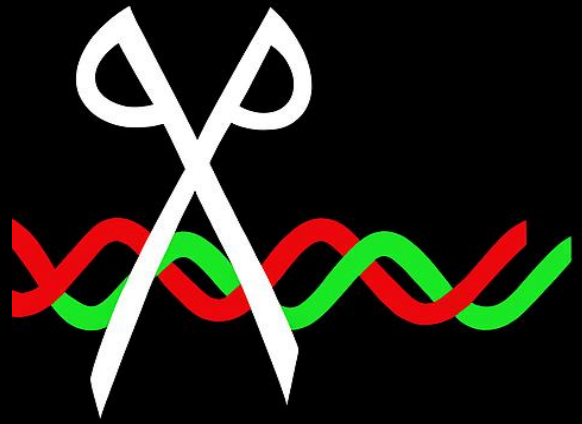
CLASS- 12th // BIOLOGY

BIOTECHNOLOGY:

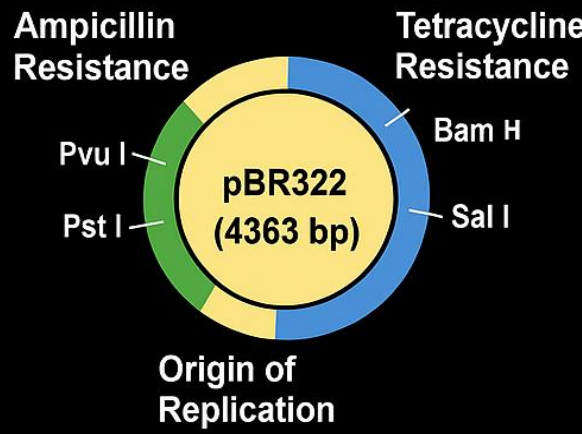
PRINCIPLES AND PROCESSES

TOOLS OF RECOMBINANT DNA TECHNOLOGY

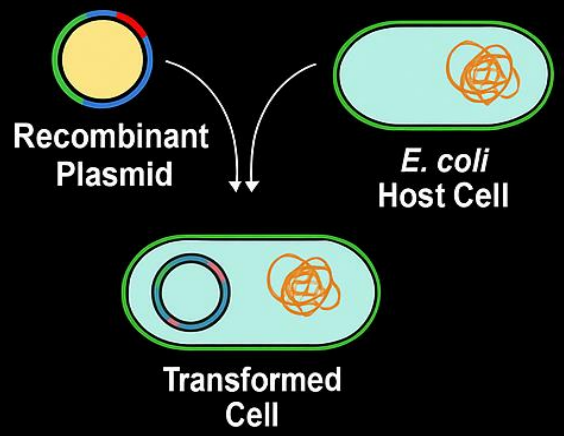
TOOLS OF RECOMBINANT DNA TECHNOLOGY



Restriction Enzymes "molecular scissors'



Cloning Vectors



Competent Host

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TOOLS OF RECOMBINANT DNA TECHNOLOGY

Restriction Enzymes ('molecular scissors')

These are the enzymes which cut DNA at specific sites into fragments.

They belong to a class of enzymes called nucleases.

In 1963, two enzymes responsible for restricting growth of bacteriophage in E. coli were isolated. One enzyme added methyl groups to DNA. The other (restriction endonuclease) cut DNA.

More than 900 restriction enzymes have been isolated from over 230 strains of bacteria.

