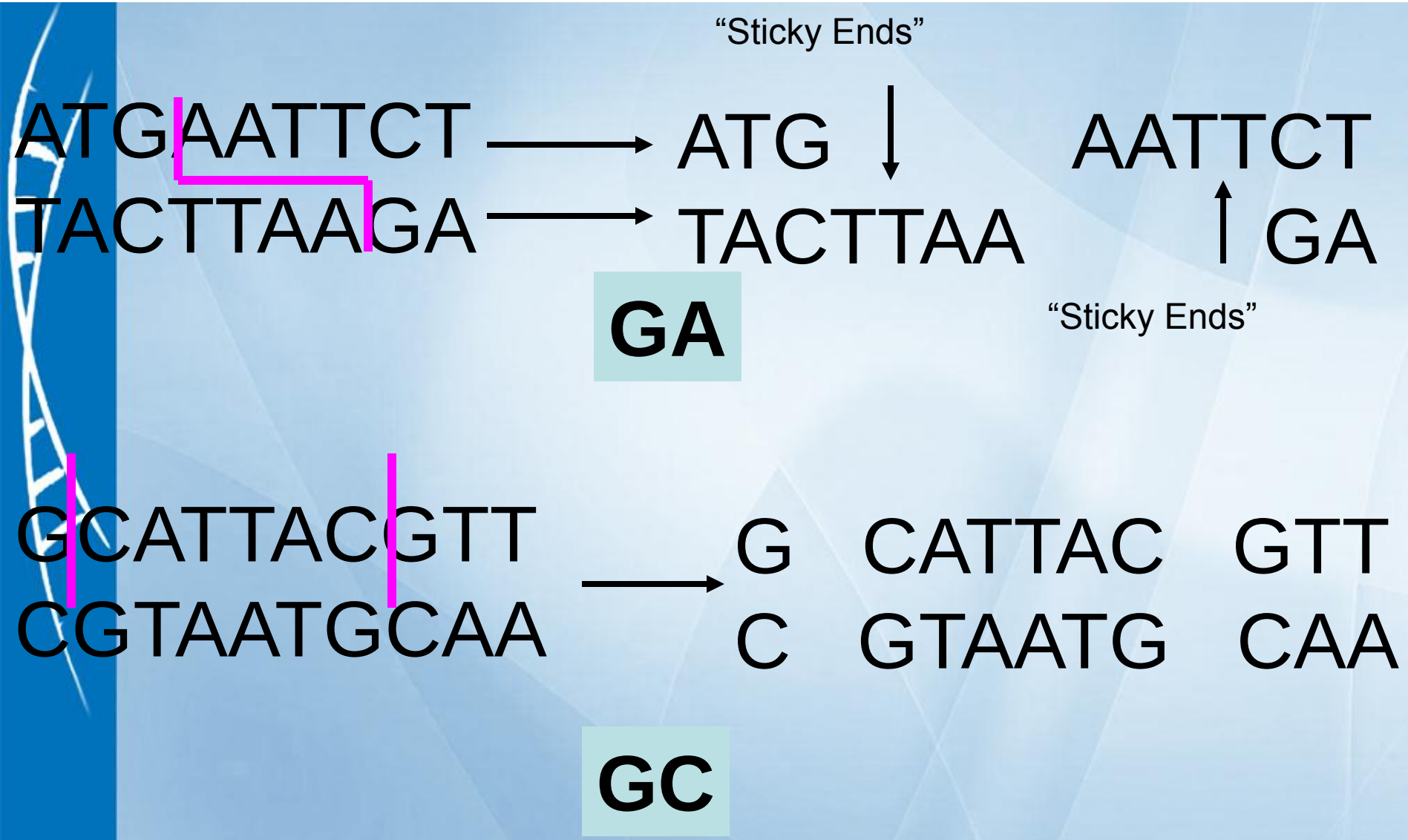
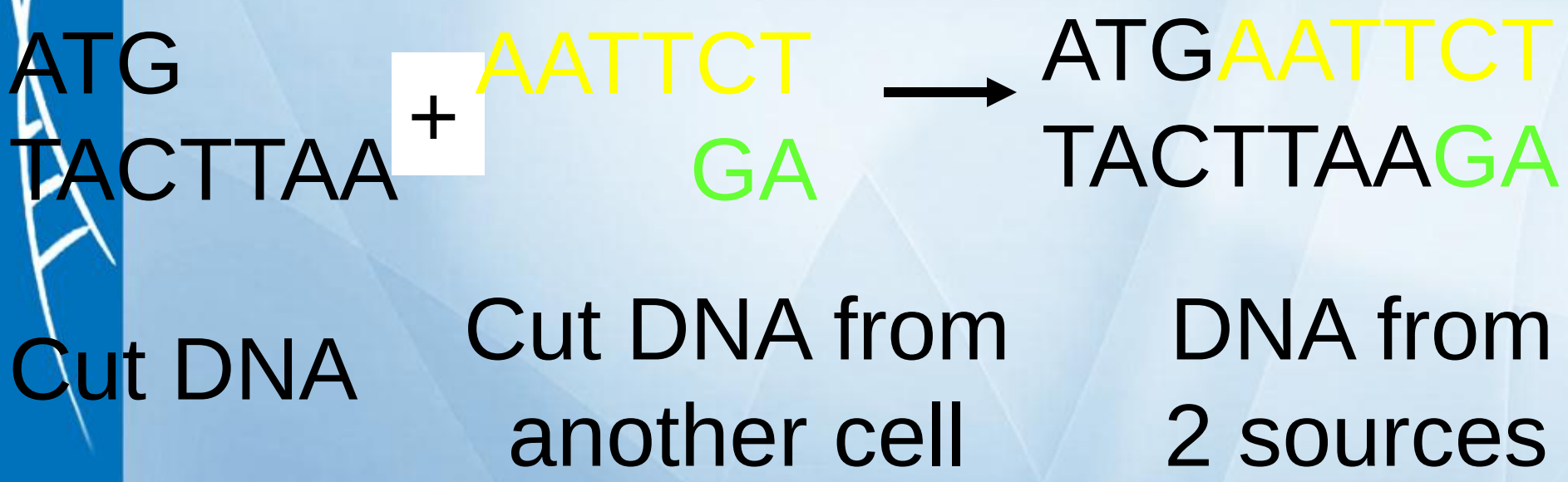
What base sequences were these **Restriction Enzymes** specific for?

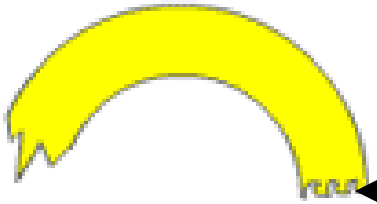


How is DNA Modified?

Step 3: The cut DNA sequence is then combined with the DNA of another organism.



What do we call DNA that comes from 2 sources?

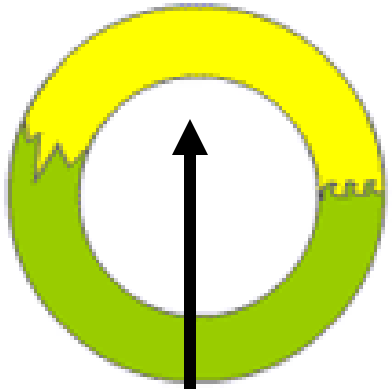


Source 1

A restriction enzyme has cut out this gene.

