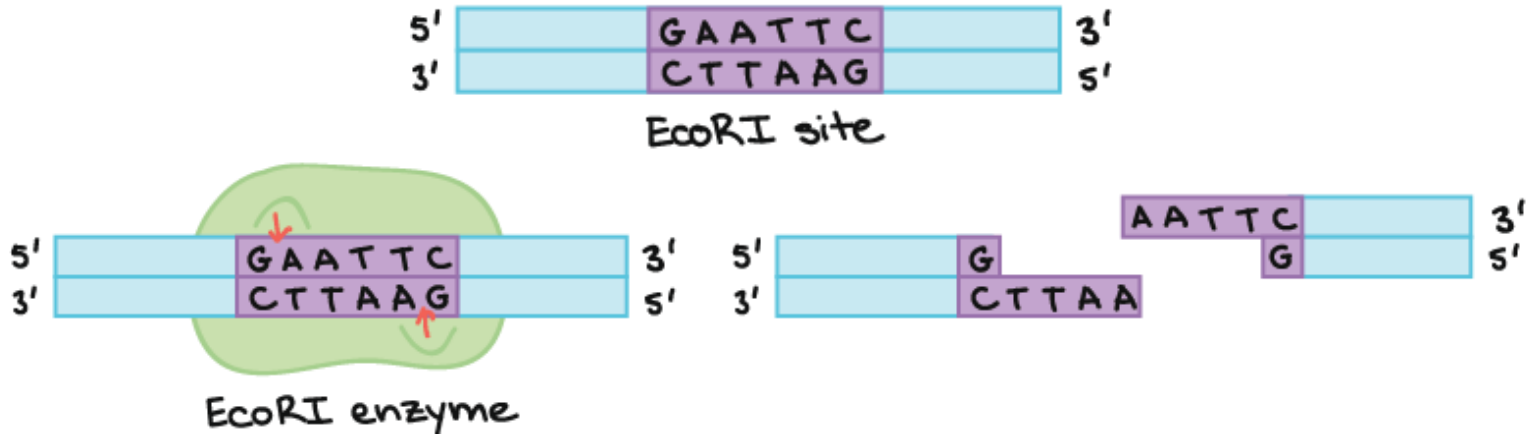
Genetics Engineering (Zoo-455)

Restriction enzymes & DNA ligase

Lecture-3

Restriction enzymes:

- Restriction enzymes were discovered in **bacteria** in 1978.
- Restriction enzymes are called **restriction endonucleases,** because it cuts inside of the DNA sequences (**in the middle**), but **exonucleases** always cut at the end of DNA sequences
- They help the bacteria to **destroy viral DNA** (bacteriophages) as a defense mechanism.
- They recognize and bind to specific sequences of DNA, typically 4 to 8 base pairs in length, called **restriction sites**.
- They cut between specific bases (letters) of the double stranded DNA molecule
- As an example of how a restriction enzyme recognizes and cuts at a DNA sequence, let's consider EcoRI, a common restriction enzyme used in labs. EcoRI cuts at the following site:

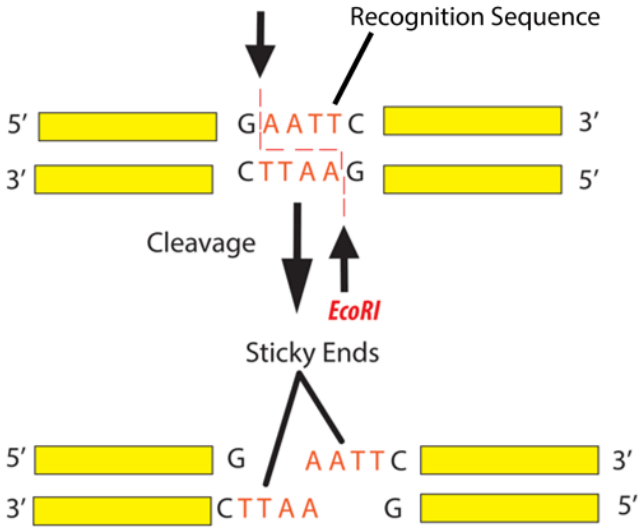


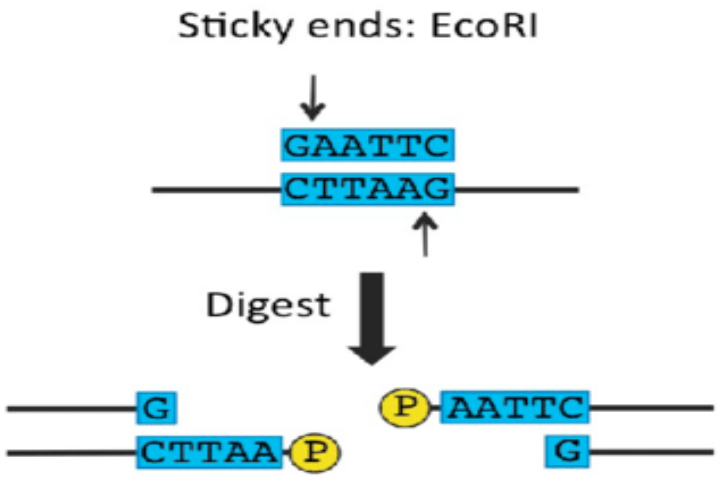
Restriction enzymes - purposes:

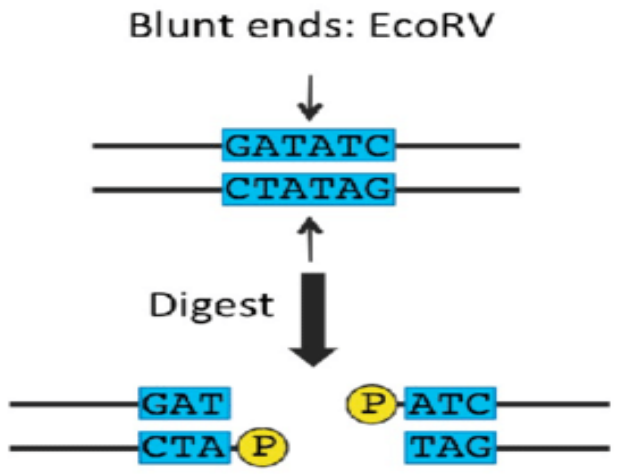
- Researchers rely on restriction enzymes to assist with many processes in laboratories around the world:
  I. Making recombinant DNA (For research, medicine and agriculture)
  II. DNA profile analysis (For disease diagnosis, family relationship testing, and forensics)

Restriction enzyme site:

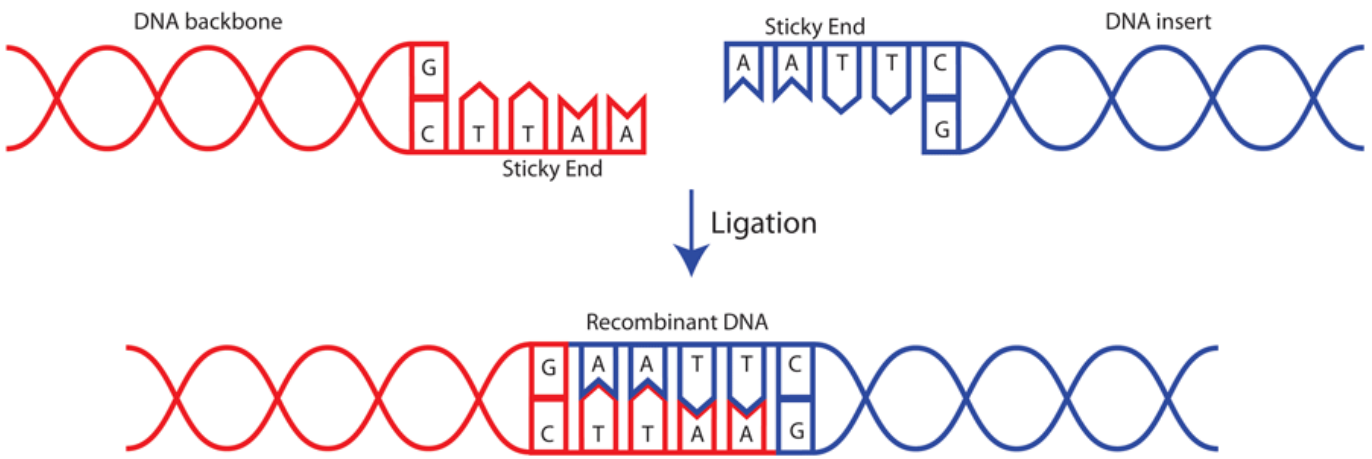
- Restriction enzymes are DNA-cutting enzymes.
- Each enzyme recognizes one or a few target sequences and cuts DNA at or near those sequences.
- Many restriction enzymes make **sticky**" or "**cohesive**" **ends** cuts, producing ends with single-stranded DNA overhangs.
- Some produce **blunt ends** with straight cuts through both DNA strands.
- If two DNA molecules have matching ends, they can be joined by the enzyme DNA ligase.

