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

Bacteriophage as Cloning Vector

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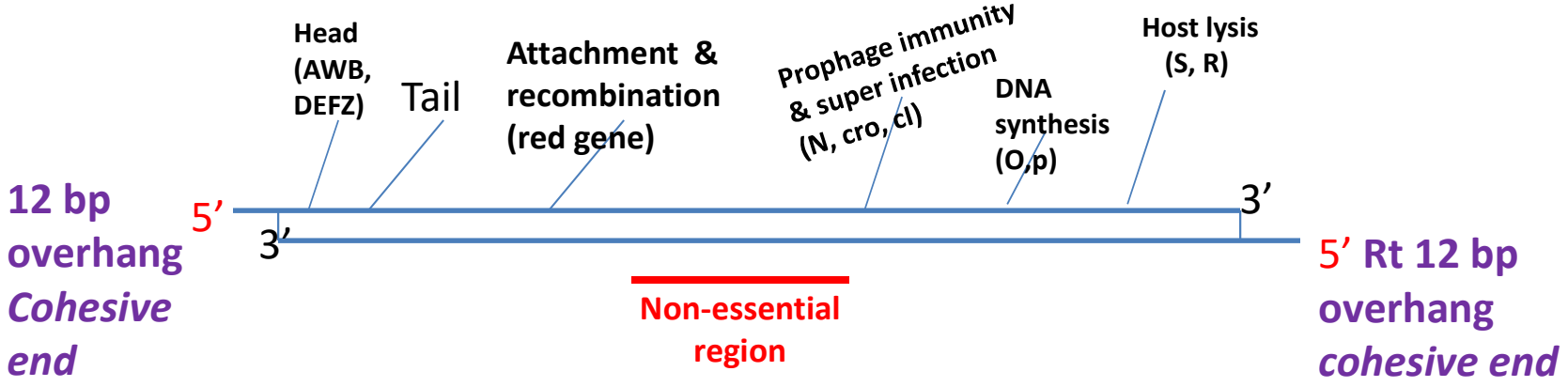
Bacteriophage

- Genetically complex
- Extensive studied virus of *E.coli*
- Developed as a vector
- Genome of lambda phage is linear duplex of about 48.5 kbp
- Sequencing of lambda phage genome was done by sanger (1982)

Simple map of lambda phage genome



- 48.5 kbp
- It has 5' 12 bp over hang complementary to each other
- 12 bp over hang is called cos site
- Cos site stick to each other when phage infect the *E.coli* to form circular structure
- Lt side genes: head, tail gene
- Central region genes are for recombination and attachment, responsible for prophage formation
- Much of this central region is Non-essential region
- This region is not essential for growth of the phage

- This non-essential region can be deleted or replaced without seriously impairing the infection growth cycle.
- Rt hand side gene are for prophage immunity to super infection (N,cro, cI), DNA synthesis (O,P) and host lysis (S, R)

