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

Shuttle Vectors

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Shuttle vector

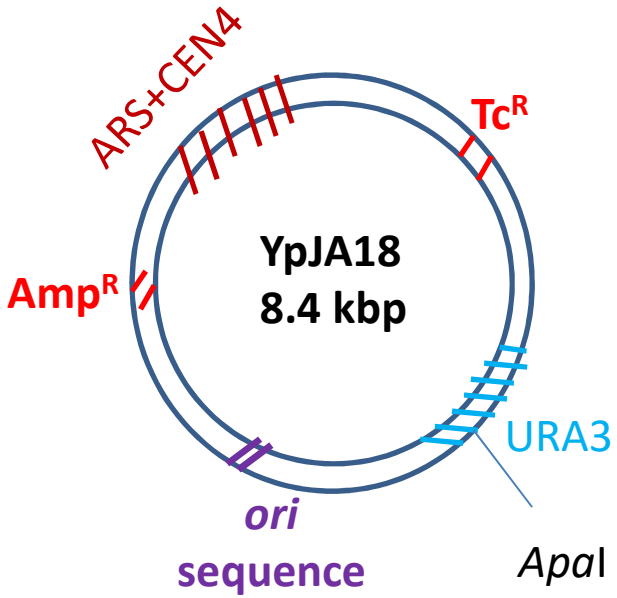
- Shuttle vector is a vector constructed to have origin of replication for two hosts (for example *E.coli* and Yeast). So, it can be used to carry a foreign DNA either prokaryote and eukaryotes
- Thus, shuttle vector has dual purpose and is constructed from both prokaryotic and eukaryotic DNA sequences.

Shuttle vector continued…

- *E.coli:*
- 1. ori sequence
- 2. selectable marker (Amp^R, Tc^R)
- Yeast:
- 1. ARS
- 2. Yeast growth promoting factors (Tryp, Ura^{3+}, Leu^{2+})

Example of shuttle vector

- ypJA18: Yanne, Jha *et. al.* (1993) at Munich University of Germany (B. Jha, Anni, Kirtler, Klaus, Eckardt and Schupp)



Genetic map of YpJA18

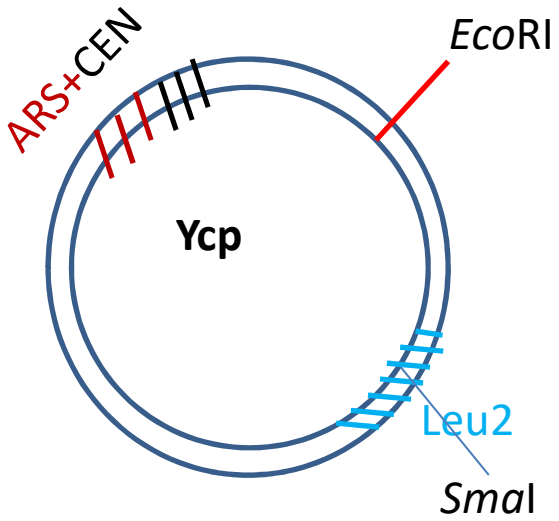
1. ARS+CEN4: Autonomous Replicating Sequence and centromere sequence derived from chromosone no. 4

