PCR Master Mix Aliquoting

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ABSTRACT

The protocol outlines how to aliquot PCR Master Mix for 5µL miniaturized amplification of 16S gene in 384-well format using the epMotion.

PROTOCOL STATUS

Working

We use this protocol in our group and it is working

MATERIALS

NAME	CATALOG #	VENDOR
Platinum Hot Start PCR Master Mix (2x)	13000014	Thermo Fisher Scientific
twin.tec PCR Plate 384	951020729	Eppendorf
epMotion Reservoir 10 mL	0030126521	Eppendorf
ep T.I.P.S 1 - 50 µL w/filter	0030015215	Eppendorf
PCR Clean Water	View	

MATERIALS TEXT

(1) ep T.I.P.S. Motion Racks 1- 50 µL w/filter
(1) epMotion reservoir 10mL
(3) twin.tec PCR Plate 384
Platinum Hor Start PCR Master Mix 2X

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.

Mix reagents

1 In a sterile 10mL reservoir, add 2,534 µl of Platinum Hot Start PCR 2X Master Mix and 3,295 µL of PCR Clean Water per set of 384 samples

2534 µl 2X PCR Master Mix **3295 µl PCR Clean Water**

Mix pipette.

Setup epMotion automation platform

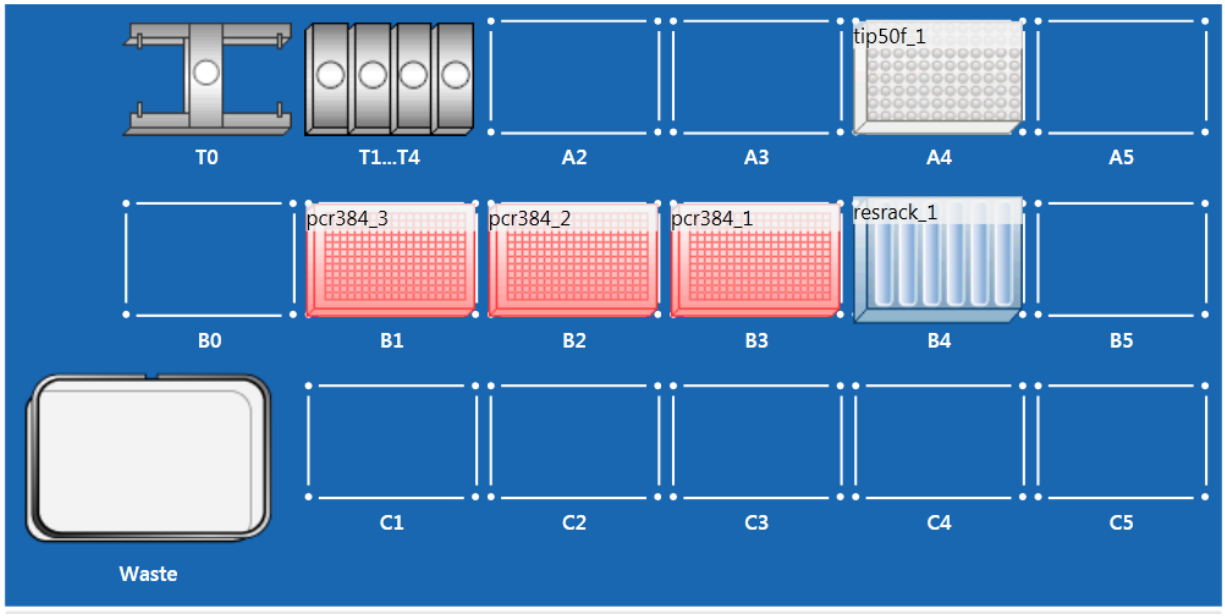
2

EQUIPMENT

epMotion 5075
Liquid Handling
Eppendorf 5075000962

Follow the diagram below while setting up the epMotion worktable.

Worktable



Place (1) box of 1-50µL epT.I.P.S. w/filters on deck slot A4
Place empty 384 PCR Primer Plates 1-3 on deck stols B1-B3
Place 10mL reservoir with PCR Master Mix in the first slot of the reservoir rack, then place reservoir rack on deck slot B4.

Execute automated protocol

3 Remove box lids and execute protocol.

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

Application_3XPCR_mastermix aliquot 4ul_384eppendorf_181003_105306.export6

The automated protocol transfers 4.6 µL of PCR Master Mix into (3) 384-well PCR plates using the multidispense feature of the epBlue software.