



The National League of POW/MIA Families

13 June 2014

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Armed Forces Medical Examiner System



Disclaimer

The opinions or assertions presented hereafter are the private views of the speaker(s) and should not be construed as official or as reflecting the views of the Department of Defense, its branches, the US Army Medical Research and Material Command or the Armed Forces Medical Examiner System

Armed Forces DNA Identification Laboratory



- Established in 1992
 - With the primary purpose of utilizing DNA methods to identifying the remains of US service members.
- Division of the Armed Forces Medical Examiner System (AFMES)
 - Current Day Deaths
- Partner with Defense Prisoner of War Missing Personnel office and Joint POW/MIA Accounting Command – Central Identification Laboratory
 - Past Accounting Mission





Laboratory Accreditations & Standards



**American Society of Crime
Laboratory Directors-Laboratory
Accreditation Board
(ASCLD - LAB Certification)**



**Federal Bureau Of Investigation
(FBI) Quality Assurance Standards
(QAS 2009)**



**DoD DA Oversight Committee
(Defense Science Board 1995)**





AFDIL's DNA Capability

Autosomal STR

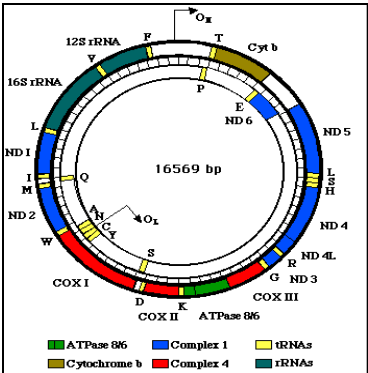
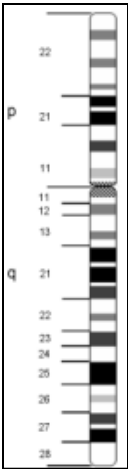
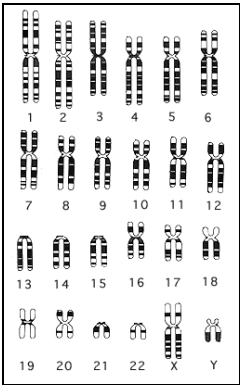
D31358 14,15
TH01 7, 8
D21S11 28,32
D18S51 12,15
Penta E 11,14
D5S818 11,---
D13S317 9,10
D7S820 10,11
D16S539 10,11
CSF1PO 10,12
Penta D 11,14
AMEL X,Y
vWA 17,19
D8S1179 13,14
TPOX 11,12
FGA 22,26

Y STR

DYS456 15
DYS389I 13
DYS390 24
DYS389II 29
DYS458 17
DYS19 15
DYS385a/b 11,14
DYS393 13
DYS391 11
DYS439 12
DYS635 24
DYS392 13
YGATA 13
DYS437 15
DYS438 12
DYS448 19

mtDNA Sequence

73 G
152 C
195 C
263 G
315.1 C
16126 C
16163 G
16186 T
16189 C
16294 T
16519 C





Comparison Of DNA Types

<u>Nuclear DNA</u>	<u>Mitochondrial DNA</u>	<u>Y- STR DNA</u>
<ul style="list-style-type: none">• High Discrimination• Two Copies/Cell• Capable of Repair & Recombination• Best Indirect Reference Are Mother Father &/Or Offspring• Several Years• Quick Turn-around• Moderate Expense	<ul style="list-style-type: none">• Limited Discrimination• Multiple Copies• No Repair Mechanism Or No Recombination• Only Indirect References From The Maternal Lineage• Decades To Centuries• Moderate Turn-around• Moderate Expense	<ul style="list-style-type: none">• Limited Discrimination• Only One Copy/Cell• No Recombination• Only Indirect References From The Paternal Lineage• Several Years• Quick Turn-around• Moderate expense



AFDIL Support Of The Joint POW/MIA Accounting Command

- Cases 25 to 140 Years Old
- Generally Fragmented
- Environmentally Challenged
- Direct References Not Available
- mtDNA, nucSTR and YSTR Analysis



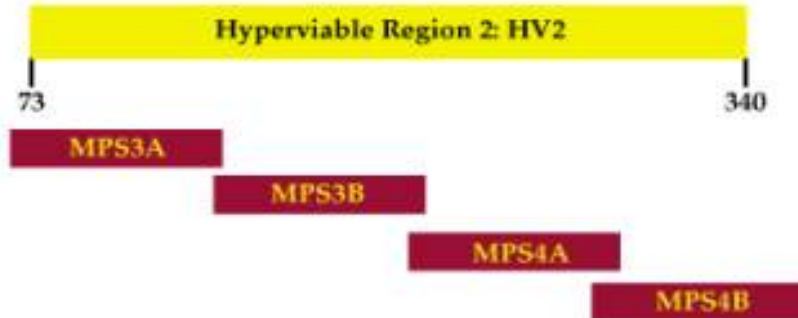
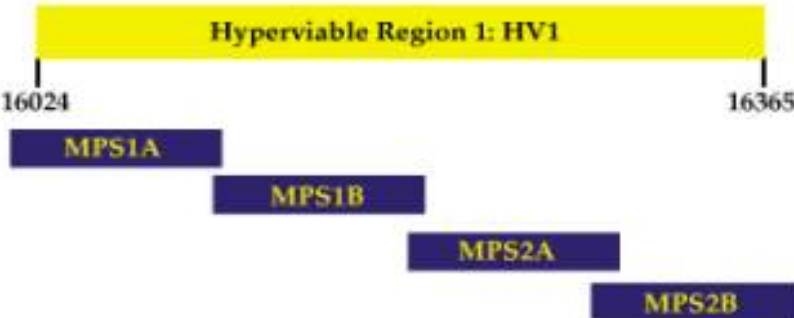


Methodology

- **JPAC-CIL Samples Are Processed On A Order Received And Priority**
 - **300+ skeletal samples in progress at any one time**
 - **~50 samples per team**
 - **Samples are reprioritized on a routine basis to meet JPAC needs.**
- **Average turn around time (TAT):**
 - **MtDNA Section → ~85 Mission Days**



