Urinary Tract Microbiota Profiling Experiments

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Note: For safety and biohazard guidelines, see the "Safety" appendix in the *Urinary Tract Microbiota Profiling Experiments Application Guide* (Pub. No. MAN0017750). Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

This document is intended as a benchtop reference for experienced users. See "Related documentation" on page 5 for resources that contain detailed instructions and troubleshooting.

Generate 384-well sample plate layouts in the OpenArray™ Sample Tracker Software

Before generating 384-well sample plate layouts, see "One-time procedures" on page 4.

1. Using a spreadsheet program, create a 96-well sample CSV file.

   CSV template files can be found at `<drive>:\Program files(x86)\AppliedBiosystems\OpenArray Sample Tracker\Examples`

2. Open the OpenArray™ Sample Tracker Software.

3. In the **Properties** screen, select **Gene Expression** for **Experiment Type**, then select the appropriate settings for **OpenArray™ Plate** and **Pipettor**.

4. In the **Samples** screen, click 📥 **Import**, then select and import your 96-well sample CSV file that you created in step 1.

5. In the **Sample Mapping** screen, confirm that the samples for a single OpenArray™ plate are assigned to one color.

   Note: If necessary, correct the **OpenArray™ Plate** and **Pipettor** settings in the **Properties** screen.

6. In the **Sample Mapping** screen, click the **384-Well Plate** tab, then click **Export ▸ Export *.csv**.

7. Select **384-Well Plate (for AccuFill)**, enter a file name, then save the exported file.

Plate layouts for the 384-well sample plates are saved to individual CSV files in the **Sample Tracker 384-well CSV Files** folder.

Set up the PCR reactions in an OpenArray™ 384-well Sample Plate

IMPORTANT! The 4 × 12 area(s) of the OpenArray™ 384-well Sample Plate being filled must match the area(s) designated in the OpenArray™ Sample Tracker Software for that set of samples.

1. Remove an OpenArray™ plate from the freezer and set it aside. Allow it to come to room temperature in its unopened sleeve (~15 minutes).

   The OpenArray™ plate must be completely thawed before transferring reactions to it from the OpenArray™ 384-well Sample Plate created in this section.

2. Gently swirl the contents of the TaqMan® OpenArray™ Real-Time PCR Master Mix to thoroughly mix. Do not invert the bottle.

3. Following the plate layout designated in the OpenArray™ Sample Tracker Software, add master mix, then DNA samples, to the wells of an OpenArray™ 384-well Sample Plate.

Component[1]	OpenArray™ Plate Format	
	18	**56**
	Volume per well	**Volume per well**
TaqMan® OpenArray™ Real-Time PCR Master Mix	2.5 µL	2.5 µL
DNA sample	2.5 µL	2.5 µL
Total reaction volume	**5.0 µL**	**5.0 µL**

[1] *(Optional)* Include the TaqMan® Urinary Tract Microbiota Amplification Control. For information about the amplification control, contact **CustomControls@thermofisher.com**.

4. Thoroughly mix each PCR reaction by pipetting up and down or by using the "mix" function on a multi-channel pipette.

5. Seal the OpenArray™ 384-well Sample Plate with an aluminum foil seal, remove the foil flap, then mark the edges of the filled 4 × 12 area with a pen.

6. Centrifuge the plate at 1,000 rpm for 1 minute.

7. Score the foil along the lines that were marked before centrifuging.

   Do not remove the foil from the scored area at this time.

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Set up the AccuFill™ instrument and software

IMPORTANT! Do not use OpenArray™ AccuFill™ System Tips that exceed the expiration date (shown on the outer box that contains the tip trays).

1. In the OpenArray™ AccuFill™ software, click **Setup and Load**.

   The **Setup Load Information** window appears.

