MSc III Sem – Biotechnology

Course – Principles of Genetic Engineering

Basic Concepts of Restriction Enzymes

- **Restriction Enzyme**, **Restriction Endonuclease**, or ***Restrictase*** is an enzyme that cleaves DNA into fragments at or near specific recognition sites within molecules known as restriction sites.
- REs are one class of the broader endonuclease group of enzymes. Commonly classified into five types, which differ in their structure and whether they cut their DNA substrate at their recognition site, or if the recognition and cleavage sites are separate from one another.
- To cut DNA, all REs make two incisions, once through each sugar-phosphate backbone (i.e. each strand) of the DNA double helix.
- These enzymes are found in bacteria and archaea and provide a defense mechanism against invading viruses. Inside a prokaryote, the restriction enzymes selectively cut up *foreign* DNA in a process called *restriction digestion*; meanwhile, host DNA is protected by a modification enzyme (a methyltransferase) that modifies the prokaryotic DNA and blocks cleavage. Together, these two processes form the restriction modification system.
- More than 3,600 restriction endonucleases are known which represent over 250 different specificities. More than 800 REs are available commercially for routine DNA modification experiments in laboratories, and they are a vital tool in molecular cloning.

History of REs

The term restriction enzyme originated from the studies of phage λ, a virus that infects bacteria, and the phenomenon of host-controlled restriction and modification of such bacterial phage or bacteriophage, First identified in work done in the laboratories of Salvador Luria, Jean Weigle and Giuseppe Bertani in the early 1950s. Bacteriophage λ that can grow well in one strain of *Escherichia coli*, for example *E. coli* C, when grown in another strain, for example *E. coli* K, its yields can drop significantly (3-5 times lower). The host cell, in this example *E. coli* K, is known as the restricting host and appears to have the ability to reduce the biological activity of the phage λ. If a phage becomes established in one strain, the ability of that phage to grow also becomes restricted in other strains.

In the 1960s, Werner Arber and Matthew Meselson showed that the restriction is caused by an enzymatic cleavage of the phage DNA, and the enzyme involved was therefore termed a restriction enzyme, which were type I restriction enzymes.

In 1970, Hamilton O. Smith, Thomas Kelly and Kent Wilcox isolated and characterized the first type II restriction enzyme, *Hind*II, from the bacterium *Haemophilus influenzae*. Restriction enzymes of this type are more useful for laboratory work as they cleave DNA at the site of their recognition sequence and are the most commonly used as a molecular biology tool. Later, Daniel Nathans and Kathleen Danna showed that cleavage of simian virus 40 (SV40) DNA by restriction enzymes yields specific fragments that can be separated using polyacrylamide gel electrophoresis, thus showing that restriction enzymes can also be used for mapping DNA. For their work in the discovery and characterization of restriction enzymes, the 1978 Nobel Prize for Physiology or Medicine was awarded to Werner Arber, Daniel Nathans, and Hamilton O. Smith.

Restriction enzymes: NOMENCLATURE

Since their discovery in the 1970s, many restriction enzymes have been identified; for example, more than 3500 different Type II restriction enzymes have been characterized. Each enzyme is named after the bacterium from which it was isolated, using a naming system based on bacterial genus, species and strain.
For example, the name of the EcoRI restriction enzyme was derived as shown:

Derivation of the EcoRI name		
Abbreviation	**Meaning**	**Description**
E	*Escherichia*	genus
co	*coli*	specific species
R	RY13	strain
I	First identified	order of identification in the bacterium

