Sequencing methods

Two basic methods for DNA sequencing :-

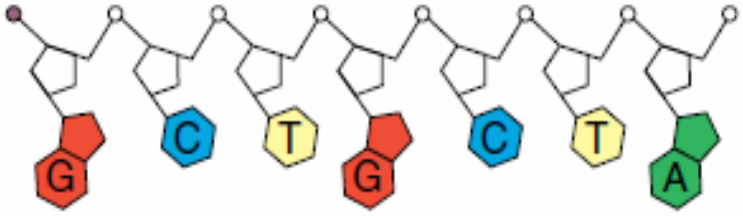
A- Chemical cleavage method (Maxam and Gilbert, 1977)

- Base-specific cleavage of DNA by certain chemicals
- Four different chemicals, one for each base
- A set of DNA fragments of different sizes
- DNA fragments contain up to 500 nucleotides

B- Enzymatic method (Sanger, 1981)

The chain cleavage reaction

DNA labeled at one end with ^{32}p



Base modification

Release or displacement of reacted bases

Strand scission

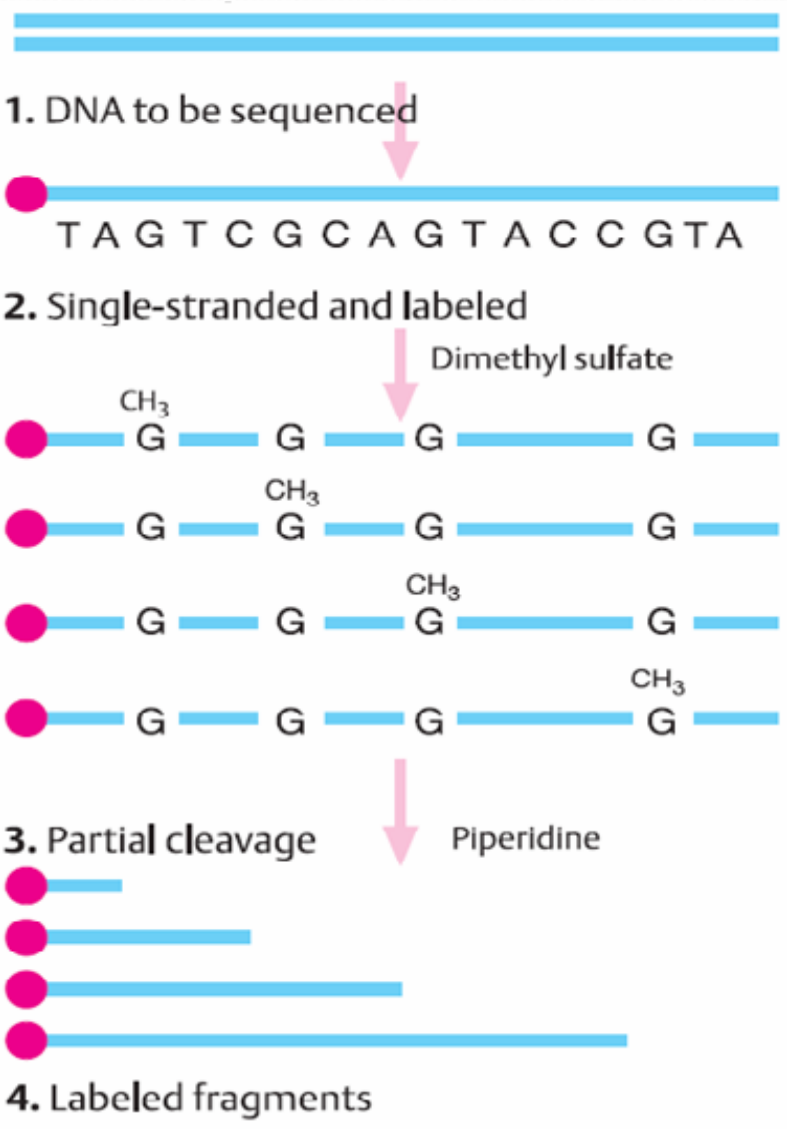
- Dimethyl sulfate (DMS) methylates G -Acid (A)

