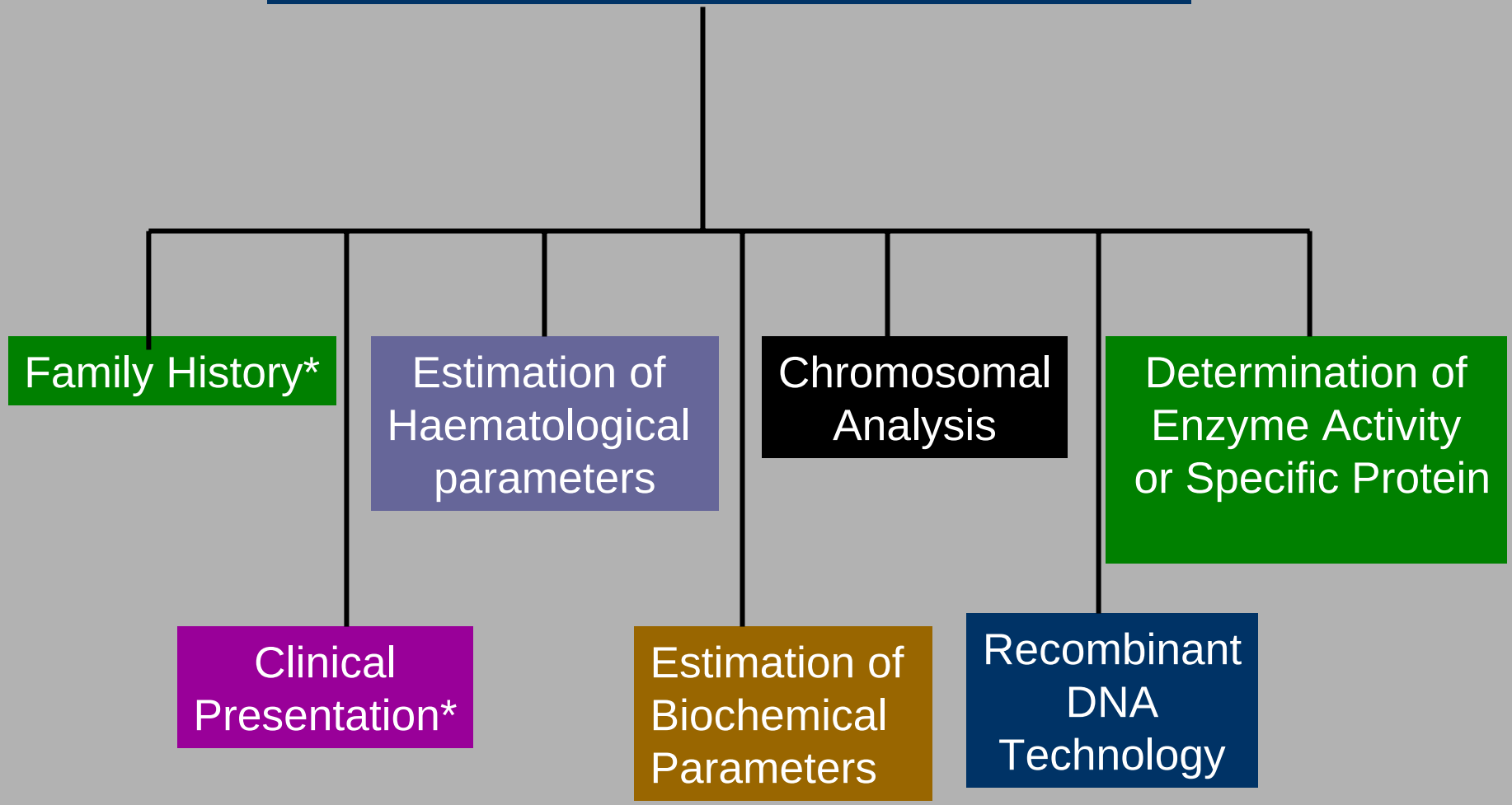
Diagnosis of Genetic Diseases

Diagnosis of Genetic Diseases



* Important for all genetic diseases

1. Family History

- Consanguinity of parents.
- Presence of other siblings with the same disorder.
- Occurrence of the disorder in other members of the family.
- Repeated abortions or still births,
- mother and fathers ages.
- Drawing punnet square helps to determine the mode of inheritance of the genetic disorders.
    - Autosomal or X-linked
    - Dominant or recessive

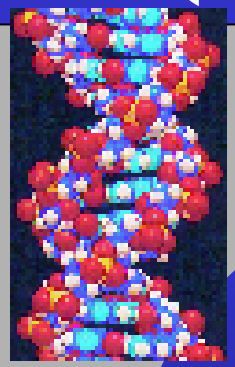
2. Clinical Presentation

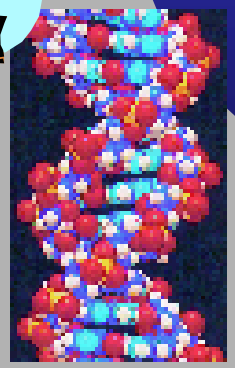
Certain clinical features are specific for a disease:

- Chronic anaemia:
  - Haemoglobinopathies
  - Thalassaemia
  - Other genetic anaemias
- Acute anaemia, under certain stressful conditions.
  - G-6-PD deficiency
- Hypoxia – sickle cell disease.
- Dependence on blood transfusion - β-thalassaemia (major)
- Severe immune deficiency – ADA deficiency.
- Emphysema - α1 anti-trypsin deficiency.
- Hypercholesterolaemia – familial hypercholesterolaemia.
- Delayed blood coagulation – Haemophilia (decrease in factor VIII or IX).
- Mental retardation – Fragile syndrome (in X chromosome) or phenylketonuria (PKU).
- Muscular weakness and degeneration – Duchenne muscular dystrophy.

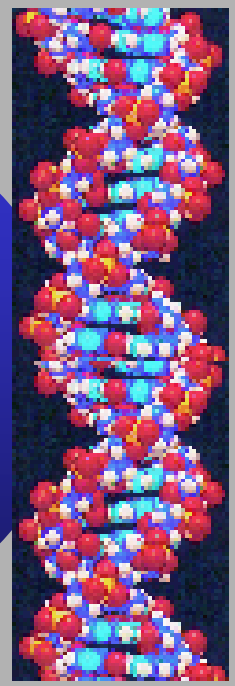
Recombinant DNA Technology
(Genetic Engineering)

Recombinant DNA Technology (Genetic Engineering)