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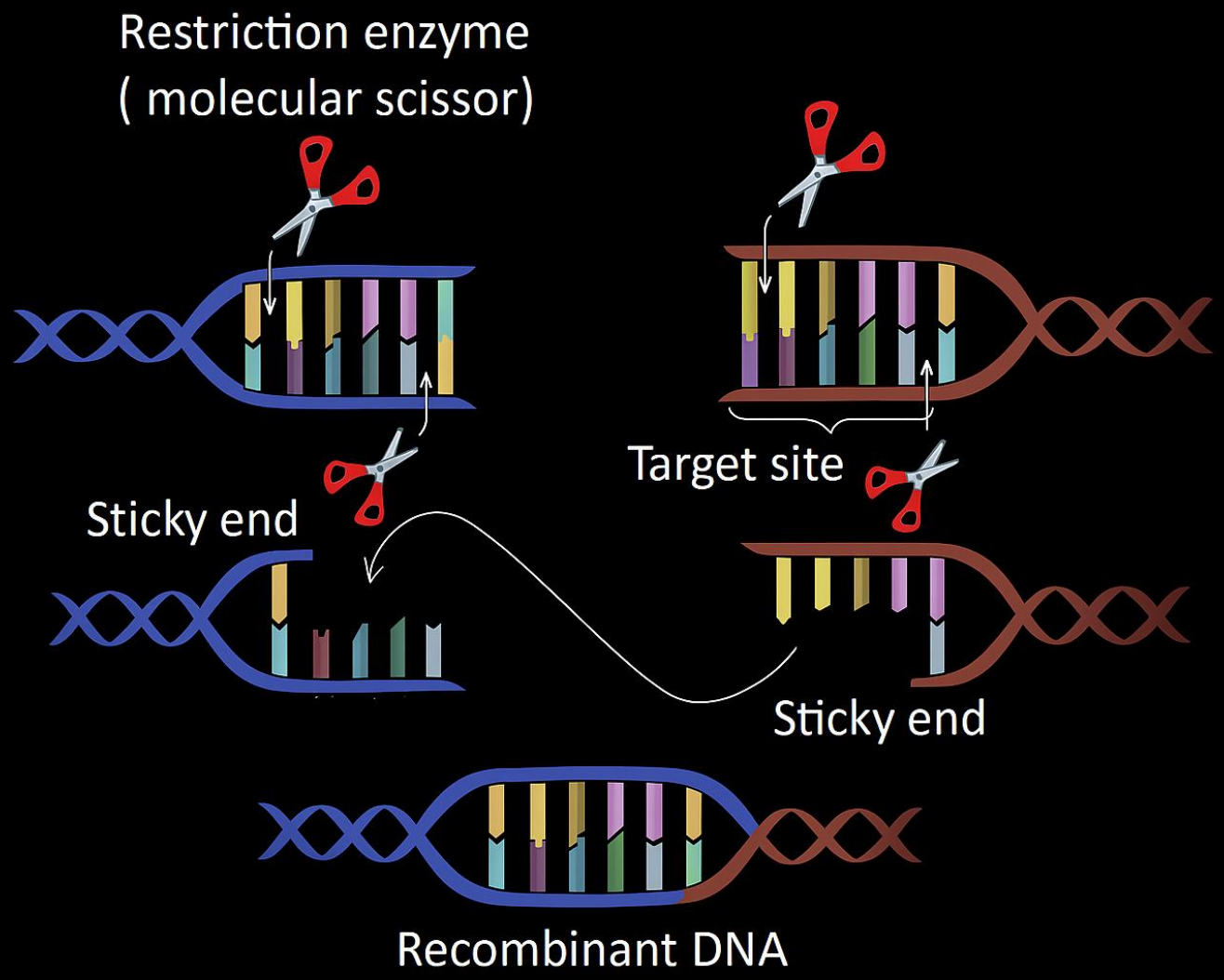

