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ISHI INTERNATIONAL SYMPOSIUM ON HUMAN IDENTIFICATION

WASHINGTON D.C. | OCT. 31-NOV. 3, 2022

November 2021

The ISHI Report

News from the World of DNA Forensics

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Letter from the Editor

Greetings dear readers,

As the year draws to a close, we would like to express our appreciation to those of you who attended ISHI 32 in Orlando and those who participated by watching the talks on demand. We are grateful for your patience as we worked through the continuously evolving circumstances affecting the logistics for the symposium. As of today, we are optimistic that ISHI 33 next fall will offer the opportunity to gather in Washington DC with many fewer restrictions.

Each year, focused topic workshops are a highlight of the International Symposium on Human Identification. If you have a topic that you would like to share with the community, we would like to hear from you! Workshop proposals are being solicited now with the deadline of January 17. Workshop chairs and speakers will receive support to attend the symposium. Find complete details including the workshop proposal form on the official symposium website, www.ishinews.com.

Look for ISHI registration to open in February. To accommodate those unable to travel to DC, we will once again offer an option to participate in a virtual fashion.

In this issue of the ISHI Report, Ken Doyle provides a recap of the topics covered at the symposium in Orlando. In addition, he interviews several up-and-coming forensic scientists with an interest in exploring the emerging field of MPS as a tool to provide useful data related to ancestry, the microbiome and ancient DNA.

Guest authors in this issue include Ellen McRae Greytak from Parabon Nanolabs who provides background on how Investigative Genetic Genealogy (IGG) is being used to solve an increasing number of cold cases. François-Xavier Laurent and Susan Hitchins of INTERPOL introduce I-Familia, a program to identify remains across borders through the use of DNA kinship matching and Regina Wells, from Kentucky State Police Central Lab explains how rapid DNA is being used at her agency to process sexual assault kits.

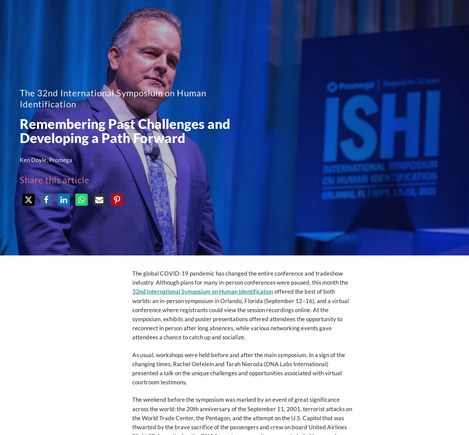
Finally, this year's team of student ambassadors describe their experiences attending ISHI 32. We will begin recruiting for a new group of students studying forensic science to attend next year's symposium in National Harbor, Maryland just outside of Washington DC in the spring of 2022. Find details on the perks of being a student ambassador and guidelines for applications on the symposium website.

We hope you enjoy the November issue. As always, we welcome your ideas and written contributions to our publication.

Stay well,

The Editorial Board

Contact ISHI



The 32nd International Symposium on Human Identification

Remembering Past Challenges and Developing a Path Forward

Ken Doyle, Promega

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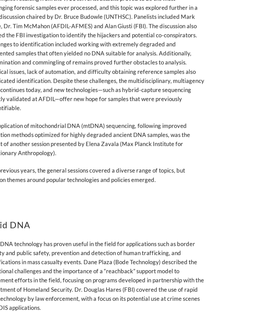
The global COVID-19 pandemic has changed the entire conference and tradeshow industry. Although plans for many in-person conferences were paused, this month the 32nd International Symposium on Human Identification offered the best of both worlds: an in-person symposium in Orlando, Florida (September 12–16), and a virtual conference where registrants could view the session recordings online. At the symposium, exhibits and poster presentations offered attendees the opportunity to reconnect in person after long absences, while various networking events gave attendees a chance to catch up and socialize.

As usual, workshops were held before and after the main symposium. In a sign of the changing times, Rachel Oefelein and Tarah Nieroda (DNA Labs International) presented a talk on the unique challenges and opportunities associated with virtual courtroom testimony.

The weekend before the symposium was marked by an event of great significance across the world: the 20th anniversary of the September 11, 2001, terrorist attacks on the World Trade Center, the Pentagon, and the attempt on the U.S. Capitol that was thwarted by the brave sacrifice of the passengers and crew on board United Airlines Flight 93. In particular, the DNA forensics community was reminded of how much technology has evolved over the years, in the efforts—still ongoing—to identify the victims of the attacks.

Remembering 9/11

To commemorate the 9/11 anniversary, the New York City Office of Chief Medical Examiner (OCME) held a special video presentation, "Staying Strong: NYC OCME Reflects on 9/11." It was only fitting that Mark Desire, Assistant Director of the OCME, deliver the first part of the keynote address at ISHI 32. He reflected on the OCME's commitment to identifying the over 20,000 samples of human remains from the World Trade Center; to date, approximately 7,100 samples remain unidentified.



In his keynote address, Desire also shared the personal story of his close brush with death, when he was hit by debris from the South Tower. The memories of that day are still fresh in his mind, and he gave the audience a compelling account of the incredible efforts made by first responders in the hours and days following the collapse of the towers. He described how the forensics community came together with other agencies and scientists from around the world in a superhuman effort to identify those that perished in the attacks.

Mary Fetchet, Founding Director of the Voices Center for Resilience (VOICES), presented the second part of the keynote address. Her organization provides long-term support and resources that promote mental health care and wellness for victims' families, responders, and survivors, while extending their efforts to assist communities in recovering from other tragedies. Like the OCME, VOICES remains committed to use their expertise beyond the work that began with 9/11.

The samples resulting from the 9/11 terrorist attacks proved to be the most challenging forensic samples ever processed, and this topic was explored further in a panel discussion chaired by Dr. Bruce Budowle (UNTHSC). Panelists included Mark Desire, Dr. Tim McMahon (AFDIL-AFMES) and Alan Giusti (FBI). The discussion also covered the FBI investigation to identify the hijackers and potential co-conspirators. Challenges to identification included working with extremely degraded and fragmented samples that often yielded no DNA suitable for analysis. Additionally, contamination and commingling of remains proved further obstacles to analysis. Logistical issues, lack of automation, and difficulty obtaining reference samples also complicated identification. Despite these challenges, the multidisciplinary, multiagency effort continues today, and new technologies—such as hybrid-capture sequencing recently validated at AFDIL—offer new hope for samples that were previously unidentifiable.

The application of mitochondrial DNA (mtDNA) sequencing, following improved extraction methods optimized for highly degraded ancient DNA samples, was the subject of another session presented by Elena Zavala (Max Planck Institute for Evolutionary Anthropology).

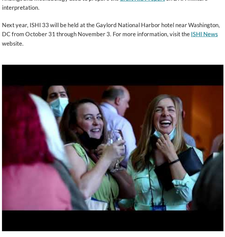
As in previous years, the general sessions covered a diverse range of topics, but common themes around popular technologies and policies emerged.

Rapid DNA

Rapid DNA technology has proven useful in the field for applications such as border security and public safety, prevention and detection of human trafficking, and identifications in mass casualty events. Dane Plaza (Bode Technology) described the operational challenges and the importance of a "reachback" support model to supplement efforts in the field, focusing on programs developed in partnership with the Department of Homeland Security. Dr. Douglas Hares (FBI) covered the use of rapid DNA technology by law enforcement, with a focus on its potential use at crime scenes in CODIS applications.

Forensic Genetic Genealogy (FGG)

Former FBI Special Agent Steve Busch (now CEO of FGG software company Indago Solutions) was instrumental in the FBI's efforts for training their staff to work with local law enforcement in applying FGG. In his presentation, he addressed common myths about the use of FGG by law enforcement agencies. He also provided more details about the FBI efforts to advance the use of FGG and gave the audience a glimpse of what the future holds for this revolutionary method.



Leading the audience through a series of epiphanies from landmark cases, Dr. Colleen Fitzpatrick (IdentifinderS International) explored the deficits in the criminal justice system and CODIS that FGG has revealed. She also discussed the influence of public opinion on the use of FGG databases to solve crime.

Dr. Lawrence Wein (Stanford University) presented a two-stage mathematical model of the genealogy process, which is the most time-consuming part of FGG. The analysis allows for the prior assessment of the difficulty of a case, ultimately helping to decrease the case workload.

