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DEPARTMENT OF BIOLOGY

Generating Human STR DNA Profiles from Blood Ingested by Leeches

ELIZABETH KNAPP
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Reviewed and approved* by the following:

Reena Roy, Ph.D.
Emeritus Professor of Forensic Science
Thesis Supervisor

Stephen Schaeffer, Ph.D.
Professor of Biology
Honors Adviser

* Electronic approvals are on file.

ABSTRACT

Aquatic crime scenes may include the presence of annelids, such as leeches, which may have ingested human blood and can potentially aid in the identification of a victim and/or suspect. In this research, human blood from one male donor was fed to 35 North American medicinal leeches. These leeches were then euthanized at various periods (0, 1, 2, 4-, 6-, 12-, and 24-hours post-feed). Blood from their midguts (crop) was amplified using three methods. First, the tip of each Copan microFLOQ® Direct Swab was dipped into the midgut of the individual leeches and amplified directly. Second, Copan microFLOQ® Direct Swabs were utilized to sample the crop, concentrating crop blood followed by direct amplification. Third, 4N6 FLOQSwabs® forensic collection devices collected, extracted, and amplified remaining crop blood. The aim was to determine if blood found in the midgut of leeches can be used in revealing human identity. This research can aid in unique forensic cases where annelids might be present at a crime scene. Autosomal STR profiles were generated using PowerPlex® Fusion 6C System and GlobalFiler™ Express. Y-STR profiles were obtained with PowerPlex® Y23 System and Yfiler® Plus amplification kits. Complete and partial, concordant, and consistent, autosomal, and Y-STR profiles were observed between 420 autosomal and 420 Y-STR profiles. All three methods can be used to generate DNA profiles from blood ingested by leeches when collected within a 24-hour period. The results indicate that blood ingested by annelids can serve as a valuable source of evidence in unique crime scene cases.

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Conflict of interest

None of the authors have any financial conflict or any disclosures that need to be declared.

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Ethical approval

All procedures were conducted following the guidelines of Institutional Biosafety Committee (IBC), Protocol # 49047 of The Pennsylvania State University, University Park, Pennsylvania.

Chapter 1

Introduction

In medieval times, a tactic known as bloodletting utilized medicinal leeches, known as *Hirudo medicinalis*, as a treatment for those who were ill.[1] These leeches convey an anticoagulant known as hirudin; a polypeptide found in their salivary glands. Hirudin prevents blood from clotting by inhibiting the action of thrombin, an enzyme that converts fibrinogen into fibrin which is an integral step in clot formation. Once the leeches hook onto human flesh, they make a small incision, and can ingest 5 mL to 15 mL of blood from one human. Though the structural skeleton is absent in leeches, they do have 34 segmented structures within their body. Frequently considered a parasite, leeches feed on blood by fastening each end of its body to the host like suction cups.[2]

The specific location of a crime scene and its surrounding ecosystem may prove advantageous to investigators. For instance, organisms such as mosquitoes can be a valuable source of forensic evidence. Researchers in Croatia fed mosquitoes human blood meal, which was then collected and analyzed to determine DNA quantification after periods of euthanization.[3] Also, in a recent study,[4] it was demonstrated that autosomal STR profiles can be obtained from the mosquito's midgut using direct amplification. Single source and mixture profiles were obtained from the mosquitoes when they were euthanized several hours after being fed human blood meal. Other researchers noted that blood ingested by mosquitoes can be used for human identification.[5-9] Furthermore, an annelid such as a leech, that is also characterized by its intake of blood, may link an individual to a crime scene. More specifically, if a suspect were to dispose of a victim's body near a pool of water, a leech could get attached to the suspect or the victim, ingesting their blood.

Once engorged, the leech may fall off the body in the surrounding area, with the possibility of leaving valuable evidence at the scene. Leeches are primarily found in habitats of freshwater but can also be discovered traveling on moist ground or in marine locations.

The authors understand that the conditions in which a leech may be discovered by investigators at a crime scene are unique, but there was at least one case in which leeches have proved vital for the purpose of exclusion of suspects in a forensic case. In 2001, an elderly woman in Tasmania was robbed at gunpoint in her home.[10] When the suspects escaped, forensic investigators were left with little evidence to pursue her aggressors; however, they discovered an engorged leech at the crime scene. Blood within the gut of the leech was analyzed and the DNA profile obtained from the body fluid was stored in a databank. Eight years later, when Peter Alec Cannon was charged with a drug offense, his DNA was analyzed. His DNA profile matched the DNA profile stored in the databank, obtained from the blood in the leech's abdomen several years earlier. On the face of this overwhelming evidence, Cannon pled guilty.[10] If discovered at a crime scene, a leech can be examined to discover the identity of the victim, the perpetrator, or bystanders at the scene, using the blood the organism had ingested.

Currently, several commercially available amplification kits are offered in the forensic community for generating STR profiles.[11] Examples of kits include direct amplification and autosomal STR amplification kits. Direct amplification kits are used mainly for generating profiles from reference (known) samples which can be deposited on substrates such as an FTA® card (Flinders Technology Associates) or similar substrates.[12,13] The autosomal STR amplification kits utilized were the PowerPlex® Fusion 6C System (Promega Corporation, Madison, WI, USA), containing 27 loci, and the GlobalFiler™ Express (Thermo Fisher Scientific, Waltham, MA),

containing 24 loci. The Yfiler® Plus Amplification Kit (Life Technologies, Foster City, CA, USA) is a 27-plex Y-STR system, containing two multicopy loci and seven rapidly mutating Y-STR loci, which allows for discrimination among related individuals. The PowerPlex® Y23 System (Promega Corporation, Madison, WI, USA) includes 23 Y-STR loci, containing a multicopy locus and two rapidly mutating loci.

Direct amplification using Copan microFLOQ® Direct Swabs and the PowerPlex® Fusion 6C System have been employed previously to determine STR DNA profiles from blood stored in the gut of mosquitoes after feeding them human blood and being euthanized at various time periods.[3] Copan microFLOQ® Direct Swabs and the GlobalFiler™ Express kit has been utilized to study various materials containing human bloodstains.[14] Direct amplification using the Yfiler® Plus and PowerPlex® Y23 System have been used previously to determine Y-STR profiles from blood deposited on various substrates, and the blood on these were amplified directly, where the substrates remained in the reaction mixture during amplification.[11]

This research project pursued several goals and objectives. First, it was postulated that autosomal and Y-STR profiles can be generated from the ingested human blood in the midgut of North American medicinal leeches. Second, we aimed to determine if profiles can be generated at different and defined time periods after the blood was ingested. Third, we hypothesized that DNA profiles can be obtained utilizing direct amplification methods with Copan microFLOQ® Direct Swabs. As demonstrated previously, the direct amplification process bypasses several steps, generating STR profiles within a short period of time.[3,15] Fourth, an additional purpose was to compare the results of direct amplifications using microFLOQ® Direct Swabs with profiles generated via the traditional method of extraction, quantification, and eventually downstream

analysis, where concordant and consistent profiles could be generated using four STR amplification kits.

Chapter 2

Materials and Methods

2.1. Sample preparation

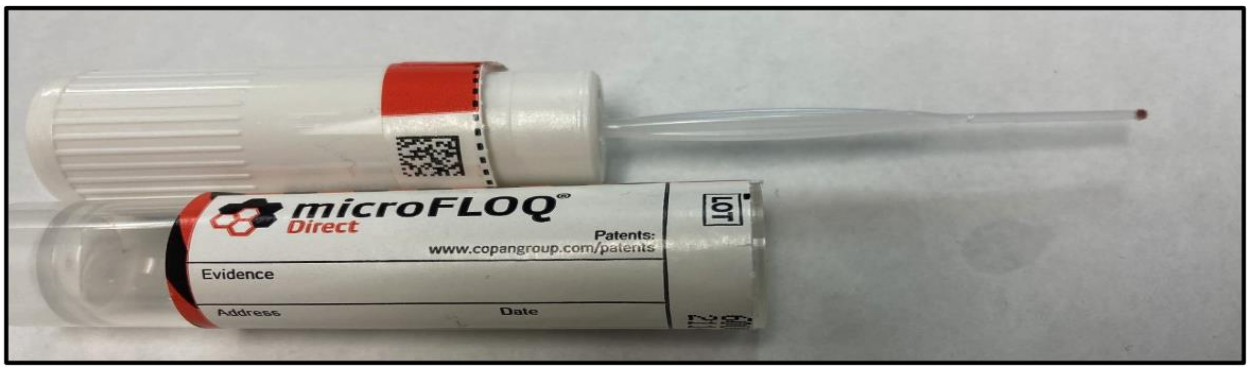
North American medicinal leeches, *Macrobdella decora*, were obtained from a commercial source (Leechtrapper, Dent, MN, USA) and whole blood from an anonymous male donor was purchased from Lee Biosolutions, Inc. (Maryland Heights, MO, USA). The leeches were kept in spring water (Walmart Inc, Bentonville, AK, USA) for storage and were moved to a separate container when being fed human blood meal.

