

Comments on DNA Analysis

Current Practice

Needed Improvements

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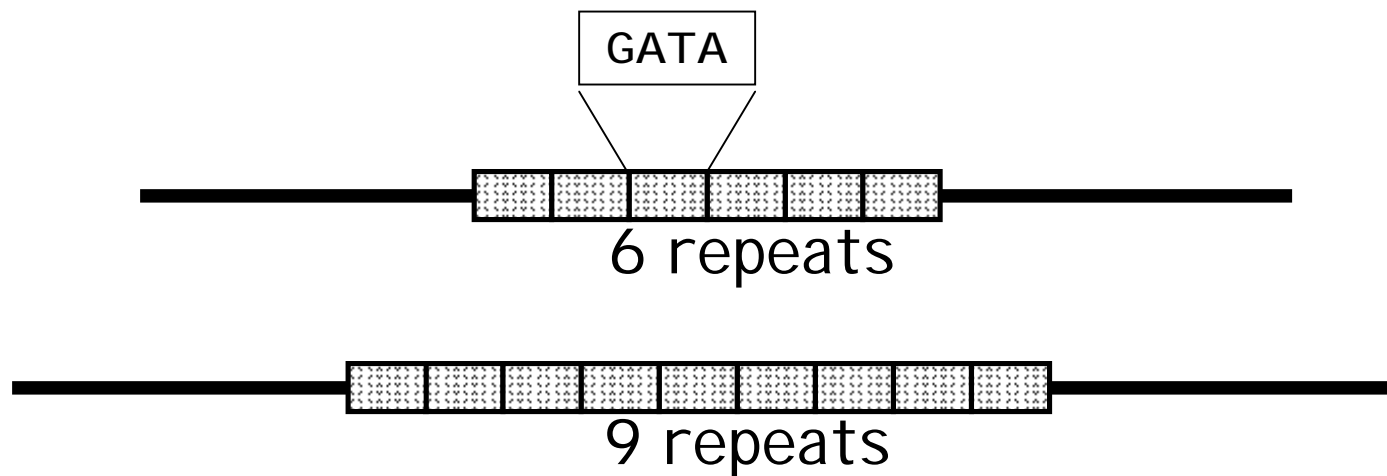
Boston Univ. School of Medicine

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Types of DNA Testing Currently in Use

- Short Tandem Repeat (STR) loci on the 44 non-gender determining (autosomal) chromosomes
 - Mini STR analysis for degraded DNA
- Short Tandem Repeat loci on the Y chromosome
 - Types shared by male relatives
- Mitochondrial DNA
 - Types shared by maternal offspring

Short Tandem Repeats (STRs)



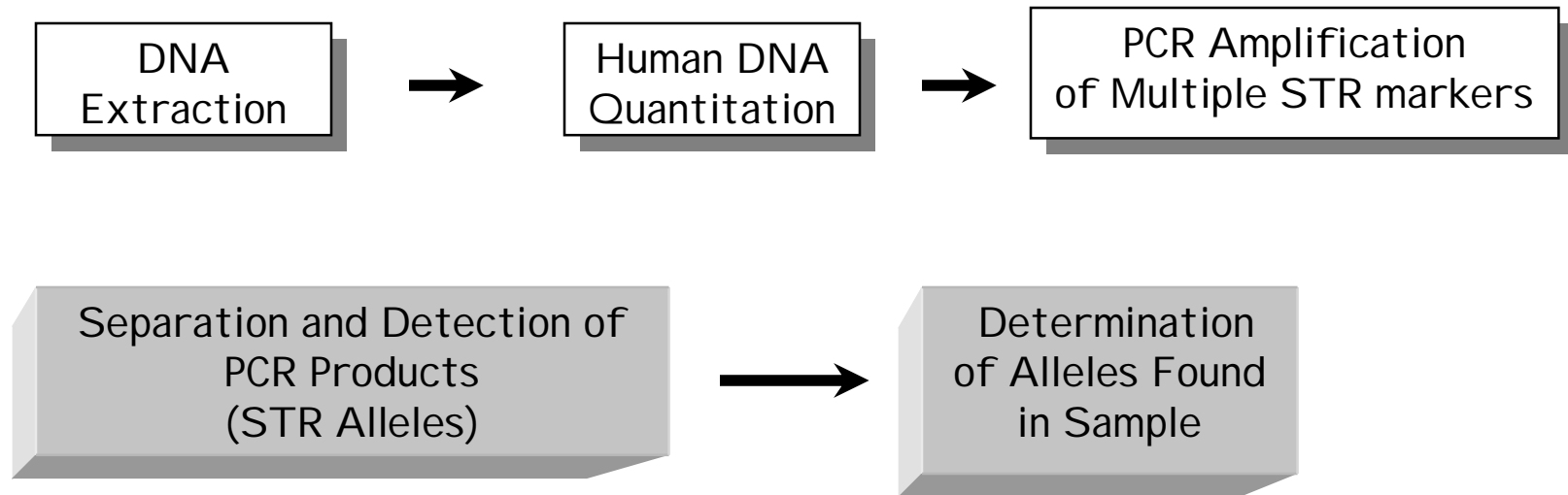
Repeat number = Type = Allele

The type (genotype) shown above is a 6, 9.

