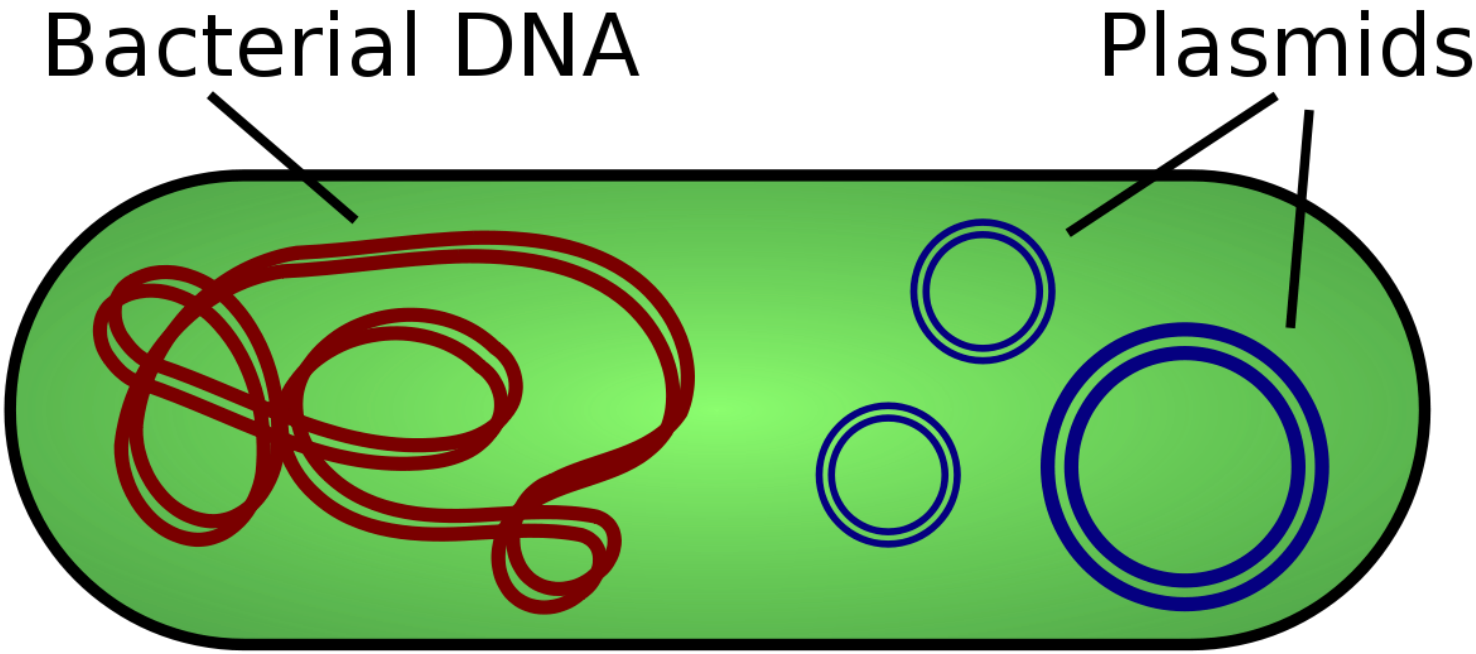
Manipulation of Purified Plasmids

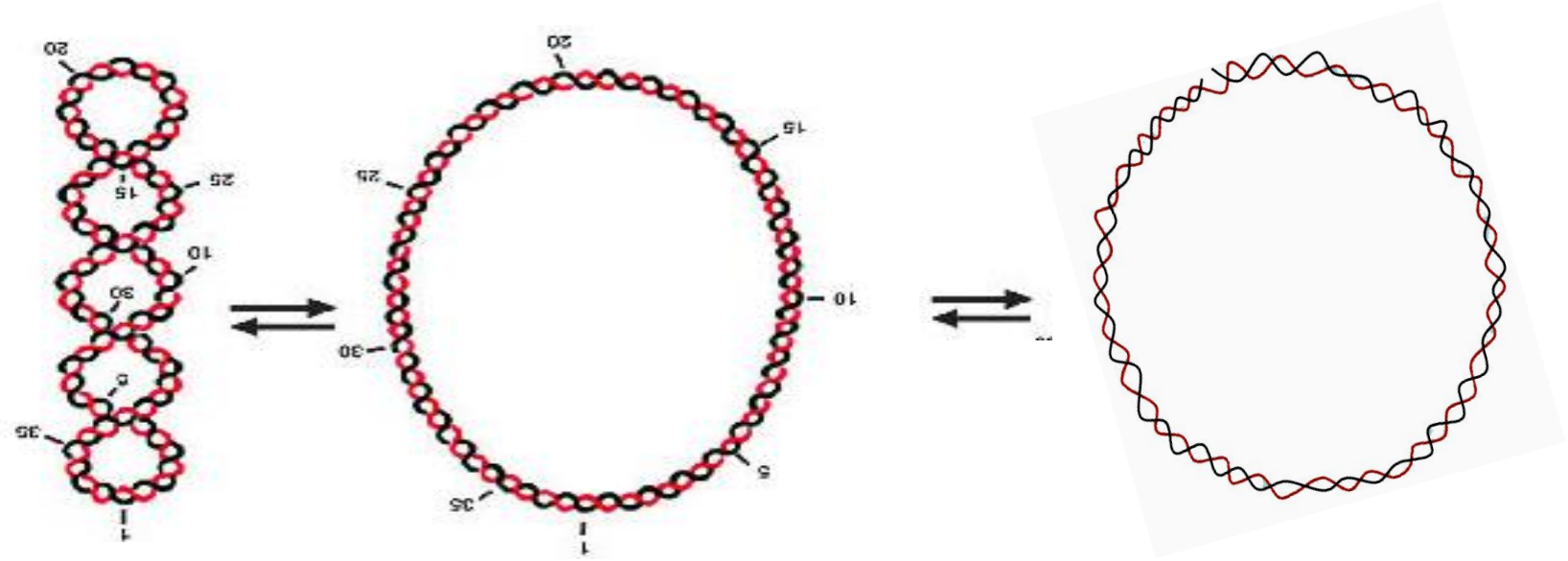
What are plasmids..??



Typical bacterial cell with chromosomal DNA and extra-chromosomal plasmids.

What are plasmids..??

- Plasmids are replicons which are stably inherited in an extra-chromosomal state in bacteria.
- Usually they exist as double-stranded circular DNA molecules.



Supercoiled plasmid

Covalently closed circle (CCC) When both strands of plasmid are intact

Open circle (OC) form When one strand of plasmid is open

