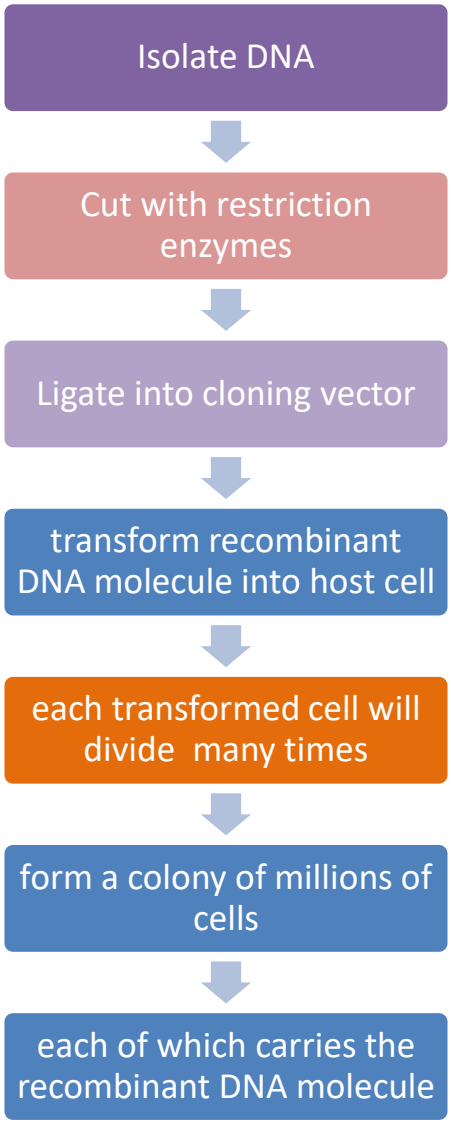
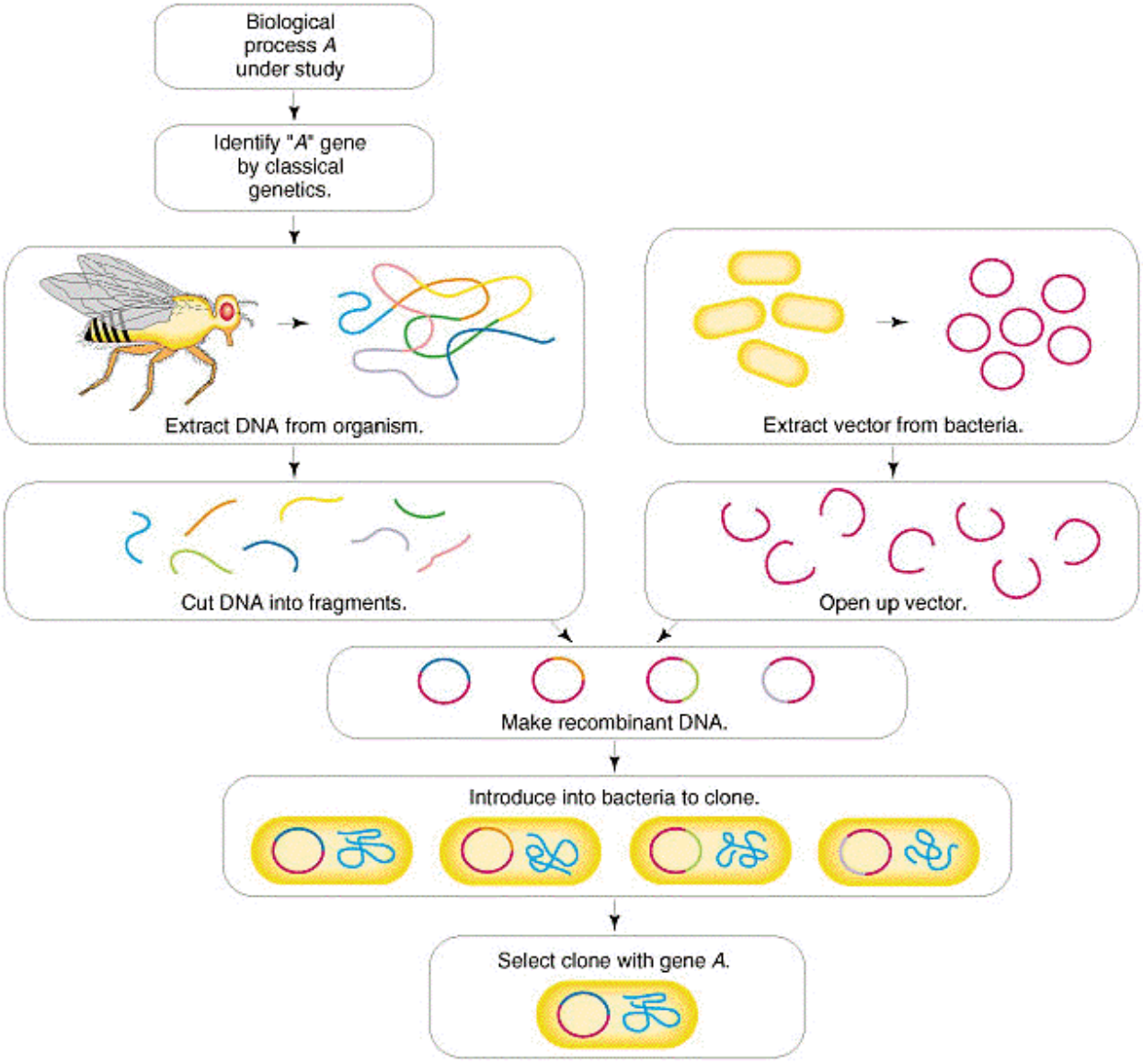
RECOMBINANT DNA TECHNOLOGY

