INTRODUCTION

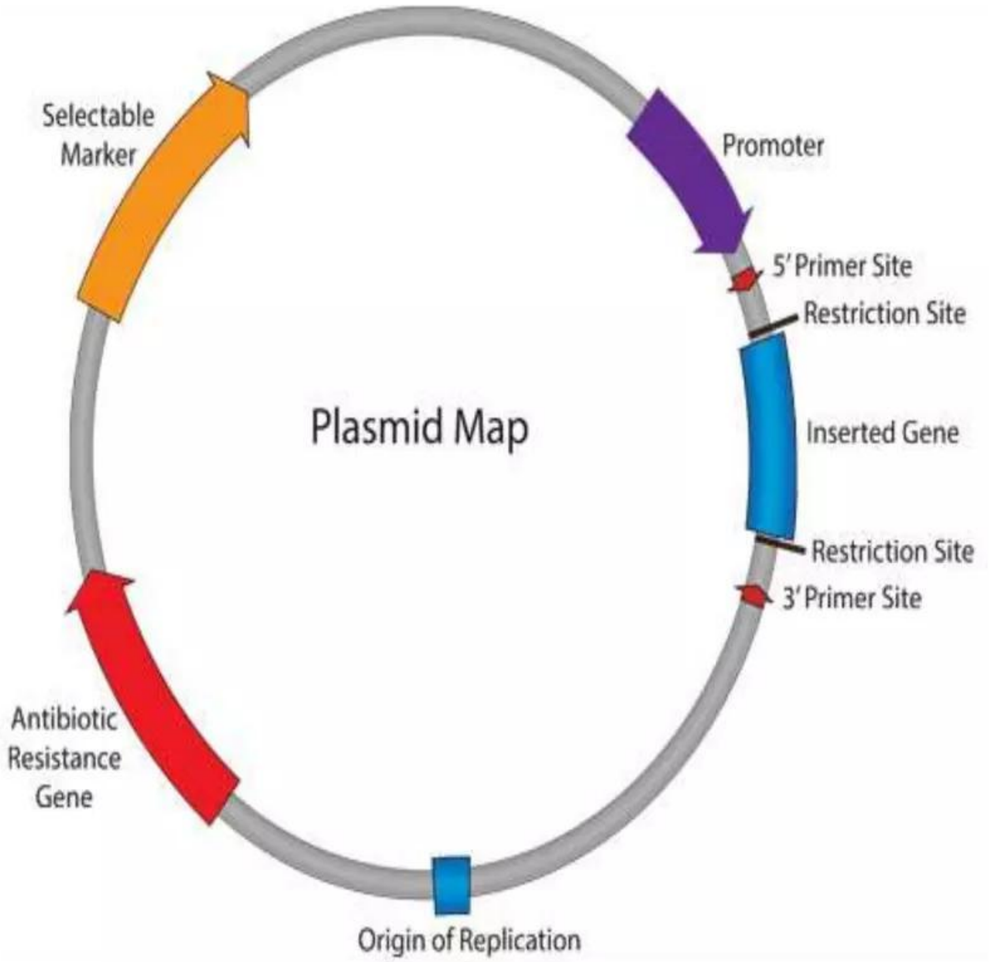
- A plasmid is a small, circular, extrachromosomal double stranded DNA that has the capacity to replicate independently.
- Discovered by Laderberg in 1952.
- It naturally occur in bacteria, however sometimes present in archaea and eukaryotes.
- The genes carried in plasmid benefit the survival of the organism by providing them with genetic advantages like antibiotic resistance etc. under certain situation or particular conditions.
- They provide mechanism for horizontal gene transfer within a population of microbes and thus provide a selective advantage under a given environmental state.

PROPERTIES

- Specific to one or a few particular bacteria.
- Replicate independently and code for their own transfer.
- Do not cause damage to cells and generally are beneficial, do not have extracellular forms and exist inside cells simply as free and typically circular DNA.
- Size ranges from 1 kbp to several mbp.
- Number of plasmids in an individual cell may vary, ranging from one to several hundreds, denoted by copy number.
- Genes carried by plasmid encodes traits for antibiotic resistance or resistance to heavy metal.
- Some produces virulence factor that help in defence or nutrient utilization.
- Plasmids can also provide bacteria with the ability to fix nitrogen.
- Some also exhibits properties like sulphur utilization, hydrocarbon degradation, drug resistance etc.

