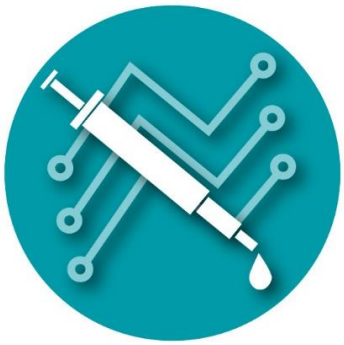

EFEVRE TECH LTD

YOUR NECESSITY, OUR INNOVATION

Automation of 16S Metagenomic Library Preparation by AMGEL technology

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Serial number : AMGEL2021N1001CY
Intended use : Laboratory automation for Research only

Contents

AUTOMATION OF 16S METAGENOMIC LIBRARY PREPARATION

Consumables / Labware calibrated on AMGEL.

Fully skirted 96-well PCR plates

a) 4ti-tude #4ti-0960
4TI FrameStar® 96 Well Skirted PCR Plate clear, wells, purple frame

b) 4ti-tude #4ti-0740 for PICO prep only

c) Alternative (calibration needed) : Eppendorf or Bio-Rad plates.

Reservoir plates

a) 4ti-tude #4ti-0131
4TI 12 Channel Reservoir Plate 21ml channels

b) Alternative (calibration needed) : NEST 12-Well Reservoirs, 15 mL – Need calibration.

96-well deep-well plates

a) ABGENE #AB-0765 #AB-0859

b) Alternative (calibration needed) : NEST 2 mL 96-Well Deep Well Plate, V Bottom

c) Alternative (calibration needed) : 4ti-tude #4ti0132.

Tips

a) Opentrons 20ul Tips
b) Opentrons 300ul Tips

Alternative (Calibration needed):

a) Opentrons 20ul Filter Tips
b) Opentrons 200ul Filter Tips.

Tubes

a) Eppendorf 1.5ml and 2ml tube

b) 15ml tubes Thermo scientific #339650 (NUNC)

Automated procedures

1. Amplicon PCR

- Duration : 0 hours, 07 minutes, 49 seconds
- Positions: Deck 2, Bioshake
- Fully automated : User actions are NOT required during process running.

2. PCR Clean Up 1

- Duration : 2 hours, 15 minutes, 28 seconds
- Positions: Deck 2, Bioshake, Magnetic module
- Fully automated : User actions are NOT required during process running.

3. Index PCR

- Duration : 0 hours, 47 minutes, 55 seconds
- Positions: Deck 1
- Fully automated : User actions are NOT required during process running.

4. PCR Clean Up 2

- Duration : 2 hours, 15 minutes, 28 seconds
- Positions: Deck 2, Bioshake, Magnetic module
- Fully automated : User actions are NOT required during process running.

5. DNA dilution 1:20

- Duration : 0 hours, 31 minutes, 34 seconds
- Positions: Deck 1
- Fully automated : User actions are NOT required during process running.

6. Pico standards

- Duration : 0 hours, 24 minutes, 07 seconds
- Positions: Deck 1
- Fully automated : User actions are NOT required during process running.

7. Pico prep

- Duration : 0 hours, 18 minutes, 58 seconds
- Positions: Deck 1
- Fully automated : User actions are NOT required during process running.

8. Normalization

- Duration : 1 hours, 30 minutes, 00 seconds
- Positions: Deck 1
- Fully automated : User actions are NOT required during process running.

9. Pooling plate A

- Duration : 0 hours, 35 minutes, 26 seconds
- Positions: Deck 1
- Fully automated : User actions are NOT required during process running.

9. Pooling plate B

- Duration : 0 hours, 35 minutes, 17 seconds
- Positions: Deck 1
- Fully automated : User actions are NOT required during process running.

9. Pooling plate C

- Duration : 0 hours, 35 minutes, 09 seconds
- Positions: Deck 1
- Fully automated : User actions are NOT required during process running.

9. Pooling plate D

- Duration : 0 hours, 35 minutes, 04 seconds
- Positions: Deck 1
- Fully automated : User actions are NOT required during process running.

Instructions for running the automated procedures.

1. Amplicon PCR _ DECK 2

1.1 Description

The Amplicon PCR procedure on AMGEL platform is preparing the Amplicon PCR plate, which is then manually loaded onto a Thermal cycler.

Amplicon PCR program on Thermal Cycler amplifies template out of a DNA sample using region of interest specific primers with overhang adapters attached.

1.2 Source

16S Metagenomic Sequencing Library Preparation Guide Page 6.

https://support.illumina.com/content/dam/illumina-support/documents/documentation/chemistry_documentation/16s/16s-metagenomic-library-prep-guide-15044223-b.pdf

1.3 Consumables

Item	Quantity	Storage
Microbial Genomic DNA (5 ng/µl in 10 mM Tris pH 8.5)	2.5 µl per sample	-15° to -25°C
Amplicon PCR Reverse Primer (1 µM)	5 µl per sample	-15° to -25°C
Amplicon PCR Forward Primer (1 µM)	5 µl per sample	-15° to -25°C
2x KAPA HiFi HotStart ReadyMix	12.5 µl per sample	-15° to -25°C

1.4 Labware setup

Please place the following labware on Liquid handling robot Deck 2:

DECK 2

Position 1:

Module/Labware : Magnetic module

Model : Opentrons GEN2

Containing : Nothing - No labware

Position 2:

Module/Labware : 96-well deep-well plate

Model : ABGENE #AB-0765

Containing : Nothing – Empty wells

Position 3:

Module/Labware : Bioshake module

Model : Qinstruments Bioshake 3000T-elm

Containing : Nothing - No labware

Position 4:

Module/Labware : Fully skirted PCR plate _ Amplicon PCR plate

Model : 4ti-tude #4ti-0960

Containing : 2.5ul microbial genomic DNA (5ng/ul in 10mM Tris pH8.5) in each well.

Position 6:

Module/Labware : Tube rack for 15ml tubes

Model : 15ml tube Thermo scientific #339650 (NUNC) on A1 of tube rack

Containing : 2385ul Master Mix (see 1.3 Consumables) for 106 samples (96+10).

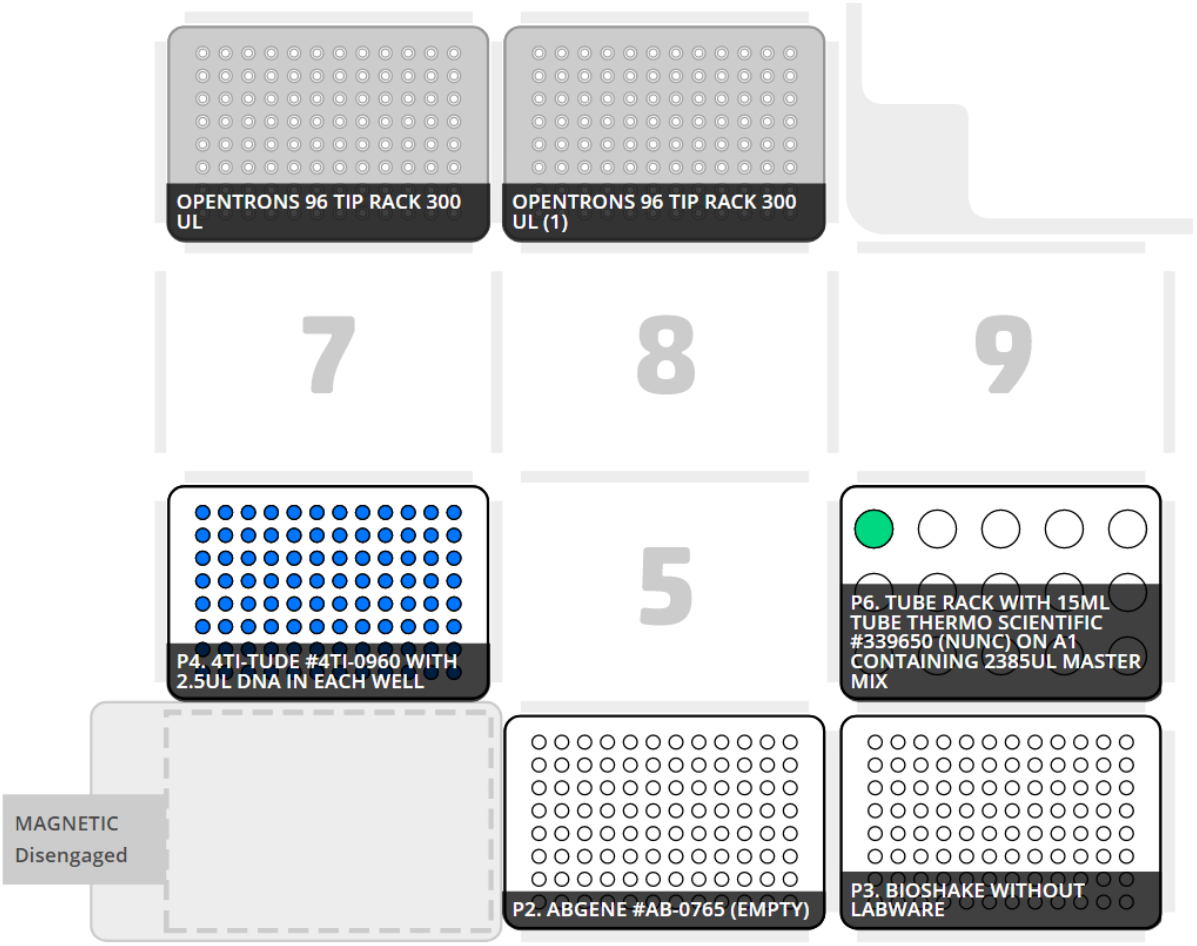
Position 10, 11:

Module/Labware : Tip rack

Model : Opentrons 300ul Tips

Containing : 96 Tips per rack

DECK 2



1.5 Run automated protocol

1.5.1 Press the Amplicon PCR button on the left-hand side of AMGEL interface and follow instructions to START the process for Amplicon PCR plate preparation.
1.5.2 The Status is now RUNNING.
1.5.3 Estimated time for user action on Status page is the walk-away duration. Please return to the platform when the walk-away duration is finished.
1.5.4 After the automated process is finished the Status changes from RUNNING to IDLE. Please do the following:

a) Remove all labware from Deck 2.
b) Empty trash bin
c) Seal the Amplicon PCR plate (P4) and spin down the liquid.

d) Transfer the Amplicon PCR plate to a thermal cycler and run the following PCR procedure:
   - 95°C for 3 minutes
   - 25 cycles of:
     - 95°C for 30 seconds
     - 55°C for 30 seconds
     - 72°C for 30 seconds
   - 72°C for 5 minutes
   - Hold at 4°C

ⓘ Source: 16S Metagenomic Sequencing Library Preparation Guide Page 7. https://support.illumina.com/content/dam/illumina-support/documents/documentation/chemistry_documentation/16s/16s-metagenomic-library-prep-guide-15044223-b.pdf

1.6 Automated Amplicon PCR protocol steps

1. Transferring Master Mix to Deep plate.
   P300
   Transferring 297ul Master mix
   From Tube on P6. A1
   To each well of P2. Deep plate Column 2.

2. Transferring Master Mix to Amplicon PCR plate.
   M300
   Distributing 22.5ul Master mix
   From P2. Deep plate Column 2
   To each well of P4. Amplicon PCR plate.

2. PCR Clean Up 1 _ DECK 2

2.1 Description

This step uses AMPure XP beads to purify the 16S V3 and V4 amplicon away from free primers and primer dimer species.

2.2 Source

16S Metagenomic Sequencing Library Preparation Guide Page 8.

ⓘ https://support.illumina.com/content/dam/illumina-support/documents/documentation/chemistry_documentation/16s/16s-metagenomic-library-prep-guide-15044223-b.pdf

2.3 Consumables

Item	Quantity	Storage
10 mM Tris pH 8.5	52.5 µl per sample	-15° to -25°C
AMPure XP beads	20 µl per sample	2° to 8°C
Freshly Prepared 80% Ethanol (EtOH)	400 µl per sample	

2.4 Labware setup

Please place the following labware on Liquid handling robot Deck 2:

DECK 2

Position 1:

Module/Labware : Magnetic module

Model : Opentrons GEN2

Containing : Nothing- No labware

Position 2:

