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Plant Genetic Engineering

Lecture no. 2

Fourth grade/ sub-dep. Fungi and Plant Sciences

2022-2023/ course no. 1

Applications of Plant Genetic Engineering

A. Crop Improvement
B. Genetically Engineered Traits: The Big Six
   1. Herbicide Resistance
   2. Insect Resistance
   3. Virus Resistance
   4. Altered Oil Content
   5. Delayed Fruit Ripening
   6. Pollen Control
C. Biotech Revolution: Cold and Drought Tolerance and Weather-Gard Genes
D. Genetically Engineered Foods
   1. Soybeans
   2. Corn
   3. Cotton
   4. Other Crops

Plant mitochondrial DNA

- chromosome size is much bigger but varies dramatically between species (200-2000 kb)
- arranged as different size circles, sometimes with plasmids.
- The plant mtDNA contains chloroplast sequences, indicating exchange of genetic information between organelles in plants.
- Much of the plant mtDNA is non-coding, but coding regions are larger than animals and fungi.
- Number of proteins synthesised not known definitely but more than in animals and yeast (probably about 50)

Plant mitochondria have specialised functions

- in leaves they participate in photorespiration
- sites of vitamin synthesis (vit C, folic acid, biotin)

Chloroplast genome

- Chloroplast DNA (cpDNA) is also known as plastid DNA (ptDNA).
- Circular double stranded DNA molecule
- Chloroplast genome size ranges 120-217kb with majority of plants fall into 120-160kb. (*Pelargonium* has a chloroplast genome size 217kb)
- contain about 100 genes to synthesize proteins
- cpDNA regions includes Large Single-Copy (LSC) & Small Single-Copy (SSC) regions, and Inverted Repeats (IR_A & IR_B).
- Conifers and a group of legumes lack Inverted Repeats.

Differences between mtDNA and nuclear DNA (N-DNA):

1. mtDNA contains is circular in shape with a molecular weight ranging from 220 to 166 x10^6 .
2. mtDNA contains more G and C contents than the N-DNA.
3. MtDNA has higher denaturation temperature than N-DNA.
4. MtDNA is shoreter and contains few coded informations than N-DNA.
5. DNApolymerase of mtDNA is different than DNApolymerase of the N-DNA.
6. MtDNA is lack of histone proteins..

Similarities between mtDNA andcpDNA :

- Both mtDNA and cpDNA are circular, double stranded and not enveloped by a membrane.
- Both are devoid of histone proteins. (histones: they are highly alkaline proteins found in eukaryote cell nuclei that package and order the DNA into structural unit called nucleosomes, and they play a role in gene regulation).
- Both lack introns.
- Both are generally AT rich genomes.

Basic steps in genetic engineering

1. Isolate the gene
2. Insert it in a host using a vector
3. Produce as many copies of the host as possible
4. Separate and purify the product of the gene

Restriction Enzymes

What are restriction enzymes?

- Molecular scissors that cut double stranded DNA molecules at specific points.
- Found naturally in a wide variety of prokaryotes
- An important tool for manipulating DNA.

History Of Restriction Enzyme

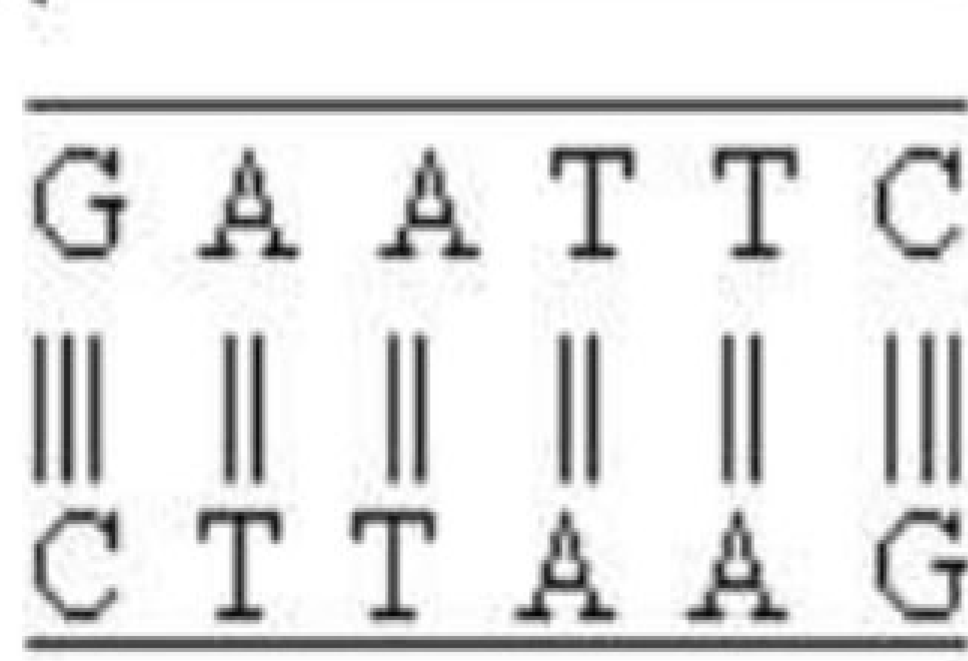
- First restriction enzyme was isoltaed in 1970 by Hindll.
- He also done the subsequent discovery and characterization of numerous restriction endonucleases.
- From then Over 3000 restriction enzymes have been studied in detail, and more than 600 of these are available commercially and are routinely used for DNA modification and manipulation in laboratories.

