

OpenArray™ Vaginal Microbiota Profiling Experiments

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Note: For safety and biohazard guidelines, see the “Safety” appendix in the *Vaginal Microbiota Profiling Experiments Application Guide* (Pub. No. MAN0015669). 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 4 for resources that contain detailed instructions and troubleshooting.

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

See “One-time procedures” on page 4 to:

- Set up optimized folder locations and software preferences
- Download the *.tpf files for the OpenArray™ plates

1. Generate the 96-well sample plate *.csv file using the 96-Well Sample Plate 1.csv template, then save it to the Sample Tracker 96-well Input folder.

Note: We recommend three technical replicates of each reaction.

2. In the Sample Tracker Software **Properties** screen, select **Gene Expression** for **Experiment Type**, then select the appropriate settings for **OpenArray™ Plate** and **Pipettor**.
3. In the **Samples** screen, click  **Import**, then select and import the sample *.csv file.
4. In the **Sample Mapping** screen, confirm that the samples for a single OpenArray™ plate are assigned to one color.
If necessary, correct the **OpenArray™ Plate** and **Pipettor** settings in the **Properties** screen.
5. In the **Sample Mapping** screen, click the **384-Well Plate** tab, then click **Export** ▶ **Export *.csv**.
6. Select **384-Well Plate (for AccuFill)**, 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 384-well plate being filled must match the area(s) designated in the OpenArray™ Sample Tracker Software for that set of samples.

1. Remove the OpenArray™ plate from the freezer and allow it to thaw in its sleeve, unopened, at room temperature (~15 minutes).

The OpenArray™ plate must be completely thawed before transferring reactions to it from the 384-well sample plate.

2. Gently shake the bottle of TaqMan® OpenArray™ Real-Time PCR Master Mix to thoroughly mix the contents. 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	OpenArray™ Plate Format		
	18	56	112 ^[1]
	Volume per well	Volume per well	Volume per well
TaqMan® OpenArray™ Real-Time PCR Master Mix	2.5 µL	2.5 µL	2.5 µL
DNA sample	2.5 µL	2.5 µL	2.5 µL
Total reaction volume	5.0 µL	5.0 µL	5.0 µL

^[1] For the 112-format, the OpenArray™ Sample Tracker Software designates two wells for each sample.

4. Seal the plate with an aluminum foil seal, remove the foil flap, mark the edges of the filled 4 × 12 area with a pen, then score the foil along those lines.

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

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

Set up the AccuFill™ instrument

IMPORTANT! Before proceeding, check the tip expiration date (shown on the outer box that contains the trays of tips). Do not use tips that exceed the expiration date.

1. In the OpenArray™ AccuFill™ software, click **Setup and Load**.
2. In the Setup Load Information window, verify that the **Use Sample Integration** checkbox is selected.
3. Click **Browse** to the right of **Sample Plate**, then select the 384-well sample plate *.csv file that was generated with the OpenArray™ Sample Tracker Software.
4. Click **Browse** to the right of the plate holder position corresponding to the OpenArray™ of interest, then select the *.tpf file corresponding to the desired OpenArray™ plate.
5. Click the corresponding 4 × 12 area of the 384-well plate, then click **Next** to open the **Setup Deck** window.
6. Ensure that:
 - Tip boxes are loaded in the AccuFill™ instrument in the displayed configuration.
 - Lids are removed from the tip boxes.
 - The waste bin in the instrument is emptied.
7. In the **Setup Deck** window, select:
 - **The tips are configured as shown above**
 - **The Waste Bin is empty**

Transfer reactions to the OpenArray™ plate (AccuFill™ instrument)

1. Prepare the items needed to seal the 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 packaging 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. Remove the OpenArray™ plate from its sleeve and place it in the plate holder of the AccuFill™ instrument.

Ensure that the bar code on the OpenArray™ plate is facing left and the serial number is facing right.
3. Using forceps, peel the foil from the filled area of the OpenArray™ 384-well Sample Plate.
4. Close the instrument door.

5. 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**
6. As soon as the Remove OpenArray Plate window appears, open the instrument door, then remove the loaded OpenArray™ plate.
7. 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 case only by the edges throughout this procedure.