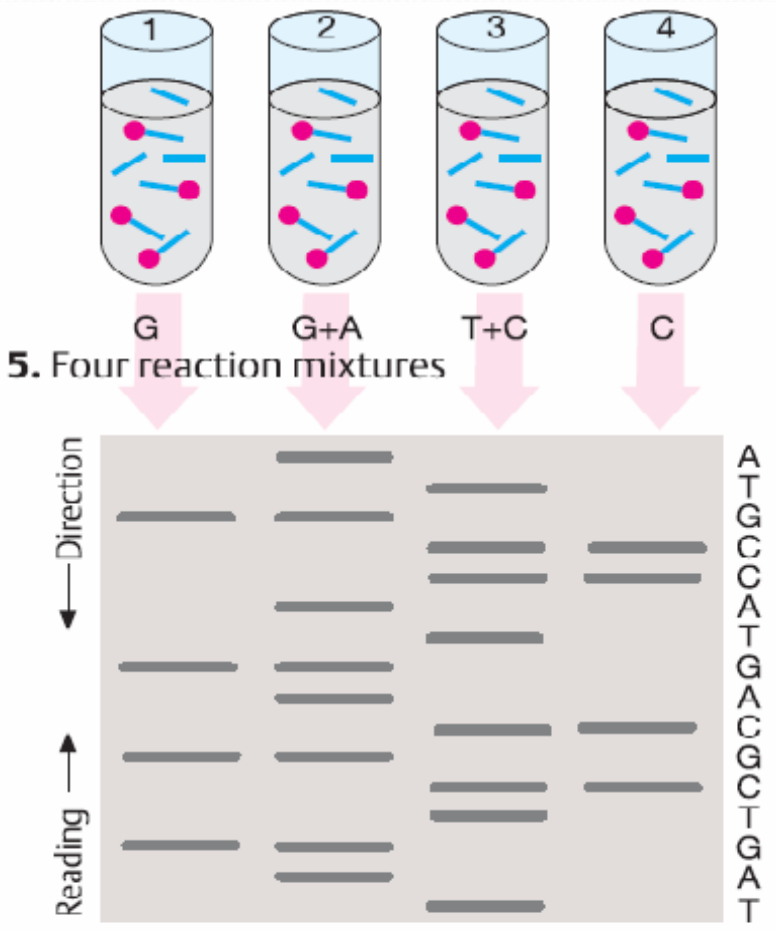
-Hydrazine (C)
- Hydrazine & NaCl (T)
- Piperidine

The fragments created by chain cleavage at guanines

^{32}pGpCpTpGpCpTpApGpGpTpGpCpCpGpApGpC
G G G G G G
^{32}p
^{32}pGpCpTp
^{32}pGpCpTpGpCpTpAp
^{32}pGpCpTpGpCpTpApGp
^{32}pGpCpTpGpCpTpApGpGpTp
^{32}pGpCpTpGpCpTpApGpGpTpGpCpCp
^{32}pGpCpTpGpCpTpApGpGpTpGpCpCpGpAp
^{32}pGpCpTpGpCpTpApGpGpTpGpCpCpC

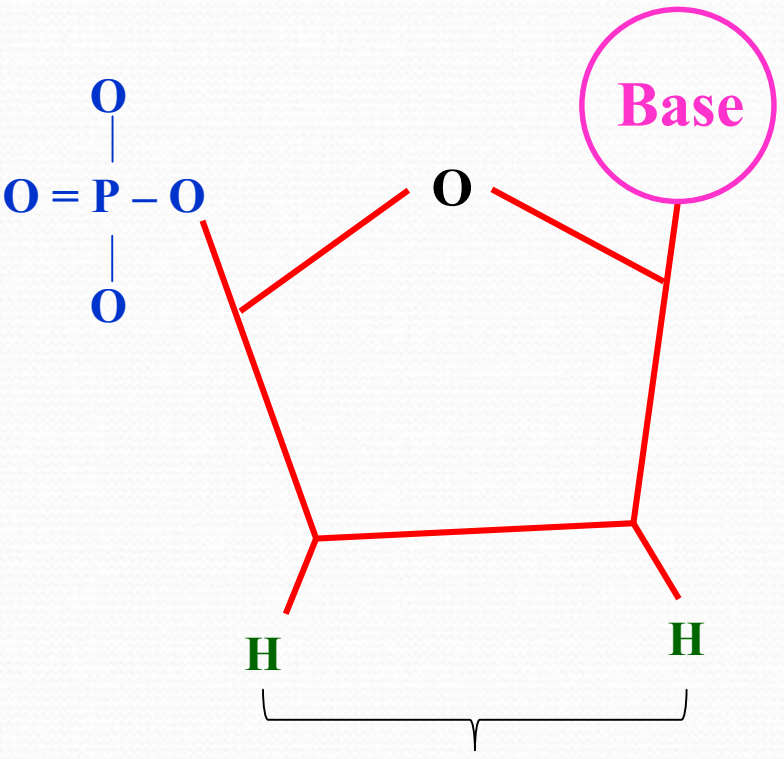
Chemical degradation method (Maxam–Gilbert method)





