Promega

Product Application

DNA Extraction From Samples Containing Gelatin Using the ReliaPrep™ Blood gDNA Miniprep System

DNA was manually purified from samples containing gelatin using the 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 K (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 the tubes.
3. Place tubes in a heat block at 60°C for 30 minutes with shaking at 600rpm. After incubation, vortex tubes with lysate to mix thoroughly.
4. Centrifuge the tubes for 10 minutes at ≥16,000 × *g* to separate any solids 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% Isopropanol. Vortex to mix.
7. Load 600µl of sample to a ReliaPrep™ Binding Column placed in a collection tube. Centrifuge for 1 minute at maximum speed. Discard flowthrough.
8. Load the rest of the sample to the ReliaPrep™ Binding column and spin for 1 minute more. Place the ReliaPrep™ Binding Column into a new collection tube.
9. Add 500µl of Column Wash Solution (CWD). Spin for 2 minutes at maximum speed. Discard the flowthrough.
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 for 1 minute at maximum speed. Discard the column and save eluate.

Results:

Sample	NanoDrop™ ONE (ng/µl)	QuantiFluor® ssDNA System (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 1. 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	RapidFinder™ Pork ID Kit	RapidFinder™ Beef ID Kit	Expected Origin of Gelatin
Aspic	+	–	Pork
Gelatin Sheet	+	–	Pork
Capsule	–	+	Unknown
Candy	+	–	Unknown

+ Amplification – No Amplification

Table 2. qPCR amplification of DNA extracted from 100mg of samples containing gelatin using ReliaPrep™ Blood gDNA Miniprep System (Cat.# A5081). Five microliters 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).