CHANDIGARH UNIVERSITY

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INSTITUTE-UNIVERSITY INSTITUTE OF ENGINEERING

ACADEMIC UNIT-II

Computer Science Engineering

Subject Name-Biology For Engineers

Subject Code- 20SZT148

CARBONIC ANHYDRASE AND RESTRICTION ENZYME

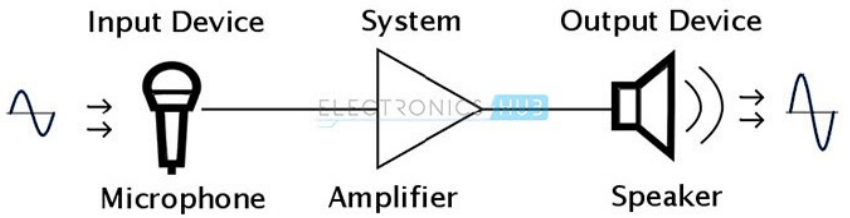
CARBONIC ANHYDRASE AND RESTRICTION ENZYME

Course Outcome

CO Number	Title	Level
CO1	It gives an idea about the about the basic cell biology.	**Understanding**
CO2	It deals with the idea of uses of biology in engineering.	**Understanding**
CO3	It provide knowledge about the uses of softwares in biology field.	**Remembering**

WHAT ARE TRANSDUCERS?

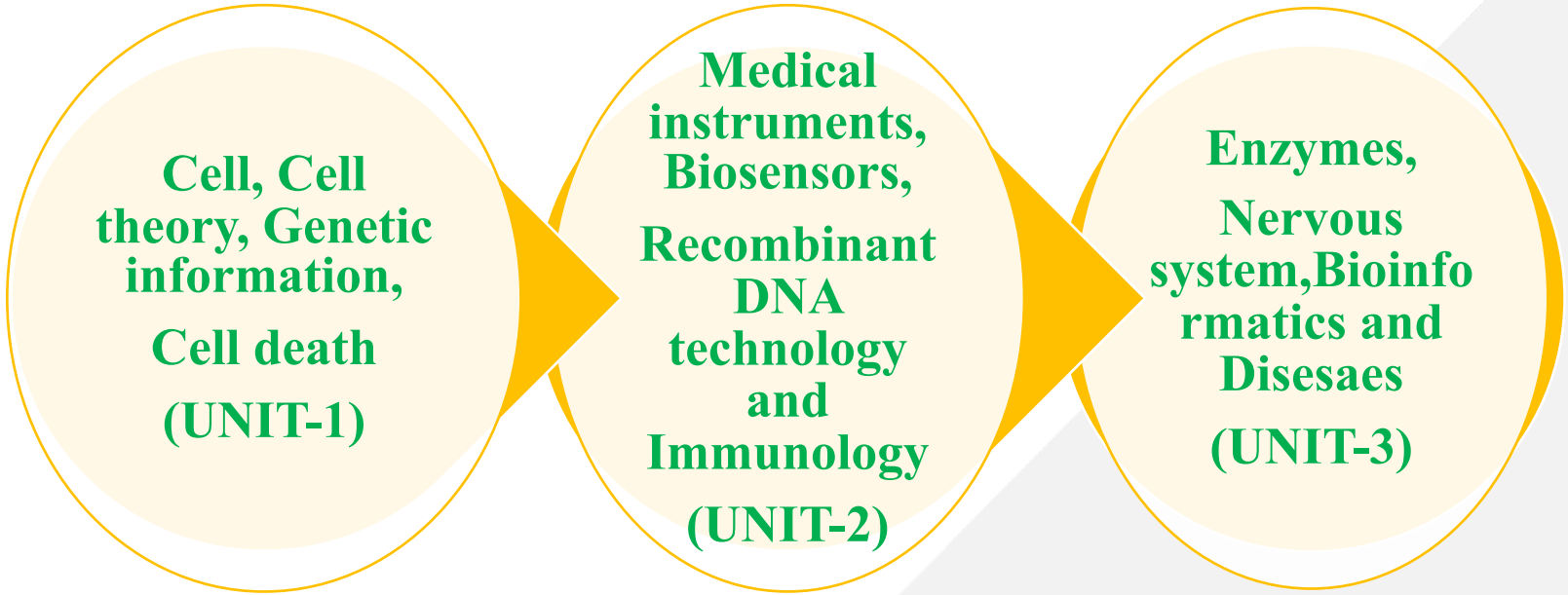
Different Types, Characteristics, Classification and Applications



Will be covered in this lecture

https://www.electronicshub.org/types-of-transducers/

BIOLOGY FOR ENGINEERS



CARBONIC ANHYDRASE

• The team is basing the development of its artificial enzyme on naturally occurring carbonic anhydrase (CA), which accelerates the conversion of carbon dioxide into carbonates.

