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AFMES, DNA IDENTIFICATION LABORATORY

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DNA FAQs

Who is AFMES-AFDIL?

The Armed Forces Medical Examiner System's-Armed Forces DNA Identification Laboratory (AFMES-AFDIL) is the operational element of the AFMES and is accredited by the American Society of Criminal Laboratory Directors- Laboratory Accreditation Board (ASCLD-LAB-International) to both ISO-17025 and FBI Quality Assurance Standards for Forensic DNA testing. AFMES-AFDIL is the Department of Defense's (DoD's) only human remains DNA testing laboratory, which under 10 U.S.C. 1471 and 10 U.S.C. 1509 is charged with providing human remains DNA testing in support of current day operations (AFMES), past accounting operations (Defense POW/MIA Accounting Agency; DPAA) and other DoD Agency missions. Additionally, through other memorandums of agreement, AFMES-AFDIL will provide DNA testing for identifying human remains or to determine the probable contributor of biological samples of human origin in criminal investigations for other Bureaus of the Federal Government and civilian medical institutions. AFMES-AFDIL's Past accounting mission is comprised of the Past Accounting Casework section and the Family Reference Specimen section which are dedicated to processing all of the DPAA and Family Reference specimens.

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What are AFMES-AFDIL's current staffing?

In 2013, AFMES-AFDIL requested funding to build our capability and capacity to support 200 identifications per year. We had asked for 32 new hires and were granted 24 starting in 2015. Due to internal efficiencies, AFMES-AFDIL was able to hire a total of 52 new employees, which allowed AFMES-AFDIL to increase testing and support the DPAA in reaching 201 identifications in 2017. To meet increased DPAA submissions for 2018 and the future, AFMES-AFDIL is currently able to meet DPAA needs with existing staff and instruments, but has requested additional funding to grow to meet DPAA's needs.

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What is DNA?

DNA (deoxyribonucleic acid) is a double stranded molecule of helical structure containing the genetic code that makes you who you are. You inherit your DNA from your parents and it determines certain characteristics such as hair color, eye color and other physical attributes.

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What is DNA composed of?

All DNA is constructed of the same four nucleotide bases: Adenine (A), Guanine (G), Cytosine (C) and Thymine (T), which are organized into two complementary helical strands. The number of Adenine bases is equal to the number of Thymine bases while the number of Guanine bases is equal to the number of Cytosine bases (A=T and G=C). For all DNA, a base-pair is considered an "Adenine base" pairing with a "Thymine base" on the opposite strand or a "Guanine base" pairing with a "Cytosine base" on the opposite strand. It may be easier to think of double stranded DNA as being arranged like a ladder where the sides of the ladder represent the individual strands of the DNA molecule and the rungs of the ladder are comprised of A-T or G-C base-pairs. The rungs of the ladder can contain either nucleotide base of the pair, and either base could be on either strand.

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What are the different types of DNA available for testing?

All human cells with a nucleus contain two types of DNA: 1) **nuclear DNA** (nucDNA), which is found within the nucleus of the cell; and 2) **mitochondrial DNA** (mtDNA), which is found within the mitochondria of the cell. Both of these types of DNA can be utilized for human identification and forensic testing.

Nuclear DNA, which is found as a single copy within all nucleated cells, is what is most commonly used for human identification and forensic DNA testing. Nuclear DNA is made up of 23 pairs of chromosomes (22 pairs of autosomes and one pair of sex chromosomes) for a total of 46 individual chromosomes. A chromosome is a discrete bundle of genetic information, with one chromosome of a chromosomal pair being inherited from your mother and one being inherited from your father. There are two types of DNA in the nucleus: autosomal DNA and Y chromosomal DNA (Y-DNA).

Strictly speaking, only autosomal DNA (auDNA) is unique to each individual, which makes it a powerful tool for DNA identifications. Autosomal DNA testing uses specific, well defined locations (or loci), which are found throughout the 22 pairs of autosomal chromosomes and the sex determining chromosomes (the X and the Y). Each locus consists of a short sequence, commonly referred to as an autosomal short tandem repeat (auSTR), and the quantity of these repeats determines the specific 'numerical value' associated with each locus. The 'numerical values' for each locus are combined to make up your 'STR profile'. You will always share half of your numerical values with your biological mother and half with your biological father, but you may not necessarily share any numerical values with your siblings (Figure 1).

