Extraction method A

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Abstract

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

Before start

Clean

Materials

☑ proteinase K containing buffer by Contributed by users
☑ organic solvents (phenol:chloroform) by Contributed by users
  MinElute columns by Qiagen
  PB buffer 19066 by Qiagen
  Buffer PE 19065 by Qiagen
  Buffer EB 19086 by Qiagen

Protocol

Extraction

Step 1.

Digestion in a proteinase K containing buffer following Gilbert et al. 2007.

� NOTES
GigaScience Database 02 Jun 2017

Gilbert MTP, Tomsho LP, Rendulic S, Packard M, Drautz DI, Sher A, et al. Whole-genome shotgun sequencing of mitochondria from ancient hair shafts. Science. American Association for the Advancement of Science; 2007;317:1927–30.

Extraction
Step 2.

Pre-digest all samples at 56 °C for 1 hour.

⏱ DURATION
01:00:00

➕ NOTES
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Perform incubation with a rotation of the samples in an oven.

Extraction
Step 3.

Replace the buffer with fresh buffer.

Extraction
Step 4.

Perform a second 12-hour digest with the fresh buffer.

⏱ DURATION
12:00:00

➕ NOTES
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Perform incubation with a rotation of the samples in an oven.

Extraction
Step 5.

Purify digests using organic solvents (phenol:chloroform) and Qiagen MinElute columns (Qiagen, Hilden, DE) as described in the following steps:

Extraction
Step 6.

Mix digests with 8x modified PB buffer (Allentoft et al. 2015).

➕ NOTES
GigaScience Database 02 Jun 2017

Allentoft ME, Sikora M, Sjögren K-G, Rasmussen S, Rasmussen M, Stenderup J, et al. Population genomics of Bronze Age Eurasia. Nature. Nature Publishing Group; Dabney J, Knapp M, Glocke I et al. (2013) Complete mitochondrial genome sequence of a Middle Pleistocene cave bear reconstructed from ultrashort DNA fragments. Proceedings of the National Academy of Sciences of the United States of America, 110, 15758–15763.2015;522:167–72.

Extraction
Step 7.

Bind the digests to the MinElute column using reservoirs as previously described (Dabney et al. 2013).

Extraction
Step 8.

Wash with 800 µl buffer PE (Qiagen).

Extraction
Step 9.

Elute in 17 µl buffer EB (Qiagen). (1/2)

Extraction
Step 10.

Incubate for 5 minutes at 37 °C prior to release. (1/2)

   ⟳ DURATION
   00:05:00

Extraction
Step 11.

Elute in 17 µl buffer EB (Qiagen). (2/2)

Extraction
Step 12.

Incubate for 5 minutes at 37 °C prior to release. (2/2)

   ⟳ DURATION
   00:05:00

Extraction
Step 13.

Prior to library construction, analyze small aliquots of each extract on an Agilent 2200 TapeStation HS chip (Agilent Technologies, Palo Alto, California, USA) for fragment size estimation and molar concentration.