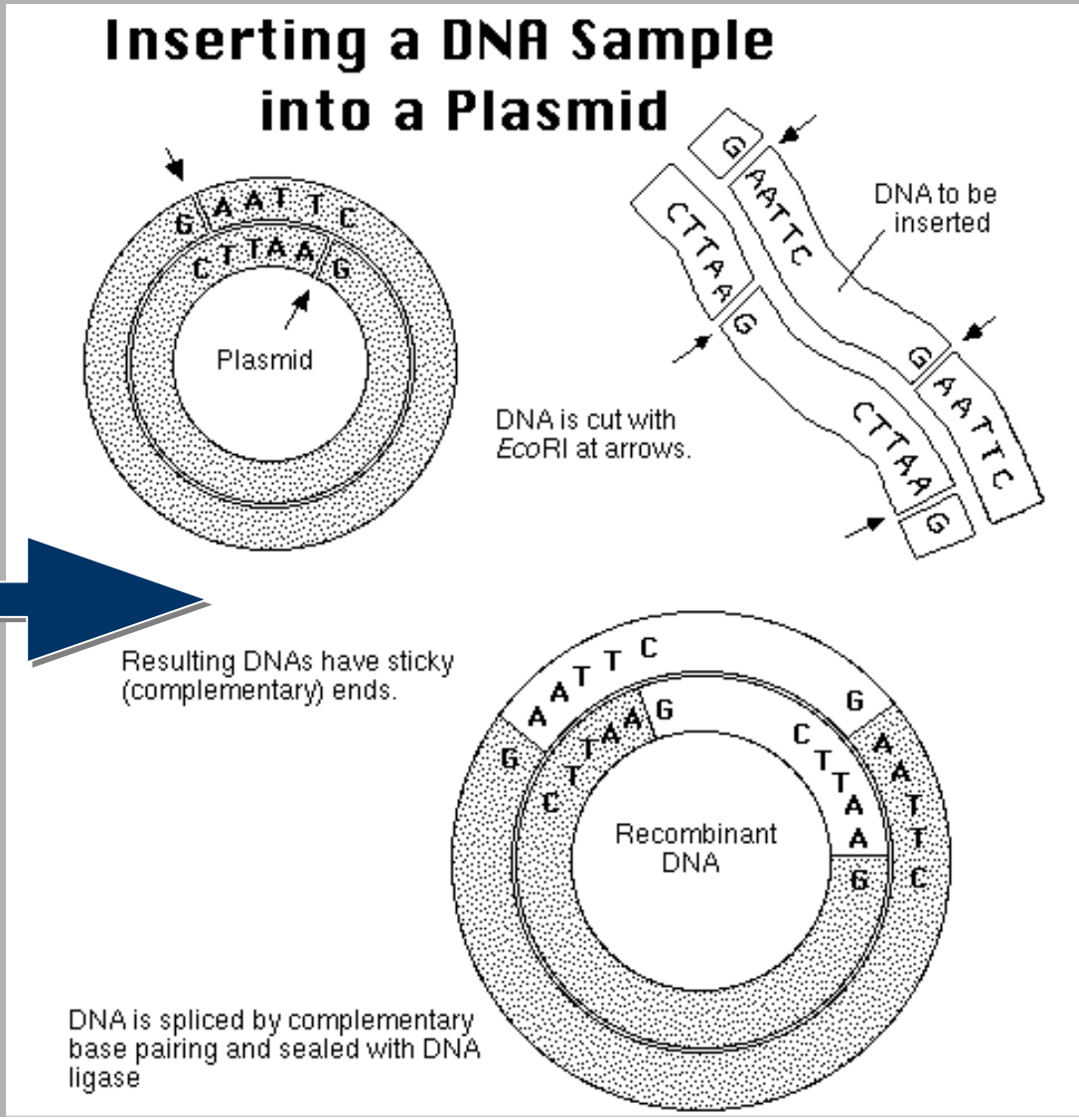


Techniques for cutting and joining DNA



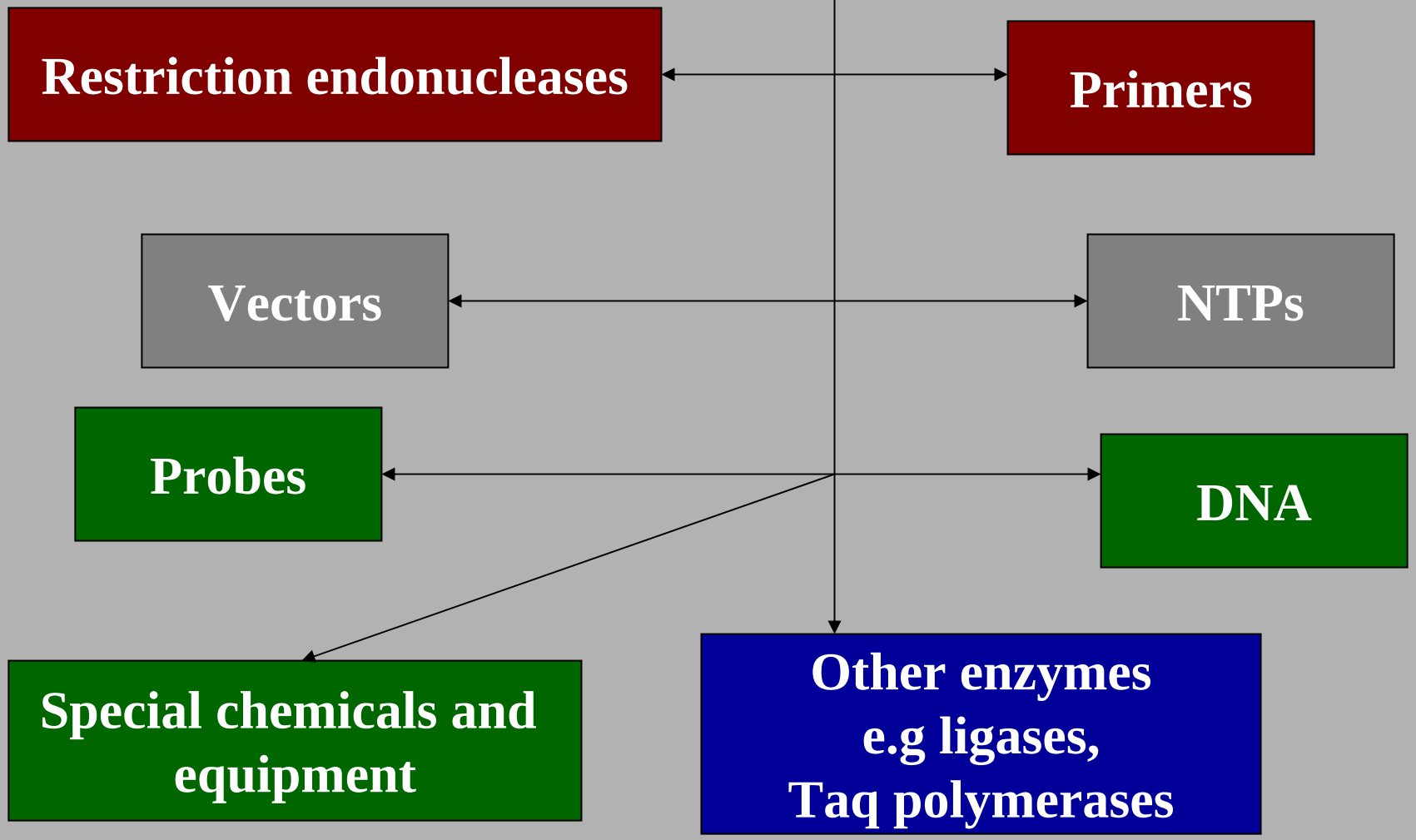
Recombinant DNA

- The DNA created by joining DNA from different origins e.g human DNA sequence of interest and bacterial or other DNA molecule.
- It is capable of duplication in the laboratory.



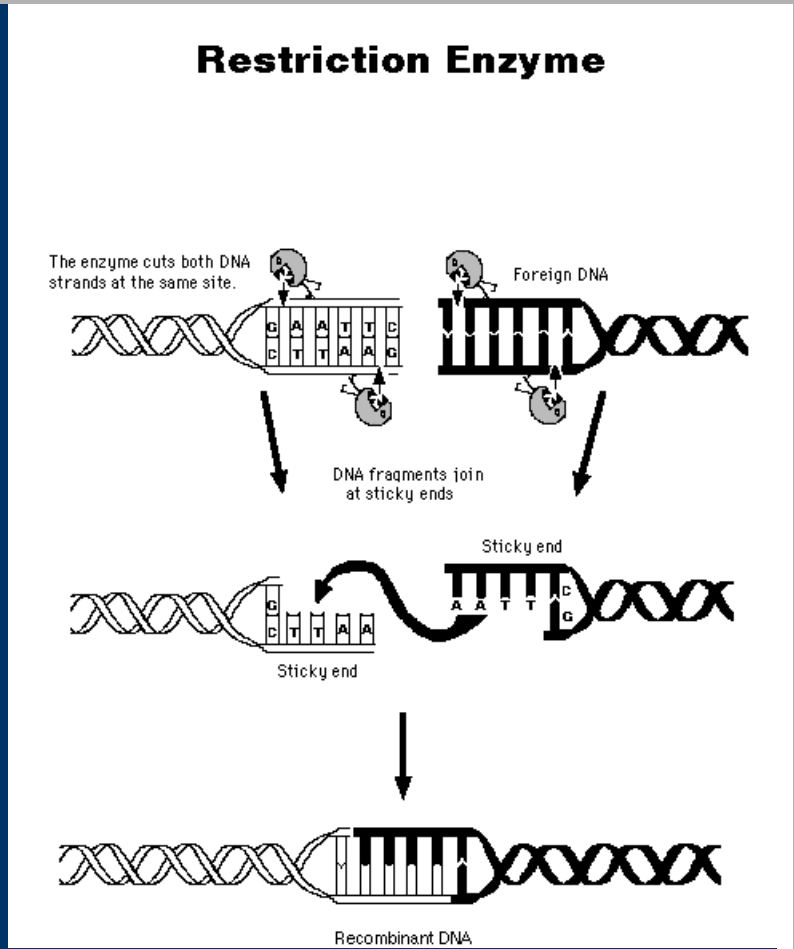
Recombinant DNA

Requirements for DNA technology



Restriction Endonuclease

- Endonucleases.
- Synthesized by procaryotes. Do not restrict host DNA.
- Recognize and cut specific base sequence of 4-6 bases in double helical DNA.
- The sequence of base pairs is palindromic i.e. it has two fold symmetry and the sequence, if read, from 5’ or 3’ end is the same.

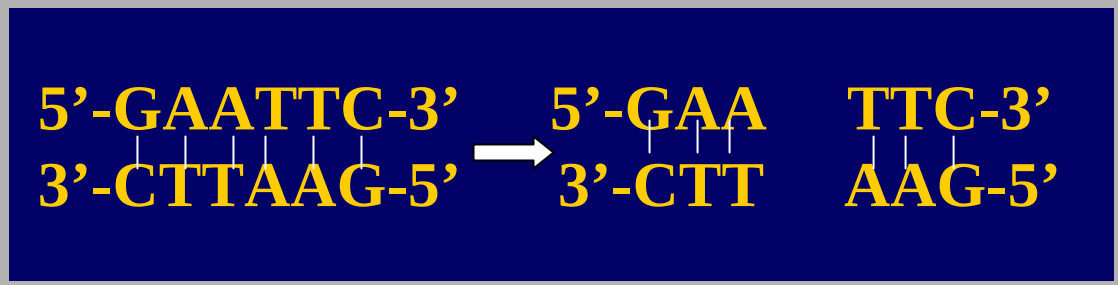


5’-GAATTC-3’
3’-CTTAAG-5’

Restriction Endonuclease

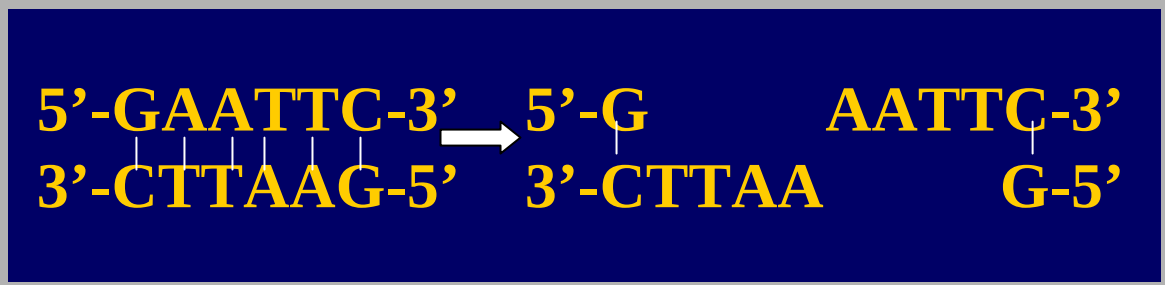
Produce either Blunt Ends or Staggered ends:

