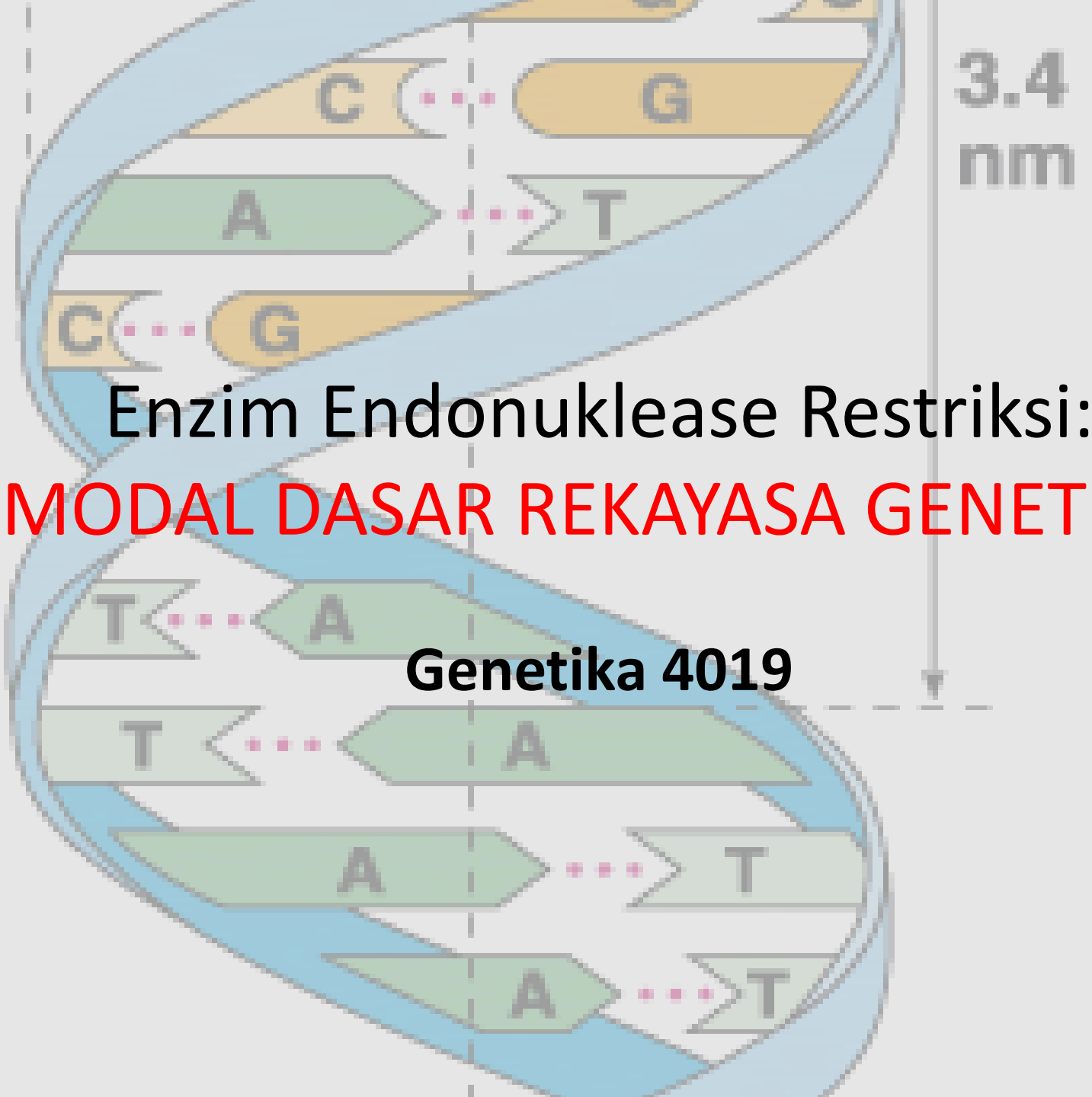
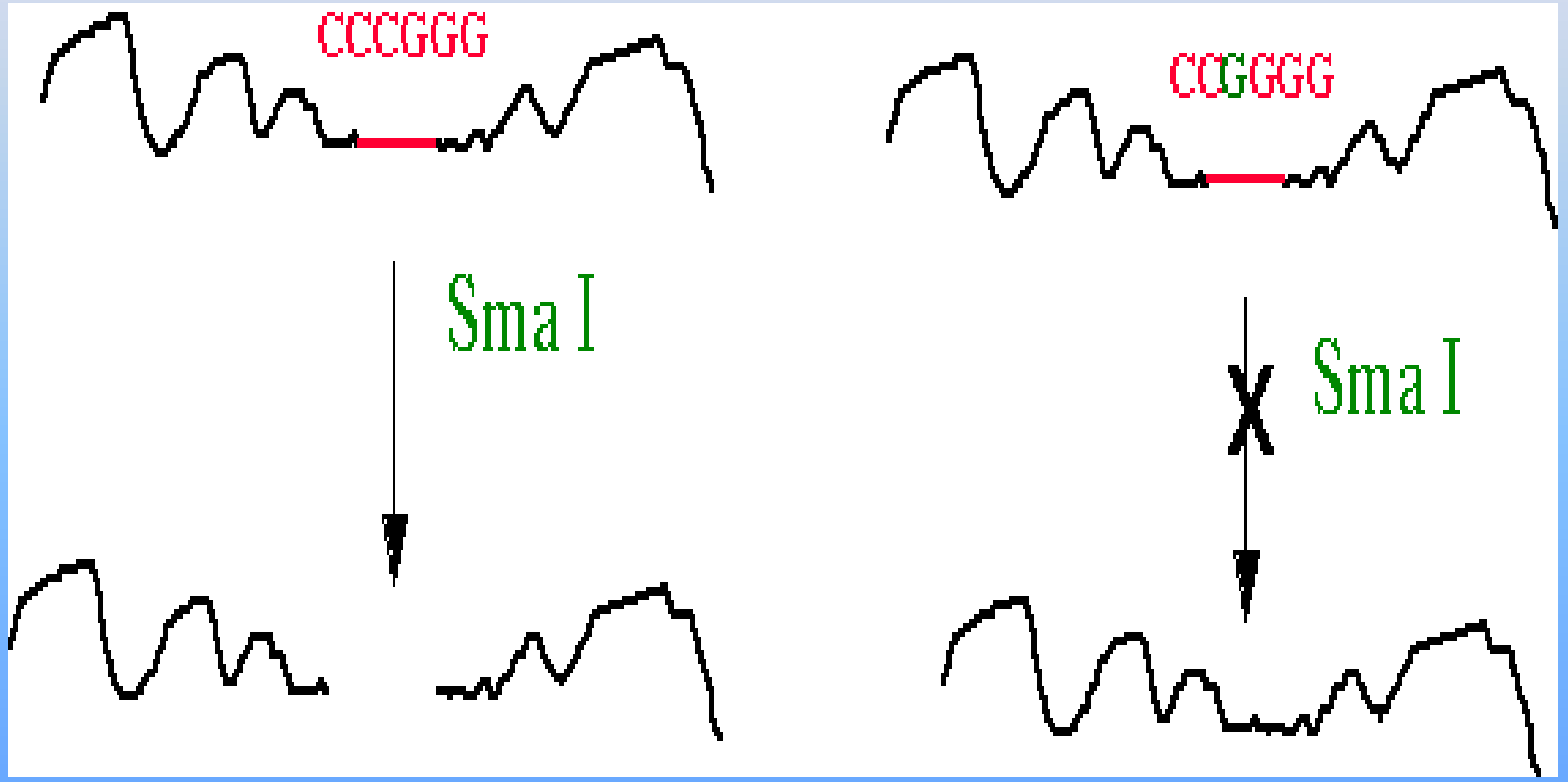