Module/Labware Option 1: Reservoir plate

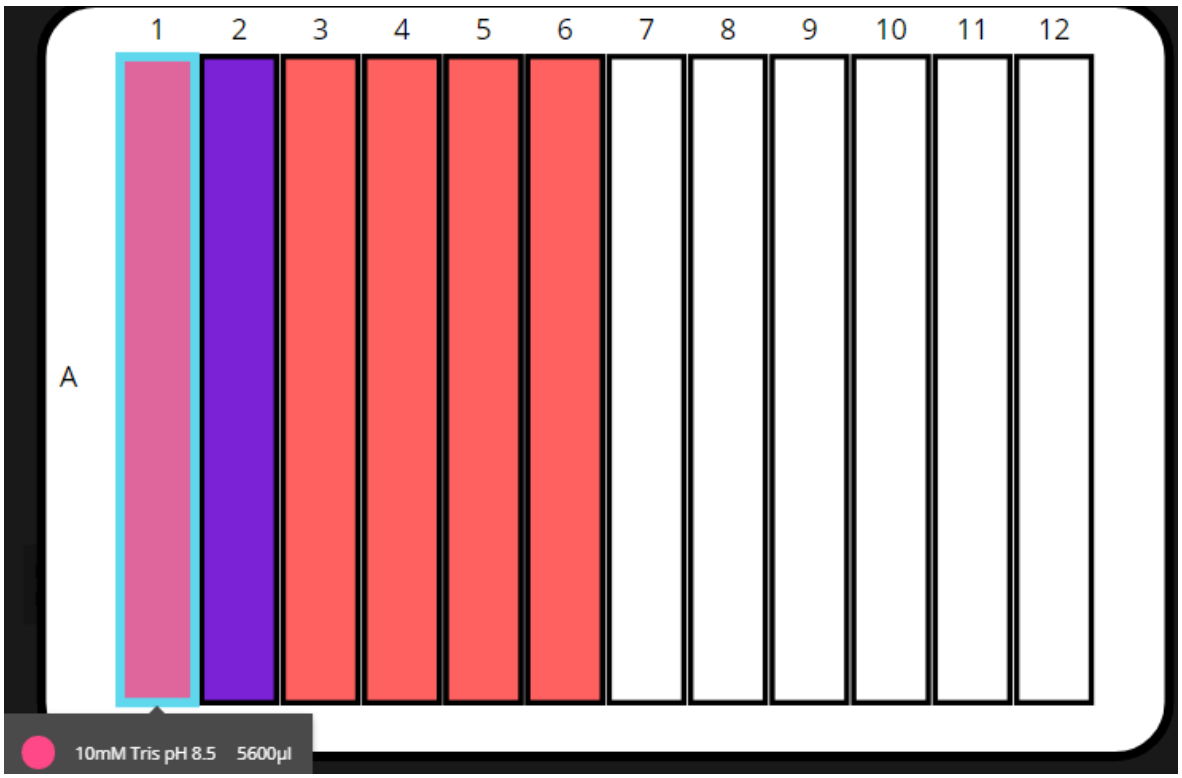
Model Option 1 : 4ti-tude #4ti-0131

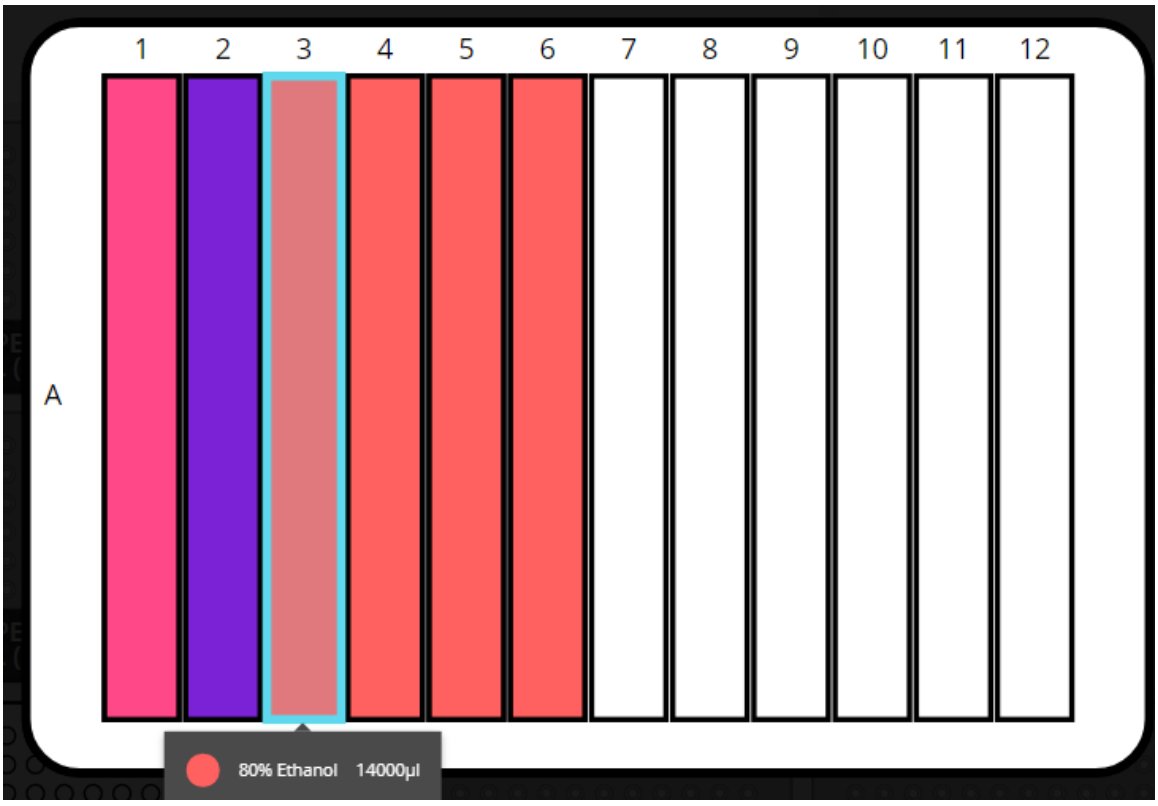
Containing :

Column 1 : 5600 ul10mM Tris pH 8.5 in column 1 of Reservoir plate

Column 2 : 5000 ul AMPure XP beads in column 2 of Reservoir plate

Columns 3, 4, 5, 6 : 14000 ul 80% Ethanol in column 3, 4, 5, 6 of Reservoir plate





Position 3:

Module/Labware : Bioshake module

Model : Qinstruments Bioshake 3000T-elm

Containing : Amplicon PCR plate (after Thermal cycler Amplicon PCR program is executed)

⚠ *Open ELM fingers before placing PCR plate on Bioshake!*

Position 4:

Module/Labware : Fully skirted PCR plate (Clean up 1 plate)

Model : 4ti-tude #4ti-0960

Containing : Nothing – Empty wells

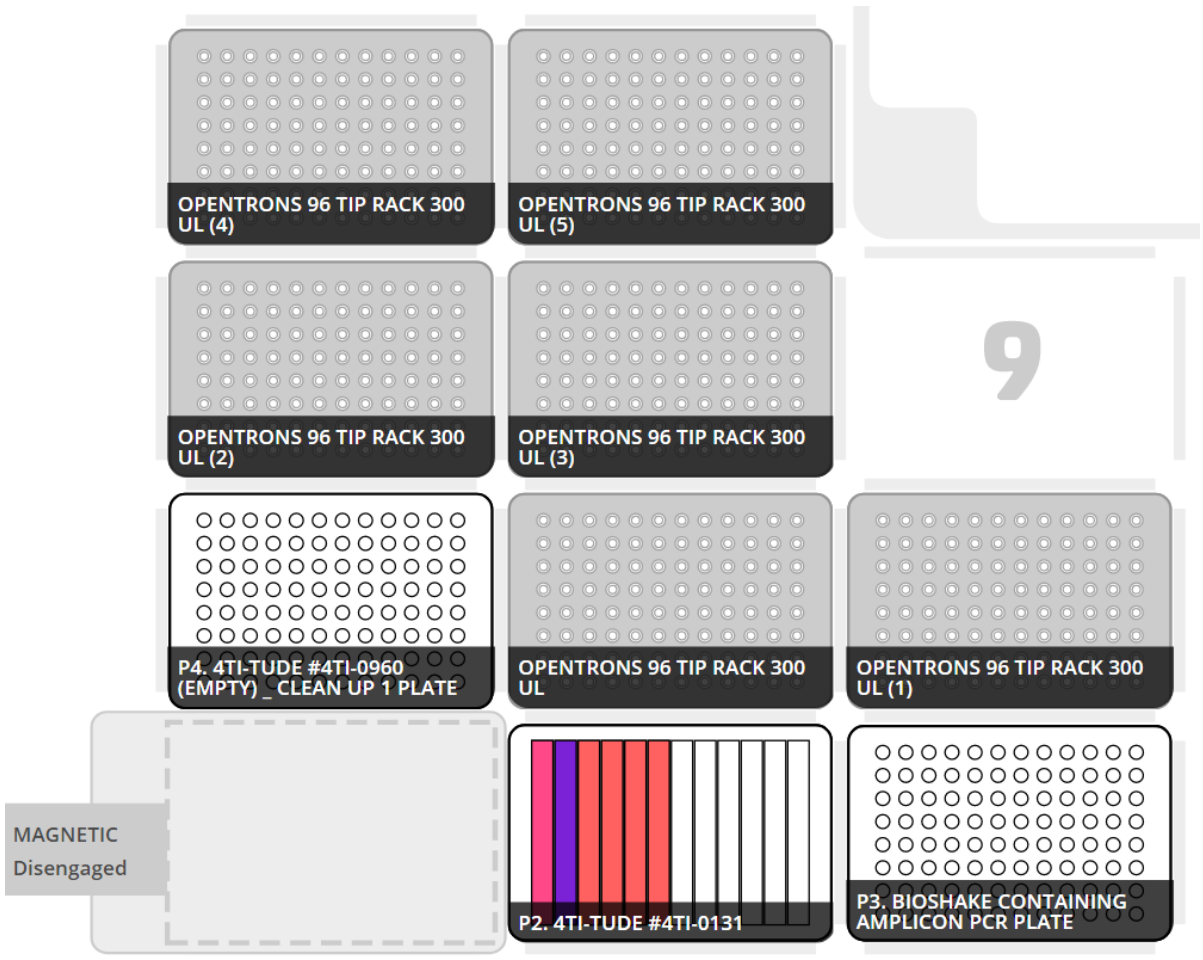
Position 5, 6, 7, 8, 10, 11:

Module/Labware : Tip rack

Model : Opentrons 300ul Tips

Containing : 96 Tips per rack

DECK 2



2.5 Run the automated protocol

2.5.1 Press the PCR clean up 1 button on the left-hand side of AMGEL interface and follow instructions to START the process for cleaning up of Amplicon PCR products (purifying the 16S V3 and V4 amplicon away from free primers and primer dimer species).

2.5.2 The Status is now RUNNING.

2.5.3 Estimated time for user action on Status page is the walk-away duration. Please return to the platform when the walk-away duration is finished.

2.5.4 After the automated process is finished the Status changes from RUNNING to IDLE. Please do the following:

a) Remove all labware from Deck 2.
b) Empty trash bin
c) Seal the Clean up 1 plate (P4) and spin down the liquid.

SAFE STOPPING POINT
If you do not immediately proceed to Index PCR, seal Clean up 1 plate with Microseal "B" adhesive seal and store it at -15° to -25°C for up to a week.

2.6 Automated PCR Clean up 1 protocol steps

1. Transferring Beads.
   M300
   Mixing by pipetting 200ul AMPure XP beads up and down for 5 repeats in P2. Reservoir/Deep well plate Column 2 before every aspirate.
   Transfer 20ul AMPure XP beads
   From P2. Reservoir plate column 2
   To TOP of each well of PCR plate on P3. Bioshake module.

2. Mixing sample and beads by shaking.
   Bioshake module
   Shaking at 1800 rpm for 5 minutes.

3. Placing Amplicon PCR plate on Magnetic module.
   Robotic arm
   Transferring the Amplicon PCR plate

From P3. Bioshake module
To P1. Magnetic module.

4. Incubating at Room temperature for 5 minutes.

5. Precipitating beads on Magnetic module for 12 minutes.
   Magnetic module
   Activated at 18mm height.

6. Disposing supernatant from precipitated beads.
   M300
   Transferring 40ul supernatant
   (*Aspirate flow rate 4.6, speed 20, Delay 10sec, Aspirate height 2.3mm from bottom*)
   From each well of Amplicon PCR plate on P1. Magnetic module
   To P2. Reservoir/Deep well plate Column 12 (Liquid Trash).

7. Washing beads (1st wash).
   a. M300
      Transferring 200ul 80% ethanol (*3mm higher than well top*)
      From P2. Reservoir/Deep well plate Columns 3-6
      To each well of Amplicon PCR plate on P1. Magnetic module.
   b. Incubation for 30 seconds at Room Temperature.
   c. M300
      Disposing 220ul supernatant from precipitated beads(*aspirate flow rate 4.6*)
      From each well of Amplicon PCR plate on P1. Magnetic module
      To P2. Reservoir/Deep well plate Column 8-11 (Liquid Trash).

8. Washing beads (2nd wash).
   a. M300
      Transferring 200ul 80% ethanol (*3mm higher than well top*)
      From P2. Reservoir/Deep well plate Columns 3-6
      To each well of Amplicon PCR plate on P1. Magnetic module.
   b. Incubation for 30 seconds at Room Temperature.
   c. M300
      Disposing 220ul supernatant from precipitated beads
      (A*spirate flow rate 4.6, Aspirate height 0.2mm from bottom*)
      From each well of Amplicon PCR plate on P1. Magnetic module
      To P2. Reservoir/Deep well plate Column 8-11 (Liquid Trash).

9. Air drying for 10 minutes.

10. Resuspending Beads in 10mM Tris pH 8.5.
    a. Disengaging Magnetic module.
    b. M300
        Transferring 52.5ul 10mM Tris pH 8.5
            From P2. Reservoir/Deep well plate Column 1
            To each well of Amplicon PCR plate on P1. Magnetic module and mixing by pipetting 40ul up and down for 25 repeats.

11. Incubating at Room temperature for 2 minutes.

12. Precipitating beads on Magnetic module for 12 minutes.
    a. Magnetic module
        Activated at 18mm height.

13. Transferring 50ul supernatant from Amplicon PCR plate on P1. Magnetic module to corresponding wells of P4. Clean up 1 plate.
        M300
            Transferring 50ul supernatant
            (*Aspirate flow rate 1, speed 10, Delay 15sec, Aspirate height 1mm from bottom*)
                From each well of Amplicon PCR plate on P1. Magnetic module
                To corresponding well (from A1 to A1) of P4. Clean up 1 plate.

14. Disengaging Magnetic module.

ⓘ SAFE STOPPING POINT

If you do not immediately proceed to Index PCR, seal Clean up 1 plate with Microseal "B" adhesive seal and store it at -15° to -25°C for up to a week.

3. Index PCR _ DECK 1

3.1 Description

The Index PCR procedure on AMGEL platform is preparing the Index PCR plate, which is then manually loaded onto a Thermal cycler.

Index PCR program on Thermal Cycler attaches dual indices and Illumina sequencing adapters using the Nextera XT Index Kit.

3.2 Source

16S Metagenomic Sequencing Library Preparation Guide Page 10.

