A88 High - Performance PCR for Multiplexing STR Loci Directly From Whole Blood

Mikko T. Koskinen, PhD, Finnzymes Oy, Keilaranta 16A, Espoo, FINLAND; Arthur J. Eisenberg, PhD, and John V. Planz, PhD, University of North Texas Center for Human ID, UNTHSC, 3500 Camp Bowie Boulevard, EAD 310E, Fort Worth, TX 76107; and Bruce Budowle, PhD, FBI Laboratory, 2501 Investigation Parkway, Quantico, VA*

After attending this presentation, attendees will have learned how a novel PCR master mix can be utilized for multiplexing STR loci directly from whole blood without a need for DNA extraction.

This presentation will impact the forensic community by demonstrating an application made possible by a novel PCR master mix. Whole blood is often used as a source of DNA for human identification and clinical diagnostics. To reduce the manipulations required for analyses, it would be desirable to be able to type the DNA from whole blood without performing an extraction. This has not been previously possible. The practical benefit is that high throughput typing can be facilitated; without a need for extraction, automation can be simplified. In addition, the higher processivity PCR reported provides more consistent yields than with other routinely used methods.

Whole blood is often used as a source of DNA for human identification and clinical diagnostics. To reduce the manipulations required for analyses, it would be desirable to be able to type the DNA from whole blood without performing an extraction. However, *Taq* DNA polymerase, the most commonly used DNA polymerase for the PCR, can be completely inhibited by even small quantities of blood. This inhibition is attributed to the presence of heme. Several strategies have been proffered to overcome this inhibition, such as inclusion of special reaction buffers and additives. Because these approaches have had mixed results, DNA purification and extraction from whole blood still remains a common and necessary practice. In this study, an application of a novel master mix for multiplex PCR amplification and typing of human STR loci directly from whole blood is reported. The master mix contains a *Pyrococcus*-like DNA polymerase that is covalently linked to an Sso7D double-stranded DNA binding protein domain. The Sso7D-DNA polymerase linkage increases the processivity of the polymerase by ~10- fold and makes it extremely tolerant to the PCR inhibitors present in blood. With the master mix, co-amplification of 17 STR loci yields high amplicon yields and full profiles without requiring a DNA extraction step. This approach has been applied to both liquid whole blood and to dried blood stains in which the latter requires only a solubilization step. A PCR can contain up to 10% (by volume) whole blood and yield full profiles with added $MgCl_2$. The benefit of using the Sso7D-*Pyrococcus*-like DNA polymerase is that high throughput typing can be facilitated. Without a need for extraction automation can be simplified. In addition, the higher processivity of Sso7D-*Pyrococcus*-like DNA polymerase provides more consistent yields than with other routinely used DNA polymerases.

High Performance PCR, Multiplex, Blood

 ** Presenting Author*