TEJASVI NAVADHITAMASTU

"Let our (the teacher and the taught) learning be radiant"

Let our efforts at learning be luminous and filled with joy, and endowed with the force of purpose

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DNA Fingerprinting

History and terminology

The original DNA fingerprinting technique was developed by Alec Jaffreys in 1985. Although the DNA fingerprinting is commonly used, a more general term ***DNA profiling*** is preferred. This is due to the fact that a wide range of tests can be carried out by DNA sequencing with improved technology.

CURRENT TECHNOLOGY OF DNA FINGERPRINTING

In the forensic analysis of DNA, the original techniques based on RFLPs and VNTRs are now largely replaced by microsatellites (short tandem repeats). The basic principle involves the amplification of microsatellites by polymerase chain reaction followed by their detection.

It is now possible to generate a DNA profile by automated DNA detection system (comparable to the DNA sequencing equipment).

DNA FINGERPRINTING OR DNA PROFILING

DNA fingerprinting is the present day ***genetic detective*** in the practice ***of modern medical forensics***. The underlying principles of DNA fingerprinting are briefly described.

The structure of each person's genome is unique. The only exception being monozygotic identical twins (twins developed from a single fertilized ovum). The unique nature of genome structure provides a good opportunity for the specific identification of an individual.

It may be remembered here that in the traditional fingerprint technique, the individual is identified by preparing an ink impression of the skin folds at the tip of the person's finger. This is based on the fact that the nature of these skin folds is genetically determined, and thus the fingerprint is unique for an individual. In contrast, the ***DNA fingerprint is an analysis of the nitrogenous base sequence in the DNA of an individual***.

1984,86 **Alec Jeffreys** discovered the technique of **DNA fingerprinting**.

1991 **Dr. Lalji Singh** at CCMB, Hyderabad has developed a new technique of DNA fingerprinting by using **BKM-DNA probe** (BKM = banded krait minor satellite). He discovered this probe while he was working on sex determination in snake, the banded krait (*Bungarus fasciatus*) for his Ph.D. work.

In India, DNA fingerprinting tests are carried out at the Centre for Cell and Molecular Biology (CCMB), Hyderabad. For this purpose, a test with the **BKM-DNA probe** (= banded krait minor satellite DNA) earlier used for identification of sex chromosomes (by **Dr. Lalji Singh**) has been found to cost one-tenth of the cost of tests used in Europe and U.S.A. Paternity dispute cases are much more common in India and most of them are referred to CCMB for DNA evidence. The first such test on DNA fingerprinting was used in June, 1989 to settle a drawn-out paternity case in Madras.

Procedure

- Restriction fragment length polymorphisms are often found in noncoding regions of DNA and are therefore frequently quite variable in humans. Two randomly chosen people will differ at many RFLPs and, if enough RFLPs are examined, no two people (with the exception of identicaltwins) will be exactly the same. The use of DNA sequences to identify a person, called DNA fingerprinting, is a powerful tool for criminal investigations and other forensic applications.

- In a typical application, DNA fingerprinting might be used to confirm that a suspect was present at the scene of a crime. A sample of DNA from blood, semen, hair, or other body tissue is collected from the crime scene. If the sample is very small, PCR can be used to amplify it so that enough DNA is available for testing. Additional DNA samples are collected from one or more suspects.