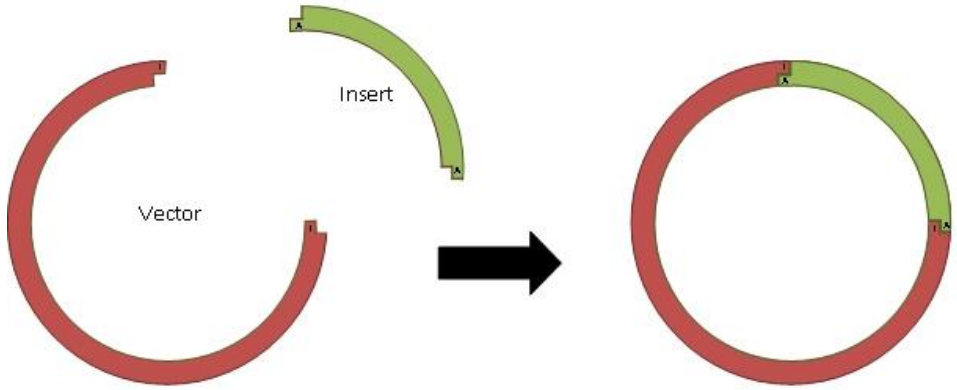






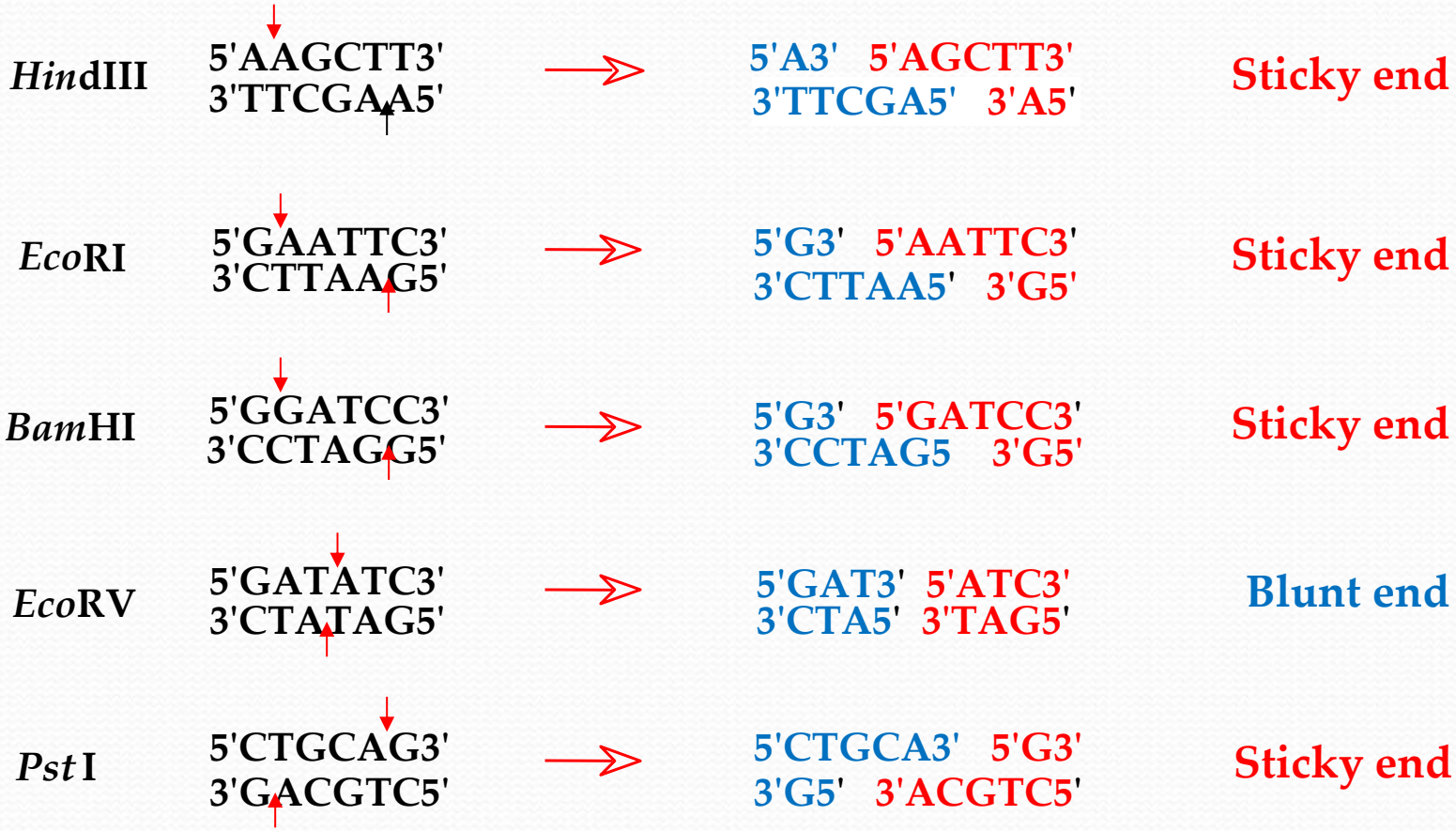
Making recombinant DNA using a restriction enzyme with sticky ends:





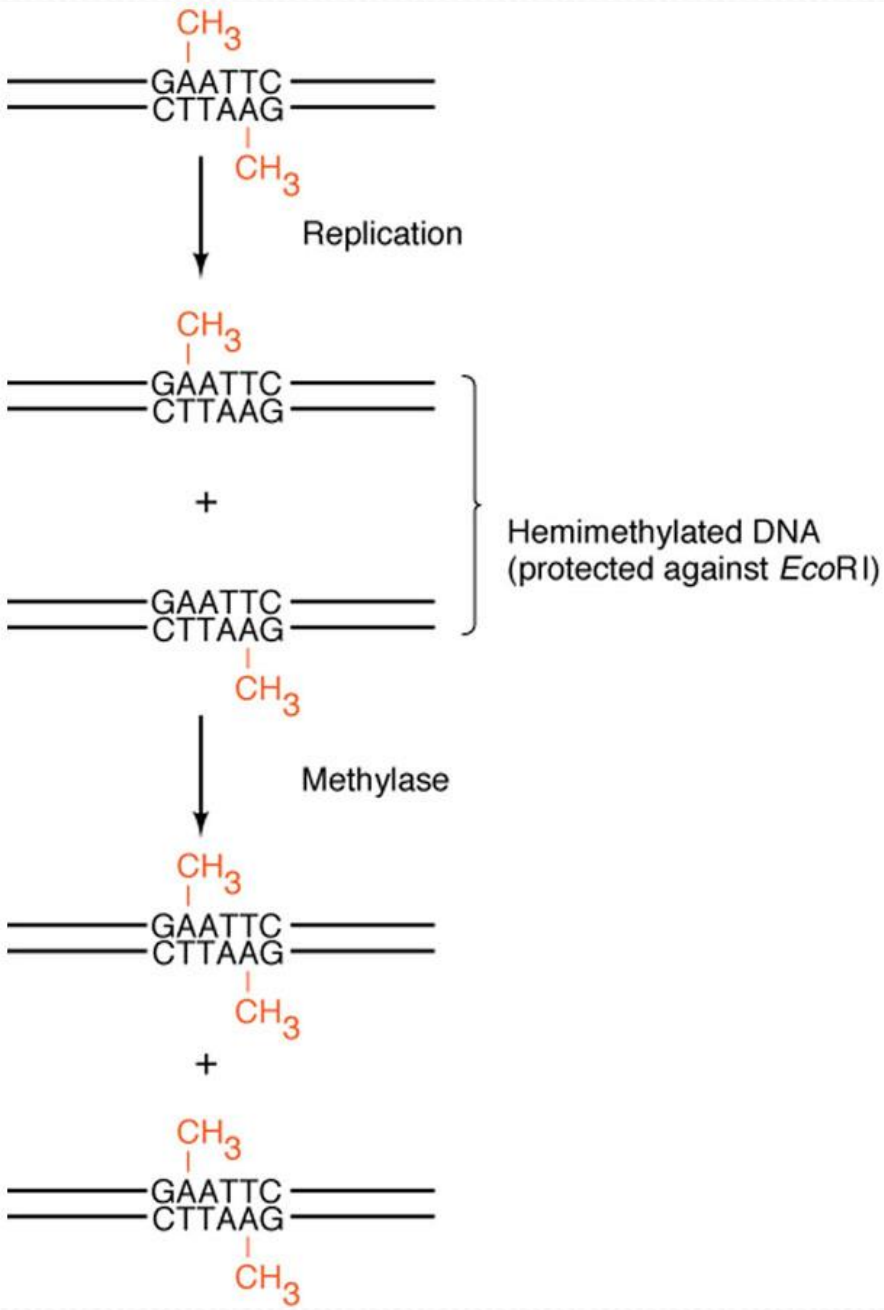
- When you cut two separate molecules of DNA with the same restriction enzyme, the fragments will have matching sticky ends.
- This is how recombinant DNA is created.

