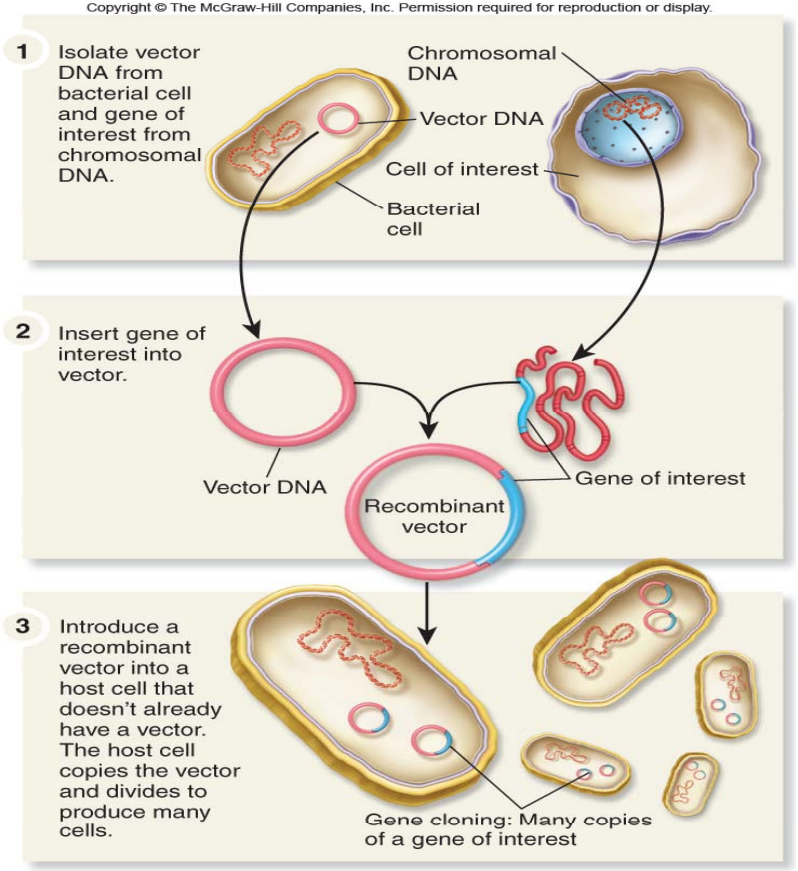
DNA CLONING
CHAPTER 20

- Recombinant DNA technology
  - Use of laboratory techniques to isolate and manipulate fragments of DNA
- Recombinant DNA contains DNA from 2 or more sources
- Once inside a host cell, recombinant molecules are replicated to produce identical copies or clones

Gene cloning

- Procedures that lead to the formation of many copies of a particular gene
- Why?
  - Want copies of a gene for study or use
  - Obtain lots of gene product- mRNA or protein



4 Gene cloning is done to achieve one of two main goals:

Producing large amounts of DNA of a specific gene	Expressing the cloned gene to produce the encoded protein
Examples • Cloned genes provide enough DNA for DNA sequencing. The sequence of a gene can help us understand how a gene works and identify mutations that cause diseases. • Cloned DNA can be used as a probe to identify the same gene or similar genes in other organisms.	*Examples* • Large amounts of the protein can be purified to study its structure and function. • Cloned genes can be introduced into bacteria or livestock to make pharmaceutical products such as insulin. • Cloned genes can be introduced into plants and animals to alter their traits. • Cloned genes can be used to treat diseases—a clinical approach called gene therapy.

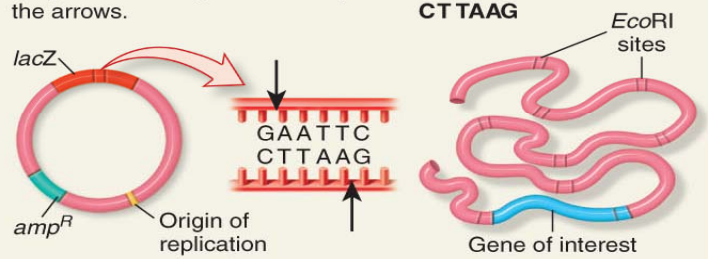
Step 1 in gene cloning

- Vector DNA acts as a carrier for the DNA segment to be cloned
- When a vector is introduced into a living cell, it can replicate making many copies
- Common vectors are plasmid or viral
- Also need the gene of interest from chromosomal DNA

Step 2

- Insert chromosomal DNA into vector
- Cut DNA using restriction enzymes or restriction endonucleases
  - Made naturally by bacteria as protection against bacteriophages
  - Cuts at specific known restriction sites
  - Most restriction sites palindromic
  - May produce sticky ends
  - DNA ligase must be used to permanently link DNA
- Result may be
  - Recircularized vector with no gene of interest inserted
  - Recombinant vector with gene of interest inserted

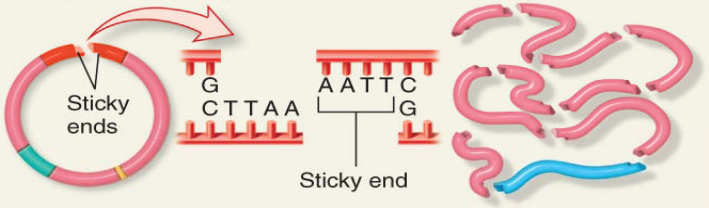
1 Cut vector and chromosomal DNA with *Eco*RI, a restriction enzyme that recognizes the sequence **GAATTC** and cuts at the arrows. **CTTAAG**



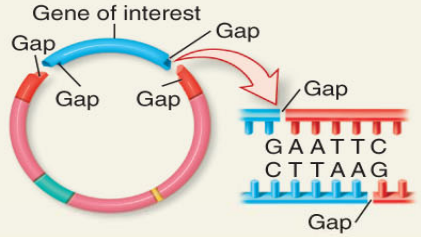
Vector DNA has one *Eco*RI site.

