Establishment of genes Library

Aims of the library

1. To represent all genes in genome.
2. To isolate specific gene.
3. To study the evolutionary relations and paternity.
4. To find the correlations in crime purposes.
5. To bullied the genes mapping.
6. Other purposes.

Types of genes libraries

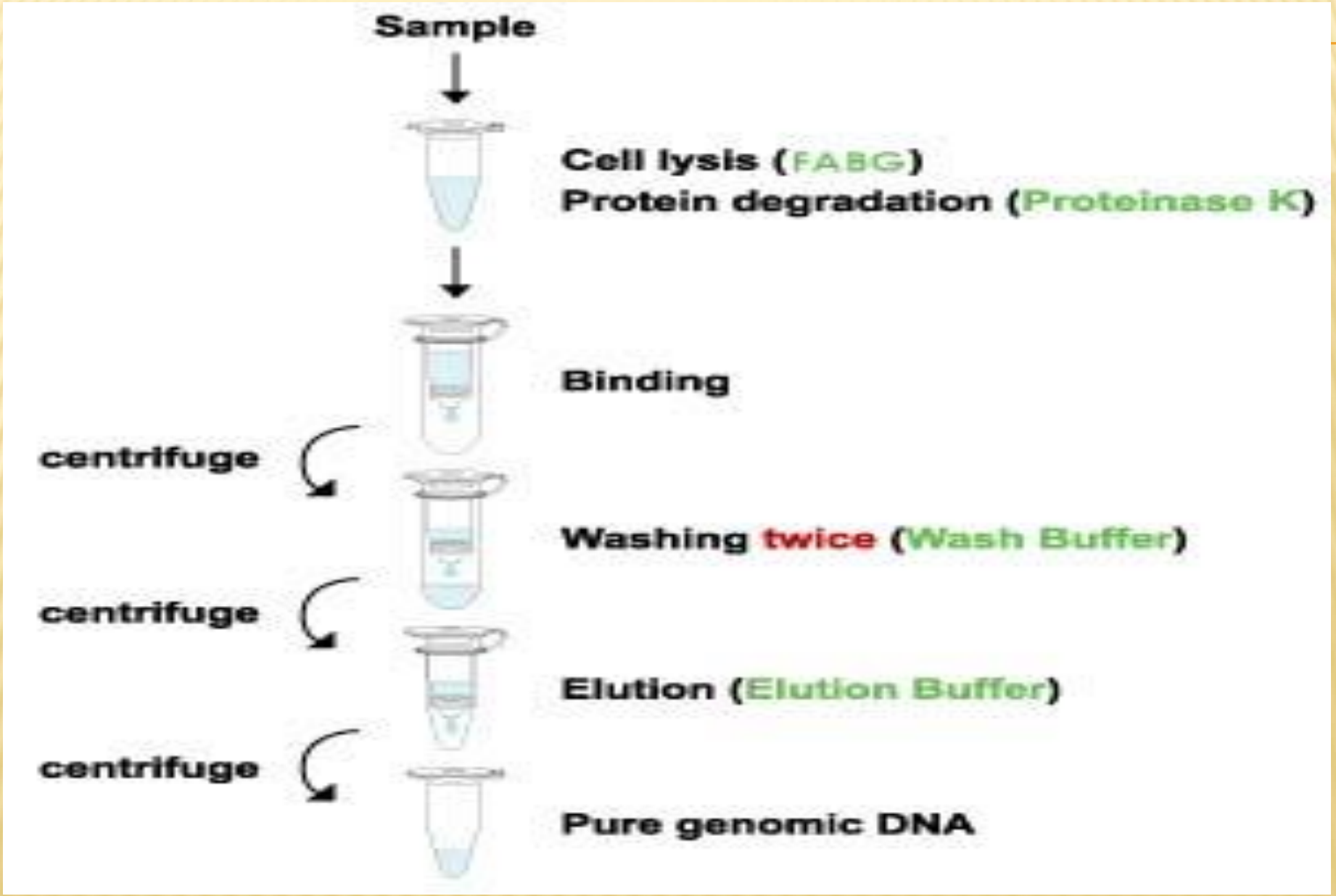
1. DNA libraries
2. cDNA libraries

DNA library establishment

Steps

1. DNA extraction.
2. DNA fragmentation by specific enzyme.
3. Isolation of specific size fragments.
4. Select the suitable vector.
5. Clone the fragments to vector.
6. Transfer the cloned into host.
7. Calculate the size and the efficiency of library.