2. URA 3- yeast growth
3. Amp^r and ori sequence derived from pBR322

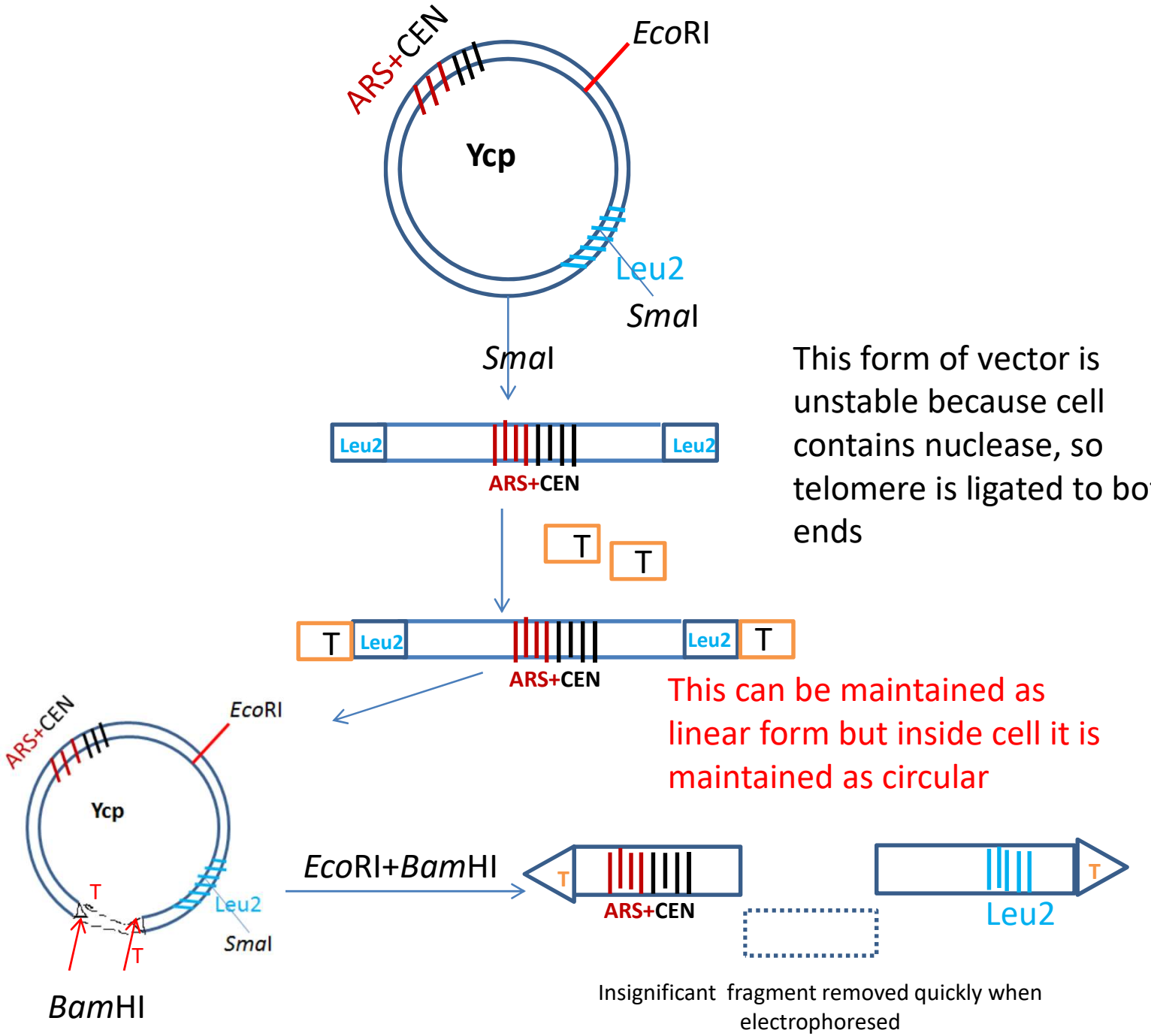
This vector is used for the study of DNA repair

