

TaqMan® OpenArray® Real-Time PCR Plates

Protocol

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About This Guide

Safety information

For general safety information, see this section and Appendix D, "Safety" on page 61.

Safety alert words

Four safety alert words appear in Applied Biosystems user documentation at points in the document where you need to be aware of relevant hazards. Each alert word—IMPORTANT, CAUTION, WARNING, DANGER—implies a particular level of observation or action, as defined below:



IMPORTANT! – Indicates information that is necessary for proper instrument operation, accurate chemistry kit use, or safe use of a chemical.



CAUTION! – Indicates a potentially hazardous situation that, if not avoided, may result in minor or moderate injury. It may also be used to alert against unsafe practices.



WARNING! − Indicates a potentially hazardous situation that, if not avoided, could result in death or serious injury.



DANGER! – Indicates an imminently hazardous situation that, if not avoided, will result in death or serious injury. This signal word is to be limited to the most extreme situations.

SDSs

The Safety Data Sheets (SDSs) for any chemicals supplied by Applied Biosystems or Ambion are available to you free 24 hours a day. For instructions on obtaining SDSs, see "SDSs" on page 69.

IMPORTANT! For the SDSs of chemicals not distributed by Applied Biosystems or Ambion contact the chemical manufacturer.

About This Guide Safety information

TaqMan® OpenArray® Real-Time PCR Plates Protocol

Section 1 Product Information

This section covers:

Product overview		9
Workflow	1	1

Product overview

The TaqMan[®] OpenArray[®] Real-Time PCR Plates allow you to perform gene expression experiments on the OpenArray[®] Real-Time PCR System. The OpenArray system uses fluorescence-based polymerase chain reaction (PCR) reagents to detect targets of interest.

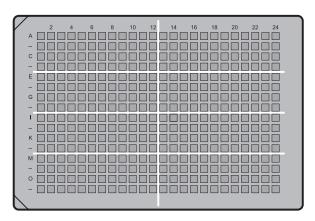
To perform gene expression experiments, the OpenArray system requires two plate types:

- OpenArray® 384-Well Sample Plate (sample plate) (this page)
- TaqMan® OpenArray® Real-Time PCR Plate (OpenArray plate) (page 10)

OpenArray® 384-Well Sample Plate

The OpenArray 384-Well Sample Plate is a 384-well reaction plate. You prepare the PCR mix and combine it with cDNA or gDNA sample in the sample plate, then use the OpenArray[®] AutoLoader to transfer the mixture from the sample plate to the OpenArray plate.

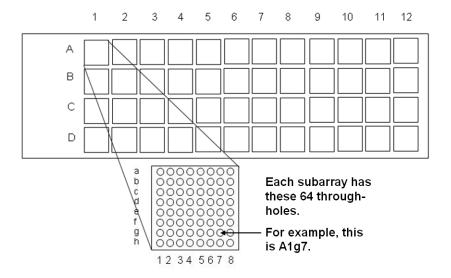
IMPORTANT! The well dimensions of the OpenArray 384-Well Sample Plates are specifically suited for use with the OpenArray AutoLoader. Applied Biosystems does not recommend the use of other microtiter plates with the AutoLoader.



TaqMan® OpenArray® Real-Time PCR Plate

The TaqMan[®] OpenArray[®] Real-Time PCR Plate is a 63-mm × 19-mm mid-density reaction plate. There are 3072 reaction through-holes in each plate. Individual through-holes are preloaded with a TaqMan[®] gene expression assay; each through-hole can accommodate a 33-nL reaction volume.

As shown in the figure below, the OpenArray plate is divided into 48 subarrays; each subarray consists of 64 through-holes. Hydrophilic and hydrophobic coatings allow reagents to be held within the through-holes.



Available TaqMan[®] assays

When you order an OpenArray plate, you select the TaqMan assays to include in the plate. The assays are dried-down and preloaded into the OpenArray plate. You can select any combination of Inventoried TaqMan® Gene Expression Assays.

Available formats

Each through-hole in an OpenArray plate contains a single assay. The number of assays in the OpenArray plate and the number of samples you can load in the plate depend on the OpenArray plate format that you order.

For ordering information, see "Required materials" on page 24.

For more information

For more information, refer to the OpenArray system product documentation suite. See "Product documentation" on page 73.

Workflow

Prepare the OpenArray® 384-Well Sample Plate (Section 2)

- 1. Prepare the cDNA or gDNA samples.
- 2. Determine the layout of the OpenArray® 384-Well Sample Plate.
- 3. Prepare the sample plate (add sample and PCR mix).



Prepare the TaqMan® OpenArray® Real-Time PCR Plate (Section 3)

- 1. Prepare for loading.
- 2. Place an OpenArray® plate in an OpenArray® AutoLoader Plate Holder.
- 3. Load the OpenArray® AutoLoader Tip Blocks.
- 4. Run the OpenArray $^{\circledR}$ AutoLoader.
- 5. Seal the OpenArray® Case.



Perform real-time imaging and analyze the run data

- 1. Load the OpenArray® plates into the OpenArray® instrument, then start the run. The OpenArray instrument performs thermal-cycling and real-time imaging.
- 2. Use the OpenArray® Real-Time qPCR Analysis Software to analyze and (if needed) modify the run data.



Note: For procedures, refer to the *OpenArray*® *Real-Time PCR System User Guide*. See "Documentation and Support" on page 73.

 $\mathsf{TaqMan}^{\circledR}$ $\mathsf{OpenArray}^{\circledR}$ $\mathsf{Real-Time}$ PCR Plates $\mathsf{Protocol}$ $\mathsf{Workflow}$

Section 2 Prepare the OpenArray[®] 384-Well Sample Plate

In this section, you prepare your cDNA or gDNA samples, prepare the OpenArray[®] 384-Well Sample Plates, then load your sample into the sample plates. In Section 3, you will use the OpenArray[®] AutoLoader to transfer sample from the prepared sample plates to TaqMan[®] OpenArray[®] Real-Time PCR Plates.

This section covers:

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Prepare the cDNA or gDNA samples	15
Determine the layout of the sample plate	17
Prepare the sample plate	20

Required materials

Item	Source	Part number
For cDNA preparation (cDNA from RNA)	1	
High Capacity cDNA Reverse Transcription Kit	Applied Biosystems	4322171
RNase-free water	Major Laboratory Supplier (MLS)	
Total RNA	User-supplied	
Incubator or thermal cycler	MLS	
For sample plate layout and preparation		
OpenArray® 384-Well Sample Plates	Applied Biosystems	4406947
(Optional) Fine-tip marker	MLS	
TaqMan [®] OpenArray [®] Gene Expression Master Mix	Applied Biosystems	4362070
5X TaqMan® REmix Solution	Applied Biosystems	4453993
(Supplied in the TaqMan® OpenArray® Real- Time PCR Accessories Kit)		
RNase-free water	MLS	
(For gDNA samples only) Albumin, bovine (BSA)†	Sigma	A7906
cDNA or gDNA sample	User-supplied	
Corning® 96 Well Microplate Aluminum Sealing Tape, Nonsterile	Corning Life Sciences	6570
For general use		
Powder-free nitrile gloves	MLS	
Lint-free wipes	MLS	
Disposable transfer pipettes	MLS	
Pipettes, P10 to P1000	MLS	
Pipette tips, 10 to 100 μL	MLS	
Centrifuge with plate adaptor	MLS	
Vortexer	MLS	

[†] For the SDS of any chemical not distributed by Applied Biosystems, contact the chemical manufacturer. Before handling any chemicals, refer to the SDS provided by the manufacturer, and observe all relevant precautions.

Prepare the cDNA or gDNA samples

Quantity of starting material

Applied Biosystems recommends the following amounts of starting material:

- **Total RNA**, for preparing cDNA 10 μ L at a concentration of 250 ng/ μ L (2.5 μ g total). To prepare cDNA from RNA, see page 16.
- **gDNA** 10 μ L at a concentration of 108 ng/ μ L.

RNA quality

Be sure that the RNA you use for gene expression experiments:

- Is extracted from the raw material of interest using an optimized protocol
- Does not contain PCR inhibitors
- Has an $A_{260/230}$ ratio between 1.7 and 1.9
- Has an A_{260/280} ratio between 1.7 and 1.9
- Is intact as visualized by gel electrophoresis
- Has an RNA Integrity Number (RIN) that is between 6.5 and 10

Prepare cDNA from RNA

If the starting material is total RNA, follow this procedure to prepare cDNA.

- 1. Thaw the High Capacity cDNA Reverse Transcription Kit components and the total RNA on ice.
- **2.** Combine the following components to prepare the reverse-transcription mix:

(Component	Volume (µL) for 1 reaction	Stock concentration	Final concentration (2× mix)
High Capacity cDNA	10× RT Buffer	2.0	10	2
Reverse Transcription Kit	10× RT Random Primers	2.0	10	2
	25× dNTP Mix	0.8	25	2
	MultiScribe [™] Reverse Transcriptase	1.0	50	5
RNase-free water		4.2		
Final volume of reverse-transcription mix		10.0		

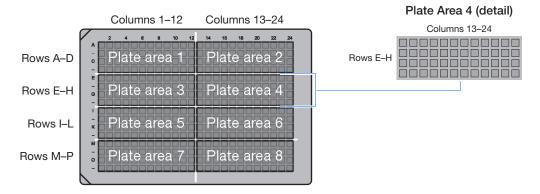
- 3. Add an equal volume of total RNA to the 2X reverse-transcription mix (10 μ L per reaction).
- **4.** Incubate at room temperature for 10 minutes.
- **5.** Incubate at 37 °C for 2 hours, place on ice for 5 minutes, then spin down.
- **6.** Incubate at 75 °C for 10 minutes, place on ice for 5 minutes, then spin down.

If needed, you can store the cDNA -20 °C for at least 2 months. Otherwise, proceed to "Determine the layout of the sample plate" on page 17.

Determine the layout of the sample plate

About the sample plate

The OpenArray 384-Well Sample Plate is divided into eight areas; each sample plate area is 12 wells × 4 wells (48 wells). During each load, the OpenArray[®] AutoLoader transfers sample from *one area* of a single sample plate to TaqMan[®] OpenArray[®] Real-Time PCR Plates.



