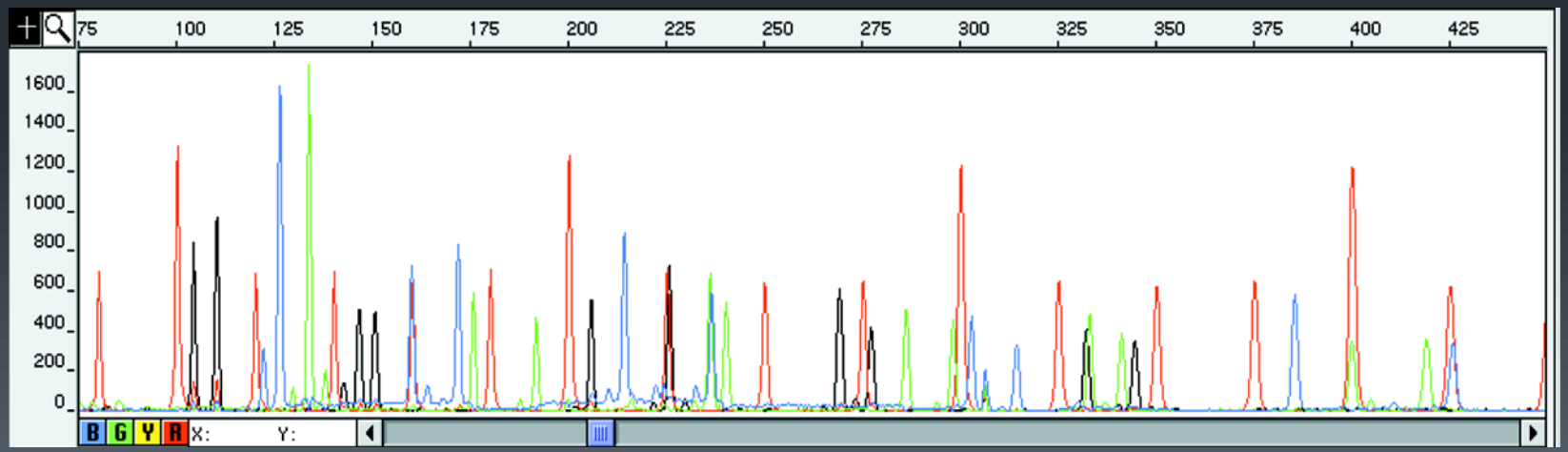
Forensic Sciences

Fatchiyah

Dept. of Biology, FMIPA, UB



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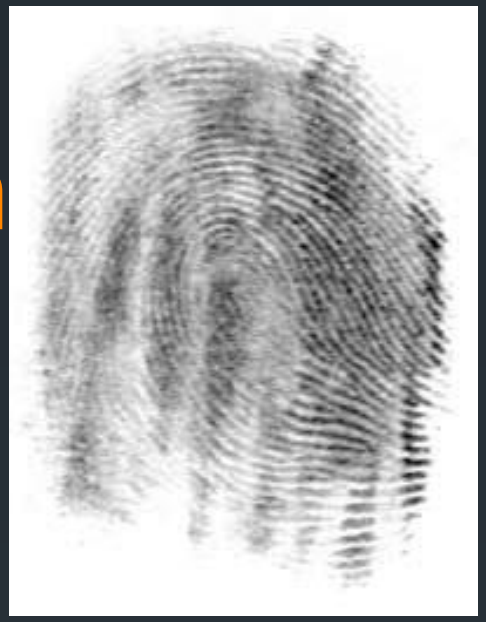
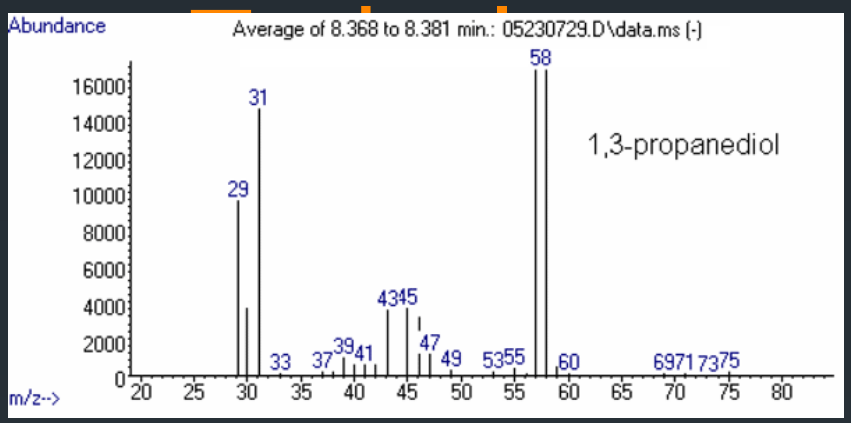


- Fingerprints
- Palm prints
- Footwear and tire impressions
- Other – ears, lips, etc.



- Blood alcohol, urinalysis, poisons
- Blood, urine, organs, tissue, vitreous humor

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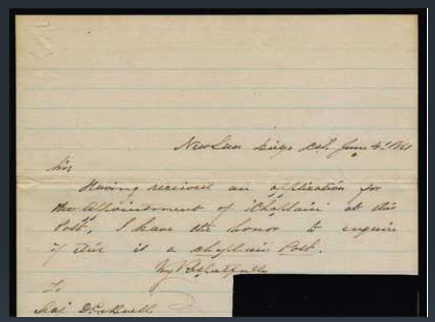
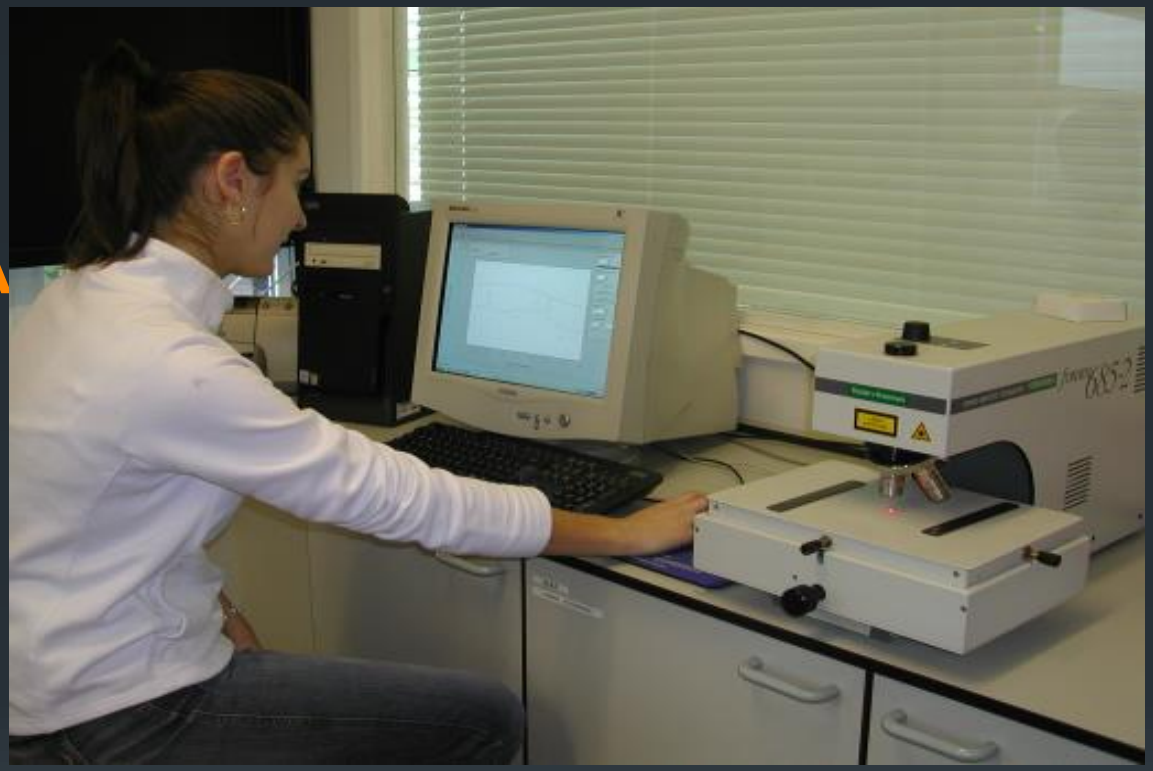
- Accurate and complete documentation of scene and evidence
- Establish spatial locations, conditions, scale

Document A

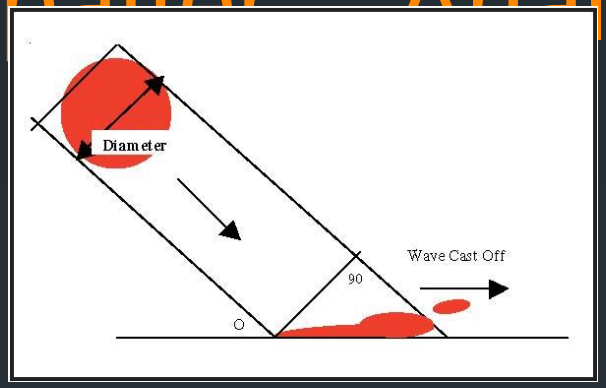


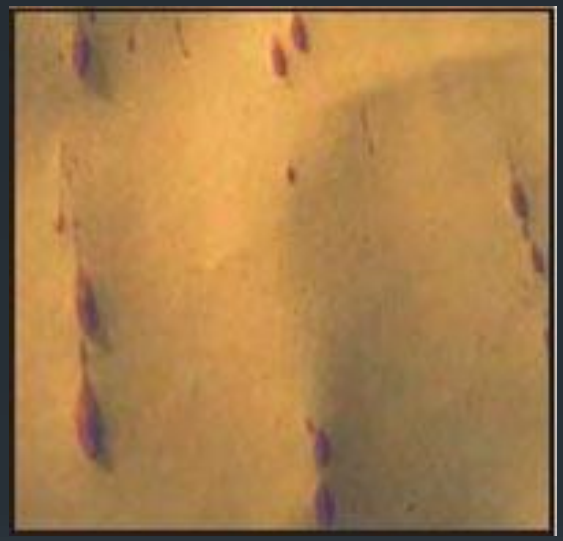


Analysis of inks using Raman Spectroscopy.

- Comparison of inks, paper, printers, copiers, and handwriting

Spatter Analysis





- Physics of flight, trigonometry used to determine origin point of blood
- Size and orientation of spatters can determine method by which stains are created

Fiber analysis

Polymer analysis on a pyrolysis gas chromatograph

- Fibers have distinct color, diameter, shape, and chemical composition
- Microscopic and chemical analysis to compare

F

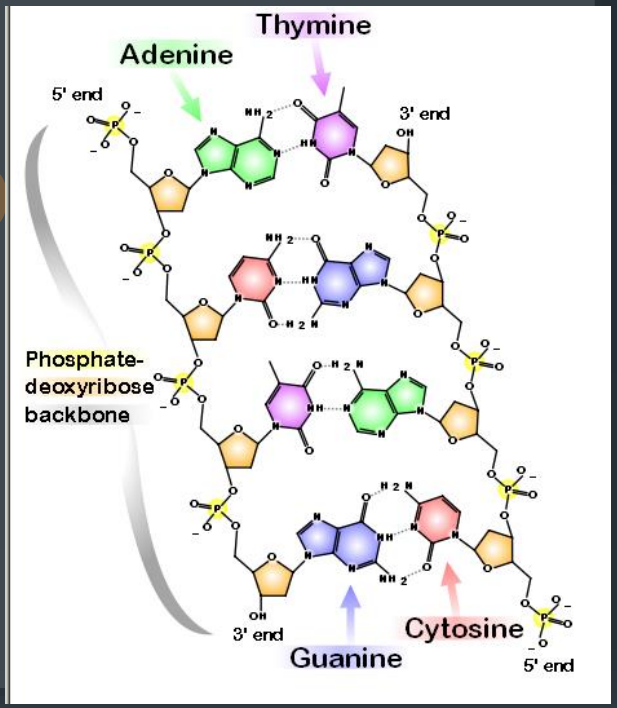
- Direct comparison of known samples and unknowns from crime
- Striations or firing pin impressions
- Also used to do tool-mark comparisons (screwdrivers, etc.)

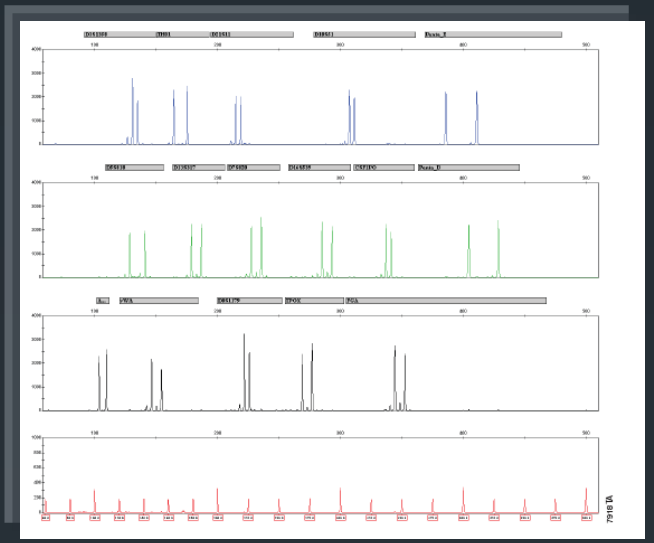
- Search for chemical signs of accelerants (gasoline, etc.)
- Test burn scenarios

ves

- Search for unique chemical traces or bomb-making materials
- Look for evidence from makers of bomb (DNA, fingerprints)

D

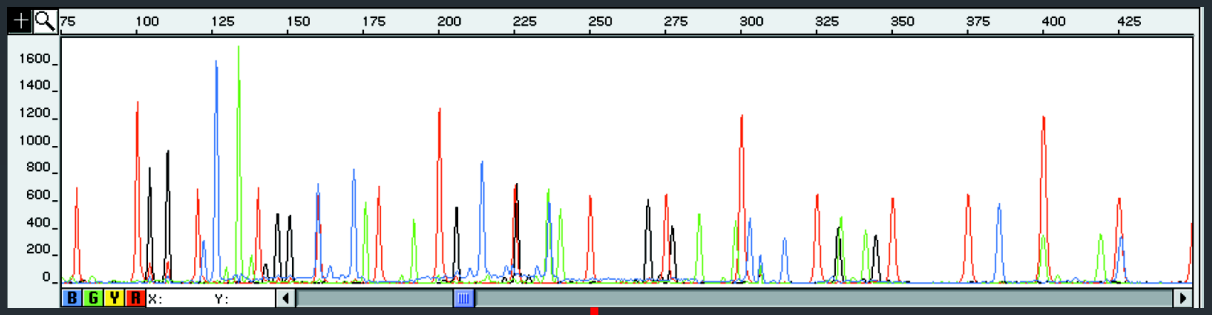

Thymine
Adenine
5' end
3' end
Phosphate-
deoxyribose
backbone
3' end
Guanine
Cytosine
5' end

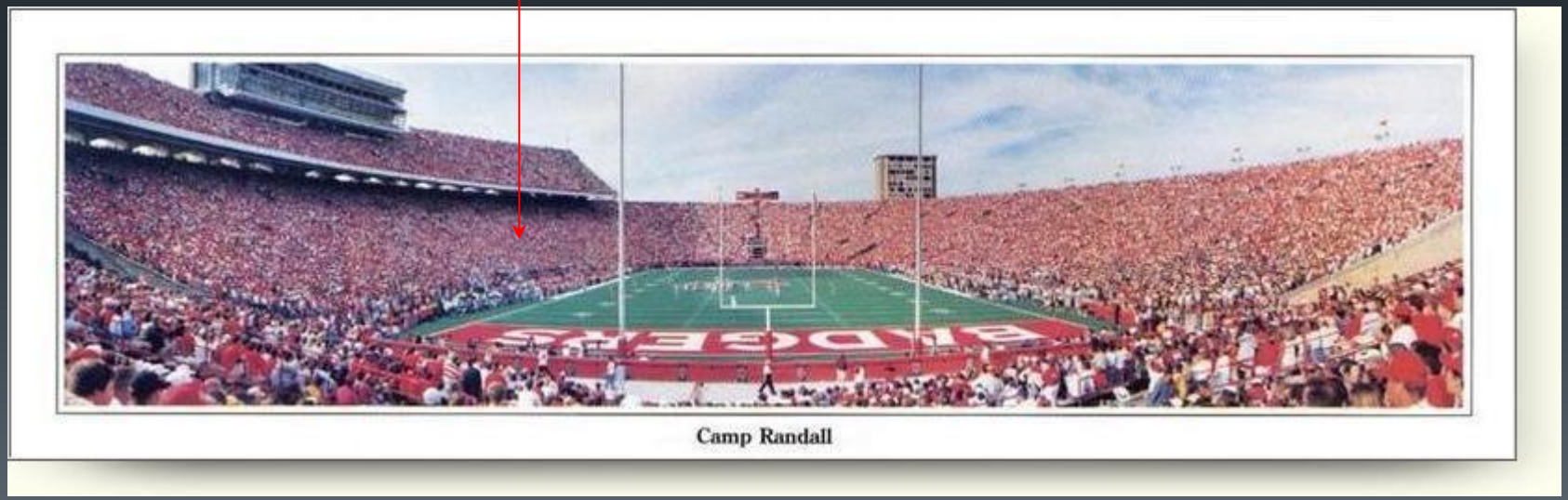


Others

- Serology (body fluids)
- Drug analysis (marijuana, cocaine, meth)
- Anthropology
- Pathology (medical examiner)

Are you just a number?