Five leeches at a time were each fed 0.5mL of blood. Each leech was subsequently washed with spring water after feeding to remove traces of blood on their exterior prior to euthanization by freezing. The leeches were euthanized at defined time periods of 0, 1, 2, 4-, 6-, 12- and 24-hours post-feed. A coding system was devised to keep track of all samples and subsequent downstream analysis. Thus, a leech fed human blood meal at 0 hr. and the first leech incised at that time was categorized as 0-1, and the second leech would be categorized as 0-2. Similar coding was created for all seven time periods. Leeches were incised to expose the blood stored in the midgut (crop) as shown in Fig. 1.

Figure 1. Pictures of two incised leeches, one showing blood contained in the midgut (A), and the other (B) where blood oozed out of the midgut after incision.

Copan microFLOQ® Direct Swabs, which contain a lysing agent, were used for direct amplification. These swabs have been used previously for research projects and by other researchers.[3,15,16] In this research, direct amplification after collecting blood directly from the midgut and after concentrating the blood (through centrifuging) from the midgut was utilized. Copan microFLOQ® Direct swabs were dipped into the concentrated samples and amplified directly. For the first direct amplification method, Method A (further described in section 2.3 Direct amplification), the tips of two microFLOQ® swabs were dipped directly into the crop blood of one leech at a time. The tips were cut with a razorblade and put into two separate thermal cycling tubes and remained in the reagent mixture during the amplification process. Fig. 2. depicts the minute quantity of blood collected from the crop area at the tip of the swab.

Figure 2. Copan microFLOQ® Direct Swab tip showing a minute amount of blood on the tip.



The tips of these swabs can collect only a minute amount of blood; therefore, the reference samples were treated similarly by taking 0.5 µL of the blood and depositing the liquid on a clean glass slide. The tips of two swabs were used to collect blood and both samples were amplified. A preliminary study prior to amplifying the blood from the midgut of leeches by direct amplification was completed with reference samples by adding either the Prep-n-Go™ Buffer[19] (Applied Biosystems™) or Low TE buffer (Invitrogen, Carlsbad, CA, USA) when amplifying the samples with Yfiler® Plus Amplification kit reagent. When reviewing the results, it was determined that Prep-n-Go™ Buffer generated a better quality of profiles when performing direct amplification using this kit. As mentioned earlier, after reviewing the results of the initial study, it was determined that Prep-n-Go™ Buffer produced a better quality of results when amplifying using this kit. When utilizing PowerPlex® Y23 System, amplification grade water was added.

2.2. STR amplification kits

Four amplification kits were chosen for this study because they can be used for direct amplification and traditional methods for obtaining DNA profiles. The tables below describe each kit and the reagents used for amplification (Tables 1 and 2). Recommended protocols, by the manufacturers[12,13,17,18], were followed except as noted: amplification reaction volume for each kit was reduced to 12.5 µL from the recommended volume of 25 µL. Reduced reaction volume using 12.5 µL has been used in research and published elsewhere for both Y-STR and for autosomal DNA analysis.[11,15] For all reactions, an appropriate amount of nuclease free, sterile water, or Prep-n-Go™ Buffer was used as needed for a total reaction volume of 12.5 µL.

PowerPlex® Fusion 6C System utilized positive control that consisted of 0.5 µL DNA supplied in the kit. The negative control was 5.0 µL sterile, nuclease free, amplification grade water (Promega Corporation), added to each swab tip in each sterile MicroAmp® 0.2 mL amplification reaction tube (Applied Biosystems™).

For GlobalFiler™ Express, 3.0 µL of Prep-n-Go™ Buffer was used as a negative control with 1.0 µL of DNA as positive control supplied in the kit.

With PowerPlex® Y23 System, the positive control of DNA contained in this kit was used as suggested, and an appropriate amount was used for each amplification. The water used in the PowerPlex® Fusions 6C System served as a negative control for the Powerplex® Y23 system and was used with all amplifications.[12]

The Yfiler® Plus Amplification kit contained control DNA and the suggested amount was used as a positive control. Prep-n-Go™ Buffer was used as a negative control.

Table 1. The autosomal amplification kits and their reaction reagents are tabulated.

Autosomal Amplification Kits	Reaction Reagents
PowerPlex® Fusion 6C System (Promega Corporation, Madison, WI, USA)	▪ 2.5 µL of PowerPlex® Fusion 6C 5X Master Mix ▪ 2.5 µL of PowerPlex® Fusion 6C Primer Pair Mix ▪ 2.5 µL of 5X AmpSolution Reagent
GlobalFiler™ Express (Thermo Fisher Scientific, Waltham, MA)	▪ 6.0 µL GlobalFiler™ Express Master Mix ▪ 6.0 µL of GlobalFiler™ Express Primer Set Mix

Table 2. The Y-STR amplification kits and their reaction reagents are tabulated.

Y-STR Amplification Kits	Reaction Reagents
PowerPlex® Y23 System (Promega Corporation, Madison, WI, USA)	▪ 2.5 µL of 5X Master Mix per reaction ▪ 1.25 µL Primer Pair Mix per reaction
Yfiler® Plus Amplification kit (Applied Biosystems™, Foster City, CA, USA)	▪ 5.0 µL of Yfiler® Plus Master Mix ▪ 2.5 µL of Yfiler® Plus Primer Pair Mix

A targeted amount of DNA (0.5 ng/µL) was added to each extracted sample. Sterile, nuclease-free, amplification grade water (Promega Corporation) was added to each reaction tube for each sample. If necessary, these samples were diluted with amplification grade water for an optimal amount of Y DNA for use with each kit.

All thermal cycling was performed using the Veriti® 96-Well Thermal Cycler (Applied Biosystems™).

2.3. Direct amplification

Copan microFLOQ® Direct swabs were used with two of the three methods (Methods A and B) pursued in this research. The purposes of the two methods are described below.

A. Method A - Tips of two Copan microFLOQ® swabs were dipped directly into the crop blood of each leech. The tips of each swab were cut and put into a sterile PCR amplification tube (Amicon® Ultra-0.5 mL 50 K Centrifugal Tubes, Merck Millipore, Billerica, MA, USA). This method ensured that samples were amplified in duplicate. Appropriate reagents were added into each tube containing the tip and then amplification was performed. For autosomal STR

amplification, 27 cycles for thermal cycling were utilized for both PowerPlex® Fusion 6C and GlobalFiler™ Express. For all amplifications with PowerPlex® Y23 System, 25 cycles were used for thermal cycling. Conversely, for Yfiler® Plus amplification kit reagents, 27 cycles were used, which is within the 24-27 recommended cycles used for thermal cycling.

B. Method B – Concentration of blood from the midgut by centrifuging. Using a pipette, 5 μL of the midgut fluid was aspirated and transferred to Amicon® Ultra-0.5 mL 50 K Centrifugal Tubes (Merck Millipore, Billerica, MA, USA). The tubes were loaded to Veriti® 96-Well Thermal Cycler (Applied Biosystems™) at 14,000 rpm for 30 minutes. After centrifuging, approximately 1.0 μL of concentrated sample remained. The tips of two Copan microFLOQ® Direct swabs were dipped in this concentrated sample and these tips with blood were processed as described in Method A. Thus, in Method B, samples were also amplified and processed in duplicate.

2.4. Capillary electrophoresis and generation of DNA profiles

After amplification of samples, DNA profiles were generated using capillary electrophoresis on a 3130xl Genetic Analyzer (Applied Biosystems™), with POP-4™ polymer and EDTA buffer (Applied Biosystems™). The data was analyzed with GeneMarker® HID, version 3.0.0 software from SoftGenetics® (State College, PA, USA). Appropriate internal lane size standards and ladders as recommended by the manufacturer of each kit were added to Hi-Di™ Formamide (Applied Biosystems™), along with the recommended volume of amplified products. Amplified products were denatured at 95° C

for 3 min and then snap chilled immediately for 2 min prior to injection. Analytical threshold was set at 60 relative fluorescence units (RFU) for separation of alleles and fragment analysis. The injection and run conditions were followed as recommended by the manufacturer.