Recombinant DNA refers to the creation of new combinations of DNA segments that are not found together in nature. The isolation and manipulation of genes allows for more precise genetic analysis as well as practical applications in medicine.

Making recombinant DNA-





Isolating DNA

1. Crude isolation of donor (foreign) DNA is accomplished by isolating cells → disrupting lipid membranes with detergents → destroying proteins with phenol or proteases → degrading RNAs with RNase → leaving DNA at the end
2. Crude isolation of plasmid vector DNA is accomplished by an alkaline lysis procedure or by boiling cells which removes bacterial chromosomal DNA from plasmid DNA.
3. To get purer DNA from either (1) or (2), crude DNA is
a) Fractionated on a CsCl2 gradient
b) Precipitated with ethanol

c) Poured over a resin column that specifically binds DNA

B. Cutting DNA

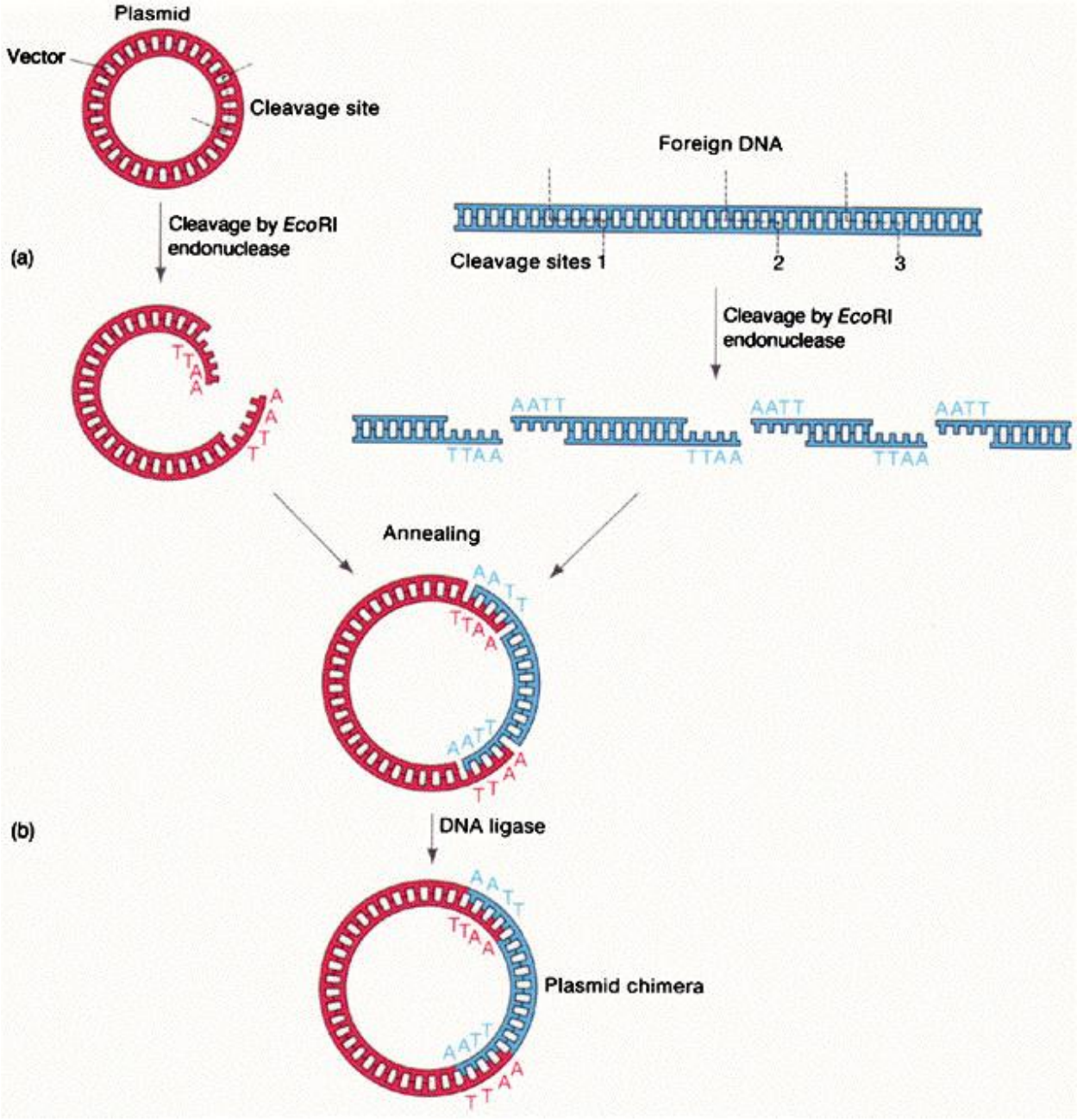
1. DNA can be cut into large fragments by mechanical shearing.
2. Restriction enzymes are the scissors of molecular genetics. Restriction enzymes (RE) are endonucleases that will recognize specific nucleotide sequences in the DNA and break the DNA chain at those points. A variety of RE have been isolated and are commercially available. Most cut at specific palindromic sites in the DNA (sequence that is the same on both antiparallel DNA strands). These cuts can be a staggered which generate "sticky or overhanging ends" or a blunt which generate flush ends.

