Workflow for DNA purification from tough specimens

Dennis Mertens,[1] Noel Anderson,[2] Gabriele Christoffel,[1] and Friederike Wilmer[1]

[1] QIAGEN GmbH, Hilden, Germany; [2] SPEX CertiPrep Ltd., Stanmore, Middlesex, United Kingdom

Overview

We present a workflow for DNA purification from tough specimens (Figure 1) which we tested with teeth, bone, tail, and skin. Tissues were ground into powder using the 6770 Freezer/Mill, and genomic DNA was purified using a QIAGEN® spin-column kit on the QIAcube®. Analysis of the purified DNA revealed high yields of DNA that was of excellent quality. The DNA was amplified in real-time PCR, and the specificity of the PCR products was analyzed using the QIAxcel, a multicapillary electrophoresis system. This workflow is intended as a guideline for easy tissue disruption, especially when handling very tough material.

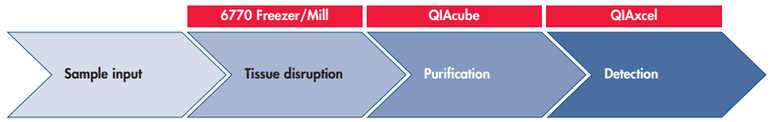

Figure 1. From biological samples to high-quality DNA. Workflow for DNA purification from tough specimens.

Introduction

Effective methods for tissue disruption and subsequent DNA purification and detection are becoming increasingly important in cases where specimens are available only in limited amounts. DNA purification from bones and teeth of crime victims or war casualties is essential if medical and dental records are not available or do not provide the required information. These tough samples, especially when fresh, are difficult to disrupt. With our workflow, grinding of bones, teeth, and other tough tissues under cryogenic conditions and subsequent DNA purification yields high-quality DNA that performs well in downstream molecular analysis.

Methods and materials

Tissue disruption

Various tough animal tissues (0.5 g) were frozen in liquid nitrogen and ground in a 6770 Freezer/Mill (SPEX CertiPrep) for 5–20 seconds at –193°C (Figure 2). The resulting powder (20 mg) was digested with proteinase K at 56°C for 1 hour.

DNA purification

Genomic DNA was purified from 20 mg tissue powder using the DNeasy® Blood & Tissue Kit, and eluted in 200 µl RNase-free water. Purification was either fully automated on the QIAcube or performed manually.

Quantitative, real-time PCR

Reactions (25 µl) were run on the ABI PRISM® 7700 using the QuantiTect® Probe PCR Kit and primers and a TaqMan® probe specific for the β-actin gene.

Capillary gel electrophoresis

To confirm the specificity of the real-time PCR, the final PCR products were analyzed by capillary gel electrophoresis using the QIAxcel. Samples were automatically loaded onto individual capillaries, and voltage was applied to separate DNA molecules (Figure 3).

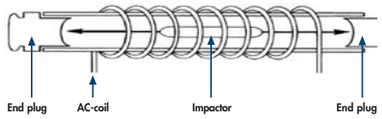

Figure 2. Tissue disruption using the 6770 Freezer/Mill. A magnetic coil rapidly shuttles an impactor back and forth to pulverize samples in liquid nitrogen against the end plugs.

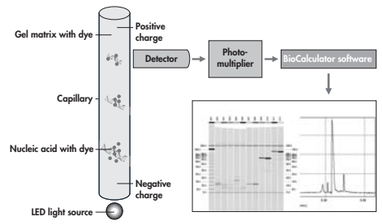

Figure 3. Capillary gel electrophoresis using the QIAxcel. Nucleic acid molecules are size separated by applying a current to a gel-filled capillary, and detected as they migrate toward the positively charged terminus. The signal data pass through a photomultiplier and are converted to an electropherogram and gel image by the BioCalculator software.

Results — tissue disruption and DNA purification

Tissue disruption using the 6770 Freezer/Mill (Figure 4A) followed by DNA purification using the QIAcube (Figure 4B) yielded high-quality DNA. Agarose gel analysis showed that DNA fragments greater than 20 kb were obtained from teeth, tail, bone, and skin (Figure 5).

Purification of genomic DNA with the QIAcube resulted in similar yields as those achieved with manual purification, demonstrating the reliability of the automated procedure (Table 1).

* DNA was quantified by measuring absorbance at 260 nm.

Table 1. Genomic DNA yields achieved using the QIAcube or manual purification*

Tissue	DNA (µg)		DNA (ng/µl)	
	Manual	QIAcube	Manual	QIAcube
Hip bone (rat)	24.5	27.5	122.4	137.4
Thigh bone (rat)	23.2	20.6	116.2	103.1
Skin, shaved (rat)	6.2	3.8	31.0	18.9
Tail (mouse)	8.4	6.3	42.0	31.6
Teeth (pig)	15.2	13.9	75.9	69.6
Teeth (dog)	5.3	4.9	26.7	24.6

Figure 4. Tissue disruption and genomic DNA purification. A The 6770 Freezer/Mill is designed to release DNA from tough biological materials, such as teeth, bone, and skin. B The QIAcube automates nucleic acid purification using QIAGEN spin-column kits, standardizing all steps from sample lysis to nucleic acid elution. Up to 12 samples are processed per run.

