7.1 Techniques for Producing and Analyzing DNA

SBI4UP

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Biotechnologies

The following technologies will be reviewed in this lesson:

1) Recombinant DNA Technology

2) Gene Cloning

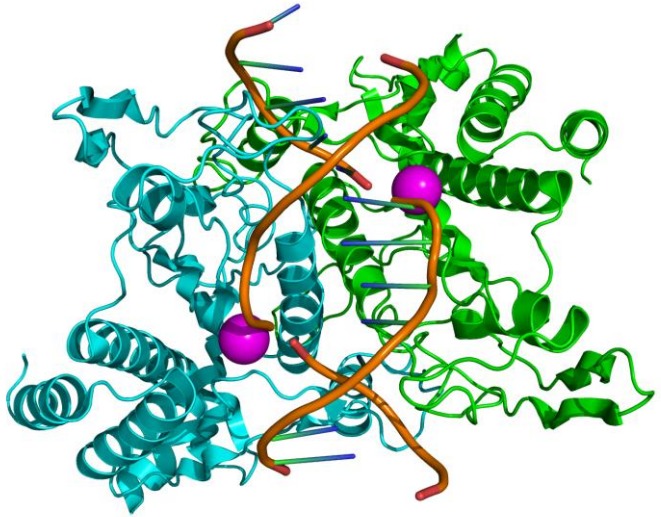
3) Polymerase Chain Reaction (PCR)

4) Gel Electrophoresis

Recombinant DNA Technology

Recombinant DNA: a molecule of DNA composed of genetic material from different sources.

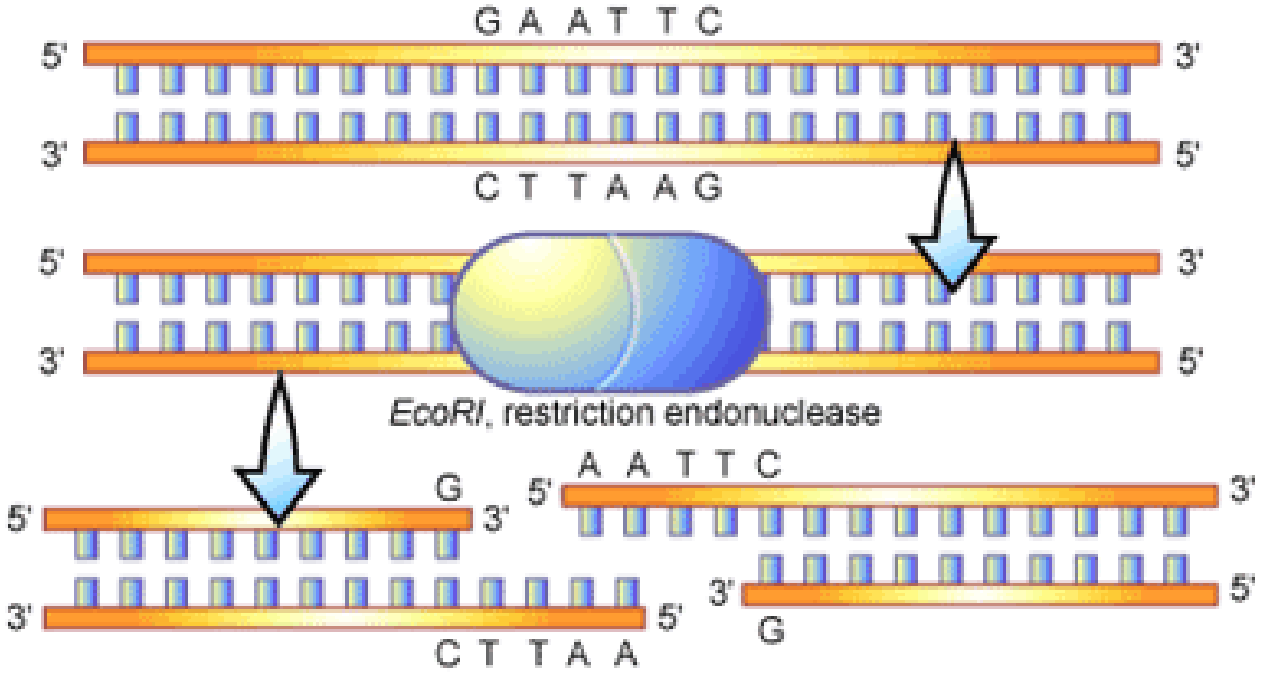
Prokaryotic organisms have synthesized many different restriction enzymes that enable them to protect themselves against viral DNA. Restriction enzymes ***cleave*** viral DNA so that it can no longer replicate within the organism.



Restriction endonuclease is an example of a restriction enzyme.

Recombinant DNA Technology

Restriction endonuclease *(enzyme)* recognizes specific nucleotide sequences and cleaves the double stranded DNA. The enzyme will cut at the restriction site of the target sequence.



Characteristics of Restriction Enzyme:

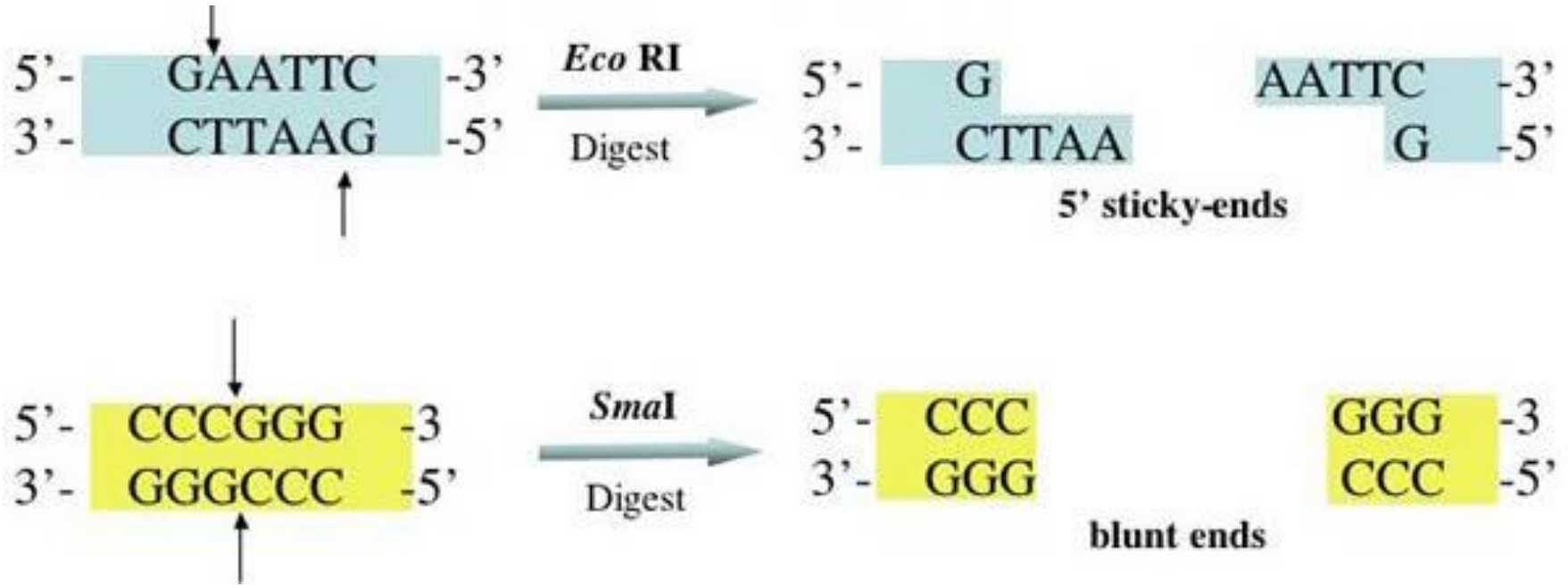
a) ***Sequence Specificity:*** Each enzyme recognizes a specific sequence which it cuts on every DNA.

b) Staggered cuts: sticky ends are left on either strand of the DNA.

Recombinant DNA Technology

Blunt ends: the ends of the DNA are fully base paired when cut by a restriction endonuclease.

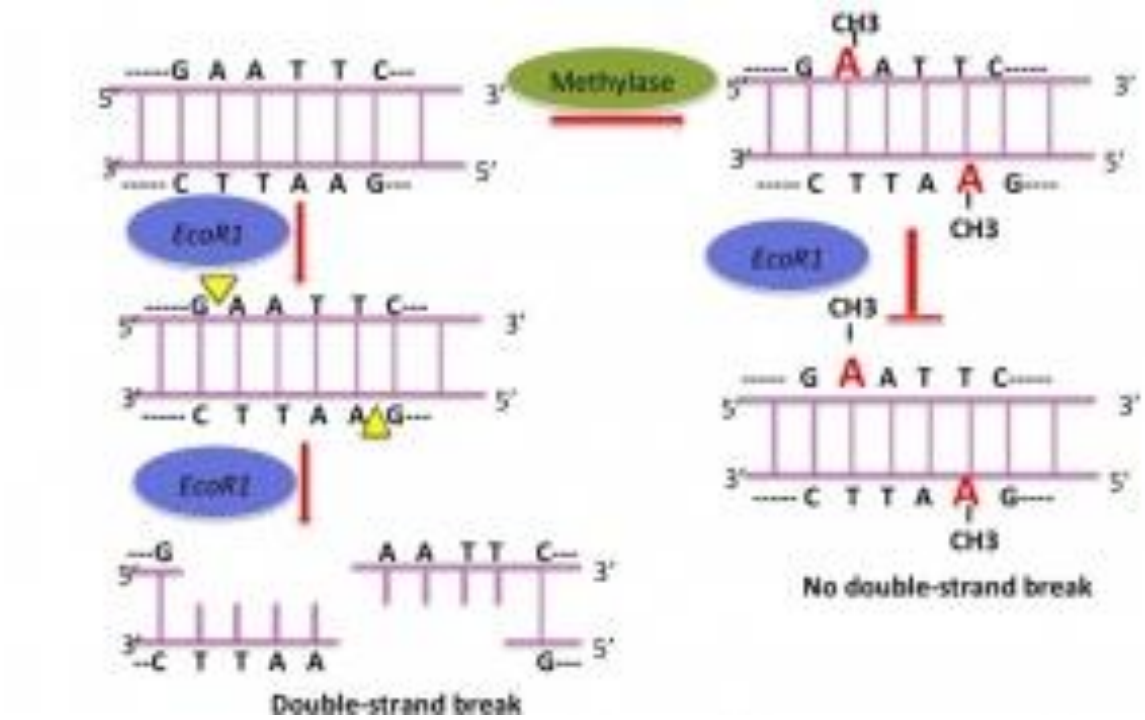
Sticky ends: the ends of the DNA have an overhang that allows them to bind to a complimentary overhang.



