A120 Modified Protocol for Extracting DNA From Bones and Teeth in Cases With Low Expectations for Success: Reliability and Validity

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After attending this presentation, attendees will be able to use a modified DNA extraction protocol useful on degraded specimens of bones, teeth, and other various tissues.

This presentation will impact the forensic science community presenting factual details that ancient DNA research shares a common problem with forensics and other approaches requiring analyses of museum and non-invasively collected specimens; the amount of endogenous DNA available in the samples is often limited. Thus, extraction techniques that retrieve as much DNA as possible from a specimen are of crucial importance.

A wide range of techniques has been published to date, all of which aim to maximize DNA yields, while minimizing the co-extraction of PCR inhibitors. Due to low levels of endogenous DNA, environmental, bacterial, and postmortem DNA damage, as well as the potential presence of environment-borne inhibitors that co-extract with DNA, the recovery of DNA data from degraded specimens can still pose a significant challenge.

Previously, DNA extraction from the dental pulp samples was performed following a modified protocol of a Total RNA isolation system, suitable for DNA extraction from samples containing only a small number of nucleated cells. The same method was used for the bone samples. The protocol was partially modified by lengthening the incubation time of the cell lysis step: each sample of dental pulp was placed, overnight, at room temperature, in a single microtube containing 350 L of SV RNA Lysis Buffer. These protocols were applied to five skeletons discovered in Canosa di Puglia (Bari, Italy), during the archaeological excavations of tombs. These protocols do not allow for a complete characterization of genetic systems; however, even though the results obtained were satisfactory considering that the bones were ancient dated between the sixth and seventh centuries.

The extraction method on bones, teeth and various tissue fragments of human remains, making some changes to previous protocols used were tested. Following this, the success of amplifying ancient DNA was estimated.

Five cases are presented: In the first case, human remains were found in the Apulian countryside in 2002. They most probably belonged to a man who disappeared in 1989 according to the results of parentage testing by forensic hemogenetic investigations performed on the remaining members of the alleged missing man's family. In the second case, human remains were found in 2006 on an Italian highway which probably belonged to a man reported as missing. Identification was made by comparing the DNA of the remains to a blood sample taken from a brother. The third case involved human remains, discovered in 2009 near a rest home for the elderly in the province of Bari, most likely belonging to an 84-year-old man who disappeared in 1995. In this case identification was carried out by comparing the genetic profile of the remains to a blood sample taken from the son of the missing man. The fourth case involved the remains of two unknown skeletonized individuals discovered two meters underground. They were discovered in the small town of Marsicovetere, in southern of Italy where a trench for an oil duct was being excavated. In this case, the remains consisted of two full human skeletonized bodies that were highly fragmented. They were presumed to be from a prehistoric period because of their extreme lightness and porosity: they were extremely fragile. The skeletons were

removed from the trench by digging around the remains and taking them out along with the soil. The last case concerned remains found in the attic of a church in Potenza in March 2010. DNA extracted from the human remains was compared with the DNA extracted from the blood of members of the missing girl's family in order to establish identification.

The modified method for extracting the DNA genome, followed by the amplification reaction has allowed for the identification of four cadavers and the typification of the fifth. Each of these cases had low expectations for success. These scenarios involved cadavers or remains of unknown origin, which were discovered many years following the time of death. Each was discovered under conditions that did not favor the maintenance of the integrity of nucleic acids.

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