applied biosystems by life technologies™

Supported Protocol: Getting Started with the QuantStudio™ 3D Digital PCR System

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Overview

Purpose

The following procedure enables you to confirm the performance of the QuantStudio™ 3D Digital PCR System either at the time of system installation or as part of future system verification using established Life Technologies™ consumables, reagents, control DNA, and reference assays.

Life Technologies™ Supported Protocols

Life Technologies™ Supported Protocols have been successfully tested and verified by our scientists on the specified instrument. Our Technical Support and Field Application Specialists have been trained to support this protocol.

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Required materials

Unless otherwise indicated, materials are available to order from our website: **www.lifetechnologies.com**.

Cat. no.	Contents	Quantity	Conc.	Storage conditions
403062	Control DNA CEPH Individual 1347-02	1 tube (180 µL)	50 ng/µL	Remove the tube from the packaging, then store it at -20°C until first use, then store multiple aliquots at -20°C.
Hs04107718_cn	TaqMan® Copy Number Assay, GLA (FAM™ dye-labeled)	1 tube (360 µL)	20X	Remove the tube from the packaging, then store it at -20°C.
4403326	TaqMan® Copy Number Reference Assay, RNase P, Human (VIC® dye-labeled)	1 tube (750 µL)	20X	Remove the tube from the packaging, then store it at -20°C.
4482710	QuantStudio™ 3D Digital PCR Master Mix	1 tube (1.5 mL)	2X	Remove the tube from the box, then store it at -20°C until first use, then store it at 2 to 8°C.
4485507	QuantStudio™ 3D Digital PCR 20K Chip Kit	1 kit, containing: • 12 Digital PCR 20K Chips • 12 Chip Case Lids • Sufficient Immersion Fluid for 12 chips	—	Store at room temperature.
4488475	QuantStudio™ 3D Digital PCR UV Sealant Kit	1 kit, containing UV-Activated Chip Sealant Syringe and tips	—	Store at room temperature.
AM9938	Nuclease-free Water	1 bottle (100 mL)	—	Store at room temperature.

Note: For safety and biohazard guidelines, refer to 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.

Workflow

The procedures for sample and reaction mix preparation and result analysis that are specific to verifying instrument performance are shown below.

Start

▼

Dilute the control DNA

▼

Prepare the digital PCR reaction by mixing control DNA, master mix, and reference assay(s)

▼

Load the PCR reaction onto a QuantStudio™ 3D Digital PCR 20K Chip, apply a lid, load the assembly with immersion fluid, then seal the loading port

▼

Thermal cycle the chip

▼

Read the Digital PCR 20K Chip using the QuantStudio™ 3D Digital PCR Instrument

▼

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

▼

Finish

Note: Refer to the *QuantStudio™ 3D Digital PCR System User Guide* (Pub. no. MAN0007720) for the workflow for performing a single digital PCR (dPCR) experiment on the QuantStudio™ 3D System. Refer to the user documentation for the AnalysisSuite™ Software for detailed information on data analysis.

Protocol

About the control DNA

The control DNA sample (CEPH Individual 1347-02) is provided at a concentration of approximately 50 ng/µL. As this concentration may vary from lot to lot, this protocol focuses on determining the concentration of the GLA target gene relative to the RNase P reference gene. Since each gene should be present at the rate of a single copy per haploid genome, the expected ratio between measurements for each assay is 1.0 ± 10%. Assuming a starting concentration of approximately 50 ng/µL when loaded onto the Digital PCR 20K Chip, this protocol targets a final concentration of 1,000 Copies/µL in the dPCR reaction.

Dilute the control DNA

The control DNA sample must first be diluted down to a limiting quantity prior to running the dPCR experiment. For this protocol, we recommend diluting the control DNA to a final concentration of ≈1,000 Copies/µL. In general, we recommend loading approximately 0.16 to 1.6 copies of the target sequence per reaction well in the Digital PCR 20K Chip, which is equivalent to 200 to 2,000 Copies/µL of the target sequence in the reaction.

1. Thaw on ice, vortex, then briefly centrifuge the control DNA.

   Note: Do not perform more than 10 freeze-thaw cycles. If you expect to freeze-thaw more than three times, we recommend that you aliquot the control DNA to minimize the number of freeze-thaw cycles.

2. Dilute the control DNA from the stock concentration to a working concentration prior to preparing the dPCR reaction mix:
   a. Using a permanent marker, label a 0.5- or 1.5-mL reaction tube.

   b. Prepare the dilution by transferring the appropriate volume of nuclease-free water, then the corresponding volume of DNA to the labeled tube as shown below.