Methods of identification

Used Since?	Identification Method	**Accuracy?**
1800	Measurement of height	1 in 4
	(Quételet's method)	
	Comparison of Pubic hair	1 in 800
Late 1800's Early 1900's	Comparison of Scalp hair	1 in 4500
Late 1800's early 1900's	Anthropometry	1 in 268 million
	(Bertillon's method)	
	Forensic odontology Teeth bite marks	1 in 2.5 billion
Evidence in Early Egypt – documented forensic use 1800's -1900's	Dactylography	?
	(Fingerprints)	
Late 1900's	DNA Fingerprinting	1 in 2 x 10^{22}
Late 1900's early 2000's	Facial recognition	?

http://lifeloom.com/I2Aggrawal.htm and

Brief History of Forensic DNA Typing

- 1980 - Ray White describes first polymorphic RFLP marker
- 1985 - Alec Jeffreys discovers multi-locus VNTR probes
- 1985 - first paper on PCR
- 1988 - FBI starts DNA casework
- 1991 - first STR paper
- 1995 - FSS starts UK DNA database
- 1998 - FBI launches CODIS database

Unique identifying characteristics

Identification vs. Expression

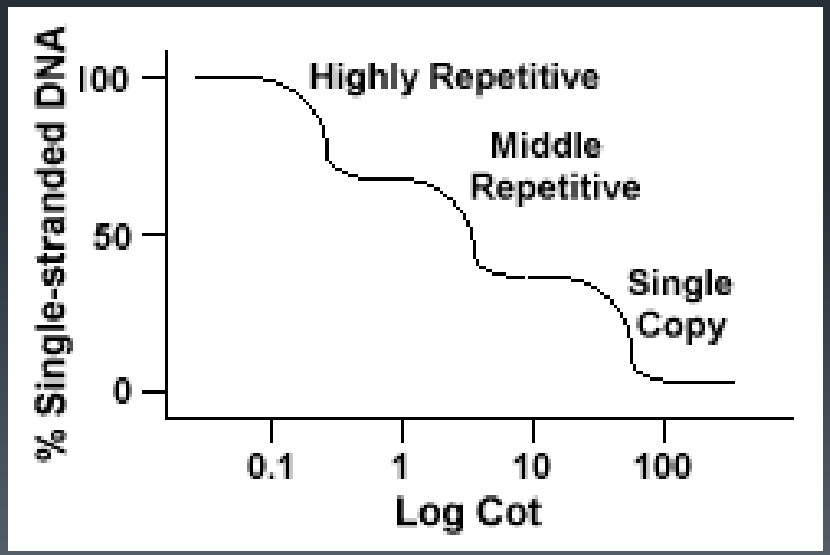
What regions of DNA would you expect to use for identification?

- Regions from genes expressing proteins?
- Other regions?
- Why

What are some of the DNA technologies used in forensic investigations?

- Restriction Fragment Length Polymorphism (RFLP)
- PCR Analysis
- STR Analysis
- Mitochondrial DNA Analysis
- Y-Chromosome Analysis

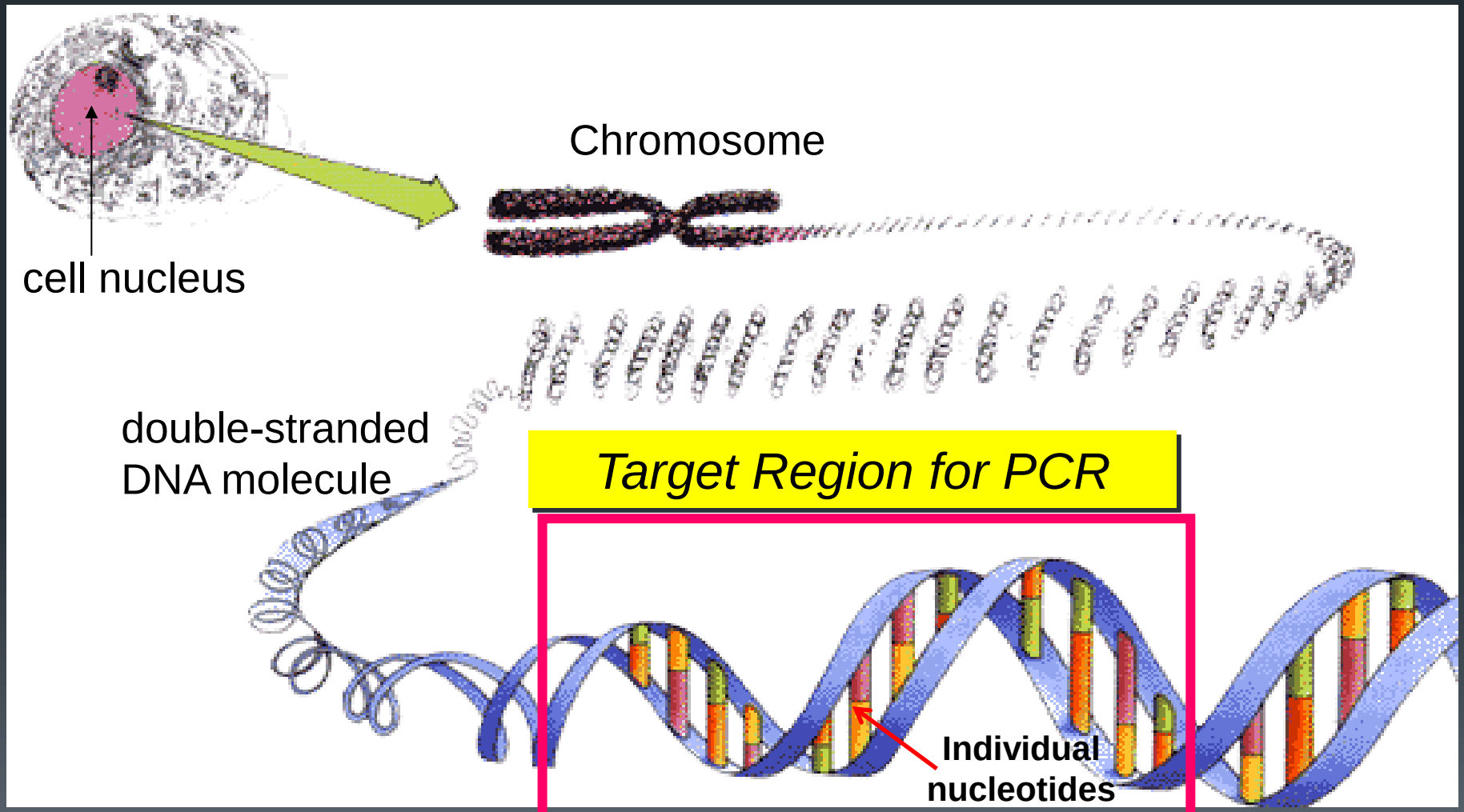
Repetitive DNA in the Human Genome



- Less than 2% codes for Proteins
- 50% of the genome contains repeated sequences
  - No apparent function
  - Recombination?
  - Formation of new genes?

- Types of repeated DNA
  - Tandomly repeated
    - Telomeres
    - Satellite (VNTRs)
    - Minisatellite (STRs)
  - Interspersed repetitive DNA
    - SINES (Alu sequences)
    - LINES
    - Transposable elements

DNA in the Cell



What are STRs?

- **S**hort **T**andem **R**epeats (STR) are repetitive sequences:
  - Tetranucleotide: AAAG AAAG AAAG AAAG
  - Trinucleotide: CTT CTT CTT CTT CTT
  - Dinucleotide: AG AG AG AG AG AG
- **Tetranucleotides** are favored in human identity
  - Good balance of “ease of interpretation” and “variability found in nature”

D18S51 “D18”
Chromosomal location FL 18q21.3

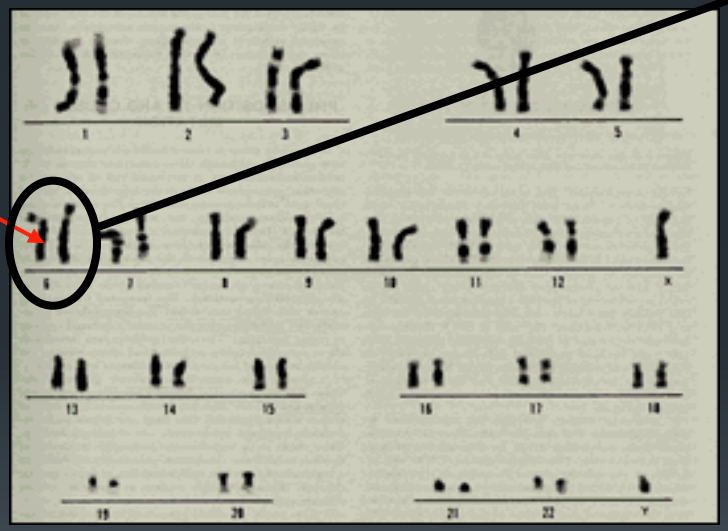
```
   1 aattgagcnc aggagtttaa gaccagcctg ggtaacacag tgagacccct gtctctaca
  61 aaaaatacaa aaatnagttg ggcatggtgg cacgtgcctg tagtctcagc
tacttgcagg 121 gctgaggcag gaggagttct tgagcccaga aggttaaggc tgcagtgagc
catgttcatg 181 ccactgcact tcactctgag tgacaaattg agaccttgtc tcagaaagaa
agaaagaaag 241 aaagaaagaa agaaagaaag aaagaaagaa agaaagaaag aaaagagagg
ggaaagaaag 301 agaaanagna aanaaatagt agcaactgtt attgtaagac atctccacac
accagagaag 361 ttaattttaa ttttaacatg ttaagaacag agagaagcca acatgtccac
cttaggctga 421 cggtttgttt atttgtgttg ttgctggtag tcgggtttgt tatttttaaa
gtagcttatc 481 caatacttca ttaacaattt cagtaagtta tttcatcttt caacataaat
acgnacaagg 541 atttcttctg gtcaagacca aactaatatt agtccatagt aggagctaat
actatcacat 601 ttactaagta ttctatttgc aatttgactg tagcccatag ccttttgtcg
gctaaagtga 661 gcttaatgct gatcgactct agag
```

The repeat sequence is aaga – this particular individual has 14 repeats

The locus is “where it’s at”

Locus—the physical position of an STR and its associated flanking sequence

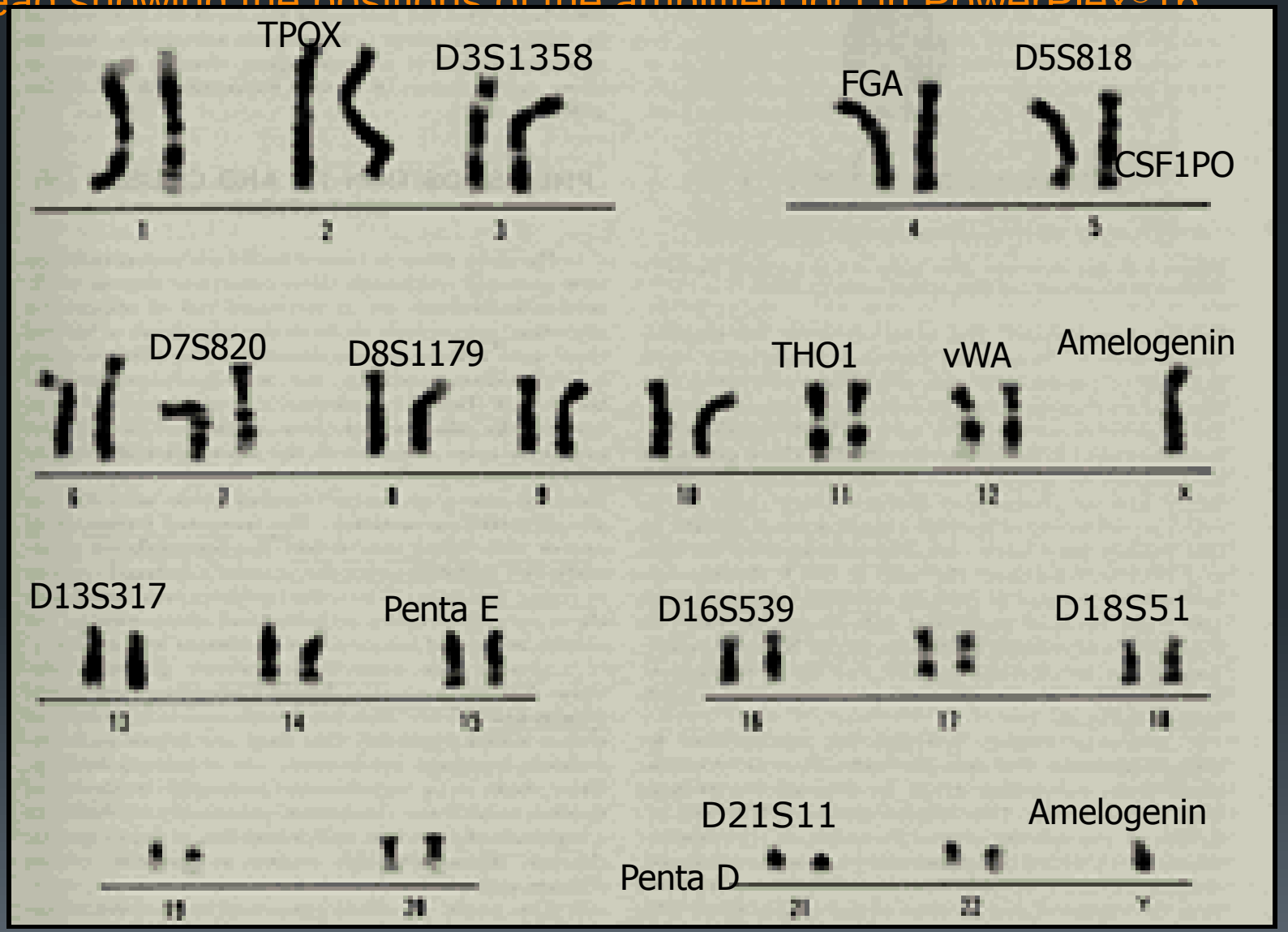
Both chromosomes of a homologous pair contain this locus



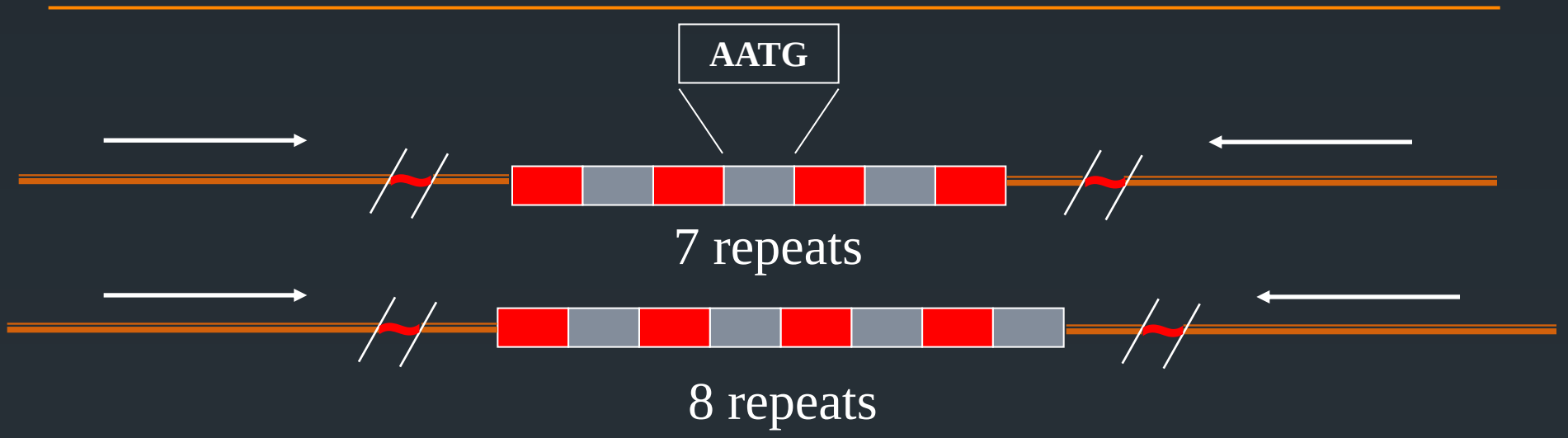
The allele contained on either chromosome can be the same or different lengths (**homozygous** or **heterozygous**)

Chromosome Spread showing the positions of the amplified loci in PowerPlex®16

The PowerPlex® 16 System amplifies 16 loci.



Short Tandem Repeats (STRs)



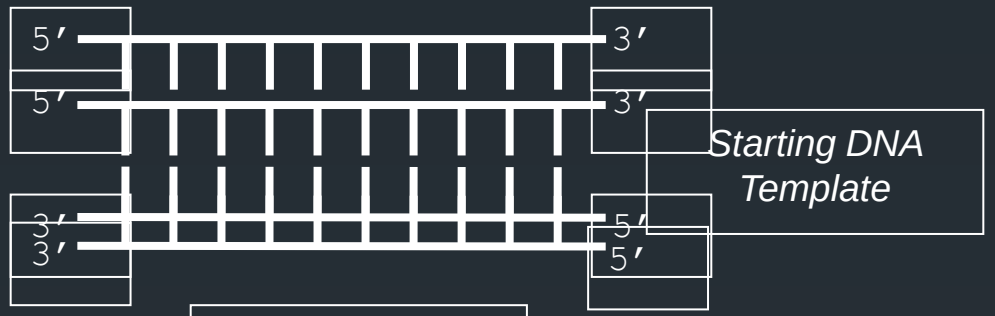
- Repeat region is variable (**polymorphic**)
  - Each variant is referred to as an **allele**
- Flanking region is constant

KEY: Alleles are distinguished by length

Homozygote = both alleles are the same length

Heterozygote = alleles differ and can be resolved from one another

DNA Amplification with PCR



Starting DNA Template

Separate strands (**denature**)

Forward primer

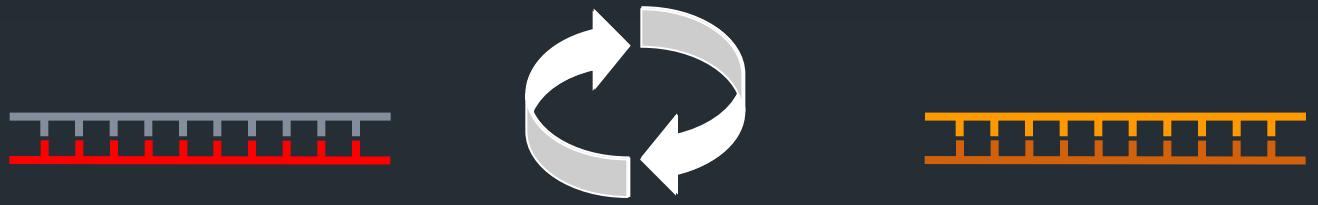
Add primers (**anneal**)

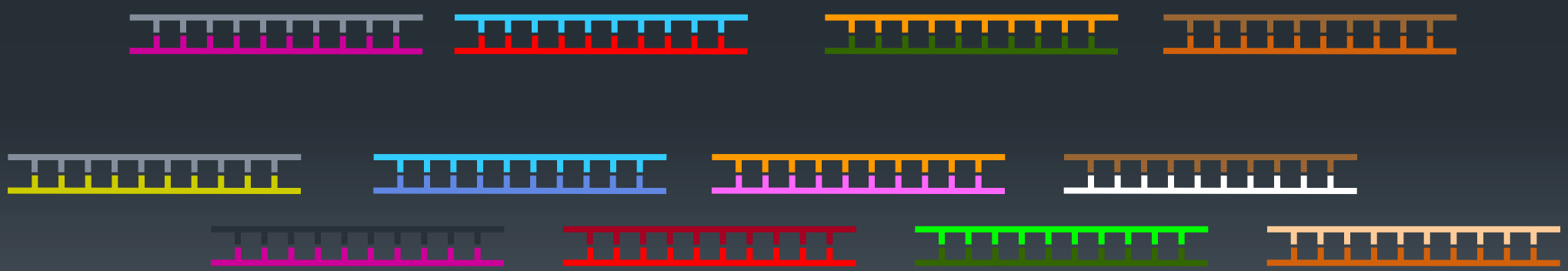
Reverse primer

Make copies (**extend primers**)

Exponential Amplification with PCR

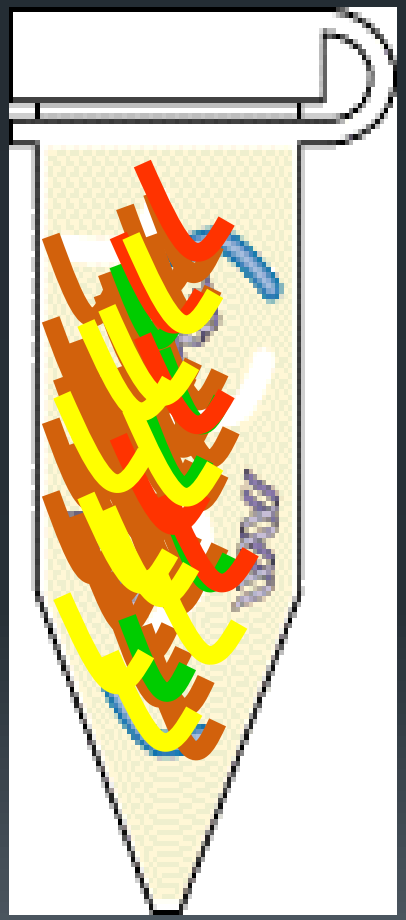
Original DNA target region





In 32 cycles at 100% efficiency, 1.07 billion copies of amplicon are made.