IMPORTANT! The way you set up the sample plates in this section depends on the format of the OpenArray plate that you will be transferring the cDNA or gDNA samples to in Section 3.

Determine the layout of the sample plate

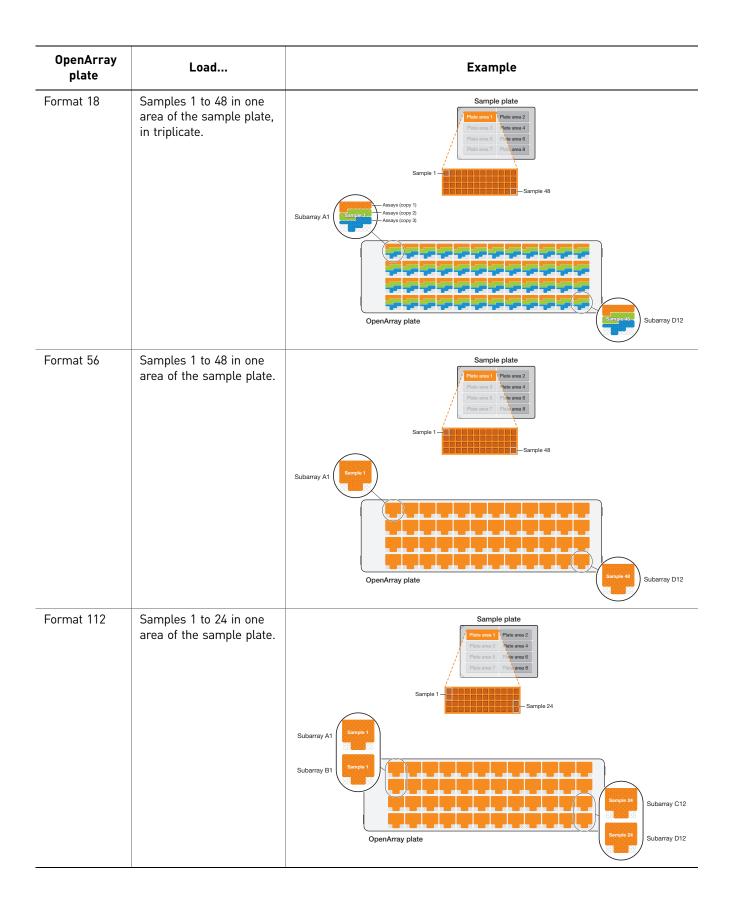
1. Label the sample plate with a unique identifier.



Note: You will enter this unique identifier when you set up the project file (*.ncx) in the OpenArray[®] Real-Time qPCR Analysis Software. Refer to the *OpenArray*[®] *Real-Time PCR System User Guide*.

- **2.** Determine which sections of the sample plate you will load with sample. Each 12-well × 4-well rectangular area corresponds to one TaqMan OpenArray Real-Time PCR Plate. You may find it helpful to mark the sample plates with a fine-tip marker.
 - IMPORTANT! Be sure to track where the samples are in each sample plate. For each sample plate, Applied Biosystems recommends creating a sample information file (*.csv). See Appendix A on page 43.

Applied Biosystems recommends the arrangements illustrated on the next page.



OpenArray plate	Load	Example
Format 168	Samples 1 to 16 in one area of the sample plate.	Sample plate Plate area 1 Plate area 2 Plate area 3 Plate area 6 Plate area 7 Plate area 8 Plate area 7 Plate area 8 Sample 1 Sample 16 Sample 16 Sample 16 Sample 16 Sample 16 Sample 16
		Subarray D10 Subarray D11 Subarray D12
Format 224	Samples 1 to 12 in one area of the sample plate.	Sample plate Plate area 1 Plate area 3 Plate area 3 Plate area 4 Plate area 7 Plate area 7 Plate area 7 Plate area 8 Sample 12 Subarray A1 Sample 12 Subarray B1 Subarray D1 Subarray D1 Subarray D1 Subarray D1 Subarray D1 Subarray D12 Subarray D12

Prepare the sample plate

Prepare the PCR mix

- **1.** Mix the TaqMan[®] OpenArray[®] Gene Expression Master Mix by gently inverting the tube 10 times.
- **2.** Combine the following components:

Component	Volume (µL) for 1 area of the sample plate [†]	Stock concentration	Final concentration	Units
For cDNA samples				
TaqMan [®] OpenArray [®] Gene Expression Master Mix	132.0	2	1	×
5X TaqMan® REmix Solution	52.8	5	1	×
(Supplied in the TaqMan® OpenArray® Real-Time PCR Accessories Kit)				
RNase-free water	15.8			
Final volume of PCR mix	200.6			
For gDNA samples				
TaqMan [®] OpenArray [®] Gene Expression Master Mix	132.0	2	1.00	×
5X TaqMan® REmix Solution	52.8	5	1.00	×
(Supplied in the TaqMan® OpenArray® Real-Time PCR Accessories Kit)				
BSA	1.3	10	0.05	mg/mL
RNase-free water	14.5			
Final volume of PCR mix	200.6			

 $^{^\}dagger$ One area of a sample plate corresponds to one TaqMan $^{\circledR}$ OpenArray $^{\circledR}$ Real-Time PCR Plate.

- **3.** Mix well by pipetting up and down.
- **4.** Proceed immediately to "Add sample and PCR mix to the sample plate" on page 21.

Add sample and PCR mix to the sample plate

- 1. At room temperature, thaw the cDNA or gDNA samples. Mix the samples by vortexing, then centrifuge for 1 minute @ 1000 rpm.
- 2. Review the concentration of the normalized samples. The recommended starting concentration for human cDNA and gDNA samples is $108 \text{ ng/}\mu\text{L}$. See Appendix B, "Calculator Tool" on page 55.
- **3.** Add the normalized cDNA or gDNA sample to the appropriate wells of the OpenArray 384-Well Sample Plate (as determined in "Determine the layout of the sample plate" on page 17). For human cDNA and gDNA samples, use the amounts listed below *per well of the sample plate*.

	Volume (µL) per sample plate well†, when transferring to						
Component	Format 18 (in triplicate)	Format 56	Format 112	Format 168	Format 224		
Normalized human cDNA or gDNA sample	1.2	1.2	1.2	1.2	1.2		

[†] One well of a sample plate corresponds to one subarray of a TaqMan® OpenArray® Real-Time PCR Plate. The number of subarrays required depends on the format of the OpenArray plate.

STOPPING POINT If needed, you can store the sample plate at room temperature for up to 1 hour before you add the PCR mix (step 4). Cover the sample plate to prevent evaporation.

4. Add the prepared PCR mix (from page 20) to each well that contains cDNA or gDNA sample.

	Volume (μL) per sample plate well [†] , when transferring to					
Component	Format 18 (in triplicate)	Format 56	Format 112	Format 168	Format 224	
PCR mix	3.8	3.8	3.8	3.8	3.8	
Total volume (sample + PCR mix)	5.0	5.0	5.0	5.0	5.0	

[†] One well of a sample plate corresponds to one subarray of a TaqMan® OpenArray® Real-Time PCR Plate. The number of subarrays required depends on the format of the OpenArray plate.

- **5.** Mix well by gently pipetting up and down.
- **6.** Cover the sample plate with sealing tape.
- 7. Centrifuge the sample plate for 1 minute @ 1000 rpm to eliminate bubbles, then place the sample plate on ice.

Proceed to Section 3, "Prepare the TaqMan® OpenArray® Real-Time PCR Plate" on page 23.

Section 3 Prepare the TaqMan® OpenArray® Real-Time PCR Plate

In this section, you use the OpenArray® AutoLoader to transfer your cDNA or gDNA samples from the OpenArray® 384-Well Sample Plates (prepared in Section 2) to TaqMan® OpenArray® Real-Time PCR Plates.

This chapter covers:

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Load the OpenArray® AutoLoader Tip Blocks	31
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Seal the OpenArray® Case	37
Guidelines for high-throughput loading	41

Required materials

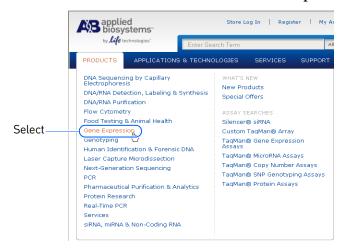
TaqMan® OpenArray® Real-Time PCR Plates

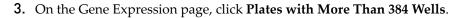
The following table provides a list of available formats for the TaqMan $^{\text{\tiny (B)}}$ OpenArray $^{\text{\tiny (B)}}$ Real-Time PCR Plates.

OpenArray plate	Part number	No. of preloaded assays	Maximum no. of samples
Format 18	4456272	18 (in triplicate)	48
Format 56	4456276	56	48
Format 112	4456268	112	24
Format 168	4456270	168	16
Format 224	4456274	224	12

Ordering the OpenArray® plates

- 1. Go to www.appliedbiosystems.com.
- 2. From the menu bar, select PRODUCTS ▶ Gene Expression.







4. Click TaqMan® OpenArray® Real-Time PCR Plates.



- **5.** (Optional) On the product page, click the **Product Description** tab for more information about the OpenArray plates.
- 6. Click the Ordering Information tab, then click Configure.



7. If you are not already logged in, the Store Log In page appears. On the Store Log In page, enter your user name and password. If you are not a registered user, click **Register Now**, then follow the prompts.



Note: If you are already logged in, the Store Log In page does not appear. Go to the next step.