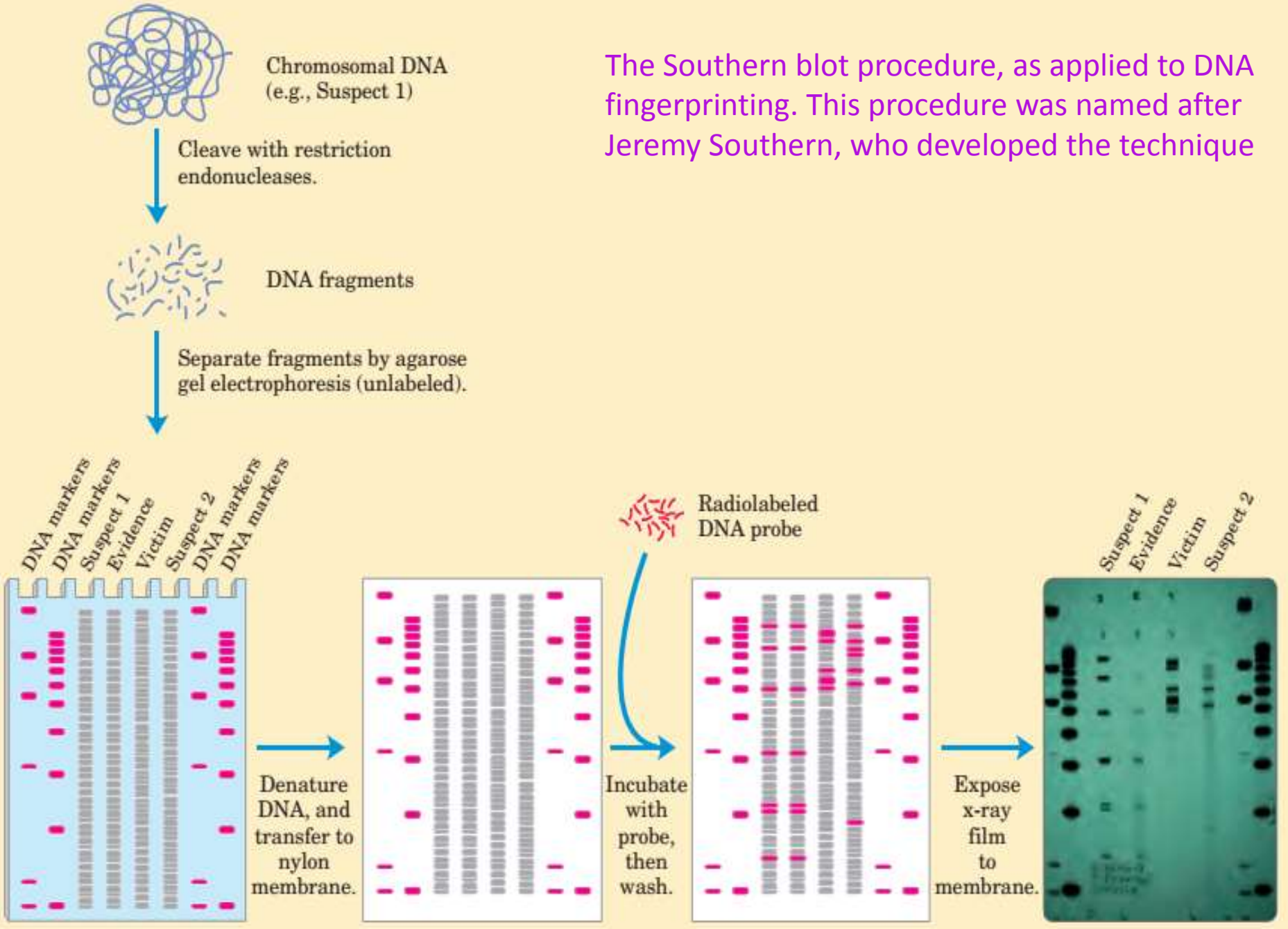
- Each DNA sample is cut with one or more restriction enzymes, and the resulting DNA fragments are separated by gel electrophoresis. The fragments in the gel are denatured and transferred to nitrocellulose paper by Southern blotting. One or more radioactive probes is then hybridized to the nitrocellulose and detected by autoradiography. The pattern of bands produced by DNA from the sample collected at the crime scene is then compared with the patterns produced by DNA from the suspects.

- 4The probes used in DNA fingerprinting detect highly variable regions of the genome; so the chances of DNA from two people producing exactly the same banding pattern is low. When several probes are used in the analysis, the probability that two people have the same set of patterns becomes vanishingly small (unless they are identical twins).

- A match between the sample from the crime scene and one from the suspect can provide evidence that the suspect was present at the scene of the crime.

- The probes most commonly used in DNA fingerprinting are complementary to short sequences repeated in tandem that are widely found in the human genome. People vary greatly in the number of copies of these repeats; thus, these polymorphisms are termed variable number of tandem repeats (VNTRs)

The Southern blot procedure, as applied to DNA fingerprinting. This procedure was named after Jeremy Southern, who developed the technique



Applications of DNA fingerprinting

The amount of DNA required for DNA fingerprint is remarkably small. The ***minute quantities of DNA*** from blood strains, body fluids, hair fiber or skin fragments ***are enough***. ***Polymerase chain reaction is used to amplify*** this DNA for use in fingerprinting. DNA profiling has wide range of applications—most of them related to medical forensics. Some important ones are listed below.

Identification of criminals, rapists, thieves etc.

Settlement of paternity disputes.

Use in immigration test cases and disputes.

In general, the fingerprinting technique is carried out by collecting the DNA from a suspect (or a person in a paternity or immigration dispute) and matching it with that of a reference sample (from the victim of a crime, or a close relative in a civil case).

DNA MARKERS IN DISEASE DIAGNOSIS AND FINGERPRINTING

The ***DNA markers are highly useful for genetic mapping of genomes***. There are four types of DNA sequences which can be used as markers.

