B110 The Recovery of the Mitochondrial Genome From Cremated Human Remains

Maureen Hickman, MS, Western Carolina University, 111 Memorial Drive, NSB 231, Cullowhee, NC 28723; Mark Wilson, PhD, Battelle, 2001 Jefferson Davis Highway, Ste 1203, Arlington, VA 22202; and Kelly Grisedale, PhD, Western Carolina University, 111 Memorial Drive, NSB 231, Cullowhee, NC 28723*

After attending this presentation, attendees will better understand how mitochondrial DNA (mtDNA) may be recovered from highly degraded samples, such as cremated remains, and analyzed using Massively Parallel Sequencing (MPS).

This presentation will impact the forensic science community by introducing a protocol that provides the means for DNA identification from severely burned human remains when other methods for identification have been exhausted or are not feasible.

The combination of a DNA extraction method designed for calcified tissues combined with a whole mitochondrial Genome (mtGenome) multiplex Polymerase Chain Reaction (PCR) assay and MPS will yield sufficient sequence information necessary for DNA identification of cremated human remains.

In cases of fires or explosions, forensic identification by traditional methods such as fingerprinting or anthropological analysis is often not possible. DNA identification of charred or incinerated remains may be the last resort for family members wishing to identify their loved ones. The recovery of DNA from burned human remains has been notoriously difficult. Some studies have been successful in typing DNA from charred remains and dental pulp; however, concerns exist regarding the quality and purity of DNA that is recovered.[1,2] Nuclear DNA in bone is often limited and PCR inhibitors can impede amplification of Short Tandem Repeats (STRs). The circular structure and subcellular sequestration of mtDNA may provide some protection from degradation, and high copy number of mtDNA per cell increases sensitivity of DNA typing assays, making it better suited for DNA identification of severely degraded samples such as burned remains.[3]

In this study, mtDNA was sequenced from an individual whose remains had been incinerated in a commercial crematorium. Ashes and small bone fragments were recovered from the cremated remains of the deceased. Bone fragments were externally cleaned with bleach and pulverized. DNA was extracted from ash and pulverized bone using a kit optimized for calcified tissues.[4] MtGenome copies from each extract were quantified, and Hypervariable region 1 (HV1) was amplified and sequenced via Sanger sequencing. Whole mtGenome was amplified using a multiplex PCR assay, and libraries were prepared and sequenced via MPS. HV1 and whole mtGenome sequences were compared to one another for concordance. All sequences were also compared to a maternally related reference to establish identity.

Results indicate that mtDNA can be successfully extracted from cremated remains. The combination of a calcified tissue-specific DNA extraction method with multiplex PCR and MPS is a useful technique to maximize recovery of genetic information from such severely compromised sample types.

Reference(s):

1. Brown, K.A., O'Donoghue, K., Brown, T.A. DNA in cremated bones from an early bronze age cemetery cairn. *Int. J. Osteoarchaeol.* 5, 181–187 (1995).
2. Tsuchimochi, T. et al. Chelating Resin-Based Extraction of DNA from Dental Pulp and Sex Determination from Incinerated Teeth with Y-Chromosomal Alphoid Repeat and Short Tandem Repeats. *Am. J. Forensic Med. Pathol.* 268–271 (2002).
3. Budowle, B. et al. Mitochondrial DNA regions HVI and HVII population data. *Forensic Sci. Int.* 103, 23–35 (1999).
4. Stray, J. et al. Extraction of high quality DNA from biological materials and calcified tissues. *Forensic Science International : Genetics Supplement Series.* 2, 159–160 (2009).

Mitochondrial DNA, Massively Parallel Sequencing, Cremated Remains

*Presenting Author