16S Illumina Primer Plate Resuspension (100 µM)

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ABSTRACT

The following protocol describes resuspension of lyophilized primers from (4) 96-Well plates shipped from Integrated DNA Technologies (IDT) at 3nmol per well using the epMotion 5075.

For information about ordering consult the Earth Micriobiome Projects website.

Barcoded primer constructs for 16S V4 region are also described in the Earth Microbiome Project website.

PROTOCOL STATUS

Working

We use this protocol in our group and it is working

MATERIALS

NAME	CATALOG #	VENDOR
ep T.I.P.S. Motion Racks 20 - 300 µL w/ filter	0030014456	Eppendorf
PCR Clean Water	View	
IDT Lyophilized Primer Plate	View	Integrated DNA Technologies
epMotion Reservoir 30 mL	960051009	Eppendorf

MATERIALS TEXT

(4) ep T.I.P.S. Motion Racks 20 -300 µL w/ filter
(4) IDT 96-Well Lyophilized Primer Plates (3 nmoles of primer per well)
(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 In a sterile 30mL reservoir, add 11.725 ml (required minimum volume) of PCR Clean Water to resuspend (4) 96-Well plates.

11.725 ml PCR Clean Water

Prepare Plates

2 Centrifuge all 96-Well primer plates to ensure lyophilized sample is at the bottom of the well. (3nmol primer per well)

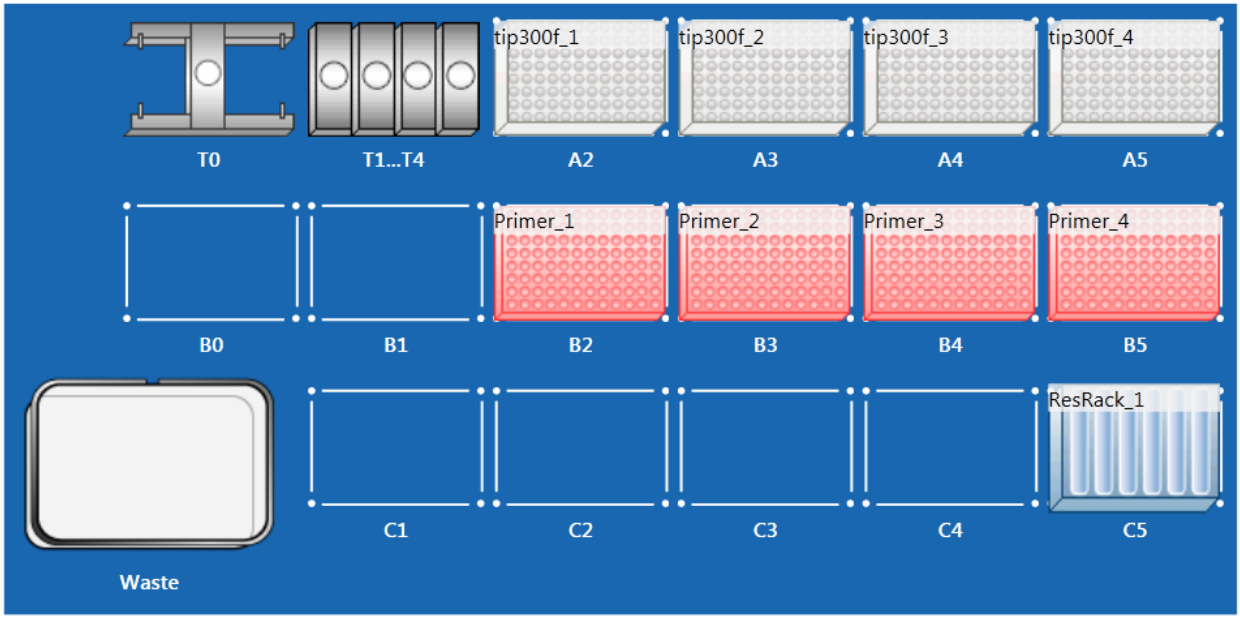
Setup epMotion automation platform

3

EQUIPMENT
epMotion 5075
Liquid Handling
Eppendorf 5075000962

Follow the diagram below while setting up the epMotion worktable.

Worktable



Place (4) boxes of ep.T.I.P.S. Motion Racks 20 - 300 µL w/ filter in slots A2-A5
Place (4) 96-Well plates of lyophilized primers from IDT in slots B2-B5
Place 30 mL reservoir with PCR Clean Water in slot 1 of the Resevoir Rack and place Rack in slot C5

Execute automated protocol

4 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_Resus_100uM_Primers_181003_105824.export6

The automated protocol transfers 30µL of PCR Clean Water into the (4) different 96-Well Primer Plates and mix pipettes for 5 cycles to resuspend lyophilized primers to a 100 µM)

Seal and store plates.

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