DNA fingerprinting

Zoo-401A, Unit V
By
Sudhansu Sekhar Nishank
Dept. of Zoology,
Utkal University

- DNA fingerprinting also called Genetic fingerprinting,, is a technique to support the identification of persons on the basis of their respective DNA profiles.

Evidence as sources of DNA

Evidence	Source	Comments
Blood	White blood cells	Good source of DNA
Semen	Sperm cells	Rich source of DNA
Hair with roots	Hair follicle cells	Good source of DNA
Skin, dandruff	Skin cells	Not good sources of DNA for routine analysis
Shed hair shafts	Adhering dead skin or follicle cells	Not usually a good source of nuclear DNA; mtDNA can be obtained
Sweat stains	Skin cells rubbed off into the sweat	Can be a good source of DNA
Vaginal fluid	Mainly liquids that may contain cells sloughed off mucosal surfaces	Good source of DNA
Nasal secretions	Mainly liquids that may contain cells sloughed off mucosal surfaces	Good source of DNA
Urine	Mainly liquids that may contain cells sloughed off mucosal surfaces	Contains few cells; not profiled routinely but may be used for serious offences
Faeces	Cells sloughed off the intestinal surfaces	Not usually a good source of nuclear DNA; mtDNA can be obtained

- Sir Alex Jefferies and his colleagues, Dr. Peter Gill and Dr. Dave Werrett, developed techniques for extracting DNA and preparing profiles using old samples of human tissue.

- Because every cell in a body shares the same DNA, cells collected by swabbing the inside of a person's cheek will be a perfect match for those found in white blood cells, skin cells, or any other tissue.

- There are two main types of forensic DNA testing: one based on restriction fragment length polymorphism (RFLP) and the other on the polymerase chain reaction (PCR)

Preparing a DNA Fingerprint

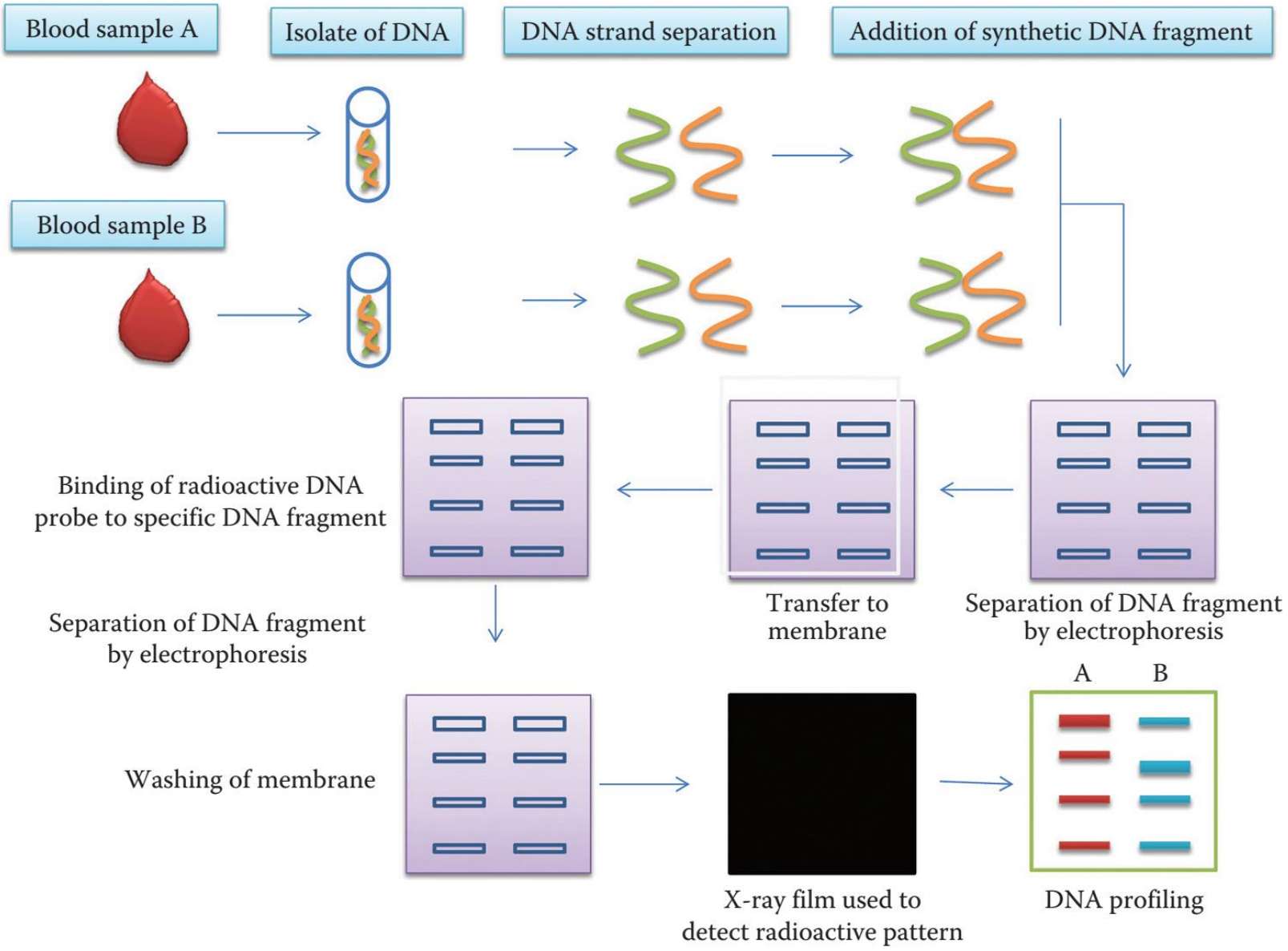
- Preparing a DNA fingerprint requires specimen collection, DNA isolation and quantification, and PCR amplification.

- DNA fingerprinting is a comparative process. DNA from the crime scene must be compared with known DNA samples from the suspect. The ideal specimen used to compare evidence is 1 mL or more of fresh whole blood treated with an anticlotting agent called ethylenediaminetetraacetic acid (EDTA).

STEPS OF DNA FINGERPRINTING

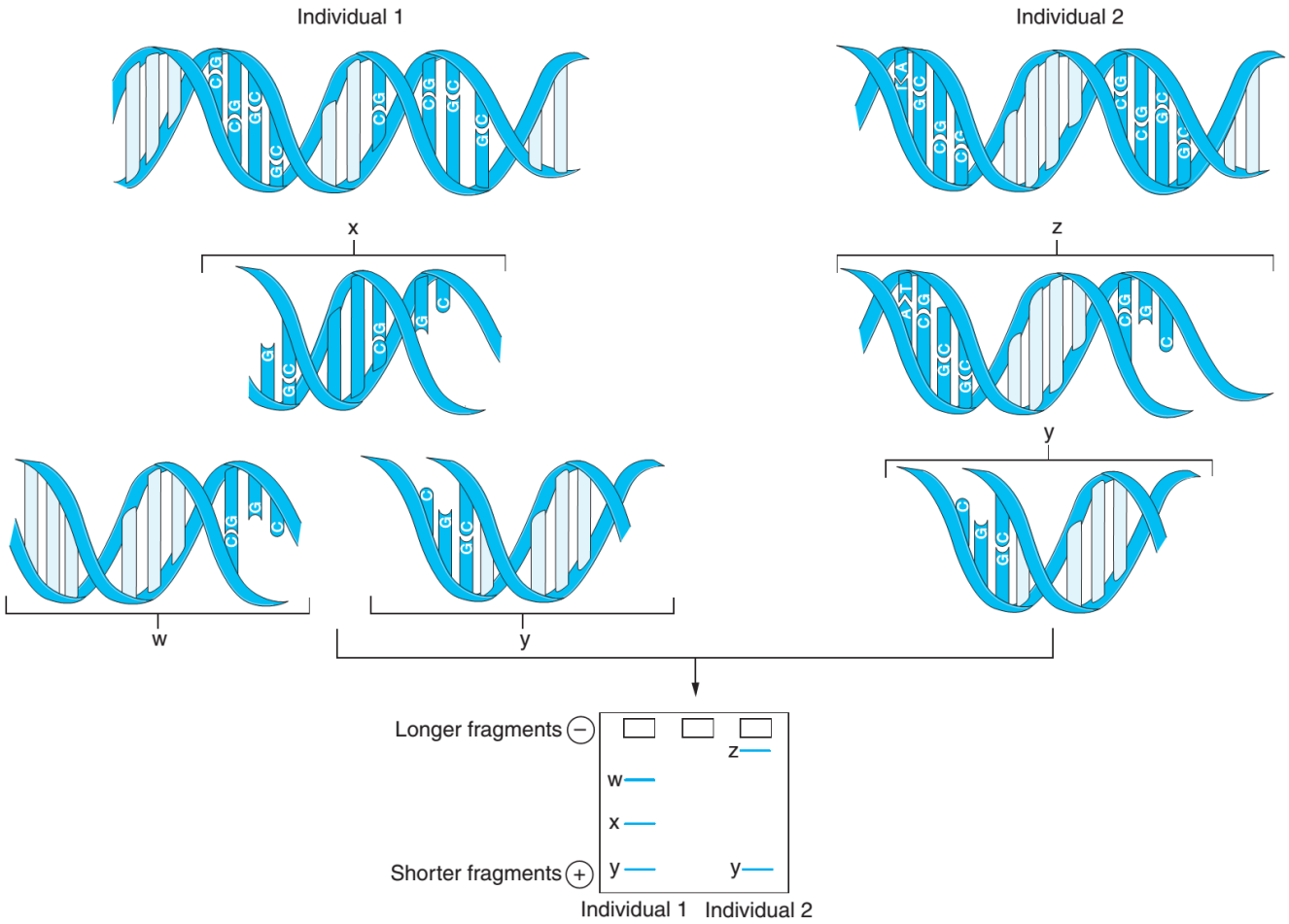
Several steps are necessary before DNA samples can be analyzed and compared. These steps are summarized as follows and then expanded upon in more detail following the summary:

1. Extraction. DNA is extracted from cells.
2. Restriction fragments. In some VNTR analyses, DNA is cut by restriction enzymes. **Restriction enzymes** recognize a unique pattern of DNA bases (restriction sites) and will cut the DNA at that specific location. Restriction fragments of varying lengths are formed when the DNA is cut.
3. Amplification. In the case of other VNTR analyses analysis, specifically chosen DNA fragments are amplified using polymerase chain reaction.
4. Electrophoresis. DNA is loaded into the wells found in an agarose gel. When an electric current is passed through the gel, the negatively charged DNA fragments (pieces of DNA) migrate toward the positive end of the gel. DNA fragments are separated by size, with the smallest DNA fragments moving the fastest through the gel.

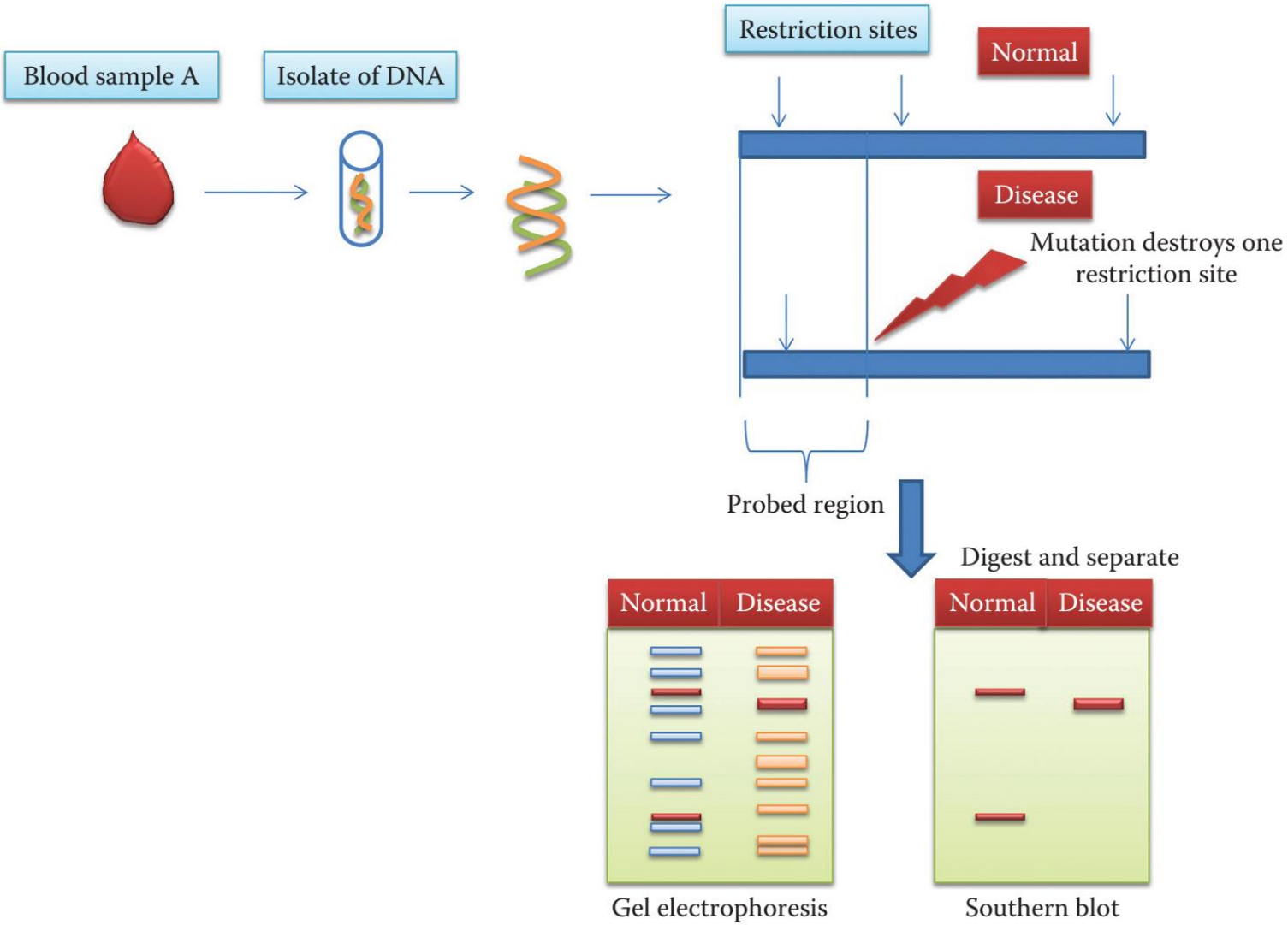


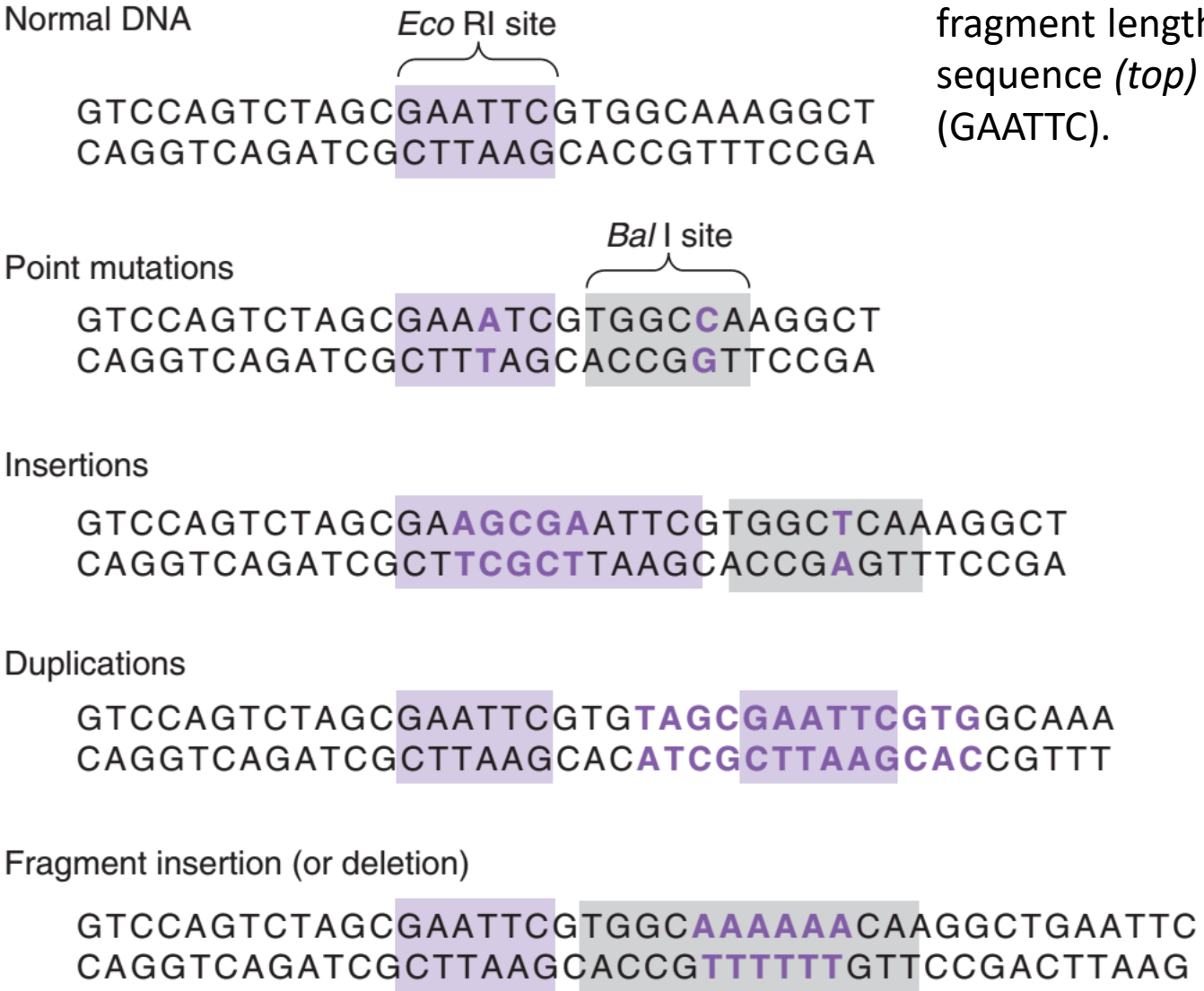
Process of DNA fingerprinting

Restriction Fragment Length Polymorphisms (RFLPs) Can Be Used to Produce DNA Fingerprints

