JUL 05, 2017

OPEN ACCESS

DOI:
dx.doi.org/10.17504/protocols.io.indcda6

External link:
<https://doi.org/10.1093/gigascience/gix053>

Protocol Citation: Faezah Mohd Salleh, Jazmin Ramos-Madriral, Fernando Penaloza, Shanlin Liu, Mikkel-Holger S Sinding, Riddhi P Patel, Renata Martins, Dorina Lenz, Jorns Fickel, Christian Roos, Mohd Shahir Shamsir, Mohammad Shahfiz Azman, Burton K Lim, Stephen J Rossiter, Andreas Wilting, M Thomas P Gilbert 2017. Extraction Method D (PRP and RM). **protocols.io** <https://dx.doi.org/10.17504/protocols.io.indcda6>

MANUSCRIPT CITATION: Salleh FM, Ramos-Madriral J, Peñaloza F, Liu S, Mikkel-Holger SS, Riddhi PP, Martins R, Lenz D, Fickel J, Roos C, Shamsir MS, Azman MS, Burton KL, Stephen JR, Wilting A, Gilbert MTP, An expanded mammal mitogenome dataset from Southeast Asia. GigaScience 6(8). doi: [10.1093/gigascience/gix053](https://doi.org/10.1093/gigascience/gix053)

Extraction Method D (PRP and RM)

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GigaScience Press

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ABSTRACT

This protocol allows for adequate DNA extraction from archival samples.

MATERIALS

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⊗ Buffer AL **Contributed by users Catalog #19075**

⊗ 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

Created: Jun 26, 2017

Last Modified: Nov 17, 2017

PROTOCOL integer ID:
6565

Extraction

- 1 Digestion of ca. 50 mg sample with Buffer ATL (Qiagen, Hilden, DE) and Proteinase K overnight at 56 °C.

🧴 50 mg Buffer ATL

Note

Incubation was performed with rotation of the samples in an oven.

- 2 After pelleting remaining material, remove the supernatant and add to Buffer AL and ethanol.
- 3 Transfer the solution to DNeasy Mini spin column (Qiagen, Hilden, DE)
- 4 Centrifuge the solution 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 20 min at 37 °C.

00:20:00 Incubation at 37 °C

- 7 Proceed elution by centrifugation at 8000 rpm for 1 min.

00:01:00 Centrifugation

- 8 Prior to library construction, analyze small aliquots of each extract on Nanodrop (Thermo Fischer Scientific, Darmstadt, DE) for estimation of DNA concentration.