
DNA Profiling

DNA: What *is* it? *Where* is it?

- **DeoxyriboNucleic Acid**
  - The genetic material found in every nucleated cell in the body
  - Has structure of a “double helix”, like a zipper or a spiral staircase
  - Made of long polymer strands of nucleotides (molecules made up of nitrogenous bases and sugar-phosphate groups)

DNA 101

- Nucleotides contain either a purine or a pyrimidine base:

  <u>Pyrimidines</u>

  - Cytosine (C) G-C, A-T
  - Thymine (T)

  <u>Purines</u>

  - Guanine (G)
  - Adenine (A)

OHT 6.4

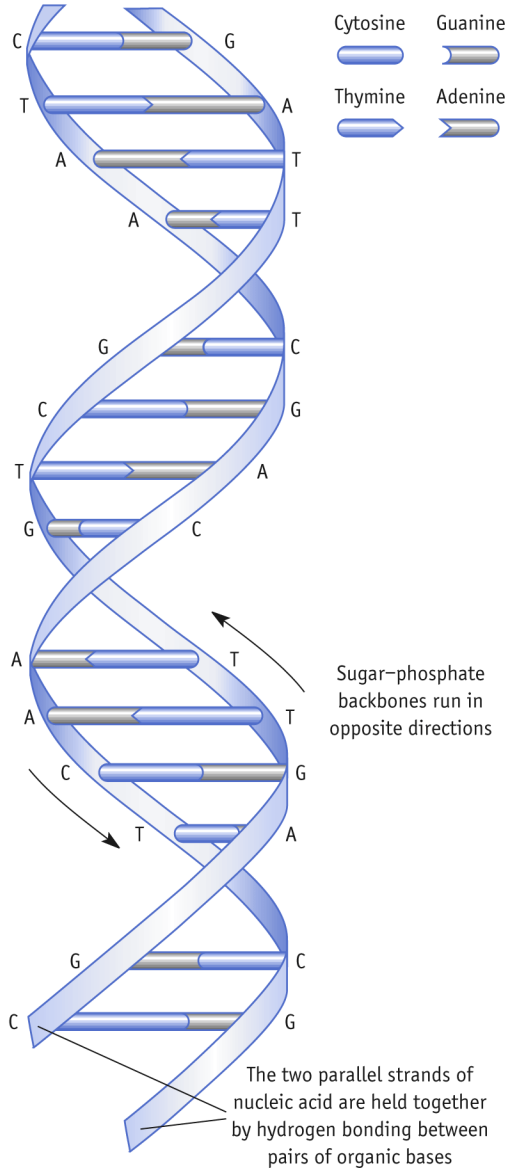
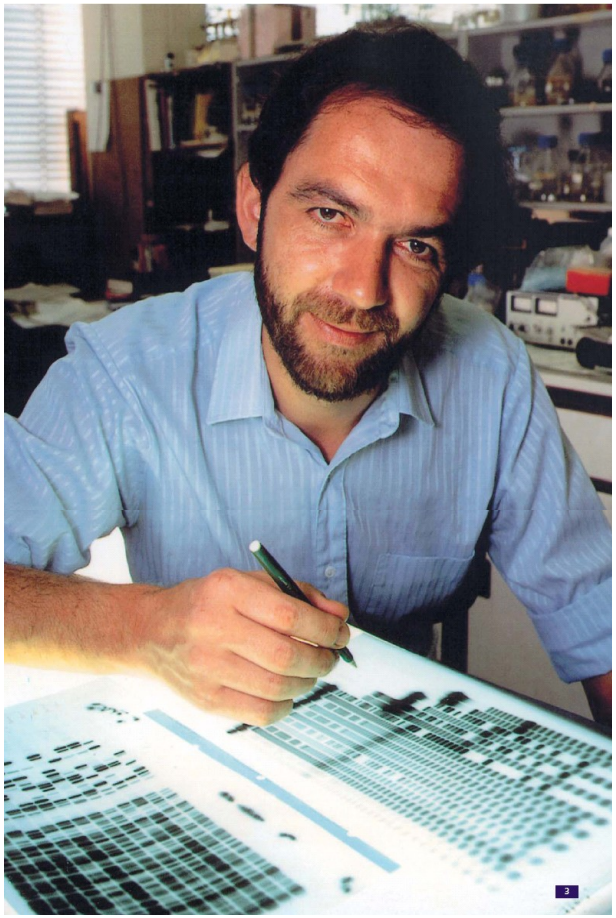


Figure 6.2 (b) DNA structure

A Historical Perspective

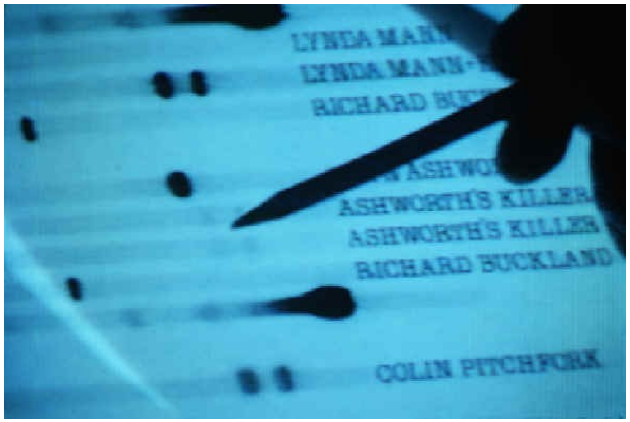
- Double helix structure discovered in 1953 by Watson & Crick, two British scientists who based their work on Rosalind Franklin's data
- Cary Mullis (PCR)
- In the course of his research on variability in human DNA, Alec Jeffreys developed a method of forensic DNA typing in 1985



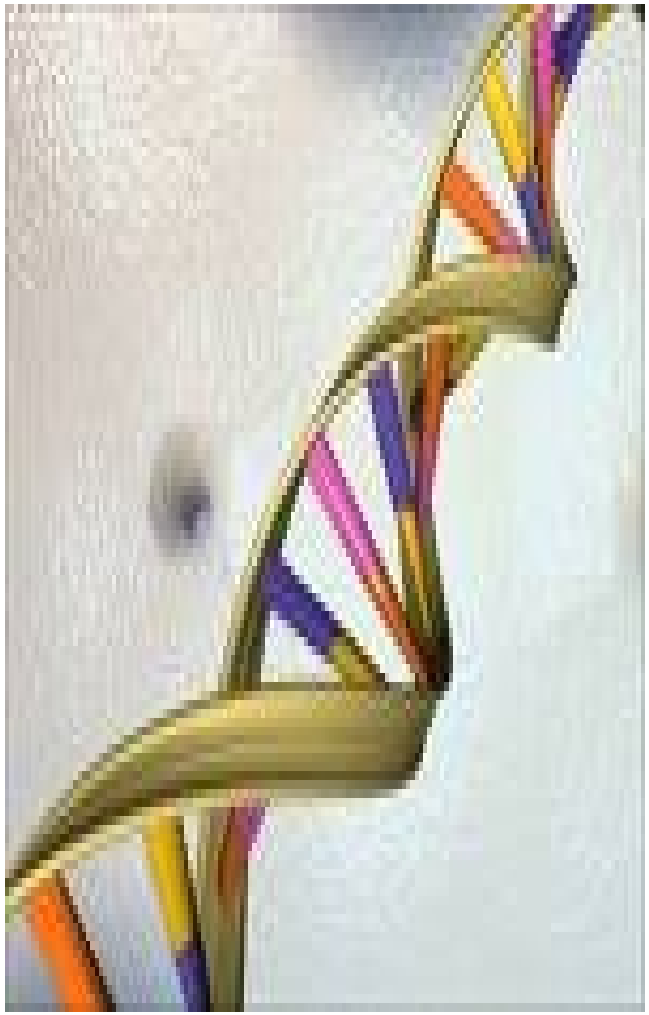
A Historical Perspective

- DNA fingerprinting first used in 1986 to catch a rapist/murderer in Scotland: Colin Pitchfork

- The Blooding (Joseph Wambaugh)