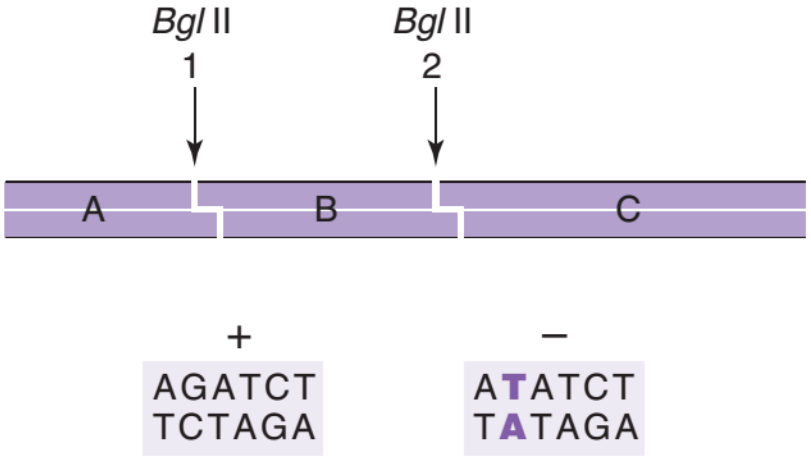


Restriction fragment length polymorphism.

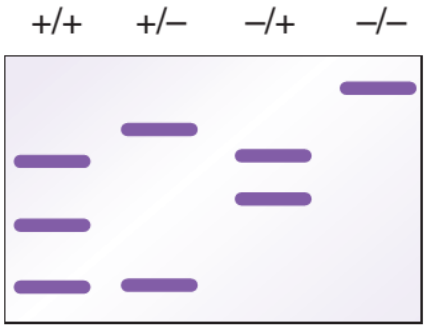




Types of DNA sequence alterations that change restriction fragment lengths. The normal sequence *(top)* has an *Eco*R1 site (GAATTC).

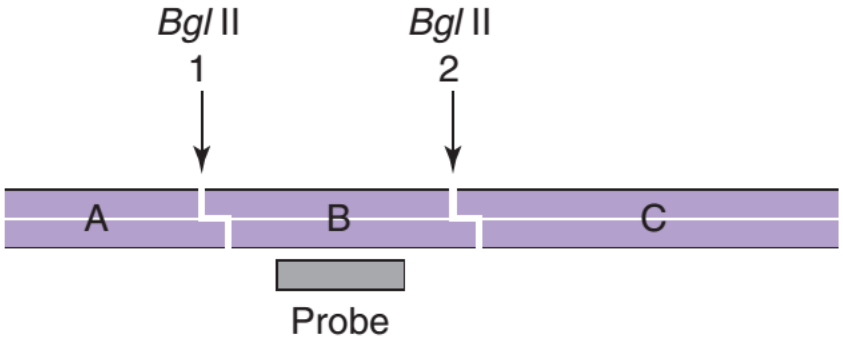


1	2	Size	Number
+	+	A, B, C	3
+	–	A, B+C	2
–	+	A+B, C	2
–	–	A+B+C	1

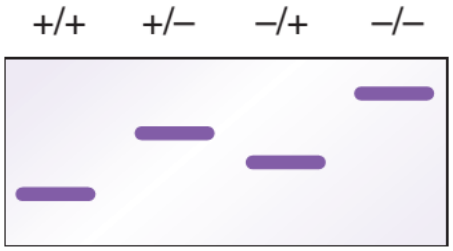


A linear piece of DNA with two polymorphic *Bgl*II restriction enzyme sites, designated as 1 and 2, will yield different fragment sizes, depending on the presence of neither, either, or both of the restriction sites.

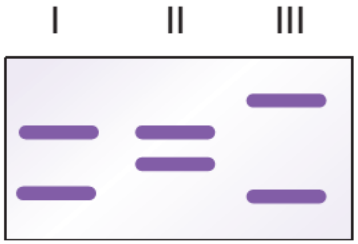
For instance, a G to T mutation will change the sequence of the normal site (+) to one not recognized by the enzyme (–). The presence or absence of the polymorphic sites is evident from the number and size of the fragments after cutting the DNA with BglII (bottom right).



1	2	Fragments visualized
+	+	B
+	–	B+C
–	+	A+B
–	–	A+B+C

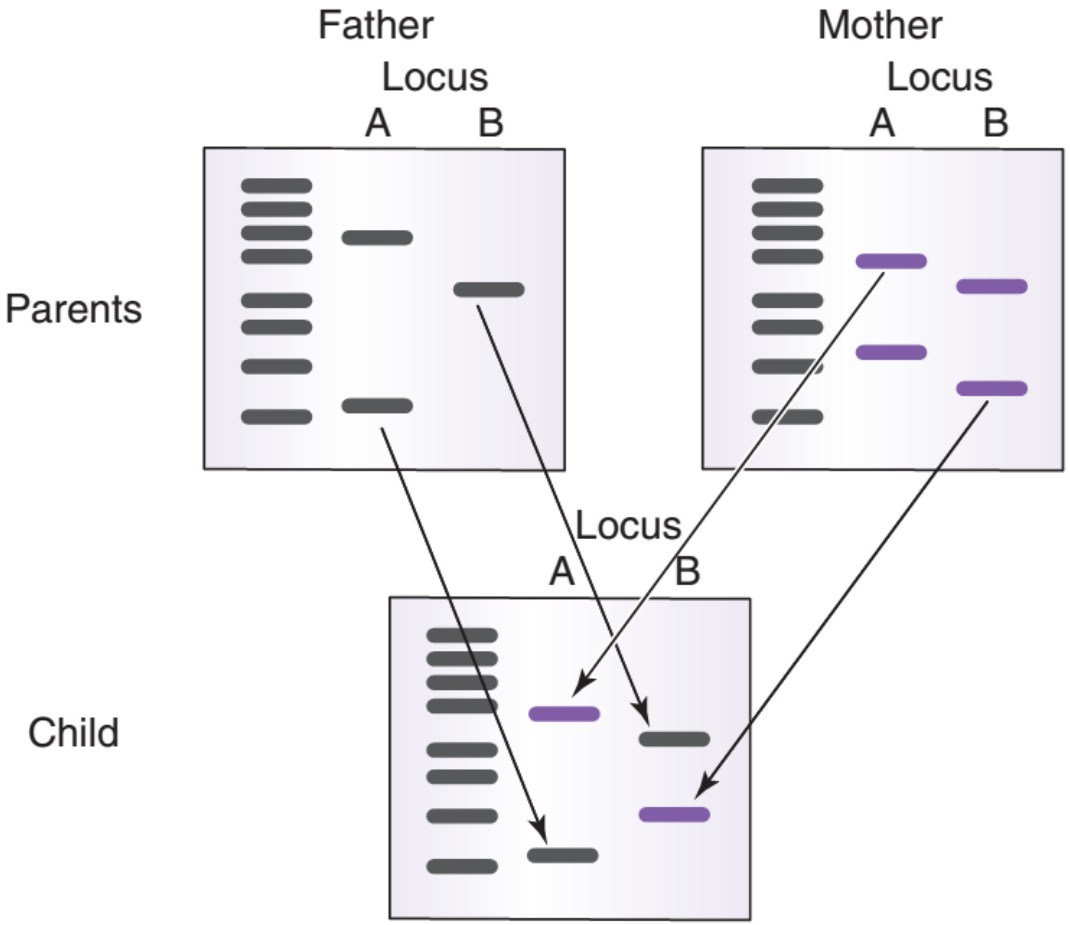


Genotype		Fragments visualized
I	++/+–	B, B+C
II	+–/–+	A+B, B+C
III	++/––	B, A+B+C



