

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

# Introduction of Cloning Vectors

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# Introduction

- Extra chromosomal genetic material found in prokaryotic cell (except yeast and lower eukaryotes), which independent from host genome and replicate independently and stably inherited.
- Plasmids are replicons, which are stably inherited in the form of extra chromosomal genetic material.

# Properties

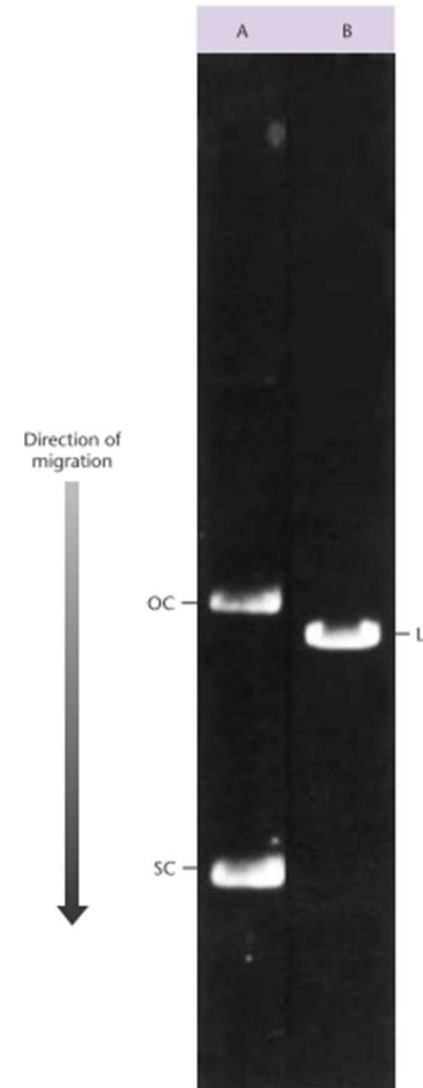
- It should have *ori* sequence
- Plasmids are stably inherited as a extra genetic material state
- Genetic homogeneity
- Plasmids have constant monomeric size
- Plasmids are dispensable entity because bacteria survive without it

# Why mitochondria and chloroplast genome not considered as a plasmid?

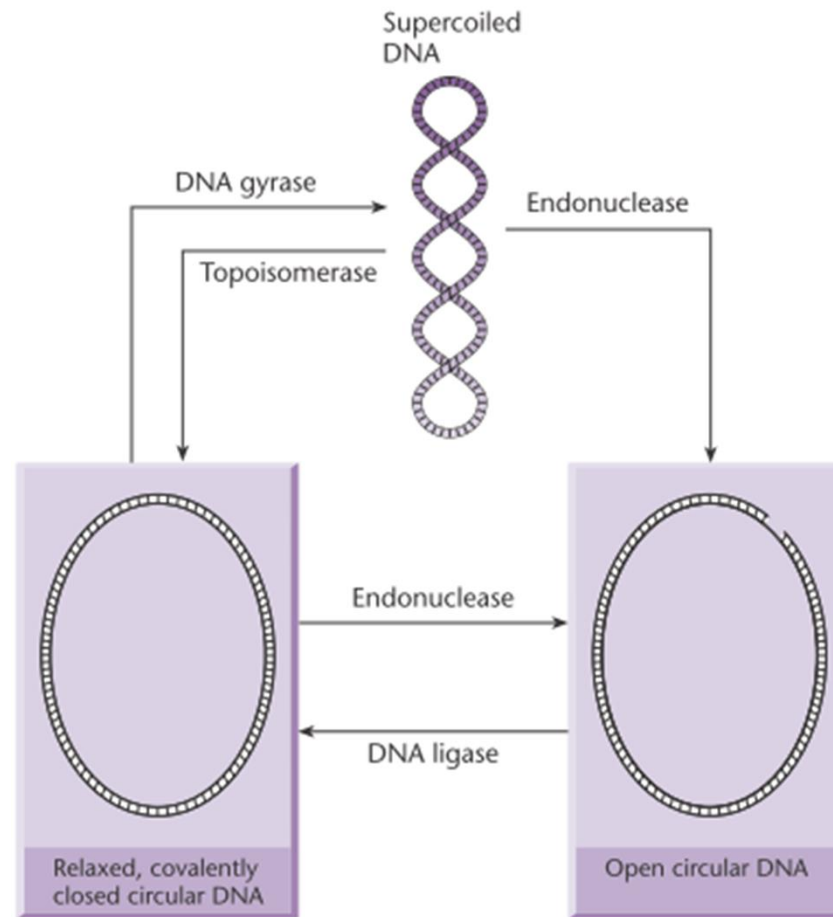
- Because:
  - 1. It has not constant monomeric size
  - 2. It has not phenotypic scorable trait
- Why lambda bacteriophage is not considered as a plasmid?
- Lambda bacteriophage has all property of plasmid. It has an additional property of getting integration into host genome.