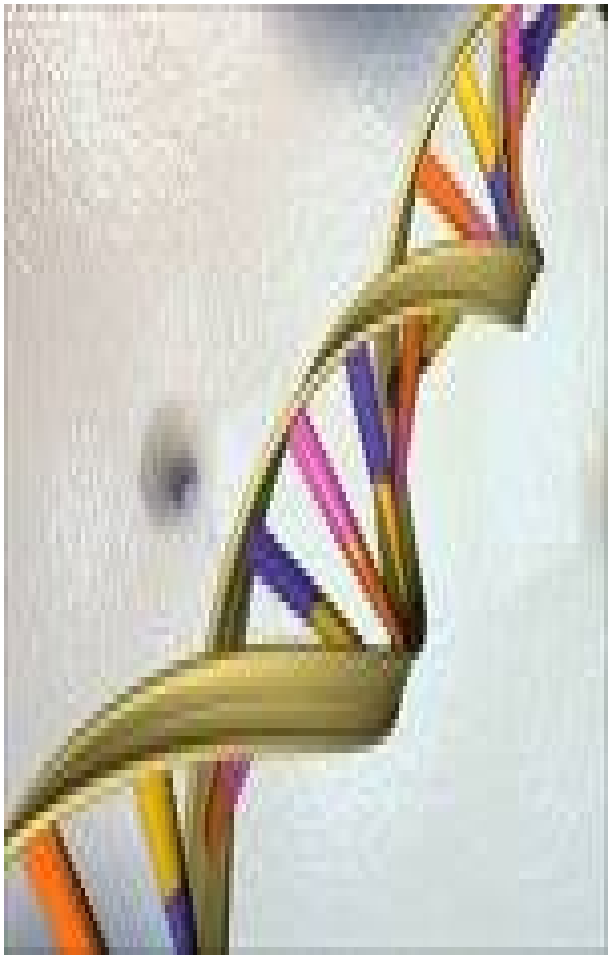
DNA 101



- There are approximately 6 billion base pairs in every nucleated cell.
- DNA is organized into *chromosomes*
- On the chromosomes, *genes* (sequences of DNA that code for a protein) are found.
- A gene is a hereditary unit that determines a particular characteristic in an organism.
- Genes are sequences of A,G,C,T *nucleotides*.
- The length and order of nucleotides determines the type of protein that is produced by that gene.

DNA 101

- The location of the gene on the chromosome is its *locus* (plural: *loci*).
- An allele is an alternate form of gene
- Each person inherits one allele from each parent
- Simplified example: eye color



DNA 101

- DNA is unique to each individual: even though we share 99.9% of our genome in common with other humans, 0.1% of 6 billion nucleotides is a significant and detectable level of difference
- Most variation exists in non-coding (“junk DNA”) regions
- Approximately 98% of our DNA is non-coding
- Mutations in the non-coding regions are tolerated and can accumulate with no effect on the organism

DNA 101

Phenotype: the physical characteristics (or exterior expression) of an organism's genetic makeup

Genotype: 1. the genetic makeup of an organism;
2. the combination of alleles present at a particular locus, or at all loci present

Humans have ~20,000-25,000 genes

Biological materials used for DNA profiling

- Blood (white blood cells)
- Hair with roots
- Saliva
- Semen (sperm cells)
- Skin, dandruff
- Sweat stains
- Vaginal fluids
- Nasal secretions
- Urine
- Feces

DNA is the same in every cell, and is robust and stable

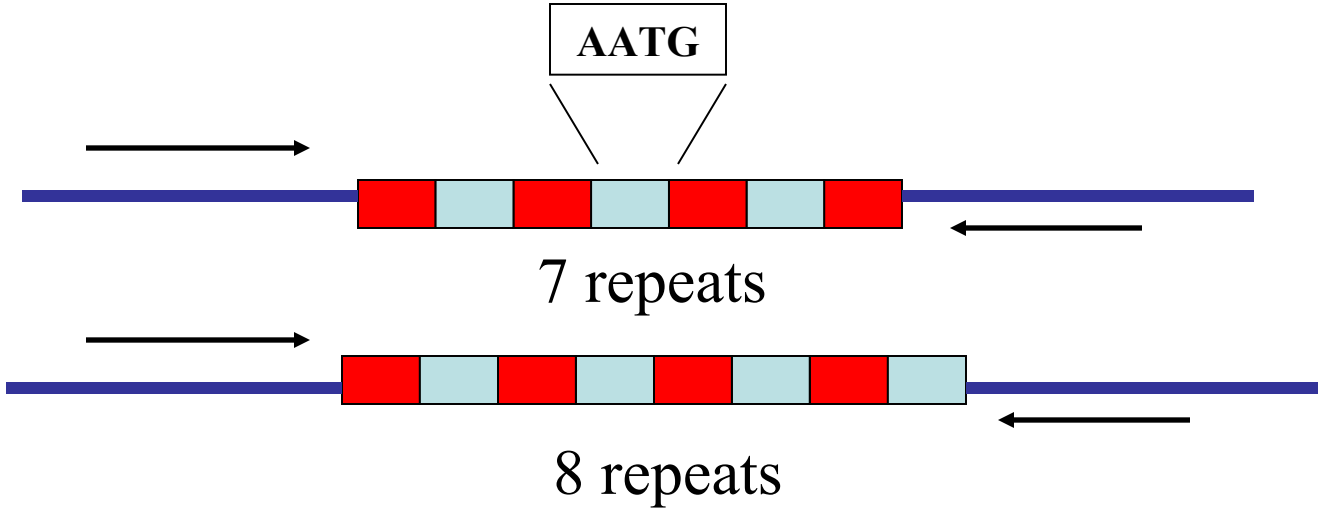
Short Tandem Repeats

A sequence of DNA, usually 4 bases long, that is repeated many times

- Short Tandem Repeats (STRs)
  - Short because the differences are short – usually 1-4 nucleotides in length
  - Tandem because they occur one after the other
  - Repeats because they are repeats of the same DNA sequence
  - ATCACTTG-GCCG-GCCG-GCCG-GCCG-ATCGACCA = 4 tandem repeats of GCCG

- Found in sections of DNA that are non-coding
- Highly variable regions
- The number of repeats may vary between individuals

Short Tandem Repeats (STRs)



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

Homozygote = both alleles have same number of repeats

Heterozygote = alleles differ in number of repeats

An Example of a STR in locus D7S280

- D7S280 is a region (locus) of human chromosome 7. Its DNA sequence, as obtained from GenBank (a public DNA database) is:

aatttttgta ttttttttag agacggggtt tcaccatgtt ggtcaggctg
actatggagt tattttaagg ttaatatata taaagggtat gatagaacac
ttgtcatagt ttagaacgaa ctaacgatag atagatagat agatagatag
atagatagat agatagatag atagacagat tgatagtttt tttttatctc
actaaatagt ctatagtaaa catttaatta ccaatatttg gtgcaattct
gtcaatgagg ataaatgtgg aatcgttata attcttaaga atatatattc
cctctgagtt tttgatacct cagattttaa ggcc

- The STR repeat sequence is gata
- Alleles of this locus have from 6 to 15 tandem repeats of the 'gata' sequence

The Process

1. Extract DNA from sample
2. Quantify the DNA
3. Amplify the DNA (PCR)
4. Separate & detect PCR products via Capillary Electrophoresis
5. Determine genotype
6. Compare to reference profiles
7. Consult population dbase & determine frequency of profile

PCR: Polymerase Chain Reaction

- Because most tissue samples from a crime scene contain very little DNA, whatever amount is present must be amplified (many copies made of) certain loci containing STR's
- In STR analysis, you want to amplify the DNA containing the tandem repeats *and only this DNA*
- The process used is called Polymerase Chain Reaction (PCR)
- PCR Machines, or thermocyclers, use repeated cycles of heating and cooling to replicate the DNA using many of the same enzymes found in cells which facilitate DNA replication naturally

PCR

- Using a thermocycler, the sample is denatured (double helix is unzipped) @ ~95°C
- Primers (oligonucleotides, typically 20 bases long) adhere to complimentary open strands
  (A-T, G-C) @ ~55°C
- New DNA strand is synthesized with Taq polymerase @ 72 °C
- Process is repeated until there are enough copies of the DNA for detection using electrophoresis

OHT 6.14

How to amplify DNA...

