Restriction Fragment Length Polymorphism (RFLP)

Dr. Kalsoom Tariq

Restriction Fragment Length Polymorphism (RFLP)

- RFLP is a technique in which organisms may be differentiated by analysis of patterns derived from cleavage of their DNA.
- Two organisms differ in the distance between sites of cleavage by a particular restriction endonuclease, the length of the fragments produced will differ when the DNA is digested with a restriction enzyme.

- The similarity of the patterns generated can be used to differentiate species(& even strains) from one another.
- Polymorphisms are inherited differences found among the individuals in more than 1% of normal population.
- An RFLP probe is a labeled DNA sequence that hybridizes with one or more fragments of the digested DNA sample after they were separated by gel electrophoresis.

Restriction Fragment Length Polymorphism

- A restriction fragment length polymorphism (RFLP) is a genetic variant that can be examined by cleaving the DNA into fragments (restriction fragments) with a restriction enzyme.
- An inherited difference due to genomic variations in the pattern of restriction enzyme digestion is known as a RFLP.
- Short, single or genomic DNA are typically used as RFLP probes.

Genomic variations

- Genome variations are differences in the sequence of DNA among individuals that donot affect the phenotype.
- the genomes of nonrelated people differ at about 1 of 1,500 DNA bases, or about 0.1% of the genome.

Variations resulting in RFLP

- Variations commonly result in RFLPs:
  - single-base changes in the nucleotide sequences (SNP),
  - tandem repeats (VNTR),
  - polymorphisms,
  - mutations.

SNP or VNTR, are simply markers, which, in most cases, have no known effect on the structure or rate of production of any particular protein.

Polymorphism

- A polymorphism is a clinically harmless DNA variation that does not affect the phenotype.
- At the molecular level, polymorphism is a variation in nucleotide sequence from one individual to another.
- Polymorphisms often occur in the intervening sequences that do not code for proteins. [Note: Only a few percent of the human genome actually encodes proteins.]
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Tandem repeats

Tandem repeats

- Polymorphism in chromosomal DNA can arise from the presence of a variable number of tandem repeats
- These are short sequences of DNA at scattered locations in the genome, repeated in tandem (one after another).
- The number of these repeat units varies from person to person, but is unique for any given individual and, therefore, serves as a molecular markers.

- Cleavage by restriction enzymes yields fragments that vary in length depending on how many repeated segments are contained in the fragment.
- Variation in the number of tandem repeats can lead to polymorphisms.

Mutation

- Mutation is a permanent change in the DNA.
- Mutations affecting germ cells cause hereditary diseases.
- Eg: Huntington’s chorea.
- Mutations affecting somatic cells donot necessarily cause hereditary diseases but are important in the genesis of cancer.

Single base changes in DNA

- About 90% of human genome variation comes in the form of single-nucleotide polymorphisms, (SNPs), that is, variations that involve just one base .
- The alteration of one or more nucleotides at a restriction site can render the site unrecognizable by a particular restriction endonuclease. A new restriction site can also be created by this mechanism.

- In either case, cleavage with an endonuclease results in fragments of lengths differing from the normal, which can be detected by DNA hybridization.
- The altered restriction site can be either at the site of a disease-causing mutation or at a site some distance from the mutation.
- Example : Sickle cell anaemia.

RFLP - Technique

- DNA sequences are cut using restriction enzyme.
- Then seperated by gel electrophoresis.
- DNA is transferred on to nitrocellulose paper (southern blotting) and labelled with probe sequences.
- Genotyping can be recognized by altered restriction fragments.

Restriction Enzymes

- **1962: "molecular scissors" discovered in bacteria**

- **Naturally produced by bacteria – restriction endonucleases**
  - **Natural function** - destroy bacteriophage DNA in bacterial cells
  - Cannot digest host DNA with methylated C (cytosine)

A restriction enzyme

- **Substrate –DNA -**recognizes one particular nucleotide sequence in DNA and **cuts** the DNA molecule (breaks down the bond between two nucleotides)

