PRINCIPLES OF MOLECULAR BIOLOGY

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Molecular biology

is the science that strives to understand the chemical and physical basis of biological specificity and variation

Particularly important to molecular biology techniques is a set of enzymes

1

restriction endonucleases

(also called restriction enzymes)

recognize and cleave DNA

at specific DNA sequences

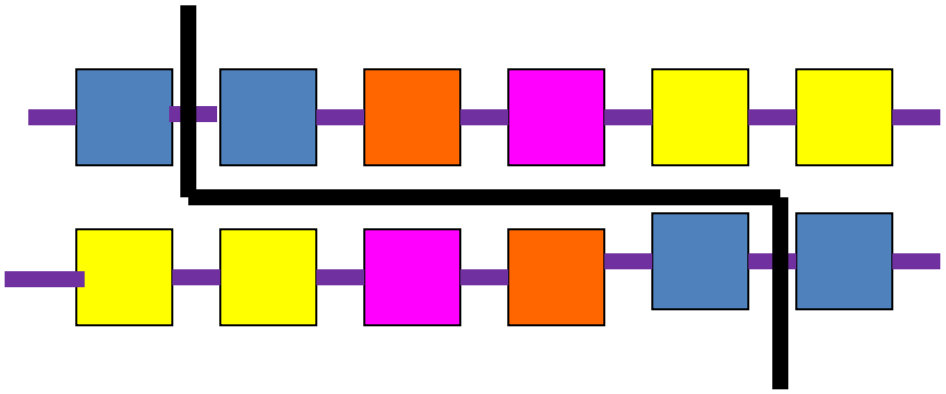
to generate a set of smaller fragments

Two types of restriction endonucleases

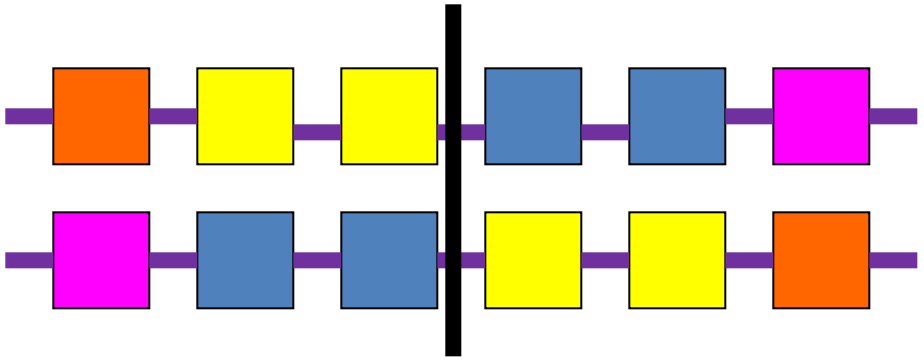
- **One give unpaired strands which are referred to as sticky ends because they can base-pair with each other or with complementary sticky ends of other DNA fragments.**

- **Other restriction endonucleases cleave both strands of DNA at the opposing bonds, leaving no unpaired bases on the ends, often called blunt ends.**

Restriction endonucleases



Sticky ends



Blunt ends

Particularly important to molecular biology techniques is a set of enzymes

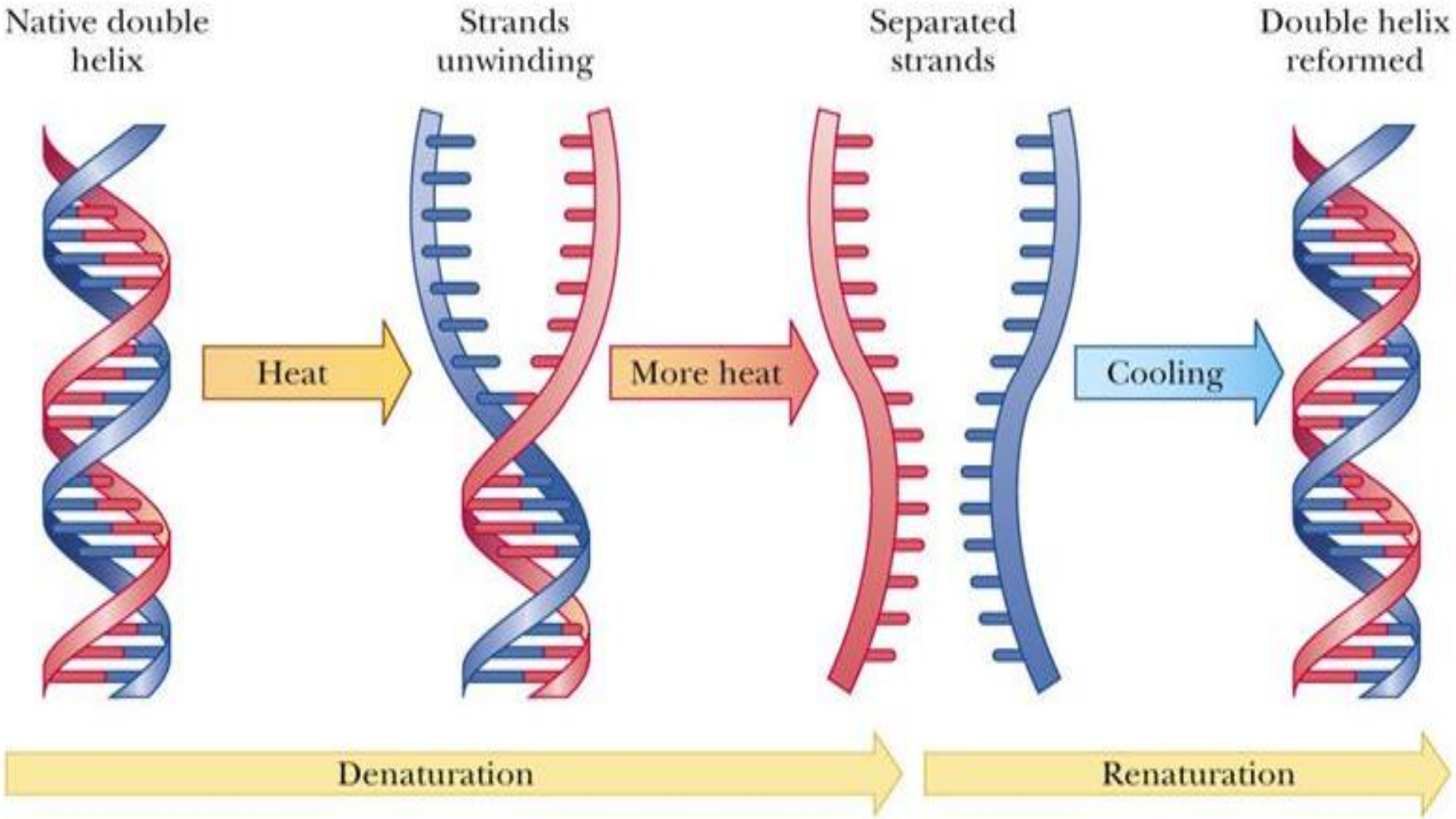
2

DNA ligases

can join the DNA fragment to link the DNA molecules together

Denaturation of DNA

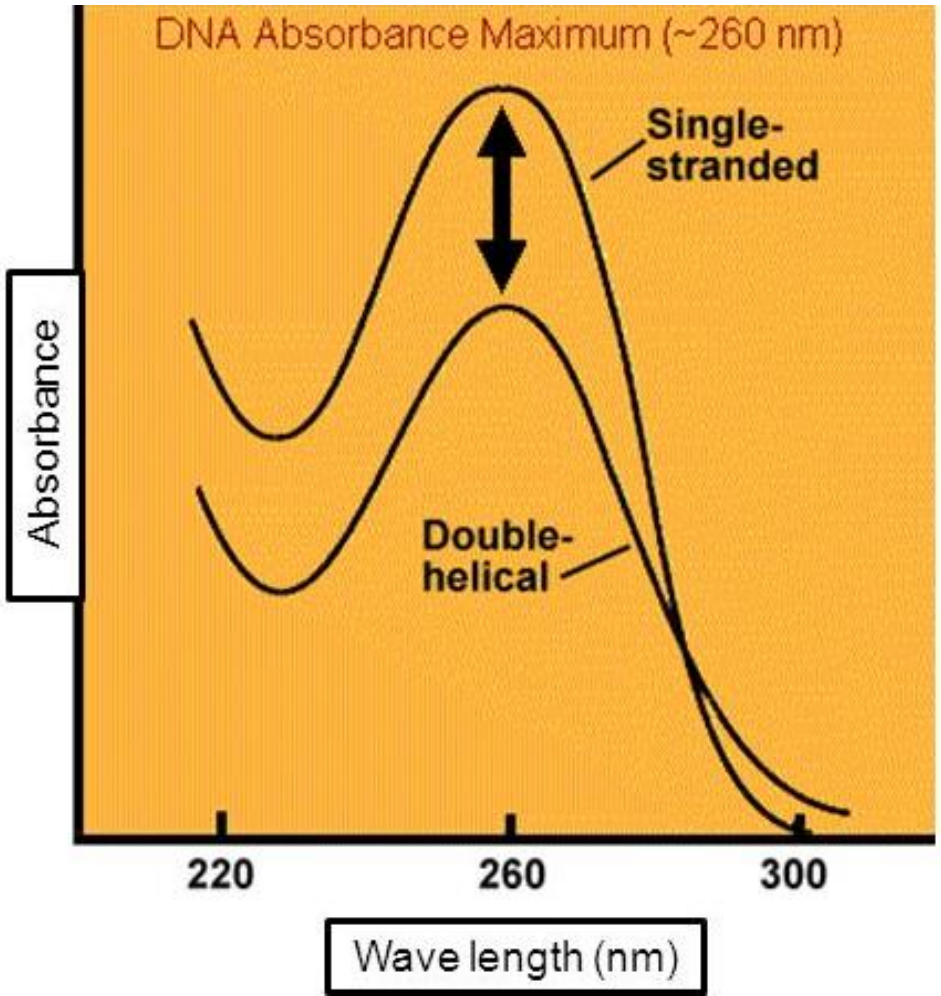
The double-stranded structure of DNA can be separated into two strands in solution by increasing the tº or decreasing the salt concentration



Disruption of the hydrogen bonds between paired bases causes unwinding of the double helix to form two single strands.