Scientists prefer the sticky ends when constructing a recombinant DNA

Recombinant DNA Technology

All bacterial cells can produce their own restriction endonucleases which enables them to breakdown bacteriophage DNA *(viral DNA)* and prevent them from being transcribed when they invade their cells.



By adding methyl to the recognition sites of endonucleases, the enzyme will not destroy its own DNA. Bacteriophage DNA does not contain methyl groups and thus is destroyed.

Recombinant DNA Technology

Features of Bacterial cells and restriction endonucleases that are useful in recombinant technology:

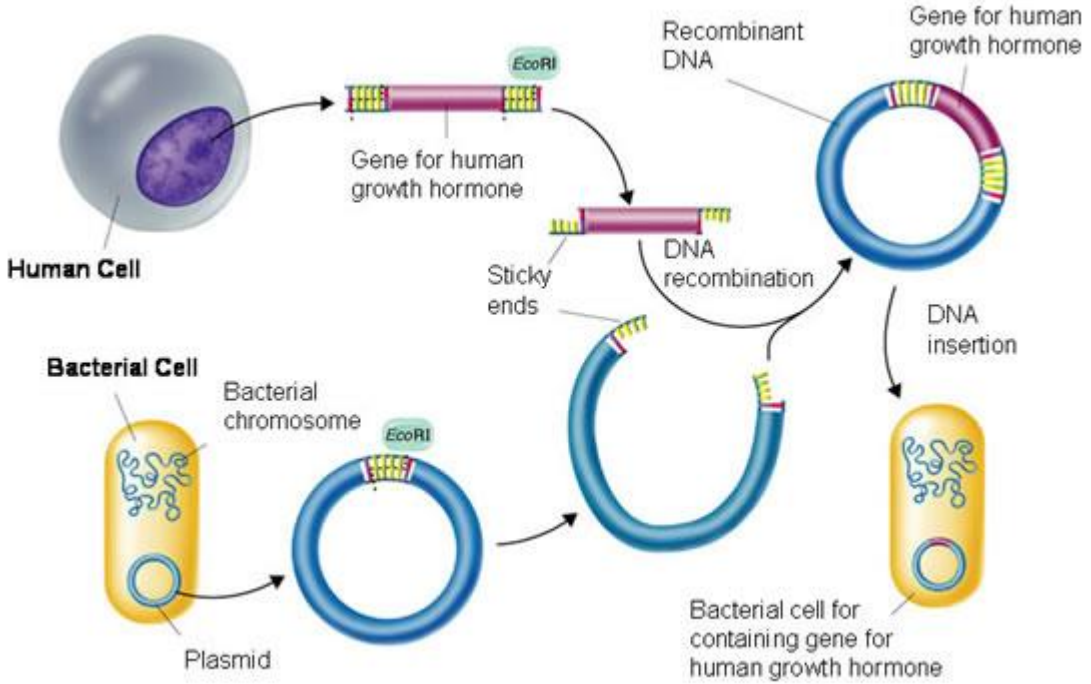
1) Enzymes can produce sticky ends

2) By adding a methyl group to a recognition site it will not be destroyed.

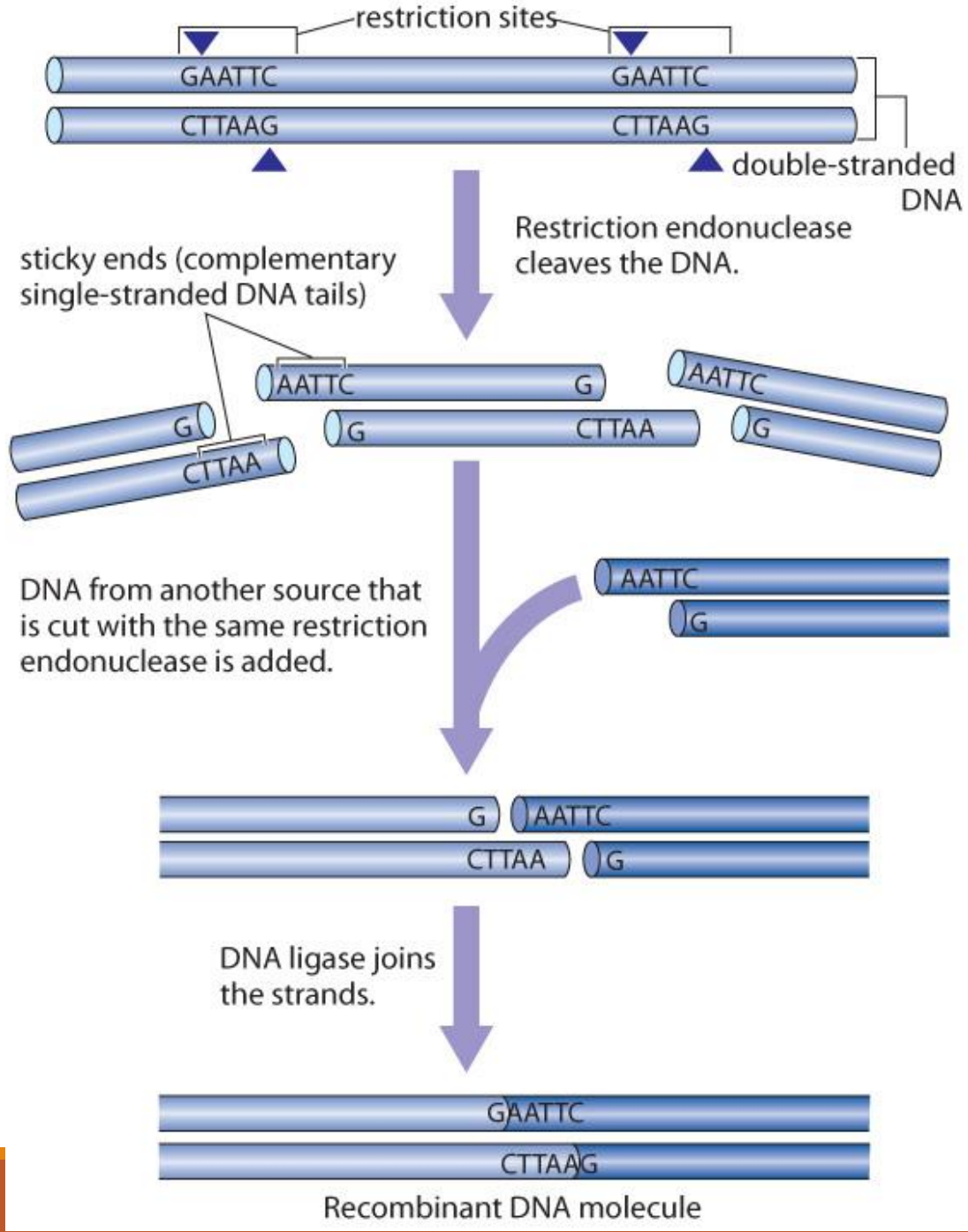
3) Most bacterial strains contain plasmids which can be used as a vector to carry genes of interest.

Recombinant DNA Technology

Restriction Endonuclease enzymes are used by scientists to combine DNA of interest to a plasmid. The sticky ends of the DNA can combine to any other DNA that also have complementary sticky ends.



Both DNA strands must be cut with the same restriction enzyme so that the sticky ends are complementary. When both pieces of DNA bind together it is known as 'Recombinant DNA'.



Step 1: Restriction enzyme are used to cleave the target sequence of the DNA of interest.

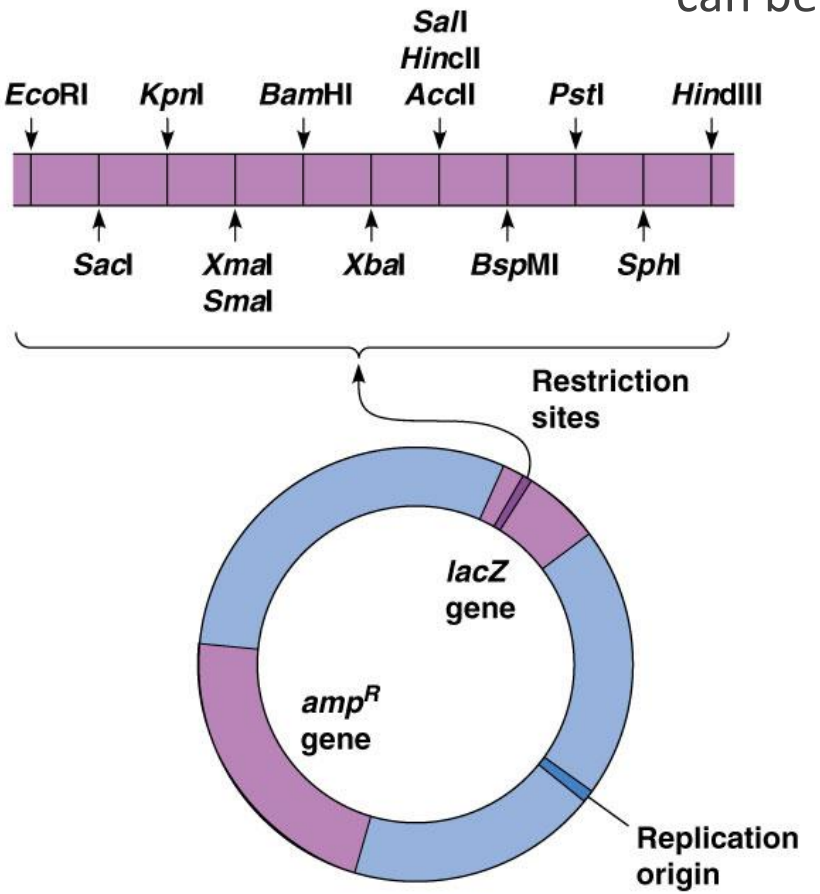
Step 2: When the target DNA sequence is removed, it produces a DNA fragment with sticky ends.