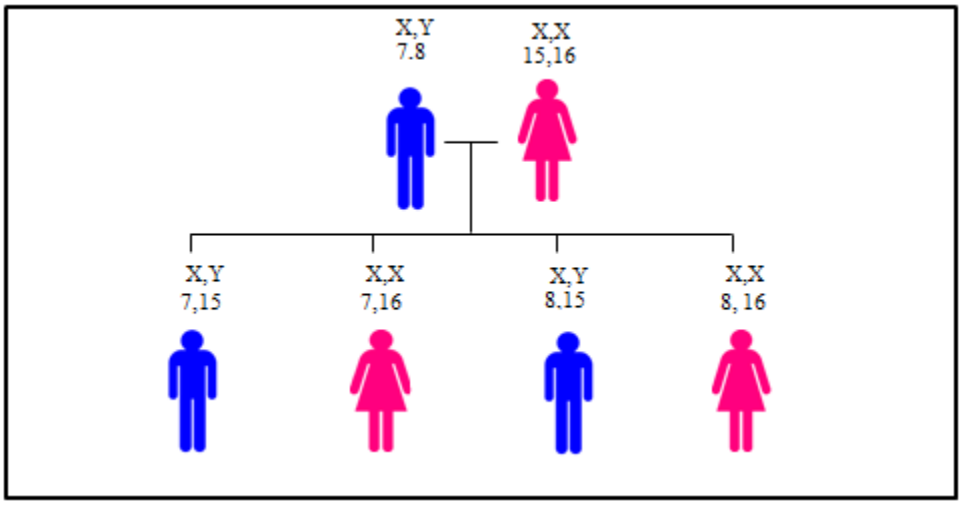

Figure 1. An example of how chromosomal, or allelic, information may be passed from parents to children. As noted, siblings do not necessarily share any allelic information.

Y-DNA analysis is only possible on male individuals, as it is an analysis of locations (or loci) on the Y-chromosome. The 23rd chromosome pair is responsible for determining the sex of an individual, with women having two X chromosomes (XX) and males having one X chromosome, which is donated from the mother, and one Y chromosome, which is donated from the father (XY). Y-DNA is found as a single copy within human nucleated cells. Y chromosomal DNA is passed from father to son thru the paternal lineage. It is extremely stable, does not change from generation to generation, and is rich in well-defined short tandem repeats. Although Y-DNA is not unique to a specific person—as all individuals in a family's paternal lineage share it—it is exceptionally useful since any male of the paternal lineage can serve as a reference.

Mitochondrial DNA (mtDNA) is the second type of DNA found within human cells that can be used for identification. It is located in the mitochondria of the cell. Within a single cell, hundreds to thousands of mtDNA molecules can be found. Mitochondrial DNA, like Y-DNA, is a lineage marker, however, it is only transmitted through the maternal line. What this means is that you and your siblings will share the same mtDNA profile as your biological mother, but, if you are a male, your children will have their biological mother's mtDNA. This sharing among a maternal lineage makes it extremely useful when dealing with cases where viable nuclear DNA references are unavailable. For example, a maternal fourth cousin will still have the same mtDNA profile as a sibling, making this type of testing invaluable as the cases extend further back in time. Mitochondrial DNA testing is different from auSTR and Y-STR testing in that instead of determining the numerical value at a specific location, the testing determines the individual's DNA base composition within a set region. When an individual's base composition is compared to a set reference, the base differences or 'polymorphisms' make up an individual's mito-type.

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Where is DNA found?

Autosomal and Y-chromosomal DNA are found within the nucleus of each cell of the body. There is a single copy of nuclear DNA, which is comprised of autosomal and Y- chromosomal DNA. Mitochondrial DNA (mtDNA) is found in the mitochondria of the cell. Mitochondria, found in the cytoplasm, or "body," of the cell, are like batteries or powerhouses—they provide energy to the cell. There are hundreds to thousands of mitochondria per cell. Each mitochondrion contains its own DNA, separate from the nucleus. Even after many years, during which time all DNA degrades to some extent, mtDNA can be found in very small fragments of biological material. If there is sufficient quality, it can be tested and a sequence can be generated.

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What DNA tests are used to identify missing service members?

Autosomal Short Tandem Repeat (auSTR) and Y chromosomal STR (Y-STR) tests are used to analyze nuclear DNA, Mitochondrial DNA control region or whole genome sequencing is used to analyze mitochondrial DNA. All thee DNA tests can be used to aid in the identification of missing service members. Because of the age and degradation of the DNA due to environmental conditions, mitochondrial DNA testing is the most sensitive and is usually the first type of DNA testing used. If the appropriate reference materials are available, autosomal DNA and Y-DNA will be tested as well. All of the DNA information can be used to calculate a combined likelihood statistic. The likelihood statistic assesses the evidential support for the identification hypothesis that the DNA from the unknown sample is biologically related to the associated references (auSTR, Y-STR and mtDNA).