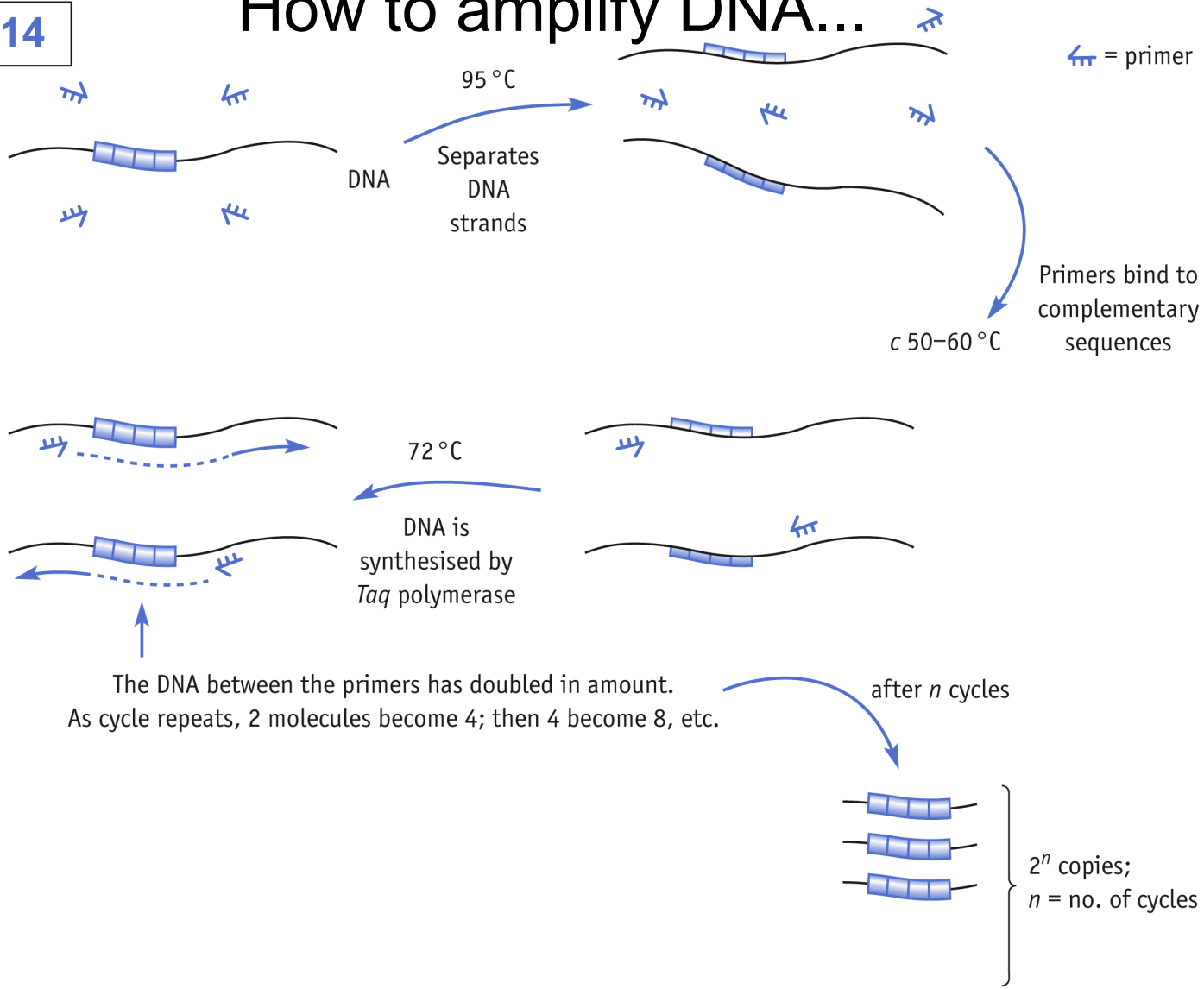


Figure 6.7 The polymerase chain reaction (PCR) (a) the basis of the process

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How to amplify DNA...

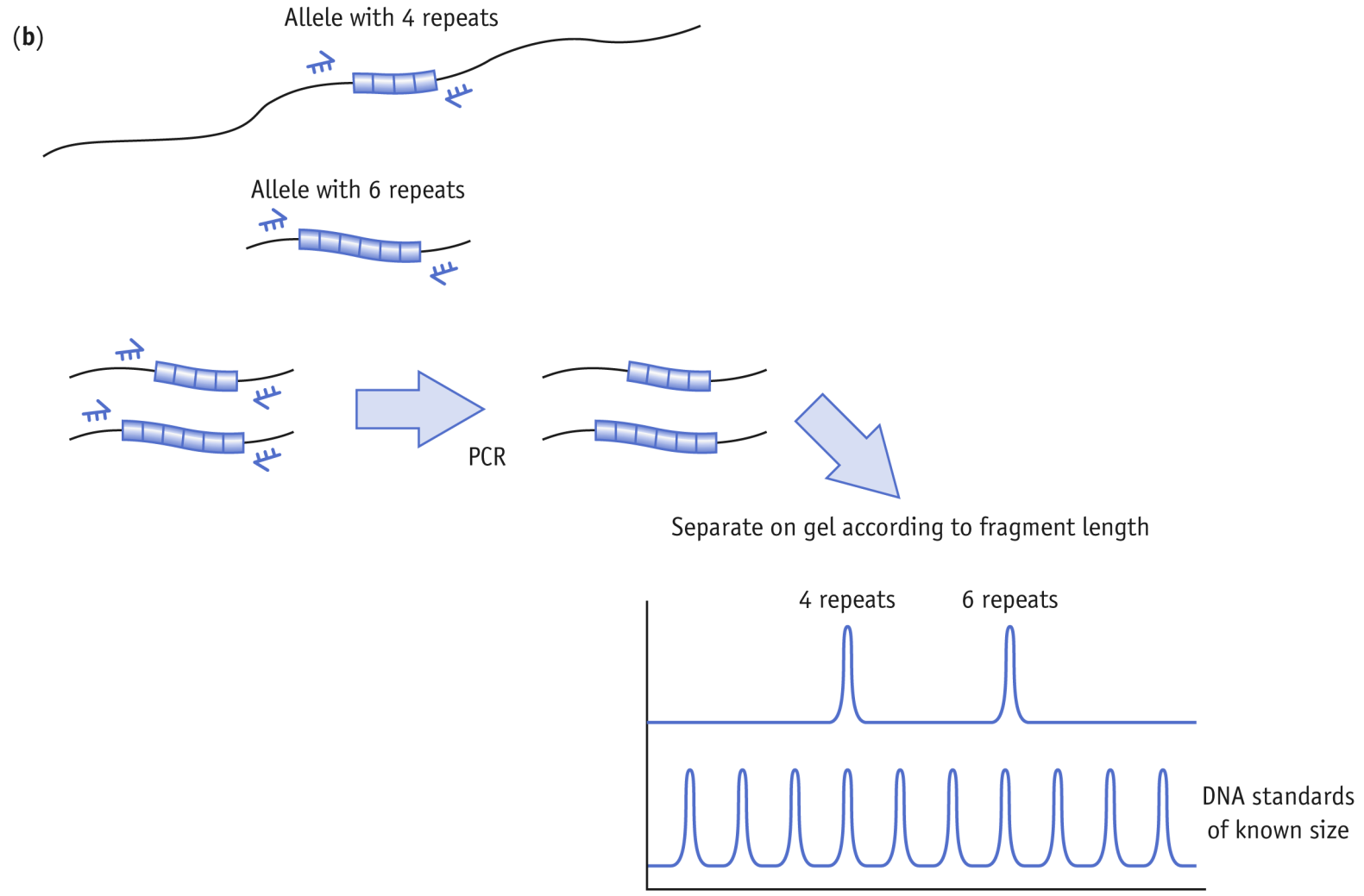


Figure 6.7 The polymerase chain reaction (PCR) (b) STR analysis using PCR

Electrophoresis

- DNA is negatively charged
- Electrophoresis is the process of moving charged particles through a gel plate by applying an electric field
- Following PCR, amplified

