DNA LIBRARIES

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Introduction

- DNA libraries are **collections** of cloned DNA fragments.
- Two types of DNA libraries

  ➡ **Genomic Library**

  ➡ **cDNA Library**

Genomic Library

- It represents an entire **genome** of an individual animal, plant, bacteria, virus under study.

- It contains **DNA sequence** representatives of an entire genome in a stable form.

- **10kb** in length------ **Prokaryotic** libraries.

- **40kb** in length------**Eukaryotic** libraries.

Construction of genomic library

It involves in the following steps:

i. Isolation of chromosomal DNA of interest.
ii. Cutting the fragments into suitable size.
iii. Cloning the fragments in the suitable vector.
iv. Screening, identification and characterization of clones.
v. Maintenance of set of clones.

❖Isolation of chromosomal DNA

- Wash the cells with tris buffer saline of pH 7.4
- Centrifuge and collect the pellet.
- TE+EDTA+Proteinase K +Sarcosyl is added to the pellet.
- Lysed cells are kept at 50 C for 3 hours.
- Phenol extraction.
- Collect aqueous extract. Dialyze it against 50mM tris-Cl.
- Treat the sample with Rnase.
- Extraction with phenol-chloroform.
- Dialyze with TE.

❖ Cutting the fragments into suitable size

- Fragments can be generated by two methods:

Mechanical Shearing

By using Restriction Enzymes

Mechanical Shearing

- Depends on the **size** of the vector.
- The ends are mostly **blunt** due to breakage across DNA strands.
- They can be repaired and made blunt if necessary with **klenow polymerase**.
- Technical difficulties.
- No exclusion caused.
- **Chromosome walking** is possible.
- Random shearing may result in **infinite fragments.**

Using Restriction Enzymes

- To produce **proper size** of fragments.

- **Sau 3A** is a commonly restriction enzyme used to give **sticky ends** which are compatible with a vector that has been cut with Bam H1.

- **Time of treatment** and **amount of enzyme** is used is very critical to get fragments of appropriate length.

Using Restriction Enzymes

- The correct size fragments are then purified by **agarose gel** or a **sucrose gradient**.

- It becomes **easier** to insert into vector.

- Drawback is, sequence of interest having restriction site which will cause more fragmentation.

❖Cloning the fragments into vector

Lambda Vectors	Cosmid Vectors
• Insert size is 20-25kb • More clones are required for library. • Easier to make. • Simpler to screening and more efficient too. • Plaque hybridization is used.	• Insert size is 45-50kb • Less clones are required. • Difficult to make. • More difficult to screening and less efficient. • Colony hybridization is used.

