DNA Analysis
In The Identification Process

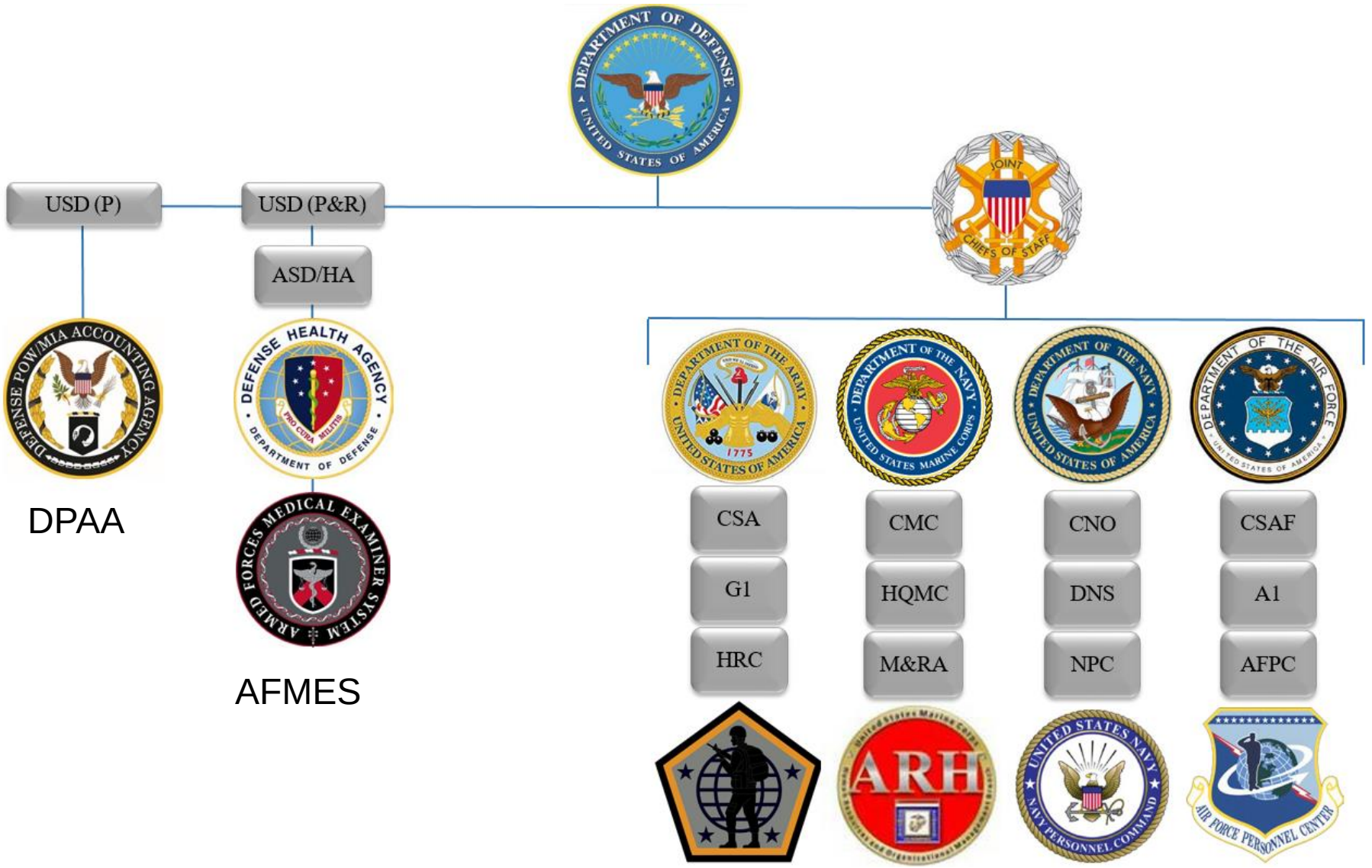
Timothy P McMahon; Ph.D
Director, DoD DNA Operations
Armed Forces Medical Examiner System

National League of POWMIA Families
Family Member Update
September 14th-16th, 2021

Purpose

- AFMES / DoD DNA Operations (AFDIL)
- DNA Introduction
- Fit For Use Tests
- Genetic Genealogy

Mission Partners = Different Parent Organizations = Different Funding



Service Causality Offices

Armed Forces DNA Identification Laboratory (AFMES-AFDIL)

- Division of the Armed Forces Medical Examiner System (AFMES)
  - Defense Health Agency (DHA)
- Established in 1990
  - Utilize DNA methods to identify the remains of US service members
- Mission Partner with the Defense POW/MIA Accounting Agency (DPAA) since 1990
- Accredited DNA Forensic Laboratory
  - American Society of Crime Laboratory Directors-Laboratory (ASCLD – ISO 17025 International Certification)
  - Federal Bureau of Investigation Quality Assurance Standards (FBI-QAS)
  - 1995 Defense Science Board

AFMES Missions Supported By AFDIL

Present Day Accounting

Past Accounting

FRS Databasing

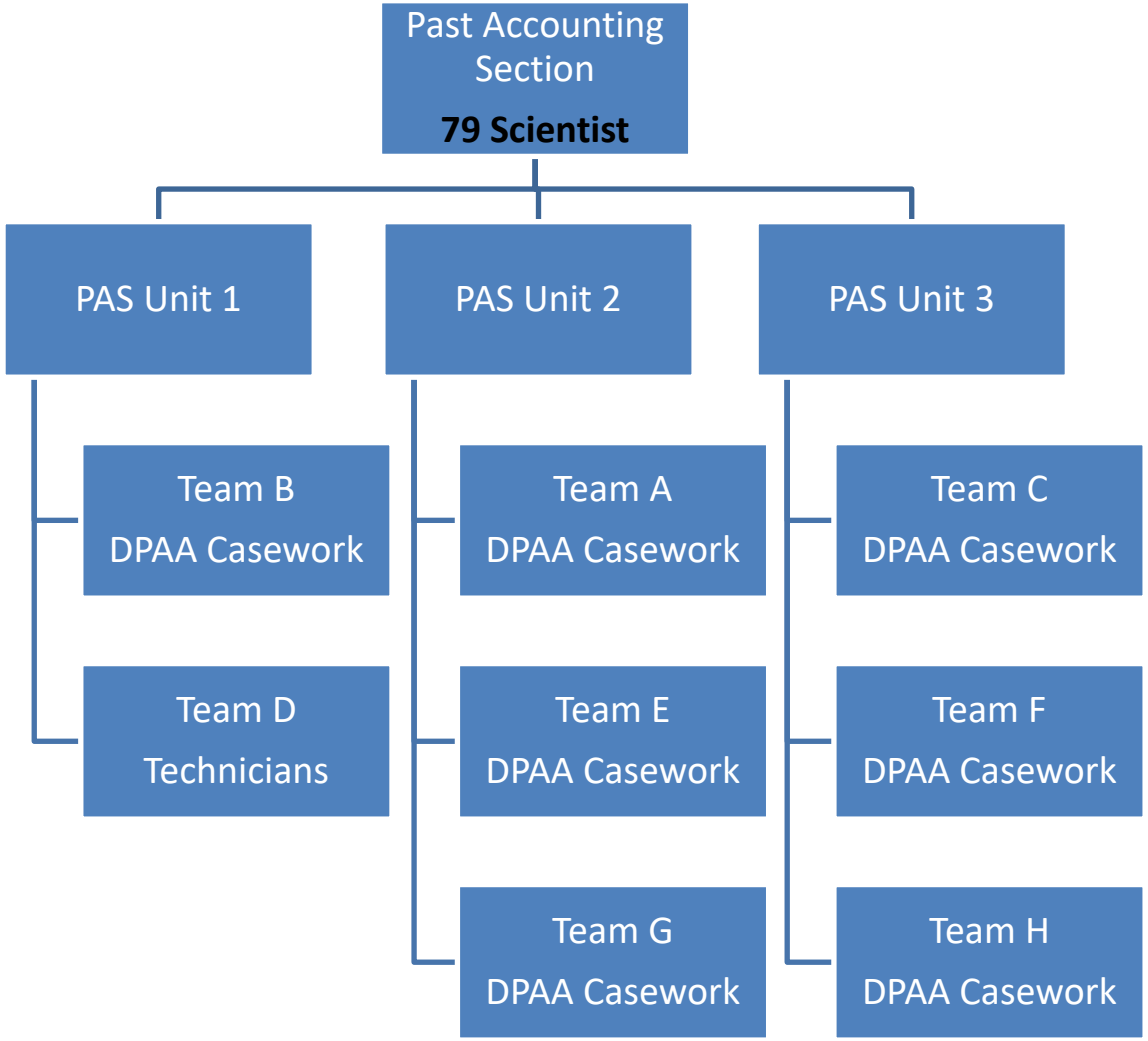
World Wide Support

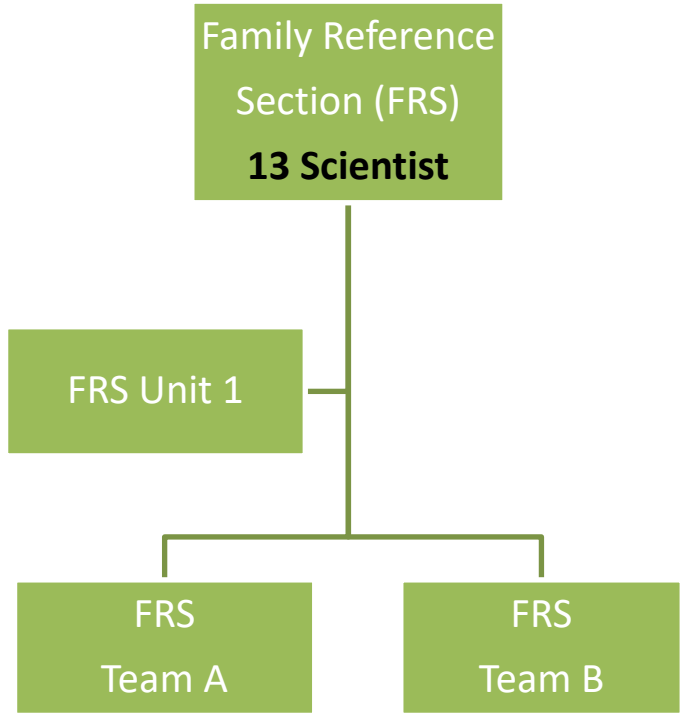
AFMES-AFDIL COVID Response

- **March 9th- June 15th 2020**
  - **Established AM and PM Shift**
  - **10% of staff per shift**
  - **One week in lab, one week reviewing from home**
- **June 15th 2020**
  - **25% of staff per shift**
  - **One week in lab, one week reviewing from home**
- **July 20th 2020 – June 12th 2021**
  - **50% of staff per shift**
- **June 12th 2021: 100% in the building**

