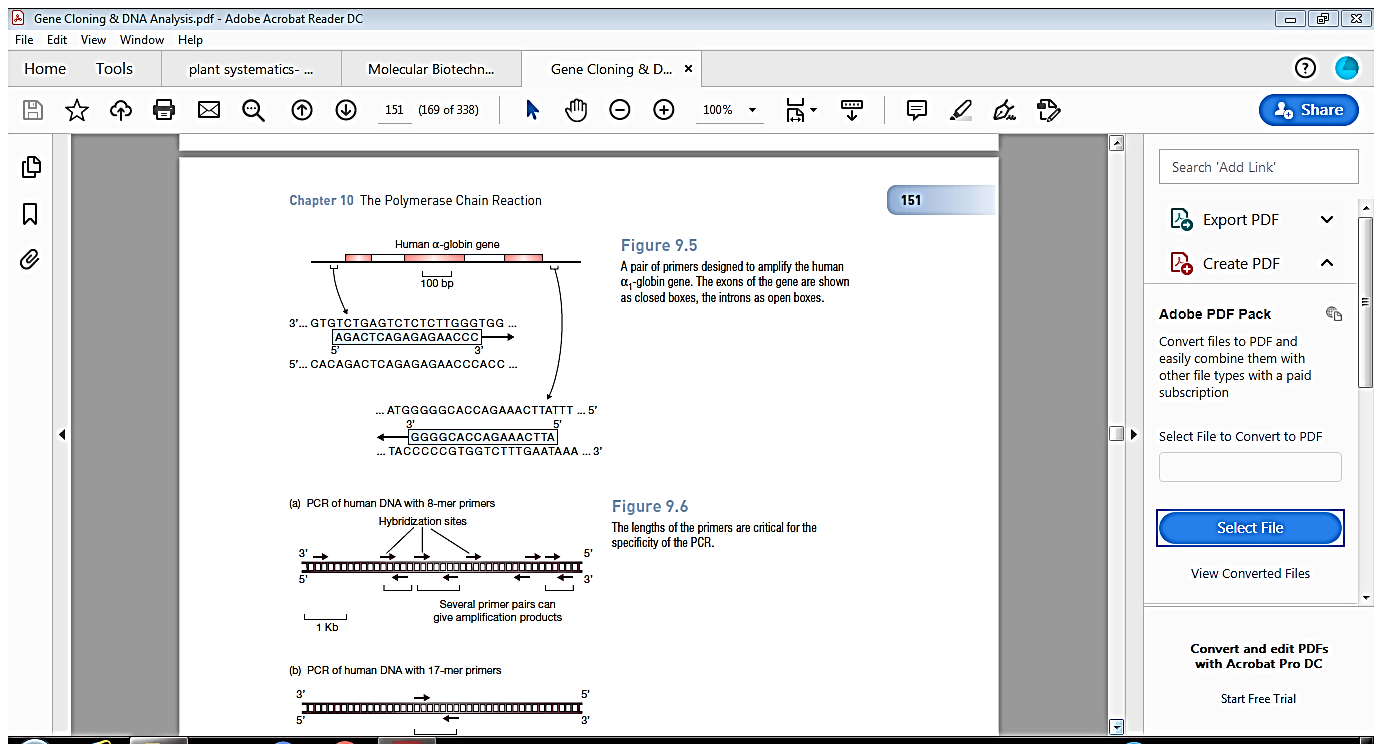
**Polymerase Chain Reaction**

PCR is an effective procedure for generating large quantities of a specific DNA sequence in vitro. This amplification, which can be more than a million-fold is achieved by a three-step cycling process. The polymerase chain reaction results in the selective amplification of a chosen region of a DNA molecule.

**Components of PCR**

* **PRIMERS-** Two synthetic oligonucleotide primers (~20 nucleotides each) that are complementary to regions on opposite strands that flank the target DNA sequence and that, after annealing to the source DNA, have their 3′ hydroxyl ends oriented toward each other. Any region of any DNA molecule can be chosen, so long as the sequences at the borders of the region are known. The border sequences must be known because in order to carry out a PCR, two short oligonucleotides must hybridize to the DNA molecule, one to each strand of the double helix. These oligonucleotides, which act as primers for the DNA synthesis reactions, delimit the region that will be amplified.

