Purpose

• Resources
  – Families
  – Funding

• Capabilities
  – AFMES/DoD DNA Operations (AFDIL)
  – DNA overview

• Assets
  – Staff
  – Emerging Technology Section
  – Where we are going
Resources
Viable Family References

Shaded gray boxes indicate a relative who is not a usable reference for DNA analysis.

Biological children of missing females can be used as a maternal mtDNA reference.

“R” represents Removed. (ex. 1st Cousin 2R means 1st Cousin twice removed)

(=) Donor for Autosomal DNA Analysis
(#) Donor for Mitochondrial DNA
(+) Donor for “Y” DNA Analysis

Descendants Not a Useable Reference
Family Reference Samples Status
As Of June 14, 2018

Total References Collected: 29,451
Families Represented: 14,387

- Korea: 15,808
  - Total Family References Received: 15,808
  - Missing Service Members: 7,401
  - 91% Representation

- SEA: 3,742
  - Total Family References Received: 3,742
  - Missing Service Members: 1,954
  - 85% Representation

- WWII: 9,354
  - Total Family References Received: 9,354
  - Missing Service Members: 4,840
  - 6% Representation

- Cold War: 194
  - Total Family References Received: 194
  - Missing Service Members: 194
  - 85% Representation

- Other: 107
  - Total Family References Received: 107
  - Missing Service Members: 107
  - 85% Representation

Legend:
- Total Family References Received By Conflict
- Missing Service Members By Conflict With A Family FRS
Family Reference Sample

- Family References Are Collected Under Informed Consent
- Only Used For Human Remains Identification
- Treated As a Medical Specimen
- Protected Under the Health Insurance Portability and Accountability Act of 1996 (HIPAA)
- FRS Database Information Is Restricted and Not Shared Or Uploaded To Any Outside Agency
- Release of Any HIPAA Information With PII Must Be With Consent of Donor
Funding

• 2013
  – Developed FY2015-2019 funding requirements.

• 2015-2019
  – ~11 mil to 19.2 mil
  – Requested 32 new hires/ 24 authorized

• 2019-2021
  – Maintenance
  – Programing for growth ????
Assets

Clippanda.com
Past Accounting Section 2018

- 52 New Hires
  - 39 New Hires
  - 12 Reallocations
Emerging Technologies Section

Demineralization Protocol

Romanov Family
Capabilities
U.S. Army Sgt. 1st Class Jean Villanuevalopez with the Defense POW/MIA Accounting Agency (DPAA) carries the American flag during a disinterment ceremony at the National Memorial Cemetery of the Pacific, Honolulu, Hawaii. The remains will be transferred to the DPAA laboratory for analysis and identification. (U.S. Marine Corps photo by Sgt. Lauren Falk)
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, and 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 in which 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.
AFMES Missions Supported
By AFDIL

Present Day Accounting

Past Accounting

www.bbc.co.uk

FRS Databasing

World Wide Support
AFMES-Armed Forces DNA Identification Laboratory

• **1988 Armed Forces Medical Examiner**
  – Aid POW/MIA recovery operations
  – DNA Forensics becomes possible

• **1990 Armed Forces DNA Identification Lab**
  – AFMES with current day identifications
  – DPAA with past accounting

• **1991 DNA testing and Identification support**
  – First Gulf War and service members recovered from Vietnam

• **1992**
  – DNA supports first Vietnam identification
  – Establishment of the Armed Forces Repository of Specimen Samples for the Identification of Remains

• **1998 Accreditation and Tomb of the Unknown**
  – First laboratory accredited for mtDNA forensics
  – Identification of the Vietnam unknown as First Lt Michael Blassie on July 19th 1998
Laboratory Accreditations & Standards

American Society of Crime Laboratory Directors-Laboratory Accreditation Board (ASCLD – ISO 17025 International Certification)

Federal Bureau Of Investigation (FBI) Quality Assurance Standards

DoD DA Oversight Committee (Defense Science Board 1995)
Points to Consider

• Every DNA result will not necessarily lead to or support a new ID
  – Too degraded
  – Commingling
  – Additional Portions
  – Local national/non-US
  – No family reference
  – Non human
Nuclear DNA
~3.2 billion base pairs (bp)

Mitochondrial DNA
16,549 bp
23 Pairs of Chromosomes

The Nuclear Genome contains approximately 25,000 Genes
Nuclear DNA Shared Between Relatives