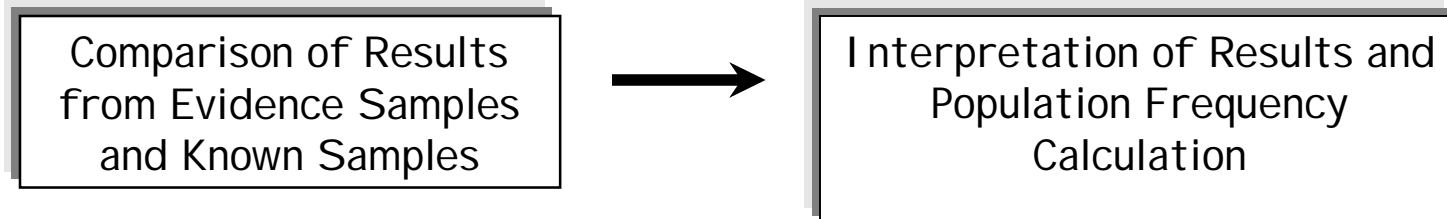
Steps in DNA Testing

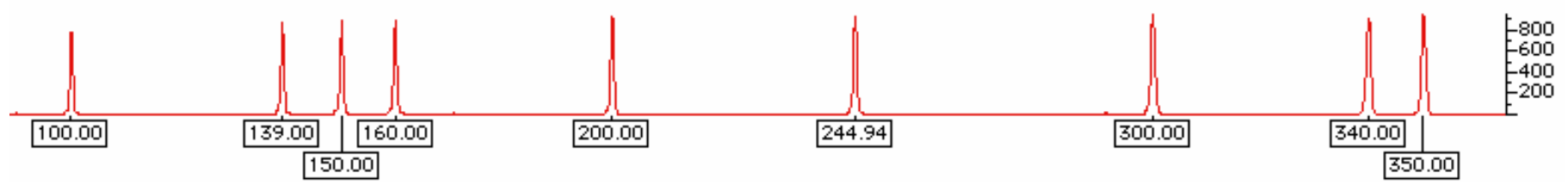
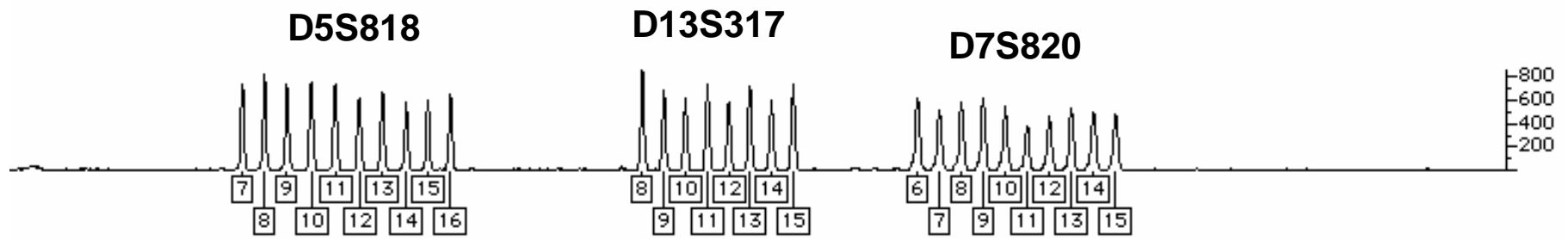
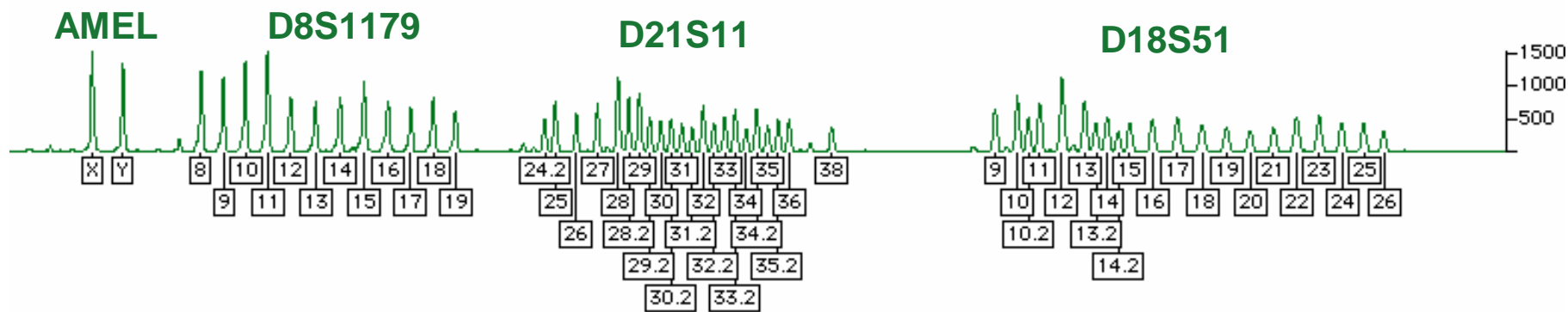
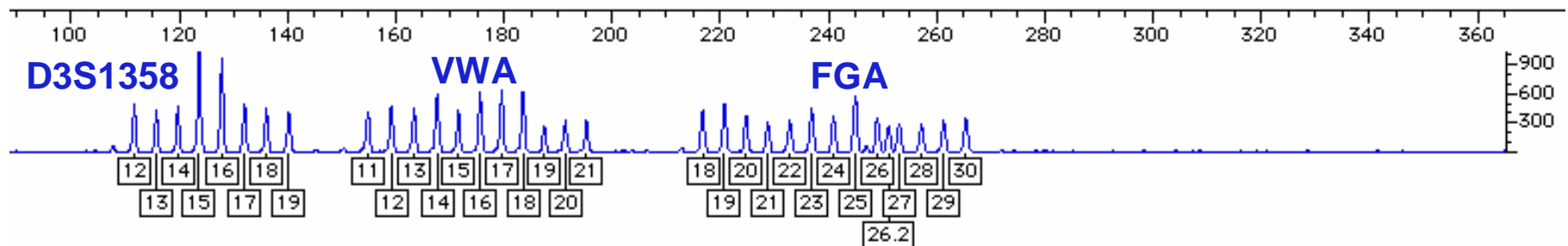
- DNA extraction from biological sample
- Quantitation of amount of human DNA obtained
- PCR amplification of the STR locations of interest
- Analysis of the PCR products using capillary electrophoresis and fluorescent detection of the DNA fragments