1. Restriction fragment length polymorphisms (***RFLPs***, pronounced as rif-lips).

2. Minisatellites or variable number tandem repeats (***VNTRs***, pronounced as vinters).

3. Microsatellites or simple tandem repeats (***STRs***).

4. Single nucleotide polymorphisms (***SNPs***, pronounced as snips).

The general aspects of the above DNA markers are described along with their utility in disease diagnosis and DNA fingerprinting.

RESTRICTION FRAGMENT LENGTH POLYMORPHISMS (RFLPs)

A RFLP represents a stretch of DNA that serves as a marker for mapping a specified gene. RFLPs are located randomly throughout a person's chromosomes and have no apparent function.

A DNA molecule can be cut into different fragments by a group of enzymes called restriction endonucleases (***See Table 27.1***). These fragments are called ***polymorphisms*** (literally means ***many forms***).

An outline of RFLP is depicted in ***Fig.27.22***. The DNA molecule 1 has three restriction sites (R_1, R_2, R_3), and when cleaved by restriction endonucleases forms 4 fragments. Let us now consider DNA 2 with an inherited mutation (or a genetic change) that has altered some base pairs. As a result, the site (R_2) for the recognition by restriction endonuclease is lost. This DNA molecule 2 when cut by restriction endonuclease forms only 3 fragments (instead of 4 in DNA 1).

As is evident from the above description, a stretch of DNA exists in ***fragments*** of various ***lengths*** (***polymorphisms***), derived by the action of ***restriction*** enzymes, hence the name restriction fragment length polymorphisms.

RFLPs in the diagnosis of diseases

If the RFLP lies within or even close to the locus of a gene that causes a particular disease, it is possible to trace the defective gene by the analysis of RFLP in DNA. The person's cellular DNA is isolated and treated with restriction enzymes. The DNA fragments so obtained are separated by electrophoresis. The RFLP patterns of the disease suspected individuals can be compared with that of normal people (preferably with the relatives in the same family). By this approach, it is possible to determine whether the individual has the marker RFLP and the disease gene. With 95% certainity, RFLPs can ***detect single gene-based diseases***.

Applications of RFLPs : The approach by RFLP is very powerful and has helped many genes to be mapped on the chromosomes. e.g. sickle-cell anemia (chromosome 11), cystic fibrosis (chromosome 7), Huntington's desease (chromosome 4), retinoblastoma (chromosome 13), Alzheimer's disease (chromosome 21).

VARIABLE NUMBER TANDEM REPEATS (VNTRs)

VNTRs, also known as minisatellites, like RFLPs, are DNA fragments of different length. The main difference is that RFLPs develop from random mutations at the site of restriction enzyme activity while VNTRs are formed due to different number of base sequences between two points of a DNA molecule. In general, VNTRs are made up of tandem repeats of short base sequences (10–100 base pairs). The number of elements in a given region may vary, hence they are known as variable number tandem repeats.

An individual's genome has many different VNTRs and RFLPs which are unique to the individual. The ***pattern of VNTRs and RFLPs forms the basis of DNA fingerprinting*** or DNA profiling.

In the ***Fig.27.24***, two different DNA molecules with different number of copies (bands) of VNTRs are shown. When these molecules are subjected to restriction endonuclease action (at two sites R_1 and R_2), the VNTR sequences are released, and they can be detected due to variability in repeat sequence copies. These can be used in mapping of genomes, besides their utility in DNA fingerprinting.

VNTRs are useful for the detection of certain genetic diseases associated with alterations in the degree of repetition of microsatellites e.g. Huntington's chorea is a disorder which is found when the VNTRs exceed 40 repeat units.

Limitations of VNTRs : The major drawback of VNTRs is that they are not evenly distributed throughout the genome. VNTRs tend to be localized in the telomeric regions, at the ends of the chromosomes.

Use of RFLPs and VNTRs in genetic fingerprinting

RFLPs caused by variations in the number of VNTRs between two restriction sites can be detected (***Fig.27.25***). The DNAs from three individuals with different VNTRs are cut by the specific restriction endonuclease. The DNA fragments are separated by electrophoresis, and identified after hybridization with a probe complementary to a specific sequence on the fragments.

