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Abstract

This protocol provides an efficient DNA extraction and purification of historical museum hides, which potentially have been chemically tanned.

Materials

MATERIALS

- MinElute PCR Purification Kit **Qiagen Catalog #**28004
- Chloroform
- Sodium Hypochlorite Solution
- 70% Ethanol
- Digestion Buffer consisting of 10 mM tris(hydroxymethyl)aminomethane hydrochloride (Tris-HCl) (pH 8.0), 10 mM NaCl, 2% w/v sodiu

Before start

Separate PCR-free facility

Extraction

1 Prior to extraction, individually vortex skin samples in a 10% commercial sodium hypochlorite solution (bleach) solution to decontaminate surface.

2 To remove the bleach subsequently vortexed the samples in 70% ethanol.

3 Finally vortex the samples in H2O.

4 Add the skin samples to 1 mL digestion buffer consisting of 10 mM tris(hydroxymethyl)aminomethane hydrochloride (Tris-HCl) (pH 8.0), 10 mM NaCl, 2% w/v sodium dodecyl sulfate (SDS), 5 mM CaCl2, 2.5 mM ethylenediaminetetraacetic acid (EDTA) (pH 8.0), 40 mM dithiothreitol (DTT), and 10% Proteinase K [Gilbert et al. 2007].

Note

Gilbert, M. T. P., Tomsho, L. P., Rendulic, S., Packard, M., Drautz, D. I., Sher, A., ... & Campos, P. F. (2007). Whole-genome shotgun sequencing of mitochondria from ancient hair shafts. science, 317(5846), 1927-1930

5 Incubate the samples for 12 hours at 56°C.

12:00:00 Incubation at 56°C

6 In order to purify the DNA from contaminants, first, mix 1mL supernatant with 1mL phenol.

1 mL Phenol

7 Vortex the sample for 20 sec.

00:00:20 Vortexing

8 Gently rotate the sample for 5 min.

00:05:00 Gentle rotation

9 Centrifuge the sample at 3000 g for 3 min.

00:03:00 Centrifugation

10 Remove approximately 1mL aqueous liquid and mix with 1mL chloroform.

1 mL Chloroform

11 Vortex the mixture for 30 sec.
00:00:30 Vortex

12 Rotate the mixture for 5 min.
00:05:00 Rotation

13 Centrifuge the mixture at 3000 g for 3 min.
00:03:00 Centrifugation

14 Remove approximately 1mL aqueous liquid and purify using the MinElute PCR Purification kit (Qiagen, Valencia, CA) according to manufacturer's instruction with a slight modification: Firstly, modify the PB buffer according to [Allentoft et al. 2015].

Note

Allentoft, M. E., Sikora, M., Sjögren, K. G., Rasmussen, S., Rasmussen, M., Stenderup, J., ... & Malaspinas, A. S. (2015). Population genomics of Bronze Age Eurasia. Nature, 522(7555), 167-172

15 Secondly, increase the volume of PB binding buffer to 10x.

16 Apply the buffer to the spin columns following the method developed by [Dabney et al. 2013], use a Zymo-Spin V reservoir (Zymo Research, Irvine, CA) to pass the large buffer volume through the MinElute column.

Note

Dabney J, Knapp M, Glocke I, et al (2013) Complete mitochondrial genome sequence of a Middle Pleistocene cave bear reconstructed from ultrashort DNA fragments. Proc Natl Acad Sci U S A 110:15758–15763

17 Prior to the final centrifugation, add 15 µL of EB buffer to the column.
15 µL EB Buffer

18 Incubate for 15 minutes at 37°C.
00:15:00 Incubation at 37°C

19 Centrifugate at 6000 g for 1 min.
00:01:00 Centrifugation

20 Quantify the extracted DNA using a Qubit fluorometer with a dsDNA high sensitivity (HS) assay (Life Technologies, Carlsbad, CA).