• Carbonic anhydrase is capable of turning carbon dioxide molecules into carbonates at a rate of one million molecules per second.

CARBONIC ANHYDRASE

- Random Mutagenesis
- Scientists plan to modify and multiply the genes encoding for carbonic anhydrases using a molecular technique called random mutagenesis.
- The researchers will then place the mutated genes in the artificial environment to see which ones are most effective at converting carbon dioxide into carbonates.

CARBONIC ANHYDRASE

- The best mutations will then be put through the modification and multiplication processes again.

- The researchers will repeat the whole process until they have isolated a mutated gene encoding for recombinant carbonic anhydrase that can convert carbon dioxide into carbonates under industrial conditions.

CARBONIC ANHYDRASE

- With the help of artificial enzymes, • carbon dioxide-converted carbonates could be used in everything • from baking soda and chalk to Portland cement and lime manufacturing

RESTRICTION ENZYME

- Enzymes that cut DNA at or near specific recognition nucleotide sequences known as restriction sites.
- Especial class of enzymes that cleave (cut) DNA at a specific unique internal location along its length.
- Often called restriction endonucleases (Because they cut within the molecule). Discovered in the late 1970s by Werner Arber, Hamilton Smith, and Daniel Nathans.

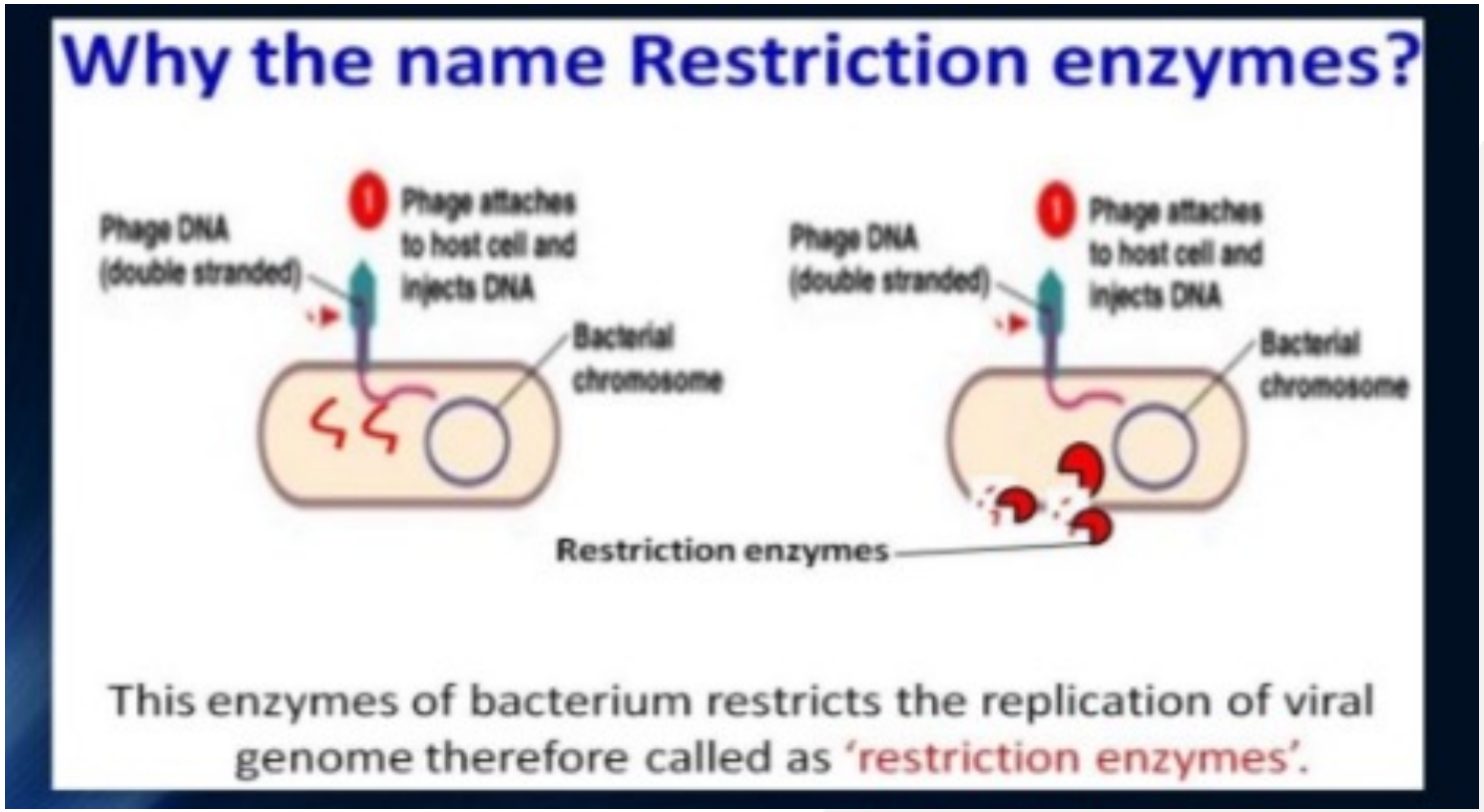
RESTRICTION ENZYME

- Essential tools for recombinant DNA technology.