Step 3: The other DNA of interest must be cut with the same enzyme to produce complimentary sticky ends.

Step 4: Both DNA restriction fragments are incubated with DNA ligase so that a covalent bond can be formed between both fragments.

Gene Cloning in Bacteria

Recombinant DNA can be used by scientists to determine particular function of the gene of interest. Considering that specificity of restriction enzymes, particular sequences of human DNA can be analyzed and reproduced.

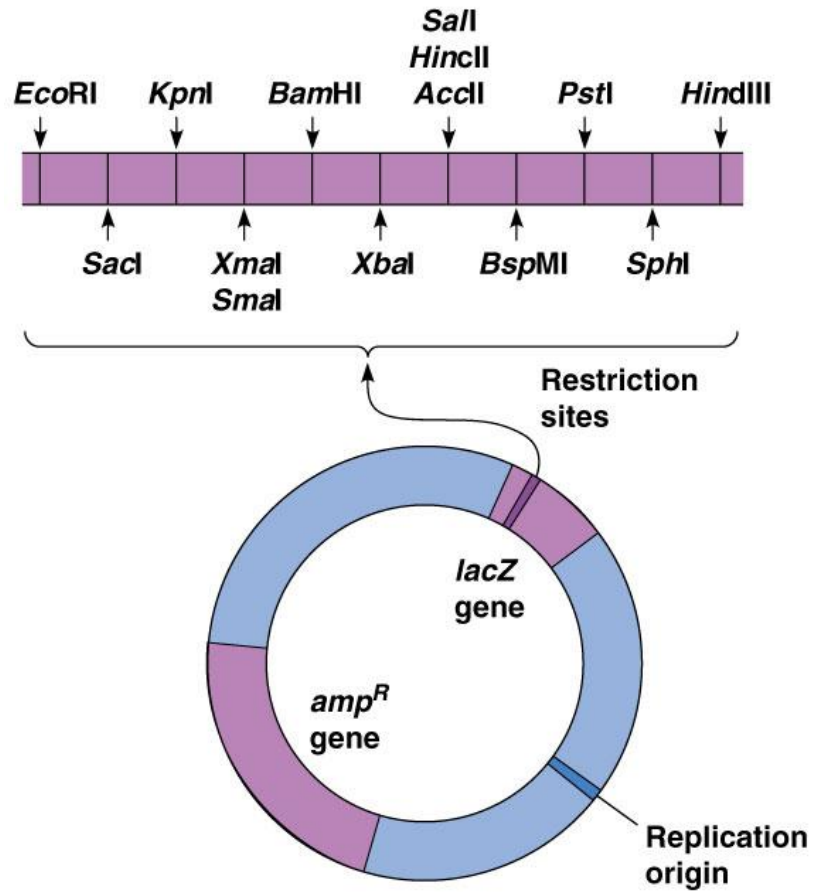


Recombinant DNA can be used to clone a gene of interest (gene cloning). In order to do so, a vector is required.

A vector can be a plasmid (from prokaryotic cells) that can carry the human genre of interest.

Vectors contain origins of replication that are independent of the bacterial DNA.

Gene Cloning in Bacteria



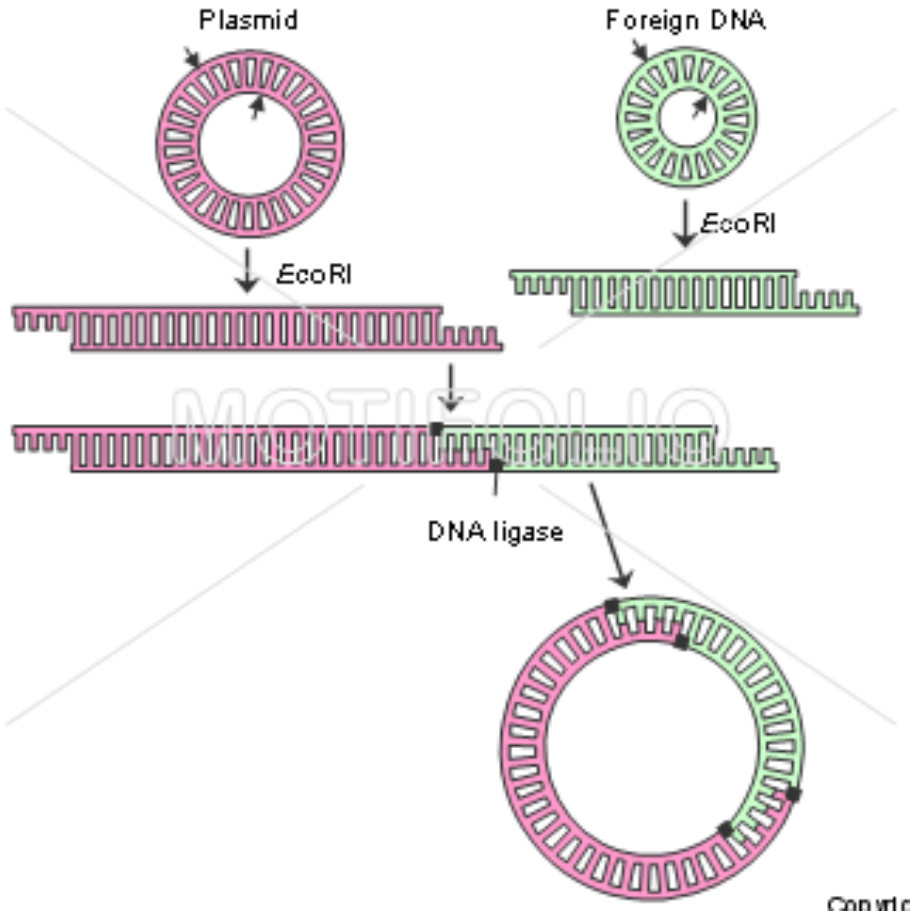
Criteria for Plasmids:

1) Must contain its own origin of replication

2) Must contain a gene resistant to a drug (ie ampicillin)

3) Must contain restriction sites that will allow the DNA of interest to bind.

Gene Cloning in Bacteria



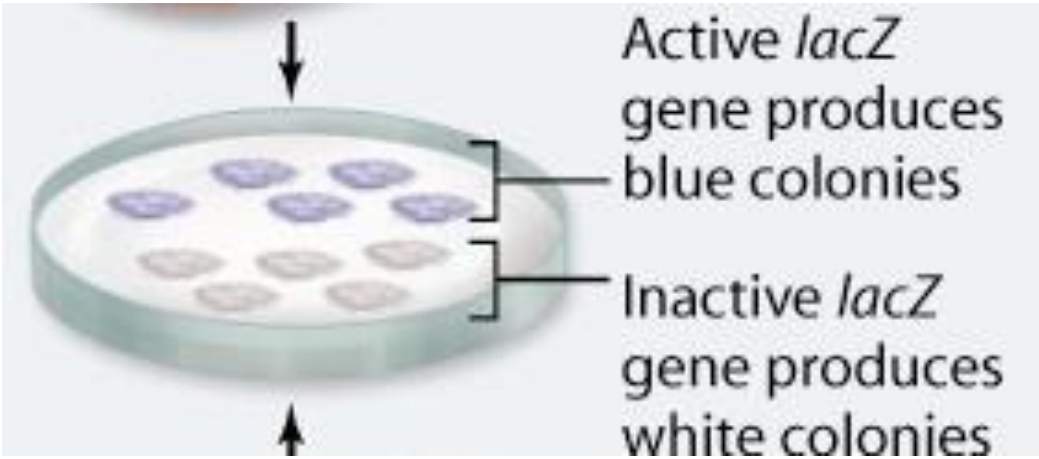
Both the DNA of interest and the vector must be cut with the same restriction enzyme so that complementary stick ends can be produced.

The bacterial medium must be treated with chemicals so that the membrane can be disrupted and the DNA of interest can enter the cytoplasm.