Using two examples, CeCe Moore (Parabon NanoLabs) demonstrated the use of FGG to solve two cold cases. The first led to the conviction of Michael DeVaughn in what was known as the Labor Day Murders. In this case, two women were viciously assaulted and murdered in Starkville, Mississippi, in 1990. The second case involved the identification of "Oscar Doe", whose remains were found in a trash can at a construction site in Anne Arundel County, Maryland, in 1985. After conventional methods had failed, a combination of DNA phenotyping, kinship analysis, and FGG supported by social media research resulted in the identification of the unknown homicide victim as Roger Kelso of Glen Burnie, Maryland. Moore subsequently presented the case study on *The Dr. Oz Show*, hoping to gather more information that would lead to the identification of Kelso's killer.

Massively Parallel Sequencing (MPS)

A decade has passed since Dr. Seth Faith introduced massively parallel sequencing (MPS) for STR typing at ISHI 22. While initial adoption of the technology was slow, it has seen increasing acceptance within the forensic community in recent years.

Dr. Jennifer Ciblar (UNTHSC) introduced a cross-platform MPS forensic workflow, using the PowerSeq® 46GY System, followed by library prep and sequencing on both the Verogen MiSeq® FGx System and the Ion Torrent Ion S5™ System. The data demonstrated good concordance across platforms and bioinformatics pipelines, while also pointing out potential methodology-based biases for the generated profiles.

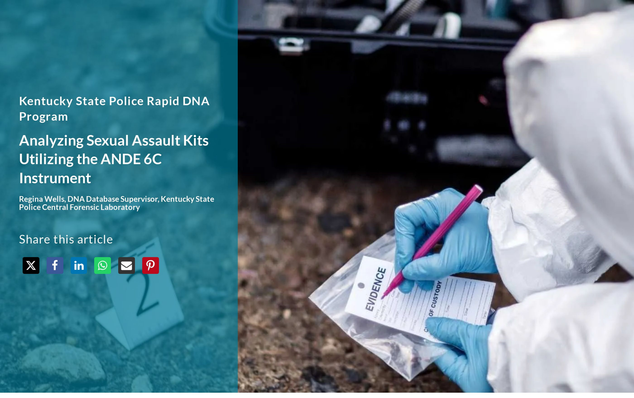
Two presentations illustrated the use of MPS for analyzing ancestry information in widely divergent applications. Laurence DeVesse (King's College London) showcased the results of genotyping 1,000 samples from five global population groups using the MiSeq FGx™ Forensic Genomics System. The analysis supplemented autosomal STR information with novel sequence variants that could be used for ancestry determination. Allison Sherier (UNTHSC) described the use of a custom targeted SNP panel for the human skin microbiome and identified ancestry-informative markers to identify the human host. The results showed that the microbiome could serve as a powerful alternative to traditional analysis, especially for samples with limited amounts of DNA. Sherier was an ISHI Student Ambassador last year and encourages her peers to check out the program.

Other Topics

Other technological innovations covered at ISHI 32 included DNA extraction from rootless hair (Dr. Ed Green, University of California–Santa Cruz), Y-STR analysis for missing persons cases (Dr. Jianye Ge, UNTHSC), an automated differential extraction method for sexual assault evidence samples (Emily Simek and Lies Janssens, Utah Bureau of Forensic Services), and proteomic analysis for human identification (Dr. Curt Hewitt, Signature Science). In addition, Sharon Bertsch McGrayer presented a fascinating history of the use of Bayes' Rule in forensic investigations (also see the related article in *The ISHI Report*, February 2021.)

Several sessions covered policy topics of interest to the entire forensics community. Lisa Grossweiler (FBI) presented the customary CODIS and NDIS update. Dr. Tom Callaghan (FBI) discussed the latest information from the Investigative Genetic Genealogy Working Group, SWGDAM. Dr. John Butler (NIST) summarized the key findings and methodology used to prepare the draft NIST report on DNA mixture interpretation.

Next year, ISHI 33 will be held at the Gaylord National Harbor hotel near Washington, DC from October 31 through November 3. For more information, visit the ISHI News website.



Kentucky State Police Rapid DNA Program

Analyzing Sexual Assault Kits Utilizing the ANDE 6C Instrument

Regina Wells, DNA Database Supervisor, Kentucky State Police Central Forensic Laboratory

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Over the past decade a spotlight has been placed on the tens of thousands of sexual assault kits that have remained untested in evidence rooms across the country. The Kentucky State Police Laboratory identified 4800 untested sexual assault kits through an audit of law enforcement agencies in the Commonwealth. This discovery prompted the Kentucky General Assembly to pass Senate Bill 63, the SAFE Act, in the spring of 2016. This sweeping legislation addressed everything from victim's rights to officer training to evidence preservation. One of the key points of the legislation placed timeframes on hospitals, law enforcement, and the Kentucky State Police laboratory concerning the handling of sexual assault kits. Requirements for the laboratory meant by July of 2020 the average turnaround time for sexual assault kit results would need to be 60 days. With this requirement in mind in December of 2018, the KSP laboratory launched a pilot project in conjunction with the ANDE company to evaluate sexual assault kit evidence using the ANDE 6C Rapid DNA instrument.

The ANDE 6C Rapid DNA system can analyze an evidence sample in under two hours however sexual assault kits are challenging due to mixtures of the victim and suspect's DNA. A pre-treatment step was developed by ANDE to separate the suspect DNA in sperm cells from the victim's DNA providing clean suspect profiles which can be searched against a database. The purpose of this study was to determine if this procedure could produce a searchable suspect profile using actual evidence samples collected during a forensic sexual assault exam. Suspect profiles obtained would then be searched against a copy of the state database to determine if a hit could be obtained between the evidence profile and an offender. This would provide an officer with an investigative lead in the early stages of the investigation rather than months after the assault.



One hundred Rapid DNA sexual assault kits were provided to sexual assault nurse examiners in three jurisdictions in Kentucky for collection during a forensic sexual assault exam. Two additional swabs for the Rapid sexual assault kit were taken along with the four swabs for the traditional sexual assault kit. The Rapid sexual assault kit came directly to the laboratory while the traditional sexual assault kit was collected by law enforcement and submitted to the laboratory following their evidence handling protocols. The Rapid sexual assault kits were analyzed as quickly as possible once received into the laboratory following the ANDE protocols. Any suspect profiles obtained from the analysis were searched against a copy of the state database, exported from CODIS, and housed in the FAIRS software. Hits obtained during this process were confirmed and reported to law enforcement as an investigative lead. The traditional sexual assault kits were fast-tracked through serology screening and DNA testing and comparisons made between the traditional DNA analysis and the results from the ANDE 6C system.

The pilot project generated fourteen searchable profiles, which at first glance appeared low. However, after serological analysis of the traditional kits, only 32 of the kits contained semen on the corresponding evidence swabs. Rapid DNA analysis produced profiles in 44% of the semen positive kits versus 59% with traditional analysis. Comparison to traditional analysis found that the ability to perform mixture interpretation and increased instrument sensitivity were the primary differences when profiles were obtained in traditional analysis only. Five database hits were obtained from the database searches including two hits identifying unknown suspects and directly leading to at least one arrest. Based on these results the laboratory concluded the ANDE 6C system can produce clean suspect profiles following the pre-treatment step when enough suspect DNA is present. This would allow the laboratory to quickly report an investigative lead to officers while the case is still on-going.

Final implementation of this program was challenging due to the lack of guidance in the FBI Quality Assurance Standards. Current rapid DNA standards are applicable to reference standards only and not crime scene samples. The laboratory's approach was to design our methods as though it would be any other DNA process that would be subject to the standards. Validation, evidence handling, reagent quality control, and proficiency testing are all modeled after our current DNA procedures. Technicians were trained to perform the pre-processing step and operate the instruments. Modified Rapid DNA analysis was validated with a trained DNA analyst reviewing all profiles and issuing reports.



During the pilot project every kit was tested immediately, no serological analysis was completed to determine if semen was present in the kit. To increase efficiency the laboratory needed a method to identify the kits that would be the most likely to provide a profile. The laboratory had been evaluating the use of Y-screen in the months prior to the project for introduction into the casework workflow. However, the concern in sensitivity had deterred the laboratory from implementing it. Knowing that there needed to be a significant amount of male DNA present to obtain a profile using the ANDE 6C, Y-screen was explored as an option. The data from the validation allowed us to determine a threshold for male DNA that needed to be present to potentially produce a searchable profile using rapid DNA analysis. Any sexual assault kit containing 5ng or more of male DNA is routed to the Rapid DNA section for analysis.

A crucial tool for a rapid DNA program to be successful is the creation of a database to search unknown profiles against to identify suspects. ANDE provides software known as FAIRS to decrypt their data but is also capable of storing and searching DNA profiles. The laboratory designates the database maintained in the FAIRS software as the Rapid SDIS. This nomenclature is used on reports along with a statement that no profiles were entered into CODIS. This is an effort to differentiate the two processes and ensure there is no confusion about where the rapid DNA profiles are being stored. CODIS is still an important tool in the laboratory to identify suspects so any kits that do not produce a hit will have traditional analysis performed for CODIS entry. This will also be the procedure for those cases entering the court system as rapid DNA analysis has not been introduced into Kentucky courts.