  DNA samples are separated

  by size through electrophoresis

- Process is automated using an

  instrument (a genetic analyzer) that reads the DNA by size -- a laser scans and detects the DNA samples as they electrophorese

OHT 6.11

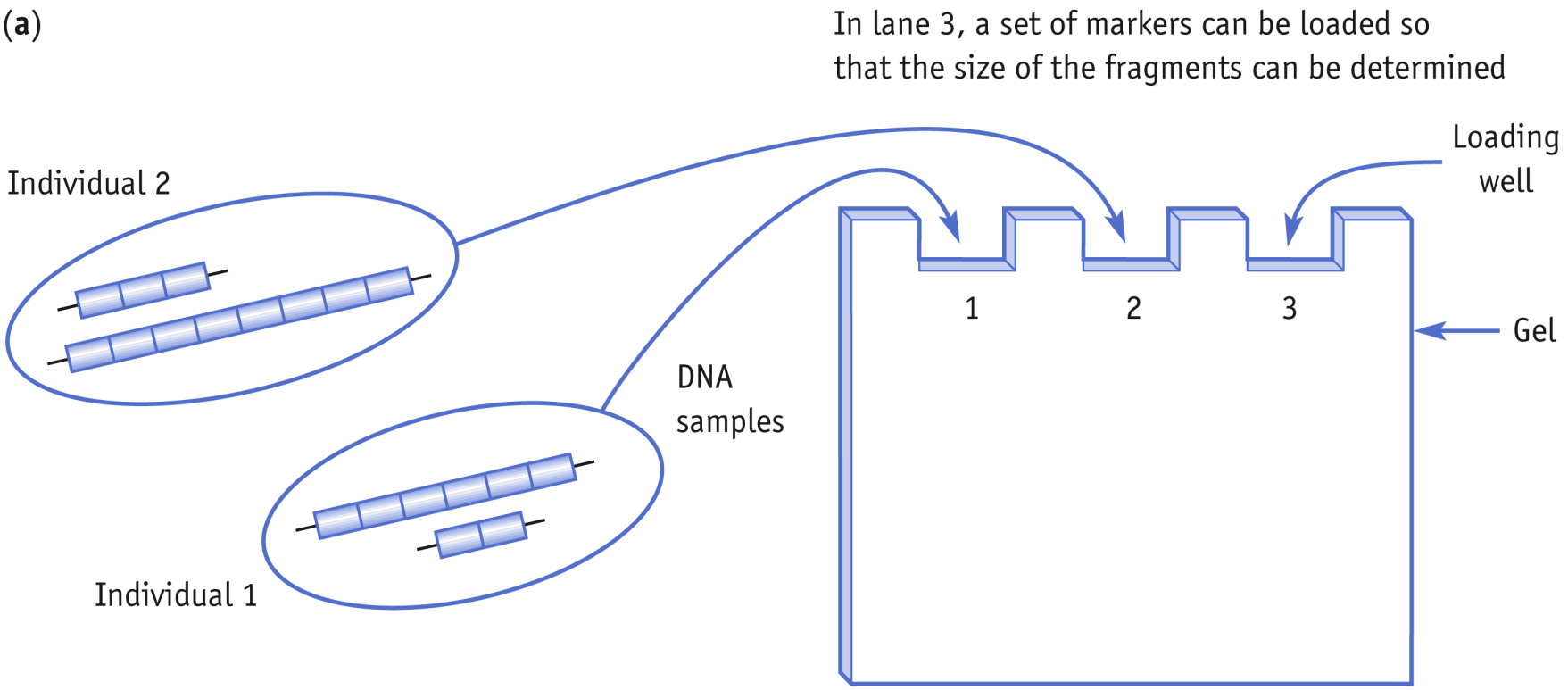


Figure 6.6 Separating DNA molecules according to their length: gel electrophoresis (a) loading the gel

OHT 6.12

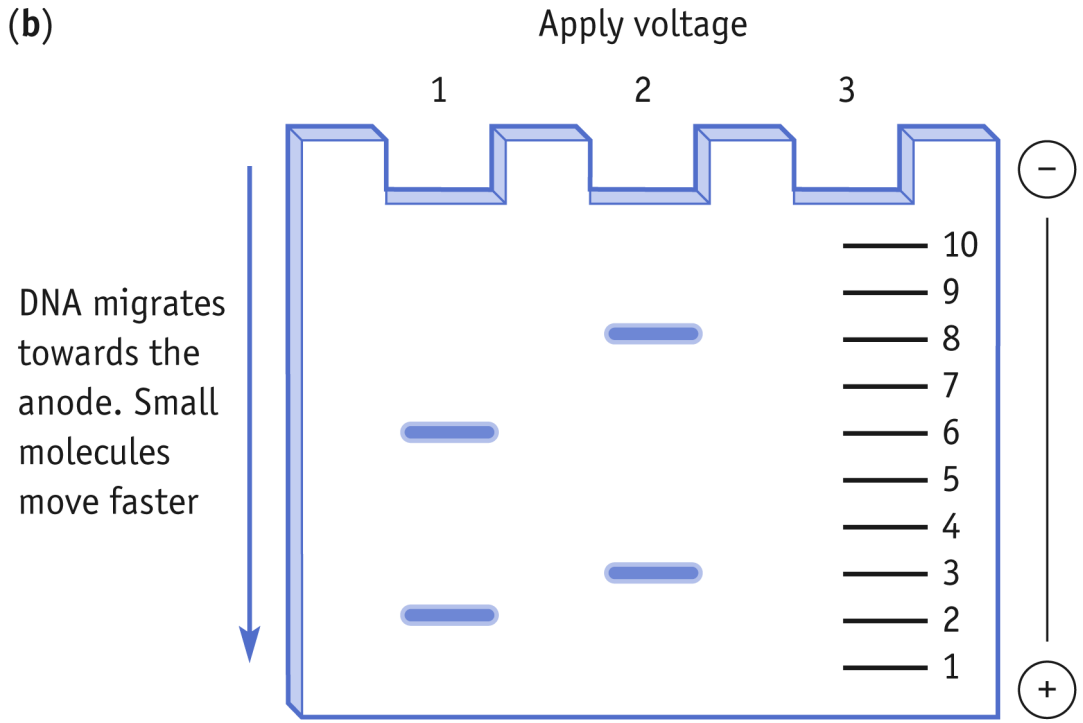
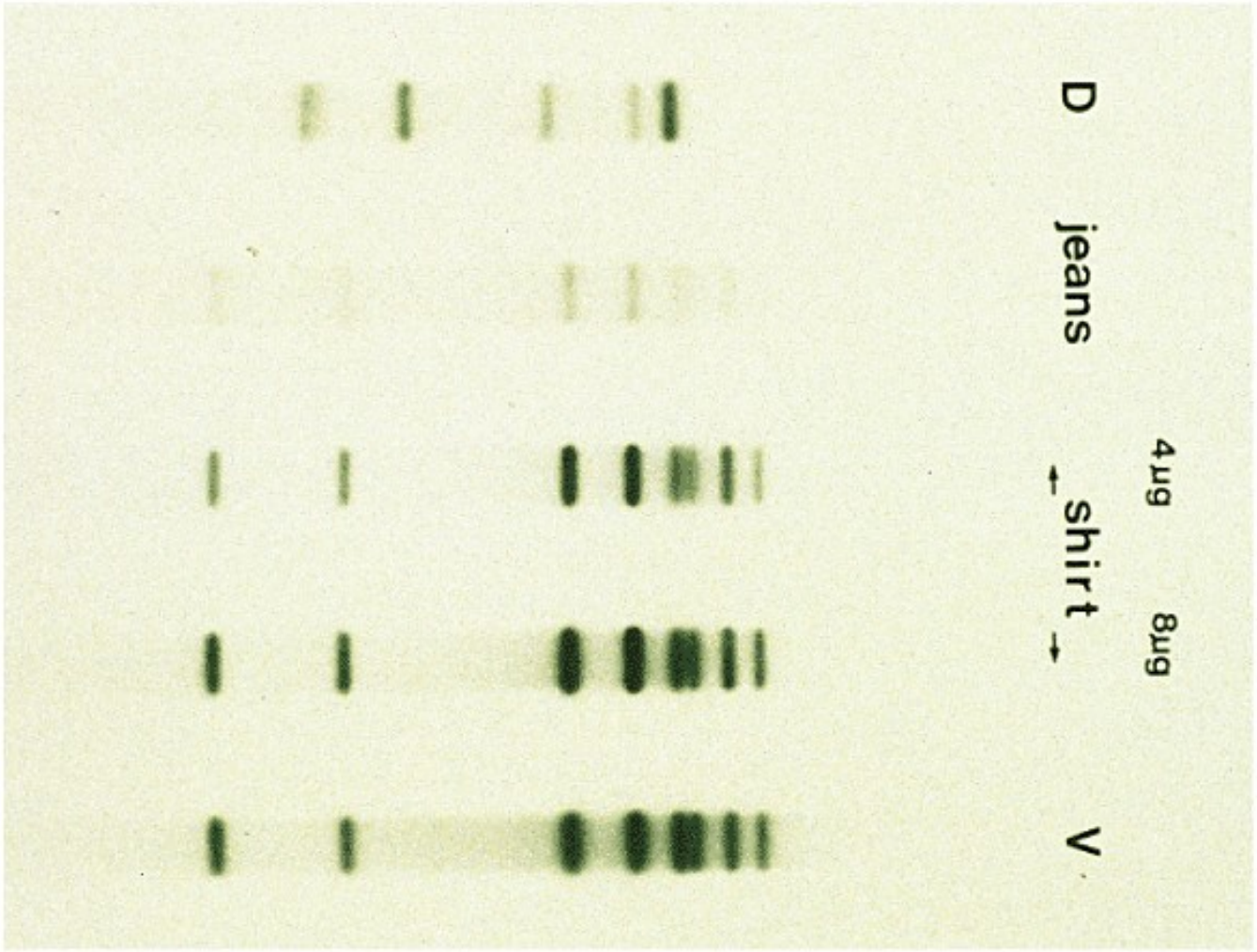