ELEMENTS OF PLASMIDS

- Origin of replication: it is the DNA sequence which directs initiation of plasmid replication by recruiting bacterial transcriptional machinery.
- Antibiotic resistance gene: these genes allows for selection of plasmid containing bacteria by providing a survival advantage to the bacterial host.
- Multiple cloning site: this is the short segment containing several restriction enzyme sites, enabling easy insertion of foreign DNA.



- Insert: it is the foreign DNA cloned into the multiple cloning site.
- Promoter region: it drives the transcription of the foreign insert.
- Selectable marker: it is used to select for cells that has successfully taken up the plasmid for the purpose of expressing the insert.
- Primer binding site: it is the site for binding of short single stranded DNA sequence, used as an initiation point for PCR amplification or sequencing of the plasmid.

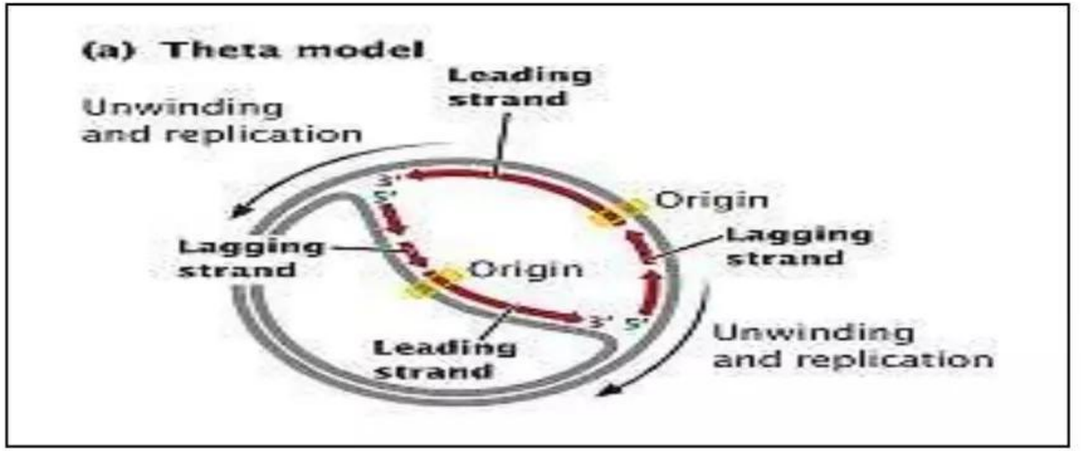
PLASMID CONFORMATIONS

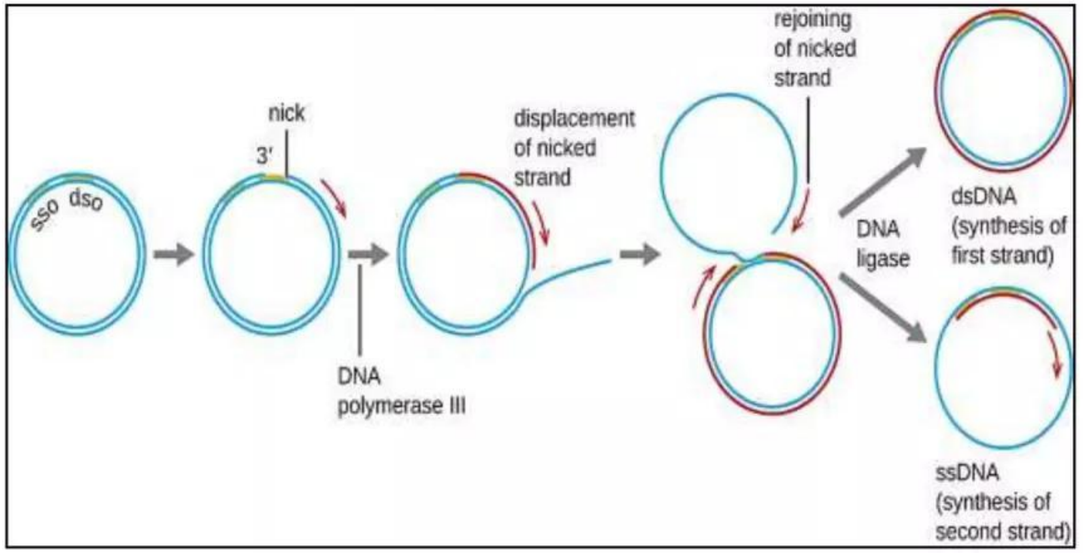
1. Nicked open circular DNA
2. Relaxed circular DNA
3. Linear DNA
4. Supercoiled or covalently closed circular DNA
5. Supercoiled denatured DNA

PLASMID REPLICATION

Plasmids replicate autonomously because they have their own replication origins.