Enzyme	Source	Recognition Sequence	Cut
EcoRI	*Escherichia coli*	5'GAATTC 3'CTTAAG	5'---G AATTC---3' 3'---CTTAA G---5'
EcoRII	*Escherichia coli*	5'CCWGG 3'GGWCC	5'--- CCWGG---3' 3'---GGWCC ---5'
BamHI	*Bacillus amyloliquefaciens*	5'GGATCC 3'CCTAGG	5'---G GATCC---3' 3'---CCTAG G---5'
HindIII	*Haemophilus influenzae*	5'AAGCTT 3'TTCGAA	5'---A AGCTT---3' 3'---TTCGA A---5'
TaqI	*Thermus aquaticus*	5'TCGA 3'AGCT	5'---T CGA---3' 3'---AGC T---5'
NotI	*Nocardia otitidis*	5'GCGGCCGC 3'CGCCGGCG	5'---GC GGCCGC---3' 3'---CGCCGG CG---5'
HinFI	*Haemophilus influenzae*	5'GANTC 3'CTNAG	5'---G ANTC---3' 3'---CTNA G---5'
Sau3AI	*Staphylococcus aureus*	5'GATC 3'CTAG	5'--- GATC---3' 3'---CTAG ---5'
PvuII*	*Proteus vulgaris*	5'CAGCTG 3'GTCGAC	5'---CAG CTG---3' 3'---GTC GAC---5'
SmaI*	*Serratia marcescens*	5'CCCGGG 3'GGGCCC	5'---CCC GGG---3' 3'---GGG CCC---5'
HaeIII*	*Haemophilus aegyptius*	5'GGCC 3'CCGG	5'---GG CC---3' 3'---CC GG---5'
HgaI	*Haemophilus gallinarum*	5'GACGC 3'CTGCG	5'---NN NN---3' 3'---NN NN---5'
AluI*	*Arthrobacter luteus*	5'AGCT 3'TCGA	5'---AG CT---3' 3'---TC GA---5'
EcoRV*	*Escherichia coli*	5'GATATC 3'CTATAG	5'---GAT ATC---3' 3'---CTA TAG---5'
EcoP15I	*Escherichia coli*	5'CAGCAGN_{25}NN 3'GTCGTCN_{25}NN	5'---CAGCAGN_{25} NN---3' 3'---GTCGTCN_{25}NN ---5'
KpnI	*Klebsiella pneumoniae*	5'GGTACC 3'CCATGG	5'---GGTAC C---3' 3'---C CATGG---5'
PstI	*Providencia stuartii*	5'CTGCAG 3'GACGTC	5'---CTGCA G---3' 3'---G ACGTC---5'
SacI	*Streptomyces achromogenes*	5'GAGCTC 3'CTCGAG	5'---GAGCT C---3' 3'---C TCGAG---5'
SalI	*Streptomyces albus*	5'GTCGAC 3'CAGCTG	5'---G TCGAC---3' 3'---CAGCT G---5'
ScaI*	*Streptomyces caespitosus*	5'AGTACT 3'TCATGA	5'---AGT ACT---3' 3'---TCA TGA---5'
SpeI	*Sphaerotilus natans*	5'ACTAGT 3'TGATCA	5'---A CTAGT---3' 3'---TGATC A---5'
SphI	*Streptomyces phaeochromogenes*	5'GCATGC 3'CGTACG	5'---GCATG C---3' 3'---C GTACG---5'
StuI*	*Streptomyces tubercidicus*	5'AGGCCT 3'TCCGGA	5'---AGG CCT---3' 3'---TCC GGA---5'
XbaI	*Xanthomonas badrii*	5'TCTAGA 3'AGATCT	5'---T CTAGA---3' 3'---AGATC T---5'

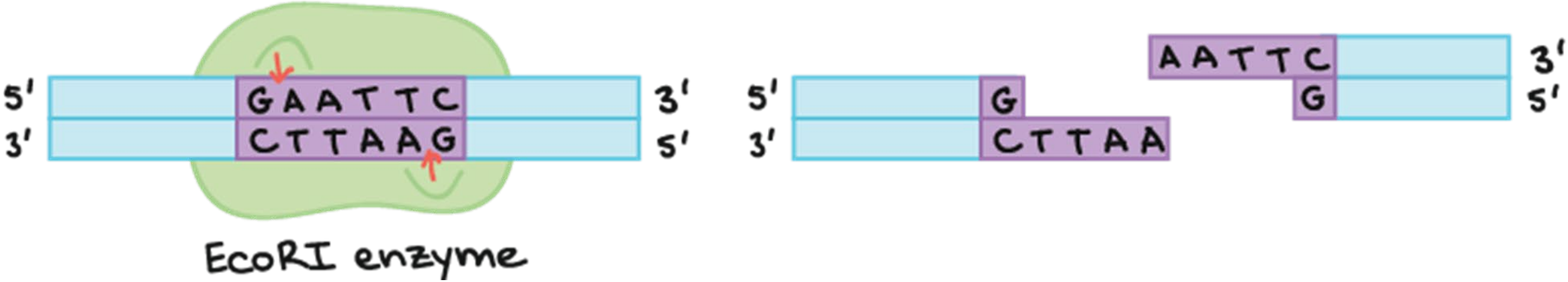
Key: * = blunt ends; N = C or G or T or A; W = A or T

Restriction enzymes working mechanism

Restriction enzymes are found in bacteria (and other prokaryotes). They recognize and bind to specific sequences of DNA, called **restriction sites**. Each restriction enzyme recognizes just one or a few restriction sites.

