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DNA Extraction from Swabbed Feces for Depositor Species Identification - 2024

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We use this protocol and it's working

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Abstract

Protocol developed by the Culver Lab at the University of Arizona to extract DNA from feces for depositor species identification

Troubleshooting

Preparation

1 **Qiagen Kit:** Qiagen DNeasy Blood and Tissue extraction Mini kit
Lab Equipment: Heat block, Vortex, Centrifuge, Pipettes, Stainless steel tray, Stainless steel Scissors, Stainless steel tweezers
Solutions and Reagents: 10x PBS (to make 1000 ml of 10X PBS: 5.56 g NaH2PO4, 23.0 g NaHPO4, 87.68 g NaCl, pH to 7.1), proteinase K, ATL buffer, AL buffer, AW1 buffer, AW2 buffer, AE buffer (provided by Qiagen kit), 100% ethanol 100% (not provided)
Consumables: 1.5 ml microcentrifuge tubes, 2 ml microcentrifuge tubes, 2 ml collection tubes, 100-1000 µl pipette tips (filter and sterile), 10-100 µl pipette tips (filter and sterile), QIAamp spin columns (provided by Qiagen kit), sterile cotton-tipped applicators
Other supplies: Parafilm, copy paper, paper towels,10% bleach solution, gloves
Check Qiagen manual "Things to do before starting"

DAY 1

2 Saturate cotton-tipped applicators with PBS buffer.
● Keep cotton-tipped applicators in PBS buffer for at least 3 minutes.

3 Pipette 25 µl of proteinase K and 300 µl of ATL buffer (from DNeasy kit) into a new, labeled 2 ml microcentrifuge tube.

4 Place a sheet of copy paper on a stainless-steel tray to analyze scat samples for swabbing. Swab the surface of the scat with PBS-saturated applicator. Cut the applicator tip and place it in the 2 ml microcentrifuge tube from step 2.
● Bleach and dry the tray and change the copy paper after each sample is processed.
● Change gloves after each scat sample is processed.
● Scissors used to cut the applicator should be submerged in 10% bleach, rinsed, and wiped dry before cutting the next applicator.

5 Incubate samples at 56°C for 24 – 48 hours.
● To reduce the risk of evaporation due to faulty caps, cover the cap of tubes with parafilm.

DAY 2

6 Remove parafilm and spin tubes for 15 seconds to remove condensation inside the tube lids.

7 Remove the applicator and add 366 µl of AL buffer. Vortex and incubate at 56°C for 1 hour.
- Scissors or tweezers used to remove the applicator should be submerged in 10% bleach, rinsed, and wiped dry before removing the next applicator.

8 Add 366 µl of 100% ethanol and mix by inverting. Continue to step 8 or store at 4°C (stable for weeks).

9 Label spin columns provided by Qiagen kit and carefully apply 500 µl of lysate from step 7 to the column without moistening the rim.

10 Centrifuge according to Qiagen DNeasy protocol >= 6000 x g (8000 rpm) for 1 min.

11 Place the columns in a new 2 ml collection tube, and discard the tube containing the filtrate.

12 Carefully open the column, apply a second aliquot of 500 µl of lysate and centrifuge according to Qiagen DNeasy protocol >= 6000 x g (8000 rpm) for 1 min.

13 Place the columns in a new 2 ml collection tube and discard the tube containing the filtrate.

14 Prepare AE buffer: Bring Buffer AE to 70°C with the heat block. Pipette 100 ul (or 200 for less concentrated DNA) of buffer AE per sample into a new tube and place it in heat block (To use at the end)

15 Add 500 µl of AW1 wash buffer to column. Centrifuge at >= 6000 x g (8000 rpm) for 1 min. Place the column in a new 2 ml collection tube. Discard the tube containing the filtrate.

16 Add 500 µl of AW2 wash buffer to column. Centrifuge at 20,000 x g (14,000 rpm) for 3 min. Place the column in a new 2 ml collection tube. Discard the tube containing the filtrate.

17 Place the columns in a new 2 ml collection tube. Centrifuge at 20,000 x g (14,000 rpm) for 1 min. (Dry spin: this step helps to eliminate the chance of possible buffer AW2 carryover). Discard the tube containing the filtrate.

18 Remove the column and place it into a labeled 1.5 ml tube. Cut off the caps.

19 Add 100 - 200 µl of AE elution buffer directly to the column and incubate at room temperature for 1 to 3 minutes.

● Elution with 100 µl (instead of 200 µl) increases the final DNA concentration in the eluate

20 Centrifuge columns at ≥ 6000 x g (8000 rpm) for 1 minute to elute DNA.

21 Transfer DNA into labeled 0.5 ml or 1.5 ml tubes.

22 Discard columns and store DNA at 4°C or -20°C for long term.

23 Disinfect countertops and equipment with 10% bleach solution.

24 **Optional:**
For maximum DNA yield, repeat elution once as described in steps 19-21 . This step leads to increased overall DNA yield. A new microcentrifuge tube can be used for the second elution step to prevent dilution of the first eluate. Alternatively, to combine the eluates, the microcentrifuge tube from step 7 can be reused for the second elution step.