Past Accounting Section

- **2013**
  - 6 teams and 45 Scientist
  - Casework and FRS processing
- **2019**
  - **92 Scientists dedicated to PAS Mission**
    - 2 New Past Accounting Teams
    - 47 New Staff
  - **New FRS section**

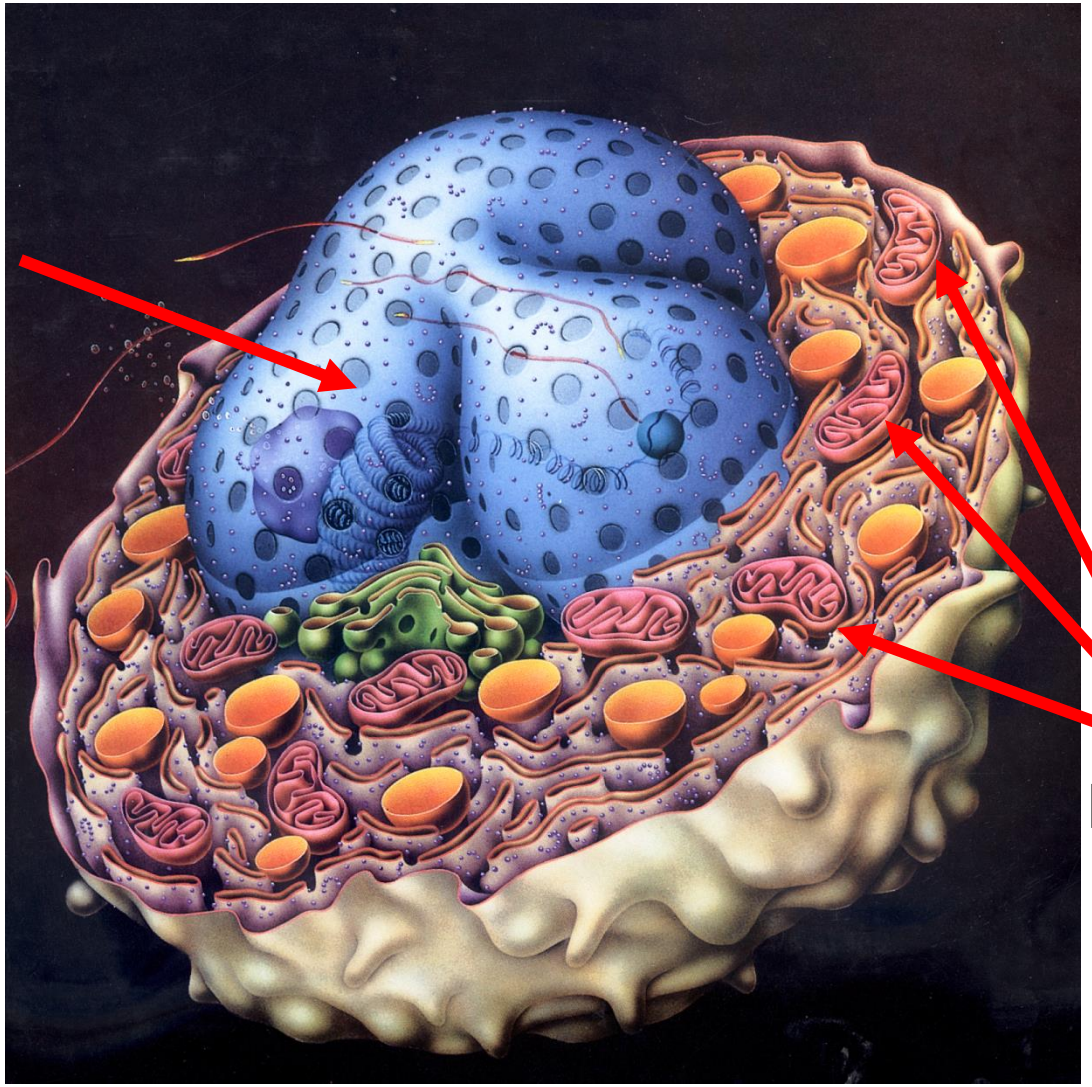




The Human DNA Genome

Nuclear DNA

~3.2 billion base pairs (bp)