C. Joining DNA

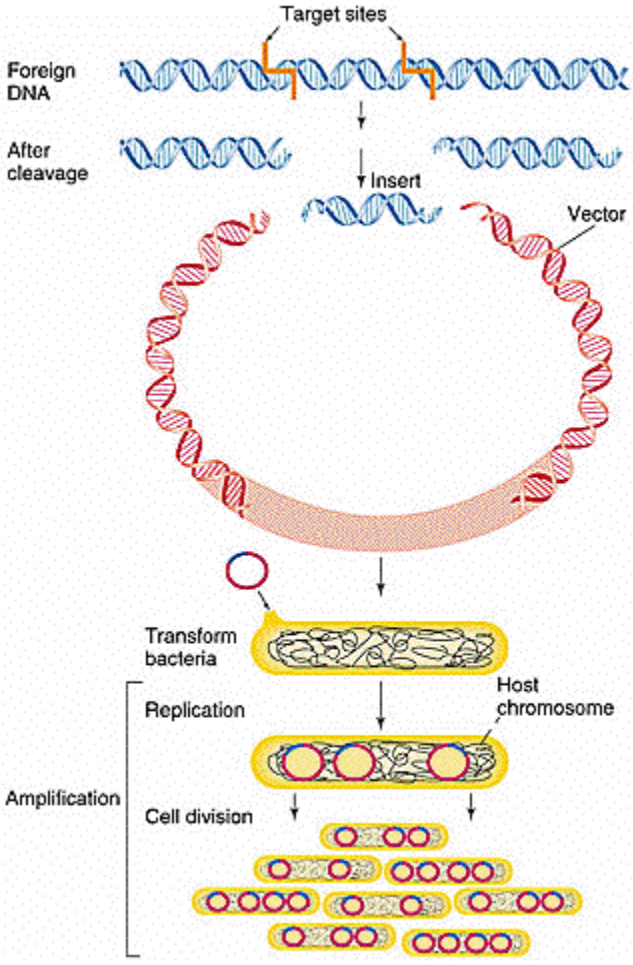
Once you have isolated and cut the donor and vector DNAs, they must be joined together. The DNAs are mixed together in a tube. If both have been cut with the same RE, the ends will match up because they are sticky. DNA ligase is the glue of molecular genetics that holds the ends of the DNAs together. DNA ligase creates a phosophodiester bond between two DNA ends.

D. Amplifying the recombinant DNA

To recover large amounts of the recombinant DNA molecule, it must be amplified. This is accomplished by transforming the recombinant DNA into a bacterial host strain. (The cells are treated with CaCl2 à DNA is added à Cells are heat shocked at 42 C à DNA goes into cell by a somewhat unknown mechanism.) Once in a cell, the recombinant DNA will be replicated. When the cell divides, the replicated recombinant molecules go to both daughter cells which themselves will divide later. Thus, the DNA is amplified.


Plasmid
Vector
Cleavage site
Foreign DNA
Cleavage by EcoRI endonuclease
(a)
Cleavage sites 1
2
3
Cleavage by EcoRI endonuclease
AATT
TTAA
Annealing
DNA ligase
(b)
Plasmid chimera

Joining DNA



Amplification of DNA

DNA clone = A section of DNA that has been inserted into a vector molecule and then replicated in a host cell to form many copies.

