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Preparation of indexing primer stock racks V.1

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MPI EVA Ancient DNA C...

Ancient DNA Core Unit
MPI EVA

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Abstract

Protocol for the preparation of tube racks in 96 SBS format containing indexing primers at a concentration of 100 µM. These stocks are needed by the Ancient DNA Core Unit of the MPI-EVA to create indexing primer aliquot plates for the automated set-up of indexing PCRs after library preparation.

Troubleshooting

Note

Lyophylized primers are dissolved to a concentration of 100 µM and transferred to FluidX tubes arranged in 96-well FluidX tube racks. P5 and P7 indexing primers are transferred to separate racks.

Materials

	Reagents/consumables	Supplier	Cat. no.
	Reagents		
	TE buffer*	self	-
	P5 and P7 indexing primers, lyophilized †	see indexing PCR protocol	-
	Consumables		
	1.0 ml external thread jacket tube	FluidX/Brooks	68-1003-11
	Filter tip, Natur, 1250 µl, low retention	Greiner Bio-One	778363
	Filter tip, Natur, 10 µl, low retention	Greiner Bio-One	771265

* See document in the Appendix for preparation of TE buffer.
† See indexing PCR protocol for primer sequences and order information.

Equipment

- Tube decapper (e.g., Aperio 8-Channel Semi- Automatic Screw Top tube rack decapper, Brooks Life Sciences, cat. no. 46-6502)
- Centrifuge for PCR plates (e.g., Eppendorf, cat. no. 5948000913)
- Table-top micro-centrifuge for 1.5 ml tubes (e.g., Carl Roth Mini-Zentrifuge ROTILABO, cat. no. T464.1)

Protocol

1. Dissolve each indexing primer in TE buffer to obtain a primer stock concentration of 100 µM. Vortex thoroughly and let stand for at least 10 minutes at room temperature.

2. Vortex the primer stock tubes again and briefly spin down.

3. To prevent liquid splashing caused by electrostatic effects, first transfer 5 µl of TE buffer to the bottom of each 1 ml FluidX tube in a FluidX tube rack. Then, add 300 µl of the 100 µM primer stock solution to each tube.

Note

[Note]
Arrange the P5 indexing primer FluidX stock tubes column-wise in a 96-well FluidX tube rack. Use the following setup:

- rack #1: P5 primers 001-096
- rack #2: P5 primers 097-192
- rack #3: P5 primers 193-288
- rack #4: P5 primers 289-384

Use the same set up for the P7 indexing primer FluidX stock tubes.

4. Scan each FluidX stock tube rack using the FluidX barcode reader and save the file here: P:\AncientDNA\indices\8bp_indices_in_FluidX

5. Store the racks at -20°C.

Appendix

Document

NAME

TE buffer

CREATED BY

Anna Schmidt

Preview