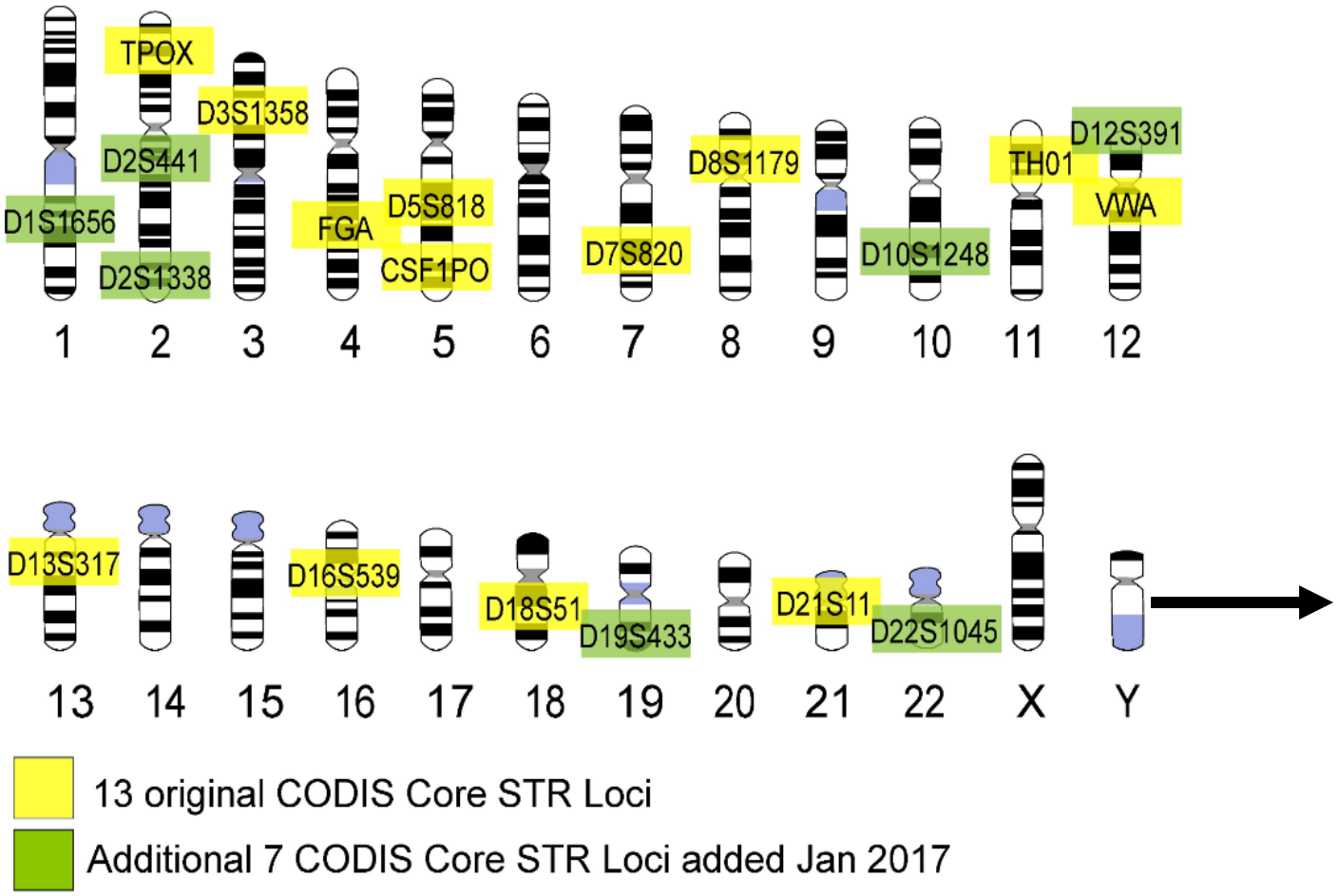


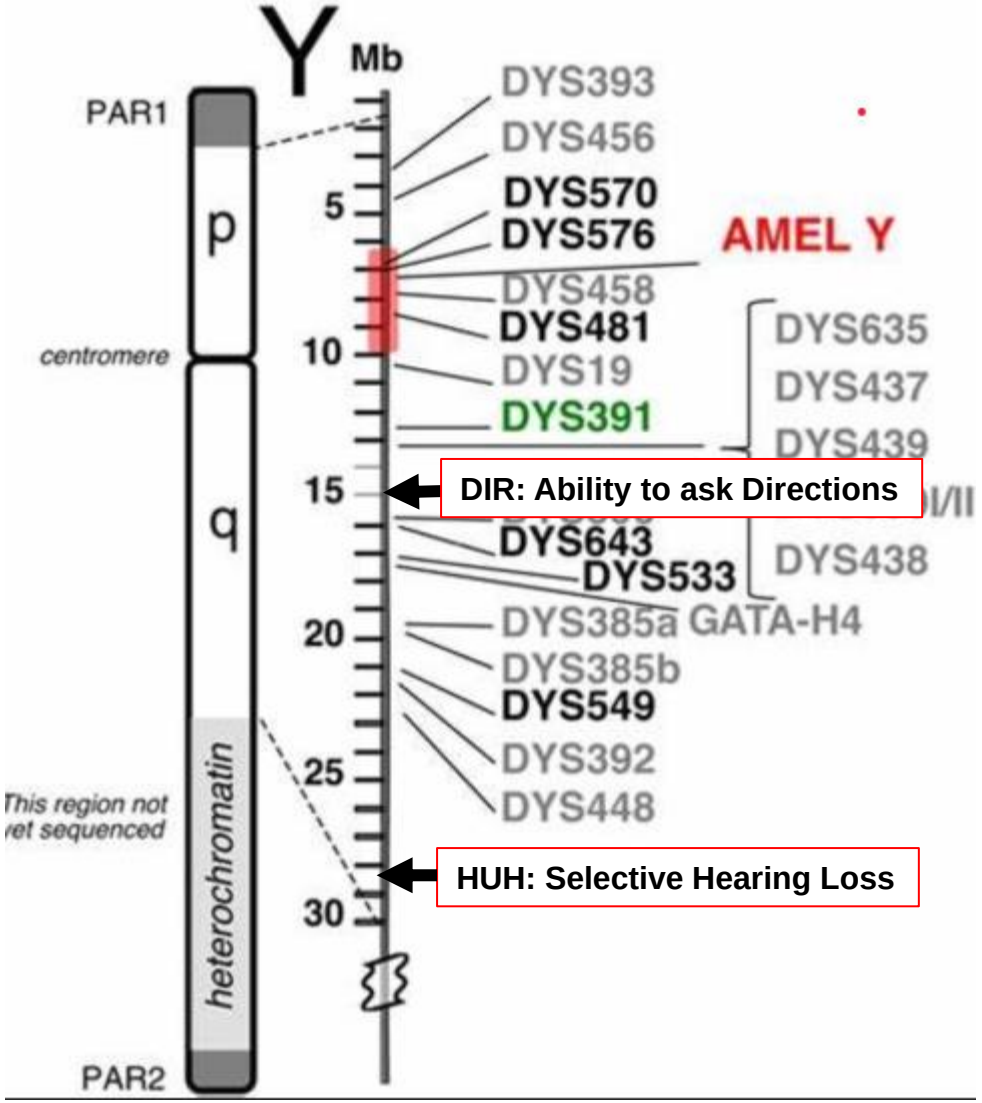
Mitochondrial DNA

16,549 bp

Nuclear DNA: 23 Pairs of Chromosomes
Short Tandem Repeats (STR)

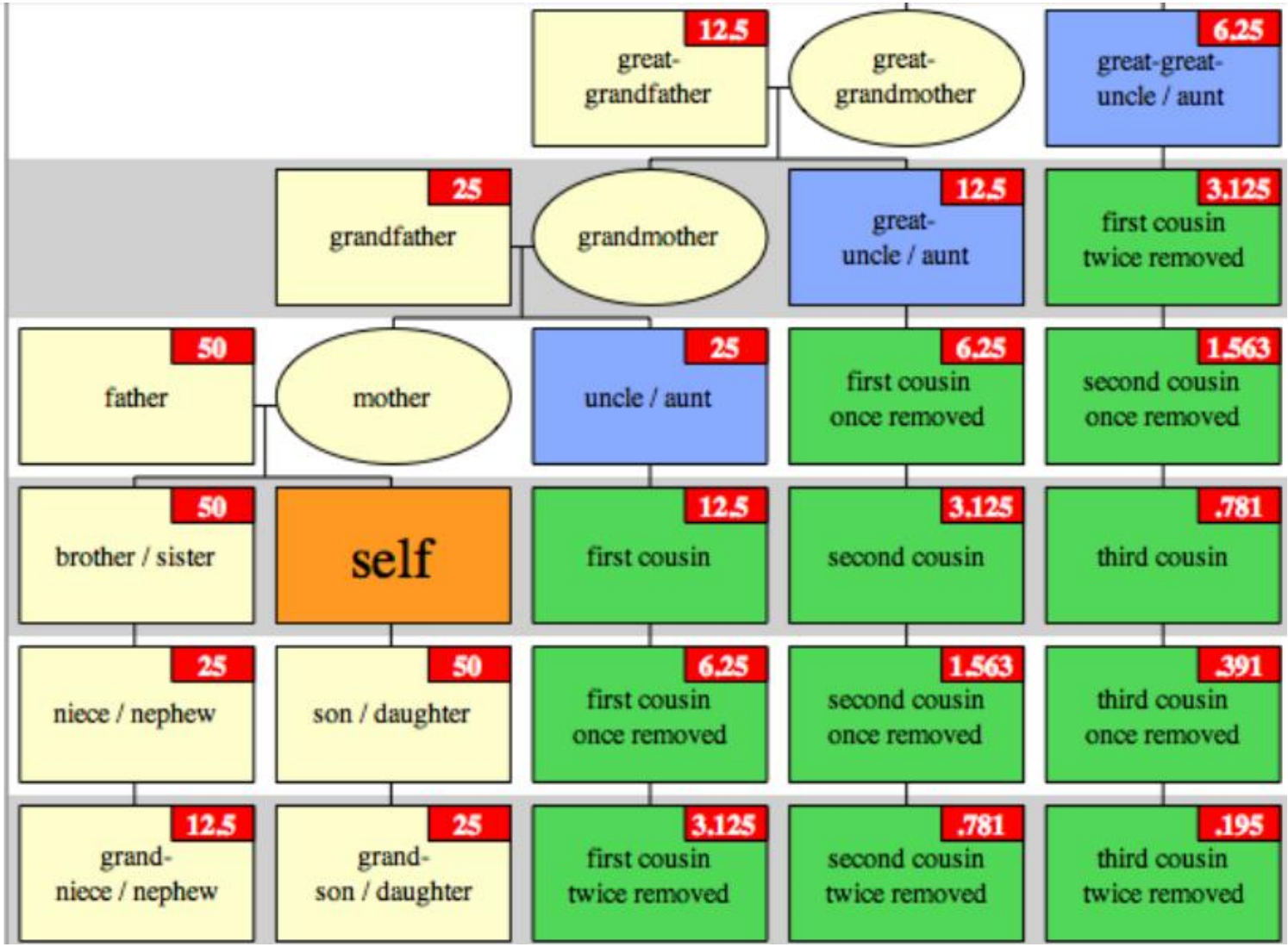
STR loci used in U.S. Combined DNA Index System (CODIS)