- Naturally produced by bacteria that use them as a defense mechanism against viral infection.
- Chop up the viral nucleic acids and protect a bacterial cell by hydrolyzing phage DNA.

RESTRICTION ENZYME

- The bacterial DNA is protected from digestion because the cell methylates (adds methyl groups to) some of the cytosines in its DNA.
- The purified forms of these bacterial enzymes are used in today's laboratories.
- Commonly classified into three types, which differ in their structure and whether they cut their DNA substrate at their recognition site, or if the recognition and cleavage sites are separate from one another.
- To cut DNA, all restriction enzymes make two incisions, once through each sugar-phosphate backbone (i.e. each strand) of the DNA double helix.

RESTRICTION ENZYME



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NOMENCLATURE

- Since their discovery in the 1970s, many restriction enzymes have been identified; for example, more than 3500 different Type II restriction enzymes have been characterized.
- Each enzyme is named after the bacterium from which it was isolated, using a naming system based on bacterial genus, species and strain.

Derivation of the EcoRI name

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

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NOMENCLATURE

- For example, the name of the EcoRI restriction enzyme was derived as shown in the box.

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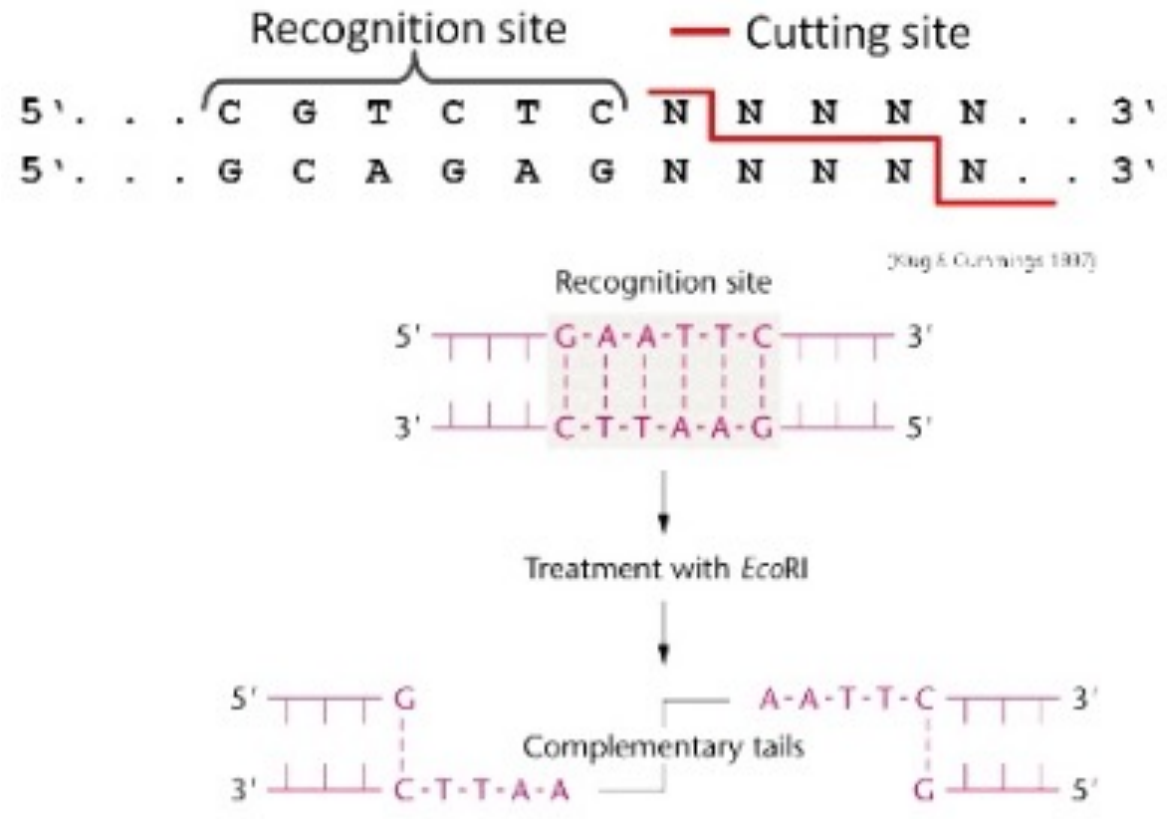