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Is there a gold standard when it comes to DNA forensic testing?

There is no such thing as a "gold standard" with regards to forensic DNA testing and its use in the identification process. Different DNA testing methods have different strengths and weaknesses when testing highly degraded samples and their use in the human remains identification process. For example, in criminal DNA forensic cases, where the goal is to identify an unknown individual from among the world's approximately 7 billion population, then autosomal STR's (nuclear DNA) from a direct reference may prove to be the most definitive method, as it is an exact match to the suspected individual. However, for identifications involving missing individuals in closed populations (specific loss incident), the combination of mtDNA and/or Y-STR and/or auSTR testing can be the most effective method. An example of a closed population is a bomber crew of 10 individuals lost during a war. The DPAA, after recovery operations, submits skeletal elements to AFMES-AFDIL for testing. AFMES-AFDIL generates 10 unique (not shared between individuals) mtDNA sequences from the submitted samples and those 10 unique mtDNA sequences match maternal references for the 10 missing service members. In this instance, since the loss incident is a closed population (limited number of people), the mtDNA sequence testing alone can support a DNA based identification. Mitochondrial DNA testing also is highly effective in compromised skeletal cases because of its durability and high-copy number per sample. Additionally, mtDNA is very effective for use in closed-population groups and in situations where autosomal (auSTR) or paternal (Y-STR) reference samples may be difficult to obtain—a pragmatic reality often not appreciated by individuals with limited experience working on compromised older cases. Nevertheless, advances developed by AFMES-AFDIL, which are employed by other DNA forensic and commercial laboratories, have made the use of nuclear DNA testing (auSTR and Y-STR)

possible with samples submitted for testing by the DPAA laboratory. Finally, recent advancements using a Next Generation Sequencing mtDNA capture assay, developed by AFMES-AFDIL allows on an as needed basis the whole mtDNA genome to be sequenced. Sequencing the whole mtDNA genome allows AFMES-AFDIL to identify a single individual from multiple individuals who share a common mtDNA control region sequence.

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Why are samples processed in duplicate in order to report out DNA results (mtDNA, auSTR, and/or Y-STR)?

In 1995, a Department of Defense Science Board (DSB) report on the "Use of DNA Technology for Identification of Ancient Remains" recognized the special complexities associated with the identification of war casualties from 50-plus years ago. The DSB composed of leading scientist from academia, the Federal Bureau of Investigation (FBI), the British Forensic Science Service (FSS) and the California Department of Justice (CAL-DOJ) determined that the forensic testing procedures employed by the Armed Forces DNA Identification Laboratory were scientifically sound and valid. Specifically cited were AFMES-AFDIL's quality assurance measures, that included the need to perform two independent DNA analyses from the same skeletal specimens tested, the use of over lapping sequencing products and the use of dedicated separate laboratory rooms. When processing specimens in duplicate, each sample is extracted twice and processed to completion with mtDNA, auSTR and/or Y-STR analysis methods. To report out the duplicate extracts, the results between the individual extracts need to be consistent with one another. If the results are not consistent the samples are reported as "Inconclusive." This differs dramatically from how modern auSTR criminal casework is processed at commercial, state and local laboratories. There a single extraction and analysis is sufficient to report out a result. Due to the low quality of these samples, it is very easy to amplify a modern contaminant over the low quality authentic DNA. It's why reproducibility of results are essential, whether testing mtDNA or nuclear DNA. Many DNA experts, who deal routinely with modern samples rather than these older samples, often underestimate the importance of this difference.

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Why are samples tested for mtDNA first rather than auSTR or Y-STRs?

In 1991, due to technological limitations in dissolving bone powder and isolating degraded DNA molecules, mtDNA, which is highly effective in compromised skeletal cases due to its durability and high-copy number per sample, was utilized to assist in the identification process. For about 15 years, AFMES-AFDIL prioritized the collection of maternal references (mtDNA) and, when

possible, nuclear (auSTRs) references in the hope that technology would evolve. The demineralization extraction protocol developed by AFMES-AFDIL in 2006, which is now employed by other DNA forensic and commercial laboratories, has made the use of nuclear DNA testing (auSTR and Y-STR) possible with samples submitted for analysis by the DPAA laboratory. Since 2006, AFMES-AFDIL has prioritized the collection of not only maternal (mtDNA) family references, but also paternal (Y-Chromosomal STR) and nuclear (auSTR) family references.

