



FORENSIC DNA ANALYSIS

Richard Saferstein, Ph.D.

Biochemical Analysis

Analysis of Biological Fluids (usually dry stains), for use in the Criminal Justice System:

1. Blood
2. Semen
3. Saliva

Is it Blood?

Appearance

Chemical Tests

1. Kastle-Meyer
2. Luminol

Kastle-Meyer (Phenolphthalein)

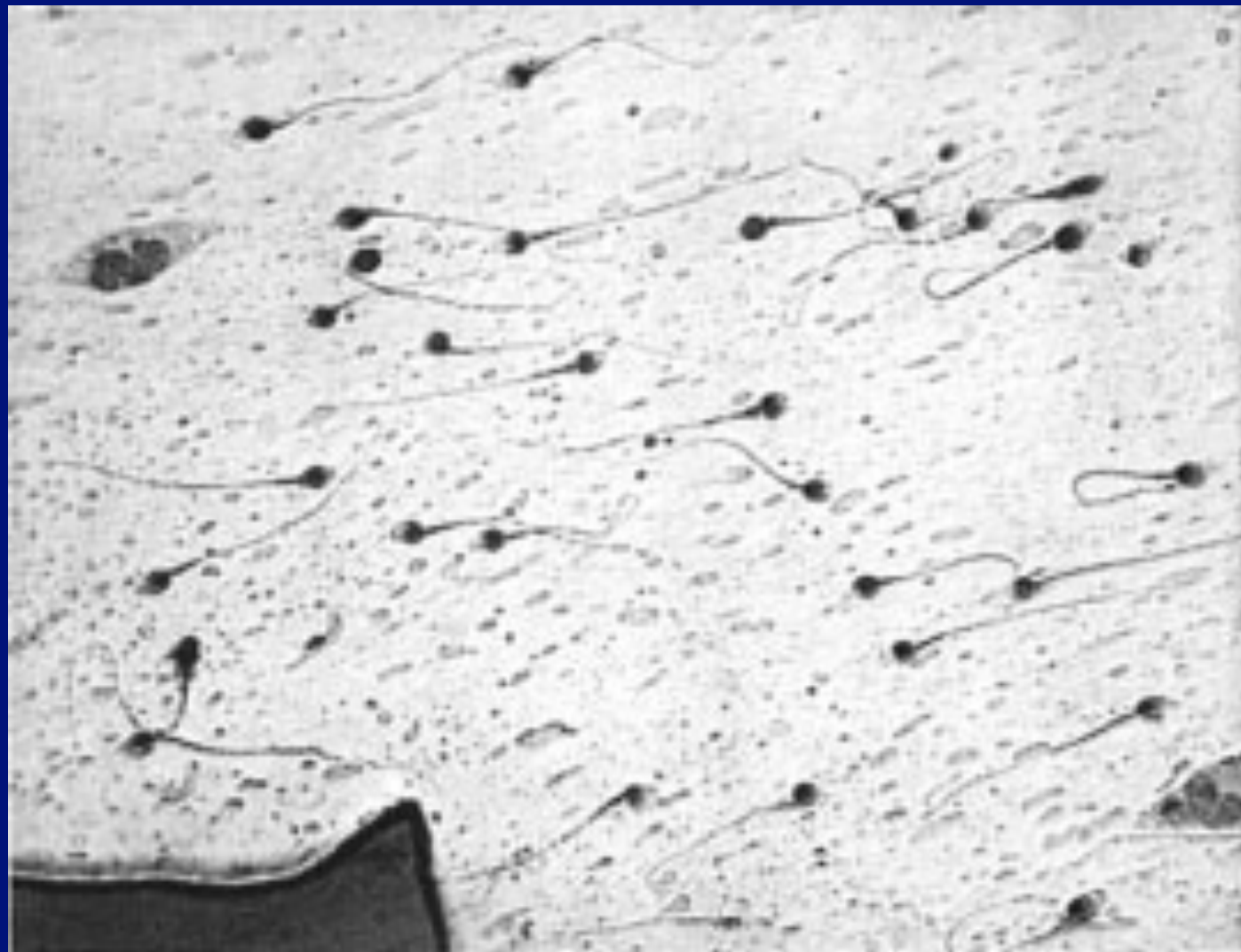
1. Simple color reaction
2. Sensitivity 1:10,000
3. False positives:
 - a. copper salts
 - b. Horseradish
 - c. strong oxidizing agents

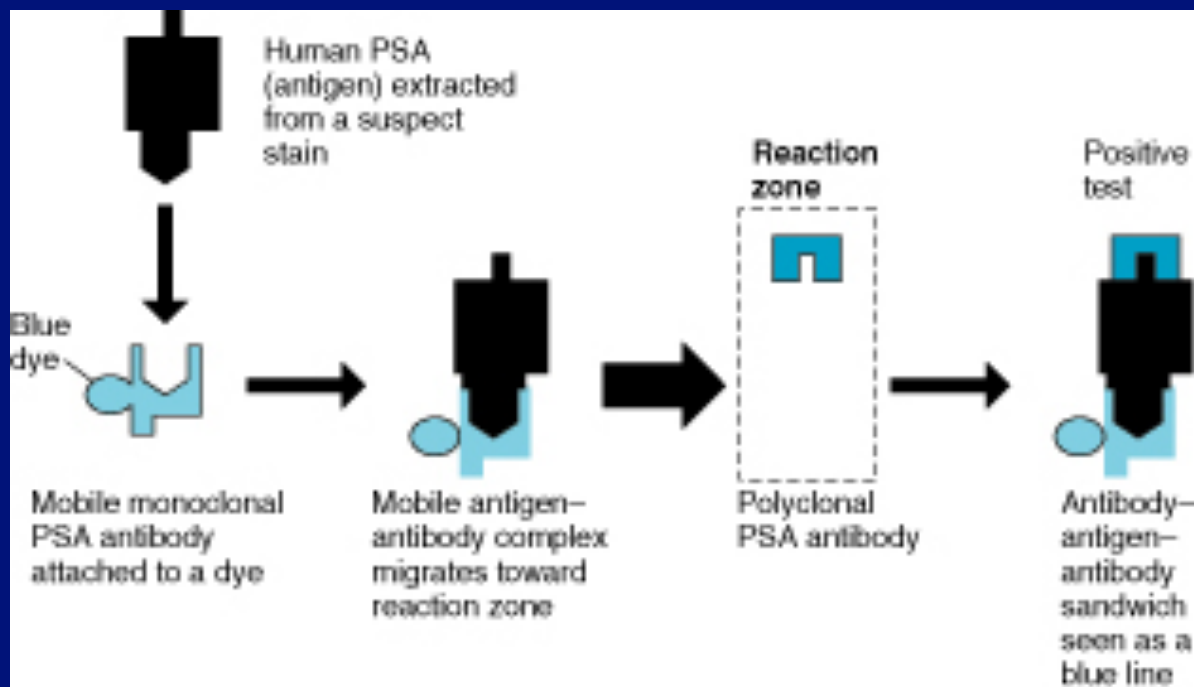
Luminol

1. Luminescent reaction
(Glow in the dark)
2. Sensitivity } 1:300,000
3. Useful for invisible bloodstains
4. False positive:
 - a. Metal
 - b. bleach

The Presumptive Tests are so sensitive that a negative result indicates that blood is absent in detectable amounts.

- Appearance and Texture
- Acid Phosphatase Test
- P30 Test
- Sperm Identification

