BEL711 Recombinant DNA Technology
Major Exam-2nd Semester 2006-07

Max. Marks: 40 **Time: 2 hr.**

Part A: 12 Marks

Q.1 a. Give a schematic diagram of the BAC vector. What is the origin of the *par* loci in the BAC vectors and what is its function? 3

b. Why does the yeast artificial chromosome contain two selectable markers, namely *TRP* and *URA3*? 2

Q.2 a. The mammalian DNA is highly methylated. Could there be a practical significance of this? 2

b. Which property of *E. coli* DNA Polymerase I is most important during nick-translation of DNA? 1

c. What are the cosmid vectors? Draw a schematic diagram of the same and show how cloning can be performed in this vector. 2

d. What is colony hybridization? How would you label a single stranded oligo (what type of enzyme, what type of radioactivity?) 2

Part B-28 Marks

1 a) Consider the following hypothetical DNA sequence, where runs of N represent the sequence complementary to primer

3' NNNNNNNNN ATTCC[150 bases]GATGGCTAGGAAATCTCGCATGGACCTTACGATTAAC 5'

If you sequence the above DNA as a template using conventional radiolabeled base, what bases will you use for chain terminating method? What would be the composition of 4 reaction mixtures? Draw the sequence ladder for first 30 bases. If you want to generate sequence information up to say 250 bases, what operation do you have to carry out and how do you read the sequence? Explain with a hypothetical gel. 4

b) What is the basic difference in the chemistry of radiolabled bases used for conventional and automated DNA sequencing methods? Mention any two advantages of automated method over the conventional one. Why it is called automated? Can you automate conventional DNA sequencing method? Justify your answer. 4

c) What is the basic difference of cycle sequencing from conventional PCR? What is the advantage of cycle sequencing? 2

2 a) What are the two major strategies for whole genome sequencing? Which of these two follows more systematic approach and why do you think it to be so? Show the major steps involved in each of them. If you are required to sequence an organism having a genome of 2000 kb, which of the above two strategies would you prefer and why? 5

b) What are contigs? How are they obtained from, say a BAC library? Suppose 5 clones of a BAC library form a contig. Experimentally, how can you find out that they really are part of a contig? You may give your answer schematically for clarity. 5

3 If you want to measure the levels of a particular gene expression at various stages of its growth by PCR, which of the PCR methods will you use and why? Briefly describe how exactly this method is carried out? Mention the main difference of this method from other PCR methods. What are the principles on which detection by SYBR green, TaqMan and Molecular Beacon are based? Can colorimetric measurement be adapted for real time PCR? 8