ⓘ https://support.illumina.com/content/dam/illumina-support/documents/documentation/chemistry_documentation/16s/16s-metagenomic-library-prep-guide-15044223-b.pdf

3.3 Consumables

Item	Quantity	Storage
2x KAPA HiFi HotStart ReadyMix	25 µl per sample	-15° to -25°C
Nextera XT Index 1 Primers (N7XX) from the Nextera XT Index kit (FC-131-1001 or FC-131-1002)	5 µl per sample	-15° to -25°C
Nextera XT Index 2 Primers (S5XX) from the Nextera XT Index kit (FC-131-1001 or FC-131-1002)	5 µl per sample	-15° to -25°C
PCR Grade Water	10 µl per sample	

3.4 Labware setup

Please place the following labware on Liquid handling robot Deck 1:

DECK 1

Position 1:

Module/Labware : Fully skirted PCR plate _ Index PCR plate

Model : 4ti-tude #4ti-0960

Containing : Nothing – Empty wells

Position 2:

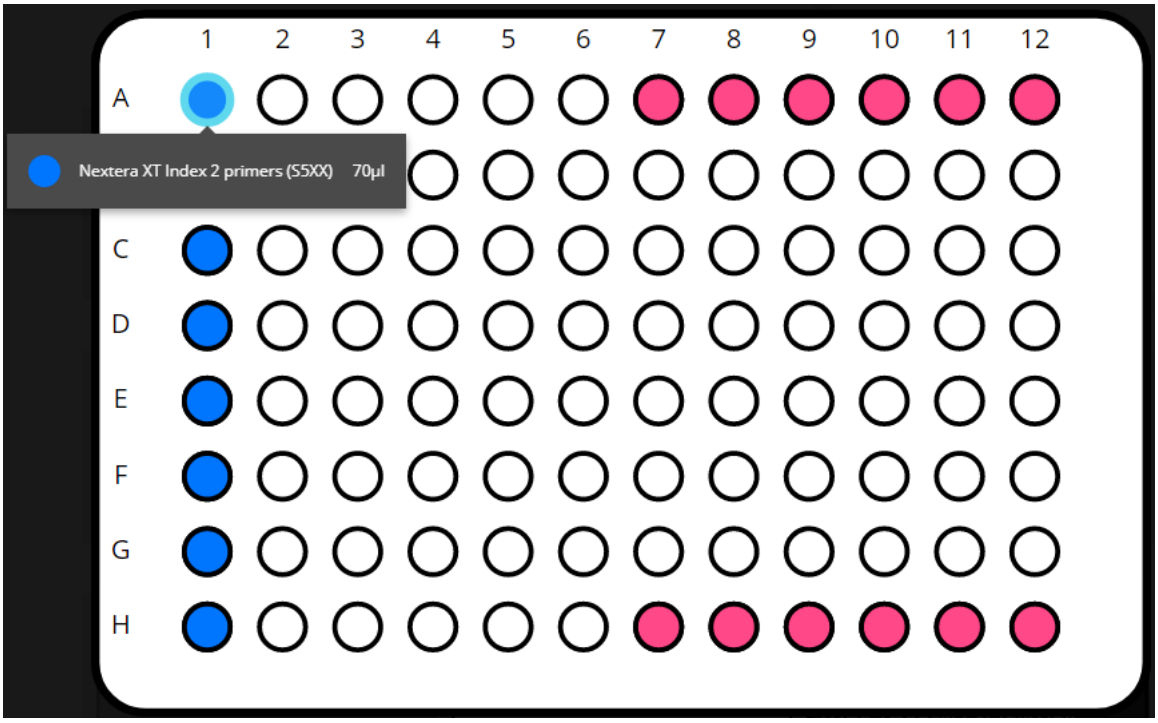
Module/Labware : Fully skirted PCR plate

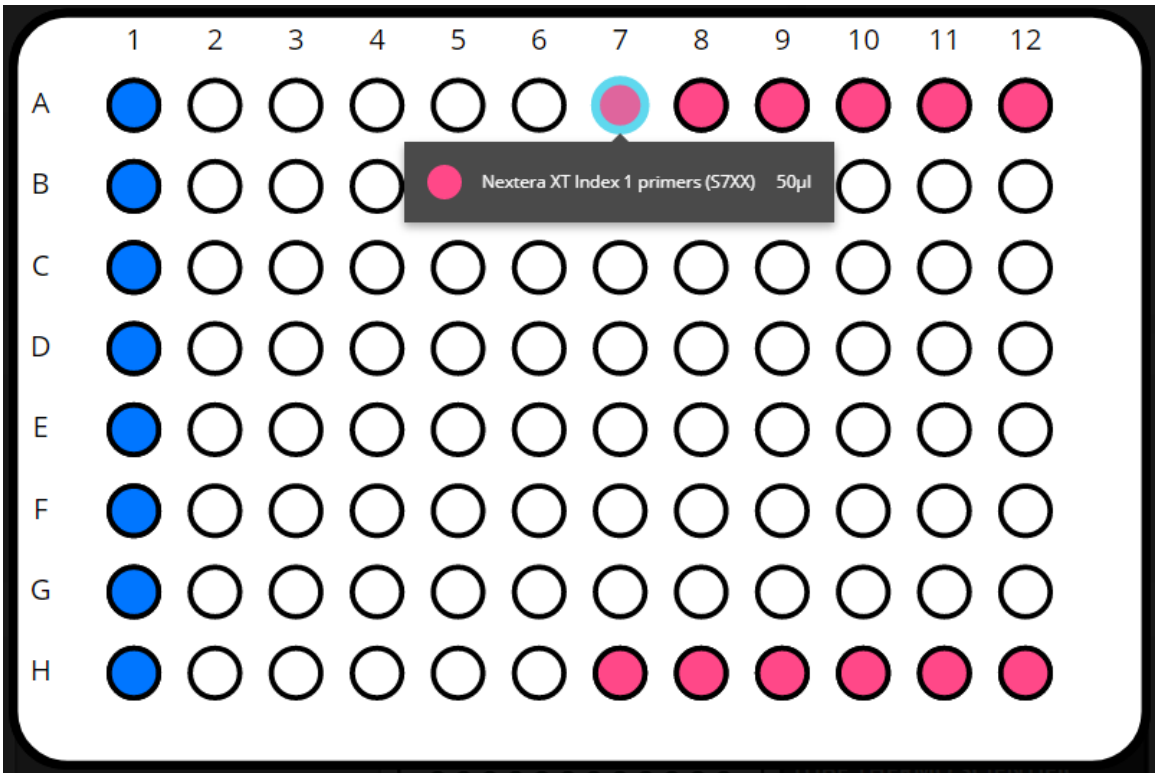
Model : 4ti-tude #4ti-0960

Containing : one set of Index primers (see 3.3. Consumables)

Column 1 : 70ul Index 2 Primers (S5XX) (white caps)

Wells A7-12 and H7-12 : 50ul Index 1 Primers (S7XX) (orange caps)





Position 5:

Fully skirted PCR plate _ Clean up 1 plate

Model : 4ti-tude #4ti-0960

Containing : Clean up 1 plate after PCR Clean up 1 procedure is finished.

Position 10:

Module/Labware : 96-well deep-well plate

Model : ABGENE #AB-0765

Containing : 470ul Master Mix in each well of Column 1 (please prepare Master Mix for 106 samples according to section 3.3 Consumables and then distribute in Column 1).

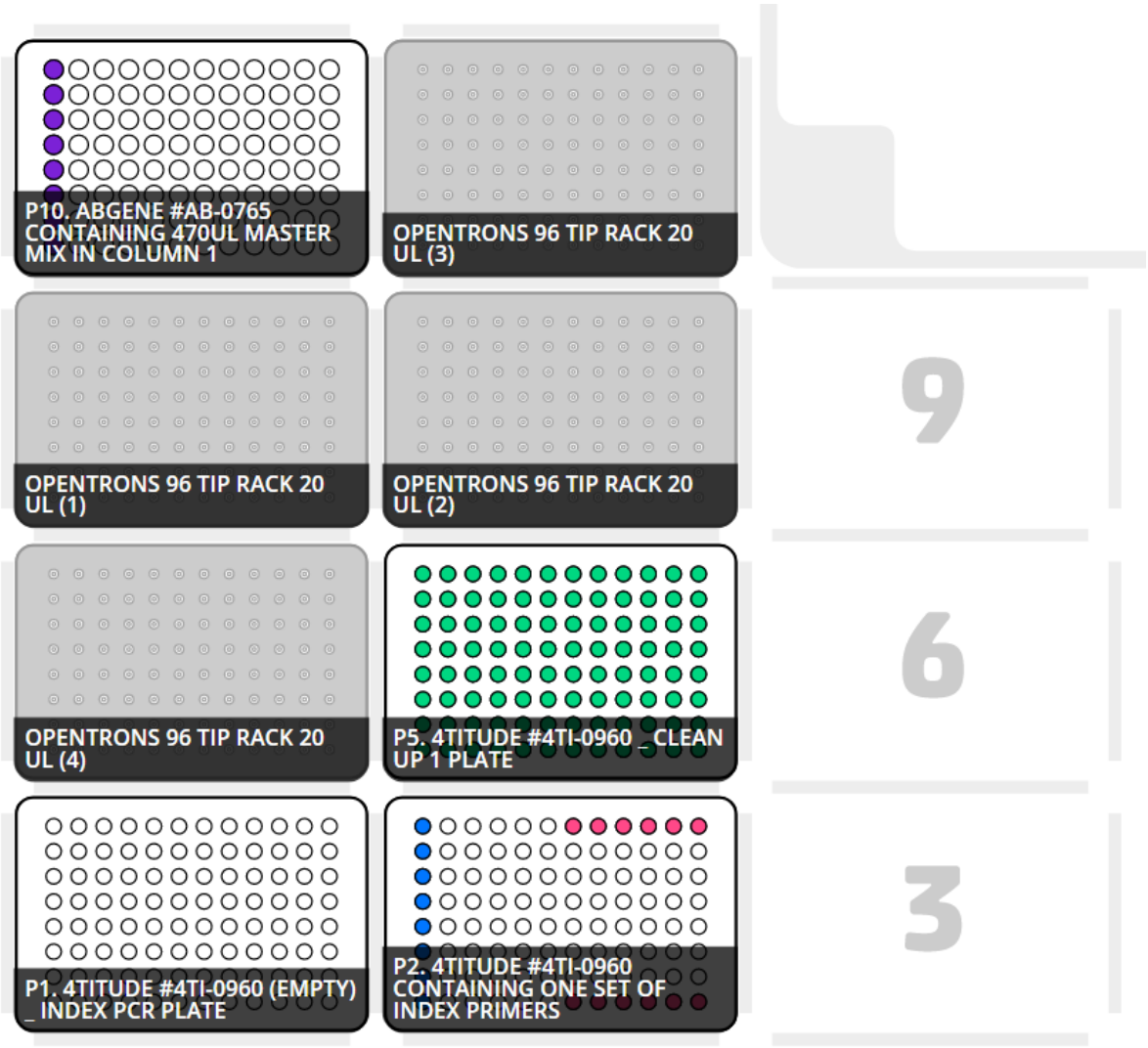
Position 4, 7, 8, 11:

Module/Labware : Tip rack

Model : Opentrons 20ul Tips

Containing : 96 Tips per rack

DECK 1



3.5 Run the automated protocol

3.5.1 Press the Index PCR button on the left-hand side of AMGEL interface and follow instructions to START the process for Index PCR plate preparation.

3.5.2 The Status is now RUNNING.

3.5.3 Estimated time for user action on Status page is the walk-away duration. Please return to the platform when the walk-away duration is finished.

3.5.4 After the automated process is finished the Status changes from RUNNING to IDLE. Please do the following:

a) Remove all labware from Deck 1.
b) Empty trash bin
c) Seal the Index PCR plate (P1), the Clean Up 1 plate (P5) and the PCR plate with stock indexes (P2) and spin down the liquid.
d) Transfer Index PCR plate to a thermal cycler and run the following program:

• 95°C for 3 minutes

• 8 cycles of:

— 95°C for 30 seconds

— 55°C for 30 seconds

— 72°C for 30 seconds

• 72°C for 5 minutes

• Hold at 4°C

ⓘ Source: 16S Metagenomic Sequencing Library Preparation Guide Page 12. https://support.illumina.com/content/dam/illumina-support/documents/documentation/chemistry_documentation/16s/16s-metagenomic-library-prep-guide-15044223-b.pdf

3.6 Automated Index PCR preparation protocol steps

1. Transferring Master Mix to Index PCR plate.
   P20
   Transferring 35ul Master mix
   From P10. ABGENE #AB-0765 Column 1
   To each well of P1. Index PCR plate.

2. Transferring Nextera XT Index 2 primers (S5XX) to Index PCR plate.
   M20
   Transferring 5ul Index 2 primers (S5XX)
   From P2. PCR plate Column 1
   To each well of P1. Index PCR plate with the following manner:
   From A1 to Row A
   From B1 to Row B
   From C1 to Row C

From D1 to Row D
From E1 to Row E
From F1 to Row F
From G1 to Row G
From H1 to Row H

3. Transferring Nextera XT Index 1 primers (S7XX) to Index PCR plate.
   P20
      Transferring 5ul Index 1 primers (S7XX)
         From P2. PCR plate A7-12 and H7-12
         To each well of P1. Index PCR plate with the following manner:
            From A7 to Column 1
            From A8 to Column 2
            From A9 to Column 3
            From A10 to Column 4
            From A11 to Column 5
            From A12 to Column 6
            From H7 to Column 7
            From H8 to Column 8
            From H9 to Column 9
            From H10 to Column 10
            From H11 to Column 11
            From H12 to Column 12

4. Transferring DNA from Clean Up 1 plate to Index PCR plate.
   M20
      Transferring 5ul DNA
         From P5. Clean up 1 plate
         To corresponding well (from A1 to A1) of P1. Index PCR plate.

4. PCR Clean Up 2 _ DECK 2

4.1 Description

This step uses AMPure XP beads to purify the 16S V3 and V4 amplicon away from free primers and primer dimer species.

4.2 Source

16S Metagenomic Sequencing Library Preparation Guide Page 13.

ⓘ https://support.illumina.com/content/dam/illumina-support/documents/documentation/chemistry_documentation/16s/16s-metagenomic-library-prep-guide-15044223-b.pdf

4.3 Consumables

Item	Quantity	Storage
10 mM Tris pH 8.5	27.5 µl per sample	-15° to -25°C
AMPure XP beads	56 µl per sample	2° to 8°C
Freshly Prepared 80% Ethanol (EtOH)	400 µl per sample	

4.4 Labware setup

Please place the following labware on Liquid handling robot Deck 2:

DECK 2

Position 1:

Module/Labware : Magnetic module

Model : Opentrons GEN2

Containing : Nothing – No labware

Position 2:

Module/Labware Option 1: Reservoir plate

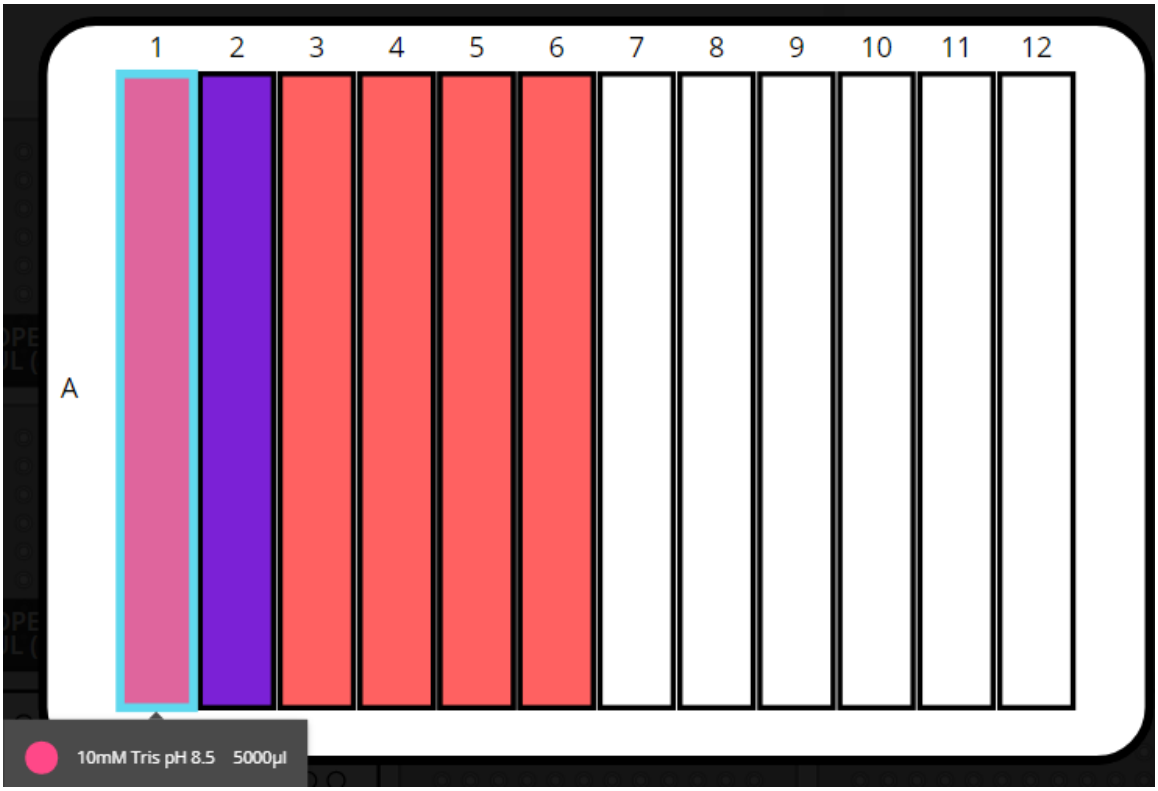
Model Option 1 : 4ti-tude #4ti-0131

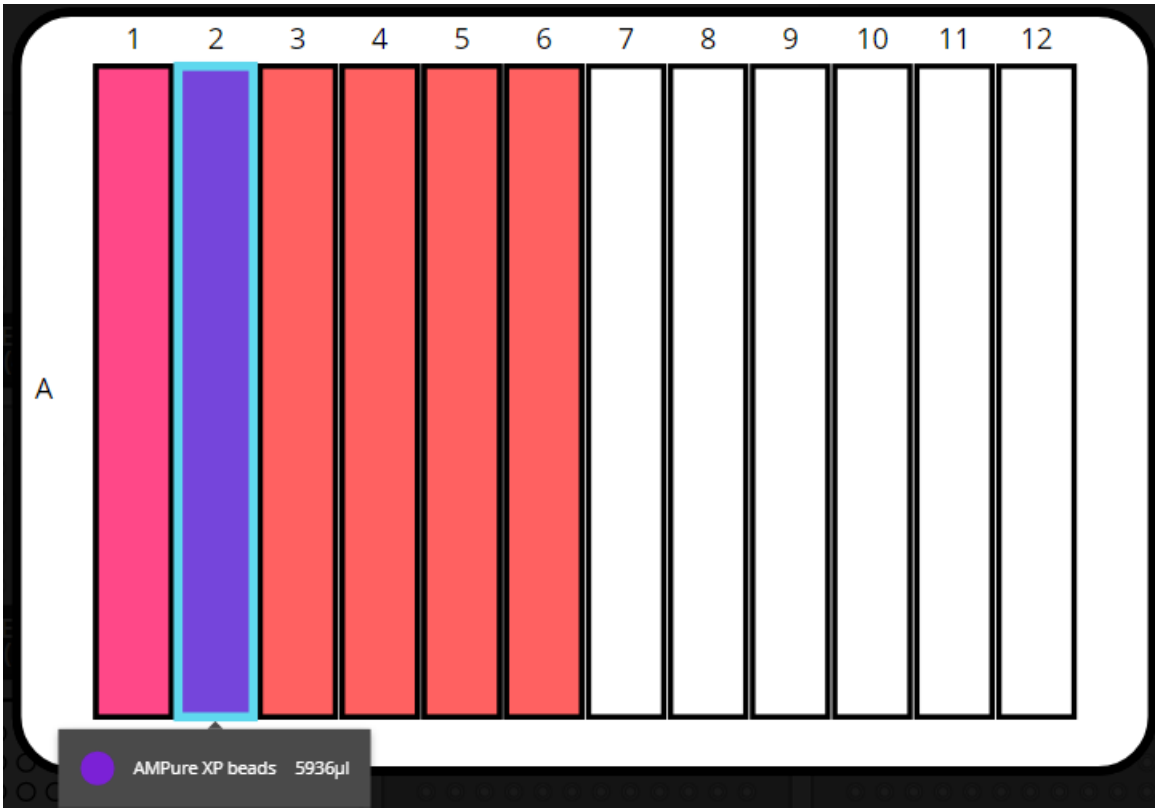
Containing :

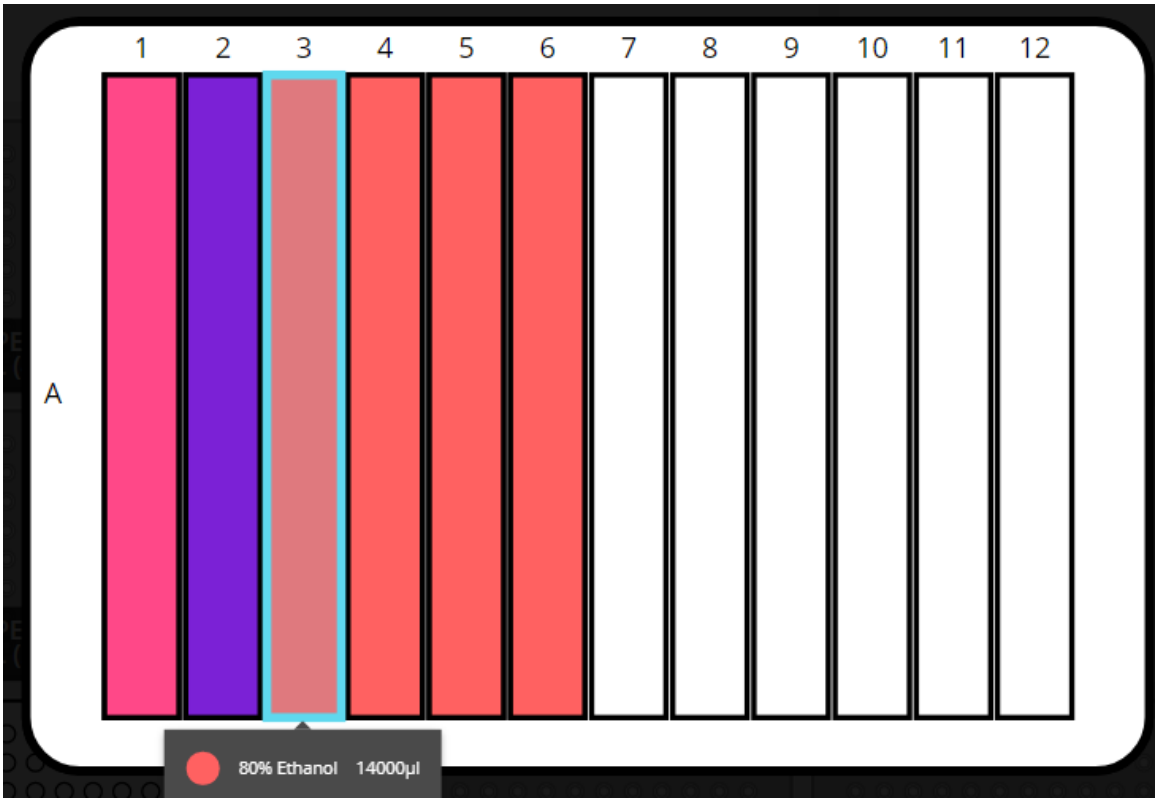
Column 1 : 5000 ul10mM Tris pH 8.5 in column 1 of Reservoir plate

Column 2 : 5936 ul AMPure XP beads in column 2 of Reservoir plate

Columns 3, 4, 5, 6 : 14000 ul 80% Ethanol in column 3, 4, 5, 6 of Reservoir plate







Position 3:

Module/Labware : Bioshake module

Model : Qinstruments Bioshake 3000T-elm

Containing : Index PCR plate (after Thermal cycler Index PCR program is executed)

⚠ *Open ELM fingers before placing PCR plate on Bioshake!*

Position 4:

Module/Labware : Fully skirted PCR plate (Clean Up 2 plate)

Model : 4ti-tude #4ti-0960

Containing : Nothing – Empty wells

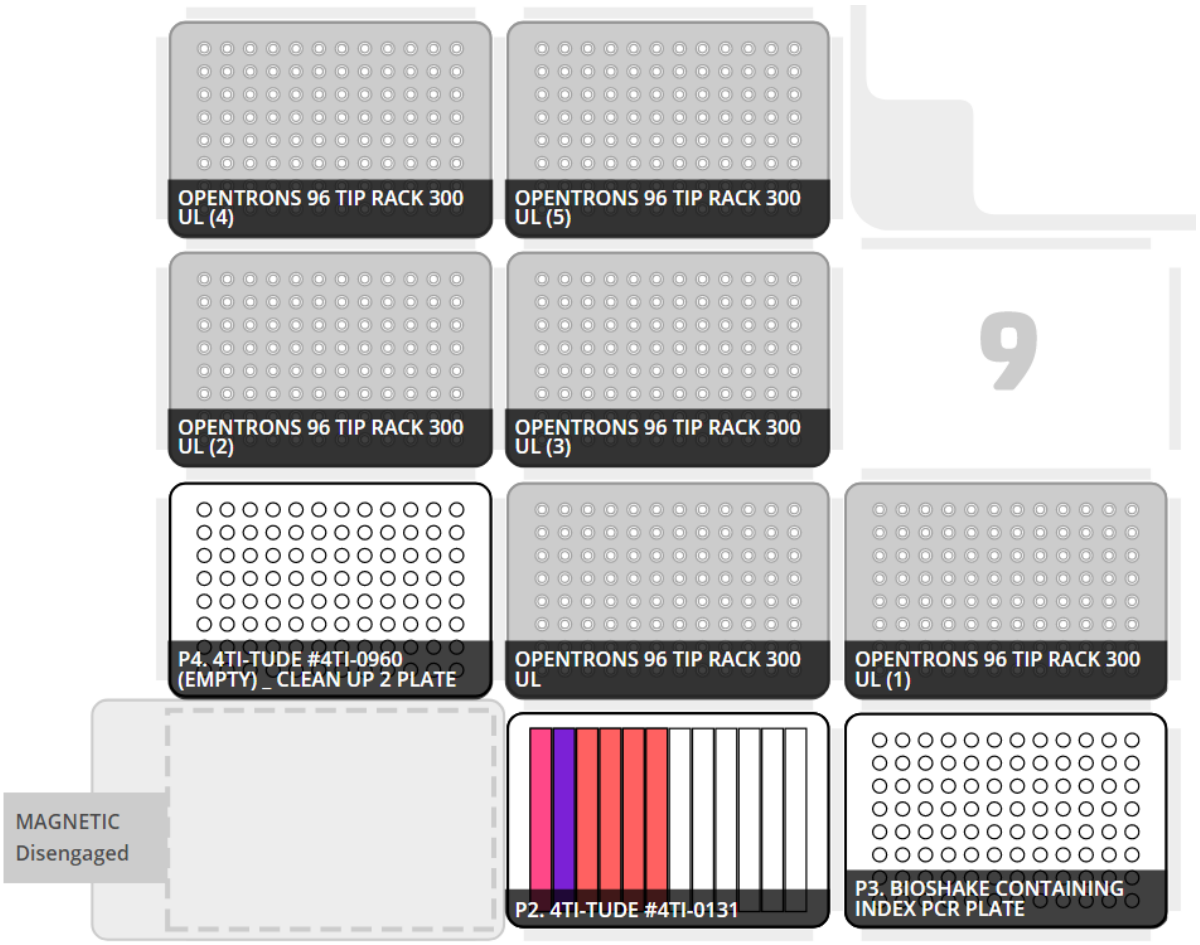
Position 5, 6, 7, 8, 10, 11:

Module/Labware : Tip rack

Model : Opentrons 300ul Tips

Containing : 96 Tips per rack

DECK 2



4.5 Run the automated protocol:

4.5.1 Press the PCR Clean Up 2 button on the left-hand side of AMGEL interface and follow instructions to START the process for cleaning up of Index PCR products (purifying the 16S V3 and V4 amplicon away from free primers and primer dimer species).

4.5.2 The Status is now RUNNING.

4.5.3 Estimated time for user action on Status page is the walk-away duration. Please return to the platform when the walk-away duration is finished.

4.5.4 After the automated process is finished the Status changes from RUNNING to IDLE. Please do the following:

a) Remove all labware from Deck 2.
b) Empty trash bin
c) Seal the Clean up 2 plate (P4) and spin down the liquid.

ⓘ SAFE STOPPING POINT

If you do not immediately proceed to next protocols, seal the Clean Up 2 plate with Microseal "B" adhesive seal and store it at -15° to -25°C for up to a week.

4.6 Automated PCR Clean Up 2 protocol steps.

1. Transferring Beads.
   M300
   Mixing by pipetting 200ul AMPure XP beads up and down for 5 repeats in P2. Reservoir/Deep well plate. Column 2 before every aspirate.
   Transfer 56ul AMPure XP beads
   From P2. Reservoir/Deep well plate column 2
   To TOP of each well of Index PCR plate on P3. Bioshake module.

2. Mixing sample and beads by shaking.
   Bioshake module
   Shaking at 1800 rpm for 2 minutes.

3. Placing Index PCR plate on Magnetic module.
   Robotic arm
   Transferring the Index PCR plate
   From P3. Bioshake module
   To P1. Magnetic module.

4. Incubating at Room temperature for 5 minutes.

5. Precipitating beads on Magnetic module for 12 minutes.
   Magnetic module
   Activated at 18mm height.

6. Disposing supernatant from precipitated beads.
   M300
   Transferring 101ul supernatant
   (*Aspirate flow rate 4.6, speed 20, Delay 10sec, Aspirate height 2.3mm from bottom*)
   From each well of Index PCR plate on P1. Magnetic module
   To P2. Reservoir/Deep well plate Column 12 (Liquid Trash).

7. Washing beads (1st wash).
   a. M300

Transferring 200ul 80% ethanol (*3mm higher than well top*)
From P2. Reservoir/Deep well plate Columns 3-6
To each well of Index PCR plate on P1. Magnetic module.

b. Incubation for 30 seconds at Room Temperature.
c. M300

Disposing 220ul supernatant from precipitated beads(*aspirate flow rate 4.6*)
From each well of Index PCR plate on P1. Magnetic module
To P2. Reservoir/Deep well plate Column 8-11 (Liquid Trash).

8. Washing beads (2nd wash).
   a. M300

Transferring 200ul 80% ethanol (*3mm higher than well top*)
From P2. Reservoir/Deep well plate Columns 3-6
To each well of Index PCR plate on P1. Magnetic module.

   b. Incubation for 30 seconds at Room Temperature.
   c. M300

Disposing 220ul supernatant from precipitated beads
(A*spirate flow rate 4.6, Aspirate height 0.2mm from bottom*)
From each well of Index PCR plate on P1. Magnetic module
To P2. Reservoir/Deep well plate Column 8-11 (Liquid Trash).