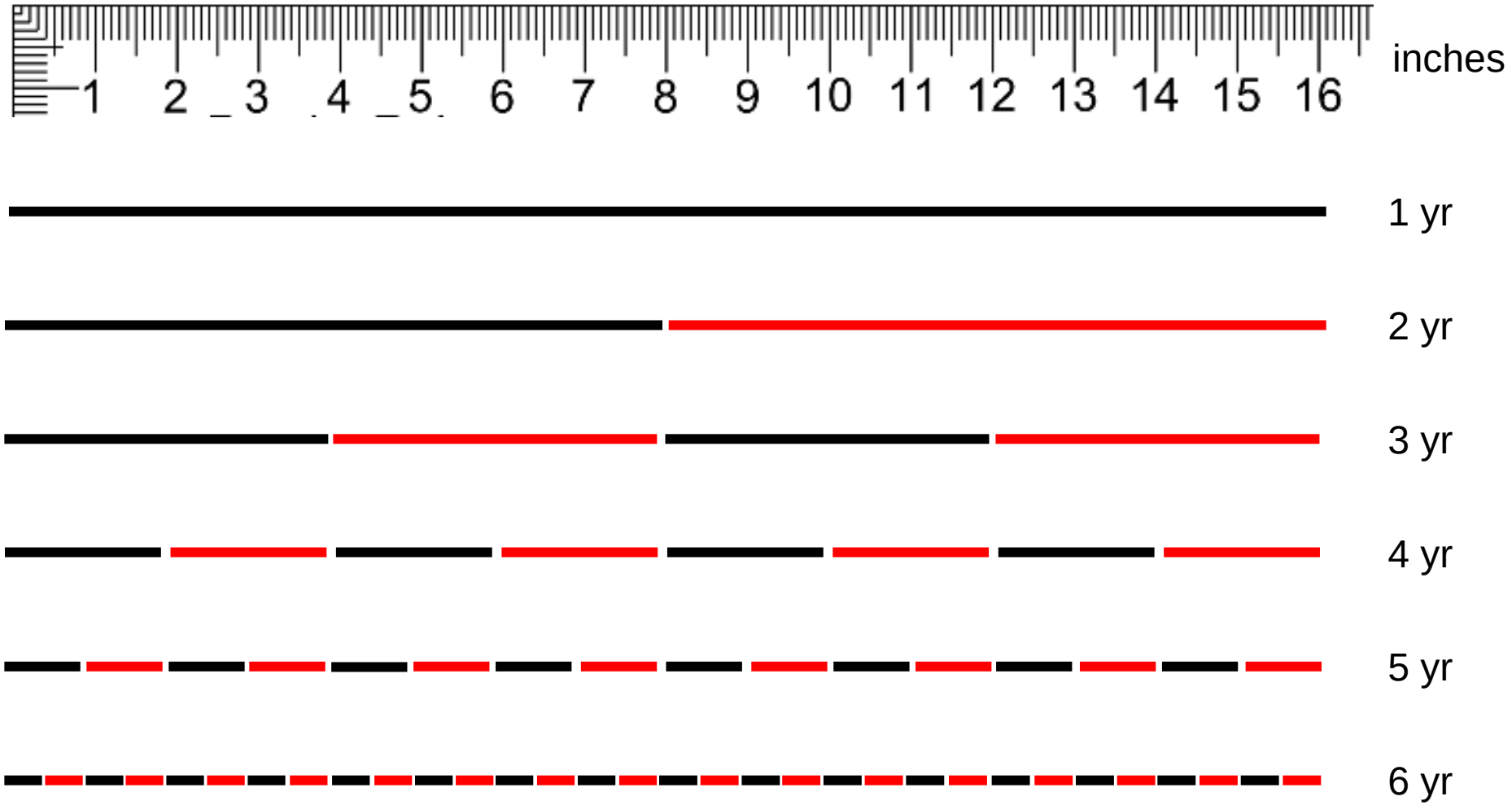
Web Image:UNTHSC.edu

Percent Nuclear DNA Shared Between Relatives



Amount of total nuclear DNA shared with ones relatives

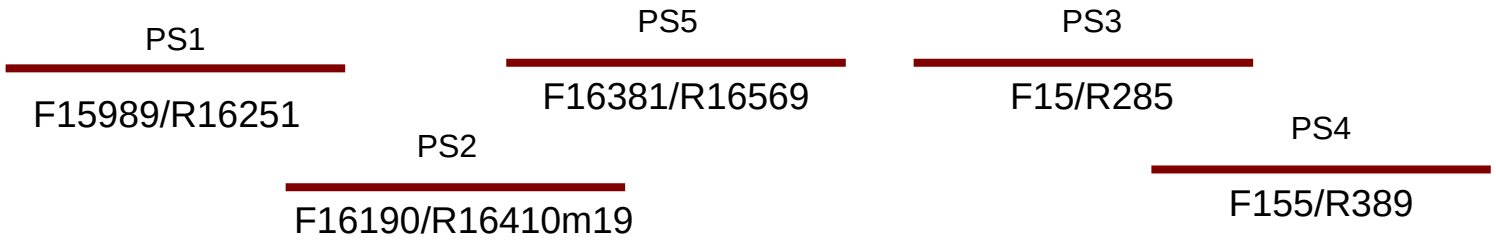
DNA Degradation: Example



1 in = 100 base-pairs

Current AFDIL Amplification Strategies

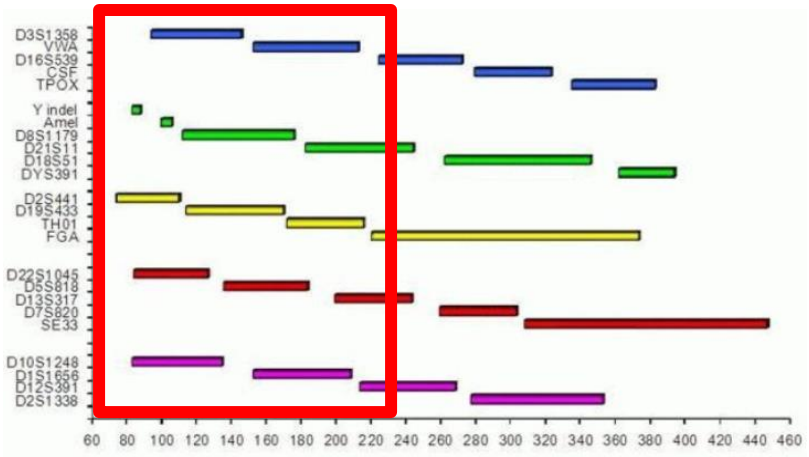
Primer sets (~200bp i.e. 2 in)



Mini-primer sets (~120bp i.e. 1 in)

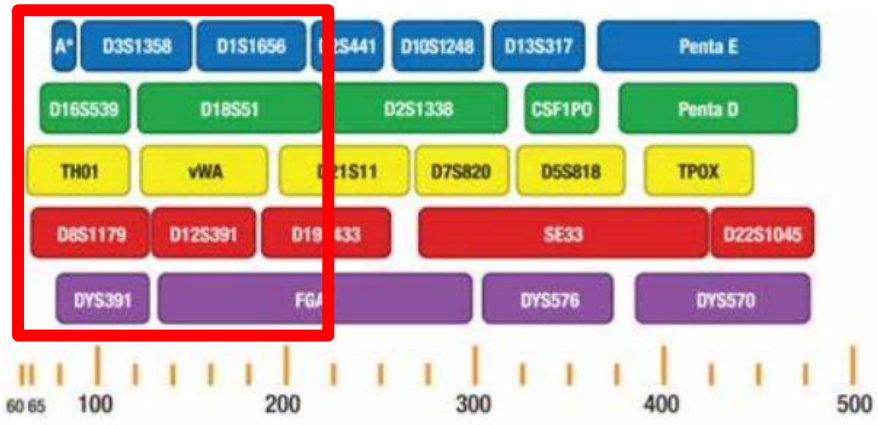
Nuclear DNA Capabilities (auSTR and YSTR)

~2.25 inch DNA or 225 bp



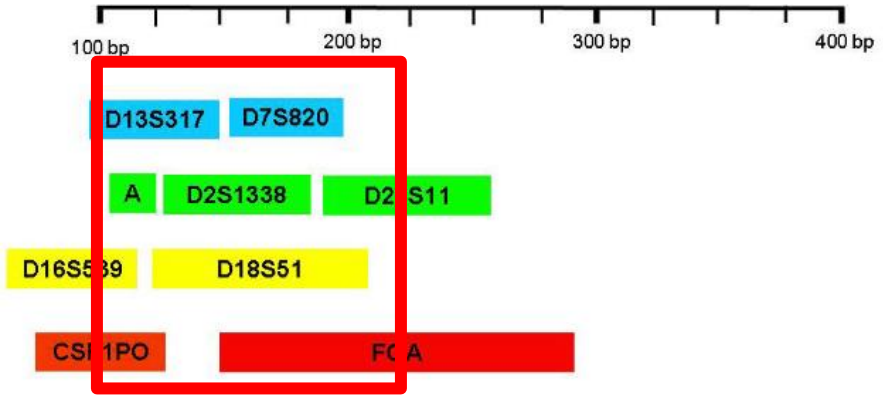
AmpFlSTR GlobalFiler

https://www.thermofisher.com/order/catalog/product/4476135



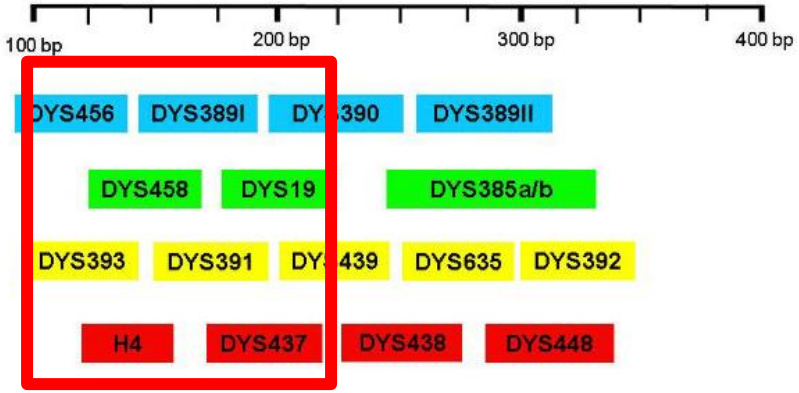
PowerPlex Fusion

https://promega.com



AmpFlSTR MiniFiler

https://strbase.nist.gov/kits/MiniFiler.htm



AmpFlSTR YFiler

https://strbase.nist.gov/kits/YFiler.htm

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 appropriate nuclear family references
- **DNA Degradation = Sample Quality**
  - Degradation = Reduction in DNA size
- **Databases are used to determine the:**
  - "uniqueness"
  - "consistency"
- **Au-STR and/or Y-STR testing performed after mtDNA**
  - Segregate
  - Increase statistical significance