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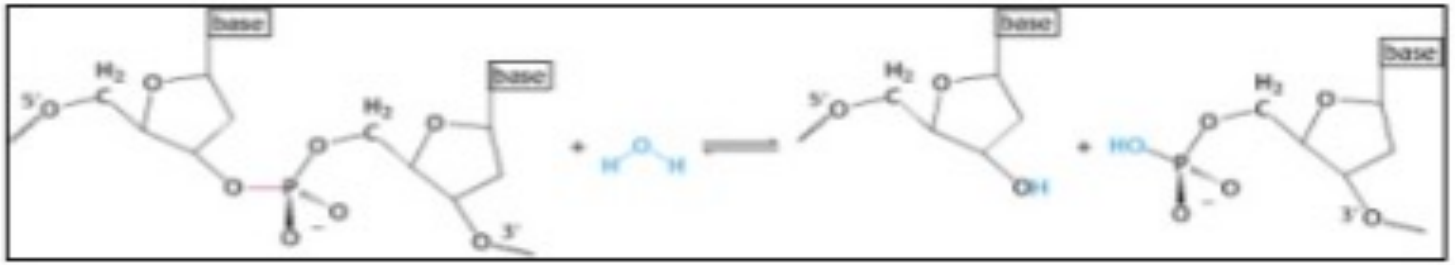
RESTRICTION ENDONUCLEASE

Mechanism of action

- Restriction Endonuclease scan the length of the DNA
- binds to the DNA molecule when it recognizes a specific sequence and makes one cut in each of the sugar phosphate backbones of the double helix – by hydrolyzing the phoshphodiester bond.
- Specifically, the bond between the 3’ O atom and the P atom is broken.

RESTRICTION ENDONUCLEASE

Direct hydrolysis by nucleophilic attack at the phosphorous atom

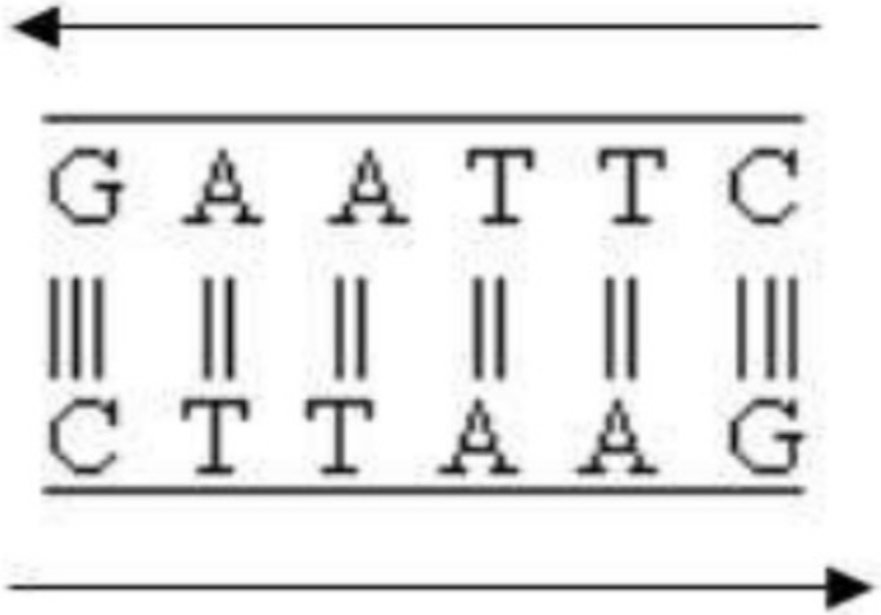


3’OH and 5’ PO4 3- is produced. Mg2+ is required for the catalytic activity of the enzyme. It holds the water molecule in a position where it can attack the phosphoryl group and also helps polarize the water molecule towards deprotonation .