9. Air drying for 10 minutes.

10. Resuspending Beads in 10mM Tris pH 8.5.
    a. Disengaging Magnetic module.
    b. M300

Transferring 27.5ul 10mM Tris pH 8.5
From P2. Reservoir/Deep well plate Column 1
To each well of Index PCR plate on P1. Magnetic module and mixing by pipetting 25ul up and down for 25 repeats.

11. Incubating at Room temperature for 2 minutes.

12. Precipitating beads on Magnetic module for 12 minutes.
    a. Magnetic module

Activated at 18mm height.

13. Transferring 25ul supernatant from Index PCR plate on P1. Magnetic module to corresponding wells of P4. Clean Up 2 plate.

M300

Transferring 25ul supernatant

(*Aspirate flow rate 1, speed 10, Delay 15sec, Aspirate height 1mm from bottom*)

From each well of Index PCR plate on P1. Magnetic module
To corresponding well (from A1 to A1) of P4. Clean Up 2 plate.

14. Disengaging Magnetic module.

SAFE STOPPING POINT

If you do not immediately proceed to next protocols, seal the Clean Up 2 plate with Microseal "B" adhesive seal and store it at -15° to -25°C for up to a week.

5. DNA dilution 1:20 _ DECK 1

5.1 Description

This step dilutes 1 ul of each DNA sample, generated after Clean Up 2 procedure, into 19 ul10mM Tris pH 8.5.

5.2 Source

Customized protocol – Dr Omirou lab

5.3 Consumables

a) Clean Up 2 plate containing 25ul purified DNA after finalization of PCR Clean up 2 process.

b) 10mM Tris pH 8.5 e.g., Qiagen #Buffer EB Cat. No. / ID: 19086

5.4 Labware setup

Please place the following labware on Liquid handling robot Deck 1:

DECK 1

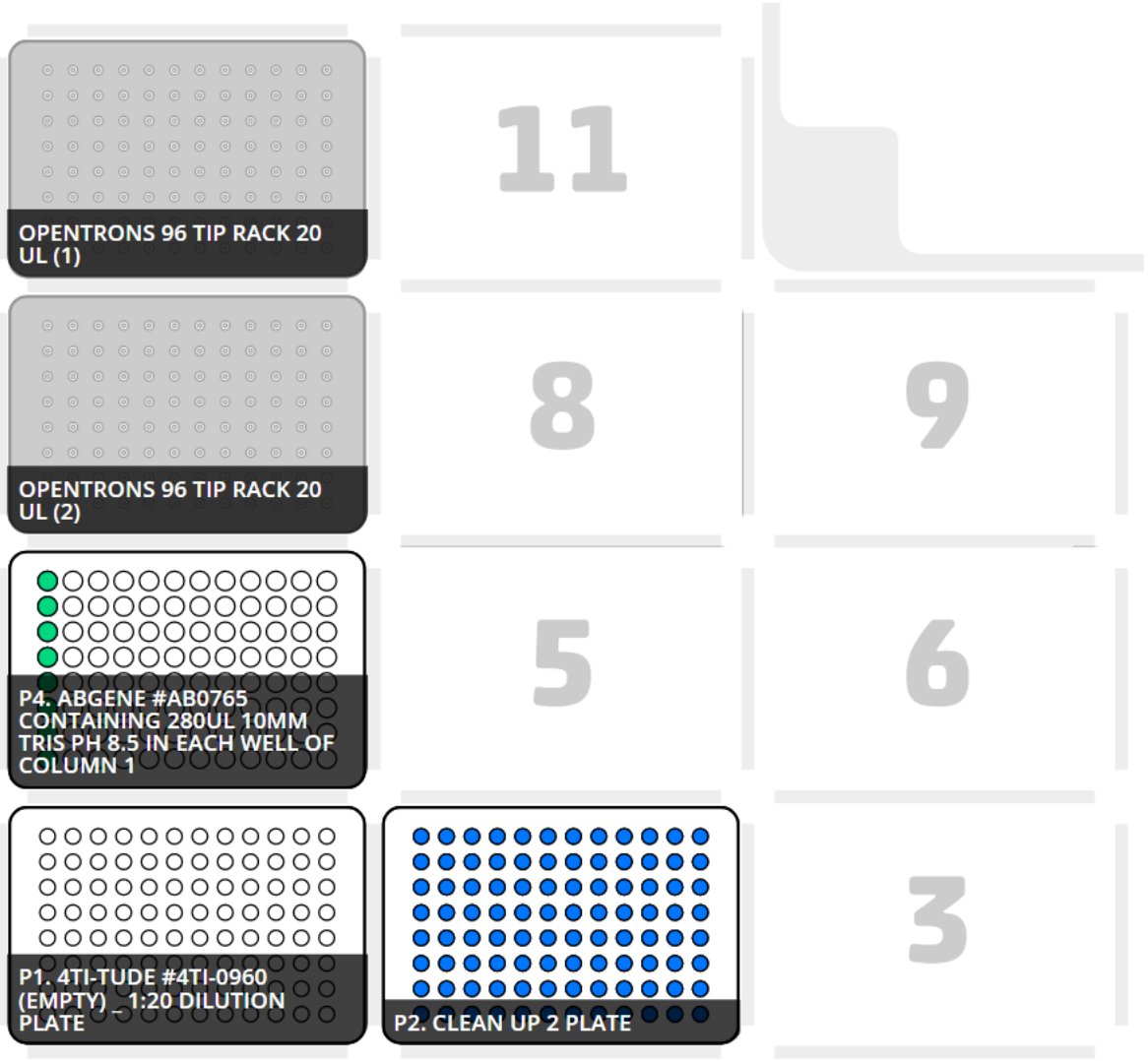
Position 1:
- Module/Labware : Fully skirted PCR plate _ 1:20 Dilution plate
  - Model : 4ti-tude #4ti-0960
    - Containing : Nothing – Empty wells

Position 2:
- Module/Labware : Fully skirted PCR plate _ Clean Up 2 plate
  - Model : 4ti-tude #4ti-0960
    - Containing : Clean Up 2 plate after PCR Clean up 2 procedure is finished.

Position 4:
- Module/Labware : 96-well deep-well plate
  - Model : ABGENE #AB-0765
    - Containing : 280ul 10mM Tris pH 8.5 in each well of column 1

Position 7,10:
- Module/Labware : Tip rack
  - Model : Opentrons 20ul Tips
    - Containing : 96 Tips per rack

DECK 1



5.5 Run the automated protocol:

5.5.1 Press the DNA dilution 1:20 button on the left-hand side of AMGEL interface and follow instructions to START the process.

5.5.2 The Status is now RUNNING.

5.5.3 Estimated time for user action on Status page is the walk-away duration. Please return to the platform when the walk-away duration is finished.

5.5.4 After the automated process is finished the Status changes from RUNNING to IDLE. Please do the following:

a) Remove all labware from Deck 1.
b) Empty trash bin
c) Seal the 1:20 Dilution plate (P1) and Clean Up 2 plate (P2) and spin down the liquid.

5.6 Automated DNA dilution 1:20 protocol steps

1. Transferring 10mM Tris pH 8.5 to 1:20 Dilution plate.
   M20
   Transferring 19ul 10mM Tris pH 8.5
   From P4. Deep well Column 1
   To each well of P1. 1:20 Dilution plate.

2. Transferring DNA from Clean Up 2 plate to 1:20 Dilution plate.
   M20
   Mixing by pipetting 5ul DNA up and down for 10 repeats in P2. Clean up plate before every aspirate.
   Transferring 1ul DNA
   From P2. Clean Up 2 plate
   To corresponding well (from A1 to A1) of P1. 1:20 Dilution plate.
   Mixing by pipetting 15ul mixture up and down for 20 repeats in P1. 1:20 Dilution plate after every dispense.
   Touch tip after every dispense.

6. Pico standards _ DECK 1

6.1 Description

This step is used for preparing the DNA sample with concentrations 0, 5, 10, 20, 25, 50, 75 and 100 ng/ul in TE buffer (Pico Kit) that will be used for creating a the standard curve in Pico DNA quantification protocol.

6.2 Source

Customized protocol – Dr. Omirou lab

6.3 Consumables

a) 100ng/ul DNA

b) TE buffer (Pico Kit)

6.4 Labware setup

Please place the following labware on Liquid handling robot Deck 1:

DECK 1

Position 4:

Module/Labware : Tip rack

Model : Opentrons 20ul Tips

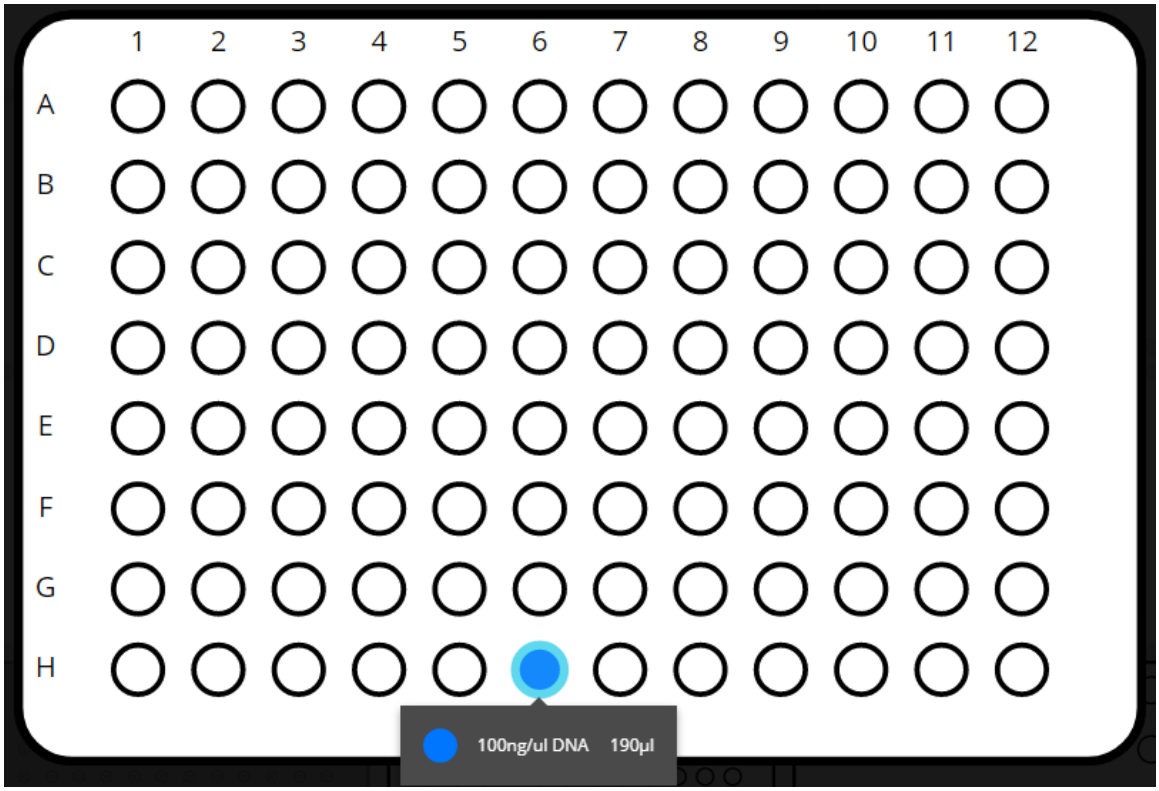
Containing : 96 Tips per rack

Position 5:

Module/Labware : Fully skirted PCR plate _ Pico standards plate

Model : 4ti-tude #4ti-0960

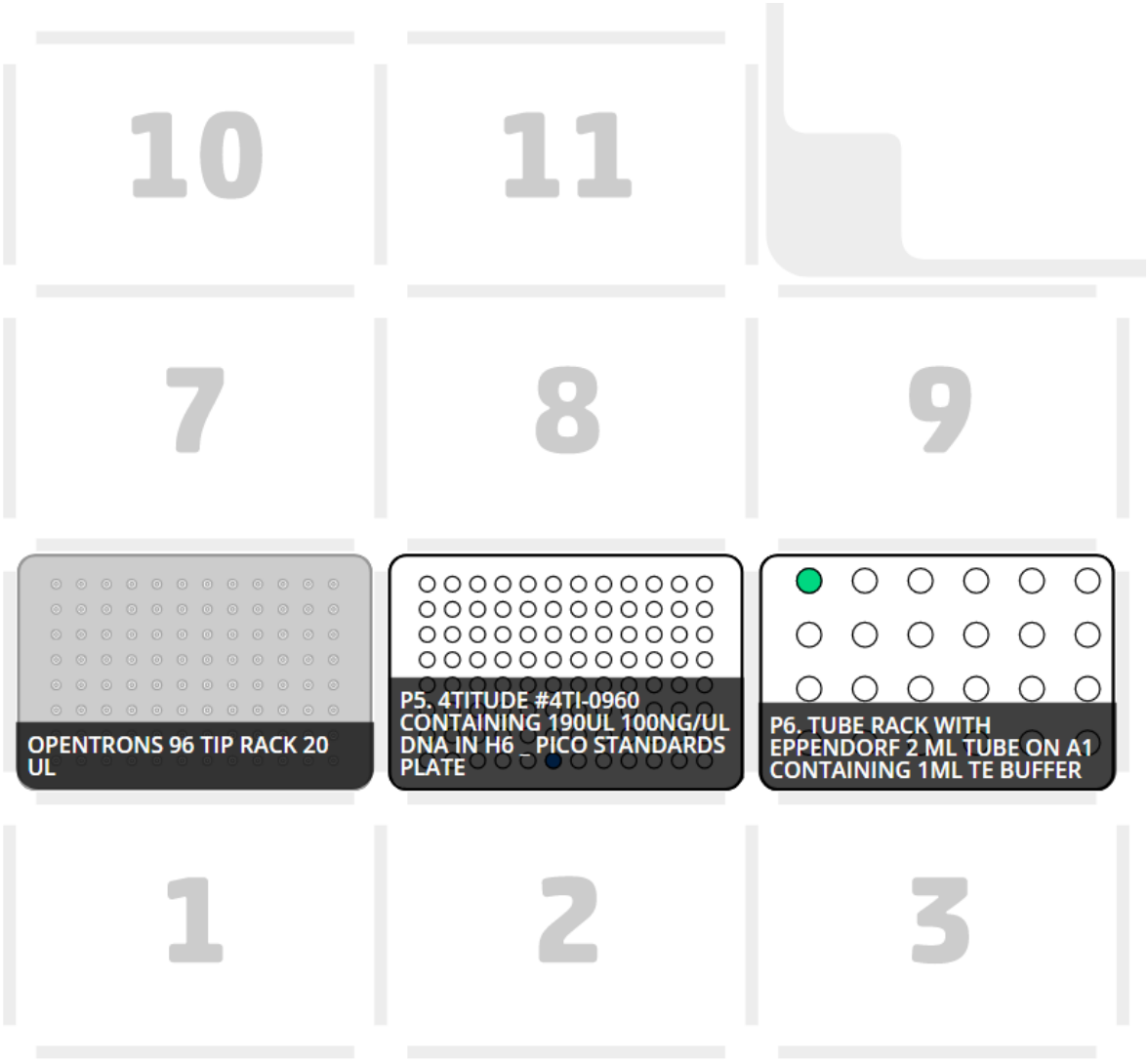
Containing : 190 ul 100ng/ul DNA in H6



Position 6:

Module/Labware : Tube rack for 1.5-2ml snap-cap tubes
Model : Eppendorf 2ml tube on A1 of tube rack
Containing : 1000ul TE buffer (Pico kit)

DECK 1



6. 5 Run the automated protocol:

6.5.1 Press the Pico standards button on the left-hand side of AMGEL interface and follow instructions to START the process.

