Extraction method C

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Abstract

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

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Before start

Clean

Materials

Monarch DNA Cleanup Columns (5ug) - 100 columns T1034L by New England Biolabs

Buffer EB 19086 by Qiagen

proteinase K containing buffer by Contributed by users

PB buffer 19066 by Qiagen

PE buffer 19065 by Qiagen

Protocol

Extraction

Step 1.

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

NOTES

GigaScience Database 02 Jun 2017

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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

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

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
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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; 2015;522:167–72.

Extraction

Step 7.

Centrifuge digests Monarch DNA Cleanup Columns (New England Biolabs, Massachusetts, USA).

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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.

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