PALLANDROMIC SEQUENCE

- The mirror like palindrome in which the same forward and backwards are on a single strand of DNA strand, as in GTAATG.
- The Inverted repeat palindromes is also a sequence that reads the same forward and backwards, but the forward and backward sequences are found in complementary DNA strands (GTATAC being complementary to CATATG).
- Inverted repeat palindromes are more common and have greater biological importance than mirror- like palindromes.

PALLANDROMIC SEQUENCE



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Ends Of Restriction Fragments

- Blunt ends

- Sticky ends

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BLUNT END

- Some restriction enzymes cut DNA at opposite base
- They leave blunt ended DNA fragments
- These blunt ended fragments can be joined to any other DNA fragment with blunt ends.
- Enzymes useful for certain types of DNA cloning experiments .

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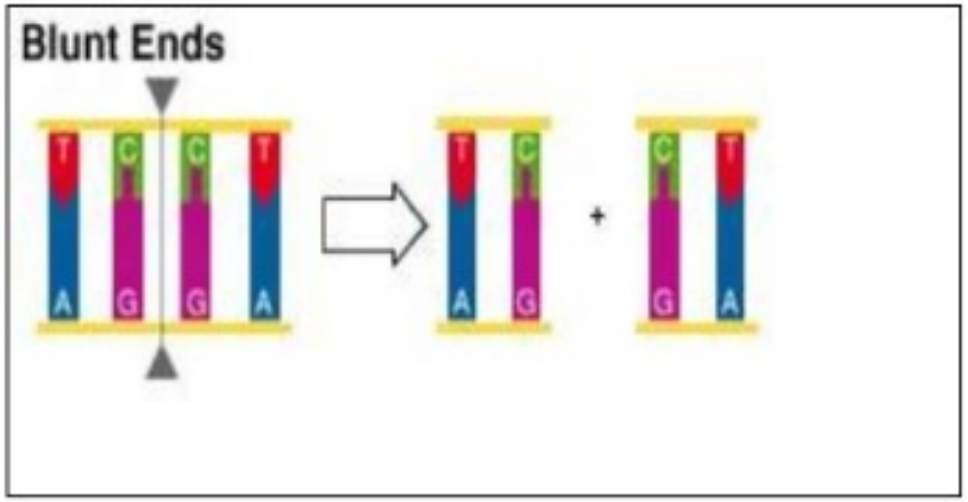

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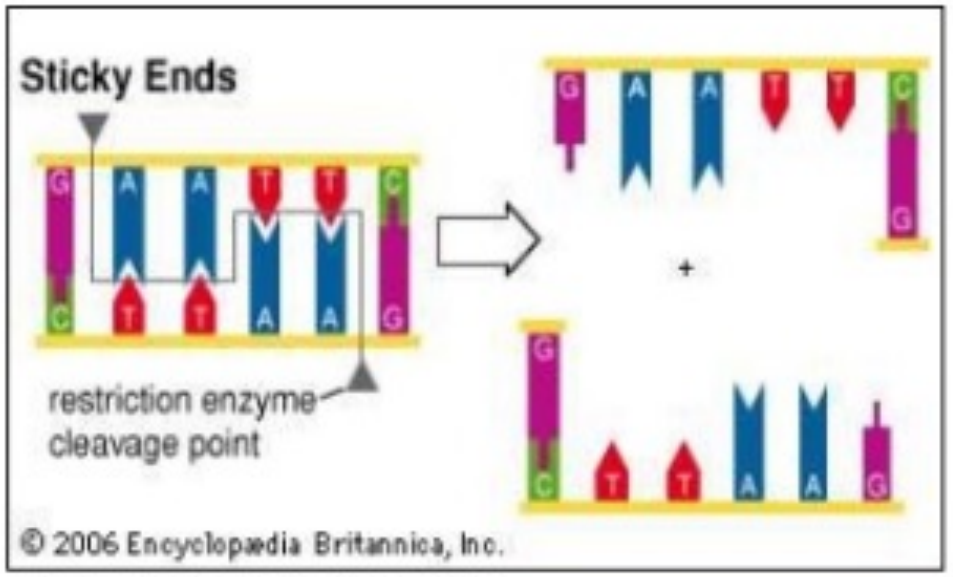
STICKY END