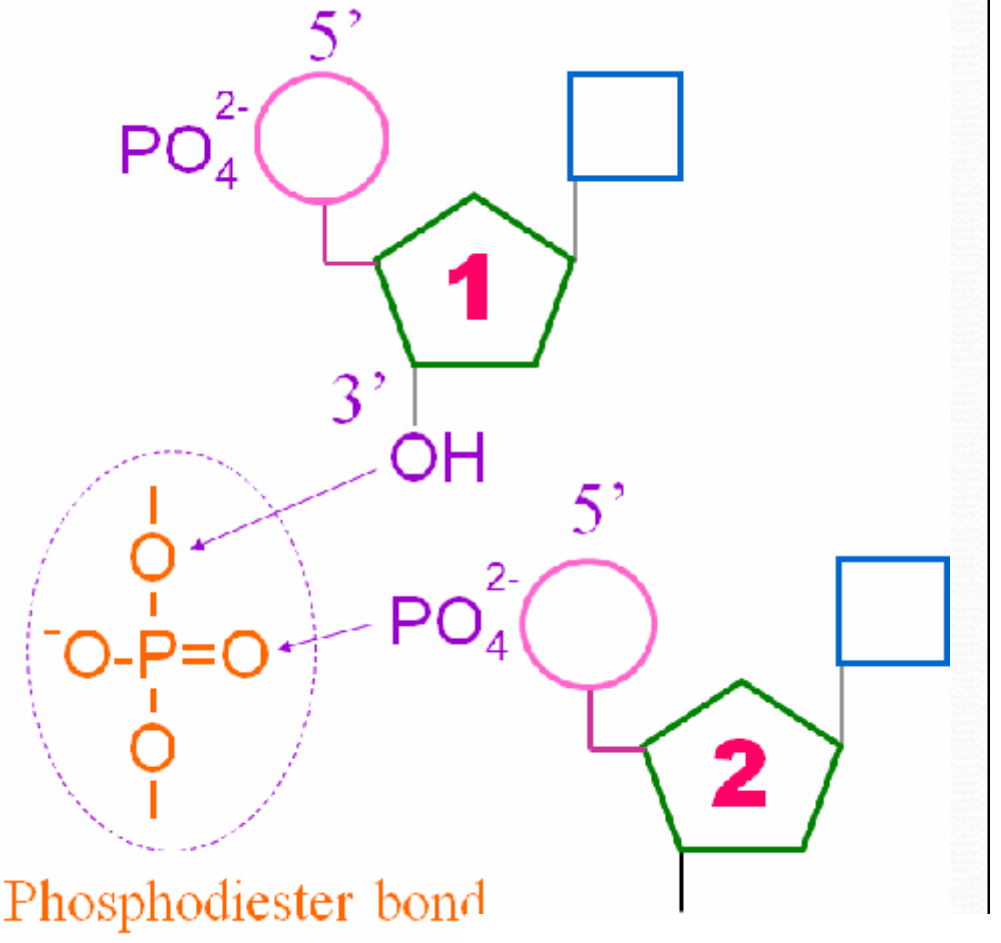
The Sanger DNA sequencing method

- Uses dideoxy nucleotides to terminate DNA synthesis.
- DNA synthesis reactions in four separate tubes
- Radioactive dATP is also included in all the tubes so the DNA products will be radioactive.
- Yielding a series of DNA fragments whose sizes can be measured by electrophoresis.
- The last base in each of these fragments is known.

