

TECHNIQUES IN MOLECULAR BIOLOGY: RESTRICTION ENZYMES

Course: Molecular Biology (BIOL333)

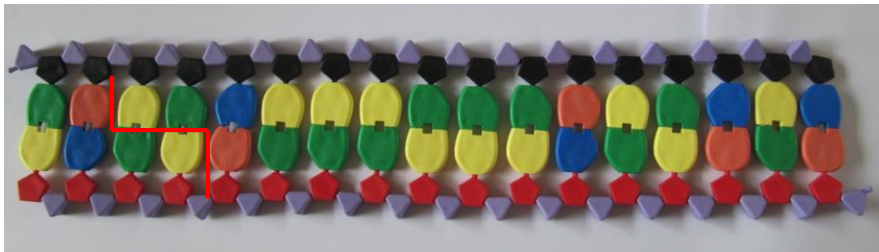
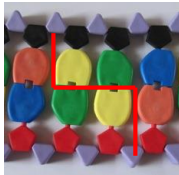
Instructor: Dr. M A Srou

Textbook:

Watson J, et al. (2014). Molecular Biology of the Gene, 7th ed.

Chap 7/ pp.147-

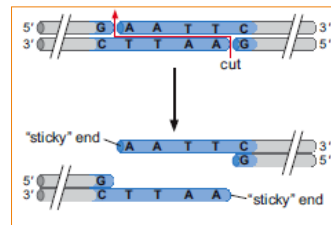
Restriction Enzymes



Restriction Enzymes (RE)

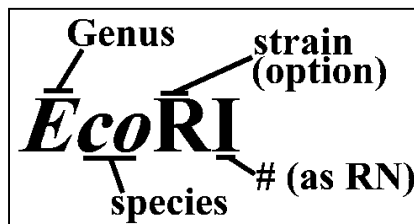
- RE: Site-specific endonucleases of prokaryotes
- Restriction enzymes = restriction endonucleases
- Recognize short (4-8 bp) target sequences called **Restriction site**, typically **Palindromic** sites

EcoRI restriction site



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



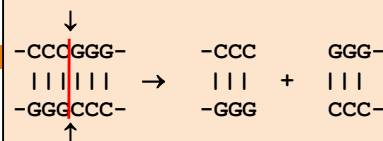
Features of Restriction Sites

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

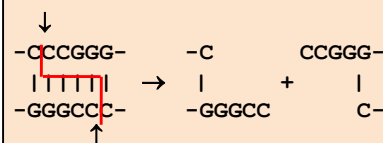
Features of Restriction Sites

- Cleavage produces 5'-PO₄ & 3'-OH
- Both strands cleaved between same residues:
 - Blunt ends (flush ends)
 - Staggered /sticky ends at RT
 - 5'-overhangs
 - 3'-overhangs

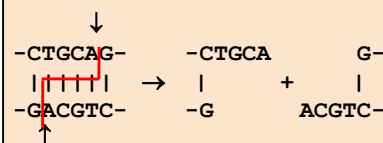
Blunt End (*Sma* I)



5' Overhang (*Xma* I)



3' Overhang (*Pst* I)

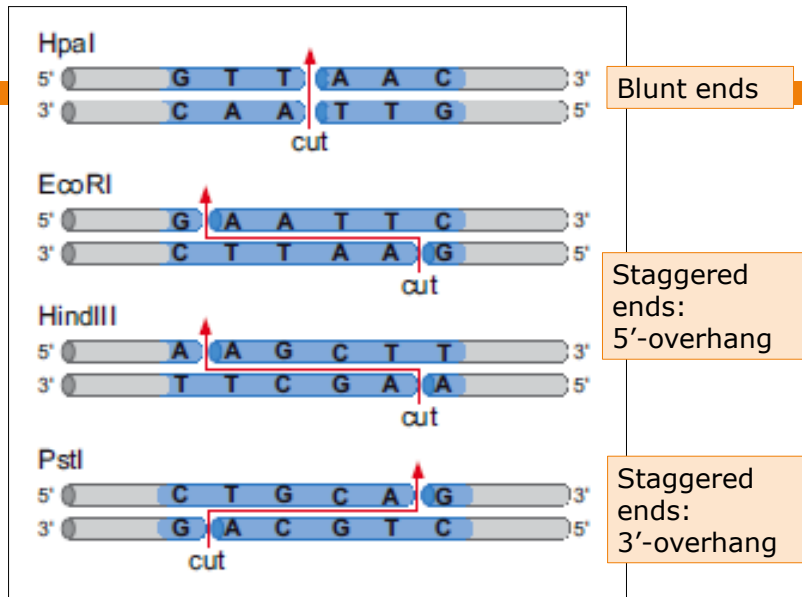


Some Res and their recognition sequences

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



REs

Isoschizomers

- *Sma*I CCC↓GGG
- *Xma*I C↓CCGGG

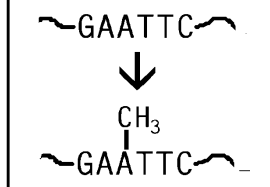
Compatible Ends

- *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 \(R-M system\)](#)

