

# DNA Analysis In The Identification Process



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Director, DoD DNA Operations  
Armed Forces Medical Examiner System

Family Member Update  
Little Rock, AK  
Nov 6<sup>th</sup>, 2020



# Purpose



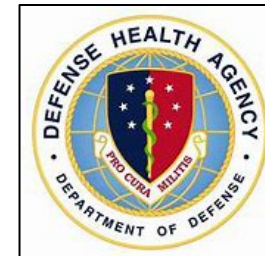
- **AFMES / DoD DNA Operations (AFDIL)**
- **DNA 101**
- **Way ahead**
- **Where you fit in**



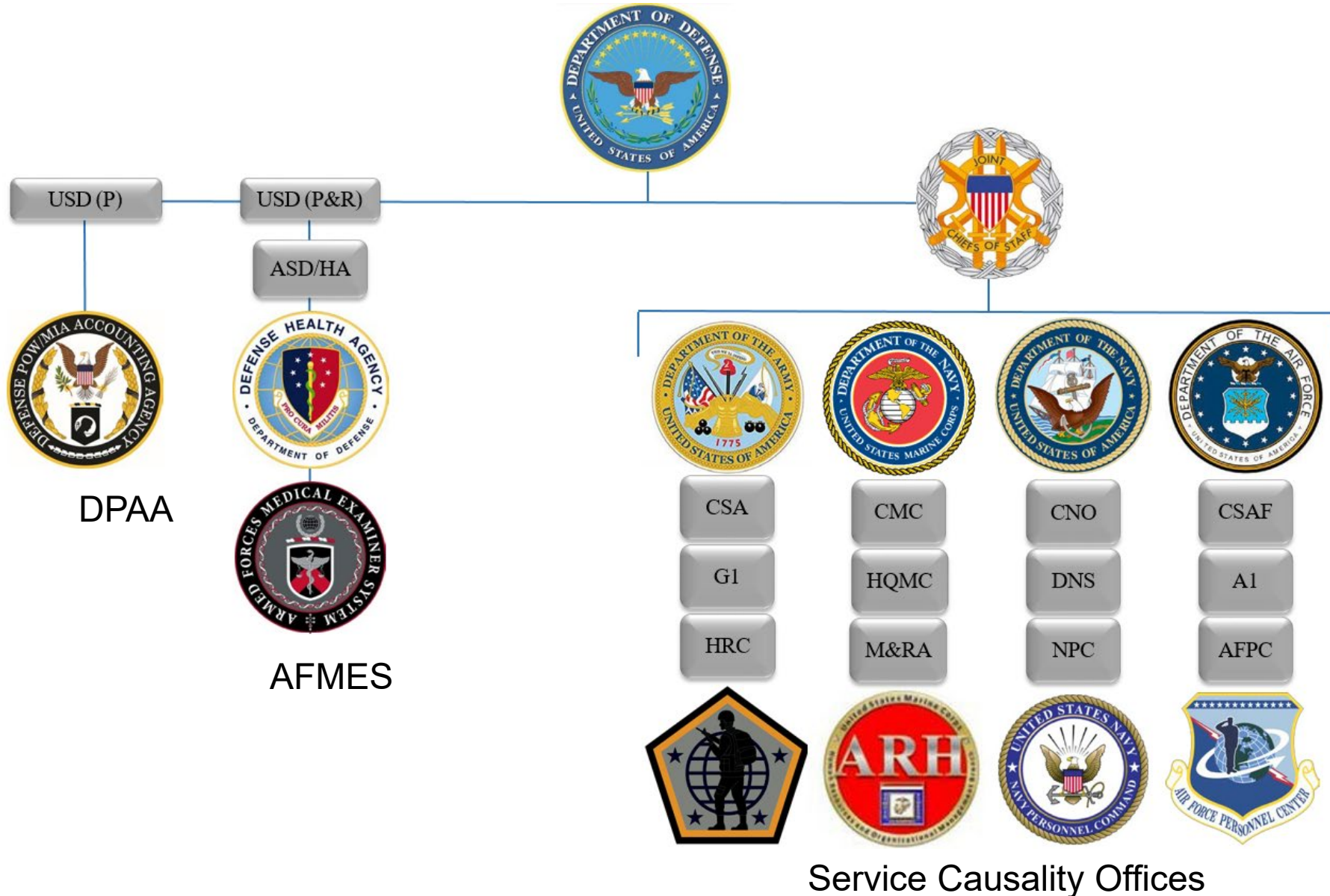
# Armed Forces DNA Identification Laboratory (AFMES-AFDIL)