Source 2

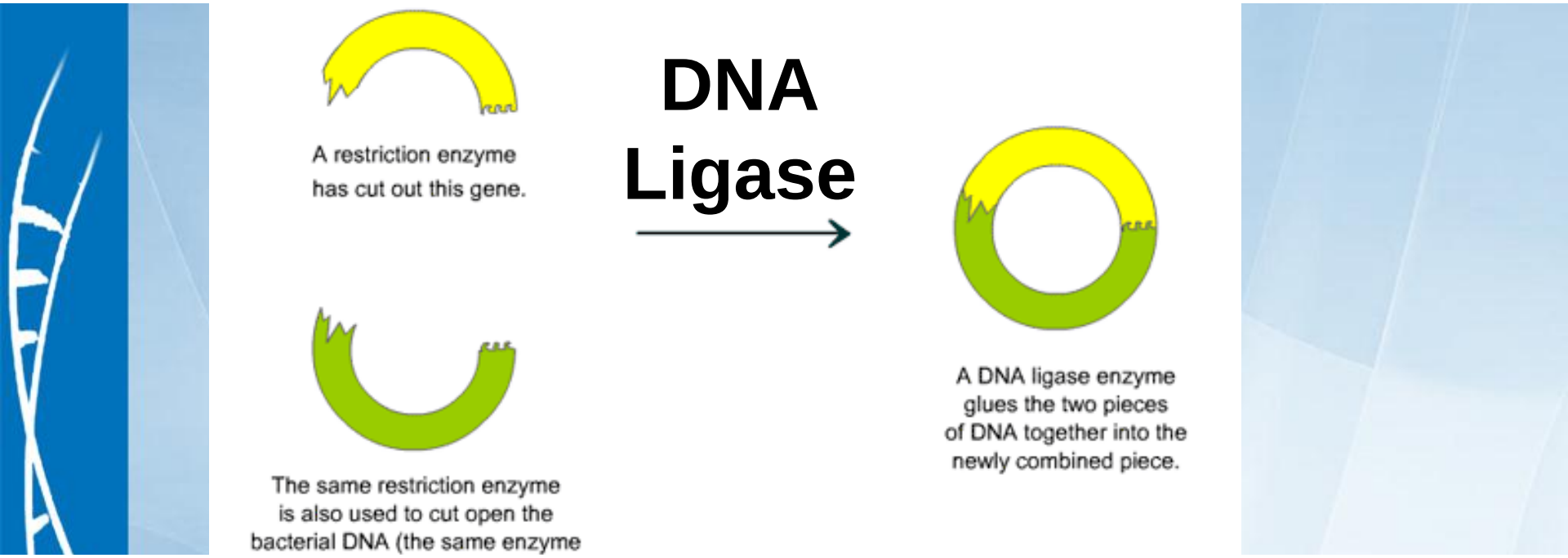
The same restriction enzyme is also used to cut open the bacterial DNA (the same enzyme gives them the same shaped ends).



A DNA ligase enzyme glues the two pieces of DNA together into the newly combined piece.

Recombinant DNA

Gene Splicing



The process that allows scientists to attach pieces of DNA segments to the DNA of other organisms is called:

GENE SPLICING

How is DNA Modified?

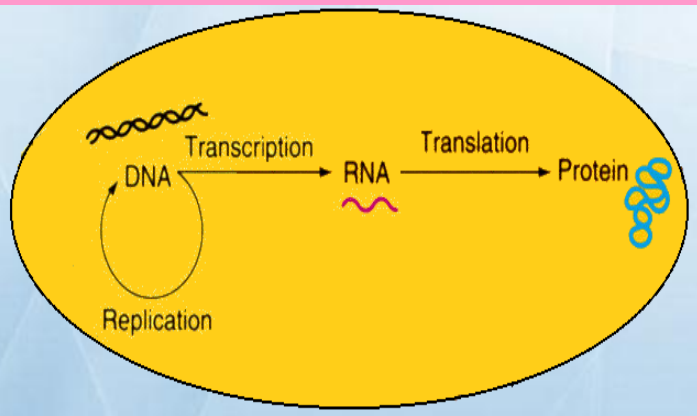
Step 4: The recombinant DNA is then placed inside a living cell.

"Cut DNA"
+
"Cut DNA"

- What will the recombined DNA do?

It will allow for the production of proteins from both sources of DNA.

Recombinant DNA



Recombinant DNA Technology

- Most DNA technology methods depend on bacteria, more specifically E. coli.
- In fact, research into the genetics of E.coli during the 1970s led to the development of **recombinant DNA technology,** a set of laboratory techniques for combining genes from different sources—even different species– into a single DNA molecule.
- It is now widely used to alter the genes of many types of cells for practical purposes.
- For example, scientists have genetically engineered bacteria to mass-produce many useful chemicals, from cancer drugs to pesticides. Furthermore, genes have been transferred from bacteria into plants and from humans to farm animals.

Recombinant DNA Technology

- To manipulate genes in the laboratory, biologists often use bacterial plasmids, which are small, circular DNA molecules that replicate separately from the much larger bacterial chromosome.
- Because plasmids can carry virtually any gene and replicate in bacteria, they are key tools for gene cloning, the production of multiple identical copies of a gene-carrying piece of DNA.

Vectors

Cloning vectors

Allowing the exogenous DNA to be inserted, stored, and manipulated mainly at DNA level.

1. **Plasmid vectors**
2. **Bacteriophage vectors**
3. **Cosmids**
4. **BACs & YACs**

Plasmid vectors

Plasmid vectors are double-stranded, circular, self-replicating, extra-chromosomal DNA molecules.

- *Advantages:*

Small, easy to handle –

Straightforward selection strategies –

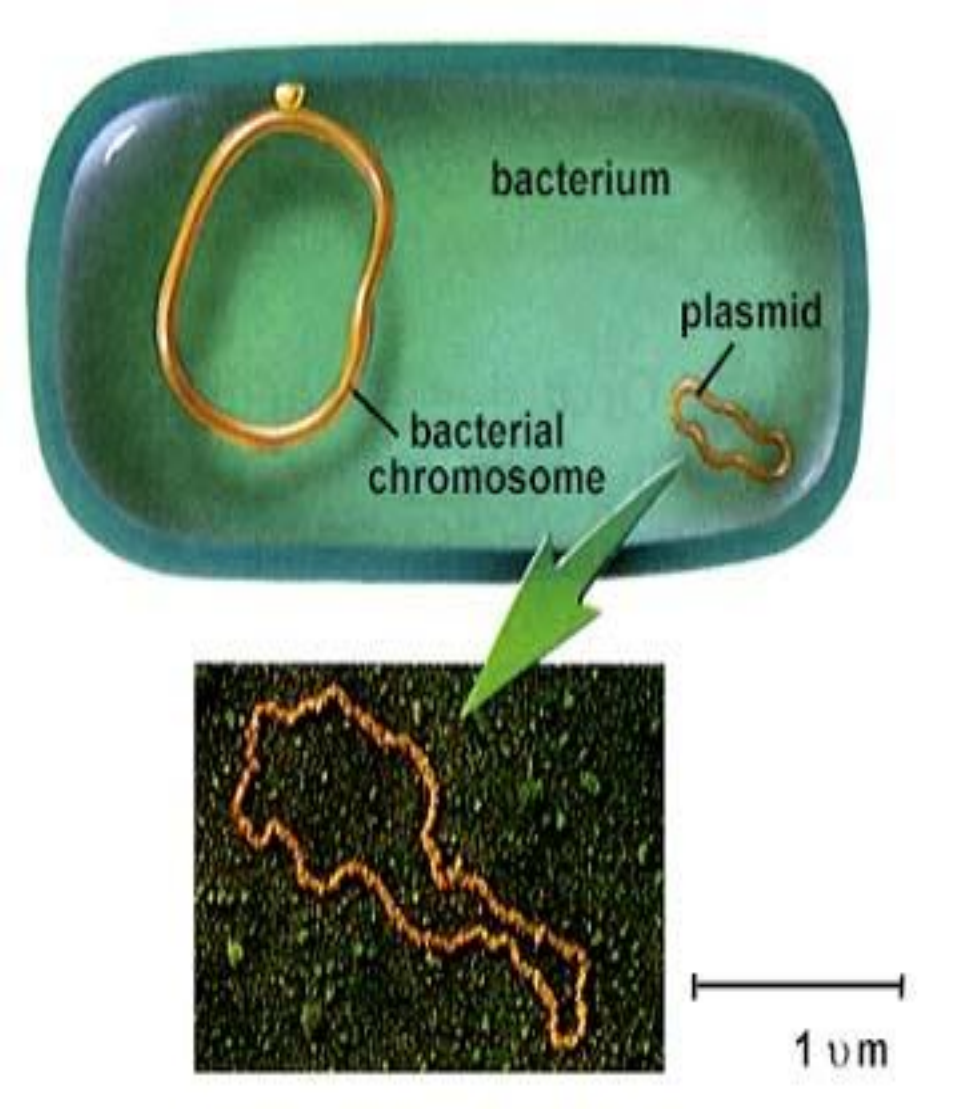
Useful for cloning small DNA fragments – (< 10kbp)

