Chapter 4

Recombinant DNA Technology

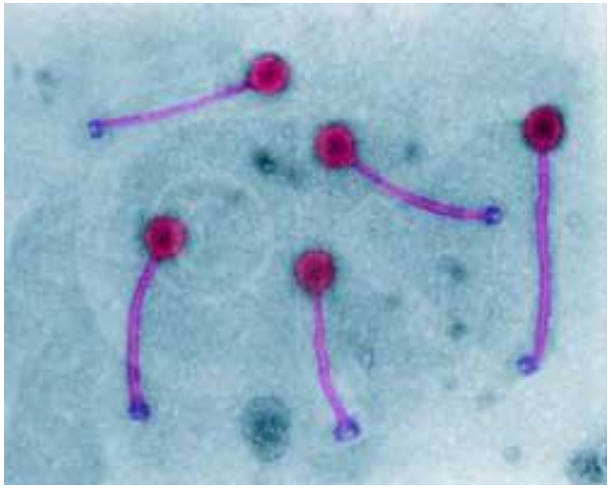
8. Vectors for Cloning Large Pieces of DNA

Insert Capacities of Vector Systems

Vector system	Host cell	Insert capacity (kb)
Plasmid	*E. coli*	0.1-10
Bacteriophage λ	*E. coli*	10-20
Cosmid	*E. coli*	35-45
Bacteriophage P1	*E. coli*	80-100
BAC	*E. coli*	50-300
P1-derived artificial chromosome (PAC)	*E. coli*	100-300
Yeast artificial chromosome (YAC)	Yeast	100-2,000
Human artificial chromosome	Cultured human cells	>2,000

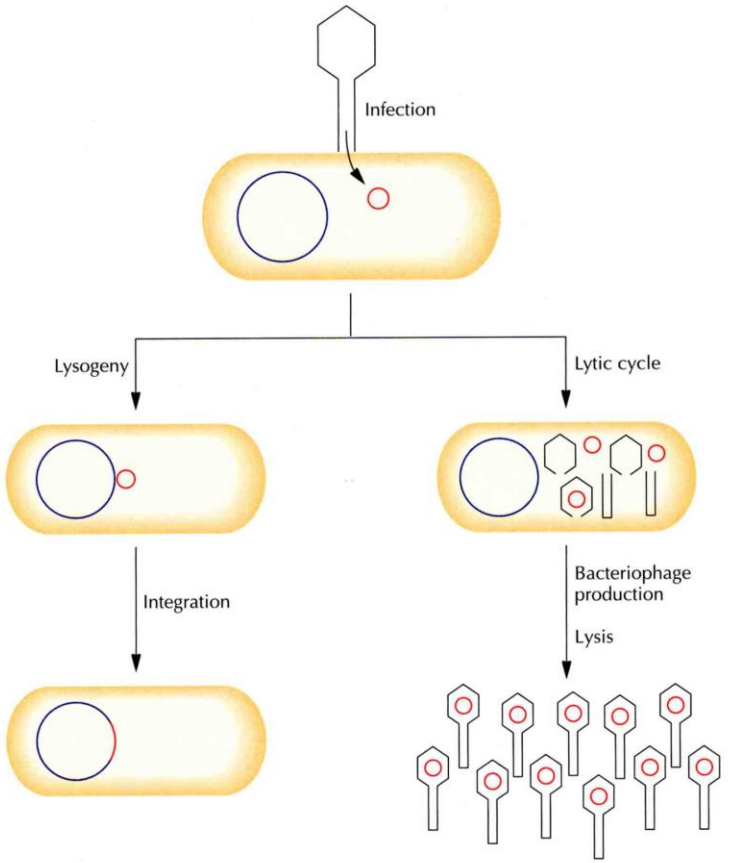
Bacteriophage λ

- 48.5 kb linear DNA
- Cohesive ends with 5’ 12 nt : cos site
  - Circularization after infection
- Lytic and lysogenic life cycle