For eg. *Eco*RI, a common restriction enzyme used in labs cuts DNA at the following site:

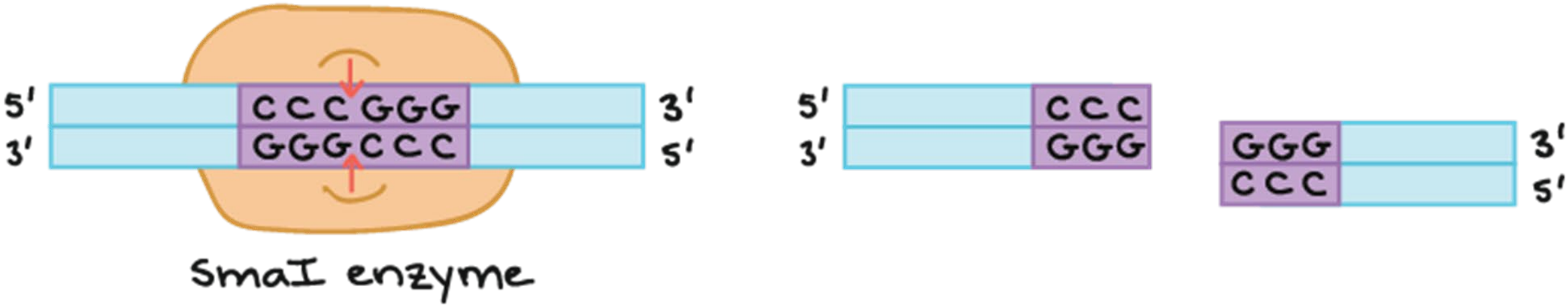
When *Eco*RI recognizes and cuts this site, it always does so in a very specific pattern that produces ends with single-stranded DNA “overhangs”:



Restriction enzymes working mechanism

If another piece of DNA has matching overhangs (for eg similar to EcoRI), the overhangs can stick together by complementary base pairing. For this reason, enzymes that leave single-stranded overhangs are said to produce **sticky ends**. Sticky ends are helpful in cloning because they hold two pieces of DNA together so they can be linked by DNA ligase.

Not all restriction enzymes produce sticky ends. Some are “blunt cutters,” which cut straight down the middle of a target sequence and leave no overhang. The restriction enzyme *Sma*I is an example of a blunt cutter:



Restriction enzymes: TYPES

Naturally occurring restriction endonucleases are categorized into **four groups (Types I, II III, and IV)** based on their composition and enzyme cofactor requirements. Yet DNA sequence analyses of restriction enzymes show great variations and more than four types. All types of enzymes recognize specific short DNA sequences and carry out the endonucleolytic cleavage of DNA with terminal 5'-phosphates. They differ in their recognition sequence, subunit composition, cleavage position, and cofactor requirements.

- **Type I enzymes** (EC 3.1.21.3) cleave at sites remote from a recognition site; require both ATP and S-adenosyl-L-methionine to function; multifunctional protein with both restriction digestion and methylase (EC 2.1.1.72) activities.
- **Type II enzymes** (EC 3.1.21.4) cleave within or at short specific distances from a recognition site; most require magnesium; single function (restriction digestion) enzymes independent of methylase.
- **Type III enzymes** (EC 3.1.21.5) cleave at sites a short distance from a recognition site; require ATP (but do not hydrolyse it); S-adenosyl-L-methionine stimulates the reaction but is not required; exist as part of a complex with a modification methylase (EC 2.1.1.72).
- **Type IV** enzymes target modified DNA, e.g. methylated, hydroxymethylated and glucosyl-hydroxymethylated DNA
- **Type V** enzymes utilize guide RNAs (gRNAs)

TYPES OF CUTS

DNA ends may be sticky or blunt based on the enzyme which cuts the DNA. The restriction enzyme belong to a larger class of enzymes called **exonucleases and endonucleases.** Exonucleases remove nucleotide from ends whereas endonuclease cuts at specific position within the DNA.

BLUNT ENDS

The simplest DNA end of a double stranded molecule is called a *blunt end*. Blunt ends are also known as non-cohesive ends. In a blunt-ended molecule, both strands terminate in a base pair. Blunt ends are not always desired in biotechnology since when using a DNA ligase to join two molecules into one, the yield is significantly lower with blunt ends. When performing subcloning, it also has the disadvantage of potentially inserting the insert DNA in the opposite orientation desired. On the other hand, blunt ends are always compatible with each other. Here is an example of a small piece of blunt-ended DNA:

```
5'-GATCTGACTGATGCGTATGCTAGT-3’
3'-CTAGACTGACTACGCATACGATCA-5'
```

