**UW Medicine - Pathology**

400-11-01-14

**DNA Isolation from Peripheral Blood or Bone Marrow Specimen Using Puregene Kit**

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

PURPOSE

To isolate high molecular weight genomic DNA from peripheral blood or bone marrow specimen for array CGH+SNP analysis.

PROCEDURE

### SPECIMEN REQUIREMENTS

### Refer to section IV-I). 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

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 400 µl of peripheral blood. After aliquoting 400 µl of blood from the lavender top tube, process rest of the blood or bone marrow sample with RBC Lysis Solution in the 15 ml tube in order to keep the white cell pellet as a backup in -80C freezer.

1. Red Blood Cell Lysis
2. Add 400 µl whole blood (or bone marrow) to a 1.5 ml tube containing 1.2 ml RBC Lysis solution. Invert to mix and gently shaking on a shaker at room temperature for 20 minutes.

**Note**: If the amount of starting material differs, add 3 times volume of RBC Lysis solution to the starting material.

1. Centrifuge for 1 min at 13,000 rpm. Remove supernatant leaving behind visible white cell pellet and 10-20 µl of residual liquid.
2. Drag the tube across a tube rack 3 times or vortex the tube vigorously to resuspend the white blood cells in the residual liquid; this greatly facilitates cell lysis below.
3. Cell Lysis

Add 400 µl Cell Lysis Solution to the tube containing the resuspended cells and vortex the tube for 10 seconds to lyse the cells. Incubate overnight at room temperature or minimum 1-2 hours. (*If cell clumps or solution is viscous, incubate at 37 ˚C for 30 minutes. If the clump of cell or the solution is still viscous, then add another 200 µl of Cell Lysis Solution*). Samples are stable in Cell Lysis Solution for at least 18 months at room temperature.

 Note: If the amount of starting material differs, add same amount of Cell Lysis Solution as the starting material to the white cell pellet.

1. RNAse A Treatment
2. Add 2 µl RNAse A Solution (100 mg/ml) to the cell lysate.
3. Mix the sample by inverting the tube 25 times and incubate at 37°C for 5 min.
4. Incubate on ice for 10 minutes
5. Protein Precipitation
6. Add 120 µl Protein Precipitation Solution to the cell lysate.
7. Vortex vigorously at high speed for 10 sec to mix the Protein Precipitation Solution uniformly with the cell lysate.
8. Centrifuge at 13,000 rpm for 5 min. The precipitated proteins will form a tight, dark brown pellet. (See Protein Precipitation section of manufacturer’s Troubleshooting Guide if pellet is not visible or tight.)
9. DNA Precipitation
10. Transfer the supernatant containing the DNA (leaving behind the precipitated protein pellet) into a clean 1.5 ml tube containing 400 µl of 100% Isopropanol.
11. Mix the sample by inverting gently 60 times until the white threads of DNA form a visible clump.
12. Centrifuge at 13,000 rpm for 5 min DNA will be visible as a small white pellet.
13. Carefully pipette out the supernatant. Add 400 µl of 70% ethanol and invert the tube several times to wash the DNA pellet.
14. Centrifuge at 13,000 rpm for 2 min. Remove ethanol by pipetting. Pellet may be loose so pipette out slowly and watch pellet.
15. Quick spin the tube to collect residual ethanol on inside the tube
16. Air dry for 5-10 minutes
17. DNA Hydration, dilution and storage
18. Add 100 µl DNA Hydration solution. Allow DNA to rehydrate at room temperature for minimum 30 minutes.
19. Flick the tubes until a transparent blob is visible in the solution.
20. Measure the concentration and purity using Nanodrop.
21. Store DNA at 4 ˚C for up to a month or at -20 ˚C for years.
	1. **Reference**

Puregene Blood Core kit manual

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 Peripheral Blood or Bone Marrow Specimen Using Puregene Kit**

Procedure / Policy Number: 400-11-01-14

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