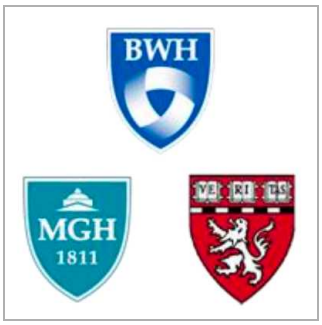

NOV 21, 2022

WORKS FOR ME 1

PAXgene Processing by RNA Extraction

COMMENTS 0

In 1 collection

DOI
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ABSTRACT

This protocol explains the Standard Operating Protocol for performing Paxgene Processing by RNA extraction.

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PROTOCOL CITATION

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COLLECTIONS

BIOSPECIMENS SOPs

KEYWORDS

paxgene, processing, RNA, extraction, ASAPCRN

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OWNERSHIP HISTORY

Feb 18, 2021	Liz Brydon	Protocols.io
May 03, 2021	Yuliya Kuras	
May 05, 2021	Yuliya Kuras	
Oct 03, 2022	Daniel El Kodsi	

PROTOCOL INTEGER ID

47408

PARENT PROTOCOLS

Part of collection
BIOSPECIMENS SOPs

GUIDELINES

PROTOCOLS NO LONGER IN USE

PURIFICATION OF MIRNA FROM RNA EXTRACTION FLOW-THROUGH
MiRNA Extraction
MATERIALS:

1. RNeasy MinElute Cleanup Kit (QIAgen, Cat #74204)
2. 15 mL falcon tubes (BD, Cat #352097)
3. 5 mL syringe reservoirs (Applied Biosystems, Cat #4344437)
4. Freezerbondz labels (Fischer Scientific, Cat #22500521)

PROTOCOL:

1. Keep the flow-through from steps 18 and 20 in RNA extraction protocol.
2. The miRNA protocol should be performed during or after completion of RNA purification to ensure consistency with previous samples. Keep flow-through covered until beginning miRNA purification.
3. Take RNeasy MinElute spin columns out from 4°C and equilibrate to room temperature (25°C) for several minutes.
4. Prepare the Qiavac 24 Plus Vacuum Manifold with one RNeasy spin column per subject. Use disposable adaptors to prevent contamination.
5. Attach a 5 mL syringe reservoir to RNeasy spin column to accommodate for the volume of miRNA flow through.
6. Combine flow-through for each subject into a 15 ml tube.
7. Add 700 µl of 100% ethanol for each 500 µl of flow through. Mix thoroughly by vortexing.
8. Pour the entire flow-through into the 5 mL syringe reservoir and turn on vacuum.
9. When all flow-through has been passed through the spin column, turn off the vacuum.
10. Add 500 µl of Buffer RPE into the spin column and turn on the vacuum until the buffer has passed.
11. Remove spin columns from the Quivac and place in a 2 ml processing tube.
12. Add 500 µl of 80% ethanol to the spin column and centrifuge at 13,000 rpm for 2 min at 25°C.
13. Place spin column in a new 2ml processing tube and discard old tube. (Remove spin column carefully so that the column does not touch the flow -through.)
14. Open lid of the spin column and centrifuge at 13,000 rpm for 5 min at 25°C.
15. Place the spin column in a low-retention 1.5 ml microcentrifuge tube.
16. Pipette 15 µl of RNase-free water directly on the spin column membrane. Close lid gently and sit for 1 min, then to elute,

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centrifuge at 13,000 rpm for 1 min at 25°C.

17. Label miRNA tube (miRNA).
18. MiRNA is logged and stored the same way as RNA.

FREEZER STORAGE

Freezers are divided into 4 shelves, with 6 racks per shelf, and 24 boxes that can be held in each shelf. In total, 576 boxes, approximately 2,160 sample sets, can be stored in one -80°C freezer. The first three shelves are designated by visit number: Shelves A1-6 (top shelf) house samples from enrollment visits, shelves B1-6 (2nd shelf) house samples from the 1st year follow-up, and shelves C1-6 (3rd shelf) house samples from the 2nd year follow-up. Shelves D1-6 contain packed red blood cell tubes (PRBC), DNA, and RNA, extracted from blood as described in the protocols above. CSF is designated between two freezers in selected racks. Freezer storage and transactions of samples are recorded in the Freezerworks Inventory software.

MATERIALS TEXT

MATERIALS:

1. PAXgene tubes from BLOOD DRAW (Two 2.5 cc PAXgene™ Blood RNA Tubes (VWR Ref# 77776-026))
2. PreAnalyix PAXgene kit (Qiagen/BD Company, Cat # 762164)
3. Freezerbondz labels (Fischer Scientific, Cat # 22500521)
4. 1.5 mL low-retention microcentrifuge tubes (Fisher Scientific, Cat #02-681-320)

SAFETY WARNINGS

Please refer to Safety Data Sheets (SDS) for health and environmental hazards. Gain all required consent and experimental approvals before beginning any procedures.