Mitochondrial DNA Amplification





Methodology

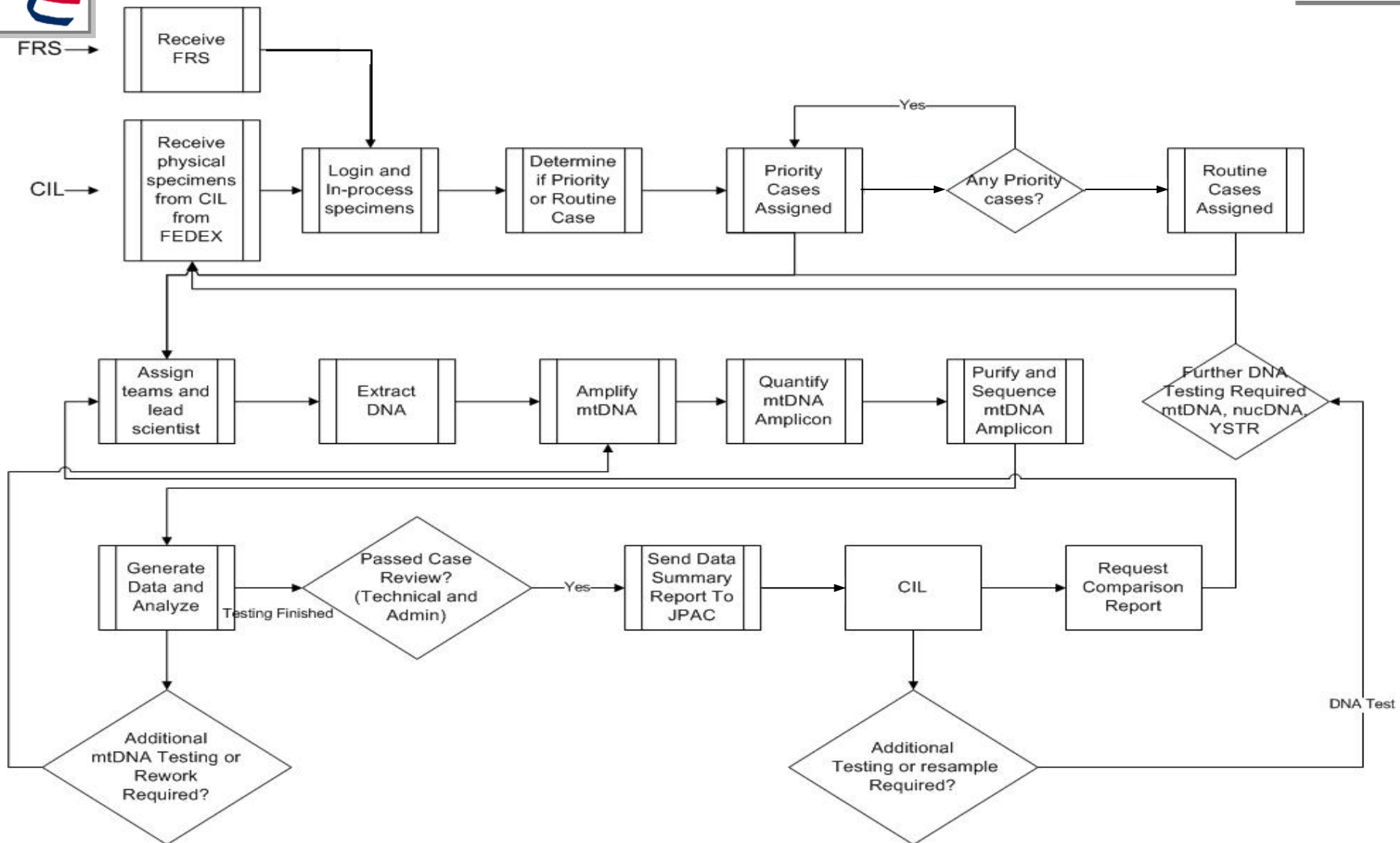
Family Reference Flow



- Accessioning – Receipt of FRS
- Extraction – 2 per specimen
- Amplification – 19 per specimen with controls
- Sequencing- mtDNA – 48 per specimen
- Data Analysis – 96 lanes of data per specimen
- Report Writing – 1 report per specimen
- Review – 3 levels per specimen
- Verification Process – Service Causality Offices
 - Additional testing
 - Recollect from Donor
 - Relationship verification
 - Name verification
 - Gender verification
 - Reports submitted to Donor

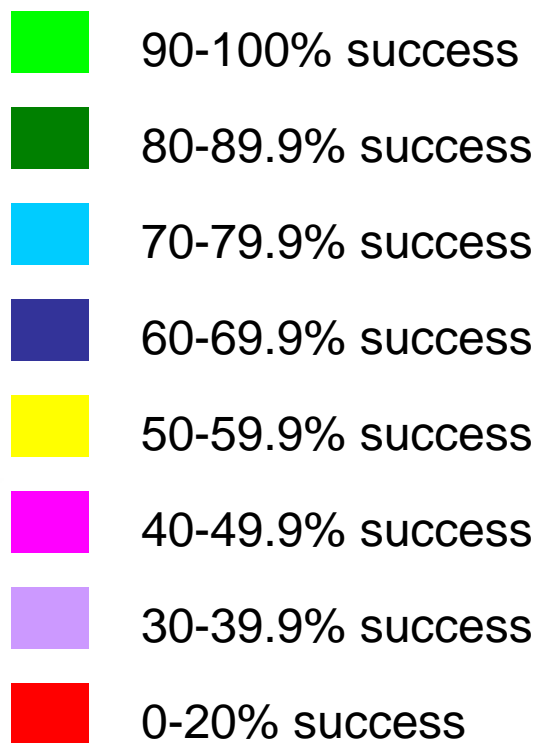
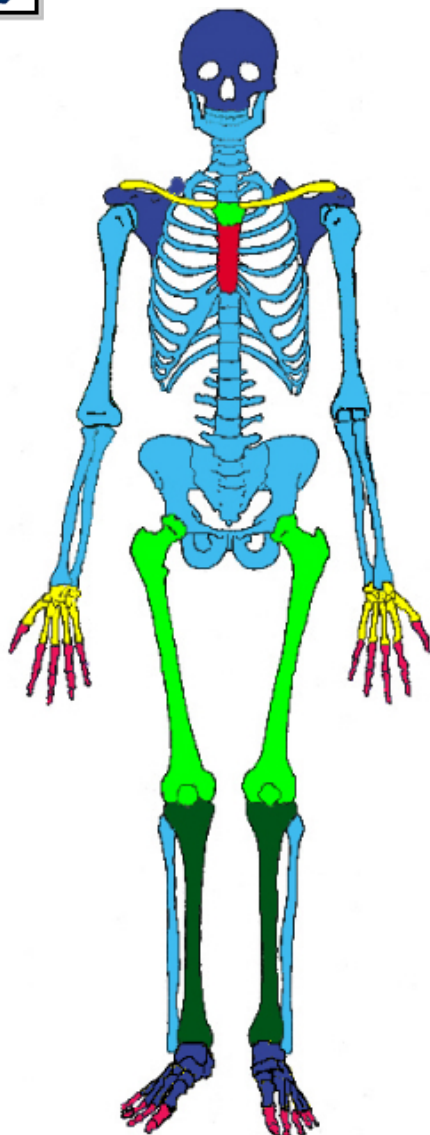