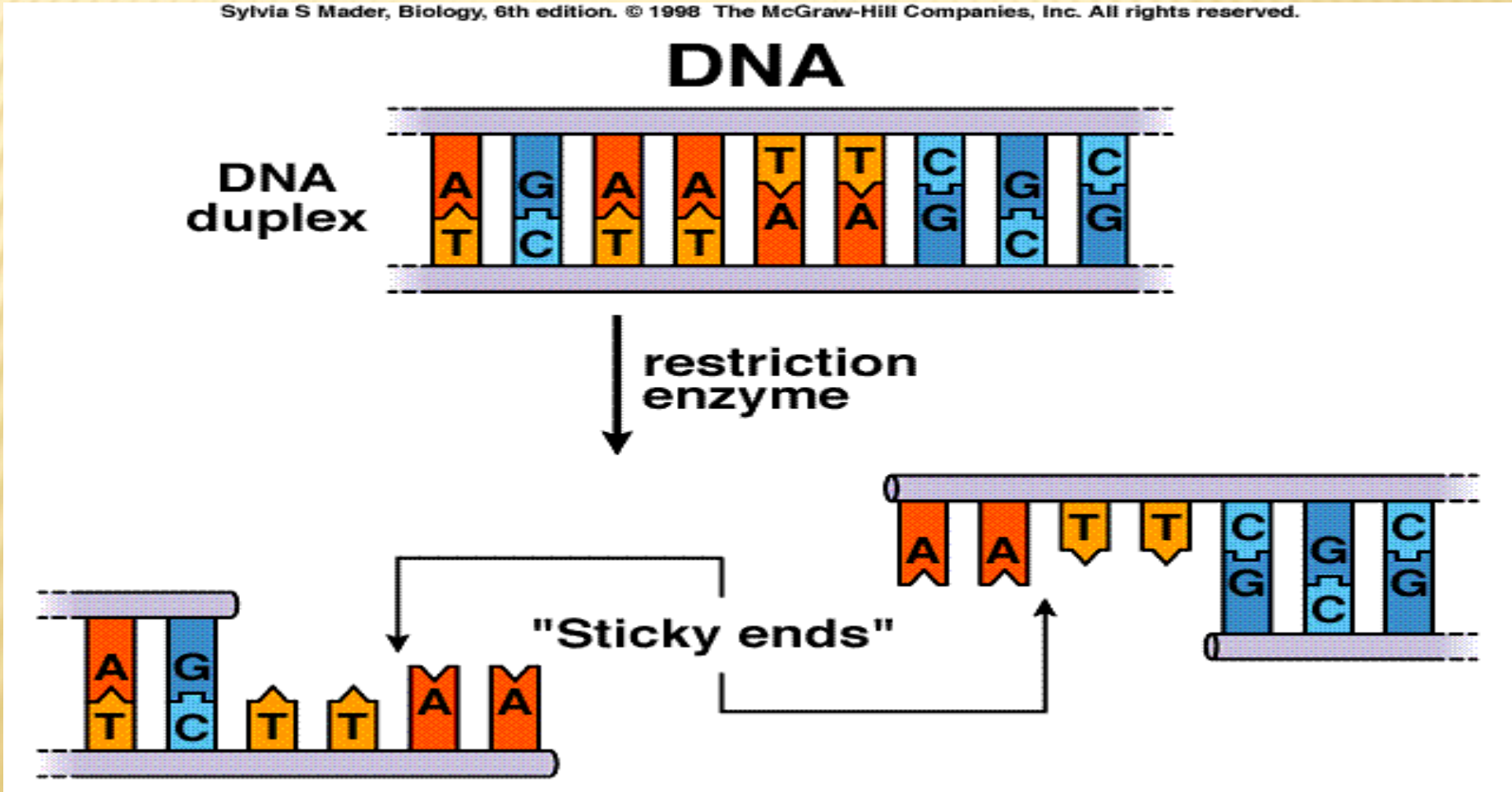
DNA Extraction



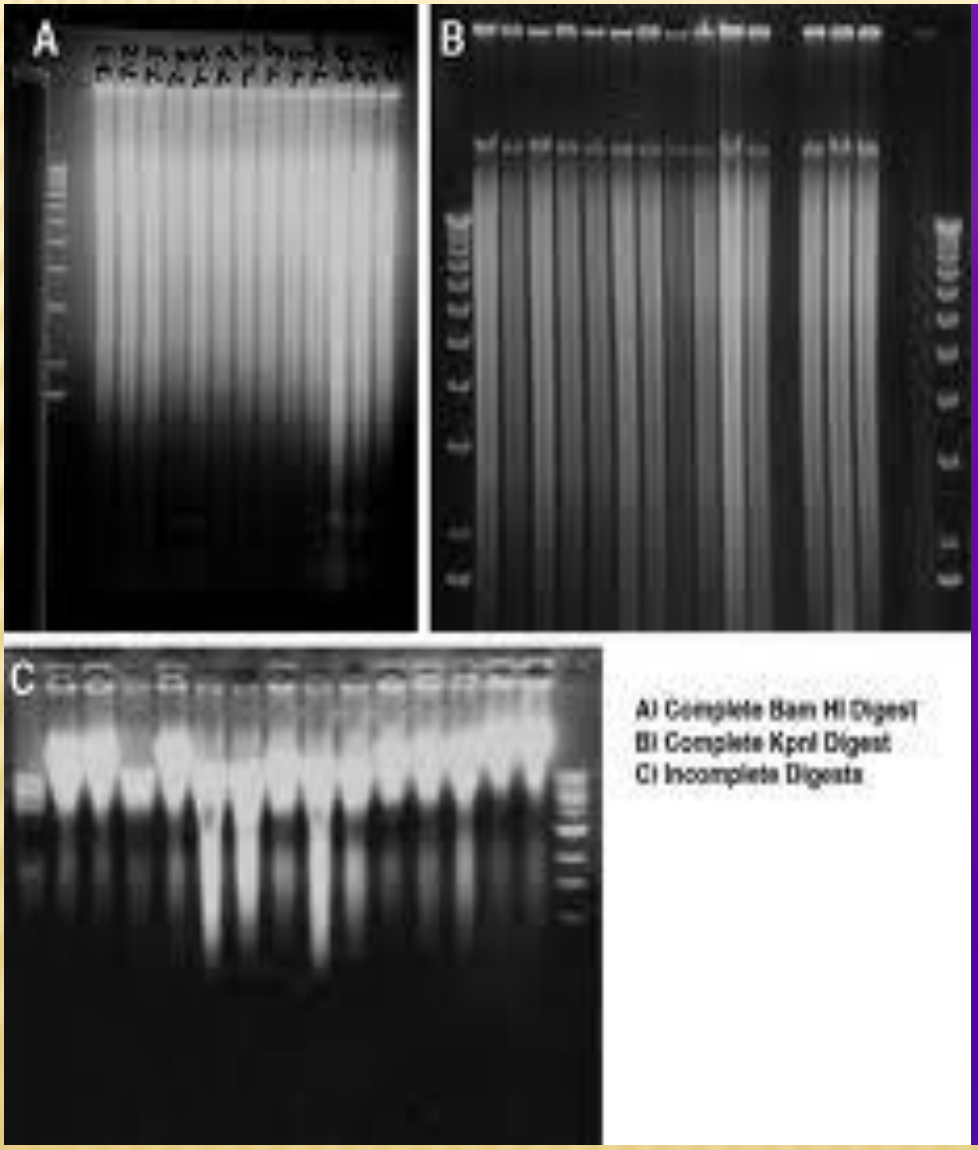
Fragmentation of DNA by restriction enzyme

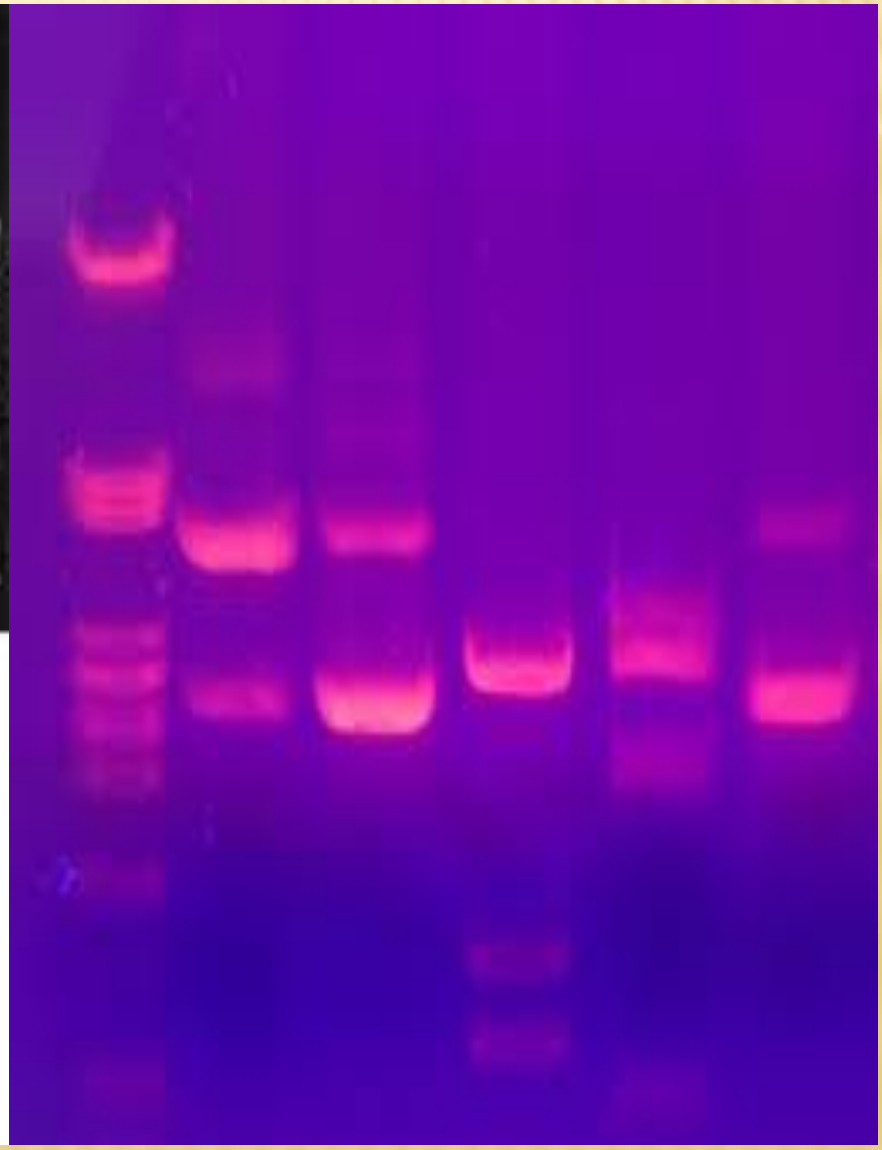
Aims?

- Complex genomes + Simple genomes



Fragmentation of DNA by electrophoresis





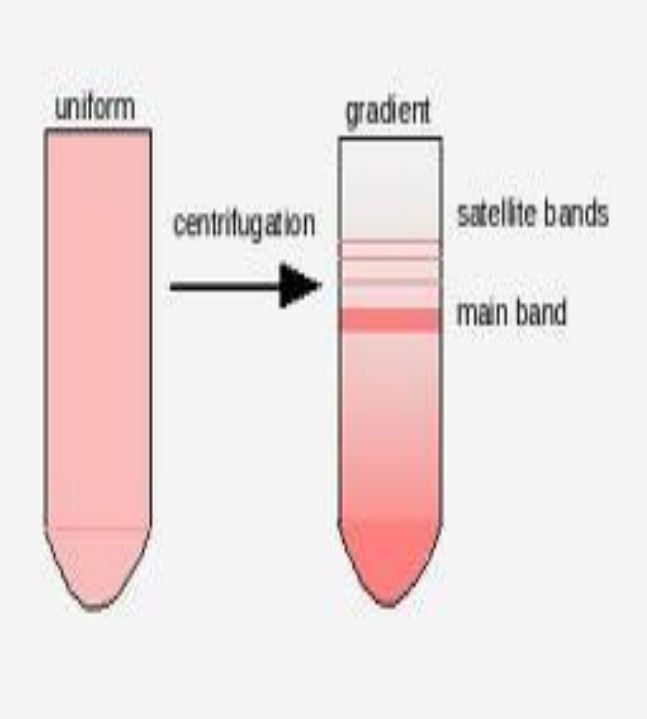
Isolation of specific size DNA fragments

