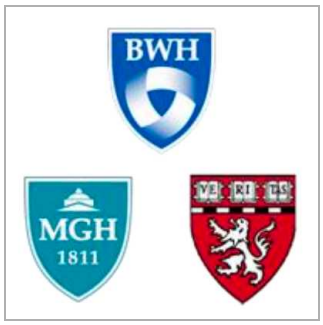

NOV 21, 2022

WORKS FOR ME 1

Automated QIAcube DNA Extraction

In 1 collection

COMMENTS 0

DOI
dx.doi.org/10.17504/protocols.io.5qpvoypkdg4o/v1

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ABSTRACT

This protocol explains the Standard Operating Protocol for automated extraction of DNA using QIAcube.

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PROTOCOL CITATION

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COLLECTIONS

BIOSPECIMENS SOPs

KEYWORDS

DNA, extraction, QIAcube, ASAPCRN

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OWNERSHIP HISTORY

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May 03, 2021	 Yuliya Kuras	
May 05, 2021	 Yuliya Kuras	
Oct 03, 2022	Daniel El Kodsi	

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PARENT PROTOCOLS

Part of collection
BIOSPECIMENS SOPs

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GUIDELINES

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. QIAcube (QIAgen)
2. Buffy Coat tube(from WHOLE BLOOD, PLASMA, BUFFY COAT PROCESSING)
3. Sample Tubes RB (QIAgen)
4. 1.5 mL low-rentention microcentrifuge tubes (Fisher Scientific, Cat #02-681-320)
5. Disposable 1000 µL tips for QIAcube (QIAgen) (Do NOT use wide-bore tips!)
6. Disposable 200 µL tips for QIAcube (QIAgen)
7. QIAamp DNA Blood Mini Kit (250) (QIAgen)
8. Rotor Adaptors 10 x 24 (QIAgen)
9. Freezerbonds Labels (Fisher Scientific, Cat #22500521)

SAFETY WARNINGS

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

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approvals before beginning any procedures.

BEFORE STARTING

Based on 12 Samples.

DNA Q/C GOALS

1. Cary Concentration Assay
   a. 260/280 = 1.8-2.0
   b. Manual Puragene Extraction: 260 µg /mL (65 µg total) of DNA/subject
   c. Automated QIAcube Extraction: 125 µg/mL (50 µg total) of DNA/subject
2. .7% Agarose Gel Electrophoresis
   a. Human DNA = 23.13 kb with λ DNA-HindIII digest (NEB)

Part A: First Elution

1 Heat water bath to 37 °C before starting.

2 Thaw Buffy Coat samples by gently agitating sample in water bath.

3 Turn on QIAcube. Open door and remove reagent tray from QIAcube. Unscrew all caps and refill reagents where necessary. Do not exceed fill line shown on side of reagent bottle. Make sure reagents are in the proper position in reagent tray.
   a. Position 1: Empty
   b. Position 2: Buffer AL
   c. Position 3: 96-100% Ethanol
   d. Position 4: Buffer AW1
   e. Position 5: Buffer AW2
   f. Position 6: Buffer AE

4 Add a fresh set of disposable 1000 µL and 200 µL tips to QIAcube.

5 Lay out rotor adaptors on holding tray labeled DNA.

6 Set up rotor adaptor by placing following materials in the proper positions:
   a. Position 1: QIAamp spin column

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i. Lid Position: L1

b. Position 2: Empty

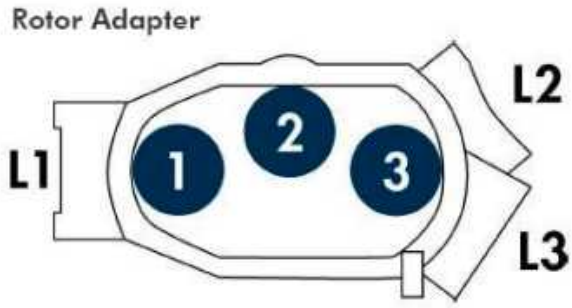
i. Lid Position: Empty

c. Position 3: 1.5 mL Eppendorf tube

i. Lid Position: L3

Note

It is very important that the lids go in the proper positions or else the QIAcube will generate an error message!



7 Load rotor adaptors in centrifuge of QIAcube.*

Note

* If there are less than 12 samples load materials according to the QIAcube loading chart found in the QIAcube binder.

8 Aliquot 200 µL Buffy Coat to Sample Tubes RB taking care to pipette up as much Buffy Coat as possible.

9 Load Sample Tubes RB into sample tray of QIAcube.*

Note

* If there are less than 12 samples load materials according to the QIAcube loading chart found in the QIAcube binder.

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10 Aliquot 284 µL QIAgen Protease into a 1.5 mL Eppendorf tube.* Place in microcentrifuge tube slot A in QIAcube.

Note

* If there are less than 12 samples load materials according to the QIAcube loading chart found in the QIAcube binder.

11 Close door of QIAcube and begin program:
DNA > QIAamp DNA Blood Mini > blood or body fluid > DNA isolation Part A

Part B: Second Elution

12 Remove rotor adaptors and place on sample holding tray.

Note

Make sure to place the adaptors in the corresponding numbered position in the sample tray that matches the number that the adaptor held in the centrifuge to prevent cross contamination of samples.

13 Move QIAamp spin column from 1.5 mL Eppendorf tube to Position 1 with lid in Position L1. (Part A step 6)

14 Remove and cap 1.5 mL tube with DNA sample and label with Freezerbondz label DNA-01.

15 Add a fresh 1.5 mL tube to rotor adaptor in Position 3 with lid in Position L3. (Part A step 6)

16 Place rotor adaptors back in the corresponding numbered position in QIAcube's centrifuge.

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17 Add a fresh set of 200 µL disposable tips.

18 Close QIAcube lid and begin program:
DNA > QIAamp DNA Blood Mini > blood or body fluid > Add Elution Part B

19 When program has completed, discard QIAamp spin column and remove 1.5 mL tube. Label tube with Freezerbondz label DNA-02.

20 Combine volume of DNA-02 to DNA-01 and pipette up and down to mix to ensure a homogenous mixture of DNA.

21 Split DNA back into two 200 µL aliquots.

22 Aliquot 3 µL DNA into a 1.5 mL tube for Cary concentration assay. (Store sample in -20 °C if not being immediately assayed.)

23 Aliquot 2 µL DNA into a PCR tube for .7% agarose gel electrophoresis to confirm for the presence and size of the DNA. (Store sample in -20 °C if not being immediately assayed.)

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