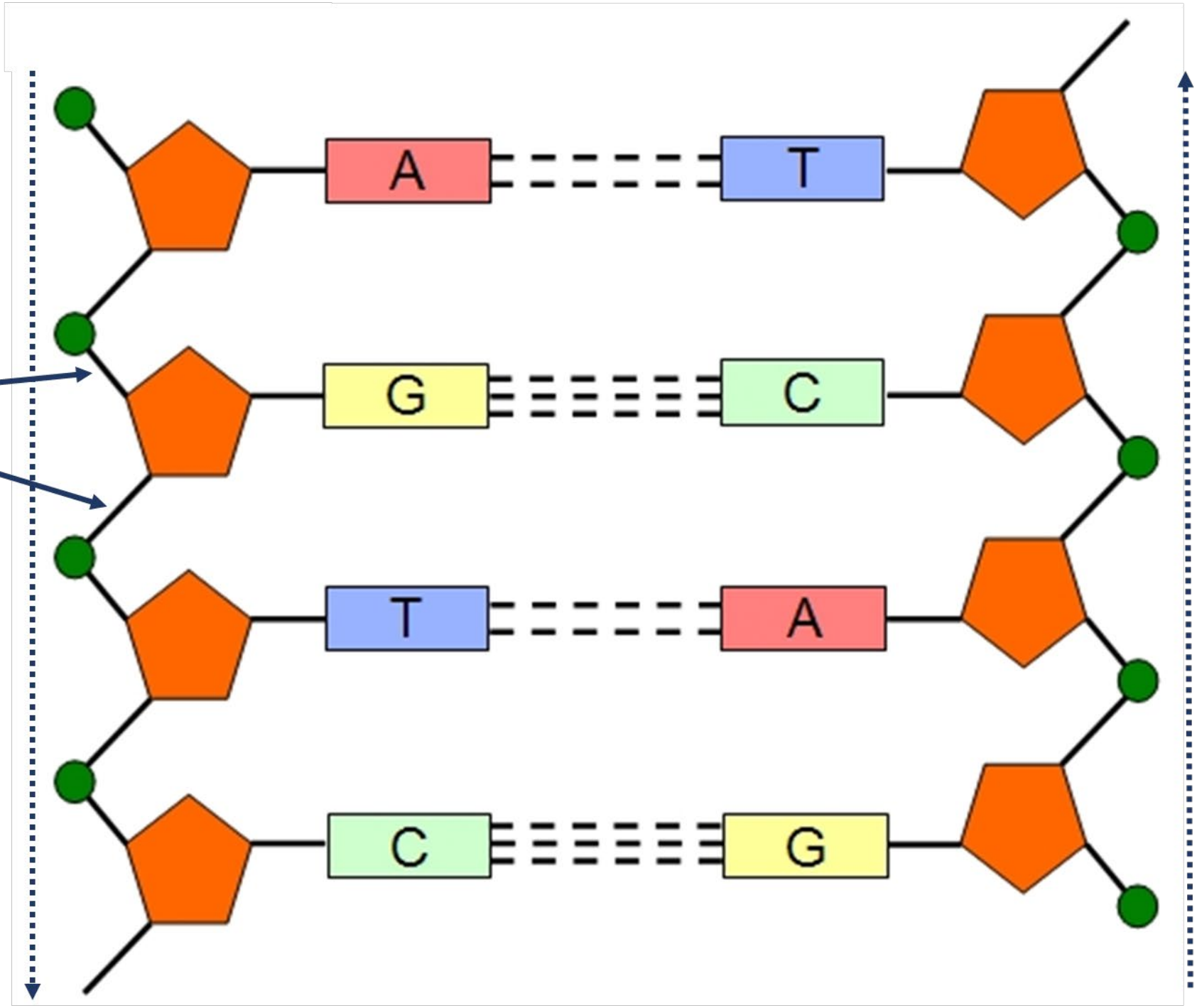
STICKY OR STAGGERED OR OVERHANGS ENDS

Longer overhangs are called *cohesive ends* or *sticky ends*. They are most often created by restriction endonucleases when they cut DNA. Very often they cut the two DNA strands four base pairs from each other, creating a four-base 3' overhang in one molecule and a complementary 3' overhang in the other. These ends are called cohesive since they are easily joined back together by a ligase. For example:

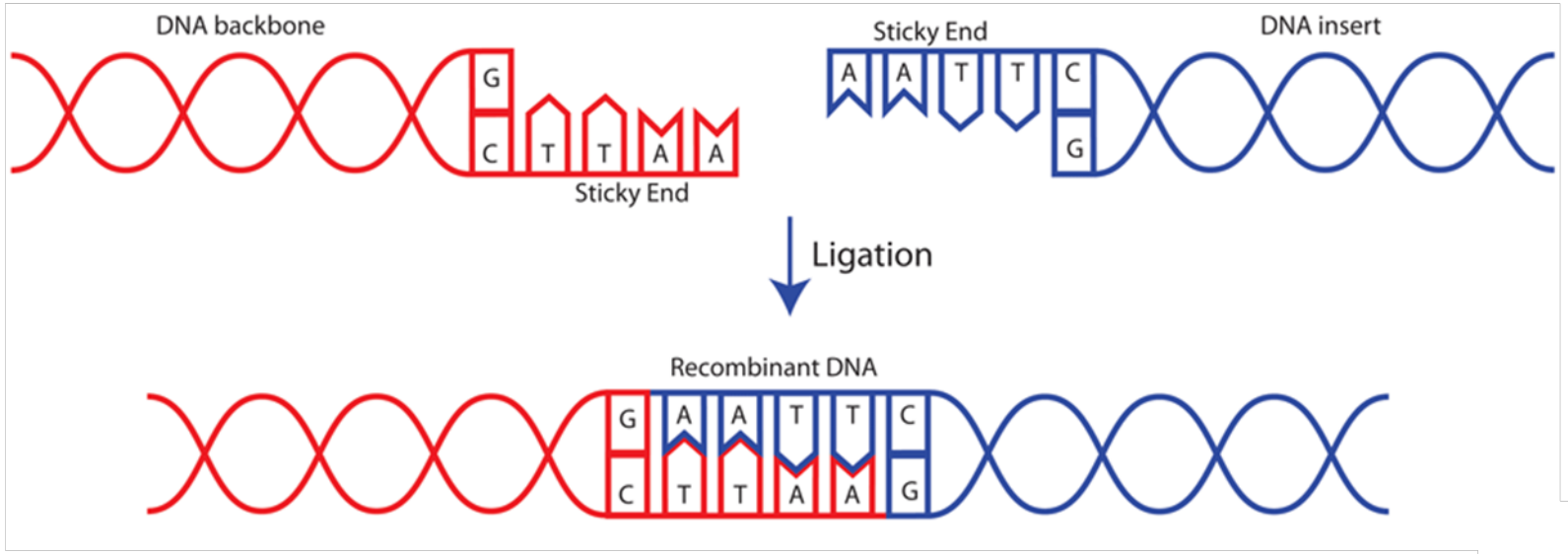
```
5'-ATCTGACT      GATGCGTATGCT-3’
3'-TAGACTGACTACG      CATACGA-5'
```

DNA Strand

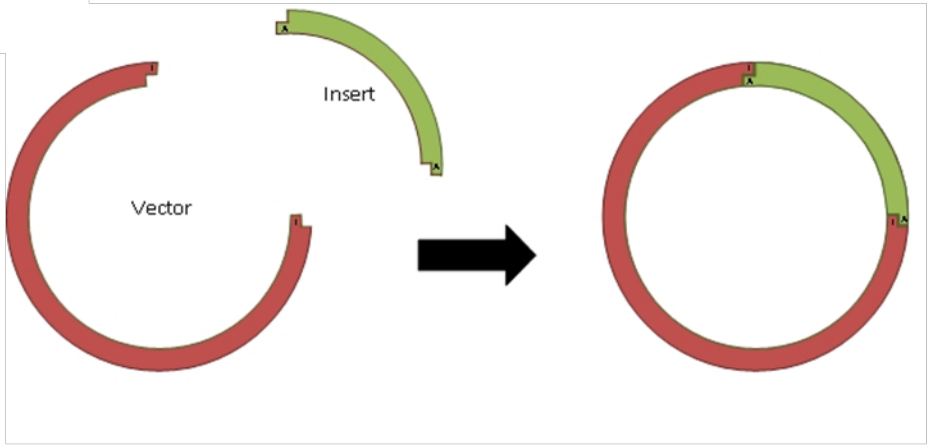
Phosphodiester bonds



Restriction fragments: sticky ends



