3X PCR Replicate Pooling

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dx.doi.org/10.17504/protocols.io.ue8ethw

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ABSTRACT

The following protocol pools miniaturized PCR replicates from (2) different 384-well plates into (1) final 384-well plate using the epMotion 5075. The miniaturized PCR reaction was done in triplicate across (3) 384-well plates and the third PCR plate will be the final storage plate for PCR products.

PROTOCOL STATUS

Working

We use this protocol in our group and it is working

MATERIALS

NAME	CATALOG #	VENDOR
twin.tec PCR Plate 384	951020729	Eppendorf
ep T.I.P.S 1 - 50 µL w/filter	0030015215	Eppendorf

MATERIALS TEXT

(3) twin.tec PCR Plate 384
(4) ep T.I.P.S. 1-50 µL Motion Racks w/filter

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 PCR plates

1 If needed, thaw PCR plates, then centrifuge them.

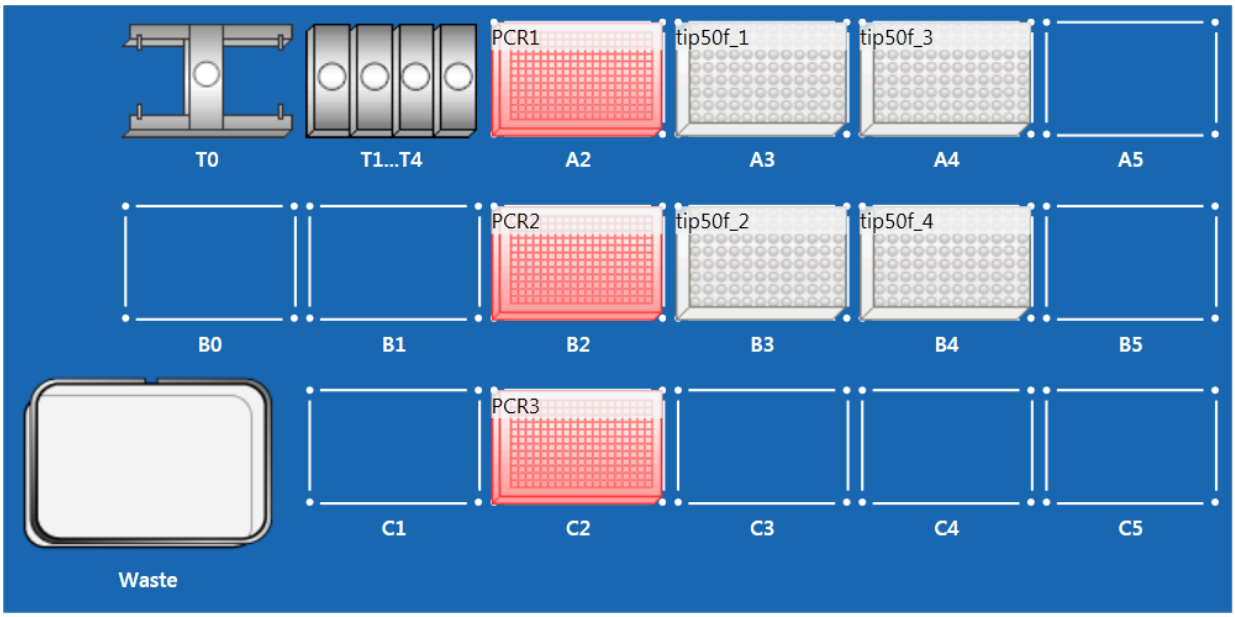
Setup epMotion automation platform

2

EQUIPMENT
epMotion 5075
Liquid Handling
Eppendorf 5075000962

Follow the diagram while setting up the epMotion worktable.

Worktable



Place (4) boxes of 1-50µL ep T.I.P.S. w/filter on slots A3-A4 and B3-B4 respectively.
Place PCR plates on slots A2, B2, and C2.

Execute automated protocol

3 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_3X_pooling_3-384 to 1-384_181003_105035.export6

The automated protocol uses the multiaspirate feature of the epBlue software to pool PCR products from plates PCR1 and PCR2 and dispense them into plate PCR3. The command aspirates 7µL from each plate which will result in some air being aspirated.