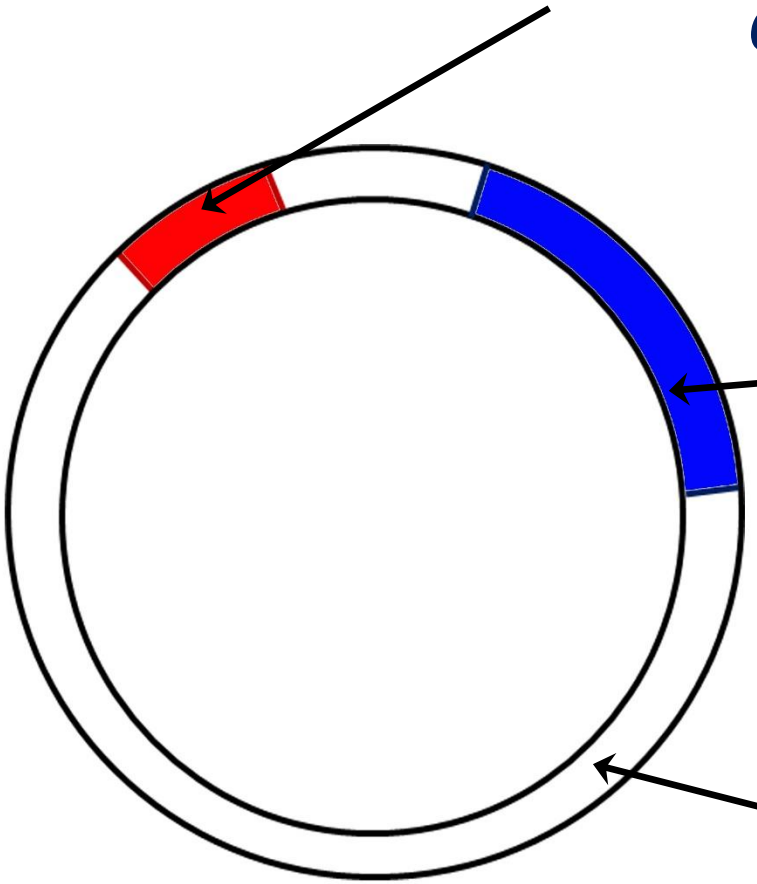
Type of plasmids

- Plasmids can be divided into two groups depending on the presence or absence of "*tra*" genes or transfer genes.
    1. Conjugative plasmids
    2. Non-conjugative plasmids

- Plasmids can also be divided into two groups based on their number of copies or abundance in a bacteria.
    1. Relaxed plasmids
    2. Stringent plasmids

- Plasmids are normally attributed to a phenotypic character in the bacterial system. However, the plasmid which is not associated with any phenotypic character is known as "cryptic plasmid".