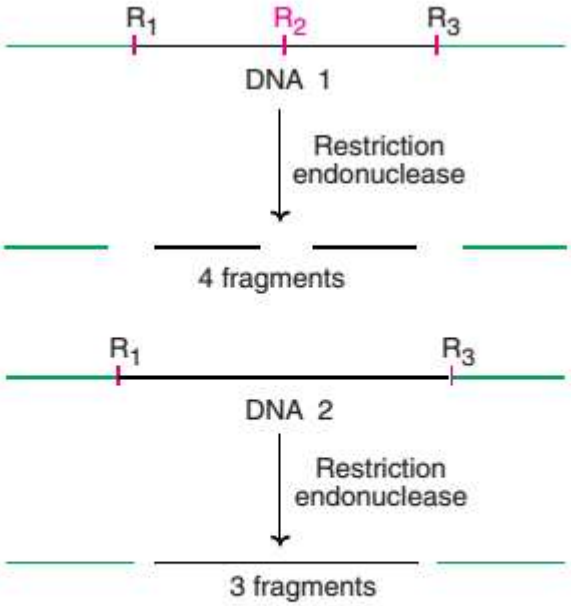


Fig. 27.22 : *An outline of the restriction fragment length polymorphism (RFLP) (R_1, R_2, R_3 represent the sites for the action of restriction endonucleases).*

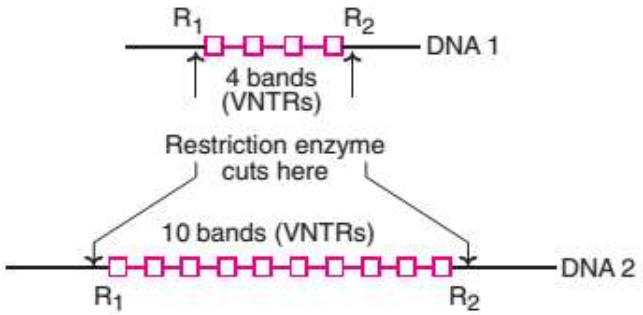


Fig. 27.24 : *A diagrammatic representation of variable number tandem repeats (VNTRs). Each band (or copy) represents a repeating sequence in the DNA (e.g. 100 base pairs each). R_1 and R_2 indicate the sites cut by a restriction enzyme.*

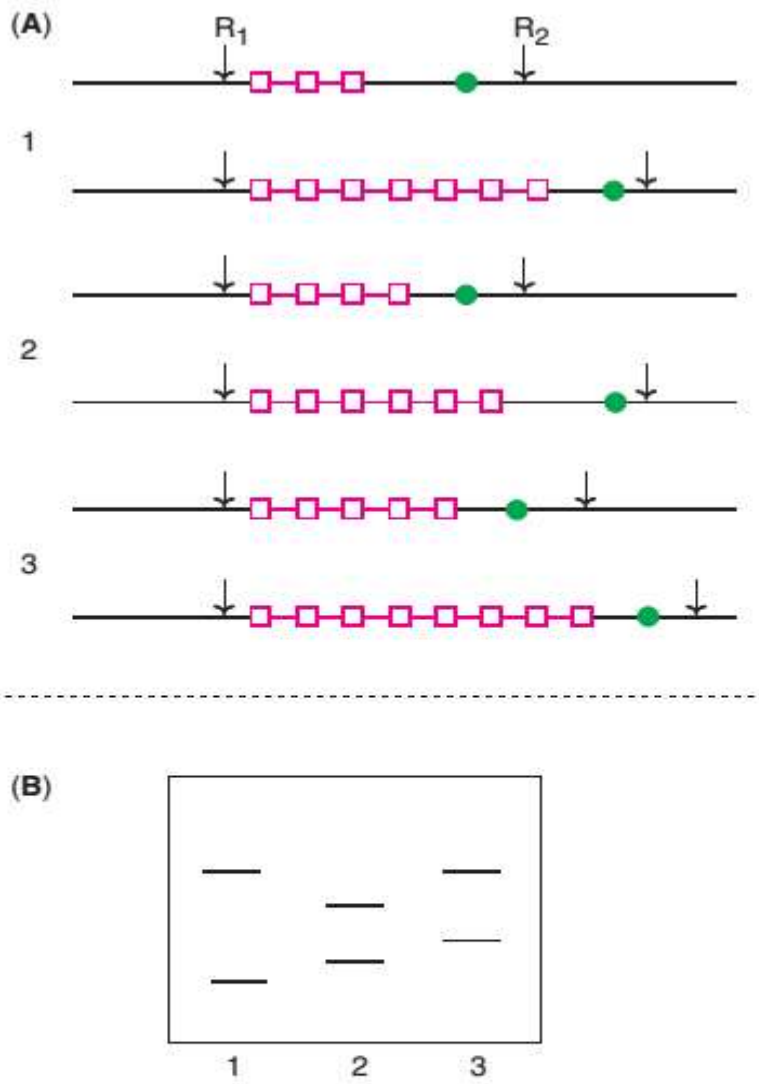


Fig. 27.25 : *Use of restriction fragment length polymorphisms (RFLPs) caused by variable number tandem repeats (VNTRs) in genetic fingerprinting (**A**) An illustration of DNA structure from three individuals (**B**) Hybridized pattern of DNA fragment with a probe complementary to the sequence shown in green circles (1, 2 and 3 represent the individuals; R_1 and R_2 indicate restriction sites; coloured squares are the number of VNTRs)*

MICROSATELLITES (SIMPLE TANDEM REPEATS)

Microsatellites are short repeat units (10–30 copies) usually composed of dinucleotide or tetranucleotide units. These simple tandem repeats (STRs) are more popular than minisatellites (VNTRs) as DNA markers for two reasons.

