Restriction enzymes are type of nucleases biology essay

Science, Biology

AIM: To digest E. coli genomic DNA with Sau 3A1 and pUC19 plasmid DNA with Bam H1.

PRINCIPLE:

Restriction enzymes are type of nucleases that cleave the sugar-phosphate bond present within the nucleic acid. The nucleotide sequence cleaved by the restriction enzymes is called recognition/restriction sites. Hence, these enzymes are called the molecular scissors. In bacteria, the restriction enzymes are components of restriction modification system which functions to protect the cell from invasion of foreign nucleic acid like bacteriophage DNA. However, restriction enzymes do not cleave self DNA. This self-protective mechanism involves methyl transferase enzyme which methylates cysteine or adenosine residues of self DNA. Generally, the restriction enzymes are endonucleases which have recognition sequence within the DNA. A " unit" of restriction enzyme is usually defined as the amount of enzyme needed to digest 1 µg of DNA under standard buffering conditions and temperature. Types of Restriction enzymes: Type 1: These exhibit both DNA restriction and modification activity. They require co-factors such as Mg+2 ions, S-adenosylmethionine and ATP for their activity. The enzyme cleaves the DNA at nonspecific sites which may be 1000 bp apart from actual recognition site. The target DNA is modified before it is cleaved. The enzymes within this group are not used for gene manipulation due to their nonspecific site of cleavage and stringent requirement of co-factors. E. g. Eco K and Eco BType 2: These enzymes cleave the DNA without modifying it and these enzymes require only Mg+2 as a cofactor. The Type II restriction endonuclease cleaves the DNA at specific site. These enzymes hence are

perfect for molecular cloning experiments. E. g. Sau 3a1 and Bam H1. Type 3: These also exhibit both DNA restriction and modification activity. These recognize specific sequence; however, these enzymes cleave the DNA 25-27 bps away from the recognition site. These require Mg+2 as a co factor. Eg. EcoP15I, EcoPI, HinfIII, and StyLTIClass II restriction enzymes generate cohesive and blunt DNA ends, all having 5´-phosphate and 3´-hydroxyl groups: Cohesive ends:- For example, ends generated by Sau 3A1: Cohesive ends:- For example, ends generated by Bam H1Blunt ends:- For example, ends generated by Hae IIISticky ends are produced by cleaving the DNA in a staggered manner in the recognition site, thus, producing single stranded DNA ends. These ends have identical nucleotide sequence. These are called cohesive ends as they can bind to the complementary ends of other DNA fragments cut by the same restriction enzyme. Restriction enzymeOrganismRecognition sequenceBlunt end/ Sticky endOptimum reaction tempInactivation tempSau 3A1Staphylococcus aureus5’GATC3’CTAGSticky25

Bam H1Bacillus amyloliquefaciens5’GGATCC3’CCTAGGSticky37oC60oCIsoschizomers and neoschizomers: Different restriction enzymes, isolated from different organisms having identical recognition sequences are called isoschizomers. Neoshizomers are isoschizomeric enzymes that cleave at different recognition site. Applications:• Prepare molecular probes• Map DNA molecules• Creating mutantsFactors affecting activity of Restriction Enzyme:

Temperature: Generally, digestions are carried out at 37°C. However, there are a few exceptions e. g., digestion with Sma I is carried out at lower temperatures (~25°C), while with Taq I at higher temperature (65°C). Buffer Systems: Tris-HCl is the most commonly used temperature dependent buffering agent. Most restriction enzymes are active in the pH range 7. 0-8. 0. Ionic Conditions: Mg2+ is required by all restriction endonucleases. Other ions (Na+/K+) may be required by different enzymes. Methylation of DNA: Methylation of adenine or cytidine residues within the recognition sequence of the restriction enzyme may affect the digestion of DNA. Star Activity:-An alteration of the specificity of restriction enzyme to cleave specific site on the DNA may occur under some non-optimal conditions. This alteration leads to the cleavage at nonspecific sites. This is called the Star activity. Non-optimal conditions: 1. High pH (> 8. 0). 2. High glycerol concentration3. High concentration of enzyme (> 100 U/µg of DNA). 4. Presence of organic solvents in the reaction (e. g., phenol, chloroform). 6. Incorrect cofactor (Mn2+, Hg2+ instead of Mg2+). To avoid star activity, optimal buffer system must be used. The concentration of enzyme must be as recommended. The DNA preparation should be free of organic solvents and unwanted salts. Requirements: Reagents: 1X restriction bufferSterile deionized waterRestriction enzymes : Sau 3A1 and BamH1Isolated DNA: E. coli DH5α genomic DNAPUC19 vectorAgarose Gel ElectrophoresisHorizontal Electrophoresis Unit with power pack1X tris-glycine bufferAgarose 0. 5-1%Tracking dye (2. 5 mL of 0. 125M Tris buffer + Glycerol-2mL + bromophenol blue-25mg + make volume up to 10mL)Staining dye (Coomassie brilliant blue R250-125mg + Methanol-50mL + G. acetic acid-

10mL + make volume up to 100mL)Destaining solution (methanol-50mL + G. acetic acid-7mL + make volume up to 100mL)Distilled waterMiscellaneous: Eppendorf tubesMicropipetteMicrotipsWaterbath at 65oCIncubatorIceProtocol: Restriction digestion of genomic DNA1. Take 1-2 µg of genomic DNA in an eppendorf tube. 2. Add 1X restriction buffer. 3. Add 5-10 units of Sau 3AI enzyme. 4. Add H2O to make up the volume. Total reaction volume should be 30 to 50 µl. Set up the reaction on ice. 5. Incubate at 37°C for 1 hour. 6. Take a control tube without the restriction enzyme. 7. Heat inactivate the enzyme by incubating at 65°C for 10 minutes. 8. Run the entire reaction volume on 1% agarose gel and extract the DNA smear of 2-8 Kb bands by a gel extraction kit. Store the DNA in water at -20°C till further use. Measure the concentration of DNA. Restriction digestion of the plasmid DNA1. Take 1 µg of pUC19 vector in an eppendorf tube. 2. Add 1X restriction buffer. 3. Add 5 units of BamHI enzyme. 4. Add H2O to make up the volume. Total reaction volume should be 30 to 50 µl. Set up the reaction on ice. 5. Incubate at 37°C for 1 hour. 6. Take a control tube without the restriction enzyme. 7. Heat inactivate the enzyme by incubating at 65°C for 10 minutes. 8. Run the entire reaction volume on 1% agarose gel and extract the DNA band of 3 Kb by a gel extraction kit. Store the DNA in water at -20°C till further use. Measure the concentration of DNA. NOTE: Always keep ice ready. Add water and buffer first. Add enzyme in last step. Use fresh tip for enzyme. Tap the tube and mix well