For the tabulated results portrayed in this project, if one of the two samples in each amplification yielded a complete profile, and the other sample generated either no profile or a partial profile, the results were counted as generating a complete profile. If the alleles in two loci or less dropped out, then these profiles were considered more informative partial profiles. When the profiles showed dropouts in more than two loci, the results are depicted as less informative profiles.

2.5. Extraction of blood from leeches and quantification of DNA (Method C)

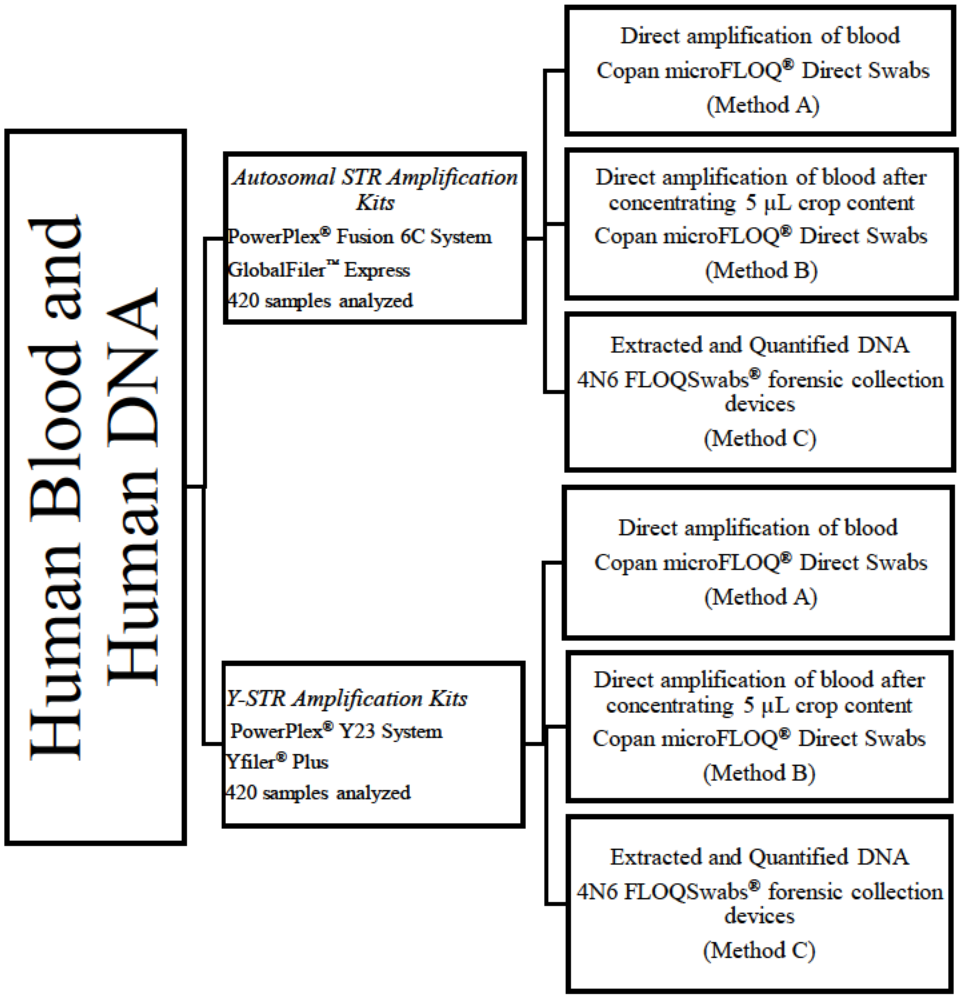
Qiagen EZ1 DNA Investigator® kit and BioRobot® EZ1 Workstation (Qiagen, Hilden, Germany) were used for extraction and quantification following the manufacturer's recommended protocol.[20] Tips of 4N6FLOQSwabs® forensic collection devices were used to collect blood from the midgut of each leech, after both Methods A and B were completed. DNA was eluted in a 50 µL volume of TE buffer and, to increase the efficiency of the amplification, the eluted DNA samples were concentrated down to a volume of approximately 30 µL in an Eppendorf Vacfuge Plus Concentrator System (Eppendorf, Hauppauge, NY, USA) with fixed speed. The concentration method took approximately 30 minutes at room temperature. Reference samples were extracted similarly, however, reference samples were not concentrated.

Quantifiler™ Trio DNA Quantification kit (Applied Biosystems™) and 7500 Real Time PCR System (Applied Biosystems™) were used to determine the quantity of DNA in

the extracted samples.[21] This kit allows the quantitation of total human and total male DNA, and assesses the quality of the DNA, particularly in degraded samples where inhibitors might be present. Recommended protocol of the manufacturer was followed.

An optimal amount of DNA was used for each amplification method. Each extracted and quantitated DNA sample was amplified in duplicate, as described earlier for direct amplification methods. The organizational structure of the methods used in the research is diagrammed in Table 3 below.

Table 3. The organizational chart depicting the workflow in this research.



Chapter 3

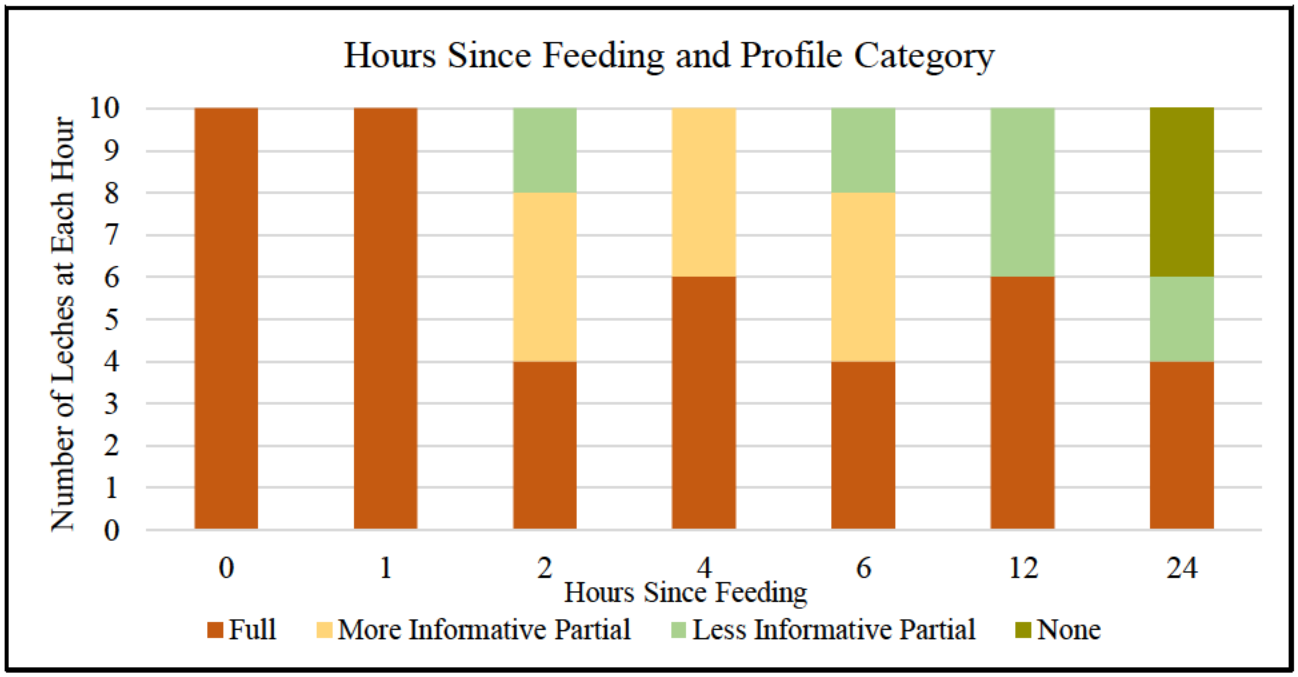
Results and Discussion

This project analyzed 35 leeches, 5 leeches per each of the 7 time points, which were fed blood from one male donor. Two swabs from each leech's crop were used for two direct amplification methods (Methods A and B), and amplification of each extracted sample (Method C). Each sample was amplified in duplicate, using two autosomal and two Y-STR amplification kits for a total of 840 samples.

Four different STR amplification kits were utilized, as not all forensic laboratories use the same kit. The researchers wanted to see if direct amplification would influence the different kits. Additionally, we wanted to confirm that if scientists did not wish to use direct amplification from a crime scene evidence sample, they can use the traditional method of extraction, quantitation, and downstream analysis using PCR amplification and capillary electrophoresis.

