

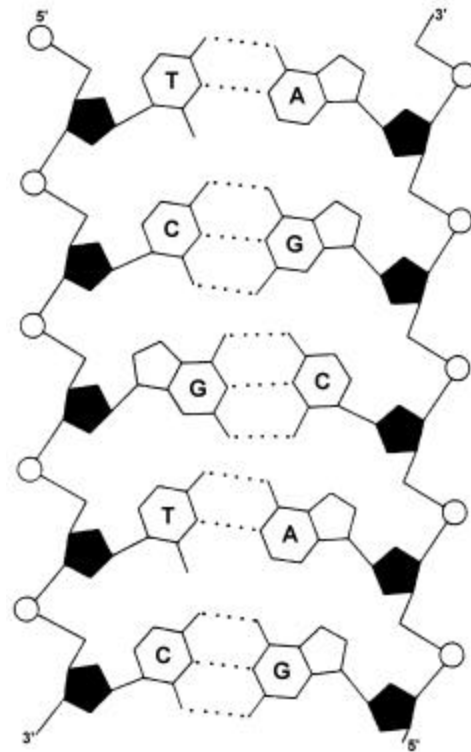
## DNA METHODS

Analysis and characterization of protozoa often requires the use of genetic, biochemical or immunological techniques. Such techniques can be used for diagnosis or the identification of species or strains which are morphologically similar. The use of restriction fragment length polymorphism (RFLP) and the polymerase chain reaction (PCR) are routinely used in molecular epidemiology studies.

### Nucleic Acid Structure and Properties

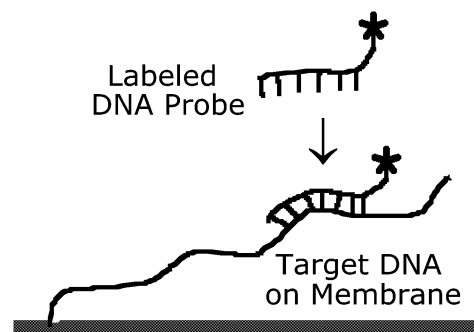
All living organisms contain DNA. It functions as the hereditary material and encodes all the information necessary to make an organism. This information is decoded into proteins by processes known as transcription and translation. The proteins are then responsible for cellular structure and function. Cells also replicate their DNA before cell division and pass on exact copies of the DNA to daughter cells.

DNA is a long double stranded polymer which forms a double helix. Each single strand is composed of nucleotides. The nucleotides come in 4 flavors (GATC). The two strands are held together by H-bonds between nucleotides on opposite strands. Only G and C can pair and only A and T can pair. The two strands of DNA are known as complementary strands. The biological importance of this is that a cell can separate the strands and use them as a template for synthesizing the complementary strand.



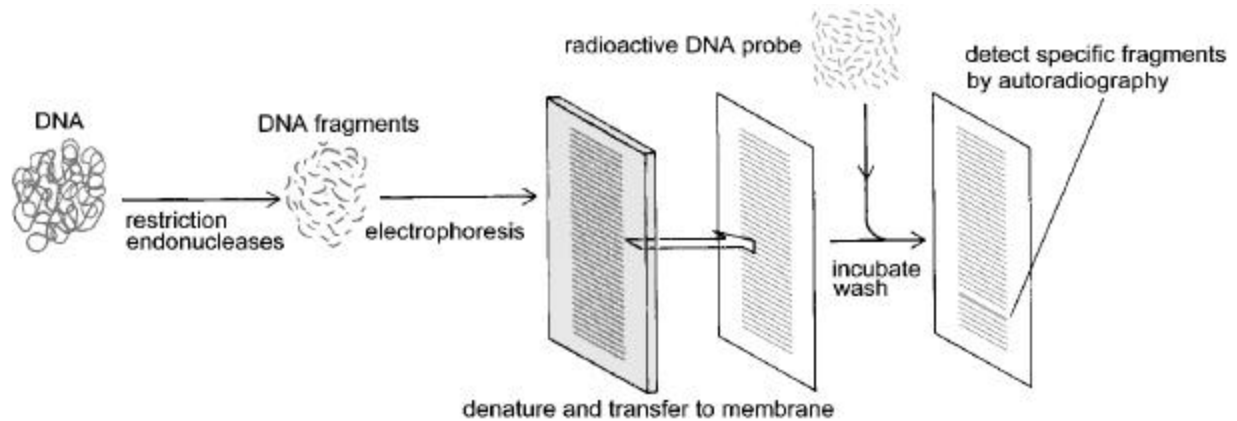
The double-strand DNA (dsDNA) molecule can also be separated into single-strands of DNA (ssDNA) in the test tube. The easiest method to accomplish this is by heating and is often referred to as melting. Equally important is the ability of ssDNA to reform dsDNA. This process is referred to as hybridization and only occurs if there is sufficient homology between the two strands.