- *Disadvantages:*

Less useful for cloning large DNA fragments – (> 10kbp)

Plasmid vectors

- Plasmids are circular DNA molecules present in the cytoplasm of the bacteria
- Capable of autonomous replication
- Can transfer genes from one cell to other
- Act as vectors in genetic engineering.
- Can also present in Yeasts



Plasmid vectors

- may encode genetic information for properties

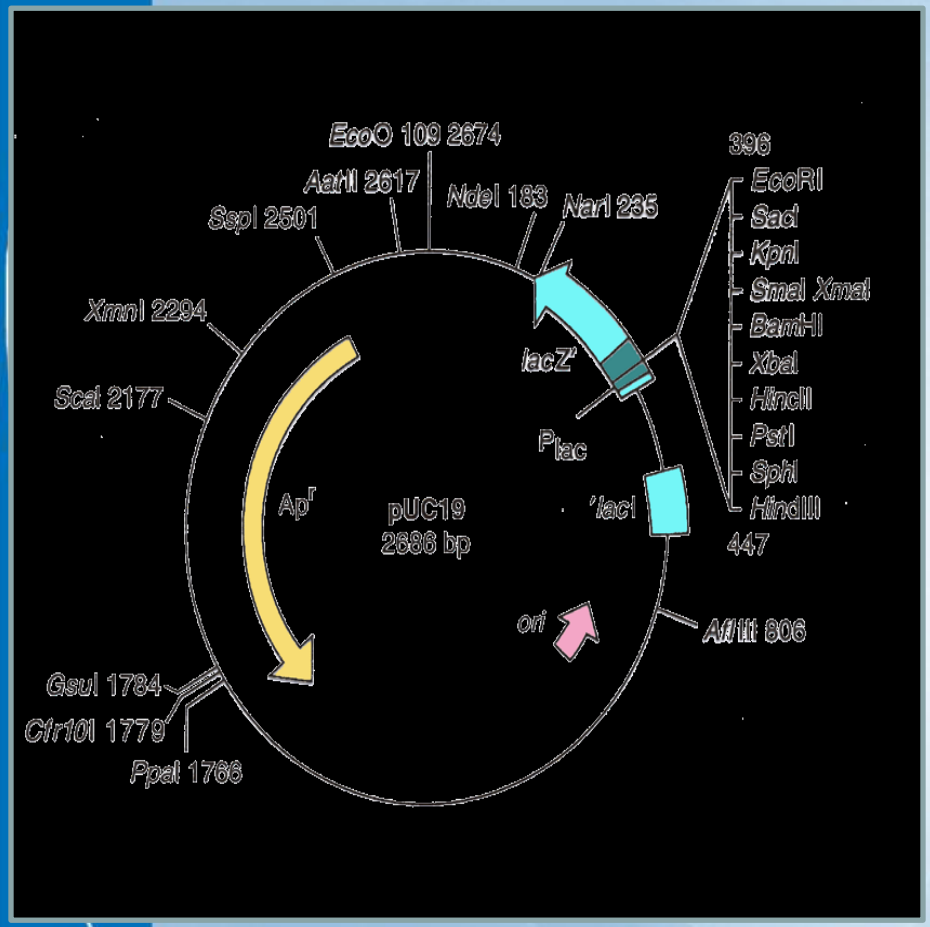
1 Resitance to Antibiotics
2 Bacteriocins production
3 Enterotoxin production
4 Enhanced pathogen city
5 Reduced Sensitivity to mutagens
6 Degrade complex organic molecules

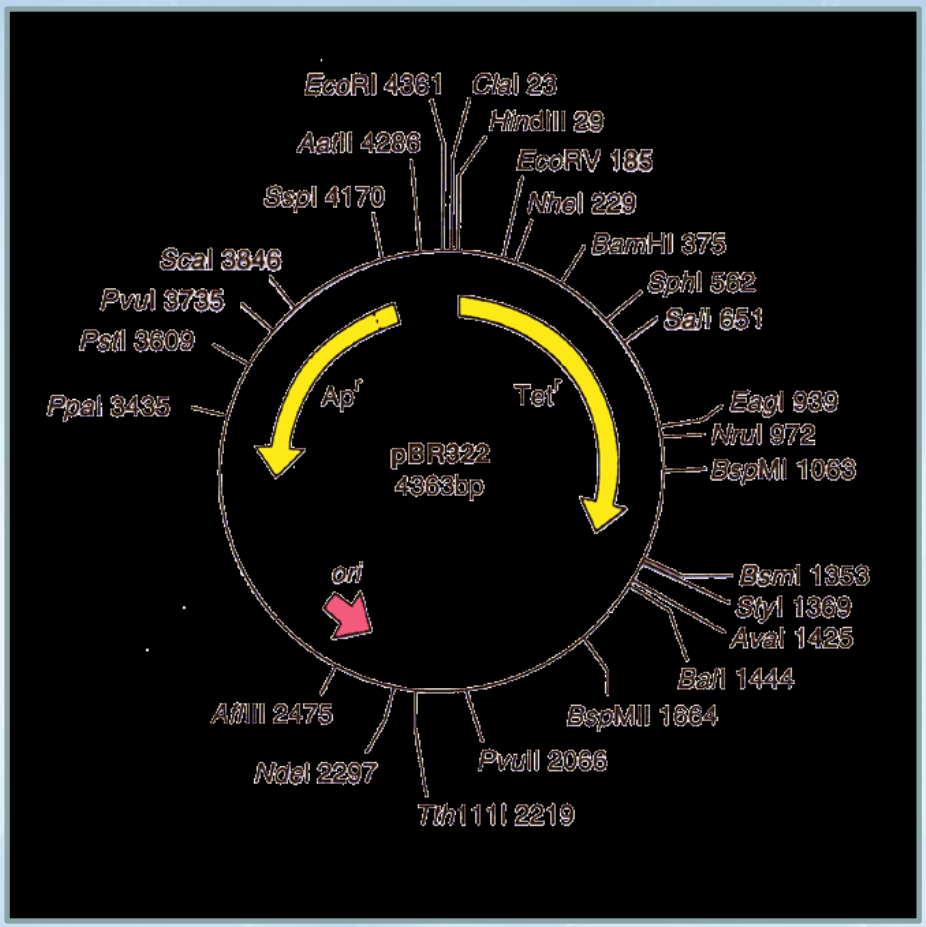
T.V.Rao MD

Plasmid vector for cloning

1. Contains an origin of replication, allowing for replication independent of host's genome.
2. Contains Selective markers: Selection of cells containing a plasmid
   twin antibiotic resistance
   blue-white screening
3. Contains a multiple cloning site (MCS)
4. Easy to be isolated from the host cell.