Based on the nature and the samples being tested (degraded) and the maturity of the mtDNA family reference database, all samples are processed initially for mtDNA to gauge the quality of the sample and to allow AFMES-AFDIL and DPAA scientists to segregate samples by mtDNA control region sequence. Once mtDNA control region profiles are obtained, and if paternal and/or nuclear references are available, Y- STR and auSTR testing is performed to help segregate samples with common mtDNA control region sequences or to aid further statistical relevance to the initial mtDNA results. When testing is complete, all of the DNA information (mtDNA and/or Y-STR/ and/or auSTR) can be used to calculate a combined likelihood statistic. The likelihood statistic assesses the evidential support for the identification hypothesis that the DNA from the unknown sample is biologically related to the associated references (auSTR, Y-STR and mtDNA).

However, if paternal and/or nuclear references are not currently available for missing service members, the DPAA-Laboratory will request the appropriate service causality office attempt to find appropriate paternal and/or nuclear references as needed. Once the reference is received at AFMES-AFDIL, its testing will begin and based on the associated case the testing may be expedited. If no paternal and/or nuclear references are available, AFMES-AFDIL has the ability to perform whole mtDNA genome sequencing on both the submitted specimen and the maternal references to identify an individual from many who share a common mtDNA control region sequence.

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Can DNA be used to determine if small fragmented remains are of human origin?

The DPAA forensic anthropologists are very good at determining whether a skeletal element is of human origin by examining various bone features. However, some highly fragmented skeletal material may not be anthropologically distinguishable as human and may require DNA testing to confirm whether DNA obtained from the skeletal element is from a human or an animal. In 2012, AFMES-AFDIL developed a DNA procedure which amplifies a specific region of the 12S ribosomal RNA located within the mitochondrial genome. This region is species specific, which allows AFMES-AFDIL to determine if the DNA is human or non-human.

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When was nuclear DNA (auSTR and Y-STR) first used in identification?

For present day accounting, nuclear auSTR DNA testing methods have solely been utilized since the early 1990s and Y-STRs since 2003. The first case that auSTRs and Y-STRs were utilized in the past conflict accounting mission was in 2006 for the identification of a service member missing from the Vietnam war. In October 1994, a Joint Task Force-Full Accounting (JTFFA) team excavated the supposed crash site, where four bone fragments were recovered. However, at the time the bone fragments were too small for mtDNA testing. AFMES-AFDIL's development and publication of the Demineralization method in 2006 offered the first chance for these samples to be analyzed. The samples were tested in duplicate and a consistent mtDNA sequence was obtained; however, there were no maternal references available for the service member. At this time, AFMES-AFDIL tested these specimens using both auSTR and Y-STR testing methods and auSTR and Y-STR results were obtained. Upon the successful generation of nuclear DNA results, references from the service member's spouse, daughter and son were obtained for comparison and the service member was identified in early 2007.

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What is the future of Forensic DNA Testing?

To understand where the science is going we need to understand where the science has been and the continued advancements that have occurred over the past 20 years. The modern DNA forensic age started in the late 1980's with the development of the Polymerase Chain Reaction (PCR), which allowed for the amplification of small amounts of DNA. In the 1990s, the first commercial 4 auSTR locus kits were released and AFMES-AFDIL developed the first mtDNA primer-sets; a commercial 7 auSTR locus kit was released in 1997 and AFMES-AFDIL simultaneously developed and published the first mtDNA mini-primer sets for processing degraded samples; a commercial 16 auSTR locus kit was released in 2001; commercial mini-STRs and Y-STR kits were released in 2005; commercial 16 auSTR locus kits optimized for inhibition were released in 2010; and commercial 20 locus kits were released in 2013. Each new commercially-developed kit brought greater discriminatory power, as well as increased chances of recovering usable DNA results from challenged samples. However, these commercial kits were optimized for criminal casework samples, which have high levels of DNA present and not the highly degraded skeletal elements processed by AFMES-AFDIL in support of the past accounting mission. During all of these changes, AFMES-AFDIL has continuously either optimized existing methods or kits, or developed new methods for processing challenged samples. The forensic DNA field is constantly evolving. AFMES-AFDIL stays abreast of all new technologies and leads the DNA forensic field in

implementing or developing these state of the art technologies. For example, in 2015, AFMES-AFDIL discussed Next Generation or Massively Parallel Sequencing instruments which can sequence your entire genome in 48 hours. This technology is no longer the future, but is currently being employed by AFMES-AFDIL to assist with the past accounting mission (refer to Does AFMES-AFDIL have a Next Generation Sequencing Method?) However, building on the functionality of NGS instrumentation, AFMES-AFDIL is currently researching Identity Single Nucleotide Polymorphisms (i-SNP) to develop an identification panel. This is similar to what commercial entities are using for developing your personal genetic profile but more definitive.