Figure 6.6 Separating DNA molecules according to their length: gel electrophoresis (b) DNA migration

Electrophoresis Autoradiograph



Defendant's blood

Blood from defendant's clothes

Victim's blood

OHT 6.13

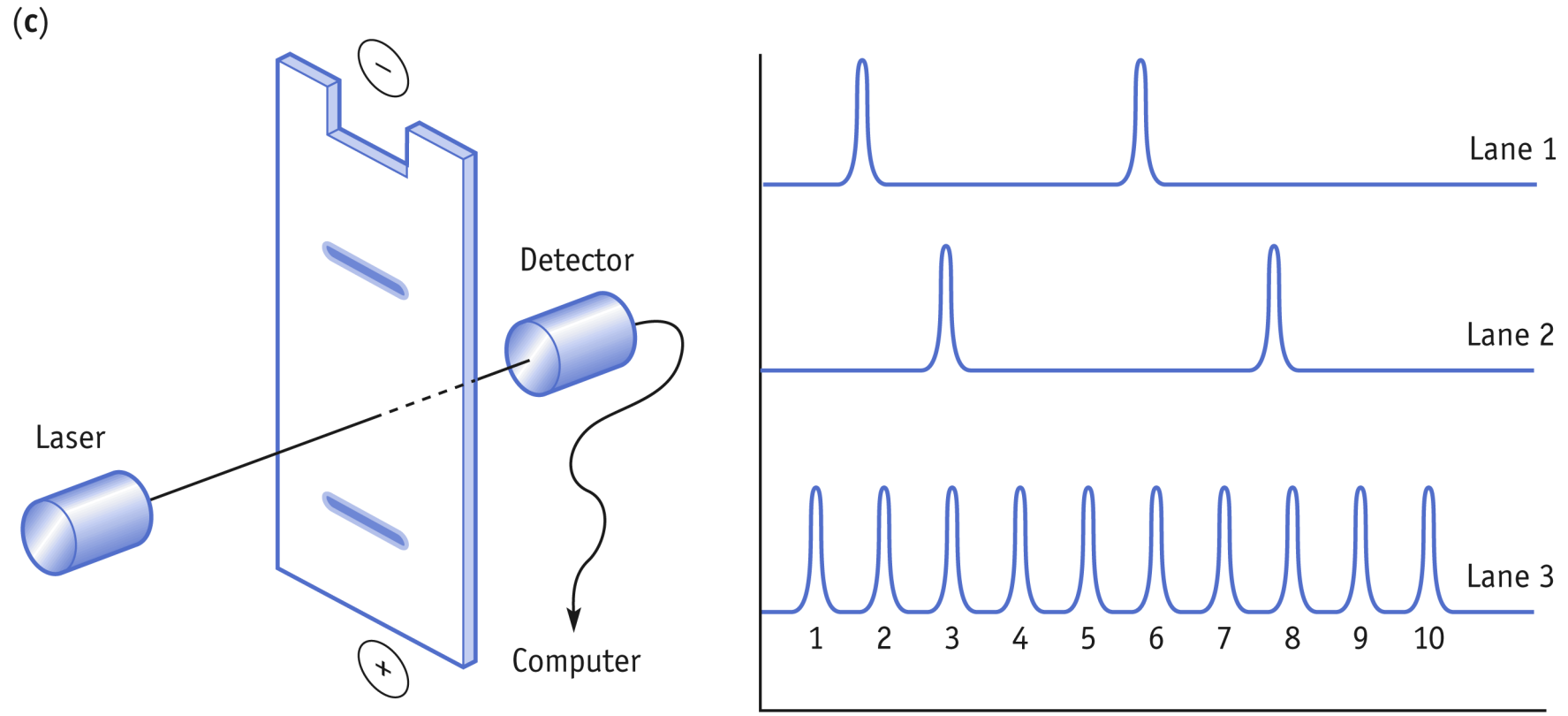


Figure 6.6 Separating DNA molecules according to their length: gel electrophoresis (c) detecting fluorescently tagged DNA by laser

