Application of PLASMIDS in Biotechnology

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INTRODUCTION

History

Independent strands of DNA were first discovered in viral cells in the late 1940's by researchers investigating how germ can fight off germs and how traits are transmitted to the offspring by phages (viruses that live in bacteria) and DNA structures without chromosomes.

Initially, various scientists gave these circular DNA structures different names, such as "pangene" or "cytogene". It was not until 1952 that Joshua Lederberg came up with the term "plasmid", which has been in use ever since. He described it as " any non essential extra chromosomal hereditary element"

A different term, 'episome', defined as 'an unnecessary genetic component that can be independent or integrated with a chromosome' was brought up by and was agreed upon throughout the world.

At that time, the name episome appeared adequate , as the F (fertility) factor which was discovered then was known to interact with *Escherichia coli* chromosome in some cases. The term was coined until the 1960's when scientists began studying other extra chromosomal molecules, specially the R-factor.

Like the F-factor, R-factor can also be transmitted between two bacteria by sexual interaction. Nevertheless , many scientists observed that, in contrast to F-elements, the data is inconsistent with the notion that R-factors may interact with chromosomes. Since then, instead of the word 'episome', we have been using the term 'plasmid' ever since.

Plasmids

Plasmids are tiny DNA particles which have known to be physically separated from chromosomal DNA. They are present bacteria. They are small, circular shaped, double-stranded DNA molecules. The size of the plasmids belong to the range 1 to 1000 kilo base pairs. Linear plasmids also exist but mostly the known plasmids which are used in its application are circular.

They are not necessary for bacteria but can offer a choice. A category of plasmids,called the colicinogenic (usually referred as 'Col'), has a role of regulating the manufacture of proteins known as colicins. A category of plasmids called the Resistance plasmids (usually referred as 'R') provide antibacterial resistance. Some Col and R factor substances can be transferred between cells. They are also capable of multiplying exponentially . Plasmids are an important tool in the field of molecular biology. Gene therapy, Cloning, PCR amplifcation, in all these processes, plasmids play a key role.

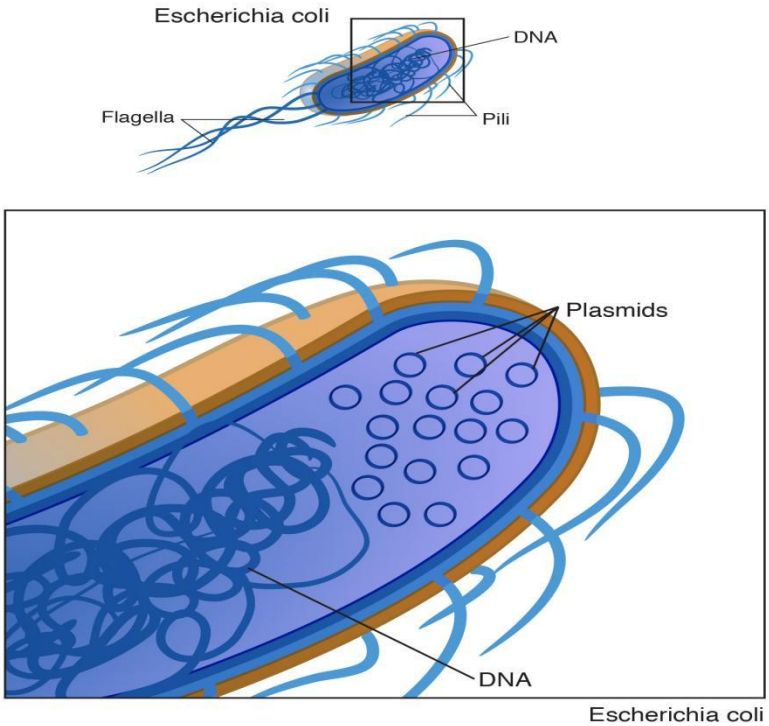

Fig 1. Circular Plasmids in *Escherichia coli*

Types of Plasmids

1) **Basis** - Ability of Plasmids to transfer to other bacteria.

Conjugative Plasmids - Bacteria reproduce by sexual conjugation . Conjugation is a phenomenon by which, genetic molecules are passed on from one cell to another by physical contact. Conjugative plasmids (F plasmids) carry the genes responsible for transmitting them to other cells. These genes are called transfer genes or tra genes that initiate the conjugation by directing the synthesis of sex pili . Plasmids may be transferred from one bacterial cell to another via the sex pili.