2. Configure the **Loading Information** pane for sample integration using the 384-well sample plate CSV file and TPF files.

   a. In the **Loading Information** pane (top section of the window), ensure that the **Use Sample Integration** checkbox is selected.

   b. Click **Browse** to the right of the **Sample Plate** field, then select the 384-well sample plate CSV file that you generated with the OpenArray™ Sample Tracker Software.

   c. Click **Browse** to the right of the **Plate Holder Position** of the OpenArray™ plate, then select the TPF file for the OpenArray™ plate.

3. In the **Select Samples to Load** pane (bottom section of the window), click the corresponding 4 × 12 area of the 384-well sample plate image, then click **Next**.

   The **Setup Deck** window is displayed.

4. In the AccuFill™ instrument, ensure that:
   • Tip boxes and tips are loaded as shown in the **Setup Deck** window.
   • The lids are removed from the tip boxes.
   • The waste bin in the instrument is emptied.

5. In the **Setup Deck** window, confirm that the deck is ready:
   • Select **The tips are configured as shown above**.
   • Select **The Waste Bin is empty**.

Transfer reactions to the OpenArray™ plate using the AccuFill™ instrument

1. Prepare the items needed to seal the loaded OpenArray™ plate.

   Note: The OpenArray™ plate must be sealed promptly after being loaded with the reactions (this section).

   a. Ensure that the QuantStudio™ 12K Flex OpenArray™ Plate Press 2.0 is ready.

   b. Gather and remove from their packaging the following: an OpenArray™ lid, plug, syringe with OpenArray™ Immersion Fluid, and syringe tip.

   c. Attach the syringe tip to the syringe and carefully push some of the fluid through the tip to remove air bubbles, then lay the syringe aside.

2. Load the OpenArray™ plate and the OpenArray™ 384-well Sample Plate into the AccuFill™ instrument.

   a. **OpenArray™ plate**—Remove the plate from its sleeve, then place the plate in the appropriate plate holder position in the instrument.

   Ensure that the barcode on the OpenArray™ plate is facing left and the serial number is facing right.

   b. **OpenArray™ 384-well Sample Plate**—Place the 384-well sample plate onto the deck of the instrument, then, using forceps, peel the foil from the filled area of the plate.

3. Close the door of the AccuFill™ instrument.

4. In the AccuFill™ software **Setup Deck** window, select the following confirmations, then click **Load**.
   • **The OpenArray Plate is in the Plate Holder**
   • **Remove foil from the highlighted section of the Sample Plate**

5. As soon as the **Remove OpenArray Plate** window appears, open the instrument door, then remove the loaded OpenArray™ plate.

6. Proceed immediately to seal the OpenArray™ plate.

   Note: For best results, seal the OpenArray™ plate within 90 seconds of completion of loading, to prevent evaporation.
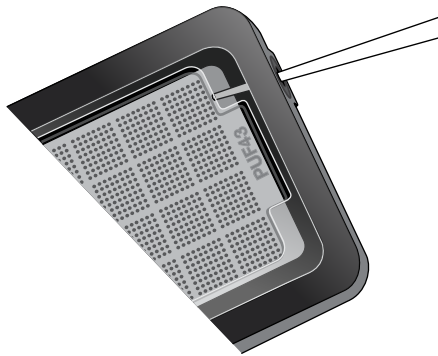
Seal the OpenArray™ plate

IMPORTANT! Handle the OpenArray™ plate and the OpenArray™ case (sealed plate + lid) only by the edges throughout this procedure.

1. Place the OpenArray™ plate (loaded with reactions) in the QuantStudio™ 12K Flex OpenArray™ Plate Press 2.0.

   Ensure that the barcode is facing left and the serial number is facing right.

2. From the OpenArray™ lid, remove the clear protective film from the *inside* of the lid and the red adhesive-protective strip from around the edge of the lid.