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.

Palindrome Sequences

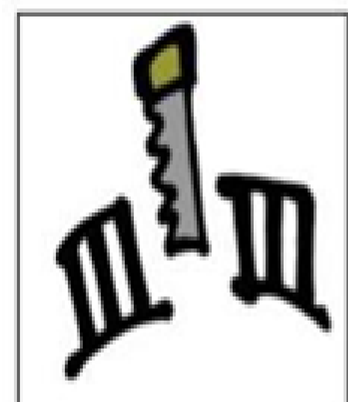
- The mirror like palindrome in which the same forward and backwards are on a single strand of DNA strand, as in GTAATG
- The Inverted repeat palindromes is also a sequence that reads the same forward and backwards, but the forward and backward sequences are found in complementary DNA strands (GTATAC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



G A A T T C
C T T A A G

Ends Of Restriction Fragments

- Blunt ends



- Sticky ends

Blunt ends

- 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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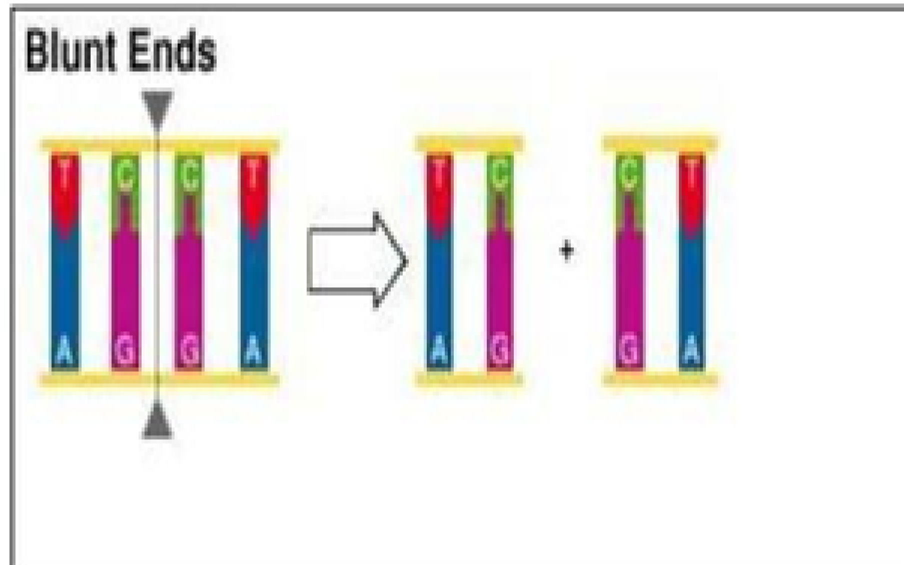
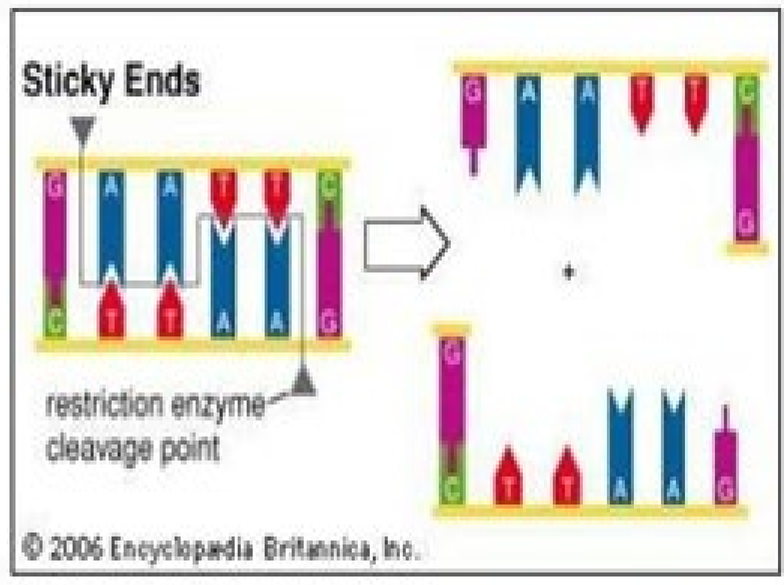


Sticky ends

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



“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

Eg: SmaI and XmaI

Xma I	Sma I
C C | C G G G	C C C G G G
G G G C C C |	G G G | C C C

NOMENCLATURE OF RESTRICTION ENZYME

- Each enzyme is named after the bacterium from which it was isolated using a naming system based on bacterial genus, species and strain.

For e.g EcoRI

The enzyme are named by the binomial nomenclature (genus) and species

First letter (uppercase) represented the genus.

 Second and third letters (lowercase) are the first two letters of the species.

 Roman numerals represent different R-M systems,

Ex: *HpaI* and *HpaII* represent the first and second type II restriction enzymes isolated from *Haemophilus parainfluenzae*.

TYPES OF RESTRICTION ENZYMES

- Restriction endonucleases are categorized into three general groups.

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

- 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.

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 Mg^{2+} as a cofactor.

Type III

- Type III restriction enzymes) 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 ENZYMES

- 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.

Thank You