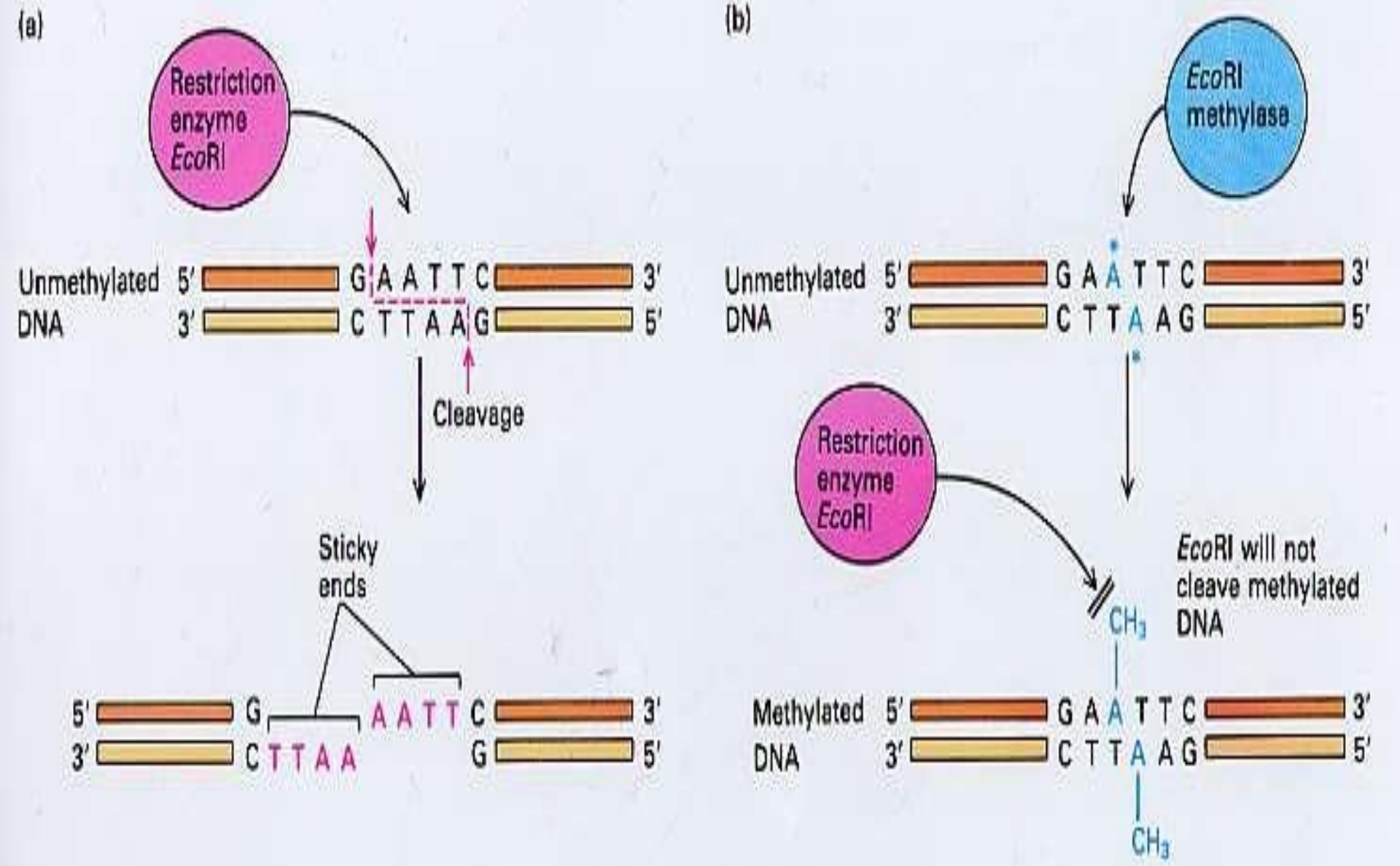
sticky ends **blunt ends**

- Prepackaged kits are available for rDNA techniques

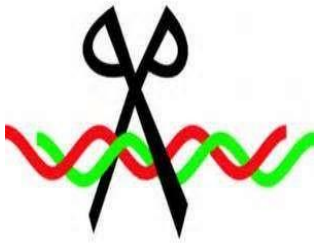
The distinguishing feature of restriction enzymes is that

- they only cut at very specific sequences of bases.
- This specific DNA sequence is called **recognition sequence.**

- A restriction enzyme requires a specific double stranded recognition sequence of nucleotides to cut DNA.
- Recognition sites are usually 4 to 8 base pairs in length.
- Cleavage occurs within or near the site.