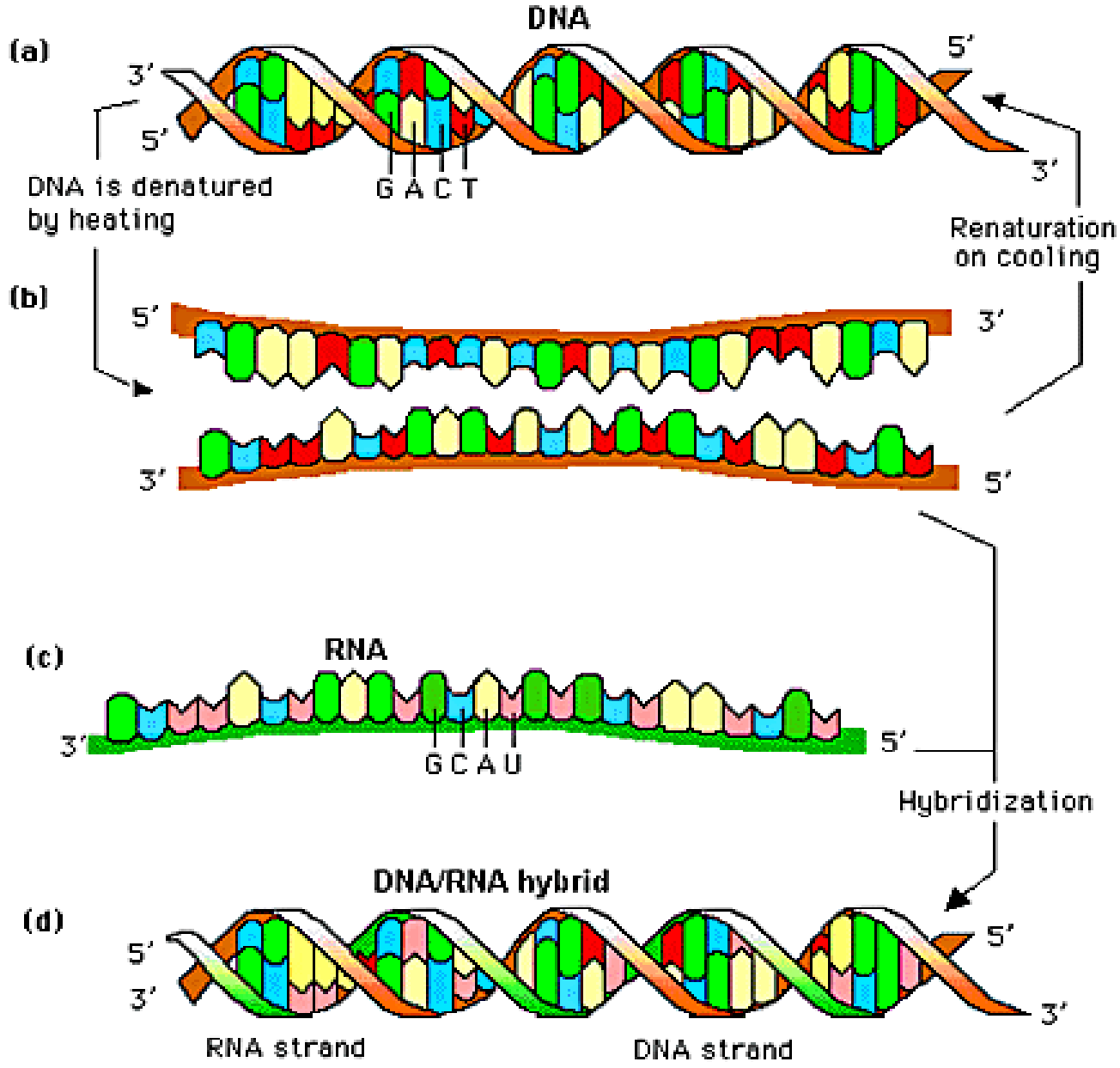
Denaturation of DNA is accompanied by increasing of optical density – a phenomenon referred as

hyperchromicity



- **Separated chains of DNA will renature when physiologic tº and or salt concentration are achieved.**

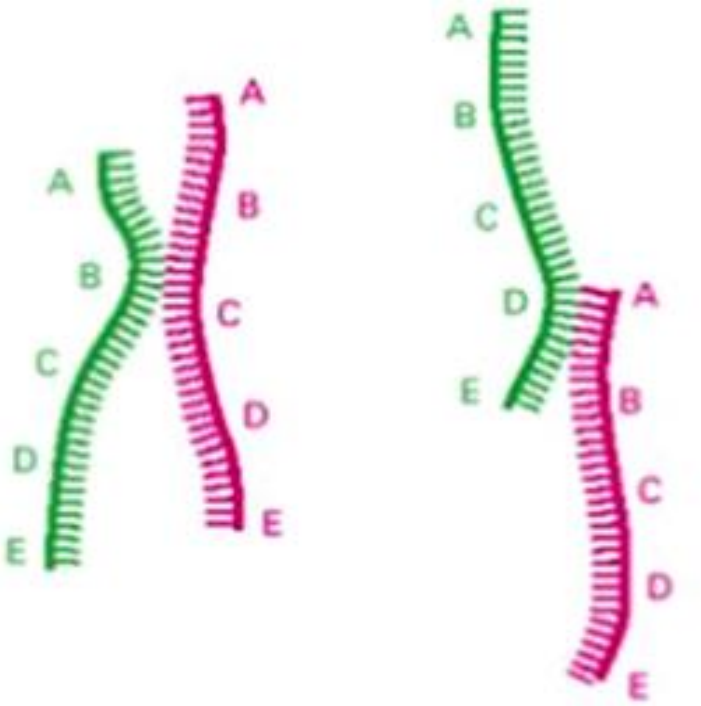
- **This process is referred as hybridization**



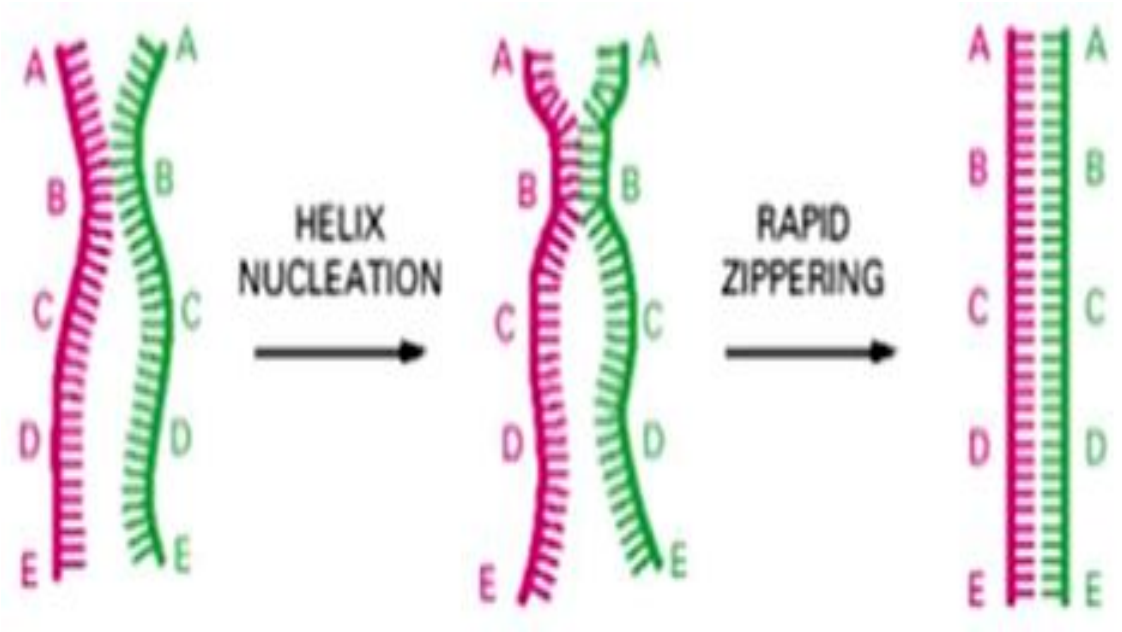
Nucleic Acid Hybridization

DNA (RNA) hybridization can be used to identify specific DNA (RNA) molecules or genes

nonpairing interactions



pairing interactions



Hybridization of nucleic acids

a process by which a piece of DNA or RNA of known nucleotide sequence is used to identify a region or fragment of DNA containing complementary sequences

Hybridization of nucleic acids

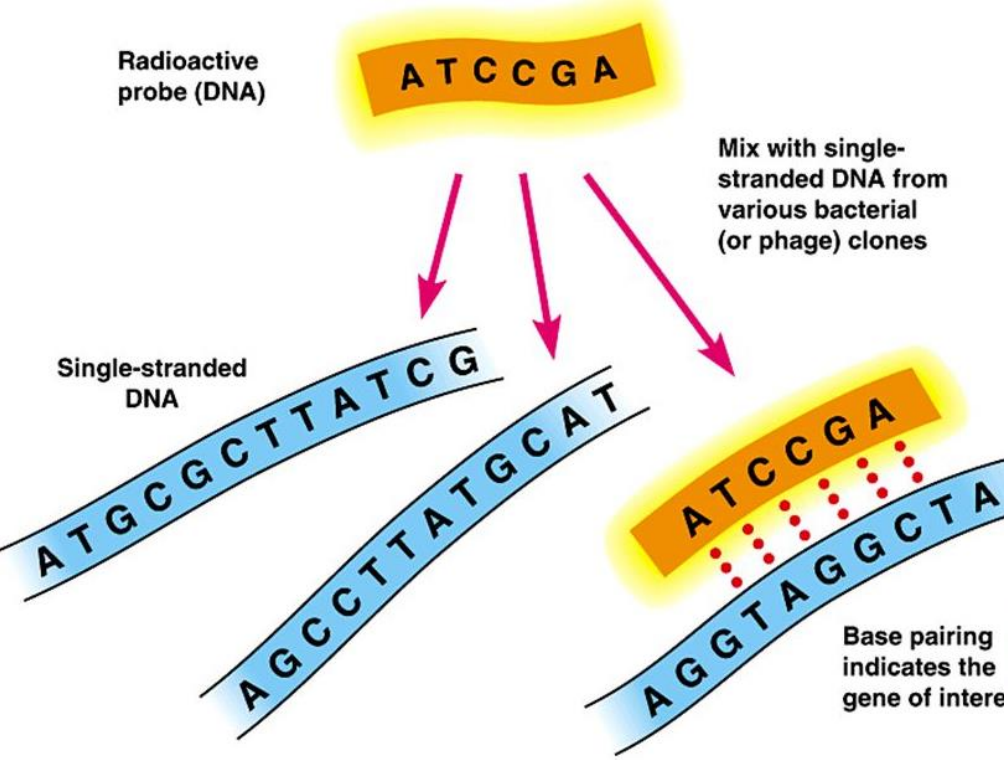
Such piece of DNA or RNA is called a **probe**.

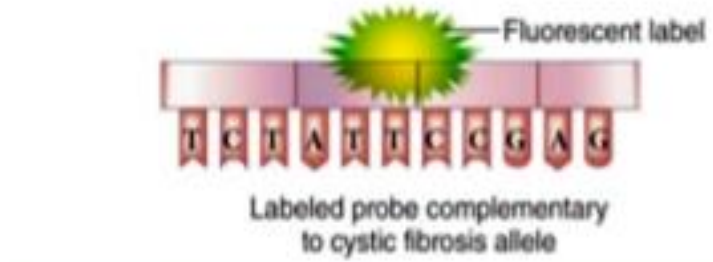
Probe will form complementary base pairings with another strand of DNA, termed the **target**.

