NOV 07, 2018

OPEN ACCESS

DOI:

[dx.doi.org/10.17504/protocols.io.uz7ex9n](https://doi.org/10.17504/protocols.io.uz7ex9n)

External link:

<https://doi.org/10.1128/mSystems.00166-18>

Protocol Citation: Rodolfo A Salido Benitez 2018. 16S Illumina Primer Plate Aliquoting (working plates 5µM Forw & Rev). **protocols.io** <https://dx.doi.org/10.17504/protocols.io.uz7ex9n>

MANUSCRIPT CITATION:

Minich JJ, Humphrey G, Benitez RAS, Sanders J, Swafford A, Allen EE, Knight R, High-Throughput Miniaturized 16S rRNA Amplicon Library Preparation Reduces Costs while Preserving Microbiome Integrity. *mSystems* 3(6). doi: [10.1128/mSystems.00166-18](https://doi.org/10.1128/mSystems.00166-18)

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

mSystems

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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 Knight Lab stores primer combos premixed in working primer plates.

MATERIALS

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✕ 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

✕ IDT Lyophilized Primer in vial Contributed by users

(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

Protocol status: Working
We use this protocol and it's working

Created: Oct 25, 2018

Last Modified: Nov 07, 2018

PROTOCOL integer ID:
17183

BEFORE START INSTRUCTIONS

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

15840 μ L PCR Clean Water

880 μ L Reverse Primer (100 μ M)

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	BRAND
5075000962	SKU

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 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_181003_105836.export6

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.