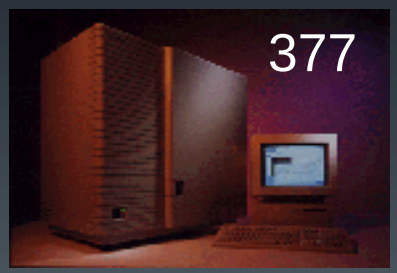
Multiplex PCR



- 16 Loci Are Copied at Once
- Sensitivities to levels less than 0.5 ng of DNA
- Ability to Handle Mixtures and Degraded Samples
- Different Fluorescent Dyes Used to Distinguish STR Alleles with Overlapping Size Ranges

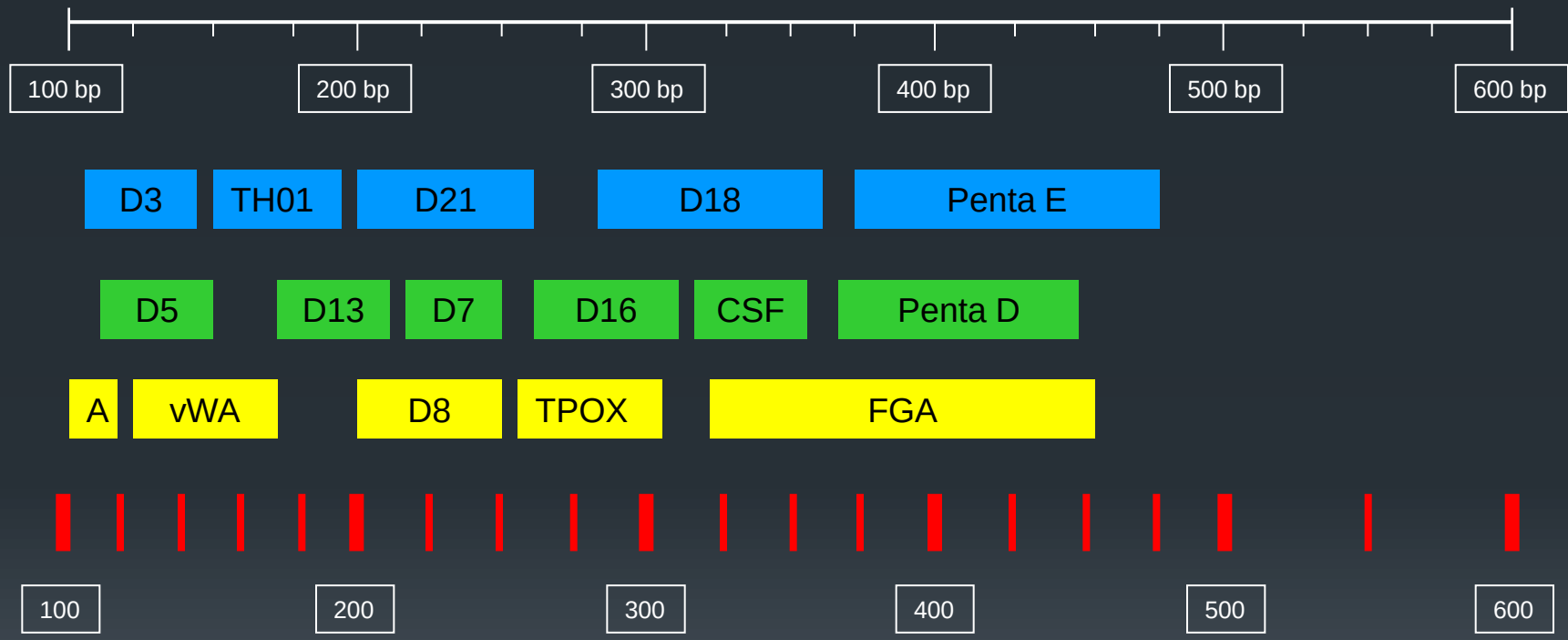
Separating and “Seeing” STR’s

- Electrophoresis
  - Separates amplification products based on size
- Fluorescent detection
  - Amplification products have a fluorescent “label” attached to the primer
  - Label is seen through excitation via a laser and corresponding emission captured with a camera

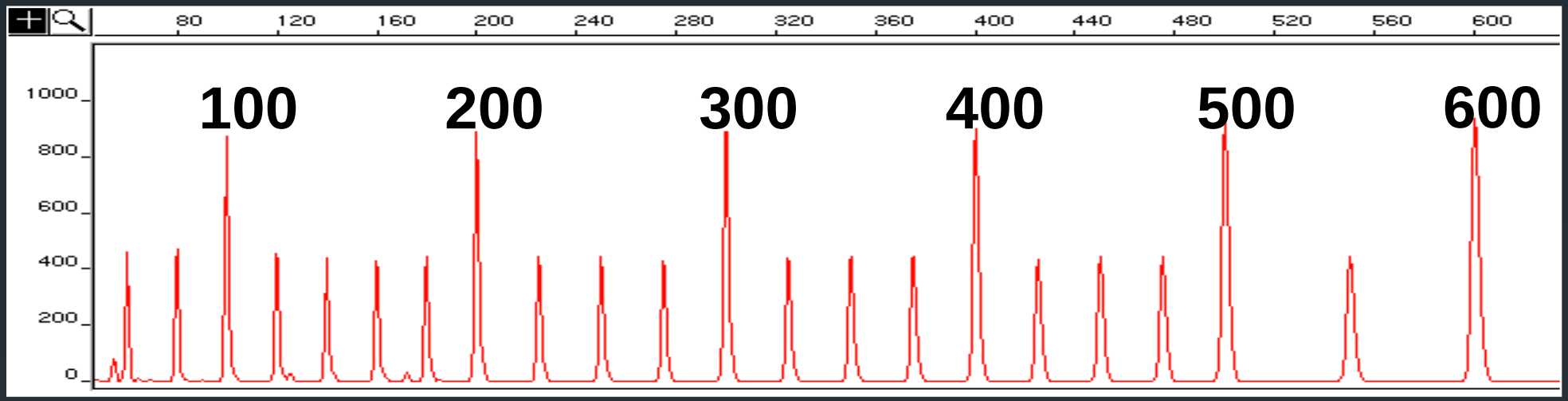


Current Forensic STR Multiplexes

PowerPlex 16



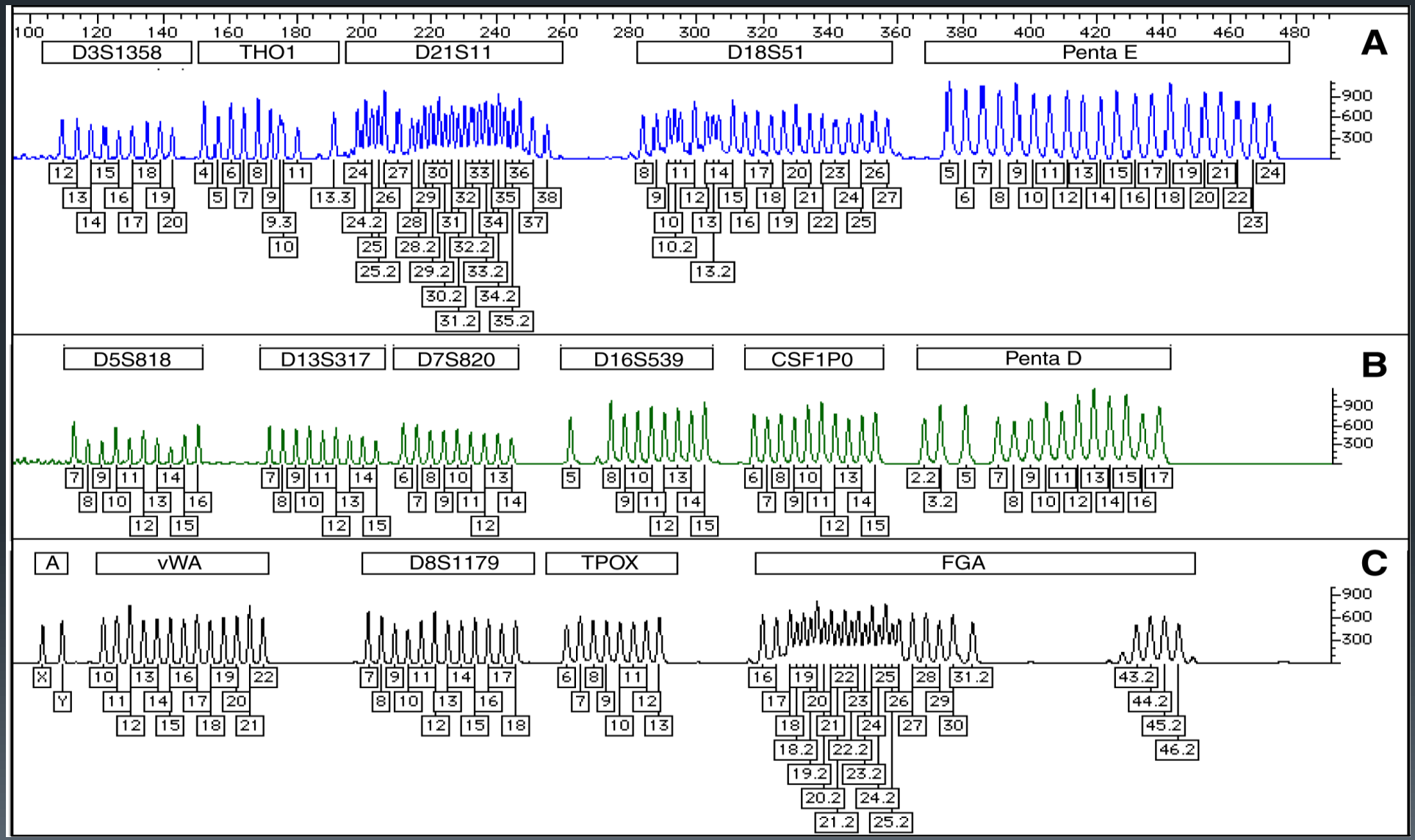
ILS600 Size Standard



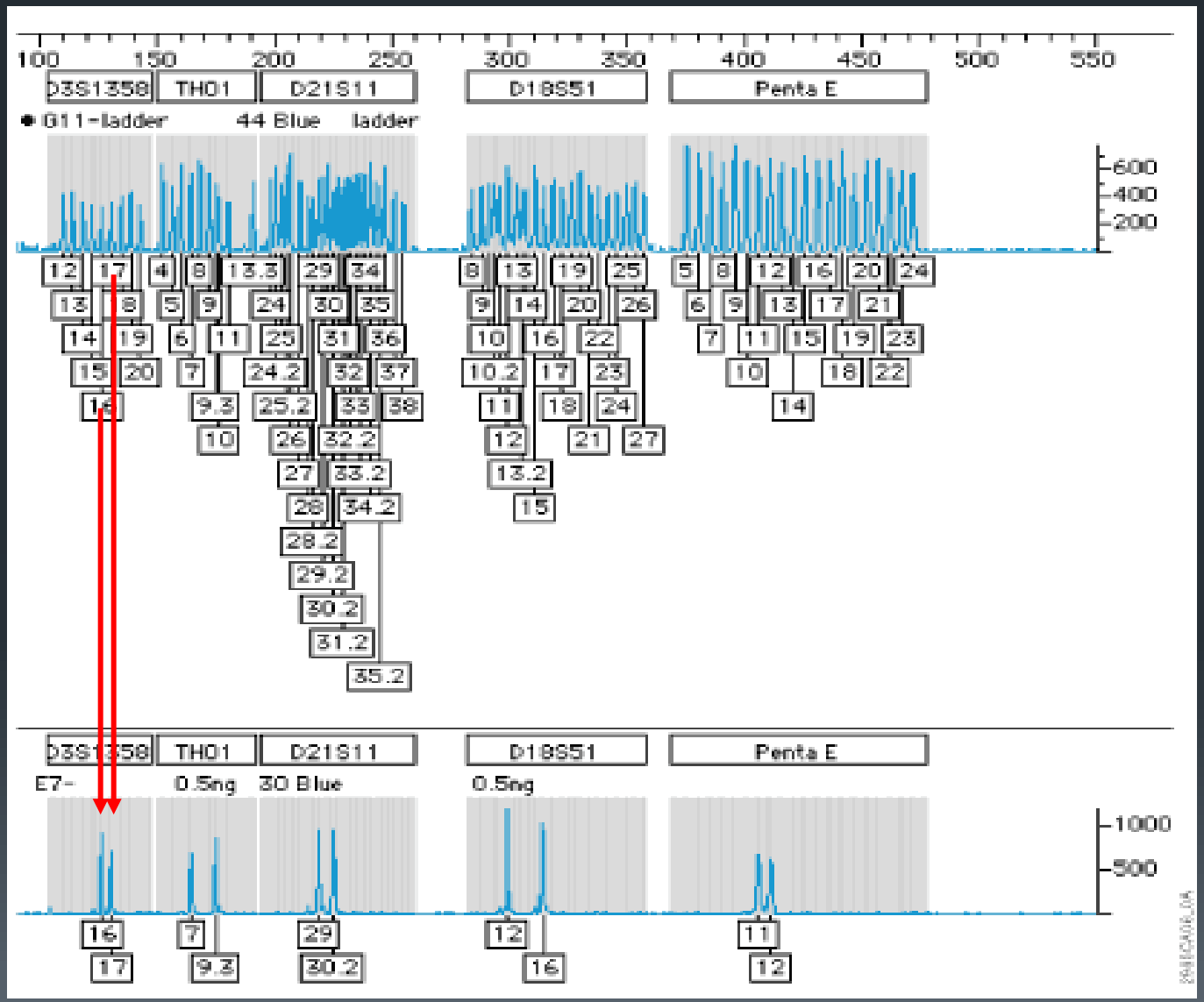
A sizing standard is used in all samples and allelic ladders

The known standard is used to determine the size of the allelic ladders and the unknown samples