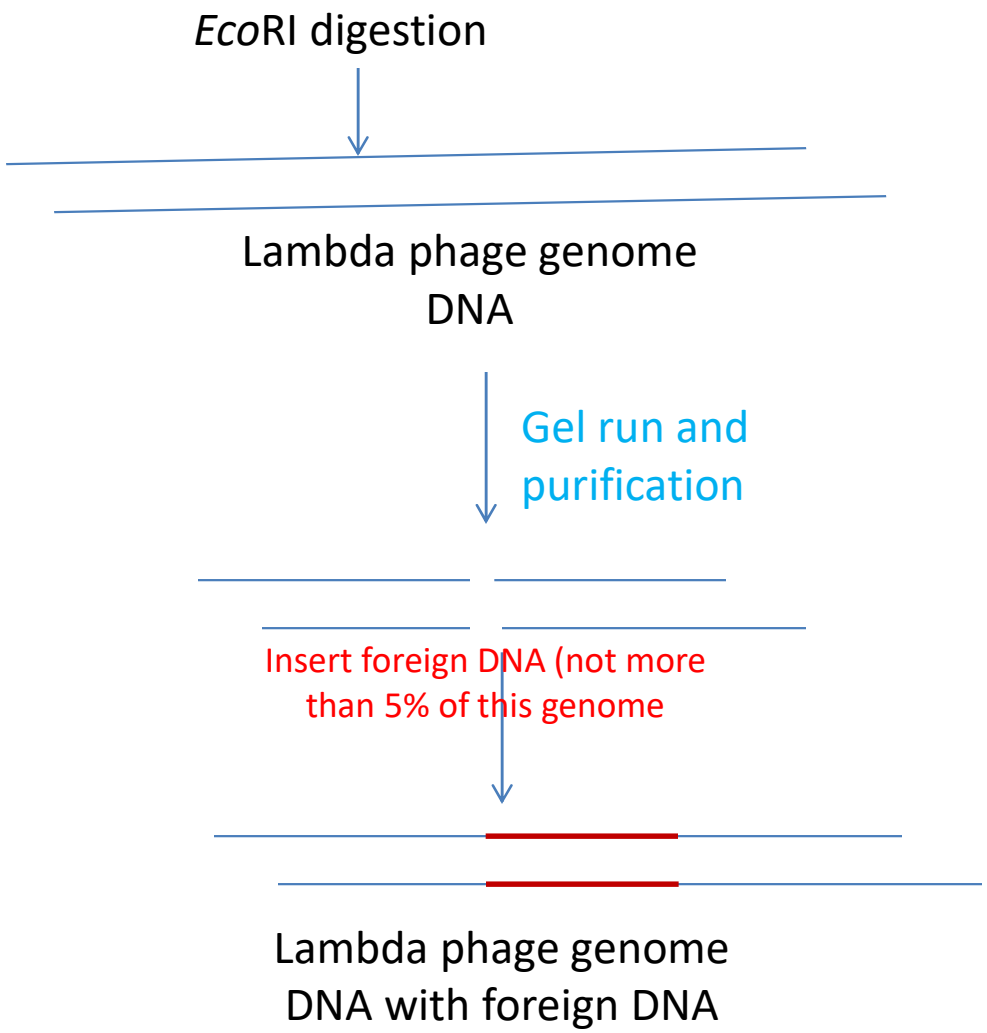
λΦ as a cloning vector

- Wild type λΦ genome contains sites for several RE
- So, it needs to be modified to develop as a vector
- One way is to cut out non-essential region using RE
- Take care not to delete more than 25% of total genome other wise phage will unable to packaged into phage particle

- Delete the non essential region (not more than 25%) this will give small plaque
- Add your desired gene (5%+25%): this will give normal plaque
- This provides advantage to positive selection for recombinant phage carrying foreign DNA
- Improved lambda phage DNA is made in laboratory means lambda DNA contains limited no. of Restriction sites

Types

- **Insertional**: Lambda phage DNA that has been cut with single RE in the centre of genome so, that two arms are generated between which foreign DNA inserted.
- Then this will go for *in vitro* packaging
- ex charon 16A

