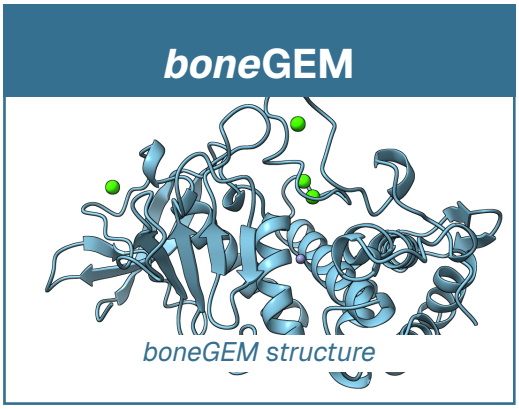
Exymes



Extracting High-Quality DNA from Bone using Exymes *bone*GEM

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Introduction

Bone is an important reservoir for DNA as one of the last remaining tissues available for the identification of remains. But bone presents complex chemical and structural challenges for DNA extraction and despite the DNA's longevity, conditions to which the bone is exposed and the length of time in these conditions greatly impacts yield. Any loss of DNA during the extraction process can therefore greatly impact outcome.

This application note describes the use of Exymes' *bone*GEM chemistry to successfully extract DNA from pig bones, resulting in high molecular weight DNA directly compatible with PCR. BoneGEM is an alternative to current methods that requires less overall processing time, no harsh chemicals or damaging pipetting steps that share DNA unnecessarily and lead to loss of material. This extraction protocol and demonstration DNA quality are described below.

Method

Bone Sampling

Pig femurs were first prepared by removing any soft tissue followed by freeze drying.

Once cleaned, bone powder was created using a Dremel® rotary tool with a 1/8" engraver attachment. Both the attachment and the bone were placed on ice prior to use. Bone powder was collected by scraping the bone with the engraver attachment at low speed, ensuring neither the sample nor attachment overheated (fig. 1). Once collected the powder was stored at -20°C.

DNA Extraction

Extraction of DNA was performed using the Exymes *bone*GEM chemistry. Approximately 10 mg of bone powder was added to a 0.2 ml PCR tube and suspended in 100 µl of *bone*GEM extraction mix (10 µl Orange+ Buffer, 2 µl *forensic*GEM and 88 µl of water). The powder was mixed by briefly vortexing and allowed to equilibrate at room temperature for 30 minutes with an additional vortex halfway through the incubation.

At completion of incubation the mix was place in a thermal cycler for DNA extraction. This was performed by incubating the samples at 75°C for 20 minutes, with a brief vortex performed 10 minutes into the 75°C incubation. The samples were then incubated at 95°C to inactive the forensicGEM enzyme.

Figure 1. Preparation of bone powder from pig femur showing the Dremel® tool, fragment of femur, and resulting bone powder.

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Visualisation of Extracted DNA and end-point PCR

Visualisation of extracted DNA was performed by agarose gel electrophoresis. DNA fragment length was assessed by comparing a neat sample to one mechanically sheared. DNA was sheared using a tuberculin syringe and needle by repeated sample aspiration.

Unsheared extracted DNA was also amplified using primers targeting porcine 16S mitochondrial DNA in Sus scrofa. These were amplified using the MyTaq HS reagents (Meridian Bioscience, USA). Each reaction mix contained 10 µl 1× MyTaq HS mix, 0.25 µM of each primer, and 2 µl of a 1:10 dilution of the extracted DNA.

The PCR was performed by with an initial denaturation temperature of 95°C for 2 minutes, and 40 cycles of 95 °C for 5 seconds and 58°C for 10 seconds. Samples were diluted 1:50 and visualised on an 1% agarose gel. DNA extracted from bone was compared to a control extraction from fresh pork muscle tissue.

Results

Exymes DNA Bone extract as a direct PCR template

The Bone DNA was amplified using primers that targeted the porcine mitochondrial 16S sequence. The results in Figure 2 demonstrate successful direct amplification from the bone extract compared to the positive control

DNA Fragment Size

DNA Fragment Length was assessed by running the extracted DNA from bone with and without manually shearing (figure 3) on an agarose gel. The gentle Exymes extraction chemistry yielded high length DNA fragments that were prevented by their size from migrating into the agarose gel, as demonstrated following manually shearing of the DNA.

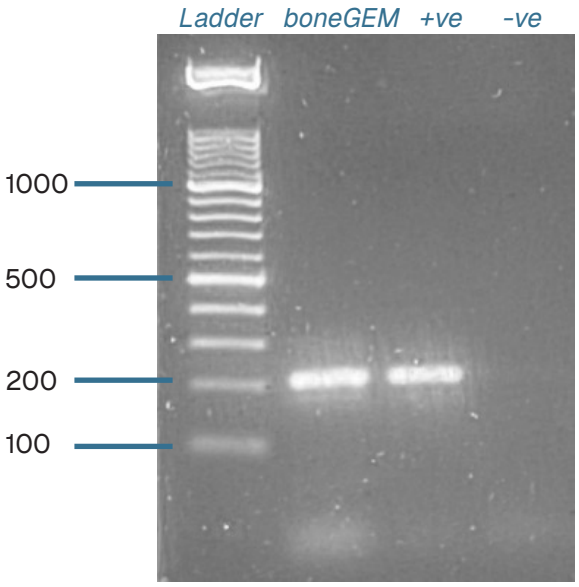

Figure 2. Successful amplification of DNA using primers targeting the 16S mitochondrial sequence. Lanes show DNA ladder, DNA amplified from bone extracted using BoneGEM, a positive control amplification, and a no template control.

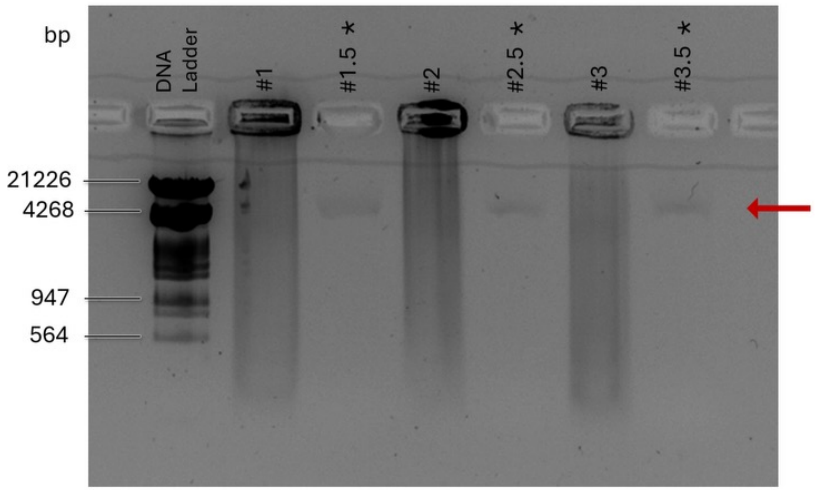

Figure 2. Gel image comparing three DNA extractions with and without manually shearing the DNA. Sample 1, 2, and 3 show the intact DNA that did not migrate through the gel. Samples 1.5, 2.5, and 3.5, denoted with an asterisk, were manually sheared. The red arrow shows the presence of a band following shearing of the DNA.

Conclusion

The BoneGEM protocol showed successful extraction of DNA from 10mg bone tissue processed as a powder from freeze dried bone.

BoneGEM provides a quick protocol that gives results with minimal handling or liquid transfer steps. From generating bone powder from a cleaned sample to PCR answer, a typical protocol takes around 2 – 3 hours. As shown by these results, the DNA is of high molecular weight and compatible with PCR.