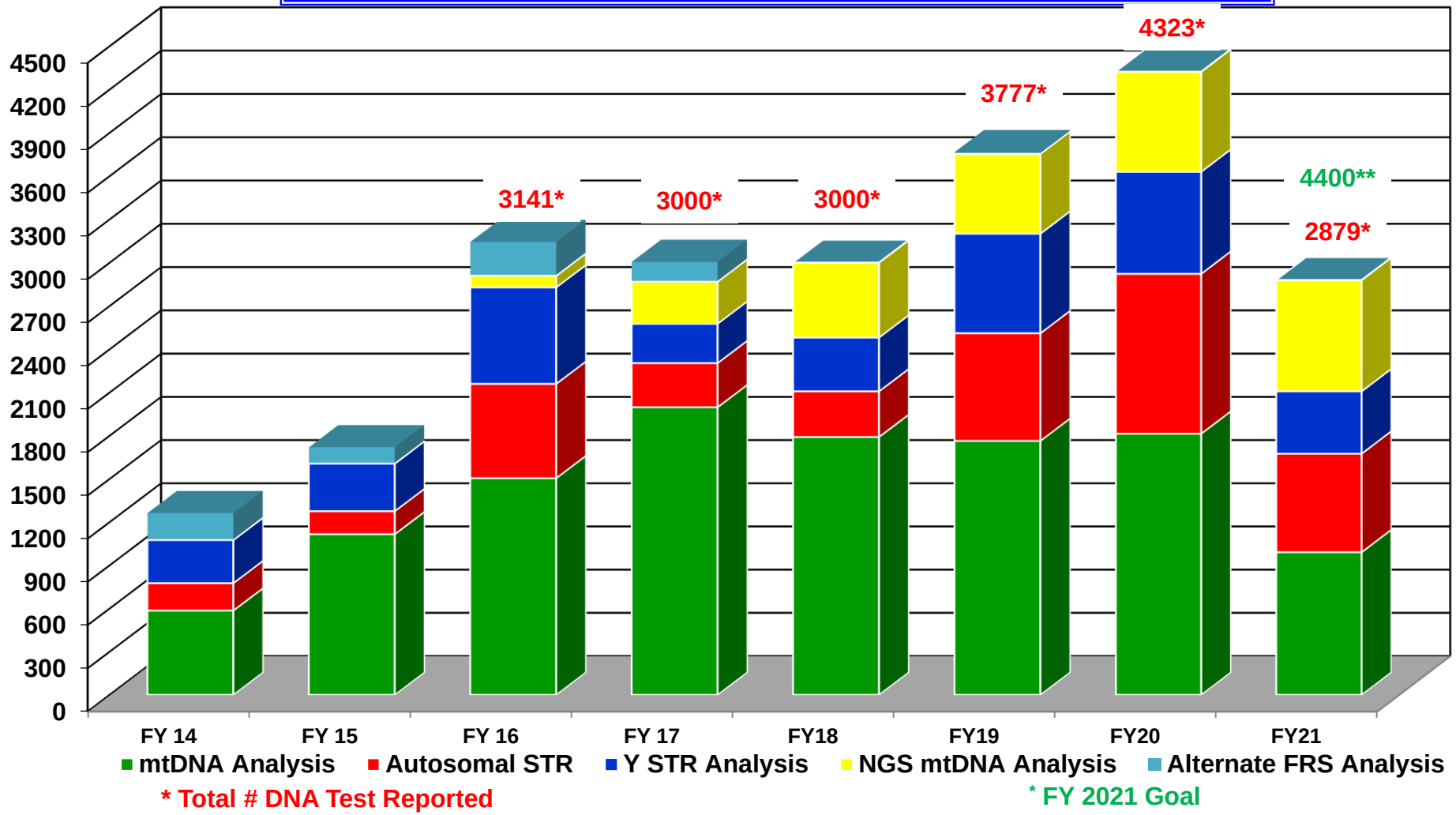
Past Accounting Process

- **DPAA-Lab samples are processed on a rolling basis**
  - 750+ skeletal samples in progress at any one time
  - Samples are reprioritized on a routine basis
    - DPAA scientist in charge of prioritizing
- **Average turn around time (TAT):**
  - Extraction to Report ~55 Mission Days

DNA Tests Reported By AFDIL

As Of July 31st , 2021

91% Success Rate Obtaining MtDNA Sequence Data
67% Success Rate Obtaining auSTR DNA Data
670% Success Rate Obtaining Y-STR DNA Data
71% Success Rate Obtaining NGS Data



DNA Reports
July 31st, 2021

FY	Believe to Be Reports	Addendum Reports	Foreign National	Total Reports
2017	154	165	26	345
2018	189	496	34	719
2019	207	448	3	658
2020	145	1088	7	1240
2021	125	819	845	971

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

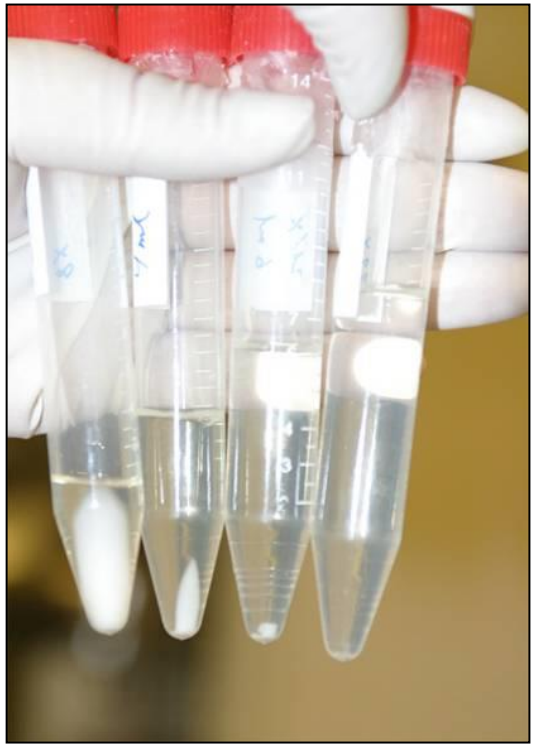
2014: Improved DNA Purification

2015: New 23 locus auSTR Kit

2016: NGS mtDNA Capture Protocol for chemically modified samples

2017:15 New Scientist and additional NGS Team

2021 NextSeq validation



Why Does DNA Take Time

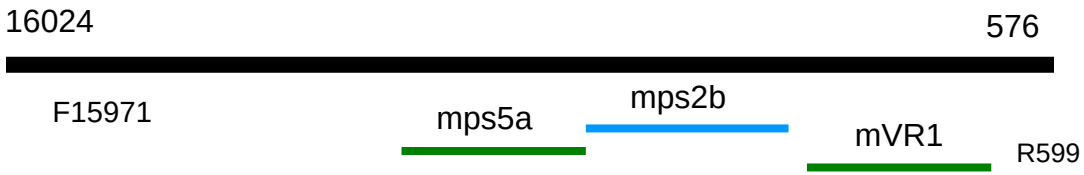
- Sample quality and DNA quantity
- Authentic sequence and confidence
- **Forensic instruments and kits geared toward**
- **FIT FOR USE**
- **AFMES has to create new testing method**
  - No commercial kits/have to develop
  - Science needs to catch up
- Family References