Plasmid vectors





Bacteriophage vectors

- *Advantages:*

Useful for cloning large DNA fragments – (10 - 23 kbp)

Inherent size selection for large inserts –

- *Disadvantages:*

Less easy to handle –

λ vectors

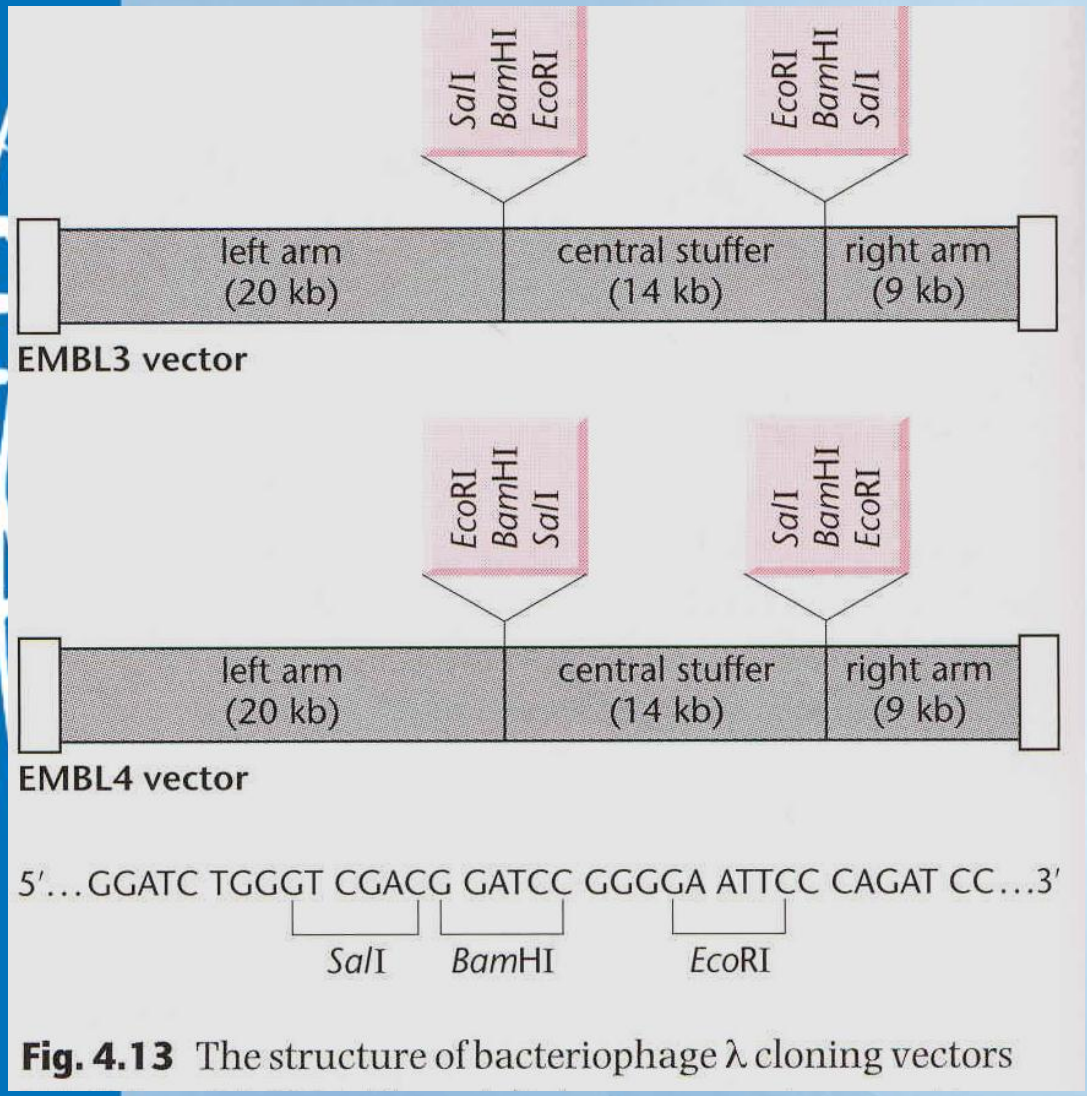
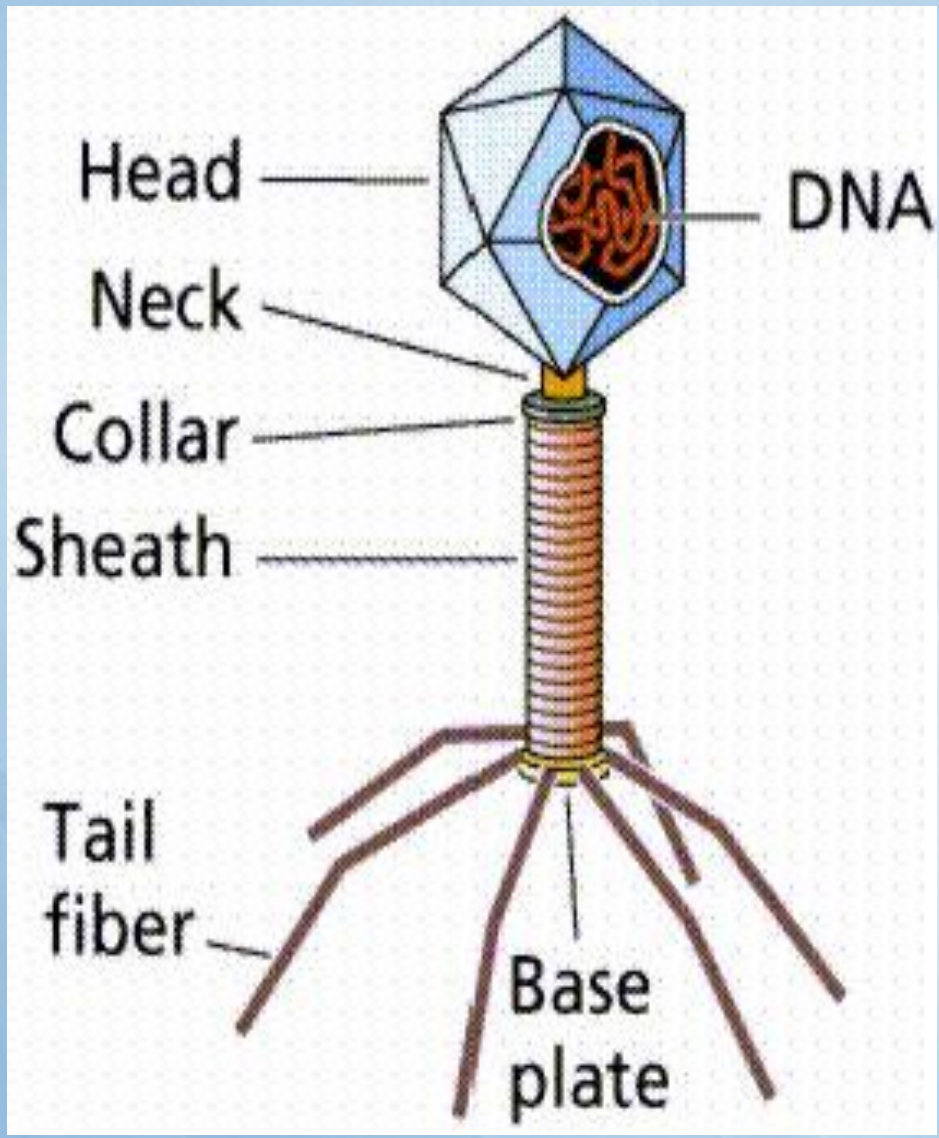
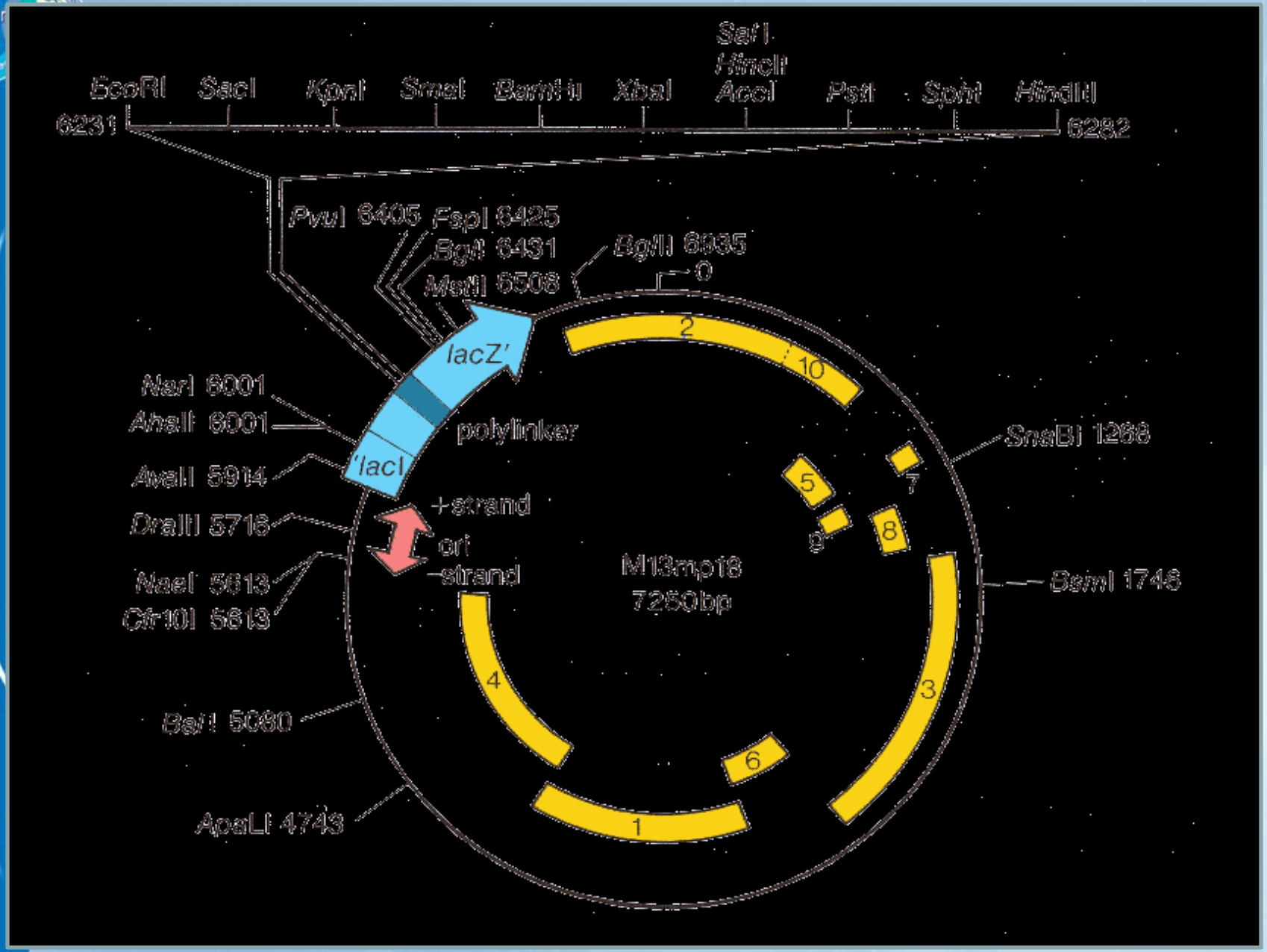