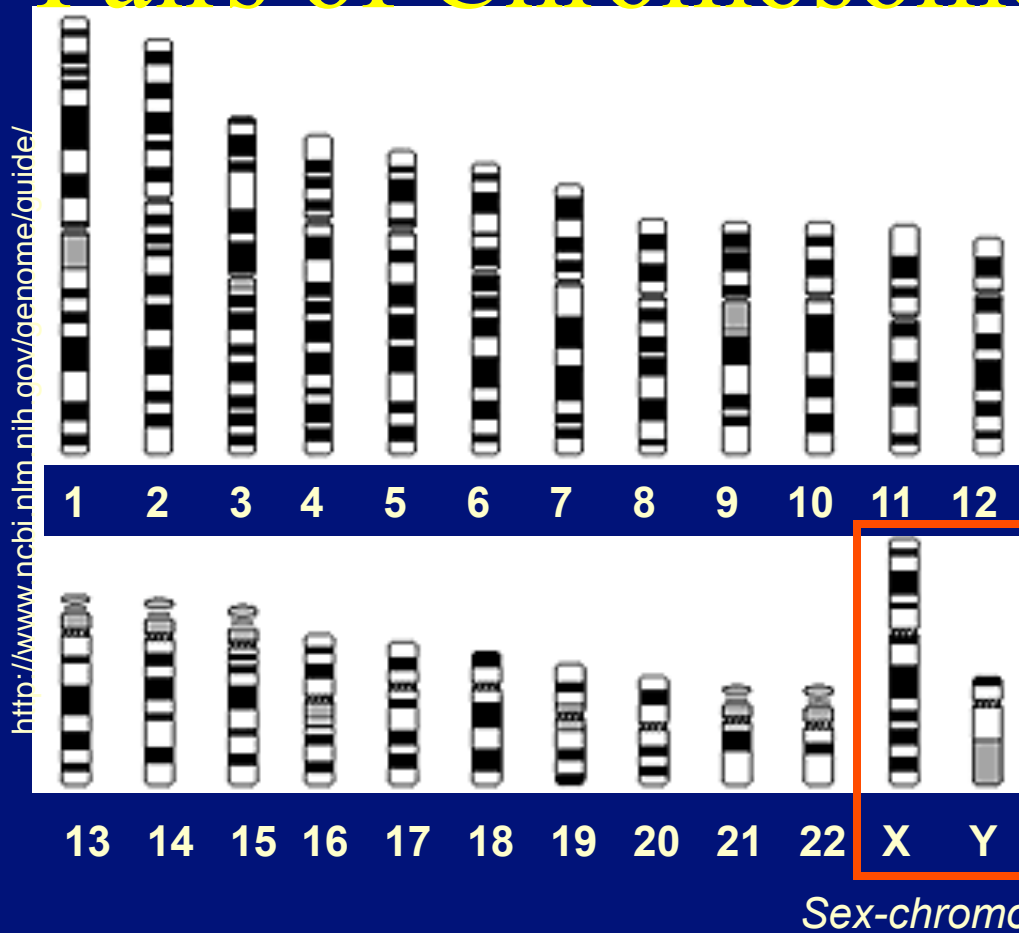


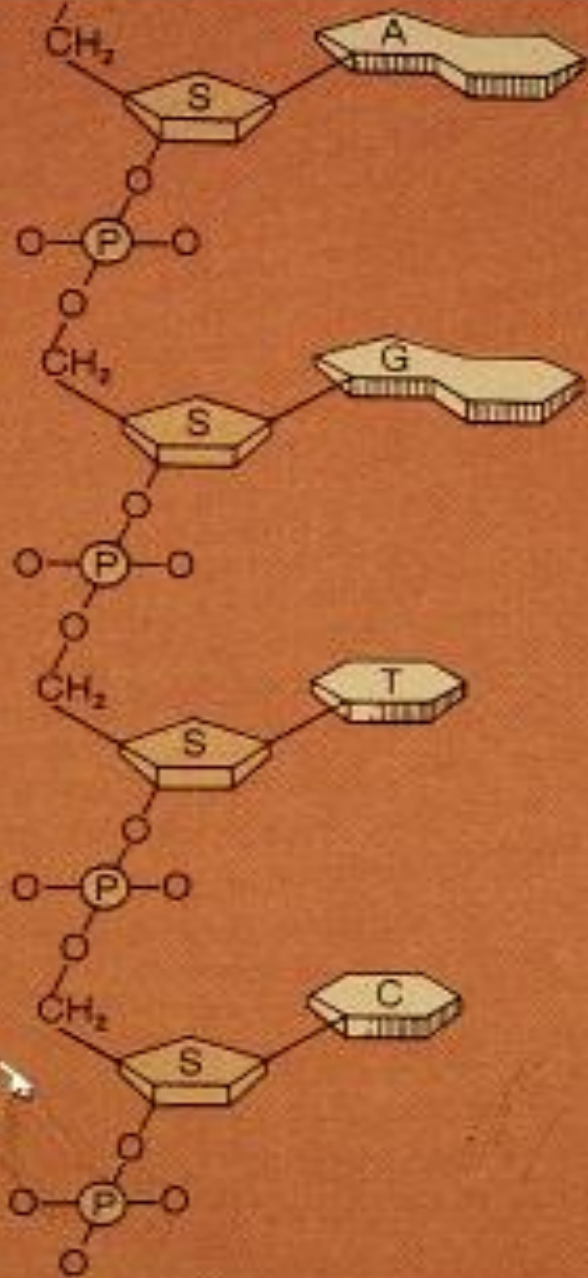


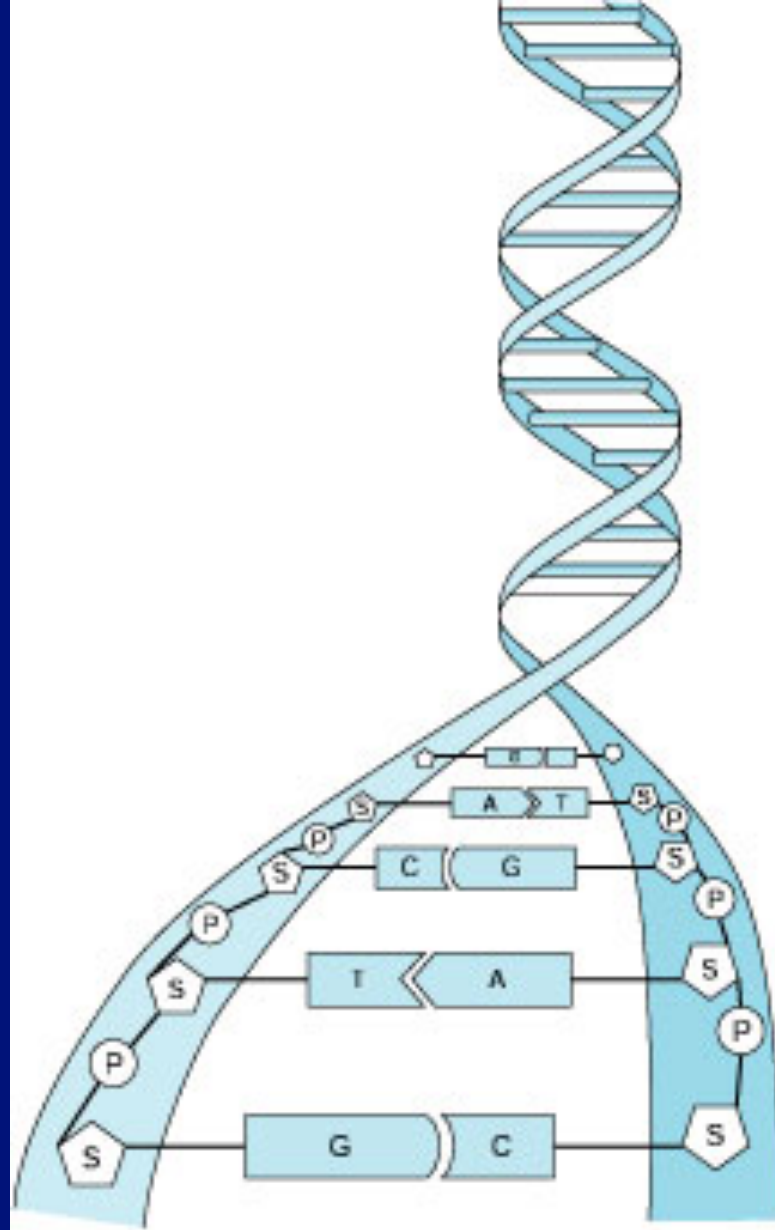
Brief History of DNA Typing

- 1980 - Ray White describes first polymorphic RFLP marker
- 1985 - Alec Jeffreys discovers multilocus 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

Human Genome Contains 23 Pairs of Chromosomes







DNA Structure

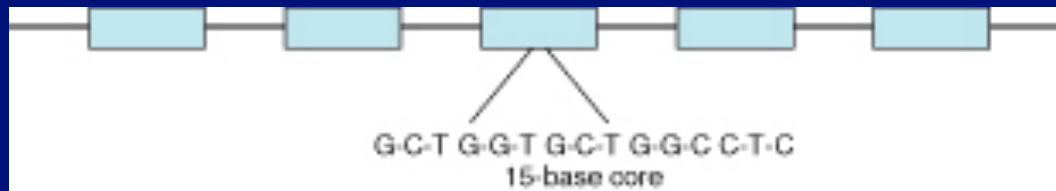
DNA is double stranded with specific pairing