- 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



# Mission Partners = Different Parent Organizations = Different Funding



# AFMES Missions Supported By AFDIL



**Present Day Accounting**



**Past Accounting**

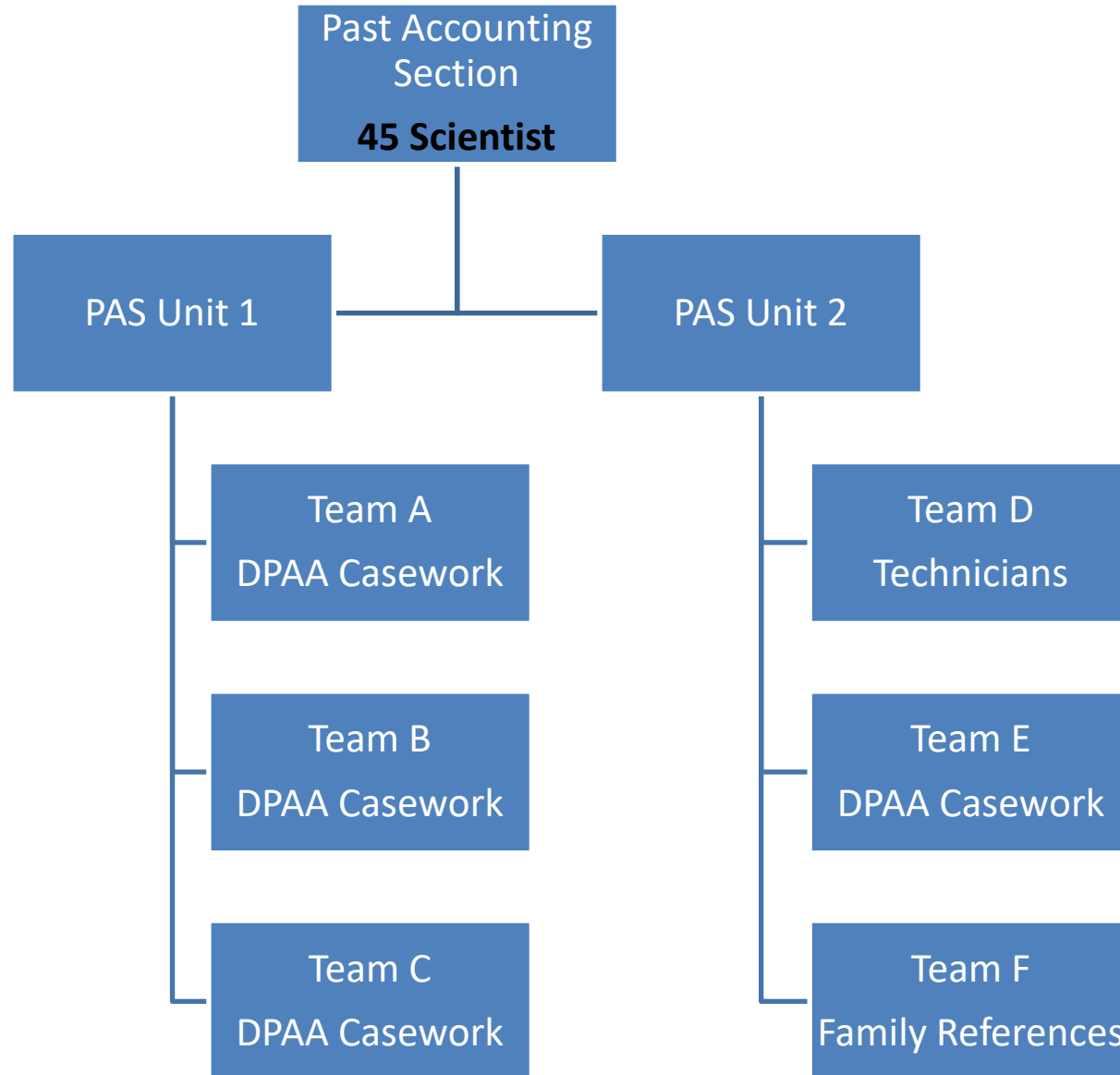


**FRS Databasing**



**World Wide Support**

# Past Accounting Section 2013



## 6 Teams

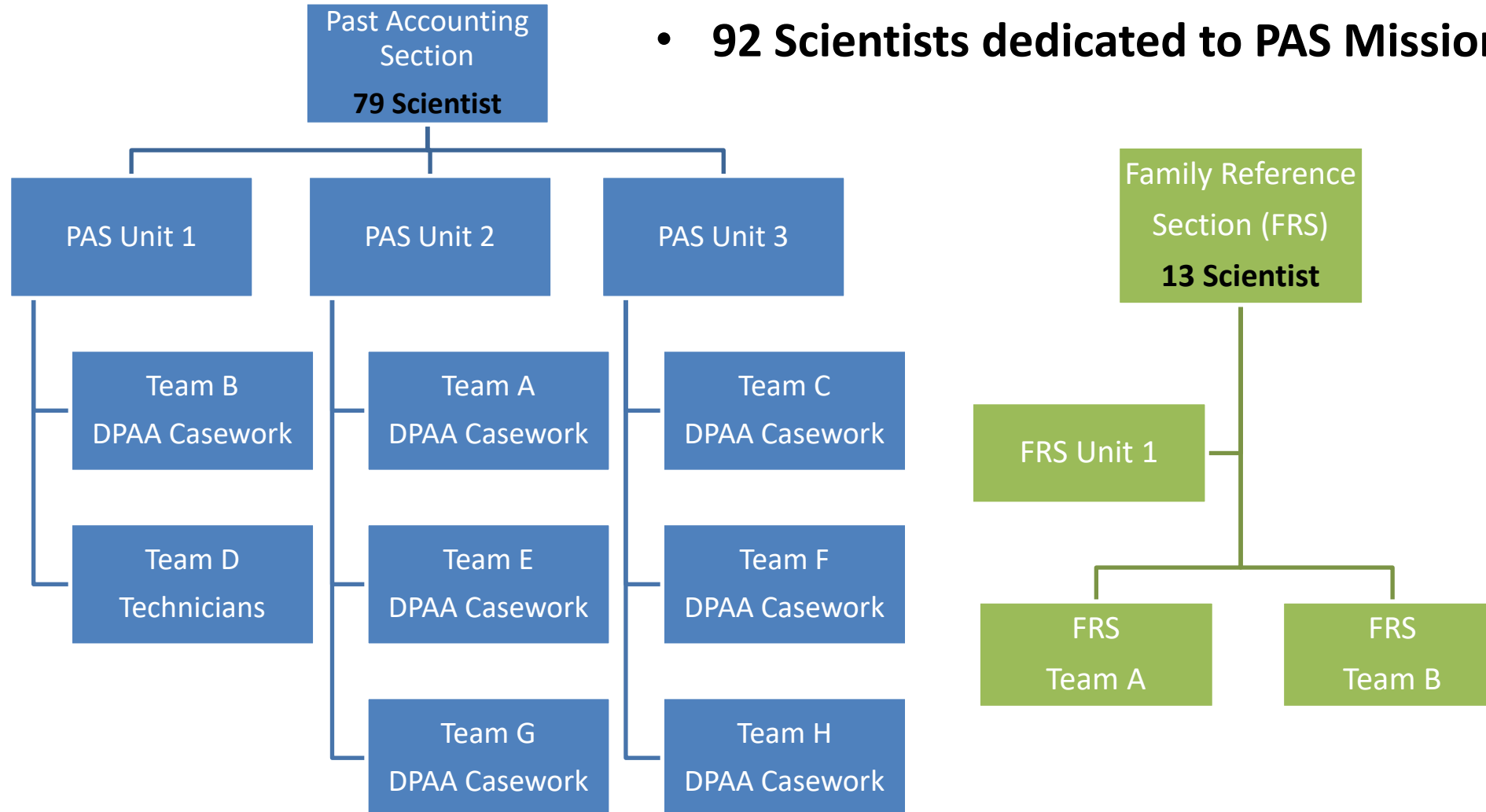
Casework

Family References

# Past Accounting Section



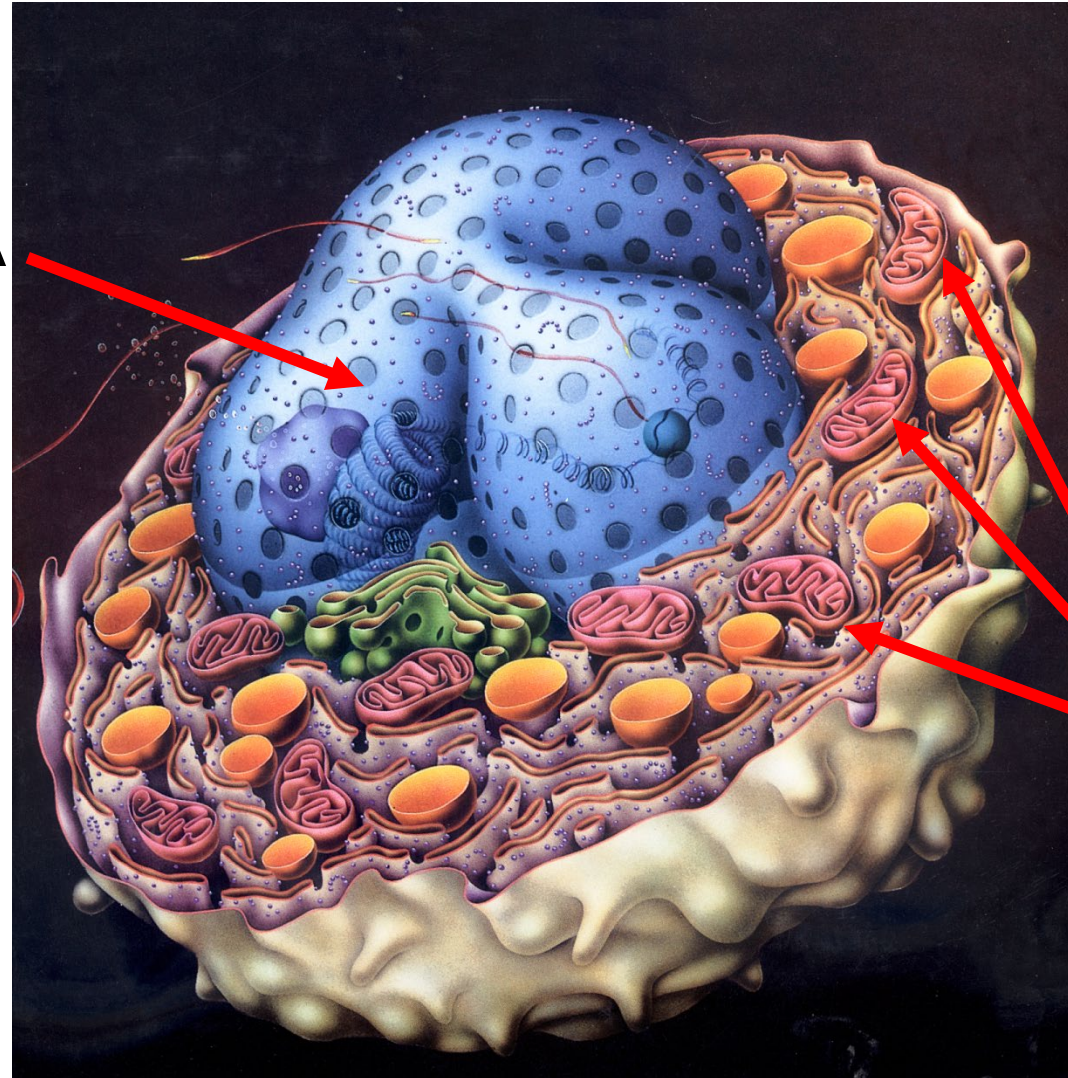
- 2 New Past Accounting Teams
- New FRS section
- 47 New Staff
- **92 Scientists dedicated to PAS Mission**