8. In the Configurator, follow the prompts to place your order.

Other consumables and equipment

Product	Source	Part number		
For loading the TaqMan [®] OpenArray [®] Real-Time PCR Plates				
OpenArray [®] Plate Guide Set	Applied Biosystems	20292		
OpenArray® AutoLoader Tip Block	Applied Biosystems	20322		
Finnpipette [®] Multichannel Digital Pipettor, 5 to 50 μL	Applied Biosystems	4452470		
OpenArray® Loader Tips	Applied Biosystems	4404571		
OpenArray® Loader Tips 10 Pack	Applied Biosystems	4404604		
OpenArray® AutoLoader Plate Holder	Applied Biosystems	20384		
OpenArray® AutoLoader	Applied Biosystems	4409360		
For sealing the TaqMan® OpenArray® Real-Time PCR Plates				
OpenArray® Accessories Kit The accessories kit contains: OpenArray® Case OpenArray® Sealing Glue OpenArray® Immersion Fluid	Applied Biosystems	4404572		
OpenArray® Case Sealing Station	Applied Biosystems	4409361		
Ethanol [†]	Major Laboratory Suppliers (MLS)			
Razor blade	MLS			
25 Slide Holder	Applied Biosystems	4407056		
OpenArray [®] Frame	Applied Biosystems	4453942		
OpenArray® Frame Adhesive	Applied Biosystems	4453940		
For general use				
Powder-free nitrile gloves	MLS			
Laboratory-grade wipes	MLS			
Tweezers or forceps	MLS			
3 Plastic bins (medium to large) for washing the tip blocks and plate holders	MLS			
(Optional) Filtered 100% compressed nitrogen gas or residue-free compressed air canister, for drying the plate holder, tip blocks, and plate guides.	MLS			
(Optional) Hand-held spray attachment for the compressed gas/air canister	MLS			

[†] For the SDS of any chemical not distributed by Applied Biosystems, contact the chemical manufacturer. Before handling any chemicals, refer to the SDS provided by the manufacturer, and observe all relevant precautions.

Storage conditions

The following materials require special storage conditions:

Item		Storage Conditions	
TaqMan [®] OpenArray [®]	Real-Time PCR Plate		
If the OpenArray plate is	Frozen, unopened	Store at -20 °C until the expiration date provided on the product label.	
	Thawed, unopened	Store at room temperature for up to 24 hours.	
	Thawed, opened	Store at room temperature for up to 1 hour.	
	Loaded and sealed	Store at room temperature, in the dark, for up to 1 hour.	
OpenArray® Immersion Fluid		See the product label for storage conditions and expiration date. After you open the package, do not store any remaining immersion fluid; use the amount required, then discard the remainder.	
OpenArray® Loader Tips		See the product label for storage conditions and expiration date. After you open the package, discard unused tips after 2 weeks.	
OpenArray® Sealing Glue		Store the glue in a dark place; ambient light can cure the glue in the tip. If the glue has been open more than 2 weeks, discard it and use a new tube.	

Prepare for loading

- 1. Be sure that the OpenArray[®] AutoLoader, OpenArray[®] Plate Guide Set, OpenArray[®] AutoLoader Tip Blocks, and OpenArray[®] AutoLoader Plate Holder are completely clean and dry. For cleaning procedures, refer to the *OpenArray[®] Real-Time PCR System User Guide*.
 - **IMPORTANT!** Residual water prevents correct loading of the samples into the TaqMan[®] OpenArray[®] Real-Time PCR Plates.
- **2.** Remove the OpenArray plates from the freezer, *but do not open the packaging*. Allow the OpenArray plates to thaw at room temperature (approximately 5 minutes).
 - Note: Unopened OpenArray plates can remain at room temperature for up to 24 hours.
 - (1) IMPORTANT! Thaw only the OpenArray plates you will need for the current loading session.
- **3.** Fill the appropriate number of OpenArray® Cases with OpenArray® Immersion Fluid:
 - **a.** Using scissors, open a container of immersion fluid.
 - **b.** Place the case in the case rack, then fill approximately 3/4 of the case with immersion fluid.
 - (1) IMPORTANT! Within 1 hour after opening the container of immersion fluid, fill the case with immersion fluid, insert a loaded OpenArray plate, then seal the case.

Proceed immediately to "Place an OpenArray® plate in a plate holder" on page 29.

Place an OpenArray® plate in a plate holder

Important guidelines for handling the OpenArray® plate

- Wear gloves that are one size smaller than the size you typically wear, to help prevent excess glove material from contacting the OpenArray plate while loading.
- Hold the OpenArray plate by the edges, at the end opposite from the barcode. Do not touch the through-holes.
- Within 1 hour after opening the plate packaging, load the OpenArray plate with sample, place the loaded plate in an OpenArray Case, then seal the case.
- If you drop a loaded OpenArray plate, discard it in the appropriate waste container.

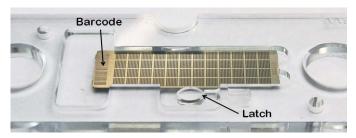
Place the OpenArray® plate in the plate holder

1. Remove a thawed OpenArray plate from its packaging.

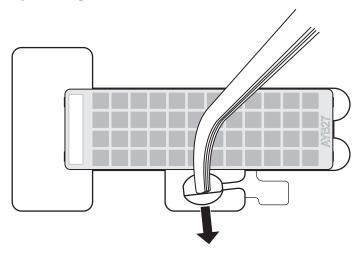


Note: You may want to save the OpenArray plate packaging, because you can scan the barcode on the package to enter the OpenArray plate serial number into the OpenArray[®] Real-Time qPCR Analysis Software. Refer to the *OpenArray*[®] *Real-Time PCR System User Guide*.

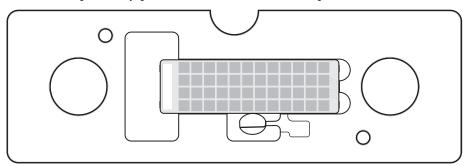
- 2. Orient the OpenArray® AutoLoader Plate Holder so that the latch is towards you.
- **3.** Orient the OpenArray plate so that the barcode faces up and to your left.



4. Pull the latch on the plate holder towards you, as shown below. The OpenArray plate drops into place. Be sure that the OpenArray plate reaches all the way to the right of the plate holder, then release the latch.



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



(1) IMPORTANT! Proceed immediately to "Load the OpenArray® AutoLoader Tip Blocks" on page 31.

Load the OpenArray® AutoLoader tip blocks

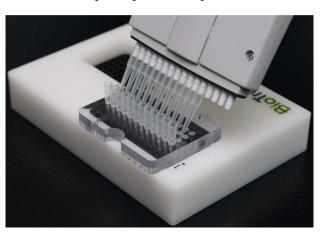
- 1. Using tweezers, peel the sealing tape from the area of the sample plate that contains the samples to be transferred.
- **2.** From the OpenArray[®] Plate Guide Set, select the plate guide that aligns with the 12-well × 4-well areas in the sample plate:
 - One plate guide is for sample plate areas 1, 3, 6, and 8 (shown below).
 - One plate guide is for sample plate areas 2, 4, 5, and 7.



- **3.** Place the plate guide over the sample plate.
 - IMPORTANT! Be sure that the plate guide sits flat on the benchtop. The plate guide should not be tilted by the sample plate beneath it. To check the plate guide position, gently slide the plate guide across the benchtop. If the plate guide is not aligned correctly, it slips toward the base of the sample plate.
- **4.** Place the tip block into the appropriate area of the plate guide. For example, place the tip block in position 1 to load the tips with samples from sample plate area 1. Sample plate area 1 includes wells A1 to A12, B1 to B12, C1 to C12, and D1 to D12. For an illustration of the sample plate areas, refer to "Determine the layout of the sample plate" on page 17.



- **5.** Using the Finnpipette[®] Multichannel Digital Pipettor (or by hand), place 12 OpenArray[®] Loader Tips in each hole of the tip block (one row). Release the tips when they are submerged.
 - (!) IMPORTANT! Do not press firmly when inserting the tips into the tip block. Let the tips drop into the tip block slots.

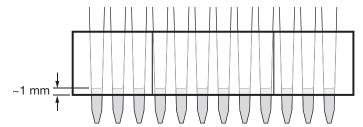


Place 12 tips into the tip block as shown. If you are using the digital pipettor, four channels will be empty.

- **6.** Slide the tip block up and down (25 to 50 times), until the tips:
 - Are filled to 1 mm above the bottom edge of the tip block.
 - Have no air bubbles.

You can remove the tip block to look at the tips. When you replace the tip block to load more sample, be sure to:

- Level the tip heights.
- Keep the tip block in the same orientation. If you turn the tip block around, the samples will mix together and become contaminated.



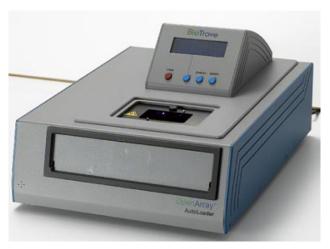
- (1) IMPORTANT! If the tips are not filled correctly, product performance may be adversely affected.
- **7.** Leave the tip block with the loaded tips in the plate guide, keeping the tips submerged in sample, until the OpenArray plate is ready for loading.



- **8.** If you are transferring samples from additional sample plates, repeat this procedure for each sample plate.
- IMPORTANT! Proceed immediately to "Run the OpenArray® AutoLoader" below.

Run the OpenArray® AutoLoader

Use the OpenArray[®] AutoLoader to load the TaqMan[®] OpenArray[®] Real-Time PCR Plates (that is, transfer samples from the sample plate onto OpenArray plates).



Set up the AutoLoader

For the following hazards, see the complete safety alert descriptions in Appendix D, "Safety":



WARNING! PHYSICAL INJURY HAZARD. Moving parts can crush and cut. Example 2 Keep hands clear of moving parts while operating the instrument.

- 1. Power on the AutoLoader. The AutoLoader homes the platform, then displays: "Welcome, AutoLoader Ready! Press Enter to Start".
- **2.** Press **ENTER**. The screen displays:

Samples/Subarray:

ENTER: #

NEXT: More Choices

where # is the number of loads the AutoLoader will perform: ONE, TWO, or **THREE**

3. Press **ENTER** to accept the current number, *or* press **NEXT** until the correct number is displayed, then press **ENTER**. Be sure to select the correct number of loads for your OpenArray plate:

OpenArray plate	No. of loads
Format 18	ONE
Format 56	ONE
Format 112	ONE
Format 168	ONE
Format 224	ONE



Note: To change the number of loads that you entered, press **CANCEL**, then at the "Continue with the Cancel" prompt, press **ENTER**.

- **4.** At the "Insert OpenArray Holder" prompt, place the OpenArray plate and plate holder on the AutoLoader platform. Be sure that the:
 - Notch in the plate holder faces the instrument.
 - Holes in the plate holder are aligned with the circular guides on the AutoLoader platform.