(a)
Restriction enzyme EcoRI
Unmethylated DNA
5′ G A A T T C 3′
3′ C T T A A G 5′
Cleavage
Sticky ends
5′ G A A T T C 3′
3′ C T T A A G 5′
(b)
EcoRI methylase
Unmethylated DNA
5′ G A A T T C 3′
3′ C T T A A G 5′
Restriction enzyme EcoRI
EcoRI will not cleave methylated DNA
CH3
Methylated DNA
5′ G A A T T C 3′
3′ C T T A A G 5′
CH3

Restriction enzymes

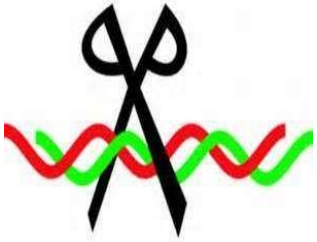


- The type of bacteria in which the enzyme is found
- The order in which the restriction enzyme was identified and isolated.

Derivation of the *Eco*RI name		
Abbreviation	Meaning	Description
E	*Escherichia*	genus
co	*coli*	species
R	RY13	strain
I	First identified	order of identification in the bacterium

TABLE 9.1 Selected Restriction Enzymes Used in rDNA Technology

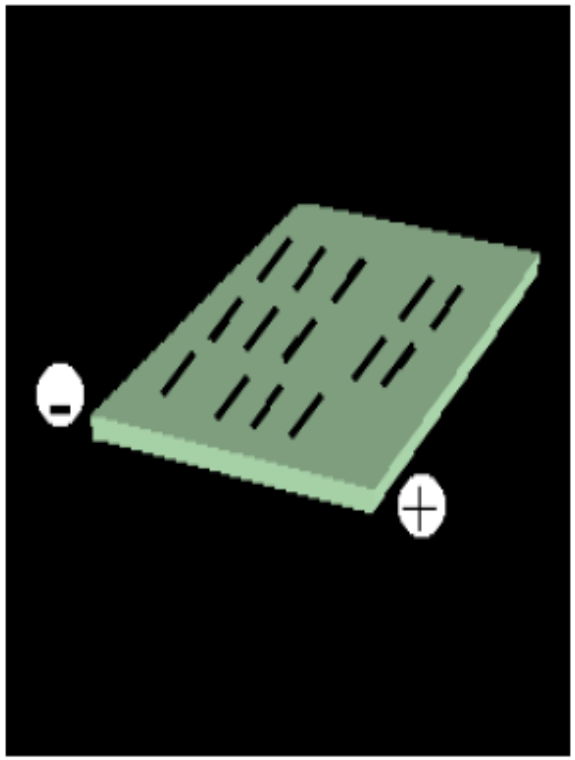
Enzyme	Bacterial Source	Recognition Sequence
*Bam*HI	*Bacillus amyloliquefaciens*	G↓G A T C C G C T A G↑G
*Eco*RI	*Escherichia coli*	G↓A A T T C C T T A A↑G
*Hae*III	*Haemophilus aegyptius*	G G↓C C C C↑G G
*Hin*dIII	*Haemophilus influenzae*	A↓A G C T T T T C G A↑A