The results of the direct amplification (Method A) with GlobalFiler™ Express Amplification kit are diagrammed in Fig. 3, displaying that it was possible to obtain complete profiles from all euthanized leeches at each period. As the bar graph shows, partial profiles were obtained from leeches which were euthanized at 2, 4-, 6-, 12-, and 24-hours post-feed. These partial profiles were categorized as more informative or less informative partial profiles depending on how many loci yielded results. All the partial profiles, even the less informative ones, as depicted in the Fig. 3 legend, were consistent and concordant with the reference samples and with complete profiles obtained from other leeches.

Figure 3. Results of the direct amplification method (Method A) with the GlobalFiler™ Express Amplification kit depicting full profiles (dark orange), more informative partial profiles, where two loci or less dropped out (yellow), less informative partial profiles, where dropouts were seen in more than two loci (light green), and no profile (dark green).



There does not appear to be any consistent pattern as to if a certain time of euthanization would yield more complete profiles. It is hypothesized that leeches consume blood at different rates, depending on their appetite and metabolism. It is postulated that environmental stress might also be a factor.

When comparing Method A to Methods B and C, two extracts from five leeches at each time point did not have a consistent pattern in quantification results with extracted blood from the midgut. Inhibition was not observed in the quantification results, while concentrated blood from the gut (Method B) and extracted blood (Method C), yielded complete profiles, which was not the case with the first method of direct amplification. It is postulated that this is due to the leeches having a great deal of water in the midgut prior to swallowing blood, however this assumption is

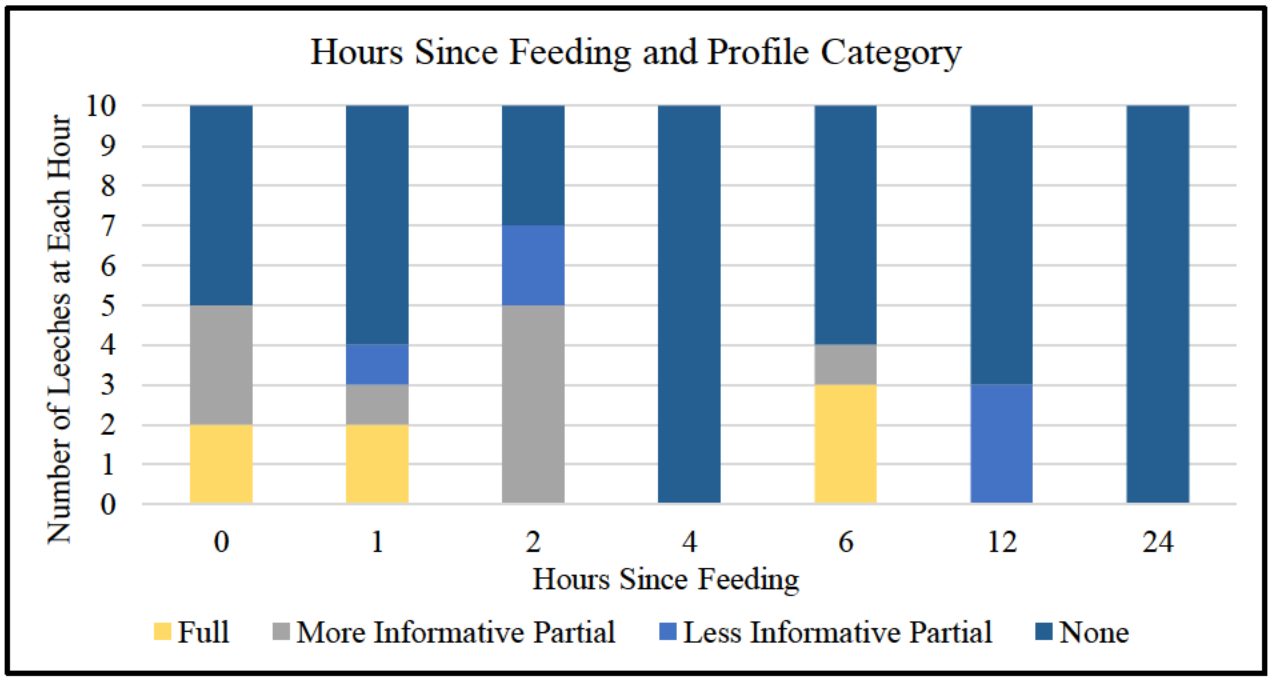
made only by observing the colors of blood in the midgut and is not supported by any experiments or the results using each kit and profiles obtained at different times. The annelids could have been affected differently by the stress of being removed from their natural environment, leading to a variance in typical feeding.

Furthermore, the results of direct amplification method with PowerPlex® Fusion 6C System Amplification kit are diagrammed in Fig. 4. With PowerPlex® Fusion 6C System, not all the leeches yielded complete profiles at each euthanization period. As the bar graph shows, partial profiles were obtained from leeches which were euthanized at 0, 1-, 2-, 6-, and 12-hours post-feed. All the partial profiles, even the less informative ones, were consistent and concordant with the reference samples and with the complete profiles obtained from other leeches. However, no profiles were obtained, even with duplicate amplification, from any of the leeches euthanized at 4- and 24-hours post-feed.

When comparing the results of the PowerPlex® Fusion 6C System and the GlobalFiler™ Express amplification kit, there does not appear to be consistency between leech samples, or the results obtained with different amplification kits when using direct amplification. For instance, leeches euthanized at 0- and 1-hour post-feed generated complete profiles with GlobalFiler™ Express, but the same samples generated a mixture of observed profiles (full, more informative partial, less informative partial and no profiles) with PowerPlex® Fusion 6C System. The

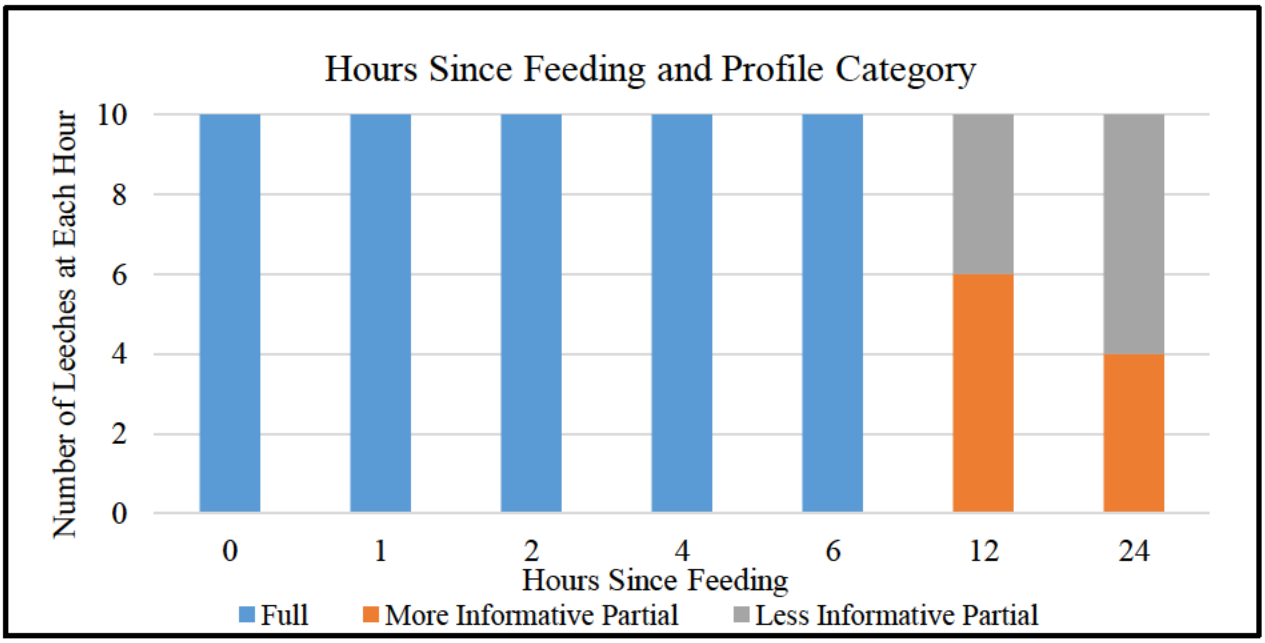
remaining euthanized samples exhibit results which showed no correlation between amplification kits and period of euthanization.