YAC vector

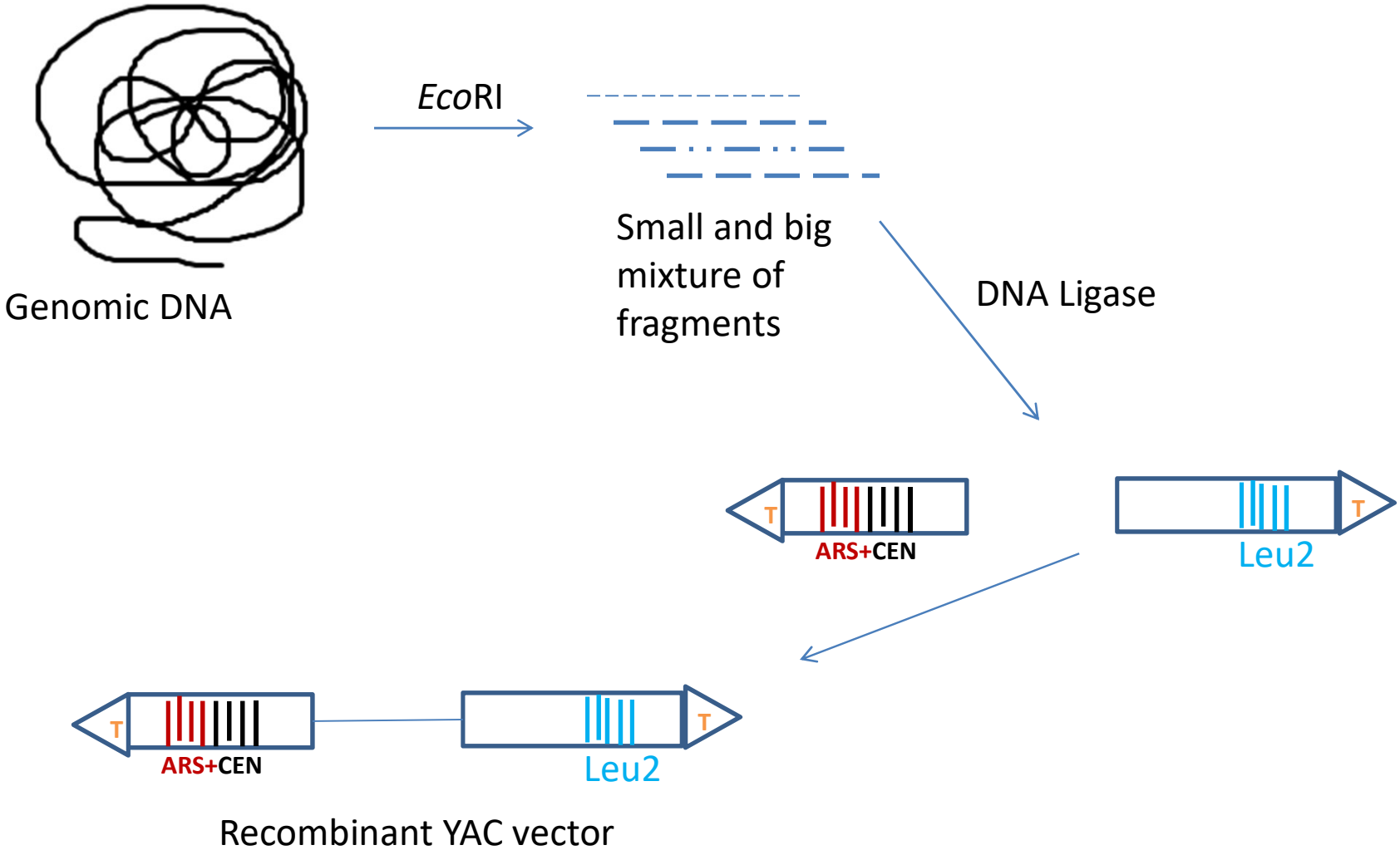
- Yeast artificial chromosome
- Contains both centromere and telomere
- Cloning capacity ~1Mbp



Map
Telomere for maintaining linear form


ARS+CEN
EcoRI
Ycp
Leu2
SmaI
SmaI
Leu2
Leu2
ARS+CEN
T
T
T
Leu2
Leu2
T
ARS+CEN
This form of vector is unstable because cell contains nuclease, so telomere is ligated to both ends
This can be maintained as linear form but inside cell it is maintained as circular
ARS+CEN
EcoRI
Ycp
T
Leu2
SmaI
T
BamHI
EcoRI+BamHI
T
ARS+CEN
T
Leu2
Insignificant fragment removed quickly when electrophoresed