Non Conjugative Plasmids - Non conjugative plasmids do not have the ability to initate conjugation. For their transfer, there has to be presence of conjugative plasmids. In the transfer of non conjugative plasmids, the physical establishment has to be done by the conjugative plasmids, once that is done, the donor can transfer non conjugative
plasmids.

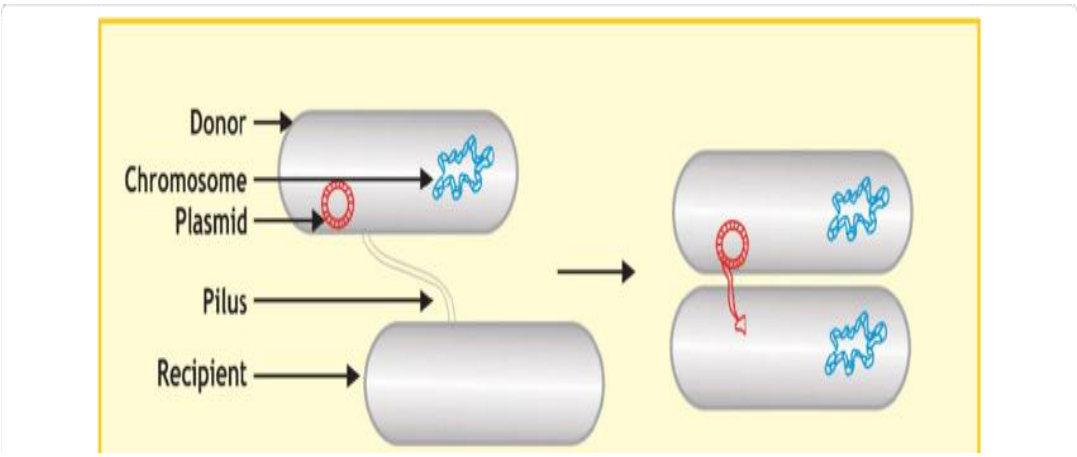

Fig 2. Transfer of plasmids between bacteria by conjugation

2) **Basis -** Classification on the basis of their function

F Plasmids - F here stands for 'Fertility'. This is a circular shaped DNA molecule which contains around 100 kbp. A part of circumference of the plasmid contains genes that regulate DNA replication. Another region, the transfer region contains the tra (transfer) genes and thereby permits genes to be moved from one cell to another by conjugation. There also exists a region which contains transposable elements (Elements in the upper right quarter of the **Fig 3.** shown below) . F Plasmids span a broad section of the conjugative plasmids. They contain the structural gene for 'Pilin'. Pilin is the pilus protein that is responsible in the formation of sex pilus. Bacteria containing them are termed as F+(F positive), and the ones which don't contain them are called F-(F negative).

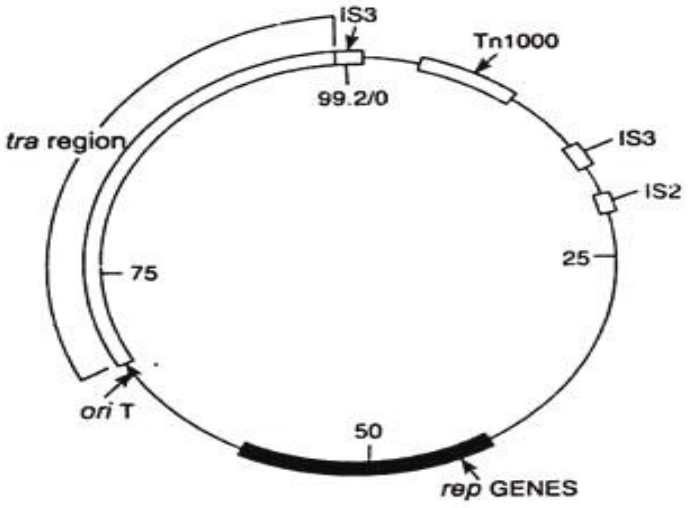

Fig 3. F Plasmid of *Escherichia coli.* Tra region conatins the transfer genes. Ori T here refers to the origin of transfer.

R Plasmids- R here stands for 'Resistance'.They confer resistance to antibiotics..R-plasmids usually contain genes which encode in enzymes that can tear down antibiotics. They are usually not connected to the host chromosome. Some R-plasmids contain genes that fight only one type and some as many as eight. Resistance plasids play an important role in microbiology and can have adverse effects on the treatment of bacterial infections.