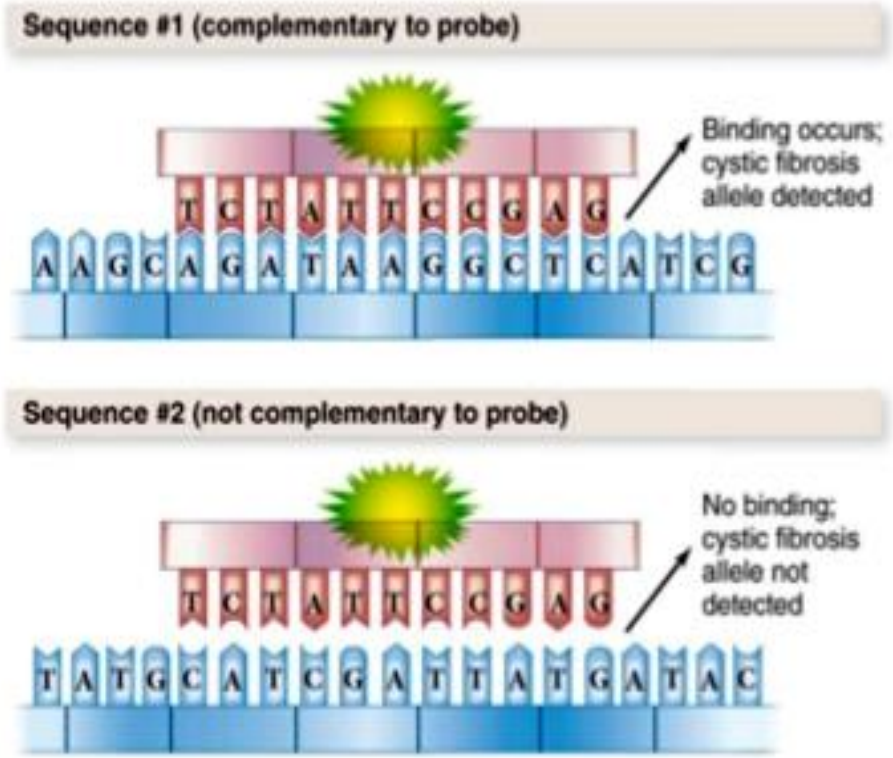
Probes must have a label to be identified.

ways in which probes can be labeled:

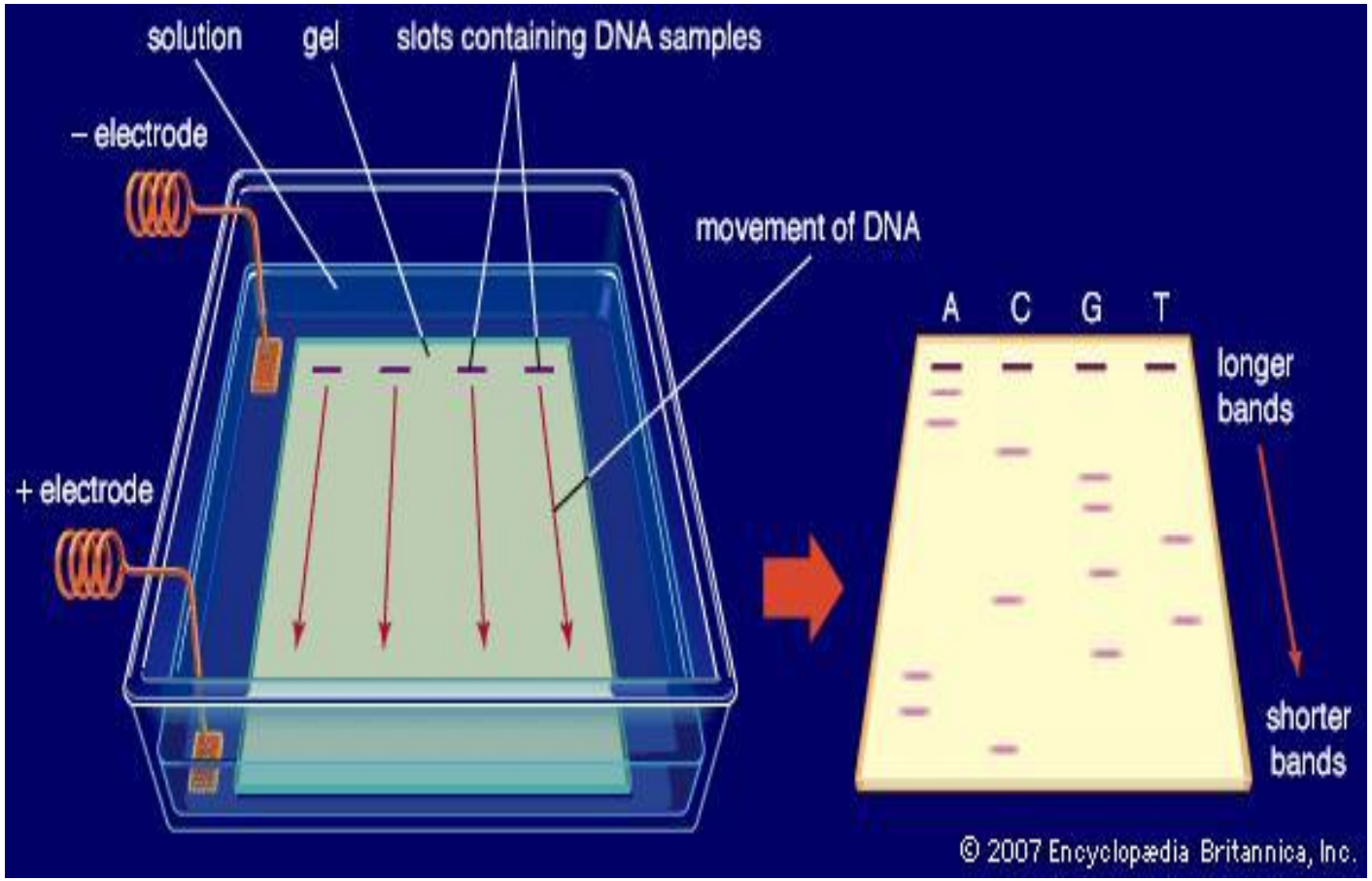
- isotopic (involving radioactive atoms)
- nonisotopic (probes with fluorescent tags or small ligand molecules).







Electrophoresis is used to separate NA fragments



Polymerase chain reaction (PCR)

process conceived by

Kary Mullis in 1983

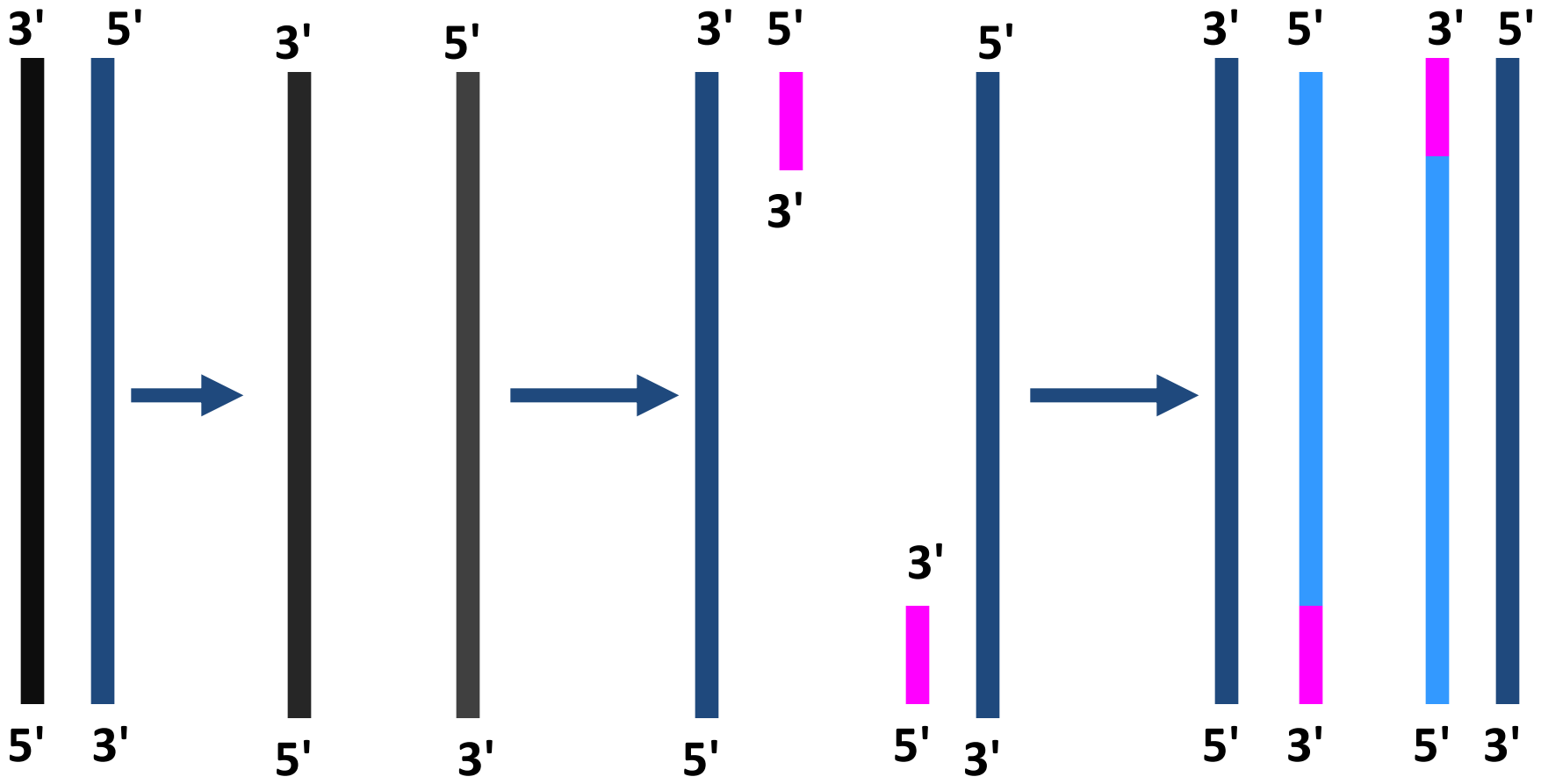
for amplification of DNA *in vitro*

The amplified DNA can be cloned directly or used in a variety of analytical procedures

The cycle of the PCR comprises three steps

- **denaturation: heating of DNA to approximately 95°C to denature it;**
- **hybrudisation: cooling of the mixture to allow heteroduplexes of primer and template to form. The temperature for this is typically in the 50 to 65°C range;**
- **elongation: DNA polymerase elongates the newly synthesized DNA strand (typically about 70°C).**

PCR procedure



Denaturation **Hybridisation** **Elongation**

PCR uses a heat-stable DNA polymerase, such as the *Taq* polymerase (derived from a bacterium that lives at 90°C), which remains active after every heating step.

Design of the primers used for PCR, such as including restriction endonuclease cleavage sites, can facilitate the subsequent cloning of the amplified DNA.

Applications of PCR:

- genetic marker typing;
- detection of point mutations;
- amplification of double-stranded DNA;
- DNA sequencing;
- genomic DNA cloning;
- genome walking;
- introduction of mutations *in vitro* to test their effect in biological systems.

