Digestion of DNA using Restriction Enzymes and Analysis by Agarose Gel Electrophoresis

Principle

Restriction endonucleases or restriction enzymes are bacterial nucleases that cleave a phosphodiester linkage within the DNA and restricts the entry of foreign DNA into the bacterial cells. They serve as a part of defence mechanism against invading viruses. Bacteria protect their DNA by modifying their own recognition sequences, usually by adding methyl (CH3) molecules to nucleotides in the recognition sequences and then relying on the restriction enzymes' capacity to recognize and cleave only unmethylated recognition sequences. The endonucleases and methylases together form the Restriction Modification system (RMS) of the bacteria.

Three categories of restriction endonucleases are : Type I, which recognize specific DNA sequences but make their cut at seemingly random sites that can be as far as 1,000 base pairs away from the recognition site; Type II, which recognize and cut directly within the recognition site; and Type III, which recognize specific sequences but make their cut at a different specific location that is usually within about 25 base pairs of the recognition site. Type II restriction enzymes can act as precise "molecular scissor" as they cleave within the specific recognition sites. Hence Type II restriction enzymes are used for cutting DNA molecules in recombinant DNA technology.

The first three letters of a restriction enzyme's name are abbreviations of the bacterial species from which the enzyme has been isolated (e.g., Eco- for E. coli and Hin- for H. influenzae), and the fourth letter represents the particular bacterial strain. Roman numerals are also used as part of the name when more than one restriction enzyme has been isolated from the same bacterial strain. So HindIII is restriction enzyme III of strain Haemophilus influenzae Rd strain. They recognize palindromic DNA sequences e.g. recognition and cleavage site of HindIII is 5'-A/ AGCTT-3'. They require Mg2+ as cofactor. Activity of restriction enzymes is represented in units where 1unit is the amount of enzyme that can cleave 1 ug of DNA in 1hr under optimal assay conditions.

Reagents Required

• □–DNA (300ng/ul)

• EcoRI/HindIII

• 10X Assay Buffer

• Sterile water

• 1X TBE (1 lit.) : 10.8 gm Tris

5.5 gm Boric Acid

2ml 0.5 (M) EDTA pH=8

• 6X Gel-loading dye(1 ml):2.5 mg Bromophenol blue

400 mg Sucrose

0.06 ml 0.5 (M) EDTA, pH=8; 0.94 ml sterile water

• Ethidium bromide(10mg/ml)

• Sterile water

Procedure

1. Place all the vials containing lambda DNA, enzyme and buffer on ice.

2. Prepare the restriction digestion mix in a microcentrifuge tube by adding the different components in the order mentioned below

Sterile water – 19 ul

□–DNA – 7ul

10X Assay Buffer – 3 ul

Enzyme – 1 ul

Total - 30ul

3. Tap the tube gently for proper mixing and incubate the mixture at 37oC for 1 hr.

4. Separately digest 4.5 ug of lambda DNA i.e. 15ul of lambda DNA as above and store it at-200C for the ligation experiment.

5. After incubation run the mix on a 1% agarose gel.

6. Prepare 500 ml 1X TBE buffer. Add 1 gm of agarose to 100 ml of 1X TBE and heat it until the agarose dissolves completely.

7. Seal the open ends of the gel tray with tape and place the comb in the proper slot.

8. Add 10 ul of ethidium bromide to the molten gel, pour the gel solution in the gel tray

and allow it to solidify at room temperature.

9. Remove the comb and tapes from the gel tray, put the gel in the buffer tank and immerse it in 1X TBE.

10. Add 6 ul of 6X gel loading dye to the digestion mix and load it in a well of the gel along with uncut lambda DNA and a marker DNA.

11. Run the gel at 100V till the dye front has traversed two-third of the gel.

12. Finally visualize the gel in a uv-transilluminator.

Observation:

Paste the gel image of the digested sample with proper labelling.

Interpretation:

• Compare the digested sample with the uncut DNA and comment whether proper digestion has taken place.

• Compare the digested fragments with the Marker and determine their sizes.

• Comment on the probable number of restriction sites present taking into consideration that lambda DNA is linear

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