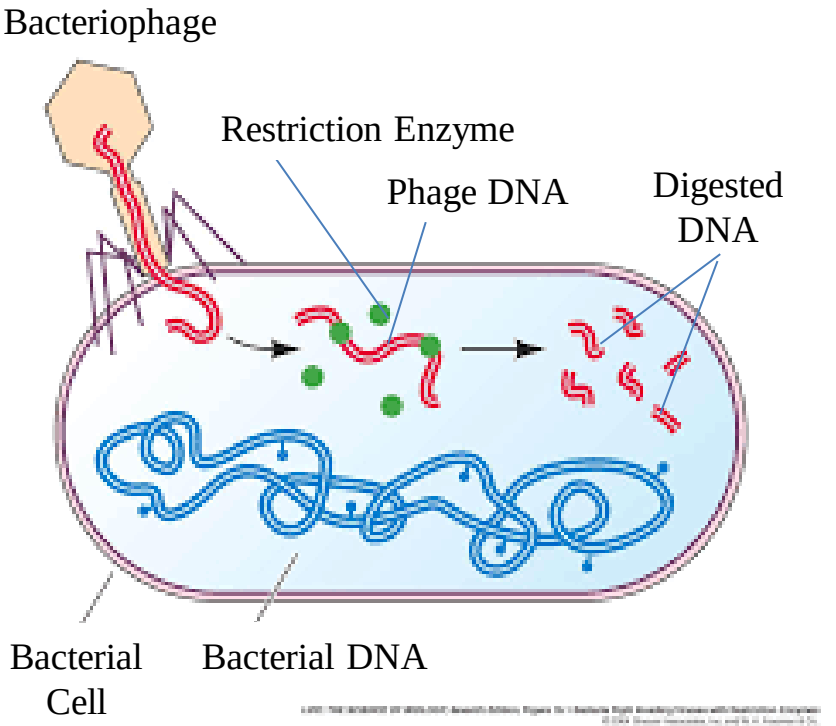
Restriction Digestion

INTRODUCTION

- Gene manipulation requires enzymes to cut DNA molecules.
- The restriction endonucleases cut at the interior part of DNA.
- These enzymes are found in bacteria and *in vivo* are involved in recognition and destruction of foreign DNA.
- Invading phage DNA for instance will be restricted by such enzymes.
- The bacteria protect their own DNA by modification process.



http://www.aun.edu.eg/molecular_biology/gene&protein/2nd%20day/5%206%20restriction%20enzymes%20and%20ligases%20[Compatibility%20Mode].pdf

Restriction Digestion:
Bacterial innate Immune System

- The essential feature of restriction endonucleases is that these enzymes recognize a particular sequence of bases.

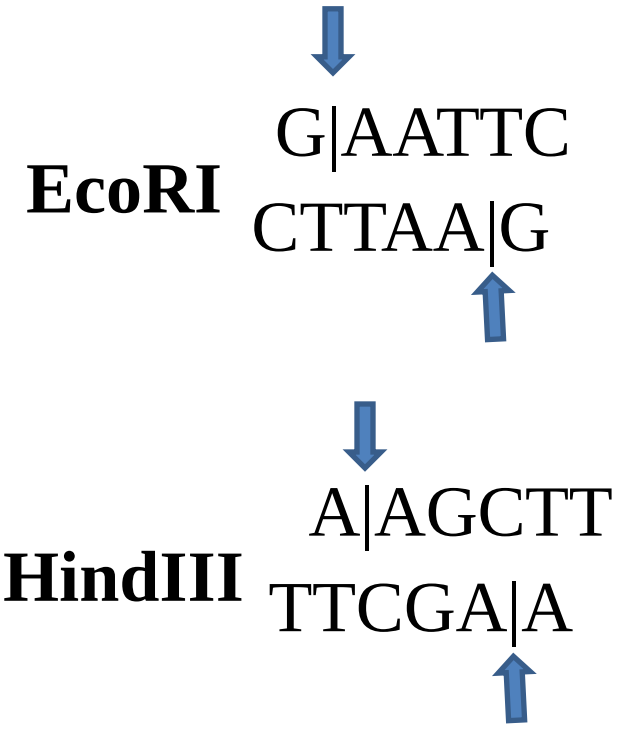
- Type II restriction enzymes cut the DNA within the recognized sequences.