- **This technology is highly sensitive: PCR can detect and amplify as little as one DNA molecule in almost any type of sample**

- **PCR is a potent tool in forensic medicine.**

- **It is also being used for detection of viral infections before they cause symptoms**

- **and for prenatal diagnosis of a wide array of genetic diseases.**

PCR equipment

Restriction fragment length polymorphisms analysis sensitivity is augmented by using PCR to amplify vanishingly small amounts of DNA.

This allows investigators to obtain DNA fingerprints from a single hair follicle, a drop of blood, a small semen sample from a rape victim, or samples that might be months or even many years old.

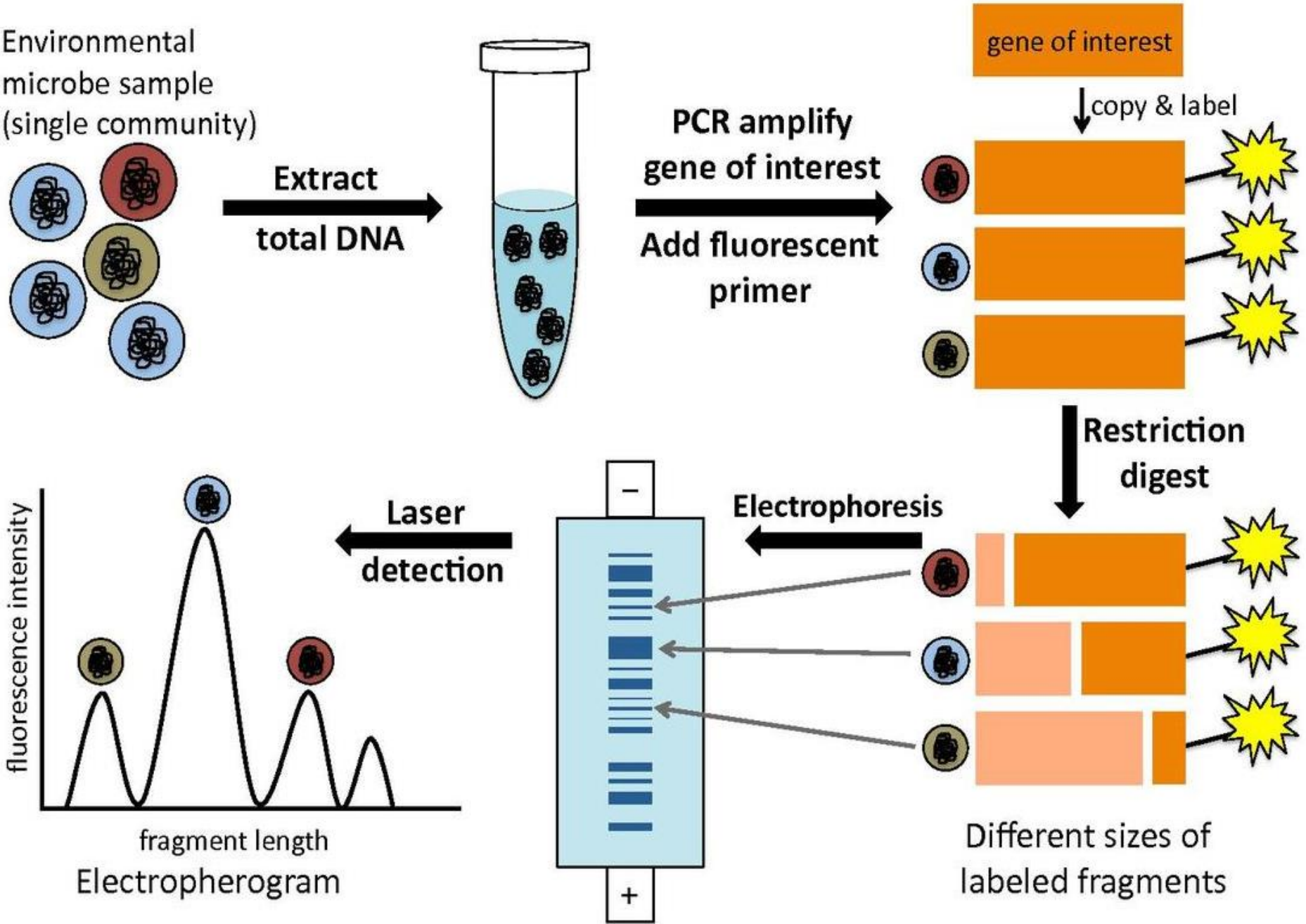
DNA fingerprinting
(DNA typing or DNA profiling)

based on **sequence polymorphisms**
(slight sequence differences between individuals).
Every individual has some differences.

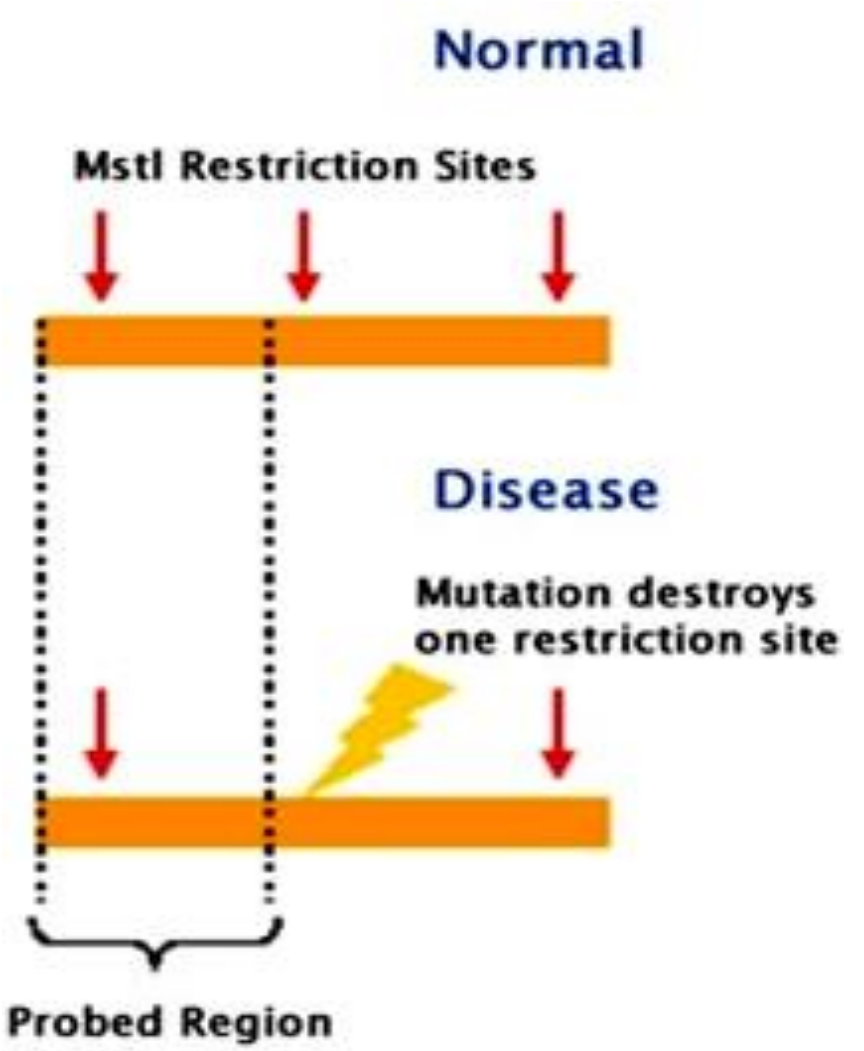
Some of the sequence changes affect recognition sites for restriction enzymes, resulting in variation in the size of DNA fragments produced by digestion with a particular restriction enzyme.

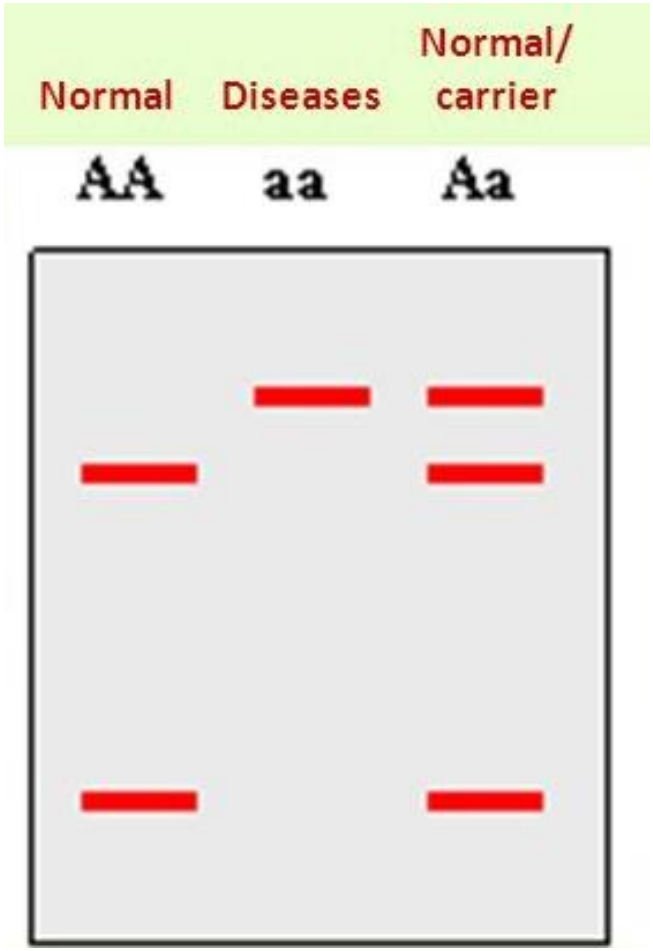
These variations are **restriction fragment length polymorphisms (RFLPs).**

