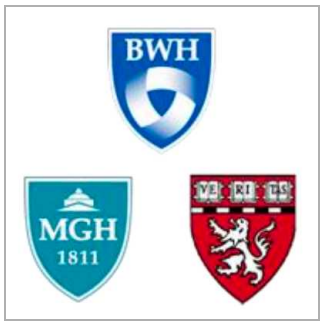

NOV 21, 2022

WORKS FOR ME 1

Manual Gentra Puragene DNA Extraction

COMMENTS 0

In 1 collection

DOI
dx.doi.org/10.17504/protocols.io.n2bvjx2nblk5/v1

Clemens Scherzer[1,2], Bradley Hyman[3,2], Charles Jennings[1,2]

[1]Brigham and Women's Hospital;
[2]Harvard Medical School;
[3]Massachusetts General Hospital

Daniel's workspace

Daniel El Kodsi

ABSTRACT

This protocol explains the Standard Operating Protocol for manually extracting DNA using Gentra Puragene.

DOI

dx.doi.org/10.17504/protocols.io.n2bvjx2nblk5/v1

PROTOCOL CITATION

Clemens Scherzer, Bradley Hyman, Charles Jennings 2022. Manual Gentra Puragene DNA Extraction. **protocols.io** https://dx.doi.org/10.17504/protocols.io.n2bvjx2nblk5/v1

COLLECTIONS

BIOSPECIMENS SOPs

KEYWORDS

DNA, extraction, gentra, puragene, ASAPCRN

CREATED

Feb 18, 2021

LAST MODIFIED

Nov 21, 2022

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

47415

PARENT PROTOCOLS

Part of collection
BIOSPECIMENS SOPs

Citation: Clemens Scherzer, Bradley Hyman, Charles Jennings Manual Gentra Puragene DNA Extraction
https://dx.doi.org/10.17504/protocols.io.n2bvjx2nblk5/v1

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. 3 mL whole blood in 6 mL EDTA tube

a. If volume is not 3 mL: Adjust for whole blood volume using ratios in protocol.
b. Whole blood is from samples collected prior to the initiation of the HBS study.
2. Gentra Puragene DNA kit (QIAgen, Cat #158445)
3. 15 mL falcon tubes (BD, Cat #9023-4981) 4. Low retention pipette tips
   a. 1000 mL low-retention tips (Bio Plastic, Inc., Cat #3606SRS)
   b. 200 uL low-retention tips (Molecular BioProducts, Cat #3932-05)
4. Low retention pipette tips:
   a. 1000 mL low-retention tips (Bio Plastic, Inc., Cat #3606SRS)
   b. 200 uL low-retention tips (Molecular BioProducts, Cat #3932-05)
5. 1.5 mL low-retention microcentrifuge tubes (Fisher Scientific, Cat # 02-681-320)

Citation: Clemens Scherzer, Bradley Hyman, Charles Jennings Manual Gentra Puragene DNA Extraction
https://dx.doi.org/10.17504/protocols.io.n2bvjx2nblk5/v1

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.

BEFORE STARTING

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)

2h 6m 35s

Manual Gentra Puregene DNA Extraction

1 Preheat water bath to 37 °C before removing whole blood samples from -80°C freezer.

2 Thaw whole blood samples by gently agitating sample in the 37°C water bath. Once thawed, reset water bath to 65 °C for last step in protocol.

3 Add 9 mL (1:3) RBC Lysis Solution to a 15 mL centrifuge tube.

4 Add 3 mL whole blood and mix by inverting 10 times.

5m

5 Incubate sample for 00:05:00 at Room temperature (15°C-25°C). Invert once during the incubation.

6 Centrifuge at 2000 x g, 25°C, 00:02:00 .

Citation: Clemens Scherzer, Bradley Hyman, Charles Jennings Manual Gentra Puragene DNA Extraction
https://dx.doi.org/10.17504/protocols.io.n2bvjx2nblk5/v1

7 Pour off and discard supernatant. Leave approximately 200 µL of residual liquid and the white blood cell pellet.

8 Vortex tube on high speed to resuspend pellet in the residual liquid.

9 Add 3 mL (1:1) Cell Lysis Solution. Vortex on high speed for 00:00:10. 10s

Note

If clumps are visible in the tube, incubate sample in 37°C water bath until homogenous.

10 Add 1 mL (1:.333) of Protein Precipitation Solution. Vortex on high speed for 00:00:20. 20s

11 Centrifuge sample at 2000 x g, 25°C, 00:05:00.

Note

If pellet does not tightly stick to the bottom of the tube, incubate on ice for 5 min and repeat centrifugation.

12 Add 3 mL (1:1) isopropanol into a clean 15 mL tube. Carefully pour the supernatant from step 11 into this tube.

Note

Be careful not to dislodge pellet during this step!

Citation: Clemens Scherzer, Bradley Hyman, Charles Jennings Manual Gentra Puragene DNA Extraction
https://dx.doi.org/10.17504/protocols.io.n2bvjx2nblk5/v1

13 Invert samples 50 times until DNA is visible as threads or a clump.

14 Centrifuge tube at 2000 x g, 00:03:00. Observe DNA as a small white pellet.

15 Carefully pour/pipette off supernatant without disturbing the pellet. Drain excess drops of supernatant by inverting on a Kimwipe, while making sure that pellet remains in the tube.

16 Add 3 mL (1:1) 70% ethanol and invert 3-5 times to wash the DNA pellet.

17 Centrifuge pellet at 2000 x g, 25°C, 00:01:00.

18 Carefully pour/pipette off ethanol without disturbing the pellet. Drain excess drops of ethanol by inverting on a Kimwipe, while making sure that pellet remains in the tube.

19 Air dry the pellet for 00:01:00. 1m

Note

Do not excessively air dry or pellet will be difficult to reconstitute. On the other hand, do not air dry the pellet for too short a period of time or residual ethanol will contaminate DNA!

20 Add 250 µL (1:100) DNA Hydration Solution. Vortex on medium speed for 00:00:05. 5s

Citation: Clemens Scherzer, Bradley Hyman, Charles Jennings Manual Gentra Puragene DNA Extraction https://dx.doi.org/10.17504/protocols.io.n2bvjx2nblk5/v1

21 Incubate DNA in 65 °C water bath for 01:00:00 in order to dissolve DNA. 1h

22 Incubate at Room temperature (25°C) Overnight on rocker. 1h

23 On the following day, DNA can be transferred to a 1.5 mL tube. Split volume in half to create two aliquots of DNA (DNA0-01 and 02).

24 Aliquot 3 µL (40x dilution) DNA into a 1.5 mL tube for Cary concentration assay. (Store in -20 °C if not being assay immediately.)

25 Aliquot 1.5 µL DNA into a PCR tube for .7% agarose gel electrophoresis to confirm presence and size of DNA. (Store in -20 °C if not being assay immediately.)

Citation: Clemens Scherzer, Bradley Hyman, Charles Jennings Manual Gentra Puragene DNA Extraction
https://dx.doi.org/10.17504/protocols.io.n2bvjx2nblk5/v1