*Eco*RI methylase



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 ($\sim 20\mu\text{l}$)
 - ▣ Digested DNA fragments are analyzed by agarose gel electrophoresis

Restriction mapping

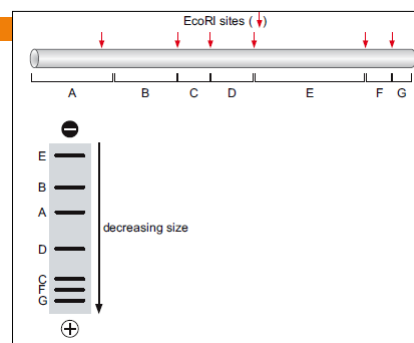
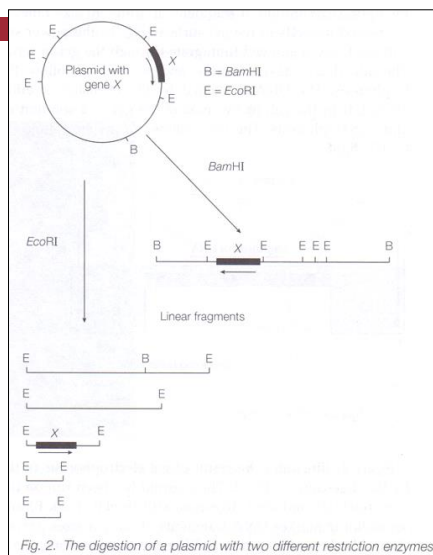


FIGURE 7-3 Digestion of a DNA fragment with endonuclease EcoRI. At the top is shown a DNA molecule and the positions within it at which EcoRI cleaves. When the molecule, digested with that enzyme, is run on an agarose gel, the pattern of bands shown is observed.

Gel electrophoresis results

DNA gel electrophoresis

Ladder	Uncut	TaqI	SmaI	TaqI & SmaI
Band 1	Band 1	Band 1	Band 1	Band 1
Band 2	Band 2			
Band 3		Band 2	Band 2	Band 2
Band 4				
Band 5		Band 3		Band 3
Band 6				
Band 7		Band 4	Band 4	Band 4
Band 8				
Band 9		Band 5	Band 5	Band 5
Band 10				
Band 11		Band 6	Band 6	Band 6
Band 12				
Band 13		Band 7	Band 7	Band 7
Band 14				
Band 15		Band 8	Band 8	Band 8
Band 16				
Band 17		Band 9	Band 9	Band 9
Band 18				
Band 19		Band 10	Band 10	Band 10
Band 20				
Band 21		Band 11	Band 11	Band 11
Band 22				
Band 23		Band 12	Band 12	Band 12
Band 24				
Band 25		Band 13	Band 13	Band 13
Band 26				
Band 27		Band 14	Band 14	Band 14
Band 28				
Band 29		Band 15	Band 15	Band 15
Band 30				
Band 31		Band 16	Band 16	Band 16
Band 32				
Band 33		Band 17	Band 17	Band 17
Band 34				
Band 35		Band 18	Band 18	Band 18
Band 36				
Band 37		Band 19	Band 19	Band 19
Band 38				
Band 39		Band 20	Band 20	Band 20
Band 40				
Band 41		Band 21	Band 21	Band 21
Band 42				
Band 43		Band 22	Band 22	Band 22
Band 44				
Band 45		Band 23	Band 23	Band 23
Band 46				
Band 47		Band 24	Band 24	Band 24
Band 48				
Band 49		Band 25	Band 25	Band 25
Band 50				
Band 51		Band 26	Band 26	Band 26
Band 52				
Band 53		Band 27	Band 27	Band 27
Band 54				
Band 55		Band 28	Band 28	Band 28
Band 56				
Band 57		Band 29	Band 29	Band 29
Band 58				
Band 59		Band 30	Band 30	Band 30
Band 60				
Band 61		Band 31	Band 31	Band 31
Band 62				
Band 63		Band 32	Band 32	Band 32
Band 64				
Band 65		Band 33	Band 33	Band 33
Band 66				
Band 67		Band 34	Band 34	Band 34
Band 68				
Band 69		Band 35	Band 35	Band 35
Band 70				
Band 71		Band 36	Band 36	Band 36
Band 72				
Band 73		Band 37	Band 37	Band 37
Band 74				
Band 75		Band 38	Band 38	Band 38
Band 76				
Band 77		Band 39	Band 39	Band 39
Band 78				
Band 79		Band 40	Band 40	Band 40
Band 80				
Band 81		Band 41	Band 41	Band 41
Band 82				
Band 83		Band 42	Band 42	Band 42
Band 84				
Band 85		Band 43	Band 43	Band 43
Band 86				
Band 87		Band 44	Band 44	Band 44
Band 88				
Band 89		Band 45	Band 45	Band 45
Band 90				
Band 91		Band 46	Band 46	Band 46
Band 92				
Band 93		Band 47	Band 47	Band 47
Band 94				
Band 95		Band 48	Band 48	Band 48
Band 96				
Band 97		Band 49	Band 49	Band 49
Band 98				
Band 99		Band 50	Band 50	Band 50
Band 100				