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BEFORE STARTING

***NOTE: Please see Appendix in guidelines for miRNA Extraction Protocol. MiRNA Extraction was discontinued 5/1/2019

RNA Q/C GOALS

1. Nanodrop Concentration Assay
   a. 260/280 > 2.0
   b. 94 µg/mL (30 µg total) of RNA/subject
2. Agilent 2100 Bioanalyzer Assay
   a. 28S/18S peaks = 1.0 -2.0
   b. RNA Integrity Number (RIN) > 7.3

1d 0h 16m 10s

PAXgene Processing BY RNA Extraction

1 Place all 2 PAXgene tubes in the 4 °C fridge from blood draw if RNA extraction is NOT to be done the next day.

1d

2 Incubate PAXgene Blood RNA Tubes for 24:00:00 at Room temperature (25°C) after blood collection or removal from 4°C before processing.

3 Prepare 55°C and 65°C heating blocks.

4 Centrifuge tubes at 4000 rpm, 25°C, 00:12:00 .

5 Remove supernatant and add 4 mL RNase-free water to each tube.

6 Close tube using a fresh secondary Hemogard closure.

10s

7 Vortex 00:00:10 until pellet is dissolved.

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8 Centrifuge at 4000 rpm, 25°C, 00:10:00. Discard supernatant.

9 Add 350 µL Buffer BR 1, cap and vortex until the pellet is visibly dissolved.

10 Pipette sample into a 1.5 ml microcentrifuge tube.

11 Add 300 µL Buffer BR2 and 40 µL proteinase K.

12 Mix by votexing briefly, then incubate for a total of 10 minutes on a heating block at 55 °C as follows: incubate for 00:05:00, briefly vortex, incubate 00:05:00. 10m

> Note
>
> **Do not mix Buffer BR2 and proteinase K together before adding to samples!**

13 Pipette lysate directly onto the membrane of a PAXgene Shredder spin column (lilac-colored) placed in a 2 ml processing tube.

14 Centrifuge at 13000 rpm, 25°C, 00:03:00.

15 Carefully pipette supernatant of the flow-through to a 1.5 microcentrifuge tube without disturbing pellet.

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16 Add 350 µL 100% ethanol, mix by votexing, and centrifuge 1000 x g, 00:00:02 , 1-2sec at 500-1000 x g to remove drops from inside of the tube lid.

Note

Do not centrifuge any longer to avoid pelleting of nucleic acids.

17 Pipette 700 µL sample into the PAXgene RNA spin column (pink) placed in a 2 ml processing tube, and centrifuge at 10000 rpm, 25°C, 00:01:00.

18 Place the spin column in a new 2 ml processing tube.

19 Pipette the remaining sample from step 16 into the spin column and centrifuge at 10000 rpm, 25°C, 00:01:00.

20 Place spin column in a new 2 ml processing tube.

21 Add 350 µL Buffer BR3 into the spin column and centrifuge at 10000 rpm, 25°C, 00:01:00.

22 Place spin column in a new 2 ml processing tube and discard old tube.

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23 Add 10 µL DNase I stock solution to 70 µL Buffer RDD in a 1.5 ml microcentrifuge tube and mix by gently flicking the tube (do not vortex). Centrifuge briefly to collect residual liquid on sides.

5m

24 Add 80 µL DNase I incubation mix directly onto the membrane of the spin column, and place on benchtop (20°C - 30°C, Room temperature) for 00:05:00 .

25 Pipette 350 µL Buffer BR3 into the spin column and centrifuge at 10000 rpm, 25°C, 00:01:00 .

26 Place spin column in a new 2 ml processing tube and discard old tube.

27 Add 500 µL Buffer BR4 (diluted in 1:4 in 100% ethanol) into the spin column, and centrifuge at 10000 rpm, 25°C, 00:01:00 .

28 Place spin column in a new 2 ml processing tube and discard old tube.

29 Add another 500 µL Buffer BR4 to the spin column and centrifuge at 10000 rpm, 25°C, 00:01:00 .

30 Place spin column into a new 2 ml processing tube and discard old tube. Centrifuge at 13000 rpm, 25°C, 00:03:00 .

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31 Place spin column into a new 1.5 ml microcentrifuge tube and discard old tube.

32 Add 41 µL Buffer BR5 directly onto spin column membrane and sit for 00:01:00. 1m

33 Centrifuge for 10000 rpm, 25°C to elute the RNA.

34 Repeat step 32 with 41 µL Buffer BR5 and the same microcentrifuge tube: sit for 00:01:00. 1m
Centrifuge for 10000 rpm, 25°C to elute the RNA.

35 Combine all two elutes into one tube. Split volume in half between two tubes and label (RNA-01 and 02).

36 Place the aliquots in the 65 °C heat block for 00:05:00 without shaking. After incubation, chill immediately On ice. 5m

37 Aliquot 3 µL RNA into a 1.5 mL tube for Nanodrop concentration assay.

38 Aliquot 2 µL RNA into a PCR tube for Agilent 2100 Bioanalyzer assay. (Store in -20 °C if not being assay immediately.)

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RNA Sample Storage

39 Scan and position RNA in the Freezerworks Inventory Program.

40 Store in corresponding -80°C freezer.

Note

Split and store RNA-01 and RNA-02 aliquots in separate freezers in case of freezer failure.

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