Enzim Endonuklease Restriksi:

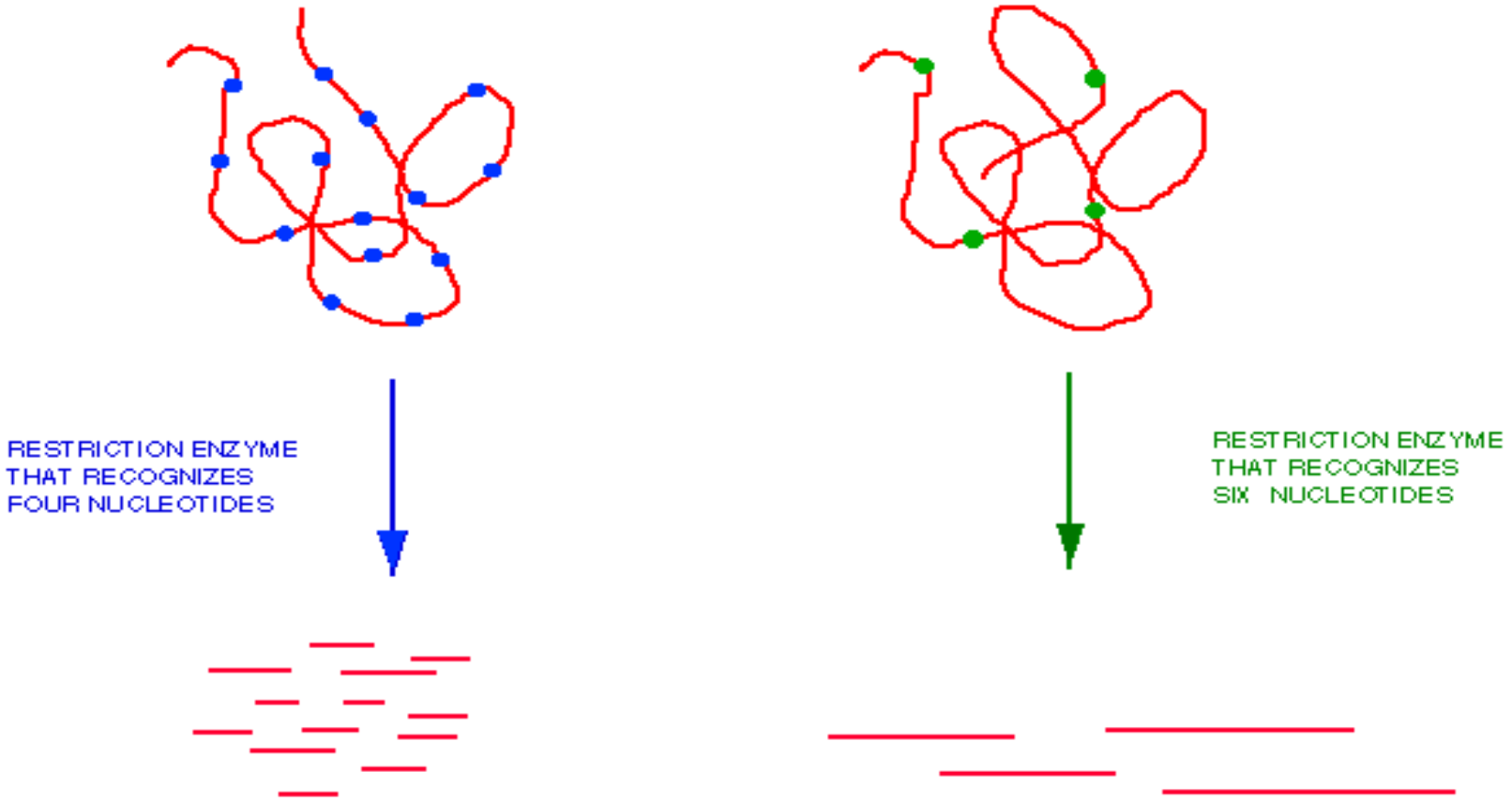
MODAL DASAR REKAYASA GENETIKA

Genetika 4019

Enzim Restriksi hanya memotong DNA pada urutan basa (nukleotida) yang "dikenalinya"

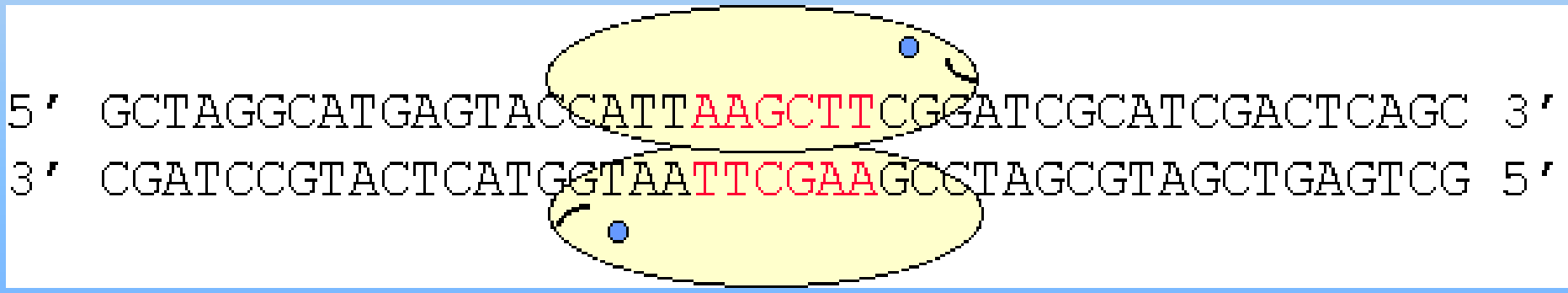


A restriction enzyme with a four nucleotide recognition sequence cuts long DNA more frequently and produce smaller DNA fragments than a restriction enzymes with a six nucleotide recognition sequence.

