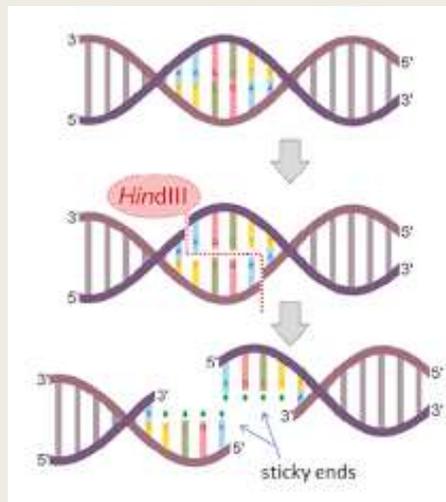


RESTRICTION ENDONUCLEASES

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Restriction Enzymes

- ❑ Restriction enzymes are endonucleases (**Endo** (inside), **nuclease**(cuts nucleic acid), which catalyze the cleavage of the phosphodiester bonds within both strands of DNA.
- ❑ They require Mg^{+2} for activity and generate a 5 prime (5') phosphate and a 3 prime (3') hydroxyl group at the point of cleavage.

- ❑ The distinguishing feature of restriction enzymes is that they only cut at very specific sequences of bases. This specific DNA sequence is called **recognition sequence**.
- ❑ Restriction enzymes are traditionally classified according to the subunit composition, cleavage position, sequence-specificity and cofactor requirements.

- A restriction enzyme requires a specific double stranded recognition sequence of nucleotides to cut DNA.
- Recognition sites are usually 4 to 8 base pairs in length.
- Cleavage occurs within or near the site.

Enzyme Activity

Scanning

GGACGCTAGCTGAT**GAATTC**GCATCGGATCCGAATCCGCTCTTTCAA
CCTGCGATCGACTA**CTTAAG**CGTAGCCTAGGCTTAGGCGAGAAAGTT

Recognition Sequence

GGACGCTAGCTGAT**GAATTC**GCATCGGATCCGAATCCGCTCTTTCAA
CCTGCGATCGACTA**CTTAAG**CGTAGCCTAGGCTTAGGCGAGAAAGTT

Cleavage

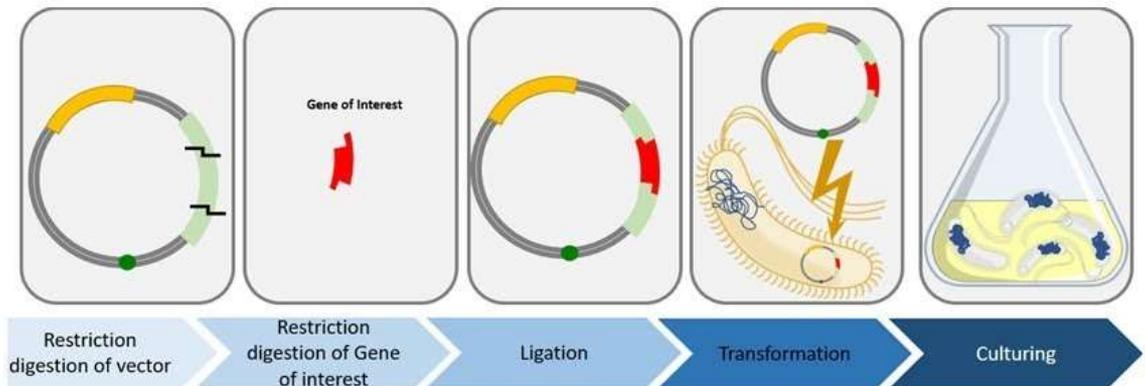
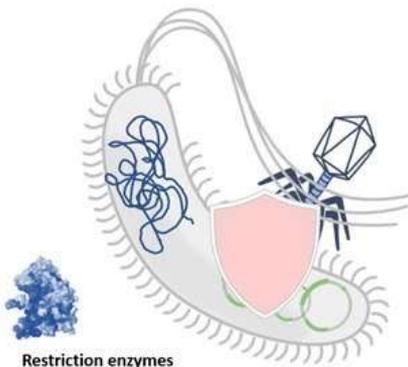
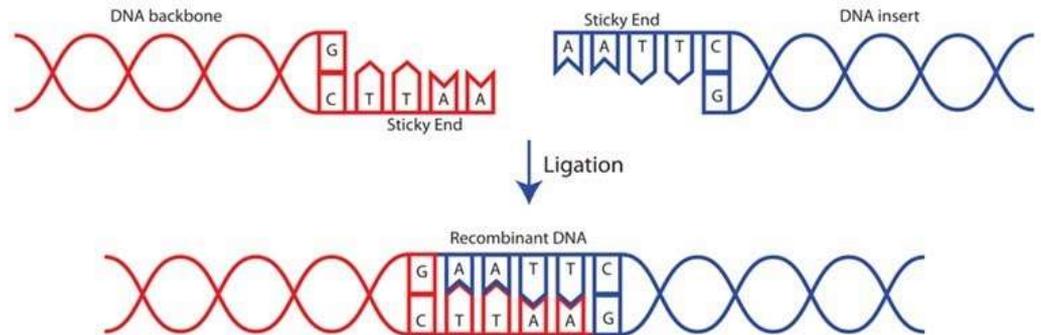
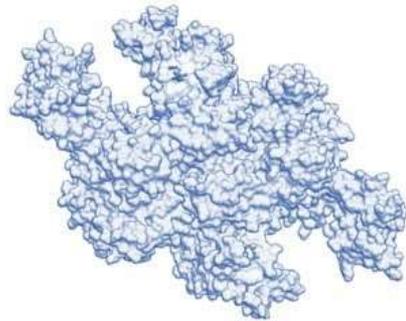
GGACGCTAGCTGAT**G** **AATTC**GCATCGGATCCGAATCCGCTCTTTCAA
CCTGCGATCGACTA**CTTAA** **G**CGTAGCCTAGGCTTAGGCGAGAAAGTT