OHT 6.2

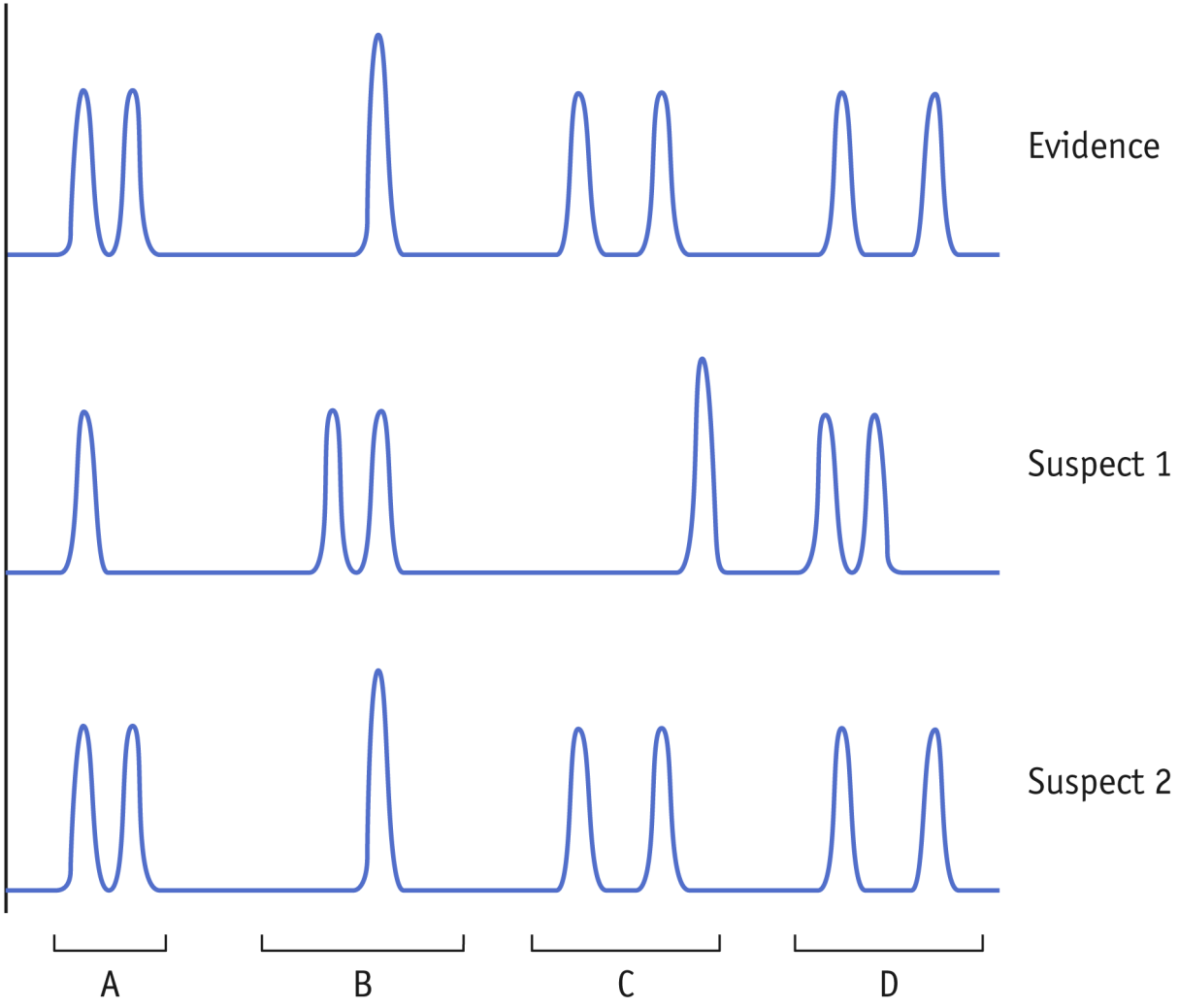


Figure 6.1 (continued) Simplified examples of DNA profiles

OHT 6.16

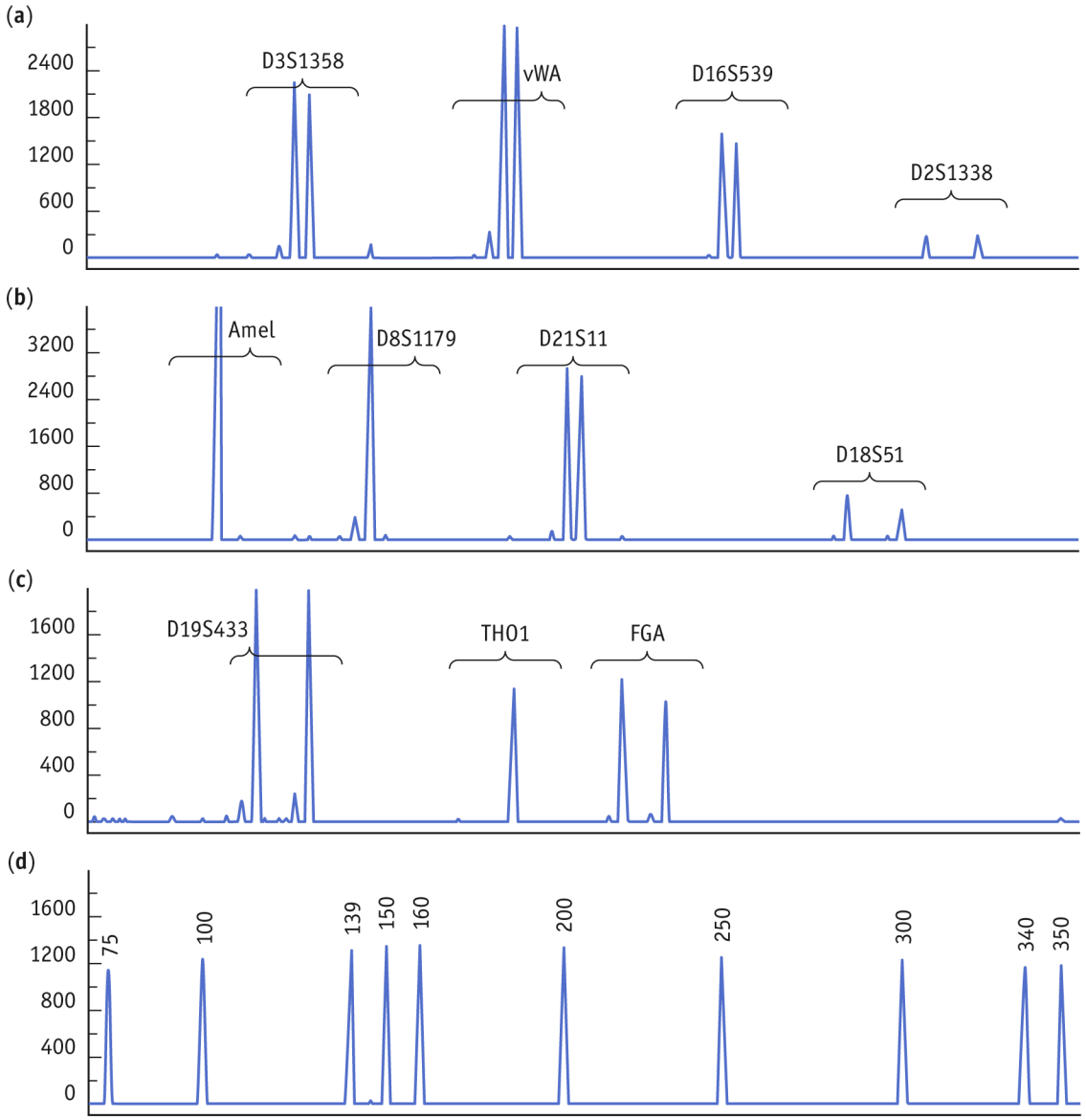
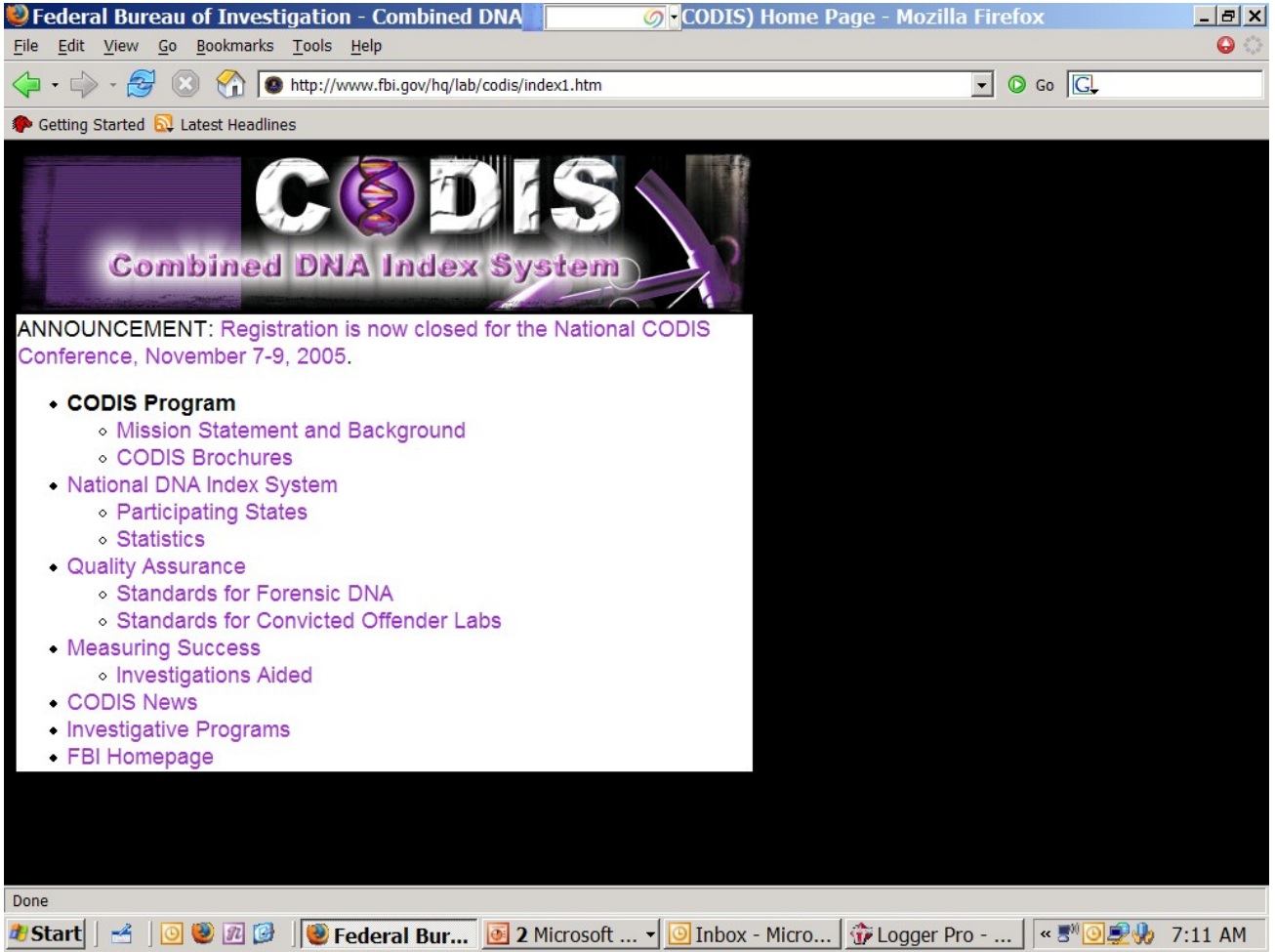


