**UW Medicine - Pathology**

400-11-01-12

**DNA Isolation from Formalin-Fixed Paraffin-Embedded (FFPE) Tissue Using Agilent’s Procedure to Isolate Genomic DNA (gDNA) from FFPE**

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| Adopted Date: Oct. 12, 2011Review Date: Oct. 21, 2012Revision Date: Oct. 21, 2012Under Revision: |

PURPOSE

To isolate high molecular weight genomic DNA from formalin-fixed paraffin-embedded (FFPE) tissue specimen for microarray analysis.

###

PROCEDURE

### Specimen Requirements

Please see Specimen Requirement.

### Material and Equipment

* + 1. Thermocycler
		2. Shaking thermomixer 37 ˚C and 55 ˚C
		3. Microcentrifuge
		4. 50˚C water bath
		5. 70˚C and 90˚C heat block
		6. Sterile polypropylene 2 ml and 1.5 ml eppendorf tubes
		7. Micropipettors (Rainin P10-P1000)
		8. Sterile pipette tips with barrier filters
		9. Ice bucket or Stargene benchtop coolers
		10. Spectrophotometer Nanodrop 2000c

### Reagents and Solutions

1. Puregene Blood Core C (Cat#158389)
2. PBS
3. 10% Tween 20
4. 1M NaSCN
5. Proteinase K
6. RNAse A (Qiagen Cat# 158924)
7. DNAse-, RNAse-, and Protease-free H20 (5 Prime Cat#2900132)
8. Qiagen DNeasy Blood & Tissue Kit (Cat# 69504)
9. 70% and 100% ethanol

### Procedures

This procedure is optimized for 5 sections of 20 micron FFPE section containing 1 cm2 of tissue for Agilent 1x1M, 2x400K, or 4x180k feature array

Paraffin Removal:

i. Equilibrate a heat block or water bath to 90 ˚C and a thermomixer to 37 ˚C.

ii. Place 5 of 20-micron FFPE sections into the 2 ml nuclease-free microcentrifuge tube with screw cap.

iii. Add 480 µl PBS and 20 µl of 10 % Tween 20 to the FFPE sections in the tube

iv. Put the sample tube to a circulating water bath or heat block at 90 ˚C for 10 minutes.

v. Spin immediately for 15 minutes at 13,000 rpm in a microcentrifuge.

vi. Place the sample tube on ice for 5 minutes.

vii. Remove wax disc with a pipette tip. Remove and discard the supernatant without disturbing the pellet.

viii. Add 1 ml of 100 % ethanol to the pellet and vortex briefly.

ix. Spin for 5 minutes at 13,000 rpm in a microcentrifuge.

x. Remove ethanol without disturbing the pellet and dry the pellet in the SeepVac on medium heat for 3~10 minutes.

xi. Add 400 µl 1M NaSCN to the dry pellet and briefly mix on a vortex mixer.

xii. Transfer the sample tube to a thermomixer at 37 ˚C and incubate overnight at 450 rpm.

**b**. Proteinase K Treatment:

i. Equilibrate a thermomixer to 55 ˚C.

ii. Transfer the sample tube to a microcentrifuge and spin at 13,000 rpm for 20 minutes.

iii. Remove and discard the supernatant without disturbing the pellet.

iv. Add 400 µl PBS to the pellet and vortex briefly.

v. Transfer the sample tube to a microcentrifuge and spin at 13,000 rpm for 20 minutes.

vi. Remove and discard the supernatant without disturbing the pellet.

vii. Add 360 µl of Qiagen buffer ATL.

viii. Add 40 µl of proteinase K, mix well on a vortex, and incubate in a thermomixer at 55 ˚C overnight at 450 rpm.

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

x. Add 40 µl of proteinase K, mix well on a vortex, and incubate in a thermomixer at 55 ˚C for approximately 6 to 8 hours at 450 rpm.

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

xii. Add 40 µl of proteinase K, mix well on a vortex, and incubate in a thermomixer at 55 ˚C overnight at 450 rpm.

**c**. gDNA Extraction:

i. Equilibrate a heat block or water bath to 70 ˚C.

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

iii. Add 8 µl of RNAse A (100 mg/ml), mix by flicking the tube and incubate at room temperature for 2 minutes. Transfer the sample tube to a microcentrifuge and spin for 30 seconds at 6,000 x g to drive the contents off the walls and lid.

iv. Add 400 µl Buffer AL, mix thoroughly on a vortex and incubate on a heat block at 70 ˚C for 10 minutes. Transfer the sample tube to a microcentrifuge and spin for 30 seconds at 6,000 x g to drive the contents off the walls and lid.

v. Add 440 µl 100 % ethanol and mix thoroughly on a vortex. Transfer the sample tube to a microcentrifuge and spin for 30 seconds at 6,000 x g to drive the contents off the walls and lid.

vi. Place two DNeasy Mini spin columns in two clean 2 ml collection tubes. Split the entire sample mixture onto two DNeasy Mini spin columns (approximately 660 µl each).

vii. Spin in a microcentrifuge for 2 minute at 10,000 rpm. Discard the flow-through and collection tube. Place the DNeasy Mini spin columns in fresh 2 ml collection tubes.

viii. Add 500 µl Buffer AW1 onto each spin column, and centrifuge for 1 minute at 10,000 rpm. Discard the flow-through and collection tube. Place the DNeasy Mini spin columns in fresh 2 ml collection tubes.

ix. Add 500 µl 80% ethanol onto each column, and spin in a microcentrifuge for 6 minutes at 13,000 rpm to dry the column membrane. Discard the flow-through and collection tube.

x. Place the DNeasy Mini spin column in a clean 1.5 ml microcentrifuge tube, and add 50 µl of nuclease-free water directly to the center of each spin column.

xi. Let stand at 37˚C for 1 minute, and then spin in a microcentrifuge for 1 minute at 10,000 rpm to elute the DNA. Repeat once.

xii. Combine the purified DNA from the same sample in one microcentrifuge tube with a final total volume of 100 µl.

xiii. Measure gDNA concentration and purity using Nanodrop, and analyze on an agarose gel

**REFERENCE**

 Agilent ULS Labeling for Blood, Cells, Tissues or FFPE Chapter 2 DNA Isolation from FFPE Tissues

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: **DNA Isolation from Formalin-Fixed Paraffin-Embedded (FFPE) Tissue Using Agilent’s Procedure to Isolate Genomic DNA (gDNA) from FFPE**

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