A study material for M.Sc. Biochemistry (Semester: III) Students
on the topic (CC-13; Unit I)

Genomic DNA Library

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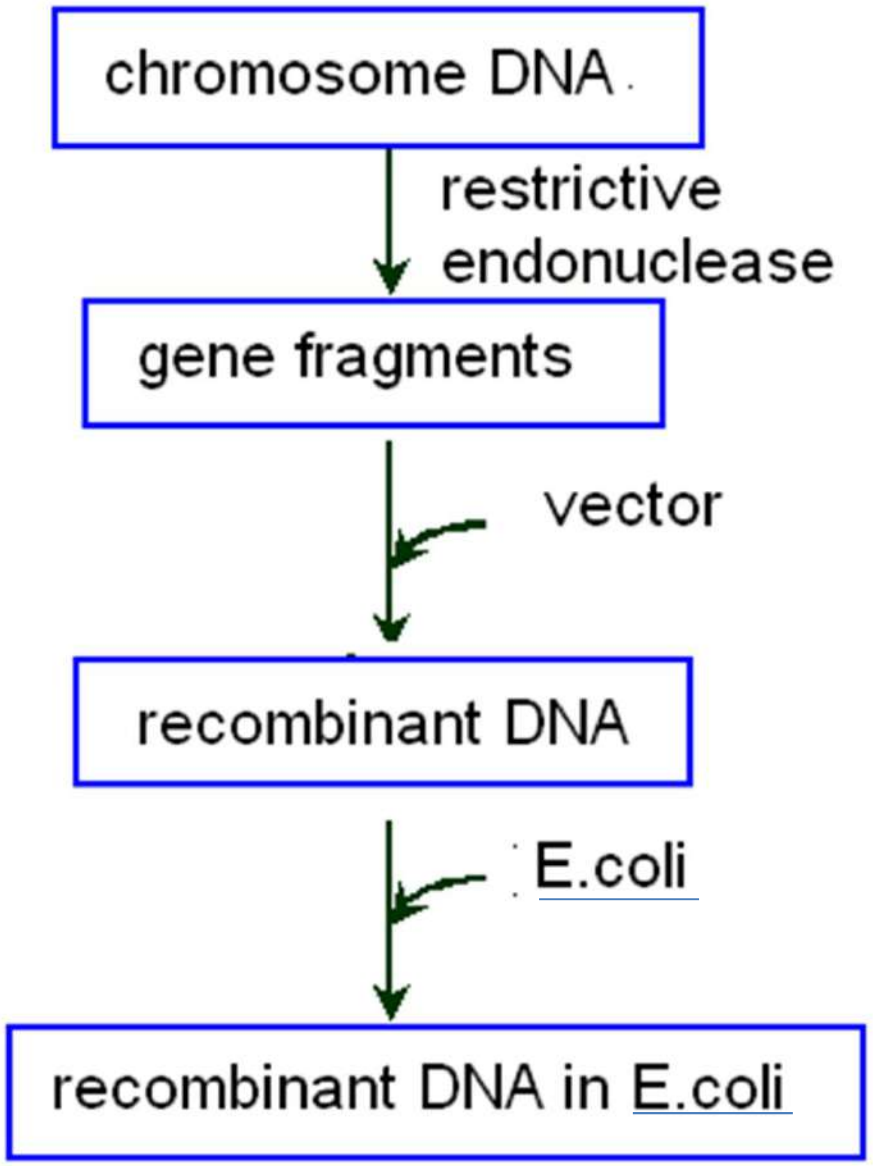
The genomic DNA library is a collection of the comprehensive DNA fragments representing the entire genome of a species.

Cloning of entire genome is called Shotgun experiment.



Genomic DNA libraries

1. **Purify genomic DNA: prokaryotes or eukaryotes;**
2. **Fragment this DNA : physical shearing and restriction enzyme digestion;**
3. **Clone the fragments into vectors;**
4. **Transfer the constructed vectors into recipient cells;**
5. **Culture and amplify the clones;**
6. **Screen the clone of target.**

To make a representative genomic libraries , genomic DNA must be purified and then broken randomly into fragments that are correct in size for cloning into the chosen vector.

Purification of genomic DNA :

Eukaryotes :**prepare** **cell nuclei**

remove protein, lipids and other unwanted macro-molecules by protease digestion and phase extraction.

Prokaryotes :**extracted DNA directly from cells**

Break DNA into fragments randomly:

Physical shearing :

pipeting, mixing or sonicaion

Restriction enzyme digestion:

partial digestion is preferred to get a greater lengths of DNA fragments.