Purpose: making recombinant DNA



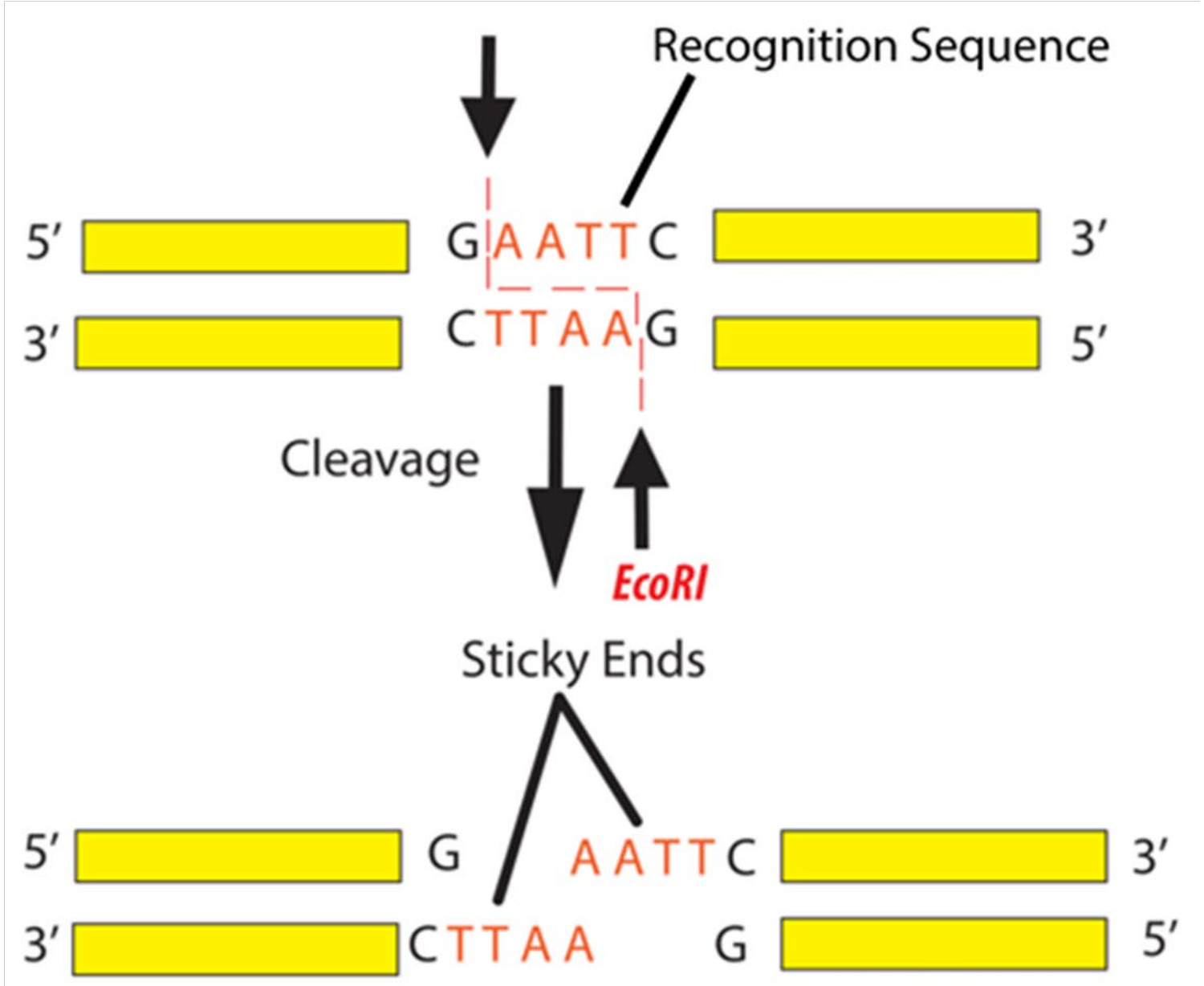
When you cut two separate molecules of DNA with the same restriction enzyme, the fragments will have matching sticky ends. This is how recombinant DNA is created.