3. Seat the lid on the OpenArray™ plate with the lid adhesive against the plate.

4. Engage the press mechanism until the green flashing light changes to a steady green light (~20 seconds).

5. Disengage the press, then remove the OpenArray™ case (sealed plate + lid).

6. While holding the case by its edges, insert the prepared syringe tip into the port in the case, then carefully inject immersion fluid until the case is filled.

Note: Minimize creation of air bubbles when you dispense the fluid; one small air bubble in the case is acceptable.

The syringe tip must be in front of the array when filling the case with immersion fluid.

7. While holding the case *vertically*, remove the syringe tip, insert the screw end of the OpenArray™ plug into the port of the case and rotate clockwise until the black handle breaks off.

IMPORTANT! To avoid leaking of immersion fluid, hold the case *vertically* and rotate the plug slowly.

If the plug handle breaks off prematurely, use a Phillips #0 screwdriver to complete this step.

8. Clean the case with a laboratory wipe that has been thoroughly sprayed with ethanol, then dry the case with a clean laboratory wipe.

Run the OpenArray™ plate on the QuantStudio™ 12K Flex instrument

1. On the QuantStudio™ 12K Flex instrument touchscreen, touch ⏏ to extend the instrument tray arm.

2. Remove the clear protective film from the outside of the OpenArray™ case (sealed plate + lid).

3. Place the OpenArray™ case on the tray arm plate adapter.
   - Support the case from underneath the tray arm to prevent the case from slipping through the adapter.
   - Ensure that the plate barcode and serial number are facing the front of the instrument.

4. Touch ⏏ to retract the instrument tray arm.

5. In the 🏠 Home screen of the QuantStudio™ 12K Flex Software, in the **Run** pane, click **OpenArray**.

6. In the **Select Instrument** pane, select your instrument.

7. Click **Get Plate IDs** to import the barcode of the OpenArray™ plate.

Once the OpenArray™ serial number appears, the loaded TPF file corresponding to the plate should appear in the **Setup File** field.

If the TPF file does not appear, click **Browse**, then select the correct loaded TPF file from the **Loaded TPF** folder.

8. *(Optional)* Click **Browse** to change the **Experiment File Location**.

9. *(Optional)* Change the software-determined **Experiment File Name**.

10. Click **Start Run**.

Note: The instrument pauses at 41 or 42 seconds prior to the end of the run. Wait for the system to complete the run before opening the EDS file.

11. Transfer the EDS file from the instrument to an accessible location for analysis.

12. Check the QC images for loading issues or leaks.

Check the QC images

Check the QC images before analysis. Images can be viewed using ImageJ, an open source software available from the NIH (visit **imagej.nih.gov/ig**). For additional information, see *Urinary Tract Microbiota Profiling Experiments Application Guide* (Pub. No. MAN0017750).

1. In the QuantStudio™ 12K Flex Software 📊 **Export** screen:
   a. Click **Browse** to create a uniquely-named folder for the QC images export.
   b. Click **Export QC Images** (bottom of screen).

IMPORTANT! Create a new folder for images each time; exporting a second run to the same folder overwrites the images.

2. View the following ROX™ image to check for loading quality issues:
   - POST-READ_CHANNEL_4.tiff

3. Check for leaks or other displaced sample issues.
   a. View the following spotfinding images:
      - s02_c001_t03_p0001_m1_x2_e1_cp#_spotfind.tiff
      - s02_c040_t03_p0001_m1_x2_e1_cp#_spotfind.tiff

   Note: The "cp#" in the image file name refers to the array position (1–4) within the instrument.

   b. If a problem is found, view the following pre-run spotfinding image to determine if the issue existed even before cycling (this is useful for troubleshooting):
      - s00_c001_t01_p0001_m2_x3_e1_cp#_spotfind.tiff

4. View the following FAM™ images to check for any fluorescent abnormalities and to confirm any problem seen in the spotfinding images:
   - STAGE2_CYCLE1_CHANNEL_1.tiff

- STAGE2_CYCLE40_CHANNEL_1.tiff

5. Note any abnormalities found, as well as all other potentially relevant information related to the setup of the run.

One-time procedures

Set up default folders and software preferences

Set up the default file locations and preferences before using the OpenArray™ AccuFill™ system for the first time. You must be logged in as an administrator.