Fig. 4.13 The structure of bacteriophage λ cloning vectors

- *Left arm*: head & tail proteins –
- *Right arm*: DNA synthesis – regulation – host lysis –
- *Deleted central region*: integration & excision – regulation –

Bacteriophage

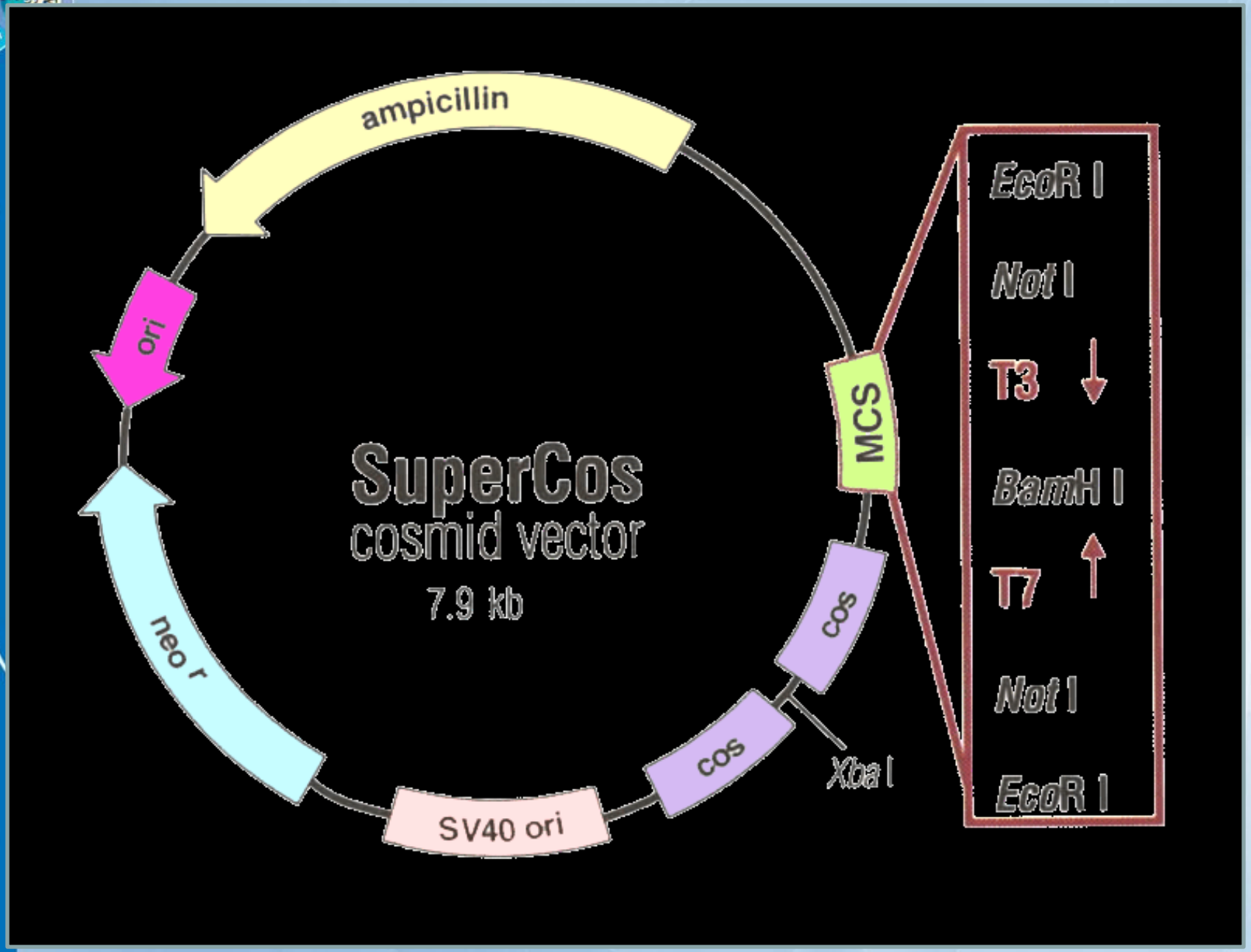



EcoRI
SacI
KpnI
SmaI
BamHI
XbaI
SalI
HincII
AccI
PstI
SphI
HindIII
6231
6282
PvuI 6405
FspI 6425
Bgl 6431
MscI 6508
BglII 6935
0
lacZ'
'lacI
polylinker
NarI 6001
AhaII 6001
AvaII 5914
DraIII 5715
NaeI 5613
Cfr10I 5613
BsaI 5060
ApaLI 4743
+strand
ori
–strand
M13mp18
7250bp
SnaBI 1268
BsmI 1746
1
2
3
4
5
6
7
8
9
10

Cosmid vectors

Combine the properties of plasmid vectors with the useful properties of the l *cos* site

- *Advantages:*
  - Useful for cloning very large DNA fragments (32 - 47 kbp)
  - Inherent size selection for large inserts
  - Handle like plasmids
- *Disadvantages:*
  - Not easy to handle very large plasmids (~ 50 kbp)

