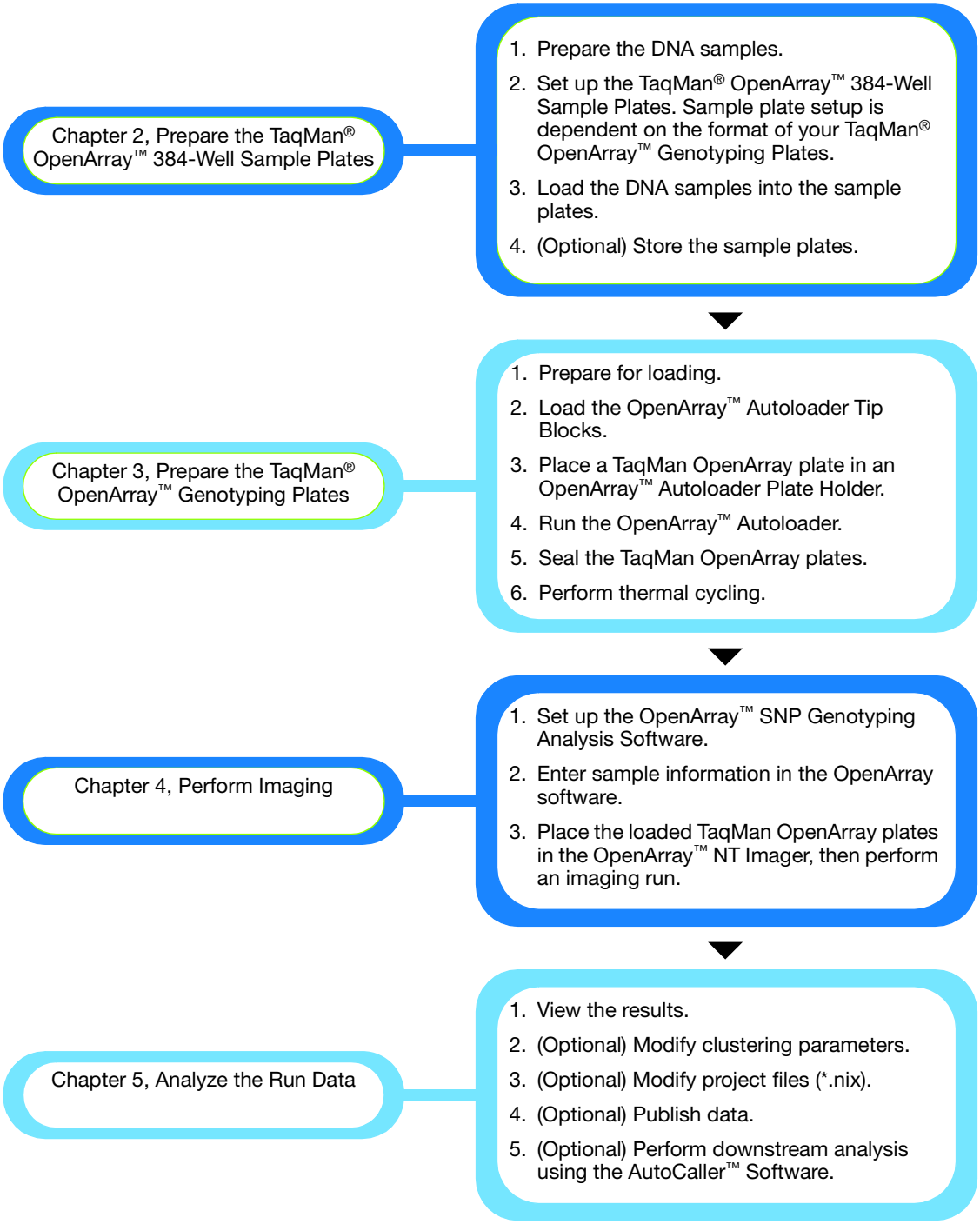
TaqMan® OpenArray™ Genotyping System Workflow

Note: For safety and biohazard guidelines, refer to the "Safety" section in the *TaqMan® OpenArray™ Genotyping System User Guide* (PN 4377476). For all chemicals in **bold red** type, read the MSDS and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Overall Workflow

For detailed procedures, see the referenced chapter numbers in the *TaqMan® OpenArray™ Genotyping System User Guide.*



Prepare the TaqMan® OpenArray™ 384-Well Sample Plate

1 Prepare the DNA samples

Be sure that the quality and quantity of DNA meet the guidelines recommended in the "Prepare the DNA samples" section of the *TaqMan® OpenArray™ Genotyping System User Guide*.

