Agilent

Oligonucleotide Array-Based CGH for Genomic DNA Analysis – Enzymatic Labeling For Blood, Cells, or Tissues

Quick Reference Guide for 4-pack microarrays

This Quick Reference Guide is an abbreviated version of publication G4410-90010, "*Oligonucleotide Array-Based CGH for Genomic DNA Analysis - Enzymatic Labeling, For Blood, Cells, or Tissues (with a High Throughput option) Protocol*." It contains instructions for enzymatic labeling of gDNA from blood, cells, or frozen tissues and hybridization of that labeled gDNA to Agilent SurePrint CGH and CGH+SNP microarrays in a 4-pack (i.e., 4 microarrays/slide) format.

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

Before You Begin

Using this Guide

This Quick Reference Guide is intended for experienced users who are already familiar with processing Agilent Oligo CGH Microarrays arrayed on 4-pack slides. If you are a new user, refer to publication G4410-90010, *Oligonucleotide Array-Based CGH for Genomic DNA Analysis - Enzymatic Labeling, For Blood, Cells, or Tissues (with a High Throughput option) Protocol*, which is the full-length version of this Quick Reference Guide. The full-length protocol includes additional instructions and details, as well as procedural notes, information on kit contents, required materials and equipment, and troubleshooting tips.

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.**
- **Agilent 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.**

Step 1. DNA Quantitation and Quality Analysis

After you have isolated genomic DNA (gDNA) from your test samples and, if applicable, your reference samples, verify the quantity and quality of the DNA samples using fluorometry, UV-VIS spectrophotometry, or agarose gel electrophoresis.

Fluorometry is the recommended approach. Use the Qubit dsDNA BR Assay Kit at room temperature (22°C to 28°C) to determine the gDNA concentration. Use 1 to 20 µL of your DNA sample for the Qubit dsDNA BR assay.

Step 2. Sample Preparation

This section provides instructions on the *direct method* for sample preparation, which uses the Agilent SureTag or SureTag Complete DNA Labeling Kit for restriction digestion of gDNA. Digest an equal amount of reference gDNA.

The direct method requires at least 0.5 µg of starting gDNA. If you have <0.5 µg of gDNA, use the amplification method for sample preparation described in the full-length protocol (publication G4410-90010).

Note on heat fragmentation For many samples, you can skip the restriction digestion step and, instead, fragment the gDNA by performing a longer incubation at 98°C during sample labeling. See **step 3** in **"Fluorescent Labeling of gDNA."** Importantly, if the samples are to be hybridized to SurePrint G3 CGH+SNP microarrays, then heat fragmentation is not an option, and you must perform restriction digestion on the gDNA samples.

1 Set up the restriction digest reaction tubes. Add 0.5–1.0 µg of gDNA to each tube, then add enough water to each reaction to bring the volume to 20.2 µL.

2 Prepare the Digestion Master Mix by mixing the components in **Table 1**. Mix well by pipetting up and down.

Table 1 Digestion Master Mix

Component	Per reaction (µL)	× 16 rxns (µL) including excess	× ___ rxns (µL)
Nuclease-Free Water	2.0	34	
10× Restriction Enzyme Buffer	2.6	44.2	
BSA	0.2	3.4	
Alu I	0.5	8.5	
Rsa I	0.5	8.5	
Final volume of Digestion Master Mix	**5.8**	**98.6**	

3 Aliquot 5.8 µL of Digestion Master Mix to each reaction tube of gDNA. Mix well by pipetting up and down.

4 Incubate the samples in a circulating water bath, heat block, or thermal cycler.
- 37°C for 2 hours
- 65°C for 20 minutes
- 4°C until ready to proceed to **"Step 3. Sample Labeling"**, or store at –20°C.

5 *Optional*. Take 2 µL of the digested gDNA and run on a 0.8% agarose gel. The majority of the digested products should be 200 bp to 500 bp in length.

1 month **Optional Stopping Point** Digested gDNA can be stored up to one month at –20°C.

Step 3. Sample Labeling

Treat the test/reference sample pairs identically during labeling. Minimize light exposure throughout the procedure.

Fluorescent Labeling of gDNA

1 Spin the samples in a centrifuge for 1 minute at 6,000 × g.

If you did not perform restriction digestion on the samples, add enough nuclease-free water to each sample to bring the volume to 26 µl.

2 Add 5 µL of Random Primers to each sample to make a total volume of 31 µL (or 29 µL if the optional agarose gel step on **page 3** was done). Mix well by pipetting up and down gently.

3 Incubate samples in a circulating water bath, heat block, or thermal cycler.

- 98°C for 3 minutes (with restriction digestion) or 10 minutes (without restriction digestion)
- 4°C (or ice) for 5 minutes

4 Spin the samples for 1 minute at 6,000 × g.

5 Prepare the Labeling Master Mixes by mixing the components in **Table 2** on ice in the order indicated. Prepare one cyanine-3 and one cyanine-5 Labeling Master Mix.

