DNA Analysis Techniques

1. Measuring DNA length
2. Copying DNA
3. Cutting and Pasting DNA
4. Probing DNA
5. DNA Cloning

Measuring DNA Length

Gel Electrophoresis
separating DNA segments according to size

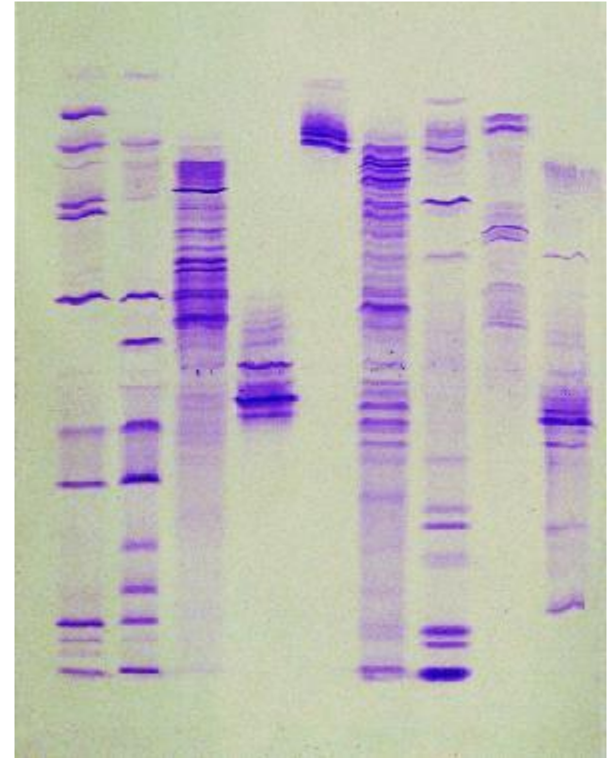
- The DNA sequence can be read by a technique called ***gel electrophoresis*** which separates DNA molecules into groups depending on their lengths.
- Gel electrophoresis has high resolution; even fragments which differ by a single nucleotide can be separated.
- The sample molecules are placed in a gel under the influence of an electric field.

Gel Electrophoresis(contd.)

- The DNA or RNA molecules (which are slightly negatively charged) can migrate towards the positive electric field.
- The speed of migration is inversely proportional to the length of the molecule; longer molecules move slow, shorter move faster.
- All molecules are initially placed at the top of the 'well' and after a few hours, the molecules move to different locations depending on its length.
- If the molecules are labeled with radioactive isotopes, their positions can be photographed on a film.

Gel Electrophoresis(contd.)

- A copolymer of mannose and galactose, agaose, when melted and recooled, forms a gel with pores sizes dependent upon the concentration of agarose