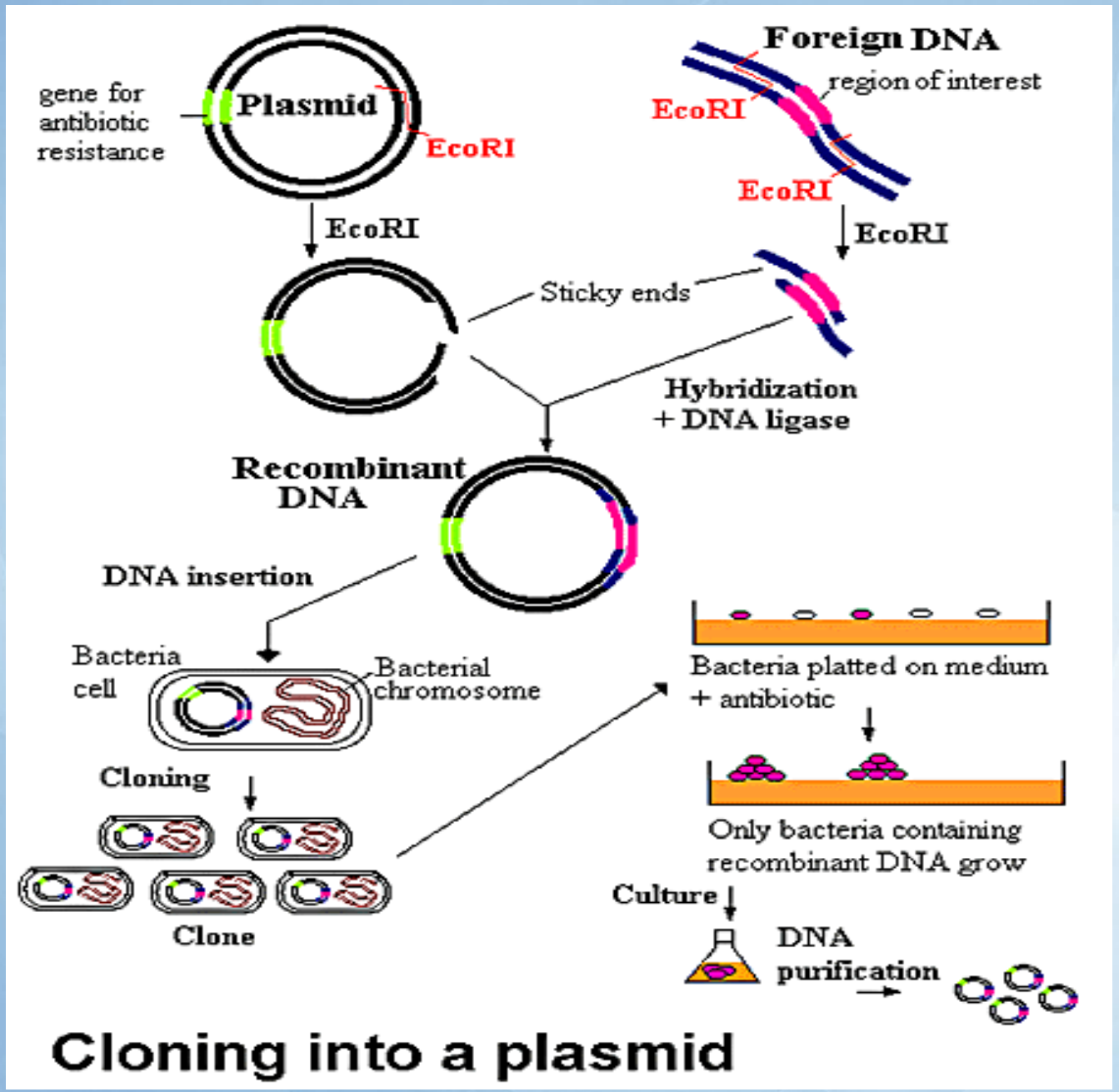

ampicillin
ori
neo r
SV40 ori
cos
cos
Xba I
MCS
SuperCos
cosmid vector
7.9 kb
EcoR I
Not I
T3
BamH I
T7
Not I
EcoR I

Recombinant DNA Technology

- Overview of gene cloning:
  - 1. the procedure begins when a plasmid is isolated from a bacterium and
  - 2.DNA carrying a gene of interest is obtained from another cell.
    - The gene of interest could be, for instance, a human gene encoding a protein of medical value or a plant gene conferring resistance to pests.
  - 3. A piece of DNA containing the gene is inserted into the plasmid. The resulting plasmid now consists of recombinant DNA, DNA in which genes from two different sources are combined in vitro into the same DNA molecule.
  - 4. Next, a bacterial cell takes up the plasmid through transformation.
  - 5. This recombination bacterium then reproduces to form a clone of cells (a group of identical cells descended from a single ancestral cell), each carrying a copy of the gene.
- Cloned genes can be used directly or to manufacture protein products.

Recombinant DNA Technology

- Gene-cloning methods are central to genetic engineering, the direct manipulation of genes for practical purposes.
- Genetic engineering has launched a revolution in biotechnology, the use of organisms or their components to make useful products.



Cloning into a plasmid

Restriction Enzymes

- For the gene cloning procedure to occur, a piece of DNA containing the gene of interest must be cut out of a chromosome and “pasted” into a bacterial plasmid.
- The cutting tools are bacterial enzymes called restriction enzymes.
- In nature, these enzymes protect bacterial cells against intruding DNA from other organisms or viruses.
- They work by chopping up the foreign DNA, a process that *restricts* foreign DNA from surviving in the cell.

The bacterial cell’s own DNA is protected from – restriction enzymes through chemical modification by other enzymes.

Restriction Enzymes

- Hundreds of different restriction enzymes have been identified and isolated. Each restriction enzyme is very specific, recognizing a particular short DNA sequence (usually four to eight nucleotides long).
- Once the DNA sequence is recognized, the restriction enzyme cuts both DNA strands at specific points within the sequence.

Restriction enzymes

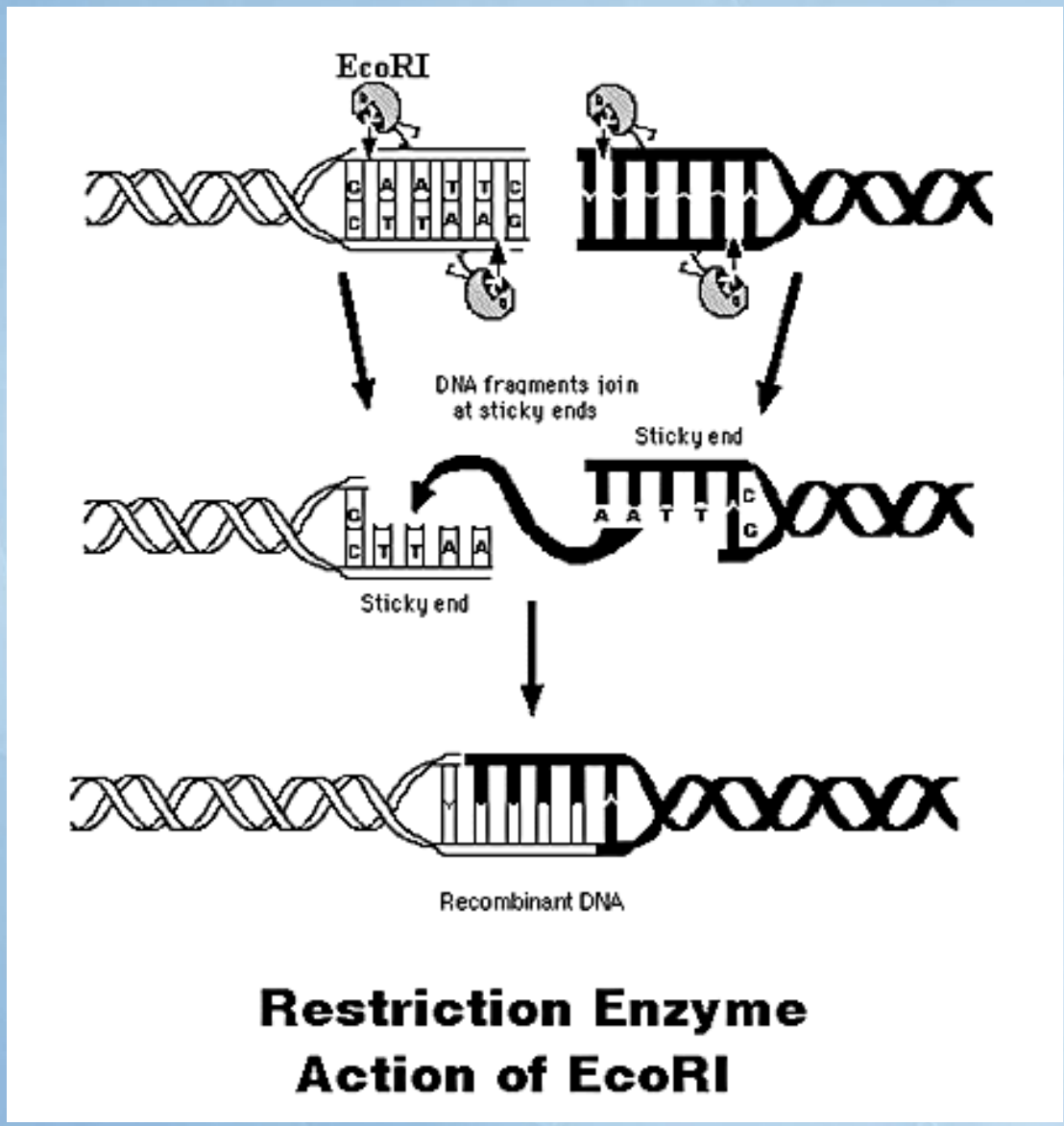
- Creating recombinant DNA using a restriction enzyme and DNA ligase (Figure 12.2):

1. we start with a piece of DNA containing one recognition sequence for a particular restriction enzyme from E.coli. In this case, the restriction enzyme will cut the DNA strands between the bases A and G within the sequence, producing pieces of DNA called restriction fragments.