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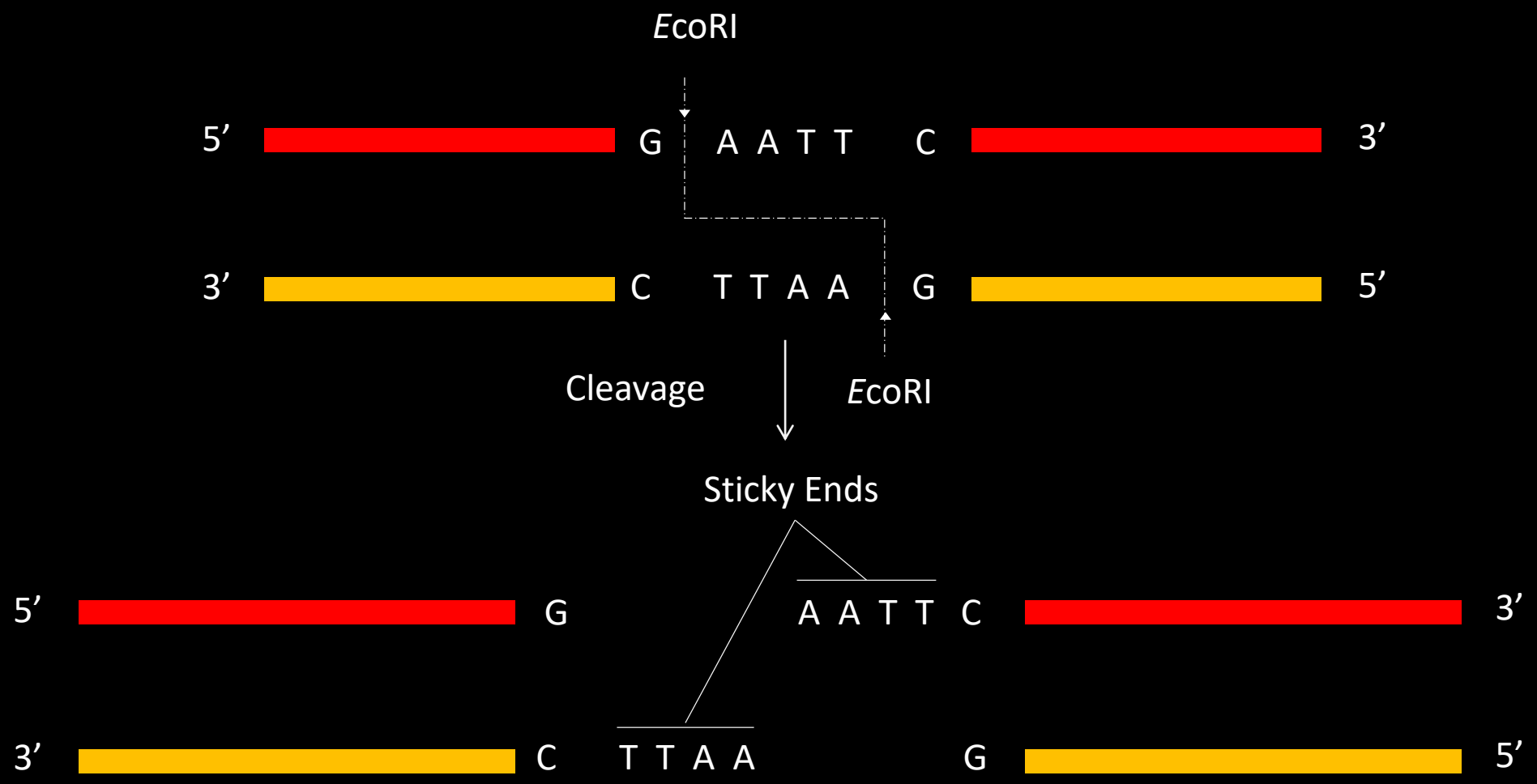

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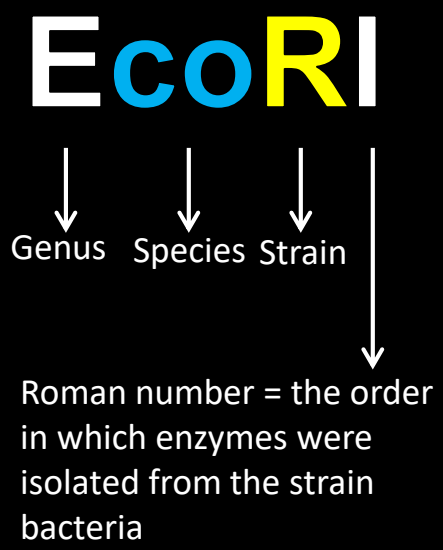
TOOLS OF RECOMBINANT DNA TECHNOLOGY

Restriction Enzymes

First letter indicates genus.