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What new technology is AFMES-AFDIL developing?

With the success of commercial ventures like Ancestry.com and 23 and Me, AFMES-AFDIL looked at using the same commercial assay that these companies use. The commercial assay did not work with the degraded DNA samples associated with the past accounting mission. However, one major issue was observed. The assay does not provided definitive identification based on the SNPs used. Although they can provide investigative leads as to who you may be related to, the individual will need to verify the relationship through their family tree. A perfect example of this was the recent news about how these databases were used to identify the "Golden State Killer." However, once traditional autosomal STR's, the same auSTR's used at AFMES-AFDIL, were performed on the crime scene sample and a reference from the Golden State Killer. He was exonerated as the profiles did not match. Two years ago, noting the weakness in the commercial assay, AFMES-AFDIL in conjunction with Parabon and through the Department of Defense Office of the Deputy Assistant Secretary of Defense for Emerging Capabilities and Prototyping identified a core set of i-SNPs that can be used to establish identity. Currently, AFMES-AFDIL is working with Parabon to optimize the analysis software as well as develop a capture assay that will allow for an increased recovery of the targeted i-SNPs. The goal of this project is to increase the pool of viable family references coupled with establishing a nuclear identification.

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What is Next Generation or Massively Parallel Sequencing?

DNA forensics is at a pivotal junction in its development as the traditional STR or sequencing strategies are soon to be replaced by massively parallel sequencers also known as next generation sequencers (NGS). These are instruments that can sequence the entire human genome (~3 billion base-pairs; for additional information refer to question "What is DNA made of?") within 48 hours.

Commercial kits have been released that allow for the simultaneous detection of auSTRs, ancestry single nucleotide polymorphism (SNPs), and Y-STRs. However, these kits were developed for use with traditional forensic samples that have a lot of high quality non-damaged DNA present. AFMES-AFDIL was part of three member forensic laboratory testing group that took one of the commercially available NGS testing kits (auSTR, YSTR, and SNPs) and performed all of the testing necessary to meet the National DNA Index System's validation requirements. These results were written up and submitted to the FBI for approval for use in criminal casework. AFMES-AFDIL determined that although the commercial kit was fit for use with high quality non-damaged DNA, it did not meet the needs of AFMES-AFDIL for testing with highly damaged DNA such as those submitted by the DPAA. However, AFMES-AFDIL is actively utilizing the strength of NGS testing and developing methods that are fit for use to meet DPAA needs (refer to what is the future of Forensic DNA Testing?).

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Does AFMES-AFDIL have any Next Generation Sequencing Instruments?

AFMES-AFDIL purchased several NGS instruments three years ago to assist with the development of an mtDNA control region sequencing method, well ahead of any other forensic laboratory within the United States. Currently, AFMES-AFDIL has five NGS instruments dedicated to sequencing past accounting mission specimens.

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What is AFMES-AFDIL using Next Generation Sequencing Instruments for?

AFMES-AFDIL has developed a whole mtDNA genome NGS sequencing method for use with chemically modified and/or highly degraded samples. This method will allow AFMES-AFDIL to obtain reproducible results from samples that have average DNA fragments of less than 50 base-pairs, which is half the size of current mtDNA testing methods used for testing past accounting samples. This is the reason why for the last 16 years there has been little to no success with chemically modified samples (Korea Punchbowl) with existing testing methods.

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Does AFMES-AFDIL have a Next Generation Sequencing method?

YES. AFMES-AFDIL was the first and currently the only DNA forensic testing laboratory in the United States with a forensically validated NGS sequencing method. In 2016, AFMES-AFDIL finished the forensic validation of a Next Generation Sequencing mtDNA capture assay for use with chemically modified or highly degraded samples. This method was validated to meet the FBI's Quality assurance and ISO-17025 forensic laboratory standards and has passed to external accreditation reviews. AFMES-AFDIL to date has processed more than 600 samples using its NGS mtDNA capture assay.