Blunt Ends



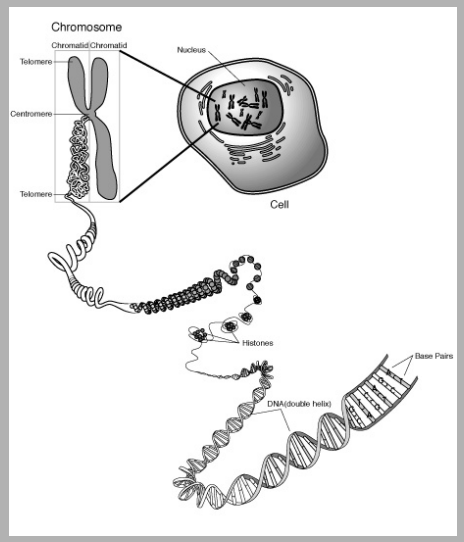
or

Staggered Ends



Uses of Restriction Endonuclease

- Obtaining DNA fragments of interest.
- Gene mapping.
- Sequencing of DNA fragments.
- DNA finger printing
- Recombinant DNA technology
- Study of gene polymorphism.
- Diagnosis of disease.
- Prenatal diagnosis



Sources of DNA

Genomic DNA

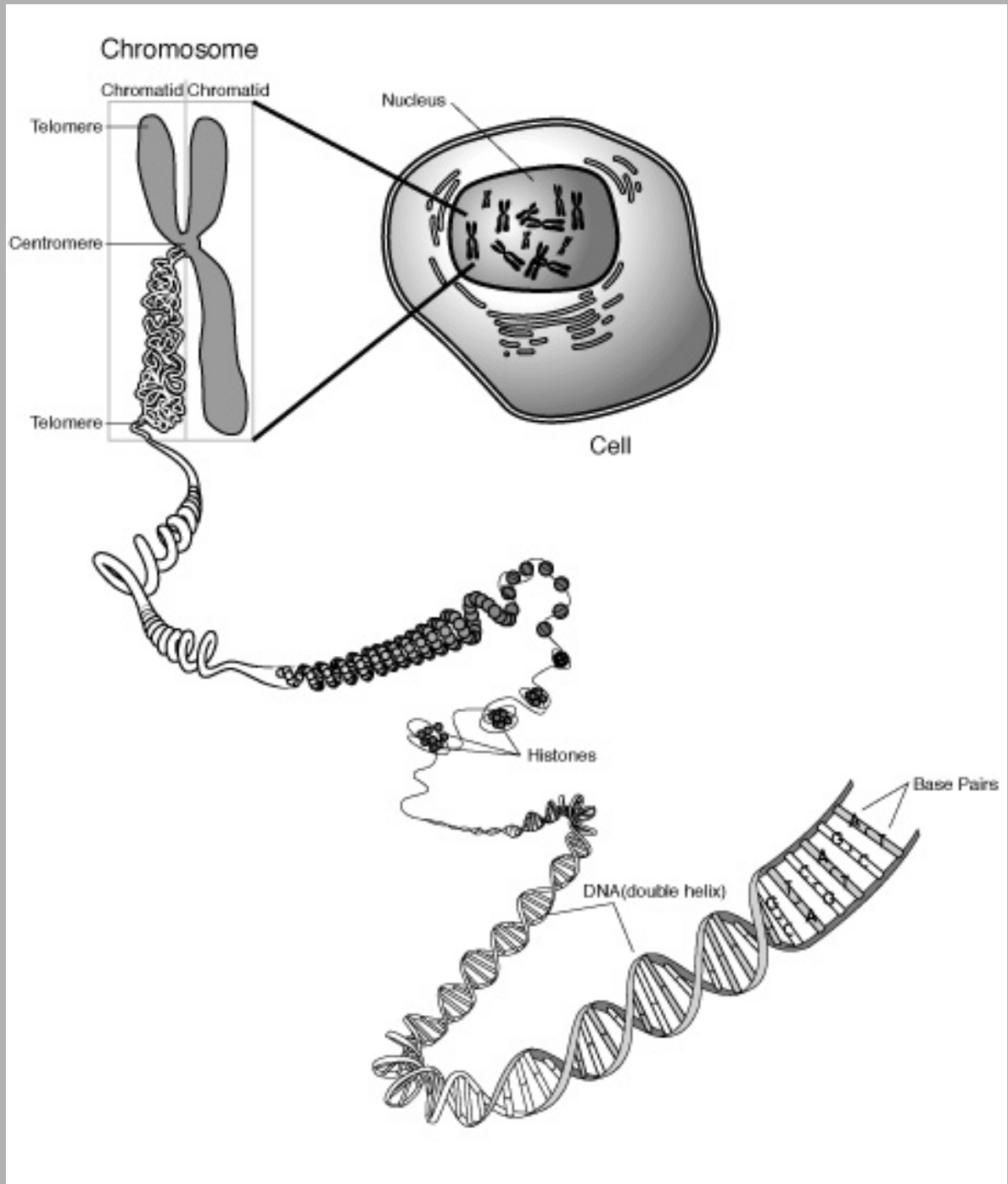
DNA extracted from cells

Synthesis of DNA

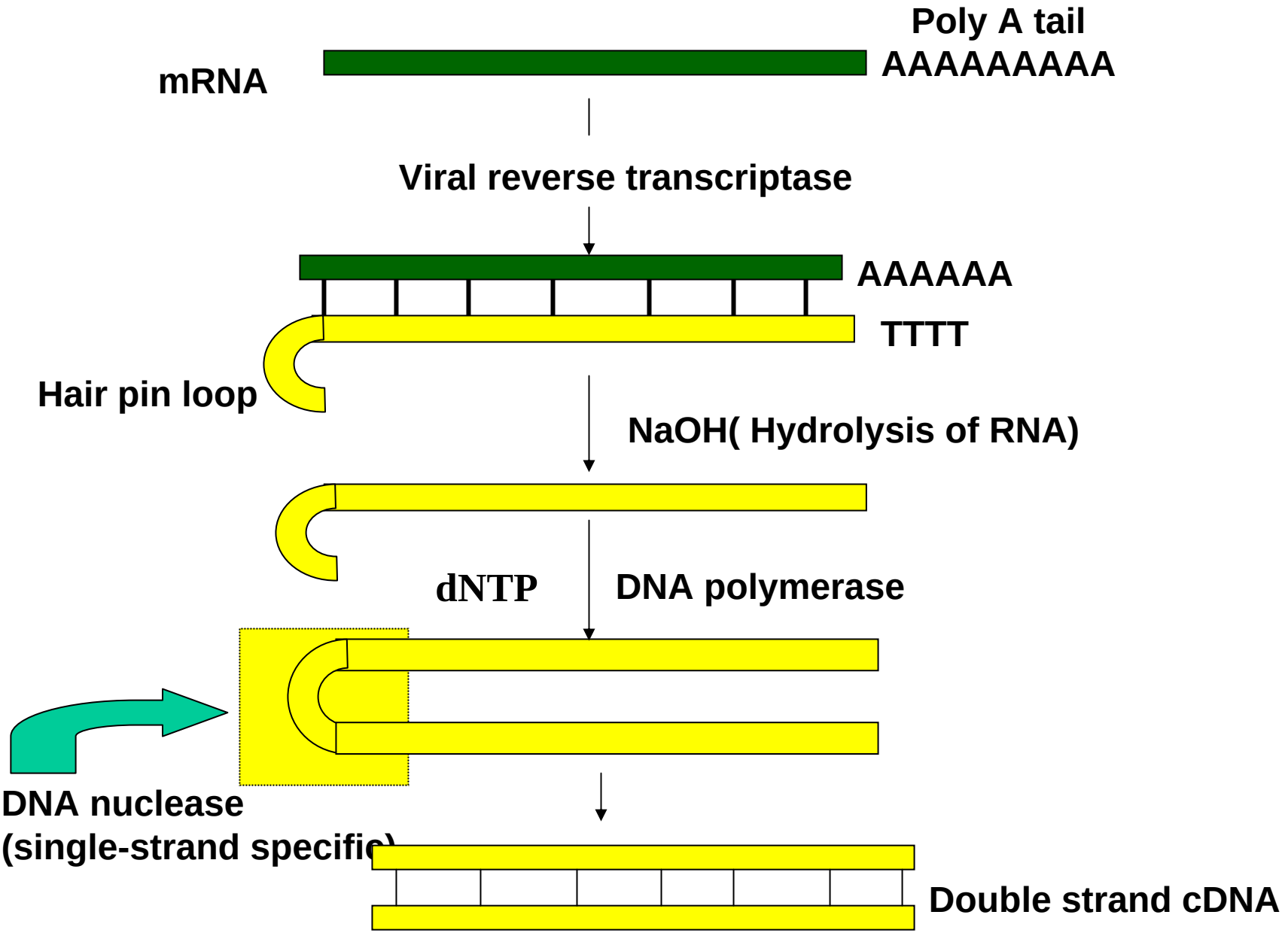
Using DNA synthesiser

cDNA

Synthesised from mRNA using reverse transcriptase


Chromosome
Chromatid
Chromatid
Telomere
Centromere
Telomere
Nucleus
Cell
Histones
Base Pairs
DNA(double helix)