# The Human DNA Genome



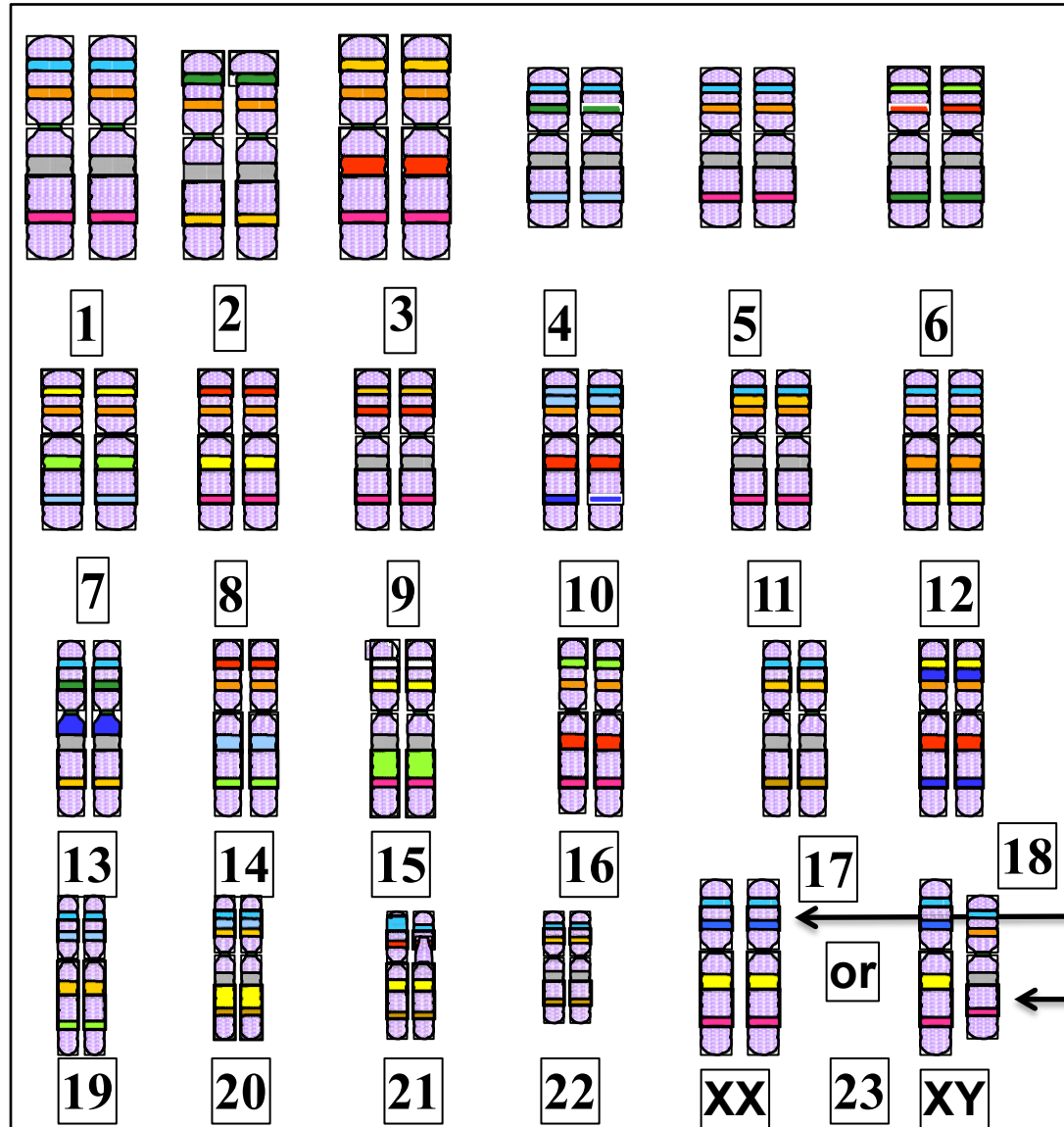
**Nuclear DNA**  
~3.2 billion  
base pairs  
(bp)



