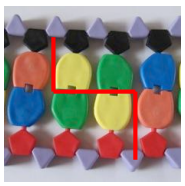
TECHNIQUES IN MOLECULAR BIOLOGY:
RESTRICTION ENZYMES

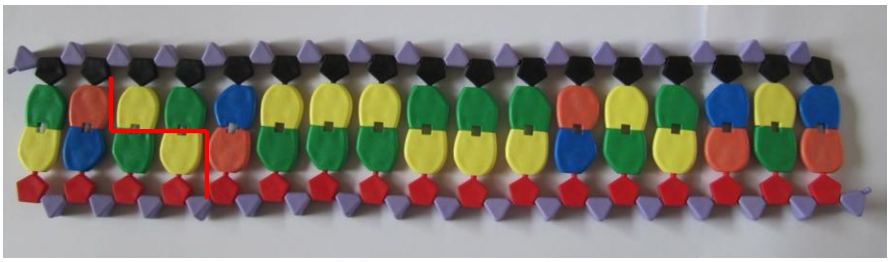
Course: Molecular Biology (BIOL333)

Instructor: Dr. M A Srour

Textbook:
Watson J, et al. (2014). Molecular Biology of the Gene, 7th ed.
Chap 7/ pp.147-

Restriction Enzymes





part of Restriction-modification system (R-M system)

we have 3 type but "the immune system of bacteria"

"type2" use

Restriction Enzymes (RE)

endo type nuclea

قطع من الوسط at specific site Not randomly

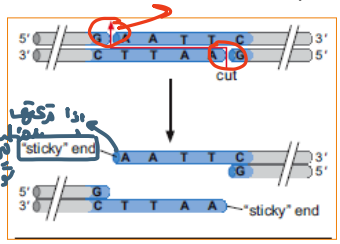
- RE: Site-specific endonucleases of prokaryotes
- Restriction enzymes = restriction endonucleases
- Recognize short (4-8 bp) target sequences called **Restriction site**, typically ***Palindromic*** sites

ROTATOR

Roman number

EcoRI restriction site

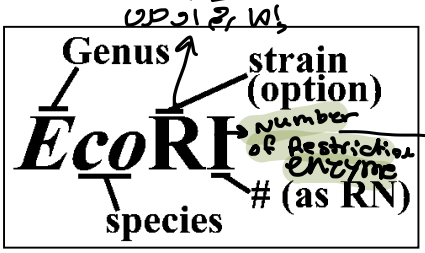
5′...GAATTC...3′
3′...CTTAAG...5′



enzyme Ligase

Restriction Enzymes (RE) are common

- Type II REs cuts adjacent or within restriction sites
- Type II enzymes are powerful tools in molecular biology → gene cloning
- RE are named after the bacterial species/strain from which it was isolated



I → Roman Number

"RE" هي أول تم التعرف عليها من E.coli

* UGA → stop codone → Selin , Cicten amino acid 21

Features of Restriction Sites

- Typically 4-8 bp & palindromic
- Frequency of RS: 4^4=256 bp, 4^6=4096 bp, 4^8=~65000 bp
- Degeneracy permitted by some enzymes
- Some Res are sensitive to methylation

1bp A/T C TG / G AC

A CTG / T GAC — CH_3

Diffrent codone → one amino acid: CCG, CCT, CCA, CCC — proline

Lift → Right

Features of Restriction Sites

- Cleavage produces 5'-PO_4 & 3'-OH
- Both strands cleaved between same residues:
  - Blunt ends (flush ends)
  - Staggered /sticky ends at RT
    - 5'-overhangs
    - 3'-overhangs

Blunt End (*Sma* I)

```
    ↓
-CCCGGG-        -CCC      GGG-
 ||||||    →     |||   +   |||
-GGGCCC-        -GGG      CCC-
    ↑
```

5' Overhang (*Xma* I)

```
  ↓
-CCCGGG-       -C        CCGGG-
 ||||||    →    |     +      |
-GGGCCC-       -GGGCC        C-
      ↑
```

3' Overhang (*Pst* I)

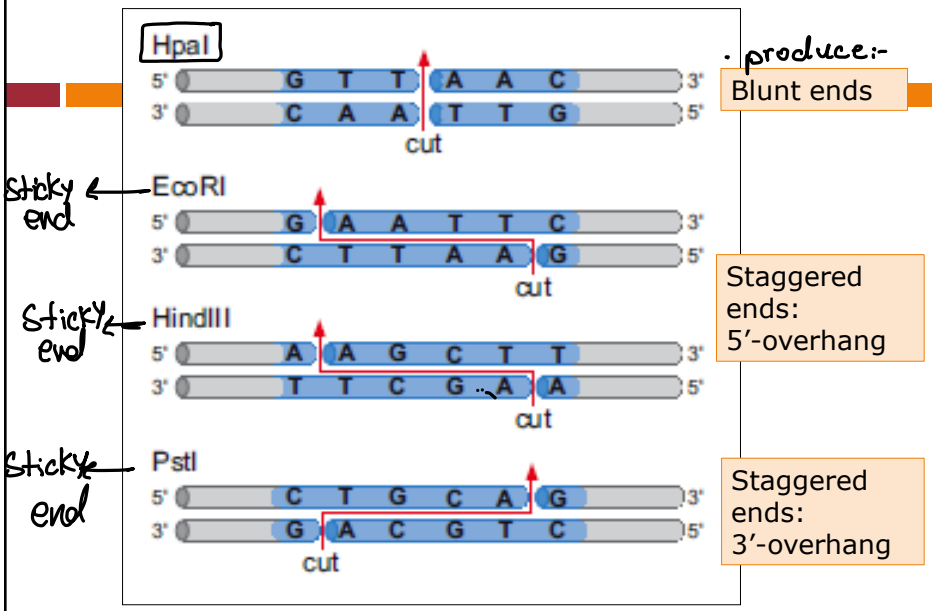
```
      ↓
-CTGCAG-       -CTGCA      G-
 ||||||    →    |     +     |
-GACGTC-       -G      ACGTC-
  ↑
```

