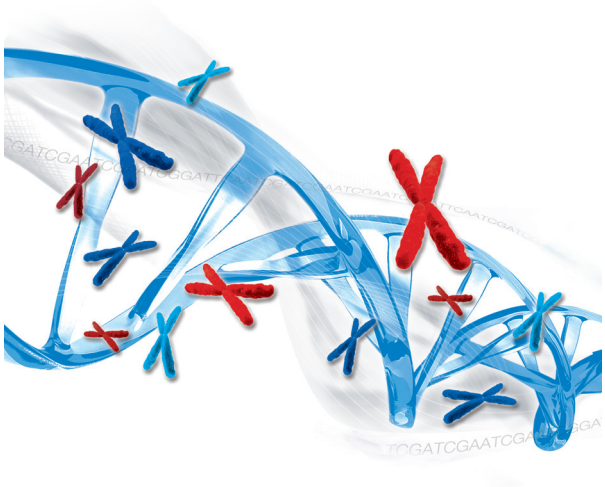
Oligonucleotide Array-Based CGH for Genomic DNA Analysis - ULS Labeling

For Blood, Cells, or Tissues, or FFPE

Protocol

For Research Use Only. Not for use in diagnostic procedures.

Version 4.0, July 2021



Notices

Manual Part Number

G4410-90020 Revision B0
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Safety Notices

CAUTION

A **CAUTION** notice denotes a hazard. It calls attention to an operating procedure, practice, or the like that, if not correctly performed or adhered to, could result in damage to the product or loss of important data. Do not proceed beyond a **CAUTION** notice until the indicated conditions are fully understood and met.

WARNING

A WARNING notice denotes a hazard. It calls attention to an operating procedure, practice, or the like that, if not correctly performed or adhered to, could result in personal injury or death. Do not proceed beyond a WARNING notice until the indicated conditions are fully understood and met.

In This Guide…

This guide describes the recommended operational procedures to analyze DNA copy number variations using Agilent 60-mer oligonucleotide microarrays for array-based comparative genomic hybridization (aCGH) analysis. This protocol is specifically developed and optimized to non-enzymatically label DNA from blood, cells, tissues or FFPE samples and is quick, cost-efficient and highly reproducible.

Note that this protocol does *not* support analysis of CGH+SNP microarrays. Refer to the Agilent Oligonucleotide Array-Based CGH for Genomic DNA Analysis (Enzymatic Labeling for Blood, Cells, or Tissues) Protocol (publication G4410-90010).

1 Before You Begin

This chapter contains information (such as procedural notes, safety information, required reagents and equipment) that you should read and understand before you start an experiment.

2 DNA Isolation

This chapter describes the method to isolate genomic DNA (gDNA) from blood, cells, frozen tissues, or FFPE samples prior to labeling.

3 Sample Labeling

This chapter describes the steps to chemically label the gDNA samples with fluorescent dyes through ULS technology.

4 Microarray Processing and Feature Extraction

This chapter describes the steps to hybridize, wash and scan Agilent CGH microarrays and to extract data using the Agilent Feature Extraction Software for use in Agilent CytoGenomics and Genomic Workbench.

5 Troubleshooting

This chapter contains potential causes for above-threshold DLRSD (Derivative Log Ratio Standard Deviation). A poor DLRSD score reflects high probe-to-probe log ratio noise.

6 Reference

This chapter contains reference information related to the protocol.

What’s new in 4.0

- Updated document look and feel.
- Instructions on handling the newly redesigned “secure fit” slide boxes in which the microarray slides are shipped. Before opening the box for the first time, see **““Secure Fit” Slide Box Opening Instructions"** on page 84.
- Expanded instructions and new images in the **Microarray Processing and Feature Extraction** procedures to help avoid common problems and optimize hybridization of your sample to the microarray.
- Updated web addresses for Agilent materials.
- Updated **Safety Notes**.
- Removed microarray scanning instructions for the Agilent scanner B.
- Removed instructions for the high-throughput option that used the Genomic DNA High-Throughput ULS Labeling Kit and 96-well purification module to remove non-reacted ULS-Cy dye.

What’s new in 3.4

- Guidelines for adjusting heat fragmentation times are expanded.

Content

1 Before You Begin

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

Procedural Notes

- To process SurePrint G3 CGH+SNP microarrays, refer to the *Agilent Oligonucleotide Array-Based CGH for Genomic DNA Analysis (Enzymatic Labeling for Blood, Cells, or Tissues) Protocol* (publication G4410-90010).
- Follow the procedure described in this document to isolate gDNA from blood, cells, frozen tissues, or FFPE samples.
- If the DNA isolation procedure described in this document cannot be followed, make sure that the DNA is free of RNA and protein contamination. and is in one of the following buffers compatible with ULS labeling:
  - TE-buffer (10 mM Tris-HCl, 1 mM EDTA, pH 7.5 or pH 8)
  - 10 mM LiCl
  - 10 to 100 mM Na acetate
  - 10 mM NaCl
- To prevent contamination of reagents by nucleases, always wear powder-free laboratory gloves, and use dedicated solutions and pipettors with nuclease-free aerosol-resistant tips.
- Maintain a clean work area.
- Do not mix stock solutions and reactions containing gDNA or enzymes on a vortex mixer. Instead, mix the solutions and reactions by gently tapping the tube with your finger.
- Avoid repeated freeze-thaw cycles of solutions containing gDNA or enzymes.
- When preparing frozen reagent stock solutions for use:
  1. Thaw the aliquot as quickly as possible without heating above room temperature.
  2. Mix briefly on a vortex mixer, and then spin in a microcentrifuge for 5 to 10 seconds to drive the contents off the walls and lid.
  3. Store on ice or in a cold block until use.
- In general, follow Biosafety Level 1 (BL1) safety rules.

Safety Notes

CAUTION Wear appropriate personal protective equipment (PPE) when working in the laboratory.

WARNING

- Cyanine reagents are considered hazardous by the OSHA Hazard Communication Standard (29 CFR 1910.1200). Contains material that causes damage to the following organs: kidneys, liver, cardiovascular system, respiratory tract, skin, eye lens or cornea, stomach. May be harmful if swallowed. Avoid contact with eyes, skin and clothing.
- 2× HI-RPM Hybridization Buffer is considered hazardous by the OSHA Hazard Communication Standard (29 CFR 1910.1200). Contains material that causes damage to the following organs: skin, central nervous system. May be harmful if swallowed. Avoid contact with eyes, skin and clothing.
- Triton is harmful if swallowed. Risk of serious damage to eyes. Wear suitable PPE. Triton is a component of the Agilent 2× HI-RPM Hybridization Buffer.
- Acetonitrile is a flammable liquid and vapor. Harmful if inhaled, swallowed, or contacts skin. Target organs: liver, kidneys, cardiovascular system, and CNS.
- Stabilization and Drying Solution is considered hazardous by the OSHA Hazard Communication Standard (29 CFR 1910.1200). Flammable liquid and vapor. Keep away from heat, sparks and flame. Keep container closed. Use only with adequate ventilation. This solution contains material which causes damage to the following organs: kidneys, liver, cardiovascular system, upper respiratory tract, skin, central nervous system (CNS), eye, lens or cornea.

Agilent Oligo CGH Microarray Kit Contents

Store microarray kit at room temperature. After the microarray foil pouch is opened, store the microarray slides at room temperature (in the dark) under a vacuum desiccator or N_2 purge box. Do not store microarray slides in open air after breaking foil.

Catalog SurePrint HD and G3 CGH Microarray Kits

- Five 1-inch × 3-inch, 1-pack and 2-pack microarray slides
- Three 1-inch × 3-inch, 4-pack and 8-pack microarray slides

Design files can be downloaded from www.agilent.com/genomics/suredesign.

See the tables that follow for available designs. For more information on CGH designs, go to www.agilent.com.

Table 1 Catalog CGH Microarray Kits - Human

Part Number	Description
G4447A	SurePrint G3 Human CGH Microarray Kit 1×1M (5 slides)
G4824A-021529	SurePrint G3 Human CGH Microarray Slide 1×1M
G4448A	SurePrint G3 Human CGH Microarray Kit 2×400K (5 slides)
G4825A-021850	SurePrint G3 Human CGH Microarray Slide 2×400K
G4449A	SurePrint G3 Human CGH Microarray Kit 4×180K (5 slides)
G4826A-022060	SurePrint G3 Human CGH Microarray Slide 4×180K
G4450A	SurePrint G3 Human CGH Microarray Kit 8×60K (5 slides)
G4827A-021924	SurePrint G3 Human CGH Microarray Slide 8×60K
G4423B-016266	SurePrint G3 Human CGH 244A Supplemental Slide 1×244K
G5955A	SurePrint G3 Human CGH ISCA v2 Microarray Kit 8×60K (3 slides)
G4411B	Human Genome CGH 244A Microarray Kit 1×244K (5 slides)
G4423B-014693	Human Genome CGH 244A Microarray Slide 1×244K
G4412A	Human Genome CGH 105A Microarray Kit 2×105K
G4425B-014698	Human Genome CGH 105A Microarray Slide 2×105K
G4413A	Human Genome CGH Microarray Kit 4×44K (3 slides)
G4426B-014950	Human Genome CGH Microarray Slide 4×44K

Table 2 Catalog CNV Microarray Kits - Human

Part Number	Description
G4506A	SurePrint G3 Human CNV Microarray Kit 1×1M (5 slides)
G4824A-023642	SurePrint G3 Human CNV Microarray Slide 1×1M
G4507A	SurePrint G3 Human CNV Microarray Kit 2×400K (5 slides)
G4825A-021365	SurePrint G3 Human CNV Microarray Slide 2×400K
G4423B-018897	SurePrint G3 Human CNV Microarray Slide, Slide 1 of 2, 1×244K
G4423B-018898	SurePrint G3 Human CNV Microarray Slide, Slide 2 of 2, 1×244K
G4417A	Human CNV Association Microarray Kit 2×105K (5 slides)
G4425B-022837	Human CNV Association Microarray Slide 2×105K

Table 3 Catalog CGH Microarrays- Mouse

Part Number	Description
G4838A	SurePrint G3 Mouse CGH Microarray Kit 1×1M (5 slides)
G4824A-027414	SurePrint G3 Mouse CGH Microarray Slide 1×1M
G4839A	SurePrint G3 Mouse CGH Microarray Kit 4×180K (3 slides)
G4826A-027411	SurePrint G3 Mouse CGH Microarray Kit 4×180K
G4415A	Mouse Genome CGH Microarray Kit 1×244K (5 slides)
G4423B-014695	Mouse Genome CGH Microarray Slide 1×244K
G4416A	Mouse Genome CGH Microarray Kit 2×105K (5 slides)
G4425B-014699	Mouse Genome CGH Microarray Slide 2×105K

Table 4 Catalog CGH Microarrays - Rat

Part Number	Description
G4840A	SurePrint G3 Rat CGH Microarray Kit 1×1M (5 slides)
G4824A-027065	SurePrint G3 Rat CGH Microarray Slide 1×1M
G4841A	SurePrint G3 Rat CGH Microarray Kit 4×180K (3 slides)
G4826A-027064	SurePrint G3 Rat CGH Microarray Slide 4×180K
G4435A	Rat Genome CGH Microarray Kit 1×244K (5 slides)
G4423B-015223	Rat Genome CGH Microarray Slide 1×244K
G4436A	Rat Genome CGH Microarray Kit 2×105K (5 slides)
G4425B-015235	Rat Genome CGH Microarray Slide 2×105K

Table 5 Catalog CGH Microarrays - Model Organism/Non-Human

Part Number	Description
G4826A-024419	SurePrint G3 Rhesus Macaque CGH Microarray Kit 4×180K (5 slides)
G4826A-024422	SurePrint G3 Chimpanzee CGH Microarray Kit 4×180K (5 slides)
G4826A-025242	SurePrint G3 Bovine CGH Microarray Kit 4×180K (5 slides)
G4826A-025522	SurePrint G3 Canine CGH Microarray Kit 4×180K (5 slides)
G4826A-025843	SurePrint G3 Rice CGH Microarray Slide 4×180K
G4423B-019553	Chicken Genome CGH Microarray, 1x244K

Unrestricted SurePrint HD and G3 CGH Microarrays

- One, two, four or eight microarrays printed on each 1-inch × 3-inch glass slide
- Number of microarray slides vary per kit and per order

Design files can be downloaded from www.agilent.com/genomics/suredesign.

See the tables that follow for available designs.

Table 6 Unrestricted CGH Microarrays - Human

Part Number	Description
G4826A, AMADID 031748	Unrestricted SurePrint G3 CGH ISCA v2 Microarray, 4×180K
G4827A, AMADID 031746	Unrestricted SurePrint G3 CGH ISCA v2 Microarray, 8×60K
G4425B, AMADID 031750	Unrestricted HD CGH ISCA v2 Microarray, 2×105K
G4426B, AMADID 031747	Unrestricted HD CGH ISCA v2 Microarray, 4×44K

Custom SurePrint HD and G3 Microarrays

- One, two, four or eight microarray(s) printed on each 1-inch × 3-inch glass slide
- Number of microarrays varies per kit and per order

See the tables that follow for available formats.

Table 7 Custom SurePrint G3 CGH Microarrays

Part Number	Description
G4123A	SurePrint G3 Custom CGH Microarray, 1×1M
G4124A	SurePrint G3 Custom CGH Microarray, 2×400K
G4125A	SurePrint G3 Custom CGH Microarray, 4×180K
G4126A	SurePrint G3 Custom CGH Microarray, 8×60K

Table 8 Custom SurePrint HD CGH Microarrays

Part Number	Description
G4423A	Custom HD-CGH Microarray, 1×244K
G4425A	Custom HD-CGH Microarray, 2×105K
G4426A	Custom HD-CGH Microarray, 4×44K
G4427A	Custom HD-CGH Microarray, 8×15K

Required Equipment

Table 9 Required equipment

Description	Vendor and part number
200-µL Thin-Wall Tube	Agilent p/n 410091 or equivalent
Agilent Microarray Scanner Bundle for 1×244K, 2×105K, 4×44K or 8×15K, *or* for 1×1M, 2×400K, 4×180K or 8×60K	 Agilent p/n G4900DA or G2565CA Agilent p/n G4900DA or G2565CA
Hybridization Chamber, stainless	Agilent p/n G2534A
Hybridization gasket slides, 5-pack (20 and 100 packaging sizes are available) for 1-pack microarrays *or* for 2-pack microarrays *or* for 4-pack microarrays *or* for 8-pack microarrays	 Agilent p/n G2534-60003 Agilent p/n G2534-60002 Agilent p/n G2534-60011 Agilent p/n G2534-60014
Hybridization oven; temperature set at 65°C	Agilent p/n G2545A
Hybridization oven rotator for Agilent Microarray Hybridization Chambers	Agilent p/n G2530-60029
Ozone-barrier slide covers (box of 20)*	Agilent p/n G2505-60550
UV-Transilluminator with SYBR photographic filter	Alpha Innotech p/n AlphaImager 2000 or equivalent
1.5-mL RNase-free Microfuge Tube (sustainable at 98°C)	Thermo Fisher Scientific p/n AM12400 or equivalent
96-well PCR plate†	Agilent p/n 401334 or equivalent
Tall Chimney PCR plate†	Fisher Scientific p/n 14-230-242
Magnetic stir plate (×1 or ×3)‡‡	Corning p/n 6795-410 or equivalent
Magnetic stir plate with heating element	Corning p/n 6795-420D or equivalent
Thermal cycler with heated lid	Eppendorf p/n 950000015 or equivalent
Microcentrifuge	Eppendorf p/n 5430 or equivalent
E-Gel Opener‡	Thermo Fisher Scientific p/n G5300-01
E-Gel Simple Runner Electrophoresis Device‡	Thermo Fisher Scientific p/n G8000
Qubit 4 Fluorometer**	Thermo Fisher Scientific p/n Q33226
Thin wall, clear 0.5 mL PCR tubes**	Thermo Fisher Scientific p/n Q32856 *or* VWR p/n 10011-830

Table 9 Required equipment (continued)

Description	Vendor and part number
Sterile storage bottle	Nalgene 455-1000 or equivalent
UV-VIS spectrophotometer	NanoDrop 8000 or 2000, or equivalent
P10, P20, P200 and P1000 pipettes	Pipetman P10, P20, P200, P1000 or equivalent
1.5 L glass dish	Pyrex p/n 213-R or equivalent
Vacuum Concentrator ††	Thermo Scientific Savant SpeedVac p/n DNA130-115 or equivalent
Magnetic stir bar, 7.9 × 38.1 mm (×2 or ×4)‡‡	VWR p/n 58948-150 or equivalent
250 mL capacity slide-staining dish, with slide rack (×3 or ×5)‡‡	Wheaton p/n 900200 *or* Thermo Fisher Scientific p/n 121
Circulating water baths or heat blocks set to 37°C 56°C, and 90°C (for DNA extraction), and 37°C and 95°C (for sample preparation for hybridization)	
Ice bucket	
Clean forceps	
Powder-free gloves	
Sterile, nuclease-free aerosol barrier pipette tips	
Timer	
Vacuum desiccator or N_2 purge box for slide storage	
Vortex mixer	

* Optional. Recommended when processing arrays with G2565CA scanner in environments in which ozone levels are 5 ppb or higher.

