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QNO1 :Difference bw linker and adaptor

ANS: The main difference between linker and adaptor is that former having blunt ends while later have one cohesive end. Chemically synthesized adaptor molecules which have a preformed cohesive end can be used to insert blunt ended foreign DNA into vector. Adaptors

QNO 2: Difference bw type II and Type II ?

ANS: Most of the useful R-M system is Type II. Type II enzymes recognize a defined sequence and cut within it. Type I systems have similar cofactors and structure to type II but restriction occurs at a distance from recognition site that limits their usefulness.

Qno 3: WRITE the Mechanism of wga?

Ans: Whole genome amplification (WGA) is a group of procedures that allow amplification to occur at many locations in a genome.

Qno4: Write the Mechanism of DNA ligase DNA ligase ?

Ans: An enzyme that creates a phosphodiester bond between the 3' end of one DNA segment and the 5' of another. DNA ligase from *E. coli* and phage T4 encode enzyme, DNA ligase, which seals single stranded nicks between adjacent nucleotides in a duplex DNA chain. i). Joining covalently annealed cohesive ends by DNA ligase. ii). Joining blunt-ended fragments by DNA ligase from phage T4 infected *E. coli*. iii). The third utilizes the enzyme terminal deoxynucleotidyltransferase to synthesize homopolymeric 3' single stranded tails at the ends of fragments.

DNA ligase can repair the nicks produced after the association of cohesive ends of DNA strands.

Qno 5: Write the Limitations of pcr?

Ans: limitations

Contamination risk Primer complexities Primer-binding site complexities

Amplifies rare species

Detection methods

Qno6: write the Western blotting procedure Procedure?

Ans: 1- Sample preparation 2- Gel Electrophoresis 3- Blotting (or transfer) 4- Blocking 5- Antibody probing 6- Detection Procedure 1- The choice of extraction method depends primarily on the sample and whether the analysis is targeting all the proteins in a cell or only a component from a particular subcellular fraction. 2- Samples are loaded into separate wells. A protein marker is also loaded. The separated protein mixtures are transferred to a solid support for further analysis.

3- Transfer can be done in wet or semi-dry conditions. Semi-dry transfer is generally faster. Wet transfer is recommended for large proteins, >100 kD. If you need any other queries, then freely to ask us.

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4- Blocking is a very important step in the immunodetection phase of Western blotting because it prevents non-specific binding of antibody to the blotting membrane.

Qno6: write the Properties of DNA probe ?

Ans: PROBES –PROBES: Labeled material to detect a target. For DNA: 20-30 nucleotides, complementary to a region in the gene or DNA. RADIOACTIVE PROBE - P32: Sensitive Relatively cheap Hazardous Radioactive waste disposal regulations should be followed NON-RADIOACTIVE PROBE –BIOTIN: Sensitive Relatively expensive.. HYBRIDIZATION OF PROBES: The binding between single stranded labeled probe to a complementary nucleotide sequence on the target DNA.. PROBES: Labeled material to detect complementary region in the gene or DNA

QNO7:Write the Nomenclature of restrictions enzyme. ?

Ans:Cutting DNA molecules--Nomenclature : A suitable system was proposed by Smith and Nathans (1973). The species name of host organisms is identified by the first letter of genus and first two letters of specific epithet E. coli = Eco, H. influenzae = Hin Lecture No. 36

Strain identification is written as EcoK . In case, host strain has several restriction and modification systems, these are identified by roman numerals, for example, in case of H. influenzae, HindI, HindII, HindIII etc. All restriction enzymes have general name endonuclease R and modificationmethylase M followed by the system name, for example, in case of H. influenzae. R. HindIII or M. HindIII Examples of restriction endonuclease nomenclature

Qno7:Calculate restriction sites every four, six, eight.?

Ans: . Four base recognition site occurs every 4⁴ (256) bp Six base recognition site occurs every 4⁶ (4096) bp Eight base recognition site occurs 4⁸ (65,536) bp.

Qno8)write the note Mini-primer PCR ?

Ans:MINI-PRIMER PCR

Mini Primer PCR uses a thermostable polymerase (S-Tbr) that can extend from short primers as short as 9 or 10 nucleotides. This method permits PCR targeting to smaller primer binding regions, and is used to amplify conserved DNA sequences, such as the 16S (or eukaryotic 18S) rRNA gene.PCR that can extend from short primers.

Qno10:. Write the first step of RT-PCR?

ANS:First step of RT-PCR - first strand reaction □Synthesis of cDNA using oligo dT primers (37°C) one hour.
□Second strand reaction - digestion of cDNA:RNA hybrid (RNaseH)- □Standard PCR with DNA oligo primers.

QNO10:. What is the purpose of blank control and negative control in PCR reaction?

ANS:BLANK REACTION: Controls for contamination contains all reagents except DNA template.
NEGATIVE CONTROL: Controls for specificity of the amplification reaction contains all reagents and a DNA template lacking the target sequence.

