

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)

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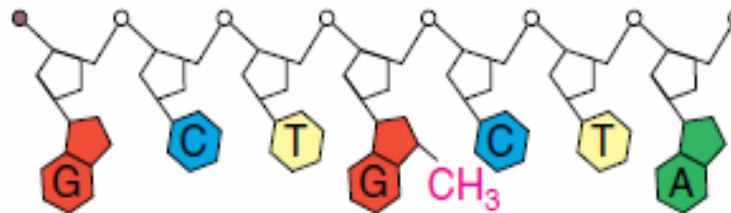
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The chain cleavage reaction

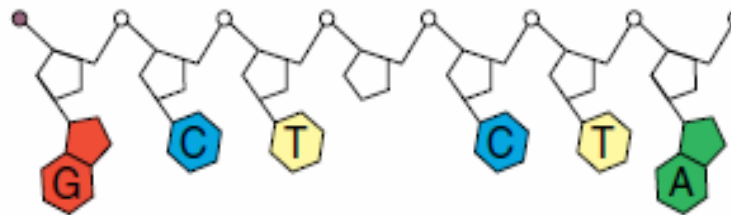
DNA labeled at one end with ^{32}P



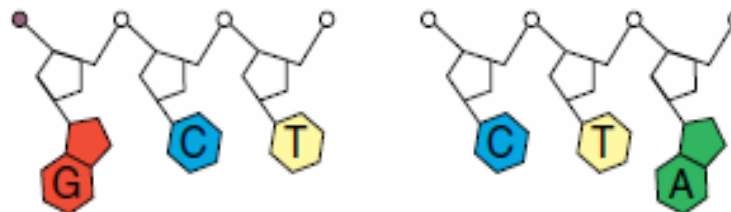
Base modification



Release or displacement of reacted bases



Strand scission



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

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

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The fragments created by chain cleavage at guanines

$^{32}\text{pGpCpTpGpCpTpApGpGpTpGpCpCpGpApGpC}$
G G G G G G

^{32}p

$^{32}\text{pGpCpTp}$

$^{32}\text{pGpCpTpGpCpTpAp}$

$^{32}\text{pGpCpTpGpCpTpApGp}$

$^{32}\text{pGpCpTpGpCpTpApGpGpTp}$

$^{32}\text{pGpCpTpGpCpTpApGpGpTpGpCpCp}$

$^{32}\text{pGpCpTpGpCpTpApGpGpTpGpCpCpGpAp}$

$^{32}\text{pGpCpTpGpCpTpApGpGpTpGpCpCpC}$

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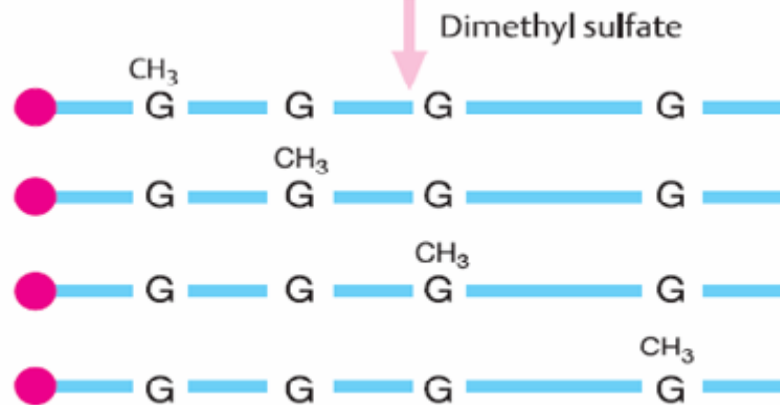
Chemical degradation method (Maxam-Gilbert method)