6.5.2 The Status is now RUNNING.

6.5.3 Estimated time for user action on Status page is the walk-away duration. Please return to the platform when the walk-away duration is finished.

6.5.4 After the automated process is finished the Status changes from RUNNING to IDLE. Please do the following:

a) Remove all labware from Deck 1.
b) Empty trash bin
c) Seal the Pico standards plate (P5) and spin down the liquid.

6.6 Automated PICO standards protocol steps

1. Preparing Pico standard 0 ng/ul.

    P20

        Transferring 60ul TE buffer
            From P6. Tube on A1
            To A6 of P5. Pico standards plate.

2. Preparing Pico standard 5 ng/ul.

    P20

        Transferring 57ul TE buffer
            From P6. Tube on A1
            To B6 of P5. Pico standards plate.

3. Preparing Pico standard 10 ng/ul.

    P20

        Transferring 54ul TE buffer
            From P6. Tube on A1
            To C6 of P5. Pico standards plate.

4. Preparing Pico standard 20 ng/ul.

    P20

        Transferring 48ul TE buffer
            From P6. Tube on A1
            To D6 of P5. Pico standards plate.

5. Preparing Pico standard 25 ng/ul.

    P20

        Transferring 45ul TE buffer
            From P6. Tube on A1
            To E6 of P5. Pico standards plate.

Mixing by pipetting 20ul mixture up and down for 2 repeats in P5. Pico standards plate E6 after dispensing.
Touching tip.

6. Preparing Pico standard 50 ng/ul.

P20

Transferring 30ul TE buffer
From P6. Tube on A1
To F6 of P5. Pico standards plate.

7. Preparing Pico standard 75 ng/ul.

P20

Transferring 15ul TE buffer
From P6. Tube on A1
To G6 of P5. Pico standards plate.

8. Mixing by pipetting 20ul 100ng/ul DNA up and down for 20 repeats to homogenize the DNA sample.

P20

Transferring 20ul 100ng/ul DNA for 20 repeats
From H6 of P5. Pico standards plate
To H6 of P5. Pico standards plate.

9. Preparing Pico standard 5 ng/ul.

P20

Transferring 3ul 100 ng/ul DNA
From P5. Pico standards plate H6
To B6 of P5. Pico standards plate.
Mixing by pipetting 20ul mixture up and down for 20 repeats in P5. Pico standards plate B6 after dispensing.
Touching tip.

10. Preparing Pico standard 10 ng/ul.

P20

Transferring 6ul 100 ng/ul DNA
From P5. Pico standards plate H6
To C6 of P5. Pico standards plate.

Mixing by pipetting 20ul mixture up and down for 20 repeats in P5 Pico standards plate C6 after dispensing.
Touching tip.

11. Preparing Pico standard 20 ng/ul.

P20

Transferring 12ul 100 ng/ul DNA
From P5. Pico standards plate H6
To D6 of P5. Pico standards plate.
Mixing by pipetting 20ul mixture up and down for 20 repeats in P5. Pico standards plate D6 after dispensing.
Touching tip.

12. Preparing Pico standard 25 ng/ul.

P20

Transferring 15ul 100 ng/ul DNA
From P5. Pico standards plate H6
To E6 of P5. Pico standards plate.
Mixing by pipetting 20ul mixture up and down for 20 repeats in P5. Pico standards plate E6 after dispensing.
Touching tip.

13. Preparing Pico standard 50 ng/ul.

P20

Transferring 30ul 100 ng/ul DNA
From P5. Pico standards plate H6
To F6 of P5. Pico standards plate.
Mixing by pipetting 20ul mixture up and down for 20 repeats in P5. Pico standards plate F6 after dispensing.
Touching tip.

14. Preparing Pico standard 75 ng/ul.

P20

Transferring 45ul 100 ng/ul DNA
From P5. Pico standards plate H6
To G6 of P5. Pico standards plate.
Mixing by pipetting 20ul mixture up and down for 20 repeats in P5. Pico standards plate G6 after dispensing.

Touching tip.

15. Mixing by pipetting to homogenize each one of the Standards.

M20

Transferring 20ul of each standard for 20 repeats
From P5. Pico standards plate Column 1
To P5. Pico standards plate Column 1.

6.7 Pico standards plate (P5) layout

A6: 0 ng/ul

B6: 5 ng/ul

C6: 10 ng/ul

D6: 20 ng/ul

E6: 25 ng/ul

F6: 50 ng/ul

G6: 75 ng/ul

H6: 100 ng/ul

7. Pico prep _ DECK 1

7.1 Description

This step is transferring in triplicate 1ul from each DNA sample generated by Process 5. DNA Dilution 1:20 and in triplicate 1ul of each Pico standard sample generated by Process 6. Pico standards into PCR plates suitable for Pico analysis (Pico A, B, C, D, see layout at 7.5).

7.2 Source

Customized protocol – Dr. Omirou lab

7.3 Consumables

a) 1:20 Dilution plate : DNA generated by Process 4. PCR clean up 2 that was then diluted 20 times in 10mM Tris pH 8.5 by Process 5. DNA Dilution 1:20.

b) Pico standards plate: 60ul DNA samples with known concentrations: 0, 5, 10, 15, 20, 25, 50, 75, 100 ng/ul in wells A6, B6, C6, D6, E6, F6, G6, H6 respectively generated by Process 6. Pico standards.

7.4 Labware setup

Please place the following labware on Liquid handling robot Deck 1:

DECK 1

Position 1:

Module/Labware : Fully skirted PCR plate _ 1:20 Dilution plate

Model : 4ti-tude #4ti-0960

Containing : PCR_clean_up_2-generated DNA which was diluted 20 times in 10mM Tris pH 8.5 (see process 5)

Position 5:

Module/Labware : Fully skirted PCR plate _ Pico standards plate

Model : 4ti-tude #4ti-0960

Containing : 60ul DNA samples with known concentrations: 0, 5, 10, 15, 20, 25, 50, 75, 100 ng/ul in wells A6, B6, C6, D6, E6, F6, G6, H6 respectively (see process 6)

Position 7, 9, 10, 11:

Module/Labware : Fully skirted PCR plate _ Pico A, B, C, D respectively.

Model : 4ti-tude #4ti-0740

Containing : Nothing – Empty wells

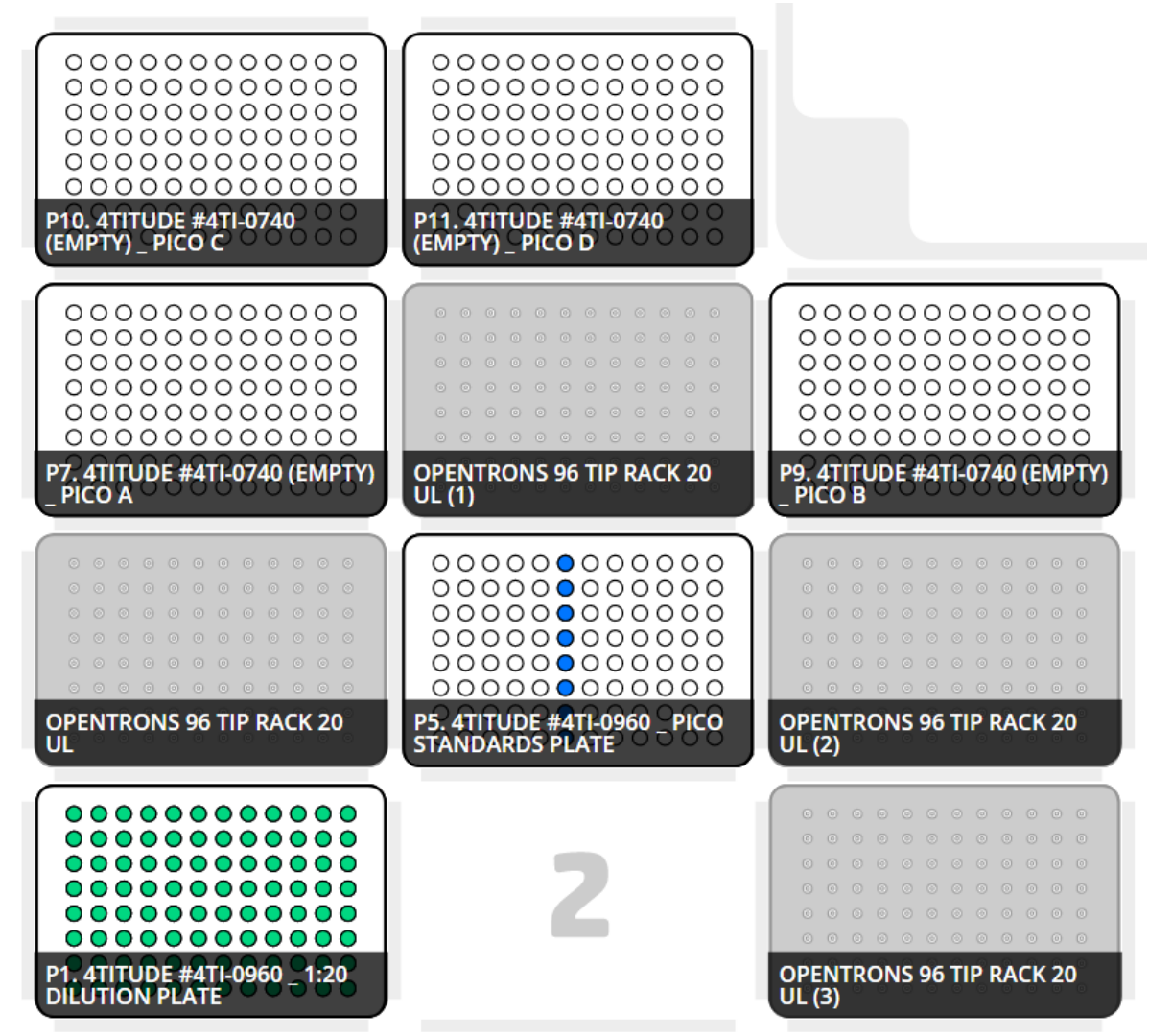
Position 3, 4, 6, 8:

Module/Labware : Tip rack

Model : Opentrons 20ul Tips

Containing : 96 Tips per rack

DECK 1



7. 5 Run the automated protocol

7.5.1 Press the Pico prep button on the left-hand side of AMGEL interface and follow instructions to START the process.

7.5.2 The Status is now RUNNING.

7.5.3 Estimated time for user action on Status page is the walk-away duration. Please return to the platform when the walk-away duration is finished.

7.5.4 After the automated process is finished the Status changes from RUNNING to IDLE. Please do the following:

a) Remove all labware from Deck 1.
b) Empty trash bin
c) Seal the 1:20 Dilution plate(P1), Pico standards plate (P5), Pico A (P7), Pico B (P9), Pico C (P10) and Pico D (P11) and spin down the liquid.

7.6 Automated PICO prep protocol steps

*Dispense flow rate maximum.

*Dispense 0.1mm from bottom.

1. Transferring in triplicate1ul Pico Standards to Pico plates A, B, C and D.

M20

Transferring 1ul Pico standards

From P5. Pico standards plate Column 6

To corresponding wells (from A to A) in each Column 1, 2 and 3 in P7, 9, 10 and 11 Pico plate A, B, C and D.

Blowout 5mm from top.

Touching tip 5mm from top.

2. Transferring in triplicate1ul Sample from P1. 1:20 Dilution plate Column 1 to P7. Pico plate A columns 4,5,6.

M20

Transferring 1ul Sample

From P1. 1:20 Dilution plate Column 1

To corresponding wells (from A to A) in each Column 4, 5 and 6 in P7. Pico plate A.

Blowout 5mm from top.

Touch tip 5mm from top.

3. Transferring in triplicate1ul Sample from P1. 1:20 Dilution plate Column 2 to P7. Pico plate A columns 7,8,9.

M20

Transferring 1ul Sample

From P1. 1:20 Dilution plate Column 2

To corresponding wells (from A to A) in each Column 7, 8 and 9 in P7. Pico plate A.

Blowout 5mm from top.

Touch tip 5mm from top.

4. Transferring in triplicate1ul Sample from P1. 1:20 Dilution plate Column 3 to P7. Pico plate A columns 10,11,12.

M20

Transferring 1ul Sample

From P1. 1:20 Dilution plate Column 3

To corresponding wells (from A to A) in each Column 10, 11 and 12 in P7. Pico plate A.

Blowout 5mm from top.

Touch tip 5mm from top.

5. Transferring in triplicate1ul Sample from P1. 1:20 Dilution plate Column 4 to P9. Pico plate B columns 4,5,6.

M20

Transferring 1ul Sample

From P1. 1:20 Dilution plate Column 4

To corresponding wells (from A to A) in each Column 4, 5 and 6 in P9. Pico plate B.

Blowout 5mm from top.

Touch tip 5mm from top.

6. Transferring in triplicate1ul Sample from P1. 1:20 Dilution plate Column 5 to P9. Pico plate B columns 7,8,9.

M20

Transferring 1ul Sample

From P1. 1:20 Dilution plate Column 5

To corresponding wells (from A to A) in each Column 7, 8 and 9 in P9. Pico plate B.

Blowout 5mm from top.

Touch tip 5mm from top.

7. Transferring in triplicate1ul Sample from P1. 1:20 Dilution plate Column 6 to P9. Pico plate B columns 10,11,12.

M20

Transferring 1ul Sample

From P1. 1:20 Dilution plate Column 6

To corresponding wells (from A to A) in each Column 10, 11 and 12 in P9. Pico plate B.

Blowout 5mm from top.

Touch tip 5mm from top.