Types Of Restriction Enzymes

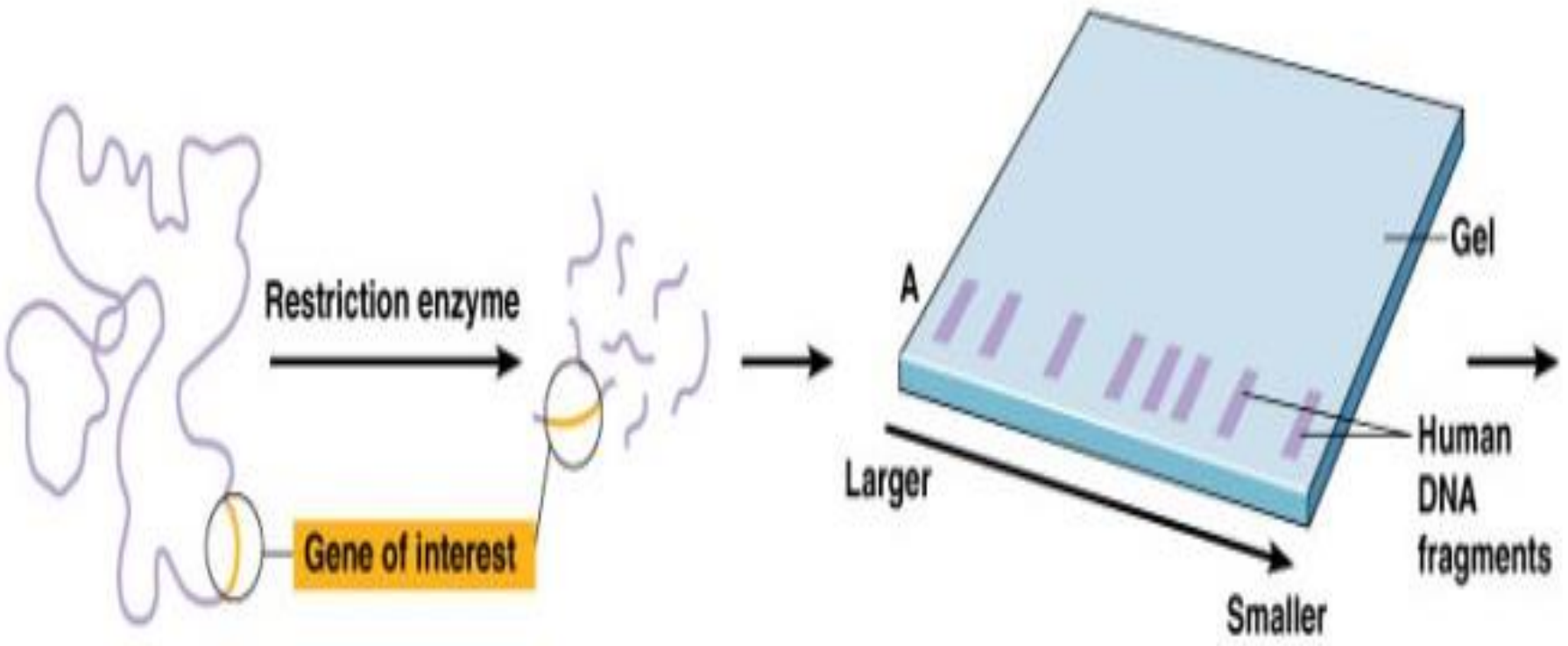
Naturally occurring Restriction endonucleases categorized into four groups (Types I, II III, and IV) based on their composition and cofactor requirements, the nature of their target sequence, And the position of their DNA cleavage site relative to the target sequence.

All types of enzymes recognize specific short DNA sequence & carryout the endo nucleolytic cleavage of the DNA to give specific fragments with terminal 5'-phosphates.