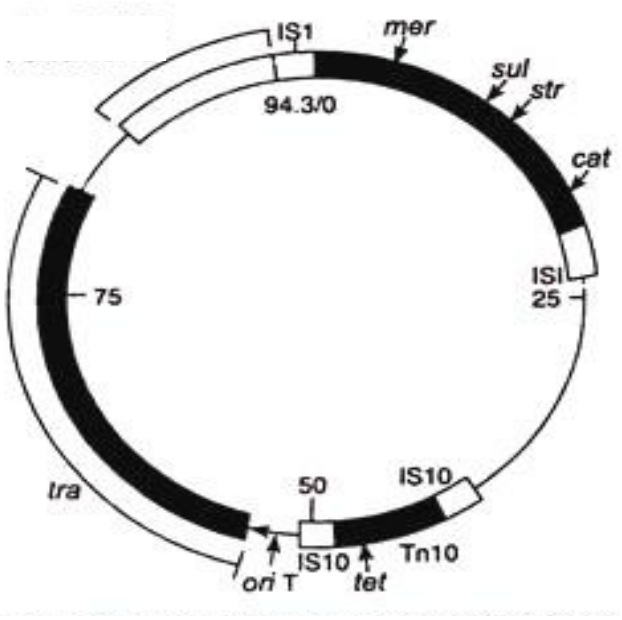

Fig 4. R100(A resistance plasmid.)

Virulence Plasmids - Virulence plasmids confer pathogenicity(the ability to cause disease). They are responsible for making a bacteria pathogenic so that it can fight the host defence.

This enables bacteria to grow within individuals and attack the host by replication. *Escherichia coli* is known to have virulence plasmids.It is present in the human gut, however strains of it are responsible for causing symptoms such as diarrhea , puking etc. *Salmonella enterica,* a gram negative bacteria also contains virulence plasmids.

Metabolic Plasmids - These are also known as 'degradative' plasmids. As the name suggests, these plasmids assist the host in digesting compounds which are not normally known to exist naturally such as camphor ,aromatic compounds(like toluene and xylene) and also some sugars.

Col Plasmids - 'Col' here refers to a bacteriocin (proteins that inhibit the growth of similar strains) named 'Colicin'. Col plasmids contain genes that are responsible for producing colicin.. Deterioration of DNA and RNA can also be done by these type of plasmids. Their action is known against similar strains. These protect the host bacterium by killing other bacteria . For example, *Escherichia coli* contains ColE1 plasmid.

APPLICATIONS

Plasmids in Gene Therapy - Plasmids play a significant role in gene therapy.They are widely used to inject human genes into the fight against disease. They are easy to use and their duplication in the bacterial cell is easy. They have the ability to fine-tune the damaged cells and to initiate therapeutic genes into them. They represent the simplest form of vectors(DNA molecules used to carry foreign material into another cell).They are found in almost all types of bacteria where they often contain antibiotic-resistant proteins. A gene therapy plasmid has a resistance gene which acts against antibiotics..

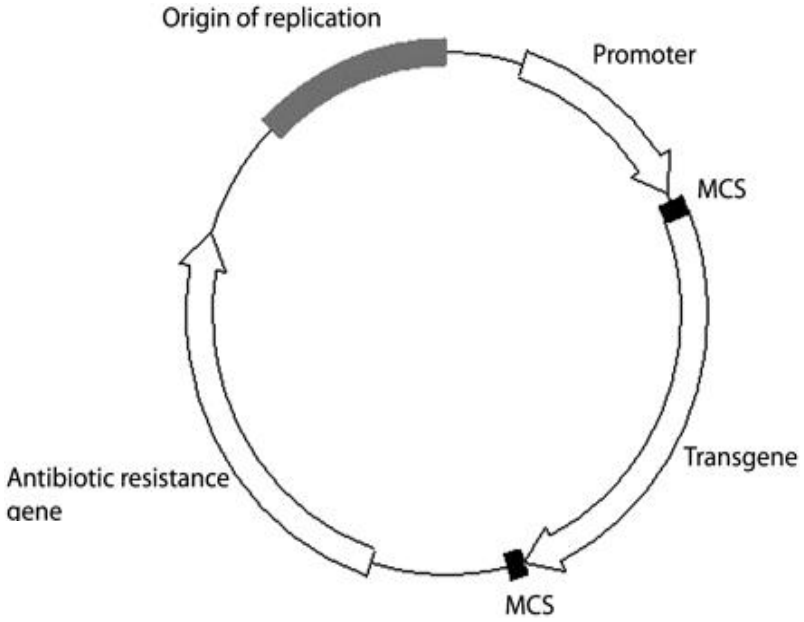

Fig 5. A model for a general plasmid used in gene therapy. MCS (also called the polylinker) is known as the Multiple cloning site. It allows insertion of gene .