- Most plasmids in gram-negative bacteria replicate in a manner similar to the replication of bacterial chromosome involving initiation at the replication origin site and bidirectional replication around the DNA circle giving a theta (Θ) intermediate.
- Most plasmids of gram-positive bacteria replicate by a rolling circle mechanism.





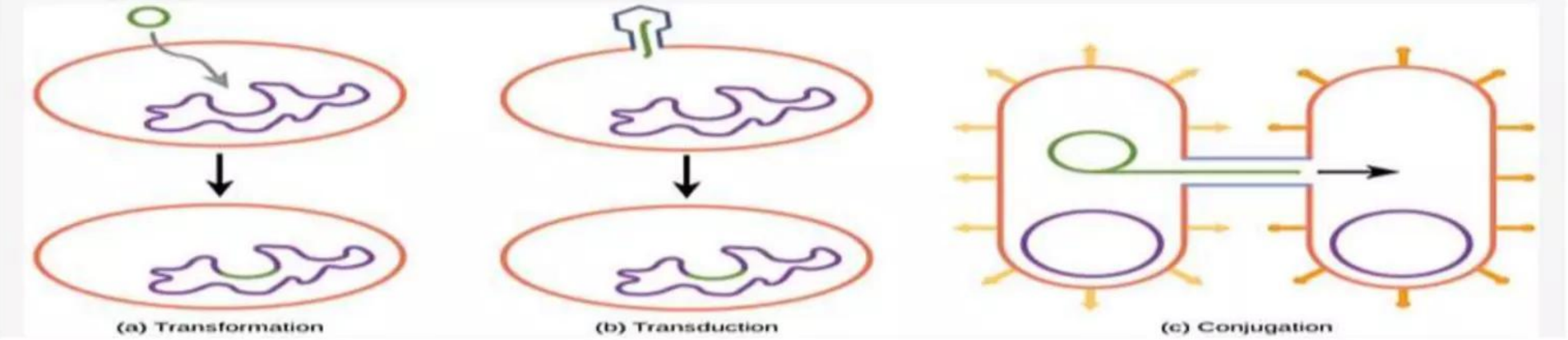
MODE OF PLASMID TRANSFER

The genetic information encoded in a plasmid of bacteria is transferred across a broad range of microorganism via-

1.Transformation: requires competent cells which are ready to accept extracellular plasmid and further stable replication inside host cell.

2.Transduction: plasmid mediated gene transfer through bacteriophages.(can be generalised or specialised)

3.Conjugation: transfer through cell to cell contact of donor and recipient cell, requires DNA metabolism of donor cell.



CLASSIFICATION

Based on their functions, plasmids are classified as follows-

- F-PLASMID (fertility plasmid)
  - contain ‘tra’ gene, capable of conjugation.
- R-PLASMID (resistance plasmid)
  - contain genes that provide resistance against antibiotics or poisons.
- Col PLASMID
  - contain genes that codes for bacteriocins (proteins that can kill bacteria).

DEGRADATIVE PLASMID

they enable the digestion of unusual substances like toluene, salicylic acid etc.