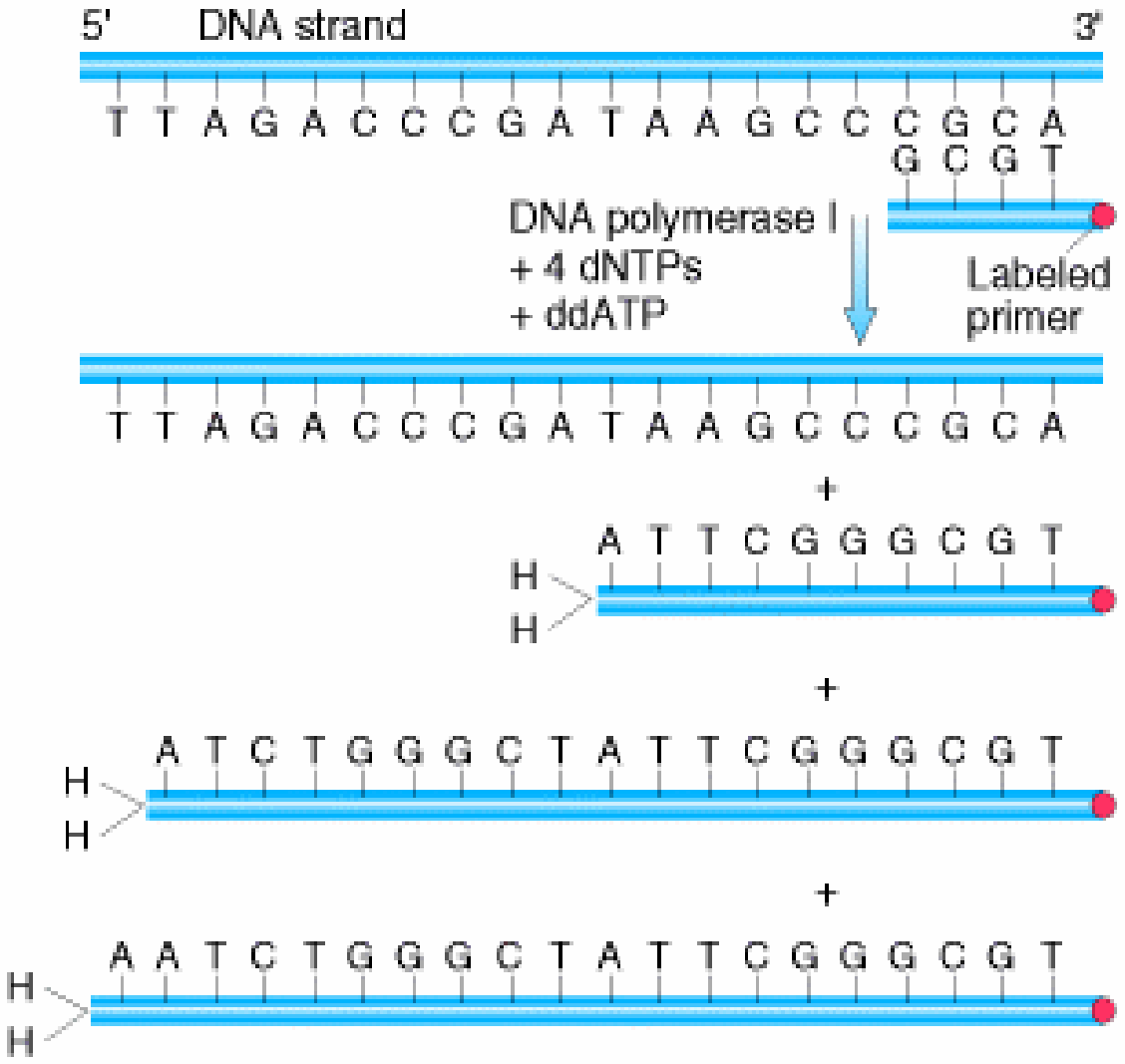


2’, 3’ dideoxy nucleotide

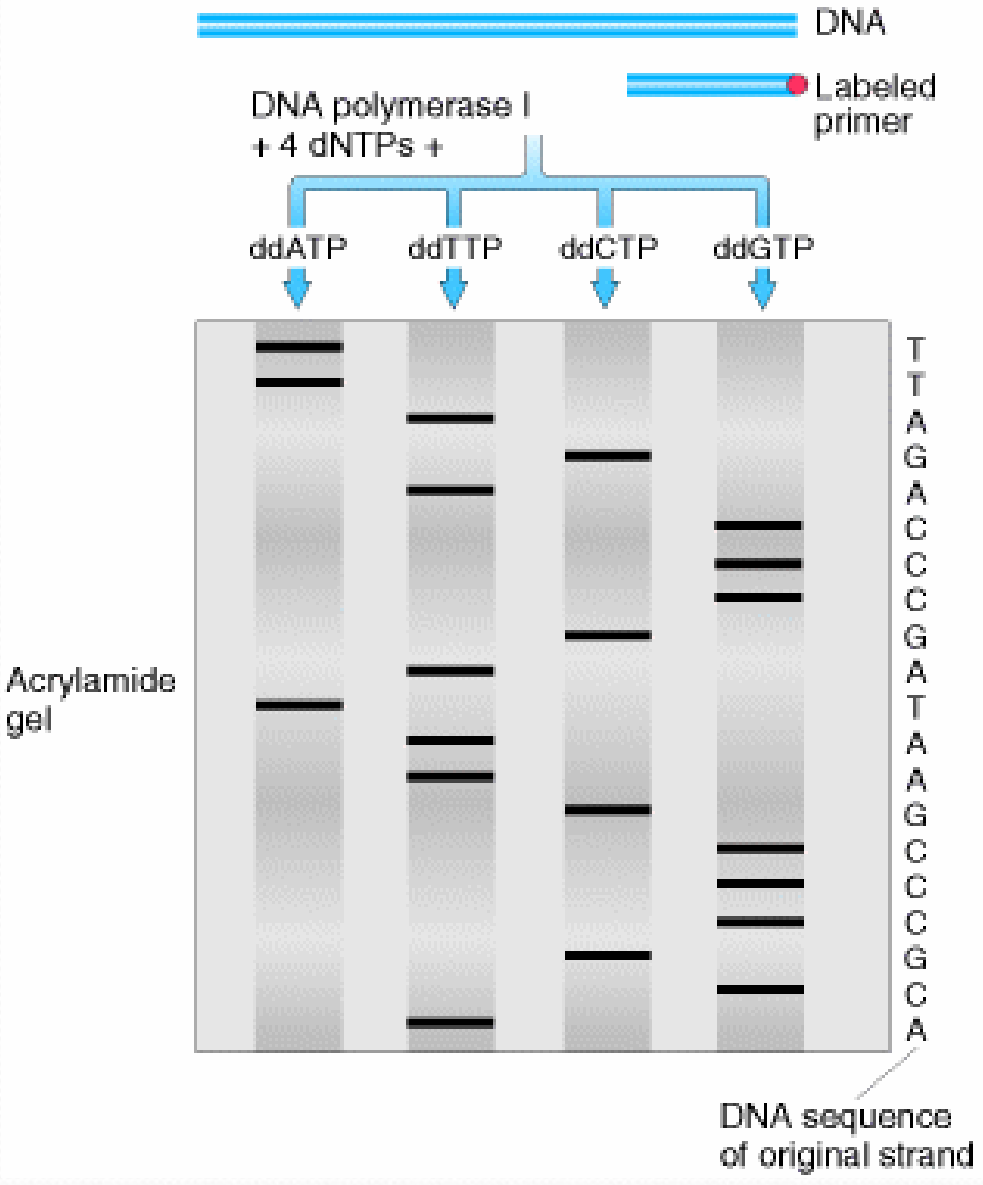
Can not form phosphodiester bound with next coming dNTP



The dideoxy sequencing method (Sanger method)



A labeled primer is used to initiate DNA synthesis. The addition of four different dideoxy nucleotides randomly arrests synthesis.



The resulting fragments are separated elect
and subjected t

Automated DNA sequencing

-The primer extension reactions are run in the same way as in the manual method

-Reaction carried out in one tube and all possible products are actually produced