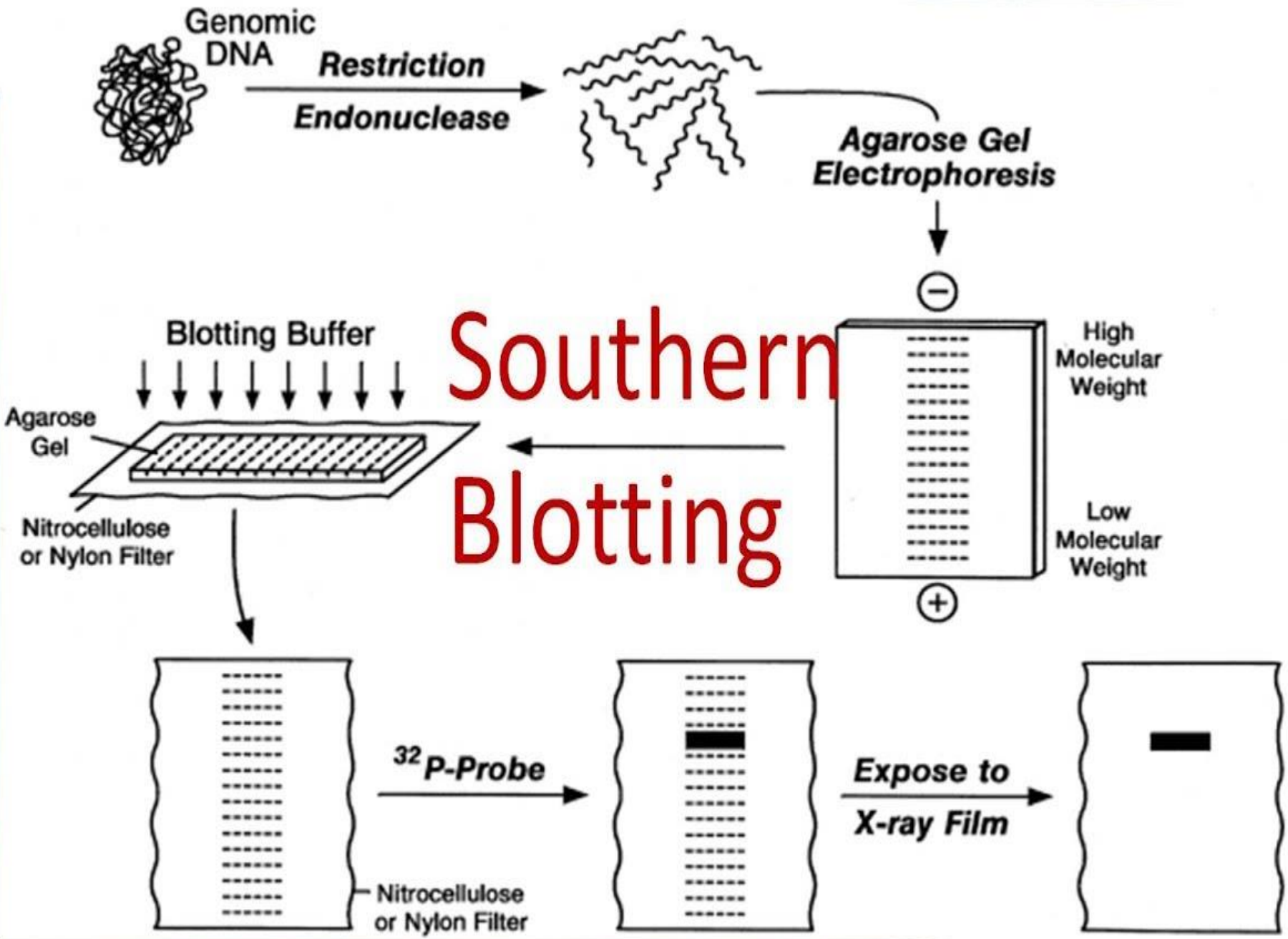
The samples of DNA that have been treated with restriction enzymes are placed in separate lanes on a slab of electrophoretic gel across which is placed an electric field. The fragments migrate towards the positive electrode, the smaller fragments moving faster than the larger fragments, thus separating the DNA samples into distinct bands.