Table 2 Labeling Master Mix

Component	Per reaction (µL)	× 8rxns (µL) including excess	× ___ rxns (µL)
Nuclease-Free Water	2.0*	17*	
5× Reaction Buffer	10.0	85	
10× dNTPs	5.0	42.5	
Cyanine 3-dUTP *or* Cyanine 5-dUTP	3.0	25.5	
Exo (-) Klenow	1.0	8.5	
Final volume of Labeling Master Mix	**19.0 or 21.0**	**161.5 or 178.5**	

* Do *not* add Nuclease-Free Water if you skipped the optional agarose gel step (**step 5** on **page 3**).

6 Add 19 µL (or 21 µL) of the appropriate Labeling Master Mix to each sample for a total volume of 50 µL. Mix well by gently pipetting up and down.

7 Incubate the samples in a circulating water bath, heat block, or thermal cycler.

- 37°C for 2 hours
- 65°C for 10 minutes to inactivate the enzyme
- 4°C until ready to proceed, or store at –20°C in the dark.

Optional Stopping Point Labeled DNA can be stored up to one month at –20°C in the dark.

Purification of Labeled gDNA

Purify labeled gDNA using the purification columns provided with the SureTag and SureTag Complete DNA Labeling Kits. Keep cyanine-3 and cyanine-5 labeled gDNA samples separated during purification.

1. Spin the labeled gDNA samples in a centrifuge for 1 minute at 6,000 × g.
2. Add 430 µL of 1×TE (pH 8.0) to each gDNA sample.
3. For each gDNA sample to be purified, place a column into a 2-mL collection tube and label the column appropriately. Load each sample onto a column.
4. Cover the column with a cap and spin for 10 minutes at 14,000 × g at room temperature. Discard the flow-through and place the column back in the collection tube.
5. Add 480 µL of 1×TE (pH 8.0) to each column. Spin for 10 minutes at 14,000 × g at room temperature. Discard the flow-through.
6. Invert the column into a fresh 2-mL collection tube that has been appropriately labeled. Spin for 1 minute at 1,000 × g at room temperature to collect purified sample.

   The volume per sample will be approximately 20 to 32 µL.
7. Add 1×TE (pH 8.0) or use a concentrator to bring the sample volume to that listed in **Table 4**.
8. Take 1.5 µL of each sample to determine yield and degree of labeling or specific activity on a NanoDrop 8000 or 2000 UV-VIS Spectrophotometer. Blank the instrument with 1×TE (pH 8.0).

   Refer to **Table 3** for expected yield of labeled gDNA and specific activity after labeling and purification, when starting with high quality gDNA. The cyanine-3 and cyanine-5 yield after labeling should be the same.

Table 3 Expected Yield and Specific Activity after Labeling and Clean-up

Input gDNA (µg)	Yield (µg)	Specific Activity (pmol/µg)
0.5	8 to 13	20 to 60
1	9 to 14	20 to 60

9. In a fresh tube or plate well (1.5-mL tube, 200-µL tube, tall chimney PCR plate, or 96-well PCR plate) combine test and reference sample using the appropriate cyanine-5-labeled sample and cyanine-3-labeled sample for a total mixture volume listed in **Table 4**.

Table 4 Sample volume and total mixture volumes

Cyanine 3 or Cyanine 5 sample volume after purification	Total mixture volume after Nanodrop and combining Cyanine 3 and Cyanine 5 samples
21 µL	39 µL

Optional Stopping Point Purified, labeled DNA can be stored up to one month at –20°C in the dark.

Step 4. Microarray Hybridization

Preparation of labeled gDNA for hybridization

1 Mix the components in **Table 5** to prepare the Hybridization Master Mix. Use Cot-1 DNA from the appropriate species.

Table 5 Hybridization Master Mix

Component	Volume (µL) per hybridization	× 8rxns (µL) including excess	× ___ rxns (µL)
Cot-1 DNA (1.0 mg/mL)	5	42.5	
10× aCGH Blocking Agent	11	93.5	
2× HI-RPM Hybridization Buffer	55	467.5	
Final Volume	71	603.5	

2 Add 71 µL of Hybridization Master Mix to each tube of labeled gDNA to make a total volume of 110 µL/tube.

3 Mix the samples by pipetting up and down, then briefly spin in a centrifuge.

4 Incubate the samples in a circulating water bath, heat block, or thermal cycler.
- 98°C for 3 minutes
- 37°C for 30 minutes

5 Remove sample tubes from the water bath, heat block, or thermal cycler. Spin at 6000 × g for 1 minute. The samples are ready to be hybridized.

CAUTION **The samples must be hybridized immediately. If not, keep the temperature of hybridization sample mixtures as close to 37°C as possible.**

Preparation of the hybridization assembly

For detailed instructions on proper technique, refer to the full-length protocol (publication G4410-90010).

1 Remove a gasket slide from its packaging and, with tweezers, remove the protective film.

2 Insert the gasket slide into the chamber base with the barcode label resting over the base's barcode guide.

3 Slowly dispense 100 µL of sample mixture across the surface of each gasket well. Do not let the pipette tip touch the gasket or the glass.

