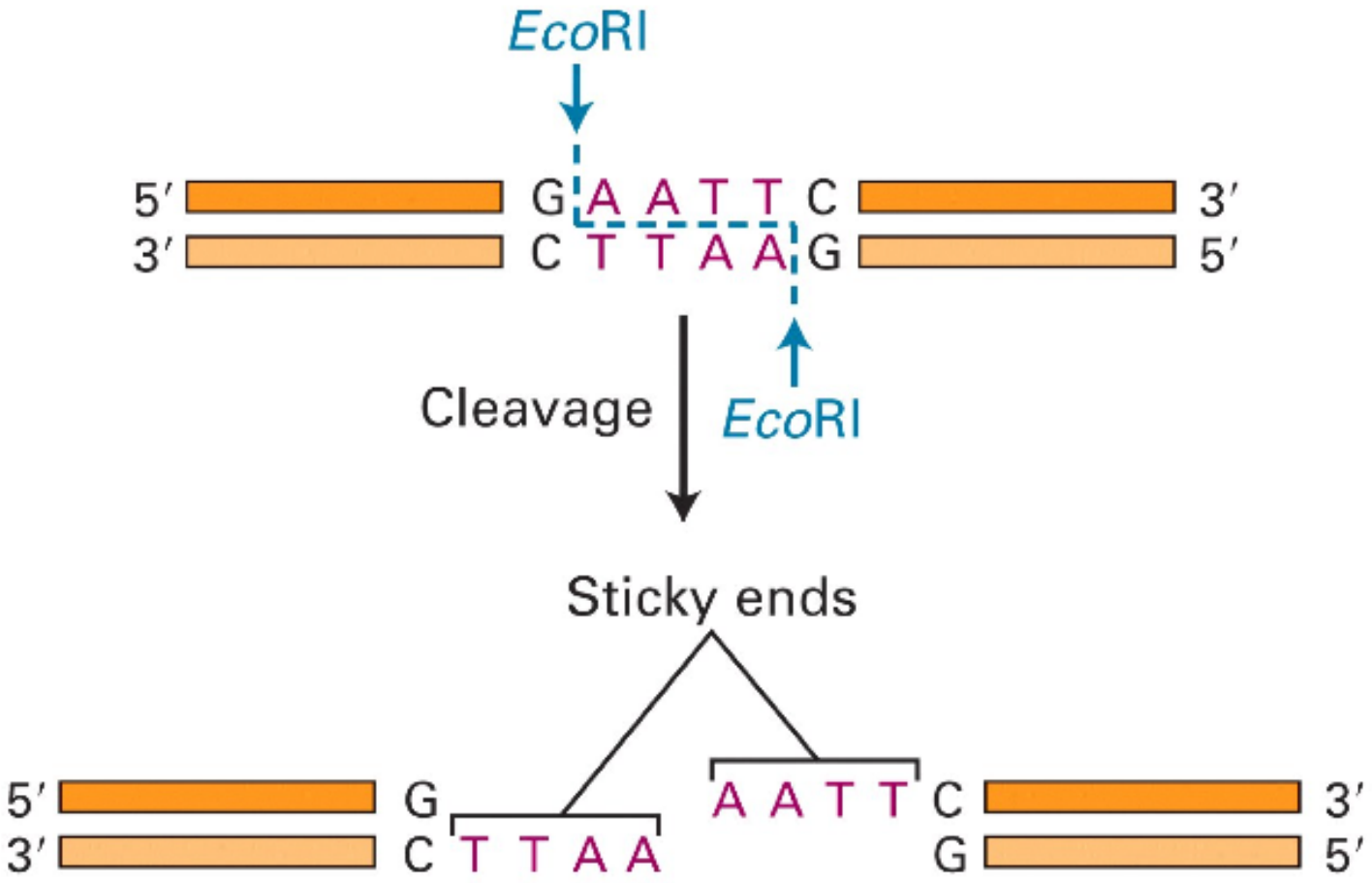
Cutting and Pasting a DNA Sequence

Molecular Biology Laboratory Techniques : Cutting a DNA Sequence

- **Cutting a DNA Sequence** -- Restriction Enzymes
- DNA is a long molecule. In order to be able to sequence it piece by piece, a biological pair of scissors is needed.
- Around 1973, Smith et. Al. made a startling discovery in the course of their study of defense mechanism of bacterial cells from viral attack.
  - Observation: certain bacteria produced enzymes that can cut or break a double stranded DNA at specific points.
  - These proteins, called **restriction enzymes** can catalyze the **hydrolysis** of DNA (the process of breaking a molecule by adding water) at specific points called **restriction sites** that are determined by a specific sequence of base pair.

