

Respiratory Tract Microbiota Profiling Experiments v2

OpenArray™ Plates and OpenArray™ AccuFill™ Software v1.2

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Note: For safety and biohazard guidelines, see the “Safety” appendix in the *Respiratory Tract Microbiota Profiling Experiments v2 using OpenArray™ Plates Application Guide* (Pub. No. MAN0019506). 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 6 for resources that contain detailed instructions and troubleshooting.

Note: This document includes instructions for OpenArray™ AccuFill™ Software v1.2. For instructions for OpenArray™ AccuFill™ Software v2.0, see *Respiratory Tract Microbiota Profiling Experiments v2 Using OpenArray™ Plates and OpenArray™ AccuFill™ Software v2.0 Quick Reference* (Pub. No. MAN0026009).

Perform preamplification and dilute samples

Perform preamplification

Start with isolated nucleic acid. For the nucleic acid isolation procedure, see *Respiratory Tract Microbiota Profiling Experiments v2 using OpenArray™ Plates Application Guide* (Pub. No. MAN0019506).

Note: Preamplification of the TrueMark™ Respiratory Panel 2.0 Amplification Control is not recommended.

1. Prepare the PreAmp Reaction Mix: Combine the following components for the number of required reactions plus 10% overage, then mix thoroughly by pipetting up and down.

Component	Volume per reaction
TaqPath™ 1-Step RT-qPCR Master Mix, CG	2.5 µL
TrueMark™ Respiratory Panel 2.0 PreAmp Primers ^[1]	2.5 µL

^[1] The TrueMark™ Respiratory Panel 2.0 PreAmp Primers contains primers for all respiratory tract microbiota assays plus the TaqMan™ Universal RNA Spike In/Reverse Transcription (Xeno) Control and the RNase P RPPH1 gene, but does not contain primers for the TaqMan™ Universal Extraction Control Organism (*B. atrophaeus*).

2. Distribute the PreAmp Reaction Mix, then nuclease-free water or sample nucleic acid to the appropriate wells of a 96-well plate.

Component	Sample reaction	No-template control (NTC) reaction
PreAmp Reaction Mix	5 µL	5 µL
Sample DNA or NEC	5 µL	—
Nuclease-free water	—	5 µL
Total volume per reaction	10 µL	10 µL

3. Seal the plate with adhesive film.
4. Gently vortex the plate for 10 seconds to mix, then briefly centrifuge to bring contents to the bottom of the wells.
5. Place the plate in a thermal cycler that is programmed with the following thermal cycling conditions, then start the run.

Stage	Step	Temperature	Time
Hold	UNG incubation ^[1]	25°C	2 minutes
Hold	Reverse transcription	50°C	30 minutes
Hold	Activation	95°C	2 minutes
Cycling (14 cycles)	Denaturation	95°C	15 seconds
	Annealing/Extension	60°C	2 minutes
Hold	Inactivation	99.9°C	10 minutes
Hold	—	4°C	Hold

^[1] Heat-labile UNG is completely inactivated during the initial ramp to 95°C.

6. Store the plate on ice until dilution for PCR (see “Dilute the preamplified sample” on page 2).

Dilute the preamplified sample

To determine dilution volumes, first determine the total volume of diluted preamplified sample that is required for PCR (see “Prepare and run respiratory tract microbiota profiling experiments with OpenArray™ plates” on page 2). We recommend that you prepare only the volume of diluted preamplified sample that is required for your experiment. The undiluted preamplified sample can be stored at –20°C long term.

1. Vortex, then briefly centrifuge the plate that contains the completed preamplification sample reactions.
2. Remove the adhesive film from the plate.
3. Prepare a 1:10 dilution of the preamplified samples in a new 96-well plate.
 - a. Transfer the desired volume of the preamplified samples to a new 96-well plate (for example, 2 µL).
 - b. Add the appropriate volume of nuclease-free water to each sample and control well (for example, 18 µL).
4. Seal the plate with new adhesive film.
5. Vortex the plate for 10 seconds, then briefly centrifuge.
6. Proceed directly to prepare the OpenArray™ Plates (see “Prepare and run respiratory tract microbiota profiling experiments with OpenArray™ plates” on page 2).

Seal the plate that contains the unused portion of the undiluted preamplified samples, then store at –20°C.

Prepare and run respiratory tract microbiota profiling experiments with OpenArray™ plates

One-time procedures

Set up default folders and software preferences

Set up the default file locations and preferences before using the QuantStudio™ 12K Flex OpenArray™ AccuFill™ System for the first time.