- Each enzyme has its own characteristic recognition sequence and it may be 4 to 7 bases long with dyad symmetry.

- With the availability of nearly 50 restriction enzymes commercially, it is now possible to construct the physical map of genes after digestion of the DNA with different restriction enzymes and subsequent of DNA fragments on agarose gel.

PRINCIPLE

- Restriction enzyme EcoRI is isolated from *E. coli*
- EcoRI recognizes and cleaves the sequence 5”-GAATTC-3”
- EcoRI generates cohesive or sticky ends
- HindIII isolated from Haemophilus influenza
- It cleaves the sequence 5”-AAGCTT-3”
- HindIII generates cohesive or sticky ends.



Recognition sequence and cutting sites are shown by “|” and ⬆

NOMENCLATURE OF RE

EcoRI

E = *Escherichia*	genus name
co = *coli*	species name
R = strain RY12	strain
I = Roman numeral one	First Enzyme isolated from this species

HindIII

H = *Haemophilus*	genus name
in = *influenzae*	species name
d = serotype d	serotype
III = Roman numeral Three	Third Enzyme isolated from this species

TYPES OF RESTRICTION ENZYME

Three types of restriction enzyme

- **TYPE I**
- **TYPE II**
- **TYPE III**

Restriction Enzymes	Cleavage sites	Location of Methylase	Common Examples
TYPE I	Random Away from recognition site	Present with Endonuclease on a single protein	EcoKI EcoAI CfrAI
TYPE II	Very specific Within the recognition site	Separate proteins	EcoRI BamHI HindIII
TYPE III	Random Away from recognition site	Present with Endonuclease on a single protein	EcoPI Hinf III EcoP15I

TYPE I RE

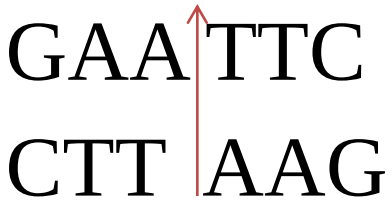
- They are complex enzymes which act as endonuclease and methylase function
- They require ATP,Mg+2
- They are single, multi-functional enzyme which recognizes 15bp in length and cleavage site is 1000bp
- They show specificity for recognition but not for cleaveage
- They produce heterogeneous fragments
- Not used in gene cloning techniques
- Examples: EcoK, EcoB

TYPE II RE

- Most common RE used in gene cloning
- They are simple enzyme having single polypeptide
- They have separate methylase and endonuclease activity
- The recognition and cutting site is the same one
- They generally recognize six nucleotide
- Some enzymes also recognize 4,5,or 8bp
- Examples: PvuI, PvuII, EcoRI

Cutting site of RE Type II

The recognition sequences for Type II RE form palindromes with rotational symmetry .