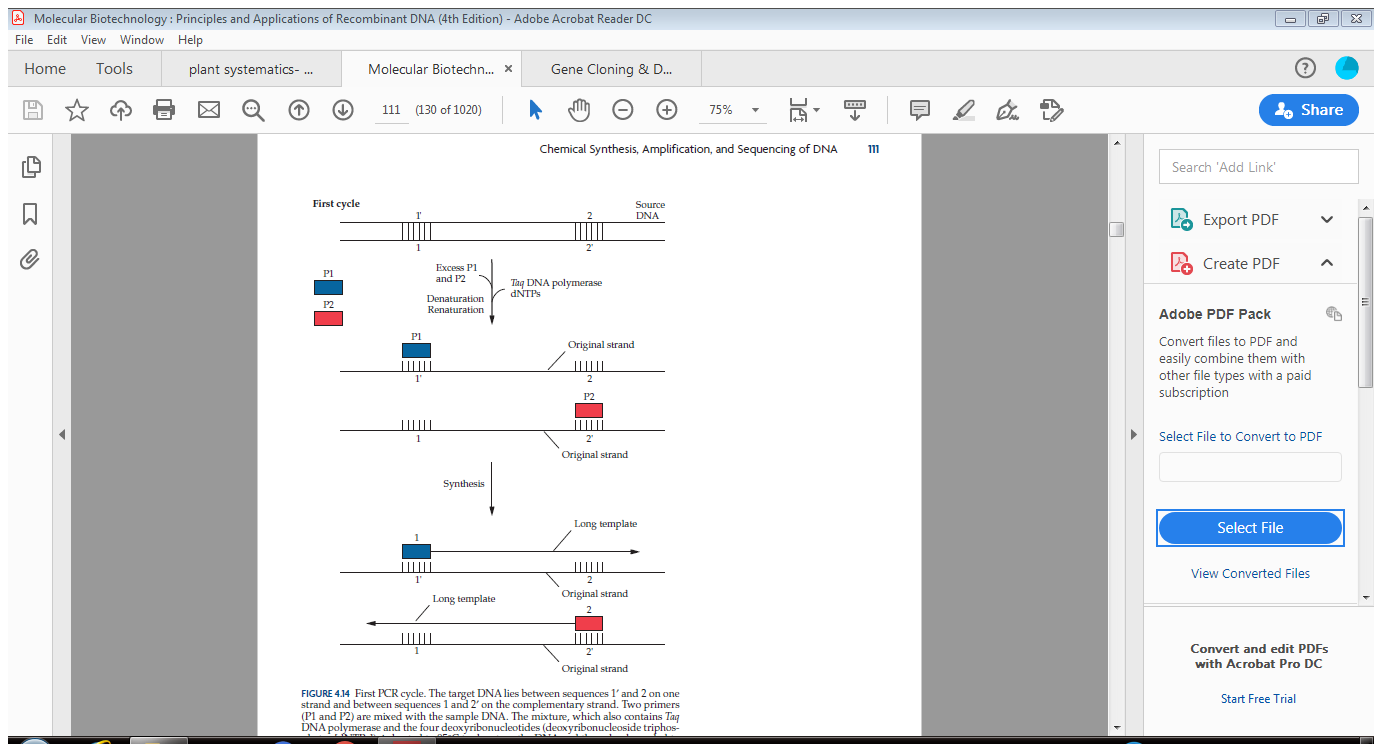
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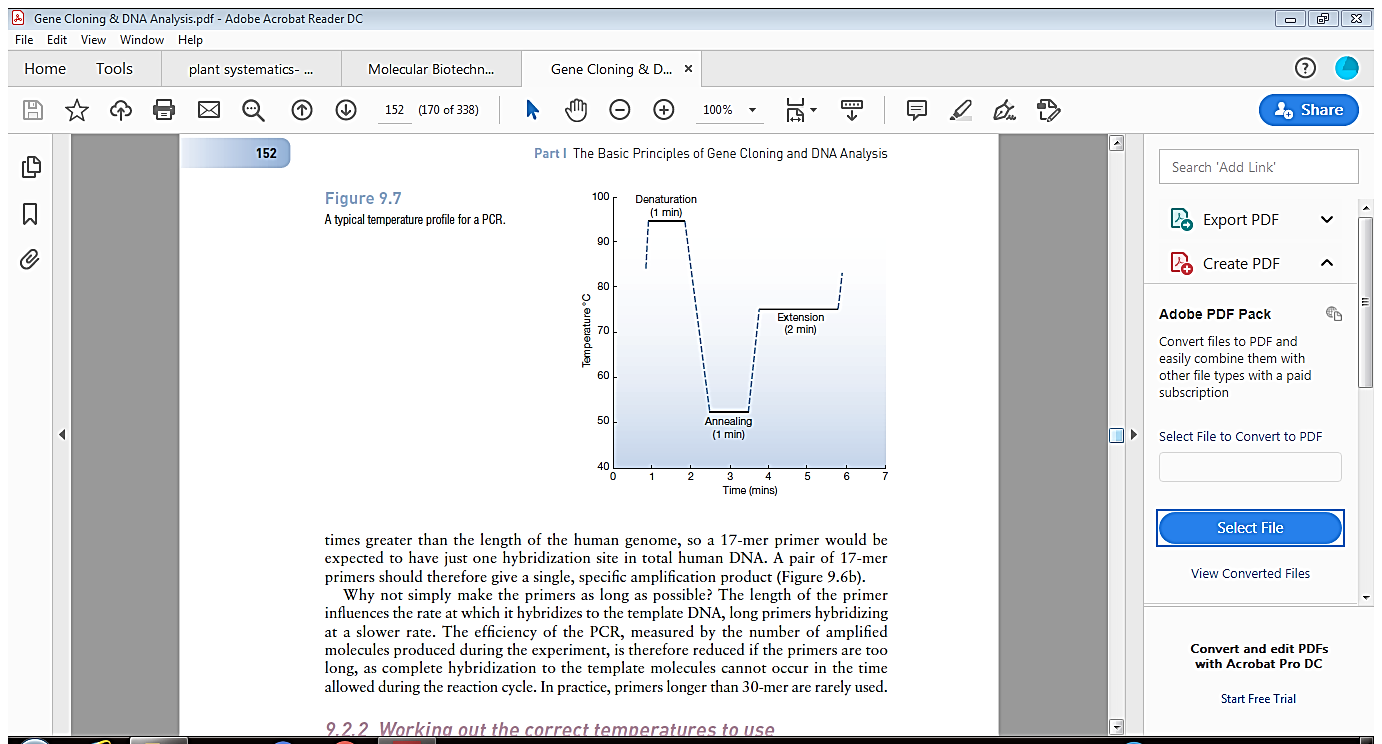
* **a template sequence in a DNA sample that lies between the primer-binding sites and that can be from 100 to ~35,000 bp in length.**
* **Thermostable DNA polymerase that can withstand being heated to 95°C or higher and that copies the DNA template with high fidelity;**
* **and the four deoxyribonucleotides (dATP,dGTP, dTTP, dCTP).**