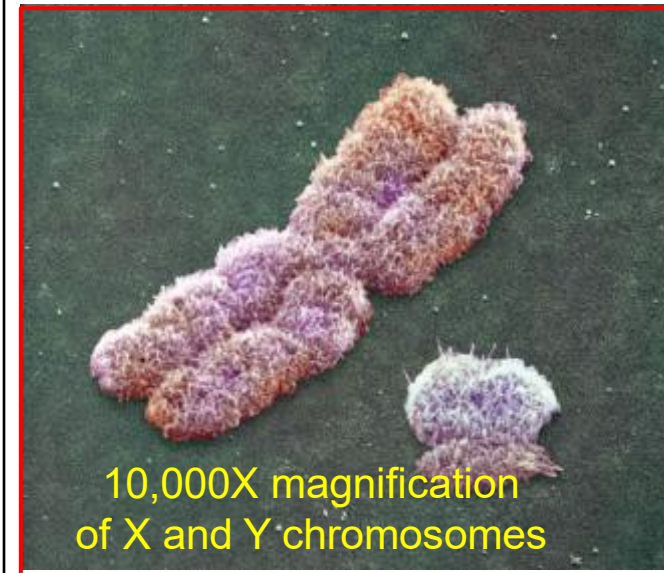
**Mitochondrial  
DNA**  
16,549 bp



# 23 Pairs of Chromosomes



The Nuclear Genome contains approximately 25,000 Genes

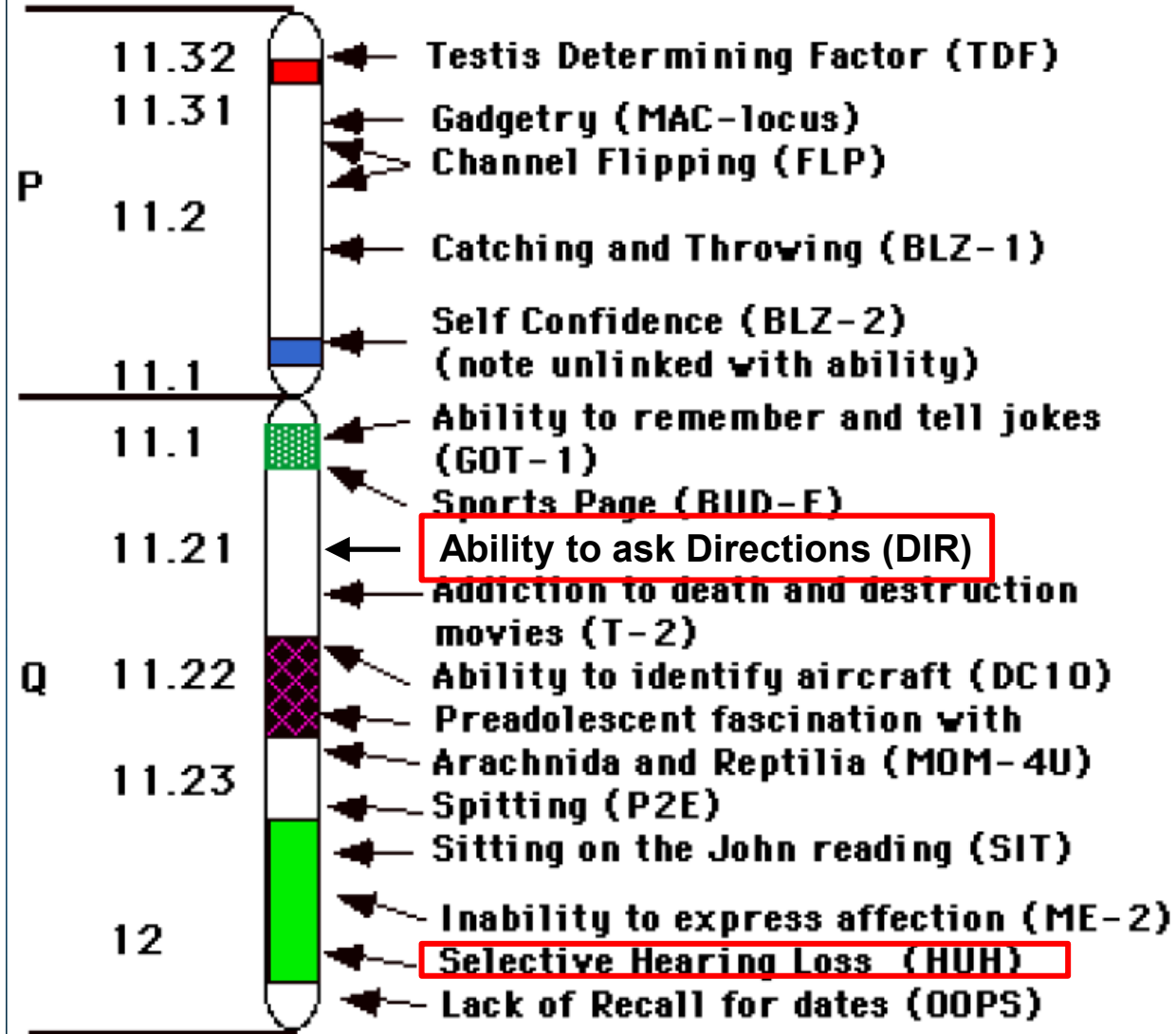


Female

Male

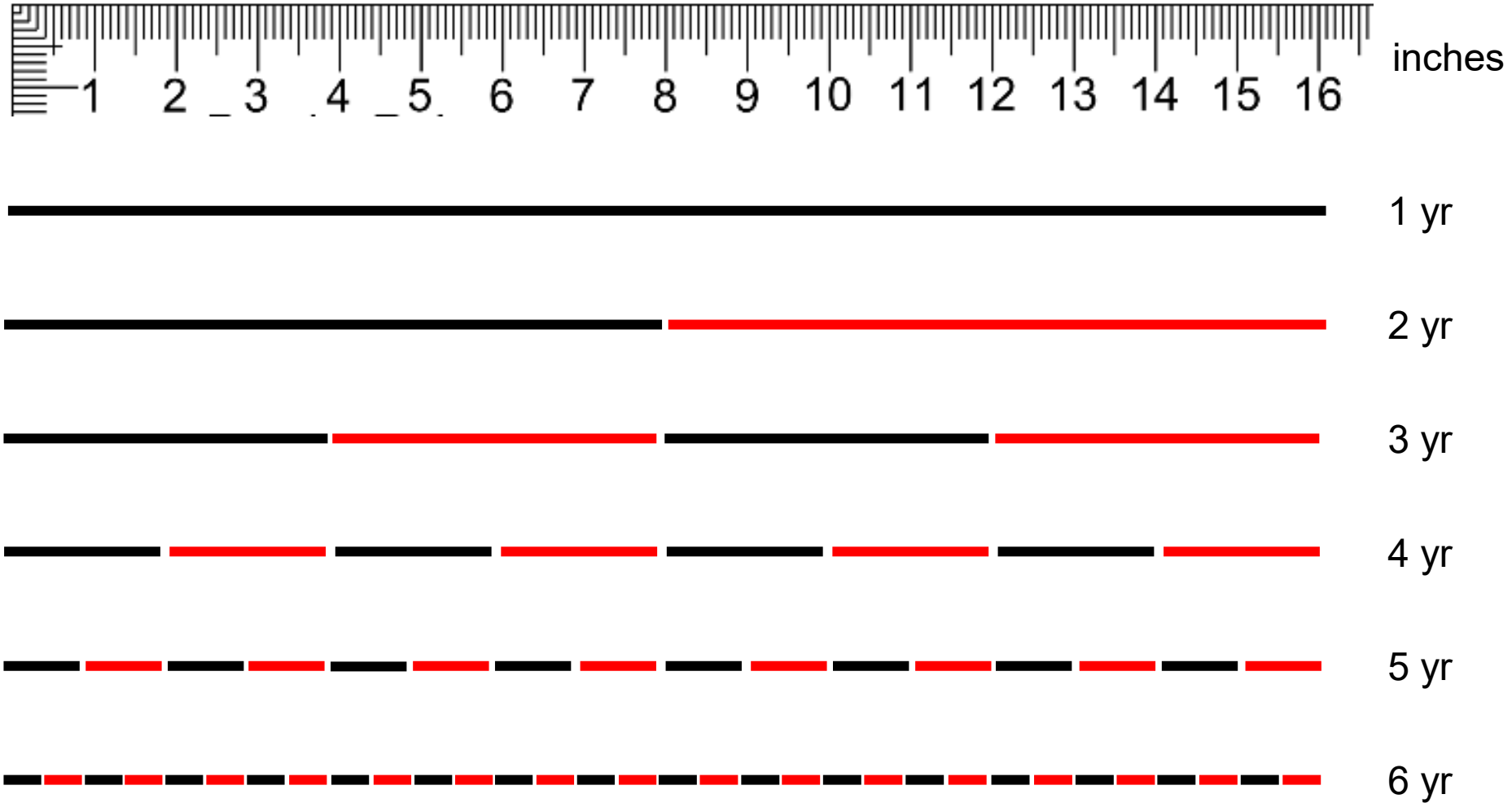
Chromosome #23

# Y Chromosome map



- Jane Gitschier, UCSF Science, 261, 679 (Aug. 93)

# DNA Degradation: Example

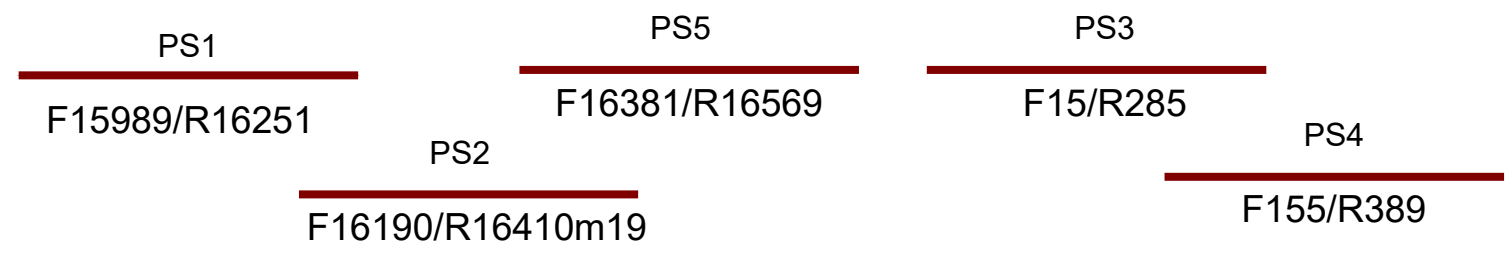


1 in = 100 base-pairs

# Current AFDIL Amplification Strategies



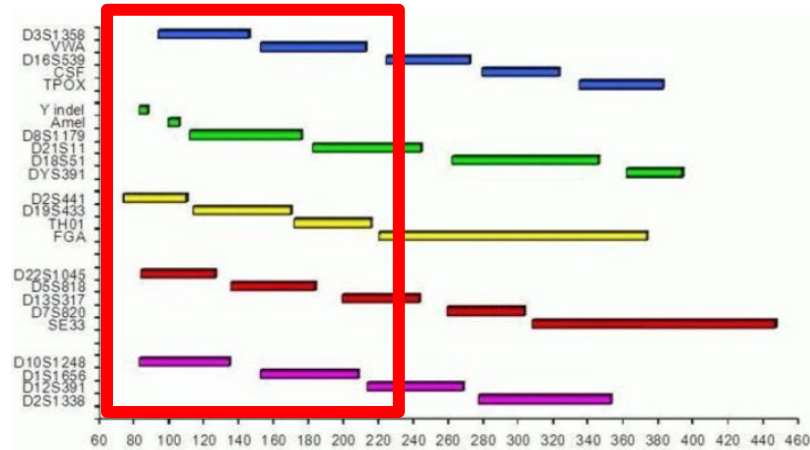
Primer sets (~200bp i.e. 2 in)