Recognition Sequences and Cutting Sites of Selected Restriction Endonucleases



- *Hin*dIII was discovered in ***Haemophilus influenzae***, d (strain designation), III (3rd restriction enzyme isolated from *H. influenzae)*
- *Eco*RI *was discovered in* ***Escherichia coli,*** strain R
- *Bam*HI was discovered in ***B****acillus* ***amy****loliquefaciens*
- *EcoRV was discovered in* ***Escherichia coli***
- *Pst*I was discovered in ***P****rovidencia* ***st****uartii*

- Methylation of the bacterial DNA **prevents** the restriction enzyme from **cutting** the host genome, as the enzyme can only recognize and cleave **unmethylated DNA**.
- This protective mechanism allows the bacteria to differentiate between its own DNA (**methylated**) and foreign DNA (**unmethylated**).
- As a result, the restriction enzyme selectively targets and cleaves foreign DNA without harming the bacterial genome.



Example sequence. Cut with EcoRI.

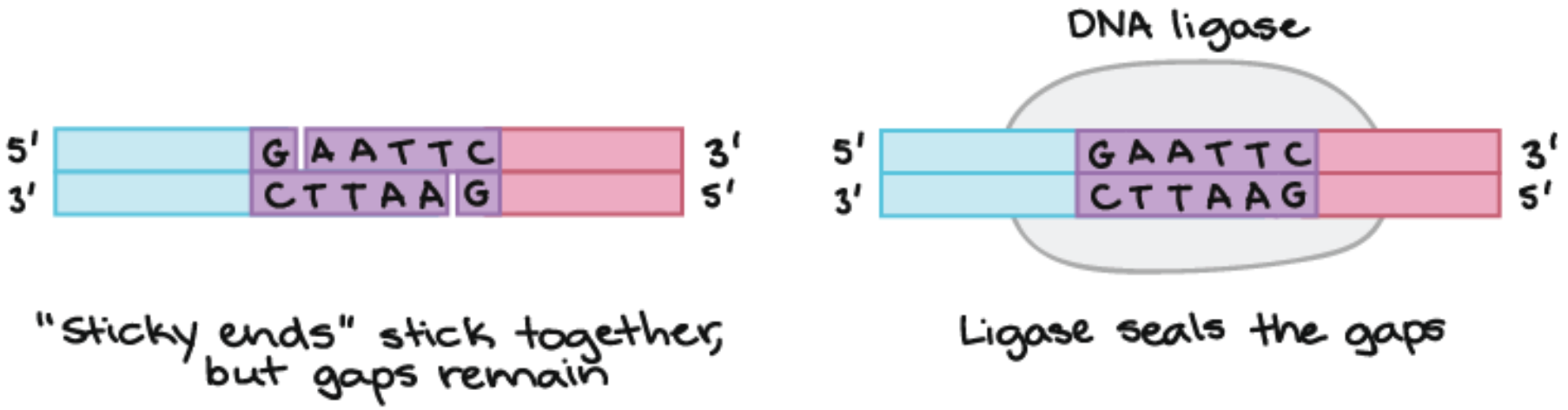
T C C A G C T G G A C G A A T T C T T C A G A T G A A T T C A A A