Biological Function

- ❑ Restriction enzyme is part of the cell's restriction-modification system in bacteria.
- ❑ The phenomenon of restriction modification in bacteria is a small scale immune system for protection from infection by foreign DNA.
- ❑ Bacteria can protect themselves only after foreign DNA has entered their cytoplasm (as bacteriophages).

Biological Function

Restriction enzyme



Biological Role of RE

- ❑ Restriction Modification System -restriction enzymes are paired with methylases.
- ❑ Methylases are enzymes that add methyl groups to specific nucleotides within the recognition sequence. The methylation prevents recognition by the restriction enzyme.
- ❑ Therefore, the restriction enzyme within a cell doesn't destroy its own DNA. However the restriction enzyme can destroy foreign DNA which enters the cell such as bacteriophage.

Naming of Restriction enzymes

- Restriction enzymes are named according to the organism from which they are isolated.
- This is done by using the first letter of the genus followed by the first two letters of the species and additional letter or number represent the strain or serotypes.
- Only certain strains or sub-strains of a particular species may produce restriction enzymes.

Example of restriction enzymes

EcoRI *Escherichia coli* R

G/AATTC

BamHI *Bacillus amyloliquefaciens* H

G/GATCC

HindIII *Haemophilus influenzae* Rd

A/AGCTT

PstI *Providencia stuartii*

CTGCA/G

PmeI *Pseudomonas mendocina*

GTTT/AAAC

Recognition Sequences

EcoRI G/AATTC

BamHI G/GATCC

HindIII A/AGCTT

PstI CTGCA/G

PmeI GTTT/AAAC

HincII GTY/RAC

FunII G/AATTC

Digestion Conditions

- Xbal

- *Buffer 2: (10 mM Tris-HCl, 10 mM MgCl₂, 50 mM NaCl, 1 mM DTT, pH 7.9 at 25 °C.*
- *100 µg/ml BSA*
- *Incubate at 37°*
- *1 Unit digest 1 µg DNA in 1 hour*
- *Heat inactivate 65° for 20min*

Typical RE Reaction

20 μl reaction.

10 μl DNA (~1 μg total)

7 μl water

2 μl 10X reaction buffer

1 μl RE 10units/ μl

Incubate 1 hour at appropriate temperature

Note:

1. 10 fold excess enzyme ensures complete digestion.
2. Enzyme should never exceed 1/10th of reaction.
3. BSA is often recommended because it stabilizes the enzyme.
add 0.2 μl of BSA stock for 20 μl reaction.
4. For plasmid minipreps – add 1 μl RNase the last 5 min of digestion.

