Rare mutation analysis using the QuantStudio 3D Digital PCR System

Introduction

Rare somatic mutations can be detected using Applied Biosystems™ TaqMan® dPCR Liquid Biopsy Assays combined with digital analysis on the Applied Biosystems™ QuantStudio™ 3D Digital PCR System. This quick reference protocol provides instructions specific to this application. For detailed instructions on preparing and performing a digital PCR (dPCR) experiment, refer to the QuantStudio 3D Digital PCR System User Guide (Pub. No. MAN0007720).

Ordering information

Product	No. of reactions	Conc.	Volume	Cat. No.
Custom TaqMan Assay	12	20X	10 µL	4383547
TaqMan dPCR Liquid Biopsy Assays	450	40X	165 µL	A44177

The reaction setup below is recommended for rare allele detection and accommodates the running of two technical replicates. For mutation rates lower than 1%, additional chips may be required to achieve robust detection.

Reaction mix preparation for dPCR sample replicates (1 sample, 2 chips)

Material	Volume*	Stock	Final
QuantStudio 3D Digital PCR Master Mix	17.4 µL	2X	1X
TaqMan dPCR Liquid Biopsy Assays**	1.7 µL	20X	1X
Diluted genomic DNA†	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	–	–

* Volumes include 20% excess to compensate for volume loss during pipetting.
** Dilute to 20X prior to reaction mix preparation.
† Depending on sample source, required input DNA amount may vary.

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Thermal cycling protocols

For use with the Applied Biosystems™ ProFlex™ PCR System

If not using the 3D default template from ProFlex v1.1.4 firmware, you must create a thermal cycling protocol and verify the ramp rates. The following thermal cycling protocol table (Table 1) should be displayed on your instrument screen (ramp rates are not displayed on the main screen).

For use with the Applied Biosystems™ GeneAmp™ PCR System 9700

For the GeneAmp PCR System 9700, you do not need to adjust the cover temperature or the ramp rates. The following thermal cycling protocol table (Table 2) should be displayed on your instrument screen.

Table 1.

Stage 1	Stage 2		Stage 3		Cover temp.	Reaction volume
96.0°C	60.0°C	98.0°C	60.0°C	10.0°C	70.0°C	1 nL* (33 nL for firmware older than v1.1.4)
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 (Hold)	39x (Cycles)		1x (Hold)			

* The reaction volume on the instrument display (**1 nL**) does not refer to the reaction volume on the chip and should not be changed.

Table 2.

Stage 1	Stage 2		Stage 3		Reaction volume
96.0°C	56.0°C*	98.0°C	60.0°C	10.0°C	20 µL**
10 min	2 min	30 sec	2 min	∞	
1x (Hold)	39x (Cycles)		1x (Hold)		

* In stage 2, an anneal/extend temperature of 56.0°C is recommended, which is lower than the standard protocol described in the QuantStudio 3D Digital PCR System User Guide.
** The reaction volume on the instrument display (**20 µL**) does not refer to the reaction volume on the chip and should not be changed.

Find out more at **thermofisher.com/dpcr-raremutation**

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