Application Note

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# Viral Detection using Agilent Brilliant III Master Mixes on the Applied Biosystems QuantStudio Real-Time PCR System


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## Abstract

Researchers at the Oregon Health and Science University's Vaccine and Gene Therapy Institute are interested in characterizing viral pathogenesis in mammalian hosts to facilitate the development of appropriate therapeutics for infection and disease treatment. To assess these questions, quantitative PCR (qPCR) is used to quantify viral load in infected cells and tissues from infected animals. Primers are used to target unique regions within the viral genome to specifically detect viral RNA within the host.

A protocol was developed to detect O'nyong'nyong virus (ONNV), a single-stranded, positive-sense RNA virus, in mouse samples using the Agilent Brilliant III qPCR SYBR Green and probe-based reagent kits on the Applied Biosystems (ABI) QuantStudio 7 Real-Time PCR 384-well system. The assay used in the protocol specifically detects sequences within the 3' region of the ONNV genome in mice that have been infected at a specific titer. The assay involves establishing a reproducible standard curve starting at five million copies, with the ability to detect as few as 50 viral copies of ONNV per sample.[1] Dilutions performed showed similar slopes and good linearity, and the amplified targets gave similar Ct values in triplicates.

## Materials and Methods

### Collection, processing, extraction, and preparation of nucleic acid from mouse tissue

Mice were infected with ONNV at 1 x $10^3$ plaque forming units (PFUs) in the right foot pad. Right ankle muscle and connective tissues were harvested at day five post infection. Total nucleic acids were extracted using Promega's Maxwell RSC 48-sample automated purification system and the Maxwell RSC Viral TNA extraction kit (Promega cat# AS1330). Samples were eluted in 100 µL of RNase-free water.

For the cDNA reactions, 11 µL of the resuspended sample was transcribed using Random Primer Mix (New England Biolabs cat# S1330S) and reverse transcriptase SuperScript IV (Invitrogen cat# 18090050) following the manufacturer's protocol. Either Agilent AffinityScript multiple temperature or Agilent AccuScript high-fidelity reverse transcriptases may also be used for reverse transcription.

### qPCR

To detect the presence of ONNV viral genomes, real-time PCR was performed on a QuantStudio 7 Flex Real-Time PCR system, and data was analyzed using the Applied Biosystems Design and Analysis software v2.8.0.

Briefly, 5 µL of cDNA was used as template for each of the qPCR reactions using either the Brilliant III Ultra-Fast qPCR master mix (part number 600880) or Brilliant III Ultra-Fast SYBR Green qPCR master mix (part number 600882) according to the manufacturer's protocol. The samples were run in triplicate, including the no template control (nuclease-free water) and the standard curve. The standards were made from serial dilutions of a plasmid containing the viral genome at a known concentration: 5,000,000, 500,000, 50,000, 5,000, 500, and 50 copies. Each assay was performed three separate times using the ABI StepOnePlus PCR cycling program indicated in each of the Agilent enzyme-specific protocols (see Table 1).

The primers and probes were designed to amplify the 3' region of the ONNV genome. The primer sequences are as follows: forward (upstream) primer: CCCACAGCATGGCAAAGAAC; reverse (downstream) primer: CTGGCGGCATATGCACTTCT; probe: FAM-ACGTACGTCCATACCACAG-MGB.

### For Brilliant III Ultra-Fast qPCR master mix:

**1x reaction**

- 10.0 µL of 2X master mix
- 0.3 µL of experimental probe (final concentration 150 nM)*
- 0.6 µL of upstream primer (final concentration 300 nM)*
- 0.6 µL of downstream primer (final concentration 300 nM)*
- 0.3 µL of the diluted reference dye (final concentration of 300nM)*
- 3.2 µL of nuclease-free water
- 5.0 µL of cDNA
- 20.0 µL total per well

### For Brilliant III Ultra-Fast SYBR Green qPCR master mix:

**1x reaction**

- 10.0 µL of 2X SYBR Green qPCR master mix
- 0.6 µL of upstream primer (final concentration 300 nM)*
- 0.6 µL of downstream primer (final concentration 300 nM)*
- 0.3 µL of the diluted reference dye (final concentration 30nM)*
- 3.5 µL of nuclease-free water
- 5.0 µL of cDNA
- 20.0 µL total per well

**Table 1.** Agilent Brilliant III qPCR SYBR Green and probe-based reagent kit PCR cycling protocols on the QuantStudio 7 Flex Real-Time PCR system.