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



~2.25 inch DNA or 225 bp



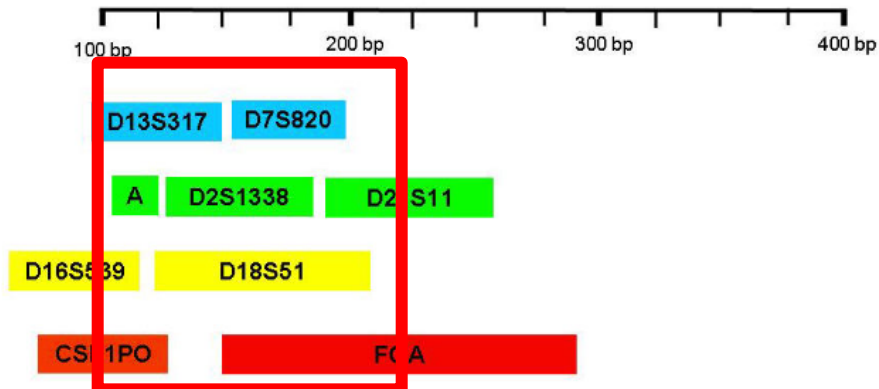
**AmpFISTR GlobalFiler**

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



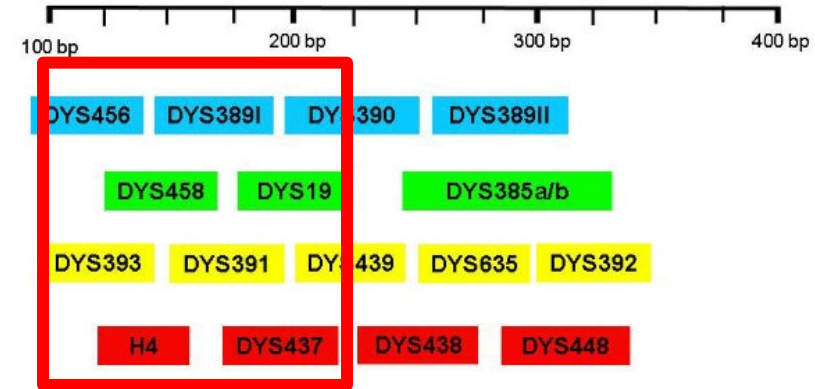
**PowerPlex Fusion**

<https://promega.com>



**AmpFISTR MiniFiler**

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



**AmpFISTR YFiler**

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

# Percent Nuclear DNA Shared Between Relatives



Amount of total nuclear DNA shared with ones relatives

# 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

# 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





# AFMES-AFDIL COVID Response



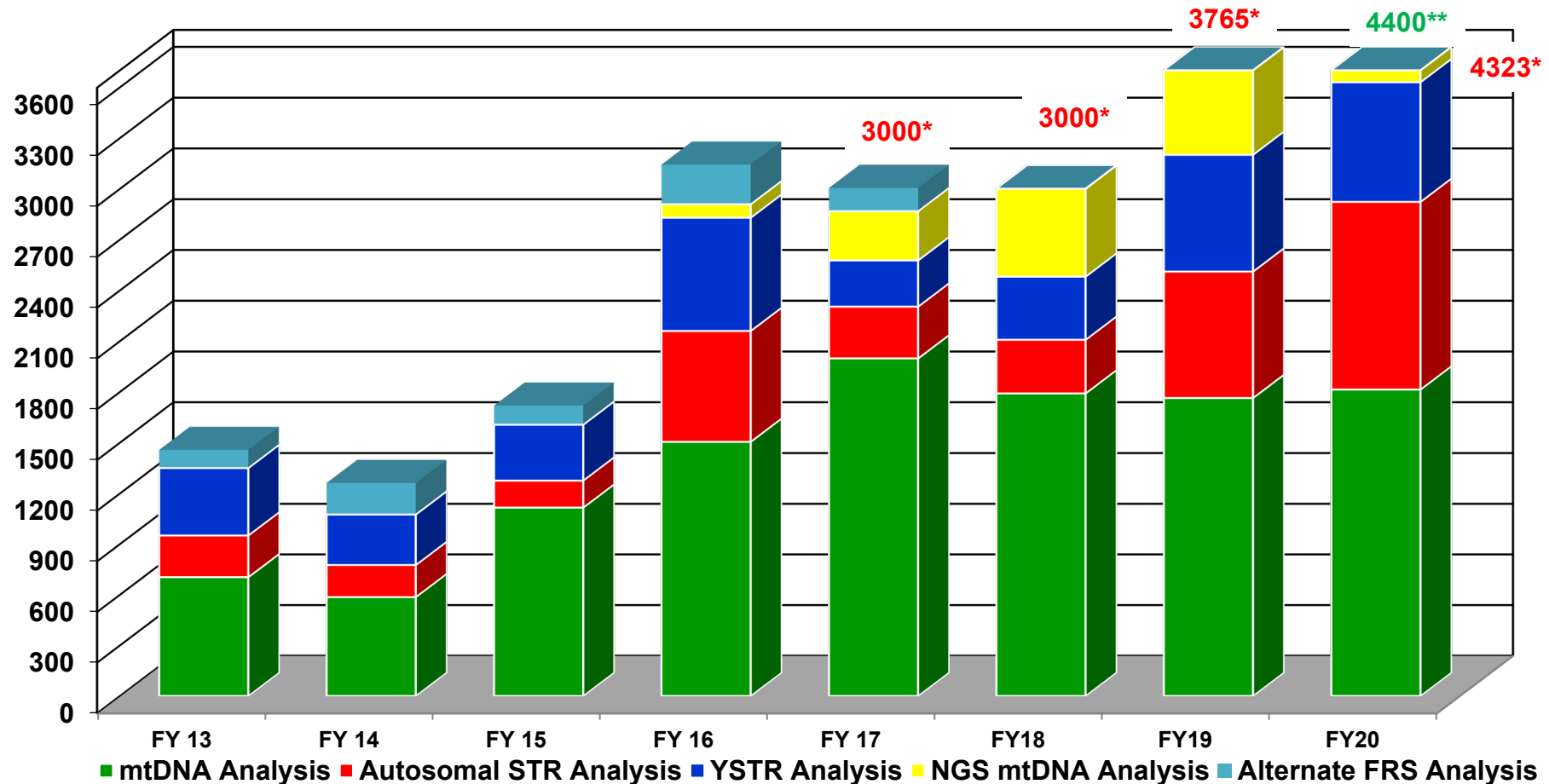
- **March 9<sup>th</sup>- March 13<sup>th</sup>**
  - **Established AM and PM Shift**
  - **50% of staff per shift**
- **March 16<sup>th</sup>- March 20<sup>th</sup>**
  - **10% of staff per shift**
  - **One week in lab, one week reviewing from home**
- **March 23<sup>rd</sup> – June 15<sup>th</sup>**
  - **25% of staff per shift**
  - **One week in lab, one week reviewing from home**
- **July 20<sup>th</sup> – Present**
  - **50% of staff per shift**

# DNA Tests Reported By AFDIL

As Of Sept 30<sup>th</sup>, 2020



**93% Success Rate Obtaining MtDNA Sequence Data**  
**80% Success Rate Obtaining Au-STR DNA Data**  
**70% Success Rate Obtaining Y-STR DNA Data**  
**68% Success Rate Obtaining NGS Data**



\* Total # DNA Test Reported

\*\* FY 2020 Goal



# DNA Reports

## September 30<sup>th</sup>, 2020



<b>FY</b>	<b>Believe to Be Reports</b>	<b>Addendum Reports</b>	<b>Foreign National</b>	<b>Total Reports</b>
<b>2013</b>	<b>55</b>	<b>120</b>	<b>39</b>	<b>214</b>
<b>2014</b>	<b>75</b>	<b>57</b>	<b>51</b>	<b>183</b>
<b>2015</b>	<b>71</b>	<b>77</b>	<b>32</b>	<b>180</b>
<b>2016</b>	<b>133</b>	<b>65</b>	<b>6</b>	<b>204</b>
<b>2017</b>	<b>154</b>	<b>165</b>	<b>26</b>	<b>345</b>
<b>2018</b>	<b>189</b>	<b>496</b>	<b>34</b>	<b>719</b>
<b>2019</b>	<b>207</b>	<b>448</b>	<b>3</b>	<b>658</b>
<b>2020</b>	<b>145</b>	<b>1088</b>	<b>7</b>	<b>1240</b>

# Why Does DNA Take Time

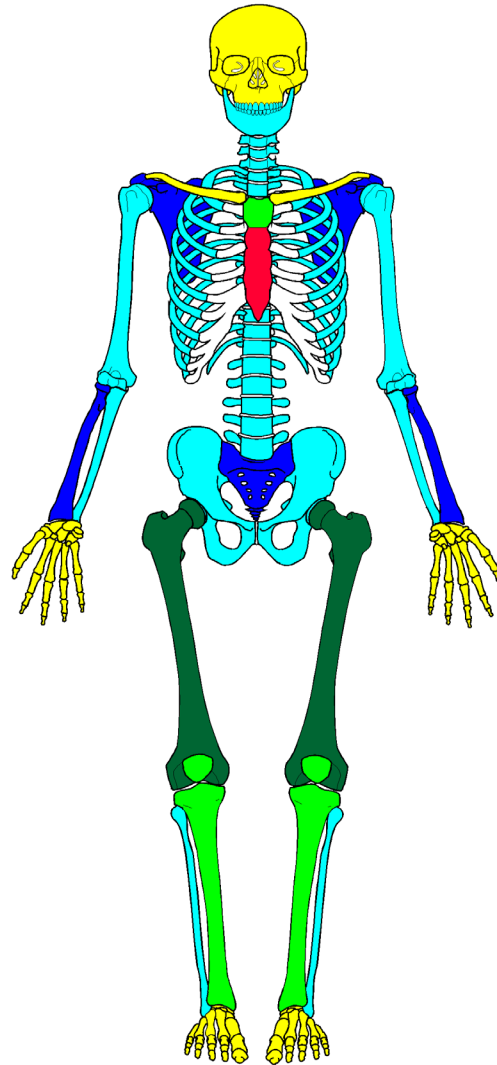


- Sample quality and DNA quantity
- Authentic sequence and confidence
- **Forensic instruments and kits geared toward**

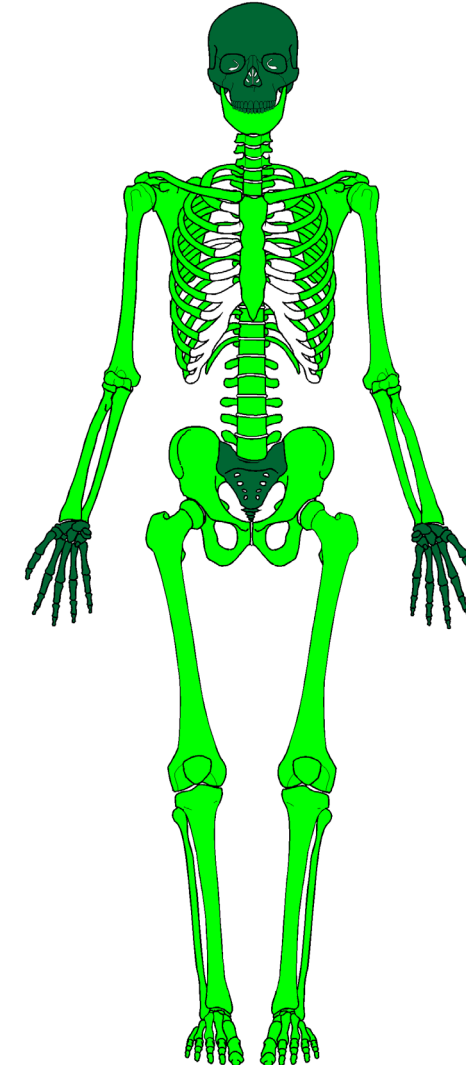
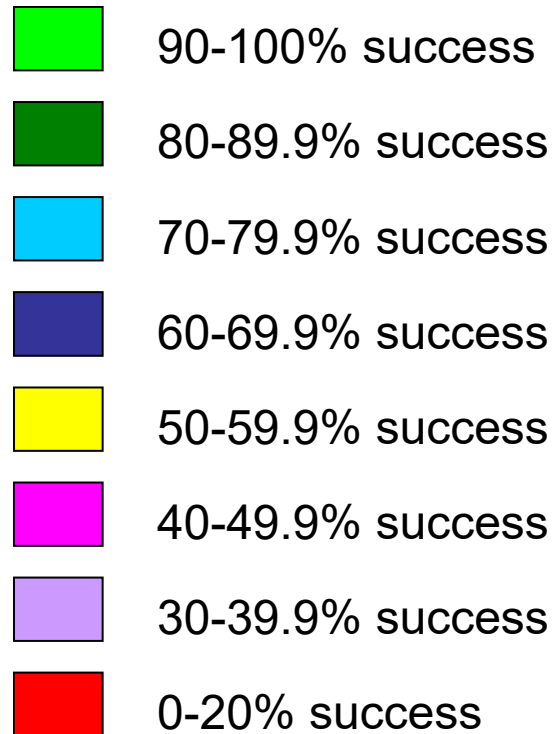
- **FIT FOR USE**

