DNA/RNA Co-Isolation with Qiagen AllPrep DNA/RNA Mini Kit and *mir*Vana (Total & Small RNAs)

This protocol applies to: Acute Myeloid Leukemia (AML) and Acute Myeloid Leukemia Induction Failure (AML-IF)

The protocol herein describes the procedures used by Fred Hutchinson Cancer Research Center to process disease tissues for RNA and/or DNA subsequently used for characterization in the NCI's TARGET initiative. All nucleic acid samples used in TARGET projects were quality tested for consistency using picogreen quantification and SSTR genotyping methods, regardless of where the nucleic acid was originally extracted.

I. PRINCIPLE

The Qiagen AllPrep DNA/RNA Mini kit is a fast method of simultaneously purifying DNA and RNA from leukocytes for downstream analysis. The kit is a column-based method where lysate is passed through the DNA spin column to selectively isolate DNA and then through an RNeasy spin column to isolate RNA. Subsequent wash steps remove any residual contaminants and salts that may interfere with downstream application. Finally an elution step into a desired volume provides the bound DNA or RNA to be eluted into a stabilizing buffer.

II. SPECIMEN

A. Types

White blood cells resuspended in fetal calf serum with 10% DMSO obtained by Ficoll separation at a concentration of 1 x 10^7 cells/ml.

B. Handling Conditions

Follow standard precautions when handling all cell suspensions.

C. Sample Preparation

Peripheral blood and marrow specimens are ficolled for white blood cells and then resuspended in 1 ml Fetal calf serum with 10% DMSO and stored in liquid nitrogen.

D. Indications for Study

DNA and RNA are isolated from white blood cells to perform molecular testing on Acute Myeloid Leukemia specimens.

III. REQUIRED EQUIPMENT, SUPPLIES, AND REAGENTS

A. Equipment

Microcentrifuge with rotor for 2ml tubes
Centrifuge with rotor for 15ml conical tubes

B. Supplies

Sterile, RNase-free pipet tips
15-mL Polypropylene tubes
Microcentrifuge tube rack
Racks to hold 15-ml polypropylene tubes
Ice bucket and ice
Permanent-ink colored markers
Lab coat
Gloves

C. Reagents

Qiagen AllPrep DNA/RNA Mini Kit (cat# 80204)
QiaShredder Columns (cat# 79654)
14.3M ß-mercaptoethanol (ß-ME)
100% ethanol
70% ethanol
Phosphate Buffered Saline (PBS)

IV. WARNINGS / PRECAUTIONS

All cell suspension samples should be handled using standard precautions. Laboratory personnel should wear gloves and lab coat during sample processing.

V. REAGENT PREPARATION (INCLUDING STORAGE CONDITIONS)

A. ß-ME must be added to Buffer RLT Plus before use. Add 10ul ß-ME/ml Buffer RLT Plus. Dispense in a fume hood and wear protective clothing. Buffer RLT Plus is stable at room temperature for 1 month after addition of ß-ME.