E. Vectors

1. Requirements for a cloning vector

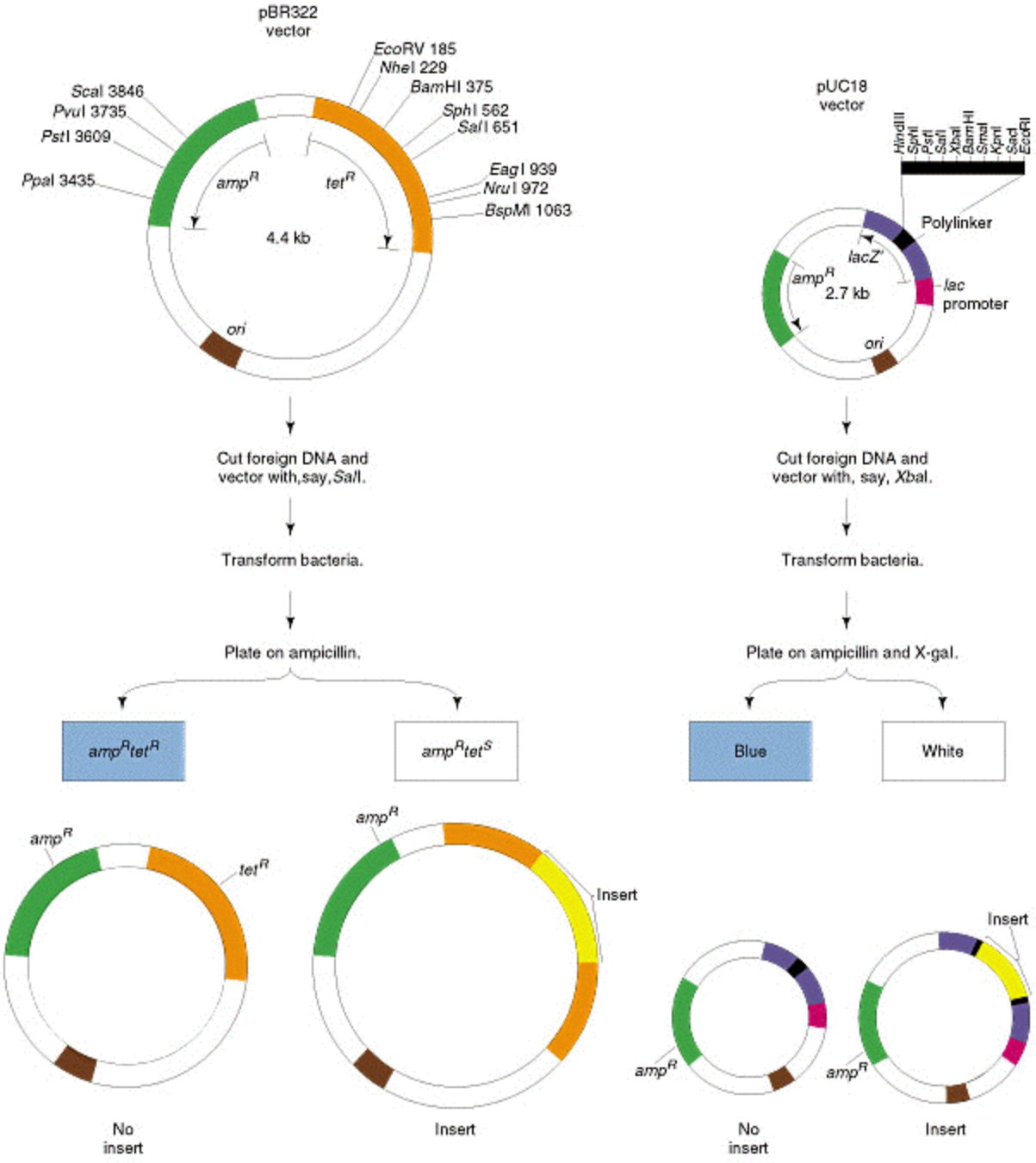
a) Should be capable of replicating in host cell

b) Should have convenient RE sites for inserting DNA of interest

c) Should have a selectable marker to indicate which host cells received recombinant DNA molecule

d) Should be small and easy to isolate

2. Bacterial plasmids are small, circular DNA molecules that are separate from the rest of the chromosome. They replicate independently of the bacterial chromosome. Useful for cloning DNA inserts less that 20 kb (kilobase pairs). Inserts larger than 20 kb are lost easily in the bacterial cell.



3. Bacteriophage lambda (45 kb) contains a central region of 15 kb that is not required for replication or formation of progeny phage in *E. coli*. Thus, lambda can be used as a cloning vector by replacing the central 15 kb with 10-15 kb of foreign DNA. This is done as follows: mix RE cut donor DNA and lambda DNA in test tube à ligate à use *in vitro* packaging mix that will assemble progeny phage carrying the foreign DNA à infect *E. coli* with the phage to amplify.

4. Cosmids are hybrids of phages and plasmids that can carry DNA fragments up to 45 kb. They can replicate like plasmids but can be packaged like phage lambda.
5. Expression vectors are vectors that carry host signals that facilitate the transcription and translation of an inserted gene. They are very useful for expressing eukaryotic genes in bacteria.
6. Yeast artificial chromosomes (YACS) are yeast vectors that have been engineered to contain a centromere, telomere, origin of replication, and a selectable marker. They can carry up to 1,000 kb of DNA. Since they are maintained in yeast (a eukaryote), they are useful for cloning eukaryotic genes that contain introns. Also, eukaryotic genes are more easily expressed in a eukaryotic host such as yeast.
7. Bacterial artificial chromosomes (BACS) are bacterial plasmids derived from the F plasmid. They are capable of carrying up to 300 kb of DNA.