Promega

Product Application

Manual DNA extraction from food samples

DNA was successfully purified manually from food samples using ReliaPrep™ Blood gDNA Miniprep System.

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: 50mg to 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.

Further information can be found by e-mailing 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 (Cat. #A505C)
- Heat block
- Microcentrifuge
- Isopropanol 100%.
- 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 30min. After incubation, vortex to mix. Centrifuge samples for 10min at ≥16,000 × g.
4. Transfer 300µl of clear supernatant to a clean 1.5ml microtube.
5. Add 300µl of CLD Buffer to the cleared supernatant and mix. Add 600µl of 100% Isopropanol and vortex.
6. Load 600µl of sample to a ReliaPrep™ Binding column placed in a collection tube. Centrifuge for 1min at maximum speed. Discard flow through.
7. Load the rest of the sample to the ReliaPrep™ Binding column and spin for 1min more. Place Binding Column into a new collection tube.
8. Add 500µl of Colum Wash Solution (CWD). Spin 2min at maximum speed. Discard the flow through.
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. Spin 1min at maximum speed. Discard the column and save eluate.

Results:

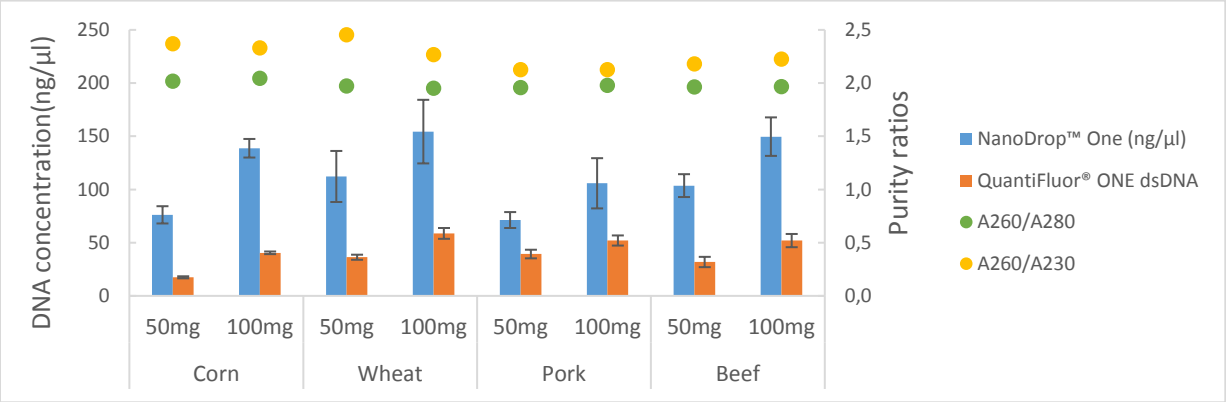

Figure 1. DNA concentration and purity ratios obtained for DNA extracted from 50 or 100mg of food samples using the ReliaPrep™ Blood gDNA Miniprep System. DNA concentration and purity ratios were assessed by absorbance with NanoDrop™ One Spectrophotometer and by using the QuantiFluor® ONE dsDNA System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

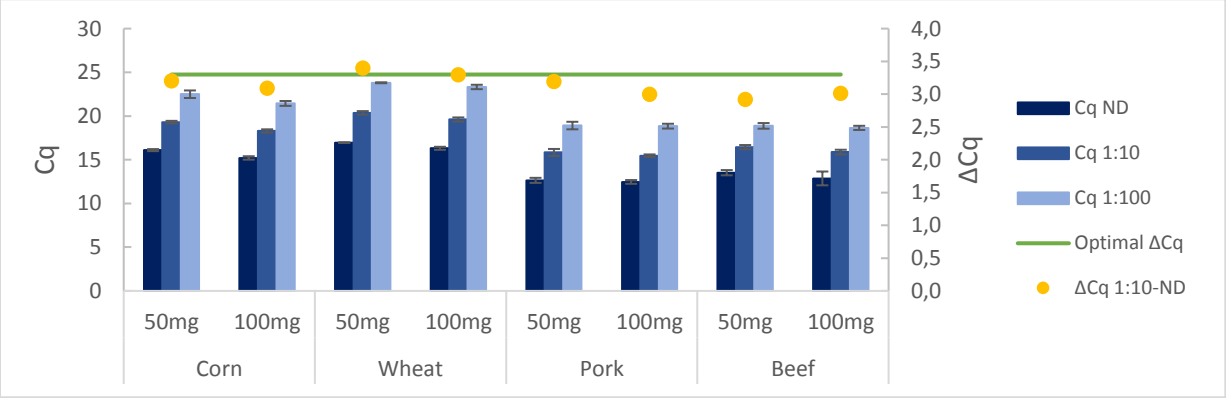

Figure 2. qPCR amplification results for DNA extracted from 50 or 100mg of food samples using ReliaPrep™ Blood gDNA Miniprep System. Cq and ΔCq values for 2µl of the eluted DNA amplified using GoTaq® qPCR Master Mix (Cat. #A6001) and universal plant primers[1] or pork and beef primers[2] in a final volume of 20µl (final concentration of primers 500nM). A ΔCq value of 3.3 indicates not presence of qPCR inhibitors compounds.

Reference:

1. Wang et al.: *Universal endogenous gene controls for bisulphite conversion in analysis of plant DNA methylation.* Plan Methods 2011 7:39.
2. López-Andreo et al: *Identification and quantitation of species in complex DNA mixture by real-time polymerase chain reaction.* Analytical Biochemistry, 73-82, 2005.