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

Restriction Enzymes I

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Restriction Enzyme

- Arbor discovered the enzymes that were able to cut the DNA into small fragments.
- Hamilton and Smith and associates isolated a RE from H. influenzae and called it *Hind*II, which able to cut viral DNA at specific sites producing a fragments with 5’ and 3’ terminals (5’pPupApCp and pGpTpPy-3’)
- “Restriction Enzymes are an endonuclease that recognizes the specific sequences of DNA and cut them”

Properties of RE

- Active against foreign DNA
- Inactive against its own DNA
- Act upon active target sequence
- Cut the dimeric bond
- All REs act on two fold rotational symmetry of DNA

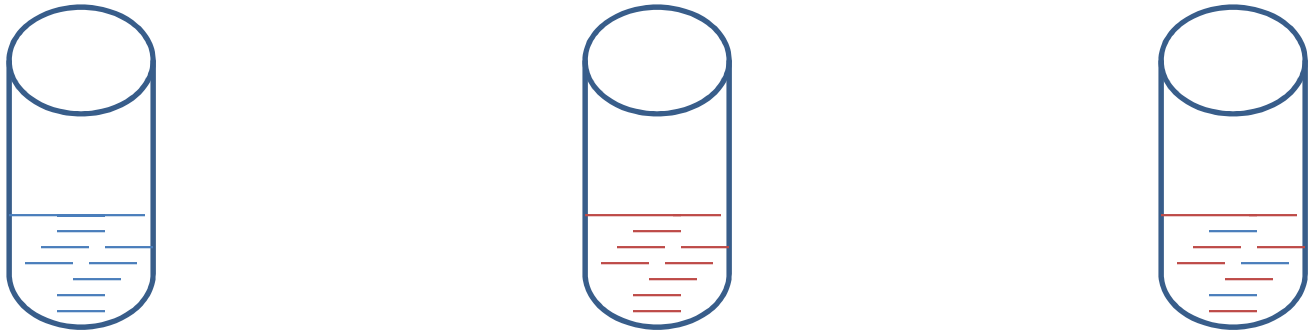
Types of RE

- Type I
- Type II
- Type III
- Type IIs

Type I

- In 1968 Messelson and Yuan discovered the RE in *E.coli* strain.
- The protein responsible for degradation of DNA
- Expt.:

E. coli protein Extract contains RE



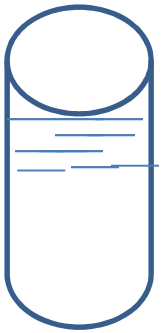
1. ET contained normal **not modified DNA**

2. ET contained **modified DNA** (Methylated DNA)

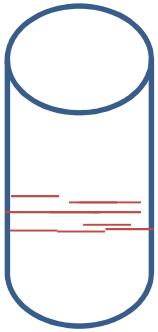
3. ET contained both normal and **modified DNA**

Result

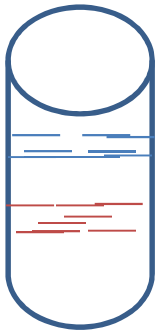
Sucrose gradient centrifugation



1. DNA fragmented



2. DNA not fragmented



3. Intact DNA and fragmented

Conclusion:

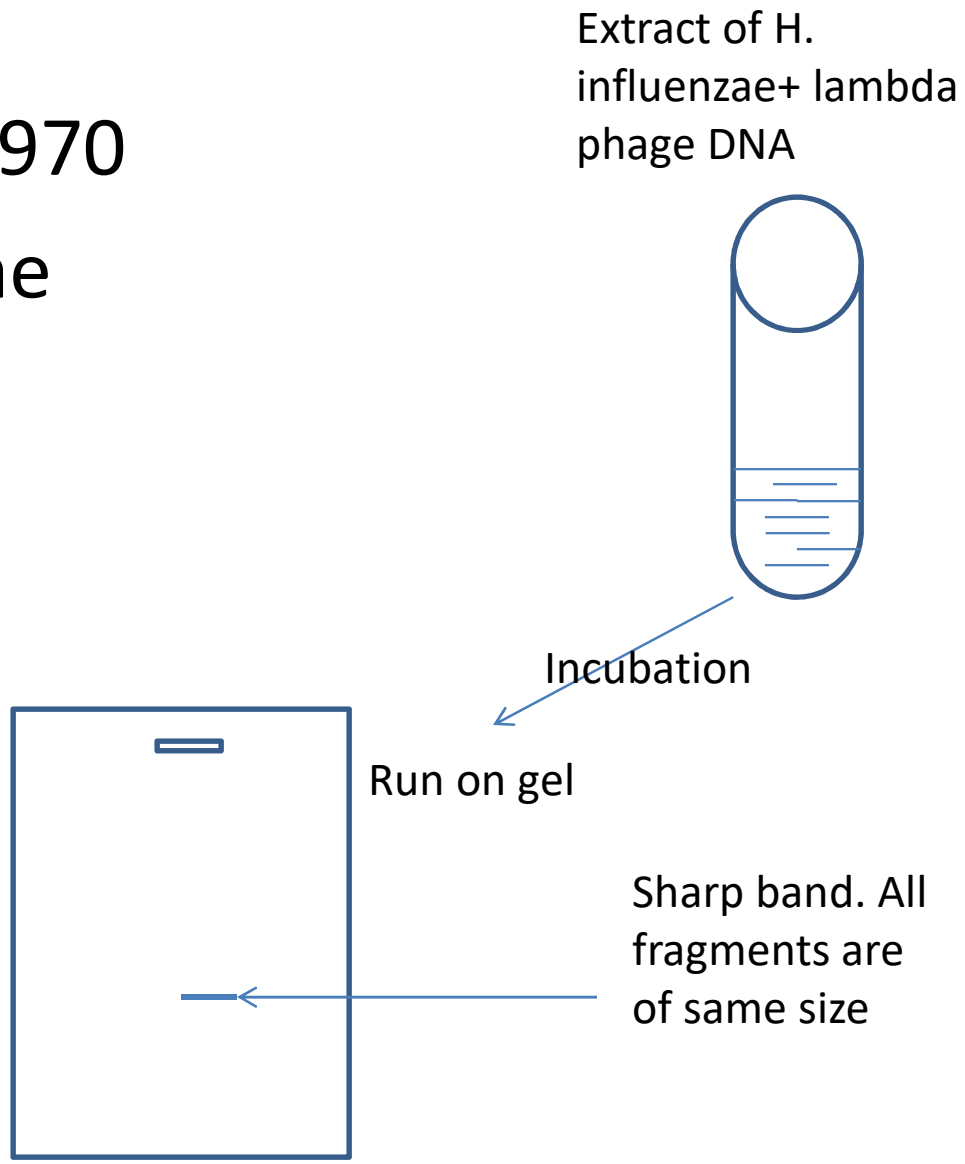
***E. coli* protein extract has ability to cut the normal DNA but not the modified DNA. They called these protein RE.**

Type I RE properties

- Discovered by Messelson and Yuan in the year 1968.
- The active enzyme consists of 2 restriction subunits, 2 modification subunits and 1 recognition subunits
- These subunits are the products of *hsd*R, *hsd*M and *hsd*S genes
- Recognize the specific sequence by cut at non specific sites.
- Type I RE was not suitable for GE because:
- 1. It moves randomly along the DNA about 1000-5000 nts
- 2. cuts only one strand of the DNA
- 3. produces gap of 75 nts
- 4. No evidence that enzyme is true catalytic
- 5. requires large amount of ATP and Mg^{++}
- 6. Biochemistry of type I RE is complex because role of S-adenosyl methionine remains unclear
- 7. same enzyme modified the DNA nucleotides before cut the DNA that's why it produces cut away from recognition site

Type II RE

- Kelly and Smith 1970
- From H. influenzae
- Experiment:



Conclusion: ***H. influenzae*** **has ability to cut DNA at specific site and generate fragment of defined and discrete size.**

Property of Type II RE

- It has two different enzymes, one for methylation and other for Restriction
- R and M are mediated by separate enzymes so it is possible to cleave DNA in (-)ve of modification subunit
- Suitable for GE because:
- 1. Cut at specific site
- 2. No ATP and S-adenosyl methionine requires
- 3. Only Mg^{++} requires

Difference →

Type I	Type II
Non specific in cleavage	Cleavage at specific site
MW 30,000 dalton	20,000 to 100,000 dalton
Requires ATP, Mg^{++}, S-adenosyl as co-factor	Requires only Mg^{++} only
Contain non identical subunit Ex. *Eco*B1, *Eco*K	Contain two identical subunit Ex. *Eco*RI, *Hin*dIII,I,II *Pst*I etc

Type III RE properties

- Intermediate property of Type I and Type II RE
- It has one Enzyme with 2 different subunits; 1-M and 1- R
- Restriction site is asymmetric
- Cleaves dsDNA at a defined site
- Cleavage site is 24-26 bp down stream from recognition site
- Requires Mg^{++}
- Partial requirement of adenosyl-methionine
- Ex. EcoP15, Eco571

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