Second two letters indicate species of the prokaryotic cell from which they were isolated.

E.g. EcoRI comes from E. coli RY 13.



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TOOLS OF RECOMBINANT DNA TECHNOLOGY

Restriction Enzymes

Exonucleases

They remove nucleotides from the ends of the DNA.

Endonucleases

They cut at specific positions within DNA.
They bind to specific recognition sequence of the DNA and cut the two strands at specific points.

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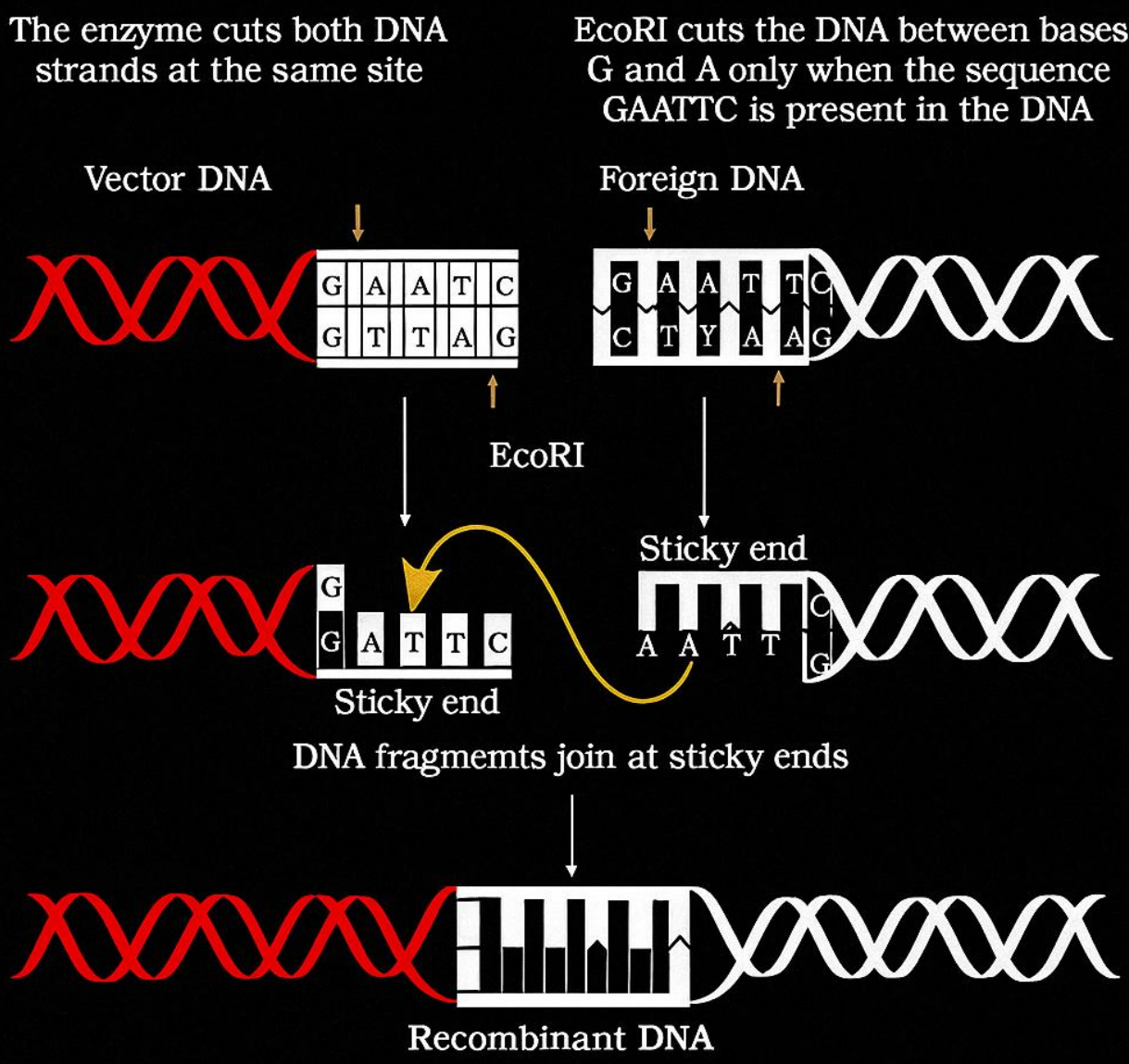

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First restriction endonuclease is Hind II.