5. Gently push the plate holder all the way down. Be sure that there is no rocking motion when you apply pressure.



6. Press **ENTER** to send the platform to the load position, then press **ENTER** again.

- 7. At the "Please Insert Tip Block #1" prompt, insert the loaded tip block (from step 7 on page 32):
 - **! IMPORTANT!** Insert the loaded tip block only when prompted. Otherwise, the AutoLoader will calibrate and return to the Welcome screen.
 - **a.** Place the loaded tip block above the OpenArray plate.
 - **b.** Align the tip block with the metal guide pins on the AutoLoader.
 - **c.** Bring the tip block straight down, without tilting it. Slowly position the tip block over the metal guide pins.
 - **IMPORTANT!** Perform this step slowly and evenly to prevent improper sample loading (for example, too much sample or not enough sample).
- **8.** Be sure that the tip heights are level:
 - Gently slide your finger across the tops of the tips so that the tip heights are level.

or

- Gently rest another tip block on top of the tips until the tip heights are level, then remove the tip block.
- IMPORTANT! For the AutoLoader to properly load the OpenArray plate with sample, the tip heights must be level.

Load the sample

- IMPORTANT! Be sure that you have prepared the OpenArray Cases before performing this procedure (see step 3 on page 28). After an OpenArray plate is loaded with sample, you must immediately place the plate into a case.
- IMPORTANT! After you press ENTER (step 1 below), you cannot stop the AutoLoader. To stop the AutoLoader before you begin loading sample, press STOP now. The AutoLoader ends the current operation, calibrates, then returns to the Welcome screen. If you are prompted to remove the tip block, you must remove the tip block to proceed.
- 1. On the AutoLoader, press ENTER. The AutoLoader transfers the samples to the the OpenArray plate. Each tip fills 64 through-holes in one subarray, as follows:

OpenArray plate	OpenArray plate subarray locations
Format 18	Through-holes A1 through H8
Format 56	
Format 112	
Format 168	
Format 224	

- **2.** At the "Please Remove Tip Block. Hit Enter to Continue" prompt, remove the tip block:
 - **a.** Slowly pull the tip block straight up, without any rocking motion. To prevent rocking, it may be helpful to hold the tip block with your index finger and thumb, and press your remaining fingers against the AutoLoader surface.



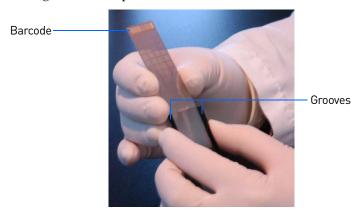
Note: To ensure that the samples are uniformly loaded in the OpenArray plate, remove the tip block slowly and evenly.

- b. Press ENTER.
- **3.** Follow the prompts to remove the plate holder from the AutoLoader platform.
- **4.** Remove the OpenArray plate from the plate holder:
 - a. Place the plate holder on a flat surface.
 - **b.** With one hand, push the latch down.
 - c. With the other hand, grasp the edge of the OpenArray plate and lift it out.
 - IMPORTANT! Hold the OpenArray plate by the edges, at the end opposite from the barcode. Do not touch the through-holes. If you drop a loaded OpenArray plate, discard it in the appropriate waste container.
- (IMPORTANT! To prevent evaporation of the samples, proceed *immediately* to "Seal the OpenArray® Case" on page 37.

Seal the OpenArray® Case

Insert the loaded OpenArray® plate into a prepared OpenArray® Case

- 1. Hold the OpenArray plate by its edges, at the end opposite from the barcode, with the barcode facing up.
 - (1) IMPORTANT! Do not touch the through-holes. If you drop a loaded OpenArray plate, discard it in the appropriate waste container.
- 2. Slide the OpenArray plate into a prepared OpenArray Case. Be sure that the:
 - OpenArray plate barcode is at the top of the case and facing the black side of the case.
 - Plate aligns with the grooves in the case. Misalignment may cause surface rubbing, loss of samples, and/or contamination.



- 3. Push the OpenArray plate all the way down into the case, using tweezers if needed. The bottom of the plate should touch the bottom of the case; there should be ~1 cm of space at the top.
- **4.** The immersion fluid should be level with the OpenArray plate. If needed, add or remove immersion fluid with a pipette.

Seal the OpenArray® Case

Use the OpenArray[®] Sealing Glue and the OpenArray[®] Case Sealing Station to seal the OpenArray Case so that immersion fluid does not leak during the real-time imaging run.

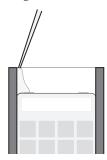
IMPORTANT! If the glue has been open more than 2 weeks, discard it and use a new tube. Store the glue in a dark place; ambient light can cure the glue in the tip.

For the following hazards, see the complete safety alert descriptions in Appendix D, "Safety":

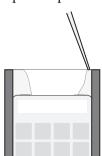


WARNING! ULTRAVIOLET LIGHT HAZARD.

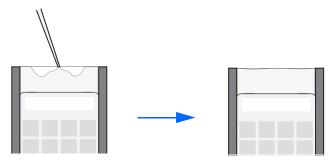
- 1. Fill the case with glue:
 - **a.** Place a drop of glue on one edge of the OpenArray Case opening, then fill the opening until the glue reaches the top of the case. Angle the tip so that the glue reaches the inside rail.



b. Repeat step 1a on the other edge of the case opening.



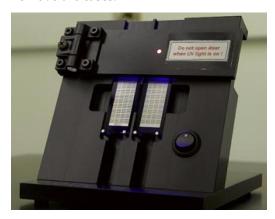
c. Continue adding glue on each side until the glue runs together in the middle. Fill the case to the top. Be sure that both the left and right sides are covered with glue.



- **2.** If you see air bubbles in the immersion fluid underneath the glue, use a small pipette to carefully aspirate the bubbles.
- **3.** With a lint-free wipe, wipe any excess glue from the surface of the glass.

4. Cure the glue:

- **a.** Place the case(s) in the sealing station so that the barcode faces out, then close the door. You can place up to two cases at a time in the sealing station.
- **b.** Turn the switch to **ON**, allow the glue to cure for 3 minutes, then turn the switch to **OFF**.
- **c.** Open the door, turn the case(s) over, then repeat step 4b.
- d. Remove the cases.



Clean the OpenArray® Case

- IMPORTANT! Be sure to clean each OpenArray Case thoroughly. Dust, glue, or excess sample on the case may interfere with thermal uniformity and may fluoresce. While cleaning, do not squeeze the case; gently hold the case to ensure that the glass does not touch the OpenArray plate through-holes.
- 1. If there is any glue on a case, carefully remove the glue with a razor blade. Be sure not to scratch the glass.
- 2. Moisten a lint-free wipe with ethanol, then gently wipe the case surface.
- **3.** Allow the case to air-dry, or spray each side of the case with compressed air for 2 seconds.

Frame the OpenArray® Case

To ensure that the loaded OpenArray Case tightly contacts the heat block in the OpenArray instrument, attach an OpenArray[®] Frame to the case.

- 1. Be sure that the frame is clean (there should be no residual adhesive from previous runs).
- **2.** Place the loaded OpenArray Case on a lint-free wipe, with the black side of the case facing up.
- **3.** Using scissors, cut two ~3-mm rectangles from the OpenArray[®] Frame Adhesive.

4. Using tweezers, remove the backing from one side of the frame adhesive, then stick the adhesive to the frame: one piece of adhesive in the middle of each long side.



- **5.** Using tweezers, remove the backing from the other side the frame adhesive.
- **6.** Align two corners of the frame with two corners of the case, then bring the other corners of the frame down onto the case. Flatten the frame against the case for 10 seconds.



Proceed to real-time imaging and analysis with the OpenArray[®] instrument. For procedures, refer to the *OpenArray*[®] *Real-Time PCR System User Guide*.



Note: After the loaded OpenArray plate is sealed, you can store it at room temperature, in the dark, for up to 1 hour.

Guidelines for high-throughput loading

For optimal efficiency when loading large numbers (>6) of OpenArray plates, follow the guidelines below.

Before you begin loading

- If possible, obtain a tip block for each OpenArray plate to load during the high-throughput loading session.
- Be sure that all the tip blocks are clean and dry, then stack the tip blocks next to the AutoLoader. For cleaning procedures, refer to the *OpenArray® Real-Time PCR System User Guide*.
- Fill all OpenArray Cases with immersion fluid, then place the cases in a vertical slide rack.
- Insert all of the OpenArray plates into plate holders, then stack the plate holders to one side.
- Load the tip blocks with cDNA or gDNA samples. Be sure that the tips are filled to 1 mm above the bottom edge of the tip block and that there are no air bubbles.

During and after loading

- To help avoid mistakes when entering sample information in the OpenArray[®] Real-Time qPCR Analysis Software, run the OpenArray plates in the AutoLoader in alphanumeric order (per the OpenArray plate serial number).
- Seal the OpenArray Cases as time permits. You can:
 - Seal all the cases at once, after the loading session is completed.
 - Seal the cases in batches, while other OpenArray plates are being loaded.
 - IMPORTANT! To avoid evaporation, insert the OpenArray plate into a case and cover it with immersion fluid immediately after loading. However, the cases can be left unsealed for up to 8 hours.
- Use a carrying case to transport several loaded OpenArray plates from the case sealing station to the OpenArray[®] instrument.
- After loading is complete, you can use a large bin to clean several tip blocks at a time. For cleaning procedures, refer to the OpenArray[®] Real-Time PCR System User Guide.

 $\label{eq:condition} \begin{tabular}{ll} TaqMan @ OpenArray @ Real-Time PCR Plates Protocol \\ \textit{Guidelines for high-throughput loading} \end{tabular}$

Create a sample information file (*.csv) for sample tracking

This appendix covers:

Overview	43
Use the Sample Tracking Tool	44
Use a spreadsheet or simple text program	49
Export sample information from an existing *.ncx file	51

Overview

Most researchers maintain stocks of cDNA or gDNA samples in individual tubes or in 96-well stock plates. However, samples to be used with the OpenArray[®] Real-Time PCR System must be transferred first to an OpenArray[®] 384-Well Sample Plate, and then to a TaqMan[®] OpenArray[®] Real-Time PCR Plate.

