

Name : .....

Roll No. : .....

Invigilator's Signature : .....

**CS/M.Tech (MBT)/SEM-1/MBT-101/2009-10**

**2009**

**ADVANCED RECOMBINANT DNA TECHNOLOGY**

Time Allotted : 3 Hours

Full Marks : 70

*The figures in the margin indicate full marks.*

*Candidates are required to give their answers in their own words  
as far as practicable.*

**GROUP – A**  
**( Multiple Choice Type Questions )**

1. Choose the correct alternatives for any *ten* of the  
following : 10 × 1 = 10

i) Eukaryotic RNA polymerase I is specialized to transcribe  
which of the following ?

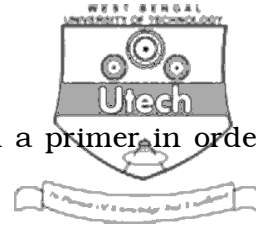
- a) mRNA
- b) tRNA
- c) Ribosomal RNA
- d) Mitochondrial RNA
- e) None of these.



- ii) The part of the bacterial RNA polymerase responsible for recognizing the promoter is the
- a) Alpha subunit
  - b) Rho protein
  - c) DNA Pol. III
  - d) Sigma subunit
  - e) Beta subunit.
- iii) Which of the following is false about the *E.coli* Lac operon ?
- a) It is polycistronic
  - b) It is an example of negative control
  - c) The presence of lactose acts as an inducer
  - d) The repressor binds to the operator
  - e) The *mRNA* for the repressor binds to the operator.
- iv) The RNA primer is removed from the Okazaki fragment by
- a) DNA Pol. I
  - b) DNA Pol. II
  - c) DNA Pol. III
  - d) RNA polymerase
  - e) Exonuclease.



- v) Which of the following enzyme(s) can remove or insert supercoil twists into circular DNA ?
- a) Topoisomerases
  - b) DNA Pol. II
  - c) Spliceosomes
  - d) Helicase
  - e) None of these.
- vi) Repressor molecules bind to the
- a) promoter
  - b) enhancer
  - c) operator
  - d) alpha subunit
  - e) reverse transcriptase.
- vii) Which of the following contains zinc fingers ?
- a) DNA Pol. II
  - b) Transcription factors
  - c) TFIIA
  - d) TFIIID
  - e) Reverse transcriptase.



viii) Which of the following does not need a primer in order to function ?

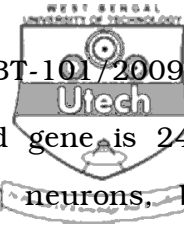
- a) DNA Pol. I
- b) DNA Pol. II
- c) DNA Pol. III
- d) RNA polymerase.
- e) Reverse transcriptase.

ix) The *lac* operon is transcribed when

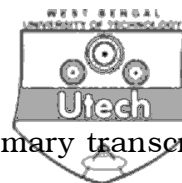
- a) lactose is present and glucose is absent
- b) cAMP concentrations in the cell are high
- c) the cAMP-CAP protein is bound to the *lac* promoter region
- d) the *lac* repressor is bound to allolactose or a similar shaped molecule
- e) all of these.

x) The *trp* operon is transcribed when

- a) tryptophan concentrations in the cell are high
- b) the *trp* repressor is bound to tryptophan or a similar shaped molecule
- c) tryptophan is bound to its aporepressor
- d) the appropriate corepressor is absent
- e) all of these.



- xi) The *mRNA* for this Alzheimer's related gene is 2400 nucleotides long when isolated from neurons, but 2900 nucleotides long when isolated from glial cells. Genomic DNAs isolated from the two cell types show the identical nucleotide sequence. Which of the following mechanisms best accounts for the difference in the sizes of the *mRNAs* ?
- a) Site-specific recombination
  - b) Transposition
  - c) Alternative splicing
  - d) Post-translational modification
  - e) Variations in terminal glycosylation.
- xii) A rho-independent transcription terminator sequence in prokaryotic DNA has
- a) a GC-rich region followed by a string of uracils
  - b) a GC-rich region followed by a string of adenines.
  - c) several stop codons
  - d) the sequences TTGACA and TATATT separated by about 17 bases
  - e) the sequences AAUAAA and a poly-A tail up to 250 nucleotides long, separated by about 20 bases.



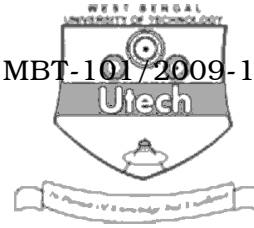
xiii) When an *mRNA* is generated from a primary transcript in eukaryotic cells, which part or parts ~~is/are~~ removed before transport out of the nucleus ?