Kentucky's Rapid DNA program came online July 15th. Only 22 rapid sexual assaults kits have been submitted thus far to the laboratory. 23% of the kits that have been Y-screened have qualified for rapid DNA analysis. Currently the laboratory receives approximately 950 sexual assault kits in a year. If this number holds true, the rapid DNA program could potentially process over 200 of these sexual assault kits. The average turnaround time from time the kit arrives at the laboratory until the report is complete is currently seven days. While the sexual assault kit program was the primary focus for implementing rapid DNA the laboratory validated its use for other casework samples. Blood and saliva evidence as well as human remains testing are all currently being analyzed on the ANDE 6C as part of the Kentucky State Police's rapid program.

As with any new technology in forensic science there are challenges in designing and implementing a program such as this. There were no other laboratories to share protocols and procedures with or provide answers when questions arose. With no clear-cut guidance in standards and accreditation documents our laboratory as well as our auditors have had to find a way to apply current standards to this program. As the technology advances and more laboratories implement their own programs these impediments should resolve over time. With only months of casework under our belt, the Kentucky State Police Laboratory has already processed and reported results in timeframes that were previously outside of our reach demonstrating the work is worth the reward.

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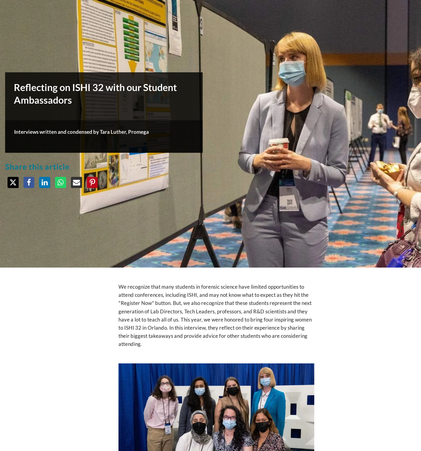

Reflecting on ISHI 32 with our Student Ambassadors

Interviews written and condensed by Tara Luther, Promega

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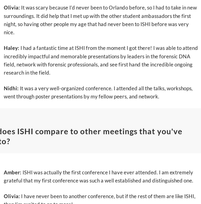
We recognize that many students in forensic science have limited opportunities to attend conferences, including ISHI, and may not know what to expect as they hit the "Register Now" button. But, we also recognize that these students represent the next generation of Lab Directors, Tech Leaders, professors, and R&D scientists and they have a lot to teach all of us. This year, we were honored to bring four inspiring women to ISHI 32 in Orlando. In this interview, they reflect on their experience by sharing their biggest takeaways and provide advice for other students who are considering attending.



What were your first impressions of the conference?

Amber: I immediately noticed how well organized the conference was! The set-up of the different rooms made for a quick walk between the presentations and vendors. The online schedule was super easy to navigate and allowed me to make sure I did not miss any of the presentations or events I was interested in. Overall, it couldn't have been a smoother experience and I enjoyed every minute of it!

Olivia: It was scary because I'd never been to Orlando before, so I had to take in new surroundings. It did help that I met up with the other student ambassadors the first night, so having other people my age that had never been to ISHI before was very nice.

Haley: I had a fantastic time at ISHI from the moment I got there! I was able to attend incredibly impactful and memorable presentations by leaders in the forensic DNA field, network with forensic professionals, and see first hand the incredible ongoing research in the field.

Nidhi: It was a very well-organized conference. I attended all the talks, workshops, went through poster presentations by my fellow peers, and network.

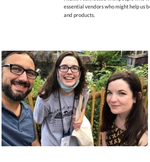
How does ISHI compare to other meetings that you've been to?

Amber: ISHI was actually the first conference I have ever attended. I am extremely grateful that my first conference was such a well established and distinguished one.

Olivia: I have never been to another conference, but if the rest of them are like ISHI, then I'm excited to go to more!

Haley: By far and away, ISHI was the best meeting I've attended. Everyone was friendly and willing to answer questions about their professional experiences, the speakers were outstanding, and the location couldn't have been better.

Nidhi: I was glad to attend a conference related to human identification which is closely related to my research. Also attending the conference in person was a bonus.





What was your favorite part of the conference?

Amber: My favorite part of the conference was stopping by all the vendor booths. I learned about many new technologies both in and outside of my discipline and had great conversations with many companies. When looking into some companies and products further, I found many webinars and resources that I am using to continue learning outside of the classroom.

Olivia: I really liked CeCe Moore's presentation on the Starkville, Mississippi homicide case that she helped solve with Parabon. I live right next to Mississippi and have been to Starkville so I enjoyed listening about a case in a town that I was familiar with.

Haley: My favorite part of the conference was attending workshops. These smaller spaces allowed for a more comfortable environment to ask questions and meet new people.

Nidhi: I was fortunate to have been selected as an ISHI student ambassador. This gave me a great opportunity to present and talk about research. I also got the opportunity to interview Mark Desire, Assistant Director at Chief Medical Examiner's Office. To sit and chat with him about his experience in the field was an invaluable experience. Special Thanks to the ISHI Organizing committee.

Did you make any new connections with attendees that will assist in your research?

Amber: I met Brian Young and Luigi Armogida from NicheVision in person for the first time! Together with NicheVision, Olivia D'Angelo, Dr. Michael Marciano, professor Jonathan Adelman, and I have been working on a project about motif specific stutter modeling. After having many phone calls with Brian and Luigi over the past year, it was great to finally put a face with the names.

Olivia: I'd like to think so. I met a lot of people from different DNA laboratories and a lot of different investigative genetic genealogists from different companies.

Haley: Yes! I asked a number of people for advice regarding my research objectives and was happy to receive some helpful feedback. I can't wait to try some of their methods out!

Nidhi: I connected with people who work in the field of Forensic Genealogy and a few essential vendors who might help us better our research work with their experience and products.

What is one thing that you learned while at the conference?

Amber: I definitely learned a lot at the conference through all the presentations I attended. I think I learned the most about Investigative Genetic Genealogy (IGG). This was a discipline I did not know much about and through the presentations I learned how it works and how it can be very effective in solving cold cases.

Olivia: The keynote presentation was about 9/11 and what they are doing to identify the still unidentified victims of the tragedy (roughly 40%). I learned a lot about how difficult those DNA extractions have been and how dedicated OCME is to return these remains to their families. I was only a few months old (born at the end of July 2001) when 9/11 occurred so I do not remember 9/11 when it occurred but I could sense the emotions from everyone in the room who were there or watched it on TV when it happened.

Haley: Networking is less intimidating that I thought it would be. The opportunity to speak with leading researchers and forensic practitioners will not only have an impact on my research, but it has also made me more excited to join the forensic DNA community!

Nidhi: I received good feedback through my poster presentation at the conference. I feel sharing your work with the forensic community and receiving their feedback was a great learning curve for me.

What are your biggest takeaways?

Amber: My biggest takeaway from the conference was to talk and have conversations with the professionals you meet. Everyone I spoke with, whether it was at my poster, at a booth, or even at Disney World, was extremely kind and willing to share their experiences and guidance with me. I am so grateful to have connected with so many scientists and professionals in the field and I hope to see them again at the next ISHI!

Olivia: I think I came out with a lot of new connections and friends. It certainly was memorable.

Haley: My biggest takeaway from ISHI is that forensic DNA research is the field I want to be in long-term, because I've never before attended a meeting in which everything I heard was what I wanted to do. I learned so much at ISHI (and had fun). I can't wait to attend next year!

Nidhi: Learning about how the forensic community is working together to solve problems at hand with ongoing innovation and research was my biggest takeaway from the conference.

Do you have any advice for other students or attendees considering attending ISHI?

Amber: My advice is to make the most out of the experience by attending as many events as you can! Attend every presentation and visit every booth even if you believe the topic doesn't interest or apply to you. I truly learned the most from and was most fascinated by these presentations and vendors and can apply that knowledge to my current internship and my research.

Olivia: Network. Make connections. Put yourself out there.

Haley: Apply to be an ISHI Student Ambassador! It was so cool to meet other students with similar interests and have the opportunity to attend ISHI. I can't recommend it enough!

Nidhi: Please do participate in the upcoming ISHI ambassador competition. It was an incredible experience. If you are planning to attend a conference related to forensic DNA analysis opt for ISHI.