IMPORTANT! To ensure accurate data results, you must correctly track the sample IDs from plate to plate (stock plate to sample plate to OpenArray plate).

Applied Biosystems recommends that you create a sample information file (*.csv) to track your samples. Prior to imaging the OpenArray plates, you can import the *.csv file into the OpenArray[®] Real-Time qPCR Analysis Software.

You can create a sample information file in one of three ways:

- (Recommended) Use the Sample Tracking Tool (page 44)
- Use a spreadsheet or simple text program (page 49)
- Export sample information from an existing *.ncx file (page 51)

Include no template controls

Applied Biosystems strongly recommends that you include at least one no template control (NTC) per OpenArray plate. NTCs serve as negative controls, and they are useful in data analysis. When adding NTCs to the 96-well stock plate, place one NTC in each section of the stock plate to ensure that the NTCs are plated in the correct location in the OpenArray plate. Also follow this procedure for any positive controls (for example, CEPH DNA).

Use the Sample Tracking Tool

About the Sample Tracking Tool

The Sample Tracking Tool is a spreadsheet created with the Microsoft[®] Excel[®] Software that Applied Biosystems provides during training.

Applied Biosystems recommends that you use the Sample Tracking Tool to convert the sample IDs to the appropriate format. When you use the tool, you only need to enter the sample IDs once, then the process of transferring the sample information from plate to plate is automatic.

Standard vs. modified spreadsheet

Applied Biosystems provides a standard spreadsheet in the Sample Tracking Tool. The standard spreadsheet applies to the Format $64~(64\times48)~\text{TaqMan}^{\circledR}$ OpenArray $^{\circledR}$ Genotyping Plate. You need to modify the standard spreadsheet to create the correct *.csv file for the TaqMan OpenArray Real-Time PCR Plates. How you modify the spreadsheet depends on:

- The TaqMan OpenArray Real-Time PCR Plate format that you are loading the samples into (Format 18, 56, 112, 168, or 224)
- The method that you use to transfer the samples



Note: If you are unfamiliar with the Excel software, contact Applied Biosystems Technical Support for help with modifying the spreadsheet.

Example in this section

The standard spreadsheet was used for the example experiment illustrated in this section. The example experiment used:

- The Format 64 (64 × 48) TaqMan[®] OpenArray[®] Genotyping Plate.
- A 12-channel pipette with 9-mm spacing. The 384-well sample plates have 4.5-mm spacing; due to the 9-mm spacing of the pipette, sample was added to every other well in the 384-well sample plate. That is, all samples from row A of the 96-well plate were transferred to the odd-numbered wells of row A in the 384-well sample plate; all samples from row B of the 96-well plate were transferred to the even-numbered wells of row A in the 384-well sample plate, and so on.

Edit the spreadsheet

- 1. Open the Sample Tracking Tool to start the Excel software.
- 2. Select the Entry for Samples List Format tab.



3. Enter the sample IDs next to the appropriate well locations. You can enter up to four 96-well plates per spreadsheet (four 96-well plates are equal to one 384-well sample plate).

	А	В	С	_
1	96 well plate #	Well Location	Sample ID	_
2	1	A01	N1	
3	1	A02	N2	
4	1	A03	N3	
5	1	A04	N4	
6	1	A05	N5	
7	1	A06	NTC	
8	1	A07	N6	
9	1	A08	N7	
10	1	A09	N8	
11	1	A10	N9	
12	1	A11	N10	
13	1	A12	NTC	
14	1	B01	N11	
15	1	B02	N12	
16	1	B03	N13	
17	1	B04	N14	
18	1	B05	N15	
19	1	B06	N16	
20	1	B07	N17	
21	1	B08	N18	
22	1	B09	N19	
23	1	B10	N20	
24	1	B11	N21	
25	1	B12	N22	

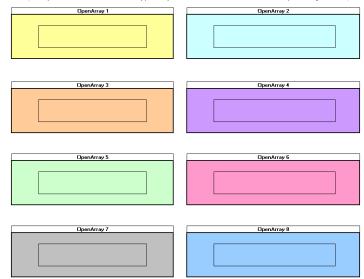
For each 96-well plate in the spreadsheet, the sample IDs are color-coded in groups of six, using alternate colors.

4. Select the **Entry for Samples – Plate Format** tab, then confirm that the three plate views correspond to the layout of your stock gDNA plate:

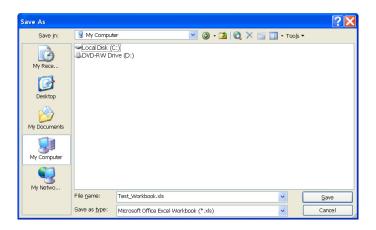
Plate view												E	xaı	mp	le										
96-well plate views		1		2		3		4	ı	5	5	6	;	7		8		9		10)	1	1	1	2
(up to four 96-well plates)	A	N1		N2	2	N	3	N	4	N	5	N	гс	N	6	N7	,	N	3	N	9	N	10	N	С
	В	N11		N1	2	N1	13	N′	14	N1	15	N'	16	N1	7	N1	8	N1	9	N2	20	N	21	N	22
	С	N23	3	N2	4	N2	25	N2	26	N2	27	N2	28	N2	9	N3	0	N3	1	N3	32	N	33	N	34
	D	N35	5	N3	6	N3	37	N	38	N3	39	N ₂	10	N4	11	N4	2	N4	3	N4	14	N	45	N	16
	E	N47	,	N4	8	N4	19	N	50	NE	51	N	52	NE	3	N5	4	N5	5	NE	66	N	57	N	58
	F	N59	9	N6	0	N6	51	Ne	62	Ne	63	Ne	64	Ne	65	N6	6	N6	7	Ne	8	N	69	N	70
	G	N71	1	N7	2	N7	73	N	74	N7	75	N	76	N7	7	N7	8	N7	9	NE	80	N	81	N	32
	н	N83	3	N8	4	N8	85	N	36	N	37	Po	s	N	88	N8	9	N9	0	NS)1	N	92	P	s
384-well plate view		1	2	3		5		7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
(one 384-well plate)	А		N11		N12	N3	N13	N4	N14	N5			N16		N17	N7	N18	N8	N19	N9	N20			NTC	
	В	—	_		_	N25	_	N26	N38	N27	N39	N28	-	N29		N30	N42	N31			N44	N33	-	N34	N46
	С		_	N48 N72		N49 N73	_	N50 N74	N62 N86	N51 N75	N63 N87	N52 N76	-	-	N65 N88	N54 N78	N66 N89	N55 N79	N67 N90	N56 N80	N68 N91	N57 N81	N69 N92	N58 N82	N70 Pos
	D	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	F	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	G	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	н	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	J	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	К .	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	L M	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	M	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	0 P	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
OpenArray plate views (up to	ļ,																								
eight OpenArray plates)		1 2	3	4	5 E	enArra 6 7	- 8	9		11 12		2	_	4 5	6		8	9 10	11 1 NTC	12					
	A B	N1 N11 N23 N35	N2 N24	\rightarrow	_	_	4 N14 6 N38	-	N15 N N39 N		_	$\overline{}$	N7 N30 N	_	_	-	N20 N								
	С	N47 N59																							
	D	N71 N83	N72	N84	N73 N8	85 N7	4 N86	N75	N87 N	76 Pc	n77	N88	N78 N	189 N7	9 N90	N80	N91 N	181 N9	2 N82	Pos					
					Оре	enArra	ıy 3								Open	Array 4									
		0 0	3	0	0 0		8	9		0 0	0	0	_	4 5 0 0	6	7	_	9 10		12 0					
	В	0 0	0	0	0 0		_	+		0 0	_	-	-	0 0	_	0	-	0 0		0					
	C D	0 0	0	0	0 0	_	_	-	_	0 0	_	0	_	0 0	_	0	_	0 0		0					
		1 2	3	4	5 E		- 8			11 12		2		4 5	6	Array 6	8	9 10		12					
	В	0 0	-	0	0 0	_		-	_	0 0		0	_	0 0	_	0	_	0 0		0					
	С	0 0	-	0	0 0	_		0	_	0 0	_	0	-	0 0	_	0	_	0 0	-	0					
		1 2	3	4	5 E		- 8			11 12		2		4 5	6		8	9 10		12					
	_ A	0 0	0	0	0 0	_	_	+	_	0 0	_	0	_	0 0	_	0	_	0 0		0					
		0 0	n	0	0 0	ը ի ո		1 0 1	0 -	0 0	1 0	0	0	0 0	0	0	0	0 0	1 0	0 '					
	В	0 0	0	0	0 0			-	_	0 0	_	0	-	0 0	_	0	_	0 0		0					

5. (Optional) Enter barcodes to track the eight plate areas of the 384-well sample plate to the eight OpenArray plates. The barcode is located on the OpenArray plate and on its packaging.

(For your records, scan or type in your barcodes into the corresponding boxes)

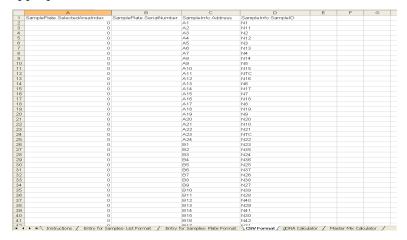


- **6.** Save your changes to a new spreadsheet file (*.xls):
 - a. Select File ▶ Save As.
 - **b.** Browse to a save location, enter a file name, then select *.xls as the file type.
 - c. Click Save.

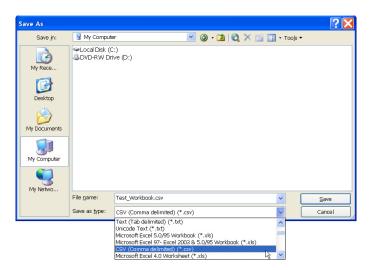


Generate the *.csv file

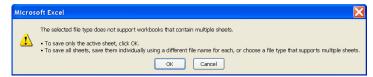
1. In the saved spreadsheet (from step 6 on page 47), select the CSV Format tab, then confirm that all of the information you entered (including sample ID, OpenArray plate barcode and, if applicable, sample plate barcode) appears as text in the appropriate columns.



- **2.** Save the CSV Format page as a *.csv file:
 - a. Select File ▶ Save As.
 - **b.** Browse to a save location, enter a file name, then select *.csv as the file type.
 - c. Click Save.