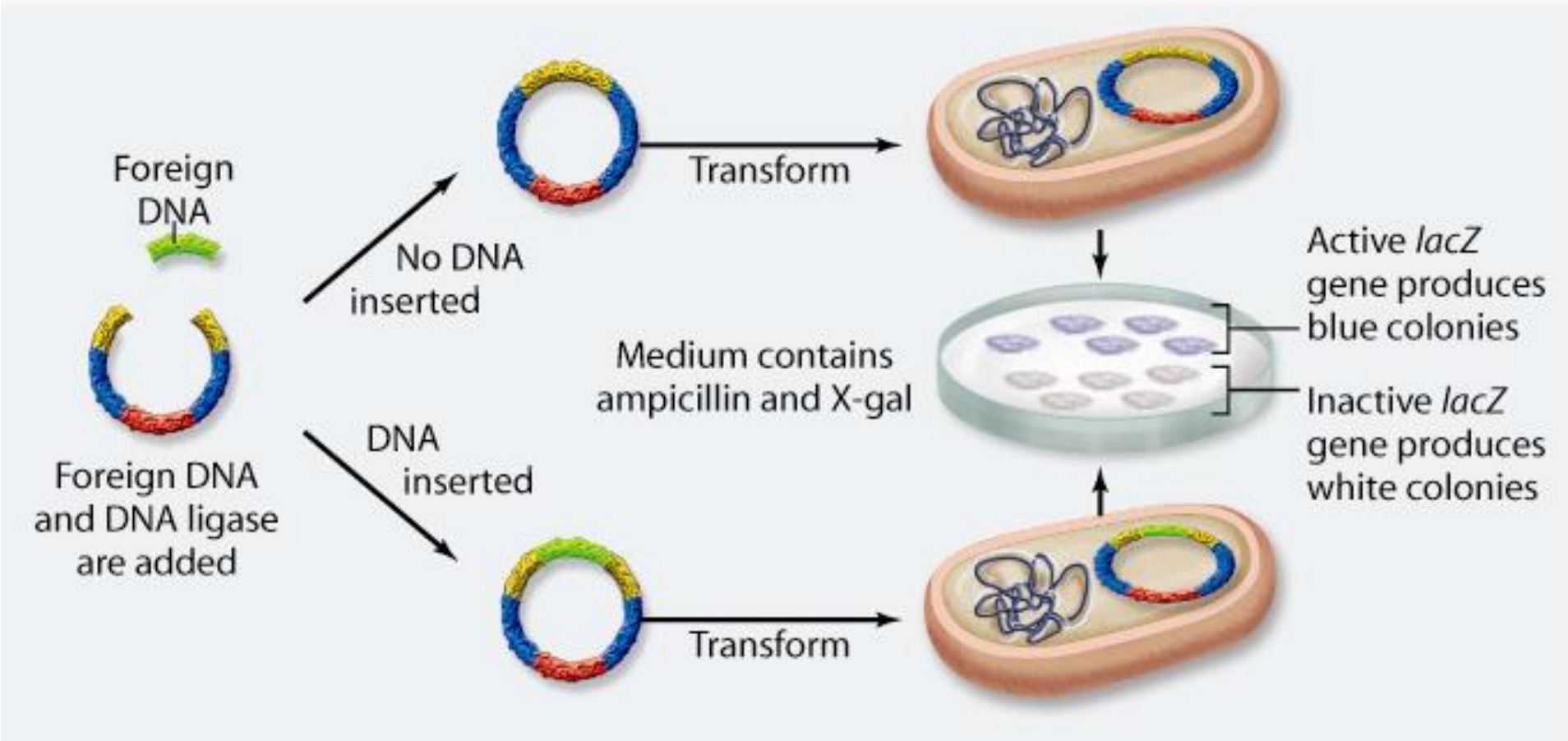
Gene Cloning in Bacteria

Two types of bacteria are grown on a petri dish containing ampicillin and an X-gal (galactose) which causes bacterial colonies to turn blue when broken down by active lacZ gene.

Through the process of elimination, scientists are able to identify the colonies that have integrated the gene of interest. They are isolated and grown on a separate culture.

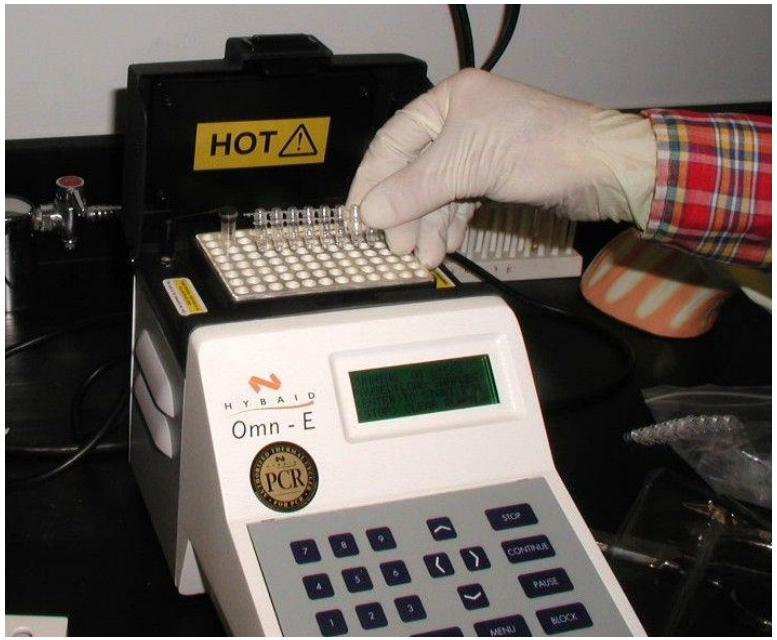


Recap of Gene Cloning in Bacteria

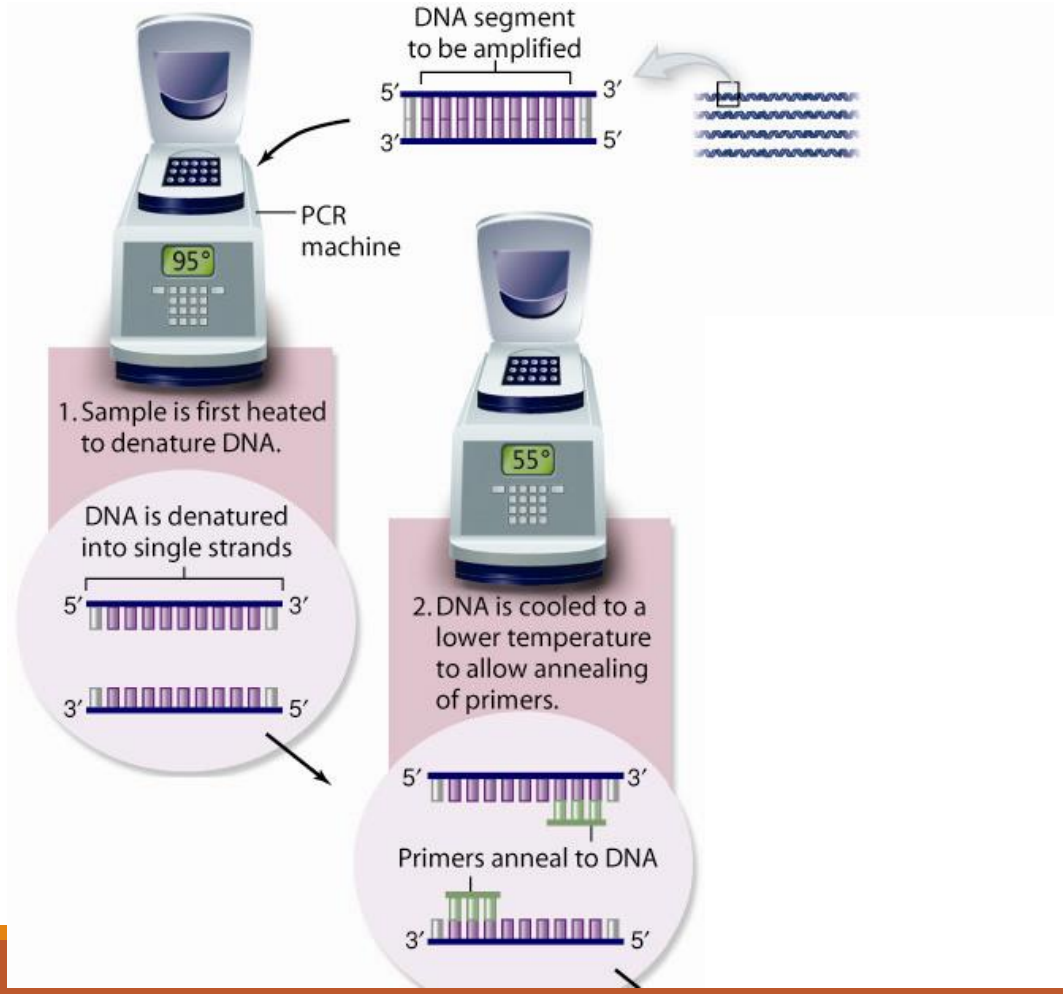


The Polymerase Chain Reaction (PCR)

PCR is a biotechnology used by scientists to produce large amounts of DNA (i.e DNA amplification). This technology also enables scientists to purify a small fragment of DNA for analysis purposes.



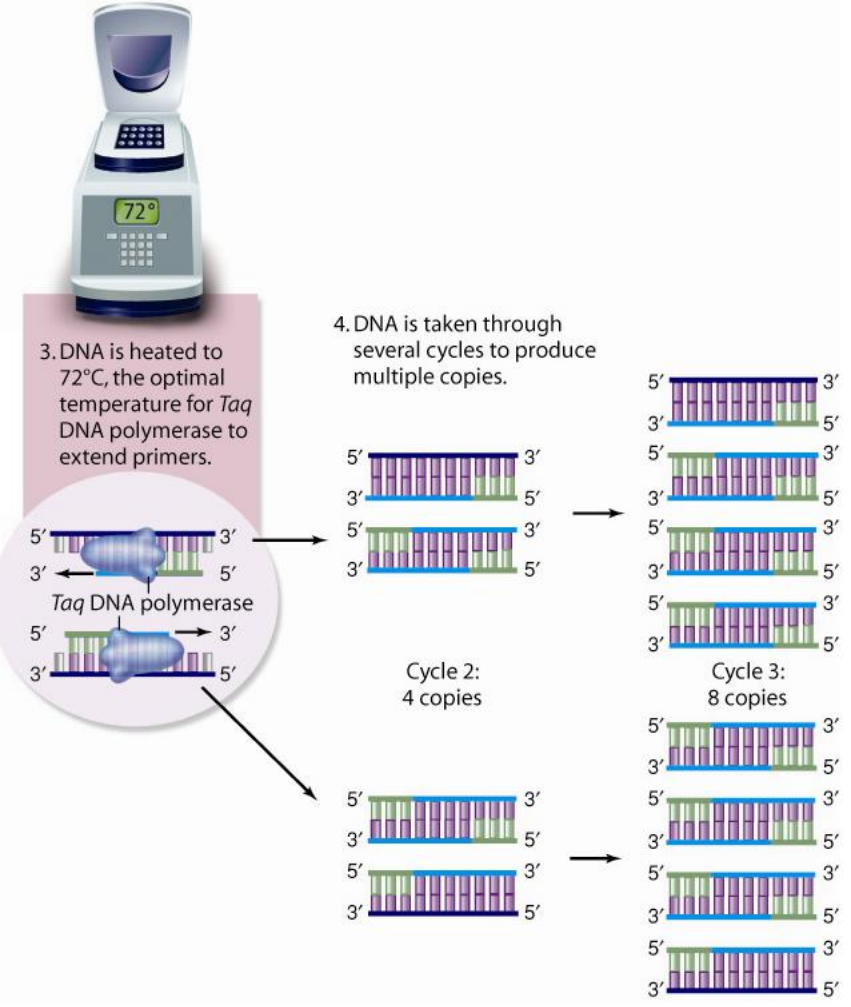
Step by Step PCR Procedure



Step 1: double stranded DNA is denatured in high temperature (95^{o}C).

