QuantStudio™ 3D Digital PCR System

Catalog Number A29154

Pub. No. MAN0008159 Rev. E.0

Note: For safety and biohazard guidelines, see the "Safety" appendix in the *QuantStudio™ 3D Digital PCR System User Guide* (Pub. No. MAN0007720). Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Operational workflow

The following shows a single experiment workflow on the QuantStudio™ 3D Digital PCR System. The procedures for sample and/or consumable preparation and result analysis can vary depending on the specific experiment that you are performing.

Start

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Set up the dPCR reaction by mixing sample, master mix, and assay(s).

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Load the dPCR reaction onto a QuantStudio™ 3D Digital PCR 20K Chip v2, apply the lid, load the assembly with Immersion Fluid, then seal the loading port.

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Perform the PCR using the ProFlex™ 2 × Flat PCR System or Dual Flat Block GeneAmp™ PCR System 9700.

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Read the chip using the QuantStudio™ 3D Digital PCR Instrument.

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Review the results on the instrument touchscreen.

▼

Store or discard the chip.

▼

Analyze the data using the QuantStudio™ 3D AnalysisSuite™ Software.

▼

Finish

Prepare the dPCR reaction mix for each sample

Prepare the PCR reactions for loading on the QuantStudio™ 3D Digital PCR 20K Chip v2.

1. Thaw the following at room temperature, and ensure that the tubes are at room temperature before using:
   - QuantStudio™ 3D Digital PCR Master Mix v2
   - TaqMan™ Assay(s)
2. Prepare the genomic DNA sample:
   a. Dilute the DNA sample as needed so that the concentration of target sequence in the final reaction is between 200 and 2,000 copies/µL.
   b. Vortex, then briefly centrifuge the DNA sample.
   c. Using a permanent marker, label a 0.5- or 1.5-mL reaction tube with the sample name.
3. When the master mix has warmed to room temperature, gently invert the tube 10 times (or gently vortex on low-medium speed).
4. In a 0.5- or 1.5-mL low-bind tube, prepare the following dPCR reaction mix at room temperature. Scale the volumes appropriately, depending on the number of samples.

Table 1 dPCR reaction mix—example amounts

The example amounts assume that you are running two chips per human gDNA sample at 10 ng/µL.

Item	Amount		
	1 sample/ 2 chips[1]	Stock	Final
Master mix	17.4 µL	2X	1X
TaqMan™ Assay(s), 20X (primer/probe mix)	1.7 µL	20X	1X
Diluted genomic DNA[2]	3.5 µL	10 ng/µL	1 ng/µL
Water	12.2 µL	—	—
Total volume	**34.8 µL**	—	—
Volume to load per chip	14.5 µL	—	—

[1] Volumes include 20% excess to compensate for volume loss from pipetting.
[2] Depending on sample source, required input DNA amount may vary.

5. Mix well by gently pipetting up and down (or gently vortex on low-medium speed).

6. Transfer 34.8 µL of the dPCR reaction mix to the labeled reaction tube.

7. Cap the reaction tube, briefly centrifuge, then immediately proceed to load the chips.

IMPORTANT! For optimal results, load the chips as soon as possible after setting up the reactions. If you placed the reactions on ice, warm them to room temperature before loading.

Load the chips using the QuantStudio™ 3D Digital PCR Chip Loader

For instructions on loading chips manually, see the *QuantStudio™ 3D Digital PCR System User Guide* (Pub. No. MAN0007720).

Prepare the chip loading workspace

1. Plug in and power on the chip loader.

2. Fold back the chip loader arm, then wait until the status light illuminates solid green, indicating that the chip nest has reached operating temperature (≤20 minutes depending on room temperature).

3. Remove the following consumables from their packaging and place them on a clean, dry, lint-free surface:
   - Chip lid
   - QuantStudio™ 3D Digital PCR Sample Loading Blade

Prepare the syringe containing the Immersion Fluid

1. Remove the Immersion Fluid syringe, plunger, and tip from the packaging.

2. Before uncapping the syringe, gently pull back the plunger 1-2 mm and release it to break any resistance that may have formed during storage.

3. Remove the cap from the syringe, then attach the syringe tip by pushing it into place.

4. Carefully depress the plunger until Immersion Fluid flows from the tip of the assembled syringe. Place it on a clean surface and proceed to the next steps.

Load and seal the chips

Before using the QuantStudio™ 3D Digital PCR Chip Loader, wait until the status light illuminates solid green, indicating that the chip nest has reached operating temperature.

1. Open the package containing a QuantStudio™ 3D Digital PCR 20K Chip v2. Gently grasp the chip by its sides and load it face-up into the chip nest.

