H45 Using Amplification of Bacteriophage Lambda DNA to Detect PCR Inhibitors in Skeletal DNA

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The goals of this presentation are to present to the forensic anthropological community a technique to detect the presence of PCR inhibitors in skeletal DNA. Using a mixture of a small amount of concentrated skeletal DNA and dilution of bacteriophage lambda DNA, the analyst can successfully detect PCR inhibitors early in the process and add additional purification steps to remove them from the skeletal DNA.

The analysis of DNA from skeletal remains is becoming a useful tool for forensic anthropologists. Genetic analysis complements the osteological examination and aids in the identification process. However, analysis of DNA samples obtained from bone may be challenging due to the presence of contaminating molecules that can later interfere with the process of DNA fingerprinting. These inhibitory substances may not be removed with conventional methods used to extract purified skeletal DNA. The cause of PCR inhibition may vary among samples because the different taphonomic forces that effect bone degradation also have an affect on skeletal DNA. Soil components, such as iron or tannins, may leach into the bone tissue during diagenesis and co-purify with the DNA contained within the skeletal remains. PCR inhibitors may also be the maillard products of sugar reduction or humic acids, which are a mixture of substances produced by the decay process. In the case of PCR analysis, the presence of these inhibitory molecules may result in a failure to amplify a decedent's DNA sequence needed for identification. Genetic analysis is expensive, time consuming, and destructive to the skeletal remains. If PCR inhibitors can be detected early in the process of genetic analysis, it can reduce the amount of skeletal remains lost to unsuccessful amplification. This paper will present a simple technique that can be used to detect PCR inhibitors in skeletal DNA.

A series of dilutions were performed using lambda DNA in order to find a concentration that would be both sensitive and not saturating to the inhibitors. A preliminary amplification of lambda DNA was then used as a quality control indicator for the skeletal DNA. The lambda DNA was mixed with a small amount of concentrated bone DNA and lambda specific primers. The mixture was then subject to 35 rounds of PCR at 95°C for one minute, 62°C for one minute, and 72°C for one minute. Inhibitors carried through the DNA purification process may block the amplification of the lambda DNA, and a failure to amplify the lambda DNA from the mixture indicates the presence of inhibitors in the skeletal DNA samples. Bacteriophage lambda DNA was chosen for this study because it is inexpensive and abundant in molecular laboratory facilities. The DNA tested for the inhibitors was extracted from the femora of 6 different historic human skeletal specimens. These DNA samples previously failed as templates for PCR using human specific primers. As a control, DNA from a human skeletal specimen that previously was successfully subjected to PCR analysis for human specific DNA fragments was also analyzed.

Sampling of bone tissue for genetic analysis is becoming an important aspect of the identification process in forensic anthropology. The skeletal material is valuable to the anthropologist and a minimal amount should be destroyed in the DNA extraction process. Contaminating molecules introduced into the skeletal remains during decomposition may complicate genetic analysis. With the early detection of inhibitors, additional purification steps may be added to remove them from the sample and save valuable amounts of skeletal DNA from loss due to fruitless PCR amplification. The results of this experiment show a concentration that is effective in detecting the PCR inhibitors found in some skeletal DNA samples.

Skeletal DNA, PCR Inhibitors, Lambda DNA

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