pDNA(p here stands for plasmid) is capable of accepting large segments of genomic DNA .The structure of the plasmid is straight forward and allows for the production of regulatory factors which contribute to genetic transfer . Usually plasmids have 1 transfer gene,however some EC's(Expression cassettes) are capable of containing multiple proteins without any limitations of size. An expression cassette is a component of vector DNA and it consists of a gene and a regulatory sequence.

Cloning vectors - The cloning vectors which are used preferably throught the globe are genetically engineered plasmids.Cloning is usually done first using *Escherichia coli.* The plasmids are made in such a way that they can be as efficient as possible . These vectors are usually less in length than natural plasmids which are found in *E. coli* .

Plasmid based vectors not only incorporate the nucleotide sequence but, the origin of the replication, the drug-resistant component, and the place where the outer DNA fragments are placed .The ORI (origin of duplication) is the direct DNA chain of around 50 - 100 basic bases which are essential in the replication of the plasmids. ORI is the region where the replication is initiated.

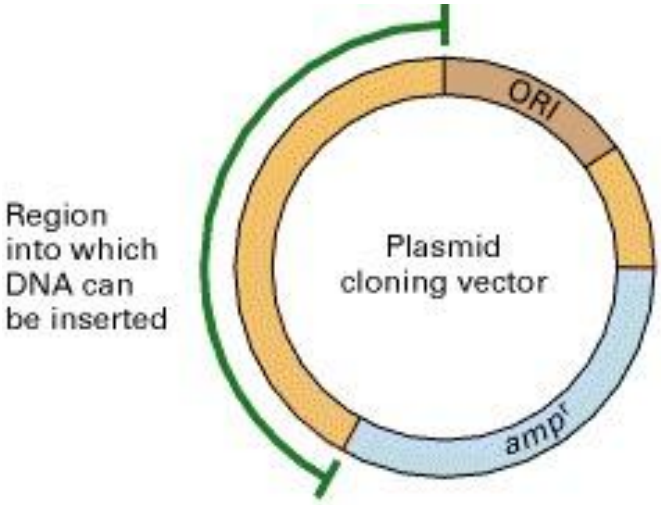

Fig 6. A cloning vector derived from a plasmid

As the replication process is started in the ORI, it pursues to surround the plasmid unaccompanied by its nucleotide sequence. Because of this,the DNA sequences which enter in these plasmids are replicated with the remaining plasmid DNA.

By the conversion of *E. coli* from these plasmids, all the resistance incorporating cells which have mutated from the original cells will posses plasmids having the same DNA sequence. Hence, the DNA which was first duplicated in the cell is transferred into multiple cells as all these come from one mutated parent.

DNA which is first put into the parent plasmid is called ' synthetic DNA'. The synthesis of DNA permits pieces with a similar nucleotide sequence to be segregated. DNA sequencing is therefore a powerful, yet simple way to clean up a particular piece of DNA in a complex piece of fragmentation and to produce a large number of these fragments of interest.