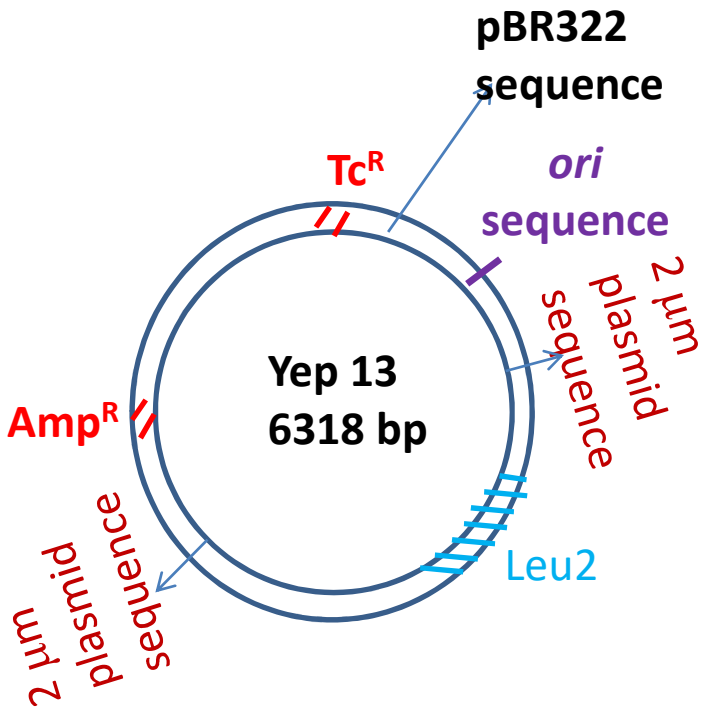
YAC as a vector



- Advantage of YAC vector takes up DNA fragment of ~1Mbp
- Disadvantage:
- Cloning efficiency is low(1000 clones/μg against 10^6 to 10^7 clones/μg of cosmid), thus impractical to generate complete genomic library

Yeast Episomal Plasmid (YEp)

- Naturally occurring plasmid found in some strain of yeast
- Present in several copy
- It's other name is 2μm plasmid
- 6318 bp long
- pBR322 sequence present
- Ori sequence from 2 micro meter plasmid
- Yep 13 vector pBR322 sequence contains 1. origin of replication for *E.coli* host. 2. tet^r and amp^r selectable marker genes and 3. LEU2 yeast gene, which encodes an enzyme involved in leucine biosynthesis

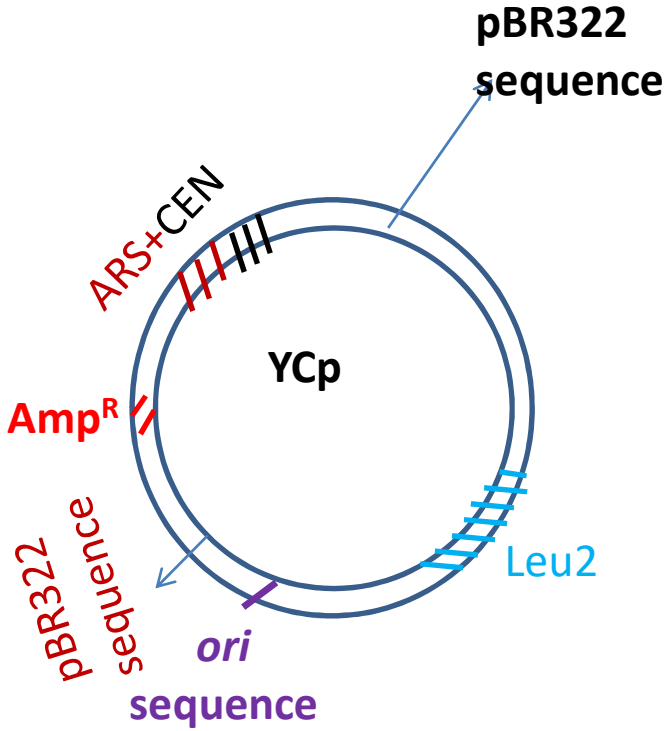


Yeast Replicating Plasmid (YRp)

- Also called ARS vector because contains ARS from yeast chromosome
- Example is YRp vector is YRp 7, which is 5.8 kb in size
- Made up of pBR322 sequence and yeast TRP1 gene
- YRp vectors are less efficient in transformation than YEp vectors; they yield 1 to 10 thousand transformants per mg of plasmid DNA
- YRp 7 is ten times less stable than YEps
- YRps are present at 3-100 copies per cell
- **Exhibit extreme mitotic and meiotic instability**
- As a result YRp vectors are rarely used now.

