Promega

Product Application

Manual DNA Extraction from Food Samples

Manual DNA purification from food samples using the ReliaPrep™ Blood gDNA Miniprep System followed by amplification using GoTaq® qPCR Master Mix.

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

Analyses: Quantitation by absorbance and with fluorescent dye; qPCR amplification

Sample Type(s): Ground seed (corn and wheat) and meat samples (pork and beef)

Input: 50–100mg

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

Users are responsible for determining suitability of the protocol for their application.

For further information, e-mail technical services at: techserv@promega.com

Materials Required:

- ReliaPrep™ Blood gDNA Miniprep System (Cat.# A5081)
- CTAB Buffer (Cat.# MC1411)
- RNase A Solution (Cat.# A7973)
- Proteinase K (PK) Solution (Part# A505C)
- heat block
- microcentrifuge
- 100% isopropanol
- Elution Buffer (Cat.# A8281)
- GoTaq® qPCR Master Mix (Cat.# A6002)
- QuantiFluor® ONE dsDNA System (Cat.# E4871)

Protocol:

1. For seed samples: Add 1ml of CTAB Buffer, 20µl of RNase A Solution and 40µl of Proteinase K (PK) Solution to each tube containing up to 100mg of sample. Vortex until completely suspended.
2. For meat samples: Add 600µl of CTAB Buffer, 2µl of RNase Solution and 30µl of Proteinase K (PK) Solution to each tube containing up to 100mg of sample. Vortex until completely suspended.
3. Place samples in a heat block at 65°C (seed samples) or 60°C (meat samples) for 30 minutes. After incubation, vortex to mix. Centrifuge samples for 10 minutes at ≥16,000 × *g*.
4. Transfer 300µl of clear supernatant to a clean 1.5ml microcentrifuge tube.
5. Add 300µl of CLD Buffer to the cleared supernatant and mix. Add 600µl of 100% isopropanol and vortex.
6. Load 600µl of sample onto a ReliaPrep™ Binding Column placed in a collection tube. Centrifuge for 1 minute at maximum speed. Discard flowthrough.
7. Load the rest of the sample to the ReliaPrep™ Binding Column and centrifuge for 1 minute. Place Binding Column into a new collection tube.
8. Add 500µl of Column Wash Solution (CWD). Centrifuge for 2 minutes at maximum speed. Discard the flowthrough.
9. Repeat Step 7 twice for a total of three washes.
10. Place Binding Column in a labeled elution tube. Add 100µl of Elution Buffer to the Binding Column. Centrifuge for 1 minute at maximum speed. Discard the column and save eluate.

Results:

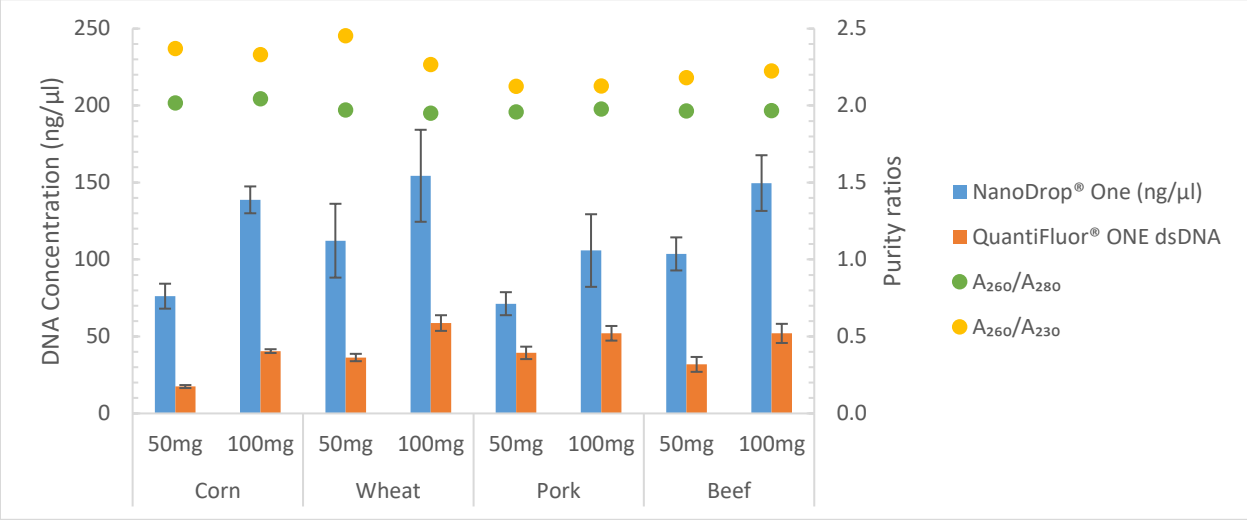

Figure 1. DNA concentration and purity ratios obtained for DNA extracted from 50mg or 100mg of food samples using the ReliaPrep™ Blood gDNA Miniprep System. DNA concentration and purity ratios were assessed by absorbance with NanoDrop® One Spectrophotometer and by using the QuantiFluor® ONE dsDNA System (Cat.# E4871). Error bars indicate standard deviation (N=3).

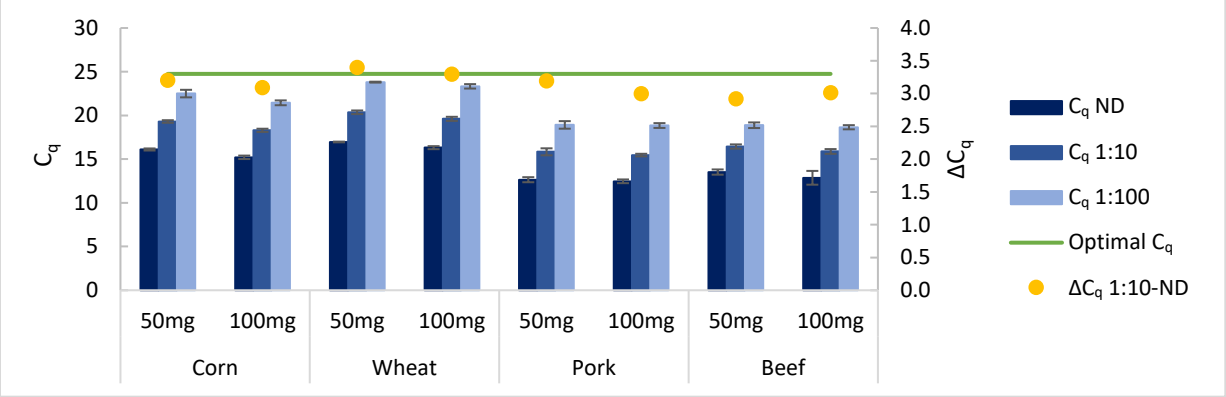

Figure 2. qPCR amplification results for DNA extracted from 50mg or 100mg of food samples using ReliaPrep™ Blood gDNA Miniprep System. C_q and ΔC_q values for 2µl of the eluted DNA amplified using GoTaq® qPCR Master Mix (Cat.# A6001) and universal plant primers (1) or pork and beef primers (2) in a final volume of 20µl (final concentration of primers 500nM). A ΔC_q value of 3.3 indicates no qPCR inhibitor compounds are present.

References:

1. Wang, J. *et al.* (2011) Universal endogenous gene controls for bisulphite conversion in analysis of plant DNA methylation. *Plant Methods* **7**, 39.
2. López-Andreo, M. *et al.* (2005) Identification and quantitation of species in complex DNA mixture by real-time polymerase chain reaction. *Anal. Biochem.* **339**, 73–82.