Extraction method C (FMS)

Faezah Mohd Salleh,Jazmin Ramos-Madrigal,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

Abstract

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

Citation: Faezah Mohd Salleh,Jazmin Ramos-Madrigal,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 Extraction method C (FMS). **protocols.io**

dx.doi.org/10.17504/protocols.io.inbcdan

Published: 05 Jul 2017

Before start

Separate PCR-free facility

Materials

MinElute PCR Purification Kit 28004 by Qiagen

- Chloroform by Contributed by users
- Sodium Hypochlorite Solution by Contributed by users
- 70% Ethanol by Contributed by users
- Digestion Buffer consisting of 10 mM tris(hydroxymethyl)aminomethane hydrochloride (Tris-HCl) (pH 8.0), 10 mM NaCl, 2% w/v sodiu by Contributed by users

Protocol

Extraction

Step 1.

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

Extraction

Step 2.

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

Extraction

Step 3.

Finally vortex the samples in H2O.

Extraction

Step 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].

NOTES

GigaScience Database 26 Jun 2017

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

Extraction

Step 5.

Incubate the samples for 12 hours at 56°C.

Extraction

Step 6.

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

AMOUNT

1 ml Additional info: Phenol

Extraction

Step 7.

Vortex the sample for 20 sec.

Extraction

Step 8.

Gently rotate the sample for 5 min.

Extraction

Step 9.

Centrifuge the sample at 3000 g for 3 min.

Extraction

Step 10.

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

AMOUNT

1 ml Additional info: Chloroform

Extraction

Step 11.

Vortex the mixture for 30 sec.

Extraction

Step 12.

Rotate the mixture for 5 min.

Extraction

Step 13.

Centrifuge the mixture at 3000 g for 3 min.

Extraction

Step 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].

NOTES

GigaScience Database 26 Jun 2017

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

Extraction

Step 15.

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

Extraction

Step 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.

NOTES

GigaScience Database 26 Jun 2017

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

Extraction

Step 17.

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

AMOUNT

15 µl Additional info: EB Buffer

Extraction

Step 18.

Incubate for 15 minutes at 37°C.

Extraction

Step 19.

Centrifugate at 6000 g for 1 min.

Extraction

Step 20.

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