VIRULENCE PLASMID

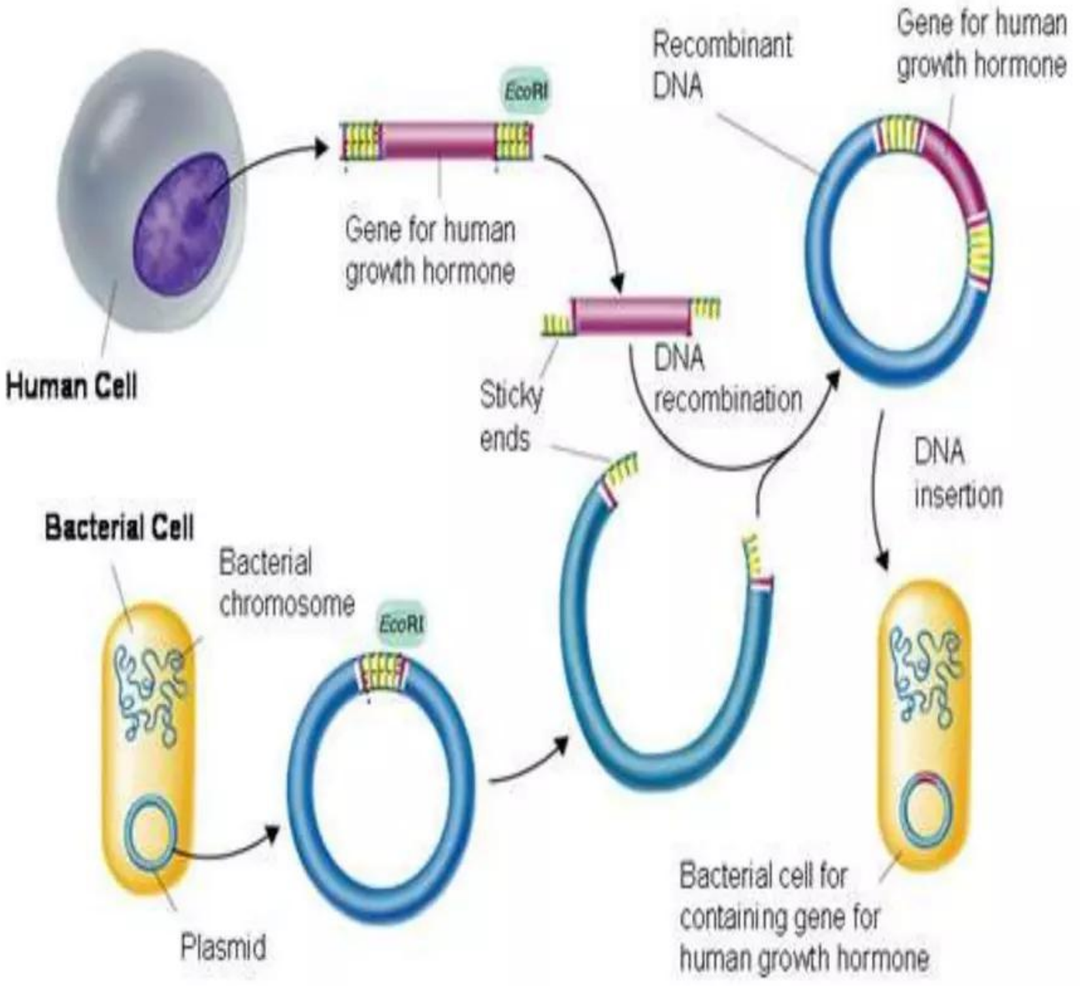
they turn the bacteria into pathogen.

SUICIDE PLASMID

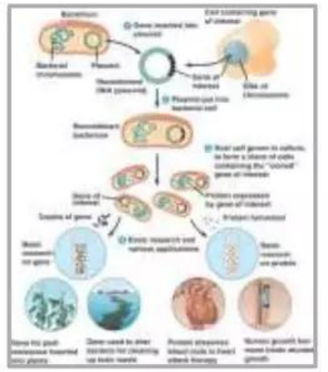
gets transferred to another bacterial cell but don’t replicate further.

PLASMIDS AND RECOMBINANT DNA TECHNOLOGY

- Recombinant DNA technology is joining together of DNA molecules from two different species that are inserted into a host organism to produce new genetic combinations that are of value to science, medicine, agriculture, and industry.
- Artificially constructed plasmids are used as vectors in genetic engineering to clone or amplify or express particular genes.
- When a single recombinant DNA molecule composed of vector and inserted DNA molecule is introduced into host cell, the inserted DNA is reproduced along with the vector, producing large number of recombinant DNA molecule that include the fragment of DNA molecule originally linked to the vector



USES



1. CLONING
2. PROTEIN PRODUCTION
3. GENE THERAPY
4. GENETIC DISEASE MODELS
5. PLASMID DNA VACCINES

IMPORTANCE OF PLASMID

- Easy to work with due to convenient size for physical isolation and manipulation, easy to create and modify plasmids containing the genetic element that one is interested in.
- Independent origin of replication allows plasmid replication in the cell to proceed independently from direct chromosomal control.
- Multiple copy number makes them to be present in the cell in several copies so that amplification of the plasmid DNA becomes easy
- Presence of selectable markers such as antibiotic resistance genes, which make detection and selection of plasmid-containing clones easier.

