A study material for M.Sc. Biochemistry (Semester: III) Students
on the topic (CC-13; Unit I)

Restriction Enzymes II

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Difference

Property	Type I	Type II	Type III
Protein structure	Bifunctional, 3 subunits	Separate endonuclease and Methylase	Bifunctional, 2 subunits
Recognition site	Asymmetrical ($TGAN_8$)	Short sequence palindromic (4-8 bp)	asymmetrical
Cleavage site	>100bp from recognition site	Close to recognition site or same	24-26 bp downstream of recognition site
ATP needed	yes	No	yes
Mg^{++}	yes	yes	yes

Type IIs RE

- Two different enzymes but recognition sequence is asymmetric
- Cleavage occurs on one side of the recognition sequence up to 20 bp away.

Other Restriction system

- homing endonuclease (DNase) derived from introns.
- Asymmetric recognition sequences
- Tolerate some sequences degeneracy with in their recognition sequence

Table 3.1 Characteristics of the different types of endonucleases.

System	Key features
Type I	One enzyme with different subunits for recognition, cleavage, and methylation. Recognizes and methylates a single sequence but cleaves DNA up to 1000 bp away
Type II	Two different enzymes which both recognize the same target sequence, which is symmetrical. The two enzymes either cleave or modify the recognition sequence
Type III	One enzyme with two different subunits, one for recognition and modification and one for cleavage. Recognizes and methylates same sequence but cleaves 24–26 bp away
Type IIs	Two different enzymes but recognition sequence is asymmetric. Cleavage occurs on one side of recognition sequence up to 20 bp away

Nomenclature of RE

- Nathans and Smith proposed nomenclature system
- Features:
- *Eco*RI
- E=First letter of RE name derived from genus first letter of microbes (written in italics)
- Next two letters derived from species name of microbe from where RE is isolated (written in italics)
- Then strain subscript of bacterium has been taken "R" (non italic)
- When a particular host strain has several different R-M systems these are identified by roman numerals eg. *Hin*dI, *Hin*dIII
- R.*Hin*dII, where R is Restriction
- M.*Hin*dIII, where M is methylase
- All REs have the general endonuclease, R but in addition carry the system name eg. Endonuclease R. *Hin*dIII etc.
- Similarly modification enzymes are named Methylase by the system name ex. M. *Hin*dIII

Derivation of the EcoRI name

Abbreviation	Meaning	Description
E	Escherichia	genus
co	*coli*	species
R	RY13	strain
I	First identified	order of identification in the bacterium

Style of cleavage

- 2 types: 1. **Blunt** and 2. **Cohesive**
- Blunt/flush end: cut both strands of DNA at the same position
- No overhang of nucleotides
- Used to join with any fragment of having blunt end
- Ex. *Hae*III, SmaI, *Hpa*I, *Hin*dII, *Hae*III, *Alu*I etc

RE	Recognition site	Cleavage product
*Hae*III	GG ↓ CC	--GG CC--
*Sma*I	CCC ↓ GGG	--CCC GGG--

Cohesive/Staggered/Sticky end

- RE leaves overhang of nucleotides after cutting

RE	Recognition site	Cleavage product
*Eco*RI	5’G↓AATTC 3’ 3’CTTAA↑G 5’	-5’G AATTC3’- -3’CTTAA G5’-
*Hin*dIII	5’A↓AGCTT 3’ 3’T TCGA↑A 5’	-5’A ACCTT 3’- -3’TTGGA A 5’

The recognition sequences for some of the most frequently used restriction endonucleases.

ENZYME	ORGANISM	RECOGNITION SEQUENCE*	BLUNT OR STICKY END
*Eco*RI	*Escherichia coli*	GAATTC	Sticky
*Bam*HI	*Bacillus amyloliquefaciens*	GGATCC	Sticky
*Bgl*II	*Bacillus globigii*	AGATCT	Sticky
*Pvu*I	*Proteus vulgaris*	CGATCG	Sticky
*Pvu*II	*Proteus vulgaris*	CAGCTG	Blunt
*Hind*III	*Haemophilus influenzae* R_d	AAGCTT	Sticky
*Hin*fI	*Haemophilus influenzae* R_f	GANTC	Sticky
*Sau*3A	*Staphylococcus aureus*	GATC	Sticky
*Alu*I	*Arthrobacter luteus*	AGCT	Blunt
*Taq*I	*Thermus aquaticus*	TCGA	Sticky
*Hae*III	*Haemophilus aegyptius*	GGCC	Blunt
*Not*I	*Nocardia otitidis-caviarum*	GCGGCCGC	Sticky
*Sfi*I	*Streptomyces fimbriatus*	GGCCNNNNNGGCC	Sticky

*The sequence shown is that of one strand, given in the 5′ to 3′ direction. "N" indicates any nucleotide. Note that almost all recognition sequences are palindromes: when both strands are considered they read the same in each direction, for example:

*Eco*RI

5′–GAATTC–3′
||||||
3′–CTTAAG–5′

Two conditions

- **5’ overhang**: RE cleaves the DNA asymmetrically leaves ssDNA bases. If single strand DNA end with 5’ phosphate the enzyme said to produce 5’ overhang. Ex.