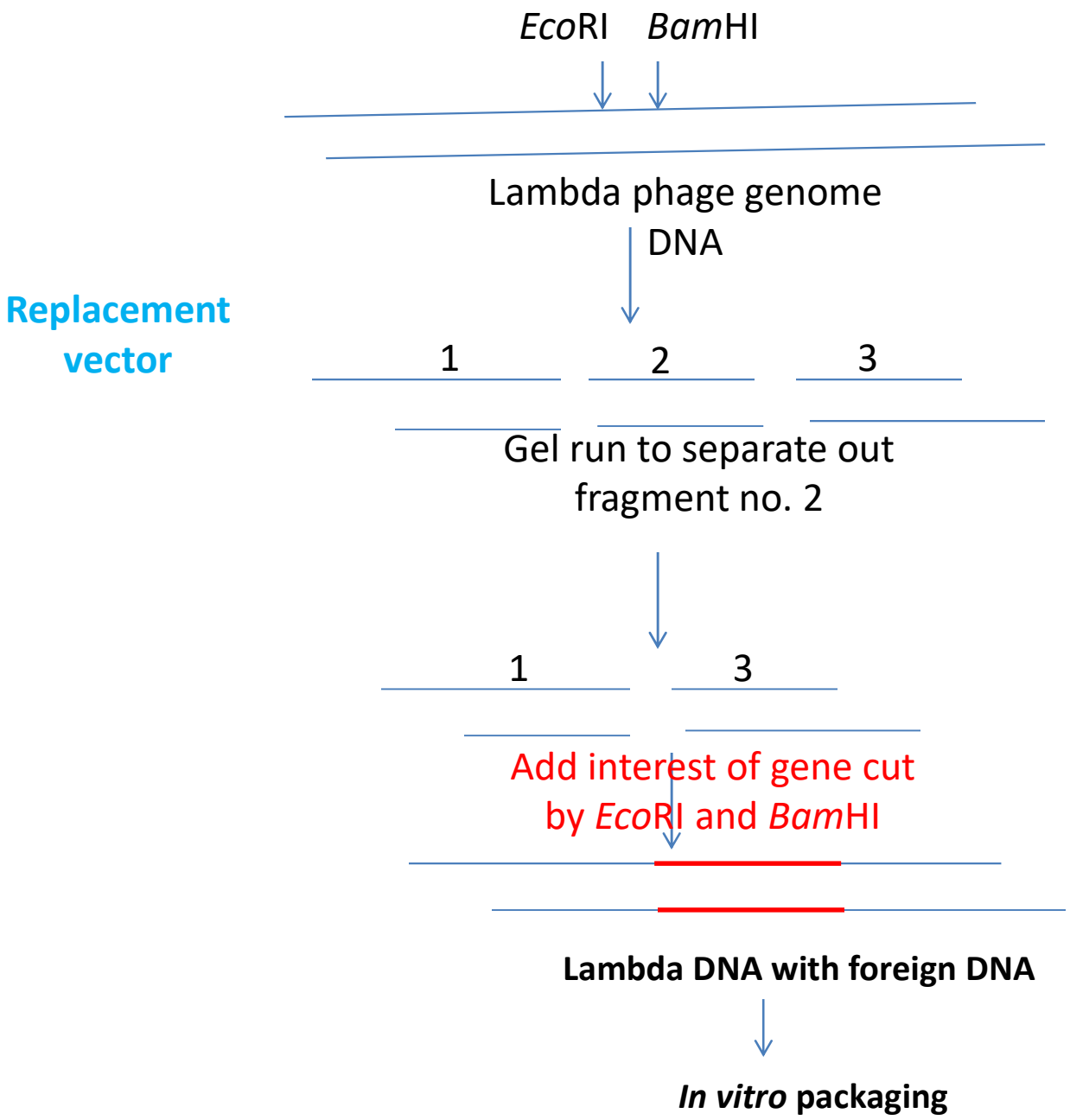


Insertional vector: Derived wild lambda phage contain unique Restriction site at non-essential region where break is done and desired gene/DNA fragment can be inserted. This will go for the *in vitro* packaging. Ex. Charon 16A

Charon is a greek mythology word means old ferryman who conveyed the spirits of the dead across the river styx.