2. The staggered cuts yield two double-stranded DNA fragments with single-stranded ends, called “sticky ends.” Sticky ends are the key to joining DNA restriction fragments originating from different sources. These short

Restriction Enzymes

- 3. a “foreign” piece of DNA from another source is now added. This “foreign” piece of DNA has single-stranded ends identical in base sequence to the sticky ends on the original DNA.
  - The “foreign” DNA has ends with this particular base sequence because it was cut from a larger molecule by the same restriction enzyme used to cut the original DNA.
- 4. The complementary ends on the original and “foreign” fragments allow them to stick together by base-pairing.
  - The union between foreign and original DNA fragments is made permanent by the “pasting” enzyme DNA ligase.
  - This enzyme, which the cell normally uses in DNA replication, catalyzes the formation of covalent bonds between adjacent nucleotides, sealing the breaks in the DNA strands.
- 5. The final outcome is a stable molecule of recombinant DNA.



Restriction Enzyme Action of EcoRI

Cloning Genes in Recombinant Plasmids

- Consider a typical genetic engineering challenge: a molecular biologist at a pharmaceutical company has identified a human gene that codes for a valuable product: a hypothetical substance called protein V that kills certain human viruses.
- The biologist wants to set up a system for making large amounts of the gene so that the protein can by manufactured on a large scale.

Cloning Genes in Recombinant Plasmids

Steps to a way to make many copies of the gene using the techniques of recombinant DNA technology:

1. The biologist isolates two kinds of DNA: the bacterial plasmid that will serve as the vector (gene carrier), and the human DNA containing gene V.

In this example, the DNA containing the gene of – interest comes from human tissue cells that have been growing in laboratory culture. The plasmid comes from the bacterium E.coli.

Cloning Genes in Recombinant Plasmids

2. The researcher treats both the plasmid and the human DNA with the same restriction enzyme.

- An enzyme is chosen that cleaves the plasmid in only one place.
- The human DNA, with thousands of restriction sites, is cut into many fragments, one of which carries gene V. In making the cuts, the restriction enzyme creates sticky ends on both the human DNA fragments and the plasmid.
- The figure on p. 234 shows the processing of just one human DNA fragment and one plasmid, but actually millions of plasmids and human DNA fragments (most of which do not contain gene V) are treated simultaneously.

Cloning Genes in Recombinant Plasmids

- 3. The human DNA is mixed with the cut plasmid. The sticky ends of the plasmid base-pair with the complementary sticky ends of the human DNA fragment.
- 4. the enzyme DNA ligase joins the two DNA molecules by covalent bonds, and the result is a recombinant DNA plasmid containing gene V.
- 5. The recombinant plasmid is added to a bacterium. Under the right conditions, the bacterium takes up the plasmid DNA by

Cloning Genes in Recombinant Plasmids

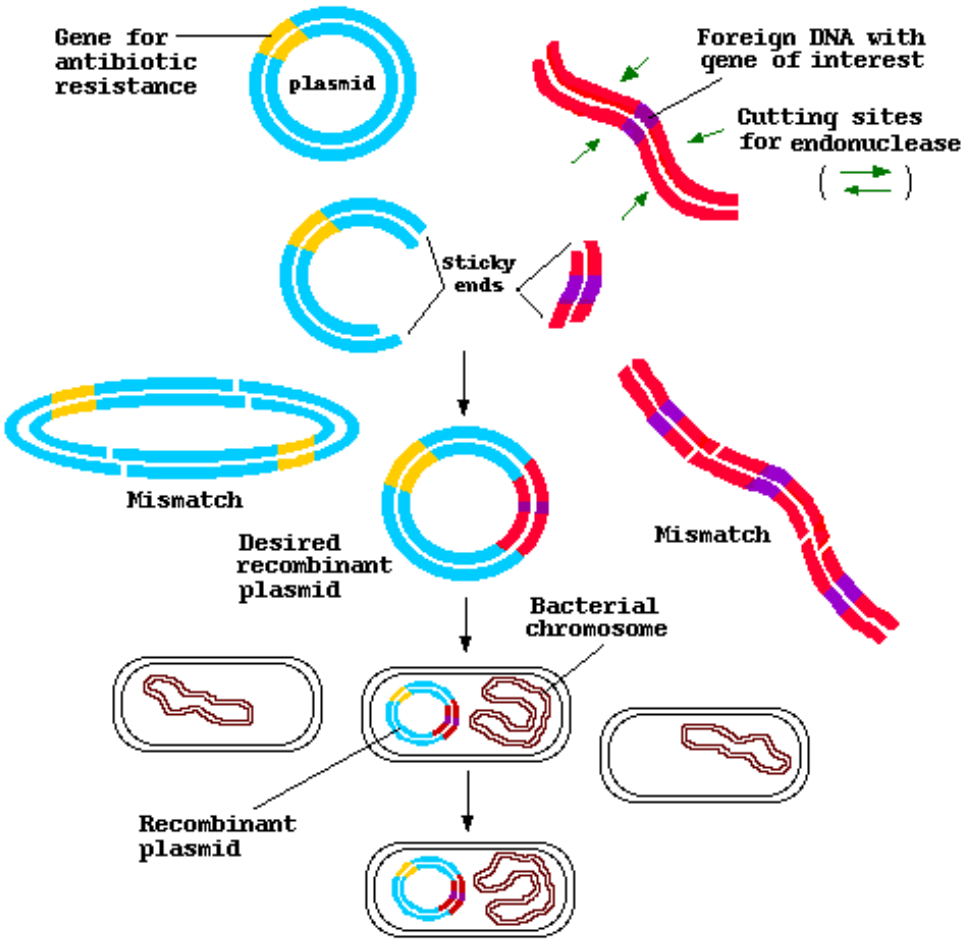
- 6. This step is the actual gene cloning. The bacterium is allowed to reproduce, forming a clone of cells that all carry the recombinant plasmid.

In our example, the biologist will grow a cell – clone large enough to produce protein V in marketable quantities.

