit from a fully integrated and tested system that improves productivity while you reduce microarray experiment variability.

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.

Updated protocols for all of Agilent's microarray products are available at www.agilent.com/chem/dnamanuals-protocols.

Before You Begin

Make sure you read and understand the information in this chapter and have the necessary equipment and reagents listed before you start an experiment.

Safety Notes

WARNING

- **Observe standard laboratory safety procedures when working in the laboratory.**
 - **The edges of the gasket slides may be sharp. Please handle carefully.**
-

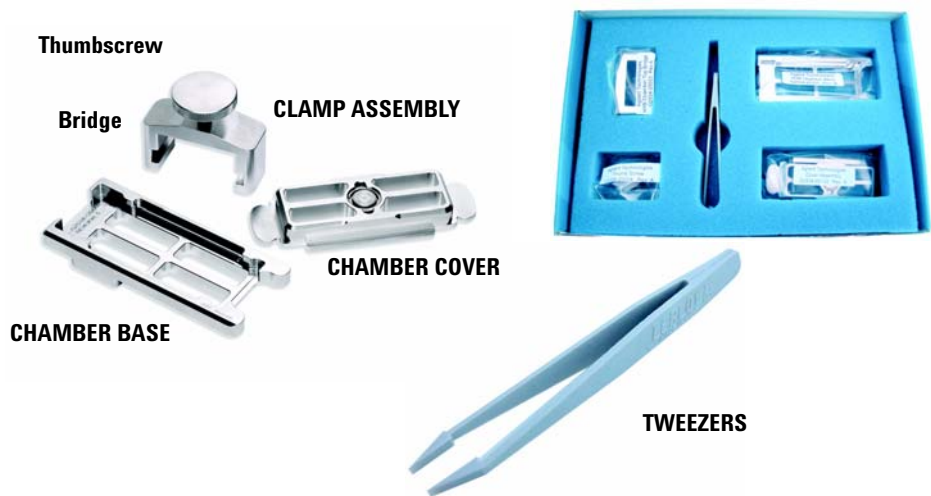
Agilent Microarray Hybridization Chamber Kit (G2534A) Contents

Table 1 Hybridization Chamber Kit Content

Component	Description
Hybridization Chamber Assembly	Chamber base, chamber cover, clamp assembly and tweezers
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CAUTION






Store protected in a storage box or drawer. Do *not* stack or drop chamber components or irreparable harm can be done to the precision stainless steel hybridization surfaces.



Equipment

Refer to the microarray-processing protocol for more required equipment, reagents, hardware and software.

Table 2 Disposable gasket slides for Agilent microarray hybridization chambers

Description	Part Number.	Description.
Microarray hybridization chamber, stainless steel 	G2534A	
Gasket slide, 1 microarray/slide format 	G2534-60003 G2534-60008 G2534-60005	5 slides/kit 20slides/kit 100 slides/kit
Gasket slide, 2 microarrays/slide format 	G2534-60002 G2534-60009 G2534-60006	5 slides/kit 20slides/kit 100 slides/kit
Gasket slide, 4 microarrays/slide format 	G2534-60011 G2534-60012 G2534-60013	5 slides/kit 20slides/kit 100 slides/kit
Gasket slide, 8 microarrays/slide format 	G2534-60014 G2534-60015 G2534-60016	5 slides/kit 20slides/kit 100 slides/kit

Procedures

This section contains the instructions to use the hybridization chamber. Refer to your microarray protocol for details.

CAUTION

The precision-engineered stainless steel parts are vulnerable to damage. Use caution when you assemble, disassemble, wash and store the chamber assembly.

To prepare to load the microarray hybridization chamber

- 1 Check that the work area is clean and free from potential contaminants.
- 2 Get the needed quantity of labeled chamber assemblies and packaged gasket slides to process samples. Disassemble chambers, if needed, and line up chamber bases, chamber covers and clamp assemblies.



- 3 Prepare pipettes and tips for sample loading.

- 4 To better track samples, label your chamber bases. Mark them with a black waterproof marking pen.



To remove gasket slide from its packaging

- 1 With tweezers, carefully lift up the corner of the clear plastic covering and slowly pull back the protective sheath.
- 2 With clean, powder-free gloved fingers, remove the gasket slide from its package. Handle the slide only from its edges.
- 3 Immediately place in the chamber base to avoid any potential contamination from surrounding surface materials.