Double Digest

Enzyme	Supplied NEBuffer	% in B1	% in B2	% in B3	% in B4
Sacl	NEBuffer 1 + BSA	100	50	10	100
Sacll	NEBuffer 4	25	75	10	100
Sall	NEBuffer 3 + BSA	0	0	100	0
Sapl	NEBuffer 4	75	50	0	100
Sau3AI	NEBuffer 1 + BSA	100	50	10	100
Sau96I	NEBuffer 4	50	100	100	100
Sbfl	NEBuffer 4	75	50	0	100

Double Digest Option

1. Mix the enzymes in the same compatible buffers
2. Conduct sequential digest

Caution: some enzymes display star activity in certain buffers which causes them to digest the DNA at sites other than the standard recognition site.

Many recognition sequences are **palindromic**.
For example,

5' GAATTC 3'
3' CTTAAG 5'

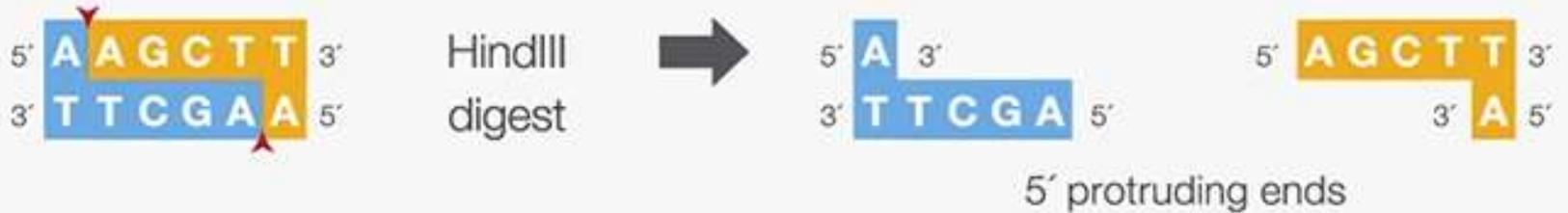
palindromic: read the same in the opposite
direction

Not all restriction endonucleases cut symmetrically and leave blunt ends
Sticky and Blunt end cutters

Many endonucleases cleave the DNA backbones in positions that are not directly opposite each other or can make staggered cuts, which produce single stranded “sticky-ends”

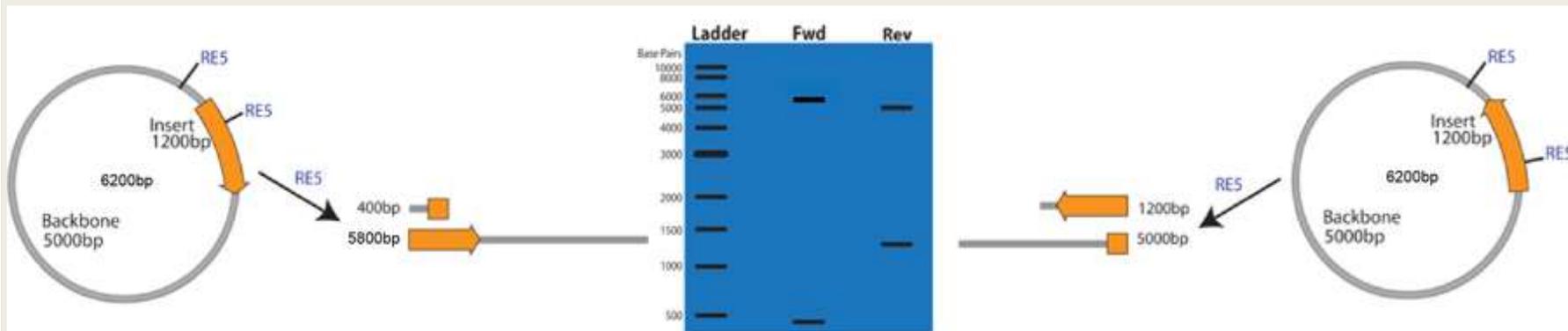
DNA from different sources can be spliced easily because of these **sticky-end overhangs**

Example of RE that produce sticky end cutters:



How to determine orientation of the insert

Digest with enzymes to cut internal site and external site—analyze by electrophoresis