AFDIL CASE FLOW



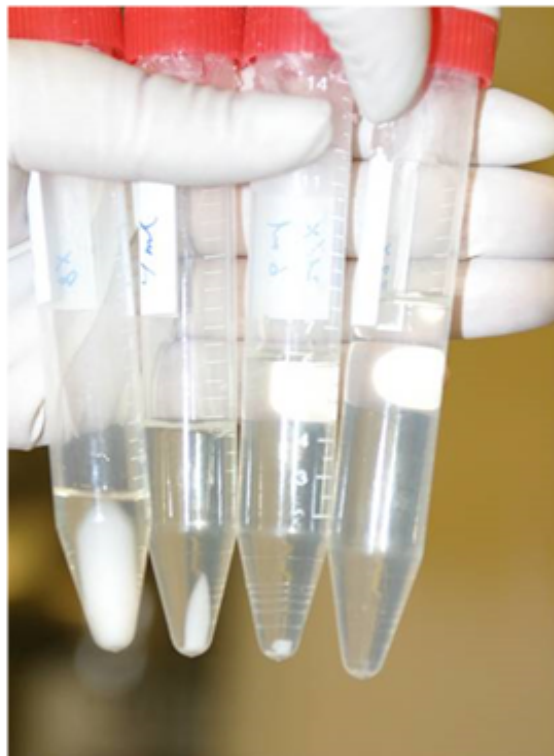


Standard Protocol Success





Demineralization Modifications to Casework



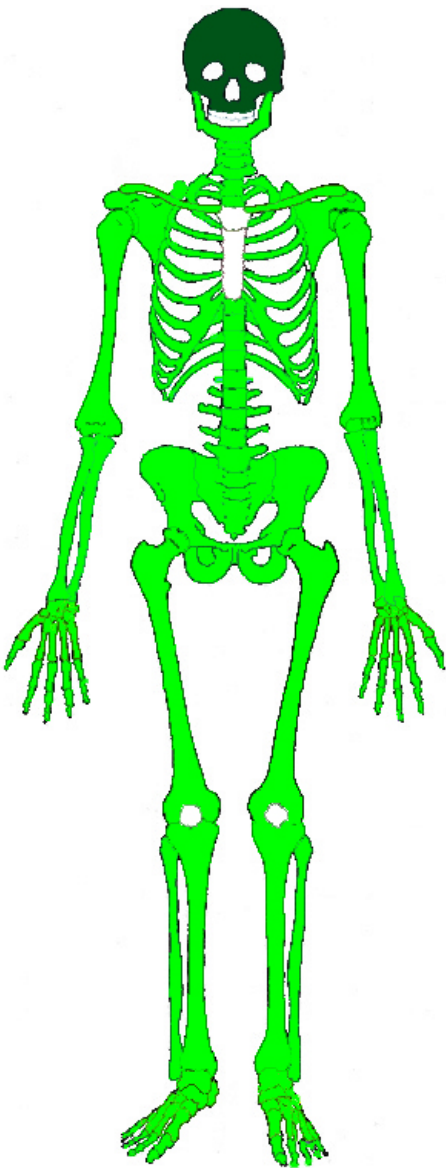
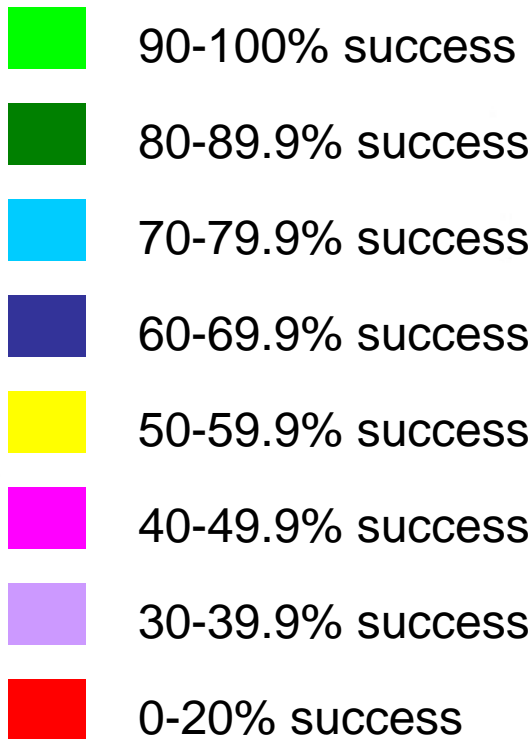
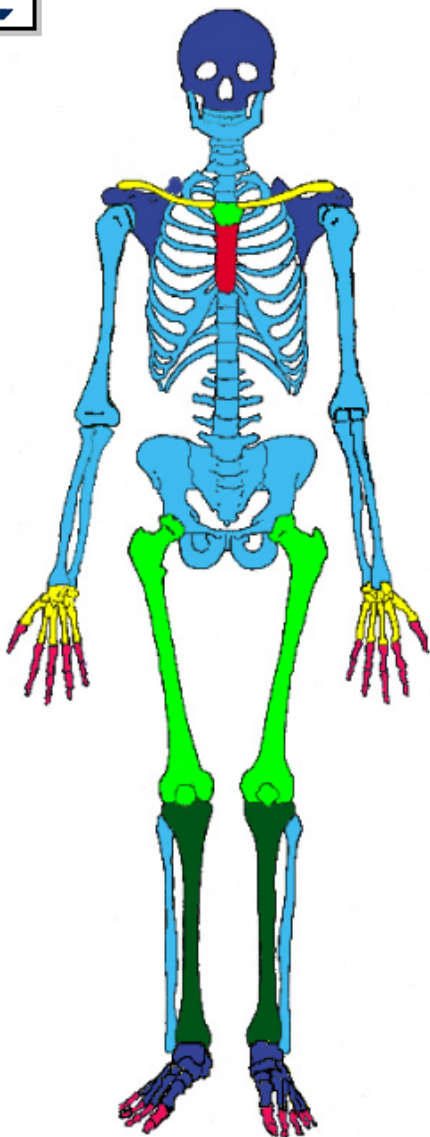
- Fundamental chemistry change to the buffer but no other procedural changes.
- Ten-fold reduction of amount of bone necessary for extraction.

2.0-2.5g bone  0.1g bone

O.M. Loreille et al., High efficiency DNA extraction from bone by total demineralization, Forensic Science International: Genetics; Volume 1, Issue 2, pp191-195.