Allelic Ladder

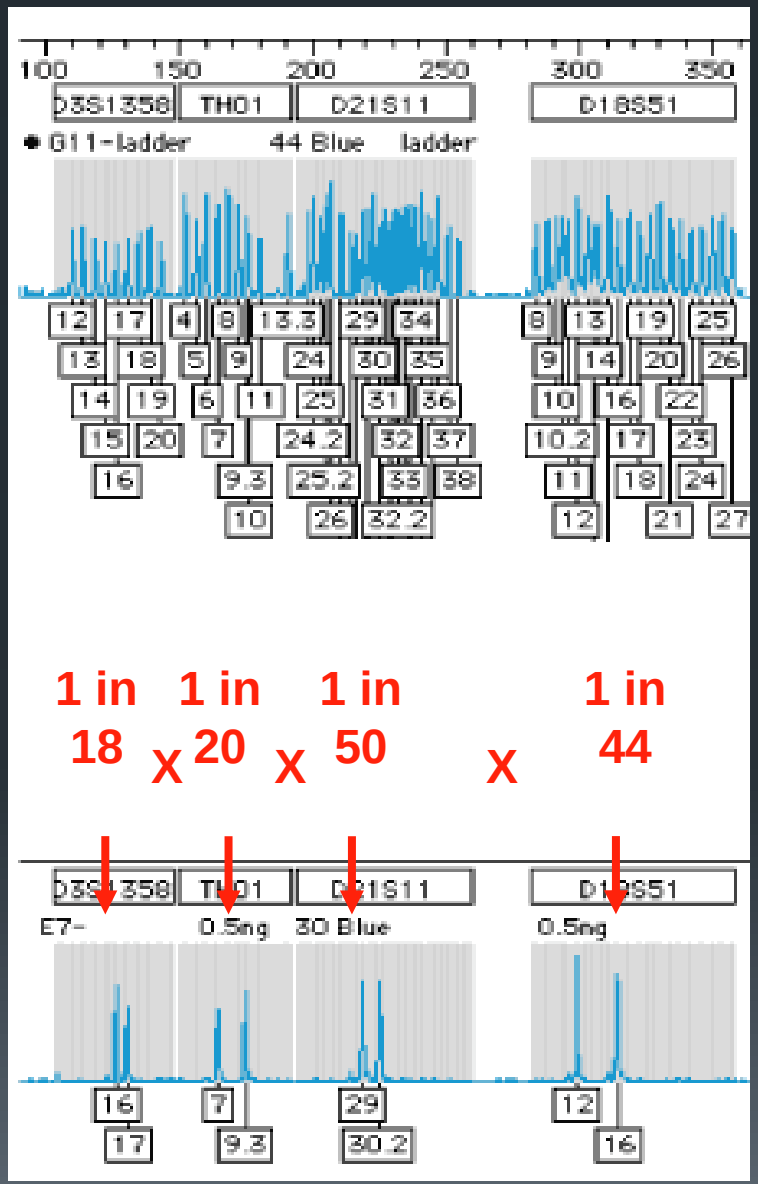


Allele Calls



Discrimination power through multiplexing

Allele possibilities



Hypothetical likelihood of occurrence

1 locus: 1 in 18
2 loci: 1 in 360
3 loci: 1 in 18000
4 loci: 1 in 792000

9 loci: ~1 in 10^{10}

16 loci: ~1 in 10^{17}

Current World Pop: ~6.3 billion

Sample Genotype

DATA ANALYSIS

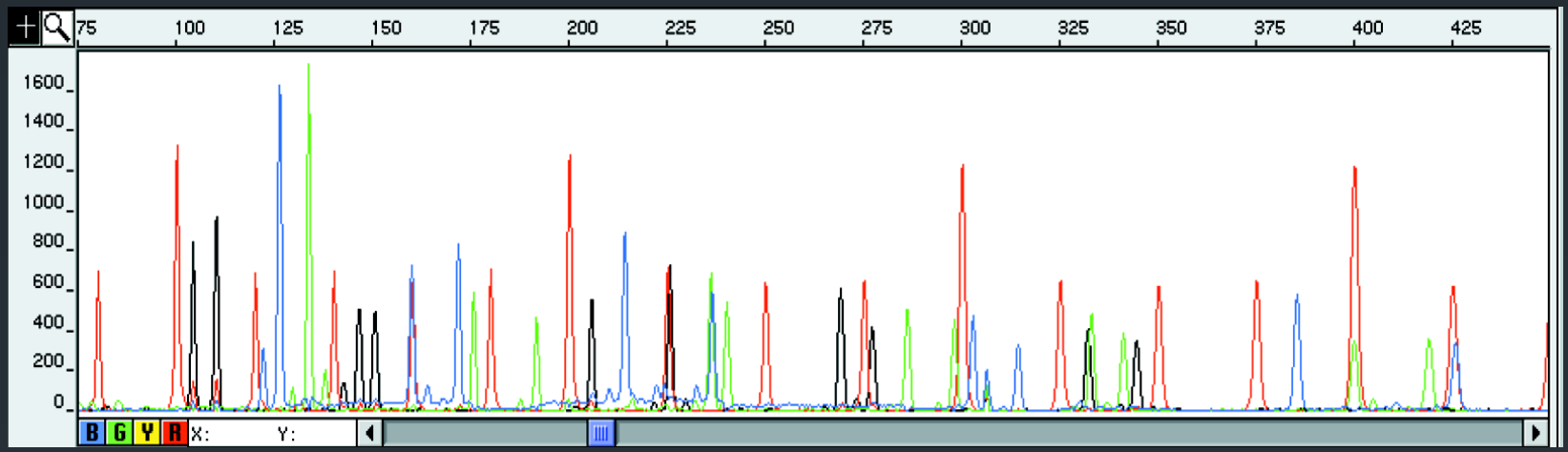
- Controls
  - Negative control devoid of amplification products
  - Compare positive control 9947a with locus-specific ladder
- STR Allelic Ladders
  - Comparison with samples allows precise assignment of alleles
- Fluorescent Ladder (CXR)
  - Internal Size Standard

Human Identity Testing Applications

- Forensic cases: matching suspect with evidence
- Paternity testing: identifying father
- Convicted felon DNA databases
- Missing persons investigations
- Mass disasters -- putting pieces back together
- Historical investigations
- Military DNA “dog tag”

Complete STR Profile

DNA from Small Stains/challenging samples



- 0.1µl blood stain on denim
- 1/5 of eluted material used for amplification

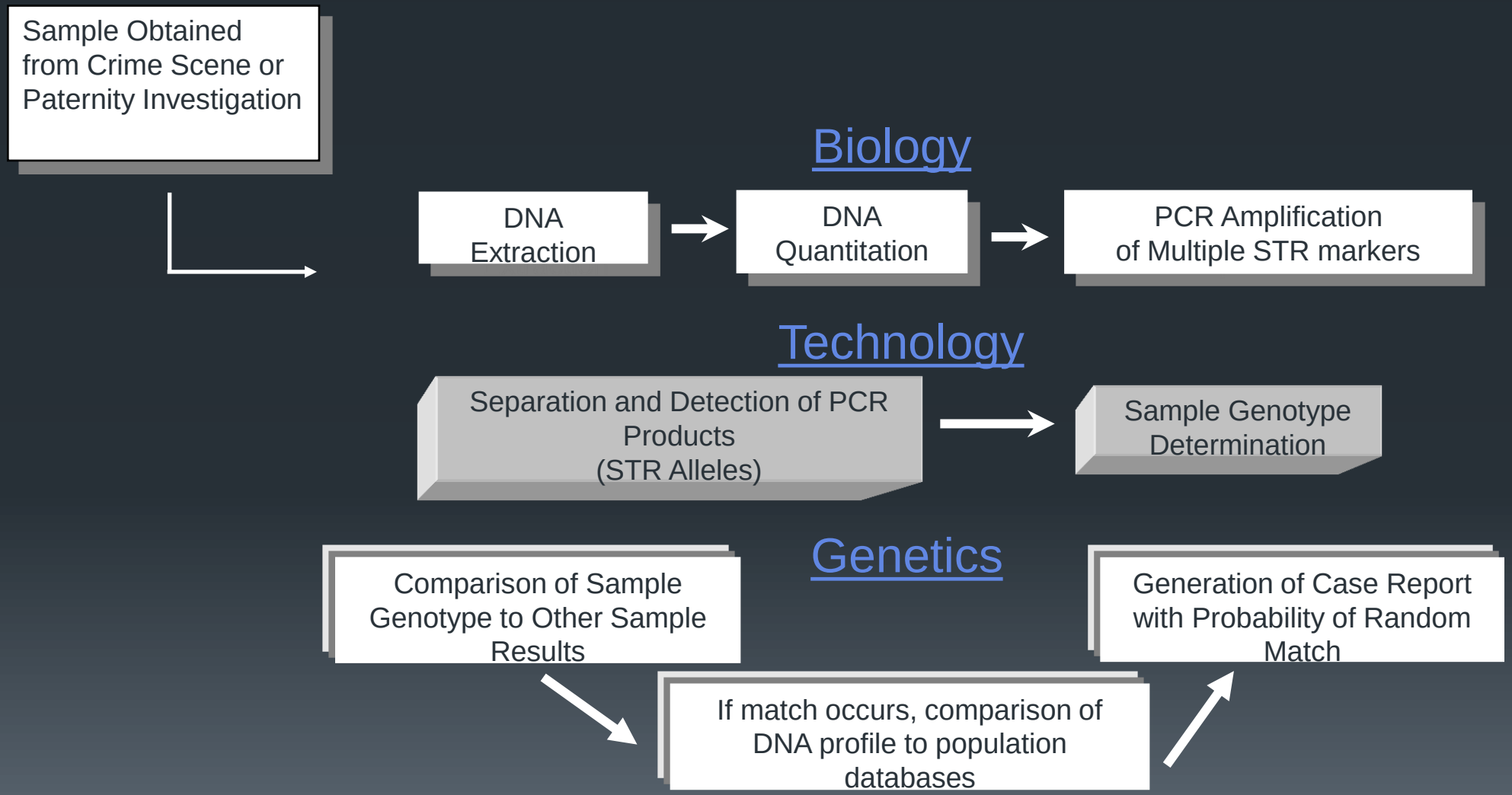
DNA Use in Forensic Cases

- Most are rape cases (>2 out of 3)
- Looking for matches between evidence, victim, and suspect
- Must compare DNA profiles

Challenges

- Mixtures must be resolved if present
- DNA is often degraded
- Inhibitors to PCR and sample contamination are often present

Steps in DNA Sample Processing



Sources of Biological Evidence

- Blood
- Semen
- Saliva
- Urine
- Hair
- Teeth
- Bone
- Tissue

DNA extraction

- Samples can have extremely small amounts of DNA
- Available Technologies for DNA Isolation
  - Phenol:Chloroform Extraction (Homebrew)
  - Chelex (ReadyAmp™)
  - FTA® Paper
  - Qiagen
  - DNA IQ™ System
  - DNA IQ™ Reference Sample Kit for Maxwell® 16

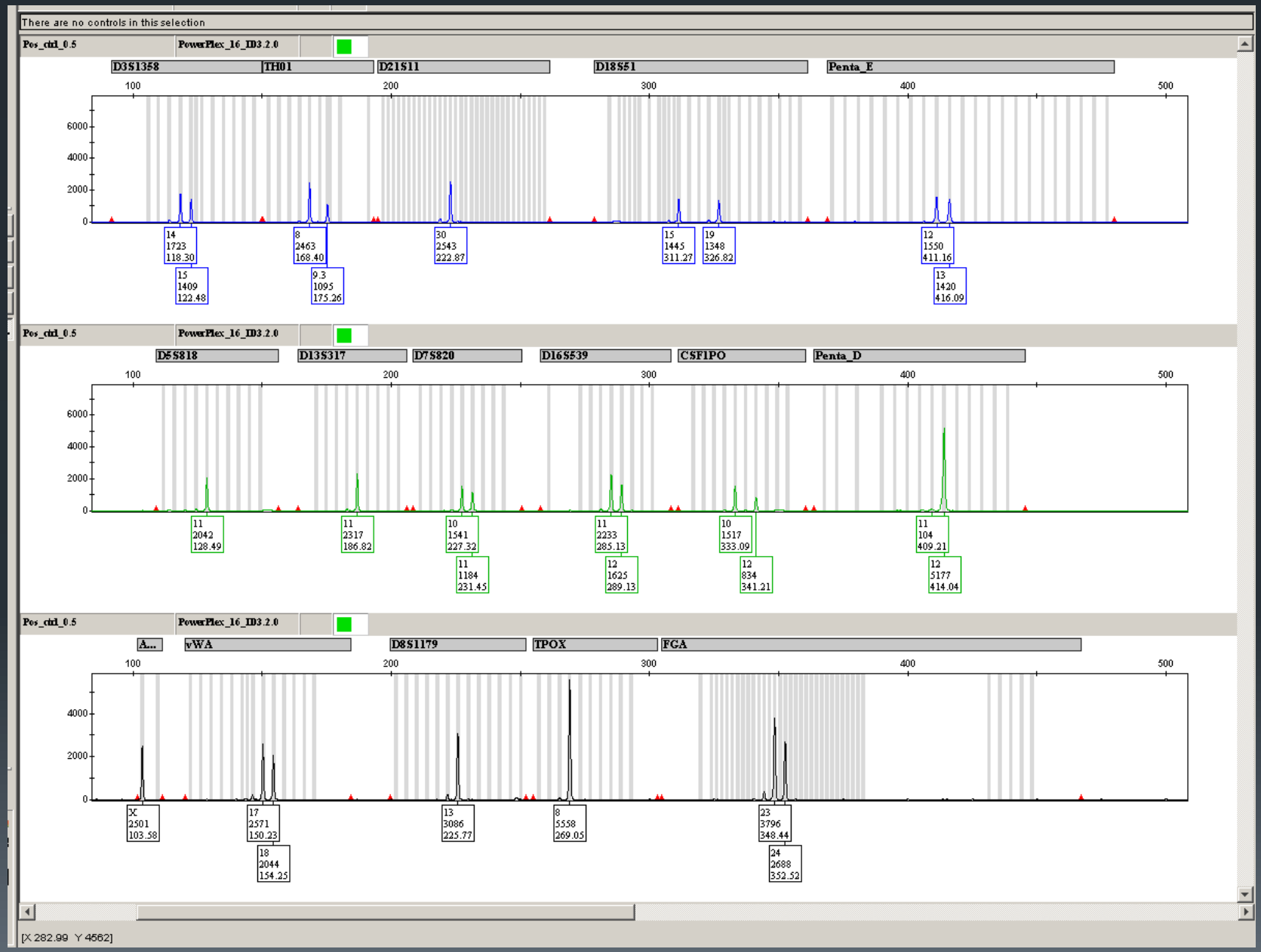
DNA Quantitation

- Forensic labs in the US are required by law to quantitate the amount of Human DNA in crime scene samples
- How might this be done?

Regulation of Labs

- Forensic
  - FBI
    - Standards for Combined DNA Index System (CODIS) labs
      - http://www.fbi.gov/hq/lab/codis/index1.htm
    - The Scientific Working Group for DNA Analysis Methods(SWGDAM) publishes guidelines
- Paternity
  - American Association of Blood Banks (AABB)
    - http://www.aabb.org

PowerPlex® 16



Statistical Analysis

- Analysis based on population statistics and data
- Probability that the evidence matches the suspect

Allele Frequency Data

F13A01[b]			
	Caucasian-American	African-American	Hispanic-American
Allele	**Frequency**		
3.2	0.085	0.087	0.225
4	0.041	0.076	0.113
5	0.208	0.342	0.227
6	0.287	0.131	0.164
7	0.329	0.195	0.227
8	0.017	0.067	0.014
9	0.000	0.009	0.000
10	0.000	0.005	0.000
11	0.000	0.009	0.007
12	0.002	0.011	0.000
13	0.005	0.032	0.005
14	0.017	0.021	0.005
15	0.010	0.014	0.007
16	0.000	0.002	0.007
Allele Frequencies			
Homozygotes	0.237	0.225	0.203
Heterozygotes	0.763	0.775	0.797
Total Samples	207	218	222
Forensic Statistics			
Matching Probability (general)	0.098	0.061	0.07
Expressed as 1 in ...	10.2	16.4	14.3
Matching Probability (siblings)			
Expressed as 1 in ...			
Power of Discrimination			
PIC			
Paternity Statistics			
Power of Exclusion	0.533	0.554	0.594
Typical Paternity Index	2.11	2.22	2.47

References and resources

- http://www.cstl.nist.gov/biotech/strbase/intro.htm (some information in this presentation is from this ppt)
- http://www.promega.com/applications/hmnid/ (Promega Human identity testing products)
- http://www.promega.com/profiles/ (Profiles in DNA)
- http://journalsip.astm.org/JOURNALS/FORENSIC/jofs_home.html (Journal of Forensic Science)
- http://appliedbiosystems.com Supplier of Human identification systems

COLLECTION OF TOUCH DNA FROM EVIDENCE

When:

- At the Crime Scene by Law Enforcement
  (e.g. door knobs, counters, windows)
- Forensic Laboratory by analyst
  (if other testing is needed)

Where: - Areas of contact (e.g. grips, slide, trigger, magazine, cartridge cases; fired vs. unfired)

- Any touched object…

(but be cautious regarding objects accessible to the general public)

DNA Profiles from Weapons and the DNA Database

- The weapon must be associated with a crime
  - seized vs. surrendered.
- The weapon cannot be seized from the suspect's person or property.
- Cannot use a "possession" sample as an alternate way to get a suspect's known profile into the DNA Database.

TOUCH DNA EVIDENCE : COLLECTION SUGGESTIONS…….

Proper collection of "touch" DNA evidence:

Collection protocol:

-Wear latex gloves (change frequently)
-Disposable face masks/supplies
- Clean instruments with bleach and alcohol

How : -Swab using sterile swab/solution

Collection of Touch DNA Evidence

1. Contamination is a significant possibility.

2. Impact of contamination is false exclusion of suspect or artificial mixtures.

How to minimize:

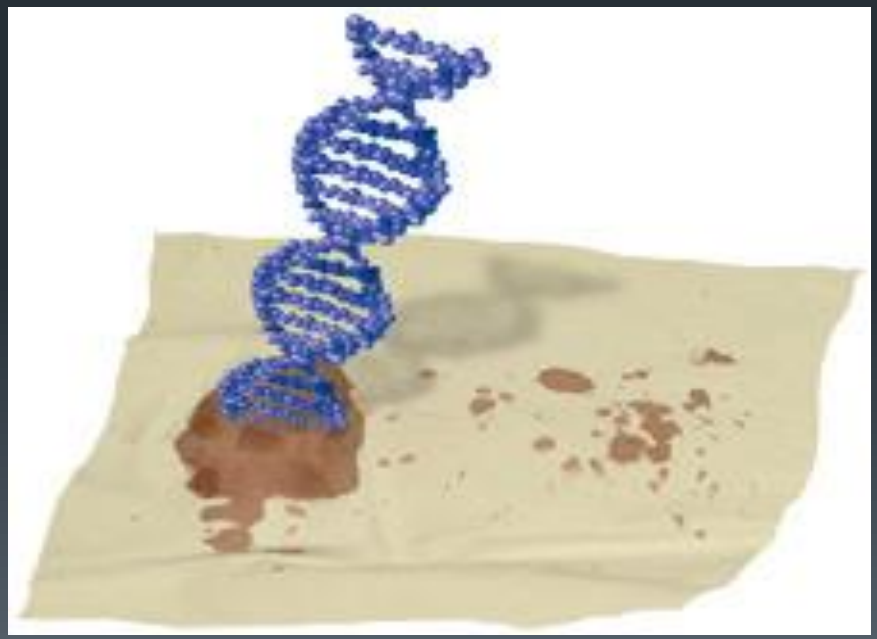
Gloves, Masks, Disposable Instruments, procedure (no talking over evidence!!!)

