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Extraction method A (FMS and CR)

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Faezah Mohd Salleh¹, Jazmin Ramos-Madriral¹, Fernando Penalzoza¹, Shanlin Liu¹, Mikkel-Holger S Sinding¹, Riddhi P Patel¹, Renata Martins¹, Dorina Lenz¹, Jorns Fickel¹, Christian Roos¹, Mohd Shahir Shamsir¹, Mohammad Shahfiz Azman¹, Burton K Lim¹, Stephen J Rossiter¹, Andreas Wilting¹, M Thomas P Gilbert¹

¹Natural History Museum of Denmark, Copenhagen, Denmark

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Faezah Mohd Salleh

ABSTRACT

This protocol allows for adequate DNA extraction from fresh tissue samples.

MATERIALS

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⊗ Buffer AW1 **Qiagen Catalog #19081**

⊗ Buffer AW2 **Qiagen Catalog #19072**

⊗ Buffer AE **Qiagen Catalog #19077**

⊗ Buffer ATL **Qiagen Catalog #19076**

⊗ Ethanol **Contributed by users**

BEFORE START INSTRUCTIONS

Separate PCR-free facility

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Protocol status: Working

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6559

Extraction

- 1 Cut tissue into small pieces.
- 2 Digestion of ca. 25 mg sample with Buffer ATL (Qiagen, Hilden, DE) and Proteinase K overnight at 56 °C.
25 mg Sample
Note
Incubation was performed on a thermomixer with 800 rpm
- 3 After pelleting remaining material, remove supernatant and added to Buffer AL and ethanol.
- 4 Transfer the solution to DNeasy Mini spin column (Qiagen, Hilden, DE) and centrifuge at 8000 rpm.
- 5 DNA purification following manufacturer's protocol with Buffer AW1 and Buffer AW2 (Qiagen, Hilden, DE).

- 6 Prior to elution, incubate DNA in the membrane with elution Buffer AE for 5 min at room temperature.

00:05:00 Incubation at RT

- 7 Elution proceeds by centrifugation at 8000 rpm for 1 min.

00:01:00 Centrifugation

- 8 Measure DNA concentration on Nanodrop (Thermo Fischer Scientific, Darmstadt, DE).