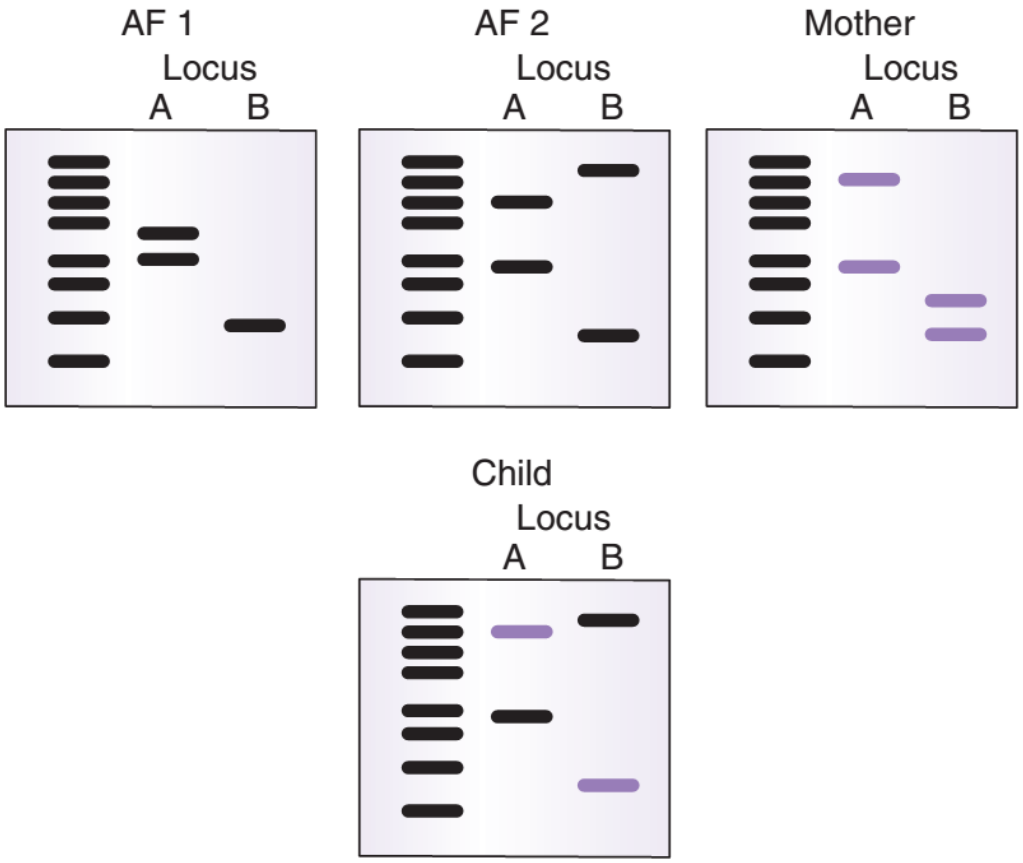
Using a Southern blot to probe for RFLP. With the same region shown in previous slide Figure , only the fragments with complementary sequences to a probe to the B region (top) can be visualized. The bottom panel shows a diploid genotype where homologous chromosomes carry different RFLP alleles.

RFLP and Parentage Testing



RFLP inheritance. Two different genetic regions, or loci, are shown, locus A and locus B. There are several versions or alleles of each locus. Note that the father is heterozygous at locus A and homozygous at locus B. The alleles in the child will be a combination of one allele from each parent.

RFLP and Parentage Testing



Two alleged fathers (AFs) are being tested for paternity of the child whose partial RFLP profile is shown in the bottom gel. The mother ' s alleles are shown in green. One AF (AF1) is excluded from paternity because he cannot supply the child ' s paternal allele at locus B.

- Warm, moist conditions may accelerate DNA degradation, rendering it unsuitable for RFLP testing in a relatively short period of time. Because of this, RFLP is not used as often for DNA testing as it once was.
- Instead, DNA profiling depends on inactive portions of DNA that contain repeated sequences of between 1 and 100 base pairs. These sequences, called variable number tandem repeats (VNTRs).
- Every person has some VNTRs that were inherited from his or her mother and father.
- No person has VNTRs that are identical to those of either parent (this could only occur as a result of cloning). Instead, the individual's VNTRs are a combination of repeats of those of the parents' DNA regions in tandem.
- The uniqueness of an individual's VNTRs provides the scientific marker of identity known as a DNA fingerprint.

- DNA fingerprints produced by PCR are usually restricted to detecting the presence of microsatellites (Figure 2), which are one- to six-nucleotide repeats dispersed throughout chromosomes.
- The small size of these repeated segments has resulted in the term short tandem repeat, or STR.
- In 1990, the United States Federal Bureau of Investigation (FBI) began a process of extensive analysis that culminated in the selection of 13 independently assorting human STR markers to form the core of the FBI's Combined DNA Index System (CODIS)
- National DNA Index System (NDIS) uses CODIS genetic markers as a basis for comparison of DNA information

Use of CODIS marker in Forensic Genetic Analysis

- A CODIS marker must meet four critical criteria:
- First, a CODIS STR must have a known chromosome location, and its location must ensure that the STR assorts independently of all other CODIS markers.
- The second criterion for CODIS STR markers is that they must have multiple alleles in all populations examined.
- Third, the STR markers selected for CODIS must carry alleles that can be consistently, reliably, and accurately amplified by PCR
- The final criterion for CODIS STRs is that their PCR products must distinguish alleles from one another clearly enough for automated PCR amplification and gel electrophoresis to reliably identify each allele.
- Most CODIS STRs are located in noncoding regions of the genome.
- These noncoding STRs are designated by gene labels reading "D*S***." **e.g. D1S1656**. The "D" indicates that the STR is encoded in DNA, the number following D is the chromosome on which the STR is located, and the "S" indicates that the repetitive sequence of the STR is a "single" repetitive sequence, meaning that the STR is found just once in the genome.

Forensic Analysis Using CODIS

- In criminal cases where the DNA of a suspect is compared with a sample from a crime scene, the genotypes for as many of the CODIS markers as possible are compared to discover whether any mismatches are present.
- The principle at work in these comparisons is the principle of exclusion, meaning that if just one of the STR markers analyzed fails to match between a suspect and the crime scene or reference sample, the suspect is excluded as the source of the crime scene or reference DNA.

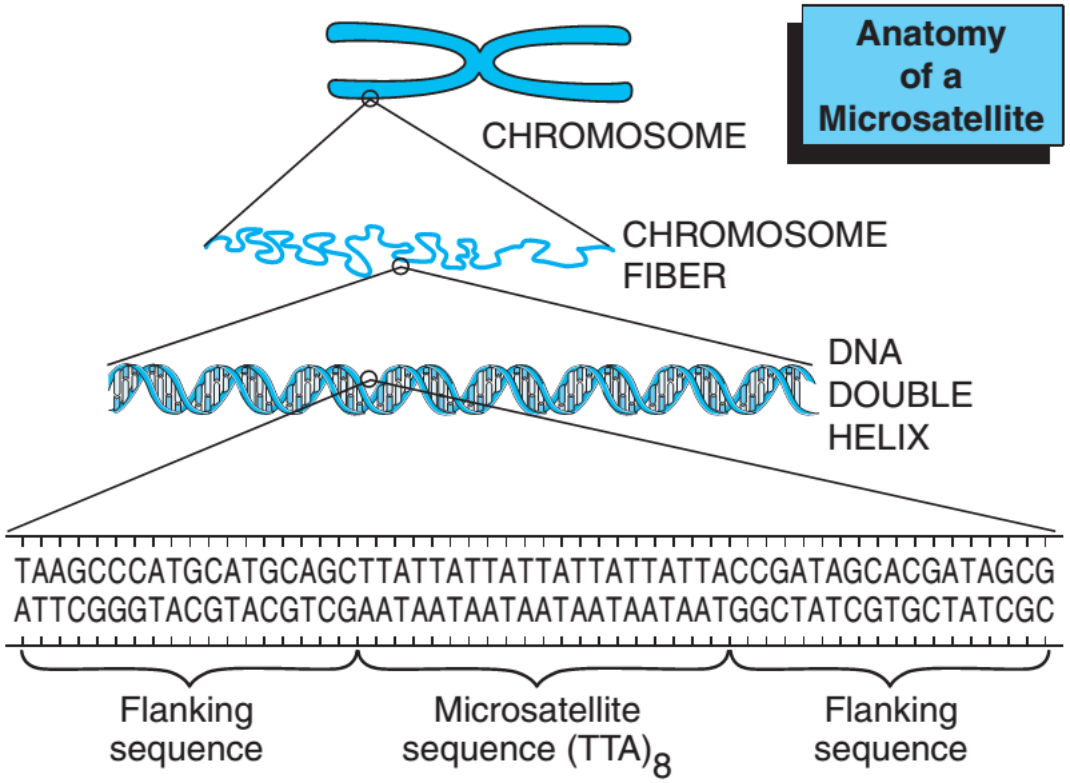


FIGURE 2 Anatomy of a DNA Microsatellite A microsatellite is a variable number of repeated nucleotides (like TTA) that occur in specific locations in the genome. Using primers for the flanking regions on the ends of the microsatellite allows for amplification using PCR (from both directions).
Individual inherit a specific number of these repeats from their parents, but the number of repeats varies for unrelated individuals, forming a distinct pattern or DNA fingerprint.