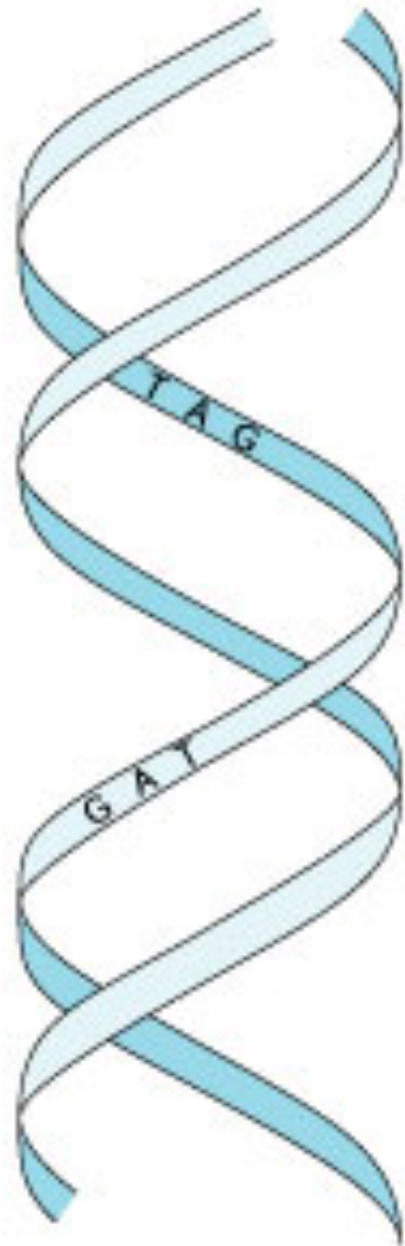
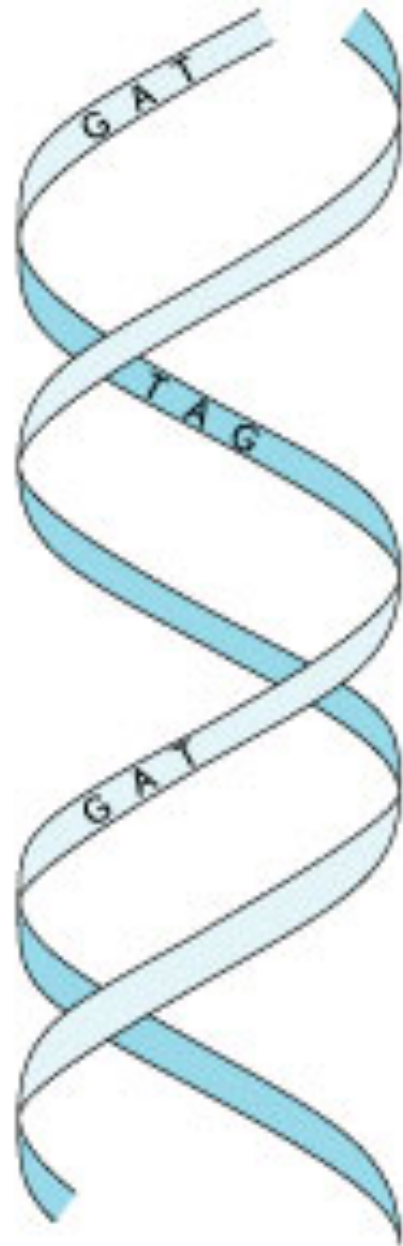
C-A-G-T-T-A-A-A-G-T-T-C-G-T-C-G-G-
G-T-C-A-A-T-T-T-C-A-A-G-C-A-G-C-C-

DNA Profiling Technology

- Restriction Fragment Length Polymorphism (RFLP)
- Polymerase Chain Reaction (PCR)
 - Traditional Short Tandem Repeats (STRs)
 - Y-STRs
- Mitochondrial DNA

RFLP





VNTR LOCUS

Tandem repeats determine size of fragment

Ex: core sequence is G C T G G T G C T G G C C G
(14 MER)

ALLELE A 100 repeats of core sequence

ALLELE B 1000 repeats of core sequence

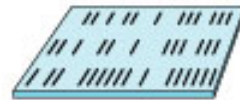
A B



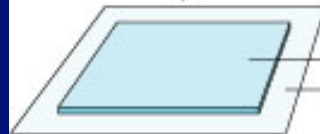
no. of repeats of core sequence determines polymorph



DNA is cut into fragments by a restriction enzyme

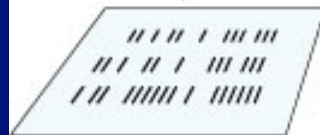


DNA fragments are separated into bands by electrophoresis; three rows of bands are shown to represent blood or semen samples from three sources

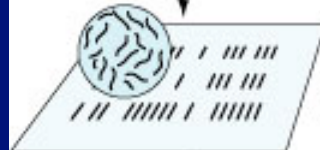


Gel
Membrane

The DNA band pattern on the electrophoretic gel is transferred to a nylon membrane



Nylon membrane with DNA fragments positioned exactly as they were on the gel



A radioactive DNA probe is added to the membrane where it binds to specific fragments

