**UW Medicine - Pathology**

400-11-01-04

DNA Labeling with Random Priming for Array CGH Procedure

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| Adopted Date: 10/12/09Review Date: 02/14/10Revision Date: 07/2010 |

PURPOSE

To label the gDNA samples and the normal control reference DNA in different fluorochromes using a NimbleGen Dual-Color DNA Labeling Kit through random priming with Cy primers.

PROCEDURE

### Specimen Requirements

1. 500 ng patient gDNA
2. 500 ng normal control gDNA (gender matched)

### Equipments and Materials

1. Spectrophotometer NanoDrop ND-1000
2. Heat block capable of temperatures to 98°C
3. MJ Research Thermal Cycler
4. Microcentrifuge (12,000 x g capability)
5. SpeedVac Thermo Savant
6. Vortex mixer
7. Pipetmen p10, p20, p200, p1000
8. Aerosol-resistant sterile pipette tips
9. Sterile microcentrifuge tubes, 1.6 ml, 0.6 ml, 0.2 ml
10. Ice buckets
	1. **Reagents**

NimbleGen Dual-Color DNA Labeling Kit for 24 Cy3 and Cy5 labeling reactions (full reaction) or 48 Cy3 and Cy5 labeling reactions (half reaction).

1. Nuclease-free Water (2 x vial 1)
2. Random Primer Buffer (vial 2)
3. Cy3-Random Nonamers (vial 3)
4. Cy5-Random Nonamers (vial 4)
5. Klenow Fragment (3'->5' exo-) 50U/μL (vial 5)
6. 10mM dNTP Mix (vial 6)
7. Stop Solution (0.5M EDTA) (vial 7) plus 5M NaCl

Additional Reagnets:

1. β-Mercaptoethanol (Sigma Aldrich 25ml M3148)
2. Absolute Ethanol (Sigma Aldrich 500ml E702-3)
3. Isopropanol (Sigma Aldrich 500ml I-9516)

### Safety Precautions:

* 1. **Wear gloves** and take precautions to avoid sample contamination.
	2. Cy dyes are **light sensitive**. Be sure to minimize light exposure of the dyes during use and store in the dark immediately after use.
	3. Cy dyes are **ozone sensitive**. Take the necessary precautions to keep atmospheric ozone levels below 20ppb (parts per billion).
	4. Cy dyes are **humidity sensitive**. Take the necessary precautions to keep humidity levels below 40%.

### Procedures

To use a NimbleGen Dual-Color DNA Labeling Kit. Be aware of the following when using these kit contents:

* Aliquot dNTPs and Cy primers into single-use amounts.
* 5M NaCl could precipitate. Vortex or heat if necessary.

***Note***:

* 1. Purified, unamplified, and unfragmented genomic DNA (gDNA) is required for optimal sample labeling and hybridization.
	2. Pairs of samples intended for hybridization to the same array should be labeled in parallel using Cy3-Random and Cy5-Random Nonamers from the same kit (or multiple kits from the same lot).
	3. Label test samples with Cy3 and normal control (reference) samples with Cy5.
1. Prepare the following solution in a 1.5ml microcentrifuge tube in the fume hood. Prepare fresh buffer each time when primers are re-suspended.

Random Primer Buffer (vial 2) 1.1ml

β-Mercaptoethanol\* 2μl

**Total 1.102ml**

 \* Do not use bottles of β-Mercaptoethanol that have been opened for more than 6 months.

1. Briefly centrifuge Cy3-Random and Cy5-Random Nonamers (vials 3 and 4, respectively) because some of the product could have dislodged during shipping. Dilute the primers in 1050μl each of Random Primer Buffer with β-Mercaptoethanol. Add the remainder of the buffer into each random primer tube (about 50μl to each tube). Aliquot to 40μl in 0.2ml thin-walled PCR tubes and store at -20°C, protected from light.

***Note:*** Do not store diluted primers longer than 4 months*.*

1. Assemble the test and reference DNA samples in separate 0.2ml thin-walled PCR tubes. Use Cy3 primers for patient test DNA sample and Cy5 primers for normal control reference DNA sample.

**Component Test Sample Reference Sample**

 **Full Half Full Half**

gDNA Sample 1μg 500ng 1μg 500ng

Diluted Cy3-Random Nonamers from step 2 40μl 20μl -- --

Diluted Cy5-Random Nonamers from step 2 -- -- 40μl 20μl

Nuclease-free water (vial 1) To volume (80μl) (40μl) (80μl) (40μl)

**Total 80μl 40μl 80μl 40μl**

***Important:*** double check the label for patient sample on the tube (use waterproof marker). **Record the amount of gDNA used for each test sample on the aCGH Labeling Worksheet.**

1. Heat-denature samples in a thermocycler at 98°C for 10 minutes. Hot start the incubation. Set up a separate timer for 10 min with an alarm. Prepare an ice-water bath. Quick chill in an ice-water bath for 2-5 minutes. ***Important:*** Quick-chilling after denaturation is critical for high-efficiency labeling.
2. Prepare the following dNTP/Klenow master mix for each sample prepared in step 4 during the 98°C incubation. ***Important:*** Keep all reagents and dNTP/Klenow master mix on ice at all times. Do not vortex after addition of Klenow.