Figure 4. Results of the direct amplification method (Method A) with the PowerPlex® Fusion 6C System Amplification kit depicting full profiles (yellow), more informative partial profiles, where two loci or less dropped out (gray), less informative partial profiles, where dropouts were seen in more than two loci (light blue), and no profile (dark blue).



The results of the direct amplification method – Method A – with Yfiler® Plus Amplification kit are depicted in Fig. 5, where complete profiles were observed up to 6 hours post-feed. As the bar graph shows, partial profiles were obtained from leeches which were euthanized at 12 and 24 hours.

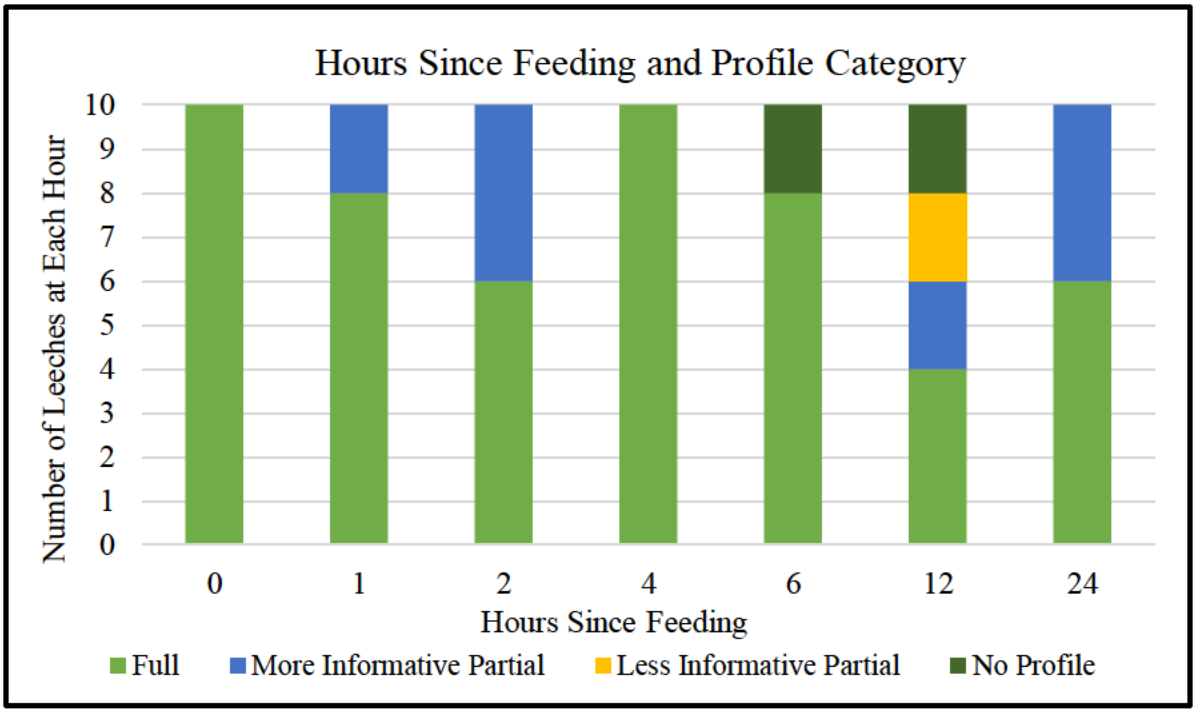
Figure 5. Results of the direct amplification method (Method A) with the Yfiler® Plus Amplification kit depicting full profiles (blue), more informative partial profiles, where two or less loci dropped out (orange), and less informative partial profiles, where two or less loci dropped out (orange), and less informative partial profiles, where dropouts were seen in more than two loci (gray).



When using PowerPlex® Y23 System amplification kit with this method of direct amplification, the results were slightly different. While complete profiles were obtained from all the leeches euthanized at 0 hr. and at 4 hr., several partial profiles were obtained from samples at other periods post-feed, as depicted in Fig. 6. At 1, 2, and 24 hrs. post-feed, only complete or more informative partial profiles were observed, whereas at 6 hrs. post-feed, only complete profiles or no profiles were generated with this method of amplification. The profiles at 12 hrs. post-feed

consisted of complete, more informative partial, less informative partial, or no profiles from some of the leeches.

Figure 6. Results of the direct amplification method (Method A) with PowerPlex® Y23 System amplification kit depicting full profiles (green), more informative partial profiles, where two or less loci dropped out (light blue), less informative partial profiles, where dropouts were seen in more than two loci (yellow) and no profile (dark green).



While the amount of blood ingested by the leeches is important when extracting the blood stored in the crop, it is important to note that direct amplification requires an extremely minute amount of blood. The amount of DNA, or the number of white blood cells, are unknown. The direct amplification with Method A, without concentration, yielded complete STR profiles from several leeches, particularly with the Yfiler® Plus Amplification kit. Similar results were also observed between GlobalFiler™ Express and PowerPlex® Fusion 6C System. It is therefore safe to assume that the minute quantity of blood picked up by the tip of the Copan microFLOQ® Direct

Swabs had enough human DNA to yield complete profiles, and in many cases, more informative partial profiles. With PowerPlex® Fusion 6C System and GlobalFiler™ Express, there was a complete absence of alleles in a few of the samples.

Comparing the results of all STR amplification kits, the generated profiles were consistent and concordant between and within the samples, and this was true even when partial profiles were compared. In the forensic community, allelic dropouts are observed regularly depending on the type of sample utilized, particularly if the alleles are larger because of DNA degradation. We noticed the same phenomenon in this project where direct amplification was performed. Samples amplified with Yfiler® Plus Amplification kit, showed that alleles at loci DYS389II, DYS627, DYS391, DYS518, DYS449 and DYS533 dropped out most often in less informative partial profiles. Interestingly, in some profiles, only two loci, DYS389II and DYS389I (both in blue channel dye) dropped out more often; thus, these were designated as more informative partial profiles when using this kit. Similar phenomena were observed in autosomal STR profiles. No attempts were made to vary parameters such as annealing, increasing injection time, or implementing post-amplification clean-up for incomplete profiles, as the authors wanted to keep the parameters the same for all methods.

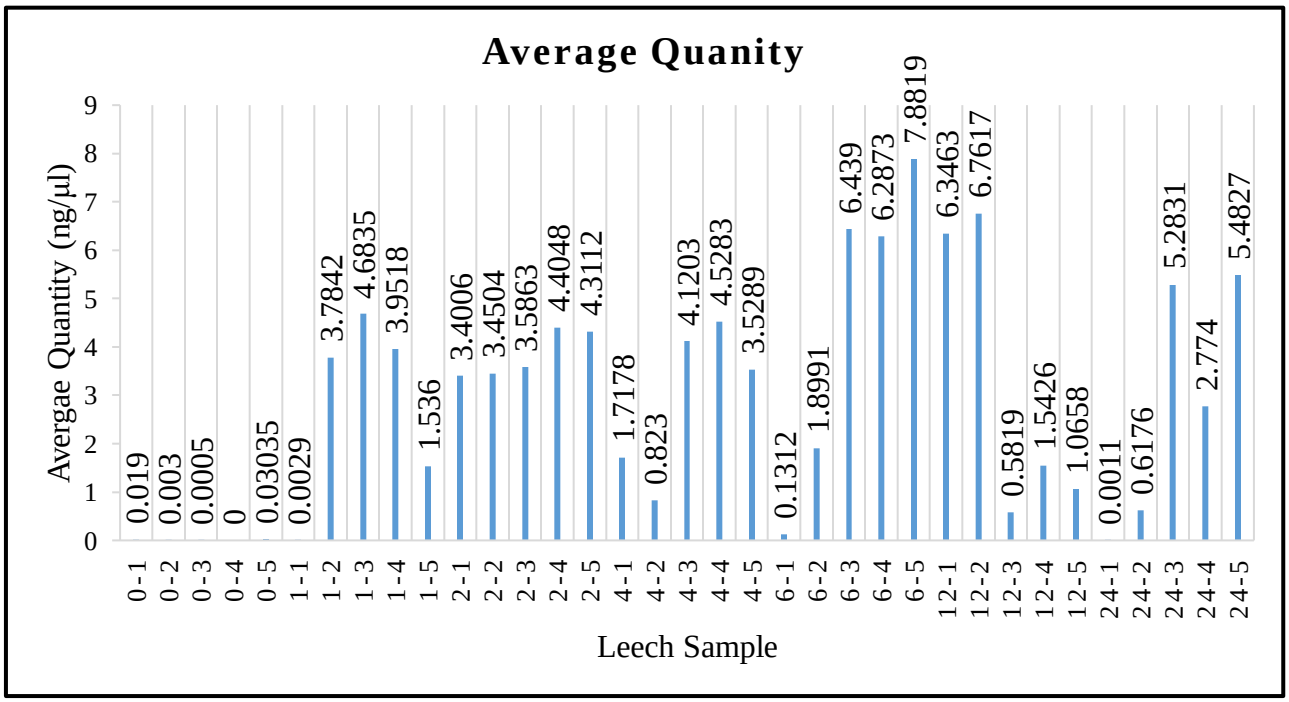
After reviewing the results of direct amplification with Method A, 5 µL of the content of the midgut was concentrated to approximately 1.0 µL to increase the quality and quantity of complete profiles. The rationale for concentrating all samples, even those that gave complete profiles with Method A, was to analyze all samples the same way and to note if there was consistency between both Methods A and B. Copan microFLOQ® Direct Swabs, in duplicate, were used to collect these concentrated samples and amplified using both kits. This method yielded

complete profiles from all the samples (data not shown), when amplified with each STR kit. Profiles generated were consistent and concordant between all amplification kits, with reference samples, between and within all samples, and with data generated using Method A.