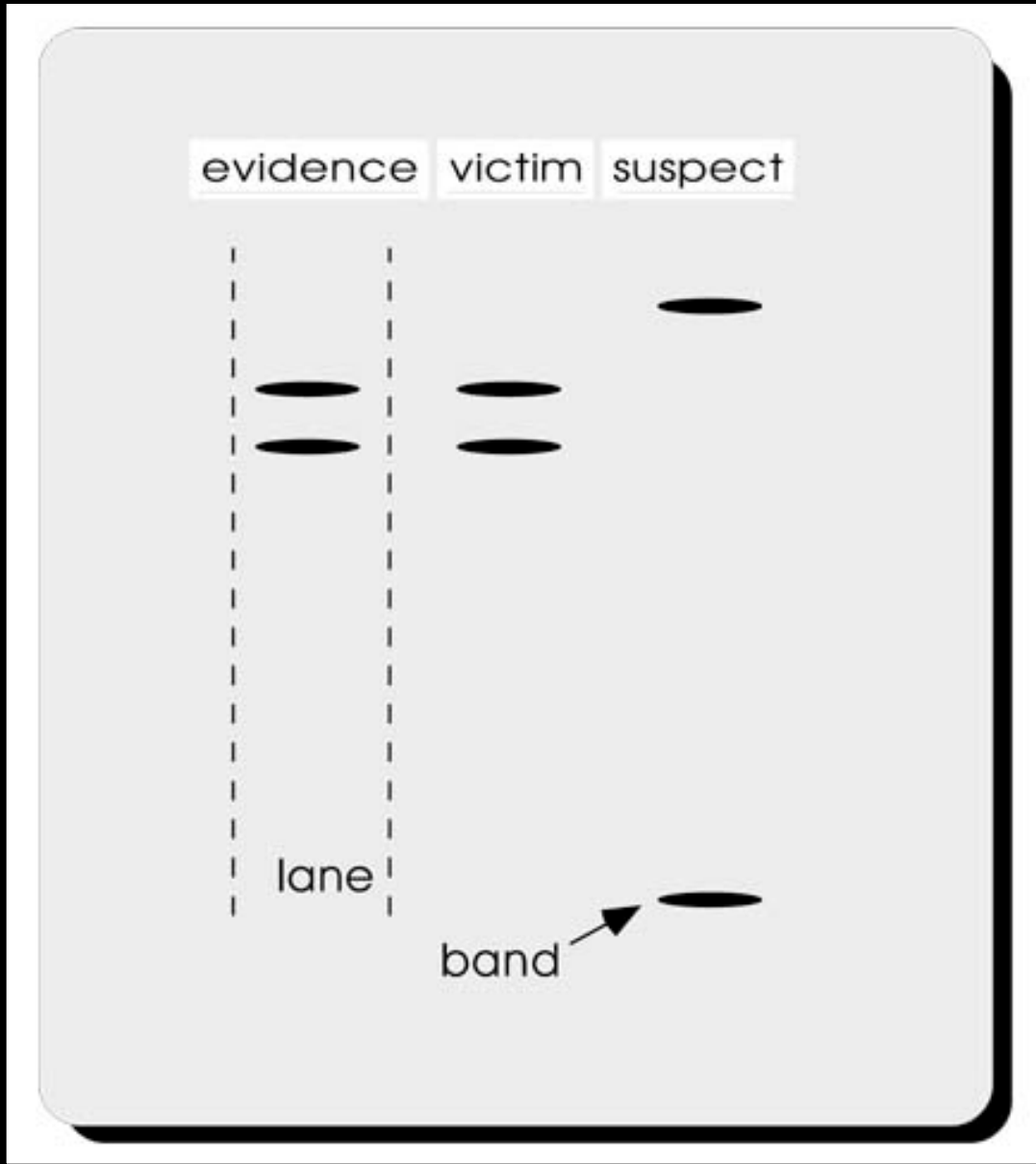


X-ray film
Membrane

X-ray film is placed next to the membrane to detect the radioactive pattern

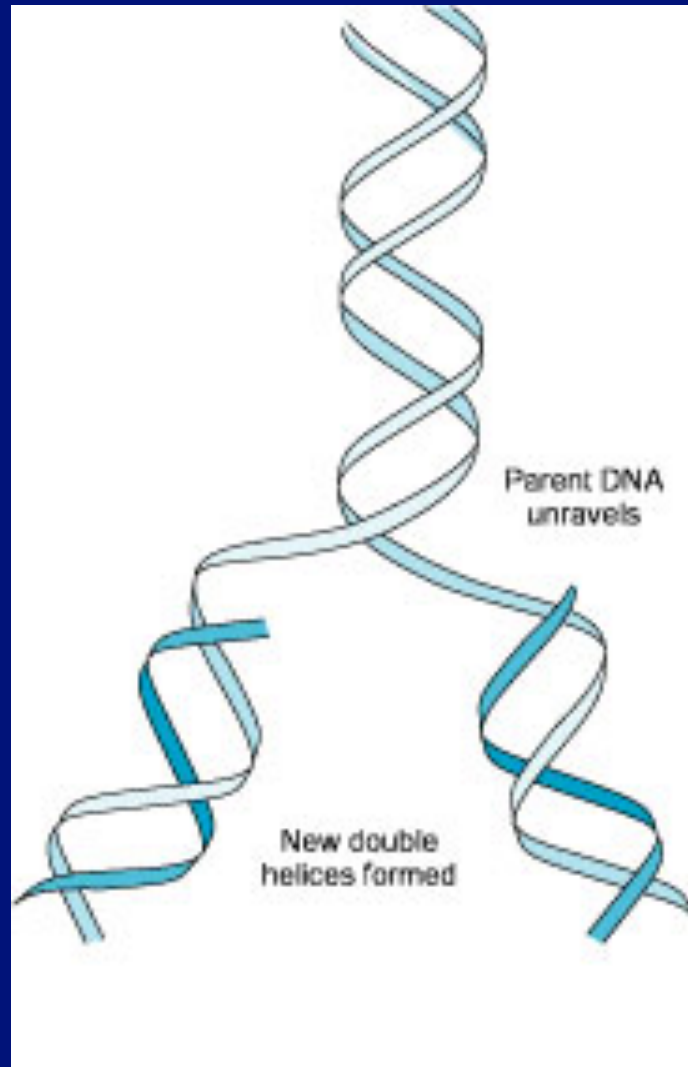


Developed X-ray film showing DNA fragments that combined with radioactive probe

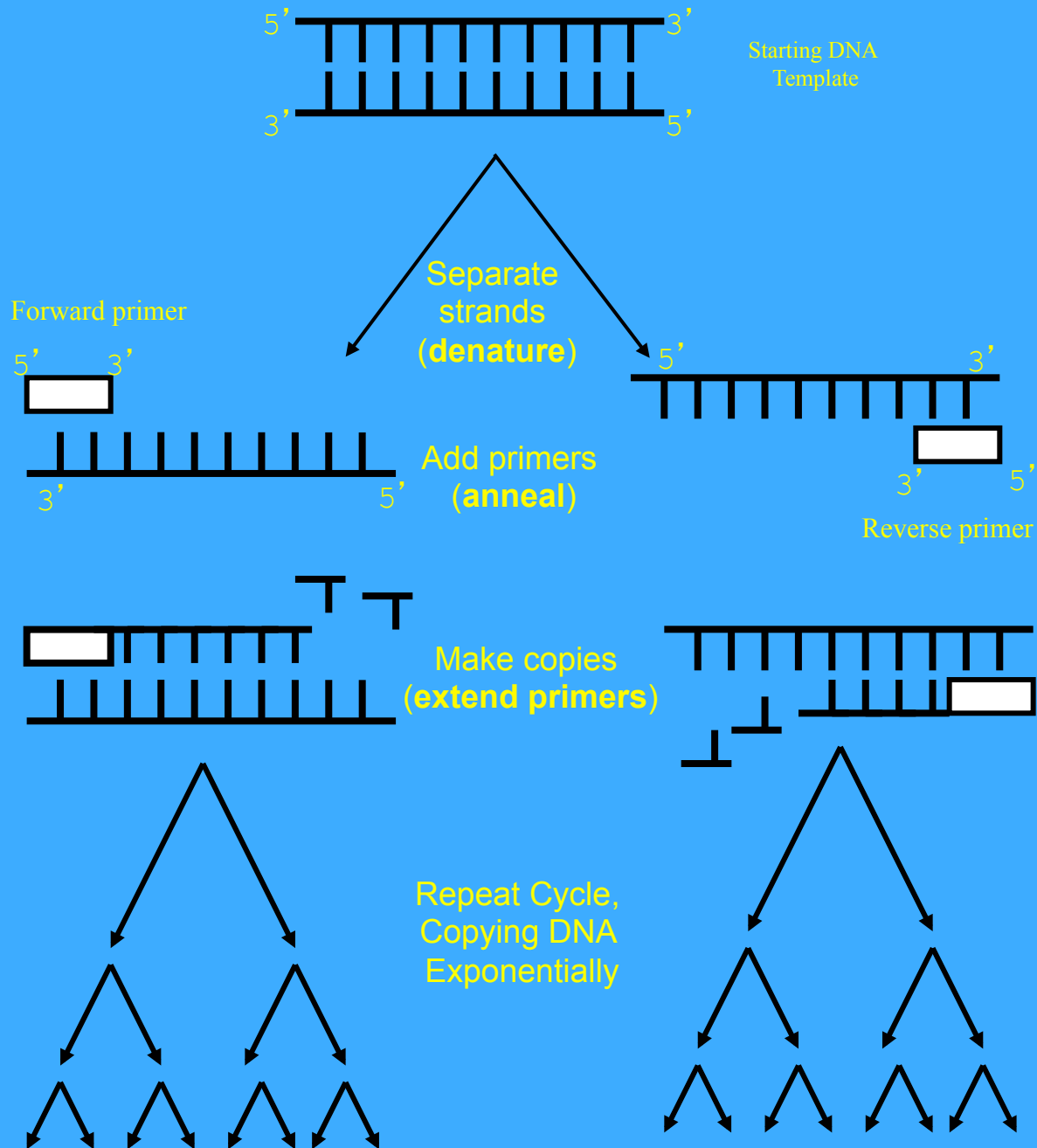


PCR

- Duplicates or amplifies a strand of DNA many millions of times.
- Sensitivity is 0.25-1.0 nanograms of DNA.
- One Hundred times more sensitive than RFLP.



PCR Process



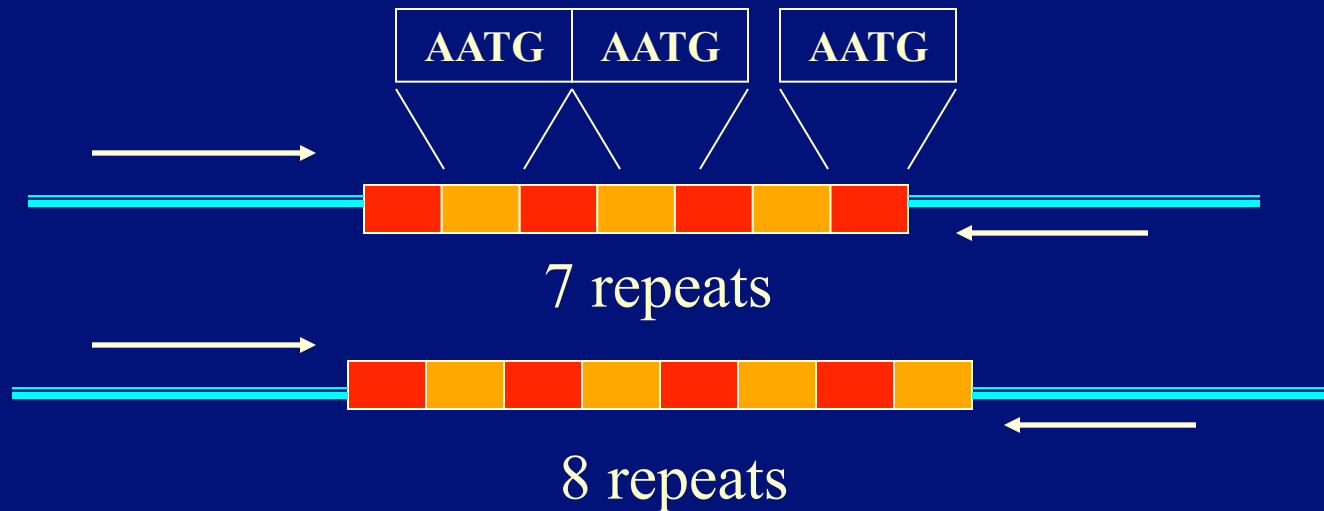
Number of Target Molecules Created

Cycle Number	Number of Double-stranded Target Molecules
1	0
2	0
3	2
4	4
5	8
6	16
7	32
8	64
9	128
10	256
11	512
12	1024
13	2048
14	4096
15	8192
16	16,384
17	32,768
18	65,536
19	131,072
20	262,144
21	524,288
22	1,048,576
23	2,097,152
24	4,194,304
25	8,388,608
26	16,777,216
27	33,544,432
28	67,108,864
29	134,217,728
30	268,435,456
31	536,870,912
32	1,073,741,824