- On your computer, complete the following tasks:
  a. Create the following four folders in a convenient location on the same computer drive as the AccuFill™ software:
    - TPF Files
    - Sample Tracker 96-well Input
    - Sample Tracker 384-well CSV Files
    - Loaded TPF Files
  b. *(Optional)* Navigate to the following folder on your computer, copy the `96-Well Sample Plate 1.csv` template file (provided with the OpenArray™ Sample Tracker Software), then paste the template file into the **Sample Tracker 96-well Input** folder.

    <drive>:`\Program files(x86)\AppliedBiosystems\OpenArray Sample Tracker\Examples`

- In the OpenArray™ Sample Tracker Software, select **View ▸ Preferences**, then enter the following preferences:

Field	Selection
Experiment Type	**Gene Expression**
OpenArray™ Plate	Select the OpenArray™ format that will be run most often; for example, **Gene Expression – 56**
Pipettor	**Fixed** or **Adjustable** tip spacing
Import Data Directory	**Sample Tracker 96-well Input**
Export Data Directory	**Sample Tracker 384-well CSV Files**

- In the AccuFill™ software, select **Instrument ▸ Edit Preferences**, then:
  a. Select **Require Sample Integration**.

b. Select the indicated folders.

AccuFill™ folder	Default folder	Folder contents
OpenArray™ Plate File Input Folder	**TPF Files**	TPF files for the OpenArray™ plates; contain assay name and location
Sample Plate File Folder	**Sample Tracker 384-well CSV Files**	CSV 384-well sample plate layout files
Loaded OpenArray™ Plate File Folder	**Loaded TPF Files**	Integrated TPF files that are generated during processing with the AccuFill™ software.

- In the QuantStudio™ 12K Flex Software, select **Tools ▸ Preferences ▸ OpenArray**, then select the **Loaded TPF Files** folder for the software **Setup Folder**.

  Note: If the QuantStudio™ 12K Flex Software is not on the same computer as the AccuFill™ software, transfer the loaded TPF files to the computer running the QuantStudio™ 12K Flex Software.

Download TPF files

Set up the optimized folder locations and software preferences before downloading TPF files.

To download TPF files for custom OpenArray™ plates, you need the **Lot#** and the **Serial#** from the packaging of each OpenArray™ plate.

1. Go to **thermofisher.com/OA-platefiles**.

2. From the **Select Your Product** dropdown list, select **TaqMan® OpenArray™ Custom Gene Expression/Genotyping Plates**.

3. Select the desired option for downloading either only the TPF files or both the TPF files and the AIF files.

4. Enter the **Lot#** and the **Serial#**, then click **Submit**.

   Note: The **Serial#** is case-sensitive.

5. Save the TPF files to the desktop **TPF Files** folder.

   Note: Do not create sub-folders in the **TPF Files** folder. The software cannot access sub-folders.

Documentation and support

Related documentation

Document	Pub. No.
Urinary Tract Microbiota Profiling Experiments Application Guide	MAN0017750
TaqMan® Urinary Tract Microbiota Amplification Control Product Information Sheet	MAN0017753
QuantStudio™ 12K Flex Real-Time PCR System: OpenArray™ Experiments User Guide	4470935
OpenArray™ Sample Tracker Software Quick Reference	4460657
OpenArray™ AccuFill™ System User Guide	4456986

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- Product documentation, including:
  - User guides, manuals, and protocols
  - Certificates of Analysis
  - Safety Data Sheets (SDSs; also known as MSDSs)

  Note: For SDSs for reagents and chemicals from other manufacturers, contact the manufacturer.

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A.0	06 August 2018	New document.

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