8. Transferring in triplicate1ul Sample from P1. 1:20 Dilution plate Column 7 to P10. Pico plate C columns 4,5,6.

M20

Transferring 1ul Sample
From P1. 1:20 Dilution plate Column 7
To corresponding wells (from A to A) in each Column 4, 5 and 6 in P10. Pico plate C.

Blowout 5mm from top.

Touch tip 5mm from top.

9. Transferring in triplicate1ul Sample from P1. 1:20 Dilution plate Column 8 to P10. Pico plate C columns 7,8,9.

M20

Transferring 1ul Sample
From P1. 1:20 Dilution plate Column 8
To corresponding wells (from A to A) in each Column 7, 8 and 9 in P10. Pico plate C.

Blowout 5mm from top.

Touch tip 5mm from top.

10. Transferring in triplicate1ul Sample from P1. 1:20 Dilution plate Column 9 to P10. Pico plate C columns 10,11,12.

M20

Transferring 1ul Sample
From P1. 1:20 Dilution plate Column 9
To corresponding wells (from A to A) in each Column 10, 11 and 12 in P10. Pico plate C.

Blowout 5mm from top.

Touch tip 5mm from top.

11. Transferring in triplicate1ul Sample from P1. 1:20 Dilution plate Column 10 to P11. Pico plate D columns 4,5,6.

M20

Transferring 1ul Sample
From P1. 1:20 Dilution plate Column 10
To corresponding wells (from A to A) in each Column 4, 5 and 6 in P11. Pico plate D.

Blowout 5mm from top.

Touch tip 5mm from top.

12. Transferring in triplicate1ul Sample from P1. 1:20 Dilution plate Column 11 to P11. Pico plate D columns 7,8,9.

M20

Transferring 1ul Sample

From P1. 1:20 Dilution plate Column 11

To corresponding wells (from A to A) in each Column 7, 8 and 9 in P11. Pico plate D.

Blowout 5mm from top.

Touch tip 5mm from top.

13. Transferring in triplicate1ul Sample from P1. 1:20 Dilution plate Column 12 to P11. Pico plate D columns 10,11,12.

M20

Transferring 1ul Sample

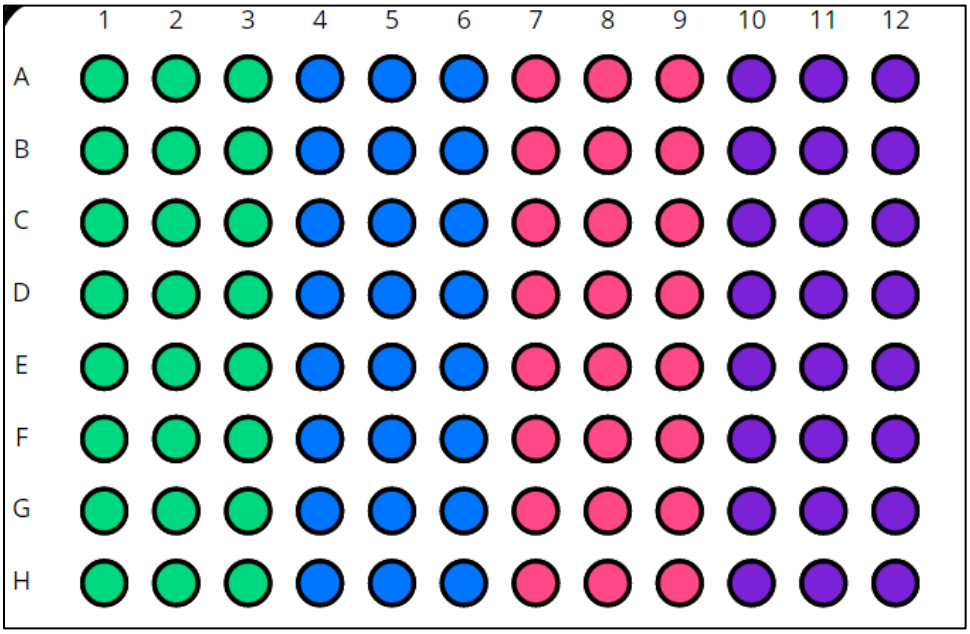
From P1. 1:20 Dilution plate Column 12

To corresponding wells (from A to A) in each Column 10, 11 and 12 in P11. Pico plate D.

Blowout 5mm from top.

Touch tip 5mm from top.

7.7 Layout of Pico A, B, C, D plates



Green : Standards in triplicate from Pico Standards plate Column 6.

Blue : Samples in triplicate from 1:20 Dilution plate Column 1, 4, 7, 10 transferred into Pico A, B, C, D plate, respectively.

Pink : Samples in triplicate from 1:20 Dilution plate Column 2, 5, 8, 11 transferred into Pico A, B, C, D plate, respectively.

Purple : Samples in triplicate from 1:20 Dilution plate Column 3, 6, 9, 12 transferred into Pico A, B, C, D plate, respectively.

8. Normalization _ DECK 1

8.1 Description

This step dilutes concentrated final DNA library (after Clean Up 2 and DNA Dilution 1:20 processes) using Resuspension Buffer (RSB) or 10 mM Tris pH 8.5 to 4nM final concentration (in Normalized DNA plate).

8.2 Source

16S Metagenomic Sequencing Library Preparation Guide Page 16.

ⓘ https://support.illumina.com/content/dam/illumina-support/documents/documentation/chemistry_documentation/16s/16s-metagenomic-library-prep-guide-15044223-b.pdf

8.3 Consumables

a) 1:20 Dilution plate : DNA generated by Process 4. PCR clean Up 2 that was then diluted 20 times in 10mM Tris pH 8.5 by Process 5. DNA Dilution 1:20.

b) 1100 ul of 10 mM Tris pH 8.5.

8.4 Labware setup

Please place the following labware on Liquid handling robot Deck 1:

DECK 1

Position 1:

Module/Labware : Fully skirted PCR plate _ 1:20 Dilution plate

Model : 4ti-tude #4ti-0960

Containing : PCR_Clean_Up_2-generated DNA diluted 20 times in 10mM Tris pH 8.5 (see process 5)

Position 2:

Module/Labware : Fully skirted PCR plate _ Normalized DNA plate

Model : 4ti-tude #4ti-0960

Containing : Nothing – Empty wells

Position 6:

Module/Labware : Tube rack for 1.5-2ml snap-cap tubes

Model : Eppendorf 2ml tube on A1 of tube rack

Containing : 1100ul 10 mM Tris pH 8.5

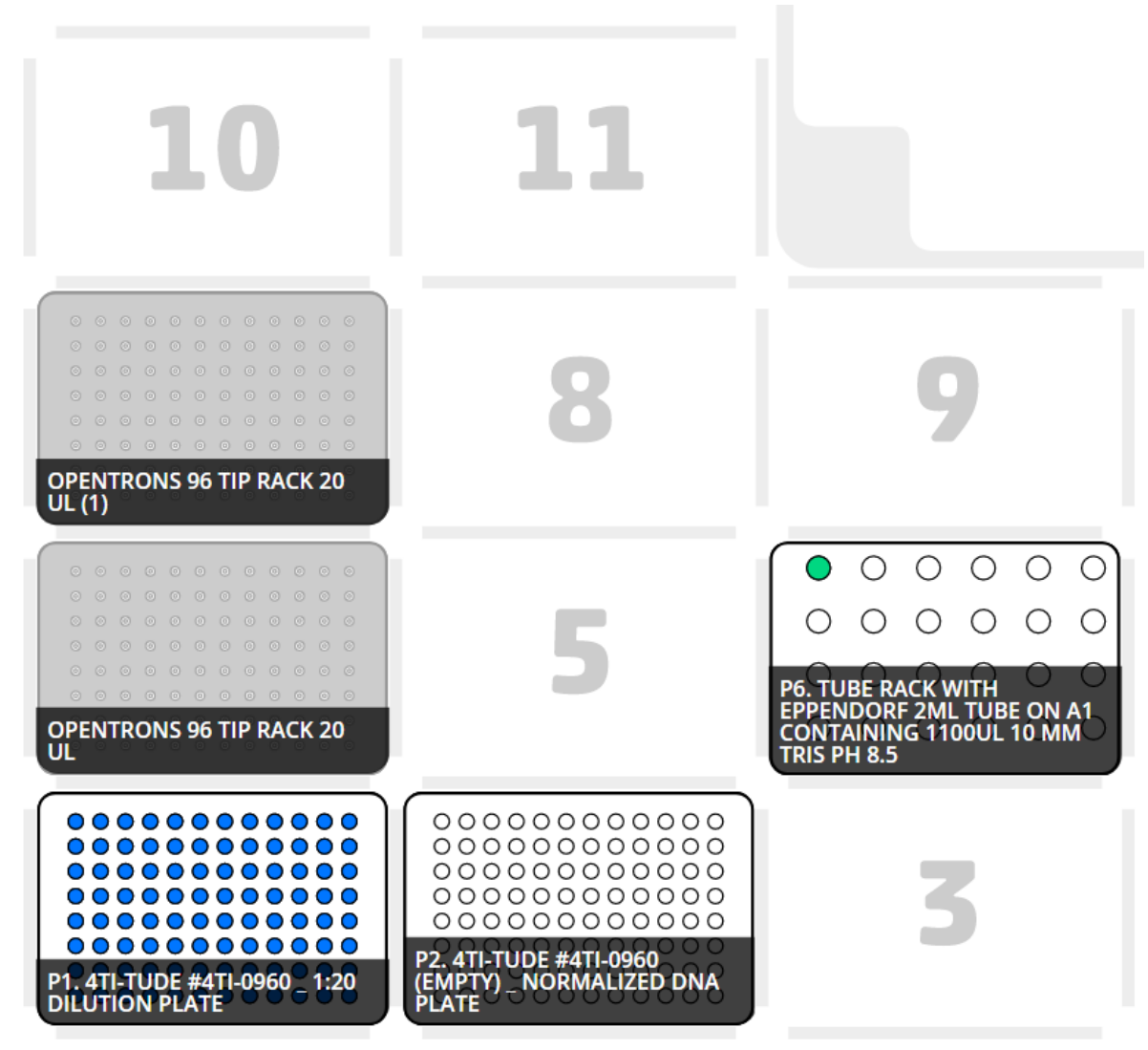
Position 4, 7:

Module/Labware : Tip rack

Model : Opentrons 20ul Tips

Containing : 96 Tips per rack

DECK 1



8. 5 Run the automated protocol

8.5.1 Press the Normalization button on the left-hand side of AMGEL interface.

8.5.2 Prepare 2 CSV files each containing ***transferring volume (ul) of Dilution buffer (CSV 1) and DNA (CSV 2) with the following layout:

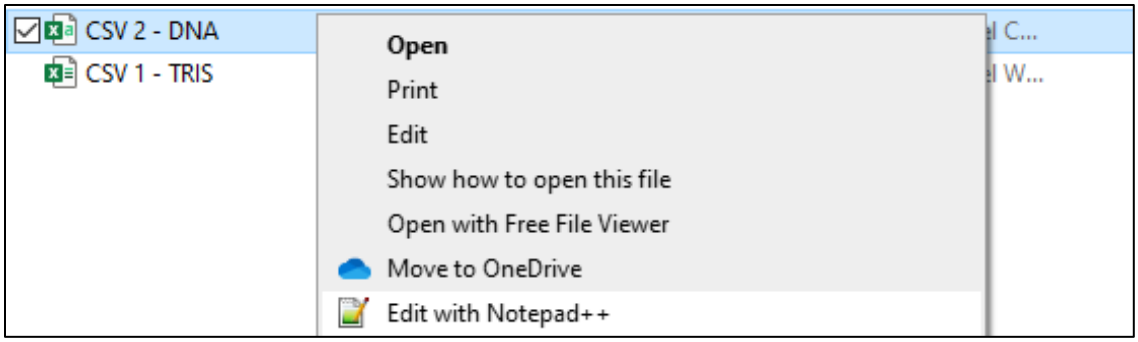
	A	B	C	D	E	F	G	H	I	J	K	L
1	A1	A2	A3	A4	A5	A6	A7	A8	A9	A10	A11	A12
2	B1	B2	B3	B4	B5	B6	B7	B8	B9	B10	B11	B12
3	C1	C2	C3	C4	C5	C6	C7	C8	C9	C10	C11	C12
4	D1	D2	D3	D4	D5	D6	D7	D8	D9	D10	D11	D12
5	E1	E2	E3	E4	E5	E6	E7	E8	E9	E10	E11	E12
6	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12
7	G1	G2	G3	G4	G5	G6	G7	G8	G9	G10	G11	G12
8	H1	H2	H3	H4	H5	H6	H7	H8	H9	H10	H11	H12

***transferring volume (ul) is the volume in ul that the liquid handler will transfer from each DNA sample (from the 1:20 Dilution plate) into the Normalized DNA plate and the volume of Dilution buffer (e.g., Tris) that the liquid handler will transfer into the Normalized DNA plate and mix to each DNA sample to have the desired final concentration (e.g., 4nM).

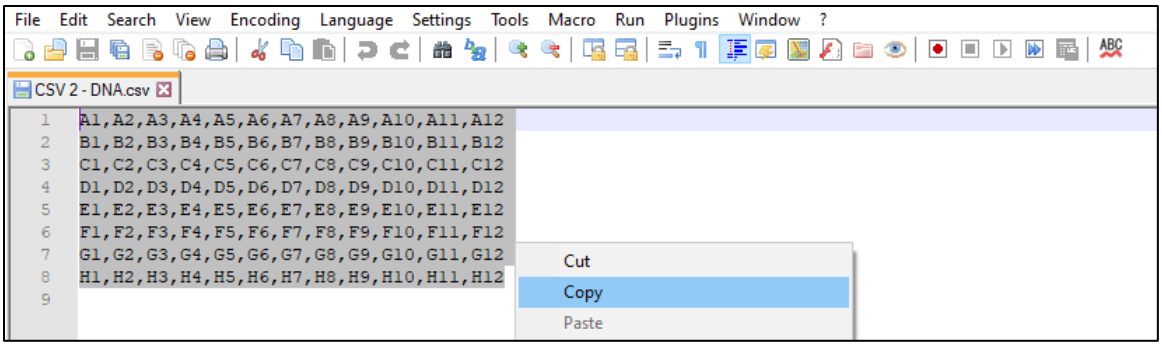
- Correct format of transferring volume : Volume in ul + dot + one decimal (e.g., 1.8 for 1.8 ul)
- The recommended final volume can be any volume between 10 - 20ul.
- If any value entered is lower than 1ul the liquid handler will load 1ul. In such cases it is recommended that you substitute all values lower than 1ul with Zero (0ul) and load the required volume manually.
- If any value entered in any table is zero, then the liquid handler will not transfer anything to the new plate (Normalized DNA plate).