CAUTION

- Do not remove gasket slide from protective sleeve until ready for use.
 - Do not slice or cut open the gasket slide protective packaging.
 - Handle only the edges of the slide.
-

To insert the hybridization gasket slide into the chamber base.

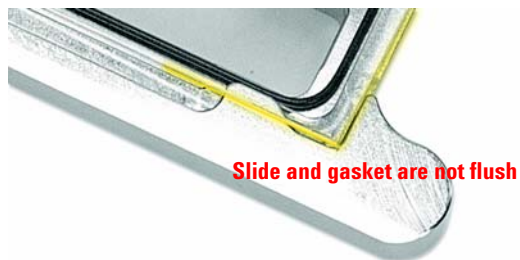
- 1 Hold the slide with the label reading “Agilent” face up, which puts the gasket side of the slide up.



- 2 Locate the four chamber base guideposts and rectangular barcode guide in the chamber base.
- 3 Position the gasket slide between the 4 chamber base guide posts (see figure below) with the “Agilent Label” resting over the base’s rectangular barcode guide.
- 4 Gently place the gasket slide into the chamber base.



- 5 Make sure the gasket slide rests flush against the chamber base. Re-adjust to a flush position against the chamber base if needed.



To load the sample

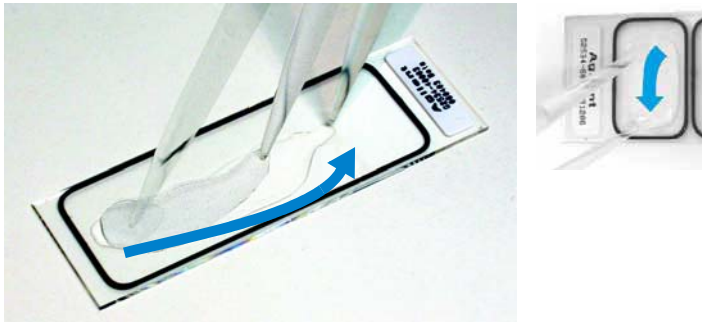
- 1 Select the correct volume of hybridization mixture to load onto the gasket slide surface. Refer to the microarray processing protocol for hybridization solution preparation steps and volume.

The labeled target and hybridization solution will be loaded onto the gasket slide, not the microarray slide.

- 2 Start close to one end of space contained within the gaskets.
- 3 Slowly dispense your sample while you move your pipette to the opposite end of the well so that the sample is distributed across the space within the gaskets.

Do not touch the slide.

This action is called “drag and dispense”. This method helps to distribute the sample evenly across the surface and avoids the spillover of sample over the gasket edge.



These images are for demonstration purposes only. Always put the backing slide in the chamber base before you dispense the hybridization solution.

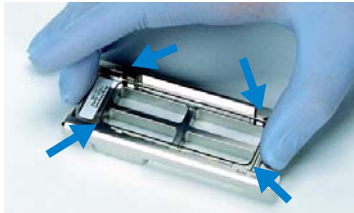
To add the microarray slide

- 1 With gloved fingers, grab a microarray from its slide holder between your thumb and index finger, *numeric barcode side facing up and Agilent label facing down*, and carefully lower it on top of the gasket slide.

Barcode ends of both slides must line up at rectangular end of the chamber base.



- 2 Use the four 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.



CAUTION

Do not drop the array slide onto the gasket slide as this increases the chances of sample mixing between gasket wells. Make sure that the oligo microarray is not exposed.

Quickly reposition the slide if needed. Once correctly placed, do not attempt to move the chamber and sandwiched slides as this can cause leakage of the hybridization solution.

To assemble the chamber

- 1 Place the chamber cover, correct side facing up, onto the chamber base which contains both “sandwiched” slides.



- 2 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.



- 3 Keep the chamber assembly flat on the lab bench to avoid spillage of the hybridization solution.

4 Firmly tighten the thumbscrew fully.

The slides will not be harmed by hand-tightening.



CAUTION

If you do not completely tighten the thumbscrew, hybridization solution can leak in during hybridization.

Do not use tools to tighten the thumbscrew. The use of pliers or other tools can damage the parts and will void the warranty.

5 Rotate the final assembled chamber in a *vertical orientation*, clockwise, 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.



6 Inspect for good bubble formation.

- Hold the chamber vertically and inspect for stray or small bubbles that do not move as you rotate the chamber.
- Use the “large mixing bubble” to dislodge small stray or stationary bubbles.