Restriction Enzyme *Eco*RI: basic mechanism

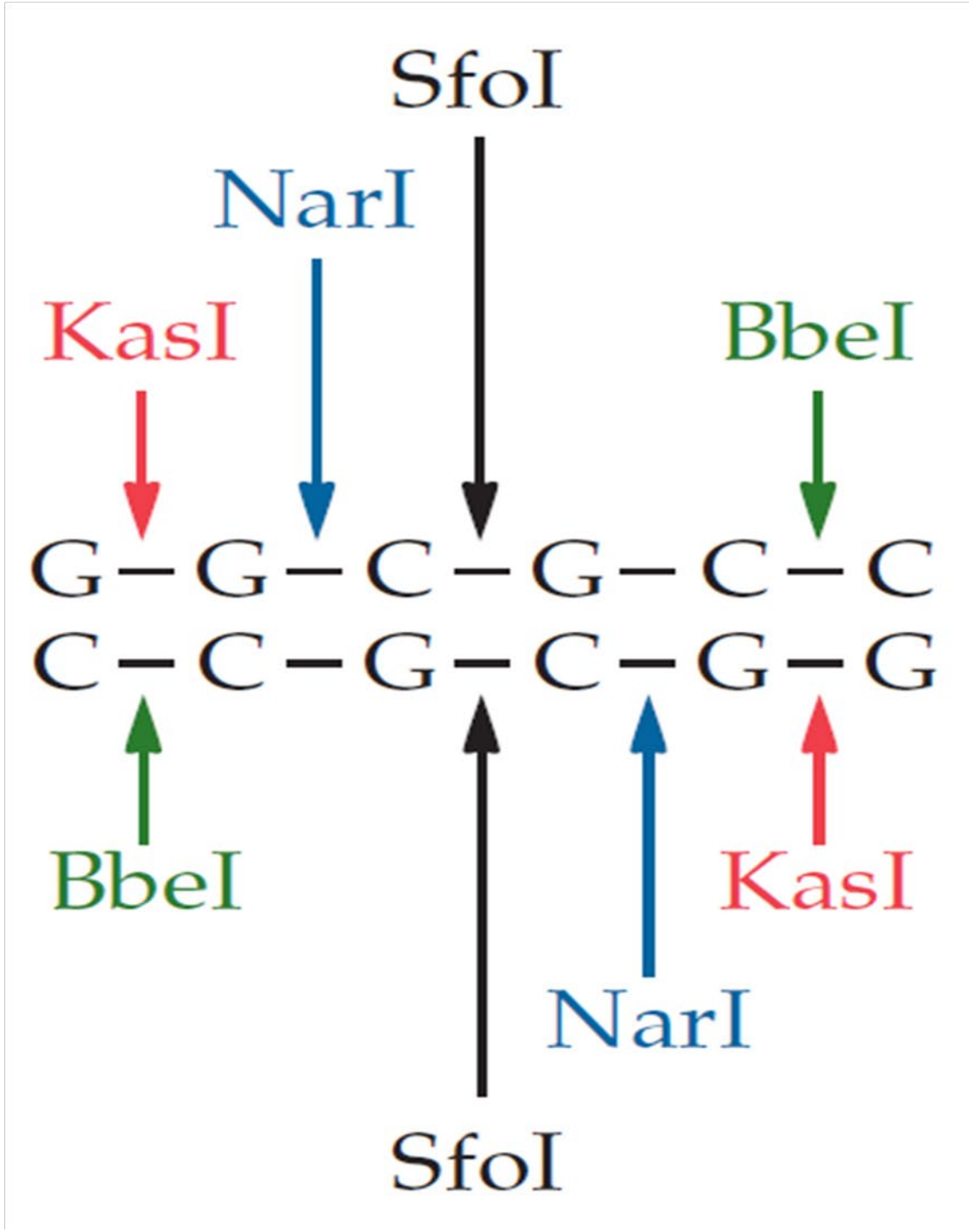
EcoRI was discovered in ***E. coli*** bacteria.

EcoRI cuts at the following sequence: GAATTC

The resulting pieces of DNA are called “restriction fragments.”



Neoschizomers: four restriction endonucleases bind to the same recognition site and cleave at different positions.



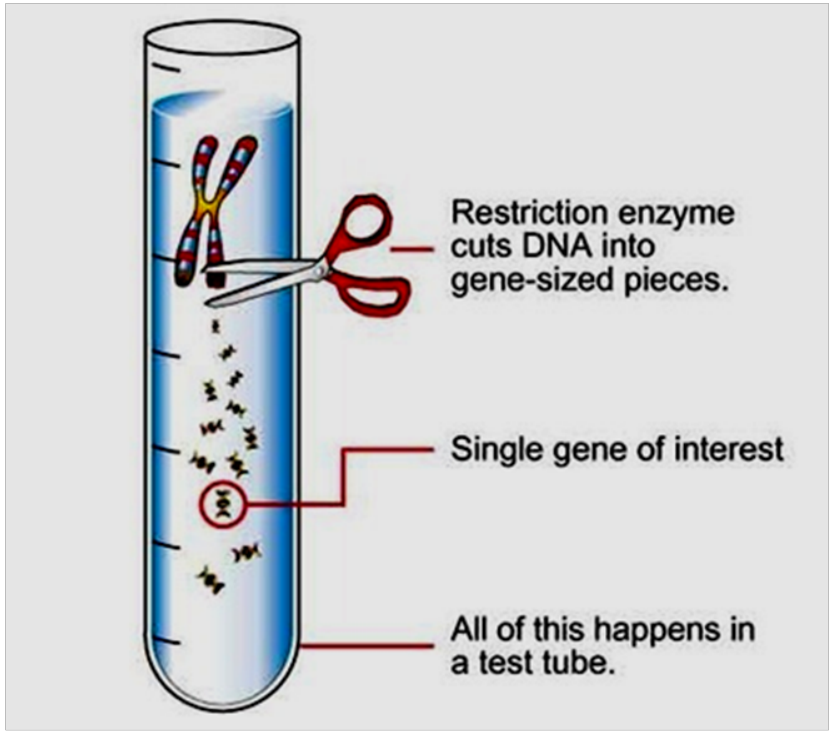
Restriction Enzymes

1962: “molecular scissors” discovered in bacteria

An enzymatic immune system that recognizes and destroys foreign DNA in bacteria

Restrict viruses - Viral genome is destroyed upon entry

Endonuclease - Endo (inside), nuclease (cuts nucleic acid)

