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Sub-microliter Primer and gDNA Dispense

mSystems

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

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Abstract

The following protocol describes the acoustic droplet ejection dispenses needed for a minitaturized 16S PCR reaction using the Echo 550. The protocol expects (1) 384-Well 16S Illumina Primer Plate in an echo qualified Low Dead Volume (LDV) plate, (1) 384-Well Extracted gDNA in an echo qualified Polypropylene (PP) plate, and (3) 384-Well PCR Plates in twin.tec PCR plates.

Materials

MATERIALS

384-Well Low Dead Volume (LDV) Microplate **Catalog #**LP-0200

384-Well Polypropylene Microplate **Catalog #**P-05525

twin.tec PCR Plate 384 **Eppendorf Catalog #**951020729

(1) 384-Well Low Dead Volume (LDV) Echo Qualified Microplate.
(1) 384-Well Polypropylene (PP) Echo Qualified Microplate.
(3) 384-Well twin.tec 384-Well PCR Plates

Before start

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 Plates

1 Thaw and centrifuge primer, gDNA, and PCR plates.

Safety information

Centrifugation speed for echo qualified Low Dead Volume (LDV) plates **must not** exceed 1500rpm.

2

Note

The following steps are optional. The Knight Lab performs them to ensure enough source material is present in source plates before executing the protocol.

Survey source plates to ensure the Primer and gDNA source plates have enough material to execute the protocol to completion.

Open Echo 550 Liquid Handler software. Go to Diagnostics tab. Click Source Plate Out. Place plate to be surveyed in source plate tray ensuring that the instrument has the appropriate plate insert, then click Source Plate In. Select appropriate plate profile when prompted. Under Miscellaneous, click the dropdown menu and select Survey, then click Launch. A window will pop up. Click Go in the new window to start the plate survey.

Note

384-Well Polypropylene (PP) plate expects the 2.10 mm insert
The Knight Lab uses the following plate profile for the PP plate: 384PP_AQ_BP2_HT
Working range of volumes for the PP plate is 15 - 65 µL.

384-Well Low Dead Volume (LDV) plate expects the 4.50 mm insert
The Knight Lab uses the following plate profile for the LDV plate: 384LDV_AQ_B2_HT
Working range of volumes for the LDV plate is 3-12 µL

Execute acoustic droplet ejection transfers

3

Equipment	
Echo 550	NAME
Liquid Handling	TYPE
Labcyte	BRAND
GEN-27	SKU

Execute the following Echo 550 protocol to transfer 16S primers. You'll need the Echo Plate Reformat software.

Mini-PCR_primer_dispense.epr

The protocol will transfer 200 nL of primers from each source well of the LDV source plate to the corresponding destination well for a total of 3 copies of the twin tec PCR destination plates.

4 Execute the following Echo 550 protocol to trasnfer gDNA.

Mini-PCR_gDNA_dispense.epr

The protocol will transfer 200 nL of gDNA from each source well of the PP source plate to the corresponding destination well for a total of 3 copies of the same twin tec PCR destination plates used in last step.

Seal plates

5 Seal all source plates with storage aluminum foils.

Seal all destination plates with thermo-cycler compatible aluminum foils and centrifuge them.