Step 2: The temperature is lowered (55^{o}C) so that DNA primers can anneal to the 3’ end of both DNA strands.

Step by Step PCR Procedure



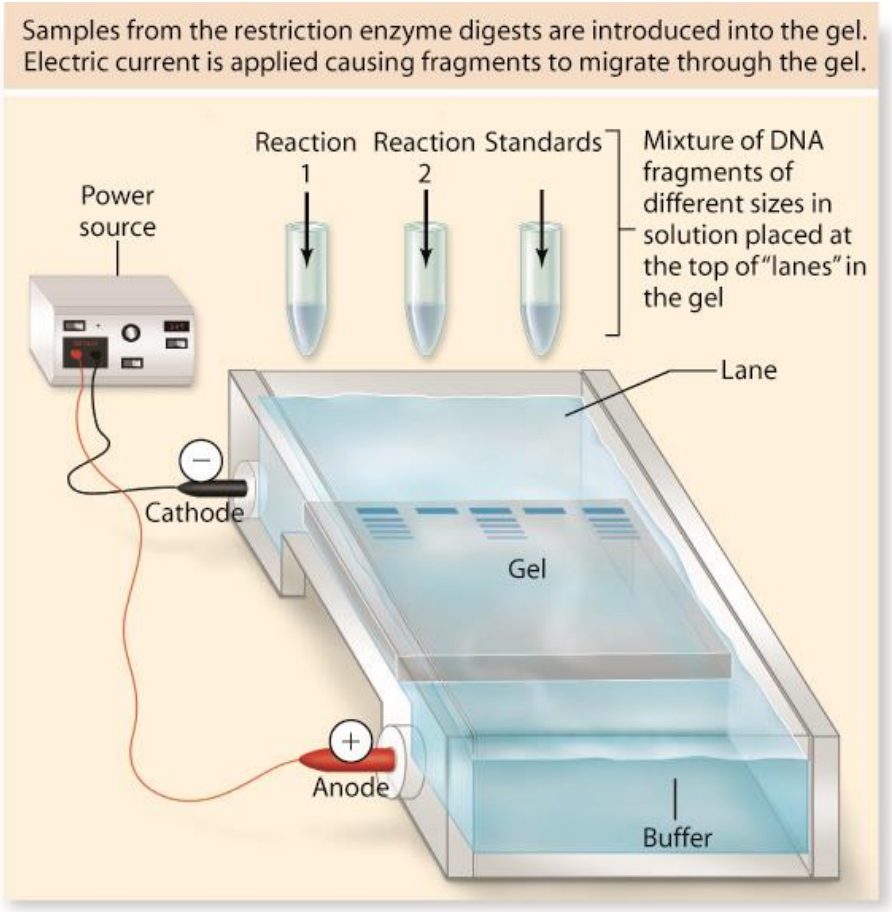
Step 3: Temperature is increased to 72°C and Taq polymerase is added to the sample. Taq polymerase adds free nucleotides to the primers that are complementary to the template DNA strand.

Step 4: The steps 1-3 are repeated multiple times (30 – 40 cycles). Each round of replication generates two new DNA strands. This DNA is amplified exponentially.

Gel Electrophoresis

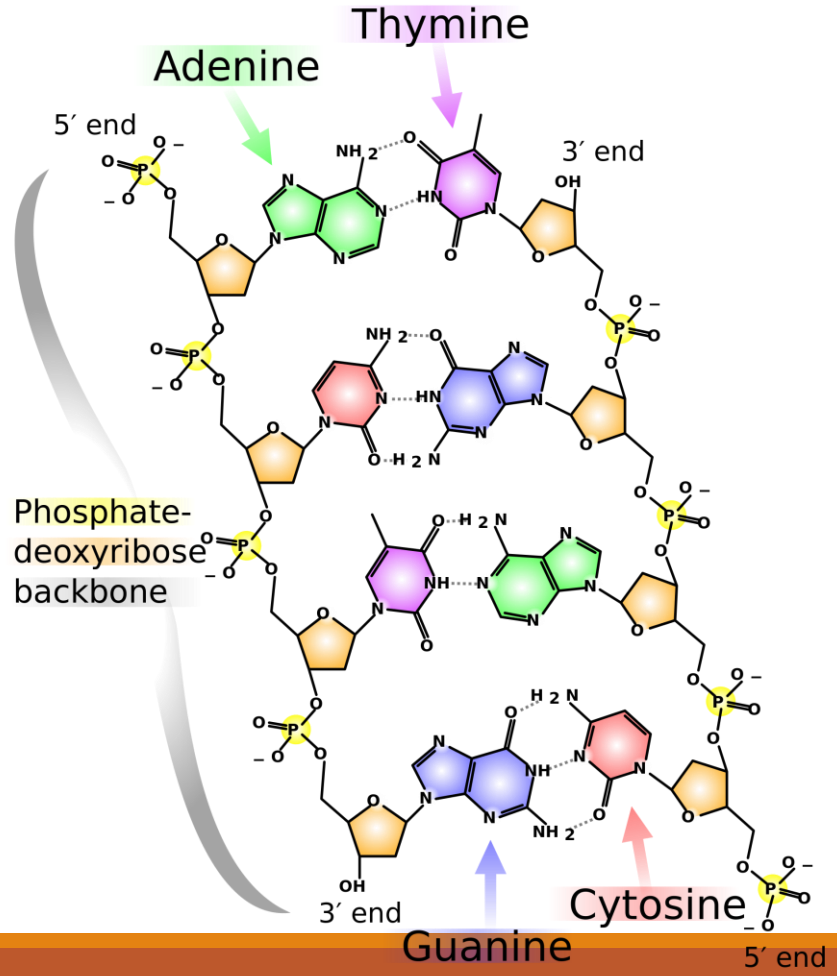
Once restriction enzymes are used to break the DNA into fragments, the desired DNA fragment can be identifies through gel electrophoresis.

Gel Electrophoresis: technology that separates charged molecules on the basis of sorting through a gel meshwork.



This technology takes advantage of the chemical properties of DNA to separate the fragments.

Gel Electrophoresis



Properties of DNA:

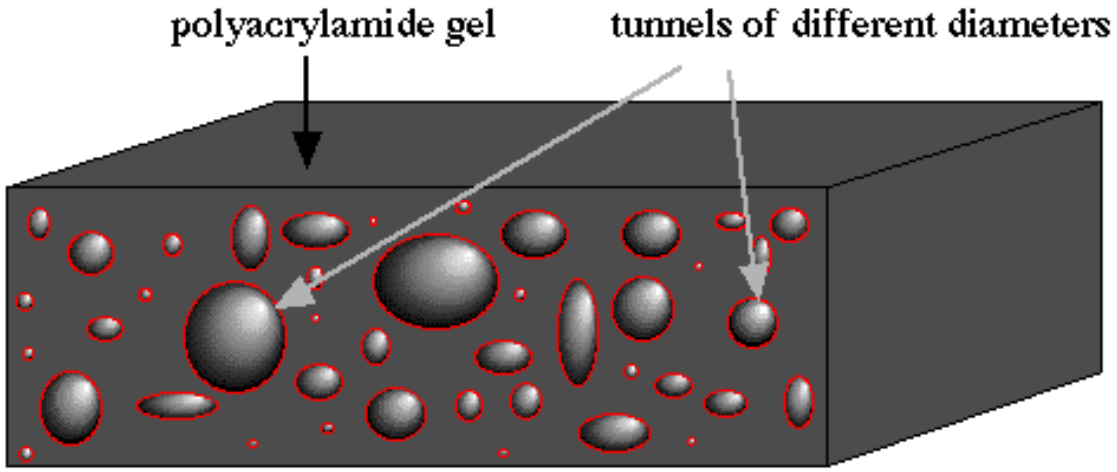
1) DNA is negatively charged due to the phosphate group on the backbone

2) The molar mass of each nucleotide is approximately the same.

As a result of these two properties, each nucleotide has the same charge-to-mass ratio. The only difference between both fragments is the length of the fragment.

Gel Electrophoresis

The smaller DNA fragments have smaller molecular mass and travel through the gel at a greater speed. The smaller fragments are able to travel trough the pores of the gel much easier than larger fragments.



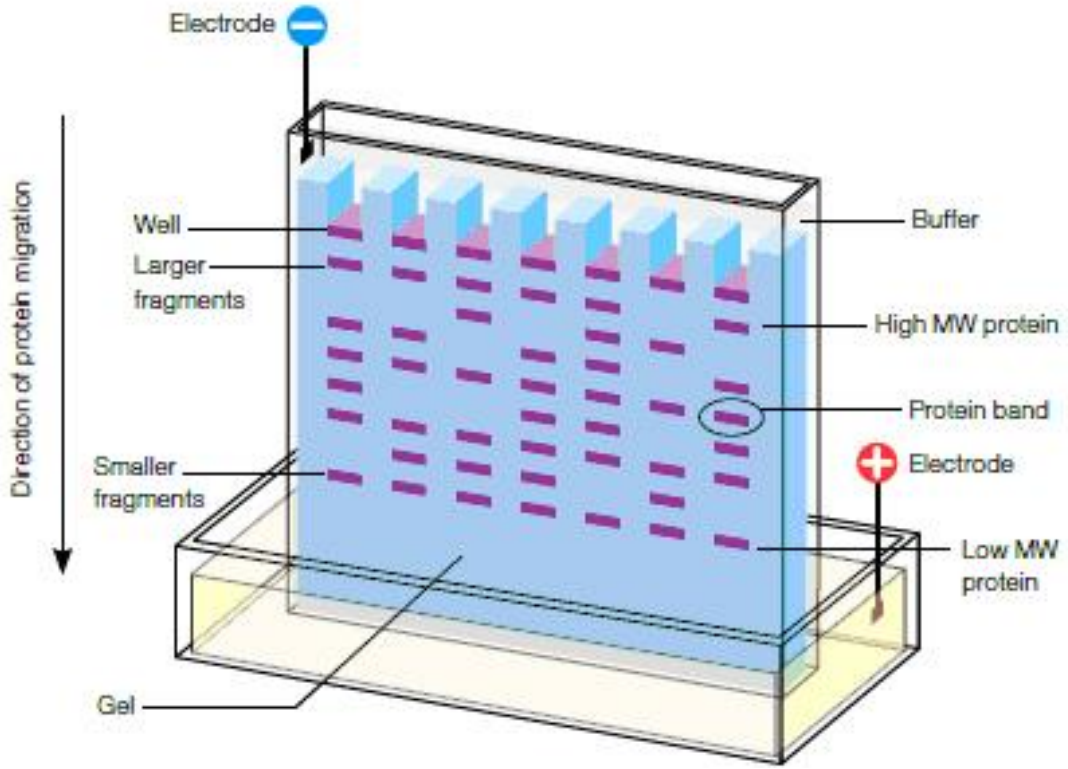
A buffer solution such as ***agarose or a polyacrylamide gel*** is added to the box. The buffer creates pores of different diameters.