- **AFMES has to create new testing method**
  - No commercial kits/have to develop
  - Science needs to catch up
- Family References

# 2006:New Method: Demineralization



**Pre-Demineralization**



**Post- Demineralization**

- **FY13: Old Purification Process**

- Y-STR: 24%
- auSTR: 26%
- mtDNA Sequencing: 90%

- **Improved DNA Purification Process**

<b>Success rates by analysis</b>	<b>FY16</b>	<b>FY17</b>	<b>FY18</b>	<b>FY19</b>	<b>FY 20</b>
mtDNA Sequencing	92%	94%	92%	94%	93%
Autosomal STR	48%	46%	61%	63%	80%
Low Copy Y STR	47%	51%	57%	66%	70%

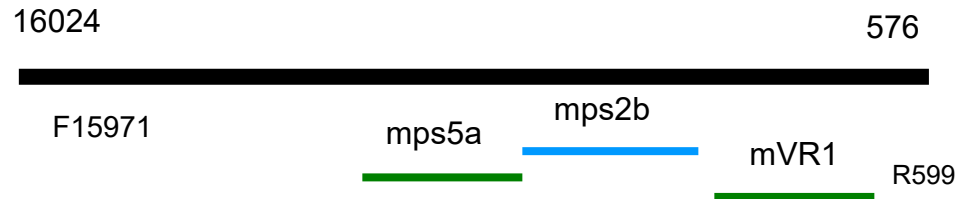
# 2016: NGS Korea Punchbowl



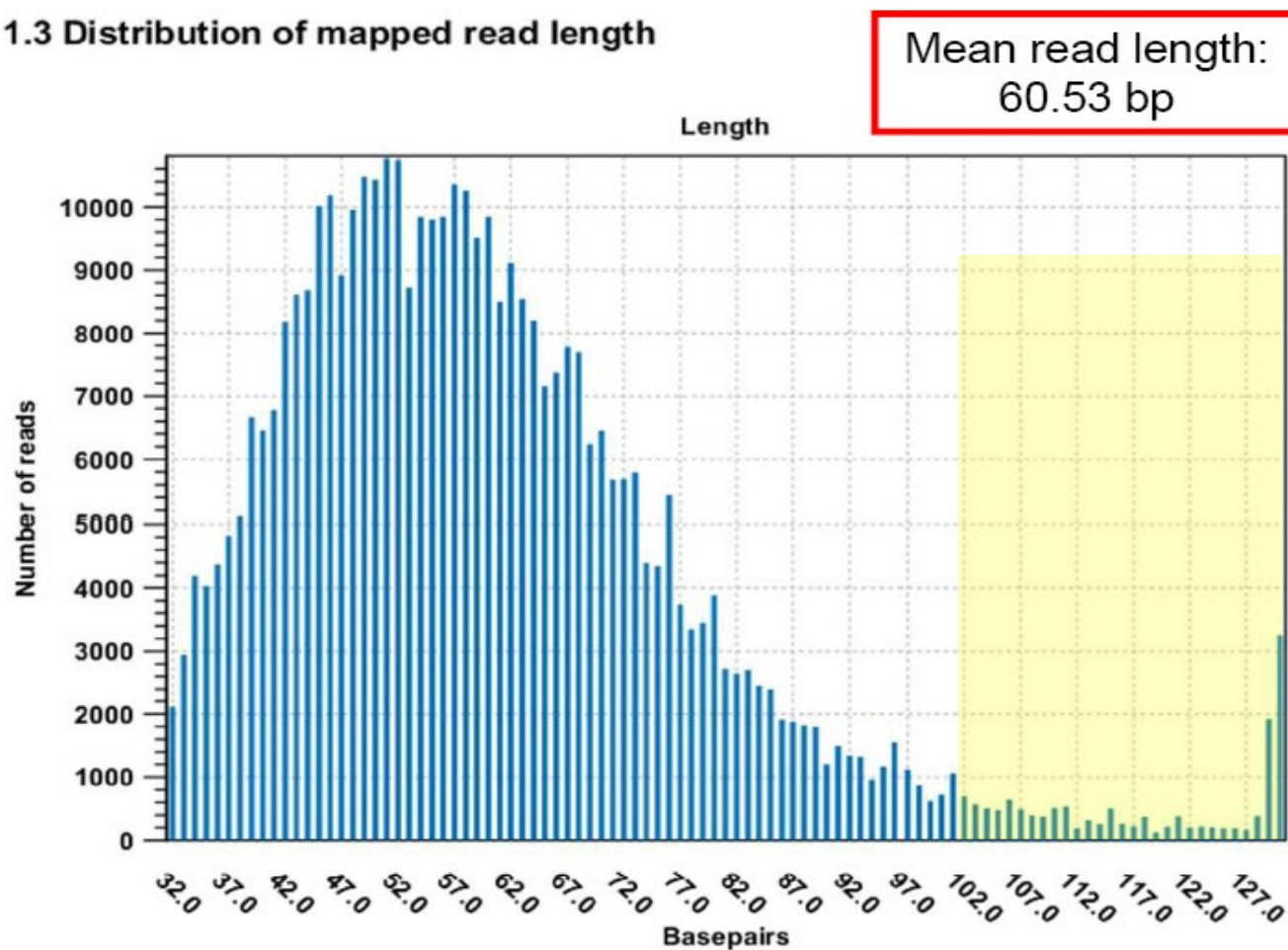
**Success Rates: Normal mtDNA**  
**Testing**  
**6% mtDNA**  
**0% STRs**

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

# DNA Fragment Size: Typical Bone Submission



## 1.3 Distribution of mapped read length



Mini-primer sets  
(~120bp)

