



### B85 Human Identity From the Mosquito Midgut Using GlobalFiler™ Express

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**Learning Overview:** After attending this presentation, attendees will better understand the evidentiary potential mosquitoes have in the forensic community. Complete and partial Short Tandem Repeat (STR) profiles generated from mosquitoes that fed on human blood will demonstrate to the DNA analysts that identification of a person can be obtained from the blood meal, even after several hours of ingestion.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by informing attendees on possible methods and materials that can be used to utilize the minute quantities of blood present in the mosquito's midgut. The use of such a unique substrate could greatly impact the forensic science field.

There are currently three main forms of the *Anopheles stephensi* mosquito: typical, intermediate, and mysorensis. Despite the intermediate and mysorensis forms having poor vector status, the typical form is found in many urban areas and is known to spread various diseases such as malaria. While these insects are generally considered as pests and carriers of such disease, the female mosquitoes can be useful in forensic cases as they feed on human blood. Nucleated white blood cells found in the blood stored in the midgut of the mosquitoes can be useful in identifying the donor on which the female mosquito has fed. In the context of a forensic case, the ingested blood could help identify a suspect or link a victim to a crime scene.

Generation of Short Tandem Repeat (STR) DNA profiles by direct amplification is useful for identification of the donor of the blood that was ingested by the mosquito. Commercially available direct amplification kits allow for detection of the donor of a body fluid and bypasses the labor-intensive and time-consuming steps needed for extraction and quantification of DNA.

Previous research in this laboratory using another direct amplification kit indicated that DNA stored within the white blood cells of the mosquito gut starts to degrade after eight hours post-feed, and the DNA is completely degraded 72 hours post-feed. Partial profiles can still be obtained after eight hours of ingestion of blood. In this current study, mosquitoes were fed on warm blood meals and euthanized at zero hours, four hours, and eight hours post-feed. Several mosquitoes were euthanized at each time interval having fed on a single donor. A mixture of blood from two donors was also used in this study, and these mosquitoes were euthanized at the same time intervals.

This project utilized the GlobalFiler™ Express PCR Amplification Kit to perform direct amplification and generation of STR profiles. To assist in the direct amplification process, COPAN® microFLOQ® Direct Collection Devices were used for puncturing the midgut of each mosquito. These swabs contain a nylon-fiber tip with a lysis agent that helps to break open the nucleated white blood cells. Once a minute quantity of blood was collected on the tips, the tips were cut and placed into the Polymerase Chain Reaction (PCR) amplification tubes where they remained immersed in the direct amplification reagents during the thermal cycling process. A blood sample from each donor was used as reference to compare the results of profiles generated from the midguts of the mosquitoes.

This research indicated that DNA profiles obtained from the mosquitoes using GlobalFiler™ Express amplification kit were consistent between and within each donor. In addition, both donors could be identified in the mixture profiles. Full profiles for all sample types were obtained for zero hours, four hours, and eight hours post-feed. Additionally, it is hypothesized that variability between mosquitoes, both in their feeding and digestion patterns, affected the quality of the profiles obtained. In conclusion, DNA contained in a single or mixed human blood meal ingested by mosquitoes can be useful in determining the donors' identity using the GlobalFiler® Express PCR Amplification Kit. This is a robust method to establish the presence of one or more individuals at a location of interest, within a specific time window.

#### GlobalFiler™ Express, Mosquitoes, Direct Amplification