TECHNIQUES IN MOLECULAR BIOLOGY:

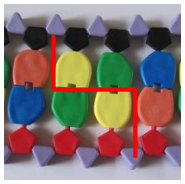
RESTRICTION ENZYMES

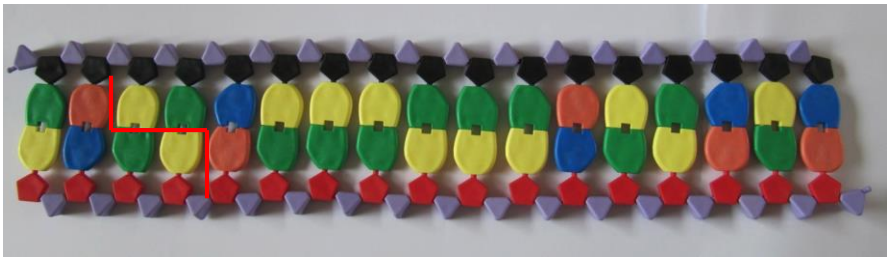
Course: Molecular Biology (BIOL333)

Instructor: Dr. M A Srour

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

Restriction Enzymes



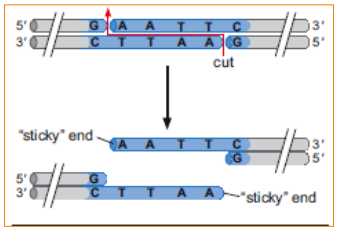


Restriction Enzymes (RE)

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

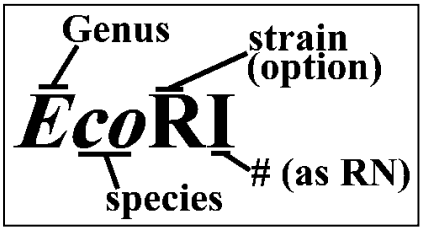
EcoRI restriction site

5′...GAATTC...3′
3′...CTTAAG...5′



Restriction Enzymes (RE)

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

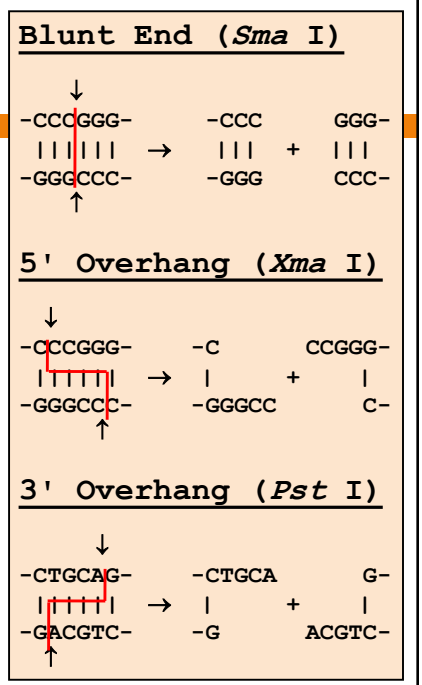


Features of Restriction Sites

- 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

Features of Restriction Sites

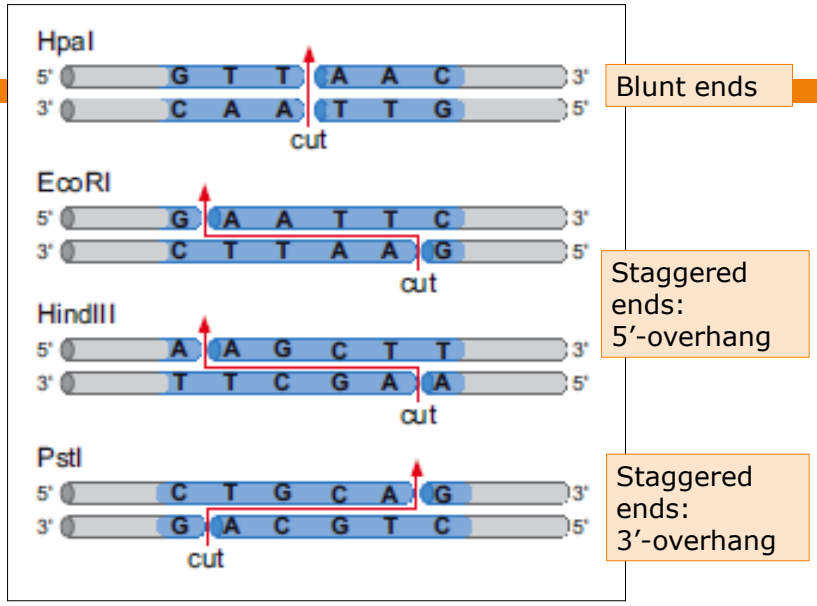
- Cleavage produces 5'-PO_4 & 3'-OH
- Both strands cleaved between same residues:
  - Blunt ends (flush ends)
  - Staggered /sticky ends at RT
    - 5'-overhangs
    - 3'-overhangs