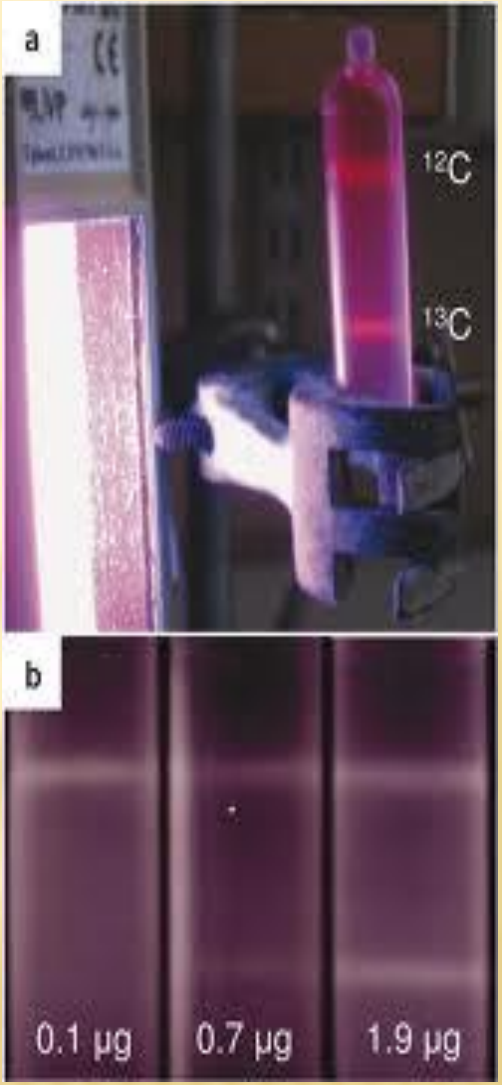
Methods:

1. Ultra centrifugation.
2. Low melting agaros gel electrophoresis

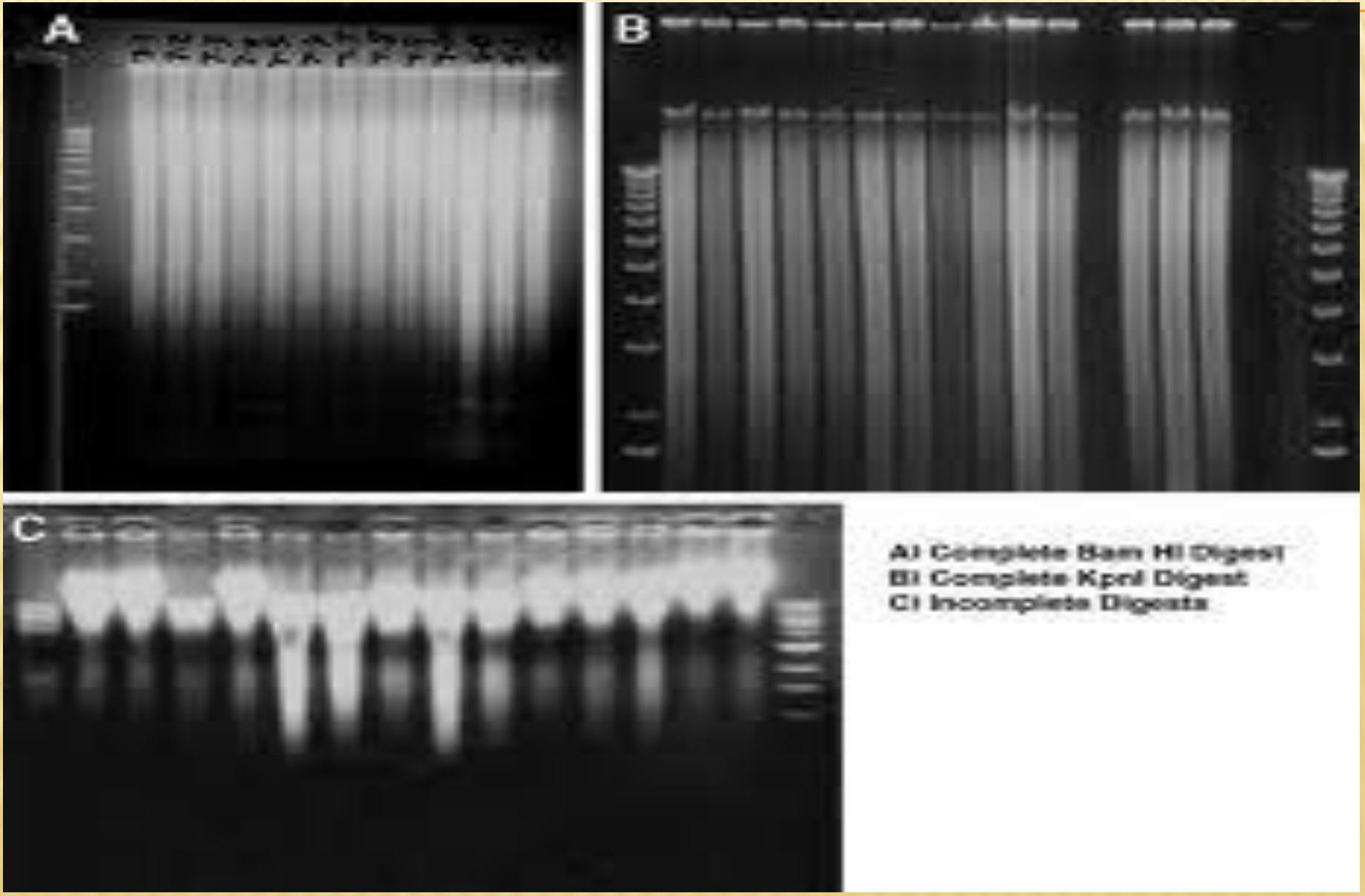
1. Ultra centrifugation

1. Ultra centrifugation

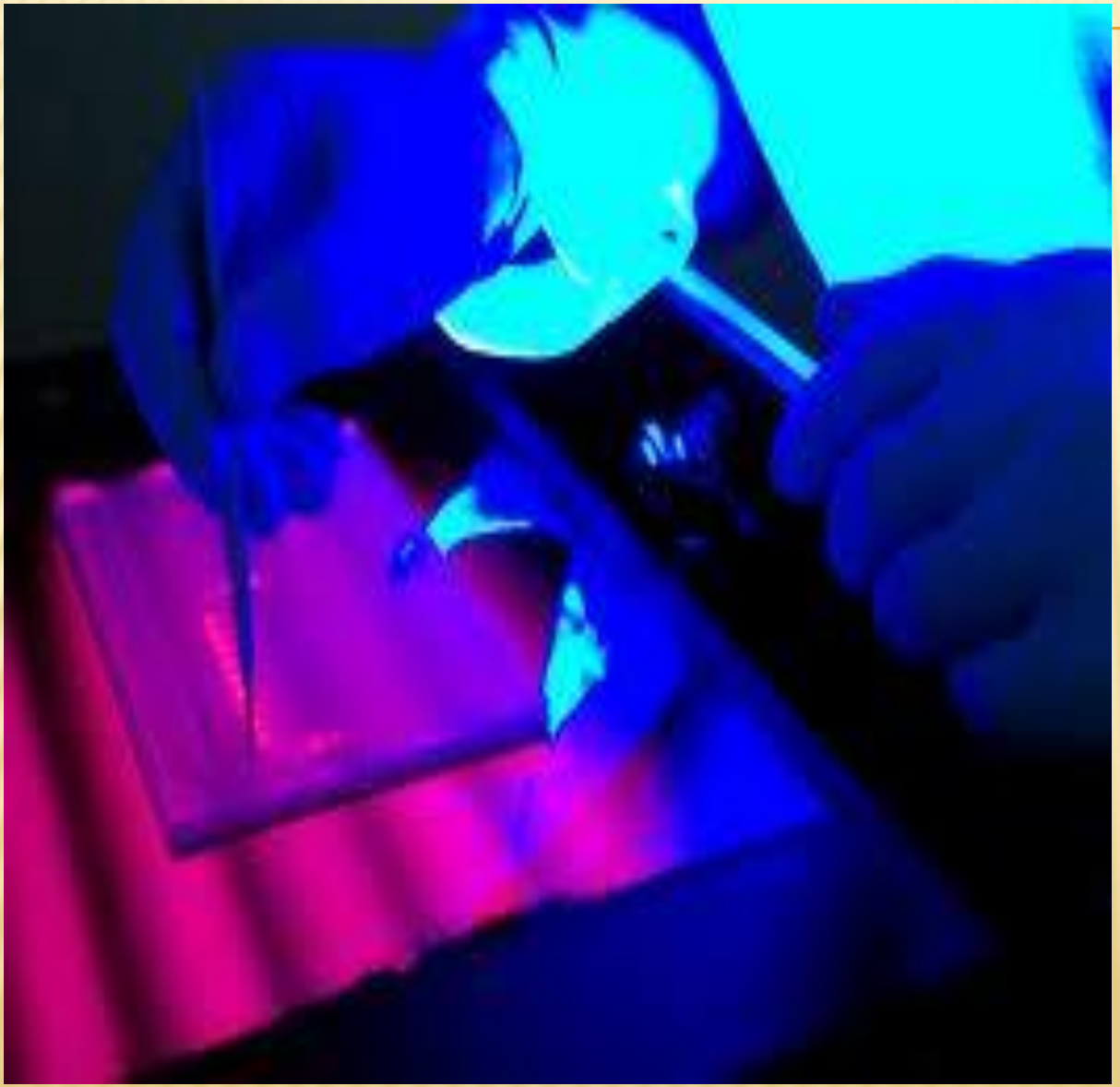


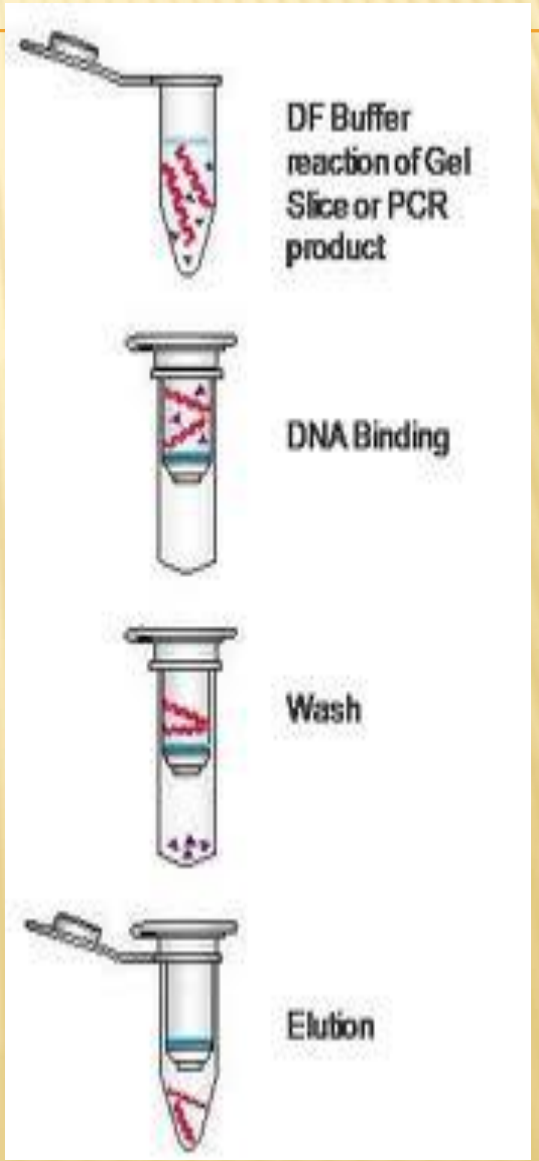


2. Low melting agarose gel electrophoresis

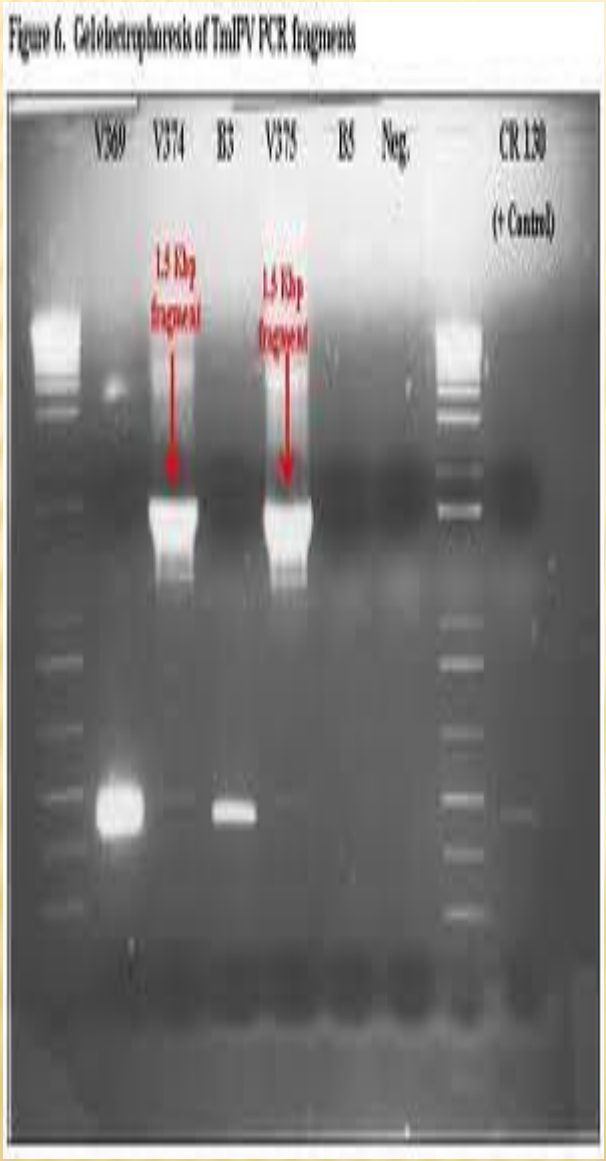


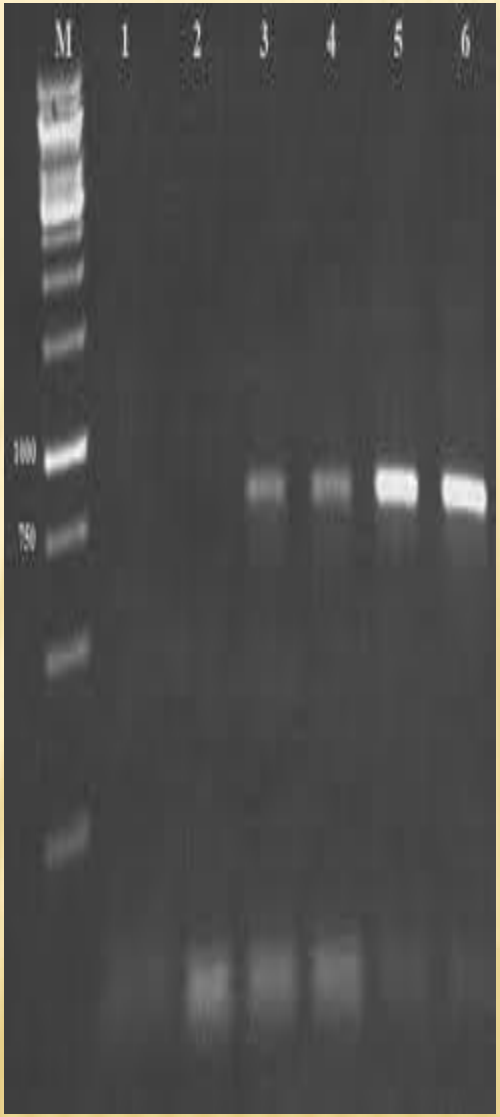
Isolation of specific DNA fragments

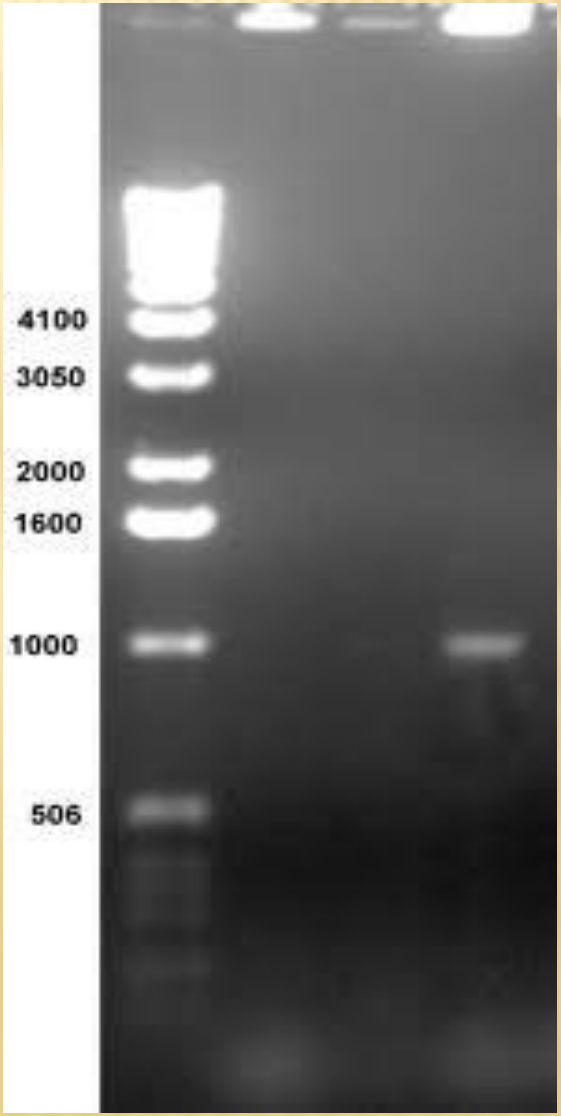




Checking the size of the isolated fragments





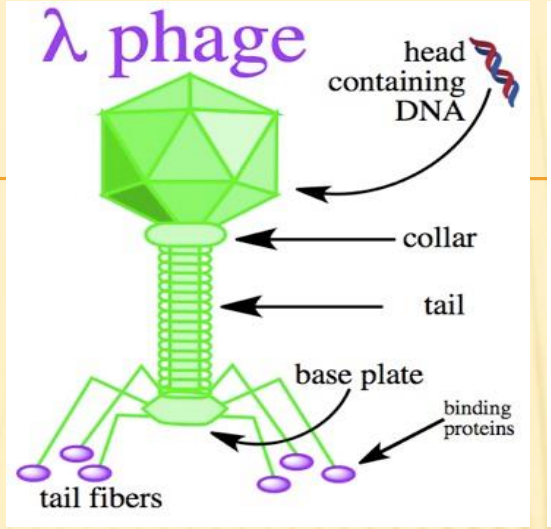


Vector selection for cloning:

1. Stability
2. High copy number
3. Easy to extract
4. Accepted by host
5. With good capacity
6. Easy to identify- with marker

Types of Vectors

- Plasmids
- Phages
- Cosmid
- Viruses
- Artificial Chromosomes



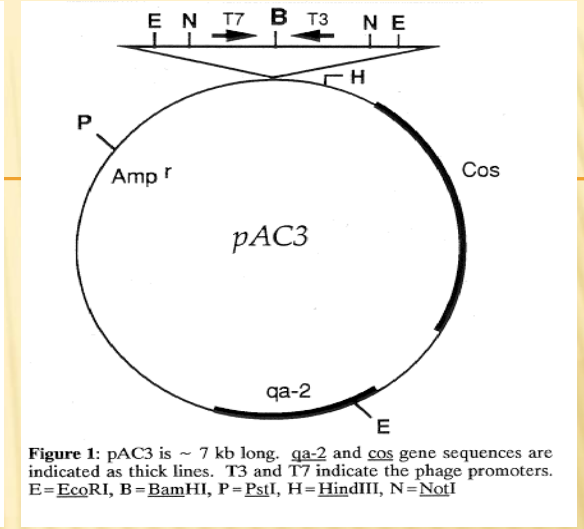
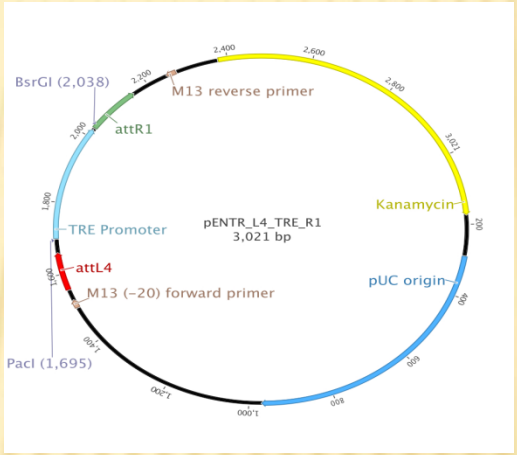
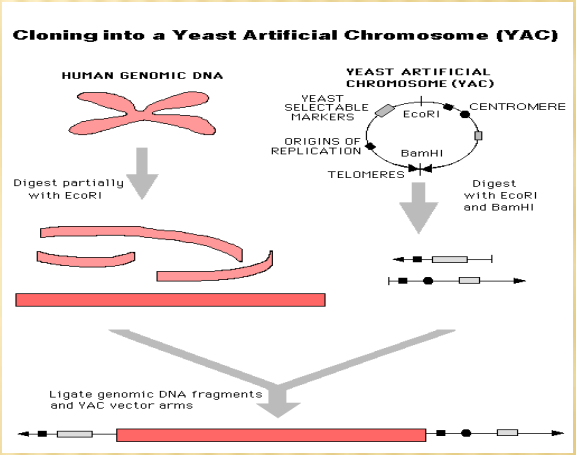


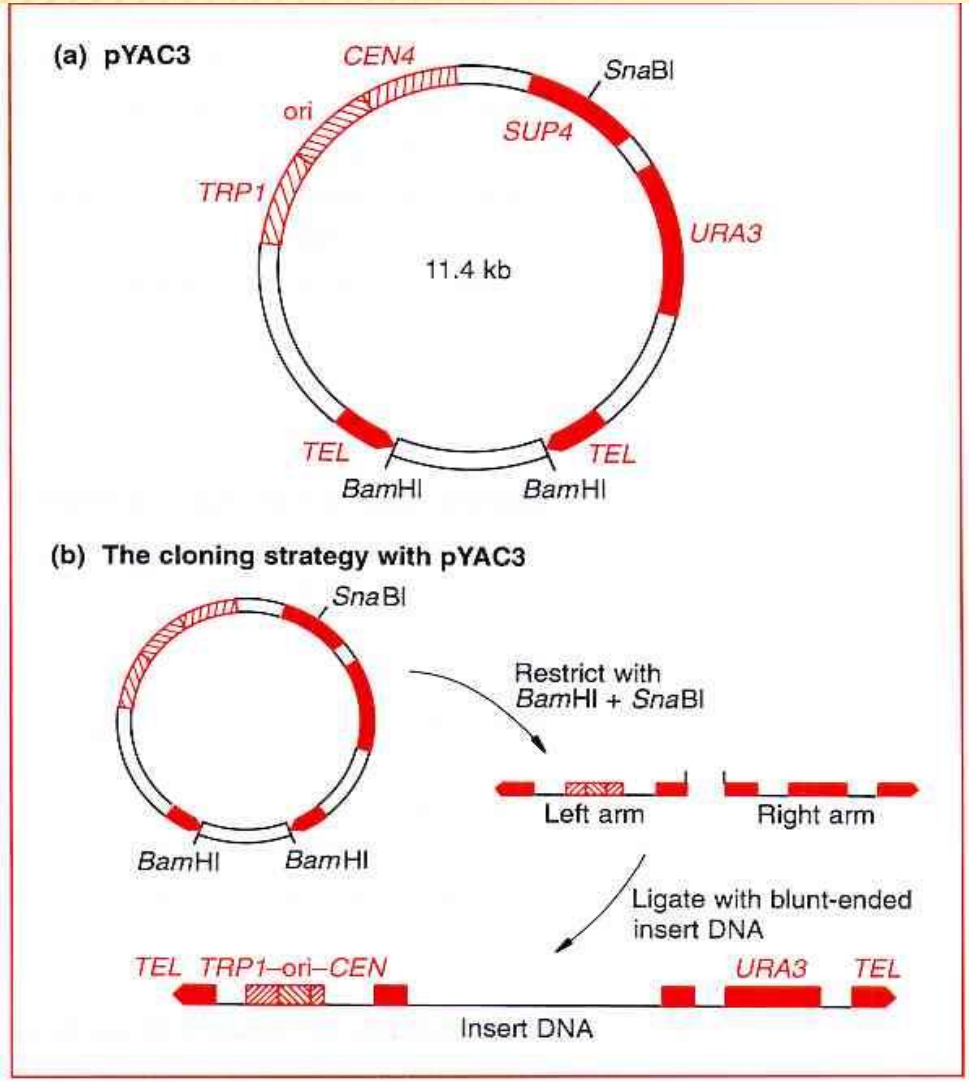
Figure 1: pAC3 is ~ 7 kb long. qa-2 and cos gene sequences are indicated as thick lines. T3 and T7 indicate the phage promoters. E = EcoRI, B = BamHI, P = PstI, H = HindIII, N = NotI