hydrogen bond → NOT phosphodester bond

Some Res and their recognition sequences

Enzyme	Sequence	Cut frequency	Site/ type of ends
Sau3A	5'- | GATC-3'	0.25 kb	Tetrameric site/ sticky
EcoRI	5'-G | AATTC-3'	4 kb	Hexameric site / sticky
NotI*	5'-GC | GGCCGC-3'	65 kb	Octomeric site / sticky
SmaI	5'-CCC | GGG-3'	4 kb	Hexameric site / blunt

Source: Sau3A: Staphylococcus aureus; EcoRI: Escherichia coli; NotI: Nocardia otitidis-caviarum; SmaI: Serratia marcescens.
*Methylation sensitive/ cleavage blocked at all sites by methylation



. produce:-
Blunt ends
Staggered ends: 5'-overhang
Staggered ends: 3'-overhang

REs

Isoschizomers

Different restriction enzyme that recognize the same sequence "same Restriction site"

- *Sma* I CCC↓GGG
- *Xma* I C↓CCGGG

Compatible Ends

تترك نهايات متشابهة

These ends can be ligated easily

تجارب الاستنساخ الجيني

- *Pst* I CTGCA↓G
- *Nsi* I ATGCA↓T

"استثناء يعني ممكن يرجعوا يلتزقوا مع بعض"

Functions of Restriction Enzymes

- Function to protect bacteria from phage (virus) infection
- Why REs do not destroy the host cell's own DNA? → answer → زي جهاز مناعة تبع البكتيريا.
- Almost all REs are paired with Methylases that recognize & methylate the same DNA sites
- The two enzymes RE & Methylase are collectively called a Restriction-Modification system (R-M system)

[*Eco*RI methylase]

~GAATTC~

↓

CH_3 ← Methylation

~GAATTC~

Applications of REs

- Restriction mapping & RFLP analysis (discussed later??)
- Cloning: Insertion of DNA fragments into cloning vectors

الاستنساخ

- Restriction or digestion of DNA by RE
  - Usually done in the appropriate buffer and temperature, in a small volume (~20µl)
  - Digested DNA fragments are analyzed by agarose gel electrophoresis

used vector gene cloning -

small, circular DNA molecules found in bacteria

process of determing the location of RE cut sites within a DNA molecules.

Restriction mapping

1. plasmid circular DNA cut with one or more restriction enzymes

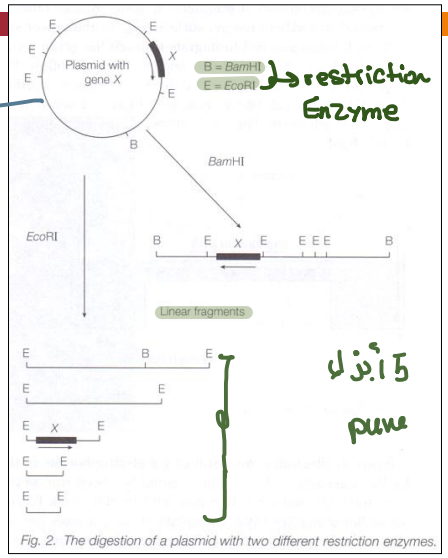


Fig. 2. The digestion of a plasmid with two different restriction enzymes.

"after cut plasmid by Restriction enzyme"

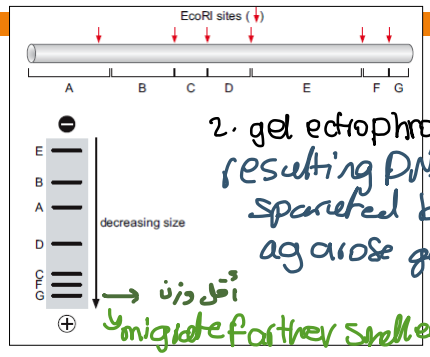


FIGURE 7-3 **Digestion of a DNA fragment with endonuclease EcoRI.** At the top is shown a DNA molecule and the positions within it at which EcoRI cleaves. When the molecule, digested with that enzyme, is run on an agarose gel, the pattern of bands shown is observed.

is technique based on size chary

2. gel ectrophoresis:- resulting DNA fragments separated by size using agarose gel electrophoresis

3 - DATA Analysis:- position RE cut sites

Restriction Map

Gel electrophoresis results