8.5.3 Save as CSV 1 the transferring volumes of the Dilution buffer (e.g., dH2O, Tris etc.). Save as CSV 2 the transferring volumes of each DNA samples (1:20 Dilution plate).

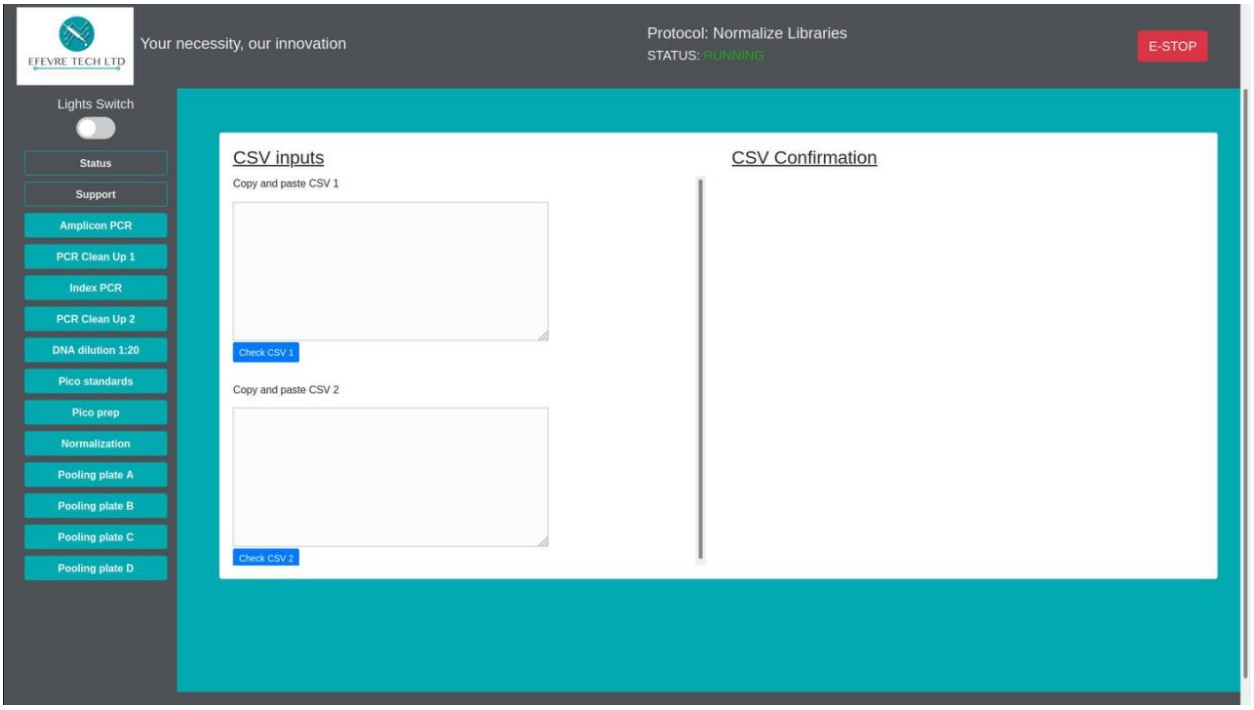
8.5.4 Open each CSV file by right click and Edit with Notepad.



8.5.5 Copy values (volume in ul).



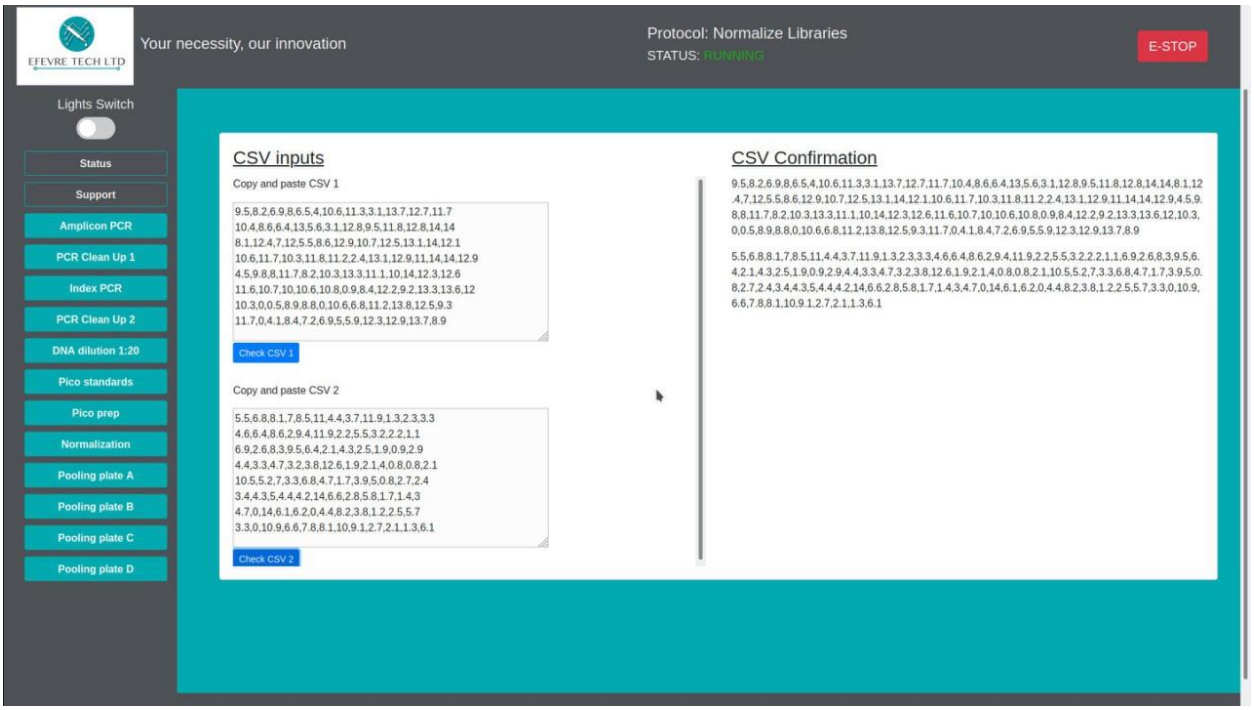
8.5.6 Paste values (volume in ul) in appropriate box (CSV 1 for dilution buffer / CSV 2 for DNA).



8.5.7 Check tables for missing values and incorrect characters (e.g., letters, comma before for decimals) by pressing Check CSV 1 and Check CSV 2 buttons.

- Correct format of values accordingly : Volume in ul + dot + one decimal (e.g., 1.8 for 1.8 ul)

- If no errors are contained within the tables. Please validate that all values are correct before proceeding with the process.



8.5.8 Follow instructions to START the process.

8.5.9 The Status is now RUNNING.

8.5.10 Estimated time for user action on Status page is the walk-away duration. Please return to the platform when the walk-away duration is finished.

8.5.11 After the automated process is finished the Status changes from RUNNING to IDLE. Please do the following:

a) Remove all labware from Deck 1.
b) Empty trash bin
c) Seal the 1:20 Dilution plate and Normalized DNA plate and spin down the liquid.

8.6 Automated Normalization protocol steps

1. Transferring Dilution buffer volumes as submitted in CSV 1 by user.
   P20
   Transferring CSV 1 volumes
   From P6. Tube on A1

To indicated by user wells in P2. Normalized DNA plate.
Touching tip.

2. Transferring Dilution buffer volumes as submitted in CSV 2 by user.
   P20
   Mixing of each sample in P1. 1:20 Dilution plate by pipetting 3ul up and down for 10 repeats.
   Transferring CSV 2 volumes
   From P1. 1:20 Dilution plate
   To corresponding (from A1 to A1) wells in P2. Normalized DNA plate.
   Mixing by pipetting 5ul mixture up and down for 20 repeats in P2. Normalized DNA plate after dispensing.
   Touching tip.

9. Pooling plate _ DECK 1

9.1 Description

Aliquot 2 µl of diluted Normalized DNA from each library and mix aliquots for pooling libraries with unique indices.

9.2 Source

16S Metagenomic Sequencing Library Preparation Guide Page 16.

ⓘ https://support.illumina.com/content/dam/illumina-support/documents/documentation/chemistry_documentation/16s/16s-metagenomic-library-prep-guide-15044223-b.pdf

9.3 Consumables

Normalized DNA plates A, B, C or D : 4nM DNA generated by Process 8. Normalization.

9.4 Labware setup

Please place the following labware on Liquid handling robot Deck 1:

DECK 1

Position 5:

Module/Labware : Fully skirted PCR plate _ Normalized DNA plate A-D

Model : 4ti-tude #4ti-0960

Containing : PCR_clean_up_2-generated DNA diluted 20 times in 10mM Tris pH 8.5 (see process 8)

Position 6:

Module/Labware : Tube rack for 1.5-2ml snap-cap tubes

Model : Eppendorf 2ml tube on A1 of tube rack

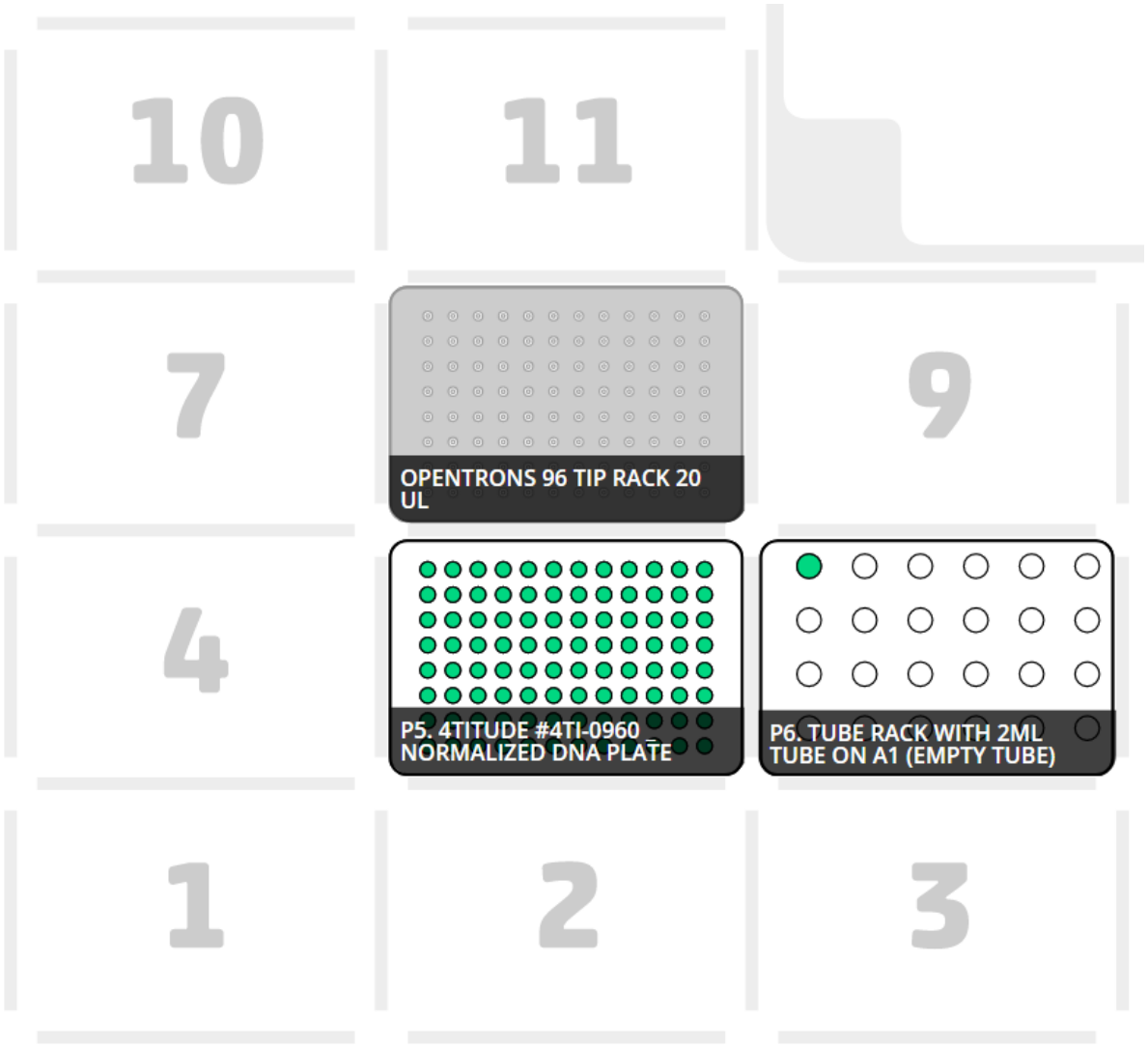
Containing : Nothing – Empty tube

Position 8:

Module/Labware : Tip rack

Model : Opentrons 20ul Tips

Containing : 96 Tips per rack



9. 5 Run the automated protocol

- The automated Pooling process for 384 samples (or less) is segmented in four distinct buttons.
- Each button executes the same process for a different Normalization plate (A-D).
- Only the dispensing height changes between processes Pooling plate A-D.

9.5.1 Please use the Pooling plate A-D buttons as follows:

Run Pooling plate A procedure when the automated Pooling process is used to transfer the first set of 96 samples (Normalization plate A) into the 2ml collection tube.

Run Pooling plate B procedure when the automated Pooling process is used to transfer a second set of 96 samples (Normalization plate B) into the same 2ml collection tube.

Run Pooling plate C procedure when the automated Pooling process is used to transfer a third set of 96 samples (Normalization plate C) into the same 2ml collection tube.

Run Pooling plate D procedure when the automated Pooling process is used to transfer a fourth set of 96 samples (or 48 samples) (Normalization plate D) into the same 2ml collection tube.

9.5.2 The Status is now RUNNING.

9.5.3 Estimated time for user action on Status page is the walk-away duration. Please return to the platform when the walk-away duration is finished.

9.5.4 After the automated process is finished the Status changes from RUNNING to IDLE. Please do the following:

a) Remove all labware from Deck 1.
b) Empty trash bin
c) Seal the Normalized DNA plate A, B, C or D (P5) and spin down the liquid.
d) Close lid of tube containing Pooled libraries and use 16S Metagenomic Sequencing Library Preparation Guide for next steps.

9.6 Automated Pooling plate protocol steps

Transferring 2ul from each well of P5. Normalized DNA plate to 2ml tube.

P20
Transferring 2ul Normalized DNA
From P5. Normalized DNA plate
To Tube on P6. Tube rack A1
Blowout.
Touching tip.

Troubleshooting

In case of emergency stop of automated procedure or device malfunction :

Phone : +0035799799808

Email : info@efevretech.com

- Maintenance information

  On-site check-up every 4-6 months

  Remote access to system (if allowed by user)

- Repair information

  In case of emergency stop of automated procedure or device malfunction :

  Phone : +0035799799808

  Email : info@efevretech.com

- Contact details
  info@efevretech.com