| QuantStudio 7 Flex Real-Time PCR System | Brilliant III Ultra-Fast SYBR Green Kit (P/N 600882) | | | Brilliant III Ultra-Fast qPCR Probe Kit (P/N 600880) | | |
|---|---|---|---|---|---|---|
| | Cycles | Temperature | Duration of Cycle | | Temperature | Duration of Cycle |
| PCR Cycling | 1x | 95 °C | 3 minutes | 1x | 95 °C | 3 minutes |
| | 40x | 95 °C | 5 seconds | 40x | 95 °C | 5 seconds |
| | | 60 °C | 10 seconds | | 60 °C | 10 seconds |
| Melt Curve Analysis | Continuous | 95 °C | 15 seconds | | | |
| | | 60 °C | 1 minute | | | |
| | | 95 °C | 1 second | | | |

** These concentrations have been optimized by the end user for their specific use.*

# Results

### ABI QuantStudio amplification and detection of ONNV using the Brilliant III Ultra-Fast qPCR and SYBR Green qPCR master mixes.

The cDNA samples were analyzed across six 10-fold serial dilutions down to 50 copies. Brilliant III qPCR kits capable of detecting down to one copy of viral genome per sample.[3] This analysis covers an extensive detection range in the two qPCR assays using the different Brilliant III Ultra-Fast master mixes.

The linear fit of the standards was excellent, with $R^2$ value for the Brilliant III Ultra-Fast SYBR Green qPCR master mix equal to 0.997, and the $R^2$ value for the Brilliant III Ultra-Fast qPCR master mix equal to 0.983. These values demonstrate exceptional linearity and high sensitivity of the two assay reagents. In addition, both master mixes showed a high efficiency of on target amplification, 94.294% and 79.419% respectively.