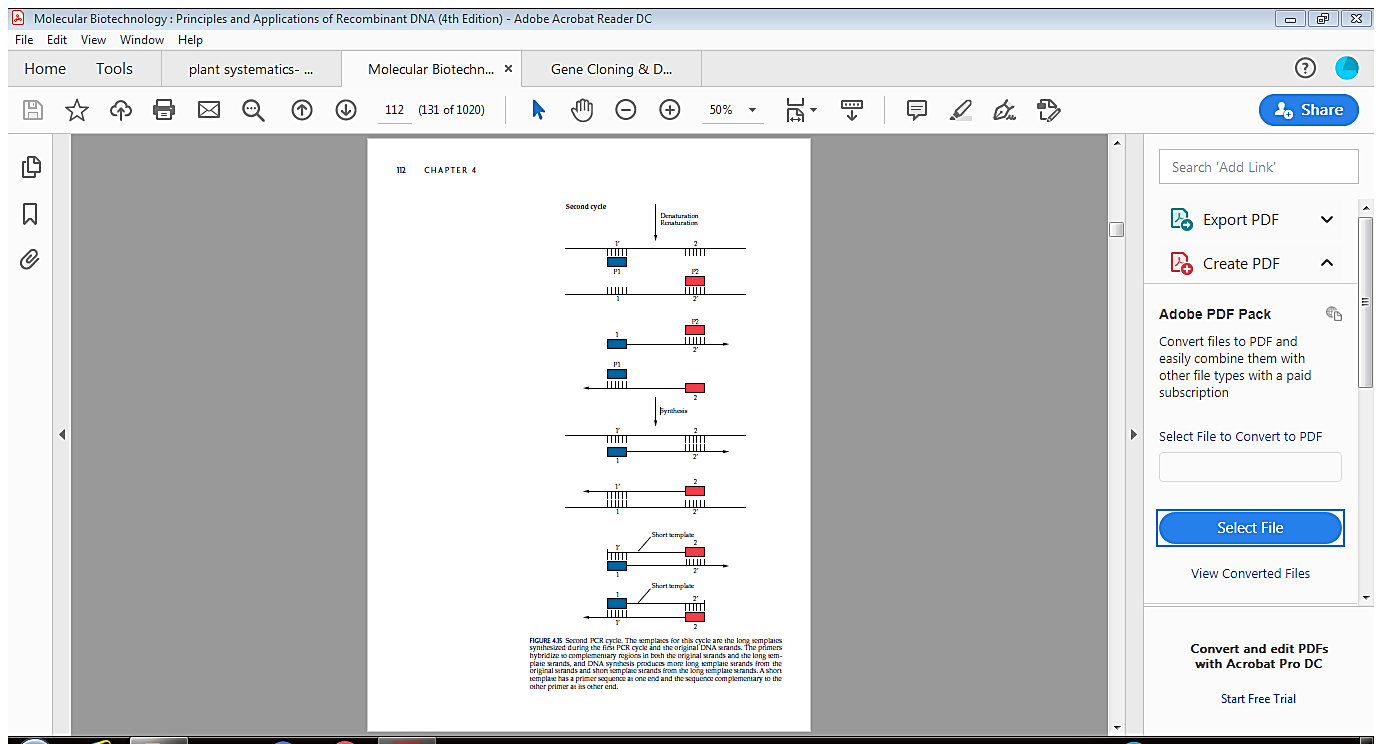
**The process of Polymerase chain reaction involves three steps**