Figure 6.8 A modern DNA profile

Where do the data go?

- CODIS – Combined DNA Index System



CODIS

- Looks at 13 loci

- All forensic laboratories that use the CODIS system can contribute DNA profiles to the CODIS database.

  – The **Forensic Index** contains DNA profiles from crime scene evidence.

  – The **Offender Index** contains DNA profiles of individuals convicted of sex offenses (and other violent crimes) with many states now expanding legislation to include other felonies.

Is the profile unique?

- For a DNA profile to be considered useful in identification, it must be unique or rare -- otherwise it would describe too many people

- The uniqueness of a profile is determined using frequency databases (frequencies of alleles in a given population) and probability law

- Each STR site index is an independent event, so use probability law: "the probability that two independent events may happen together is the product of their individual probabilities"

For example…

- A typical DNA case involves the comparison of two samples – an unknown or *evidence* sample, such as semen from a rape, and a known or *reference* sample, such as a blood sample from a suspect.

- If the DNA profile obtained from the two samples are indistinguishable (they "match"), that is evidence for the court that the samples have a common source – in other words, that the suspect is the source of the semen.

For example…

- How strong is the evidence?
- If the DNA profile consists of a combination of traits that figure to be extremely rare, the evidence is very strong that the suspect is the contributor.
- If the combination of traits is not so rare, it is easier to imagine that the suspect's profile matches only by chance.

For example…

Therefore it is essential to know the likelihood that a match would occur by chance. It is easiest to illustrate by example how probability is determined.

For example…

Locus	Alleles	#Times Observed	Dbase size	Frequency	Formula	Number
CSF1PO	10 11	109 134	432	p=.25 q=.31	2pq	.16
TPOX	8 8	229	432	p=.53	p2	.28
THO1	6 7	102 64	428	p=.24 q=.15	2pq	.07
vWA	16 16	91	428	p=.21	p2	.05

Use the following formula to determine the frequency of this profile: p2+2pq+q2=1 (where p = frequency of the dominant allele and q = frequency of the recessive allele). Frequency is multiplied by 2 at heterozygous loci and squared at homozygous loci.

Analysis

- Using probability law, multiply the frequencies together:

  16x.28x.07x.05 = .00016 = ~ 1/8000

- This number becomes much smaller when you multiply the frequencies of alleles at more loci.

- When you multiply the frequencies of 13 loci, the probability of 2 unrelated people having exactly the same DNA profile becomes **astronomically small.**

A Sample Profile

Locus	D3S1358	vWA	FGA	D8S1179	D21S11	D18S51	D5S818
Genotype	15, 18	16, 16	19, 24	12, 13	29, 31	12, 13	11, 13
Frequency	8.2%	4.4%	1.7%	9.9%	2.3%	4.3%	13%

Locus	D13S317	D7S820	D16S539	THO1	TPOX	CSF1PO	AMEL
Genotype	11, 11	10, 10	11, 11	9, 9.3	8, 8	11, 11	X Y
Frequency	1.2%	6.3%	9.5%	9.6%	3.52%	7.2%	(Male)

- By combining the frequency information for all 13 CODIS loci, the frequency of this profile would be .0000000001421055
- In other words, the probability that this profile matches by chance (and not because it *is this person's profile*) is 1 in 7.7 quadrillion -- a number greater than the number of people on earth by BILLIONS!

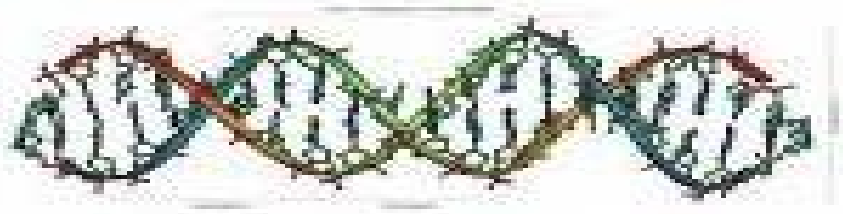
mtDNA & Y-STR

Mitochondrial DNA

- 37 genes, 16569 bp
- 100-10,000 copies per cell (significantly more than the 2 copies of nuclear DNA) make it useful for analyzing degraded samples
- Circular structure
- Non-coding D loop used for testing
- Inherited maternally

Y-STR

- Short tandem repeats located on Y chromosome
- Useful for resolving mixtures between males & females
- Inherited paternally



Some Considerations

- Although DNA is relatively stable, it does denature or get destroyed through enzyme action, from bacteria or through oxidation
- Therefore, samples should be collected quickly and preserved (usually in a buffer and by freezing) if possible
- Care should also be taken not to cross contaminate during collection -- including from collector
- Blood is also a potential pathogen, so care must be taken to avoid exposing oneself to blood borne viruses like Hep B, tuberculosis or HIV

Some uses of DNA Profiling

- Forensic work on crime scenes
- Parentage testing
- Victim identification in mass disasters
- Animal identification- e.g. racehorse paternity, endangered species poaching
- Conservation biology and evolutionary studies

DNA Profiling can solve crimes

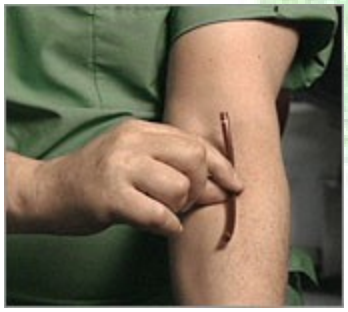
- The DNA profile is compared with those of the victim and the suspect.
- If the profile matches the suspect, it provides strong evidence that the suspect was present at the crime scene (**Note: this does not necessarily prove he or she committed the crime).**
- If the profile doesn't match the suspect, then that suspect may be eliminated from the inquiry.

As the technology gets smarter, so too do the criminals

- A physician in Canada eludes authorities for years
- Accused of drugging and sexually assaulting patients, DNA profiles from semen samples from the assaulted women do not match Dr. Schneeberger
- Blood was drawn on 3 occasions in 1992, 1993 and 1996, but never came back as a match
- Finally police obtain blood from a finger prick, swabbed the inside of his cheek and took hair samples
- The results matched the DNA from the semen of the victims
- How did he get away with it?

As the technology gets smarter, so too do the criminals

- On the previous 3 occasions, blood was drawn from the same arm
- The last time the blood was drawn, the technician stated that the blood looked brown and “old”
- Schneeberger had surgically implanted a piece of rubber tubing in his arm and filled it with stored blood from a patient



- DNA is also used in the identification of remains recovered in mass disasters

The Angel of Death: Josef Menegle

- Josef Mengele was a Nazi war criminal notorious for grotesque human experiments that he carried out at the Auschwitz concentration camp.
- After the Second World War he fled from the Allies and escaped to South America. The fugitive succeeded in living out the rest of his days without being caught.
- In 1985 investigators went to the cemetery of Nossa Senhora do Rosario in the small Brazilian town of Embu to dig up the skeleton of a man who had been drowned in a swimming accident six years previously.
- Using DNA extracted from blood provided by Mengele's wife and son, it was concluded that it was more than 99.94% certain that the skeleton was Mengele's.