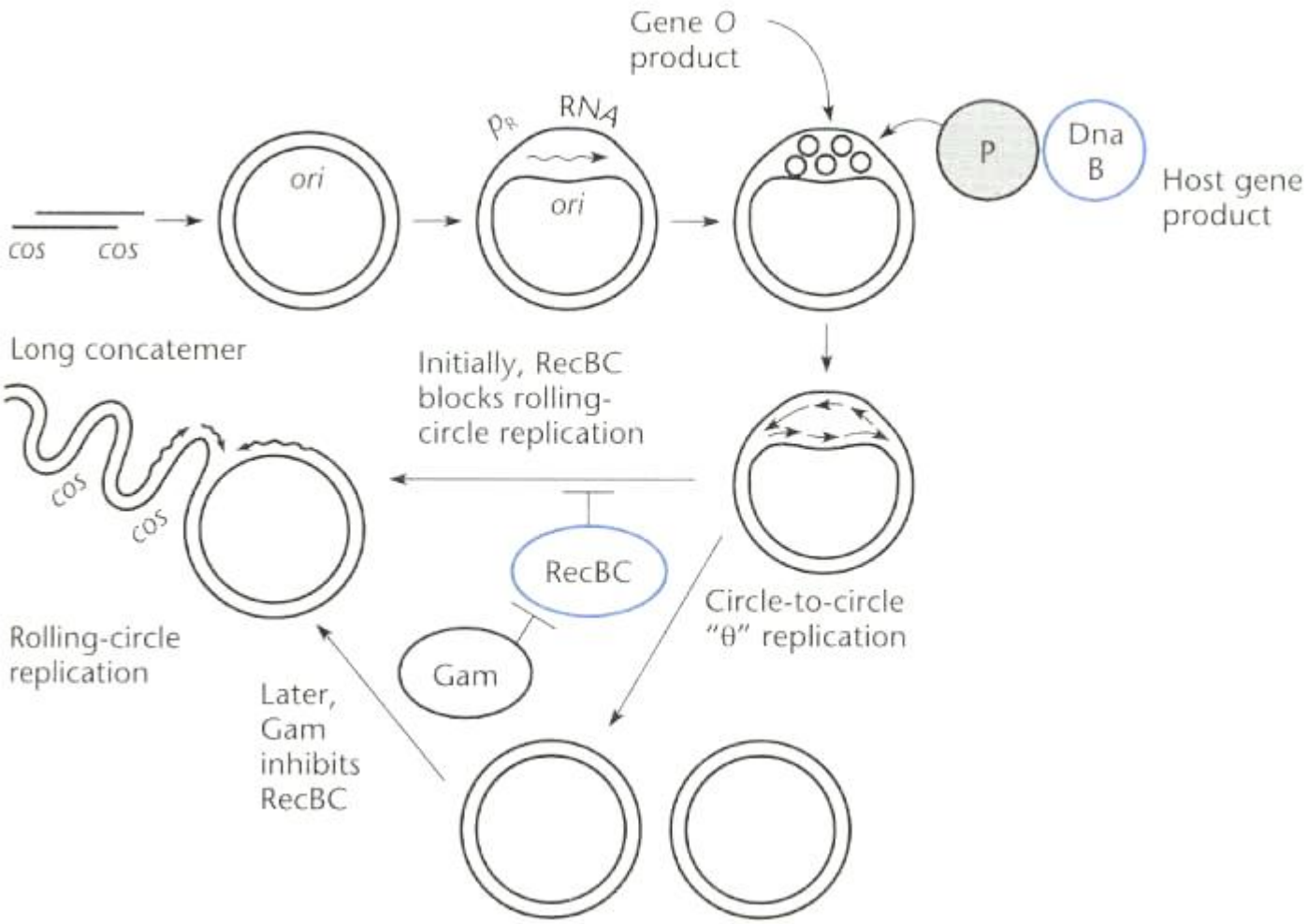


Life Cycle of Bacteriophage l

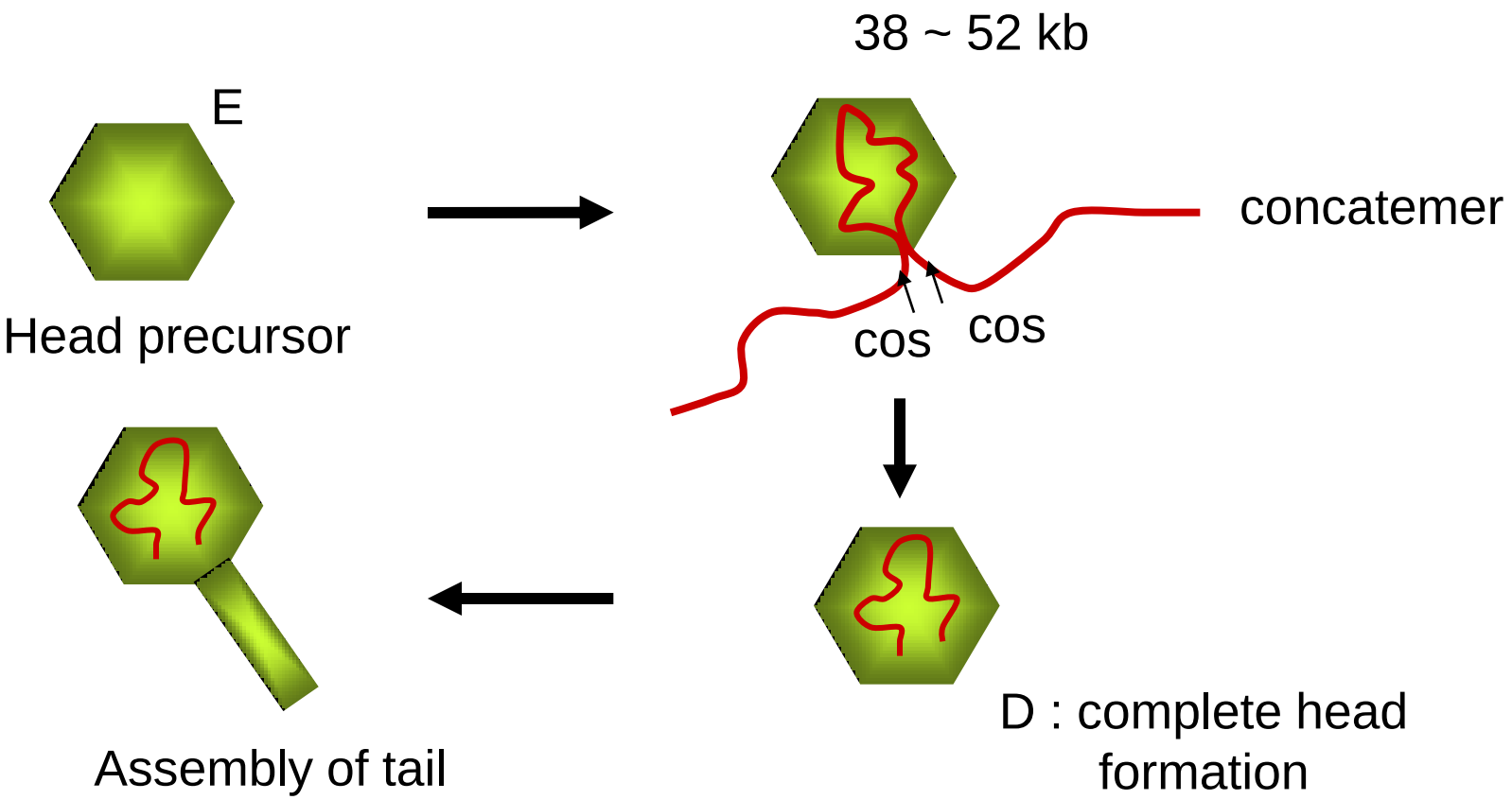
- Lytic cycle
  - Cell lysis and release of phage particles
- Lysogenic cycle
  - Prophage state
    - Integration of DNA into host genome (lysogen)
  - Induction of lytic cycle by nutrient or environmental stress



