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

 [mSystems](#)

DOI

<https://dx.doi.org/10.17504/protocols.io.uz7ex9n>

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Protocol status: Working

We use this protocol and it's working

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Protocol Integer ID: 17183

Keywords: illumina primer plate aliquoting, resuspended primer, working primer plate, primer, knigh lab stores primer combo, working plates 5µm forw, 100µm in stock plate, stock plate, plate, working plate, following protocol outline, 100µm

Abstract

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

Materials

MATERIALS

✕ Eppendorf twin.tec® PCR 96-well plate, skirted **Eppendorf Catalog #951020401**

✕ ep T.I.P.S. Motion Racks 20 - 300 µL w/ filter **Eppendorf Catalog #0030014456**

✕ ep T.I.P.S 1 - 50 µL w/filter **Eppendorf Catalog #0030015215**

✕ epMotion Reservoir 30 mL **Eppendorf Catalog #960051009**

✕ IDT Resuspended Primer Plates (100µM) **Integrated DNA Technologies, Inc. (IDT)**

✕ IDT Lyophilized Primer in vial

(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 start

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 (100 μM)

Prepare Plates

- 3 Thaw and centrifuge 96-Well resuspended primer plates.
- 4 Appropriately label destination plates.

Setup epMotion automation platform.

5

Equipment

epMotion 5075

NAME

Liquid Handling

TYPE

Eppendorf

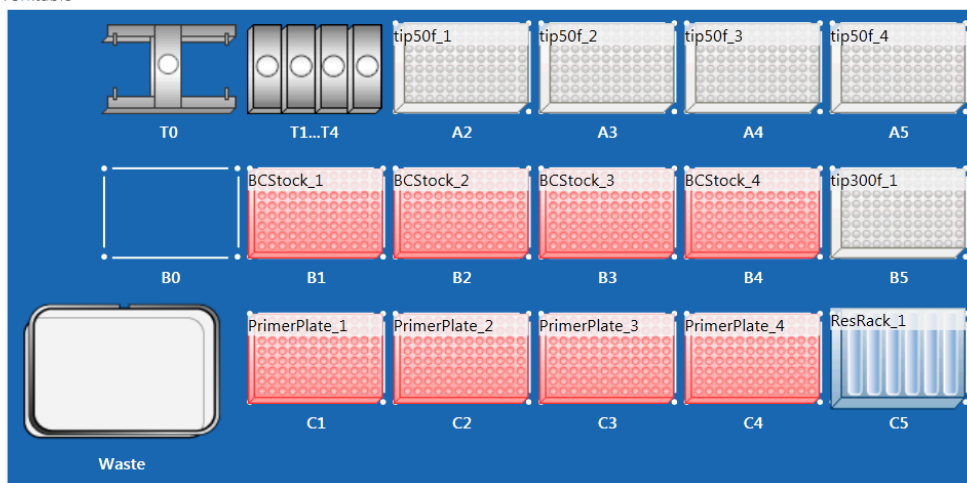
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SKU

Follow the diagram bellow while setting up the epMotion worktable

Worktable



Place (4) boxes of ep.T.I.P.S. Motion Racks 1 - 50 μ L w/ filter in slots A2-A5
Place (1) box of ep. T.I.P.S. Motion Racks 20 - 300 μ L w/ filter in slot B5
Place (4) 96-Well plates of resuspended primers (100 μ M) 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 μ M) in slot 1 of the Reservoir 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_181...

The automated protocol transfers 38 μ L of reverse primer (5.26 μ M) into the (4) different 96-Well Primer Plates using the multidispense feature of the epBlue software.
Then it transfer 2 μ L of barcoded forward primers (100 μ M) 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.