Extraction method B

SARAH SIU TZE MAK, SHYAM GOPALAKRISHNAN, CHRISTIAN CAROE, CHUNYU GENG, SHANLIN LIU, MIKKEL-HOLGER S SINDING, LUKAS F K KUDERNA, WENWEI ZHANG, SHUJIN FU, FILIPE G VIEIRA, MIETJE GERMONPRÉ, HERVÉ BOCHERENS, SERGEY FEDOROV, BENT PETERSEN, THOMAS SICHERITZ-PONTEN, TOMAS MARQUES-BONET, GUOJIE ZHANG, HUI JIANG, M THOMAS P GILBERT

Abstract

This protocol provides an efficient DNA extraction and purification of ancient bones.

Citation: SARAH SIU TZE MAK, SHYAM GOPALAKRISHNAN, CHRISTIAN CAROE, CHUNYU GENG, SHANLIN LIU, MIKKEL-HOLGER S SINDING, LUKAS F K KUDERNA, WENWEI ZHANG, SHUJIN FU, FILIPE G VIEIRA, MIETJE GERMONPRÉ, HERVÉ BOCHERENS, SERGEY FEDOROV, BENT PETERSEN, THOMAS SICHERITZ-PONTEN, TOMAS MARQUES-BONET, GUOJIE ZHANG, HUI JIANG, M THOMAS P GILBERT Extraction method B. **protocols.io**

dx.doi.org/10.17504/protocols.io.iadcaa6

Published: 14 Jun 2017

Before start

Clean

Materials

- Monarch DNA Cleanup Columns (5ug) - 100 columns T1034L by New England Biolabs
- Buffer EB 19086 by Qiagen
- PB buffer 19066 by Qiagen
- K-urea buffer by Contributed by users
- PE buffer 19065 by Qiagen

Protocol

Extraction

Step 1.

Digestion in a proteinase K-urea buffer following Ersmark et al. 2016.

NOTES

GigaScience Database 02 Jun 2017

Ersmark E, Klütsch C, Chan Y, Dalén L, Sinding MHS, Gilbert T, et al. From the past to the present: Wolf phylogeography and demographic history based on the mitochondrial control region. Frontiers in ecology and the environment; 2016;4:134.

Extraction

Step 2.

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

DURATION
01:00:00

NOTES
GigaScience Database 02 Jun 2017

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
GigaScience Database 02 Jun 2017

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

Extraction

Step 5.

Centrifuge samples at 6000 xG for 1 minute.

DURATION
00:01:00

Extraction

Step 6.

Mix 500 µl supernatant 1:8 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.

Purify samples with Monarch DNA Cleanup Columns (New England Biolabs, Massachusetts, USA).

Extraction

Step 8.

Bind DNA to the column.

Extraction

Step 9.

Wash with 800 µl buffer PE (Qiagen).

Extraction

Step 10.

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

Extraction

Step 11.

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

DURATION

00:05:00

Extraction

Step 12.

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

Extraction

Step 13.

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

DURATION

00:05:00

Extraction

Step 14.

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.