**UW Medicine - Pathology**

400-11-01-06

Hybrid and Label Procedure

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| Adopted Date: 10/12/09Review Date: 10/19/09Revision Date: 02/2010 |

PURPOSE

To co-hybridize labeled gDNA of the test sample and the normal control reference sample onto the microarray chip slide and wash before scanning the slides for the detection of the gains and losses in the test sample genome.

PROCEDURE

### Equipments and Materials:

1. Roche NimbleGen Hybridization System 4
2. Accessory kit of NimbleGen Hybridization Systems:
	1. Precision Mixer Alignment Tool (PMAT)
	2. Mixer Disassembly Tool
	3. Mixer Brayer
	4. System Verification Assemblies
	5. Replacement O-rings
	6. Forceps
3. Microarray Dryer (Array-it)
4. Heat block capable of temperatures to 98°C
5. Heat block of 42°C
6. Microcentrifuge
7. Gilson Microman M100 Pipette
8. Gilson Microman CP100 pipette tips
9. NimbleGen HX3 Mixer for 3x720K arrays
10. Compressed Gas Nozzle
11. Desiccator
12. NimbleGen Array Processing Accessories kit:
	1. Slide Rack
	2. Wash Tanks
	3. Slide Containers

### Reagents

NimbleGen Hybridization Kit:

* 2X Hybridization Buffer
* Hybridization Component A
* Alignment Oligo

NimbleGen Sample Tracking Control: 12 Sample tracking Controls

NimbleGen Wash Buffer Kit:

* 10X Wash Buffer I
* 10X Wash Buffer II
* 10X Wash Buffer III
* 1M DTT
* VWR or Mol Bio grade (5’prime #250020) reagent grade water (VWR, RC915025)

### Procedure

***Note:*** The **Alignment Oligo** and **Sample Tracking Controls (STCs)** provided in the NimbleGen Hybridization and Sample Tracking Control Kits, respectively, are labeled with Cy dyes, which are sensitive to photo bleaching and freeze-thawing. After thawing stock tubes for the first time, aliquot the Alignment Oligo and STCs into single-use volumes and freeze at -20°C. Protect tubes from light.

**Step 1. Prepare Samples**

* 1. Set the Hybridization System to 42°C. With the cover closed, allow at least 2 hours for the temperature to stabilize.

***Note:*** Be aware that the temperature of the Hybridization System may fluctuate during stabilization.

* 1. Re-suspend the dried sample pellet by adding 5.6μl Sample Tracking Control to Cy-labeled Sample from step 23 in DNA Labeling protocol. Each sample to be hybridized to 3x135K array should be resuspended in a **unique STC**. **Record which STC is used for each test sample on aCGH Hybridization Worksheet.**
	2. Mix well and place tubes at 42°C heat block for about 5 minutes for complete sample resuspension. Vortex well and spin to collect contents in bottom of the tube.
	3. Using components from a NimbleGen Hybridization Kit, prepare the hybridization solution master mix. The amount listed below is sufficient to hybridize all 3 subarrays on a slide. To hybridize multiple slides, adjust the amounts accordingly.

**Hyb Solution Master mix 3x135K**

2X Hybridization Buffer(vial 1)35μl

Hybridization Component A (vial 2) 14μl

Alignment Oligo (vial 3) 1.4μl

**Total 50.4μl**

* 1. Add the appropriate amount of hybridization solution to each sample pair according to the following table:

Re-suspended sample from step 2 5.6μl

Hybridization solution from step 4 14.4μl

**Total 20 μl**

* 1. Vortex well (approximately 15 seconds) and spin to collect contents in bottom of the tube. Incubate at 95°C for 5 minutes, protected from light.
	2. Place tubes at 42°C (in the Hybridization System sample block or heat block) for at least 5 minutes or until ready for sample loading. Vortex and spin prior to loading.

**Step 2. Prepare Mixers**

1. Remove HX3 mixer from its package for Nimblegen 3x135K array.

***Note:*** For best results, use a compressed gas nozzle to gently blow compressed nitrogen or argon gas across the mixer and slide to remove any dust or debris. Load samples within 30 minutes of opening the vacuum-packaged mixer to prevent the formation of bubbles during loading and/or hybridization.