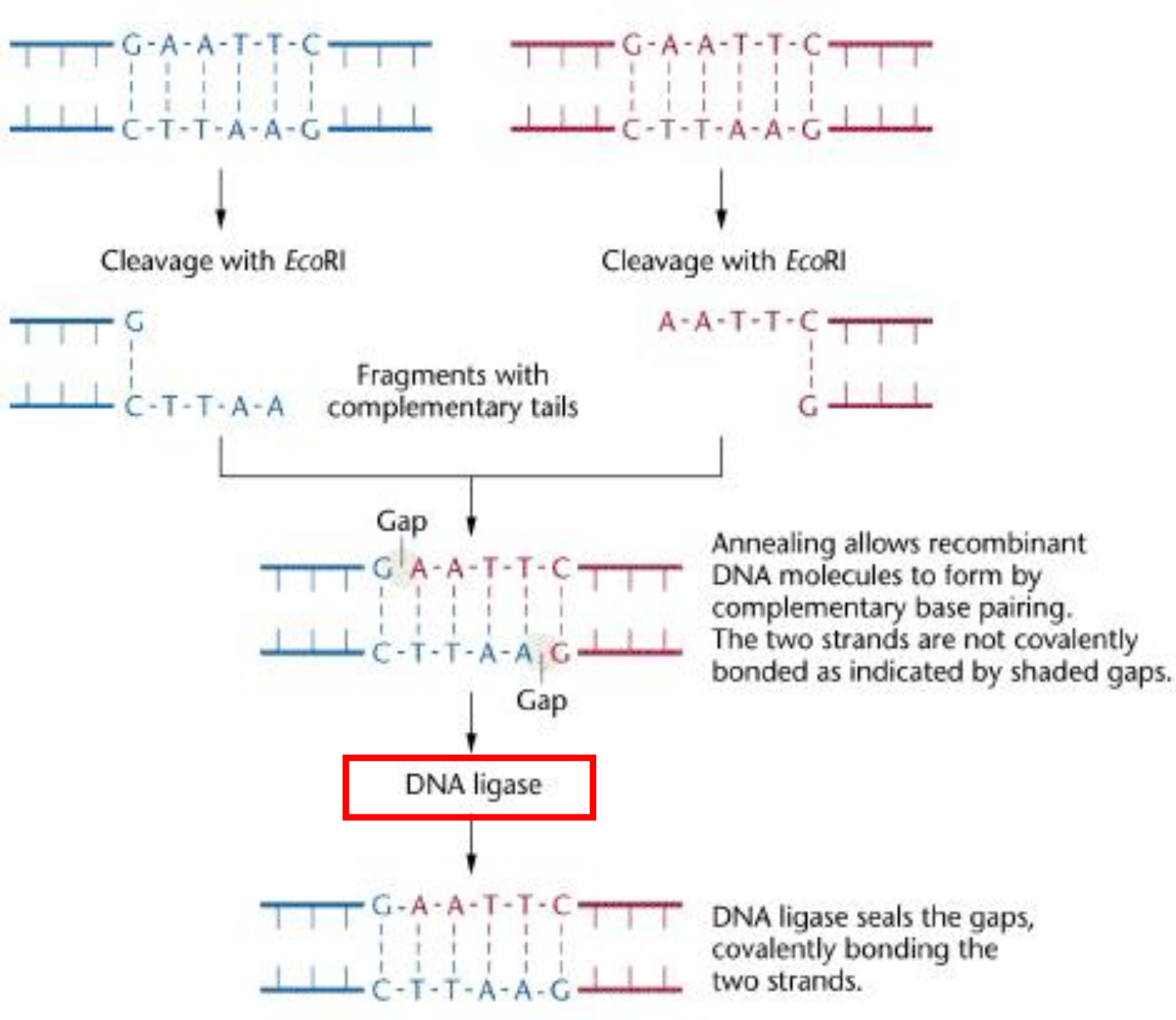
A G G T C G A C C T G C T T A A G A A G T C T A C T T A A G T T T