To quantify human genomic DNA:

a. Create and set up a sequence detector plate document.

b. Prepare the reaction plate. Use the TaqMan® RNase P Detection Reagents Kit (PN 4316831) and TaqMan® DNA Template Reagents Kit (PN 401970).

Use at least three replicates of each standard or sample, and use all five DNA standards to ensure an accurate standard curve is generated. The range of known copy number should bracket anticipated copy numbers of the unknown samples on the same reaction plate.

c. Run the reaction plate on an Applied Biosystems Real-Time PCR System using the following thermal cycling conditions:

	AmpliTaq® Gold enzyme activation	**PCR**	
	HOLD	CYCLE (40 cycles)	
		Denature	Anneal/extend
Time	10 min	15 sec	1 min
Temp	95 °C	92 °C	60 °C

d. Generate a standard curve to quantify the amount of DNA in each sample.

e. Normalize all the human genomic DNA samples to a working stock concentration of 50 ng/µL, ~15,000 copies/µL.

The acceptable starting concentration for human DNA samples is 50 ng/µL. To calculate the starting concentration for other species, see the "DNA Calculator" section of the *TaqMan® OpenArray™ Genotyping System User Guide*.

2 Set up the sample plates

a. Label each TaqMan® OpenArray™ 384-Well Sample Plate with a unique identifier.

b. Determine how to arrange the samples in each sample plate, based on the format of the TaqMan® OpenArray™ Genotyping Plate that you will transfer the samples to.

c. (Recommended) Create a sample information file (*.csv) to track where the samples are in the sample plate.

3 Load DNA samples into the sample plate(s)

a. At room temperature, thaw the DNA samples. Mix by vortexing, then centrifuge for 1 min @ 1000 rpm.

b. Review the concentration of the normalized genomic DNA samples.

c. Mix the TaqMan® OpenArray™ Master Mix by gently inverting the tube 10 times.

d. Add the master mix and the normalized DNA samples to the sample plate. For human DNA samples, use the amounts listed below *per well of the sample plate*:

Component	**Volume (µL) per well‡, when transferring to...**		
	Format 16	**Format 32**	**All other formats**
Normalized human DNA sample Starting concentration = 50 ng/µL	1.5	2.0	2.5
TaqMan® OpenArray™ Master Mix, 2×	1.5	2.0	2.5
Total volume	**3.0**	**4.0**	**5.0**

‡ 1 well of a sample plate corresponds to 1 subarray of a TaqMan OpenArray Genotyping Plate.

e. Mix well by gently pipetting up and down, then cover the sample plate with sealing tape.

f. Centrifuge the sample plate for 1 min @ 1000 rpm to eliminate bubbles.

Prepare the TaqMan® OpenArray™ Genotyping Plates

1 Prepare for loading

a. Be sure that the OpenArray™ Plate Guide Set, OpenArray™ Autoloader Tip Blocks, and OpenArray™ Autoloader Plate Holder are completely clean and dry.

b. Fill the appropriate number of TaqMan® OpenArray™ Genotyping Cases with **TaqMan® OpenArray™ Immersion Fluid**.

c. Remove the TaqMan OpenArray plates from the freezer, *but do not open the packaging*. Allow the TaqMan OpenArray plates to thaw at room temperature (approximately 5 min).

2 Load the OpenArray™ Autoloader Tip Blocks

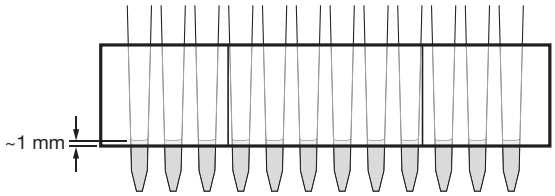
a. Peel the sealing tape from the area of the sample plate that contains the samples to be transferred.

b. From the OpenArray™ Plate Guide Set, select the plate guide that lines up with the 12-well × 4-well areas in the sample plate, then place the plate guide over the sample plate.

c. Place the tip block into the appropriate area of the plate guide.

d. Using the Finnpipette Multichannel Digital Pipettor (or by hand), place 12 TaqMan® OpenArray™ Loader Tips in each hole of the tip block (one row). Release the tips when they are submerged.

e. Slide the tip block up and down (approximately 25 to 50 times), until the pipettes are filled to 1 mm above the bottom edge of the tip block. Visually inspect the tip block to ensure that the tips are filled to 1 mm above the bottom edge of the tip block and that there are no air bubbles.