Southern blotting



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.

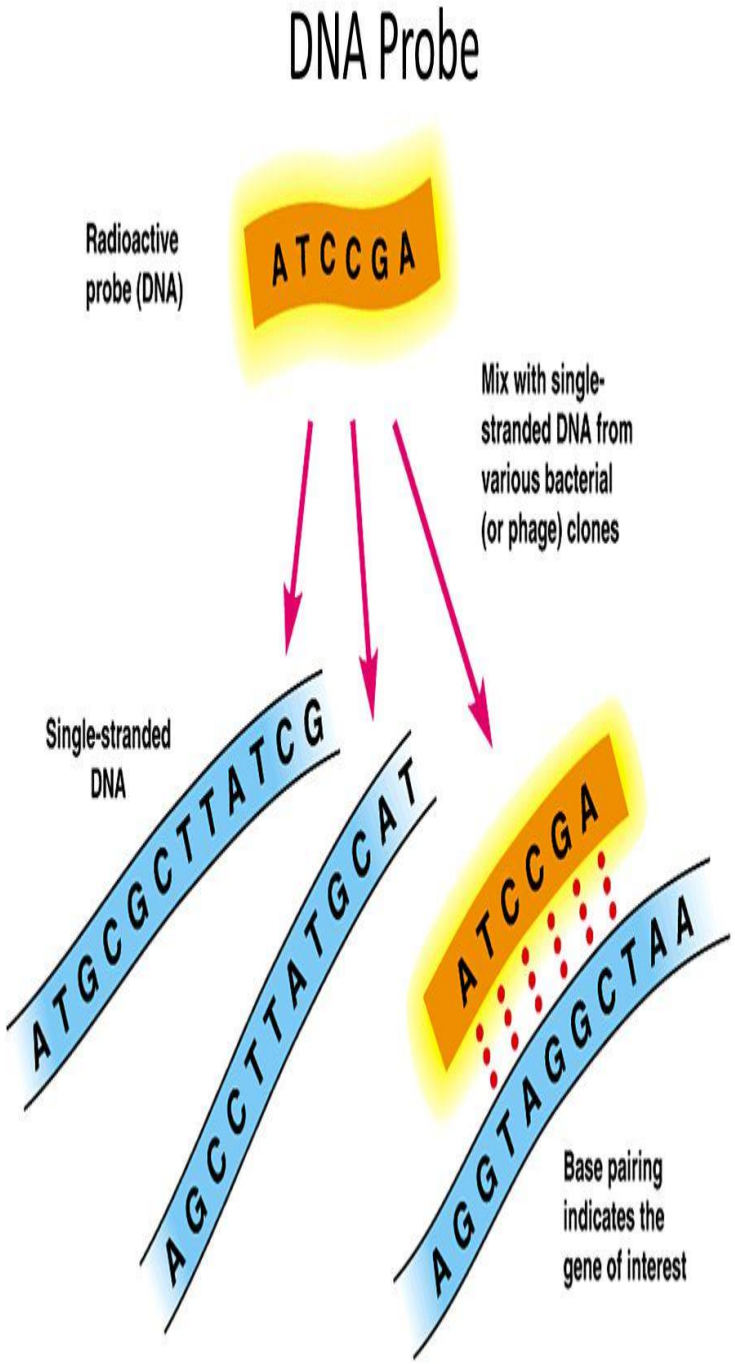

Genomic DNA
Restriction Endonuclease
Agarose Gel Electrophoresis
Blotting Buffer
Agarose Gel
Nitrocellulose or Nylon Filter
High Molecular Weight
Low Molecular Weight
Southern Blotting
32P-Probe
Nitrocellulose or Nylon Filter
Expose to X-ray Film

DNA probes

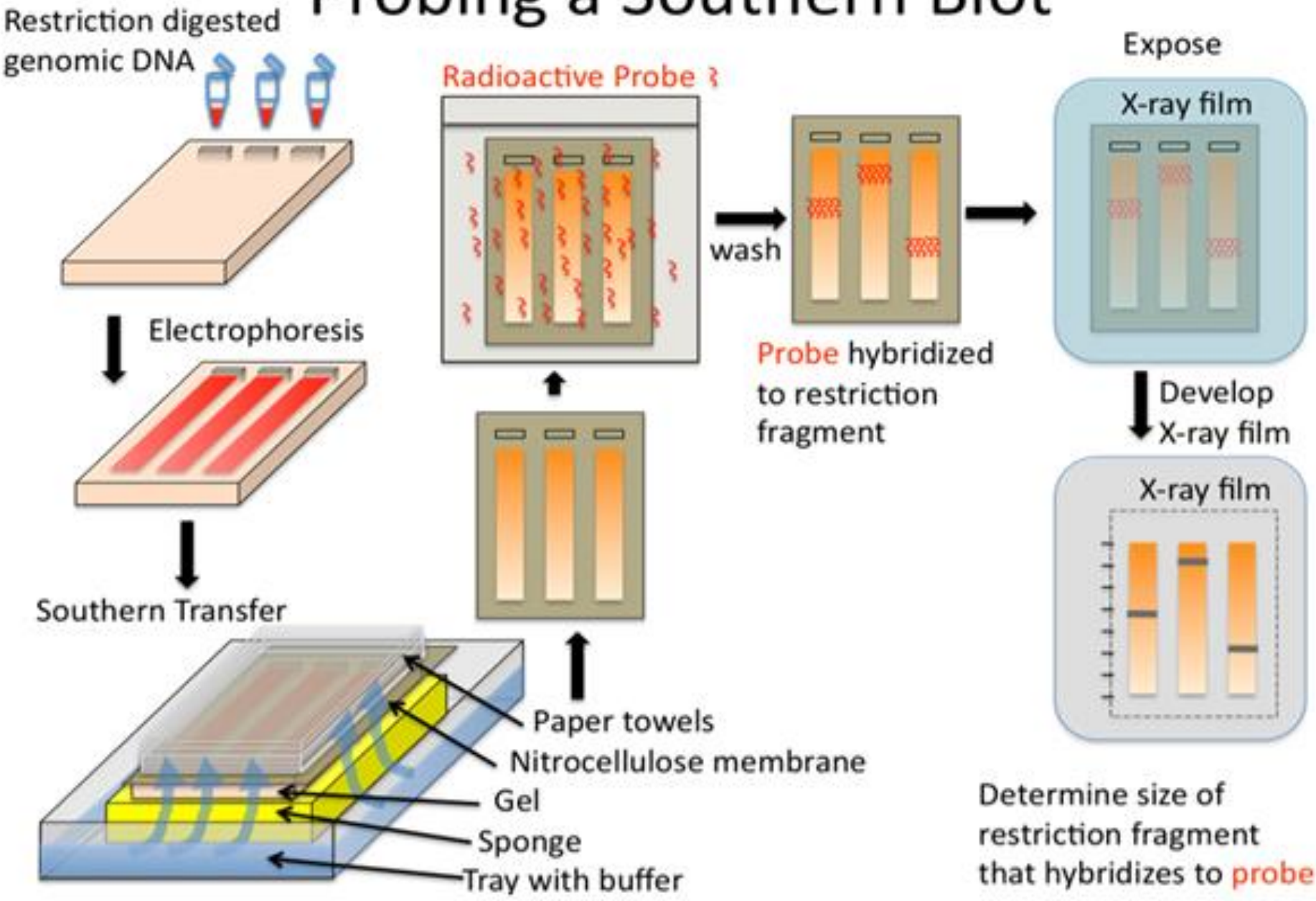
- A **DNA probe is a short length of single stranded DNA** that has a complementary base sequence to the gene you want to extract

