MCCRD-SOP0002: Purification of Genomic DNA and Total RNA Tumor Tissue Using Qiagen AllPrep DNA/RNA Mini Kit

Effective Date: 12/1/2014

Please check for revision status of the SOP at

https://pdmr.cancer.gov/sops/

PDMR NCI Patient-Derived Models Repository
An NCI Precision Oncology Initiative℠ Resource

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VERSION INFORMATION

1. Change History

Revision	Description
	Internal SOP used by MOCHA Laboratory
12/1/2014	Standardize SOP for posting to PDMR internal site for use by designated NCI intramural laboratories

1.0 PURPOSE/SCOPE

This procedure is a modification of the Qiagen AllPrep DNA/RNA Mini Kit (Qiagen). MOCHA will use this protocol to extract genomic DNA and total RNA from frozen tissue using the Qiacube. The RNA and DNA extracted using this protocol will be suitable for downstream applications such as gene expression profiling and next generation sequencing. **This SOP is for research purposes only and no clinical samples will be processed using this SOP. Any deviation from this SOP will be noted but will not be formally documented.**

This procedure is to be used to extract nucleic acids from the samples (macro- and micro-dissected enriched tissue) for the Patient-Derived Models Repository effort.

2.0 SAFETY

2.1 Lab coats, safety glasses, and gloves must be worn at all times when handling hazardous or sensitive equipment, samples, reagents, and materials. These safety measures must also be followed when in close proximity to those who are working with these items.

2.2 Sample - Insulated gloves should be worn when handling any dry ice or liquid nitrogen vapor, both of which can cause severe burns to skin while handled

2.3 Sample - Dry ice and liquid nitrogen should be allowed to sublimate or evaporate at room temperature in an appropriate cooler or dewar, ideally in a chemical fume hood, or in open air, away from ventilation sources at a minimum. Do not discard of either in trash or sink.

3.0 EQUIPMENT

3.1 Reagents

3.1.1 AllPrep DNA/RNA Mini Kit (50), Qiagen, Cat# 80204

3.1.2 1.5 ml Eppendorf DNA/RNA LoBind Tubes, Eppendorf, Cat# 022431021

3.1.3 2.0 ml Eppendorf Safe Lock Tubes, Eppendorf, Cat# 022363352

3.1.4 1000ul Pipet and tips, Rainin, Cat# L1000

3.1.5 2.8mm ceramic beads in 2mL tubes RNase/DNase-Free, Omni International, Cat# 19-627

3.1.6 Ethyl Alcohol

3.1.7 Quant-iT™ dsDNA Broad-Range (BR) Assay Kit, Molecular Probes, Cat# Q33130

3.1.8 Quant-iT™ RNA Broad-Range BR Assay Kit, Molecular Probes, Cat# Q10213

3.1.9 96 well Black Flurotrac Plates, Griener Bio-one, ref#655076

3.2 Equipment

3.2.1 Bead Ruptor 24 Homogenizer, Omni International

3.2.2 Qiacube, Qiagen

3.2.3 Spectramax M2, Molecular Diagnostics

3.3 AUTOMATION METHODS

3.3.1 As per the protocol described.

4.0 PROCEDURE

4.1 Sample disruption and homogenization - Tumor samples are prepared in the Histology Lab.

4.2 For each OCT tumor sample, pre-chill a 2ml tube containing the 2.8mm ceramic beads and tumor fragment on dry ice.

4.3 Transfer up to no more than 24 tubes to a room temperature rack.

4.4 Add 360ul RLT Plus Lysis buffer with 2ME prepared as described in the protocol for the AllPrep DNA/RNA Mini Kit.

4.5 Place each sample in the Bead Ruptor and run Program 2 at room tempaerature.

4.5.1 Speed = 4

4.5.2 Time each cycle = 0:25

4.5.3 Cycles = 2

4.5.4 Time interval or Dwell between each cycle = 0:10

4.6 Remove samples and spin 4 minutes full speed at 4º C.

4.6.1 Transfer lysate to appropriately labeled 2.0 ml Safe Lock Eppendorf tubes.

4.6.2 Samples may be stored @-80ºC for processing at a later date or may be used immediately.

4.7 Extraction with Qiacube and Allprep RNA/DNA Mini Kit: Standard Part A

4.7.1 Place listed buffers in the bottle and tip rack in designated positions:

Position	Reagent
1	70% ethanol
2	Buffer RW1
3	Buffer RPE
4	Buffer AW1
5	Buffer AW2
6	Buffer EB

4.7.2 Load Disposable Filter-Tips, 1000ul.

4.7.3 Place bottle and tip rack in Qiacube

4.7.4 Load Rotor Adapters

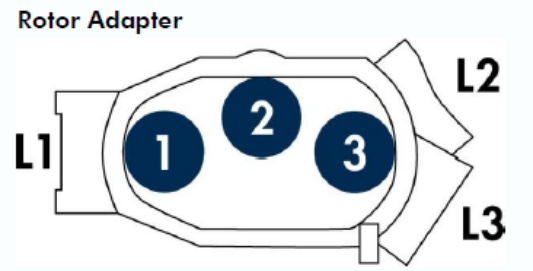
4.7.4.1 Up to twelve samples may be run with this protocol.

4.7.4.2 Use loading rack with Rotor Adapters evenly divided between positions 1-6 and 7-12, according to the number of samples to be processed. If necessary, add dummy Rotor Adaptor for balance while centrifuging.

4.7.4.3 Label Rotor Adapters and 1.5 ml collection tubes with sample ID number.

- Add the letter D to the collection tubes in addition to the sample ID number.