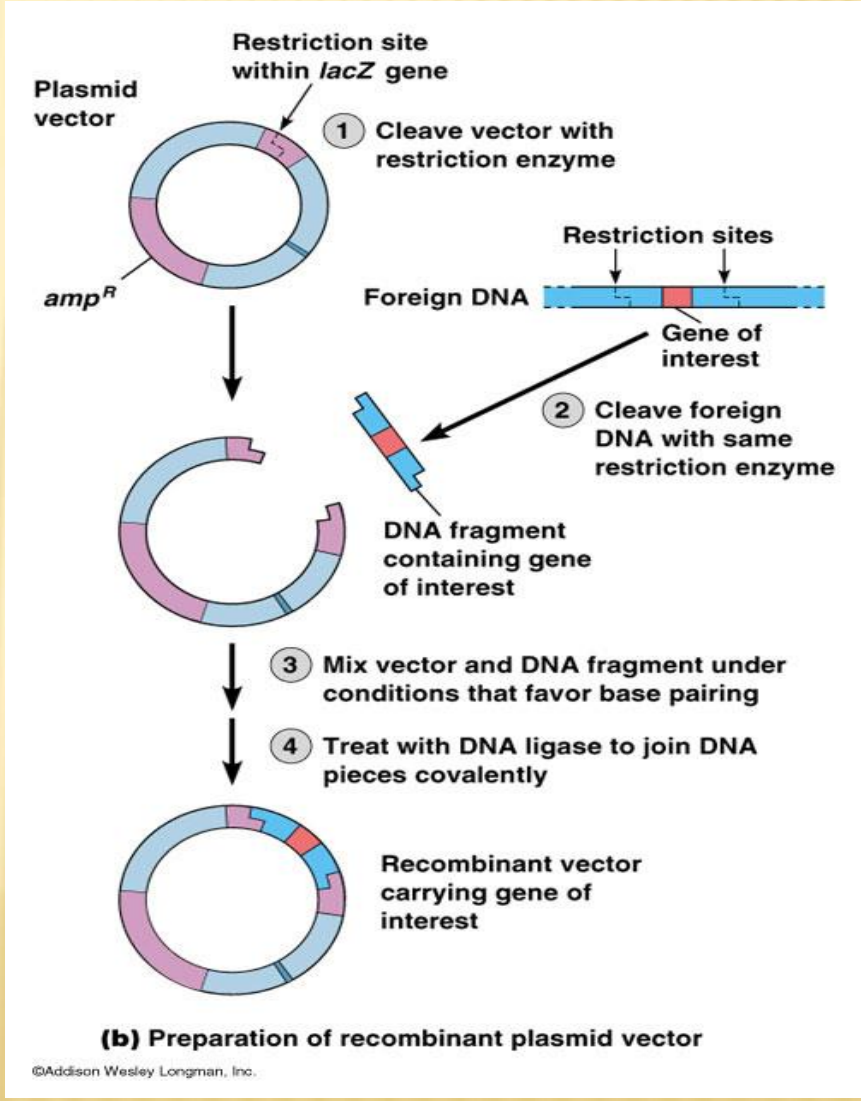




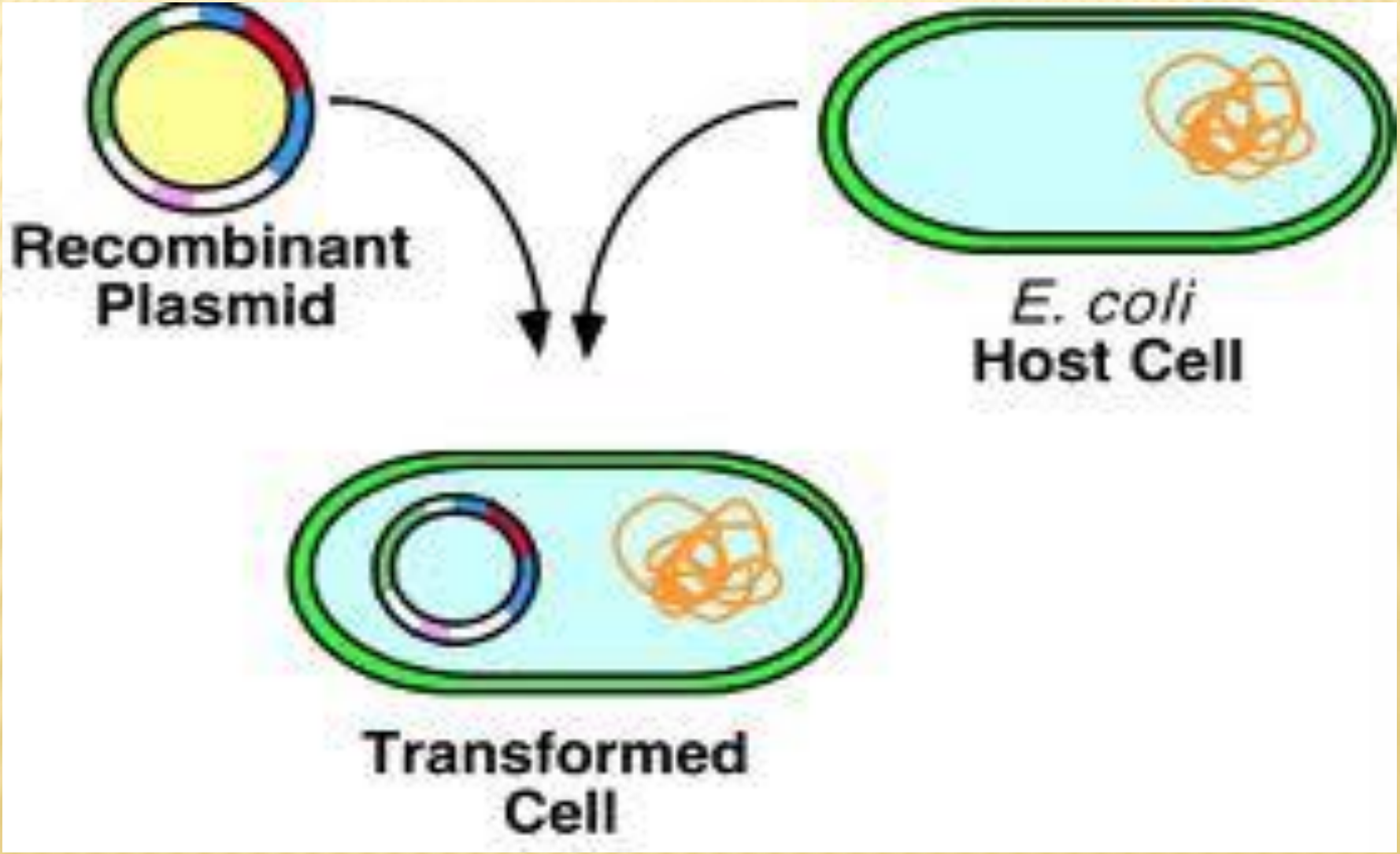
- Opening Vector -

- Ligation -

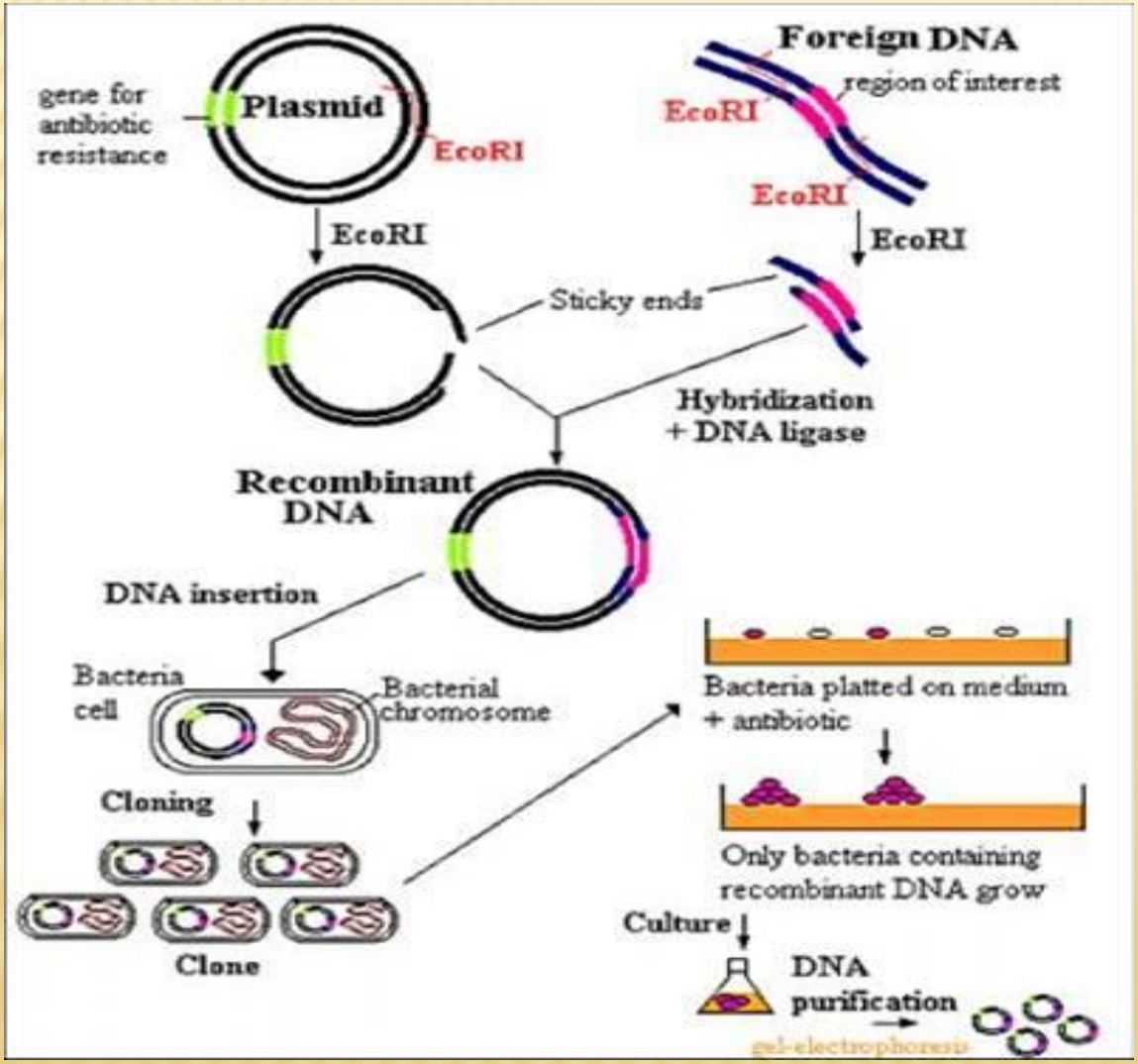




- Transformation



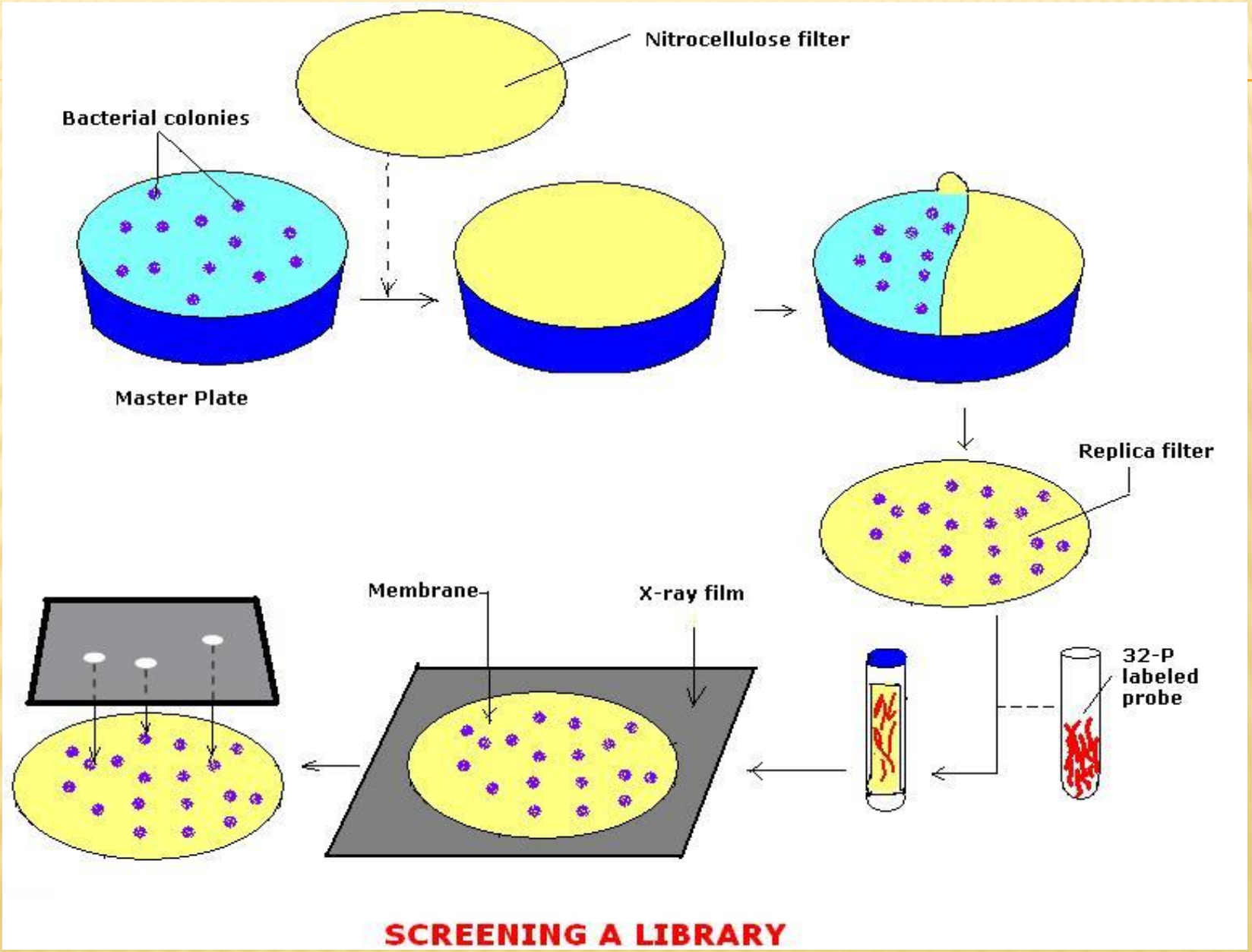
- Calculation of library size and efficiency



Isolation of DNA fragment with specific gene:

USING PROBE, SOUTHERN BLOTTING and HYBRIDIZATION

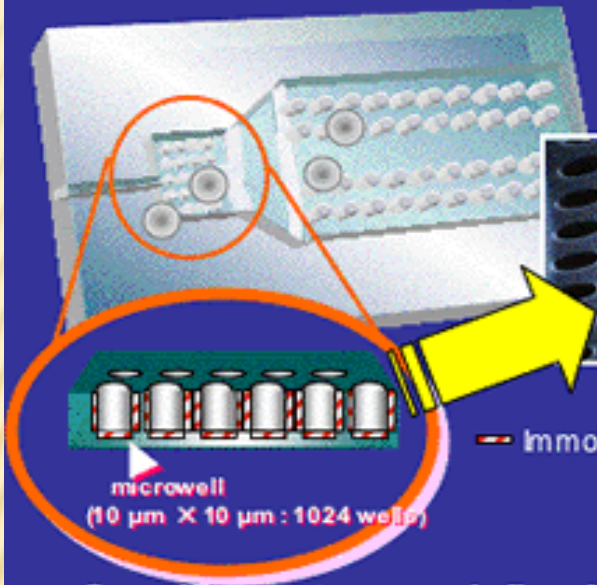
- **Probes are Labeled genes**
  either with:
    - **-- isotopes**
    - **-- fluorescent part**