IMPORTANT! If the tips are not filled correctly, product performance may be adversely affected.

f. Leave the tip block with the loaded tips in the plate guide, keeping the tips submerged in sample, until the TaqMan OpenArray plate is ready for loading.

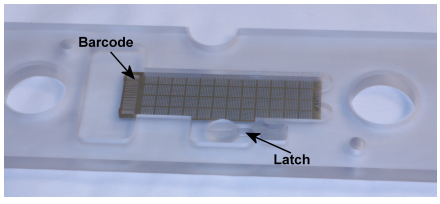
3 Place a TaqMan® OpenArray™ plate in a plate holder

IMPORTANT! Hold the TaqMan OpenArray plate by the edges. Do not touch the through-holes.

IMPORTANT! Load the TaqMan OpenArray plate with sample, place the loaded plate in a TaqMan OpenArray Genotyping Case, then seal the case *within 1 hour after opening the plate packaging*.

a. Remove a thawed TaqMan OpenArray plate from its packaging.

b. Orient the plate holder so that the latch is towards you, then orient the TaqMan OpenArray plate so that the barcode is facing up and to your left.



c. Pull the latch on the plate holder towards you to drop the TaqMan OpenArray plate into place. Be sure that the TaqMan OpenArray plate is all the way to the right, then release the latch.

d. With clean forceps, push the TaqMan OpenArray plate flat. Push the forceps against all four corners and the edges, carefully avoiding the through-holes. The numbered side of the TaqMan OpenArray plate should be flush with the plate holder.

4 Run the OpenArray™ Autoloader

WARNING! PHYSICAL INJURY HAZARD. Moving parts can crush and cut. Keep hands clear of moving parts while operating the instrument.

Set up the Autoloader:

a. Power on the Autoloader, then press **ENTER** at the prompt. The following message appears:

Samples/Subarray:
ENTER: # [where # is the number of loads the Autoloader will perform: *ONE*, *TWO*, or *THREE*]
NEXT: More Choices

b. Press **ENTER** to accept the current number, *or* press **NEXT** until the correct number appears, then press **ENTER**. Be sure to select the correct number of loads for your TaqMan OpenArray plate.

c. At the prompt, place the TaqMan OpenArray plate and plate holder on the Autoloader platform. The notch in the plate holder should face the instrument.

d. Gently push the plate holder all the way down, then press **ENTER** to send the platform to the load position.

e. At the prompt, insert the loaded tip block:
   - Place the loaded tip block above the TaqMan OpenArray plate.
   - Align the tip block with the metal guide pins on the Autoloader.
   - Bring the tip block straight down, without tilting it. Slowly position the tip block over the metal guide pins.

   IMPORTANT! Insert the loaded tip block only when prompted.

f. Gently slide your finger across the tops of the tips so that the tip heights are level.

   IMPORTANT! In order for the Autoloader to properly load the TaqMan OpenArray plate with sample, the tip heights must be level.

Load the sample:

a. On the Autoloader, press **ENTER**. The samples in each tip are loaded in the appropriate through-holes.

b. At the prompt, remove the tip block: Slowly pull the tip block straight up, without any rocking motion, then press **ENTER**.

   IMPORTANT! To ensure the samples are uniformly loaded in the TaqMan OpenArray plate, remove the tip block slowly and evenly.
   Note: If you programmed the Autoloader to perform two or three loads, you are prompted to remove the current tip block and insert the next tip block.

c. Follow the prompts to remove the plate holder from the Autoloader platform.

d. Place the plate holder on a flat surface, then remove the TaqMan OpenArray plate by pushing the latch down and carefully lifting the TaqMan OpenArray plate.

5 Seal the TaqMan® OpenArray™ plates

WARNING! ULTRAVIOLET LIGHT HAZARD. Looking directly at a UV light source can cause serious eye damage. Never look directly at a UV light source. Follow the manufacturer's recommendations for the appropriate eyewear and clothing.

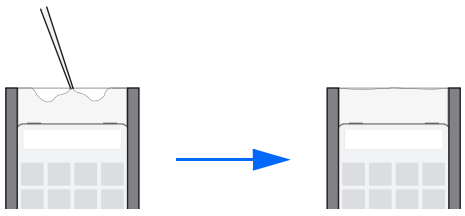
a. Hold the TaqMan OpenArray plate with the barcode facing up.

b. Slide the TaqMan OpenArray plate into a TaqMan OpenArray Genotyping Case so that the plate sits between the rails on the inside edges of the case. Be sure that the plate barcode is at the top of the case and facing the black, painted side of the case.

c. Push the TaqMan OpenArray plate all the way down into the case.

d. If needed, adjust the level of immersion fluid with a transfer pipette. The immersion fluid should be approximately 1 mm above the TaqMan OpenArray plate.

e. Fill the case with **TaqMan® OpenArray™ Sealing Glue**: Alternately add glue in each side of the case, until the glue runs together in the middle, then fill the case to the top. Be sure that both the left and right sides are covered with glue.