Identification of Contamination:

Know the DNA profiles of:

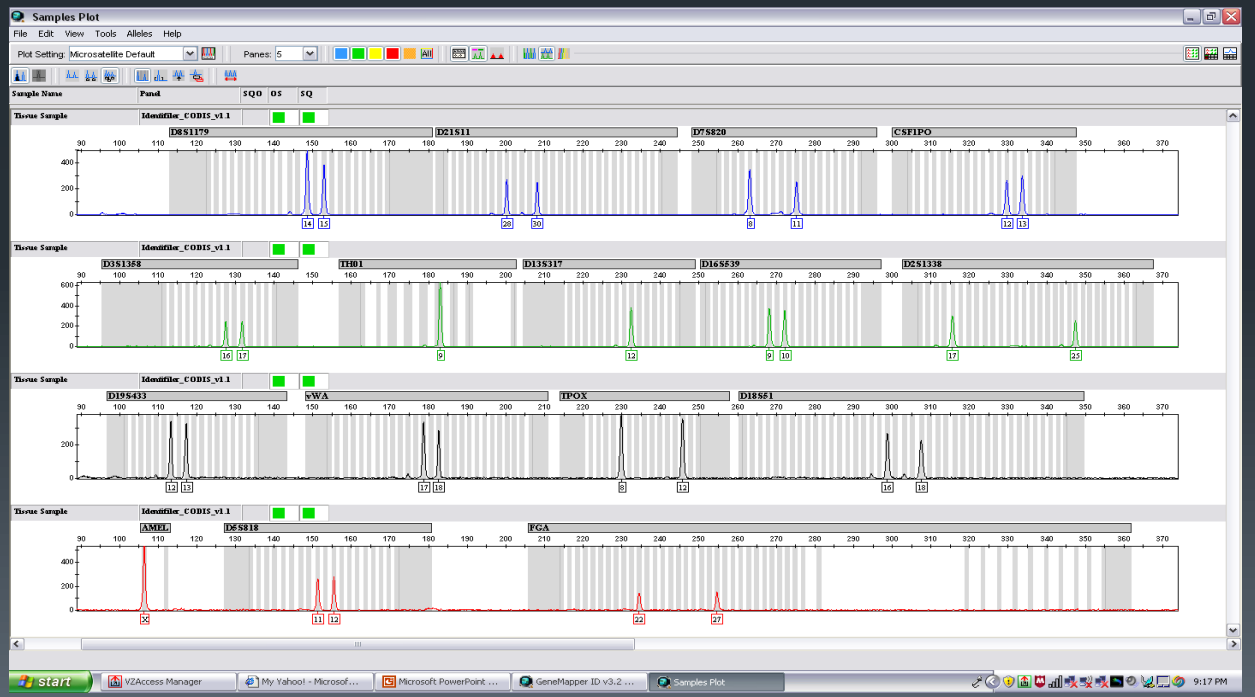
First Responders, Major Crime Squads, and Laboratory Personnel

Forensic DNA Testing



How do we go from this . . .

. . . To this?



Evidence Collection

With increasingly sensitive DNA tests, proper collection protocols are more critical.

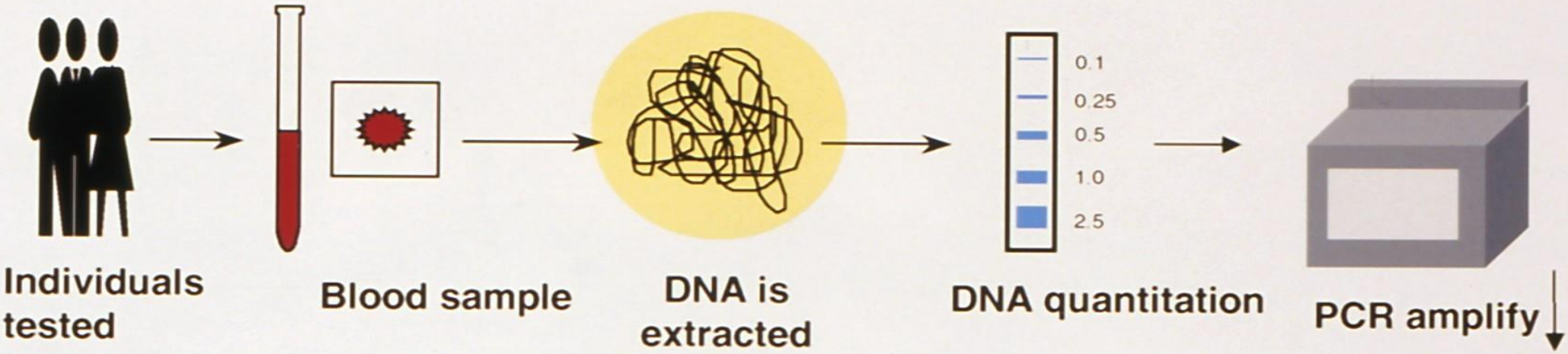
Standard measures: Gloves, disposable supplies, etc. consider masks—especially for low yield samples.

Clean any non-disposable instruments with bleach and alcohol.

Elimination swabs from people at the scene answers standard defense question.

Collect evidence to avoid/minimize mixtures especially with certain samples.

DNA analysis using STRs and the DNA Database



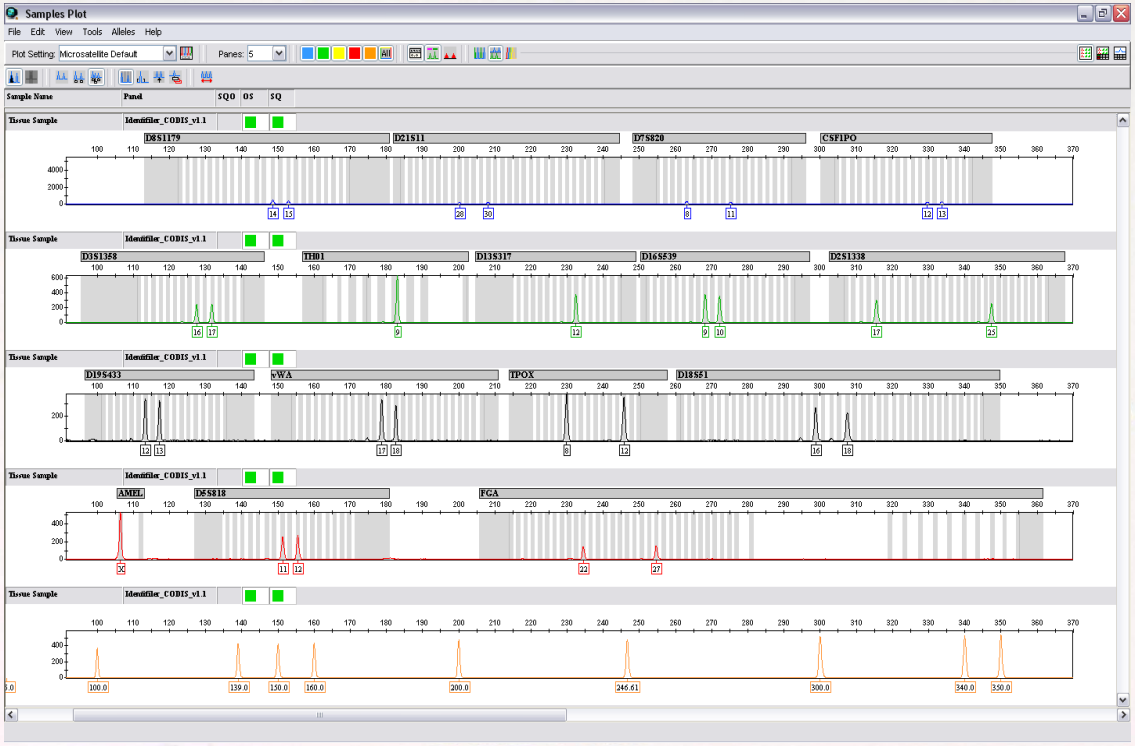
Individuals tested

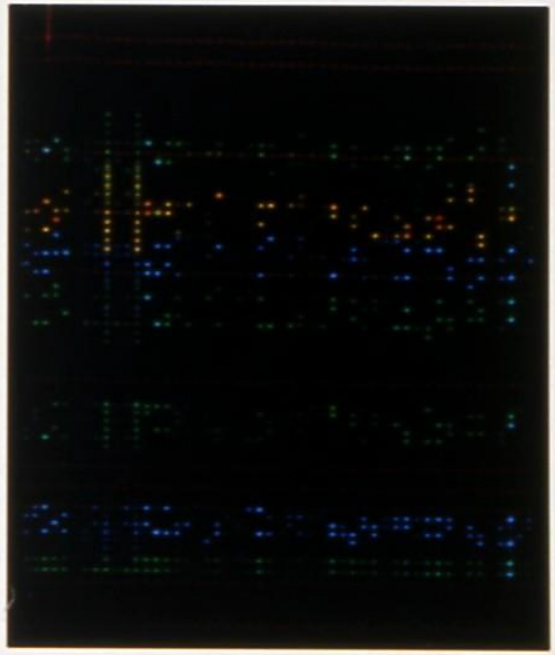
Blood sample

DNA is extracted

DNA quantitation

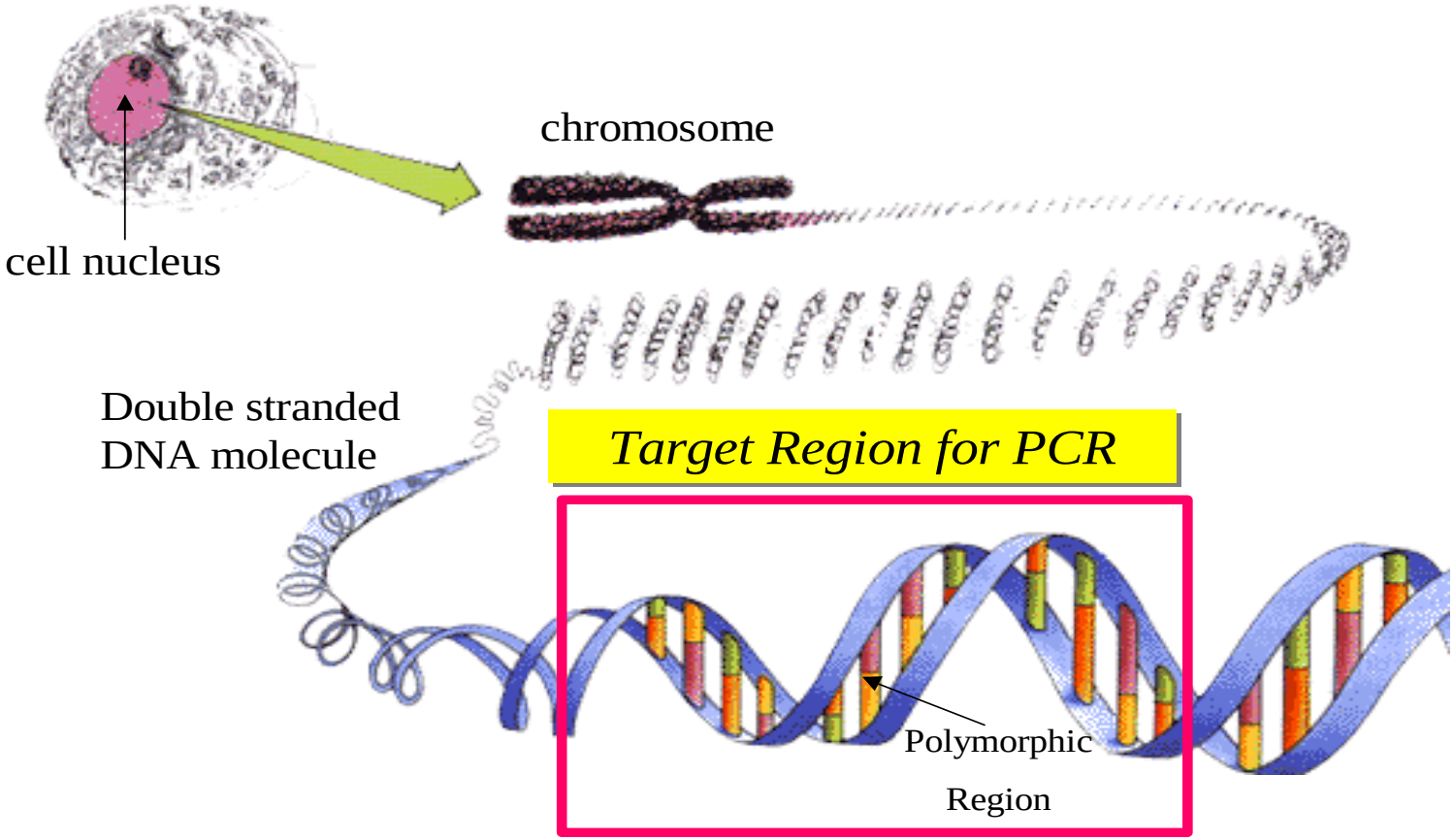
PCR amplify





DNA profiles

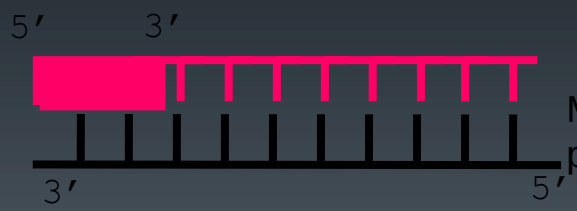
DNA in the Cell



DNA Amplification with the Polymerase Chain Reaction (PCR)

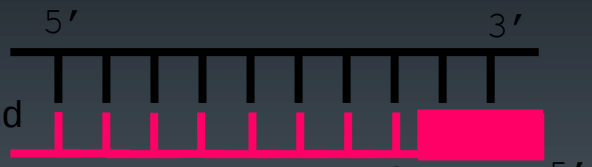
Separate strands (denature)

Forward primer



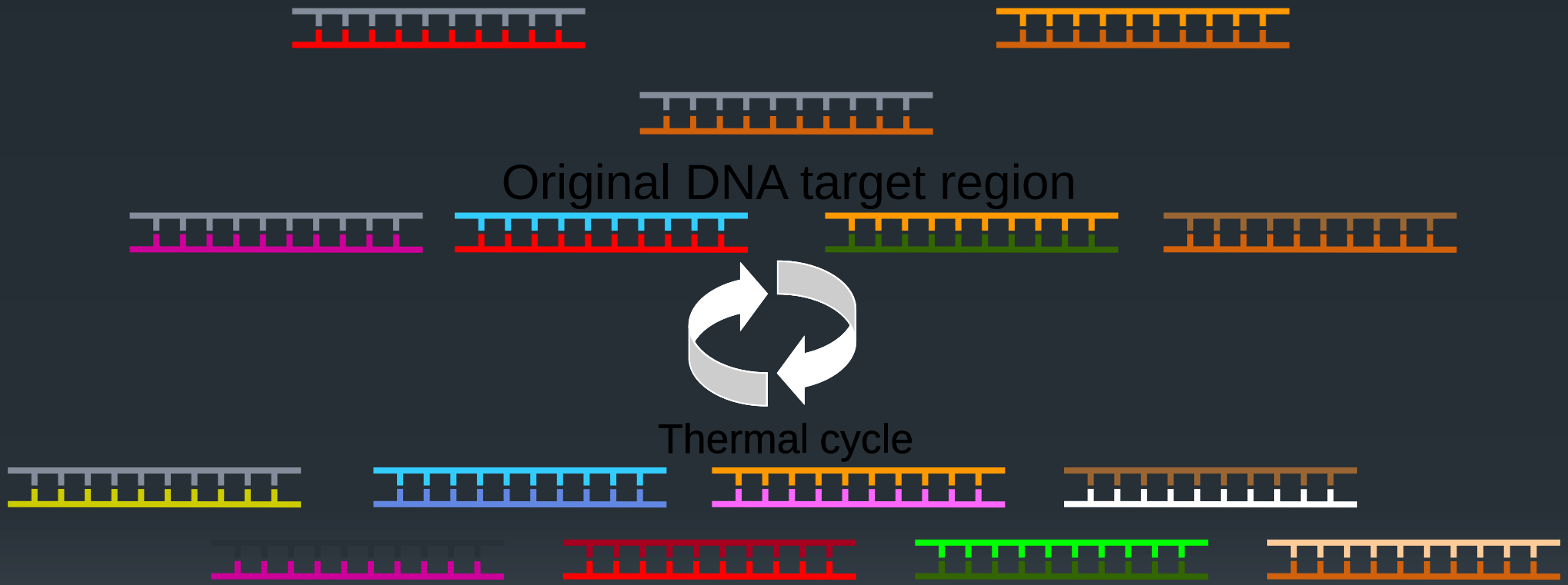
Add primers (anneal)

Make copies (extend primers)



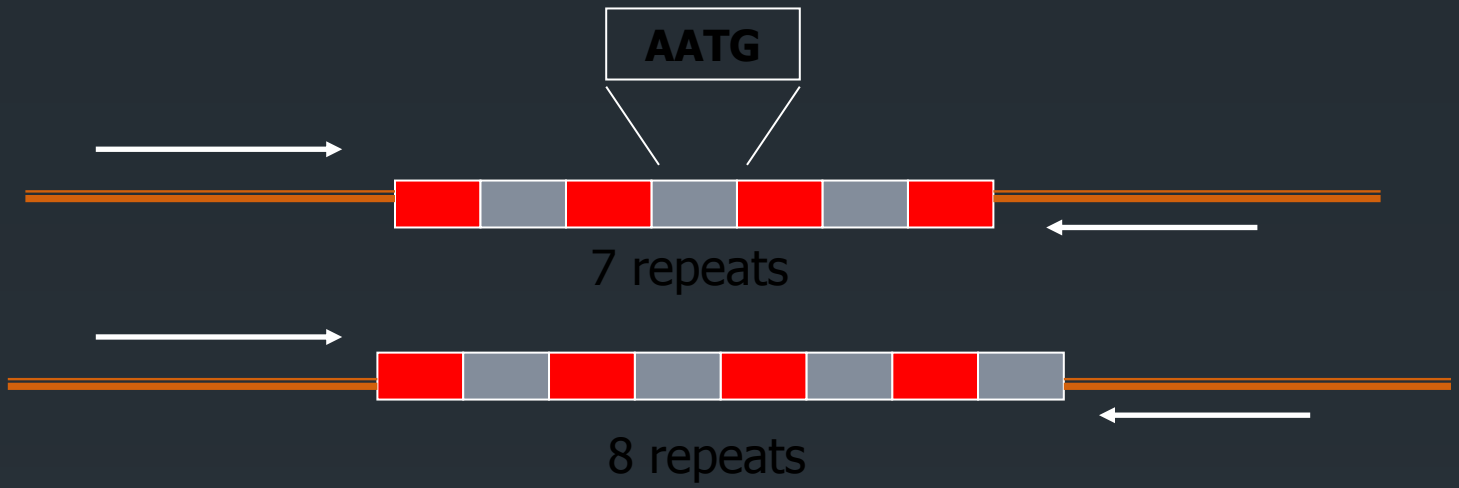
Reverse primer

PCR Copies DNA Exponentially through Multiple Thermal Cycles



In 32 cycles at 100% efficiency, 1.07 billion copies of targeted DNA region are created

Short Tandem Repeats (STRs)



Repeat number varies between alleles. PCR primers bind to flanking regions that are constant.

Homozygote = Two copies of same allele.

Heterozygote = Two different alleles.

