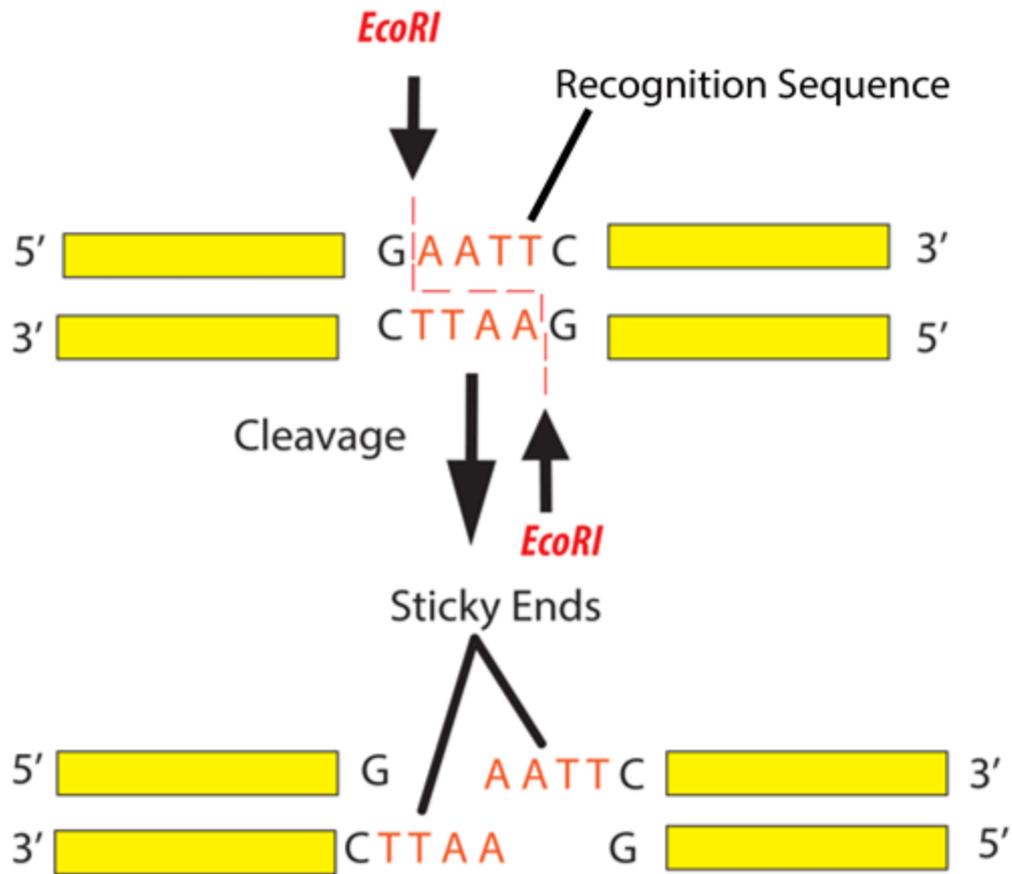


Molecular cloning and genetic engineering

- Molecular cloning is a set of experimental methods in molecular biology that are used to assemble recombinant DNA molecules and to direct their replication within host organisms
- Genetic engineering is the direct manipulation of an organism's genes using biotechnology. It is a set of technologies used to change the genetic makeup of cells, including the transfer of genes within and across species boundaries to produce improved or novel organisms.
- Both technologies utilize a variety of enzymes that act upon nucleic acids