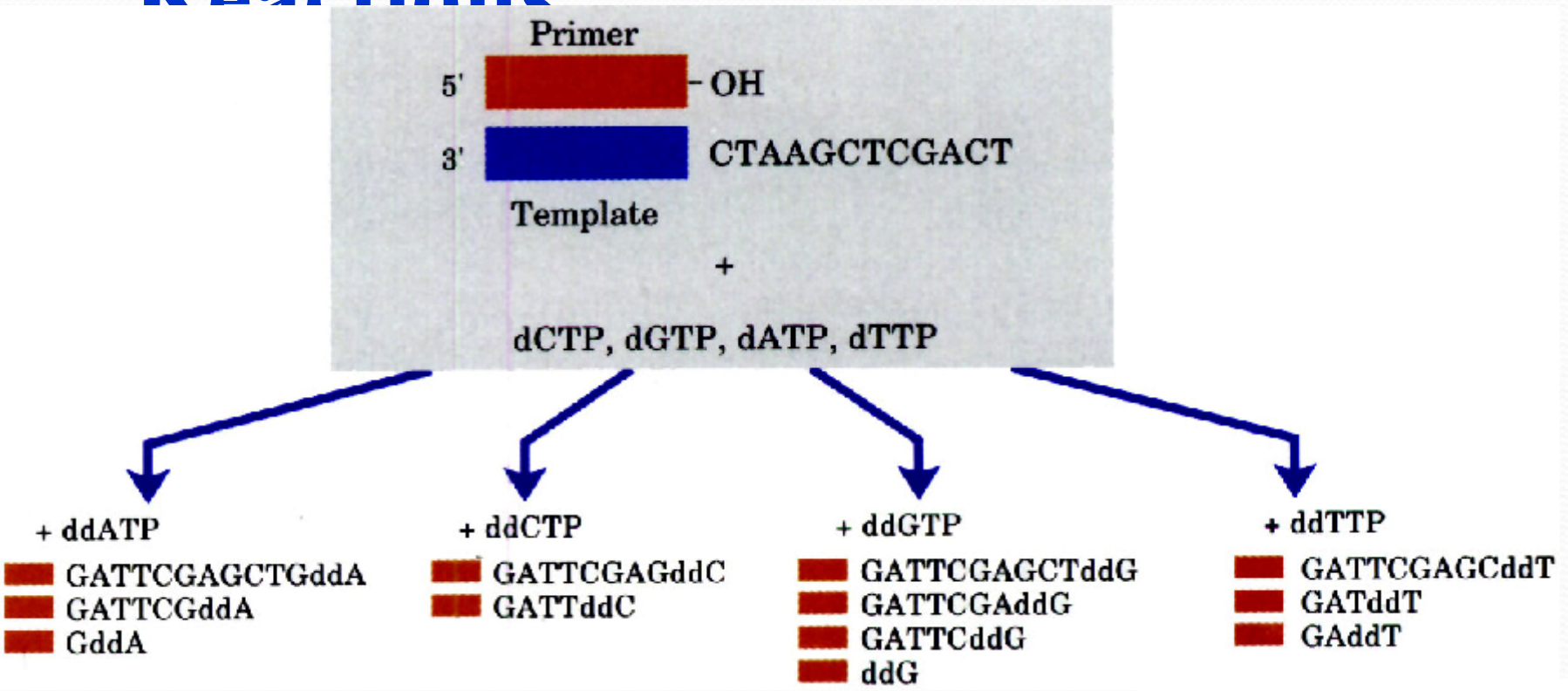
- The various reaction products separate according to size on gel electrophoresis

-The bands are color-coded according to the termination reaction that produced them

-A laser scanner excites the fluorescent tag on each band as it passes by, and a detector analyzes the color of the resulting emitted light

- Each colored peak is a plot of the fluorescence intensity of a band as it passes through the laser beam