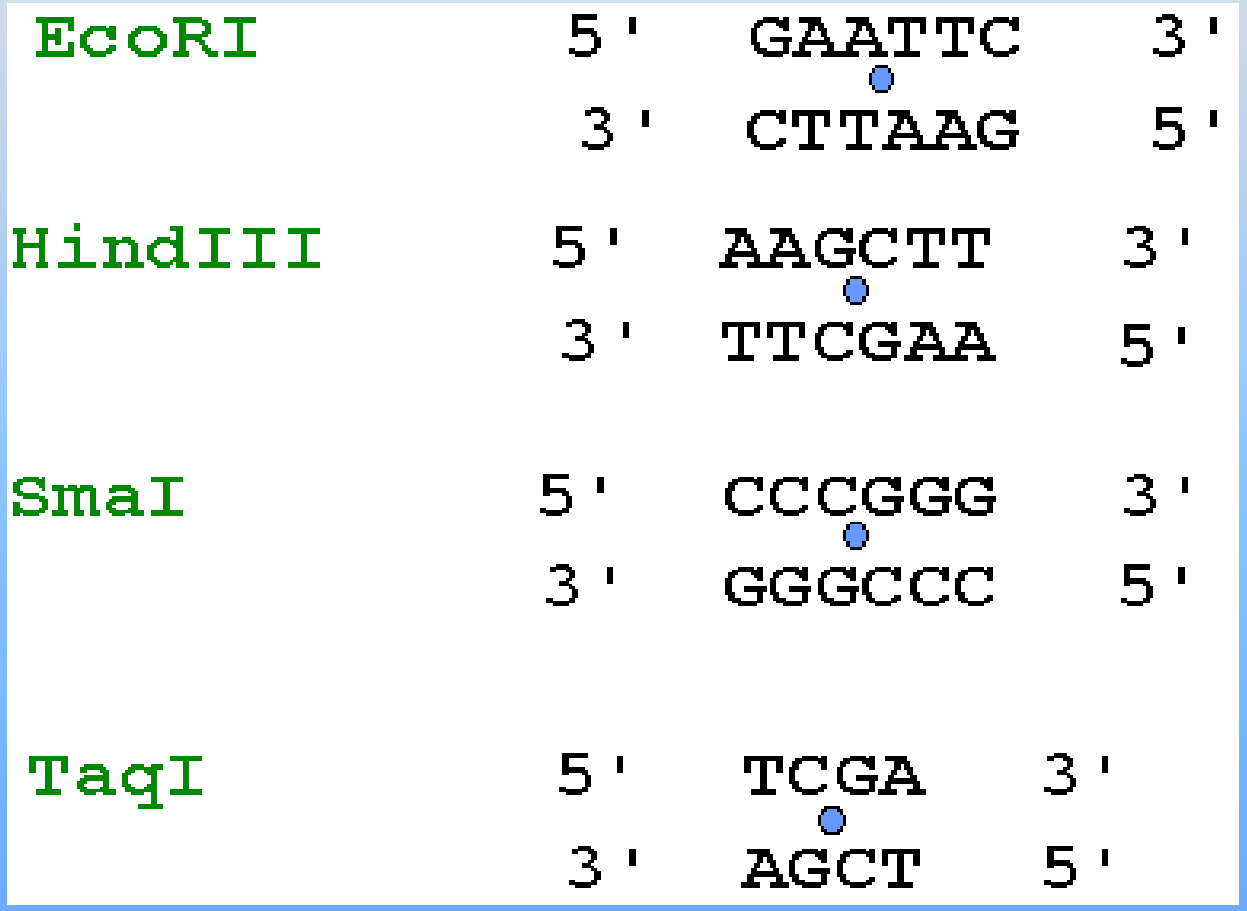


Any given four nucleotide long recognition site occurs in DNA, on average, at a distance of 256 (4^4) nucleotides. Any given six nucleotide long sequence occurs, on average, at a distance of 4096 (4^6) nucleotides. Any given eight nucleotide long sequence occurs, on average, at a distance of 65536 (4^8) nucleotides

RESTRICTION ENZYMES often act as dimers; each restriction enzyme subunit recognizes THE SAME (5'->3') nucleotide sequence in complementary DNA strands:



As a result, restriction enzymes usually recognize palindromic sequences:



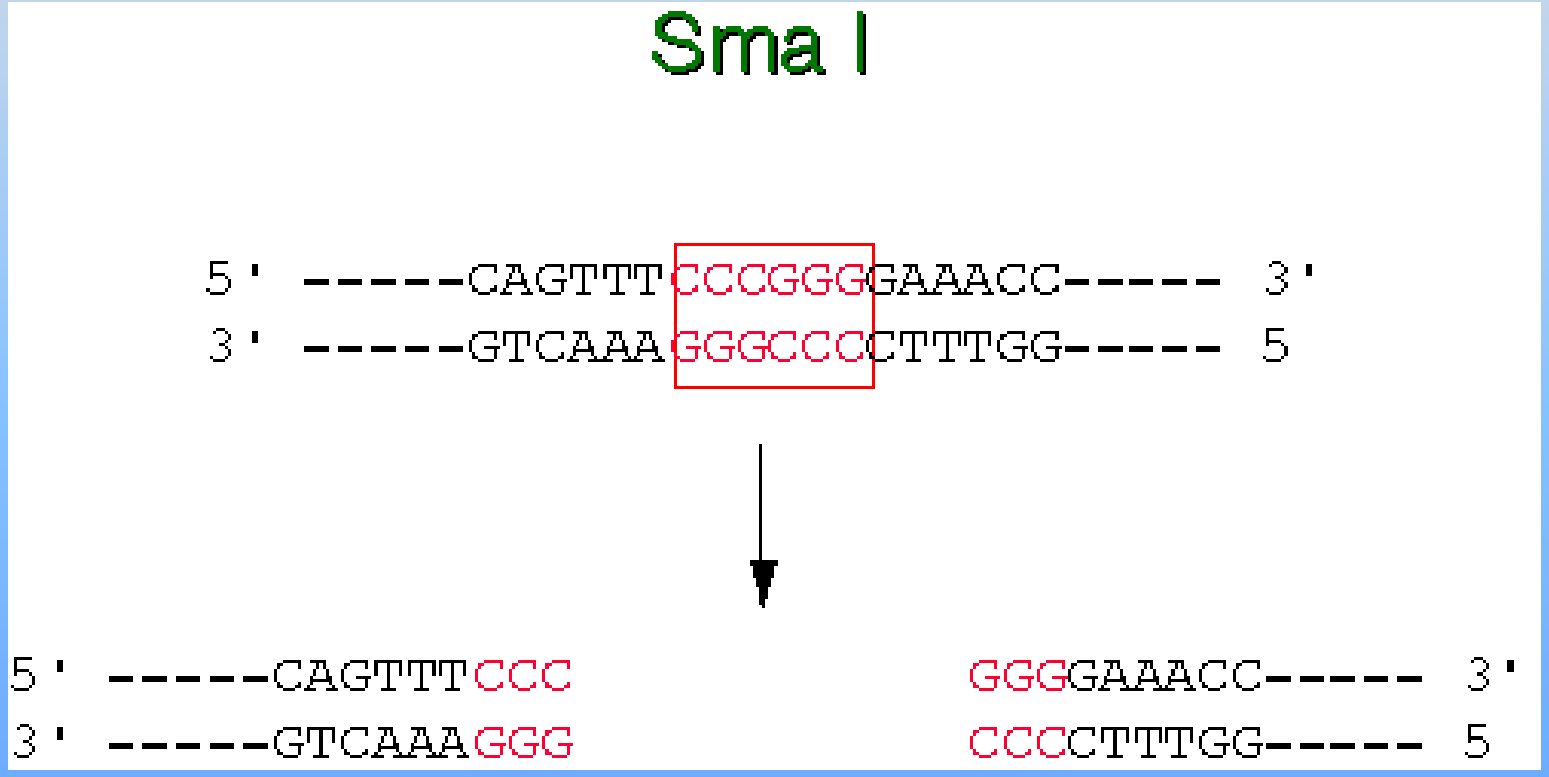
EcoRI=E. coli RY13 (R.N. Yoshimori).