2. Press down on the chip nest lever to open the clamp, and place the chip in the nest. Release the lever to lock the chip into place.

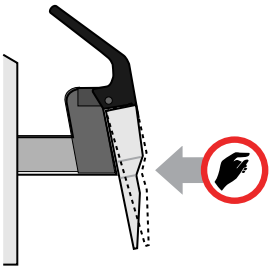
3. Press the sample loading blade lever, then install the QuantStudio™ 3D Digital PCR Sample Loading Blade into the loader head.

4. Grasping the lid by its sides, peel away the red protective film from the back of the chip lid. Avoid contact with the exposed sticky surface.

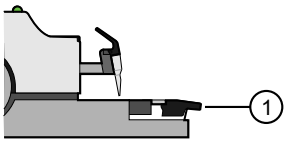
5. Press the lid nest button, and carefully place the lid with the sticky side up into the nest in the correct orientation. Slowly release the button to lock the lid in place.

6. Briefly vortex and centrifuge the prepared dPCR reaction (from "Prepare the dPCR reaction mix for each sample" on page 1), then carefully transfer 14.5 µL of the solution into the sample loading port of the loading blade. If the reactions were placed on ice, allow them to warm prior to loading.

7. Ensure that the loading blade is firmly seated on the loader arm.



8. Press the dark grey/black loading button to load the chip.



① Loading button

The status light flashes green during the loading sequence, and displays solid green when finished.

9. After loading, hold the Immersion Fluid syringe at an angle over the chip surface without touching the surface, and *slowly* add several drops of fluid directly onto the chip so that the fluid covers the entire surface. After dispensing, remove any fluid from the edges of the chip case with a low-lint wipe that has been sprayed with isopropanol.

10. Rotate the loader arm so that the chip lid solidly contacts the chip. Firmly press down on the arm for 15 seconds to ensure a tight seal (you can count each flash of the status light, which flashes at 1-second intervals).

11. Press the lid nest button to release the chip lid, then lift and return the loader arm to its original position.

Fill the chip case with Immersion Fluid

1. Hold the chip and lid assembly by its edges at a 45° angle so that air can escape from the fill port as you fill it.
2. Hold back the top half of the chip lid label to expose the fill port.
3. Carefully dispense Immersion Fluid into the port until the chip case contains an air bubble slightly larger than the fill port (<2–3 mm in diameter). Rotate the chip slightly to expose any hidden bubble. If a bubble larger than 2–3 mm is present, add additional fluid.
4. Using a low-lint wipe, remove any excess Immersion Fluid from the chip case to ensure optimal imaging.

Seal the chip case with the label

1. Gently pull back the top half of the label on the chip lid.
2. Remove the label backing and press the label firmly over the fill port for 5 seconds to ensure a tight seal.
3. Gently run your fingers over the entire label to seal the remainder of the label.
4. Inspect the sealed chip for leaks, bubbles, and correct lid orientation.
5. Store the prepared chip in a clean, dry, dark location until you are ready to load it onto the thermal cycler.

Thermal cycle the chips

Perform the PCR

1. Open the heated cover of the thermal cycler and wipe the surface of both sample blocks using a low-lint wipe to ensure that they are clean and dry.
2. Confirm that the Tilt Base is installed and Chip Adapters are installed in *both* sample blocks (even if you are using only one block).
3. Place the chips onto the sample block so that the fill ports on the chips are positioned toward the *front* of the thermal cycler. The fill port must be elevated during thermal cycling to ensure that any bubbles float to the top of the case.

   IMPORTANT! If you are thermal cycling less than 24 chips, load according to the following guidelines:

   - Load the right sample block first, placing at least 1 chip on the right sample block.
   - Balance the load between the left and right blocks so that the pressure applied by the heated cover and thermal pads is uniform across all of the loaded chips.
4. Lay the Thermal Pads over the chips.
5. Close and engage the heated cover of the thermal cycler.
6. Use the thermal cycler to select and start the pre-programmed run for the chips. See the user guide for your thermal cycler for more information on running methods.

Table 2 ProFlex™ 2 × Flat PCR System PCR Method

PCR protocol					Cover temp.	Rxn. vol.
Stage 1	Stage 2		Stage 3			
96.0°C	60.0°C	98.0°C	60.0°C	10.0°C	70.0°C	1 nL[1]
1.6°C/sec	1.6°C/sec	1.6°C/sec	1.6°C/sec	1.6°C/sec		
0:10:00	0:02:00	0:00:30	0:02:00	∞		
1x	39x		1x			

[1] 33 nL for firmware earlier than v1.1.4. The reaction volume on the instrument display does not refer to the reaction volume on the chip and should not be changed.