Cloning Genes in Recombinant Plasmids

- This cloning procedure, which uses a mixture of fragments from the entire genome of an organism, is referred to as the “shotgun” approach.
- Thousands of different recombinant plasmids are produced in step 3, and a clone of each is made during steps 5 and 6.
- The complete set of plasmid clones, each carrying copies of a particular segment

Plasmid Insertion



Genomic Library

- Each bacterial clone from the procedure we previously discussed consists of identical cells with recombinant plasmids carrying one particular fragment of human DNA.
- The entire collection of all the cloned DNA fragments from a genome is called a genomic library.
  - Various DNA segments represent thousands of "books" that are "shelved" in plasmids inside bacterial cells.
  - A typical cloned DNA fragment is big enough to carry one or a few genes, and together the

Genomic Library

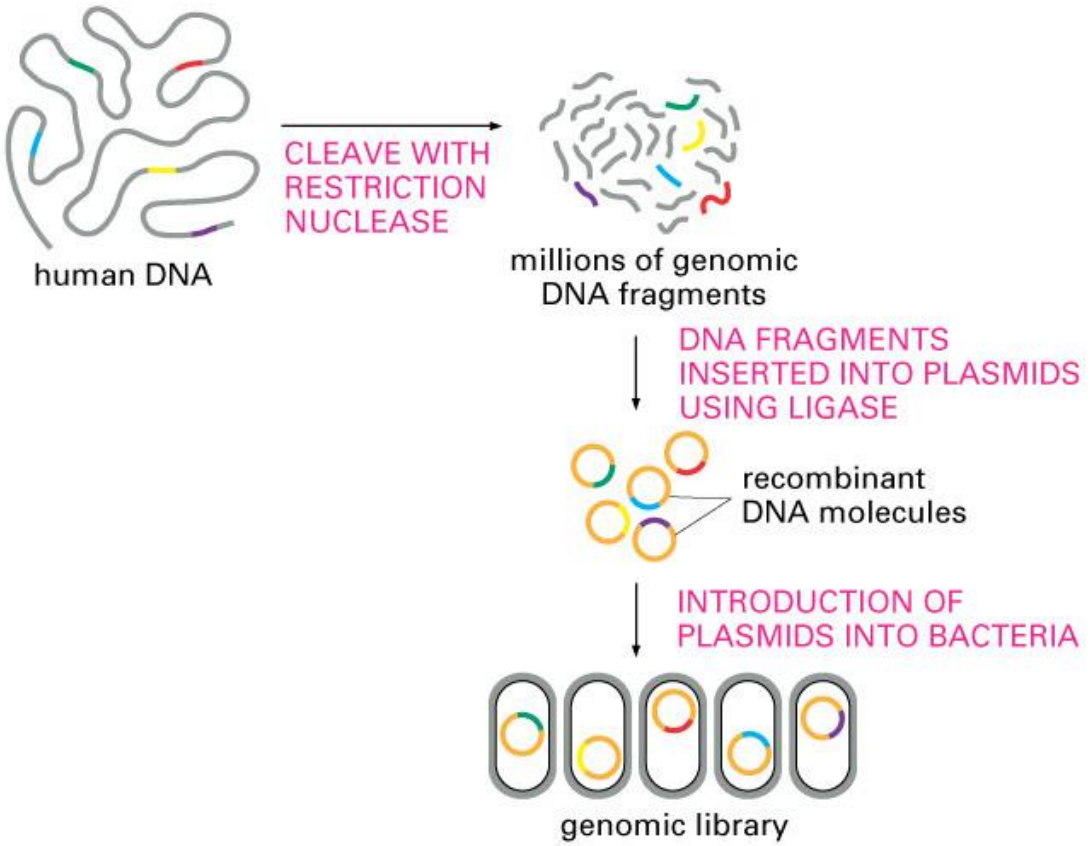


Figure 10-23 Essential Cell Biology, 2/e. (© 2004 Garland Science)

Genomic Library

- Bacterial plasmids are one type of vector that can be used in the cloning of genes, but not the only type. Phages can also serve as vectors.
- When a phage is used, the DNA fragments are inserted into phage DNA molecules. The recombinant phage DNA can then be introduced into a bacterial cell through the normal infection process.
- Inside the cell, the phage DNA replicates and produces new phage particles, each carrying the foreign DNA.
- A collection of phage clones can constitute a second type of genomic library.

Reverse transcriptase

- Rather than starting with an entire eukaryotic genome, a researcher can focus on the genes expressed in a particular kind of cell by using its mRNA as the starting material.:

1. the chosen cells transcribe their genes and –
2. process transcripts to produce mRNA. –
3. the researcher isolates the mRNA and makes single-stranded DNA transcripts from it using the enzyme reverse transcriptase, which is obtained from retroviruses. –
4. enzymes are added to break down the mRNA and –

Reverse transcriptase

- Complementary DNA (cDNA) is the DNA that results from this procedure.
  - It represents only the subset of genes that were transcribed into mRNA in the starting cells.
  - Among other purposes, a cDNA library is useful for studying the genes responsible for the specialized functions of a particular cell type, such as brain or liver cells.
  - And because cDNAs lack introns, they are shorter than the full versions of the genes,

Mass-Produced Gene Products

- Recombinant cells and organisms constructed by DNA technology are used to manufacture many useful products, chiefly proteins.
- Most of these products are made by cells grown in culture.
- By transferring the gene for a desired protein in a bacterium, yeast, or other kind of cell that is easy to grow, one can produce large quantities of proteins that are present naturally in only minute amounts.

Mass-Produced Gene Products

- Bacteria are often the best organisms for manufacturing a protein product.
  - Major advantages of bacteria include the plasmids and phages available for use as gene-cloning vectors and the fact that bacteria can be grown rapidly and cheaply in large tanks.
  - Furthermore, bacteria can be readily engineered to produce large amounts of particular proteins and in some cases to secrete the protein products into their growth medium, which simplifies the task of collecting and purifying the products.

A number of proteins of importance in human

Mass-Produced Gene Products

- Although there are many advantages to using bacteria, it is sometimes desirable or necessary to use eukaryotic cells to produce a protein product.
  - Often times, the yeast Saccharomyces cerevisae, which is used in making bread and beer, is the first-choice eukaryotic organism for protein production.
  - Yeast are easy to grow, and can take up foreign DNA and integrate it into their genomes like E.coli.
  - Also have plasmids that can be used as gene vectors, and are often better than bacteria at

Mass-Produced Gene Products

- The cells of choice for making some gene products come from mammals.
  - Genes fro these products are often cloned in bacteria as a preliminary step.
  - For example, the genes for two proteins that affect blood clotting, Factor VIII and TPA, are cloned in a bacterial plasmid before transfer to mammalian cells for large-scale production.
    - Many proteins that mammalian cells secrete are glycoproteins, proteins with chains of sugars attached.
    - Because only mammalian cells can attach the sugars correctly, mammalian cells must be used to make these products.

Mass-Produced Gene Products

- Recently, pharmaceutical researchers have been exploring the mass production of gene products by whole animals or plants rather than cultured cells.
  - For example, using recombinant DNA technology, genetic engineers can add a gene for a desired human protein to the genome of a mammal in such a way that the gene's product is secreted in the animal's milk.
  - Sheep are being used to carry a gene for a human blood protein that is a potential

DNA technology and the pharmaceutical industry and medicine

- DNA technology and gene cloning are widely used to produce medicines and to diagnose disease:

Therapeutic hormones –

- Human insulin and human growth hormone

Diagnonsis and Treatment of disease –

- Pinpoint genetic disease alleles
- Diagnosis HIV

Vaccines –

- Hepatitis B

Nucleic Acid Probes

- Often the most difficult task in gene cloning is finding the right “shelf” in a genomic library—that is, identifying a bacterial or phage clone containing a desired gene from among all those created.
  - If bacterial clones containing a specific gene actually translate the gene into protein, they can be identified by testing for the protein product. –
  - However, this is not always the case. Fortunately, researchers can also test directly for the gene itself. –

Nucleic Acid Probe

- Methods for detecting genes directly depend on base pairing between the gene and a complementary sequence on another nucleic acid molecule, either DNA or RNA.
- When at least part of the nucleotide sequence of a gene is already known or can be guessed, this information can be used to advantage.
  - For example, if we know that a hypothetical gene contains the sequence TAGGCT, a biochemist can synthesize a short single strand of DNA with the complementary sequence (ATCCGA) and label it with a radioactive isotope or fluorescent dye.
  - This labeled, complementary molecule is called a nucleic acid probe because it is used to find a specific gene or other nucleotide sequence within a mass of DNA.

Nucleic Acid Probe

- Refer to p. 238 Figure 12.8 for the procedure of how a probe works.

DNA Microarray

- Besides hunting for one specific gene, nucleic acid probes can be used to perform large-scale analyses that determine which of many genes are active (transcribed) in particular cells at particular times.
- This technique relies on DNA microarrays:
  - DNA microarray is a glass slide carrying thousands of different kinds of single-stranded DNA fragments arranged in an array (grid).
  - Each DNA fragment is obtained from a particular gene; a single microarray thus carries DNA from thousands of genes.