Replication of Bacteriophage λ

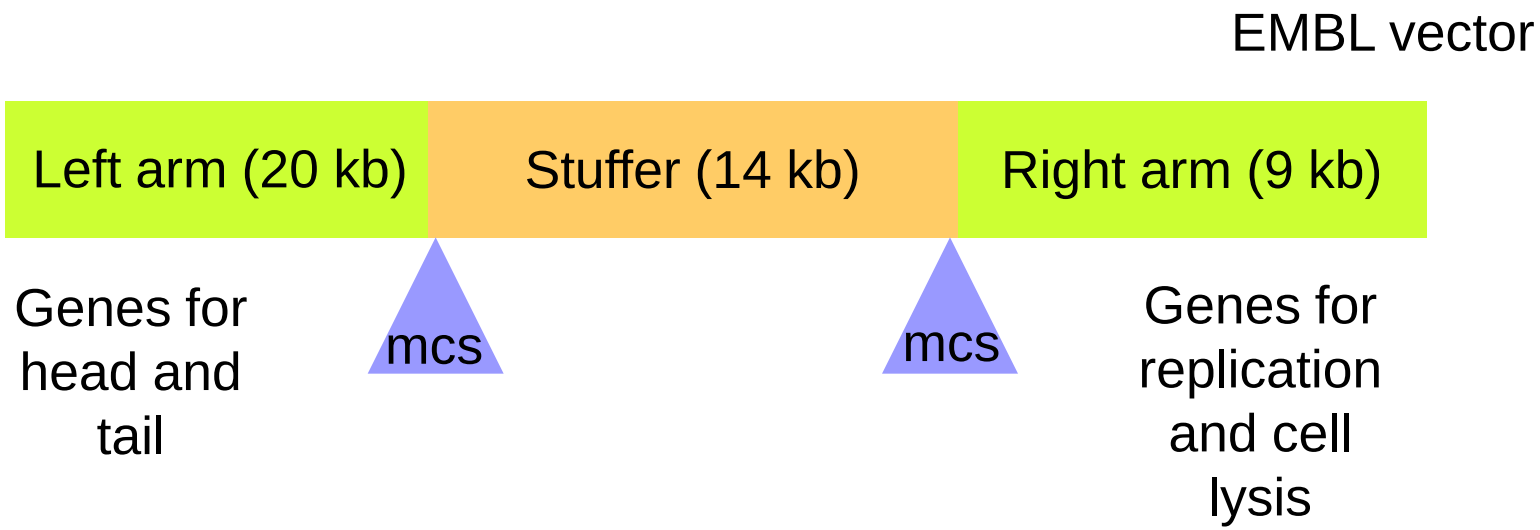


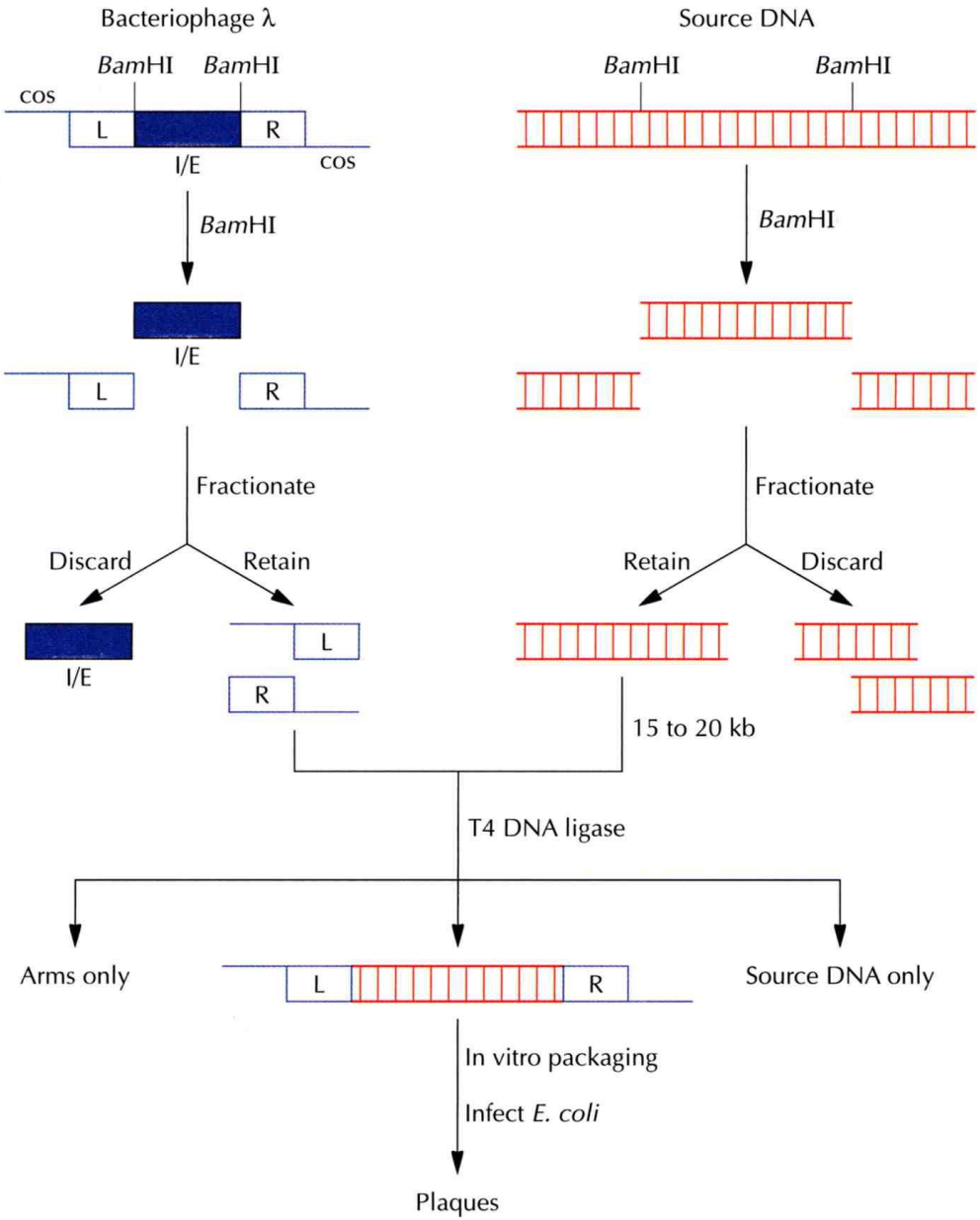
Packaging of Bacteriophage λ DNA



Bacteriophage λ Vector

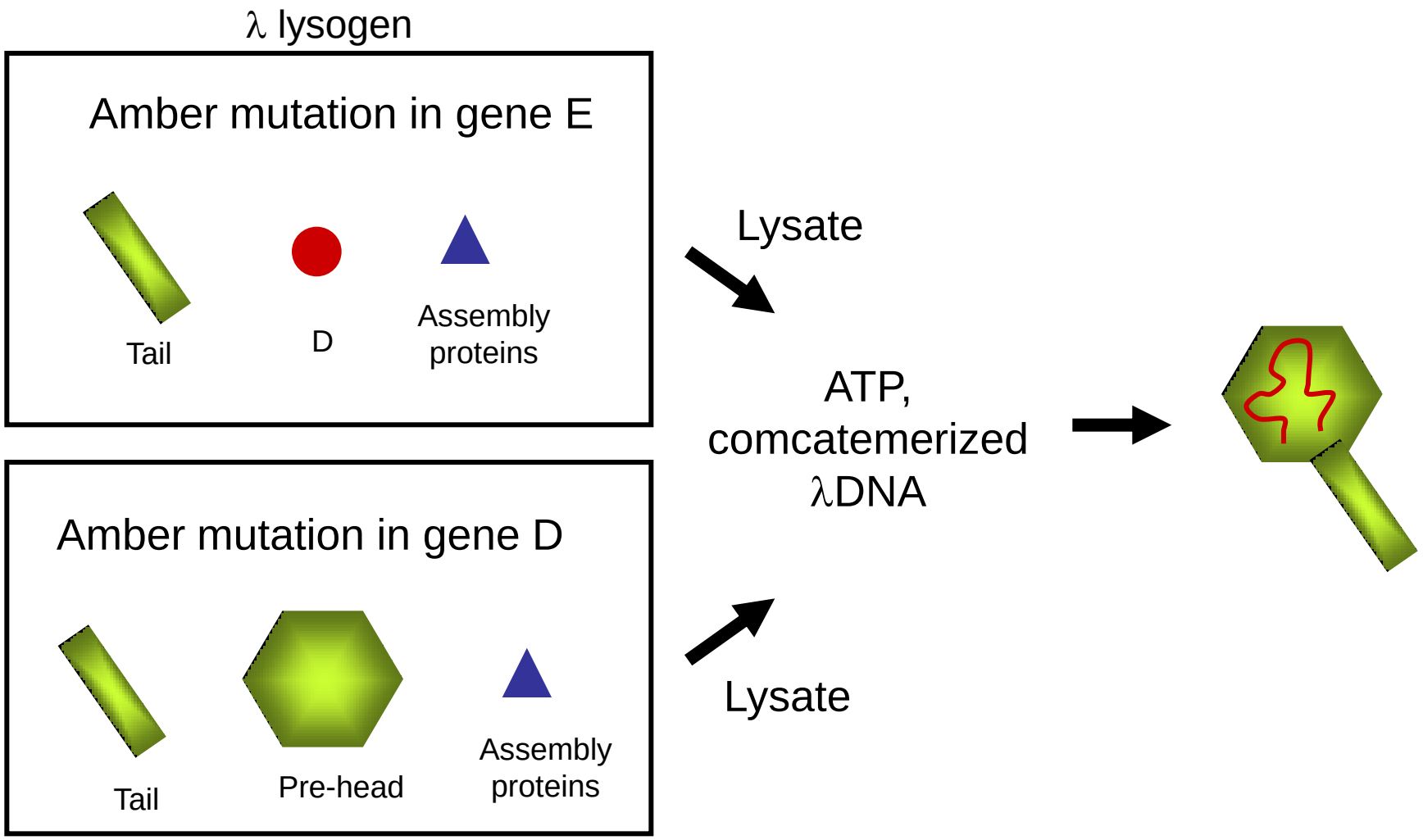
- Insertion or replacement
- Plaque formation after up to 25% deletion
  - Integration-excision (I/E) region
- λ Vectors




Bacteriophage λ
BamHI
BamHI
cos
L
R
I/E
cos
BamHI
I/E
L
R
Fractionate
Discard
Retain
I/E
L
R
Source DNA
BamHI
BamHI
BamHI
Fractionate
Retain
Discard
15 to 20 kb
T4 DNA ligase
Arms only
L
R
Source DNA only
In vitro packaging
Infect E. coli
Plaques