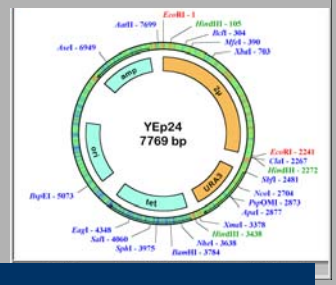
cDNA Synthesis



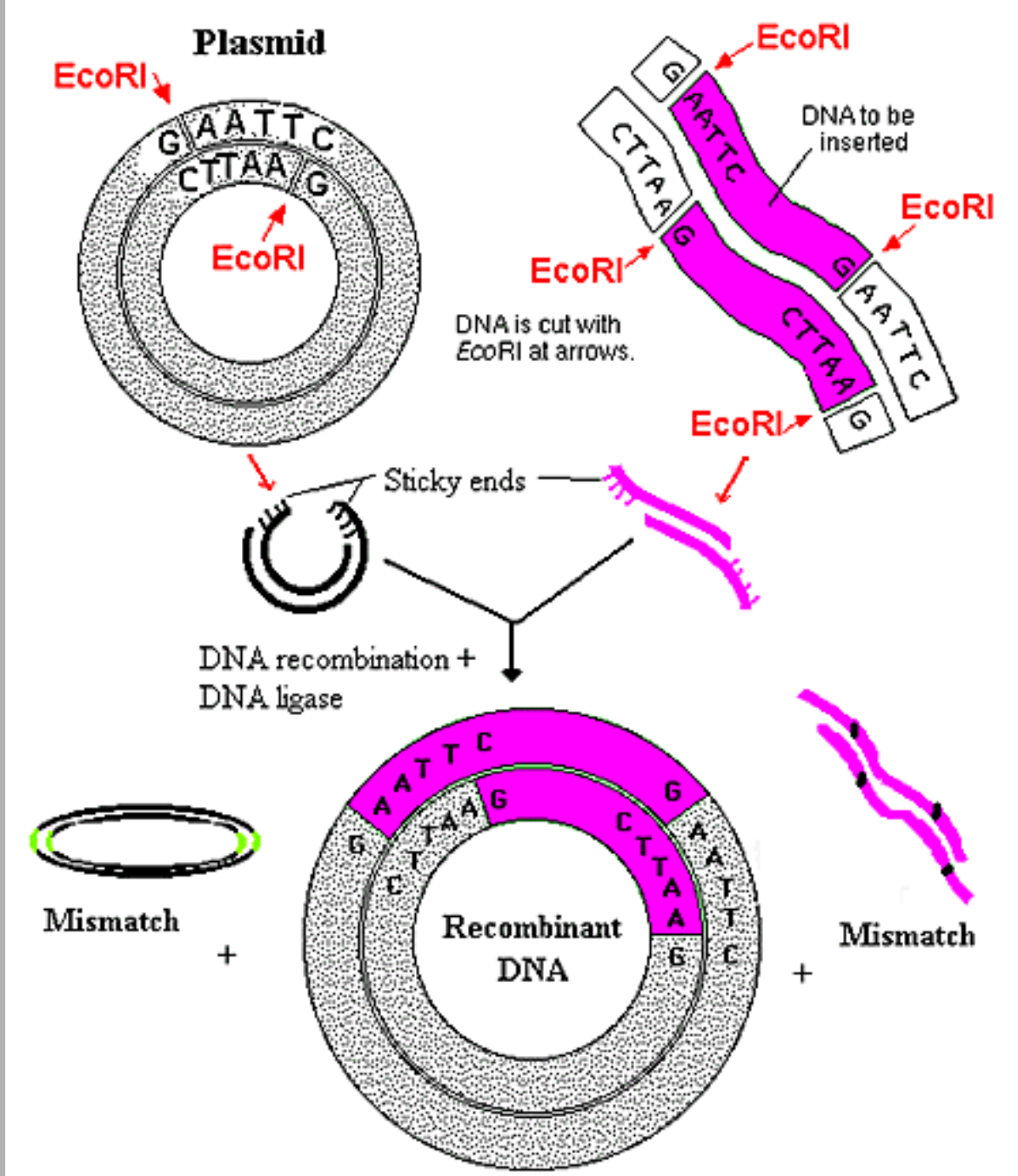
Vectors → Cloning vesicles

- DNA molecules.
- Can replicate in a host e.g bacterial cells or yeast.
- Can be isolated and re-injected in cells.
- Presence can be detected.
- Can be introduced into bacterial cells e.g. E. coli.
- May carry antibiotic resistance genes.

Types of vectors

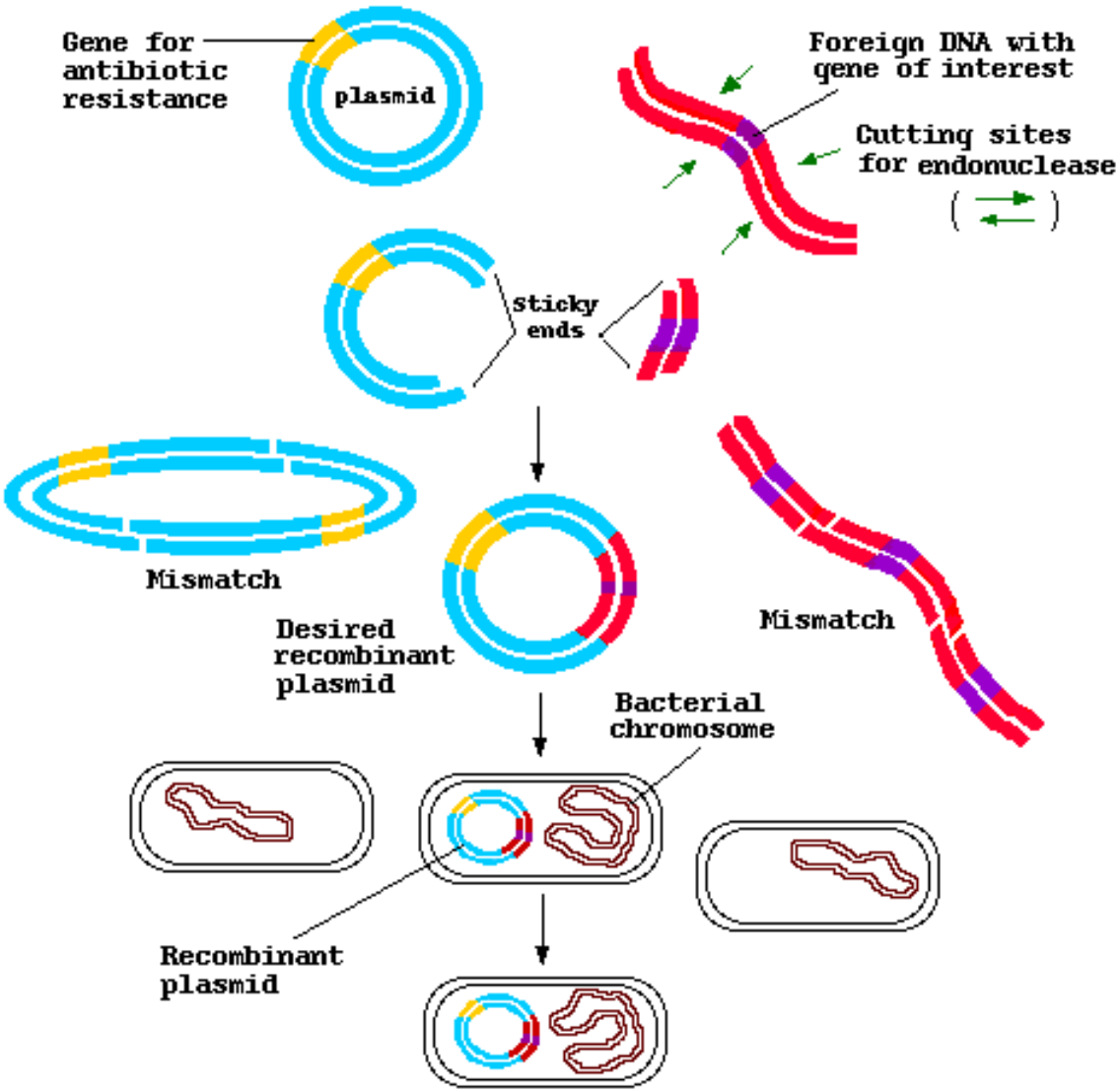


Type	Insert size
I. Plasmid : circular, double stranded cytoplasmic DNA in procaryotic e.g. PBR 3 of Ecoli.	• <5-10 kb.
II. Bacteriophage lambda: a bacterial virus infects bacteria.	• Upto 20kb.
III. Cosmids: a large circular cytoplasmic double stranded DNA similar to plasmid.	• Upto 50kb.
IV. Yeast Artificial Chromosomes (YAC)	•~100-1000kb.