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How does the mtDNA capture assay work?

When extracting DNA from a DPAA specimen, AFMES-AFDIL recovers all human and non-human (bacterial etc.) DNA. With highly degraded or chemically modified, the amount of bacterial DNA far exceeds the amount of human DNA. Current NGS methods allowed for the recovery of human DNA, but it was lost in the amount of bacterial DNA that was co-sequenced. To enrich for the human DNA, AFMES-AFDIL developed probes or baits to capture the human mitochondrial DNA. Think of fishing, if you have one hook with bait you catch one fish, but if you have 1000's of hooks you can catch 1000's of fish. The 1000's of human mtDNA specific baits developed by AFMES-AFDIL for this method, allowed AFMES-AFDIL to capture and enrich for the human mtDNA over the bacterial DNA, which allowed AFMES-AFDIL to generate results for the first time from chemically modified or treated specimens. The NGS mtDNA capture method is not a commercially available method, but is a method solely developed by AFMES-AFDIL to assist with the identification of your loved ones. The method is time consuming and through-put was initially four samples per month. With the addition of new personnel and instruments, AFMES-AFDIL has increased processing to over 18 samples per month. AFMES-AFDIL is constantly refining the testing method and looking at new instruments to increase through-put. Although initially developed for chemically modified samples, this method has also been used with highly degraded samples from Vietnam, World War II and Korea.

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What other advantages does the NGS mtDNA capture assay give AFMES-AFDIL?

The NGS mtDNA capture assay sequences the whole mtDNA genome, which allows individuals who share a common mtDNA control region sequence to be segregated. AFMES-AFDIL's traditional mtDNA sequencing method, known as Sanger Sequencing, only looks at the mtDNA

control region. The mtDNA control region is only approximately 1200 base-pairs out of the full 16,569 base-pairs. The mtDNA control region has been used since 1991 to assist in the identification process as it has a high degree of variation between individuals. Its weakness is that there are common sequences among the different populations. For example, about 7.7% of all Caucasians share a common mtDNA control region sequence. The NGS mtDNA capture assay allows for the sequencing of the whole mtDNA genome and where individuals may have a common mtDNA control region sequence, they differ across the whole genome.

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What effect do environmental conditions have on DNA?

All environmental conditions (e.g. heat, humidity, acidic soil), post-mortem effects (e.g. high fragmentation, burning, chemical fixation via embalming techniques), and time will degrade (break down) DNA into smaller fragments (pieces). The DNA is still present in the sample; however, the DNA molecule has been reduced to extremely small fragments, making it difficult to test. Extreme heat (e.g. cremation) can completely destroy any DNA within a sample, and certain chemical treatments (e.g. embalming) may prevent DNA from being recovered from a sample.

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Why is DNA important to the missing personnel accounting process?

DNA is an integral part of the identification effort. This is particularly true when the skeletal elements are highly fragmented or commingled. DNA testing allows DPAA anthropologists to sort recovered elements into discrete groups, allowing for more efficient anthropological analysis. In other cases, where the skeleton has been broken into small fragments, anthropology may be limited in what it can tell about the samples and DNA will be the primary method of identification.

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How is DNA used to identify missing service members?

DNA can be used by the DPAA to support the anthropological and archaeological results from the missing service member's recovered skeletal remains, along with any associated circumstantial evidence to identify an individual. DNA on its own can be used as the primary means of

identification. In addition, DNA analysis can be used to sort sets of highly commingled samples, and it is important to remember that not all DNA testing leads to new identifications. Exclusions are just as important as inclusions in the search for missing service members. By eliminating potential matches, work can be focused on other persons for whom we may not have reference materials. DNA analysis is not limited to these tasks, and as technological advances occur, DNA may be used in new and different ways.

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Can DNA analysis provide definitive proof of my family member's identity?

In some instances, DNA analysis can provide a means of scientific identification, or definitive proof of your loved one's identity. For example, if alternative reference materials (i.e. eye glasses, envelopes, service covers) are available from the missing service member, nuclear DNA may be able to be recovered from those materials and tested with an auSTR kit to provide a direct auSTR reference for the missing service member. When and if an auSTR result is generated from the skeletal samples recovered by the DPAA, the auSTR DNA profiles from the direct reference and the skeletal sample can be compared. If auSTR DNA profiles match exactly at a statistically significant number of loci, this will result in a positive identification. This scenario is what happens with current day military identifications. However, in most instances, the DNA reference materials on hand are from relatives of the missing person. This provides what is known as a presumptive identification. Regardless of the type of DNA testing method (auSTR, Y-STR or mtDNA sequencing), there is a statistical likelihood that the samples tested belong to your missing service member. When applicable, scientists at the AFMES-AFDIL will combine all of the available DNA testing information and calculate a combined likelihood statistic. The likelihood statistic assesses the probability that the DNA from the unknown sample is biologically related to the associated references (auSTR, and/or Y-STR and/or mtDNA profile)

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Who is eligible to donate DNA reference material?