1. Microsatellites are evenly distributed throughout the genome.

2. PCR can be effectively and conveniently used to identify the length of polymorphism.

Two variants (alleles) of DNA molecules with 5 and 10 repeating units of a dimer nucleotides (GA) are depicted in ***Fig.27.26***.

By use of PCR, the region surrounding the microsatellites is amplified, separated by agarose gel electrophoresis and identified.

SINGLE NUCLEOTIDE POLYMORPHISMS (SNPs)

SNPs represent the positions in the genome where some individuals have one nucleotide (e.g. G) while others have a different nucleotide (e.g. C). There are large numbers of SNPs in genomes. It is estimated that the human genome contains at least 3 million SNPs. Some of these SNPs may give rise to RFLPs.

SNPs are highly useful as DNA markers since there is no need for gel electrophoresis and this saves a lot of time and labour. The detection of SNPs is based on the oligonucleotide hybridization analysis (***Fig.27.27***).

An oligonucleotide is a short single-stranded DNA molecule, synthesized in the laboratory with a length not usually exceeding 50 nucleotides. Under appropriate conditions, this nucleotide sequence will hybridize with a target DNA strand if both have completely base paired structure. Even a single mismatch in base pair will not allow the hybridization to occur.

DNA chip technology is most commonly used to screen SNPs hybridization with oligo-nucleotide (***See*** p. 593).

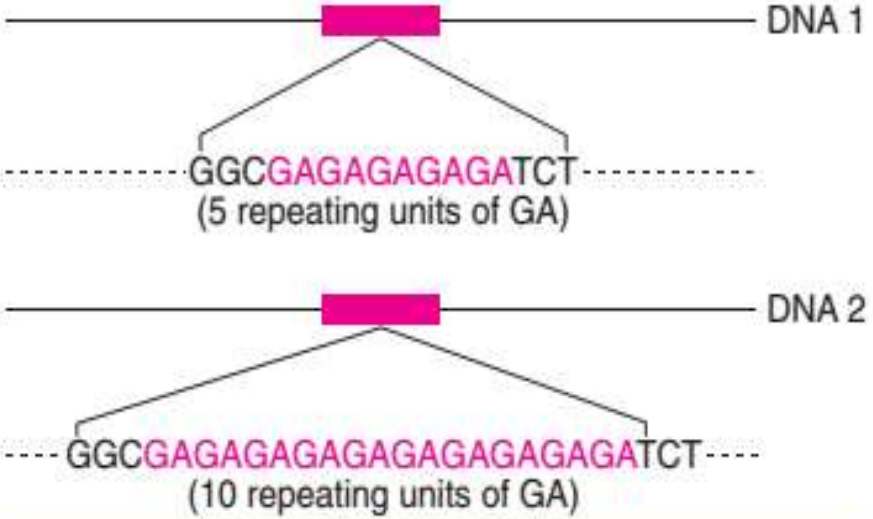


Fig. 27.26 : *Two alleles of DNA molecules representing 5 and 10 dimer repeating units.*