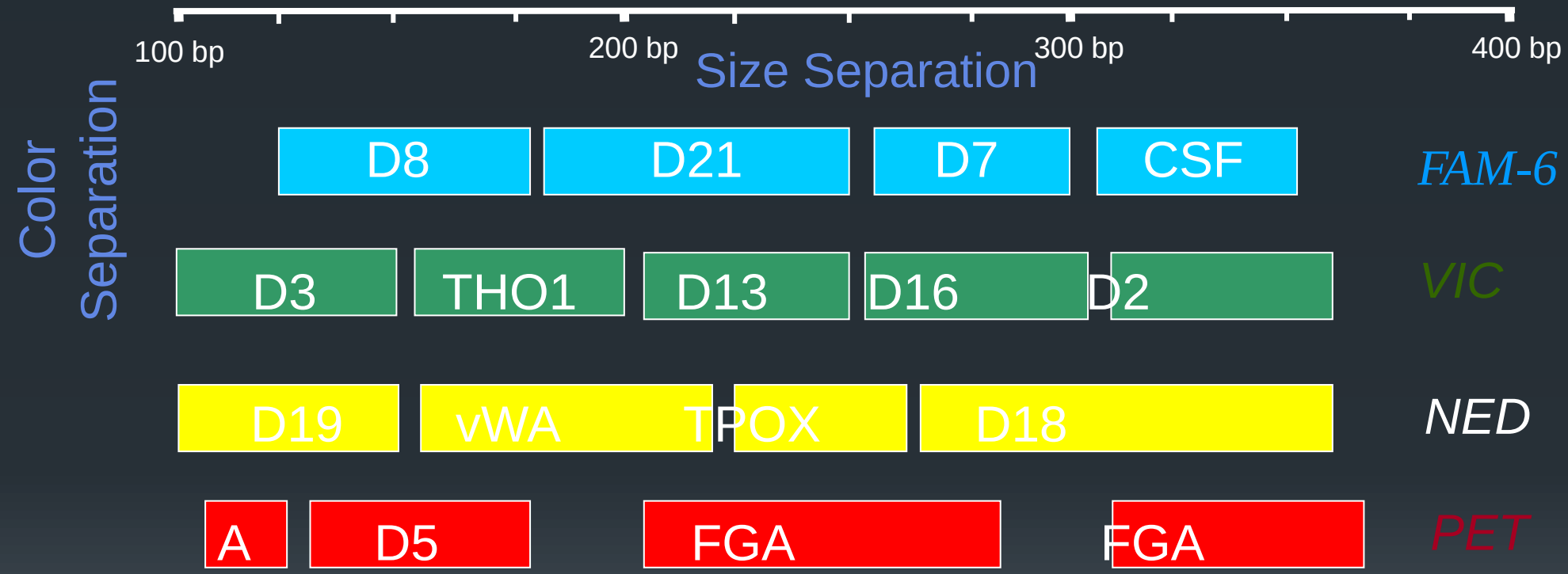
Multiplex PCR

- 15 STR Markers Can Be Amplified in 1 reaction.
- Sensitivity = less than 250 pg of DNA.
- Ability to Handle Mixtures and Degraded Samples.
- Different Fluorescent Dyes Used to Distinguish STR Alleles with Overlapping Size Ranges.

Example of Forensic STR Multiplex Kit

AmpFlSTR® Identifiler™

Kit available from PE Biosystems (Foster City, CA)

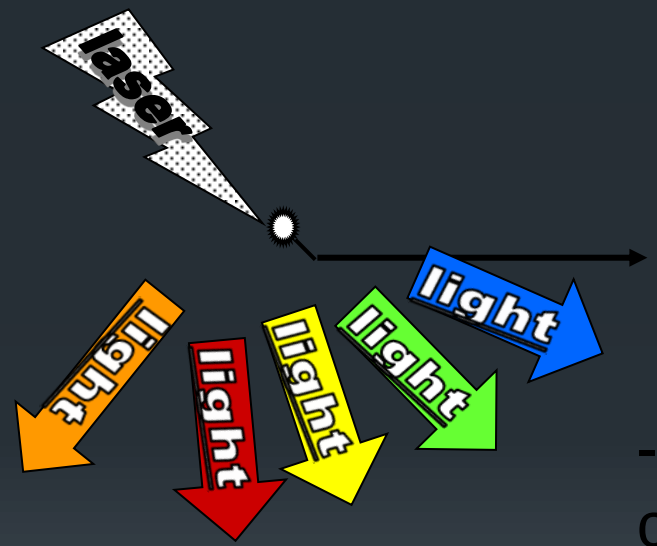


15 STRs amplified along with sex-typing marker amelogenin in a single PCR reaction.

LIZ-internal lane standard

Fluorescent STR Analysis

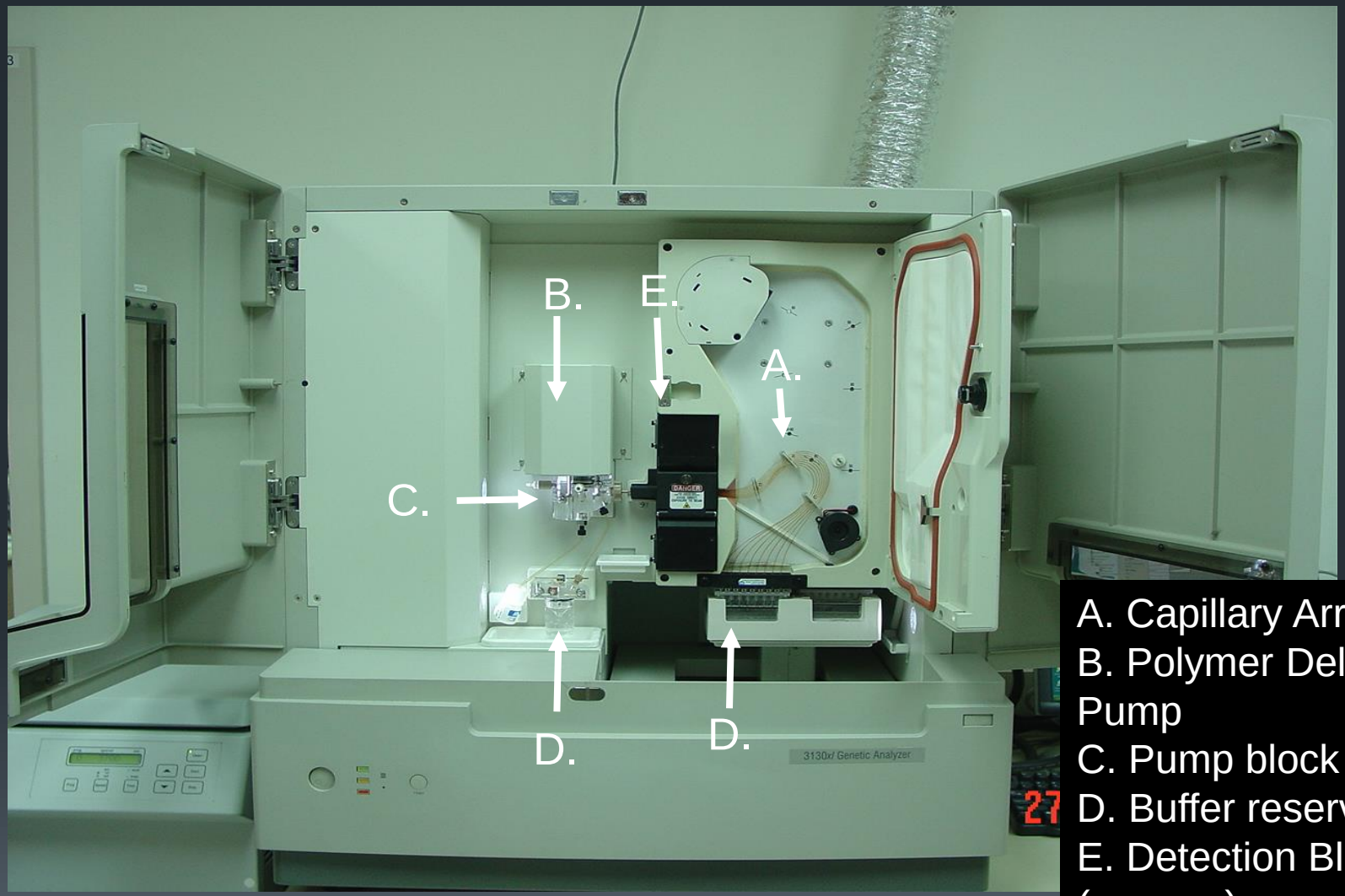
-Fluorescent dye tags on the primers



-Visualize emitted light with a digital camera.

-Collect and analyze data with computer.

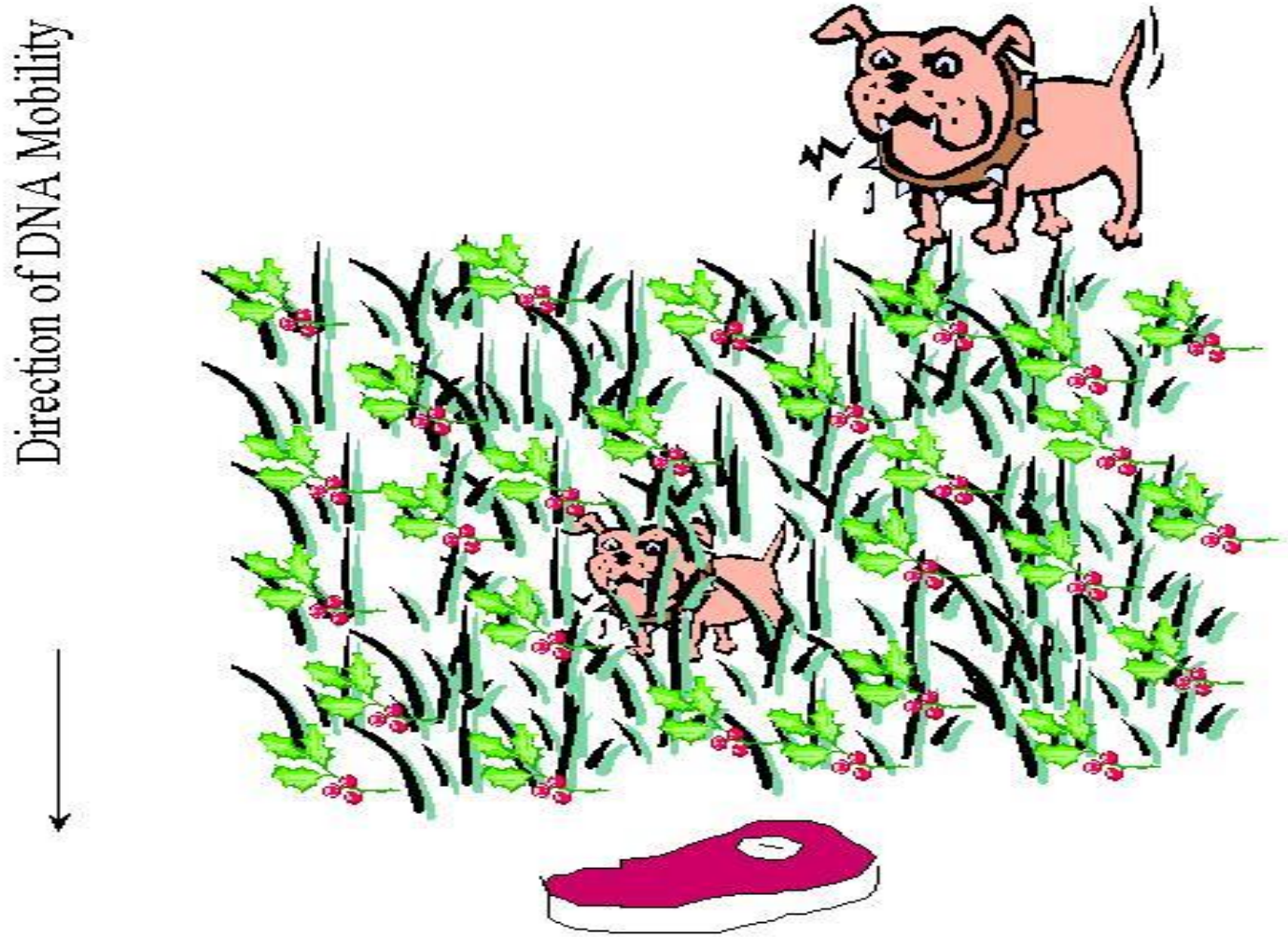
Capillary Electrophoresis



A. Capillary Array
B. Polymer Delivery Pump
C. Pump block
D. Buffer reservoirs
E. Detection Block (camera)

Size Separation of DNA

(Dog in a Thicket Analogy)

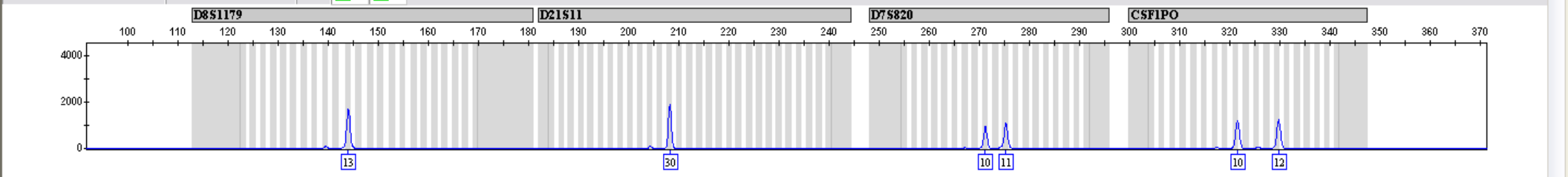


Samples Plot

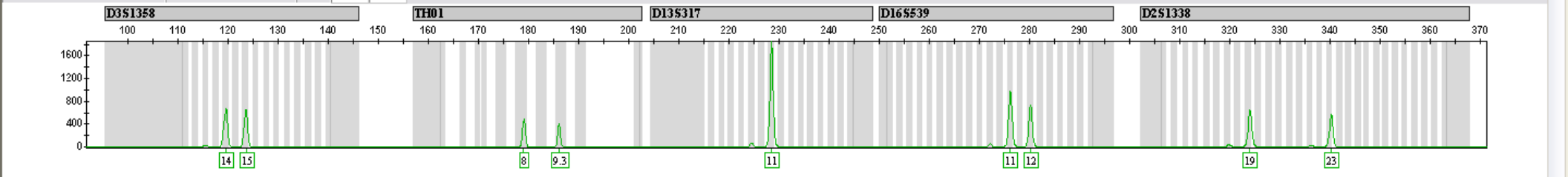
File Edit View Tools Alleles Help

Plot Setting: Microsatellite Default | Panes: 4 | All

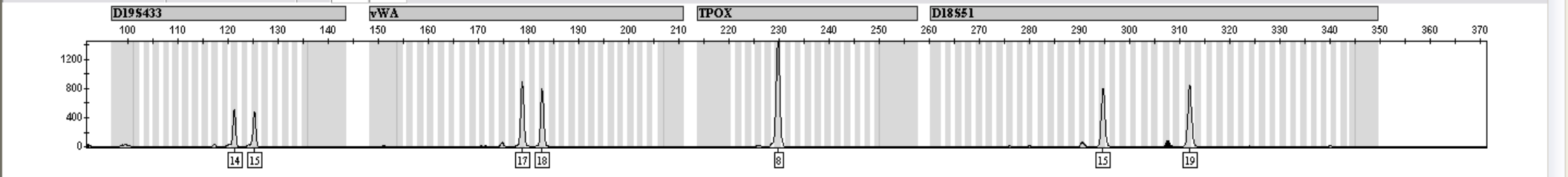
Sample Name	Panel	SQO	OS	SQ
Amplification_Positive_MIDPCR	Identifiler_CODIS_v1.1			



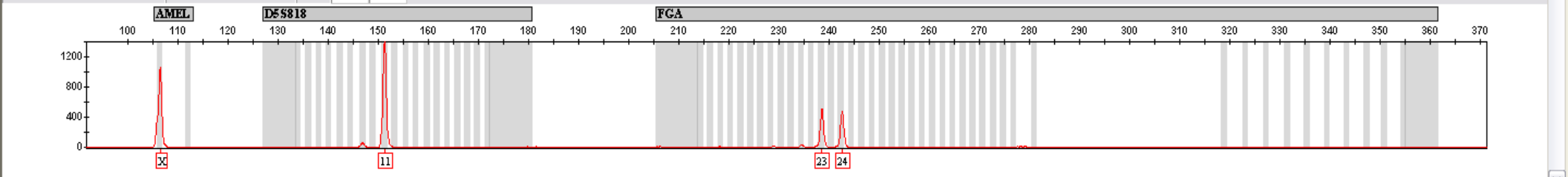
Amplification_Positive_MIDPCR	Identifiler_CODIS_v1.1		