It cuts DNA by recognizing a specific sequence of six base pairs.

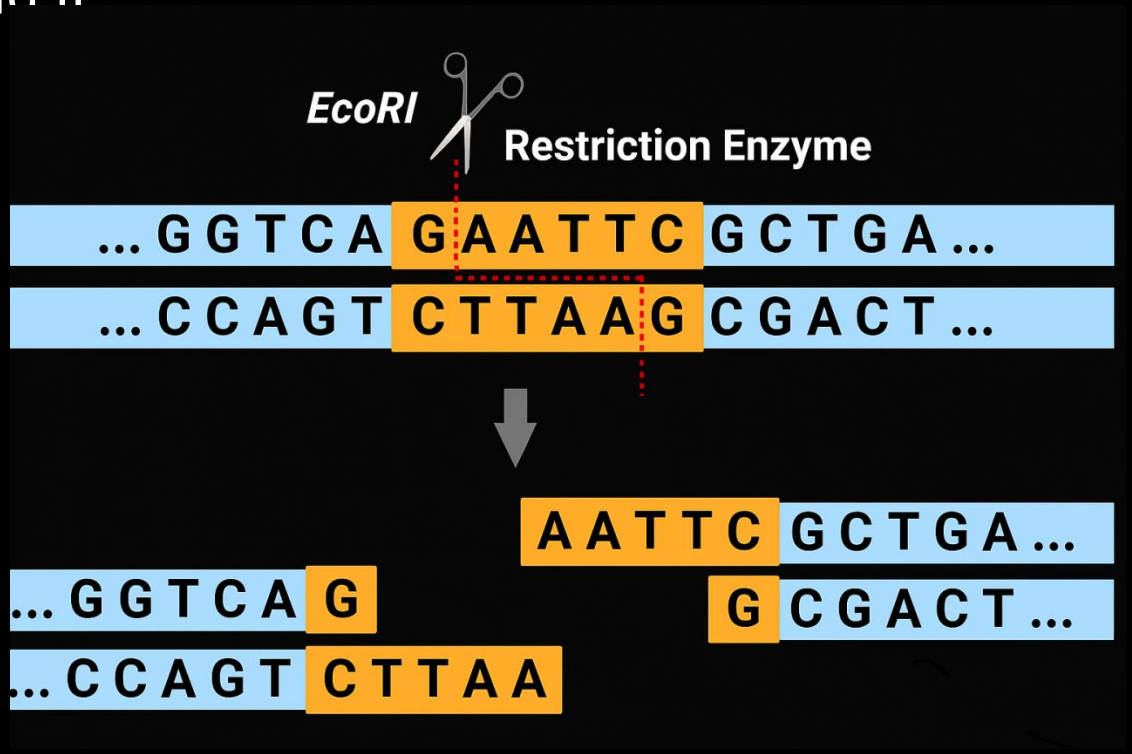
This is called the recognition sequence for Hind II

Hind II

Recognition site of Hind II

5'... GT Py | Pu AC ... 3'

3'... CA Pu | Py TG ... 5'