5	Seal the TaqMan® OpenArray™ plates	**f.** Cure the glue with the OpenArray™ Case Sealing Station. **g.** Clean the case. **IMPORTANT!** Be sure to clean each TaqMan OpenArray Genotyping Case thoroughly. Dust, glue, or excess sample on the case may interfere with thermal uniformity and can fluoresce. While cleaning, do not squeeze the case; gently hold the case to ensure the glass does not touch the TaqMan OpenArray plate through-holes.
6	**Perform thermal cycling**	Perform thermal cycling on a thermal cycler that has been qualified for use with the TaqMan OpenArray plates.

Perform imaging

1	**Set up the software**	**a.** Start the OpenArray™ NT Imager, then start the OpenArray™ SNP Genotyping Analysis Software. **b.** Insert the CD that shipped with your TaqMan OpenArray plate into the computer, then open the folder that contains the plate setup file (*.spf) for your TaqMan OpenArray plate. **c.** Copy the plate setup file to the **PLATEFILES** folder on your computer.
2	**Enter the serial number and sample information**	**a.** In the OpenArray software, open a project file (*.nix), then click **Image** to open the Input Plate Serial Numbers dialog box. **b.** Click **Locate File**, then browse to and open the plate setup file (*.spf) that corresponds to the TaqMan OpenArray plate. The software automatically displays the serial number in the Plate Serial Number field. **c.** Click **Edit** to open the Sample Information dialog box. **d.** For each load number, enter the unique identifier for each sample plate, then select the appropriate 12-well × 4-well area of the sample plate. **e.** Enter sample information for each sample. You can: – Enter sample information manually – Import sample information from a *.csv file
3	**Perform imaging**	**a.** Place the TaqMan OpenArray plate(s) into the OpenArray NT Imager. Be sure that: – The plate position in the OpenArray NT Imager matches the position you entered in the software – The barcode is facing up and to the right, and that the TaqMan OpenArray plate is flush with the right and back edges. **b.** Close the OpenArray NT Imager lid and door. **c.** In OpenArray software, in the Input Plate Serial Numbers dialog box, click **Image**. **IMPORTANT!** Do not open the OpenArray NT Imager door during the imaging run. The run is complete when: (1) The blue indicator light on the instrument is off; and (2) In the software, the system indicator circle turns green and data appears. **d.** When the run is complete, save the project file (*.nix), then remove the TaqMan OpenArray plate(s).

Analyze the run data

1 View the results

After an imaging run, the OpenArray software automatically calls the genotypes for each TaqMan OpenArray plate in the run. To view the results of the automatic analysis:

a. Open a project file (*.nix).

b. In the Assays pane, select the assay ID to view.

c. View data in the Assays pane, Samples pane, and Scatter Plot.

2 (Optional) Modify clustering parameters

If the automatic calls are not suitable for your experiment, you can modify the clustering parameters as follows:

- Set *Don't Call* samples.
- Set outliers.
- Adjust stringency
- Adjust tolerance
- Adjust genotyping clusters
- Draw genotyping clusters
- Exclude genotyping clusters from analysis

IMPORTANT! Modifications to clustering parameters are made only to the assay you are viewing, not to the entire project. To change the default settings for the entire project, you need to modify the project file (see below).

3 (Optional) Modify project files

Project files (*.nix) are the files you view and modify in the OpenArray software. You can modify project files as follows:

- Add plate data files (*.spd).
- Remove plate data files (*.spd).
- Set project parameters.

4 (Optional) Publish data

Publish data for use in reports, spreadsheets, and so on. You can:

- Copy and paste Scatter Plots.
- Export genotype tables.
- Export *.csv files.

5 (Optional) Perform downstream analysis

In the OpenArray software, select **File ▸ Export to Applied Biosystems AutoCaller™...** to export project files (*.nix) to Applied Biosystems AutoCaller™ Software. For information on performing downstream analysis with the AutoCaller software, refer to the *Applied Biosystems AutoCaller™ Software User Guide* (PN 4385543).

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