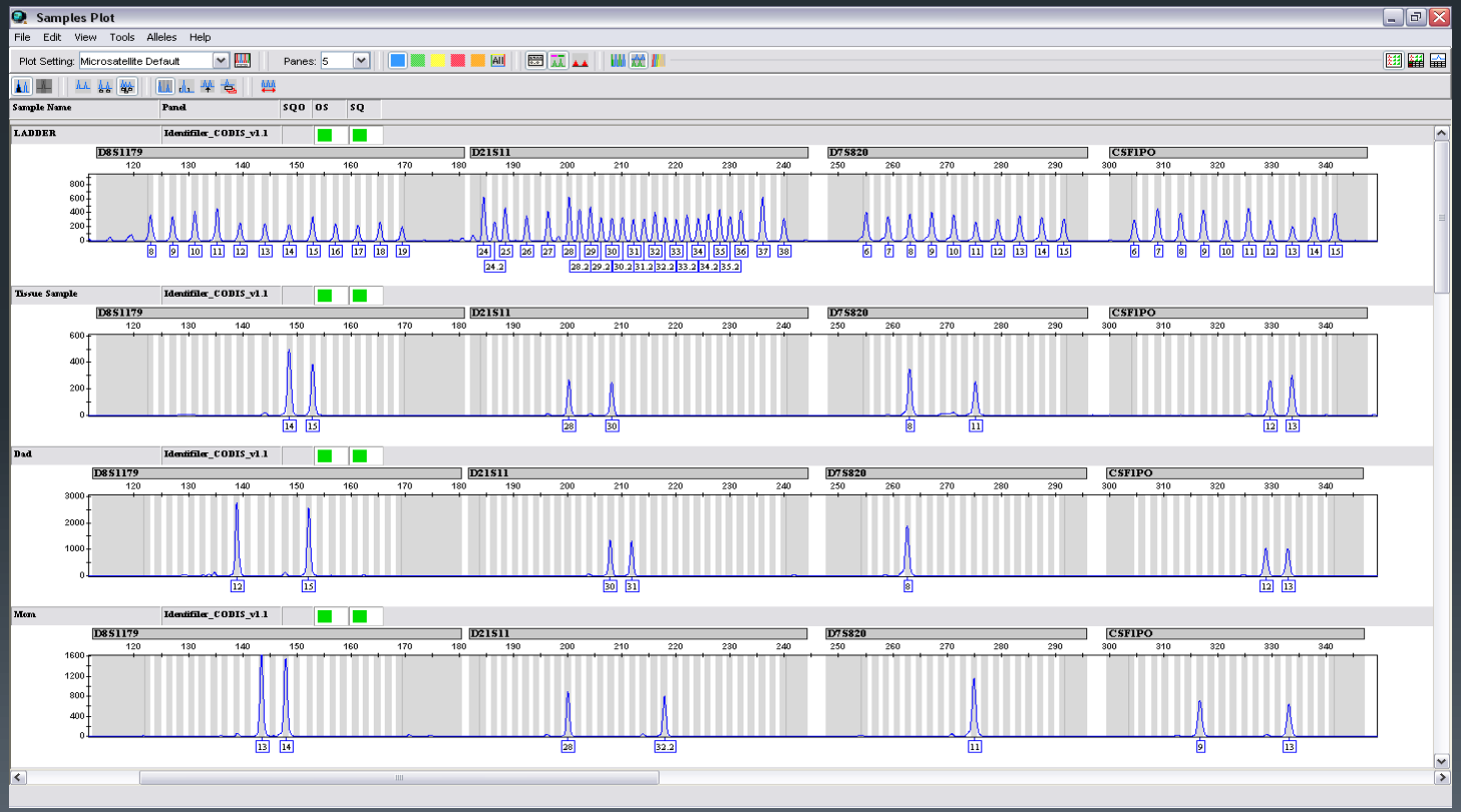
Amplification_Positive_MIDPCR	Identifiler_CODIS_v1.1		



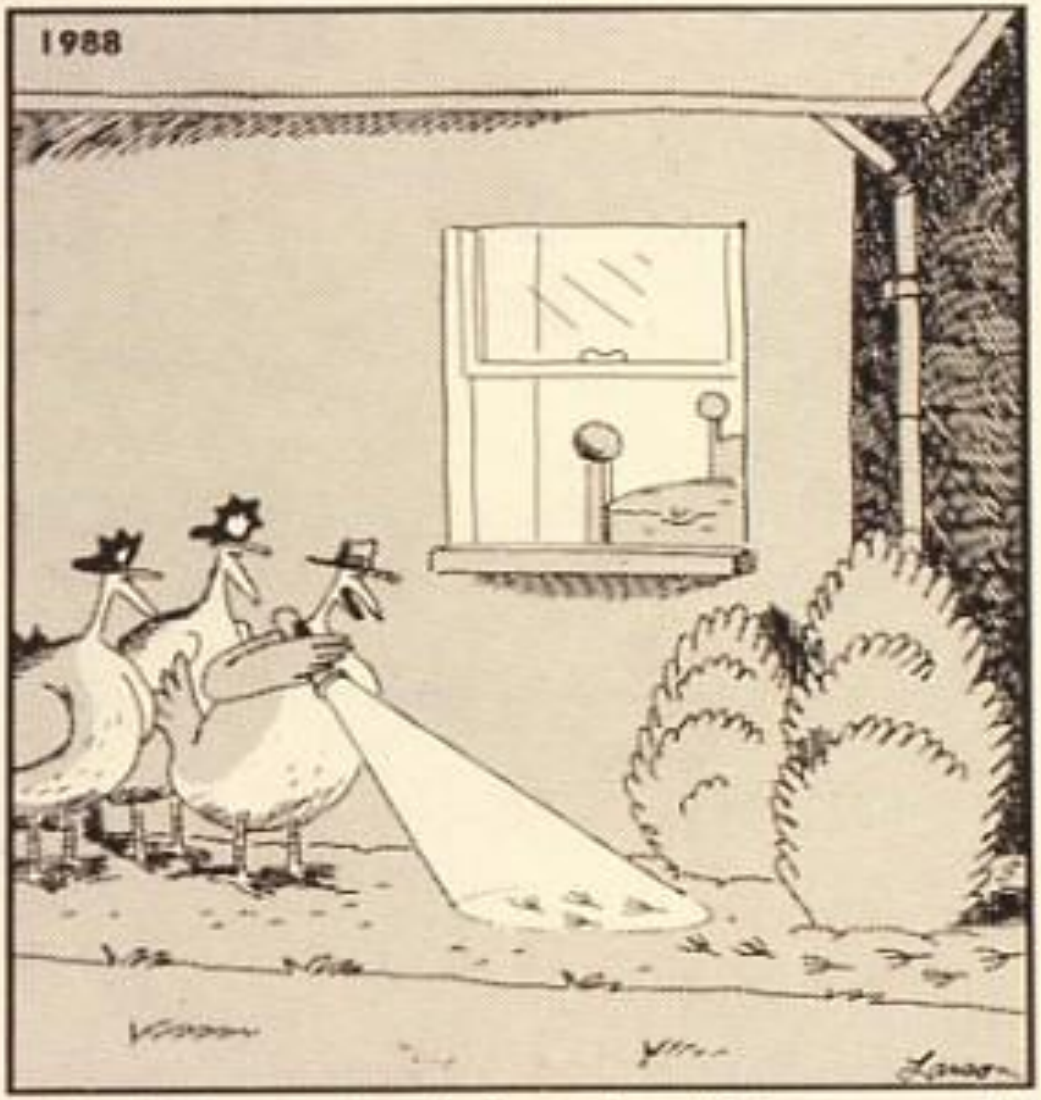
Amplification_Positive_MIDPCR	Identifiler_CODIS_v1.1		



Forensic DNA Analysis



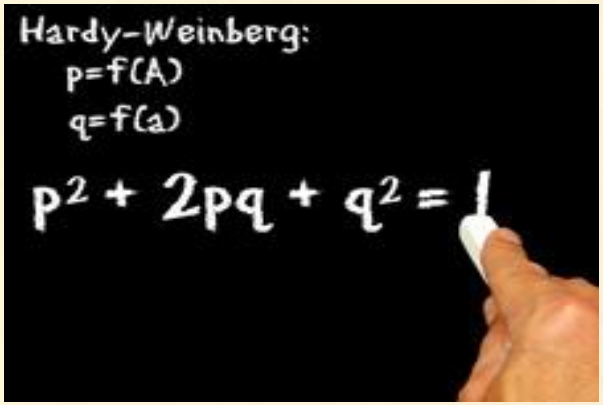
Forensics: Pattern Comparisons



"Aha! The murderer's footprints! 'Course, we all leave tracks like this."

The FAR SIDE

March 15 WEDNESDAY

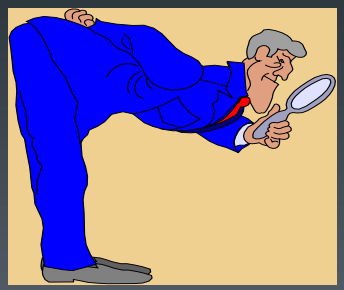


Forensic DNA Analysis

Evidentiary DNA profile(s) are generated from samples submitted to Forensic Lab.

Known profile(s) of suspect/victim (blood or buccal) are compared to DNA profiles from instant case.

Evidentiary profiles entered into CODIS database. Suspect's profile is not entered into CODIS database.

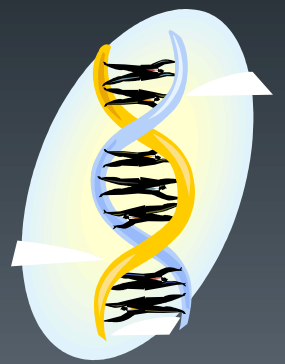


DNA MIXTURES

- Common in Forensic DNA testing.
  Sexual Assault samples-intimate swabs, clothing.

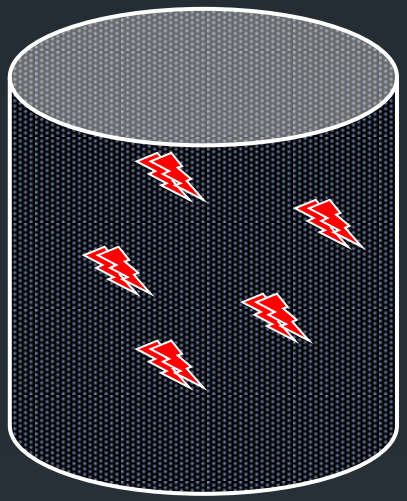
- Mixtures of victim & suspect(s).
  -How many people?
  -Previous consensual partners?
  -Contamination: scene, collection, lab?

- Mixture not always detected at all tests.

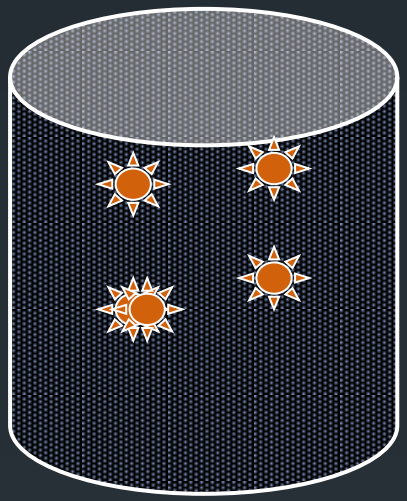


DNA Profile Detection

DNA Profile Detection



Profile A Detected

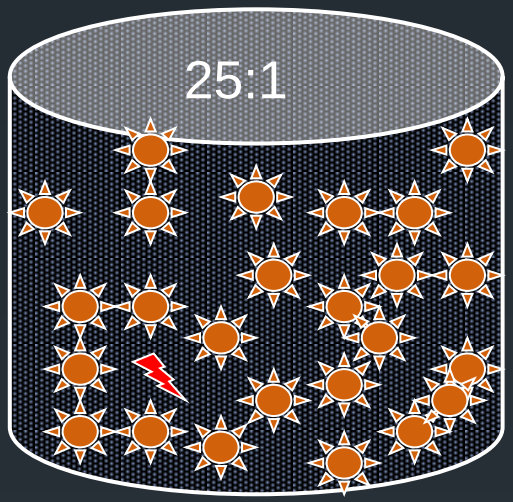


Profile B Detected

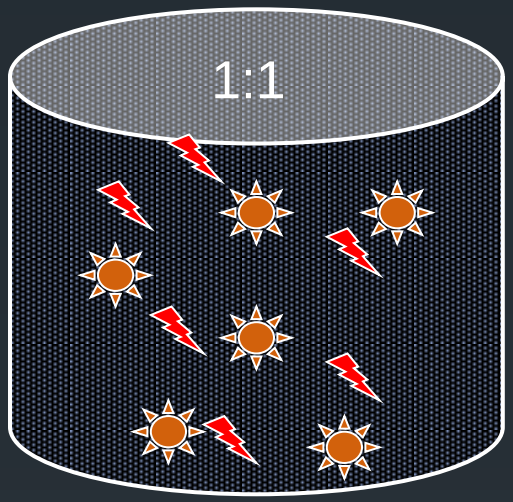
Factors:

1. Quantity of DNA
2. Quality of DNA

Mixture Detection?



Only Profile B Detected



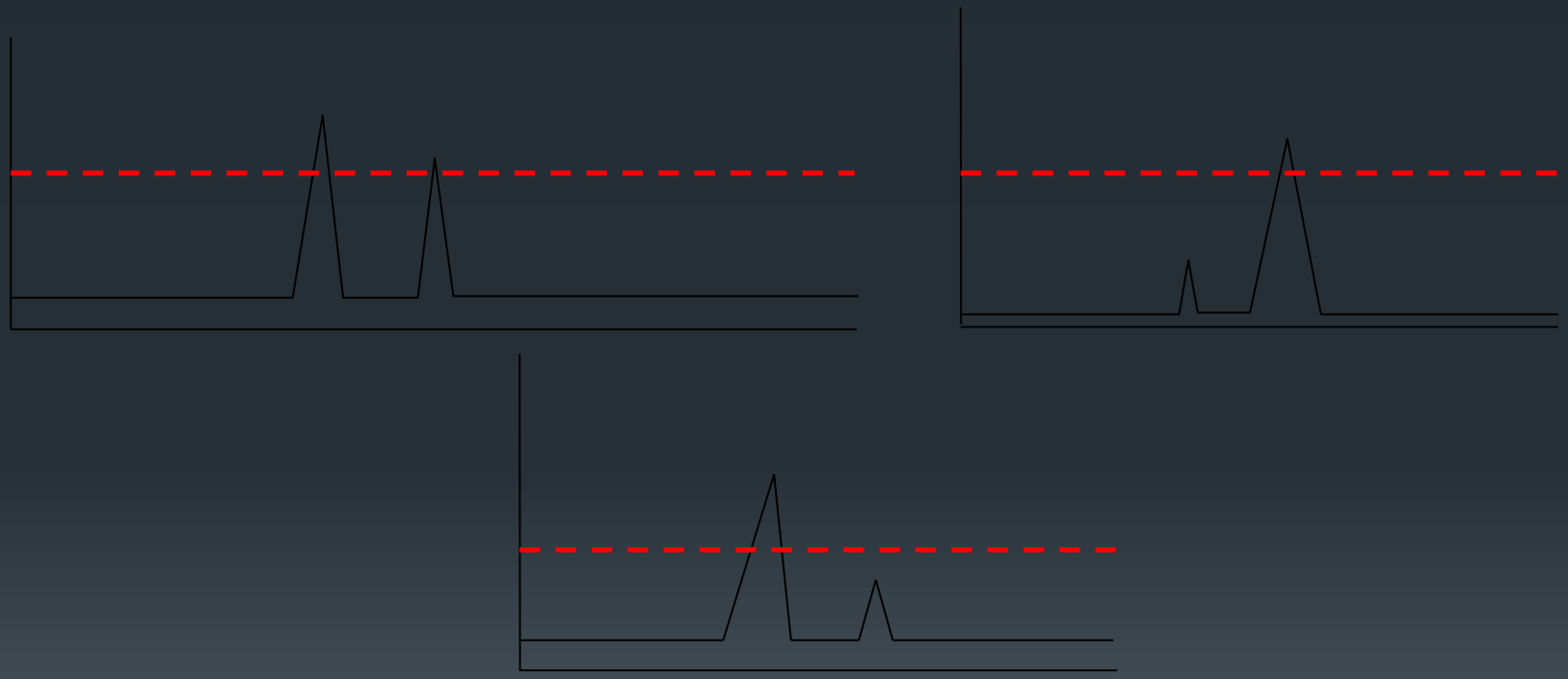
Profiles A and B Detected

Factors:

1. Quantity
2. Quality
3. Ratio

Stochastic Fluctuation

- Stochastic = chance.
- Result of PCR founder effect and chance allele sampling.



If you amplify small amounts of DNA (LCN PCR), can see stochastic effects.

The Meaning of a DNA Match?

1. Person A is the source of the DNA profile from the evidence.

2. The identical twin of person A is the source of the DNA profile.

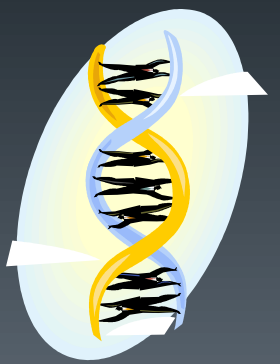
or

3. Another person who coincidentally has the same profile as person A is the source of the DNA profile from the evidence.

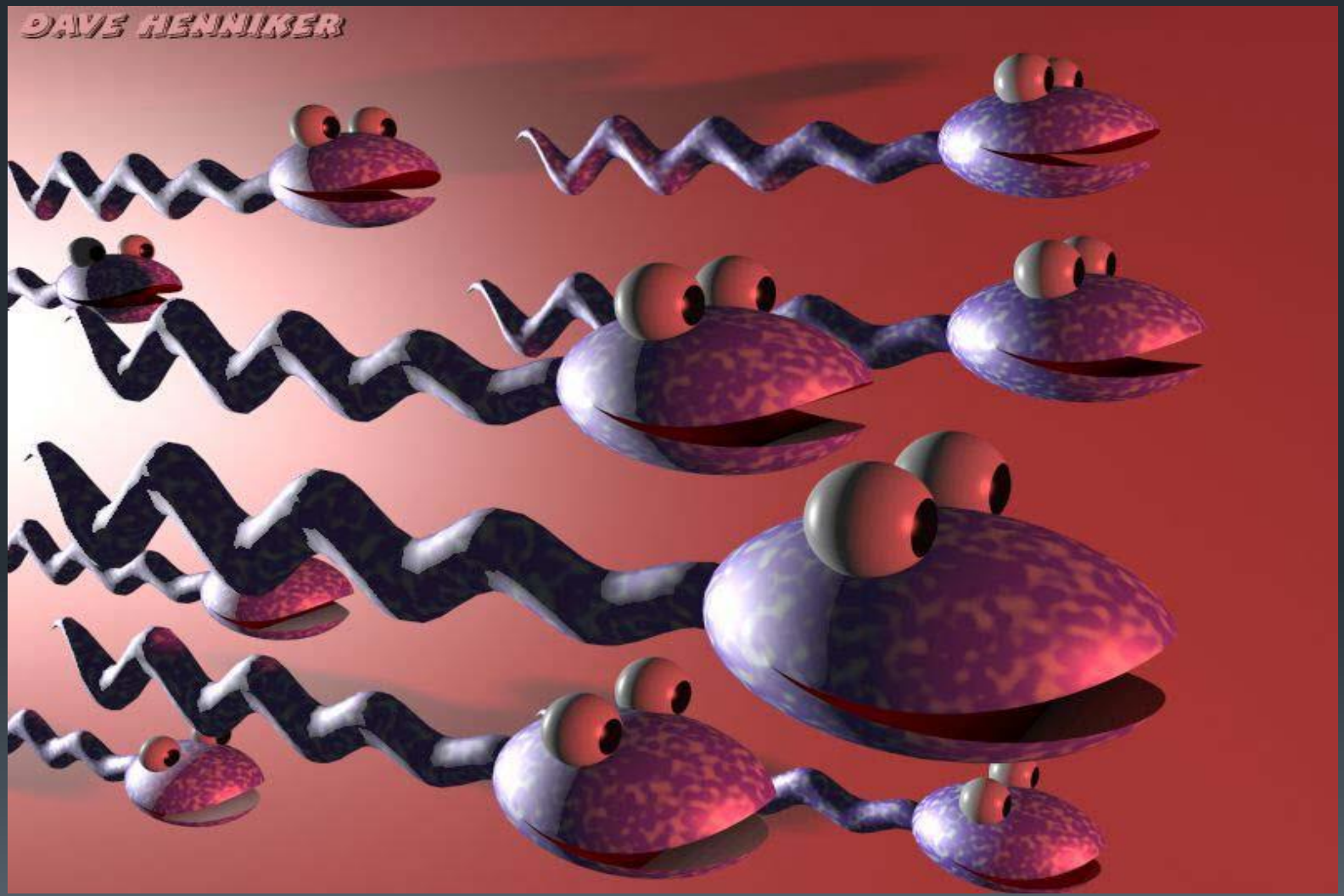
= the random match probability

DNA Conclusions

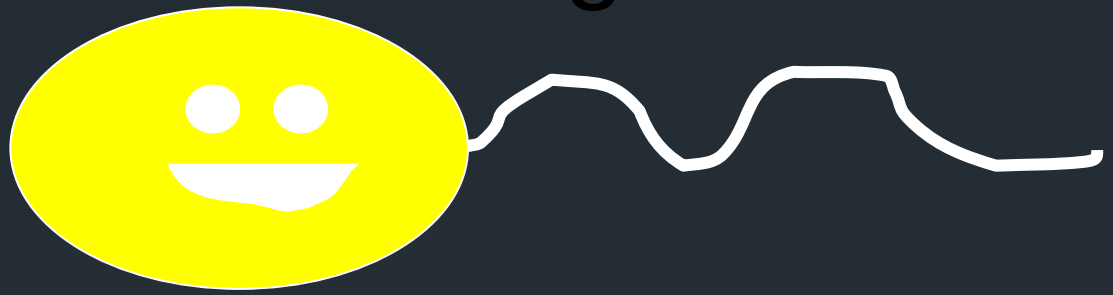
1. Included – source or contributor
2. Excluded – source or contributor
3. CBE – source or contributor
4. Inconclusive
5. Insufficient data