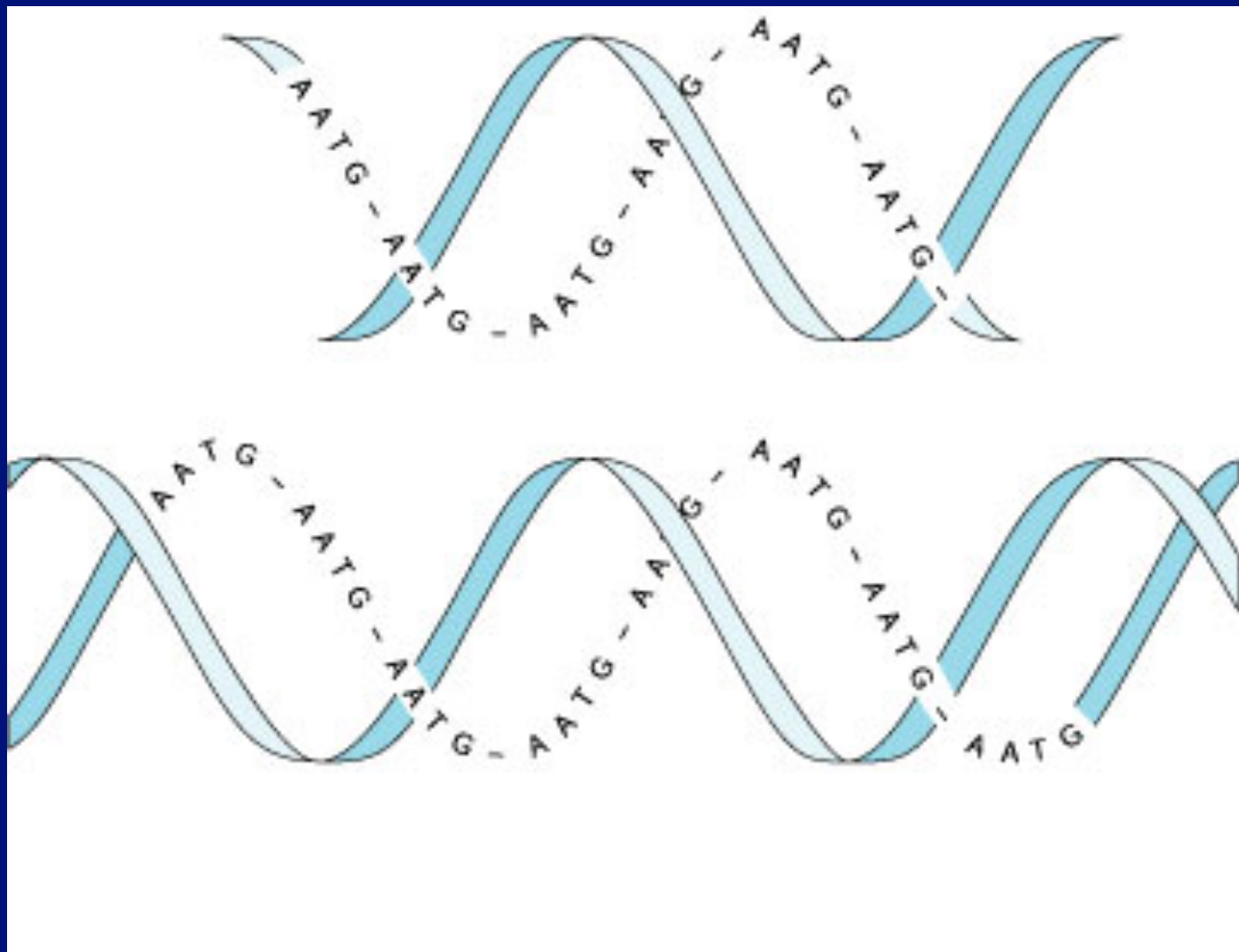
Where does low copy number start?

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

Short Tandem Repeats (STRs)



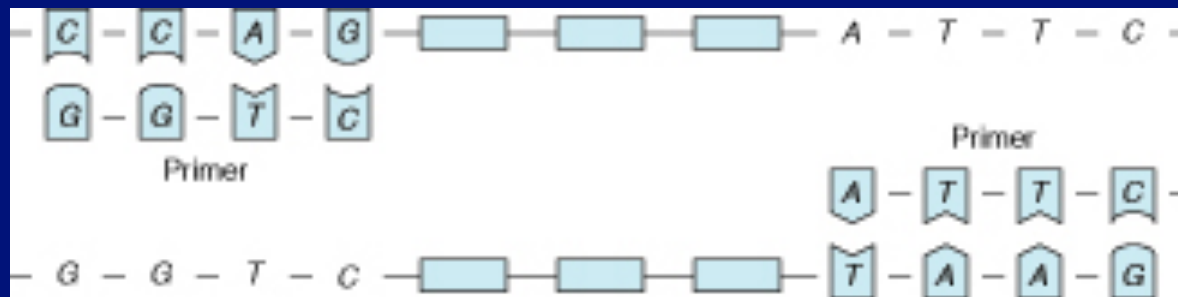
the repeat region is variable between samples while the flanking regions where PCR primers bind are constant

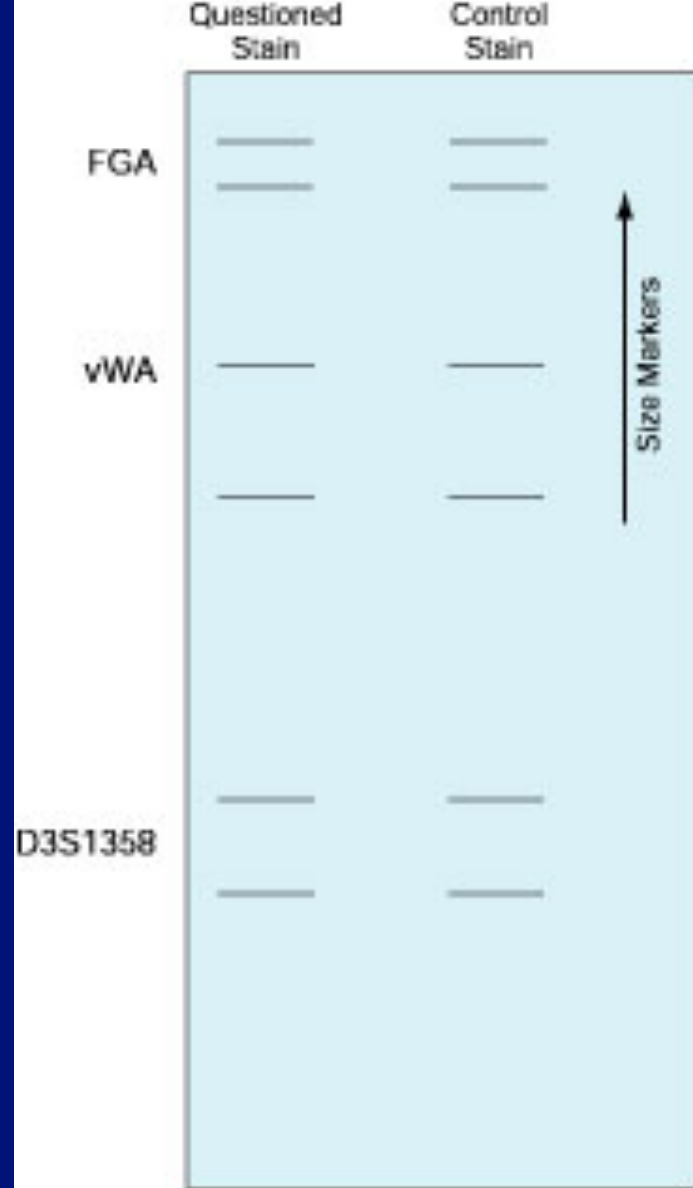




Multiplex PCR

- 15 Markers Can Be Copied at Once
- Sensitivities to levels less than 1 ng of DNA
- Ability to Handle Mixtures and Degraded Samples