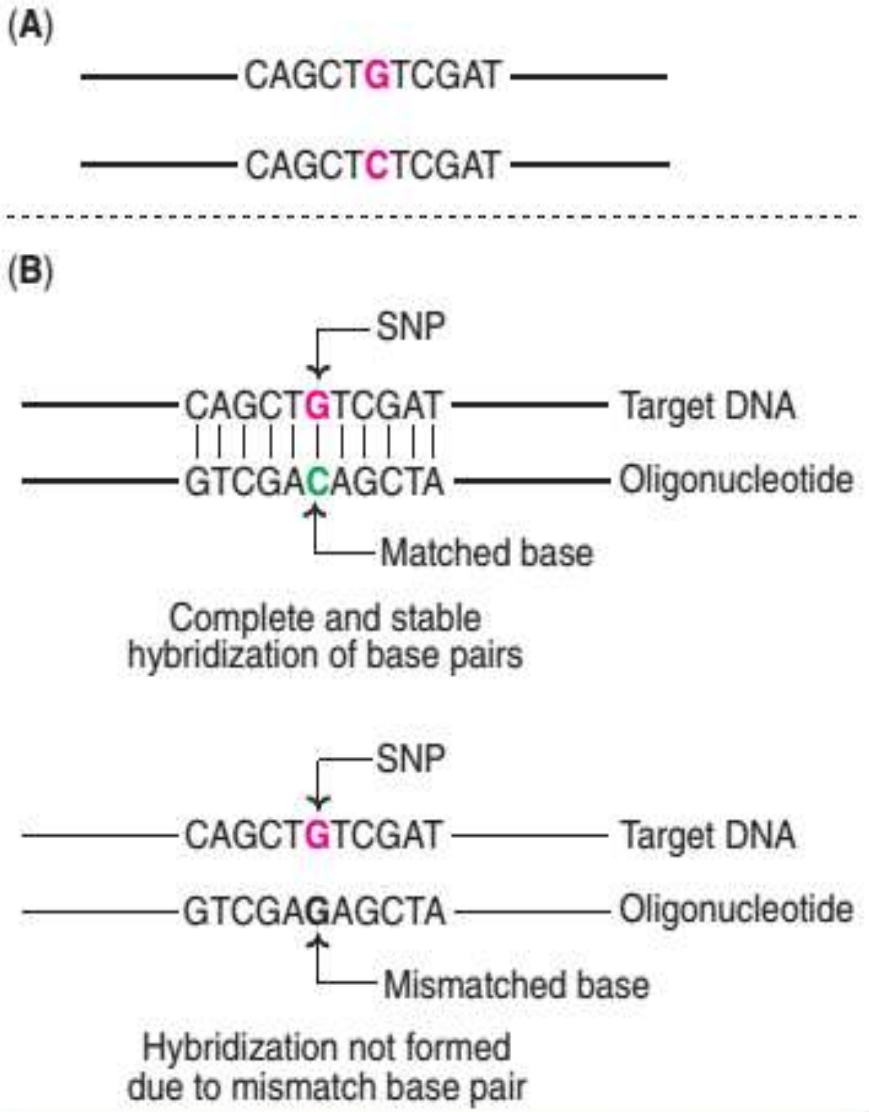


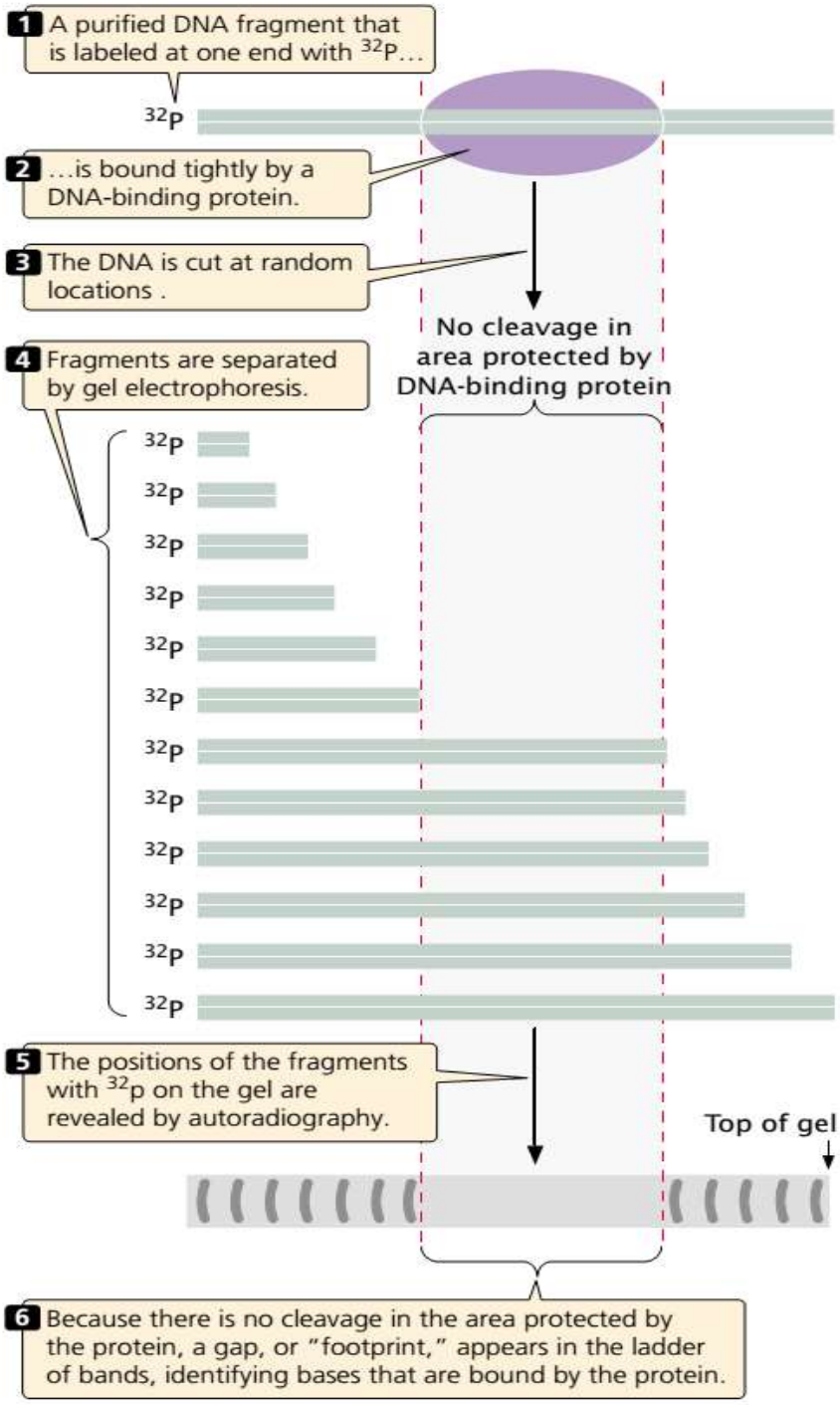
Fig. 27.27 : *(**A**) An illustration of single nucleotide polymorphism (SNP) (**B**) Oligonucleotide hybridization to detect SNP.*

