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WORKS FOR ME

1

Salivary DNA Extraction

In 1 collection

COMMENTS 0

DOI

dx.doi.org/10.17504/protocols.io.3byl4k598vo5/v1Clemens Scherzer^{1,2}, Bradley Hyman^{3,2},
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Daniel's workspace



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ABSTRACT

This protocol explains the Standard Operating Protocol for extracting salivary DNA.

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PROTOCOL CITATION

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COLLECTIONS ⓘ

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KEYWORDS

DNA, extraction, salivary, saliva, ASAPCRN

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OWNERSHIP HISTORY

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PARENT PROTOCOLS

Part of collection

[BIOSPECIMENS SOPs](#)

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. OGR-600 Oragene DISCOVER saliva collection kit
2. prepIT-L2P (catalog #: PT-L2P)
3. DNA storage buffer: TE (10mM Tris-HCl, 1mM EDTA, pH 8.0) or similar solution
4. 70% and 100% ethanol

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)

4h 26m

Salivary DNA Extraction

- 1 Mix sample in the DNA Genotek kit by inversion and gentle shaking.



- 1.1 Weigh sample in original tube and subtract 6.81g to estimate saliva volume provided – amount of saliva collected is directly proportional to the amount of DNA recovered.

- 2 Incubate the sample at **50 °C air incubator** for minimum **02:00:00**.

2h



Note

Alternatively: Can incubate in **50 °C water bath** **Overnight** the night before extraction or minimum **01:00:00**.



- 3 Transfer the entire sample to a 15mL falcon tube by pouring.

- 3.1 Note volume of sample.

Volume of
Sample:


- 4 Add 1/25th volume of PT-L2P and mix by vortexing for a few seconds.



5 Incubate  On ice for  00:10:00 .

10m




6 Centrifuge  4500 rpm, Room temperature, 00:10:00 , max speed .




7 Carefully transfer most of the clear supernatant with a pipette to a fresh 50mL centrifuge tube.

7.1 Discard the pellet.

8 Add 1.2x volume of  Room temperature 100% ethanol to the clear supernatant. Mix gently by inversion 10x.



9 Let the sample stand at  Room temperature for  00:10:00 to allow DNA to fully precipitate.

10m



10 Centrifuge  4500 rpm, Room temperature, 00:10:00 , max speed .



11 Carefully pipette off the supernatant and discard it.

11.1 Avoid disturbing DNA pellet.

12 Add  1 mL 70% ethanol to the tube without disturbing the pellet.





- 12.1 Let stand at Room temperature for 00:01:00, then gently swirl and completely remove the ethanol without disturbing the pellet.

1m



- 12.2 Let stand at Room temperature for 00:05:00 to allow remaining ethanol to evaporate.

5m



- 13 Rehydrate the DNA by adding 300 μ L TE solution.



- 13.1 Mix by pipetting up and down 10x using wide-bore pipette tips.



- 14 Incubate at Room temperature for 01:00:00 or 4 °C Overnight.

2h



- 15 Mix by pipetting up and down 10x using wide-bore pipette tips, then transfer the rehydrated DNA to 2 fresh 1.5mL low-retention microcentrifuge tubes (150uL each) for storage at -80 °C.




Note

Aliquots are labeled "DNA-SAL-01" and "DNA-SAL-02".

DNA Sample Storage

- 16 Scan DNA samples into the Freezerworks Inventory Program and position in corresponding freezer.

- 17 Separate and store each DNA aliquot (DNA-01 and DNA-02 or DNA-SAL-01 and DNA-SAL-02) in two different



local -80°C freezers.