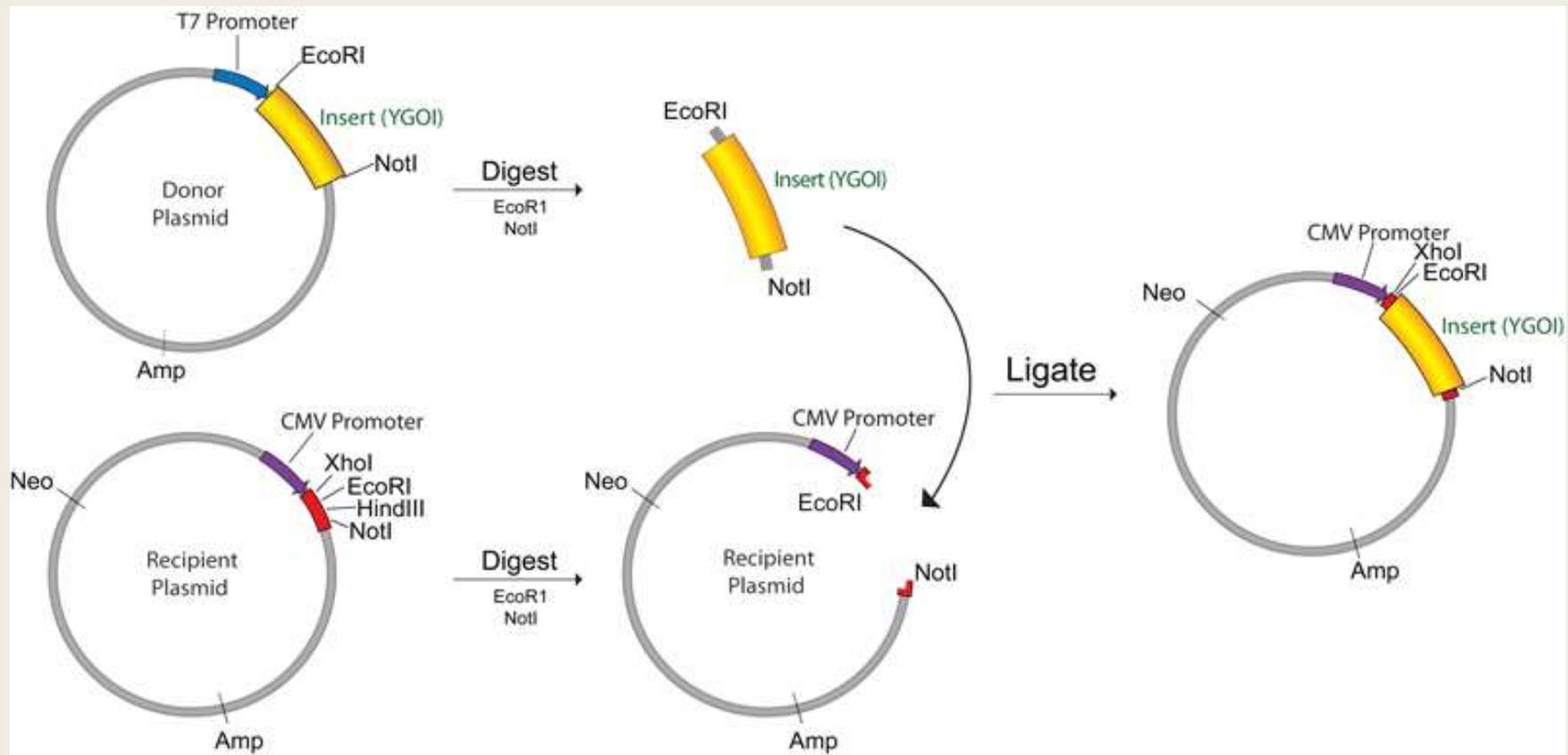


Uses of Restriction Enzyme

1. Recombinant DNA technology

- ❑ Discovery of enzymes that cut and paste DNA make genetic engineering possible.
- ❑ Restriction enzyme cuts DNA and generates fragments
- ❑ Ligase joins different DNA fragments
- ❑ DNA fragments from different species can be ligated (joined) to create **Recombinant DNA**