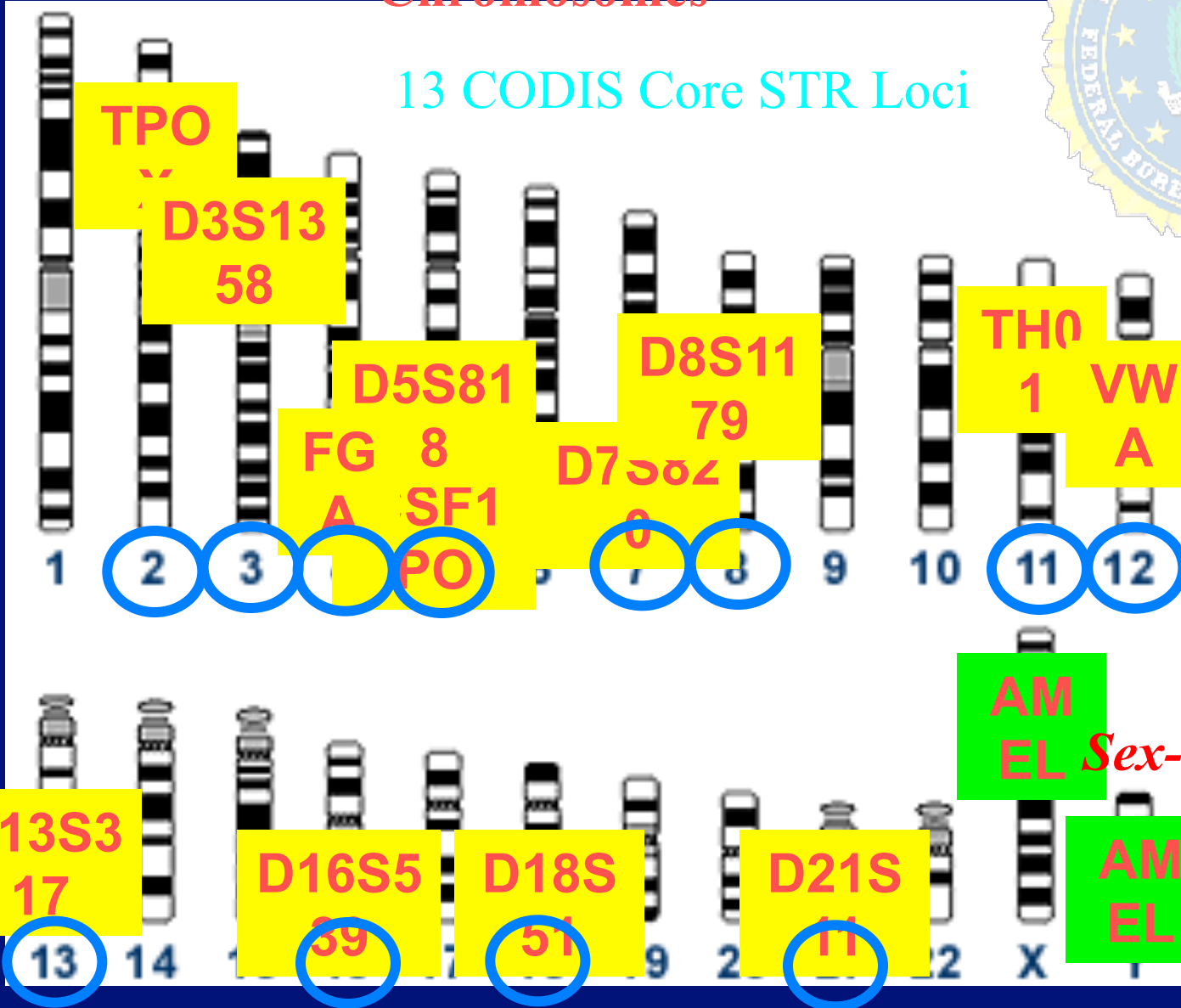




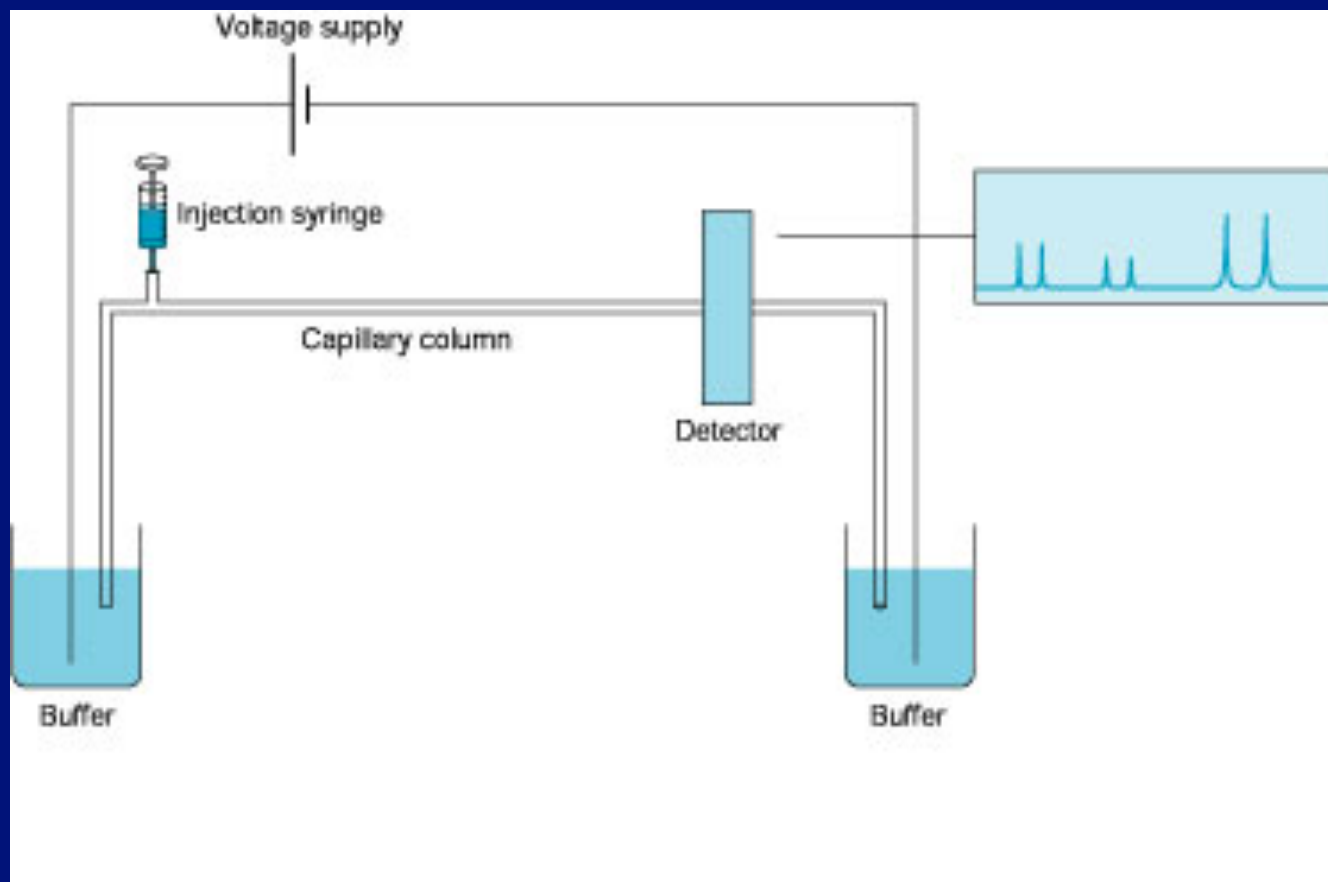
Position of Forensic STR Markers on Human Chromosomes