4 Place a microarray slide on top of the gasket slide with the numeric barcode facing up and the Agilent label facing down. Do not move the hybridization chamber once the microarray slide has been placed.

5 Place the chamber cover onto the chamber base.

6 From the rounded corner of the chamber base, slip the clamp onto the chamber base and cover until it stops in the center. Keep the chamber assembly flat on the lab bench at all times.

7 Firmly tighten the thumbscrew on the clamp.

8 Rotate the final assembled chamber in a *vertical orientation*, clockwise, 2 to 3 times. If necessary, rotate and/or tap the assembled chamber to dislodge any small or stationary bubbles.

Hybridization

1 Load the assembled chamber into the oven rotator rack, starting from the center of the rack (i.e., position 3 or 4).

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

3 Hybridize at 67°C for 24 hours.

During hybridization, perform **"Prewarming of Agilent Oligo aCGH/ChIP-on-Chip Wash Buffer 2 (overnight)"**.

Prewarming of Agilent Oligo aCGH/ChIP-on-Chip Wash Buffer 2 (overnight)

Prewarm the Agilent Oligo aCGH/ChIP-on-Chip Wash Buffer 2 while the 24-hour hybridization is in-progress.

1 Add the required volume of Agilent Oligo aCGH/ChIP-on-Chip Wash Buffer 2 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 5. Microarray Washing

NOTE The microarray wash instructions provided here use only Agilent Oligo aCGH/ChIP-on-Chip Wash Buffers 1 and 2 to wash the slides. If ozone levels in your laboratory exceed 10 ppb, then refer to the full-length protocol (publication G4410-90010) for instructions on Wash Procedure B.

Table 6 summarizes the wash procedure.

Table 6 Wash conditions

	Dish	Wash buffer	Temperature	Time
Disassembly	#1	Agilent Oligo Wash Buffer 1	Room temperature	
1st wash	#2	Agilent Oligo Wash Buffer 1	Room temperature	5 minutes, with stirring
2nd wash	#3	Agilent Oligo Wash Buffer 2	37°C	1 minute, with stirring

Refer to the full-length protocol (publication G4410-90010) for instructions on cleaning slide-staining dishes, slide racks, and stir bars.

1 Prepare the three slide-staining dishes using the preparation instructions provided in **Table 7**.

Table 7 Preparation of slide-staining dishes for washing

Dish	Preparation
#1	1 Fill completely with Agilent Oligo Wash Buffer 1 at room temperature.
#2	1 Add a slide rack and a magnetic stir bar. 2 Fill with enough Agilent Oligo Wash Buffer 1 at room temperature to cover the slide rack. 3 Put dish on a magnetic stir plate.
#3	1 Put the prewarmed 1.5 L glass dish on a magnetic stir plate with heating element. 2 Put slide-staining dish #3 into the 1.5 L glass dish. 3 Fill the 1.5 L glass dish with pre-warmed Milli-Q ultrapure water. 4 Fill the slide-staining dish approximately three-fourths full with Agilent Oligo Wash Buffer 2 (warmed to 37°C). 5 Add a magnetic stir bar. 6 Turn on the heating element and maintain temperature of Wash Buffer 2 at 37°C. Monitor with a thermometer.

2 Remove one hybridization chamber from the oven and resume rotation of the others.

3 With the hybridization chamber assembly on a flat surface, remove the clamp assembly and the chamber cover.

4 Remove the microarray-gasket sandwich from the chamber base, keeping the microarray slide numeric barcode facing up. Without letting go of the slides, submerge the microarray-gasket sandwich into slide-staining dish #1 containing Wash Buffer 1.

5 Use forceps to pry the slides apart on the barcode end, gently twisting the forceps until the slides separate. Then, quickly transfer the microarray slide to the slide rack in slide-staining dish #2.

6 Repeat **step 2** through **step 5** for up to four additional slides in the group.

7 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.

8 At the end of the 5-minute wash, transfer the entire slide rack to slide-staining dish #3, which contains Agilent Oligo aCGH/ChIP-on-Chip Wash Buffer 2 at 37°C.

9 Activate the magnetic stirrer. Adjust the setting to get thorough mixing without disturbing the microarray slides.

10 Wash microarray slides in slide-staining dish #3 for at least 1 minute and no more than 2 minutes.

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

12 Repeat **step 1** through **step 11** for the next group of five slides using fresh Wash Buffer 1 and fresh Wash Buffer 2 warmed to 37°C.

13 Scan slides immediately. If necessary, store slides in orange slide boxes in a N_2 purge box, in the dark.

Step 6. Microarray Scanning and Analysis

1 Put the slides into the appropriate slide holders for your microarray scanner system and proceed with scanning.

2 Analyze the scanned images using the Agilent Feature Extraction software.

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

Array/Sample tracking on microarrays

Use the form below to make notes to track your samples.

Arrays

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

Barcode Number _____

In This Book

This quick reference guide contains instructions for enzymatic labeling of gDNA from blood, cells, or frozen tissues and hybridization of that labeled gDNA to Agilent SurePrint CGH and CGH+SNP microarrays in a 4-pack (i.e., 4 microarrays/slide) format.

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