Detection of an RFLP

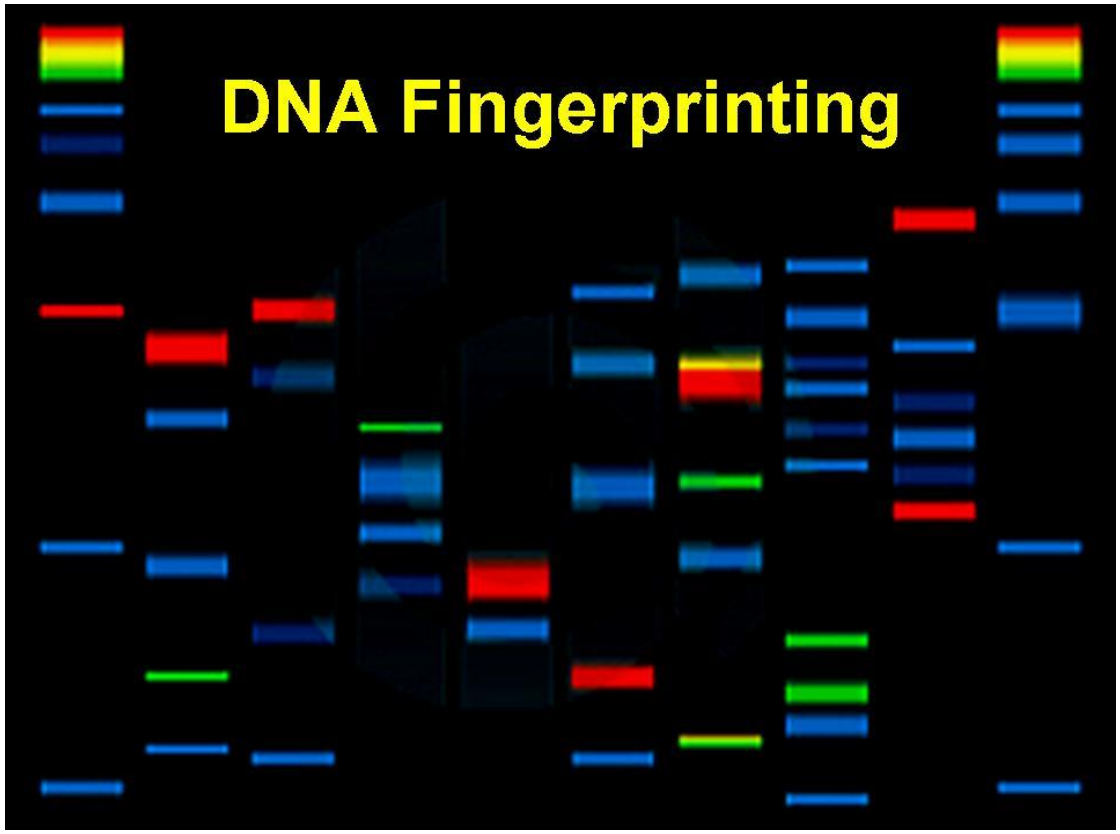


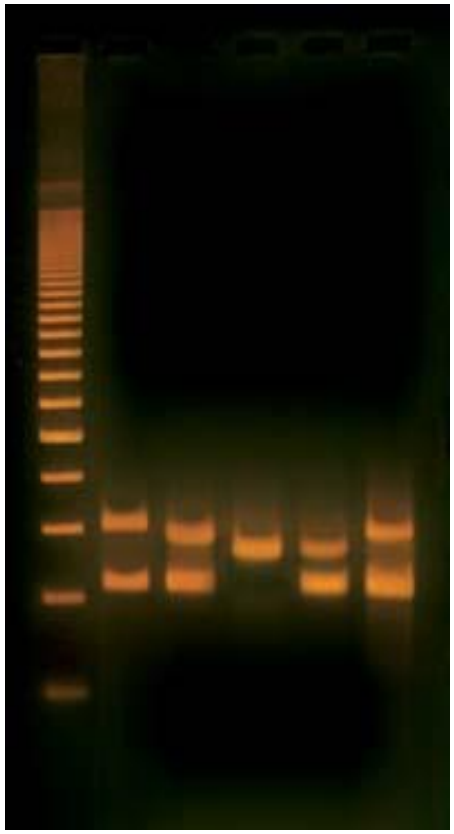
Sickle-Cell Anemia





In this disease, the person must have both copies of the chromosomes mutated. If a person has one mutated copy, the person is a carrier.


DNA Fingerprinting



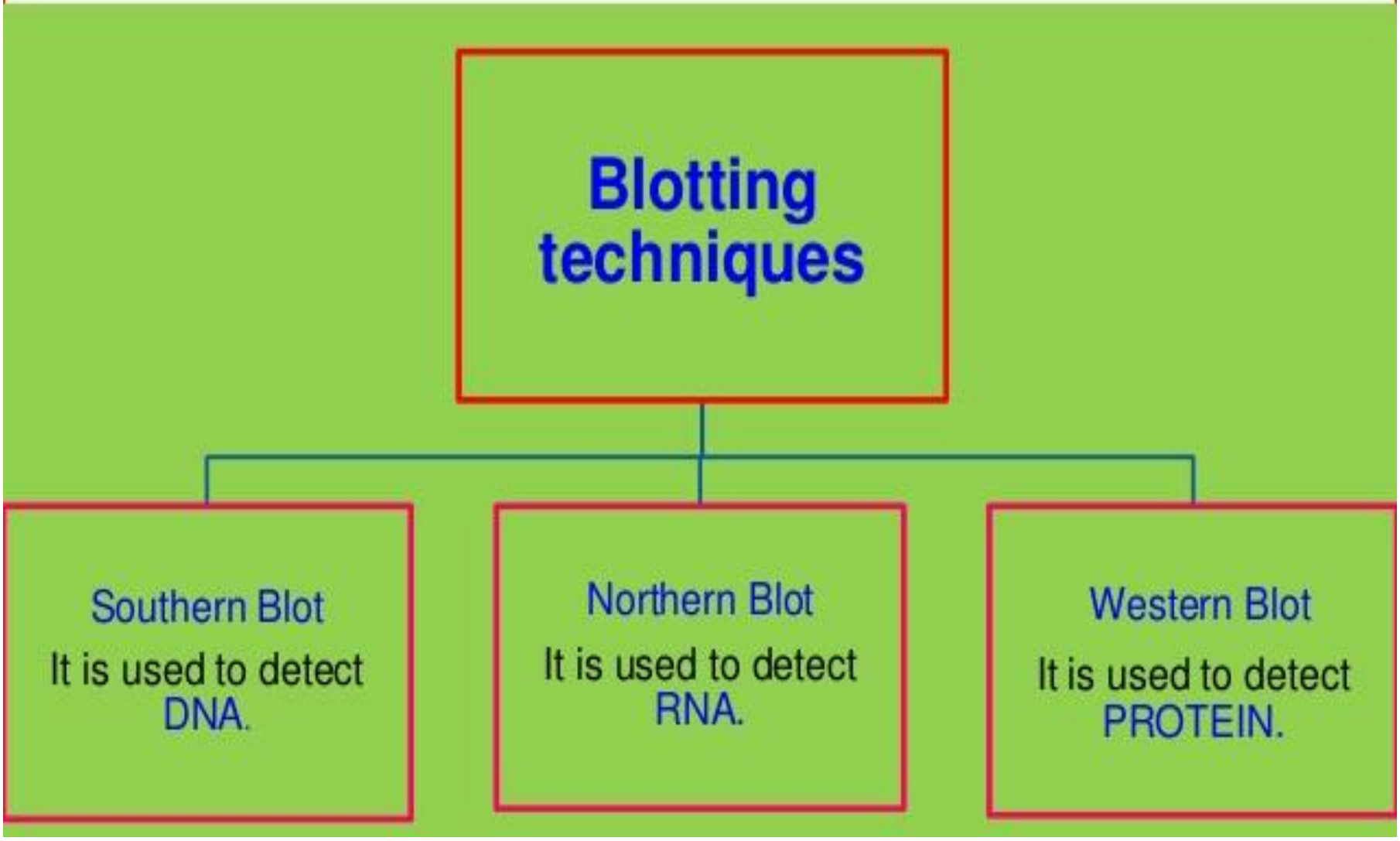
The detection of RFLPs relies on a specialized hybridization procedure called

Southern blotting

The blot transfer procedure

used for visualization of specific proteins and DNA or RNA fragments

Types of blotting tecniques

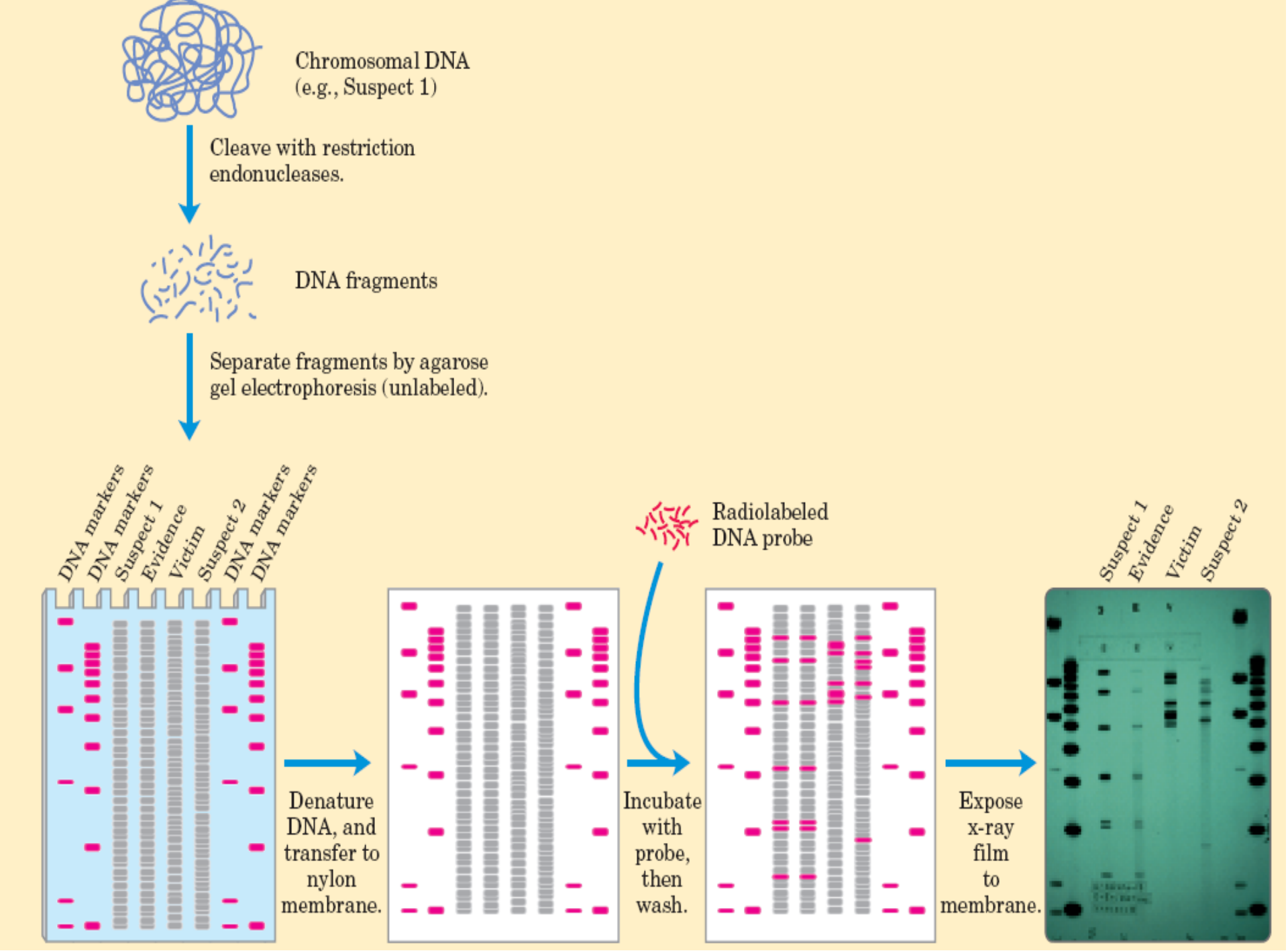


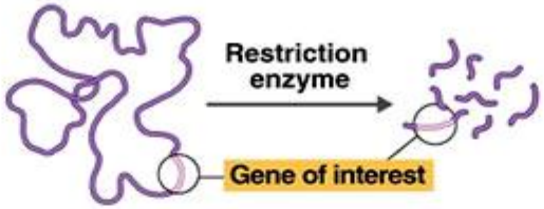
Blots used in molecular biology

Blot	*Probe*	*Target*
Southern	**nucleotide**	**DNA**
Northern	**nucleotide**	**RNA**
Western	**antibody**	**protein**