Hind=Haemophilus influenzae

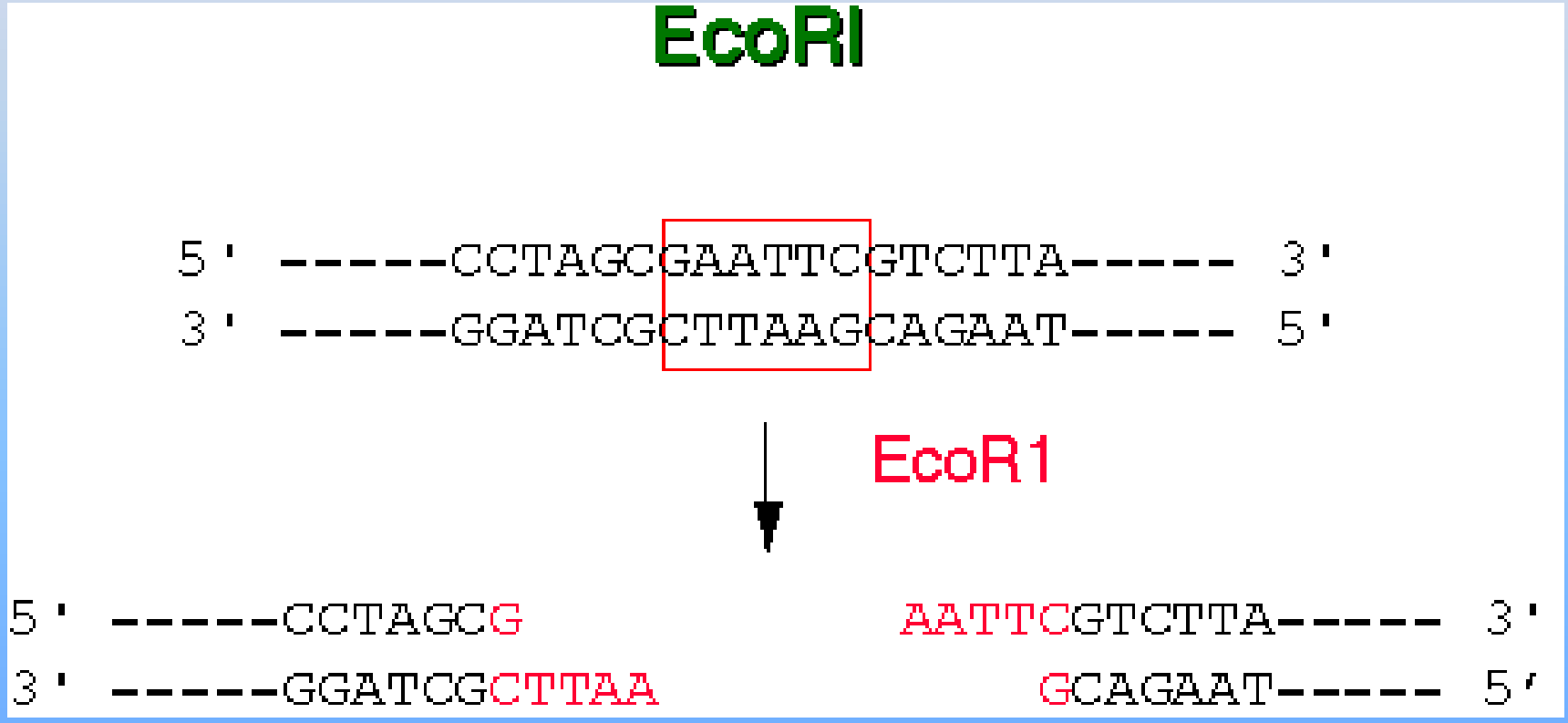
Sma=Serratia marcescens

Taq=Thermus aquaticus

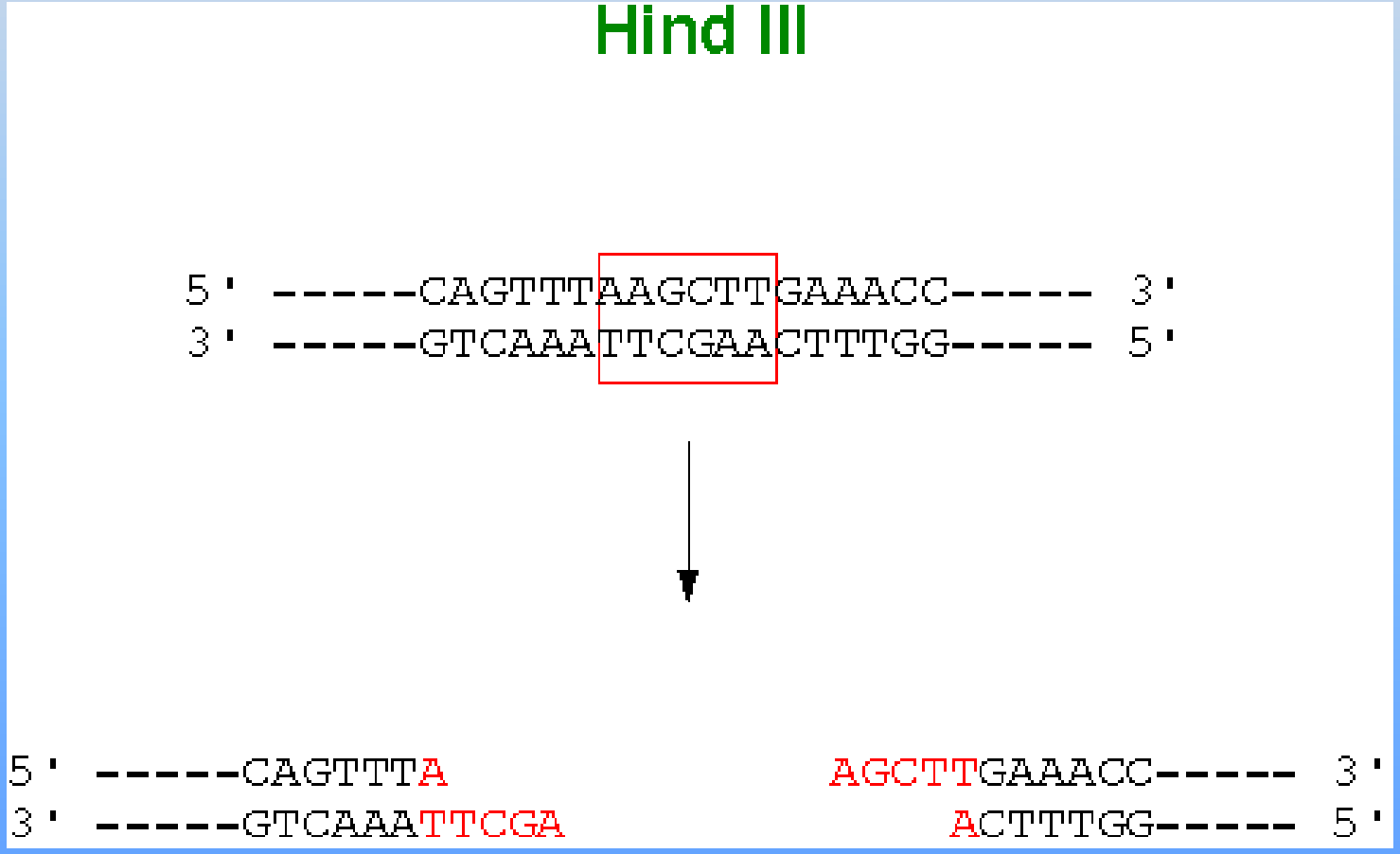
Some restriction enzymes, for example, Sma I, cut both DNA strands in the middle of the recognition sequence and produce "blunt-end" DNA fragments:



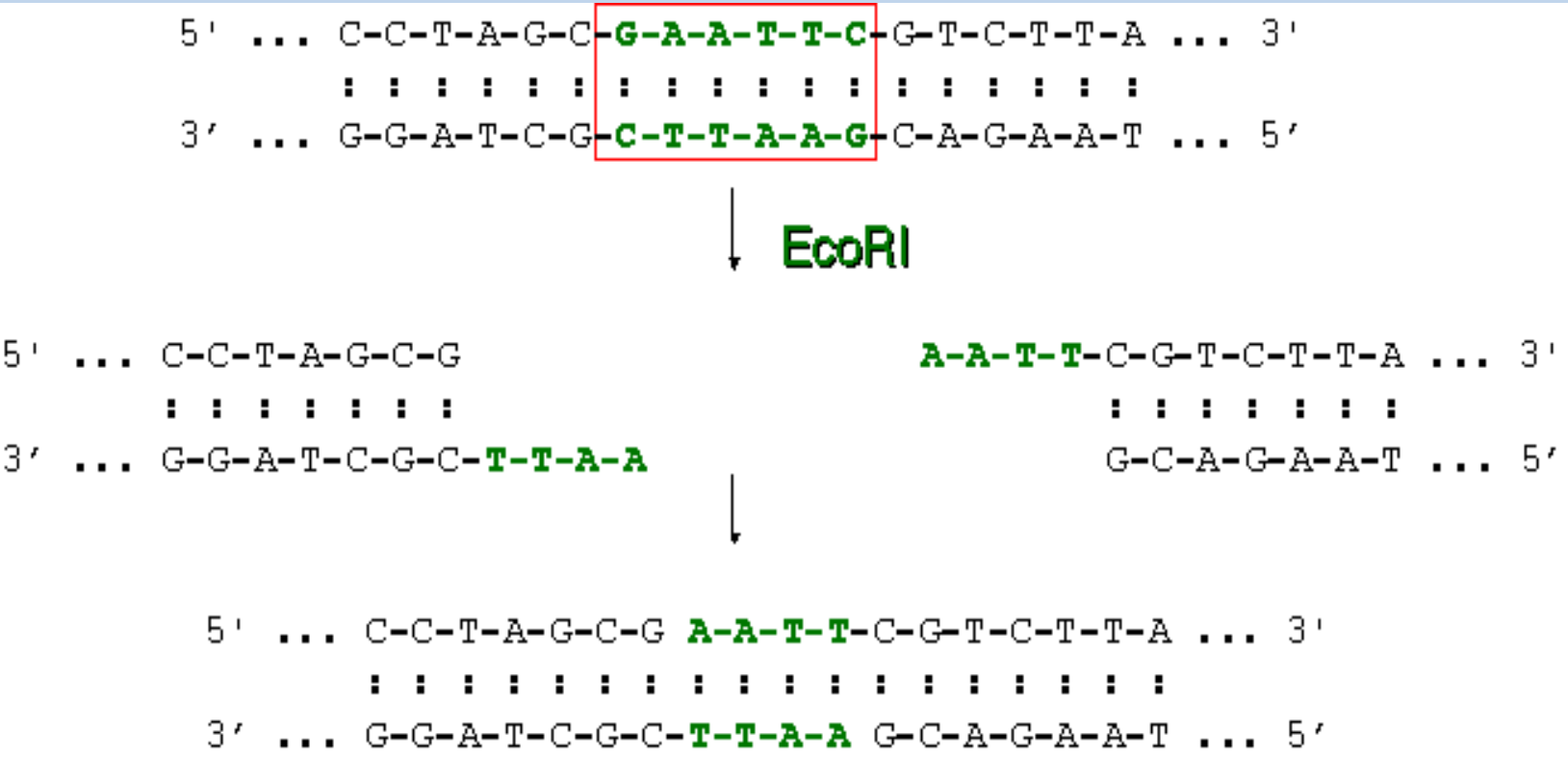
Many restriction nucleases produce staggered cuts in DNA which leave short single stranded "tails" at the ends of DNA fragments: (1)



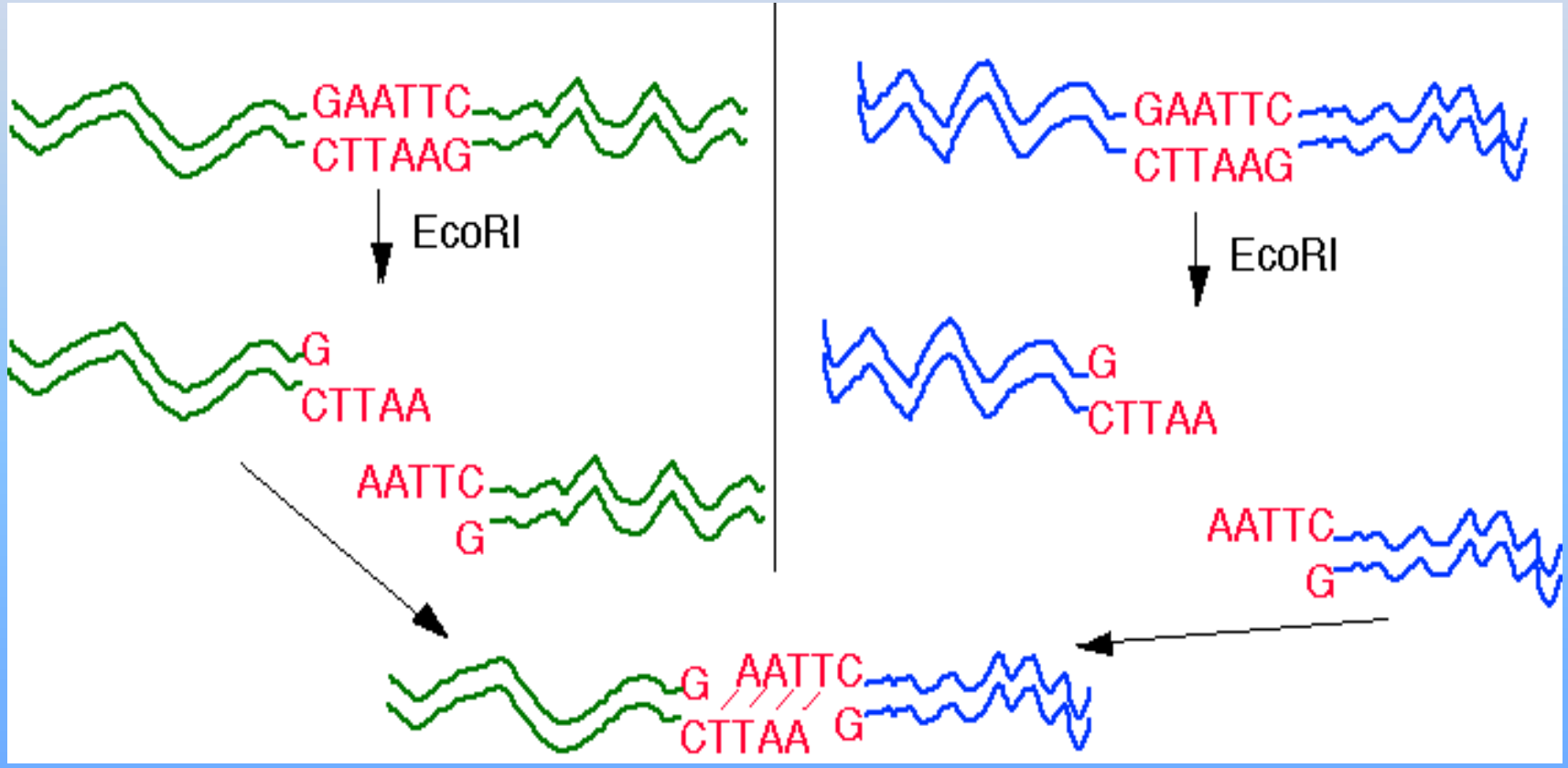
Many restriction nucleases produce staggered cuts in DNA which leave short single stranded "tails" at the ends of DNA fragments: (2)