Are you interested in becoming an ISHI ambassador? We will again be looking for students to represent ISHI in 2022. Stay tuned for more information, including important dates and how to apply to future issues of the magazine and on our social media channels.

Chosen applicants will be awarded free registration (including workshops) to the 33rd International Symposium on Human Identification.

This contest is open to students currently enrolled in a forensic science (or similarly named program) at the undergraduate or graduate levels. Applicants must have a public Facebook and Instagram profile to allow for sharing of content and will be asked to provide an original written essay and video recording with their submission.

Winners will be selected by a group of reviewers from the ISHI Committee. Those selected will be asked to provide:

- A 500-word blog post before the ISHI symposium
- A handful of social media posts before the symposium introducing themselves to the ISHI audience
- A scientific poster presentation at ISHI
- One Twitter post each day of the ISHI conference
- An on-site interview at the symposium with the ISHI media team
- A handful of photos taken at the event
- A 500-word blog post after the symposium about what was learned at the meeting

I-Familia: Identifying Missing Persons Globally Through International DNA Kinship Matching

François-Xavier Laurent, PhD, DNA Database Manager, INTERPOL
Susan Hitchin, PhD, DNA Unit Coordinator, INTERPOL

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The identification of human remains belonging to missing persons persists as a challenging process in forensic genetics. When unidentified human remains are discovered, a comparison between the post-mortem DNA profile (obtained from the remains), and the ante-mortem DNA profile (conventionally obtained from personal items of the missing person or from a previous medical sample) is the most reliable method for identification [1].

A number of countries have established national missing person DNA programmes, which are very effective when the disappearance of individuals and the discovery of unidentified human remains occur within the same country. However, many countries have evolved missing person investigations and unidentified human remains that cannot be identified using their national systems alone. International cooperation in missing person investigations is therefore highly advised in light of increased global migration, the consequences of growing transnational crime and human trafficking, the vulnerability of migrants and refugees, and the latter's high risk for being victims of a criminal act.

INTERPOL, the world's largest international police organization, is mandated to assist in international investigations, including missing persons, by connecting its 194 member countries. The Organization works primarily with national law enforcement agencies via the INTERPOL National Central Bureau in each country but can also cooperate with other international entities involved in Disaster Victim Identification situations and missing person cases. INTERPOL provides the legal framework and technical infrastructure for a secure exchange of information and access to its 19 databases, thereby enabling police around the world to collaborate (**Figure 1**).



Figure 1: INTERPOL's 19 databases.

Created in 2002, the INTERPOL DNA database currently contains more than 250,000 profiles contributed by 86 member countries. National Central Bureaus and international entities can submit a DNA profile from offenders, crime scenes, missing persons and unidentified human remains, with an automated search result provided within minutes. There is no nominal data attached to the profile and member countries retain ownership of their information, in line with INTERPOL's data processing rules. Countries can also choose with whom they wish to make their data available for comparison. The INTERPOL DNA database has enabled investigators around the world to link offenders to different types of crime including rape, murder and armed robbery as well as to formally identify missing persons found deceased outside their national borders from where they were reported missing.

However, in many cases, direct DNA matches are not possible as ante-mortem DNA profiles are either unavailable or insufficient to confirm the missing person's identity. This could be due to the lack of available personal items or medical records, often key to missing person investigations, or due to the displacement of populations and the destruction or lack of property. Consequently, in the majority of cases, ante-mortem DNA data can only be obtained through relatives donating biological samples to the requesting authorities.

While most laboratories have the capacity and experience to perform relatively simple kinship testing, such as paternity tests, the evaluation of complex kinship scenarios is far more challenging [2]. Specialized computer software is often required to undertake comparisons of ante-mortem and post-mortem data to perform complex kinship calculations with large datasets of DNA profiles. This software computes likelihood ratios (LR), which are the optimal basis for statistical decisions, regardless of whether there is a hypothesis about prior probabilities [3]. Using specific allele frequencies from the reference population to which the missing person belongs, the fit of a missing person for any given pedigree (e.g. parent, child, or sibling of the missing person) is measured by comparing two hypotheses; H1 and H2. H1 supports that the individual is related to a defined pedigree, whereas H2 states that they are unrelated [2]. Although this method is relatively straightforward to implement in a national environment where the missing person and the unidentified human remains are reported within the same country, many challenges needed to be resolved before applying this method to an international configuration.

INTERPOL has addressed these challenges, and in June 2021, officially launched its 19th database, I-Familia, dedicated to international DNA kinship matching for missing persons (**Figure 2**).

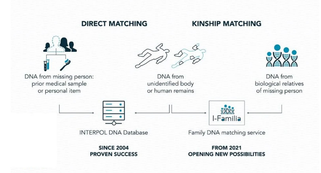

Figure 2: The DNA comparison process for missing person investigations using the INTERPOL DNA Database and I-Familia.

I-Familia, which stands for "INTERPOL Family Associated Matching to Identify Lost Individuals Abroad" is a pioneering service that is free-of-charge and available to all 194 member countries to help find potential biological relationships between DNA profiles from biological relatives of missing persons and unidentified human remains globally. I-Familia is composed of three components. First, a dedicated DNA database hosts anonymized DNA profiles from the biological relatives of missing persons and from the unidentified human remains. This is a stand-alone database with no links to other INTERPOL databases housing criminal data. Secondly, the state-of-the-art forensic DNA matching software BONAPARTE [4] is used to handle LR computations for any family pedigree with the available DNA data (autosomal STRs, Y-STRs, mitochondrial DNA) with all unidentified human remains DNA profiles. Thirdly, interpretation guidelines have been established to help users with the LR interpretation and the decision-making process that leads to the rejection or reporting of a potential biological relationship.

DNA kinship matching, based on autosomal STR profiles, requires the use of allele frequencies from the appropriate reference population. As information on the ancestry of DNA profiles is very often lacking or incorrectly reported, I-Familia enables LR computations using worldwide allele frequencies [5] and a theta correction factor to account for the degree of relatedness of alleles within a closely shared ancestry. To streamline the decision-making process and the review of potential biological relationships, extensive pedigree simulations were carried out to accurately determine optimal LR thresholds. Based on the 10 most frequently encountered scenarios in missing person investigations (depending on the availability of DNA profiles from biological relatives such as parents, children or siblings) and the number of overlapping STR loci (ranging between 6 and 24 loci), the LR thresholds help prevent the reporting of false-positive matches while minimizing the rejection of false-negative matches. Interpretation tables, specifically designed to reflect the expected number of adventitious matches for each type of match, are extremely useful to determine the best outcome (review the match, reject the match or require additional DNA information).

When the strength of the DNA evidence is sufficient, and ante-mortem and post-mortem data are compatible (if available), a potential biological relationship report is issued to both sources of data who can then cooperate on a bilateral basis to confirm the identification using their national procedures (**Figure 3**).

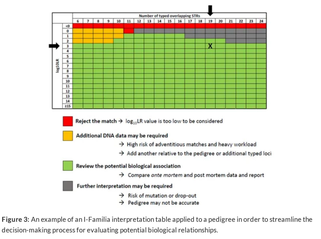

Figure 3: An example of an I-Familia interpretation table applied to a pedigree in order to streamline the decision-making process for evaluating potential biological relationships.

I-Familia has already been successful to help member countries with the identification of missing persons found in another country from where they were reported missing. The first confirmed match was found between the children's DNA profiles of an Italian missing person and that of a body found in the Adriatic Sea by the Croatian police in 2004, therefore closing a case that had gone cold 16 years earlier.

By late 2021, over 12,000 active Yellow Notices – international police alerts for missing persons – had been issued by the INTERPOL General Secretariat highlighting the need for greater international cooperation. I-Familia is a humanitarian tool which, enabled by INTERPOL's global reach, opens up vast new possibilities to identify missing persons and to provide families with answers.

With the purpose of providing scientific credibility and transparency to our member countries, the INTERPOL DNA Unit has recently published an article on the developmental validation of our interpretation guidelines for international DNA kinship matching [6]. This article describes the scientific rationale behind I-Familia and will allow forensic DNA experts around the world to replicate INTERPOL's method and use this process nationally in the event of a missing person investigation for whom the genetic ancestry is unknown or uncertain.

For any additional information on I-Familia, please consult the I-Familia brochure [7] or contact the INTERPOL DNA unit at DNA@interpol.int.