DNA FOOT PRINTING

DNA FOOT PRINTING

DNA with protein bound is resistant to digestion by DNase enzymes. When a sequencing reaction is performed using such DNA, a protected area, representing the "footprint" of the bound protein, will be detected because nucleases are unable to cleave the DNA directly bound by the protein

DNA footprinting can be used to determine which DNA sequences are bound by binding proteins.



Procedure

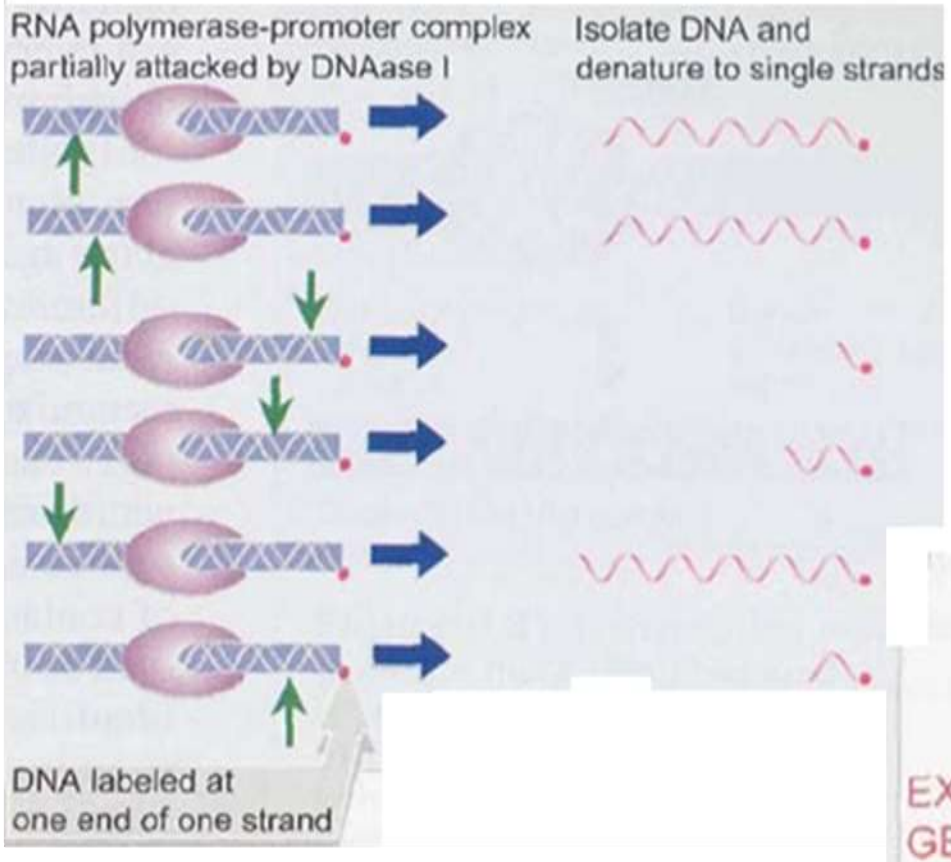
DNA footprinting: Many important DNA sequences serve as binding sites for proteins; for example, consensus sequences in promoters are often binding sites for transcription factors. DNA footprinting can be used to determine DNA sequences bound by such proteins.