In vitro packaging of Bacteriophage λ DNA

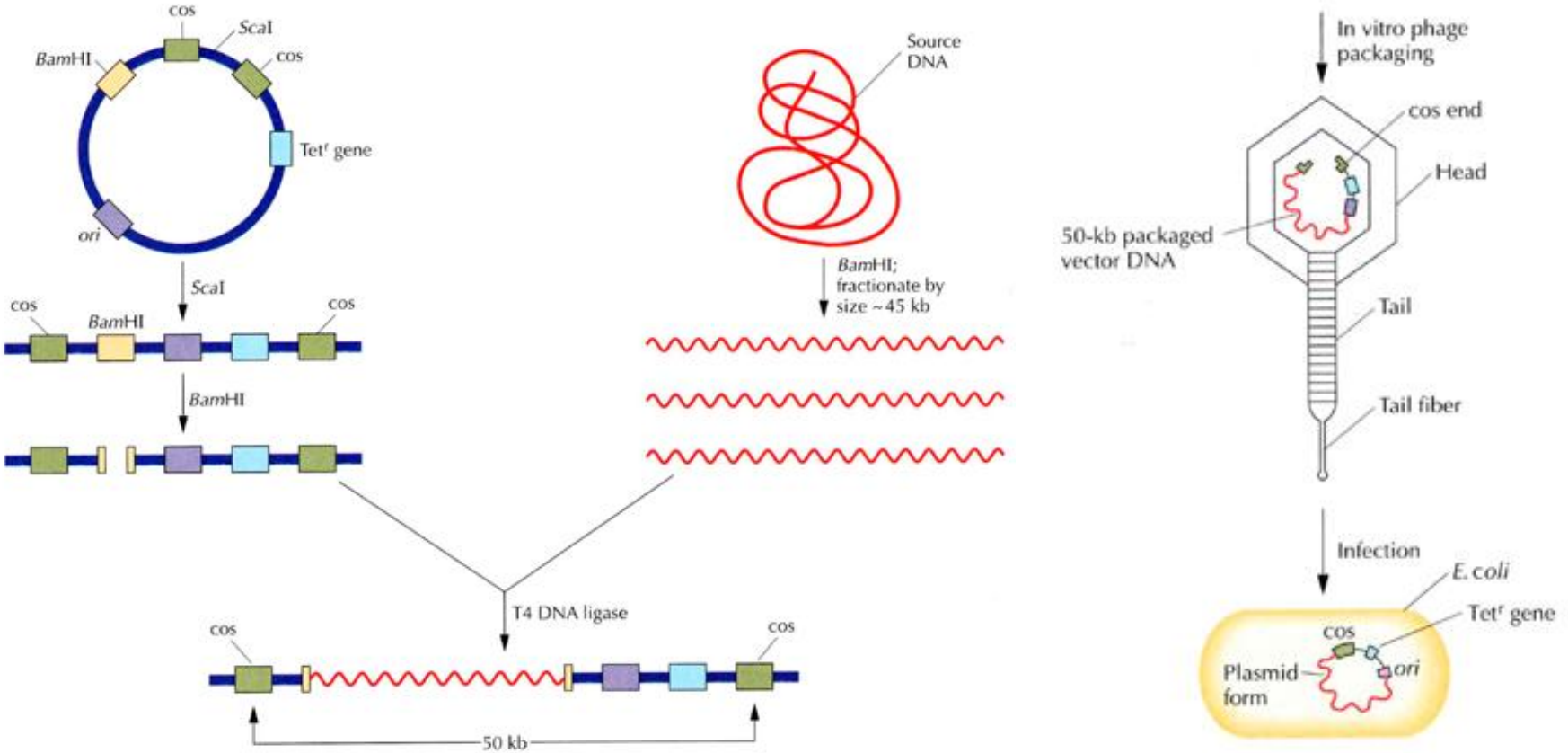
- Transfection of λ DNA: 10^5 plagues/μg DNA
- In vitro packaging and infection : 10^6 plagues/μg DNA