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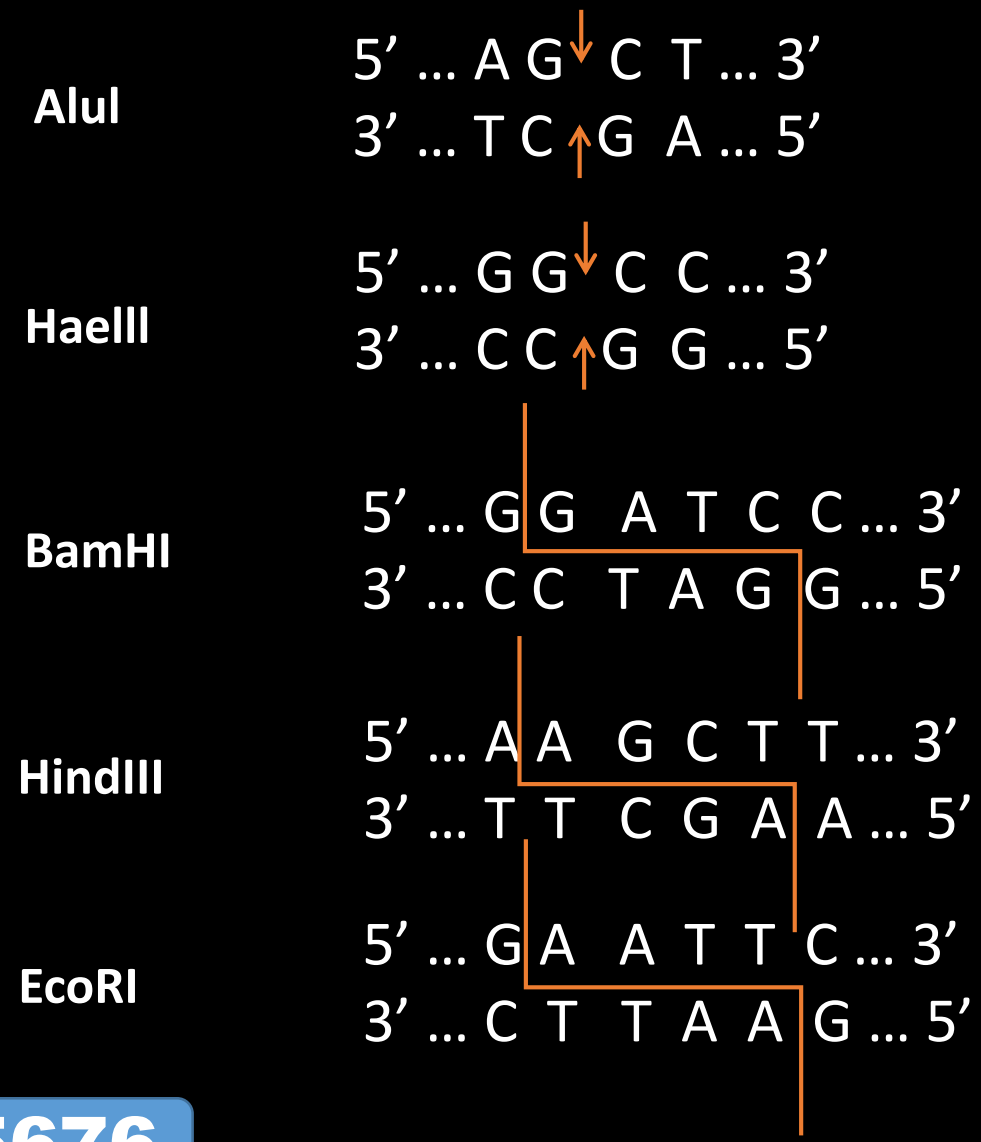
Recognition sites of various types of restriction endonucleases

AluI and HaeIII produce blunt ends BamHI HindIII and EcoRI produce "sticky ends



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TOOLS OF RECOMBINANT DNA TECHNOLOGY

Restriction Enzymes

Restriction endonuclease recognizes a specific palindromic nucleotide sequences. It is a sequence of base pairs that read the same on two strands in 5'→ 3' and in 3' → 5‘ directions.

5‘ -- GAATTC – 3’
3’ -- CTTAAG – 5’

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Restriction Enzymes ('molecular scissors')

Restriction enzymes cut the strand a little away from the center of palindrome sites, but between the same two bases on the opposite strands. This leaves single stranded overhanging stretches at the ends. They are called sticky ends.

Sticky ends form H-bonds with their complementary cut counterparts. This stickiness facilitates action of DNA ligase.