Y-DNA Typing



Y Chromosome Testing



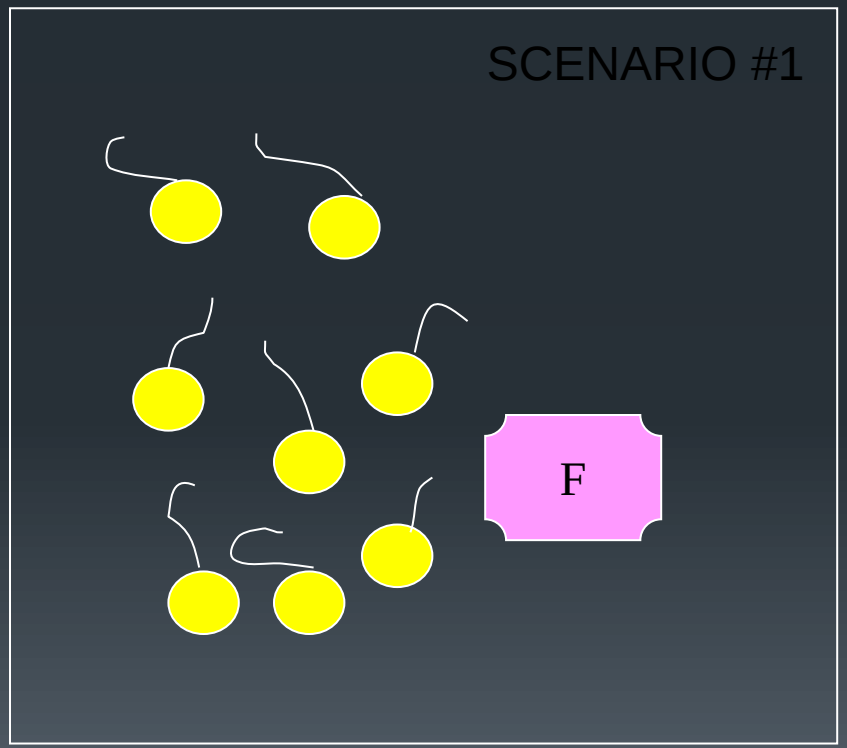
- Paternal inheritance.
- Detects male component of a mixture.
- Less discriminating than standard DNA testing. Statistics = counti
  (linkage).
- Important for detecting the semen donor in sexual assault mixtures.

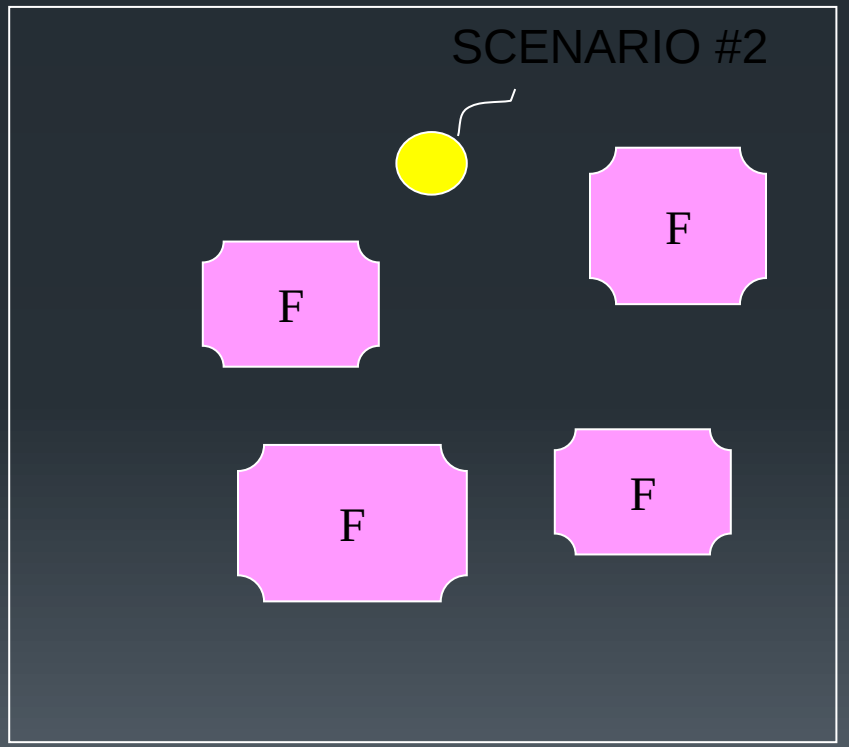
When to Use Y-STR Testing

- Sexual assaults by vasectomized or azoospermic males (no sperm left behind for differential extraction)
- Extending length of time after assault for recovery of perpetrator's DNA profile (greater than 48 hours)
- Male-female mixtures
- Other bodily fluid mixtures (blood-blood, skin-saliva)
- Gang rape situation to include or exclude potential contributors
- When you want to double the amount of DNA for the PCR Reaction.

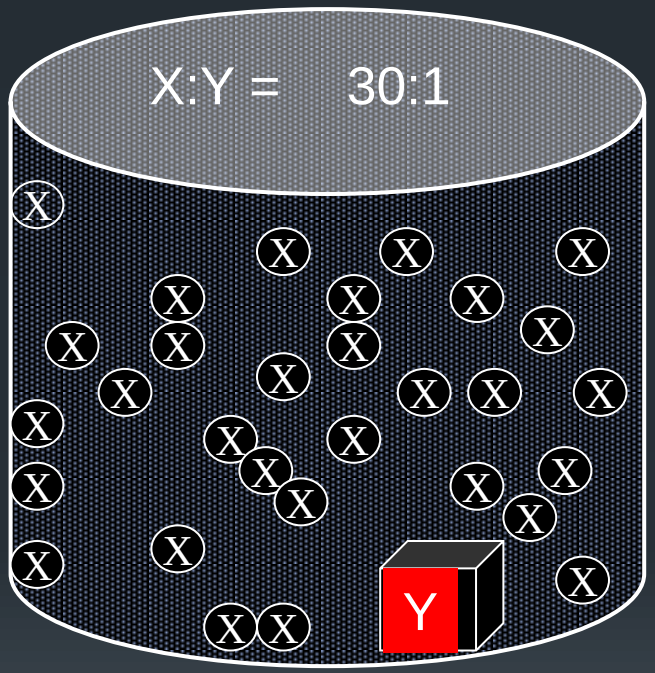
Y-STRs

"detects male component of a mixture"





Y-STRs



Y Profile Detected

Disadvantages of the Y-Chromosome

- Loci are not independent of one another and therefore rare random match probabilities cannot be generated with the product rule; must use haplotypes (combination of alleles observed at all tested loci)

- Paternal lineages possess the same Y-STR haplotype (barring mutation) and thus fathers, sons, brothers, uncles, and paternal cousins cannot be distinguished from one another

- Not as informative as autosomal STR results
  - **More like addition (10 + 10 + 10 = 30) than multiplication (10 x 10 x 10 = 1,000)**

Forensic Advantages of Y-STRs

- **Male-specific amplification** extends range of cases accessible to obtaining probative DNA results (e.g., fingernail scrapings, sexual assault without sperm)
- **Technical simplicity due to single allele profile**; can potentially recover results with lower levels of male perpetrator DNA because there is not a concern about heterozygote allele loss via stochastic PCR amplification; number of male contributors can be determined
- **Courts have already widely accepted STR typing**, instrumentation, and software for analysis (Y-STR markers just have different PCR primers)
- **Acceptance of statistical reports using the counting method** due to previous experience with mtDNA
- **Double the Genomic DNA** within the PCR Amplification reaction.

A Haplotype

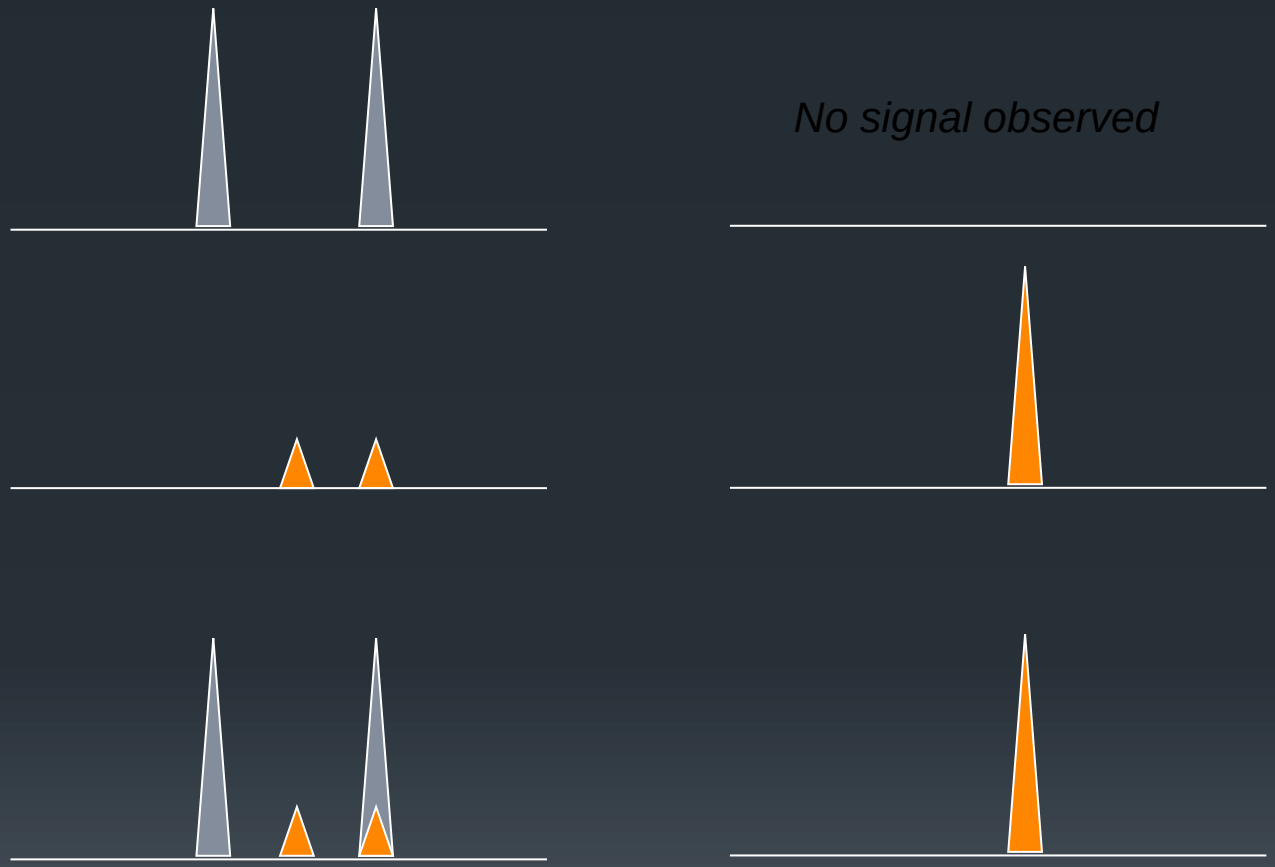
- Although 17 loci are typed
- They are linked and are treated as one “super” locus
- A haplotype essentially is an allele
- The more alleles at a locus, generally the lower the effect of substructure on statistical calculations

Y-STRs can permit simplification of male DNA identification in sexual assault cases

Female Victim DNA Profile

Male Perpetrator DNA Profile

DNA Profile from Crime Scene



Autosomal STR Profile

Y-Chromosome STR Profile

Butler, J.M. (2005) *Forensic DNA Typing, 2nd Edition,* Figure 9.2, ©Elsevier Science/Academic Press

$$p + 1.96\sqrt{\frac{(p)(1-p)}{N}}$$

$p^2 + 2pq + q^2 = 1$

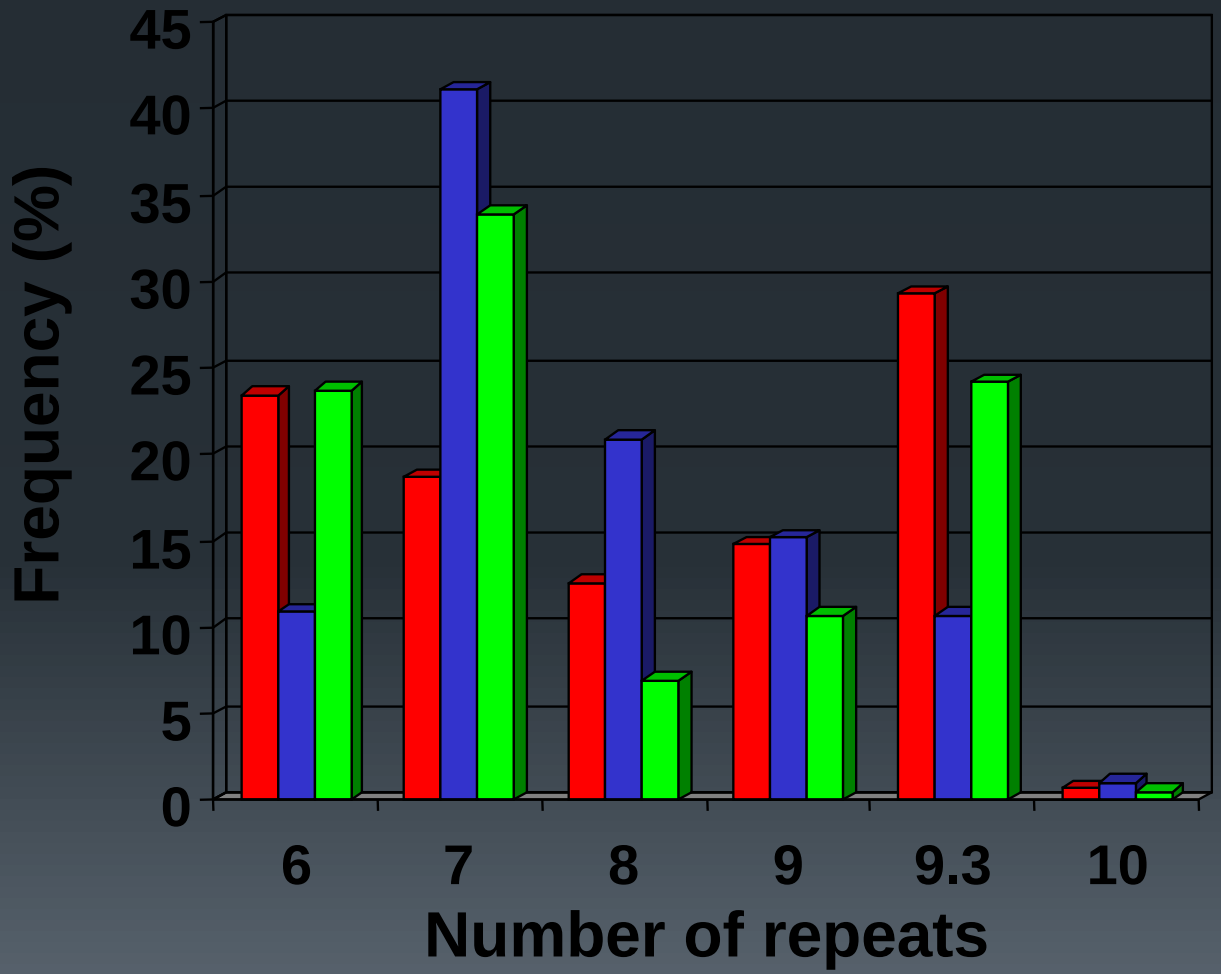
$1-\alpha^{1/N}$

Forensic DNA Statistics

AA + 2AB +2AC+ BB+ 2BC + CC = 1

P = .5 x .5 x .5 x .5 x .5 = 1/32

STR Allele Frequencies



Locus: TH01

- **Caucasians (N=427)**
- **Blacks (N=414)**
- **Hispanics (N=414)**

**Proc. Int. Sym. Hum. ID (Promega)* 1997, p. 34.

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2. The identical twin of person A is the source of the DNA profile.

or

3. Another person who coincidentally has the same profile as person A is the source of the DNA profile from the evidence.

= the random match probability

Random Match Probability

Is not:

Defense Fallacy.

A) Therefore, everyone else with the same genotype has an equal chance of being guilty.

B) Therefore, every possible genotype in a mixture has an equal chance of having committed the crime.

Random Match Probability

Is not:

Prosecutor's Fallacy.

A) There is only a 1 in 100 million chance that the DNA profile came from someone else.

B) There is only a 1 in 100 million chance that the defendant is not guilty.

RMP is not:

1. The probability that someone else is guilty.
2. The probability that someone else left the DNA.
3. The probability that the defendant is not guilty.