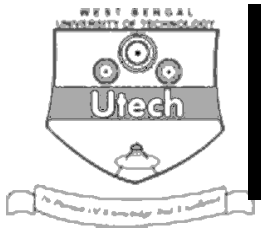
*r*DNA TECHNOLOGY (SEMESTER - 2)

CS/MBT, PHMB, PHMC/SEM-2/MBT/PHMB/PHMC-201/09



1. .....
Signature of Invigilator

2. .....
Signature of the Officer-in-Charge

Reg. No.

Roll No. of the Candidate

CS/MBT, PHMB, PHMC/SEM-2/MBT/PHMB/PHMC-201/09
ENGINEERING & MANAGEMENT EXAMINATIONS, JUNE – 2009
*r*DNA TECHNOLOGY (SEMESTER - 2)

Time : 3 Hours] [Full Marks : 70

INSTRUCTIONS TO THE CANDIDATES :

1. **This Booklet is a Question-cum-Answer Booklet.** The Booklet consists of **32 pages**. **First page** of the Booklet shows Instructions to the Candidates. The **questions** of this concerned subject commence from **Page No. 3**.
2. You have to answer the questions in the space provided marked 'Answer Sheet'. Write on both sides of the paper.
3. Fill in your Roll No. in the box provided as in your Admit Card before answering the questions.
4. Read the instructions given inside carefully before answering.
5. You should not forget to write the corresponding question numbers while answering.
6. Do not write your name or put any special mark in the booklet that may disclose your identity, which will render you liable to disqualification. Any candidate found copying will be subject to Disciplinary Action under the relevant rules.
7. Use of Mobile Phone, Calculator or Log table is totally prohibited in the examination hall.
8. You should return the booklet to the invigilator at the end of the examination and should not take any page of this booklet with you outside the examination hall, which will lead to disqualification.
9. Rough work, if necessary is to be done in this booklet only and cross it through.

No additional sheets are to be used and no loose paper will be provided

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FOR OFFICE USE / EVALUATION ONLY

Marks Obtained

Question Number											***Total Marks***	***Examiner's Signature***
Marks Obtained												

.....
Head-Examiner/Co-Ordinator/Scrutineer

32010 (01/06)

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ENGINEERING & MANAGEMENT EXAMINATIONS, JUNE – 2009

*r*DNA TECHNOLOGY

SEMESTER – 2

Time : 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

Objective Type (Compulsory)

1. Answer the following : 10 × 1

i) Which group for Endonucleases (restriction enzymes) comprise two different enzymes for modification or cleavage yet recognize the same symmetrical target sequence ?

ii) Which viruses are used as high-efficiency vectors for long-term, stable gene expression ?

iii) What two vital features would you consider important in order to maximize high-level transgene expression in animal cells ?

iv) Name two techniques to study protein-protein interactions.

v) Name an appropriate method to determine a full length *m*RNA.

vi) Give examples of neoschizomers.

vii) Name an appropriate means to terminate a growing DNA chain.

viii) The DNA shown below is from the 3′ end of the β-globin gene, which is mutated in sickle cell anemia (autosomal recessive). Which bands would be seen in a Southern blot of DNA from normal subjects digested with EcoRI and hybridized with Probe A ? (Select the right option)

a) 6 kb band only
b) 6 kb band and 10 kb band
c) 4 kb band, 6 kb band and 10 kb band
d) 4 kb band and 10 kb band
e) 4 kb band only.

ix) A particular RFLP is diagrammed below. 'E' represents invariant EcoRI restriction sites. '✳' represents polymorphic EcoRI sites. The dark box represents the location of a particular DNA probe 'A'. What are all the possible alleles (*i.e.* size of bands) seen on a Southern blot probed with 'A' ? (Select the right option)

a) 1 kb, 2 kb, 3 kb, 4 kb, 5 kb, 6 kb
b) 1 kb, 3 kb, 4 kb, 6 kb
c) 3 kb, 4 kb, 6 kb
d) 2 kb, 3 kb, 6 kb
e) None of these.

x) Choosing from the list below, which is a reasonable sequence of steps for cloning a piece of foreign DNA into a plasmid vector ?

1. Transform competent cells
2. Select from the lack of antibiotic resistance gene #1 function
3. Select for the plasmid antibiotic resistance gene #2 function
4. Digest vector and foreign DNA with EcoRI, which inactivates antibiotic resistance gene #1
5. Ligate the digested DNA together.

a) 4, 5, 1, 3, 2

b) 4, 5, 1, 2, 3

c) 1, 3, 4, 2, 5

d) 3, 2, 1, 4, 5

e) None of these.

GROUP - B

Answer any *six* questions. 6 × 10 = 60

2. a) What is the alpha complementation test ?

b) Illustrate very briefly the phenomena of restriction-modification (R-M) of a phage λ on *E.coli C* and E.coli *K*.

c) Outline he steps involved in screening a *c*DNA library in λ phage. 4 + 3 + 3

3. a) Explain schematically the cloning of genomic fragments in any one high capacity vector that you know of.

b) Many gene products (proteins) when overexpressed may be toxic to the host cell. Explain with a suitable example how this problem may be overcome with special reference to genes that express under the control of viral promoters.

c) Name two appropriate vector systems used for expressing proteins fused to protein tags. 4 + 4 + 2

4. a) Merely increasing annealing temperature may not be sufficient to reduce non-specific amplification of undesired products during PCR. Suggest any suitable means (with proper examples) to conduct PCR in order to reduce non-specific product amplification.

b) What is RNAase protection assay and why is it preferred over conventional Northern blotting experiments ?

c) In Real time PCR, which of the two methods, Sybr green or Taqman probe, is more reliable in monitoring DNA synthesis/product formation and why ? 3 + 3 + 4

5. a) Some cancers are caused by the overexpression of a "normal" protein. What therapeutic strategy can be used to treat this type of disease ?

b) Viral-based vectors may be utilized to correct the defect in patient's cells. Briefly explain a method which seems practical.

c) How would you achieve site-directed mutagenesis using PCR techniques ? 3 + 3 + 4

6. a) Distinguish between primer walking and chromosome walking ?

b) What are reporter genes and how are they useful for promoter analysis ? Explain with a suitable example.

c) How would you select cells that are stably transfected ? 4 + 4 + 2

7. a) How would you check protein-protein interaction using an appropriate hybrid system ?

b) Explain schematically how you would introduce foreign DNA using embryonic stem cells.

c) Illustrate with a suitable example how a transgene expression can be targeted to specific tissues. 4 + 3 + 3

8. a) What are heterologous expression systems and what are their main uses ?

b) Single-stranded DNA is used as a template in one of the methods in site-directed mutagenesis. What is that method ?

c) In an expression construct, the junction between the promoter and coding sequence can be made in two functionally different ways, that is, transcriptional or translational fusions. Describe what are meant by a transcriptional fusion and a translational fusion. What are their relative advantages and disadvantages ?

d) How would you purify recombinant proteins and check for purity ? 3 + 1 + 3 + 3

9. a) What is a microsatellite ? Give a very brief description.

b) What type of DNA methods are RAPD and RFLP ?

c) Describe briefly how RAPDs differ from more standard applications of this type of method ?

d) Describe the technique of DNA footprinting in brief. 2 + 4 + 2 + 2

END