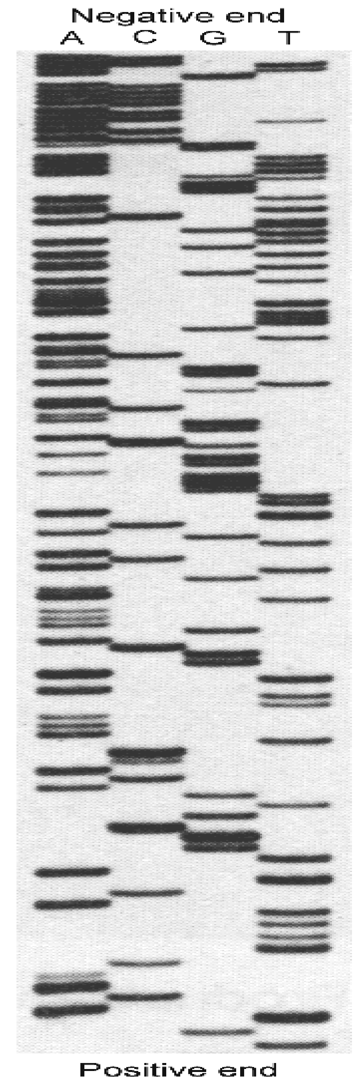


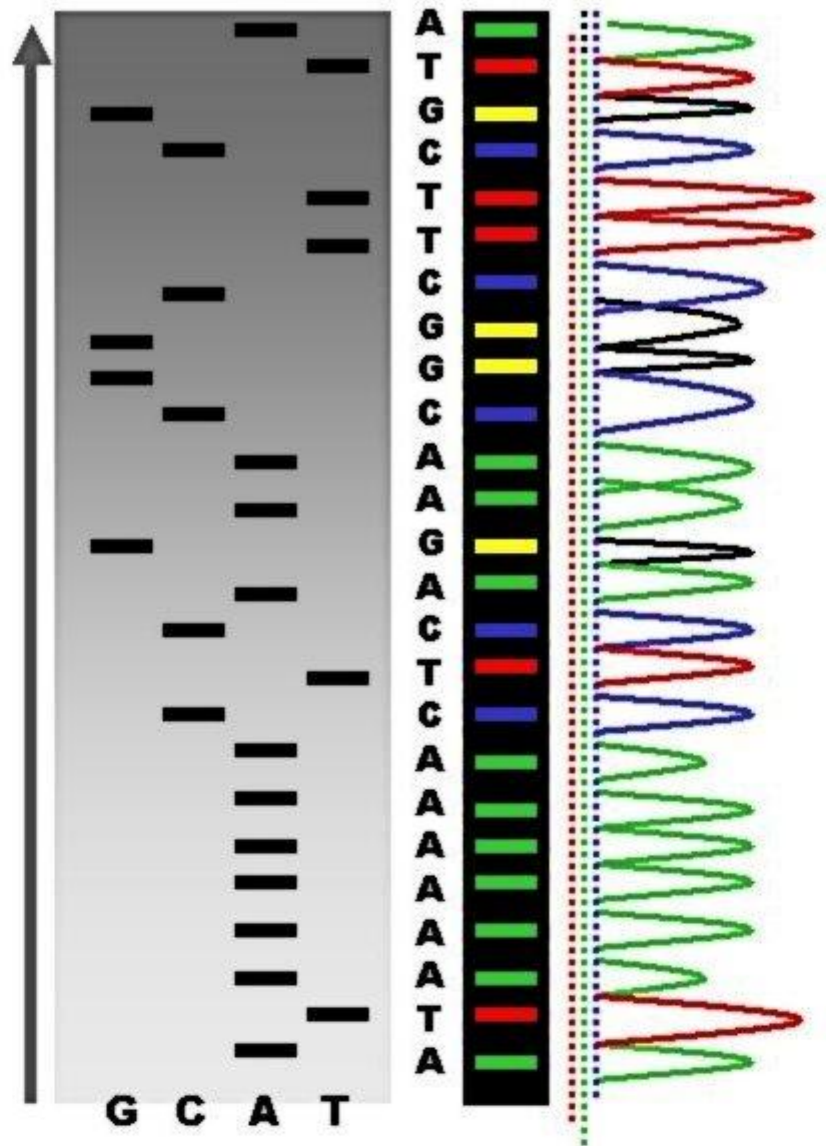
- The phosphate backbone of DNA is highly negatively charged, therefore DNA will migrate in an electric field
  - The size of DNA fragments can then be determined by comparing their migration in the gel to known size standards.

Electrophoresis

– DNA or RNA molecules are charged in aqueous solution and move to a definite direction by the action of an electric field.

– The DNA molecules are either labeled with radioisotopes or tagged with fluorescent dyes. In the latter, a laser beam can trace the dyes and send information to a computer.