1. DNA to be sequenced



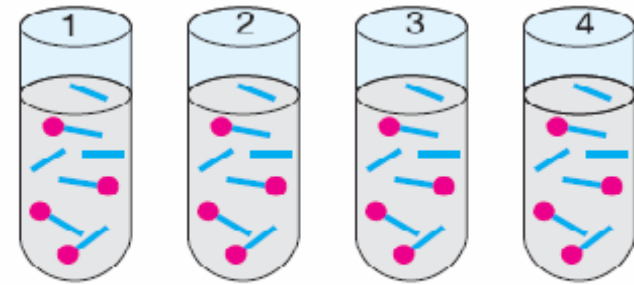
2. Single-stranded and labeled



3. Partial cleavage



4. Labeled fragments



5. Four reaction mixtures



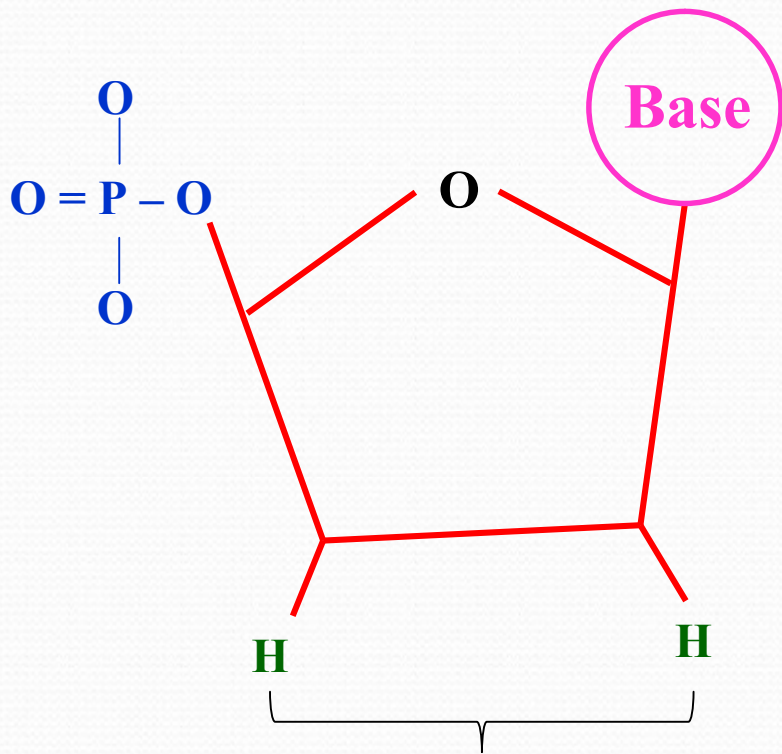
6. Gel electrophoresis

7. Determined sequence

TAGTCG

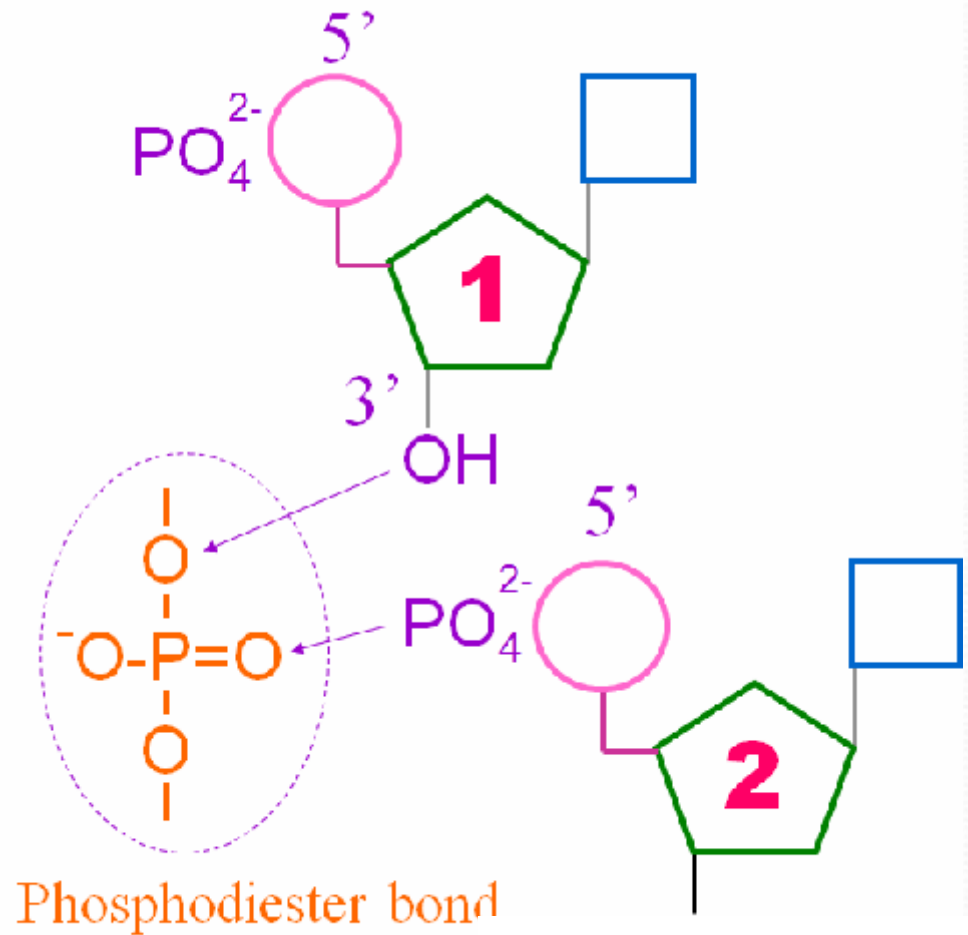
The Sanger DNA sequencing method

- 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 bond with next coming dNTP

