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Library positive controls (LPCs) prepared on the Bravo NGS Workstation

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ABSTRACT

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

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Note

LPCs consist of 0.1 pmol oligonucleotide CL104 in 30 µl EBT buffer in FluidX screw-cap tubes. This document describes the preparation of a complete tube rack containing 96 LPCs.

Materials

	Reagent/consumable	Supplier	Catalogue number	Decontamination *
	Reagents			
	EBT buffer	self	-	UV
	0.1 µM oligonucleotide CL104 †	Sigma/Merck	-	-
	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	-
	Falcon tube 50 ml	Greiner Bio-one	210261	-

* Decontamination of buffers should be performed as detailed in the documents in the Appendix.
† Order oligonucleotide CL104 at 0.2 µmol synthesis scale (Sigma/Merck, desalted). Dissolve in TE buffer at a concentration of 100 µM (see document in the Appendix for preparation of TE buffer). Prepare 0.1 µM working dilution in TET buffer (see document in the Appendix for preparation of TET buffer). Sequence: 5'-Pho-TCGTCGTTTGGTATGGCTTCATTCAGCTCCGGTTCCCAACGATCAAGGCGAGTTACATGA-Pho-3'

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. In a 50 ml Falcon tube, combine 29 ml EBT with 1 ml 0.1 µM CL104. Mix properly. EBT buffer should be decontaminated using UV treatment. Instructions for UV-decontamination are provided in the Appendix.

2. Get a new FluidX 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 LPCs 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 oligo dilution will be re-used in all runs. Adapt the buffer volume accordingly. 30-50 ml are enough to prepare up to 4 racks of LPCs.

3. Switch on the Bravo system. Switch on light and ventilation ("Betrieb") inside the robot hood.

4. 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".

5. Select the proper settings in the form file:
- buffer volume (30 µl)
- plate type for buffer transfer (96 Ay Brooks_FluidX 1ml tubes)

6. Fill reservoir with approx. 30 - 50 ml oligo dilution and place it into the position in the Bravo deck indicated by the form file.

7. Unpack 1 Bravo tip box and place it into the position in the Bravo Deck indicated by the form file.

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

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

10. Start Run by clicking the "Run" Button (30 µl of oligo dilution are now added to each tube).

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

Note

[Note]

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

Note

[Documentation]

Label the FluidX rack with tube content (Library Positive 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 rack file with rack ID, tube content, date and initials.

12. Store LPCs at -20 °C until used.

Appendix

Document

NAME

TET buffer

CREATED BY

Sarah Nagel

PREVIEW

Document

NAME

UV decontamination of reagents/buffers

CREATED BY

Elena Essel

PREVIEW

Document

NAME

EBT buffer

CREATED BY

Anna Schmidt

PREVIEW

Document

NAME

TE buffer

CREATED BY

Anna Schmidt

PREVIEW