Inserting a DNA Sample into a Plasmid

Plasmid Insertion



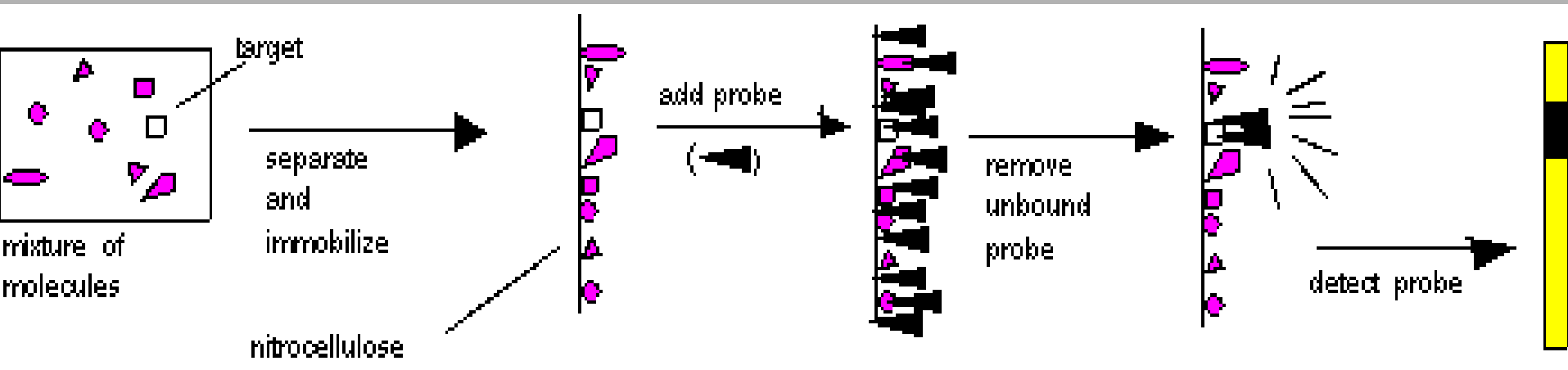
Probes

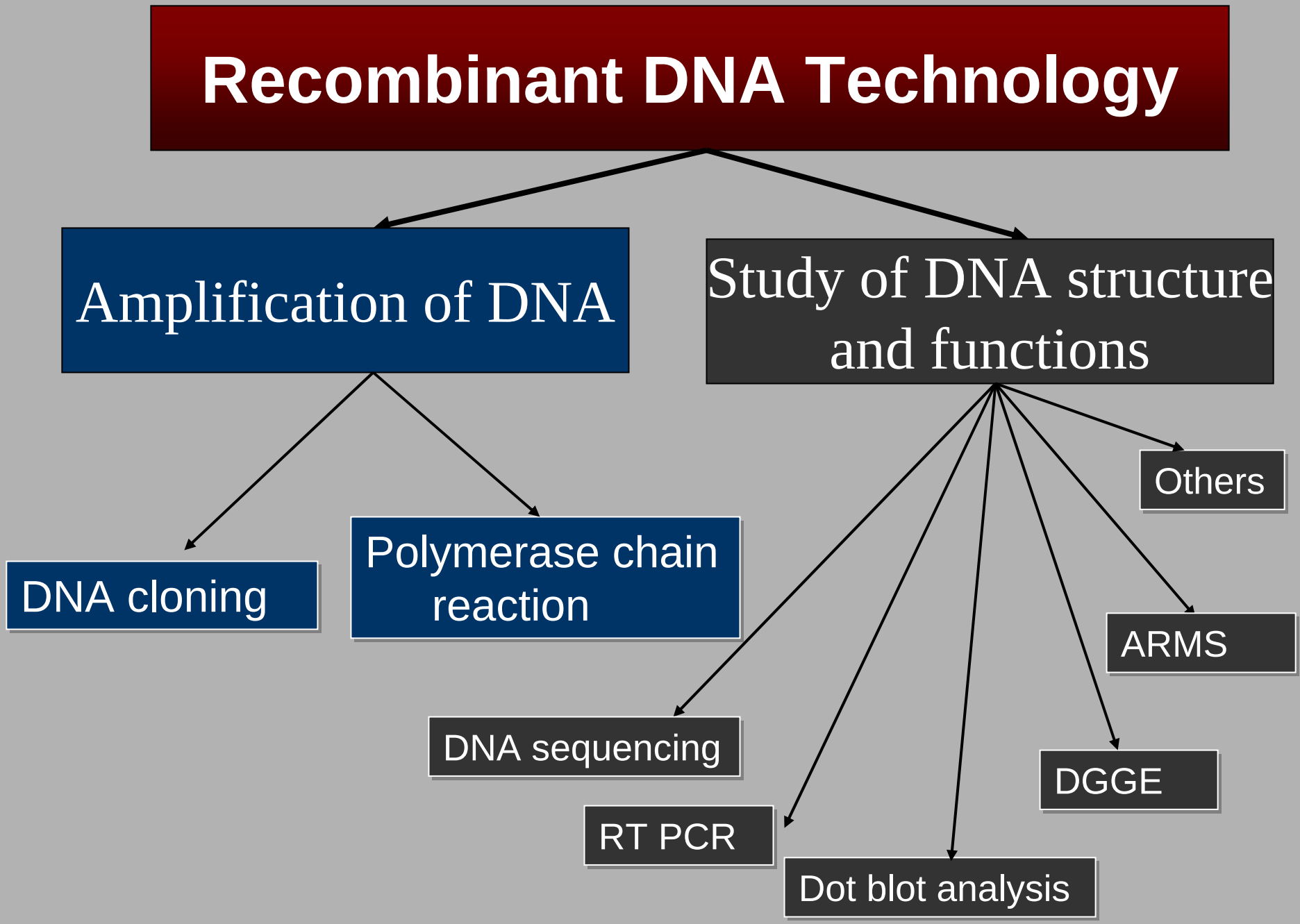
Cloned or synthetic nucleic acids used for DNA:DNA or DNA:RNA hybridization reactions to hybridize to DNA of interest.