- a) The 3' poly-A tract
- b) The 3' and 5' non-coding regions
- c) The exons
- d) The 5' cap structure
- e) The introns.

**GROUP – B**  
**( Short Answer Type Questions )**

Answer any *three* of the following. 3 × 5 = 15

- 2. How can you use SOEing in site directed mutagenesis ?  
Explain with a diagram.
- 3. How can you use PCR in deletion and addition ? Explain with a diagram.
- 4. Write two applications for each of the following techniques :
  - a) Chromatin immunoprecipitation
  - b) Methyl interference assay.



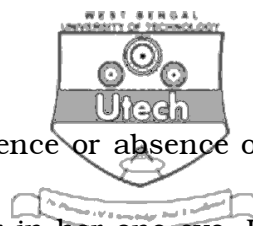
- c) Protein truncation assay
  - d) Transgenics
  - e) Gene silencing.
5. Gene products may be RNA or protein. Describe three gene products that are RNA. How do they function in the cell ?

**GROUP – C**

**( Long Answer Type Questions )**

Answer any *three* of the following.  $3 \times 15 = 45$

6. a) In a real time PCR experiment no upregulation of signal was seen although the gene was supposed to be upregulated in microarray experimentation. What could be the problems in the components of the Real-Time PCR reaction ?
- b) Alpha amanitin is found in poisonous mushrooms and irreversibly binds RNA polymerase in eukaryotic cells. Why do people die when they eat poisonous mushrooms ?

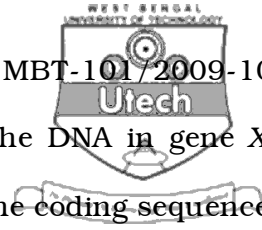


- c) A patient is being tested for the presence or absence of retinoblastoma disease in the tumour in her one eye. It has been found that she has mutation of that gene in that tumour. How will you determine whether that mutation is hereditary or spontaneous ?

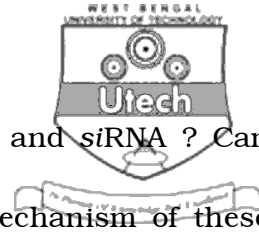
7. What is the function of the following enzymes or proteins in DNA replication ?

Primase, ligase, DNA polymerase 5' to 3' polymerizing activity, DNA polymerase 3' to 5' editing function, DNA polymerase 5' to 3' exonuclease activity, initiator protein, helicase, single stranded DNA binding protein, clamp protein, topoisomerase.

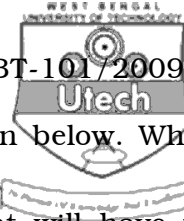
Describe what would happen during DNA replication if a mutation occurred in the genes encoding each of these proteins. ( i.e., How would a mutation in the topoisomerase gene affect DNA replication ? )



8. a) Imagine that a mutation occurs in the DNA in gene X that changes a trp codon ( UGG ) in the coding sequence to a stop codon ( UGA ). In which case ( i or ii ) will this mutation in gene X be the most deleterious to the cell ?
- i) The original trp codon is located at the beginning of the coding sequence for the protein X.
  - ii) The original trp codon is located at the end of the coding sequence for the protein X.
  - iii) Explain your answer, telling what will happen during translation and how this will affect the function of protein X.
- b) With the aid of a cDNA probe, Southern blotting revealed presence of a single copy of a chromosomal gene, whereas, Northern blotting indicated presence of two transcripts of distinct sizes. Western blotting, using a monoclonal antibody raised against the protein coded for by the cDNA, indicated the presence of three components in an extract of the cell line used for preparation of the cDNA. Explain these findings.



9. What are the differences between *miRNA* and *siRNA* ? Can you explain with proper flowchart the mechanism of these two RNAs ? Why the size of the *siRNA* is the way it is ?
10. a) A DNA molecule has the restriction map shown below when digested by *EcoRI* restriction endonuclease ( numbers refer to relative distance of each cut from the left end of the molecule ). When analysed by agarose gel electrophoresis, instead of the expected five bands, the second pattern shown is observed. Explain the possibilities if you heat/do not heat the digested sample to 65° C.



- b) There is a typical *E.coli* promoter shown below. Which arrow indicates the strand of DNA that will have the same sequence as the newly synthesized RNA ( with  $T = U$  ) ?

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