Mar 07, 2024 Version 1

Library negative controls (LNCs) prepared on the Bravo NGS Workstation V.1

DOI
dx.doi.org/10.17504/protocols.io.261ged17wv47/v1

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MPI EVA Ancient DNA C...

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Document Citation: Anna Schmidt, Sarah Nagel, Matthias Meyer 2024. Library negative controls (LNCs) prepared on the Bravo NGS Workstation. **protocols.io https://dx.doi.org/10.17504/protocols.io.261ged17wv47/v1**

Created: January 09, 2024

Last Modified: April 10, 2024

Document Integer ID: 93178

Keywords: lnc, library negative control, bravo ngs workstation protocol for the preparation, negative control, bravo ngs workstation protocol, bravo ngs workstation, fluidx tube, protocol for the preparation, part of the library preparation workflow, ancient dna core unit of the mpi, library preparation workflow, ancient dna core unit, library preparation

Funders Acknowledgements:
Max Planck Society
Grant ID: -

Abstract

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

Troubleshooting

Note

LNCs consist of 30 µl EBT buffer in FluidX screw-cap tubes. This document describes the preparation of a complete tube rack with 96 LNCs.

Materials

	Reagent/consumable	Supplier	Catalogue number	Decontamination *
	Reagents			
	EBT buffer †	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 EBT buffer.

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. Aperio 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 tubes in the rack.

Note

[Note]

Optional: Usually up to 4 racks of LNCs are prepared at once. In case you want to prepare more than 1 rack, get the number of new FluidX 96-tube racks you need, scan them with the FluidX barcode reader and label the racks according to the details described below.

The protocol needs to be started independently for each FluidX rack. Take a fresh pipette tip box for each run. The EBT buffer reservoir will be re-used in all runs. Adjust the buffer volume accordingly (minimum buffer volume in the reservoir should be 30 ml; 50 ml are enough to fill up to 4 FluidX 96-tube racks).

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 (30 µl)
- plate type for buffer transfer (96 Ay Brooks_FluidX 1ml tubes)

5. Fill reservoir with approx. 30 ml EBT (UV decontaminated) and place it into the position in the Bravo deck indicated by the form file.

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

7. Open all tubes in the FluidX 96-tube rack using the decapper and keep the lids by placing them in a 96-format clean 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 indicated by the form file.

9. Start Run by clicking the "Run" Button (30 µl of EBT 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

[Note]

Optional: Put a fresh tip box and a fresh FluidX 96-tube rack into the Bravo Deck and start the protocol again if you want to prepare another rack of LNCs.

Note

[Documentation]

Label the FluidX 96-tube rack with tube content (Library Negative Controls), date and initials. If more than one rack is prepared, also add "rack x (consecutive number) of x (total amount of prepared racks)", e.g. "rack 3/4".

Save the document containing the tube IDs under "P:\AncientDNA\FluidXBarCodeReader\FluidX_Data\Stock_Buffer_FluidXTubes" and label the file with the date (YYYY.MM.DD), rack ID, tube content and initials of the worker, e.g. "20220111_FH00246461_EPCs_AS".

11. Store LNCs at -20 °C until used.

Appendix

Document

NAME

UV decontamination of reagents/buffers

CREATED BY

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Preview

Document

NAME

EBT buffer

CREATED BY

Anna Schmidt

Preview