In a typical DNA-footprinting experiment, purified DNA fragments are labeled at one end with a radioactive isotope of phosphorus, ^{32}P. An enzyme or chemical that makes cuts in DNA is used to cleave the DNA randomly into subfragments, which are then denatured and separated by gel electrophoresis.

The positions of the subfragments are visualized with autoradiography. This procedure is carried out both in the presence and in the absence of a particular DNA-binding protein.

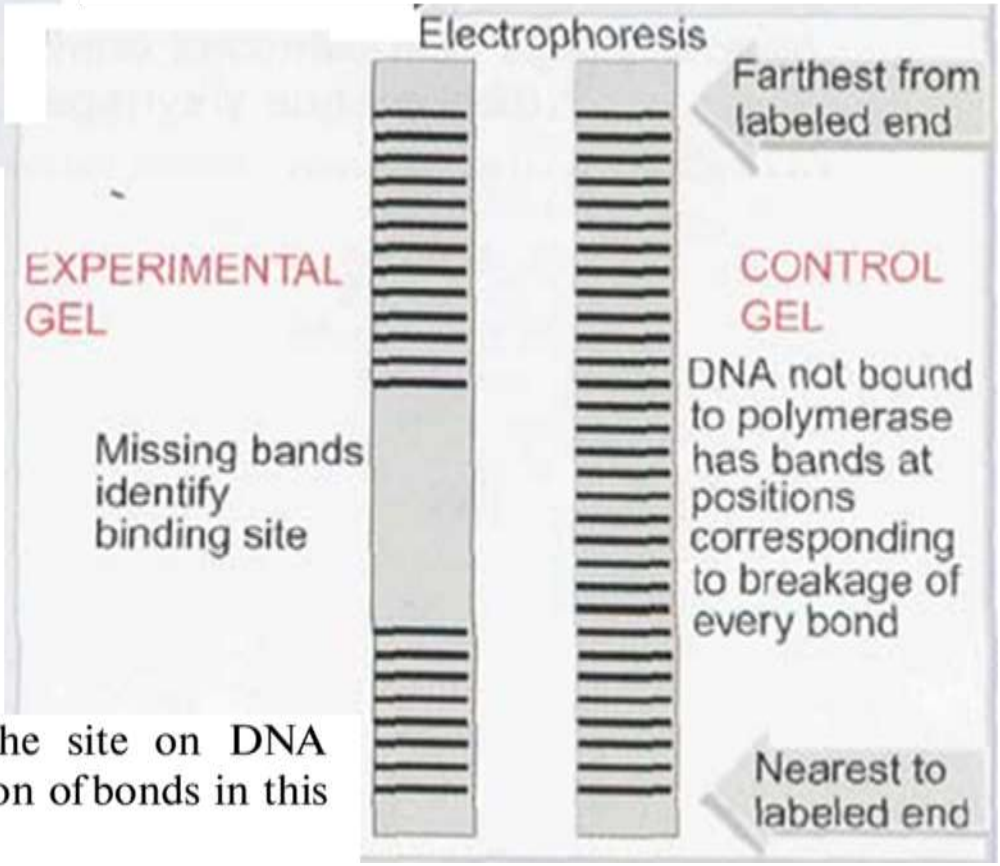
When the protein is absent, cleavage is random along the DNA, producing a continuous "ladder" of bands on the autoradiograph. When the protein is present, it binds to specific nucleotides and protects their phosphodiester bonds from cleavage. Therefore, there is no cleavage in the area protected by the protein, and no labeled fragments terminating in the binding site appear on the autoradiograph.

Their omission leaves a gap, or "footprint", on the ladder of bands (see Figure 18.20), and the position of the footprint identifies those nucleotides bound tightly by the protein



The ability of RNA polymerase (or indeed any protein) to recognize DNA can be characterized by footprinting. A sequence of DNA bound to the protein is *partially* digested with an endonuclease to attack individual phosphodiester bonds within the nucleic acid. Under appropriate conditions, any particular phosphodiester bond is broken in some, but not in all, DNA molecules. The positions that are cleaved are recognized by using DNA labeled on one strand at one end only. The principle is the same as that involved in DNA sequencing; partial cleavage of an end-labeled molecule at a susceptible site creates a fragment of unique length.

Electrophoresis



Foot printing identifies DNA binding sites for proteins by their Protection against nicking.

Footprinting is a technique for identifying the site on DNA bound by some protein by virtue of the protection of bonds in this region against attack by nucleases.

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