Amount of total nuclear DNA in common with ones relatives
# Comparison of DNA Testing Methods

<table>
<thead>
<tr>
<th>Mitochondrial DNA</th>
<th>Y- DNA</th>
<th>Autosomal DNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Limited discrimination</td>
<td>Limited discrimination</td>
<td>High discrimination</td>
</tr>
<tr>
<td>Multiple copies/cell</td>
<td>Only one copy/cell; from the father</td>
<td>Two copies/cell</td>
</tr>
<tr>
<td>References from any maternal relative</td>
<td>References from any paternal relative</td>
<td>Best reference are mother, father, siblings &amp;/or offspring</td>
</tr>
<tr>
<td>Decades to centuries</td>
<td>Several years</td>
<td>Several years</td>
</tr>
<tr>
<td>Slower turn-around</td>
<td>Quick turn-around</td>
<td>Quick turn-around</td>
</tr>
</tbody>
</table>
Why Lead With mtDNA Analysis

- Due to sample quality mtDNA analysis offers the greatest chance of success for these types of remains
  - 1000’s of mtDNA copies compared to single nuclear copy
  - Maturity of the mtDNA family reference database
  - Limited availability of nuclear family references

- Databases are used to determine the:
  - “uniqueness”
  - “consistency”

- Au-STR and/or Y-STR testing performed after mtDNA
  - Segregate
  - Increase statistical significance
Past Accounting Process

• DPAA-Lab samples are processed on a rolling basis
  – 700+ skeletal samples in progress at any one time
  – Samples are reprioritized on a routine basis
    • DPAA scientist in charge of prioritizing
    • AFDIL sends Data summary to DPAA
    • DPAA request Comparison and BTB reports

• Average turn around time (TAT):
  – Extraction to Report ~55-85 Mission Days
DNA Tests Reported By AFDIL
As Of May 31, 2018

92% Success Rate Obtaining MtDNA Sequence Data
57% Success Rate Obtaining Au-STR DNA Data
58% Success Rate Obtaining Y-STR DNA Data
41% Success Rate Obtaining NGS Data
<table>
<thead>
<tr>
<th>FY</th>
<th>Believe to Be Reports</th>
<th>Addendum Reports</th>
<th>Foreign National</th>
<th>Total Reports</th>
</tr>
</thead>
<tbody>
<tr>
<td>2012</td>
<td>60</td>
<td>103</td>
<td>57</td>
<td>220</td>
</tr>
<tr>
<td>2013</td>
<td>55</td>
<td>120</td>
<td>39</td>
<td>214</td>
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<tr>
<td>2014</td>
<td>75</td>
<td>57</td>
<td>51</td>
<td>183</td>
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<tr>
<td>2015</td>
<td>71</td>
<td>77</td>
<td>32</td>
<td>180</td>
</tr>
<tr>
<td>2016</td>
<td>133</td>
<td>65</td>
<td>6</td>
<td>204</td>
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<tr>
<td>2017</td>
<td>154</td>
<td>165</td>
<td>26</td>
<td>345</td>
</tr>
<tr>
<td>2018</td>
<td>98</td>
<td>281</td>
<td>25</td>
<td>404</td>
</tr>
</tbody>
</table>
Notable Casework Advances

1992: First Use of MtDNA for Past Accounting Casework
2006: Demineralization Buffer
2010: 12S rRNA: Human vs Non-Human
2013: Low Copy Number Y-STR testing
2014: Improved DNA Purification
2015: New 23 locus auSTR Kit
2016: NGS mtDNA Capture Protocol for chemically modified samples
2017: 15 New Scientist and additional NGS Team
2006: Demineralization Success

- 90-100% success
- 80-89.9% success
- 70-79.9% success
- 60-69.9% success
- 50-59.9% success
- 40-49.9% success
- 30-39.9% success
- 0-20% success

Pre-Demineralization

Post-Demineralization
2010: 12S rRNA Gene Testing

- Used to verify if a sample is human
  - 567 samples tested since 2011
  - 250 samples (44%) were non-human
  - Reduces time processing non-viable samples
• FY13: Old Purification Process
  – Y-STR: 24%
  – auSTR: 26%
  – mtDNA Sequencing: 90%