Single stranded tails produced in the result of DNA cleavage by restriction nucleases are known as cohesive or "sticky" ends. They can form complementary base pairs with the DNA ends produced by the same restriction enzyme.

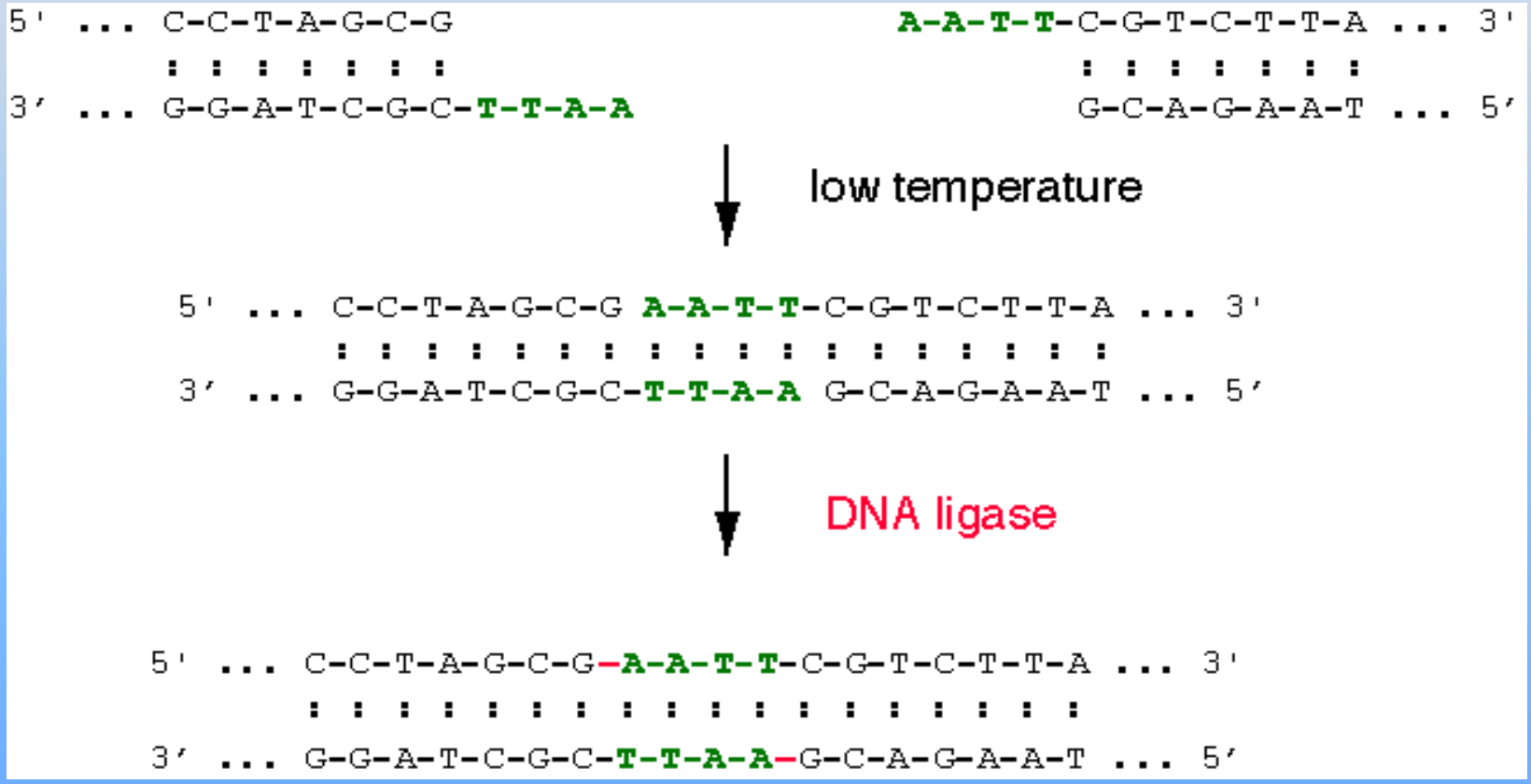


Sticky ends of different DNA fragments produced by the same restriction enzyme can base-pair to each other. Linking together such fragments will produce a recombinant DNA molecule.