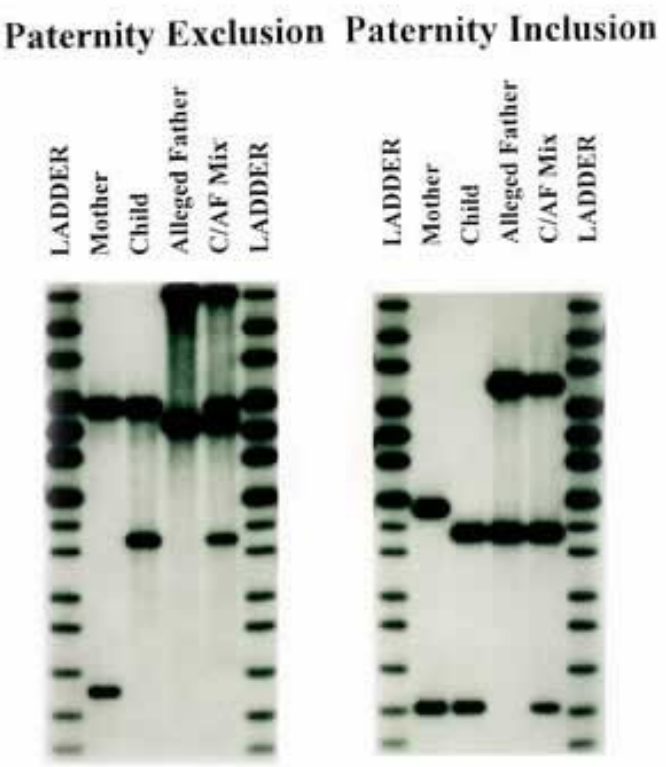
Paternity Cases

- Who's your daddy?

1.

2.

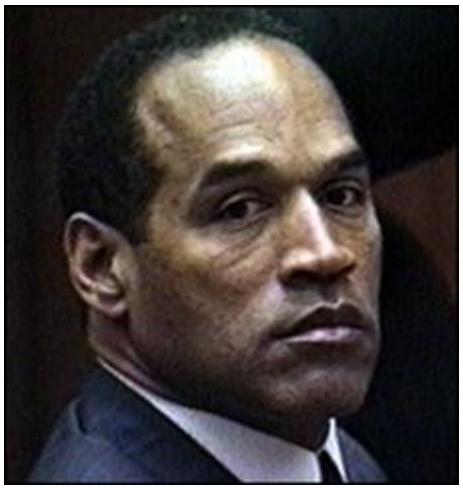
1. 2.

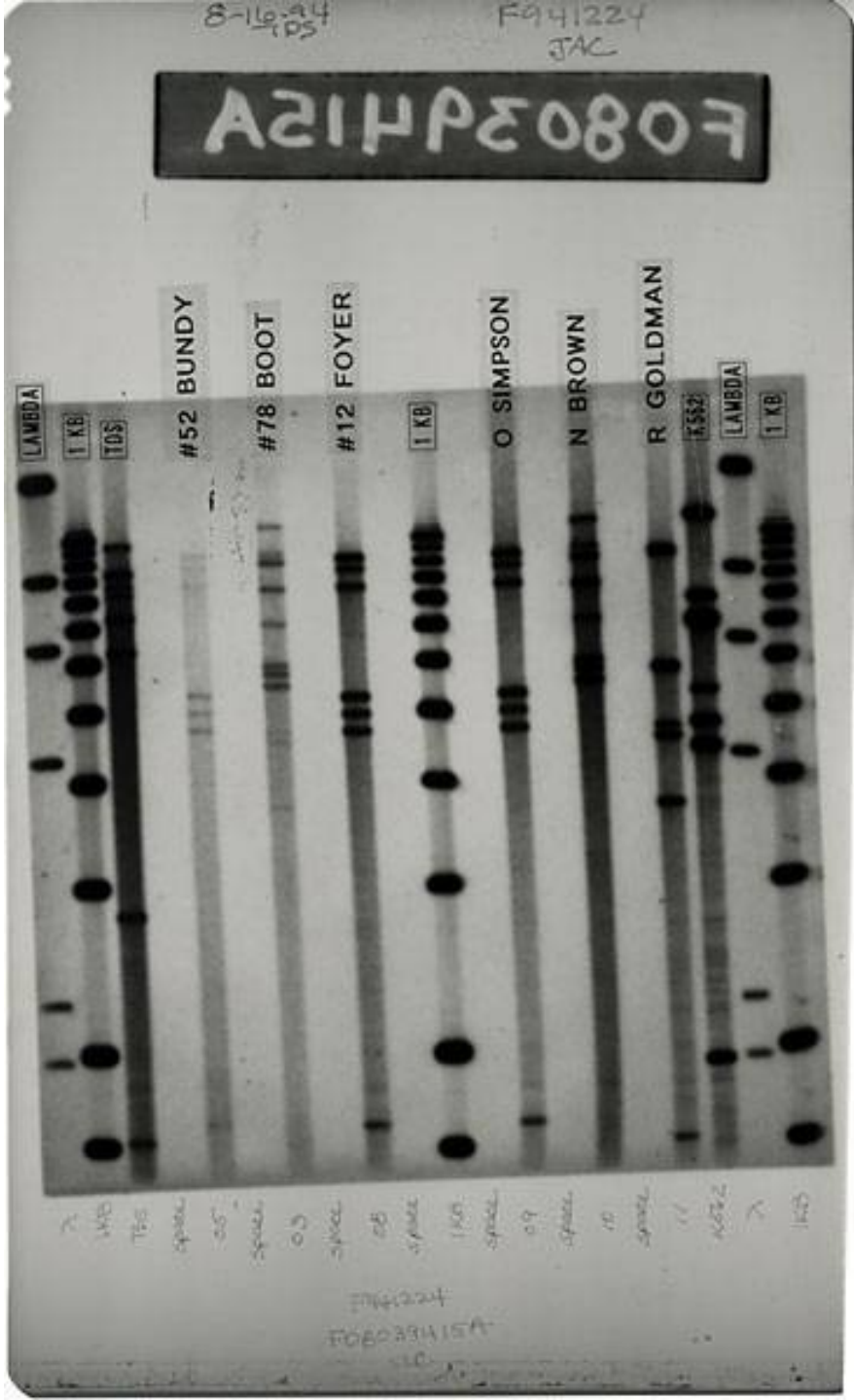


Paternity Cases

DNA Marker	Mother	Child	Alleged Father
D21S11	28, 30	28, 31	29, 31
D7S820	9, 10	10, 11	11, 12
TH01	14, 15	15, 15	15, 16
D13S317	7, 8	7, 9	8, 9
D19S433	14, 16.2	14, 15	15, 17

OJ Simpson




LAMBDA
1 KB
TDS
#52 BUNDY
#78 BOOT
#12 FOYER
1 KB
O SIMPSON
N BROWN
R GOLDMAN
LAMBDA
1 KB

Exoneration

- Kirk Bloodsworth
  - Convicted in 1985 for the rape and strangulation of a 9-year old girl and sent to death row
  - In 1992, defense attorneys were successful in having a dime-sized semen stain on the girl's underpants tested against Bloodsworth's DNA
  - He was exonerated

Exoneration

DNA Profiling

- **"I didn't understand the DNA stuff at all. To me, it was just a waste of time. It was way out there and carried absolutely no weight with me at all."**
- Post-trial commentary from a juror in the O.J. Simpson trial: V. Bugliosi, Outrage (New York: Dell Publishing, 1996).

- **"In a forensic setting, ... an innocent suspect has little to fear from DNA evidence, unless he or she has an evil twin."**
- N. Risch & B. Devlin, "On the Probability of Matching DNA Fingerprints" (1992) 255 Science.