Molecular Biology



Genetics, Population Genetics

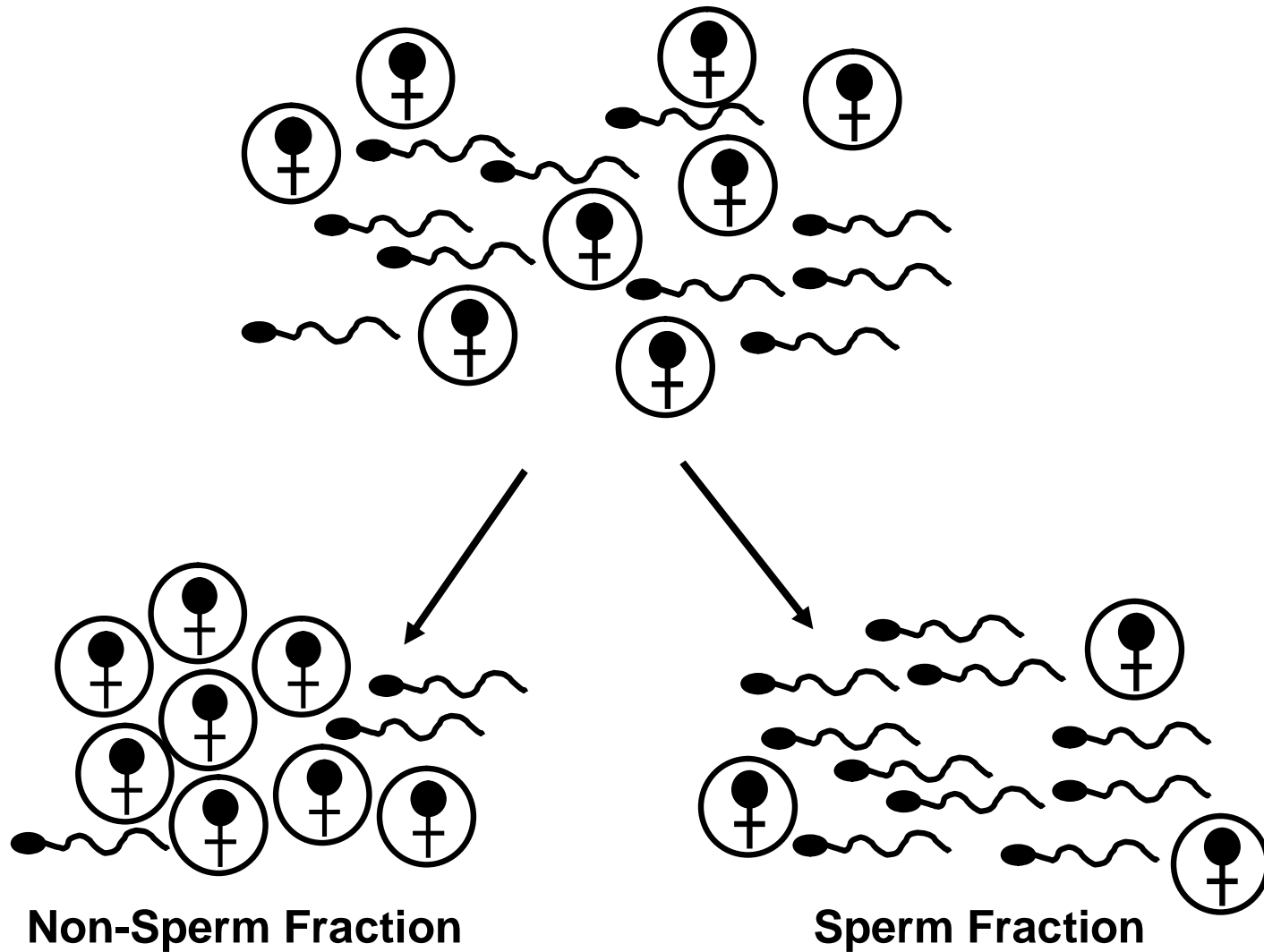




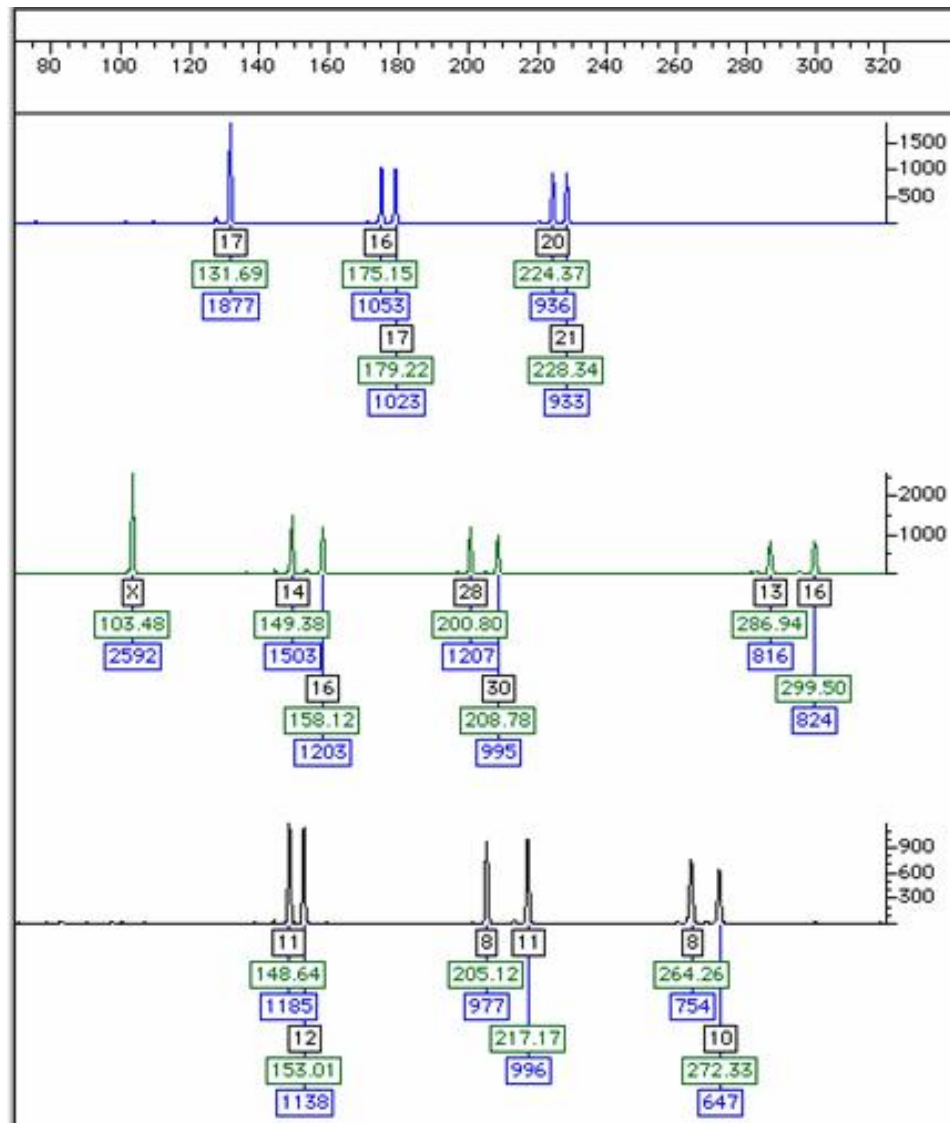
Random Match Probability

- For single source profiles that match at all loci this is usually the number presented to the jury
- This is usually stated as:
 - What is the frequency that another person would be found who by chance would have the DNA profile which is common to the known person and the evidence
 - That number is approximately 1 in X (often a very large number)
 - Numbers are typically presented for several racial/ethnic groups