13 CODIS Core STR Loci

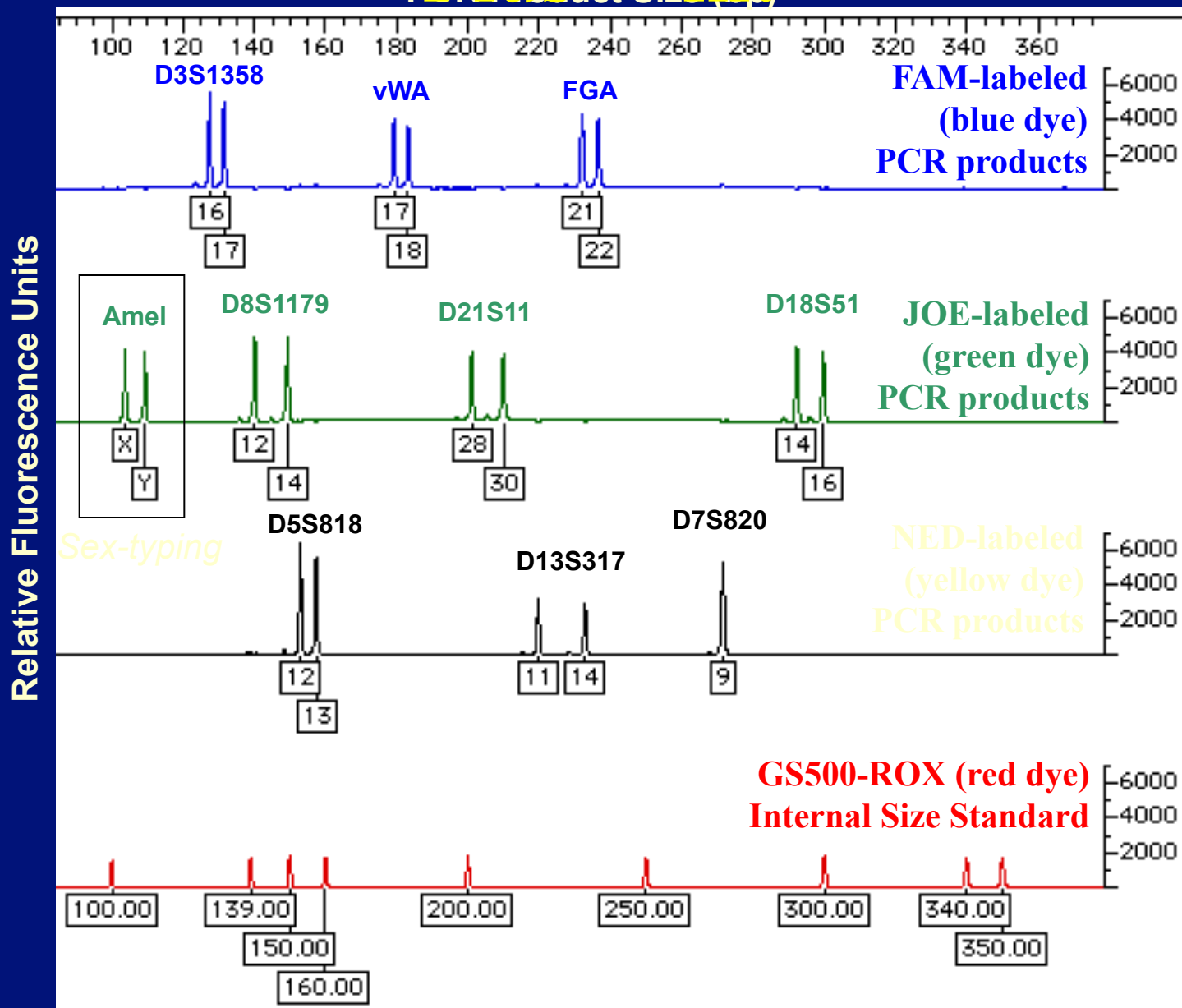


Sex-typing



Genotype Results with Profiler

Plus™ kit



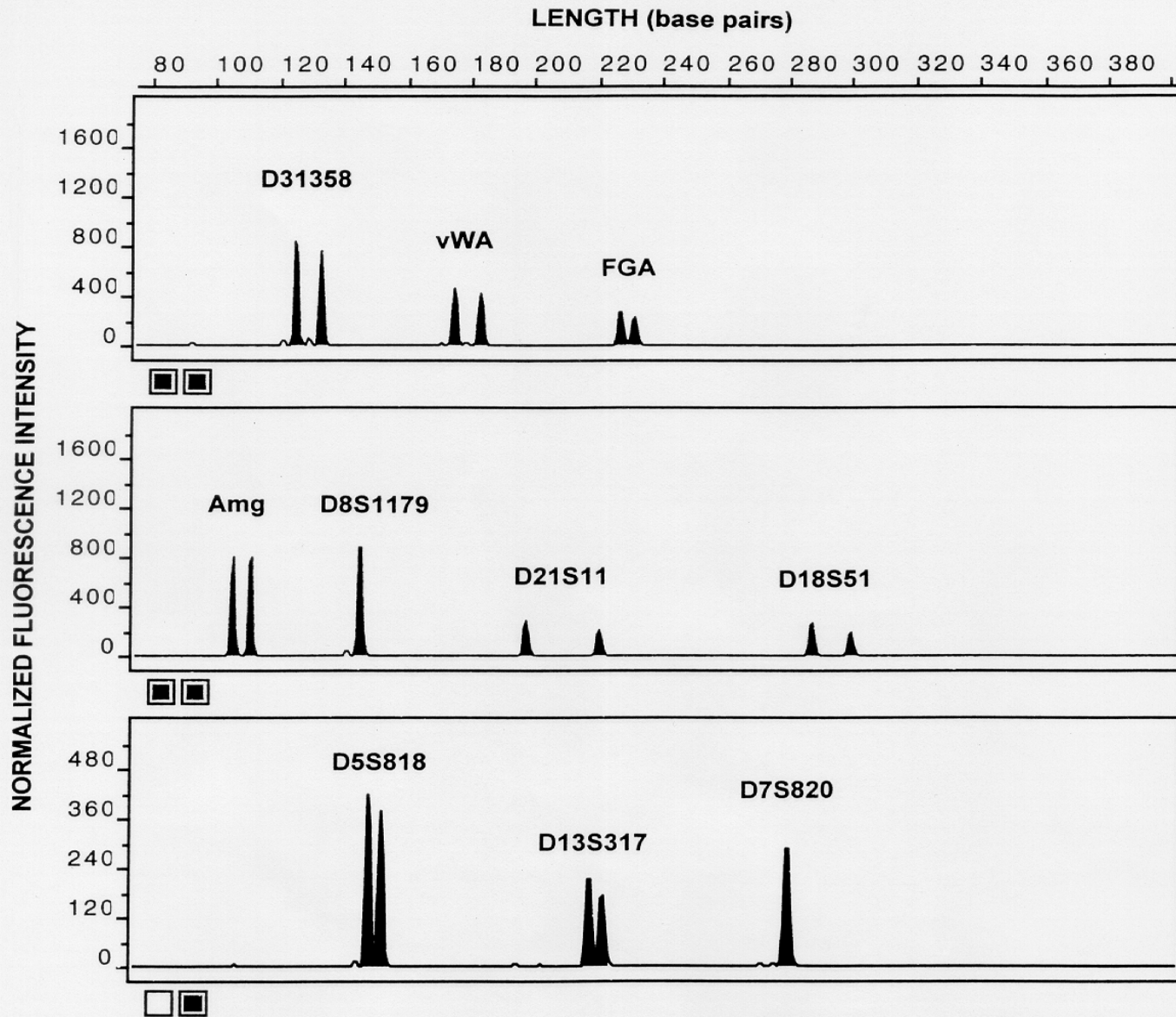


Figure 4: Example of a typical, full DNA profile. Generated from subject A after 10 seconds of handling the plastic knife handle.

STR Advantages

- Short strands of base pair repeats allows for the typing of degraded DNA.
- PCR-based
 - good for minimal samples
- Routine analysis of 13 different STR's
 - allows for the individualization of biological evidence
- Easy to interpret mixtures
- Easy to automate
 - high throughput

The 13 CODIS STRs and Their Probability of Identities

STR	African-American	U.S. Caucasian
D3S1358	0.097	0.080
vWA	0.074	0.068
FGA	0.036	0.041
THO1	0.114	0.080
TPOX	0.091	0.207
CSF1PO	0.079	0.128

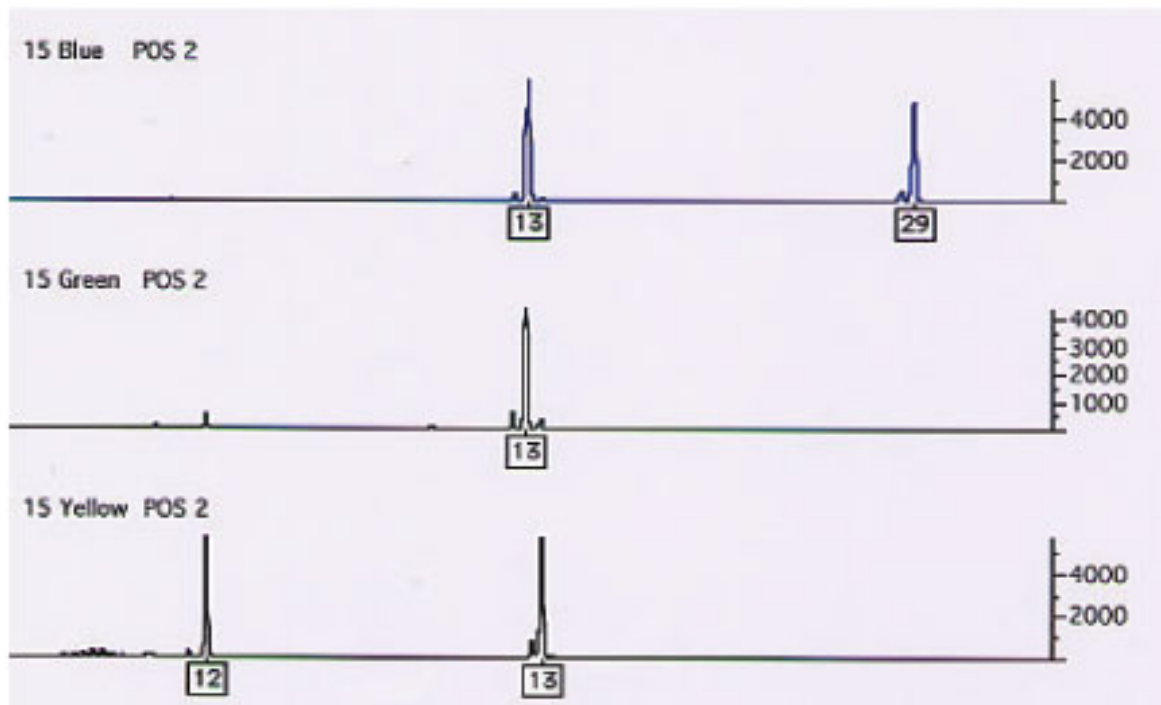
The 13 CODIS STRs and Their Probability of Identities...(cont' d)

STR	African-American	U.S. Caucasian
D5S818	0.121	0.166
D13S317	0.139	0.081
D7S820	0.087	0.067
D8S1179	0.080	0.069
D21S11	0.042	0.041
D18S51	0.032	0.032
D16S539	0.076	0.091

Evidence	Possible Location of DNA on the Evidence	Source of DNA
Baseball bat or similar weapon	Handle, end	Sweat, skin, blood, tissue
Hat, bandanna, or mask	Inside	Sweat, hair, dandruff
Eyeglasses	Nose or ear pieces, lens	Sweat, skin
Facial tissue, cotton swab	Surface area	Mucus, blood, sweat, semen, ear wax
Dirty laundry	Surface area	Blood, sweat, semen
Toothpick	Tips	Saliva
Used cigarette	Cigarette butt	Saliva
Stamp or envelope	Licked area	Saliva
Tape or ligature	Inside/outside surface	Skin, sweat

Evidence	Possible Location of DNA on the Evidence	Source of DNA
Bottle, can, or glass	Sides, mouthpiece	Saliva, sweat
Used condom	Inside/outside surface	Semen, vaginal or rectal cells
Blanket, pillow, sheet	Surface area	Sweat, hair, semen, urine, saliva
"Through and through" bullet	Outside surface	Blood, tissue
Bite mark	Person's skin or clothing	Saliva
Fingernail, partial fingernail	Scrapings	Blood, sweat, tissue