If you are a maternal relative (maternal mother, maternal aunt, brother, sister [mtDNA testing]), a paternal relative (father, brother, paternal uncle, paternal cousin [Y-STR testing]), or a nuclear relative (father, mother, brother or sister [auSTR testing]) of a missing service member, you are eligible to donate a DNA reference material. Depending on your relationship to the missing service member you could be a reference for multiple testing methods (mtDNA, auSTR and/or Y-STR). For example, if the father is the missing service member and is survived by his son, his son is an eligible

nuclear (auSTR) and paternal (Y-STR) reference. AFMES-AFDIL is attempting to collect at least 2 mtDNA references, 2 Y-STR and 2 auSTR references for each missing service member.

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How do I donate DNA as a Family Reference Sample (FRS)?

The process of donating a DNA reference sample is easy, painless and free-of-charge. If you are the relative of a missing service member, you should contact your Service Casualty Office (SCO) for information on how to provide a DNA sample. The SCO will mail to your home, a DNA donor kit that contains a donor consent form, instruction form, three buccal (cheek) swabs and a shipping envelope. All you have to do is fill out the paperwork, rub the inside of your cheek with the swabs, place the swabs back in their containers and affix the label. The collected samples are then placed in a pre-addressed and pre-paid envelope and mailed to AFMES-AFDIL at Dover AFB, Delaware. That's it! It's a completely painless process.

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I am not eligible to give a reference, how can I help?

If you are a family member that is not eligible for a DNA donation, there are still ways you can help. First, you can work with your SCO to assist with identifying other known living family members who may be eligible for donating an autosomal, maternal or paternal DNA reference. However, in special instances, when there are no references on file for a missing service member, AFMES-AFDIL may be able to utilize alternative references such as baby hair and teeth. In these instances, contact your SCO with information on what alternative references you may have so that they can discuss with AFMES-AFDIL. If deemed appropriate for testing, the SCO will coordinate with you on how to send them to AFMES-AFDIL. Once testing is complete, the items will be returned to you. It is important to note that DNA testing can be a destructive process and AFMES-AFDIL will attempt to limit the damage to alternative references. For example, with envelopes, AFMES-AFDIL will try not damage the letter from your loved one, but may need to possibly take a cutting of the envelope flap or cut the stamp off to get at the potential DNA. Similarly, with service covers, AFMES-AFDIL will swab the inside of the hat band and, if needed, may cut a small piece of the hat band if the swabbing does not work. It is for this reason that AFMES-AFDIL will only test these alternative references as a last resort when no other viable reference is available.

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Why do I have to give you three cheek swabs?

Three swabs are collected to provide us with extra samples should we need them. When you wipe the inside of your cheek with the swab, you are scrubbing off some cheek cells onto the swab. Sometimes, only a few cells are collected on a single swab and we need to test the second or even a third swab. We prefer to have multiple samples rather than disturb you to collect another swab.

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Will my DNA be tested for genetic diseases?

No. Nuclear DNA is tested using commercial auSTR and Y-STR kits that look only at locations in the DNA that are not associated with any genetic diseases. Mitochondrial DNA is tested in what is known as the ‘control region’. This region also does not contain any markers for known genetic diseases.

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Will my DNA be compared to or included in criminal databases?

No. Although there is a federal criminal National DNA Index System (NDIS) maintained by the Federal Bureau of Investigation (FBI), your DNA information will be kept on separate secure servers maintained and controlled at AFMES-AFDIL. Your DNA profiles will not be released to any other agencies for any other purposes, nor will it be uploaded to any other federal forensic databases.

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If I provide a DNA sample, is my DNA and personal identity information protected?

