**UW Medicine - Pathology**

400-11-01-18

**Microarray Slide Washing**

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| Adopted Date: Nov. 12, 2011Review Date: Nov. 21, 2011Revision Date: Nov. 2011, Jan. 2012Under Revision: November, 2012 |

PURPOSE

To decrease the background noise by wash out the un-hybridization probe in order to get a high Signal/Noise ratio.

PROCEDURE

### Material and Equipment

1. Incubator set at 37C
2. slide holder
3. ozone-barrier slide cover
4. slide container
5. Magnetic stir bar
6. Magnetic stir plate

### Reagents

1. Agilent Oligo aCGH Wash Buffer 1 (Agilent Cat# 5188-5221)
2. Agilent Oligo aCGH Wash Buffer 2 (Agilent Cat#5188-5222)
3. Mili-Q water
4. Distil water

### Procedures

1. **Washing Preparation**
2. Checking working environment

***Note:*** The microarray wash procedure must be done in environments where ozone levels are 5 ppb or less. If ozone levels are between 5 and 10 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 listed in Agilent Technical Note 5989-0875EN.

Before you begin, determine which wash procedure to use:

**Table 12.** Wash procedure to follow

|  |  |  |
| --- | --- | --- |
| **Ozone level in****your lab** | **Wash Procedure** | **Ozone-Barrier****Slide Cover** |
| < 5 ppb | ppb “Wash Procedure A (without Stabilization and Drying Solution)” on page 76 | No |
| > 5 ppb < 10 | ppb “Wash Procedure A (without Stabilization and Drying Solution)” on page 76 | Yes |
| > 10 ppb | “Wash Procedure B (with Stabilization and Drying Solution)” on page 78 | Yes |

***Note:***

1. 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.
2. Always use clean equipment when conducting the wash procedures.
3. Use only dishes that are designated and dedicated for use in Agilent oligo aCGH experiments.
4. Clearing with Mili-Q Water Wash
5. Rinse slide-staining dishes, slide racks and stir bars thoroughly with high-quality Milli-Q ultrapure water before use and in between washing groups.
6. Run copious amounts of Milli-Q ultrapure water through the slide-staining dishes, slide racks and stir bars.
7. Empty out the water collected in the dishes at least five times. Repeat step 1 and step 2 until all traces of contaminating material are removed.
8. Prewarm Agilent aCGH/ChIP-on-Chip Wash Buffer II (Overnight) s
9. The temperature of Agilent Oligo aCGH/ChIP-on-Chip Wash Buffer 2 must be at 37°C for optimal performance.
10. Add the volume of buffer required to a disposable plastic bottle and warm overnight in an incubator or circulating water bath set to 37°C.
11. Put a slide-staining dish into a 1.5 L glass dish three-fourths filled with Milli-Q ultrapure water and warm to 37°C by storing overnight in an incubator set to 37°C.
12. **Microarray Washing Procedure**

***Note:***

1. 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).
2. Following the Table of wash condition lists the wash conditions

**Table 13.** Wash conditions

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Step** | **Dish** | **Washing Buffer** | **Temperature** | **Time** |
| Disassembly |  #1 | Agilent Wash Buffer 1 | Room temperature | seconds |
| 1st wash | #2 | Agilent Wash Buffer 1 | Room temperature | 5 minutes |
| 2nd wash | #3 | Agilent 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. Put a slide rack into slide-staining dish #2. 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. Put this dish on a magnetic stir plate.
3. Put the prewarmed 200 ml glass dish filled with water and containing slide-staining dish #3 on a magnetic stir plate with heating element. Fill the slide-staining dish #3 approximately three-fourths full with Agilent Oligo aCGH/ChIP-on-Chip Wash Buffer 2 (warmed to 37°C). Add a magnetic stir bar. Turn on the heating element and maintain temperature of Agilent Oligo aCGH/ChIP-on-Chip Wash Buffer 2 at 37°C; monitor using 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.
6. Put the hybridization chamber assembly on a flat surface and loosen the thumbscrew, turning counter-clockwise.
7. Slide off the clamp assembly and remove the chamber cover.
8. 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.
9. 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.
10. With the sandwich completely submerged in Agilent Oligo aCGH/ChIP-on-Chip Wash Buffer 1, pry the sandwich open from the barcode end only. Do this by slipping one of the blunt ends of the forceps between the slides and then gently twist the forceps to separate the slides. 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.

***Note***: Touch only the barcode portion of the microarray slide or its edges!

1. Repeat step 4 through step 6 for up to additional slides in the group. A maximum of two disassembly procedures yielding two microarray slides is advised at one time in order to facilitate uniform washing.
2. When all slides in the group are put into the slide rack in slide-staining dish #2, stir using setting 4 for 5 minutes. Adjust the setting to get good but not vigorous mixing. Cover the container from light
3. Transfer slide rack to slide-staining dish #3 containing Agilent Oligo aCGH/ChIP-on-Chip Wash Buffer 2 at 37°C, and stir using setting 4 for 1 minute.
4. Slowly remove the slide rack trying to minimize droplets on the slides. It should take 5 to 10 seconds to remove the slide rack.
5. Discard used Agilent Oligo aCGH/ChIP-on-Chip Wash Buffer 1 and Agilent Oligo aCGH/ChIP-on-Chip Wash Buffer 2.
6. 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 pre-warmed to 37°C.
7. **Assemble Slide into a Slide holder for Scan**
8. Carefully place the end of the slide without the barcode label onto the slide ledge.
9. Gently lower the microarray slide into the slide holder. Make sure that the active microarray surface faces up (Agilent), toward the slide cover.
10. Close the plastic slide cover, pushing on the tab end until you hear it click. For more detailed instruction, refer to the *Agilent G4900DA SureScan Microarray Scanner System User Guide*.
11. Scan slides immediately to minimize impact of environmental oxidants on signal intensities. If necessary, store slides in the original slide boxes in a N2 purge box, in the dark.

**Figure 2** Slide in slide holder for SureScan microarray scanner

* 1. **Reference**

Agilent Arrays-based CGH for genomic DNA analysis protocol version 7.1 p. 70-76.

Written By: Director Approval:

(Signature and Date) (Signature and Date)

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**UW Medicine - Pathology**

**Cytogenetics and Genomics**

**SIGNATURE PAGE FOR POLICIES AND PROCEDURES**

Procedure / Policy Title: **Microarray Slide Washing**

Procedure / Policy Number: 400-11-01-18

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