† Optional. Depending on microarray format, may be used as a container for combining the ULS-Cy5-labeled sample and the ULS-Cy3-labeled sample.

‡ For use with Thermo Fisher Scientific E-gels.

** Optional.

†† Optional. Depends on microarray format used.

‡‡ The number varies depending on if wash procedure A or B is selected.

Table 10 Optional equipment for DNA extraction from tissue or FFPE samples.

Description	Vendor and part number
Thermal shaker	Eppendorf Thermomixer p/n 2231000574 or equivalent

Required Reagents

Table 11 Required reagents for gDNA isolation

Description	Vendor and part number
Phosphate Buffered Saline pH 7.4 (PBS)	VWR p/n 97062-818
E-Gel General Purpose Agarose Gels, 1.2%	Thermo Fisher Scientific p/n G5518-01
SYBR Gold Nucleic Acid Gel Stain	Thermo Fisher Scientific p/n S11494
SYBR photographic filter	Thermo Fisher Scientific p/n S7569
TrackIt 1 Kb DNA Ladder	Thermo Fisher Scientific p/n 10488085
DNase/RNase-free distilled water	Thermo Fisher Scientific p/n 10977-015
Qubit dsDNA BR Assay Kit, for use with the Qubit fluorometer (100 assays)*	Thermo Fisher Scientific p/n Q32850
RNase A (100 mg/mL)	Qiagen p/n 19101
DNeasy Blood & Tissue Kit	Qiagen p/n 69504
Proteinase K (>600 mAU/mL, solution)	Qiagen p/n 19131
Sodium thiocyanate (NaSCN)†	Sigma-Aldrich 467871-50G
Ethanol (95% to 100% molecular biology grade)	Sigma-Aldrich p/n E7023-6×500ML
Tween 20†	Sigma-Aldrich p/n P9416-50ML

* Optional.

† Optional components if isolating DNA from FFPE samples.

Table 12 Required reagents for ULS sample prep and labeling

Description	Vendor and part number
Genomic DNA ULS Labeling Kit	Agilent p/n 5190-0419
Genomic DNA Purification Module* (Pack of 10 additional KREApure columns and collection tubes)	Agilent p/n 5190-0418
DNase/RNase-free distilled water	Thermo Fisher Scientific p/n 10977015
For possible use as a reference sample: • Human Genomic DNA *or* • Mouse Genomic DNA *or* • Rat Genomic DNA	 • Promega p/n G1521 (female) or p/n G1471 (male) • Jackson Labs p/n 000664 (female and male) • Harlan Sprague Dawley (custom)

* Optional. The Genomic DNA ULS Labeling Kit includes enough KREApure columns and collection tubes to process five 1-pack microarrays.

Table 13 Required reagents for hybridization and wash

Description	Vendor and part number
Oligo aCGH/ChIP-on-chip Wash Buffer Kit *or* Oligo aCGH/ChIP-on-chip Wash Buffer 1 *and* Oligo aCGH/ChIP-on-chip Wash Buffer 2	Agilent p/n 5188-5226 Agilent p/n 5188-5221 Agilent p/n 5188-5222
Stabilization and Drying Solution*	Agilent p/n 5185-5979
Oligo aCGH/ChIP-on-chip Hybridization Kit	Agilent p/n 5188-5220 (25) or p/n 5188-5380 (100)
Cot-1 DNA (1.0 mg/mL) • Human Cot-1 DNA *or* • Mouse Cot-1 DNA *or* • Rat Hybloc	 Agilent p/n 5190-3393 Thermo Fisher Scientific p/n 18440-016 Applied Genetics p/n RHB
DNase/RNase-free distilled water	Thermo Fisher Scientific p/n 10977015
Milli-Q ultrapure water	Millipore
Acetonitrile*	Sigma-Aldrich p/n 271004-1L

* Optional components recommended if wash procedure B is selected.

Required Hardware and Software

- Refer to the Agilent Scanner manual and Agilent CytoGenomics or Feature Extraction manuals for minimum memory requirements and other specifications. Go to www.agilent.com.

2 DNA Isolation

The Agilent array-based Comparative Genomic Hybridization (aCGH) application uses a "two-color" process to measure DNA copy number changes (CNC) in an experimental sample relative to a reference sample. The type of sample used as a reference is a matter of experimental choice; however, many experimenters use normal commercial gDNA as a reference sample.

This chapter describes the Agilent recommended procedure for isolating gDNA from blood, cells, frozen or FFPE tissues using the **DNeasy Blood & Tissue Kit**.

NOTE

Agilent cannot guarantee microarray performance and does not provide technical support to those who use non-Agilent protocols in processing Agilent microarrays.

"DNA Isolation" on page 19

"Sample Labeling" on page 33

"Microarray Processing and Feature Extraction" on page 43

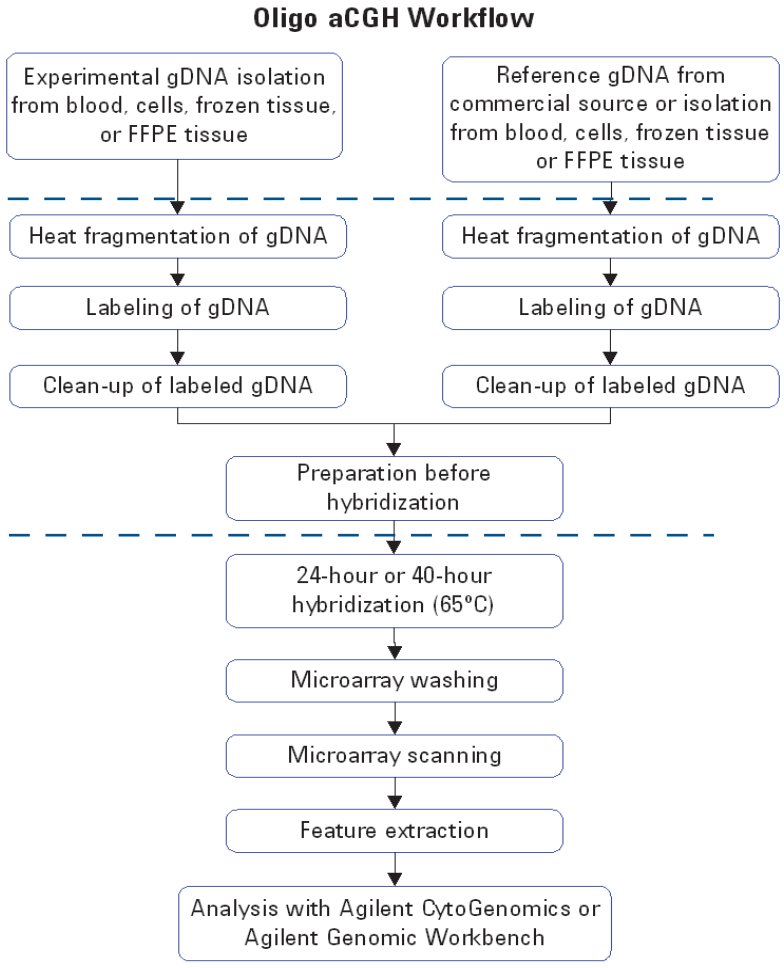

Figure 1. Workflow diagram for sample preparation and microarray processing.

Blood, Cells or Frozen Tissues

This section describes the recommended procedure to isolate gDNA from blood, cells or frozen tissues using the **DNeasy Blood & Tissue Kit**.

Table 14 Minimum required gDNA amount from blood, cells or frozen tissues

Microarray format	gDNA input amount requirement (ng)
1-pack	1500
2-pack	1000
4-pack	500
8-pack	250

Step 1. gDNA Extraction

Use reagents from the **DNeasy Blood & Tissue Kit**.

1. Equilibrate a thermomixer and heat block or water bath to 56°C.
2. For blood with nonnucleated erythrocytes (mammals):
   a. Put 20 µL of **Proteinase K** into the bottom of a **1.5-mL RNase-free Microfuge Tube**.
   b. Add 50 to 100 µL of anticoagulated blood.
   c. Add enough **Phosphate Buffered Saline pH 7.4 (PBS)** to make a total volume of 220 µL.
   d. Go to **step 7**.
3. For blood with nucleated erythrocytes (such as chicken):
   a. Put 20 µL of **Proteinase K** into the bottom of a **1.5-mL RNase-free Microfuge Tube**.
   b. Add 5 to 10 µL of anticoagulant blood.
   c. Add enough **Phosphate Buffered Saline pH 7.4 (PBS)** to make a total volume of 220 µL.
   d. Go to **step 7**.

4 For cells:

   a Spin a maximum of 5×10^6 cells in a centrifuge for 5 minutes at 300 × g. Resuspend the pellet in 200 µL of **Phosphate Buffered Saline pH 7.4 (PBS)**.

   b Add 20 µL of **Proteinase K**.

   c Go to **step 7**.

5 For frozen tissue:

   a Cut up to 25 mg frozen tissue (up to 10 mg for spleen tissue) into small pieces and put into a **1.5-mL RNase-free Microfuge Tube**.

   b Add 180 µL of **Buffer ATL**.

   c Add 20 µL of **Proteinase K**.

   d Mix well on a vortex mixer.

   e Incubate in a thermomixer at 56°C shaking at 450 rpm until the tissue is completely lysed.

      Lysis time varies depending on the type of tissue processed. Usually lysis is complete in 1 to 3 hours. If it is more convenient, samples can be lysed overnight.

   f Let the sample cool to room temperature and spin in a microcentrifuge for 30 seconds at 6,000 × g to drive the contents off the walls and lid.

   g Go to **step 7**.

6 For further purification of extracted DNA:

   a Take a maximum 25 µg of DNA.

   b Add enough **Phosphate Buffered Saline pH 7.4 (PBS)** to make a total volume of 220 µL.

   c Add 20 µL of **Proteinase K**.

7 Add 4 µL of **RNase A (100 mg/mL)**, mix on a vortex mixer, and incubate for 2 minutes at room temperature. Spin in a microcentrifuge for 30 seconds at 6,000 × g to drive the contents off the walls and lid.

8 Add 200 µL of **Buffer AL** to each sample, mix thoroughly on a vortex mixer, and incubate at 56°C for 10 minutes in a heat block or water bath. Spin in a microcentrifuge for 30 seconds at 6,000 × g to drive the contents off the walls and lid.

9 Add 200 µL of 100% **Ethanol** to each sample, and mix thoroughly on a vortex mixer. Spin in a microcentrifuge for 30 seconds at 6,000 × g to drive the contents off the walls and lid.

10 Transfer the sample mixture onto a **DNeasy Mini Spin Column** in a **2 mL Collection Tube**. Spin in a centrifuge at 6,000 × g for 1 minute. Discard the flow-through and collection tube. Put the **DNeasy Mini Spin Column** in a new **2 mL Collection Tube**.

11 Before using for the first time, prepare **Buffer AW1** by adding 100% **Ethanol** to the **Buffer AW1** bottle (see bottle label for volume). Mark the appropriate check box to indicate that ethanol was added to the bottle.

12 Add 500 µL **Buffer AW1** onto the column, and spin in a microcentrifuge for 1 minute at 6,000 × g. Discard the flow-through and collection tube. Put the **DNeasy Mini Spin Column** in a new **2 mL Collection Tube**.

13 Prepare a fresh 80% **Ethanol** solution by adding 40 mL 100% **Ethanol** to 10 mL of **DNase/RNase-free distilled water**.

CAUTION

Do *not* use Buffer AW2 supplied with the DNeasy Blood & Tissue Kit for the subsequent step because salt from Buffer AW2 will interfere with the subsequent labeling reaction. This is especially important if you need to do a concentration step before labeling.

14 Add 500 µL of 80% **Ethanol** onto the column, and spin in a centrifuge for 3 minutes at 20,000 × g to dry the DNeasy membrane. Discard the flow-through and collection tube.

15 Put the **DNeasy Mini Spin Column** in a clean **1.5-mL RNase-free Microfuge Tube**, and pipette 200 µL of **DNase/RNase-free distilled water** directly onto the center of the DNeasy column membrane.

16 Incubate at room temperature for 1 minute, and then spin in a microcentrifuge for 1 minute at 6,000 × g to elute the DNA.

17 Repeat elution with **DNase/RNase-free distilled water** once as described in **step 15** and **step 16**. Combine the duplicate samples in one microcentrifuge tube for a final volume of 400 µL.

NOTE

If long term storage is needed, store DNA that was eluted in water at -20°C. Make small aliquots before you freeze the DNA so as to avoid repeated freeze-thaw cycles.

Step 2. gDNA Quantitation and Quality Analysis

Accurate assessment of gDNA quantity and quality are crucial to the success of an Agilent Oligo aCGH experiment. High quality gDNA should be free of contaminants such as carbohydrates, proteins, and traces of organic solvents, and should also be intact with minimal degradation. gDNA isolated from FFPE samples typically exhibits varying degrees of degradation depending on the age of the tissue and the paraffin embedding protocol used. See **"FFPE Tissues"** on page 28 for details on how to isolate gDNA from FFPE tissues.

Use Quant-iT dsDNA Broad-Range Assay Kit to measure the concentration of double-strand DNA by fluorometry. Use the NanoDrop ND-1000 UV-VIS Spectrophotometer (or equivalent) to assess gDNA concentration and purity. Use agarose gel electrophoresis to assess gDNA intactness and the average molecular weight for each sample.

NOTE

Agilent recommends the use of a fluorometric quantitation method for the highest quality data.

Fluorometry

Use the **Qubit dsDNA BR Assay Kit** at room temperature (22°C to 28°C). Temperature fluctuations can affect the accuracy of the assay.

1 Set up **Thin wall, clear 0.5 mL PCR tubes** for the two standards plus the number of samples you are processing.

2 Make a **Qubit working solution**.

For each standard and sample to be quantified, mix the components in **Table 15** together on a vortex mixer for 2 to 3 seconds.

Table 15 Qubit working solution

Component	Amount
Qubit dsDNA BR reagent	1 µL
Qubit dsDNA BR buffer	199 µL

3 Load 190 µL of **Qubit working solution** into the two **Thin wall, clear 0.5 mL PCR tubes** labeled for the standards.

4 Load 180 to 199 µL of **Qubit working solution** into the tubes labeled for your samples.

5 Add 10 µL of **Qubit dsDNA BR standard #1** or **Qubit dsDNA BR standard #2** to the appropriate tube.

6 Add 1 to 20 µL of your DNA sample to the appropriate tubes.

7 Mix the content of all the tubes on a vortex mixer for 2 to 3 seconds. Be careful not to create bubbles.

8 Incubate the tubes at room temperature for 2 minutes.

- To calibrate the Qubit:
  - **a** On the home screen of the Qubit 1.0, use the up or down arrow to select **dsDNA Broad Range Assay** as assay type, and then press **GO**. The standard screen is automatically displayed.
  - **b** Select **Run new calibration**, and then press **GO**.
  - **c** Insert the tube with the first standard into the Qubit Fluorometer, close the lid and press **GO**. After the reading is done, remove the standard.
  - **d** Insert the tube with the second standard into the Qubit Fluorometer, close the lid, and press **GO**. After the reading is done remove the standard.

  The calibration is complete after the second standard has been read.
- To measure sample concentration:
  - **a** After the calibration is complete, insert a sample and press **GO**.
  - **b** When the measurement is complete (approximately 5 seconds later), make a note of the reading.
  - **c** The result is displayed on the screen. The number displayed is the concentration of the nucleic acid in the assay tube.
  - **d** Remove the sample from the instrument, insert the next sample, and press **GO**.
  - **e** Repeat sample readings until all samples have been read.
  - **f** Calculate the concentration of your original sample.