1. Create the following four folders in a convenient location on the same computer drive as the OpenArray™ AccuFill™ Software:
 - TPF Files
 - Sample Tracker 96-well Input
 - Sample Tracker 384-well CSV Files
 - Loaded TPF Files
2. (Optional) Copy a template file into the OpenArray™ Sample Tracker Software folder.
 - Navigate to this folder on your computer: <drive>:\Program Files (x86)\Applied Biosystems\OpenArray Sample Tracker\examples.
 - Copy the 96-Well Sample Plate 1.csv template file, which is provided with the OpenArray™ Sample Tracker Software.

- Paste the template file into the Sample Tracker 96-well Input folder.

3. 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, such as Gene Expression – 112 .
Pipettor	Fixed or Adjustable tip spacing
Import Data Directory	Sample Tracker 96-well Input
Export Data Directory	Sample Tracker 384-well CSV Files

4. In the OpenArray™ AccuFill™ Software, select **Instrument ▶ Edit Preferences ▶ Require Sample Integration**, then select the folders indicated in this table:

OpenArray™ AccuFill™ Software folder	Default folder	Folder contents
OpenArray Plate File Input Folder	TPF Files	TPF files for the OpenArray™ Plates, with 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 generated during processing with the OpenArray™ AccuFill™ Software.

5. 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 OpenArray™ AccuFill™ Software, transfer the loaded TPF files to the computer running the QuantStudio™ 12K Flex Software.

Obtain TPF files

Go to [thermofisher.com/OA-platefiles](https://www.thermofisher.com/OA-platefiles) to obtain the TPF files for the TrueMark™ Respiratory Panel 2.0, OpenArray™ Plate.

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

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 2.

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

CSV template files can be found at <drive>:\Program Files (x86)\Applied Biosystems\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 (OpenArray™ AccuFill™ Software v1.2)

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.

(Optional) Use the TrueMark™ Respiratory Panel 2.0 Amplification Control in place of diluted preamplified sample, as a positive amplification control sample.

Component	Volume per well	Volume per sample
TaqMan™ OpenArray™ Real-Time PCR Master Mix	2.5 µL	5.0 µL
Diluted preamplified sample	2.5 µL	5.0 µL
Total reaction volume	5.0 µL	10.0 µL

Note: An overage is recommended when preparing the components.

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 1,200 × g 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.

Set up the OpenArray™ AccuFill™ Instrument and the OpenArray™ AccuFill™ 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 in the Sample Tracker 384-well CSV Files folder.
 - c. Click **Browse** to the right of the **Plate Holder Position** of the OpenArray™ Plate, then select the TPF file for the OpenArray™ Plate in the TPF Files folder.

- 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.

- In the OpenArray™ 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.
- 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 OpenArray™ AccuFill™ Instrument

- Prepare the items needed to seal the loaded OpenArray™ Plate.

Note: The OpenArray™ Plate must be sealed promptly after being loaded with the reactions, as described here.

- Ensure that the QuantStudio™ 12K Flex OpenArray™ Plate Press 2.0 is ready.
 - Gather and remove from their packaging the following: an OpenArray™ Lid, plug, syringe with OpenArray™ Immersion Fluid, and syringe tip.
 - Attach the syringe tip to the syringe, carefully push some of the fluid through the tip to remove air bubbles, then lay the syringe aside.
- Load the OpenArray™ Plate and the OpenArray™ 384-well Sample Plate into the OpenArray™ AccuFill™ Instrument.
 - 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.
 - OpenArray™ 384-well Sample Plate**—Place the 384-well sample plate onto the deck of the instrument, then use forceps to peel the foil from the filled area of the plate.
 - Close the door of the instrument.
 - In the OpenArray™ AccuFill™ Software **Setup Deck** window, select the following confirmations:
 - The OpenArray Plate is in the Plate Holder**
 - Remove foil from the highlighted section of the Sample Plate**
 - Click **Load**.
 - As soon as the **Remove OpenArray Plate** window appears, open the instrument door and remove the loaded OpenArray™ Plate.

- Proceed immediately to seal the OpenArray™ Plate.

See “Seal the OpenArray™ Plate” on page 4.

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

Seal the OpenArray™ Plate

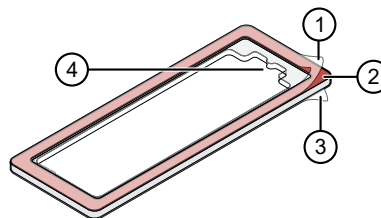
IMPORTANT! Throughout this procedure, handle the OpenArray™ Plate and the OpenArray™ Case only by the edges.