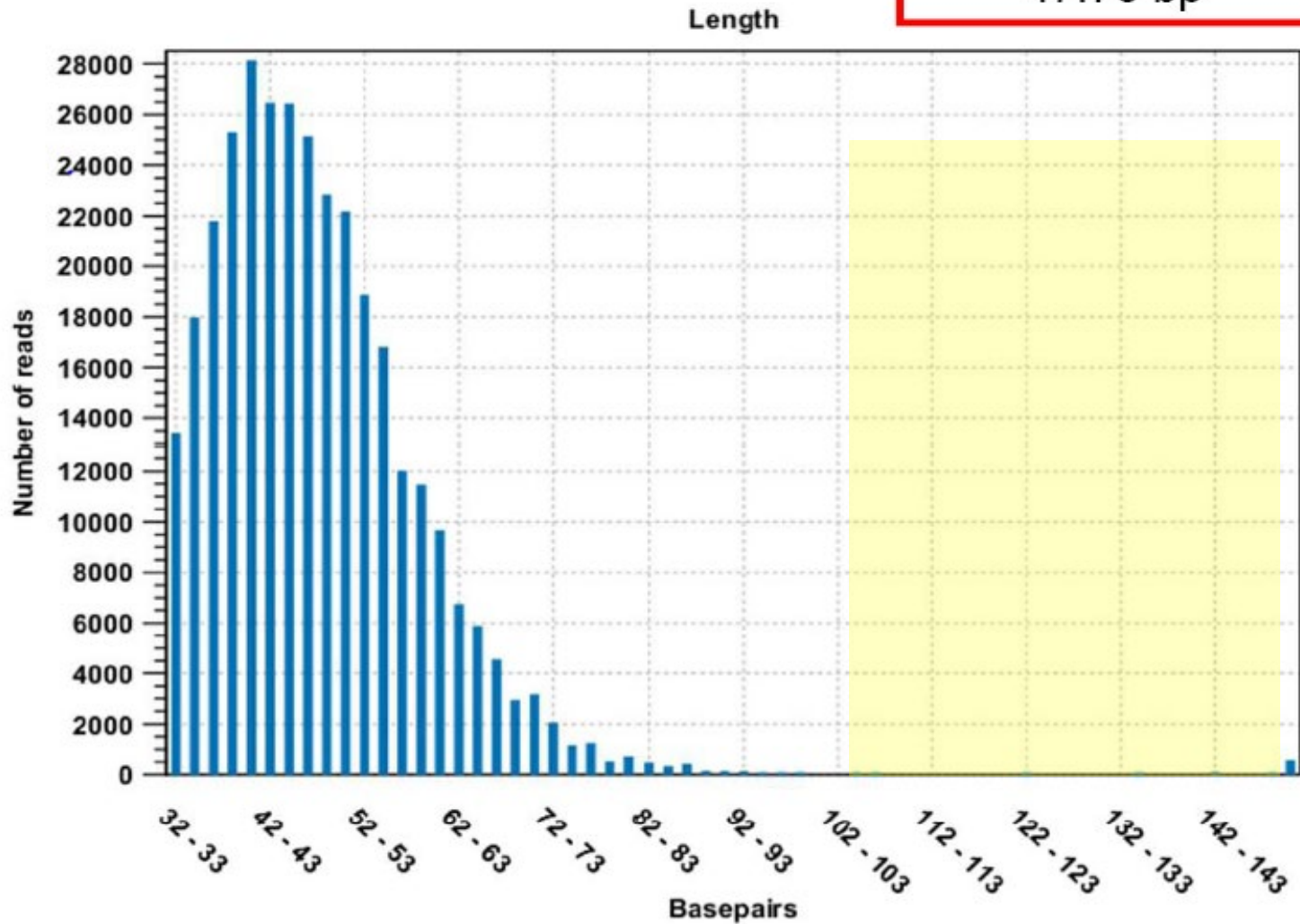


# DNA Fragment Size: Highly Degraded Submission

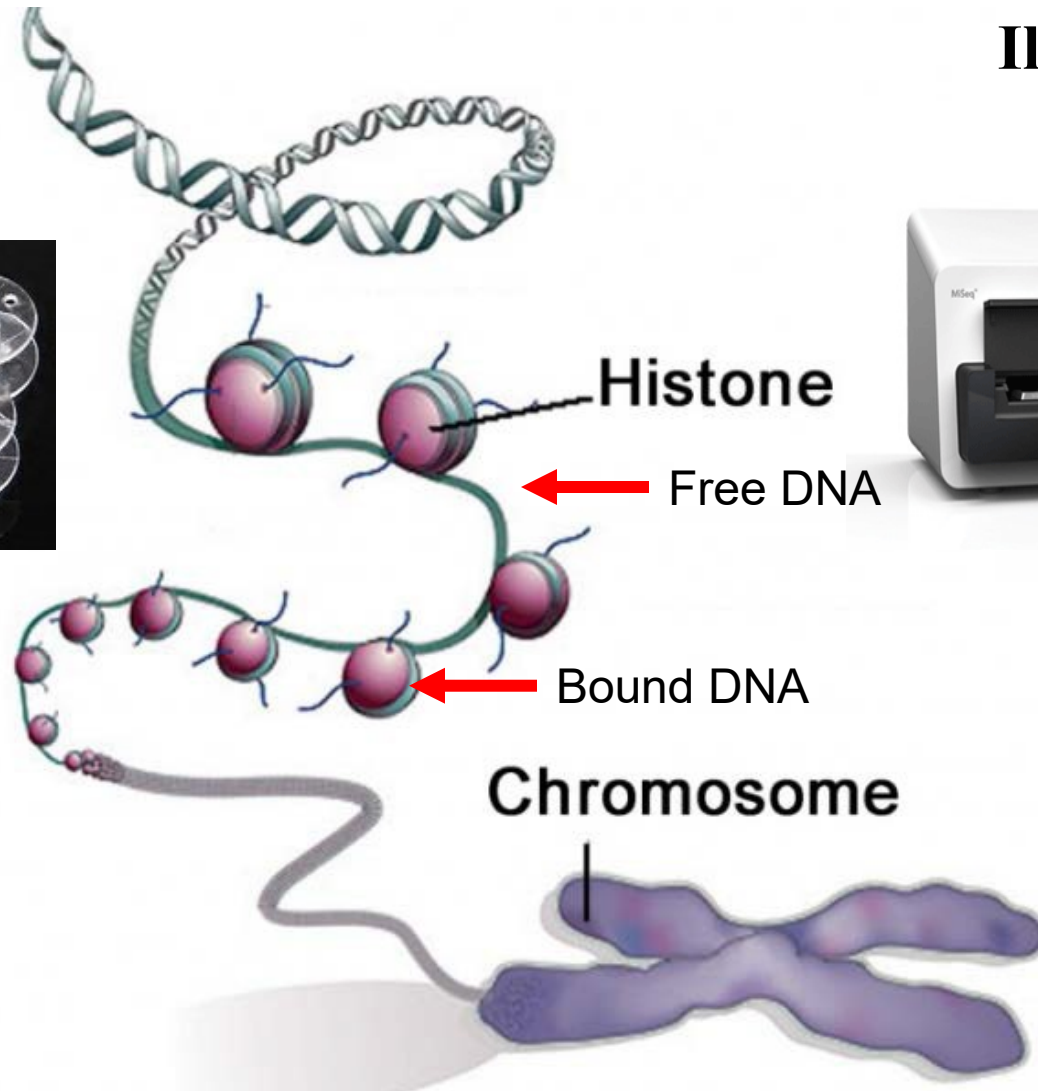


## 1.3 Distribution of mapped read length

Mean read length:  
47.76 bp



# DNA/Protein Fixation Issue



## Illumina MiSeq



Fiscal Year	16	17	18	19	20	Overall
NGS Analyses Reported	78	291	519	553	693	2134
NGS Analyses Reported with Data	22	129	247	331	468	1197
Percent Success Rate	28%	44%	40%	60%	68%	56%
Average Samples per Month	11	24	43	46	58	
Whole Genome Family References			93	279	179	551

- **DNA Reports**

- 389 DNA Comparison Reports
- 168 First Time Named Reports
- 221 Addendum Reports

- **Processing Capacity**

- New Teams
- New Instruments





# Emerging Technologies: New Method Development

# Approaching CSI Shows



Public Law 115-50  
115th Congress

## An Act

To establish a system for integration of Rapid DNA instruments for use by law enforcement to reduce violent crime and reduce the current DNA analysis backlog.

Aug. 18, 2017  
[H.R. 510]

*Be it enacted by the Senate and House of Representatives of the United States of America in Congress assembled,*

Rapid DNA Act  
of 2017.  
42 USC 13701  
note.

### SECTION 1. SHORT TITLE.

This Act may be cited as the "Rapid DNA Act of 2017".

### SEC. 2. RAPID DNA INSTRUMENTS.

12, 2018, 2:27 p.m.

CAMP FIRE

By RONG-GONG LIN II

## Rapid DNA analysis is being used to identify dozens of California fire victims



The ANDE Rapid DNA instrument - FBI NDIS Approved

## ANDE Corporation's Rapid DNA Identification System First to Receive FBI Approval Under New Standards June 2018

DNA results in under 2 hrs

# RAPID Instruments



- PROS
  - Developed for Swabs
  - High Copy Samples
  - Fast
  - Booking Stations/Family assistance Centers
  - Adoptable to other samples
    - Bone, Cigarettes, tissue

Cow



- CONS
  - Modern samples only
  - Fresh Bone/buccal swabs
  - STR based only
  - 7 samples/low throughput