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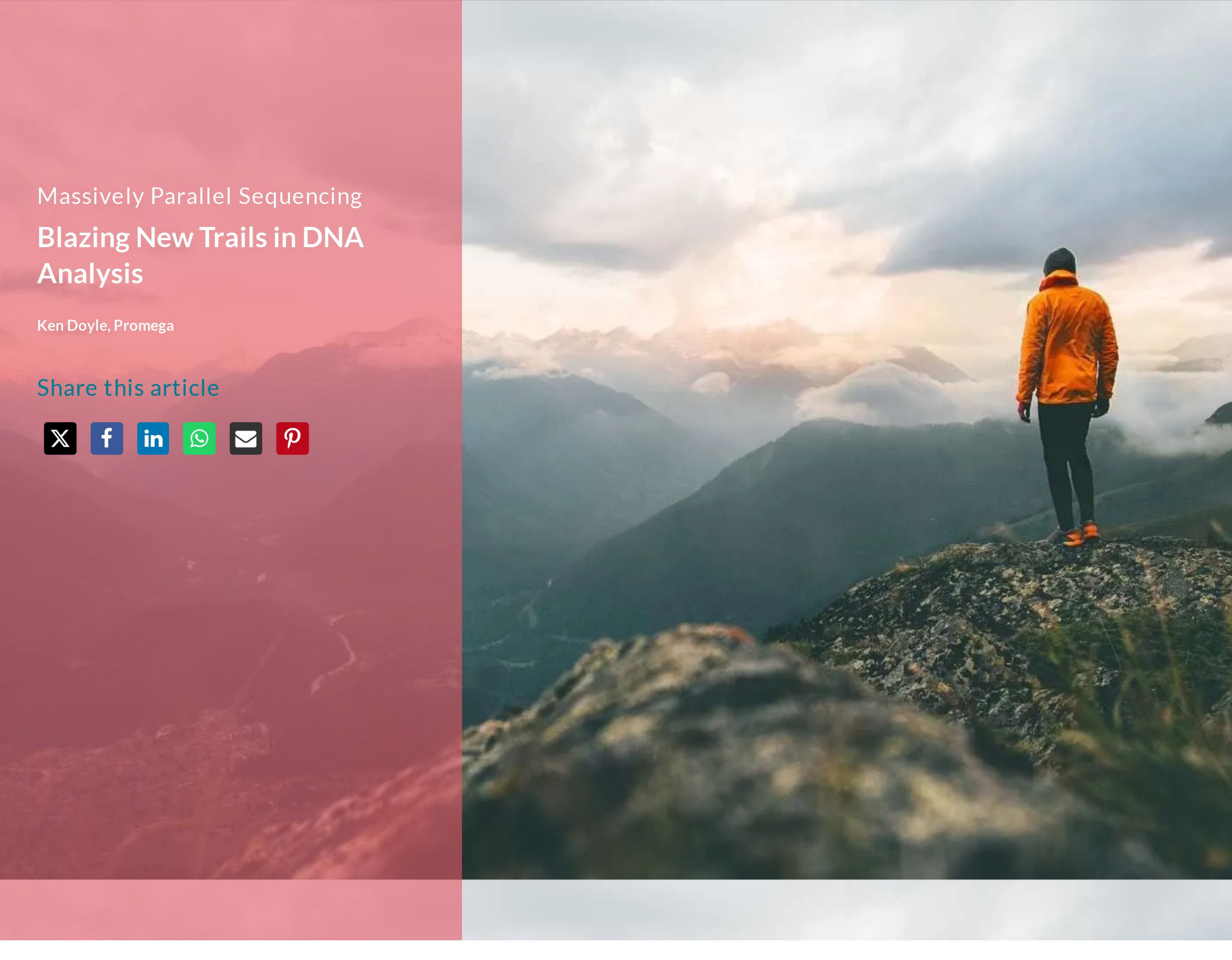

Massively Parallel Sequencing

Blazing New Trails in DNA Analysis

Ken Doyle, Promega

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Analysis of short tandem repeats (STRs) by capillary electrophoresis remains the most popular method for human identification in forensic laboratories. However, interest in massively parallel sequencing (MPS) is growing rapidly, as reflected in the increasing number of MPS-related presentations and posters at the International Symposium on Human Identification (ISHI) over the past few years.

MPS enables high-throughput analysis of forensic samples, and MPS-based methods can provide information on many more loci compared to conventional STR analysis, thereby offering greater discriminatory power. MPS is especially useful for processing challenging samples, like mixtures or degraded DNA. Modifications to a typical sequencing workflow, such as hybridization-capture sequencing, have made it possible to obtain DNA profiles from samples that previously yielded no useful information by conventional STR analysis. Recently, a hybridization-capture MPS workflow was validated by the Armed Forces Medical Examiner System's Armed Forces DNA Identification Laboratory (AFMES-AFDIL) and used to identify historical human remains (see *Technological Innovation Aids the Identification of Fallen Soldiers*).

At ISHI 32, held in Orlando, Florida (and virtually), several presentations and posters discussed a variety of human identification applications for MPS. Three presentations by graduate students are highlighted here.

DNA Analysis of Historical Remains

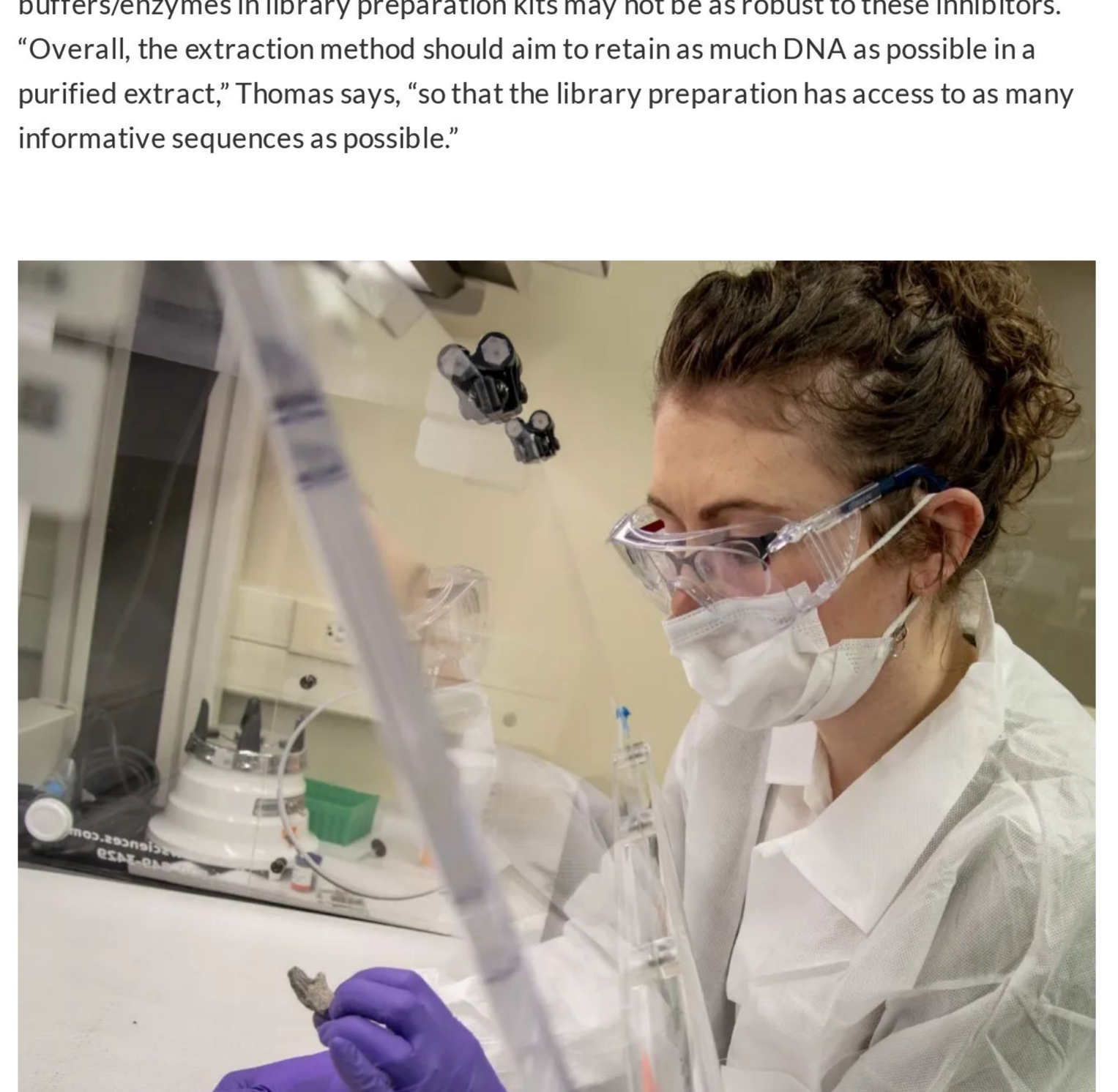
MPS has proved valuable in working with historical human remains and other highly degraded samples. Typically, mitochondrial DNA (mtDNA) analysis is used for identification of older samples, because mtDNA is more abundant than nuclear DNA. Forensic scientists at AFMES-AFDIL routinely work with skeletal samples that have been compromised by chemical treatment or otherwise degraded over time. A vital process in the analysis workflow is the initial extraction step that is used to obtain DNA from the samples.