Yes. Your DNA reference packet is treated as a medical record and as such is protected by the Health Insurance Portability and Accountability Act (HIPAA). Once received, your DNA sample will be assigned a specific unique AFMES-AFDIL casework number so that no personal identifying information (PII) is associated with the DNA sample as it is processed in the laboratory. Once the

profile has been generated, the sequence data is entered into AFMES-AFDIL's secured family reference database, which associates your name to the AFMES-AFDIL case number. This database is used only within AFMES-AFDIL, cannot be shared with other agencies and access to the database is limited to scientific staff that have completed formal training. Access is further controlled by computer permissions and tied to a scientist's government common-access-card. The computer system validates the user every time they log into the system and if the user does not have permission to access that database, the computer system will not allow access to that information. In addition, the DNA sample and profiles are considered medical records and therefore are collected under a signed informed donor consent form that explains the obligations that AFMES-AFDIL has to you and your samples. As mentioned above, you are also protected by the HIPAA. Due to HIPAA and the informed donor consent form, AFMES-AFDIL can only release a DNA profile to the donor who signed the consent form and must protect your profile, sample and PII.

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How long does the Government keep my DNA sample and profile on file?

AFMES-AFDIL maintains your reference materials indefinitely. As different types of testing become available, your reference materials may be tested again as needed. Your DNA profile will be maintained in the database indefinitely or until the overall mission is deemed complete.

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Can personal items be submitted as an alternative reference to buccal swabs?

Ideally, buccal swabs from living family members are preferred, but in certain instance where there are no living relatives, alternative references such as eye glasses, watches, hearing aids, hats, envelopes, baby hair and teeth may be utilized by AFMES-AFDIL. If you have any of these items, or believe you have something else that might be helpful, please contact your SCO for information on how to send these in for testing. However, the DNA associated with these references are highly degraded and will need to be processed under casework conditions (duplicate processing), which is labor intensive and slower than buccal swab processing. Once testing is complete, the items will be returned to you.

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Will the alternative Reference (i.e. watch, eyeglasses) be returned to me?

If you submit a personal item—such as an envelope or a lock of hair—as a reference for either your missing loved one or a deceased family member, we will return those materials to you as soon as testing has been completed. Please be aware that although the sample may be in our procession for an extended period of time, it has been photo-documented, given an AFMES-AFDIL case number, and stored securely in a limited access evidence room under optimal environmental conditions to prevent contamination or further degradation. However, it is important to note that DNA testing is destructive and AFMES-AFDIL will attempt to limit the damage to the alternative reference. In some instances, a small portion of the alternative reference may be used in the testing process. For example, with a hat, AFMES-AFDIL will swab the inside of the hat band first and then may cut a small piece of the hat band if the swabbing does not work. It is for this reason alone that AFMES-AFDIL will only test these alternative references as a last resort when no other viable reference is available.

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Why do I have to give you swabs if I'm sending in other personal materials?

Sometimes family members are not eligible donors themselves, but may have personal materials either from the missing service member or other viable mtDNA, auSTR or Y-STR donors. These materials can be baby hair, clothing, envelopes or hairbrushes. When you provide materials such as these, we will ask you to provide a swab from you as the donor for what is known as an 'exclusion' reference. Many of these personal materials are often handled extensively. We need to be able to eliminate your DNA from the DNA profile we obtain from the materials.

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What percentage of missing personnel have FRS associated with them?

As of June 2018, the percent of missing service members that have some type (mtDNA, auSTR, or Y-STR) of a family reference on file varies by the conflict:

- Vietnam War – 85%
- Korean War – 92%
- Cold War – 85%
- WWII –6%

Efforts for family reference collection are ongoing. If you or someone you know is a valid DNA reference donor for a missing service member please contact the respective service casualty office for information on participating. For World War II collections, family references are being collected on an as needed base due to the fact that a large number of missing individuals were lost in deep water losses and are not recoverable at this time.

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How does Present day accounting differ from Past accounting?

For present day accounting, all samples are compared to a direct blood reference to the deceased individual. Due to the success of DNA testing on past accounting samples, the United States Government, in 1992, established the Armed Forces Repository of Specimen Samples (AFRSSIR) to house and protect DNA blood reference cards for all active duty military, Reserve and National Guard personnel. The references and samples associated with present day accounting contain high amounts of DNA and only require single auSTR amplification for the reference and sample for a DNA comparison report to be issued. This differs from the past accounting mission, in which direct references are not an option in most cases and maternal, paternal and nuclear family references are needed to aid in the identification process. In addition, due to the low quality of the skeletal samples submitted by the DPAA for testing, all mtDNA, auSTR and Y-STR analyses must be performed at a minimum in duplicate in order to make a comparison. Duplication of the results is essential whether testing mtDNA or nuclear DNA as it is very easy to amplify a modern contaminant over the low quality authentic DNA.

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