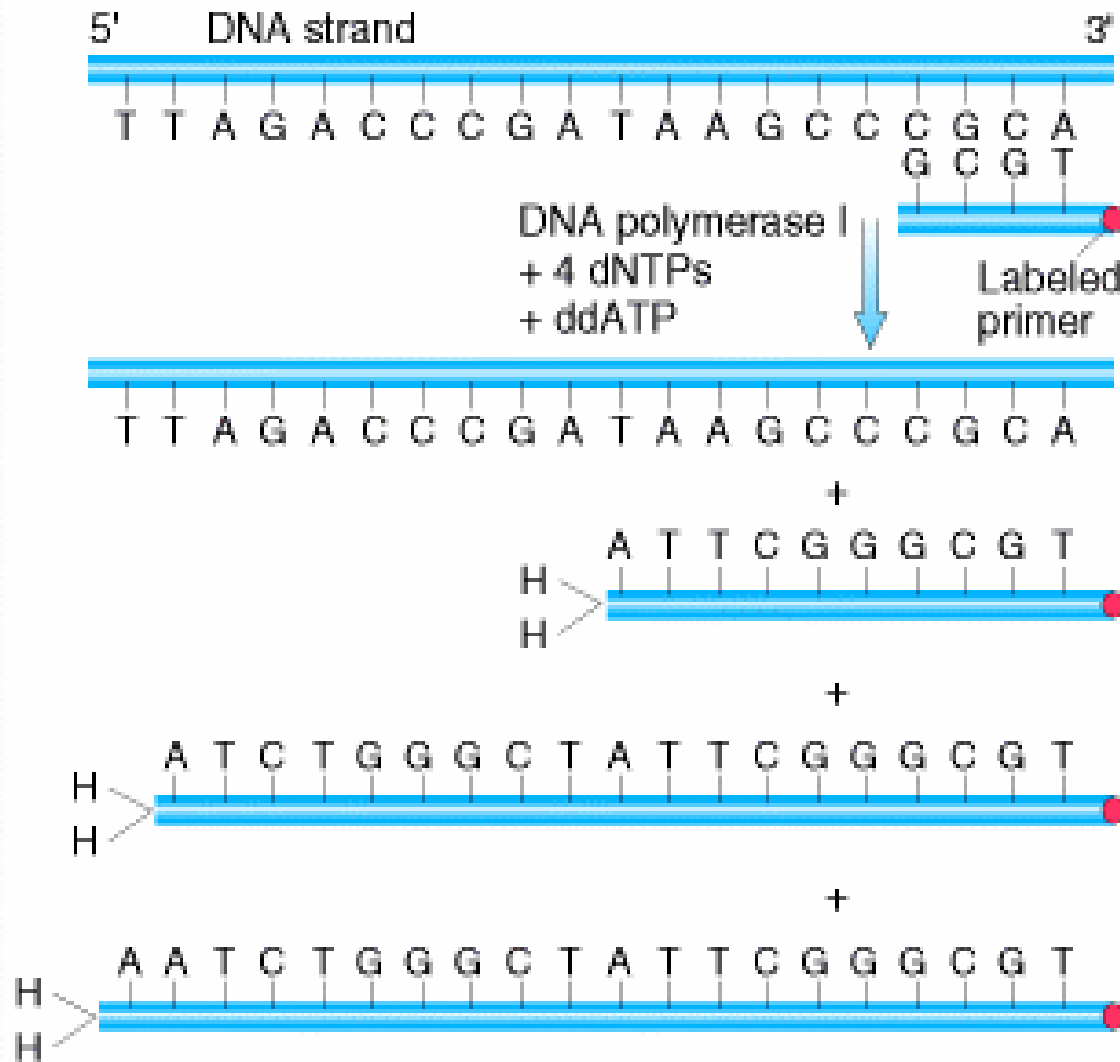


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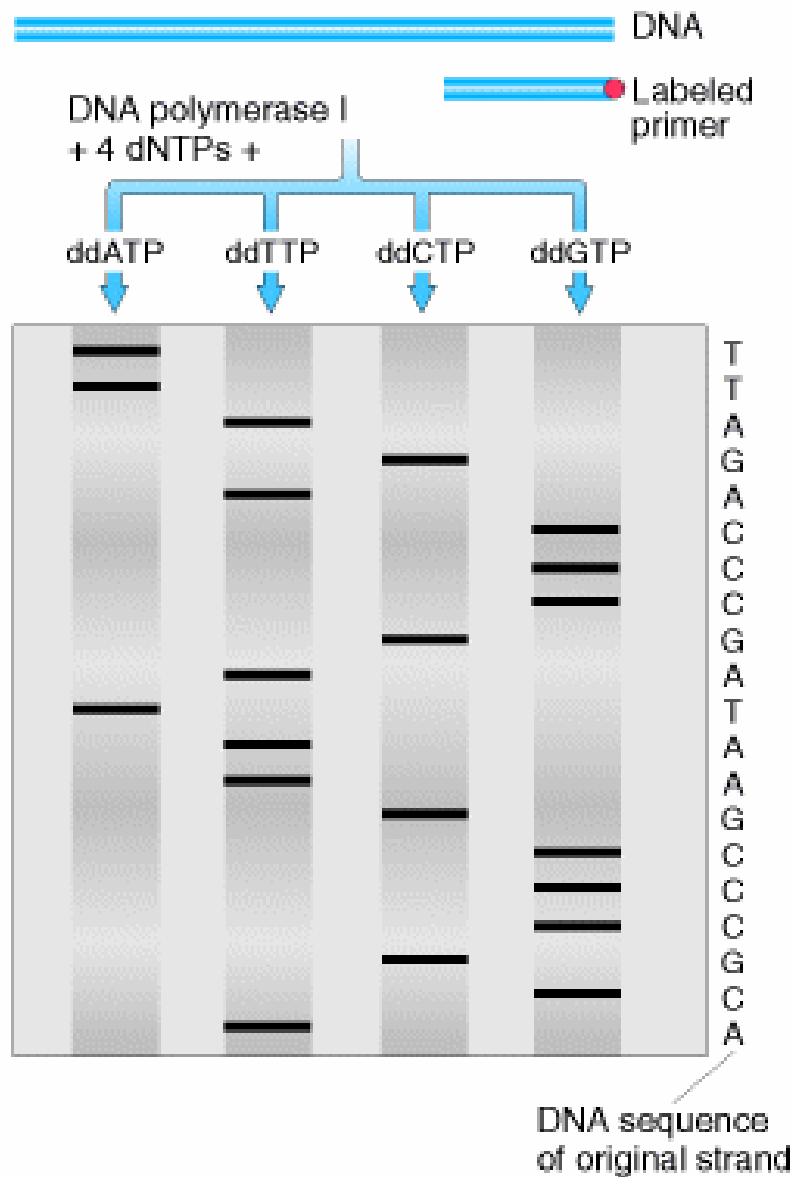
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The dideoxy sequencing method (Sanger method)



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



The resulting fragments are separated electrophoretically and subjected to autoradiography.

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

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-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

