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Extraction positive controls (EPCs) V.1

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Abstract

Protocol for the preparation of extraction positive controls (EPCs) in FluidX tubes. EPCs are used as part of the DNA extraction workflow of the Ancient DNA Core-Unit of the MPI-EVA.

Note

The lysate used for generating the EPCs was produced by lysing several grams of bone powder from a Pleistocene dolphin bone in a large volume of lysis buffer. All EPCs consist of 150 µl aliquots of this pooled lysate in FluidX screw-cap tubes. This document describes the preparation of a complete tube rack containing 96 EPCs.

Materials

	Reagent/consumable	Supplier	Catalogue number	Decontamination
	Reagents			
	Dolphin test lysate*	self	-	-
	Consumables			
	FluidX screw-cap tubes in FluidX 96-tube rack	Brooks Life Sciences	68-1003-11	-
	Filter tip, natur, 300 µl, low retention	Greiner Bio One	775353	-
	Disposable reagent reservoir, sterile, 25 ml	Roth	HT66.1	-

*Instructions for lysate preparation are provided in the protocol "Bravo workstation: automated ancient DNA extraction part I: lysate preparation". The protocol is included in the Appendix. The dolphin test lysate was prepared in a large scale volume (500 ml). Therefore the protocol was adapted accordingly.

Equipment

- FluidX rack barcode reader (e.g. Brooks Life Sciences, cat. no 20-4018)
- Tube decapper (e.g. Aperio 8-Channel Semi- Automatic Screw Top tube rack decapper, Brooks Life Sciences, cat. no. 46-6502)
- Multichannel pipette 8 × 300 µl (e.g. VWR International GmbH, cat. no. 55019-52)

Protocol

1. Get a new FluidX 96-tube rack and use the FluidX barcode reader to read the bottom barcodes of all empty tubes in the rack and the rack ID in the front.
2. Thaw the dolphin test lysate stock aliquot (50 ml), shake it and pour ~20 ml into a fresh reservoir.



3. Open the first column of FluidX screw-cap tubes in the sample rack with the decapper and pipette 150 μ l dolphin test lysate from the reservoir into the tubes using a multichannel pipette. Carefully close the tubes and open the next column.

Note

[Note]

Since there is only one column opened at a time the lids can stay on the decapper during the pipetting steps.

4. Fill the tubes in the rack column-wise until all tubes are filled.

Note

[Note]

Optional: repeat steps 1 - 4 in case you want to prepare another rack of EPCs. The reservoir containing the lysate can be re-used for all racks.

Note

[Documentation]

Label the FluidX rack with tube content (Extraction Positive Controls), date and initials.

Save the document(s) with rack and tube IDs under "P:\AncientDNA\FluidX BarCodeReader\FluidX_Data\Stock_Buffer_FluidXtubes" and label the rack file with rack ID, tube content, date and initials.

5. Store EPCs at -20 °C until used.

Appendix



Protocol

NAME

Ancient DNA extraction part I: lysate preparation

CREATED BY

Ancient DNA Core Unit

Preview