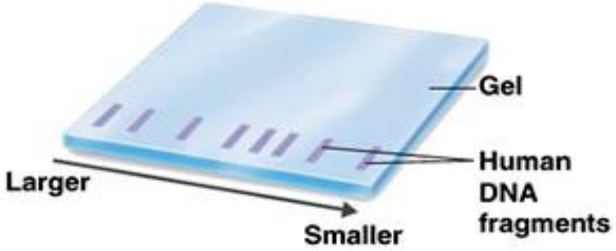
Southern blotting (mane steps)

- **DNA fragments from digestion of DNA by restriction endonucleases are separated electrophoretically**
- **denatured by soaking the agarose gel in alkali**
- **blotted to a nylon membrane to reproduce the distribution of fragments in the gel**
- **the membrane is immersed in a solution containing a labeled DNA probe**
- **Probe identification reveals the fragments to which the probe hybridizes**

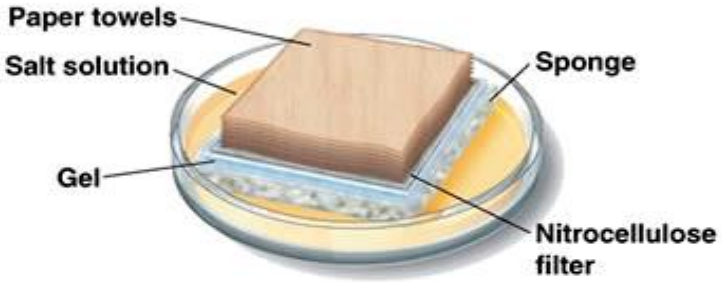

Chromosomal DNA
(e.g., Suspect 1)
Cleave with restriction endonucleases.
DNA fragments
Separate fragments by agarose gel electrophoresis (unlabeled).
DNA markers
DNA markers
Suspect 1
Evidence
Victim
Suspect 2
DNA markers
DNA markers
Denature DNA, and transfer to nylon membrane.
Radiolabeled DNA probe
Incubate with probe, then wash.
Expose x-ray film to membrane.
Suspect 1
Evidence
Victim
Suspect 2



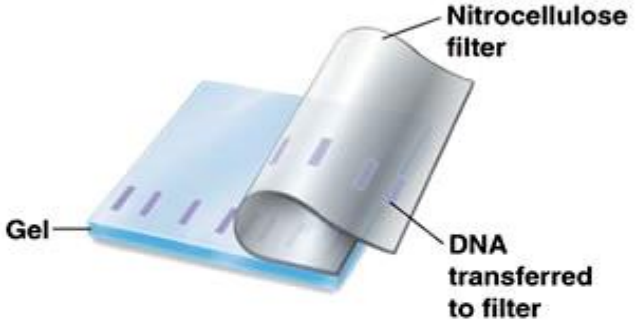
1 DNA containing the gene of interest is extracted from human cells and cut into fragments by restriction enzymes.



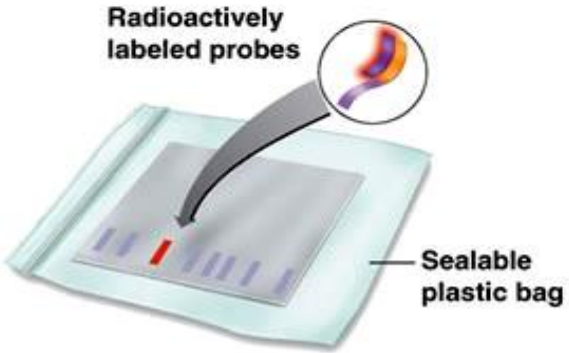
2 The fragments are separated according to size by gel electrophoresis. Each band consists of many copies of a particular DNA fragment. The bands are invisible but can be made visible by staining.



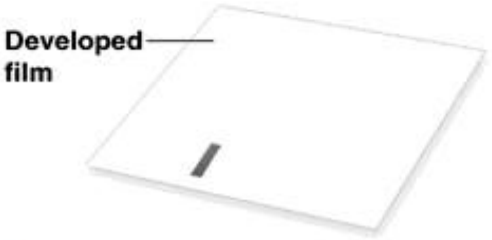
3 The DNA bands are transferred to a nitrocellulose filter by blotting. The solution passes through the gel and filter to the paper towels.



4 This produces a nitrocellulose filter with DNA fragments positioned exactly as on the gel.



5 The filter is exposed to a radioactively labeled probe for a specific gene. The probe will base-pair (hybridize) with a short sequence present on the gene.



6 The filter is then exposed to X-ray film. The fragment containing the gene of interest is identified by a band on the developed film.