- DNA or RNA.
- cDNA.
- Labeling of probes:
  - ^{3}H
  - ^{32}P

  Radioactive

Hybridization

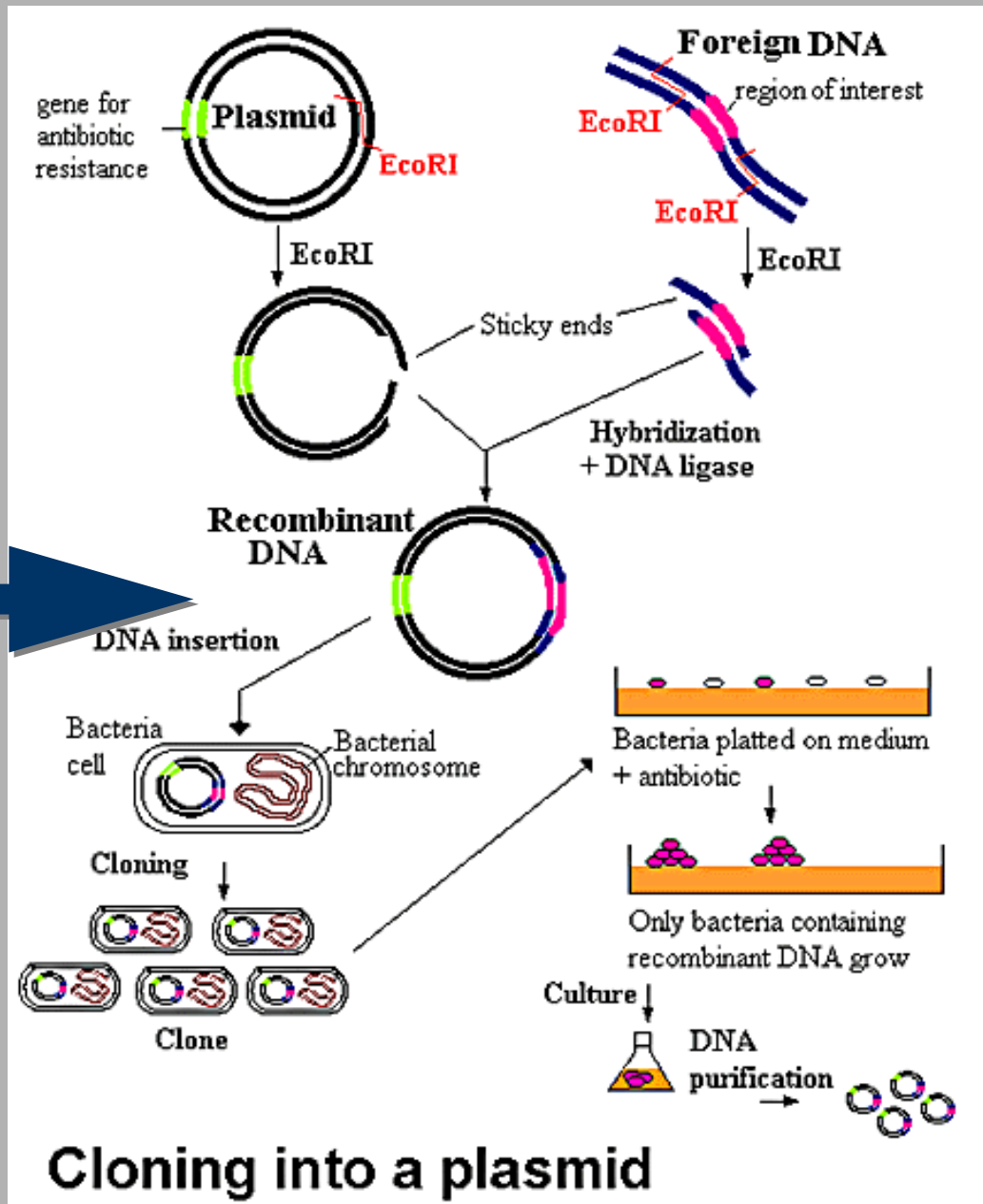


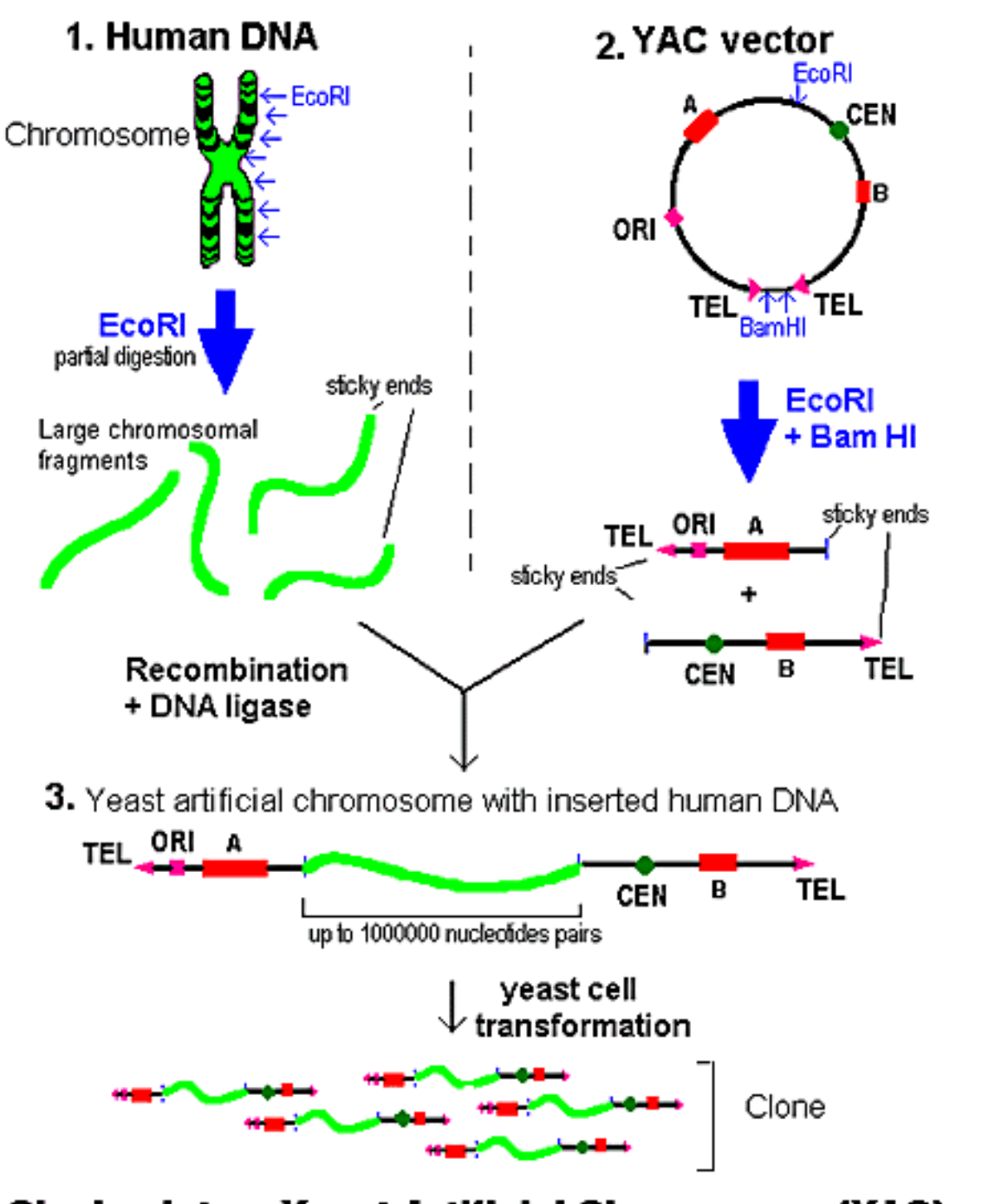

Recombinant DNA Technology
Amplification of DNA
Study of DNA structure and functions
DNA cloning
Polymerase chain reaction
Others
ARMS
DNA sequencing
DGGE
RT PCR
Dot blot analysis

Principles of Molecular Cloning

Involves:

- Isolation of DNA sequence of interest.
- Insertion of this DNA in the DNA of an organism that grows rapidly and over extended period e.g. bacteria.
- Growing of the bacteria under appropriate condition.
- Obtaining the pure form of DNA in large quantities for molecular analysis.



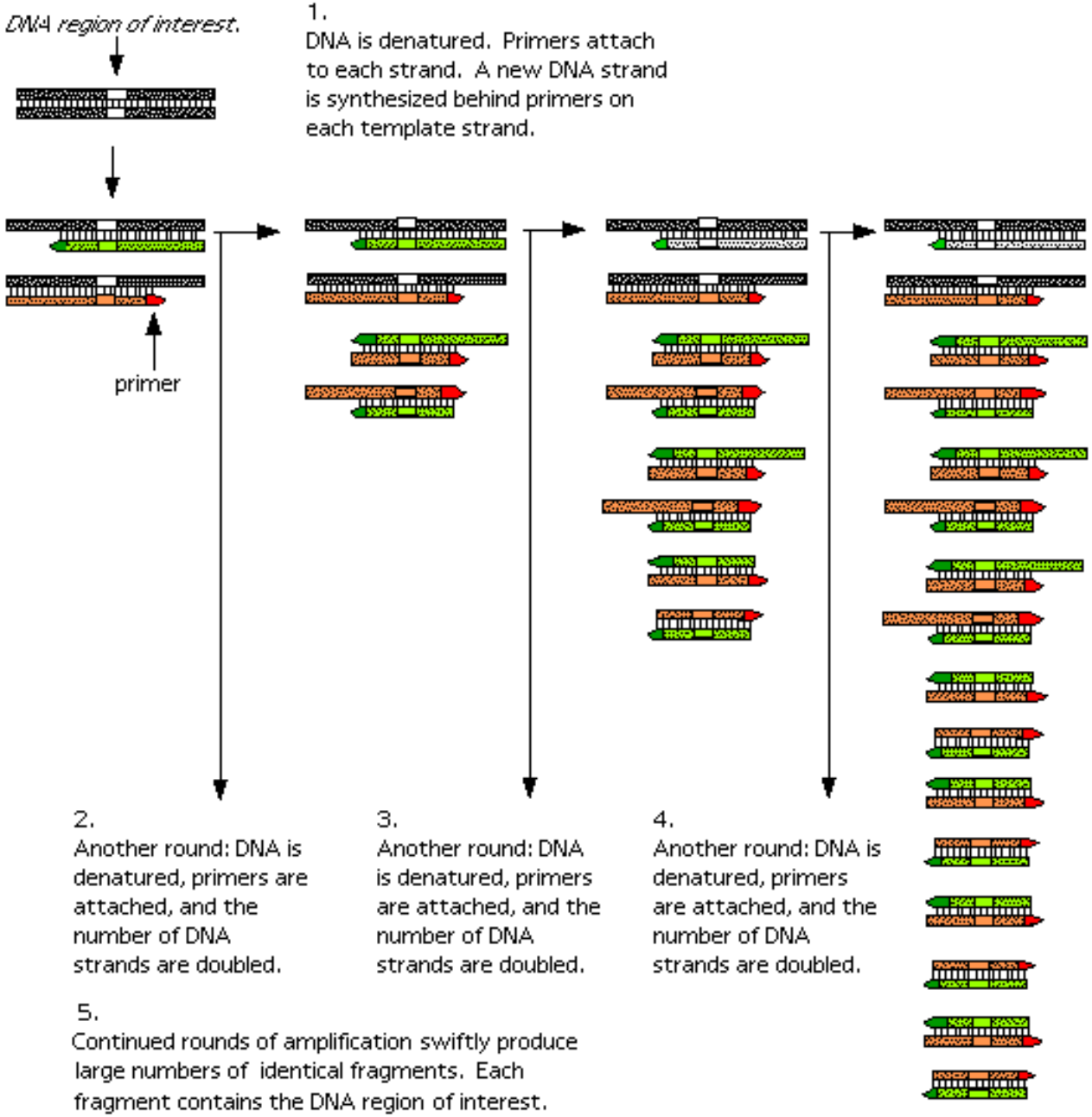


Cloning into a Yeast Artificial Chromosome (YAC)

Polymerase Chain Reaction (PCR)

- Method to amplify a target sequence of DNA or RNA several million folds.
- Developed by Saiki et al in 1985.
- Based on Enzymatic amplification of DNA fragment flanked by primers i.e. short oligonucleotides fragments complimentary to DNA. Synthesis of DNA initiates at the primers.

POLYMERASE CHAIN REACTION



1.
DNA is denatured. Primers attach to each strand. A new DNA strand is synthesized behind primers on each template strand.