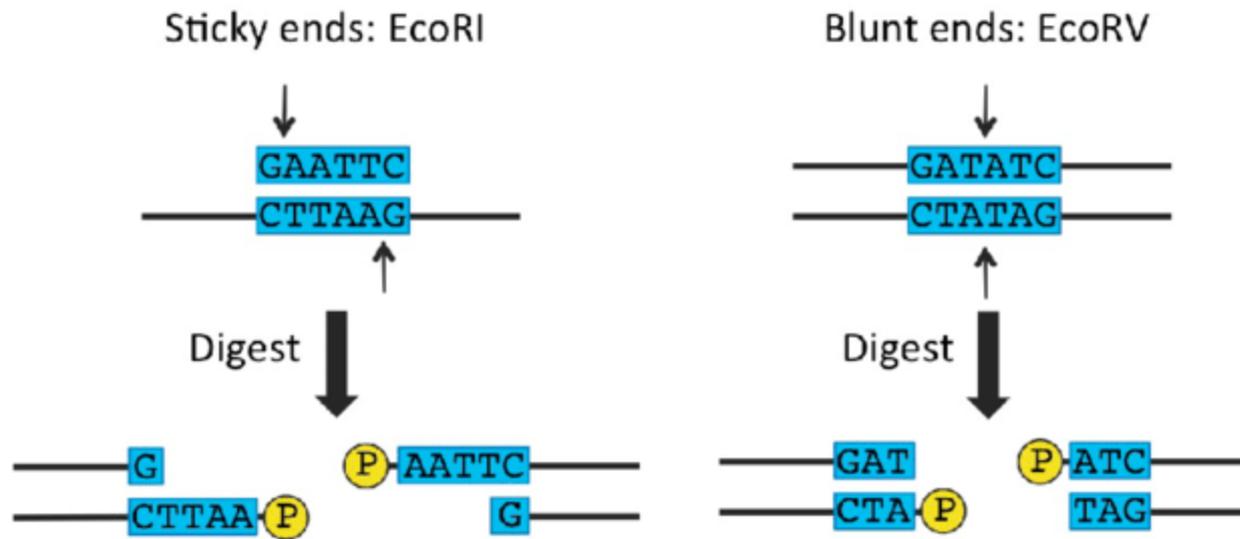
Restriction endonucleases

- In nature there found many different enzymes called *restrictases* or, more properly, *restriction endonucleases*. These enzymes recognize specific short sequences within target DNA molecules. If that sequence is found the restrictase will cut the DNA molecule by introducing breaks (nicks) into both strands of DNA



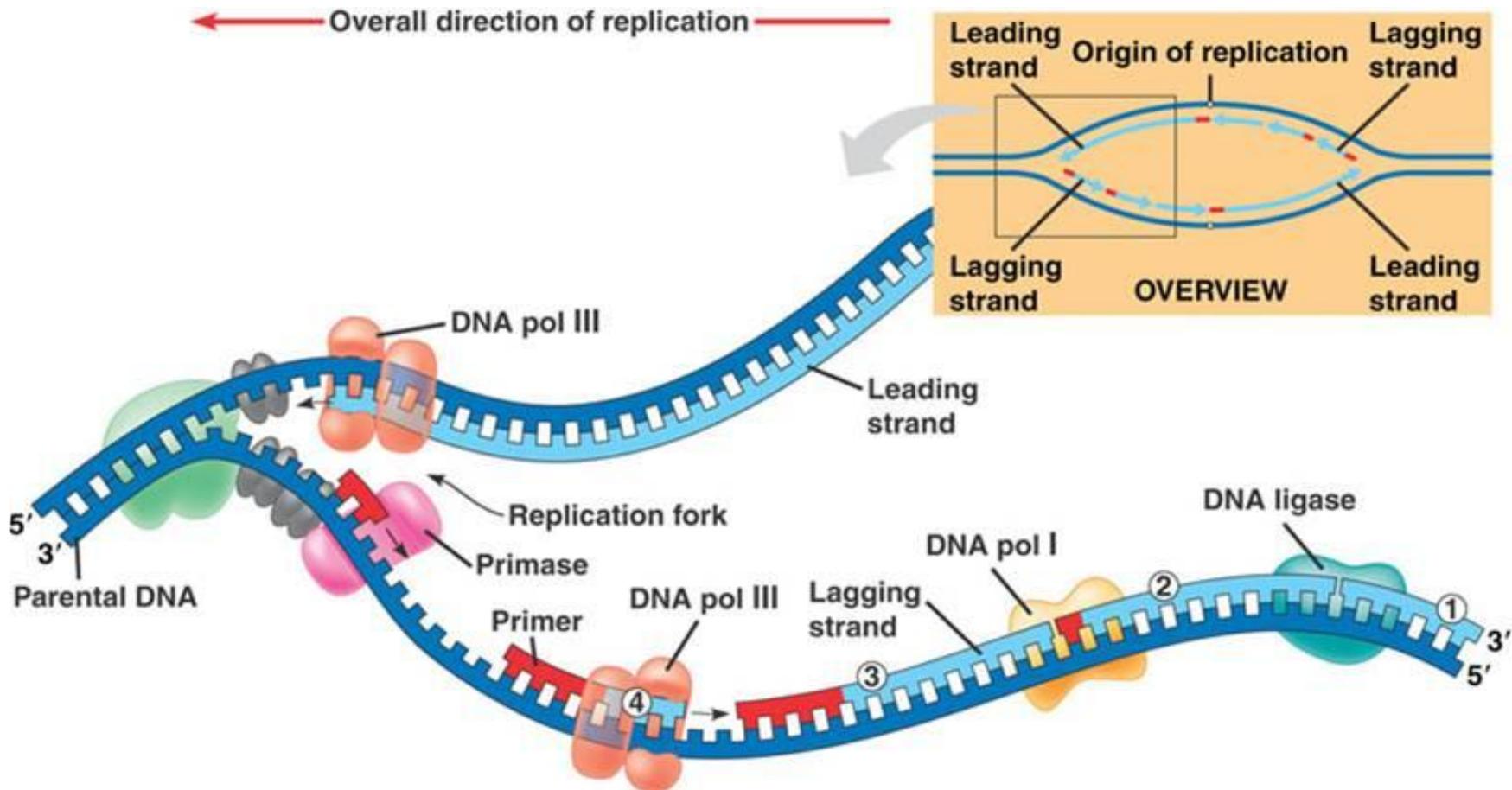
Hundreds of different restrictases recognizing unique DNA sequences were discovered. They are widely used for DNA analysis and engineering.