**dNTP/Klenow Master Mix: Recipe per Sample All Array Formats**

 **Full Half**

10mM dNTP Mix (vial 6) 10μl 5μl

Nuclease-free Water (vial 1) 8μl 4μl

Klenow Fragment (3'->5' exo-) 50U/μl (vial 5) 2μl 1μl

**Total 20μl 10μl**

1. Add 20μl (full rxn) or 10μl (half rxn) of the dNTP/Klenow master mix prepared in step 5 to each of the denatured samples prepared in step 4. Keep on ice.

**Components All Array Formats**

 **Full Half**

Reaction volume from step 4 80μl 40μl

dNTP/Klenow Master Mix from step 5 20μl 10μl

**Total 100μl 50μl**

1. Mix well by pipetting up and down 10 times or flicking the tube until well mixed.

***Important****:* Do not vortex after addition of Klenow*.*

1. Quick-spin to collect contents in bottom of the tube.
2. Incubate for 2 hours at 37°C in a thermocycler with heated lid, protected from light.
3. During 2 hr incubation, label a set of 1.6 ml tubes with patient/case ID for adding isopropanol in step13.
4. Stop the reaction by addition of the Stop Solution (0.5M EDTA).

**Component All Array Formats**

 **Full Half**

Reaction volume from step 6 100μl 50μl

Stop Solution (0.5M EDTA) (vial 7) 10μl 5μl

**Total 110μl 55μl**

1. Add 5M NaCl (vial 8) to each tube. Make sure NaCl is mixed well and not precipitated.

**Component All Array Formats**

  **Full Half**

Reaction volume from step 10 110μl 55μl

5M NaCl (vial 8) 11.5μl 5.75μl

**Total 121.5μl 60.75μl**

1. Vortex briefly, spin, and transfer the entire contents to a 1.5ml microcentrifuge tube containing isopropanol.

**Component All Array Formats**

  **Full Half**

Reaction volume from step 11 121.5μl 60.75μl

Isopropanol 110μl 55μl

**Total 231.5μl 115.75μl**

***Note:*** Up to 4 reactions containing the same sample can be combined in a 1.5ml tube and precipitated together. If combined, be sure to scale the isopropanol volume appropriately.

1. Vortex well. Incubate for 10 minutes at room temperature, protected from light.
2. Centrifuge at 12,000 x g for 10 minutes. Remove supernatant with a pipette. Pellet should be pink (Cy3) or blue (Cy5) depending on the dye.
3. Rinse pellet with 500μl 80% ice-cold ethanol. Dislodge pellet from tube wall by flicking the tube a few times.
4. Centrifuge at 12,000 x g for 2 minutes. Remove supernatant with a pipette.
5. Dry contents in a SpeedVac on medium heat for 5 minutes, protected from light. **STOP POINT**: Proceed to step 19, or store labeled samples at -20°C (up to 1 month), protected from light.
6. Spin tubes briefly prior to opening. Rehydrate each pellet in 25μl Nuclease-free Water (vial 1) per reaction.
7. Vortex for 30 seconds and quick-spin to collect contents in bottom of the tube. Continue to vortex or let sit at room temperature, protected from light, for approximately 5 minutes or until the pellet is completely rehydrated, then vortex again and quick-spin.
8. Quantitate each sample using the NanoDrop Nucleic Acid module. Print out Nanodrop report.
9. Based on the concentration, calculate the volume of the test sample and reference sample required for each hybridization per the following table and combine both test and reference samples in a 1.5ml tube:

**Sample Requirements 3x135K Array**

Test sample 31μg

Reference sample 31μg

1. Dry contents in a SpeedVac on medium heat for 45 minutes, protected from light.
2. **STOP POINT:** Proceed to Chapter 4, or store labeled samples at -20°C (up to 1 month), protected from light.

REFERENCES

* 1. NimbleGen Arrays User’s Guild: CGH Analysis v5.1 p. 15-19.

Written By: Director Approval:

(Signature and Date) (Signature and Date)

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 Cytogenetic Supervisor

**UW Medicine - Pathology**

 **Cytogenetics - UWMC**

**SIGNATURE PAGE FOR POLICIES AND PROCEDURES**

Procedure / Policy Title: DNA Labeling with Random Priming for Array CGH Procedure

Procedure / Policy Number: 400-11-01-04

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