DNA Extraction from Bone Fragments without Pulverization

Current methods for the extraction of DNA from bone fragments often include a pulverization step due to the hardness of the sample [1]. Pulverization is tedious and time-consuming, is an open process and thus inherently unsafe, and offers an increased risk of cross-contamination with other samples because the pulverizing equipment is re-used. Subsequent to pulverization, current extraction methods often require such complex procedures as decalcification, phenol-chloroform extraction, the use of chaotropic agents, and silica beads. In an effort to increase the safety, speed, simplicity, and efficiency of DNA extraction from bone fragments, Pressure BioSciences, Inc. (PBI) has developed a novel extraction system based on a new, patented technique called Pressure Cycling Technology (PCT). This PCT-based system eliminates the requirement for pulverization and the addition of most harsh chemicals currently required for DNA extraction from bone. Consequently, the PCT Sample Preparation System (PCT SPS) offers a safer, more rapid, simpler, and more efficient method for bone DNA extraction than other methods in use today.

Pressure Cycling Technology (PCT)

PCT uses alternating cycles of high and low pressures to induce cell lysis. Cell suspensions or tissues, such as bone, are placed in specially designed, single-use processing containers (PULSE Tubes) and are then subjected to alternating cycles of high (up to 35,000 PSI) and ambient pressures in a pressure-generating instrument (Barocycler). Maximum and minimum pressures, the time at each pressure level, and the number of cycles is defined using a programmable logic controller. The reaction chamber of the Barocycler instrument is temperature controlled using a peripheral circulating water bath. Safety features in the PCT System design significantly reduce risk of exposure to the researcher to pathogens and cross-contamination of samples [2].

Methods

Pig bone was used as a model system for process development. To eliminate the possibility of DNA from soft surface tissue, the bone was prepared for extraction by first removing tissue with a razor; the bone was shattered with a hammer to expose and remove internal soft tissue, and then washed with 10% bleach for 5 min followed by extensive washing with water. Bone fragments of approximately 250 mg were then made by shattering the bone with a hammer.

To optimize the release of DNA from bone fragments by PCT, a series of time course experiments were conducted by incubating bone fragments in various acids for 0 min to 12 hours at ambient pressure prior to PCT treatment. Optimum DNA release was obtained with a 60 min incubation in 1% acetic acid (pH 4.8), followed by PCT treatment (see Figure 2). Negative controls were incubated in buffer, but not exposed to PCT.

DNA released from PCT-processed bone was compared to DNA released from bone that had been first chilled in liquid nitrogen and subsequently pulverized using a standard tissue pulverizer (BioSpect Products, Bartlesville, OK). Each extract was purified using a QIAGEN DNeasy Kit (Germantown, MD) according to the manufacturer's instructions. The resulting DNA was amplified by PCR using primers for pig β-actin DNA. PCR products were analyzed using a 2100 BioAnalyzer (Agilent, Palo Alto, CA). Yield of DNA was determined semi-quantitatively by integrating the DNA PCR products obtained using the BioAnalyzer.

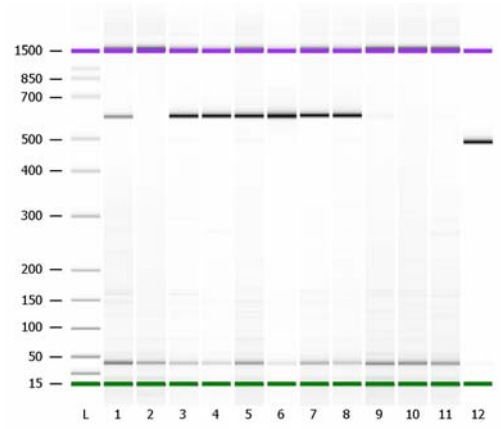

Figure 1. PCR products examined using the Agilent BioAnalyzer. Lanes 1-8 were from samples incubated with acetic acid (pH 4.9) and saturated EDTA for 60 min and followed by 10 cycles of PCT at 4°C. Lanes 9-11 were incubated with acetic acid for 1 hr but no PCT. Lanes 12 was a PCR positive control.