3. At the prompt, click **OK** to save only the active sheet.



4. If you are prompted to keep the workbook in the current format (*.csv), click **Yes**.



The software saves and closes the new *.csv file. Prior to imaging the OpenArray plates, you can import the *.csv file into the OpenArray software, as described in the *OpenArray*® *Real-Time PCR System User Guide*.

Use a spreadsheet or simple text program

- 1. Open a new file in a simple text program or in a spreadsheet program (such as Microsoft[®] Excel[®] Software).
- 2. In Row 1, enter column headings, as described below.
 - a. Include the following columns:

Column heading	Column description
SampleInfo.Address	The well address of the sample on the sample plate (for example, A1)
SampleInfo.SampleID	Identifying information for the sample (user-defined)
SampleInfo.Description	A description of the sample (user-defined)

- **b.** (Optional) Add new columns (user-defined) to the right of the required columns.
- **3.** In the remaining rows, enter information for each sample in the sample plate. Follow these guidelines:
 - Include all samples and all NTCs in the sample plate.
 - Enter information for only one sample per row.



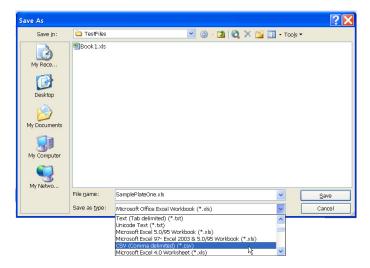
Note: You can have a maximum of 385 rows: One row for the column headings, and up to 384 rows with sample information.

- 4. Save the spreadsheet as a *.csv file:
 - a. Select File ▶ Save As.
 - **b.** Browse to a save location, enter a file name, then select *.csv as the file type.



Note: Applied Biosystems recommends that you name the file with the same unique identifier as the sample plate.

c. Click Save.



5. At the prompt, click **OK** to save only the active sheet.



6. If you are prompted to keep the workbook in the current format (*.csv), click **Yes**.



The software saves and closes the new *.csv file. Prior to imaging the OpenArray plates, you can import the *.csv file into the OpenArray software, as described in the *OpenArray*® *Real-Time PCR System User Guide*.

Export sample information from an existing *.ncx file

Select the sample information to export

- 1. Start the OpenArray® Real-Time qPCR Analysis Software. The software displays a new (empty) project file (*.ncx).
- 2. Select **File** Open, then browse to and select the project file (*.ncx) of interest.

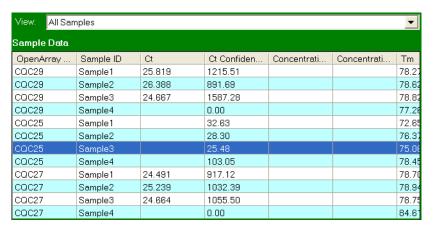


Note: Applied Biosystems does not recommend exporting sample information from one type of OpenArray plate format to create a *.csv file for a different type of format. For example, to create a *.csv file for Format 18, select a *.ncx file that contains sample information from a Format 18 OpenArray plate.

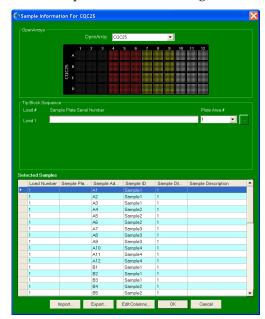
3. In the Sample Data pane, select any sample from an OpenArray plate that contains the information to export.



Note: You can export sample information from only one OpenArray plate at a time. If you select more than one sample, the software will export information for the OpenArray plate that contains the last sample you selected.



4. In the Settings pane, click **Edit Sample Info**.



5. In the Sample Information dialog box, click **Export**.

6. In the Export Sample Plates dialog box, browse to a save location, enter a file name, then click **Save**. The software exports the sample information to a *.csv file.



Note: The software exports only the rows required for the selected plate areas. The software does not export all 384 rows for each of the sample plates.

Open the *.csv file

- 1. Open the new *.csv file, using a spreadsheet or simple text program (such as Microsoft® Excel® Software). The *.csv file should include the column headings and data exported from the software.
- **2.** (Optional) Edit the sample information in the following columns:

Column heading	Column description
SampleInfo.SampleID	Identifying information for the sample (user-defined)
SampleInfo.Description	A description of the sample (user-defined)



Note: The software exports several columns. Do not alter the column headings or data for any of the remaining exported columns.

3. (Optional) Add new columns (user-defined) to the right of the exported columns.

4. Save and close the file. If you are prompted to keep the workbook in the current format (*.csv), click **Yes**.



Prior to imaging the OpenArray plates, you can import the *.csv file into the OpenArray software, as described in the *OpenArray*[®] *Real-Time PCR System User Guide*.

Appendix A Create a sample information file (*.csv) for sample tracking *Export sample information from an existing *.ncx file*

Calculator Tool

This appendix covers:

About the Calculator Tool	55
Calculate the starting concentration of gDNA	55
Calculate the volume of master mix and water	57

About the Calculator Tool

Before you load the OpenArray[®] 384-Well Sample Plate with samples (page 13), calculate the required:

• Starting concentration of the genomic DNA (gDNA) sample (this page)



Note: The *starting concentration* is the concentration of the gDNA sample prior to adding it to the sample plate.

• Volume of master mix and (if needed) water (page 57)

The Calculator Tool is a spreadsheet created with the Microsoft[®] Excel[®] Software. You can use the tool to quickly calculate the required amounts of gDNA and master mix. Applied Biosystems provides the Calculator Tool during training.

Calculate the starting concentration of gDNA

Applied Biosystems recommends that you add 250 haploid copies of gDNA to each through-hole of a TaqMan[®] OpenArray[®] Real-Time PCR Plate. To calculate the required concentration:

- 1. Determine the genome size in megabases (Mb) or the picogram (pg) quantity:
 - **Genome size** For humans, 1 haploid copy of human genome is equal to 3300 Mb.
 - **Picogram (pg) quantity (C-value)** For humans, the pg quantity is 3.3.



Note: To obtain the genome size or pg quantity for other species, go to **www.genomesize.com**, or use another trusted source.

- **2.** Browse to and open the Calculator Tool to start the Excel software.
- 3. Select the gDNA Calculator tab.

- **4.** In the spreadsheet, enter the:
 - Genome size in the yellow box.
 - Picogram quantity in the blue box.

How d	o I determine t	he star	ting co	ncentration ⁴	?		
Step 1a:	Enter the size of the g	enome (in t	terms of lea	ngth) in the yellow b	ox, or		
Step 1b:	Enter the pg quantity	of hapliod g	enome fror	n www.genomesize	.com (C value)	in the blue bo	ОX
Step 2:	Convert the size of 1 h	aploid copy	from leng	th to mass			
Step 3:	Convert the mass of 1	haploid co	by from pg	to ng			
Step 4:	Multiply the mass of 1	haploid co	py by the #	of copies required	per through-ho	le (250 copies	s)
Step 5:	Divide the mass of 250	copies by	the volume	e per through-hole			
Step 6:	Multiply the concentra	tion of gDN	A required	per through-hole by	the dilution fa	ctor constant	(2)
Step 7:	Result = required star	ing concen	tration in (ng/uL) (round to the	nearest whole	number)	
					Result	Result	Units
Genome	Size (enter number)				0		Mb
Conversi	on from length to ma	ss		978Mb = 1pg	0.0000	0.0000	pg
Conversi	on from pg to ng			1pg = 0.001ng	0.0000	0.0000	ng
Multiply r	nass by # of copies			250 copies	0.0000	0.0000	ng
Divide m	ass by through-hole v	olum e		0.033uL	0.0000	0.0000	ng/uL
Multiply	concentration by dilu	ion factor		2	0.0000	0.0000	ng/uL
Result = I	Required starting ng/	uL				0	ng/uL

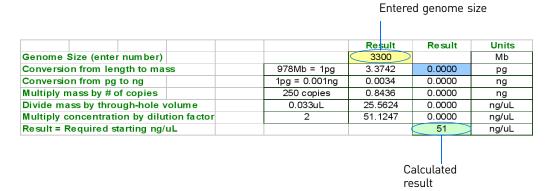
The Calculator Tool calculates the required starting concentration (in $ng/\mu L$) of the gDNA sample, rounded to the nearest whole number.

Example

The figures below show the results for human DNA.



Note: The final result may vary slightly due to rounding off by the calculator.



Entered pg quantity

		Result	Result	Units
Genome Size (enter number)		0		Mb
Conversion from length to mass	978Mb = 1pg	0.0000	3.3000	pg
Conversion from pg to ng	1pg = 0.001ng	0.0000	0.0033	ng
Multiply mass by # of copies	250 copies	0.0000	0.8250	ng
Divide mass by through-hole volume	0.033uL	0.0000	25.0000	ng/uL
Multiply concentration by dilution factor	2	0.0000	50.0000	ng/uL
Result = Required starting ng/uL			50	ng/uL
		•	Calculated result	

Calculate the volume of master mix and water

The Master Mix Calculator aids in determining:

- The volume of gDNA and water required per subarray
- The total volume of master mix and water required for the project
- 1. Browse to and open the Calculator Tool to start the Excel software.
- 2. Select the Master Mix Calculator tab.
- **3.** In the spreadsheet, enter the required values in Steps 1 through 3.



Note: The tool automatically enters the gDNA value from the gDNA Calculator.

How n	nuch Ma	aster M	ix shou	ıld I prepar	e?	
Step 1:		number of				0
Step 2:	Enter the	overage for	pipetting er	ror (e.g., 10% = 1	.1)	0
Step 3:	Enter your	gDNA con	centration			0
		•		Starting	uL per	Total uL (including
Compone	ent			Concentration	Reaction	overage)
TaqMan	Ope nArray	Master M	x	2x	2.50	0.0
Nuclease	Free Wate	er			#DIV/0!	#DIV/0!
gDNA*				50	#DIV/0!	
Total Vo	ume				5.00	0.0
	*Takes th	e ng/uL fr	om the gD	NA calculator		

The Calculator Tool calculates the required volumes.