Sequencing of DNA

chain termination method devised by

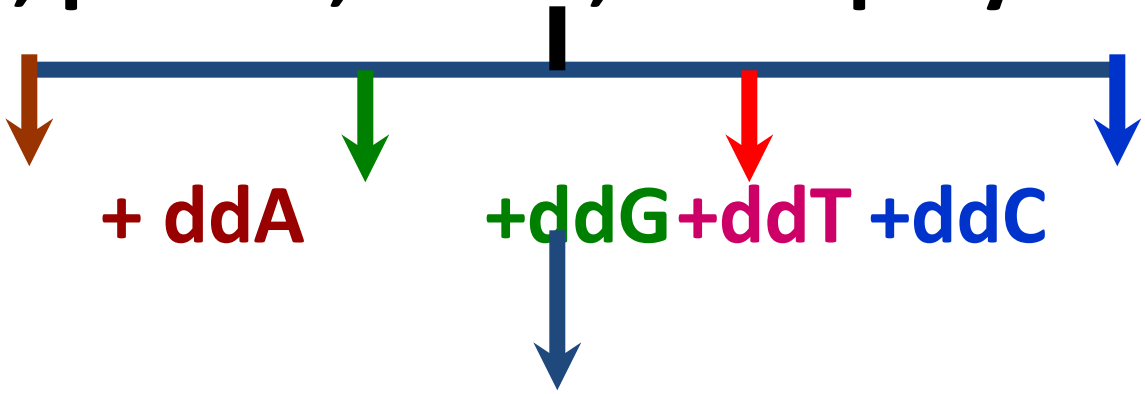
Sanger

Sequencing of DNA

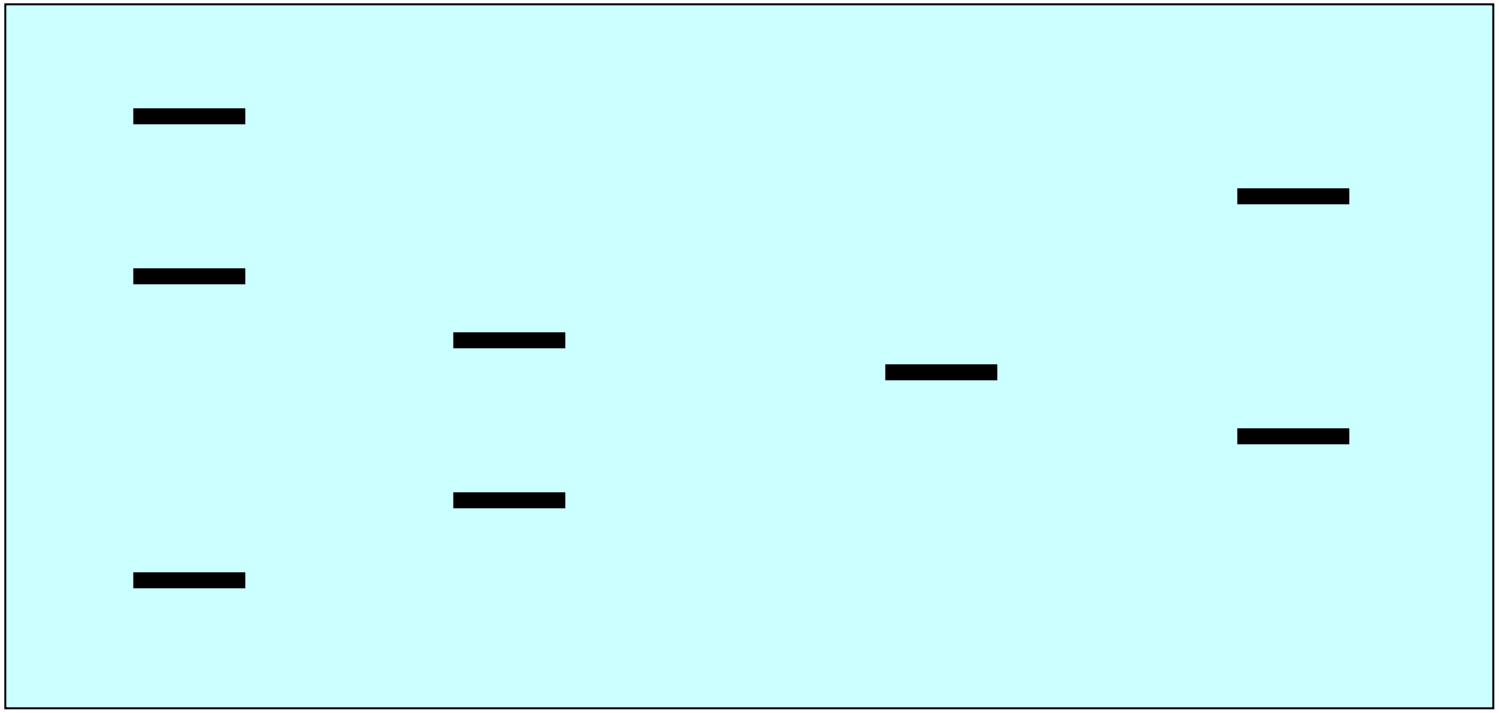
employ specific dideoxynucleotides

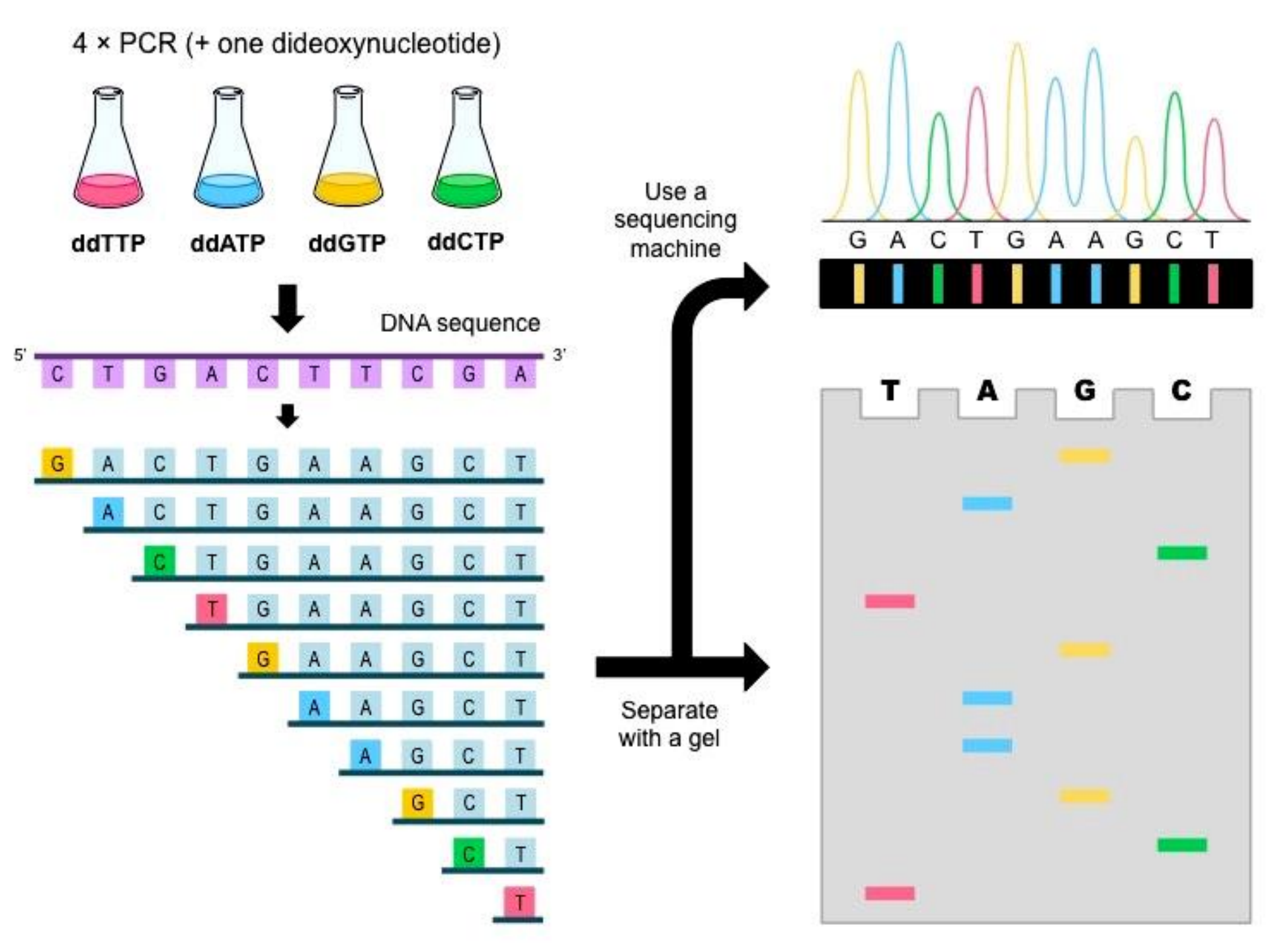
that terminate DNA synthesis at specific nucleotides