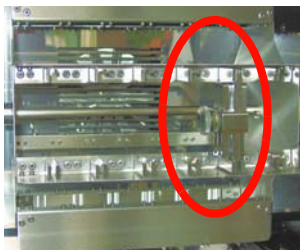
- If the small stray or stationary bubbles persist, gently tap the assembled chamber on a firm surface. Rotate the chamber on its sides as you tap. Inspect again and repeat if needed until the small stray or stationary bubbles dissipate.



Figure 1 The slide on the left shows stray or stationary bubbles, which must be removed before hybridization. The slide on the right shows a single large mixing bubble, which moves freely around the chamber when rotated. Multiple bubbles are acceptable, as long as they move freely when you rotate the chamber.

To load assembled chambers into the hybridization oven

- 1 Load each assembled chamber into the oven rotator rack. Refer to the figures below for correct orientation.
- 2 Close the door and adjust the rotator speed to match what is specified in your microarray processing protocol.



Correct



Incorrect

To transfer microarray slides to slide rack

- 1 Prepare the wash staining dishes as described in microarray processing protocol.
- 2 Fill dishes with wash solutions.
- 3 Check that the slide racks, stir bars and magnetic stirrer are ready to go.



- 4 Put a pair of blunt end tweezers next to the *first* wash staining dish for quick disassembly of slides.
- 5 Remove 1 microarray hybridization chamber assembly from the oven. Leave the remaining assemblies in the oven to minimize the loss of heat in the chambers during the disassembly process.
- 6 Put the hybridization chamber assembly on a flat surface and loosen the thumbscrew, turning *counterclockwise*.



- 7 Slide off the clamp assembly and remove the chamber cover.
- 8 With gloved fingers, grab the slides from their ends and remove the “sandwiched slides” from the chamber base.

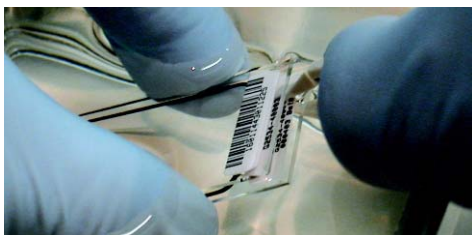


- 9 Keep the oligo microarray slide *numeric barcode face up* as you quickly transfer and submerge the sandwiched slides into the FIRST wash staining dish.



- 10 With the “sandwiched slides” completely submerged in the wash solution, pry the two slides apart from the barcode end only.

To do so, slip one of the blunt ends of the tweezers between the slides and then *gently* turn the tweezers upward or downward to separate the slides.



11 Let the gasket slide drop to the bottom of the wash staining dish.

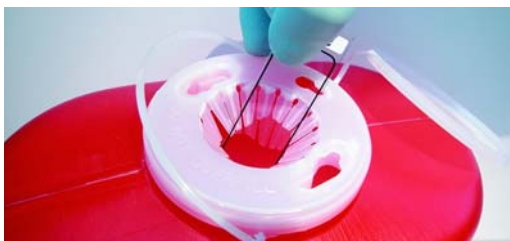


12 Remove the microarray slide quickly and put it into the slide rack in the *second* wash staining dish. Minimize exposure of the slide to air.



13 Repeat disassembly procedure until all slides are in the rack.

14 When all gasket slides have been removed, dispose of them in a “sharp objects” disposal container.



To clean the chamber assemblies

CAUTION

The chamber assemblies are precision-engineered and are vulnerable to damage when mishandled. Do not stack, pile, or throw the assemblies.

- 1 Line up each chamber assembly part side by side in a wash basin.

Refer to the figures below for the correct and incorrect ways to handle the chamber assembly parts. Do not stack, pile, throw or otherwise mishandle the chamber assembly parts.



- 2 Use tap water to clean the chamber assembly parts.

CAUTION

Do not use deionized water or you will corrode the stainless steel surface.

- 3 Thoroughly rinse with copious amounts of water.

CAUTION

Make sure cleaning solution is thoroughly rinsed. Any remaining solution can cause damage to the stainless steel surface.

- 4 Dry each part completely.

5 Assemble and store appropriately.

- Line up neatly in a drawer, or store in a protective plastic container.
- Store gasket slides in original shipping container or in a protected container.
- Store the chamber assemblies and gasket slides in a nuclease-free environment to avoid contamination.

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In This Guide

This guide contains information to use the Agilent G2534A Microarray Hybridization Chamber with Agilent DNA Microarray protocols.

Agilent microarray hybridization is enabled by SureHyb technology.

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