Selection of restriction enzyme

1. **Ends produced (sticky or blunt) &**
   The cleaved ends of the vector to be cloned

***Sma*I:** 5'-CCC/GGG-3', Blunt cutter

*Bam*HI: 5'-G/GATCC 3', sticky end cutter

2. **Let's assume *Bam*HI RE is used to generate fragment of Human Genomic DNA (2.8×10^6 Kb). The average length of fragment is generated by this RE is 4^6=4096 bp**
3. **In other word we have to generate large no. of recombinants, which together contain a complete collection of all (or nearly all) of the DNA sequences in the entire human genome. Such collection from which we draw the desired clone is called Gene library or gene bank.**

No. of Clones

- **How many clones are required that represents the whole genome library?**
- **This can be calculated by using Carbon and Clarke (1976) formulae**
- **N=ln(1-P)/ln(1-a/b), where N= no. of clones require to generate the whole gene library, P= Probability of including gene sequence in a random genomic DNA library, and a= average size of fragment cloned in the vector and b= total size of the genome.**

Table 6.1

Number of clones needed for genomic libraries of a variety of organisms.

SPECIES	GENOME SIZE (bp)	NUMBER OF CLONES*	
		17 kb FRAGMENTS†	35 kb FRAGMENTS‡
E. coli	4.6×10^6	820	410
Saccharomyces cerevisiae	1.8×10^7	3225	1500
Drosophila melanogaster	1.2×10^8	21,500	10,000
Rice	5.7×10^8	100,000	49,000
Human	3.2×10^9	564,000	274,000
Frog	2.3×10^{10}	4,053,000	1,969,000

*Calculated for a probability (p) of 95% that any particular gene will be present in the library.

†Fragments suitable for a replacement vector such as λEMBL4.

‡Fragments suitable for a cosmid.

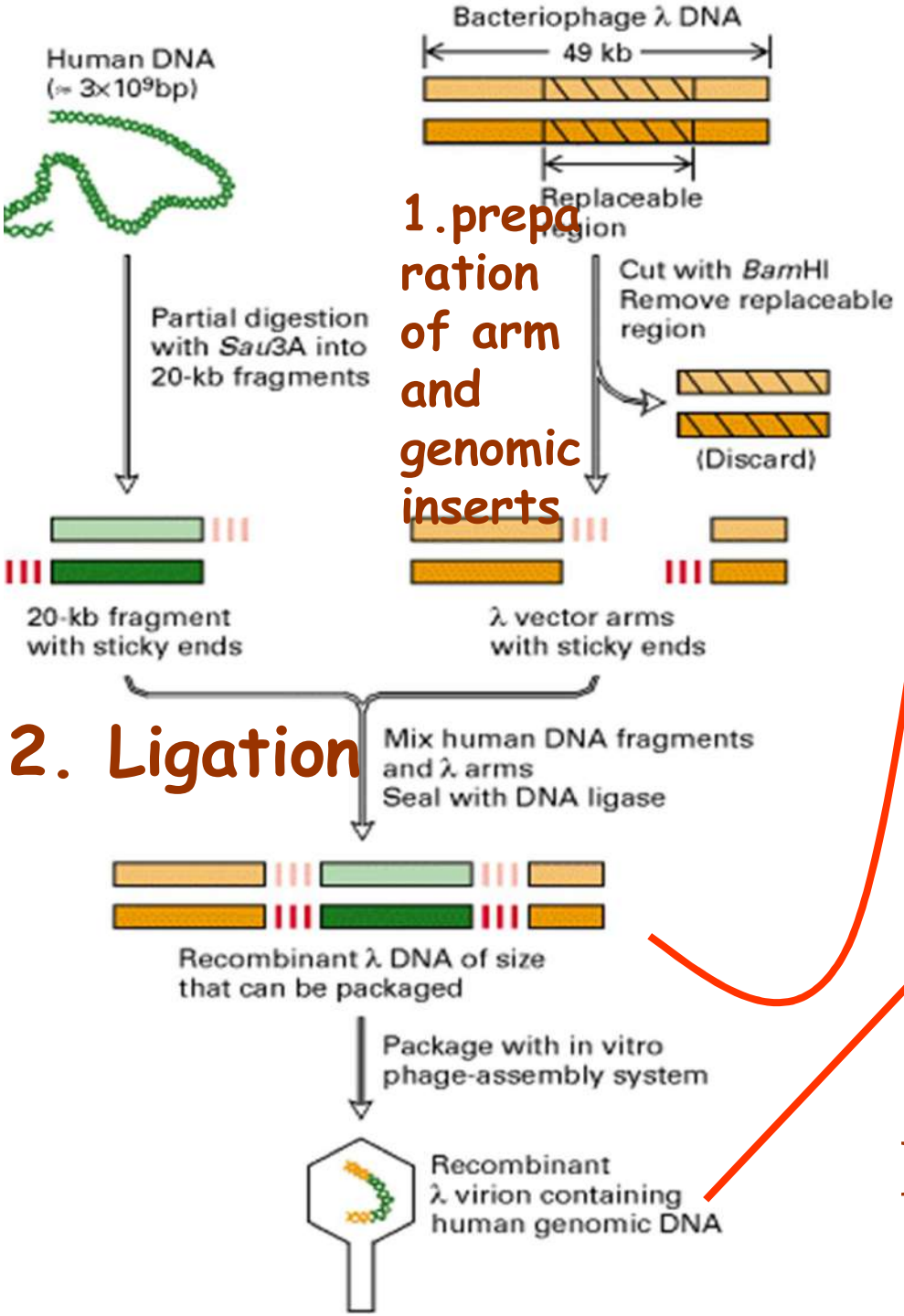
Vectors

According to genome's size,we can select a proper vector to construct a library .