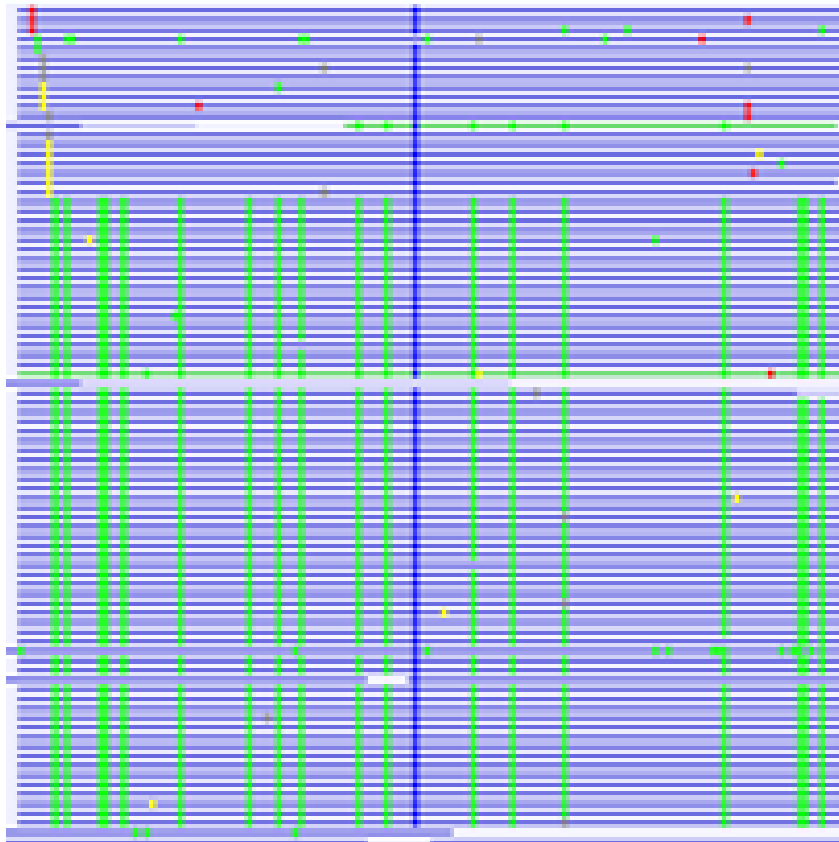


# Emerging Technologies

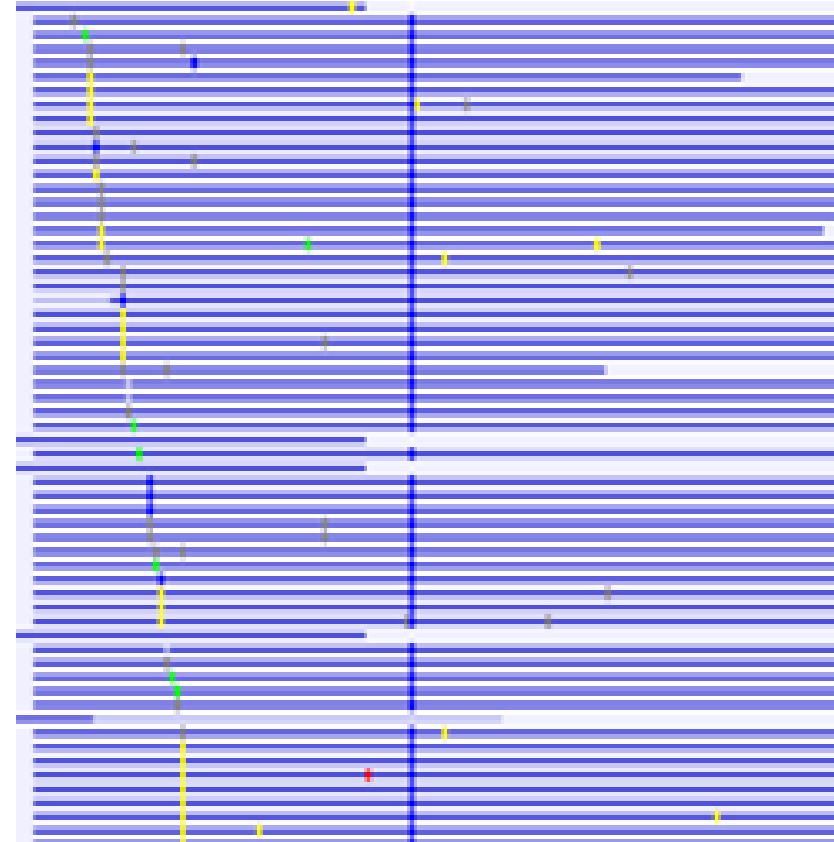


- **Repairing DNA Damage Due to Degradation**

**Unrepaired**



**FFPE Repaired**



# Emerging Technologies



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



## Golden State Killer

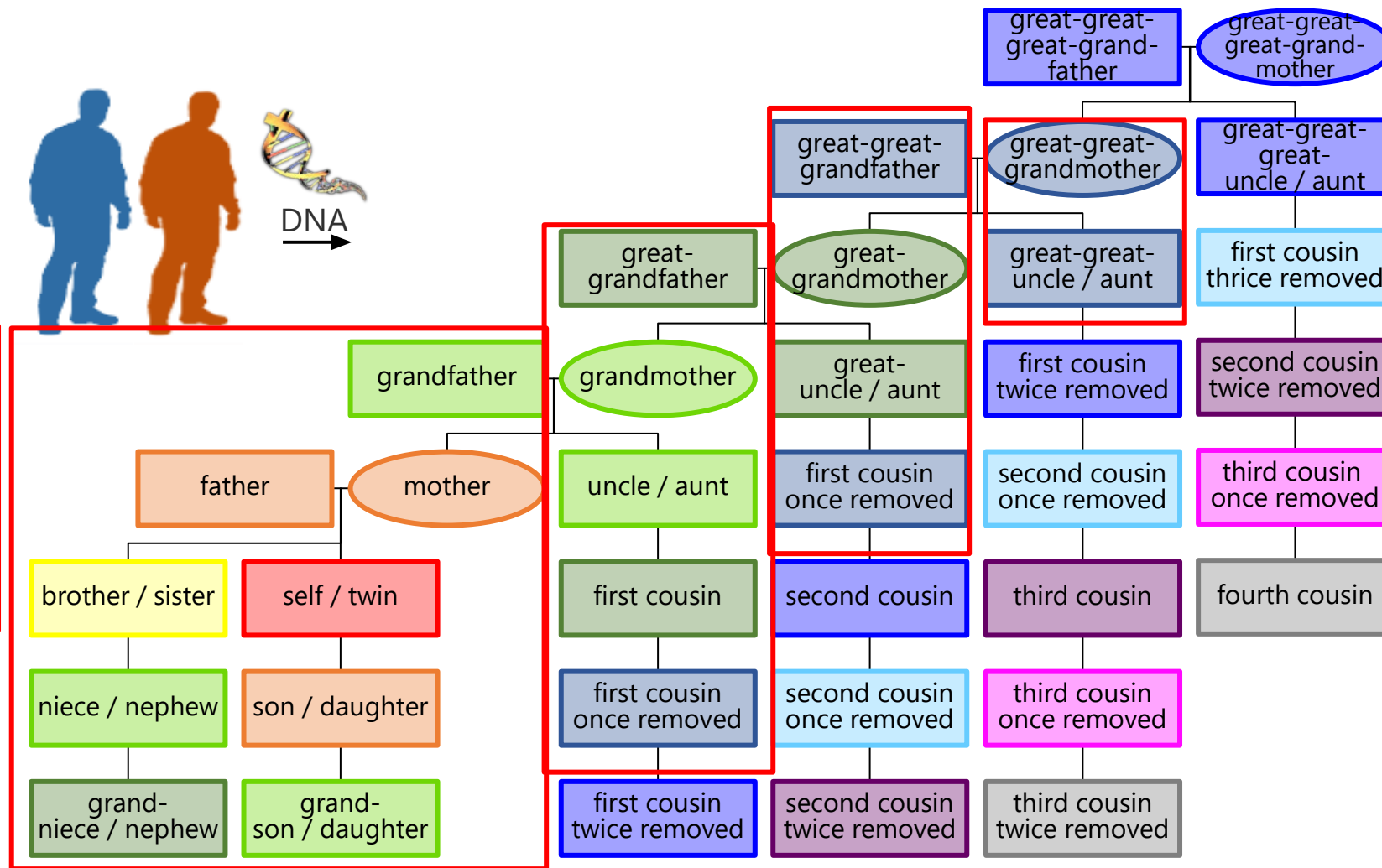
- 1975-1986: Crime spree (murders, rapes, burglaries.
- Rape case samples frozen: DNA Technology had to catch up.
- GedMatch Open Database started in 2011
  - Allows matching independent of vendor results
  - Only shows matches and not raw data
- Can this work for Past Accounting
  - Not in current commercial form
  - DNA too damaged
  - Develop method that will

# Degrees of Relatedness



## Relationship

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



- **No viable mtDNA, YSTR, or auSTR references available**
  - Millions of identity and kinship targets in the nuclear genome
    - Ancestry.com and 23andMe
    - Commercial test did not work
  - As little as 15,000 targets can identify unrelated from related out to a 4<sup>th</sup> degree relative
    - Great-Great Uncle or First Cousin 1 removed: 6.25% nuclear DNA
  - Parabon developed custom software to work with DPAA samples
  - AFDIL developed method to capture SNPs

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



# Modern Approach to DNA-Assisted Identification



- Maximize discrimination power of the mtDNA
- Faster processing using automation
- Kinship Single Nucleotide Polymorphism analysis
  - Improved nuclear kinship statistics
  - Increases ability to utilize more distant relations as nuclear references.

**SUPPORT MORE IDENTIFICATIONS FASTER**

# 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



**Research and Innovation**

**Armed Forces Medical Examiner System**

- Office of the Armed Forces Medical Examiner
- DoD DNA Operations
- Forensic Toxicology

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MHSRS 2018

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Health IT Research and Innovation Strategy

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Innovation

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Medical Research and Development

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Military Health System Studies Inventory Tool

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Privacy, Information

## Armed Forces Medical Examiner System

The Armed Forces Medical Examiner System (AFMES) is committed to being the Department of Defense's (DoD's) leader in providing medical-legal services and emerging technologies essential for the readiness, sustainability and survivability of our service members. AFMES provides the DoD and other federal agencies comprehensive forensic investigative services, to include forensic pathology, DNA Forensics, forensic toxicology, and medical mortality surveillance. AFMES is not only the single worldwide medical examiner system, but it also supports the entire U.S. federal government.

### Mission

Investigate deaths, Identify the fallen, Improve readiness

### Vision

Be the global leader in comprehensive and innovative medicolegal services enhancing the readiness, sustainability, and survivability of those we serve.

- Office of the Armed Forces Medical Examiner
- [DoD DNA Operations](#) ←
- Division of Forensic Toxicology

**Contact Us**

The Armed Forces Medical Examiner System  
115 Purple Heart Drive  
Dover Air Force Base, DE 19902

[Email Us](#)

DSN: 366-8648

**Phone (During Duty Hours):** 1-302-346-8648

**To Report a Death After Hours:** 1-202-409-6811

Fax: 1-302-346-8819


**Related Links**

[Autopsy Diagrams/Paperwork](#)

[Frequently Asked Questions about Medical-Legal Examinations](#)

[iSalute](#)

<https://health.mil/afmes>



## DEFENSE POW/MIA ACCOUNTING AGENCY

FULFILLING OUR NATION'S PROMISE

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CONTACT

U.S. Army Sgt. 1st Class Jean Villanueva Lopez 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)

- USRJC
- FAQS
- FACT SHEETS
- PUBLICLY RELEASED DOCUMENTS
- DNA ←
- VFW 2016 PRESENTATION

<http://www.dpaa.mil/>



Director DoD DNA Operations: [Timothy.P.McMahon10.civ@mail.mil](mailto:Timothy.P.McMahon10.civ@mail.mil)