Elena I. Zavala, PhD Student at the Max Planck Institute for Evolutionary Anthropology, presented the results of a research project to compare DNA extraction and MPS library preparation methods for historical samples. The project was carried out in collaboration with AFMES-AFDIL, including FS2 Research Analyst Jacqueline T. Thomas, (SNA International LLC contractor supporting AFMES-AFDIL).

Conventional STR analysis often fails with historical human remains for several reasons. The samples may be chemically treated for preservation, which results in permanent changes to the DNA. In addition, the DNA may be highly fragmented and contaminated with both non-human DNA from the environment and more recent human DNA. "This means that DNA fragments of interest are limited and are likely too small for conventional methods," Thomas explains. "Conventional methods utilize PCR, requiring DNA fragments that are approximately 100 base pairs and larger, while many of our historical samples average 60 base pairs and lower." MPS-based analysis can overcome the size limitations, as sequencing libraries can be prepared successfully from very small DNA fragments.

Extracting usable DNA from historical remains—typically, from bones or teeth—poses its own challenges. "It is important to have a very sterile environment, starting with sampling and powdering of the bone samples, to avoid introducing modern contaminants," Thomas says. Several variables need to be optimized for successful DNA extraction, including the amount of bone powder extracted, the composition and relative volume of the buffer used to demineralize the bone powder, and the temperature and time for demineralization. Thomas notes that demineralization of powdered bone or teeth should yield a crude extract that has released as much DNA as possible from the bone cells, or osteocytes, without causing further damage to the DNA.

The method used to purify DNA from the crude extract is also critical. Some popular DNA purification methods rely on size-exclusion spin columns, in which DNA fragments smaller than a certain size or molecular weight limit are eliminated. If such a column normally designed to purify DNA for STR analysis is used with historical DNA samples, the process may yield little or no usable DNA, because the STR analysis workflow is based on larger DNA fragments. In addition, Thomas says, "the purification step of the extraction must sufficiently purify out inhibitors that could interfere with the various enzymes used in library preparation." Although many of the same inhibitors may be present in historical and modern samples, the buffers/enzymes in library preparation kits may not be as robust to these inhibitors. "Overall, the extraction method should aim to retain as much DNA as possible in a purified extract," Thomas says, "so that the library preparation has access to as many informative sequences as possible."



Shannon Ryan, AFMES-DoD DNA Operations DNA analyst, sands a vertebra to get rid of any external contaminant before DNA extraction. (U.S. Air Force photo by Staff Sgt. Nicole Leidholm, provided by AFMES).

The research team tested several forensic DNA extraction protocols with or without a DNA repair step using uracil DNA glycosylase (UDG), a protocol optimized for ancient DNA, and a combined forensic-ancient DNA extraction protocol. After evaluating the sequencing data, the ancient DNA extraction protocol produced the highest proportion of informative sequences. This protocol is currently being tested at AFMES-AFDIL for casework samples.

Zavala's research compared three different library preparation methods: the KAPA Hyper Prep kit, which has been used extensively at AFDIL; the SRSLY™ kit (Claret Bioscience); and an automated protocol, specifically developed for ancient DNA samples at the Max Planck Institute (MPI). The KAPA kit requires double-stranded input DNA, while the other two methods can prepare libraries from single-stranded DNA.

Since the DNA from the samples tested was highly fragmented, it's likely that a large proportion of it was single-stranded; therefore, the KAPA kit did not produce the most complete results with these samples. "This is why we wanted to test the effectiveness of the two single-stranded DNA library preps for this sample set," Zavala says. Sequencing data showed that both single-stranded library preparation methods produced a significantly higher proportion of informative sequences, compared to the double-stranded protocol.

Zavala expects that hybridization-capture sequencing, which can be applied to both mtDNA and nuclear DNA, could increase future success rates for identification of ancient DNA samples. She also sees the potential for increasing throughput and decreasing per-sample costs by automating workflows.

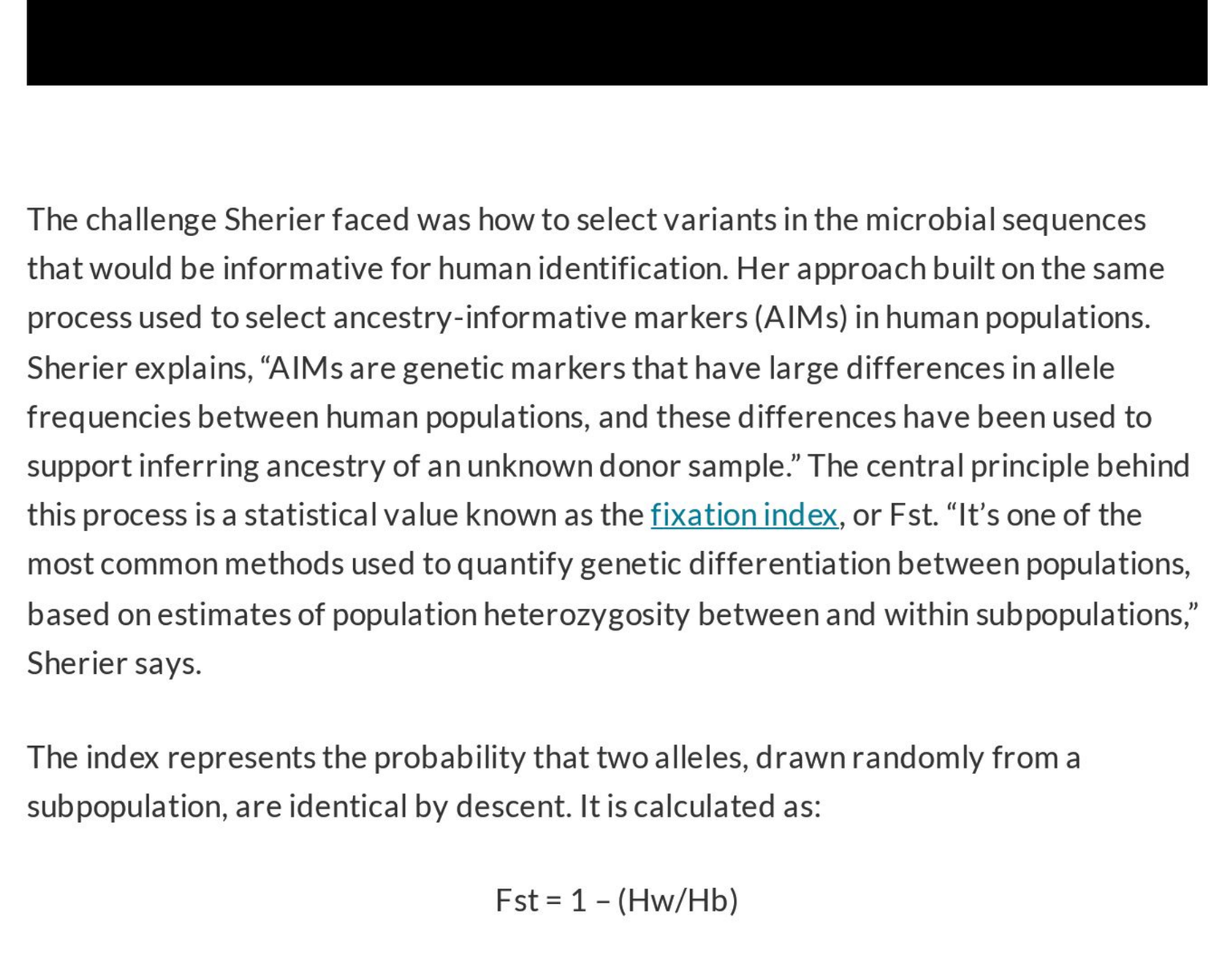
Using the Skin Microbiome for Human Identification

The skin is the largest human organ, and it plays host to a diverse collection of microorganisms, collectively known as the skin microbiome. Multiple factors affect the composition of the skin microbiome, but a core group of microorganisms tend to remain fairly stable. Understanding the interaction between the skin microbiome and skin cells has guided key discoveries in fields from cosmetology to dermatology.

Now, the skin microbiome is showing promise in yet another field—that of human identification. Allison Sherier discussed the latest results of her research into this novel forensic application. Sherier is a PhD candidate at the Center for Human Identification, University of North Texas Health Science Center, and she was an ISHI Ambassador in 2019.