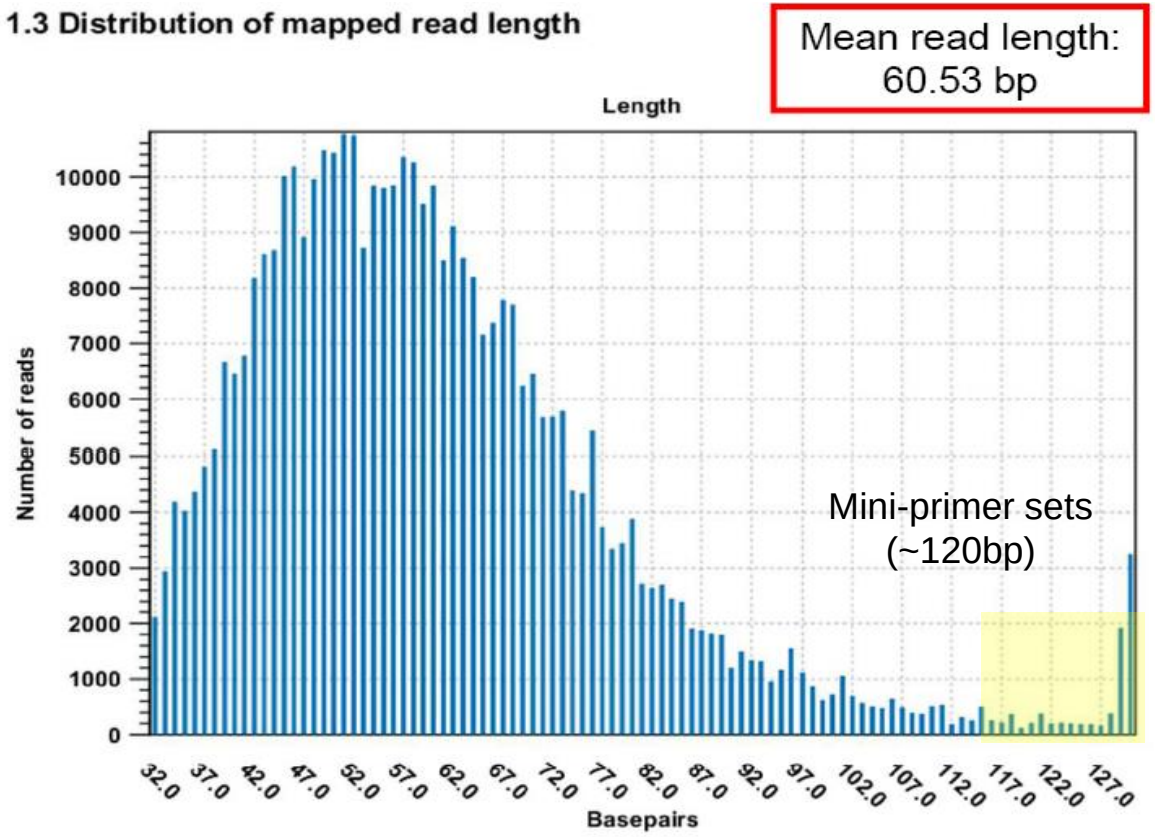
2016: NGS Korea Punchbowl

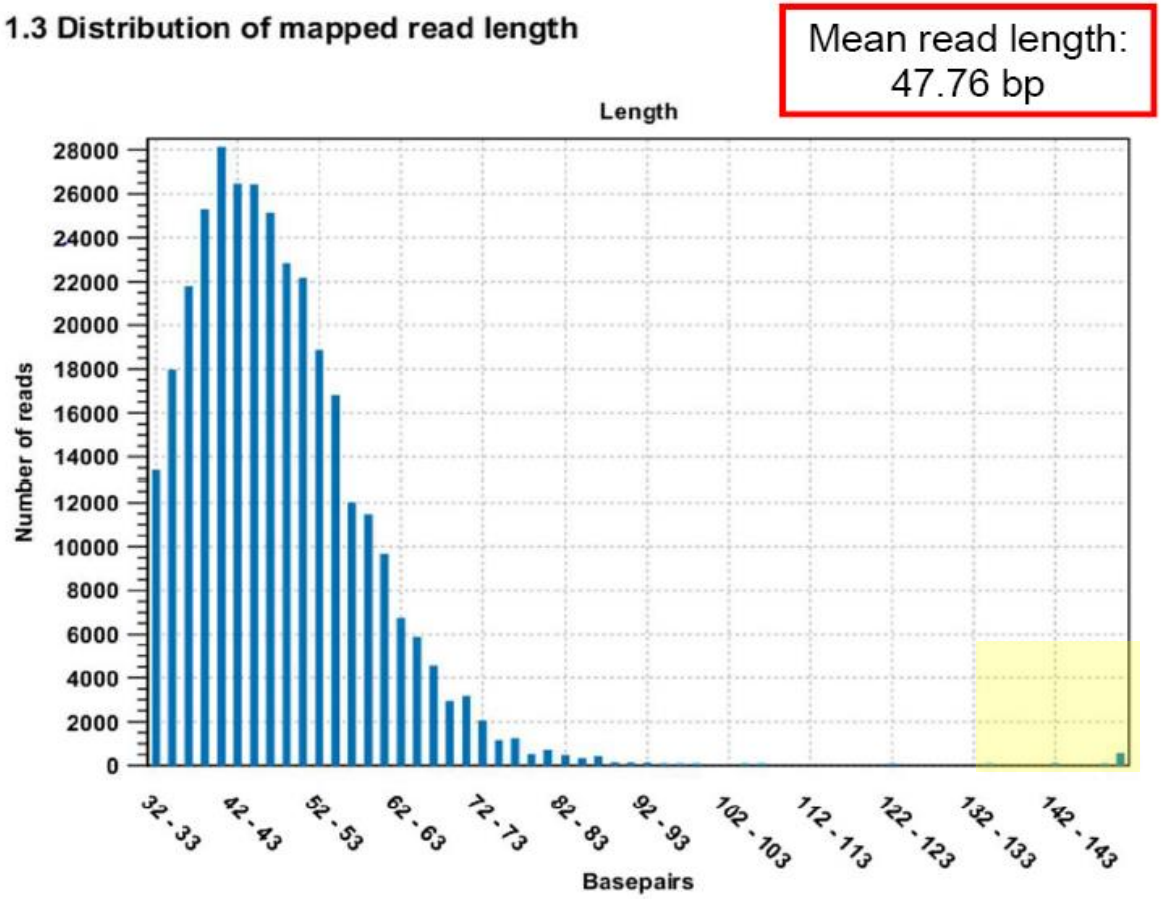
Success Rates: Normal mtDNA Testing
6% mtDNA
0% STRs

- **Kokura Mortuary**
- **Chemically Modified**
  - 40-50% formaldehyde: Bad for DNA
- **16 year project by AFMES-AFDIL**

DNA Fragment Size: By Bone Submission

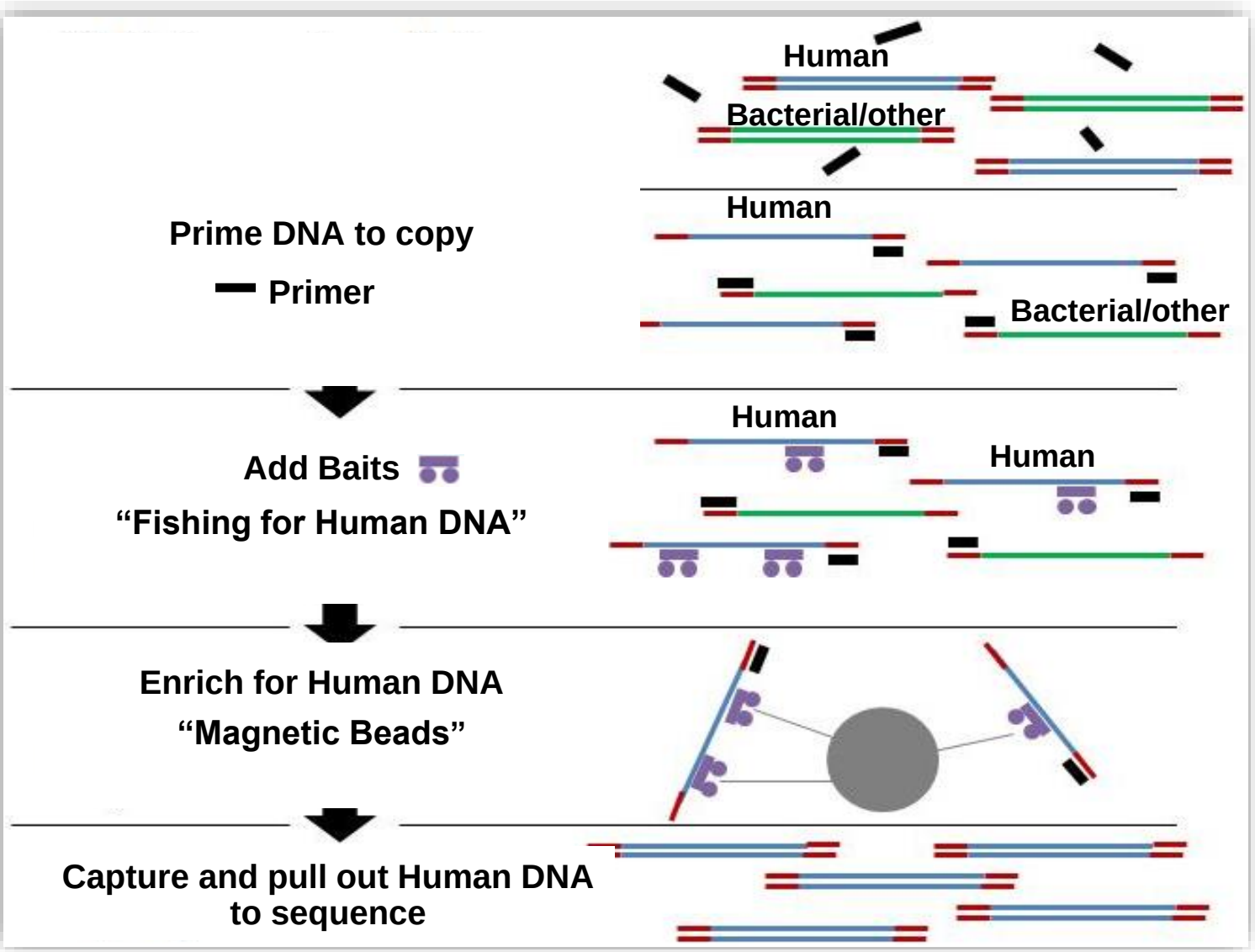






Human mtDNA Capture Method

Capture Method



Contents lists available at ScienceDirect
Forensic Science International: Genetics
journal homepage: www.elsevier.com/locate/fsigen

Research paper

Performance evaluation of a mitogenome capture and Illumina sequencing protocol using non-probative, case-type skeletal samples: Implications for the use of a positive control in a next-generation sequencing procedure

Charla Marshall[a,b,*], Kimberly Sturk-Andreaggi[a,b], Jennifer Daniels-Higginbotham[a,b], Robert Sean Oliver[a,b], Suzanne Barritt-Ross[a,b], Timothy P. McMahon[a]

Contents lists available at ScienceDirect
Forensic Science International: Genetics
journal homepage: www.elsevier.com/locate/fsigen

Case report

Mitochondrial DNA haplogrouping to assist with the identification of unknown service members from the World War II Battle of Tarawa

Charla Marshall[a,b,c,☆], Rebecca Taylor[d], Kimberly Sturk-Andreaggi[a,b,e], Suzanne Barritt-Ross[a], Gregory E. Berg[d], Timothy P. McMahon[a]

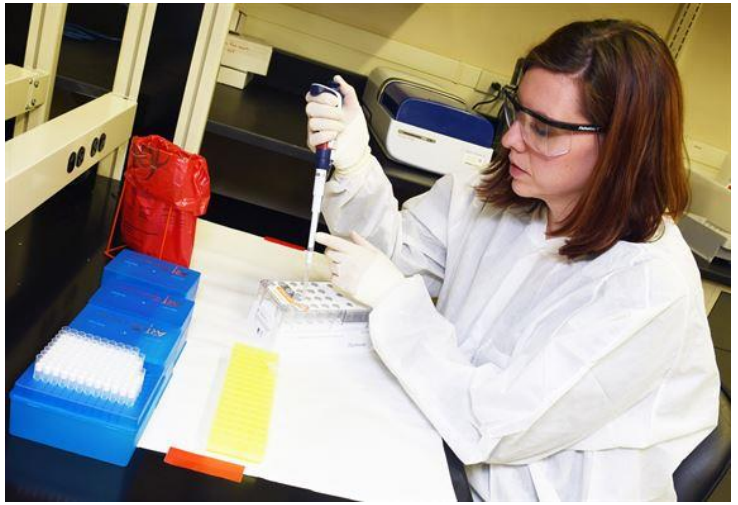
Gorden et al., 2021 FSI:Genetics

NGS Success

Fiscal Year	16	17	18	19	20	21	Overall
NGS Analyses Reported	78	291	519	553	693	771	2905
NGS Analyses Reported with Data	22	129	247	331	468	548	1745
Percent Success Rate	28%	44%	40%	60%	68%	71%	60%
Average Samples per Month	11	24	43	46	58	74	
Whole Genome Family References			93	279	179	130	681

- **DNA Reports**
  - 473 DNA Comparison Reports
  - 202 First Time Named Reports
  - 271 Addendum Reports

- **Processing Capacity Increase**
  - New Teams
  - New Instruments



http://www.airforcemedicine.af.mil/Media-Center/Display/Article/1226667/next-generation-dna-sequencing/

NextSeq 550

4X Increase

MiSeq: 6 samples per run per instrument
31 hours

Next Seq: 24 samples per run per instrument
15 hours

Emerging Technologies:
New Method Development

FORENSIC GENETIC GENEALOGY/INVESTIGATIVE GENETIC GENEALOGY

Forensic Genetic Genealogy/Investigative Genetic Genealogy

- Genetic Genealogy:
  - Who am I related to
  - What potential medical issues may I have
  - What is my heritage
- Commercial Databases
  - Ancestry.Com, 23 and Me, MyHeritage, FamilyTreeDNA, and GEDmatch
- Criminal Cold Case
  - Golden State Killer (April 2018)

Genetic Genealogy Cont.