The Qubit Fluorometer gives a value for the Qubit dsDNA BR assay in µg/mL. This value corresponds to the concentration after your samples were diluted into the assay tube. To calculate the concentration of your sample, use this equation:

$$\text{Sample concentration} = QF\ value \times (200/y)$$

where

QF value = the value given by the Qubit Fluorometer

y = the volume of sample you added to the assay tube.

UV-VIS Spectrophotometry

1 In the Nanodrop program menu, select **Nucleic Acid Measurement**, and then select **Sample Type** to be **DNA-50**.

2 Use 1.5 µL of **DNase/RNase-free distilled water** to blank the instrument.

3 Use 1.5 µL of each gDNA sample to measure DNA concentration. Record the gDNA concentration (ng/µL) for each sample. Calculate the yield as

$$\text{Yield (µg)} = \frac{\text{DNA Concentration (ng/µL)} \times \text{Sample Volume (µL)}}{1000\ \text{ng/µg}}$$

4 Record the A_{260}/A_{280} and A_{260}/A_{230} ratios. High-quality gDNA samples have an A_{260}/A_{280} ratio of 1.8 to 2.0, which indicates the absence of contaminating proteins. Scanning the absorbance from 220-320 nm will show whether contaminants exist that affect absorbance at 260 nm. Check the absorbance scans for a peak at 260 nm and an overall smooth shape as shown in **Figure 2**. The ideal 260/230 ratio for pure DNA is >1.0.

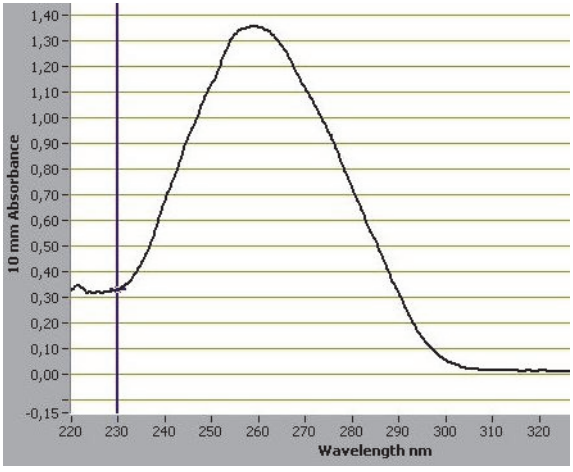

Figure 2. Typical spectrum of pure DNA

Agarose Gel Electrophoresis

1. Load 20 ng of gDNA for each sample in 10 µL of **DNase/RNase-free distilled water** in the well of a single-comb **E-Gel General Purpose Agarose Gels, 1.2%**. (You do not need to add loading buffer in this system).
2. As a control, load 20 ng of commercial **Human Genomic DNA** in 10 µL of **DNase/RNase-free distilled water** in one of the wells of the E-Gel.
3. Mix 5 µL of **TrackIt 1 Kb DNA Ladder** with 95 µL of deionized water and load 10 µL of the diluted ladder in one of the wells of the E-Gel.
4. Run the gel for 30 minutes as described in Invitrogen's instructions.
5. Open the gel cassette with **E-Gel Opener** as described in Invitrogen's instructions.
6. Stain the gel with **SYBR Gold Nucleic Acid Gel Stain** (diluted 1:10,000 by adding 10 µL of **SYBR Gold Nucleic Acid Gel Stain** to 100 mL of **DNase/RNase-free distilled water**) in a plastic tray for 15 minutes.
7. Visualize the gel on the UV-transilluminator using a **SYBR photographic filter**.

FFPE Tissues

This section describes the recommended procedure to isolate gDNA from formalin-fixed paraffin-embedded (FFPE) samples and is based on the method described by van Beers et al. (Br J Cancer. 2006 Jan 30; 94(2):333-7) using the **DNeasy Blood & Tissue Kit**. Determine the number of FFPE sections needed for your experiment based on the estimates summarized in **Table 16**. One 20 micron FFPE section containing 1 cm^2 of tissue is estimated to generate a minimal yield of 500 ng of gDNA.

Table 16 Estimated number of 20 micron FFPE sections needed per microarray

Microarray format	gDNA input amount requirement (ng)	Estimated number of 20 micron FFPE sections
1-pack	2000	4 to 5
2-pack	1000	3
4-pack	500	2
8-pack	250	1

Step 1. Paraffin Removal

1. Equilibrate a heat block or water bath to 90°C and a thermomixer to 37°C.
2. Put up to 5 20-micron FFPE sections into a **1.5-mL RNase-free Microfuge Tube**.
3. Prepare 10% **Tween 20**, by adding 100 µL **Tween 20** to 900 µL of **DNase/RNase-free distilled water**. The solution can be prepared in advance and stored up to 6 months at room temperature.
4. Add 480 µL **Phosphate Buffered Saline pH 7.4 (PBS)** and 20 µL 10% **Tween 20** to the FFPE sections in the **1.5-mL RNase-free Microfuge Tube**.
5. Transfer the sample tube to a circulating water bath or heat block at 90°C. Incubate at 90°C for 10 minutes.
6. Spin immediately for 15 minutes at 10,000 × g in a microcentrifuge.
7. Put the sample tube on ice for 2 minutes.

8 Remove the resulting wax disc with a pipette tip or tweezers. Remove and discard the supernatant without disturbing the pellet.

9 Add 1 mL of 100% **Ethanol** to the pellet and vortex briefly.

10 Spin for 5 minutes at 10,000 × g in a microcentrifuge.

11 Remove **Ethanol** without disturbing the pellet and let the sample tube sit at room temperature with the lid open until residual ethanol has completely evaporated.

12 Prepare a 1M NaSCN solution by adding 10 g of **Sodium thiocyanate (NaSCN)** to 123 mL of **DNase/RNase-free distilled water**. The solution can be prepared in advance and stored up to 1 month at room temperature.

13 Add 400 µL 1M **Sodium thiocyanate (NaSCN)** to the dry pellet and briefly mix on a vortex mixer.

14 Transfer the sample tube to a thermomixer at 37°C. Incubate overnight at 37°C while shaking at 450 rpm.

Step 2. Proteinase K Treatment

Use reagents from the **DNeasy Blood & Tissue Kit**.

1 Equilibrate a thermomixer to 56°C.

2 Transfer the sample tube to a microcentrifuge. Spin for 20 minutes at 10,000 × g.

3 Remove and discard the supernatant without disturbing the pellet.

4 Add 400 µL **Phosphate Buffered Saline pH 7.4 (PBS)** to the pellet and vortex briefly.

5 Spin again for 20 minutes at 10,000 × g in a microcentrifuge.

6 Remove and discard the supernatant without disturbing the pellet.

7 Add 360 µL of **Buffer ATL**.

8 Add 40 µL **Proteinase K**, mix well on a vortex mixer, and incubate overnight in a thermomixer at 56°C shaking at 450 rpm.

9 Transfer the sample tube to a microcentrifuge. Spin for 30 seconds at 6,000 × g to drive the contents off the walls and lid.

10 Add 40 µL **Proteinase K**, mix well on a vortex mixer, and incubate in a thermomixer for approximately 6 to 8 hours at 56°C shaking at 450 rpm.

11 At the end of the day, transfer the sample tube to a microcentrifuge and spin for 30 seconds at 6,000 × g to drive the contents off the walls and lid.

12 Add 40 µL **Proteinase K**, mix well on a vortex mixer and incubate overnight in a thermomixer at 56°C shaking at 450 rpm.

Step 3. gDNA Extraction

1 Equilibrate a heat block or water bath to 56°C.

2 Let samples cool to room temperature and spin in a microcentrifuge for 30 seconds at 6,000 × g to drive the contents off the walls and lid.

3 Add 8 µL of **RNase A (100 mg/mL)**, mix on a vortex mixer, and incubate for 2 minutes at room temperature. Transfer the sample tube to a microcentrifuge and spin for 30 seconds at 6,000 × g to drive the contents off the walls and lid.

4 Add 400 µL **Buffer AL**, mix thoroughly on a vortex mixer, and incubate in a circulating water bath or heat block at 56°C for 10 minutes. Transfer the sample tube to a microcentrifuge and spin for 30 seconds at 6,000 × g to drive the contents off the walls and lid.

5 Add 440 µL 100% **Ethanol**, and mix thoroughly on a vortex mixer. Transfer the sample tube to a microcentrifuge and spin for 30 seconds at 6,000 × g to drive the contents off the walls and lid.

6 Put two **DNeasy Mini Spin Columns** in two clean **2 mL Collection Tube**s. Split the entire sample mixture onto two **DNeasy Mini Spin Columns** (i.e. 660 µL each).

NOTE

Use two **DNeasy Mini Spin Columns** per sample to prevent clogging.

7 Spin in a microcentrifuge for 1 minute at 6,000 × g. Discard the flow-through and collection tube. Put the **DNeasy Mini Spin Column** in a fresh **2 mL Collection Tube**.

8 Before using for the first time, prepare **Buffer AW1** by adding 100% **Ethanol** to the **Buffer AW1** bottle (supplied; see bottle label for volume). Mark the appropriate check box to indicate that ethanol was added to the bottle.

9 Add 500 µL **Buffer AW1** onto each spin column, and spin in a centrifuge for 1 minute at 6,000 × g. Discard the flow-through and collection tube. Put the **DNeasy Mini Spin Column** in a fresh **2 mL Collection Tube**.

10 Prepare a fresh 80% ethanol solution by adding 40 mL 100% **Ethanol** to 10 mL of **DNase/RNase-free distilled water**.

CAUTION

Do not use Buffer AW2 supplied with the DNeasy Blood & Tissue Kit for the subsequent step because salt from Buffer AW2 will interfere with the subsequent labeling reaction. This is especially important if you need to do a concentration step before labeling.

11 Add 500 µL of 80% **Ethanol** onto each column, and spin in a microcentrifuge for 3 minutes at 20,000 × g to dry the column membrane. Discard the flow-through and collection tube.

12 Put the **DNeasy Mini Spin Column** in a clean **1.5-mL RNase-free Microfuge Tube**, and add 50 µL of **DNase/RNase-free distilled water** directly to the center of each spin column.

13 Let stand at room temperature for 1 minute, and then spin in a microcentrifuge for 1 minute at 6,000 × g to elute the DNA.

14 Combine the purified DNA from the same sample in one microcentrifuge tube for a final total volume of 100 µL.

Measure gDNA concentration and purity, and analyze on an agarose gel as described in **"Step 2. gDNA Quantitation and Quality Analysis"** on page 24.

NOTE

If long term storage is needed, store DNA that was eluted in water at -20°C. Make small aliquots before you freeze the DNA so as to avoid repeated freeze-thaw cycles.

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3 Sample Labeling

The **Genomic DNA ULS Labeling Kit** offers a one-step non-enzymatic procedure to differentially label gDNA samples with fluorescent dyes.

The **Genomic DNA ULS Labeling Kit** contains sufficient two-color labeling reaction reagents for five microarray slides of all formats. It also contains sufficient KREApure purification columns and collection tubes to process five 1-pack microarrays. For use with 2-pack, 4-pack or 8-pack microarrays, order additional columns and tubes by purchasing the **Genomic DNA Purification Module (Pack of 10 additional KREApure columns and collection tubes)** (p/n 5190-0418).

For Agilent's Oligo aCGH application, the experimental sample is labeled with one dye while the reference sample is labeled with the other dye. The “polarity” of the sample labeling is a matter of experimental choice. The norm is for the test sample to be labeled with Cy5 and the reference with Cy3.

You use equal amounts of gDNA for both the experimental and reference channels. The required gDNA input amount depends on the microarray format used (see **Table 17** on page 35).

Step 1. Preparation of gDNA Before Labeling

CAUTION

gDNA samples need to be clean of salt and other (wash) buffer components as well as divalent cations (such as Mg^{2+}) which can disturb the subsequent labeling efficiency. Follow the DNA isolation procedure described in Chapter 2, "DNA Isolation". Failure to clean samples thoroughly will result in unsatisfactory microarray results.

If the DNA isolation procedure described in this document cannot be followed make sure that the DNA is free of RNA and protein contamination and is in one of the following buffers compatible with ULS labeling:

- **Nuclease-free water (for best ULS performance)**
- **TE-buffer (10 mM Tris-HCl, 1 mM EDTA, pH 7.5 or pH 8)**
- **10 mM LiCl**
- **10 to 100 mM Na acetate**
- **10 mM NaCl**

If needed, repurify already isolated DNA and start from step 6 on page 22 in the previous chapter.

Make sure that the gDNA is completely in solution by pipetting up and down. If needed, incubate at 37°C for 30 minutes. If the gDNA concentration is > 350 ng/µL, dilute 1:2 in water or any of the recommended buffers, and then requantitate to make sure quantitation is accurate.

1 Estimate the average molecular weight for each gDNA sample based on the agarose gel analysis (see **"DNA Isolation"** on page 19).

2 If the gDNA concentration is less than those listed in **Table 17**, concentrate the sample using a concentrator (such as Speed Vac) before you continue to the heat fragmentation.

   You can concentrate the gDNA to dryness and resuspend in water to the final volume listed in **Table 17**. Do not excessively dry the gDNA because the pellets will become difficult to resuspend.

Table 17 gDNA Input Amount Required and Volume per Microarray

Microarray format*	gDNA input amount (ng)†	Volume of gDNA (µL)	Minimum gDNA concentration (ng/µL)
1-pack (non-FFPE samples)	1500	16.5	91
1-pack (FFPE samples)	2000	16	125
2-pack	1000	17	59
4-pack	500	8	62.5
8-pack	250	8	32

* Input gDNA requirements and volumes are the same for both FFPE and non-FFPE samples for the 2-pack, 4-pack, and 8-pack arrays.

† You can use more gDNA, but you will also need to use more ULS dye. Always use a ratio of 1 µL ULS dye per microgram gDNA.

3 Put the appropriate amount of gDNA and **DNase/RNase-free distilled water** in **200-µL Thin-Wall Tube** or plate to achieve the volumes listed in **Table 17**.

NOTE

Process samples that have the same average molecular weight together. For example, do not put DNA isolated from FFPE samples and non-FFPE samples in the same 96-well plate.

Step 2. Heat Fragmentation

1 Incubate the gDNA at 95°C in a thermal cycler with heated lid for the time period indicated in **Table 18** to fragment the gDNA.

2 Transfer the sample tubes to ice and incubate on ice for 3 minutes. You can also hold at 4°C for 3 minutes in a thermal cycler.

3 Spin in a microcentrifuge for 30 seconds at 6,000 × g to drive the contents off the walls and lid.

Store heat-fragmented DNA on ice until ready for labeling.

Table 18 Length of heat fragmentation

Average molecular weight	Sample type	Fragmentation time
> 10 KB	Intact gDNA	10 minutes
> 7 KB	Some fresh FFPE samples	5 minutes
< 7 KB	Most FFPE samples	No fragmentation

NOTE

Adjust the fragmentation time of the intact reference gDNA so that the average molecular weight is the same as that of the gDNA isolated from FFPE tissues (< 7 kb). This recommendation is based on the method described by Craig JM *et al*. "DNA fragmentation simulation method (FSM) and fragment size matching improve aCGH performance of FFPE tissues," PLoS One 2012; 7(6).

Step 3. ULS Labeling

NOTE

In every labeling reaction, always use a ratio of 1 µL ULS dye per 1 microgram DNA.

ULS-Cy3 and ULS-Cy5 are light sensitive. Minimize light exposure throughout the labeling procedure.

1 Prepare one Cy3 and one Cy5 Labeling Master Mix by mixing the components in **Table 19** through **Table 23** on ice, based on your microarray format and sample type. Avoid pipetting volumes less than 2 µL to ensure accuracy.