In her presentation, Sherier summarized previous studies that have shown the skin microbiome composition remains relatively stable for up to 3 years. It is also an abundant source of DNA: a skin swab typically contains DNA equivalent to only four human diploid cells but enough microbial DNA to allow accurate identification. For casework samples, microbial DNA profiling can provide valuable information that complements human DNA profiling. "In a case where nuclear DNA is left behind but it is too little or too degraded to obtain a complete profile," Sherier says, "the microbial profile collected on the same swab may be able to provide supportive information for human identification."

Typically, microbiome characterization is performed using 16S ribosomal RNA gene sequencing such as in metagenomic studies. While this approach can provide relatively rapid and simple identification of bacterial species, it has its limitations when bacterial DNA is used for human identification. "These limitations include insufficient resolution at the species and strain level, copy number variation, inaccurate phylogenetic predictions, sample preparation bias and PCR bias," Sherier says. As an alternative, she recommends whole-genome sequencing (WGS). However, in some situations, WGS does not cover the entire genome of all the microorganisms present in a skin microbiome sample. For Sherier's research, she used a targeted genome sequencing panel known as hidSkinPlex. This custom panel enriches sequencing libraries in characteristic sequences for 286 microorganisms commonly found in the skin microbiome. "The hidSkinPlex allows for high power of discrimination for human identification, by focusing on specific and informative skin microorganisms," Sherier says.



The challenge Sherier faced was how to select variants in the microbial sequences that would be informative for human identification. Her approach built on the same process used to select ancestry-informative markers (AIMs) in human populations. Sherier explains, "AIMs are genetic markers that have large differences in allele frequencies between human populations, and these differences have been used to support inferring ancestry of an unknown donor sample." The central principle behind this process is a statistical value known as the fixation index, or Fst. "It's one of the most common methods used to quantify genetic differentiation between populations, based on estimates of population heterozygosity between and within subpopulations," Sherier says.

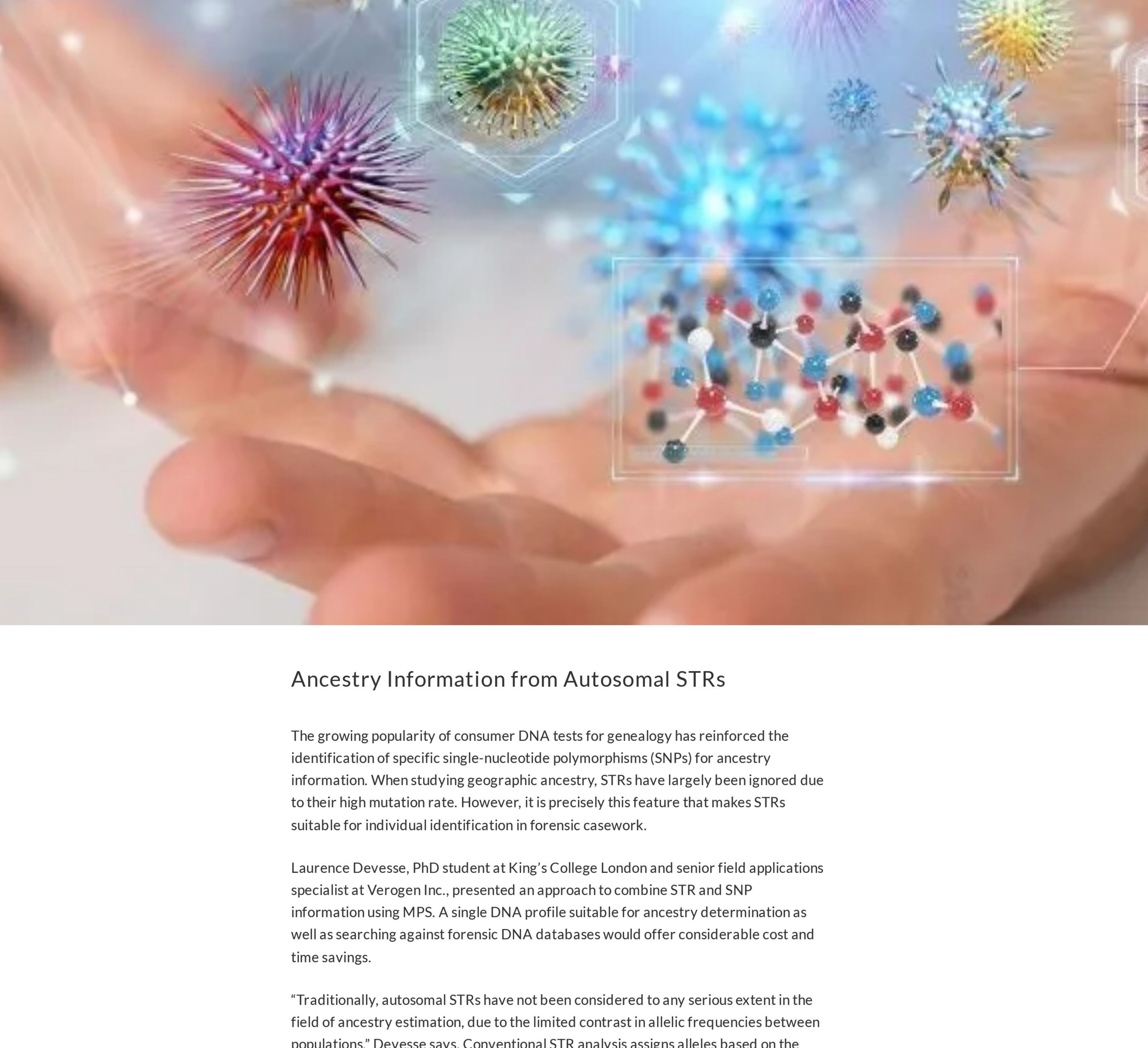
The index represents the probability that two alleles, drawn randomly from a subpopulation, are identical by descent. It is calculated as:

$$Fst = 1 - (Hw/Hb)$$

where Hw is the mean number of pairwise differences within a population, and Hb is the mean number of pairwise differences between two populations. Alleles from two completely identical populations would have Fst = 0, while two completely different populations (i.e., with no shared alleles) would result in Fst = 1. In the case of Sherier's research, each population is the skin microbiome from a single individual. "Fst may provide insight into whether two microbial alleles are identical by descent between two microbial populations," Sherier says.

For Sherier's project, the research team collected skin swabs from the non-dominant hand of 51 individuals in triplicate, extracted DNA, enriched the samples prior to library preparation using the hidSkinPlex panel, and sequenced the libraries using an Illumina MiSeq® system. Pairwise Fst comparisons were conducted between all samples, and the data were analyzed to classify unknown samples to the individual that they most resembled. The accuracy of classification ranged from 88% to 95%, suggesting that AIMs in targeted microorganisms can improve the accuracy of human identification.

Sherier notes that it is premature to compare the accuracy of identification using the skin microbiome to conventional human identification analysis using STR markers. "After reliable markers are selected, and an analysis panel is developed, then more research can be done on the stability and transfer rate of the human skin microbiome," she says.



Ancestry Information from Autosomal STRs

The growing popularity of consumer DNA tests for genealogy has reinforced the identification of specific single-nucleotide polymorphisms (SNPs) for ancestry information. When studying geographic ancestry, STRs have largely been ignored due to their high mutation rate. However, it is precisely this feature that makes STRs suitable for individual identification in forensic casework.

Laurence Devesse, PhD student at King's College London and senior field applications specialist at Verogen Inc., presented an approach to combine STR and SNP information using MPS. A single DNA profile suitable for ancestry determination as well as searching against forensic DNA databases would offer considerable cost and time savings.

"Traditionally, autosomal STRs have not been considered to any serious extent in the field of ancestry estimation, due to the limited contrast in allelic frequencies between populations," Devesse says. Conventional STR analysis assigns alleles based on the length of the PCR amplicon—i.e., the number of "repeats" contained at the STR locus as revealed by capillary electrophoresis. "If we see two peaks," Devesse says, "we assume it's a heterozygote profile." In other words, there are two different sized alleles at that locus. "If we see one peak, we assume it's homozygous, so both alleles are exactly the same." However, when these same samples were analyzed by MPS, Devesse discovered that there were, in fact, sequence variations within the repeats, even though the amplicons were the same length. Thus, MPS revealed allelic variation that was missed by conventional STR analysis using capillary electrophoresis.

For example, at locus D12S391, Devesse observed 7 different versions of an allele 20 across 200 samples from the white British population. Sequence variations showed up both within the repeat region and the flanking regions of STRs. Devesse points out the need to understand and characterize these variants, and she also notes that, "we need frequencies for all of these new alleles that we're finding."