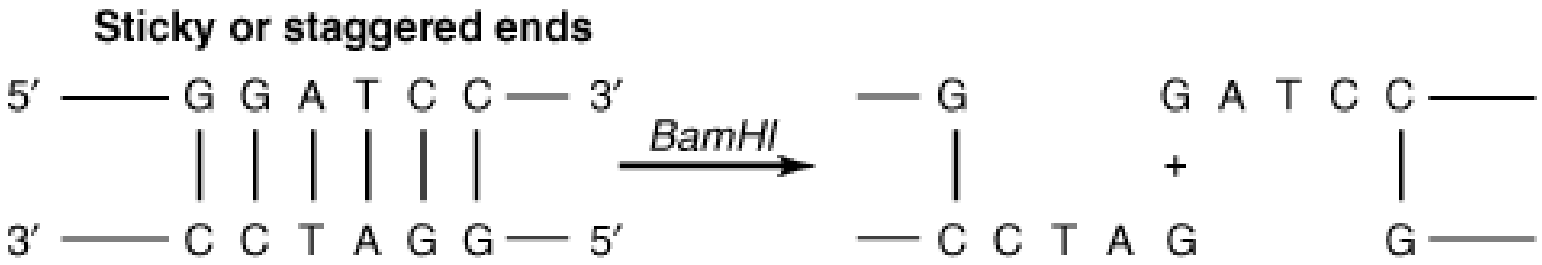


EcoRI recognition site

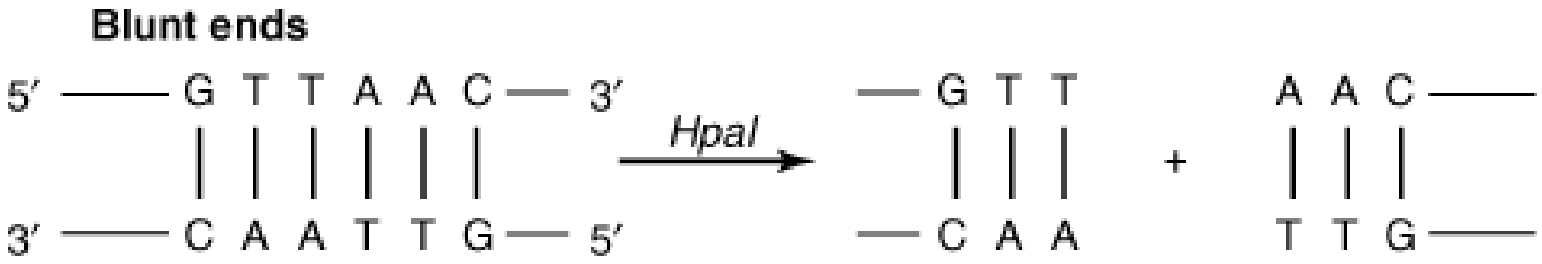
Cutting site of RE Type II

Cleave DNA to generate different "ends"

- Staggered cut



- **Blunt end**



TYPE III RE

- There are two subunits
- One is for recognition and modification another for nuclease activity.
- They require ATP and Mg^{2+}
- The problem is that the recognition sites are assymetric non-palindromic
- The single strand ends produce by TYPE III differ from each other and cannot recombine at random
- They lack ATPase activity
- Example: HgaI, MboII

Requirements for Restriction Digestion

How to do Restriction Digestion??

Need for Restriction Digestion

MATERIALS REQUIRED

- Isolated DNA
- Restriction Buffer (10x) Sodium acetate
- Ethanol
- Sterile double distilled water
- Restriction enzymes
- Agarose
- Gel running buffer
- Ethidium Bromide
- Agarose gel electrophoresis unit

Other requirements:

- Micropipette
- Microcentrifuge tubes
- DNA marker
- bucket with crushed ice,
- pipette tips

Preparation for Restriction Digestion

Recipes (µl)	1	2	3	5
Distilled Water	16	16	14	14
10x buffer	2	2	2	2
Marker DNA	2	-	-	-
Sample DNA	-	2	2	2
Hind III	-	-	2	-
Bam HI	-	-	-	2
Total volume (µl)	20	20	20	20

Procedure

1. Label microfuge tubes 1 to 4 and arrange them open in a rack.
2. Bring all reactants on ice.
3. DNA samples stored frozen are thawed quickly and brought on ice.
4. Prepare the following reaction mixes by carefully pipetting into the bottom of microfuge tubes.
5. Fasten the cap on each tube.
6. Mix contents of tubes carefully.
7. Centrifuge all the microfuge tubes for 2 second to settle the contents at the bottom of tubes.
8. Incubate $30^{O}C$ in water bath for 60 minutes or longer if necessary.