Table 3 GeneAmp™ PCR System 9700 PCR Method

PCR protocol					Run speed	Rxn. vol.
Stage 1	Stage 2		Stage 3			
96.0°C	56.0°C	98.0°C	60.0°C	10.0°C	Std.	20 µL[1]
10 min	2 min	30 sec	2 min	∞		
1x	39x		1x			

[1] The reaction volume on the instrument display does not refer to the reaction volume on the chip and should not be changed.

Unload the thermal cycler

You can remove the chips from the thermal cycler immediately after the final extension step at 60°C is complete and the temperature of the block is ≤25°C. Alternatively, the chips can remain on the block for up to 24 hours at 10°C.

CAUTION! PHYSICAL INJURY HAZARD. During operation, the sample block may reach temperatures of 100°C. Before removing chips, wait until the block reaches room temperature.

1. If you programmed the thermal cycler to perform a 10°C hold after cycling, do the following to prevent condensation:
   a. Confirm the final extension step at 60°C is complete, then stop the run.
   b. Allow the thermal cycler to sit for at least 10 minutes with the heated covered closed.
2. Open the heated cover to expose the chips.
3. Remove the thermal pads from the sample block and set them on a clean, dry surface.
4. Remove the Chip Adapters from the sample block and place them on a clean, dry surface. Remove the chips from the adapters and allow them to equilibrate to room temperature.
5. Inspect each chip for leaks or potential problems. Using a low-lint wipe, remove any condensation or Immersion Fluid from the chip surface by wiping in one direction. If necessary, use a low-lint wipe sprayed with isopropanol to remove any dried residue. Make sure the surface is thoroughly clean.

Image the chips

IMPORTANT! Before imaging, confirm that the latest firmware is installed on the QuantStudio™ 3D Digital PCR Instrument.

1. If you selected **USB** in the Data Destinations screen (as described above), insert a USB device into the USB port on the front of the instrument.
2. Open the chip tray and load the chip face-up into the tray, with the chip ID and fill port toward the front of the instrument.
3. Confirm that the chip is correctly aligned, then close the tray to begin chip detection and imaging. You can monitor the progress of the run in the touchscreen, which counts down the time remaining.
4. *(Optional)* During the run, touch the **Add Prefix** field to enter a prefix for each experiment (.eds) file name.
5. The instrument is done imaging when the touchscreen displays the Analyzing Chip screen. At this point, you can either wait for the instrument to complete the analysis and display the results, or you can open the chip tray and remove the chip.

Manufacturer:
Life Technologies Holdings Pte Ltd |
Block 33 |
Marsiling Industrial Estate Road 3 |
#07-06, Singapore 739256

Products:
QuantStudio™ 3D Digital PCR Instrument
ProFlex™ 2 × Flat PCR System
Dual Flat Block GeneAmp™ PCR System 9700
QuantStudio™ 3D Digital PCR Chip Loader

Manufacturer:
Life Technologies Corporation |
6055 Sunol Blvd |
Pleasanton, CA 94566

Products:
QuantStudio™ 3D Digital PCR 20K Chip v2

Life Technologies Corporation | 7335
Executive Way | Frederick, MD 21704 | USA

Products:
QuantStudio™ 3D Digital PCR Master Mix v2

For descriptions of symbols on product labels or product documents, go to **thermofisher.com/symbols-definition**.

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

Revision history: Pub. No. MAN0008159

Revision	Date	Description
E.0	18 March 2020	Removed the v1 chip and master mix. Updated template and legal information. Updated "Prepare the dPCR reaction mix for each sample" on page 1. Updated thermal cycling parameters.
D.0	15 July 2015	Updated for v2 chips, lids, and master mix. Also updated with changes to instrument firmware, including adjusting for the different well volumes between v1 and v2 chips. Includes improvements to the chip loading and imaging workflows.
C.0	February 2015	Updated for firmware and software revisions, including changes to instrument networking, thermal cycling, chip analysis, maintenance, software access, and computer requirements. Updated to latest corporate boilerplate. Updated general sample and chip preparation, system installation, troubleshooting, and parts and materials.
B.0	April 2014	Added support for the ProFlex™ PCR System and revised the chip loading instructions.
A.0	December 2013	Updated the manual chip preparation and added procedures for chip preparation using the QuantStudio™ 3D Digital PCR Chip Loader and wireless network installation.
02	June 2013	Updated general chip preparation and instrument networking.
01	March 2013	Initial version