When cut by the same restriction enzyme, the DNA fragments have the same kind of sticky-ends. They are joined by DNA ligases.

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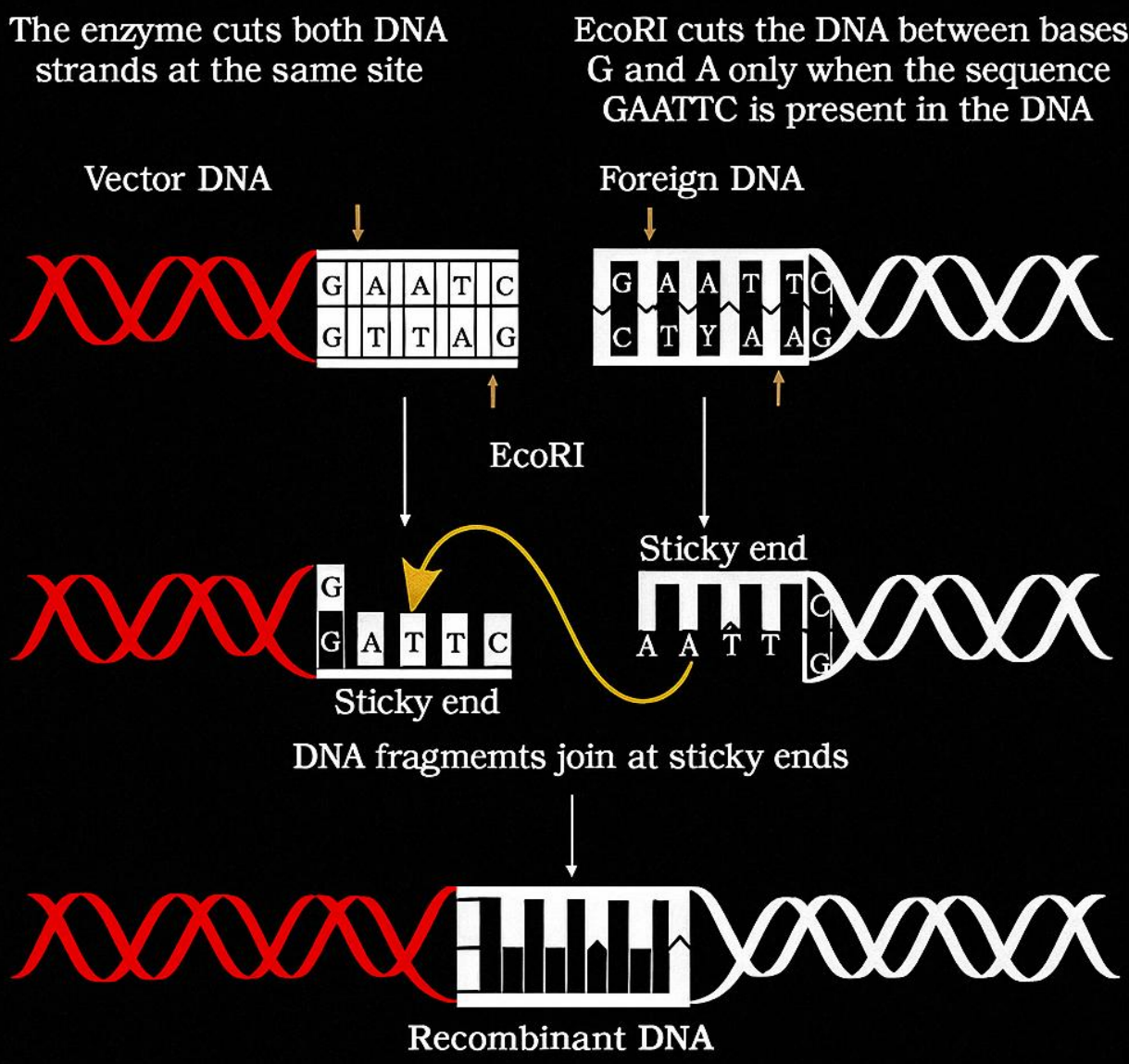