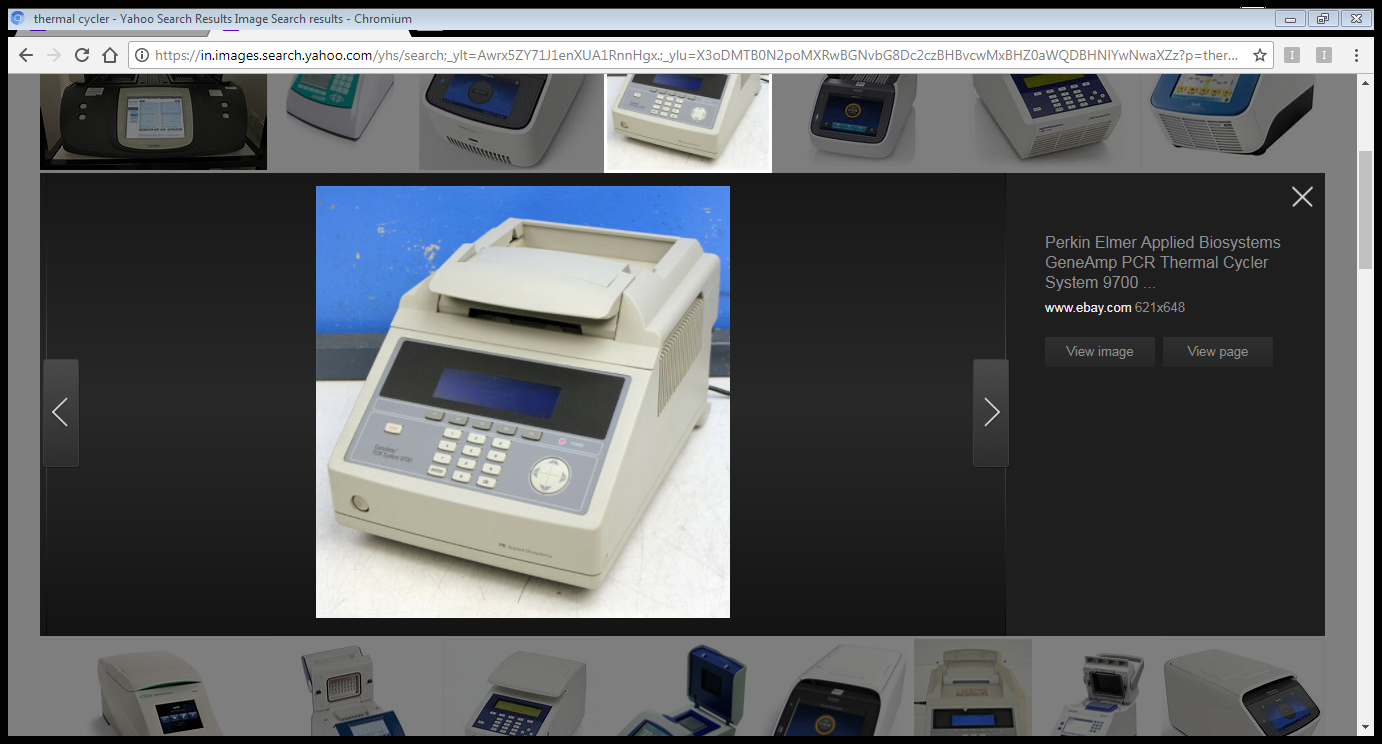
1. **Denaturation. The first step in the PCR amplification system is the thermal denaturation of the DNA sample by raising the temperature within a reaction tube to 95°C. In addition to the source template DNA, this reaction tube contains a vast molar excess of the two oligonucleotide primers, a thermostable DNA polymerase (e.g., Taq DNA polymerase, isolated from the bacterium *Thermus aquaticus*), and four deoxyribonucleotides. The temperature is maintained for about 1 minute.**
2. **Renaturation. For the second step, the temperature of the mixture is slowly lowered to ~55°C. During this step, the primers base pair with their complementary sequences in the DNA sample.**
3. **Synthesis. In the third step, the temperature is raised to ~75°C, which is optimum for the catalytic functioning of Taq DNA polymerase. DNA synthesis is initiated at the 3′ hydroxyl end of each primer and uses the source DNA as a template.**

