

Total number of printed pages – 8

B. Tech
CPBT 8307

Sixth Semester Examination – 2008

DNA TECHNOLOGY

Full Marks – 70

Time : 3 Hours

Answer Question No. 1 which is compulsory and any **five** from the rest.

The figures in the right-hand margin indicate marks.



1. Answer the following questions : 2 × 10
- (a) What is *mic* RNA ? How it differs from *si* RNA ?
- (b) What is the function of Alkaline Phosphatase and Agarase in recombinant DNA technology ?

- (c) Draw graphical sketch of pBR-322 vector along with its gene annotation.
- (d) An *Eco* R-I enzyme has cleaved a genomic DNA of 3.2×10^4 bp by incubating at 37°C for 2 hours. The GC content of the genome is 50%. Calculate the frequency of restriction cleavage and number of restriction fragments generated at the end of this experiment assuming it a complete digestion.
- (e) Differentiate between 'southern blotting' and 'northern blotting'.
- (f) How many number of targeted DNA fragment will be generated by using PCR amplification of 30 cycles, where the initial copy number of template DNA is 20 ?

P.T.O.

CPBT 8307

2

Contd.

- (g) What do you mean by DNA profiling ?
Name two co-dominant Marker system(s)
used in DNA profiling.
- (h) What is isoschizomer ? Name the example
of isoschizomer with suitable explanations.
- (i) An Oligo-nucleotide probe of 50 bp length
labeled with [α - ^{32}P] dCTP has hybridized
with hybrid nylon membrane containing
Hind-III digested human genomic DNA.
Estimate the number bands will be visualized
on an autoradiogram.
- (j) Define Attenuation. Name the operon
system where it regulates transcription.
2. (a) What do you mean by genomic-DNA
library ? Briefly explain the steps involved
during the construction of genomic library
using λ -replacement vector. 2+5

- (b) How many number of genomic clone is
required for 95% coverage of the genome,
if the cloning vector is cosmid and genome
size is 4×10^4 mbp. 3
3. What is ribozyme ? How many types of
ribozymes are there ? Briefly explain the structure
and mode of action of hammerhead
ribozyme with reference to its applications in
auto-immune diseases. 1+2+7
4. (a) Give a brief account on the methods and
strategies used for the expression of
cloned gene with emphasis on recovery
and purification of recombinant proteins.
3+4
- (b) Sketch the structural differences of
eukaryotic and prokaryotic gene using
line diagram. 3

5. (a) Explain the methods of DNA labeling and detection systems using [α - 32 P] dCTP and digoxigenin as nucleotide label.

4+3

- (b) One μ g of λ -phage DNA is used for a nick translation reaction, 25 μ Ci of labeled [α - 32 P] dCTP are used in a 50 μ l reaction. Following treatment with DNA Polymerase -I, one μ l sample is withdrawn and diluted into a total volume of 100 μ l with TE buffer. Five μ l of this dilution are spotted on to a glass fiber filter disc to determine total cpm in the reaction and another 5 μ l are used in a TCA precipitation procedure to determine the amount of radioisotope incorporation. The TCA precipitated sample is collected on a glass fiber disc and washed with TCA and ethanol. Both

filters are dried and counted by liquid scintillation. The filter disc representing the total cpm gives 19000 cpm and TCA precipitated sample gives 11600 cpm. Calculate the percentage of incorporation and estimate the specific activity of the labeled nucleotide product. 3

6. Write down short notes on any *two* of the following : 5 \times 2

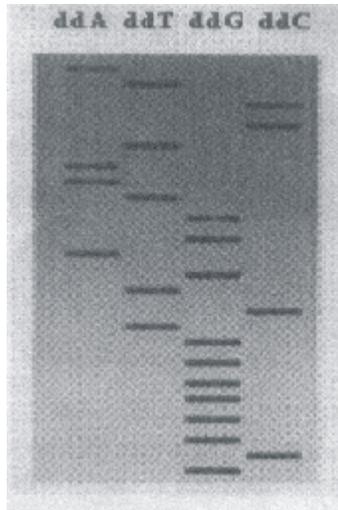
- (a) YAC as cloning vector.
(b) Role of Human genome project in gene therapy.
(c) Post transcriptional processing of m-RNA

7. (a) Briefly explain the Sanger's method of DNA sequencing. How this method was

automated for the sequencing of BAC clones during human genome project ?

4+3

- (b) The following is a part out come autoradiogram of dideoxy sequencing experiment. What is the sequence including polarity of the SS-DNA that served as template for generating this pattern. 3



8. (a) What is Site directed mutagenesis ? Briefly explain the strategies used for creating mutants of target gene using Cassette mutagenesis and PCR based mutagenesis. 6
- (b) What is IBSC ? What role it plays in India with reference to recombinant product development and transgenic research ? 4