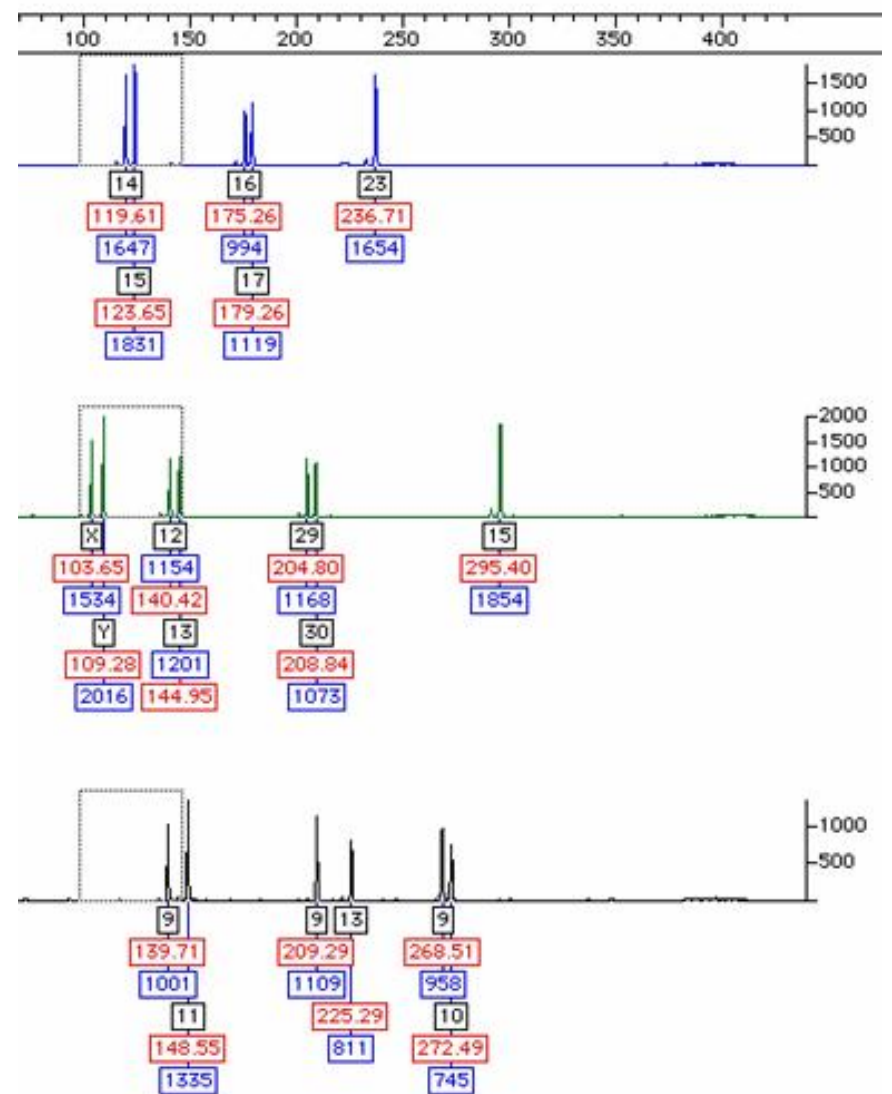
Differential Extraction



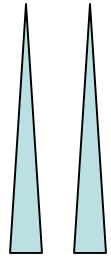
non-sperm fraction



sperm fraction

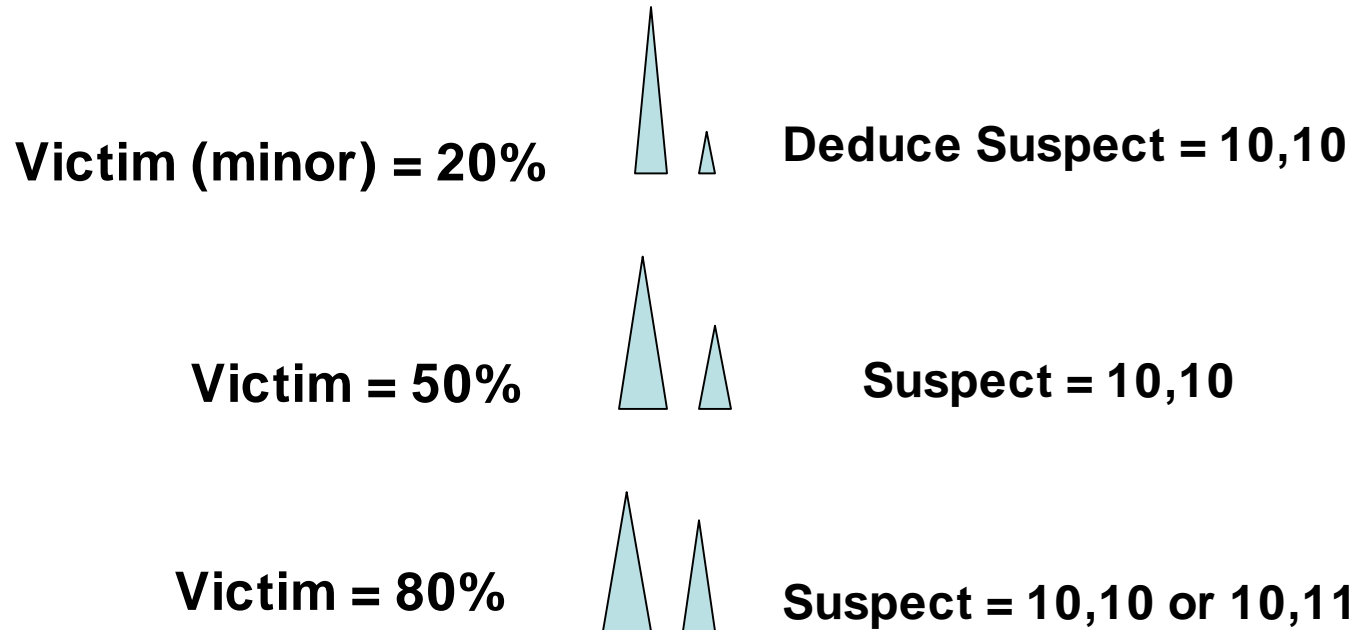


Complications: Ability to deduce male contributor depends of amount of victim's DNA is present in the mixture



10,11
victim

Evidence Profile: 10,11



Standards for DNA Testing Cover these Areas

- QA Program
- Organization and management
- Personnel qualifications
- Facilities
- Evidence control
- Validation
- Analytical procedures
- Equipment
- Reports
- Technical review of reports
- Proficiency testing
- Corrective action procedures
- Audits and safety

Accreditation

ASCLD/LAB

329 Laboratories

180 State

99 Local

22 Federal

18 Private

10 International

FQS-I

47 Laboratories

Best Practices

- There are standards that require particular controls and QA procedures that are considered “best practice”
- There are not best practices for:
 - DNA extraction
 - DNA amplification conditions

As an Example:

- Why is there no best practice standard for DNA extraction:
 - Different sample types necessitate the use of different procedures.
 - Many procedures work well.
 - There is no single procedure which is best for all sample types

What Could be Improved

- Still need technical improvements in:
 - Automatable form of separation of sperm from other cells in sexual assault evidence
 - Separation of male and female cells of the same cell type
 - Increasing DNA yield from some sample substrates