Material	Volume	DNA conc.
Nuclease-free water	8.3 µL	—
Control DNA	16.7 µL	Stock: ≈15,000 Copies/µL (or 50 ng/µL)
Total	25 µL	Working: 10,000 Copies/µL (or 10X)

3. Cap the tube, vortex, then briefly centrifuge the diluted control DNA.

Prepare the dPCR reactions

In this procedure, you will prepare the dPCR reactions for the control DNA sample and reference assays that you will load into the Digital PCR 20K Chip. The volumes of the protocol have been adjusted so that three replicates (chips) are run for the example sample and assay combination.

1. Remove the following from the freezer and allow them to thaw at room temperature:
   - QuantStudio™ 3D Digital PCR Master Mix
   - TaqMan® Copy Number Assay, GLA
   - TaqMan® Copy Number Reference Assay, RNase P

2. Gently invert the tube of Digital PCR Master Mix 10 times.

3. Vortex, then briefly centrifuge the diluted control DNA and the assays.

4. In an appropriate reaction tube, combine the dPCR reaction mix components as shown in the following table.

Material	Working conc.	Final conc.	Volume (µL)	
			Per chip	3 chips [1]
Digital PCR Master Mix	2X	1X	7.25	26.1
GLA Assay (FAM™ dye-labeled primer/probe mix)	20X	1X	0.725	2.61
RNase P Reference Assay (VIC® dye-labeled primer/probe mix)	20X	1X	0.725	2.61
Diluted Control DNA	10X	1X	1.45	5.22
Nuclease-free Water	—	—	4.35	15.66
Total volume	**—**	**—**	**14.5**	**52.2**

[1] Includes 20% excess volume to account for pipetting errors.

5. Mix well by gently pipetting up and down.

6. Cap the reaction tube, then centrifuge for 1 minute at 1,000 rpm to eliminate any bubbles from the reaction. Proceed immediately to loading the Digital PCR 20K Chips.

IMPORTANT! For optimal results, load the Digital PCR 20K Chips as soon as possible after setting up the reactions.

Load and run the reactions

Refer to the *QuantStudio™ 3D Digital PCR System User Guide* (Pub. no. MAN0007720) for general guidelines and information on the following:

- Loading and sealing the Digital PCR 20K Chips
- Preparing the thermal cycler and thermal cycling the chips
- Imaging and analyzing the chips using the QuantStudio™ 3D Instrument

Expected analysis results

Summary

A calculated FAM™/VIC® dye ratio of 1.0 ± 10% confirms the performance of the QuantStudio™ 3D Digital PCR System. More information on the expected analysis results are shown below.

IMPORTANT! If you do not receive the expected results when using the described protocol, troubleshoot the chip image in the software as described in the user documentation for the QuantStudio™ 3D System and the AnalysisSuite™ Software. If you cannot troubleshoot the results, then contact Technical Support.

Example of expected analysis results

Figure 1 and Figure 2 below show an example of the expected results when using the described protocol. The control DNA was diluted to a working concentration as described in "Dilute the control DNA" on page 4, loaded in a total volume of 14.5 µL to each replicate Digital PCR 20K Chip, and amplified using both FAM™ and VIC® dye-labeled reference assays.

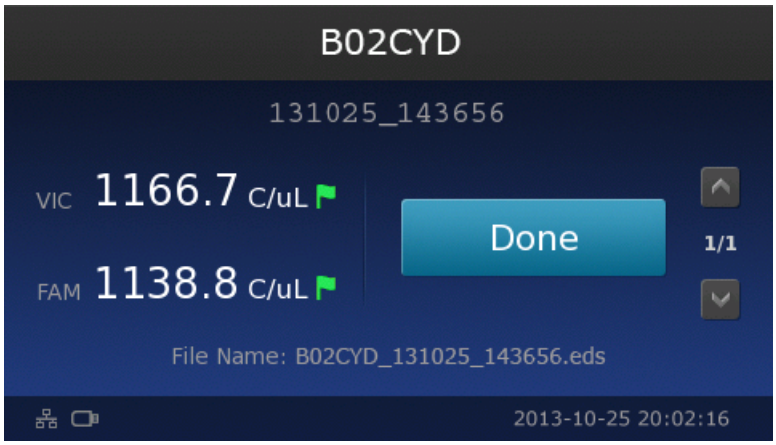

Figure 1 Analysis results in the QuantStudio™ 3D Instrument touchscreen

Each data point in Figure 2 was analyzed using the default quality threshold and 95% confidence level in the QuantStudio™ 3D AnalysisSuite™ Software and is displayed by the assigned call, based on the target (dye) signal detected in a reaction well of the chip.