Basic structure of plasmids



Ori (Origin of replication)

Other encoded proteins

Area open for genetic manipulations

A typical natural bacterial plasmid

Copy numbers of plasmids

- The copy number of a plasmid is determined by regulating the initiation of plasmid replication.
- Two major mechanisms of control of initiation have been recognized: regulation by antisense RNA and regulation by binding of essential proteins to iterons.
- Most of the cloning vectors in current use carry an ori region derived from plasmid Col E1 and copy-number control is mediated by antisense RNA.
- Plasmids can also present as multiple copies of same plasmid known as multimeric plasmids. However, at times multimeric plasmids may lead to segregative instability in the bacteria due to the same number of plasmid origins but fewer plasmid molecules.

Plasmid incompatibility

- Plasmid incompatibility is the inability of two different plasmids to coexist in the same cell in the absence of selection pressure.
- Groups of plasmids which are mutually incompatible are considered to belong to the same incompatibility (Inc) group.
- Plasmids will be incompatible if they have the same mechanism of replication control.
- Over 30 incompatibility groups have been defined in *Escherichia coli*.

Purification of plasmids

- The most used method is the “alkaline-lysis” method.
- In this method, the bacteria cells are harvested via centrifugation and then subjected to the lysis process in a lysis buffer.
- The lysis buffer typically consists of a strong base such as NaOH (sodium hydroxide), a detergent such as SDS (sodium dodecyl sulfate), and even at times enzymes like lysozyme.
- The alkaline environment and SDS help the bacteria cells to break releasing Plasmids along with other cell debris, including chromosomal DNA.

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- Then, sudden change in pH and neutralization by the use of high concentration of salts such as Potassium acetate (CH_3CO_2K) results in the reannealing of the covalently closed plasmid, while the chromosomal DNA and other debris still remain denatured and insoluble.

- In the next step, all these debris along with the chromosomal DNA can be separated by centrifugation.

- Finally, the plasmid can be isolated by the alcohol precipitation and then can be stored by dissolving it in an appropriate buffer.

- The other practiced methods for plasmid isolation are Cesium chloride (CsCl) centrifugation method, boiling method, and use of various commercial kits.

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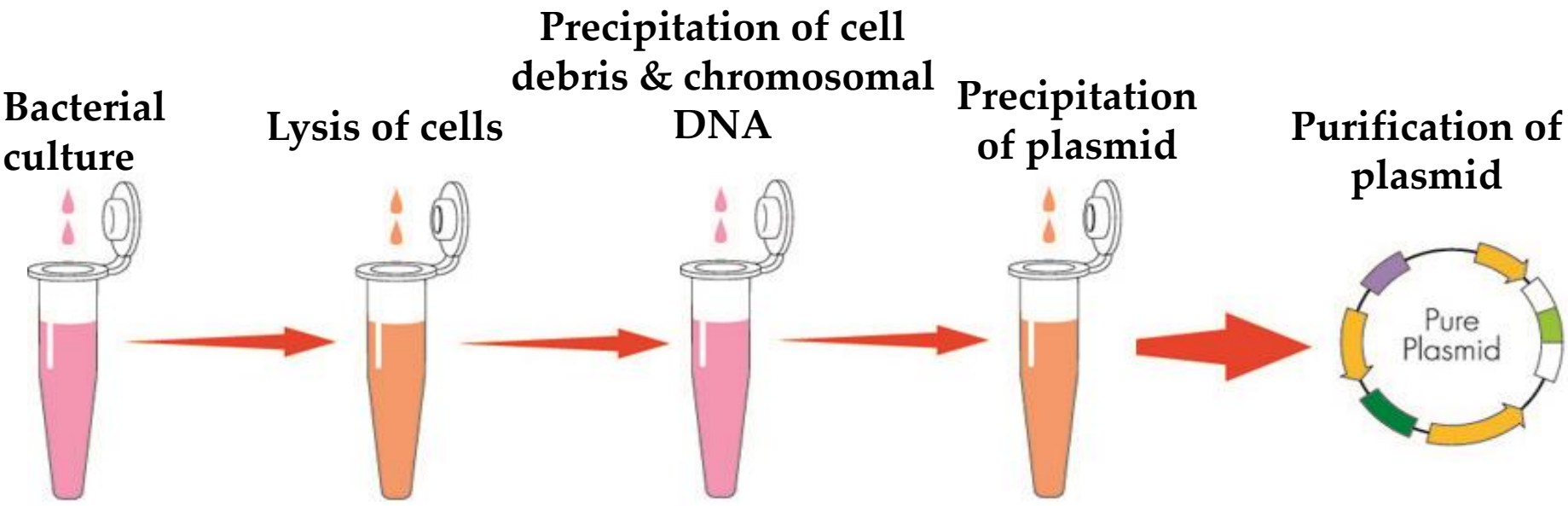