Vectors	Plasmid	λ phage	cosmid	YAC
insert (kb)	10	23	45	1000

The most commonly chosen genomic cloning vectors are λ replacement vectors which must be digested with restriction enzymes produce the two λ end fragment or λ arms between which the genomic DNA will be Incorporated.

λ replacement vector cloning



3. Packing with a mixture of the phage coat proteins and phage DNA-processing enzymes

4. Infection and formation of plaques

Library constructed

Maniatis strategy for producing a representative gene library

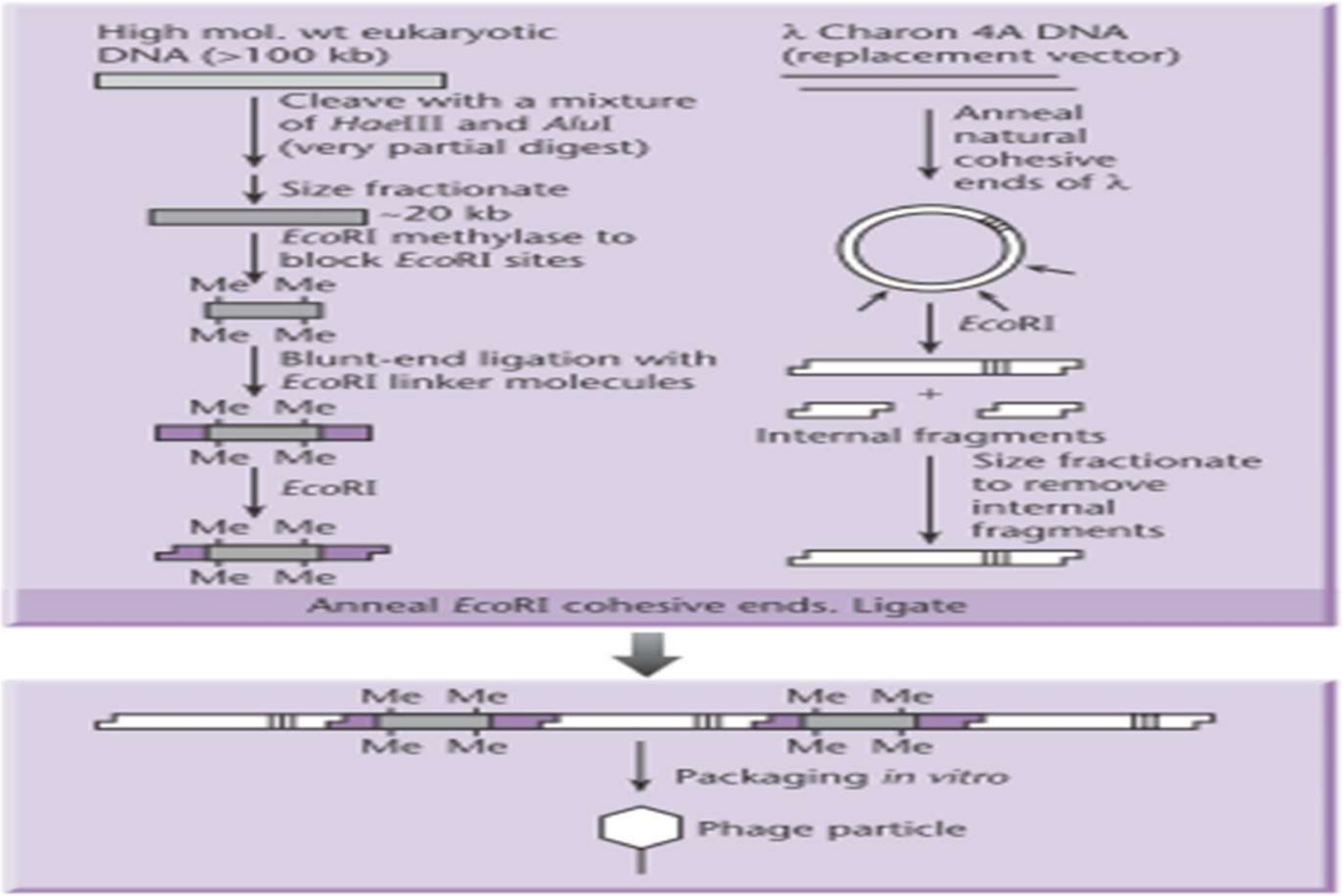


Fig. 6.2 Maniatis' strategy for producing a representative gene library.

Acknowledgement and Suggested Readings:

1. Gene Cloning and DNA Analysis: An Introduction; Sixth Edition ; T. A. Brown; Wiley – Blackwell Publications
2. Principles of Gene Manipulation; Sixth Edition; Sandy B Primrose, Richard M Twyman and Robert W. Old; Wiley – Blackwell Publications
3. Biotechnology: Applying the Genetic Revolution; David P. Clark and Nanette J. Pazdernik; Academic Press (Elsevier)

Thanks