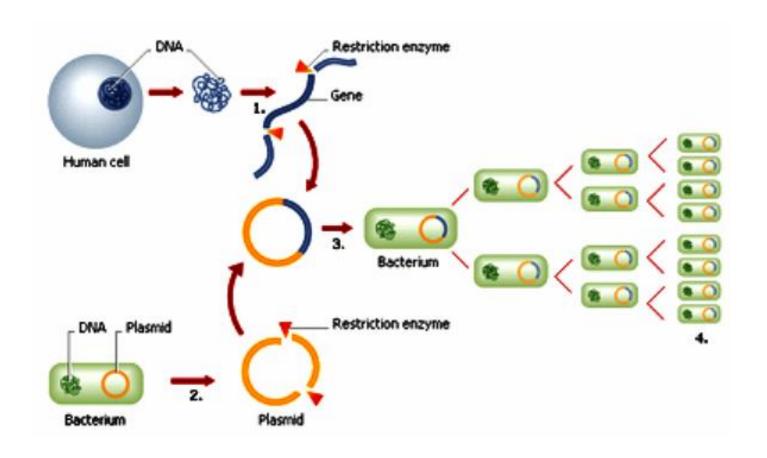
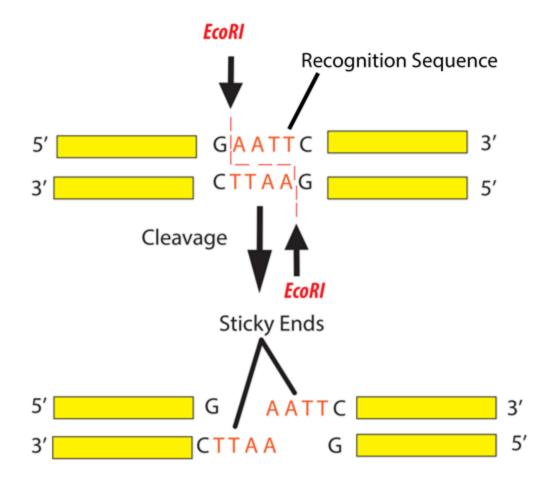
DNA cloning

- 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

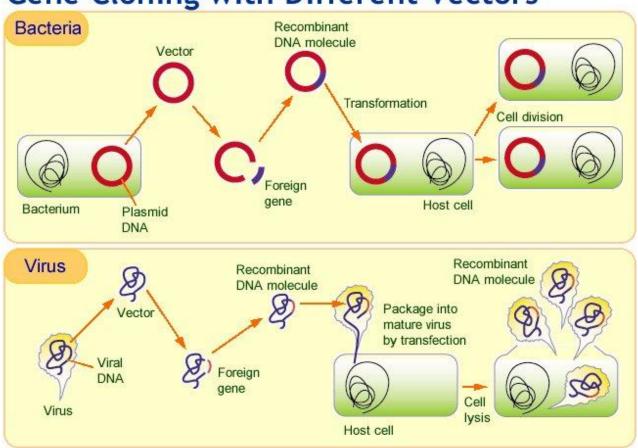
- Creating multiple copies of the same DNA molecule for studies, e.g. DNA sequencing
- Manipulation with cloned DNA molecules, e.g. in vitro mutagenesis
- Expression of the cloned gene in the host organism





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

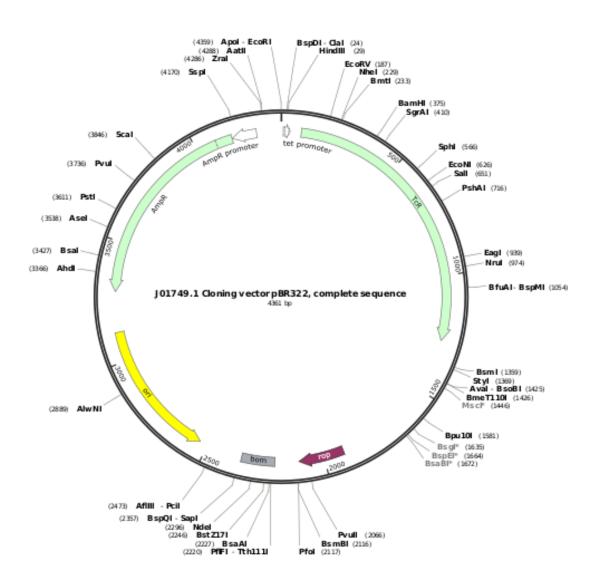
Gene Cloning with Different Vectors



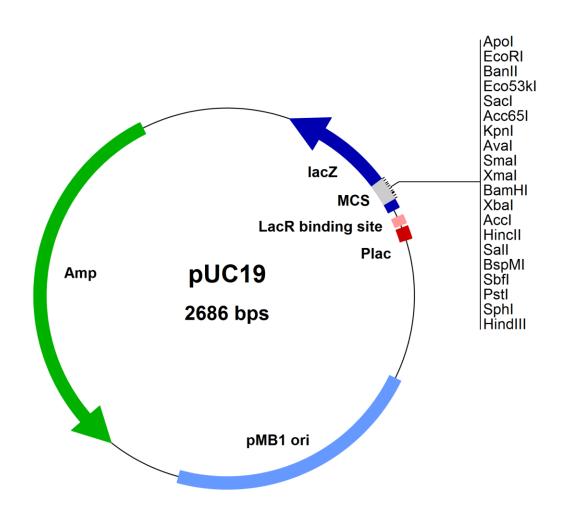
Plasmid vector

- 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"



pUC19 Multiple Cloning Site (MSC_

pUC19



5' GITGIAN AND GACGGC CAGTGA ATTICGA GCT CGG TAC CCG GGG ATC CTC TAG AGT CGA CCT GCA GGC ATG CAA GCT TGG CGT ANTICAT GGT CAT AGC TGTTTC CTG 3'
3' CANCATTITIC CTG CCG GTC ACTITAA GCT CGA GCC ATG GGC CCC TAG GAG ATC TCA GCT GGA CGT CCG TAC GTT CGA ACC GCA TTAGTA CCAGTA TCG ACANAAG GAC 5'
LacZ ← Leu Val Val Val Ala Leu Ser Asn Ser Ser Pro Val Arg Pro Asp Glu Leu Thr Ser Arg Cvs Ala His Leu Ser Pro Thr He Met Thr Met

M13/pUC reverse sequencing primer (-26),17-mer