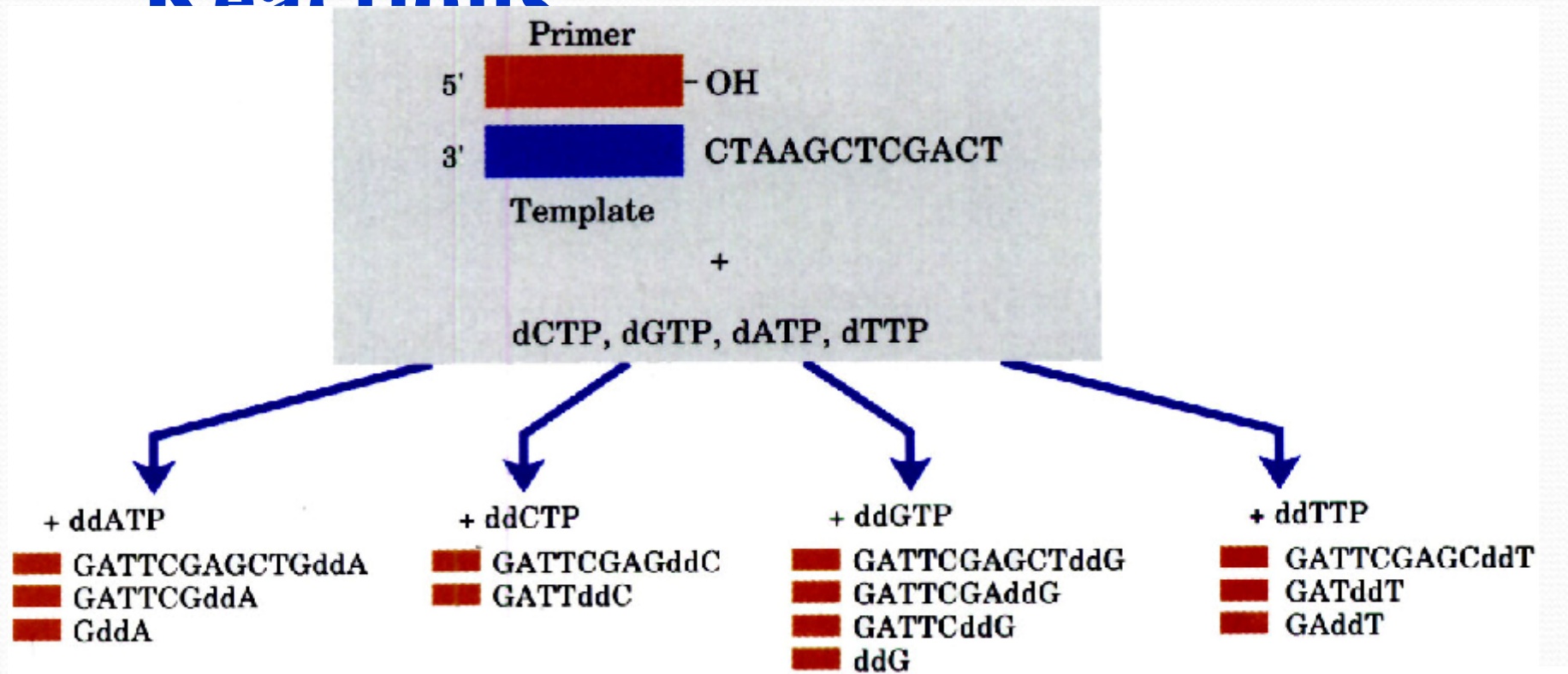
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DNA Sequencing Reactions

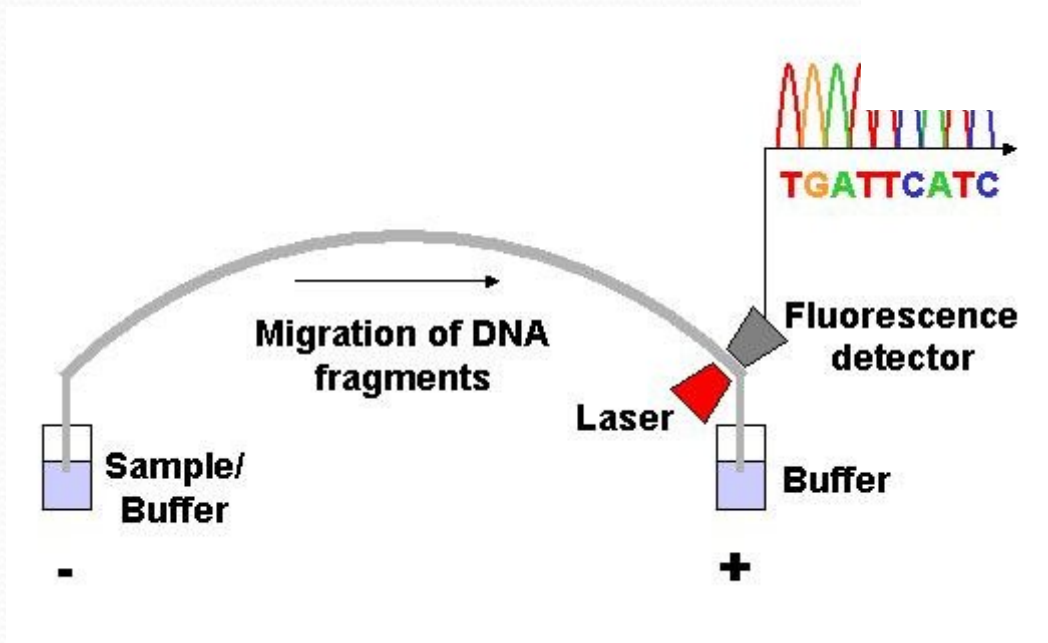
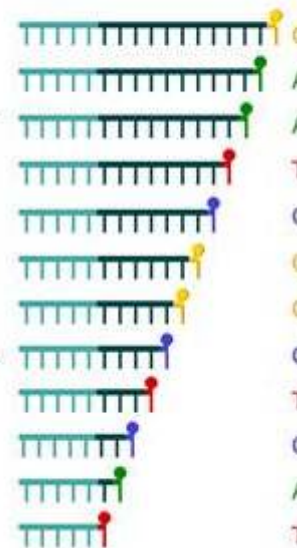
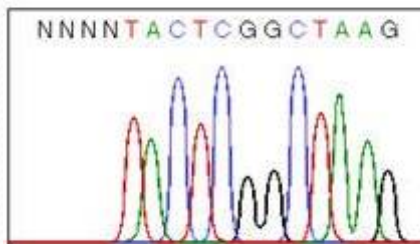