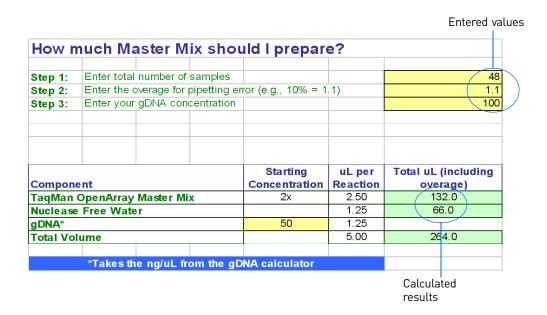


Note: The calculated results are the volumes required for the overall project, *not* the volumes required per subarray.

Example

The figure below shows the results for 48 samples, a 10% overage, and a stock gDNA concentration at $100 \text{ ng/}\mu\text{L}$ (thus, requiring a dilution).

Since the gDNA is double the required concentration, only 1.25 μ L of gDNA is required; 1.25 μ L of nuclease-free water is required to bring the final sample volume to 2.5 μ L.



Prevent Contamination

This appendix covers:

General guidelines	59
PCR good laboratory practices.	59

General guidelines

PCR assays require special laboratory practices to avoid false positive amplifications. The high throughput and repetition of these assays can lead to amplification of one DNA molecule.



Note: After a TaqMan[®] OpenArray[®] Real-Time PCR Plate has been sealed in an OpenArray[®] Case, it is less likely to spread contamination than other types of reaction plates.

PCR good laboratory practices

When preparing samples for PCR amplification:

- Wear a clean lab coat (not previously worn while handling amplified PCR products or used during sample preparation) and clean gloves.
- Change gloves whenever you suspect that they are contaminated.
- Maintain separate areas and dedicated equipment and supplies for:
 - Sample preparation
 - PCR setup
 - PCR amplification
 - Analysis of PCR products
- Never bring amplified PCR products into the PCR setup area.
- Open and close all sample tubes carefully. Try not to splash or spray PCR samples.
- Keep reactions and components capped as much as possible.
- Use a positive-displacement pipette or aerosol-resistant pipette tips.
- Clean lab benches and equipment periodically with 10% bleach solution.

Appendix C Prevent Contamination

PCR good laboratory practices

Safety

This appendix covers:

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Instrumentation safety

Symbols on instruments

Electrical symbols on instruments

The following table describes the electrical symbols that may be displayed on Applied Biosystems instruments.

Symbol	Description
	Indicates the On position of the main power switch.
0	Indicates the Off position of the main power switch.
பு	Indicates a standby switch by which the instrument is switched on to the Standby condition. Hazardous voltage may be present if this switch is on standby.
Φ	Indicates the On/Off position of a push-push main power switch.
÷	Indicates a terminal that may be connected to the signal ground reference of another instrument. This is not a protected ground terminal.
	Indicates a protective grounding terminal that must be connected to earth ground before any other electrical connections are made to the instrument.
~	Indicates a terminal that can receive or supply alternating current or voltage.
=	Indicates a terminal that can receive or supply alternating or direct current or voltage.

Safety symbols

The following table describes the safety symbols that may be displayed on Applied Biosystems instruments. Each symbol may appear by itself or with text that explains the relevant hazard. These safety symbols may also appear next to DANGERS, WARNINGS, and CAUTIONS that occur in the text of this and other product-support documents.

Symbol	Description	
<u></u>	Indicates that you should consult the manual for further information and to proceed with appropriate caution.	
Ź	Indicates the presence of an electrical shock hazard and to proceed with appropriate caution.	
<u>M</u>	Indicates the presence of a hot surface or other high-temperature hazard and to proceed with appropriate caution.	

Symbol	Description	
*	Indicates the presence of a laser inside the instrument and to proceed with appropriate caution.	
	Indicates the presence of moving parts and to proceed with appropriate caution.	
	Indicates the presence of a biological hazard and to proceed with appropriate caution.	
	Indicates the presence of a radiological hazard and to proceed with appropriate caution.	
K	Indicates the presence of a slipping hazard and to proceed with appropriate caution.	
	Indicates the presence of an ultraviolet light and to proceed with appropriate caution.	

Environmental symbols on instruments

The following symbol applies to all Applied Biosystems electrical and electronic products placed on the European market after August 13, 2005.

Symbol	Description	
	Do not dispose of this product as unsorted municipal waste. Follow local municipal waste ordinances for proper disposal provisions to reduce the environmental impact of waste electrical and electronic equipment (WEEE).	
	European Union customers: Call your local Applied Biosystems Customer Service office for equipment pick-up and recycling. See www.appliedbiosystems.com for a list of customer service offices in the European Union.	

Locations of safety labels on instruments

Hazard symbol	English	Français
	CAUTION! UV LIGHT HAZARD. UV light may harm your skin and eyes. Keep at least 25 cm distance.	ATTENTION! Dangers liés aux rayons UV. Les rayons UV peuvent endommager votre peau et vos yeux. Gardez une distance de plus de 25 cm.





General instrument safety



WARNING! PHYSICAL INJURY HAZARD. Use this product only as specified in this document. Using this instrument in a manner not specified by Applied Biosystems may result in personal injury or damage to the instrument.



WARNING! PHYSICAL INJURY HAZARD. Using the instrument in a manner not specified by Applied Biosystems may result in personal injury or damage to the instrument.



CAUTION! For safety information related to the centrifuge and thermal cycler, refer to the manufacturer's documentation.

Moving and lifting the instrument



CAUTION! PHYSICAL INJURY HAZARD. The instrument is to be moved and positioned only by the personnel or vendor specified in the applicable site preparation guide. If you decide to lift or move the instrument after it has been installed, do not attempt to lift or move the instrument without the assistance of others, the use of appropriate moving equipment, and proper lifting techniques. Improper lifting can cause painful and permanent back injury. Depending on the weight, moving or lifting an instrument may require two or more persons.



CAUTION! Do not tip the OpenArray[®] instrument on end. Tipping damages the instrument hardware and electronics and is an unsafe practice.

Moving and lifting stand-alone computers and monitors



WARNING! Do not attempt to lift or move the computer or the monitor without the assistance of others. Depending on the weight of the computer and/or the monitor, moving them may require two or more people.

Things to consider before lifting the computer and/or the monitor:

- Make sure that you have a secure, comfortable grip on the computer or the monitor when lifting.
- Make sure that the path from where the object is to where it is being moved is clear of obstructions.
- Do not lift an object and twist your torso at the same time.
- Keep your spine in a good neutral position while lifting with your legs.
- Participants should coordinate lift and move intentions with each other before lifting and carrying.
- Instead of lifting the object from the packing box, carefully tilt the box on its side and hold it stationary while someone slides the contents out of the box.

Operating the instrument

Ensure that anyone who operates the instrument has:

- Received instructions in both general safety practices for laboratories and specific safety practices for the instrument.
- Read and understood all applicable Safety Data Sheets (SDSs). See "About SDSs" on page 69.

Cleaning or decontaminating the instrument



CAUTION! Before using a cleaning or decontamination method other than those recommended by the manufacturer, verify with the manufacturer that the proposed method will not damage the equipment.

Physical hazard safety

Ultraviolet light



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

Compressed gases



WARNING! EXPLOSION HAZARD. Pressurized gas cylinders are potentially explosive and can cause severe injury if not handled properly. Always cap the gas cylinder when it is not in use and attach it firmly to the wall or gas cylinder cart with approved brackets or chains.

Moving parts



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

Solvents and pressurized fluids



WARNING! PHYSICAL INJURY HAZARD. Always wear eye protection when working with solvents or any pressurized fluids.



Electrical safety



WARNING! ELECTRICAL SHOCK HAZARD. Severe electrical shock can result from operating the OpenArray[®] instrument without its instrument panels in place. Do not remove instrument panels. High-voltage contacts are exposed when instrument panels are removed from the instrument.

Power



WARNING! ELECTRICAL HAZARD. Grounding circuit continuity is required for the safe operation of equipment. Never operate equipment with the grounding conductor disconnected.



WARNING! ELECTRICAL HAZARD. Use properly configured and approved line cords for the voltage supply in your facility.



WARNING! ELECTRICAL HAZARD. Plug the OpenArray[®] platform components into properly grounded receptacles with adequate current capacity.

Overvoltage rating

The OpenArray® platform has an installation (overvoltage) category of II, and is classified as portable equipment.

Barcode scanner laser safety

Laser classification

The barcode scanner included with the OpenArray $^{\circledR}$ platform is categorized as a Class 2 (II) laser.

Laser safety requirements

Class 2 (II) lasers are low-power, visible-light lasers that can damage the eyes. Never look directly into the laser beam. The scanner is designed to prevent human access to harmful levels of laser light during normal operation, user maintenance, or during prescribed service operations.



WARNING! LASER HAZARD. Class 2 (II) lasers can cause damage to eyes. Avoid looking into a Class 2 (II) laser beam or pointing a Class 2 (II) laser beam into another person's eyes.

Workstation safety

Correct ergonomic configuration of your workstation can reduce or prevent effects such as fatigue, pain, and strain. Minimize or eliminate these effects by configuring your workstation to promote neutral or relaxed working positions.



CAUTION! MUSCULOSKELETAL AND REPETITIVE MOTION HAZARD.

These hazards are caused by potential risk factors that include but are not limited to repetitive motion, awkward posture, forceful exertion, holding static unhealthy positions, contact pressure, and other workstation environmental factors.

To minimize musculoskeletal and repetitive motion risks:

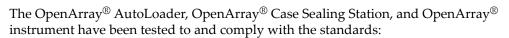
- Use equipment that comfortably supports you in neutral working positions and allows adequate accessibility to the keyboard, monitor, and mouse.
- Position the keyboard, mouse, and monitor to promote relaxed body and head postures.

Safety and electromagnetic compatibility (EMC) standards

This section provides information on:

- U.S. and Canadian safety standards
- Canadian EMC standard
- European safety and EMC standards
- Australian EMC Standards

U.S. and Canadian safety standards



UL 61010-1:2004, 2nd Edition/CSA-C22.2 No. 61010-1, "Safety Requirements for Electrical Equipment for Measurement, Control and Laboratory Use: Part 1: General Requirements."