Y-PLEX™5 Positive Control



Common Sources of Touch DNA

From Hands:

Gloves

Knife handles

Weapon handles

Firearm grips

Plastic bag handles

Steering Wheels

Rope

Shoe laces

Electrical cords

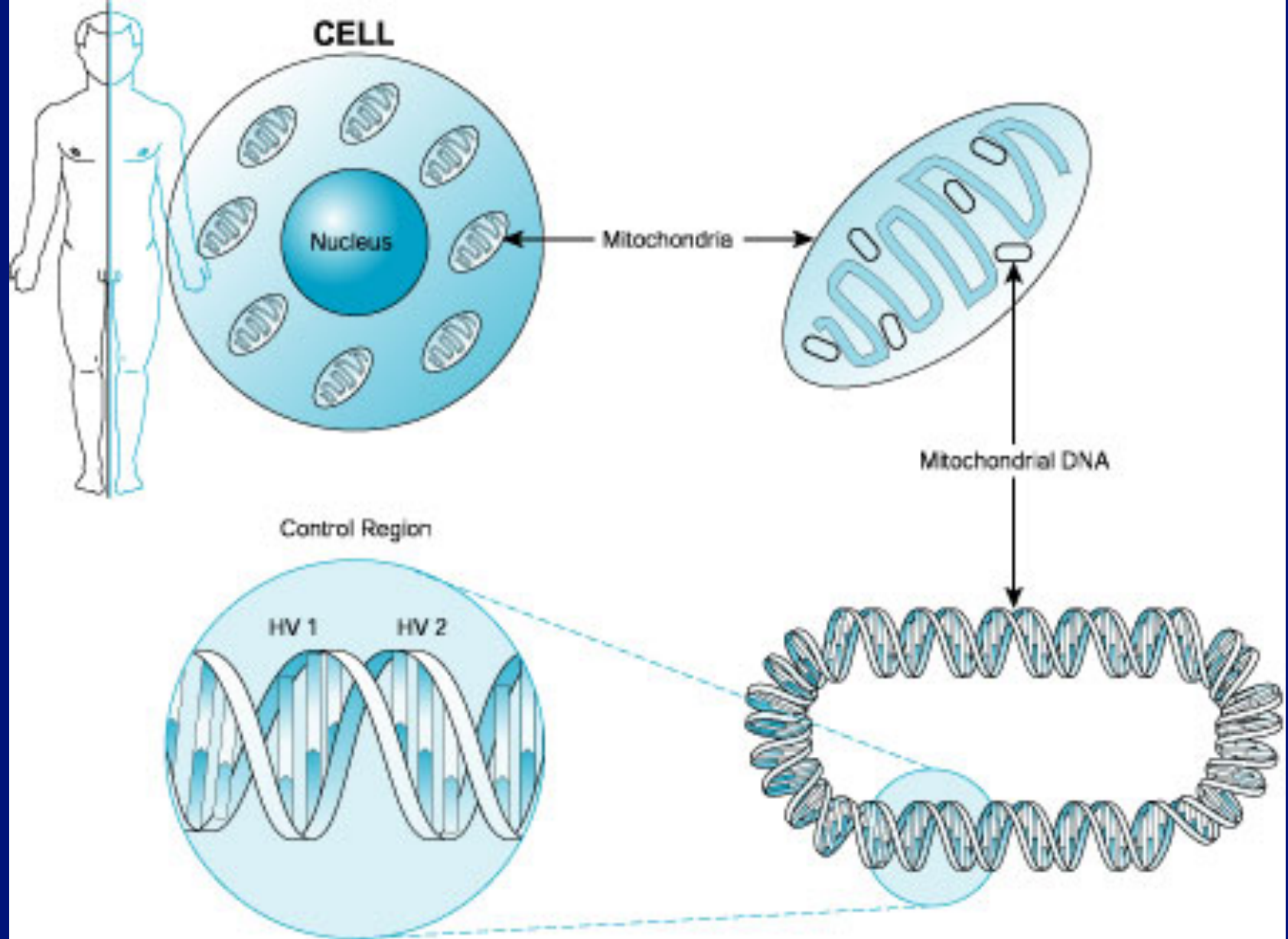


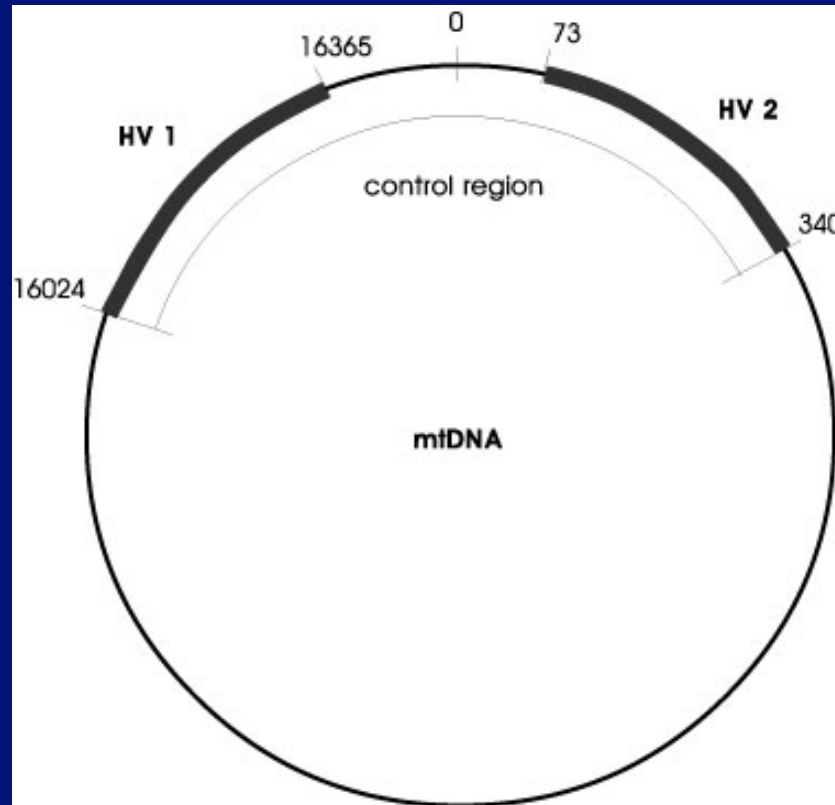
DNA ISSUES-LCN

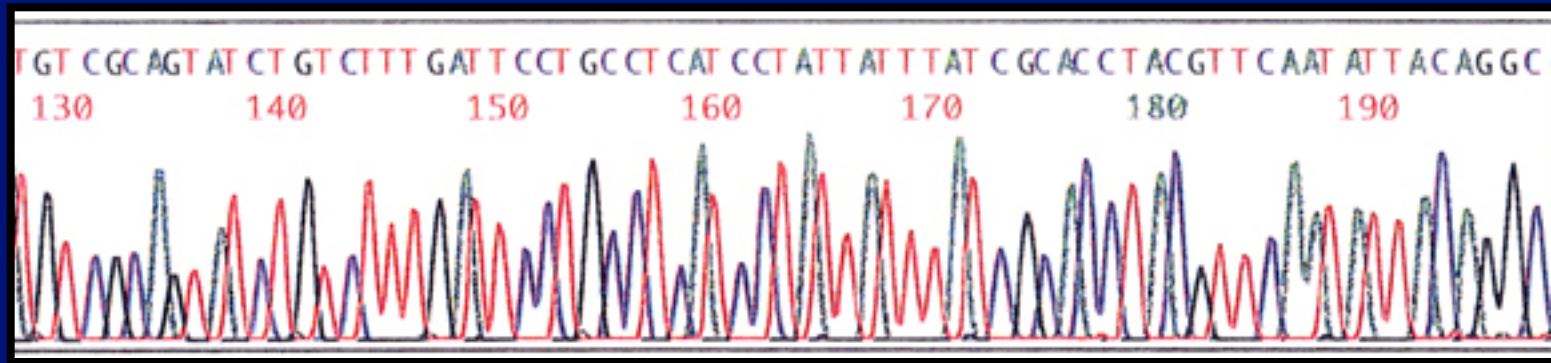
- Less than 100 nanograms DNA
- Allelic dropin
- Allelic dropout
- Peak Imbalance
- Replicate amplifications required

Substrate Control Issues

Failure to analyze substrate controls
in situations involving casual and
intimate contact







HAIR COMPARISONS REQUIRE A DNA DETERMINATION

Eleven Percent of Microscopic Hair Comparisons Conducted at the FBI Between 1996 and 2000 were shown to be Incorrectly Matched when Confirmed by DNA Analysis

DNA Hair Profiling Technology

- Nuclear Short Tandem Repeats (STRs)

 - Follicular Tissue

 - Anagen Root

- Mitochondrial DNA

 - Hair Shaft

How to Avoid Contamination of Biological Evidence

- Wear disposable latex gloves and a face mask when collecting biological evidence.
- Collect a substrate control for possible subsequent laboratory examination.
- Pick up small items of evidence such as cigarette butts and stamps with clean forceps.
- Always package each item of evidence in its own well-ventilated container.