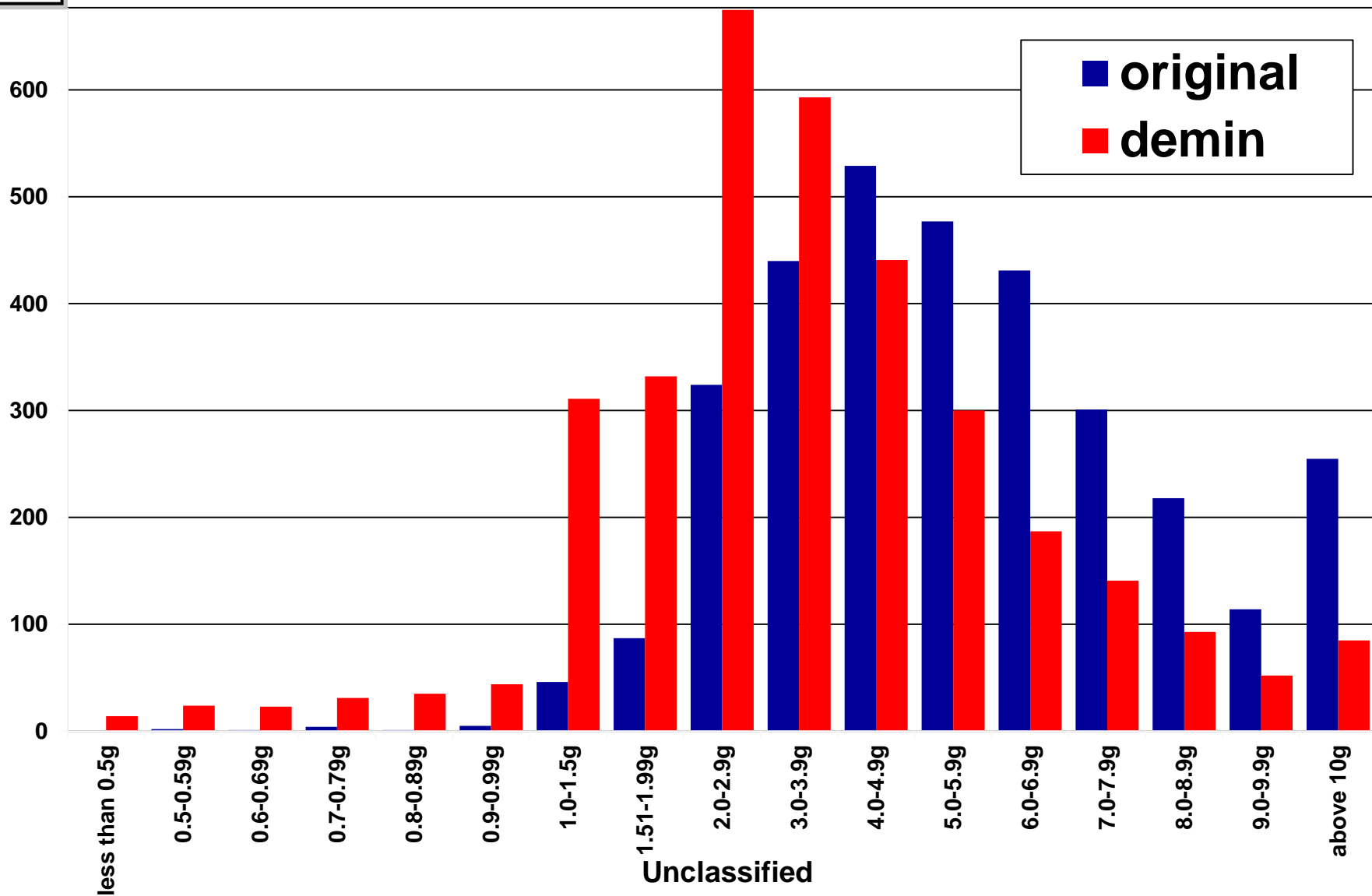
Demineralization Success



Unclassified

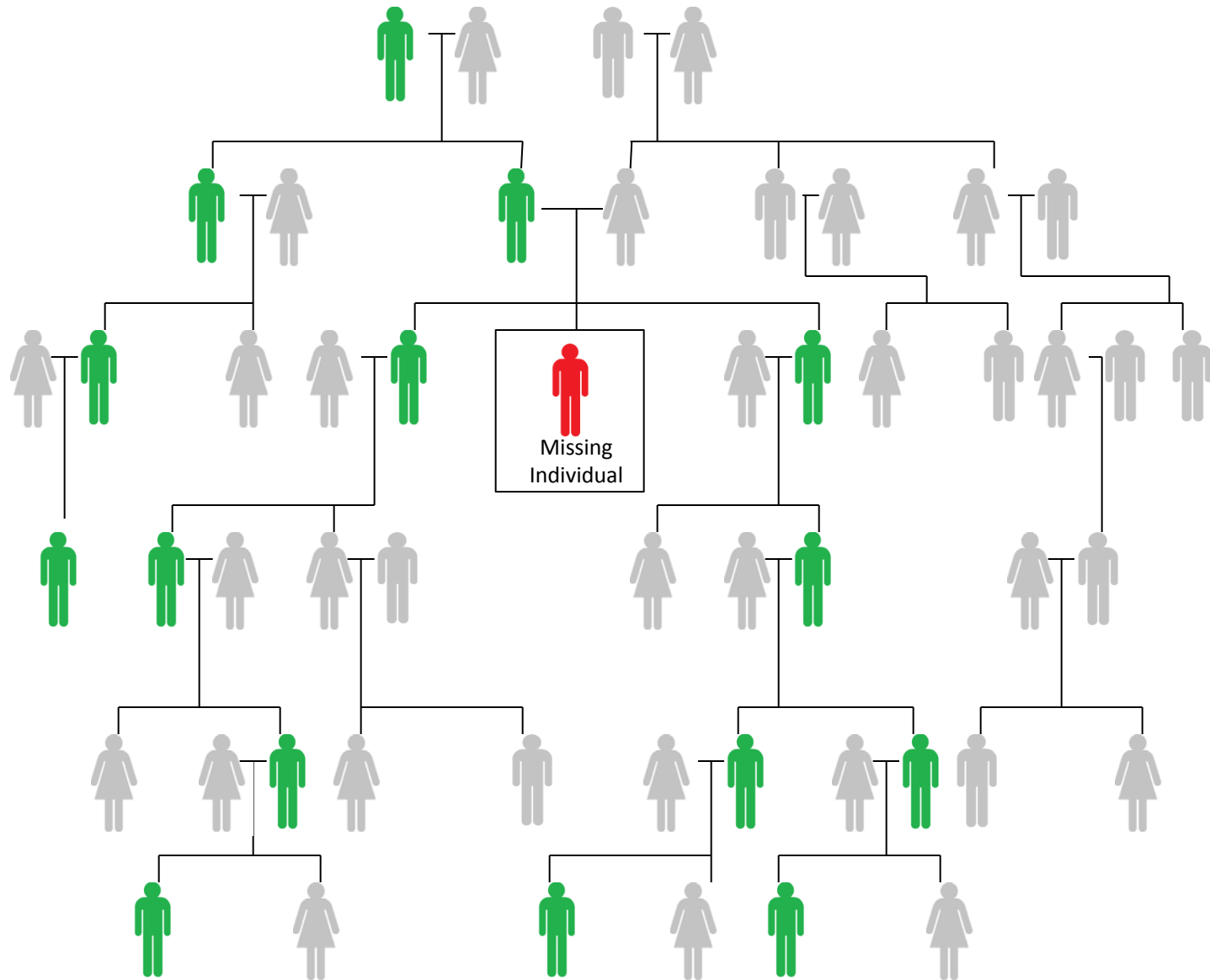


Samples Submitted By Weight





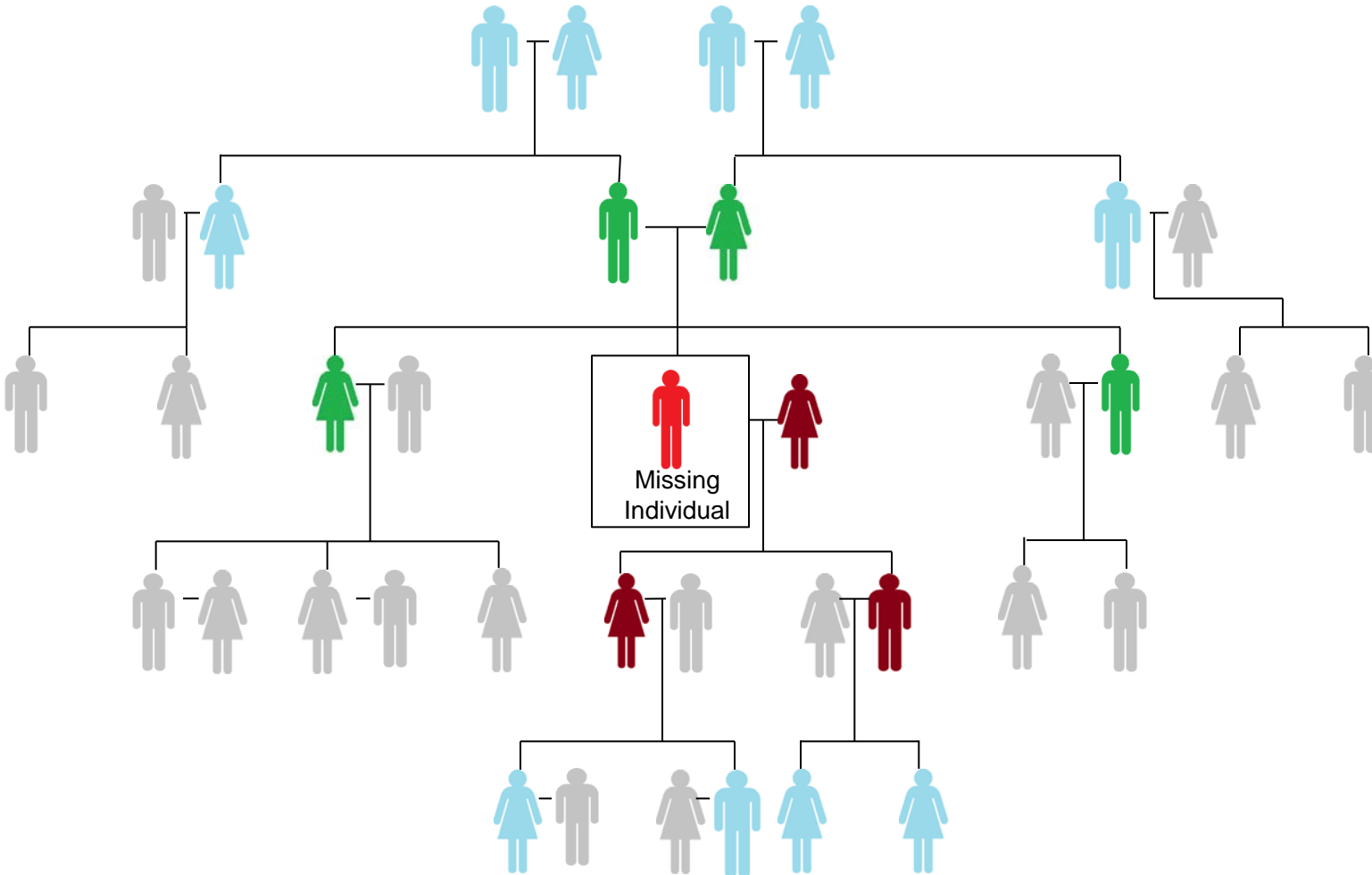
Paternal (Y-STR) Family References



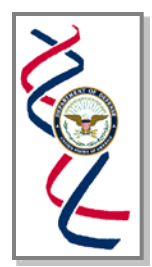
Unclassified



Nuclear DNA (Au-STR) Family References



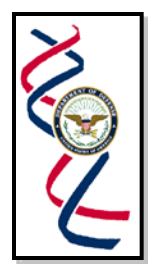
Unclassified



2013-2014 Casework Processing Advances



- Low Copy Number Y-STR testing
 - Smaller amounts of DNA can be used to increase success of obtaining Y-STR profiles
- Improvements to demineralization of bone
 - Remove more contaminants/inhibitors to testing
 - Increased nuclear and Y STR results
- Additional nuclear DNA kits
 - Expand the number of STR loci available for testing and increase success of obtaining a nucDNA profile