Table 19 Labeling Master Mix (for 1-pack microarray using non-FFPE samples)

Components	Per reaction (µL)	× 8 rxns (µL) (including excess)	× 24 rxns (µL) (including excess)	× 48 rxns (µL) (including excess)
ULS-Cy 3 Reagent or **ULS-Cy 5 Reagent**	1.5	12.75	37.5	75
10× Labeling Solution	2	17	50	100
Final volume of Labeling Master Mix	**3.5**	**29.75**	**87.5**	**175**

Table 20 Labeling Master Mix (for 1-pack microarray using FFPE samples)

Components	Per reaction (µL)	× 8 rxns (µL) (including excess)	× 24 rxns (µL) (including excess)	× 48 rxns (µL) (including excess)
ULS-Cy 3 Reagent or ULS-Cy 5 Reagent	2	17	50	100
10× Labeling Solution	2	17	50	100
Final volume of Labeling Master Mix	**4**	**34**	**100**	**200**

Table 21 Labeling Master Mix (for 2-pack microarray, non-FFPE and FFPE samples)

Components	Per reaction (µL)	× 8 rxns (µL) (including excess)	× 24 rxns (µL) (including excess)	× 48 rxns (µL) (including excess)
ULS-Cy 3 Reagent or ULS-Cy 5 Reagent	1	8.5	25	50
10× Labeling Solution	2	17	50	100
Final volume of Labeling Master Mix	**3**	**25.5**	**75**	**150**

Table 22 Labeling Master Mix (for 4-pack microarray, non-FFPE and FFPE samples)

Components	Per reaction (µL)	× 8 rxns (µL) (including excess)	× 24 rxns (µL) (including excess)	× 48 rxns (µL) (including excess)
DNase/RNase-free distilled water	0.5	4.25	12.5	25
ULS-Cy 3 Reagent or ULS-Cy 5 Reagent	0.5	4.25	12.5	25
10× Labeling Solution	1	8.5	25	50
Final volume of Labeling Master Mix	**2**	**17**	**50**	**100**

Table 23 Labeling Master Mix (for 8-pack microarray, non-FFPE and FFPE samples)

Components	Per reaction (µL)	× 8 rxns (µL) (including excess)	× 24 rxns (µL) (including excess)	× 48 rxns (µL) (including excess)
DNase/RNase-free distilled water	0.75	6.38	18.75	37.5
ULS-Cy 3 Reagent or ULS-Cy 5 Reagent	0.25	2.13	6.25	12.5
10× Labeling Solution	1	8.5	25	50
Final volume of Labeling Master Mix	**2**	**17**	**50**	**100**

2 Add the appropriate amount of Labeling Master Mix to each PCR tube containing the gDNA to make a total volume as listed in **Table 24**. Mix well by gently pipetting up and down.

Table 24 Amount of Labeling Master Mix to add

Microarray format*	Volume of Labeling Master Mix	Volume of gDNA	Total volume
1-pack (non-FFPE samples)	3.5 µL	16.5 µL	20 µL
1-pack (FFPE samples)	4 µL	16 µL	20 µL
2-pack	3 µL	17 µL	20 µL
4-pack	2 µL	8 µL	10 µL
8-pack	2 µL	8 µL	10 µL

* Required master mix amounts are the same for FFPE and non-FFPE samples for the 2-pack, 4-pack, and 8-pack arrays.

3 Transfer PCR tubes or plates to a thermal cycler with heated lid and incubate at 85°C for 30 minutes.

4 Transfer the samples to ice and incubate on ice for 3 minutes. You can also hold at 4°C for 3 minutes in a thermal cycler.

5 Spin in a microcentrifuge for 1 minute at 6,000 × g to drive the contents off the walls and lid.

Labeled gDNA can be stored on ice until dye removal using the **Agilent-KREApure purification column**.

6 **For 4-pack microarray samples only**: add 10 µL of **DNase/RNase-free distilled water** to each PCR tube to make a total volume of 20 µL.

CAUTION **Do not add DNase/RNase-free distilled water to the 8-pack microarray samples as dilution of the samples will prevent accurate measurement of gDNA concentration and Degree of Labeling by Nanodrop.**

Step 4. Removal of non-reacted ULS-Cy

Non-reacted ULS-Cy3 or ULS-Cy5 can interfere with the subsequent microarray experiment and increase background noise if they are not efficiently removed prior to hybridization. The **Agilent-KREApure purification column** effectively removes non-reacted ULS dye.

CAUTION **Do not use the KREApure column if the column appears to be dried out.**

NOTE Use the same microcentrifuge speed and length for all three spinning steps (**step 4**, **step 7** and **step 11**).

1. Resuspend **Agilent-KREApure purification column** material by briefly mixing on a vortex mixer.
2. Loosen cap ¼ turn and snap off the bottom closure.
3. Place the **Agilent-KREApure purification column** in a **Collection tube**.
4. Spin the **Agilent-KREApure purification column** in a microcentrifuge for 1 minute at maximum speed (minimum 16,000 × g).
5. Discard the cap, flow-through, and place the **Agilent-KREApure purification column** back into the same **Collection tube**.
6. Add 300 µL **DNase/RNase-free distilled water** to the **Agilent-KREApure purification column**.
7. Spin again in a microcentrifuge for 1 minute at maximum speed (minimum 16,000 × g).
8. Discard the flow-through and collection tube.
9. Transfer the **Agilent-KREApure purification column** to a clean **1.5-mL RNase-free Microfuge Tube**.

10 Add ULS-labeled gDNA (20 µL or 10 µL for 8-pack microarray samples) onto the **Agilent-KREApure purification column**.

11 Spin in a microcentrifuge for 1 minute at maximum speed (minimum 16,000 × g) to collect the purified labeled gDNA in the collection tube.

12 Take 1.5 µL of each sample to determine the yield and degree of labeling. See **"To determine yield, degree of labeling or specific activity"** on page 41.

13 Combine the appropriate ULS- Cy5-labeled sample and ULS- Cy3-labeled sample for a total volume of 37 µL (for 1-, 2-, or 4-pack microarrays) or 17 µL (for 8-pack microarrays) and bring to the volumes indicated in **Table 25** on page 40. Use one of the appropriate containers listed in **Table 25**.

For 4-pack and 8-pack microarrays, use a vacuum concentrator to concentrate the combined Cy5- and Cy3-labeled gDNA mixture to the Total Mixture Volume indicated in **Table 25**.

If needed, you can concentrate the combined Cy5- and Cy3-labeled gDNA mixture to dryness and resuspend in water to the final volume in **Table 25**. Do not excessively dry the samples because the pellets will become difficult to resuspend.

Table 25 Total Mixture Volumes

Microarray	Cy3 or Cy5 sample volume after purification	Volume after Nanodrop and combining	Total mixture volume	Container
1-pack	20 µL	37 µL	37 µL	**1.5-mL RNase-free Microfuge Tube**
2-pack	20 µL	37 µL	37 µL	**1.5-mL RNase-free Microfuge Tube** or **Tall Chimney PCR plate**†
4-pack	20 µL	37 µL	concentrate to 22 µL	**1.5-mL RNase-free Microfuge Tube**, **Tall Chimney PCR plate**†, or **96-well PCR plate**
8-pack	10 µL	17 µL	concentrate to 9 µL	**1.5-mL RNase-free Microfuge Tube**, **Tall Chimney PCR plate**†, or **96-well PCR plate**

Labeled gDNA can be stored in the dark on ice until ready for hybridization, at 4°C for up to one month, or at - 20°C for long term storage (avoid freeze-thaw cycles).

To determine yield, degree of labeling or specific activity

Use the NanoDrop 8000 or 2000 UV-VIS Spectrophotometer to measure the yield, degree of labeling or specific activity.

1. From the main menu, select **MicroArray Measurement**, then from the **Sample Type** menu, select **DNA-50**.
2. Use 1.5 µL of 1× labeling solution (dilute 10× labeling solution 1:10) to blank the instrument.
3. Use 1.5 µL of each labeled gDNA sample for quantitation. Measure the absorbance at A_{260} nm (DNA), A_{550} nm (Cy3), and A_{650} nm (Cy5).
4. Calculate the Degree of Labeling or Specific Activity of the labeled gDNA:

$$\text{Degree of Labeling} = \frac{340 \times \text{pmol per } \mu\text{L dye}}{\text{ng per } \mu\text{L gDNA} \times 1000} \times 100\%$$

$$\text{Specific Activity*} = \frac{\text{pmol per } \mu\text{L dye}}{\mu\text{g per } \mu\text{L gDNA}}$$

*pmol dyes per µg gDNA

Note that the Specific Activity is Degree of Labeling divided by 0.034.

5. Record the gDNA concentration (ng/µL) for each sample. Calculate the yield as

$$\text{Yield (µg)} = \frac{\text{DNA concentration (ng/µL)} \cdot \text{Sample Volume (µL)}}{1000 \text{ ng/µg}}$$

As a general guideline, an optimal Cy5 degree of labeling lies between 0.75% and 2.5% and an optimal Cy3 degree of labeling lies between 1.75% and 3.5%, with a Cy3 minus Cy5 range between 1% and 2%. Because the ULS-labeling does not copy or amplify the input DNA, the yield after the labeling should be the same as the input amount of DNA.

3 Sample Labeling

To determine yield, degree of labeling or specific activity

4 Microarray Processing and Feature Extraction

Microarray processing consists of hybridization, washing, and scanning.

Feature Extraction is the process by which data is extracted from the scanned microarray image (.tif) and translated into log ratios, allowing researchers to measure DNA copy number changes in their experiments in conjunction with Agilent CytoGenomics or Genomic Workbench Software.

Microarray Hybridization

Before you begin, make sure you read and understand **""Secure Fit" Slide Box Opening Instructions"** on page 84 and **"Microarray Handling Tips"** on page 86.

To practice hybridization, prepare a 1:1 **2× HI-RPM Hybridization Buffer** and water mix and use a microscope slide or used microarray slide, and a gasket slide. You can use the same slide to practice wash and placement of slide in the slide holder.

Step 1. Prepare the 100× aCGH Blocking Agent

1. Add 135 µL of **DNase/RNase-free distilled water** to the vial containing lyophilized **10× aCGH Blocking Agent** (supplied with **Oligo aCGH/ChIP-on-chip Hybridization Kit**).
2. Mix briefly on a vortex mixer and leave at room temperature for 60 minutes to reconstitute sample before use or storage.
3. Cross out "10×" on the label on the blocking agent vial and write "100×".

You are actually making a 100× aCGH Blocking Agent, so you need to relabel the vial of lyophilized blocking agent as such.

The 100× aCGH Blocking Agent can be prepared in advance and stored at -20°C.

Step 2. Prepare the labeled gDNA for hybridization

1. Equilibrate water baths or heat blocks to 95°C and 37°C or use a thermal cycler.
2. Prepare the Hybridization Master Mix by mixing the components in the **Table 26** through **Table 30** according to the microarray format:

Table 26 Hybridization Master Mix for 1-pack microarray, non-FFPE and FFPE samples

Component	Volume (µL) per hybridization	× 8 rxns (µL) (including excess)	× 24 rxns (µL) (including excess)	× 48 rxns (µL) (including excess)
DNase/RNase-free distilled water	37.8	321.3	945	1,890
Cot-1 DNA (1.0 mg/mL)*	50	425	1,250	2,500
100× aCGH Blocking Agent†	5.2	44.2	130	260
2× HI-RPM Hybridization Buffer†	260	2,210	6,500	13,000
Final Volume of Hybridization Master Mix	353	3,000.5	8,825	17,650

* Use **Cot-1 DNA (1.0 mg/mL)** from the appropriate species.

† Supplied with **Oligo aCGH/ChIP-on-chip Hybridization Kit**

Table 27 Hybridization Master Mix for 2-pack microarray, non-FFPE and FFPE samples

Component	Volume (µL) per hybridization	× 8 rxns (µL) (including excess)	× 24 rxns (µL) (including excess)	× 48 rxns (µL) (including excess)
DNase/RNase-free distilled water	0.4	3.4	10	20
Cot-1 DNA (1.0 mg/mL)*	25	212.5	625	1,250
100× aCGH Blocking Agent†	2.6	22.1	65	130
2× HI-RPM Hybridization Buffer†	130	1,105	3,250	6,500
Final Volume of Hybridization Master Mix	158	1,343	3,950	7,900

* Use **Cot-1 DNA (1.0 mg/mL)** from the appropriate species.

† Supplied with **Oligo aCGH/ChIP-on-chip Hybridization Kit**

Table 28 Hybridization Master Mix for 4-pack microarray, non-FFPE and FFPE samples

Component	Volume (µL) per hybridization	× 8 rxns (µL) (including excess)	× 24 rxns (µL) (including excess)	× 48 rxns (µL) (including excess)
Cot-1 DNA (1.0 mg/mL)*	5	42.5	125	250
100× aCGH Blocking Agent†	1	8.5	25	50
2× HI-RPM Hybridization Buffer†	55	467.5	1,375	2,750
Final Volume of Hybridization Master Mix	61	518.5	1,525	3,050

* Use **Cot-1 DNA (1.0 mg/mL)** from the appropriate species.

† Supplied with **Oligo aCGH/ChIP-on-chip Hybridization Kit**

Table 29 Hybridization Master Mix for 8-pack microarray, non-FFPE and FFPE samples

Component	Volume (µL) per hybridization	× 8 rxns (µL) (including excess)	× 24 rxns (µL) (including excess)	× 48 rxns (µL) (including excess)
Cot-1 DNA (1.0 mg/mL)*	2	17	50	100
100× aCGH Blocking Agent†	0.5	4.25	12.5	25
2× HI-RPM Hybridization Buffer†	22.5	191.25	562.5	1,125
Final Volume of Hybridization Master Mix	25	212.5	625	1,250

* Use **Cot-1 DNA (1.0 mg/mL)** from the appropriate species.

† Supplied with **Oligo aCGH/ChIP-on-chip Hybridization Kit**

3 Add the appropriate volume of the Hybridization Master Mix to the **1.5-mL RNase-free Microfuge Tube** or plate well containing the labeled gDNA to make the total volume listed in **Table 30**.

Table 30 Volume of Hybridization Master Mix per hybridization, non-FFPE and FFPE samples

Microarray format	Volume of Hybridization Master Mix	Total volume
1-pack	353 µL	390 µL
2-pack	158 µL	195 µL
4-pack	61 µL	83 µL
8-pack	25 µL	34 µL

4 Mix the sample by pipetting up and down, and then quickly spin in a centrifuge to drive the contents off the walls and lid.

5 Incubate the samples:

   a Transfer sample tubes to a circulating water bath or heat block at 95°C. Incubate at 95°C for 3 minutes.

   b Immediately transfer sample tubes to a circulating water bath or heat block at 37°C. Incubate at 37°C for 30 minutes.

   or

   Transfer sample tubes to a thermal cycler. Program the thermal cycler according to the following table and run the program:

Table 31 Thermal cycler program

Step	Temperature	Time
Step 1	95°C	3 minutes
Step 2	37°C	30 minutes

6 Remove sample tubes from the water bath, heat block or thermal cycler. Quickly spin in a centrifuge to drive the contents off the walls and lid.

7 Bring the **Agilent-CGHblock** (supplied with the **Genomic DNA ULS Labeling Kit**) to room temperature.

   Make sure that the **Agilent-CGHblock** is completely equilibrated to room temperature before you continue.

8 Add the appropriate volume of **Agilent-CGHblock** to each **1.5-mL RNase-free Microfuge Tube** or plate well containing the labeled gDNA and Hybridization Master Mix to make the final volume of hybridization sample mixture listed in **Table 32**.

   Mix well by pipetting up and down.

CAUTION **The addition of Agilent-CGHblock to the hybridization is needed to eliminate background noise on the microarray. The Agilent-CGHblock contains components that cannot be heated to 95°C.**

Table 32 Volume of Agilent-CGHblock per hybridization, non-FFPE and FFPE samples

Microarray format	Volume of Agilent-CGHblock	Final volume of hybridization sample mixture
1-pack	130 µL	520 µL
2-pack	65 µL	260 µL
4-pack	27 µL	110 µL
8-pack	11 µL	45 µL

9 Quickly spin in a centrifuge to drive the contents off the walls and lid.

The samples are ready to be hybridized.

CAUTION **The samples must be hybridized immediately after labeling. If not, keep the temperature of hybridization sample mixtures as close to 37°C as possible on a heat block, thermal cycler or in an oven.**

Step 3. Prepare the hybridization assembly

Refer to the *Agilent Microarray Hybridization Chamber User Guide* (publication G2534-90004) for in-depth instructions on how to load samples, assemble and disassemble chambers, as well as other helpful tips. This user guide can be downloaded from the Agilent Web site at www.agilent.com.

Before you begin, make sure you read and understand **""Secure Fit" Slide Box Opening Instructions"** on page 84 and **"Microarray Handling Tips"** on page 86.

Remove gasket slide from its packaging

NOTE

- 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 gasket slide.
- Prior to use, inspect gasket slides for visible gaps or cuts through the gaskets or any debris within the hybridization areas as these are indications of instability. Do not use gasket slides that have these features.

