International Congress Series 1261 (2004) 553–555

www.ics-elsevier.com

Novel method of DNA extraction from bones assisted DNA identification of World Trade Center victims

T. Bille, R. Wingrove, M. Holland, C. Holland, C. Cave, J. Schumm*
The Staff of The Bode Technology Group

The Bode Technology Group, Research and Development, 7364 Steel Mill Drive, Springfield, VA 22150, USA

Abstract. DNA identification of many mass fatality victims of the 11 September 2001 attack on the World Trade Centers (WTC) in New York City required development of new analytical methods. Development of novel STR multiplex sets with improved performance using challenged DNA samples is described in an accompanying paper. Here we describe modifications and improvements to procedures used to extract DNA from bone fragments found at the site.

Keywords: World Trade Center; Bone; Short tandem repeat; PCR

1. Introduction

The WTC DNA Identification Project required an effective high-throughput method of DNA extraction from bones. Many of the WTC samples had been abused not only by the enormous force of the towers collapse, but also by the long-lasting fires and substantial exposure to water that lasted for many weeks afterward. The solution came in two phases. Phase I included implementation of more traditional DNA extraction methods re-designed to accommodate the high-throughput requirements. Phase II focused on protocol changes to improve DNA yield and lower retention of inhibiting substances.

2. Results and discussion

Phase I included adaptation to a rapid DNA extraction method for 12849 WTC bone fragments. Once tissue was removed from the surface of each bone fragment with a scalpel and the bone was cleaned with bleach, we employed a drill to remove a portion of the outer surface of the bone. This outer material was discarded as potential surface contamination. A clean drill bit was used to pulverize an inner portion of bone.

* Corresponding author. Tel: +1-703-644-1200x303; fax: +1-703-644-2319.
E-mail address: james.schumm@bodetech.com (J. Schumm).

0531-5131/
doi:10.1016/S0531-5131(03)01831-4

Table 1

Results of initial STR analysis (AmpFlSTR Profiler Plus and COfiler)	WTC victim remains (100% = 12,849)	AA Flight 587 victim remains (100% = 442)
Full STR Profile (13 loci)	27.2%	73.1%
High Partial STR Profile (7–12 loci)	17.4%	11.8%
Low Partial STR Profile (1–6 loci)	21.1%	7.2%
No STR Profiles (0 loci)	34.3%	7.0%

Approximately 25–50 mg of this inner powder was extracted in a 96-well format using a modification of the QIAamp® 96 DNA Blood Kit (QIAGEN, Valencia, CA). This extraction includes a lysis step in the presence of SDS and Proteinase K followed by the bind-wash-elute QIAGEN technology [1].

STR results using this method were incomplete due to a combination of poor yield and possible inhibition of amplification. The same method was employed on remains of victims of American Airlines flight 587 that occurred in New York during this period. The much higher success rate with these materials reassured us that the method itself was very effective, but that the sample quality was much more challenging with the WTC samples (Table 1).

Phase II incorporated modifications to the Phase I extraction procedure to provide higher sample yield and quality. Treatment of the bone powder with EDTA assisted decalcification of the bone. Then overnight cell lysis without proteases was followed by a 3-h Protease K digestion step. The digested material was then captured onto a QIAamp membrane, washed, and eluted. Additional details of the method may be found in Holland et al. [1]. These modifications allowed use of up to 150 mg bone powder for extraction and provided an extracted product that was more amenable to PCR amplification.

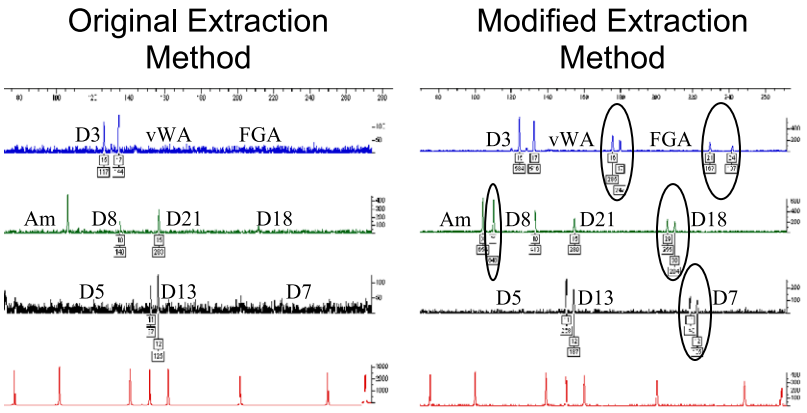

Fig. 1. Separate portions of one bone fragment sample were extracted using the original modified QIAamp® 96 DNA Blood Kit protocol or the modified extraction method. Products of each extraction were amplified with AmpFlSTR Profiler Plus. New alleles observed following amplification of DNA extracted with the new method are marked with ovals.

A total of 5335 samples that originally generated either Low Partial STR Profiles (1–6 loci in 13 attempted) or No STR Profiles were re-extracted with the improved method and re-amplified with the Profiler Plus and COfiler multiplex systems, respectively. Of these, 2.9% (i.e., 154) provided Full STR Profiles, 7.3% (i.e., 388) High Partial STR Profiles, 14.0% Low Partial STR Profiles, and 78.5% generated No STR Profiles. Thus, 542 new profiles were generated using the second extraction with the modified method. An example of an individual bone sample extracted with the two methods, respectively, and amplified with Profiler Plus is illustrated in Fig. 1.

In sum, high-throughput methods for extraction of DNA from bone fragments for STR analysis have been developed and then improved.

Reference

[1] M.M. Holland, C.A. Cave, C.A. Holland, T.W. Bille, Croatian Medical Journal 44 (3) (2003) 264–272.