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

<table>
<thead>
<tr>
<th>MATERIALS</th>
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<tbody>
<tr>
<td>Eppendorf twin.tec® PCR 96-well plate, skirted Eppendorf Catalog #951020401</td>
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<tr>
<td>ep T.I.P.S. Motion Racks 20 - 300 µL w/ filter Eppendorf Catalog #0030014456</td>
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<tr>
<td>ep T.I.P.S 1 - 50 µL w/ filter Eppendorf Catalog #0030015215</td>
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<tr>
<td>epMotion Reservoir 30 mL Eppendorf Catalog #960051009</td>
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<tr>
<td>IDT Resuspended Primer Plates (100µM) Integrated DNA Technologies</td>
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<tr>
<td>IDT Lyophilized Primer in vial Contributed by users</td>
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(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 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.

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.

3. Thaw and centrifuge 96-Well resuspended primer plates.

4. Appropriately label destination plates.
Follow the diagram below while setting up the epMotion worktable

![Diagram of 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

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*)

The automated protocol transfers 38µL of reverse primer (5.26µM) into the (4) different 96-Well Primer Plates using the multidispense feature of the epBlue software. Then it transfers 2µL of barcoded forward primers (100µM) from IDT stock plates into the (4) different 96-Well Primer Plates (working plates).

**Seal and store plates.**

Remove plates from worktable and seal with storage aluminum foils.