Digestion with a restrictase will result in a DNA fragment ending with sticky ends or with blunt ends

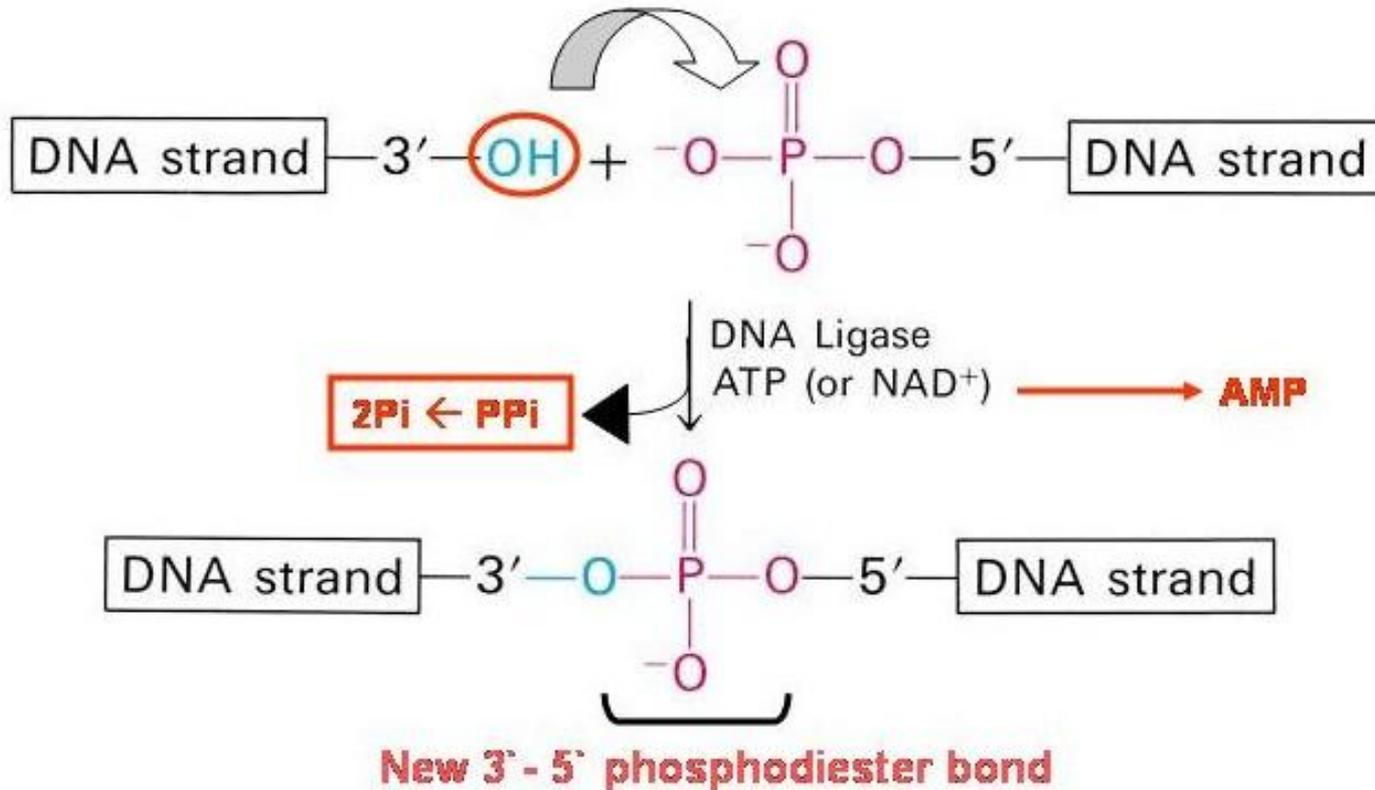


DNA ligase

- *DNA ligase* is an enzyme, joins DNA strands together by catalyzing the formation of a phosphodiester bond.
- In other words DNA ligase repairs nicks in dsDNA. One of the important functions of this enzyme is joining Okazaki fragments together.