- The first such enzyme discovered called ***EcoRI*** could **cleave** or **digest** DNA molecules between *G* and *A* whenever it encountered the sequence 5'-*GAATTC-3'*.
  - Note that the sequence is its own reverse complement, i.e., if you read the single strand in the 3'-5' direction, you get the same sequence *GAATTC.* Such reverse complement sequences are called **palindromes.**
  - So, whenever such a sequence appears in one strand, it also appears in the other strand. Since the cuts are made in both strands between *G* and *A*, the remaining DNA pieces have **sticky ends.**

Molecular Scissors



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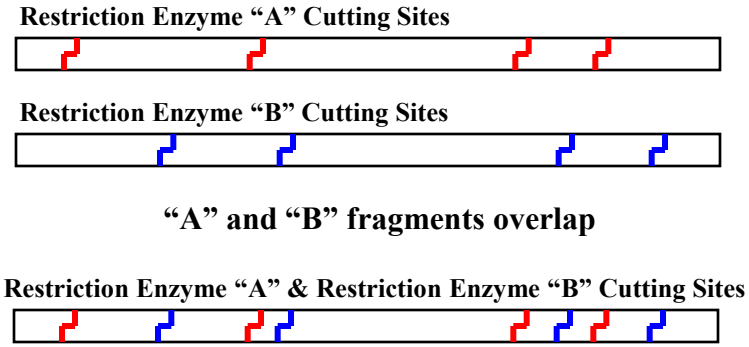
Cutting DNA

- Restriction Enzymes cut DNA
  - Only cut at special sequences
- DNA contains thousands of these sites.
- Applying different Restriction Enzymes creates fragments of varying size.



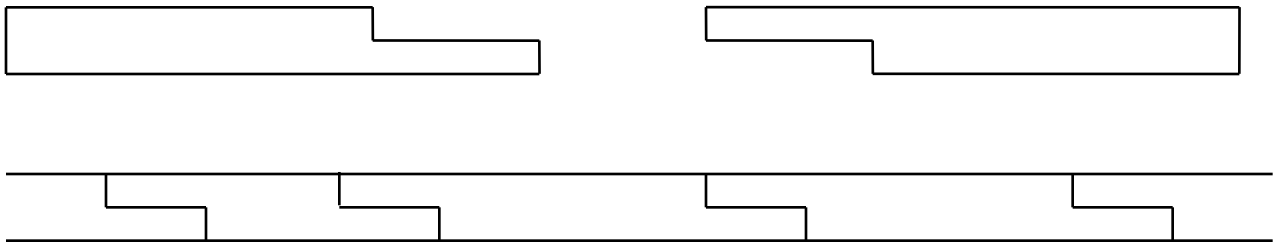
Example

5' ……GAATTC……3'

3'…….CTTAAG……5'

5'……..G AATTC……3'

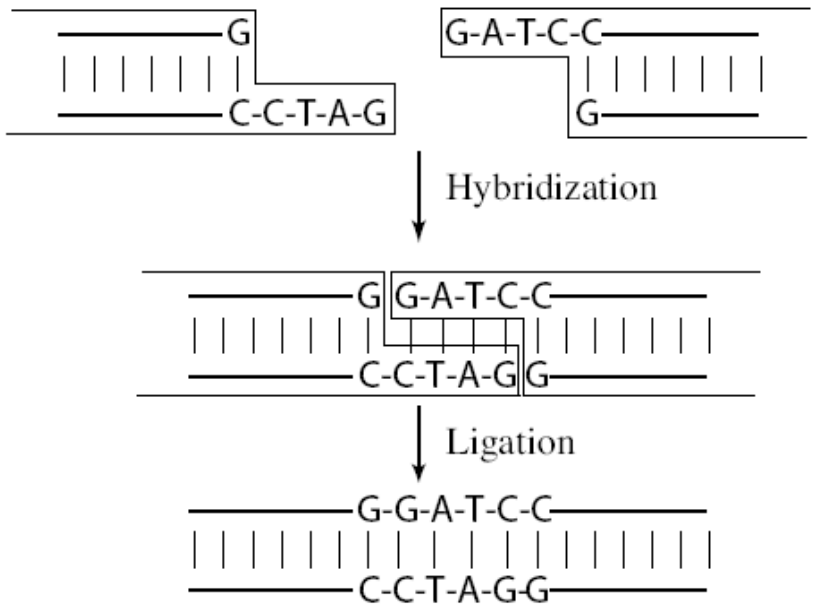
3'……..CTTAA G……5'