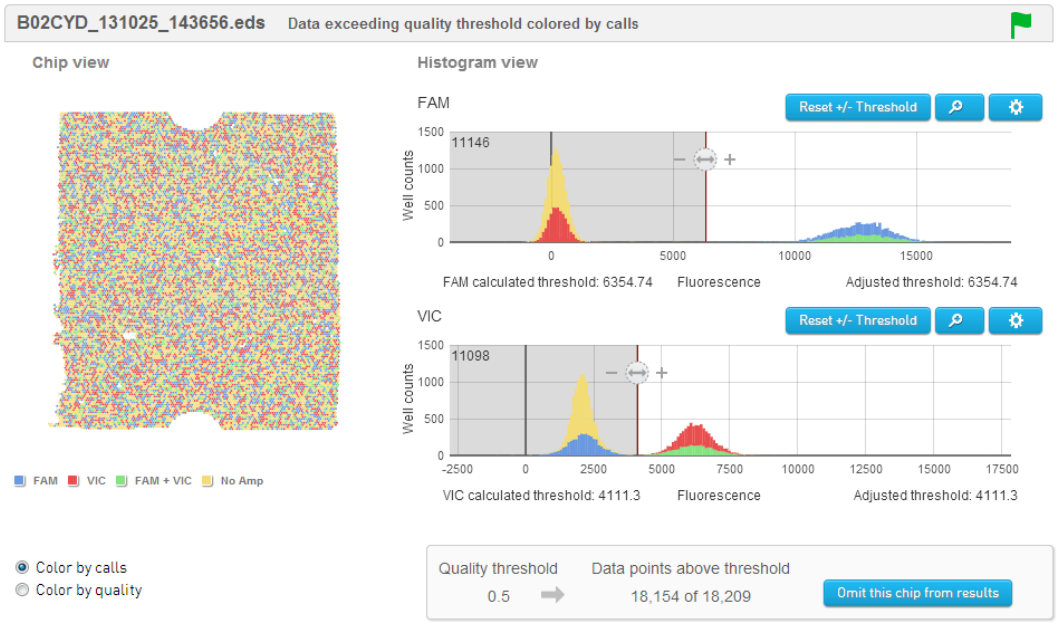

Figure 2 Analysis results in the AnalysisSuite™ Software

Passing criteria

Passing criteria for all replicate chips include:

- A (green) data quality flag in the instrument touchscreen or in the Review Quality tab of the AnalysisSuite™ Software

  Note: A (yellow) data quality flag may indicate possible problems with the consumables or workflow that are independent of instrument and reagent performance.
- Uniform spatial distribution of calls across the chip, as shown in the Chip view of the AnalysisSuite™ Software

  Note: In order to calculate a quality value for each reaction well in a chip, the AnalysisSuite™ Software assesses the distribution of positive and negative calls across the chip and compares the expected clustering of high FAM™ or VIC® dye signal *at a given concentration* against the observed clustering. Reaction wells forming groups of positives that cluster differently from what is expected at the estimated concentration are assigned lower quality values. Reaction wells that do not meet the default quality value threshold of 0.5 are automatically excluded from further analysis.
- Bi-modal (two peak) distribution of both FAM™ and VIC® dyes with clear separation between the positive and negative peaks, as shown in the Histogram view of the AnalysisSuite™ Software
- Assuming there are no significant workflow errors (including DNA concentration, dilution, or pipetting):
  - Copies/µL (C/µL) for both FAM™ and VIC® dyes is within ± 10% of the mean concentration
  - FAM™/VIC® dye ratio is within ± 10% of the expected 1.0 ratio

    Note: To calculate the dye ratio, you can use either the C/µL values shown in the instrument touchscreen or the Copies/µL values shown in the Results or Replicates tab of the AnalysisSuite™ Software.

Obtaining support

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

www.lifetechnologies.com/support

At the website, you can:

- Access worldwide telephone and fax numbers to contact Technical Support and Sales facilities
- Search through frequently asked questions (FAQs)
- Search for user documents, SDSs, vector maps and sequences, application notes, formulations, handbooks, certificates of analysis, citations, and other product support documents
- Obtain information about customer training
- Download software updates and patches

Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale found on Life Technologies' website at **www.lifetechnologies.com/termsandconditions**. If you have any questions, please contact Life Technologies at **www.lifetechnologies.com/support**.

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Headquarters
5791 Van Allen Way | Carlsbad, CA 92008 USA | Phone +1 760 603 7200 | Toll Free in USA 800 955 6288
For support visit **lifetechnologies.com/support** or email **techsupport@lifetech.com**

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