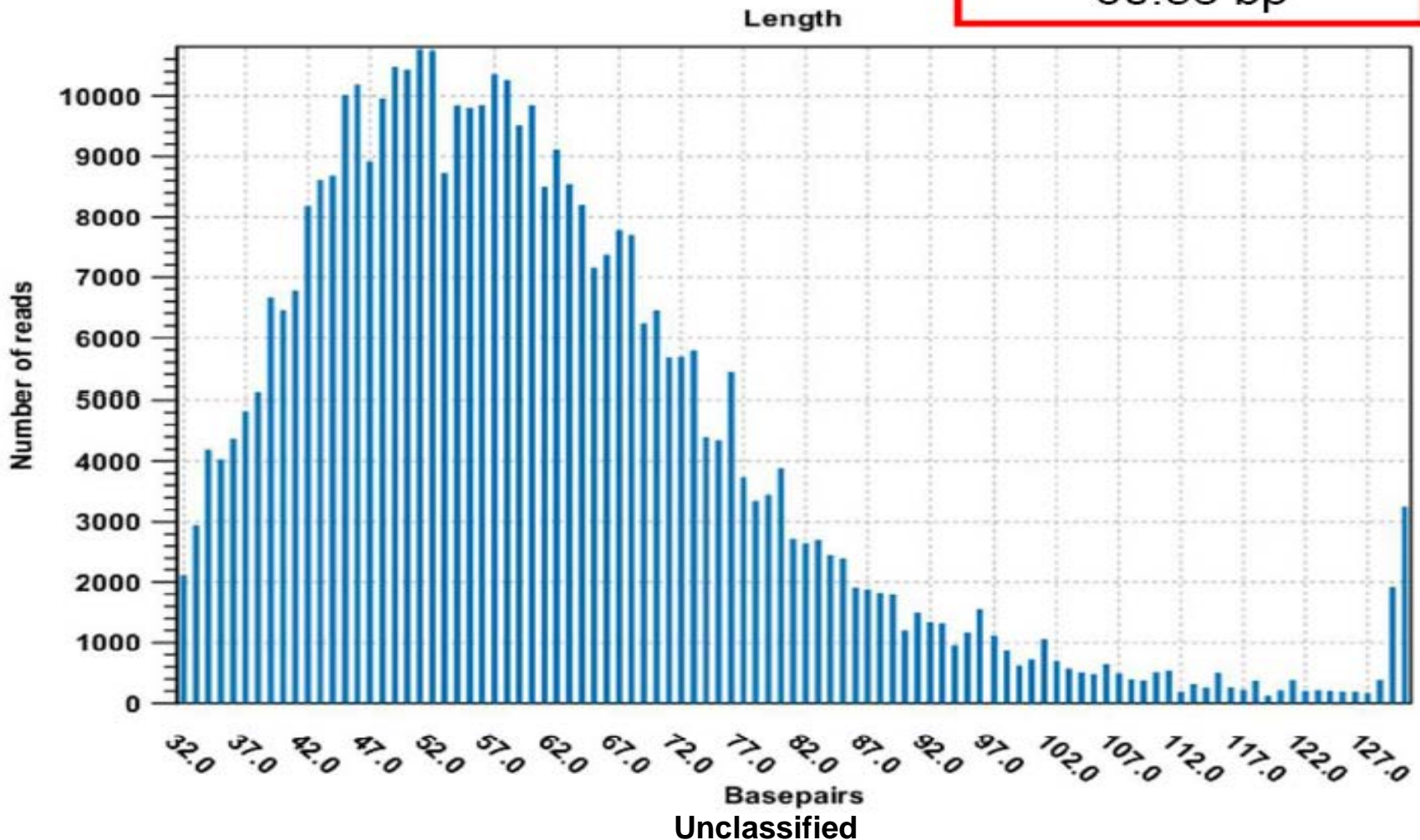


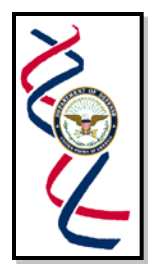
DNA Fragment Size: Typical Bone Submission



1.3 Distribution of mapped read length

Mean read length:
60.53 bp

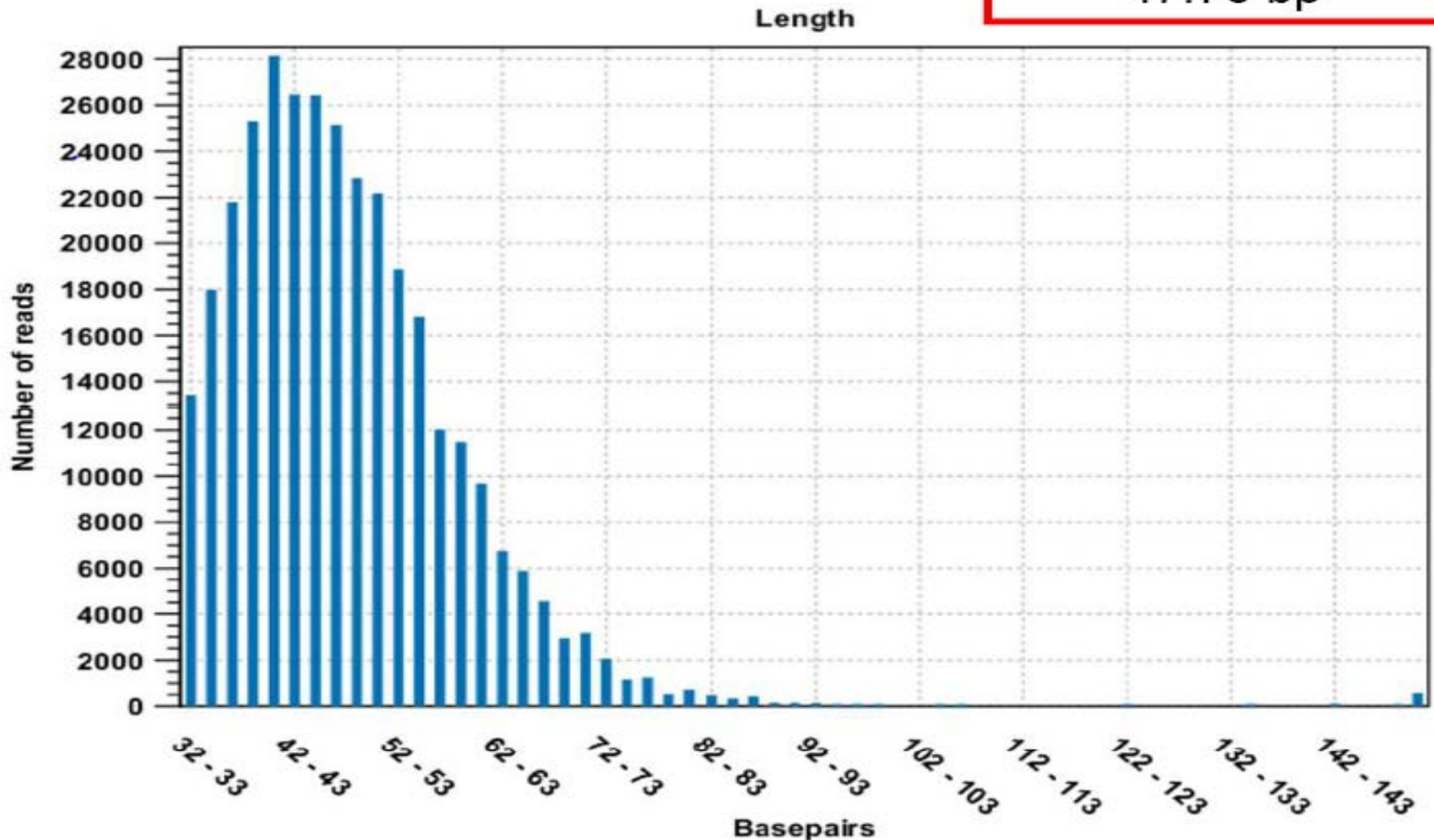




DNA Fragment Size: Highly Degraded

1.3 Distribution of mapped read length

Mean read length:
47.76 bp



Unclassified



Next Generation Technology

Pacific Biosciences RS

MiSeq



mtDNA Single Molecule Analysis

- 3rd Generation Sequencing System
- Targets Actual DNA Template Without Requiring Amplification of DNA Product

mtDNA Sequence

- Amplification
 - Sequencing
 - Capillary Electrophoresis
 - Fluorescent Detection
 - Number of Bases
 - Order of Bases
- **ATGCCTAGCAG**