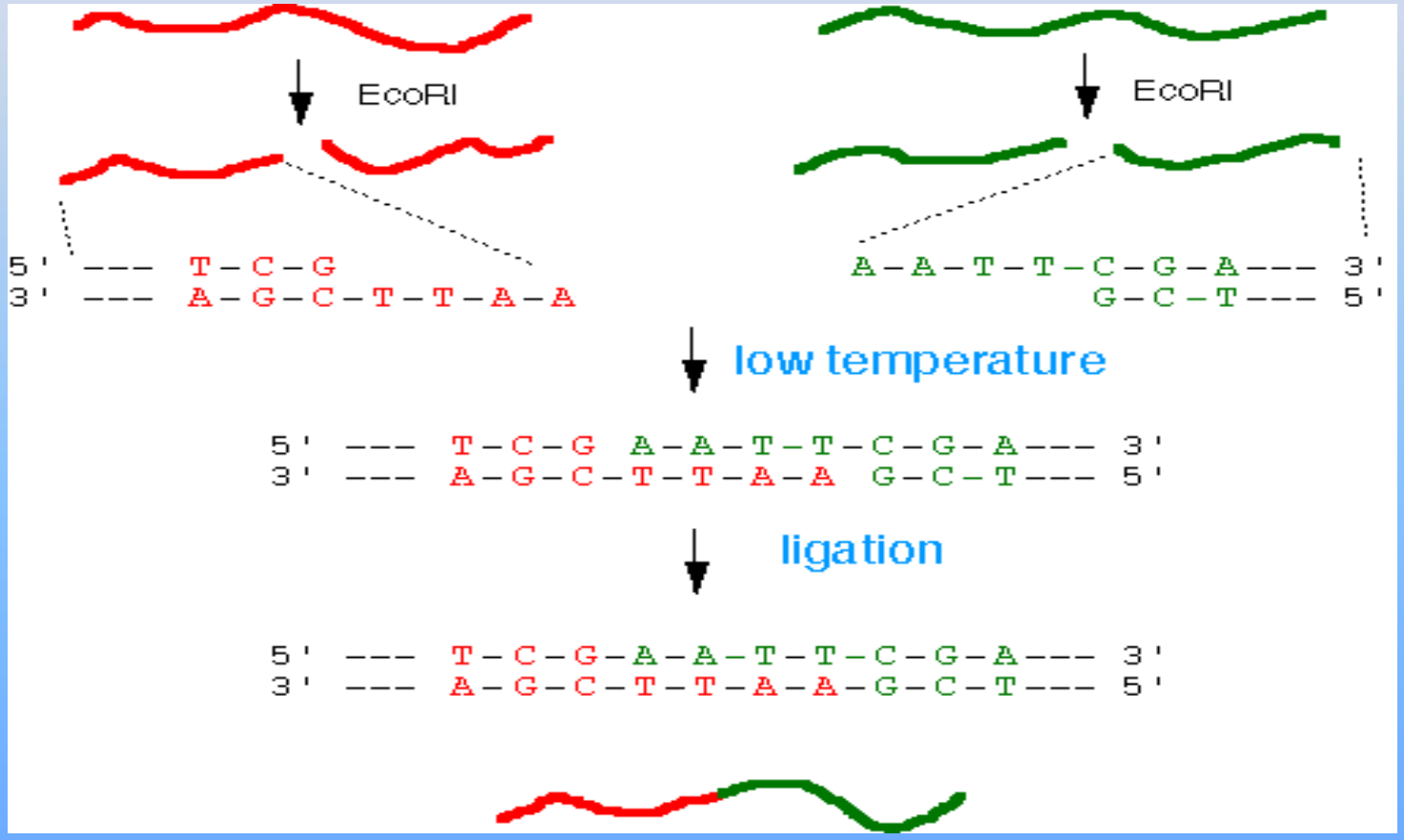


DNA LIGASE

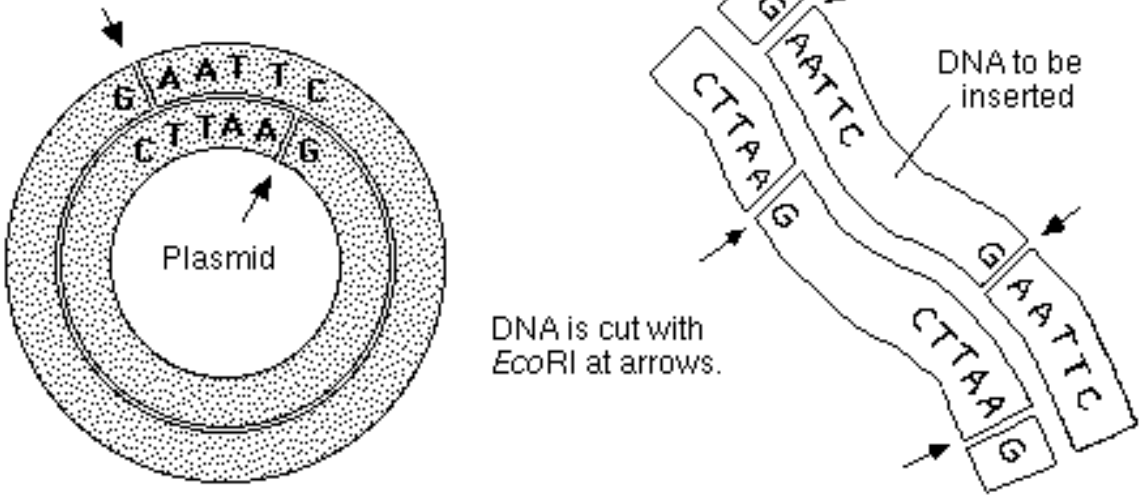
DNA ligase is an enzyme that can connect two DNA fragments together.



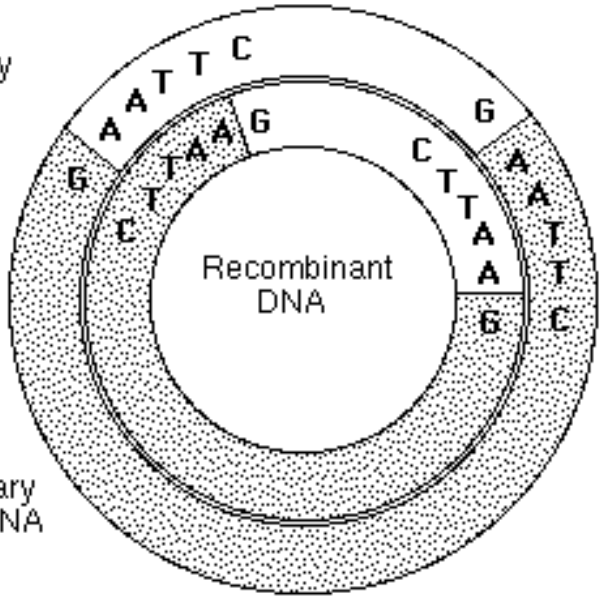
Combining together fragments of different DNA molecules we can produce recombinant DNA