• Improved DNA Purification Process

<table>
<thead>
<tr>
<th>Success rates by analysis</th>
<th>FY14</th>
<th>FY16</th>
<th>FY17</th>
<th>FY18</th>
</tr>
</thead>
<tbody>
<tr>
<td>mtDNA Sequencing</td>
<td>92%</td>
<td>92%</td>
<td>94%</td>
<td>92%</td>
</tr>
<tr>
<td>Autosomal STR</td>
<td>48%</td>
<td>48%</td>
<td>46%</td>
<td>57%</td>
</tr>
<tr>
<td>Low Copy Y STR</td>
<td>48%</td>
<td>47%</td>
<td>51%</td>
<td>57%</td>
</tr>
</tbody>
</table>
2015: New Autosomal STR Kit

- Provides enhanced performance with degraded DNA

- Eleven loci less than 250 bp
  - Current mini-STR kit has 8 loci below 250 bp

- 2 Years of mandated FBI requirement
2016: NGS Korea Punchbowl

- Kokura Mortuary
- Chemically Modified
  - 40-50% formaldehyde: Bad for DNA
- 16 year project by AFMES-AFDIL
DNA/Protein Fixation Issue

Illumina MiSeq

http://www.precisionnutrition.com/wordpress/wp-content/uploads/2009/12/Figure-1-Histones-1024x1022.jpg
2016 NGS mtDNA Capture Protocol

- Samples processed in duplicate
  - Slow process, 2 weeks per sample
  - Initial: 3 to 5 samples per month
- First report issued March 17, 2016
- Optimize and increasing through-put
  - 5 instruments now on line
  - Second Team Trained
  - Grow to support ~20 Analysis per month
NGS Success

- Overall: 660 samples in 2 yrs
  - 273 reportable sequence (41.3%)
  - 390 inconclusive (58.7%)
- Vietnam War (Non-Chemically Treated)
  - 2/7 reportable sequence (29%)
  - Highly degraded – post fire
- Korean War (Punchbowl)
  - Prior success with Sanger: 6%
  - 62/151 reportable sequence (41%)
- WWII- Tarawa
  - 78/235 reportable sequence (33.5%)
- NGS data included in 92 DNA reports - 60 BTB (first time named) reports

http://www.airforcemedicine.af.mil/Media-Center/Display/Article/1226667/next-generation-dna-sequencing/
How Does This Help You and DPAA

• Reduced time spent processing samples

• Reduced reagent costs due to increased success rates
  – Allowed for hiring 24 new staff 2 years ahead of plan
  – Authorized 15 new scientists in FY17; No increase in budget
    • 39 new scientists

• Increased number of Analysis reported to DPAA
  – 2015: 1,700 samples reported
  – 2016: 2,100 goal for FY16; 3,142
  – 2017: 3000 goal for FY17; 3,005
Current Problems and Gaps
Current Problems/Gaps

- Environmental Influences
  - DNA Damage due to degradation

Unrepaired

FFPE Repaired
Current Problems/Gaps

• Same mitotype and no auSTR or YSTR results generated
  – Whole mtDNA genome sequencing
  – Individuals with common mtDNA distinguishable
  – Implemented October 2016
  – Grant to expand whole mtDNA database: better statistics
Current Problems/Gaps

• No viable mtDNA, YSTR, or auSTR references available
  – Millions of identity and kinship single nucleotide polymorphisms (SNPS) across nuclear genome
    • Ancestry.com/23andMe
    • Commercial test did not work
  – As little as 15,000 SNPS can identify unrelated from related 4th degree relative
  – Parabon developed custom software to work with DPAA samples
  – AFDIL developed method to capture SNPs
  – Opens up reference pool and will establish identity

Parabon Awarded U.S. Department of Defense (DoD) Contract to Aid Identification of Unknown Remains from Past Conflicts

Snapshot™ Kinship Analysis to Be Enhanced for Toughest Missing Personnel Cases
1095 Out of 15000 SNPs Recovered

Default Model (15K)

Dynamic Model (Actual SNPs)

All FRS unrelated

Related to FRS 6 (93.5%)

Related to

FRS 6

Data courtesy of Ellen Greytak (Parabon)
We Need **YOU**

To

Help Us Make the

Unknown Known
Director DoD DNA Operations: Timothy.P.McMahon10.civ@mail.mil