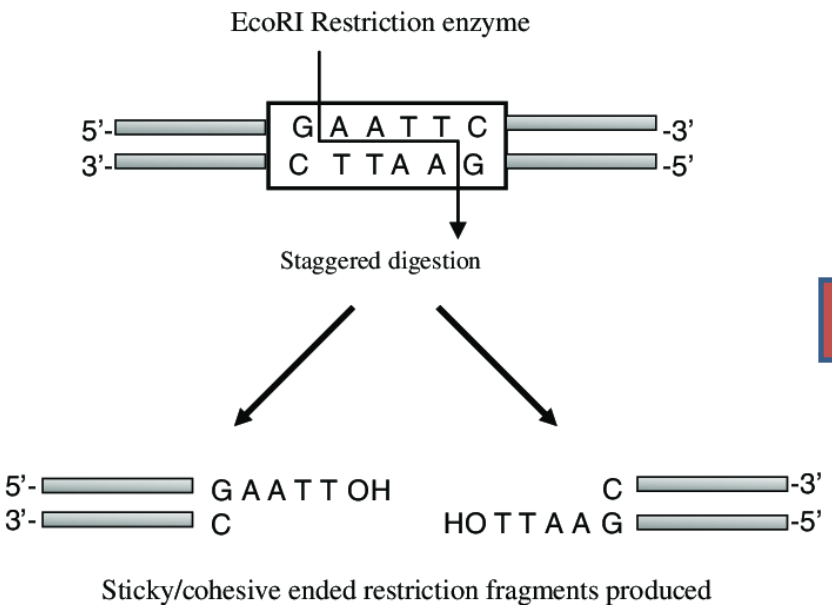
9. To the digested reaction mixture, add 2 µl of 3 M sodium acetate and 50 µl absolute ethanol for precipitation. Keep it at -20^{O}C for 20 minutes.

10. Recover the DNA by centrifugation for 15 minutes.

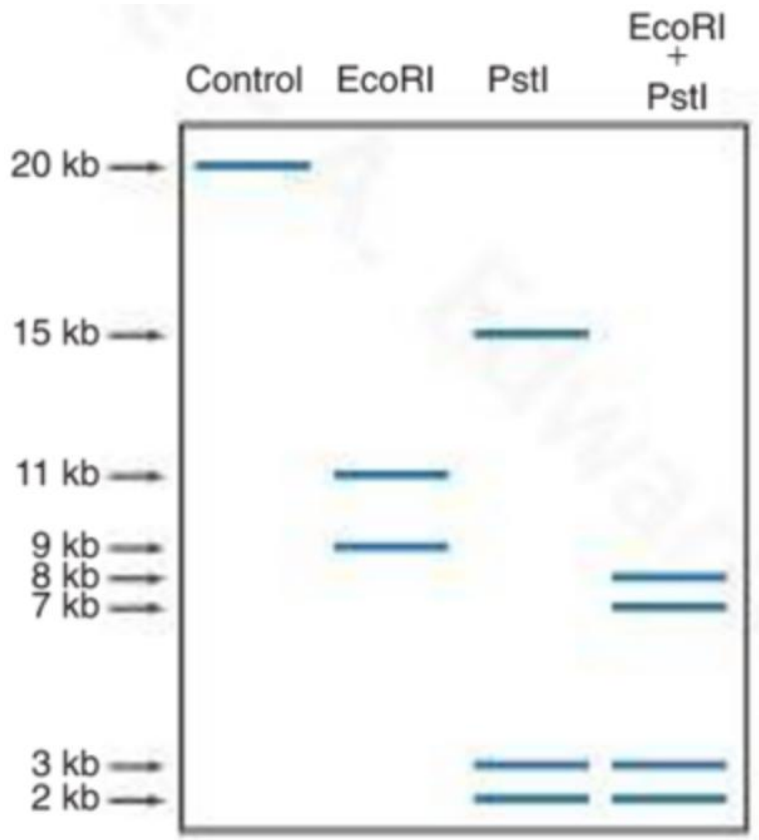
11. Wash the pellets twice with 70 % ethanol.
    Remove supernatant.

12. Air dry the pellets. Dissolve the pellets in 20 µl TE buffer and store at -40^{O}C

13. The digested DNA fragments can be separated through electrophoresis.



Sticky/cohesive ended restriction fragments produced



Application

- Restriction enzyme digestion is a commonly used technique for molecular cloning
- It is also used to quickly check the identity of a plasmid by diagnostic digest
- Study of mutation and population-wide polymorphism
- Used in Linkage Mapping
- Used in DNA probe preparation
- DNA fingerprinting and Paternity test (RFLP Method). RFLP stands for Restriction Fragment Length Polymorphism
- Preparation of Expression Vector

PRECAUTION

Make sure that the restriction enzyme does not exceed more than 10% of the total reaction volume, otherwise the glycerol and the EDTA in the enzyme storage buffer may inhibit digestion process.