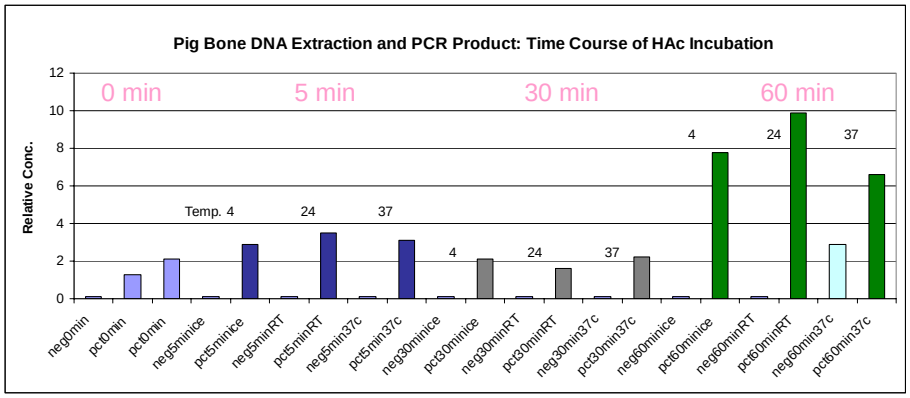

Figure 2. Optimization of the release of DNA from bone by PCT. Incubation ranged from 0, 5, 30 and 60 min. Temperatures were set at 4°, 24° or 37°C. Relative concentration was obtained based on the PCR products analyzed using the Agilent BioAnalyzer.

Results and Discussion

Results indicate that PCT is essential for the release of DNA from bone if pulverization is not conducted (See Figures 1 and 2). DNA was detected only in those samples subjected to PCT regardless of the time and temperature of incubation, except for incubation for 60 min at 37°C (See Figure 2). These data indicate that sufficient decalcification may occur at these conditions to release some DNA without PCT; however, when the sample is subjected to the same conditions and also processed by PCT, additional DNA is released.

It should be noted that bone fragments that were PCT-treated appeared intact although DNA had been released. To verify that PCT extraction was complete, bone fragments were pulverized post-PCT treatment and were extracted using a DNeasy tissue kit. No additional DNA was detected by PCR amplification from these samples, suggesting that the majority of DNA had already been released by PCT. DNA released from bone fragments by PCT was similar in quantity and quality to DNA released by the standard pulverization method. In the experiment shown in Figure 1, 7 out of 8 bone fragment samples yielded β-actin PCR products.

The PCT Sample Preparation System offers the forensic scientist the ability to more rapidly extract DNA from bone for further analysis, as the PCT treatment can be typically accomplished in approximately 2-4 hrs as compared to the standard extraction method of 24 hrs or more. The PCT SPS eliminates the pulverization step, and the need for strong organic solvents, while providing the advantage of performing the DNA extraction in a closed system. In addition, the PULSE Tube can be used as a storage and transportation device, making it ideal for maintaining an unquestionable chain of custody from the crime scene to the laboratory. The PULSE Tube also reduces the risk of cross-contamination for the sample, while providing a significant increase in the level of safety for the user. The Barocycler automates the sample extraction process, which makes the PCT SPS safe, fast, efficient, and reproducible. Other advantages of the PCT SPS are shown in Table 1.

Table 1. Comparison of Conventional and PCT Extraction Methods for Bone Fragments

METHOD	CONVENTIONAL	PCT
Equipment Requirement	• Pulverizer • Liquid nitrogen	• Barocycler • PULSE Tubes
Typical Steps	• Collect bone fragments • Pulverizing • Incubation • DNA purification	• Collect bone fragments • Incubation • PCT process • DNA purification
Process Time	24 -32 hours	2 - 4 hours
Features	• Gold standard • Low set-up cost	• Time saving • Ease of use • Safety • Chain of Custody • Efficiency • Reproducibility

References

[1] Thomas, MG, and L. Jane Moore. (1997). *BioTechniques,* 22:402.

[2] Schumacher RT et al. (2002). *Am. Laboratory*, 34:38-43.

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