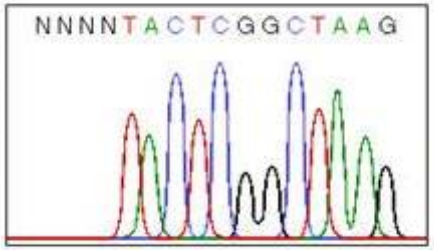
DNA Sequencing Reactions

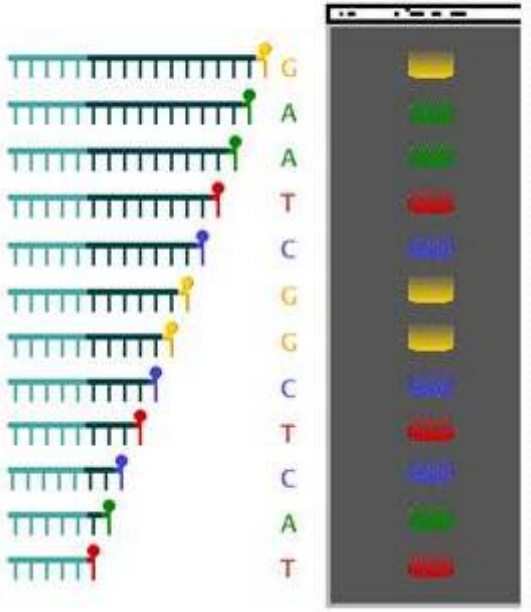


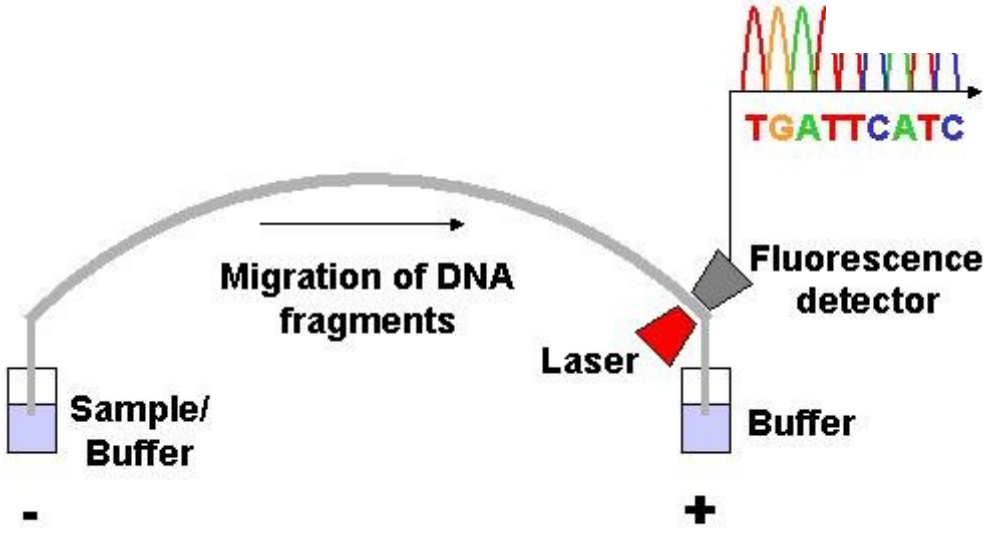
Gel electrophoretic Fractionation

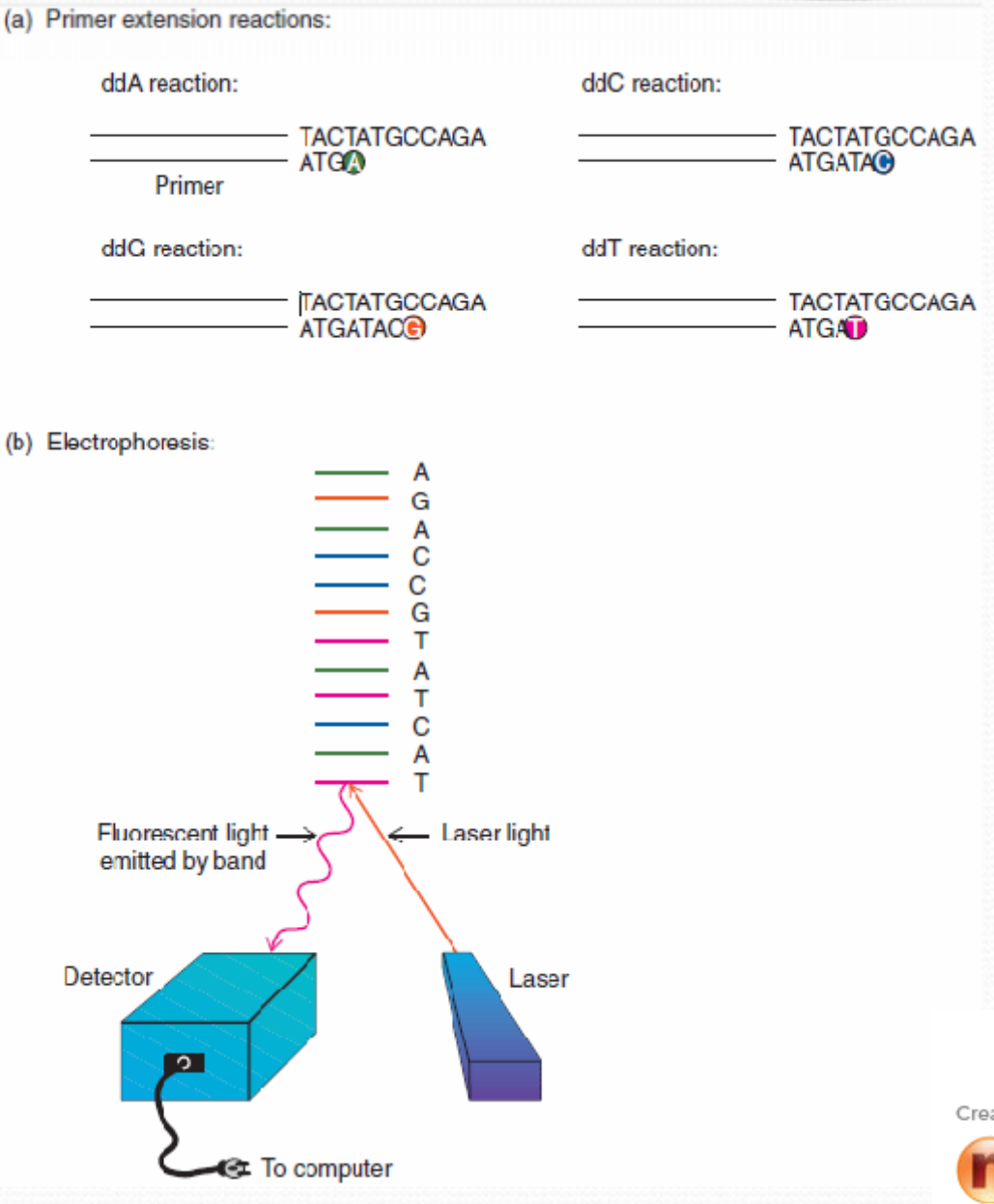