Devesse genotyped approximately one thousand samples from five global population groups using the MiSeq FGx™ System (Verogen). Her team prepared sequencing libraries using the ForenSeq™ DNA Signature Prep Kit, which targets 27 autosomal STRs, 7 X STRs, 24 Y STRs and 94 SNPs simultaneously, when using Primer Mix A. "What we see is that across all the markers studied, across all populations, we have a huge increase in the number of discernible alleles observed," she says.

Further analysis brought new insights. "I started noticing just how many of these autosomal alleles only appeared to be present in one of the population groups I was studying," Devesse says. At D12S391, she observed 38 alleles that only appeared in one population group. Most of these population-specific variants were sequence-based, not length-based. "My question was: can we predict differences in populations using sequence-specific STR alleles?"

After mapping allele frequencies, Devesse identified sets of common alleles that could indeed be used for population identification, both on their own and in combination with ancestry-informative SNPs. Previous studies by other researchers using capillary electrophoresis data suggested that doing so required a much larger marker set: up to 779 non-CODIS STR loci for adequate population differentiation. However, Devesse points out that doing so "is often not really feasible in the context of a forensic investigation."

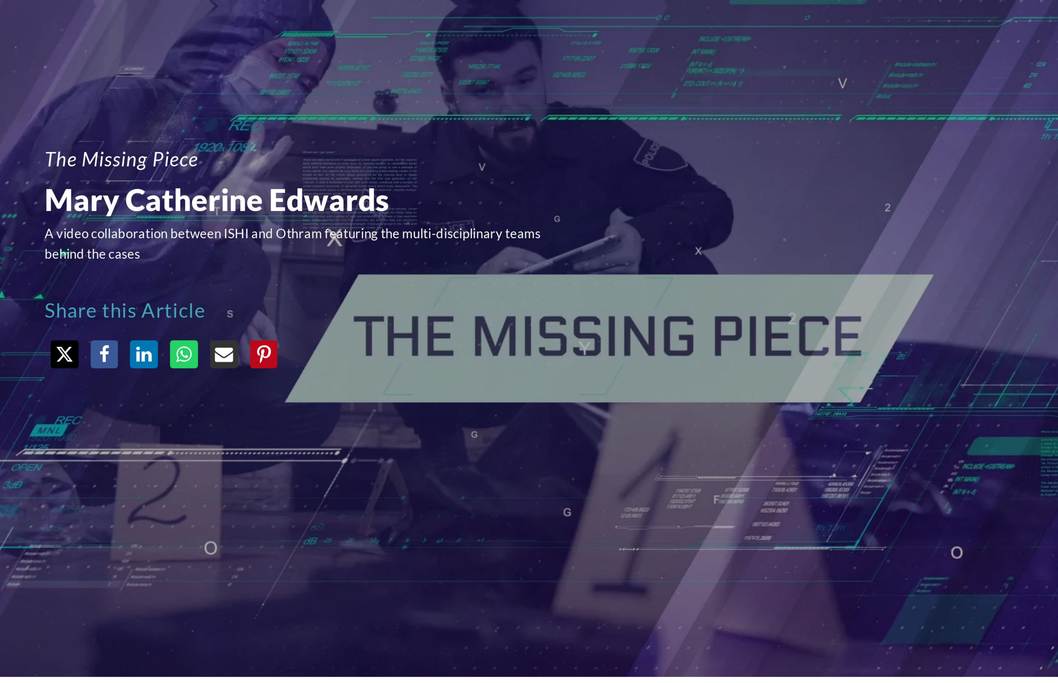
Attempting to narrow the scope, Devesse found that the majority of samples could be assigned to the correct population group using data from just 27 autosomal STRs. Next, she looked at whether information from ancestry-informative SNPs could be combined with autosomal STR data. She reanalyzed a subset of the DNA samples using a primer set that included 56 ancestry-informative SNPs. The addition of ancestry-informative SNP data improved ancestry assignment from autosomal STRs, but the improvement was not significant in all population groups. "The most interesting finding," Devesse says, "is that over 85% of the samples were assigned to the correct population cluster…based on autosomal STR sequencing data alone." She adds, "The ability to predict ancestry on a continental basis from STR results alone is an exciting, untapped resource that MPS analysis makes possible."

What's Next?

Future developments in MPS workflows should further lower the costs to make large-scale typing more accessible. Sherier points to the need for more user-friendly software and bioinformatics pipelines. Increasing acceptance of these methods will also require "better teaching and training tools, such as virtual reality, to educate current forensic scientists and future forensic scientists," she concludes.

One innovation Zavala would like to see is an efficient method to assess the quality of skeletal remains before beginning DNA extraction. "A way to cheaply and quickly screen remains, so that the correct analytical techniques are applied, would revolutionize the way samples are handled," she says.

For ancestry testing using STRs, Devesse says the main advance that will accelerate its uptake in forensics is the user-friendliness of ancestry prediction software. "The data are often there; they just need interpreting and reporting."



The Missing Piece

Mary Catherine Edwards

A video collaboration between ISHI and Othram featuring the multi-disciplinary teams behind the cases

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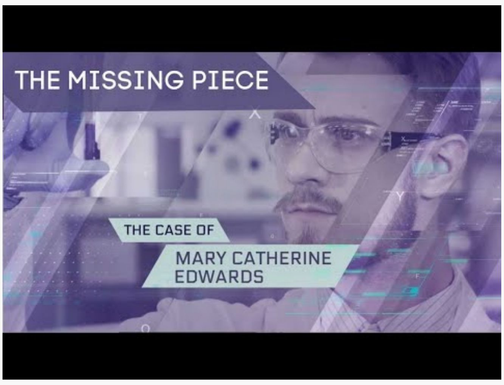
For years, victims were listed on cold case files in law enforcement evidence boxes. While never forgotten, limitations in forensic science prevented their cases from being closed. Families waited decades for answers and justice while their killers remained unidentified.

In 2018, a new tool for crime solving rose to prominence as the notorious Golden State Killer was finally unmasked; forensic genetic genealogy. To begin, investigators upload their unknown DNA profile to the site, which generates a list of the closest genetic matches, based on how many centimorgans (cM) of DNA are shared with the unknown person. Next, investigators will begin to build back the unknown person's family tree until a common ancestor is uncovered. Then, they will build the family trees forward, identifying every descendent possible, using traditional genealogy techniques. Once the trees have been fully built out, investigators look to identify candidate persons that might match the unknown person. Once a person of interest has been identified, new DNA can be collected to compare to the original forensic sample.



In January 1995, the parents of Mary Catherine Edwards, a "well-loved" Beaumont, Texas school teacher, went to her home to check on her. Mary lived alone, had not responded to calls and her parents were concerned. Upon arrival, they found she had been drowned in her bathroom. Beaumont detectives later determined that the Mary had been sexually assaulted before her murder. Through the years, investigators worked the case and all available leads. DNA from an unknown male was found at the crime scene and a STR profile was produced, however it returned no matches in a CODIS search. Traditional forensic DNA testing was used to exclude many people over the years and eventually all leads were exhausted and the case went cold.

In 2020, the Texas Rangers and Beaumont PD renewed efforts on the case and offered a $6000 reward for information leading to the arrest of the person responsible for Mary Catherine Edwards' sexual assault and murder. In addition, the Texas Rangers Unsolved Crimes Investigation Program and Beaumont Police Department teamed up with Othram to leverage advanced DNA testing and forensic genetic genealogy to help uncover new investigative leads that would identify the unknown person from the crime scene. There was remaining evidence from the 1995 crime and several items were sent to Othram, where lab scientists eventually developed a DNA extract that was suitable for advanced DNA testing. Processing the DNA, a mixture of an unknown male and the victim, the Othram team leveraged Forensic-Grade Genome Sequencing® to produce an enhanced DNA profile that could be used for forensic genetic genealogy.

The Texas Rangers Unsolved Crimes Investigation Program, working with Beaumont PD, lead the investigative effort leveraging family trees and public records to identify the unknown male from the crime scene. Eventually, the unknown man was identified as Clayton Foreman. Although he was not a person of interest in the original investigation, he knew the victim from high school.

Clayton had been residing in Reynoldsburg, Ohio. With the assistance of the Jefferson County District Attorney's Office an arrest warrant was obtained. The Jefferson County Sheriff's Office Aviation Unit flew investigators to Reynoldsburg, Ohio where Foreman was arrested and later extradited to Jefferson County.

On Wednesday, September 22, 2021, Foreman was indicted for the Capital Murder of Mary Catherine Edwards.

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