*Bam*HI 5’ G↓GATCC 3’

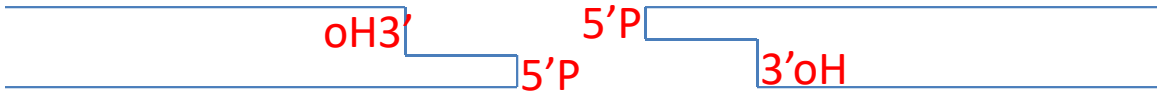
3’ C CTAG↑G 5’

*Bam*HI

5’G 5’pGATCC3’

3’CCTAGp5’ 3’$_{OH}$AAG5’

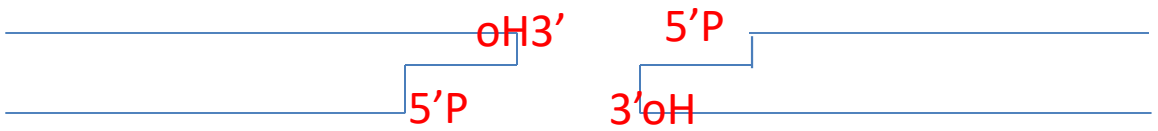
5’ phosphate exposed and 3’ OH recessed



3’ overhang: example *Sac*I

5’ GAGCTC 3’ —*Sac*I→ 5’-GAGCT-3’ + 5’ ||||C 3’
3’ CTCGAC 5’ 3’ C|||-5’ 3’ TCGAG 5’

5’ P recessed and 3’ OH exposed



If the ss bases end with a 3’OH , enzyme is said to be leave 3’ overhang. Example is *Sac*I

Some terms

- **Target sites (Recognition site): The RE cut large DNA molecules into shorter fragments at specific nucleotide sequences referred as recognition or target site.**
- **Type II RE recognizes and break DNA with in particular sequences of tetra-, penta-, hexa or hepta nucleotides, which have two fold rotational symmetry. For example *Eco*RI cuts at the positions indicated by in arrows in the target sequence**

5’ G↓AATTC 3’

- ***Not*I, *Pac*I recognizes 8bp sequence (5’GC↓GGCCGC 3’)**
- **Majority of enzymes uses 6 bp target sites**

Ambiguous in recognizing the restriction site or target site
Example is *Hin*dII GG(C/T)↓(G/A)AC
*Hin*dII recognizes the target site with some ambiguity. The RE cut the both either C/ T, or G/A nucleotide in target site. This type of RE has ambiguity in recognizing or cutting the target site

Isoschizomers: More than one RE recognizes the same target site. Example:
*Sph*I (CGTAC↓G) and *Bbu*I(CGTAC↓G).

Neoschizomers: two different RE recognize the same target sequence but break phosphate group between two different nucleotides. Example is *Sma*I and *Xma*I
*Sma*I 5’ GGG↓CCC 3’ and *Xma*I (5’G↓GCCCC 3’). Both recognizes the same target site but produces different cleavage product. So *Xma*I and *Sma*I is neoshcizomers

Hybrid site: two different RE recognizes the two different target site but the resulting protuding ss region is complementary to each other.
Example is ***Age*I and *Ava*I**

***Age*I** **A↓CCGGT produces A CCGGT and *Ava*I C↓CCGGG produces C CCGGG**
TGGCC↑A TGGCC A G GGCC↑C GGGCC C

- **Star activity:** Under extreme condition such as high pH, RE are able to cut sequences which are similar but not identical to their defined recognition sequence called SA.
- Ex *Eco*RI N↓AATTN (N is any nucleotide) *Eco*RI cleaves (G↓AATTC)

Some useful information

- Nucleases are enzymes that cut, shorten or degrade the nucleic acid molecules
- *Bal*31 exonuclease removes nucleotides from both strands of DSD.
- Ecoli *exoIII* degrade just one strand of a double stranded DNA molecules
- *DNase*I cuts both single and dsDNA molecule
- **Alkaline phosphatase**: removes phosphate group from 5’ end
- **Polynucleotide kinase** adds phosphate group to free 5’ termini
- **TTase** (from calf thymus) which adds one or more deoxynucleotides on to the 3’ terminus of a DNA molecule
- **Acid pyrophophatase** removes the cap structure from the mRNA
- **HK alkaline phosphatase** removes the 5’-phosphate group from N.a.
- **mRNA guanyl transferase** adds 5’ cap structure to the mRNA
- **Polynucleotide phosphorylase** adds ribonucleotides to the 3’OH terminus of mRNA

Acknowledgement and Suggested Readings:

1. Gene Cloning and DNA Analysis: An Introduction; Sixth Edition ; T. A. Brown; Wiley – Blackwell Publications
2. Principles of Gene Manipulation; Sixth Edition; Sandy B Primrose, Richard M Twyman and Robert W. Old; Wiley – Blackwell Publications
3. Biotechnology: Applying the Genetic Revolution; David P. Clark and Nanette J. Pazdernik; Academic Press (Elsevier)
4. Restriction Endonucleases; Alfred M. Pingoud; Springer Publications

Thanks