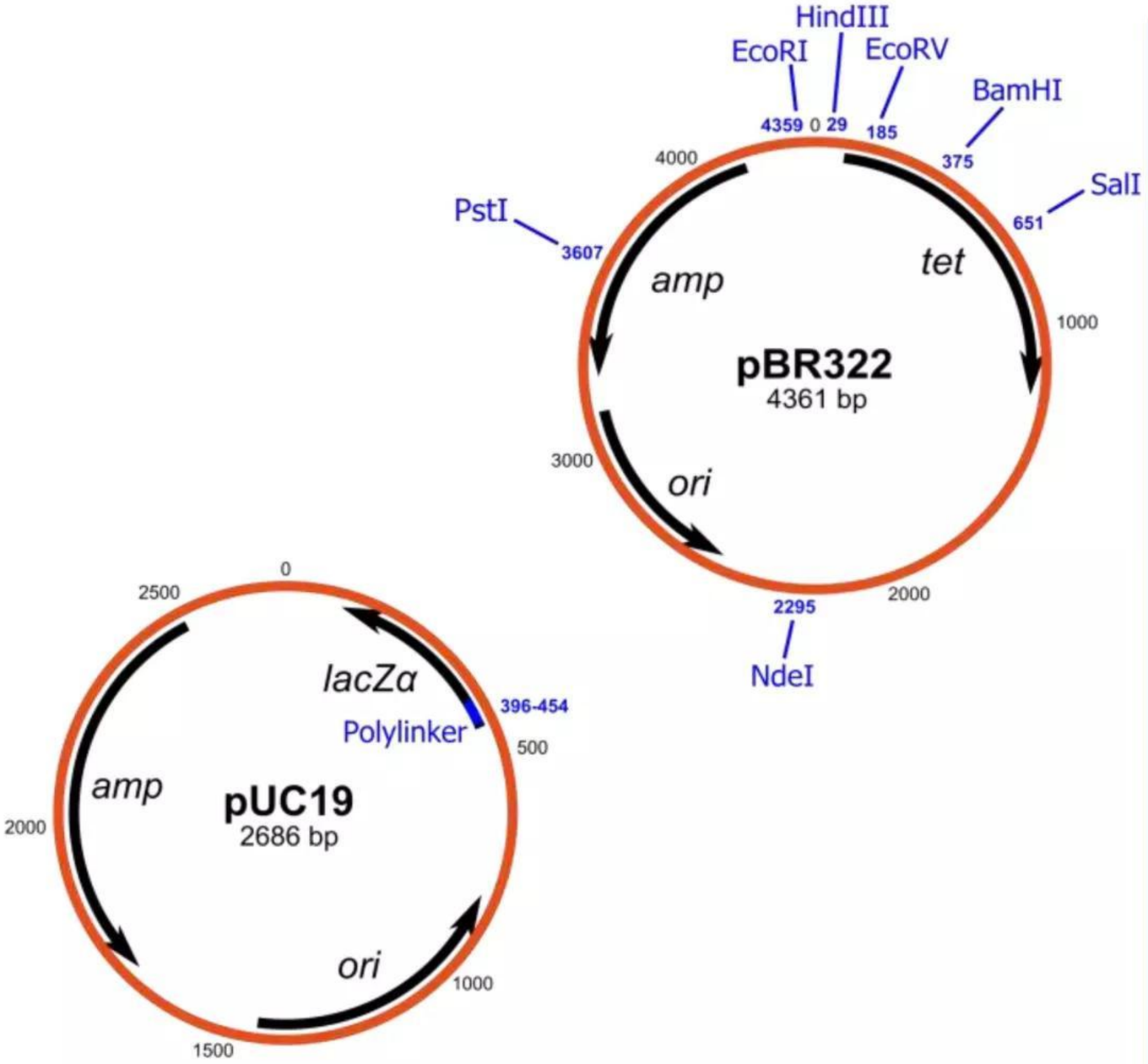
Continued…

- Stable for long term either as purified DNA or within bacterial cell preserved as glycerol stocks.
- Functional in many species including plants, worms, mice and even cultured human cells and useful for a diverse set of applications like investigation of promoters, small RNAs or other genetic elements.

DRAWBACK: Less useful for cloning large segment of DNA (>10kbp).

EXAMPLES

- pBR322
- pBR327
- pBR325
- pBR328
- pUC8
- pUC9
- pUC12
- pUC13



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THANK YOU..!!