A dye is also added to the DNA fragment solution so that it can be visible when added to the buffer solution.

Gel Electrophoresis

A negative charge is applied to the well where the DNA solution is injected and a positive charge is applied on the opposite end. Due to DNA's negative charge, it will migrate towards the positively charged end of the box.

The smaller fragments will travel much quicker towards the positively charged end of the box. The heavier fragments will remain closer to the wells where they were first injected.

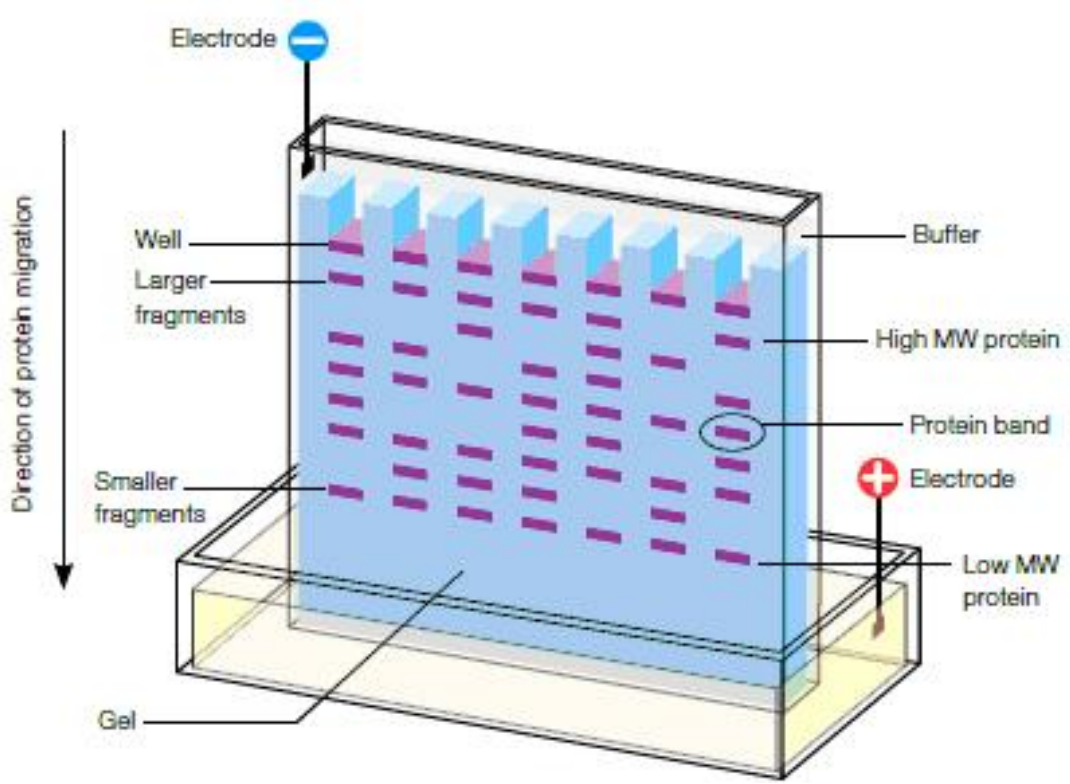


Gel Electrophoresis

When using a particular restriction enzyme to cleave the DNA, there is a characteristic pattern that is associated with that enzyme. Molecular markers must be used on the well to compare the sizes of the DNA fragments from the solution.

The fragments from the DNA solution are them compared to the molecular marker to find the gene of interest. This area can be detected by looking at the bands on the gel.

The desired fragment can then be isolated and purified for further study.



Checking for Understanding

What is the main function of restriction enzymes?

A) to join DNA fragments at the 3’ ends

B) to join DNA fragments at the 5’ ends

C) to cut DNA at specific sites within the DNA

D) to cut DNA from the 5’ or 3’ ends

E) to cut DNA at the 5’ ends

Checking for Understanding

Which enzyme catalyzes the joining of fragments to form recombinant DNA?

A) DNA polymerase

B) helicase

C) DNA ligase

D) Restriction endonuclease

E) RNA polymerase

Checking for Understanding

What is the name of the process that introduces DNA into bacterial cells?

A) gene cloning

B)translocation

C) translation

D) transcription

E) transformation

Checking for Understanding

Why are bacteria the most common hosts used in gene cloning?

A) bacteria can produce selectable markers

B) bacteria have the capacity to perform all post-translational modification of human proteins

C) bacteria have mechanisms to prevent the degradation of foreign proteins

D) bacteria are resistant to many antibiotics

E) bacteria grow quickly in large amounts and are inexpensive to maintain

Checking for Understanding

During PCR, why is the DNA sample initially heated to a high temperature for approximately 95^{o}C?

A) heating causes the DNA sample to be denatures into two separate strands

B) heating allows DNA polymerase to bind to DNA

C) Heating allows RNA polymerase to bind to DNA

D) Heating allows the annealing of primers to DNA

E) Heating allows the extensions of DNA

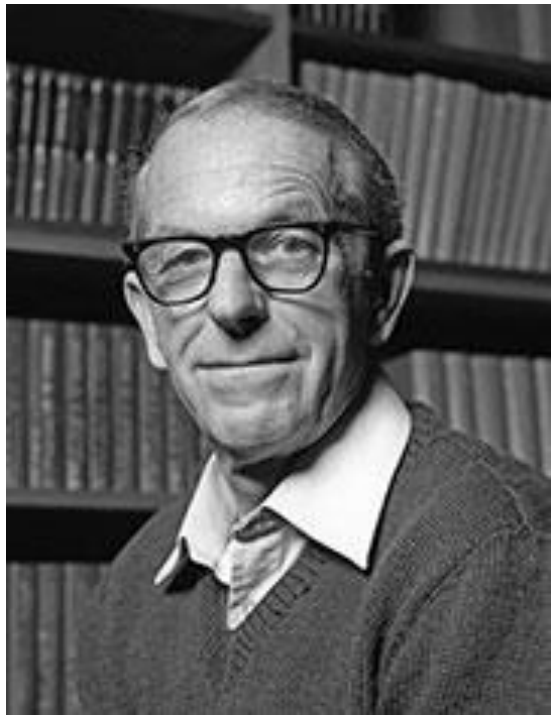
Homework

Textbook: p. 291 # 1-6

DNA Sanger Sequencing

DNA sequencing is a method commonly used by scientists to determine the nucleotide sequence for a particular gene. The Sanger sequencing method enables Frederick Sanger to determine the entire genome of a bacteriophage (5386 b.p).

Sanger took into account the properties of DNA and DNA replication and developed a method to determine nucleotide sequences.



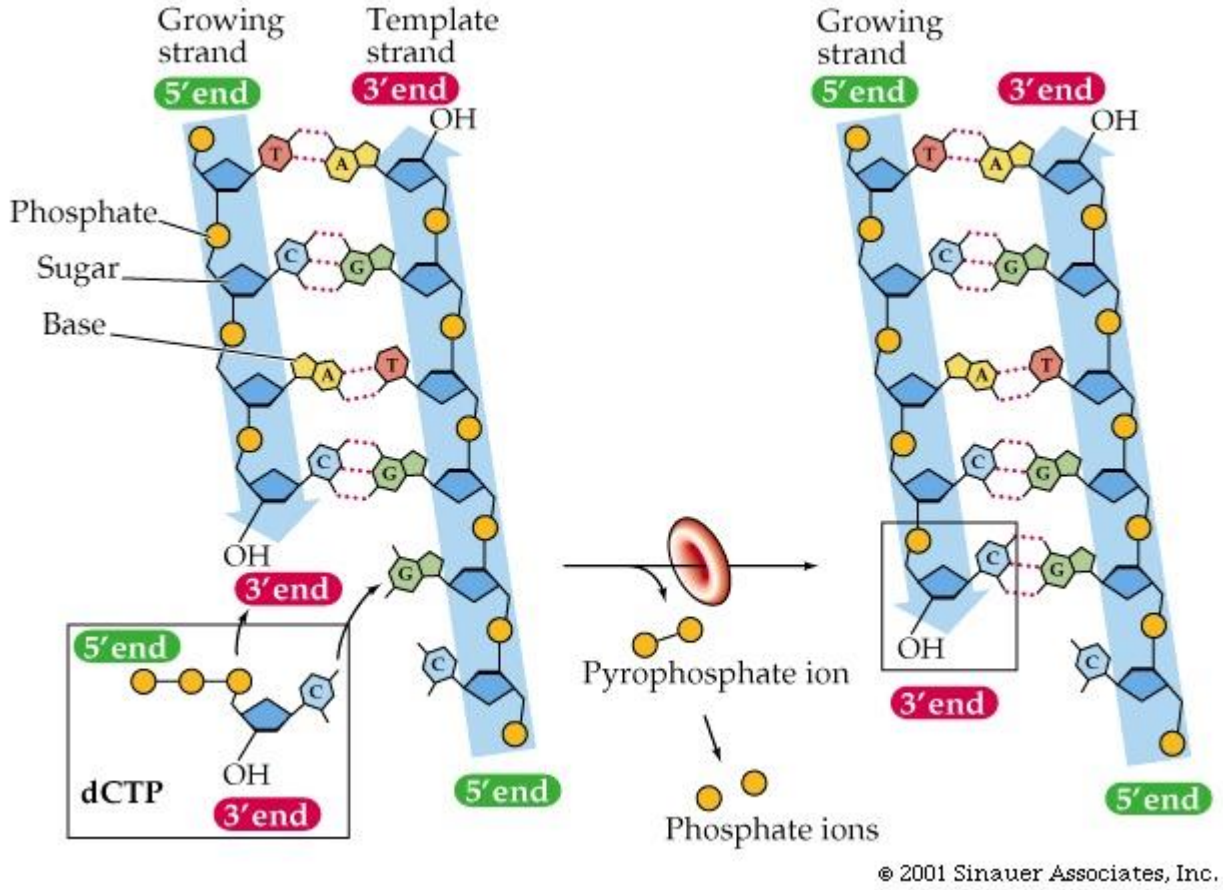
DNA Sequencing – Overall Method

DNA replication is the process whereby both DNA strands are used as template to create new, identical copies of the DNA sequence.

