

# **Virtual University of Pakistan**

## **Bio203**

### **Methods in Molecular Biology**

**Midterm past papers solved**

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## Bio203

timing: 10:30

My paper

1) multiplex PCR enables the simultaneous amplification in many targets on interest in .....

### One reaction

2) Per amplification specificity is ensured by -----(**polymerase enzyme**)

3) to clone large genes not possible with conventional PCR.( Long pcr)

4) Allele-specific PCR used for identifying of SNPs.

5) **In Situ PCR (ISH)** is a polymerase chain reaction that actually takes place inside the cell on a slide.

6) Assembly PCR is the synthesis of long DNA structures by performing PCR on a pool of long **oligonucleotides** .

7) In sucide is typically used in **polegenetics**.

8) PCR used to produce a unique fingerprints of amplified product lengths is called (**INTER SEQUENCE SPECIFIC PCR** )

9) RT pcr is widely used in ----( **expression profiling**).

10) in nested pcr -----used to amplify a fragment.( **.two pairs of primer**).

11) in molecular technique the RAPD stand for( **.Random Amplified Polymeric DNA**).

12) Denaturation temperature:Reduces double stranded molecules to single stranded, **90–96oC, 20-45 seconds**.

13) primer may occur due to the Hybridization of primer each other.

14) Taq polymerase most commonly used polymerase and code for (**Thermus aqauticus**)..

15) WGA is teachnique in which amplifications proceed \_\_\_in genome.( **Directly at any location**)

16) In annealing temp is lowered to \_\_\_ for 20 - 40 sec.( **40 - 65**)

17) PRIMERS: Primers are single-stranded **18–30 bp** long.

18) ..... Controls for contamination contains all reagents except DNA template.(**Blank reaction**).

19) Source of **Taq polerases** the *Thermus aquaticus*

20) Set of each primers work on the A. **high temp**

## 21) What is WGA technique

Ans: Whole Genome Amplification (WGA) is a method for robust amplification of an entire genome, starting with nanogram quantities of DNA and resulting in microgram quantities of amplified products. WGA has become an invaluable method for preserving limited samples of precious stock material, particularly when using WGA methods that have been developed to amplify material from a single cell

## 22) RAP stands for?

Ans:

### 23) use of methylation in pcr

**Ans:** 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, 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 used in quantitative PCR provides information about methylation state of a given CpG island.

### 24) chemical reagents which is use in conventional PCR and also give reason why they are use

**Ans:** DNA template

- ☐ Primers
- ☐ Buffer
- ☐ MgCl<sub>2</sub>
- ☐ Deoxynucleotide triphosphates
- ☐ DNA polymerase
- ☐ Distilled water

### 25) Write the extension rate and source of Taq, Polymerase Vent and Pfu

<b>Ans:</b> 1) Taq pol	extension rate 75	Source <i>T. aquaticus</i>
2) Vent	extension rate >80	Source <i>Thermococcus Litoralis</i>
3) Pfu	extension rate 60	Source <i>Pyrococcus furiosus</i>

### 26) Differentiate the methodology and principles of Allele specific and AFLP PCR

**Ans: 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.

### **ALLELE SPECIFIC PCR**

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

This diagnostic or cloning technique is used to identify or utilize singlenucleotide Polymorphisms (SNPs).

**Bio203.**

28-6-2018.

10:30am

### **Qno.1.steps of ligation mediated PCR?**

**Ans:** 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 fragments.
- ☐ DNA sequencing
- ☐ Genome walking
- ☐ DNA footprinting

### **Qno.2.uses of methylation specific PCR?**

**Ans:** Answer above

### **Qno.3.negative control and blank reaction ?**

**Ans:** NEGATIVE CONTROL

- ☐ Controls for specificity of the amplification reaction contains all reagents and a DNA template lacking the target sequence.

## BLANK REACTION

- ☐ Controls for contamination contains all reagents except DNA template.

**Qno.4. write the extension rate and source of taq polymerase,pfu,vent?**

**Ans:** Answer above

**Qno.5. Write the chemical reagents used in PCR.give reason why they are used?**

**Ans:** Answer above

**Qno.6. methodology and principle of allelic specific PCR and AFLP PCR ?**

**Ans:** Answer above

All objective from past papers.

## Bio203

**1) What is methylation pcr**

**Ans:** Answer above

**2) chemical reagents which is use in conventional PCR and also give reason why they are use**

**Ans:** Answer above

**3) Write the extension rate and source of Taq. Polymerase Vent and Pfu**

**Ans:** Answer above

**4) Differentiate the methodology and principles of Allele specific and AFLP PCR**

**Ans:** Answer above

**5) FIRST STEP OF RT PCR**

**Ans:** REVERSE TRANSCRIPTASE PCR

- ☐ 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 .

### 6) the name and length of polymerase used mini PCR

**Ans:** Mini Primer PCR uses a thermostable polymerase (S-Tbr) that can extend from short primers as short as 9 or 10 nucleotides.

#### MCQ

- 1) Multiplex PCR enables the simultaneous amplification in many targets on interest in ..... ans) **One reaction**
- 2) RT pcr is widely used in (**expression profiling**).
- 3) . Controls for contamination contains all reagents except DNA template.(**Blank reaction**).
- 4) 2) Denaturation temperature: Reduces double stranded molecules to single stranded, **90–96oC, 20-45 seconds**.
- 5) in nested pcr -----used to amplify a fragment.( **.two pairs of primer**).
- 6) PRIMERS: Primers are single-stranded **18–30 bp** long.
- 7) LONG PCR - to clone( **large genes not**) possible with conventional PCR
- 🕶️ **SOURTH BLOTING USED TO DETECT**
- 9) PCR can be perform to amlify
- 10) TAq polymerase optimum activity tem **70 to 80 c**

Bio203, timing 5:30

What is WAG technique

use of methylation in pcr

chemical reagents which is use in conventional PCR and also give reason why they are used

Differentiate the methodology and principles of Allele specific and AFLP PCR

Principle of ligated mediated Pcr

Negative control and blank reaction

Write 1st step of RT-PCR(2)

Uses of methylation PCR(2)

**Describe denaturation step of PCR(3)**

**Ans:** □ First regular cycling event and consists of heating the reaction to 93°C - 98°C for 20-45 seconds.

- It causes melting of DNA template yielding single strands of DNA.

#### **Denaturation temperature**

↓ Reduces double stranded molecules to single stranded

↓ 90–96oC, 20-45 seconds

### **ALU-PCR(3)**

**Ans:** The use of primers from a commonly repeated segment is called Alu-PCR, and can help to amplify sequences adjacent (or between) these repeats.

Mini-primers(5)

Chemical reagents of PCR and their uses?..(5)

**BIO203**

**23 June 2018. 12:00 pm**

**20 mcq's + 6 questions**

#### **1) how template is different in RT-PCR from conventional PCR?**

**Ans:** In conventional PCR the template is DNA while in RT-PCR the template we use is RNA.

#### **2) methodology of QRT-PCR.**

**Ans:** QRT-PCR methods use fluorescent dyes, such as Sybr Green, or fluorophore-containing DNA probes, such as TaqMan, to measure the amount of amplified product in real time.

#### **3) write first step of RT-PCR.**

**Ans:** 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.

#### **4) write methodology of assembly PCR.**

**Ans:** 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.

#### **5) write the names of chemical reagents used in standard PCR and also write their uses.**

**Ans:** Answer above mentioned.

#### **6) differentiate between methodology and application of ALFP-PCR and Allele Specific PCR.**

**Ans:** Answer above mentioned