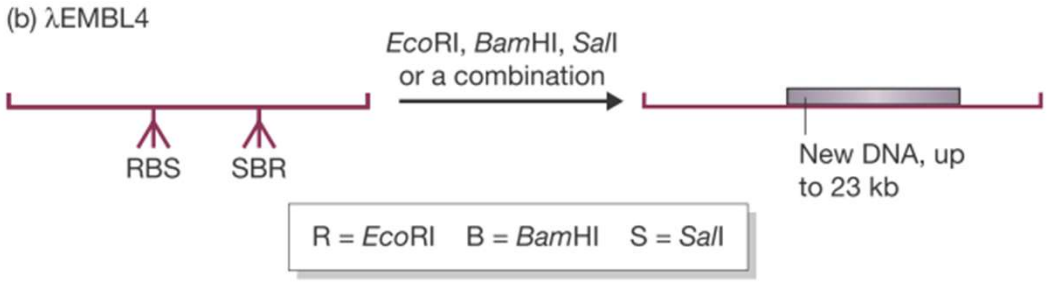
Replacement vector

- Derivative of wild type phage have therefore been produced that have a pair of sites, defining a fragment that can be removed and replaced by foreign DNA called replacement vector.
- Example is EMBL3, EMBL4


*Eco*RI *Bam*HI
Lambda phage genome
DNA
Replacement vector
1
2
3
Gel run to separate out fragment no. 2
1
3
Add interest of gene cut by *Eco*RI and *Bam*HI
Lambda DNA with foreign DNA
***In vitro* packaging**

Cloning with λEMBL4

- λEMBL4 is an example of replcement vector.
- λEMBL4 (below Figure b) can carry up to 20 kb of inserted DNA by replacing a segment flanked by pairs of EcoRI, BamHI, and SalI sites.
- Any of these three restriction endonucleases can be used to remove the stuffer fragment, so DNA fragments with a variety of sticky ends can be cloned.
- Recombinant selection with λEMBL4 can be on the basis of size, or can utilize the Spi phenotype.



- Many vector derivatives of both the insertional and replacement types were produced by several groups of researchers early in the development of rDNA technology (e.g., Thomas et.al. 1974, Murray and Murray 1975, Blattner et.al. 1977 and Leder et. al. 1977)
- Most of these vectors were constructed for use with *Eco*RI, *Bam*HI, or *Hin*dIII, but their application could be extended to other endonucleases by the use of linker molecules.

In vitro packaging

- Placing the recombinant lambda phage DNA in a phage coat allows it to be introduced into the host bacteria by normal process of phage infection, *i.e.* phage adsorption followed by DNA injection.
- Depending upon the details of the experimental design, packaging *in vitro* yields about 10^6 plaques per μg of vector DNA after the ligation reaction.

