Promega

Product Application

DNA extraction from samples containing gelatin using ReliaPrep™ Blood gDNA Miniprep System

DNA was manually purified from samples containing gelatin using ReliaPrep™ Blood gDNA Miniprep System. Extracted DNA was suitable for speciation applications.

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

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

Sample Type(s): Aspic, gelatin sheet, capsule and candy.

Input: Up 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 (PK) solution (Cat. #MC5005)
- Elution Buffer (Cat. #A8281)
- Isopropanol 100%.
- Frozen mortar and pestle
- Heat block

Protocol:

1. Grind sample with a frozen mortar and pestle.
2. Add 600µl of CTAB Buffer, 2µl of RNase A Solution and 30µl of Proteinase K (PK) Solution to each tube containing up to 100mg of sample. Tap and vigorously vortex tubes.
3. Place in a heat block at 60°C for 30 min with shaking at 600pm. After incubation, vortex tubes with lysate to mix thoroughly.
4. Centrifuge the tubes 10 min at ≥16,000 × g to separate any solid and oils.
5. Transfer 300µl of cleared lysate to a clean 1.5ml microtube.
6. Add 300µl of CLD Buffer (Cell Lysis Buffer) to supernatant. Add 600µl of 100% of Isopropanol. Vortex to mix.
7. Load 600µl of sample to a ReliaPrep™ Binding column placed in a collection tube. Centrifuge for 1 min at maximum speed. Discard flow through.
8. Load the rest of sample to the ReliaPrep™ Binding column and spin for 1 min more. Place the ReliaPrep™ Binding Column into a new collection tube.
9. Add 500µl of Column Wash Solution (CWD). Spin 2 min at maximum speed. Discard the flow through.
10. Repeat step 9 twice, for a total of three washes.
11. Place the ReliaPrep™ Binding Column in a labeled elution tube. Add 50µl of Elution Buffer to the ReliaPrep™ Binding Column. Spin 1 min at maximum speed. Discard the column and save eluate.

Results

Table 1. DNA concentration and purity ratios of DNA extracted from 100mg of samples containing gelatin using ReliaPrep™ Blood gDNA Miniprep System (Cat. #A5081). DNA concentration and purity ratios were assessed by absorbance on the NanoDrop™ One Spectrophotometer and using the QuantiFluor® ssDNA System (Cat. #E3190).

Sample	NanoDrop™ (ng/µl)	QuantiFluor® ssDNA (ng/µl)
Aspic	132.38 ± 4.69	71.40 ± 2.61
Gelatin sheet	7.58 ± 2.63	0.36 ± 0.03
Capsule	22.26 ± 1.83	20.52 ± 1.88
Candy	7.21 ± 0.85	0.63 ± 0.38

Table 2. qPCR amplification of DNA extracted from 100mg of samples containing gelatin using ReliaPrep™ Blood gDNA Miniprep System (Cat. #A5081). 5µl of extracted DNA at 10ng/µl (7.58 and 7.21ng/µl for gelatin sheet and candy respectively) was amplified using RapidFinder™ Pork ID (Thermo Fisher ref. A24392) and RapidFinder™ Beef ID (Thermo Fisher ref. A24391).
Results: + (amplification), - (no amplification).

Sample	RapidFinder™ Pork ID kit	RapidFinder™ Beef ID kit	Expected origin of gelatin
Aspic	+	-	Pork
Gelatin sheet	+	-	Pork
Capsule	-	+	Unknown
Candy	+	-	Unknown