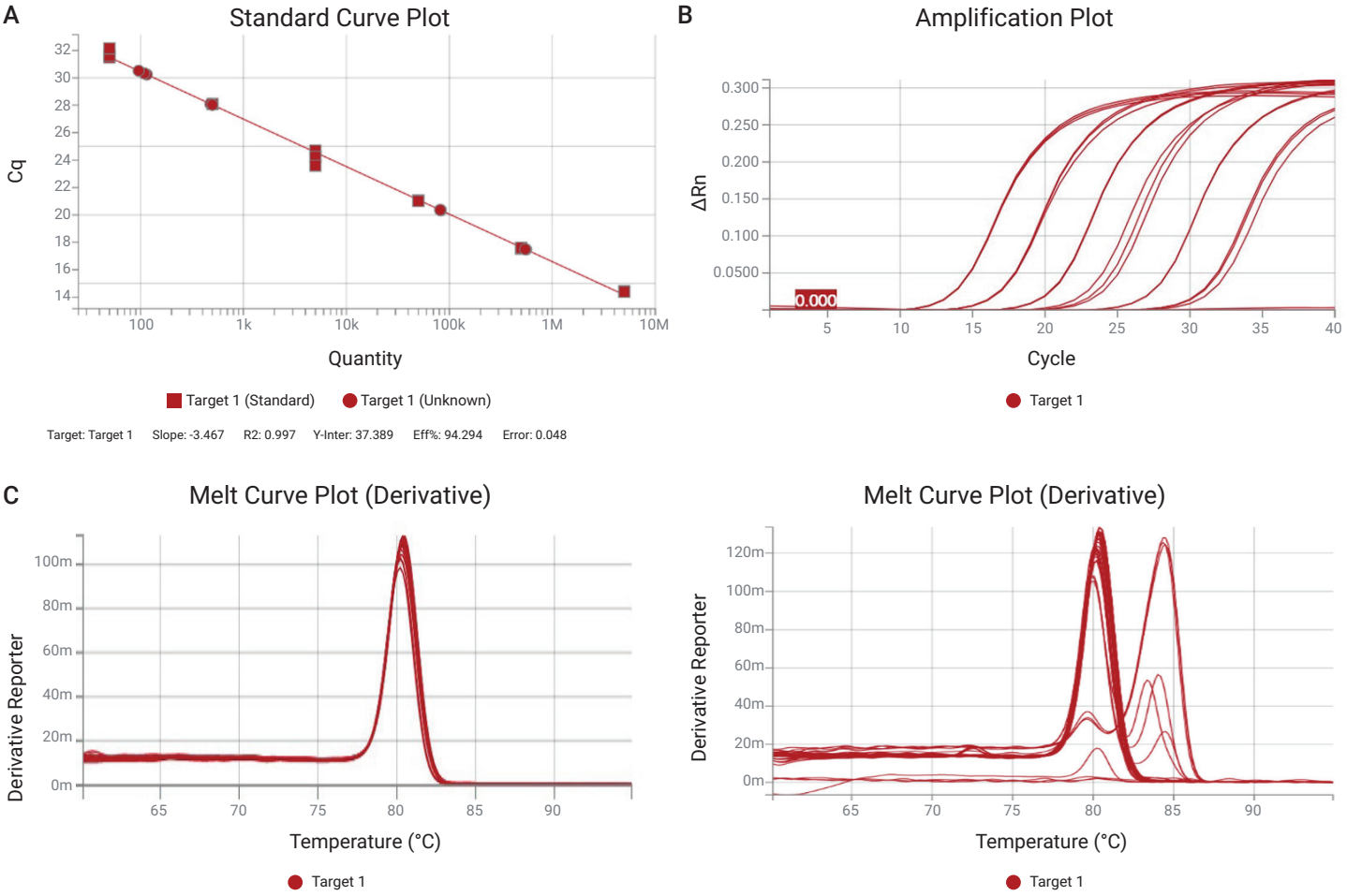


**Figure 1. Agilent Brilliant III Ultra-Fast SYBR Green qPCR master mix.** A) Standard Curve Plot. All four unknown sample concentrations fit the linear curve of the standards. B) Amplification Plot. Each triplicate standard concentration has a discrete amplification curve. C) Melting Curve Plots. Left, standards. Right, samples. The two peaks in the dissociation curve plot indicate target and nonspecific amplification/primer dimerization. The first peak represents the dissociation curves for the standards. The second peak is approximately 5 °C higher than the standard curve and represents a secondary target in the unknown samples. Changing the annealing temperature, using HPLC purified primers, or selecting a different set of unique primers can generate melt curves with one peak only.

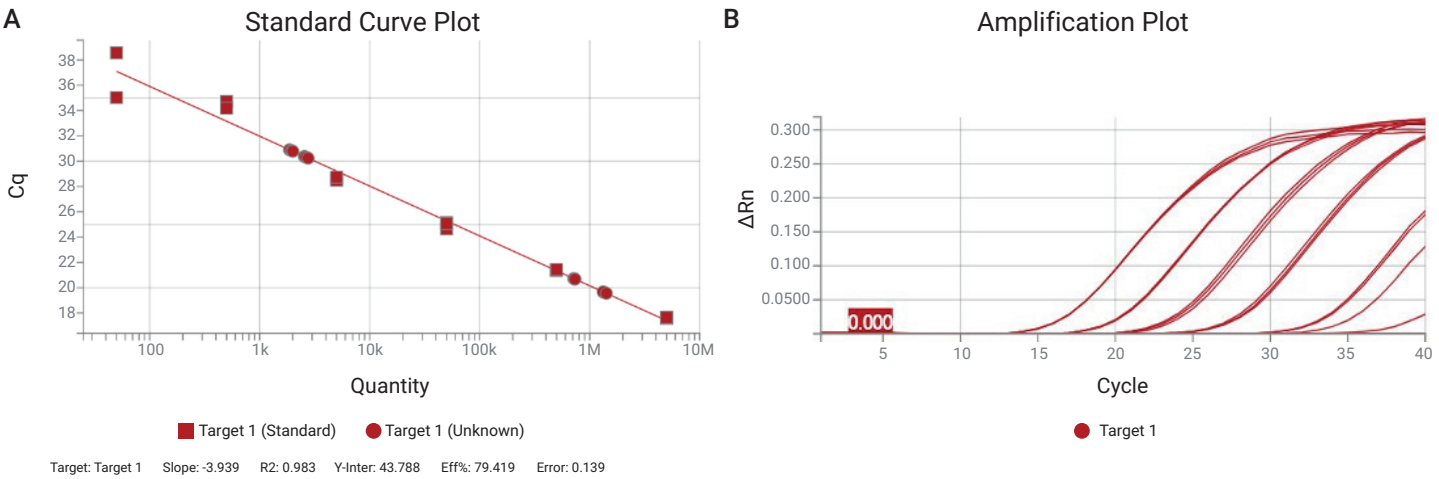


**Figure 2. Agilent Brilliant III Ultra-Fast qPCR master mix.** A) Standard Curve Plot. All four unknown sample concentrations fit to the linear curve of the standards. B) Amplification Plot. Each triplicate standard concentration has a discrete amplification curve.

## Conclusion

Given the need for continuous analysis of gene expression and quantification of various nucleic acid sequences within research settings and non-diagnostic environments, having options for a robust and accurate enzyme is important. Demonstrated in this application note, the Agilent Brilliant III Ultra-Fast qPCR master mixes, when coupled with the ABI QuantStudio instruments, can accurately detect various pathogenic viral genomes, including ONNV, in a reliable and reproducible manner. This detection can be achieved using either a probe-based or SYBR Green master mix with a melt curve method.

## References


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2. *Brilliant III Ultra-Fast SYBR Green qPCR Master Mix Instruction Manual*. https://www.agilent.com/cs/library/usermanuals/public/600882.pdf
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### For additional background information


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