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🌐 Bravo workstation: Preparation of indexing primer aliquots in 384-well plates V.1

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Abstract

Protocol for the preparation of 384-well PCR plates containing 10 μ l primer aliquots of either P5 or P7 indices for indexing PCR at a concentration of 10 μ M. These aliquot plates are needed by the Ancient DNA Core Unit of the MPI-EVA for the automated set-up of indexing PCRs after library preparation. Primers are distributed to the 384-well plates in four 96-well quadrants.

Troubleshooting

Note

The protocol is divided into two parts: (i) the preparation of 10 μM primer dilutions, and (ii) the distribution of these dilutions to 384-well PCR plates. Nine 384-well plates are prepared at once. For high-throughput, it is advisable to perform three rounds of the protocol to obtain 2×27 primer aliquot plates.

Materials

	Reagents/consumables	Supplier	Cat. no.
	Reagents		
	Water, HPLC-grade	Sigma Aldrich/Merck	1153332500
	FluidX tube racks containing 4 \times 96 P5 and P7 indexing primer stocks in 1.0 ml external thread jacket tubes (100 μM)*	self	-
	Consumables		
	96LT 250 μl sterile, filtered tips	Agilent	19477-022
	HTS deep well reservoirs	Kisker	97813
	NUNC 96 deepwell plate	NUNC	736-0600
	Hard-Shell 384-well PCR plates, thin wall, skirted, clear/clear	Bio-Rad	HSP3801
	External Thread screw caps, orange, in re-usable carrier	FluidX/Brooks	68-53111-50N
	Microseal B Adhesive seals, optical	Bio-Rad	MDB1001

* See document in the Appendix for preparation of P5 and P7 primer stock racks.

Equipment

- Bravo-B NGS workstation G5522A with 96-channel LT pipette head
- Hood for NGS workstation B with HEPA-filtered ventilation system and strip curtain (custom-built, optional)

- Tube decapper (e.g. Apero 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)

Protocol

1. Thaw the FluidX tube racks containing the P5 and P7 indexing primer stocks overnight in the refrigerator. Before proceeding, ensure that all indexing primers are fully thawed.

Note

[Documentation]

Note the detailed information (date, initials) of the stock primers used in the respective field in Labfolder.

2. Switch on the required components of the Bravo system. The external cooling device is not needed in this protocol. Switch on light and ventilation ("Betrieb") inside the robot hood.
3. Log into the VWworks software using the administrator account (password "a"). Load the first part of the primer plate preparation protocol under "S:\Bravo_protocols\MPI-EVAN-homebrew\forms\Reagent_preparation\Part1_Aliquot_Stock_8bp_Indices_96NuncPlate.VWForm". Initialize the system.
4. Fill a buffer reservoir with 40 ml water and set up the Bravo deck as indicated by the form file. A total of eight tip boxes are required. These will be used throughout the protocol as prompted by the robot.
5. Briefly vortex the stock primer FluidX tube racks and spin them down in a plate centrifuge. Carefully open the tubes with the fluidX decapper and put the rack onto the indicated position of the robot deck.
6. Select the required volume for the transfer of stock primer. The volume required for running the protocol once (nine plates) is 10 μ l.
7. Start the run by clicking the button "Start aliquot stock index primers" and follow the instructions by VWorks. The software will ask for the respective stock primer FluidX tube rack and a fresh tip box. Click "continue" each time you provided the required consumables.

Note

[Note]

In the first part of the protocol, the robot will prepare a 10 μM dilution of the stock primers by combining water and the necessary volume of stock primer to the wells of a 96-well NUNC plate. The transfer of stock primers will be performed consecutively for all four 96-well FluidX tube racks containing either the P5 or P7 indexing primers to four NUNC plates.

Run time is approx. 30 min.

8. Once the transfer is complete carefully close the respective stock primer FluidX tube rack with clean FluidX screw caps using the FluidX decapper and store the rack at $-20\text{ }^{\circ}\text{C}$ in the freezer.
9. Go to step 7 and repeat until all four 96-well stock primer FluidX tube racks have been used.
10. Discard used tips into the laboratory trash and dispose of the empty tip boxes in the large waste bin located on the left side of the cooling unit.
11. Load the second part of the primer plate preparation protocol under "S:\Bravo_protocols\MPI-EVAN-homebrew\forms\Reagent_preparation\Part2_Aliquot_8bp_indices_384wellPlate.VWForm".
12. Set up Bravo deck as indicated by the form file.
13. Unpack 36 Bravo tip boxes. Remove the lids and load tip boxes into stackers 1-3 of the BenchCel.
14. Start the run by clicking the "Start aliquot index primers" button and follow the instructions by VWorks.

Note

[Note]

In the second part of the protocol, the robot will combine the 10 μM primer dilutions from the four 96-well NUNC plates to one 384-well plate by aliquoting 10 μl of the respective primer dilution to each well. Nine 384-well primer plates will be prepared. Run time is approx. 45 min.

Note

[Labeling]

Label the 384-well plates with batch and aliquot number, date and your initials. The batch number is sequential and increases with each new preparation of primer dilution plates. The aliquot number corresponds to the number of 384-well plates prepared from each batch (aliquot 1-9).

15. Carefully seal the 384-well aliquot plates with optical Microseal B Adhesive foil and store them at -20°C.
16. Remove all plastic ware from the robot. Discard used tips into laboratory trash and dispose of the tip boxes in the large waste bin located on the left side of the cooling unit.
17. Clean the robot by wiping the Bravo deck and the benches using tissues and ethanol. Switch off the robot and restart the computer. Switch off the computer screen.

Bravo electronic protocol files

Available on Github: <https://github.com/adnacore/Bravo-workstation-single-stranded-DNA-library-preparation-ssDNA2.0/tree/v1.0>

Appendix

Document for Preparation of P5 and P7 stock primer plates

Document

NAME

Preparation of indexing primer stock racks

CREATED BY

Ancient DNA Core Unit

Preview