❖Screening, identification and characterization

There are three method can be used for screening

i. Hybridization with probe followed by detection

ii. Immunological screening of protein product

iii. Screening of protein activity

❖Screening, identification and characterization

- **Hybridization technique:**

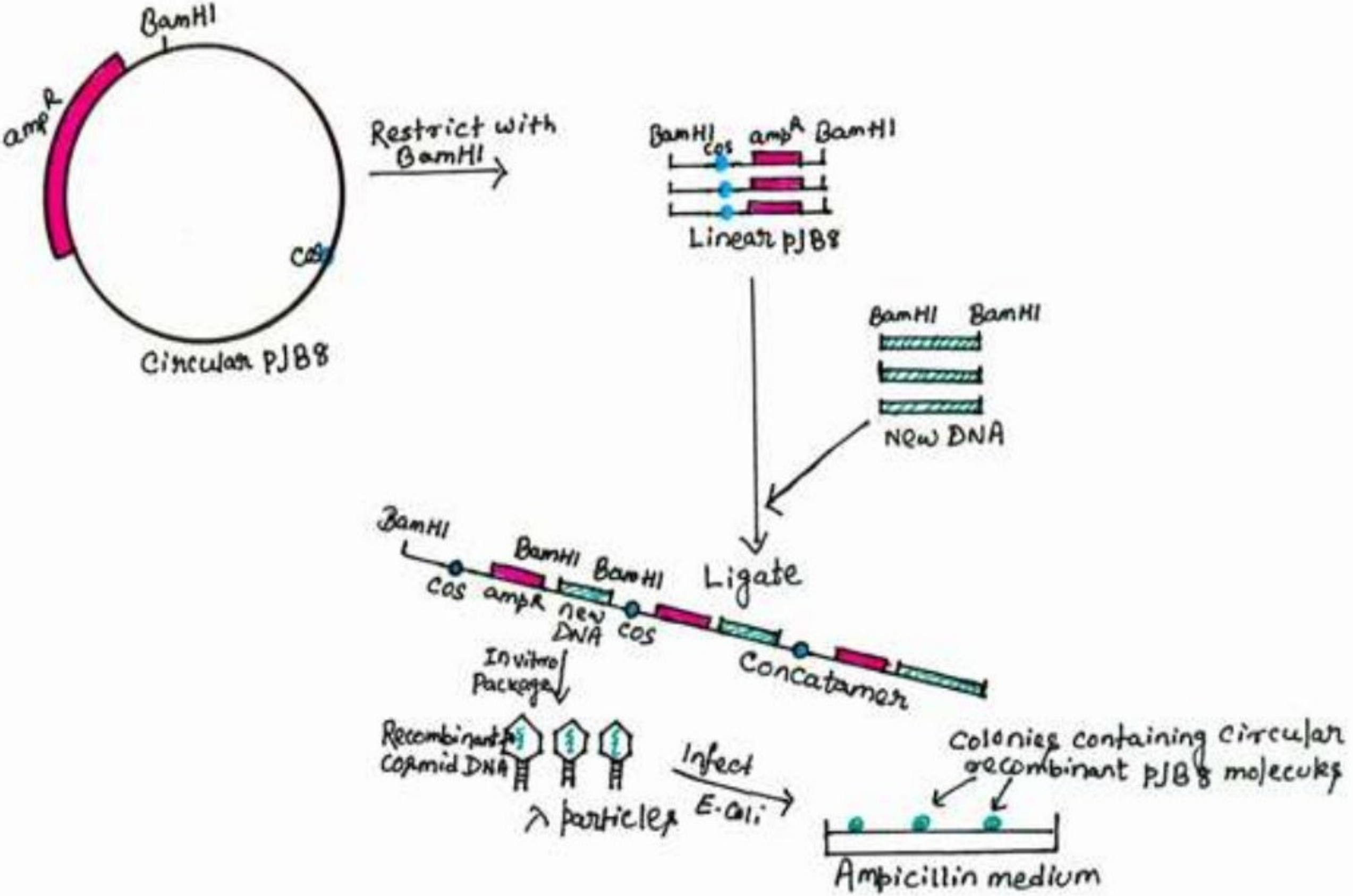
  Colonies of transformed cells can be screened for particular DNA with radioactive or nonradioactive **labelled probes**.

- **Immunological Screening:**

  Primary antibody is added to the colonies and then the enzyme labelled antibody is added. Conversion of colourless to coloured products. The **coloured colony** indicates that the presence of DNA.

- **Screening of protein activity:**

  A plate assay can be detect the clone processing the particular **protein activity**. It can be screened by transferring on minimal media.


BamHI
ampR
cos
Circular pJB8
Restrict with BamHI
BamHI cos ampR BamHI
Linear pJB8
BamHI BamHI
New DNA
Ligate
BamHI BamHI BamHI
cos ampR new DNA cos
Concatamer
In vitro Package
Recombinant cosmid DNA
λ particles
Infect E. coli
Colonies containing circular recombinant pJB8 molecules
Ampicillin medium

cDNA Library

- It represents the compete **mRNA** complement of a single type of cell.

- cDNA libraries are useful for the study of **tissue specific gene expression**.

- cDNA library is a **collection of cDNA's**, prepared from mRNA of a particular cell and these cDNAs are then cloned and maintaining in either plasmid or phage.

cDNA Library

- Generally cDNA library are not made using **prokaryotic mRNA**, because it is very **unstable**.

- cDNA library is mostly made by **eukaryotic mRNA** because it is **easy** to done cDNA and express the encoded protein in E.coli.

❖Construction of cDNA library

It involves in the following steps:

i. Synthesis of cDNA from eukaryotic functional mRNA.
ii. Cloning cDNA population in suitable vectors.
iii. Screening of cDNA library.
iv. Maintenance of clones.

❖Synthesis of cDNA from mRNA

- Purified mRNA are taken along with the enzyme **reverse transcriptase** and four deoxyribonucleotides are in the reaction mixture.

- Reverse transcriptase cause formation of **complementary DNA strand** with hairpin loop at end.

- Second DNA strand is synthesized by **klenow fragment** of E.coli DNA polymerase which uses first DNA strand as template..

❖Synthesis of cDNA from mRNA

- At the end of second strand synthesis, treatment with **T4 DNA polymerase** ensures that linkers can be blunt end ligated to the cDNA.
- After the reaction is complete, the sample is treated with enzyme **Rnase** which degrades mRNA molecule. **S1 nuclease** is used to open hairpin ends.

❖ Cloning the fragments into vector

- cDNA can be cloned by **blunt end ligation** into a **plasmid** vector to form library (0.5-10kb size cDNA).

- Alternatively it can be cloned into **phage** (Greater number of clones).

- Packaging of cDNA bearing phage or transformation of E.coli with plasmids bearing to form library.

❖Screening, identification and characterization

- Procedures are similar to the genomic library.

- cDNA library can be screened either by **hybridization** or **immunological assay** to identify the clones.