PCR Optimization for Ancient DNA from Bone

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Saremi M.A(1),Ph.D. Tavallaei M(1),Ph.D. Zeinali S(2), Saremi M(3) (1) Imam Hussein university, Faculty of science, Tehran, Iran. (2) Pasteur Institute of Tehran, Iran. (3) Reference Laboratories of Iran- Research Center. Using of molecular methods such as DNA fingerprinting has a special place in identifying of anonymous people and corpses, and has obviated deficiencies of old methods. This method has more importance when apparent characteristic of a person has been ruined in different events, or just some parts of bodies or bones which has remained from previous years are available for identifying in this case just genetically composition or DNA is permanent. According to specific and immortal characters of DNA and also impossibility of finding two identical persons in using of this method for identifying, therefore DNA could be one of the best evidences in this matter. For this purpose, after extraction of DNA from samples by choosing molecular markers and using molecular methods such as PCR, specific places in DNA must be reproduced to obtain personal profile by examination and sequence determination. In this research, tissues such as tooth and bone were used for identifying of corpses, which were buried for a long time. In these specimens, environmental factors have special effects on macro morphology and micro morphology of a tissue and also DNA is cut to pieces intensively in old tissues because of bacterial analysis, automatic autolysis that is consequent of Hydrolase's and Nuclease's release from cells. Therefore basis of this kind of examination should be on using of small parts of gene such as STRs in DNA. Presenting of inhibitors in compounds of bone tissue, Soil of burial place , and materials that enter in cycle of DNA rescued during this process, cause decrease of quality and quantity of DNA disentanglement and ability of doing PCR reaction. Recent examination in this research showed that an equal DNA extraction methods could not suitable for all kinds of samples of old bones, because composition and condition of samples change by influence of different environmental factors. Assay of samples by electronic microscope also indicates. Many differences between percentage of their components. Performance of calcification causes increasing of quantity and quality efficiency of disentangled DNA. Analysis of results of PCR reaction on different samples , which had been disentangled in different ways shows positive effect of passing time on samples of old bones, which have extracted by Silica and Promega Kit methods. This probably indicates presence of inhibitors in extract of disentanglement which it is amount or activity is decreased by passing of time. Examination of quantity of disentangled DNA by spectrophotometer indicates the highest output of DNA disentanglement in order of Silica, Promega kit, Phenol - Chloroform and Chelex methods. Despite of performance of changes and optimization in all circumstances of PCR reaction, some samples of old bones were not suitable for PCR. Therefore the greatest restriction for performance of DNA fingerprinting with samples of old bones is access to suitable disentangled aDNA, which is avoided from inhibitor and exotic factors.