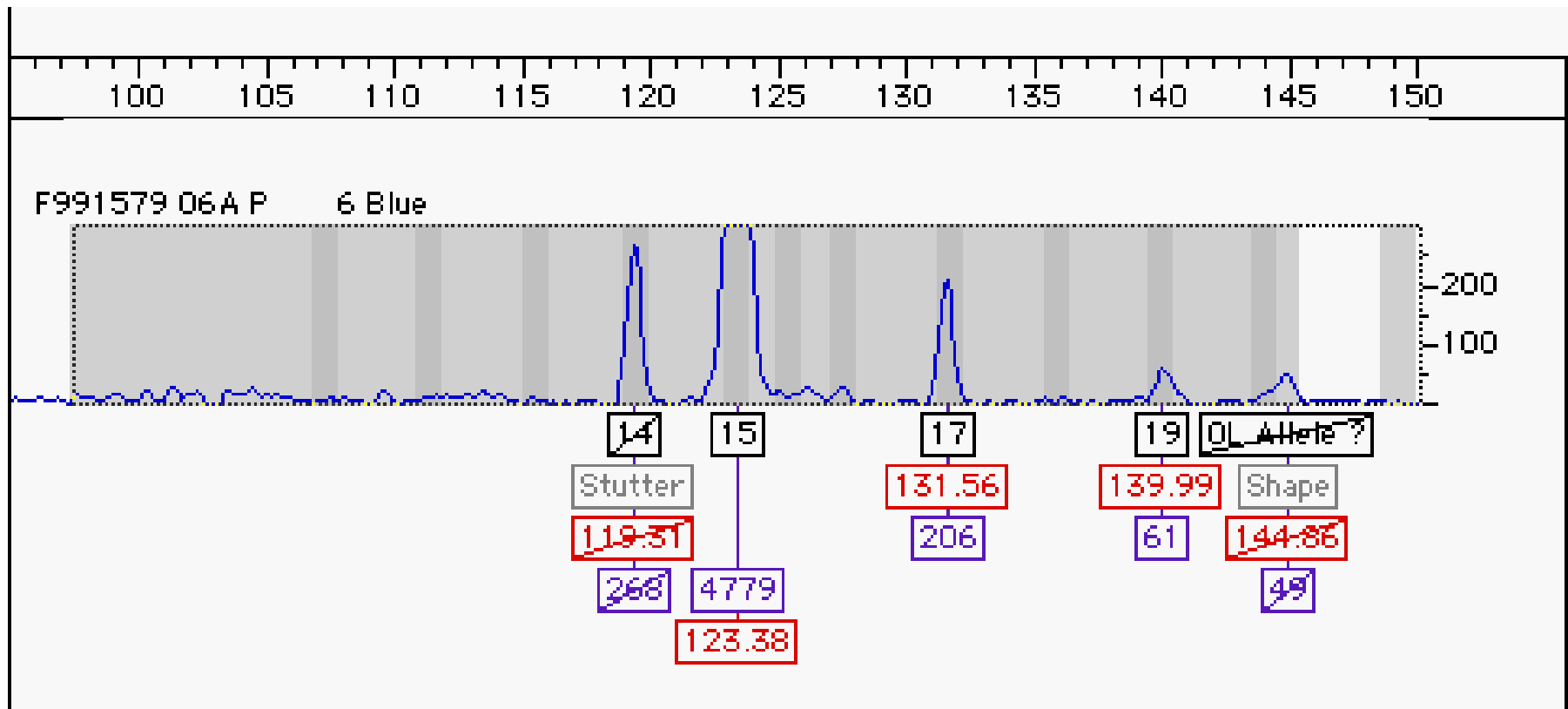
What Else Could be Improved

- Use of expert systems for analysis of convicted offender samples
- Use of expert systems for analysis of complex mixtures

D3S1358

Primary type: 15

Other alleles: 17 and (19)



What Else Could be Improved

- Better techniques for amplification of degraded DNA
- Increased awareness of problems associated with low copy number samples

Where does low copy number start?

<100 pg template DNA

(Butler, 2001, Fregeau & Fourney 1993, Kimpton *et al* 1994)

Amount of DNA	~ # of cells
1ng	143
0.5ng	71
0.25ng	36
0.125ng	18
0.0625ng	9