Yeast centromere plasmid vector (Ycp)

- Also called mini chromosomes vectors because contains centromere sequences, replicate once during cell division and distributed to daughter cell like true chromosomes
- A typical minichromosome vector contains: 1. An ARS, 2. CEN sequence, 3. LEU2 selectable marker for yeast, and from bacterial plasmid 4. ori sequence and 5. amp^r selectable marker
- Ycp is stable vector it maintains stably one no. in per cell
- YCps are used as general yeast cloning vectors, and are the basis of YAC vectors

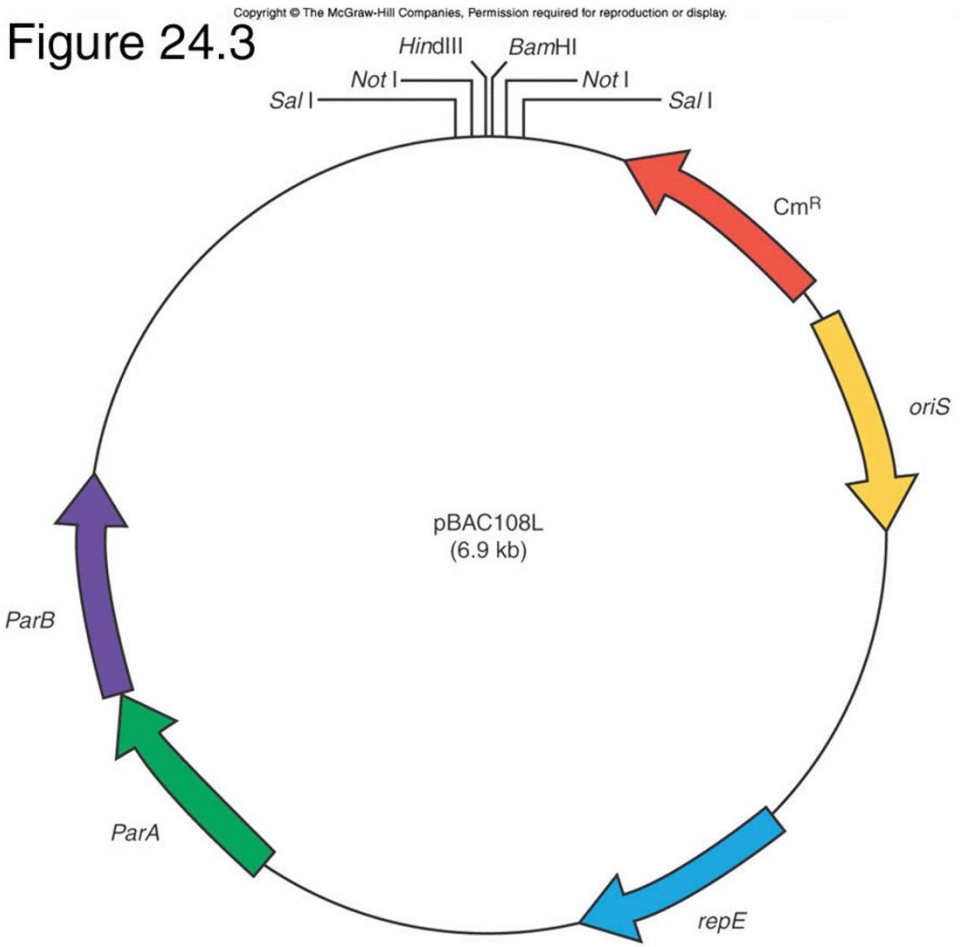


BAC

- Allow cloning of ~300 Kb fragment
- constructed in year 1992
- Based on F’ conjugation factor of *E.coli* for chromosomal transfer and exists as extrachromosomal element
- BAC vector pBAC108L
- pMB0131

pBAC108L map

- Ori S and repE for self replication
- OriS replicon is based on the F (fertility) factor of E. coli
- Initiation factor RepE (also known as RepA) mediates assembly of a replication complex at OriS.
- parA and parB genes for partition (segregation during cell div.) maintain copy no. 1 or 2 per *E.coli* genome
- MCS flanked by T7 and SP6 promoters
- Chloroapmphenicol for selection marker
- Advantage: upto ~300 kb fragment cloned
- Disadvantage: Not able to take more than 300 kb fragment



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