- The probe is "labelled"
  - E.g. with nucleotides containing an isotope of phosphorous, ^{32}P, which emits beta radiation

- When the probe is mixed with DNA fragments it forms hydrogen bonds with stretches of DNA complementary to its own base sequence (**annealing**)



Probing a Southern Blot



Clinical applications

- DNA Fingerprinting.
- Disputed parenthood.
- In human population genetics, geographical isolates and comparison of genetical makeup of related species.
- Genetic diseases —

Sickle cell anaemia.

Direct diagnosis of sickle cell disease using RFLP.

- In the case of sickle cell disease, the mutation that gives rise to the disease is actually one and the same as mutation that gives the rise to the polymorphism.
- Deletions or insertions of DNA larger than 50 bp can often be detected by the Southern blotting procedure and PCR.

Others

- The gene for the X-linked disorder, Duchenne-type muscular dystrophy, was found using RFLP.
- The defect in Huntington disease was localized to the terminal region of the short arm of chromosome 4 by RFLP.
- The defect that causes polycystic kidney disease is linked to the α-globin locus on chromosome 16 was found by RFLP.

CLONING

What Are Clones?

- **Clones**
  - Genetically identical molecules, cells, or organisms all derived from a single ancestor

- **Cloning**
  - The production of identical copies of molecules, cells, or organisms from a single ancestor

Advantages of Cloning

- Cloning to produce children-genetically virtually identical to pre-existing individual

- Cloning for Biomedical Research
- Cloning for better plants and animals
-

Types of cloning

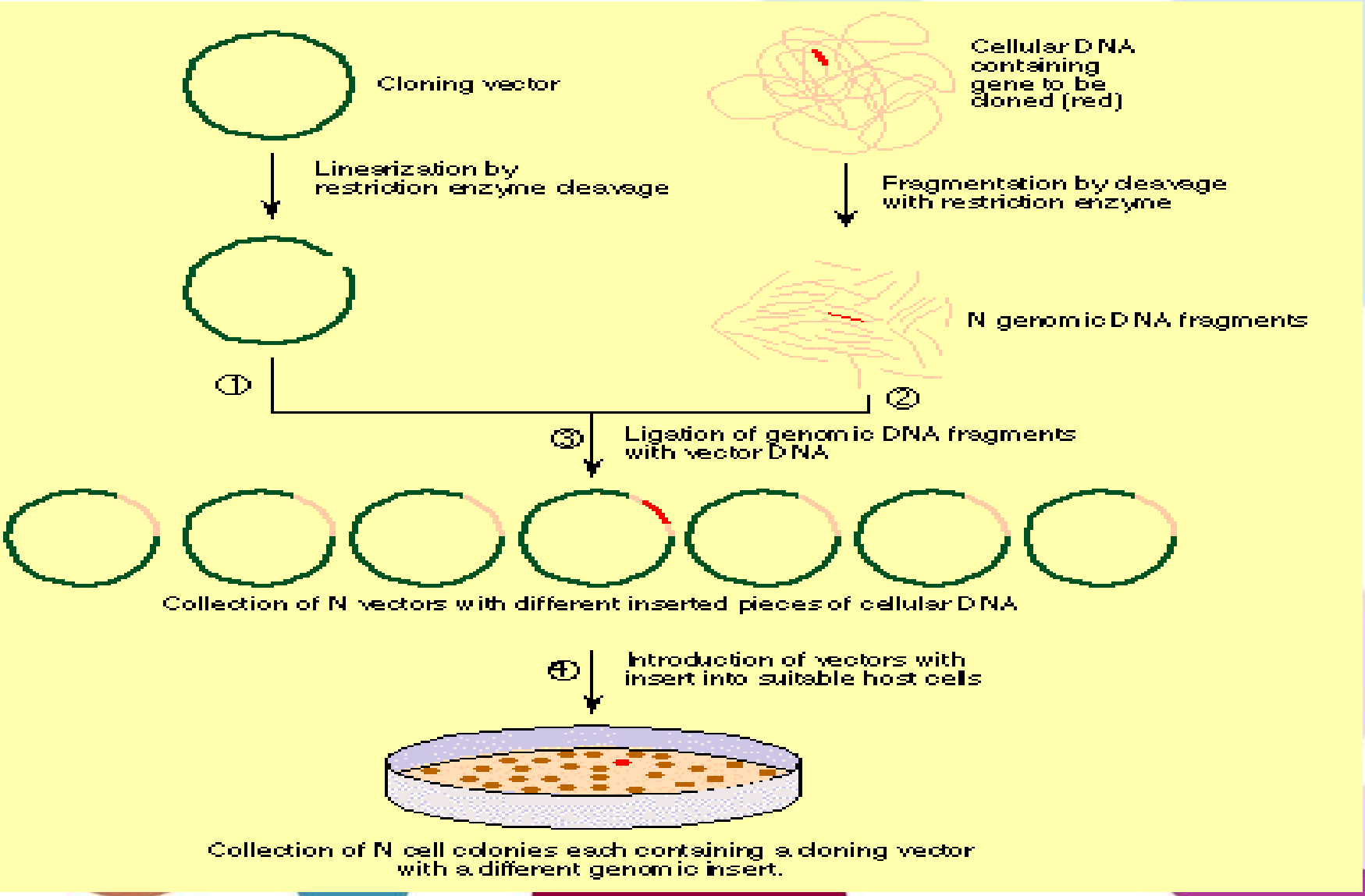
- Gene cloning
- Reproductive cloning
- Therapeutic cloning