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



Blunt ends

Staggered ends: 5'-overhang

Staggered ends: 3'-overhang

REs

Isoschizomers

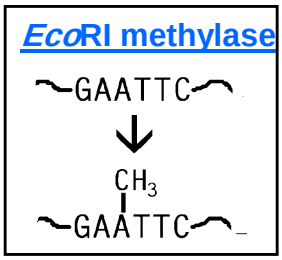
- ***Sma* I CCC↓GGG**
- ***Xma* I C↓CCGGG**

Compatible Ends

- ***Pst* I CTGCA↓G**
- ***Nsi* I ATGCA↓T**

Functions of Restriction Enzymes

- Function to protect bacteria from phage (virus) infection
- Why REs do not destroy the host cell's own DNA?
- 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)



Applications of REs

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

- 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

Restriction mapping

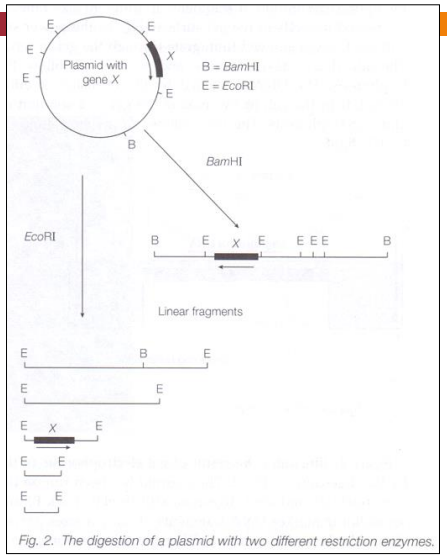


Fig. 2. The digestion of a plasmid with two different restriction enzymes.

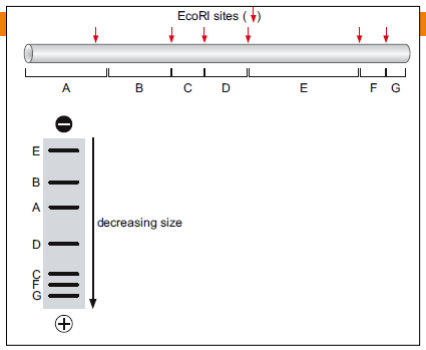
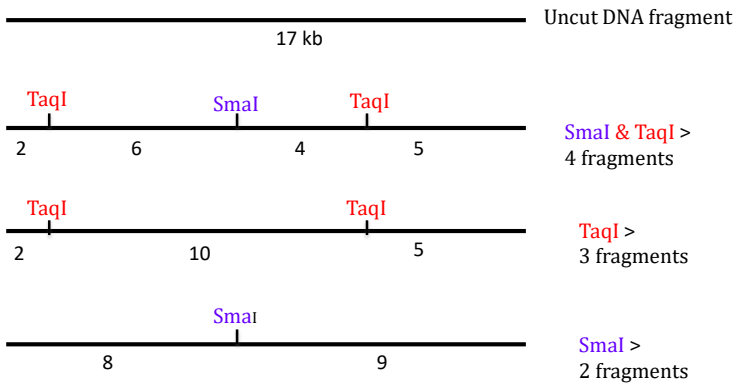


FIGURE 7-3 Digestion of a DNA fragment with endonuclease EcoRI. At the top is shown a DNA molecule and the positions within it at which EcoRI cleaves. When the molecule, digested with that enzyme, is run on an agarose gel, the pattern of bands shown is observed.

Restriction Map



Gel electrophoresis results

DNA gel electrophoresis