1 With tweezers, carefully lift up the corner of the clear plastic covering and slowly pull back the protective film.

Figure 3. Removal of clear plastic covering

2 With clean, powder-free gloved fingers, remove the gasket slide from its package. Handle the slide only on its edges.

To avoid any potential contamination from surrounding surface materials, immediately insert the gasket slide in the chamber base using the instructions below.

Insert the gasket slide into the chamber base

1 Hold the gasket slide so that the barcode label is facing towards you. This side of the slide is the gasket side.

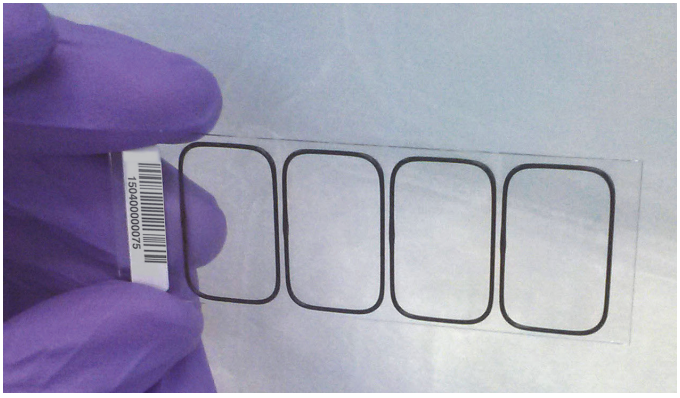

Figure 4. Gasket slide, gasket side

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 5**) with the barcode label resting over the base's rectangular barcode guide.

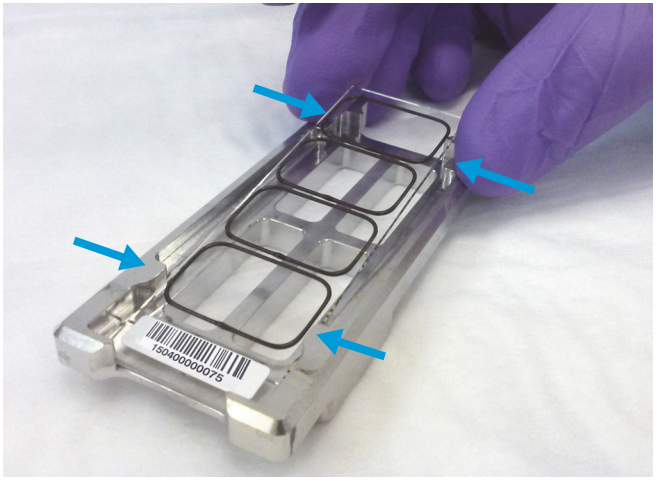

Figure 5. Chamber base, guide posts denoted with arrows

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.

Figure 6. Correct positioning of gasket slide in chamber base

Load the sample

1 Slowly dispense the appropriate volume of hybridization sample mixture onto the gasket well in a "drag and dispense" manner (described below). For multi-pack microarray formats (2-pack, 4-pack or 8-pack microarray), load all gasket wells before you add the microarray slide.

- 490 µL (for 1-pack microarray)
- 245 µL (for 2-pack microarray)
- 100 µL (for 4-pack microarray)
- 40 µL (for 8-pack microarray)

The "drag and dispense" method helps to distribute the sample evenly across the surface of the well and avoids spillover of sample over the gasket edge. Start with the pipette tip near the top edge of the well. *Do not directly touch the gasket or the glass with the pipette tip*. Then, dispense the mixture while you move your pipette tip to the opposite end of the well so that the sample is distributed across the well space. Avoid creating large air bubbles as you dispense the mixture as they could lead to spillover.

This image is for demonstration purposes only. Always put the gasket slide in the chamber base before you dispense the hybridization sample mixture.

Figure 7. Drag and dispense method – Start dispensing when the pipette tip is near the top of the well. Finish dispensing when the pipette tip is near the bottom of the well.

CAUTION **Keep the temperature of hybridization sample mixtures as close to 37°C as possible. To do this, process them in small batches and/or put them on a heat block, thermal cycler or in an oven.**

Add the microarray slide

1 Remove a microarray slide from the slide storage box between your thumb and index finger, *numeric barcode side facing up and Agilent label facing down*.

2 Use the four chamber base guideposts and rectangular end of the base to position the microarray slide as you lower it to within 3 mm (1/8") above the gasket slide, making sure the microarray slide is not tilted with respect to the gasket slide. Barcode ends of both the gasket slide and the microarray slide must line up at the corners of the chamber base. Once positioned, gently rest the microarray slide on the lower gasket slide. Refer to **Figure 8** for proper technique on holding the microarray slide with both hands.

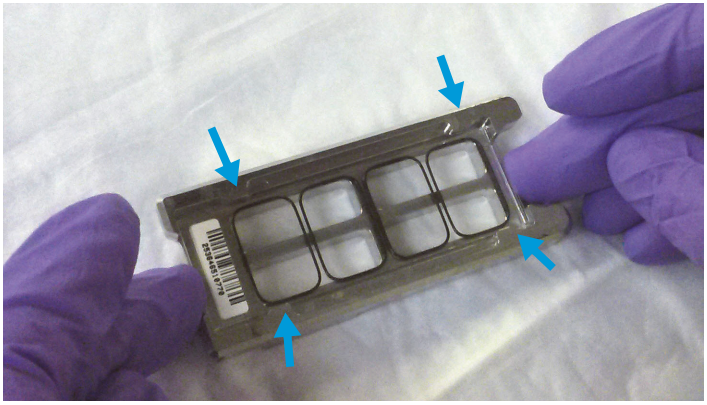

Figure 8. Chamber base with gasket and microarray slide applied, guide posts denoted with arrows

CAUTION **Do not drop the microarray slide onto the gasket slide as this increases the chances of sample mixing between gasket wells.**

Once placed, do not attempt to move the chamber and sandwiched slides as this can cause leakage of the hybridization solution.

Assemble the chamber

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

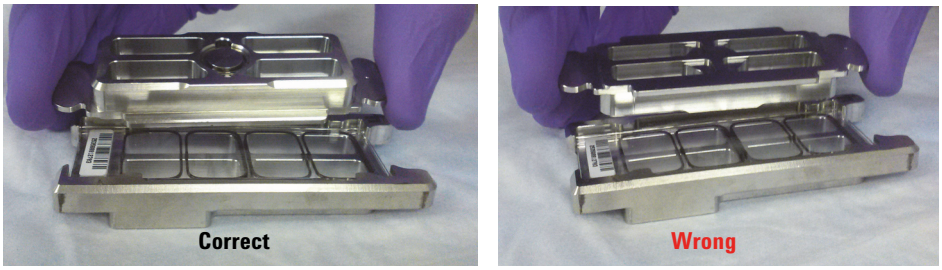

Figure 9. Chamber cover in correct (left) and incorrect (right) orientations

2 From the rounded corner of the chamber base, slip the clamp onto the chamber base and cover until it stops firmly in place, resting at the center of the two pieces.

Keep the chamber assembly flat on the lab bench to avoid spilling the hybridization solution.

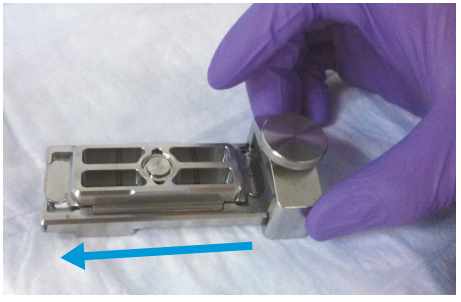

Figure 10. Slipping the clamp onto the chamber base

3 Firmly tighten the thumbscrew fully.

The slides will not be harmed by hand-tightening.

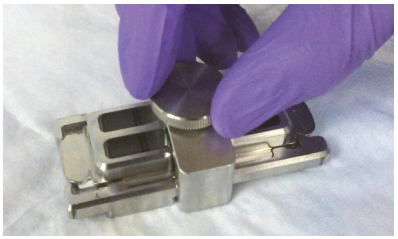

Figure 11. Tightening of the thumbscrew on the clamp

CAUTION

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

4 Rotate the final assembled chamber in a *vertical orientation*, clockwise, 2 to 3 times to wet the gaskets (see **Figure 12**).

Rotation helps ensure that the hybridization solution will coat the entire surface of the microarray during the incubation process.

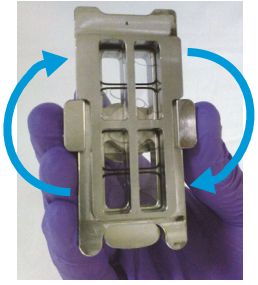

Figure 12. Rotation of the final assembled chamber

5 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.

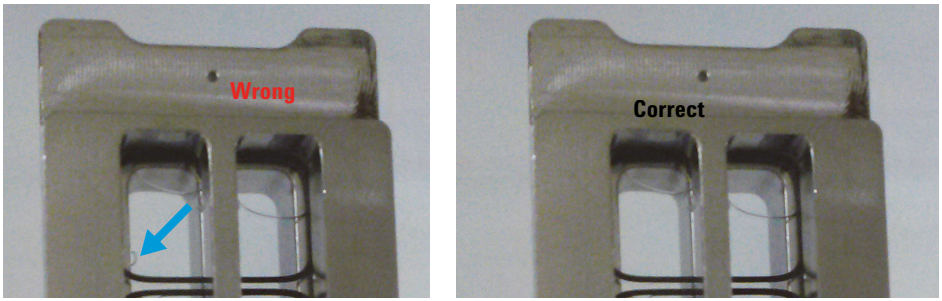

Figure 13. The slide on the left shows a stray, stationary bubble (denoted with arrow), which must be removed before hybridization. The slide on the right shows only large mixing bubbles, which move freely around the chamber when rotated. Bubbles are acceptable, as long as they move freely when you rotate the chamber.

Step 4. Hybridize

1 Load the assembled chamber into the oven rotator rack, starting from the center of the rack (position 3 or 4 when counting from left to right). Refer to the figure below for correct and incorrect orientations.

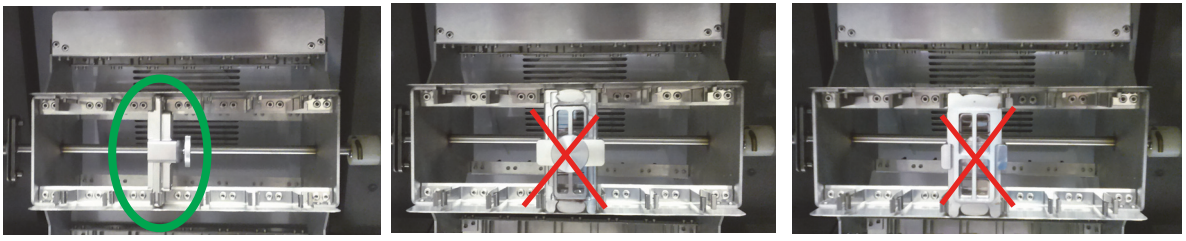

Figure 14. Assembled chambers in correct (left) and incorrect (middle and right) orientations

2 Close the door and set the rotator speed to 20 rpm.

3 Hybridize at 65°C for:

- 24 hours for blood, cell and tissue samples (4-pack and 8-pack microarrays)
- 40 hours for blood, cell and tissue samples (1-pack and 2-pack microarrays)
- 40 hours for FFPE samples (1-pack, 2-pack, 4-pack and 8-pack microarrays)

CAUTION If you are not loading all the available positions on the hybridization rotator rack, be sure to *balance* the loaded hybridization chambers on the rack similar to a centrifuge to prevent unnecessary strain on the oven motor.

CAUTION You must calibrate the hybridization oven regularly for accuracy of the collected data. Refer to *Agilent G2545A Hybridization Calibration Procedure* (publication G2545-90002) for more information.

CAUTION The Agilent Oligo aCGH/ChIP-on-Chip Wash Buffer 2 that is used in the microarray wash procedure needs to be warmed overnight. While you are waiting for the microarray slides to hybridize, do the steps in "Step 1. Prewarm Agilent Oligo aCGH/ChIP-on-Chip Wash Buffer 2 (overnight)" on page 58.

Microarray Wash

NOTE

The microarray wash procedure must be done in environments where ozone levels are 5 ppb or less. For Scanner C, if ozone levels are between 5 to 10 ppb in your laboratory, use the Agilent Ozone Barrier Slide Cover. SureScan microarray scanner uses a slide holder with a built-in ozone barrier. If ozone levels exceed 10 ppb, use the **Stabilization and Drying Solution** together with the ozone barrier.

You can also use Carbon Loaded Non-woven Filters to remove ozone from the air. These filters can be installed in either your HVAC system, or as part of small Ozone Controlled Enclosures. These free-standing enclosures can be installed either on a lab bench or as a walk-in room within your lab. These products are available through filter suppliers.

Before you begin, determine which wash procedure to use:

Table 33 Wash procedure to follow

Ozone level in your lab	Wash Procedure	Ozone-Barrier Slide Cover
< 5 ppb	**"Wash Procedure A (*without Stabilization and Drying Solution)"*** on page 60	No
> 5 ppb < 10 ppb	**"Wash Procedure A (*without Stabilization and Drying Solution)"*** on page 60	Yes
> 10 ppb	**"Wash Procedure B (*with Stabilization and Drying Solution)"*** on page 64	Yes

CAUTION

Do not use detergent to wash the staining dishes as some detergents may leave fluorescent residue on the dishes. If you do, you must ensure that all traces are removed by thoroughly rinsing with Milli-Q ultrapure water.

- Always use clean equipment when conducting the wash procedures.
- Use only dishes that are designated and dedicated for use in Agilent oligo aCGH experiments.

Step 1. Prewarm Agilent Oligo aCGH/ChIP-on-Chip Wash Buffer 2 (overnight)

The temperature of **Agilent Oligo aCGH/ChIP-on-Chip Wash Buffer 2** must be at 37°C for optimal performance.

1 Add the volume of buffer required to a **Sterile storage bottle** and warm overnight in an incubator or circulating water bath set to 37°C.

2 Put a slide-staining dish with a lid, a **1.5 L glass dish**, and one to two liters of **Milli-Q ultrapure water** in an incubator or water bath set at 37°C to warm overnight.

Step 2. Wash with Milli-Q ultrapure water

Rinse slide-staining dishes, slide racks and stir bars thoroughly with high-quality **Milli-Q ultrapure water** before use and in between washing groups.

1 Run copious amounts of **Milli-Q ultrapure water** through the slide-staining dishes, slide racks and stir bars.

2 Empty out the water collected in the dishes at least five times.

3 Repeat **step 1** and **step 2** until all traces of contaminating material are removed.

Step 3. Clean with Acetonitrile (Wash Procedure B Only)

Acetonitrile wash removes any remaining residue of **Stabilization and Drying Solution** from slide-staining dishes, slide racks and stir bars that were used in previous experiments with **"Wash Procedure B (*with Stabilization and Drying Solution)"** on page 64.

WARNING

Do Acetonitrile washes in a vented fume hood. Acetonitrile is highly flammable and toxic.

1. Add the slide rack and stir bar to the slide-staining dish, and transfer to a magnetic stir plate.
2. Fill the slide-staining dish with 100% **Acetonitrile**.
3. Turn on the magnetic stir plate and adjust the speed to a setting of 4 (medium speed).
4. Wash for 5 minutes at room temperature.
5. Discard the **Acetonitrile** as is appropriate for your site.
6. Repeat **step 1** through **step 5**.
7. Air dry everything in the vented fume hood.
8. Continue with the **Milli-Q ultrapure water** wash as previously instructed.

Step 4. Prewarm Stabilization and Drying Solution (Wash Procedure B Only)

The **Stabilization and Drying Solution** contains an ozone scavenging compound dissolved in **Acetonitrile**. The compound in solution is present in saturating amounts and may precipitate from the solution under normal storage conditions. If the solution shows visible precipitation, warming of the solution will be necessary to redissolve the compound. Washing slides using **Stabilization and Drying Solution** showing visible precipitation will have profound adverse affects on microarray performance.

WARNING **The Stabilization and Drying Solution is a flammable liquid. Warming the solution will increase the generation of ignitable vapors. Use gloves and eye/face protection in every step of the warming procedures.**

WARNING **Do not use a hot plate, oven, an open flame or a microwave. Do not increase temperature rapidly. Warm and mix the material away from ignition sources.**

WARNING **Failure to follow the outlined process will increase the potential for fire, explosion, and possible personal injury.**

1 Put a clean magnetic stir bar into the **Stabilization and Drying Solution** bottle and recap.
2 Partially fill a plastic bucket with hot water at approximately 40°C to 45°C (for example from a hot water tap).
3 Put the **Stabilization and Drying Solution** bottle into the hot water in the plastic bucket.
4 Put the plastic bucket on a magnetic stirrer (*not a hot-plate*) and stir.
5 The hot water cools to room temperature. If the precipitate has not all dissolved replenish the cold water with hot water.
6 Repeat **step 5** until the solution is clear.
7 After the precipitate is completely dissolved, allow the solution to equilibrate to room temperature prior to use.