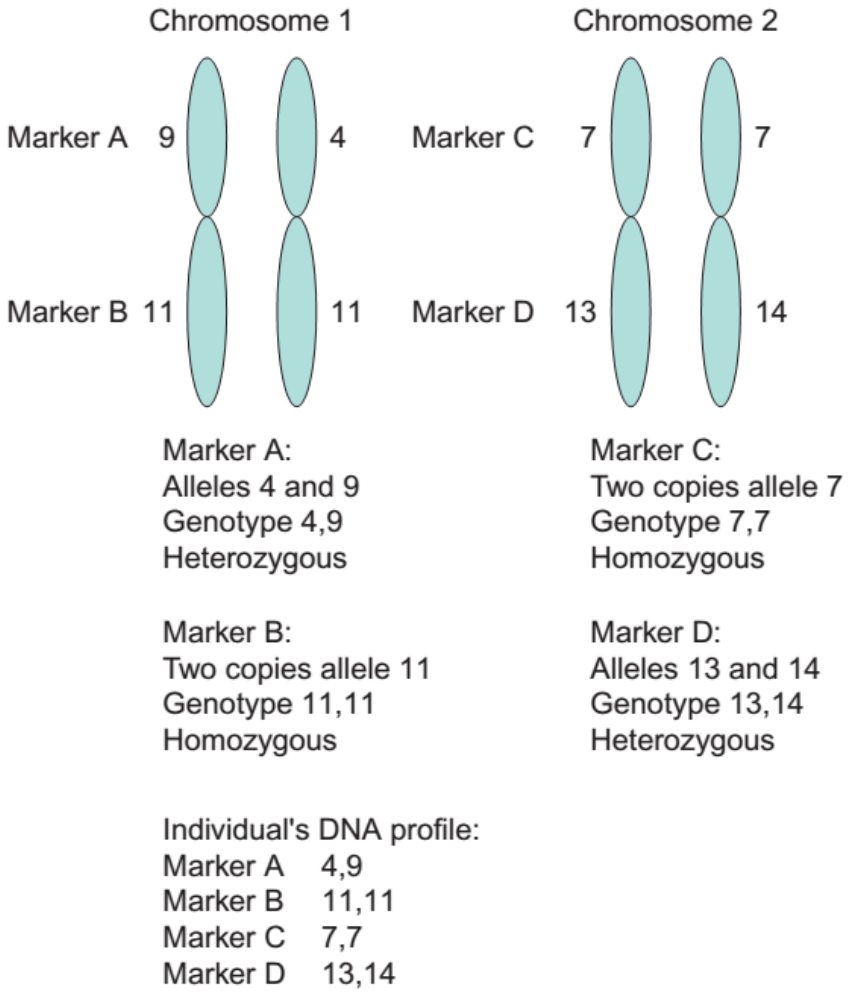
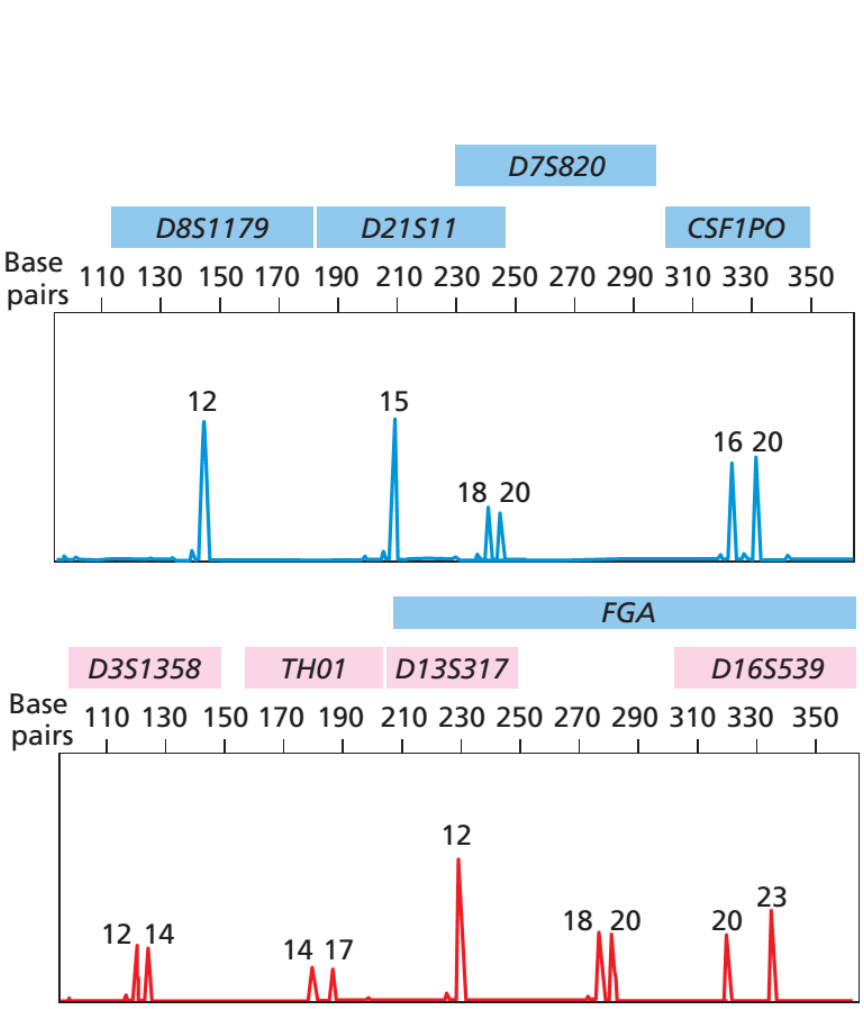


FIGURE 8.1 Relationships among chromosomes, markers, alleles, genotypes, and DNA profiles. Chromosome 1 has two alleles for marker A: 9 and 4, which are named for the number of repeats of a specific repeated sequence that exists at that position in the chromosome 1 DNA. The genome is heterozygous for the repeat at marker A because the alleles have different numbers of specific repeats. marker C on chromosome 2 is homozygous at marker C because each allele has seven copies of the specific repeated sequence.



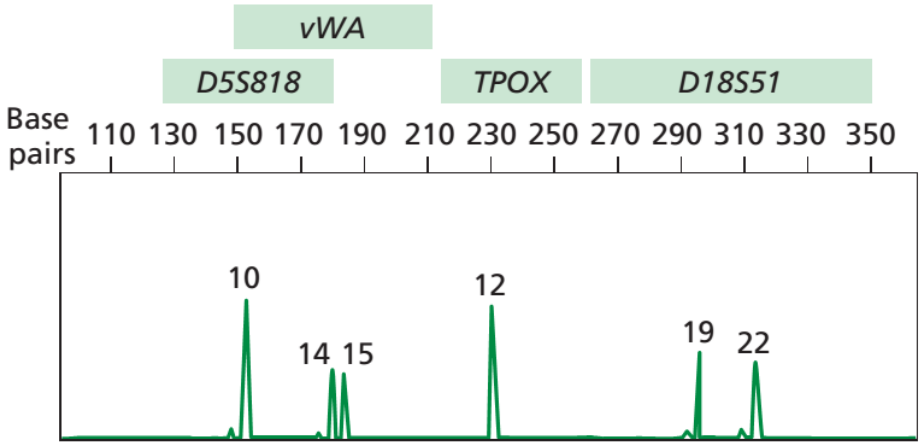


Figure E.1 An example of DNA profile results for the 13 original CODIS STRs. Homozygous STRs have one peak and heterozygous STRs have two peaks. Alleles are determined by the migration of DNA fragments along the base pair scale.