- **Golden State Killer**
  - 1975-1986: Crime spree (murders, rapes, burglaries.
  - Rape case samples frozen: DNA Technology had to catch up.
  - Direct to Consumer Testing/Genealogist/GEDmatch
    - Samples sent to lab
    - Results used by Genealogist to identify potential suspects

- Being utilized in cold case by criminal DNA forensic labs

Genetic Genealogy Cont.

- Cold Case Investigative Genetic Genealogy
  - Crime Laboratory develops and STR profile from the evidence sample
  - Crime laboratory uploads perpetrators STR profile to CODIS, No hit
  - Police Investigators have no leads, case is cold
  - Extracted DNA if of high enough concentration sent to Direct to consumer testing laboratory.
    - Laboratory Develops Single Nucleotide polymorphism profile
    - Searches Commercial Database that allows for criminal searches
    - Identifies either the perpetrator or relatives
    - Genealogist uses public records (marriage, death, births) to provide investigative leads
  - Police investigators take leads and identify perpetrator and get DNA sample to compare to STR results

Genetic Genealogy Cont.

- Key Points to understand
  - 23AndMe, Ancestry.com, MyHeritage have in place stringent policies that prohibit law enforcement agencies from using these databases for investigative purposes, whether for criminal or missing persons.
  - FamilyTreeDNA and GEDmatch allow for law enforcement searches of their SNP based nuclear databases, but individuals have the right to opt out of these searches.
  - Unlike CODIS where individuals are entered according to strict state guidelines (all arrestees, all felons..) genetic genealogy databases (GGD) are populated by individuals who sole interest is their ancestry and lineage.
  - The problem with searching GGD's is that the privacy of all of an individual's relatives are at risk as well as the identification of unknown adoptions or paternity issues

Genetic Genealogy Cont.

- Key Points to understand Continued
  - Genetic genealogy techniques utilize commercial kits that test for over 700,000 single nucleotide polymorphism (SNPS) and the more SNPs a person has in common with another person, the more closely they are related.
  - Unlike STRs, which do not contain any disease prediction markers, SNPs utilized in the commercial assays do contain disease prediction markers.
  - 2017 AFMES-DNAops tested a commercial SNP kit with a typical DPAA sample.
    - Recovered ~25K SNPs out of 850K SNPs
    - Not enough to identify the known relative from an unknown relative
  - 2017 AFMES-DNAops working through a DoD funded project with PARBON, started the development of a SNP assay and analytical software, specifically designed to work on the chemically treated and highly degraded DNA associated with DPAA cases.
  - Project finished June 2021 and is currently undergoing extensive internal validation process that meets all Federal Bureau of Investigation Quality Assurance Standards and ISO-17025 Forensic Accreditation Standards

Kinship Analysis from Nuclear SNPs

- Part of a OSD-ATL funded Defense Forensic Enterprise research project.
- Collaborative effort with Parabon NanoLabs, Inc.
  - Develop a SNP capture assay for use with AFMES-AFDIL chemically treated and non-chemically treated DPAA samples
- Collaborator responsibilities:
  - AFMES-AFDIL is will develop a method to obtain sufficient SNP data
  - Parabon will optimize the software specifically for this data

Degrees of Relatedness

Relationship

- Identical Twins
- Parent-Offspring
- Full Siblings
- 2nd Degree Relatives
- 3rd Degree Relatives
- 4th Degree Relatives
- 5th Degree Relatives
- 6th Degree Relatives
- 7th Degree Relatives
- 8th Degree Relatives
- 9th Degree Relatives

Slide obtained courtesy of Ellen Greytak (Parabon)

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

Why SNP Capture?

- DNA length in degraded samples prevents use of commercial SNP assays
- Capture procedure is already in use at AFDIL
  - Mitogenome sequencing of challenging samples
- Incorporation of more SNPs
  - Needed for distant relations

Forensic Science International: Genetics 31 (2017) 198–206

Contents lists available at ScienceDirect

Forensic Science International: Genetics

journal homepage: www.elsevier.com/locate/fsigen

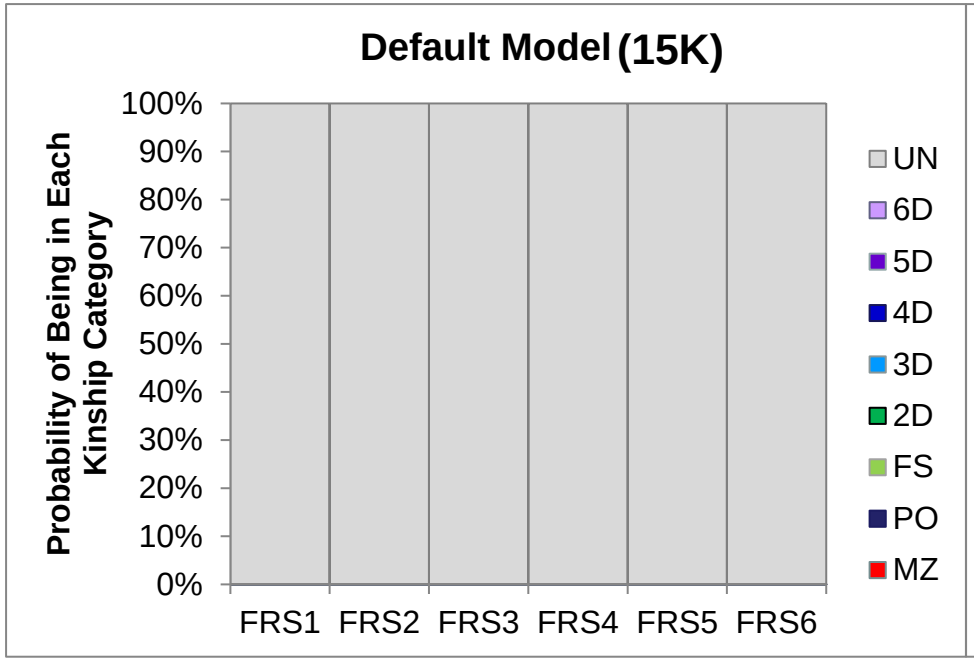
Research paper

Performance evaluation of a mitogenome capture and Illumina sequencing protocol using non-probative, case-type skeletal samples: Implications for the use of a positive control in a next-generation sequencing procedure

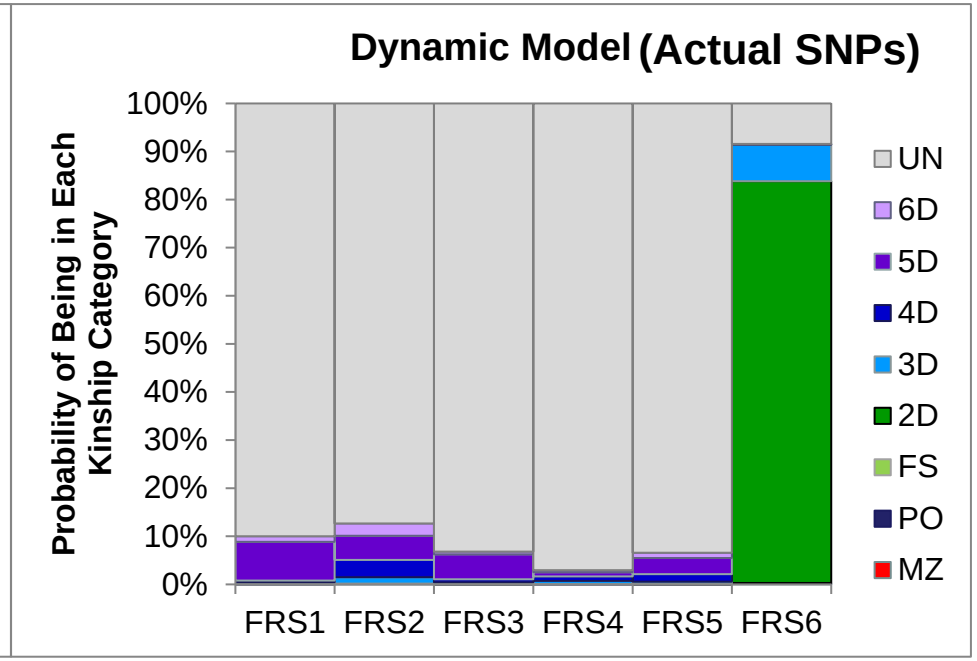
Charla Marshall[a,b,*], Kimberly Sturk-Andreaggi[a,b], Jennifer Daniels-Higginbotham[a,b], Robert Sean Oliver[a,b], Suzanne Barritt-Ross[a,b], Timothy P. McMahon[a]