2.
Another round: DNA is denatured, primers are attached, and the number of DNA strands are doubled.

3.
Another round: DNA is denatured, primers are attached, and the number of DNA strands are doubled.

4.
Another round: DNA is denatured, primers are attached, and the number of DNA strands are doubled.

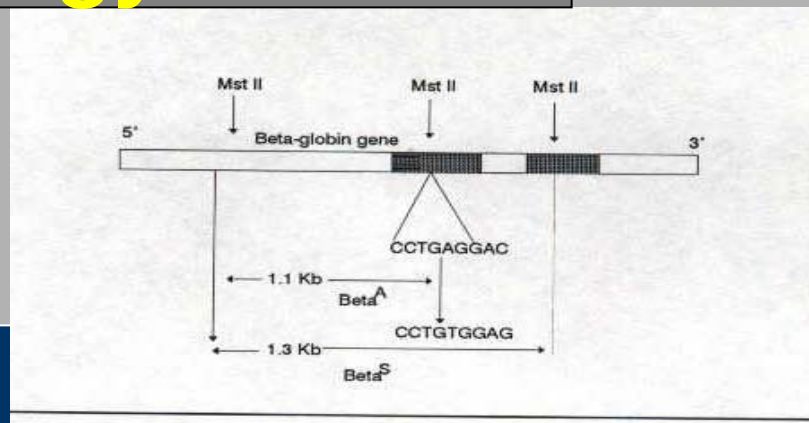
5.
Continued rounds of amplification swiftly produce large numbers of identical fragments. Each fragment contains the DNA region of interest.

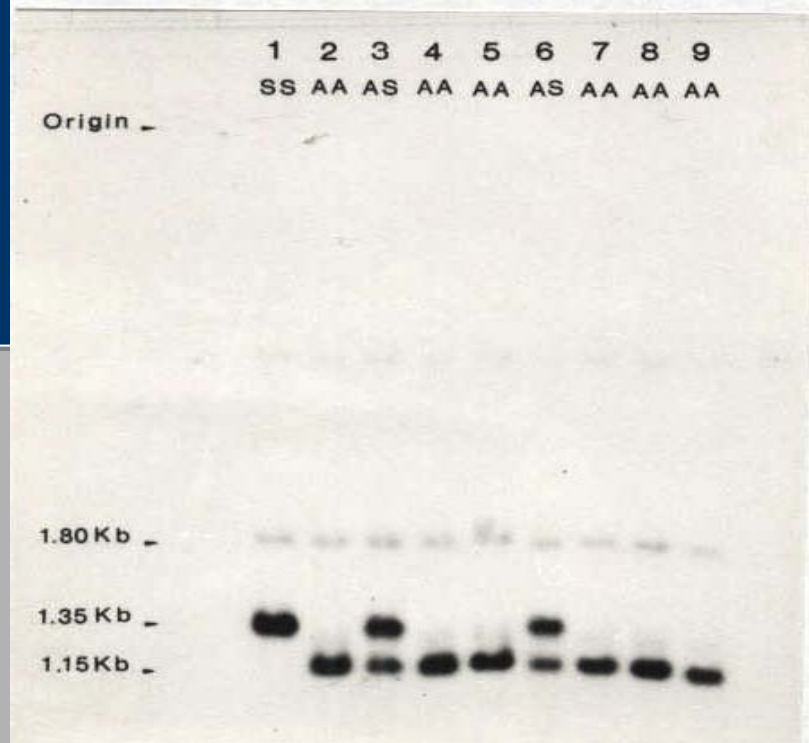
Application of PCR

- Diagnosis of genetic disease by amplification of the gene of interest, followed by detection of mutation.
- Detection of infectious agent e.g. bacteria and viruses.
- DNA sequencing.
- In forensic medicine.

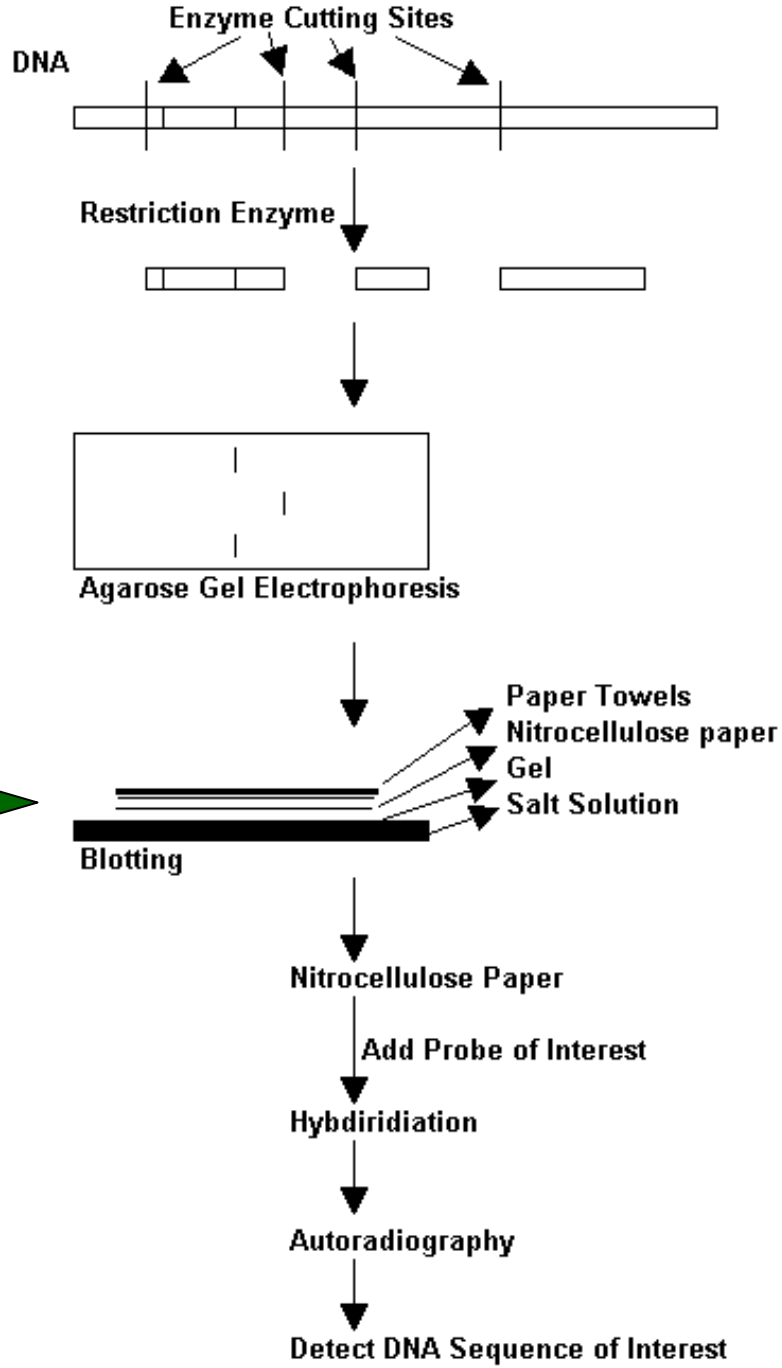
Application of Recombinant DNA Technology