7. What is ALLELE -PCR and write it purpose?

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Allele-specific PCR used for identifying of SNPs. It requires prior knowledge of a DNA sequence, including differences between alleles. Uses primers whose 3' ends encompass the SNP. PCR amplification under stringent

AMPLIFICATION WITH SNP SPECIFIC PRIMER: Successful amplification with an SNPspecific primer signals presence of the specific SNP in a sequence.

conditions is much less efficient in the presence of a mismatch between template and primer.

ALLELE SPECIFIC PCR: This diagnostic or cloning technique is used to identify or utilize single-nucleotide polymorphisms (SNPs).

Qno11: What is southern blot and how DNA cleaved?

ANS:leukocytes. DNA is cleaved into many fragments by restriction enzyme (e.g, BamH1, EcoR1 etc) . The resulting fragments are separated on the basis of size by electrophoresis. The DNA fragments are denatured and

transferred to nitrocellulose membrane for analysis. The labeled probe is added to the blocked membrane in buffer and incubated for several hours to allow the probe molecules to find their targets. Blot is incubated with wash buffers containing NaCl and detergent to wash away excess probe. Radioactive probes enable autoradiographic detection. Southern blot is used to detect the presence of a particular piece of DNA in a sample by a molecular probe.

Qno12:Write a note on Mutation identification?

MUTATIONS IDENTIFICATION BY SOUTHERN BLOTTING MUTATION IDENTIFICATION: The presence of a mutation affecting a restriction site causes the pattern of bands to differ from those seen in a normal gene. A change in one nucleotide may alter the nucleotide sequence so that the restriction endonuclease fails to recognize and cleave at that site. Digestion of genomic DNA to DNA fragments. Size-separation of the fragments. In situ denaturation of the DNA fragments. Transfer of denatured DNA fragments into a solid support (nylon or nitrocellulose). Hybridization of the immobilized DNA to a labeled probe (DNA, RNA). Detection of the bands complementary to the probe (e.g. by autoradiography). Estimation of the size & number of the bands generated after digestion of the genomic DNA will be different. Mutations can be identified by Southern blotting

Qno13: Use of asymmetric PCR?

Ans:Asymmetric PCR is used to amplify one strand of the original DNA more than the other. It is used in some types of sequencing and hybridization probing where having only one of the two complementary strands is ideal. PCR is carried out as usual, but with a great excess of one primers for the chosen strand. Due to the slow amplification later in the reaction after the limiting primer has been used up, extra cycles of PCR are required. It finds use in some types of sequencing and hybridization probing where having only one of the two complementary strands is required.

Qno14:What is the use of methylation PCR?

Methylation-specific PCR is used to identify patterns of DNA methylation at CpG islands in genomic DNA. Target DNA is first treated with sodium bisulfite, which converts unmethylated cytosine bases to uracil,

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which is complementary to adenosine in PCR primers. Two amplifications are then carried out on the bisulfite-treated DNA: One primer set anneals to DNA with cytosines (corresponding to methylated cytosine), and the other set anneals to DNA with uracil (corresponding to unmethylated cytosine). MSP: MSP used in quantitative PCR provides information about methylation state of a given CpG island.

Qno15: Why we use Southern blotting technique?

Ans: Because a **Southern blot** is a method used in molecular biology for detection of a specific DNA sequence in DNA samples. **Southern blotting** combines transfer of electrophoresis-separated DNA fragments to a filter membrane and subsequent fragment detection by probe hybridization.

Qno16: Nested PCR primers?

Ans: Two pairs (instead of one pair) of PCR primers are used to amplify a fragment. First pair amplifies a fragment similar to a standard PCR. Second pair of primers nested primers (as they lie / are nested within the first fragment).

Qno17: write a note on colony PCR? 5

The screening of bacterial or yeast clones for correct ligation or plasmid products. **METHODOLOGY:** The screening of bacterial or yeast clones for correct ligation or plasmid products. Pick a bacterial colony with an autoclaved toothpick, swirl it into

Transfer 20 µl of the supernatant into a new microfuge tube. Take 1-2 µl of the supernatant as template in a 25 µl PCR standard PCR reaction. The screening of bacterial or yeast clones for correct ligation or plasmid products.

Qno18: conventional PCR reagents and their use?

Ans: A technique widely used in Molecular Biology and Biotechnology. Its name is from one of its key component - DNA polymerase. As PCR progresses, DNA generated is itself used as template for replication

1. Multiplex-PCR consists of multiple primer sets within a ----- PCR mixture to produce amplicon of varying sizes that are specific to different DNA sequences. (single PCR)
2. The PCR is preceded by a reaction using reverse transcriptase to convert -(-----) (RNA into DNA)
3. Real Time PCR is a technique in which ----- bind to specific target regions of amplicons to produce fluorescence during PCR. (fluoroprobes)
4. Allele-specific PCR used for identifying of ----- (SNPs)
5. Colony PCR. (The screening of bacterial or yeast clones for correct ligation or plasmid products.)
6. ----- is a polymerase chain reaction that actually takes place inside the cell on a slide (IN SITU PCR)