# Facts of plasmid

- Most of the plasmids are circular DNA duplex
- But some plasmids are exceptionally linear as found in *Borrelia*, *Streptomyces*.
- Plasmids may exist in 3 native forms
- 1. covalently close circular DNA
- 2. open circular DNA: one strand of the DNA is nicked
- 3. supercoiling Plasmid DNA



# Interconversion of plasmids form



**Fig. 4.1** The interconversion of supercoiled, relaxed covalently closed circular DNA and open circular DNA.

# Intercalating agent

- Ethidium bromide
- 10 mg/ml
- Carcinogen

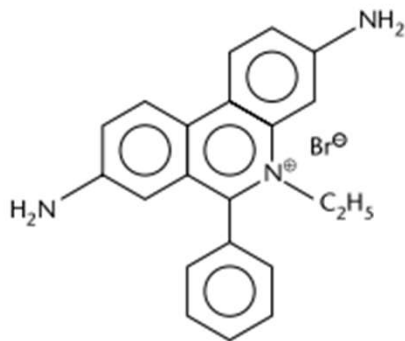


Fig. 2.3 Ethidium bromide.

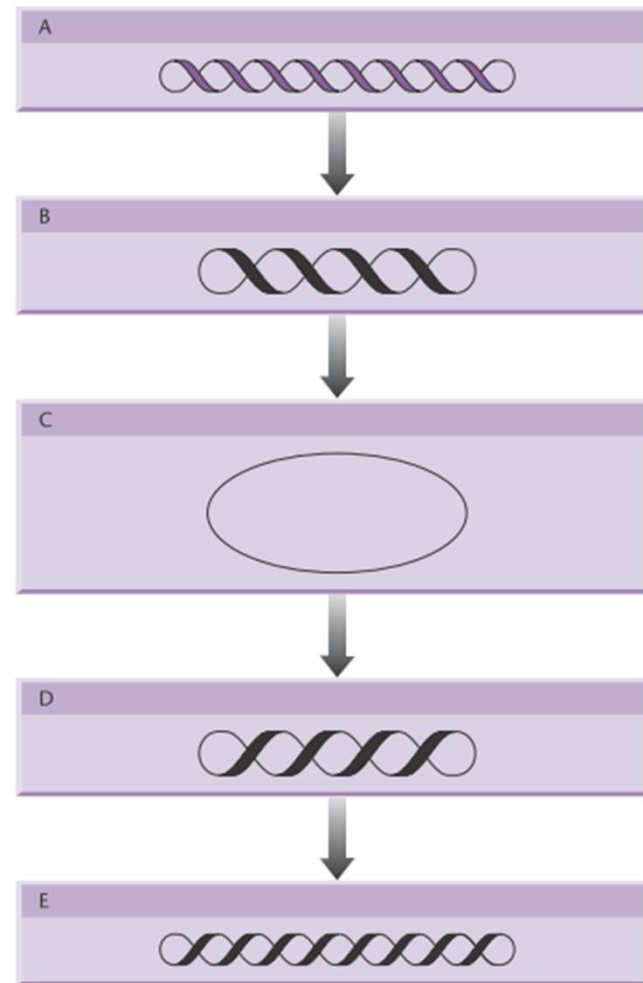


Fig. 4.3 Effect of intercalation of ethidium bromide on supercoiling of DNA. As the amount of intercalated ethidium bromide increases, the double helix untwists, with the result that the supercoiling decreases until the relaxed form of the circular molecule is produced. Further intercalation introduces excess turns in the double helix, resulting in supercoiling in the opposite sense (note the direction of coiling at B and D). For clarity, only a single line represents the double helix.

# Classification

- A. on the basis of property of plasmid:
  - a. **cryptic plasmid**: Plasmids having no any attributable phenotypic trait.
  - b. **typical plasmid**: besides the cryptic plasmids all plasmids are typical plasmid.  
Typical plasmids have several phenotypic trait:

## Some phenotypic trait exhibited by plasmid carried genes

Antibiotic resistance

Antibiotic production

Heavy Metal resistance

Host controlled restriction and modification



B. On the basis of presence of or absence of *tra* gene:

- a. Conjugative plasmid: *tra* gene containing plasmid. *Tra* gene facilitate the conjugation.
- b. Non-conjugative: *tra* gene lacking plasmid

C. On the basis of no. of Plasmid:

1. **Relax plasmid:** Plasmid present in multiple copies of the same type are called relax plasmid. Generally relax plasmids are tiny.
2. **Stringent plasmid:** if plasmid is present in a few no. (1-3) then the plasmid is called stringent plasmid
3. If the stringent plasmid is present in only one copy in the cell then it is called single copy plasmid. Single copy plasmid is used for the study of DNA repair.

## D. On the basis of occurrence

- **Natural plasmid:** found naturally in the MO.  
Ex pSC101, RSF 2124 etc
- **Artificial plasmid:** not natural or constructed in the laboratory. Ex. pBR322, pUC 8/9 etc.

## 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