Promega

Product Application

Reliaprep™ isolation of viral DNA from raw and processed food products containing pork

Isolate viral DNA from raw and processed pork products using the Reliaprep™ purification protocol

Kit: Reliaprep™ Blood gDNA Miniprep System (Cat. #A5081)

Analyses: GoTaq® Probe qPCR (Cat. #A6102)

Sample Type(s): Raw pork shoulder meat and processed pork products (pork jerky and Chinese sausage)

Input: 100mg of pork sample

This protocol was developed by Promega Applications Scientists and is intended for research use only.

The user is responsible for determining its suitability in the user's application.

Further information can be found in Technical Manual #TM330, available at: **www.promega.com/protocols**

or by e-mailing technical services at techserv@promega.com

Materials Required:

- Reliaprep™ Blood gDNA kit (Cat. #A5081)
- Cell Lysis Buffer (CLD, Cat. #A1731)
- Proteinase K solution (Cat. #MC5008)
- 100% Isopropanol
- Thermomixer
- Microcentrifuge
- 1.5ml tube

Protocol:

1. Add 300µl of CLD and 40µl of Proteinase K to each 1.5ml tube containing 100mg of pork sample resuspended in 300µl PBS.
2. Vortex vigorously on maximum speed to for 10 seconds to mix.
3. Incubate samples at room temperature for 10 minutes.
4. Place samples in a standard heat block at 56°C for additional 10 minutes.
5. Centrifuge for 10 minutes at 16,000 *x g* to separate any solid or oils.
6. Add 600µl of 100% isopropanol to the cleared sample supernatant. Vortex for 10 seconds to mix.
7. Load lysate to binding column/collection tube assembly.
8. Centrifuge at maximum speed for 1 minute.
9. Add 500µl CWD and centrifuge at maximum speed for 2 minutes. Discard the flow-through.
10. Repeat step 9 twice for a total of 3 washes.
11. Place column in a clean Elution Tube. Add 100µl of Nuclease-Free Water to the column.
12. Centrifuge at maximum speed for 1 minute.

Result

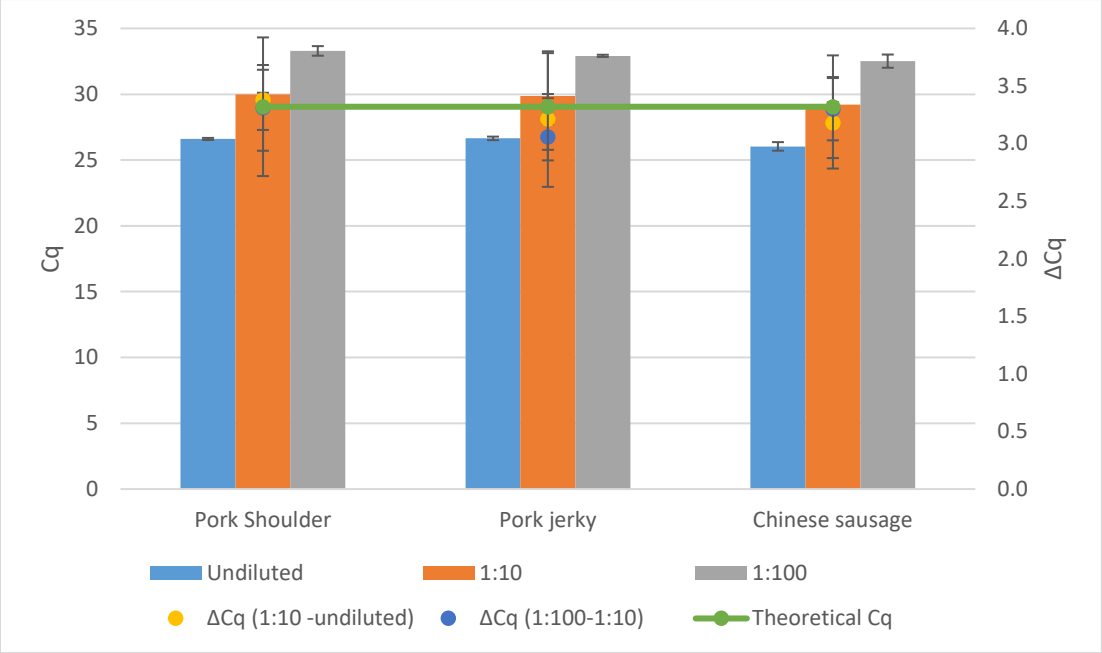

Figure 1. qPCR amplification of DNA isolated from different pork products spiked with Lambda phage. $5x10^5$ PFU of lambda phage was diluted at a ratio of 1:10 or 1:100 and spiked into three different pork containing samples. Viral DNA was detected using Real-Time PCR GoTaq® probe qPCR system (Cat. #A6101) with lambda phage specific primers and probe. Cq values of lambda viral DNA recovered from different pork containing products was shown. There is approximately a 3.3 Cq difference between the undiluted and diluted virus spiked samples, indicating the purification efficiency was linear. Undiluted DNA eluate prepared from 100mg raw pork shoulder was diluted at 1:10 and amplified with lambda phage specific primers and probe to examine the amplification efficiency. Average values from three experiments ± STD are shown.