DNA replication requires:

- DNA template
- Short s.s radioactively labelled primer
- DNA polymerase
- Nucleoside triphosphate

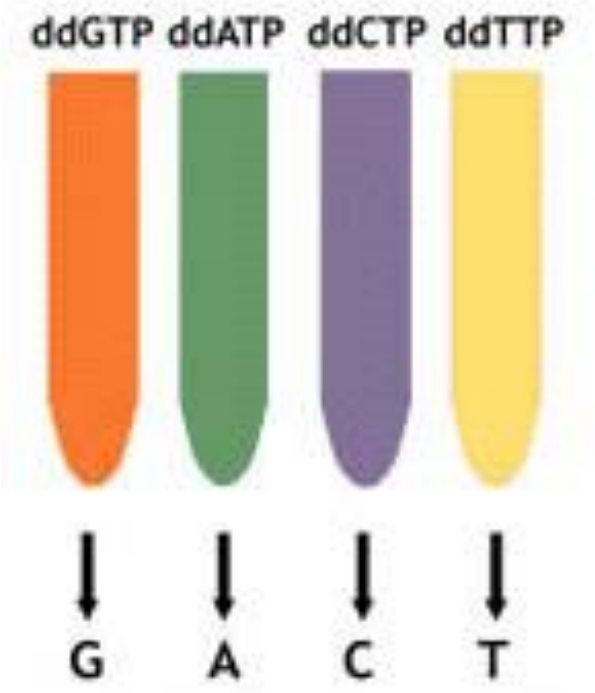
DNA Sequencing – Overall Method



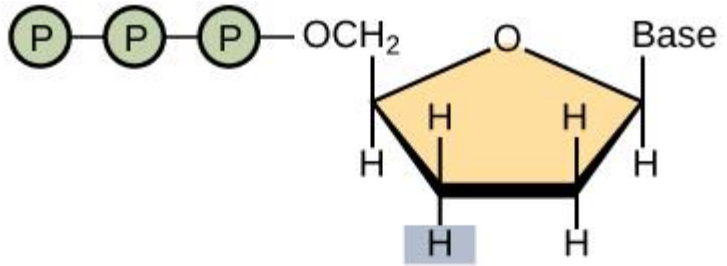
- Within 4 tubes contain the same DNA primer (radioactive), and the 4 different types of nitrogenous bases
- The DNA polymerase, will add the free floating nitrogenous bases from the tube to the growing DNA strand
- Two phosphate groups must be removed from the nitrogenous base and a phosphodiester bond is created between the sugar and the phosphate of the previous nucleotide.

DNA Sequencing – Overall Method

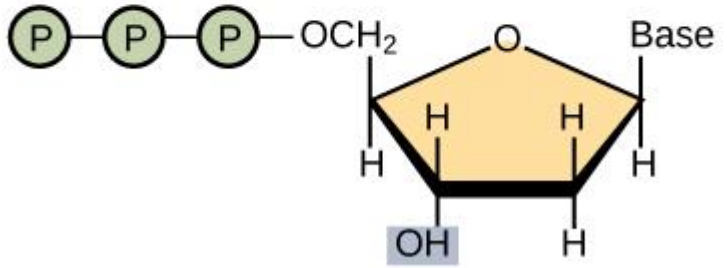
Within the 4 reactions tubes, a radioactively labelled dideoxy analogue of one of the deoxynucleoside triphosphates (dNTPs) is added in low concentration.



Dideoxynucleotides lack a –OH group at the 2' and 3' carbons on the ribose sugar. As a result, DNA synthesis terminates when one of the four possible dideoxynucleotides are incorporated.



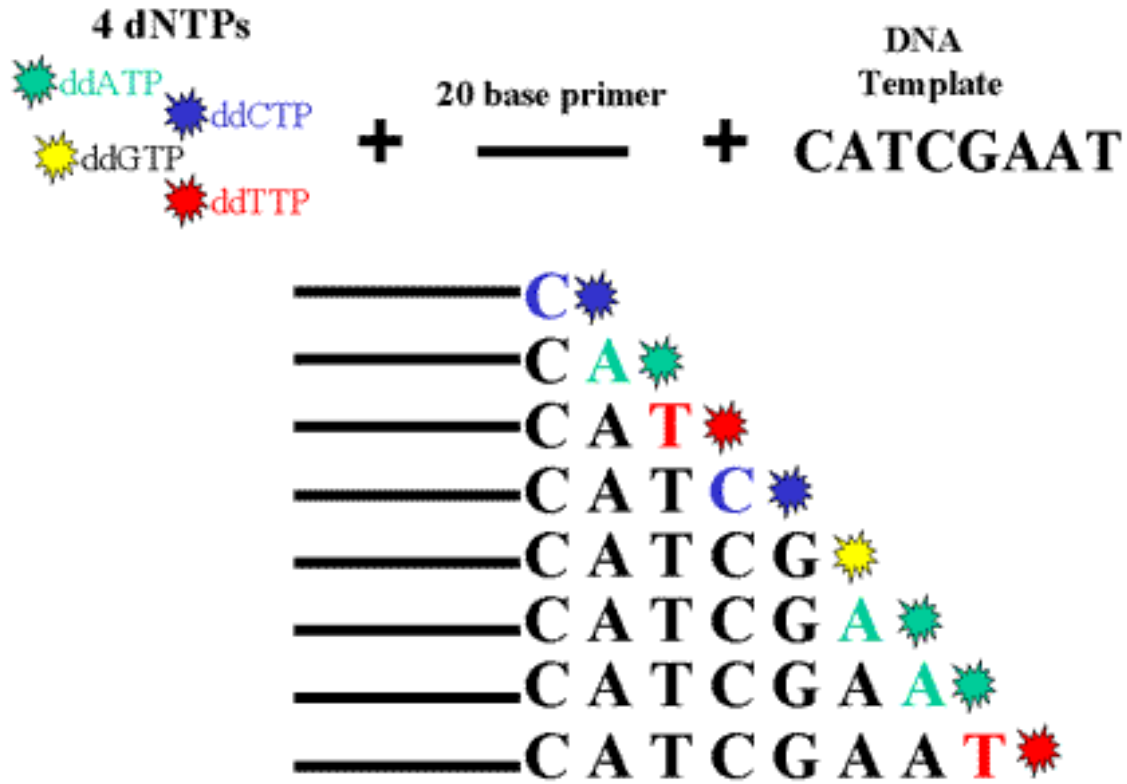
Dideoxynucleotide (ddNTP)



Deoxynucleotide (dNTP)

DNA Sequencing – Overall Method

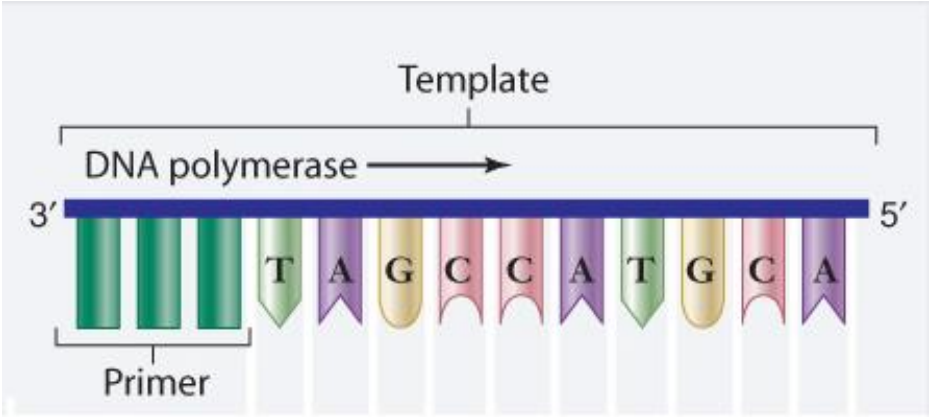
Considering that only a small portion of the dNTPs are dideoxy analogue, different lengths of DNA fragments can be created.



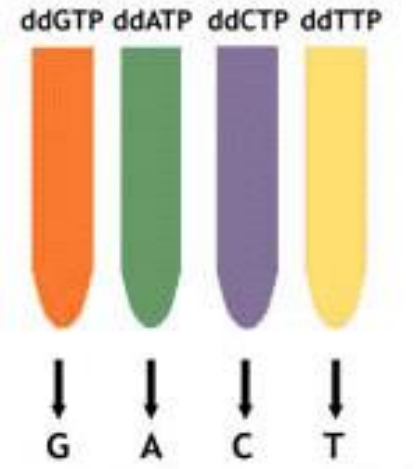
Different length of DNA fragments are created due to the dideoxy's ability to terminate DNA replication within the reaction tube. The different lengths allow it to be separated by gel electrophoresis.

Steps in DNA Sequencing

Step 1: DNA is denatured into s.s stranded DNA and the radioactively labelled primer is added to a reaction tube. This provides a 3'-OH for replication.



Step 2: S.S DNA annealed with the primer are added to four separate reaction tube along with dNTPs. DNA polymerase and one of four dideoxynucleotides is added to each reaction tube.