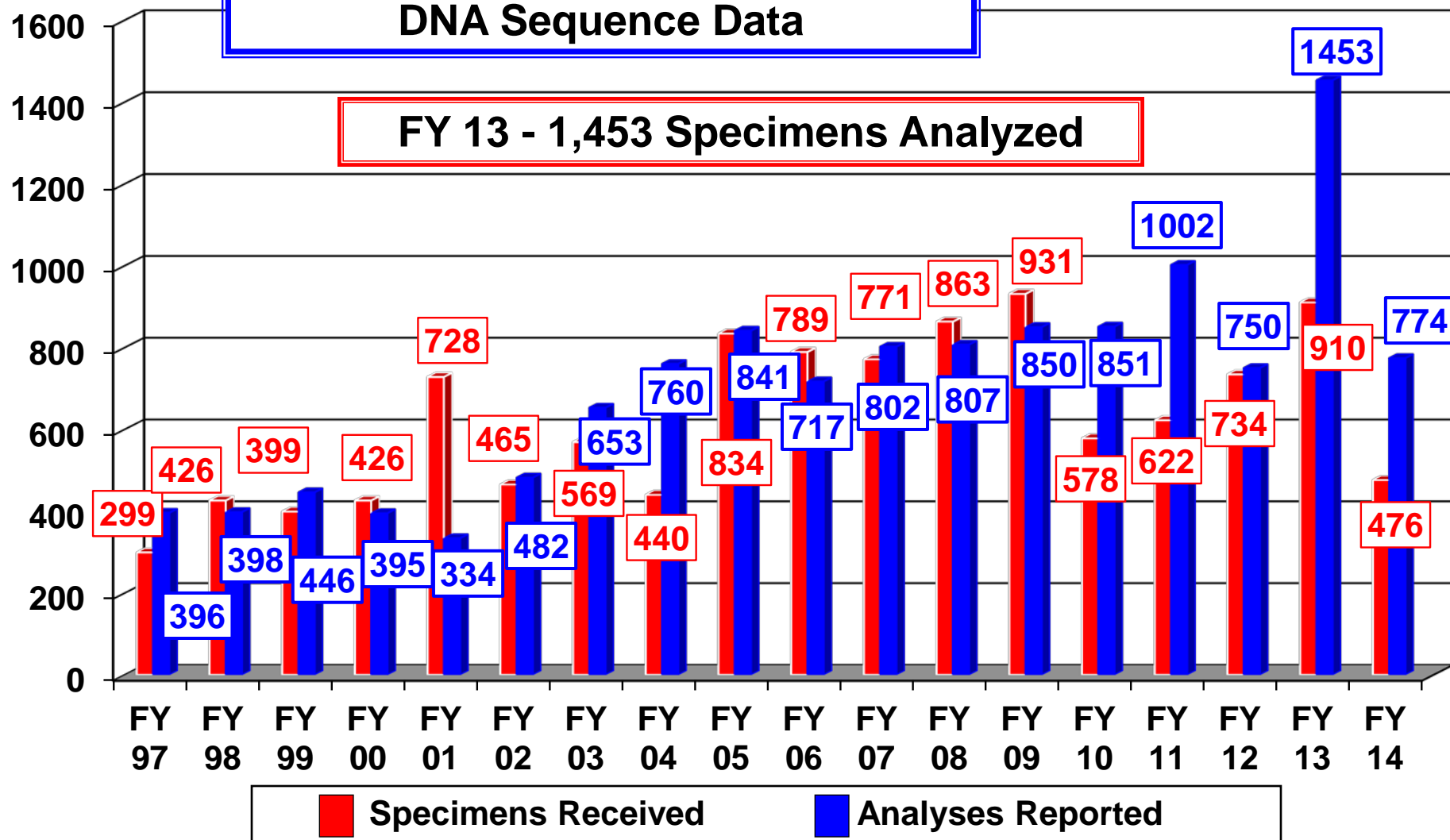
Composite Kits

- auSTRs
- YSTRs
- Ancestry Markers
- SNPS

JPAC Samples Received & Reported By AFDIL As Of 30 May 2014

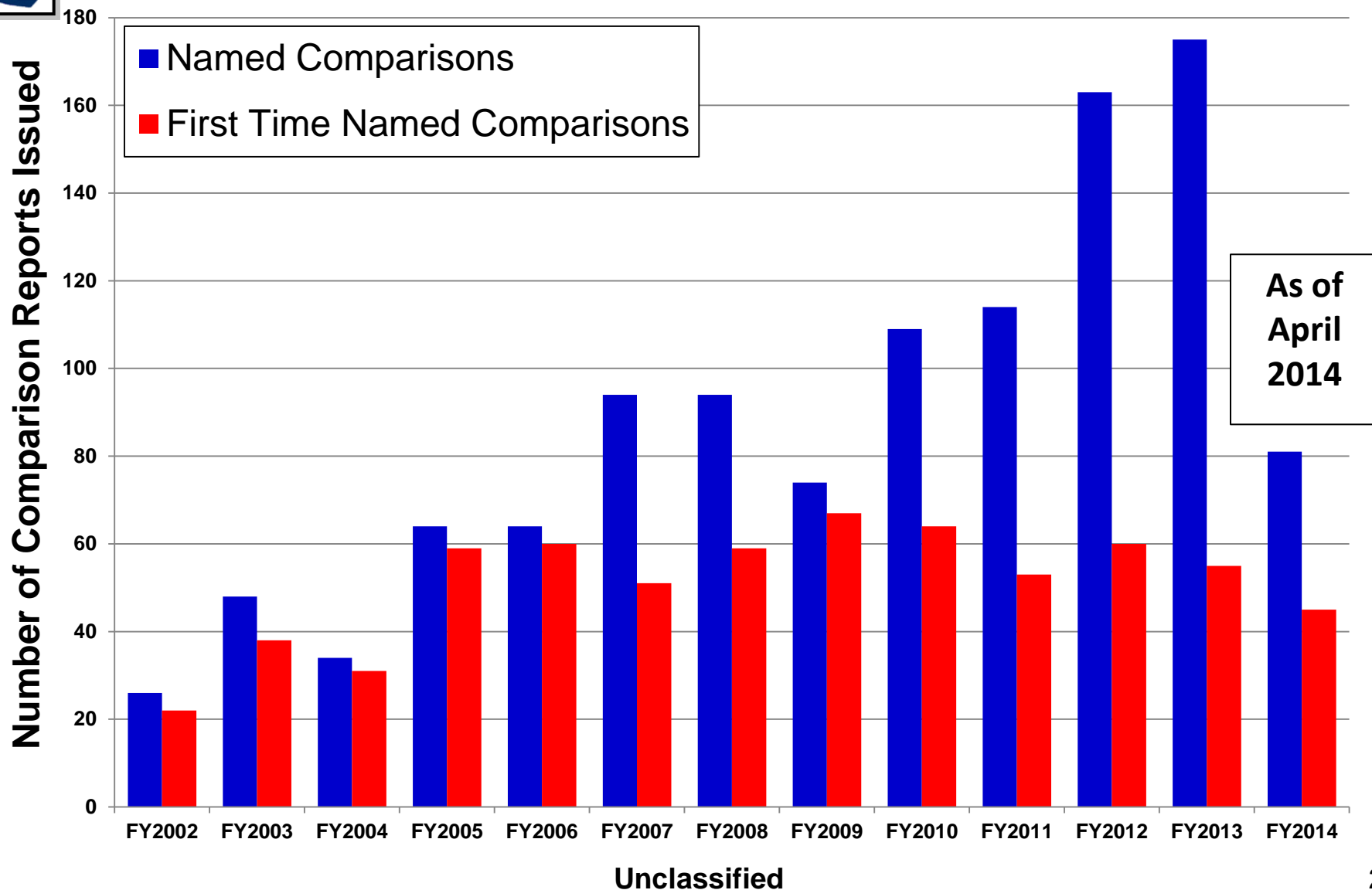
**91% Success Rate For Obtaining
DNA Sequence Data**

FY 13 - 1,453 Specimens Analyzed



Unclassified

Named Comparison Reports

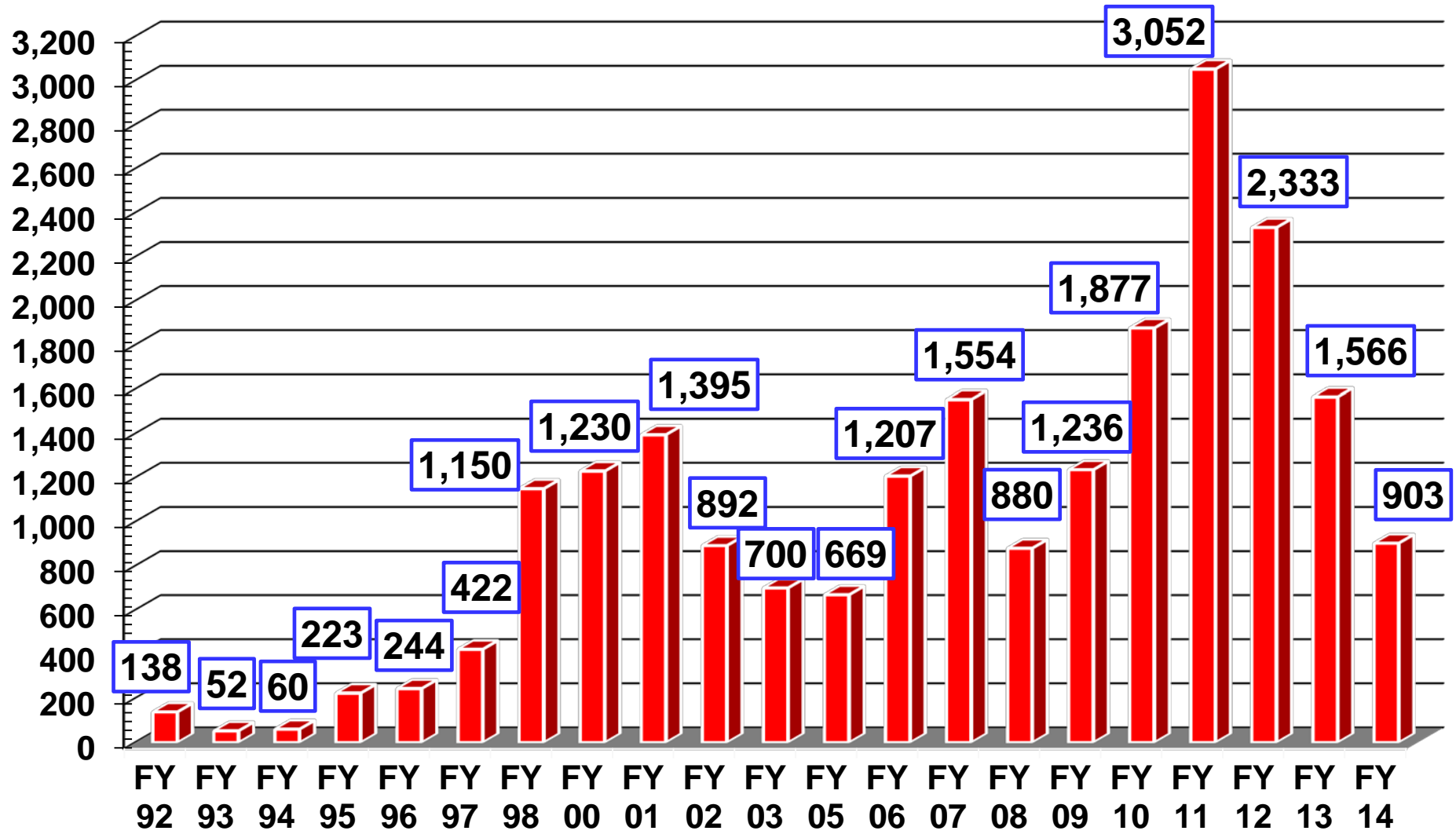




Family Reference Samples Received



As Of 30 May 2014 – 23,116 References





Summary

- Accreditation = Defined forensic standards
- Reproducibility = Validity of Results
- Success rates = Unifying metric
- mtDNA, nucSTR and YSTR capability
- mtDNA, nucSTR and YSTR FRS databases
- Blind Searches of Database
- Statistical Software = Kinship Analysis
- Research = Constant innovation



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