1. Position the Precision Mixer Alignment Tool (PMAT) with its hinge on the left. Open the PMAT (Figure 1).
2. While pushing back the plastic spring with a thumb, place the slide in the base of the PMAT so that the barcode is on the right and the corner of the slide sits against the plastic spring. The NimbleGen logo and barcode number should be readable. Remove your thumb and make sure the spring is engaging the corner of the slide and the entire slide is registered to the edge of the PMAT to the rightmost and closest to you. In addition, be sure that the slide is lying flat against the PMAT. Gently blow compressed nitrogen or argon gas across the mixer and slide to remove dust.
3. Place the PMAT so that the mixer side is on the bottom. Snap the mixer onto the two alignment pins on the lid of the PMAT with the tab end of the mixer toward the inside hinge and the mixer’s adhesive gasket facing outward (Figure 1). Make sure to align the slide correctly in the PMAT. Incorrectly aligned slides may result in inaccurate attachment of the mixer and may affect the array features, or may not fit well into the Mixer Disassembly Tool used to remove the mixers after hybridization*.*
4. Using forceps, remove the backing from the adhesive gasket of the mixer and close the lid of the PMAT so that the gasket makes contact with the slide.
5. Lift the lid by grasping the long edges of the PMAT while simultaneously applying pressure with a finger through the window in the lid of the PMAT to free the mixer-slide assembly from the alignment pins.



**Figure 1: PMAT with X1 Mixer and Slide.**

1. Remove the mixer-slide assembly from the PMAT.
2. Place the mixer-slide assembly on the back of a 42°C heating block to facilitate adhesion of the mixer to the slide.
3. Rub the Mixer Brayer over the mixer with moderate pressure to adhere the adhesive gasket and remove any bubbles. For HX3 mixer, first use a corner of the Mixer Brayer to rub the borders between the subarrays and then rub around the outside of the subarrays. The adhesive gasket will become clear when fully adhered to both surfaces.
4. Place the mixer-slide assembly in the slide bay of the Hybridization System.

**Step 3. Load & Hybridize Samples**

* + - 1. Refer to the appropriate diagram below when loading sample:

 

**Figure 2: HX3 Mixer and Slide for a 3x135K Array Vent Ports**

* + - 1. Keep the following in mind before loading sample:
				* Leave residual volume in the sample tube to avoid bubbles. The volumes listed in the table below account for this additional amount.
				* After aspirating the designated sample volume, inspect the pipette tip for air bubbles. Dispense and reload the pipette if bubbles exist.

Keep the following in mind when loading sample:

* + - * + Keep the pipette tip perpendicular to the slide to avoid possible leakage at the fill port (Figure 2).
				+ Apply gentle pressure of the tip into the port to ensure a tight seal while loading the sample.

 **Component**

 Sample Loading Volume 18μl

 Pipette Tip CP100

* + - 1. Using the appropriate Gilson Microman pipette, slowly dispense the appropriate sample volume into the fill port. Load samples and seal mixer ports as described below for each array format:

Load sample into the fill port. Dry any overflow from the fill and vent ports with kimwipe after loading the array. For 3x135K arrays, it is not unusual for small bubbles to form in the corners of the mixer-slide assembly during loading. These bubbles will dissipate upon mixing and will not compromise the data.

Use one mixer port seal to cover both the fill and vent ports on HX3 mixers, filling and sealing one chamber at a time. Press the mixer port seal, using uniform pressure across the seal to adhere.

Use forceps to press the mixer port seal around the fill and vent ports to ensure it is adhered in those areas.

* + - 1. Close the bay clamp.
			2. Turn on the Mixing Panel on the Hybridization System, set the mix mode to B, and press the mix button to start mixing. Confirm that the Hybridization System recognizes the slide in each occupied bay (its indicator light becomes green).
			3. Approximately 10 minutes after starting the Hybridization System:
				* Ensure the mix mode is set to B.
				* Ensure a green light is displayed for all occupied stations.
			4. Hybridize sample at 42°C to the 3x135K array(s) for 40 - 72 hours (usually 48 hours) incubation time.

***Note:*** Roche NimbleGen has found that longer hybridization times result in higher signal-to-noise ratios, which may be beneficial for some experiments.

**Step 4. Wash Hybridized Arrays**

***Important:*** To ensure high quality data, it is important to proceed through all the washing and drying steps without interruption. If using a microarray dryer that dries one slide at a time, wash only one slide at a time.

* 1. Locate the components of the NimbleGen Wash Buffer Kit and NimbleGen Array Processing Accessories .

***Important:*** Prior to the first use of the Wash Buffer Kit, prepare 1M DTT solutions by adding 1.2ml of water (vial 5) to each tube of dry DTT (vial 4). After reconstitution, store the 1M DTT solutions at -15ºC to -25ºC.