Note: The OpenArray™ Case consists of the sealed OpenArray™ Plate and the OpenArray™ Lid.

- Place the newly loaded OpenArray™ Plate in the QuantStudio™ 12K Flex OpenArray™ Plate Press 2.0.

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

- 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.



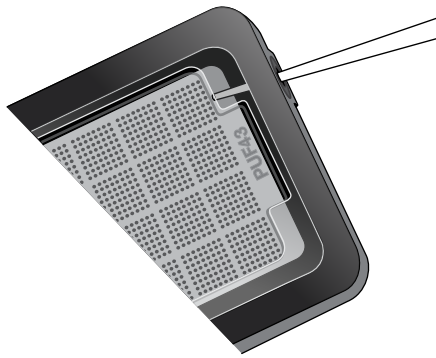
OpenArray™ Lid

- Protective film on inside of the lid (remove before *sealing*)
- Red adhesive-protective strip (remove before *sealing*)
- Protective film on the outside of the lid (remove before *running*)
- Notched end (align with serial number on plate)

- Seat the lid on the OpenArray™ Plate with the lid adhesive against the plate.
- Engage the press mechanism until the green flashing light changes to a steady green light (approximately 20 seconds).
- Disengage the press and remove the OpenArray™ Case.
- While holding the case by its edges, insert the prepared syringe tip into the port in the case, then carefully inject OpenArray™ Immersion Fluid until the case is filled.

Note:

- Minimize creation of air bubbles when you dispense the fluid.
- Leave a small bubble at the fill point to prevent fluid leaks during the instrument run.



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, then 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. If needed, 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 quality-control images

Check the quality-control (QC) images before analysis. Images can be viewed using ImageJ, an open-source software available from the NIH at imagej.nih.gov/ig.

1. In the QuantStudio™ 12K Flex Software  **Export** screen, click **Browse**, then create a uniquely-named folder for the QC images export.

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

2. Click **Export QC Images** at the bottom of the screen.
3. View the following ROX™ image to check for loading quality issues:
 - POST-READ_CHANNEL_4.tiff
4. Check the following spotfinding images for leaks or other displaced sample issues.
 - 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 array positions 1 through 4 within the instrument.
5. If a problem is found, view the following pre-run spotfinding image to determine whether the issue existed before cycling:
 - s00_c001_t01_p0001_m2_x3_e1_cp#_spotfind.tiff
6. View the following FAM™ images to check for fluorescent abnormalities and to confirm any problem seen in the spotfinding images:
 - STAGE2_CYCLE1_CHANNEL_1.tiff
 - STAGE2_CYCLE40_CHANNEL_1.tiff
7. Note any abnormalities found, as well as all other potentially relevant information related to the setup of the run.

Related documentation

Document	Pub. No.
<i>Respiratory Tract Microbiota Profiling Experiments v2 using OpenArray™ Plates Application Guide</i>	MAN0019506
<i>Respiratory Tract Microbiota Profiling Experiments v2 Using OpenArray™ Plates and OpenArray™ AccuFill™ Software v2.0 Quick Reference</i>	MAN0026009
<i>TrueMark™ Respiratory Panel 2.0, OpenArray™ Plate Product Information Sheet</i>	MAN0019510
<i>TrueMark™ Respiratory Panel 2.0 Amplification Control Product Information Sheet</i>	MAN0019505
<i>QuantStudio™ 12K Flex Real-Time PCR System: OpenArray™ Experiments User Guide</i>	4470935
<i>OpenArray™ Sample Tracker Software Quick Reference, for OpenArray™ AccuFill™ Software v1.2</i>	4460657
<i>OpenArray™ AccuFill™ System User Guide, for OpenArray™ AccuFill™ Software v1.2</i>	4456986
<i>QuantStudio™ 12K Flex OpenArray™ AccuFill™ System User Guide, for OpenArray™ AccuFill™ Software v2.0</i>	MAN0025669

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Revision history: Pub. No. MAN0019509

Revision	Date	Description
B.0	6 December 2021	<ul style="list-style-type: none">• A note was added to specify that this document is for OpenArray™ AccuFill™ Software v1.2.• The volumes that are provided for setting up PCR reactions had the 10% overage removed (an overage is still recommended, to be determined and calculated by the user).
A.0	5 January 2021	New document.

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