Sticky ends

- Most restriction enzymes make staggered cuts
- Staggered cuts produce single stranded "sticky-ends"



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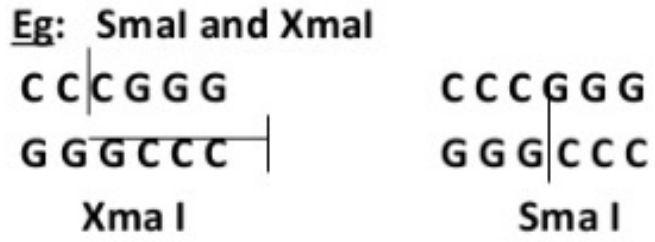
STICKY END

- Sticky Ends" Are Useful DNA fragments with complimentary sticky ends can be combined to create new molecules which allows the creation and manipulation of DNA sequences from different sources.

ISOSCHIZOMERS & NEOSCHIZOMERS

- Restriction enzymes that have the same recognition sequence as well as the same cleavage site are Isoschizomers
- Restriction enzymes that have the same recognition sequence but cleave the DNA at a different site within that sequence are Neoshizomers



TYPES OF ENZYME

TYPES OF RESTRICTION ENZYMES

- Restriction endonucleases are categorized into three general groups.

- **Type I**
- **Type II**
- **Type III**

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TYPES OF ENZYME

- These types are categorization based on:

➢ Their composition.
➢ Enzyme co-factor requirement.
➢ the nature of their target sequence.
➢ position of their DNA cleavage site relative to the target sequence.

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TYPE I

- Capable of both restriction and modification activities
- The co factors S-Adenosyl Methionine(AdoMet), ATP, and mg+are required for their full activity.
- Contain: two R(restriction) subunits two M(methylation) subunits one S(specifity) subunits
- Cleave DNA at random length from recognition sites

TYPE II

- These are the most commonly available and used restriction enzymes
- They are composed of only one subunit.
- Their recognition sites are usually undivided and palindromic and 4-8 nucleotides in length, they recognize and cleave DNA at the same site.
- They do not use ATP for their activity
- they usually require only Mg2+ as a cofactor.

TYPE III

- Recognize two separate non-palindromic sequences that are inversely oriented. • They cut DNA about 20-30 base pairs after the recognition site.
- These enzymes contain more than one subunit.
- And require AdoMet and ATP cofactors for their roles in DNA methylation and restriction

TYPE IV

- Cleave only normal and modified DNA (methylated, hydroxymethylated and glucosyl- hydroxymethylated bases).
- Recognition sequences have not been well defined
- Cleavage takes place ~30 bp away from one of the sites

APPLICATION OF RESTRICTION ENZYME

- They are used in gene cloning and protein expression experiments.
- Restriction enzymes are used in biotechnology to cut DNA into smaller strands in order to study fragment length differences among individuals (Restriction Fragment Length Polymorphism – RFLP).
- Each of these methods depends on the use of agarose gel electrophoresis for separation of the DNA fragments.

CONCLUSION

Till now we have discuss:

- Carbonic anhydrase is capable of turning carbon dioxide molecules into carbonates at a rate of one million molecules per second.
- The bacterial DNA is protected from digestion because the cell methylates (adds methyl groups to) some of the cytosines in its DNA.
- The co factors S-Adenosyl Methionine(AdoMet), ATP, and mg+are required for their full activity

ASSESSMENT PATTERN

Assessment Pattern	Total Marks
1st Hourly Test	36
2nd Hourly Test	36
Surprise Test	12
Assignment (3)	10
Quiz	4
End Semester Examination	60

REFERENCES

- C.B.Powar, 2010.Cell Biology.5th Ed,Himalyan Publishing House.
- Leshie Cromwell, Fred.J. Weibell and Erich.A.Pfeiffer. 2003. Biomedical instrumentation and measurements. 2nd edition, PHI.
- John G. Webster 1998. Medical Instrumentation: Applications and Design, 3rd edition, Jon Wiley and Sons, New York.
- Jeremy M. Berg, John L. Tymoczko and Lubert Stryer. 2006. "Biochemistry," 6th Ed. W.H. Freeman and Co. Ltd.
- Robert Weaver. 2012 "Molecular Biology," 5th Edition, MCGraw-Hill.
- Jon Cooper, , **2**004. "Biosensors A Practical Approach" Bellwether Books.
- Martin Alexander, 1994 "Biodegradation and Bioremediation," Academic Press.

THANK YOU

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