Inserting a DNA Sample into a Plasmid



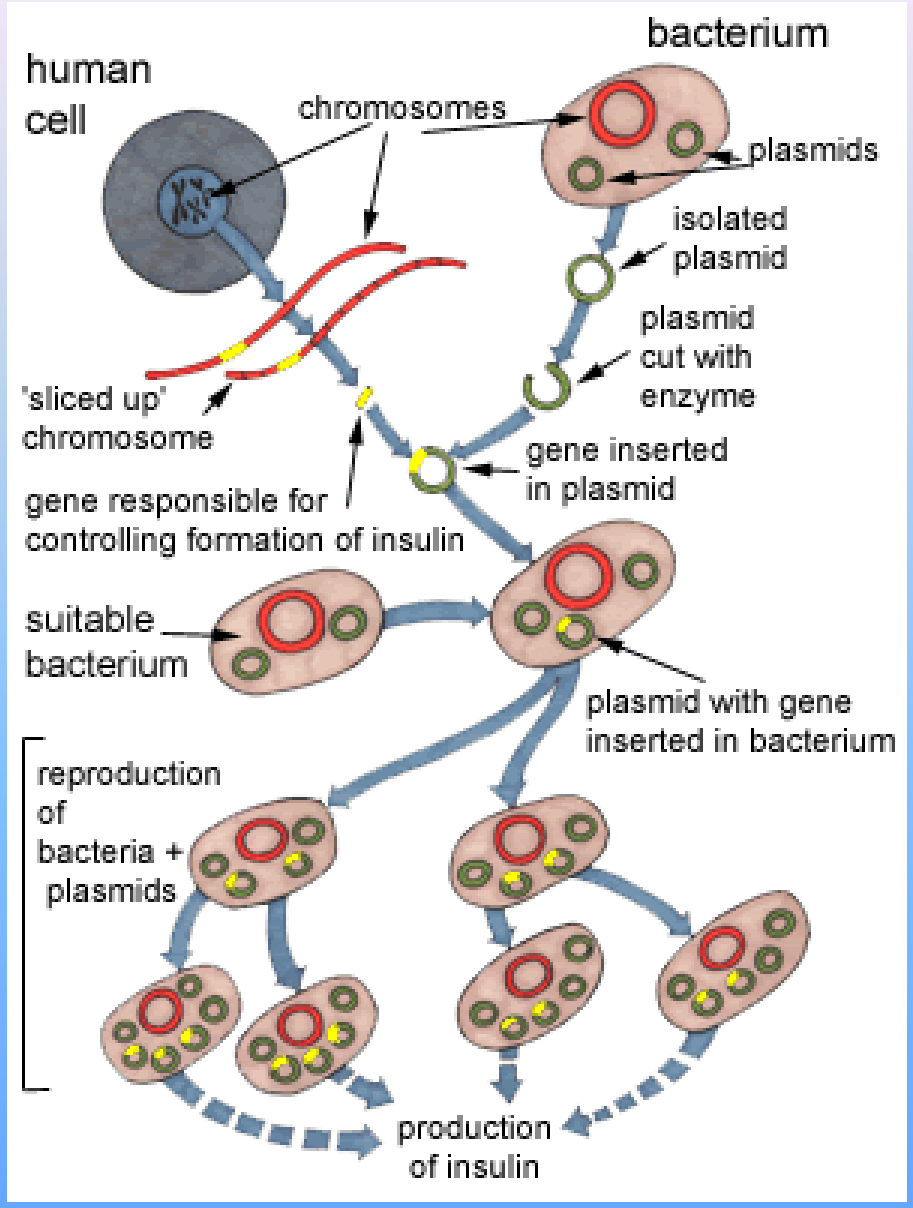
Resulting DNAs have sticky (complementary) ends.

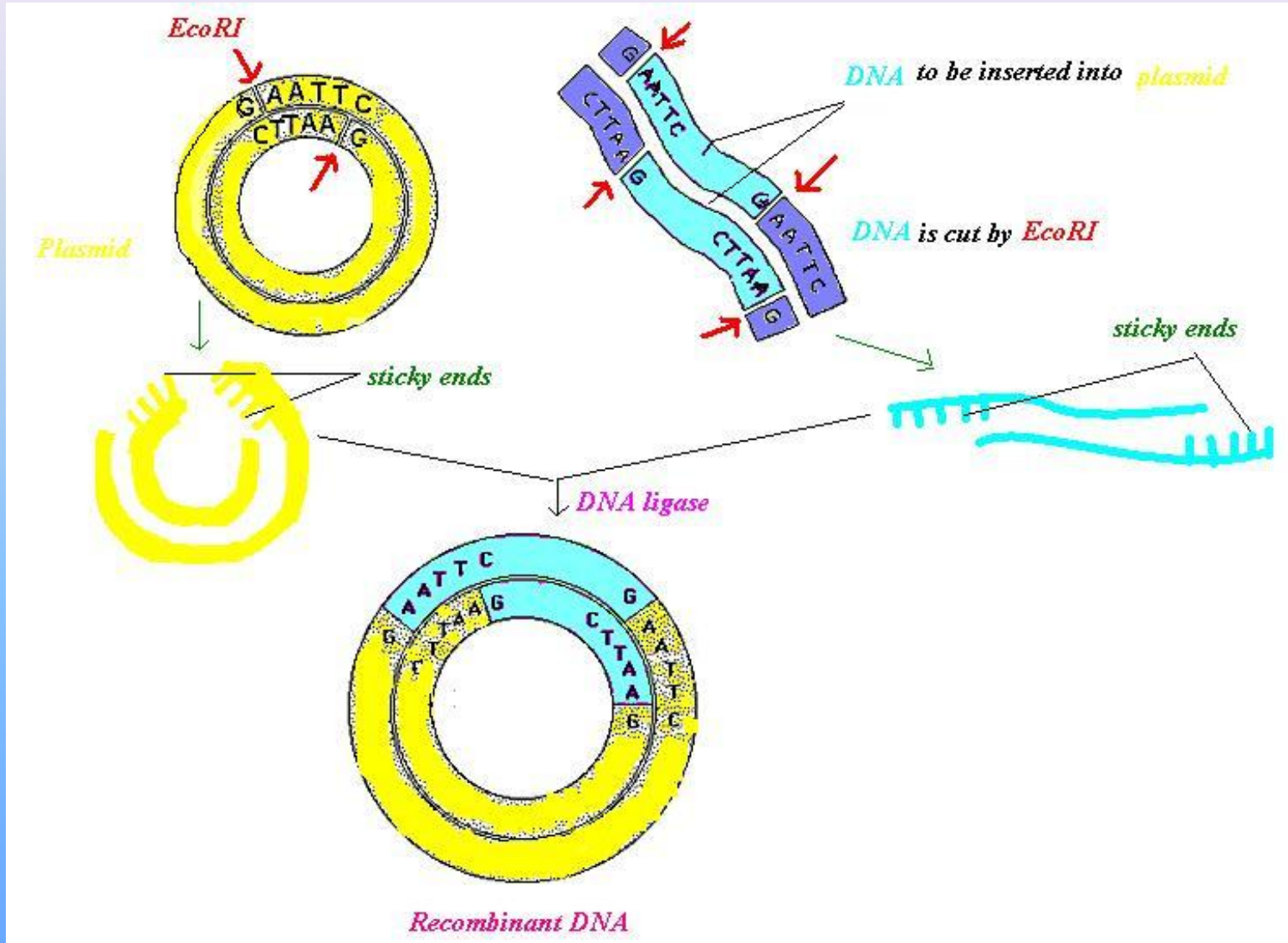


DNA is spliced by complementary base pairing and sealed with DNA ligase

Contoh aplikasi pada produksi insulin

1. Gen manusia yang mengekspresikan insulin diisolasi
2. Plasmid dari bakteri diisolasi untuk kemudian diinkubasi dengan enzim restriksi
3. Gen manusia disisipkan kepada plasmid
4. Plasmid “dibiakkan” dalam sel bakteri (cloning gen)
5. Sel bakteri mengekpresikan gen insulin manusia, hasilnya (insulin manusia?)
6. Pada contoh di atas, apakah enzim restriksi yang digunakan pada sel manusia sama dengan yang digunakan pada plasmid?




EcoRI
G AATTC
CTTAA G
Plasmid
sticky ends
DNA to be inserted into plasmid
DNA is cut by EcoRI
sticky ends
DNA ligase
Recombinant DNA

TUGAS INDIVIDUAL

1. Carilah sepuluh macam lagi endonuklease restriksi
2. Bagaimana urutan basa sebagai gugus palindrom untuk setiap enzim yang telah anda pilih