A119 Useful Methods to Overcome the Interference of Humic Acid With STR Typing

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This presentation will impact the forensic science community by offering useful methods to improve purification and STR amplification of DNA in samples containing HA.

It is important for a successful STR amplification to remove PCR inhibitors as well as obtaining appropriate amounts of DNA. DNA samples obtained from forensic evidence may contain PCR inhibitors such as HA, hematin, and urea. These PCR inhibitors can interfere with PCR amplification and thus may inhibit the detection of STR loci. One of the main PCR inhibitors is HA. This substance is frequently observed when DNA is extracted from old bone exposed to the soil, because HA is easily co-extracted and purified with DNA.

An attempt to increase STR amplification of DNA samples containing humic acid (HA) was conducted. This study was conducted with two purposes. One purpose was to find a useful purification method through the comparison of three commercially available DNA isolation kits, and the other purpose was to overcome the interference of residual HA with DNA STR amplification. For purification tests, 0.25 ng/µl DNA samples containing different concentrations (10-1,000 ng/µl) of HA were used. The efficiency of three commercial kits (QIAquick® PCR Purification kit (Qiagen), QIAamp® DNA Investigator kit (Qiagen) and Prepfiler™ Forensic DNA Extraction kit (Applied Biosystems) were compared to the amplified STR loci using a AmpFlSTR® Identifiler® PCR Amplification kit (Applied Biosystems). Sixteen full loci were identified by the QIAquick® kit at 76 ng/µl HA (final concentration in the Identifiler), 11.5 loci by the Prepfiler™ kit and no loci identified by the QIAamp® kit at 38 ng/µl HA.

The interference of residual HA with STR amplification was overcome by the use of TaKaRa Ex Taq™ Hot Start Version (Takara) versus of AmpliTaq Gold® DNA Polymerase (Applied Biosystems). Eilert and Foran have shown that Ex Taq™ HS has advantages in assaying skeletal remains.[1] The usefulness of the STR amplification by different units (1-5 U) of both polymerases was also examined. Furthermore, 400 ng/µl bovine serum albumin (BSA) was added to the STR amplification. The Ex Taq™ HS was more resistant to HA than the AmpliTaq Gold®. Samples containing 19 ng/µl HA resulted in seven loci identified using one U Ex Taq™ HS, while no loci were identified using one U AmpliTaq Gold®. Full loci were identified using increasing the Ex Taq™ HS unit from one to two U. Full loci were also identified when BSA was added to the STR amplification containing one U Ex Taq™ HS. An increase in units of Taq and the addition of BSA improved the usefulness of STR amplification when assessed by using the AmpliTaq Gold®.

In summary, the QIAquick® PCR Purification kit appears to be more useful in removing HA than the QIAamp® DNA Investigator and Prepfiler™ Forensic DNA Extraction kits. TaKaRa Ex Taq™ Hot Start Version appears to be more resistant to HA than AmpliTaq Gold® DNA Polymerase. An increase in units of Taq and the addition of BSA may help to overcome HA interference.

Reference:

1. Eilert KD, Foran DR. Polymerase resistance to polymerase chain reaction inhibitors in bone. J Forensic Sci. 2009;54:1001-7.

Forensic DNA, Humic Acid, STR Amplification

After attending this presentation, attendees will learn useful methods to overcome the negative effect of humic acid (HA) on STR typing for bones buried in the soil.

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