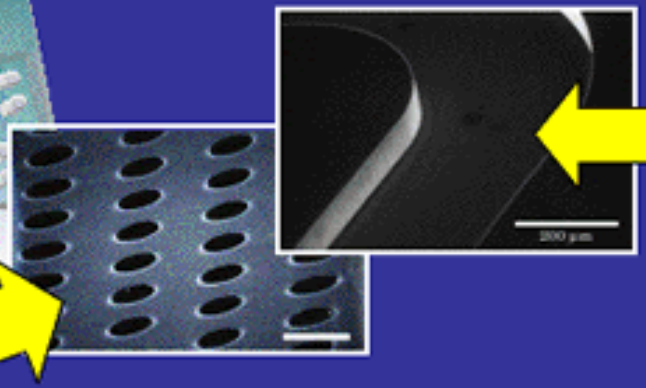


SCREENING A LIBRARY

Further Construction of our Biochip

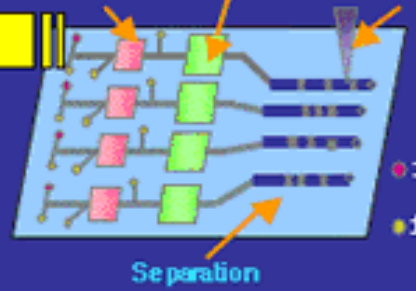
- Detection of pathogen using microchip



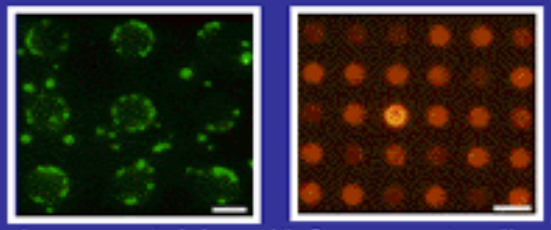


Immobilization of antibody

- Microdevice for analysis of DNA



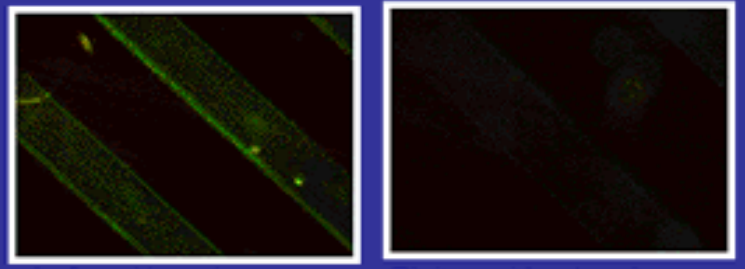
Specific capture on defined area



Immuno staining with fluorescent antibody
Left : pathogenic protozoan captured in microwell
Right : microwell immobilized with antibody