Assume sample is a 1:1 mixture of two sources:

Amount of DNA	Total Cells in sample	~ # of cells from each component
1ng	143	71
0.5ng	71	36
0.25ng	36	18
0.125ng	18	9
0.0625ng	9	4

Assume sample is a 1:3 mixture of two sources:

Amount of DNA	~ # of cells from major component	~ # of cells from minor component
1ng	107	36
0.5ng	53	18
0.25ng	27	9
0.125ng	12	4
0.0625ng	7	2

Assume sample is a 1:9 mixture of two sources:

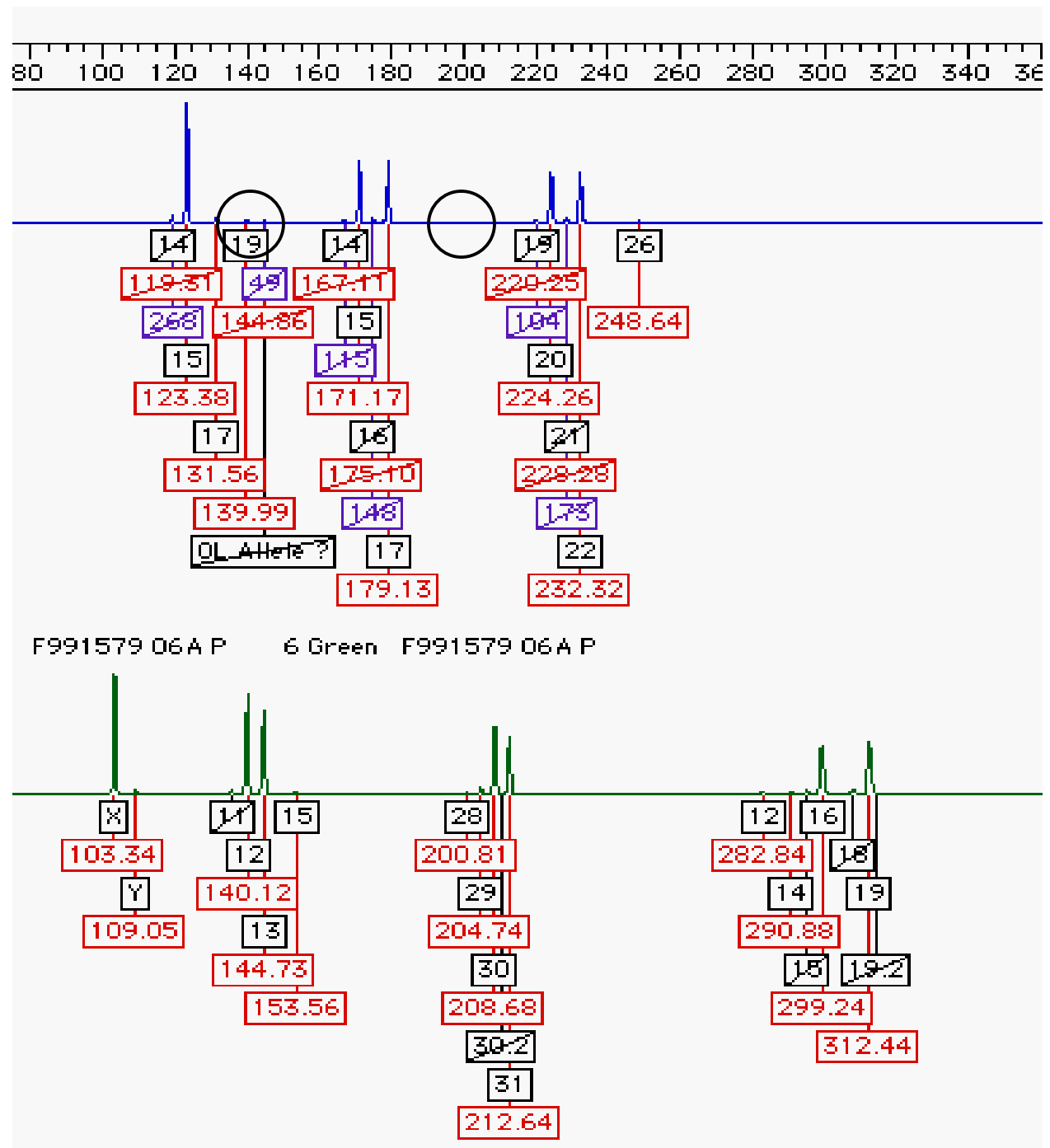
Amount of DNA	~ # of cells from major component	~ # of cells from minor component
1ng	129	14
0.5ng	64	7
0.25ng	32	4
0.125ng	16	2
0.0625ng	8	1