1. Place the filled OpenArray™ plate in the QuantStudio™ 12K Flex OpenArray™ Plate Press 2.0.

Ensure that the bar code is facing left and the serial number is facing right.
2. Remove the clear plastic sheets from the top and the bottom of the lid, remove the red protective film around the edge of the OpenArray™ lid, then seat the lid on the OpenArray™ case in the plate press.
3. Engage the press mechanism until the green flashing light changes to a steady green light (~20 seconds).
4. Disengage the press, then remove the OpenArray™ case.
5. While holding the OpenArray™ case by the 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.

6. While holding the case vertically, remove the syringe tip, insert the screw end of the OpenArray™ plug into the port 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.

7. 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(s) on the QuantStudio™ 12K Flex instrument

1. On the instrument touchscreen, touch  to extend the loading arm, and place the OpenArray™ plates on the plate adapter.

Ensure that the plate barcode and serial number are facing the front of the instrument.

2. Touch  to retract the loading arm.
3. In the  **Home** screen of the QuantStudio™ 12K Flex Software, select **Run** ▶ **OpenArray**.
4. In the **Select Instrument** pane, select your QuantStudio™ instrument.
5. Click **Get Plate IDs** to import the barcode(s) of the OpenArray™ plate(s).
Once the OpenArray™ serial numbers appear, the loaded *.tpf files corresponding to each plate should appear in the **Setup File** field.
If not, click **Browse**, then select the correct loaded *.tpf file from the Loaded TPF folder.
6. (Optional) Click **Browse** to change the QuantStudio™ **Experiment File Location**.
7. (Optional) Change the software-determined **Experiment File Name**.
8. 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.
9. Transfer the *.eds file from the instrument to an accessible location for analysis.
10. Check the QC images for loading issues or leaks.

Check the QC images

Check the QC images before analysis. For additional information, see *Vaginal Microbiota Profiling Experiments Application Guide* (Pub. No. MAN0015669).

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.

Recover from layout errors in the 384-well sample plate

After the AccuFill™ procedure, you can recover from plate layout errors that were made during setup of the reactions in the 384-well sample plate. See the *OpenArray™ Sample Tracker Software Quick Reference* (Pub. No. 4460657) for additional information.

1. Create a corrected sample *.csv file.
2. Repeat “Generate 384-well sample plate layouts in the OpenArray™ Sample Tracker Software” on page 1, but select **OpenArray Plate X (for QuantStudio)** when exporting from the OpenArray™ Sample Tracker Software.
3. Import the corrected *.csv file into the QuantStudio™ 12K Flex Software.
Note: You can import either before starting the run or after the run is complete.

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.

1. 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
2. (Optional) Navigate to <drive>:\Program files(x86)\AppliedBiosystems\OpenArray Sample Tracker\Examples, copy the 96-Well Sample Plate 1.csv file, then paste it in 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; for example, Gene Expression – 56
Pipettor	Fixed or Adjustable
Import Data Directory	Sample Tracker 96-well Input
Export Data Directory	Sample Tracker 384-well CSV Files

4. 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.

5. In the QuantStudio™ 12K Flex instrument software, select **Tools ▶ Preferences ▶ OpenArray**, then select the **Loaded TPF Files** folder for the software **Setup Folder**.

Note: If the instrument software is not on the same computer as the AccuFill™ software, transfer the loaded *.tpf files to the computer running the instrument software.

Download *.tpf files

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

For custom OpenArray™ plates, you need the **Lot#** and the **Serial#** from the packaging of each OpenArray™ plate.

1. At www.thermofisher.com/OA-platefiles, select **TaqMan® OpenArray™ Custom Gene Expression/Genotyping Plates** in the **Select Your Product** dropdown list.
2. Select a download option:
 - **I want to download all available TPF & AIF files**
 - **I want to download a specific TPF file**
3. Enter the **Lot#** and the **Serial#**, then click **Submit**.

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

4. 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.

Related documentation

Document	Pub. No.
<i>Vaginal Microbiota Profiling Experiments Application Guide</i>	MAN0015669
<i>QuantStudio™ 12K Flex Real-Time PCR System: OpenArray™ Experiments User Guide</i>	4470935
<i>OpenArray™ Sample Tracker Software Quick Reference</i>	4460657
<i>OpenArray™ AccuFill™ System User Guide</i>	4456986

The information in this guide is subject to change without notice.

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A.0	28 June 2016	New document.

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