G32 Bone Extraction Procedure for Nuclear DNA Analysis Used in World Trade Center Human Identification Project

Zoran M. Budimlija, MD, PhD, Bianca Nazzaruolo, MA, Sheila M. Estacio, BS, Mechthild K. Prinz, PhD, and Robert C. Shaler, PhD, Office of Chief Medical Examiner, Department of Forensic Biology, 520 First Avenue, New York, NY*

The goal of this presentation is to demonstrate a highly effective method to extract and purify amplifiable nuclear DNA from severely compromised bone samples

The described extraction strategy was established for human bones recovered from the World Trade Center mass disaster site after September 11, 2001. Morphologically, the samples were in different states of preservation - ranging from very good with preserved bone marrow, to semi-burned, and completely burned. The heat and friction forces at the disaster site had reduced many of the bones to small, severely damaged pieces. Each sample was individually assessed visually as to the amount of the bone tissue to be taken for the extraction procedure.

Osteocytes (mature bone cells), with their central, nuclear containing region are completely surrounded by the bone matrix. The bone matrix is permeated by an extensive and complex system of lacunae (cavities occupied by the cell bodies of osteocytes) and canaliculi (narrow channels that radiate from the lacunae). The predominant organic component is collagen, while the inorganic matrix is calcium phosphate in the form of hydroxyapatite, which accounts for about 75% of the bone mass. The philosophy of this protocol is to use as much bone tissue as needed, as determined by the quality of the sample, to obtain a sufficient amount of DNA, by “untrapping” the DNA containing osteocytes from compact and spongy bone matrix.

In order to minimize contamination with any external DNA each bone specimen was cleaned vigorously using a series of disposable scalpels and brushes. After sonication in a 5% Terg-a-zyme solution, the outer surface was sanded down (using a Dremel tool equipped with a disposable emery disk), until the outer surface was completely free of dirt and debris. This step was followed by an additional Terg-a-zyme and H_2O wash step; then the bone specimen was cut into approximately 0.5x0.5x0.5 cm pieces, frozen in liquid nitrogen, and ground in a MicroMill Grinder (Scienceware, Bel-Art Products, Pequannock, NJ) into a dust. The mill was cleaned vigorously after each sample.

Depending on the condition of the bone sample, the bone dust was divided into 50 ml conical tubes (2 g of bone dust per tube). 4g for good specimen, 6g, 8g, and 10g for more compromised samples. Each dust aliquot was incubated in 3 ml of organic incubation buffer (shaken at 56°C, overnight). The DNA was extracted using Phenol-chloroformisoamylalcohol (24:24:1) in 1.5mL Phase Lock Gel tubes (Eppendorf, Hamburg, Germany) and Microcon 100 microconcentrators (Amicon, Inc., Beverly, MA). The extracts were further subdivided into smaller aliquots. As the final step all aliquots were recombined and concentrated further using Microcon 100 vials. Samples were quantified using QuantiBlot (ABI, Foster City, CA), and amplified with the PowerPlex 16 (Promega, Madison, WI) multiplex. The resulting DNA profiles were analyzed and interpreted following the standard procedure established for the WTC Human Identification Project.

Usable STR profiles with a sufficient number of loci were obtained in ~ 75% of cases. Approximately 50% of all analyzed cases even had more than 13 loci typed. The procedure has been successful for nuclear DNA based identifications even if the bones were in an extremely poor condition. The approach of dividing samples into smaller aliquots and then recombining the extracts later allows for the processing of a large amount of bone matrix. This also allows the use of the Eppendorf Phase Lock Gel technology while avoiding clogged Microcon membranes.

Nuclear DNA, Bones, Mass Disaster

 ** Presenting Author*