What Else Could be Improved

- Repository for information regarding contamination arising from consumables
- Rigorous discussion in US regarding application and interpretation of results from low copy number samples
- Routine use of quantitation of both total genomic DNA and total male DNA to avoid amplification of samples with greater than 10:1 mixtures

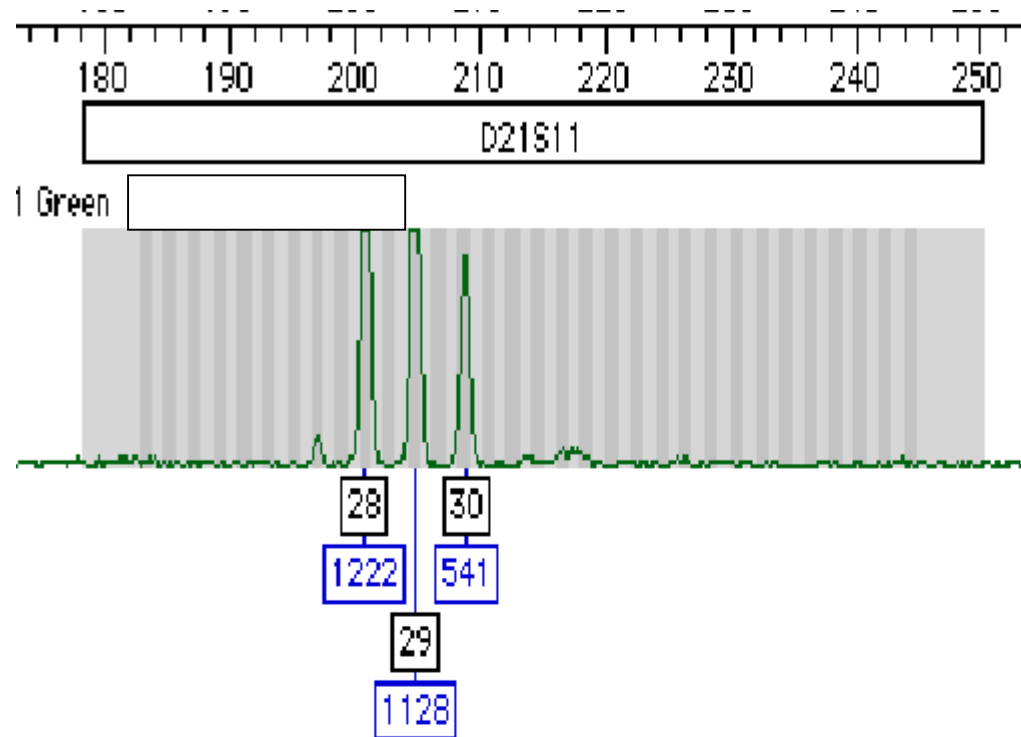
Is the 19 at D3 real?

This would imply that there could be a second contributor.



Victim = 28,29: Second contributor either 28,30 or 29,30

LMA result: 28,30 = 55%, 29,30 = 39%



Victim = 8,9: Second contributor either 7,9 or 7,7

LMA result: 7,9 = 70%, 7,7 = 30%