B. Add appropriate amounts of ethanol to Buffer RPE, Buffer AW1, and Buffer AW2 as indicated on the bottles.

C. All other reagents included in AllPrep DNA/RNA Mini kit should be kept at room temperature.

VI. QUALITY CONTROL

N/A

VII. PROCEDURE – STEPWISE

1. Label 6 rows of columns and/or tubes with specimen barcode. Make sure labels are pre-printed for RNA and DNA elution tubes (label should include COG study #, COG registration #, specimen barcode and specimen location) Double check that all tubes are in the correct order in each row. Row 1: Qiashredder column. Row 2: AllPrep DNA Spin column. Row 3: empty 2ml collection tube (not labeled). Row 4: RNeasy spin column. Row 5: clean 1.5 ml collection tube with cryolabel affixed to the front (for RNA elution). Row 6: clean 1.5 ml collection tube with cryolabel affixed to the front (for DNA elution). Also, prepare a 15ml polypropylene tube with 5ml of PBS for each sample to be extracted.
2. Remove cryovials from liquid nitrogen and place on ice. Quick-thaw the samples at 37°C. Pipet thawed cells into 15ml tubes containing PBS. Centrifuge samples at 1200 rpm for 5 minutes.
3. Pour off the supernatant and resuspend the cell pellets in 600ul of Buffer RLT Plus and pipet up and down to mix. Pipet the lysates onto the corresponding labeled Qiashredder spin columns (row 1) and spin for 2 minutes at maximum speed. (Note: the homogenized lysate may be frozen at -70°C for months without degradation).
4. Remove tubes from centrifuge, place in rack and recheck that the barcodes match the next row of labeled tubes.
5. Discard Qiashredder columns and pipet lysates onto AllPrep DNA spin columns (row 2). Centrifuge for 30 seconds at 10,000 rpm.
6. Remove tubes from centrifuge, place in rack and recheck that the barcodes match the next row of labeled tubes.
7. Remove the AllPrep DNA spin column and place into the empty 2ml collection tube (row 3). Set aside tubes until step 17.
8. Add 600ul of 70% ethanol to the flow-through from step 5 and mix well by pipetting. Transfer 700ul of the sample to the RNeasy spin column in row 4. Centrifuge for 15 seconds at 10,000 rpm and discard the flow-through.
9. Load the remaining amount of sample from step 8 onto the RNeasy spin column and centrifuge for 15 seconds at 10,000 rpm. Discard the flow-through.

10. Add 700ul Buffer RW1 to the RNeasy spin column and centrifuge for 15 seconds at 10,000 rpm to wash the column. Discard the flow-through.
11. Add 500ul Buffer RPE to the RNeasy spin column and centrifuge for 15 seconds at 10,000 rpm to wash the column. Discard the flow-through.
12. Add 500ul Buffer RPE to the RNeasy spin column and centrifuge for 2 minutes at 10,000 rpm to remove any residual ethanol from the column. Remove tubes from centrifuge, place in rack and recheck that the barcodes match the next row of labeled tubes.
13. Carefully remove the column from the collection tube and prevent any contact with the flow-through so ethanol is not carried over to the elution step. Place the RNeasy column into a labeled 1.5 ml collection tube (row 5).
14. Add 30ul RNase-free water directly to the column and centrifuge for 1 minute at 10,000 rpm to elute the RNA.
15. Repeat step 14 with another 30ul of RNase-free water.
16. Discard RNeasy spin column and place samples on ice during the subsequent DNA extraction part of the protocol.
17. Add 500ul Buffer AW1 to the AllPrep DNA spin columns in step 7 to wash the column. Centrifuge for 15 seconds at 10,000 rpm. Discard the flow-through.
18. Add 500ul Buffer AW2 to the AllPrep DNA spin columns. Centrifuge for 2 minutes at maximum speed to remove any residual ethanol from the column.
19. Carefully remove the column from the collection tube and prevent any contact with the flow-through so ethanol is not carried over to the elution step. Place the DNA spin column into a labeled 1.5 ml collection tube (row 6).
20. Add 100ul Buffer EB directly to the column, incubate at room temperature for 1 minute and centrifuge for 1 minute at 10,000 rpm to elute the DNA.
21. Repeat step 20 with another 100ul of Buffer EB.
22. Discard AllPrep DNA spin column.

VIII. INTERPRETATION / ANALYSIS / DOCUMENTATION

A. Record following information onto DNA worksheet for database: pt Reg number, pt Barcode, location, concentration, date extracted, study number, date received, and specimen type.

B. Briefly vortex RNA and DNA samples and quantify using the NanoDrop spectrophotometer.

C. If necessary, remove an aliquot of RNA for Agilent Bioanalyzer (3ul) before storing sample at -80°C for quality assessment.

IX. REFERENCES

1. Qiagen AllPrep DNA/RNA Mini Kit: *Simultaneous Purification of Genomic DNA and Total RNA from Animal Cells*. November 2005.