**All steps in a PCR cycle are carried out in an automated block heater that is programmed to change temperatures after a specified period of time. One cycle generally lasts from 3 to 5 minutes.**

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**Thermal Cycler**

**DNA Fingerprinting**

The DNA from a biological sample left at the scene of a crime can be analyzed and compared with the DNAs of likely suspects. A match between evidence and a particular individual is helpful to the prosecution.

Distinguishing individuals with DNA analysis is called **DNA fingerprinting** (DNA typing).

One of the approaches to establish relationship is by using minisatellites present on human DNA.

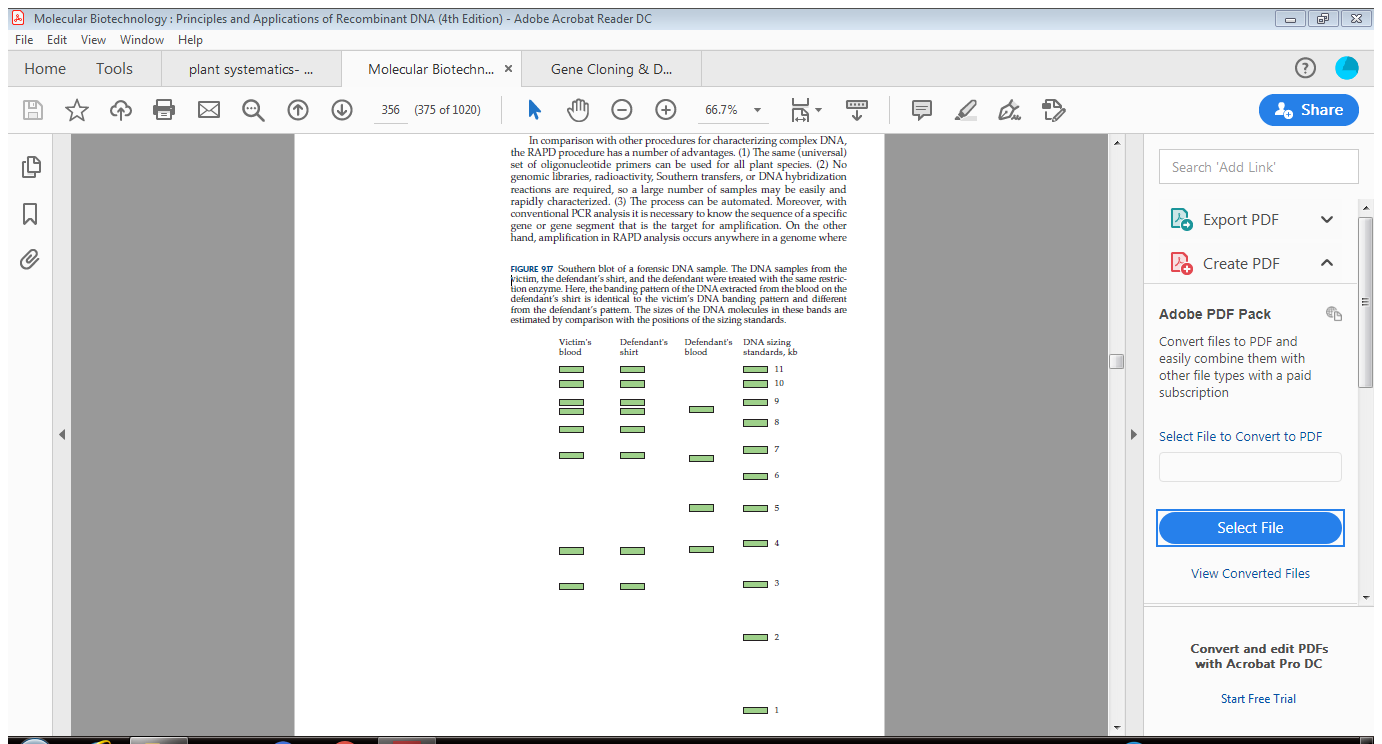
The probes for this type of analysis consists of human minisatellite DNAs, sequences that occur throughout the human genome and consist of tandemly repeated sequences. The lengths of the repeats range from 9 to 40 bp, and the numbers of repeats in the minisatellites range from about 10 to 30. A minisatellite DNA sequence at a specific chromosome location can have different lengths in different individuals. This variability is due to either a gain or a loss of tandem repeats, probably during DNA replication.

Minisatellite DNA does not encode any proteins. Unrelated individuals generally have minisatellites that differ in length, but children inherit one set of minisatellite DNA sequences from each parent.

**Process**

The sample DNA is digested with a restriction enzyme, and the fragments are separated on an agarose gel and transferred by blotting them onto a nylon membrane. The membrane is hybridized sequentially with four or five separate labeled minisatellite DNA probes, each of which recognizes a distinct DNA sequence. After each hybridization reaction, the bands in which the probe has bound to the digested DNA sample are visualized by autoradiography, and the banding pattern for each sample is noted.