- DNA ligase can also join blunt ends of DNA fragments



DNA ligase



Molecular cloning

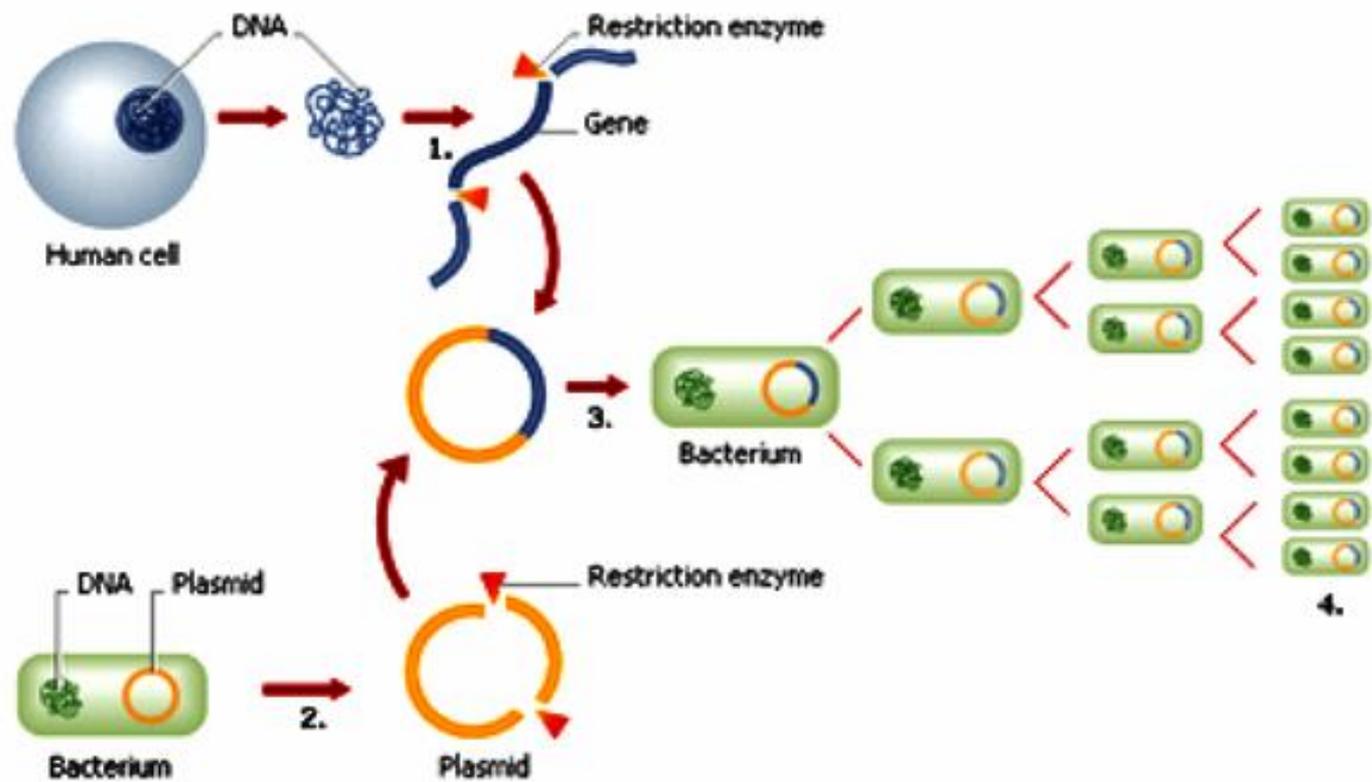
- Molecular cloning of DNA is a procedure that employs creation of recombinant DNA molecules and their subsequent replication in host organisms
- In this case “cloning” means creation of a population of host cell carrying identical DNA molecules including the recombinant one