* 1. Before removing the mixer-slide assemblies from the Hybridization System, prepare Washes I, II, and III according to the following tables. Note that you prepare two containers of Wash I.

**Washing Multiple Slides Wash I Washes I, II, and III**

VWR Water 243ml 243ml

10X Wash Buffer I, II, or III 27ml 27ml

1M DTT solution from step 1 27μl 27μl

**Total 270ml 270ml**

**Washing One Slide Wash I Washes I, II, and III**

VWR Water 243ml 24.3ml

10X Wash Buffer I, II, or III 27ml 2.7ml

1M DTT solution from step 1 27μl 2.7μl

**Total 270ml 27ml**

* + - * + Ensure that this dish is shallow and wide enough to accommodate the mixer-slide assembly loaded in the Mixer Disassembly Tool. This dish must also be small enough to ensure that the Mixer Disassembly Tool is completely submerged in the wash solution.
				+ If washing multiple slides, prepare the washes in the wash tanks. If washing only one slide, prepare the washes in the slide containers.
	1. To facilitate the removal of the mixer, heat the shallow dish containing Wash I to 42°C. Roche NimbleGen recommends measuring the temperature of Wash I at every use. Keep the remaining three wash solutions at room temperature.
	2. Insert the Mixer Disassembly Tool into the shallow dish containing warm Wash I. If you will be washing multiple slides, insert a slide rack into the wash tank containing Wash I at room temperature.
	3. Remove a mixer-slide assembly from the Hybridization System and load it into the Mixer Disassembly Tool immersed in the shallow dish containing warm Wash I (Figure 3).

***Important:*** Do not allow the mixer-slide assembly to cool before removing the mixer. Keep power on to the Hybridization System’s heat block and mixer system during mixer-slide disassembly, and transfer each mixer-slide assembly one at a time to Wash I for immediate removal of the mixer.

* 1. With the mixer-slide assembly submerged, carefully peel the mixer off the slide. It is important to hold the Mixer Disassembly Tool flat while removing the mixer and to avoid any horizontal movement or scraping with the mixer across the slide. Do not touch the array surface of the slide.

***Important****:* The mixer is extremely flexible. Peel the mixer off slowly to avoid breaking the slide.

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**Figure 3: Using the Mixer Disassembly Tool to Remove a Slide from a Mixer**

* 1. Working quickly, discard the mixer and remove the slide from the Mixer Disassembly Tool.
	2. Gently agitate the slide for 10 - 15 seconds in the shallow dish containing warm Wash I to quickly remove the hybridization buffer.

***Note:* It is important for achieving good array uniformity to quickly and evenly wash the hybridization buffer off the slide surface as soon as the mixer is removed**.

* 1. If washing multiple slides, transfer the slide with the barcode at the top into a slide rack in the wash tank that contains Wash I. If washing one slide, transfer the slide into a slide container that contains Wash I. Agitate vigorously for 10 - 15 seconds. Slide rack users: To ensure high quality data, make sure the microarray area of the slide remains wet at all times during all wash steps.
	2. Wash for an additional 2 minutes in room temperature Wash I with vigorous, constant agitation. If washing multiple slides, move the rack up and down with enough agitation to make foam appear. If washing one slide, shake the slide container at least 20 times every 10 seconds.

***Important:*** At several times during the wash, rock the wash tank so the wash solution covers and cleans the top of the slide(s).

* 1. Quickly blot the rack, or edges of the slide if only washing one slide, several times on the edge of the slide container. Transfer the slide(s) to Wash II and wash for 1 minute with vigorous, constant agitation. If washing multiple slides, rock the wash tank so the wash solution covers and cleans the tops of the slide(s).

 ***Important:* Do not allow slides to dry between wash steps.**

* 1. Transfer the slide(s) to Wash III and wash for 15 seconds with vigorous, constant agitation. If washing multiple slides using the slide rack, rock the wash tank so the wash solution covers and cleans the tops of the slide(s).
	2. Remove the slide from Wash III and place the slide array side up in a Microarray High Speed Centrifuge (Arrayit). Spin dry for 1 minute.
	3. Remove the slide from the Arrayit. Use a compressed gas nozzle to gently blow compressed nitrogen or argon gas across the mixer and slide to remove any dust or debris.

***Note:*** When not in use, store the dried slide in its original slide case in a dark desiccator.

Proceed immediately to the steps for scanning the arrays, protected from light and ozone

REFERENCES

1. NimbleGen Arrays User’s Guild: CGH Analysis v5.1 p. 21-33.

Written By: Director Approval:

(Signature and Date) (Signature and Date)

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**UW Medicine - Pathology**

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