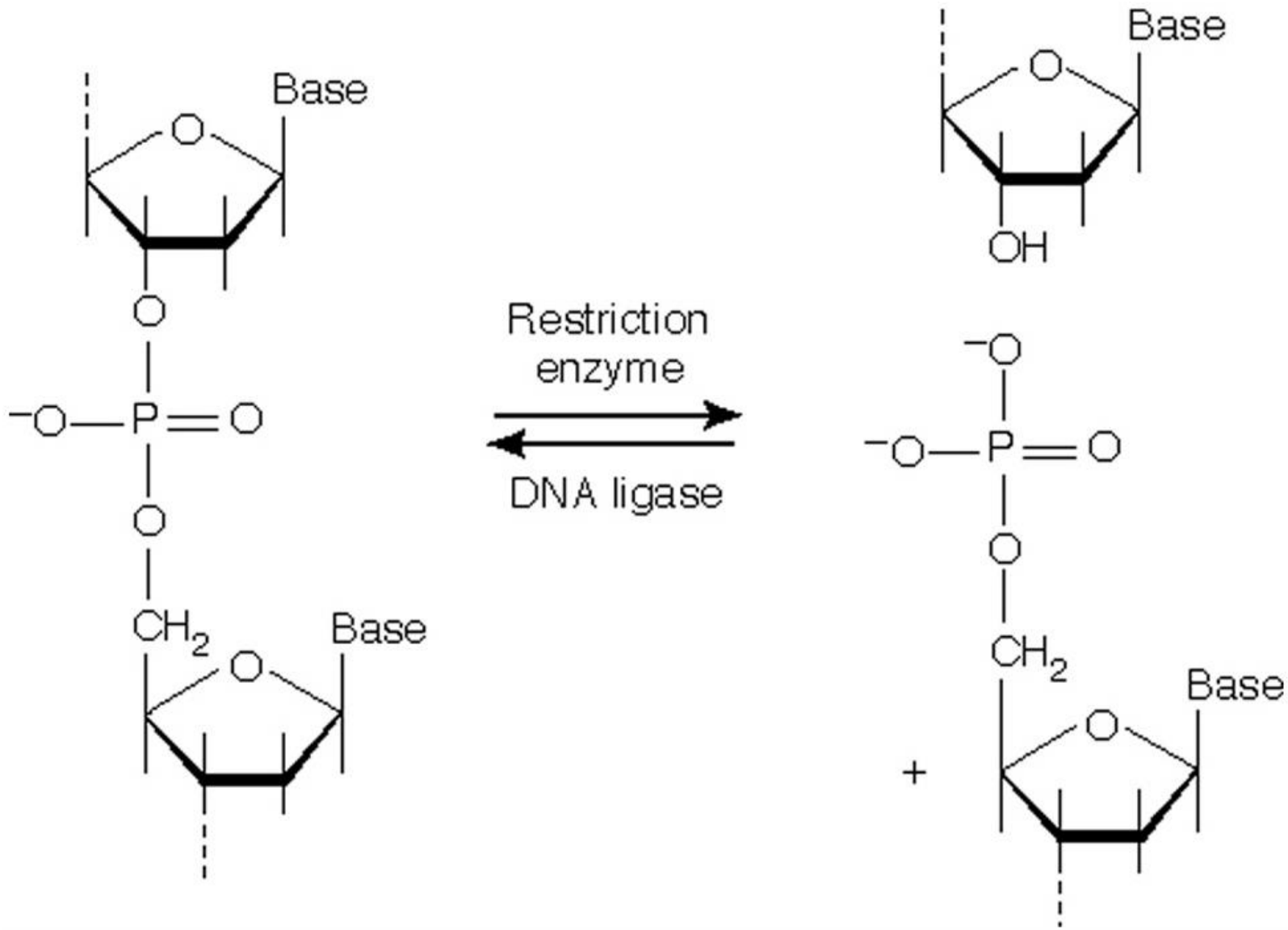
- Number of fragments: 3
- Size of each fragment: 12 bp, 9 bp, 4 bp

DNA ligase:

- DNA ligase is a DNA-joining enzyme.
- If two pieces of DNA have matching ends, ligase can link them to form a single molecule of DNA.
- In DNA cloning, restriction enzymes and DNA ligase are used to insert genes and other pieces of DNA into plasmids.




G-A-A-T-T-C
C-T-T-A-A-G
G-A-A-T-T-C
C-T-T-A-A-G
Cleavage with EcoRI
Cleavage with EcoRI
G
C-T-T-A-A
A-A-T-T-C
G
Fragments with complementary tails
Gap
G A-A-T-T-C
C-T-T-A-A G
Gap
Annealing allows recombinant DNA molecules to form by complementary base pairing. The two strands are not covalently bonded as indicated by shaded gaps.
DNA ligase
G-A-A-T-T-C
C-T-T-A-A-G
DNA ligase seals the gaps, covalently bonding the two strands.


Base
Base
OH
Restriction
enzyme
DNA ligase
Base
Base
+

Questions?