Gene cloning

- A term used to describe a collection of DNA fragments derived from the genome of an organism and cloned randomly into suitable cloning vectors (plasmids, phages,viral
- genomes).

  The term **genomic DNA clone** or **chromosomal DNA clone** then refers to an individual cell carrying a cloning vector with one of the cellular DNA fragments or to a phage isolate with a specific DNA insert.

Gene Cloning



Reproductive Cloning

- Reproductive cloning is the production of a genetic duplicate of an existing organism. A human clone would be a genetic copy of an existing person.
- Some oppose reproductive cloning because of safety considerations. Animal cloning is seldom successful. Many scientists believe that reproductive cloning can never be made safe. Human reproductive cloning would also threaten the psychological well-being of cloned children, open the door to more powerful genetic manipulation Technologies and raise other social and ethical concerns.

- Dolly defied scientific convention. With her birth on 5th July 1996, her makers had done the impossible - cloned an animal from a cell taken from an adult mammal.

  When Dolly was announced to the world on 22nd February 1997 she became global front page news. Press and public flocked to her home at the Roslin Institute outside Edinburgh to catch a glimpse of the world's most famous sheep.

Dolly the Cloned sheep

Therapeutic Cloning

- Which creates embryonic Stem Cells. Researchers hope to use theses cells to grow healthy tissue to replace injured or diseased tissues in the human body.

Animals Cloned so Far

- Tad pole
- Sheep
- Goats
- Cows
- Mice
- Pigs
- Cats
- Rabbits
- Human

Reasons for cloning

- Infertility
- Rejuvenation
- Reverse heart attacks
- Plastic, reconstructive, and
- Cosmetic surgery
- Defective genes
- Down's syndrome
- Tay-Sachs disease

Reasons for cloning (Cont.)

- Liver failure
- Kidney failure
- Leukemia
- Cancer
- Cystic fibrosis
- Spinal cord injury
- Testing for genetic disease
- Alzheimer’s and Parkinson’s

Risks of Cloning

- Expensive
- Highly inefficient (2%)
- Unethical
- Higher rates of tumor growth
- Reduce Genetic Diversity
- Programming errors in genetic material from donor cell

Why is DNA Cloning Important?

- DNA clones are used to find genes, map them, and transfer them between species

- Cloning technology is used to find carriers of genetic disorders, perform gene therapy, and create disease-resistant plants.

Keep In Mind

- *Cloned plants and animals are used in research, agriculture, and medicine*

THANK YOU