1. Recombinant DNA technology

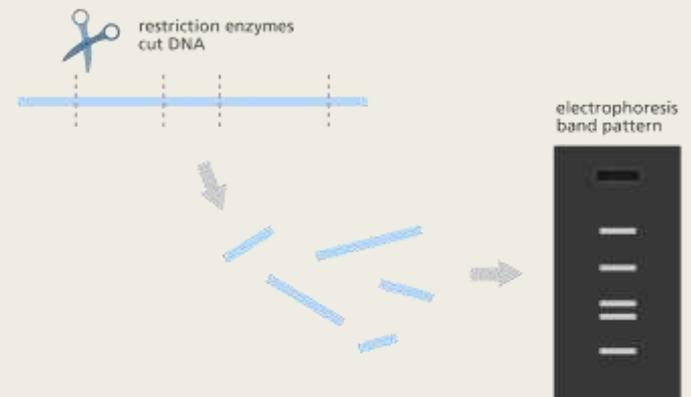


2. Cloning

Replicates a sequence inserted into a host cell

3. DNA restriction mapping

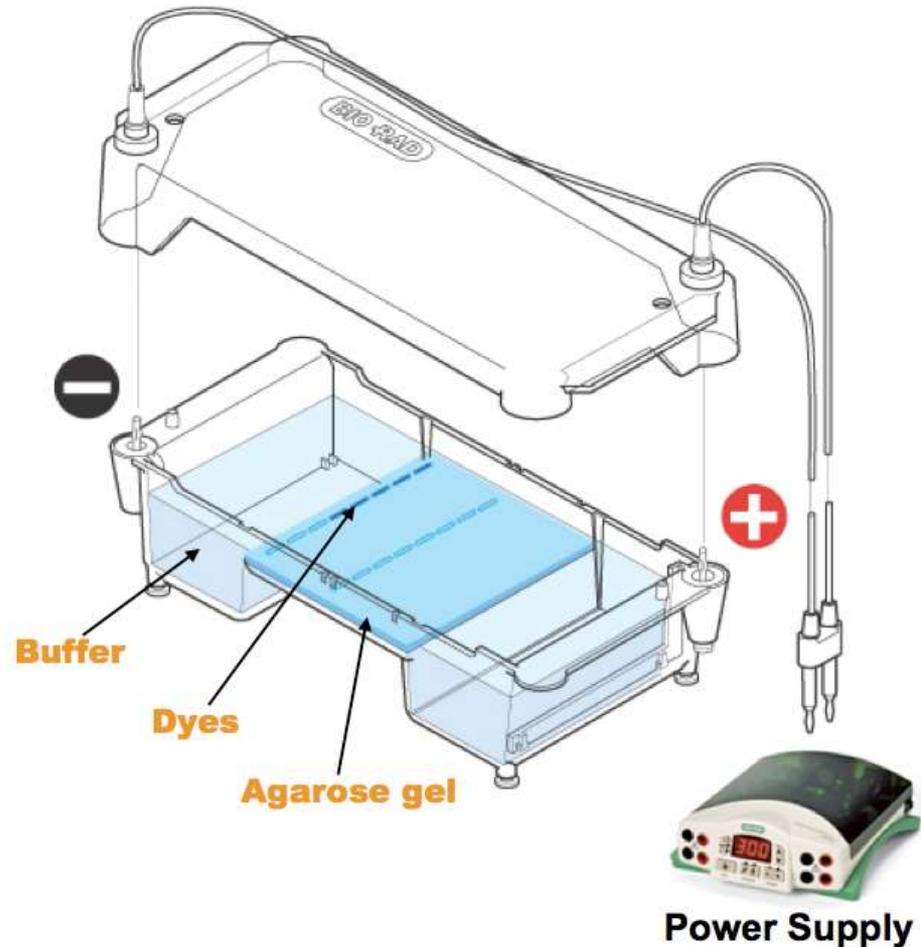
The location of the restriction enzyme cleavage sites on the DNA molecule.



Separating Restriction Fragments, I

Agarose Electrophoresis Loading

- **Electrical current carries negatively-charged DNA through gel towards positive (red) electrode**



Separating Restriction Fragments, II

Agarose Electrophoresis Running

- **Agarose gel sieves** DNA fragments according to size
 - Small fragments move farther than large fragments