Purposes of DNA cloning

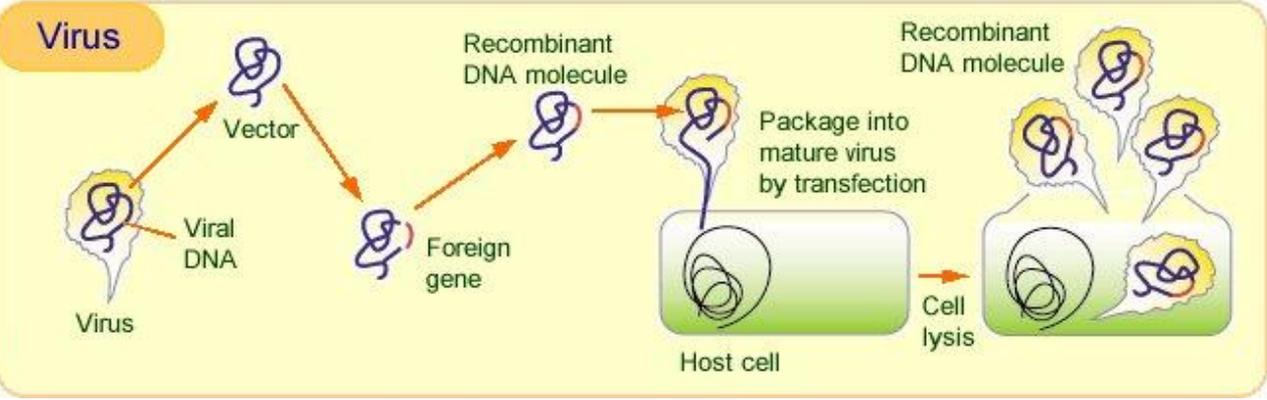
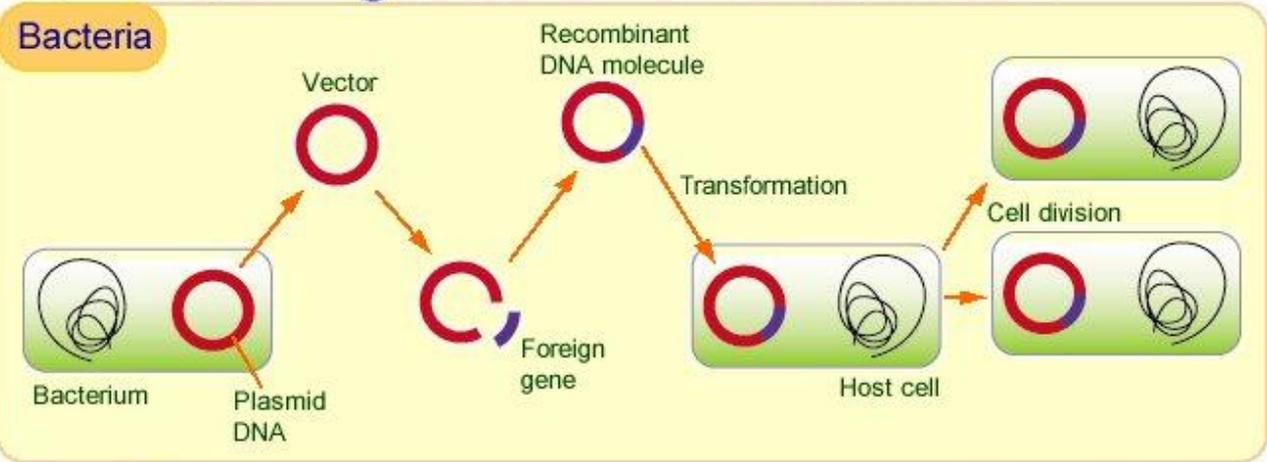
- Creating multiple copies of the same DNA molecule for studies, e.g. DNA sequencing
- Manipulation with cloned DNA molecules, e.g. *in vitro* mutagenesis
- Expression of the cloned gene in the host organism