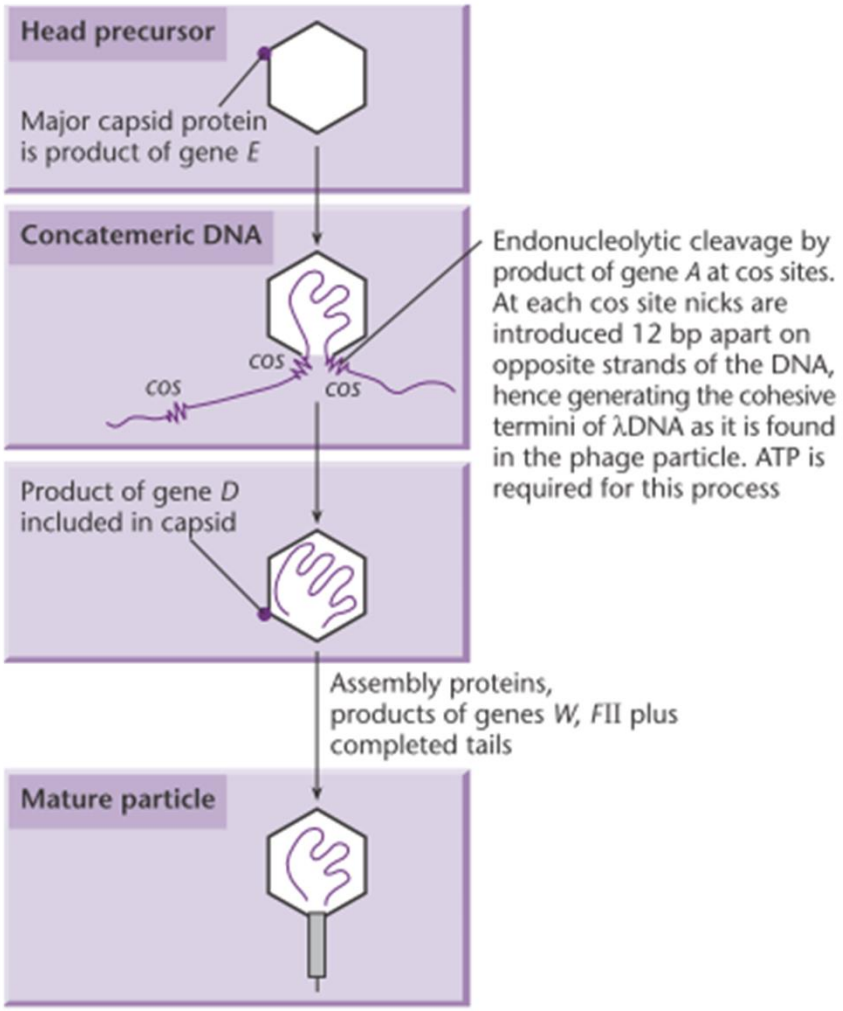


Fig. 4.14 Simplified scheme showing packaging of phage-λ DNA into phage particles.

Concatamer: of DNA consists of a series of unit genomes repeated in tandem.

Identification of recombinant Phages

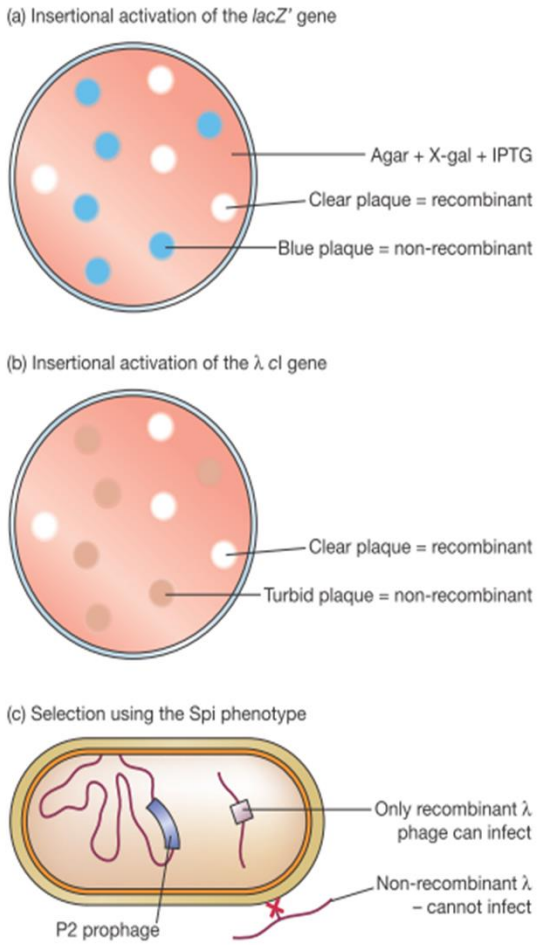
- **Insertional inactivation of *lacz* gene:** Blue and opaque screening
- **Insertional inactivation of *cI* gene:** wt phage plaque looks turbid and recombinant phage plaque looks clear
- **Selection using *Spi* phenotype** (**S**ensitive to **P**2 prophage **i**nhibition)

  spi$^+$ sensitive to P2 prophage

  spi$^-$ not sensitive to P2 prophage

Recombinant phages DNA are so modified that become spi$^-$ allow their growth in P2 prophage containing *E.coli* where as non recombinant cann't.

Figure 5.13
Strategies for the selection of recombinant phage.



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