Figure E.1 illustrates example results obtained from the electrophoretic analysis of the 13 original CODIS markers. For each gene, one peak indicates a homozygous genotype and two peaks indicate a heterozygous genotype. For example, the sample shown is homozygous for *D8S1179* and for *D21S11*, and it is heterozygous for *FGA, D7S820,* and *CSF1PO*. Overall, this sample is homozygous for five genes and heterozygous for the other eight genes. The horizontal axis indicates the fragment length. Near each peak in the figure is a number indicating the number of repeats in each DNA fragment. These numbers are used to designate the different alleles for each STR. In this instance, the sample is homozygous *12/12* for *D8S1179,* homozygous *15/15* for *D21S11*, heterozygous *18/20* for *FGA*, and so on through the 13 STR genes.

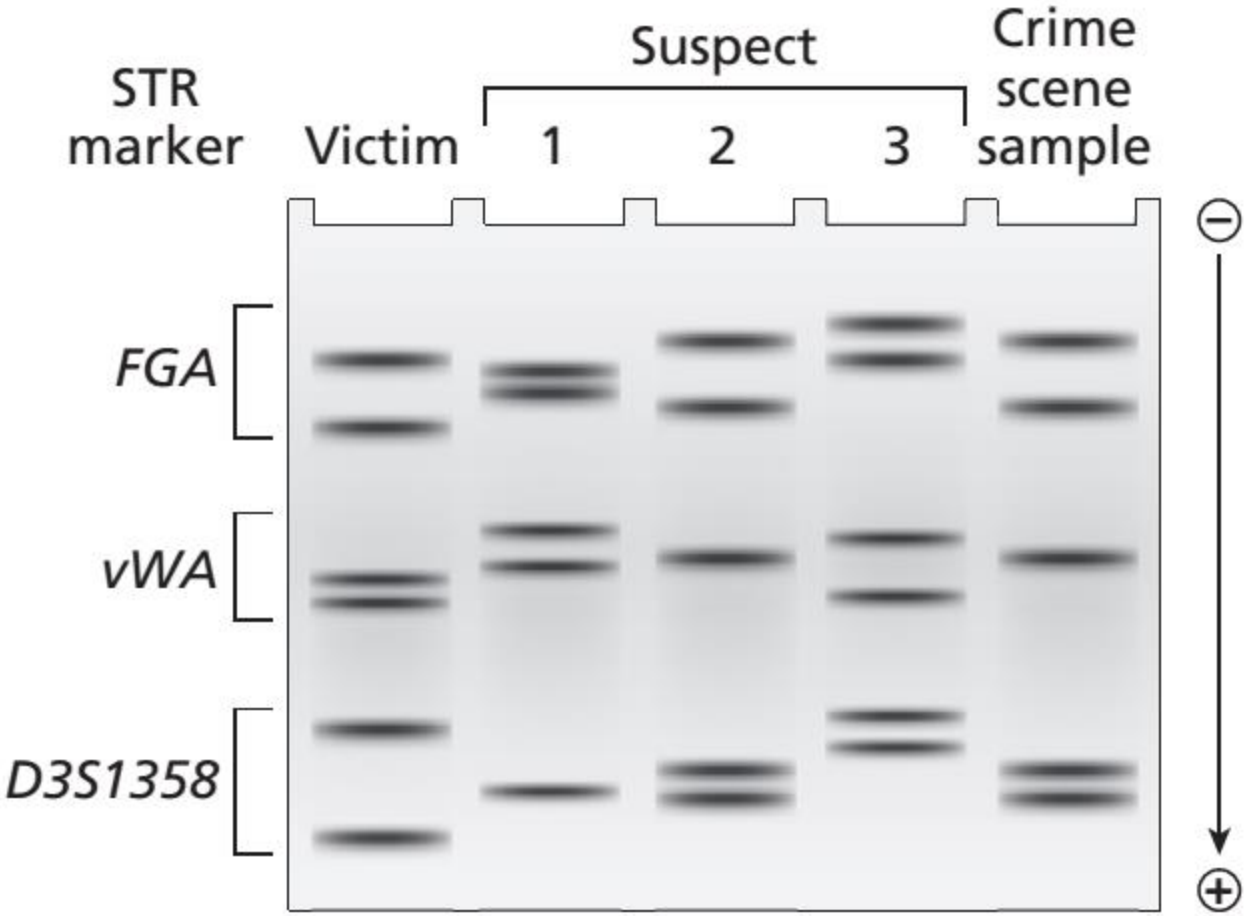


Figure E.2 **STR marker comparison.** Three suspect samples, a victim sample, and a crime scene sample are compared for three STRs to determine whether a suspect profile matches the crime scene profile. Suspects 1 and 3 are excluded based on mismatches, but Suspect 2 is not excluded.

More number of STRs are analyzed for suspect2 for further confirmation

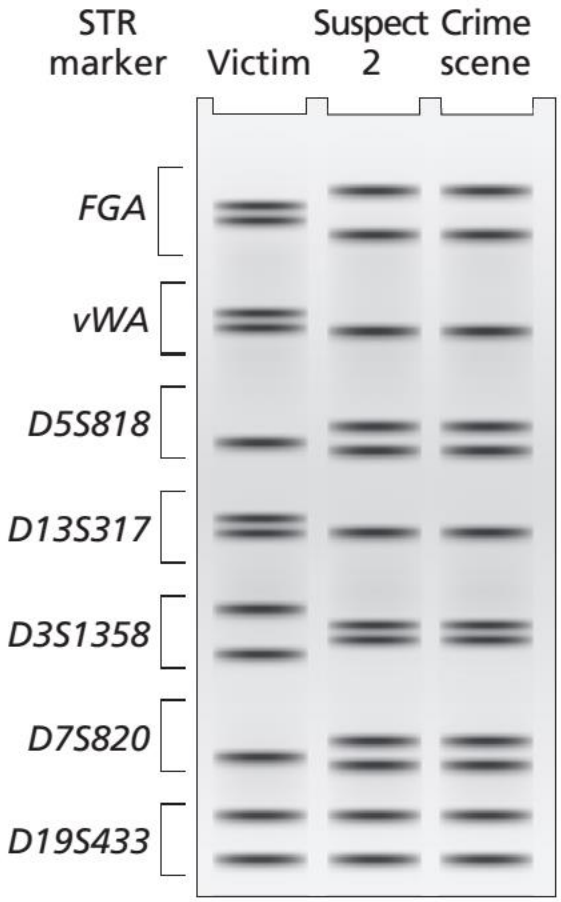


Figure E.3 Expanded STR marker comparison for seven markers. Suspect 2 is not excluded by analysis of additional STRs. The probability of a match is calculated using population frequencies of STR alleles.

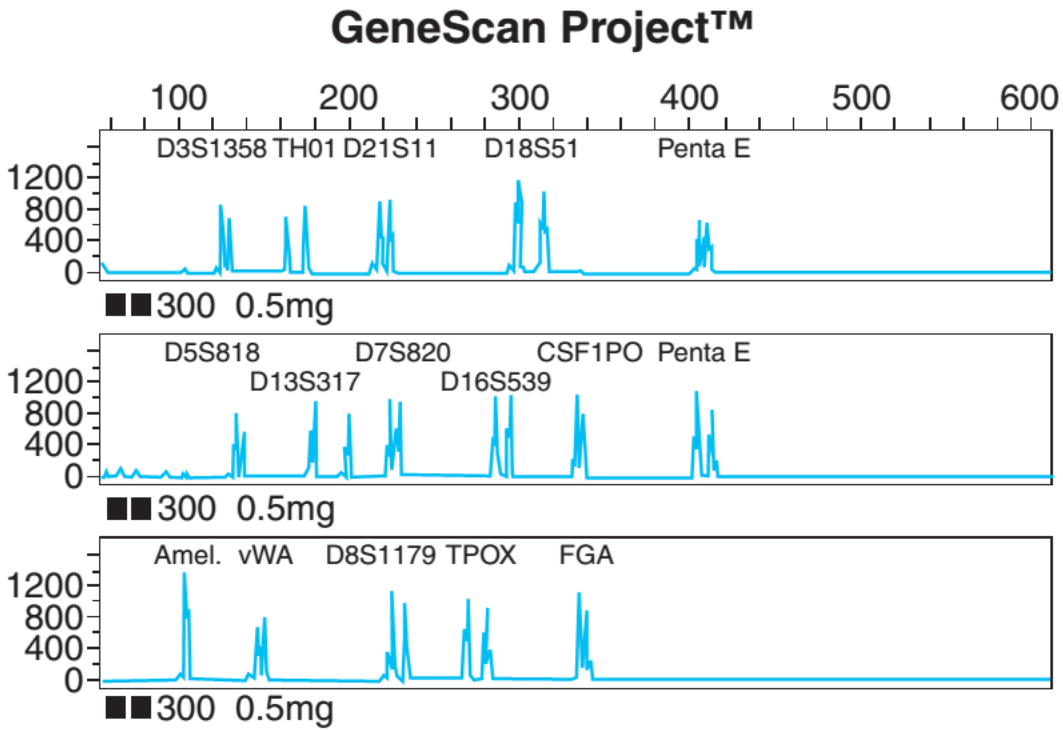


FIGURE 5 Gene Scan Display of all 13 STRs accepted by CODIS Utilizing capillary electrophoresis to detect the amplified STR microsatellite DNA sequences, it is possible to determine the number of STRs. By comparing the patterns obtained as evidence, it can be determined if the DNA patterns are exactly the same or different. Note that none of the DNA fingerprints shown are exact matches.