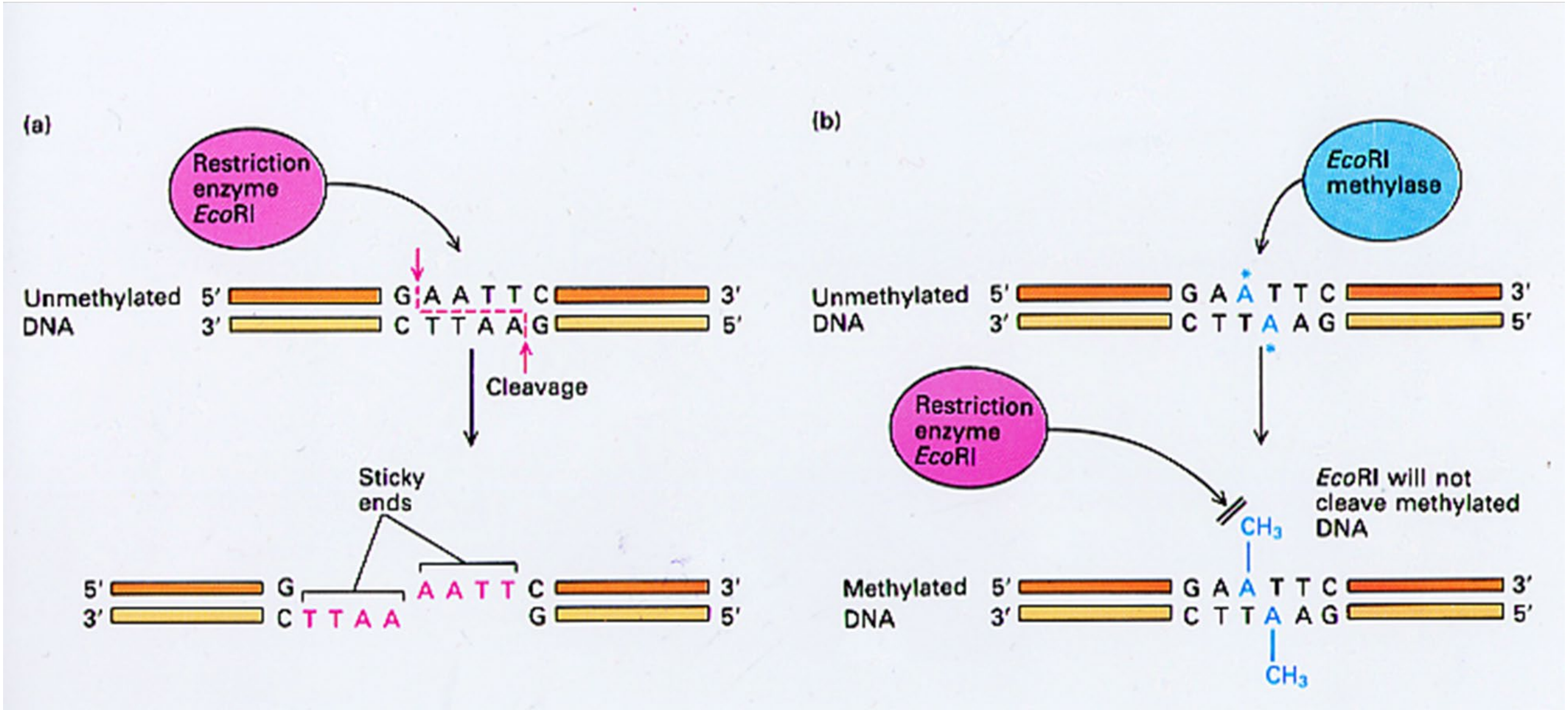


Restriction-modification (R-M) system – protects bacteria from own Endonuclease activity

Methyltransferase activity: protects host DNA from cleavage by the restriction enzyme. Methylate one of the bases in each strand

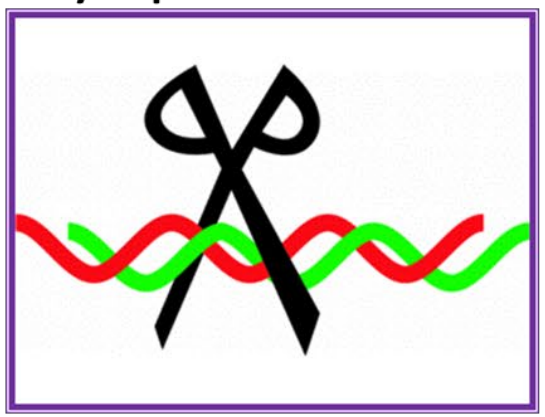
Restriction enzyme and its cognate modification system constitute the R-M system

Methylation



Restriction Enzymes: applications

Researchers rely on **restriction enzymes** to assist with many processes in laboratories around the world:



1. Making recombinant DNA and appraising success

- For research, medicine and agriculture

2. DNA profile analysis

- For disease diagnosis, paternity/family relationship testing, and forensics

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