Profiles generated using extracted DNA and both amplification kits (Method C)

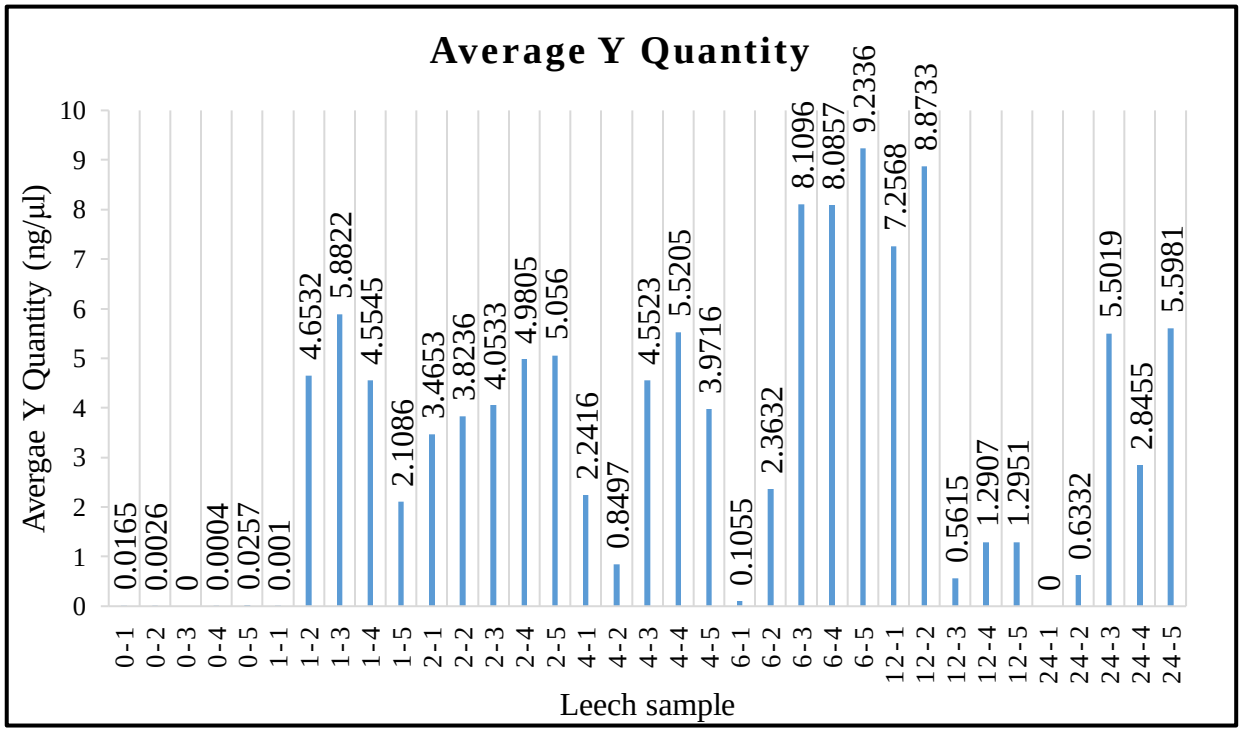
All samples generated from the traditional methods of extraction and quantification prior to amplification yielded complete profiles. We do not believe that it was due to the removal of inhibitors such as heme since previous research with direct amplification and blood[3,16] yielded complete profiles. It is possible that other PCR inhibitors were removed or that all extracted samples were also concentrated prior to amplification. The extracted samples from 35 leechees were quantified, and the results are depicted below in Fig. 7.

Figure 7. The average quantity of DNA (ng/µl) from extracted and concentrated samples quantified with the Quantifiler™ Trio DNA Quantification kit, from 35 leeches (two swabs per leech for a total of 70 samples) euthanized at 0, 1, 2, 4, 6, 12, and 24-hour post-feed, shown by a bar graph.



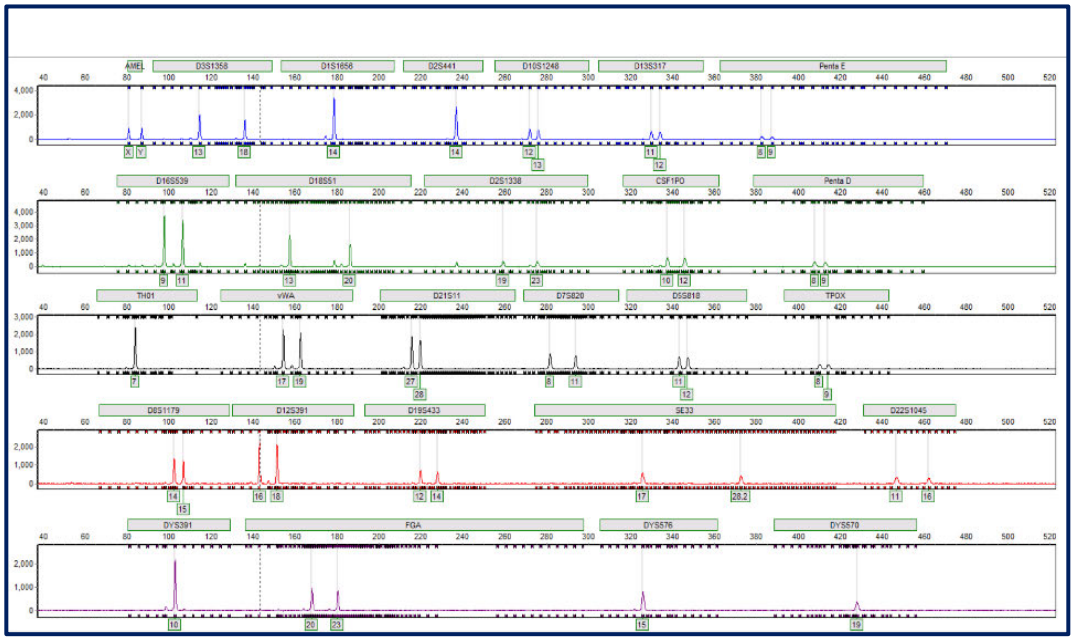
Results of Quantifiler™ Trio DNA Quantification kit indicate that the lowest average quantity of DNA were identified in the following samples: 0-3 and 0-4. Complete profiles were generated even when quantification data indicated the absence of DNA. The highest quant result was found in sample 6-5 with a value of 7.8819 ng/µl when averaging duplicate samples together. All samples using extracted DNA yielded complete profiles (data not shown) and the profiles were concordant and consistent with the profiles generated by direct amplification using both methods (A and B), as described previously, and with profiles from reference samples.

Figure 8. The average quantity of Y DNA (ng/µl) from extracted and concentrated samples quantified with the Quantifiler™ Trio DNA Quantification kit, from 35 leeches (two swabs per leech for a total of 70 samples) euthanized at 0, 1, 2, 4, 6, 12, and 24-hour post-feed, shown by a bar graph.



Results of Quantifiler™ Trio DNA Quantification kit, as indicated in Fig. 8., that the lowest Y quant results were identified in the following samples: 0-3, and in 24-1. The highest Y quant result was found in sample 6-5 with a value of 9.2336 ng/µl when averaging duplicate samples together. It should be noted that all quantified samples, irrespective of Y quant values, were amplified. All samples using extracted DNA yielded complete profiles (data not shown) and the profiles were concordant and consistent with the profiles generated by direct amplification using both methods (A and B), as described previously, and with profiles from reference samples. Below is a profile generated with autosomal amplification kit PowerPlex® Fusion 6C System, depicted in Fig. 9, from leech 24-1 using Method B.