Steps in DNA Sequencing

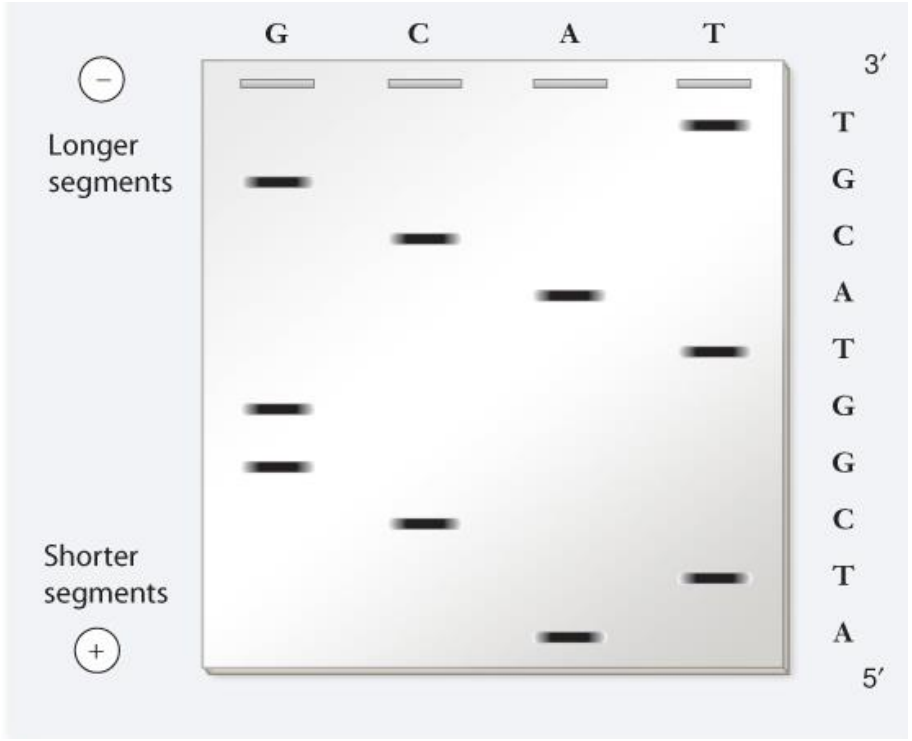
Step 3: The reaction proceeds and DNA polymerase begins to add the nucleotides onto the growing DNA chain. Due to the dideoxynucleotides, different fragments are produced.

The corresponding radioactive dideoxy will be found at the end of each newly synthesized DNA fragment in the reaction tube.

Steps in DNA Sequencing

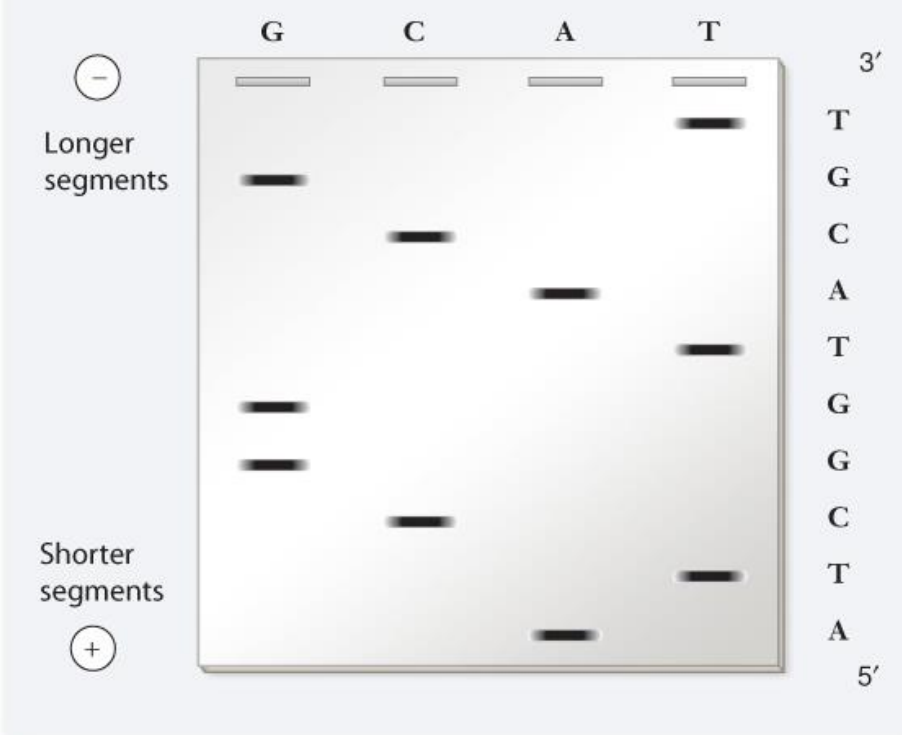
Step 4: Each of the reaction tubes are separated on the gel electrophoresis with a polyacrylamide gel. Depending on the size of the DNA fragments, they will travel across he gel at different speed towards the positive end of the gel.

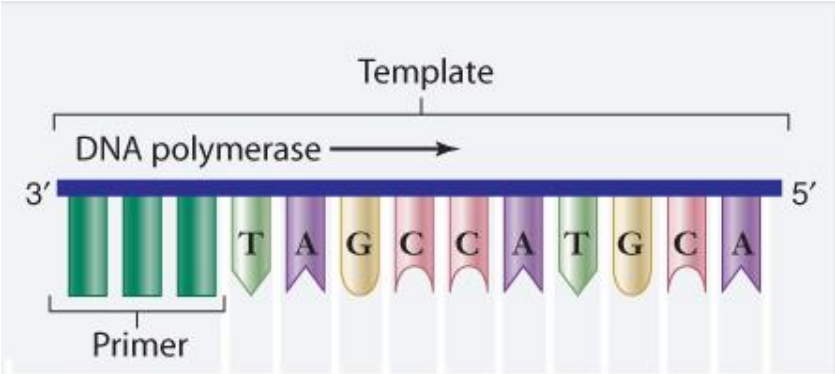
Once the fragments have run along the gel, it is exposed to X-ray film through autoradiography. This highlights the radioactive tags of dideoxynucleotides.



Steps in DNA Sequencing

Step 5: Scientists then compare the fragments within the gel and analyze the length of the fragments along with their radioactive labels. Through comparison, scientists are able to identify the location of each nucleotide within the DNA sequence.





The DNA sequence identified on the gel electrophoresis is the complementary sequence to the original template strand.

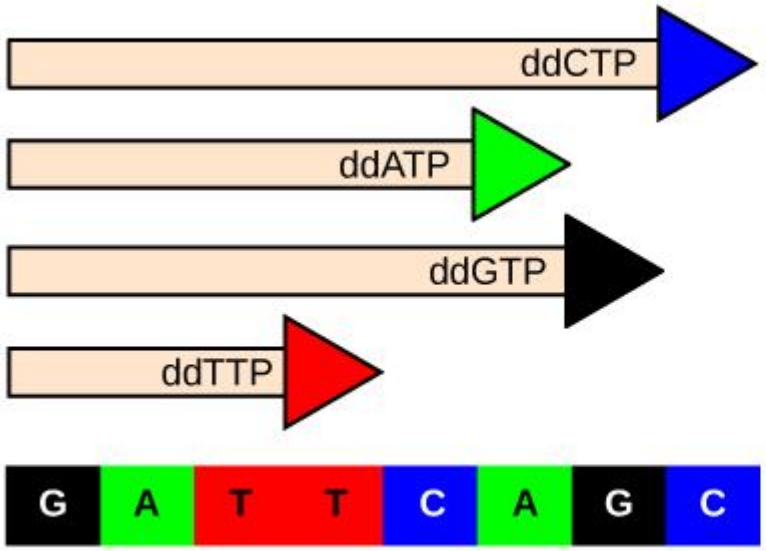
New Sequencing Methods

New and efficient methods were developed for the Human Genome Project. The old Sanger sequencing was limiting for scientists could only read 300 nucleotides at one and it took time to create and separate the four reaction tubes.

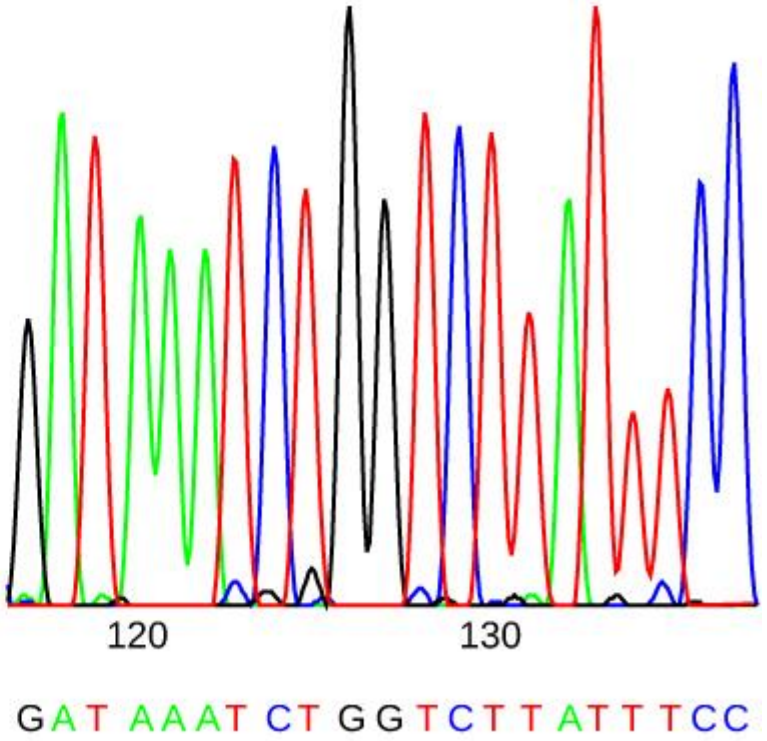
New Method:

- More bases can be read
- Dideoxynucleotides are labelled with their own color of dye tags
- All dideoxynucleotides are added to one reaction tube only
- In gel electrophoresis, only one lane is required for the only reaction tube and a laser lights up the tags in the gel
- Photodetectors are used to identify the colour and the fragments can be analyzed.

New Sequencing Methods



Dye–labeled dideoxynucleotides are used to generate DNA fragments of different lengths



Each peak can be interpreted as the following nucleotide in the DNA sequence. Once scientists have identified the newly synthesized DNA strand, they must determine the complementary sequence to identify the sequence of the gene they have isolated.

Homework

Textbook: pg. 300 # 1, 4, 7, 8 & 9