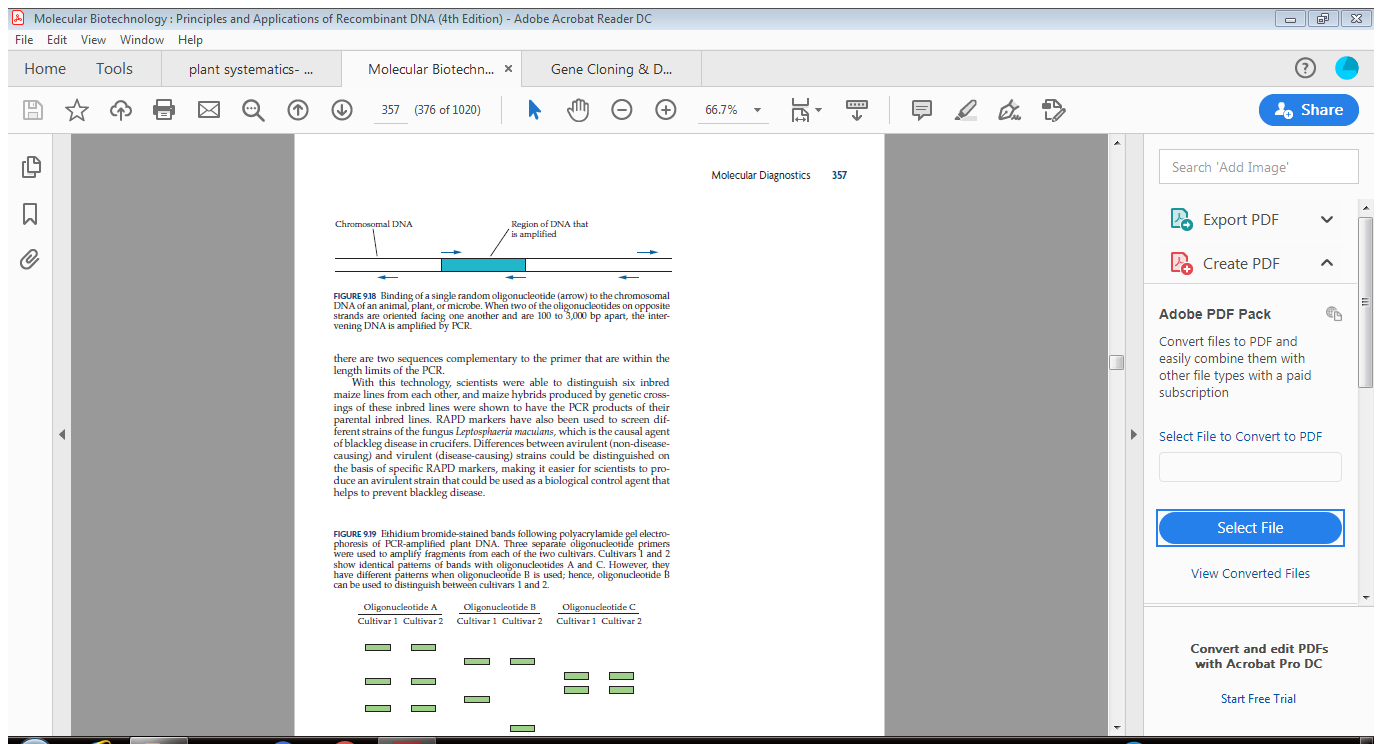
A minisatellite DNA pattern (fingerprint) represents the repertoire of the lengths of some of these sequences in an individual. Because of the extensive variability in human minisatellite DNA sequences, the chance of finding two individuals in the population with the same DNA fingerprint is about 1 in 105 to 1 in 108. Therefore, individuals’ DNA banding patterns based on minisatellite DNA sequences are almost as unique as their fingerprints.

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Another approach is by using RAPD method

Random amplified polymorphic DNA (RAPD) markers may be used for this purpose. With this procedure, an arbitrary oligonucleotide primer, usually 9 to 10 bp long, that does not contain any palindromic sequences and has a G+C content of 50 to 80% is added to a sample of plant chromosomal DNA;

Because of its short sequence, the added oligonucleotide will pair with the chromosomal DNA at many sites, sometimes including opposite strands on the target DNA. When the 3′ ends of the oligonucleotides on opposite strands of the DNA face each other, the DNA in between can be amplified.