Figure 9. This complete profile was generated utilizing PowerPlex® Fusion 6C System from leech 24-1, where the sample was euthanized 24 hours after feeding and was the first leech in the group of five leeches. Method B, where 5 µL of blood from the midgut was concentrated and then directly amplified using Copan microFLOQ® Direct Swabs, was followed.



Chapter 4

Conclusion

In this research, we demonstrated that it was possible to generate STR profiles from human blood ingested by an annelid. Direct amplification methods, and the traditional method of extraction and downstream analysis, generated consistent and concordant profiles. Three methods were utilized in this research. The first utilized Copan microFLOQ® Direct Swabs, dipped directly into the blood in the crop, yielded complete, more informative partial, and less informative partial profiles. When 5 µL of concentrated blood from the crop was amplified using the second method, complete, consistent, and concordant profiles were obtained. The traditional method, using 4N6FLOQSwabs® forensic collection devices, yielded complete and consistent profiles from all extracted samples. All profiles were consistent with reference samples.

It is possible that differences in results are simply a function of sampling, considering only a very minute amount of blood was used for direct amplification. However, two samples were taken from each midgut. The results obtained with PowerPlex® Fusion 6C System, GlobalFiler™ Express, PowerPlex® Y23 System and Yfiler® Plus Amplification kits were consistent. The overlapping loci yielded consistent data even in less informative partial profiles.

Although successful in our generation of complete DNA profiles utilizing each method and each STR amplification kit, there were several limitations of the research project. For instance, the amount of white blood cells being extracted from each leech's midgut and the association between stress and intake of blood by the annelids are unknown. This research project compares to research with other vectors, such as mosquitoes, where generation of STR DNA profiles and sequencing of DNA was successfully completed.[3]

Appendix A

Supplemental Figures

Figure 10. This complete profile was generated utilizing GlobalFiler™ Express from leech 2-2, where the sample was euthanized 2 hours after feeding and was the second leech in the group of five leeches. Method C, where blood from the midgut (crop) was collected on 4N6FLOQSwabs® forensic collection devices, extracted and amplified, was followed.

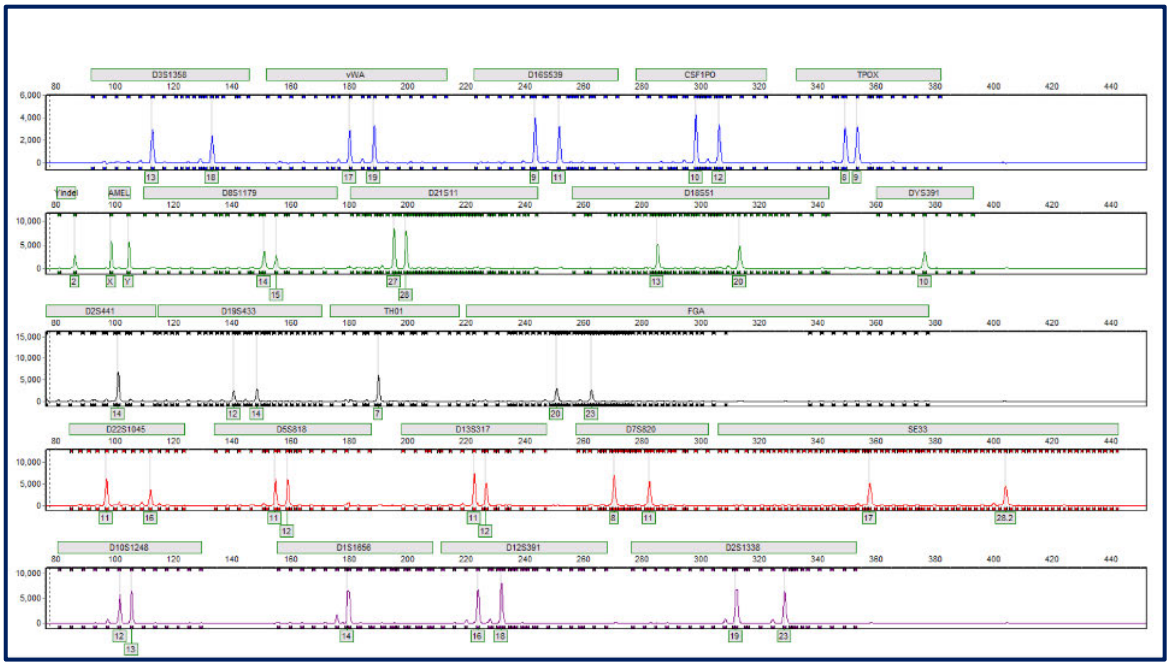


Figure 11. This is a complete Y-STR profile generated using PowerPlex® Y23 System from leech 24-2, where the sample was euthanized 24 hours after feeding and was the second leech in the group of five leeches. Method A was used, where blood from the midgut (crop) was collected directly on Copan microFLOQ® Direct Swabs and amplified directly.

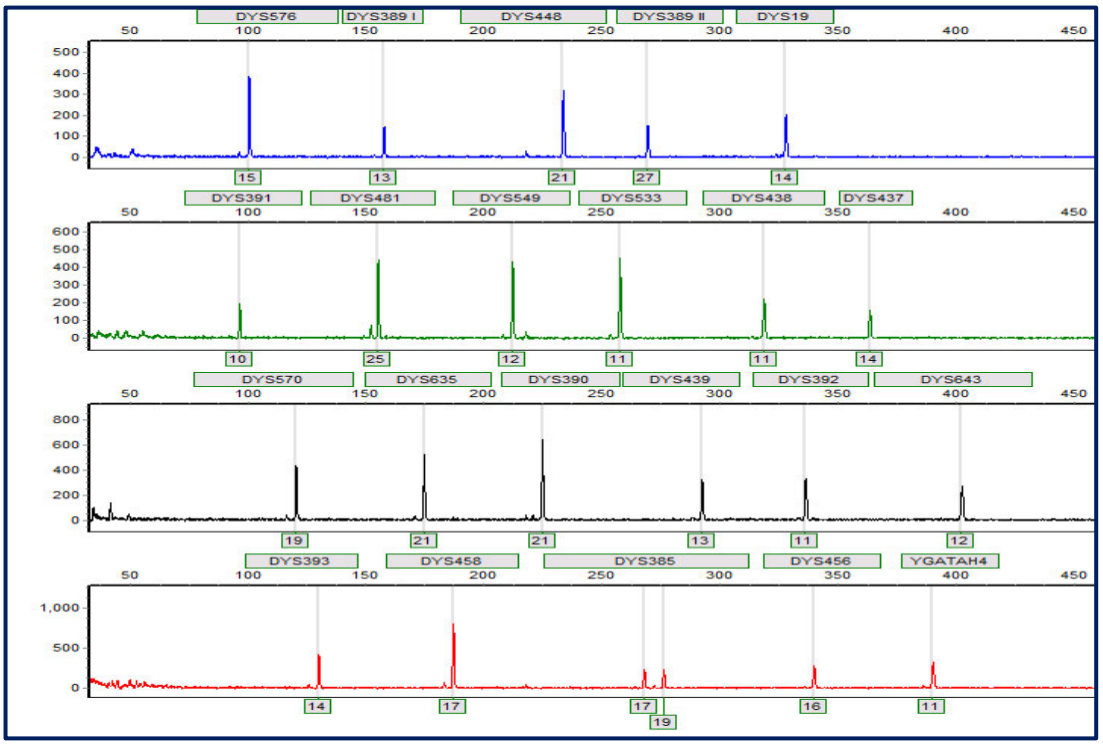


Figure 12. This is a complete Y-STR profile generated using PowerPlex® Y23 System from leech 1-3, where the sample was euthanized at 1 hour after feeding and was the third leech in the group of five leeches. Method A was used, where blood from the midgut (crop) w was collected directly on Copan microFLOQ® Direct Swabs and amplified directly.