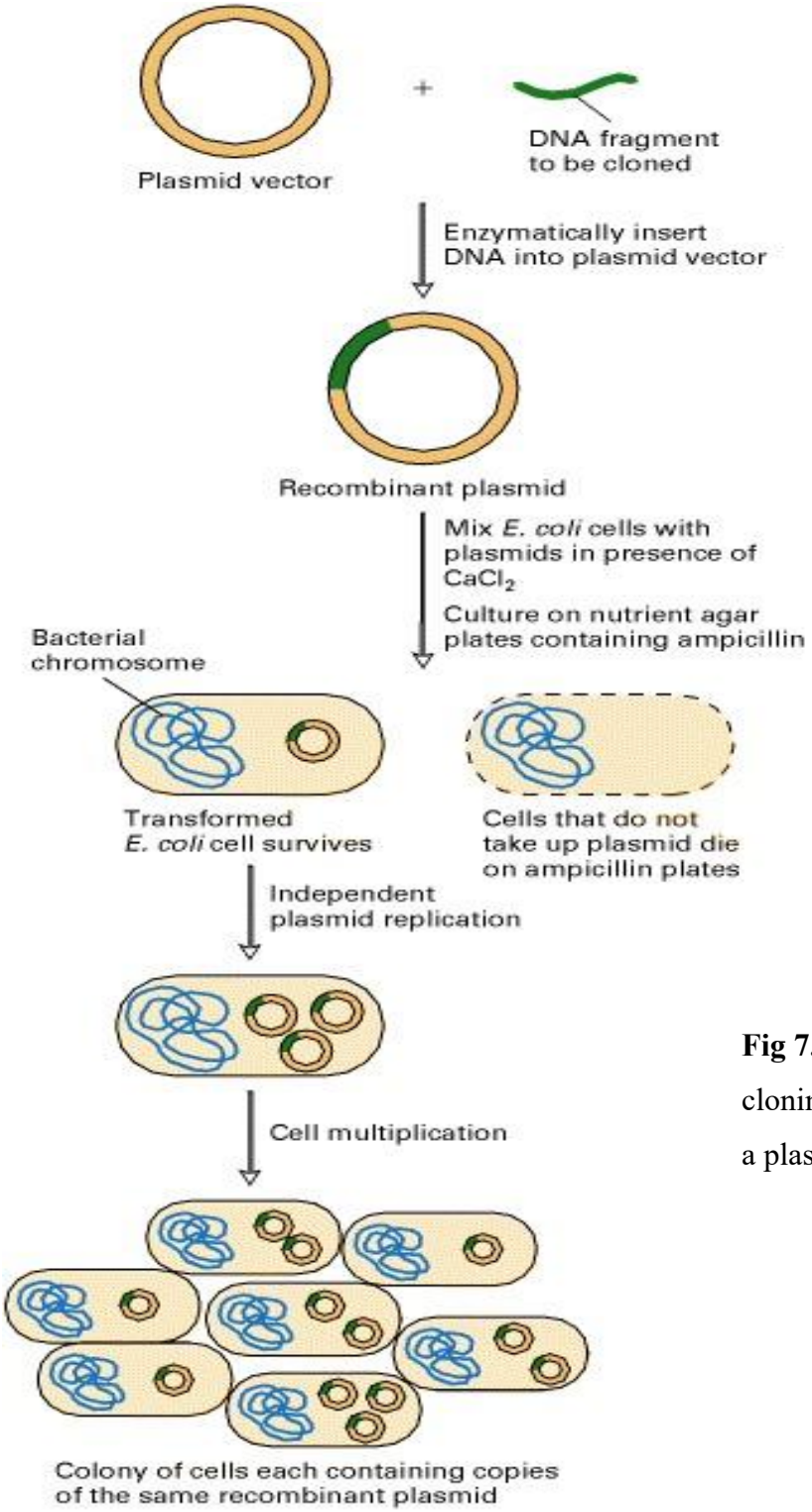

Fig 7. General procedure for cloning a DNA fragment in a plasmid vector.

PCR Amplification - The polymerase chain reaction (PCR) is reaction by which we can amplify DNA fragments i.e. make multiple copies of it. PCR enables us to produce copies even from a very less amount of DNA. This is performed in the beginning stages of DNA sequencing and is used to check the presence certain genes so that we can identify pathogens , in turn , also producing DNA profiles.

The pDNA which is fully purified can be taken as a template in the PCR reaction for amplification of certain DNA sequences.

