Bio203 past papers for mid term

Subject: Bio203 (Past papers)

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QNO1) Write first step of RT-PCR? (2 marks)

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.

2) Write down the principle of conventional PCR?(2)

ANS; PCR: 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

3) What is use of Blank reaction and negative control in a PCR reaction? (2)

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.

Q4)Write the extention rate and source of Taq. Polymerase Vent and Pfu? (3 marks) 5.

ANS; Polymerase Extension Rate (nt/sec) Source Taq pol 75 T. T. aquaticus Vent >80 Thermococcus

Vent >80 Thermococcus litoralis

Pfu 60 Pyrococcus furiosus

5) Write down the principle of QRT-PCR? (3)

ANS;Quantitative real-time PCR is often confusingly known as RT-PCR (Real Time PCR) or RQ-PCR. QRT-PCR or RTQ-PCR are more appropriate contractions. QRT-PCR methods use fluorescent dyes, such as Sybr Green, or Q-PCR is commonly used to determine whether a DNA sequence is present in a sample and the number of its copies in the sample. fluorophore-containing DNA probes, such as TaqMan, to measure the amount of amplified product in real time.

6) Write note on Ligation mediated PCR? (3

ANS;METHODOLOGY: Uses small DNA oligonucleotide 'linkers' (or adaptors) that are first ligated to fragments of the target DNA. PCR primers that anneal to the linker sequences are then used to amplify the target fragment

Q7) Write the chemicals used in convential PCR and also reason of used of these chemicals? (5

ANS; MAJOR INGREDIENTS: Microfuge tube, Thermal cycler, DNA template, Primers, Buffer, MgCl2, Distilled H2o, Deoxynucleotide triphosphates, DNA polymerase,

MICROFUGE TUBES: These are small cylindrical plastic tubes with conical bottoms. THERMOCYCLER: The thermocycler works on the principle of Peltier effect, which raises and lowers the temperature of the block in a pre-

programmed manner. DNA POLYMERASES: Taq polymerase, Pfu polymerase, Vent polymerase.

TEMPLATE CAN BE DNA OR RNA: Template DNA, RNA in case of reverse transcriptase

These include DNA cloning for sequencing, gene cloning and manipulation, gene mutagenesis; construction of DNA-based phylogenies, or functional analysis of genes; diagnosis and monitoring of hereditary diseases; amplification of ancient DNA.

QNO8) Differeniate the methodology and principles of Allele specific and AFLP PCR?

ANS; 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).

AFLP PCR

METHODOLOGY: Genomic DNA is digested with one or more restriction enzymes. tetracutter (MseI) and a hexacutter (EcoRI). Ligation of linkers to all restriction fragments. Pre-selective PCR is performed using primers which match the linkers and restriction site specific sequences. Electrophoretic separation and amplicons on a gel matrix, followed by visualization of the band pattern. AFLP is a highly sensitive PCR-based method for detecting polymorphisms in DNA. AFLP can be also used for genotyping individuals for a large number of loci.

QNO9)Write the reagent components of pcr ?

Ans; MAJOR INGREDIENTS: Microfuge tube, Thermal cycler, DNA template, Primers, Buffer, MgCl2, Distilled H2o, Deoxynucleotide triphosphates, DNA polymerase,

Qno10))how template is different in RT- Pcr from conventional Pcr?

RT-PCR is widely used in expression profiling, to determine the expression of a gene. To identify the sequence of an RNA transcrip. 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.

Q11)Write the method of assembly PCR? (3)

ANS; Assembly PCR used to assemble two or more pieces of DNA into one piece. PRINCIPLE: Assembly PCR is the synthesis of long DNA structures by performing PCR on a pool of long oligonucleotides with short overlapping segments, to assemble two or more pieces of DNA into one piece. METHODOLOGY: It involves an initial PCR with primers that have an overlap and a second PCR using the products as the template that generates the final full-length product. ASSEMBLY PCR: Assembly PCR used to assemble two or more pieces of DNA into one piece.

Q12) Write first step of RT-PCR?

ANS; the 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.