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Gel electrophoretic Fractionation

Cycle Sequencing

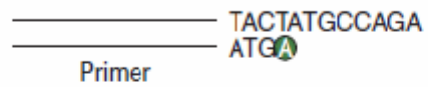
The simulated gel image is read from bottom to top, starting with the smallest fragment.



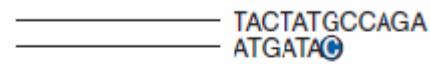
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(a) Primer extension reactions:

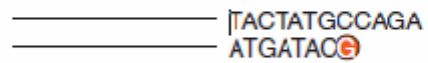
ddA reaction:



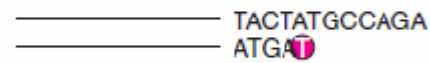
ddC reaction:



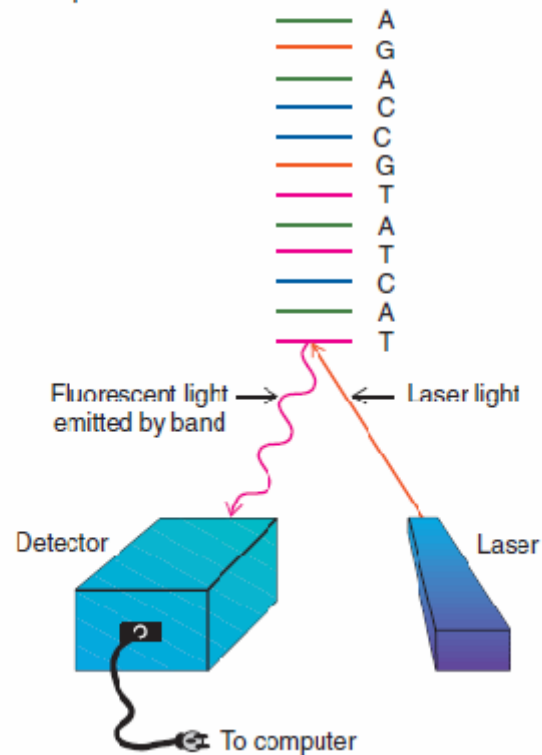
ddG reaction:



ddT reaction:



(b) Electrophoresis:



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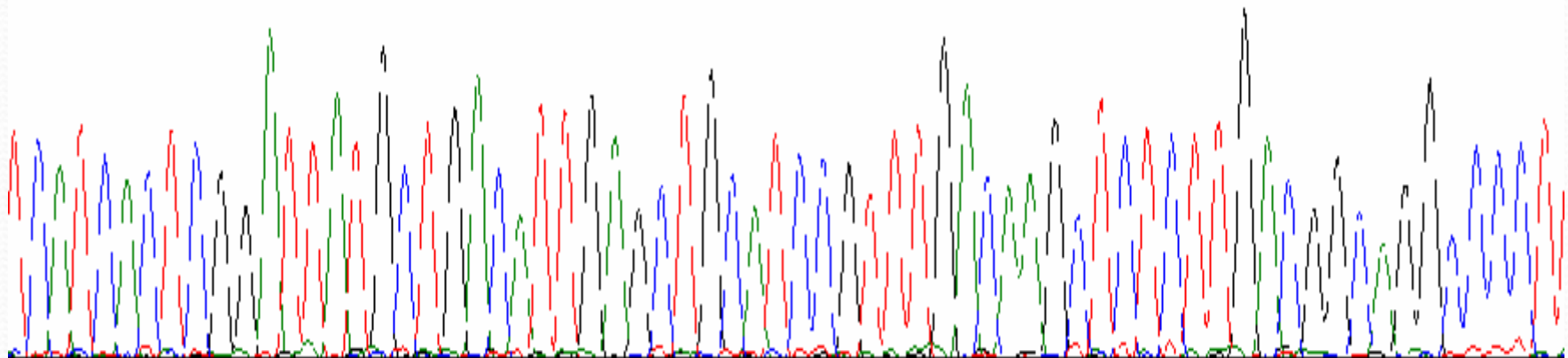
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Printout of an automated DNA sequencing

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

255 260 265 270 275 280 285 290 295 300 305 310 315 320

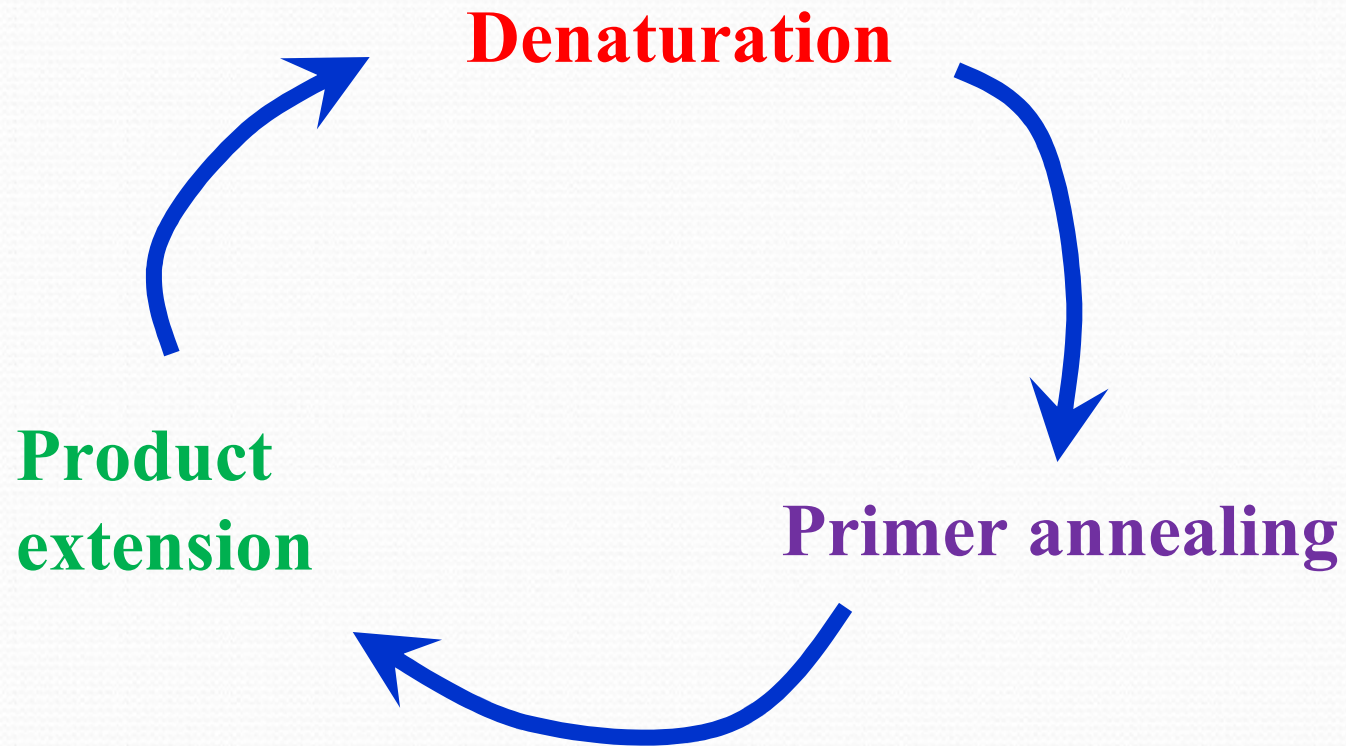


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Set up cycle sequencing



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