Typical Process of Plasmid Isolation

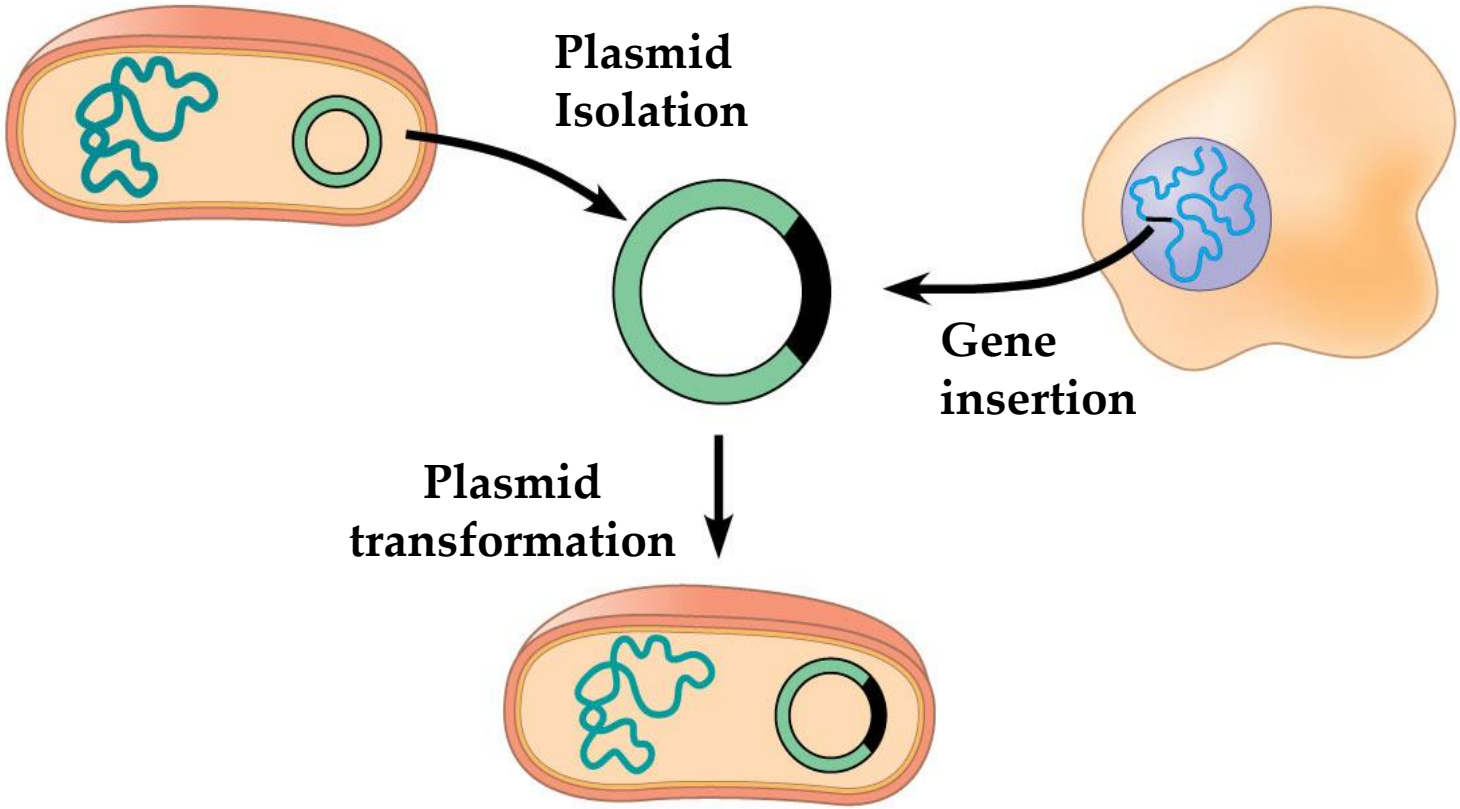
Manipulation of plasmids

- Once the purified plasmid is obtained, it's then manipulated for as per different necessary downstream processes.

- Plasmid manipulation majorly involve the changing of its original molecular structure and arrangement by adopting various biotechnological methods, including restriction digestion, adapter ligation, and DNA joining.

- Then the chimeric (recombinant) plasmid will be transferred into particular host cells in a process known as transformation.

- Often, the plasmid manipulation makes the first step of gene cloning, where a foreign DNA fragament or gene is inserted to a modified plasmid.



Typical Process of Gene cloning using modified a plasmid