Cycle Sequencing

The simulated gel image is read from bottom to top, starting with the smallest fragment.

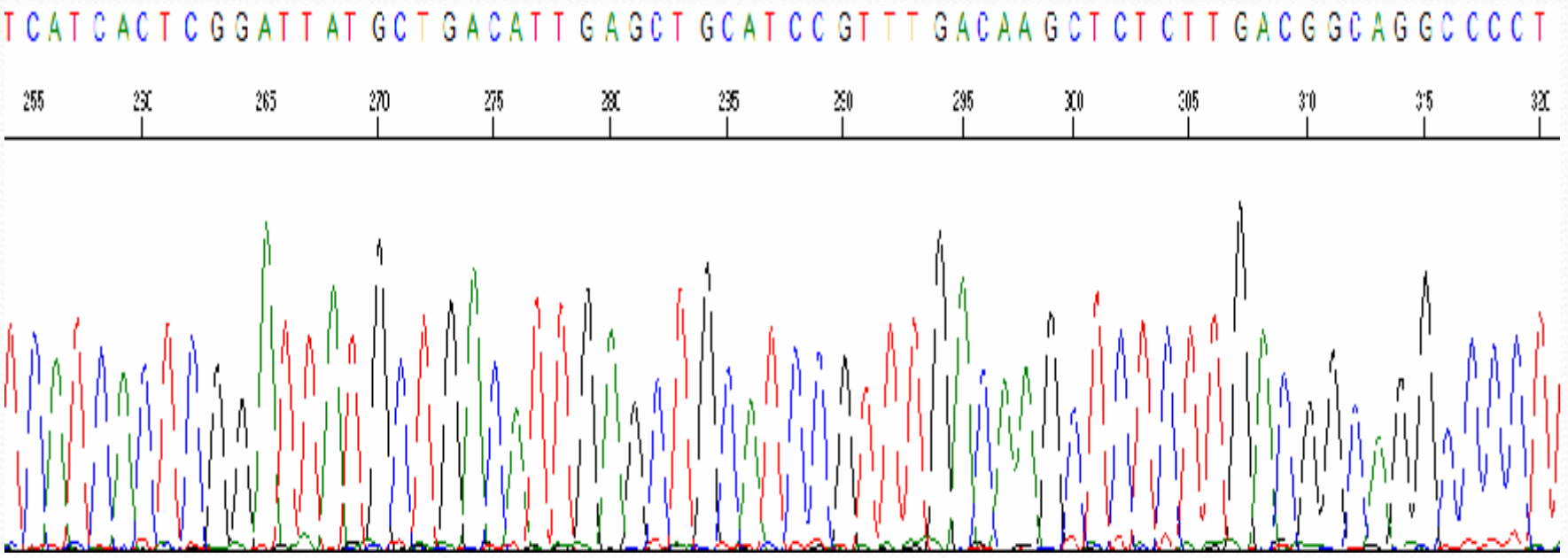








Printout of an automated DNA sequencing



Set up cycle sequencing