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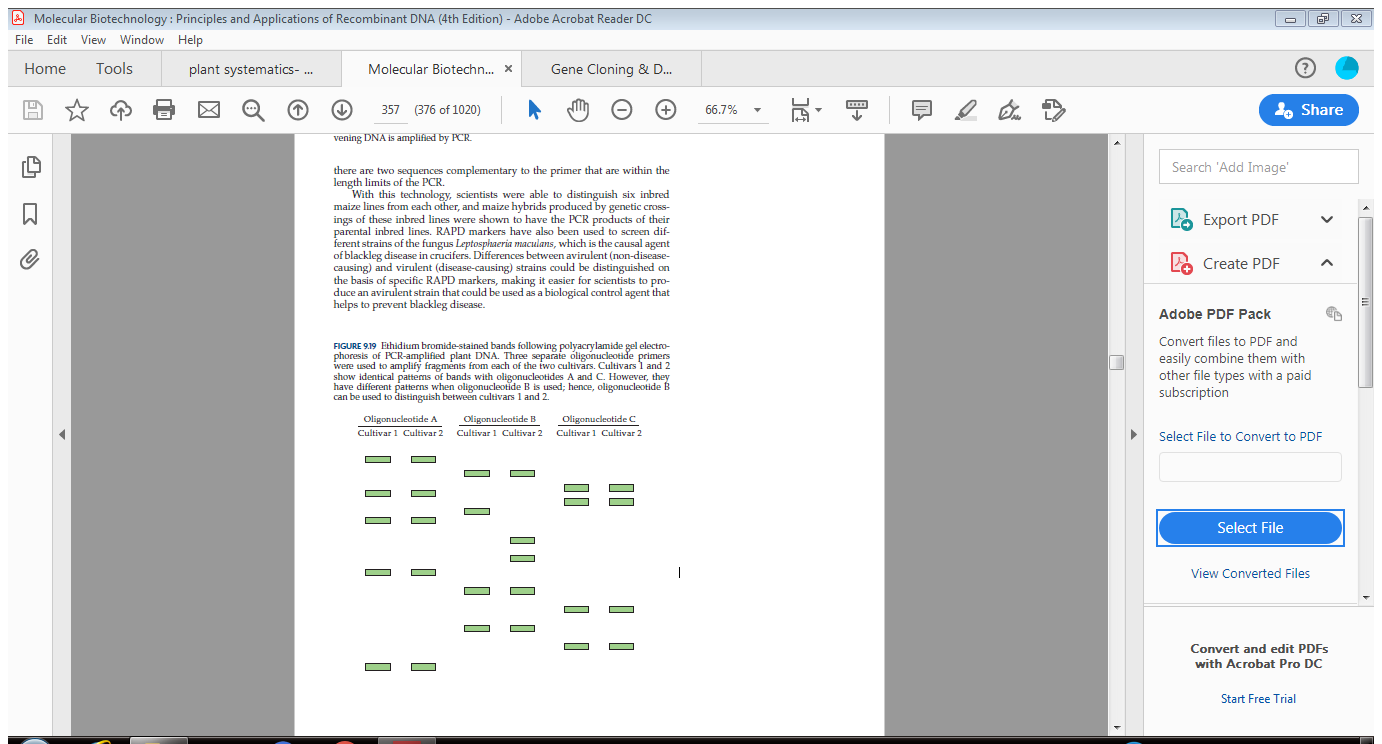
Whenever a primer can hybridize to both strands of the target DNA in the proper orientation and the two sites are about 100 to 3,000 bp from each other, the intervening DNA region will be amplified via PCR. The DNA fragments of characteristic size that are produced can be visualized following polyacrylamide gel electrophoresis. The number of amplified DNA fragments in a sample is dependent on the primer and the genomic DNA used.

Each time that the same primer is used with the same target DNA, the amplified products will be the same. A single nucleotide substitution in a primer will result in a complete change in the RAPD pattern. Thus, the RAPD fingerprints of different plant cultivars can be compared when the same set of oligonucleotide primers is used.

In comparison with other procedures for characterizing complex DNA, the RAPD procedure has a number of advantages. (1) The same (universal) set of oligonucleotide primers can be used for all plant species.

(2) No genomic libraries, radioactivity, Southern transfers, or DNA hybridization reactions are required, so a large number of samples may be easily and rapidly characterized.

(3) The process can be automated.



Application of RAPD

* To distinguish inbred lines of plants from each other and from parent line. RAPD is used to distinguish between variety is based on difference in DNA sequence.
* To establish difference between virulent and non virulent strains of pathogen.
* RAPD molecular marker used in the direct selection of desirable trait. Molecular marker linked to the trait of interest can be screened for at any stage in the breeding programme.
* RAPD and other molecular markers have great value in the selection for desirable trait in long-lived species which takes long time for maturity and show phenotypic character. For example, avacado (*Persea americana*) fruit quality can be assessed in seedling itself using RAPD molecular marker.
* RAPD markers have been used to identify several disease resistant genes in plants. The rp94 gene is responsible for resistance to stem rust (*Puccinia graminis*) in barley. RAPD markers identified to link to this gene. Similarly, RAPD markers linked to heat smut resistance gene have been characterized.

**Reference**

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