Chromosomal DNA and restriction enzyme cutting sites

Pasting DNA

- Two pieces of DNA can be fused together by adding chemical bonds
  - Hybridization – complementary base-pairing
  - Ligation – fixing bonds with single strands



- The sticky ends themselves are naturally complementary to each other.
- This favors re-linking with another DNA piece cut with the same enzyme with the help of another glue enzyme called **ligase**.
- It is also possible to mix DNA from two different sources that have both been cut by using the same restriction enzyme.
- This allows combining fragments from two distinct DNA. Thus, restriction and ligase enzymes are nature's way of providing "cut and paste" editing facility for DNA sequences and have been used in **genetic engineering for recombinant DNA**.
- Even for the same DNA, the cut pieces may join together in different combinations generating overlapping DNA fragments. These are also recombinant DNA and can be cloned for further processing.

- There are also restriction enzymes that do not create sticky ends, they create **blunt ends.**
- Such blunt cuts can also be ligated with other blunt-ended DNA molecules. In particular, small oligonucleotides can be legated at the blunt ends to have almost arbitrary combination of DNA ends.
- Since the discovery of *EcoR*I, more than 300 restriction enzymes have been found in other bacterial species and have been used in laboratories.
  - These are mostly 4-, 6- or 8-cutters;
  - it is rare to find an odd cutter since the palindromes must be of even length.
  - Finally, the restriction enzymes are sometimes called **endonucleases** because they cut the DNA in the middle of the sequence. There are enzymes called **exonulceases** that cut a DNA from only one end.

Discovering Restriction Enzymes

- *Hin*dII - first restriction enzyme – was discovered accidentally in 1970 while studying how the bacterium *Haemophilus influenzae* takes up DNA from the virus
- Recognizes and cuts DNA at sequences:
  - GTGCAC
  - GTTAAC

Discovering Restriction Enzymes

Werner Arber **Daniel Nathans** **Hamilton Smith**

Werner Arber – discovered restriction enzymes

Daniel Nathans - pioneered the application of restriction for the construction of genetic maps

Hamilton Smith - showed that restriction enzyme cuts DNA in the middle of a specific sequence

"My father has discovered a servant who serves as a pair of scissors. If a foreign king invades a bacterium, this servant can cut him in small fragments, but he does not do any harm to his own king. Clever people use the servant with the scissors to find out the secrets of the kings. For this reason my father received the Nobel Prize for the discovery of the servant with the scissors".

Daniel Nathans' daughter
(from Nobel lecture)

Recognition Sites of Restriction Enzymes

Enzyme	Source Microorganism	Recognition Site*	Ends Produced
BamH II	*Bacillus amyloliquefaciens*	↓ -G-G-A-T-C-C- -C-C-T-A-G-G- ↑	Sticky
*Eco*RI	*Escherichia coli*	↓ G A A T T C C T T A A G ↑	Sticky
*Hin*dIII	*Haemophilus influenzae*	↓ -A-A-G-C-T-T- -T-T-C-G-A-A- ↑	Sticky
*Kpn*I	*Klebsiella pneumonia*	↓ -G-G-T-A-C-C- -C-C-A-T-G-G- ↑	Sticky

Uses of Restriction Enzymes

- Recombinant DNA technology
- Cloning
- cDNA/genomic library construction
- DNA mapping
- Stem Cell Research

Restriction Maps

- A map showing positions of restriction sites in a DNA sequence
- If DNA sequence is known then construction of restriction map is a trivial exercise
- In early days of molecular biology DNA sequences were often unknown
- Biologists had to solve the problem of constructing restriction maps without knowing DNA sequences