H29 Using Restriction Enzymes to Reduce the Inhibitory Properties of Bacterial DNA on PCR Amplification of Human DNA Sequences

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This presentation suggests a protocol to successfully amplify a human DNA sequence (huTh01) from historic skeletal DNA samples that previously produced little or no Polymerase Chain Reaction (PCR) amplification. The results of this study demonstrate the potent inhibitory effects of DNA, obtained from a mixed population of soil microorganisms, on PCR amplification of huTH 01 (a short tandem repeat or STR) from human skeletal DNA. However, the PCR-inhibitory properties of soil microbial DNA was reduced by hydrolysis with a restriction enzyme.

When DNA is recovered from bone samples, PCR amplification of specific informative DNA sequences is often inhibited. Difficulties in amplification of DNA may be due to diffusible inhibitors from the soil and surrounding burial environment (that co-purify with the skeletal DNA) or non-diffusible inhibition (due to covalent modification of the template DNA). One hypothesized source of diffusible inhibition is non-human DNA contamination whose source is predominantly the bacteria that colonized the bone during decomposition. The circular bacterial DNA chromosome is less prone to degradation than linear human chromosomal DNA, and from bone samples is usually purified in great excess compared to human DNA. The physical presence of the abundant bacterial DNA could conceivably interfere with PCR amplification by 1) reducing the ability of a human-specific primer to anneal to its complementary sequence (mispriming on the abundant bacterial DNA); and 2) saturating the active sites of the Taq DNA polymerase making little or no enzyme available for binding to the minute amounts of human DNA present.

DNA extracted and purified from a mixed population of soil microorganisms (predominantly bacteria) was added to contemporary human DNA in a series of graduated ratios: 0.76 - 9.5 mg bacterial DNA was added to 0.112 mg of human DNA. The combined human and bacterial DNA samples were then PCR amplified using a primer pair designed to select for amplification of the short tandem repeat, TH 01 (huTH 01). PCR products were resolved by electrophoresis through a 10% poly acrylamide gel and visualized by ethidium bromide staining. The results indicated that DNA from a mixed population of soil bacteria was a potent inhibitor of PCR amplification from human DNA templates. All masses of bacterial DNA tested resulted in complete inhibition of PCR amplification of huTH 01.

In an attempt to remove the inhibitory effects of the bacterial DNA on the PCR amplification of huTH 01, the restriction enzyme *Pst 1* was used to hydrolyze the bacterial DNA before adding it to human DNA in the pre-PCR reaction cocktail. Aliquots of human DNA were combined with different amounts of either hydrolyzed bacterial DNA and or intact bacterial DNA. At a ratio of 2.7:1 (mg: mg) of hydrolyzed bacterial DNA to human DNA, amplification of hu TH 01 was not inhibited. At this same ratio, intact bacterial DNA was inhibitory to huTH 01 amplification. When bacterial DNA: human DNA ratios fell below 1.3:1 (mg: mg), the bacterial DNA did not inhibit PCR amplification of huTH 01 whether hydrolyzed or intact. When bacterial DNA to human DNA ratios of 11.4:1 (mg: mg) were tested, both hydrolyzed and intact bacterial DNA inhibited PCR amplification of huTH 01. Thus, there is a window at which hydrolysis of the bacterial DNA with *Pst 1* will enable PCR amplification to occur.

To test the potential of pretreatment of historic DNA templates with restriction enzyme, human DNA from three different historic bone samples was hydrolyzed using *Pst 1* restriction enzyme. This DNA, along with control bone DNA not treated with *Pst 1*, was subjected to PCR amplification of huTH 01. Amplification occurred for all three samples of bone DNA samples tested; however, more huTH 01 was amplified after pretreatment of the bone DNA with *Pst 1*.

These results have a practical application. Often when specific, informative DNA sequences cannot be amplified using bone DNA as a template, the amount of DNA used in the PCR reaction is decreased, to try to reduce or eliminate inhibitory effects of diffusible inhibitors such as foreign DNA. However, this method also dilutes the already limited amount of human DNA that can be amplified; if the human DNA is highly degraded to begin with (as is the case with historic samples) dilution may reduce the templates below the lower limit needed for PCR amplification. Attempts to separate the abundant bacterial DNA from the pg quantities of human DNA introduce the risk of contamination with contemporary DNA (from additional handling of samples) or the loss of the historic human DNA. However, results suggest that with the use of restriction enzymes, the original amount of bone DNA may be maintained or even increased; therefore a better chance of amplification and/or a higher yield of PCR product may be obtained.

Historic DNA, DNA Profiling, PCR Inhibitors

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