Canadian EMC standard

The OpenArray[®] AutoLoader, OpenArray[®] Case Sealing Station, and OpenArray[®] instrument have been tested to and comply with ICES-001, Issue 3: "Industrial, Scientific, and Medical Radio Frequency Generators."

European safety and EMC standards





The OpenArray® AutoLoader, OpenArray® Case Sealing Station, and OpenArray® instrument meet European requirements for safety (Low Voltage Directive 73/23/EEC). This instrument has been tested to and complies with standards EN 61010-1:2001, "Safety Requirements for Electrical Equipment for Measurement, Control and Laboratory Use, Part 1: General Requirements."

The OpenArray[®] instrument has been tested to and complies with the standard:

EN 60825-1, "Radiation Safety of Laser Products, Equipment Classification, Requirements, and User's Guide.



EMC

This instrument meets European requirements for emission and immunity (EMC Directive 89/336/EEC). This instrument has been tested to and complies with standard EN 61326 (Group 1, Class B), "Electrical Equipment for Measurement, Control and Laboratory Use – EMC Requirements."

Australian EMC Standards



The OpenArray® AutoLoader, OpenArray® Case Sealing Station, and OpenArray® instrument have been tested to and comply with standard AS/NZS 2064, "Limits and Methods Measurement of Electromagnetic Disturbance Characteristics of Industrial, Scientific, and Medical (ISM) Radio-frequency Equipment."

Chemical safety

General chemical safety

Chemical hazard warning



WARNING! CHEMICAL HAZARD. Before handling any chemicals, refer to the Safety Data Sheet (SDS) provided by the manufacturer, and observe all relevant precautions.



WARNING! CHEMICAL STORAGE HAZARD. Never collect or store waste in a glass container because of the risk of breaking or shattering. Reagent and waste bottles can crack and leak. Each waste bottle should be secured in a low-density polyethylene safety container with the cover fastened and the handles locked in the upright position. Wear appropriate eyewear, clothing, and gloves when handling reagent and waste bottles.

Chemical safety guidelines

To minimize the hazards of chemicals:

- Read and understand the Safety Data Sheets (SDSs) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials. (See "About SDSs" on page 69.)
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing). For additional safety guidelines, consult the SDS.
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with adequate ventilation (for example, fume hood). For additional safety guidelines, consult the SDS.
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer's cleanup procedures as recommended in the SDS.
- Comply with all local, state/provincial, or national laws and regulations related to chemical storage, handling, and disposal.

SDSs

About SDSs

Chemical manufacturers supply current Safety Data Sheets (SDSs) with shipments of hazardous chemicals to new customers. They also provide SDSs with the first shipment of a hazardous chemical to a customer after an SDS has been updated. SDSs provide the safety information you need to store, handle, transport, and dispose of the chemicals safely.

Each time you receive a new SDS packaged with a hazardous chemical, be sure to replace the appropriate SDS in your files.

Obtaining SDSs

The SDS for any chemical supplied by Applied Biosystems is available to you free 24 hours a day. To obtain SDSs:

- 1. Go to www.appliedbiosystems.com, click Support, then select SDS.
- **2.** In the Keyword Search field, enter the chemical name, product name, SDS part number, or other information that appears in the SDS of interest. Select the language of your choice, then click **Search**.



- **3.** Find the document of interest, right-click the document title, then select any of the following:
 - **Open** To view the document
 - **Print Target** To print the document
 - Save Target As To download a PDF version of the document to a destination that you choose



Note: For the SDSs of chemicals not distributed by Applied Biosystems, contact the chemical manufacturer.

Chemical waste safety

Chemical waste hazards



CAUTION! HAZARDOUS WASTE. Refer to Safety Data Sheets and local regulations for handling and disposal.



WARNING! CHEMICAL WASTE HAZARD. Wastes produced by Applied Biosystems instruments are potentially hazardous and can cause injury, illness, or death.



WARNING! CHEMICAL STORAGE HAZARD. Never collect or store waste in a glass container because of the risk of breaking or shattering. Reagent and waste bottles can crack and leak. Each waste bottle should be secured in a low-density polyethylene safety container with the cover fastened and the handles locked in the upright position. Wear appropriate eyewear, clothing, and gloves when handling reagent and waste bottles.

Chemical waste safety guidelines

To minimize the hazards of chemical waste:

- Read and understand the Safety Data Sheets (SDSs) provided by the manufacturers of the chemicals in the waste container before you store, handle, or dispose of chemical waste.
- Provide primary and secondary waste containers. (A primary waste container
 holds the immediate waste. A secondary container contains spills or leaks from
 the primary container. Both containers must be compatible with the waste
 material and meet federal, state, and local requirements for container storage.)
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing). For additional safety guidelines, consult the SDS.
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with adequate ventilation (for example, fume hood). For additional safety guidelines, consult the SDS.
- Handle chemical wastes in a fume hood.
- After emptying a waste container, seal it with the cap provided.
- Dispose of the contents of the waste tray and waste bottle in accordance with good laboratory practices and local, state/provincial, or national environmental and health regulations.

Waste disposal

If potentially hazardous waste is generated when you operate the instrument, you must:

- Characterize (by analysis if necessary) the waste generated by the particular applications, reagents, and substrates used in your laboratory.
- Ensure the health and safety of all personnel in your laboratory.
- Ensure that the instrument waste is stored, transferred, transported, and disposed of according to all local, state/provincial, and/or national regulations.
 - IMPORTANT! Radioactive or biohazardous materials may require special handling, and disposal limitations may apply.

Biological hazard safety

General biohazard



WARNING! BIOHAZARD. Biological samples such as tissues, body fluids, infectious agents, and blood of humans and other animals have the potential to transmit infectious diseases. Follow all applicable local, state/provincial, and/or national regulations. Wear appropriate protective equipment, which includes but is not limited to: protective eyewear, face shield, clothing/lab coat, and gloves. All work should be conducted in properly equipped facilities using the appropriate safety equipment (for example, physical containment devices). Individuals should be trained according to applicable regulatory and company/ institution requirements before working with potentially infectious materials. Read and follow the applicable guidelines and/or regulatory requirements in the following:

- U.S. Department of Health and Human Services guidelines published in Biosafety in Microbiological and Biomedical Laboratories found at: www.cdc.gov/biosafety/publications/index.htm
- Occupational Safety and Health Standards, Bloodborne Pathogens (29 CFR§1910.1030; www.access.gpo.gov/ nara/cfr/waisidx_01/ 29cfr1910a_01.html).
- Your company's/institution's Biosafety Program protocols for working with/ handling potentially infectious materials.

Additional information about biohazard guidelines is available at: www.cdc.gov



Appendix D Safety

Chemical safety

Documentation and Support

Product documentation

Portable document format (PDF) versions of the documents listed in this section are available at www.appliedbiosystems.com

Note: To open the PDF versions, use the Adobe Acrobat Reader software available from **www.adobe.com**

OpenArray® Real-Time PCR System

Document	Description	
OpenArray [®] Real-Time PCR System Troubleshooting Guide	Provides troubleshooting information for the OpenArray® Real-Time PCR System.	4458839
OpenArray [®] Real-Time PCR System User Guide	Provides procedures for imaging and analyzing OpenArray [®] plates. Provides maintenance information for the OpenArray [®] Real-Time PCR System.	4458837
OpenArray [®] Real-Time PCR System Quick Reference Card: Workflow	Describes the overall workflow and provides brief procedures for performing gene expression experiments with the OpenArray® Real-Time PCR System.	4458838
OpenArray [®] Real-Time PCR System Quick Reference Card: Using the Sample Tracking Tool	Describes how to use the Sample Tracking Tool to create sample information files (*.csv) and import the files into the OpenArray® Real-Time qPCR Analysis Software.	4460957
SYBR [®] OpenArray [®] Real-Time PCR Plates Protocol	Provides procedures for preparing the SYBR® OpenArray® Real-Time PCR Plates.	4458869
TaqMan [®] OpenArray [®] Real-Time PCR Plates Protocol	Provides procedures for preparing the TaqMan [®] OpenArray [®] Real- Time PCR Plates.	4458840

TaqMan® OpenArray® Genotyping System

Document	Description	Part number
TaqMan [®] OpenArray [®] Genotyping Getting Started Guide	Provides procedures for performing genotyping experiments with the TaqMan® OpenArray® Genotyping System.	4377476
TaqMan [®] OpenArray [®] Genotyping Plates Ordering Guide	Provides ordering information for the TaqMan® OpenArray® Genotyping Plates.	4400403
TaqMan [®] OpenArray [®] Genotyping System Quick Reference Card: Using the Sample Tracking Tool	Describes how to use the Sample Tracking Tool to create sample information files (*.csv) and import the files into the OpenArray® SNP Genotyping Analysis Software.	4460657
TaqMan [®] OpenArray [®] Genotyping System Quick Reference Card: Workflow	Describes the overall workflow and provides brief procedures for performing genotyping experiments on the TaqMan® OpenArray® Genotyping System.	4400402
TaqMan [®] OpenArray [®] Genotyping System Site Preparation Guide	Provides information on preparing the customer site for the TaqMan [®] OpenArray [®] Genotyping System.	4401171
TaqMan [®] OpenArray [®] Genotyping Troubleshooting Guide	Provides troubleshooting information for the TaqMan [®] OpenArray [®] Genotyping System. To be used in conjunction with the <i>TaqMan</i> [®] OpenArray [®] Genotyping Getting Started Guide.	4401671

Related documentation

To obtain the documents listed in this section or additional documentation, see "Obtaining support" on page 75.

Genotyping experiments

Document	Part number
Application Note: DNA Genotyping from Human FFPE Samples – Reliable and Reproducible	137AP04-01
Bioinformatic Evaluation of a Sequence for Custom TaqMan® SNP Genotyping Assays	4371003
Ordering TaqMan® SNP Genotyping Assays Quick Reference Card	4374204
TaqMan [®] SNP Genotyping Assays Protocol	4332856
User Bulletin: Human DNA Sample Quantification Protocol Using the RNase P Kit	4342582

Obtaining support

For the latest services and support information for all locations, go to:

www.appliedbiosystems.com

At the Applied Biosystems web site, you can:

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