SNP Capture Results

1095 Out of 15000 SNPs Recovered



All FRS unrelated



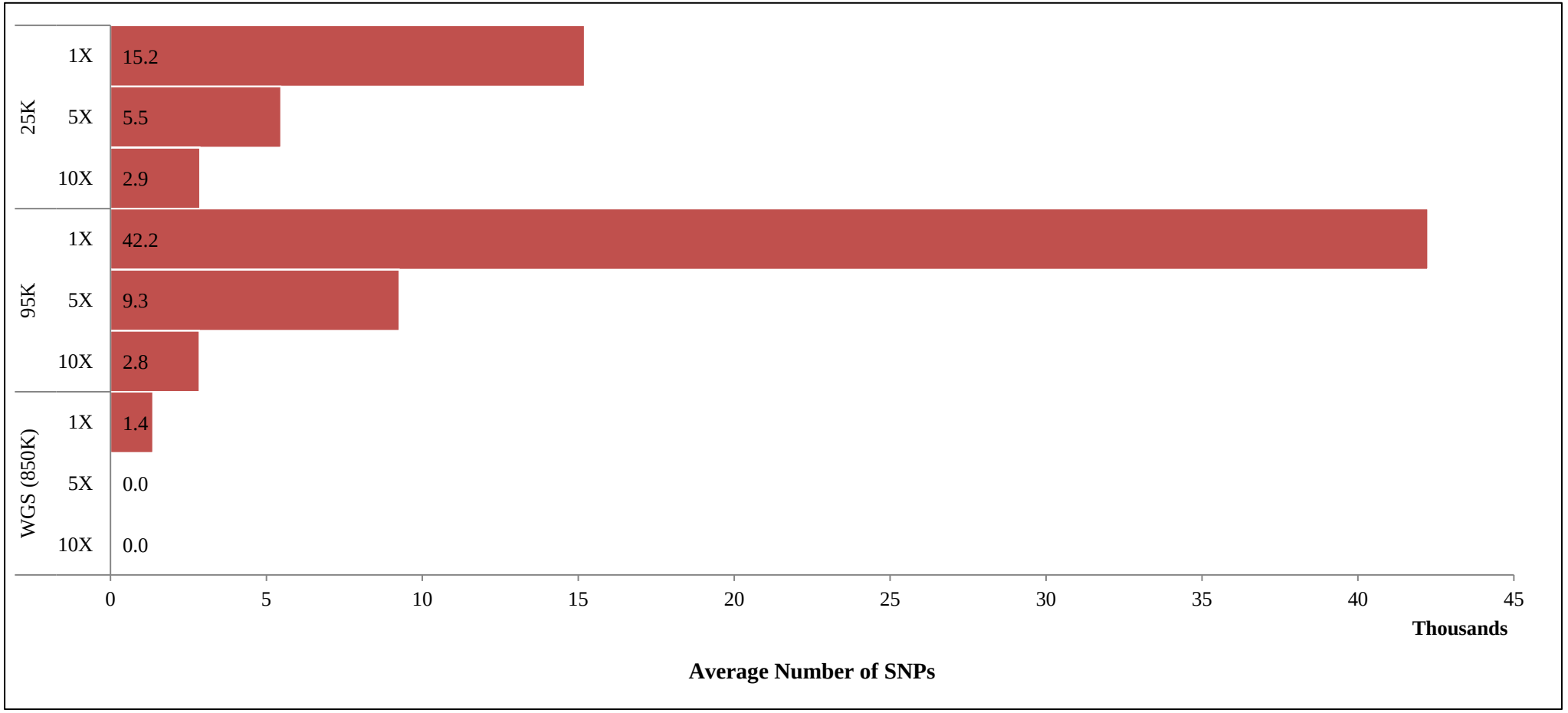
Related to FRS 6 (93.5%)

Related to	Actual relationship
FRS 6	Brother (FS)

Data courtesy of Ellen Greytak (Parabon)

SNP Capture Recovery

Based on using either 25K or 95K of the 100K identity SNPs in commercial kits



Increasing analysis thresholds decreases number of comparable data (SNPs)

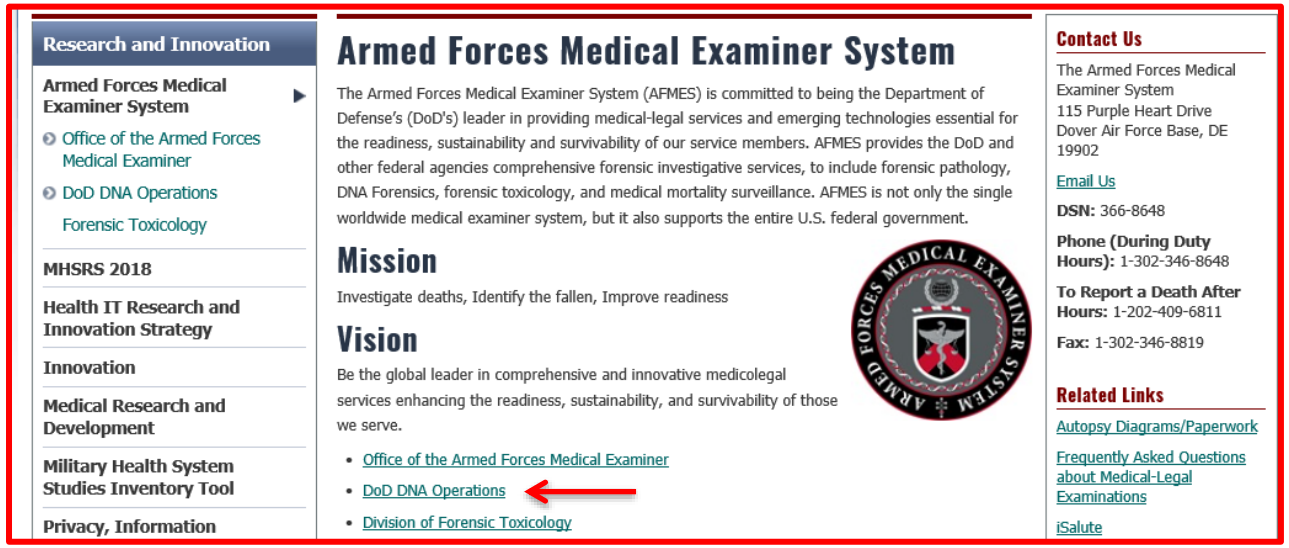
SNP Likelihood Approach

- Genotype likelihood scores applied
  - Determines what the likelihood of the data is authentic or artifact
- Maximizes use of all available data
- Helps prevent the possibility of incorrect genetic profiles
- New to the forensic field and reason for in depth validation

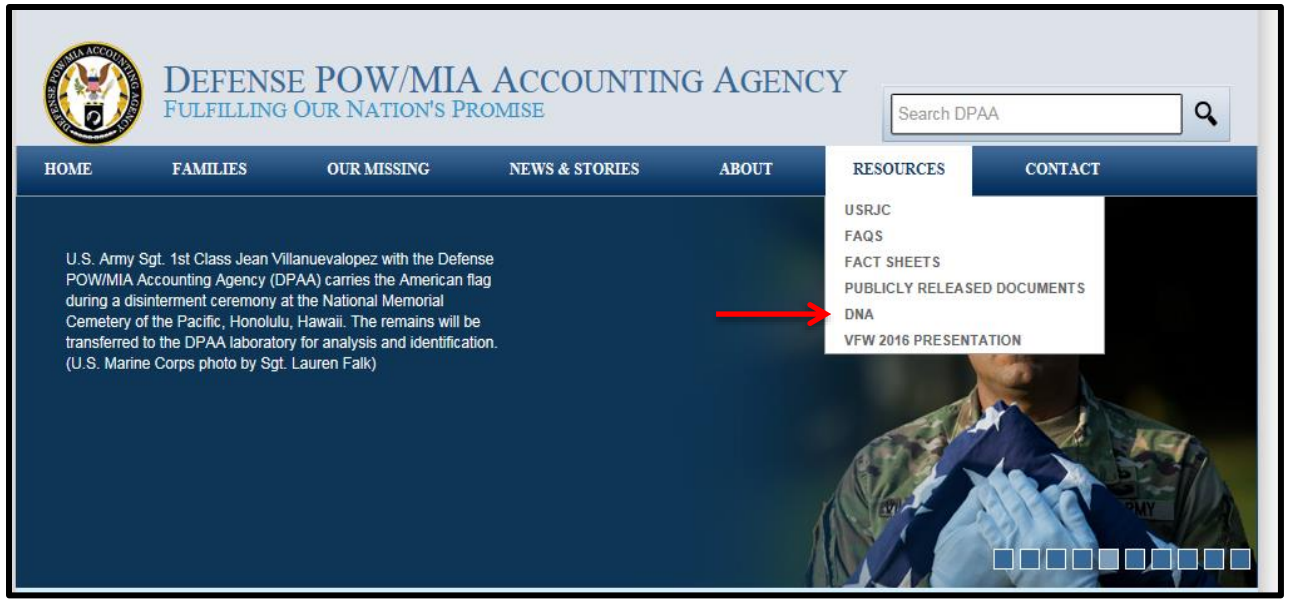
Family Reference Sample Collections

- **YOU Are The Key To The Identification Process**
- **Family References Are Collected Under Informed Consent With the Donor and Can Only Be Used For Human Remains Identification**
- **All FRS Samples Are Treated As a Medical Specimen**
- **Protected Under the Health Insurance Portability and Accountability Act of 1996 (HIPAA) For Personally Identifiable Information (PII) To Release of Information**
- **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**

DNA FAQ



https://health.mil/afmes



http://www.dpaa.mil/

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