1. Clinical Chemistry:
   - Diagnosis of disease e.g. sickle cell anaemia by Mst II.
   - Prenatal diagnosis





Southern Blotting



Pathogenesis of α-Thalassaemia

Withdraw blood

Extract DNA

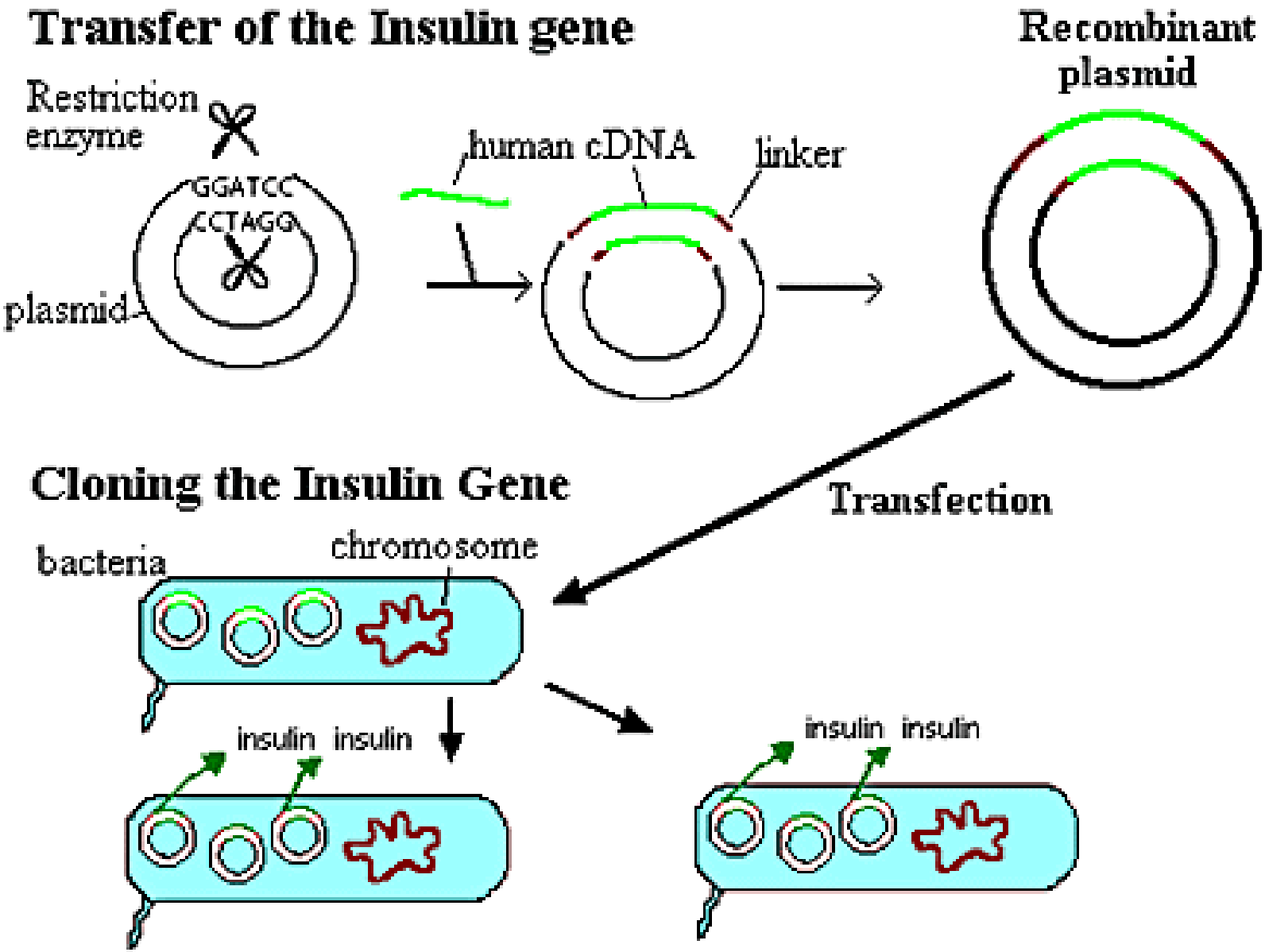
Treat with BglII

Electrophoresis

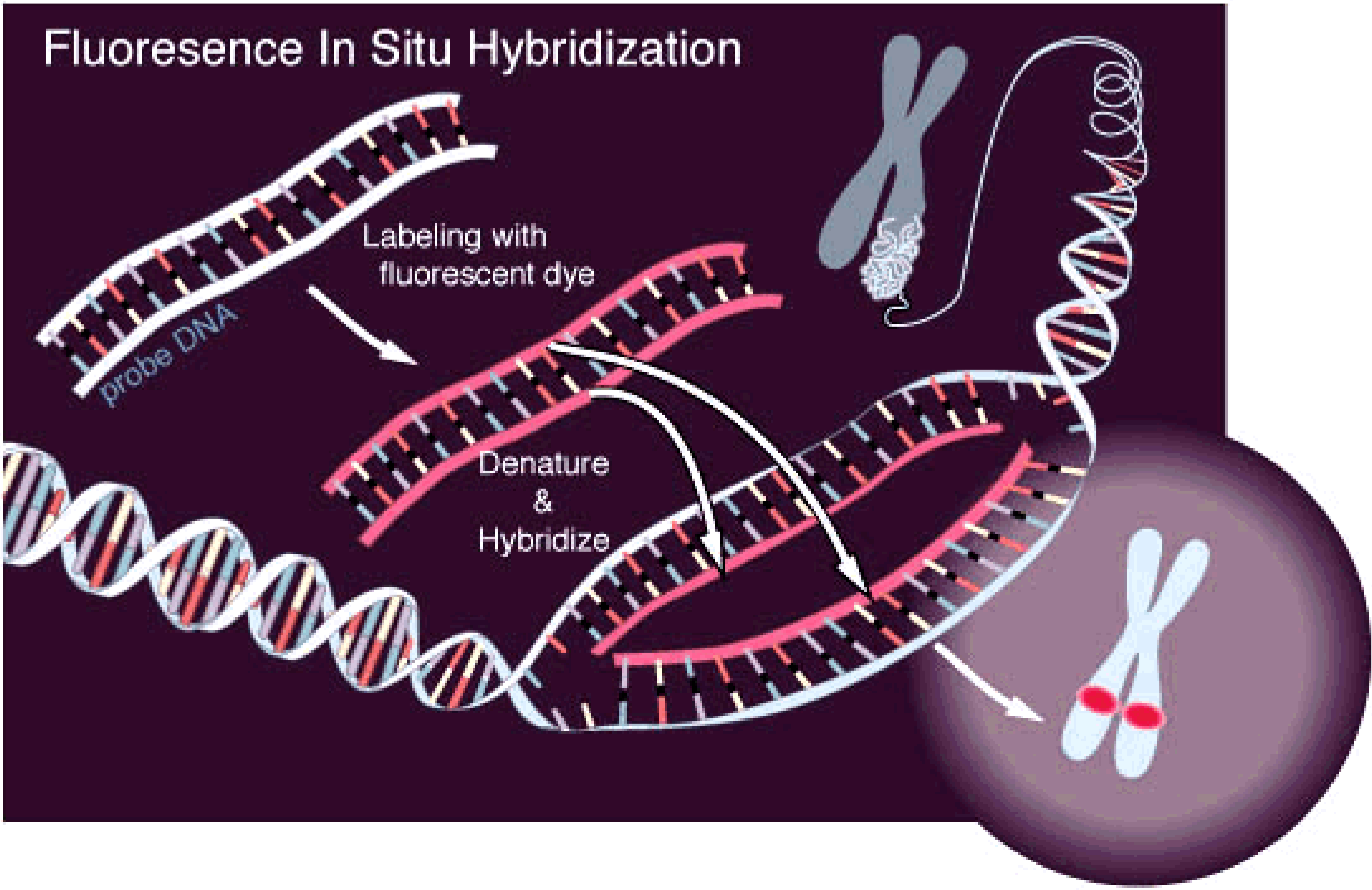
Southern Blotting

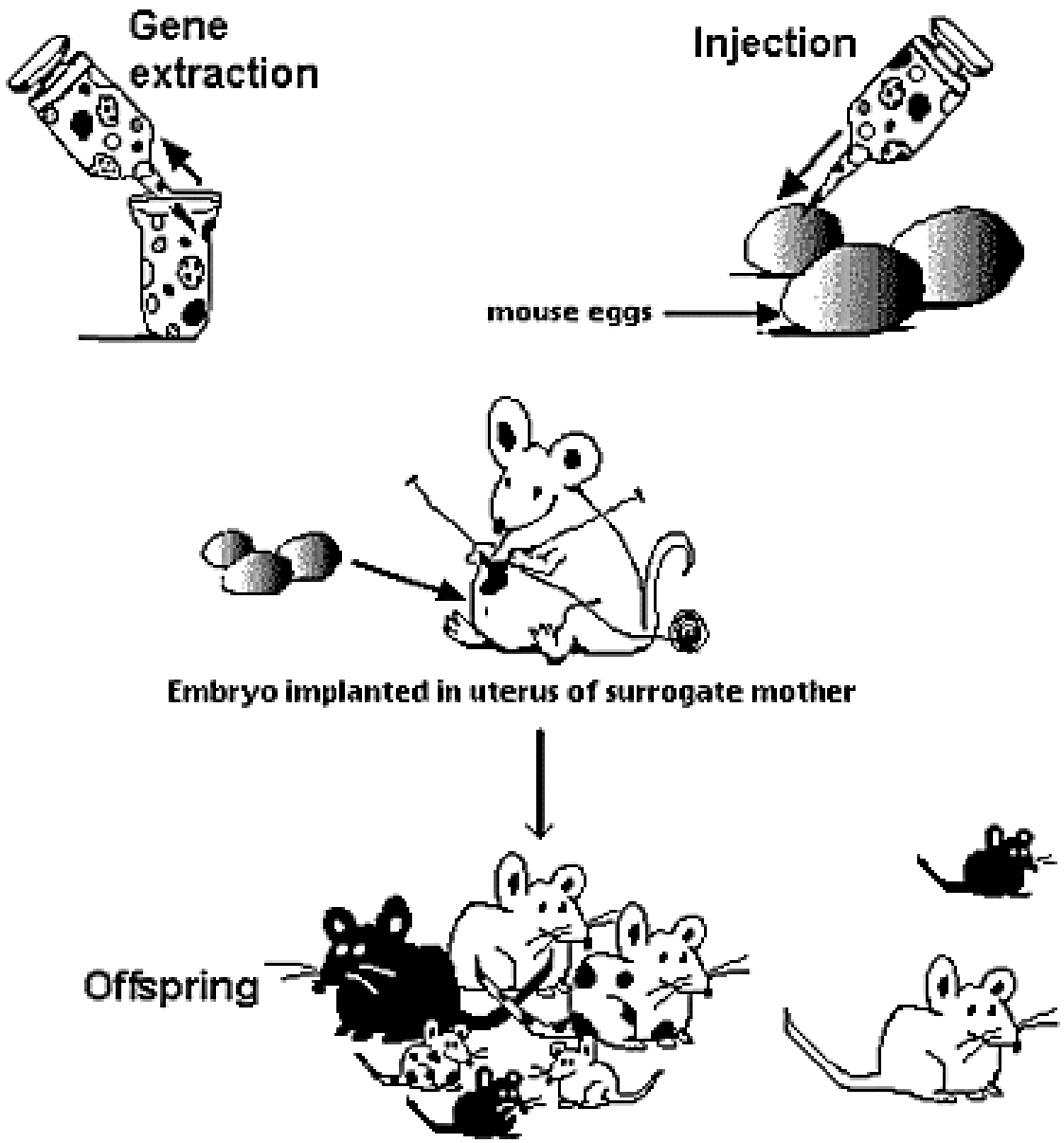
Visualize

2. Human Genetics
   - Mutations in genes causing hereditary disease e.g. diagnosis of fibrosis Channes disease.
3. Forensic Medicine
   - Analysis of stains of blood, semin.
4. Virology
   - Detection of viral diseases e.g. hepatitis
5. Microbiology
   - Using specific gene probes for detection of E.coli
6. Cytology, Histology and Pathology
   - Used in detection of tumor.
7. Synthesis of protein in bacterial
   - Insulin
   - GH
   - Somatostatin
   - Interferon



Transfer and cloning of the Insulin gene


Fluoresence In Situ Hybridization
Labeling with
fluorescent dye
probe DNA
Denature
&
Hybridize



Transgenic Mice