**UW Medicine - Pathology**

400-11-01-11

**DNA Isolation from Cell Culture or Amniotic Fluid Using Puregene Kit**

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

PURPOSE

To isolate high molecular weight genomic DNA from cell culture or direct amniotic fluid 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 40 -50 µl of cell pellet from two of T-25 flask of cell culture or direct amniotic fluid.

a. Cell Lysis

1. For cultured cells, collect the media in a 15 ml centrifuge tube. Rinse flask 2 times with versene at 37°C (3 ml each) and collect the rinses in the same centrifuge tube.
2. Add 1 ml of trypsin-EDTA at 37°C to the flask. When the cells are detached (check in microscope), transfer cells in trypsin to the same centrifuge tube.
3. Then feed the flasks with 10 ml media if back up cultures are needed
4. For direct amniotic fluid (AF), transfer 10 ml AF in 15 ml centrifuge tube and continue step v.
5. Spin tube at approximately 13,000 rpm for 10 minutes, discard the supernatant, resuspend the cells, then add 10 ml PBS to wash the cells and centrifuge at 2,000 x g for 10 minutes.
6. Remove supernatant leaving behind visible cell pellet and 50 - 100 µl of residual liquid.
7. Flick the tube to resuspend the cells in the residual supernatant; this greatly facilitates cell lysis below.
8. 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 solution is viscous, incubate at 37 ˚C for 30 minutes. If 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.

b. Proteinase K and RNAse Treatment:

i. Add 2 µl Proteinase K (20 mg/ml) to the cell lysate.

ii. Mix the sample well by pipetting up and down 15 times (avoid bubble)and incubate at 55°C for 1 hour.

iii. Add 2 µl RNAse A (100 mg/ml) to the solution.

iv. Mix the sample well by pipetting up and down 15 times (avoid bubble) and incubate at 37°C for 5 minutes.

1. Protein Precipitation
2. Add 120 µl Protein Precipitation Solution to the cell lysate.
3. Vortex vigorously at high speed for 10 sec to mix the Protein Precipitation Solution uniformly with the cell lysate.
4. 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.)
5. DNA Precipitation
6. 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.
7. Mix the sample by inverting gently 60 times until the white threads of DNA form a visible clump.
8. Centrifuge at 13,000 rpm for 5 min DNA will be visible as a small white pellet.
9. Carefully pipette out the supernatant. Add 400 µl of 70% ethanol and invert the tube several times to wash the DNA pellet.
10. Centrifuge at 13,000 rpm for 2 min. Remove ethanol by pipetting. Pellet may be loose so pipette out slowly and watch pellet.
11. Quick spin the tube to collect residual ethanol on inside the tube
12. Air dry for 5-10 minutes
13. DNA Hydration, dilution and storage
14. Add 100 µl DNA Hydration solution. Allow DNA to rehydrate at room temperature for minimum 30 minutes.
15. Flick the tubes until a transparent blob is visible in the solution.
16. Measure the concentration and purity using Nanodrop.
17. Store DNA at 4 ˚C for up to a month or at -20 ˚C for years.

REFERENCE

Puregene Blood Core kit manual

Written By: Director Approval:

(Signature and Date) (Signature and Date)

­­­­­­­­­­­­­­­­­\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

Technologist: Yuhua Liu and Yang Zhou Yajuan Liu PhD, FACMG

**UW Medicine - Pathology**

**Cytogenetics and Genomics**

**SIGNATURE PAGE FOR POLICIES AND PROCEDURES**

Procedure / Policy Title: **DNA Isolation from Cell Culture or Amniotic Fluid Using Puregene Kit**

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

|  |  |  |
| --- | --- | --- |
| **STAFF NAME**: (printed) | **STAFF SIGNATURE** | **DATE REVIEWED** |
| Chen, Xiaoqin |  |  |
| Darrin, Delores |  |  |
| DeHoogh-Grigsby, Debi |  |  |
| Donovan, Chris |  |  |
| Kraus, Jean |  |  |
| Liu, Yuhua |  |  |
| McInnis, Donna |  |  |
| Mohapatra, Itu |  |  |
| Morgan, Catherine |  |  |
| Pilger, Carrie |  |  |
| Staley, Rong |  |  |
| Stampalia, Ann |  |  |
| Villiers, Catherine |  |  |
| Vogel, Jared |  |  |
| Wang, Sharon |  |  |
| Waychoff, Emma |  |  |
| Whalen, Sara |  |  |
| Zhou, Yang |  |  |