It is possible to design a DNA probe which will only recognize DNA from a particular species, strain, or even individual. The general procedure for detecting DNA is to isolate DNA from material containing the pathogen, denature and immobilize the target DNA, and then hybridize it with a probe. The probe will contain a detectable label, such as radioactivity, enzyme, etc. The two major sources of probes are cloned genes of interest or synthetic oligonucleotides.



## Southern Blotting and RFLP

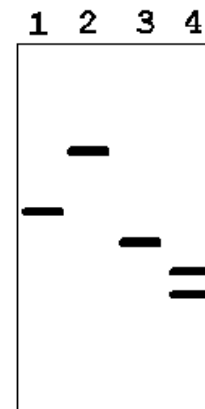
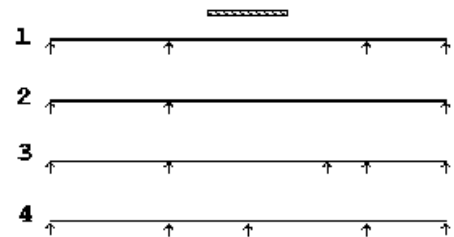
One problem with detecting specific DNA sequences within an organism's genomic DNA is the size and amount of chromosomal DNA found within a cell. Therefore, it is often necessary to cut DNA into smaller fragments before analysis. Restriction endonucleases (or restriction enzymes) reproducibly cut DNA at precise sequences. For example, EcoRI cleaves DNA at GAATTC sequences. Genomic DNA treated with a restriction enzyme will be cut into randomly sized pieces. The various DNA fragments can be separated according to size by gel electrophoresis. The DNA fragments are then transferred to a membrane support. Incubation of the membrane with a labeled probe will result in hybridization of the probe to the DNA fragment. Detection of restriction fragments by this method is known as Southern blotting.



The fact that restriction enzymes cut DNA very precisely allows for the possible detection of single base mutations due to the gain or loss of a restriction site. Therefore DNA from different species, strains or even individuals may exhibit different sizes of restriction fragments. This is known as restriction fragment length polymorphism, or RFLP.

## PCR

A major problem with RFLP analysis is that a relatively large amount of relatively pure DNA is needed. It will rarely be possible to obtain sufficient DNA from clinical specimens to carry such an analysis. Furthermore, the Southern blotting technique is rather laborious and takes several days to carry out. The polymerase chain reaction, or PCR, amplifies DNA fragments up to a million fold. This amplification allows the detection of a few copies of a unique DNA sequence from relatively impure samples. In addition, the PCR technique is relatively simple to carry out and can usually be carried out in a single day.



PCR is carried out by mixing the target DNA with oligonucleotide primers, DNA polymerase and nucleotides and placing the mixture in a thermocycler. DNA polymerase is an enzyme that synthesizes DNA from nucleotides using ssDNA as a template. The oligonucleotide primers are complementary to the target DNA and are the starting point of DNA synthesis. The thermocycler is an instrument which can rapidly and accurately change temperatures.

The first step of PCR is to heat the sample and denature the target DNA. The sample is then cooled so that the primers can anneal to the target DNA. The DNA polymerase will replicate the ssDNA beginning at with the annealed primer. The sample is reheated and the procedure repeated. The newly synthesized DNA strands are able to serve as template resulting in a geometric amplification of the DNA segment between the primers.

Normally enzymes, such as DNA polymerases, are destroyed at the temperatures required for DNA melting. This problem is resolved by using DNA polymerases isolated from organisms found in hot springs or deep ocean vents. For example, the *Taq* polymerase is isolated from *Thermus aquaticus*, a bacterium found hot springs, and has a temperature optimum of 74°. Furthermore, the *Taq* polymerase is stable at the temperatures necessary for DNA denaturation.

The amplified DNA is analyzed by gel electrophoresis. A single band, or amplicon, the of the size corresponding to the distance between the primers should be present. As in the case of RFLP analysis it may be possible to detect size heterogeneity between species, strains, or individuals. The amplified DNA can also be analyzed by Southern blotting using a labeled DNA probe to increase sensitivity or to further distinguish genotypes. PCR products can also be sequenced.