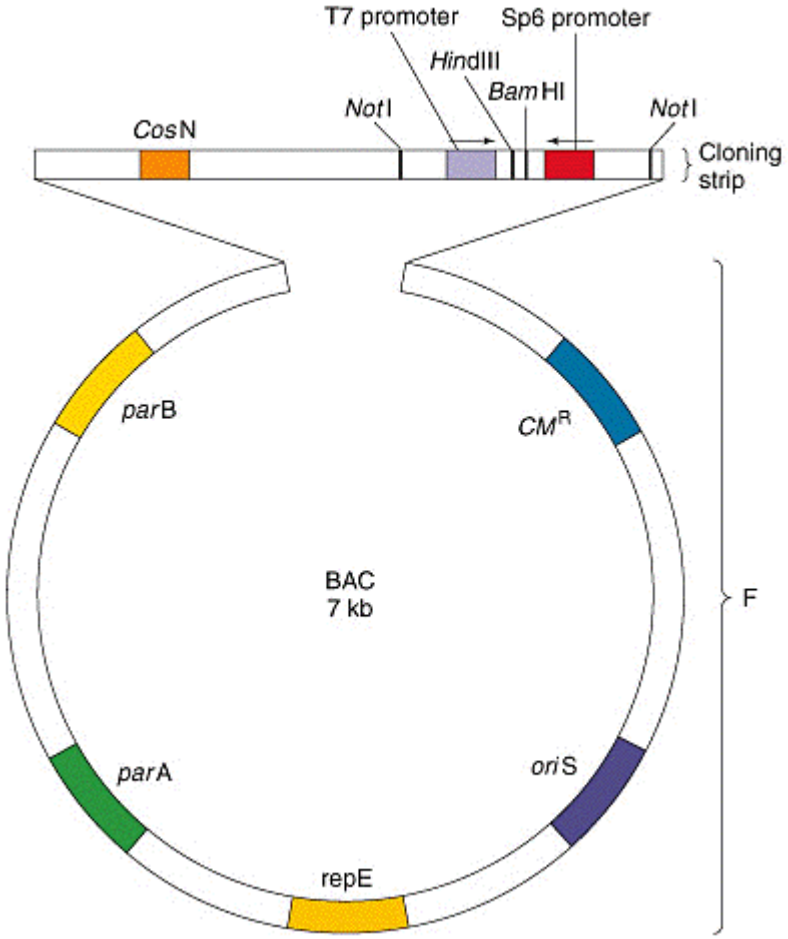
Cosmid Vector

- Cosmid vector
  - Plasmid with λ cos sites
- λ vector vs. cosmid vector
  - DNA Size for packaging: 38~52 kb (75-105% of λ DNA)
    - λ Phage vectors:
      - Limitation for the deletion of essential genes
    - Cosmid vectors:
      - Accommodate 33-47 kb DNA in 5 kb cosmid vector

Cloning of Genomic DNA into Cosmid Vector



BAC: Bacterial Artificial Chromosome

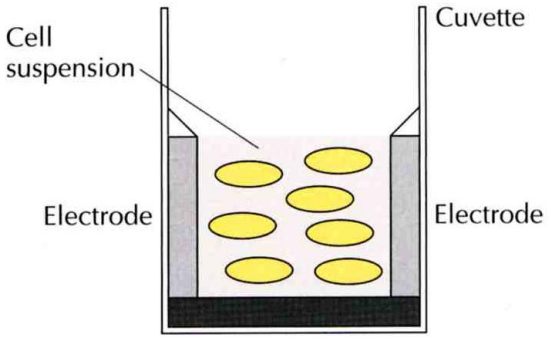


- Derived from E. coli F' (single copy sex factor) Plasmid

- Used to generate genomic library with average size of 125 kb

Genetic Transformation of Prokaryotes

- Chemical method: $CaCl_2$ and heat shock
  - Transformation frequency : 10^{-3}
  - Transformation efficiency : 10^7 to 10^8/mg DNA
- Electroporation
  - Electric field-mediated membrane permeabilization
  - *E.coli*: electric pulse of 25μF, 2.5 kV, 200 ohms for 4.6 ms
  - Transformation efficiency : 10^6 (~136 kb) to 10^9 (~3 kb) /mg DNA



Conjugation

- Mobilization of DNA by conjugative plasmid
- Supply of the mobilizing function by its own plasmid or helper plasmid
  - Mixing three strains and selection on minimal medium with kanamycin