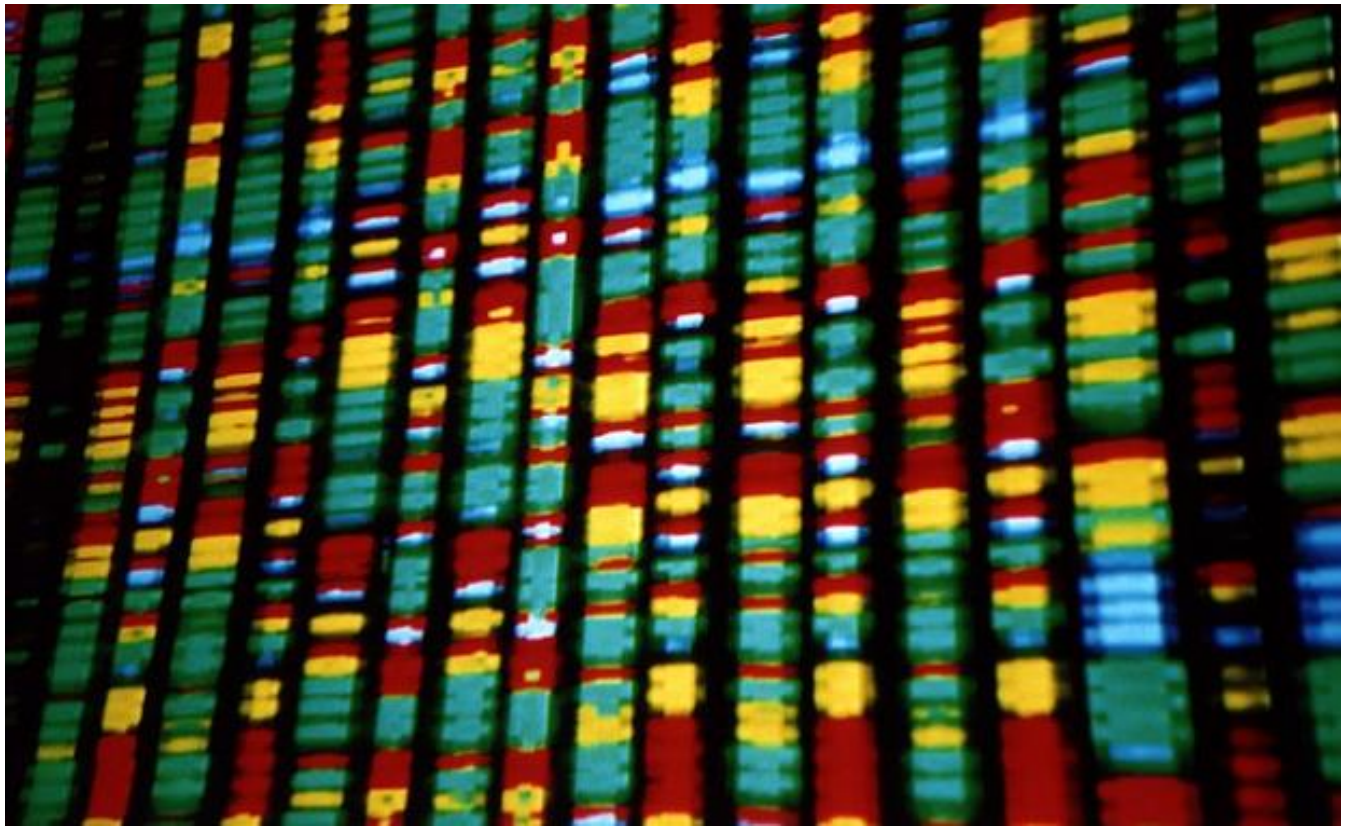
DNA, primer, dNTP, DNA-polymerase



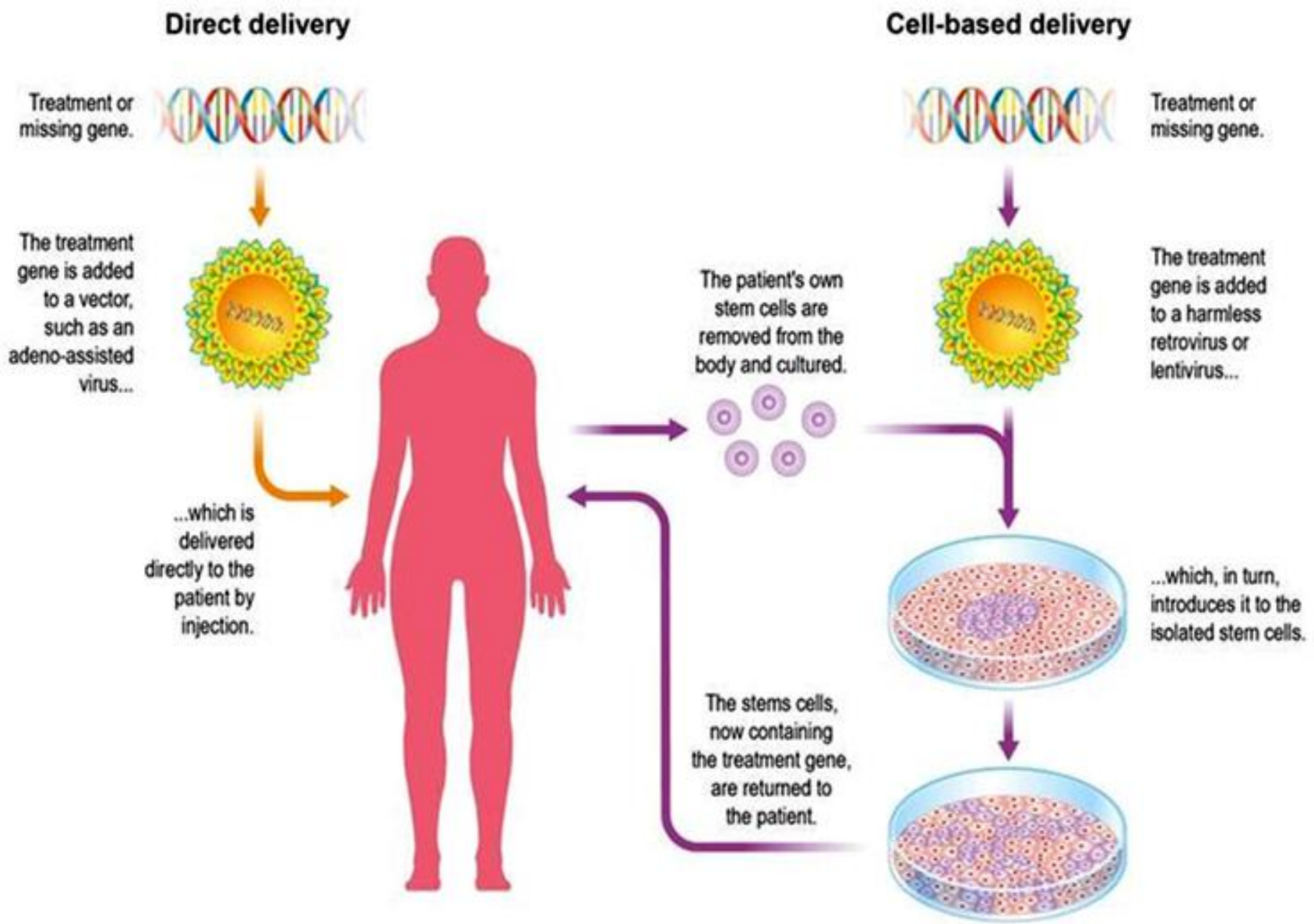
Population of DNA fragments

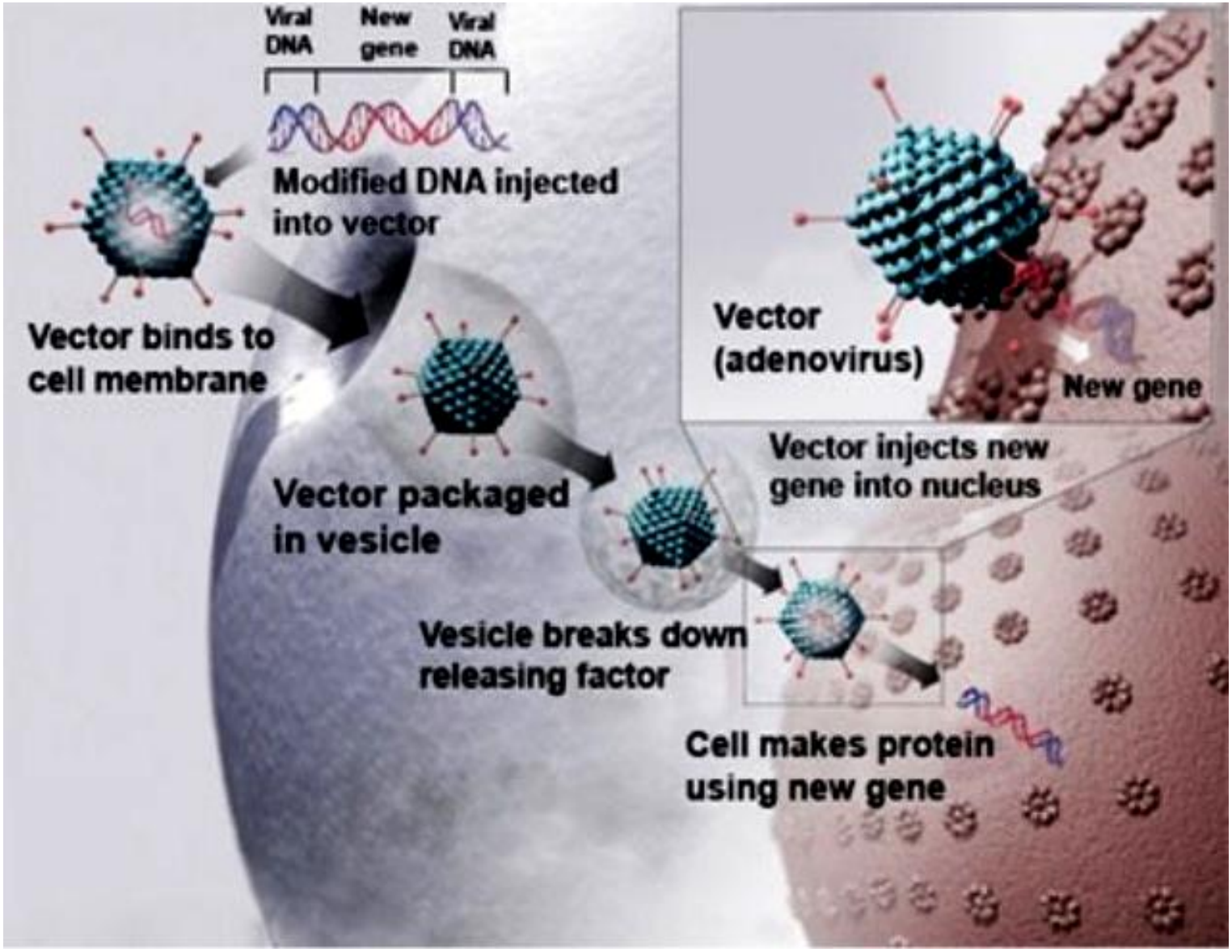



4 × PCR (+ one dideoxynucleotide)
ddTTP
ddATP
ddGTP
ddCTP
DNA sequence
5'
3'
C T G A C T T C G A
G A C T G A A G C T
A C T G A A G C T
C T G A A G C T
T G A A G C T
G A A G C T
A A G C T
A G C T
G C T
C T
T
Use a sequencing machine
Separate with a gel
G A C T G A A G C T
T A G C



Gene therapy




Viral DNA
New gene
Viral DNA
Modified DNA injected into vector
Vector binds to cell membrane
Vector packaged in vesicle
Vesicle breaks down releasing factor
Cell makes protein using new gene
Vector injects new gene into nucleus
Vector (adenovirus)
New gene

DNA amplification and cloning

approaches to the amplification of DNA are:

- **<u>cell-based DNA cloning</u>:**

  DNA is amplified *in vivo* by a cellular host

- **enzyme-based DNA cloning (cell-free): this method is represented by the <u>polymerase chain reaction (PCR)</u> and involves *in vitro* DNA amplification.**

DNA Cloning

A *clone* is an identical copy

this term originally applied to cells of a single type, isolated and allowed to reproduce to create a population of identical cells.

DNA Cloning involves

- **separating a specific gene or DNA segment from a chromosome**
- **attaching it to a small molecule of carrier DNA**
- **replicating this modified DNA in host cell.**

Cloning of DNA entails five general procedures

1. ***Cutting DNA at precise locations.***

   Sequence-specific endonucleases provide the necessary molecular scissors.

2. ***Selecting a small molecule of DNA capable of self-replication.***

   These DNAs are called cloning vectors (a vector is a delivery agent). They are typically plasmids or viral DNAs.

3. Joining two DNA fragments covalently.
The enzyme DNA ligase links the cloning vector and DNA to be cloned. Composite DNA molecules comprising covalently linked segments from two or more sources are called recombinant DNAs.

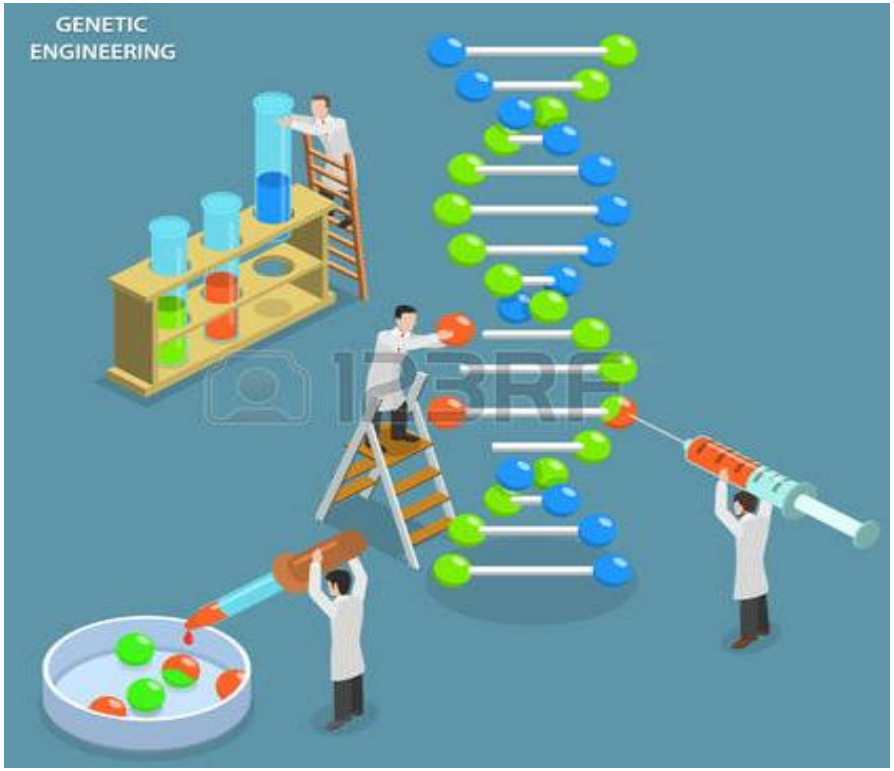
4. Moving recombinant DNA from the test tube to a host cell **that will provide the enzymatic machinery for DNA replication.**

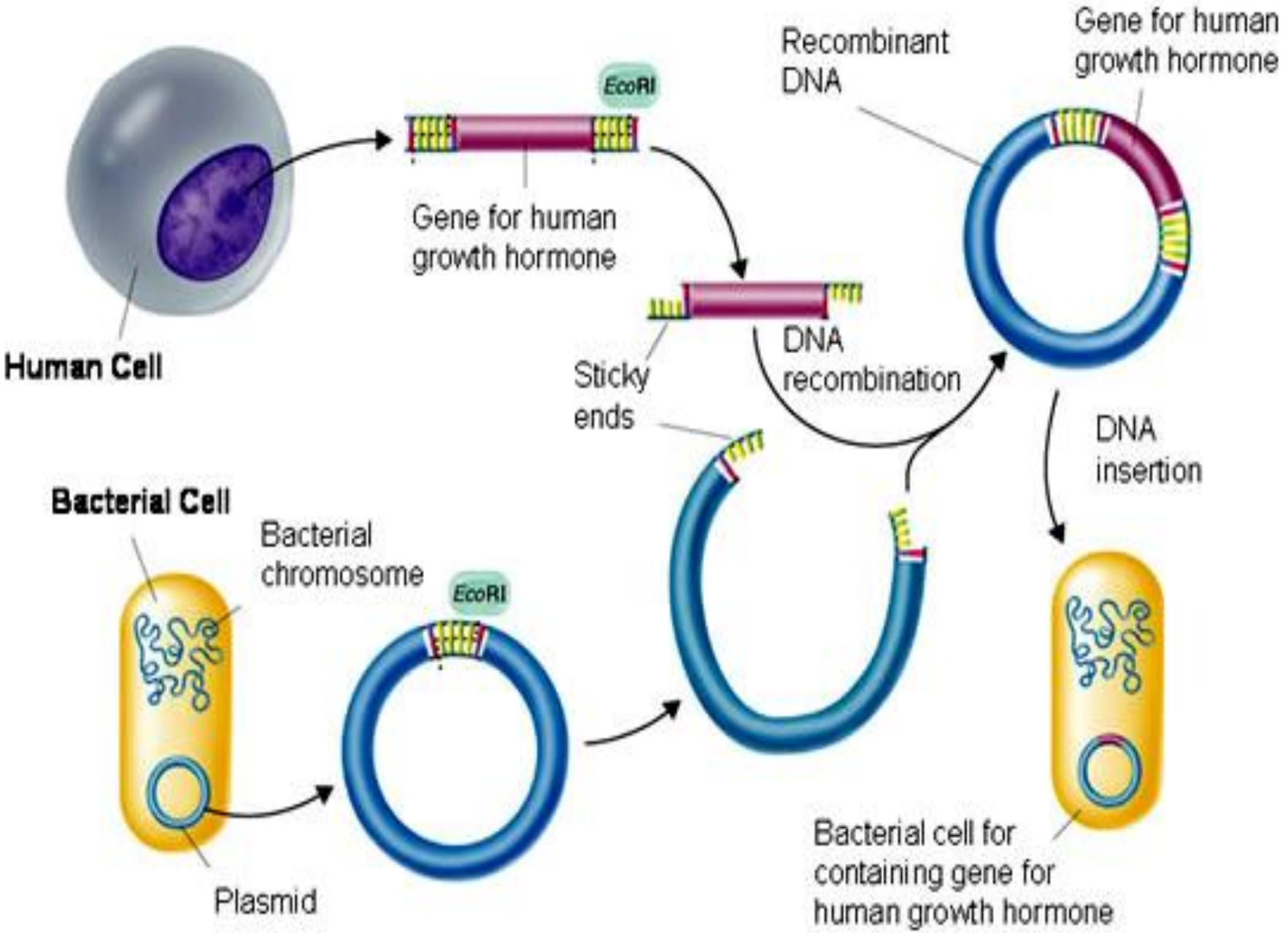
5. Selecting or identifying host cells that contain recombinant DNA.

The methods used to accomplish these and related tasks are collectively referred to

as recombinant DNA technology or, more informally,

genetic engineering




Human Cell
EcoRI
Gene for human growth hormone
Sticky ends
DNA recombination
Recombinant DNA
Gene for human growth hormone
DNA insertion
Bacterial Cell
Bacterial chromosome
EcoRI
Plasmid
Bacterial cell for containing gene for human growth hormone