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Purification negative controls (PNCs) prepared on the Bravo NGS Workstation V.1

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Abstract

Protocol for the preparation of purification negative controls (PNCs) in FluidX tubes using the Bravo NGS Workstation. PNCs are used as part of the DNA extraction workflow of the Ancient DNA Core Unit of the MPI-EVA.

Note

PNCs consist of 150 µl extraction buffer stock solution in FluidX screw-cap tubes. This document describes the preparation of a complete tube rack containing 96 PNCs.

Materials

	Reagent/consumable	Supplier	Catalogue number	Decontamination *
	Reagents			
	Extraction buffer stock solution (w/o Proteinase K) [†]	self	-	UV
	Consumables			
	FluidX screw-cap tubes in FluidX 96-tube rack	Brooks Life Sciences	68-1003-11	-
	Bravo 96LT 250 µL, Sterile, Filtered Tips	Agilent	19477-022	-
	HTS deep well reservoir	Kisker	97813	-

* Decontamination of buffers should be performed as detailed in the documents below.

† See document in the Appendix for preparation of extraction buffer stock solution.

Equipment

- Bravo-B NGS workstation G5522A with 96-channel LT pipette head
- FluidX rack barcode reader (e.g. Brooks Life Sciences, cat. no 20-4018)
- Tube decapper (e.g. Apero 8-Channel Semi- Automatic Screw Top tube rack decapper, Brooks Life Sciences, cat. no. 46-6502)

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. Switch on the Bravo system. Switch on light and ventilation ("Betrieb") inside the robot hood.
3. Log into the VWworks software using the administrator account (password "a"). Load the buffer transfer protocol under



"S:\Bravo_protocols\MPI-EVAN-homebrew\forms\Reagent_preparation\BufferTransfer_to_FluidX_Tubes.VWForm".

4. Select the proper settings in the form file:

- Buffer volume (150 µl)
- Plate type for buffer transfer (0.8 ml FluidX tubes)

5. Fill the reservoir with approx. 30 - 50 ml extraction buffer stock solution (UV decontaminated) and place it on the position in the Bravo deck as indicated by the form file.

6. Unpack 1 Bravo Tip box and place it on the position in the Bravo deck as indicated by the form file.

7. Open the FluidX screw-cap tubes in the sample rack with the decapper and keep the lids by placing them in a clean 96 lid holder. You can find this holder next to the decapper.

8. Place the uncapped FluidX 96-tube rack into the position of the Bravo Deck as indicated by the form file.

9. Start Run by clicking the "Run" button (150 µl of extraction buffer stock solution buffer are now added to each tube).

10. After run has finished, close the FluidX tubes using the decapper. Use the lids from the lid holder.

Note

[Documentation]

Label the FluidX 96-tube rack with tube content (Purification Negative Controls), date and initials.

Save the document containing the tube IDs under "P:\AncientDNA\FluidXBarcodeReader\FluidX_Data\Stock_Buffer_FluidXTubes" and label rack file with rack ID, tube content, date and initials.

11. Store PNCs at -20 °C until used.

Appendix



Document

NAME

UV decontamination of reagents/buffers

CREATED BY

Elena Essel

Preview

Document



NAME

Extraction buffer stock solution (w/o Proteinase K)

CREATED BY

Ancient DNA Core Unit

Preview