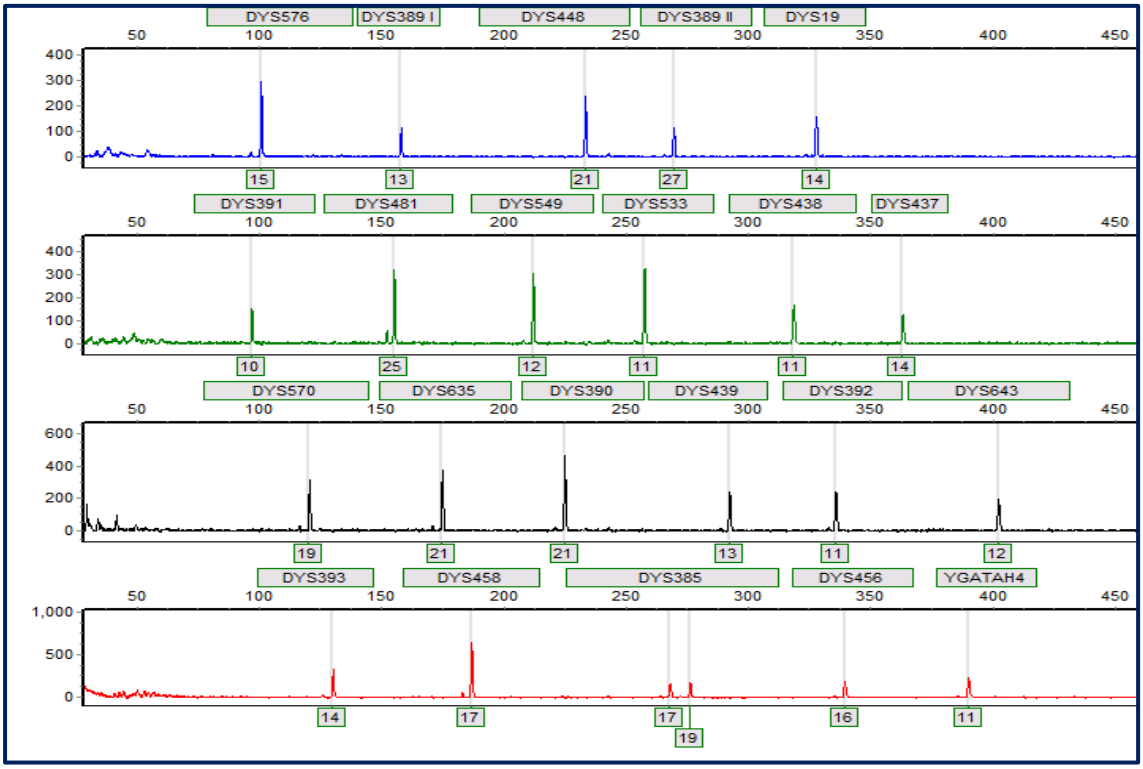
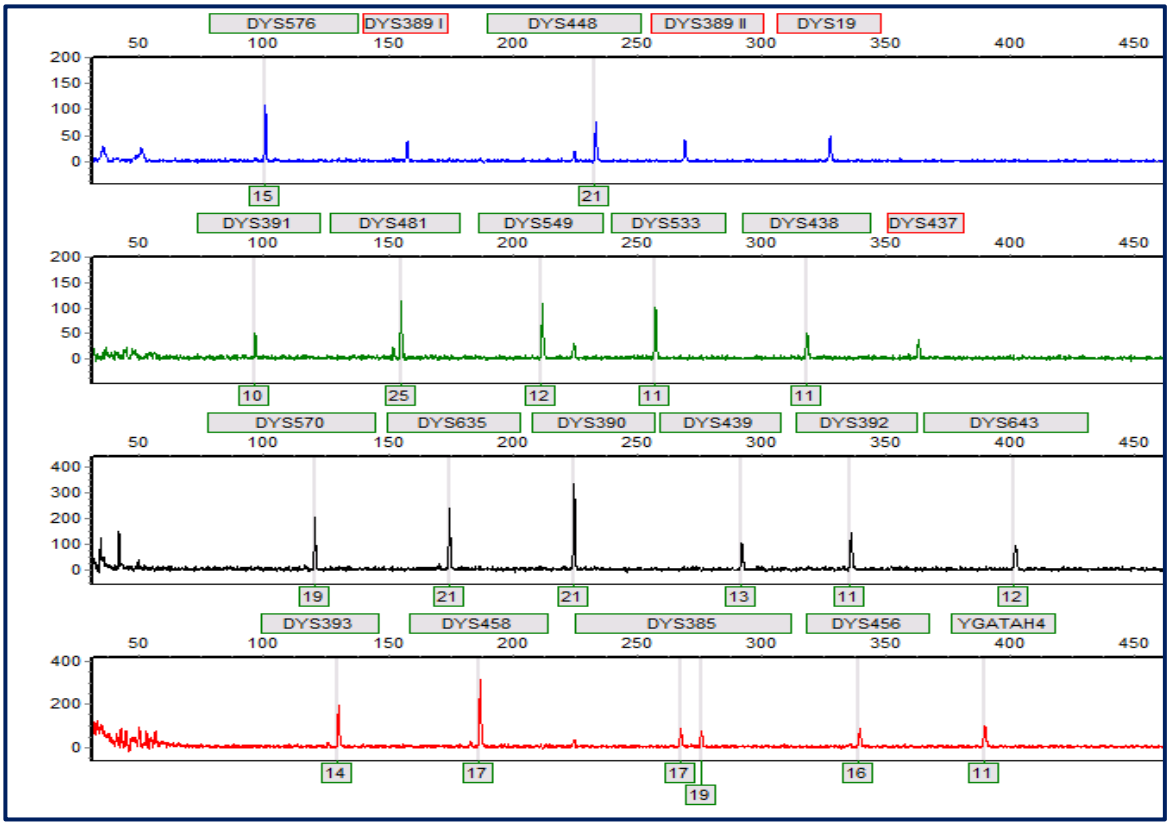


Figure 13. This is a partial Y-STR profile generated using PowerPlex® Y23 System from leech 1-5, where the sample was euthanized at 1 hour after feeding and was the fifth leech in the group of five leeches. Method A was used, where blood from the midgut (crop) was collected directly on Copan microFLOQ® Direct Swabs and amplified directly.



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ACADEMIC VITA

EDUCATION

The Pennsylvania State University — **University Park, PA**
Eberly College of Science – Schreyer Honors College — *August 2020 - December 2023*
Bachelor of Science in Biology
[redacted]

PUBLICATION

Research Principal Investigator: Reena Roy, Ph.D. — **University Park, PA**
Manuscript published in Canadian Society of Forensic Science (CSFS) Journal *as First Author* — *February 14th, 2023*

- "Generating human STR DNA profiles from blood ingested by leeches," published by CSFS Journal
- The research fulfilled the Thesis requirement of the Schreyer Honors College

RESEARCH AND PROFESSIONAL EXPERIENCE

The Pennsylvania State University – Course Assistant for Forensic 421W — **University Park, PA**
Department of Biochemistry and Molecular Biology — *January 2023 – May 2023*

- Graded assignments, restocked laboratory equipment, and maintained the cleanliness of the classroom environment
- Used knowledge of forensic laboratory etiquette to aid students in their class research assignments

Research Principal Investigator: Reena Roy, Ph.D. — **University Park, PA**
Determining Y-STR Profiles from Leeches — *August 2021 – February 2023*

- Leeches were fed human blood meal while Copan microFLOQ® Direct swabs and STR amplification kits were utilized to compare reference profiles to software-generated STR profiles to determine profile completeness
- Three different methods were utilized in the research that was performed using novel techniques

Newport, RI
Speaker at the 2021 Northeastern Association of Forensic Scientists (NEAFS) Annual Meeting — *November 3rd, 2021*

- Presented research entitled, "Determining Y-STR Profiles from Leeches Using Copan microFLOQ® Direct Swabs and the Yfiler® Plus Amplification Kit"
- Answered the inquiries of over 100 Conference attendees through a presentation

SKILLS

- PCR amplification (PowerPlex® Fusion 6C System, GlobalFiler™ Express, PowerPlex® Y23 System, and Yfiler® Plus amplification kits); DNA quantification (Quantifiler™ Trio)

LEADERSHIP AND INVOLVEMENT

Schreyer Scholar Ambassadors — **University Park, PA**
Executive Director — *Elected in September 2022*

- Selected via a competitive interview process
- Tasked with recruiting new ambassadors and planning events for the Honors College

ServeState Organization — **University Park, PA**
Active Member — *September 2021 - Present*

- Attend weekly meetings and obtain 25 hours of service per semester to remain an active organization member
- Participate in organizing four service events for the organization per semester

HONORS

- Dean's List all semesters, Eberly College of Science Undergraduate Research Grant 2021 and 2022, Schreyer Honors College Study Abroad Grant 2022 and 2023