A
T
G
C
T
T
C
G
G
C
A
A
G
A
C
T
C
A
A
A
A
A
A
T
A
G C A T

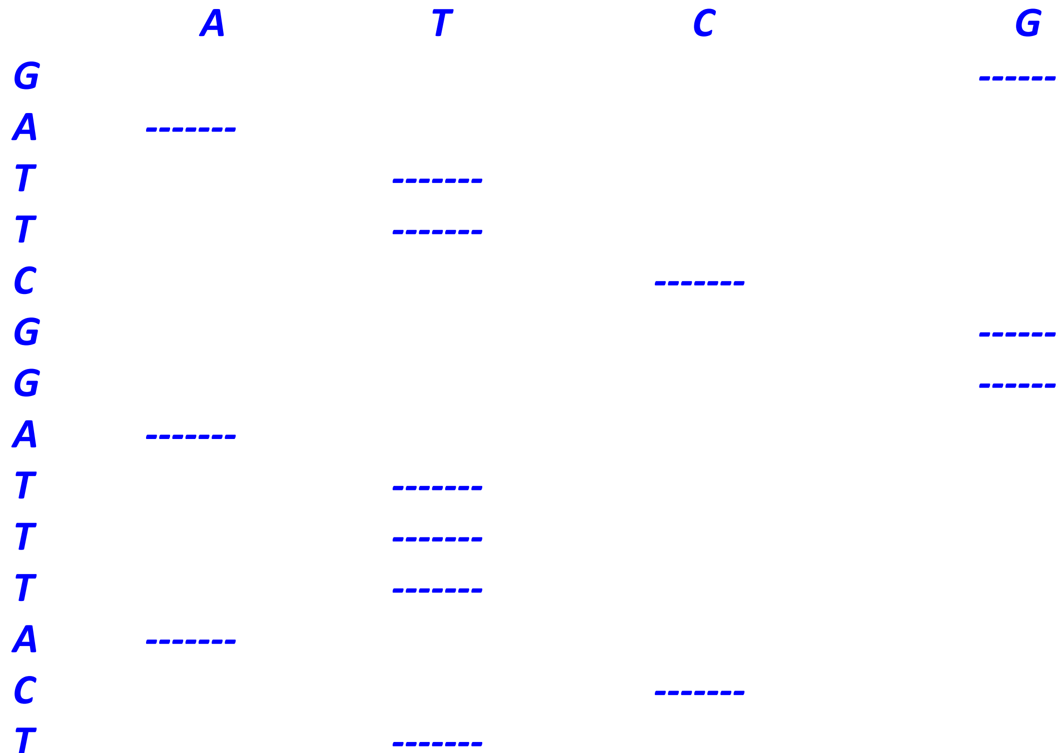
DNA Sequencing

- DNA or a RNA molecule can be sequenced using these techniques as follows.
  - Given a DNA molecule, obtain all fragments that end in a single letter *A.*
  - Similarly, obtain all sequences ending in *T, C* and *G.*
  - For example, if the sequence is GATTCGGATTTACT the fragments that end in *T* are *GAT,GATT, GATTCGGAT, GATTCGGATT, GATTCGGATTT* and the whole sequence *GATTCGGATTTACT.*

Automated Sequencing

- In modern automated sequencing, the primer is replaced by a different fluorescent probes
- and the signals from the probes are detected by special detectors.
- These sequences are then placed in four wells, the *A*-well, the *T*-well, the *C*-well and the *G*-well and subjected to electric field simultaneously.
- We can conclude the precise sequence of the original fragment.

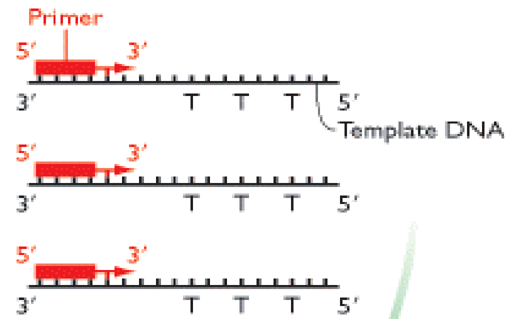
- The figure below illustrates the principle. We assume here that the positive terminal is on the top and the shorter fragments leave their mark near the top.



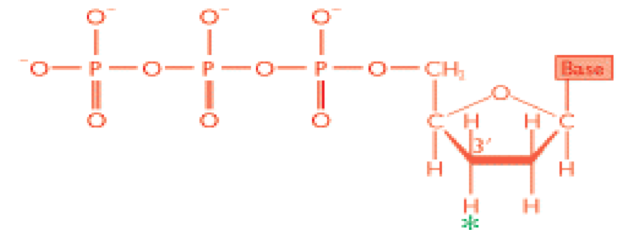
- If you now read the horizontal bars from top to bottom corresponding to the wells, you will get the entire sequence *GATTCGGATTTACT*
- For further details, see http://web.utk.edu/~khughes/main.htm
- The gel electrophoresis technique was developed in 1970 by Maxam and Gilbert and Sanger. Since the method obtained the DNA fragments by **chemical degradation of part of the sequence**, it was not very reliable. A more efficient and reliable method is to use PCR which we describe next.

Chain termination method

(A) Initiation of strand synthesis

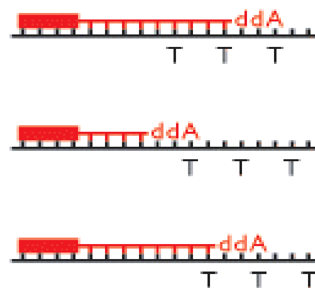


(B) A dideoxynucleotide

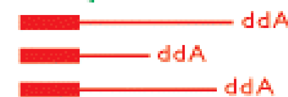


* Position where the –OH of a dNTP is replaced by –H

(C) Strand synthesis terminates when a ddNTP is added



The 'A' family



(D) The resulting autoradiograph

DNA sequence

Automatic DNA sequencing