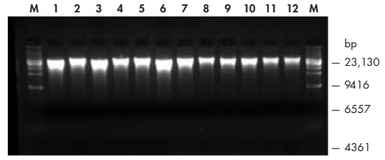

Figure 5. Purification of high-quality DNA. Genomic DNA purified from various animal tissues was analyzed on an 0.8% agarose gel in 1× TBE (7 µl from 200 µl eluate loaded per lane). **1–2**: dog teeth; **3–4**: pig teeth; **5–6**: mouse tail; **7–8**: rat thigh bone; **9–10**: rat hip bone; **11–12**: shaved rat skin; **M**: QIAGEN GelPilot Lambda *Hin*dIII Marker (odd numbers denote DNA purified using the QIAcube; even numbers denote DNA purified manually).

Results — quantitative, real-time PCR

Genomic DNA purified in our workflow was used as template in a real-time PCR assay for β-actin (Table 2). C_T values of less than 30 were obtained with all samples (Figure 6), demonstrating the suitability of the purified genomic DNA for molecular analysis.

Genomic DNA purified with the QIAcube provided similar C_T values as those achieved with manually purified genomic DNA (Figure 6). This confirms the reliability of automated purification with the QIAcube.

Table 2. Amount of DNA in 5 µl eluates used as template in real-time PCR

Tissue	Manual purified DNA (ng)	DNA purified using the QIAcube (ng)
Hip bone (rat)	611.9	686.9
Thigh bone (rat)	580.8	515.6
Skin, shaved (rat)	155.2	94.6
Tail (mouse)	210.0	157.9
Teeth (pig)	379.6	347.9
Teeth (dog)	133.5	123.2

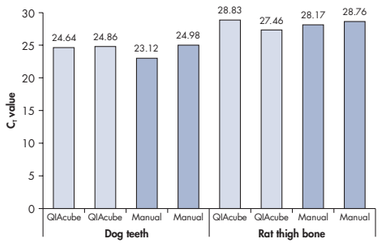

Figure 6. Reliable C_T values in real-time PCR. Genomic DNA purified from dog teeth and rat thigh bone were analyzed using a real-time PCR assay for β-actin. The C_T values obtained with DNA purified using the QIAcube were comparable to those obtained with DNA purified manually.

Results — capillary gel electrophoresis

The PCR products from the real-time PCR assays were analyzed on the QIAxcel. The resulting electropherogram and gel image revealed that all samples contained a single peak or band (Figures 7 and 8), demonstrating the specificity of the assays. Twelve samples (<0.1 µl each) were run in parallel on the QIAxcel in less than 10 minutes.

Figure 7. Electropherogram showing specific PCR product. Genomic DNA purified from dog teeth was used as template in a real-time PCR assay for β-actin. The final PCR product was analyzed on the QIAxcel using a QIAxcel DNA High Resolution Gel Cartridge and AM 0112 Alignment Marker 15 bp/5 kb. Detection was carried out using the OM500 method (5 kV per 500 seconds separation time). The central peak indicates the specific PCR product. The left- and right-hand peaks indicate the alignment markers.

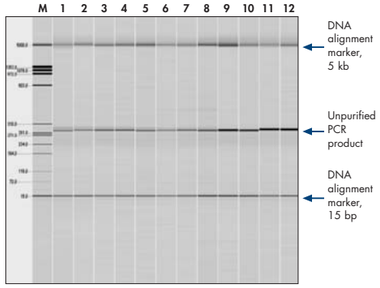

Figure 8. Gel image showing specific PCR products. Genomic DNA purified from various animal tissues was used as template in a real-time PCR assay for β-actin. The final PCR products were analyzed on the QIAxcel as described in Figure 7. **1–2**: shaved rat skin; **3–4**: rat hip bone; **5–6**: rat thigh bone; **7–8**: mouse tail; **9–10**: pig teeth; **11–12**: dog teeth; **M**: Phi X 174 *Hae*III marker (odd numbers denote DNA purified using the QIAcube; even numbers denote DNA purified manually).

Conclusions

- The combination of the 6770 Freezer/Mill and the QIAcube provides an effective, reliable method for tissue disruption and DNA purification at low-throughputs of 1–12 samples per run.
- Fully automated DNA purification with the QIAcube delivers the same DNA yields as manual purification.
- Our workflow facilitates disruption of tough tissues and yields high-quality DNA suitable for downstream molecular analyses.
- DNA purified using our workflow performs well in real-time PCR, showing low C_T values.
- The QIAxcel allows fast analysis of unpurified PCR products and at a high resolution.

Optimized protocols for sample disruption prior to manual or automated purification of nucleic acids are available from QIAGEN: www.qiagen.com .

More information about the 6770 Freezer/Mill can be found at: www.spexcertiprep.co.uk .

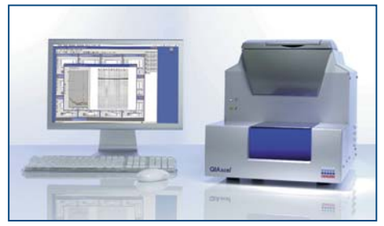

Figure 9. Capillary gel electrophoresis. The QIAxcel with BioCalculator analysis software.

The QIAcube and QIAxcel are intended for research applications. No claim or representation is intended for their use to provide information for the diagnosis, prevention, or treatment of a disease.

The DNeasy Blood & Tissue Kit, QuantiTect Kits, and QIAxcel Kits are intended for research use. No claim or representation is intended to provide information for the diagnosis, prevention, or treatment of a disease.

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