STR Analysis

- After STRs are amplified by PCR, the alleles are separated and detected using capillary electrophoresis, which separates the lengths of amplified DNA, allowing the number of repeats in each of the two alleles on homologous chromosomes to be determined.
- The number of repeats within an STR is referred to as an allele.
- For instance, the STR known as D7S820, found on chromosome 7, contains between 5 and 16 repeats of GATA. Therefore, there are 12 different alleles possible for the D7S820 STR sequence.
- An individual with D7S820 alleles 10 and 15, for example, would have inherited a copy of D7S820 with 10 GATA repeats from one parent and a copy of D7S820 with 15 GATA repeats from the other parent.

STR Analysis

Case Mockup: Results

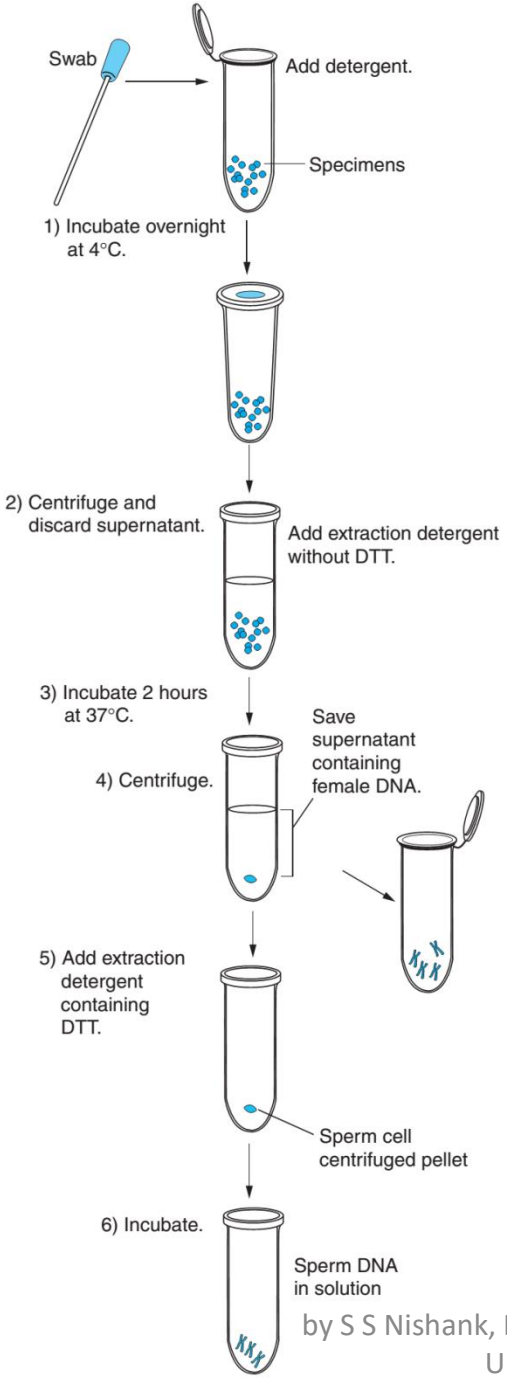
Sample ID	D3S1358	VWA	FGA	AMEL	D8S1179	D21S11	D18S51	D5S818	D13S317	D7S820
Jane Doe, Reference Sample	15, 16	16, 19	18, 22	X	11, 12	30, 31.2	14	9, 12	9, 12	8, 11
Dorothy Smith, Reference Sample	15, 18	16, 17	20, 21	X	10, 13	31, 31.2	12, 16	10, 12	11, 12	8
Knife Blade Stain A	15, 16	16, 19	18, 22	X	11, 12	30, 31.2	14	9, 12	9, 12	8, 11
Knife Blade Stain B	15, 16	16, 19	18, 22	X	11, 12	30, 31.2	14	9, 12	9, 12	8, 11

Conclusions: Dorothy Smith CAN be excluded from contributing to the DNA found on the knife blade stains A and B. Jane Doe CANNOT be excluded from contributing to the DNA found on the knife blade stains A and B.

STR Analysis

FIGURE 6 Who Is Excluded by DNA Comparison? The DNA profiles shown below were taken from a crime scene. Knife stains A and B were found on the blade of a knife used in the crime, while stain C was found on the handle of the knife. All three stains were amplified for DNA analysis. Two individuals were tested for a match to the DNA profiles. Read the conclusion from the figure and see if you agree with the analysis.

STR Analysis



Isolation of Sperm DNA or Vaginal Cell DNA from Mixed Sources

STR Analysis

Paternity Dispute

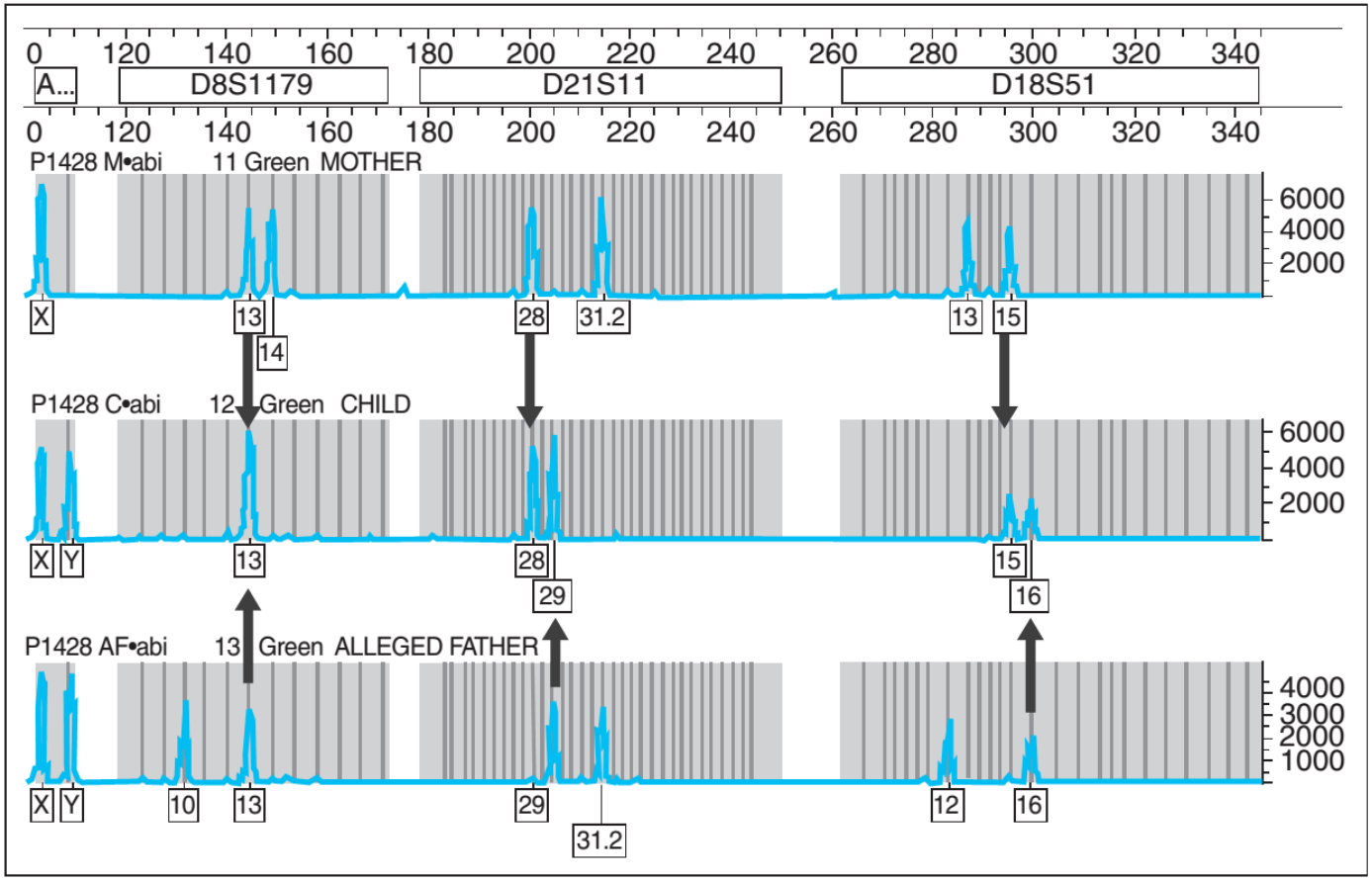


FIGURE 9 Paternity Dispute A man has claimed not to be the father of a child, but DNA allelic comparison shows that the child received one allele from each parent. Follow the arrows from the parents to the child to see how each parent contributed one of the two alleles (bands) that show in the DNA fingerprint of the child.