CAUTION **Do not filter the Stabilization and Drying Solution, or the concentration of the ozone scavenger may vary.**

Step 5. Wash microarrays

Wash Procedure A (*without* Stabilization and Drying Solution)

Always use fresh **Agilent Oligo aCGH/ChIP-on-Chip Wash Buffer 1** and **Agilent Oligo aCGH/ChIP-on-Chip Wash Buffer 2** for each wash group (up to five slides).

Table 34 lists the wash conditions for the Wash Procedure A without **Stabilization and Drying Solution**.

Table 34 Wash conditions

	Dish	Wash buffer	Temperature	Time
Disassembly	#1	**Agilent Oligo aCGH/ChIP-on-Chip Wash Buffer 1**	Room temperature	
1st wash	#2	**Agilent Oligo aCGH/ChIP-on-Chip Wash Buffer 1**	Room temperature	5 minutes
2nd wash	#3	**Agilent Oligo aCGH/ChIP-on-Chip Wash Buffer 2**	37°C	1 minute

1 Completely fill slide-staining dish #1 with **Agilent Oligo aCGH/ChIP-on-Chip Wash Buffer 1** at room temperature.

2 Prepare dish #2:

   a Put a slide rack into slide-staining dish #2.

   b Add a magnetic stir bar. Fill slide-staining dish #2 with enough **Agilent Oligo aCGH/ChIP-on-Chip Wash Buffer 1** at room temperature to cover the slide rack.

   c Put this dish on a magnetic stir plate.

3 Prepare dish #3:

   a Put the prewarmed **1.5 L glass dish** on a magnetic stir plate with heating element.

   b Put the slide-staining dish #3 into the **1.5 L glass dish**.

   c Fill the **1.5 L glass dish** with pre-warmed **Milli-Q ultrapure water**.

   d Fill the slide-staining dish #3 approximately three-fourths full with **Agilent Oligo aCGH/ChIP-on-Chip Wash Buffer 2** (warmed to 37°C).

   e Add a magnetic stir bar.

   f Turn on the heating element and maintain temperature of **Agilent Oligo aCGH/ChIP-on-Chip Wash Buffer 2** at 37°C. Monitor with a thermometer.

4 Remove one hybridization chamber from the incubator and resume rotation of the others. Record whether bubbles formed during hybridization and if all bubbles are rotating freely.

5 Prepare the hybridization chamber disassembly.

   a Put the hybridization chamber assembly on a flat surface and loosen the thumbscrew, turning counter-clockwise.

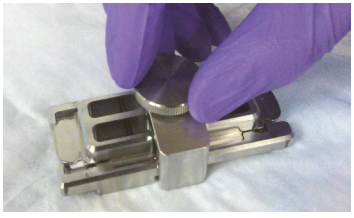

Figure 15. Loosening of the thumbscrew

   b Slide off the clamp assembly and remove the chamber cover.

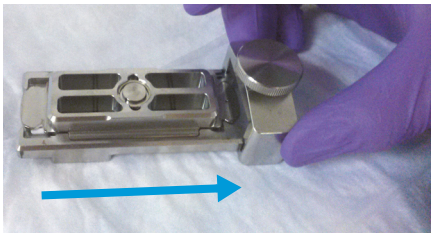

Figure 16. Removal of the clamp

c With gloved fingers, remove the microarray-gasket sandwich from the chamber base by lifting one end and then grasping in the middle of the long sides. Keep the microarray slide numeric barcode facing up as you quickly transfer the sandwich to slide-staining dish #1.

d Without letting go of the slides, submerge the microarray-gasket sandwich into slide-staining dish #1 containing **Agilent Oligo aCGH/ChIP-on-Chip Wash Buffer 1**.

6 With the sandwich completely submerged in **Agilent Oligo aCGH/ChIP-on-Chip Wash Buffer 1**, pry the sandwich open from the barcode end only:

a Slip one of the blunt ends of the forceps between the slides.

b Gently twist the forceps to separate the slides.

c Let the gasket slide drop to the bottom of the staining dish.

Remove the microarray slide, grasp it from the upper corners with thumb and forefinger, and quickly put into slide rack in the slide-staining dish #2 containing **Agilent Oligo aCGH/ChIP-on-Chip Wash Buffer 1** at room temperature. Minimize exposure of the slide to air. *Touch only the barcode portion of the microarray slide or its edges!*

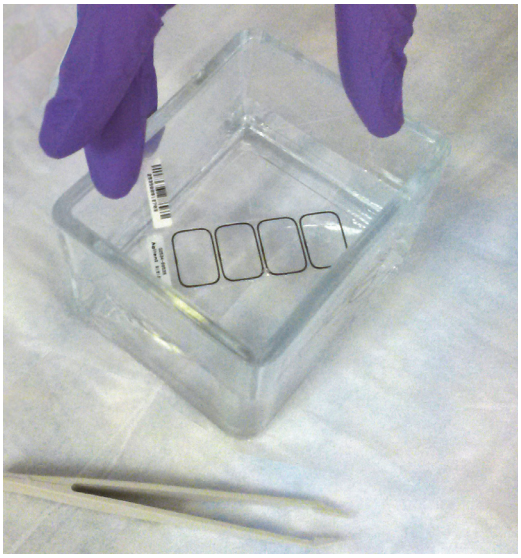

Figure 17. Removal of the microarray slide from the staining dish

7 Repeat **step 4** through **step 6** for up to four additional slides in the group. A maximum of five disassembly procedures yielding five microarray slides is advised at one time in order to facilitate uniform washing.

8 When all slides in the group are put into the slide rack in slide-staining dish #2, stir at 350 rpm for 5 minutes. Adjust the setting to get good but not vigorous mixing.

9 Wash the slides in **Agilent Oligo aCGH/ChIP-on-Chip Wash Buffer 2**:

   a Transfer slide rack to slide-staining dish #3, which contains **Agilent Oligo aCGH/ChIP-on-Chip Wash Buffer 2** at 37°C:

   a Activate the magnetic stirrer.

   b Wash microarray slides for at least 1 minute and no more than 2 minutes.

   Adjust the setting to get thorough mixing without disturbing the microarray slides.

10 Slowly remove the slide rack trying to minimize droplets on the slides. It should take 5 to 10 seconds to remove the slide rack.

11 Discard used **Agilent Oligo aCGH/ChIP-on-Chip Wash Buffer 1** and **Agilent Oligo aCGH/ChIP-on-Chip Wash Buffer 2**.

12 Repeat **step 1** through **step 11** for the next group of five slides using fresh **Agilent Oligo aCGH/ChIP-on-Chip Wash Buffer 1** and **Agilent Oligo aCGH/ChIP-on-Chip Wash Buffer 2** warmed to 37°C.

13 Scan slides immediately to minimize the impact of environmental oxidants on signal intensities. If necessary, store slides in orange slide boxes in a N_2 purge box, in the dark.

Wash Procedure B (*with* Stabilization and Drying Solution)

Cyanine reagents are susceptible to degradation by ozone. Use this wash procedure if the ozone level exceeds 10 ppb in your laboratory.

Always use fresh **Agilent Oligo aCGH/ChIP-on-Chip Wash Buffer 1** and **Agilent Oligo aCGH/ChIP-on-Chip Wash Buffer 2** for each wash group (up to five slides).

The **Acetonitrile** (dish #4) and **Stabilization and Drying Solution** (dish #5) below may be reused for washing up to 4 batches of 5 slides (total 20 slides) in one experiment. Do not pour the **Stabilization and Drying Solution** back in the bottle.

CAUTION

The Stabilization and Drying Solution must be set-up in a fume hood. Put the Wash Buffer 1 and Wash Buffer 2 set-up areas close to, or preferably in, the same fume hood. Use gloves and eye/face protection in every step of the washing procedure.

Table 35 lists the wash conditions for the Wash Procedure B with **Stabilization and Drying Solution**.

Table 35 Wash conditions

	Dish	Wash Buffer	Temperature	Time
Disassembly	#1	**Agilent Oligo aCGH/ChIP-on-Chip Wash Buffer 1**	Room temperature	
1st wash	#2	**Agilent Oligo aCGH/ChIP-on-Chip Wash Buffer 1**	Room temperature	5 minutes
2nd wash	#3	**Agilent Oligo aCGH/ChIP-on-Chip Wash Buffer 2**	37°C	1 minute
Acetonitrile wash	#4	**Acetonitrile**	Room temperature	10 seconds
3rd wash	#5	**Stabilization and Drying Solution**	Room temperature	30 seconds

1 In the fume hood, fill slide-staining dish #4 approximately three-fourths full with **Acetonitrile**. Add a magnetic stir bar and put this dish on a magnetic stir plate.

2 In the fume hood, fill slide-staining dish #5 approximately three-fourths full with **Stabilization and Drying Solution**. Add a magnetic stir bar and put this dish on a magnetic stir plate.

3 Do **step 1** through **step 9** in **"Wash Procedure A (*without Stabilization and Drying Solution)"** on page 60.

4 Remove the slide rack from **Agilent Oligo aCGH/ChIP-on-Chip Wash Buffer 2** and tilt the rack slightly to minimize wash buffer carry-over. Quickly transfer the slide rack to slide-staining dish #4 containing **Acetonitrile**, and stir at 350 rpm for 10 seconds.

5 Transfer slide rack to slide-staining dish #5 filled with **Stabilization and Drying Solution**, and stir at 350 rpm for 30 seconds.

6 Slowly remove the slide rack trying to minimize droplets on the slides. It should take 5 to 10 seconds to remove the slide rack.

7 Discard used **Agilent Oligo aCGH/ChIP-on-Chip Wash Buffer 1** and **Agilent Oligo aCGH/ChIP-on-Chip Wash Buffer 2**.

NOTE

The **Acetonitrile** and the **Stabilization and Drying Solution** may be reused for washing of up to four batches of five slides (that is, total 20 microarray slides) in one experiment. Pour the **Stabilization and Drying Solution** to a different marked bottle, and protect from light with other flammables. After each use, rinse the slide rack and the slide-staining dish that were in contact with the **Stabilization and Drying Solution** with **Acetonitrile** followed by a rinse in **Milli-Q ultrapure water**.

8 Repeat **step 1** through **step 7** for the next group of five slides using fresh **Agilent Oligo aCGH/ChIP-on-Chip Wash Buffer 1** and **Agilent Oligo aCGH/ChIP-on-Chip Wash Buffer 2** prewarmed to 37°C.

9 Dispose of **Acetonitrile** and **Stabilization and Drying Solution** as flammable solvents.

Step 6. Put slides in a slide holder

Scan slides immediately to minimize impact of environmental oxidants on signal intensities. If necessary, store slides in the original slide boxes in a N_2 purge box, in the dark.

For SureScan microarray scanner

1. Before you insert the slide into a slide holder, inspect the slide holder for any dust or fingerprints. If found, remove the dust or fingerprints with compressed air or a soft, dust-free cloth. If the slide holder is scratched, worn, or damaged, has a lid that does not close tightly, or has a hinge that does not move freely, discard the slide holder and select a different one.
2. Place the slide holder on a flat surface, with the clear cover facing up, and the tab on the right. This helps to ensure that you have the slide aligned properly when you insert it into the slide holder.
3. Gently push in and pull up on the tabbed end of the clear plastic cover to open the slide holder.

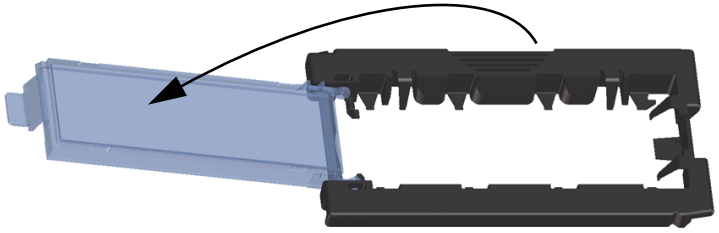

Figure 18. Opening the slide holder

4. Insert the slide into the holder.
   a. Hold the slide at the barcode end and position the slide over the open slide holder. Make sure that the active microarray surface faces up with the barcode on the left, as shown in **Figure 19**.
   b. Carefully place the end of the slide without the barcode label onto the slide ledge. See **Figure 19**.

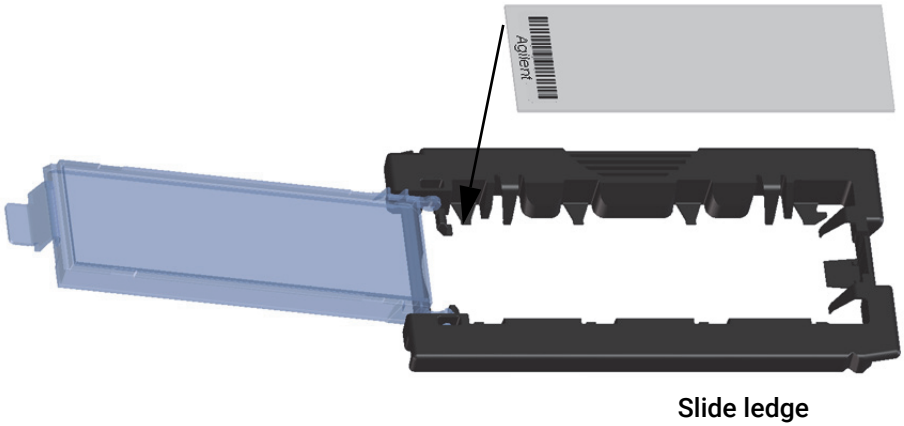

Figure 19. Inserting slide into the slide holder

c Gently lower the slide into the slide holder. See **Figure 20**.

d Close the plastic slide cover, pushing on the tab end until you hear it "click." This moves the slide into position in the holder.

CAUTION An improperly inserted slide can damage the scanner.

e Gently push in and pull up on the tabbed end of the clear plastic cover to open it again and verify that the slide is correctly positioned.

f Close the plastic slide cover, gently pushing on the tab end until you hear it "click". See **Figure 21**. Make sure that the slide holder is completely closed.

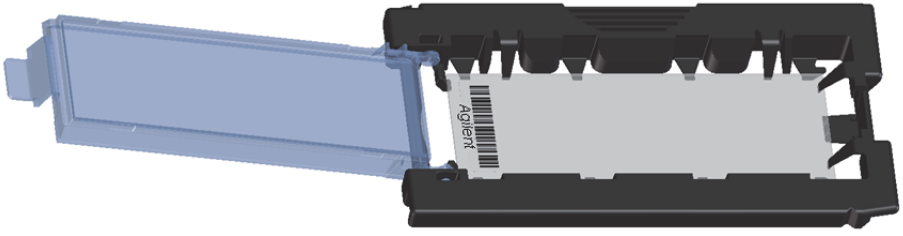

Figure 20. Slide inserted in slide holder – cover open

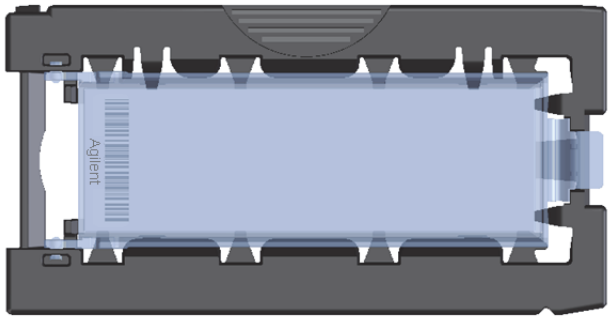

Figure 21. Slide inserted in slide holder – cover closed

For Agilent Scanner C

- In environments in which the ozone level exceeds 5 ppb, immediately put the slides with active microarray surface ("Agilent"-labeled barcode) facing up in a slide holder. Make sure that the slide is not caught up on any corner. Put an ozone-barrier slide cover on top of the array as shown in **Figure 22**. Refer to the *Agilent Ozone-Barrier Slide Cover User Guide* (publication G2505-90550), included with the slide cover, for more information.