Cloning vectors

- Cloning experiments often utilize DNA molecules called *cloning vectors*.
- A cloning vector is a small piece of DNA that can be stably maintained in an organism, and into which a foreign DNA fragment can be inserted.
- Thus a foreign DNA fragment is maintained as a part of a *recombinant DNA molecule* consisting of a vector and the fragment.



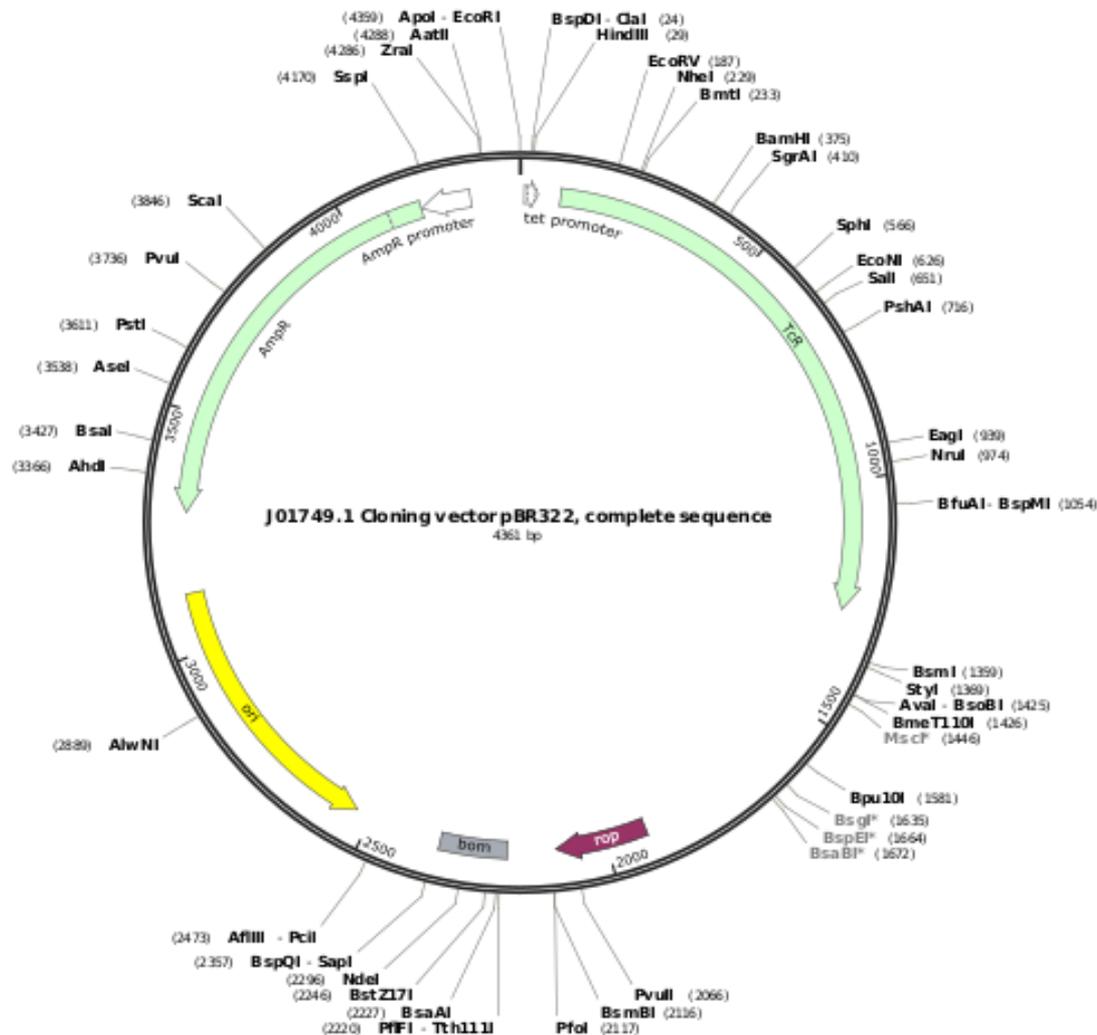
Gene Cloning with Different Vectors



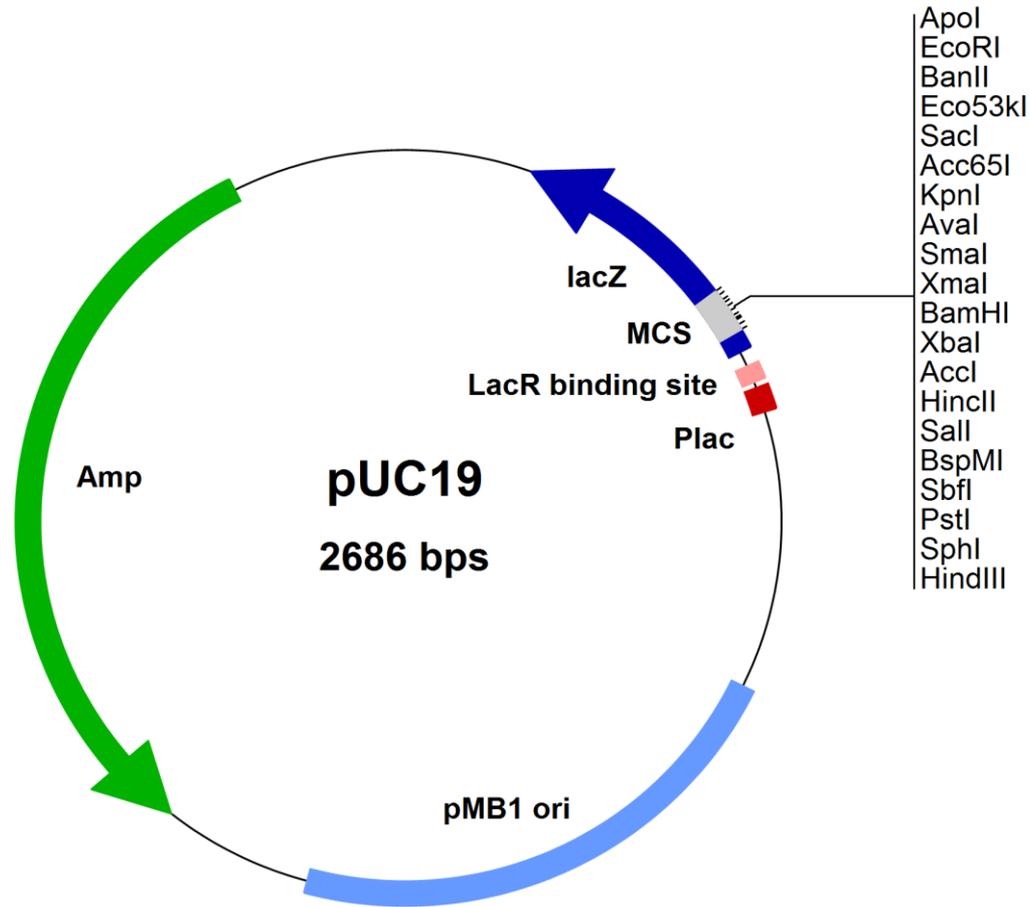
Plasmid vector

- Must replicate in the host cell
- Must have a selective marker
- Preferably should have a way to select recombinant molecules

