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Positive test extract (PTE) aliquots

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Abstract

This document describes the preparation of FluidX tubes containing positive test extract (PTE). It is part of the library preparation workflow of the Ancient DNA Core Unit of the MPI-EVA.

Note

A test extract prepared from animal or human material can be used as an additional positive control for in-depth testing of the performance of single-stranded library preparation, for example when testing new batches of oligonucleotides. This test extract is prepared in large volume in order to suffice for many experiments.

Materials

	Reagent/consumable	Supplier	Catalogue number	Decontamination *
	Reagents			
	EBT buffer	self	-	UV
	text extract	self	-	-
	Consumables			
	FluidX screw-cap tubes in Fluidx 96-tube rack	Brooks Life Sciences	68-1003-11	-
	Pipetting reservoir, 25 ml	Sigma Aldrich	Z680354	-

* Decontamination of reagents and consumables should be performed as detailed in the documents below.

Equipment

- FluidX rack barcode reader (Brooks Life Sciences, cat. no. 20-4018)
- multichannel pipette (e.g. Thermo Scientific Finnpipette, cat. no. 4661050N)

Protocol

1. In a 50 ml Falcon tube, prepare PTE dilution by combining 12.5 ml EBT with 2.5 ml test extract. Mix properly.

Note

[Documentation]

Note the lot numbers, extract ID, date and initials written on the reagents in Labfolder (orange fields).

2. Get a new FluidX rack and use the FluidX barcode reader to read the bottom barcodes of all tubes in the rack.

Note

[Documentation]

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.

3. Carefully open the first row of the FluidX rack with the FluidX decapper. Keep the clean lids on the decapper to use them later for closing the tubes.

4. Pour required amount of PTE dilution into a fresh reservoir.

5. Pipette 30 µl of PTE dilution into each of the 8 FluidX tubes using a multichannel pipette.

6. Carefully close the FluidX tubes and open the next row. Repeat this step until all tubes of the rack are filled.

Note

[Labeling]

Label the FluidX rack with tube content (Positive Test Extract), 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".

Note

[Note]

Optional: Get a new FluidX rack and repeat the whole procedure if you want to prepare another rack of PTEs. The PTE dilution in the reservoir can be re-filled and re-used.

7. Store PTE aliquots at -20 °C until used.

Appendix

Document

NAME

EBT buffer

CREATED BY

Anna Schmidt

PREVIEW

Document

NAME

UV decontamination of reagents/buffers

CREATED BY

Elena Essel

PREVIEW