A73 Acetylated Versus Non-Acetylated BSA When Processing Inhibited Samples

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The goal of this presentation is to demonstrate the differences between using acetylated and non-acetylated bovine serum albumin (BSA) in the amplification of mitochondrial DNA (mtDNA), particularly when working with inhibited and/or ancient skeletal remains and blood.

This presentation will impact the forensic science community by increasing the success rate of obtaining DNA sequence data from inhibited samples by using proper amounts of non-acetylated BSA during polymerase chain reaction (PCR) amplification.

One of the primary missions of the Armed Forces DNA Identification Laboratory (AFDIL) is to identify the remains of military personnel missing from previous United States armed conflicts. In many cases, the skeletal remains recovered have been exposed to harsh environmental conditions and inhibitory factors causing the osseous structure of the remains and total genomic DNA within the bones to be highly degraded due to the condition of the skeletal remains, mtDNA testing is routinely performed. Many samples have also been subjected to surrounding stressors, such as high/low pH and high levels of humic acid that can cause DNA recovered from the remains to be inhibited. During PCR amplification of these samples, it is important to know how to overcome potential inhibition. BSA can be added to PCR amplifications to help minimize inhibition by acting as a blocking agent. There are two types of BSA available, acetylated and non-acetylated. Publications that describe the amplification of ancient DNA often recommend BSA as an additive but almost never stipulate which type. Preparations of BSA usually contain high amounts of nucleases, so BSA is often treated with acetic anhydride in order to inactivate nucleases. However, this acetylation can modify the binding characteristics of BSA by transferring the acetyl group from the BSA protein to the polymerase, therefore minimizing the effectiveness in overcome inhibition. Non-acetylated BSA is a highly purified version of BSA that is tested for DNase, RNase, endonucleases, protease, peroxidase, and alkaline phosphatase activity, the absence of which is vital to maintain DNA or RNA integrity.[1]

The two types of BSA were tested to determine which one provided the greatest success when processing inhibited samples. The optimized concentrations of acetylated BSA and non-acetylated BSA were used in separate PCR amplifications against the common inhibitors humic acid and hematin, found in soil and blood, respectively. Humic acid and hematin solutions were prepared in house at concentrations ranging from 7.5ng/µl to 20ng/µl for humic acid and 10µM to 50µM for hematin. The sample used for the PCR amplification setup was high quality DNA of a known sequence. The samples were spiked with various concentrations of the inhibitors. For the master mixes containing acetylated and non- acetylated BSA, 2µl of Taq was added per sample. In addition, a master mix was prepared containing non-acetylated BSA with 1µl of Taq per sample. Amplifications using acetylated BSA failed to overcome inhibition at the varying concentrations of humic acid and hematin. However, all amplifications involving non-acetylated BSA, at both1µl and 2µl of Taq per sample, were successful. The two types of BSA were then compared using samples displaying low quantitative values. Similar to previous results, the non-acetylated BSA outperformed acetylated BSA during the PCR amplification of the samples.

Results obtained from the comparison of the acetylated and non- acetylated BSA, demonstrate that non-acetylated BSA should be used for the processing of degraded and/or inhibited mtDNA samples.

The opinions and assertions contained herein are solely those of the authors and are not to be construed as official or as views of the United States Department of Defense or the United States Department of the Army.

Reference:

[1] http://www.ambion.com/catalog/CatNum.php?AM2616

Bovine Serum Albumin, Inhibition, MtDNA and STR

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