4.7.4.4 Add AllPrep DNA Mini Spin column and 1.5 collection tube to the rotor adapters at the positions designated in the diagram and table.



Position	Labware	Lid Position
1		L1
2	Allprep DNA spin column (cut off lid before placing into rotor adapter)	L2
3	1.5 ml safe lock eppendorf tube	L3

4.7.5 Load Disposable Filter-Tips, 1000ul

4.7.6 Load prepared Rotor Adapters into centrifuge.

4.7.7 Load up to twelve lysate samples from **Sample disruption and homogenization** above into the blue shaker rack with attached caps stored in the storage slot. The samples should be loaded in the identical order that the collection tubes are ordered in the centrifuge.

4.7.7.1 Turn Qiacube on.

4.7.7.2 On the touchscreen select RNA.

4.7.7.3 Scroll down until AllPrep DNA RNA Mini appears and press Select.

4.7.7.4 Select “Animal tissues and cells”.

4.7.7.5 Select Custom part A.

4.7.7.6 Select “Edit modified”.

4.7.7.7 Select “Elution Volume”, choose 50 ul and Save.

4.7.7.8 Select “Back”.

4.7.7.9 Select “Start”.

4.7.7.10 Follow the steps on the screen to set up the Qiacube.

4.7.7.11 Press “Start”.

4.7.7.12 Remain with the instrument until the loading check is complete and address any issues that may be found.

4.7.7.13 After the run is complete remove rotor adapters to loading rack at positions matching those in the centrifuge. Remove collection tube/column and discard column. Close tube and store on ice.

4.8 Extraction with Qiacube and Allprep RNA/DNA Mini Kit: Standard Part B

4.8.1 Add 1000ul disposable filter-tips so that tip racks are full.

4.8.2 Check volumes of reagents in bottle rack.

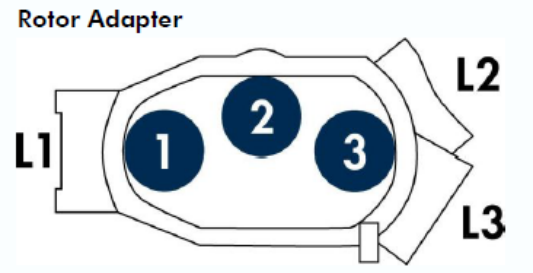
4.8.3 Load Rotor Adapters

4.8.3.1 Use rotor adapters in positions identical to that in the Part A.

4.8.3.2 Label Rotor Adapters and 1.5 ml collection tubes with sample ID number.

- Add the letter R to the collection tubes in addition to the sample ID number.

4.8.3.3 Add RNeasy Min spin columns and 1.5 ml collection tubes to the rotor adapters at the positions designated in the diagram and table.



Position	Labware	Lid Position
1	RNeasy spin column (cut off lid before placing into rotor adapter)	L1
2		L2
3	1.5 ml safe lock eppendorf tube	L3

4.8.3.4 Turn Qiacube on.

4.8.3.5 On the touchscreen select RNA.

4.8.3.6 Scroll down until AllPrep DNA RNA Mini appears and press Select.

4.8.3.7 Select “Animal tissues and cells”.

4.8.3.8 Select Custom part A.

4.8.3.9 Select “Edit modified”.

4.8.3.10 Select “Elution Volume”, choose 50 ul and Save.

4.8.3.11 Select “Back”.

4.8.3.12 Select “Start”.

4.8.3.13 Follow the steps on the screen to set up the Qiacube.

4.8.3.14 Press “Start”.

4.8.3.15 Remain with the instrument until the loading check is complete and address any issues that may be found.

4.8.3.16 After the run is complete remove rotor adapters to loading rack at positions matching those in the centrifuge. Remove collection tube/column and discard column. Close tube and store on ice.

4.8.4 Remain with the Qiacube until loading verification is complete.

4.8.5 After the run is complete remove rotor adapters to loading rack at positions matching those in the centrifuge. Remove collection tube/column and discard column. Close tube and store on ice.

4.8.6 Discard all disposables and turn Qiacube off.

4.9 Use the SpectraMax M2^{e} and the Quant-it DNA and RNA BR Kits to determine nucleic acid concentrations.

4.10 Remove Quant-it reagents from 4^{o}C storage and bring to room temperature; Liquefy the dye(s) at 37°C prior to use.

4.11 Make working solution by diluting Quant_iT dsDNA BR reagent 1:200 in Quant_iT dsDNA BR buffer.

4.12 In duplicate, add 10 ul of each Quant-It dsDNA BR standards to separate wells of black fluotrac plate(s).

4.13 In duplicate, add 2 ul of each sample to separate wells of black fluotrac plate(s).

4.14 Run plate on the Spectramax M2 microplate reader with Softmax 6.0 software using the following settings:4

4.14.1 DNA BR

4.14.1.1 Ex = 480

4.14.1.2 Cutoff = 530

4.14.1.3 Em = 530

4.14.1.4 6 flashes / read

4.14.1.5 Read from top

4.14.1.6 Shake Once

4.14.1.7 Calibrate off

4.14.2 For RNA Assay Kit, Broad Range

4.14.2.1 Ex = 630

4.14.2.2 Cutoff = 665

4.14.2.3 EM = 68017

4.14.2.4 6 flashes/read

4.14.2.5 Read from top

4.14.2.6 Shake Once

4.14.2.7 Calibrate Off

4.15 Determine concentration using standard curve

4.15.1 Label and store NA samples at 80°C.