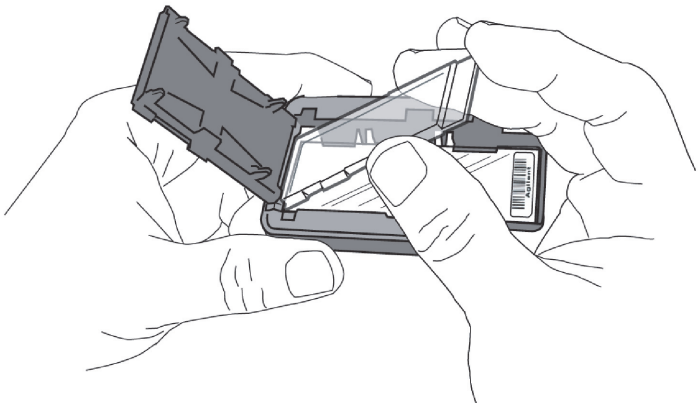

Figure 22. Inserting the ozone-barrier slide cover

- In environments in which the ozone level is below 5 ppb, put the slides with Agilent barcode facing up in a slide holder.

Microarray Scanning

Step 1. Scan the microarray slides

A SureScan or Agilent C microarray scanner is required for G3 microarrays.

Agilent provides support for Agilent microarrays scanned on select non-Agilent scanners. Please see "Feature Extraction Compatibility Matrix for Non Agilent scanners" for scanner compatibility and settings (**www.agilent.com/cs/library/usermanuals/Public/G1662-90043_ScannerCompatibilityMatrix.pdf**).

However, Agilent can guarantee the quality of data only if the data comes from Agilent microarrays scanned on Agilent scanners.

Agilent SureScan Microarray Scanner

1 Put assembled slide holders into the scanner cassette. Refer to **Figure 23** and **Figure 24**.

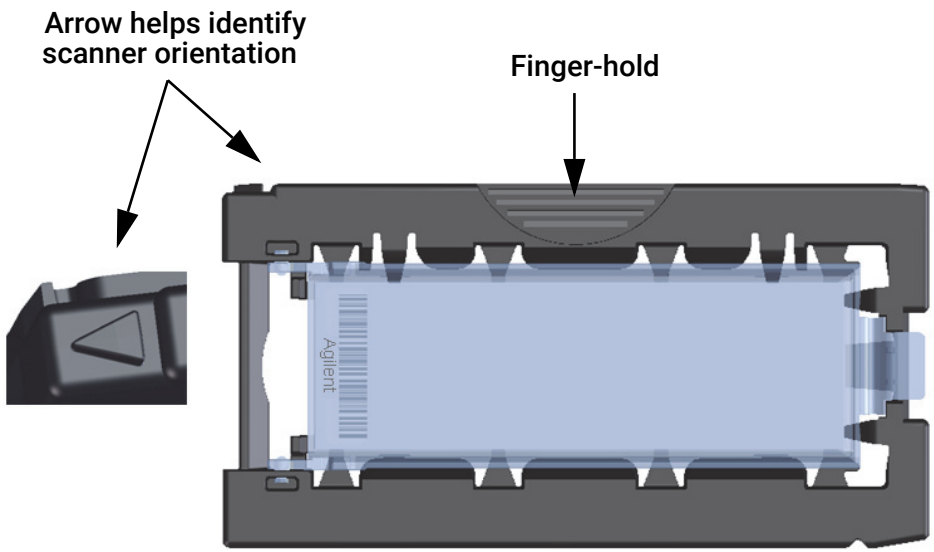

Figure 23. Slide holder helps you to insert slides correctly

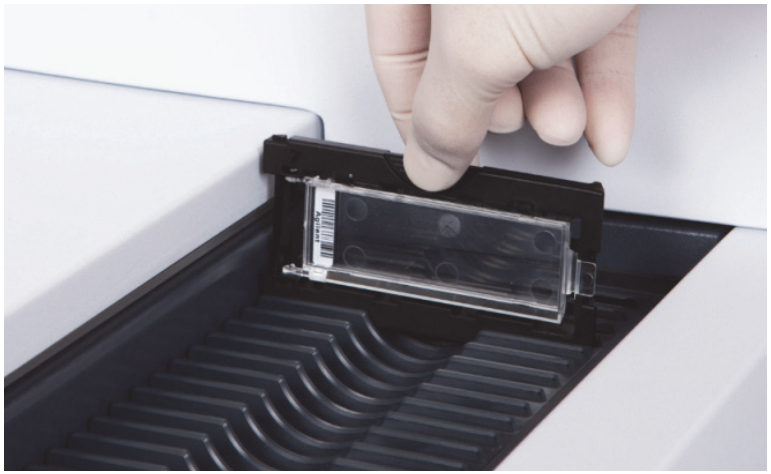

Figure 24. Inserting slide holder into cassette

2 Select **Protocol AgilentG3_CGH** for G3 microarrays. Select P**rotocol AgilentHD_CGH** for HD microarrays.

3 Verify that the Scanner status in the main window says **Scanner Ready**.

4 Click **Start Scan**.

Agilent C Scanner Settings

1 Put assembled slide holders with or without the ozone-barrier slide cover into scanner carousel.

2 Select **Start Slot *m* End Slot *n*** where the letter *m* represents the Start slot where the first slide is located and the letter *n* represents the End slot where the last slide is located.

3 Select **Profile AgilentG3_CGH** for G3 microarrays. Select **Profile AgilentHD_CGH** for HD microarrays.

4 Verify scan settings. See **Table 36**.

Table 36 C Scanner Scan Settings

	For HD Microarray Formats	For G3 Microarray Formats
Dye channel	R+G (*red and green*)	R+G (*red and green*)
Scan region	Agilent HD (61 x 21.6 mm)	Agilent HD (61 x 21.6 mm)
Scan resolution	5 µm	3 µm
Tiff file dynamic range	16 bit	16 bit

Table 36 C Scanner Scan Settings

	For HD Microarray Formats	For G3 Microarray Formats
Red PMT gain	100%	100%
Green PMT gain	100%	100%
XDR	<No XDR>	<No XDR>

5 Check that Output Path Browse is set for desired location.

6 Verify that the Scanner status in the main window says **Scanner Ready**.

7 Click **Scan Slot *m-n*** on the Scan Control main window where the letter *m* represents the Start slot where the first slide is located and the letter *n* represents the End slot where the last slide is located.

Step 2. Analyze microarray image

- After scanning is completed, extract features and analyze.

Feature extraction is the process by which data is extracted from the scanned microarray image (.tif) and translated into log ratios, allowing researchers to identify aberrations in their samples.

Use the Agilent Feature Extraction (FE) software.

Agilent provides Feature Extraction software as a standalone program and as an integral part of CytoGenomics software (Windows version only).

- Use the Windows version of Agilent CytoGenomics for automated and streamlined analysis of human samples. During the extraction and analysis process, Agilent CytoGenomics generates feature extraction files, QC and aberration reports.
- To use Agilent CytoGenomics on a Mac computer, first use Feature Extraction on a computer that is running Windows to extract features. Feature Extraction does not run on Mac computers.
- For non-human samples, use Feature Extraction (available for Windows only) to extract features, and then use Agilent Genomic Workbench to run an analysis workflow on the extracted features.

Microarray QC Metrics for high DNA quality samples

These metrics are only appropriate for samples analyzed with Agilent CGH microarrays by following the standard operational procedures provided in this user guide. These metrics are reported in the Feature Extraction QC report generated by Feature Extraction (standalone or as included in the Agilent CytoGenomics software). They can be used to assess the relative data quality from a set of microarrays in an experiment. In some cases, they can indicate potential processing errors that have occurred or suggest that the data from particular microarrays might be compromised. Many factors can influence the range of these metrics, including the microarray format (1-pack, 2-pack, 4-pack or 8-pack), biological sample source, quality of starting gDNA, experimental processing, scanner sensitivity, and image processing. The value guidelines presented below represent the thresholds that Agilent has observed when analyzing samples using this protocol.

Table 37 QC metric thresholds for ULS labeling

	Blood and Cell Samples	Tissue Samples	FFPE Samples
BGNoise	<15	<15	<15
Signal Intensity	>90	>90	>90
Signal to Noise	>20	>20	>10
Reproducibility	<0.2	<0.2	<0.2
DLRSD	<0.2	<0.3	<0.4

QC Report - Agilent Technologies : 2 Color CGH

Date	Tuesday, August 28, 2018 - 08:39	Sample(red/green)	Agilent Female_dbSNP141/Agilent Female_dbSNP141
User Name	digoidin	FE Version	5.0.0.1
Image	SG15165041_252983031233_S001 [1_2]	BG Method	Detrend on (NegC)
Protocol	CytoCGH_0500_4x_Nov17 (Read Only)	Multiplicative Detrend	True
Grid	029830_20151217	Dye Norm	Linear
Saturation Value	65526 (r), 65526 (g)		
DyeNorm List	NA		
No of Probes in DyeNorm List	NA		

Spot Finding of the Four Corners of the Array

Grid Normal

Outlier Numbers with Spatial Distribution
1064 rows x 170 columns

*Red FeaturePopulation
*Green FeaturePopulation
*Red Feature NonUniform
*Green Feature NonUniform

Feature	Red	Green	Any	%Outlier
Non Uniform	121	81	136	0.08
Population	121	101	189	0.10

Evaluation Metrics for CytoCGH_QCMT_4x_Nov17
Excellent (10) ; Good (3)

Metric Name	Value	Excellent	Good	Evaluate
IsGoodGrid	1.00	>1	NA	<1
AnyColorPrcntFeatNonU...	0.08	<1	1 to 5	>5
DerivativeLR_Spread	0.10	<0.20	0.20 to 0.30	>0.30
gRepro	0.07	0 to 0.10	0.10 to 0.20	<0 or >0.20
g_BGNoise	7.16	<10	10 to 20	>20
g_Signal2Noise	68.13	>100	50 to 100	<50
g_SignalIntensity	487.80	>400	200 to 400	<200
rRepro	0.06	0 to 0.10	0.10 to 0.20	<0 or >0.20
r_BGNoise	5.01	<10	10 to 20	>20
r_Signal2Noise	80.31	>100	50 to 100	<50
r_SignalIntensity	402.04	>400	200 to 400	<200
RestrictionControl	0.97		0.80 to 1	<0.80 or >1
LogRatioImbalance	0.00	-0.26 to 0.26	(-0.75 to -0.2...	<-0.75 or >0.75

• Excellent • Good • Evaluate

Histogram of Signals Plot (Red)

Histogram of Signals Plot (Green)

Figure 25. Feature Extraction QC Report, page 1

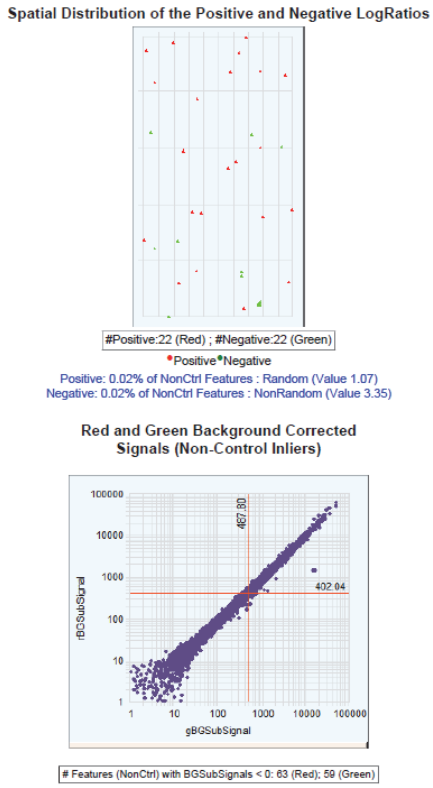

Figure 26. Feature Extraction QC Report, page 2

5 Troubleshooting

This chapter contains potential causes for above-threshold DLRSD (Derivative Log Ratio Standard Deviation). A poor DLRSD score reflects high probe-to-probe log ratio noise.

If you have a low OD260/230 or OD260/280 value

A low OD260/230 value can indicate contaminants, such as residual salt or organic solvents (which would inhibit enzyme). A low OD260/280 value indicates residual protein. Either condition can result in low specific activity (pmol dye/µg DNA) or Degree of Labeling. See **"To determine yield, degree of labeling or specific activity"** on page 41.

- ✔ Repurify the DNA using the Qiagen DNeasy protocol. See **"DNA Isolation"** on page 19. This procedure includes a proteinase K treatment.
- ✔ If you must do a phenol/chloroform DNA extraction, do not get too close to the interface.

  Phenol has an absorbance maximum of 270–275 nm, which is close to that of DNA. Phenol contamination mimics both higher purity and higher yields, because of an upward shift in the OD260 value.
- ✔ Make sure to calibrate the spectrophotometer with the appropriate buffer.
- ✔ The ULS labeling is very sensitive to salt contamination. Use 80% EtOH instead of AW2 in the DNA extraction step and elute in water.
- ✔ The ULS Labeling also labels proteins and RNA that compete for dye. Make sure that the DNA is free of proteins and RNA. See **"DNA Isolation"** on page 19.

If you have poor sample quality due to residual RNA

The input amount of DNA for the experimental sample must be the same as for the reference sample. RNA absorbs at the same wavelength as DNA. Therefore, RNA-contaminated sample can result in a DNA overestimation.

- ✔ Repurify the DNA using the Qiagen DNeasy protocol. See **"DNA Isolation"** on page 19. This procedure includes a RNase A treatment.
- ✔ The ULS labeling also labels RNA that will hybridize to the probe.

If you get poor sample quality due to degradation

For non-FFPE samples: On a 1 to 1.5% agarose gel, intact gDNA should appear as a compact, high-molecular weight band with no lower molecular weight smears. Degraded DNA results in biased labeling.

- ✔ Check DNA on a 1 to 1.5% agarose gel. If DNA that was isolated from cells, blood or frozen tissue is degraded, then repurify the DNA using the Qiagen DNeasy protocol. See **"DNA Isolation"** on page 19.
- ✔ Make sure that the DNA is not over-fragmented prior to labeling. Possible causes are incorrect temperature or length of incubation at 95°C, or evaporation (use a thermal cycler with heated lid). Make sure most of the heat fragmented products are between 1000 and 3000 bases in length.

If the estimated concentration is too high or low

The input amount of DNA for the experimental sample must be the same as for the reference sample. Precipitated DNA or DNA that is at a very high concentration cannot be quantitated accurately.

Contaminants such as organic solvents and RNA also absorb at 260 nm, which results in an inaccurate DNA quantitation.

- ✔ Make sure that the gDNA is completely in solution by pipetting up and down. If needed, incubate at 37°C for 30 minutes. If the gDNA concentration is > 350 ng/µL, dilute 1:2 in water (or any of the recommended buffers). Quantitate again to make sure quantitation is accurate.
- ✔ Different DNA isolation methods can create different quantitation artifacts, the risk of assay noise is higher when the experimental and reference DNA samples are isolated from different sources. If you used a spectrophotometer (such as the Nanodrop) for the initial measurement, also use a double-stranded DNA-based fluorometer (such as the Qubit) to verify.
- ✔ If needed, repurify the DNA using the Qiagen DNeasy protocol. See **"DNA Isolation"** on page 19.

If you have low yield not due to poor sample quality

Possible sample loss during clean-up after labeling.

✔ See **"Step 4. Removal of non-reacted ULS-Cy"** on page 39 to remove unreacted dye. Many other columns result in the loss of shorter fragments.

If you have low specific activity or degree of labeling not due to poor sample quality

Low specific activity or degree of labeling can result from sub-optimal labeling conditions such as wrong temperatures or times, volume mistakes, or too much exposure to light or air.

✔ Double check incubation times and temperatures (use a calibrated thermometer), and use a thermal cycler with heated lid.

✔ Evaporation can be a problem when you process samples at high temperatures. Make sure that sample tubes are well closed or use plate seals to avoid evaporation.

✔ Make sure that the pipettors are not out of calibration.

✔ Make sure that the gDNA, reagents, and master mixes are well mixed. Tap the tube with your finger or use a pipette to move the entire volume up and down. Then spin in a microcentrifuge for 5 to 10 seconds to drive the contents off the walls and lid. Do not mix the stock solutions and reactions that contain gDNA on a vortex mixer.

If you have post-labeling signal loss

Signal loss can be due to wash or hybridization conditions that are too stringent, or degradation of the Cyanine 5 signal.