Chromosomal DNA has many *Eco*RI sites.

The restriction enzyme opens up the vector and cuts the chromosomal DNA into many fragments with short single-stranded regions called sticky ends.

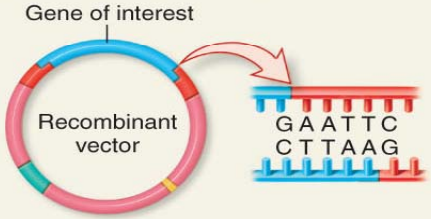


2 Allow sticky ends to hydrogen-bond with each other due to complementary sequences.



In this example, a fragment of DNA carrying the gene of interest has hydrogen-bonded to the vector. Four gaps are found where covalent bonds in the DNA backbone are missing.

3 Add DNA ligase to close the gaps by catalyzing the formation of covalent bonds in the DNA backbone.

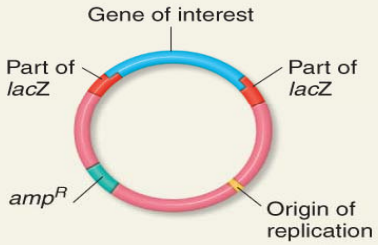


Step 3 – actual cloning

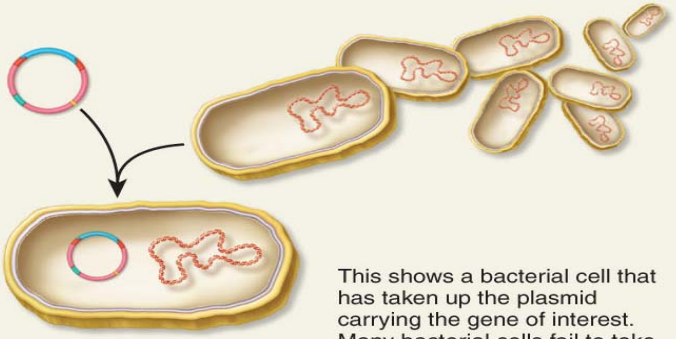
- Goal for recombinant vector to be taken up by bacteria
  - Some will take up a single plasmid
  - Most cells fail to take up a plasmid
- Vector carries a selectable marker
  - Presence of antibiotics selects for cells expressing amp^R gene – contains plasmid
  - amp^R gene codes for b-lactamase that degrades ampicillin, which normally kills bacteria

- After treatment, only cells with the plasmid will grow on plates treated with ampicillin
  - To eliminate recircularized vectors from further examination, lacZ gene part of vector
  - Insertion of chromosomal DNA disrupts lacZ gene
  - lacZ codes for b-galactosidase which cleaves colorless X-Gal into a blue dye
    - Recircularized plasmids will form blue colonies
    - Recombinant vectors will form white colonies

1 Mix plasmid DNA with many *E.coli* cells that have been treated with agents that make them permeable to DNA.

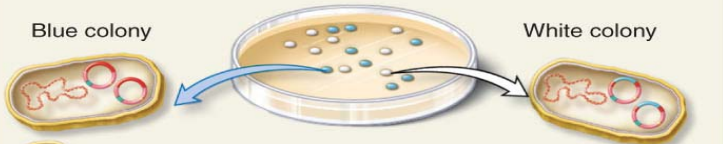


In this example, the gene of interest was inserted into a plasmid. This separates the *lacZ* gene and renders it nonfunctional. It is also possible for any other chromosomal DNA fragment to be inserted into the plasmid, or the plasmid may recircularize without an insert.



This shows a bacterial cell that has taken up the plasmid carrying the gene of interest. Many bacterial cells fail to take up a plasmid.

2 Plate cells on media containing X-Gal and ampicillin. Incubate overnight. Note: The *ampR* gene allows bacteria to grow in the presence of ampicillin. The *lacZ* gene encodes β-galactosidase that degrades X-gal to produce a blue color.



Each bacterial colony is derived from a single cell; so all the cells in a colony are genetically identical.

Recircularized vector without an insert—*lacZ* gene functional and produces blue color

Recombinant vector with an insert—*lacZ* gene is nonfunctional

DNA library

- Treatment of chromosomal DNA with restriction enzymes yields tens of thousands of different fragments
- DNA library- collection of many recombinant vectors each with a fragment of chromosomal DNA
- 2 types of common DNA libraries
  - Genomic – inserts derived from chromosomal DNA
  - cDNA – use reverse transcriptase to make DNA from mRNA of interest (complementary DNA) - lacks introns so simpler to use