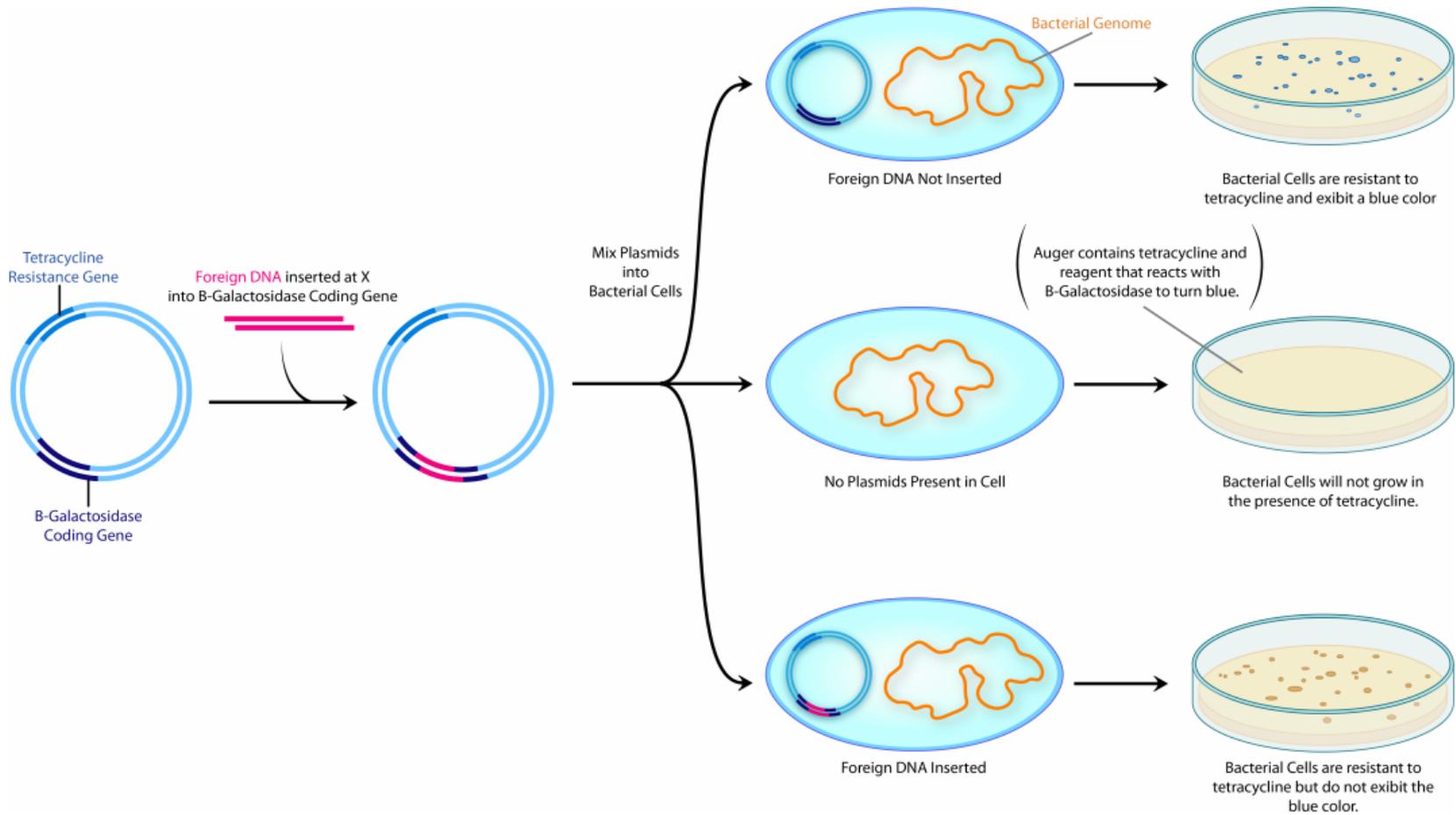
pBR322 - one of the first vectors



pUC19- vector for “blue-white selection”



“blue-white selection”



“blue-white selection”



pUC19 Multiple Cloning Site (MSC_

pUC19

