16S Illumina Primer Plate Aliquoting (working plates 5µM Forw & Rev)

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ABSTRACT

The following protocol outlines aliquoting resuspended primers, stored at 100µM in stock plates, into working primer plates at a conentration of 5µM for both forward (barcoded) and reverse primers, using the epMotion 5075. The Knigh Lab stores primer combos premixed in working primer plates.

PROTOCOL STATUS

Working

We use this protocol in our group and it is working

MATERIALS

NAME	CATALOG #	VENDOR
Eppendorf twin.tec® PCR 96-well plate, skirted	951020401	Eppendorf
ep T.I.P.S. Motion Racks 20 - 300 µL w/ filter	0030014456	Eppendorf
ep T.I.P.S 1 - 50 µL w/filter	0030015215	Eppendorf
epMotion Reservoir 30 mL	960051009	Eppendorf
IDT Resuspended Primer Plates (100µM)	View	Integrated DNA Technologies
IDT Lyophilized Primer in vial	View	

MATERIALS TEXT

(4) IDT 96-Well Resuspended Primer Plates (100 µM)
(1) IDT Lyophilized Reverse Primer in vial.
(4) 96-Well Eppendorf twin.tec plates
(4) ep T.I.P.S. Motion Racks 1 - 50 µL w/ filter
(1) ep T.I.P.S. Motion Racks 20 -300 µL w/ filter
(1) epMotion Reservoir 30 mL

BEFORE STARTING

Please wear at least the minimum required personal protective equipment.
Ensure that all necessary kit components are available as well as user-supplied consumables.
Remove nuclease and nucleotide contamination from work surfaces and instruments prior to starting using an appropriate solution, such as RNase AWAY™ (Thermo Scientific™ catalogue: 700511), followed by wiping with 70% to 100% molecular biology grade ethanol to remove additional contaminants.

Prepare reagents

1 Resuspend Reverse Primer from lyophilized sample vial to a final concentration of 100 µM

2 In a sterile 30mL reservoir, add 15,840 µL of PCR Clean Water and 880 µL of Reverse Primer (100 µM) to make (4) 96-Well primer working plates.

15840 µl PCR Clean Water | 880 µl Reverse Primer (100µM)

Prepare Plates

3 Thaw and centrifuge 96-Well resuspended primer plates.

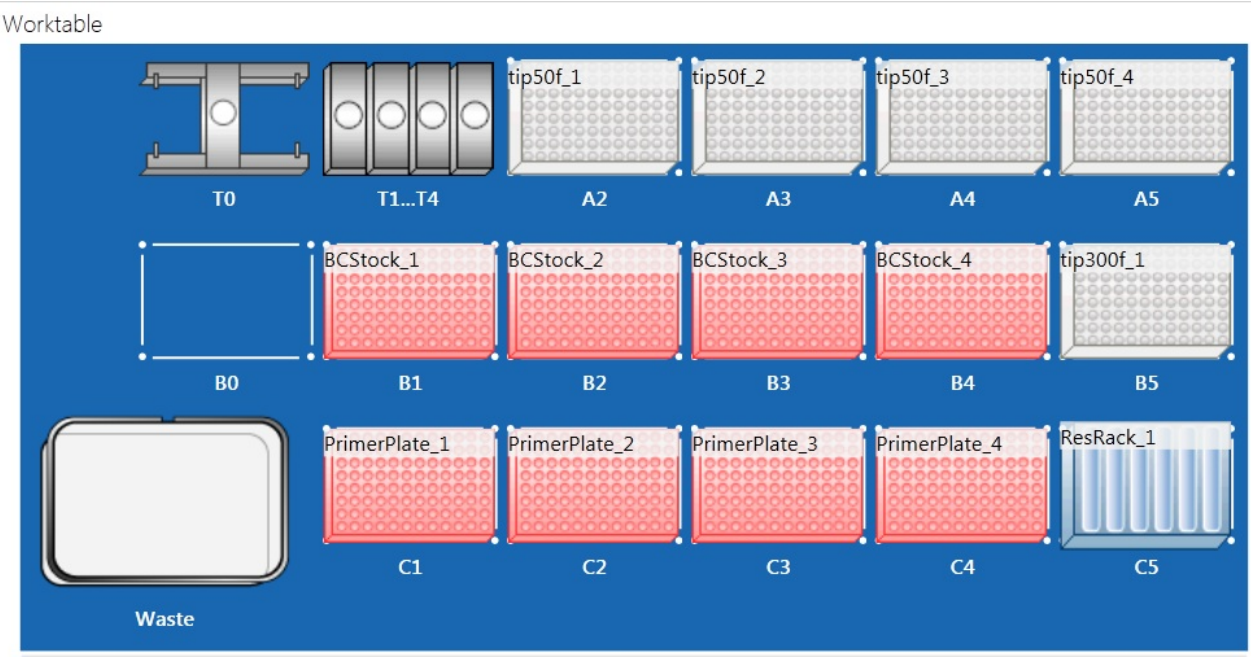
4 Appropriately label destination plates.

Setup epMotion automation platform.

5

EQUIPMENT
epMotion 5075
Liquid Handling
Eppendorf 5075000962

Follow the diagram bellow while setting up the epMotion worktable



Place (4) boxes of ep.T.I.P.S. Motion Racks 1 - 50 µL w/ filter in slots A2-A5
Place (1) box of ep. T.I.P.S. Motion Racks 20 - 300 µL w/ filter in slot B5
Place (4) 96-Well plates of resuspended primers (100µM) from IDT in slots B1-B4
Place (4) 96-Well twin.tec plates in slots C1-C4.
Place 30 mL reservoir with reverse primer (5.26 µM) in slot 1 of the Resevoir Rack and place Rack in slot C5

Execute automated protocol

6 Remove box lids and plate foils and execute protocol.

(Protocol must be imported to epBlue software prior to attempting to execute it. *epBlue 40.6 or later*)

Application_4_5uM_Primer_40ul_181003_105836.export6

The automated protocol transfers 38µL of reverse primer (5.26µM) into the (4) different 96-Well Primer Plates using the multidispense feature of the epBlue software.
Then it transfer 2µL of barcoded forward primers (100µM) from IDT stock plates into the (4) different 96-Well Primer Plates (working plates)

Seal and store plates.

7 Remove plates from worktable and seal with storage aluminum foils.