Automated and High Throughput Detection

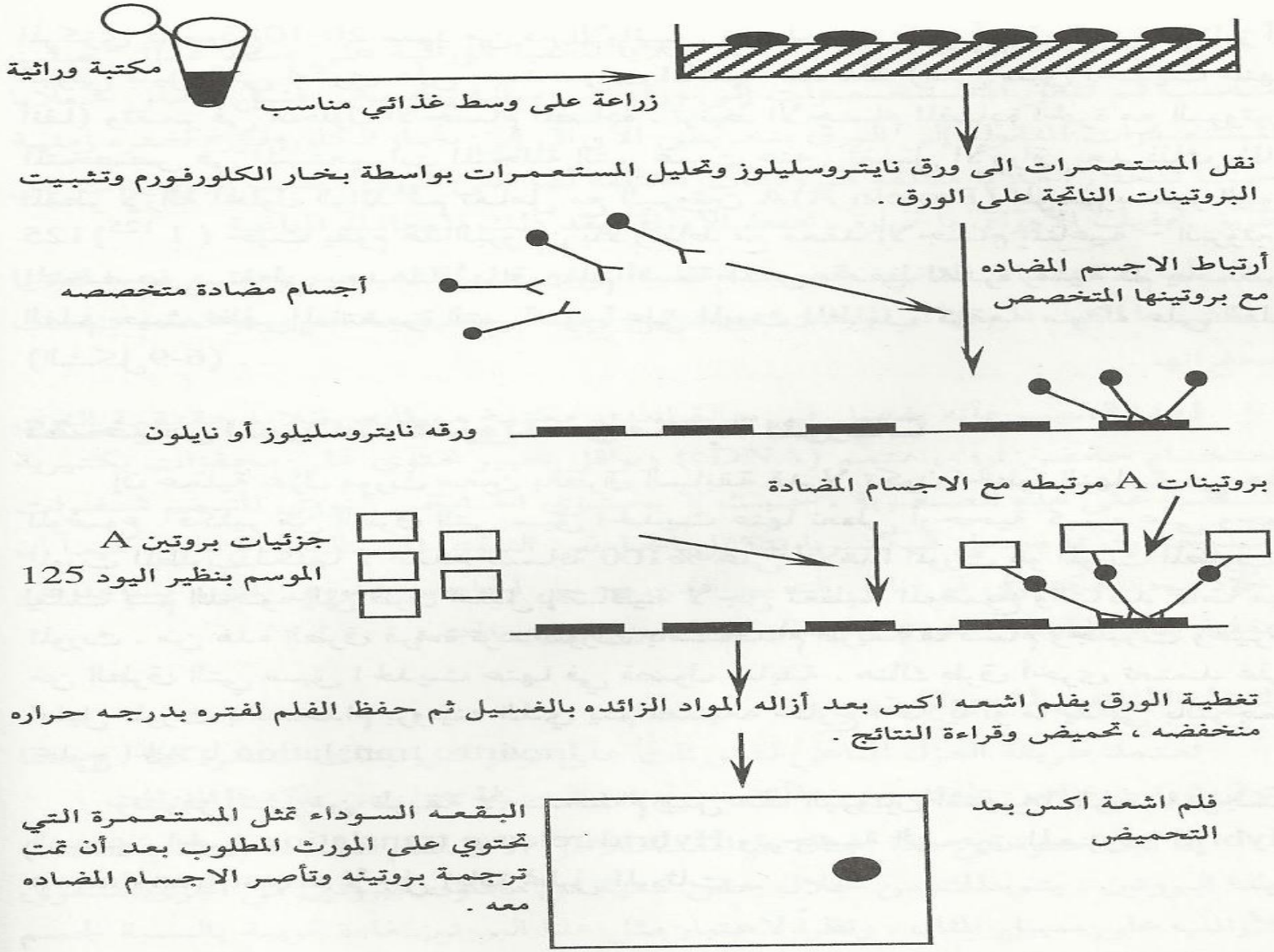
Adsorption of DNA on chip with amino residue



Left : with amino group
Right : without amino group

DNA adsorbed on chip and stained with fluorescent dye

Construction of Simple System with Modification of Surface



(الشكل9-6) : استخدام الأجسام المضادة المتخصصة في تشخيص البروتين المشفر من مورث معين مطلوب عزله من مكتبة مورثات.

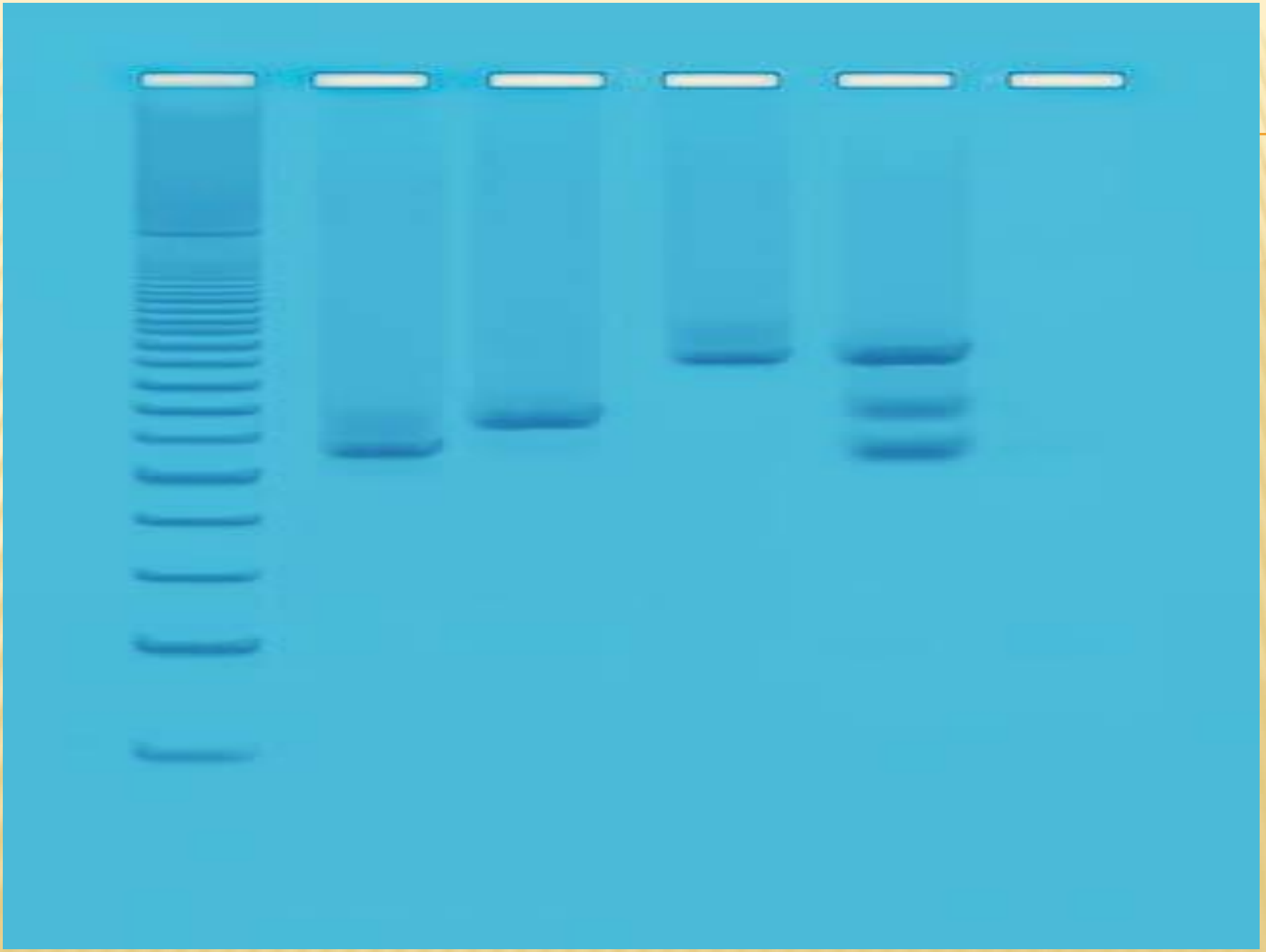
- Isolation of the correct colonies

- Grow the colonies to increase the number of cloned plasmid.

- Extract the cloned plasmids.

- Isolate the DNA fragment from plasmid by RE.

- Use the DNA fragment for further work.



Thank you for listening