B154 Studies of PCR Inhibition

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After attending this presentation, attendees will be able to identify several problematic matrices for the DNA typing of bloodstains and to evaluate the usefulness of a variety of techniques for alleviating PCR inhibitory substances.

This information could be of assistance to crime labs to use as a reference for dealing with specific PCR inhibitor problems encountered with STR analysis of casework material.

Bloodstains were prepared on 15 separate matrices; titanium, blue denim jeans, lead, steel, drift wood, spruce 2x4, pressure treated spruce 2x4, maple tree bark, ocean beach sand, garden sand, compost soil, rock, dried leaf, soiled dry leaf, and fresh grass clippings. DNA was prepared from the bloodstains using a standard proteinase K digestion in buffer and organic extraction followed by a Centricon ® spin procedure.

The most widely used method to avoid PCR inhibition is to use the PCR facilitator, bovine serum albumin (BSA). Most forensic labs rely on manufacturers' kits for STR amplification. Both Applied Biosystems (AB) and Promega have included BSA in their STR kit amplification reactions. We decided to use the D1S80 AMFLP kit from AB (the last few kits available from this discontinued product line), which does not include BSA, as a model system to assess the effects of BSA on relieving inhibition. DNA extracted from bloodstains on the 15 different matrices was tested and examined for PCR inhibition. The DNA extracted from bloodstains on titanium sheet metal, denim jeans, driftwood, 2x4 spruce, maple tree bark, ocean beach sand, compost soil, rock, soiled dry maple leaf and grass clippings exhibited D1S80 inhibition. The addition of non-acetylated BSA (at 160µg/ml, New England BioLabs) relieved inhibition of DNA extracts from blood on the titanium sheet metal, the denim, the rock, the dried maple leaf and the grass clippings. The addition of acetylated BSA (Molecular Biology Grade, Sigma) did not relieve inhibition. The results were then compared to results from the AB AmoF*/*STR Profiler Plus™ kit. All DNA extracts from bloodstained matrices that displayed inhibitory substances which could not be relieved by BSA for the D1S80 also showed inhibited STR results.

There were 5 DNA extracts that did not show relief of PCR inhibition by BSA. These were driftwood, 2x4 spruce, maple tree bark, ocean beach sand and compost rich soil. Several different techniques were used in an attempt to reduce or alleviate the inhibition observed in both the D1S80 and Profiler Plus results. These included extra Centricon® or Microcon® spins, sepharose beads, chelex®, non-human DNA on beads, QIAamp® Stool mini kit, DNeasy® Plant mini kit, diffusion in LMT agarose as well as other methods.

While some techniques were either effective or partially effective for DNA extracted from any one particular matrix, there was no universal solution for all matrices. A summary of the methods that improved results for the problem matrices will be presented.

Data on the potential of DNA IQ to remove inhibitors from DNA extracts and from bloodstains on inhibitory matrices will also be presented.

Since there was no single solution, a test was devised to determine if the inhibitory substance would be present in the final extract after extracting an unstained matrix control. The extract was mixed with control DNA and PCR inhibition was observed. The mixed inhibitory extract-control DNA sample could be used to test for the most successful method to relieve inhibition without wasting the limited critical evidence sample. This approach could be useful in very special cases.

STRs, PCR Inhibitors, Bloodstains

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