Use of Mitochondrial genes in DNA fingerprint

- Older biological samples that lack nucleated cellular material, such as hair, bones, and teeth, cannot be analyzed with STR and RFLP, but they can be analyzed with mtDNA.
- Comparing the mtDNA profile of unidentified remains with the profile of a potential maternal relative can determine whether they share the same mtDNA profile and are related.
- Since mtDNA remains virtually the same from generation to generation, changing only about 2% to 4% every million years due to random mutation.
- Consequently relationships can be traced through the unbroken maternal line, as shown in Figure.
- It has been shown that forensic scientists can amplify the HV1 and HV2 regions of the mtDNA, and then sequence each region and compare single nucleotide differences to a reference sample.
- Also, mtDNA that is maternally inherited come from the mother and can be directly linked to maternal relatives and can be used as match references to establish family relationships through the mother's side.

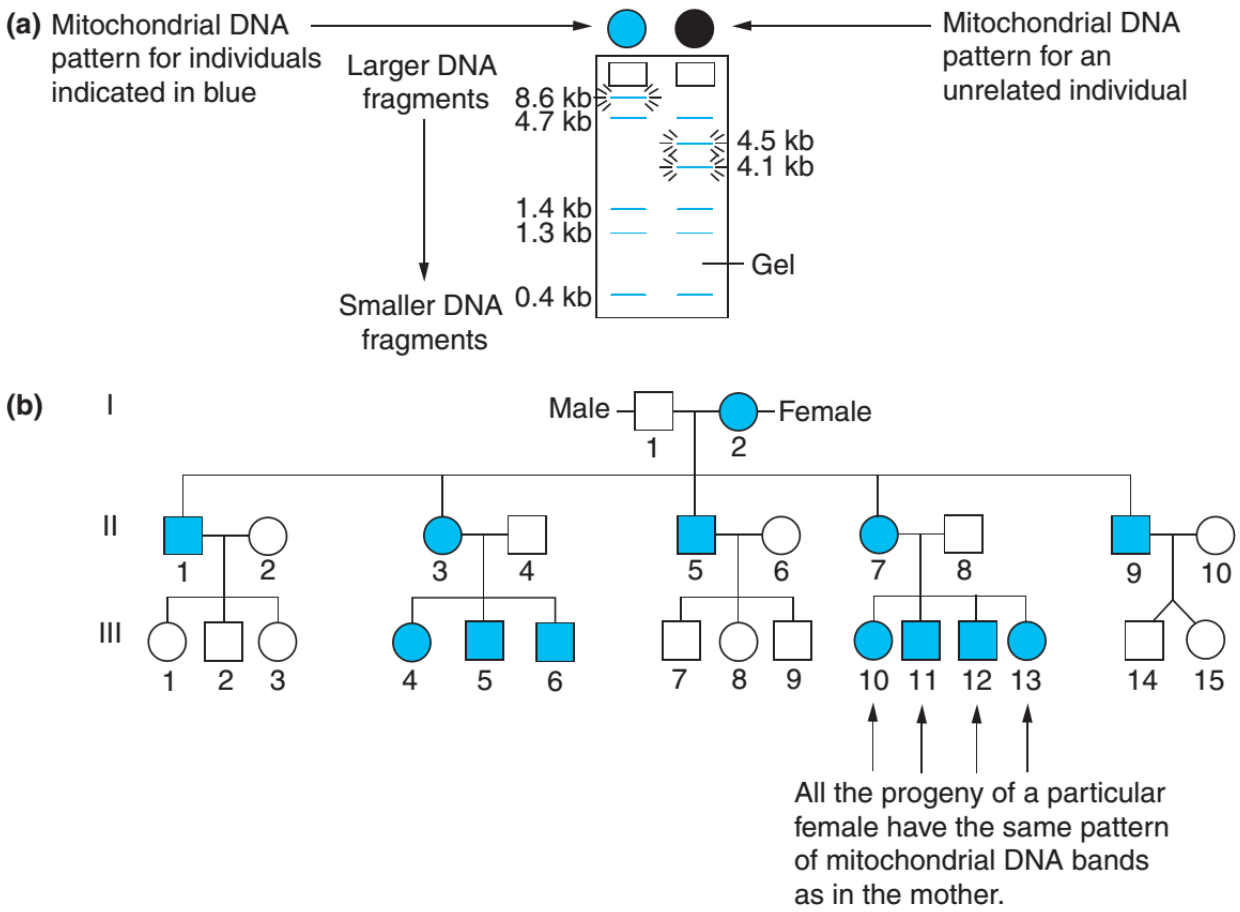


FIGURE 11 Maternal DNA Pattern of Inheritance Key genes for mitochondrial function (cell respiration) are located in a small DNA ring in human mitochondria. Because mitochondria are contributed by the egg (only) before fertilization, DNA can be traced through the maternal line with fingerprinting of the mitochondrial DNA. Some genes in mitochondria show variations due to mutations (see top of gel at 8.6, 4.7 vs. 4.5, 4.1 kilobases); others do not.

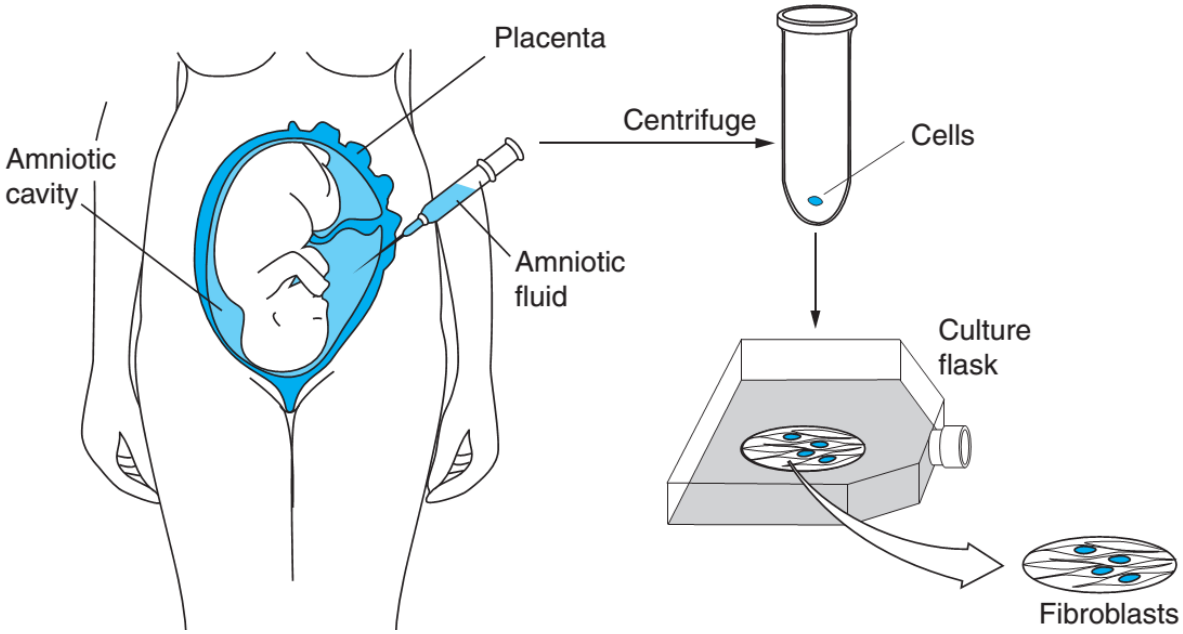


FIGURE 10 DNA for Paternity Testing For disputed paternity suits, it is possible to draw a few fetal cells from the fluid that surrounds the fetus (amniotic fluid) without harming the growing fetus. When cultured, these cells can be a source for DNA extraction and fingerprinting. When compared with the DNA of the suspected father, exclusion or inclusion of the individual can be determined.

DNA fingerprinting by Y chromosome

- Y-STR analysis can help in the identification of paternally related males.
- The use of Y-chromosome-specific PCR primers can improve the chances of detecting low levels of the perpetrator's DNA in a high background of the female victim's DNA.

Applications of DNA fingerprinting

Identifying Plants through DNA profile