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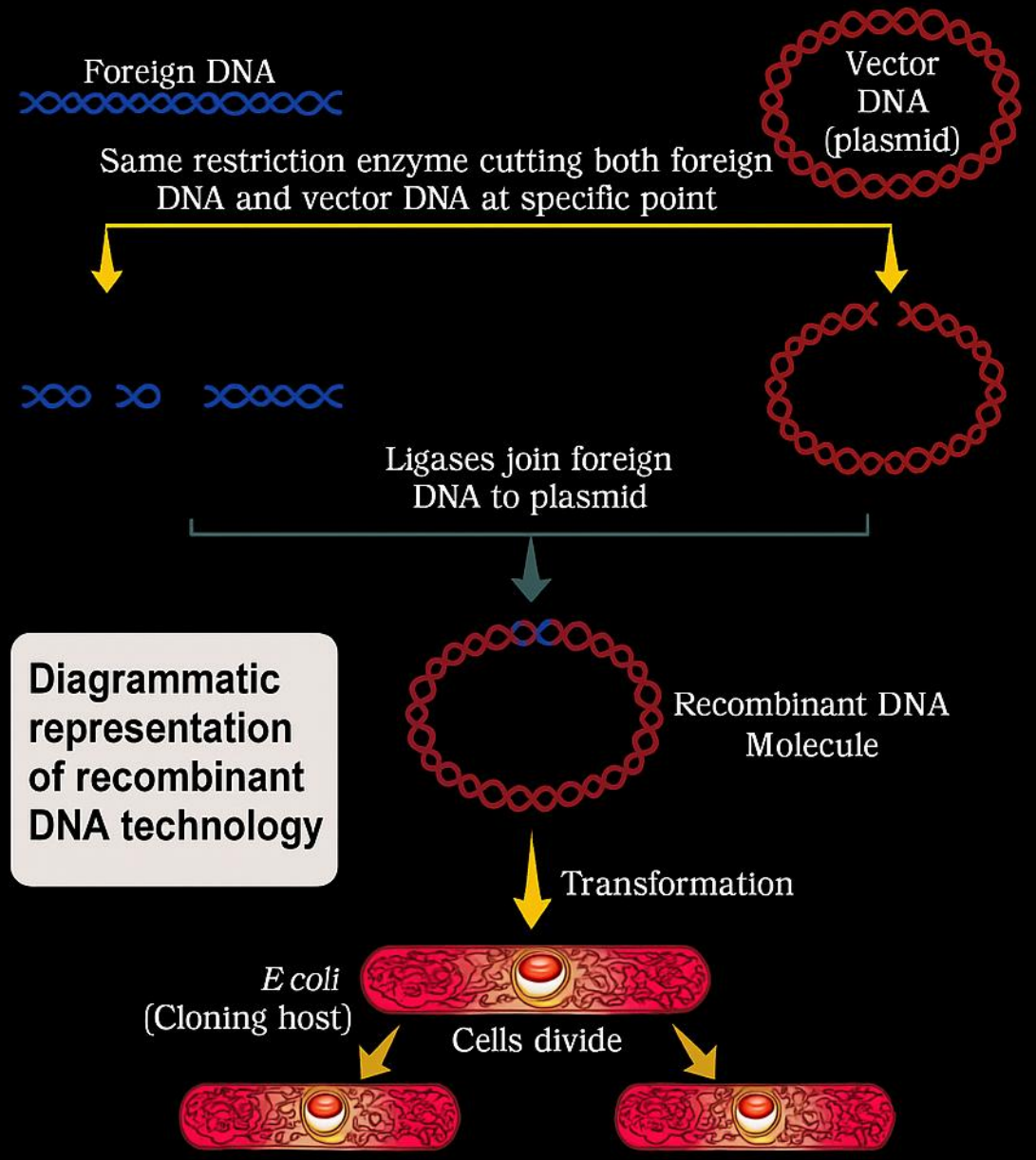

Diagrammatic representation of recombinant DNA technology

Thanks for watching!