Cyanine 5 signal degradation can be caused by ozone or NOx compounds coming from pollution and/or compressors and centrifuges. Cyanine 5 signal degradation can result in less red signal around the edges of the features, a visible gradient of significant Cy5/Cy3 positive ratios and more significant Cy5/Cy3 negative ratios – especially on the left side of the slide and on slides scanned later in a batch.

- ✔ Check that the oven temperature is 65°C. If needed, recalibrate the hybridization oven. Follow the steps in *Agilent G2545A Hybridization Calibration Procedure* (publication G2545-90002).
- ✔ Check that the temperature of Wash 2 is 37°C.
- ✔ Check that Wash 2 was not accidentally used instead of Wash 1.
- ✔ Wash and scan slides in an ozone controlled environment (<5 ppb), such as an ozone tent.
- ✔ Use small batches that can be washed and scanned in about 40 minutes to minimize exposure to air.
- ✔ For Agilent Scanner C or B, use the Agilent Ozone-Barrier Slide Cover (p/n G2505-60550). The SureScan scanner has built-in ozone protection.
- ✔ Use the **Stabilization and Drying Solution** as described in **"Wash Procedure B (*with Stabilization and Drying Solution*)"** on page 64.

If you have high BGNoise values

High BGNoise can cause lower signal-to-noise values (see **Table 37** for thresholds) and higher DLRSD values. BGNoise is defined as the standard deviation of the signals on the negative controls. If the BGNoise is high, examine the microarray image for visible non-uniformities. High BGNoise is often introduced during hybridization steps or washes.

✔ Make sure that the oven is calibrated. Follow the steps in *Agilent G2545A Hybridization Calibration Procedure* (publication G2545-90002).

Sample hybridization at incorrect temperatures affects the stringency of the hybridization.

✔ Make sure that wash dishes, racks and stir bars are clean. Do not use tap water or detergents to clean wash equipment. If needed, rinse wash equipment with acetonitrile followed by rinses with MilliQ water.

✔ If needed, wash the slides with acetonitrile:

1. In the fume hood, fill a slide-staining dish approximately three-fourths full with acetonitrile.
2. Add a magnetic stir bar and put this dish on a magnetic stir plate.
3. Put the slides in a slide rack and transfer the slide rack to the slide-staining dish containing acetonitrile, and stir at 350 rpm for 1 minute.
4. Slowly remove the slide rack and scan the slides immediately.

If you have poor reproducibility

Poor reproducibility (see **Table 37** for thresholds), defined as high CVs of signals of replicated probes may indicate that the hybridization volume was too low or that the oven stopped rotating during the hybridization. Only very high scores on this metric will affect the DLRSD.

✔ When setting up the gasket-slide hybridization sandwich dispense the hybridization sample mixture slowly in a “drag and dispense” manner to prevent spills.

✔ Check that the oven is rotating.

6 Reference

This chapter contains reference information that pertains to this protocol.

Reagent Kit Components

The contents of the reagent kits used in this protocol are listed here.

Table 38 DNeasy Blood & Tissue Kit

Component
DNeasy Mini Spin Column
2 mL Collection Tube
Buffer ATL
Buffer AL
Buffer AW1
Buffer AW2
Buffer AE
Proteinase K

Table 39 Qubit dsDNA BR Assay Kit

Component
Qubit dsDNA BR reagent
Qubit dsDNA BR buffer
Qubit dsDNA BR standard #1
Qubit dsDNA BR standard #2

Table 40 Genomic DNA ULS Labeling Kit

Component
Agilent-KREApure purification column
Collection tube
Agilent-CGHblock
10× Labeling Solution
ULS-Cy 5 Reagent
ULS-Cy 3 Reagent

Table 41 Oligo aCGH/ChIP-on-chip Hybridization Kit

Component
2× HI-RPM Hybridization Buffer
10× aCGH Blocking Agent

Table 42 Oligo aCGH/ChIP-on-chip Wash Buffer Kit

Component
Agilent Oligo aCGH/ChIP-on-Chip Wash Buffer 1
Agilent Oligo aCGH/ChIP-on-Chip Wash Buffer 2

"Secure Fit" Slide Box Opening Instructions

Agilent now ships all microarray slides in a newly designed "secure fit" slide box. The instructions below describe how to remove the slide box from the shipping pouch, how to open the slide box, and how to properly remove a microarray slide.

1 Use scissors to cut below the seal and remove box from its foil pouch.

After breaking foil on microarray pouch, store microarray slides in the slide box at room temperature (in the dark) under a vacuum desiccator or nitrogen purge box.

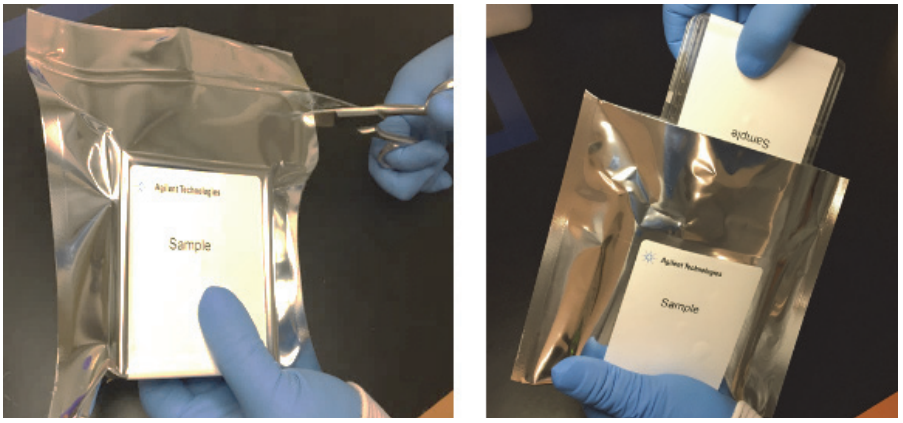

Figure 27. Opening foil pouch (left) and removing the slide box (right)

2 Place the slide box on a flat surface. While stabilizing the box from the top with one hand, use a sharp edge to cut the sealing tape on both sides of the box before opening.

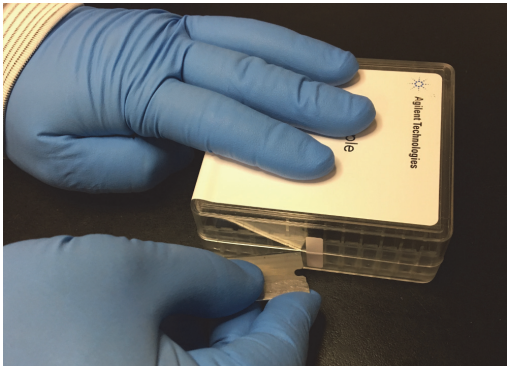

Figure 28. Cutting the sealing tape

3 With one hand, firmly hold the base of the box on the sides with the indentations (or dimples) for added grip.

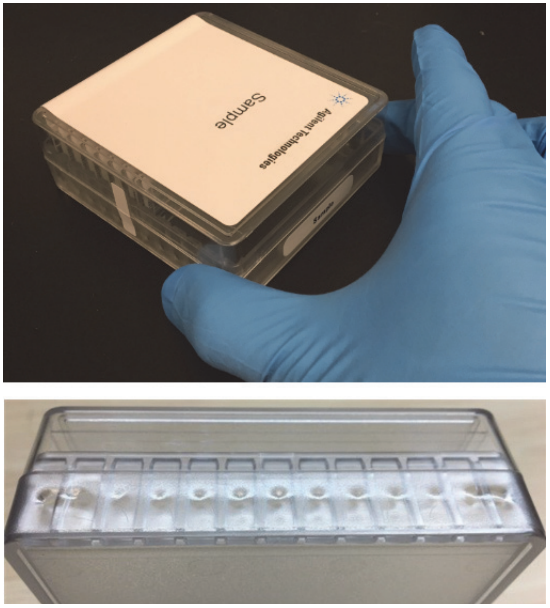

Figure 29. Gripping the base at the indentations (top) and close-up of the indentations (bottom)

4 Using your free hand, grasp the lid and gently lift it away from the base as if it is hinged. Set the lid aside.

Depending on your preference, you can reverse the hand positions so that the left hand holds the base while the right hand grasps the lid.

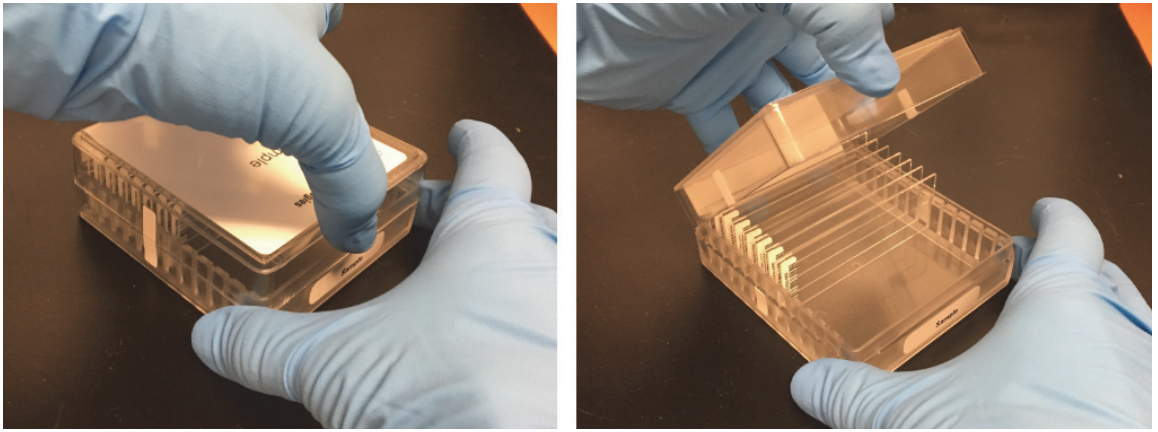

Figure 30. Grasping the lid (left) and lifting the lid from the base (right)

Microarray Handling Tips

Each microarray is printed on the side of the glass slide containing the "Agilent"-labeled barcode. This side is called the "active" side. The numeric barcode is on the inactive side of the slide.

CAUTION

You must familiarize yourself with the assembly and disassembly instructions for use with the Agilent Microarray Hybridization Chamber (G2534A) and gasket slides. Practice slide kits are available.

In this "processing and hybridization" procedure, the hybridization mixture is applied directly to the gasket slide, and not to the active side of the oligo microarray. Instead, the active side of the oligo microarray is placed on top of the gasket slide to form a "sandwich slide" pair.

To avoid damaging the microarray, always handle glass slides carefully by their edges. Wear powder-free gloves. Never touch the surfaces of the slides. If you do, you may cause irreparable damage to the microarray.

Never allow the microarray surface to dry out during the hybridization process and washing steps.

Agilent Microarray Layout and Orientation

Agilent oligo microarray (1 microarray/slide format) as imaged on the Agilent microarray scanner

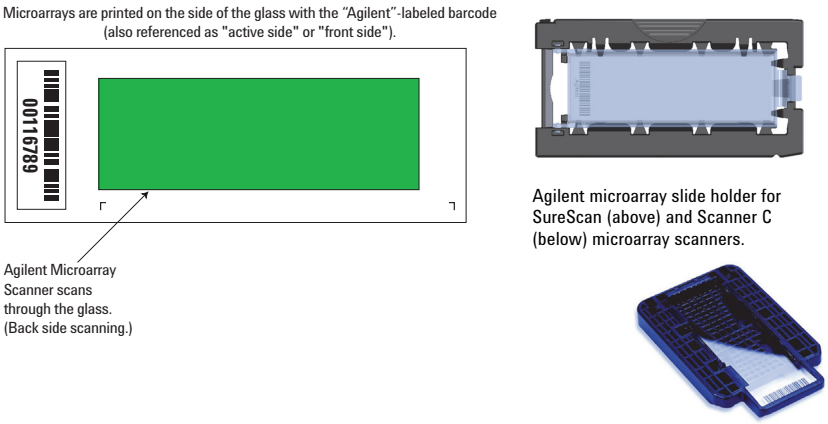

Figure 31. Agilent microarray slide and slide holder

Agilent oligo microarrays formats and the resulting "microarray design files" are based on how the Agilent microarray scanner images 1-inch x 3-inch glass slides. Agilent designed its microarray scanner to scan through the glass slide (back side scanning). The glass slide is securely placed in an Agilent microarray slide holder with the "Agilent" labeled barcode facing the opening of the slide holder (on SureScan Microarray Scanner) or facing the inside of the slide holder (Scanner C). In this orientation, the "active side" containing the microarrays is protected from potential damage by fingerprints and other elements. Once securely placed, the numeric barcode, non-active side of the slide, is visible from the outside of the slide holder.

Figure 31 depicts how the Agilent microarray scanner reads the microarrays and how this relates to the "microarray design files" that Agilent generates during the manufacturing process of its *in situ*-synthesized oligonucleotide microarrays. Thus, if you have a scanner that reads microarrays from the "front side" of the glass slide, the collection of microarray data points will be different in relation to the "microarray design files". Therefore, please take a moment to become familiar with the microarray layouts for each of the Agilent oligo microarrays and the layout information as it pertains to scanning using a "front side" scanner.

Non-Agilent Front Side Microarray Scanners

When scanning Agilent oligo microarray slides, the user must determine:

- If the scanner images the microarrays by reading them on the “front side” of the glass slide (“Agilent”-labeled barcode side of the slide) and
- If the microarray image produced by the non-Agilent scanner is oriented in a “portrait” or “landscape” mode, and “Agilent”-labeled barcode is on the left-side, right-side, up or down, as viewed as an image in the imaging software (see **Figure 32**).

This changes the feature numbering and location as it relates to the “microarray design files”.

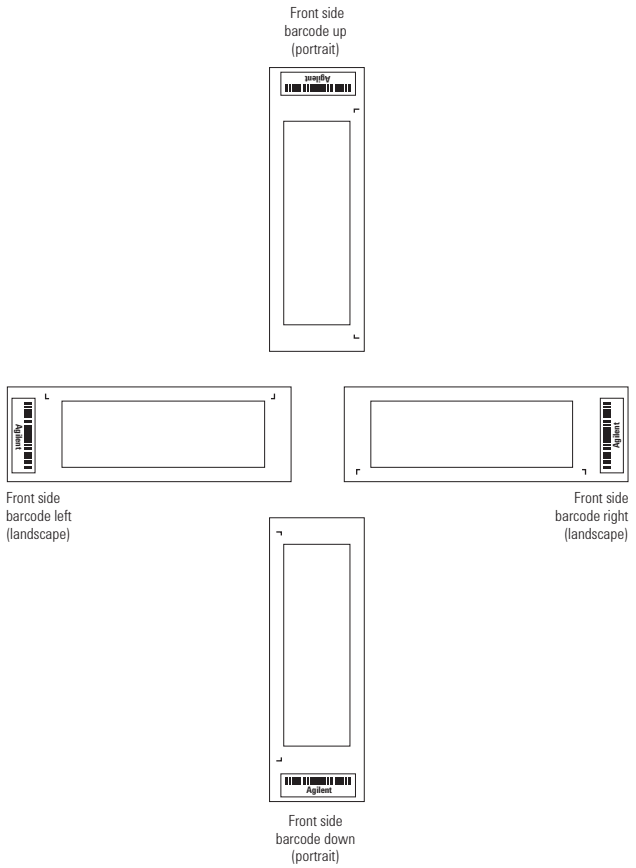

Figure 32. Microarray slide orientation

Array/Sample tracking on microarray slides

Use the forms below to make notes to track your samples on microarray slides.

Position the gasket slide in the SureHyb chamber base with the label to the left and load the samples: top row, left to right, then lower row, left to right. The array suffix assignments from Feature Extraction will then occur in the order shown.

Arrays

	Array 1_1	**Array 1_2**
BARCODE	**Sample:**	**Sample:**

Barcode Number _____

Figure 33. 2-pack microarray slides

Arrays

	Array 1_1	Array 1_2	Array 1_3	Array 1_4
BARCODE	Sample:	Sample:	Sample:	Sample:

Barcode Number _____

Figure 34. 4-pack microarray slides

Arrays

	Array 1_1	Array 1_2	Array 1_3	Array 1_4
BARCODE	Sample:	Sample:	Sample:	Sample:
	Sample:	Sample:	Sample:	Sample:
	Array 2_1	Array 2_2	Array 2_3	Array 2_4

Barcode Number _____

Figure 35. 8-pack microarray slide

In This Book

This guide contains information to run the Oligonucleotide Array-Based CGH for Genomic DNA Analysis – ULS Labeling for Blood, Cells, or Tissues protocol.

www.agilent.com

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Agilent