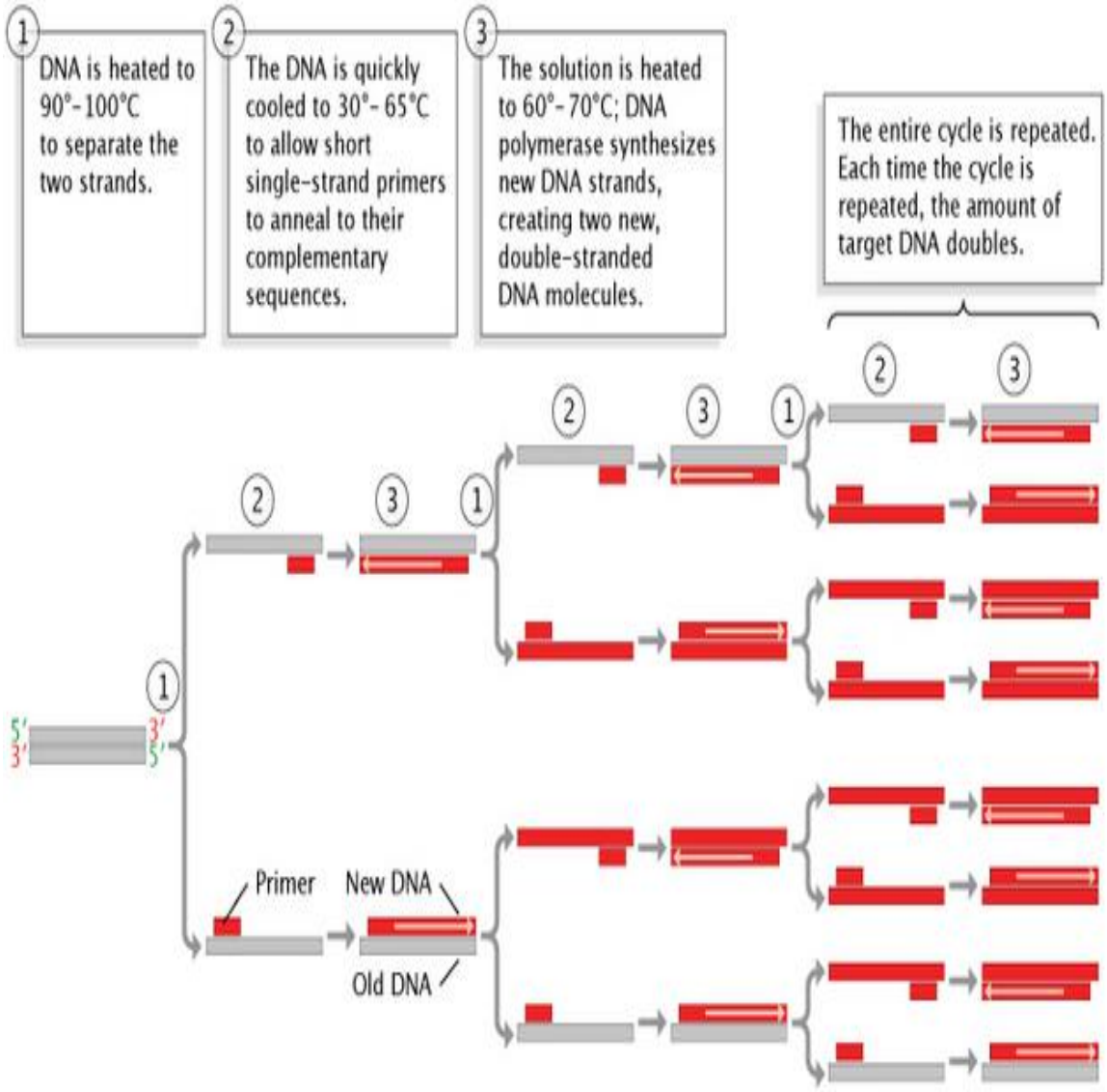

Fig 8. The polymerase chain reaction. (This process is usually repeated for 25-40 times)

REFERENCES

1)Lederberg, Joshua, Luigi L. Cavalli, and Esther M. Lederberg. “Sex Compatibility in Escherichia coli.” *Genetics* 37.6 (1952): 720–730.

2)Brock Biology of Microorganisms FIFTEENTH EDITION Madigan • Bender • Buckley • Sattley • Stahl ISBN 10: 1-292-23510-1 ISBN 13: 978-1-292-23510-3

3)Willey, J. M., Sherwood, L., Woolverton, C. J., & Prescott, L. M. (2008). Prescott, Harley, and Klein's microbiology. New York: McGraw-Hill Higher Education.

4)Molecular Cell Biology, 4th edition - Harvey Lodish, Arnold Berk, S Lawrence Zipursky, Paul Matsudaira, David Baltimore, and James Darnell. New York: W. H. Freeman; 2000. ISBN-10: 0-7167-3136-3

5)Gene cloning and DNA analysis : an introduction / T.A. Brown.—6th ed. p. cm. ISBN 978-1-4051-8173-0 (pbk. : alk. paper) – ISBN 978-1-4443-3407-4

6)Molecular Biology of the Cell, 4th edition
Bruce Alberts, Alexander Johnson, Julian Lewis, Martin Raff, Keith Roberts, and Peter Walter. New York: Garland Science; 2002.